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The commercialization of new products and processes made possible through biotechnology requires a coordinated coupling of unit operations in order to develop efficient processes. The usual object of a process is to convert relatively inexpensive raw materials into more valuable products. The aim of this exercise was to study the feasibility of the fermentative production of pyrrolo-quinoline quinone, (PQQ), a coenzyme, from methanol. Besides this, a synthetic route for the manufacture of PQQ was also investigated. The amount of PQQ to be produced annually was arbitrarily decided to be 100 kg for which 40200 kg methanol is required.

Fermentation is carried out with the organism *Pseudomonas* Nl-1 in a large fermenter having a work volume of about 50 m$^3$. Pre-fermentation is carried out in a smaller fermenter having a work capacity of 2.5 m$^3$. Since each main fermentation takes three days and keeping 200 production days per year as capacity, a total of 67 fermentation cycles are accomplished. The method of fermentation is fed batch and the downstream processing is continuous.

The production cost of PQQ amounts to Dfl 31.50 per gram. The present market price of PQQ is about Dfl 3200 per gram. The rendement on investment is estimated to be 17%.

The waste flow includes 50 m$^3$ of spent medium, 123 kg dry weight of biomass, which can be further treated and used as cattle fodder, and 1.86 kg of purged water, containing sodium chloride and methanol, which originates from the ion exchange unit.
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CONCLUSIONS AND RECOMMENDATIONS

The fermentative production of PQQ from methanol is certainly more attractive than via the chemical synthesis. The cost price of PQQ has been estimated to be Dfl 31.50 per gram; this value lies far below the present production price of chemically synthesized PQQ which is about Dfl 500 per gram. The rendement on investment has been calculated to be 17% assuming the selling price to be twice the cost price.

Production of PQQ via a chemical route was also investigated and a cost price, solely based on raw material investment, was estimated to be Dfl 8.80 per gram. However, considering the complex character of the synthetic route, the cost price of PQQ would certainly be higher than the one estimated for the fermentative process. Nevertheless, we recommend that a detailed study should be undertaken to determine the actual cost price of PQQ via the synthetic route.

A poster has been accepted, based fundamentally upon this study, to be presented in the forthcoming International Symposium on PQQ and Quinoproteins, being held from 5-7 September, 1988, in Rijswijk.

During the concluding stages of our study we came across an abstract article, authored by Japanese scientists, that claimed to have achieved a production level of 600 mg PQQ per litre of the culture medium. This value is 20 times higher than the one we have taken as the starting point of our study. If this new level of production can be maintained, the consequences become quite evident. The cost price of PQQ would further decrease and the main fermenter would not be required anymore as the pre-fermenter is capable of handling the bulk fermentation provided the annual amount of production is kept the same. However, we are not in the position to give an exact estimate of the cost price as, once again, the basic biokinetic data is not available and, moreover, the composition of the medium is not mentioned. Nevertheless, if the fermentation conditions remain identical, we expect that the cost price of PQQ would decrease at least by a factor of three.
Pyrrolo-quinoline quinone (henceforth PQQ) is the semi-systematic name of the compound 2,7,9-tricarboxy-1H-pyrrolo [2,3-f] quinoline-4,5-dione, which constitutes the prosthetic group in the enzyme methanol dehydrogenase and some other oxidoreductases occurring in bacteria. Oxidoreductases are a class of enzymes that transfer redox equivalents from or to their substrates.

The first indication of the possible existence of a novel cofactor, other than NAD(P)^+ and flavin, active in cells emerged in 1953 [1]. After an extensive amount of work, this cofactor was established to be PQQ. The structure of the derivative of the cofactor was elucidated in 1979 by applying X-ray diffraction technique [2]. The structure of PQQ, along with two other biologically relevant redox forms, the semiquinone and the quinol of PQQ, are shown in figure 2.1.

![Figure 2.1](image_url)

PQQ is widely distributed in nature. Its occurrence is not restricted to prokaryotes. Recently, it has been discovered in mammalian amine oxidases and lysyl oxidase isolated from human placenta. Considering that similar enzymes exist in yeast, fungi, and plants, it can be concluded that PQQ is a functional cofactor present in both prokaryotes and eukaryotes [3].

As well-known co-factors such as flavins and nicotinamide nucleotides of many oxidoreductases are vitamins (appendix 1), it is emphatically thought of that PQQ, too, might exhibit vitamin character. Since the discovery of PQQ in amine oxidases which play an important physiological role in regulation of cell division and cross-linking of collagen, provision with PQQ in the form of vitamin may, therefore be essential. Furthermore, the natural occurrence of PQQ in table vinegars (originating from PQQ-producing bacteria) has been established. Exaggerated craving for products...
made from vinegar by some women during early months of pregnancy may after all have a biological rationale explanation. As the level of placental amine oxidases increases during pregnancy, there is an increased demand for PQQ which is somehow translated into an urge to consume products that contain relatively large amounts of this cofactor [4].

PQQ has also been identified as a growth-stimulating substance for some microorganisms at it reduces the lag-phase in the growth cycle [5,6].

PQQ is synthesized chemically in Delft and commercialized by FLUKA AG, Buchs, Switzerland. However, it is also commercially produced by fermentation and sold by Ube Industries Ltd, and Mitsubishi Chemical Industries in Japan.

Microbial production of PQQ is less complicated as compared to the chemical method. Several bacteria excrete PQQ in their culture medium [7,8], especially when grown on alcohols. This study was undertaken to ascertain the feasibility of PQQ production via microbial fermentation of methanol on an industrial scale. Furthermore, a detailed study of one of the synthetic routes was done.
Introduction

Chemical synthesis of PQQ has been achieved in different ways by various research groups [3]. The fundamental aspects of the following study are based upon the route as reported by Mackenzie et al [9]. The yield obtained via this route is 36.5% on mole basis with 4-amino salicylic acid as the starting compound.

Synthesis of PQQ is a complex sequence of multiple organic reactions requiring various reactants, solvents and catalysts. These solvents and catalysts must be recovered in order to make the industrial process commercially attractive. Nevertheless, the recovery processes considerably increase the initial investment and makes the product on the whole expensive. Furthermore, some of the solvents and catalysts are potential pollutants and consequently the waste treatment becomes an invariable part of the industrial process. At present commercially available PQQ is prepared chemically, but on a laboratory scale. A small amount is manufactured by the subfaculty of Enzymology at the Delft University of Technology.

The Mackenzie Route: a general description

The Mackenzie method in general involves eight major steps. The starting compound, 4-amino-salicylic acid, is converted into benzaldehyde which in turn is transformed into indole followed by annulation of the pyridine ring ultimately resulting in the formation of PQQ.

The conversion of benzaldehydes into indoles is achieved in two steps via the corresponding azidocinnamates. The initial benzaldehyde (5) is prepared (Scheme 1) from methyl-4-acetamido-2-hydroxy-benzoate (3), obtained by esterification and N-acetylation of commercially available 4-amino-salicylic acid. The benzaldehyde undergoes O-alkylation and reduction of the ester forms the corresponding benzyl alcohol with lithium aluminium anhydride and reoxidation to the benzaldehyde with barium manganate.
The benzaldehyde is then condensed with methyl azidoacetate to give the azidocinnamate (6b), thermolysis of which in boiling xylene results in the formation of the expected indole (7b). Elaboration of the indole to the required tricyclic pyrroloquinoline (9b) is achieved via acid methanolysis of the indole, followed by the reaction of the resulting aniline with commercially available dimethyl-2-oxoglutaconate in a Doebner von Miller type quinoline synthesis (Scheme 2).

Scheme 2. (a, R = H; b, R = CH₂Ph) Reagents: i, MeO₂CCH₂N₂, NaOMe, MeOH; ii, xylene, reflux; iii, MeOH, HCl, heat; iv, MeO₂COCH=CHCO₂Me, CH₂Cl₂, then H⁺.

Thus, the key tricyclic intermediate (9b) is obtained in four steps from the benzaldehyde and the introduction of the ortho-quinoline unit by oxidation of the central ring gives the desired compound, PQQ. The tricyclic intermediate undergoes catalytic hydrogenolysis (Scheme 4) to form the corresponding phenol (21), the oxidation of which yields the quinone triester (13). The triester then subsequently undergoes hydrolysis with excess amount of sodium hydroxide resulting in the formation of PQQ (1).

Scheme 4. Reagents: i, BBr₃, CH₂Cl₂, then MeOH, H⁺; ii, H₂, Pd-C, MeOH; iii Bu₄N(COPh)WO₃, CH₂Cl₂-MeOH (9:1); iv, HCl(OMe)₃, MeOH, H⁺, then aq. K₂CO₃, 85°C, then HCl to pH 2.5.
The process was studied in detail and scaling up of the synthesis scheme was done so as to produce 100 kg of PQQ annually. A process scheme is drawn (figure 3.1) to give an idea of the production unit. Tables 3.1 and 3.2 summarises the quantities and the costs of reactants and solvents required in the manufacture. One assumption, however, is that all solvents and catalysts are 95% recoverable. On this basis the price of PQQ is calculated to be Dfl 8.40 per gram. Investment on equipment, energy expenditure, labour costs and other miscellaneous finances were not determined. Despite the fact that chemical synthesis has some advantages such as a compact process plant and reasonable output yield, it is evidently characterized by a few disadvantages. A large number of, and some expensive, raw materials are required, some of which are potential pollutants. Since the process involves several steps, a large amount of equipment is needed. A refrigeration system is essential as some of the reaction are carried out at as low as -15 °C.
<table>
<thead>
<tr>
<th>REACTANT</th>
<th>AMOUNT</th>
<th>PRICE (Dfl)/UNIT</th>
<th>TOTAL</th>
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<tr>
<td>Sulphuric acid</td>
<td>215.4 l</td>
<td>1.58/1</td>
<td>340.00</td>
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<tr>
<td>Aminosalicylic acid</td>
<td>114 kg</td>
<td>5.92/kg</td>
<td>675.00</td>
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<tr>
<td>Sodium bicarbonate</td>
<td>2670 kg</td>
<td>0.57/kg</td>
<td>1513.00</td>
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<tr>
<td>Acetanhydride</td>
<td>76.6 kg</td>
<td>7.60/kg</td>
<td>582.00</td>
</tr>
<tr>
<td>Potassium carbonate</td>
<td>480.0 kg</td>
<td>1.56/kg</td>
<td>749.00</td>
</tr>
<tr>
<td>Benzylbromide</td>
<td>148.0 l</td>
<td>22.17/1</td>
<td>3281.00</td>
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<tr>
<td>Lithium aluminium hydride</td>
<td>49.1 kg</td>
<td>200.00/kg</td>
<td>9820.00</td>
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<td>Barium manganate</td>
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<td>412.00/kg</td>
<td>630360.00</td>
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<tr>
<td>Sodium nitride</td>
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<td>Methyl chloroacetate</td>
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<td>3.17/kg</td>
<td>761.00</td>
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<tr>
<td>Cerium (IV) ammonium nitrate</td>
<td>894.0 kg</td>
<td>69.00/kg</td>
<td>61686.00</td>
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<tr>
<td>Dimethyl-2-oxo-glutaconate</td>
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<td>1200.00/kg</td>
<td>90840.00</td>
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<tr>
<td>Sodium hydroxide</td>
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<td>1.33/kg</td>
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<td>15.50/kg</td>
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<td></td>
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</tr>
<tr>
<td>SOLVENT</td>
<td>AMOUNT</td>
<td>PRICE (Dfl)/UNIT</td>
<td>TOTAL</td>
</tr>
<tr>
<td>--------------------</td>
<td>--------</td>
<td>----------------</td>
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<tr>
<td>Methanol</td>
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<tr>
<td>Ethanol</td>
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<tr>
<td>Aceton</td>
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<td>Tetrahydrofuran</td>
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<tr>
<td>Chloroform</td>
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<tr>
<td>Ammonium chloride</td>
<td>70.0 kg</td>
<td>0.60/kg</td>
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<tr>
<td>Ethylacetate</td>
<td>1115.0 l</td>
<td>3.83/kg</td>
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<tr>
<td>Xylene</td>
<td>1230.0 l</td>
<td>3.40/l</td>
<td>4182.00</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>1030.0 l</td>
<td>3.83/l</td>
<td>3940.00</td>
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<tr>
<td>Methylchloride</td>
<td>118.0 l</td>
<td>11.45/l</td>
<td>1351.00</td>
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<tr>
<td>Acetonitrile</td>
<td>157.0 l</td>
<td>9.00/l</td>
<td>1413.00</td>
</tr>
<tr>
<td><strong>TOTAL (Dfl)</strong></td>
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<td></td>
<td><strong>24401.00</strong></td>
</tr>
</tbody>
</table>
4.1 Microbiology and metabolism

Several bacteria excrete PQQ into their culture medium, especially when grown on alcohols. This novel co-enzyme was originally discovered and identified in methanol dehydrogenases of methylotrophic bacteria. Methylotrophic bacteria are a group of organisms that are able to use methane derivatives as their sole source of carbon and metabolic energy and so can sustain growth on methane, methanol and other organic compounds that lack carbon-carbon bonds. Since they are not autotrophic, they rely exclusively on their ability to oxidise these compounds. In addition to methanol dehydrogenases from various methanol-grown microorganisms [10], alcohol [11], aldehyde [11] and glucose [11,12] dehydrogenases of acetic acid bacteria and glucose dehydrogenase of Acenitobacter calcoaceticus [13] and Pseudomonas fluorescence [11] have been revealed to be PQQ-dependent hydrogenases. Methylamine dehydrogenase of Pseudomonas AM1 has been shown to be an PQQ-enzyme in which the PQQ is attached to the enzyme protein via a covalent bond [14].

