Biological Industrial waste-water treatment minimizing biomass Production and maximizing biomass Concentration

J.J. Heijnen

Biological Industrial waste-water treatment
minimizing biomass Production and
maximizing biomass Concentration

Biological Industrial waste-water treatment minimizing biomass Production and maximizing biomass Concentration

PROEFSCHRIFT ter verkrijging van
de graad van doctor in de
technische wetenschappen aan
de Technische Hogeschool Delft,
op gezag van de Rector Magnificus,
prof. ir. B.P.Th. Veltman,
in het openbaar te verdedigen
ten overstaan van het College van Dekanen
op donderdag 22 maart 1984
te 16.00 uur door
JOSEPH JOHANNES HEIJNEN,
geboren te Tegelen,
scheikundig ingenieur

Delft University Press/1984

Dit proefschrift is goedgekeurd door de promotor
Prof. dr. ir. N.W.F. Kossen

Aan Mam en Pap
Aan mijn vrouw Netty
Aan mijn kinderen Linda en Karin

DANKWOORD

Dit proefschrift is een weergave van een groot deel van het onderzoek naar een optimale biologische zuiveringstechniek voor het afvalwater van Gist-Brocades te Delft zoals dit is uitgevoerd tussen 1977 en 1981.

Bij dit onderzoek zijn, naast de promovendus, mensen betrokken uit alle geledingen van Gist-Brocades, die ieder op hun wijze hebben bijgedragen.
Allereerst natuurlijk de Raad van Bestuur en de Directie van de R&D-organisatie van Gist-Brocades die met hun voortdurend enthousiasme deze ontwikkeling sterk hebben gestimuleerd.
Het onderzoek zelf is op voortreffelijke wijze uitgevoerd binnen R&D op laboratorium- en pilot-schaal alwaar collega stafleden, analisten en proces-operators, onder vaak moeilijke omstandigheden mede zorg droegen voor de experimenten met het afvalwater.
De analyses ten behoeve van de experimenten waren groot in aantal en verscheidenheid en moesten soms apart ontwikkeld worden.
Niettemin zorgden de instrumentatie en analyse-afdelingen van R&D en KWL voor snelle service van bestaande analyses en voor de ontwikkeling van nieuwe analyses.
De beschreven afvalwaterreactoren moesten worden gebouwd op laboratorium- en pilot-schaal waarbij de inbreng van de instrumentatie-afdelingen en van procesontwerp en proces-engineeringsafdelingen van Gist-Brocades van onschatbare waarde was.
Ten behoeve van toekomstige implementatie van de onderzoeksresultaten in de praktijk waren er veelvuldige en vruchtbare contacten met staf-medewerkers van de productiebedrijven van Gist-Brocades.
Voor de 3 octrooi-aanvragen die uit dit onderzoek voortkwamen is bekwaam zorg gedragen door de afdeling Octrooien en Merken van Gist-Brocades.

Naast de inbreng van de vele Gist-Brocades medewerkers is de bijdrage van de overheid van grote waarde geweest, zowel door middel van een forse financiële steun door het toenmalige Ministerie van Volksgezondheid, Ruimtelijke Ordening en Milieuhygiëne als door middel van een deskundige inbreng via de "Begeleidingscommissie".

BIOLOGICAL INDUSTRIAL WASTE-WATER TREATMENT MINIMIZING BIOMASS PRODUCTION AND MAXIMIZING BIOMASS CONCENTRATION

1. INTRODUCTION

One of the severe problems for fermentation industries today is the handling of the strongly polluted waste-waters which are produced from fermentation processes and downstream processing of the fermentation broths.

Gist-Brocades (GB) is one of the major fermentation companies in the world which has its main production facility at Delft in the Netherlands. At this location bakers yeast, penicillin and semi-synthetic penicillins are produced.

The waste-waters from the bakers yeast production are evaporated and the resulting slurry of salts and proteins is sold as fodder additive. The biomass from the penicillin production process is mostly sold as a protein source. However, there still remains a concentrated waste-water which is loaded with carbohydrates, proteinaceous matter, organic acids, organic solvents, sulphates and suspended organic material like e.g. penicillin mycelium and yeast cells. This waste-water is also subject to large variations in COD concentration, flow rate, pH and temperature. A further complication is the presence of inhibitive compounds in the waste-water. A characterization of the waste-water is given in Table 1.1 together with the concentration ranges for the several compounds. As an illustration of the variability of the waste-water strength 5 minute analysis of Total Organic Carbon (TOC) values are recorded in Figure 1.1. The variation in daily COD and N values can be seen in Figure 1.2. Figure 1.3 gives a typical example of the variations in temperature and pH.

Because Delft is situated in the most densely populated part of the Netherlands the biological waste-water treatment process has to meet high standards of especially odours. Furthermore the available space at the production plant is very limited, which means that the waste-water treatment plant should occupy a small area.

Therefore a process is required which can treat the waste-water in a small volume high performance reactor where odours can be controlled more easily than in a large volume low performance reactor.

volume	8000 à 12000 m^3/day
flowrate	300 - 600 m^3/hr
temperature	32 - 38°C ; peakvalues 20 - 45°C
pH	6 - 8 ; peakvalues 3 - 12
COD	4000 - 12000 mg/ltr
BOD	2400 - 7200 mg/ltr
Kjeldahl-N	300 - 800 mg/ltr
sediment	0 - 100 ml/ltr
suspended dry matter	0 - 8 gr/ltr
SO_4^{2-}	300 - 1100 mg/ltr
phosphate as P_2O_5	≤ 75 mg/ltr
solvents - COD	400 - 1200 mg/l
solvents	ethanol, butanol, butylacetate, methanol, aceton, propanol, methyl iso-butylketon, iso-butanol

Table 1.1
Composition of the waste-water from Gist-Brocades
(all numbers are daily averaged values)

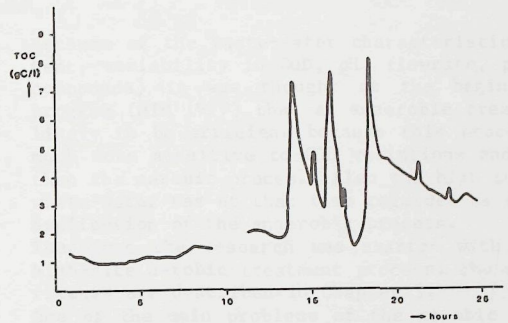


Figure 1.1
Variability in total organic Carbon content of GB waste-water
(analysis each 5 min.)

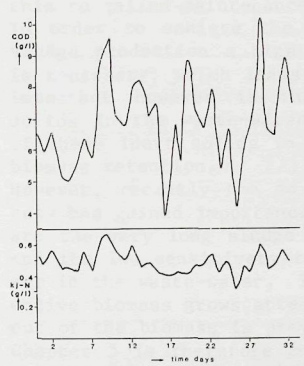


Figure 1.2
Daily average COD and N analyses in GB waste-water

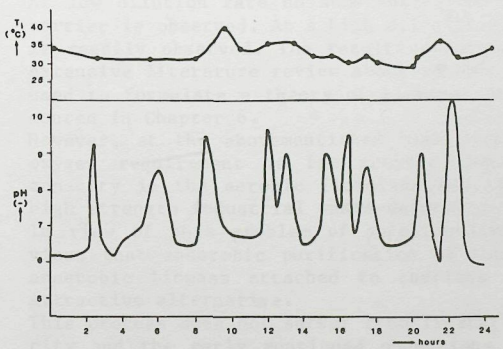


Figure 1.3
Variation in pH and temp. in GB waste-water

2. SCOPE OF THE THESIS

Because of the waste-water characteristics (high sulphate content, variability in COD, pH, flowrate, presence of inhibitive compounds) it was thought at the beginning of the research program (mid 1977) that an anaerobic treatment process was not likely to be efficient because this process is regarded to be much more sensitive to COD variations and inhibitive compounds than the aerobic proces. Also the high sulphate content of the waste-water was at that time regarded as a major problem in the application of the anaerobic process.
Therefore the research was started with the development of a high-rate aerobic treatment process. The results of these experiments are described in Chapter 3.
One of the main problems of the aerobic treatment process appeared to be the gigantic masses of surplus sludge production. Model calculations showed that probably the surplus sludge could be eliminated completely by ensuring that all the substrate is used for cell-maintenance processes, rather than for growth. The calculations and the experiments to substantiate this so called maintenance concept are described in Chapter 4.
In order to achieve the aerobic purification without surplus sludge production a high biomass concentration in the reactor is necessary, which leads to large areas for the settler. More important however is that the presence of inert suspended solids in the waste-water would lead to a steady accumulation of these inert solids in the reactor if a settler is used for biomass retention.
However, recently the so called biological fluidized bed-process has gained importance. The main advantages of this reactor are the very long sludge retention time which can be achieved and the low sensitivety towards the presence of suspended matter in the waste-water. In such a biological fluidized bed the active biomass grows attached to a heavy carrier and thus washout of the biomass is prevented.
Chapter 5 is therefore concerned with the experiments for the cultivation of aerobic biomass which is attached to a carrier and the development of an aerobic fluidized bed reactor. It was found that the liquid dilution rate is a major variable for the establishment of a stable biological fluidized bed reactor.
At low dilution rate no substantial biolayer formation to any carrier is observed. At a high dilution rate biomass attachment is readily observed. The results obtained in Chapter 5 and an extensive literature review about micro-organism attachment are used to formulate a theory of biomass attachment which is presented in Chapter 6.
However, at the abovementioned high liquid dilution rate, the oxygen requirement is far greater than the oxygen transfer capacity in the aerobic fluidized bed reactor, especially for high strength industrial waste-waters.
In view of this problem of aeration-limitation it became obvious that anaerobic purification of the GB-waste water using anaerobic biomass attached to carriers would provide a very attractive alternative.
This process does not suffer from limitations in aeration capacity and the early mentioned objections against the anaerobic process appeared to be less serious than originally assumed (COD, pH and flowrate variations, high sulphate content, inhi-

bitive compounds).
From Chapter 6 it was concluded that an anaerobic fluidized bed reactor should be possible.
The experiments to develop such an anaerobic fluidized bed reactor are described in Chapter 7.
In Chapter 8 a comparative evaluation of the different treatment processes from Chapter 3, 4, 5 and 7 is presented.
For clarities sake the reader is referred to Figure 2.1 which shows schematically the connections between the several subjects which are discussed in this thesis.

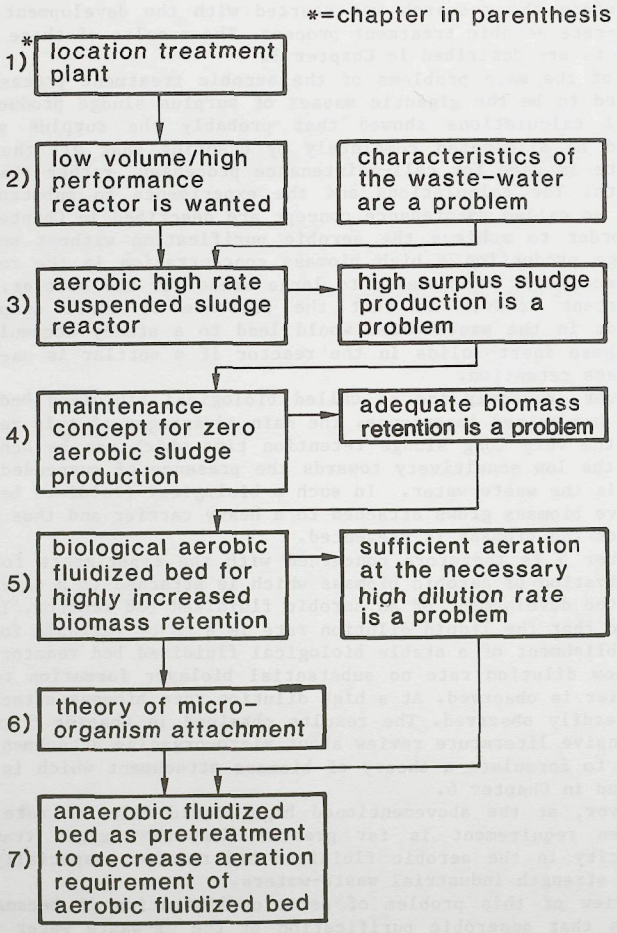


Figure 2.1
Scope of the Thesis.

3. AEROBIC HIGH RATE BIOLOGICAL TREATMENT OF THE GB WASTE-WATER

3.1. INTRODUCTION

From general accepted experience it is known that amongst the biological waste-water treatment processes (aerobic, anaerobic) the aerobic treatment is the most reliable and stable with the highest COD purification efficiency. Therefore it was decided to start with a study of the aerobic treatment process for the purification of the highly variable GB waste-water. This Chapter is concerned with the results of a both theoretical and experimental study. The aspects which are included in the reported research are the purification efficiency of COD, BOD, TOC under conditions of different liquid residence times, different reactor loadings (due to variations in liquid residence times and COD variations), different temperatures, and different waste water pre-treatments like hydraulic buffering, presettling or pH control.
The reactor temperature was varied because of lower cooling costs at the higher temperatures.
Furthermore the sludge settling was studied in order to estimate the maximum attainable biomass concentration in the aerobic reactor for different sedimentation areas.
Finally the oxygen uptake was measured in order to estimate the necessary oxygen transfer capacity, the heat production in the aerobic reactor and the biomass production.
For design purposes a model description of the aerobic treatment process is developed, the parameters of which will be estimated from the reported experiments.

3.2. MODEL FORMULATION

3.2.1 GENERAL INTRODUCTION

The aerobic treatment process consists of an aerobic fermentation reactor and a sedimentation bassin.
A general flowsheet is presented in Figure 3.1.
The waste-water to be treated flows into an aeration vessel with flow Φ_i.
The waste concentration is C_{si}.
In the aeration vessel the mixed liquor suspended solids concentration is C_x and the substrate concentration is C_s.
The temperature in the reactor is T °C. The oxygen needed for the aerobic process is introduced through air injection which also controls the CO_2 ventilation.
Together with the fresh waste-water a flow Φ_r of recycled sludge from the sedimentation vessel with a solids concentration of C_u is pumped into the aeration vessel.
From the aeration vessel a flow $\Phi_r + \Phi_i$ is pumped into the sedimentation vessel. The overflow $\Phi_i - \Phi_w$ of the sedimentation vessel is a more or less clear effluent with a low solids concentration C_e.
The underflow $\Phi_r + \Phi_w$ consists of a concentrated solid suspension, with solids concentration C_u which is separated in a small sludge waste flow Φ_w and a major sludge recycle flow Φ_r to the aeration vessel.

This sludge waste flow has to be maintained in order to obtain a stable sludge concentration C_x in the aeration vessel and a more or less clear overflow from the sedimentation vessel.

In this aerobic waste-water purification process there are 3 important subprocesses:
- the fermentation process for the transformation of soluble wastes into CO_2 and sludge
- the sedimentation of the sludge
- the transfer of O_2 from the air to the broth in the aeration vessel

In the next sections a model description will be proposed for each of these three subprocesses and after this the resulting complete model of the aerobic purification process will be discussed.

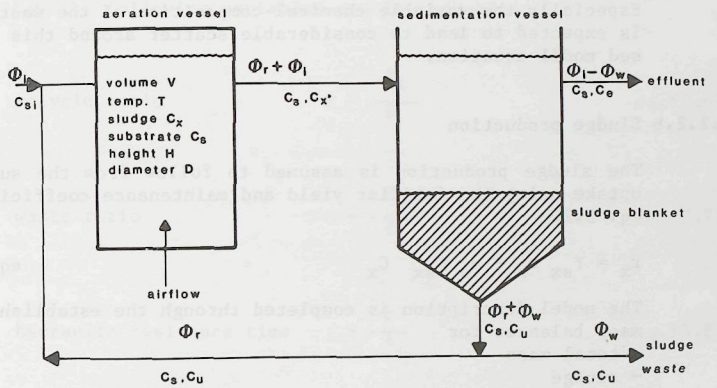


Figure 3.1
Description of the aerobic treatment process with sludge recycle

3.2.2. MODEL FOR THE AEROBIC WASTE-WATER FERMENTATION

In the available literature one is confronted with a multitude of more or less complex fermentation models. For a review the reader is referred to Roels and Kossen (Ref.3.1).
With regard to waste-water treatment processes however the following very simple description is generally used (see Andrews Ref. 3.2.).

3.2.2.a **Substrate uptake**

The following equation is proposed:

$$r_s = q_s^m \frac{C_s \cdot C_x}{K_s + C_s} \qquad \text{eq.(3.1)}$$

This is the familiar Michaelis-Menten equation, which has been formulated for r_s and not for r_x as eq.(3.1a). The choice for r_s circumvents the conceptual problems of the required negative substrate concentration if r_x is negative in eq.(3.1a).

$$r_x = \mu^{MAX} \frac{C_s \cdot C_x}{K_s + C_s} \qquad \text{eq.(3.1a)}$$

The use of the Michaelis-Menten eq.(3.1) in a mixed substrate/mixed culture process as the waste-water treatment process is of course a gross simplification.

The obtained model parameters K_s and q_s^m can therefore not be extrapolated beyond the limits of the present experiments. Especially the variable chemical composition of the waste-water is expected to lead to considerable scatter around this idealised model equation.

3.2.2.b Sludge production

The sludge production is assumed to follow from the substrate uptake using the familiar yield and maintenance coefficients in eq. 3.2.

$$r_x = Y_{sx}\, r_s - m_s\, Y_{sx}\, C_x \qquad \text{eq.(3.2)}$$

The model description is completed through the establishment of mass balances for
- total mass
- sludge
- substrate

3.2.2.c Total mass balance

Because of the low organic C-content of the waste-water (C ≈ 3 gr C/ltr) and the short liquid residence time (≤ 6 hours) in the aeration vessel the C-loss to CO_2 and the water loss due to evaporation can be neglected in the total mass balance.
In the activated sludge process there are three flows which can be varied independantly, Φ_i, Φ_r and Φ_w, and all the other flows do follow from straight total mass balances as indicated in Figure 3.1.

3.2.2.d Sludge balance

It is assumed that the biomass production in the sedimentation vessel does not significantly contribute to the sludge balance since the substrate concentration is small relative to the original substrate concentration. Furthermore it has been assumed that there is no loss in biomass activity during its passage through the settling tank. The steady state sludge balance around the aeration vessel then runs as

$$\Phi_r\, C_u + V r_x = (\Phi_r + \Phi_i)\, C_x \qquad \text{eq.(3.3)}$$

In this equation it is assumed that the influent waste-water Φ_i contains no suspended solids.

The steady state sludge balance around the sedimentation vessel runs as

$$(\Phi_r + \Phi_i)\, C_x = (\Phi_i - \Phi_w)\, C_e + (\Phi_r + \Phi_w)\, C_u \qquad \text{eq.(3.4.)}$$

If it is assumed that the sedimentation vessel is operated under such conditions that no overloading occurs, C_e may be put equal to 0. The conditions under which this assumption is valid will be regarded in Chapter 3.2.3.c on sludge settling. Equation 3.4 reduces then to

$$(\Phi_r + \Phi_i)\, C_x = (\Phi_r + \Phi_w)\, C_u \qquad \text{eq.(3.5.)}$$

Now the following variables are introduced :

recycle ratio $$r = \frac{\Phi_r}{\Phi_i} \qquad \text{eq.(3.6)}$$

waste ratio $$w = \frac{\Phi_w}{\Phi_i} \qquad \text{eq.(3.7)}$$

hydraulic residence time $$\tau = \frac{V}{\Phi_i} \qquad \text{eq.(3.8)}$$

sludge growth rate $$\mu = \frac{r_x}{C_x} \qquad \text{eq.(3.9)}$$

From equation 3.3 and 3.5 the following relation may be obtained after elimination of C_u and after appropriate substitutions from eq. 3.6 - 3.9.

$$\mu = \frac{w\,(1 + r)}{(w + r)} \cdot \frac{1}{\tau} \qquad \text{eq.(3.10)}$$

If C_e is not equal to zero the calculation of μ should proceed according to the more complex equation 3.10a, which follows from eq.(3.3) and eq.(3.4).

$$\mu = \frac{1 + \left\{ \frac{1}{w} - 1 \right\} \frac{C_e}{C_u}}{\frac{\tau\,(w + r)}{w\,(1 + r)} + \frac{\tau\,(1 - w)}{w\,(1 + r)} \cdot \frac{C_e}{C_u}} \qquad \text{eq.(3.10a)}$$

3.2.2.e Substrate balance

The steady state mass balance on substrate around aeration plus settling vessels runs as

$$\Phi_i C_{si} - \Phi_i C_s = V r_s \qquad \text{eq.(3.11)}$$

Elimination of Φ_i with eq.3.8 leads then to

$$r_s = \frac{C_{si} - C_s}{\tau} \qquad \text{eq.(3.12)}$$

Untill now there are 4 equations available eq.(3.1),(3.2), (3.10),(3.12) with 4 unknown variables r_s, r_x, C_s,C_x.

After some algebraic manipulations the following equations are obtained for C_s and C_x.

$$C_s = \frac{K_s}{\left\{ \frac{m_s}{q_s^m} + \frac{w\,(1+r)}{(w+r)\,\tau\,Y_{sx}\,q_s^m} \right\}^{-1} - 1} \qquad \text{eq.(3.13)}$$

$$C_x = \frac{Y_{sx}\,(C_{si} - C_s)}{\frac{w\,(1+r)}{(w+r)} + m_s\,Y_{sx}\,\tau} \qquad \text{eq.(3.14)}$$

The hydraulic residence time τ cannot be diminished indefinitely, because at some instant the hydraulic outflow of sludge through the sludge waste stream will be larger than the sludge growth in the aeration vessel.
Such a situation is called the "wash out conditon". The steady state which is associated with this condition is $C_x = 0$. From eq.(3.14)it follows then that $C_s = C_{si}$.

Substitution of this value of C_s into eq.(3.13) leads then to the following expression for the minimum hydraulic residence time τ_{min}.

$$\tau_{min} = \frac{w\,(1+r)}{(w+r)\left\{ Y_{sx}\,q_s^m\,\frac{C_{si}}{K_s + C_{si}} - m_s\,Y_{sx} \right\}} \qquad \text{eq.(3.15)}$$

Because, especially in industrial waste-water $C_{si} \gg K_s$ and bearing in mind that $Y_{sx}\,(q_s^m - m_s) = \mu^{MAX}$, eq.(3.15) can be reduced to

$$\tau_{min} = \frac{w\,(1+r)}{(w+r)\,\mu^{MAX}} \qquad \text{eq.(3.16)}$$

At first sight it follows from eq.(3.16), that τ_{min} can be lowered indefinitely if the wasteratio w is decreased. However all the above calculations are based on an adequately operating sludge sedimentation vessel. If however the waste ratio is decreased the solids concentration C_x will increase (according to eq.(3.14)), and therefore the solidsload of the sedimentation tank increases and this will result at some instant in overloading of the sedimentation vessel. The sludge will then leave the system in the effluent waste-water stream in stead of through the sludge waste-stream. Therefore it is obvious that there are limits for the solids load of the settling tank which can be realised in practice. This limiting solids load will follow from a model of the sludge settling process which will be outlined in the next Chapter.

3.2.3. MODEL FOR THE ACTIVATED SLUDGE SETTLING TANK.

In Figure 3.2 an idealised schematic picture is provided for the sludge settling tank.
The sludge/waste-water mixture which leaves the aeration tank with a flowrate $\Phi_i + \Phi_r = (1 + r)\,\Phi_i$, in which the solids concentration is C_x, is pumped into the settling tank.
This flow is then divided in an upwards and a downwards flow in the settling tank. The downwards flow is equal to the sum of the recycle and sludge wasteflow, $(r+w)\,\Phi_i$, and the upward flow follows then immediately from a mass balance and is equal to $(1-w)\,\Phi_i$.
The settlingtank has an area A, the sludge concentration in the overflow is C_e and the sludge concentration in the recycle/ sludge wasteflow is C_u.

In general a settling tank has 2 functions:
1 Clarification of the overflow.
2 Sludge thickening of the underflow.

Clarification takes place in the upper part of the settling tank and sludge thickening occurs in the lower part of the tank, where both parts are separated at the point of entrance of the mixed liquor as indicated in Figure 3.2.
In the next sections a model description of the settling tank will be provided based on the mass flux theory of Kynch (Ref. 3.3). Beyond this, a model is needed to describe the settling behaviour of the sludge to complete the description of the settling tank. Therefore this sludge settling model will be first dealt with in the following section.

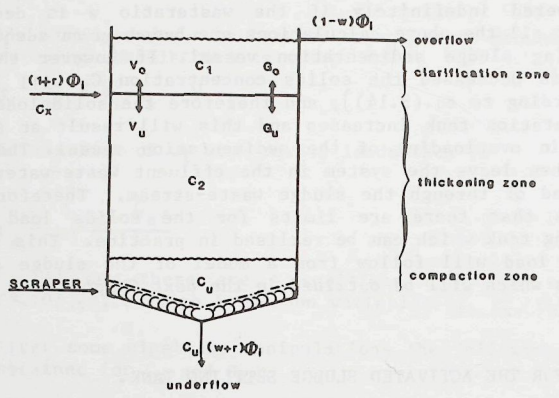


Figure 3.2
Description of the settling tank

3.2.3a. Model for the sludge settling

It is generally assumed that the settling velocity of the sludge is only a function of the sludge concentration (Ref. 3.4, 3.5).
Thus it is assumed that the direct solids interaction can be neglected.
This interaction does of course occur at the microbe-microbe level due to microbial flocculation. However the interaction between flocs is far less. Therefore solids interaction can be neglected if flocculation time is much smaller than the solids retention time in the settler. This is generally the case as flocculation occurs within minutes whereas the solids retention time is much longer (≥ 30 minutes).
According to Ref. 3.4 and 3.5 the following equation 3.17 gives a good correlation between settling velocity V and solids concentration C

$$V = a\ C^{-b} \qquad \text{eq.(3.17)}$$

It is obvious that according to eq (3.17) $V \to \infty$ if $C \to 0$.
This equation is therefore only valid at the higher solids concentration range, which does occur in the thickening zone of the settling tank.
The parameter values a and b in equation (3.17) are a = 30 → 100 for different sludge types and b = 2.25, (Ref. 3.5 and 3.6) and therefore eq (3.17) can be written as

$$V = (30 \to 100)\ C^{-2.25} \qquad \text{eq.(3.18)}$$

The description of the sludge settling as given above is only valid for one specific sludge, because each sludge has its own value of a in eq. (3.17).

However the settling properties of the sludge do vary with time. The most widely used parameter to express this variability is the Sludge Volume Index (SVI).

A possible way to incorporate in eq.(3.18) the variability of the sludge settling properties is eq (3.19).

$$V = a_1 \left\{ \frac{C}{\left\{ \frac{1000}{SVI} \right\}} \right\}^{-2.25} \qquad \text{eq.(3.19)}$$

The rationale behind eq.(3.19) is that the SVI value is equivalent to the effective sludge volume per gram dry matter and thus SVI * C is a measure of the sludge volume in the settling sludge suspension. Therefore eq.(3.19) gives the settling velocity of the solids suspension as a function of the effective solids volume fraction.
The constant in eq. (3.19) can be roughly estimated from published settling data of sludge with different SVI values (Ref. 3.7). Processing of these data, where SVI varied between 50-250, gives

$$V = (\ 0.17 \pm 0.04\ ) \left\{ \frac{C * SVI}{1000} \right\}^{-2.25} \qquad \text{eq.(3.20)}$$

It is stressed once more that this equation is not valid for very dilute sludge suspensions and should only be applied in the thickening zone of the settling tank.

3.2.3.b Mass flux analysis in the thickening zone

In the thickening zone there is a downward linear velocity V_u which can be calculated from the massflow according to eq.(3.21), where A is the surface area of the settling tank

$$V_u = \frac{(\ w + r\ )\ \Phi_i}{A} \qquad \text{eq.(3.21)}$$

The solids in the thickening zone are transported downwards due to
- convective movement of the liquid
- gravity settling of the solids

The convective solids flux is equal to $V_u * C$.
The gravity solids flux is equal to V * C, where V is the settling velocity of the sludge suspension according to eq.(3.20).

Thus the total solids flux G_u in downward direction can be calculated from eq.(3.22).

$$G_u = 0.17 * C \left\{ \frac{C * SVI}{1000} \right\}^{-2.25} + V_u C \qquad \text{eq.(3.22)}$$

This solids flux is equal to the amount of total solids which leaves the settling tank through the underflow, which is equal to eq.(3.23):

$$G_u = V_u \, C_u \qquad \text{eq.(3.23)}$$

If the settling tank is in stable operation, all solids are removed through the underflow, thus $C_e = 0$.
This last assumption is dealt with in detail in Chapter 3.2.3.c. C_u can then be calculated from the solids mass balance around the settling vessel (eq. 3.4), which results in

$$C_u = \frac{(1 + r)}{(w + r)} C_x \qquad \text{eq.(3.24)}$$

Because C_x, w, r and V_u are known, the solids concentration in the settling tank can be calculated from eq.(3.22 - 3.24). This is the concentration C_2 in Figure 3.2. The above calculations are only valid if the settling tank is in a stable operation, which usually means that the solids flux G_u is not to high.
In practice one wishes to have the highest possible G_u, because this leads then to small area's of the settling tank. However for dispersed systems there exists a limit in the dispersed mass flux (Wallis Ref. 3.8). Higher dispersed mass fluxes do lead to instability phenomena like flooding in bubble columns, bubble formation in homogeneously gas/solids fluidized beds, and also to solids carry over from thickening zone into clarification zone in settling tanks. This last phenomenon leads to solids loss in the overflow.
This solids loss leads then to a decreased solids concentration in the aeration tank. If the overloading of the settling tank is very heavy, the very substantial decrease in C_x may result in a substrate load of the sludge which is higher than the maximum substrate load q_s^m, and this leads then to a collapse of the purification efficiency.

According to Wallis (Ref. 3.8) the stability criterion for stable settler operation is given by eq.(3.26)

$$V_u \geq \frac{-d\,[\,C * V\,]}{d\,C} \qquad \text{eq.(3.26)}$$

Thus the settling tank operation becomes unstable at

$$V_u = \frac{-d\,[\,C * V\,]}{d\,C} \qquad \text{eq.(3.27)}$$

Substitution of V from eq.(3.20) in eq.(3.27) and performing the differentiation gives eq.(3.28)

$$V_u = 0.17 * 1.25 \left\{ \frac{C * SVI}{1000} \right\}^{-2.25} \qquad \text{eq.(3.28)}$$

If C is eliminated from eq. (3.28) and (3.22) the following relation is obtained for the downwards mass flux under limiting stability conditions

$$G_u^1 = \frac{905}{SVI} * V_u^{0.556} \qquad \text{eq.(3.29)}$$

Thus if the downwards solids flux is increased beyond the value of eq. (3.29) the settling tank operation becomes unstable and there occurs carry-over of solids to the overflow.

3.2.3.c Mass flux analysis in the clarification zone

In the clarification zone there is an upward linear velocity V_o which can be calculated from the overflow rate, according to equation (3.30), where A is the surface area

$$V_o = \frac{(1 - w)\,\Phi_i}{A} \qquad \text{eq.(3.30)}$$

The solids in the clarification zone are transported due to two processes:
- convective upward movement
- gravity settling of the solids downwards.

The convective solids transport is $V_o C$ and the gravity solids transport is $-VC$ where V is the settling velocity of the sludge suspension.
In the dilute solids suspensions of the clarification zone eq.(3.20) is not applicable, because $V \to \infty$ if $C \to 0$.
Another equation which is often used (ref. 3.4) for the relation between V and C is eq.(3.31)

$$V = V^{MAX} \exp(-k\,C) \qquad \text{eq.(3.31)}$$

In this equation $V \to V^{MAX}$ if $C \to 0$.
With equation (3.31) the total solids mass flux G_o in the clarification zone can be calculated as eq.(3.32).

$$G_o = V_o\,C - V^{MAX}\,C \exp(-k\,C) \qquad \text{eq.(3.32)}$$

This solids mass flux is equal to the amount of total solids which leaves the settling tank through the overflow which is equal to :

$$G_o = V_o\,C_e \qquad \text{eq.(3.33)}$$

Elimination of G_o from eq.(3.32) and (3.33) gives eq.(3.34).

$$V_o\,(C - C_e) = V^{MAX}\,C \exp(-k\,C) \qquad \text{eq.(3.34)}$$

The solution of this equation, which gives the concentration C_1 in the clarification zone, is most easily performed grafically according to Figure 3.3.

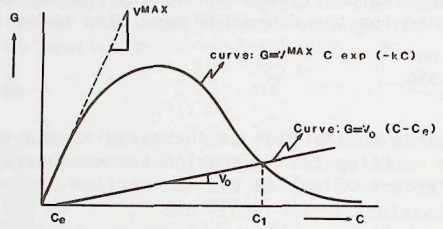


Figure 3.3
Graphical solution of eq.(3.34)

In this Figure 3.3 two relations are represented, whose intersection point at concentration C_1 is the solution of eq.(3.34). At concentration point C_1 however it is obvious that $V \geq \frac{dG}{dc}$ which according to Wallis (Ref. 3.8) is an unstable condition. This means that eq.(3.34) as such has no stable solution. However on a closer look it appears that a second solution of eq. (3.34) is possible at C = 0 for C_e = 0 which is also a stable solution. This means that the clarification zone in stable operation contains no solids.
If the stability criterion of Wallis is then applied, to calculate at which V_o value the clarification zone becomes unstable, this is found to occur at $V_o \geq V^{MAX}$ values.

3.2.4 COMBINATION OF THE FERMENTATION MODEL AND THE SLUDGE-SETTLING MODEL

The maximum solids flux for a settling tank is given by eq.(3.29). From simple mass considerations this solids flux can be shown to be equal to

$$G_u = \frac{(1+r)\,\Phi_i\,C_x}{A} \qquad \text{eq.(3.35)}$$

From eq.(3.35), eq.(3.21) and eq.(3.29) the following eq.(3.36) for C_x can be obtained

$$C_x = \left\{ \left(\frac{\Phi_i}{A} \right)^{0.444} \frac{(1+r)}{(w+r)^{0.556}} \frac{SVI}{905} \right\}^{-1} \qquad \text{eq.(3.36)}$$

Elimination of C_x from eq.(3.36) and eq.(3.14) and replacement of the term w(1+r)/(w+r) with eq.(3.10) results in the following eq.(3.37) for the liquid residence time in the aerated fermentor which can be maintained without overloading the settling tank:

$$\tau = \frac{(C_{si} - C_s)}{\left(\frac{\mu}{Y_{sx}} + m_s \right)} * \left(\frac{\Phi_i}{A} \right)^{0.444} \frac{1+r}{[w+r]^{0.556}} \frac{SVI}{905} \qquad \text{eq.(3.37)}$$

Subsequent substitution of the term $\left\{ \frac{\mu}{Y_{sx}} + m_s \right\}$ with the help of the eq.(3.1) and (3.2) results in eq.(3.38).

$$\tau = \frac{(C_{si} - C_s)(K_s + C_s)}{q_s^m \quad C_s} * \left(\frac{\Phi_i}{A} \right)^{0.444} \frac{(1+r)}{(w+r)^{0.556}} \frac{SVI}{905} \qquad \text{eq.(3.38)}$$

The term $\frac{1+r}{(w+r)^{0.556}}$ in eq.(3.38) can be approximated by a constant value of 1.94 for $0 \leq r \leq 2.0$ and $0 \leq w \leq 0.3$, as shown in table 3.1

r \ w	0	0.05	0.1	0.2	0.3
2	2.04	2.01	1.99	1.94	1.89
1	2.0	1.96	1.90	1.81	1.73
0.5	2.20	2.09	1.99	1.83	1.70

Table 3.1 Value of $\frac{1+r}{(w+r)^{0.556}}$

Therefore eq.(3.38) can be written as eq.(3.39)

$$\tau = \frac{(C_{si} - C_s)(K_s + C_s)}{q_s^m \quad C_s} \left(\frac{\Phi_i}{A} \right)^{0.444} \frac{SVI}{905} * 1.94 \qquad \text{eq.(3.39)}$$

From eq.(3.39) it appears that the required residence time of the waste-water in the aeration tank depends on

-surface area A of the settling tank; an increase in surface area leads to lower liquid residence times
-sludge settling quality expressed as the parameter SVI; a bad

settling sludge with high SVI value's leads to higher τ-values.

- -the maximum sludge activity q_s^m; a highly active sludge with high q_s^m values leads to low τ-values.
- -The substrate concentration C_{si} in the polluted waste-water; a highly polluted waste-water , with high C_{si} values leads to high τ values.
- -The substrate concentration C_s in the purified waste-water; if a high degree of purification is required with low C_s values the τ values do increase. It also appears from eq. (3.39) that the condition of $C_s = C_{si}$ (no purification at all, or "wash-out condition") is reached at $\tau = 0$. This means that, due to sludge recycle, the wash-out situation can never be reached, which is of course quite logical.

Eq.(3.39) gives the minimum allowable τ-value, or the maximum allowable waste-water flow rate Φ_i into the aeration tank (because $\tau = \frac{V}{\Phi_i}$), before the settler is overloaded. Decrease of τ (or increase of Φ_i) beyond this value leads to a decreased biomass concentration C_x in the aeration tank (according to eq.(3.36)).
Therefore the sludge substrate load is increased because C_x is lower and the substrate load in the reactor is higher (τ was decreased).This increased sludge load means a less efficient purification because C_s rises. However because of the small value of K_s the decrease in purification efficiency is at first insignificant.
If however the τ-value is decreased more and more the sludge load eventually reaches q_s^m, and then the purification efficiency collapses because C_s does increase very much.
Therefore from eq. (3.39) a critical τ-value, τ_{crit}, can be calculated by realizing that in such a case $C_s \gg K_s$ and from eq. (3.39) it follows then:

$$\tau_{crit} = \frac{\left(C_{si} - C_s \right)}{q_s^m} \left\{ \frac{\Phi_i}{A} \right\}^{0.444} \frac{SVI}{905} * 1.94 \qquad \text{eq.(3.40)}$$

Combination of eq. (3.39) and (3.40) gives the following relation between τ_{crit} and τ

$$\frac{\tau}{\tau_{crit}} = \frac{K_s + C_s}{C_s} \qquad \text{eq.(3.41)}$$

This equation is presented graphically in Figure 3.4. It can be seen that for $\frac{\tau}{\tau_{crit}} \leq 2$ the C_s-values do increase very rapidly. This means that for τ-values in this regime, the purification efficiency decreases very rapidly if an hydraulic shock load occurs due to e.g. raining. In practice therefore it is advisable that $\tau \geq 2\tau_{crit}$, which leads then to a stable process operation.

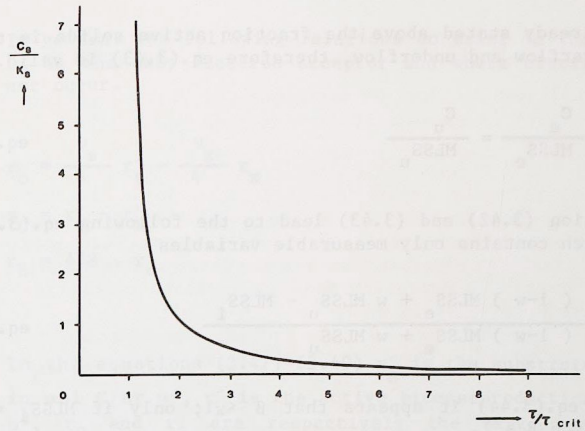


Figure 3.4
Relation between effluent substrate concentration and hydraulic residence time

3.2.5 EFFECT OF SUSPENDED SOLIDS IN THE WASTE-WATER ON THE MODEL DESCRIPTION

In the previous description of the aerobic activated sludge process with sludge recycle it has been assumed that the waste-water contains only soluble substrate. From Table 1.1 in Chapter 1 it is obvious that the GB waste-water contains a large amount of suspended solids. Because the research aims at a high rate aerobic process with short liquid residence times it can be assumed that these suspended solids are not degraded in any appreciable extent. As a consequence these influent solids can be treated as inert solids which do enter the system and leave the system unchanged. It is further assumed that there is no difference in settling rates between inert solids and biologically active solids, because these inert solids are mainly biological like yeast and mycelium.

This last assumption means that the mixed solids in the activated sludge system contain a fraction β of active solids which is the same in the aeration tank, the settling tank and the waste sludge. The fraction β can then be calculated from the appropriate mass balance on total solids (inert and active solids) over the total installation (reactor and settler).
This balance runs as eq.(3.42).

$$\Phi_i * MLSS_i = (1-w) \Phi_i \left\{ MLSS_e - C_e \right\} + w \Phi_i \left\{ MLSS_u - C_u \right\} \qquad \text{eq.(3.42)}$$

In this equation (3.42), C is the concentration of active solids, MLSS is the concentration of total solids and hence (MLSS - C) is the concentration of inert solids.

As already stated above the fraction active solids is the same in overflow and underflow, therefore eq.(3.43) is valid.

$$\beta = \frac{C_e}{MLSS_e} = \frac{C_u}{MLSS_u} \qquad \text{eq.(3.43)}$$

Equation (3.42) and (3.43) lead to the following eq.(3.44) for β which contains only measurable variables

$$\beta = \frac{(1-w)\,MLSS_e + w\,MLSS_u - MLSS_i}{(1-w)\,MLSS_e + w\,MLSS_u} \qquad \text{eq.(3.44)}$$

From eq.(3.44) it appears that $\beta \leq 1$; only if $MLSS_i = 0$ the fraction $\beta = 1$.
The consequences of the presence of inert solids on the model description in Chapter 3.2 - 3.4 are relatively simple.
Because β is the same in overflow and underflow and in the aeration tank the calculation of μ according to eq.(3.10) or (3.10a) is not affected.
The settling vessel however is loaded with a mixture of active and inert solids and hence the maximum active solids flux is only a fraction β of the maximum total solids flux according to equation (3.29). This then leads to modified eq.(3.39) and (3.40) which are given by eq.(3.45) and (3.46)

$$\tau = \frac{(C_{si} - C_s)}{\beta\, q_s^m} * \frac{(K_s + C_s)}{C_s} \left\{ \frac{\Phi_i}{A} \right\}^{0.444} \frac{SVI}{905} * 1.94 \qquad \text{eq.(3.45)}$$

$$\tau_{crit} = \frac{(C_{si} - C_s)}{\beta\, q_s^m} \left\{ \frac{\Phi_i}{A} \right\}^{0.444} \frac{SVI}{905} * 1.94 \qquad \text{eq.(3.46)}$$

The only modification is the change of q_s^m into $\beta\, q_s^m$.
Therefore the direct consequence of the presence of inert solids is the increase of required liquid residence time.

3.2.6. OXYGEN REQUIREMENT, CO_2-PRODUCTION, HEAT-PRODUCTION AND OXYGEN TRANSFER

In general the oxygen requirement, the CO_2-production and heat-production can be calculated from substrate uptake and biomass-production using the method of elemental and enthalpy balances. For a detailed discussion of this technique the reader is referred to a review of Roels (Ref. 3.14).

In general the following relations do exist in this case where O_2 is the only electron acceptor and where nitrification does not occur.

$$r_o = \frac{\nu_s}{4} r'_s - \frac{\nu_x}{4} r_x \qquad \text{eq.(3.47)}$$

$$r_c = r'_s - r'_x \qquad \text{eq.(3.48)}$$

$$r_H = 478 \cdot r_o \qquad \text{eq.(3.49)}$$

In the equations (3.47)-(3.49) r'_s is the substrate consumption in mol C/hr m^3, r'_x is the active biomassproduction in mol C/hr m^3, r_o and r_c are respectively the oxygenconsumption and the CO_2 production in mol/m^3hr and r_H is the heatproduction in kJ/m^3 hr. ν_s and ν_x are the degrees of reduction of substrate and biomass (Ref. 3.14). Furthermore it is known that the organic fraction of biomass has a fairly constant C-content of 48% and that ν_x can be regarded to be 4.19 (Ref. 3.14). The problem is then to find a value for ν_s and a way to convert r'_s to the more conventional units of kg/m^3 hr (either in COD, BOD or TOC).
The most appropriate procedure is to characterize the waste substrate in terms of COD and a parameter α, which is equal to the weight of C per weight of COD. After some algebra the following relations are obtained.

$$\nu_s = 4 * \frac{12}{32\,\alpha} \qquad \text{eq.(3.50)}$$

$$r_o = \frac{r_{COD}}{0.032} - \frac{4.19}{4} * \frac{0.48}{0.012} r_x \qquad \text{eq.(3.51)}$$

$$r_c = \frac{\alpha\, r_{COD}}{0.012} - \frac{0.48}{0.012} r_x \qquad \text{eq.(3.52)}$$

Equations (3.51) and (3.52) can be used in two ways, either to calculate the oxygenconsumption and CO_2 production from measured COD and biomass conversion rates or to calculate the biomass conversion rate from measured CO_2, O_2 and COD conversion rates.
The heat production can be calculated by elimination of r_o from eq. (3.51) and eq. (3.49)
This then leads to eq. (3.53), where $Y_{COD} = r_x/r_{COD}$

$$r_H = 15000\,[\,1 - 1.34\,Y_{COD}\,]\,r_{COD} \qquad \text{eq.(3.53)}$$

Because $r_H = 4190 \, (T - T_i) / \tau$ and $r_{COD} = \frac{C_{si} - C_s}{\tau} = \frac{\Delta C_{COD}}{\tau}$

eq.(3.53) can be used to calculate the waste-water temperature rise ($T-T_i$) in absence of cooling from eq.(3.54)

$$T - T_i = 3.6 \, \Delta C_{COD} \, [1 - 1.34 \, Y_{COD}] \qquad \text{eq.(3.54)}$$

If it is assumed that the biomass yield on COD is about 0.4 it can be calculated that the removal of 1 g COD/ltr gives rise to 1.6°C increase in reactor temperature. In industrial waste-waters with COD concentrations of up to 10 g/ltr, the temperature rise can thus be up to 16°C.
It is therefore important to operate the aeration tank on the highest possible temperature, in order to cut down in cooling equipment and cooling water requirements. When the oxygen requirement is known there remains the problem of O_2-transfer. Surveying the literature on O_2 transfer in aerobic industrial waste-water treatment processes it became apparent, that there is a trend towards a high bubble columnreactor for the aeration of the waste-water (Turmbiologie Ref. 3.19, ICI deep shaft Ref. 3.18, Biohochreactor Ref.3.16). This trend is understood because waste-water reactors tend to become very large (up to thousands of m^3)and hence stirred vessels do suffer from the construction problems of very large stirrers and stirrer drives.Other important advantages of tall bubble colums are the relative small area and the high efficiency of O_2-utilisation wich leads to lower air consumption (see also below). Furthermore it is indicated in a recent review about mass transfer by Heijnen and Van 't Riet (Ref. 3.15) that the oxygen transfer efficiency in kg O_2/kWhr is not different for stirred vessels and bubble columns in low viscous systems. It appears that a very simple design rule can be applied for bubble columns, wich was formulated by Jackson and co-workers (Ref. 3.10 and 3.11). This design rule is that per meter of unaerated liquid height there is a maximum removal of 0.55 % O_2 from the sparged air in the bubble column, irrespective of the air superficial velocity. This means for example that in a bubble column of 20 m. liquid height the minimum O_2content of the air in the reactor outlet 21 - 20 * 0.55 = 10 %.
This design rule is only valid for coalescing systems like the present waste-water studied.
The maximum (C_{OL} = 0) oxygen transfer is then directly coupled to the maximum air superficial velocity which can be allowed due to gas hold-up and/or foaming problems which do occur at high gas superficial velocities. This maximum velocity is about V_{sg} = 360 m/hr (Ref. 3.15).

The maximum oxygen transfer rate OTR can therefore be calculated from eq (3.55).

$$OTR = V_{sg} * \frac{V}{H} * 44.6 * 0.55 * 10^{-2} * H \qquad \text{eq.(3.55)}$$

From equation (3.55) one can see immediately that the required air flow (which is $V_{sg} \times \frac{V}{H}$) to achieve a certain OTR decreases proportional to an increase in reactor liquid height H.

The oxygen uptake rate OUR is directly related to the substrate loading of the reactor and can be calculated from eq.(3.56).

$$OUR = \Phi_i * (C_{si} - C_s) * Y_{so} \qquad \text{eq.(3.56)}$$

Y_{so} is the oxygen requirement per unit substrate (mol O_2 per kg substrate).
Combination of eq.(3.55) and (3.56) leads to eq.(3.57) which gives the minimum required superficial gasvelocity to aerate the aerobic fermentor where waste-water with a substrate concentration C_{si} is treated in τ hours residence time and a waste concentration C_s results.

$$V_{sg} = \frac{(C_{si} - C_s) * Y_{so}}{\tau} * 4.08 \qquad \text{eq.(3.57)}$$

As already mentioned there is a certain maximum V_{sg} value which can be applied without problems of gas-hold up or foaming. This value is around V_{sg} = 360 m/hr. (Ref.3.15).
This means that there will be a certain minimum liquid residence time from the aeration point of view, because of limited aeration capacity in bubble columns.
If a common value of Y_{so} = 20 mol O_2/kg COD is used as parameter in eq (3.57) the curve in Figure 3.5 can be calculated for the minimum liquid residence time for various values of (C_{si} - C_s) and gas superficial velocities.
From these calculations it appears that for an appreciable COD conversion (4 - 6 gr COD/ltr waste-water) the waste-water residence time in the bubble column cannot be lowered beneath 1 to 3 hours because of limiting O_2 transfer from gas → liquid.

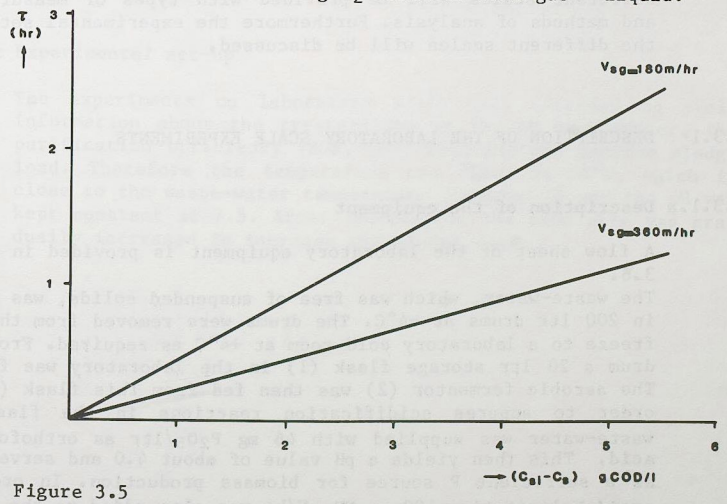


Figure 3.5
Required liquid residence time due to limited aeration in the bubble column

3.3 MATERIALS AND METHODS

The aerobic waste-water treatment experiments have been conducted on laboratory scale (3.5 ltr.), pilot scale (200 - 300 ltr) and semi-technical scale (3600 ltr).
The laboratory experiments where performed in the ICI laboratory at Billingham, England.
On the laboratory scale the biological purification of the waste-water was studied. This waste-water was free of suspended solids (to avoid clogging of peristaltic pumps) and was taken from 200 l storage vessels at - 4°C.
On the pilot scale more realistic conditions were studied. The waste-water was pumped directly from the sewer into the treatment installation after a rough sedimentation stage where only fast settling solids were removed. Therefore this waste-water was highly variable (see Chapter 1) and contained considerable amounts of suspended solids. On the semi-technical scale the pre-settling stage of the sewer waste-water was ommitted. The reason for this ommission is that for an eventual large scale treatment plant space is a limiting factor (see Chapter 1). Because settling tanks occupy large areas, it was important to study the effect of the omission of the presettling tank on the aerobic waste-water treatment process. Moreover the semitechnical scale is a tall bubble column contrary to the laboratory scale and the pilot scale which are stirred tanks. The bubble column is chosen for the ultimate proces because of the small area which is needed and the relative low air consumption (see also Chapter 3.2.6).
In this Chapter 3.3. a description of the equipment used on the different scales will be provided with types of measurements and methods of analysis. Furthermore the experimental set-up at the different scales will be discussed.

3.3.1 DESCRIPTION OF THE LABORATORY SCALE EXPERIMENTS

3.3.1.a Description of the equipment

A flow sheet of the laboratory equipment is provided in Figure 3.6.
The waste-water, which was free of suspended solids, was stored in 200 ltr drums at -4°C. The drums were removed from the deep freeze to a laboratory cold room at +4°C as required. From this drum a 20 ltr storage flask (1) in the laboratory was filled. The aerobic fermentor (2) was then fed from this flask (1). In order to suppres acidification reactions in the flask the waste-water was supplied with 46 mg P_2O_5/ltr as orthofosforic acid. This then yields a pH value of about 4.0 and serves also as a sufficient P source for biomass production. In order to avoid N-limitation 100 mg NH_3-N/kg was also added to the waste-water. This waste-water was then fed to a 5 ltr laboratory fermentor with a working column of 3.5 ltr (H=0.3m, D=0.15m). From this aeration vessel the broth flowed to a degasification unit (3) and subsequently to a sedimentation tank of 0.15 m diameter (4). From this settling tank the waste-water flowed

into the sewer. The sludge was partly recycled to the aeration tank with a recycle ratio r=1.0, and partly the sludge was wasted. The amount of sludge waste (waste ratio w) was adjusted to obtain a selected constant solids concentration in the aeration vessel (2). In practice this proved to be very difficult due to delays in the solids concentration analysis. This then led to large variations in solids concentration in the aeration vessel. The aeration was achieved through variable air/oxygen sparging and a stirrer at 500 rpm, in such a way that the dissolved oxygen concentration was kept above 40% air saturation. The back pressure in the fermentor was atmospheric. The pH in the aeration tank was kept constant through the addition of 3N NaOH solution. The temperature was controlled by water recirculation through a coil within the fermentor. Only peristaltic pumps were used. The fermentor was inoculated with aerobic sludge from a sewage treatment plant.

3.3.1.b Measurements and analyses

The influent was sampled daily and the effluent was also sampled every day, provided that the operation during the previous 24 hours had been satisfactory (e.g. no mechanical failures).
Each sample was then analysed for BOD_5, COD and TOC (Total Organic Carbon). Each day the aeration vessel was sampled for suspended solids content. All analyses were performed according "Standard Methods" (Ref. 3.12).
Also the Sludge Volume Index (SVI) of the sludge was measured regularly (Ref. 3.13).

3.3.1.c Experimental set-up

The experiments on laboratory scale were intended to yield information about the treatability of the GB waste-water, the purification efficiency (BOD, COD, TOC) and the maximum sludge load. Therefore the temperature was fixed at 35°C, which is close to the waste-water temperature (Chapter 1) and the pH was kept constant at 7.5. After inoculation the feed rate was gradually increased to increase the sludge load.

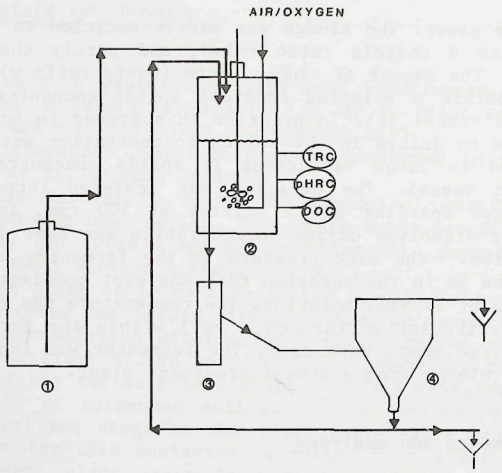


Figure 3.6
Flowsheet laboratory equipment

3.3.2 DESCRIPTION OF THE PILOT-SCALE EXPERIMENTS

3.3.2.a Description of the equipment

The flow sheet of the pilotscale equipment is provided in Figure 3.7.

