THESES

belonging to the dissertation

Aerobic Heterotrophic Biodegradation in Polluted Drains and Sewers

The drain and sewer as dual-phase biological reactors

Ye-Shi Cao

November 1994

1. Much effort has been spent on improvement of the respirometer design for precise measurement of benthic respiration, but little attention has been paid to the function of suspended micro-organisms which can be quite competitive when compared to the function of the benthic phase.

James (1974) The measurement of benthic respiration. Wat. Res. *8, 955-959.*
Hickey C.W. (1988) Benthic chamber for use in river: testing against oxygen balance. J. Env. Eng. ASCE, *11, 825-845.*

2. Many experiments have been conducted to explore the effect of reactor design on biofilm ecology and composition. However, because such studies often spend more attention to the reactors' geometry than to reactor process characteristics like flow pattern and concentration gradient, many of their conclusions cannot be rationalized.

Morgan J.M., Evison L.M. and Forster C.F. (1991) Changes of microbial ecology in anaerobic digesting treating ice-cream wastewater during starting-up. Wat. Res. *5, 639-653.*

3. One crucial hypothesis used by Siegrist and Gujer (1985) leading to the conclusion of the existence of convective or flow-induced transport in the upper regions of the biofilms is by assuming that the real contact surface between liquid and biofilm equals the projected area. Actually, this often is unlikely especially in the case of filament dominated biofilm.

Siegrist H. and Gujer W. (1985) Mass transfer mechanisms in a heterotrophic biofilm. Wat. Res. *19, 1369-1378.*

4. Thanks to Danckwerts' contribution to the theory of residence time distribution in vessels, it is possible to study the hydraulic situation in equipment of different scales. Therefore, if there were a Nobel price in chemical engineering, it should be awarded to him.

Danckwerts P.V. (1953) Chem. Eng. Sci. *2, p.1.*

5. The oxidation ponds can be an appropriate technology only when the algae could be removed from the effluent; in many cases it is not feasible, however.

6. Reduction of excess sludge production is an increasing important reason why anaerobic processes such as UASB and EASB reactors and aerobic biofilm reactors such as biofilters and air-lift suspension reactors are drawing strong interest.

7. Low-cost technology needs an as thorough understanding with regard to the processes involved as the so-called advanced technology, because of the demanding requirement of easy maintenance and operation by unskilled operators.

8. Nowadays "sustainable development" is at the focus of many discussions, but preconditions to achieve it are a healthy economic policy and social stability.

9. Chinese environmental protection policy is almost perfect. But enforcement of regulations and public involvement are two issues critical for its effective implementation.

10. Along with the popularity of computers and software, mathematical modelling and simulation are becoming a fashion. However, only seeking data that fit nicely black boxes, rather than use them to investigate the physical background and composing processes, can make this kind of work comparable to playing computer games.

11. Engineers should not be confused with regard to the difference between a piece of leaf and the forest.

12. The following famous dialogue, written by Larzhi, an ancient Chinese philosopher, tells us the limitation of the efforts made to explore the truth of the nature and social life.

    "How exciting the fish is!", people standing on the river bank sighed.
    "How can you know the fish's feeling?", one person asked.
    The answer was, "You are not a fish, how you know it is not happy."

13. The Dutch and Chinese are similar, to some extent, on the way they spend money; it might be one of the advantages for Chinese studying in the Netherlands.

AEROBIC HETEROTROPHIC BIODEGRADATION IN POLLUTED DRAINS AND SEWERS

THE DRAIN AND SEWER AS DUAL-PHASE BIOLOGICAL REACTORS

Aerobic Heterotrophic Biodegradation in Polluted Drains and Sewers

The drain and sewer as dual-phase biological reactors

DISSERTATION
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to be defended in public
on Monday, 7 November 1994 at 10:30 h

by

YE-SHI CAO

born in Shanghai, People's Republic of China
Master of Science in Chemical Engineering

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This dissertation has been approved by the promotor and co-promotor:
Prof. dr ir J.J. Heijnen, TU Delft
Prof. dr ir G.J.F.R. Alaerts, IHE Delft

Other committee members:
Chairman: Rector Magnificus, TU Delft
Co-chairman: Rector, IHE Delft
Prof. dr ir K.C.A.M. Luyben, TU Delft
Prof. dr P. Denny, IHE Delft
Prof. dr C. Polprasert, Asian Intitute of Technology
Prof. dr ir W. Verstraete, University Gent

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CONTENTS

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"Recall the resource while drinking". I hope this piece of work can be a happy gift to my teachers in the primary and secondary schools and Universities in China where I received my education. I do feel indebted to many of my previous teachers; their working attitude and thinking deeply impressed and largely shaped me, and encouraged me to overcome obstacles.

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Finally, my thanks from the heart go to my virtuous wife Xinghong and my lovely son Xiaoyi, and I wish this piece of work can be a compensation for their sacrifice for this study.

ABSTRACT

Cao Y.S. (1994) *Aerobic heterotrophic biodegradation in polluted drains and sewers - The drain and sewer as dual-phase biological reactors.* Doctoral thesis, Technical University of Delft (TUD)/International Institute for Infrastructural, Hydraulic and Environmental Engineering (IHE), 1994, Delft, The Netherlands. 131 pages, 1 appendix.

Drains, sewers, and polluted shallow aquatic systems such as rivers, streams, and lagoons are characterized by the fact that biomass is not only suspended in the liquid but also attaches on the solid material surface. Understanding of the processes in these dual-phase systems is essential to water quality management, wastewater treatment plant design and operation, the study of the feasibility of enhancing biodegradation in-line, or using sewers as part of wastewater treatment plants. So far a systematic, mechanistic approach in the investigation on such dual-phase systems is lacking.

In this study, three reactors are adopted to study aerobic heterotrophic biodegradation in drains and sewers including intrinsic and process kinetics, microbial community and mathematical modelling. They are a batch reactor representing a plug flow, and the CSTR and the channel reactor representing two types of well-mixed reactor configurations. The flow velocity and shear stress in the channel reactor are one order of magnitude smaller than in the other two reactors. To distinguish the relative function of the two biomass types the system was investigated by allocating the biomass separately to liquid and solid phases.

The results demonstrated that the parameters such as suspended biomass concentration X (mg/L), its maximum growth rate μ_{max} (day^{-1}), biofilm oxygen up-take rate BUR ($gO_2/m^2.day$), and the specific area WA/V (m^{-1}) are essential to understand and describe dual-phase system processes. These should be considered in experimental design, in mathematic model development and in simulation.

The measurements indicate that the intrinsic specific oxygen consumption activity of suspended biomass (based on dry weight) is 50-100% higher than that of the biofilm biomass. The inert materials including extracellular polymeric substance (EPS) accumulation in biofilm is supposed to be the main reason. The apparent biofilm activity can be further reduced by mass transfer resistance. This leads to the conclusion that suspended biomass can play a significant role even under so low concentrations that it is hardly detected as turbidity. The traditional one chamber design of the respirometer could result in an overestimation of benthic respiration activity, and should be improved.

Comparison of the experimental results illustrates that a plug flow favours floc-forming bacteria, while a well mixed reactor favours filamentous growth. No effect of shear stress on biofilm

composition was found in the ranges of stress applied here. The reactor type and shear stress each exert their effect on biofilm morphology, composition and kinetics. A conceptual model is proposed for the interaction between reactor type and shear stress, and biofilm morphology, composition and kinetics.

A mathematic model, mainly describing oxygen consumption, with two variations in the kinetics, *i.e.* the first and zero order with respect to soluble organics, and containing relevant parameters in both liquid and solid phases, has been developed.

The study indicates that in-line purification can be enhanced by increasing specific biofilm area, or recycling activated sludge into the lines, depending on the conditions. However, from a more comprehensive point of view an integration and optimization of soluble COD, nitrogen and phosphate removal should be sought.

Obviously, the relevance of the results from this study is not only limited to drains or sewers, but can improve the understanding of the processes in natural or man-made shallow aquatic systems such as lakes, lagoons and wetlands, and parts of bioreactors.

Key words sewers, drainage, self-purification, streams, deposit, sediment, respirometers, oxygen consumption, biodegradation, suspension growth, biofilm, biofilm morphology, shear stress, population dynamics, filamentous bacteria, *Sphaerotilus natans*, reactors, hybrid reactors, mixed-culture reactors, kinetics, mass transfer, mathematical modelling.

Chapter 1

Kinetics, Biocenosis, and Modelling of Aerobic Dual-Phase Systems - an Introduction

1. INTRODUCTION

1.1. Biodegradation in drain and sewer systems

Wastewater conveyance systems such as sewers, drains, and polluted shallow aquatic systems such as some rivers, streams, and lakes, are characterized by the fact that microorganisms not only are suspended in the liquid ("liquid phase") but also attached to the solid surface area of *e.g.* walls ("solid phase") (Fig.1a). Pollutants can be discharged at a single or at multiple points (Figs.1b,c).

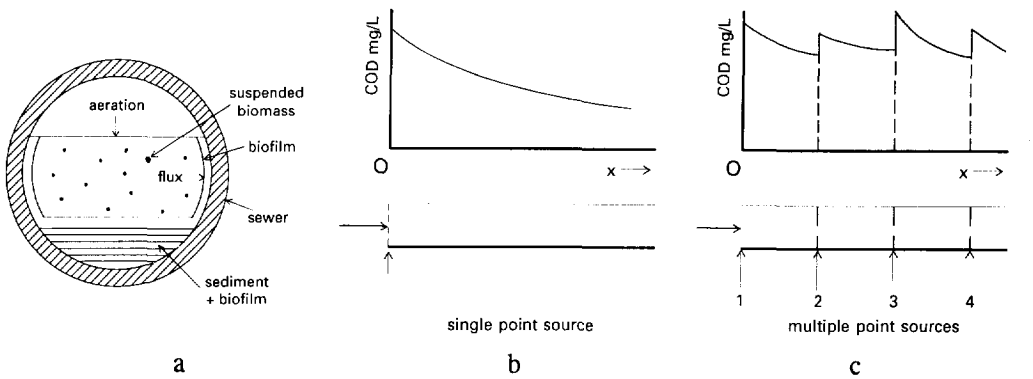

Fig.1 Diagram of a drain system and pollutant profile. x: path length along drain. a. Liquid and solid phase; b. COD profile under single point discharge; c. COD profile under multiple point discharge.

The suspended biomass may consist of a suspension in the form of individual cells or very small aggregates with a limited number of cells (dispersed condition), or of flocs suspended in the liquid like activated sludge in an aeration tank (flocculated condition). It can originate from biomass detached from the biofilm due to erosion or sloughing, or from multiplication in the suspended state.

The attached biomass may, on the other hand, take the form of biofilm (or benthic biofilm) consisting exclusively of organic material grown on the smooth walls and bottom of the sewer or drain, or on the riverbed's gravel or stone; it may in fact also take the form of a sediment *i.e.* attached biomass mixed with freshly settled mineral or organic or old (stabilized) organic matter, when the water flow velocity is low and the solid phase support is soil or sand. It is reported that biofilm comprises almost 90% of the total biomass amount in the biosphere (Flemming, 1993) and 90-99% of total biomass in the polluted shallow rivers and streams (Wuhrmann, 1972). Because of mass transfer resistance for oxygen, aerobic and anaerobic layers can occur simultaneously within one biofilm.

One of the differences between these two benthic types is that conceptually, the first biomass type takes substrate (and oxygen) from the liquid while the latter could take substrate from the benthos itself in addition to from the liquid phase. The biofilm oxygen up-take rate (flux),

BUR (gO_2/m^2.day) results from biological activity while oxygen consumption of the sediment termed SOD (sediment oxygen demand, gO_2/m^2.day) consists of BSOD, the sediment oxygen demand resulting from biological respiration by all living organisms in the sediment, and CSOD, the sediment oxygen demand resulting from chemical oxidation of reduced substances such as sulfide, ferrous compounds, *etc.* However, biotechnology literature tends to approach all these aggregate types as biofilm. In this thesis BUR is adopted to express oxygen up-take rate (flux) for two types of benthic phase.

Polluted water, which is also turbid, like domestic sewage, is intuitively thought to carry bacteria in the liquid phase. Inside a sewer also the bacteria in the slimy biofilm layer on the bottom and wall (solid phase) grow on the substrate in the sewage, which explains why such a system can be considered dual-phase. The moderately polluted drains and shallow rivers, which contain fairly transparent water, are conventionally thought to be exclusively dominated by their biofilm or benthic activity.

A series of physical, chemical and biological processes occur in sewers, drains and rivers. In gravity sewers significant amounts of pollutants (*e.g.* about 6-25% of BOD) can be biodegraded in-line aerobically and/or anaerobically (Pomeroy and Parkhurst, 1972; Hogendoorn-Roozemond, 1984; Saldanha and de Ribeiro, 1991; Raunkjær *et al.*, 1993). Biodegradation also occurs in a pressure sewer under anaerobic condition (Hvitved-Jacobsen *et al.*, 1994), as also testified by the large quantities of CH_4 generated in gravity sewers; in the 19th and early 20th century London lit many of its street gas lamps with collected "sewage gas". The activities of the respective microbial communities are comparable with those of activated sludge (Lemmer *et al.*, 1994). Research has been carried out on the production of H_2S and sulphuric acid by the biofilm, and on aeration in gravity sewers to prevent sewer corrosion (Nielsen and Hvitved-Jacobsen, 1988; Jensen and Hvitved-Jacobsen, 1991). A review on the microbiological wastewater transformation in sewers was given by Nielsen *et al.* (1992).

In polluted shallow streams the active benthic biofilm is found downstream of wastewater discharges especially when the wastewater originates from food processing, paper and pulp industry, domestic pollution, *etc.* (Kittrell and Kochtitzky, 1947; Roberts, 1977; Srinanthakumar and Amirtharajah, 1983). These benthic biofilms are often called sewerage fungus and may consist of *Sphaerotilus natans*, a typical filamentous bacteria with relatively small value of the saturation constant K_s in the Monod equation featuring high activity already at low substrate concentration; this is a so called K-strategy growth characteristic, in contrast to the r-strategy growth characteristic of floc-forming bacteria with commonly a larger K_s value featuring high activity only at high substrate concentration (Kappeler and Gujer, 1992). Comparatively high rates of self-purification in shallow polluted rivers (depth < 1.5 m), for example as compared with deep rivers (depth > 1.5 m), were found to be related to the benthic phase (Rutherford and O'Sullivan, 1974; Avnimelech *et al.*, 1981; Boyle and Scott 1984; Velz, 1984; Hickey, 1988a); the oxygen profile along the rivers (oxygen sag curve) becomes steep and the minimum oxygen concentration (oxygen sag

point), where the biodegradation rate is equal to the aeration capacity, can get lower (Fig.2). This would pertain not only to organic pollutants, but also to N and P transformation and removal (Tuffey *et al.*, 1974; Curtis *et al.*, 1975; Velz, 1984; Thomann and Mueller, 1987; Wyer and Kay, 1989). As oxygen and organic pollutants content are the two most important parameters in water quality, many studies focus on aerobic heterotrophic biodegradation processes.

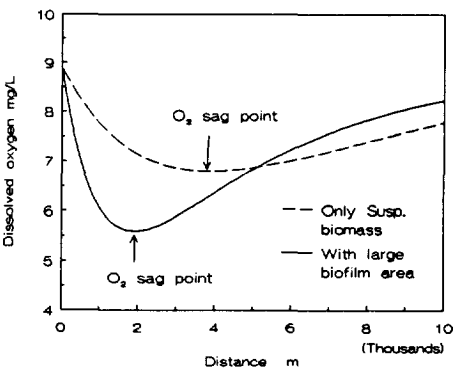

Fig.2 Oxygen profiles of the rivers with and without substantial effect of benthic biomass

For shallow lakes and microphyte-dominated systems, the release of nutrients from the sediment can under certain circumstances be the major nutrient source for photosynthetic growth; the interface and exchange processes between the water and sediment are crucial to the understanding and prevention of eutrophication (Sweerts, 1990).

Given the above the concept emerged to consider sewers as part of the wastewater treatment scheme or plant, and enhance self-purification *e.g.* through supplying oxygen (Pomeroy and Parkhurst, 1972) or adding activated sludge into the sewer (Green *et al.*, 1985). On the other hand, attempts have been made to enhance self-purification capacity in drains by immobilizing cells on the surface of the walls and bottoms (Phaup and Gannon, 1967; Wuhrmann, 1972; Curtish *et al.*, 1974; Liu *et al.*, 1986; Zhang *et al.*, 1986; Karnchanawrong and Polprasert, 1991). Carrier materials have been placed into oxidation ponds as well to favour attached biomass growth to attempt to achieve higher treatment efficiency (Reynolds *et al.*, 1973; Wang *et al.*, 1991; Baskaran *et al.*, 1992).

Biofilm-based wastewater treatment reactors have been developed because of their high biomass concentration retained in the system and because of assumed operational advantages (aeration does not have to occur by expensive mechanic aerators, active sludge needs not be recycled, and the large sedimentation tank is not necessary). Some reactors contain both attached and suspended biomass in comparable amounts, such as the Rotating Biological Contactor (RBC), whose suspended biomass can amount to 15% of total biomass (Pan and Hartmann, 1992), and Turbulent Beds in which suspended biomass accounts for 25-35% of

total biomass (Lazarova *et al.*, 1993a). These reactors are sometimes termed hybrid (Lee, 1991) or mixed-culture reactors (Lazarova *et al.*, 1993b).

Characteristically, the systems mentioned above are a reactor kind differing from the newly developed Biofilter or Air Lift Suspended Reactor (ASR), whose specific biofilm area amounts to 3000-5000 m^2/m^3; hence the very large biofilm is likely to dominate the reactor, and the role of suspended biomass is negligible. On the other hand, the systems differ from activated-sludge type reactors (including *i.a.* carrousel, oxidation ditch and sequential batch reactors) as well, for which the biofilm function is likely to be negligible. The dual-phase systems, as mentioned, comprise suspended and attached growth. Most research has been carried out on the activated sludge and biofilm processes; the majority of sewage treatment reactors are considered to be typically single-phase, dominated by the suspended biomass or the biofilm. Most of the few studies on the dual-phase systems are of empirical nature, based on simplifying assumptions or lacking a mechanistic approach.

1.2. Salient conclusions from other research

Upon reviewing literature the following conclusions relating to the significance and mechanisms of the self-purification in sewers and drains are drawn:

- Recent efforts have been made to formulate a new rational water quality management strategy to integrate sewers, treatment plants and receiving water bodies as proposed by Lijklema *et al.* (1993). The biodegradation in wastewater collection systems, and polluted shallow aquatic systems is related to physical, chemical and microbiological processes and can to a significant degree determine the wastewater composition and the loading of the subsequent treatment plants and receiving waterbodies, and hence directly influences the water quality of receiving waterbodies. A thorough understanding of the biodegradation in these systems is meaningful to the water quality management.

- Investigations on the processes of the above mentioned systems would allow more rational design and operation of wastewater treatment plants (Marsalek *et al.*, 1993).

- The feasibility should be explored of enhancing in-line self-purification in wastewater collection systems, which will contribute to a decrease in the loading of the subsequent treatment plants, to the extent that the collection systems can be regarded as a partial alternative to wastewater treatment. This would likely pertain primarily to sub-tropical and tropical zones where consistently high sewage temperature would be conducive to self-purification, and to developing countries where much municipal sewage is collected and conveyed by open and flat drains rather than closed, sloping gravity sewers.

- Many wastewater reactors (including treatment systems, as well as the microbial self-purification occurring in the natural and engineered locations, such as sewers and rivers)

are considered single-phase biofilm dominated. Given the complexity of the biofilm processes involved, knowledge on the kinetics and reactor process mechanics is still limited. This is especially true in the context of the dual-phase concept, where disperse suspended biomass may be less hindered by mass transfer resistance (diffusion limitation) than biofilm.

2. MICROBIAL PROCESSES IN THE SOLID AND LIQUID PHASE

2.1. Solid phase

2.1.1. Biofilm processes Reports on the biofilm conditions in sewer system are limited. In Denmark it was found that the attached biomass in gravity sewers is commonly mixed with settled organic and inert materials; it has a thickness of 1-3 mm. Biofilms exclusive of settling organic or other matter exist in sewage pressure mains and have a thickness of 0.1-0.5 mm (Nielsen *et al.*, 1992). In river systems benthic biofilms are formed on the bottom and bank consisting of gravel or cobbles when flow velocity exceeds a certain value, *e.g.* 0.3 m/s (Imhoff, 1967), even under low contamination concentration, *e.g.* 1 mgBOD/L, as reported by Curtis *et al.* (1971) and 0.1-0.5 mgBOD/L, as reported by Roberts (1977). The area coverage ratio of the biofilm area over the whole river bottom is related to the size of gravel and cobble in the bottom (Novotny, 1969). For biofilm development it is a precondition that reactors keep the retention time (reciprocal of dilution rate) smaller than the maximum growth rate of microorganisms (μ_{max}) because of nutrient competition between suspended and attached biomass (Heijnen, 1984).

Microbial, physical and chemical processes occurring in the biofilm are complex and interrelated. As shown in Fig.3 three sub-processes are involved (1) the external diffusion, *i.e.* substrate and reaction products diffusion from the liquid bulk to the biofilm surface, or back, through the boundary layer on the interface between the biofilm and liquid, (2) the reaction within the biofilm (intrinsic kinetics), and (3) the internal diffusion of the substrate and the reaction products within the biofilm. Different biodegradation and physical-chemical reactions take place within different layers: in the aerobic upper layer aerobic heterotrophic biodegradation, nitrification, and chemical reactions (*e.g.* between oxygen and H_2S and CH_4, the products of anaerobic processes) take place; denitrification, sulfate reduction, organic acidogenesis and methanogenesis including breakdown of organic molecules to low-molecular weight compounds in fermentation processes occur in the anaerobic zone underneath. In the anaerobic zone the compounds are utilized by other bacterial groups using other electron-acceptors than oxygen following a characteristic sequence (successively NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-} and HCO_3^-), which is governed by the energy yield of each individual process, and the system's redox potential. It was supposed that part of substrate flux could be consumed (or mineralized) in the anaerobic zone (Mergaert *et al.*, 1992). However, it appears that there

is no quantitative picture yet obtained concerning the relative function to biodegradation between aerobic and anaerobic processes in thick biofilms.

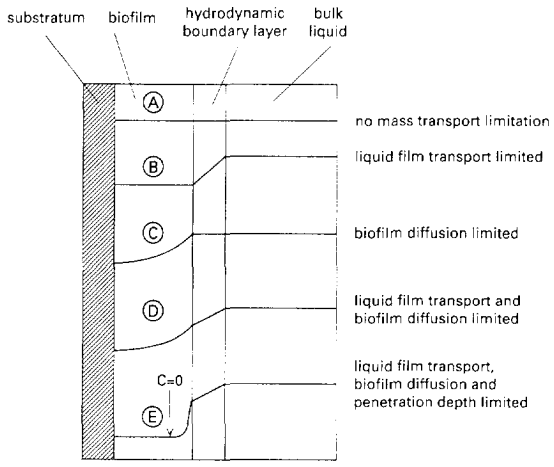

Fig.3 External and internal mass transfer on biofilm surface (C-substrate concentration) (Adapted from: van Loosdrecht, 1993)

A: Virtually no limitation of transport in the liquid boundary layer.

B: Strong mass transfer limitation in the boundary layer, no limitation in the fully penetrated biofilm.

C: No limitation in the boundary layer, limitation in the fully penetrated biofilm.

D: Substrate transport limitation in both the boundary layer and the biofilm.

E: As D but also conversion limitation because the biofilm is not fully penetrated by the substrate.

2.1.2. External mass transfer Under common conditions the kinetics of biofilm processes is water flow velocity dependent when flow velocity and Reynolds number are below certain values (*e.g.* under laboratory conditions when velocity < 0.8 m/s, LaMotta, 1976b; or $Re < 5000$, Hartmann, 1967). Field data underscored this (Hickey, 1988c; Saldanha and de Ribeiro, 1991). Mixing characteristics and mass transfer from the liquid bulk to multiple gravel layers in drains were described (Gantzer *et al.*, 1989; Nagaoka and Ohgaki, 1992). Generally speaking one can consider that high flow velocity improves mass transfer and hence bacterial growth, but high water flow velocity results in high shear stress which increases biomass erosion from the biofilm. It was reported that low velocity (*e.g.* 0.03 m/s) appears to favour biomass accumulation (Liu *et al.*, 1993).

The boundary layer between biofilm and bulk liquid was physically observed and measured by using microsensors; its thickness is reported to be between 100-300 μm in trickling filters (Chen *et al.*, 1981; Revsbeck and Jørgensen, 1986; Kuenen *et al.*, 1988). The turbulence (eddy) diffusion mechanism was proposed based on the roughness concept of classic fluid mechanics to explain the observed high flux of oxygen over the boundary layer into the biofilm especially for filamentous film; however, as shown in Fig.4, such surface would

normally be irregular in shape and morphology as compared with the typical floc-forming bacteria dominated "smooth" and dense film. Filamentous biofilms are typical for sewage conditions. It has been noticed that mass transfer could be intensified due to the "irregular" surface, and "eddy" or "turbulence" diffusion on the surface are supposed to be the main reason. Apparently, the area difference of real interface and the projected area (the plane perpendicular to the diffusion flow through which diffusion takes place) can be as large as one order of magnitude, the large interface area of irregular surface biofilm can strongly intensify external mass transfer process. The fact that the diffusivity coefficients for filaments dominated biofilm, calculated by Fick's first law, are larger than in bulk water was supposed to be evidence of the turbulence diffusion (Siegrist and Gujer, 1985; Hickey, 1988a). However, measurement revealed that the boundary layer for the rough filamentous biofilm is thicker than that of smooth biofilm (Kuenen *et al.*, 1986), and that in fact the bulk liquid movement can prevail at the water-biofilm interface (Lewandowski *et al.*, 1993). Both findings would rather contradict the proposed turbulence flow condition on the biofilm surface. This seems to indicate that the classic roughness concept is not applicable to surface process of irregular biofilm, and that the hydraulic situation is even more complicated than expected.

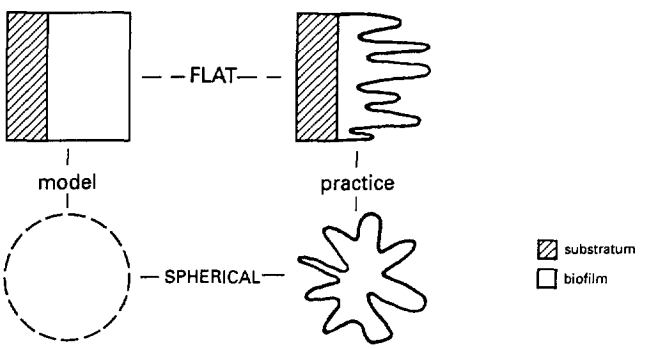

Fig.4 Diagram of irregular biofilm surface both for biofilm and floc.
(Adapted from: van Loosdrecht, 1993)

2.1.3. Internal mass transfer The methodologies used in the investigation of diffusion in porous media have been adopted for the study of the internal mass transfer inside biofilms. The experimental approaches include tracer experiment (Bhamidimarri and Greenfield, 1988), diffusion chambers (Matson and Characklis, 1976; Siegrist and Gujer, 1985), and oxygen profile measurement (Bungay *et al.*, 1969; Kuenen *et al.*, 1986). Most derived diffusivity values typically are 60-90% of the values in water; a few, mainly in irregular rough filament dominated biofilm, are higher as mentioned before. A critical assumption in the calculation of the diffusivity coefficients is that the projected area is equal to real mass transfer area; however, in real sewage grown biofilms it can be substantially higher. This applies not only to biofilm but also to filamentous flocs as shown in Fig.3. By using *i.a.* confocal laser microscopy, a recent study demonstrated the occurrence of three dimensional liquid movement including bulk flow within the biofilm or between biofilm-clusters (Lewandowski

et al., 1993). The hydraulic behaviour observed above and inside biofilms appears much more sophisticated than on rigid media like mineral catalysts.

2.1.4. Kinetics The intrinsic kinetic constants are derived by two approaches: (1) from free cell systems, which can be further divided into original liquid free cell systems (Williamson and McCarty, 1974) and homogenized biofilm (Sata and Toda, 1983; Hiemstra, 1983; Hickey, 1988; Karnchanawrong and Polprasert, 1990); and (2) by regression analysis of the measured oxygen concentration profiles inside biofilms (Bungay *et al.*, 1969; de Beer, 1990; Sweerts, 1990). In both cases biofilms are considered homogeneous, and consisting of more or less active living cells; the approaches don't distinguish between living cells and inert materials. Equations were developed for the prediction of the limiting concentration of either electron acceptor or donor for biofilm systems (Williamson and McCarty, 1974; Castalodi and Malina, 1982).

The macro-kinetics of the overall biofilm performance is investigated by fluxes, *e.g.* biofilm oxygen uptake rate BUR ($gO_2/m^2.day$). Generally, dissolved oxygen concentration in the liquid bulk is the limiting factor to the biological conversion in the biofilm processes of trickling filters and rotating biological contactors due to the constraint of the oxygen saturation value. Similar phenomena are found in natural biofilms. However, on the other hand, it was reported that filament-dominated biofilms are less sensitive to the oxygen effect, *e.g.* BUR is a function of 0.28 power of DO for filamentous film as compared to 1.55 power of DO for surface smooth biofilms under the DO range of 2 to 9 mg/L (Hickey, 1988a), no evident correlation was found between field sewer BUR and DO values when DO varies between 2-7 mg/L (Saldanha and de Ribeiro, 1991), and even near independence of BUR from short term DO change (2-6 mg/L) was reported for the biofilms grown on sewage (Nielsen *et al.*, 1992; Norsker *et al.*, 1994). This may be attributed to the loose structure and separated swaying filamentous bundles of filament-dominated biofilm, both reduce the effect of the concentration(s) in the liquid bulk on the conversion. A convincing explanation is still missing. For BUR of sediment it was reported that no effect of DO has been found when oxygen is above 2 mg/L or at the level of 3-8 mg/L (Fillos and Molof, 1972; Bowman and Delfino, 1980). Less living microorganisms in sediment as compared with biofilm is supposed to be the reason. All this indicates that the oxygen effect on bioconversion is related to types of biofilm and its active content. Also, it is related to the complex interaction of both external and internal mass transfer on and inside of the irregular biofilm, and explains the deviation of some biofilm kinetics from the widely used biofilm kinetics of half order, where BUR increases when increasing the limiting substrate concentration for partial penetration, and zero order, where BUR reaches constant value BUR_{max} for full penetration of biofilm or under constant limiting substrate condition (Harremoës, 1978), although it works quite well in the description of many biofilm processes.

BUR can be measured by repirometers in the field (James, 1974; Bowman and Delfino, 1980) or using batch or continuous flow reactors through oxygen balance calculation, and by measuring oxygen profiles of benthic biofilm or sediment samples by microsensor in the

laboratory. Literature values (Table 1) are diverse, and so far no comprehensive analysis of the data has been offered. BUR values of sediments are smaller than BUR values of biofilms because of much less living biomass contents in a typical sediment. The BUR values also depend on the limiting substrate concentration although they can reach maximum activity at a low concentration. The values measured by microsensors can be 10-50% less than actual values because they miss the respiration of the abundant infauna (Revsbeck and Jørgensen, 1986). Also, the different substratum structure of biofilms as opposed to irregular heterogenous riverbeds provide for substantially differing mass transfer areas. Similarly, as will be discussed later on, neglecting the role of suspended biomass in the liquid phase can equally result in large deviation of BUR values.

TABLE 1 Literature values of Benthic Oxygen Uptake Rate BUR

Type of biofilm	Temp. °C	BUR $gO_2/m^2.day$	Measurement method	Reference
sewer slime	25	5.3-33.6	mass balance	Pomeroy and Parkhurst (1972)
sewer slime (in pressure sewer)	15	16.7	mass balance	Boon and Lister (1975)
sewer slime	25	16.8	mass balance	Saldanha and de Ribeiro (1991)
trickling filter biofilm	22	6.22	microsensor, BOD 520 ppm	Chen and Bungay (1981)
trickling filter biofilm	16-22	4.6	microsensor	Revsbech and Jørgensen (1986)
trickling filter biofilm	20	4.2-6.4	microsensor	Kuenen *et al.* (1986)
rotating contactor biofilm	12-20	10-16	mass balance	Bovendeur *et al.* (1990)
benthic biofilm in polluted river	15-21	3.4-14.9	modified respirometer	Boyle and Scott (1984)
benthic biofilm in polluted river		2.04	mass balance in channel	Srinanthakumar and Amirtharajah (1983)
filamentous biofilm on gravels	18-24	57-70	respirometer	Hickey (1988b)
biofilm on polypropylene	18-24	3.8-8.2	respirometer	Hickey (1988b)
sediment	20	0.15	respirometer	Bowman and Delfino (1979)
deposit	22	4.7-7.4	laboratory flow chamber	Fillos and Molof (1972)

Pomeroy and Parkhurst (1972) and Hickey (1988a,b) developed equations to correlate BUR to oxygen concentration in the bulk liquid, and to the water flow velocity. But as mentioned and analyzed in the former part not all the field data can be satisfactorily described by these kinetics. Attempts have been made to find the relationship between BUR and the biomass amounts expressed as biomass dry weight (Reynolds *et al.*, 1973), ATP content (Hickey, 1988a), *etc.*, but the results rarely are convincing, perhaps due to the complex composition of biofilm. A Monod-like equation was found to approximately describe the macroscale kinetics at the level of the reactor (Hartmann, 1967).

2.2. Liquid phase

In-line purification in sewers and drains is primarily related to readily biodegradable substrate and hydrolysis products, *e.g.* volatile fatty acids (VFA) (Henze, 1992). Efforts recently have been made on the characterization of the chemical composition and on biomass groups in domestic wastewater. One approach has been to distinguish soluble, particulate and inert substrate, as advocated by the IAWQ activated sludge process model No.1 (Henze *et al.*, 1987; Henze, 1992). In domestic wastewater the readily biodegradable organic substrate concentration is about 20% of total COD *i.e.* around 80 mg/L as COD. A large fraction of the organic pollutants is in particulate form, *e.g.* it was reported that approximately 63-70% of organic material is associated with particles larger than 0.1 μ (Levine *et al.*, 1985). It is also useful to distinguish in wastewater carbohydrates, proteins, fats, *etc.*, and investigate the biodegradation of each of them (Raunjkær *et al.*, 1994). It was found that carbohydrate typically is the most easily biodegradable substrate (Nielsen *et al.*, 1992), the readily biodegradable and colloid COD is decomposed very fast even under low oxygen concentration condition (micro-aerophilic condition), but the reaction rate drops significantly under anaerobic condition particularly for the colloid COD (Wang *et al.*, 1994).

The suspended heterotrophic biomass in domestic wastewater accounts for about 15-20% of total COD, and ranges between 45-100 mg/L as COD (Henze, 1992); however, little information can be found on their existing forms and size distribution. The same holds for the low suspended biomass concentration in polluted rivers (10-20 mg(VSS)/L, according to Srinanthankumar and Amirthrajah, 1983; 16-30 mg(VSS)/L, according to Løkkegaard *et al.*, 1994), although much work has been devoted to the pathogenic bacteria and viruses. Because the suspended bacteria in waste- and river water usually are in the form of single bacteria or very small aggregates (dispersed condition) and often relatively few, and hence not visible like *e.g.* the flocs in activated sludge, they are generally neglected.