On denaturation of methanol dehydrogenase, two PQQs per enzyme molecule are found in the extract [3]. It was postulated that the following equilibria exist for the semi-quinone in this enzyme:

\[ 2 \text{PQQH}^+ \rightleftharpoons \text{PQQ} + \text{PQQH}_2 \rightleftharpoons \text{(PQQ + PQQH}_2 \text{) complex.} \]

However, this hypothesis was later scrutinized [15]. For the enzyme methanol dehydrogenase, in vitro, a reaction scheme has been proposed (figure 4.1) in which the role of the activator NH$_3$ and that of one-electron acceptors is indicated [3].

![Figure 4.1](image_url)
Several of the intermediates in the redox cycle have been isolated: $\text{MDH}_{\text{red}}$, the fully reduced form containing $\text{PQQH}_2$; $\text{MDH}_{\text{oxl}}$, the enzyme form as it is usually isolated, containing $\text{PQQH}_2$; $\text{MDH}_{\text{ox}}$, the fully oxidized, labile form containing PQQ which can be converted with carbonyl group reagents, for example, cyanide, into $\text{MDH}_{\text{ox}2}$.

4.1.1 Bioenergetical role in bacteria

In principle, bacterial dehydrogenases can be localized at the inside or the outside of the cytoplasmic membrane. For proton translocation efficiencies, the relationship with substrate transport and the site of coupling to the respiratory chain should also be taken into account. Methanol dehydrogenase [16], methylamine dehydrogenase [17] and glucose dehydrogenase [18] in gram-negative bacteria are all situated at the outside of the cytoplasmic membrane [3]. The quinoprotein dehydrogenases studies in bacteria are coupled to different sites in the respiratory chain. This indicates that bacteria not only have bioenergetical and kinetic flexibility by branching their respiratory chains with different types of cytochromes, but also by their capacity to produce different kinds of dehydrogenases to oxidize a particular substrate.
4.2 Assumptions

Fundamental aspects of the production process are based upon a Japanese patent application. This application discloses a new process for the production of PQQ by culturing a bacterium belonging to the genus *Pseudomonas*. A maximum level of PQQ accumulation, 30 mg/l, in the culture medium is achieved after 50 hours of fermentation in a 30 liter fermenter performed in fed batch with methanol as the substrate.

However, due to the lack of basic biokinetic data, a number of assumptions have been made in order to do the calculations. Few of these assumptions are based upon figure 4.2. This figure represents a profile of the batch fermentation, wherein an amount of PQQ accumulated in the medium reaches a maximum point during period from the late logarithmic phase to the early stationary phase. The biomass increase in the batch process is ten times the initial concentration during which 26.1 mg/l of PQQ accumulates in the medium in which the methanol concentration is 1% by weight. In the fed-batch process 30 mg/l of PQQ accumulates. Assuming that the PQQ concentration increases linearly with the biomass concentration, then the increase of fed-batch biomass is eleven times the initial concentration.

![Figure 4.2](image-url)

Figure 4.2
It is assumed that besides PQQ no other product is excreted into the medium. Furthermore, the amount of PQQ formed is negligible with respect to the substrate consumption.

Growth kinetics of the organism follows the equation of Pirt, with no product formation [19].

\[ r_s = \frac{1}{\gamma_s} \cdot r_x + m_s \cdot C_x \]  \hspace{1cm} (4.1)

where \( r_x = \mu C_x \)

The value for \( \mu_{\text{max}} \) is derived from figure 4.3, which relates the influence of methanol concentration on the specific growth rate of the organism, *Methylomonas methanolica* [20]. This bacteria is assumed to be the model organism for the determination of the specific growth rate of *Pseudomonas* Nl-1. The \( \mu_{\text{max}} \) value determined is 0.5 h\(^{-1}\). Moreover, it is evident from the figure that the concentration of methanol should be kept below 4 g/l in order to maintain a specific growth rate of 0.5 h\(^{-1}\).
The maintenance coefficient on substrate, $m_s$, is estimated to be

$$0.05 \frac{\text{mol substrate}}{\text{mol biomass} \cdot \text{h}}$$

This value is an average of maintenance coefficients of different Pseudomonas species growing on methanol [19].

The netto yield of biomass on substrate, $Y_{sx}$, is 0.67 mol/mol. Method of determination remains the same as that for maintenance coefficient.

Considering that the global demand for PQQ at this moment is not very large and furthermore, PQQ is itself utilised in minute quantities, an annual production of 100 kg is arbitrarily decided. Keeping 200 production days per year in mind and 50 hours needed per fermentation, it can easily be calculated that a total number of fermentations required are 66 with 1.515 kg PQQ per run being accumulated in 50 m$^3$ of the fermentation medium. However, it is assumed that 99% of PQQ is recovered from the culture medium and this requires an extra fermentation run.

The results of the 30 litre fed batch fermenter given in the patent are scaled up for a large fermenter producing 1.510 kg of PQQ per fermentation. The fermentation time, however, remains 50 hours.

### 4.3 Rheology of the medium

It is assumed that the fermentation medium exhibits Newtonian fluid behavior similar to many other bacterial fermentation media. The viscosity of the medium is assumed to be constant at $10^{-3}$ Pa.s throughout the fermentation. The density is similar to that of water i.e 1000 kg/m$^3$.

### 4.4 Physical properties of PQQ

PQQ is a brick red compound which easily dissolves in water (solubility of the sodium salt in water is at least 20 g/l at 20 °C). The solution shows green fluorescence, but colour and fluorescence are temperature dependent, the reason being that PQQ is partially hydrated in water. PQQ is not corrosive and explosion dangers do not exist. PQQ is extremely stable even at temperatures as high as 300 °C.

Methanol is one of the raw materials that is stored in bulk quantity. Limits of inflammability of methanol in air are as follows:

- **lower limit**: 6.72% on volume basis
- **upper limit**: 36.50% on volume basis
4.5 Raw materials and wastes

Raw materials required for the production process include methanol, fermentation salts (see composition of medium), Amberlyst A21 as ion exchanger and sodium chloride.

The waste flow includes approximately 50 m$^3$ of water that and a small amount of purge of water containing sodium chloride and methanol from the ion exchange section. The biomass that remains over after centrifugation can be used as fodder in the form of single cell protein.

4.5.1 Medium composition

Medium composition as described in the patent is used in the main fermenter. However, nitrate is not added as the organism is unable to reduce it. Medium composition is as follows: (all in wt %)

\[
\begin{align*}
\text{(NH}_4\text{)}_2\text{SO}_4 & : 0.2 \% \\
\text{K}_2\text{HPO}_4 & : 0.1 \% \\
\text{MgSO}_4\cdot7\text{H}_2\text{O} & : 0.02 \% \\
\text{H}_3\text{BO}_4 & : 0.00005 \% \\
\text{CuSO}_4\cdot5\text{H}_2\text{O} & : 0.000004 \% \\
\text{MnSO}_4\cdot\text{H}_2\text{O} & : 0.00002 \% \\
\text{(NH}_4\text{)}_2\text{MoO}_4 & : 0.00002 \% \\
\text{ZnSO}_4\cdot7\text{H}_2\text{O} & : 0.00004 \% \\
\text{CoCl}_2\cdot2\text{H}_2\text{O} & : 0.0015 \% \\
\text{KCl} & : 0.004 \% \\
\text{FeSO}_4\cdot7\text{H}_2\text{O} & : 0.0001 \%
\end{align*}
\]

The pH is regulated at 7.0 and the temperature of fermentation is 30 °C.

4.6 Preparation of pre-culture

A pure culture of *Pseudomonas* NI-1 is initially cultured in series of conical flasks of increasing volume and consequently transformed into the pre-fermenter. Since the aim of this fermentation is to cultivate a sufficient amount of biomass in order to inoculate the main fermenter, salt concentration is doubled to account for the extra growth. It is assumed that the extra salt does not negatively effect the growth of the organism.
Duration of the main fermentation is about 50 hours and is performed in fed batch. Methanol is added from the second hour onwards until the 42nd hour in order to facilitate an exponential growth of the biomass. During the last 8 hours of the fermentation methanol addition is kept constant as now only the maintenance of the cells is desired rather than their multiplication. Considering that PQQ is excreted during the period of late exponential phase and stationary phase of their growth cycle, the organisms are maintained in their stationary phase.
5. **Description of the process**

The sterilization section provides sterile media for several processes, among which for the presently discussed PQQ fermentation process.

Tapwater and a salt solution are mixed and after heat exchange with the already sterilized medium, are pushed through a steam injector. The temperature of the medium is raised to 125 °C and subsequently transported into either the pre-culture or main fermenter. The temperature of the medium drops to 55 °C as a result of heat exchange with the incoming raw material.

Methanol is added into the pre-culture fermenter once the medium has been cooled to 30 °C. Following inoculation with the lab-culture, the fermentation medium is aerated for 20 hours with controlled methanol supplementation to facilitate rapid biomass production. Simultaneously, the main-fermenter is prepared for the operation and methanol is added. The pre-culture is pumped into the main fermenter and the broth is aerated and fed with methanol for 50 hours.

On completion of the fermentation, the biomass is separated from the fluid by centrifugation with a disk bowl centrifuge. This operation takes about two hours. The biomass can be further processed (by sterilization or spray drying), together with biomass formed in other processes, to serve as fodder in the form of SCP.

The supernatant is brought into a large tank, which has almost the same volume as the main fermenter. This vessel contains pretreated Amberlyst A 21, an ion-exchanger which has a strong affinity for PQQ. The contents of the tank are slowly stirred to ensure complete binding of PQQ with the ion-exchanger. Amberlyst A 21 is then separated by a sieve, washed with tapwater and poured into a separation column. The waste water contains inorganic matter (according to assumptions) and is transported to other parts of the plant to be treated.

A flow of eluting agent dissolves the PQQ into the liquid phase and is subsequently brought in a crystallization tank. The acidity of the solution is lowered to pH 2 with 6 N hydrochloric acid and left over for a night. The PQQ crystals are consequently separated with a sieve and dried at a temperature of about 60 °C to give the end product. The remaining eluent is neutralized with 6 N sodium hydroxide. Excess eluent containing water, methanol and sodium chloride is purged, while rest of the liquid is recirculated and prepared for a new process.
6.1 Sterilization

6.1.1 Fermenter Sterilization

Sterilization is understood as the elimination of all microorganisms and the inactivation of viruses present in or on a product. According to this definition sterility is an absolute concept and day-to-day practice has shown that this theoretical goal cannot always be achieved, depending upon the respective conditions. Consequently, a contamination probability of $10^{-6}$ is accepted for the calculation of the required lethality of a sterilization process.

For the sterilization of the fermenter superheated steam is introduced into the fermenter for 30 minutes. Thickness of the wall of the fermenter [26]:

$$d = \frac{p \cdot T}{2 \cdot G} = \frac{3 \cdot 3.25}{2 \cdot 740} \approx 6.6 \text{ mm}$$

To be on the safe side, wall thickness of 1 cm is optioned.

Heat transfer coefficient

$$l/U = \frac{1}{\alpha_1} + \frac{d}{\lambda} + \frac{1}{\alpha_2} \quad (6.15)$$

$$\alpha_1 = \alpha_2 = 10 \text{ W/m}^2 \text{ K}$$

$$\lambda \text{ (for steel)} = 11 \text{ W/mK}$$

$$U \approx 5 \text{ W/m}^2 \text{ K}$$

Energy required for sterilization can now be calculated. This is divided into three parts: (for the main-fermenter)

(i) steam required to fill the fermenter (fermenter at 2 bar, 120°C):

$$M = \text{volume of fermenter} \ast \text{density of steam}$$

$$= 70 \text{ m}^3 \ast 1.122 \text{ kg/m}^3$$

$$\approx 78.5 \text{ kg steam}$$

amount of energy released:

$$Q_{\text{heat}} = M \ast (H_{1,133} - H_{1,120} + c_p,160 \ast \Delta T + \Delta H_{\text{cond},133}) \quad (6.16)$$
Putting the values in equation 6.16, gives the total amount of energy released.

\[ Q_{\text{heat}} = 1.82 \times 10^8 \text{ J} \]

(ii) Heat needed to warm up the fermenter from 25°C to 120°C

\[ Q_{\text{warm up}} = V_{\text{steel}} \times \rho_{\text{steel}} \times c_p \times \Delta T \]  

\[ V_{\text{steel}} = d_{\text{wall}} \times (2\pi rH + 2\pi r^2) \]

\[ = d_{\text{wall}} \times (\pi DH + 0.5\pi D^2) \]

where \( H = 7.3 \text{ m} \)
\( D = 3.5 \text{ m} \)

\[ V_{\text{steel}} = 0.9951 \text{ m}^3 \]

Total heat needed for warming up:

\[ c_p = 486 \text{ J/kgK} \]  [28]
\[ \rho_{\text{steel}} = 7750 \text{ kg/m}^3[28] \]

\[ Q_{\text{warm up}} = 0.9951 \times 7750 \times 486 \times 95 \]

\[ = 3.561 \times 10^8 \text{ J} \]