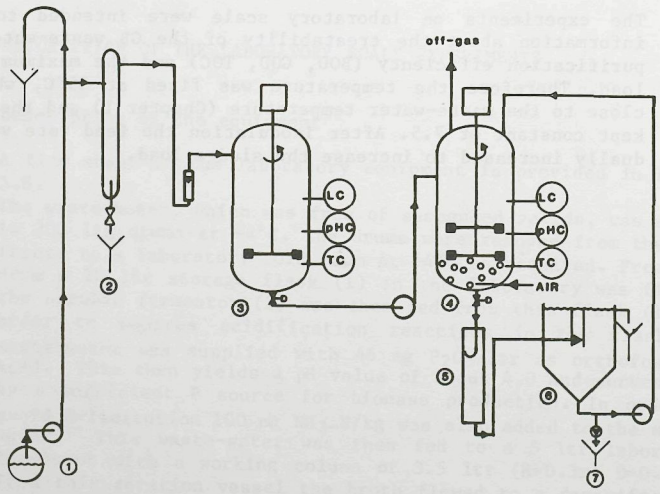


Figure 3.7
Flow sheet pilot plant equipment

The waste-water is transported with a centrifugal pump from sewer (1) to the researchfacilities. The flow rate is 1800 kg/hr and the transport time is 13 minutes. Most of the waste-water bypasses the pilot equipment. The waste-water to be treated (50-150 kg/hr) flows into a settling column (2) (Height 210 cm diameter 22.5 cm, area 0.038 m^2, volume 80 ltr). The sediment in this column is wasted periodically. From this settling column the waste-water flows through a rotameter into buffertank (3)(height 1.6 m diameter 0.625 m, area 0.31 m^2, total volume 500 ltr, working volume variable 40-300 ltr). The working volume is kept constant manually with a level indicator. In this buffertank (3) pH can be controlled and a fixed amount of 86 mg P_2O_5/ltr waste-water is provided in order to avoid possible P-limitation. For homogenisation the buffertank is equiped with a 6-bladed standard turbine stirrer of 25 cm diameter. The temperature is controlled by jacket-cooling/heating. Then the waste-water is pumped from the buffertank (3) into the aeration vessel (4) which has identical dimensions as the buffertank. This flow is controlled by a magnetic flow meter/control valve combination. The aeration vessel is equiped with a double eight bladed turbine stirrer of 28 cm diameter. Also the pH, temperature and broth weight are controlled. In case of excessive foaming the foam alarm at 500 ltr volume releases the anti-foam pluronic L81 addition. For O_2 transfer air is sparged under the turbine stirrer.
From the aeration vessel the broth flows in a degasification vessel (5) (height 120 cm, diameter 30.5 cm, area 0.073 m^2, volume 85 ltr). The broth is fed tangentially at 10 cm under the liquid surface. This degasification vessel also serves as a buffer for the discontinuous release of broth from the aeration vessel (actuated through the weight control system in the aeration vessel). From vessel (4) the degasified broth flows into the settling tank (6) (area 0.28 m^2).
From the settling tank the clear waste-water overflows into the sewer and the settled sludge is recycled with a centrifugal pump to the aeration vessel (5). The surplus sludge is wasted in the sewer at point (7). The aeration vessel (4) was inoculated with aerobic sludge of the municipal waste-water plant of Leiden.

3.3.2.b Measurements and analyses

The composition of the waste-water varies continuously. Therefore the waste-water influent in the aeration vessel and the overflow of the settling tank are sampled frequently. A peristaltic pump is operated 1 minute in each 15 minutes and the sampled waste-water (influent or effluent) is then pumped into a storage vessel of 10 ltr inside a refrigerator at 7°C.
From this storage vessel well mixed samples are prepared each 24 hrs for subsequent analysis. From these 24 hr averaged samples of influent and effluent centrifuged samples are analysed for BOD_5, COD, Kjeldahl-N and P_2O_5, and untreated samples are analysed for Kjeldahl-N, suspended solids and P_2O_5.Apart from these semi-continuous samples grab samples are collected at regular intervals. These grabsamples are taken from the aeration vessel (every 8 hours) and from the sludge recycle (every 24 hours). In the grabsample of the aeration vessel suspended solids dry matter is analysed and also the SVI value

is determined. In the grabsample of the sludge recycle only suspended solids dry matter is analysed.
Furthermore the different flow rates (waste-water feed, recycle sludge, waste sludge) are controlled each 8 hours. The air-flow rate is controlled with a rotameter, and the off-gas is analysed for O_2 (Oxygor-analysor from Maihak Germany).

The BOD_5 analysis is more precisely the BOD_5^{20}(at) analysis. This is the biological oxygen demand after 5 days at 20°C with added allyl thioureum to suppress nitrification according to NEN 3235/5.4.
The COD analysis was done according to NEN 3235/5.3.
The Kjeldahl-N analysis was performed according to Technicon Autoanalysor Methodology (Industrial Method 28-69A(1969)). The P_2O_5 analysis was done according to Ref. 3.22. The suspended solids analysis in the influent and effluent waste-water, in the aeration vessel and in the return sludge was performed according to Ref. 3.12. The SVI value was determined according to NEN 3235/4.5.
During this study experiments were also performed to determine the settling velocity of sludge as a function of sludge concentration and SVI value. The sludge settling velocities were obtained by observing the sludge level in a 1 ltr glasscylinder (h = 30 cm, d = 6.5 cm) as a function of time. The settling velocity was determined graphically as the tangent of the sludge level curve at t = 0.

3.3.2.c Experimental set-up

The experiments at the pilot scale lasted 4750 hours, and this time can be divided into 4 main periods where different aspects were studied, as indicated in Table 3.2. The operational conditions in the buffertank (3) and the aeration tank (4) are listed in Tables 3.3 and 3.4.
The applied liquid residence time τ in the aeration tank, sludge recycle ratio r and sludge waste ratio w are given in Figures 3.8 A,B,C.

The experiments from 0-1361 hours are meant to check the results of the laboratory at 35°C under more realistic conditions of highly variable waste concentration and the presence of substantial amounts of suspended solids. The aerobic process was during that period studied with regard to substrate uptake kinetics, the minimum waste-water residence time, purification efficiency, sludge production, oxygen requirements and SVI values.

The experiments from 1361-2358 hours were performed at 45°C to study the effect of higher reactor temperature (leading to lower cooling costs) on the purification process.

The experiments from 2358-3976 hours aimed at the most practical process configuration at 35°C; thus a small buffervolume, no pH control, a high CO_2 concentration in the off-gas which is expected in a large scale bubble column type reactor, application of hydraulic shock loads.

The procedure for shockloading was as follows. During a day $1.5\Phi_i$ was applied for the first 8 hours, $1.0\Phi_i$ was applied for the next 8 hours and $0.5\Phi_i$ was maintained for the last 8 hours.

In the last period the temperature was again increased from 35-40°C.
Furthermore the sludge settling was studied in order to verify eq. (3.20).

PERIOD	TIME (hrs.)	RESEARCHOBJECTIVES
1	0-1361	Determination of minimum liquid residence time at 35°C and check of lab.scale results
2	1361-2358	Effect of change of reactortemperature from 35°C to 45°
3	2358-3976	Application of realistic conditions at 35°C, which means a small buffervolume (with no temperature or pH control or agitation), no pH control in aeration tank, low aeration rate and application of hydraulic shock loads (2944 - 3445 hrs.).
4	3976-4750	Application of realistic conditions at 40°C.

Table 3.2
Research objectives during pilot-scale experiments

Weight(kg)		Temp °C		pH -		agitation (rpm)		Back pressure $10^5 N/m^2$	
Time(hrs)		Time(hrs)		Time(hrs)		Time(hrs)		Time(hrs)	
0- 645	200	0-1361	35	0- 428	6.5	0-2046	100	0-4750	1.0
645-1361	300			428-2046	6.0				
1361-2046	200	1361-1690	45						
		1690-2046	35						
2046-2130	135	2046-4750	*	2046-4750	*	2046-4750	0		
2130-4750	40-60								

* no control is applied

Table 3.3
Operational conditions in the buffer tank (3) of the pilot scale equipment.

Weight(kg)		Temp °C		pH -		agitation (rpm)		Back pressure $10^5 N/m^2$		Air flow Nm^3/hr	
Time(hrs)		Time(hrs)		Time(hrs)		Time(hrs)		Time(hrs)		Time(hrs)	
0- 457	300	0-1361	35	0-2358	7.5	0-4750	300	0- 188	1.0	0-2358	20
457-1774	200	1361-2358	45	2358-4750	*			188-1675	2.0	2358-4750	2.1-
1774-4750	300	2358-3976	35					1675-2358	1.5		3.4
		3976-4750	40					2358-2520	2.0		
								2520-4750	1.3		

* no control is applied

Table 3.4
Operational conditions in the aeration tank (4) of the pilot scale equipment.

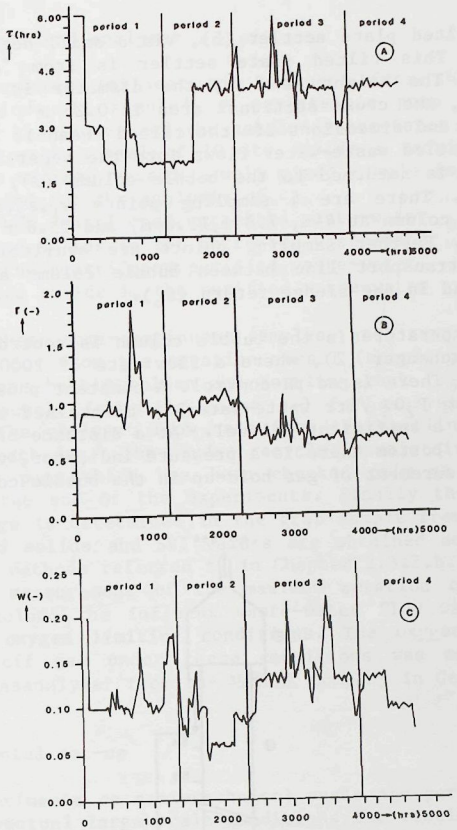


Figure 3.8
(A) waste-water liquid residence time,
(B) sludge recycle ratio,
(C) waste ratio during pilot-scale experiments

3.3.3 DESCRIPTION OF THE SEMI-TECHNICAL SCALE EXPERIMENTS

3.3.3.a Description of the equipment

The flow sheet of the semi-technical scale process is given in Figure 3.9. The waste-water is pumped directly from the sewer (1) into the bubble column (3). The maximum liquid height (unaerated) in this bubble column is 18.3 m, the column diameter is 0.495 m, the area is then 0.192 m^2 and the volume is 3520 ltr. On top of the column is a gas disengaging section (4) of 0.7 m diameter, area 0.38 m^2 and 1.2 m high. The temperature in the column is controlled with the external heatexchanger (2).
For oxygen transfer air is sparged through a ringsparger of 0.2 m diameter which contains 120 holes of 4 mm diameter. The waste-water sludge mixture flows out of the bubble column into

the tilted plate settler (5), via a small degasification space (6). This tilted plate settler is from "Alton Eco Supply B.V.". The height is 3 m, the diameter is 0.56 m x 0.45 m square, the cross sectional area is 0.25 m^2, the tilting angle is 62° and dimensions of the tilted channels are 37 x 37 mm. The settled waste-water flows into the sewer and the thickened sludge is returned to the bubble column (3), except the waste sludge. There are 4 sampling points s_1, s_2, s_3, s_4, along the bubble column at 4 m, 7.8 m, 11.6 m, and 15.8 m above the column bottom. Further sampling points are available in the waste-water transport line between bubble column and settling tank (s_5) and in the sludge return (s_6).

The temperature in the bubble column is controled with a plate heat exchanger (2), where a flow rate of 2000 ltr/hr is maintained. There is no pH-control. A constant phosphoric acid feed of 84 mg P_2O_5 /ltr waste-water is maintained which is added to sustain a sufficient P-level. At a distance of 0.4 m above the reactor bottom there is a pressure indicator, which is used for the measurement of gas hold-up in the bubble column.

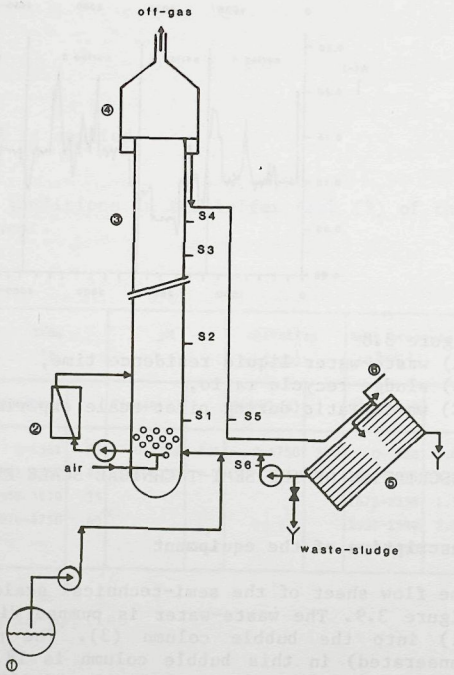


Figure 3.9
Flow sheet semi-technical equipment

3.3.3.b Measurements and analyses

The waste-water influent and effluent from the settler are semi-continuously sampled. A peristaltic pump is operated 1 minute in 15 minutes and the sampled waste-water is then pumped into a storage vessel of 10 ltr inside a refrigerator (4°C). From this storage vessel a well mixed sample is prepared each day and used for the analysis of
- COD in the centrifuged untreated waste-water
- suspended solids in the untreated waste-water
- COD in the centrifuged purified waste-water
- suspended solids in the purified waste-water

Besides these semi-continuous samples, grab samples are collected each day from the aeration vessel (sampling point s_2), in between the bubble column and settling tank (s_5) and from the sludge recycle (sampling point s_6) for analysis of suspended solids. The effluent flow rate from the settling tank is controlled each day. The sludge recycle is maintained by a variable flow pump which has been checked only at the start and towards the end of the experiments. Finally the SVI value of the sludge is determined in the grab sample from s_2. The COD, suspended solids and SVI values are obtained according to the analysis methods referred to in Chapter 3.3.2.b.
For the measurement of the maximum aeration capacity of the bubble column the influent waste-water flow was increased to achieve oxygen limiting conditions. The oxygen concentration in the off gas under these conditions was measured with a OXYGOR gasanalyser from the MAIHAK Company in Germany.

3.3.3.c Experimental set-up

The experiments on semi-technical scale are performed to check under eventual large scale conditions the results of the pilot scale stirred vessel, which were obtained in the final period 4 (Table 3.2).

Therefore the following conditions were applied
- liquid residence time is 4 hours, Φ_i = 750 ltr/hr
- recycle ratio r = 0.75
- 40°C as reactor temperature
- no pH control

Furthermore it was thought advantageous to avoid the pre-settling of the waste-water. Therefore the bubble column was fed with unsettled waste-water from the sewer. During the experiment the air-flow rate was maintained at 26.1 Nm^3/hr from 0-787 hrs and at 39.3 Nm^3/hr from 787-1435 hrs.
This is equivalent to V_{sg} = 0.036 m/s and V_{sg} = 0.055 m/s, and ensured sufficient oxygen transfer capacity.

Again because of the limited space available at Delft it was decided to test the tilted plate settler for sludge recycle as a space saving alternative for the conventional settler.

The parameters of interest in the purification experiments are
- suspended solids content in the bubble column
- treatment efficiency
- SVI value of the sludge in the bubble column
- oxygen transfer capacity of the bubble column
- mixing in the bubble column
- performance of the tilted plate settler

3.4 RESULTS AND DISCUSSION

In this section the results will be presented for the different scales of experiments. Where ever appropriate, the results will be compared.

3.4.1 LAB-SCALE EXPERIMENTS

3.4.1.a General results

The results of the experiment at 35°C are presented in the Figures 3.10A-H.
It can be seen that the influent substrate concentration has varied widely, which is due to the use of different waste-water storage vessels (Figure 3.10A). The irregularities in sludge wasting led to a large variation in suspended solids concentration in the aeration vessel, as shown in Figure 3.10B. A too high waste ratio at 700-750 hours decreased the fermenter suspended solids concentration to such an extent (from 25→5 gDM/l) that the purification efficiency decreased sharply (Figure 3.10E, F, G). Otherwise the purification process was remarkable stable as evidenced by the high removal efficiencies of BOD, COD and TOC (Figures 3.10E,F,G), despite the very short liquid residence times in the aeration vessel (as short as 0.9 hr, see Figure 3.10C) and the very high COD reactor load (up to 140 kg COD/m^3 reactor day, see Figure 3.10D). However even at this very high COD load of the reactor, there were no signs of reactor overloading. The sludge which was produced had very good settling properties as can be seen from the very low SVI values of 20-50 ml/gDM (see Figure 3.10H).
This well settling sludge led then to a high suspended solids concentration in the aeration vessel and is therefore an important factor for the high reactor COD loads which were attained.
During the experiments bulking sludge or filamentous growth was never observed.
The average results, for quick reference to other scale experiments, are listed in Table 3.5.

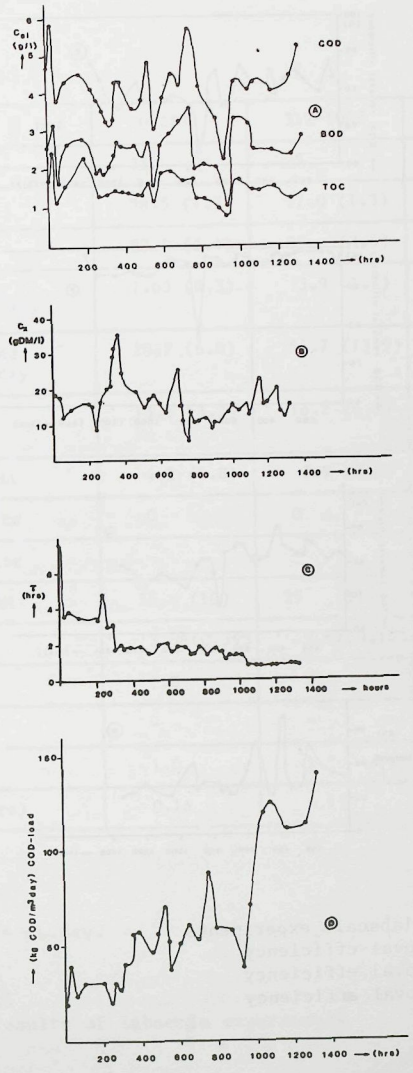


Figure 3.10
Results of labscale experiments
(A) influent substrate concentration
(B) solids concentration in reactor
(C) waste-water residence time
(D) applied COD load

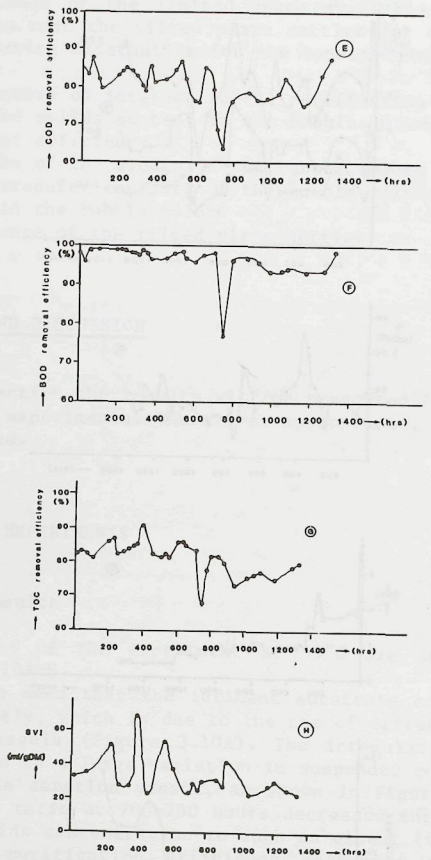


Figure 3.10
Results of labscale experiments
(E) COD removal efficiency
(F) BOD removal efficiency
(G) TOC removal efficiency
(H) SVI

hrs	0-288	312-1008	1056-1344
Temp. °C	35	35	35
BOD eff. %	98.5 (1.3)	97.0 (1.3)	95.8 (1.8)
COD eff. %	83.6 (2.4)	80 (3.5)	80.8 (4.7)
Sludge load g COD/g DM day	1.63 (0.3)	3.9 (1.7)	7.1 (1.9)
Reactor load kg COD/m^3 day	28.7 (6.0)	54.7 (13.9)	121 (11)
$MLSS_{FERM}$ g DM/ltr	15.1 (3.3)	16.2 (6.9)	16.1 (3.6)
C_{si} gCOD/ltr	4.2 (0.8)	3.9 (0.8)	4.3 (0.5)
$MLSS_i$ g/ltr	0	0	0
$MLSS_e$ g/ltr	-	-	-
SVI ml/g DM	36 (10)	35 (14)	26 (3)
τ hr	3.56(0.59)	1.67(0.18)	0.87(0.06)
r (-)	1.0	1.0	1.0
w (-)	-	-	-
β (-)	1.0	1.0	1.0
τ_{crit} (hrs)	0.16	0.19	0.23

( ) = st.dev.

Table 3.5
Average results of labscale experiments

3.4.1.b Estimation of model parameters

From the data, which are presented in the Figures 3.10A,E,F,G the relation between COD, BOD and TOC conversion can be found. In Figure 3.11 the relation between specific sludge BOD load (q_{BOD}) and specific sludge COD load (q_{COD}) is shown. From this relation it follows that the conversion of 1 g BOD corresponds to the conversion of 1.3 g COD.

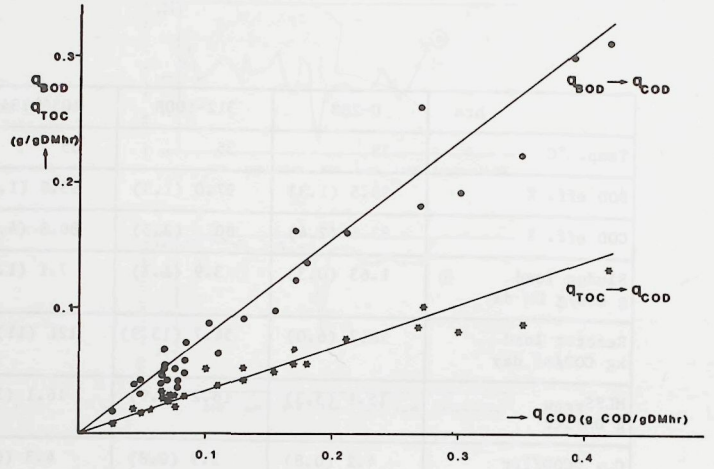


Figure 3.11
Relation between the specific conversion rates of COD and TOC or BOD on labscale

Also in Figure 3.11 the relation between the specific sludge C-load (q_{TOC}) and q_{COD} is shown. From this relation it follows that the conversion of 1 g COD corresponds to the conversion of 0.35 g carbon.
The following relation between BOD, COD and TOC conversion does therefore hold for the treatment of GB-waste-water at 35°C

1 g BOD = 1.3 g COD ≈ 0.46 g TOC

This result can be used to calculate the value of α (see chapter 3.2.6), which leads to

α = 0.46/1.3 = 0.35 g C/g COD.

Because sludge production and CO_2/O_2-conversionrates were not measured the yield of biomass on substrate can not be calculated. However from BOD conversion and biomass concentrations the parameters of the substrate uptake equation eq.(3.1) can be estimated. The results are presented in Figure 3.12. It is found that the maximum sludge activity q_s^m is 0.37 ± 0.05 gBOD/gDM.hr and K_s = 0.198 ± 0.04 gBOD/ltr.
It is clear that there is a large scatter of the data in Figure 3.12. As already mentioned in Chapter 3.2.2.a this is most probably due to the highly variable composition of the waste-water (variable with regard to different chemical compounds and not with regard to variable concentrations).

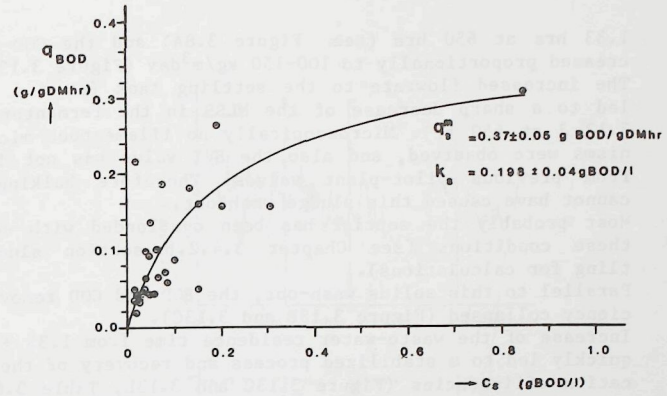


Figure 3.12
Relation between specific BOD conversion rate and BOD concentration on labscale

With the above mentioned model parameters the critical liquid residence time according to eq. 3.46 can be calculated. It has been assumed that the fraction active biomass in the sludge (β) is equal to 1, due to the absence of suspended solids in the waste-water. The results are listed in Table 3.5. It is evident that the critical liquid residence time is very low and far below the applied τ. Therefore a very stable process is expected, which agrees with the findings mentioned in Chapter 3.4.1.a.

3.4.2 PILOT-SCALE EXPERIMENTS

3.4.2.a General results

The overal results of the experiments on pilot scale are presented in the Figures 3.13A-L and Table 3.6 .
As already mentioned in Chapter 3.3.2.c the experiments are divided in 4 periods, wherein different aspects of the aerobic purification were studied. In the following the general results of these different periods will be discussed.

Period 1: 0 - 1345 hr

The prime purpose of the experiments in this period was to check the laboratory results of Chapter 3.4.1. Therefore the waste-water was fed into the aeration tank (35°C) via a large buffertank (holding time equal to liquid residence time in the aeration vessel) and the pH was controlled at 7.5. Contrary to the labscale experiments the waste-water still contained an appreciable amount of suspended solids. During this period no routine suspended solids measurements were performed, but the analysis done after 2358 hr indicates a suspended solids content of 0.5-2 gDM/ltr (Figure 3.13I) The liquid residence time in the aeration tank was sequentially decreased from 6 hrs →

1.33 hrs at 650 hrs (see Figure 3.8A) and the COD-load increased proportionally to 100-150 kg/m^3day (Figure 3.13B).
The increased flowrate to the settling tank (at τ = 1.33 hr) led to a sharp decrease of the MLSS in the fermentor (Figure 3.13 J at 650 hr). Microscopically no filamentous micro-organisms were observed, and also the SVI value was not different from previous pilot-plant values. Therefore bulking sludge cannot have caused this sludge wash-out.
Most probably the settler has been overloaded with solids at these conditions (see Chapter 3.4.2.b section **sludge settling** for calculations).
Parallel to this solids wash-out, the BOD and COD removal efficiency collapsed (Figure 3.13E and 3.13C).
Increase of the waste-water residence time from 1.33 → 2.9 hrs quickly led to a stabilized process and recovery of the purification efficiencies (Figure 3.13C and 3.13E, Table 3.6). The SVI value of the sludge during this period was remarkably constant at about 70 ml/gDM (Figure 3.13L). The purification results at 35°C and some other process variables at different liquid residence times are listed in Table 3.6.

Period 2: 1414-2326 hr

According to Chapter 3.2.6 the heat production in the aerobic treatment process is considerable. A higher fermentor temperature is therefore favorable with regard to cooling costs. Therefore the experiments were continued with a fermentor temperature of 45°C instead of 35°C as in period 1. The liquid residence time was 2 hrs from 1367 - 1800 hrs and 4 hrs from 1800 - 2358 hrs.
It appears from Figure 3.13C and 3.13E that the removal efficiency of COD and BOD is about 10% lower than under comparable conditions of liquid residence time or sludge load at 35°C (see also Table 3.6).
The SVI value tended to increase (see Figure 3.13L), and it was also observed microscopically that at 45°C a much higher number of non-flocculating micro-organisms was present than at 35°C. This obviously led to a higher suspended solids content in the purified waste-water at 45°C compared to 35°C (see Figure 3.13H and Table 3.6).
Because of this result the reactor temperature was again changed to 35°C for the next period 3.

Period 3: 2424-3950 hr

During this experimental period the process operation was shifted towards more practical conditions, again at 35°C. The changes included (see also Table 3.2) a virtual elimination of the hydraulic buffer before the aeration tank, a low air sparging rate with subsequent high CO_2-content in the off gas from the aeration tank, no pH-control, and application of hydraulic shock loads (in the period 3.2, see Table 3.6).
From the Figures 3.13A to 3.13L and from Table 3.6 the following results are mentioned.

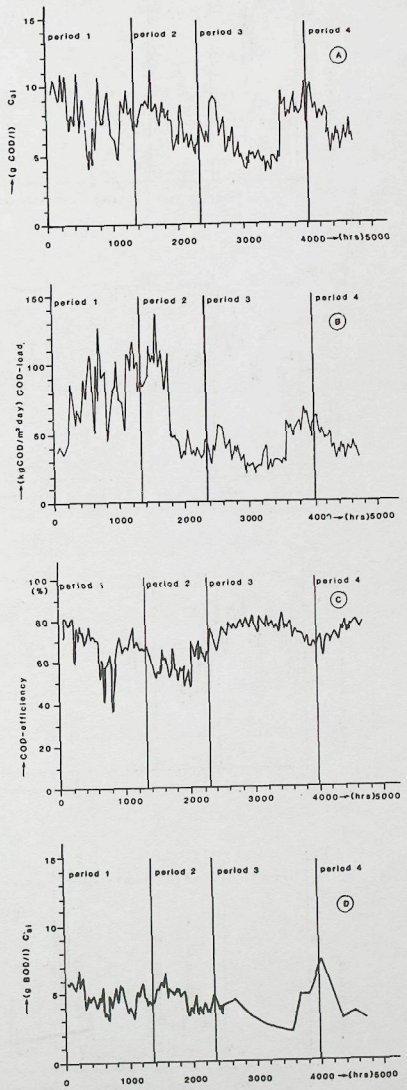


Figure 3.13
Results of pilot-scale experiments
(A) influent COD concentration
(B) applied COD load
(C) COD removal efficiency
(D) influent BOD concentration

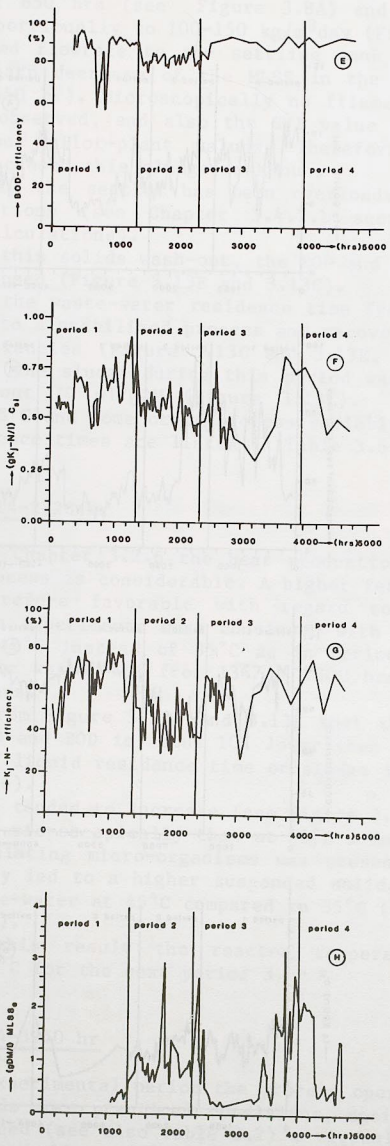


Figure 3.13
Results of pilot-scale experiments
(E) BOD removal efficiency
(F) influent Kjeld-N concentration
(G) N-removal efficiency
(H) suspended solids concentration in effluent waste-water

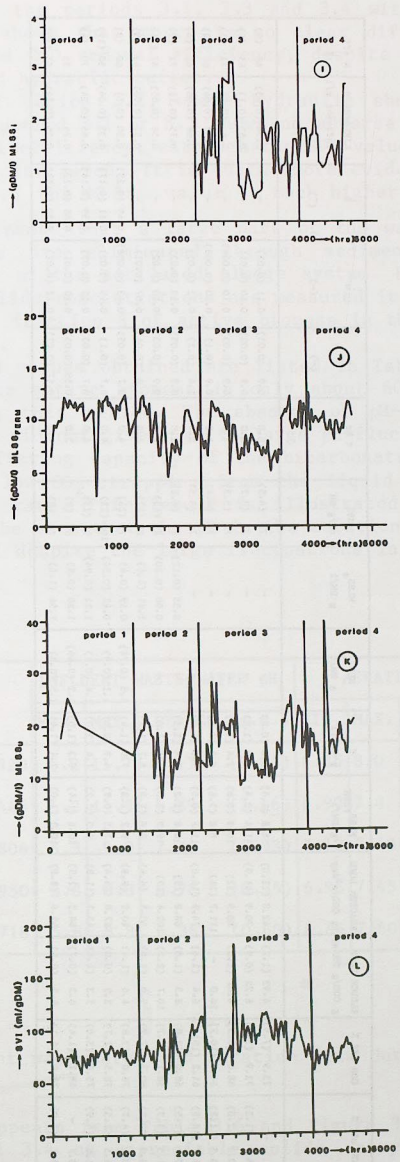


Figure 3.13
Results of pilot-scale experiments
(I) suspended solids concentration in influent waste-water
(J) suspended solids concentration in aeration tank
(K) suspended solids concentration in sludge recycle
(L) SVI

PERIOD	HOURS	TEMP °C	BOD eff %	COD eff %	SLUDGE LOAD g COD/g DM/day	REACTOR LOAD kg COD/m³/day	$MLSS_{PERM}$ g DM/l	C_{s1} g COD/l	$MLSS_1$ g DM/l	$MLSS_e$ g DM/l	SVI ml/g DM	τ hr	r (-)	w (-)	β (-)	KJELDAHL N influent g/l	N eff. %
1.1	0- 241	35	-	75.9 (7.9)	4.93 (1.2)	42.8 (11.0)	8.8 (1.4)	9.6 (0.9)	-	-	74 (4.5)	6.0	0.9 (0.03)	0.11 (0.03)	-	0.56 (0.03)	48 (9)
1.2	265- 457	35	94.7 (1.2)	72.9 (3.8)	6.22 (0.9)	70.9 (11.9)	11.4 (0.4)	8.8 (1.6)	-	-	71 (8.2)	3.0	0.9 (0.02)	0.10 (0.01)	-	0.62 (0.1)	65 (5)
1.3	481- 625	35	90.3 (2.6)	68.0 (8.3)	8.07 (1.8)	88.5 (22)	10.9 (0.8)	7.4 (1.8)	-	-	72 (5.3)	2.0	0.9 (0.03)	0.10 (0.004)	-	0.6 (0.1)	73 (5)
1.4	649- 769	35	76.8 (11.2)	54.3 (10.2)	10.0 -	111.7 (36)	8.6 (3.6)	6.2 (1.8)	-	-	78 (8.4)	1.3	0.9 (0.1)	0.10 (0.008)	-	0.52 (0.06)	60 (12)
1.5	793- 937	35	84.7 (16.9)	61.2 (15)	6.4 (1.4)	74.9 (16.6)	11.7 (0.6)	9.0 (1.3)	-	-	79 (5.5)	2.9	1.4 (0.2)	0.12 (0.03)	-	0.68 (0.05)	67 (4)
1.6	961-1345	35	90.6 (1.5)	68.0 (3.0)	8.3 (1.8)	88.8 (19)	10.8 (1.2)	7.4 (1.5)	-	0.35 (0.12)	75 (6.1)	2.0	0.9 (0.05)	0.13 (0.03)	-	0.75 (0.15)	72 (9)
2.1	1414-1790	45	81.5 (3.3)	58.1 (5.2)	10.7 (2.2)	102.4 (13)	9.9 (2.0)	8.5 (1.0)	-	0.90 (0.20)	76 (13)	2.0	0.9 (0.03)	0.10 (0.02)	-	0.56 (0.05)	47 (11)
2.2	1774-2326	45	82.0 (3.5)	60.3 (7.2)	5.0 (1.8)	40.3 (6.6)	8.6 (1.8)	6.7 (1.1)	-	1.61 (1.6)	87 (17)	4.0	1.0 (0.04)	0.07 (0.01)	-	0.52 (0.08)	42 (9)
3.1	2424-2920	35	88.0 (4.7)	76.0 (3.8)	4.6 (1.4)	40.9 (8.6)	9.2 (1.8)	7.0 (1.4)	1.6 (0.74)	0.59 (0.4)	79 (26)	4.0	0.7 (0.05)	0.13 (0.008)	0.48 (0.25)	0.50 (0.1)	60 (8)
3.2	2944-3445	35	89.5 (2.5)	78.5 (2.7)	3.9 (0.8)	27.8 (4.4)	7.3 (0.9)	4.9 (0.4)	1.1 (0.9)	0.42 (0.34)	100 (11)	4.0	0.7 (0.1)	0.15 (0.02)	0.61 (0.17)	0.35 (0.04)	50 (19)
3.3	3469-3806	35	91.6 (4.9)	75.8 (3.0)	5.3 (1.4)	45.3 (11.8)	9.0 (2.2)	7.4 (2.0)	1.4 (0.4)	1.22 (0.94)	91 (13)	4.0	0.7 (0.03)	0.15 (0.03)	0.59 (0.14)	0.65 (0.24)	70 (4)
3.4	3830-3950	35	88.7 -	69.2 (1.4)	6.3 (0.7)	64.5 (2.5)	10.6 (1.4)	8.3 (1.0)	1.2 (0.46)	1.68 (0.8)	81 (9)	3.0	0.55 (0.05)	0.10 (0.01)	0.68 (0.13)	0.74	55
4	3974-4718	40	92.1 (2.0)	74.0 (4.1)	4.5 (0.9)	44.6 (8.8)	10.0 (1.0)	7.4 (1.4)	1.5 (0.5)	1.56 (1.6)	74 (8)	4.0	0.67 (0.02)	0.12 (0.02)	0.50 (0.19)	0.56 (0.14)	65 (10)

( ) = standard dev.

Table 3.6
Average results of pilot-scale experiments

Comparison of the periods 3.1, 3.3 and 3.4 with periods 1.1, 1.2 and 1.3 shows that there is no clear difference in SVI value, BOD and COD removal efficiency, despite the absence of pH-control and hydraulic buffer.
Comparison of period 3.2, where hydraulic shock loads were tested, with period 3.1 or 3.3 shows no adverse effects on COD or BOD removal efficiency, although the SVI value of the sludge seems to have increased. It is furthermore evident that during period 3.1-3.3 the $MLSS_i$ value is much higher than the $MLSS_e$ value, which means that a large part of the waste-water suspended solids are eliminated through sedimentation in the settling tank of the activated sludge system. Because in this period all solids concentrations are measured it is possible to calculate the fraction β of active biomass in the total solids with eq. 3.44.
The average β values obtained are listed in Table 3.6. It appears that the active biomass is only about 60% of the total solids during this period. The absence of pH-control in the aeration tank did not give rise to large pH-fluctuations due to the large buffering capacity of the bicarbonate buffer, which is due to a low CO_2-stripping from the liquid because of the low air flow rate. This result is illustrated in Table 3.7, where it can be seen that the pH in the aeration tank is fairly stable at 7.2 despite the large fluctuations in influent waste water pH.

PERIOD	hrs	INFLUENT WASTE-WATER pH			AERATION TANK pH		
		MIN.	MAX.	AVERAGE (s.d.)	MIN.	MAX.	AVERAGE (s.d.)
3.1	2424-2920	6.2	11.7	7.13 (0.93)	6.85	8.0	7.25 (0.14)
3.2	2944-3445	5.2	11.5	6.89 (1.16)	6.90	7.8	7.24 (0.14)
3.3	3469-3806	6.3	9.0	7.25 (0.93)	7.10	7.40	7.27 (0.09)
3.4	3830-3950	5.8	8.8	6.70 (0.75)	6.95	7.45	7.24 (0.09)
4	3974-4718	5.6	8.8	6.35 (0.60)	6.85	7.60	7.20 (0.15)

Table 3.7
pH in influent wastewater and aeration tank during pilot scale experiments

Finally it appears from Table 3.6 and Figure 3.13H that there is in period 3.4 an appreciable solids carry over into the effluent in the settling tank. This indicates an overloading of the settling tank with solids under the conditions during period 3.4.

Period 4: 3974-4718 hr

In order to reduce the cooling costs the reactor temperature was again increased but now to 40°C.
From the Figures 3.13A-L and from Table 3.6 it appears that this temperature rise to 40°C does not lead to a decrease in BOD or COD treatment efficiency (compare period 4 to period 3.3). Also the SVI value is very much the same in period 4 and period 3.3.
From Table 3.7 it can be seen that also in this period the pH in the aeration vessel remained nearly constant, despite large fluctuations of the pH in the influent waste-water.

3.4.2.b Estimation of model parameters

- **relation between BOD conversion and COD conversion**

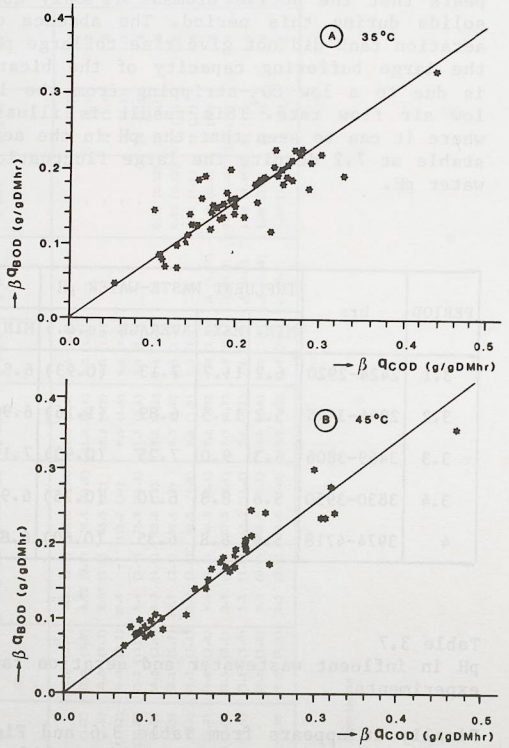


Figure 3.14
Relation on pilot scale between specific conversion rates of COD and BOD at (A) 35°C and (B) 45°C

In the Figures 3.14A and 3.14B the relation between specific conversion rates of COD and BOD is shown for two temperatures, 35°C and 45°C. The BOD and COD conversion rates are calculated from total solids in the aeration tank, and therefore are not corrected for the presence of inert suspended solids.

Hence Figure 3.14A and 3.14B show the relation between βq_{BOD} and βq_{COD}, and the value of the tangent is not affected through the presence of this inert suspended matter.
It appears for 35°C that 1 g BOD = 1.3 g COD, which is exactly the same relation as was found on labscale (Figure 3.11).
However for 45°C the relation is different and results in 1 g BOD = 1.17 g COD.
It is stressed that these relations only give the relation between BOD and COD conversion and not the relation between BOD and COD concentration in the untreated or treated waste-water.
Because for 40°C only a few BOD analyses were done, the relation between COD and BOD conversion for this temperature is not available.

- **substrate uptake kinetics**

The substrate uptake is modeled with the Michaelis-Menten equation (eq.(3.1)).
In the Figures 3.15A and 3.15B the relation between q_{BOD} and C_{BOD} is shown for 35°C and 45°C. The number of BOD analyses at 40°C was to small to allow for a significant correlation. In order to be able to compare with the labscale experiments q_{BOD} is calculated from the active biomass, which is only 60% of the total solids in the aeration tank (see Chapter 3.4.2.a, period 3).

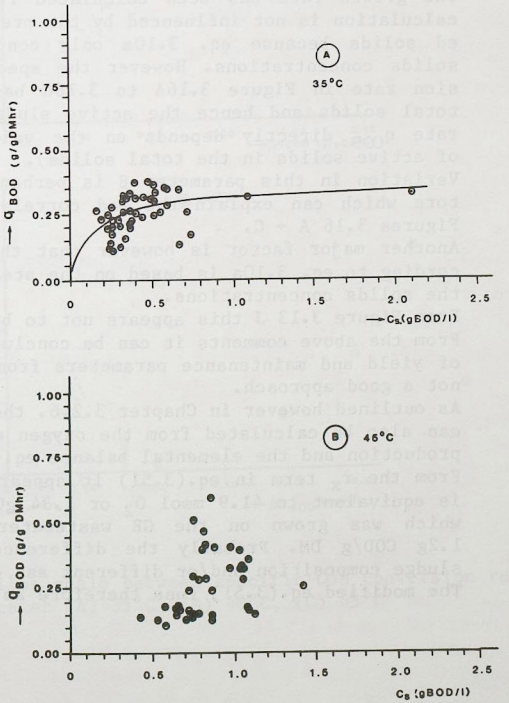


Figure 3.15
Relation on pilot scale between specific BOD conversion rate and BOD concentration at (A) 35°C and (B)45°C

From the correlation presented in Figure 3.15A the following parameters for 35°C are obtained, although there is a large scatter:

$q_s^m = 0.36 \pm 0.05$ g BOD/g DM hr

$K_s = 0.149 \pm 0.055$ g BOD/l

From Figure 3.15B it is obvious that at 45°C there is no good correlation between the q_{BOD} and the BOD concentration. This is not surprising because from Table 3.6 it appeared that at this high temperature the BOD removal efficiency is substantially lower than at 35°C. This means that several wastewater compounds which are not degradable at 45°C are readily degraded in the BOD analysis. Thus the BOD concentration is not a correct measurement of the degradable substrate at 45°C.

- **sludge production kinetics**

In order to estimate substrate yield and maintenance values of the sludge, the relation between growth rate and specific substrate (COD) conversion is shown in the Figures 3.16A to 3.16C for 35, 40 and 45°C.
The growth rate has been calculated from eq. 3.10a and this calculation is not influenced by the presence of inert suspended solids because eq. 3.10a only contains ratio's of total solids concentrations. However the specific substrate conversion rate in Figure 3.16A to 3.16C has been calculated from total solids and hence the active sludge substrate conversion rate q_{COD} directly depends on the value of β (the fraction of active solids in the total solids).
Variation in this parameter β is perhaps one of the main factors which can explain the bad correlations presented in the Figures 3.16 A ÷ C.
Another major factor is however that the calculation of μ according to eq. 3.10a is based on the steady state assumption of the solids concentrations.
From Figure 3.13 J this appears not to be true.
From the above comments it can be concluded that the estimation of yield and maintenance parameters from the sludge balance is not a good approach.
As outlined however in Chapter 3.2.6. the yield and maintenance can also be calculated from the oxygen uptake or carbondioxyde production and the elemental balance eq.(3.51) and (3.52).
From the r_x term in eq.(3.51) it appears that 1 gDM of sludge is equivalent to 41.9 mmol O_2 or 1.34 gCOD. However the sludge which was grown on the GB wastewater has a COD value of 1.2g COD/g DM. Probably the difference is due to different sludge composition and/or different ash content.
The modified eq.(3.51) runs therefore as eq.(3.51a).

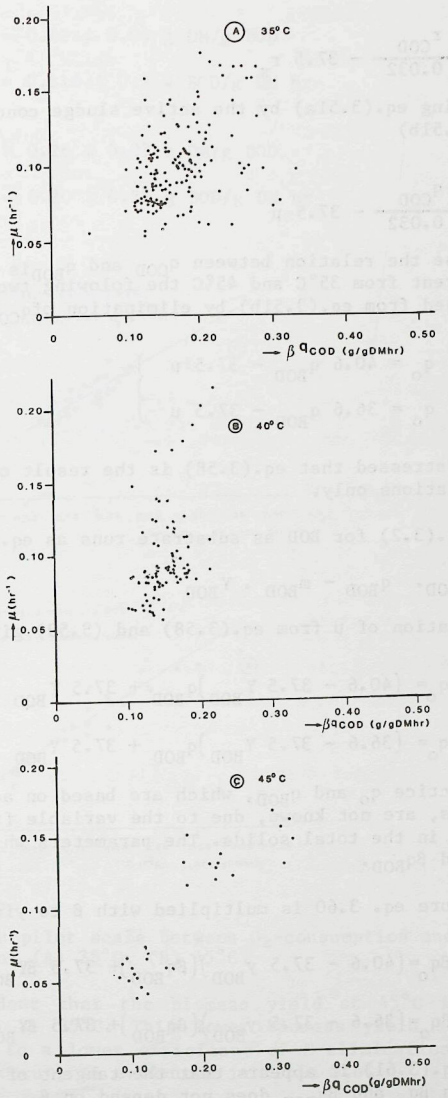


Figure 3.16
Relation on pilot scale between specific COD conversion rate and growth rate at (A) 35°C, (B) 40°C, (C) 45°C

$$r_o = \frac{r_{COD}}{0.032} - 37.5\ r_x \qquad \text{eq.(3.51a)}$$

Dividing eq.(3.51a) by the active sludge concentration leads to eq.(3.51b)

$$q_o = \frac{q_{COD}}{0.032} - 37.5\ \mu \qquad \text{eq.(3.51b)}$$

Because the relation between q_{COD} and q_{BOD} is different from 35°C and 45°C the folowing two equations are obtained from eq.(3.51b) by elimination of q_{COD}

$$\left.\begin{aligned} 35°C: q_o &= 40.6\ q_{BOD} - 37.5\ \mu \\ 45°C: q_o &= 36.6\ q_{BOD} - 37.5\ \mu \end{aligned}\right\} \qquad \text{eq.(3.58)}$$

It is stressed that eq.(3.58) is the result of stoichiometric calculations only.

The eq.(3.2) for BOD as substrate runs as eq.(3.59)

$$\mu = Y_{BOD} \cdot q_{BOD} - m_{BOD} \cdot Y_{BOD} \qquad \text{eq.(3.59)}$$

Elimination of μ from eq.(3.58) and (3.59) gives eq.(3.60)

$$\left.\begin{aligned} 35°C: q_o &= (40.6 - 37.5\ Y_{BOD})q_{BOD} + 37.5\ Y_{BOD} \cdot m_{BOD} \\ 45°C: q_o &= (36.6 - 37.5\ Y_{BOD})q_{BOD} + 37.5\ Y_{BOD} \cdot m_{BOD} \end{aligned}\right\} \qquad \text{eq.(3.60)}$$

In practice q_o and q_{BOD}, which are based on active biomass, are not known, due to the variable fraction of active solids in the total solids. The parameters which are known are βq_o and βq_{BOD}.

Therefore eq. 3.60 is multiplied with β to yield eq.(3.61)

$$\left.\begin{aligned} 35°C: \beta q_o &= (40.6 - 37.5\ y_{BOD})(\beta q_{BOD}) + 37.5\ \beta Y_{BOD} \cdot m_{BOD} \\ 45°C: \beta q_o &= (36.6 - 37.5\ y_{BOD})(\beta q_{BOD}) + 37.5\ \beta Y_{BOD} \cdot m_{BOD} \end{aligned}\right\} \qquad \text{eq.(3.61)}$$

From eq.(3.61) it appears that the tangent of the relation between βq_o and βq_{BOD} does not depend on β. This means that Y_{BOD}, calculated from eq.(3.61) is not disturbed by variable values of β.

The relations between βq_o and βq_{BOD} for 35°C and 45°C are shown in the Figures 3.17 A and B. From the slope and intercept values the following values of Y_{BOD} and m_{BOD} are obtained, where it is assumed that β = 0.6 (see Chapter 3.4.2.a, period 3):

35°C Y_{BOD} = 0.42 ± 0.04 g DM/g BOD

m_{BOD} = 0.116 ± 0.2 g BOD/g DM hr

45°C Y_{BOD} = 0.26 ± 0.03 g DM/g BOD

m_{BOD} = 0.20 ± 0.22 g BOD/g DM hr

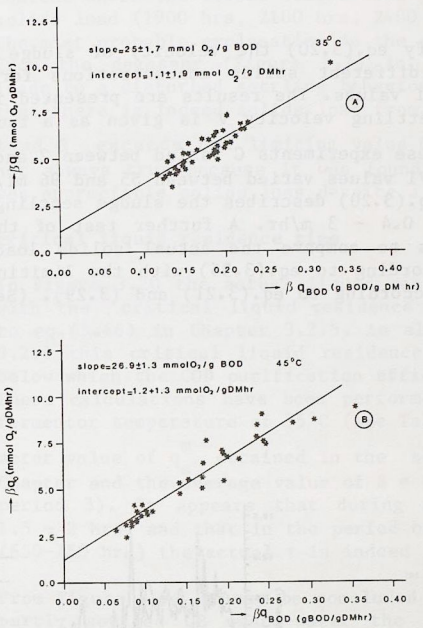


Figure 3.17
Relation on pilot scale between O_2-consumption and BOD conversion rate at (A) 35°C, (B) 45°C.

It is evident that the biomass yield at 45°C is appreciably lower than at 35°C. This lower biomass yield at 45°C should also lead to a lower efficiency of N-elimination, which seems indeed to occur, as evidenced in Table 3.6.

As to the maintenance values, no interpretation should be given to their numerical values, due to the large standard deviation in this parameter.
This large standard deviation for the maintenance parameter is not uncommon. Even under very carefully controlled experiments with pure cultures m_s tends to have a large standard deviation (Ref.3.17), which is in the present study even larger due to the influence of a variable value of β.
The above value of the sludge yield at 35°C on BOD which is consumed, compares reasonable with the value of 0.60 ± 0.18 gDM/gBOD, which can be calculated from a sludge balance in the periods 3.1 to 3.4 of Table 3.6.

- **oxygen requirements**

From the slope of the lines in Figure 3.17A and B an oxygen requirement of 0.8 kg O_2/kg BOD at 35°C and of 0.86 kg O_2/kg BOD at 45°C can be calculated.
With the relations between BOD and COD conversion, found in the first section of this Chapter, the oxygen requirements can also be expressed as 0.62 kg O_2/kg COD at 35°C and 0.74 kg O_2/kg COD at 45°C.

- **sludge settling**

In order to verify eq.(3.20) the velocity of sludge settling was measured at different solids concentrations for sludges with different SVI values. The results are presented in Figure 3.18 where the settling velocity V is given as a function of $(C/\frac{1000}{SVI})$. In these experiments C varied between 2 and 10 g DM/ltr and SVI values varied between 55 and 96 ml/g DM.
It appears that eq.(3.20) describes the sludge settling reasonable between V = 0.4 - 3 m/hr. A further test of the sludge settling model is to compare the actual solids load of the settling tank according to eq.(3.35) with the limiting solids load calculated according to eq.(3.21) and (3.29). (See Figure 3.19).

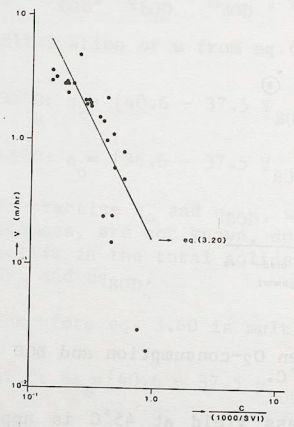


Figure 3.18
Relation between sludge settling velocity and $\left\{C/\frac{1000}{SVI}\right\}$

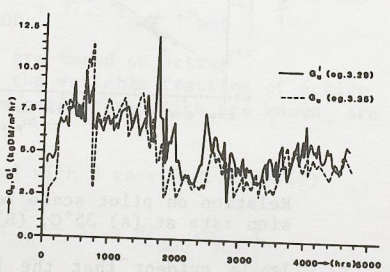


Figure 3.19
Actual and calculated limiting solids load in settling tank on pilot scale.

Periods where the actual solids load is larger then the limiting solids load should show a high suspended solids content in the treated waste-water. According to Figure 3.19 the actual solids load is much larger than the limiting solids load at 600-700 hrs., wich is so serious that the sludge is lost from the aeration tank (see Figure 3.13J).

Suspended solids in the effluent were measured from 1100 hrs onward (see Figure 3-13H).
During most of the experiments the actual solids load is smaller than the limiting solids load.
However at 1500 hrs, at 2700 hrs and from 3600-4200 hrs the actual solids load is larger or nearly equal to the limiting solids load.
According to Figure 3.13H this does indeed coincide with a high suspended solids content of the treated waste-water.
Nevertheless there are peaks of high effluent suspended solids content where the actual solids load is well below the limiting solids load (1900 hrs, 2100 hrs, 2400 hrs, 3500 hrs).
The most probable explanation is the carry-over of air bubbles from the degasser (Figure 3.7) in the settling tank, which leads to a disturbed settling behaviour. In summary it appears that there is indeed solids loss from the settler if the solids load G_u exceeds the limiting value G_u^1. The reverse is however not always true because it was found several times that there was appreciable solids loss despite a low solids load.

- **critical liquid residence time**

In Figure 3.20 the actual liquid residence time τ is compared with the critical liquid residence time calculated according to eq.(3.46) in Chapter 3.2.5. As already outlined in Chapter 3.2.4 this critical liquid residence time is the limit for τ below which the COD purification efficiency collapses rapidly.
These calculations have been performed for the periods with a fermentor temperature of 35°C (see Table 3.6), using the parameter value of q_s^m obtained in the second section of this Chapter and the average value of β = 0.6. (see Chapter 3.4.2.a period 3). It appears that during most of the time τ_{crit} = 1.5 - 2 hrs, and that in the period of serious biomass wash-out (650-750 hrs) the actual τ is indeed below τ_{crit}.

From Figure 3.20 it may be concluded that for the treatment of partly settled GB waste-water the critical liquid residence time in the pilot-plant is 1.5 - 2 hrs. In order to obtain a good process stability a liquid residence time of about 2 times τ_{crit} is required (see Chapter 3.2.4) and thus for the pilot plant a τ-value of 3 - 4 hrs is required.
With these calculations in mind the liquid residence time on pilot-scale in period 2 to 4 (see Table 3.6) and on semitechnical scale (Chapter 3.4.3) were chosen between 3 and 4 hours.

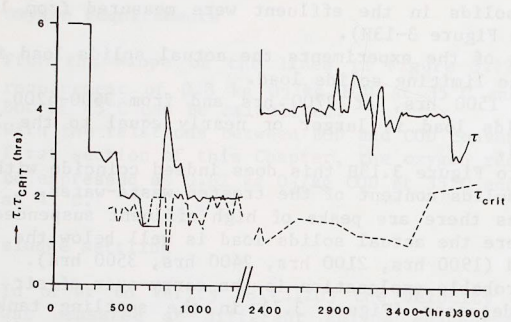


Figure 3.20
Actual liquid residence time and calculated critical liquid residence time on pilot scale.