Concerning the external diffusion resistance of suspended biomass aggregates, given the individual cell diameter of 1-5 μm, an aggregate diameter of typically 10-150 μm, oxygen concentration difference between that at the boundary layer and the biomass surface greater than 2 mg/L, and other typical values for relevant parameters, it can be calculated that the maximum possible oxygen fluxes through the liquid-biomass interface can exceed the overall

intrinsic aerobic activity. Hence, external mass transfer limitation has no significant influence on the net reaction rate in many cases (LaMotta, 1976a). Actually the irregular shape of activated sludge (Fig.3) can provide extraordinary mass transfer area, *e.g.* it was found that the specific surface area measured (typically 100-200 m^2/g.dry sludge) of the activated sludge flocs with the sizes of 10-70 μm can be one to two orders of magnitude higher than the geometric floc surface area, indicating a porous floc structure (Andreadakis, 1993). Internal diffusion, on the other hand, depends on the diameter and structure of the aggregates determined diffusivity inside them, and the substrate concentration in the liquid bulk. Literature suggests critical sizes of the suspended biomass aggregates or flocs above which diffusion limitation starts playing a role, *e.g.* 26 μm for nitrification, as reported by LaMotta and Shieh (1979a), and 16-18 μm for aerobic heterotrophic biodegradation, as reported by Green *et al.* (1985). Denitrification can happen in the core of the activated sludge floc where anaerobic or anoxic conditions exist (Grady and Lim, 1980) as a consequence of oxygen diffusion limitation from the (aerated) bulk liquid into the floc.

To some degree, large suspended aggregates like activated sludge flocs can phenomenologically be considered similar to biofilm because the cells are immobilized in dense floc material instead of in a biofilm, and diffusion limitation occurs due to the thickness of the bacterial aggregates. However, the microflora, composition and structure of the flocs may differ from those of biofilm, even in the same reactor, so it is not obvious that both systems can be treated the same way. In literature the suspended flocculated biomass is consistently classified as suspended biomass and not as biofilm (Flemming, 1993).

The Monod equation is a main kinetic equation used for describing bacterial growth, and is valid for liquid phase (and solid phase) biodegradation both for intrinsic and process kinetics. In the latter case, the mass transfer influence would reflect in the magnitude of the saturation constant K_s. Henze *et al.* (1987) suggested an equation expressing the concentration effect of electron acceptor and donor on kinetics for both carbon substrate and for nitrogen removal. A first order kinetic equation with respect to substrate concentration can be used to describe oxygen consumption and BOD removal rate in river and ponds; the biomass concentration is lumped together with the kinetic constants in this black-box approach.

A batch reactor was adopted to investigate the biodegradation in the liquid in sewers (Pomeroy and Parkhurst, 1972; Saldanha and de Ribeiro, 1990). The oxygen level appeared to have little effect on the reaction rate when its concentration is above a certain value (typically 1 mg/L (Pomeroy and Parkhurst, 1972). The age and temperature of sewage influence the reaction rate (Pomeroy and Parkhurst, 1972; Sollfrank *et al.*, 1992). Literature values of oxygen consumption rate due to the suspended biomass in gravity sewers are 0.3-7 mg/L.h at 25^{0}C (Pomeroy and Parkhurst, 1972), 15 mgO_2/L.h at 15^{0}C (USEPA, 1974), 3-6 mgO_2/L.h for sewerage with age 10 and 30 minutes, respectively (Saldanha and de Ribeiro, 1990), and 5, 10 and 15 mgO_2/L.h for wastewater in main sewer with age 1, 2 and higher than 3 h at 20^{0}C (Boon and Lister, 1975). It was reported that the biodegradation rate for a settled sample is only half that of an unsettled one (Pomeroy and Parkhurst, 1972); most

likely a large part of the biomass is attached to the surface of particulate matter. For the description of oxygen consumption in rivers the field BOD measurement and the regression analysis of field oxygen sag curve data are widely used for kinetics investigation.

Research on the factors contributing to the development of microbial community, pioneered by Chudoba (1973), has been conducted particularly aimed at preventing sludge bulking in the aeration tank of activated sludge processes. It suggests that: (1) soluble substrate stimulates filamentous growth, while particulate substrate stimulates floc-forming growth; and (2) a plug flow favours floc-forming bacterial growth, while well-mixed flow favours filamentous growth; this so-called kinetic selection is due to the concentration effect on bacterial growth as related to the bacterial k- or r-growth strategy.

To summarise, biofilm and suspended biomass processes have been successfully studied by applying knowledge of process technology and microbiology. However, a thorough understanding to the various bio- and chemical reactions, flux balance within thick biofilms, and mass transfer mechanism on the "irregular" biofilm surface are still missing. For polluted aquatic systems the real effect of suspended biomass concentration is ignored, and to a certain extent a black box approach is adopted.

3. MICROBIAL COMMUNITY

3.1. Biocenosis in sewers, drains and rivers

Efforts have been made by microbiologists and process engineers to correlate microbial community composition and system performance in order to achieve a better understanding of the characteristics of the system and its sub-processes. Various methods have been developed and used to assess the microorganism groups in activated sludge (chemoheterotrophs, nitrifiers and denitrifiers, as summarised by Kristensen *et al.*, 1992); many methods have been developed to measure biomass activity such as using determination of ATP (Hickey, 1988a), phospholipids (Zhang and Bishop, 1993b; Hooijmans *et al.*, 1994), tetrazolium dye reduction (INT) (Blenkinsopp and Lock, 1990), oxygen profile (Kuenen *et al.*, 1986), and enzyme activity (Lemmer *et al.*, 1994). A recent review was given by Lazarova *et al.* (1993).

The ecological community in sewers is very complex consisting of aerobic heterotrophic, facultive and anaerobic bacteria. A high population density of several groups, such as heterotrophs, ammonifying, nitrate reducing and anaerobic bacteria were found in sewers, and the heterotrophic activity of sewer biofilm based on dry weight appeared to be in the same range or even higher than those in high loading activated sludge aeration tanks (Bv: 0.8

kg/kg.day) (Lemmer *et al.*, 1994). In heavily polluted drains the microbial composition appeared to correspond to the pollution situation, *i.e.* the more serious the pollution is, the higher the amount of heterotrophs albeit with reduced species diversity, and vice versa (Zhang *et al.*, 1986).

In transparent moderately polluted rivers, as well as in wastewater stabilization lagoons, heterotrophs (H) and phototrophs (P) co-exist. Almost no algae tend to be found in seriously polluted sections of rivers; the relative amount of heterotrophs to phototrophs decreases downstream of the discharge point into the stream. The ratio P/H was suggested as an index expressing the heterotrophic biodegradation capacity (Wuhrmann, 1972). Nevertheless, oxygen production by photosynthesis, in which the benthic phase plays an important role as base for many phototrophs, is an important source for oxygen supply in shallow rivers. Oxygen production by algae in the benthic phase could be 1-4 gO_2/m^2.day; it can be up to 3 times higher than that in the water phase due to the enrichment of algae in the benthos, and as such higher than the stream's aeration capacity (Boyle and Scott, 1984), and in some cases 1-3 times higher than the oxygen consumption by heterotrophic respiration (Meir *et al.*, 1970). Therefore, the symbiosis of heterotrophs and algae can enhance, from the oxygen supply point of view, heterotrophic biodegradation. However, algae cannot contribute directly to heterotrophic biodegradation, but rather they would fix carbon dioxide, the product of heterotrophic biodegradation, and create new organic matter that ultimately will pollute the water again, as their removal from the effluent is problematic. Active nitrifying bacteria occur in the section where C-concentration is low (Karnchanawrong and Polprasert, 1991). On the other hand, Zhang and Bishop (1993a) reported that under high organic pollution condition nitrification can be inhibited by limited oxygen supply but not by organic substrate.

3.2. Specific activity of suspended and attached biomass

The volatile (organic) fraction of a biofilm can be considerably less than the volatile fraction of a suspended microbial cell aggregate (>90% of volatile solids), primarily because of the inorganic constituents absorbed, entrapped or precipitated within the biofilm matrix (Christensen and Characklis, 1989). The inorganic fraction is especially high in biofilm accumulated in natural aquatic systems where silt, clay, and other sediment are entrapped in the biofilm matrix. As an example, Lemmer *et al.* (1994) reported that volatile fraction of sewer biofilm can vary between 0.29-0.86 (g/g). Concerning the biomass, it is necessary to measure the biomass activity per unit of mass for the suspended and attached cells in order to quantify the relative function of the two phases in the dual-phase system. Generally, the specific activities of both biomass types are different. Common hypotheses are that this difference is caused by the different microenvironment.

It has been suggested that the biofilm cells would be more active than the suspended ones because of typical morphological advantages, *e.g.* by being more hydrophobic and more susceptible to plasmid transfer, thus displaying higher antibiotic resistance (Kölbel-Boelke

and Hirsch, 1989; Flemming, 1993). Some reported field data are consistent with these hypotheses; *e.g.* enzyme activity of sewer biofilm (based on volatile weight) is higher than that of activated sludge (Lemmer *et al.*, 1994), 130% higher activity of nitrification was found with attached than with suspended biomass (Audic *et al.*, 1984), and about 50% higher activity of biofilm biomass than suspended biomass in a RBC (Pan and Hartmann, 1992). It can be argued, however, that in the first two cases the biomass was not cultured in the same reactor, and in the last case that the derived kinetic constants are anyway well beyond the normal range.

On the other hand Siebel and Characklis (1991) reported the same activity for the suspended and attached biomass when excluding the EPS (extracellular polymeric substance) content from biomass dry weight. The same results were reported for the nitrification activity of the two biomass types (Chudoba and Pannier, 1993), and for the nitrogen removal activity in an air-lift suspension reactor (Tijhuis *et al.*, 1993). Nouvion *et al.* (1987) reported marked difference in the active fraction in the two biomass types, *i.e.* 256-726 ng(ATP)/mgVSS for a biofilm, and less than 600-1000 ng(ATP)/mgVSS for activated sludge. Such seemingly contradictory pictures can possibly be explained by a not fully consistent definition of biomass by researchers, the different growth conditions and the way of expressing and measuring activity.

3.3. Species distribution in and structure of biofilm

The fact that biofilms have a diverse speciation is well known. Mathematical models have been developed to describe the species distribution in biofilm (Wanner and Gujer, 1984; Kissel *et al.*, 1988), but the heterogeneous distribution of species, and the structure can only since recently be experimentally studied *in vivo* by micro-slicing and microsensor.

The microbial species distribution was determined in different biofilms; *e.g.* in RBCs filaments gather on the surface film layer creating larger pore diameters (typically 480 μm), while single cocci concentrate in the bottom film layer and create smaller pores (*e.g.* 40 μm) in a biofilm with thickness of around 1000 μm (Zahid and Ganczarczyk, 1993). In a trickling filter biofilm, a bacteria and algae symbiotic consortium was encountered (Kuenen *et al.*, 1986), and in the biofilm in an air-lift suspension reactor heterotrophs were found to concentrate on the outer layer of the film while nitrifying bacteria concentrate in the inner layer (Tijhuis *et al.*, 1993). Obviously, anaerobic and reductive consortia will tend to concentrate in the lower, anaerobic or anoxic layers.

A pronounced difference of living cell distribution was found, for example, between a biofilm of thickness >500 μm, where 82-89% of dry weight in the top layer was active biomass (measured by INT) and 5-11% in the bottom layer; in a biofilm of thickness <500 μm, 83-86% of dry weight in the top layer was active biomass, against 57-63% in the bottom layer. Therefore, the living bacteria in the top layer may essentially all be metabolically

active. However, in the bottom layer only 1/4 to 1/7 of total dry weight could be metabolically active (Zhang and Bishop, 1993b). This means that the overall activity of the living cells in the top layer can be substantially higher than that in the bottom layer, and hence the activity of the suspended biomass eroded from the biofilm surface can be much higher than that of the average biofilm biomass. The biomass activity gradient also is connected to the inert material distribution in the biofilm. The average EPS content in organic biofilm amounts to 50-90% of total organic material (Bakke *et al.*, 1984; Flemming, 1993), although the term EPS here may include other inert materials. The inert content of biofilm seems much higher than what is reported for suspended biomass; for the latter few literature sources can be found. These literature data are not fully consistent with Zhang and Bishop (1993b). However, both figures indicate that possibly only minor part of total dry weight of biofilm consists of living cells which can effectively contribute to biodegradation. Adding the disadvantage of mass transfer resistance in biofilms, an even smaller portion of living cells would be in a position to fully contribute to these processes. This illustrates that the total biomass amount is in itself not a rational criterion to compare the relative importance of the two types of biomass in one reactor, and why studies on biofilm reactor is oriented to developing thin (100 μm) active biofilm on carrier material surface.

Similarly, a clear distribution of physical properties occurs in the biofilm as well, *e.g.* Zhang and Bishop (1993b) reported that the porosity is 70-90% in the top layer and 35-60% in the bottom layer. Clearly, the biofilm is highly heterogeneous in composition and structure. The competition for substrate and oxygen in the biofilm is supposed to be the main reason for such stratified structure. The common assumption of a homogeneous biofilm, therefore, is an over-simplification, and at present it is not yet described to which extent such simplification can influence calculation results on real-life systems.

3.4. Reactor type, shear stress and biofilm composition and structure

Though still insufficiently investigated, this topic is crucial to biofilm and dual-phase systems particularly when the latter would be at first hand considered to be biofilm dominated. Morgan *et al.* (1991) reported that treatment of ice-cream wastewater by four biofilm reactors with different shear stress yielded the conclusion that the biofilm reactor design had no effect on biofilm ecology. Shin *et al.* (1991) found from aerobic upflow sludge blanket reactors treating synthetic wastewater that high shear stress resulted in a compact structure and inhibition of filamentous growth of aerobic granular sludge. Kugaprasatham *et al.* (1992) found higher turbulence resulted in higher areal biomass density, and a smoother surface of the nitrifying biofilm. Lazarova *et al.* (1993) used four biofilm reactors to explore the factors which affect the biofilm composition; they found that with higher hydraulic stress, the biofilm contains more EPS and inert contents, as well as heterotrophs. It was concluded that the shear stress determined biofilm composition and its physical properties. In both studies, the biofilm loading, which involves water flow velocity and organics concentration, and not the organics concentration was chosen as a state parameter in the experiments, which could

lead to confusion on the possible separate effect from either the concentration or the shear stress on biofilm properties.

4. DESCRIPTION OF THE DUAL-PHASE SYSTEMS

4.1. Relative contribution of the two phases to aerobic respiration

For sewer systems, the biodegradation in the liquid and solid phase has been noticed; an equation for oxygen consumption was derived by regression analysis on field data (Pomeroy and Parkhurst, 1972; Nielsen *et al.*, 1992). No criterion was developed to describe their relative importance, although it was surmised that the biofilm could be more important in biodegradation in sewers with small diameter because of its higher wetted surface to volume ratio (Saldanha and de Ribeiro, 1991).

Open-air and in-door channels at different scale were adopted to simulate biodegradation in sewers and drains; the role of suspended biomass usually was ignored and the analysis only limited to the benthic phase (Dias *et al.*, 1968; Curtis *et al.*, 1971), even in case the recorded suspended biomass amount was comparable with that of the attached biomass (Liu *et al.*, 1986).

The tendency to ignore suspended biomass (or to lump everything in one phase) is even more obvious in the studies on organic pollutant biodegradation in shallow aquatic systems (Rutherford and O'Sullivan, 1973; Bowman and Delfino, 1980; Avnimelech *et al.*, 1981; Singathaman and Arithamnan, 1983), as well as for N removal (Tuffer *et al.*, 1974; Williams and Lewis, 1986), probably because of suspended (dispersed) biomass being almost invisible to the eye. The only exception, to the author's knowledge, is Boyle and Scotter (1984) who developed a two-chamber respirometer that allows to account for the role of suspended biomass in oxygen consumption, and they found its function was comparable with benthic biofilm.

Fig.5 shows a typical repirometer with only one chamber used to measure BUR in water quality management studies in the field; the open bottom is only exposed to the benthic phase during field measurement. Over the past two decades a series of improvements have been made mainly to compensate better for hydraulic effects on BUR measurement (James, 1974; Bowman and Delfino, 1980; Hickey, 1988c). However, it is almost impossible to reproduce the field hydraulic situation within the chamber, because of the small chamber size as compared to the length required for sufficient boundary layer development (Nowell and Jumars, 1985). Moreover, one-chamber design implies that the measured BUR values also reflect the function of any biomass suspended due to vigorous stirring in the chamber. To prevent this influence a pre-sedimentation has been suggested to allow the resuspended

biomass to settle down, although the biomass, particularly the colloidally dispersed would remain in the liquid. A second constraint is that biofilm can grow on the inside wall of the chamber if the measurement in the field lasts longer than 24 h. The same problems also hold for measurement of the benthic respiration using a batch or continuous reactor in the laboratory. As BUR are crucial parameters in calculation and mathematical modelling, these constraints should be clearly addressed and quantified, and the measurement procedure adapted and standardized.

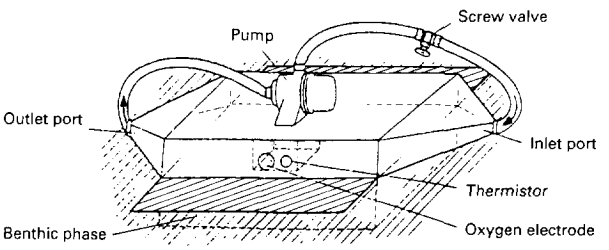

Fig.5 Diagram of a respirometer (James, 1984).

To date a relatively mature understanding has been achieved on macro-scale of biodegradation in single-phase processes, but not of the dual-phase systems. The main efforts made so far tend to simplify the dual-phase systems as single phase, or to lump suspended biomass with the biofilm function.

4.2. Mathematical modelling

Empirical and semi-mechanistic mathematical models have been developed to describe systems that are in fact to a larger or lesser extent dual-phase processes. In many cases efforts were made to modify the Streeter-Phelps equation (1925), an early water quality model in which only the liquid phase was considered and oxygen consumption is expressed by the first order kinetics with respect to COD or BOD. These models can be classified into four types:

(1) the suspended biomass is totally neglected, and the models only have one item describing benthic phase contribution to aerobic respiration and organic removal (Rutherford and O'Sullivan, 1974; Srinanthakumar and Amirtharajah, 1983), as well as for nitrogen removal (Williams and Lewis, 1986);

(2) the benthic phase is described by empirically choosing the values of kinetic constants in the Streeter-Phelps model (*e.g.* for carbon removal $k=0.1\text{-}0.5\ day^{-1}$ for deep rivers, with depth > 1.5 m, and $k=0.5\text{-}3.0\ day^{-1}$ for shallow rivers, with depth ≤ 1.5), and a

similar approach is applied to nitrification (Thomann and Mueller, 1987);

(3) in addition to the original kinetic constant an extra item is added to express the benthic contribution in the Streeter-Phelps equation (Thomas, 1948; Velz, 1984), this kinetic constant being correlated to the depth of water (Wolf, 1977), or slope or roughness of the river bed (Novotny, 1969).

(4) both suspended and attached biomass functions have been taken into account by separated items in the models such as stream quality model QUAL II (Norton *et al.*, 1974) where the first-order kinetics with respect to BOD was adopted for suspended biomass respiration together with a term containing stream height as the denominator and an empirical chosen constant as the numerator to express the attached biomass function.

The common characteristics in the model approaches of cases (1) to (3) are that no separate suspended biomass concentration appears and the two phases are lumped or BUR are coupled with its specific area. Therefore, they are empirical rather than mechanistic; for example they cannot distinguish pollutant sources either in water or from the benthos. This means they may offer a good fit for local conditions but cannot be extrapolated for broader water quality management purposes. As for the applied kinetics it is known that the first order kinetics is applicable only under low substrate concentration condition, while the zero order approximation holds for higher concentration range. Recently some attention has been paid to the weak points mentioned above. Lee (1992) developed a model concerning two biomass types competing for a single substrate in a well mixed reactor under steady state condition; the suspended biomass and limiting substrate concentrations and the fraction of substrate used by suspended growth can be solved by iteration approach. In the latest extension of the MOUSE programme dealing with water quality in sewer systems (Garsdal *et al.*, 1994) the suspended biomass respiration is expressed by an equation similar to that in the IAWQ Wastewater Treatment Model No.1 (Henze *et al.*, 1987) where suspended biomass concentration is described as a fraction of BOD, and the attached biomass function is expressed by half order kinetics with respect to oxygen concentration.

5. SCOPE OF THE RESEARCH

5.1. Purposes of the research

As can be seen from the above, the study on the aerobic biodegradation in the dual-phase systems such as sewer and drain draws from water quality management, wastewater treatment technology, and from the fundamental understanding of the functioning of suspended and

attached biomass in aquatic environments with medium to high organic pollution degree. The main focal points of this study are aerobic heterotrophic biodegradation by these two biomass types, though the role of the biofilm will be emphasized, because its function is receiving increasing attention. This study will increase the understanding to the processes in drains and sewers, improve the design and operation of the following treatment plants, and help to formulate a rational water quality management strategy which integrating wastewater transportation, treatment and final discharge. Apparently, this study has a special meaning to the developing countries in (sub-)tropical climates where the warm sewage in the sewers and drains enhance biodegradation, and where various types of oxidation ponds are adopted for treatment purposes. It will help to explore the feasibility of in-line sewage treatment, although it should be kept in mind that from a long-term point of view, a choice between soluble COD removal in-line (even in the treatment plants) and COD concentration for the sake of nitrogen and phosphate removal should be sought. The main objectives of this study are:

(1) to identify the key parameters of the dual-phase systems such as biofilm oxygen up-take rate BUR, specific biofilm area, suspended biomass concentration and its activity, in relation to the study of suspended biomass function, and of the relative importance of the two phases, under varying conditions.

(2) to study the process mechanisms including mass transfer (external and internal), and kinetics (intrinsic and process) in wastewater collection systems and in lagoons with low flow velocity and shear stress; and to set up mass balances for oxygen and soluble carbon, which is essential to estimate oxygen supply requirement and sludge production;

(3) to explore the feasibility of enhancing purification in-line through intensifying biomass respiration capacity as an alternative of wastewater treatment mainly in tropic and sub-tropical countries;

(4) to study the influence of reactor type and shear stress on biofilm morphology, composition and kinetics as this is very important to the understanding of the operation of biofilm dominated dual-phase systems such as sewers with small diameter and biofilm reactors;

(5) to develop a conceptual and mathematical model describing dual-phase systems and processes in sewer and drain based on the previous experimental work.

5.2. Experimental design

The general research approach taken is to divide the dual-phase system into its liquid and solid phase parts, then to investigate them separately. Three reactor types fed by the same

synthetic wastewater and operated at the same temperatures 20°C and 28°C corresponding to sub-tropical climate were adopted in this study. To simulate a long sewer or drain experiencing a course of substrate concentration decrease (Fig.1b), a batch reactor was used representing a plug flow. To simulate a drainage system with essentially constant substrate, oxygen and biomass concentration as a sewer reach with multiple point discharges (Fig.1c) or a well mixed lagoon, two other reactor configurations, *i.e.* the CSTR and channel reactor would represent a continuous well-mixed tank. The flow velocity and shear stress on the biofilm surface in the batch and CSTR are comparable with those in a sewers; in the channel reactor they are similar to a drain with mild slope or a pond system, which is one order of magnitude smaller than for the batch and CSTR. Macro-balances on oxygen and soluble carbon are carried out on the reactors for the study of reactor performance, defined as oxygen consumption and substrate (glucose) decomposition rate and their fluxes into the biofilm, and of the flow velocity and temperature effect on the performance.

The suspended biomass function was distinguished in the case of the batch reactor from the that of biofilm by parallel experiments, one with both phases and one with only suspended biomass, and in case of the CSTR and channel reactors by the batch wise operated Biological Oxygen Monitor (BOM) to measure the suspended biomass activity the intrinsic kinetics. The microbial community is studied by observation under light, SEM (Scanning Electronic Microscope) and TEM (Transmitted Electronic Microscope) microscopy, and by biological activity measurement by use of the BOM. The experimental results from the three reactors are compared to investigate the relationship between flow pattern, shear stress, biofilm morphology, microbial composition, and kinetics. A conceptual model is developed based on the experimental results, which is applied to a new mathematical model; however, this model is developed only to carry out a response analysis, but it has not been validated against field data.

An introduction to the experimental set-up including schematic diagrams of the three reactors and the calculation equations used is attached (Appendix).

5.3. Organization of the thesis

Chapter 1 reviews the literature on this topic. *Chapter 2* presents the results for the batch reactor with high shear stress on the biofilm surface, which was used to simulate the plug flow. The suspended biomass function was distinguished from the biofilm by using identical parallel reactor experiments, one with both phases and one with only suspended biomass. *Chapter 3* introduces the experimental results of the CSTR reactor, in which three different biofilm specific area values were adopted and the shear stress on biofilm surface is the same magnitude as for the batch reactor. The suspended biomass function was investigated by the Biological Oxygen Monitor (BOM). *Chapter 4* presents the channel experimental results; the average flow velocity and shear stress are one order of magnitude smaller than those in the other two reactor types. *Chapter 5* presents a new model concept describing the interaction

between reactor type, shear stress on the biofilm population, morphology and kinetics, based on the comparison of the three reactor results. According to the experimental results, the mathematical model is developed in *Chapter 6*. The summary and conclusions are given in *Chapter 7*.

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Chapter 2

Using Batch Reactor to Study Oxygen Consumption and Organics Decomposition in Drainage Systems with Attached Biofilm *

ABSTRACT Oxygen consumption and organics decomposition in drainage systems with attached biofilm were investigated using batch reactors. In order to distinguish the relative contribution to these processes of suspended biomass and attached biofilm parallel experiments were performed. The reactors with low and high glucose concentrations simulated lightly polluted rivers and sewers, respectively. The big discrepancy of benthic oxygen up-take rate (BUR, $gO_2/m^2.day$) data in literature can result from different biofilm morphology, benthic configuration, and neglecting the suspended biomass function in the measurement by using conventional one chamber respirometer. The biofilm played a major role in the biodegradation only when the suspended biomass concentration was below a critical value. The biodegradation in the liquid was much temperature sensitive than for the biofilm due to different controlling mechanisms.. The biofilm ecological community was dominated by filaments (mostly like *Sphaerotilus natans*) but coccus bacteria coexisted with them.

KEY WORDS self-purification; wastewater treatment; drainage; streams; sewers; biodegradation; biofilm; bacteria.

INTRODUCTION

Drainage systems commonly refer to channels or sewers which transport wastewater to treatment plants or disposal sites. Attached biomass also called "benthic biofilm" in literature, grows on the surface of the bottom or on the wall (wetted area) especially when these bottoms and walls consist of rock, gravel, *etc.* Lightly polluted streams and rivers are in principle comparable with drainage and sewerage networks, although the substrate load is an order of magnitude higher in sewers and many drainage channels. With an increasing wetted area per unit volume of water the importance of the suspended biomass in the water to the total biodegradation of the system can be expected to be decreased. The terms "shallow

* Y.S. Cao, G.J. Alaerts, C.M. Hooijmans and H.J. Lubberding (1992) *Wat. Sci. Tech.* **26**, 683-692. Revised version.

river" (for a typical depth < 1.6 m) and "deep river" (for a typical depth > 1.6 m) have been introduced to imply systems with a minor or major influence of the suspended biomass as compared to the attached biomass (Thomann and Mueller, 1987). The benthic biofilm, the immobilised phase in the system, consists to considerable extent of microorganisms, including bacteria, algae, protozoa, *etc.* They obtain oxygen for respiration from the overlying water, the liquid phase in the system. The related biodegradation of organics, has attracted attention both with respect to the description of the phenomena and possible application.

The suspended biomass oxygen uptake rate (SUR, $gO_2/m^3.d$) and the benthic biofilm oxygen uptake rate (BUR, $gO_2/m^2.d$) have been introduced and investigated earlier, and BUR/SUR (m) or "equivalent depth" was considered as the additional depth of water required to give the same local oxygen uptake rate if the benthic biofilm was removed, expressing the relative contribution from the two phases. To get reliable data for SUR and BUR, respirometers used for benthic oxygen demand determination, have been modified and used with different sizes and configurations mostly only featuring one combined chamber (Hickey, 1988), only one exception with two chambers separating the two phases of the system (Boyle and Scott, 1984). Also microsensors have been also used to measure the oxygen uptake rate of biofilms (Revsbech and Jørgensen, 1986; Kuenen *et al.*, 1986). As Table 1 shows BUR values are very diverse, and these substantial discrepancies have not been explained satisfactorily.

For lightly polluted streams and rivers it has been claimed that the benthic biofilm plays the dominant role, with the contribution from the liquid phase smaller or negligible (Srinanthakumar and Amirtharajah, 1983; Velz, 1984). Field and lab data for self-purification in sewer systems indicate that benthic biofilm contributes substantially to the biodegradation process (Pomeroy and Parkhurst, 1972; Saldanha and de Ribeiro, 1991). One problem for this sewer system research is the accurate measurement of the aeration coefficient; empirical equations were used for determining oxygen mass transport rate introducing some uncertainty into the results. In attempts to increase its applicability, self-purification has been tried to be improved by increasing the area of the biofilm in the system (Wuhrmann, 1966; Liu and Zhang, 1988; Karnchanawong and Polprasert, 1990).

However, upon review of the available literature it appears that most sources provide scarce or incomplete information on the characteristics of the studied system. This pertains *i.a.* to the kinetic parameters used for "shallow river" and "deep river" simulation; on the other hand biofilm kinetics has been studied extensively (Harremoës, 1978). Similarly, the degree of competition and interdependency between suspended biomass and biofilm, and the representative of experimental conditions leading to the conclusion that the benthic biofilm makes a main contribution to the biodegradation process, needs further study. Few researchers apparently considered the relative concentration of the suspended biomass against attached biomass a determining factor. Therefore, this research intends to study the hypothesis that the relative amount of attached and suspended biomass and relevant parameters affect their respective contribution to biodegradation.

TABLE 1 Literature Values for Benthic Oxygen Uptake Rate (BUR)

Description of biofilm	Temp °C	BUR $gO_2/m^2.day$	Method	Reference
Benthic biofilm in polluted river	15-21	3.4-14.9	modified respirometer	Boyle *et al.* (1982)
Benthic biofilm in polluted river	18-23	2.0	mass balance in channel	Srinanthakumar *et al.* (1983)
Benthic biofilm on granulate mat	18-24	28.0	respirometer	Hickey (1988)
Filamentous biofilm on gravel	18-24	57-70	respirometer	Hickey (1988)
Biofilm on polypropylene	18-24	3.8-8.2	respirometer	Hickey (1988)
Sewer slime	25	16.8	mass balance in sewer	Saldanha *et al.* (1991)
Trickling filter biofilm	22	6.2	microsensor substrate 520 ppm	Chen *et al.* (1981)
Trickling filter biofilm	16-22	4.6	microsensor	Revesbech *et al.* (1986)
Trickling filter biofilm	20	4.2-6.4	microsensor	Kuenen *et al.* (1986)
Rotating contactor biofilm (RBC)	12-20	10-16	mass balance in lab RBC	Bovendeur *et al.* (1990)

As the geometric characteristics of channels feature a large ratio of length over width, combined with negligible back-mixing, the flow pattern for most drainage systems is approximately a plug flow. Therefore a batch reactor could be used to simulate "deep" and "shallow river" processes, varying the amounts of suspended and attached biomass as well as of substrate. The "deep river" case is represented by a batch reactor (respirometer) with only suspended biomass; the "shallow river" reactor contains in addition a large attached biomass.

The general equation for the oxygen mass balance of a micro-volume in a batch reactor simulating a plug flow is as follows, with the first right-hand term the aeration and the second the total oxygen uptake rate (TUR, $gO_2/m^3.d$):

$$\frac{d(C^*-C)}{dt} = k_L a(C^*-C)-TUR$$

Where:

C^*, C - saturation oxygen concentration and oxygen concentration in the bulk of the solution, gO_2/m^3,
k_La - aeration coefficient, day^{-1},
t - time, day.

For a batch reactor with a attached biofilm ("shallow river"), oxygen is consumed by the suspended biomass and the biofilm; therefore TUR can be divided into two parts:

$$TUR = SUR+\frac{BUR*WA}{V}$$

where:

SUR - oxygen uptake rate by the suspended biomass, $gO_2/m^3.day$,
BUR - oxygen uptake rate by biofilm, $gO_2/m^2.day$,
WA - wetted area, the area covered by biofilm, m^2,
V - volume of the water, m^3.

For a "deep river" system, WA≅0, TUR=SUR. In case of the "shallow river" TUR expresses the oxygen uptake rate of the "shallow river" reactor containing the attached biofilm as well as the suspended biomass.

In order to distinguish the contribution of the suspended and attached biomass with respect to oxygen consumption and organics decomposition, two reactors, one with biofilm and suspended biomass and the second with suspended biomass only, are operated in parallel; in both reactors suspended biomass concentration, substrate concentration, and temperature are kept nearly identical. TUR and SUR can be obtained from these reactors using numeric integration or the finite difference method when the aeration constants and oxygen concentration curves are available.

The accumulated amount of oxygen consumed can be calculated from following equations:

$$\int_o^t TUR\ dt \quad \text{or} \quad \int_o^t SUR\ dt = (C_0 - C_t) + k_L a \int_o^t (C^* - C)\, dt$$

where:

C_0, C_t- oxygen concentrations at the beginning (t=0) and at time t.

The oxygen yield coefficient $Y_{o/s}$ (gO_2/gglu) is then obtained from

$$Y_{O/S} = \frac{\int_o^t TUR\ dt}{S_o - S} \quad \text{or} \quad Y_{o/s} = \frac{\int_o^t SUR\ dt}{S_o - S}$$

S_o, S - substrate concentrations at the beginning (t=0) and at time t, g/m^3.

MATERIALS AND METHODS

Apparatus Beakers of 10 l volume were used as parallel reactors. For the "shallow river", biofilm was present on the surface of polypropylene substratum which was placed on the bottom of the reactor; other experiments were carried out with additional biofilm on the vertical wall surface of the reactor. The WA/V ratios thus obtained are 2.1 m^{-1} and 22 m^{-1}, respectively. the former resembles real life drainage systems with a flat bottom, whilst the latter resembles systems with an irregular bottom consisting of gravel or cobbles. For comparison purpose experiments with trickling filter media stones placed on the bottom of the reactor were carried out as well.

The reactors were placed in a water bath with a temperature 20 ± 1^0C (C^*=9.1 mg/L) and 28 ± 1^0C (C^*=7.8 mg/L). A mechanical stirrer was used for reaeration. The aeration coefficients k_La were determined, and the applied k_La values were 1.95 ± 0.18 day^{-1} and 9.0 ± 0.8 day^{-1} at rotational velocity 220 and 440 rpm, respectively. both at 20 °C. The k_La values at 28 °C were 2.14 day^{-1} and 10.2 day^{-1}, respectively. The oxygen distribution indicated the reactor was well mixed. During the running time the reactors were covered to shield them from sunlight to prevent algal growth.