(iii) Heat lost during sterilization:

\[ Q_{\text{lost}} = U \times A \times \Delta T \times t \]  

\[ A = 99.51 \text{ m}^2 \]
\[ t = 30 \times 60 = 1800 \text{ s} \]
\[ \Delta T = 120 - 25 = 95^\circ \text{C} \]

\[ = 5 \times 99.51 \times 95 \times 1800 \]

\[ = 8.51 \times 10^7 \text{ J} \]

Extra energy needed is:

\[ Q_{\text{extra}} = 3.561 \times 10^8 + 8.51 \times 10^7 - 1.820 \times 10^8 \]

\[ = 2.592 \times 10^8 \text{ J} \]

Therefore, extra steam required is:

\[ M_{\text{extra}} = 2.592 \times 10^8 / 2.33 \times 10^6 \]

\[ \approx 111.23 \text{ kg steam} \]

Total steam required for sterilization is:

\[ M_{\text{total}} = 78.5 + 111.23 \approx 190.00 \text{ kg steam.} \]
Similarly, the energy required to sterilize the pre-fermenter can be calculated.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of fermenter</td>
<td>3.5 m³</td>
</tr>
<tr>
<td>Diameter</td>
<td>1.30 m</td>
</tr>
<tr>
<td>Height</td>
<td>2.64 m</td>
</tr>
<tr>
<td>Thickness of wall</td>
<td>3.5 mm</td>
</tr>
<tr>
<td>Volume of steel</td>
<td>0.0470 m³</td>
</tr>
</tbody>
</table>

Steam required to fill the fermenter = 3.3 kg
Heat liberated due to filling = $7.69 \times 10^6$ J
Heat needed to warm up fermenter (from 25°C to 120°C) = $1.682 \times 10^7$ J
Loss of heat during sterilization = $1.21 \times 10^7$ J
Total amount of steam needed for sterilization ≈ 9.00 kg

Total steam requirement for the sterilization of the two fermenters is:

$190.00 + 9.00 = 199$ kg steam

6.1.2 Medium sterilization

Thermal death kinetics:

Microbial destruction is a probabilistic phenomenon that follows first order kinetics as described by the equation:

$$\frac{dN}{dt} = -kN$$  \hspace{1cm} (6.1)

where $N =$ number of viable cells
$t =$ time in seconds
$k =$ death rate constant (s⁻¹)

Integration of the above equation gives the following relationship:

$$\ln \frac{N}{N_0} = -kt \quad \text{or} \quad N = N_0 \exp(-kt)$$  \hspace{1cm} (6.2)

The effect of temperature on thermal destruction may be described by the Arrhenius relationship:

$$k = k_0 \exp(-E_A/RT)$$  \hspace{1cm} (6.3)

$k_0$ and $E_A$ are characteristic for different types of microorganisms. For the case of media preparation for fermentation or bioconversion, these values are taken for the organism *Bacillus stereothermophilus*. 

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\[ E_A = 287.2 \text{ kJ/mol} \quad \text{and} \quad k_0 = 5.6 \times 10^{38} \text{ min}^{-1} \quad [21] \] 
\[ k_0 = 1.0 \times 10^{37} \text{ sec}^{-1} \]

If the sterilization temperature is 125°C, the value of \( k \) is:
\[
k = 1 \times 10^{37} \times \exp(-287200/(8.314 \times 398.15)) = 0.21 \text{ sec}^{-1}
\]

The number of viable organisms present in tap-water is assumed to be in the order of \( 10^9 / \text{m}^3 \) [22]. Amount of medium to be sterilized is 50 m\(^3\), thus \( N_0 = 50 \times 10^9 \). If the number of organism surviving after sterilization is assumed to be 1/100, then
\[
N/N_0 = 1/100/(50 \times 10^9)
\]
\[
= 2 \times 10^{-13}
\]

\( N/N_0 \) is regarded as the design criterion for sterilization.

Thus, from equation (6.2), the time required for sterilization can be calculated.

\[
\ln (2 \times 10^{-13}) = -0.21 \times t
\]
\[
t = 139 \text{ sec} = 2.3 \text{ min}
\]

This residence time is achieved by allowing the culture medium to flow through a properly insulated pipe. Since the flow characteristics are not ideal, a longer pipe is required than if calculated on the basis of the average residence time.

The influence of axial dispersion on the elimination of microorganisms is illustrated in Appendix 2 [22]. The length of the sterilization pipe is calculated by trial and error method.

Mass flow, \( \dot{m} \), through pipe is 47680 kg/2 hr or 6.900 kg/s.

At 125°C, the density of the medium is 943.8 kg/m\(^3\).

Thus the volumetric flow rate is:
\[
\dot{V} = 6.900 / 943.8
\]
\[
= 0.00731 \text{ m}^3/\text{s}
\]

Diameter of the pipe is assumed to be 0.155 m

Mean velocity of the flow is:
\[
\bar{v} = \dot{V} / A
\]
\[
A = \pi r^2
\]
\[
= 1.887 \times 10^{-2} \text{ m}^2
\]
\[
\bar{v} = 0.387 \text{ m/s}
\]

Reynolds number is:
\[
Re = \bar{v} \cdot p \cdot d / \eta
\]
\[
\eta = 2.19 \times 10^{-4} \text{ Pa.s}
\]
\[
= 2.57 \times 10^5
\]
From appendix 3 [22] the value of \( \frac{D}{(v \cdot d)} \) is found.

\[
\frac{D}{(v \cdot d)} = 0.24
\]

\[
D = 1.3 \times 10^{-2} \text{ m}^2/\text{s}
\]

Peclet number, \( Pe \) is:

\[
Pe = \frac{\bar{v} \cdot L}{D} = (\frac{D}{v \cdot d})^{-1} \cdot \frac{L}{d}
\]

\( = 26.88 \times L \)  

\( Nr = k \cdot L / \bar{v} \)  

From Appendix 2 [22] the optimal value of \( L \) can be found.

\( L = 50 \text{ m} \)

Total length of pipe (including recycle) = 65 m

Diameter of pipe = 0.155 m

Wall thickness of pipe = 2.11 \times 10^{-3} \text{ m}

Total volume of steel in pipe = 6.7 \times 10^{-2} \text{ m}^3

Energy needed to warm up pipe from 25°C to 125°C:

\[
\Phi_H = V_{\text{steel}} \cdot c_{\text{p(steel)}} \cdot \rho_{\text{steel}} \cdot \Delta T
\]

\( c_{\text{p(steel)}} = 486 \text{ J/Kg K} \)

\( \rho_{\text{steel}} = 7750 \text{ kg/m}^3 \) [28]

\( \Phi_H = 2.40 \times 10^7 \text{ J} \)

From here it can be calculated that the amount of steam required for warming up the pipe is 10.3 kg.

Amount of medium to be sterilized:

Total volume = Amount of water + water due to + \( V \) in fermenter + sterilization

\( 50 = 0.2 + V_1 + V_{\text{medium}} \)

\( 49.8 \text{ m}^3 = V_1 + V_{\text{medium}} \)
Thus, 49800 kg of medium has to be sterilized in two hours.

\[ \dot{\varphi}_{\text{out}} = 6.90 \text{ kg/s} \]

By setting up an enthalpy balance over the steam injector, the mass flow of steam required for the sterilization of the medium can be calculated.

\[
\begin{align*}
\dot{\varphi}_{\text{out}} \cdot H_{L,125^\circ C} & = \dot{\varphi}_{\text{steam}} \cdot H_{v,190^\circ C,3\text{atm}} + (\dot{\varphi}_{\text{out}} - \dot{\varphi}_{\text{steam}}) \cdot H_{L,94^\circ C} \\
6.90 \cdot 525 & = \dot{\varphi}_{\text{steam}} \cdot 2856.525 + (6.90 - \dot{\varphi}_{\text{steam}}) \cdot 393.45
\end{align*}
\]

\[ \dot{\varphi}_{\text{steam}} = 0.369 \text{ kg/s} \]

\[ \dot{\varphi}_{\text{in}} = 6.90 - 0.369 = 6.531 \text{ kg/s} \]

Total steam entering the system in two hours:

\[ = 0.369 \cdot 2 \cdot 3600 = 2657 \text{ kg} \]

Thus, considering that two media sterilization start ups per cycle are required, total steam utilised for sterilization is 2678 kg.

Volume of condensed steam \( \approx 2.678 \text{ m}^3 \)

Therefore, volume of medium (mixture of tap water and concentrated salt solution) can be calculated from equation (6.10b):

\[ 49.8 - 2.678 = 47.122 \text{ m}^3 \]

6.1.3 Air sterilization

Filtration of compressed air used in aerobic fermentation has been employed very commonly as the most practical and economical solution for sterilization of air. Filtration devices provide a fair statistical probability of organism retention as long as they are properly maintained and care is taken to control the quality and velocity of the inlet air. The criteria for selecting an air filtration system include the efficacy of organism retention provided by the air filters and a low pressure drop across the filter depth. These properties are well provided by filters made of polyvinyl alcohol (PVA) coated with heat resistant silicon to enable it to withstand repeated steam sterilizations.

PVA filter \([22]\)

For main fermenter

\[ V = \varphi_v \cdot t \] \hspace{1cm} (6.12)
\[ \dot{V} = 593 \text{ m}^3/\text{h}; \quad t = 50 \text{ h} \]

\[ V = 29650 \text{ m}^3. \]

Assuming air contains \( 10^4 \) organisms per \( \text{m}^3 \) [22].

Fraction of organisms filtered:
\[ \eta = 1 - \frac{N}{N_0} \]

\[ N_0 = 2.965 \times 10^4 \times 10^4 \]

\[ N = 1 \]

\[ \eta = 0.999999996 \]

\( \eta \) is regarded as the collection efficiency. At an optimal superficial air velocity of 1 m/s, 0.999995 fraction of organisms are trapped using a PVA filter of thickness of \( 3 \times 10^{-3} \text{ m} \) (Appendix 4). Thickness of filter needed to achieve an efficiency of 0.999999996 is:
\[ d = 3 \left( \log 3.37 \times 10^{-9} \right) / \log(1 - 0.999995) \]

\[ d = 5.788 \text{ mm} \approx 6 \text{ mm}. \]

From Appendix 5 [23], the pressure drop over the filter is determined. The condition for this is that the superficial velocity of air remains 1 m/s. This is possible if the cross sectional area of the filter is 0.113 m\(^3\).

\[ \Delta P = 6.00 \times 1.37 \times 10^3 \]

\[ \approx 0.1 \text{ bar}. \]

The value \( 1.37 \times 10^3 \text{ N/m}^2/\text{mm} \) filter is obtained from the graph in appendix 5. The pressure drop across a filter (for superficial gas velocity of 1 m/s) is given to be 70 cm H\(_2\)O.

\[ 70 \text{ cm H}_2\text{O} = 6864.48 \text{ N/m}^2 \]

For a filter of 5 mm thickness, the pressure drop per mm of the filter is:

\[ 6864.48/5 = 1.37 \times 10^3 \text{ N/m}^2/\text{mm filter.} \]

The power of the compressor can now be calculated assuming the efficiency of the compressor to be 0.7. The pressure drop across the filter present at the exit of the fermenter for the outgoing air is assumed to be 0.1 bar. Pressure drop over the fermenter is 0.5 bar.
Total fall in pressure \( \Delta P_{\text{ferm}} + 2 \cdot \Delta P_{\text{PVA}} \)
\[ = 0.5 + 2 \cdot 0.1 = 0.7 \text{ bar} \]

Adiabatic power of the compressor is calculated using the following formula [24]:

\[
W = \varphi_{\text{air}} \cdot \frac{\kappa}{(\kappa - 1)} \cdot R \cdot T_1 \cdot \frac{X}{\eta}
\]

\[ X = \left[ 1 - \left( \frac{P_2}{P_1} \right)^{\kappa - 1/\kappa} \right] \]

\[ P_1 = 1 \text{ bar} \]
\[ P_2 = 1.7 \text{ bar} \]
\[ \varphi_{\text{air}} = 6.73 \text{ mol/s} \]

\[ = 6.73 \cdot 1.395/0.395 \cdot 8.314 \cdot 0.16203/0.7 \]

\[ W = 13.70 \text{ kW} \]

Due to compression, the temperature of air increases. The adiabatic temperature discharge of air can be calculated [25].

\[ T_2 = T_1 \cdot \left( \frac{P_2}{P_1} \right)^{\kappa - 1/\kappa} \]

\[ T_2 = 73.4 \degree \text{C} \]

The above calculations for the pre-fermenter are completely analogues.

PVA filter for pre-fermenter:

\[ V = \varphi_v \cdot t \]

\[ \varphi_v = 212 \text{ m}^3/\text{hr} \]
\[ t = 20 \text{ hr} \]

\[ V = 4240 \text{ m}^3 \]

Assuming air contains \( 10^4 \) organisms per m\(^3\), the thickness of the filter needed to achieve an efficiency of 0.999999975 is calculated to be 5 mm.

The pressure drop over the filter (for a superficial gas velocity of 1 m/s) is 0.07 bar. For practical purposes the pressure drop is estimated to be 0.1 bar. The cross sectional area of the filter is 0.046 m\(^2\).