3.4.2.c Comparison to the lab scale experiments

The substrate uptake kinetic parameters K_s and q_s^m obtained at labscale and pilot scale at 35°C are virtually identical (see Chapter 3.4.1.b and 3.4.2.b). In Figure 3.21 the joint correlation between q_{BOD} and C_{BOD} for lab-scale and pilot-scale is given for the experiments at 35°C. This leads to

q_s^m = 0.38 ± 0.03 g BOD/g DM hr

K_s = 0.183 ± 0.04 g BOD/l

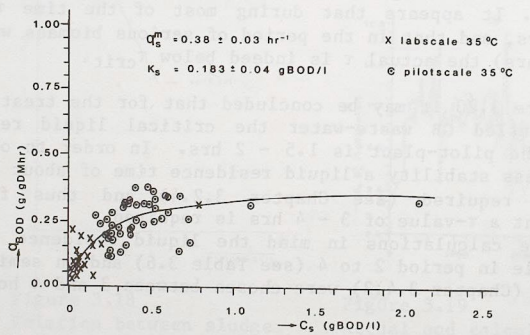


Figure 3.21
Relation on pilot-scale and lab-scale at 35°C between specific BOD conversion rate and BOD concentration.

The relation between BOD and COD conversion rates at 35°C on labscale and pilot scale are the same as can be seen from the Figures 3.11 and 3.14A.

The absence of an hydraulic buffer and pH control has obviously no influence on the proces performance as evidenced by the identical substrate uptake parameters on labscale and pilot scale at 35°C.
A very important difference between labscale and pilot scale is the presence of suspended solids in the waste-water on pilot scale.
The fraction of active solids (β) in the sludge on pilot scale is about 0.6 compared to 1.0 on labscale.
This presence of suspended solids in the waste-water might offer also an explanation for the much higher SVI value of the sludge on pilot scale than on labscale.
From Table 3.6 it appears that SVI on pilot scale is about 78 ml/g DM and from Table 3.5 SVI is about 30 ml/g DM on labscale.
Finally it appeared that on pilot scale the liquid residence time could not be decreased below about 2 hrs because of sludge wash-out.
Contrary, on labscale the liquid residence time could be decreased to 0.87 hrs, without any sign of sludge wash-out (Table 3.5).
This discrepancy can be explained with the concept of τ_{crit}. From eq.(3.46) in Chapter 3.2.5 it appears that τ_{crit} increases if the fraction active solids β decreases, if the SVI value of the sludge increases and if the substrate concentration C_{si} increases.
In Table 3.8 both scales are compared for the τ_{crit} value, calculated with the appropriate values according to eq.(3.46).

	q_s^m g COD/g DM hr	τ hrs	β –	C_{si} g COD/l	C_s g COD/l	$\frac{\Phi_i}{A}$ m/hr	SVI ml/gDM	τ_{crit} hr
lab.scale	0.49	0.87	1.0	4.2	0.76	0.23	30	0.23
pilot scale (period 1.4 Table 3.6)	0.49	1.3	0.6	6.2	1.98	0.54	78	1.83

Table 3.8
Comparison between labscale and pilot scale for τ_{crit} at 35°C.

The q_s^m in Table 3.8 is expressed in gr COD/g DM hr and is calculated from the BOD-based value and the relation between BOD and COD conversion rates at 35°C (1 g BOD = 1.3 g COD).
From Table 3.8 it appears indeed that τ_{crit} on labscale is much smaller than on pilot scale. The difference is particularly due to the absence of suspended solids in the waste-water, a lower SVI-value of the sludge and a lower substrate concentration on labscale. Finally it appeared on the pilot scale that an increase of the fermentor temperature to 40°C does not lead to a decreased purification efficiency, however an increase to 45°C leads to substantially lower purification efficiencies (Table 3.6).

3.4.3. SEMI-TECHNICAL EXPERIMENTS

3.4.3.a General results

The results of the semi-technical experiments are presented in the Figures 3.22A - 3.22G, and the average values of the specific parameters are listed in Table 3.9 for direct comparison with Table 3.5 and 3.6 of labscale and pilot scale experiments.

The dips in purification effiency in Figure 3.22B are due to oxygen limitation during the experiments for the determination of the maximum aeration capacity of the bubble column.

From Figure 3.22D it appears that the suspended solids content in the overflow of the tilted plate settler is rather high and lies mostly considerably above the raw waste-water suspended solids (Figure 3.22C). This is due to the fact that during this experiment the sludge waste flow was maintained zero (w = o) to maximize solids content of the aeration tank. Therefore the surplus sludge leaves the reactor through the settler overflow.

Towards the end of the experiment (1250 hr) it was found that the recycle flow had decreased (probably pump wear) and the pumpspeed had to be increased 60 % to restore the original recycle flow of 560 ltr/hr. Therefore the sludge recycle has probably varied between 350 and 560 ltr/hr. The recycle ratio has then varied between 0.46 - 0.75 .

Despite these uncertainties in the sludge recycle it appears from the solids analysis in the aeration tank (Figure 3.22E), in the sludge recycle (Figure 3.22F) and in the settler overflow (Figure 3.22D) that there has been an efficient sludge retention in the system. The sludge retention will be discussed more extensively in the following Chapter (3.4.3.b).

The results listed in Table 3.9 compare well with the pilot scale results listed in Table 3.6 during period 4 (also at 40°C).

On both scales the same COD purification efficiency at comparable sludge and reactor COD load is obtained.

This means that the waste-water can be treated aerobically without presettling, hydraulic buffering or pH control in a high bubble column.

The fraction active solids β in the total solids in the fermentor was calculated with eq.(3.44) and is 0.36 according to Table 3.9. This is considerably lower than during the pilot scale experiments and is due to the absence of the presettling treatment of the raw waste-water.

The SVI value of 102 ml/gDM on semi-technical scale seems to be higher than the average SVI value of 78 ml/gDM on pilot scale. This result tends to substantiate the finding that a sludge with a low fraction of active biomass has a high SVI value (see Chapter 3.4.2.c). This phenomenon might also be the reason of the low SVI-value of the sludge between 1000 and 1150 hrs (see Figure 3.22G) during which period the influent waste-water suspended solids are exceptionally low (see Figure 3.22C).

In Figure 3.23 the SVI value of the sludge is related to the active biomass fraction of the sludge. It appears very clearly that at smaller β values the SVI value of the sludge increases rapidly. The mechanisms behind this phenomenon however do remain obscure.

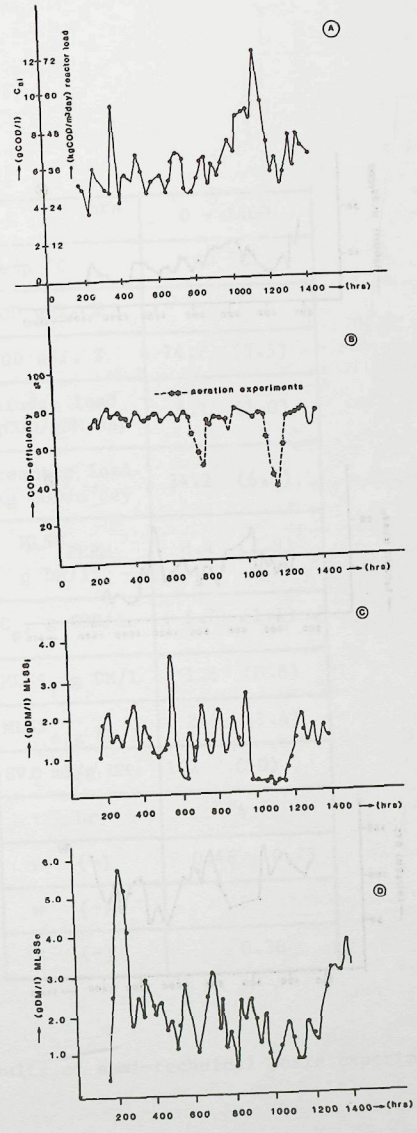


Figure 3.22
Results of the semi-technical scale experiment.
(A) influent COD concentration and applied reactor COD load.
(B) COD removal efficiency.
(C) suspended solids concentration in influent waste-water
(D) suspended solids content in effluent waste-water

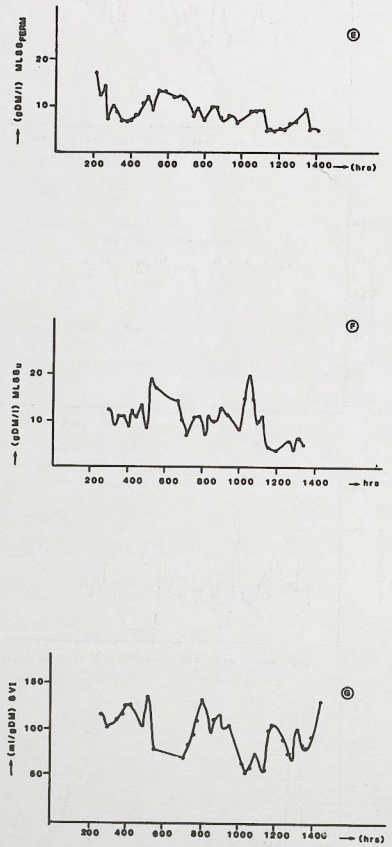


Figure 3.22
Results of semi-technical scale experiment.
(E) suspended solids concentration in aeration tank
(F) suspended solids concentration in sludge recycle
(G) SVI

hrs	0 - 1400
Temp °C	40 °C
BOD eff. %	-
COD eff. %	74.7 (3.5)
sludge load gCOD/gDM day	4.1 (1.0)
reactor load kg COD/m^3day	34.2 (6.6)
$MLSS_{FERM}$ g DM/1	8.4 (2.9)
C_{si} g COD/1	5.7 (1.1)
$MLSS_i$ g DM/1	1.6 (0.6)
$MLSS_e$ g DM/1	2.5 (1.4)
SVI ml/g DM	102 (20)
τ hr	4
r (-)	0.46 - 0.75
w (-)	0
β (-)	0.36

( ) st. dev.

Table 3.9
Average results of semi-technical scale experiments

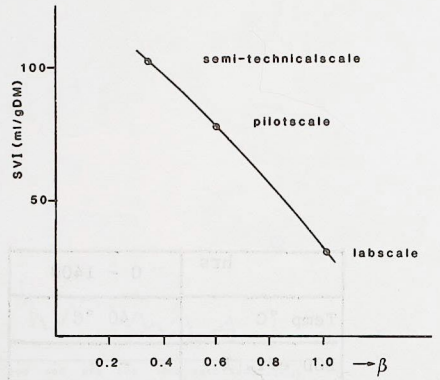


Figure 3.23
Relation between SVI value of activated sludge and the fraction active biomass (β) in the sludge

3.4.3.b Technological aspects

As already mentioned in Chapter 3.3.3.c the experiments on semi-technical scale should provide information regarding mixing, oxygen transfer capacity, sludge settling in and performance of the tilted plate settler. In the next sections each of these subjects will be dealt with.

- **mixing in the bubble column**

The mixing in bubble columns can be regarded as the ideal mixing state (Ref. 3.15).
This was shown in one way through the absence of temperature differences between top and bottom of the column (which is not insulated) and in a second way through the absence of differences in suspended solids concentrations in the bubble column (see Table 3.10).

- **aeration in the bubble column**

During two periods (850 - 930 hrs and 1100 - 1200 hrs) the maximum aeration capacity of the bubble column fermentor was determined. The results, expressed as percentage O_2 depletion per meter unaerated liquid height, and the air hold-up are listed in Table 3.11 at different air flow rates.

It can be seen that the correlation, mentioned in Chapter 3.2.6, of 0.55% O_2/m for the calculation of oxygen transfer is indeed applicable for the waste-water/sludge system studied. During these experiments, which were carried out at zero dissolved oxygen concentration, it appeared that bulking sludge was present in the fermentor. This bulking sludge could be eliminated through aeration above zero dissolved oxygen concentration.

$MLSS_{FERM}$ g DM / l				
sampling point / time hrs	S_1 4.1 m above bottom	S_2 7.9 m above bottom	S_3 11.9 m above bottom	S_4 15.9 m above bottom
187	15.9	16.8	15.2	14.4
211	11.9	12.2	12.4	11.9
235	13.9	14.3	13.8	13.5
259	7.6	7.4	7.3	6.6
285	9.1	9.6	8.4	8.6
331	6.5	6.8	6.4	6.1

Table 3.10
Sludge concentration at different heights in bubble column

air flow rate (Nm^3/hr)	V_{sg} m/hr	O_2 depletion (% O_2)	static liquid height m	gas hold-up %	% O_2/m
5.0	26	-	17.4	4	-
10.0	52	9.36	16.9	7	0.55
15.1	79	9.16	16.6	9	0.55
20.1	105	8.34	16.35	10	0.51
29.7	155	8.15	15.0	18	0.54
39.3	205	7.91	14.2	22	0.56
54.0	281	-	14.3	21	-

Table 3.11
Gas hold-up and oxygen transfer in the bubble column

- sludge retention by the tilted plate settler

In Chapter 3.4.3.a it was already mentioned that the sludge recycle from the tilted plate settler has varied between 350 and 560 1/hr.
This tilted plate settler has been operated at conditions of sludge overloading during the whole experiment in order to determine the limiting sludge load G_u^1 .
Because of the variable sludge recycle however only an estimation of this limiting sludge load G_u^1 is possible. From the above mentioned recycle flows and the settler area of 0.25 m^2 it follows that V_u has varied between 1.4 and 2.3 m/hr. From Figure 3.22F an average underflow solids concentration C_u = 12.0 gDM/1 can be calculated during this experiment. Therefore G_u^1 has varied between 17 and 27 kg DM/m^2 hr under the present experimental conditions in the tilted plate settler (V_u = 1.4 - 2.3 m/hr). This value compares very favorably with G_u^1 = 5 - 8 kg DM/m^2 hr which was found in the conventional settler used in the pilot scale experiments (Figure 3.19). It seems therefore that the tilted plate settler is superior to the conventional settler for the sludge retention.
This better performance of the tilted plate settler has also been found by other workers (Ref. 3.23 - 3.24).
Furthermore a modified eq (3.29) can be calculated for the tilted plate settler. Using the above mentioned values of G_u^1 and the corresponding V_u-values and bearing in mind that the sludge has a SVI-value of 102 ml/g DM (table 3.9) the following equation can be estimated

$$G_u^1 = \frac{1700}{SVI} V_u^{0.556} \qquad \text{eq.(3.29a)}$$

Furthermore the remarkable feature was noted during this experiment that the solids content between reactor and settler (sampling point S_5) was always lower than the solids concentration in the aeration tank.
Some measurements are listed in Table 3.12.

time (hrs)	187	211	235	259	285	331
suspended solids in S_5 g DM/1	8.6	11.0	9.3	6.0	1.2	5.8
suspended solids in bubble column g DM/1	15.6	12.1	13.9	7.2	8.9	6.5

Table 3.12
Suspended solids concentration in the bubble column and in sampling point S_5

This phenomenon is probably caused by preferential solids retention around the liquid outflow opening in the aeration tank (Figure 3.9).

3.4.3.c Comparison to labscale and pilot scale experiments

The results listed in Table 3.9 can be compared with the pilot scale experiments listed in Table 3.6 (period 4).
There is fair agreement for COD purification and sludge load. The SVI-value at semi-technical scale is however apreciably higher than at pilot scale or labscale. As already outlined in Chapter 3.4.3.a this seems to be related to the active biomass fraction of the sludge (Figure 3.23).
The active biomass fraction β is very low at semi-technical scale due to high concentrations of suspended solids in the raw waste-water (β= 0.36).
Therefore the substrate conversion rate of the active biomass can be calculated from Table 3.9 as q_{COD} = 0.37 g/gDM hr.
The value of q_{BOD} follows then from the interpolated relation between COD and BOD conversion rates (chapter 3.4.2.b) and is q_{BOD} = 0.30 g/gDM hr.
This value of q_{BOD} is very close to the maximum value of q_s^m = 0.38 g BOD/g DM hr. which was found at labscale and pilot scale at 35°C. (Chapter 3.4.2.c).
Bearing in mind that a good purification was obtained on semi-technical scale it must be concluded that q_s^m at semi-technical scale is higher then at lab/pilot-scale. The reason for this is that at semi-technical scale a temperature of 40°C was maintained in the reactor contrary to lab/pilot scale (35°C).
Finally it is illustrative to calculate the τ_{crit} from eq.(3.46). However this equation needs a modification because of the use of a tilted plate settler. Recalling the eq.(3.29a) in the preceding Chapter 3.4.3.b, it can be shown that the number 905 in eq.(3.46) is replaced by 1700.
Another problem is that the value for q_s^m at 40°C is not known exactly, but as already said it appears that q^m at 40°C is appreciably higher than at 35°C.
Using as a rule of thumb that a temperature rise of 10°C doubles the rate of a biological process it follows that q_s^m at 40°C is 1.4 times the q_s^m value at 35°C.
Using q_s^m = 0.49 g COD/g DMhr at 35°C (Chapter 3.4.2.c) this leads to q_s^m = 0.69 g/g DMhr at 40°C.
From these modifications, and applying the appropriate values listed in Table 3.9, a τ_{crit} can be calculated for the semi-technical scale experiments as τ_{crit} = 3.3 hr, which can be compared with the applied τ = 4 hr.

3.4.4. SUMMARY OF THE RESULTS AT LAB-,PILOT-, AND SEMI-TECHNICAL SCALE

The GB waste-water, characterized in Chapter 1, can be treated aerobically in a stable proces without presettling or hydraulic buffering or pH control in a tall bubble column at 40°C with a hydraulic residence time of 4 hrs and an average reactor load of 34 kg COD/m^3 day with a COD-efficiency of 75% (Table 3.9).
The tilted plate settler which was used at semi-technical scale could handle a 3-4 times higher solids load compared to the conventional settlers used at lab-, and pilot-scale.
The COD removal efficiency is not improved if hydraulic buffering or pH control or a fermentor temperature of 35°C are provided; a fermentor temperature of 45°C however leads to a low COD removal efficiency (Table 3.6).
An important aspect is the presence of suspended solids in the waste-water. A high suspended solids concentration leads to a sludge with a low fraction active biomass. This then leads to high substrate loads of the active biomass and a low BOD/COD removal efficiency (compare Table 3.6 for the pilot scale experiments with substantial suspended solids in the waste-water and Table 3.5 for the lab scale with no suspended solids in the waste-water). A second consequence is the deterioration of the sludge settling properties when the fraction active solids decreases (Figure 3.23). At lab scale with no suspended solids in the waste-water a SVI value of 30 ml/g DM was found, at pilot scale with partial suspended solids presettling SVI = 76 ml/g DM and at semi-technical scale SVI = 102 ml/g DM for the raw waste-water.
Furthermore it appears that the proposed sludge settling model describes within practical limits sludge settling and the sludge settler (Chapter 3.4.2.b). The concept of the critical hydraulic residence time τ_{crit}, developed in Chapter 3.2.4, is an useful tool in the comparison of the experiments on different scales and for the estimation of the required liquid residence time. The substrate uptake parameters are only measured at 35°C and the same results are found for lab-scale and pilot-scale experiments. The maximum conversion rate q_s^m of the active biomass at 35°C is 0.38 g BOD/g DM.hr or 9.1 g BOD/g DM.day. The K_s value is 0.183 g BOD/ltr (Chapter 3.4.2.c). At 40°C the q_s^m value seems to be appreciably higher (Chapter 3.4.3.c).
The yield of active biomass is 0.42 g DM/g BOD at 35°C and 0.26 g DM/g BOD at 45°C (Chapter 3.4.2.b.).
The maintenance coefficient could not be estimated with acceptable accuracy.
The oxygen requirements are 0.80 kg O_2/kg BOD at 35°C and 0.86 kg O_2/kg BOD at 45°C. The substrate conversion in the aerobic proces is measured as COD, BOD and TOC. The following relations are found to hold:
35°C : 1 g BOD = 1.3 g COD = 0.46 g TOC
45°C : 1 g BOD = 1.17 g COD
It is stressed that all conversion rates or yield values are based on converted substrate and not on added substrate.
The tall bubble column reactor can be regarded as ideally mixed with regard to temperature and sludge content.

The oxygen transfer capacity in the bubble column agrees with general correlations found in literature and is 0.55% O_2/m at zero dissolved oxygen concentration.
During all the experiments at lab-scale, pilot-scale or semi-technical-scale sludge bulking was never observed onder normal operating conditions. However under O_2-limitation conditions bulking sludge quickly appeared.

3.5 MODEL CALCULATIONS FOR A FULL SCALE TREATMENT PLANT

- general assumptions

With the previous results a calculation can be performed regarding the dimensions of a large scale treatment plant which embodies an aerobic bubble column reactor and a settling tank (without tilted plates).
The following design values are specified:

- Φ_i = 420 m^3/hr.
- C_{si} = 8 kg COD/m^3 or 4.8 kg BOD/m^3
- waste-water temperature is 35°C
- The temperature is assumed to be 40°C in the reactor

As model parameters the following values are used

- maximum substrate conversion rate q_s^m = 0.38 g BOD/g DM hr. This is probably a conservative value because this value applies to the experiment of 35°C

- half velocity BOD concentration K_s = 0.183 kg BOD/m^3

- oxygen requirement is 0.83 kg O_2 per kg BOD removed. This value is the average of the values found at 35°C and 45°C experiments (Chapter 3.4.2.b)

- sludge production is 0.34 kg sludge per kg BOD removed. This is the average sludge production found at 35°C and 45°C (Chapter 3.4.2.b)

The hydraulic residence time τ is assumed to be 2 τ_{crit}
As shown in Chapter 3.2.4 this ensures process stability with regard to sludge wash-out.

Three cases will be considered:
- β = 1 and SVI = 30 which corresponds to fully pre-settled waste-water
- β = 0.6 and SVI = 76 which corresponds to partly settled waste-water
- β = 0.36 and SVI = 102 which corresponds to unsettled raw waste-water

- required reactor volume and settling area

From eq.(3.41) in Chapter 3.2.4. it follows that $C_s = K_s$ because it has been assumed that $\tau/\tau_{crit} = 2$
Therefore $C_s = 0.183$ kg BOD/m^3.

From eq.(3.46) the following relation (3.62) between reactor volume V and settling area A is obtained bearing in mind that $\tau_{crit} = 0.5\ \tau$

$$0.5 \left\{\frac{V}{\Phi_i}\right\} = \frac{C_{si} - C_s}{\beta\ q_s^m} \cdot \left\{\frac{\Phi_i}{A}\right\}^{0.444} \cdot \frac{SVI}{905} 1.94 \qquad \text{eq.(3.62)}$$

Introducing the appropriate values: $C_{si} = 4.8$ gBOD/l , $C_s = 0.183$ gBOD/ltr, $q_s^m = 0.38$ gBOD/gDMhr and $\Phi_i = 420$ m^3/hr leads to eq. (3.63)

$$V = 320 \frac{SVI}{\beta} \frac{1}{A^{0.444}} \qquad \text{eq.(3.63)}$$

In Figure 3.24 the relation between reactor volume and required settling area is shown for the three aforementioned cases. It is evident that especially for unsettled waste-water (case 3) the reactor volume and settler area become very large. In this regard it seems therefore especially important to explore in more detail the possibilities of the tilted plate settler and the use of a presettler which removes partially the suspended solids from the waste-water. (compare case 3 to case 2).

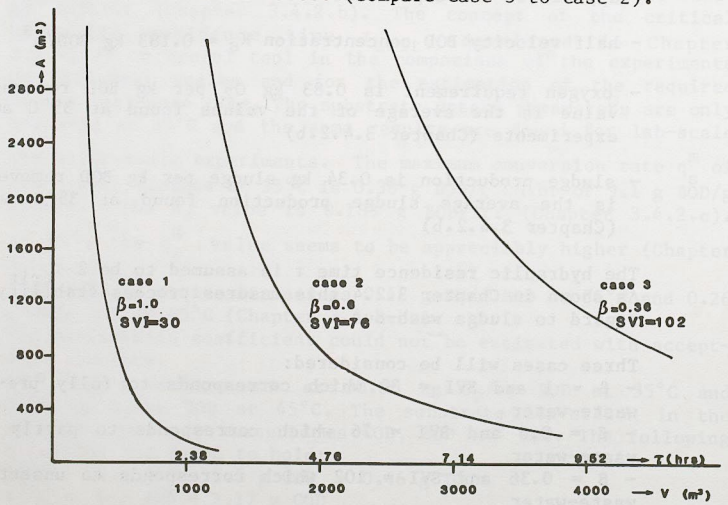


Figure 3.24
Relation between required full scale reactor volume and settling area.

- purification efficiency

As already mentioned in the previous section
$C_s = 0.183$ kg BOD/m^3.
This means a BOD purification efficiency of 96 % based on $C_{si} = 4.8$ kg BOD/m^3.

Although the BOD-purification efficiency of 96% is very satisfying with regard to deminishing the costs for discharging the waste-water, it should be born in mind that a BOD concentration of 183 mg/l in the treated waste-water does not meet the required standard of 20 mg BOD/l for discharge on surface waters. Therefore further treatment is necessary, i.e. in the municipal sewage plant or in a nitrification process.
During the purification 4.62 kg BOD are removed, which corresponds to the removal of 4.62 * 1.30 = 6.01 kg COD/m^3 which leads to a COD purification efficiency of 75 % based on C_{si}= 8 kg COD/m^3.

- oxygen requirement and minimum air requirement for a bubble column reactor

The oxygen requirement is 0.83 kg O_2/kg BOD removed (see first section of this Chapter). This then leads to an oxygen requirement of 0.83 * 0.96 *4.8 * 420=1607 kg O_2/hr or 1208 Nm^3O_2/hr.
Using the correlation of 0.55 % O_2/m for the oxygenconcentration the following minimum amount of air can be calculated for different liquid heights in the bubble column

H= 5m ΔO_2= 2.75% , air = $\frac{1208}{0.0275}$ = 44000 Nm^3/hr

H=10m ΔO_2= 5.5 % , air = $\frac{1208}{0.055}$ = 22000 Nm^3/hr

H=20m ΔO_2= 11 % , air = $\frac{1208}{0.11}$ = 11000 Nm^3/hr

If it is assumed that the maximum linear gas velocity in the bubble column is 10cm/sec (due to reasons of foaming) a minimum reactor volume of 611m^3 can be calculated for the different reactor heights from the above calculated minimum airflow rates. Therefore, if the reactor volume is chosen less than 611m^3 (or $\tau \leq 1.45$hr) the proces will be oxygen transfer limited (see Chapter 3.2.6 for extensive calculations on this subject).

- sludge production

The sludge yield being 0.34 kg sludge per kg BOD removed, the total sludge production can be calculated as
0.34 x 0.96 x 4.8 x 420 = 658 kg DM/hr = 15.8 ton DM/day.
This surplus sludge is available as a thickened slurry of about 20 kg DM/m^3 (see Figure 3.13K). This means that there remains to be treated a daily sludge slurry of 790m^3.

- **heat production**

As calculated above the oxygen requirement is 1607 kg O_2/hr = 50 kmol O_2/hr
From eq.(3.49) in Chapter 3.2.6 the heat production can then be calculated as $5 \times 10^4 \times 478 = 24 \times 10^6$ kJ/hr. A part of this heat is used to increase the waste-water temperature of 35°C to the reactor temperature of 40°C. This requires $(40-35) \times 420 \times 10^3 \times 4.19 = 8.8 \times 10^6$ kJ/hr.
Therefore there remains a heat flow of 15.2×10^6 kJ/hr to be cooled away.
This is an approximate result because there are other heat sources which are however of a minor magnititude and mostly cancel each other (like waterevaporation into the sparged air and energy dissipation from the compressed air).

3.6 CONCLUSION

As is shown in the previous Chapters the aerobic treatment of the GB waste-water poses no problems with regard to process stability or purification efficiency.
After such an aerobic treatment process there remains however the formidable problem of surplus sludge treatment and disposal. According to Ref. 3.20 the costs of surplus sludge treatment and disposal are about equal to the costs of the aerobic treatment which does generate these surplus sludge masses.
Therefore it might be interesting to explore ways to reduce the surplus sludge production in aerobic treatment processes. One possibility is to use the so called maintenance concept (Ref. 3.21), which states that the biomass yield decreases if the sludge load is decreased. If this sludge load reaches the maintenance sludge-load there should be no more sludge production.
In the next chapter several aspects concerning such a process will be evaluated and experiments will be described to substantiate the applicability of the maintenance concept in aerobic waste-water treatment of the highly variable GB-waste-water.

SYMBOLS

A	area settling tank	m^2
a	constant in equation (3.17)	
a_1	constant in equation (3.19)	
b	constant in equation (3.17)	
C	suspended solids concentration	$kgDM/m^3$
C_1	suspended solids concentration in the clarification zone of the settling tank	$kgDM/m^3$
C_2	suspended solids concentration in the thickening zone of the settling tank	$kgDM/m^3$
C_x	concentration active biomass in aeration tank	$kgDM/m^3$
C_u	concentration active biomass in recycle flow	$kgDM/m^3$
C_e	concentration active biomass in overflow of settling tank	$kgDM/m^3$
C_s	substrate concentration in aeration tank	kg/m^3
C_{si}	substrate concentration in waste-water entering into the aeration tank	kg/m^3
D	diameter of aeration tank	m
G	solids load	$kgDM/m^2hr$
G_o	solids load of clarification zone in the settling tank	$kgDM/m^2hr$
G_u	solids loads of thickening zone in the settling tank	$kgDM/m^2hr$
G_u^l	limiting solids load in the thickening zone of the settling tank	$kgDM/m^2hr$
H	unaerated liquid height of aeration tank	m
k	constant in eq. (3.31)	m^3/kg DM
K_s	Michaelis- Menten constant	kg/m^3
m_s	maintenance coefficient of active biomass on substrate	kg/kgDMhr
m_{BOD}	maintenance coefficient of active biomass on BOD	kgBOD/kgDMhr
$MLSS_i$	total suspended solids in the waste-water feed to the aeration tank	$kgDM/m^3$
$MLSS_{FERM}$	total suspended solids in the aeration tank	$kgDM/m^3$

$MLSS_e$	total suspended solids in the overflow waste-water from the settling tank	$kgDM/m^3$
$MLSS_u$	total suspended solids in the recycle flow	$kgDM/m^3$
OTR	oxygen transfer rate	mol/hr
OUR	oxygen uptake rate	mol/hr
q_s^m	maximum substrate conversion rate of active biomass	kg/kgDMhr
q_{COD}	COD-conversionrate of active biomass	kgCOD/kgDMhr
q_{BOD}	BOD-conversionrate of active biomass	kgBOD/kgDMhr
q_o	O_2-conversion rate of active biomass	mol O_2/kgDMhr
r_s	substrate conversion rate in aeration tank	kg/m^3hr
r_x	active biomass production rate in aeration tank	$kgDM/m^3hr$
r_o	oxygen consumption rate	$molO_2/m^3hr$
r_c	carbon dioxyde production rate	$molCO_2/m^3hr$
r_{COD}	COD conversion rate	$kgCOD/m^3hr$
r_H	heat production rate	kJ/m^3hr
r'_s	substrate conversion rate	$molC/m^3hr$
r'_x	biomass production rate	$molC/m^3hr$
r	recycle ratio	-
SVI	sludge volume index	ml/g DM
T	temperature of aeration tank	°C
T_i	temperature of waste-water feed into aeration tank	°C
V	volume aeration tank	m^3
V	settling velocity of sludge	m/hr
V^{MAX}	constant in eq. (3.31)	m/hr
V_o	upwards superficial liquid velocity in the clarification zone of the settling tank	m/hr
V_u	downwards superficial liquid velocity in the thickening zone of the settling tank	m/hr

V_{sg}	superficial gasvelocity in aeration vessel, calculated under standard conditions of temperature and pressure	m/hr
w	waste ratio	-
Y_{sx}	yield of active biomass on substrate	kgDM/kg
Y_{so}	oxygen requirement per unit substrate conversion	mol O_2/kg
Y_{BOD}	yield of active biomass on BOD conversion	kgDM/kgBOD
Y_{COD}	yield of active biomass on COD conversion	kgDM/kgCOD
α	carbon content of converted COD	kgC/kgCOD
β	fraction of active biomass in total suspended solids	-
Φ_r	recycle flow rate	m^3/hr
Φ_w	waste sludge flow rate	m^3/hr
Φ_i	waste-water feed flow rate	m^3/hr
μ	active biomass growth rate	hr^{-1}
μ^{MAX}	maximum growth rate of active biomass	hr^{-1}
τ	residence time of waste-water in aeration tank	hr
τ_{min}	minimum residence time of waste-water in aeration tank before biomass wash-out occurs	hr
τ_{crit}	critical residence time of waste-water in aeration tank below which the purification efficiency collapses	hr
ν_s	degree of reduction of substrate	-
ν_x	degree of reduction of active biomass	-

ABBREVIATIONS

BOD	biological oxygen demand
COD	chemical oxygen demand
C_2	acetic acid
C_3	propionic acid
C_4	butyric acid
iC_4	iso-butyric acid
C_5	valeric acid
iC_5	iso-valeric acid
DM	dry matter
FA	Fatty acids
GB	Gist-Brocades
GLS	gas/liquid/solid
PE	population equivalent
SEM	scanning electron microscope
SP	sampling point
SVI	sludge volume index
TOC	total organic carbon
VSS	volatile suspended solids

REFERENCES

3.1 J.A. Roels, N.W.F. Kossen,
Progres in Industrial Microbiology,
Ed. M.J. Bull (Elsevier, Amsterdam,1978), vol. 14, p 95,

3.2 J.F. Andrews,
Biotechnol. and Bioeng., Symp. No.2, 5-33, (1971),

3.3 G.J. Kynch,
Trans. Faraday. Soc. 48, (1952), 166,

3.4 R.I. Dick, K.W. Young,
27th Ind. Waste Conf. (1972), part 1, 33,

3.5 R.O. Ball, R.F. Smullen,
30th Ind. Waste Conf. (1975), 448,

3.6 R.I. Dick,
Filtration and Separation, (1972), 177

3.7 M.D.J. White,
Progr. Wat. Technol., 8, (1977), 515,

3.8 G.B. Wallis,
One dimensional two-fase flow
McGraw Hill, (1969)

3.9 J.H. Sherrard, E.D. Schroeder,
30th Ind. Waste Conf. (1975), 14

3.10 J.J. Urza,M.L. Jackson,
Ind. Eng. Chem. Process Des. Dev., 14, (1975), 106,

3.11 M.L. Jackson, J. Chen.
A.I.Ch.E.J., 24, (1978),

3.12 Standard methods for the analyses of raw, potable waste-waters,Dept. of the Environment 1972,

3.13 M.J.D. White,
Settling of activated sludge,
Technical Report TR 11,
Water research Centre, Stevenage,

3.14 J.A. Roels,
Biotechnol. and Bioeng., 22, (1980), 2457,

3.15 J.J. Heijnen, K. van 't Riet,
Accepted in The (Bio)Chemical Engineering Journal (1984).

3.16 A. Bauer,
Progr. Water Technol., 12, (1980), 665,

3.17 J.W. de Kwaadsteniet, J.C. de Jager, A.H. Stouthamer,
J. Theor. Biol., 57, (1976), 103,

3.18 D.A. Hines, N. Baily, J.C. Ousby, F.C. Roesler,
Symp. Institution of Chem. Eng.,
York University, 1975.

3.19 Patent NL 7613506,

3.20 A.C. Middleton, A.W. Lawrence,
Biotechnol. and Bioeng., 16, (1974), 807,

3.21 D. Herbert,
Symp. Int. Congr. Microbiol., 6, (1958), 381,

3.22 Spectrophotometrics. data for colorimetric analysis,
London, Butterworths, (1963), 426,

3.23 E. Rubin, E. Zahavi,
A.I.Ch.E.Symp. Ser. 71, (1975), 151,

3.24 G. Sarmiento, O.T.H. Ullherr,
The Can. J. Chem. Eng., 56, (1978), 705,

4.0 AEROBIC HIGH RATE TREATMENT OF GB WASTE-WATER WITHOUT SURPLUS SLUDGE PRODUCTION

4.1. INTRODUCTION

One conclusion of the previous Chapter 3 was that the massive sludge production in the aerobic waste-water treatment process is a serious drawback.
In principle however there exists the possibility to diminish the surplus sludge production by making use of the so called maintenance phenomenon in micro-organisms. In the next Chapters this maintenance concept will be discussed extensively and the experimental verification of the concept with the GB-waste-water will be presented.

4.2. THE MAINTENANCE CONCEPT FOR NO SURPLUS SLUDGE PRODUCTION IN THE AEROBIC WASTE-WATER TREATMENT PROCESS

The concept of substrate for maintenance in micro-organisms was introduced by Pirt (Ref. 4.1). It simply states that microbes do need a certain flow of energy to maintain their cellular integrity. This energy can be obtained from the conversion of substrate or cell-compounds. As a consequence the microbe cannot produce biomass if it receives only the amount of substrate which produces, after oxydation, just the energy flow for maintenance.
Mathematically this can be formulated by putting $\mu = 0$ which leads to eq.(4.1) (from eq. 3.10 and 3.14 in the previous Chapter 3).

$$C_x = \frac{C_{si} - C_s}{m_s \tau} \qquad \text{eq.(4.1)}$$

In theory the $\mu = 0$ condition means that all the biomass which flows out of the bioreactor should be separated from the liquid, and returned to the bioreactor. The biomass concentration in the reactor reaches then a steady state concentration according to eq (4.1) (see Figure 4.1).

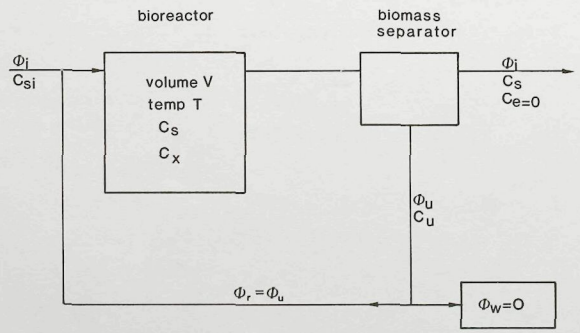


Figure 4.1
Description of the aerobic treatment process with no surplus sludge production

From this eq.(4.1) it follows that the steady state level of the biomass concentration in the reactor under maintenance condition decreases if the substrate concentration C_{si} decreases, if the hydraulic residence time τ increases and if the maintenance coefficient m_s increases. It is obvious that a high value of m_s is advantageous because this leads to a lower τ value at fixed C_{si} and C_x values. From Ref. 4.2 it appears that temperature has a demonstrated major influence on m_s for pure cultures under carbon and energy limitation; the maintenance coefficient rises steeply with increasing temperature. In Table 4.1 an indication of maintenance values for axenic cultures from Ref. 4.2 at different temperatures is provided. It should be borne in mind however that the variation of maintenance values for different micro-organisms at the same temperature is very large, up to a factor 2 (Ref. 4.2).

Temperature °C	m_s g COD/ g DM hr
5	0.0126
10	0.0149
15	0.0182
20	0.0218
25	0.0261
30	0.0310
35	0.0367
40	0.0431
45	0.050

Table 4.1
Estimated substrate maintenance values at different temperatures (Ref. 4.2).

Therefore the reactor temperature should be chosen as high as possible. Especially for the usually warm industrial waste-waters such a high temperature can readily be obtained.
Sofar the theory about the maintenance concept is straightforward and poses no problems, especially because Gaudy and coworkers (Ref. 4.3 and 4.4) have demonstrated the applicability of the maintenance concept for aerobic waste-water treatment on laboratory scale (6 ltr). These experiments however were conducted with an artificial waste water (glucose, salts medium) with a constant substrate concentration.
In the present investigation however the industrial waste-water is highly variable in substrate concentration (Chapter 1). This leads then to a fluctuating biomass concentration in the reactor, which means that biomass starvation will occur. Such a starvation leads to suspended solids from cell-lysis like cell walls, and this inert solids fraction can build up in the reactor.If this occurs the solids concentration in the reactor will

never reach a stable level and a waste flow of solids must be established.
Another complication is that in the usual aerobic process with surplus sludge production there exists the possibility to remove recalcitrant soluble organic compounds through adsorption to or incorporation in the surplus biomass. In a reactor under maintenance condition this cannot occur because there is no surplus sludge and hence it is possible that the COD removal efficiency is lower in such a reactor.
Furthermore the industrial waste-water is likely to contain certain amounts of heavy metals. These metals do accumulate in the biomass. Under conditions of surplus sludge production the metal concentration reaches a stable level because of sludge wasting. If there is no surplus sludge production it is possible that the metal concentration in the biomass reaches toxic levels which results in a collapse of the purification process.
Finally there exists the possibility of a continuous rapid selection for micro-organisms with the lowest m_s values. This means that the biomass concentration in the reactor increases beyond realistic levels and biomass production will occur.
Because of the above considerations it was considered necessary to evaluate experimentally the application of the maintenance concept on the GB waste-water.
Besides the considerations given above there are some additional aspects which should be kept in mind regarding an experimental reactor under maintenance condition:

- There is no removal of soluble organic N through incorporation in the surplus biomass. This is disadvantageous regarding the purification in terms of P.E.* . It is however possible that the slow growing nitrifying micro-organisms will develop in a reactor under maintenance condition because of the in principle sufficient sludge age.
- There is no removal of soluble COD through fixation in surplus biomass. This means that all the soluble COD must be oxidised. Therefore it is expected that the oxygen requirement per unit COD removed increases compared to the process with surplus sludge production. This also means that the heatproduction increases per unit COD removed.
- The waste-water which is fed to the reactor should not contain inert suspended solids because these solids will accumulate in the reactor and this leads to an ever increasing solids concentration in the reactor.
- The biomass separation (see Figure 4.1) can be done by gravity settling (previous Chapter 3) or centrifugation (Ref. 4.3 and 4.4).
  Because at this moment nothing is known about the settling properties of sludge under maintenance conditions a centrifugal separation is advisable to ensure a stable operation of the biomass separator.

* P.E. = population equivalent, which is the amount of pollution produced by 1 person in 1 year.

4.3 MATERIALS AND METHODS

4.3.1 DESCRIPTION OF THE EQUIPMENT

The flow sheet of the experimental equipment is shown in Figure 4.2. The waste-water is pumped directly from the sewer (1) to the pilot plant where the experiments are performed. In this pilot plant feed-tank (2) is loaded each day with a fresh batch of waste-water (2500 kg which requires about 2 hours pumping from the sewer). Before doing so however the residue of the old batch of waste-water from the previous day is removed and the empty feed tank (2) is steam sterilized at 120°C for 45 minutes. This is done to minimize residual bacterial activity in the feed tank (2).
In order to enable filtration of the raw waste-water (to eliminate the suspended solids, see Chapter 4.2) 0.5% (weight) of Dicalite superaid was added. The addition of Dicalite did not alter significantly the soluble COD content of the waste-water.
The temperature in feed tank (2) was controlled at 15-17°C.
The waste-water is subsequently filtered in a filterpress (3) of 60 l volume and 1.6 m^2 area and a clear waste-water is obtained. Also the filterpress is cleaned in a daily routine.
The clear waste-water flows then in a buffertank (4). This tank is used to store freshly filtered waste-water to enable continuous operation of the aerobic fermentor during the daily periods of cleaning and loading of the feed tank (2) and filterpress (3).
The minimum weight of waste-water in buffertank (4) is 60 kg and the maximum is 300 kg. The temperature is 15-17°C. During the experiments this tank was never cleaned and there could be observed some microbial activity which led to a slightly turbid waste-water feed into the fermentor (5).
The fermentor is equipped with a double eight bladed turbine stirrer of 28 cm diameter. The temperature and mixed liquor weight can be controlled. The mixed liquor weight is controlled by an automatic bottomvalve and the feed of the clear waste-water to the fermentor is controlled with a magnetic flow meter. From the fermentor (5) the mixed liquor flows into a degasification tank (6) of 105 ltr volume. The degasified mixed liquor is then sent to the solids separator (7). This flow of the mixed liquor is automatically controlled with a magnetic flow meter.
The solids separator (7) is a Westfalia SKOG 205 nozzle centrifuge with 1.5 kW motor, equipped with two nozzles of 0.5 mm diameter and run at 7600 rpm. To control the separation of the solids, thickened sludge can be returned to the centrifuge.
The thickened sludge from the centrifuge flows in a small sludge buffertank (8) from where it can be returned to the fermentor (5) or centrifuge (7). The liquid hold-up in tank (8) was varied during the experiments.
Clear water from the centrifuge is pumped into the sewer (9) with a prescribed flow rate.
The surplus clear liquid from the centrifuge, which was variable in flow, was returned to tank (6). The centrifuge was cleaned regularly (about once in two days).

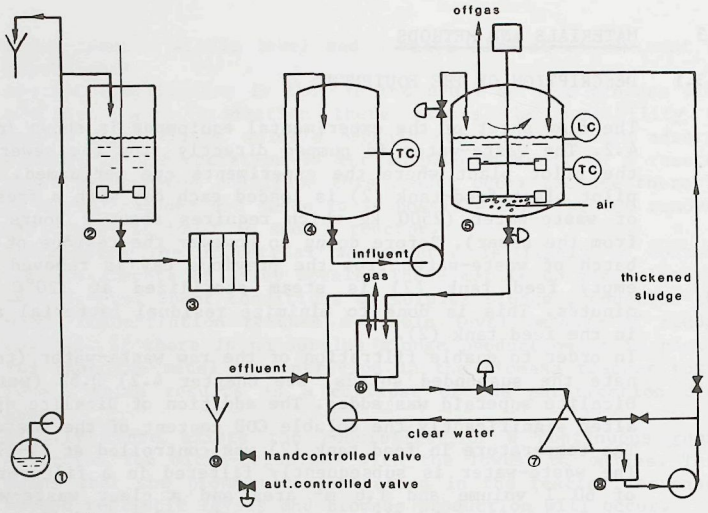


Figure 4.2
Flow sheet of the experimental equipment for no-surplus sludge production during aerobic waste-water treatment.

4.3.2. MEASUREMENTS AND ANALYSES

The influent waste-water flow into the aeration tank and the clear effluent flow from the centrifuge were sampled semi-continuously. A peristaltic pump is operated once each 15 minutes and pumps 80 ml waste-water sample in a 10 ltr container inside a refrigerator. From this container a well mixed sample is prepared each day. These 24 hr-averaged samples are analysed each day for suspended solids and COD (after centrifugation).
The aeration vessel is sampled once a day to obtain the suspended solids concentration in the reactor.
The off-gas from the aeration tank is continuously analysed for O_2 and CO_2 using an Oxygor O_2 analyser and an Unor CO_2 analyser from Maihak.
Sometimes the 24 hr averaged centrifuged liquid samples were analysed for Kjeldahl-N, BOD, NH_3 and Total Organic Carbon (TOC), and the grabsample from the aeration tank was used to measure the Sludge Volume Index (SVI-value) of the sludge.
Furthermore the different flow rates of liquid and air, the reactor temperature and pH, and the operation of the centrifuge were checked each 8 hr. The above mentioned analyses were performed as described in Chapter 3.3.2.b.
The TOC was determined using a TOCsin analyser from Phase Separations (England). The NH_3 was analysed using an NH_3-electrode from E.I.L. .

4.3.3. EXPERIMENTAL CONDITIONS

As already stated in Chapter 4.2 the maintenance condition can in principle be reached by simply returning all the biomass to the aeration vessel. The purpose of this experiment was therefore to start the aerobic fermentation with total biomass

recycle and to observe the building up and eventual leveling off of the suspended solids concentration in the aeration tank.
The following operational conditions were maintained during the experiment which lasted 1969 hrs.

- **fermentor**

The waste-water flow to the fermentor (5) was maintained at 30 l/hr. The liquid volume in the fermentor was 300 ltr, which leads to a hydraulic residence time of 10 hrs. The temperature in the fermentor was 40°C, the airflow was 2.52 Nm^3/hr from 0-962 hrs and 3.78 Nm^3/hr from 962-1969 hrs, the backpressure was maintained at 0.3 ato, and the stirrer speed was maintained at 300 rpm. The power input of the stirrer is 4600 W/m^3.
The gas-superficial velocity at the above mentioned airflow rates and airpressures is 0.19 cm/s from 0-962 hrs and 0.28 cm/s from 962-1969 hrs.
Under these conditions the oxygentransfer parameter $k_L A$ can be estimated as 120 hr^{-1} (Ref. 4.8), which gives rise to an estimated maximum oxygen transfer capacity of 30 mmol/kg hr in the fermentor. There was no pH control in the fermentor.
To control foaming the nonconsumable antifoam pluronic L81 was used.

- **solids separator**

The control of the solids separator was performed in two different ways.
For the period 0-1102 hrs the following mode of operation was maintained.
The mixed liquor flow from tank (6) to the centrifuge was 95 l/hr. The clear water surplus from the centrifuge to tank (6) averaged about 10 l/hr, and the clear waterflow into the sewer (9) is 30 l/hr.
The flow of thickened sludge to the fermentor is 55 l/hr. The liquid hold-up in the sludge buffertank (8) was 0 ltr. The degree of solids separation was controlled through the return flow to the centrifuge of thickened sludge. This mode of operation led frequently to insufficient solids separation due to clogging of the hand controlled valve for the return of thickened sludge to the centrifuge. Therefore for the period of 1102-1969 hrs the simplified following mode of operation was maintained.
There was no return of thickened sludge to the centrifuge. The feed flow rate from tank (6) to the centrifuge was chosen at such a rate that a clear water flow of 30 l/hr from the centrifuge was obtained. This was achieved at a flowrate of 140 l/hr. The return of thickened sludge from the centrifuge to the fermentor was 110 l/hr, and the liquid hold-up in the sludge buffertank (8) was 10 ltr. This last mode of operation of the sludge separator was much easier and led to less solids loss.

4.4. RESULTS

4.4.1. RELATION BETWEEN COD, BOD AND TOC CONVERSION

In Figure 4.3A and 4.3B the relations between measured elimination of COD, BOD and TOC are shown.

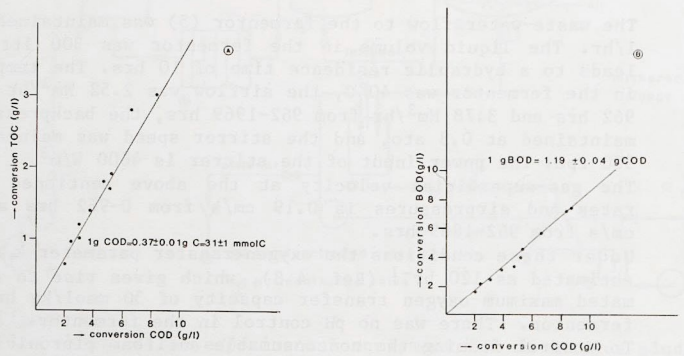


Figure 4.3
Relation between the conversion of
(A) TOC and COD, (B) BOD and COD

It follows then that the next relations hold for the present experiments

1 g BOD = 1.19 ± 0.04 g COD
1 g COD = 0.37 ± 0.01 g TOC = 31 ± 1 mmol C

These relations compare well with the values found in the previous Chapter 3.
(see Chapter 3.4.2.b and Chapter 3.4.1.b).

4.4.2. STABILITY OF THE REACTOR UNDER MAINTENANCE CONDITION

The prime purpose of this experiment was to observe wether stable process operation with regard to biomass concentration is possible. In Figure 4.4A the measured biomass concentration in the fermentor is shown. In this same figure the expected biomass accummulation (calculated from COD conversion and a biomass yield of 0.3 g/g COD) in case of a normal biomass yield is shown.
It is obvious that the measured biomass accummulation is far less and that the biomass content of the reactor does not increase steadily but shows a tendency to level off towards a steady state value as expected in Chapter 4.2. The solids content of the influent and effluent waste-water are shown in Figure 4.4B and 4.4C respectively. It is evident that in both flows the solids content is very low and nearly equal, which points already to the absence of surplus sludge production.This aspect will be discussed more extensively in section 4.4.4.

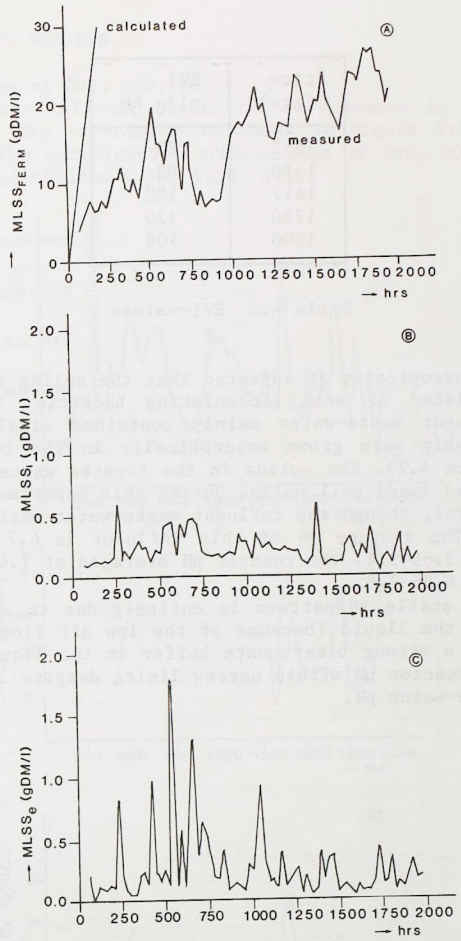


Figure 4.4
Results on reactor stability
(A) solids concentration in the reactor
(B) solids concentration in the influent waste-water
(C) solids concentration in the effluent waste-water

It is also evident from Figure 4.4C that the improved operation of the centrifugal solids separator after 1102 hrs results in less solids loss in the effluent.
The sludge settling property is not extremely good, and must be regarded as moderate as shown by the measured SVI value of about 100 ml/gr DM (Table 4.2).

time (hrs)	SVI ml/g DM
1552	80
1590	85
1617	100
1750	120
1800	108

Table 4.2 SVI-values

Microscopically it appeared that the solids from the bioreactor consisted of well flocculating bacteria. The solids in the influent waste-water mainly contained single bacteria (which probably were grown anaerobically in the buffertank (4), see Figure 4.2). The solids in the treated waste-water mainly contained lysed cell walls. During this experiment there was no pH control, though the influent waste-water varied considerably in pH. The average pH of this influent is 6.7 ± 0.83 with peaks from 5.65-9.2. The reactor pH averaged at 7.4 ± 0.24 with peaks from 6.85-7.8.

This stable pH-pattern is entirely due to a low CO_2-stripping from the liquid (because of the low air flow rate). This gives then a strong bicarbonate buffer in the liquid which maintains the reactor pH within narrow limits despite large variations in waste-water pH.

4.4.3. PURIFICATION RESULTS

4.4.3.a **Purification of COD, BOD, TOC**

The substrate influent concentration measured as COD, BOD and TOC during the experiment is shown in Figure 4.5A, 4.5B and 4.5C, and the purification efficiencies of COD, BOD en TOC are shown in the Figures 4.5D, 4.5E, 4.5F.

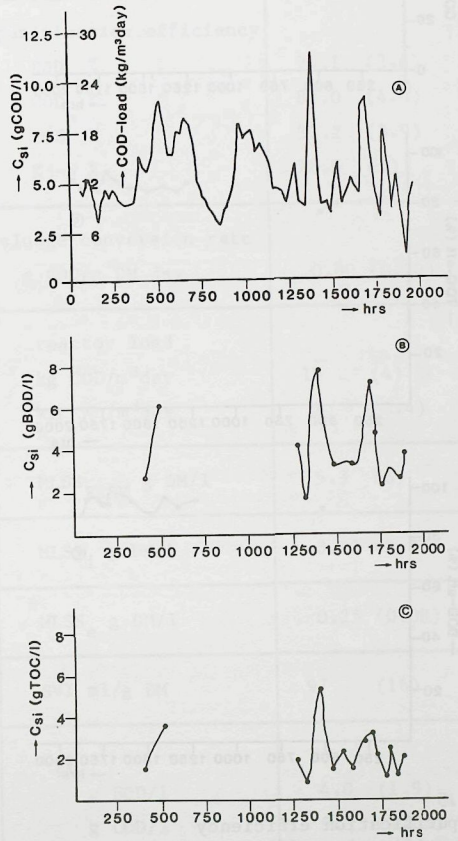


Figure 4.5
(A) Influent waste-water COD concentration and reactor COD load
(B) Influent waste-water BOD concentration
(C) Influent waste-water TOC concentration

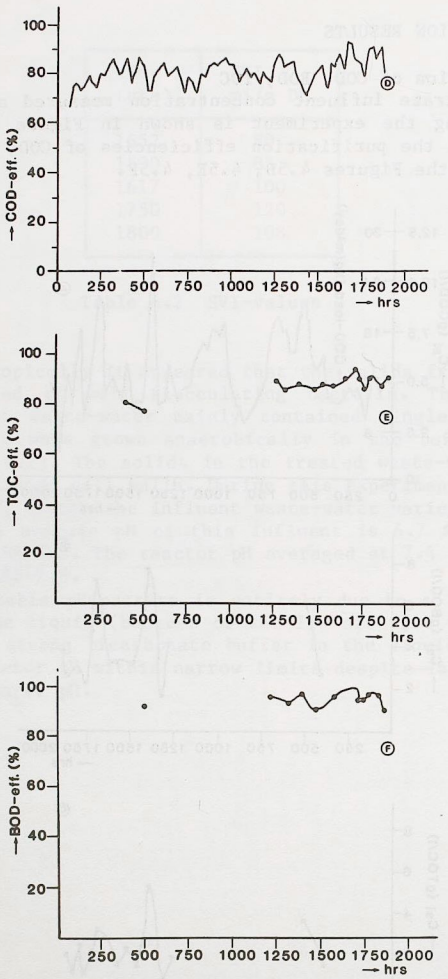


Figure 4.5
(D) COD purification efficiency
(E) BOD purification efficiency
(F) TOC purification efficiency

It is evident that there is a large variation in substrate concentration, but that nevertheless the purification efficiency remains high. The average results are summarized in Table 4.3. It can be seen that BOD and COD removal efficiencies are comparable and equal to the process with surplus sludge production (compare Table 3.5).

hrs	0-1969
Temperature °C	40
purification efficiency	
BOD %	95.1 (2.6)
COD %	82.0 (4.0)
TOC %	87.2 (3.9)
Kj-N %	78.0 (5)
sludge conversion rate g COD/g DM day	0.80 (0.20)
reactor load	
kg COD/m^3day	13 (4)
kg N /m^3day	1 (0.4)
$MLSS_{FERM}$ g DM/1	15.3 (6)
$MLSS_i$ g DM/1	0.22 (0.07)
$MLSS_e$ g DM/1	0.25 (0.08)
SVI ml/g DM	97 (16)
C_{si}	
g BOD/1	4.0 (1.9)
g COD/1	5.3 (1.83)
g TOC/1	2.23 (1.16)
g Kj-N/1	0.40 (0.15)
τ hrs	10

( )= standard deviation

Table 4.3
Average results of the aerobic purification without surplus sludge production

4.4.3.b Oxygen uptake and CO_2 production

In Figure 4.6 the oxygen uptake and carbon dioxyde production during the experiment is shown.