Wastewater Synthetic wastewater was used in the experiments. The relative amount of chemicals is presented in Table 2. Glucose was the only carbon source. The ratio of C:N:P was 14.7:4.3:1 similar to domestic wastewater. Trace elements were added in 10 ml volume per 20 l reaction solution. The ranges of the initial glucose concentration (S_0) 10 mg/l, 20 mg/l, and 40 mg/l were used to simulate lightly polluted streams or rivers, and the ranges of 75 mg/l and 100 mg/l were used to simulate sewers and drains.

TABLE 2 Composition of the Synthetic Wastewater

Macro nutrient	(mM)	Trace elements	(mg/L)
$C_6H_{12}O_6$ (glucose)	18.307	$ZnSO_4$	700
		$NaMoO_2.2H_2O$	100
NH_4Cl	28.0	$MnSO_4$	1000
		$CuSO_4.7H_2O$	50
K_2HPO_4	2.9	$CoCl_2.6H_2O$	30
		KI	100
$MgSO_4.7H_2O$	12.0	$KAl(SO_4)_2.12H_2O$	100
		EDTA	975

Biofilms and biomass The polypropylene substratum was placed in a trickling filter treating domestic wastewater (Gouda, The Netherlands) to get biofilm developed on its surface. To prevent algal growth, the polypropylene was shielded from sunlight. After 3-4 weeks these sheets and some stones of the filter media, were taken to the laboratory; of the polypropylene one side was cleaned and the other side was rinsed very carefully to remove large organisms and particulate materials. They were put into the reactors with the glucose solution for adaption during 2-3 weeks before the formal experiment was started.

The suspended biomass for the reactor simulating the "deep river" with only suspended biomass in the liquid phase, was taken from the "shallow river" reactor. The initial concentrations were adjusted to a value in the range 5-12 mg/l, based on the established correlation between turbidity and suspended biomass concentration.

Procedure For each specific glucose concentration the experiment with biofilm was repeated at least three times, and only the data obtained after the first two runs were used, because experiments indicated that then the oxygen consumption rate had reached a reproducible maximum value. The deviation of the minimum oxygen concentration (sag point) in the oxygen profile was thereafter less than 0.2 mgO_2/l for the first 7 hours in the following runs, and this was considered acceptable.

After every run the substratum with biofilm was taken out of the reactor and rinsed very gently with distilled water to remove old biomass to prevent sloughing during the experiment, and to control the initial suspended biomass concentration in the liquid phase (X_0). At the same time the vessel was also cleaned with distilled water to prevent biomass growth on the glass surface of the reactor.

The duration of every experiment was 22 hours. Oxygen was measured continuously. Samples of glucose and biomass were taken hourly for the first seven hours. To avoid unreproducible deviation at the beginning of the experiments, values of TUR and SUR were

taken as the averages of the first hour values. The oxygen yield coefficient was calculated from the first seven hour data.

Analysis Most of the analyses were according to standard methods (Am. Publ. Health Assoc., 1985). Dissolved oxygen (DO) was measured continuously with a probe (Microprocessor Oximeter, OXI 196). Glucose was measured by high-pressure ion chromatography (HPIC, Dionex, series 4500i). The biomass concentration in the liquid phase was obtained from the total TOC (Model 700, TOC Analyzer, O.I.Corporation) of the liquid sample minus the glucose TOC and TOC in the tap water. To arrive at the dry weight the values were multiplied by 2 since typically 50% of cell material consists of carbon (Hiemstra, 1983). The samples of biofilm were taken in a representative way from several places on the substratum, and their density was calculated from the VSS and TOC of the biomass scraped from the known surface area on the substratum. The biofilm thickness measurement was according to Bakke and Ollson (1986).

RESULTS AND DISCUSSION

More than forty experiments were carried out in four different reactor configurations reflecting "shallow" and "deep" river: (1) SB reactor with suspended biomass in the water only; (2) BB2.1 reactor with biofilm on the bottom of the reactor and suspended biomass ($WA/V=2.1\ m^{-1}$); (3) STB reactor with "active" filter media (stones) of 3-5 cm diameter from the trickling filter, placed on the bottom of the reactor, and with suspended biomass ($WA/V=2.1\ m^{-1}$); (4) BB22 reactor with biofilm on the bottom, as well as on the wall of the reactor and suspended biomass ($WA/V=22\ m^{-1}$).

Oxygen Consumption Patterns

Fig. 1 is a typical oxygen consumption curve for the SB system. The reaction rate gradually increases to a maximum then decreases, as also reported *e.g.* by Edeline and Lambert (1979). As glucose is decomposed the suspended biomass concentration increases from the beginning to the end of the experiment (data not shown), and this should be the cause for the consumption rate increase. This evolution indicates that first order kinetics might not be acceptable to describe the oxygen consumption rate as a black box phenomenon (which neglects changes in biomass concentration).

At the other extreme, for the BB22 reactor's "shallow river" system ($WA/V=22\ m^{-1}$) the oxygen profile (oxygen sag curve) features an oxygen deficit not-linearly proportional to the substrate concentration; however the time or distance needed to reach the minimum oxygen concentration is hardly influenced. Significantly, oxygen consumption rates are quite different

from the SB reactors "deep river" with respect to oxygen consumption rate against time as shown in Fig.2. The rate gradually decreases with proceeding glucose decomposition. The difference can be related to the different controlling mechanisms, as will be mentioned later.

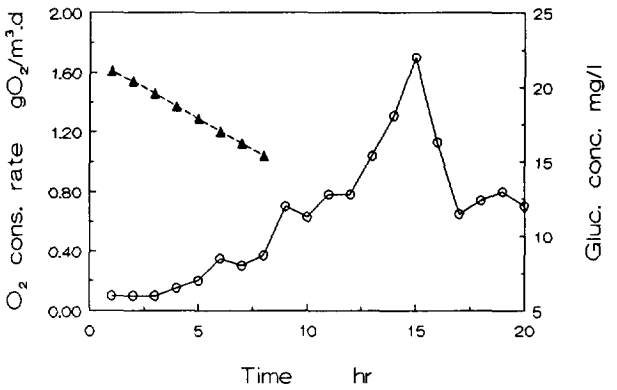

Fig.1 Oxygen consumption rate and glucose concentration in SB reactors.
S_0=21.2 mg/l, X_0=7.2 mg/l, at 20 °C. ○ O_2 consumption rate, ▲ gluc. conc.

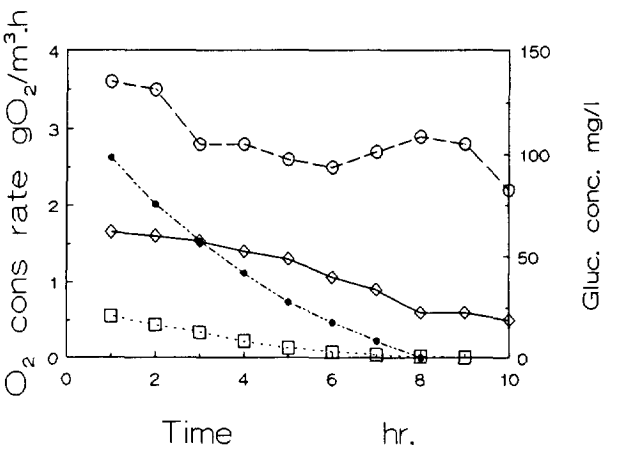

Fig.2 Oxygen consumption rate and glucose concentration in BB22 reactors at 20°C.
◇ oxygen consumption rate of BB22A, □ glucose conc. of BB22A (BB22A: S_0= 20.8 mg/l, X_0=7.0 mg/l). ○ oxygen consumption rate of BB22B, ● gluc. conc. of B22B (BB22B: S_0=100.5 mg/l, X_0=8.0 mg/l).

$Y_{o/s}$ for the two systems range from 0.20 to 0.60, but the averages 0.33 ± 0.10 and 0.31 ± 0.14 (mean $\pm$ SD) differ not significantly. However, $Y_{o/s}$ is calculated based on the first seven hours

data, during which the suspended biomass of SB reactors are still in their growth phase; the same pertains to most of the BB2.1 reactors. In the BB22 and STB reactors on the other hand after about two hours already the glucose had almost entirely decomposed bringing the biomass in the endogenous phase. The oxygen coefficient obtained here for the "shallow river" system is less than the literature value 0.60 (Ohgaki *et al.*, 1978). pH varied between 6.9 and 7.8, but no systematic influence of this factor was detected.

Contributions of Suspended and Attached Biomass

Table 3 summarizes the data. SUR and TUR values are the averages of the corresponding values from the experimental data. BUR is calculated based on the average values of SUR and TUR.

TABLE 3 Summary of Experimental Conditions and Results in Biofilm Systems

No	S_o mg/L	Temp °C	X_o mg/L	WA/V m^{-1}	SUR $gO_2/m^3.d$	TUR $gO_2/m^3.d$	BUR $gO_2/m^2.d$	BUR/SUR m^{-1}
1	9.5-11.4	20	6.8	2.1	2.9	9.9	3.3	1.6
2	19.2-25.4	20	7.2	2.1	3.0	10.4	3.4	1.7
3	19.2-25.4	20	7.2	22	2.8	40.8	1.7	0.6
4	19.2-25.4	28	8.0	22	18.4	93.6	4.0	0.2
5	30-45	20	5.7	2.1	5.2	18.0	6.1	1.2
6	30-45	20	11.8	2.1	10.1	22.3	5.8	0.6
7	30-45	20	5.7	22	5.2	76.8	3.2	0.6
8	75-86	20	5.9	2.1	8.6	24.4	7.5	0.9
9	98-115	20	7.4	2.1	9.0	23.4	6.9	0.7
10	98-115	20	7.4	22	7.0	91.4	3.8	0.5
11	98-115	28	7.6	22	28.2	235.3	9.1	0.3
12*	21	20	7.2	2.1	6.8	55.2	23.4	3.4

* STB reactor, real WA/V $\approx$ 16 m^{-1}.

Two factors determining the nutrient competition between suspended biomass and biofilm, are the interface area between biomass and water, and the mass transfer resistance for nutrients diffusing from the water to the surface of the bacteria and inside bacterial aggregates. The suspended bacterial cells or flocs are likely to provide less mass transfer resistance compared with the relatively thicker biofilm. Therefore suspended biomass

concentration and the wetted area per unit volume of water (WA/V) are taken as the relevant parameters to determine the relative contribution to the biodegradation of the system. Comparing TUR and SUR data in Table 3 it can be stated that the biofilm generally speaking plays a major role in the glucose decomposition, as shown by Fig. 3. However, in our experiments when WA/V=2.1 m^{-1}, if $X_0 > 12$ mg/l, the dominance of the biofilm declines as shown by experiments No.6 (Table 3). Contrary to what could be understood from the existing literature, overall oxygen consumption rate and the relative contribution between suspended biomass and biofilm are very sensitive to the suspended biomass concentration.

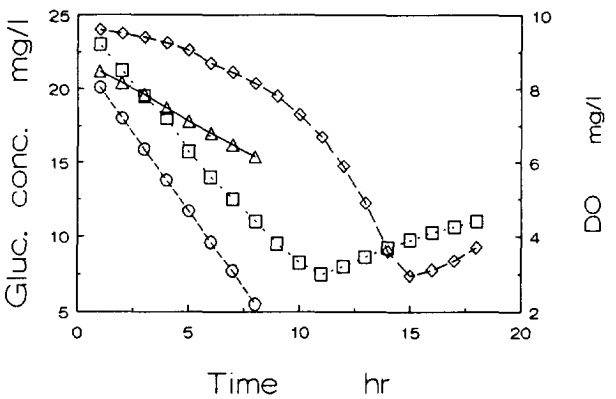

Fig. 3 Oxygen and glucose concentrations of "shallow river"and "deep river" simulation at 20 °C. ◇ DO of SB, ▵ gluc. conc. of SB (SB: S_0=21.2 mg/l; X_0=7.6 mg/l); □ DO of BB2.1, ○ gluc. conc. of BB2.1 (BB2.1: S_0=20.1 mg/l; X_0=7.2 mg/l).

To measure and calculate BUR value, commonly the wetted area used for calculation has been taken as the area covered by the respirometer. In fact this area does not reflect the real interface area where reactions take place, as it overlooks the morphology and configuration of the system. Therefore in order to distinguish from real wetted area the former should be better called "flat projected wetted area". The systematic use of this latter, less specified variable hampers the comparison of BUR between different sources. This is clearly illustrated by experiments No.2(BB2.1) and No.12(STB) in Table 3, with the same S_0, X_0 and flat projected wetted area (WA/V=2.1 m^{-1}), but the BUR and BUR/SUR for experiment No.12 (with the "active filter media" from the trickling filter) are 23.4 $gO_2/m^2.d$ and 3.4 m, *i.e.* 6 and 2 times larger than these of experiment No.2 (with polypropylene substratum), respectively. due to the larger difference of the actual wetted area for these two reactors. For the STB reactors the real WA/V is about 16 m^{-1}, less than WA/V 22 m^{-1} of the BB22 reactors; nonetheless their TUR values are larger than the corresponding values of the BB22 reactors, *e.g.* experiment No.3(BB22) (Table 3). The reasons for this discrepancy are not yet clarified. The effect of difference in biofilm morphology was noticed in the literature

(Hickey, 1988, 1989), but the importance of morphology of the systems was not emphasised, although it appears so strongly influence results.

BUR values for polypropylene substratum here ranged 3-9 $gO_2/m^2.d$ (Table 3) comparable to the literature values of 4-16 $gO_2/m^2.d$ determined by the microsensor and the mass balance over the rotating biofilm contactor (Table 1). This seems logical, as the microsensor takes measurement in a point, and therefore is independent of the definition of wetted area. Also, the rotating biofilm contactor and the polypropylene substratum used here are basically flat surfaces. The BUR values reported here, however, are smaller than Hickey's field data for filamentous biofilm grown on gravel surface (Table 1). This can be due to the large true wetted area per unit volume of water created by the gravel (no size range given). Also, Hickey used only one chamber in his respirometer with a high flow velocity (0.2-0.8 m/sec) leading to sloughing of biomass thereby substantially increasing X and inadvertent inclusion of the corresponding SUR into the reported BUR.

SUR values of 2.8-5.2 $gO_2/m^3.d$ (20 °C) simulating slightly polluted rivers are in the same range as those in literature, *e.g.* 1.5-8.2 $gO_2/m^3.d$ (Boyle and Scott, 1984), but values simulating sewers are near the lower limit reported in literature values. This may be caused by the higher heterotrophic and substrate concentrations, *i.e.* 40-80 mg/l and 100-200 mg/l (BOD_5), respectively in sewers. The BUR and BUR/SUR values of 3.3-23.4 $gO_2/m^2.d$ and 1.2-3.4 m for low glucose concentrations (Table 3) are comparable to literature values except some of Hickey's (1988), *e.g.* BUR=2.04 $gO_2/m_2.d$ (Srinanthakumar and Amirtharajah, 1983), 3.4-14.9 $gO_2/m^2.d$ and BUR/SUR=1.5-6.7 m (Boyle and Scott ,1984). For the sewer simulation our TUR/SUR values are 4-8 higher than 2.57 (Pomeroy and Parkhurst, 1972) and comparable with 6-8 (Saldanha and de Ribeiro, 1991). This difference relates to different WA/V values, the greater in ours and Saldanha and de Ribeiro than in Pomeroy and Parkhurst's system. Generally, SUR, BUR and TUR increase when glucose concentration increases.

Temperature effect and controlling mechanisms

When temperature rises from 20°C to 28°C, SUR for "deep river" simulation increases by a factor 4-5, whilst TUR for "shallow river" simulation increases only about 3 times. This difference reflects the difference in the apparent activation energy of the limiting step of the two systems (Levenspiel, 1972). The "deep river" processes with its predominant suspended biomass are likely more determined by reaction kinetics, whilst diffusion appears to be the limiting step for "shallow river" processes dominated by attached biomass. As a consequence, the "shallow river" would be much less influenced by temperature changes. Therefore the relative contribution of the attached biofilm in the overall biodegradation is bound to decrease substantially when the temperature rises. This is borne out for the case of the "shallow river" simulation (with WA/V=22 m^{-1}, S_0=19.2-25.4 mg/l, Table 3) where the values of TUR/SUR and BUR/SUR are 14.5 and 0.6 m^{-1}, respectively at 20 ^{0}C, and 5.0 and

0.2 m^{-1} at 28°C. This means that the relative contribution of the biofilm decreases by 65%. As to the case of S_0=98-115 mg/l, the ratio values are 13.1 and 0.54 m^{-1} at 20°C, and 8.3 and 0.3 m^{-1} at 28°C. Also here the relative contribution of the biofilm decreases by about 40%. Notwithstanding this pronounced drop in its contribution, the biofilm still determines oxygen consumption under these experimental conditions.

Effect of the Parameter WA/V

A larger WA/V ratio enhances the overall oxygen consumption rate (TUR) as well as the contribution by the biofilm. Fig. 4 shows that glucose and oxygen concentrations decrease much faster if WA/V=22 m^{-1} as compared to the case WA/V=2.1 m^{-1}. Therefore from a practical point of view it can be concluded that self-purification efficiency can indeed be improved by increasing biofilm area. However, it was also shown that adequate oxygen supply is crucial especially during the start period of the experiment to avoid an initial period of anaerobiosis. The lower oxygen concentration of the longer period might be due to the strong endogenous respiration because of large amounts of substrate stored in the body of the bacteria. In practice maintaining certain dissolved oxygen concentration is necessary to prevent sulfite reduction and sewer corrosion.

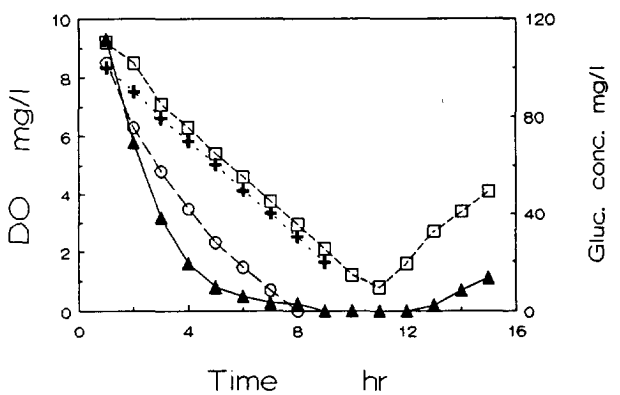

Fig.4 Effect of WA/V on oxygen and glucose profile at 20°C. □ DO of BB2.1, + gluc. conc. of BB2.1 (BB2.1: S_0=100.2 mg/l, X_0=7.9 mg/l); ▲ DO of BB22, ○ gluc. conc. of BB22 (BB22 : S_0=102.3 mg/l, X_0=8.4 mg/l).

Species in the Microbial Community

The composition of the microbial community was investigated with light microscope, Transmission Electronic Microscope (TEM), and Scanning Electronic Microscope (SEM) (Photo 1). The dominant bacteria were mostly like filamentous *Sphaerotilus*, sometimes

called "sewage fungus". In contrast to what was discovered in the biofilm in a flow-through channel (Cao *et al.*, in preparation) coccus bacteria co-existed with *Sphaerotilus* in the batch reactor biofilm. The filaments' size and length were smaller and shorter than those in the immobilised phase which could be due to local differences in velocity gradients and shear stress.

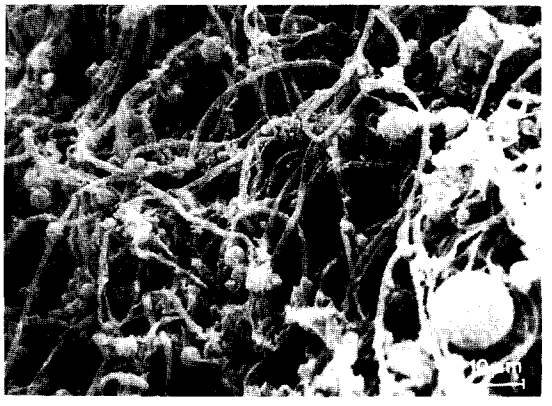

Photo 1 Scanning Electron Micrograph of the outside of filamentous *Sphaerotilus* and some coccus bacteria in the biofilm. Bar indicates 10 μm (SEM-picture taken by Jeol JXA 50A)

Biofilm dry density on the polypropylene substratum at the beginning of the experiment is 8.04 gTOC/m^2 as maximum value, 2.19 gTOC/m^2 as minimum value, and 3.45$\pm$1.1 gTOC/m^2 as average value. The average thickness is 281$\pm$67 μm . After three months running the average values are viz. 5.56$\pm$1.1 gTOC/m^2, 13.8$\pm$2.9 gVSS/m^2, and VSS/TSS = 84.2$\pm$5.2 %. A thickness has been reached of 1.22 mm as maximum value, 243 μm as minimum, and 692$\pm$181 μm as average. All the values derived from 6 samples. Density is comparable to Hickey (1988), but the biofilm thickness is lower than what is commonly discovered in field situation with polluted rivers where thickness is 2-10 mm, partly due to sedimentation of settleable particles. Biofilms in lab conditions appear much more compact than in the field.

CONCLUSIONS

1. The oxygen consumption rate due to aerobic degradation of organic material in the drainage and sewerage systems ("shallow rivers") with a biofilm benthic layer on the bed or bottom differs from "deep river" systems with predominantly suspended biomass. The mechanisms which control the processes, are different: in the case of the biofilm

dominated " shallow river", the rate is likely to be determined by mass transfer diffusion, whereas in the case of the "deep river" with a more pronounced role of the suspended biomass, the rate is determined by reaction kinetics.

2. The attached biofilm makes a significant contribution to the biodegradation *esp.* when the true wetted area per unit volume of water (WA/V) is large. But this conclusion holds only when the suspended biomass concentration is below a certain range. In this experiment the critical value of X_0 is about 12 mg/l when WA/V=2.1 m^{-1}, they are typical values for polluted shallow aquatic systems. Therefore the suspended biomass concentration is an equally important parameter determining the relative contribution of suspended biomass and attached biomass.

3. The values of SUR, TUR, and BUR increase as glucose concentration increases. A poor temperature-dependence was found with the biofilm dominated system. As a consequence the relative contribution to the overall oxygen consumption by the biofilm decreases by typically 40-65% when temperature rises from 20 to 28 ^{0}C. The oxygen coefficient of both systems is approximately 0.31.

4. Self-purification can be improved through increasing the true wetted area of the system. Adequate oxygen supply is the condition for application of this process.

5. The biofilm ecological community was dominated by filaments although cocci co-exist with them.

6. The experiments with only suspended biomass helped to distinguish the function of two biomass types, but the operation was not satisfactory due to low biomass concentration. A new approach for the study of liquid phase reaction should be considered.

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Chapter 3

Biomass Activity and Kinetics in an Aerobic Suspended/Attached Biomass CSTR *

ABSTRACT A lab CSTR with varying specific biofilm areas was adopted to investigate aerobic heterotrophic biodegradation in mixed (dual phase) systems having suspended and attached biomass such as shallow rivers, sewers and drains. The biomass activity in the suspension and biofilm was determined, and intrinsic kinetic parameters were derived; the specific activity of the suspended biomass was almost two times higher most likely due to accumulation of inert extracellular polymeric substance (EPS) in the biofilm. Under the experimental circumstances the relative contribution of suspended biomass with respect to oxygen consumption increased from about 4.7% to 45% when the specific biofilm area decreased from 20.2 m^{-1} to 2.2 m^{-1}. The experiment indicated that the parameters describing dual phase systems like suspended biomass concentration, its specific activity, oxygen up take rate of biofilm and its specific area, should be included in conceptual and mathematic water quality models.

KEY WORDS mixed system, suspended biomass, biofilm, kinetics, activity, population dynamics, filamentous bacteria, *Sphaerotilus natans*

INTRODUCTION

The term "mixed system" has been introduced to describe the dual phase systems where biomass is distributed over both liquid and solid phases, such as shallow polluted rivers or streams whose bottom consists of gravel and cobble, sewers and drainage channels, and rotating biological contactors (RBC). A review of the literature on the biological aspects of wastewater treatment suggests that the function of the suspended biomass in polluted shallow rivers as well as in biofilm-based treatment reactors has been ignored because often more than 90% of the biomass is present in the solid phase, and the suspended biomass

* Y.S. Cao and G.J. Alaerts (1993) *Proc. 2nd International Conference On Biofilm Reactors*, IAWQ/CFRP/AGHTM/EWPCA, Paris, September 28 - October 1, 1993, p.699 - 704.

concentration is so low that it is barely detectable by the eye. Respirometers measuring benthic oxygen up-take rate BUR ($gO_2/m^2.day$) neglect the suspended biomass function and therefore can use the single chamber equipment. This may, however, lead to poor understanding of the processes, and explains partly the discrepancy among BUR values in literature (Cao *et al.*, 1992). A clear picture is yet to be established about the relative importance of the suspended and attached biomass in sewer systems with regard to biodegradation, although self-purification is one of the elements influencing the design and operation of the subsequent treatment plants or the water quality of receiving water bodies.

Our study attempts to better understand aerobic heterotrophic biodegradation in mixed systems, considering microbial speciation, biomass activity, intrinsic and process kinetics. As the behaviour and characteristics of mixed systems differ from those of dispersed and biofilm systems, determining parameters need to be identified. Particular attention here was paid to the suspended biomass contribution under different conditions, the characteristics of the intrinsic and process kinetics, and the possible relationship between them. Intrinsic kinetics are described by specific oxygen consumption activity q_{o2} (gO_2/gbiom.day), Monod constants μ_{max} (day^{-1}) and K_s (mg/L), and process (reactor) kinetics by oxygen up-take rate BUR and k_s (mg/L). The CSTR (lab scale) was adopted because of its convenience of operation and mathematical description. Three specific biofilm areas in the reactor were created. The reactor performance was investigated through the mass balance of oxygen and glucose the carbon source in the wastewater.

MATERIALS AND METHODS

Wastewater and biomass seed As readily biodegradable substrate and the product of fast hydrolysis compounds are widely present in sewers (Henze, 1992), glucose was selected as the carbon and energy source in the synthetic wastewater; the nutrient nitrogen was from NH_4^+. The relative amounts of main nutrients were C:N:P=14.7:4.3:1 (by weight). The whole nutrient compositions are given in Cao and Alaerts (1992).

Biofilm growing on polypropylene substratum was cultured in a trickling filter treating domestic wastewater (Gouda, The Netherlands) for more than four weeks. The suspended biomass in the reactor consisted of eroded material from the biofilm.

Reactors Biofilms of different surface area WA (m^2) were mounted on the wall and bottom of the reactor, a plastic cylinder with effective volume V=5 L, resulting in three specific biofilm areas: WA/V=2.1, 10.8 and 20.2 m^{-1}. The influent glucose concentration were 20-100 mg/L. The flow rates were 2.5 to 7.5 L/h, thus the dilution rates applied were 0.5-1.5 h^{-1}. For most experiments, the blade stirrer rotated at 160 rpm corresponding to a peripheral velocity of 0.59 m/sec. Assuming an average flow velocity 0.3 m/s it created an average

shear stress of 1.0 N/m^2 on the biofilm surface which is typical for sewers. For detailed calculation see Cao and Alaerts (1993). The aeration coefficients were determined and calculated under varying conditions. Each run lasted 6 times hydraulic retention time, sufficient to reach a pseudo steady state with respect to oxygen consumption rate. Biomass dry weight was expressed via TOC measurement (TOC 700, O-I-Corp.) multiplied by a factor of 2 because of the fact that typically about half of the dry cell material comes from carbon (Roels, 1983). Dry biomass TOC was equal to the total sample TOC minus glucose TOC calculated from glucose measurement (HPIC, Dionex 4500i) and background TOC in the tap water which was used in the synthetic wastewater preparation.

Part of the wetted surface (clean surface) was not fully covered with polypropylene substratum *e.g.* under the conditions of WA/V=10.8 and 2.2 m^{-1}, and biomass accumulated on the surface of this part during the run period. To obtain a precise value for the contribution of the biofilm on the polypropylene substratum substitutes with size 3*3 cm were fixed on the wall, and accumulated biomass was quantified by determining the weight difference of the plates after drying at 108°C for 3 h before and after each run. It was found that the accumulated biomass ranged 200-600 mg/m^2; dilution rate and glucose concentration appeared to be the two governing factors. Its contribution was negligible when the WA/V values was 10.8 m^{-1}, but it contributed 15-20% of the total oxygen consumption of the CSTR when WA/V=2.2 m^{-1} in this experiment. The oxygen consumption of the reactor was calculated from the oxygen concentration difference in the influent and the effluent and the aeration capacity; it consisted of the contributions (1) from the biofilm on the polypropylene substratum which was equal to its oxygen up-take rate BUR multiplied by the specific area WA/V, (2) from the suspended biomass, which equalled its concentration X (mg/L) multiplied by its specific activity q_{o2}, and (3) from the accumulated biomass which was calculated from its amounts and its specific activity. BUR were obtained by knowing the parameters mentioned above, and as such is distinguished from the function of suspended and accumulated biomass. The calculation of the glucose decomposition rate R_g ($ggluc/m^2.day$) followed the same procedure as for oxygen, the relative contribution of suspended and biofilm biomass was based on the oxygen consumption. All runs were carried out at least in duplicate.

NH_4^+ concentrations measured indicated the oxygen consumption due to nitrification was negligible; no anaerobic processes could be detected by methanogenic activity experiment. Therefore the oxygen consumption was related to aerobic carbon biodegradation.

Specific activity measurement The specific activities of suspended, accumulated and biofilm biomass for oxygen consumption were determined by use of the Biological Oxygen Monitor (BOM) (Fig.1). Reaction takes place in a chamber of 2-2.5 mL volume; the medium composition is the same as used in the CSTR. The connection between the chamber and atmosphere is so small that aeration can be neglected. It is operated batch-wise; oxygen depletion (measured by a YSI 5331) during the first 10 min is used for calculation. Operation temperatures were 28±1°C and 20±1°C, and pH was 7.0-7.4. To get consistent and

representative samples from the CSTR, the reactor was run continuously for one week under dilution rate 1 h^{-1} and influent glucose concentration 100 mg/L before the BOM experiment was carried out. The suspended biomass sample was obtained by centrifuging the suspended biomass containing water at 3000 rpm for 3 min and then dilute it to the desired concentration. The accumulated biomass and biofilm on polypropylene substratum were scraped from the substratum and then homogenised (MSE Sonipre 150) for 2 min to bring them in suspension. The biomass concentration in the BOM was 50-70 mg/L. As homogenization and stirring inside of the BOM avoided mass transfer resistance, the activities measured are intrinsic and so are the kinetics derived from them (Cao and Alaerts, 1993).

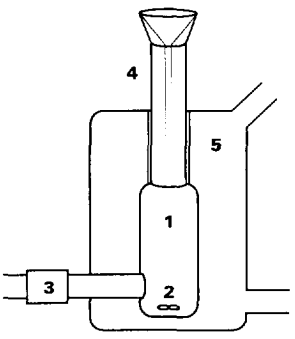

Fig.1 Diagram of Biological Oxygen Monitor 1. reactor chamber, 2. stirrer, 3. oxygen electrode, 4. stopper, 5. water batch.

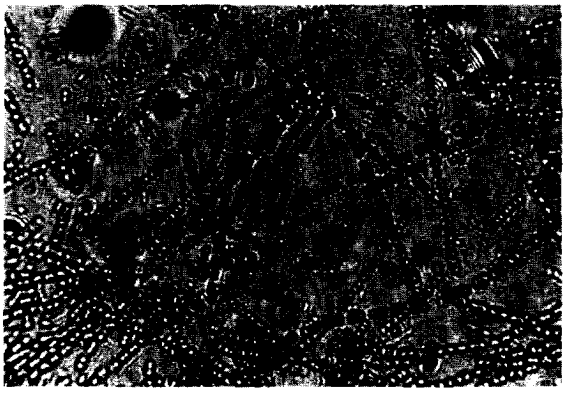

Photo 1 Cell morphology of the dominant filament under microscope after gram staining.

RESULTS AND DISCUSSION

Microbial community and morphology

The dominant species of the microbial community in both phases are filaments, mostly likely because of the selective pressure of glucose the only soluble carbon substrate. This community was totally different from the original trickling filter biofilm. As shown in Photo 1, the characteristics of the cell morphology of the dominant filaments are: diameter 1.2-2 μm, length 2-5 μm and clear cross wall observed under light microscope. The sheath can be found under the Transmission Electronic Microscope (TEM). Gram test was negative, and sulfur storage test showed the granules inside the cells were PHB. Therefore the dominant filamentous species was identified as *Sphaerotilus natans* (Eikelboom and van Buijsen, 1983), a type of bacteria sometimes called "sewage fungus", as it is often found in sewers and polluted rivers. The biofilm looked grey-white and a 1-2 mm surface film was visible. The

thickness and densities D_b (g/m^2) were 1.1±0.2(6) mm, 5.9±0.9(6) gTOC/m^2 *i.e.* 11.9 gbiom/m^2, respectively. Most of the suspended biomass are in the form of individual cells.

Specific activities and Monod constants

The equation $q_{o2}=q_{o2max}*C_g/(K_s+C_g)$ (q_{o2max}-maximum specific activity, gO_2/gbiom.day; K_s-saturation constant, mg/L; C_g-glucose concentration, mg/L) was applied to describe the observed data in Fig.2. The attached biomass activity reaches a constant value already at low glucose concentrations. The suspended biomass is more active than the attached biomass. Considering the predominance of *S. natans* and the source of biomass, a major reason for this could be the accumulation of extracellular polymeric substance (EPS) in the biofilm, which can be as high as 50 to 90% of the total carbon in biofilm (Bakke *et al.*, 1984) decreasing biofilm active content. Apparently, the kinetic constants can not be extrapolated directly from dispersed systems to immobilised systems.

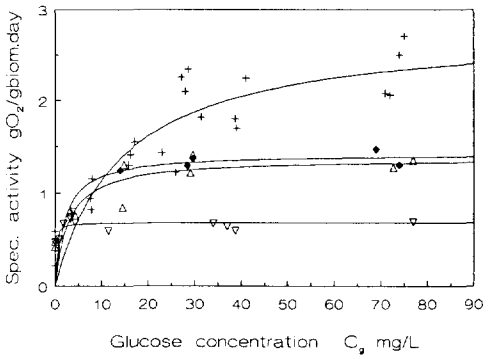

Fig.2 Specific intrinsic activity of suspended and attached biomass at 20°C and 28 °C (measured by the BOM). + suspended biomass at 28°C, Δ attached biomass at 28°C, ◆ suspended biomass at 20°C, ∇ attached biomass at 20°C.

Table 1 summarises the relevant data including the Monod constant values μ_{max} as calculated by equation $\mu_{max}=Y_{x/s}*q_{o2max}/(1-Y_{x/s})$ and accepting 1 ggluc=1.06 gCOD, $Y_{x/s}$=0.50 gbiom/gCOD from $Y_{x/s}$=0.67 gbiom-COD/gCOD the literature for *S. natans* (Kappeler and Gujer, 1992) and 1 gbiom=1.37 gCOD (Heijnen, 1993). The derived intrinsic kinetic constants are of the same order of magnitude as $\mu_{max}=2$ day^{-1}, $K_s=1$ mg/L at 20°C found for *S. natans* by Kappeler and Gujer (1992).