The pressure drop across the exit filter is, similar to that in the main fermenter, is assumed to be 0.1 bar. The drop in pressure over the fermenter is 0.2 bar.

\[ N_0 = 4.24 \cdot 10^7 \text{ organisms} \]
Design criteria $= 2.36 \times 10^{-8}$
Filter thickness $= 5$ mm
Pressure fall $= 0.07 \text{ bar} \approx 0.1 \text{ bar}$
Total pressure fall $= 0.2 + 2 \times 0.1 = 0.4 \text{ bar}$
Compression power $= 3.0 \text{ kW}$
Discharge temperature $= 55.1^\circ\text{C}$
6.2 Heat recovery

It is evident that heat recovery in media sterilization can be profitable. However, regarding the particular case of medium sterilization for the PQQ process, it has been calculated that heat recovery is only profitable if there is a steep increase in the present energy prices (see section cost evaluation). On the basis of this conclusion, it is decided to use the sterilization unit for other fermentations as well. Assuming that a fermentation plant has several simultaneously running processes, a sterilization unit fifteen times the capacity of the PQQ plant is considered.

The basis of heat exchanger calculations can be found in [24]. In this case, a heat exchanger with fluids on both sides will be used. Necessary physical parameters are viscosity \( \eta \), density \( \rho \), heat exchange coefficient \( \lambda \) and heat capacity \( c_p \) of both the hot and cold fluid phases. The heat exchange coefficient and viscosity can be approximated with the following equations [27]:

\[
\eta = 10^{-3} \times \exp(0.580 - 2.520 \theta + 0.909 \theta^2 - 0.264 \theta^3) \text{ Pa.s} \quad (6.19)
\]

\[
\lambda = 0.5607 + 0.200 (T / 100) - 0.0795 (T / 100)^2 \text{ W/m K} \quad (6.20)
\]

(\text{this equation is slightly wrong in [27], instead of 0.0795, a value of 0.795 is used})

If \( c_p \) is considered constant for the relevant temperature range, the Prandtl number

\[
Pr = \eta \times c_p / \lambda \quad (6.21)
\]

can also be calculated. Results of these equations for a temperature range of 20 to 100 °C can be found in appendix 6a.

Besides this the heat exchange coefficient \( \lambda_m \) of the metal pipe (steel 304) must be known (from [28]). With these parameters and thereof calculated parameters, the necessary heating surface can be estimated. As several temperature ranges for heat exchange had to be calculated, a computer
program has been developed (BBC basic), based on a flowsheet in [1]. This program with an example of the eventually calculated heat exchanger can be found in appendix 6b.

The heat stream $\Phi_h$ to be exchanged depends on the temperature difference that must be created between in- and outflow of the hot phase, which is the sterilized medium. Thus,

$$\Phi_h = \Phi_{m,w} \times c_p \times (t_{wi} - t_{wo})$$

$t_{wi} = 125 \, ^\circ C$, $t_{wo} = 55 \, ^\circ C$.

$\Phi_h$ must be exchanged with the cold medium flow $\Phi_{m,c}$. Then

$$t_{c,o} = \frac{\Phi_h}{c_p \cdot \Phi_{m,c}} + t_{c,i}$$

with $t_{c,i} = 20 \, ^\circ C$.

$\Phi_{m,c}$ depends on the amount of steam injection following heat exchange.

If low pressure steam of 190 °C and 3 bar (dewpoint 133 °C and not 140 °C, as mentioned in the handout) is used, the amount of steam required to heat cold fluid to 125 °C is calculated as follows:

Total enthalpy of steam at 190 °C $H_{190}^g \approx H_{133}^g + c_{160}^{p,g} \times \Delta T$

then $\Phi_{m,w} \times H_{125}^1 = \Phi_{\text{steam}} \times H_{190}^g + \Phi_{m,c} \times H_t^1$, 

$$\Phi_{\text{steam}} = \Phi_{m,w} - \Phi_{m,c} \text{ and } t = t_{c,o}$$

Equation 6.24 can be rearranged to give $\Phi_{m,c}$ as a function of total enthalpy at temperature $t_{co}$.

$$\Phi_{m,c} = \Phi_{m,w} \times \left( \frac{H_{190}^g - H_{125}^1}{H_{190}^g - H_t^1} \right)$$

Applying trial and error for $\Phi_{m,c}$ and $t_{c,o}$, using equations 6.23 and 6.25 results in a temperature of 94.0 °C and from [30]

$H_{125}^1 = 524.5 \times 10^3 \, J/kg$, $H_{133}^g = 2723 \times 10^3 \, J/kg$, $c_{160}^{p,g} = 2082 \, J/kg \, K$. 

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\[ H_{1}^{94.0} = 339.5 \times 10^{3} \text{ J/kg, } \dot{\phi}_{w} = 6.9 \text{ kg/s} \]

gives \[ \dot{\phi}_{c}^{94.2} = 6.531 \text{ kg/s and } \dot{\phi}_{\text{steam}} = 0.369 \text{ kg/s} \]

The average temperature is 57 °C for the cold, and 90 °C for the hot phase. The physical properties at these temperatures are:

<table>
<thead>
<tr>
<th>Property</th>
<th>Cold Phase</th>
<th>Hot Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \rho )</td>
<td>984.7 kg/m(^3)</td>
<td>965.3 kg/m(^3)</td>
</tr>
<tr>
<td>( \eta )</td>
<td>4.89 \times 10^{-4} \text{ Pa.s}</td>
<td>3.15 \times 10^{-4} \text{ Pa.s}</td>
</tr>
<tr>
<td>( \lambda )</td>
<td>0.649 W/m K</td>
<td>0.676 W/m K</td>
</tr>
<tr>
<td>Pr</td>
<td>3.15</td>
<td>1.95</td>
</tr>
<tr>
<td>( c_{p} )</td>
<td>4191 J/kg K (t = 73 °C)</td>
<td></td>
</tr>
<tr>
<td>( \lambda_{m} )</td>
<td>11 W/m K</td>
<td></td>
</tr>
</tbody>
</table>

The heat exchanger is entirely constructed of steel 304, a material which is strongly resistant to corrosion, and is commonly used (besides steel 316) in fermentation technology. The tubings used have an outer diameter of 2.5 cm and a thickness of 0.25 cm. The heat exchanging area needed to satisfy the input parameters is estimated to be 250 m\(^2\), consisting of two heat exchangers coupled in series. More specific information can be found in the equipment list, and the calculations, mentioned earlier, in appendix 6b.
6.3 Main fermenter

As discussed before in section 4, the assumptions concerning the main fermenter are based on the Ameyama patent application and specific the kinetic parameters from other sources. Although the information is quite rudimentary, it is indeed possible to calculate the initial biomass concentration.

6.3.1 Substrate supplementation

An advantage of fed batch is that there is the possibility to control the biomass growth by substrate limitation. Biomass growth can be decreased by reducing substrate supplementation. Thus, by adding substrate in a certain sequence, the growth curve can be determined. Excretion of PQQ appears to be stimulated as the stationary phase approaches, as suggested in the Ameyama growth curve. Furthermore, initial biomass growth must be exponential, otherwise it is possible that biomass growth may remain insufficient during rest of the fermentation. Thus, fermentation starts in batch with enough substrate available to the organisms.

The period between the exponential and stationary phase can either be a linear or a so called "S" curve. Both possibilities are shown in figure 6.1. It was arbitrarily decided to use the linear growth curve since this characteristic is relatively simpler to describe in form of a model and furthermore, insufficient information was available to exclude either of the curves. Three supplementations regions can be distinguished.

1. a certain amount of substrate is supplied at once. The organisms are allowed to grow exponentially, until practically all substrate is consumed;
2. supplementation is started and gradually increased with time:
   \[ \Phi_s = \Phi_{s,\text{start}} \times (1 + A \times t); \]
3. supplementation is kept constant, which is only sufficient to facilitate maintenance.

At the start of this project it was assumed that 100% recovery of PQQ was possible, and that twohundred production days were needed. Since each run takes three days, 66 runs were performed with the amount of PQQ per run
being 1.515 kg. If the final concentration is 30 mg/l, then 50.5 m³ broth is needed.

Later on, 99% recovery of PQQ seemed more realistic, and an extra run was required. Also, the endvolume is not exactly 50.5 m³. At the end of this section corrected results for the expected endvolume are given.

It is assumed that PQQ production and methanol consumption are linearly related, if considered for complete fermentation. Therefore,

\[
1/2.61 \times 10^{-3} \times 1.515 = 580.5 \text{ kg}
\]

methanol is needed to produce 100 kg of PQQ. This is equal to 18.12 kmole methanol. Methanol consumption must be split among the exponential, linear and stationary growth phases. Consumption can be expressed by the following equations. Indices are explained in figure 6.2. This figure represents the relation between time, growth phase and biomass concentration.
exponential phase: \( r_x = \mu_{\text{max}} \times C_x \)

\[ r_s = (1 / Y_{sx} \times \mu_{\text{max}} + m_s) \times C_x \]  

(4.1a)

linear phase: \( r_x = \) constant

\[ t = t_1, \ C(t) = C_{x,1}; \ t = t_2, \ C(t) = C_{x,2} = 11 \times C_{x,0} \Rightarrow \]

\[ r_x = \frac{dc(t)}{dt} = \Delta C_x / \Delta t = (C_{x,2} - C_{x,1}) / (t_2 - t_1) \Rightarrow \]

\[ r_s = 1 / Y_{sx} \times (C_{x,2} - C_{x,1}) / (t_2 - t_1) + m_s \times C_x \]  

(6.26)

stationary phase: \( r_x = 0 \Rightarrow \)

\[ r_s = m_s \times C_{x,2} \]  

(6.27)

Integration of the above equations (appendix 7) and iteration of them for total methanol consumption over the three regions results in a value for \( C_{x,0} \). The time durations finally chosen for exponential, linear and stationary growth phases are respectively 2, 40 and 8 hours. For these time durations an initial biomass concentration of 10.9 mole/m³ is calculated (appendix 8).

It is important to emphasize that, although the different optimization parameters (biomass concentration, oxygen consumption etc.) are treated in a certain sequence, the solution for them can only be found in an iterative way, i.e. by using algorithms. Therefore, a program has been developed to perform this optimization. This program is inserted in appendix 8.
6.3.2 Oxygen uptake and consumption, process times

Although it is relatively easy to calculate the biomass concentration, using boundary conditions as mentioned in section 4.2, the realization of this concentration depends on the possibility to supply enough oxygen into the broth.

In aerobic fermentation systems, oxygen transfer from the gas phase to the surface of the microbial cell is of primary importance. Neglecting the diffusion limitations through air and the biomembrane, oxygen transport depends upon the diffusion limitation at the gas-liquid boundary \( k_l \) and the available exchange area \( a \).

Under conditions where oxygen consumption exceeds oxygen uptake, growth limitation due to oxygen occurs, which consequently leads to die off of the biomass and decrease in the production of PQQ. The oxygen consumption rate can be described by the following equation [19]:

\[
\frac{r_{O_2}}{r_{x}} = \frac{1}{Y_{ox}} \times \frac{r_{x}}{r_{x}} + \frac{m_{ox}}{m_{s}} \times C_{O_2}^{\infty}
\]

with \( Y_{ox} = 4 \times Y_{sx} / (y_{s}^1 - y_{x}^1 \times Y_{sx}) \) and \( m_{o} = \frac{1}{4} \times m_{s} \times y_{s}^1 \)

The generalized degrees of reduction are, in case of growth on ammonia:

\[
y_{s}^1 = y_{CH_40}^1 = 4 \times 1 + 1 \times 4 + -2 \times 1 = 6
\]

\[
y_{x}^1 = y_{CH_4.80.5N_0.2}^1 = 4 + 1.8 + -1 + -3 \times 0.2 = 4.2
\]

Maximal oxygen consumption takes place at the final stage of exponential growth, in case of main fermentation. Then:

\[
\frac{r_{O_2}}{r_{x}} = \frac{1}{Y_{ox}} \times \mu_{max} \times C_{x_1} + m_{s} \times C_{x_1}
\]

On the other hand, oxygen uptake is expressed by:

\[
\Phi_{O_2} = k_{1}a \times (C_{O_2}^{\infty} - C_{O_2}) =
\]

\[
k_{1}a = \frac{\Phi_{O_2}}{(C_{O_2}^{\infty} - C_{O_2})}
\]

where \( C_{O_2}^{\infty} \) is the saturated oxygen concentration in the liquid at 1 atm pressure. It is assumed that the oxygen solubility in pure water can be used. This assumption is supported by the positive effects on oxygen
solubility caused by the higher pressure in the fermenter, and by some salt ions in the medium [33].