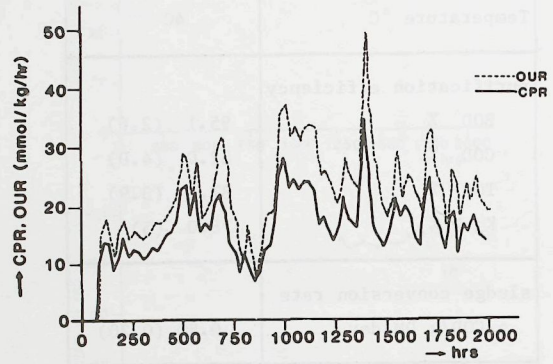


Figure 4.6
Oxygen uptake rate and Carbon dioxyde production rate during purification process

It can be seen that the oxygen consumption is always higher than the carbondioxyde production. This is probably caused by nitrification reactions, which will be discussed in the following section. It is also evident that there is a strong correlation between O_2 uptake/CO_2 production and substrate concentration in the waste-water (Compare Figure 4.6 and Figure 4.5A).
The relations between COD removal and O_2 consumption or CO_2 production are shown in Figure 4.7A and 4.7B.
The following relations do result

1 g COD = 36±4 mmol CO_2

1 g COD = 51±5 mmol O_2 = 1.63±0.16 g O_2

The last relation can be compared with the relation for the system with surplus sludge production (Chapter 3.4.2.b) which states that 1 g COD = 0.68 g O_2.
It can be concluded therefore that treatment of the GB-waste-water in a reactor under maintenance condition requires more than twice the amount of oxygen than treatment in a reactor with surplus sludge production.
Clearly this is due to the fact that now all of the COD is oxydized and not partially assimilated and furthermore extra oxygen is used for nitrification as will be discussed in the next section.

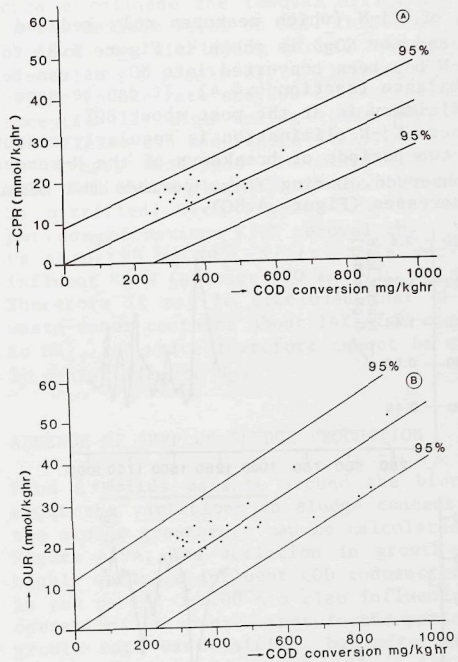


Figure 4.7
(A) relation between COD conversion and CO_2-production
(B) relation between COD conversion and O_2-consumption

4.4.3.c Elimination of Kjeldahl-N

The elimination of Kj-N (which measures only reduced nitrogeneous compounds and not NO_3^-) is shown in Figure 4.8A to 4.8C. Probably the Kj-N has been converted into NO_3 as can be deduced from an oxygen balance (section 4.4.4). It can be seen that the purification efficiency is at the most about 80%.
In the period where Kj-N elimination is regularly measured (1300-2000 hrs) two periods of breakdown of the N-removal efficiency can be observed. During these periods the measured NH_3 concentration increases (Figure 4.8C).

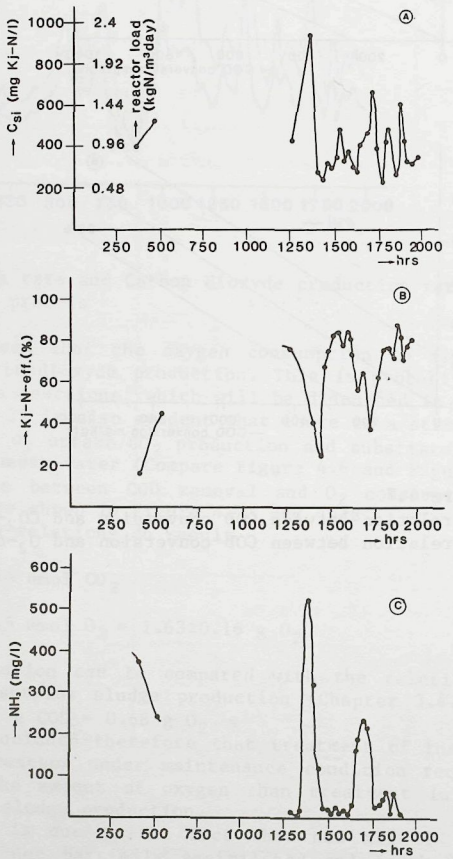


Figure 4.8
Purification results for Kjeldahl-N
(A) Kjeldahl-N concentration in the influent waste-water and reactor N-load
(B) Kjeldahl-N purification efficiency
(C) Ammonia concentration in the purified waste-water

The reason for this collapse is not known exactly, but after such a collapse the removal efficiency increases very rapidly to the maximum level of 80%. This does not point to a toxification of the nitrifying flora. Most probably the decreased N-removal is a result of O_2-limitation, (Ref. 4.5), because the oxygen uptake rate and COD concentration in the waste-water are exceptionally high during these periods (compare Figure 4.8B with Figure 4.6 and Figure 4.5A).
As already mentioned the maximum Kj-N removal efficiency is 80%. This means that 20% of the Kj-N in the waste-water cannot be nitrified. From Figure 4.8C it can be seen that during periods of maximum Kj-N removal the residual NH_4^+-concentration is about 25 mg NH_4^+ -N/ltr. This amounts to about 6% of the influent Kj-N (average 400 mg N/l, see Table 4.3).
Therefore it may be concluded that the Kjeldahl-N in the GB-waste-water contains about 14% of N-compounds which do not lead to NH_4^+, and which therefore cannot be nitrified and subsequently denitrified to N_2.

4.4.4. ABSENCE OF SURPLUS SLUDGE PRODUCTION

From a solids balance around the bioreactor, taking into account the variations in sludge concentration in the fermentor, the sludge growthrate can be calculated. The result is shown in Figure 4.9A. The variation in growth rate is a result of the highly variable influent COD concentration (Figure 4.5A) and is in the period 0-1100 hrs also influenced by improper centrifuge operation. It appears that in the period 0-1100 hrs the average growth rate was positive, but after improved operation of the centrifuge (see Chapter 4.3.3) at 1102 hrs the growth rate is zero, which implies no surplus sludge production.
The absence of surplus sludge production can also be illustrated from a carbon balance. In Chapter 4.4.3.b. it was shown that in the conversion of 1 g COD, 36±4 mmol of CO_2 is produced during this experiment. In Chapter 4.4.1 it was shown that the conversion of 1 gr COD is equal to the elimination of 31±1 mmol C from the waste-water.
Hence it is obvious that, within the limits of accuracy, the C-elimination from the waste-water is virtually equal to measured CO_2 production, and thus there cannot have been any significant surplus biomass production.
A third way to show the absence of sludge production is to consider the COD balance. In theory one expects that in the absence of sludge production and nitrification the removal of 1 g COD leads to an oxygen consumption of 1 gr O_2 (see also eq. (3.51)). In Chapter 4.4.3.b it was shown that the conversion of 1 g COD required 1.63±0.16 g O_2. According to Table 4.3 the average COD elimination is 0.82*5.3 = 4.35 g COD/ltr and the average N elimination is 0.78*0.4 = 0.31 g N/ltr.
The nitrification proces requires 4.57 g O_2/g N and therefore 0.31*4.57 = 1.42 g O_2 per liter waste-water is used for nitrification. Per gram of eliminated COD this is equal to 1.42/4.35 = 0.33 g O_2.
This leads then to the conclusion that for the oxidation of 1 g waste-water COD (1.63 ± 0.16) - 0.33 = 1.30 ± 0.16 g O_2 is consumed.

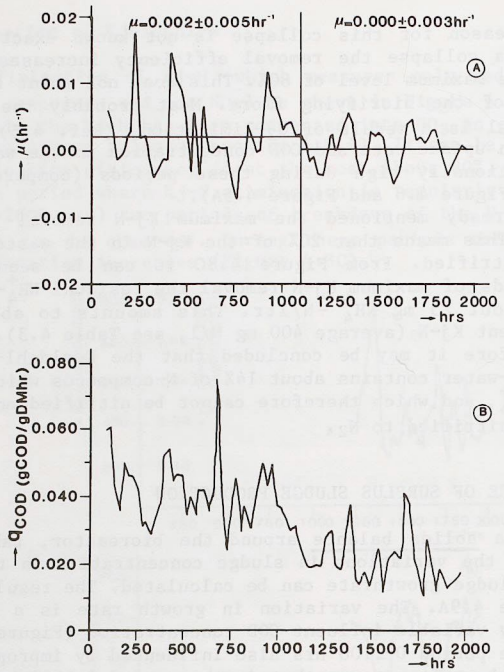


Figure 4.9
Sludge parameters during purification
(A) growthrate
(B) specific COD conversion rate of the sludge

Thus compared to the expected theoretical ratio of 1.0 , this means that within the limits of accuracy no waste-water COD has been used for surplus sludge production.
It should also be noticed that both CO_2 and O_2 flows due to COD conversion are higher than the stoichiometric values. The most likely explanation is that there were small systematic errors in airflow or waste-water flow measurements.

4.4.5. SPECIFIC SUBSTRATE CONVERSION RATE DURING THE EXPERIMENT

The conversion rate q_{COD} during the course of the experiment is shown in Figure 4.9B. It appears that after 1100 hrs the q_{COD} value is lower than before 1100 hrs.
Most probably this is caused by the higher growth rate (Figure 4.9A) in the period of 0-1100 hrs (due to operational difficulties with the centrifuge). On visual inspection of Figure 4.9A and 4.9B there appears to be no correlation between q_{COD} and μ. But application of linear regression reveals the statistical significant relation shown in Figure 4.10, which results in eq.(4.2)

$$q_{COD} = (0.5 \pm 0.15)\ \mu + (0.029 \pm 0.003) \qquad \text{eq.(4.2)}$$

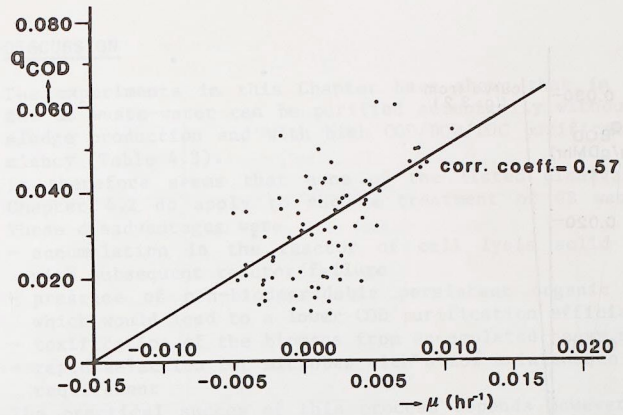


Figure 4.10
Relation between q_{COD} and μ

From this correlation the yield and maintenance parameters can be calculated as

$m_{COD} = 0.029 \pm 0.003$

$Y_{COD} = 0.5 \pm 0.15$

The calculated maintenance coefficient is in line with the maintenance values which are found for aerobic micro-organisms (Table 4.1) at 40°C. Also the biomass yield value does compare well with the values found in the previous Chapter 3.
Therefore from the presented data in Figure 4.9B one cannot conclude that there is a selection for micro-organisms with a very low maintenance requirement.

The relation between q_{BOD} and the concentration BOD is shown in Figure 4.11. Despite a considerable scatter of the data there is an increase in effluent BOD concentration when q_{BOD} increases. The curve in Figure 4.11 represents the relation between q_{BOD} and C_{BOD} found for the system with surplus biomass production (Chapter 3, Figure 3.21). It is remarkable that despite the low growth rate of the mixed population the substrate affinity of this population has decreased.
Mostly the opposite is found (Ref. 4.9). A possible explanation is that in a reactor under maintenance conditions lysis products are present in the liquid and that the microbes do have a much lower affinity for these compounds.

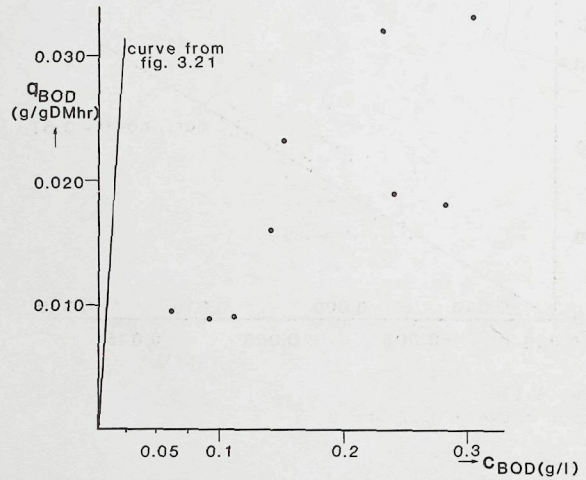


Figure 4.11
Relation between q_{BOD} and C_{BOD}

4.5 DISCUSSION

The experiments in this Chapter have shown that in principle the GB waste-water can be purified aerobically without surplus sludge production and with high COD/BOD/TOC purification efficiency (Table 4.3).
It therefore seems that none of the listed disadvantages in Chapter 4.2 do apply to such a treatment of GB waste-water. These disadvantages were

- accumulation in the reactor of cell lysis solid products, with subsequent reactor failure
- presence of non-biodegradable persistent organic compounds which would lead to a lower COD purification efficiency
- toxification of the biomass from accumulated heavy metals
- rapid selection for microbes with a low maintenance requirement

The practical succes of this process depends however upon two critical requirements.
The first requirement is that the suspended solids which are present in the untreated waste-water should not accumulate in the reactor. This means then generally a settling tank for the separation of primary sludge.
The second requirement is a solids separation technique which enables a total return of the biomass at a biomass level of 15-25 g DM/ltr in the bioreactor (see Figure 4.4A).
During the present experiments a centrifuge has been used for solids separation. However a centrifuge is not suitable for a full scale operation, due to the excessive energy requirements and capital costs. The centrifuge used in the present study has a 1.5 kW motor and obtained a solids removal efficiency of 98% (average feed solids concentration is 15.3 g DM/l and average clear water solids concentration is 0.25 g DM/l, see Table 4.3) at a flowrate of 30 l/hr. This implies a power consumption of 50 kWh/m^3 of waste-water. According to Ref. 4.6 large scale separation of sludge by a nozzle centrifuge requires about 10 kWh/m^3, where a solids removal efficiency of 80% was obtained. Considering the large difference in solids removal efficiency, there is a reasonable agreement in power consumption. Using these data, a centrifugal power requirement for full scale treatment of the GB waste-water (420 m^3/hr) can be estimated between 4200 and 21000 kW. Such an energy requirement leads to a centrifugal energy cost of Df 14.40 - Df 70.- per PE, which should be compared to the cost of Df 30/PE in 1981 for discharge of the untreated waste-water. A second method is to use a settling tank for the separation and return of the biomass.
The relation between the necessary reactor volume and settler area can then be calculated from eq.(3.37), using the following parameters for a reactor under maintenance condition (Table 4.3):
C_{si} = 5.3 gCOD/l, C_s = (1-0.82)*5.3 = 0.95 g COD/l, μ = 0 hr^{-1}, m_s = 0.029 g COD/g DM hr, Φ_i= 420 m^3/hr, SVI = 97 ml/g DM.

The result is listed in Table 4.4.

settler area A, m^2	liquid residence time τ, hrs	reactor volume V m^3
500	28.4	11930
1000	21.1	8862
2000	15.5	6510
4000	11.2	4704
16000	6.2	2604

Table 4.4
Relation between reactor volume and settler area for the aerobic purification process without surplus sludge production.

It can be seen that in order to realize the maintenance condition of the process very large settler area's and reactor volumes are required. However as already stated in Chapter 1 there is only very limited space available at the Gist-Brocades production location in Delft. Therefore a settling tank for biomass separation is unfavorable.
A third possibility to achieve the necessary retention of biomass in the reactor is to use techniques where the biomass is attached to carriers. In the past several different reactor systems have been developed where the attachment properties of micro-organisms are exploited.
The oldest reactor configurations are the trickle filter (Ref. 4.10), using a stationary carrier, and the rotating disc contactor (Ref. 4.11), using a rotating carrier.
Both reactor types do have a relatively low specific carrier-surface (m^2 of surface per m^3 of reactor volume) of 100-200 m^2/m^3. Because of this low surface area, the substrate flux is high and the biolayers do become very thick (1 - 5 mm). At such a thickness diffusion-limitation is very serious and the active biomass is only a small fraction of the total biomass (Ref. 4.11).
From the above arguments one may conclude that the specific carrier surface should be much higher to achieve thinner biolayers which are not diffusion limited.
A simple way to achieve high specific carrier surface areas is to maintain in suspension a sufficient amount of small diameter solid particles.
An early example of such a reactor system is the SSS-process (Ref. 4.12) where very small (diameter $\ll$ 0.2 mm) solid particles are kept in suspension in the aeration tank by the gas-flow.
These carrier particles are integrated in the flocculated sludge and the settling rate of the particle/sludge floc unit is increased compared to the flocs without particles. This then leads to higher biomass concentrations in the reactor or smaller settlers.

However this system still behaves like a flocculated sludge system and especially the waste-water sediment is still incorporated in the floc.
A more recent reactor system is the so called fluidized bed reactor (Ref. 4.7) where carrier particles are kept in suspension by liquid fluidization. The diameter of the carrier particle is chosen in such a way that a high specific surface area (leading to small diameter carriers) is combined with a high settling velocity (leading to larger diameter carriers).
The optimum carrier diameter seems to be in the range of 0.2-2 mm and carrier attached biomass concentrations of 30 - 60 g VS/l are maintained in the fluidized bed reactor at liquid superficial velocities of 10-40 m/hr (Ref. 4.7).
Such biomass concentrations would be more than sufficient to obtain the maintenance condition of the reactor at the hydraulic residence time used in the present study.
An additional important advantage of such reactors is that the superficial liquid velocities which are applied in the settler section of such reactors can be very high, due to the extreme fast settling carriers which are covered with biomass. In practice these liquid velocities are 10-40 m/hr (Ref. 4.7). This velocity is so high that suspended solids in the influent waste-water are merely washed through the reactor and cannot accumulate in the attached biolayers, which would lead to a lower activity of the organic solids (see Chapter 3 for this effect of inert sediment). This means that a primary settling tank is not necessary, although a final settling tank can be necessary for removal of this sediment.
It therefore seems that for the practical realization of aerobic purification without surplus sludge production the aerobic fluidized bed reactor is very attractive.

4.6. CONCLUSION

The aerobic treatment of the GB waste-water without surplus sludge production is in principle possible. A key problem is to use an economic method for biomass retention.
The biological fluidized bed, where biomass is attached to a heavy carrier, seems to be ideally suited to achieve the necessary biomass retention.
The next Chapter 5 will deal therefore with the development of such a reactor.

SYMBOLS

A	area settling tank	m^2
C_x	concentration biomass in aeration tank	$kgDM/m^3$
C_u	concentration biomass in recycle flow	$kgDM/m^3$
C_e	concentration biomass in the overflow of the solids separator	$kgDM/m^3$
C_s	substrate concentration in aeration tank	kg/m^3
C_{si}	substrate concentration in waste-water feed to the aeration tank	kg/m^3
C_{BOD}	BOD concentration	kg/m^3
CPR	Carbondioxyde production rate	mol/m^3hr
Kj-N	Kjeldahl nitrogen concentration	kg/m^3
m_s, m_{COD}	substrate maintenance coefficient	kgCOD/kgDMhr
$MLSS_{FERM}$	total suspended solids concentration in the reactor	$kgDM/m^3$
$MLSS_i$	total suspended solids in the waste-water feed to the aeration tank	$kgDM/m^3$
$MLSS_e$	total suspended solids in the overflow waste-water from the solids separator	$kgDM/m^3$
OUR	Oxygen uptake rate	mol/m^3hr
q_{COD}	specific COD conversion rate	kg/kgDMhr
q_{BOD}	specific BOD conversion rate	kg/kgDMhr
SVI	sludge volume index	ml/gDM
T	temperature of aeration tank	^0C
V	volume aeration tank	m^3
Y_{COD}	yield of biomass on COD	kgDM/kgCOD
Φ_i	waste-water feed flow rate	m^3/hr
Φ_r	waste-water recycle flow rate	m^3/hr
Φ_w	waste sludge flow rate	m^3/hr
μ	growth rate of the biomass	hr^{-1}
τ	hydraulic residence time in aeration tank	hr

REFERENCES

4.1 S.J. Pirt,
Proc.Roy.Soc.London B, 163, (1965), 224

4.2 J.J. Heijnen, J.A. Roels,
Biotechnol. and Bioeng., 23, (1981), 739

4.3 A.F. Gaudy, M. Ramanathan, P.Y. Young and T.V. DeGeare,
J.W.P.C.F., 42, (1970), 165

4.4 A.F. Gaudy, P.Y. Young, A.W. Obayashy,
J.W.P.C.F., 43, (1971), 40

4.5 G.Knowles, A.L. Downing, M.J. Barret,
J. Gen. Microbiol., 38, (1965), 263

4.6 M.R. Stumpf, W.H. Harper,
27th Ind. Waste Conf., (1972), 904

4.7 P.F. Cooper, D.H.V. Wheeldon,
Ann. Conf. Instn. Water Poll. Control,
Torquay, september 13, (1979)

4.8 K. van 't Riet,
Ind. Eng. Chem. Process. Des. Dev., 18, (1979), 357

4.9 J.G. Kuenen,
H_2O, 15, (1982), 562

4.10 F.B. de Walle, E.S.K. Chain,
Biotechnol. and Bioeng, 18, (1976), 1275

4.11 L.T. Mulcahy, W.K. Shieh, E.J. Lamotta,
Progr. Wat. Technol., 12, (1980), 143

4.12 K. Rietema, S.P.P. Ottengraf, H.P.E. v.d. Venne,
Biotechnol. and Bioeng., 13, (1971), 911

5 DEVELOPMENT OF A BIOLOGICAL AEROBIC FLUIDIZED BED REACTOR

5.1 INTRODUCTION

In Chapter 4 it was concluded that the condition of no-surplus biomass production in an aerobic waste-water treatment process can be reached provided that there is a sufficient biomass retention. An economic way to achieve such biomass retention might be its attachment to carriers in a so-called biological fluidized bed. Such a reactor has the additional advantage that the suspended solids in the waste-water do not accumulate in the reactor but are washed through. The research for the development of such an aerobic fluidized bed reactor is divided into two parts. The first part will focus on the conditions under which the biomass will attach to carriers and the second part will exploit these results and deal with the development of a reactor where these biomass covered carriers are used for waste-water purification.

5.2 CONDITIONS FOR THE ATTACHMENT OF AEROBIC BIOMASS TO CARRIERS UNDER TURBULENT CONDITIONS

5.2.1 LITERATURE SURVEY ABOUT THE ATTACHMENT OF MICRO-ORGANISMS TO SURFACES

The attachment of micro-organisms to surfaces is not well understood.
According to Ref. 5.1 several forces can be responsible for microbial attachment. These forces can be divided into weak and strong forces. Attachment through weak forces occurs with electro-static interactions, Van der Waals forces and hydrophobic interactions.
Attachment through strong forces is the result of polymer bridging between micro-organisms and the carrier surface.
Because the attached biomass is intended to be used in an aerated buble column it is required that the biomass should stay attached under turbulent conditions. This means that the strong forces of polymer bridging are necessary for sufficient attachment under these turbulent conditions.
The nature of the attachment forces listed above makes it clear that the attachment strength will be dependent upon the type of microbe and of surface type. Apart from the above mentioned approach, based on force analysis, (Ref. 5.1) it is also usefull to survey existing situations and reactors where attached micro-organisms do occur or are exploited.
Attached microbes do occur in a wide spectrum of situations ranging from tissue cell cultures (Ref. 5.2), ship walls (Ref. 5.3), to trickle filters (Ref. 5.4). Furthermore it has been shown that sludge flocs can attach to suspended solid particles which lead then to an enhanced settling rate of the sludge on solids in the settling tank (SSS process, Ref. 5.5). Also biological fluidized beds have been developed for denitrification and C-oxydation (Ref. 5.6 to 5.9) by the Ecolotrol Company and Dorr-Oliver. In these processes however a high turbulence, due to gasformation or air-sparging leads to destruction of the biolayers around

the particles. In the aerobic C-oxydation process this phenomenon is even exploited to scoure off surplus biomass to prevent clogging. Contrary to these findings however there is another group of researchers at the Oak Ridge National Laboratory (Ref. 5.10 to 5.13) who have developed aerobic biological fluidized beds using air sparging. From this limited survey we may conclude that attachment of micro-organisms to carrier surfaces is possible under specific conditions and that the available evidence seems conflicting.
Nevertheless it seems that the following parameters are of importance
- type of microbe
- type of carrier surface
- amount of turbulence in the reactor

5.2.2 MATERIALS AND METHODS

Given the available evidence listed in Chapter 5.2.1 as experimental set-up was chosen the variation of the type of carrier and the degree of turbulence in the reactor and to study the resulting biolayer development in the aerobic fluidized bed reactor

5.2.2.a **Description of the equipment**

The flow sheet of the experimental equipment is shown in Figure 5.1.
Filtered waste-water is pumped from a 2.5m^3 storage vessel (1) into a bubble column (3). The storage vessel is loaded every day with a fresh batch of waste-water according to the procedure described in Chapter 4.3.1.
The bubble column contains a lower stainless steel section (440cm high, 25cm diameter), a middle section of quickfit glass (150cm high and 22.5cm diameter) and a top section for liquid overflow and gasdisengagement. The total unaerated liquid volume is then 215 + 60 = 275 ltr.
The air is introduced at the bottom of the column through a multiple orifice sparger (224 holes of 1mm diameter) and is controlled with a rotameter. The temperature in the reactor is controlled by a flow of cold or warm water which flows over the outside lower stainless steel part of the reactor. The pH is controlled with H_3PO_4 addition in the column. The pH did not vary too much and it was sufficient to check the pH manually every 8 hours.
The waste-water is introduced at the bottom of the column through one simple pipe (3mm diameter) at 2cm above the column bottom and is controlled with a rotameter which is cleaned daily. Tap water can be introduced, if necessary, through the bottomvalve of the column. To maintain the column temperature, when cold tap water was used, it appeared to be necessary to inject steam in the bottomvalve. The amount of tap water was controlled with a rotameter.
The water leaving the bubble column flows through a sandtrap (4) of 0.2m^2 into the sewer. The sandtrap was necessary to detect any losses of carrier material or biomass covered carrier particles.

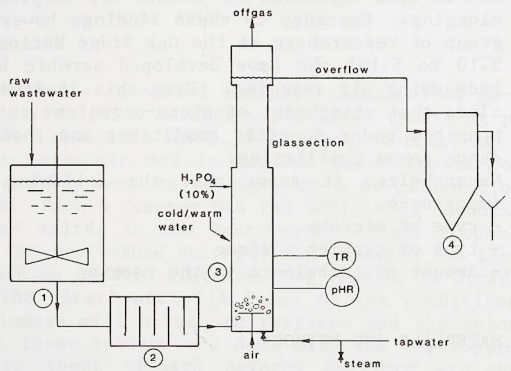


Figure 5.1
Flow sheet of experimental equipment for cultivation of sand attached biomass

5.2.2.b Measurements and analyses

Each 8 hrs reactor temperature, pH and the water flows were measured manually.
Each day the carriers in the lower part of the bubble column were examined microscopically, after thoroughly washing with tap-water, for microbial attachment. The biomass covered sand was analysed after washing with tap water for density (by weighing a specified volume), for N-content, COD-content, P-content and dry matter content using the analyses described in Chapter 3.3.2.b and for dry matter ash content (by heating at 600°C until constant weight). The influent and effluent waste-water was analysed for COD in the daily averaged sample according to the procedures outlined in Chapter 4.3.2. Each day a grabsample from the bubble column (taken at 440cm above the bottom of the column) was analysed for non-attached suspended solids concentration according to the procedures outlined in Chapter 4.3.2.

5.2.2.c Experimental conditions

During the whole experimental period (from April 5,1979 to August 6, 1979) the reactor temperature was maintained at 40°C and the pH was controlled at 6.5 - 8.0. The air-flow rate was varied and is listed in Table 5.1. Because it was reasonable to assume that the waste-water contained a sufficient wide variety of micro-organisms (including micro-organisms which can attach to surfaces) no inoculation was used. The experiment was started with a waste-water flow of 25 1/hr, which gives a liquid residence time of 11 hours. This is close to the residence time of 10 hours which was used in Chapter 4 to reach maintenance conditions and no surplus biomass production.

5.2.3 RESULTS

5.2.3.a Conditions which lead to microbe attachment

The variation in carrier type and in fluidization and the resulting attachment is listed in Table 5.1. It can be seen that a large variety of carrier materials and a large variation in fluidization and superficial gasvelocity (which is proportional to turbulence) does not lead to any biolayer formation (period 1 - 7). In setting up the experiments listed in Table 5.1 in the periods 1 - 7 it was assumed that micro-organisms with attachment properties to any particular carrier surface would become automatically the dominant population in the bubble column with these carrier particles. The main reason for this assumption was that these attaching micro-organisms would in principle never leave the reactor. From Table 5.1 it is evident that biolayer formation did never occur in the periods 1-7, although there was sufficient microbial activity which could be seen as suspended flocculated biomass in the reactor and as a constant COD purification efficiency of about 70% (see Figure 5.2.B - 5.2.C).

period number	date (1979)	Carrier material	Dilution rate hr^{-1}	gas superficial velocity cm/s	fluidization	biolayer formation
1	5/4	sand, 0.1-0.3mm, 40kg	0.09	2.7	+	no
2	17/4	sand, 0.1-0.3mm, 40kg	0.09	5.4	++	no
3	1/5	glassballotini 0.25-0.32mm,10kg	0.09	5.4	++	no
4	9/5	glassballotini 0.25-0.32mm,10kg	0.09	10.8	+++	no
5	22/5	glassballotini 0.1-0.12mm, 10kg	0.09	2.7	++++	no
6	26/5	active Carbon 0.25-1 mm, 5kg	0.09	2.7	++++	no
7	10/7	eiffellava < 1mm, 6.3kg	0.09	2.7	++++	no
8	25/7	sand, 0.1-0.3mm, 40kg	2.4	2.7	+++	yes

Table 5.1
Effect of carrier type, amount of fluidization and dilutionrate on biolayer formation

One possible explanation might be that the waste-water itself does not contain any micro-organisms with attachment properties. This seems unlikely, regarding the abundance of attached micro-organisms in nature (Chapter 5.2.1).

Another possibility is that there is a competition between attaching and non-attaching micro-organisms in the bubble column. A most certain method to give the attaching micro-organisms the possibility to use their attachment advantage is to bring the reactor under wash-out conditions which means that

$$D \geq \mu^{MAX} \qquad \text{eq.(5.1)}$$

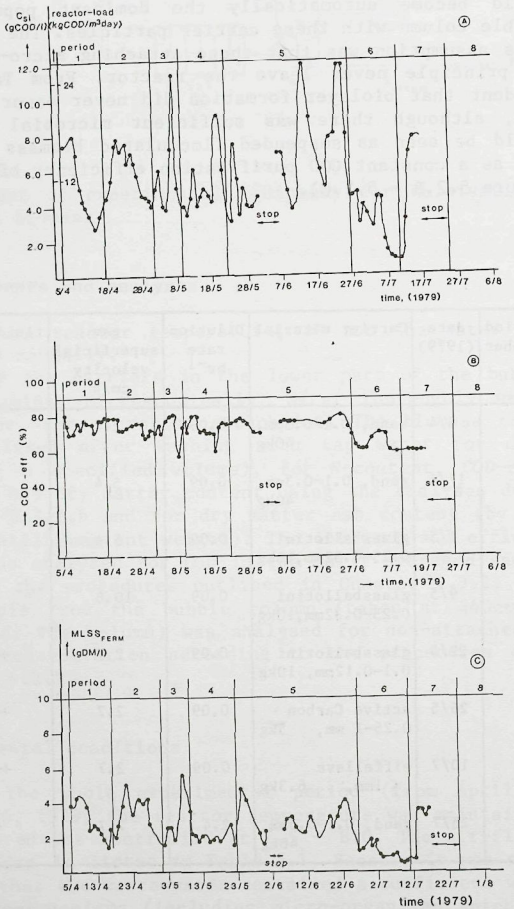
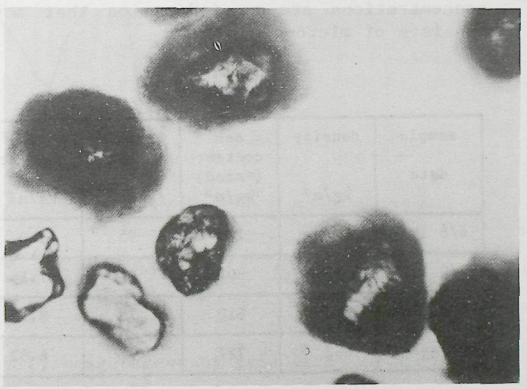
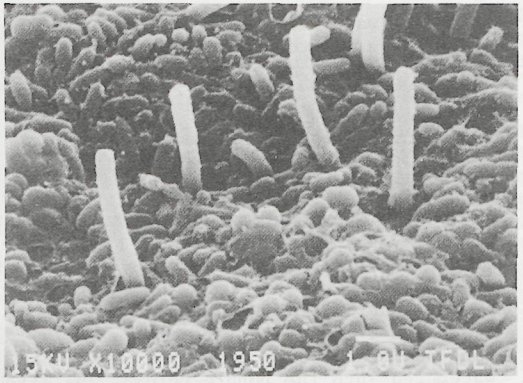


Figure 5.2
Results of waste-water purification in period 1-8 (Table 5.1)
(A) influent COD concentration and reactor COD-load
(B) COD purification efficiency
(C) concentration suspended biomass in the reactor

Under the wash-out conditions the attaching micro-organisms can stay in the reactor and do not suffer from any competition from non-attaching micro-organisms because these are washed out.
The μ^{MAX} from the suspended micro-organisms in the GB-waste-water can be estimated from Chapter 3.4.2.b at $\mu^{MAX} = 0.24 hr^{-1}$ at 40°C. Because we are dealing with mixed microbial populations, this μ^{MAX} value must be regarded only as indicative for the maximum growth rate of the culture. The $D = 0.09 hr^{-1}$ used in the periods 1 -7 clearly allows suspended growth. In order to check the hypothesis of equation (5.1) the dilution rate was increased very much to $D=2.4\ hr^{-1}$ by addition of 660 l/hr of tapwater to the waste-water flow of 25 l/hr used in period 1 to 7. The use of tapwater to increase the dilution rate leads also to a large decrease in COD concentration in the water fed into the bubble column. The effect of COD concentration on biomass attachment seems however not to be present because in the periods 1 to 7 (Table 5.1) the COD concentration in the waste-water has varied between 700 and 12000 mg/ltr (see Figure 5.2.A). The diluted COD concentration in period 8 was about 300mg/ltr.
Also the original carrier (sand 0.1-0.3mm) was used again.
It then appeared that the carriers were covered very quickly with thick biolayers within one week. An example can be seen in Picture 5.1, from which it appears that the thickness of the biolayer is about 50-100µ. Picture 5.2 shows a SEM picture of the biolayer surface and one can observe a very dense packing of microbes.



Picture 5.1
Light microscopic view of sand grains covered with aerobic biolayer, magnification 100 x



Picture 5.2
SEM picture of the surface of aerobic biolayer on sand
Bar is 1 micrometer.

5.2.3.b Characterisation of the biomass covered sand

The biomass covered sand was analysed in several ways. The results are given in Table 5.2. It should be stressed that these analyses do relate to settled biomass covered sand which was washed thoroughly with tap water.
From Table 5.2 it appears that 1 ltr of biomass covered sand contains about 77 g organic dry matter and the N, P and COD concentrations are an indication that most of this material consists of micro-organisms.

sample date	density kg/m³	ash content (=sand) kg/m³	organic matter kg/m³	N content kg/m³	P content kg/m³	COD content kg/m³
6/8 1979	-	769	95	5.0	2.0	-
15/8 1979	1590	801	61	7.16	-	73.3
16/8 1979	1660	610	75	8.30	6.1	111.9
average	1662	727	77	6.82	4	93

Table 5.2
Analysis results of biomass covered sand particles (settled volume).

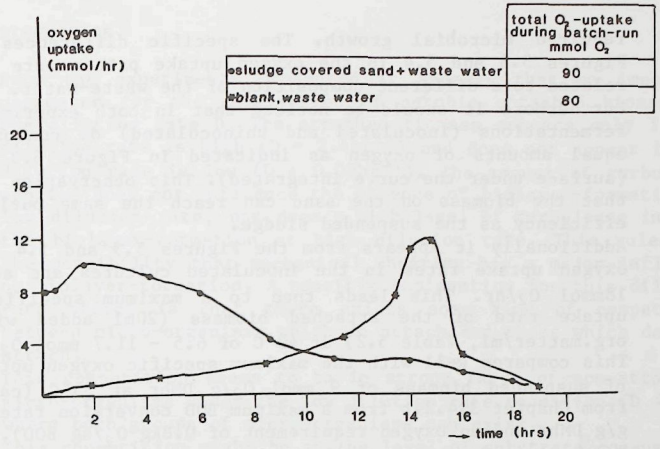


Figure 5.3
Aerobic activity measurement of sand attached biomass

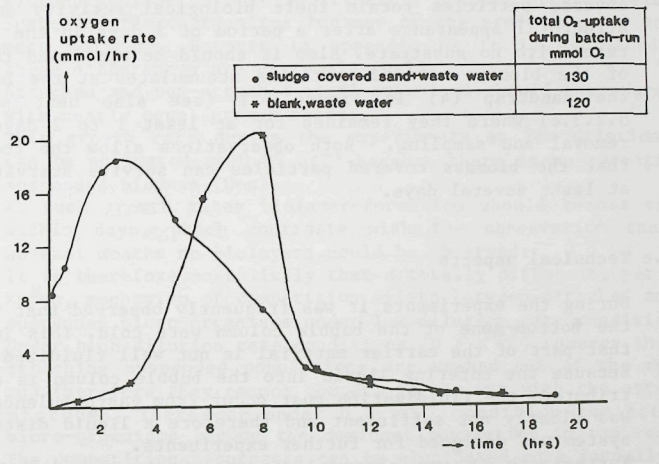


Figure 5.4
Aerobic activity measurement of sand attached biomass

The presence of micro-organisms on the sand was definitively shown by incubating 20ml of biomass covered sand in a 3 ltr stirred batch lab fermentor (40°C, pH=7.0, 200rpm, 1 VVM aeration) and measuring the oxygen uptake. This oxygen uptake was then compared with a blanc which received the same waste-water but no biomass covered sand. This blank also showed microbial activity due to micro-organisms present in the waste-water. The results of two such experiments are shown in Figure 5.3 and 5.4. It can be seen that the inoculated fermentor shows an immediate high O_2-uptake, while the uninoculated fermentor requires a certain time to reach measurable O_2-uptake due to

required microbial growth. The specific differences in the Figures 5.3 and 5.4 in the oxygen uptake pattern are probably related to a different composition of the waste-water.
Furthermore it should be noticed that in both experiments the fermentations (inoculated and uninoculated) do consume about equal amounts of oxygen as indicated in Figure 5.3 and 5.4 (surface under the curve integrated). This observation suggests that the biomass on the sand can reach the same purification efficiency as the suspended sludge.
Additionally it appears from the Figures 5.3 and 5.4 that the oxygen uptake rates in the inoculated cultures are about 10-18mmol O_2/hr. This leads then to a maximum specific oxygen uptake rate of the attached biomass (20ml added with 77mg org.matter/ml, Table 5.2) at 40°C of 6.5 - 11.7 mmol O_2/g DMhr. This compares well with the maximum specific oxygen uptake rate of suspended biomass of 9 mmol O_2/g DMhr at 35°C (calculated from Chapter 3.4.2.b from a maximum BOD conversion rate of 0.36 g/g DMhr and an oxygen requirement of 0.8kg O_2/kg BOD).
Furthermore it was found from simple settling experiments that the settling velocity of the biomass covered sand particles is about 50m/hr.
Finally it should be mentioned that the washed aerobic biomass covered particles retain their biological activity and their structural appearance after a period of 3 days in the refrigerator with no substrate. Also it should be mentioned that most of the biomass covered particles accumulated at the bottom of the sandtrap (4) in Figure 5.1 (see also next paragraph 5.2.3.c) where they remained for at least 1 to 2 days before removal and sampling. Both observations allow the conclusion that the biomass covered particles can survive starvation for at least several days.

5.2.3.c Technical aspects

During the experiments it was frequently observed that parts of the bottom-zone of the bubble column were cold. This indicates that part of the carrier material is not well fluidized.
Because the entering liquid into the bubble column is not distributed, the fluidization must occur from gasturbulence. This was clearly not sufficient and therefore a liquid distribution system was designed for further experiments.
Furthermore it was observed in experimental period 8 (Table 5.1) that the biomass covered sand particles did not accumulate in the bubble column, but large amounts were found in the sand trap ((4) in Figure 5.1)). This indicates that despite a settling velocity of 50 m/hr and a far lower superficial liquid velocity in the bubble column of 14.4m/hr, the biomass covered sand particles are washed out of the column. From visual observation it was concluded that the wash out is due to the turbulent liquid motions in the bubble column, which are of the order of bubble rise velocity, i.e. 25cm/s = 900m/hr.
For an efficient particle retention the reactor should therefore be equipped in further experiments with a settling zone where the air bubbles are excluded.

5.2.4 DISCUSSION

From the experiments presented it appears that an important parameter for the cultivation of aerobic attached biomass is the dilution rate D. The attached biomass appears only if the dilution rate is high ($D = 2.4hr^{-1}$) and does not appear if the dilution rate is low ($D = 0.09hr^{-1}$). The amount of turbulence applied does not influence the absence of biolayer-formation at low dilution rate, nor does a high level of turbulence inhibit the biolayer-formation at high dilution rate. This rules out the possibility that mechanical shearing has a major influence on biolayer-formation. A possible explanation for this dilution rate effect is that there exists some kind of competition between micro-organisms which do attach and those which do not attach.
This competition seems to be in strong favor of non-attaching micro-organisms under the low dilution rate condition ($D \leq \mu^{MAX}$) where both groups of micro-organisms can exist.
This competition might be at the level of substrate consumption rate or substrate utilisation rate. In principle the attached microbes on the carrier do suffer from some diffusion limitation compared to suspended micro-organisms. Also the attached micro-organisms do have in principle a lower growth yield than suspended micro-organisms because of the production of attachment polymers. However in appendix 5A it is estimated that these differences are extremely small.
Attached and non-attached micro-organisms should therefore grow with nearly equal growth rates.
This growth rate during the experiments at low dilution rate can be estimated at 0.09 hr^{-1} because there is no retention of suspended biomass ($D=\mu$).
At such growth rates biolayer-formation should become visible within days, which contrasts with the observation that for several months no biolayers could be observed.
It is therefore most likely that a totally different, yet unknown, mechanism of competition exists between attached and suspended micro-organisms under low dilution rate conditions.
Under high dilution rate conditions ($D \geq \mu^{MAX}$) however the non-attaching suspended populations are washed out of the reactor and therefore they cannot compete in any way with the attaching microbes. Therefore under $D \geq \mu^{MAX}$ condition the attached micro-organisms become the dominant population in the reactor.
The competition hypothesis can be elucidated more formally with Figure 5.5.
Set (1) in Figure 5.5 contains all micro-organisms which are present in the waste-water to be treated. Set (2), which is part of set (1), contains all micro-organisms which can grow on the compounds in the waste-water under the applied conditions of pH and temperature and O_2-concentrations.
Therefore all the micro-organisms within set 2 will accumulate in the reactor under $D \leq \mu^{MAX}$ conditions.

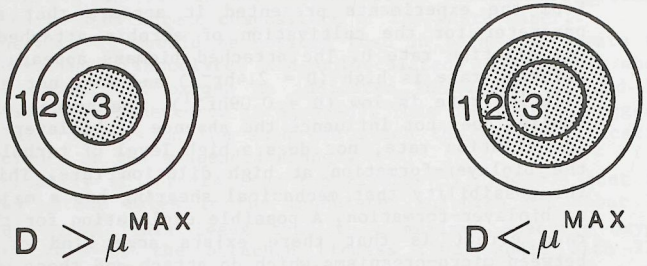


Figure 5.5
Microbial sets present in the reactor

Set (3), which is also part of set (1) and (2) contains all micro-organisms which can attach to the carrier used, under the turbulence conditions applied in the reactor. Set (3) in Figure 5.5 contains then all micro-organisms which will accumulate in the reactor if the $D \geq \mu^{MAX}$ condition is applied.
The fact that a high dilution rate is required to cultivate attached biomass, leads to very high oxygen demands in the aerobic reactor. This oxygen demand is likely to exceed the oxygen transfer capacity of the bubble column.
Based on eq.(3.57) of Chapter 3, Figure 5.6 can be calculated, which shows the maximum allowed dilution rate before underaeration occurs, for the process without surplus sludge production. A COD conversion efficiency of 82% is assumed (Table 4.2), and an oxygen requirement per kg converted COD of 1.63kg is used (Chapter 4.4.3.b). The superficial gas velocity in the bubble column is 10 cm/sec.
From this Figure 5.6 it then appears that under conditions where attachment occurs ($D = 2.4 hr^{-1}$) only a very diluted waste-water ($C_{si} \leq 0.8$ g COD/ltr) can be treated. At first sight this leads to the conclusion that GB waste-water ($C_{si} = 8$ g COD/ltr) cannot be treated.
However a possible solution might be to cultivate attached biomass with strongly diluted waste-water (as practised in period 8 of Table 5.1. At the moment that the reactor is well filled with attached biomass, the dilution water is shut off and the undiluted waste-water is fed into the reactor at a much decreased dilution rate, maintaining the same COD load to the reactor as in the period with diluted waste-water.
Based on the $D \geq \mu^{MAX}$ hypothesis it is expected that the attached biomass is outcompeted by non-attached growing micro-organisms. This change in population might however not take place in a well purifying, well mixed attached biomass reactor. The substrate concentration in such a reactor is very low (because of the efficient purification) and uniform (because of well mixed conditions). This means that the non-attaching micro-organisms cannot grow at their μ^{MAX}-value. This could lead then to wash out of the non-attaching population under $D \leq \mu^{MAX}$ condition, and the attached population would remain stable.

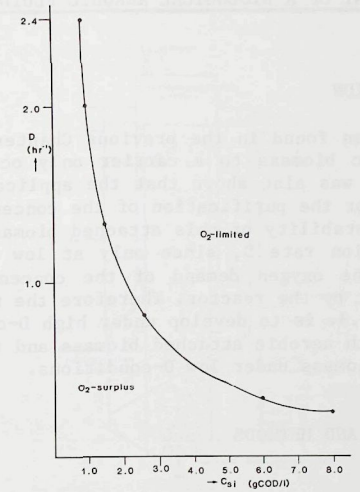


Figure 5.6
Aeration limitation in a bubble column as function of dilution rate and waste-water substrate concentration

5.2.5 CONCLUSION

It has been found that cultivation of attached aerobic biomass on a carrier depends upon the application of a high dilution rate in the reactor.
A competition hypothesis between attaching and non-attaching microbial populations has been proposed leading to the wash-out condition

$$D \geq \mu^{MAX} \qquad \text{eq.(5.1)}$$

This condition of a high dilution rate for aerobic attached biomass cultivation leads at first sight to the conclusion that the attached aerobic biomass process cannot be used for a concentrated waste-water because of aeration limitation. There exists however the possibility that attached aerobic biomass remains stable under low dilution rate conditions if a growth limiting substrate concentration is maintained. This possibility will be explored in the next Chapter 5.3.

5.3 DEVELOPMENT OF A BIOLOGICAL AEROBIC FLUIDIZED BED REACTOR

5.3.1 INTRODUCTION

It has been found in the previous Chapter 5.2. that attachment of aerobic biomass to a carrier only occurs at high dilution rates. It was also shown that the application of such attached biomass for the purification of the concentrated GB waste-water requires stability of this attached biomass under conditions of low dilution rate D, since only at low dilution rates in the reactor the oxygen demand of the concentrated GB waste-water can be met by the reactor. Therefore the main objective of this Chapter 5.3. is to develop under high D-conditions a reactor filled with aerobic attached biomass and to study the stability of this biomass under low D-conditions.

5.3.2 MATERIALS AND METHODS

5.3.2.a **Description of the equipment**

The bubble column shown in Figure 5.1. was modified in several aspects according to the comments in Chapter 5.2.3.c. Firstly a distributor for the inflowing water was designed to assure uniform fluidization. This distributor consists of a hollow ring which is located at 1.5cm from the bottom of the bubble column. This hollow ring has a diameter of 15cm and a tube diameter of 2cm. There are 8 equally spaced holes of 8mm diameter in this ring through which the water is ejected downwards to the bottom of the column (see Figure 5.7).
The water which flows into the bubble column consists of waste-water (measured with a magnetic flow meter) and tap water (which is measured with a rotameter) to create the conditions of high dilution rate (see Chapter 5.2.3.a) which leads to the formation of biomass layers on the sand. The conditions of low dilution rate to test the stability of the attached biolayers (maintaining hydraulic conditions and COD-load of the reactor) were created by shutting off the tapwater and by recirculation of an equal amount of water which leaves the reactor (forced recirculation in Figure 5.7). This forced recirculation was controlled by a variable speed pump.
The air sparger in the bubble column was the same as described in Chapter 5.2.2.a. Secondly a separator was designed which effects the separation of the gas-liquid-solid mixture in the bubble column and the transport of the settled solids into the reactor. The design is shown in Figure 5.7 .
The gas-liquid-solid mixture in the top of the bubble column ((1) in Figure 5.7) is separated in gas, which flows through the off-gas opening and a liquid-solid mixture which flows downwards through a circular annulus ((2) in Figure 5.7). In this settler a gravity separation of solids and liquid is achieved.
The liquid flows upward over a weir into a circular duct ((4) in Figure 5.7).

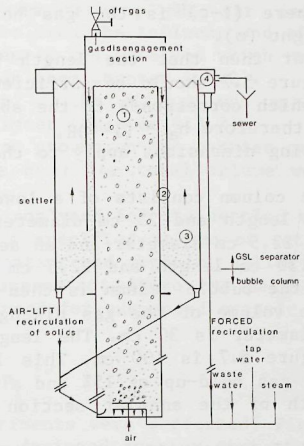


Figure 5.7
Air lift separator for the separation of gas-liquid-solids in the biological aerobic fluidized bed reactor

From this duct the liquid leaves the reactor into the sewer or part can be recirculated with a variable speed pump (to replace the tap water flow as described above) to the bottom of the bubble column. The solids settle to the bottom of the settler and are transported back to the bottom of the bubble column due to the presence of an air-lift liquid recirculation (see Figure 5.7). This air-lift recirculation exists because of a difference in hydrostatic pressure between the bubble column and the settler. This pressure difference is due to the presence of gasbubbles in the bubble column and the absence of gasbubbles in the settler and solids transport lines into the bubble column.
The control of the air-lift recirculation rate is important with regard to the fluidization of the sand in the bubble column and the entrainment of air-bubbles in the annulus (2) in Figure 5.7 .
One possibility for control is to install a valve in the air-lift circuits. This seems however not advisable because the solids with biolayers also pass through this valve with probable shearing of the biolayers and blocking of the valve. An alternative possibility is to impose a backpressure on the aerated leg of the air-lift. This backpressure is measured with a U-tube manometer connected to the gas disengagement section in Figure 5.7 . If such a backpressure is applied the liquid level in the annulus (2) in Figure 5.7 decreases to the same extent e.g. a backpressure of 0.1 atm leads to a liquid level decrease in the annulus of 1.0 meter.
Theoretically the maximum needed backpressure to stop the recirculation (neglecting frictional losses) is equal to the difference in aerated and unaerated liquid pressure in the bubble column.

The highest backpressure, expressed as meters liquid height, occurs in the situation without carrier and is then equal to $(1-\varepsilon)H$, where $(1-\varepsilon)$ is the gas holdup and H is the bubble column height (m).
It is clear then that the length h_a of the annulus section (2) in Figure 5.7 should be sufficient to allow a liquid level decrease which corresponds to the above mentioned maximum back pressure; therefore $h_a \approx (1-\varepsilon)H$.
The following dimensions apply to the separator and the bubble column.
The bubble column consists of a lower stainless steel section of 440 cm length and 25 cm diameter, a glassection of 80 cm length and 22.5 cm diameter and an aerated section in the separator of 130 cm length and 22.5 cm diameter. The total empty volume of the bubble column is then 300 ltr and the length is 6.5 m. The volume of the gas disengagement section is 35 ltr and its diameter is 30 cm. The length of the annular section (2) in Figure 5.7 is 100 cm. This length has been calculated assuming a gas hold-up of 15% and a bubble column height of 6.5 m The width of the annular section is 4 cm and the area is 0.033 m^2.
The length of the settling section (3) in Figure 5.7 is 130 cm and its external diameter is 50 cm. The area for upward liquid flow in the settler is 0.126 m^2 and the total settler volume is 205 ltr.
The solids from the settler are transported back into the bubble column through 6 holes of 2.5 cm diameter in the conical settler bottom (see Figure 5.7).
These holes are connected with 6 tubes of 2.5 cm diameter which merge together after 1.5 m into a tube of 5 cm diameter.
Through this 5cm tube of 4m length the solids are transported back into the bubble column at 55cm above the bottom. These large diameter tubes were chosen especially to minimize pressure losses in the air-lift. The air-lift flow rate was measured with a Magnetic Mass flow meter which was incorporated into the 5cm diameter tube mentioned above. This Magnetic flow meter presented a severe flow restriction because the circulating liquid flowed through a measuring section of 15cm long and 1.0 cm diameter.
The temperature of the reactor is controlled by surplus direct steam injection into the liquid distributor and cooling of the surplus heat by an automatically controlled cooling water flow along the outside of the stainless steel section of the bubble column.
The pH is controlled by addition of H_3PO_4 solution. In this reactor there are 4 sampling points. Three of these are situated along the bubble column itself (at 50, 150 and 400cm above the bottom which are noted as sp1, sp2, sp3) and one (sp4) is situated in the air-lift circulation line of 5 cm diameter directly below the merging point of the 6 tubes of 2.5 cm diameter.
The waste-water which is used is pumped directly from the sewer to the pilot-plant and is fed into the bubble column after a partial removal of suspended solids in a settler (hydraulic loading of 1.0 m/hr).
Finally it should be mentioned that the waste-water which leaves the reactor passes through the sand trap (4) (see Figure 5.1).

5.3.2.b Measurements and analyses

Each 8 hrs the temperature, pH, air and waterflows were checked. Furthermore from the influent and effluent waste-water a 24 hr averaged sample was obtained which was analysed for COD and suspended solids (see Chapter 4.3.2).
The oxygen content of the off-gas was analysed continuously with an Oxygor analyser (Chapter 4.3.2).
Whenever the biomass growth on the carrier could be seen by eye daily grab samples of 1 ltr total volume were taken from the reactor in sp1-sp4.
These samples were analysed for the settled volume of biomass covered carriers. A specified volume of settled particles was analysed for ash-content and organic solids content.
Furthermore the supernatant above the settled biomass covered particles was analysed for suspended organic solids.