TABLE 1 Biomass intrinsic activities, kinetic constants and reactor oxygen flux

	Temp	q_{o2max}	μ_{max}	K_s	WA/V	BUR_{max}	k_s	η
	°C	$gO_2/gbiom$	day^{-1}	mg/L	m^{-1}	$gO_2/m^2.d$	mg/L	%
Susp. biom.	28	2.8±0.3(24)	2.7	14.6±4.7(24)				
Susp. biom.	20	1.4±0.1(5)	1.4	2.1±1.5(5)				
Att. biom.	28	1.4±0.2(10)	1.3	2.9±2.0(10)				
Att. biom.	20	0.7±0.6(11)	0.7	0.1±0.3(11)				
BUR	28				22	13.9±0.5(14)	0.4±0.1(14)	85.9
BUR	28				10.8	14.9±0.8(11)	0.3±0.2(11)	92.1
BUR	20				22	8.4±0.9(11)	0.3±0.4(11)	104

Oxygen up-take kinetics of reactor biofilm

As shown in Fig.3, BUR reaches a plateau at C_g=5-10 mg/L, which suggests that the macroscopic biofilm kinetics follow zero order kinetics in the range of $C_g > 10$ mg/L, analogous to biofilm intrinsic activity. The equation $BUR = BUR_{max} * C_g/(k_s + C_g)$, similar to the Monod equation is proposed to calculate the biofilm oxygen flux data (Table 1). Here the small k_s value found corresponds to that of K_s for biofilm intrinsic kinetics, K-strategy characteristics typical for the dominance of filaments, illustrating the relationship between intrinsic biomass kinetics and overall reactor performance. For further discussion see Cao and Alaerts (1993). The average BUR at 28°C for the reactor with WA/V=10.8 m^{-1} is about 1 $gO_2/m^2.day$ higher than for WA/V=22 m^{-1}; this may be due to generally higher oxygen level 3-5 mg/L occurring in the reactors with WA/V=10.8 m^{-1} and those at 20°C, against 0.8-2.5 mg/L in the reactor with WA/V=22 m^{-1} at 28°C. BUR values fall in the range of reported literature values (Cao and Alaerts, 1992). BUR is maximum when the biofilm surface loading exceeds 150 $ggluc/m^2.day$ (Fig.5). This figure might be a reference value for the design of a biofilm reactor under the condition of this study. The effectiveness factor η for the oxygen penetration into the biofilm was calculated by the equation $\eta = BUR_{max}/(q_{o2max} * D_b)$. The high η values (Table 1) suggested nearly complete oxygen penetration.

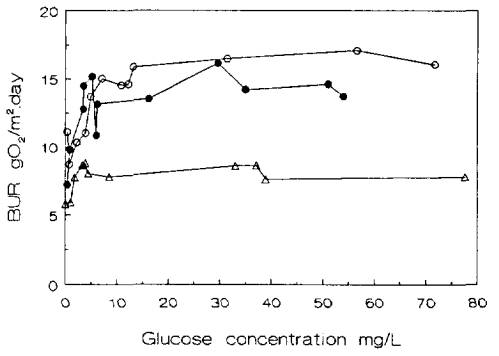

Fig.3 Oxygen flux BUR as function of effluent glucose concentration at 20°c and 28°C.
○ WA/V=10.8 m^{-1} at 28°C, ● WA/V=22 m^{-1} at 28°C, Δ WA/V=10.8 m^{-1} at 20°C.

Suspended biomass contribution

The average of suspended biomass concentration X in the reactor was 12 mg/L. Fig.4 shows that its relative contribution to the overall oxygen consumption rate, as calculated from $X*q_{o2}*V/(BUR*WA+X*q_{o2}*V)$, increases from (on average) 4.7% to 11.3% and 45% when WA/V decreases from 20.2 m^{-1} to 10.8 m^{-1} and 2.2 m^{-1} at 28°C. In the reactor with WA/V=10.8 m^{-1} X reached occasionally 20-30 mg/L then contributing around 30% to oxygen consumption. Consequently, as the range of WA/V of typical respirometers is 4-7 m^{-1}, neglecting the suspended biomass must result in overestimation of the BUR values. For gravity sewers as WA/V value ranges 2-10 m^{-1}, depending on diameter and water depth, and X ranges 30-60 mg/L (Henze, 1992) the contribution of suspended biomass in aerobic metabolisation can be quite substantial. Apparently, the process characteristics of a mixed system firstly depend on the dominance of suspended or attached biomass.

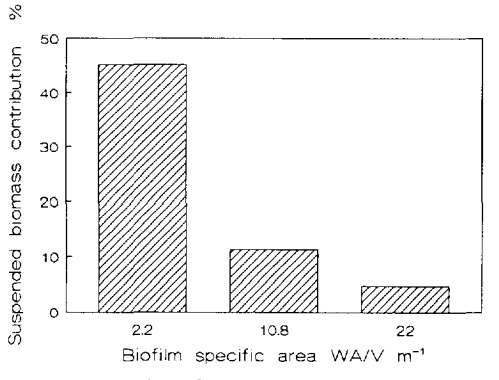

Fig.4 Suspended biomass contribution of oxygen consumption under varying WA/V at 28°C.

Velocity and temperature effects

When influent glucose concentration was 100 mg/L, dilution rate 1 h^{-1} and at 28°C as the peripheral stirrer velocity doubled from 37 cm/sec to 74 cm/sec through increasing the stirrer speed, the glucose concentration in the effluent decreased from 35.1 to 6.3 mg/L whereas the oxygen concentration in the bulk of liquid remained 1-2 mg/L. BUR increased from 11.1 to 19.7 gO_2/m^2.day and glucose flux from 65.3 to 115.9 ggluc/m^2.day, indicating fluxes responsed proportionally.

The temperature effect coefficient on BUR from 20°C to 28°C (BUR_{max28}/BUR_{max20}) is approximately 1.7; it is smaller than 2 for the homogenised biomass ($=q_{o2max28}/q_{o2max20}$). The apparent activation energies calculated by Arrhenius law were approximately 53 KJ/mol and 64 KJ/mol, respectively, which is consistent with the experimental result of the velocity effect because kinetic processes are more temperature sensitive than mass transfer processes due to their relatively larger apparent activation energy.

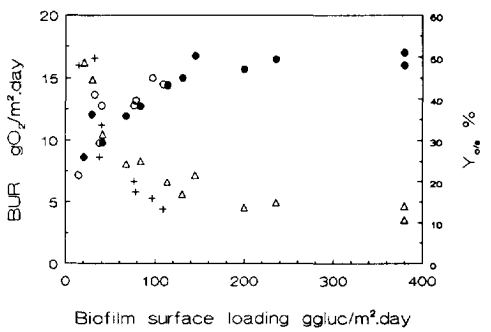

Fig.5 relationship between BUR, $Y_{o/s}$ and biofilm surface loading at 28°C.
○ BUR at WA/V=22 m^{-1}; ● BUR at WA/V=10.8 m^{-1}; + $Y_{o/s}$ at WA/V=22 m^{-1};
Δ $Y_{o/s}$ at WA/V=10.8 m^{-1}.

Yield coefficients

Fig.5 shows that the observed $Y_{o/s}$ ($=BUR/R_g$) of the biofilm decreases from 50 to 10% when the biofilm surface loading increases from 50 to 400 ggluc/m^2.day suggesting that for each glucose molecule less oxygen would be required when surface loading increased. Glucose concentration measured by HPIC and TOC was essentially identical; this showed that formation of organic intermediate products or metabolites was negligible. In contradiction to what electron balance would predict, $Y_{x/s}$ ($=X/(C_{gi}-C_{ge})$, C_{gi}, C_{ge}-glucose concentrations in the influent and effluent) exhibited the same trend as $Y_{o/s}$, even allowing for the accumulation of biomass on the clean surface. Trulear and Characklis (1984) attributed this to uncompleted oxidation resulting in less oxygen needed for one mole carbon compound to

be decomposed, and the formation of intermediate compounds under high surface loading condition. Ohgaki *et al.* (1978) surmised it is due to lack of space for biofilm growth leading to "energy uncoupled growth". The problem may to be related to the inappropriate definition of steady state achieving constant oxygen consumption rate which does not fully support the assumption of steady state biofilm growth; as has been recently reported (Kugaprasatham *et al.*, 1992) biofilm still continues to grow even after stable effluent concentration is established for more than 60 running days. Therefore, it is not proper to calculate the biomass production coefficient based only on information on the liquid phase. This area needs further investigation.

CONCLUSIONS

The microbial community both in liquid and solid phases is dominated by filamentous bacteria identified as *Sphaerotilus natans*. The intrinsic Monod constants for suspended and attached biomass are μ_{max}= 2.68 day^{-1}, K_s=14.6 mg/L, and μ_{max}=1.32 day^{-1}, K_s=2.9 mg/L at 28^{0}C, respectively. Suspended biomass is much more active than attached biomass mostly likely due to the accumulation of EPS in the biofilms. The apparent activation energies for suspended and attached biomass processes were around 64 KJ/mol and 53 KJ/mol, respectively. When the average suspended biomass was 12.4 mg/L, the suspended biomass contribution to overall oxygen consumption could be as high as 45% when the biofilm specific area WA/V was 2.2 m^{-1}. Therefore, its importance should be taken into account when modelling polluted rivers and sewers. The reactor performance is related to its intrinsic kinetic characteristics, although no deterministic model relating intrinsic biomass to overall reactor kinetics could be established as yet. The observed $Y_{o/s}$ of biofilm decreases from 50 to 10% when biofilm surface loading increases from 50 to 400 ggluc/m^2.day, further investigation is needed for carbon balance.

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Chapter 4

Aerobic Biodegradation and Microbial Population in a Channel with Suspended and Attached Biomass *

ABSTRACT A well-mixed in-door recirculating channel was adopted to investigate aerobic heterotrophic biodegradation kinetics and microbial community in drainage systems with suspended and attached biomass. The biofilm function was distinguished from the suspended biomass by using the Biological Oxygen Monitor (BOM) to measure suspended biomass activity. The microbial community was overall dominated by filaments (mainly *Sphaerotilus natans*) due to the selection pressure of the soluble organic substrate; the biofilm was surface film-dominated because of the low flow velocity and shear stress. The channel processes were dominated by the biofilm function. However, it was found that the intrinsic Monod kinetic constants were μ_{max}=2.8 day^{-1}, K_s=1.8 mg/L for the suspended biomass, 1.4 day^{-1}, 2.7 mg/L for the biofilm biomass, both at 28°C; and 0.76 day^{-1}, 2.5 mg/L, 0.52 day^{-1} and 2.9 mg/L respectively for the two types of biomass, both at 20°C. The specific activity of suspended biomass was 50-100% higher than the biofilm biomass, but no significant difference was found between surface and base film biomass. Biofilm oxygen up-take rates (BUR) were around 25 gO_2/m^2.day at 28°C, 18 gO_2/m^2.day at 20°C, and velocity dependent. The observed oxygen consumption coefficient of biofilm $Y_{o/s}$ (gO_2/ggluc) decreased from 50 to 10% when biofilm surface loading increased from 50 to 300 ggluc/m^2.day. The relationship between the biomass population, intrinsic kinetics and biofilm performance is discussed.

KEY WORDS sewer, drain, channel reactor, biofilm, shear stress, biomass activity, oxygen consumption, population dynamics, kinetics, filamentous bacteria, *Sphaerotilus natans*

INTRODUCTION

Biodegradation in sewers and drains is an important element for design and operation of subsequent treatment plants, for water quality of receiving water bodies and nitrogen removal (Nielsen *et al.*, 1992). One characteristic of these systems is that microorganism distribute over both the liquid and solid material surface, and form "dual-phase" systems. Early research studied, *e.g.* the effects of NH_4Cl, glucose and oxygen on the attached growth (Dias

* Y.S. Cao and G.J. Alaerts (1994), accepted by *Wat. Sci. Tech.* for publication.

et al., 1968), and the ecological characteristics of biofilm (Curtis *et al.*, 1971); recent attempts have aimed at enhancing biodegradation capacity by immobilizing cells in drains (Liu *et al.*, 1986) and lagoons (Wang *et al.*, 1991) or by adding activated sludge to the sewer (Green *et al.*, 1985). Literature, so far, tended to focus on the biofilm function, neglecting the role of existing suspended biomass, or lumping the two types of biomass together into the biofilm function. While engineers are interested in system performance and microbiologists in the community's ecology. A thorough understanding of these dual-phase systems and processes is lacking, and the relative importance of the phases is yet to be clarified.

This study pertains to the processes in drains with lesser slope and in oxidation ponds (lagoons) with or without parking materials to favour attached biomass growth. In both cases lower flow velocities prevail. The reactor characteristics of drains and sewers can be complex. Drains and sewers feature to some extent plug flow characteristics with respect to geometric dimensions, and the limited backmixing (together with full lateral mixing). More importantly, in many cases, the high number of waste discharges into the drain along its length causes the actual concentration of water contamination to be relatively constant over its length; this corresponds rather to the behaviour of a continuous stirred tank reactor (CSTR). The sessile biomass, therefore, will tend to have the same characteristics in the beginning and at the end of the drain as it is essentially exposed to a constant quality liquid phase. In the present study the drain or sewer was simulated by a short channel of which inlet characteristics (feed of wastewater) and recirculation flow rate (that determines hydraulic regime in the channel) can be regulated, and which is expected to function as a CSTR. Study focusses on microbial population characteristics in the two phases, including speciation, activity and kinetics; and reactor behaviour under varying conditions, including biofilm oxygen up-take rate BUR, velocity and temperature effect. The approach consists of dividing the system into liquid and solid phase then investigating each of them separately by using the Biological Oxygen Monitor (BOM), and finally integrating them. The reactor performance was studied by system mass balance, and the specific activity and intrinsic kinetics by use of the Biological Oxygen Monitor (BOM).

MATERIALS AND METHODS

Wastewater and biomass seed Glucose was selected to represent readily biodegradable carbon substrate and the fast hydrolysis products which are relevant to biodegradation in sewers and drains, and as carbon and energy source for bacterial growth. The nitrogen nutrient was NH_4^+, and the relative amounts of main nutrients were C:N:P=14.7:4.3:1 (by weight). For whole nutrient composition, refer to Cao *et al.* (1992).

Biofilm growing on polypropylene substratum was cultured in a trickling filter treating domestic wastewater (Gouda, The Netherlands) for more than 4 weeks. The suspended biomass in the reactor consisted of eroded biomass from the biofilm. To prevent phototrophic growth, the substratum was shielded from sunlight.

Recirculating channel The channel was made of thick plywood with trapezoidal cross-section (bottom and top width 0.12 and 0.33 m), length 3.0 m, depth 0.3 m, of which the water depth was 0.18-0.2 m (Photo 1). The water volume V was 130 L. Biofilm on the polypropylene substratum was mounted on the bottom and the side walls resulting in a specific biofilm area 10.4 m^{-1}. The channel was covered to avoid growth of phototrophic organisms. Recirculation of part of the effluent created mixing and reaeration, and governed the flow condition in the channel. The influent flow rates Q were 65-195 L/h, thus the dilution rates (=Q/V) were 0.5-1.5 h^{-1}. The average water flow velocity in the channel is 0.07 m/sec (limited by the recirculating pump capacity) and the average shear stress on the biofilm surface 0.07 N/m^2, both of them one order of magnitude smaller than the typical range for sewer systems. For the detailed calculation see Cao and Alaerts (1993). A tracer (NaCl) experiment and concentration profile measurements confirmed that the channel can be regarded as a well mixed reactor. To decrease and distinguish the oxygen consumption resulting from the biofilm developing inside the recirculation pipe and pump during each run, cleaning was carried out regularly, and sample plates were located inside the pipe to quantify, before and after each run, accumulated biomass by weighing after heating at 108 ^0C for 2 h. For the details of the channel and its operation, see Cao and Alaerts (1994a).

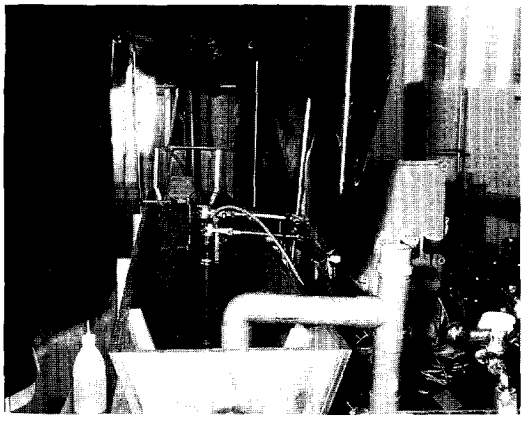

Photo 1 Channel reactor

The oxygen consumption has three origins: (1) the contribution by suspended biomass which was calculated from its concentration and specific activity q_{o2} (gO_2/gbiom.day) as measured by the BOM; (2) the contribution by the biomass accumulated inside the pipe and pump which was calculated from the accumulated biomass amount and its activity as measured by the BOM as well; and (3) the contribution by the biofilm, on polypropylene substratum, is

equal to the biofilm oxygen up-take rate BUR ($gO_2/m^2.day$) times the biofilm's specific area. BUR values were calculated through the oxygen balance of the channel. The same procedure was applied for glucose decomposition rate of biofilm R_g ($ggluc/m^2.day$). For calculation details, refer to Cao and Alaerts (1994a).

The ammonia concentrations in the influent and the effluent indicated that less than 10% of total oxygen consumption was due to nitrification; a methanogenic activity experiment indicated that the anaerobic contribution was negligible. So the oxygen consumption in the channel was aerobic carbon conversion (see also p.81).

Biomass dry weight was expressed *via* TOC measurement (TOC 700, O-I-Corp) multiplied by a factor of 2 because typically about half of the dry cell material comes from carbon (Roels, 1983). Biomass TOC was equal to the sample TOC minus glucose TOC calculated from glucose measurement (HPIC, Dionex 4500i) and background TOC in the tap water which was used in the synthetic wastewater preparation. The biofilm thickness measurement was according to Bakke and Olsson (1986).

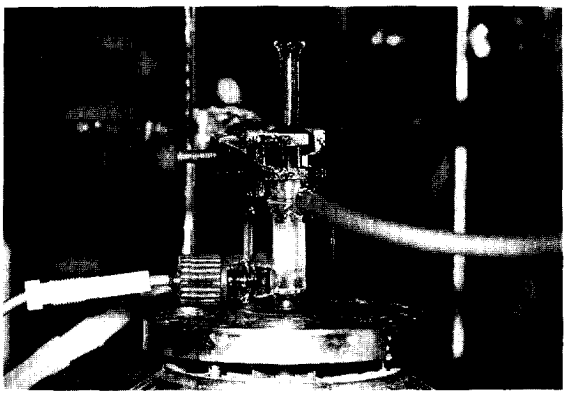

Photo 2 Biological Oxygen Monitor (BOM)

Specific activity measurement The Biological Oxygen Monitor (BOM) was used to measure specific activity of oxygen consumption of the suspended and the biofilm (including the accumulated) biomass (Photo 2). It operates batch wise, the medium components were the same as the wastewater used in the experiments. The oxygen depletion rate in the first 10 min was used for calculation. As stirring inside the BOM and homogenization of suspended and biofilm biomass started before the measurement, excluding thereby mass transfer resistance, the activity as well as the kinetic constants derived from the activity data, could be regarded as intrinsic. For details of the BOM operation and the sample preparation, refer to Cao and Alaerts (1994a).

RESULTS AND DISCUSSION

Microbial community and biofilm morphology

The microbial community of the channel reactor was overall dominated by filaments both in biofilm and suspended biomass, totally different from the initial community sampled from the trickling filter (Cao *et al.*, 1991), this illustrates the selection pressure of glucose, as only soluble carbon substrate on the ecological community of the biofilm system.

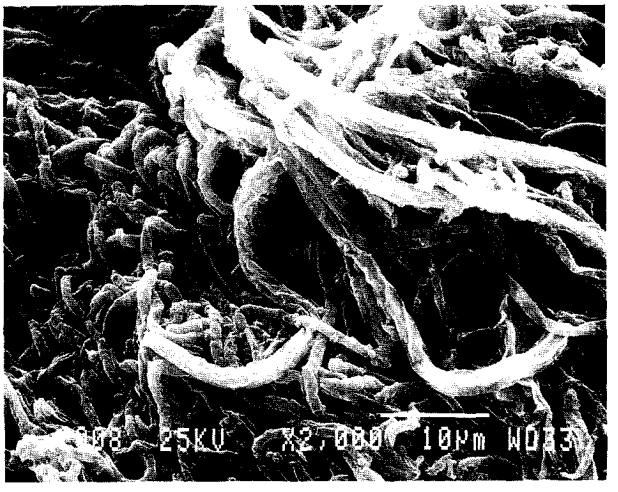

Photo 3A SEM picture of the surface biofilm

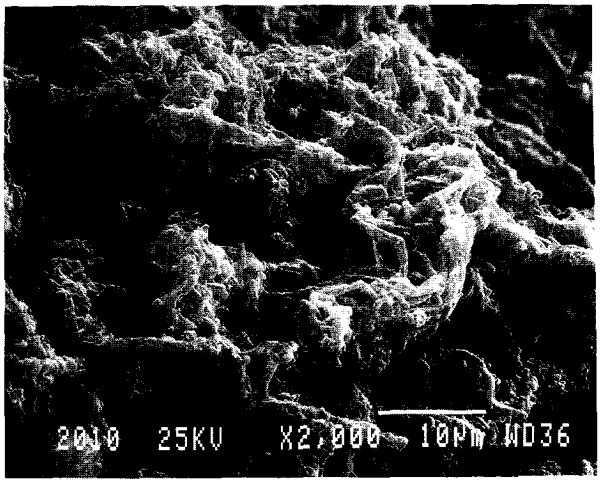

Photo 3B SEM picture of the base biofilm

The cell morphology of the dominant filament was: rod shape cell of 1.2-2 μm diameter, 2-5 μm length and clear cross wall observed under light microscope. False branches appeared sometimes. The sheath could be observed under Transmission Electronic Microscope (TEM). Gram staining and S-test (sulphur storage test) were negative, and adding ethanol to biomass showed the granules inside of the cell were poly-β-hydroxybutyrate (PHB). Therefore, the dominant filament was identified as most probably *Sphaerotilus natans* (Eikelboom and van Buijsen, 1983) a bacterium also called sewage fungus found in sewers and polluted streams. In contrast to the sludge flocs in the aeration tank of activated sludge processes, most suspended biomass was in the form of individual cells or as very small aggregates originating from erosion of the biofilm.

The biofilm was dominated by surface film, which provided a huge mass transfer area for interface processes. Photos 3A and 3B show that, the base film looked more compact in structure than the surface film. The base and surface biofilms could be easily separated by a modest water jet; this means the suspended solids concentration can be quite high under conditions with fluctuating hydraulic shear stress in the drains and lagoons with low average flow velocity. The average biofilm thickness was $3.1 \pm 0.71(6)$ mm (mean $\pm$ SD(sample No)), and areal density $19.8 \pm 2.7(6)$ gbiom/m^2. These high values, and the surface film dominance, resulted from the low average bulk flow velocity and its low shear stress. The favourable effect of low flow velocity, and hence limited erosion, on biofilm growth overtook its negative influence on nutrient diffusion rate from the bulk to biofilm.

Specific activities and Monod constants

Fig.1 displays the pronounced activity difference between the suspended and biofilm biomass at 28^{0}C and 20^{0}C, as measured in the BOM. The intrinsic specific activity of the suspended biomass in the systems was typically 50 (at 20^{0}C) to 100% (at 28^{0}C) higher than that of attached biomass. Considering the predominance of filaments and the biomass seed source, it is likely that extracellular polymeric substance (EPS) and other inert material accumulated in the biofilm are the main cause of the difference between the two biomass types. By assuming suspended biomass consists totally of living cells and suspended and attached cells have the same heterotrophic activity, it can be estimated that inert material occupies about 40% of total dry weight of biofilm; this is slightly lower than the 70-95% proposed as ratio of EPS to organic matter in biofilm (Flemming, 1993).

The EPS (and inert) content of biomass is of critical importance to define biomass activity. The biomass occurs as (1) suspended growth in the liquid bulk, either as individual cells (dispersed system) or flocs like the activated sludge, and (2) biofilm grown on the substratum surface. The EPS content of suspended biomass seems less than in biofilm, despite the differences of the microflora between flocs and biofilm have not yet been adequately elucidated (Flemming, 1993). In the biofilm, the inert material content in the surface layer (near the liquid interface) can be one order of magnitude less than near the substratum

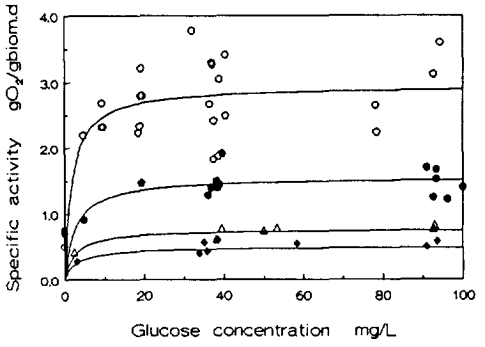

Fig.1 Specific activity of biomass: ○ suspended, ● biofilm at 28°C; Δ suspended, ♦ biofilm at 20°C

(Zhang and Bishop, 1993); thus biomass detached from the biofilm surface will be more active per weight unit than the biomass which is over its full thickness detached from the substratum and whose specific activity represents the average activity of the complete biofilm. Therefore, the suspended biomass specific activity is higher than the biofilm's when it was detached from the biofilm surface, or they are of similar magnitude when sloughing happened, or the biofilm was very thin. The comparison between the dilution rates applied and the cell multiplication time $\theta = \ln 2/\mu$ (Table 1) indicate that the suspended biomass in this study originated from the biofilm surface and not from suspended growth. Pan and Hartmann (1992) reported an opposite phenomenon, but their results based on different expressions of living cell contents were not consistent and derived kinetic constants were well beyond the normal range. Mass transfer resistance is lower for individual dispersed cells, and for the flocs and biofilm depends on the size or thickness, as well as on substrate concentrations in the liquid bulk.

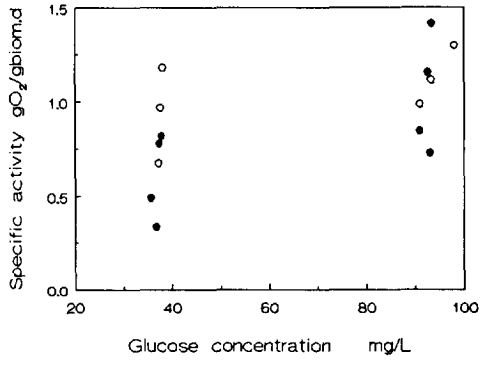

Fig.2 Specific activity of surface film and base film biomass at 28°C. ○ surface film, ● base film

Fig.2 suggests no statistically significant difference exists between the specific activities of the surface and base film biomass. This is most likely due to the base film being thicker than a critical value (*e.g.* 500 μm), where the major EPS content gradient occurs (Zhang and Bishop, 1993), the predominance of *S. natans,* and the deep oxygen penetration which was confirmed by the methanogenic activity experiment (absence of anaerobic conditions).

The specific oxygen consumption is described by $q_{o2}=q_{o2max}*C_g/(K_s+C_g)$; q_{o2max} and K_s, determined by nonlinear regression analysis, are given in Table 1. The Monod constant μ_{max} was calculated by $\mu_{max}=Y_{x/s}*q_{o2max}/(1-Y_{x/s})$, with $Y_{x/s}=0.67$ gbiom-COD/gCOD (Henze, 1987), 1 g biomass approximately being equivalent to 1.37 gCOD (based on dry bacterial composition $CH_{1.8}O_{0.5}N_{0.2}$), and 1 g glucose to 1.06 gCOD.

From the above it emerges that, firstly, suspended biomass can be quite competitive with attached biomass in many cases because of its significantly higher specific activity. (*e.g.* in sewers with large diameter or drains with large width, and in polluted shallow aquatic systems, especially when suspended solids concentration is high or biofilm specific area low); and that, secondly, caution is needed when extrapolating the kinetic constants of free cells to immobilized systems. See also Cao and Alaerts (1993), Cao and Alaerts (1994a).

Macroscopic oxygen consumption kinetics

The suspended biomass concentration was 8-15 mg/L in most of the experiments, and its contribution to total oxygen consumption rate was on average 12%. The accumulated biomass in the pipe was 200-600 mg/m^2 and its contribution was less than 10%. This suggests that the biofilm played a major role in the channel processes.

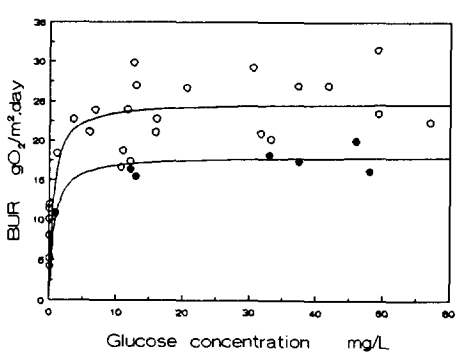

Fig.3 Relationship of BUR and glucose concentration in the effluent; • at 20°C, o at 28°C

Fig.3 shows the BUR at 20 and 28°C as a function of glucose concentration in the effluent C_g, with pH 6.8-7.4, and dissolved oxygen concentration in the effluent at 0.4-2 mg/L and

4-7 mg/L, respectively. The BUR values were around 25 gO_2/m^2.day at 28°C and 18 gO_2/m^2.day at 20°C; they are above the average literature values but still in their range (Cao *et al.*, 1992), for which the great surface area offered by filament dominated surface film is supposed to be the main reason. Characteristically, they reach a saturated state (plateau) already at glucose concentration 5-10 mg/L; this suggests that the biofilm has high activity even at low glucose concentration. A Monod-like equation $BUR=BUR_{max}*C_g/(k_s+C_g)$ was applied to describe these data, the regressed BUR_{max} and k_s are given in Table 1.

Referring to Figs.1 and 3, both q_{o2} and BUR reach a plateau value at low glucose concentration of about 10 mg/L. The k_s values were less than K_s, supposedly because oxygen was limiting in the channel while glucose was limiting in the BOM measurement. Typically, the saturation constant under conditions of limiting soluble carbon substrate is greater than under conditions of limiting oxygen for aerobic heterotrophic growth (Henze, 1987). However, the analogy between micro- (intrinsic) and macro- (reactor) kinetics indicates that this biomass features maximum activity under low glucose concentration, which is typical for k-strategy growth of filamentous organisms (Kappeler and Gujer, 1992).

The intrinsic and reactor activities of the biofilm are different because of mass transfer resistance; the effectiveness factor η of the biofilm (Table 1) is the ratio of both activities and can be calculated by $\eta=BUR_{max}/(q_{o2max}*D_b)$ where D_b is biofilm areal density. The coefficients contain the external mass transfer resistance. The η value at 20°C is greater than at 28°C, because the intrinsic activity decreases fast with decreasing temperature. A high value indicates deep oxygen penetration, here due to the loose filamentous biofilm structure despite the biofilm being as thick as 3 mm.

TABLE 1 Specific activities of biomass and oxygen fluxes into biomass

	Temp	q_{o2max}	μ_{max}	K_s	BUR_{max}	k_s	η
	°C	gO_2/gbiom.d	d^{-1}	mg/L	gO_2/m^2.d	mg/L	%
Susp. biomass	28	2.9±0.2(26)*	2.8	1.8±1.4(26)			
Atta. biomass	28	1.5±0.1(15)	1.4	2.7±2.5(15)			
Susp. biomass	20	0.79±0.1(6)	0.76	2.5±0.8(6)			
Atta. biomass	20	0.54±0.1(6)	0.52	2.9±1.9(6)			
BUR	28				24.9±1.5(30)	0.6±0.4(30)	83.8
BUR	20				18.1±0.7(7)	0.7±0.3(7)	100

* Mean±SD (Sample No.)

Velocity and temperature effect

Under the operating conditions of influent glucose concentration 100 mg/L, dilution rate 1 h^{-1}, temperature 28°C, average bulk water velocity in the channel increasing from 3.7 to 11 cm/sec, oxygen ranged 0.4-3 mg/L, and BUR increased from 23.3 to 30.2 $gO_2/m^2.day$ (Fig.4). The glucose concentration in the effluent decreased from 49 to 12 mg/L, and R_g increased from 89.7 to 121.9 $ggluc/m_2.day$. The channel processes were controlled by external mass transfer.

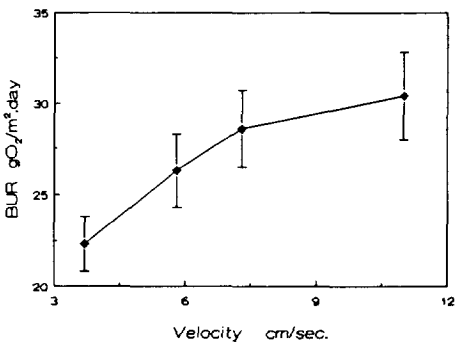

Fig.4 Effect of bulk water velocity on BUR at 28°C

The temperature effect coefficients of the biofilm for a temperature change from 20°C to 28°C were 1.37 for BUR ($=BUR_{max28}/BUR_{max20}$) and about 3.0 for q_{02} ($=q_{02max28}/q_{02max20}$); the suspended biomass processes were much more temperature-dependent than those of the biofilm. Calculated by Arrhenius law, the apparent activation energy of biofilm oxygen uptake was 29.5 KJ/mol and for suspended biomass processes 110 KJ/mol, the relative magnitude is similar to the values found in other reactor (Cao and Alaerts, 1993a). The difference between these values is consistent with the common fact that suspended biomass processes are rather kinetics controlled and with a higher apparent activation energy, whereas the mass transfer process influencing biofilm processes is generally less temperature sensitive and with a lower apparent activation energy. This activation energy difference is a support for the specific activity as measured by the BOM, being intrinsic, and consistent with the biofilm processes being velocity controlled, it also explains the reason for the lower η values at 20°C than at 28°C.

Yield coefficients

The observed oxygen consumption coefficient of the biofilm, $Y_{o/s}$ ($=BUR/R_g$) at 28°C decreases from 50% to 10% when the biofilm surface loading increases from 50 to 300 $gglu/m^2.day$ (Fig.5), which means less oxygen is needed for one mole carbon compound to be decomposed under higher biofilm surface loading condition. The same phenomenon was

reported by other researchers and was also found in other reactor (Cao and Alaerts, 1993a). The biomass production coefficient showed the same trend as the oxygen coefficient. It can be surmised that the assumption of steady state as defined by steady oxygen consumption rate is not fully identical to real steady state; biomass could continue its growth even after a steady effluent was established, as reported also by Kugaprasatham *et al.* (1992). Also, nutrient storage inside the attached cells could occur under high loading condition. However, further investigation is necessary to understand the implications of the oxygen and carbon balance.

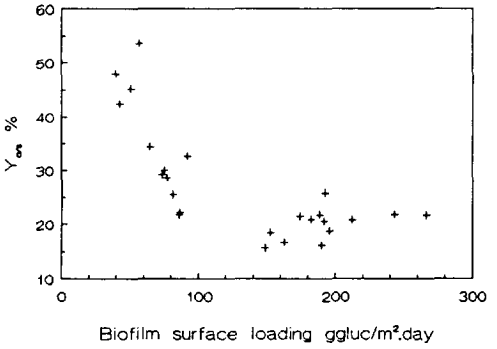

Fig.5 Relationship between oxygen consumption coefficient $Y_{o/s}$ and biofilm surface loading at 28°C

CONCLUSIONS

- Filaments, mainly *S. natans,* dominated the microbial community both in the liquid and in the biofilm because of the selection pressure of the soluble carbon substrate. The biofilm was dominated by surface film due to low shear stress. The biofilm thickness was 3.1 ± 0.71 mm, and areal density 19.8 ± 2.7 gbiom/m^2 (dry weight). Most of suspended biomass was in the form of individual cells.