Several correlations are described in literature for \( k_{1a} \) (volumetric mass transfer coefficient) of coalescing bubbles in stirred vessels, namely:

\[
k_{1a}^0 = 2.6 \times 10^{-2} \times (\frac{P_g}{V_1})^{0.4} \times v_s^{0.5} \quad (s^{-1}) \quad [32] \tag{6.30}
\]

for \( V_1 \leq 2.6 \text{ m}^3 \), \( v_s \leq 0.04 \text{ m/s} \) and \( 500 < \frac{P_g}{V_1} < 10000 \text{ W/m}^3 \)

and

\[
k_{1a}^0 = 2.7 \times 10^{-2} \times (\frac{P_g}{V_1})^{0.43} \times v_s^{0.57} \quad (s^{-1}) \quad [33, 34] \tag{6.31}
\]

for \( V_1 \leq 900 \text{ m}^3 \), \( v_s \leq 0.018 \text{ m/s} \) and \( 10 < \frac{P_g}{V_1} < 16700 \text{ W/m}^3 \)

A disadvantage of both the above equations is that any correlation with pressure is not taken into account, the pressure deviating quite distinctively with height in larger vessels. Both \( k_{1a} \) correlations predict values that deviate 20 to 40% from the measured ones. Expression 6.31 is used to calculate the gassed stirring power value required to satisfy \( k_{1a} \) in the main fermenter:

\[
\frac{P_g}{V_1} = \left(\frac{k_{1a}^0}{2.7 \times 10^{-2} \times v_s^{-0.57}}\right)^{2.33}
\]

\( \frac{P_g}{V_1} = \) \( \left(\frac{k_{1a}^0}{2.7 \times 10^{-2} \times v_s^{-0.57}}\right)^{2.33} \quad [32] \) \( \tag{6.32} \)

The broth temperature is 30 °C, which requires a correction of \( k_{1a} \) (as calculated from equation 6.29) for temperature:

\[
k_{1a} = k_{1a} \times 1.022^{(20-t)}
\]

\( k_{1a} = k_{1a} \times 1.022^{(20-t)} \quad [33, 34] \) \( \tag{6.33} \)

The maximum \( k_{1a} \) required must be calculated, \( k_{1a} \) depending on the maximum oxygen consumption that occurs. \( \Phi_{O_2} \) then must be related to \( r_{O_2} \). As mentioned earlier, \( k_{1a} \) prediction with equation 6.31 has a high deviation. The same applies to \( \frac{P_g}{V_1} \), which is calculated from a rearranged form of equation 6.31, namely 6.32. This deviation must be considered for further calculations.

\( k_{1a} \) of 6.29 has the dimension \( s^{-1} \), so \( 1/k_{1a} \) can be regarded as a time constant for oxygen uptake, called \( r_{O_2, u} \). The same can be applied to oxygen consumption, expressed by:

\[
\tau_{O_2, c} = \frac{C_{O_2, 1}}{r_{O_2}}
\]

\( \tau_{O_2, c} = \frac{C_{O_2, 1}}{r_{O_2}} \)

It is decided that \( \tau_{O_2, u} \) must be at least half of \( \tau_{O_2, c} \). If \( \Phi_{O_2} \) is equal to \( r_{O_2} \), then:
Thus, for maximum oxygen consumption $k_{la}$ is equal to:

$$k_{la}^{\text{max}} = \frac{r_{O_2}^{\text{max}}}{(\frac{1}{2} C_{O_2,1})} \quad (6.29a)$$

$C_{O_2,1}$ depends on the oxygen concentration in the gas phase. Air contains about 21 mole% oxygen. If $\Phi_2$ takes a fraction $x_{O_2,g}$ from the total amount of oxygen available in air, $\Phi_{air,in}$ is approximated by:

$$\Phi_{air,in} = \frac{\Phi_2}{x_{O_2,g}} \times 100 / 21 \quad (6.34)$$

and the average oxygen percentage by (appendix 9):

$$% \bar{O}_2 = 21 \times (1 - \frac{1}{2} x_{O_2,g}) \quad (6.35)$$

Average saturated oxygen concentration in liquid is calculated by:

$$C_{O_2,1}^{*} = \frac{\% \bar{O}_2}{21} \times C_{O_2,1,\text{air,1 atm}}$$

The mole fraction of oxygen in water $x_{O_2,1}$ is related to partial pressure $p_{O_2}$ by:

$$p_{O_2} = x_{O_2,1} \times H \quad (6.36)$$

where $H$ is the Henry coefficient for oxygen in water, being equal to 4.75×10^{-4}. The partial pressure $p_{O_2}$ is expressed in atm [25]. If total pressure is 1 atm, density of water is approximately 1000 kg/m³ and $p_{O_2}$ 0.21 atm, then:

$$C_{O_2,1,\text{air,1 atm}}^{*} = 1000 / M_{H_2O} \times 0.21 / 4.75 \times 10^{-4} \approx 2.45 \times 10^{-6} \text{ mole/l}$$

If pressure is taken as 1 bar, then the value is 0.242 mole/m³.

Besides oxygen, substrate too has a certain process time for consumption, $\tau_{s}$, which is expressed by:

$$\tau_{s} = C_{s} / r_{s}^{\text{max}}$$
The substrate concentration is generally very low, and estimated to be 0.01 g/l or 0.312 mole/m$^3$. $r_{\text{max}}^s$ is achieved at the end of exponential or linear growth. To avoid a situation in which either oxygen or substrate depletion occurs in parts of the fermenter, mixing time must be shorter than both of the other process times in the fermenter.

Mixing time is a function of the vessel geometry $H/T$, stirrer diameter $D$, stirring number $N$, Powernumber $N_p$ and stirrer height $H_r$ [33]:

$$t_m = 0.6 / N \times (T / D)^3 \times (H / T) / \{N_p \times (H_r / D)^2 \}^{\frac{1}{3}}$$

$$N = 0.6 / t_m \times (T / D)^3 \times (H / T) / \{N_p \times (H_r / D)^2 \}^{\frac{1}{3}}$$

(6.37)

Powernumber $N_p$ is a function of the stirrer type in use and of the Reynolds number Re. If Re is higher than 5000, $N_p$ remains constant. $N_p$ has a value of six, in the case of one Rushton stirrer, and has a value of twelve for two stirrers on one shaft. A requisite to use the latter is that stirrers are properly mounted in the fermenter, about a distance $D$ from the bottom and with a spacing of $D$ to 2 $D$ between the stirrers.

The mixing time must be corrected for aeration, where power consumption is less for a certain stirrer number:

$$t_{m,g} = t_m / P_{g/\Phi_o}$$

(6.38)

$P_{g/\Phi_o}$ is estimated from figure 6.3 [36], using aeration number $N_q$:

$$N_q = \Phi_{v,air,in} / N / D^3$$

**Figure 6.3: Power aeration curves**

![Figure 6.3: Power aeration curves](image-url)
Once oxygen and substrate process times are estimated, it is possible to calculate \( N \) for the necessary \( t_{m,g} \), using equations 6.37 and 6.38. \( N \) is also correlated to the power consumption \( P_g \) by:

\[
P_g = \frac{P_g}{P_o} \times N_p \times \rho \times N^3 \times D^5
\]  

(6.39)

\( P_g \) can be calculated as a function of increasing stirrer diameters for the same \( t_{m,g} \). The result obtained from equation 6.39, divided by liquid volume \( V_l \), can be compared with the result from equation 6.32.

The above outline has been used to develop a program (appendix 8), with the help of which several parameters can be calculated. Using this program it is calculated that:

\[
\begin{align*}
C_{x,0} &= 10.9 \text{ mole/m}^3 \\
\dot{\phi}_{25,\text{humid}} &= 593 \text{ m}^3/\text{h} \\
S_{\text{V,air in},1} &= 0.1 \text{ atm} \\
P_g &= 78.8 \text{ kW} \\
N &= 98 \text{ rpm} \\
D &= 1.20 \text{ m} \\
\tau_s &= 40.2 \text{ s} \\
\tau_{O_2,c} &= 26.8 \text{ s} \\
\tau_{m,g} &= 25 \text{ s} \\
T &= 3.50 \text{ m} \\
H_l &= 1.5 \times T = 5.25 \text{ m}
\end{align*}
\]

The fermenter is filled to a fraction of 0.7 of its total volume, to allow gas hold-up and foam formation. The total fermenter volume is 70 m\(^3\), and its height is 7.28 m. In this case one methanol supply tube is sufficient, which is located near one of the stirrers in the fermenter.

6.3.3 Heat exchange

Heat is produced and exchanged with the surroundings during fermentation. Following heat sources are relevant to the considered systems, the main and pre fermentation [33]:

1. heat produced due to stirring

\[
P_g = \frac{P_g}{P_o} \times N_p \times \rho \times N^3 \times D^5 = Q_s
\]  

(6.39)

2. heat produced due to oxygen consumption [19]

\[
Q_{O_2} = 460 \times r_{O_2} \times V_l
\]  

(6.40)

3. heat from hot air injection [19]

\[
Q_a = \dot{\phi}_{m,air} \times c_{p,air} \times \Delta T
\]

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This heat must be exchanged with the surroundings. There are three relevant ways for heat removal:

4. heat loss through walls to air

\[ Q_w = -U_{wall} \times A_{wall} \times (t_{ferm} - t_{surr}) \]  \hspace{1cm} (6.42)

5. heat loss due to water evaporation

\[ Q_{vap} = -\Delta H_{vap,H_2O} \times \dot{m}_{vap,H_2O} \]  \hspace{1cm} (6.43)

It is possible to calculate \( \dot{m}_{vap,H_2O} \), if outflowing air is assumed to be saturated with water vapor (appendix 10).

6. heat loss due to exchange with cooling water flowing through an annulus or coiled tubes. It is decided to use an annulus, if the available exchange area is sufficient at the inner site of the fermenter. Suitable exchange area exists of a part at the inner wall, which has liquid contact with the broth. Then [33,37]:

\[ Q_{he} = -U \times A \times \Delta T_{ln} \]  \hspace{1cm} (6.44)

\[ = -\dot{m}_{cool} \times c_{p,cool} \times (t_{cool,out} - t_{cool,in}) \]  \hspace{1cm} (6.44a)

and \( U = 1 / (1 / \alpha_{medium} + d_{wall} / \lambda_{wall} + 1 / \alpha_{annulus}) \)

with \( \alpha_{medium} = \lambda_{med} / T \times 0.6 \times \left[ N \times D^2 \right]^{0.3} \times \left( c \times \eta / \lambda_{med} \right)^{0.3} \)  \hspace{1cm} (6.45)

and \( \alpha_{annulus} = Nu \times \lambda / d_h \)

\[ Nu = \left[ Nu^\infty \times (1 + 0.14 \frac{d_i}{d_h}) \times 0.19 \times \left( \frac{Re \times Pr \times d_h}{l} \right)^{0.8} \right] \left[ 1 + 0.117 \times \frac{Re \times Pr \times d_h}{l} \right]^{0.467} \times \left( \frac{Pr}{Pr_w} \right)^{0.11} \]

\[ d_h = d_a - d_i \]

Figure 6.4

if \( Re = \rho \times v \times d_h / \eta < 2300, \) \( Pr = 0.1 \) to 1000, \( d_i / d_a = 0 \) to 1 and \( d_h = d_a - d_i \) = hydraulic diameter (figure 6.4).
If Re > 2300 then

\[ \text{Nu} = 0.86 \times \left( \frac{d_a}{d_i} \right)^{0.16} \times \text{Nu}_{\text{tube}} \]  

(6.47)

\text{Nu}_{\text{tube}} \text{ is given in Gb 4 of [37]. Reynolds is expressed as a function of massflow and hydraulic diameter:}

\[ v = \frac{\dot{\phi}}{A} = \frac{\dot{\phi}}{(4 \times \pi \times \left[ \left( \frac{d_i}{2} \right)^2 - \frac{d_h^2}{4} \right])} \]

\[ \Rightarrow \text{Re} = 4 \times \rho \times \frac{\dot{\phi}}{A} \times \frac{d_h^2}{(\pi \times d_h \times (d_h + 2 \times d_i) \times \eta)} \]

Equation 6.44 is rearranged in the following form:

\[ U \times \Delta T_{\text{ln}} = \frac{Q_{\text{he}}}{A} \]  

(6.44b)

If \( Q_{\text{he}} \) and \( A \) are known, the product of \( U \) and \( \Delta T_{\text{ln}} \) must be equal or greater than the result of the right hand side of 6.44b. The cooling capacity is sufficient in that case. \( \Delta T_{\text{ln}} \) and \( \phi_{\text{w,cool}} \) can be calculated, using the outflowing cooling water temperature as a variable. Subsequently, Re and Nu are calculated, which eventually results in a value for \( \alpha_{\text{annulus}} \) and \( U \) (appendix 11, example for main fermenter).

The exchange area depends on the amount of gas hold-up in the liquid. The gas fraction \( \varepsilon_g \) is estimated by [33]:

\[ \varepsilon_g = 0.13 \times \left[ \frac{P_g}{\rho_g} \right]^{\frac{1}{3}} \times \frac{\dot{\phi}_v}{\dot{\phi}_s} \]  

(6.48)

Thus, total volume is \( V_1/[1 - \varepsilon_g] \) and exchange area is:

\[ A = 1 \times \pi \times d_i = V_1 \times 4 \times d_i / \left[ \left( 1 - \varepsilon_g \right) \times T^2 \right] \]  

(6.49)

The wall thickness of the main fermenter is 0.01 m, and the space between inner and outer wall 0.01 m, thus \( d_i \) is 3.52 m, \( d_h \) is 0.02 m and \( d_a \) is 3.54 m. The results for the main fermenter are (appendix 12):

\[ Q_s = 78.8 \text{ kW} \]
\[ Q_a = 8.3 \text{ kW} \]
\[ Q_{\text{vap}} = -4.9 \text{ kW} \]
\[ Q_{\text{he}} = -208.3 \text{ kW} \]
\[ Q_{\text{w}} = -1.1 \text{ kW} \]
\[ \varepsilon_g = 0.1 \quad A = 63.9 \, \text{m}^2 \]
\[ \text{abs} \left( \frac{Q}{A} \right) = 3260 \, \text{kW} \]

Results for the annulus are:

\[ \Phi_{m,\text{cool}} = 18.5 \, \text{kg/s} \quad \bar{v} = 0.166 \, \text{m/s} \]
\[ t_{\text{out,cool}} = 22.7 \, ^\circ\text{C} \quad \alpha_{\text{annulus}} = 712 \, \text{W/m}^2/\text{K} \]
\[ \Delta P = 0.5 \, \text{bar (primarily static pressure)} \]

### 6.3.4 Water and methanol loss due to evaporation

It is assumed that air has ideal gas behavior under fermentation conditions. Inflowing air has a temperature of 25 °C, a humidity of 70% and a pressure of 1 atm. The mole flow of dry air is calculated from equation 6.34. The actually incoming air flow is somewhat greater, because of water vapor present in the air. Outflowing air has a humidity of 100%, a temperature of 30 °C and a pressure of 1.1 atm. Subtraction of the outflowing and incoming mole flow of water vapor results in the water loss per unit of time. Calculations are given in appendix 10. The water loss for the main fermenter is 407.3 mole/hr or 7.34 kg/hr, which is equivalent to 367 kg for a period of fifty hours.