5.3.2.c Experimental set-up

Two kinds of experiments were performed. First the technological aspects of the designed reactor were studied with the system air/tapwater/sand.
These aspects included gas hold-up and air-lift recirculation as a function of superficial gas velocity and the effect of backpressure on the air-lift recirculation rate.
Also the amount of sand loss was measured by trapping this sand (see Chapter 5.2.2.a).
During these technological experiments 80 kg sand of 0.1-0.3 mm was present in the reactor. The total flow of water through the reactor was maintained at 0.85 m^3/hr and the forced recirculation from settler overflow into the bubble column was maintained zero.
Secondly the biological fluidized bed experiment with waste-water was performed at 40°C, the pH was maintained between 6-8 and 80 kg sand of 0.1-0.3 mm was used as carrier in the bubble column.
The tapwater flow was maintained at 0.850 m^3/hr in the period of high dilutionrate and was maintained zero in the period of low dilutionrate.
The forced recirculation from the settler overflow into the bubble column was maintained at zero level in the period of high dilutionrate and was maintained at 0.850 m^3/hr in the period of low dilutionrate.
The waste-water flow was set at 100 l/hr at the start of the experiment and was changed during the experiment in such a way that the oxygen content of the off-gas was always larger than 19.5%. This ensured that the reactor was well above oxygen limiting conditions (see paragraph 5.3.3.a). The air flow rate was 20Nm^3/hr (V_{sg} = 11 cm/s) which was lowered during the experiment to 10 Nm^3/hr (V_{sg} = 5.5 cm/sec) because of foaming problems.
At the end of the experiment with the biological fluidized bed, the reactor was overloaded with COD in order to create oxygen limiting conditions for the assesment of the maximum oxygen transfer capacity. Finally it is important to note that during this purification experiment the Magflow meter was not present in the air-lift because the narrow passage of 1 cm led to

blocking with biomass covered particles. The airlift recirculation was maintained at its maximum rate during the waste-water experiment by maintaining the backpressure in the gas disengagement section of the bubble column at atmospheric level.

5.3.3. RESULTS

5.3.3.a Technological aspects of the fluidized bed reactor

In Figure 5.8 the gas hold-up is shown for a plain bubble column (no sand, no air-lift circulation) and a bubble column with sand added. It is obvious that the presence of sand lowers the gas hold-up appreciably. The relative difference is 10-20%. This effect is well known (see Ref. 5.18) and is caused by enhanced bubble coalescence. Furthermore it can be expected that the gas hold-up is lowered still more due to the air-lift liquid circulation (see Ref. 5.17). This effect is caused by the higher absolute rise velocity of the bubbles.
The lower gas hold-up in the fluidized bed reactor should lead to a proportional decrease in aeration capacity. This aeration capacity was measured at the end of the waste-water purification experiment which will be discussed in the next Chapter 5.3.3.b. The method for determination of the aeration capacity was the same as used for the semi-technical bubble column (Chapter 3.4.3.b).
This measurement showed that at a superfical gasvelocity of V_{sg} = 0.055 m/sec the maximum aeration capacity of the sand fluidized bed reactor amounts to 0.44% O_2/m of unaerated liquid height. This is considerably less than the 0.55% O_2/m found in the semi-technical bubble column (Table 3.11), but it is in approximate agreement with the lower gas hold-up mentioned above. From these aeration data it can then be calculated that the reactor will be oxygen transfer limited if the oxygen concentration in the off-gas reaches 21- 0.44*6.5*0.80 = 18.7% (assuming a gas hold-up of 20%).
From visual observation it was clear that the air-lift did indeed transport very smoothly the settled sand from the settler into the bottom of the bubble column. The amount of sand loss from the reactor was measured by collecting this washed out sand in the sandtrap ((4) in Figure 5.1).The loss amounted to 100 g sand/day at V_{sg} = 0.05 m/sec, which is small compared with the 80000 g of sand present in the reactor.
The air-lift circulation rate was measured as a function of gas superficial velocity and back pressure over the aeration section of the air-lift. The results are shown in Figure 5.9. It clearly appears that the air-lift circulation rate increases very much as V_{sg} increases, and it decreases rapidly as backpressure increases. The backpressure needed to stop the air-lift circulation follows from a simple force balance. The friction losses can be neglected because only the case of zero circulation rate is considered.
Therefore the neccesary backpressure is equal to the difference of gasboyancy pressure and gravity pressure of the sand in the air-lift circuit.

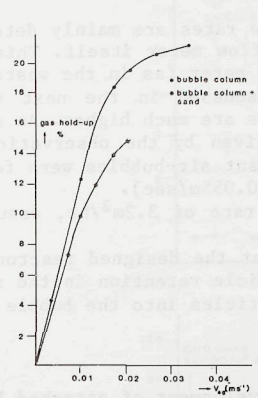


Figure 5.8
Gas hold-up in the bubble column with and without sand.

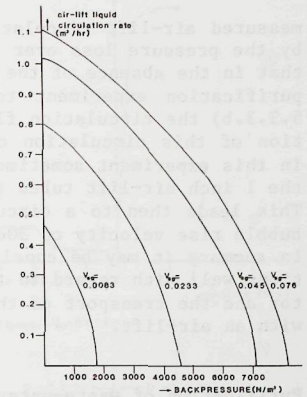


Figure 5.9
Air-lift liquid circulationrate as a function of gas superficial velocity and backpressure in the aerated part of the air-lift.

At the liquid flow rate of 0.85 m^3/hr which is fed into the column the expansion of the sand is 50 %. Therefore the volume of the fluidized sand is 80 ltr (calculated from a sand mass of 80 kg and a settled bulk density of the sand of 1500 kg/m^3). Because the air-lift enters the column at 55 cm above the bottom only 80-27=53 ltr of fluidized sand are present in the air-lift. The total mass of the sand is $\frac{53}{80} * 80 = 53$ kg.
The gravitational pressure of this amount of fluidized sand in water is $\frac{53}{2.6} * (2.6 - 1) * 9.8 * \frac{1}{0.05} = 6392$ N/m^2.
In this calculation a specific sand density of 2.6 kg/ltr is assumed and the reactor area is 0.05 m^2. At a gasvelocity of 0.023 m/s the gas hold-up can be estimated as 17% (Figure 5.8). This gives in the air-lift of 6 m length a gas buoyancy pressure of 6 * 0.17 * 1000 * 9.8 = 9996 N/m^2. The calculated backpressure to stop the air-lift is then 9996-6392=3604 N/m^2. From Figure 5.9 it appears that a backpressure of 4500 N/m^2 is needed. Considering the assumptions in the calculations this is a reasonable agreement.
The amount of circulation at no backpressure is also a strong function of gas superficial velocity.
For example at V_{sg}=0.076 m/s the circulation rate is 1.1m^3/hr. However it should be stressed that the circulation flow suffers a severe pressure loss in the Magflow meter (with which this flow is measured). As already mentioned in Chapter 5.3.2.a this Magflow forms a restriction of 1.0 cm diameter. The pressure loss in this restriction at a flow rate of 1.1m^3/hr can then be calculated (from $\Delta p = \frac{1}{2}\rho v^2$) to be 6180 N/$m^2$. From Figure 5.9 it appears that at V_{sg}=0.076m/s the available driving pressure for the air-lift (measured as the backpressure to reduce circulation to 0) is about 8000N/m^2. It is then clear that the

measured air-lift circulation flow rates are mainly determined by the pressure loss over the Magflow meter itself. This means that in the absence of the Magflow meter (as in the waste-water purification experiment to be discussed in the next Chapter 5.3.3.b) the circulation flow rates are much higher. An estimation of this circulation can be given by the observation that in this experiment sometimes stagnant air-bubbles were found in the 1 inch air-lift tubes (at V_{sg}=0.055m/sec).
This leads then to a circulation rate of 3.2m^3/hr, assuming a bubble rise velocity of 30cm/sec.
In summary it may be concluded that the designed reactor functions well with regard to the particle retention in the separator and the transport of these particles into the bubble column with an air-lift.

5.3.3.b Purification of waste-water and development of attached biomass under conditions of high dilution rate

In Figure 5.10 the dilution rate which was applied during the experiment is shown (0 - 337hr). The average dilution rate is 3.2hr^{-1} and the variations are mostly due to variations in waste-water flow.

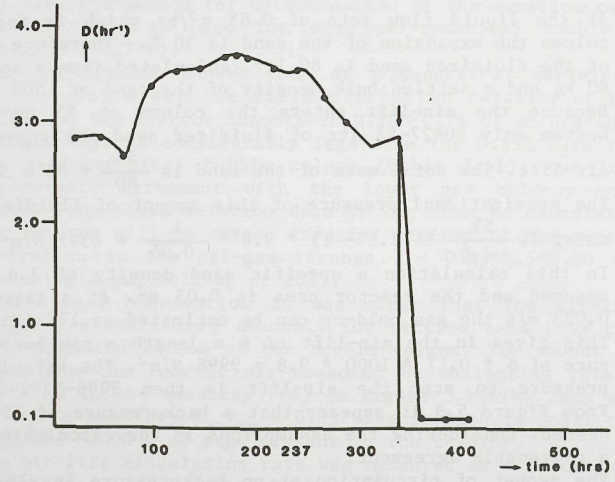


Figure 5.10
Dilution rate applied in the waste-water purification experiment in the biological aerobic fluidized bed reactor.

The dilution rate was calculated from the waterflow through the reactor and the volume of the aerated bubble column only (without the unaerated settler).
The arrow in Figure 5.10 - 5.13 indicates the change from high dilution rate to low dilution rate. The effect of this change will be discussed in the next section 5.3.3.c. The influent COD concentration is shown in Figure 5.11A and the COD load of the

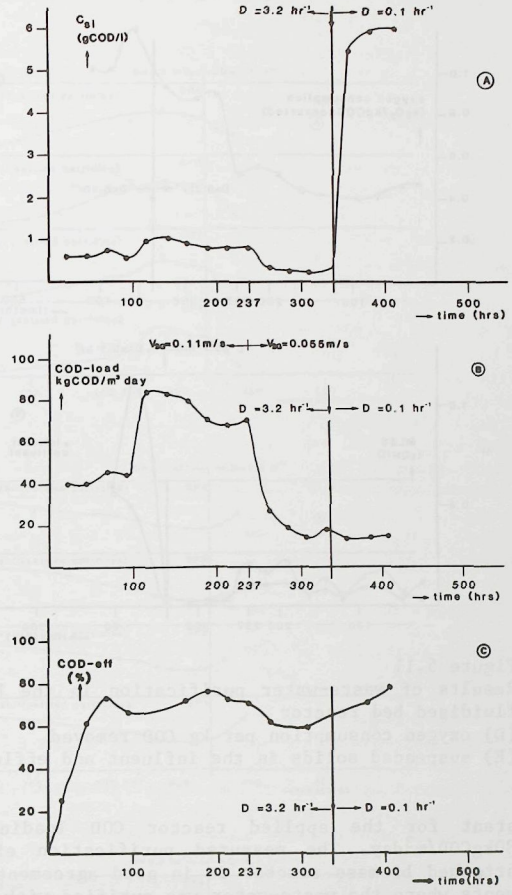


Figure 5.11
Results of waste-water purification in the biological aerobic fluidized bed reactor
(A) influent COD concentration
(B) applied COD load
(C) COD removal efficiency

reactor (calculated from 300ltr reactorvolume) is shown in Figure 5.11B. It can be seen that the reactor COD load averaged about 60kgCOD/m^{3}day until 237hrs. From thereon the COD load averaged about 15kg/m^{3}day. The reason for this decrease was that at the high superficial air velocity of 0.11m/s before 237hrs some foaming problems occured. Therefore the air superficial velocity was deminished to 0.055m/sec from 237hrs on.
Because of the concomittant decrease in aeration capacity the reactor COD load had to be decreased also.
The COD purification efficiency is shown in Figure 5.11C. It appears that the reactor reaches very quick a COD purification efficiency of 70%. This purification efficiency is about con-

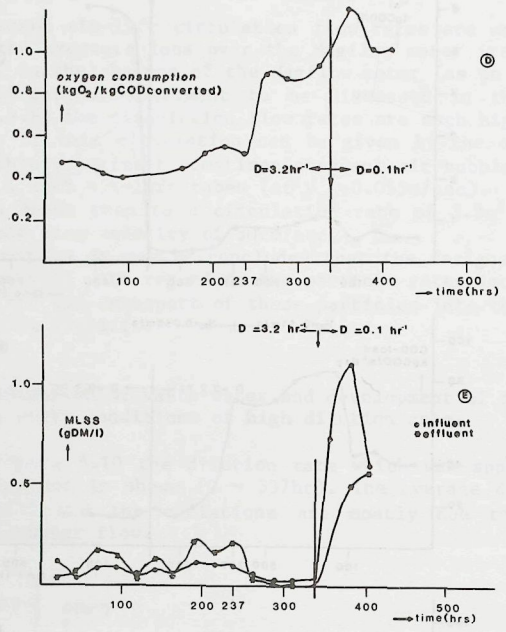


Figure 5.11
Results of waste-water purification in the biological aerobic fluidized bed reactor
(D) oxygen consumption per kg COD removed
(E) suspended solids in the influent and effluent

stant for the applied reactor COD loadings between 15 - 80kgCOD/m^3day. The measured purification efficiency in this attached biomass reactor is in good agreement with the experiments where the waste-water was purified with suspended biomass at 40°C, Chapter 3(Table 3.6, period 4) and Chapter 5.2.3.a (Figure 5.2B).
The oxygen consumption per kg COD which is removed is shown in Figure 5.11D. Under high reactor COD loadings (0 - 237hrs) the oxygen requirement is about 0.5kgO_2/kgCOD. This value compares reasonably with the oxygen requirement of about 0.65kgO_2/kgCOD measured in the suspended biomass system (Chapter 3.4.2.b).
Under low reactor COD loading (237 - 334hrs) the oxygen consumption rises quickly to about 1kgO_2/kgCOD. Such a high value of oxygen consumption indicates the absence of surplus biomass production (provided it is assumed that nitrification reactions do not occur).
This absence of surplus biomass production is due to the extreme low specific biomass COD conversion rate in this period which can be calculated at q_{COD} = 0.029 g COD/g DMhr from a COD conversion rate of 10.5kg/m^3day and an attached biomass concentration of 15 g VSS/ltr (see below). This value of q_{COD} is equal to the maintenance requirement at 40°C of suspended

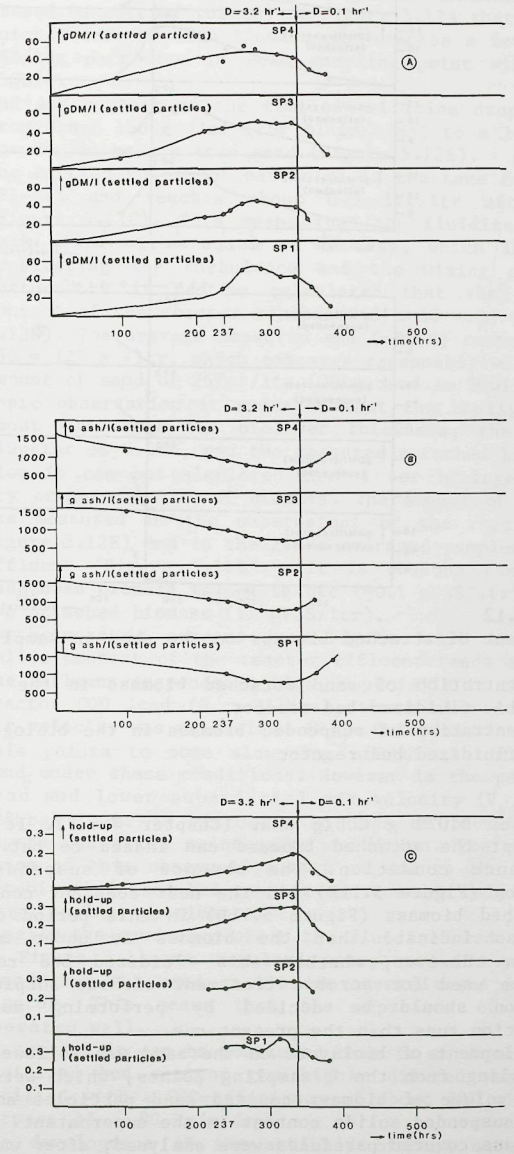


Figure 5.12
Development of attached biomass on the sand in sampling points SP1-SP4
(A) organic dry matter content of settled biomass covered sand particles
(B) sand content of settled biomass covered sand particles
(C) settled volume of biomass covered sand particles per reactor volume

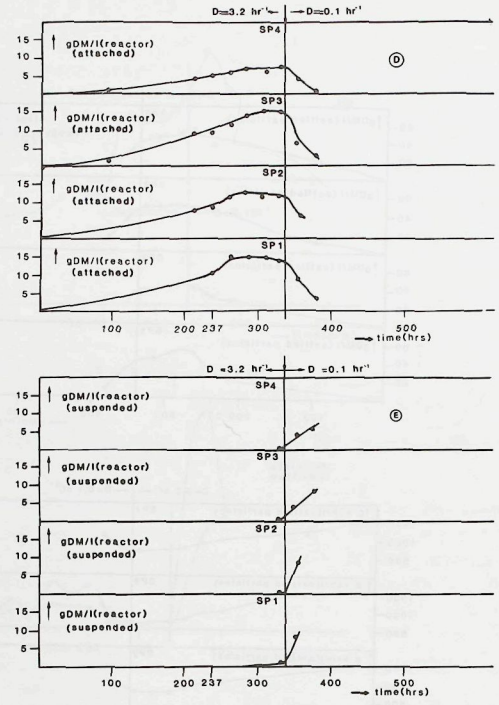


Figure 5.12
Development of attached biomass on the sand in sampling points SP1-SP4
(D) concentration of sand attached biomass in the biological aerobic fluidized bed reactor
(E) concentration of suspended biomass in the biological aerobic fluidized bed reactor

biomass of 0.029 g COD/g DMhr (Chapter 4.4.5). It therefore seems that the attached biomass can indeed be put under the maintenance condition. The absence of suspended solids production (Figure 5.11E) and the near constant concentration of attached biomass (Figure 5.11D) in this period of low COD load also indicate that the biomass is under maintenance condition. However, whether this fluidized bed reactor can indeed be used for aerobic treatment without surplus biomass production should be decided by performing much longer purification runs than the present one.
The development of biolayers on the sand was followed by regular sampling from the 4 sampling points, which were analysed for the volume of biomass covered sand particles and for the organic suspended solids content in the supernatant.
The biomass covered particles were analysed, after washing with tap water, for ash (=sand) content and organic matter content. The results are shown in Figure 5.12A - 5.12D.
The sampling points, SP1, SP2, SP3 are located at different heights along the bubble column (50, 150 and 500 cm above the bottom) and SP4 is located in the air-lift circulation (see also paragraph 5.3.2.a).

It can be seen from the organic matter content of the particles (based on settled volume) in Figure 5.12A that the sand becomes quickly covered with biomass and reaches a level of about 50 g VSS/ltr particles in each sampling point within about 200 - 250hrs.
The ash content of the settled particles drops in this period from about 1500g /ltr (for plain sand) to a level of 750g /ltr for the biomass coated sand (Figure 5.12B).
The settled volume of particles is the same for sampling point SP1-SP3 and reaches about 0.3 ltr/ltr after about 230hrs (Figure 5.12C). This means that the fluidized bed reactor is well mixed with regard to biomass, which is not surprising considering the turbulence and the mixing of the air lift.
Furthermore it can be calculated that the attached biomass content of the reactor reaches 0.3 x 50 = 15 g VSS/ltr (Figure 5.12D). The average measured ash (=sand) content is then 0.3 x 750 = 225 g /ltr, which compares reasonably well with the added amount of sand of 267g /ltr (80kg sand in 300ltr). From microscopic observation it appeared that the biolayer thickness was about 40μ. From the biolayer thickness, the average carrier diameter of 0.2mm, and the measured attached biomass concentration it can be calculated that 1 ltr biolayer contains 100 g dry organic matter of biomass. The amount of suspended solids was measured in the supernatant of the reactor samples (see Figure 5.12E) and in the 24hrs averaged samples of influent and effluent (Figure 5.11E). It is obvious that the amount of suspended biomass is negligible (≈0.1 gVSS/ltr) compared with the attached biomass (15 gVSS/ltr).
Furthermore it appears from Figure 5.11E that the suspended solids content of the reactor effluent tends to be higher than the influent suspended solids, especially in the period of high reactor COD load (0 - 237hrs), where also a high superficial air velocity was maintained (V_{sg}= 0.11m/s).
This points to some sloughing off of the biolayers from the sand under these conditions. However in the period of low COD-load and lower superficial air velocity (V_{sg}=0.055m/s, 237 - 338hrs) there is virtually no loss of biomass from the biolayers under the maintenance condition. The possible significance of this observation with regard to the operation of the aerobic fluidized bed reactor can however only be determined in long lasting experiments.
Finally the sand-trap after the fluidized bed reactor (number 4 in Figure 5.1) was regularly inspected for particles. It was found that the sand or biomass covered sand did not leave the reactor. This means that the separator of liquid-solid-gas operated well.
In summary it can be concluded that in an aerobic biological fluidized bed reactor the highly diluted waste-water can be purified with a COD-efficiency of about 70% with reactor loads between 15 - 80 kg/m^{3}day.
The development of the attached biomass under high dilution rate conditions and under high turbulence conditions proceeds quickly and there is no wash-out of the biomass covered sand particles from the reactor. Also some indications have been obtained that the aerobic fluidized bed reactor without surplus biomass production is possible.

5.3.3.c Stability of the attached biomass under conditions of low dilution rate

As already argued in Chapter 5.2.4 the stability of the attached biomass under conditions of low dilution rate is of crucial importance for the application of the aerobic fluidized bed reactor to the undiluted GB waste-water.
Therefore at 337hrs (in the experiment described in the previous Chapter 5.3.3.b) the tap water flow was replaced by an equal forced recirculation from the reactor effluent.
Therefore the hydraulic conditions and the COD load to the reactor (Figure 5.11B) did not change. Also the purification efficiency or oxygen uptake did not change (Figure 5.11C and Figure 5.11D).
The dilution rate D did however decrease from $3.2hr^{-1}$ to $0.1hr^{-1}$. The effect of this change in dilution rate was very dramatic. Within 24hrs the biolayers on the sand were destroyed and high concentrations of suspended biomass could be measured (see Figure 5.12A - E and Figure 5.11E).
It is therefore clear that the attached biolayers are not stable at low dilution rates and that the change from an aerobic attached microbial population to the aerobic suspended microbial population is a very fast process.

5.3.4 DISCUSSION

It has been found that attached aerobic biomass in a highly turbulent fluidized bed reactor is only formed under conditions of high dilution rate D (Chapter 5.2) and that a change from high D to low D in a well purifying fluidized bed reactor leads to a very fast destruction of the attached biomass (Chapter 5.3.3.c). This destruction is nearly complete within 24hrs after the change from high D to low D. Microscopic observations revealed that the biolayers on the sand were virtually cut to pieces.
It was unlikely that this was brought about by diffusion limitation or biomass starvation because hydraulic and feeding conditions and oxygen uptake in the reactor had not changed.
Another possibility, inspired from the microscopic observations, is however that suspended micro-organisms, which are not washed out of the reactor at low D, attack enzymatically the biolayer and hydrolyse the attachment biopolymers in search of food under the substrate limited conditions in the fluidized bed reactor.
In order to substantiate this hypothesis a literature survey about attached micro-organisms and the biodegradability of attachment polymers has been carried out (Chapter 6).
A problem, which results from the $D \geq \mu^{MAX}$ condition for attachment, is that the concentrated GB waste-water cannot be treated in the aerobic fluidized bed reactor at $D = 3.2hr^{-1}$ because of aeration limitation.
One possibility is then to perform series of fluidized bed experiments to determine the minimum D value which still leads to stable biolayers. A far more attractive alternative is however to develop a methane generating fluidized bed reactor, using the present findings, where the methanogenic biomass grows attached to the sand. This alternative has two main advantages: There is no aeration limitation and the amount of surplus biomass is inherently very much lower than in the aerobic purification process. The only disadvantage is however that methanogenic bacteria have been studied far less intensively than aerobic bacteria with respect to attachment and that the anaerobic waste-water purification is generally regarded as a difficult process. Therefore special attention was paid to attachment of methanogenic bacteria in the aforementioned literature survey.

5.4 CONCLUSION

It has been found that attached aerobic biomass is only formed at a high dilution rate. Once formed, the attached biomass is destroyed very rapidly under conditions of low dilution rate.
A competition mechanism between attaching and non-attaching micro-organisms is proposed to explain this phenomenon. This mechanism leads to the condition of $D \geq \mu^{MAX}$ for the development of attached biomass. The required high dilution rates to maintain the biofilm in the fluidized bed reactor implicates that only diluted GB waste-water can be treated because of aeration limitation. It has been shown that such diluted GB waste-water can be treated in the air-lift fluidized bed reactor with 70 % COD efficiency and COD loads of 15-80 kg/m^3day. Also some indications have been obtained that in such a fluidized bed reactor the aerobic purification without surplus sludge production can be realised.
Because of the destruction of the attached biolayer at low dilution rate, the aerobic treatment of the undiluted GB waste-water in a fluidized bed reactor at low D-values (because of limited aeration capacity) seems problematic.
A logical alternative for the treatment of the concentrated GB waste-water would be the development of an anaerobic methane-generating biological fluidized bed which is not hampered by the aeration limitation or possible high surplus sludge production. Before doing so it seems however wise to perform a literature study about attachment of micro-organisms, bearing in mind the effect of dilution rate on attachment and the proposed enzymatic competition mechanism between attaching and non-attaching micro-organisms.

APPENDIX 5A

In the competition for substrate the attached population has inherently two disadvantages compared to the freely suspended population under conditions of a dilution rate where both populations can exist ($D \leq \mu^{MAX}$).
First the attached micro-organism suffers from some diffusion limitation because of the diffusion layer around the carrier upon which the microbe is attached. This means that the substrate concentration for the attached micro-organism is lower than for the suspended micro-organism in the liquid bulk.
The magnitude of this concentration difference can be estimated as follows.
It is assumed that a carrier with diameter d_c is covered with a monolayer of microbes having a diameter d_m.
The total substrate consumption of these microbes is then:

$$\text{substrate consumption} = n q_{BOD}^{MAX} \qquad \text{eq.(5A-1)}$$

q_{BOD}^{MAX} is the substrate consumption per cell and n is the cell number on the carrier surface.
n can be assumed to be equal to

$$n = \frac{\pi d_c^2 \times d_m}{\frac{\pi}{6} d_m^3} = 6 \frac{d_c^2}{d_m^2} \qquad \text{eq.(5A-2)}$$

The substrate transport due to molecular diffusion can be calculated as

$$\text{substrate transport} = \pi d_c^2 * k_L \Delta C_{BOD} \qquad \text{eq.(5A-3)}$$

In eq.(5A-3) ΔC_{BOD} is the concentration difference between substrate in the liquid bulk and the substrate near the carrier surface.
k_L is the transport coefficient which can be calculated in this case from equation (5A-4) (assuming Sh = 2)

$$k_L = \frac{2 \mathbb{D}}{d_c} \qquad \text{eq.(5A-4)}$$

Equalization of substrate consumption and substrate transport (5A-1 and 5A-3) and using 5A-2 and 5A-4, the following equation for ΔC_{BOD} is obtained

$$\Delta C_{BOD} = \frac{d_c}{d_m^2} \frac{q_{BOD}^{MAX}}{\mathbb{D}} * \frac{3}{\pi} \qquad \text{eq.(5A-5)}$$

Substitution of the following numerical values:

$q_{BOD}^{MAX} = 10^{-17}$ g BOD/cell sec ($\equiv$ 0.37 g BOD/g DM hr, Chapter 3.4.2.b)
$\mathbb{D} = 2 * 10^{-9}$ m^2/sec

$d_c = 2 * 10^{-4}$ m

$d_m = 10^{-6}$ m

leads to a value of $\Delta C_{BOD} = 1.05$ g BOD/m^3.

This is still a very small value compared to the estimated K_s value of 149 g BOD/m^3 (Chapter 3.4.2.b) which has been found for suspended waste-water populations. This means that even under severe substrate limited conditions the substrate concentration for suspended or attached micro-organisms is virtually the same.
Secondly the attached micro-organisms need to synthesize a certain amount of attachment polymers. According to References 5.14 - 5.16 the amount of attachment polymers is about 1 - 3% of cell dry matter.
This means that, assumed that attached and suspended micro-organisms do have the same substrate uptake capacity, the attached micro-organisms do have a 1 - 3% lower growth yield and therefore a 1 - 3% lower growth rate.
The assumed equal substrate uptake capacity of attached and suspended micro-organisms is substantiated by the result reported in paragraph 5.2.3.b that the maximum specific O_2 uptake rate for biolayers and flocs grown on GB waste-water compare well.

SYMBOLS

C_{si}	substrate concentration in waste-water feed in aeration tank	kg/m^3
ΔC_{BOD}	difference in substrate concentration between bulk and carrier surface	$g\ BOD/m^3$
D	dilution rate	hr^{-1}
$\mathbb{D}$	diffusion coefficient	m^2/s
d_c	carrier diameter	m
d_m	microbe diameter	m
h_a	annulus height in air-lift separator	m
H	liquid height of bubble column	m
MLSS	mixed liquor suspended solids	$kg\ DM/m^3$
rpm	revolutions of stirrer per minute	min^{-1}
Sh	Sherwood number	-
vvm	volume air per volume reactor per minute	min^{-1}
V_{sg}	gas superficial velocity	m/s
$(1-\varepsilon)$	gas hold-up	-
q_{BOD}^{MAX}	maximum specific conversion rate of BOD	g BOD/g DMhr
q_{COD}	specific conversion rate of COD	g COD/g DMhr
μ^{MAX}	maximum growth rate of micro-organism in the reactor	hr^{-1}

REFERENCES

5.1 K.C. Marshall,
Water pollution Microbiology,2, (1978), 51
Ed. J. Mitchell, Wiley New York

5.2 A.L.van Wezel, C.A.M. van der Velden-Groot,
Process Biochemistry, (1978), 6

5.3 K.C. Marshall, R. Stout, R. Mitchell,
J. Gen. Microbiology, 68, (1971), 33

5.4 M.F. Kong, P.Y. Young,
Biotechnol. and Bioeng., 21, (1979), 417

5.5 K. Rietema, S.P.P. Ottengraf, H.P.E. van de Venne,
Biotechnol. and Bioeng., 13, (1971), 911

5.6 J.S. Jeris, C. Beer, J.A. Mueller,
J. Wat. Pollut. Control. Fed., 46, (1974), 2118

5.7 J.S. Jeris, R.W. Owens, R. Hickey, F. Flood,
J.W.P.C.F., 49, (1977), 816

5.8 J.E. Butts, J.E. la Padula, K.A. Sanderson,
Proc. 32th Ind. Waste Conf., (1977), 454

5.9 K.P. Becker,
Chemie-Ing.-Techn., 51, (1979), 549

5.10 C.D. Scott, C.W. Hancher,
Biotechnol. and Bioeng., 18, (1976), 1393

5.11 J.A. Klein, D.D. Lee,
Biotechnol. and Bioeng., symp. no. 8, (1978), 379

5.12 W.W. Pitt, C.W. Hancher, H.W. Hsu,
A. I. Ch. E. Symp. Series, no 181, 74, (1978),119

5.13 D.D. Lee, C.D. Scott, C.W. Hancher,
J.W.P.C.F., 51, (1979), 974

5.14 T. Hayashi, M. Kozaki,
J. Gen. Appl. Microbiol. 26, (1980), 245

5.15 Y Tago, K. Aida,
Appl. Environm. Microbiology, 34, (1977), 308

5.16 M.W. Tenney, F.H. Verhoff,
Biotechnol. and Bioeng., 15, (1973), 1045

5.17 J.J. Heijnen, K. van 't Riet,
To be published in The (Bio)Chemical Engineering Journal (1984)

5.18 K. Oestergard and P. Fosbol,
The Chemical Engineering Journal, 3, (1972), 105

6 FUNDAMENTAL ASPECTS OF MICROBIAL ATTACHMENT AND BIOLAYERFORMATION

6.1 INTRODUCTION

In the previous Chapter 5 it was found that the dilution rate D is an important parameter in the cultivation of aerobic attached biomass. It was found that under very turbulent conditions aerobic biomass grows attached to sand at D=3.2 hr^{-1} and D=2.4 hr^{-1} and that attachment does not occur at D=0.1 hr^{-1}. Moreover it appeared that attached aerobic biomass which was cultivated at D=3.2 hr^{-1} was destroyed within 1 day if D was changed to 0.1 hr^{-1} (keeping substrate load of the biomass and hydraulic conditions in the reactor at a constant value).
To explain this phenomenon the hypothesis of competition between suspended biomass and attached biomass was proposed as an important factor. The proposed competition mechanism is that suspended micro-organisms can hydrolyse the attachment polymers of attached micro-organisms.
This leads then to suspended biomass at $D \leq \mu^{MAX}$ and to a dominance of attached biomass at $D \geq \mu^{MAX}$ because suspended micro-organisms are washed out.
For more extensive discussions about these results one is referred to Chapter 5.2.4 and 5.3.4
The necessity to maintain a high dilution rate in an attached biomass reactor renders the aerobic treatment of concentrated waste waters impractical because of aeration limitation (Chapter 5). Therefore it was concluded in Chapter 5 that the development of a reactor employing attached methanogenic biomass was very attractive. Before doing so however it seems fruitfull to gather more information about attachment of micro-organisms in general. This Chapter 6 is therefore mainly concerned with a literature review about microbial attachment and the following topics will be discussed:

- **Occurrence of attachment of micro-organisms**

  It is quite important to get an impression about the extension of set 3 in Figure 5.5. For example it is important to know whether methanogenic micro-organisms possess attachment properties.

- **Description of the process of microbial attachment**

  This should provide information how microbes carry out their attachment.

- **Role of biopolymers in microbial attachment**

  In order to understand microbial attachment it is important to know something about the chemistry of these polymers, their production by microbes, their susceptibility to enzymatic degradation.

- **Influence of dilution rate on microbial attachment**

  It is interesting to see whether the effect of dilution rate, found in Chapter 5, on microbial attachment has also been reported for other systems.

- **Influence of the presence of non-attaching micro-organisms on biolayer formation**

  During the process of biolayer formation under $D \geq \mu^{MAX}$ conditions, there will always be present a certain amount of non-attaching suspended micro-organisms. These organisms do originate from the seed material from which the attaching micro-organisms are selected and they are often present in the wastewater which is fed into the reactor. Regarding the proposed mechanism that suspended micro-organisms can interfere with the attachment process it is quite important to pay attention to this aspect.

- **Physical parameters of biolayers**

  This information is concerned with biomass content, biolayer thickness, or diffusion limitation in different biolayers.

- **Dynamics of biolayer formation of a mixed culture**

  This is important for the start-up and the steady state of an attached biomass reactor.

6.2 OCCURRENCE OF ATTACHMENT OF MICRO-ORGANISMS

Attachment of micro-organisms to surfaces can be observed widely in nature and in man-made constructions. It is useful to make a division with regard to the kind of surface where upon the microbe attaches.

6.2.1 ATTACHMENT TO MACROSCOPIC INORGANIC SURFACES

With macroscopic is meant a large surface relative to microbial dimensions. From literature it then appears that attached microbes can be found:

- in drinking water circuits (Ref. 6.1)
- on shipwalls, marine constructions like drilling platforms (Ref. 6.2, 6.3)
- in cooling water circuits and cooling towers in powerplants (Ref. 6.4)
- in soil (Ref. 6.5)
- on the bottom of fast flowing streams (Ref. 6.6)

If the microbes were not attached to the bottom, most rivers would be sterile because microbes would be washed to the sea without significant growth

- on the teeth in the mouth (Ref. 6.7)
- on the walls of sewers (Ref. 6.8)
- in ore leaching processes (Ref. 6.9)
- on the wall of microbial fermentors (Ref. 6.10 and 6.11). Most often (unwanted) microbial attachment is observed in continuous flow culture, where it complicates interpretation of the experiments. Such "wall growth" has been observed within a wide spectrum of microbial cultures like aerobes (Ref.6.12), acidifying anaerobes (Ref. 6.13), methanogenic anaerobes (Ref. 6.14)
- on carriers in so-called biological expanded or fluidised bed reactors (Ref. 6.15). The microbes grow attached to a heavy carrier and wash-out is therefore impossible. These reactors have been developed for aerobic, nitrifying, denitrifying and methanogenic microbial cultures (Ref. 6.15). Types of carriers used are sand, coal, plastic, metal.
- in waste water treatment processes like trickling filters and rotating biological contactors, where the microbes grow attached to the filter or to the rotating disc (Ref. 6.16 and 6.17).

It is obvious from this list that microbial attachment seems to occur very frequently to about any macroscopic inorganic surface and for many microbial cultures, including anaerobic methanogenic ones.

6.2.2 ATTACHMENT TO MACROSCOPIC ORGANIC SURFACES

Contrary to Paragraph 6.2.1 where the surface is inorganic, large amounts of microbes can also be attached to biological organic surfaces. These surfaces are for example:

- mouth tissue, like tongue (Ref. 6.7, 6.18)
- wall of stomach or gut (Ref. 6.8, 6.19). These microbes do assist the anaerobic digestive processes which occur in the stomach or gut.
- plant material (Ref. 6.8, 6.20)
- skin (Ref. 6.8)
- plant roots (Ref. 6.8), where microbes are involved in nitrogen fixation.

In general the microbial populations attached to organic tissue can be divided into autochtonous populations, where a symbiosis exists between host tissue and microbe, and into pathogenic

populations where the microbe exerts a negative influence upon the host tissue (Ref. 6.22). Recently it has become very clear that pathogenesis is often related to attachment properties of pathogens (Ref. 6.21, 6.22)

6.2.3 ATTACHMENT TO MICROSCOPIC ORGANIC SURFACES

Contrary to Chapter 6.2.2, where microbes attach to organic tissues there also exists the phenomenon that microbes attach to each other to form flocs or pellets. Flocculation of microbes is widely known and can occur for almost any micro-organism (Ref. 6.8, 6.23). Pellet formation by microbes has been studied only recently (Ref. 6.24) and occurs in denitrifying cultures, acidifying cultures and methanogenic cultures.

In summary it appears that attachment of microbes to surfaces is a very general phenomenon, and it is perhaps not too pertinent to state that in principle most microbes can attach to surfaces. In this context it is illustrative to note that about 70% of a large number of microbial isolates possessed attachment properties (Ref. 6.5) and that a non-attaching microbe became attaching after a natural mutation (Ref. 6.25 and 6.26).

6.3 DESCRIPTION OF THE PROCESS OF MICROBIAL ATTACHMENT

According to Marshall (Ref. 6.27) surfaces do posses inherently higher substrate concentrations than the bulk liquid. The effect is most pronounced in nature where substrate concentration is very low ($\leq$ 1 mg/ltr), but e.g. in waste water treatment reactors the effect is negligible. In practice most surfaces are colonised by microbes from the bulk-liquid (Ref. 6.27). The process of colonisation occurs in several phases according to Marshall (Ref. 6.27), phase 1 of adsorption and phase 2 of attachment.

6.3.1 PHASE 1: ADSORPTION OF MICROBES

The adsorption of microbes to a surface is the result of action between several opposing forces. These forces are electrostatic interaction, London - Van der Waals forces, interfacial forces (Ref. 6.28).
The above listed forces are weak and very dependent upon distance between microbe and surface. Therefore the equilibrium is reached at a definite distance between microbe and surface. There is no direct contact between microbe and surface.
This adsorption proces is purely physical, without any biological action and its time constant is very short (from minutes to 1 hr)(Ref. 6.29).
Another feature is that the adsorption is a reversible process and finally there is an equilibrium between adsorption and

desorption of microbes. It has been observed that the relation between suspended microbes and adsorbed microbes follows the well known Langmuir adsorption isotherm (Ref. 6.29).
Furthermore it has been observed that adsorption goes never beyond a mono-layer of microbes (Ref. 6.29). This is quite logical because the second layer of microbes does not interact anymore with the surface but with the first layer of microbes, which is in principle a totally different interaction.
In general it can be concluded that the adsorption of a microbe to a surface is specifically dependent on the molecular characteristics of the microbial cell-wall and the surface. Another microbe, another surface or another liquid medium do all have an influence upon the adsorption forces and therefore the adsorption process is influenced. It has also been shown that the physiological condition of the microbe influences the adsorption. Microbes in a logarithmic growth phase do adsorb much better than microbes in the stationary growth phase (Ref. 6.29, 6.30). Also temperature, pH and ionic strength influence the adsorption through their influence on the adsorption forces (Ref. 6.31).
In summary it seems that adsorption of microbes to surfaces does always occur to some extent and that adsorption stops at a monolayer of microbes. Application of such monolayers of microbes on a carrier in a reactor is therefore impractical because the amount of biomass is far too low and because the microbes are easily desorbed due to the weak forces involved. However after adsorption to a surface microbes do generally create some form of molecular bonding between microbe and surface. This will be discussed in the next chapter as phase 2 of the attachment process (Ref. 6.27).

6.3.2 PHASE 2: ATTACHMENT OF THE MICROBE TO THE SURFACE

As already mentioned in the previous Chapter 6.3.1. the microbes can, after adsorption, attach themselves firmly to the surface with some form of molecular bonding.
In general there seem to be two classes of bonding:

- specific bonding between cell-wall polymers and surface polymers (Ref. 6.28, 6.32)

- aspecific bonding of the microbe to the surface with a cell-wall connected polymeric matrix, the so-called glycocalyx (Ref. 6.33).

The specific bonding is typical for the microbe and the surface involved, and therefore mostly a monolayer on the surface is formed (Ref. 6.34, Ref. 6.30). This type of bonding is very much influenced by surface properties, medium composition and cell-wall composition of the microbe (Ref. 6.32). It can be observed in cell tissue cultures on micro beads (Ref. 6.35) or cell-cell interactions like microbes on the tongue (Ref. 6.18) or pathogenic infections (Ref. 6.21 and 6.22).
It has been reported that specific proteins, called lectins, are involved in the specific bonding (Ref. 6.33).
Finally it should be mentioned that this specific attachment process is irreversible and that its time constant is much

longer than for adsorption because biological activity is needed for the production of bonding molecules. Also this type of attachment seems not suitable to obtain high concentrations of attached biomass on a carrier because of the high specificity of the attachment and because the attachment seems not to go further than a mono-layer.
However the aspecific glycocalyx type of attachment seems ideally suited for our purposes of achieving high biomass concentrations on a carrier. It has been shown recently (Ref. 6.33) that many microbes can produce, after adsorption, a polymeric substance which glues the microbes to the surface and to each other. This glue surrounds the whole cell and leads eventually to a thick biolayer which consists of the glue matrix (glycocalyx) wherein the microbes are embedded. The glycocalyx can be made visible under an electron microscope only after special treatment of the sample, because otherwise the dehydration of the sample leads to a collapse of the glycocalyx matrix (Ref. 6.21). The attachment which is achieved is very strong and irreversible. The time constant of this process is again much longer than for adsorption because biological activity is needed for the production of the glycocalyx polymers. It is also quite important to note that this attachment process is not specific, the glycocalyx glue attaches to any surface (Ref. 6.33). This surface can be macroscopic (inorganic or organic) or microscopic (organic) as listed in Chapter 6.2. In most of the systems listed in Chapter 6.2. the role of the glycocalyx in the microbial attachment has been confirmed.
Summarising it can be concluded that attachment of microbes to any surface or to each other can proceed readily and that the formation of strong biolayers or flocs-pellets depends to a large extent upon the formation of glycocalyx polymers. It is therefore of the utmost importance to gain knowledge about chemistry, production and stability of the glycocalyx polymers. This topic will therefore be dealt with in the next Chapter 6.4.

6.4 CHARACTERISATION AND PRODUCTION OF GLYCOCALYX BIOPOLYMERS

As already mentioned in the previous Chapter 6.3. it is important with regard to biolayers, to understand the chemistry, production and stability of glycocalyx polymers.

6.4.1 CHEMISTRY OF GLYCOCALYX POLYMERS

The polymers from the glycocalyx are polysaccharides with acid residuals. These acids are acetic acid, pyruvic acid, uronic acid (Ref. 6.33, 6.36, 6.37) and therefore the glycocalyx is negatively charged at neutral pH. There are also indications that metal-ions like Ca^{2+} and Mg^{2+} are essential for the formation of the glycocalyx (Ref. 6.38, 6.37, 6.39). These metal-ions are probably necessary for bridging between the acid residuals of the polysaccharides to create the 3-dimensional network of the glycocalyx.

The polysaccharides are well known products of microbial metabolism. They can be divided in homo-polysaccharides like starch, cellulose, dextran, curdlan, levan or heteropolysaccharides like alginate or xanthan (Ref. 6.39, 6.40, 6.41). Most of these polysaccharides are viscous and water soluble, while the glycocalyx polysaccharides are clearly not soluble. The homo-polysaccharides can be hydrolysed readily by known enzymes like cellulase, dextranase (Ref. 6.7, 6.42, 6.43). The heteropolysaccharides cannot be hydrolysed as readily by enzymes (Ref. 6.7, 6.44) and only a few enzymes have been described for this hydrolysation (Ref. 6.41). Contrary to this it has been found that aerobic metabolism of complex microbial polysaccharides by enrichment cultures proceeds rapidly (Ref. (6.90).
It has further been shown that addition of hydrolising enzymes to attached micro-organisms leads to rapid detachment (Ref. 6.43, 6.49).

6.4.2 PRODUCTION OF EXTRA-CELLULAR POLYSACCHARIDES BY MICRO-ORGANISMS

6.4.2.a Function of polysaccharides

The function of extra-cellular polysaccharides remains relatively obscure.
This is especially so for the soluble viscous polysaccharides which were listed in the previous Chapter 6.4.1. The microbes do spent a considerable part of their substrate in this polysaccharide production, which is inefficient with regard to growth. Indeed sometimes it has been observed that the polysaccharide producing strain is eliminated from a continuous culture by a non-producing mutant which grows better (Ref. 6.45), but this does not always occur (Ref. 6.46). A possible explanation for the production of soluble polysaccharides might be that although the polysaccharides are soluble under fermentation conditions they do form an insoluble glycocalyx in the natural environment of the microbe.
In this regard it is significant that it has been found that the soluble polysaccharide Xanthan forms an insoluble gel when it is mixed with galactomannan (Ref. 6.89). The galactomannan is the main polymer in plant cell walls, which is the natural environment for the attached growth of the Xanthan producing micro-organism Xanthomonas campestris. Attachment seems therefore to occur through gel formation of Xanthan and galactomannan.
Reasons for the production of these unsoluble glycocalyx polysacharides around the microbe can be found in literature:

- the glycocalyx forms a protective coat around the microbes against poisonous agents, phage attack, attack by other micro-organisms (Ref. 6.33)

- the glycocalyx forms the glue with which the microbe attaches itself to the wall of stomach, gut, mouth, urine tract, to the bottom of water streams (Ref. 6.33, 6.47). All these systems have in common that their dilution rate D is much higher than the μ^{MAX} of the microbes. Therefore only microbes which can produce an attaching glycocalyx can survive in these $D \geq \mu^{MAX}$ systems.

Summarising the main function of the glycocalyx polymers seems to be protection against wash-out, predators and a hostile environment.

6.4.2.b Production of polysaccharides in pure cultures; influence of substrate, limitation and growth rate

The production of viscous polysaccharides by microbes has been studied extensively (Ref. 6.39, 6.41, 6.48).
It appears that microbes can convert the substrate with high efficiency into the polysaccharide, with yields upto 0.6. It has also been shown that polysaccharides can be produced not only from carbohydrates but also from glycerol, acetate or methanol (Ref. 6.48). Furthermore it has been shown that N, P or S limitation sometimes leads to more polysaccharide production (Ref. 6.48), which is regarded to be due to a version of overflow metabolism. However even under C-limitation high yields of polysaccharides are observed. It is also reported that micro-organisms generally cannot hydrolyse enzymatically their own polysaccharides (Ref. 6.39). From continuous culture experiments it is found that the specific polysaccharide production of the microbes can increase, decrease or remain constant with increasing dilution rate (Ref. 6.48, 6.45, 6.46).
Summarising it appears that polysaccharides can be produced by microbes from a wide spectrum of substrates and under a wide variety of environmental conditions including low or high dilution rates. Therefore the attachment of a micro-organism through polysaccharide secretion would seem to be possible under any dilution rate. The evidence for this conclusion stems only from pure cultures. In this regard it is illustrative that it has been found that the polysaccharides produced by a microbe cannot be detected if this microbe is placed in an undefined mixed culture (Ref.6.44, 6.42, 6.43). Presumably this is because of enzymatic hydrolysis, by the mixed population, of the polysaccharides. From the above it can be expected that microbial attachment of pure cultures can occur under any dilution rate and that microbial attachment of an undefined mixed culture requires $D \geq \mu^{MAX}$ to ensure wash-out of the microbes which can degrade the attachment polysaccharides.
This apparent effect of dilution rate on microbial attachment needs however more substantiation, which will be dealt with in the next section 6.5.

6.5 ROLE OF THE DILUTION RATE IN MICROBIAL ATTACHMENT

In Chapter 6.4 it became obvious that the dilution rate D in the reactor is expected to have some influence upon the microbial attachment to surfaces in the reactor. A survey has therefore been performed among continuous culture studies for men-

tioned attachment of micro-organisms to surfaces and its relation to the dilutionrate. It is stressed that such attachment was mostly considered a nuisance which disturbed the experiments; it was never the main objective of the reported research. A separation should then be made between attachment in pure cultures and in undefined mixed cultures.

6.5.1 EFFECT OF DILUTION RATE ON ATTACHMENT OF A PURE CULTURE

Pure cultures have been described sparsely with regard to reactor-wall attachment phenomena in continuous flow reactors and most work has been performed in batch cultures. However the available literature has been summarised in Table 6.1.
It appears that attachment or clumping of micro-organisms can indeed occur at all D-values for some microbes while for others attachment or clumping requires $D \geq \mu^{MAX}$. In this regard it is important to note that the attachment disappears if the dilution rate is changed to $D \leq \mu^{MAX}$. This result is in fair agreement with the finding that polysaccharide production can occur under any D-value or that it increases with increasing D-value (Chapter 6.4.2.b).

Reference	microbe	substrate	μ^{MAX} hr^{-1}	dilution rate for attachment to reactor wall	dilution rate for cell clumping
6 50/6.51	Hyphomicrobium	methanol/ aerobic	0.15	all D-values	-
6.52	Pseudomonas putida	asparagine/ aerobic	0.61	all D-values	-
6.53	Torula sphaerica	-	-	all D-values	-
6.54	Sphaerotilus natans	glucose/ aerobic	0.5	$D > \mu^{MAX}$	-
6.55	Methylmonas mucor	Methanol aerobic	1.2	-	$D > \mu^{MAX}$
6.56	Streptococcus mutans	glucose/ anaerobic	0.5	$D > \mu^{MAX}$	$D > \mu^{MAX}$
6.57	Streptococcus cremoris	glucose/ anaerobic	0.56	$D > \mu^{MAX}$	
6.57	Streptococcus lactis	glucose/ anaerobic	0.56	$D > \mu^{MAX}$	

Table 6.1
Attachment of pure cultures in Continuous Flow Culture, influence of dilution rate and μ^{MAX}.

6.5.2 EFFECT OF DILUTION RATE ON ATTACHMENT OF AN UNDEFINED MIXED CULTURE

Attachment of undefined mixed cultures has been described occasionally.
According to Ref. 6.58 a denitrifying mixed culture in a fluidized bed reactor grows in suspension at low flowrate (and therefore low D) and attachment becomes dominant when the flowrate is increased (and therefore D in increased). According to Ref. 6.59 an aerobic mixed culture in a fluidized bed reactor grows in suspension when $D \leq 0.12\ hr^{-1}$ and there is attached growth when $D \geq 0.12\ hr^{-1}$. Furthermore the dilution rate in the reported biological fluidized beds with attached growth (Ref. 6.15) is mostly larger than $D = 2\ hr^{-1}$. This is much higher than the μ^{MAX} in these systems.
With respect to the Rotating Disc Contactors (Ref. 6.91), where the biomass grows attached to the rotating discs, it appears that the dilutionrate which is applied to the first stage mostly exceeds $D=1\ hr^{-1}$, which is higher than the μ^{MAX} value in these systems. In addition it has been found that for certain waste-waters in this reactor suspended growth of the biomass occurs when the dilutionrate is shifted from high to low values (Ref. 6.92, 6.93).
Apart from these studies a number of continuous culture experiments in stirred vessels has been performed. The results are listed in Table 6.2. In the first four cases in Table 6.2 only one dilution rate was tested which was however much larger than the μ^{MAX} in the system. In all cases a biolayer at the reactor wall was observed. In the other studies listed in Table 6.2. a whole range of D-values was tested, and it appeared that always when $D \geq \mu^{MAX}$ suspended growth was changed into attached growth or clumping. It is therefore evident that for undefined mixed cultures there is a very strict requirement ($D \geq \mu^{MAX}$) in order to get attached growth.
The most probable reason for this strict condition is that at $D \leq \mu^{MAX}$ in these systems non-attaching suspended micro-organisms can accumulate in the reactor and these suspended microbes can hydrolyse the attachment polymers of attaching microbes. Indeed this type of interference between attached and non-attached microbes has been indicated in several systems. According to Ref. 6.69 Sphaerotilus natans grows in attached form at $D = 0.6\ hr^{-1}$. Addition of a suspended waste water population leads to disappearance of Sph. natans. According to Ref. 6.53 Torula sphaerica grows attached to the wall, and addition of Escherichia coli leads to rapid sloughing from the wall of T. sphaerica. According to Ref. 6.70 a firmly attached yeast (to the stomach wall) was removed after addition of lacto bacilli.
In summary we may conclude that the available evidence for the establishment of attached growth is in agreement with the competition mechanism between attaching and non-attaching microbial populations as proposed in Chapter 5. It can also be concluded that the nature of the surface to which the mixed culture microbes should attach is not important because of the non-specific character of the glycocalyx attachment.

reference	substrate	μ^{MAX} (hr^{-1})	C_{si} (mg/l)	Dilution rate hr^{-1}	Dilution rate for	
					Attachment of microbes to reactor wall	Clumping of microbes
6.60	complex/ aerobic	0.03	20	0.5	+	
6.61	glucose/ aerobic	0.28	27-440	8.0	+	
6.62	glucose/ aerobic	0.18	2-200	6.0	+	
6.63	glucose/ aerobic	0.03	0.02	0.25	+	
6.64	phenol/ aerobic	0.6	100-800	1-6	D>0.6	
6.65	pyrite/ aerobic	0.03	-	0.05-0.20	D>0.03	
6.12	glucose/ aerobic	0.50	1000	0-0.65	D>0.50	
6.66	glucose/ aerobic	0.50	3000	0-0.67	D>0.50	
6.13	glucose/ anaerobic	0.50	-	0-1.1	D>0.50	
6.68	glucose/ anaerobic	0.33	10000	0.23-2.3		D>0.74
6.68	glucose/ anaerobic	0.11	50000	0.05-0.67		D>0.14

Table 6.2
Attachment of undefined mixed cultures in Continuous Flow Culture, influence of dilution rate and μ^{MAX}.

6.6 INFLUENCE OF THE PRESENCE OF NON-ATTACHING MICRO-ORGANISMS ON BIOLAYER FORMATION

The formation of a mixed culture biolayer requires the presence of a sufficiently wide population of micro-organisms from which the microbes with attaching properties can be selected. This implicates that under $D \geq \mu^{MAX}$ condition set 1 in Figure 5.5 should contain a wide variety of microbes. For this purpose one can then apply an inoculum which contains the suitable micro-organisms. This inoculum can be already present in the waste-water itself or it can be added from an external source (e.g. existing waste water plants). The addition of an external inoculum can be batchwise at the start of the experiment or continuously during the start-up period.
However, in any case, there will be a certain amount of non-attaching microbes in the reactor, together with the desired microbes which possess attaching properties. These non-attaching microbes can in principle interfere with biolayer formation because of hydrolysis of attachment polymers. In general it can therefore be expected that an inoculum has two effects on biolayer formation:

- an increasing mixed inoculum concentration leads to a higher concentration of available microbes with attaching properties and therefore the biolayer develops faster

- an increasing mixed inoculum concentration leads to a higher concentration of non-attaching microbes which can hydrolyse the polymers of attaching microbes, and biolayer formation is slower or even inhibited

It is obvious from the above that it can be expected that there exists an optimum inoculum concentration. No inoculum gives no biolayer, a very large inoculum gives also no biolayer because of the hydrolysing action from non-attaching microbes.
The above considerations are supported by the work of Bryers (Ref. 6.71) who studied the effect of suspended biomass concentration on biolayer formation in an aerobic mixed culture reactor were $D \geq \mu^{MAX}$. Figure 6.1 shows the biolayer growth-rate as a function of suspended biomass concentration in the reactor.
At both a low and high suspended biomass concentration the biofilm growth rate tends to be low, where-as an optimum seems to occur between 12-24 mg/l of suspended biomass for the experimental system used by Bryers.