- The intrinsic Monod constants of suspended biomass were $\mu_{max}=2.8$ day^{-1}, $K_s=1.8$ mg/L at 28°C and 0.76 day^{-1}, 2.5 mg/L at 20°C, and for attached biomass $\mu_{max}=1.4$ day^{-1}, $K_s=2.7$ mg/L at 28°C and 0.52 day^{-1}, 2.9 mg/L at 20°C. The specific activity of suspended biomass was 50-100% higher than the attached biomass; the high EPS content in and its distribution over the biofilm were supposed to be the main reason. This illustrates that suspended biomass could contribute substantially to the whole respiration process especially when they are in the form of individual cells. Generally, the kinetic constants of free cells cannot be extrapolated directly to an immobilized system. No

significant activity difference was found between surface and base biofilm biomass, which is due to the typical biofilm structure of filamentous organisms.

- Biofilm played a major role in the channel processes in this study, and this was flow velocity-dependent. BUR was 25 gO_2/m^2.day at 28°C, and 18 gO_2/m^2.day at 20°C. The small values of both K_s and k_s were consistent with the dominance of filaments in the channel, and illustrate the associated relationship between intrinsic kinetics, microbial population, macroscopic kinetics and reactor performance.

- The temperature effect coefficient for a temperature change from 20°C to 28°c was about 1.7 for the biofilm oxygen up-take, and about 3 for suspended biomass. The respective apparent activation energy was 29.5 KJ/mol and 110 KJ/mol; the suspended biomass processes were more temperature sensitive than those of the biofilm.

- The apparent oxygen consumption coefficients of biofilm $Y_{o/s}$ (gO_2/ggluc) decreased from 50% to 10% when biofilm surface loading increased from 50 to 300 ggluc/m^2.day. Further investigation is necessary to determine the implication of the oxygen and carbon balance.

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Chapter 5

Influence of Reactor Type and Shear Stress on Aerobic Biofilm Morphology, Population and Kinetics *

ABSTRACT The influence of flow pattern on the microbial community of biofilm systems is relevant for process analysis, reactor selection and microbial ecology control. The interaction between reactor type, shear stress, microbial population, intrinsic kinetics, and biofilm performance for biofilm systems was studied by comparing experimental results of three reactors: a batch reactor representing plug flow, and a CSTR and an indoor channel reactor with recirculation, two configurations of continuous stirred tank reactors but with different shear stress. Cocci co-existed with filaments (mainly *Sphaerotilus natans*) in the batch reactor biofilm, whereas filaments predominated in the other two reactors. The values of the Monod constants μ_{max} and K_s of the biofilm biomass of the batch reactor were 2.8 day^{-1} and 54 mg(glucose)/L at 28°C, whereas in the CSTR and channel they were 1.3 day^{-1} and 2.9 mg/L, and 1.5 day^{-1} and 2.7 mg/L, resp. The oxygen consumption coefficients of biofilm decreased from 50 to 10% (gO_2/gglu), when biofilm surface loading increased from 50 to 400 gglu/m^2.day in the CSTR and channel reactors. An analogy was found between intrinsic microbial kinetics and the corresponding macroscopic biofilm kinetics. A conceptual model of these interactions was proposed.

KEY WORDS biofilm, biofilm reactor, flow pattern, kinetics, biofilm morphology, shear stress, mass transfer, population dynamics, *Sphaerotilus natans*

INTRODUCTION

Biofilm systems such as trickling filters, rotating biological contactors (RBC), air-lift suspension reactors (ASR), turbulent beds, *etc.* have been widely studied for wastewater treatment because of the high biomass concentration retained in the reactors and the presumed simplicity of biomass control. Naturally developed biofilms as found on sewer walls (Pomeroy and Parkhurst, 1972; Saldanha and de Ribeiro, 1991; Nielsen *et al.*, 1992)

* Y.S. Cao and G.J. Alaerts (1994), accepted by *Wat. Res.* for publication.

and on the bottom of polluted rivers (Roberts, 1977; Srinanthakumar and Amirtharajah, 1983; Hickey, 1988) have attracted attention as well because of unexpected high rates of organic decomposition. This in-line biological activity partly determines the wastewater composition and lowers the loading of the subsequent treatment plant or water body. Hence, it influences the design and operation of the plants and the water quality of the receiving water, especially since the retention time of the wastewater in the collection system can be of the same order as in the treatment plants. In-line biodegradation is becoming an essential component in the management and planning approach of integrating sewers, treatment plants and receiving water. To enhance purification capacity, attempts have been made to immobilize additional biomass in the drainage channels (Liu *et al.*, 1986; Karnchanawrong and Polprasert, 1990) where the water velocities are typically one order of magnitude lower than in sewers, or on the surface of carrier materials like in lagoons (Wang *et al.*, 1990).

Various configurations of biofilm reactors used in wastewater treatment and water quality monitoring can be classified according to the reactor type (flow pattern). Trickling filters, rotating biological contactors in series, sewers and drainage channels are characterized to some extent by plug flow behaviour; batch-wise operated respirometers used for measuring benthic oxygen up-take rate and sediment oxygen demand are simulating plug flow. On the other hand, fluidised beds, air-lift suspension reactors, and annular reactors with high recirculation rate, and lagoon compartments, are better described as completely mixed systems.

Investigations pioneered by Chudoba (Chudoba *et al.*, 1973) on the effects of flow pattern on the microbial community aimed at preventing sludge bulking in activated sludge processes. It was concluded that (i) the high substrate concentration in the inlet of a plug flow reactor favours floc-forming bacterial growth while the low concentration prevailing in the subsequent well-mixed reactor favours filamentous bacterial growth (kinetic selection) (Houtmeyers *et al.*, 1980), and (ii) the substrate type present in the influent also affects the microbial community composition, *i.e.* soluble material stimulates filamentous bacterial growth, while particulate substrate favours floc formers (Verachtert *et al.*, 1980). Reports on the influence of reactor type on biofilm systems are few. Morgan *et al.* (1991) studied ice cream wastewater treatment by four biofilm reactors and concluded reactor design does not substantially affect the microbial ecology. Lazorova *et al.* (1993) found that hydrodynamic conditions (shear stress) are the determining factor for the contents of living cells and the composition of the microbial community of biofilms. However, understanding of this topic is still limited.

Biofilm macroscopic (process, reactor, or apparent) kinetics were investigated *i.a.* by Atkinson *et al.* (1968) and Harremöes (1978). The external (from the bulk liquid to biofilm surface) and internal (within biofilm itself) mass transfer were studied successfully by using classical theories of fluid mechanics and chemical engineering (LaMotta, 1976). A concept resulting from applying Fick's first diffusion law to mass transfer over biofilm, is turbulence or eddy diffusion particularly over the "rough" (irregular) biofilm surface (Siegrist and

Gujer, 1985; Hickey, 1988; Nielsen *et al.*, 1992). The contribution to overall aerobic respiration by disperse suspended biomass originating from eroded biofilm has been quantified by Cao and Alaerts (1993); it can be of the same order of magnitude as the biofilm's contribution.

The objectives of this study are to investigate the effects of reactor type and shear stress on several main characteristics of biofilm systems, including the microbial community composition, biomass activity, intrinsic kinetics, and biofilm performance, *i.e.* mass transfer and flux. An attempt was made to distinguish the effects of reactor type and shear stress separately.

MATERIALS AND METHODS

Experiment set-up

The biofilm in a batch reactor experiences a substrate concentration gradient in time, and with respect to the liquid is a plug flow; because the gradient develops faster than the biomass can adjust, the biomass itself is in a transitional state, comparable to biomass in a selector up-front an activated sludge system, in a carousel, or in a sewer when subjected to the hourly fluctuation of the sewerage composition. A batch reactor, a continuous stirred tank reactor (CSTR) and an indoor recirculating channel reactor were employed in our experiments to simulate plug flow and (in the latter two) continuous well mixed conditions, resp. The velocity and shear stress on the channel biofilm are substantially smaller than in the other two reactors. Glucose was selected as the only, soluble carbon substrate in the synthetic wastewater representing readily biodegradable substrate, fermentation and hydrolysis products which are concomitant with (and also a consequence of) on-going self-purification in drainage systems. The study is composed of two parts: (i) microbial ecology characteristics, *i.e.* population composition, specific activity excluding mass transfer effect as measured by the Biological Oxygen Monitor (BOM), and the intrinsic kinetics derived thereof; (ii) biofilm macroscopic kinetics, *i.e.* oxygen and glucose fluxes into the biofilms and mass transfer processes. Distinction was made experimentally between the contributions of disperse suspended biomass and biofilm to the conversion processes. Synthetic wastewater was prepared with glucose, and ammonia as the nitrogen source. The ratio of C:N:P was 14.7:4.3:1 (by weight). For the other nutrients, see Cao *et al.* (1992). Biofilm was cultured on polypropylene substratum in a trickling filter treating domestic wastewater (Gouda, The Netherlands) in the period of October-November. To prevent photoautotropic growth (algae), the polypropylene substratum was shielded from light, and only the biofilm on the bottom side of the polypropylene substratum was used. After more than one month the biofilm was taken to the lab for three weeks of adaptation to the synthetic wastewater and experimental conditions before the formal experiments started.

Reactors

Reaction temperatures were controlled at 28±1°C, and 20±°C, by water bath or heat exchanger.

Batch reactor: consists of glass beaker with height 30 cm, internal diameter 24 cm and effective volume V=10 L. The biofilm on polypropylene substratum was mounted on the wall yielding a specific biofilm area WA/V=2.1 m^{-1}. The initial feed glucose concentration was 10-100 mg/L. To distinguish the functions of suspended biomass and biofilm, parallel experiments but with only suspended biomass in the reactor were run, with the suspended biomass taken from the corresponding steady state reactor with biofilm, and kept at the same concentration. A paddle stirrer with diameter d=6 cm was used for mixing and aeration. The peripheral velocity of 0.69 m/s (at the distance 9 cm from the reactor wall) corresponded to a stirring speed $\mathbb{N}$ of 220 rpm. The average Reynolds number Re of 157 was calculated by $Re=\mathbb{N}d^2/\nu$ (Oldshue, 1983), where the kinematic viscosity of water $\nu=8.36*10^{-5}$ m^2/s at 28°C. The aeration coefficient k_La determined was 7.95 day^{-1} at 220 rpm and 20°C and its value at 28°C was calculated from $k_La_{28}=k_La_{20}*1.047^{(28-20)}$. Dissolved oxygen was proven to be uniformly distributed over the volume; aeration was not affected by suspended biomass below 35 mg/L. To exclude photoautotropic growth (algae), reactors were covered with black polythene sheet. To adapt the biofilm, the experiment was repeated at least three times for each glucose concentration, and the data of the third run was used. For details of the reactor and its operation, see Cao *et al.* (1992).

CSTR: consists of plastic beaker (PVC) with height 21 cm, internal diameter 17.5 cm and effective reactor volume V=5 L. The biofilm was mounted on the wall and bottom resulting in WA/V=20.2 m^{-1}. The area not covered by the polypropylene substratum, like the stirrer surface, termed "other surface" WA_{accu}, only occupied less than 7% of total submerged surface area, and hence the modest accumulation of biomass on WA_{accu} was neglected. Glucose and other nutrients were fed through separate tubes, which were cleaned regularly to prevent bacterial contamination (Gabb *et al.*, 1989). For most experiments stirring by a stirrer with diameter 7 cm, was at 160 rpm corresponding to a peripheral velocity of 0.59 m/s (at 5 cm from the reactor wall); average Re was 156. The aeration coefficient k_La determined was 17.0 day^{-1} at 160 rpm and 20°C. The aeration coefficients at other rotational rates were determined as well. Most influent glucose concentrations ranged 10-100 mg/L. The influent flow rates were 2.5 to 7.5 L/h, thus the dilution rates D were 0.5-1.5 h^{-1}. The operating period for every run was taken as six times the hydraulic retention time; constant effluent concentrations of oxygen and glucose indicated that a pseudo steady state was reached at the end of the run. Each run was repeated at least twice. Oxygen concentration and pH were continuously monitored, and glucose and biomass samples were taken hourly.

Indoor channel: The channel, made of thick plywood, had a trapezoidal cross-section (top and bottom width 0.33 and 0.12 m), length 3.0 m, depth 0.3 m of which water depth 0.18-0.2 m, and water volume V=130 L. The influent flow rates were 65-195 L/h. An in- and outlet chamber of 0.25 m length each, separated from the channel by perforated plates, were provided to mix influent and recirculate liquid, and to prevent short circuiting. The biofilm

on polypropylene substratum was mounted on the bottom and the wall yielding WA/V=10.4 m^{-1}. Biofilm sampling plates (18*8 cm) were located in the middle and near both ends. The influent glucose concentrations, feed pattern, dilution rates, operating period, monitoring of oxygen and pH, and sampling frequency were similar to the CSTR. The flow due to recirculation of part of the effluent to the inlet created mixing and aeration. As the recirculation rate was app. 100 times larger than the influent flow rate, the flow velocity in the channel was governed by recirculation and could be controlled *via* the recirculating pump. Corresponding to the recirculating flow of 10 m^3/h in most experiments, the net bulk velocity u was 0.07 m/s in the channel, one order of magnitude smaller than in the batch reactor and the CSTR. Re= 66 as calculated by $Re=u\chi/\nu$ where χ is hydraulic radius (Freach, 1986). Tracer experiment (by injecting sodium chloride, then measuring conductivity response) for the determination of mixing time, and concentration profiles of oxygen and glucose measured both in longitudinal and vertical directions, indicated the channel can be regarded as a well mixed reactor despite its low Re value. k_La was 17.1 day^{-1} at recirculation flow 10 m^3/h and 20°C. Coefficients under other recirculation rates were determined as well. To obtain the precise values of oxygen and glucose flux into the biofilm, J_{02} and J_g, the biomass accumulated inside of recirculating pipe and pump (WA_{accu}) was kept as small as possible. In addition to cleaning the inside of the pipe after every run, extra sampling plates were put inside the pipe to quantify the accumulated biomass for correction purposes.

The Blasius eqn $f=0.078*Re^{-0.25}$ and eqn $f=2\tau_s/u^2\rho$ (Bird *et al.*, 1960) were used to calculate the average friction factor f and the average shear stress τ_s on the biofilm surface in the three reactors (Table 1) where water density $\rho=1000$ kg/m^3. Considering the velocity gradient from the stirrer tip to the biofilm surface, the average velocity u was taken as half of the stirrer tip velocity for both the batch reactor and CSTR. As shown in Table 1, the velocity and shear stress in the batch reactor and CSTR are similar and reflect conditions in sewers. They are one order of magnitude larger than for the channel reactor.

TABLE 1 Reactor type and shear stress on biofilm surface

Reactor	Re	Reactor type	Average flow velocity cm/s	Shear stress N/m^2
Batch	157	plug flow	35[a]	1.34
CSTR	156	well-mixed	30[b]	0.99
Channel	66	well-mixed	7.3[c]	0.07

[a] at 220 rpm.
[b] at 160 rpm.
[c] at recirculating flow rate 10 m^3/h.

Specific activity measurement

The Biological Oxygen Monitor (BOM) was used for the measurement of biomass specific activity q_{o2} (gO_2/gbiom.day); intrinsic kinetic constants of biofilm and suspended biomass were derived therefrom. It is made of glass and operated batch wise (Fig.1). Reaction takes

place within the chamber with a volume 2-2.7 mL, and it is closed free from bubbles with a tightly fitting stopper. The expansion capillary of the stopper is open to the atmosphere, but is so long and limited in contact surface that aeration during the measurement is negligible. The medium components were the same as wastewater used in the experiment. To overcome external mass transfer resistance the suspension is mixed with a magnetic stirrer rotating at 350 rpm. Oxygen depletion is monitored continuously by an oxygen electrode (YSI 5331) through an amplifier (V-t2, class 2.5). The electrode was calibrated for oxygen concentration and dynamic response by the standard oxygen meter (WTW OXI 196) every 5 hours.

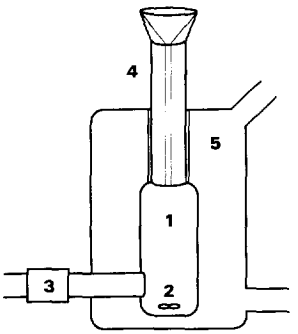

Fig.1 Schematic diagram of the Biological Oxygen Monitor (BOM).
1. reactor chamber; 2. stirrer; 3. oxygen electrode; 4. stopper; 5. water batch.

To let biomass reach a representative condition, the three reactors providing the biomass samples were run for more than 6 days at 28°C as follows before taking samples for the BOM experiment: the initial feed glucose concentration of the batch reactor was 90-100 mg/L, as were the influent concentrations of the other two reactors; the biomass samples of the batch reactor were taken after a 6-7 hour run with the glucose concentration being around 30-40 mg/L; the CSTR and channel reactors were operated under $D=1\ h^{-1}$ and effluent glucose concentration 30-40 mg/L. The biofilm samples were scraped from 6 representative places on the substratum, then washed with sterile water at least two times. To eliminate internal mass transfer resistance, samples were homogenized for 2 min (MSE Sonipreo 150), then centrifuged twice at 3000 rpm for 5 min before measuring their carbon content, and finally diluted to a certain concentration. For suspended biomass one sample aggregated 4 sub-samples taken during the last hour of each run. To keep biomass fresh the samples were aerated for a time at the appropriate temperature before the measurement started. Sample preparation was completed in less than 4 h. After synthetic wastewater was injected into the chamber and a stable temperature reached, the biomass suspension was injected with a syringe. The relative volume of wastewater to biomass suspension was 4-6. Biomass concentration ranged 40-70 mg/L. The slope of oxygen versus time in the first 10 min was taken for calculation. Taking the biomass concentrations, the specific activity of biomass under known glucose concentration C_g was calculated. The BOM was cleaned with sterile water after every measurement. For each glucose concentration, the measurement was

repeated at least twice. Because of preliminary homogenization and stirring, the specific activity and the derived kinetics can be considered intrinsic. The active component and the inert material of the biofilm were not distinguished, so extracellular polymeric substance (EPS) present was weighed as biomass.

Analytical methods

Oxygen was measured by oxygen meters (WTW OXI 196) and glucose concentration by high-pressure ion-chromatography (HPIC) (Dionex 4500i). The other analyses were according to Standard Methods (APHA, 1985). A cellulose filter membrane with 0.45 μm pore diameter was used to separate biomass from soluble organic in the wastewater. The filtered liquor contained glucose and organic originating from the tap water. Biomass was expressed through carbon content as measured by TOC (TOC 700, O-I-Corporation), multiplied by a factor 2 since typically 50% of dry cell material consists of carbon (Roels, 1983). The biomass TOC was considered equal to the difference of the total TOC of the sample minus glucose TOC (calculated from glucose measurement) and background TOC of the water. Measurement of methanogenic activity of biofilm was according to Manual Lab Work Wageningen Agricultural University (1991) and the biofilm thickness measurement according to Bakke and Olsson (1986). The identification of bacteria was according to Eikelboom and van Buijsen (1983).

RESULTS AND DISCUSSION

MICROBIAL POPULATION CHARACTERISTICS

Microbial community

The microbial composition of the trickling filter biofilm, originally dominated by floc forming bacteria, changed considerably in the three reactors when exposed to the new wastewater composition and operating conditions. As demonstrated in Photos 1a-c, filaments prevailed in the biofilm communities in the two reactor types, stimulated by the soluble carbon substrate. This demonstrates selection pressure by the wastewater composition on the biofilm microbial community as also reported by Morgan *et al.* (1991). However, the extent of filamentous growth and the microbial composition of the three biofilms differed. Cocci co-existed with the filaments in the batch reactor (Photo 1a), whereas the filaments nearly exclusively dominated CSTR and channel reactor biofilms (Photos 1b,1c). Similar phenomena have been reported for activated sludge processes: plug flow favours dense flocs with less filamentous bacteria, whereas many continuous well-mixed flow reactors suffer from less dense "bulking sludge" caused by filaments (Houtmeyers *et al.*, 1980). In contrast to the

filamentous organisms, cocci were rarely encountered in the suspended state and seem to attach preferentially to established biofilm. Suspended biomass, emerging from the eroding biofilm, was 8-15 mg/L.

The dominant filamentous bacteria had rod-shaped cells with diameter 1.2-2 μm and length 2-5 μm under light microscope and were surrounded by a sheath which could be identified by Transmission Electron Microscopy. Trichrome and constriction were manifest; false branches appeared sometimes. Gram test was negative. Granules inside cells could sometimes be observed; sulphur test and the addition of ethanol confirmed the granules were poly-β-hydroxybutyrate (PHB) particulates. Based on this the dominant filament was identified as most likely *Sphaerotilus natans* (Eikelboom and van Buijsen, 1983), favoured by glucose substrate (Gabb *et al.*, 1989), and often found as "sewage fungus" in sewers, and in polluted streams receiving effluent of paper and food processing plants containing mainly easily biodegradable carbohydrate. Also other filaments notably one resembling Type 021N, were encountered in the biofilms of the three reactors, though to a much lesser extent. The dominating species in the suspended phase was the same filament as in the biofilm. Higher organisms such as *Colpidium colpodem, Nematoda, Actinopoda* and *Rotifa* were regularly observed in the three reactors but mainly within the biofilms.

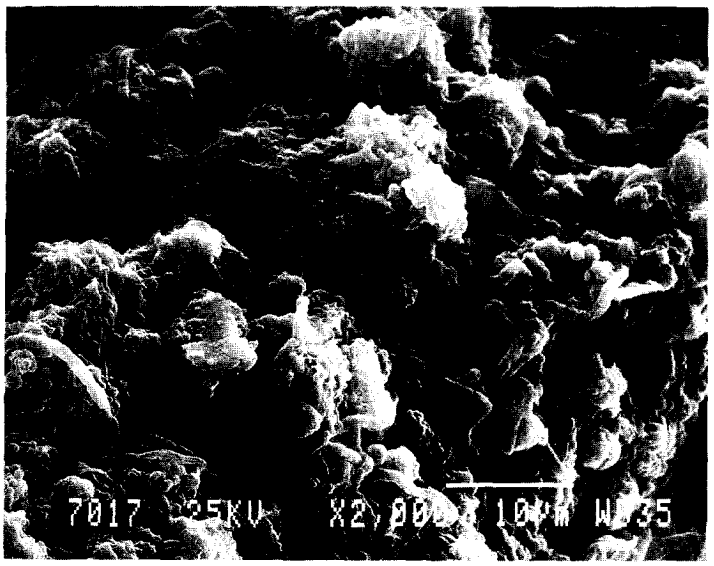

Photo 1a SEM picture of the microbial community of the batch reactor biofilm.

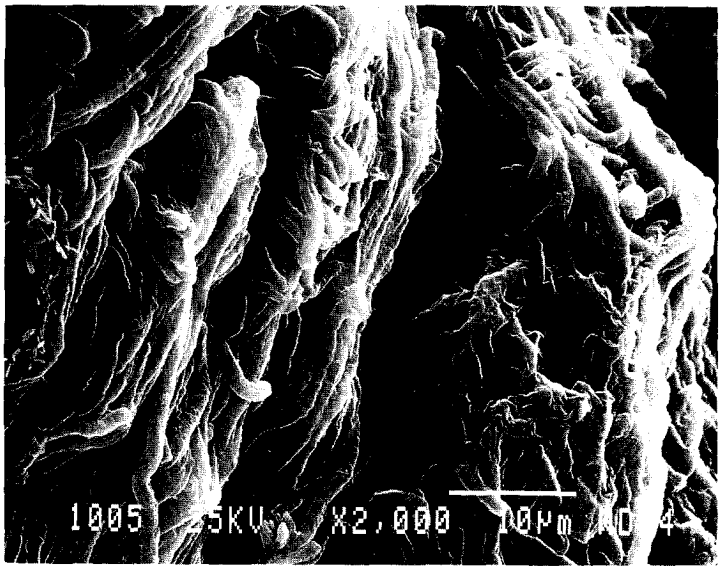

Photo 1b SEM picture of the microbial community of the CSTR biofilm.

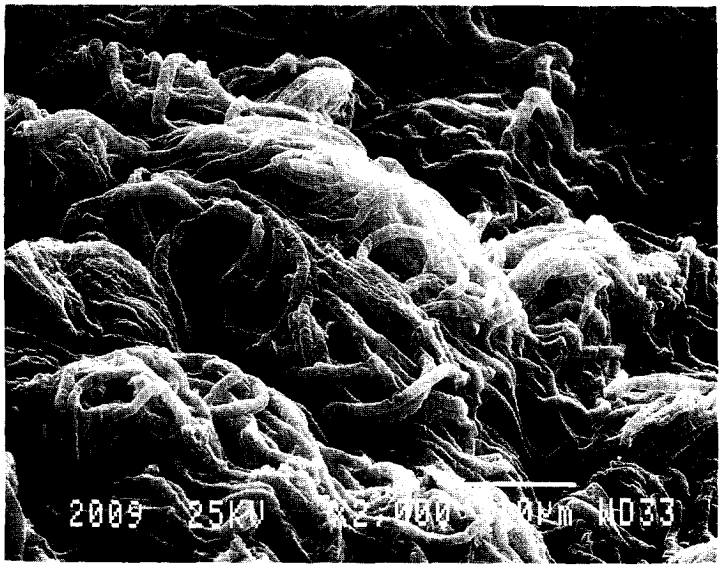

Photo 1c SEM picture of the microbial community of the Channel reactor biofilm.

Specific activity and intrinsic kinetics

The activity measured by the BOM showed that oxygen limitation occurred only when dissolved oxygen < 1 mg/L. Given the BOM procedure, this indicated the activities measured were glucose limited. The difference in activities of suspended biomass with and without preliminary homogenization was not significant, indicating that homogenization did not substantially affect the activity under the study's conditions. Hence the activities determined by BOM and the derived kinetics represent the biofilm intrinsic properties.

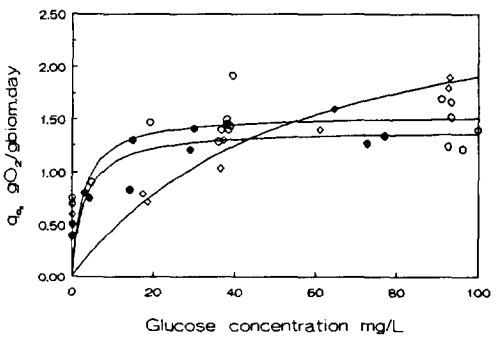

Fig.2 Specific activities of the biofilms in the three reactors at 28°C. ◇ Batch reactor biofilm • CSTR biofilm ○ Channel reactor biofilm

Fig.2 displays the relationship between specific activity q_{o2} (gO_2/gbiom.day) and glucose concentration C_g (mg/L) at 28°C for the biofilm biomass in the three reactors. The q_{o2} values of the batch reactor biomass increase steadily with increasing glucose concentration, whereas q_{o2} values of the other two reactor biofilms reach a plateau value at $C_g \approx 25$ mg/L. Table 2 contains the Monod constants q_{o2max} (gO_2/gbiom.day) and K_s (mg/L) calculated by nonlinear regression from the activity data by the Monod equation $q_{o2}=q_{o2max}*C_g/(K_s+C_g)$; the maximum specific growth rate μ_{max} was derived from following data and equations, *i.e.* 1 gglu=1.06 gCOD, 1 gbiom≈1.37 gCOD (based on the dry bacterial composition formula $CH_{1.8}O_{0.5}N_{0.2}$), $Y_{x/s}$=0.67 gbiom-COD/gCOD (Henze *et al.*, 1987) and $\mu_{max}=Y_{x/s}*q_{o2max}/(1-Y_{x/s})$. The values of μ_{max} and K_s for the batch reactor biofilm are greater than for the other two biofilms; the same trend was found for the data at 20°C as well (Cao and Alaerts, 1993; Cao and Alaerts, 1994a). This means that the microbial consortium in the batch reactor biofilm grows faster under higher glucose concentration, a characteristic for r-strategy growth, typical for floc forming bacteria (Kappeler and Gujer, 1992). The values of μ_{max} and K_s for the biofilm consortia in the CSTR and channel reactor are virtually identical, and the values mean that the biomass exhibits high activity even under low glucose concentration compared with that of the batch reactor biomass, which is characteristic for K-strategy

growth typical for filaments (Kappeler and Gujer, 1992). These kinetic constants concur with the observations on the microbial community composition in the three biofilms.

TABLE 2 Specific activities and Monod kinetic constants of the three biofilms at 28°C

Reactor	q_{o2max}	μ_{max}	K_s
	$gO_2/gbiom.d$	day^{-1}	mg/L
Batch	2.9±1.1(10)*	2.8	54±42(10)
CSTR	1.4±0.2(10)	1.3	2.9±2(10)
Channel	1.6±0.1(15)	1.5	2.7±2.6(15)

* Mean±SD (sample number)

The BOM measurements also showed that in the three reactors the q_{o2} values of disperse suspended biomass were much higher (almost two times) than those of biofilms. The EPS accumulation and its distribution in the different layers of the biofilms are assumed to be the main reason. This activity difference is of great significance for the experimental approaches to obtain exclusively biofilm-related kinetic constants, and for the processes and oxygen uptake measurements in so called "dual-phase" systems with both suspended (often visually not detectable) and attached biomass such as some wastewater treatment plants and polluted shallow aquatic systems. Detailed data and discussion are given elsewhere (Cao and Alaerts, 1993; Cao and Alaerts, 1994a; Cao and Alaerts, 1994b). Accepting as typical values $\mu_{max}=3$ day^{-1}, $K_s=1$ mg/L and $C_g=15$ mg/L, that cell multiplication time θ is about 9 h as calculated from $\theta=\ln 2/\mu$ where $\mu=\mu_{max}/(K_s+C_g)$, and comparing this with the retention time in the three reactors, it can be concluded that most suspended biomass originated from biofilm erosion.

Biofilm morphology and structure

The biofilm's physical appearance and structure were different in the three reactors. The batch biofilm looked slimy with a brown colour and the surface film was not identifiable; the CSTR and channel reactor biofilms were grey-white. As shown in Table 3 the volumetric density of the batch reactor biofilm was 1.5 and 2.5 times that of the CSTR and channel reactor biofilms, respectively; it was the most compact. Considering the similar magnitude of the shear stresses on the surface of the batch reactor and CSTR biofilms (Table 1), their different colour, thickness and density result from differences in their microbial composition, which highlights the effect of reactor type on biofilm morphology. For the CSTR biofilm the filaments with a length of 0.5-1.5 mm were clearly discernable at the surface; the areal density of the channel biofilm was highest and the length range of its filaments was 2-4 cm which looked like "cotton flower" (Photo 2). Apparently, this difference in filament length

and biofilm morphology is directly related to the different shear stresses on each biofilm surface, which determined the biofilm areal density and the ratio of surface to base biofilm.

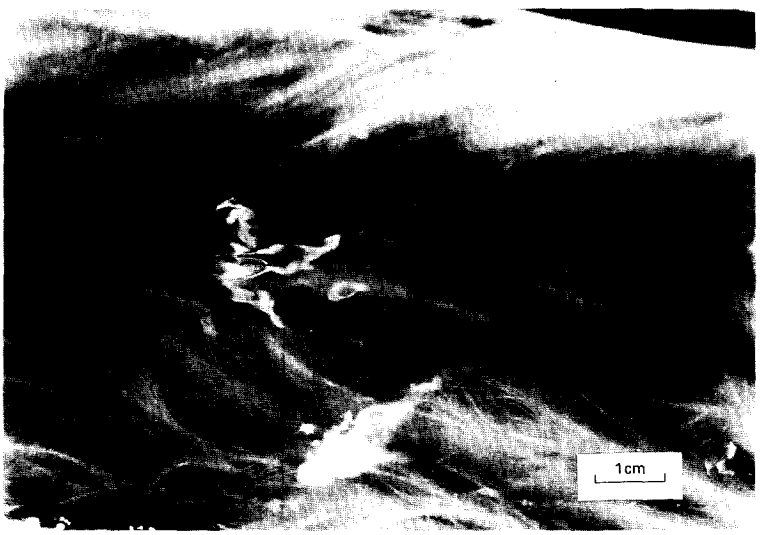

Photo 2 Morphology of the channel reactor biofilm.

BIOFILM PERFORMANCE

Calculation procedure

The calculation procedure for oxygen consumption and glucose decomposition rates differs for the batch and continuous well mixed reactors, but are both based on mass balance for oxygen and glucose. The initial reaction rate approach was adopted for the batch reactor. The slope of the oxygen depletion curve and the decrease rate of glucose concentration over the first hour of the experiment were used to calculate total oxygen consumption rate TUR (g/m^3.day) and glucose decomposition rate R_{gtot} (g/m^3.day) by eqns (1) and (2), where $\Delta t = 1$ h, and ΔC and ΔC_g are the decreases of oxygen and glucose concentration, resp., over the first hour. The average of the glucose concentrations at the beginning and after one hour was taken as the initial glucose concentration C_g corresponding to the reaction rates calculated.

$$TUR = \frac{k_L a}{\Delta t} \int_0^t (C^* - C)\, dt - \frac{\Delta C}{\Delta t} \qquad (1)$$

$$R_{gtot} = -\frac{\Delta C_g}{\Delta t} \quad (2)$$

For the CSTR and channel reactors under pseudo steady state, the equations used for calculation were

$$TUR = D^*(C_{oi}-C_{oe}) + k_La^*(C^*-C_{oe}) \quad (3)$$

$$R_{gtot} = D^*(C_{gi}-C_{ge}) \quad (4)$$

The fluxes of oxygen and glucose J_{o2} and J_g ($gO_2/m^2.day$) were calculated after having obtained TUR and R_{gtot}. For a reactor containing suspended biomass, biofilm on polypropylene substratum WA, and biomass accumulating on WA_{accu}, TUR ($gO_2/m^3.day$) is the sum of the three oxygen up-take rates sources, *viz.* suspended biomass oxygen up-take rate SUR ($gO_2/m^3.day$), J_{o2}*WA/V ($gO_2/m^3.day$), and oxygen up-take rate by biomass accumulated on WA_{accu}, J_{o2accu}*WA_{accu}/V ($gO_2/m^3.day$). Therefore:

$$J_{o_2} = \left[TUR - SUR - \frac{J_{o_2accu} * WA_{accu}}{V} \right] * \frac{V}{WA} \quad (5)$$

SUR was derived experimentally from the parallel experiments without biofilm for the batch reactor (Cao *et al.*, 1992), and from the suspended biomass concentration and its q_{o2} ($gO_2/gbiom.day$) measured by the BOM for the CSTR and channel reactors. The principle of measuring oxygen consumption rate in the parallel batch reactor and in the BOM is the same, and the BOM equipment is easier to operate. For the batch reactor the biomass accumulation during the first hour was negligible, and as mentioned in Material and Methods the same situation held for the CSTR. In case of the channel reactor, it was quantified from the accumulated amounts of biomass (Cao and Alaerts, 1994a) and its q_{o2} measured by the BOM. J_g ($ggluc/m^2.day$) was calculated in the same way as J_{o2}. The glucose decomposition rate R_{gtot} was calculated by eqn (2) for the batch reactor and eqn (4) for the CSTR and channel reactor. The contributions of suspended and attached biomass to total glucose conversion can be distinguished based on the oxygen consumption data.