It is quite reasonable to expect a considerable loss of methanol by evaporation during fermentation, due to its volatility. The maximum amount of methanol present in the liquid occurs at the beginning of fermentation, where 1500 moles or 48 kg of methanol is added to 50 m³ of medium, which is equivalent to a concentration of 0.1 % w/v. Assuming that during two hours the methanol amount remains 48 kg (which is not the case), for instance by regular addition of methanol, the total loss estimates 50 moles (appendix 13). The methanol concentration is much lower during the following 48 hours, namely 0.001 % w/v. Thus, the total loss per run is about 50 moles on a total of more than 18000 moles, which can safely be assumed to be negligible.

### 6.3.5 Total mass change during fermentation

Considering the fermenter as a black box, the following relevant flows are entering or leaving the system:
entering: methanol
    oxygen (for respiration)

leaving: water (in air)
    carbondioxide (from respiration)

It is assumed that total air flow is constant, thus oxygen consumption and carbondioxide production are equal. Although in reality the total air flow is not constant (for maximal oxygen consumption the difference between in- and outflow is about 550 moles/hour, see appendix 14), it serves as a good approximation to calculate the water loss (as shown in 6.3.4). The following totals are calculated (appendix 10), if accumulated for 50 hours:

<table>
<thead>
<tr>
<th></th>
<th>kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>oxygen</td>
<td>+687</td>
</tr>
<tr>
<td>methanol</td>
<td>+581</td>
</tr>
<tr>
<td>water</td>
<td>-367</td>
</tr>
<tr>
<td>carbondioxide</td>
<td>-557</td>
</tr>
<tr>
<td>total</td>
<td>+344</td>
</tr>
</tbody>
</table>

If $p$ is $1000 \text{ kg/m}^3$, then $V_{1,\text{end}}$ is $50.34 \text{ m}^3$, and the total amount of PQQ produced per run is $1.51 \text{ kg}$. This total becomes $1.50 \text{ kg}$ after down stream processing with 99% recovery.
6.4 Pre-culture fermenter

6.4.1 Fermentation criteria and process parameters

The aim of pre-culture fermentation is to produce sufficient biomass for the initiation of the main fermentation. Process optimization is based on efficient methanol use for biomass production. Therefore, a stationary phase must be prevented. Using 2.5 m$^3$ pre-fermentation medium, the final biomass concentration must be 218.2 mole/m$^3$ or 0.5 % dryweight/volume if an initial concentration of 10.9 mole/m$^3$ is required in the main fermenter. Because of the low dry weight content at the end of pre-fermentation, it is possible to achieve a twenty fold increase of biomass.

Both exponential and linear growth are used in the pre fermentation, total fermentation time being 20 hours. Theoretically, most efficient methanol use is achieved when only linear growth is used (results from simulations with modified program of main fermentation, appendix 15), but this is only possible if the organisms are not inhibited by substrate limitation in their initial phase. To be on the safe side, exponential growth is used during the first period of fermentation. The criterium to switch from exponential to linear growth is that oxygen consumption at the end of both exponential and linear growth must be equal. This criterium actually means that the calculated air injection and stirring power are required at two points during fermentation. The main fermentation only requires the upper limits for stirring and aeration at one time.

Applying the mentioned criterium, an exponential growth duration of 2.7 hours is calculated. The total amount of methanol consumed is 34 kg per run. The oxygen depletion of the inflowing air must be lower (4%) than in the main fermentation, as otherwise $k_{l\text{max}}^m$ is not achieved unless extremely high stirring powers are used. Stirring is realized with two Rushton stirrers on one shaft, the same stirrer type and number as used in the main fermenter. Process parameters are calculated to be:

- $\dot{V}_{\text{air, in}}^{25, \text{ humid}} = 212 \text{ m}^3/\text{hr}$
- $P_g = 2.05 \text{ kW}$
- $N = 309.4 \text{ rpm}$
- $D = 0.55 \text{ m}$
- $T = 1.29 \text{ m}$
- $\tau_s = 33.2 \text{ s}$
- $\tau_{O_2,c} = 20.4 \text{ s}$
- $\tau_{m,g} = 4.9 \text{ s}$
- $H_1 = 1.93 \text{ m}$
6.4.2 Heat exchange

The heat flows are similar to the ones of the main fermentation (appendix 12).

1. \( Q_s = \dot{P}_{\text{g}} = 2.05 \text{ kW} \)
2. \( Q_{O_2} = 9.08 \text{ kW} \)
3. \( Q_a = 1.70 \text{ kW} \)
4. \( Q_w = -0.18 \text{ kW} \)
5. \( Q_{\text{vap}} = -1.76 \text{ kW} \)
6. \( Q_{\text{he}} = -10.89 \text{ kW} \)

\[ \varepsilon_g = 0.154 \]
\[ l = 2.28 \text{ m} \]
\[ A_{\text{he}} = 9.25 \text{ m}^2 \]
\[ \text{abs} \left( \frac{Q_{\text{he}}}{A_{\text{he}}} \right) = 1178 \text{ W/m}^2 \]
\[ \dot{\Phi}_{\text{m,cool}} = 0.39 \text{ kg/s} \]
\[ t_{\text{out,cool}} = 26.6 ^\circ \text{C} \]
\[ \alpha_{\text{annulus}} = 212 \text{ W/m}^2/\text{K} \]

6.4.3 Mass change during fermentation

The mass flows considered are the same as mentioned in 6.3.4, and calculations are identical (appendix 10). The results accumulated for 20 hours are:

- oxygen : + 33.6 kg
- methanol : + 34.0 kg
- water : - 52.4 kg
- carbondioxide : - 24.0 kg +
- total : - 8.8 kg
6.5 Processing

6.5.1 Centrifugation

The most common type of centrifuge utilized to separate the biomass from the culture medium is the disk bowl centrifuge. Feed is admitted to the centre of the bowl near its floor and rises through a stack of sheet-metal disks spaced 0.4 to 3 mm apart. The purpose of the disk is primarily to reduce the sedimentation distance, since a solid particle must travel only a short distance before it reaches the underside of one of the disks. Once there, it is in effect removed from the liquid and due to centrifugal force, it continues to move outwards until it is deposited on the wall of the bowl.

To process a volumetric flow of 25 m$^3$/h, two centrifuges are required ($\varphi_v = 0.00347$ m$^3$/s); the dimensions of the centrifuge should be as follows [25]:

- Bowl diameter $D = 24$ in (609.6 mm)
- Rotation $N = 4240$ rpm ($\omega = 444$ rad/s)
- Centrifugal force $= 5500$ g
- Number of disks $= 144$
- Throughput capacity $= 4.5 - 45$ m$^3$/h

Applying Stokes Law, the sedimentation velocity of the particle under the influence of gravity can be calculated.

$$v_g = (\rho_p - \rho_f) \times \frac{d^2}{\rho_p} \times g / 18 \eta$$

(6.24)

If the diameter of the particle (bacteria) is $1.5 \times 10^{-6}$ m, the sedimentation velocity is $1.23 \times 10^{-7}$ m/s

The acceleration factor, $\zeta$, can now be calculated:

$$\zeta = \omega^2 r / g = 6129$$

The Sigma ($\Sigma$) value of the disk centrifuge is estimated using the following relation:

$$\Sigma = \zeta \times A$$

(6.25)

where $A = \text{area equivalent of the rotor (m}^2\text{)}$

By using the relation, $\varphi_v = v_g \cdot \xi \cdot A$, where $\varphi_v$ is the volumetric flow rate, the value of $A$ is determined.

Putting in the values gives $\Sigma = 2.83 \times 10^4$ m$^2$. 

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By keeping the ratio of $\varphi_v/\Sigma$ constant, the experimental results obtained on a smaller scale can be extrapolated to that of the practical requirements.

Using the following relation, the power consumption per centrifuge is estimated:

$$ P = \varphi_v \times \omega^2 \times r^2 \times \rho $$

$$ P = 63.5 \text{ kW} $$

6.5.2 Ion exchanger [29]

An ion exchanger is required for the isolation of PQQ from the supernatant of the culture medium. Due to its high adsorption capacity for PQQ, Amberlyst A21 has been extensively used for large scale extraction. Amberlyst A21 is a weakly basic macroporous anion exchanger with the following properties:

- Active group $= -N(CH_3)_2$
- Exchange capacity $= 1.6 \text{ eq/l ion exchanger}$
- Porosity $\varepsilon = 0.48$
- Particle size $d_p = 0.48 \text{ mm}$
- Density $= 0.65 \text{ gm/ml}$

The ion exchanger is slowly stirred along with the supernatant. PQQ binds to the ion exchanger which is separated from the supernatant with the help of a sieve. The ion exchanger is manually poured into a column filled with water. Water is then allowed to flow out resulting in a formation of a neatly packed bed.

If a superficial fluid velocity of 5 m/h is accepted and the ratio $H/D = 2.1$, from here it follows that the diameter of the column $D = 0.33 \text{ m}$ and the height $H = 0.7 \text{ m}$.

PQQ is then subsequently eluted from the ion exchanger with a solution of 1N NaCl in methanol water (1:1 v/v). Considering that about four bed volumes of eluent are required, it can be calculated that the 114 litres of the solution contains 60 kg of water and 47.4 kg of methanol [30]. 3.51 kg of sodium chloride is added (it is assumed that the salt dissolves only in water and not in methanol) to form a solution of 1N. During elution 13.56 eq of NaCl binds to PQQ; this amount is equal to 0.79 kg. The ion exchanger is recycled to be used again. Time required for elution is about 35 minutes. The Kozeny equation is used in calculating the pressure drop through the column:
\[ v = \frac{1 \times \varepsilon^3 \times 1 \times AP}{K \times S^2 (1-\varepsilon)^2 \eta \times H} \]  \hspace{1cm} (6.26)

\( H = \text{column height} = 0.7 \text{ m} \)
\( \rho = \text{fluid density} = 1000 \text{ kg/m}^3 \)
\( \eta = \text{fluid viscosity} \approx 1 \times 10^{-3} \text{ Ns/m}^2 \)

\( K'' = \text{Kozeny constant} = 5 \)

\[ S = \text{specific area of the particle} = \frac{\pi \times dp^2}{6} = \frac{\pi \times dp^3}{6} dp \]

\[ = 12500 \text{ m}^{-1} \]

Inserting the values in the eqn. 6.26 gives a pressure drop of 0.02 bar which can assumed to be negligible.

The flow containing PQQ is brought into a crystallization tank in which regulated amounts of 6M HCl is added to reduce the pH to 2.0 in order to protonate PQQ. For this acidification 2.36 litres of 6M HCl is required. During the process 0.79 kg of NaCl is regenerated and 2.077 kg of water is left behind containing 0.0219 kg of HCl. The protonated form of PQQ is separated after crystallization from the solution with the help of a sieve. At this point PQQ contains a small amount of water and methanol. It is assumed that 0.3 kg of water binds to PQQ (1/5th of the weight of PQQ) and 0.23 kg of methanol (this is the amount present in 0.3 kg of water).

The acidic solution is neutralized with 6M NaOH forming NaCl and water. In this reaction 0.0351 kg of NaCl and 0.0108 kg of water are formed. 0.098 kg of water is present in the NaOH solution.

On setting up a mass balance it can be seen that 1.9 kg of extra water has entered the system leading to a total of 61.9 kg containing 47.1 kg methanol and 3.5 kg NaCl. A part of this flow has to be purged to come to the exact value of 60 kg water. Therefore, 1.9 kg of water plus 0.1 kg salt and 1.44 kg methanol is purged. The remaining solution is recycled to eluting agent storage tank, V 15. To get the original concentration of salt and methanol, 0.09 kg of salt and 1.7 kg of methanol has to be added into the tank. The exact calculations for the above values are given in appendix 18.
This section deals with design aspects that have not yet been discussed earlier, but do require some attention.

In the design, a number of pumps have been used. Majority of these pumps are of centrifugal type. However, some pumps are of different characteristic. Fine suspensions, such as suspension containing PQQ crystals and broth, are not resistant against high shear stresses that are produced by the rotor of the centrifugal pump. For these special cases, membrane pumps are ideal. The pumping chambers of the membrane pumps are hermetically sealed off and hence can be used for transferring sterile fluids as the probability of contamination is quite low. Furthermore, methanol pumping is also achieved with a membrane pump as explosion risk is considerably lower as compared to the centrifugal pumps. For situations where no special conditions are imposed, centrifugal pumps are preferably employed as they are relatively inexpensive and are quite reliable.