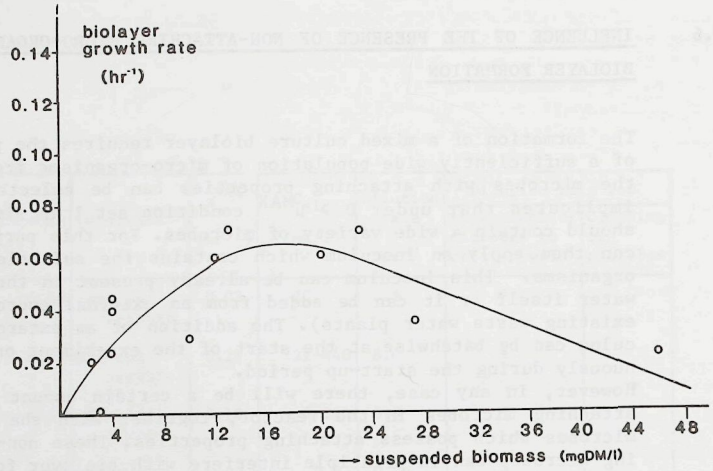


Figure 6.1
Effect of suspended micro-organisms on biolayer formation (adapted from. J.D.Bryers (Ref.6.71)).

6.7 PHYSICAL PARAMETERS OF BIOLAYERS

The most important parameter in biolayers is the biomass concentration which can be reached. According to Ref. 6.4 the biomass concentration in the biolayer is dependent on the turbulence in the liquid phase. A low turbulence gives biolayers with low biomass concentration, and high turbulence leads to high concentrations of biomass in the biolayer. The reason for this effect might be either selection of certain attaching microbes which can tolerate high turbulence or squeezing out of the water from he biolayer due to turbulent forces. Typical values of the biomass concentration in biolayers are around 50-100 kgVSS/m^3. This value holds for aerobic biolayers (Ref. 6.61, 6.62, 6.72, 6.73), for denitrifying biolayers (Ref. 6.74, 6.75) and for methane producing biolayers (Ref. 6.76).
Another important parameter is the thickness of the biofilm. A thick biolayer suffers from diffusion limitation of substrate or O_2. Measurements have shown that diffusion limitation becomes serious with regard to O_2 at thicknesses larger than 100-200 µm (Ref. 6.17, 6.78). Substrate limitation seems to occur at a thickness larger than 100 µm (Ref. 6.61, 6.62, 6.77).
It should be born in mind however that these limits are only orders of magnitude because in each system the limiting diffusion depth is governed by kinetic parameters like maximum substrate conversion rate and effective diffusion coefficients. (See for detailed calculations Ref. 6.58 and 6.79).

6.8 DYNAMICS OF BIOLAYER DEVELOPMENT OF A MIXED CULTURE

6.8.1 GENERAL FEATURES

If a carrier surface is contacted with a liquid which contains nutrients and an undefined mixed microbial culture, the micro-organisms will adsorb at the carrier surface (phase 1, Chapter 6.3.1). Because of excess nutrients the adsorbed micro-organisms will start growing and attaching micro-organisms will produce their glycocalyx. If the dilution rate in the reactor is maintained at $D \geq \mu^{MAX}$ there can be no interference from non-attaching, glycocalyx hydrolising, micro-organisms because these are washed out of the reactor.
However the concentration of suspended micro-organisms which is fed into the reactor (from the inoculation or from for example the waste water feed) should also not be too high, as indicated in Chapter 6.6.
Under these conditions the surface will therefore be covered with a biolayer of growing, glycocalyx producing, microbes. Furthermore it has been observed that in the proces of biolayer formation there occurs encapsulation of non-attaching microbes in the glycocalyx matrix (Ref.6.67, 6.7, 6.23, 6.50, 6.51,6.26) This encapsulation occurs in the plaque of the teeth (Ref. 6,7, 6.80), in aerobic biolayers growing on methane or methanol (Ref. 6.50 and 6.51), in an aerobic biolayer cultivated on the pesticide Dalapon (Ref. 6.26), and in a denitrifying biolayer (Ref. 6.81). Furthermore it has been observed that the encapsulated micro-organisms do form micro-colonies within the biolayer due to growth (Ref. 6.33, 6.6, 6.82, 6.83).
It will be clear that such non-attaching encapsulated micro-organisms can persist in large numbers in the biolayers if they do not hydrolyse the encapsulating glycocalyx matrix. If however the non-attaching encapsulated micro-organisms do hydrolyse the glycocalyx their number in the biolayer will greatly decrease because they will detach from the biolayer and subsequently be washed out of the reactor because $D \geq \mu^{MAX}$.
The residual number of these hydrolysing micro-organisms in the biolayer will be determined by the equilibrium between encapsulation rate and hydrolysis rate.
From the above it is clear that a biolayer generally consists of a population of attaching and encapsulated non-attaching micro-organisms which can grow on the nutrients present in the liquid. Between the biolayer micro-organisms there will exist the usual exchange of primary products or vitamines (Ref. 6.26, 6.84).
Due to continuous substrate supply the biolayer will become thicker. However at a particular biolayer thickness there will be diffusion limitation. This leads to nutrient starvation of some part of the biolayer and eventually this part is dislodged from the surface (Ref. 6.58, 6.60, 6.61, 6.62). The exposed surface is subsequently covered with new biolayers.
The exact reason for the biolayer detachment is not known, but the experience with the aerobic fluidised bed reactor in Chapter 5 indicates that it seems to be possible to stop net biolayer growth at 40 µ thickness (maintenance condition) without detachment of the biolayer.

For practical applications a biolayer should contain all microbes which are needed. For example for an aerobic purification process the biolayer should contain all microbes to degrade the waste-water components. Most generally this means that fast-growing heterotrophes are needed for oxydation of C-compounds and slow-growing nitrifiers for the NH_3 oxydation. In the anaerobic purification process the biolayer should contain the fast growing acidifiers and the slow growing acetogenic and methanogenic populations.
So generally there can exist a large difference in μ^{MAX} for the biolayer mixed culture microbes which are needed. In the following Chapters some possible consequences of equal μ^{MAX} or different μ^{MAX} will be discussed.

6.8.2 BIOLAYER DEVELOPMENT OF A MIXED CULTURE WITH MICROBES HAVING ABOUT EQUAL μ^{MAX}

This is the most simple case, because the biolayer will develop as outlined in the previous Chapter 6.8.1. All the necessary microbes in the biolayer do increase with about equal rate. After some time substrate limitation is reached because of steadily increased attached biomass in the reactor and from there on the conversion efficiency of the substrate remains constant.
Examples of this kind of microbial biolayers are:

- aerobic heterotrophic C-oxydation
- denitrification
- acidification

The μ^{MAX} value of these microbes is about 0.3-0.5 hr^{-1} at 35°C (see Table 6.1 and 6.2).
Therefore it can be expected that a start up at
$D \geq 0.3\text{-}0.5\ hr^{-1}$ for the above mentioned processes results in a rapid biolayer formation and good conversion efficiency after start-up. Indeed it appeared from Chapter 5 that the aerobic biolayer formation was completed under this condition after about 1 week and from Ref. 6.85 - 6.87 it appears that a denitrifying attached biolayer is fully grown after 1-3 weeks.

6.8.3 BIOLAYER DEVELOPMENT OF A MIXED CULTURE WITH MICROBES HAVING HIGHLY DIFFERENT μ^{MAX}

A well known example for such a case is the anaerobic methane fermentation. In general there are 3 populations needed for a methane fermentation. These populations are (Ref. 6.88)

- acidifying population which converts the waste into fatty acids and H_2; the acids are acetic acid, butyric acid and propionic acid. μ^{MAX} of this population is about 0.3-0.5 hr^{-1}. These microbes can also hydrolyse biopolymers.

- acetogenic population, which converts the propionic acid and butyric acid into acetate and H_2. The μ^{MAX} of this population is low, about 0.01 hr^{-1}, and their biomass yield is also low. These microbes cannot hydrolyse biopolymers.

- methanogenic population, which converts acetate or H_2/CO_2 to CH_4. The μ^{MAX} of this population is about 0.05 for H_2/CO_2 as substrate and 0.01 hr^{-1} for acetate as substrate. The biomass yield is very low and biopolymers cannot be hydrolysed.

It is obvious that there exists a fast growing population of acidifiers and a slow growing acetogenic and methanogenic population. This means that in general the carrier surface is very quickly covered with an acidifying biolayer which produces much more fatty acids than can be converted to CH_4 by the few methanogens and acetogens present in this biolayer. It should be noted that the dilution rate D should be chosen in accordance with the highest μ^{MAX} of the various populations in order to enforce attachment of all these populations. The methanogenic and acetogenic micro-organisms will attach themselves (see Chapter 6.2.1), or will be encapsulated in the biolayer of acidifying micro-organisms and subsequently they will grow and increase in number in the biolayer. However due to the large difference in μ^{MAX} between acidifiers and acetogens/methanogens, there will be an accumulation of fatty acids which necessitates a pH control to maintain the pH near to 7.0 which is optimal for methanogenesis. The rapid increase of attached acidifiers which results in increase of biolayer thickness will eventually lead to substrate limitation and the growth rate of this population will steadily decrease. Meanwhile the acetogenic/methanogenic populations can still grow with their μ^{MAX}, due to excess fatty acids. Eventually the biolayer will consist of a balanced population of acidifiers, acetogens and methanogens which can convert all substrate to CH_4. The above description of biolayer formation supposes however that the biolayer remains attached. This will probably only happen if the biolayer is not allowed to become very thick. This means that one needs a low substrate load of the biolayer.
A totally different way to operate in the case of highly different μ^{MAX} values is to separate fast and slow growing populations. This means a first stage where the substrate is acidified by attached acidifying microbes (according to Chapter 6.8.2) and a second stage where the acetogens and methanogens grow attached to the carrier and convert the fatty acids into methane. The biolayer in the second stage only contains acetogens/methanogens because acidifiers cannot grow in this stage due to lack of substrate. It should again be noted that in this second stage the dilution rate probably should be larger than μ^{MAX} of the acidifiers in the first stage, because otherwise the attachment polymers of acetogens/methanogens can be hydrolysed by acidifying micro-organisms which are fed into the second stage from the first stage.

6.9 CONCLUSION

From the available literature it appears that attachment of micro-organisms to surfaces or to each other is the rule rather than the exception. Attaching microbes can be found in a wide spectrum of populations like aerobes, denitrifiers, acidifiers and methanogens.

Mostly the attachment is brought about by the so-called glycocalyx, which consists of a polysaccharide structure. The attachment can lead to biolayers on surfaces or to pellets of biomass. The attaching surface properties seem not important, because the attachment through the glycocalyx is aspecific.

Furthermore it became evident that the condition $D \geq \mu^{MAX}$ to obtain attached undefined mixed cultures seems absolutely necessary. The reason for this condition is that there probably exists a competition between non-attaching and attaching micro-organisms. The non-attaching microbes appear to hydrolyse the attachment polymers of the attaching microbes.

Additionally it appears that the biolayer formation is very rapid under the $D \geq \mu^{MAX}$ condition.

In the case of highly different μ^{MAX} values of the necessary microbial populations in the biolayer a separation of the populations can be profitable.

This means that for the anaerobic methane fermentation both one-stage and two-stage processes should be studied with respect to biolayer formation in each stage.

REFERENCES

6.1 H.F. Ridgway, B.H. Olson
Appl. and Environm. Microbiol. 41, (1981), 274

6.2. P.S. Meadows, J.G. Anderson
Prog. Ind. Microbiology 15, (1979), 207

6.3. W.A. Corpe
In: Adhesion in biological systems
Ed. R.S. Manly, Academic Press (1970), 73

6.4. W.G. Characklis
Biotechnol. and Bioeng. 23, (1981), 1923

6.5. T. Hattori, R. Hattori
CRC Critical Rev. of Microbiology, 4, (1976), 423

6.6. G.G. Geesey, W.T.Richardson, H.G. Yeomans, R.T. Irvin, J.W. Costerton
Can. J. Microbiology 23, (1977), 1733

6.7. R.J. Gibbons, J. van Houte
Ann. Rev. Microbiol. 29, (1975), 19

6.8. H.N. Newman
Microbios 9, (1974), 247

6.9. S.G. Ash
In "Adhesion of micro-organisms to surfaces", (1979), 57
Ed. D.C. Ellwood, J. Melling, P. Rutter.
Academic Press

6.10. H.H. Topiwala, G. Hamer
Biotechnol. and Bioeng. 13, (1971), 919

6.11. H.E. Kubitschek
Introduction to research with continuous cultures, Prentice Hall Inc., (1970), 22

6.12. S.Y. Chiu, L.T. Fan, I.C. Kao, L.E. Erickson
Biotechnol. and Bioeng. 14, (1972), 179

6.13 H.E. Patterson
Appl. Microbiology 29, (1975), 437

6.14. A.W. Lawrence, P.L. McCarty
J.W.P.C.F., (1969), R1

6.15. P.F. Cooper, D.H.V. Wheeldon
Annual Conf. Instn. Water Poll. Control, Torquay, September 13, (1979)

6.16. Ju-Chang Huang, V.T. Bates
Proc. 34th Ind. Waste Conf. (1979), 483

6.17. T.G. Tomlinson, D.H.M. Snaddon
Air Water Poll. Int. J. 10, (1966), 865

6.18 R.J. Gibbons
In: Microbial adhesion to surfaces
Ed.R.C. Berkeley, J.M. Lynch, J. Melling, P.R. Rutter, B. Vincent, Ellis Horwood Limited,(1980), 351

6.19. R.P. McCowan, K.J. Cheng, C.B.M. Bailey, J.W. Costerton
Appl. Environm. Microbiol. 35, (1978), 149

6.20. A. Martini, F. Federici, G. Rossini
Can. J. Microbiol. 26, (1980), 856

6.21. J.W. Costerton, R.T. Irvin, K.J. Cheng
CRC Crit. Rev. in Microbiol. 8, (1981), 303

6.22. K.J. Cheng, R.T. Irvin, J.W. Costerton
Can. J. Microbiol. 27, (1981), 461

6.23. R.H. Harris, R. Mitchell
Ann. Rev. Microbiol. 27, (1973), 27

6.24 G. Lettinga, A.F.M. van Velzen, S.W. Hobma, W. de Zeeuw, A. Klapwijk
Biotechnol. and Bioeng. 22, (1980), 699

6.25. R.J. Munson, B.A. Bridges
J. Gen. Microbiol. 37, (1964), 411

6.26. A.T. Bull, C.M. Brown
In: Int. Rev. of Biochemistry, Microbial Biochemistry, 21
Ed. J.R. Quayle, (1979), 178
University Park Press, Baltimore

6.27. K.C. Marshall
In: Microbial adhesion to surfaces
Ed.R.C. Berkeley, J.M. Lynch, J. Melling, P.R. Rutter, B. Vincent.
Ellis Horwood Limited, (1980), 187

6.28. H.J. Rogers
In "Adhesion of micro-organisms to surfaces", (1979), 29
Ed. D.C. Ellwood, J. Melling, P. Rutter
Academic Press

6.29 M. Fletcher
Can. J. Microbiol. 23, (1977), 1

6.30 D.G. Zvyagintsev, V.S. Guzev, I.W. Guzeva
Microbiology 46, (1977), 245

6.31 M. Fletcher
In: Microbial Adhesion to Surfaces
Ed.R.C. Berkeley, J.M. Lynch, J. Melling, P.R. Rutter, B. Vincent, Ellis Horwood Limited,(1980), 197.

6.32 D.C. Ellwood, A. Robinson
In: Continuous Culture of Cells, Volume II,
Editor P.H. Calcott, CRC Press Inc. (1980), 39

6.33 J.W. Costerton, G.G. Geesey, K.J. Cheng
Scientific American 238, (1978), 86

6.34 R. Hattori
J. Gen. Appl. Microbiol. 18, (1972), 319

6.35 D. Levine, D. Wang, W. Thilly
Biotechnol. and Bioeng. 21, (1979), 821

6.36 W.A. Corpe
Dev. Ind.Microbiol. 11, (1970), 402
American Institute of Biological Sciences

6.37 I.W. Sutherland
In: Microbial Adhesion to Surfaces
Ed.R.C. Berkeley, J.M. Lynch, J. Melling, P.R. Rutter, B. Vincent, Ellis Horwood Limited,(1980), 329

6.38 K.C. Marshall, R. Stout, R. Mitchell,
J. Gen. Microbiol. 68, (1971), 337

6.39 J.F. Wilkinson
Bacteriological Rev. 22, (1958), 46

6.40 D.A. Powell
In: Microbial polysaccharides and polysaccharases
Ed: R.C.W. Berkeley, G.W. Gooday, D.C. Ellwood,
Academic Press, (1979), 117

6.41 I.W. Sutherland,
Adv. Microbiol. Physiology, 8, (1972), 143

6.42 C. Napoli, F. Dazzo, D. Hubbell,
Appl. Microbiology, 30, (1975), 123

6.43 M.H. Deinema, L.P.T.M. Zevenhuizen,
Arch. Microbiology, 78, (1971), 42

6.44 Y. Tago, K. Aida,
Appl. and Environm. Microbiol., 34, (1977), 308

6.45 F.A.Mian, T.A. Jarman, R.C. Righelato,
J. of Bact., 134, (1978), 418

6.46 A.G. Williams, J.W.T. Wimpenney,
J. Gen. Microbiol., 104, (1978), 47

6.47 R.J. Gibbons, J. van Houte
Infection and immunity, 3, (1971), 567

6.48 J.W. Sutherland, D.C.Ellwood,
In "Microbial Technology, Current state, future prospects", (1979), 107
Ed. A.T. Bull, D.C. Ellwood, C. Ratledge
Cambridge University Press

6.49 B.A. Friedman, D.R.Dugan, R.M.Pfister, C.C. Remsen,
J. of Bacteriology, 98, (1969), 1328

6.50 T.G. Wilkinson, G. Hamer,
Biotechnol. and Bioeng., 16, (1974), 251

6.51 T.G. Wilkinson, G. Hamer,
J. Chem. Technol. and Biotechnol., 29, (1979), 56

6.52 G. Molin
Eur. J. Appl. Microbiol. and Biotechnol., 13, (1981), 102

6.53 H.R. Bungay III, M.L. Bungay,
Adv. Appl. Microbiol., 10, (1968), 269

6.54 N. Yoshikawa, Yo Takiguchi,
Appl. and Environm. Microbiol., 38, (1979), 200

6.55 K.T. Tam, R.K. Finn,
In: "Extracellular microbial polysaccharides",
Ed. P.A. Sandford, A. Laskin,
Washington, ACS, (1979), 58

6.56 D.C.Ellwood, J.R. Hunter, V.M.Longyear,
Arch. Oral Biology, 19, (1974), 659

6.57 P.L. Rogers, L. Bramall, I.J. McDonald,
Can J. Microbiol., 24, (1978), 372

6.58 B. Atkinson, J.J. Davies,
Transactions Instn. Chem. Engrs, 50, (1972), 208

6.59 P.M. Sutton, D.F. Langley, K.E. Warner, Ki Park
34th Ind. Waste Conf., (1979), 450

6.60 W.M. Sanders,
Air Water Poll. Int. J., 10, (1966), 253

6.61 B.H. Kornegay, J.F.Andrews,
22nd Ind. Waste Conf., (1967), 620

6.62 E.J. Lamotta,
Appl. and Environm. Microbiol., 31, (1976), 286

6.63 R.D. Hamilton, K.M. Morgan, J.D.H. Strickland,
Can J. Microbiol., 12, (1966), 995

6.64 U. Pawlovsky, J.A. Howell,
Biotechnol. and Bioeng., 15, (1973), 897

6.65 K.B. Mehta, N.W. Leroux,
Biotechnol. and Bioeng., 16, (1974), 559

6.66 M. Ramanathan, A.F. Gaudy,
Biotechnol. and Bioeng., 11, (1969), 207

6.67 K. Rietema, S.P.P. Ottengraf, H.P.E. v.d. Venne
Biotechnol. and Bioeng., 13, (1971), 911

6.68 R.J. Zoetemeyer,
Ph D thesis, University of Amsterdam, The Netherlands, 1982.

6.69 F.F. Dias, N.C. Dondero, M.S. Finstein,
Appl. Microbiology, 16, (1968), 1191

6.70 D.C. Savage,
J. of Bacteriology, 98, (1969), 1278

6.71 J.D. Bryers,
Ph. D. Thesis, Rice University, Houston, U.S.A. (1980)

6.72 D.D. Lee, C.D. Scott, C.W. Hancher,
J.W.P.C.F., 51, (1979), 974

6.73 O.A. Sehic,
In "Biological fluidized bed treatment of water and wastewater", (1981), 179
Ed. P.F. Cooper, B. Atkinson
Ellis Horwood limited

6.74 C.D. Scott, C.W. Hancher,
Biotechnol. and Bioeng., 18, (1976), 1393

6.75 J.E. Hawkins, D.A. Stott, R.L. Stokes, A. Clennet,
In "Biological fluidized bed treatment of water and wastewater", (1981), 357
Ed. P.F. Cooper, B. Atkinson
Ellis Horwood limited

6.76 L. v.d. Berg, K.J. Kennedy,
Biotechnol. Letters, 3, (1981), 165

6.77 L.T. Mulcahy, W.K. Shieh, E.J. La Motta,
Biotechnol. and Bioeng., 23, (1981), 2403

6.78 K. Fujce, T Tsukamoto, H. Kubota,
J. Ferment. Techn., 57, (1979), 539

6.79 P. Harremoës,
In "Water Pollution Microbiology", 2,(1978), 71
Ed. R. Mitchell
John Wiley and Sons

6.80 R.M. McCabe, J.A. Donkersloot,
Inf. and Immunity, 18, (1977), 726

6.81 G. Yull Rhee, G.W. Fuhs,
J.W.P.C.F., (1978), 2111

6.82 K.J. Cheng, J.W. Costerton,
Can. J. Microbiology, 26, (1980), 104

6.83 K.J. Cheng, J.P. Foy, R.N. Coleman, C.P. Milligen, J.W. Costerton,
Appl. and environm. Microbiology, 41, (1981), 298

6.84 S.Z. Sudo,
Appl. and environm. Microbiology, 33, (1977), 450

6.85 J.E. Hawkins, P.F. Cooper, M.R. Seaman,
In "New Processes of waste-water treatment and recovery",
Ed. G. Mattock, Ellis Harwood Ltd., Sussex, (1978), 107

6.86 R.B. Gauntlett,
In "Biological fluidized bed treatment of water and waste-water", (1981), 48
Ed. P.F. Cooper, B. Atkinson
Ellis Horwood limited

6.87 P.F. Cooper, D.H.V. Wheeldon,
In "Biological fluidized bed treatment of water and waste-water", (1981), 121
Ed. P.F. Cooper, B. Atkinson
Ellis Horwood limited

6.88 A.J.B. Zehnder,
In "Water Pollution microbiology", 2, (1978), 349
Ed. R. Mitchell, John Wiley and Sons

6.89 D.A. Powell,
In "Microbial polysaccharides and polysaccharases", (1979), 117
Ed. R.C.W. Berkeley, C.W. Gooday, D.C. Ellwood
Academic Press

6.90 A.W. Obayashi, A.F. Gaudy
Proc. 27th Ind. Waste-water Conference, (1972), 324

6.91 First national symposium/workshop on rotating biological contactor technology
Champion, Pennsylvania, (1980)
Ed. D. Smith, R.D. Miller, Y.C. Wu

6.92 D.F. Kincannon, S. Groves
In "First national symposium/workshop on rotating biological contactor technology"
Champion, Pennsylvania, (1980), 433
Ed. D. Smith, R.D. Miller, Y.C. Wu

6.93 J.W. Hudson, J.P. Smith, F.G. Pohland
In "Proceedings 31th Ind. Waste Conf.", (1976), 193

7 DEVELOPMENT OF THE ANAEROBIC BIOLOGICAL FLUIDIZED BED REACTOR

7.1 INTRODUCTION

In Chapter 5 it was found that the direct treatment of the concentrated GB-waste-water in the aerobic fluidized bed reactor is impossible. This was due to limitations in oxygen transfer capacity at the high waste-water dilution rate which is necessary for stable biomass attachment.
However from the literature survey about microbial attachment in Chapter 6 it was concluded that methanogenic and acidifying microbes possess attachment capacities. Also, in parallel research, it was found that the GB waste-water can be purified anaerobically at about 65-70% soluble COD efficiency in an upflow reactor (Ref. 7.1) at a COD-load of about 8 kg/m^3day, and an hydraulic residence time of 16 hr (unpublished results). From this result is was concluded that the anaerobic fluidized bed reactor, utilising methanogenic biomass attached to sand, could be a highly efficient reactor for the purification of the GB waste-water.
Such an anaerobic reactor would have the following general advantages:

- there is no oxygen transfer limitation and therefore the dilution rate can be chosen freely in accordance with requirements for attachment of biomass (Chapter 5 and 6)
- the biomass yield in anaerobic processes is inherently low and therefore the surplus sludge problem is minimised
- there is no oxygen consumption, but methane production. Therefore this process is energy producing, contrary to the highly energy intensive aerobic processes

Compared to the anaerobic fluidized bed reactor, which utilises biolayers attached to carriers for the treatment of waste-water, the conventional anaerobic reactors which utilise flocculent biomass do have the following disadvantages:

- the retention of the anaerobic biomass in the conventional reactor is a major problem. In the anaerobic contact process an external sludge settler and sludge recirculation is used. In the upflow reactor an internal sludge settler is used. Due to the poor settling capacity of the sludge the liquid velocities in the settler should be below 1m/hr (Ref. 7.1) in these reactors and the sludge concentration rarely exceeds 10g DM/ltr. In the anaerobic filter the sludge seems to be trapped between the filter packing, but a serious drawback is the plugflow character of this reactor (Ref. 7.2), which makes this process unsuitable for waste-waters with a highly fluctuating composition.
  However in the fluidized bed reactors the methanogenic biomass grows attached to a heavy carrier. This leads to a fast settling, highly concentrated particle suspension in the reactor. This means that a high sludge concentration in the reactor can be combined with high upward velocity of the liquid in the reactor.
- In the above mentioned conventional reactors the sludge is retained in the reactor by gravity settling or filter entrapment.

  This means that suspended solids which are present in the waste-water do accumulate in the reactor. This gives then a mixed liquor suspended solid with a very low fraction active biomass (Ref. 7.1). In the fluidized bed reactor such waste-water solids can be expected to be washed out due to the high liquid velocity and therefore a higher sludge activity does result.
- Attachment of the methanogenic biomass to the heavy carrier provides a controlled method to produce an extremely good settling sludge. Well settling methanogenic sludge particles without carrier have also been formed during anaerobic treatment of sugar beet waste-water in an upflowreactor (Ref. 7.1), but the conditions for their formation are not well understood.

In the development of such an anaerobic fluidized bed reactor several aspects seem to be important as argued in Chapter 6.8 where the dynamics of biolayer formation were discussed.
These aspects are:

- which dilution rate is necessary to obtain biolayer formation
- what is the effect of suspended micro-organism concentration from the inoculum on attachment of microbes
- does the biolayer thickness increase indefinitely or can there be a steady state at constant thickness
- does microbial pre-acidification of the waste-water have a beneficial effect on biolayer development of the methanogenic biomass

In the next Chapters these aspects for attached biolayer formation for waste-water and acidified waste-water will be studied.
Subsequently the results of a well purifying fluidized bed reactor utilising these attached biolayers will be described.

7.2 CONDITIONS FOR THE ATTACHMENT OF METHANOGENIC BIOMASS TO A CARRIER USING NON-ACIDIFIED GB WASTE-WATER

7.2.1 MATERIALS AND METHODS

7.2.1.a **Description of the equipment**

The flow sheet of the experimental equipment is shown in Figure 7.1. Raw waste-water was pumped directly from the sewer (1) to a presettling tank (2) where part of the suspended solids was removed (area 2.4 m^2 and hydraulic surface load 0.8-1 m/hr). This partly settled waste-water was fed into the fluidized bed reactor (3). This reactor was the same as described in Chapter 5.3.2.a. However the entrance into the reactor of settled sludge from the settler has been moved from 550 mm above the reactor bottom to 3750 mm above the reactor bottom. This change was thought to be necessary because the gas-lift power in the anaerobic reactor is much less than in the aerobic reactor, due

to much lower gas velocities. Alkali (solution 10% $KHCO_3$) was added to the waste-water feed for pH control, if necessary.
The reactor temperature was controlled by flowing hot water along the outside of the reactor. The effluent from the reactor (3) flowed into a settler (4)(to trap washed-out carriers) of 0.16 m^2 area.
The biogas was measured and subsequently combusted in a flare.
During part of the experiments an inoculum was fed into the liquid recycle continuously. The inoculum was taken from the effluent of a conventional anaerobic upflow reactor treating the same waste-water as fed to the fluidized bed reactor (3). Tapwater was sometimes used to dilute the waste-water. This tapwater was fed also into the liquid recycle. The flow rate of this liquid recycle was controlled in such a way that the fluidized bed level was maintained in the quickfit section (between 440 and 520 cm above reactor bottom).
Towards the end of the experiments the gas-lift settler was replaced by a much simpler device, as shown in Figure 7.2, because of a continuously plugged sludge recycle. An inverted cone was used to separate the bubbles from the liquid, and the carriers settle around the cone and fall back into the reactor. The liquid hold-up of the reactor changed then from 500 ltr to 280 ltr. It should be stressed that this simple device contained no liquid weir, but that the liquid leaves the reactor through a 3" hole in the reactor wall.
For sampling of the reactor content, the column contains 3 sampling points called sp1, sp2, sp3, at 50, 150 and 400 cm above the reactor bottom.

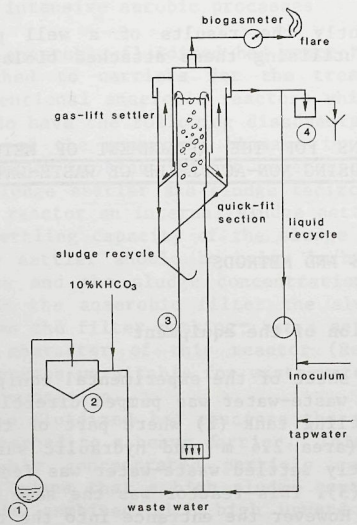


Figure 7.1
Flow sheet of the fluidized bed reactor fed with non-acidified GB waste-water

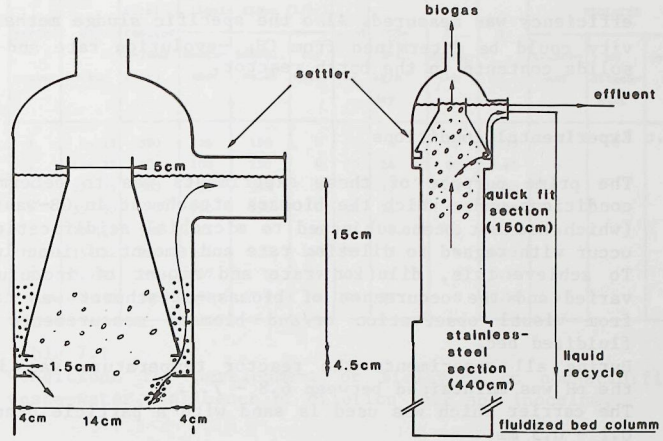


Figure 7.2
Modified separator for the fluidized bed reactor fed with non-acidified GB waste-water

7.2.1.b Measurements and analyses

The influent and effluent of the fluidized bed reactor was sampled semi-continuously as described in Chapter 4.3.2.
The influent was analyzed for COD after centrifuging.
The effluent was analyzed after centrifuging for COD and fatty acids content.
The fatty acid analysis was the titrimetric method after Hobma e.a. (Ref. 7.3).
The COD analysis was according to standard methods (Ref. 7.8).
The gasproduction was measured with a gasmeter (Ermaf B.V., G4),and the gascomposition was determined for CO_2 and CH_4 on a gaschromatografic column (Porapak Q (80-100 mesh), H_2-gas,0°C).
The pH was measured continuously in the effluent of the reactor and was checked each 8 hrs for drift.
The reactor temperature was measured continuously and was also checked each 8 hrs.
The various flow rates (waste-water, tap-water, inoculum, recycle-water) were checked each 8 hrs . The composition of the fluidized bed was measured incidentally during the experiments in samples from sp3. This sample was analysed for volatile attached solids and ash content according to the procedures outlined in Chapter 5.2.2.b .
The attachment of biomass to the carrier was examined frequently in a sample from sp3 under a microscope and could also be detected in the quick-fit glass section of the reactor.Finally the carriers with attached biolayers were tested for their methane producing capacity. For this purpose a fluidized bed sample was washed thorougly with tap-water and added to a 1 ltr batchreactor (37°C, pH 7-7.5) . This batchreactor was then fed with 0.7 ltr settled waste-water. After CH_4 formation from the waste-water had ceased, in a second run acetic acid was added as a substrate. During the experiments CH_4 production was measured in a Mariotte flask and COD and acetic acid removal

efficiency was measured. Also the specific sludge methane activity could be determined from CH_4 -evolution rate and organic solids contents in the batch reactor.

7.2.1.c Experimental conditions

The prime purpose of these experiments was to determine the conditions under which the biomass attachment in GB-waste-water (which had not been subjected to microbial acidification) does occur with regard to dilution rate and amount of inoculum.
To achieve this, dilution rate and amount of inoculumn were varied and the occurrence of biomass-attachment was concluded from visual observation or/and biomass measurements in the fluidized bed.
During all experiments the reactor temperature was 37°C and the pH was maintained between 6.8 - 7.2.
The carrier which was used is sand with a particle diameter of 0.1 - 0.3 mm.
The soluble COD-concentration of the waste-water remained fairly constant at about 5 g COD/ltr during the whole experimental period.
The inoculum variation was achieved by varying inoculum flow rates. This inoculum contained about 1 g VSS/ltr with a methane producing capacity of 0.5 g CH_4-COD/g VSS day.

7.2.2. RESULTS

7.2.2.a The conditions for biolayer formation

The experiments are divided in 6 periods where different conditions were applied as shown in Table 7.1.

PERIOD 1
The experiment was started in period 1 with a large inoculum flow and a small waste-waterflow (period 1, Table 7.1). This resulted in a dilutionrate of 0.35 hr^{-1} and a reactor load of 6 kg COD/m^3 day. Under these conditions a very low CH_4-production could be observed (Figure 7.3A), but attachment of biomass to the sand was not observed. Most probably the CH_4 production was due to the inoculum biomass.

PERIOD 2
Therefore in period 2 the waste-waterflow was increased (Table 7.1) which resulted in D = 0.6 hr^{-1} and a quick (within 2 days) appearance of attached biomass in the glass section of the reactor. The attachment led to a rapid expansion of the fluidized bed and therefore the superficial velocity had to be decreased (Figure 7.3B). Also a rapid increase in gas production could be observed (Figure 7.3A).

period number	day number	total reactor volume (ltr)	liquid flows (l/hr) waste-water	inocul. water	tap water	sand kg	COD-load kg/m^3 day	% inoculum	D hr^{-1}	BIOLAYER attachment	no attachment	destruction
1	0- 12	500	25	150	0	90	6	86	0.35		+	
2	12- 22	500	100	150	0	90	24	60	0.60	+		
3	22- 35	500	50	150	0	90	12	75	0.40			+
4	35- 60	500	150	150	0	90	36	50	0.60	+		
5	70-115	500	25	0	100	35	6.0	0	0.25	+		
6.1	115-132	280	25	0	100	135	12	0	0.45	+		
6.2	132-141	280	50	0	50	135	24	0	0.36	+		
6.3	141-160	280	100	0	0	135	36	0	0.36	+		

Table 7.1
Conditions for development of biolayers on non-acidified GB waste-water, influence of dilution rate and inoculum.

PERIOD 3
The subsequent decrease of waste-waterflow in period 3 resulted in a decreased dilution rate (Table 7.1).
Under these conditions the biolayers were destroyed rapidly with a concomittant decrease in methane production (Figure 7.3A).

PERIOD 4
An increase of the waste-waterflow in period 4 led again to rapid visible biolayer formation under these conditions of high dilution rate (Table 7.1).
The biomass content in the fluidized bed (Table 7.2) was 19 gr VSS/ltr.

day	biomass concentration g VSS/ltr	sand concentration g /ltr	ash content %	biolayer thickness µm [1]	period number
56	19	66	78	103	4
70	12.5	20	61	160	5
85	7	7	50	200	5
91	5.8	4.2	42	233	5
125	10	92	90	57	6.1
155	28	42	60	164	6.3

[1]) calculated according to Appendix 7A with d_p=200µm.

Table 7.2
Fluidized bed composition in sp3 cultivated with non-acidified GB waste-water

From the ash content (=sand) and the organic solids content a biolayer thickness can be calculated assuming an organic solids content in the biolayer of 100 kg VSS/m^3. This calculation is shown in Appendix 7A and it follows then that the average biolayer thickness is 100 µm.

The CH_4 production increased (Figure 7.3A) and due to fluidized bed expansion the superficial liquid velocity had to be decreased considerably in period 4 (Figure 7.3B).
From the results in period 1-4 it can be concluded that, in the presence of about 0.5 - 1.0 g VSS/ltr of suspended micro-organisms from the inoculum, biolayer formation only occurs at a dilution rate $D \geq 0.6 hr^{-1}$ and that attached biolayers are readily destroyed again at dilution rates lower than $D = 0.6 hr^{-1}$.

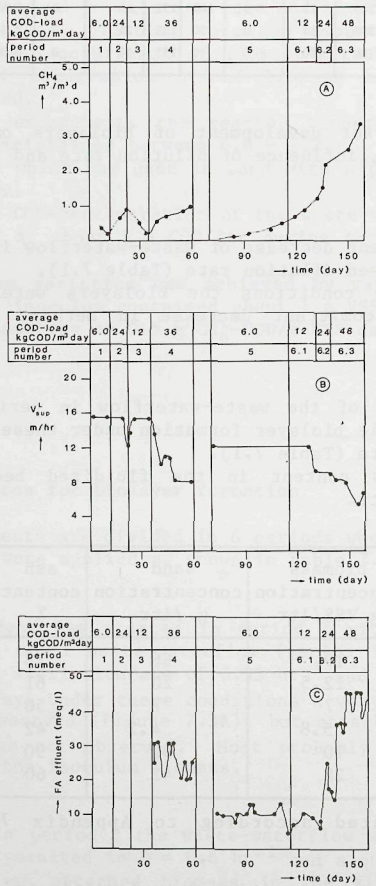
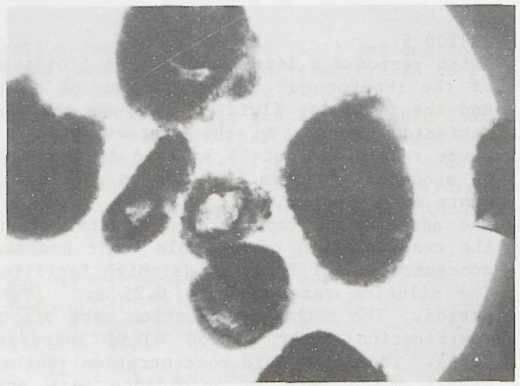


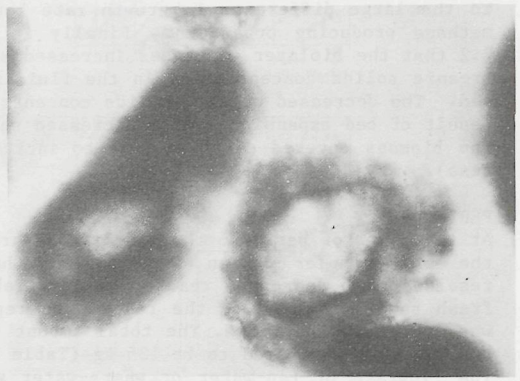
Figure 7.3
Results of a methanogenic fluidized bed reactor fed with non-acidified GB waste-water during period 1-6
(A) Methane production
(B) Applied superficial liquid velocity in the fluidized bed reactor
(C) Fatty acid concentration (titrim.) in the effluent of the fluidized bed reactor

PERIOD 5
After period 4 a large part of the fluidized bed was washed out of the reactor due to malfunction of the inoculum flow pump, and the remaining fluidized bed was toxified by a peak of bacteriocidal agents in the waste-water. It was estimated that there remained about 35 kg sand in the reactor. It was decided to stop the inoculum flow and to add tapwater to the inflowing waste water in period 5.
The addition of tapwater had several advantages like lowering the concentration of possible toxic compounds and lowering the concentration of fatty acids which facilitates pH-control.
The dilution rate was $D = 0.25 hr^{-1}$ (Table 7.1) during this period. The methane production rate started at zero (due the toxification after period 4)and increased steadily (Figure 7.3A). The fatty acid concentration reached from the beginning levels which were in accordance with complete acidification (see Figure 7.3C). This indicated that the development of acidifying biolayers proceeds much faster than methane producing biolayers. As already argued in Chapter 6.8.3 this is due to the large difference in growth rate between acidifying and methane producing organisms. Finally it appeared from Table 7.2 that the biolayer thickness increased steadily and that the organic solids concentration in the fluidized bed also decreased. The decreased organic solids concentration was probably a result of bed expansion due to decreased settling velocities of the biomass covered carriers (due to increased biolayer thickness).

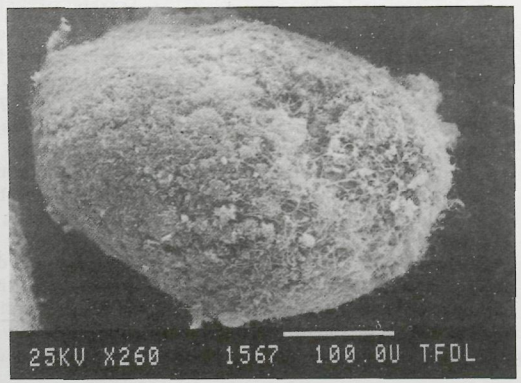
PERIOD 6
At the start of period 6 the gas-lift separator was replaced by the much simpler design described in Chapter 7.2.1.a. This resulted in a decreased reactor volume (Table 7.1). Also 100kg fresh sand was added to the reactor to replace the sand which was lost after period 4. The total amount of sand in the reactor was then estimated to be 135 kg (Table 7.1).
The flowrate of tap-water or waste-water was not changed initially and due to the decreased reactorvolume, the dilution-rate and the reactor COD-load both increased (Table 7.1) compared to period 5.
From visual observation it appeared that the newly added sand was quickly covered with biolayers which led to increased fluidized bed volume and subsequent reduction in liquid superficial velocity to maintain a constant volume (Figure 7.3B). Also the CH_4-production increased rapidly (Figure 7.3A). The subsequent decrease of tap-water and increase of waste-water (Table 7.1) led then to more CH_4-production (Figure 7.3A), a decreased superficial velocity to maintain a constant fluidized bed volume of 240 ltr (Figure 7.3B), an increased biomass concentration in the fluidized bed and an increasing biolayer thickness (Table 7.2).
A sample of the fluidized bed from sp3 at day 145 was thoroughly washed and the particles were then photographed under a light microscope and a Scanning Electon Microscope (SEM). The results are shown in Pictures 7.1-7.4a.



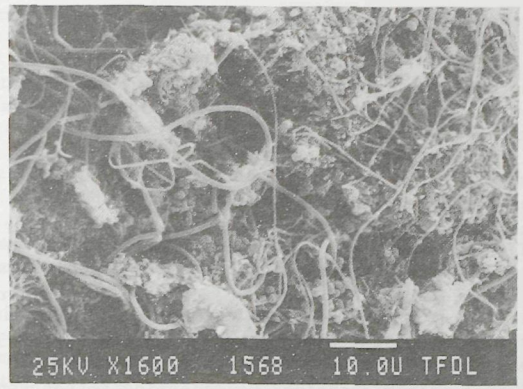
Picture 7.1
Light microscopic view of sand grains covered with methanogenic biolayers
(non-acidified GB waste-water, magnification 60 x)



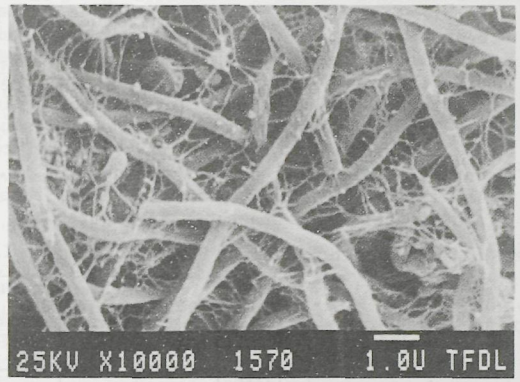
Picture 7.2
Light microscopic view of sand grain covered with methanogenic biolayers
(non-acidified GB waste-water, magnification 150 x)



Picture 7.3
SEM picture of sand grain covered with methanogenic biolayer
(non-acidified GB-waste-water), bar is 100 micrometer



Picture 7.4
SEM picture of the surface of a methanogenic biolayer
(non-acidified GB waste-water), bar is 10 micrometer



Picture 7.4a
SEM picture of the surface of a methanogenic biolayer showing presence of fibrillar material between microbes
(non-acidified GB waste-water), bar is 1 micrometer

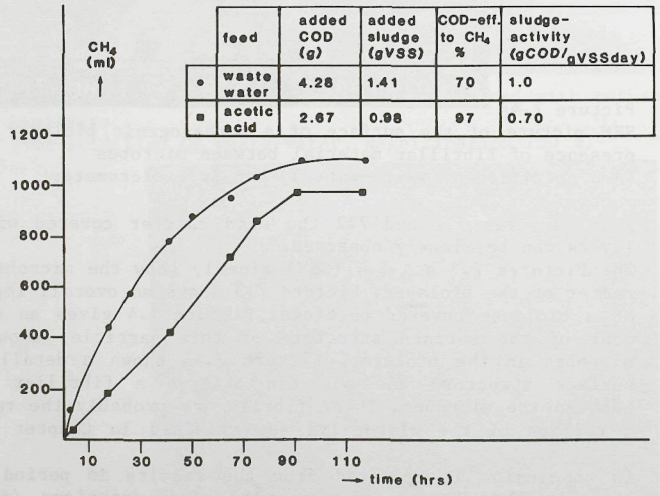
From Pictures 7.1 and 7.2 the sand carrier covered with biolayers can be clearly observed.
The Pictures 7.3 and 7.4 (SEM) clearly show the microbial character of the biolayer. Picture 7.3 shows an overall impression of a biolayer covered particle. Picture 7.4 gives an enlargement of the surface structure of this particle, showing the microbes in the biolayer. Picture 7.4a shows a detail of the surface structure and one can observe a fibrillar network between the microbes. These fibrils are probably the result of a collaps of the glycocalyx as explained in Chapter 6.3.2 .

In conclusion it appeared from the results in period 1 to 6 that in the absence of suspended micro-organisms (from the inoculum) the biolayers did develop at $D > 0.25\ hr^{-1}$, whereas under our experimental conditions in the presence of these suspended microorganisms $D > 0.6\ hr^{-1}$ was required to obtain attachment (Table 7.1). It also appeared that the biolayers

tended to increase in thickness without reaching an equilibrium thickness (Table 7.2).
Finally it appeared from Table 7.2 that an organic solids concentration of about 25 g VSS/ltr was possible in a fluidized bed reactor where the liquid superficial velocity was maintained above 6 m/hr (Figure 7.3B).

7.2.2b Characterisation of the biolayer

In the preceding Chapter 7.2.2a it was shown that biolayers were rapidly formed with non-acidified GB waste-water.
However it appeared that, despite a considerable CH_4-production in period 6 as shown in Figure 7.3A, a large amount of fatty acids was not metabolised to CH_4 (Figure 7.3C).
A possible reason for this phenomenon could be that the biolayers mainly contained H_2 consuming methanogens and no methanogenic bacteria that convert acetic acid to CH_4. To test this possibility a sample was taken from sp3 at day 145 in the fluidized bed and was washed thoroughly with tap-water (same sample as shown in Pictures 7.1-7.4a). Due to lack of experimental facilities this sludge was stored in a refrigerator (7°C) for 5 weeks and subsequently tested in a batchreactor as described in Chapter 7.2.1b using waste-water and subsequently acetic acid as substrate. The storage period of 5 weeks probably has not significantly affected the sludge activity because it is known that methanogenic sludge retains its activity during storage over several months (Ref. 7.1).
The resulting methane production, COD removal efficiencies and sludge activities are shown in Figure 7.4.



	feed	added COD (g)	added sludge (gVSS)	COD-eff. to CH_4 %	sludge-activity ($gCOD/_{gVSSday}$)
•	waste water	4.28	1.41	70	1.0
■	acetic acid	2.67	0.98	97	0.70

Figure 7.4
Measurement of methanogenic activity of biolayers cultivated on non-acidified GB waste-water using waste-water and acetic acid as subsequent substrates.

It is evident that the biolayers are capable of GB waste-water purification with 70% COD-efficiency with a relatively high methane activity of 1.0 g COD/g VSS day. This purification efficiency of the waste-water is in good agreement with purification results in the conventional anaerobic upflow reactor (see Chapter 7.1). It also appears from Figure 7.4 that the methane activity of the sludge on acetic acid is fairly high, 0.7 g COD/gVSSday. This indicates that the methanogenic biolayer contained much methanogens which convert acetic acid into CH_4, because growth of these bacteria in the batch experiment can be neglected due to their very low growth rate (see Chapter 6.8.3). It must therefore be concluded that the accumulation of fatty acids as shown in Figure 7.3C was due to an insufficient amount of biolayers in the fluidized bed reactor and that the biolayers as such contained all the necessary micro-organisms to achieve maximum purification efficiency. The biomass concentration in the biolayer was determined in a sample of particles (day 148). After washing with tap water 1 ltr of particles contained 205 gr sand and 48 g VSS.
Assuming a void volume between the particles of 400 ml, the biolayer volume could be calculated at $1000 - 400 - \frac{205}{2.6} = 521$ ml (assuming a sand density of 2.6 g/ml).
These 521 ml biolayer did contain 48 g VSS and thus the biolayer contained 92 g VSS/ltr.
This value agreed very well with the values found by other workers (see Chapter 6.7).
In Appendix 7C it is shown that diffusion limitation of acetate transport into the biolayers has not occurred.

7.2.2.c Technological aspects of the anaerobic fluidized bed reactor

During the experiments, described in this Chapter 7.2, the following observations were made with regard to the operation of the fluidized bed reactor

- homogeneous fluidization

  It could be observed multiple times that upon reduction of the liquid superficial velocity in the fluidized bed below 6 m/hr cold regions developed in the lower part of the reactor. The reason was most probably insufficient fluidization and local formation of packed bed structures.

- functioning of the separator

  As already mentioned the gas-lift separator did not perform satisfactorily due to the clogged sludge return.
  Therefore the simple separator from Figure 7.2 was implemented on day 115.
  This design functioned well with regard to sludge return.
  However after day 140 a loss of fluidized bed material could be observed which amounted to about 100 ml particles per day.
  The reason was probably that the separator did not contain an overflow weir so that the liquid left the reactor with a high velocity through the circular opening in the reactor wall

(Figure 7.2). The liquid velocity at this outflow opening could be estimated at 26 m/hr at a flow rate of 100 l/hr.
It is therefore advisable to construct a separator which contains a weir to reduce the velocity of the liquid which leaves the reactor.

- temperature control

The supply of warm water along the outside of the reactor for temperature control was frequently badly distributed due to local scale formation.
This gave probably rise to radial temperature gradients in the fluidized bed. It is therefore advisable to control the reactor temperature by supplying heat to the influent waste-water with a heat exchanger.

- toxification of the reactor

During the reported experiments it happened once that the fluidized bed reactor was toxified by a peak of bacteriocidal compounds in the waste-water. It should be realised that the fluidized bed reactor, with its short liquid residence time, is more susceptible to these effects than the conventional large volume anaerobic reactors with long hydraulic residence times. Due to these long liquid residence times the pulse of bacteriocidal compounds cannot give rise to high concentrations in the reactor and therefore damage to the micro-organisms is limited.

- pH control

The continuous accumulation of fatty acids during the experiments neccessitated a continuous addition of alkali to the influent of the reactor. In order to maintain the reactor pH between 6.8-7.2 it appeared necessary to increase the pH of the influent to pH ≈ 8. This high pH resulted regularly in problems due to formation of deposits (presumably carbonates) and subsequent clogging of tubing and piping.

7.2.3. DISCUSSION

From the experiments with non-acidified GB-waste-water it appears that in the presence of suspended micro-organisms (inoculum) $D \geq 0.6\ hr^{-1}$ is required for the attachment of anaerobic biomass.
In the absence of inoculum $D \geq 0.25\ hr^{-1}$ is already sufficient to obtain attachment. It therefore seems that there is indeed interference of suspended micro-organisms with attachment. (see also Chapter 6.6).
The development of the biolayers proceeds rapidly. The fast attainment of complete acidification points to an early predominance of acidifying micro-organisms in the biolayer.
The subsequent steady increase in CH_4 production points to a gradual increase of the CH_4-producing populations in the biolayer. From a batch test of a matured attached biolayer it appeared that this biolayer was capable of efficient purification of the waste-water. These results agree well with the postulated process of anaerobic biolayer development in Chapter 6.8.3.

An unfavorable aspect is however that the biolayers seem to increase rapidly in thickness without reaching (within the duration of the experiments) an equilibrium thickness. These thick biolayers have several unwanted effects like decreased settling velocity and increased chance of sloughing off of the biolayers.
As already indicated in Chapter 6.8.3. an interesting alternative might be the development of methane producing biolayers using completely acidified waste-water. In that case the acidifying micro-organisms, which do have a relative high biomass yield, are excluded from the biolayer in the methane reactor. This could result in a lower rate of increase of biolayer thickness. Other advantages could be that the acidification reactor acts as a buffer with regard to adverse chemicals in the waste-water and that the pH-control of the methane reactor is easier (because the pH in the acidified influent does not need to be higher than 6.8 to obtain a reactor pH of ≥ 6.8) .
As shown in Appendix 7B it can further be expected that the methanogenic activity of the biolayers cultivated on acidified waste-water is higher than the activity of biolayers cultivated on non-acidified waste-water.

7.2.4 CONCLUSION

Biolayers in a fluidized bed reactor for the methane production on non-acidified GB waste-water are readily developed at $D \geq 0.25\ hr^{-1}$ in the absence of high amounts of suspended micro-organisms. In the presence of suspended micro-organisms $D \geq 0.6\ hr^{-1}$ is required.
These biolayers have a considerable methane producing activity of q_{COD} = 1 g COD/g VSS day on waste-water and of 0.7 g COD/g VSS day for acetic acid.
The exploration of the methanogenic biolayer formation using completely acidified GB-waste-water is recommended.

7.3 DEVELOPMENT OF BIOLAYERS OF METHANOGENIC BIOMASS TO A CARRIER USING COMPLETELY ACIDIFIED GB WASTE-WATER.

In the previous Chapter 7.2 it was found that methanogenic sand-attached biolayers could be developed on non-acidified waste-water if the appropriate dilutionrate was maintained in the reactor. A problem was however that the biolayer thickness did increase and that the pH-control during biolayer maturation caused clogging problems. Regarding these aspects, as indicated in Chapter 7.2.3, the biolayer formation using completely acidified waste-water could be advantageous. The experiments to substantiate this are dealt with in this Chapter 7.3.

7.3.1. MATERIALS AND METHODS

7.3.1a Description of the equipment

The same equipment as described in Chapter 7.2.1a, was used after some modifications which have been discussed in Chapter 7.2.2c.
These modifications were:

- The heating of the reactor was performed with a heat exchanger in the influent waste-water. Because of the high recirculationflow in the reactor a homogeneous temperature is assured.
- The separator on top of the column was replaced by a new separator, wich is described in Figure 7.5. This separator differred from the one in Figure 7.2 in that there was a weir to ensure a uniform low velocity of the liquid wich left the fluidized bed reactor.

The external diameter of this separator was 50 cm, the diameter of the weir was 45 cm and the area of the settler space was 0.14 m^2. The settled particles fall back into the fluidized bed reactor through an annular area with an opening of 4 cm.