Ammonium measurement indicated that oxygen consumption due to nitrification was always well below 12% of total oxygen consumption in all reactors. Nitrification was henceforth not considered a major contribution to oxygen consumption under the experimental circumstances. The methanogenous experiment showed the absence of anaerobic methane production. Zero to negligibly small difference was found between the glucose concentration measured by HPIC and TOC, suggesting that no organic intermediates or metabolites were formed. Therefore, it appears that under these conditions anaerobic activity was negligible in the biofilm. Sulfide production was not detected in the experiments, presumably due to prevailing aerobic condition in the biofilms, especially in the case of the CSTR and channel

reactor where almost full oxygen penetration into the biofilms was recorded. Therefore, it could be concluded that the oxygen consumption in the reactors was essentially governed by the aerobic heterotrophic carbon biodegradation.

Oxygen flux into biofilm

Fig.3 displays J_{o2} in function of the system determining glucose concentration: the initial concentration in the case of the batch reactor, and the effluent (or liquid bulk) concentration for the CSTR and channel reactor, at 28 ^{0}C and pH 6.4-7.3. The channel data have a scattered look because of the irregular sloughing of the surface film due to the loose connection with the base film. According to Castalodi and Malina (1982) the biofilm processes in the three reactors should be limited by oxygen when $C_g > 15$ mg/L, even if the dissolved oxygen concentration in the batch reactor was at 7-8 mg/L higher than in the other two reactors. The oxygen flux into the batch reactor biofilm steadily increases with increasing glucose concentration; the fluxes into the CSTR and channel biofilms quickly reach a plateau value; the same occurred at 20^{0}C as well (Cao and Alaerts, 1993; Cao and Alaerts, 1994a). The curves follow trends analogous to those of the corresponding relationship between q_{o2} and glucose concentration (Fig.2).

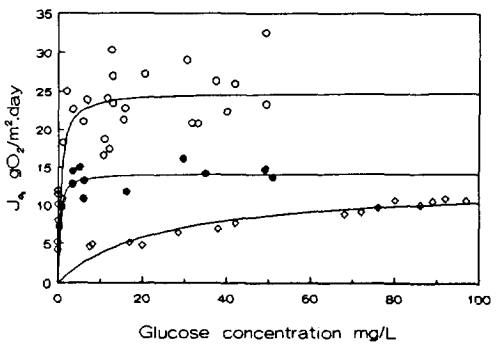

Fig.3 Oxygen flux into the biofilm of the three reactor at 28^{0}C. ◇ Flux into the batch reactor biofilm • Flux into CSTR biofilm o Flux into the channel reactor biofilm.

The curves' shape is approximated by the Monod-like equation $J_{o2}=J_{o2max}*C_g/(k_s+C_g)$. Though the mass transfer phenomenon over the biofilm remains "hidden" behind the k_s and J_{o2max} values, the constants k_s (mg/L) and J_{o2max} (gO_2/m.day,) determined by nonlinear regression analysis (Table 3), still have more a biological rather than a mathematical meaning. The k_s values are smaller than K_s (Table 2) because of the different limiting substrate: oxygen was limiting in the three reactors, whereas glucose was limiting in the BOM determination for the q_{o2} values; commonly, for aerobic heterotrophic growth the value of the half saturation constant under oxygen limitation is smaller than that under soluble organic substrate (Henze *et al.*, 1987). Comparison of the constant values of the macroscopic

kinetics (Table 3) with those of intrinsic biofilm kinetics (Table 2) shows that the batch biofilm is characterized by $k_s = 19.0$ mg/L, which is typically 31 and 47 times larger than for the CSTR and channel reactor biofilms, resp.; this is similar for the relative positions of the K_s values. Accounting for the similar bulk velocities in the batch and CSTR, and for the fact that the k_s value of the channel biofilm would be probably further decreased if its flow velocity were the same as in the other two reactors, the analogy in responses of q_{o2} and J_{o2} to glucose concentration demonstrates the associated relationship between the intrinsic kinetics and the macroscopic biofilm kinetics.

TABLE 3 Oxygen flux, biofilm morphology and macroscopic kinetic constant of the three biofilms at 28°C

Reactor	Biofilm appearance	Biofilm thickness mm	Biofilm areal density D_b gbiom/m²	Biofilm volumetric density kgbiom/m³	Oxygen flux J_{o2max} $gO_2/m^2.d$	k_s mg/L	η %
Batch	surface film not visible, brown	0.7±0.6(6)*	11.2±2.2(6)	16.0	11.6±1.0(12)	19.0±5.3(12)	54.0
CSTR	surface film visible, grey-white	1.1±0.2(6)	11.9±1.9(6)	10.8	13.9±0.5(14)	0.4±0.1(14)	80.3
Channel	surface film dominated, grey-white	3.1±0.7(6)	19.8±2.7(6)	6.4	24.9±1.5(30)	0.6±0.4(30)	80.8

* Mean±SD (sample number).

In water quality modelling, J_{o2} is expressed as biofilm or benthic oxygen up-take rate BUR. The measurement of BUR by respirometer assumes, in most cases, that all oxygen demand results from the benthic biomass and that the suspended biomass has a negligible contribution. The reported values show BUR to be 3.4-14.9 $gO_2/m^2.day$ (20°C) in a polluted river (Boyle and Scott, 1984), 70 $gO_2/m^2.day$ (14-24°C) in a rapid flowing, polluted river with a gravel bottom covered with long filamentous biofilm (Hickey, 1988), 4.2-6.4 $gO_2/m^2.day$ (20°C) for trickling filter treating domestic wastewater (measured by microsensor) (Kuenen *et al.*, 1986), 16.8 $gO_2/m^2.day$ (25°C) (Saldanha and de Ribeiro, 1991) and 23.6 $gO_2/m^2.day$ (27°C) (Pomeroy and Parkhurst, 1972) for sewers. J_{o2} values in our experiment are of the same order of magnitude as literature data. Hickey's value could be untypically high perhaps because of high suspended biomass concentration due to the high water velocity within the respirometer and because the multiple layers of gravel create a porous biofilm with a comparatively large interface.

Mass transfer resistance

The mass transfer resistance was investigated by comparing the biomass intrinsic activity, biomass areal density, and the oxygen flux. The determining factors are flow velocity, biofilm structure and interface area, the latter two determined by microbial composition and shear stress.

An overall effectiveness factor η of oxygen penetration was adopted to express the biofilm activity. η was calculated according to $\eta = \overline{J_{o_2}}/(\overline{J_{o_2}} * D_b)$ for the batch reactor where $\overline{J_{o_2}}$ and $\overline{q_{o_2}}$ are the average values taken from Fig.3 and Fig.2, and $\eta = J_{o2max}/(q_{o2max} * D_b)$ for the CSTR and channel biofilms. For the batch reactor biofilm $\eta \approx 54\%$ (only half the biofilm depth is penetrated), and for the CSTR and channel biofilms $\eta \approx 80\%$ (most of the biofilm is penetrated). The coefficients calculated contain the contribution of external mass transfer resistance (from the liquid bulk to the biofilm surface) because the flux is influenced by external mass transfer.

The batch biofilm has the largest internal mass transfer resistance as can be deduced from the different η values. Firstly, its intrinsic activity is statistically similar to that of the other two reactors in the concentration range used; secondly, its external mass transfer resistance was comparable to that of the CSTR, and less than that of the channel due to the flow velocity difference which determines the external mass transfer resistance. The compact structure of the batch biofilm, that corresponds to the large internal mass transfer resistance, is the main reason for the small value of J_{o2}. Presumably, the cocci co-existing with the filaments provide for the more compact biofilm structure.

Changes in external mass transfer were observed in the flow velocity experiments (Cao and Alaerts, 1993; Cao and Alaerts, 1994a). In contrast to the smooth and denser CSTR biofilm, microscopic observation showed the filaments in the channel biofilm to appear as loose separately swaying bundles, of which the total interface area could be tentatively calculated 10-20 times larger than their horizontally projected surface, depending on the assumed dimensions of such bundles. Given a similar concentration gradient in both reactors, a 10-20 times larger contact area of the channel biofilm surface than the smooth CSTR's, and the observed flux difference, the mass transfer coefficient over the channel reactor boundary layer appears to be around 10% of that in the CSTR, although the length of channel biofilm filaments was much greater than the typical boundary layer thickness of 100-500 μm (Kuenen *et al.*, 1986). Though precise measurement of the interface area admittedly remains precarious, the figure was based on reliable estimation. This analysis looks contradictory to the concept of eddy diffusion on the rough biofilm surface, which is based on the classic roughness theory of fluid mechanics and supported by the greater diffusivities measured over a biofilm than in water (Siegrist and Gujer, 1985; Hickey, 1988; Nielsen *et al.*, 1992); for the conventional approach, however, an essential assumption had to be adopted *i.e.* that the actual contact area between water and biofilm surface equals the horizontally projected area. Accepting the value for the interface area calculated here for the channel would lead to

diffusivities in the same order of magnitude as in water. Similarly, Kuenen *et al.* (1986) using microsensors to measure the oxygen profile in the boundary layer found a thicker rather than thinner boundary layer above rough biofilms. It is not yet clear under which conditions the roughness theory, originally developed for rigid surfaces, applies to the flexible, irregular biofilm surface as encountered in the channel reactor. However, the data of the channel biofilm illustrate the flux can be increased to the biofilm under low shear stress such as in pond systems or in the sewers in dry seasons.

Yield coefficient

The observed oxygen consumption coefficient of biofilm $Y_{o/s}$ ($gO_2/gglu=J_{o2}/J_g$) decreased from 50% to 10% when the biofilm loading increased from 50 gglu/m^2.day to 400 gglu/m^2.day for both the CSTR and channel biofilms. The same trend was found for the batch biofilm as well. Unexpectedly, the biomass production showed the same trend. Oxygen limitation, nutrient storage inside attached cells and the continued growth of biofilm even under pseudo steady state conditions could provide explanations.

CONCEPTUAL INTERACTION MODEL

The filamentous organisms dominated in all three biofilms due to the selection pressure by soluble substrate, but the extent of dominance was different in the two basic reactor types, which agreed well with the experimental results of the corresponding specific activities and intrinsic kinetics. A picture emerges similar to that of the kinetic selection by reactor types on the microbial community in activated sludge processes (Ćech *et al.*, 1984; Gujer and Kappeler, 1992). The zero-order kinetics suggested by LaMotta(1976) relates only to a well-mixed system, and no generally applicable intrinsic kinetic formulation should be expected. By comparing the thickness, density and colour of the batch reactor and CSTR biofilms, it appeared that the reactor type not only influences the microbial composition but also the biofilm morphology; for example, the compact structure of the batch biofilm was related to the presence of cocci, which affected internal mass transfer. The differences in the proportion of base and surface films, thickness and density, between the CSTR and the channel reactor biofilms illustrated shear stress effects on biofilm morphology; no significant effect of shear stress on the biofilm microbial characteristics was found. Therefore, reactor type and shear stress each exert specific effects on the microbial ecology and morphology of biofilms, and their effects should be investigated separately. The apparent biomass activity in the biofilms was retarded by mass transfer resistance and EPS accumulation. The observed analogies between microbial (intrinsic) and reactor kinetics showed the associated relationship linking microbial composition and specific activities, and overall biofilm performance. Fig.4 tentatively identifies and orders the determining system parameters and indicates the structural relationship of the interactions found in this study.

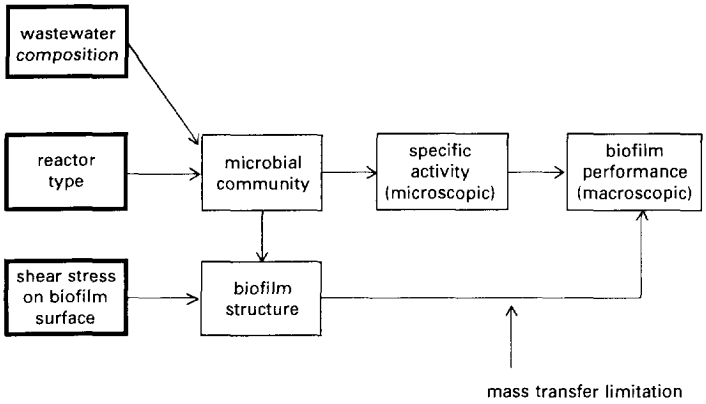

Fig.4 Interactions among reactor type, shear stress, microbial population, biofilm morphology and performance.

The qualitative difference between reactor types is caused by the differences in substrate concentration distribution in these systems. Different growth characteristic under different substrate concentrations for microorganisms is the key for the kinetic selection; substrate concentration (and type) has a pronounced influence on the microbial community composition. These points were perhaps insufficiently highlighted by Morgan *et al.* (1991) and Lazorova *et al.* (1993), as no reactor type specification and substrate concentration profile were described in their publications.

CONCLUSIONS

The batch reactor representing plug flow condition, and the CSTR and channel reactors representing the continuous well-mixed flow, were used to investigate aerobic heterotrophic biodegradation in drainage systems with attached biomass. The reactors were fed with easily biodegradable synthetic wastewater and were operated at comparable conditions. Based on the analysis of the experimental results a conceptual model for the interaction among the relevant factors is presented.

Cocci co-exist with the filaments (most likely *S. natans*) in the batch reactor biofilm, while the microbial composition is virtually the same *i.e.* nearly only filaments existing in the CSTR and channel reactor biofilms. The Monod constants μ_{max} and K_s of the batch reactor's homogenized biofilm are 2.8 day^{-1} and 54 mg/L (glucose), featuring r-strategy growth characteristics; virtually the same values, *i.e.* 1.3 day^{-1} and 2.9 mg/L, and 1.5 day^{-1} and 2.7 mg/L (glucose), are found for the CSTR and channel reactor biofilms which exhibit K-strategy characteristics. This illustrates that plug flow favours floc forming (denser) bacterial growth while a continuous well-mixed flow favours filamentous growth; the same is found

with suspended biomass in activated sludge processes. This suggests microbial population in biofilm systems and their kinetics are largely influenced by the reactor types.

Despite the shear stress on the batch biofilm surface being approximately the same as in the CSTR, the batch reactor biofilm is the most compact and thinnest in structure, due to the co-existence of cocci and filaments. This structure causes the high internal mass transfer resistance and is responsible for the smallest flux. The channel reactor biofilm is surface film-dominated, and with the highest biomass areal density and the smallest volumetric density in structure because of the smaller shear stress. The large interface area of the channel reactor biofilm is the main reason for its largest mass flux. These phenomena demonstrate the effects of the reactor type and shear stress on the biofilm morphology and on mass transfer. No effect of shear stress on the biofilm microbial composition was found. The reactor type and shear stress each exert a specific effect on biofilm systems, and their influence should be investigated separately.

The oxygen flux in the batch reactor biofilm increases steadily within a wider range of glucose concentrations, whereas fluxes in the CSTR and channel reactor biofilms reach plateau values at low glucose concentrations; this analogy to the trends of the intrinsic specific activities of the three biofilms illustrates the associated relationship between microbial population activity and reactor performance.

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NOMENCLATURE

BUR	biofilm oxygen up-take rate ($ML^{-2}T^{-1}$)
C	oxygen concentration in the reactor (ML^{-3})
C^*	saturation oxygen concentration (ML^{-3})
C_g	glucose concentration in the reactor (ML^{-3})
C_{gi}, C_{ge}	glucose concentrations in influent and effluent (ML^{-3})
C_{oi}, C_{oe}	oxygen concentrations in influent and effluent (ML^{-3})

D	dilution rate (T^{-1})
D_b	biofilm areal density (ML^{-2})
d	diameter of stirrer (L)
f	friction factor
J_{o2}	oxygen flux into biofilm on substratum ($ML^{-2}T$)
J_{o2accu}	oxygen flux into biofilm on other surface ($ML^{-2}T$)
J_{o2max}	maximum oxygen flux ($ML^{-2}T$)
k_La	overall aeration coefficient (T^{-1})
K_s	half-saturation constant of Monod equation (ML^{-3})
k_s	half-saturation constant of biofilm (Monod analogy) (ML^{-3})
$\mathbb{N}$	rotational speed of stirrer (T^{-1})
n	wetted peripheral length (L)
q_{o2}	specific oxygen activity of biomass ($MM^{-1}T^{-1}$)
q_{o2max}	maximum specific oxygen activity ($MM^{-1}T^{-1}$)
Re	Reynolds number
R_{gtot}	total glucose decomposition rate of reactor ($ML^{-3}T^{-1}$)
TUR	total oxygen up-take rate of reactor ($ML^{-3}T^{-1}$)
SUR	oxygen up-take rate of suspended biomass ($ML^{-3}T^{-1}$)
u	average water velocity in liquid bulk (LT^{-1})
V	effective reactor volume (L^3)
WA	area of biofilm on substratum (L^2)
WA_{accu}	other area covered by biomass accumulated during running period (L^2)
$Y_{o/s}$	oxygen consumption coefficient (MM^{-1})
$Y_{x/s}$	biomass yield coefficient (MM^{-1})
μ	specific growth rate (T^{-1})
μ_{max}	maximum specific growth rate (T^{-1})
θ	cell multiplication time (T)
ν	kinematic viscosity of water (L^2T^{-1})
η	effectiveness factor
τ_s	shear stress on biofilm surface ($ML^{-1}T^{-2}$)
χ	hydraulic radius (L)
ρ	water density (ML^{-3})

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Chapter 6

A Model for Oxygen Consumption in Aerobic Heterotrophic Biodegradation in Dual-Phase Drainage Systems *

ABSTRACT A mathematical model with two kinetics variations is developed for the description of oxygen consumption in aerobic heterotrophic processes in longitudinal dual-phase systems where biomass suspended in the liquid and attached on the solid material surface do occur. This pertains in particular to sewers, drains, rivers and wastewater treatment reactors. The model parameters are related to the liquid and solid phases, such as suspended biomass concentration X, its maximum growth rate μ_{max}, water volume V, biofilm oxygen up-take rate BUR, and the biofilm covered area WA. The Monod equation-based zero and first order kinetics can be applicable depending on organics concentration. Simulation demonstrates the influence of relevant parameters on the oxygen profile. It illustrates that the suspended biomass can play a critically important role, so generally, should not be neglected; the relative importance of the two biomass types is system dependent, of which a quick estimation can be made by knowing the parameters mentioned above. The potential usages of the model are discussed.

KEY WORDS modelling, simulation, sewer, drain, river, biofilm, suspended biomass, heterotroph, sediment, self-purification.

INTRODUCTION

Sewage collection systems (sewers, drains), shallow polluted aquatic systems (rivers, streams, lakes), as well as sewage lagoons and some wastewater treatment processes (such as Rotating Biological Contactors (RBC) and turbulent biofilm reactors) are characterized by the fact that active biomass is not only suspended in the liquid but that most of it is attached to the solid material's surface (Fig.1). The suspended biomass can be in the form of individual cells or very small agglomerates (dispersed condition) or of large flocs; these cells generally result from cell division in the liquid or from erosion from the established biofilm.

* Y.S. Cao and G.J. Alaerts (1994), submitted to *Wat. Res.* for publication.

The attached biomass can be a continuous film of benthic, sessile biomass, or mixed with sediment and deposit, or attached to carrier material distributed over the reactor volume. These systems can in fact be considered dual-phase systems.

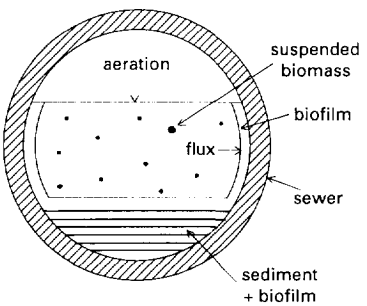

Fig.1 Conceptual scheme of aerobic dual-phase system

Biodegradation in sewers and drains makes for an essential element in the wastewater management concept integrating sewers, wastewater treatment plants and receiving water bodies (Lijklema *et al.*, 1993). The benthic phase in shallow aquatic systems plays an important role in organic mineralization, nitrogen cycling and phosphate release that largely determine the water quality. Attempts have been undertaken to enhance internal biodegradation capacity in drains and lagoons by artificial means (Qi *et al.*, 1994).

Compared with the processes of activated sludge and aerobic biofilters (biofilm), less attention has been devoted to the dual-phase systems, whose behaviour and characteristics can be expected to differ from the activated sludge processes where the suspended flocculated biomass dominates, as well as from the biofilm processes where the attached biomass dominates.

Empirical and semi-mechanistic mathematical models have been developed to describe dual-phase systems. Most of efforts made intended to modify the Streeter-Phelps equation, an early river water quality model (Streeter and Phelps, 1925), in which only the liquid phase is considered, and oxygen consumption is expressed by a first order kinetics with respect to COD or BOD. These models can be classified as following types: a. neglecting suspended biomass contribution or lumping it together with the biofilm (Srinanthakumar and Amirtharajah, 1983; Hickey, 1988); b. the benthic phase is expressed by empirically choosing values of the kinetic constants in the Streeter-Phelps model (Thomann and Mueller, 1987); c. in addition to the kinetic constant for liquid reaction an extra item is incorporated which correlates with attached biomass (Wolf, 1977), water depth, bottom slope or solid surface roughness (Novetny, 1969). Mathematical modelling work on biological transformation in sewers and drains is limited, and mainly is regressed from local data (Pomeroy and Parkhurst, 1972). Only recently attention has been paid to describe dual-phase

systems from their characteristics. Lee (1991) developed a model for a hybrid bioreactor with suspended and attached biomass competing for a single substrate. In the latest extension of MOUSE oxygen consumption and pollutant decomposition in sewers are described in terms of both suspended and attached biomass by using knowledge based on activated sludge and biofilm; a half order kinetics with respect to oxygen concentration is applied, but the model attributes the soluble BOD removal only to the suspended biomass function (Garsdal *et al.*, 1994).

To understand and describe the process of the dual-phase systems in a mechanistic way, a series of experiments have been conducted in our laboratory, focusing on oxygen consumption as master parameter. It was found that the suspended biomass concentration X, its maximum growth rate μ_{max} (both relating to the dispersed biomass), biofilm specific area WA/V, biofilm oxygen up-take rate BUR (relating to the "solid phase") are essential process parameters; the suspended biomass can play a significant role under many circumstances, even if its concentration is in so low a range, *e.g.* 10-20 mg/L, as to be difficult to be observed visually (as turbidity). Hence, both liquid and solid phase should be taken into account (Cao *et al.*, 1991; Cao and Alaerts, 1993a). It was shown that the commonly used first order kinetics with respect to organic concentration describing soluble organic substrate biodegradation only holds under low concentration, and that zero order kinetics should be adopted when carbon substrate concentration is high. For highly polluted wastewater and domestic sewage oxygen concentration doesn't affect the reaction in the liquid phase very much when the oxygen concentration is above a certain value, *e.g.* > 1 mg/L, and for biofilm when oxygen concentration is between 1-5 mg/L (Cao and Alaerts, 1993a; Norsker *et al.*, 1994) and for sediment between 1-3 mg/L as reported by Bowman and Delfino (1980). The complex of biofilm surface morphology and reactions taking place inside biofilms can be one of the reasons.

The purpose of this paper is twofold: to mathematically describe the oxygen consumption by aerobic heterotrophic degradation of soluble carbon substrate in order to analyze the behaviour of a typical dual-phase system, *i.e.* a sewer or drain of app. 10 km long; and to simulate system performance, investigate process response to parameter variation, and study the relative significance of the two biomass types under varying conditions. It is not the intention to validate the model in a real condition as theory has not sufficiently evolved to describe degradation of more complex mixtures. Because of the particular importance of the dissolved oxygen concentration to wastewater collection and shallow aquatic systems this work mainly focuses on oxygen profiles. However, the model for the calculation of the corresponding soluble organic concentration has been considered as well (see the appendix).

Assumptions

One model with two kinetics variations is developed for the description of oxygen consumption in a long sewer or drain dual-phase system with a single pollutant source under steady-state. Natural aeration takes place along the whole system length. The atmosphere above the liquid phase is supposed to consist of air. Non-ideal plug flow condition (back-mixing) is allowed and described by the longitudinal dispersion coefficient D_L, and the liquid is considered perfectly mixed over any cross-section. Biofilm is uniformly distributed on the bottom and wall surface along the longitudinal direction. Suspended biomass concentration is constant, which implies that the increment rate (by growth and biofilm detachment) of suspended biomass equals its rate of deposition.

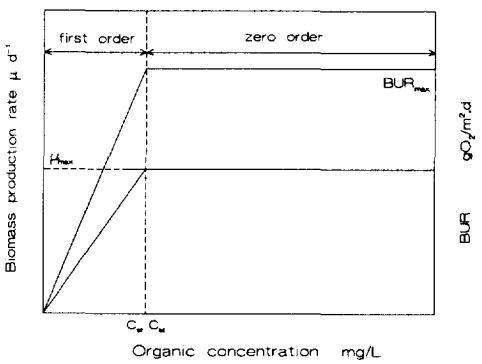

Fig.2 Zero and first order oxygen consumption kinetics

Kinetics

The kinetic equations that describe oxygen consumption are based on the modified Monod equation. Although the original equation describes cell growth, it does not necessarily describe biodegradation in a dual-phase system; however, experiments showed soluble organics decomposition as well as the concomitant oxygen consumption by biofilm can be described by zero and first order Monod-like equations (Cao and Alaerts, 1994b). The order, μ and BUR are function of soluble organic concentration in the bulk of the liquid. Therefore, in a schematized way, two kinetic models, *i.e.* of zero and first order with respect to soluble organic concentration (Fig.2), are applied to describe oxygen consumption kinetics. C_{st} and C_{bt} are the organic concentrations at which μ and BUR reach a constant value μ_{max} and BUR_{max}, and it can be assumed $C_{st} \approx C_{bt}$. When $C > C_{st}$ the zero order kinetics is applied, and the oxygen consumption rates for suspended biomass and biofilm are $Y_{o/s}*\mu_{max}*X/Y_{x/s}$, and $BUR_{max}*WA/V$, respectively; when $C < C_{st}$ the first order kinetics is applied, and the oxygen consumption rates are $Y_{o/s}*\mu_{max}*C*X/(Y_{x/s}*C_{st})$ and $BUR_{max}*C*WA/(C_{bt}*V)$, respectively. In

both cases the ratio BUR_{max}*WA/(X*μ_{max}*V) expresses physically the relative importance of biofilm over suspended biomass regarding oxygen consumption (and substrate decomposition as well). Reported C_{bt} values vary between 3-50 mg/L. Theoretically C_{bt} is greater than the Monod saturation constant K_s. In practice, the zero order kinetics would be applicable in sewers and drains, whereas the first order kinetics is applicable in moderately polluted streams or rivers. All biomass values refer to dry weight.

Derivation of equations (single point discharge)
A mass balance for dissolved oxygen (and soluble organic pollutant) was made over a micro-element (an infinitesimally thin cross-section "slice" of the drain) for developing the differential equation. To solve the mathematical equations the whole length of the system was divided into three reaches, biofilm only occurs in the reach $0 \leq x \leq L$ ("biofilm loaded reach"). The pollutant is discharged only at the point $x=0$ at the beginning of the drain as shown in Fig.3 where diffusion and the case with multiple point discharge also are displayed. For the details of the derivation, see the appendix.

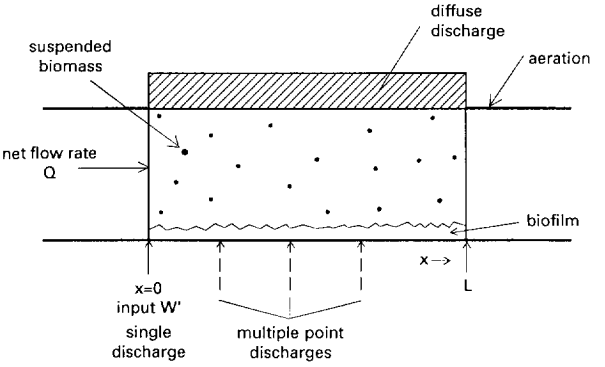

Fig.3 Conceptual scheme of the mathematical model (showing longitudinal section of drain or sewer).

Zero order model The differential equation for the biofilm loaded reach ($0 \leq x \leq L$) is:

$$\frac{d^2D}{dx^2} - \frac{u}{D_L}\frac{dD}{dx} - \frac{k_L a}{D_L}D + \frac{1}{D_L}\left(Y_{o/s}\frac{\mu_{max}X}{Y_{x/s}} + \frac{BUR_{max}WA}{V}\right) = 0$$

The terms between the brackets refer to oxygen consumption due to the suspended and the attached biomass. The solution of the equation is:

$$C_o = C_o^* - \frac{Y_{o/s}*\mu_{max}*X/Y_{x/S} + BUR_{max}*(WA/V)}{k_L a}*\left(1 - \frac{(\alpha-1)}{2\alpha}e^{j_1(x-L)} - \frac{(\alpha+1)}{2\alpha}e^{j_2 x}\right)$$

where:

$$j_1,\ j_2=\frac{u}{2D_L}(1\pm\alpha),\ \text{and}\ \ \alpha=\sqrt{1+\frac{4k_L aD_L}{u^2}}.$$

The soluble organic concentration C does not figure in this zero order kinetic model, so the point where oxygen concentration reaches the minimum (oxygen sag point), which occurs partially depending on C, is not generated in the simulation. This corresponds to highly polluted wastewater collection systems such as sewers where readily biodegradable organics concentration is still rather high at the inlet of the treatment plants.

Because of the zero order kinetics characteristic, which means that the soluble carbon concentration has no effect on the reaction rate, the mathematical solution for the oxygen profile is also valid in the case of diffuse pollution (*e.g.* runoff or pollutants released from the sediment) (Fig.3).

First order model The differential equation and the solution for the biofilm loaded reach are:

$$\frac{d^2D}{dx^2}-\frac{u}{D_L}\frac{dD}{dx}-\frac{k_L a}{D_L}D+\frac{1}{D_L}(Y_{o/s}\frac{\mu_{\max}X}{Y_{x/s}C_{st}}+\frac{BUR_{\max}}{C_{bt}}\frac{WA}{V})C_{so}\exp(sj_2x)\ =\ 0,$$

$$C_0=C_o^*-R_1*C_{so}\ \{\frac{\exp(sj_2L)}{(sj_2-j_1)(j_2-j_1)}\exp(j_1(x-L))-\frac{\exp(j_2x)}{(sj_2-j_2)(j_2-j_1)}+\frac{\exp(sj_2x)}{(sj_2-j_1)(sj_2-j_2)}\},$$

where:

$$sj_2=\frac{u}{2D_L}(1-\alpha_s),\ \ \alpha_s=\sqrt{1+\frac{4K_{tot}D_L}{u^2}},\ \ K_{tot}=\frac{\mu_{\max}X}{Y_{o/s}C_{bt}}+\frac{BUR_{\max}}{Y_{o/s}}\frac{WA}{V},$$

$$C_{so}=\frac{W'}{Q\alpha_s},\ \text{and}\ \ R_1=-\frac{Y_{o/s}\mu_{\max}X/(Y_{x/s}C_{bt})+BUR_{\max}WA/(C_{bt}V)}{D_L}.$$

Because of the first order kinetic characteristic, and the fact that the derived solution for the soluble carbon is based on single point pollution condition, in contrast to the zero order kinetic model the first order model can be only adopted for the point pollution case but not for the diffuse source case.

According to the imposed boundary conditions for the differential equations the model can only be applied to the situation where a single point discharge occurs into a "clean" stream. For field situations with multiple point sources (Fig.3), the solution for the reach can be obtained by adding the separate solutions for each source based on the superposition principle thanks to the linear character of the original equations.

Sensitivity analysis

Parameter sensitivity is tested by comparing C_o (DO) values at a fixed distance downstream from the single discharge point before and after a 20% increase of a specific parameter, meanwhile all other parameters remaining constant. Most independent parameter values are selected pertaining to the turning point in between the first and zero order curve (Fig.2). In each set of BUR_{max}, WA/V and X, μ_{max} the model shows the same sensitivity. The highest sensitivity is found to parameters that affect re-aeration (C_o^* in case of the zero order kinetic model and C_o^*, k_La in case of the first order kinetic model). The sensitivity to C_0^* suggests a substantial temperature effect. The 20% variation of D_L does not affect the oxygen profile at all in either kinetic model case. The resulting DO values are much higher in the case of the first order kinetic model, because the reaction rate of the zero order kinetics is higher than that of the first order kinetics. This also explains the substantially lower parametric sensitivity of the first order model than that of the zero order model.

SIMULATION

The following simulation focuses on the dual-phase system response with respect to oxygen consumption to a wide range of variations of the critical system parameters X, μ_{max}, BUR_{max}, WA and D_L. Simulations with same parameter values were conducted to both kinetics models for comparison purpose. All results are discussed in the next section.

X

The suspended heterotrophic biomass concentration in sewers typically amounts to 15-20% of total liquid COD (Henze, 1992); therefore, the variation of X in the simulation was chosen as 0-60 mg/L (dry weight), where the simulation with X=0 mg/L corresponds to a system without any suspended biomass. The input values of the other parameters are mentioned in the figure captions. Among them for zero order model WA/V=8 m^{-1} corresponds to sewers or drains, greater than 2 m^{-1} for the first order model corresponding to rivers.

Figs.4a,b demonstrate that oxygen profiles fall dramatically when X increases from 0 mg/L to 60 mg/L, which illustrates that the suspended biomass concentration affects the processes substantially.

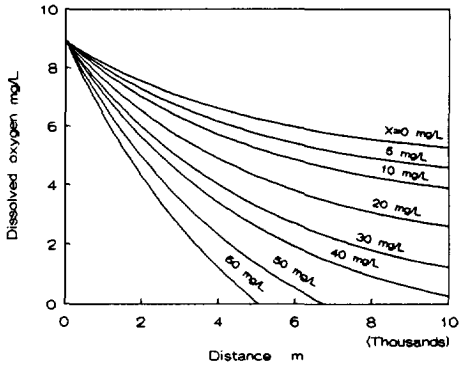

Fig.4a Effect of suspended biomass on the oxygen profile for the zero order kinetic model (representing sewer condition). Parameter values:$BUR_{max}=10$ $gO_2/m^2.d$, $WA/V=8$ m^{-1}, $\mu_{max}=3$ d^{-1}, $Y_{x/s}=Y_{o/s}=50$ % (gbiom/gCOD), u=1 m/s, $k_La=0.83$ h^{-1}, $D_L=1000$ m^2/h and $C_o^*=8.9$ mg/L.

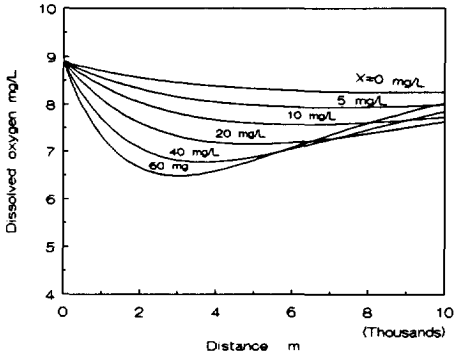

Fig.4b Effect of suspended biomass on the oxygen profile for the first order kinetic model (representing polluted river condition). Parameter values: $BUR_{max}=10$ $gO_2/m^2.d$, $WA/V=2$ m^{-1}, $C_{st}=C_{bt}=10$ mg/L, $\mu_{max}=3$ d^{-1}, $Y_{x/s}=Y_{o/s}=50\%$ (gbiom/gCOD), u=1 m/s, $k_La=0.83$ h^{-1}, $D_L=1000$ m^2/h, $W'/Q=10$ mg/L and $C_o^*=8.9$ mg/L.