Behavior of PQQ crystals is not exactly known and thus it is difficult to assume the nature of the filters required. In any case pressure filters are better than vacuum filters due to the low boiling temperature and pressure of methanol. If the crystals of PQQ are small, a paper filter or fine glass filter can be used.
(7) Mass- and heat balances.
<table>
<thead>
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<th>Voorwaarts</th>
<th>Retour</th>
<th>UIT</th>
</tr>
</thead>
<tbody>
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<td>Q</td>
<td>M</td>
<td>Q</td>
</tr>
<tr>
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<td>1053.7</td>
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<td>6.529</td>
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</tr>
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<tr>
<td>547.1</td>
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</tr>
<tr>
<td>6.900</td>
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**Massa-en Warmtebalans**

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**TOTAL**

6.900 1600.8
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<td>Q</td>
<td>M</td>
<td>Q</td>
<td>M</td>
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<td>Q</td>
<td>M</td>
<td>Q</td>
<td>M</td>
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M in kg/s
Q in kW

Stroom/Componenten staat
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<th>14 Q</th>
<th>15 M</th>
<th>16 Q</th>
<th>18 M</th>
<th>18 Q</th>
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<tbody>
<tr>
<td>sterile medium</td>
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<td>2.043</td>
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<tr>
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<th>22 M</th>
<th>23 Q</th>
<th>24 M</th>
<th>24 Q</th>
<th>26 M</th>
<th>26 Q</th>
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<td></td>
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<td></td>
<td>0.0226</td>
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<td>water</td>
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<td>0.0286</td>
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<tr>
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<td>0.021</td>
<td>877.9</td>
<td>6.971</td>
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<td></td>
<td>0.021</td>
<td>877.9</td>
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<tr>
<td><strong>Totaal:</strong></td>
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M in kg/s
Q in kW

Stroom/Componenten staat
<table>
<thead>
<tr>
<th></th>
<th>IN</th>
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<th>Retour</th>
<th>UIT</th>
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<td>Q</td>
<td>M</td>
<td>Q</td>
</tr>
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<tr>
<td></td>
<td>M</td>
<td>Q</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

M≥Q,

**BROTH**

**Biomass**

**M2Q**

**PQG + WATER**

**TOTAL**

6.992 877.9
<table>
<thead>
<tr>
<th>Componenten</th>
<th>27</th>
<th>28</th>
<th>30</th>
<th>31</th>
<th>32</th>
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</thead>
<tbody>
<tr>
<td>M in kg/s</td>
<td>M</td>
<td>Q</td>
<td>M</td>
<td>Q</td>
<td>M</td>
</tr>
<tr>
<td>PQQ</td>
<td>0.0002</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Water + salt</td>
<td>6.889</td>
<td>865.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
<td>0.0343</td>
<td>-</td>
<td>0.0344</td>
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<tr>
<td>Methanol</td>
<td>0.0263</td>
<td></td>
<td>0.0263</td>
<td>-</td>
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<td>NaCl</td>
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<td>0.0116</td>
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</tr>
<tr>
<td>Sterile medium</td>
<td></td>
<td></td>
<td></td>
<td>6.900</td>
<td>1600.8</td>
</tr>
<tr>
<td>Biomass (wet wt.)</td>
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<td></td>
<td></td>
<td></td>
<td>0.103</td>
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</tbody>
</table>

Totaal: 6.889 865.0 0.0722 - 0.0723 - 6.900 1600.8 0.103 12.9

M in kg/s
Q in kW

Stroom/Componenten staat
Mass Balances for Pre- and Mainfermenter

Mass changes have already been discussed before in section 6.3 and 6.4. However, only water has not yet been considered. It is quite evident from the overall mass balances that water is either produced or consumed during the course of fermentation. This is reflected in the following table:

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Main fermentation</th>
<th>Pre-fermentation</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>consumed (kg)</td>
<td>produced (kg)</td>
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</tr>
<tr>
<td>Methanol</td>
<td>580.5</td>
<td>-</td>
<td>34.0</td>
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<tr>
<td>Oxygen</td>
<td>686.5</td>
<td>-</td>
<td>33.6</td>
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<tr>
<td>Biomass</td>
<td>-</td>
<td>134.2</td>
<td>-</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>-</td>
<td>557.4</td>
<td>-</td>
</tr>
<tr>
<td>Total (kg)</td>
<td>1267.0</td>
<td>691.6</td>
<td>67.6</td>
</tr>
<tr>
<td>Water</td>
<td>-</td>
<td>575.4</td>
<td>-</td>
</tr>
</tbody>
</table>

Thus, in both fermentations water is produced. It is necessary to verify the results with the help of molar balances. Water can be estimated via either hydrogen or oxygen balance.

Hydrogen balance: \( 2n_{\text{H}_2\text{O}} + 1.8n_{\text{biom}} + 4n_{\text{meth}} = 0 \)

\[ n_{\text{H}_2\text{O}} = \frac{(-4n_{\text{meth}} - 1.8n_{\text{biom}})}{2} \]  \( (7.1) \)

Oxygen balance: \( n_{\text{H}_2\text{O}} + 2n_{\text{O}_2} + 0.5n_{\text{biom}} + 2n_{\text{CO}_2} + n_{\text{meth}} = 0 \)

\[ n_{\text{H}_2\text{O}} = \frac{(-2n_{\text{O}_2} + 0.5n_{\text{biom}} + 2n_{\text{CO}_2} + n_{\text{meth}})}{2} \]  \( (7.2) \)

The various molar quantities are calculated in appendix. The following table gives molar quantities that are produced or consumed.
<table>
<thead>
<tr>
<th>Reactant</th>
<th>Main fermentation</th>
<th>Pre-fermentation</th>
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<tbody>
<tr>
<td></td>
<td>consumed</td>
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</tr>
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<td>Methanol</td>
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<tr>
<td>Oxygen</td>
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<td>Biomass</td>
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<tr>
<td>Carbon dioxide</td>
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</table>

On applying these values to equations 7.1 and 7.2, the following molar quantities of water are estimated:

<table>
<thead>
<tr>
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<th>Pre-fermentation</th>
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<tr>
<td>Hydrogen balance</td>
<td>31336.0</td>
<td>1660.0</td>
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<tr>
<td>Oxygen balance</td>
<td>32964.0</td>
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</table>

There is a significant difference between the results obtained from the two balances. In the time that was left over to finish this project, we were unable to discover the error that caused this difference. However, we do suspect that the error perhaps lies in the oxygen consumption calculations. This suspicion originates from the fact that the methanol consumption is predetermined for the main fermentation. The biomass quantity is estimated using an iterative procedure, which we strongly believe is correct. Carbon-dioxide production is elementarily based upon the difference between the amount of methanol being consumed and biomass being produced. Thus, the only source of error that remains is the oxygen consumption calculations. The amount of oxygen consumed is, therefore, adjusted for the quantity of water produced by rearranging equation 7.2.

\[
    n_{O_2} = -(n_{H_2O} + 0.5 n_{biom} + 2 n_{CO_2} + n_{meth})/2 \quad (7.3)
\]

The molar amounts of oxygen calculated from equation 7.3 are:
For main fermentation: \( n_{O_2} = 639 \text{ mol} \) (deviation from old value: 3.8%).

For pre-fermentation: \( n_{O_2} = 973 \text{ mol} \) (deviation from old value: 8.4%).

Further attempts will be made to discover the error and a corrected version would be submitted once the problem is solved.
(8) Equipment list.
<table>
<thead>
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<th>V 2</th>
<th>V 4</th>
<th>V 11</th>
<th>R 14</th>
<th>V 15</th>
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<tr>
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</tr>
<tr>
<td>Stirred tank</td>
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<td>steel with epoxy coating</td>
<td>steel 316</td>
<td>steel 316</td>
<td>steel 316</td>
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<td>1</td>
<td>1</td>
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<td>600000</td>
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</table>

* aangeven wat bedoeld wordt

& 1/15th of the total price
### Apparatenlijst voor reaktoren, kolommen, vaten

<table>
<thead>
<tr>
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<th>T 20</th>
<th>R 22</th>
<th>V 23</th>
<th>V 31</th>
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<tr>
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<td>Main Fermenter</td>
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<td>1.1</td>
<td>1.1</td>
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<td>20</td>
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<td>1</td>
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<td>7000</td>
</tr>
</tbody>
</table>

* aangeven wat bedoeld wordt
<table>
<thead>
<tr>
<th>Apparaat No:</th>
<th>H 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benaming, type</td>
<td>Heat exchanger</td>
</tr>
<tr>
<td>Medium pijpen-/ mantelzijde</td>
<td>water/water</td>
</tr>
<tr>
<td>Capaciteit, uitgewisselde warmte in kw.</td>
<td>2019.4</td>
</tr>
<tr>
<td>Warmtewisselend oppvl. in m²</td>
<td>250</td>
</tr>
<tr>
<td>Aantal parallel</td>
<td>1</td>
</tr>
<tr>
<td>Abs. of eff.* druk in bar pijpen-/ mantelzijde</td>
<td>3.5/2.8</td>
</tr>
<tr>
<td>temp. in / uit in °C pijpzijde mantelzijde</td>
<td>125/55 20/94</td>
</tr>
<tr>
<td>Speciaal te gebruiken mat.</td>
<td>Steel AISI 304</td>
</tr>
<tr>
<td>Price per unit (in Dfl)</td>
<td>1976670 8</td>
</tr>
</tbody>
</table>

* aangeven wat bedoeld wordt

8 1/15th of the total price
### WARMTEWISSELAARSPECIFIKATIEBLAD

<table>
<thead>
<tr>
<th>Apparaatnummer</th>
<th>Aantal : 1. serie/parallel*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ALGEMENE EIGENSCHAPPEN</strong></td>
<td></td>
</tr>
<tr>
<td>Funktie :</td>
<td>Heat exchange between sterilized and raw medium.</td>
</tr>
<tr>
<td>Type :</td>
<td>Warmtewisselaar*</td>
</tr>
<tr>
<td>- Koeler</td>
<td></td>
</tr>
<tr>
<td>- Kondensator</td>
<td></td>
</tr>
<tr>
<td>- Verdamer</td>
<td></td>
</tr>
<tr>
<td>Uitvoering :</td>
<td>met vaste pijpplaten*</td>
</tr>
<tr>
<td>- floating-head-</td>
<td></td>
</tr>
<tr>
<td>- haarspeid</td>
<td></td>
</tr>
<tr>
<td>- dubbele pijp</td>
<td></td>
</tr>
<tr>
<td>- platenwarmtewisselaar</td>
<td></td>
</tr>
<tr>
<td>Positie :</td>
<td>horizontaal/vertikaal*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>BEDRIJFSKONDITIES</strong></th>
<th>Mantelzijde</th>
<th>Pijpziende</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Soort fluidum</strong> :</td>
<td>water + salts</td>
<td>water + salts</td>
</tr>
<tr>
<td><strong>Massastroom</strong> :</td>
<td>kg/s</td>
<td>6.531</td>
</tr>
<tr>
<td><strong>Massastroom te verdampen/kondenseren</strong>* :</td>
<td>kg/s</td>
<td></td>
</tr>
<tr>
<td><strong>Gemiddelde soortelijke warmte</strong> :</td>
<td>kJ/kg·°C</td>
<td>4191</td>
</tr>
<tr>
<td><strong>Verdampingswarmte</strong> :</td>
<td>kJ/kg</td>
<td></td>
</tr>
<tr>
<td><strong>Temperatuur IN</strong> :</td>
<td>°C</td>
<td>20</td>
</tr>
<tr>
<td><strong>Temperatuur UIT</strong> :</td>
<td>°C</td>
<td>94</td>
</tr>
<tr>
<td><strong>Druk</strong> :</td>
<td>bar</td>
<td>3.5 - 3</td>
</tr>
<tr>
<td><strong>Materiaal</strong> :</td>
<td></td>
<td>AISI 304</td>
</tr>
</tbody>
</table>

*Doorstrepen wat niet van toepassing is
<table>
<thead>
<tr>
<th>Apparaat No:</th>
<th>P 3</th>
<th>P 5</th>
<th>P 7</th>
<th>C 10</th>
<th>P 17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benaming, type</td>
<td>Centrifugal pump</td>
<td>Centrifugal pump</td>
<td>Centrifugal pump</td>
<td>Air compressor Roots blower &quot;XN3&quot; Geveke</td>
<td>Centrifugal pump</td>
</tr>
<tr>
<td>te verpompen medium</td>
<td>Salt solution</td>
<td>Methanol</td>
<td>Medium</td>
<td>Air</td>
<td>Methanol, water and salt</td>
</tr>
<tr>
<td>Capaciteit in t/d of kg/s</td>
<td>0.20</td>
<td>0.25</td>
<td>6.9</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Dichtheid in kg/m³</td>
<td>1000</td>
<td>79.14</td>
<td>1000</td>
<td>1.2</td>
<td>1000</td>
</tr>
<tr>
<td>Zuig-/persdruk in bar(abs.of eff.)</td>
<td>1/3.5</td>
<td>1/1.5</td>
<td>2.5/3.5</td>
<td>1/1.4</td>
<td>1/1.2</td>
</tr>
<tr>
<td>temp. in °C in / uit</td>
<td>20/20</td>
<td>20/20</td>
<td>55/55</td>
<td>25/55.1</td>
<td>20/20</td>
</tr>
<tr>
<td>Vermogen in kW theor./ prakt.</td>
<td>0.05/0.07</td>
<td>0.013/0.017</td>
<td>0.69/0.96</td>
<td>2.1/3.0</td>
<td>0.002/0.003</td>
</tr>
<tr>
<td>Speciaal te gebruiken mat.</td>
<td>steel 316</td>
<td>steel 316</td>
<td>steel 316</td>
<td>steel 316</td>
<td>steel 316</td>
</tr>
<tr>
<td>aantall serie/parallel</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Price per unit (in Dfl)</td>
<td>200 &amp;</td>
<td>3000</td>
<td>1534 &amp;</td>
<td>16000</td>
<td>3000</td>
</tr>
</tbody>
</table>