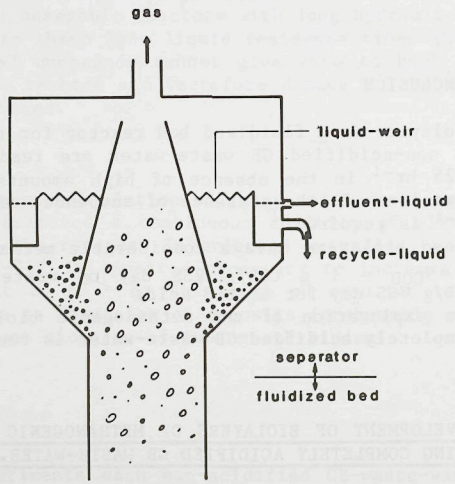


Figure 7.5
Separator of the fluidized bed reactor

The flow sheet of the modified installation is shown in Figure 7.6 .
The acidified waste-water was pumped into the heat-exchanger (6).The flow of this waste-water was controlled with a magnetic flow-meter (0-250 ltr/hr). The steamflow in the heatexchanger was controlled with a steamvalve.
To the heated waste-water the $NaHCO_3$ solution (10 % w/w) was added from reservoir (5) wich had a volume of 25 ltr . The flow of alkali was controlled by a pH-electrode in the effluent of

the fluidized bed reactor. Subsequently the waste-water was pumped into the recycle flow. This recycle flow enabled to choose the liquid superficial velocity in the fluidized bed reactor independent from the waste-water feed. This recirculation was controlled with a magnetic flow meter (0-2000 ltr/hr) and a pump (7) . The recycle flow was fed into the fluidized bed reactor through a ring distributor (8 holes, 8 mm diameter downwards flow) . The fluidized bed reactor (1) was 5.9 m high in the cylindrical section.This cylindrical section contained a stainless steel part (440 cm high, 25 cm diameter) and a quick-fit glass section on top of it (150 cm high and 22.5 cm diameter). The empty volume of the fluidized bed reactor was then 270 ltr.
The column had 3 sampling points sp1, sp2, sp3 at 50, 150 and 400 cm above the reactor bottom. The separator (2) (Figure 7.5)on top of the reactor had a liquid volume of 40 ltr. The biogas flowed through a gasmeter (3) to a flare (8). The waste-water left the separator at two points. Through the upper opening the treated water left the reactor and through the lower opening the liquid flowed to the recycle pump. The water which left the reactor flowed to a vessel (4) of 100 ltr and an area of 0.16 m^2 which functioned as a particle trap.

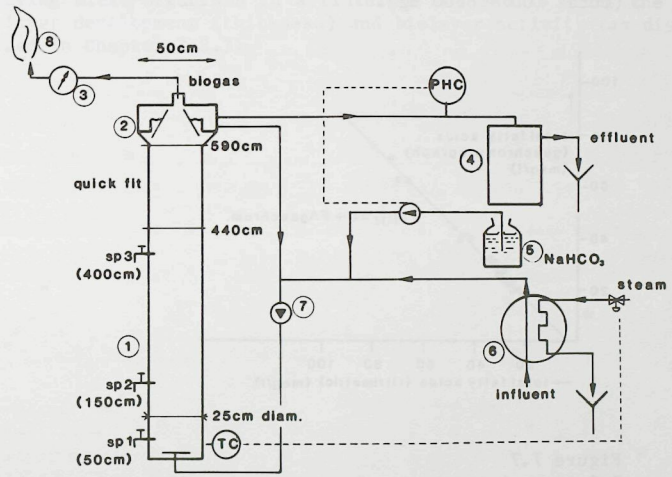


Figure 7.6
Flow sheet of methanogenic fluidized bed reactor fed with acidified GB waste-water

7.3.1b Measurements and analyses

From the influent and effluent waste-water 24 hr averaged samples were obtained. With a timer and a pump each 15 minutes 80 ml of waste-water was fed into a container in a refrigerator (7°C). From the liquid in each container a well mixed sample was prepared each day. The samples were centrifuged and the supernatant was analysed for fatty acids and bicarbonate alkalinity according to the titrimetrical method (Ref.7.3). Once a

week the fatty acid composition was analysed on a gaschromatographic column.(118°C;column with 15% sp1220/1% H_3PO_4 on chromosorb WAW 100-200 as stationary phase and N_2 as carrier phase).
Figure 7.7 shows the relation between the total fatty acids from titrimetry and from the summation of fatty acids from gaschromatography. It can be seen that there is good agreement and that the waste-water obviously contained about 4 meq/ltr of acidic groups which were not fatty acids.
The produced biogas was measured with a gasmeter (ERMAF BV, G4) and the CH_4/CO_2 composition was measured frequently on a gaschromatographic column (Porapak Q, 80-100 mesh, H_2-gas, 0°C)
Incidentally the fluidized bed was sampled and this sample was analysed for solids (drying at 105°C) and ash content (drying at 600°C).
The sand attached biolayers were also incidentally tested for their methanogenic activity in batch reactors of 3 ltr volume, 37°C.
The sample was added to 2.5 ltr solution wich contained neutralised fatty acids and also nutrients and a solution of trace elements. In the activity experiments described the feed always consisted of 2000 mg of acetic, propionic and butyric acid each (total COD = 8800 mg).

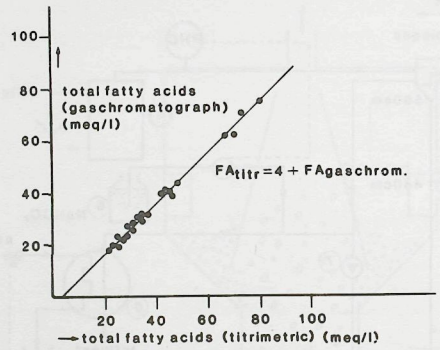


Figure 7.7
Relation between gaschromatographically and titrimetrically determined total fatty acids in acidified GB waste-water

7.3.1.c Experimental conditions

At the end of the experiment with non-acidified GB waste-water described in Chapter 7.2, the reactor was modified with regard to separator construction and temperature control. The attached methanogenic biomass which was present in the reactor at the end of the aforementioned experiment was used as batch inoculum for the fluidized bed experiment with acidified GB waste-water to be described in this Chapter 7.3.
The acidification of the GB waste-water was performed in a

fluidized bed reactor of 3600 ltr using sand (0.1-0.3 mm) as a carrier. The experimental conditions were the same as in the 300 ltr reactor experiments in period 6.3 described in Chapter 7.2.2.a (37°C, pH = 6.8 - 7.2, D = 0.33 hr^{-1}).
During the whole experiment a flow of acidified waste-water of 100 ltr/hr was maintained. Because the total reactor volume was 310 ltr this gave a dilution rate D = 0.32 hr^{-1}. Considering the results with non-acidified waste-water described in Chapter 7.2 this dilution rate was sufficiently high to allow for biolayerformation. The amount of sand (0.1 - 0.3 mm) present in the reactor was 135 kg. The reactor temperature was 37°C and the pH was maintained at $\geq$ 6.8 . The recycle flow rate was maintained at 400 ltr/hr until day 50 and 500 ltr/hr afterwards. The liquid superficial velocity was then 10 m/hr before day 50 and 12 m/hr after day 50. The hydraulic surface load in the settler was then 3.6 and 4.3 m/hr.
During the experiment each 8 hrs the liquid flows were controlled, the pH and the temperature of the reactor were measured to correct for drift of the continuous registration and the total gasproduction was measured.
The purpose of this experiment was to verify whether methanogenic/acetogenic bacteria could produce biolayers without acidifying micro-organisms in a fluidized bed and to study the biolayer development (thickness) and biolayer activity (as discussed in Chapter 7.2.3)

7.3.2 RESULTS

7.3.2.a Development of methanogenic biolayers in the fluidized bed reactor

Soon after the start of the experiment the fluidized bed volume diminished sharply. Therefore the superficial velocity of 6 m/hr which gave at the end of the previous experiment a fluidized bed volume of 240 ltr (Figure 7.3B) had to be increased after three weeks to 22 m/hr to obtain the same fluidized bed volume of 240 ltr again.

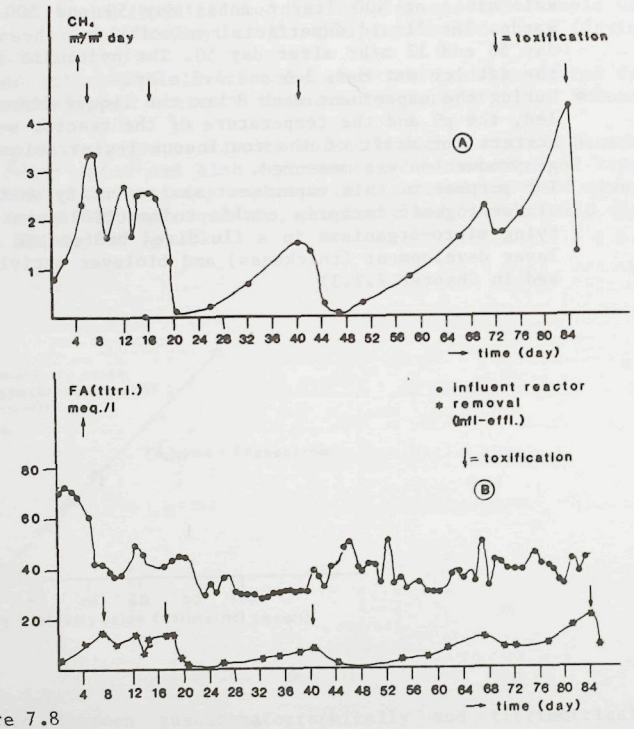
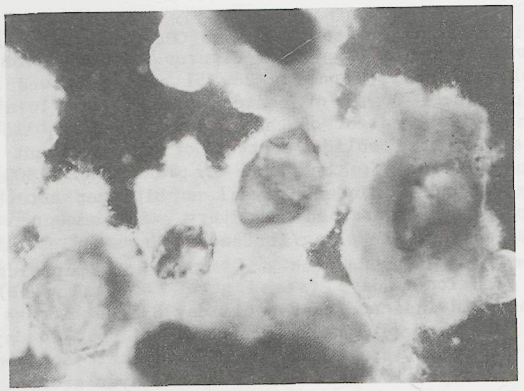


Figure 7.8
Results of the methanogenic fluidized bed reactor fed with acidified GB waste-water
(A) Methane production
(B) Total fatty acid concentration in the influent and fatty acid removal

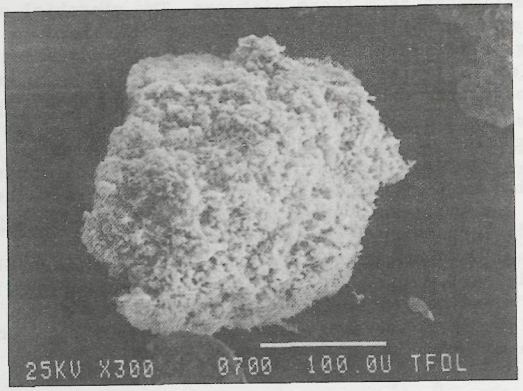
This decrease in volume was most probably due to the elimination of the acidifying biomass from the fluidized bed. With the liquid superficial velocity of 10-12 m/hr which was applied during this experiment the biolayer covered sand particles did not appear anymore in the quickfit section or in sp3 of the fluidized bed reactor before day 83. This slow growth of the biolayers was due to multiple toxifications of the micro-organisms during this experiment.

These toxifications led to near zero CH_4-production and fatty acid removal as can be seen from Figures 7.8A and 7.8B. Samples of sp1, sp2 and sp3 on day 38, 51 and 65 showed that sp1 and sp2 contained 700 ml of sediment per liter fluidized bed volume. The solids content of the fluidized bed was 1150 g/ltr. This means that the solids content of the sediment is 1742 g/ltr which is close to the value of plain sand (1500 g/ltr). Visual inspection did indeed reveal no biolayer attachment in these samples. sp3 contained only very small amounts of sand and organic solids, about 1 g/ltr each. Nevertheless there was a considerable CH_4-production and fatty acid purification on the afore mentioned days (see Figure 7.8A and 7.8B). These observations did indicate that until day 83 the biomass covered sand must have been present between sp2 and sp3. On day 83 the particles did appear in sp3 and in the quickfit section due to fluidized bed expansion as a result of biolayer growth.
Pictures of the particles on day 83 are shown in Pictures 7.5 - 7.9. The biolayer around the sand can be seen clearly on Picture 7.5 and 7.6. An average biolayer thickness of about 70μ could be estimated from these pictures. The microbial nature of the biolayer can be seen clearly in the SEM-pictures 7.7-7.9. Especially interesting is the occurrence of a filamentous segmented microbe (Picture 7.9) which resembles accurately the acetate converting methanogen Methanobacterium soehngenii (Ref. 7.9). From the increase in CH_4-production shown in Figure 7.8A a doubling time of the attached methanogenic biomass could be estimated. Assuming that detachment of biolayers could be neglected the doubling time is about 8 days. This value agreed well with the value of 9 days quoted for Methanob. soehngenii (Ref. 7.9). In summary it could be concluded that sand attached biolayers of methanogens were obtained on acidified GB waste-water applying $D = 0.32\ hr^{-1}$. The development of the biolayer thickness could not be studied because, except for the last days, the biolayer covered sand was inaccessible for visual inspection or sampling.

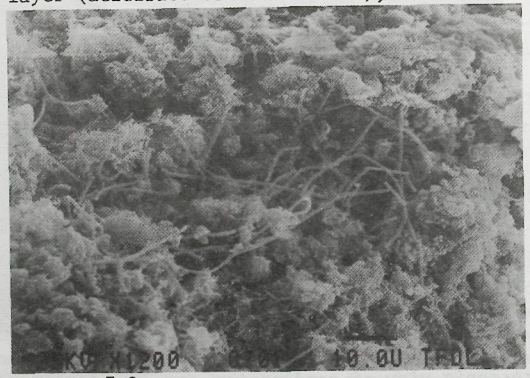
Picture 7.5
Light microscopic view of sand grains covered with methanogenic biolayers (acidified GB waste-water, magnification 120x)



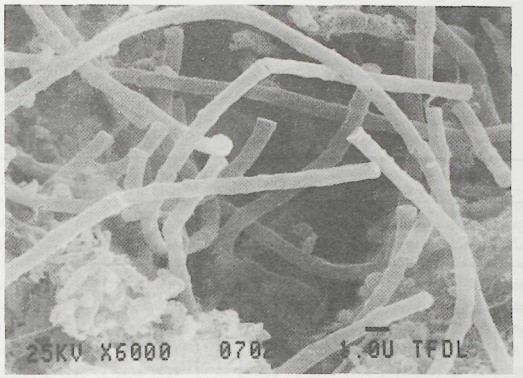
Picture 7.6
Light microscopic view of sand grains covered with methanogenic biolayers (acidified GB waste-water, magnification 300 x)



Picture 7.7
SEM picture of a sand grain covered with a methanogenic biolayer (acidified GB waste-water), bar is 100 micrometer



Picture 7.8
SEM picture of the surface of a methanogenic biolayer (acidified GB waste-water), bar is 10 micrometer



Picture 7.9
SEM picture of microbes in a methanogenic biolayer (acidified GB waste-water), bar is 1 micrometer

7.3.2.b Characterisation of the biolayer

In the previous section 7.3.2.a it has been shown that methanogenic biolayers could be developed on sand with acidified GB waste water, applying $D = 0.32\ hr^{-1}$. From the increase in CH_4-production rate a doubling time of the methanogenic biolayers of 8 days could be estimated. At the end of the experiment a biolayer thickness of 70 µm was achieved (Picture 7.5 and 7.6). In the fluidized bed reactor these biolayers produced fair amounts of methane (Figure 7.8A). As already shown in Figure 7.8B there was also a considerable purification in measured total fatty acids. A fatty acid purification of 50% was achieved at day 84 (see Figure 7.8B), where 20 meq/ltr of acids were removed from an influent concentration of 40 meq/ltr.
The fatty acid metabolism by the biolayers in the fluidized bed reactor was studied more specifically by measuring the fatty acid composition of influent and effluent. The results are shown in Table 7.3. From this Table 7.3. it appeared that the majority of the acids in the acidified GB waste-water are acetic acid, propionic acid and butyric acid. From these compounds only acetic acid and butyric acid were metabolised by the biolayers, propionic acid is not degraded. It is however a known phenomenon in methane fermentation that propionic acid is only degraded when the acetic acid concentration has reached low levels (Ref. 7.10, 7.11), because acetic acid seems to inhibit propionic acid metabolism.

day	C_2		C_3		iC_4		C_4		iC_5		C_5		FA-meq./ltr		FA-COD		FA-COD
	in mg/l	out mg/l	in mg/l	out mg/l	in mg/l	out mg/l	in mg/l	out mg/l	in mg/l	out mg/l	in mg/l	out mg/l	in meq/l	out meq/l	in mg/l	out mg/l	eff. %
1	3300	2860	400	410	85	90	660	580	30	34	56	50	70.3	62.2	5649	5055	11
4	3620	2940	390	410	87	91	660	580	33	34	66	55	75	63	6005	5152	14
7	1970	960	69	110	72	82	450	300	18	25	24	15	40	22	3237	1967	39
8	1610	940	8	10	120	120	340	190	29	31	12	11	33	20	2646	1665	37
13	2190	1600	27	23	100	110	350	240	34	36	19	18	42.5	31.6	3300	2500	24
18	2040	1550	11	12	61	67	300	210	18	17	14	12	38.6	29.4	2912	2232	23
23	1220		45		20		260		<1		3		24.1		1880		
30	1400	1200	6	8	37	36	230	180	21	19	4	6	26.9	23	2037	1734	15
37	1400	1000	10	10	72	71	260	180	19	25	<10	<10	27.7	20.1	2160	1598	26
44	2200	2200	26	38	80	8	190	170	34	46	13	17	40.7	40.3	2971	2986	0
51	1500	1420	19	42	120	100	120	83	26	30	12	13	28.5	26.9	2141	1998	7
58	1470	1280	21	26	49	49	130	100	24	23	12	11	27.3	23.9	1998	1744	13
65	2200	1200	190	190	70	68	260	150	18	19	19	16	43.6	25.8	3325	2035	39
72	1610	1260	120	130	50	62	200	160	14	18	9	10	31.5	25.6	2402	2007	16
80	1140	660	160	190	70	74	560	280	22	22	28	25	28.8	18.0	2700	1728	36
82	1660	870	20	29	65	72	470	170	16	21	12	10	34.3	17.9	2827	1473	48
83	2050	1010	5	3	50	52	330	90	21	21	25	13	41	18.8	2977	1409	53
84	1926	930	130	130	48	50	900	450	25	25	41	35	44.9	23.5	4094	2216	46

Table 7.3
Fatty acid purification in a methanogenic fluidized bed cultivated on acidified GB waste-water, C_2 is acetic acid, C_3 is propionic acid, C_4 is butyric acid, C_5 is valeric acid, iC_4 and iC_5 are iso-acids.

Finally the biolayer methanogenic activity was measured in a 2.5 ltr batch reactor with a fatty acid mixture as substrate and with fluidized bed samples from day 83 and day 75 which had been stored in a refrigerator for 8 weeks. The results are shown in Figure 7.9. It appeared that the biolayer could convert quantitatively the added acetic and butyric acid to methane and that the propionic acid was not degraded. This result was in good agreement with the purification performance of the biolayers in the fluidized bed reactor as already shown in Table 7.3. It was however also quite interesting that the methanogenic activity of the biolayers is 1 - 2 g COD/g VSS day (Figure 7.9). This was considerably higher than the value of 0.7 g COD/g VSS day found for acetic acid metabolism of biolayers cultivated on non-acidified GB waste-water (see Figure 7.4).

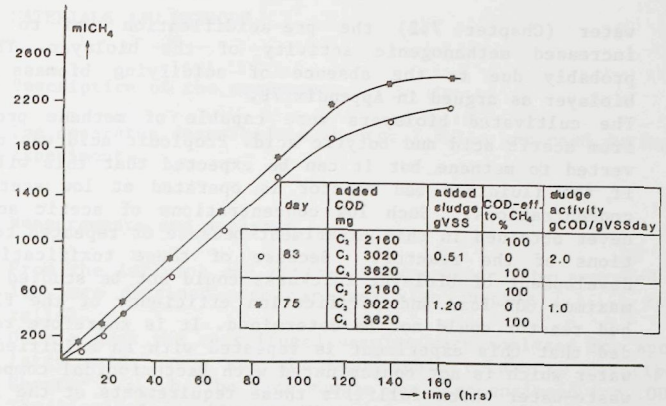


Figure 7.9
Measurement of the methanogenic activity of biolayers cultivated on acidified GB waste-water

It therefore seemed that the elimination of acidifying micro-organisms from the biolayer did indeed lead to a much higher methanogenic activity of this biolayer as calculated in Appendix 7B.

7.3.2.c Technological aspects of the anaerobic fluidized bed reactor

The modified separator of liquid/gas/solids which was used during these experiments did function very well. The sand trap (4) in Figure 7.6 did never contain any sand. Also the pH-control of the reactor did not pose any specific problems. There was however one major problem which arose during these experiments. As already mentioned it appeared from Figure 7.8A and 7.8B hat the methanogenic fluidized bed reactor was toxificated several times. This toxification could be traced back to the occasional presence of high peak concentrations of bacteriocidal compounds in the waste-water. In this regard it should be emphasized that the short liquid residence time of the fluidized bed reactor does make this reactor vulnerable to short time peaks of such compounds. It thus appeared that the presence of the acidification reactor before the methane reactor was not a sufficient way to counteract such toxification phenomena.
Therefore it must be concluded that the GB waste water must first be freed from these bacteriocidal compounds before a methanogenic fluidized bed reactor with short liquid residence times can be applied.

7.3.3 DISCUSSION

From the presented experiments it appeared that methanogenic biolayers could be cultivated on sand using acidified GB waste-water at $D = 0.32\ hr^{-1}$. Compared to the non-acidified waste-

water (Chapter 7.2) the pre-acidification led to a much increased methanogenic activity of the biolayer. This was probably due to the absence of acidifying biomass in the biolayer as argued in Appendix 7B.
The cultivated biolayers were capable of methane production from acetic acid and butyric acid. Propionic acid was not converted to methane but it can be expected that this will occur if the fluidized bed reactor is operated at low acetic acid concentrations. Such low concentrations of acetic acid were never obtained in this experiment because of repeated toxifications of the reactor. Because of these toxifications the development of biolayer thickness could not be studied and the maximum COD-load and purification efficiency of the fluidized bed reactor could not be determined. It is therefore recommended that this experiment is repeated with an acidified waste-water which is not contaminated with bacteriocidal compounds. A waste-water which fullfills these requirements at the location Delft is the waste-water from the bakersyeast production.

7.3.4 CONCLUSION

Methanogenic biolayers on sand in a fluidized bed reactor can be cultivated using acidified GB waste-water at D = 0.32 hr^{-1}. The use of acidified waste-water leads to a higher biolayer methanogenic activity and improved pH-control compared to non-acidified waste-water. Microbial acidification of the waste-water seems therefore to be beneficial. Multiple toxifications occurred during this experiment using the total GB waste-water and it is therefore recommended to repeat the experiment using acidified yeast waste-water.

7.4 DEVELOPMENT OF A METHANOGENIC FLUIDIZED BED REACTOR USING ACIDIFIED YEAST WASTE-WATER

In the previous sections 7.2 and 7.3 it was shown that under appropriate conditions attached methanogenic biolayers on sand could be obtained using non-acidified as well as completely acidified GB waste-water. It also appeared that in the case of acidified GB waste-water the obtained biolayers did have a much higher methane activity and that pH control did not suffer from clogging phenomena. However in both cases the experiments were severely hampered by toxifications from bacteriocidal compounds in the GB waste-water.
Therefore a purifying system with maximum efficiency could not be obtained in either case. To avoid the toxification phenomena it was decided to use waste-water free from bacteriocidal compounds. Such a waste-water stream is available at the Delft location from the bakersyeast production facility. This section will thus describe the anaerobic purification of pre-acidified yeast waste-water in a fluidized bed reactor.

7.4.1 MATERIALS AND METHODS

7.4.1.a Description of the equipment

The apparatus described in Chapter 7.3.1.a was used during this experiment.

7.4.1.b Measurements and analyses

From the influent and effluent waste-water 24hr averaged samples were obtained each day (see also chapter 7.3.1.b for details).
Once a week the centrifuged samples were analysed for specific fatty acids on a gas chromatographic column as described in Chapter 7.3.1.b. The same sample was also analysed for COD.
The biogasproduction was measured continuously with a gasmeter and the biogas composition was analysed once or twice a week (see Chapter 7.3.1.b). The pH was measured continuously in the effluent of the reactor and checked each 8 hrs for drift. The reactor temperature was measured continuously and also checked each 8 hrs. Finally the various flow rates (waste-water, recycle water) were recorded each 8 hrs. Incidentally the fluidized bed was sampled in sp1 and sp3 (sp2 was clogged) and the sample was analysed for solids (drying at 105 °C) and ash content (drying at 600 °C) The fluidized bed sample from sp3 was also regularly tested for its methanogenic activity as described in Chapter 7.3.1.b.

7.4.1.c Experimental conditions

At the start of the experiment the fluidized bed reactor contained the sand (135 kg) and the toxified biomass which were present at the end of the experiment with acidified GB-waste-water as described in Chapter 7.3 (see Figure 7.8A).
It was decided to use this residual methanogenic biomass as the inoculum and therefore no extra inoculation was applied. Also the same sand (0.1-0.3 mm) was used. The yeast waste-water was pumped directly from the yeast centrifuges to the pilot plant were dilution with tapwater did result in a waste-water of 4000-4500 mg COD/ltr. This dilution was applied to obtain a waste-water which did resemble the COD of the GB waste-water (see Chapter 7.2.1.c).
This waste-water was then acidified in a fluidized bed reactor of 3600ltr using acidifying biomass attached to sand (0.1-0.3 mm) as a carrier. The experimental conditions were 37°C and D = 0.55 hr^{-1} . The results of this acidifying fluidized bed reactor have been published separately (Ref. 7.13).
It is sufficient to note that the resulting waste-water was completely acidified. Also the SO_4^{2-} in the waste-water was completely reduced which resulted in about 160 mg sulfide/ltr.
This acidified, sulfide containing waste-water was then fed into the fluidized bed reactor of 310 ltr. The experiment was started with an influent flow rate of 100 ltr/hr, which gave the same dilution rate, D = 0.32 hr^{-1}, as used with acidified GB waste-water (Chapter 7.3).

The applied liquid residence time τ during the experiment is shown in Figure 7.10. Until day 71, τ was decreased steadily to maintain substrate surplus. This allowed the methanogenic microbes to grow at maximum growth rate.
After day 71 the liquid residence time was increased to achieve a good purification efficiency.

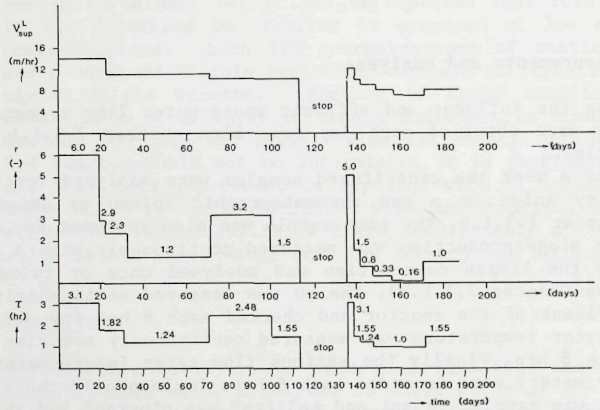


Figure 7.10
Operational parameters during methanogenic fluidized bed purification of acidified yeast waste-water: Superficial liquid velocity (V^L_{sup}), recycle ratio (r), and hydraulic residence time (τ).

The recirculation flow was varied in order to maintain the fluidized bed height at a level well below the separator. The values of the recirculation ratio r $\left\{ r = \frac{\Phi_{rec}}{\Phi_{in}} \right\}$ which were applied are shown in Figure 7.10 . During the whole experiment the reactor temperature was 37°C and the pH varied between 6.8-7.2.

7.4.2 RESULTS

7.4.2.a Development of methanogenic biolayers in the fluidized bed reactor

After 20 days of operation of the fluidized bed reactor, particles appeared in sp3. These particles amounted to 40 ml/ltr and consisted of sand coated with a biolayer. On day 27 the amount of these particles had increased to 100 ml/ltr in sp3 and on day 44 the fluidized bed became visible in the quickfit section of the reactor. This meant that the expansion of the fluidized bed due to biolayer development had reached a height of 440 cm (or a volume of 220 ltr). The fluidized bed height during the

further experiment is shown in Figure 7.11A. It can be seen that the fluidized bed height between day 44 and 111 could be maintained between 450 and 500 cm by decreasing the superficial liquid velocity in the fluidized bed (see Figure 7.11B).
However after day 140 a further decrease in V^L_{sup} was not sufficient to maintain the fluidized bed level below 500 cm and the bed expanded to 600 cm height.

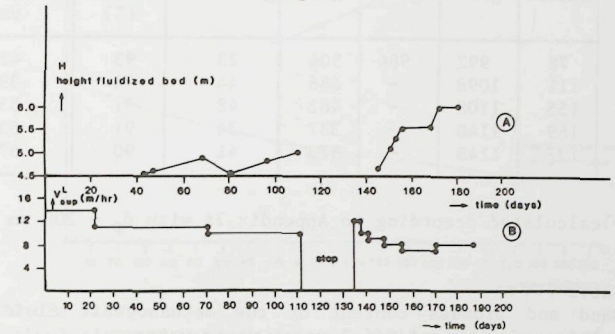


Figure 7.11
Expansion in a methanogenic fluidized bed reactor fed with acidified yeast waste-water
(A) fluidized bed height
(B) superficial liquid velocity applied

The above mentioned expansion indicated a steady build up of attached biolayers in the reactor. The fluidized bed was analysed regularly for biomass and sand content in sp1, sp2 and sp3. The results are shown in Table 7.4. Sp2 was analysed only once because of clogging. Sp1 showed that the sand content was about constant at 1100 g/ltr. Microscopic examination revealed no attachment. All samples from sp1 did contain a sediment volume of 750 ml/ltr and assuming that this was plain sand (with a settled concentration of 1500 g/ltr) this led to a calculated sand concentration of 1125 g/ltr which was in good agreement with the measurements in Table 7.4.
sp3 showed that the biological fluidized bed contained about 350gr sand/ltr (except the analysis on day 111). Furthermore it appeared that the biolayer thickness stabilised at about 50-55 µm. This thickness was in good agreement with the microscopical observations of the particles and was also close to the biolayerthickness estimated for the particles cultivated on acidified GB waste-water (Chapter 7.3.2.b). The amount of biomass which could be obtained in the fluidized bed was about 40-45 g VSS/ltr (Table 7.4). The stable thickness of the biolayer is a very favorable feature of this process because it eliminates the problems associated with very thick biolayers like flotation of the particles, agglomeration of the particles and problems of diffusion limitation.

day number	sp1	sp2	sp3			calculated biolayer[1] thickness µm
	sand g/ltr	sand g/ltr	sand g/ltr	biomass g VSS/ltr	ash content (%)	
78	992	984	304	23	93	42
111	1098	~	686	44	94	39
155	1108	~	482	48	91	53
169	1140	~	337	34	91	53
197	1148	~	370	41	90	57

[1])calculated according to Appendix 7A with d_p = 200 µm

Table 7.4
Sand and biomass content of the methanogenic fluidized bed cultivated with acidified yeast waste-water

The biomass concentration in the biolayer could be calculated from the observation that the settled particles in the sample from sp3 on day 169 occupied a volume of 750 ml/ltr. According to Table 7.4 this settled layer must then contain 450g sand/ltr and 45 g VSS/ltr.Assuming a void volume of 400 ml/ltr between the particles and applying a sand density of 2.6 g/cm^3 the biolayer volume could be calculated as 1000-400-450/2.6 = 427 ml/ltr. Therefore the biomass content of the biolayer was 45/0.427 = 105 g VSS/ltr.
The analysis of the fluidized bed listed in Table 7.4 did indicate that in the fluidized bed reactor there were two regions. Towards the bottom there was a region of plain sand without biolayers and on top of this there was the sand covered with biolayers. Because of clogging of sp2 it was impossible to detect the extension of the top region to sp2.
A crude estimate could be made assuming a constant sand concentration of 1100 g/ltr in the lower region and a constant sand concentration of 350 g/ltr in the top region after day 78 (Table 7.4). From the sand balance (total amount is 135 kg and no sand was lost) and the fluidized bed volume (from Figure 7.11A) the volume of the two regions could be calculated. The results are shown in Figure 7.12. It can be seen that at the end of the experiment an estimated volume of 235 ltr of biolayer covered sand particles was present in the reactor with a solids content of about 40-45 g VSS/ltr.

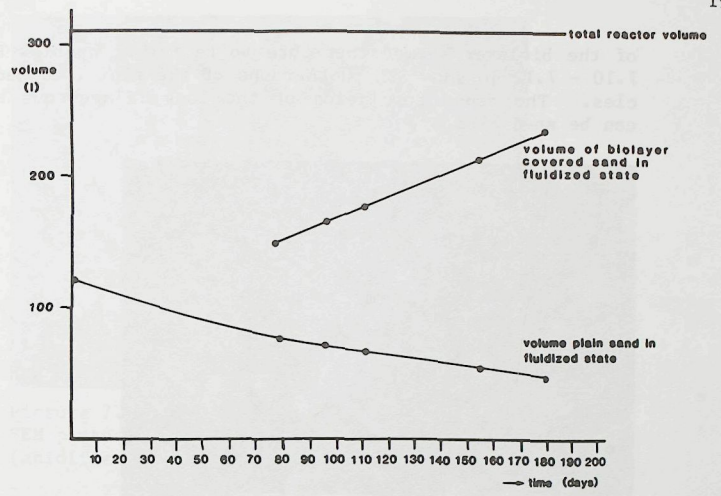


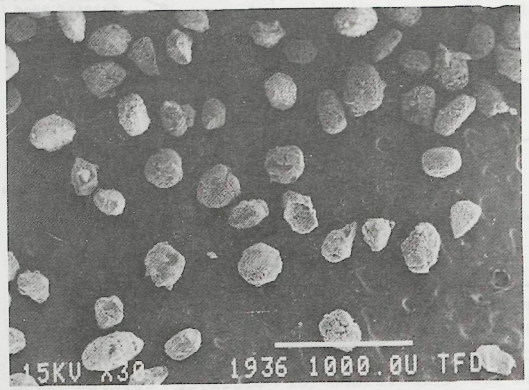
Figure 7.12
Calculation of the volume of biolayer covered sand in the methanogenic fluidized bed reactor fed with acidified yeast waste-water

From the steady increase in fluidized bed volume it could be concluded that during this experiment the equilibrium between biolayer loss (due to e.g. turbulence and friction) and biolayer growth was not yet reached. The amount of biolayer loss could be estimated from the biomass accumulation in the settler after the fluidized bed reactor ((4) in Figure 7.1). It must be stressed that this figure does not take into account the amount of non-settling solids present in the treated waste water.
It was found that from day 0-111 909 g VSS accumulated and from day 135-169 there accumulated 142 g VSS. Regarding the amount of water treated in these periods the loss of VSS amount to 2 and 1 mg VSS/ltr, which was negligible.
In summary it appeared that the methanogenic biolayers did develop very rapidly on acidified yeast waste water using a dilution rate of 0.32 - 1.0 hr^{-1}. The biolayer thickness seemed to stabilise at about 50 µm which is a very attractive feature of this process. The loss of biolayers was negligible as evidenced from still increasing fluid bed volume and detected volatile solids loss. The biomass concentration in the fluidized bed was 40 - 45 g VSS/ltr, at $v^L_{sup} \geq 7$ m/hr.
The biomass concentration in the biolayer was calculated to be 105 g VSS/ltr.

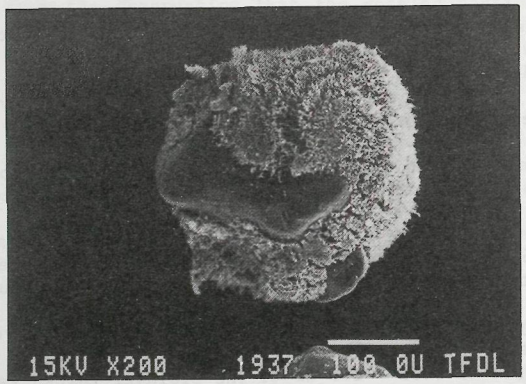
7.4.2.b Characterisation of the biolayers

As already mentioned the biolayer thickness stabilised at about 50 µm. Microscopic observation of these biolayers on day 70 and 173 revealed a high predominance of micro-organisms which resemble Methanobacterium soehngenii. The microbial population

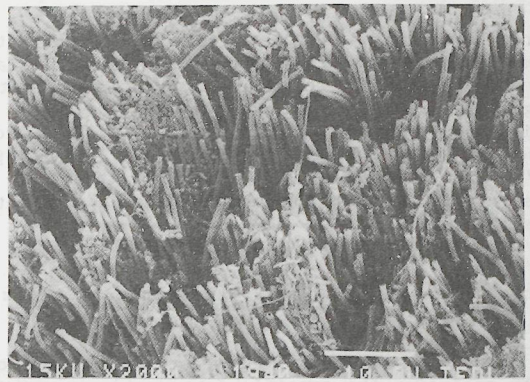
of the biolayer seemed therefore to be rather stable. Pictures 7.10 - 7.12 do show SEM photographs of the fluidized bed particles. The dense population of the long filamentous microbes can be seen clearly.



Picture 7.10
SEM picture of sand grains covered with methanogenic biolayers (acidified yeast waste-water, bar is 1 mm)



Picture 7.11
SEM picture of a sand grain covered with a methanogenic biolayer
(acidified yeast waste-water, bar is 100 micrometer)



Picture 7.12
SEM picture of the surface of a methanogenic biolayer (acidified yeast waste-water, bar is 10 micrometer)

From the increase in biogasproduction in Figure 7.14A (constant CH_4-concentration of 65%) a doubling time of the attached biomass of about 9 days could be calculated which is in good agreement with the doubling time of Methanobacterium soehngenii (see also Chapter 7.3.2.b). The activity of the biolayers on the sand from sp3 was measured twice in a batch experiment with a feed of acetic/propionic/butyric acid. The results are shown in Table 7.5. It can be seen that the methane activity of the biolayer is high (about 1.8 g COD/ g VSS day.

day	sample	methane activity g CH_4-COD/ g VSS day	removal efficiency %		
			C_2	C_3	C_4
76	sp3	1.7	100	0	100
84	sp3	1.8	100	0	100
111	settler	0.3	-	-	-

(-) = not determined
Table 7.5
Methanogenic activity of sand attached biolayers cultivated on acidified yeast waste-water

This was comparable with the values found for the biolayers grown on acidified GB waste-water (see Figure 7.9).
In these batch experiments it appeared furthermore that acetic acid and butyric acid were rapidly degraded with 100% efficiency but that propionic acid was not degraded. This is analogous to the results found for biolayers cultivated on acidified GB waste-water (Chapter 7.3.2.b) and the explanation is the same because the biolayer samples from Table 7.5 were cultivated most of the time under conditions of high acetate concentration. (Chapter 7.4.2.c.)

Furthermore the activity of the sludge found in the settler on day 111 was analysed for its methane activity. From Table 7.5 the activity appeared to be rather low. This indicated that this sludge was probably a mixture of solids which pass through the fluidized bed reactor (mainly acidification VSS) and methanogenic solids lost from the fluidized bed. In the previous Chapter 7.4.2.a it was already indicated that the loss of solids was very low; it now appears that the loss of methanogenic biomass was even lower.

7.4.2.c Purification results in the anaerobic fluidized bed reactor

The purification of the acidified yeast waste-water could be measured by analysing the specific fatty acid concentration in the influent and effluent of the reactor. This was however only allowed if in this reactor only fatty acid conversion to CH_4 only occurred. It was therefore assumed that the inflowing waste-water was totally acidified. This assumption was checked by comparing the removal of fatty acid COD (from Table 7.6) with the COD removal as calculated from the COD analysis of the same samples. The result is shown in Figure 7.13. It can be seen that there was indeed a good agreement between the two values and it could therefore be concluded that in the fluidized bed reactor only methanation of fatty acids occurred.

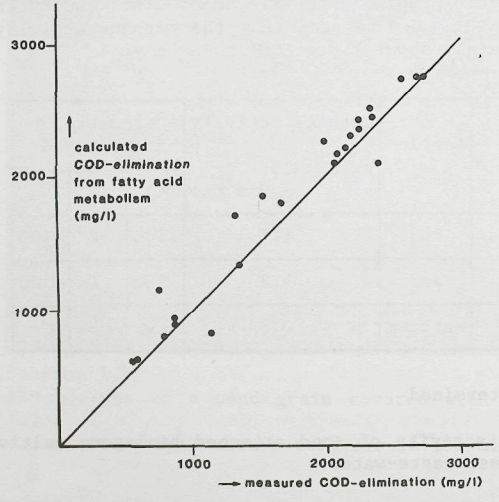


Figure 7.13
Relation between measured COD elimination and calculated COD-elimination from fatty acid analysis (Table 7.6) in acidified yeast waste-water

The applied hydraulic residence time τ, the recycle ratio r and the liquid superficial velocity V^L_{sup} in the fluidized bed reactor are shown in Figure 7.10.

day	C_2		C_3		iC_4		C_4		iC_5		C_5		FA-meq./ltr		FA-COD		FA-COD eff.
	in mg/l	out mg/l	in mg/l	out mg/l	in mg/l	out mg/l	in mg/l	out mg/l	in mg/l	out mg/l	in mg/l	out mg/l	in meq/l	out meq/l	in mg/l	out mg/l	%
14	770	350	36	35	20	18	160	54	8	8	6	5	15.4	7.1	1233	590	52
21	910	410	95	100	25	23	210	55	6	9	7	8	19.1	9.1	1570	760	52
28	680	140	37	45	21	14	170	3	8	4	7	< 3	14.0	3.1	1170	250	79
35	1600	1200	82	91	27	30	280	160	9	10	11	10	31.3	23.4	2430	1800	26
42	880	370	68	73	24	20	180	63	10	9	8	6	18.1	8.1	1860	690	63
49	2300	970	42	60	24	25	340	160	11	11	18	17	43.3	19.4	3240	1520	53
56	2000	1300	160	180	42	45	340	210	13	17	17	18	40.2	27.3	3130	2190	30
63	640	130	170	110	15	11	130	30	3	3	4	4	14.7	4.1	1220	390	68
70	1800	170	315	320	21	19	410	17	8	16	14	7	39.3	7.8	3210	780	76
77	2100	72	42	60	31	9	320	4	13	7	11	< 3	39.8	2.2	2990	210	93
84	1900	24	260	220	33	4	300	3	19	< 3	14	< 3	39.3	3.4	3083	370	88
91	1200	20	260	5	19	< 3	220	3	7	< 3	11	< 3	26.4	0.4	2138	30	99
98	2100	16	310	230	25	4	160	< 3	10	< 3	28	< 3	41.7	3.4	3120	371	88
100	2700	610	440	350	25	24	270	81	10	12	29	22	54.7	16.4	4157	1434	66
105	2200	99	390	310	21	9	48	6	9	8	13	7	43.0	6.2	3101	628	80
112	2020	49	310	120	25	5	15	< 3	9	< 3	6	< 3	38.5	2.5	2725	232	91
114	1900	30	320	198	24	5	21	< 3	11	5	8	3	36.7	3.3	2626	348	87
140	1500	56	320	53	19	13	210	7	8	5	27	5	32.3	1.9	2562	195	92
147	2000	490	320	270	21	19	62	24	9	5	23	12	38.9	12.5	2831	1040	63
154	2000	95	350	260	24	20	26	5	10	10	7	6	38.8	5.5	2783	569	80
161	1800	200	280	280	21	23	123	30	8	14	15	15	35.7	8.0	2650	790	70
168	1100	46	180	130	16	12	62	< 3	7	8	8	< 3	21.8	2.7	1617	261	84
175	1700	14	390	350	31	18	100	3	12	13	22	6	35.4	5.4	2708	616	77
182	1600	14	320	220	24	7	140	< 3	8	6	18	< 3	33.1	3.3	2535	367	86
187	1700	12	280	100	29	3	98	< 3	16	10	17	< 3	33.9	1.6	2502	183	93

Table 7.6
Fatty acid purification in an anaerobic fluidized bed reactor cultivated with acidified yeast waste-water, C_2 is acetic acid, C_3 is propionic acid, C_4 is butyric acid, C_5 is valeric acid, iC_4 and iC_5 are iso-acids

The experiment was started with D = 0.32 hr^{-1} (τ = 3.1 hr) and depending on biogasproduction the hydraulic load was increased to D = 0.81 hr^{-1} on day 71. Because of the low efficiency of fatty acid removal the hydraulic load was decreased after day 71 to D = 0.41 hr^{-1} to increase this efficiency. The biogas production (nearly constant fraction CH_4 of 65%) is shown in Figure 7.14A. The load of fatty acid COD and the conversion of these compounds is shown in Figure 7.14B, and the removal efficiency of fatty acids is shown in Figure 7.14C. Figure 7.14D finally shows the fatty acid COD in influent and effluent of the reactor. For the exact fatty acid analysis data the reader is referred to Table 7.6.

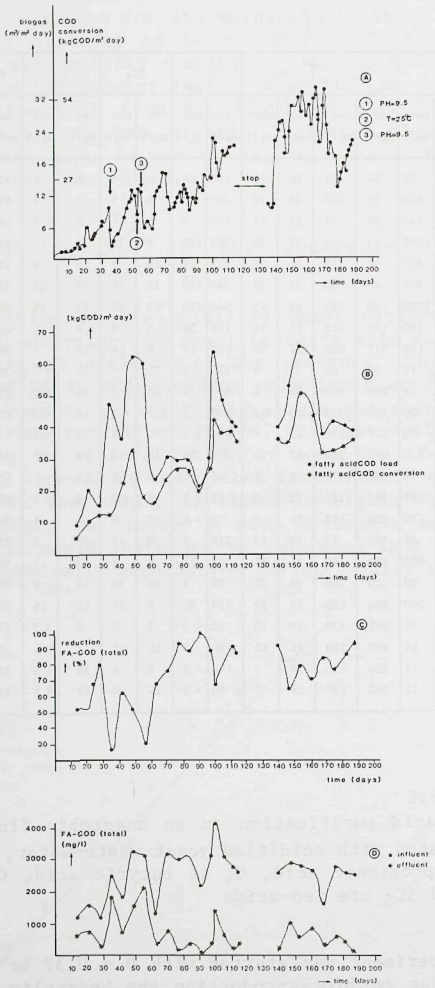


Figure 7.14
Results of the methanogenic fluidized bed reactor fed with acidified yeast waste-water
(A) biogasproduction
(B) load of total fatty acid COD and conversion of total fatty acid COD
(C) purification efficiency of total fatty acid COD
(D) total fatty acid COD concentration in influent and effluent

It appeared that the biogas production increased rapidly after start of the experiment. Between day 10 and 34 the gasproduction increased 7-fold. This indicated a doubling time of the biolayer of 9 days. On day 34 and 56 the pH in the fluidized bed increased to 9.5 due to failures in the pH-control of the acidification reactor which produced the acidified yeast waste-water. This high pH led to an immediate collapse of the gasproduction but the recovery from this shock was rapid.

A temperature decrease to 25°C on day 52 led to a 25 % lower gas production.
The fast recovery after these accidents illustrated the stability of the fluidized bed reactor.
The gasproduction in the period day 0-71 was accompanied by a considerable COD-conversion from fatty acids as shown in Figure 7.14B but due to constant overloading of the reactor the fatty acid COD removal efficiency remained low (Figure 7.14C).
After day 71 the reactor load was decreased (D=0.40 hr^{-1}) to improve the purification efficiency and it can be seen that the purification of fatty acids readily reached levels of more than 90 % (day 71-98, Figure 7.14C).
On day 98 the reactor load was increased again (D = 0.40 hr^{-1} → 0.67 hr^{-1}). From Figure 7.14C and 7.14B it appeared that the purification efficiency dropped initially and that there was an increase in effluent fatty acids COD. But between day 98 and 110 the removal efficiency of fatty acid COD steadily increased and reached again levels of 90 %.
Between day 114 and 135 the reactor was stopped by lack of acidified water.
Start up of the ractor presented no problems and within a few days the gas production had reached the pre-stop level (Figure 7.14A)
Also the purification efficiency of fatty acid COD did reach 90% again (day 140 Figure 7.14C).
On day 147 the hydraulic loading rate was again increased to D = 1 hr^{-1}. This loading was maintained until day 171 (Figure 7.10) From Figure 7.14A it can be seen that the gasproduction reached levels of 32 m^3/m^3day which corresponded to a COD conversion of 55 kg COD/m^3day.
These conversion values were confirmed from the removal of fatty acid COD in Figure 7.14B.
After an initial drop of the fatty acid removal efficiency to 65 % at day 147 there was an increase to 85 % on day 171.
After day 171 the hydraulic loading of the reactor was decreased to D = 0.60 hr^{-1}.
This was done because at D = 1.0 hr^{-1} the recycle ratio had dropped to a very low value of 0.16 (Figure 7.10). This low recycle ratio did lead to a pH gradient in the fluidized bed reactor. For example on day 156 the pH of acidified waste-water feed was 6.15, in sp1 the pH = 6.31 and in sp3 the pH = 6.97. The same pH gradient was observed on day 171.
Because such a pH gradient was not optimal for methanogens it was decided to eliminate the pH gradient.
This pH gradient disappeared after a decrease in the flow of acidified waste-water and an increase of the recycle flow on day 171 (see Figure 7.10). This recycle, which contains bicarbonate, does in fact neutralise the acidified waste-water.
The decreased loading of the reactor did lead to decreased gas production and increased fatty acid COD removal efficiency (Figure 7.14A and 7.14C).

From the evidence presented sofar it clearly appeared that the anaerobic fluidized bed reactor could reach in relative short times a conversion rate of COD as high as 55 kg COD/m^3day and biogasproduction rates as high as 32 m^3/m^3day.
This observed value of 55 kg COD/m^3day could be compared with a calculated conversion capacity from the measured biolayer activity (Chapter 7.4.2.b) of 1.8 g COD /g VSS day, a biomass content of the fluidized bed of 42.5 g VSS/ltr (Chapter 7.4.2.a) and a fluidized bed volume of 235 ltr in a reactor of a total volume 310 ltr (Chapter 7.4.2.a). This calculation results then in a conversion capacity of $\frac{42.5 * 1.8 * 235}{310}$ = 58 kg COD/m^3day.
It also appeared that the fluidized bed reactor can recover fast from "accidents".
However from Figure 7.14C it appeared that the fatty acid removal efficiency seldom reached the 100 % level.
The reason for this became clear upon analysis of the removal efficiency of the main fatty acids like acetic acid, propionic acid and butyric acid.
Figure 7.15A shows the acetic acid concentrations in the effluent of the reactor (which are also listed in Table 7.6). It is seen that acetic acid levels can be reached as low as 12 mg/l.
This means that acetic acid can be eliminated quantitatively.
Figure 7.15B shows the butyric acid concentrations in influent and effluent and it appeared also that this acid could be removed with 100 % efficiency.
Figure 7.15C shows the propionic acid removal. It was very obvious that this acid was only poorly eliminated in the course of the experiment and this was the main reason why the removal of fatty acid COD seldom reached 100 %.
As already stated in Chapter 7.3.2.b propionic acid appeared to be only degraded at low acetic acid levels (Ref. 7.10, 7.11). Therefore the correlation between acetic acid levels in the reactor effluent and the propionic acid conversion rate is shown in Figure 7.16. There appears to be a fair correlation in that propionic acid conversion was zero at high acetic acid levels (day 0-56). The zero conversion rate at day 161 was probably due to the very low recycle ratio (r = 0.16, Figure 7.10) which probably led to a strong gradient of acetic acid in the fluidized bed reactor.

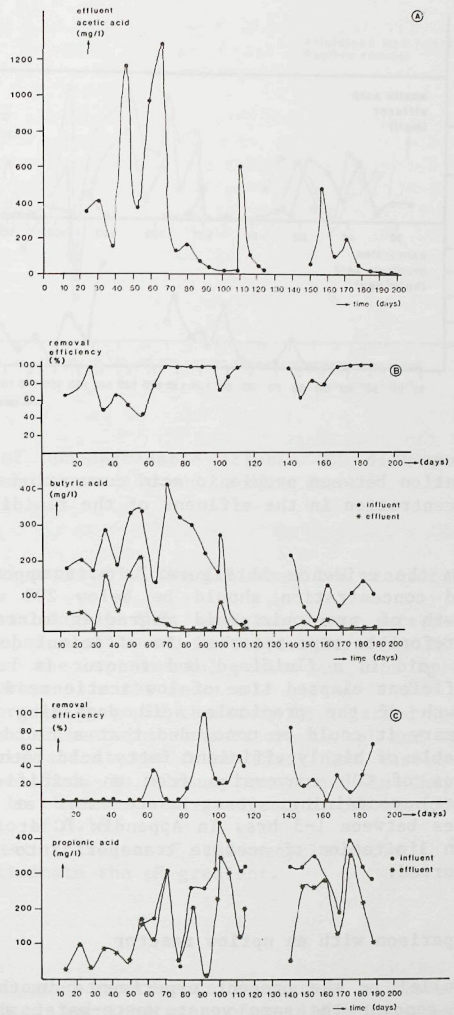


Figure 7.15
Specific fatty acid purification in the methanogenic fluidized bed reactor fed with acidified yeast waste-water
(A) acetic acid concentration in the reactor effluent
(B) butyric acid concentration in influent and effluent and its purification efficiency
(C) propionic acid concentration in influent and effluent and its purification efficiency

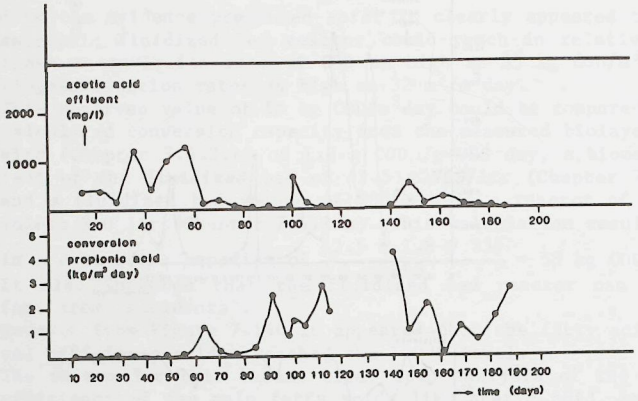


Figure 7.16
Relation between propionic acid conversion rate and acetic acid concentration in the effluent of the fluidized bed reactor

From the evidence in Figure 7.16 it appears that the acetic acid concentration should be below 200 mg/ltr to allow the growth of propionic acid degrading micro-organisms. It can therefore be expected that the efficient degradation of propionic acid in a fluidized bed reactor is realised only after a sufficient elapsed time of low acetic acid levels to allow the growth of the propionic acid degrading micro-organisms. In summary it could be concluded that a fluidized bed reactor was capable of highly efficient fatty acid methanation at very high rates of COD conversion from an acidified, sulfide (160 mg S/ltr) containing, yeast waste-water at hydraulic residence times between 1-3 hrs. In Appendix 7C it is shown that diffusion limitation of acetate transport into the biolayer has not occurred.

7.4.2.d Comparison with an upflow reactor

Parallel to the present experiments in the 310 ltr fluidized bed reactor the same yeast waste-water was fed into a 20 m^3 conventional upflow reactor at a liquid residence time of 12-16 hrs. In this upflow reactor flocculent anaerobic sludge was present. In Figure 7.17 the non-degradable COD in the effluent of this reactor is compared to the effluent of the fluidized bed reactor. The non-degradable COD is calculated from the measured COD minus the residual fatty acid COD.
It is obvious that there are no systematic differences between both reactors. However the fluidized bed reactor is to be preferred because of the much shorter purification time.

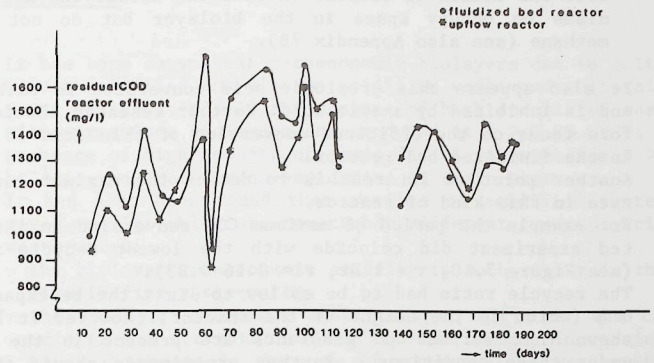


Figure 7.17
Comparison of non-degradable soluble COD in the effluent of the fluidized bed reactor and up-flow reactor

7.4.2.e Technological aspects of the fluidized bed reactor

The sand loss during this experiment was extremely low. The total amount of sand found in the settler ((4) in Figure 7.1) at the end of the experimental period was 1.58 kg (compared to the 135 kg present in the reactor).
The separator design could therefore be regarded as adequate.
During the experiment the temperature in sp1 and sp3 was regularly measured and it was found that there was no gradient in the fluidized bed. As already mentioned in Chapter 7.4.2.c there could be observed a pH-gradient in the reactor at the low recycle ratio of 0.16.
The subsequent increase of the recycle ratio to 1.0 (Figure 7.10) did eliminate the pH gradient.

7.4.3 DISCUSSION

From the presented experiments it must be concluded that anaerobic fluidized bed treatment of acidified waste-water is advantageous compared to the non-acidified waste-water treatment.
The advantages are twofold:

1. It appears that the biolayer attains a constant biolayer thickness of about 50 µm.
   This is contrary to the steady increasing biolayer thickness in non-acidified waste water (Chapter 7.3.2.a).
   The reason for this is not clear but there might be a connection with the physical appearance of the biolayer. From SEM Pictures 7.10-7.12 it seems that the filamentous methanogens are attached perpendicular to the sand surface. If these filaments do have a characteristic length this might provide an explanation of the observed constant biolayer thickness.
2. The biolayers do have a much higher methanogenic activity compared to the biolayers with additional acidifying micro-

bes. The reason is obvious in that the acidifying micro-organisms do occupy space in the biolayer but do not produce methane (see also Appendix 7B).