μ_{max}

The magnitude of μ_{max} depends on the bacterial species as well as temperature. Representative literature values are given in Table 1, and accordingly, the variation of μ_{max} in the simulation is taken from 0.5 to 8 d^{-1}. Under the given parameter values in Figs.5a,b, pronounced response of the oxygen profile to the μ_{max} variation is found. In case of the zero order kinetics the oxygen concentration at x=10,000 m drops from 5.2 mg/L to 0.2 mg/L, and in case of the first order kinetics the oxygen sag point occurs from about x=4,200 m to 2,300 m, and the oxygen concentration difference between these two points is about 2 mg/L when increasing μ_{max} from 0.5-8 day^{-1}. For further explanation, see Discussion.

TABLE 1 Literature values of μ_{max} for aerobic sewage treatment organisms

Bacterial type	μ_{max}	Substrate type	Temp	Source
	d^{-1}		^0C	
Floc-forming	4-10	acetate, glucose and	?	Jenkins (1992)
Filamentous	0.5-3.8	and domestic wastewater	?	Jenkins (1992)
Mixed heterotrophic	1-8	soluble organics in domestic wastewater	22	Kappeler and Gujer (1992)
Floc-forming	5	soluble organics in	20	Gujer and Kappeler (1992)
Filamentous	3	domestic wastewater		
Mixed community	3.7	glucose	28	Cao and Alaerts (1994a)
Filamentous	2.6	glucose	28	Cao and Alaerts (1994a)

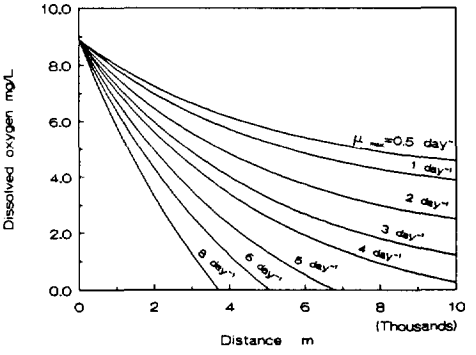

Fig.5a Effect of μ_{max} on the oxygen profile for the zero order kinetic model (for sewer condition). Parameter values: $BUR_{max}=10\ gO_2/m^2.d$, $WA/V=8\ m^{-1}$, $X=30$ mg/L, $Y_{x/s}=Y_{o/s}=50\%$ (gbiom/gCOD), $u=1$ m/s, $k_La=0.83\ h^{-1}$, $D_L=1000\ m^2/h$, and $C_o^*=8.9$ mg/L.

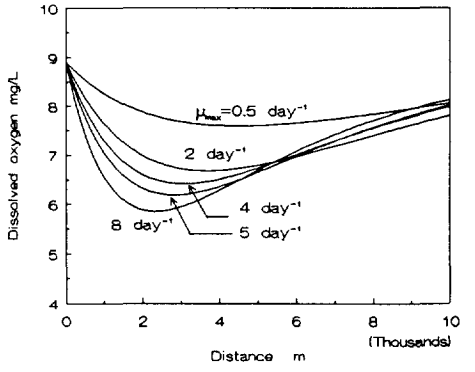

Fig.5b Effect of μ_{max} on the oxygen profile for the zero order kinetic model (for river condition). Parameter values: $BUR_{max}=10\ gO_2/m^2.d$, $WA/V=8\ m^{-1}$, $C_{st}=C_{bt}=10$ mg/L, $X=30$ mg/L, $Y_{x/s}=Y_{o/s}=50\%$ (gbiom/gCOD), $u=1$ m/s, $k_La=0.83\ h^{-1}$, $D_L=1000\ m^2/h$, $W'/Q=10$ mg/L and $C_o^*=8.9$ mg/L.

WA/V

This parameter expresses the biofilm covered area (assumed equal to wetted area WA) per unit water volume, and partly defines the biofilm contribution. It is determined by the system's geometric shape, directly related to its perimeter, and shape of cross-section and water depth for sewers, drains and rivers. Table 2 shows the calculated values for circular-section sewers. The WA/V decreases with increasing diameter and water depth. It can be seen that for sewers with $d > 1.0$ m and $H/d > 0.5$, the $WA/V < 4$ m^{-1}; for drains with rectangular cross section with width > 1.0 m and $H = 0.8$-1.0 m, $WA/V < 3$ m^{-1}. For shallow aquatic systems (depth < 1.5 m), WA/V will be smaller than the above values.

The variation range of WA/V is 0-40 m^{-1}, where $WA/V = 0$ represents the system with only suspended biomass. The dual-phase system described by zero and first order kinetics model with the parameters in Figs.6a,b appears to be highly responsive to increasing biofilm surface, meaning that the processes can be substantially intensified by increasing WA/V. In case of the zero order kinetics the oxygen concentration 5.2 mg/L at x = 10,000 m drops to zero at x = 2150 m; and in case of the first order kinetics the oxygen sag points shift from x = 5,000 m to 1,800 m, and the oxygen concentration difference between them is more than 1.5 mg/L when increasing WA/V from 0 to 40 m^{-1}.

TABLE 2 Values of WA/V (m^{-1}) for circular-section sewers with different diameter d, water depth H

H:d	d=0.25 m	d=0.50 m	d=1.0 m	d=1.5 m
0.2	33.2	16.6	8.3	5.5
0.3	23.4	11.7	5.9	4.0
0.4	18.7	9.3	4.7	3.1
0.5	16.0	8.0	4.0	2.7
0.6	14.4	7.2	3.6	2.4
0.7	13.5	6.8	3.4	2.3

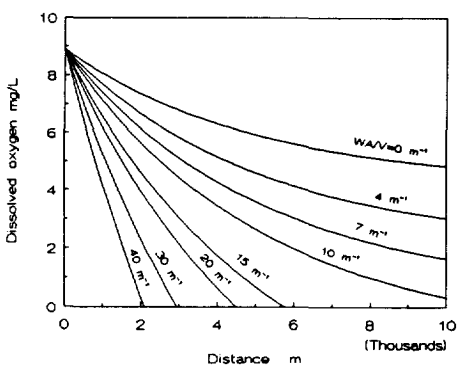

Fig.6a Effect of WA/V on the oxygen profile for the zero order kinetic model (sewer condition). Parameter values: $BUR_{max} = 10$ $gO_2/m^2.d$, $X = 30$ mg/L, $\mu_{max} = 3$ d^{-1}, $Y_{x/s} = Y_{o/s} = 50$ % (gbiom/gCOD), $u = 1$ m/s, $k_La = 0.83$ h^{-1}, $D_L = 1000$ m^2/h and $C_o^* = 8.9$ mg/L.

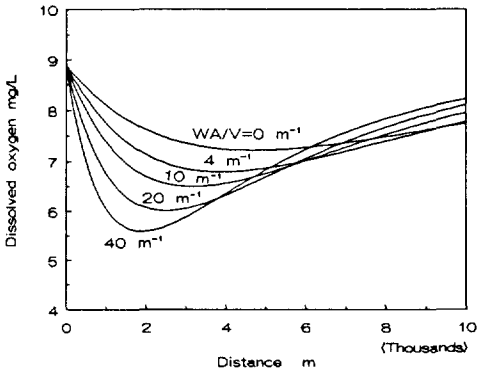

Fig.6b Effect of WA/V on the oxygen profile for the zero order kinetic model (river condition). Parameter values: BUR_{max}=10 $gO_2/m^2.d$, $C_{st}=C_{bt}$=10 mg/L, X=30 mg/L, μ_{max}=3 d^{-1}, $Y_{x/s}=Y_{o/s}$=50 %(gbiom/gCOD), u=1 m/s, k_La=0.83 h^{-1}, D_L=1000 m^2/h, W'/Q=10 mg/L and C_o^*=8.9 mg/L.

BUR_{max}

The determining factors for the magnitude of BUR (and BUR_{max}) include the concentrations of oxygen and substrate in the liquid bulk, their diffusivity coefficients within the biofilm, water flow velocity, biofilm thickness and structure. The BUR values (or BUR_{max}) in the literature vary 2-30 $gO_2/m^2.d$. A discussion on the variation is given by Cao *et al.* (1991). By definition, the parameter should exclude the suspended biomass function. In the following simulation BUR_{max} variation is between 2-20 $gO_2/m^2.d$. As Figs.7a,b show the oxygen profile is not very sensitive to the BUR_{max} variation. In case of the zero order kinetics model only less than 2 mg/L oxygen concentration at x=10,000 m drops, and in case of the first order kinetics model the oxygen concentration difference between the sag points is less than 1 mg/L when increasing BUR_{max} from 2 to 20 $gO_2/m^2.day$.

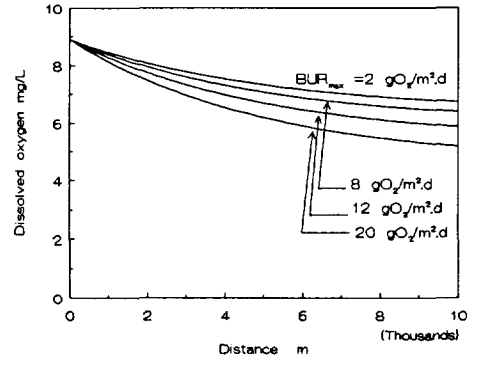

Fig.7a Effect of BUR_{max} on the oxygen profile for the first order kinetic model (sewer condition). Parameter values: WA/V=2 m^{-1}, $C_{st}=C_{bt}$=10 mg/L, X=30 mg/L, μ_{max}=3 d^{-1}, $Y_{x/s}=Y_{o/s}$=50% (gbiom/gCOD), u=1 m/s, k_La=0.83 h^{-1}, D_L=1000 m^2/h, C_o^*=8.9 mg/L.

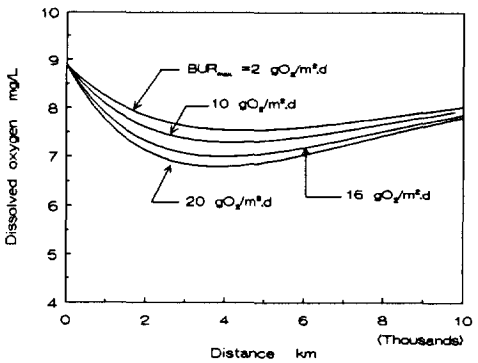

Fig.7b Effect of BUR_{max} on the oxygen profile for the first order kinetic model(polluted river condition). Parameter values: WA/V=2 m^{-1}, $C_{st}=C_{bt}=10$ mg/L, X=30 mg/L, $\mu_{max}=3$ d^{-1}, $Y_{x/s}=Y_{o/s}=50\%$ (gbiom/gCOD), u=1 m/s, $k_La=0.83$ h^{-1}, $D_L=1000$ m^2/h, W'/Q=10 mg/L and $C_o^*=8.9$ mg/L.

D_L

The literature values of D_L vary from 10 to 10^7 m^2/h or even higher. As shown in Fig.8 the oxygen profiles keep the same shape when $D_L < 10^6$ m^2/h corresponding to large river systems, and the profiles flatten out when $D_L > 10^7$ m^2/h corresponding to back-mixing situation in an estuary *i.e.* the system increasingly behaves like a well-mixed tank. The oxygen profile response to the variation of the order of magnitude of D_L in case of the first order kinetics is similar to Fig.8. The same phenomena was reported by Arceivala (1981). Considering the geometric characteristics of sewers and drains it can be concluded that the plug flow-like pattern prevails in most sewers and drains, and that a simple model neglecting D_L can be adopted, while serious back-mixing could occur in square lagoon systems.

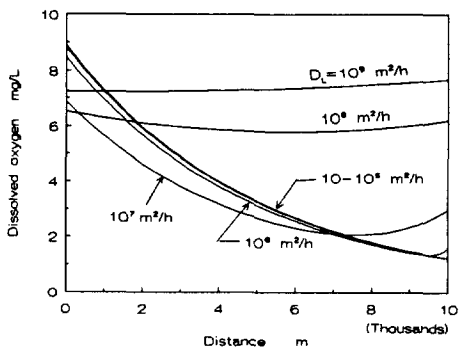

Fig. 8 Effect of D_L on the oxygen profile for the zero order kinetic model (sewer condition). Parameters values: $BUR_{max}=10$ $gO_2/m^2.d$, WA/V=8 m^{-1}, X=30 mg/L, $\mu_{max}=3$ d^{-1}, $Y_{o/s}=Y_{x/s}=50\%$ (gbiom/gCOD), $k_La=0.83$ h^{-1}, u=1 m/sec and $C_o^*=8.9$ mg/L.

Double-kinetics situation

In a system where C decreases with progressing biodegradation from a value above C_{bt} to one below, the biodegradation kinetics shifts from zero to first order. As Fig.9 displays for one typical case with $C_{x=0}$=20 mgCOD/L the kinetics shifts from the zero to the first order at x=2000 m (where C=C_{bt}=11.2 mgCOD/L). Independent parameter values are chosen like in Fig.9. The rates of oxygen consumption and COD decomposition described by first order kinetics are slower than that by zero order kinetics.

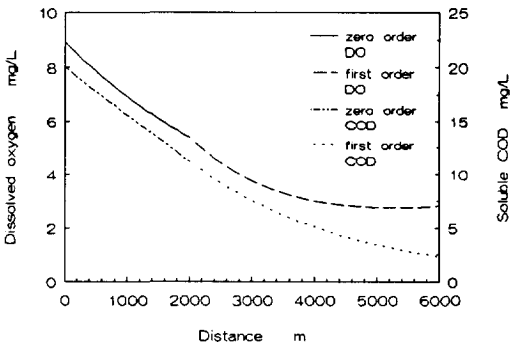

Fig.9 Comparison of the zero and first order kinetics models (sewer condition).
Parameters used: BUR_{max}=10 $g\dot{O}_2/m^2.d$, WA/V=10 m^{-1}, X=30 mg/L, μ_{max}=3 d^{-1}, $Y_{o/s}=Y_{x/s}$=50% (gbiom/gCOD), k_La=0.83 h^{-1}, u=1 m/sec, D_L=1000 m^2/h, C_o^*=8.9 mg/L, and $C_{bt}=C_{x=2000}$=11.2 mg/L.

DISCUSSION

In the simulation of the influence of suspended biomass WA/V=8 m^{-1} in the zero order model corresponds to a sewer with diameter 0.5 m and water depth 0.25 m; the value 2 m^{-1} in the first order kinetic model, corresponds to a drain with a rectangular cross section, water depth 0.5 m and width >5 m. Both simulations (Figs.4a,b) indicate that the suspended biomass function can be very important in regulating the oxygen consumption, *e.g.* dissolved oxygen concentration at x=10,000 m drops from 5.3 mg/L to 3.0 mg/L, when the suspended biomass concentration increases from 0 to 20 mg/L in case of the zero order kinetics model, and the oxygen concentration at x=10,000 m drops more than 0.5 mg/L, in the case of the first-order kinetic model, when the suspended biomass increases from 0 mg/L to 10 mg/L, which is a concentration range where the suspended biomass is almost invisible. The simulation further confirms that the suspended biomass cannot be neglected in dual-phase systems and processes (Cao *et al.*, 1991). The rationale for this relates to the fact that the apparent respiration of attached biomass is constrained by diffusion resistance, while the resistance of dispersed suspended biomass tends to be substantially less than that of biofilm;

similarly, the content of inert materials can be much higher in biofilm than in suspended biomass (Cao and Alaerts, 1993a; Cao and Alaerts, 1994a). As the WA/V values in the above simulation are in the range of 4-6 m^{-1} for conventional respirometers (James, 1974; Hickey, 1988), the simulation also illustrates the possible overestimation of the BUR (and BUR_{max}) when measured by conventional one chamber respirometer, because the measured BUR (and BUR_{max}) values contain the contribution from the suspended biomass stirred up during measurement. It can therefore be concluded that a more appropriate instrument is yet to be developed. The biological oxygen monitor (BOM) can be an appropriate equipment for the study of the biodegradation in liquid phase (Cao and Alaerts, 1994b)

As shown in Figs.5a,b a pronounced effect of μ_{max} on the oxygen profile is displayed. Smaller μ_{max} values relate to filaments, which are typically stimulated by soluble substrate; the larger values pertain to floc-forming bacteria, which are more favoured by particulate substrate. Because of different concentration profiles, a plug flow tends to favour floc-forming bacteria growth, whereas a well-mixed tank favours filamentous growth (Cao and Alaerts, 1994a). The simulation illustrates how oxygen profile may be influenced by the system's reactor configuration and microbial ecology.

Figs.6a,b show that respiration in a dual-phase system can be enhanced by increasing WA. This principle has been adopted practically in oxidation ponds (Qi *et al.*, 1994) and turbulent beds (Lazarova and Manem, 1993). In both cases carrier materials are put into the systems to allow the additional attached biomass growth to increase the process rates.

In contrast to the previous cases and unexpectedly, a ten time increase of BUR_{max} only results in an additional oxygen concentration depletion by less than 1-2 mg/L (Figs. 7a,b). It means that under the given conditions the variation of BUR_{max} does not significantly influence the aerobic conversion processes; in other words, the process is largely dominated by suspended biomass rather than by attached biomass. Therefore, not each of the parameters X, μ_{max}, BUR_{max}, and WA/V is able to intensify unconditionally the biodegradation rates in dual-phase processes. The explanation is given as follows.

According to the physical meaning of the ratio $BUR_{max}*WA/(X*\mu_{max}*V)$ for both the zero and first order kinetic models, it is obvious that when the ratio is $\gg 1$, the process is dominated by attached biomass; when the ratio is $\ll 1$, it is dominated by suspended biomass; when it is close to 1, both biomass types make a significant but almost equal contribution. However, the dominating phase can shift when a relevant system parameter changes.

In the simulation of the influence of suspended biomass (Figs.4a,b), the selected BUR_{max} and WA/V values are 10 $gO_2/m^2.day$ and 8 m^{-1} selected in the zero order model, and 10 $gO_2/m^2.day$ and 2 m^{-1} for the first order model. Correspondingly, the ratio $BUR_{max}*WA/(X*\mu_{max}*V)$ changes from $+\infty$ to 4/9 and $+\infty$ to 1/9, respectively, when increasing X from 0 to 60 mg/L, a more than 2 and 9 time respective increase of respiration activity. In both cases the system experiences a shift of dominating phase from attached to

suspended biomass. In the simulation of the influence of μ_{max} (Figs.5,b) the ratio varies from 6 to 1/3 when increasing μ_{max} from 0.5 day^{-1} to 8 day^{-1}, and the dominating phase changes from the biofilm to the suspended biomass as well, with total respiration capacity increasing 2 times. When increasing WA/V from 0 to 40 m^{-1} (Figs.6a,b) the ratio varies from 0 to 40/9, the respiration activity increases about 4 times, and the dominating phase shifts from suspended to attached biomass. In the above cases the large increase of the respiration capacity partly answers the great response of the oxygen profiles to the variations of the respective parameters. However, when BUR_{max} increases from 2 to 20 $gO_2/m^2.day$ (Figs.7a,b) the ratio changes from 1/9 to 1, resulting in doubling of respiration activity; the suspended biomass apparently still plays an essential role. This partly explains the unsensitive response. The simulations indicate that a preliminary prediction of oxygen profile response to the variation of relevant parameters can be made based on the magnitude of the ratio in case of the two kinetics types. However, a precise prediction needs the ratio of two biomass types function, and the comparison of the respiration and aeration capacities. The simulations of soluble organics profiles were conducted as well, and they are consistent with the results of oxygen consumption.

The analysis demonstrates that the aerobic degradation processes in a dual-phase systems can either be governed by suspended or attached biomass or be under the control of both of them. The ratio $BUR_{max}*WA/(V*\mu_{max}*X)$ could provide a quick estimate on the relative importance of two biomass types, which is a precondition to enhance the dual-phase processes. Considering the relationship between WA/V and the sewer diameter and water depth, it is suggested that recycling sludge into sewers is only feasible for larger sewers; suspended biomass can make an important contribution especially when WA/V and BUR are small. For example, for an aquatic system with a typical BUR_{max} =0.5 $gO_2/m^2.d$, WA/V=2 m^{-1}, X=5 mg/L and μ_{max}=3 d^{-1}, the ratio=1/15$\ll$1, thus the oxygen consumption process would be governed by suspended biomass rather than the sediment (although the latter may be important as the source pollutants as well as suspended biomass). Practically speaking, only when WA/V or BUR are substantially larger, especially in case of WA/V (*e.g.* in biofilters or air-lift reactors where WA/V can be in the range of 1000-3000 m^{-1}), then the suspended biomass function becomes negligible (<5-10% of total oxygen consumption).

As illustrated in Fig.9 the double kinetics situation can result in two different oxygen profile parts, where the difference becomes more pronounced when the organic concentration gets lower and lower. The first order kinetic model is only applicable under low organics concentration condition. The shifting point for kinetics depends on several factors, *e.g.* oxygen concentration which can be affected by aeration capacity, pollutant loads, temperature, and the characteristics of the biocenosis of the dual-phase systems. For the latter filamentous bacteria can exert high activity under low organic concentration such as 1-2 mg/L which is less than that of the floc-forming bacteria. The flow pattern (or the reactor configuration of the system) also affects the shifting kinetics, because it can determine the dominating microorganism species as mentioned before.

By varying the parameters such as u and k_La, the models can be used to estimate oxygen supply requirement, like for example, the required aeration capacity. The results can also be extrapolated to better understand bioreactor performance, and, with appropriate modifications, the models could be adopted for the description of oxygen consumption due to nitrification. Theoretically, the models can be coupled with a models concerning biofilm growth, detachment, nutrient competition *etc.*

CONCLUSIONS

Two variations of a mathematical model have been developed to describe oxygen consumption in dual-phase systems. The model parameters include suspended biomass concentration, its maximum production rate μ_{max}, the biofilm specific area, and the biofilm (or sediment) oxygen up-take rate. The zero and first order kinetics result in different oxygen profiles, and are applicable depending on organics concentration, *i.e.* the zero order kinetics pertaining to high pollution level can be adopted for sewers and drains, while the first order kinetics describes moderately polluted shallow aquatic systems.

The sensitivity of each kinetics model to changes in the parameters BUR and WA/V is essentially the same. This also holds for X and μ_{max}. Both models are most sensitive to aeration coefficient and temperature change.

The suspended biomass can play an important role in aerobic degradation even if its concentration is in such a low range as 5-15 mg/L. Commonly used respirometers neglect the suspended biomass contribution resulting in possible overestimation of the measured BUR, so an appropriate measuring instrument is yet to be developed. The parameter μ_{max} of the suspended biomass, relating to bacterial species, temperature *etc.* can largely determine the oxygen consumption, and the processes could be enhanced by increasing WA/V. However, there is no general rule on the relative contribution of the two biomass types on oxygen consumption rate; this is system dependent.

The ratio BUR_{max}*WA/(X*μ_{max}*V) can be adopted to predict the relative function of the two biomass types (suspended/dispersed and attached), when it is close to one both two biomass types play important role, when it is $\ll$ or $\gg$ one (*e.g.* $<1/3$ or $>5/3$), the process will be governed by liquid or solid phase, respectively. Also, this ratio can be used to analyze system performance, and to explore the feasibility of intensifying dual-phase processes under various conditions.

The model can be modified and extrapolated to describe the oxygen consumption due to nitrification. Similarly, the model needs to be further developed to describe biodegradation in more complex substrate mixture, in particular those containing particulate hydrolysable matter.

ACKNOWLEDGEMENTS

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APPENDIX Mathematical Derivation of the Model

To obtain analytical solutions the same approach as used by Thomann and Mueller (1987) was adopted. The whole length of the system (with x the distance along it) is divided into the "biofilm loaded" reach where attached biomass exists ($0 \leq x \leq L$), and two unloaded reaches that are imposed on either side of the biofilm loaded reach ($x \leq 0$ and $x \geq L$). The point pollutant discharge at $x=0$ as shown in Fig.10.

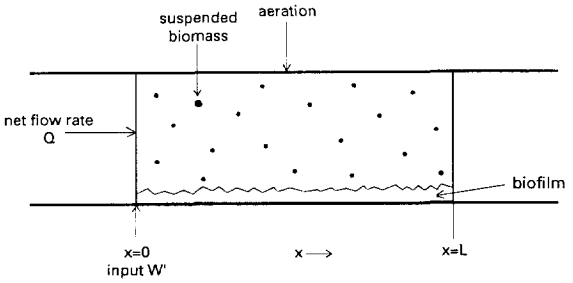

Fig.10 Conceptual diagram of the model.

Zero order model After substitution of $D=C_o^*-C_o$, the DO deficit, the differential equations governing the dissolved oxygen for the biofilm loaded reach $0 \leq x \leq L$ is:

$$\frac{d^2D}{dx^2}-\frac{u}{D_L}\frac{dD}{dx}-\frac{k_La}{D_L}D+\frac{1}{D_L}\left(Y_{o/s}\frac{\mu_{max}*X}{Y_{x/s}}+BUR_{max}*\frac{WA}{V}\right)=0.$$

It is a heterogeneous, linear, constant coefficient, second order differential equation. To obtain its analytical solution two constants need to be identified.

The equations for the other two reaches are:

$D=C_1\exp(j_1x)$, for $x \leq 0$,

$D = C_4 \exp(j_2 x)$, for $x \geq L$.

C_1 and C_4 are constants to be identified. j_1, j_2 are the roots of the characteristic equation of the differential equation for the biofilm loaded reach:

$$j_1,\ j_2 = \frac{u}{2D_L}(1 \pm \alpha), \text{ and } \alpha = \sqrt{1 + \frac{4k_L a D_L}{u^2}}.$$

The following boundary conditions are applied:

$D = 0$ at $x = \pm\infty$.

The continuity requirement at $x = 0$ and $x = L$ implies that the local values and slopes on either side of the boundary need to be equal; this allows the group of three differential equations to be solved analytically as follows:

$$C_o = C_o^* - \frac{R_1}{k_L a}\left(1 - \frac{(\alpha - 1)}{2\alpha} e^{j_1(x-L)} - \frac{(\alpha + 1)}{2\alpha} e^{j_2 x}\right), \quad 0 \leq x \leq L,$$

$$C_0 = C^* - \frac{R_1}{k_L a}\left(\frac{\alpha - 1}{2\alpha}\right)(1 - \exp(-j_1 l))\exp(j_1 x), \quad x \leq 0,$$

$$C_0 = C^* - \frac{R_1}{k_L a}\left(\frac{\alpha + 1}{2\alpha}\right)(\exp(-j_2 l) - 1)\exp(j_2 x), \quad x \geq L.$$

where:

$$R_1 = \frac{Y_{o/s}\mu_{max} X/Y_{x/s} + BUR_{max} WA/V}{k_L a}.$$

To simplify the mathematical derivation in the soluble organics COD equation it was assumed $D_L = 0$. The differential equation for the biofilm loaded reach and the boundary condition are as follows:

$$\frac{dC}{dx} = -\left(\frac{\mu_{max} X}{Y_{s/x} u} + \frac{BUR_{max} WA}{uV}\right),$$

$C = C_{s0}$, at $x = 0$.

The solution is:

$$C = C_{s0} - \left(\frac{\mu_{max} X}{Y_{s/x} u} + \frac{BUR_{max} WA}{uV}\right) x.$$

First order model The following differential equations for the oxygen balance apply to the whole length of the system:

$$\frac{d^2D}{dx^2} - \frac{u}{D_L}\frac{dD}{dx} - \frac{k_L a}{D_L}D + \frac{1}{D_L}\left(Y_{o/s}\frac{\mu_{max} X}{Y_{x/s} C_{st}} + \frac{BUR_{max}}{C_{bt}}\frac{WA}{V}\right)C_{so}\exp(sj_2 x) = 0, \quad 0 \le x \le L$$

$D = C_1' \exp(j_1 x)$, for $x \le 0$,

$D = C_4' \exp(j_2 x)$, for $x \ge L$.

C_1' and C_4' are to be identified, and j_1, j_2 are the same as for the zero order model.

By using the same approach as for the zero order kinetic model, the solutions for oxygen concentration are as follows:

$$C_0 = C_o^* - R_2 * C_{so}\left\{\frac{\exp(sj_2 L)}{(sj_2 - j_1)(j_2 - j_1)}\exp(j_1(x-L)) - \frac{\exp(j_2 x)}{(sj_2 - j_2)(j_2 - j_1)} + \frac{\exp(sj_2 x)}{(sj_2 - j_1)(sj_2 - j_2)}\right\}, \text{ for } 0 \le x \le L,$$

$$C_0 = C_0^* - \frac{R_2 C_{so}}{(Sj_2 - J_1)(J_2 - J_1)}\exp(-j_1 L)\{\exp(sj_2 L) - \exp(j_1 L)\}\exp(j_1 x), \text{ for } x \le 0,$$

$$C_0 = C_0^* - \frac{R_2 C_{so}}{(j_2 - J_1)(sJ_2 - J_2)}\exp(-j_2 L)\{\exp(sj_2 L) - \exp(j_1 L)\}\exp(j_2 x), \text{ for } x \ge L.$$

where:

$$R_2 = -\frac{Y_{o/s}\mu_{max} X/(Y_{x/s} C_{bt}) + BUR_{max} WA/(C_{bt} V)}{D_L}, \quad \alpha_s = \sqrt{1 + \frac{4K_{tot} D_L}{u^2}},$$

$$K_{tot} = \frac{\mu_{max} X}{Y_{o/s} c_{bt}} + \frac{BUR_{max}}{Y_{o/s}}\frac{WA}{V}, \text{ and } sj_2 = \frac{u}{2D_L}(1 - \alpha_s).$$

The factor $C_{so}\exp(sj_2x)$ is the solution for the soluble organic COD concentration in the reach $x>0$ as derived from the differential equation:

$$\frac{d^2C}{dx^2}-\frac{u}{D_L}\frac{dC}{dx}-\frac{1}{D_L}\left(\frac{\mu_{max}X}{Y_{x/s}C_{st}}+\frac{BUR_{max}WA}{Y_{o/s}C_{bt}V}\right)C = 0$$

with the boundary condition: $C=C_{so}$, at $x=0$

$C=0$, at $x=\pm\infty$.

The solutions are:

$C= C_{so}\exp(sj_1x)$, for $x\leq 0$.

$C= C_{so}\exp(sj_2x)$, for $0\leq x$.

According to the soluble carbon pollutants discharging at $x=0$ and at a rate W'(weight) into the stream with a volumetric flow rate Q, a mass balance for soluble COD can be made, *i.e.*

$W'= A\int_{-\infty}^{+\infty} K_{tot}C\,dx$, it leads to $C_{so}=W'/(Q\alpha_s)$.

Notation

A	cross section area, L^2
BUR	biofilm oxygen uptake rate, $ML^{-2}T^{-1}$
BUR_{max}	maximum biofilm oxygen up-take rate, $ML^{-2}T^{-1}$
C, C_{so}	soluble organic concentration and its concentration at $x=0$, ML^{-3}
C_{st}, C_{bt}	soluble organic concentration at which biomass growth rate and biofilm oxygen uptake rate reach constant values, ML^{-3}
C_0, C_0^*	oxygen concentration and its saturation concentration, ML^{-3}
d	sewer diameter, L
D	oxygen deficit, ML^{-3}
D_L	longitudinal dispersion coefficient, M^2T^{-1}
H	water depth, L
k_La	aeration coefficient, T^{-1}
K_s	half-saturation constant of Monod equation, ML^{-3}
K_{tot}	total organic decomposition rate, $ML^{-1}T$
L	length of section where attached biomass exists ("loaded" reach), L

Q	flow rate, L^3T^{-1}
q_{02}	specific oxygen consumption rate of suspended biomass, $MM^{-1}T^{-1}$
u	flow velocity, LT^{-1}
V	water volume, L^3
W'	pollutant discharge rate, MT^{-1}
WA	area covered by attached biomass, L^2
x	horizontal abscissa along drain or sewer length, L
X	suspended biomass concentration, ML^{-3}
$Y_{o/s}$	oxygen consumption coefficient, MM^{-1}
$Y_{x/s}$	biomass yield coefficient, MM^{-1}
μ	specific growth rate, T^{-1}
μ_{max}	maximum specific growth rate, T^{-1}

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Chapter 7

Summary and Conclusions

SUMMARY

Wastewater collection systems such as sewers, sewage drains, and polluted shallow aquatic systems such as rivers, streams, and lagoons are characterized by the fact that both suspended and attached biomass exist and function. They are dual-phase systems. Contrary to biofilm dominated systems such as the Biofilter, Air Lift Suspension Reactor, and to suspension growth systems such as activated sludge processes, on which many studies have been conducted, a systematic, mechanistic approach in the investigation of dual-phase systems is lacking. Yet this is essential to water quality management, wastewater treatment plant design and operation, and to the study of the feasibility of enhancing biodegradation in-line or using the sewer or drain as part of the wastewater treatment plants (Chapter 1). This study focuses on aerobic heterotrophic processes in dual-phase systems including intrinsic and process kinetics, interaction between flow pattern and shear stress and biofilm morphology and ecology, and mathematical model development.

Three reactors, fed by the same synthetic wastewater and operating at 28^0C and 20^0C, are adopted in this study. The batch reactor represents a plug flow (Chapter 2), whereas the CSTR (Chapter 3) and channel reactor (Chapter 4) represent two continuous, well-mixed configurations. The average water flow velocity and thereby the shear stress in the batch reactor and CSTR are around 0.3 m/s and 1.0 N/m^2 at the same range as in sewers, but one order of magnitude greater than in the channel reactor whose hydraulic situation corresponds to a drain with mild slope and a sewage lagoon system.

The functions of suspended biomass and biofilm are distinguished by separately determining suspended biomass activity through additional parallel experiments in the batch reactor in which only suspended biomass is available (Chapter 2), and by using the Biological Oxygen Monitor (BOM) in the case of the CSTR and channel reactor (Chapters 3,4). The intrinsic kinetic constants are derived from these activity data. Process kinetics are studied through overall mass balances of the reactors.

From experimental results with different suspended biomass concentration X (mg/L) and biofilm specific area WA/V (m^{-1}) in the reactors (Chapters 2,3), it is concluded that the parameters such as X (mg/L), its maximum growth rate μ_{max} (day^{-1}), biofilm oxygen up-take rate BUR ($gO_2/m^2.day$), and biofilm specific area WA/V (m^{-1}) are essential to understand and describe dual-phase system processes (Chapters 2,3). These parameters should be considered both in experiments, mathematical models and simulation.

Measurements indicate that the intrinsic specific oxygen consumption activity of suspended biomass (based on dry weight) is 50-100% higher than that of the biofilm biomass in both the three reactors (Chapters 3,4,5). The inert materials including extracellular polymeric substance (EPS) accumulated in biofilm is estimated to be about 40% of dry weight, and it is supposed to be the main reason for the pronounced activity difference. So the kinetics from suspension growth systems can not be extrapolated to attached growth systems. Moreover, the apparent biofilm activity can be further reduced by mass transfer resistance. This illustrates that suspended biomass can play a significant role even under so low concentration as not to be visible as turbidity. For example, in the CSTR experiment the relative contribution of suspended biomass over biofilm to oxygen consumption can be increased from 4.7% to 45% when WA/V decreases from 20.2 m^{-1} to 2.2 m^{-1}, even though the suspended biomass concentration is only in the range of 10 mg/L (Chapter 3). The traditional design of one chamber respirometer measuring BUR and SOD neglects the suspended biomass role, and the measured BUR or SOD can be largely overestimated; a standardized instrument is yet to be developed.