It also appears that propionic acid conversion is rather slow and is inhibited by acetic acid. Further research should therefore focus on the efficient conversion of this acid to methane in the fluidized bed reactor.
Another point of interest is to detect the maximum conversion rate in this kind of reactor.
For example the period of maximum COD conversion in the reported experiment did coincide with the lowest recycle ratios's (see Figure 7.10, τ = 1 hr, r = 0.16-0.33).
The recycle ratio had to be so low to limit the bedexpansion to 6 m (which is the height of the reactor). However it has been shown that serious pH gradients are present in the reactor under these conditions. Further experiments should therefore explore the use of sand with larger diameter or of particles with the same diameter but higher density to allow higher flow rates which will enable the use of an adequate recirculation.
Another point of practical interest is that the presence of an inoculum with some methanogenic micro-organisms at the start of the experiment seems to be sufficient to achieve seeding of the sand for biolayer development. It therefore seems not to be necessary to apply a continuous inoculation for long times (as used in the experiment in Chapter 7.2).
The dilution rates which were applied in this study varied between D = 0.32 hr^{-1} and D = 1.0 hr^{-1}. It is obvious that within this range biolayers do develop and are stable. However especially for treatment of more concentrated waste-waters it seems very necessary to detect the lower limit of dilution rate which still allows the development of stable biolayers. This is also relevant information with regard to allowed variation in dilution rates from varying waste-water flowrates which can occur under practical conditions.

7.4.4 CONCLUSION

It has been shown that acidified, sulfide containing, yeast waste-water can be purified anaerobically within short hydraulic residence times in a fluidized bed reactor.
During this experiment a maximum conversion rate of 55 kg COD/m^3day has been achieved at an hydraulic residence time of 1hr. The purification efficiency of the fatty acids has been shown to reach levels of more than 90%. It has been indicated that a nearly 100% purification can be achieved if propionic acid can be purified for 100% which seems to be merely a matter of time.
The biolayer on the sand does develop rapidly from the beginning and reaches a constant thickness of 50 µm.
The fluidized bed volume does increase steadily due to increasing amounts of biolayers. Typical values of biomass content in the fluidized bed are 40-45 g VSS/ltr at superficial liquid velocities of 7-9 m/hr.
The biomass in the fluidized bed has a high methanogenic activity of 1.8 g COD/g VSSday and the biolayer contains about 100 g VSS/ltr.

7.5 CONCLUSION

It has been shown that methanogenic biolayers can be cultivated on non-acidified and also on acidified waste-water (total GB or yeast waste-water). In the absence of high amounts of suspended micro-organisms biolayers are produced at D $\geq$ 0.25 hr^{-1}. In the presence of high amounts of suspended micro-organisms D $\geq$ 0.6 hr^{-1} is required for non-acidified GB waste-water.
It has also been found that the use of acidified waste-water gives results which are remarkebly different from non-acidified waste-water:
- the biolayer methanogenic activity is much higher in the case of acidified waste-water
- the biolayer thickness stabilises at 50 µm in the case of acidified waste-water contrary to the steady increasing biolayer thickness in the case of non-acidified waste-water (up to 233 µm).

Therefore the methanogenic fluidized bed reactor utilising acidified waste-water seems to be the most promising process.
This has been shown to be true.
Acidified yeast waste-water, which contains about 160 mg sulfide/ltr, can be purified at an hydraulic residence time of 1 hr. Under these conditions conversion rates of 55 kg COD/m^3day can be achieved and fatty acid removal efficiencies of about 90 % are realised.
During the experiments described in this Chapter 7 one major drawback of the fluidized bed reactor became evident. Due to its short liquid residence time the fluidized bed reactor is vulnarable to short time peaks of bacteriocidal chemicals which are sometimes present in the GB waste-water.
This means that this reactor can only be applied if the occurrence of such peaks is eliminated. There are several possibilities
- control the handling of these chemicals at the source
- install fast analysing equipment for detection of these chemicals in the waste-water
- build buffer tanks to decrease the occurrence of peaks
- a combination of these actions.

APPENDIX 7A

Calculation of biolayer thickness as a function of ash content of biolayer covered sand

Assume a carrier particle of diameter d_p and density ρ_p(= 2600 kg/m^3). This carrier is then covered with a biolayer having a thickness δ and a concentration of micro-organism C_x (kg/m^3)

The ash content ε of the dry matter of the particle can then be calculated as :

$$\varepsilon = \frac{\frac{\pi}{6} d_p^3 * 2600}{\frac{\pi}{6} d_p^3 * 2600 + \left\{ \frac{\pi}{6} (d_p + 2\,\delta)^3 - \frac{\pi}{6} d_p^3 \right\} C_x}$$

The ratio of biolayer thickness to carier diameter can then be calculated as

$$\frac{\delta}{d_p} = \frac{\left\{ \frac{(1 - \varepsilon)\ 2600}{\varepsilon\ C_x} + 1 \right\}^{1/3} - 1}{2}$$

The results in Table 7.2 and Table 7.4 have been calculated using C_x = 100 kg VSS/m^3.

APPENDIX 7B

Activity of biomass cultivated on non-acidified and acidified waste-water

It is assumed that the CH_4 fermentation proceeds along 3 reactions

a1 $C_6H_{12}O_6 + H_2O \rightarrow 2C_2H_4O_2 + 2CO_2 + 4\ H_2$

a2 $2C_2H_4O_2 \rightarrow 2\ CO_2 + 2CH_4$

a3 $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$

Reaction a1 is assumed to be performed by acidifying microbes with a yield of 0.1 g VSS/g COD (Ref. 7.4) and therefore 19.2 g VSS are produced per mol glucose.
Reaction a2 is assumed to be carried out by methanogenic bacteria with a yield of 0.04 g VSS/g COD (Ref. 7.5) and therefore 5.12 g VSS are produced.
Reaction a3 is assumed to be carried out by methanogenic bacteria with a yield of 0.09 g VSS/g COD (Ref. 7.6) and thus 5.76 g VSS is produced.

The total biomass yield in the fermentation of 1 mol glucose is then 30.1 g VSS.

This mixed culture consists of $\frac{5.12 + 5.76}{30.1} * 100 = 36\ \%$ methanogenic bacteria, which are rate limiting (Ref. 7.7), and $\frac{19.2}{30.1} * 100 = 64\ \%$ of acidifying bacteria, wich are not rate limiting.
It therefore follows that, if the methane activity of the methanogenic biomass is equal to q_{COD}, the methane activity of the methanogenic plus acidifying biomass will be equal to 0.36 q_{COD}.
In summary it can therefore be expected that the CH_4 activity for sludge cultivated on acidified waste-water is much higher then the CH_4 activity of the sludge cultivated on non-acidified waste-water.

APPENDIX 7C

Diffusion limitation in biolayers

The use of biolayers leads in general to the possibility of diffusionlimitation of the conversion of COD to biogas.
In this process the conversion of acetate to CH_4 is assumed to be the rate limiting step and therefore this reaction has to be considered. Given the evidence in Chapter 7.2 - 7.4 it appears that the main methanogenic acetate using bacterium in the biolayer is Methanobacterium soehngenii.
According to Ref. 7.9 the Michaelis-Menten constant K_s for acetate conversion is 30 g/m^3 for this micro-organism.
The biomass activity for methane production from acetate in the biolayer is 0.7 g acetate/g DM day for a biolayer cultivated on non-acidified GB waste-water (Chapter 7.2) and about 1.8 g acetate /g DM day for a biolayer cultivated on acidified GB waste-water or yeast waste-water (Chapter 7.3 and 7.4) .
The equations for the diffusion limitation problem in biolayers around spheres have been provided in Ref. 7.12 .
For zero order kinetics, diffusion limitation occurs if the biolayer thickness δ increases beyond a value compatible with eq. 7C-1.

$$f\left\{\frac{\delta}{d_p}\right\} = \left\{\frac{1}{2} + \frac{\delta}{d_p}\right\}^2 \left\{1 - \left\{\frac{1}{1 + \frac{2\delta}{d_p}}\right\}^3\right\}^2 \geq \frac{11.8\ \mathbb{D}_s\ C_s}{C_x\ q_s^{MAX}\ d_p^2}$$

eq.(7C-1)

In this equation q_s^{MAX} is assumed as zero order rate constant, which is only valid at high C_s ($C_s \geq K_s$).
For 1. order kinetics, serious diffusion limitation (an effectiveness factor smaller than 80 % is assumed as serious) occurs if the biolayer thickness δ increases beyond a thickness compatible to equation 7C-2

$$f\left\{\frac{\delta}{d_p}\right\} = \left\{\frac{1}{2} + \frac{\delta}{d_p}\right\}^2 \left\{1 - \left\{\frac{1}{1 + \frac{2\delta}{d_p}}\right\}^3\right\}^2 \geq \frac{5.3\ \mathbb{D}_s\ K_s}{C_x\ q_s^{MAX}\ d_p^2}$$

eq.(7C-2)

In this equation the first order rate constant is assumed to be $\frac{q_s^{MAX}}{K_s}$, which is the asymptot of the Michaelis-Menten equation at low C_s values ($C_s \leq K_s$).

In Table 7C-1 the value of $f\left\{\frac{\delta}{d_p}\right\}$ has been calculated for several values of $\left\{\frac{\delta}{d_p}\right\}$

$\left\{\frac{\delta}{d_p}\right\}$	$f\left\{\frac{\delta}{d_p}\right\}$
0	0
0.1	0.064
0.2	0.198
0.4	0.556
0.8	1.50
1.6	4.29

Table 7C-1

For non-acidified GB waste-water the folllowing parameter values are assumed to be valid for CH_4-production from acetate (Chapter 7.2)

$d_p = 2 * 10^{-4}$ m

$C_x = 100$ kg DM/m^3

$\mathbb{D}_s = 10^{-9}$ m^2/s

$q_s^{MAX} = 0.7$ g acetate/g DM day

$K_s = 30$ g/m^3 $= 3 * 10^{-2}$ kg/m^3

$C_s \geq 0.6$ kg/m^3

According to the experiments reported in Chapter 7.2 the maximum biolayer thickness was 233 µm (see also Table 7.2) and the acid concentration was always higher than 10 meq/l, which is 0.6 kg/m^3. Because $C_s \geq K_s$ the zero order kinetic equation 7.C-1 should be used.
Using equation 7C-1 it can be calculated that for 0. order kinetics the biolayer thickness should be smaller than 2860 µm, to prevent diffusion limitation.
For 1. order kinetics this biolayer limit would be 343 µm (eq. 7C-2). Because the actual biolayer thickness never increased beyond 233 µm (Table 7.2) it can be concluded that diffusion limitation did not occur.

For acidified waste-water the following parameters are valid for CH_4-production in a fluidized bed reactor (Chapter 7.3, 7.4).

$d_p = 2 * 10^{-4}$ m

$C_x = 100$ kg DM/m^3

$\mathbb{D}_s = 10^{-9}$ m^2/s

$q_s^{MAX} = 1.8$ g/g day $= 20.8 * 10^{-6}$ sec^{-1}

$K_s = 3 * 10^{-2}$ kg/m^3

$C_s = 15 * 10^{-3}$ kg/m^3 (Table 7.6)

Because $C_s \leq K_s$ the 1. order kinetic equation 7C-2 should be applied only. This leads then to a biolayer of 195 µm beyond which diffusion limitation occurs. As shown in Chapter 7.3 and 7.4 the actual biolayer does not increase beyond 60 µm and thus diffusion limitation does not occur.

SYMBOLS

C_s	substrate concentration	mg/l
Cx	biomass concentration in biolayer	kg VSS/m^3
$\mathbb{D}$	diffusion coefficient	m^2/s
D	dilution rate	hr^{-1}
d_p	diameter of carrier	m
H	height of the fluidized bed in the reactor	m
K_s	Michaelis-Menten constant	kg/m^3
q_{COD}	specific activity of biomass	g COD/g VSS day
q_s^{MAX}	maximum specific substrate conversion rate	s^{-1}
r	ratio of recirculation waste-water flow and influent waste-water flow	(-)
V_{sup}^L	superficial liquid velocity in the fluidized bed	m/hr
δ	biolayer thickness	m
ρ_p	density of carrier	kg/m^3
ε	percentage ash of biolayer covered sand particle	-
τ	liquid residence time	hr
Φ_i	influent waste-water flow-rate	m^3/hr
Φ_r	Recirculation waste-water flow-rate	m^3/hr

REFERENCES

7.1 G. Lettinga, A.F.M. van Velzen, S.W. Hobma, W. de Zeeuw, A. Klapwijk,
Biotechnol. and Bioeng. 22, (1980), 699

7.2 J.A. Mueller, J.L. Mancini,
30 th Ind. Waste Conf. (1975), 423
Purdue University, Lafayette

7.3 S.W. Hobma, J.B.R. van der Laan,
H_2O, 11, (1978), 465

7.4 A. Cohen, R.J. Zoetemeyer, J.G. van Andel,
H_2O, 10, (1977), 296

7.5 A.W. Lawrence, P.L. McCarty
J.W.P.C.F., 41, (1969), R1

7.6 T.G. Shea, W.A. Pretorius, R.D. Cole, E.A. Pearson,
Water Res. 2, (1968), 833

7.7 S. Ghosh, D.L. Klass
Process. Biochem. (1978), 15

7.8 Standard methods for the analyses of raw, potable waste-waters,
Department of the environment 1972

7.9 A.J.B. Zehnder, B.A. Huser, T.D. Brock, K. Wuhrmann,
Arch. Microbiol., 124, (1980), 1

7.10 F.G. Pohland, S. Ghosh,
Biotechnol. and Bioeng. Symp. No 2 (1971), 85

7.11 L. van den Berg, C.P. Lentz,
Proc. 32nd Ind. Waste Conf. Purdue (1977), 252

7.12 L.T. Mulcahy, W.K. Shieh, E.J. Lamotta,
A.I.Ch.E. Symposium Series, 77, (1981), No 209, 273

7.13 J.J. Heijnen
In Proceedings Congress "Anaerobic Wastewater Treatment",
Noordwijkerhout 23-25 november 1983, pag. 176
published by TNO, The Hague, Netherlands

8 EVALUATION OF DIFFERENT TREATMENT SCHEMES FOR THE GB WASTE-WATER AND A PROPOSAL FOR AN OPTIMAL HIGH RATE TREATMENT PROCESS

8.1 INTRODUCTION

As has been stated in Chapter 1 of this thesis the main impetus for the conduction of this research has been the fact that only little space is available in Delft for a treatmentplant of the GB-wastewater and odours must be totally absent.
Therefore a high-rate biological purification process with high efficiency was necessary. In this Chapter 8 several treatment schemes, as presented in Chapter 3, 4, 5, 7 will be compared with regard to efficiency and maximum removal rate.
This will then result in a proposal of an optimum proces configuration for the high rate biological purification of the GB-wastewater.
Finally an indication will be given about the necessary future research for the implementation of such an optimal purification process.

8.2 PURIFICATION EFFICIENCY OF THE GB-WASTEWATER USING DIFFERENT BIOLOGICAL TREATMENT SCHEMES

Purification of wastewater is generally measured as removal of oxygen requiring compounds.
In waste-water two classes of such compounds do occur:
- organic carbon compounds, the concentration of which is measured as Chemical Oxygen Demand (COD);
- reduced nitrogeneous compounds, the concentration of which is measured as Kjeldahl nitrogen (K_j-N).

Therefore both COD purification efficiency and Kj-N purification efficiency are relevant to the total purification of the waste-water. In the next sections COD and Kj-N efficiencies will be compared for the different treatment processes which have been applied (Chapter 3, 4, 5, 7) to the GB-wastewater.
The COD and Kj-N efficiencies will be based on soluble waste-water compounds because it is assumed that the waste-water sediment is not degraded (see Chapter 3) in high rate processes.

8.2.1 PURIFICATION WITH THE AEROBIC FLOCCULATED ACTIVE SLUDGE PROCESS WITH SURPLUS SLUDGE PRODUCTION

From Chapter 3 (Table 3.5, 3.6, 3.9) it appears that in this treatment process a soluble COD purification of 75-82 % can be obtained. The higher COD-efficiencies are obtained at lower sludge loads. However it also has been found (paragraph 3.4.2.b) that about 32% of this soluble COD reappears as a suspension of surplus sludge. This surplus sludge has to be disposed of in some way. The real COD purification efficiency is therefore only about (1-0.32)*0.78=53%.
The soluble Kj-N elimination in this treatment process appears to be about 50% (Table 3.6). This elimination of nitrogen is due to assimilation in the surplus biomass and therefore the real elimination efficiency of Kj-N is zero.

This means that for maximum Kj-N elimination additional sludge removal, nitrification and denitrification is required.

8.2.2 PURIFICATION WITH THE AEROBIC FLOCCULATED ACTIVE SLUDGE PROCESS WITHOUT SURPLUS SLUDGE PRODUCTION

From Chapter 4 (Table 4.3) it appears that in this treatment process a soluble COD-purification of about 82% can be obtained. Because there is no surplus sludge production this is indeed the real COD purification efficiency. The higher value of the COD purification efficiency is due to the low sludge load in this process and compares well with the highest values in the conventional process with surplus sludge production.
From Table 4.3 the Kj-N elimination efficiency appears to be about 80%. This eliminiation is due to nitrification reactions which occur in this process.

8.2.3 PURIFICATION WITH ANAEROBIC FLUIDIZED BED TREATMENT

In Chapter 7 (Paragraph 7.2.2.b) it has been indicated that in this process about 70% of the COD can be converted into CH_4. This is a real COD purification wherein the biomass production (which is very small for anaerobic processes) is already incorporated.
Compared to the aerobic treatment processes (Paragraphs 8.2.1 and 8.2.2) this purification efficiency is lower. One reason is that the GB-wastewater contains SO_4^{2-} wich is reduced to S^{2-} in the anaerobic proces. In Paragraph 7.4.1.c it has been indicated that a typical S^{2-} concentration is about 160 mg S^{2-}/l in a wastewater which contained about 4-4.5 gCOD/l. Because 1g $S^{2-} \equiv 2$g COD this sulfide concentration accounts for about 8% of COD which is still present after the anaerobic treatment of the waste-water.
An additional reason for the lower COD-efficiency is probably that the GB-wastewater contains some minor organic compounds which are not or only partially degraded under anaerobic conditions.
However both the residual S^{2-} and organic compounds can be oxydized in an aerobic posttreatment after which the COD efficiency is expected to be close to 82%, which is the maximum COD-efficiency of the aerobic proces.
In a laboratory study by A. Mulder with GB-wastewater at the Agricultural University of Wageningen (Ref. 8.1) it has indeed been found that a COD purification efficiency of 83% can be obtained in an anaerobic/aerobic purification system.
The anaerobic treatment was performed in an upflow reactor and the sulfide containing anaerobic effluent was nitrified/denitrified in an aerobic posttreatment.
The Kj-N removal efficiency in the anaerobic treatment has not been measured. Due to the low assimilation into surplus biomass in this process it is expected that Kj-N removal efficiency is very low.
During anaerobic treatment reduced nitrogeneous compounds are converted into NH_4^+. Some measurements of the NH_4^+ concentration in the effluent of the acidification fluidized bed reactor mentioned in paragraph 7.4.1.c revealed that NH_4^+-N represented

about 80% of the Kj-N concentration.
Therefore it can be expected that an additional nitrification after the anaerobic treatment will give a Kj-N removal efficiency of about 80%.
This value is close to the Kj-N efficiency in the aerobic process (8.2.2). Such a nitrification stage can of course be combined with the abovementioned oxidation of residual sulfides and organics in the anaerobic effluent.

8.2.4 CONCLUSIONS

In the previous paragraphs three biological treatment schemes for the purification of the GB-wastewater are discussed with regard to COD and Kj-N purification efficiency.

It appears that the high rate aerobic flocculated sludge waste-water treatment with surplus sludge production has a low overall COD-removal efficiency of about 53% and a Kj-N efficiency of 0%.
The aerobic flocculated sludge treatment without surplus sludge production gives a COD efficiency of 82% and a Kj-N elimination of 80%.
The anaerobic fluidized bed treatment gives a COD purification of about 70% and a low Kj-N elimination. It has been indicated however that an aerobic posttreatment of the sulfide/NH_4^+ containing anaerobic purified wastewater will increase the COD-efficiency to 83% and will give a Kj-N efficiency of about 80%.
Therefore a choice between the last two treatmentschemes will be governed by other factors like maximum treatment capacity of the reactor, size of the settling tanks and energy consumption.
The factors which determine the treatment capacity of wastewater reactors and necessary settling tanks will be outlined in the next paragraph 8.3.

8.3 FACTORS DETERMINING THE MAXIMUM CONVERSION RATE OF BIOLOGICAL PURIFICATION PROCESSES WITH GB-WASTEWATER

From the preceeding Chapters 3-7 several factors can be found which determine the maximum conversion rate of the biological purification process. These factors are:
- aeration limitation
- limited sludge activity
- limited sludge concentration.

In the next paragraphs these factors and their impact on an optimum process configuration will be discussed.

8.3.1 LIMITATIONS IN AERATION

In aerobic processes the necessary oxygen must be transferred to the reactor space. Generally air-sparging is used for this purpose.
The amount of oxygen which is needed depends on the process type. In the conventional aerobic process with surplus sludge production (Chapter 3) about 0.7 kg O_2 is needed per kg COD which is converted.

If a bubble column is used as a reactor (with an assumed maximum superficial gas velocity of 10 cm/s) it can be calculated that the maximum COD-conversion rate is about 100 kg COD/m^3day (see Figure 3.5).
In the aerobic process without surplus sludge production (Chapter 4) the oxygen consumption is about 1.6 kg O_2/kg COD. This high value is due to complete dissimilation of the COD to CO_2 and to nitrification reactions which occur. For this situation it can be calculated that the maximum COD conversion is about 40 kg COD/m^3day (Figure 5.6). It is obvious that such limitations do not occur in anaerobic processes for waste-water treatment and that therefore such a process is to be preferred (apart from the general accepted advantages as energy production and low sludge yield).

8.3.2 LIMITATIONS IN SLUDGE ACTIVITY

From Chapter 3 it became clear that for flocculated sludge systems wastewater sediment is detrimental for sludge activity. This sediment accumulates in the sludge floc and forms an integral part of the floc mass. Therefore the fraction of active biomass in the floc decreases with increasing ammounts of sediment in the wastewater.
A simple mass balance calculation, assuming no hydrolysis of the sediment and a biomass yield Y on soluble COD, leads to the following relation for the fraction active biomass β.

$$\beta = \frac{Y}{Y + \frac{C_{SED}}{C_{COD}}} \qquad \text{(eq. 8.1)}$$

C_{SED} is the concentration of sediment in the wastewater and C_{COD} is the concentration of soluble COD.
Figure 8.1 shows some calculations for β as a function of the relative sediment concentration. Two cases are considered,
- aerobic purification with Y=0.3 gDM/gCOD
- anaerobic purification with Y=0.04 gDM/gCOD

It is obvious that $\beta \ll 1$ for already moderate concentrations of sediment especially in the anaerobic process.
Because of this effect a reactor system where the waste-water sediment does not accumulate in the biomass has to be preferred.
It has already been argued in the discussion of Chapter 4 that the biological fluidized bed reactor does meet this requirement. The active biomass grows attached to the carrier and the waste-water sediment passes through the reactor because of the high superficial liquid velocities (see Chapter 5 and 7 for aerobic and anaerobic treatment of the waste-water).
A second factor which influences the sludge activity is the occurrence of consecutive-reactions in the purification process.

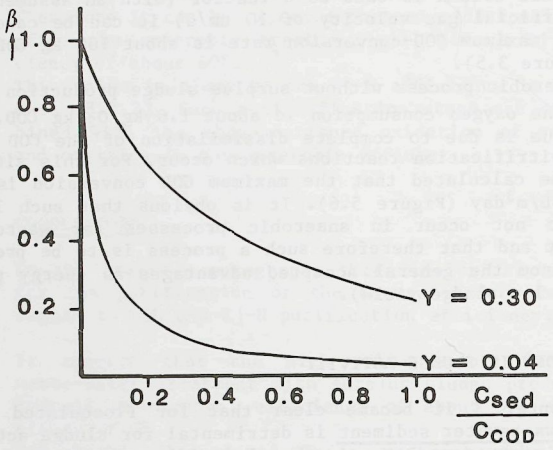


Figure 8.1
Influence of inert waste-water sediment on the fraction active biomass (β) in floc dry matter.

A typical example is the anaerobic treatment which consists of a microbial acidification and subsequent conversion of the produced fatty acids into CH_4 (see Appendix 7B).

In that case the sludge activity of the purifying CH_4 producing microbial population can be increased several fold by staging of the purification proces (see Appendix 7B and Chapter 7.3), i.e. an acidification stage and a methane production stage for the anaerobic treatment. Application of the two-stage anaerobic treatment proces in fluidized bed reactors (Chapter 7.3, 7.4) did additionally result in significant advantages with respect to pH-control and stability of the biolayerthickness (Chapter 7.4).

The third factor (see Discussion Chapter 4) which influences the biomass activity is the phenomenon of diffusion limitation. In a number of reactor systems the biomass grows as a biolayer of 1-5 mm thickness on carrier material (i.e. filterreactor, rotating disc reactor). However due to diffusion limitation the active depth of the biolayer is generally not more than 0.1-0.2 mm (Ref. 8.2). This means that in these cases only a small part of the available biomass is active. To eliminate this problem one clearly has to limit the biolayer thickness to 0.1-0.2 mm. This can be realized by supplying sufficient area in the reactor. As already argued in Chapter 4 the fluidized bed reactor with a carrier surface of 1000-5000 m^2/m^3 reactor seems ideally suited for this purpose.

In summary it may be concluded that the highest sludge activity can be obtained in a fluidized bed reactor because the waste-water sediment does not accumulate and diffusion limitation problems can be controlled. Additionally a serie of such fluidized bed reactors can be used to perform consecutive reactions like acidification, methanation and for example nitrification and denitrification.

8.3.3 LIMITATIONS IN SLUDGE CONCENTRATION

In Chapter 3, eq. 3.36 has been derived to describe the maximum biomass concentration C_X which can be obtained in the aeration tank of the active flocculated sludge process using settling tanks for sludge separation.
In this equation C_X depends on the waste-water load of the settler Φ_i/A, on the sludge index SVI, and on recycle and wasteratio r and w.

In Table 3.1 it has been shown that the effect of r and w is very small and that eq. 3.36 can be approximated by

$$C_X = \frac{905}{SVI} * 1.94 * \left\{ \frac{\Phi_i}{A} \right\}^{0.444} \qquad \text{eq. (8.2)}$$

In Figure 8.2 C_X is shown as a function of SVI and Φ_i/A. It is obvious that the maximum biomassconcentration in the aeration tank decreases sharply when the sludge index increases and when the settler area decreases (Φ_i/A higher).

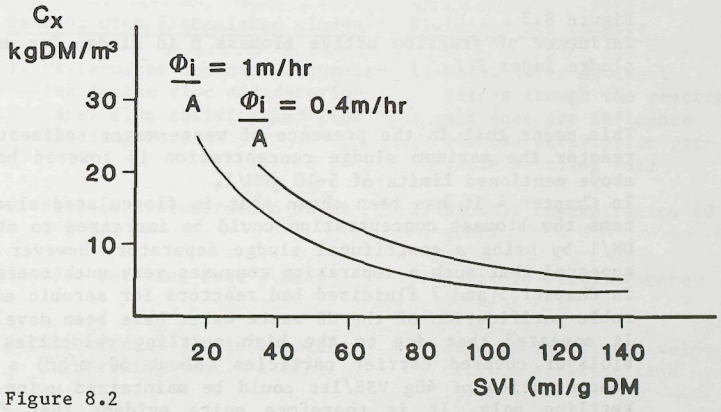


Figure 8.2
Maximum biomass concentration in a flocculated sludge reactor as a function of sludge index (SVI) and settler hydraulic loading (Φ_i/A).

For typical SVI values of 70-150 ml/gDM and a settler hydraulic loading of 0.4 m/hr the maximum flocculated biomass concentration lies between 5-10 gDM/l and the sludge settlingrate is about 5 m/hr (Figure 3.18).
The problem of limited settling capacity of flocculated sludge seems to be aggravated through the presence of waste-water sediment in the floc. In Chapter 3 it has been found for the GB waste-water that the sludge index increases rapidly if the fraction waste-water sediment in the floc dry matter increases (β smaller, See Figure 8.3.)

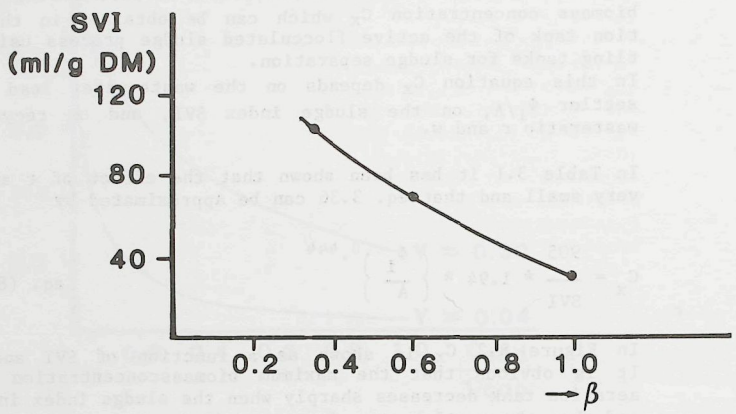


Figure 8.3
Influence of fraction active biomass β in sludge dry matter on sludge index SVI.

This means that in the presence of waste-water sediment in the reactor the maximum sludge concentration is lowered below the above mentioned limits of 5-10 gDM/l.
In Chapter 4 it has been shown that in flocculated sludge systems the biomass concentration could be increased to about 25g DM/l by using a centrifugal sludge separator. However it also appeared that such a separation consumes very much energy.
In Chapter 5 and 7 fluidized bed reactors for aerobic and anaerobic purification of the GB waste-water have been developed.
It appeared that due to the high settling velocities of the biolayer covered carrier particles (about 50 m/hr) a biomass concentration of 40g VSS/ltr could be maintained using gravity settling only. It is therefore quite evident that from the point of view of maximum biomass concentration the fluidized bed offers indeed great advantages compared to the flocculated sludge systems.

8.3.4 CONCLUSIONS

From the preceeding paragraphs 8.3.1-8.3.3 it is obvious that the fluidized bed reactor has to be preferred above the flocculated sludge reactor for biological treatment of the GB waste-water for a number of reasons which are outlined in Table 8.1.
The fluidized bed reactor is superior in biomass concentration and biomass activity and therefore reactorcapacities can be a factor 5-10 higher than in flocculated sludge reactors (Chapter 7.4.2.d).
In addition the required area of the reactor (especially the settler compartment) is much lower for the fluidized bed system due to the very high settling rate of the biomass covered carrier particles.
Finally, because of no aeration limitation problems, no energy consumption for O_2-transfer, and an inherent low sludge yield, the anaerobic fluidized bed treatment of the GB waste-water is preferred above aerobic treatment.
With respect to the fluidized bed anaerobic treatment it is advantageous to apply the two stage treatment (acidification, methanation).

Reactor with flocculated sludge	Fluidized bed reactor
1. Waste-water sediment accumulates in the floc and deteriorates floc activity and floc settling rate	1. Waste-water sediment passes trough the reactor and does not influence biomass activity or particle settling rate
2. Biomass concentration about 5 gDM/l	2. Biomass concentration 40 gDM/l
3. Floc settling rate 5 m/hr	3. Particle settling rate 50 m/hr
4. Superficial liquid velocity in settling tank $\leq$ 1 m/hr	4. Superficial liquid velocity in settling tank 10-20 m/hr
5. Reactors are large in volume and area (H/D ratio $\leq$ 1)	5. Reactors are compact, tall and slender (H/D ratio $\gg$ 1)

Table 8.1
Comparison of the fluidized bed reactor with the flocculated sludge reactor.

This choice for the anaerobic treatment of the GB waste-water does implicate however two prerequisites.

- The waste-water should be free from bacteriocidal compounds (Chapter 7.3.4) by controlling its handling at the source.
- For maximum purification efficiency with respect to COD and Kj-N a posttreatment of the sulfide and NH_4^+-containing anaerobic effluent is necessary (see Chapter 8.2.3).

In the next paragraph such a posttreatment method will be proposed and the needed future research for the full scale implementation of the proposed integral treatment system will be indicated.

8.4 PROPOSAL OF AN OPTIMUM HIGH RATE BIOLOGICAL TREATMENT SYSTEM FOR THE GB WASTE-WATER AND NECESSARY FUTURE RESEARCH

The conclusion of the paragraph 8.3.4 is that two-stage anaerobic treatment of the GB waste-water in fluidized bed reactors provides a compact small volume high rate purification process. Because of additional required sulfide and Kj-N elimination a posttreatment of the anaerobic effluent is necessary. Mulder (Ref. 8.1) has proposed an elegant treatment of the anaerobically treated GB waste-water. In addition the proposal was succesfully tested on labscale in flocculated sludge reactors using GB waste-water (Ref. 8.1).

In the next paragraph the principles of the proposed integral purification system using fluidized bed reactors instead of flocculated sludge reactors will be described.

8.4.1 DESCRIPTION OF A 4-STAGE BIOLOGICAL FLUIDIZED BED PURIFICATION PROCESS FOR THE GB WASTE-WATER.

The anaerobically treated GB waste-water contains considerable amounts of sulfide, which contributes to the COD of the water, and NH_4^+ which still needs to be eliminated by nitrification. Mulder (Ref. 8.1) has proposed to use this sulfide as the electron donor for denitrification of the nitrate produced in the aerobic nitrification stage.
This results in oxydation of HS^- to SO_4^{2-} and removal of NO_3^- to N_2 according to the reaction:

$$3H^+ + 5HS^- + 8NO_3^- \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O$$

The resulting process flowsheet is shown in Figure 8.4.

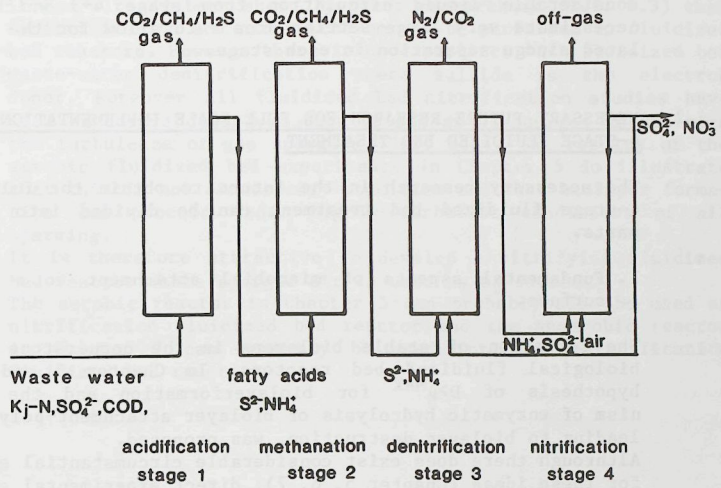


Figure 8.4
Flow sheet of the 4-stage fluidized bed purification process (acidification, methanation, denitrification, nitrification).

The waste-water is acidified in stage 1. During this acidification fatty acids are produced, NH_4^+ is produced by deammination reactions and S^{2-} is produced from SO_4^{2-}reduction. The fatty acids are subsequently metabolized to CH_4 in the methanation stage 2.
After stage 2 the waste-water still contains S^{2-} and NH_4^+. The S^{2-} is then oxydized to SO_4^{2-} by a denitrifying population (i.e. Thiobacillus denitrificans) in the denitrification stage 3. In this stage 3 the NH_4^+ in the waste-water is not significantly converted. Elimination of NH_4^+ occurs in the last stage 4 where nitrification of $NH_4^+ \rightarrow NO_3^-$ occurs. Part of the NO_3^- is recycled to stage 3 for the S^{2-} oxydation.
This treatment scheme has several advantages above a direct biological oxydation of the sulfide/ammonia containing anaerobic waste-water (Ref. 8.1).

- The sulfide containing waste-water is not brought into contact with air, as would occur if this water was oxydized in aerated reactors. This minimizes the possibility of odour problems.
- The S^{2-} oxydation occurs with NO_3^-. Compared to direct oxydation the oxygenrequirements are considerably reduced and also NO_3^- removal is obtained.
- The nitrification can be performed in a waste-water without sulfide which is toxic for nitrifiers.

It is obvious that, considering the arguments in paragraph 8.3, the preferred reactor for each stage will be a fluidized bed reactor and not a flocculated sludge reactor. This choice is even more advantageous because the 4-stage process contains a considerable liquid circulation from stage 4→3 which would necessitate very large settler area's to allow for the flocculated sludge separation in each stage.

8.4.2 NECESSARY FUTURE RESEARCH FOR FULL SCALE IMPLEMENTATION OF THE 4-STAGE FLUIDIZED BED TREATMENT

The necessary research in the future to obtain the full scale 4-stage fluidized bed treatment can be divided into several parts.

1. Fundamental aspects of microbial attachment to a carrier surface.

The formation of stable biolayers is the cornerstone of the biological fluidized bed reactors. In Chapter 5 and 6 the hypothesis of $D \geq \mu^{MAX}$ for biolayerformation and the mechanism of enzymatic hydrolysis of biolayer attachment-polymers, leading to biolayer destruction, was proposed.
Although there does exist considerable circumstantial evidence for these ideas (Chapter 5, 6, 7), direct experimental evidence should be obtained.
This research should give attention to the next topics of each attached biolayer system (acidification, methanation, nitrification, denitrification).
- analysis of polymers in the biolayer
- conditions of polymer production in the biolayer
- degradability of the polymers by micro-organisms
- factors which influence thickness of, and biomass concentration in, the biolayer.

The results of this research will provide detailed information regarding the conditions for biolayerformation and -stability for each type of biological fluidized bed reactor.

2. Scaling-up of the present pilot results of the two stage anaerobic treatment of GB waste-water.

This scaling up will be necessary in height and in width of the reactor.
A higher reactor will lead to higher liquid and gas superficial velocities and therefore to a somewhat lower biomass concentration because of more expansion of the fluidized bed. In principle this can be compensated by changing the present carrier (sand 0.1-0.3 mm) to a carrier with larger diameter and/or higher specific density.
A wider fluidized bed reactor will increase the problems of homogeneous liquid distribution (Ref. 8.3). Therefore an adequate design of the liquid distribution is necessary and research to obtain this is vital.

3. Development of pilot-scale fluidized bed purification for denitrification and nitrification using anaerobically treated GB waste-water.

It has already been shown by other researchers (Ref. 8.3) that denitrification and nitrification can be performed in fluidized bed reactors. However there is no experience with fluidized bed waste-water denitrification where sulfide is the electron donor. Moreover all fluidized bed nitrification studies have used expensive dissolved pure oxygen as O_2-sources to minimize the turbulence of gas sparging (Ref. 8.3). The results of the aerobic fluidized bed experiments in Chapter 5 do illustrate that this is not a necessary condition and that biolayer formation can proceed under highly turbulent conditions of air sparging.
It is therefore attractive to develop a nitrifying fluidized bed reactor where sparged air is used as O_2-source.
The aerobic reactor in Chapter 5 can probably also be used as nitrification fluidized bed reactor and the anaerobic reaccor in Chapter 7 can probably be used for the denitrification fluidized bed reactor.

REFERENCES

8.1 A. Mulder
Anaerobic treatment of SO_4^{2-} containing waste-water (in Dutch).
Report on the Researchproject Gist-Brocades
- Agricultural University of Wageningen, april 1982.

8.2 L.T. Mulcahy, W.K. Shieh, E.J. la Motta.
Prog.Wat.Technology 12, (1980), 143

8.3 Biological fluidized bed treatment of water and waste-water.
Ed. P.F. Cooper, B. Atkinson,
Ellis Horwood limited, (1981).

SYMBOLS

A	settler area	m^2
C_{COD}	concentration of soluble COD	kg/m^3
C_{SED}	concentration of sediment	$kgDM/m^3$
C_x	biomass concentration	$kgDM/m^3$
r	recycle ratio	-
SVI	sludge volume index	ml/gDM
w	waste ratio	-
Y	biomass yield	-
β	fraction active biomass in sludge dry matter	-
Φ_i	waste-water flow	m^3/hr

The research, reported in this Thesis, has led to the formulation the following Patent Applications by Gist-Brocades.

EPO 0024758 27/8/1979
An oxydative biological purification process of waste-water

EPO 0028846 7/11/1979
Process for preparing biomass attached to a carrier.

EPO 0090450 29/3/1982
Fluidized bed reactor for purification of waste-water.

SUMMARY

The waste-water from the Gist-Brocades fermentation plant in Delft has a high load of biodegradable material and therefore poses a serious pollution problem.
Application of low rate conventional biological treatment techniques is not possible due to limited availability of area for the treatment plant. Furthermore strict conditions are to be met with regard to odour because of nearby populated city areas.
Therefore the development of a high rate biological treatment process was undertaken.

In Chapter 3 it has been shown that aerobic flocculated sludge treatment of the highly variable waste-water from Gist-Brocades can proceed in a tall bubble column with external sludge recirculation using a settler. With regard to necessary settler area and liquid residence time in the aeration tank, a simple model description is presented.
The model provides a coupling between the biological conversion process and the settling process and results in a design equation of necessary reactor volume and settler area which ensures stable purification.
With regard to the treatment process it has been found that sediment which is present in the waste-water seriously decreases the treatment capacity because both sludge activity and sludge settling rate are diminished due to sediment incorporation in the sludge floc.
Furthermore the large surplus sludge production in this process is a serious drawback.
In Chapter 4 a principal solution has been offered to eliminate surplus sludge production in aerobic purification processes. This a achieved by feeding the micro-organisms only the amount of substrate needed for cell maintenance. Using this so called "maintenance concept" a stable purification is achieved in an aerated reactor fed with the highly variable GB waste-water where all the sludge was recirculated using a centrifugal separator. In an experimental run of 2000 hrs. no surplus sludge was produced as evidenced by carbon, solids and COD balances. Also total nitrification of the waste-water is achieved. The application of this process on full scale is however seriously hampered by the facts that centrifugal sludge separation is far too expensive, sludge separation in a settler requires to much area and that the process is expected to be only stable with waste-water which contains no sediment. This means that a presettling tank is essential.
However a biological fluidized bed reactor, where the biomass grows attached to a heavy carrier, should in principle offer adequate solutions to each of the above mentioned problems.
Sludge separation should be easy because the biomass covered carriers settle very fast and waste-water sediment should not accumulate in the reactor because of the high liquid superficial velocity (sediment is then washed through the reactor).
In Chapter 5 the development of such an aerobic fluidized bed reactor has been described. It was found that stable, strongly attached, aerobic biomass to sand in an air sparged turbulent reactor can only by obtained if the liquid residence time is very short. During this experiment several indications were obtained that also in a biological fluidized bed reactor aerobic surplus sludge production can be eliminated by applying the maintenance concept. In addition it has been shown that a large increase in liquid residence time, while maintaining

the same hydraulic conditions and COD load of the reactor, results in a fast destruction of the attached biomass. To explain this phenomenon a hypothesis has been formulated which states that non-attached micro-organisms can enzymatically hydrolyse the polymers attaching micro-organisms to carriers.
This hypothesis leads to the condition

$$D \geq \mu^{MAX}$$

for biolayer development. D is the dilutionrate of the reactor (or reciprocal liquid residence time) and μ^{MAX} is the maximum growth rate of the micro-organisms in the reactor.
If this condition is not met, $D \leq \mu^{MAX}$, biolayer formation does not occur and existing biolayers are readily destroyed. Besides the conditions for biolayer formation Chapter 5 deals with the design and functioning of a novel air sparged fluidized bed reactor.
Despite the successful development of the aerobic fluidized bed reactor this system cannot be applied to the concentrated waste-water of Gist-Brocades, due to aeration limitation which occurs at the necessary high dilution rate. However from an extensive literature survey in Chapter 6 about microbial attachment to a carriersurface it appears that the above mentioned condition $D \geq \mu^{MAX}$ probably is a generally valid condition for any mixed microbial population.
Furthermore there appears to be substantial evidence in support of the proposed hypothesis of enzymatic hydrolysis of attachment polymers.
Since anaerobic purification of waste-water does not suffer from aeration limitation, and since the biolayerformation of anaerobic bacteria probably is also governed by the $D \geq \mu^{MAX}$ condition the logical alternative of the aerobic fluidized bed system is the development of an anaerobic biological fluidized bed reactor for the purification of the concentrated waste-water of Gist-Brocades. Additional advantages, compared to aerobic treatment, are of course energy production (CH_4) and very low surplus sludge production.
Chapter 7 deals with the successful development of this system.
It has been shown that it is advantageous with respect to biolayer thickness and biolayer activity in the methane producing fluidized bed reactor to use acidified waste-water. This acidification can be performed in a first stage fluidized bed reactor with attached acidifying micro-organisms.
The methanogenic fluidized bed reactor can reach a very high conversionrate of about 55 kg COD/m^3day and is very stable because sludge wash-out is impossible. This conversion rate is about 5-10 times higher than for the conventional system with flocculated anaerobic sludge. The high conversionrate is due to a higher biomass concentration in the fluidized bed reactor (because of the high settling velocity of the biomass covered carriers) and a higher biomassactivity (because, due to the high superficial liquid velocity, waste-water sediment does not accumulate).

An evaluation in Chapter 8 of the different treatment schemes, developed in Chapters 3-7, with regard to purification efficiency and treatment capacity of the GB waste-water clearly reveals the advantages of anaerobic fluidized bed reactors (high treatment capacity, small area requirement, very stable due to efficient sludge retention). Because anaerobic treatment of the SO_4^{2-} containing Gist-Brocades waste-water results in an effluent which contains S^{2-} and NH_4^+ a post treatment is

necessary to achieve maximum purification efficiency.
This post treatment is proposed to be performed as a denitrification-nitrification two-stage fluidized bed process, where sulfide is used as electron donor in the denitrification stage.

The resulting proposed optimum treatment system for the Gist-Brocades waste-water is then a 4-stage fluidized bed system.
The stages are acidification, methanation, denitrification, nitrification.
This treatment system is expected to give the highest conversion rate, highest efficiency of COD and nitrogen removal and virtually no sludge production. Odour emissions into the environment will be absent because the compact fluidized bed reactors are completely gas-tight.
Finally a approximate indication is provided of the necessary future research items to obtain full scale implementation of this 4-stage treatment system.
Based on the presented results for anaerobic treatment of waste-water Gist-Brocades has designed and started the construction of a full scale 2-stage anaerobic fluidized bed treatment plant. The plant consists of an acidification and a methane producing fluidized bed reactor of 300 m^3 each for the treatment of 200 m^3/hr of waste-water. Start-up of the plant is scheduled in 1984.

SAMENVATTING

Het afvalwater van Gist-Brocades van het fermentatie produktie komplex te Delft is sterk verontreinigd met goed afbreekbare organische stoffen en vormt daarom een ernstig milieu probleem.
Toepassing van de bekende laag-belaste biologische zuiveringsprocessen is niet mogelijk omdat de benodigde ruimte niet aanwezig is. Bovendien moet de zuivering absoluut stankvrij zijn in verband met de zeer dichtbij gelegen woonwijken.
Om aan deze eisen tegemoet te komen was het noodzakelijk om een kompakt, hoog belastbaar, biologisch zuiveringsproces te ontwikkelen.

In Hoofdstuk 3 is aangetoond dat het sterk variërende afvalwater van Gist-Brocades aeroob kan worden gezuiverd met een hoog CZV/BZV rendement in een hoge bellenkolom met recirculatie van het vlokkig slib. Er is, ten behoeve van ontwerpdoeleinden een simpel model ontwikkeld welk de benodigde vloeistofverblijftijd in de reactor koppelt aan het beschikbare slibbezinker-oppervlak.
De zuiveringskapaciteit van het aerobe proces werd sterk nadelig beïnvloed door de aanwezigheid van organisch sediment in het afvalwater. De redenen hiervoor zijn de daling van de aktiviteit en de verslechtering van de bezinkeigenschappen van het slib bij toenemende sedimentgehaltes.
Bovendien is de enorme produktie van surplusslib een groot nadeel (in verband met een additioneel te bouwen slibverwerking).

In Hoofdstuk 4 is een principiële oplossing uitgewerkt ten einde deze surplusslib produktie in het aerobe zuiveringsproces te elimineren.
De basis van de oplossing is het zgn. "onderhoudsconcept". Volgens dit concept bestaat er voor micro-organismen een substraat belasting waarbij geen groei mogelijk is omdat alle substraat wordt verbrand ten behoeve van energieproduktie om het micro-organisme in stand te houden.
Dit concept is toegepast door gedurende 2000 uur het sterk variabele afvalwater van Gist-Brocades te zuiveren in een aerobe vlokkig slib reactor waarin met behulp van een centrifugaalscheider alle slib in de reactor werd teruggevoerd. Bij deze procesvoering blijkt dan inderdaad geen surplusslib te worden geproduceerd (gemeten via koolstof, vaste stof- en CZV balans), en het proces verloopt stabiel met een hoog rendement voor zowel CZV/BZV als N-verwijdering (nitrificatie).
Voor een praktische toepassing van dit proces is, naast een voorbezinker voor de verwijdering van afvalwatersediment, echter een aktief slibscheidings techniek nodig waarmee goedkoop een hoge slibconcentratie kan worden bereikt.
Een aantrekkelijke wijze om de voornoemde problemen op te lossen is om het aktieve slib op een zware drager te laten groeien in de vorm van biolagen in een zgn. Wervelbed reactor.
De slibscheiding is dan zeer eenvoudig (door de hoge bezinksnelheid van de drager met biolagen) en het afvalwatersediment kan zich niet in de reactor ophopen omdat de stroomsnelheid van het water hoger kan zijn dan de bezinksnelheid van het sediment.

In Hoofdstuk 5 wordt de ontwikkeling van deze aerobe wervelbed reactor beschreven. Het is aangetoond dat het (verdunde) Gist-Brocades afvalwater in een dergelijke reactor met een hoog CZV rendement wordt gezuiverd met een zeer lage slibproduktie door toepassing van het "onderhoudsconcept".

Daarnaast blijkt dat de ontwikkeling van aerobe biolagen op drager alleen gebeurt bij een korte vloeistofverblijftijd en dat bestaande biolagen zeer snel stuk gaan als de vloeistofverblijftijd wordt verlengd (bij konstante hydraulische kondities en een constante CZV-belasting van de reactor).
De verklaring voor de invloed van de vloeistofverblijftijd op de hechting van micro-organismen op dragers is gezocht in de hypothese dat niet-hechtende micro-organismen door enzymatische hydrolyse van de hechtingspolymeren de biolaagvorming van hechtende micro-organismen tegengaan. Biolagen kunnen zich dan alleen ontwikkelen als de niet-hechtende micro-organismen worden uitgespoeld. De hypothese leidt dan tot de voorwaarde van biolaagvorming

$$D \geq \mu^{MAX}.$$

De gevonden noodzaak van een korte vloeistofverblijftijd bij het bedrijven van een wervelbed reactor maakt het onmogelijk (in verband met aeratie-beperking) om het gekoncentreerde Gist-Brocades afvalwater aeroob met dit proces te zuiveren.

Echter in Hoofdstuk 6 is, aan de hand van een literatuurstudie over hechting van micro-organismen, aannemelijk gemaakt dat de gevonden voorwaarde voor biolaagvorming, $D \geq \mu^{MAX}$, een algemene geldigheid heeft.

In Hoofdstuk 7 is deze aanname getoetst door, aan de hand van het $D \geq \mu^{MAX}$ koncept een anaerobe wervelbed reactor te ontwikkelen. In een dergelijke reactor groeit het methaanslib als een dunne biolaag op een zware drager. Zowel 1- als 2-traps anaerobe zuivering in wervelbed reactoren is onderzocht. De 2-traps zuivering blijkt dan beter te voldoen. De zuiveringskapaciteit van de anaerobe wervelbed reactor is zeer hoog (55 kg CZV/m^3 dag) dankzij de zeer goede bezinking van het slib op drager (hetgeen leidt tot een hoge slibconcentratie van 40 kg O.S./m^3) en dankzij de afwezigheid van sediment ophoping in het slib van de reactor (hetgeen leidt tot een hoge slibaktiviteit van 1.8 g CZV/gOS dag).

In Hoofdstuk 8 wordt aangegeven dat, voor maximale zuivering van CZV en organische N het anaeroob gezuiverde afvalwater (met daarin nog HS^- en NH_4^+), moet worden nagezuiverd. Voor deze nabehandeling lijkt een 2-traps wervelbed systeem van denitrificatie (met HS^- als electrondrager) en nitrificatie veelbelovend.
Uiteindelijk resulteert dan een biologisch zuiveringssysteem van 4 wervelbed reactoren (verzuring, methaanvorming, denitrificatie, nitrificatie) die elk een hoge zuiveringskapaciteit hebben, weinig plaats innemen, waarin nauwelijks surplusslib zal worden geproduceerd en waarin geen stank zal ontstaan. Tot slot wordt aangegeven op welke gebieden nog onderzoek noodzakelijk is om te komen tot realisatie van het integrale 4-traps wervelbed proces op praktijkschaal.
Op basis van de hier gepresenteerde resultaten van het 2-traps anaerobe wervelbed proces op semi-technische schaal (Hoofdstuk 7) bouwt Gist-Brocades op dit moment te Delft 2 wervelbed reactoren van elk ca. 300 m^3 voor de anaerobe zuivering van 200 m^3/uur afvalwater.
Deze installatie zal in 1984 opstarten.

CURRICULUM VITAE

Joseph Johannes Heijnen is op 20 januari 1951 geboren te Tegelen in Nederland als jongste van de 8 kinderen van J.C.M. Heijnen en M.P. Heijnen-Rutten.
Na voltooiing van de HBS-B opleiding aan het "Thomas college" te Venlo is in 1968 een aanvang gemaakt met de opleiding tot Scheikundig Ingenieur aan de Technische Hogeschool te Eindhoven.
Deze studie leidde tot de toekenning van de "Unilever Chemie prijs" op 15 november 1972 en werd op 12 september 1973 met lof afgesloten met het Ingenieursexamen.
Het afstudeeronderzoek is verricht onder leiding van Prof.Dr. K. Rietema met als onderwerp "Porositeits- en drukverdeling rondom een bel in een twee-dimensionaal gefluidiseerd bed".
Op 15 oktober 1973 is de auteur in dienst getreden van Gist-Brocades alwaar de volgende funkties zijn vervuld:

- 1973-1977 Bedrijfsingenieur aan de Technisch Wetenschappelijke Staf van de pencilline gistingsfabriek.
- 1978-1982 Wetenschappelijk medewerker van de Research en Development organisatie van Gist-Brocades binnen de groep "Procesontwikkeling" alwaar is gewerkt aan antibiotica en enzym fermentaties en aan de ontwikkeling van nieuwe biologische afvalwater zuiveringstechnieken.
- 1982- Projektleider binnen de Research en Development organisatie van het onderzoek betreffende verdere ontwikkeling en toepassing van de biologische wervelbed (fluid bed) reactoren.

STELLINGEN

1. De conclusie dat remming ten gevolge van sulfide ophoping geen factor van betekenis is in de competitie tussen SO_4^{2-}-reductie of CH_4-vorming uit acetaat in experimenten waarbij het gevormde sulfide volledig wordt gestript door N_2-begassing ontbeert iedere grondslag.

   P. Schönheit e.a. Arch. Microbiol. (1982), 132, 285

2. Bij de berekening van een pH-gradient in een biofilm is ten onrechte geen rekening gehouden met buffercapaciteit van de micro-organismen zelf.

   E. Arvin, G.H. Kristensen
   Wat. Sci. Technol. (1982), 14, 833

3. The solution to pollution is dilution(rate)
   (Chapter 5, this Thesis).

4. Toepassing van de dynamische methode van klA-meting in viskeuze media moet ten strengste worden ontraden.

   J.J. Heijnen e.a. Influence of very small bubbles on the dynamic klA-measurement in viscous gas-liquid systems.
   Biotechn. and Bioeng. 22, 1945, (1980)

5. De hoge activiteit van aeroob actief slib in de Hubstrahl Reaktor is waarschijnlijk niet te danken aan een "Mechano-biochemisch effect" maar aan de selectie van een aerobe mengculture met hoge groeisnelheid onder de toegepaste condities van korte vloeistofverblijftijd.

   H. Brauer
   Biologische Abwasserreinigung im Hubstrahl Reaktor.
   GVC/VDI Tagung "Verfahrenstechnik der biologischen Abwasserreinigung" Krefeld 27/28 Oktober 1983.

6. Het publiceren van hoge efficiencies (b.v. kg O_2 overdracht per kw uur energieverbruik) is zinloos zonder het vermelden van de snelheden (b.v. kg O_2/m^3 uur).

7. Het veelgehoorde argument dat biotechnologische processen minder energie vergen en milieu-vriendelijker zijn dan chemische processen is slechts beperkt geldig.

8. De ontwikkeling van de r-DNA technieken vertoont boeiende c.q. verontrustende overeenkomsten met de ontwikkeling van de atoomsplitsingstechnieken voor wat betreft initiële euforie, secundaire huiver en (non)acceptatie door de maatschappij.