For the CSTR and the channel reactor the apparent oxygen consumption coefficients $Y_{o/s}$ (gO_2/gglu) decrease from 50 to 10% when biofilm surface loading increases from 50 to 300 gglu/m^2.day. It means that less oxygen was consumed for per mole glucose decomposition under high loading condition presumably due to incomplete oxidation. Contrary to the theory the biomass production coefficients $Y_{x/s}$ (gbiom/gglu) show the same tendency (Chapters 3,4). Nutrient storage inside attached cells, or additional EPS production can provide explanations, however, further investigation is needed.

Comparing experimental results from the three reactors (Chapter 5) indicates that the microbial communities are dominated by filaments (mainly *Sphaerotilus natans*) in the biofilms in the three reactors due to selection pressure of the soluble glucose. But cocci co-exist with filaments in the batch reactor biofilm, and filaments predominate in the other two reactors' biofilms; this is consistent with the respective intrinsic kinetic constants, *e.g.* the Monod intrinsic kinetic constants are μ_{max}=2.8 day^{-1}, K_s=54 mg(glucose)/L for the batch reactor biofilm, and 1.3 day^{-1}, 2.9 mg/L, and 1.5 day^{-1} and 2.7 mg/L for the CSTR and channel biofilms, respectively, all at 28°C. This indicates that a plug flow favours floc-forming bacterial growth, while a well-mixed tank stimulates filamentous growth. This offers the same picture as the kinetic selection principle found on activated sludge processes. The surface film dominated biofilm in the channel reactor, due to smaller water flow velocity and shear stress, provides a larger interface area, and is suggested to be the main reason for the higher oxygen and glucose fluxes. However, no effect of shear stress on biofilm community was found. A conceptual model was proposed for the interaction between reactor type and shear stress, and biofilm morphology, composition and kinetics.

A mathematical model describing oxygen consumption was developed (Chapter 6). The model contains parameters in the liquid and solid phases. The first and zero order kinetics with respect to readily biodegradable carbon substrate concentrations are applied depending

on the substrate concentration, *i.e.* the first order kinetic model is applicable to polluted aquatic systems, and the zero order kinetic model to wastewater collection systems. Simulation results show that the effects of individual parameter changes under varying conditions, and demonstrate that a dual-phase system can be governed by either the liquid or solid phase, or can be under the influence of both of them. Therefore, the relative importance of the two phases is system dependent. The ratio BUR_{max}*WA/(X*μ_{max}*V), which defines physically the relative importance of biofilm over suspended biomass regarding oxygen consumption, can give a quick estimation for process analysis. The potential use of the models was discussed.

The study indicates that in-line purification can be enhanced by increasing biomass specific area in wastewater collection systems (Chapter 2), or by recycling activated sludge into the systems (Chapter 6) depending on the dominating biomass type. Thus in-line purification can become an alternative for wastewater treatment for the developing countries especially under a (sub-)tropical climate, although extra power for reaeration, or pumping may be required. However, from a long-term point of view in-line soluble COD removal should be integrated with its potential for reuse as electron donor in nitrogen and phosphate removal when N and P removal is to be considered.

CONCLUSIONS

- Suspended biomass concentration, its specific activity, biofilm oxygen up-take rate and biofilm specific area are essential parameters to dual-phase systems and processes. Both phases should be taken into account in experiments and in mathematical models.

- The intrinsic activity of suspended biomass respect to oxygen consumption rate can be 50-100% higher than that of biofilm, presumably because of the biofilm's high fraction of inert material including extracellular polymeric substance (EPS). Apparent biofilm activity in reactors can be further reduced due to mass transfer resistance. The liquid phase can play a competitive role next to the biofilm, even when the suspended biomass concentration is in such low concentrations as 5-15 mg/L, especially when WA/V is small.

- The apparent oxygen consumption yield coefficients of biofilm decrease from 50 to 10% when biofilm surface loading increases from 50 to 300 gglu/m^2.day. Further study is needed for a complete mass balance of oxygen and carbon, and the relationship between the oxygen balance and the carbon balance.

- To biofilm systems, a plug flow stimulates floc-forming bacterial growth, while a well-mixed reactor favours filamentous growth. The same principle holds as in the kinetic selection in activated sludge. No effect of shear stress on biofilm composition was found.

- A mathematical model describing oxygen consumption in dual-phase systems has been developed. The model contains the relevant parameters in both liquid and solid phases, and the first and zero order kinetics can be chosen depending on the substrate concentration range. Qualitatively, the simulation results are consistent with the experimental discoveries.

Ye-Shi Cao
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The drain and sewer as dual-phase biological reactors.
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SAMENVATTING

Afvalwater wordt gewoonlijk via open of gesloten riolen en oppervlaktewateren zoals rivieren, stroompjes en vijvers afgevoerd. Al deze systemen worden gekenmerkt door het feit dat ze zowel gesuspendeerde aan de bodem en wand als gehechte biomassa (biofilm) bevatten, zgn. twee-fasen systemen. In tegenstelling tot systemen waarin biofilms een nadrukkelijke rol spelen (oxidatiebed, biofilter, air lift suspension reactor) en tot systemen met voornamelijk gesuspendeerde biomassa (actief slib installaties), waarover veel studies zijn uitgevoerd, is er aan twee-fasen systemen nauwelijks onderzoek gedaan. Toch is deze kennis onontbeerlijk voor waterkwaliteitsstudies, voor goed ontwerp van afvalwaterzuiverings-installaties, en om de haalbaarheid te onderzoeken van een verhoogde afbraak in het open of gesloten riool als integraal onderdeel van een afvalwaterzuivering (Hoofdstuk 1). Dit onderzoek richt zich voornamelijk op aërobe, heterotrofe processen in twee-fasen systemen, en onderzoekt de kinetiek van de processen en op het niveau van de reactor, de interactie tussen stroomsnelheid en afschuifkracht enerzijds, en morfologie en ecologie van de biofilm anderzijds, en - tenslotte - de ontwikkeling van een mathematisch model.

Het onderzoek werd uitgevoerd met drie soorten reactoren, op twee temperaturen (20°C en 28°C). De reactoren werden gevoed met hetzelfde synthetische afvalwater. In Hoofdstuk 2 wordt een batch reactor die werkt volgens het propstroom principe beschreven, terwijl twee continue, goed-gemengde systemen aan de orde komen in Hoofdstuk 3 (continuous stirred tank reactor, CSTR) en Hoofdstuk 4 (kanaalreactor).

De gemiddelde stroomsnelheid van het water en de daarmee samenhangende afschuifkracht in de batch reactor en de CSTR zijn 0.3 m/s en 1.0 N/m^2 wat ongeveer gelijk is aan die in riolen, maar een orde van grootte hoger dan in de kanaalreactor, waarin de hydraulische parameters overeenstemmen met die van een open riool met een niet al te groot verval, en met een afvalwatervijver.

De activiteiten van de gesuspendeerde biomassa en van de biofilm zijn onderscheiden door middel van parallelle experimenten: door in de batch reactor te werken met alleen aanwezigheid van gesuspendeerde biomassa (Hoofdstuk 2), en met behulp van de BOM (biologische zuurstofmeting) in de CSTR en de kanaalreactor (Hoofdstukken 3, 4). Intrinsieke kinetische constanten zijn van deze activiteiten afgeleid. De kinetiek van het geaggregeerde

proces op schaal van de reactor is bestudeerd met behulp van de massabalansen over de reactoren.

Uit de experimentele resultaten met verschillende gesuspendeerde biomassa concentratie X (mg/L) en door biofilm bezet reactor oppervlak WA/V (m^{-1}) kan geconcludeerd worden dat X (mg/L), de maximale groeisnelheid μ_{max} (dag^{-1}), de zuurstofopnamesnelheid door de biofilm BUR (Benthic oxygen up-take rate) (g O_2/m^2 dag), en het specifieke reactoroppervlak ingenomen door de biofilm WA/V (m^{-1}), zeer belangrijk zijn om de processen in een twee-fasen systeem te begrijpen en te beschrijven (Hoofdstukken 2, 3).

Metingen geven aan dat de intrinsieke, specifieke zuurstofopnamesnelheid van gesuspendeerde biomassa (gebaseerd op drooggewicht) 50-100% hoger is dan die van de biofilms (Hoofdstuk 3, 4, 5). Het inerte materiaal geaccumuleerd in biofilms, inclusief de extracellulaire polymere substantie (EPS), wordt geschat op ongeveer 40% van het drooggewicht en wordt geacht de voornaamste oorzaak te zijn voor het grote verschil in de activiteit. De kinetiek van gesuspendeerde systemen kan dus niet zonder meer geëxtrapoleerd worden naar systemen met aangehechte biomassa. Verder kan de activiteit in biofilms ook nog eens extra worden gereduceerd door diffusiebeperking. Het bovenstaande illustreert dat gesuspendeerde biomassa een significante rol kan spelen ook in concentraties die met het blote oog niet meetbaar zijn als troebeling. In de CSTR bijvoorbeeld kan de relatieve bijdrage van gesuspendeerde biomassa t.o.v. biofilms in de zuurstofopname worden verhoogd van 4.7% naar 45% wanneer WA/V afneemt van 20.2 m^{-1} naar 2.2 m^{-1}, zelfs al ligt de gesuspendeerde biomassaconcentratie in de orde van grootte van 10 mg/L (Hoofdstuk 3). Het traditionele ontwerp van een éénkamer respirometer voor de metingen van BUR en SOD (Sediment Oxygen Demand) doet geen recht aan de rol van gesuspendeerde biomassa, zodat de gemeten BUR en SOD sterk overschat kunnen worden; er zou een gestandaardiseerd apparaat ontwikkeld moeten worden.

In de CSTR en de kanaalreactor daalt de zuurstofopnamecoëfficiënt $Y_{o/s}$ (g O_2/gglu) van 50% tot 10% als de belasting op het biofilmoppervlak toeneemt van 50 tot 300 gglu/m^2.dag, hetgeen betekent dat er minder zuurstof per mol omgezet glucose wordt opgenomen onder hoge belasting, waarschijnlijk ten gevolge van onvolledige omzettingen. In tegenstelling tot de heersende theorie laat de biomassa produktiecoëfficiënt $Y_{x/s}$ (g biomassa/gglu) dezelfde tendens zien (Hoofdstukken 3, 4). Opslag van nutriënten binnen de aangehechte cellen of toename van de EPS produktie kunnen hiervoor een verklaring zijn, hoewel nader onderzoek uitsluitsel zal moeten geven.

In de drie reactortypen zijn de filamenteuze microörganismen in de meerderheid (vnl. *Sphaerotilus natans*) ten gevolge van de selectie die wordt uitgeoefend door de aanwezigheid van veel opgelost glucose. In de biofilm van de batch reactoren komen naast de filamenteuze bacteriën ook cocci voor; dit in tegenstelling tot de beide andere reactoren, die volledig gedomineerd worden door filamenten. Dit verschijnsel is te verklaren met de respectievelijke intrinsieke kinetische constanten, b.v. de Monod constanten: μ_{max} = 2.8 dag^{-1}, K_s = 54

mgglu/L voor de biofilm in de batch reactor, en 1.3 dag $^{-1}$, 2.9 g mg/L en 1.5 dag^{-1}, 2.7 mg/L voor de CSTR en de kanaalreactor; dit alles bij 28°C. Dit houdt in dat propstroom de groei van vlokvormende bacteriën bevordert, terwijl een goed gemengde tank de groei van filamenteuze bacteriën stimuleert. Dit laat hetzelfde beeld zien als het kinetisch selectieprincipe in de actief slib installaties. De biofilm in de kanaalreactor bestaat uit een heterogene, los-zwaaiende oppervlakkige laag filamenteuze organismen vanwege een lagere stroomsnelheid en afschuifkracht in het water. Dit type biofilm heeft een groter netto contactoppervlak tussen vloeistof en biofilm, hetgeen wel eens de belangrijkste rede kan zijn voor de hogere flux van zuurstof en glucose. Er werd echter geen invloed van de afschuifkracht op de biofilmsamenstelling gevonden. Een conceptueel model is ontwikkeld om de interactie tussen reactortype en afschuifkracht enerzijds, en morfologie, samenstelling en kinetiek van de biofilm anderzijds te visualiseren.

In Hoofdstuk 6 is een mathematisch model met parameters in de vloeibare (gesuspendeerde biomassa) en in de vaste fase (biofilm) ontwikkeld, die de zuurstofopname en substraatafname beschrijft. Eerste en nulde orde kinetiek met betrekking tot concentraties van gemakkelijk afbreekbare koolstofsubstraten is toegepast afhankelijk van de substraatconcentratie, d.w.z. eerste-orde kinetiek is toepasbaar voor matig vervuilde aquatische systemen en nulde-orde kinetiek voor sterk vervuilde rioolsystemen. Resultaten van zo'n simulatie tonen de invloed van de verandering van individuele parameters bij diverse omstandigheden, en laten zien dat een twee-fasen systeem gestuurd kan worden door hetzij de vloeistoffase, of de vaste fase, of door beide. Het relatieve belang van de twee fasen (vloeistof of vaste) is dan ook afhankelijk van het systeem onder studie. De verhouding BUR_{max} *WA/X*μ_{max}*V, die het relatieve belang van de zuurstofopnamesnelheid door de biofilm t.o.v. die door de gesuspendeerde biomassa definieert, laat een snelle schatting toe ten behoeve van de analyse van een proces. De mogelijke toepassing van de modellen wordt in detail besproken.

De studie geeft aan dat de zuivering in riolerings- en drainagesystemen versneld kan worden door het specifieke oppervlak van biomassa te vergroten (Hoofdstuk 2), of door actief slib terug te voeren in het systeem (Hoofdstuk 6), afhankelijk van de soort biomassa die domineert. "In-line" zuivering kan dus als een gedeeltelijk alternatief worden gezien voor afvalwaterzuivering in ontwikkelingslanden speciaal onder (sub) tropische omstandigheden, hoewel extra energie voor aëratie en voor pompen nodig zal zijn. In een bredere context echter, zou "in-line" COD verwijdering geoptimaliseerd (en beperkt) moeten worden zodat voldoende COD als electrondonor aanwezig blijft voor de verwijdering van stikstof en fosfaat.

Appendix

Experimental Set-up and Calculation Methodology

The same synthetic wastewater was adopted for the experiments of the batch, CSTR and channel reactors. The ratio C:N:P of the substrate was 14.7:4.3:1 (weight); for the detailed composition see Chapter 2. To calculate the oxygen mass balance the aeration capacity of the three reactors was determined by separate experiments. The seed biofilms grown on the polypropylene substratum were cultured in a trickling filter treating domestic wastewater, and then used in the three reactors. Biomass dry weight was expressed through carbon content (Total Organic Carbon TOC). Biomass concentration in the liquid phase was obtained from the total TOC of the liquid sample minus the glucose TOC and TOC in the tap water used for wastewater preparation, after filtration of the sample over a filter paper (0.45 μm).

BATCH REACTOR

Reactor

Beakers of 10 L volume were used as reactors. Biofilm present on the surface of the polypropylene support substratum was placed on the vertical wall surface or the bottom of the reactor depending on the required specific biofilm area (Fig.A.1). Dissolved oxygen (DO) was measured continuously with a probe. Samples of glucose and biomass in the liquid were taken hourly. For biofilm adaptation and to get reproducible results for each specific glucose concentration the experiment with biofilm was repeated at least three times, and only the data obtained after the first two runs were used. The operating temperatures were 28 ± 1 °C and 20 ± 1 °C.

Calculation procedure

The initial organic decrease rate after substrate addition was used to calculate the reaction rate. To avoid unreproducible deviation at the beginning of the experiments, values of TUR (total oxygen uptake rate, $gO_2/m^3.d$) and SUR (oxygen uptake rate by the suspended biomass, $gO_2/m^3.day$) were taken as the averages of the first hour values ($\Delta t=1$ hr). The biomass accumulated on the area not covered by the polypropylene substratum in the first hour experiment was negligible.

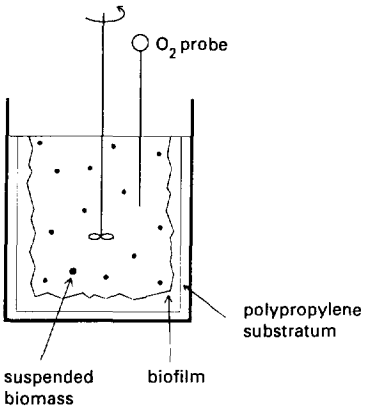

Fig.A.1 Scheme of the batch reactor

The general equations for the oxygen mass balance of a macro-volume in a batch reactor simulating a plug flow are as follows, with the first right-hand term representing the aeration and the second the TUR:

$$\frac{d(C_o^*-C_t)}{dt} = k_L a(C_o^*-C_t) - TUR \quad \text{or} \quad \frac{\Delta C}{\Delta t} = k_L a(C_o^*-C_t) - TUR \tag{1}$$

Where:

C_o^*, C_t - saturation oxygen concentration and oxygen concentration at time t in the bulk of the liquid, gO_2/m^3,
k_La - aeration coefficient, day^{-1},
t - time, day, gO_2/m^3.
ΔC - the oxygen concentration decrease after Δt time.

For a batch reactor with suspended and attached biomass, oxygen is consumed by the suspended biomass and the biofilm; therefore TUR can be divided into two parts:

$$TUR = SUR + \frac{BUR * WA}{V} \tag{2}$$

where:

BUR - oxygen uptake rate by biofilm (oxygen flux), $gO_2/m^2.day$,
WA - wetted area; the area covered by biofilm, m^2,
V - volume of the water, m^3.

According to equation (2) in case of the reactor with only suspended biomass,

TUR=SUR (3)

In order to distinguish the contribution of the suspended and attached biomass with respect to oxygen consumption and organics decomposition, two reactors, one with biofilm and suspended biomass and the second with suspended biomass only which was taken from the liquid in the reactor with biofilm, are operated in parallel; in both reactors suspended biomass concentration, substrate concentration, and temperature are kept nearly identical. TUR and SUR can be obtained from these two reactors using numeric integration or the finite difference method applied on equations (1),(2),(3).

The glucose decomposition rate of the whole reactor R_{gtot} (ggluc/m^3.day) was calculated by the equation:

$$R_{gtot} = -\frac{\Delta C_g}{\Delta t} \quad (4)$$

where:

ΔC_g - the decrease of the glucose concentration after 1 hour (Δt=1 hr), mg/L.

The contribution of the suspended and attached biomass to the total glucose conversion can again be distinguished by the parallel experimental results.

Example Parallel experiments with the conditions: $C_{go} \approx 10.0$ mg/L (C_{go}-glucose concentration at t=0), X=8.0 mg/L (X-suspended biomass concentration), T=20°C, C_o^*=9.1 mg/L, k_La=0.08 h^{-1}, and WA/V=2.1 m^{-1} for the reactor with biofilm.

Taking data from Table 1 for the first hour (Δt=1 hr), SUR=0.104 gO_2/m^3.h=2.5 gO_2/m^3.day. From Table 2 TUR=0.524 gO_2/m^3.day.

According to equation (2),

BUR=(TUR-SUR)V/WA=0.20 gO_2/m^2.h=4.8 gO_2/m^2.day.

The relative contribution of suspended and attached biomass with regarding to oxygen consumption and glucose decomposition is 0.104/(0.454-0.104)=25% and 0.3/(1.3-0.3)=30%, respectively.

Table 1 Experimental data for the reactor with only suspended biomass

Time hour	DO mg/L	$-\frac{\Delta C}{\Delta t}$ g/m³.h	$k_L a(C_o^* - C)$ g/m³.h	SUR $gO_2/m^3.h$	C_g mg/L	$-\frac{\Delta C_g}{\Delta t}$ g/m³.h
0	9.10				9.9	
		0.10	0.004	0.104		0.3
1	9.00				9.6	
		0.10	0.012	0.112		0.4
2	8.90				9.2	
		0.10	0.02	0.12		0.3
3	8.80				8.9	
		0.10	0.028	0.13		0.3
4	8.70				8.6	
		0.10	0.036	0.14		0.3
5	8.60				8.3	
		0.15	0.047	0.15		0.3
6	8.45				8.0	
		0.20	0.061	0.16		0.3
7	8.25				7.9	

Table 2 Experimental data for the reactor with biofilm and suspended biomass

Time hour	DO mg/L	$-\frac{\Delta C}{\Delta t}$ g/m³.h	$k_L a(C_o^* - C)$ g/m³.h	TUR $gO_2/m^3.h$	C_g mg/L	$-\frac{\Delta C_g}{\Delta t}$ g/m³.h
0	9.1				9.5	
		0.50	0.024	0.524		1.3
1	8.6				8.2	
		0.55	0.065	0.61		1.4
2	8.1				6.8	
		0.55	0.11	0.66		1.4
3	7.5				5.4	
		0.55	0.152	0.70		1.3
4	7.0				4.1	
		0.35	0.188	0.54		1.1
5	6.6				3.0	
		0.40	0.22	0.62		0.8
6	6.2				2.2	
		0.40	0.252	0.65		0.7
7	5.8				1.5	

CSTR AND CHANNEL REACTOR

The operating temperatures were 28±1 °C and 20±1 °C. The operating period for each run was taken as six times the hydraulic retention time, and a pseudo steady state was reached at the end of the run. Oxygen concentration and pH were continuously monitored, and glucose and biomass samples were taken hourly. Each run was repeated at least twice. To obtain the precise values of oxygen and glucose flux into the biofilm on polypropylene substratum, BUR and J_g, the biomass accumulated on the area (WA_{accu}) that is supposed to be not covered by the biofilm on the polypropylene, *e.g.* part of the internal wall in case of the CSTR, inside of recirculating pipe and pump in case of the channel, additional sampling plates were put inside the reactor and the pipe to quantify the accumulated biomass for correction purposes.

CSTR

The reactor consists of a plastic beaker (PVC) with effective reactor volume V=5 L. The influent flow rate Q were 2.5-7.5 L/h, the dilution rates D (=Q/V) applied were 0.5 to 1.5 h^{-1}. The biofilm on the polypropylene substratum was mounted on the wall or bottom (Fig.A.2), resulting in three different specific biofilm areas: WA/V=2.1, 10.8, 20.2 m^{-1}. The biomass accumulation on the "clean" surface which was not covered by polypropylene substratum could be neglected only in case of WA/V=20.2 m^{-1}.

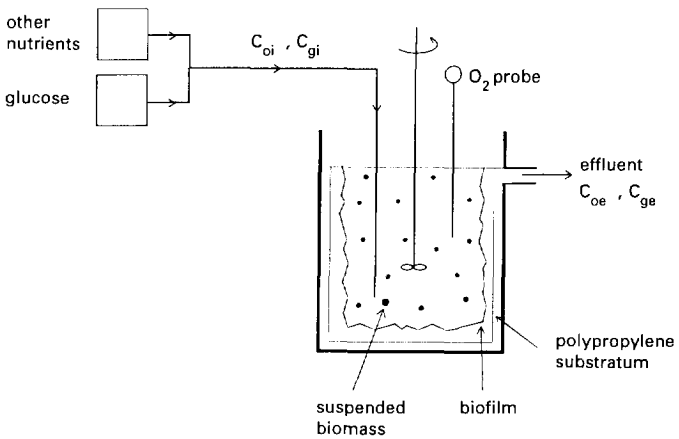

Fig.A.2 Scheme of the CSTR

Indoor channel

The channel, made of thick plywood, has a trapezoidal cross-section with the width of bottom and top 0.12 m and 0.33 m, respectively and depth 0.3 m, and length 3.0 m. The water depth in the channel was 0.18-0.2 m, and water volume V=130 L. The influent flow rates Q were 65 to 196 L/h, thus the dilution rates D (=Q/V) applied were 0.5-1.5 h^{-1}. The biofilm on polypropylene substratum was mounted on the bottom and the wall (Photo Chapter 4) (Fig.A.3) resulting in a specific biofilm area WA/V=10.4 m^{-1}. The flow due to recirculation of part of the effluent to the inlet created the required mixing and aeration. As the recirculation rate was app. 100 times larger than the influent flow rate, the flow velocity and turbulence inside the channel were governed by recirculation and could be controlled via the recirculating pump. A tracer experiment (sodium chloride) for the determination of mixing time, and concentration profiles of oxygen and glucose measured both in longitudinal and vertical directions, indicated the channel can be regarded as a well mixed reactor.

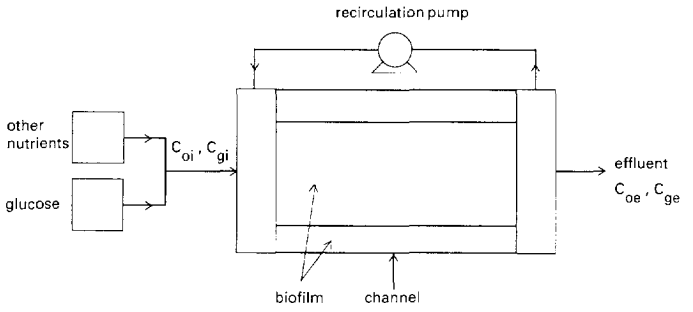

Fig.A.3 Scheme of the channel reactor

Calculation procedure

The calculation principle for the CSTR and channel is the same. The equations for oxygen consumption and glucose decomposition rates are as follows:

$$TUR = D*(C_{oi}-C_{oe}) + k_La*(C_o^*-C_{oe}) \quad (5)$$

$$R_{gtot} = D*(C_{gi}-C_{ge}) \quad (6)$$

where:

C_{oi}, C_{oe} - oxygen concentrations in the influent and effluent, mg/L,
C_{gi}, C_{ge} - glucose concentrations in the influent and effluent, mg/L.

The fluxes of oxygen and glucose BUR and J_g ($gO_2/m^2.day$) were calculated after having obtained TUR and R_{gtot}. For a reactor containing suspended biomass, biofilm on polypropylene substratum WA, and biomass accumulating on WA_{accu}, TUR ($gO_2/m^3.day$) is the sum of the three oxygen up-take rates sources: suspended biomass oxygen up-take rate SUR ($gO_2/m^3.day$), oxygen up-take rate by biofilm on polypropylene substratum BUR*WA/V ($gO_2/m^3.day$), and oxygen up-take rate by biomass accumulated on WA_{accu}, $BUR_{accu}*WA_{accu}/V$ ($gO_2/m^3.day$). Therefore:

$$BUR = (TUR - SUR - \frac{BUR_{accu} * WA_{accu}}{V}) * \frac{V}{WA}. \quad (7)$$

SUR was measured by the BOM, of which the calculation principle is the same as for the batch reactor; its details including structure and operation are given in Chapter 5. The biomass accumulation was quantified from the accumulated amounts of biomass and its q_{o2}

measured by the BOM (Chapters 3,4). J_g (ggluc/m^2.day) was calculated in the same way as BUR. The contributions of suspended and attached biomass to total glucose conversion R_{gtot} can be distinguished based on the oxygen consumption data.

Example CSTR with WA=0.054 m^2, V=0.005 m^3, WA/V=10.8 m^{-1}, dilution rate D=0.5 h^{-1}, T=28 °C, C_o^*=7.8 mg/L, k_La=0.83 h^{-1}.

The other data: C_{oi}, C_{oe} - 9.9 and 2.4 mg/L, respectively, C_{gi} and C_{ge} - 117.7 and 7.1 mg/L, respectively. Suspended biomass concentration, X - 32 mg/L, the clean area WA_{accu}=0.086 m^2, and M, the amount of accumulated biomass 390 mg/m^2; q_{o2}, the specific activity of the suspended and accumulated biomass is the same as 1.2 gO_2/gbiom.day under the conditions of glucose concentration 7 mg/L, and at 28°C.

According to equations (4),(5) for the CSTR and channel reactor,

TUR=198 gO_2/m^3.day,
R_{gtot}=1327 ggluc/m^3.day, and
SUR=X*q_{o2}=38.4 gO_2/m^3.day.

Because of the same specific activity of the accumulated biomass as the suspended biomass,

$BUR_{accu}*WA_{accu}=q_{o2}*M*WA_{accu}=1.2*0.39*0.086=0.04$ gO_2/day.

Therefore according to equation (7):

BUR=(198-38.4-0.04/0.005)/10.8=14.0 gO_2/m^2.day.

The calculation of glucose flux into biofilm R_{gb} on the polypropylene substratum is:

$R_{gb}=R_{gtot}*(BUR*WA/V)/TUR=1013$ ggluc/m^3.day.

$J_g=R_{gb}*V/WA=93.8$ ggluc/m^2.day.

The relative contribution of the suspended biomass over the biofilm regarding oxygen consumption is:

$SUR/(TUT-SUR-BUR_{accu}*WA_{accu}/V)=38.4/(198-38.4-8.0)=25.0\%$,

The relative contribution regarding glucose decomposition is the same value as for oxygen consumption by assuming the constant yield coefficients.

The yield coefficient of the biofilm $Y_{o/s}$ is $BUR/J_g=14/93.8=14\%$.

List of Symbols

A	cross section area, (L^2)
BUR	biofilm oxygen up-take rate ($ML^{-2}T^{-1}$)
BUR_{accu}	oxygen up-take rate by the biofilm accumulated during runing period ($ML^{-2}T^{-1}$)
BUR_{max}	maximum biofilm oxygen up-take rate ($ML^{-2}T^{-1}$)
Bv	feed ratio of activated sludge (MM^{-1})
C	oxygen concentration in the reactor (chapter 2); COD concentration (chapter 6) (ML^{-3})
C^*	saturation oxygen concentration (ML^{-3})
C_g	glucose concentration in the reactor (ML^{-3})
C_{gi}, C_{ge}, C_{go}	glucose concentrations in influent, effluent, and at time t (ML^{-3})
C_o, C^*_o	oxygen concentration and its saturation concentration (chapter 6) (ML^{-3})
C_{oi}, C_{oe}	oxygen concentrations in influent and effluent (ML^{-3})
C_{so}	soluble COD concentration at $x=0$ (ML^{-3})
C_{st}, C_{bt}	soluble COD concentrations at which biomass growth rate and biofilm oxygen up-take reach constant values (ML^{-3})
C_t	oxygen concentration at time t (ML^{-3})
D	dilution rate (T^{-1})
D_b	biofilm areal density (ML^{-2})
D_L	longitudinal dispersion coefficient (M^2T^{-1})
d	diameter of stirrer (L)
f	friction factor
glu	glucose concentration (ML^{-3})
H	water depth (L)
J_{o2}	oxygen flux into biofilm on polypropylene substratum ($ML^{-2}T$)
J_{o2accu}	oxygen flux into biofilm on other surface ($ML^{-2}T$)
J_{o2max}	maximum oxygen flux ($ML^{-2}T$)
J_g	glucose flux into biofilm on polypropylene substratum ($ML^{-2}T$)
k_La	overall aeration coefficient (T^{-1})
K_s	half-saturation constant of Monod equation (ML^{-3})
k_s	half-saturation constant of biofilm (Monod analogy) (ML^{-3})
M	biomass areal density on clean surface (ML^{-2})
$\mathbb{N}$	rotational speed of stirrer (T^{-1})
n	wetted peripheral length (L)
Q	flow rate (L^3T^{-1})

q_{o2}	specific oxygen activity of biomass ($MM^{-1}T^{-1}$)
q_{o2max}	maximum specific oxygen activity ($MM^{-1}T^{-1}$)
Re	Reynolds number
R_{gtot}	total glucose decomposition rate of reactor ($ML^{-3}T^{-1}$)
R_g	glucose decomposition rate due to biofilm ($ML^{-3}T^{-1}$)
S, S_o	substrate concentrations at time t and zero ($ML^{-3}T^{-1}$)
SOD	sediment oxygen demand ($ML^{-2}T^{-1}$)
SUR	oxygen up-take rate of suspended biomass ($ML^{-3}T^{-1}$)
t	time (T)
TUR	total oxygen up-take rate of reactor ($ML^{-3}T^{-1}$)
u	average water velocity in liquid bulk (LT^{-1})
V	effective reactor volume (L^3)
WA	area of biofilm on substratum (L^2)
WA_{accu}	other area covered by biomass accumulated during running period (L^2)
X, X_o	suspended biomass concentration and it value at time=0 ($ML^{-3}T^{-1}$)
x	horizontal abscissa (L)
$Y_{o/s}$	oxygen consumption coefficient (MM^{-1})
$Y_{x/s}$	biomass yield coefficient (MM^{-1})
θ	cell multiplication time (T)
μ	specific growth rate (T^{-1})
μ_{max}	maximum specific growth rate (T^{-1})
ν	kinematic viscosity of water (L^2T^{-1})
η	effectiveness factor
τ_s	shear stress on biofilm surface ($ML^{-1}T^{-2}$)
χ	hydraulic radius (L)
ρ	water density (ML^{-3})

Curriculum Vitae

The author of this dissertation was born in August 1949 in Shanghai, China. His primary and secondary education was received in No.10 School (formerly Attached School of Don Wu University), Suzhou, Jiangsu Province. As a farmer he spent five years in Kong Zan and Dan Yang counties from 1968 to 1973. From September 1973 - February 1977 he studied chemical engineering in the Department of Chemical Mechanics, in The East China University of Chemical Technology, Shanghai. After the graduation he was employed as an engineer in Zhengjiang Coke & Chemical Company until the end of 1980. Then he worked as assistant in chemical engineering in the Suzhou Institute of Silk & Textile, Suzhou. From February 1982 to November 1984, he studied in the Department of Chemical Engineering of the Nanjing Institute of Chemical Technology, Nanjing, for a master degree of engineering science. His master thesis concerns adsorption kinetics of NH_3^+ on biporous natural zeolite*. Afterwards he worked as a lecturer in the Department of Environmental Protection of the Suzhou Institute of Urban Construction and Environmental Protection (SIUCEP). In August 1985 - September 1986 he attended a post-graduate course on Environmental Science and Technology in the International Institute for Hydraulic and Environmental Engineering (IHE), Delft, The Netherlands, and obtained the post-graduate diploma on Environmental Engineering. After his return to China, he continued his work in SIUCEP and was involved in lecturing, researches and administrative works. He was a leading member to develop a new process to treat monosodium glutamate wastewater by using a UASB reactor**, and once worked for the joint project on Water Quality Planning of the Grand Canal of Southern Jiangsu Province, as a member of Chinese expert team, with the Netherlands expert team from Delft Hydraulics and DHV consultants.

In January 1990 he started work, as a research staff, in the Department of Environmental Engineering of The International Institute for Hydraulic and Environmental Engineering, Delft.

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** Cao Y.S. and Zhang H. and Li Y.Z. (1991) Anaerobic treatment of monosodium glutamate wastewater using granular sludge UASB reactor. *Envir. Chem.* **10**(4), 19-25. (in Chinese with English translation).
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International Institute for
Infrastructural, Hydraulic and
Environmental Engineering

P.O. Box 3015
2601 DA Delft
The Netherlands

Tel.: +31 15 (2)*151715
Telex: 38099 ihe nl
Cable: interwater
Fax: +31 15 (2)*122921
E-mail: whg @ ihe.nl

*) 2 included as of October '95