* aangeven wat bedoeld wordt

& 1/15th of the total price
<table>
<thead>
<tr>
<th>Apparaat No:</th>
<th>P 18</th>
<th>C 19</th>
<th>P 24</th>
<th>P 26</th>
<th>P 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benaming, type</td>
<td>Pneumatic membrane pump</td>
<td>Air compressor</td>
<td>Pneumatic membrane pump</td>
<td>Centrifugal pump</td>
<td>Centrifugal pump</td>
</tr>
<tr>
<td>te verpompen medium</td>
<td>Biomass</td>
<td>Air</td>
<td>Methanol, water, NaCl, HCl</td>
<td>Medium and biomass</td>
<td>Methanol, water and salt</td>
</tr>
<tr>
<td>Capaciteit in t/d of kg/s</td>
<td>4.5</td>
<td>0.2</td>
<td>0.1</td>
<td>7.00</td>
<td>0.1</td>
</tr>
<tr>
<td>Dichtheid in kg/m³</td>
<td>1000</td>
<td>1.2</td>
<td>+1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Zuig-/persdruk in bar(abs. of eff.)</td>
<td>1/1.7</td>
<td>1/1.7</td>
<td>---</td>
<td>1/1.5</td>
<td>---</td>
</tr>
<tr>
<td>temp. in °C in / uit</td>
<td>30/30</td>
<td>25/73.8</td>
<td>20/20</td>
<td>30/30</td>
<td>20/20</td>
</tr>
<tr>
<td>Vermogen in kW theor./ prakt.</td>
<td>---</td>
<td>9.6/13.7</td>
<td>---</td>
<td>0.35/0.49</td>
<td>---</td>
</tr>
<tr>
<td>Speciaal te gebruiken mat. aantal serie/parallel</td>
<td>steel 316</td>
<td>steel 316</td>
<td>steel 316</td>
<td>steel 316</td>
<td>steel 316</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Price per unit (in Dfl)</td>
<td>4000</td>
<td>20085</td>
<td>4000</td>
<td>23000</td>
<td>3000</td>
</tr>
</tbody>
</table>

*a aangeven wat bedoeld wordt*
## Apparatenlijst voor diversen

<table>
<thead>
<tr>
<th>Apparaat No:</th>
<th>M 1</th>
<th>M 6</th>
<th>M 9</th>
<th>M 12</th>
<th>M 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benaming, type</td>
<td>Steam injector</td>
<td>Sterilization pipe</td>
<td>Filter</td>
<td>Sieve</td>
<td>PVA Filter</td>
</tr>
<tr>
<td>Capaciteit (kg/s)</td>
<td>0.37</td>
<td>6.9</td>
<td>--</td>
<td>--</td>
<td>0.1</td>
</tr>
<tr>
<td>Abs. of eff. (\text{m}^3) druk in bar</td>
<td>3.0</td>
<td>3.0</td>
<td>--</td>
<td>--</td>
<td>1.4</td>
</tr>
<tr>
<td>temp. in °C</td>
<td>140</td>
<td>140</td>
<td>20</td>
<td>20</td>
<td>55.1</td>
</tr>
<tr>
<td>Inhoud in (\text{m}^3)</td>
<td>--</td>
<td>65</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>aantal serie/parallel</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Material of construction</td>
<td>steel 316</td>
<td>steel</td>
<td>--</td>
<td>Polyvinyl-alcohol</td>
<td></td>
</tr>
<tr>
<td>Price per unit (in Dfl)</td>
<td>800 &amp; 607</td>
<td>1000</td>
<td>2000</td>
<td>included with fermenter</td>
<td></td>
</tr>
</tbody>
</table>

\* aangeven wat bedoeld wordt

\& 1/15th of the total price
### Apparatenlijst voor diversen

<table>
<thead>
<tr>
<th>Apparaat No:</th>
<th>M 16</th>
<th>M 21</th>
<th>M 25</th>
<th>M 27</th>
<th>M 28</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Benaming, type</strong></td>
<td>Air Filter</td>
<td>Air Filter</td>
<td>Air Filter</td>
<td>Filter</td>
<td>Disk centrifuge</td>
</tr>
<tr>
<td><strong>Capaciteit (kg/s)</strong></td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>3.5</td>
</tr>
<tr>
<td><strong>Abs. of eff.</strong>&lt;sup&gt;※&lt;/sup&gt;</td>
<td>1.1</td>
<td>1.7</td>
<td>1.1</td>
<td>---</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>druk in bar</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>temp. in °C</strong></td>
<td>30</td>
<td>73.8</td>
<td>30</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td><strong>Inhoud in m³</strong></td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Material of construction</strong></td>
<td>Polyvinyl-alcohol</td>
<td>Polyvinyl-alcohol</td>
<td>Polyvinyl-alcohol</td>
<td>steel</td>
<td></td>
</tr>
<tr>
<td><strong>Price per unit</strong></td>
<td>included with fermenter</td>
<td>included with fermenter</td>
<td>included with fermenter</td>
<td>1000</td>
<td>650,000</td>
</tr>
</tbody>
</table>

<sup>※</sup> aangeven wat bedoeld wordt
<table>
<thead>
<tr>
<th>Apparaat No.</th>
<th>M 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benaming, type</td>
<td>Dryer</td>
</tr>
<tr>
<td>Capaciteit (kg/s)</td>
<td>---</td>
</tr>
<tr>
<td>Abs. of eff.* druk in bar</td>
<td>1</td>
</tr>
<tr>
<td>temp. in °C</td>
<td>60</td>
</tr>
<tr>
<td>Inhoud in m³ of afmetingen in m*</td>
<td>---</td>
</tr>
<tr>
<td>aantal serie/parallel</td>
<td>1</td>
</tr>
<tr>
<td>Material of construction</td>
<td>---</td>
</tr>
<tr>
<td>Price per unit</td>
<td>1000</td>
</tr>
</tbody>
</table>

*aangeven wat bedoeld wordt*
The accuracy of an estimate depends on the amount of design detail available, the accuracy of the cost data available and the time spent on preparing the estimate. It has been assumed that the process plant is situated in multi-complex establishment where several fermentation units are present. The costs of the steam injector, sterilization pipe, heat exchanger and the salt mixing tank are taken as 1/15th of the total price since these units are shared between several processes. An economic evaluation has been performed for the heat exchanger to predict the most economical intensity of heat recovery (appendix 16).

The fixed capital is the total cost of the plant ready for start up. It includes cost of all items of equipment and their installation, instrumentation and control systems, buildings and structures and other auxiliary facilities.

The fixed capital cost of the project on the basis of the Lang factor is described by Holland [35]. This method gives the fixed capital cost as a function of total purchase equipment. The Lang factor is calculated according to the method given in literature [35]. The factor is calculated as following:

\[ \psi_1 = 1.47 \text{ (for gas/liquid processes)} \]
\[ \psi_2 = 1 + \psi_1 + \psi_2 + \psi_3 + \psi_4 + \psi_5 \]
\[ \psi_3 = 1 + \psi_6 + \psi_7 + \psi_8 \]

The \( \psi \)-factors are obtained from the table given in literature [35]. These factors are taken as the average of the given ranges and the accuracy of the value is directly proportional to the amount of available information about the process under study. Characterization and values of the \( \psi \)-factors are given in table 9.1.

\[ \text{Lang factor} = \psi_1 \times \psi_2 \times \psi_3 = 5.42 \]

Purchase of equipment is estimated to be Dfl 3,416,060/- excluding VAT. Therefore, total equipment investment is equal to:

\[ 5.42 \times 3,416,060 = \text{Dfl 18,515,045} \]

Methanol storage tanks of various volumes were considered coupled to the number methanol deliveries per year. From the various combinations it was determined that the most economically attractive situation occurs if two deliveries per year are considered, each delivery of 20,000 kg methanol. For
this situation, a steel tank 34 m$^3$ is required. The purchase price of this tank is included in the equipment expenditure.

Table 9.1

<table>
<thead>
<tr>
<th>Factor characterization</th>
<th>Average of range</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_1$, process-piping factor</td>
<td>0.45</td>
<td>Fluid processing</td>
</tr>
<tr>
<td>$f_2$, instrumentation factor</td>
<td>0.075</td>
<td>Some automatic control</td>
</tr>
<tr>
<td>$f_3$, building factor</td>
<td>0.40</td>
<td>Mixed indoor &amp; outdoor unit</td>
</tr>
<tr>
<td>$f_4$, facility factor</td>
<td>0.0125</td>
<td>Minor additions</td>
</tr>
<tr>
<td>$f_5$, outside lines factor</td>
<td>0.10</td>
<td>Separated units</td>
</tr>
<tr>
<td>$f_6$, engineering factor</td>
<td>0.3</td>
<td>Straightforward plants</td>
</tr>
<tr>
<td>$f_7$, size factor</td>
<td>0.1</td>
<td>Small plants</td>
</tr>
<tr>
<td>$f_8$, Contingency factor</td>
<td>0.4</td>
<td>Tentative process</td>
</tr>
</tbody>
</table>

An estimate of the operating cost, the cost of producing the product, are obtained from the raw material, service requirements and capital cost estimate. The annual cost of raw materials is given in Table 9.2. The cost of utilities are given in Table 9.3.

Table 9.4 gives the total annual production costs. The yearly cost for maintenance is assumed to be 5% of the installed capital costs.

Labour costs are estimated on the basis that 4.8 personnel are required at wages of Dfl 32.50 per hour. The value of 4.8 personnel required to run the fermentation plant is derived as follows:
Two personnel are required to maintain both the pre- and the main fermenter. Since the sterilization section forms a part of the multicomplex plant, the labour for this unit is thus coupled with other processes. It is assumed that 2/15 th of the personnel time is needed for this operation. Similarly, 5/3 rd of the personnel time is required for various control functions. This value is derived from the assumption that five duty-teams manage the controls twenty-four hours a day and that each personnel is responsible for three functions. One personnel is needed as incharge of the processing unit. Thus, on summation, 4.8 personnel are needed for this process.

Depreciation is taken as 10 % of the investment value.
Selling price is assumed to be twice the production cost. Thus, profit before taxes amounts to Dfl 3,147,137.

Return on Investment (ROI) is calculated as following:

\[
\text{ROI} = \frac{3147137 \times 100}{18515045} = 17 \%
\]

The cost price of PQQ is estimated as the annual production cost divided by the total amount of PQQ produced. Thus, the cost price of PQQ is Dfl 31.50 per gram.

Internal Rate of Return (IRR) is calculated according to the method described in literature [35]. For the estimation of IRR, a small computer program is developed to perform the iterative steps (see appendix 17). IRR is determined to have a value of 10.7 %.
<table>
<thead>
<tr>
<th>MATERIAL</th>
<th>AMOUNT</th>
<th>PRICE (Dfl)/UNIT</th>
<th>TOTAL (Dfl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>METHANOL</td>
<td>40300.00 kg</td>
<td>4200/1000 kg</td>
<td>17000.00</td>
</tr>
<tr>
<td>((\text{NH}_4)\text{SO}_4)</td>
<td>6700.00 kg</td>
<td>4.55/kg</td>
<td>30485.00</td>
</tr>
<tr>
<td>K(_2)HPO(_4)</td>
<td>3350.00 kg</td>
<td>11.60/kg</td>
<td>38860.00</td>
</tr>
<tr>
<td>MgSO(_4).7\text{H}_2\text{O}</td>
<td>670.00 kg</td>
<td>7.50/kg</td>
<td>5025.00</td>
</tr>
<tr>
<td>H(_3)BO(_4)</td>
<td>1.70 kg</td>
<td>24.63/kg</td>
<td>42.00</td>
</tr>
<tr>
<td>CuSO(_4).5\text{H}_2\text{O}</td>
<td>0.134 kg</td>
<td>130.00/kg</td>
<td>17.40</td>
</tr>
<tr>
<td>MnSO(_4).\text{H}_2\text{O}</td>
<td>0.67 kg</td>
<td>63.50/kg</td>
<td>42.50</td>
</tr>
<tr>
<td>ZnSO(_4).7\text{H}_2\text{O}</td>
<td>1.34 kg</td>
<td>37.25/kg</td>
<td>49.90</td>
</tr>
<tr>
<td>CoCl(_2).2\text{H}_2\text{O}</td>
<td>39.34 kg</td>
<td>206.00/kg</td>
<td>8104.00</td>
</tr>
<tr>
<td>KCl</td>
<td>134.00 kg</td>
<td>10.80/kg</td>
<td>965.00</td>
</tr>
<tr>
<td>((\text{NH}_4)\text{Mo}<em>7\text{O}</em>{24}.4\text{H}_2\text{O})</td>
<td>0.60 kg</td>
<td>331.00/kg</td>
<td>196.80</td>
</tr>
</tbody>
</table>

TOTAL (excl. VAT) 100885.20
<table>
<thead>
<tr>
<th>Utilities</th>
<th>Amount required per fermentation</th>
<th>Price (Dfl) per unit</th>
<th>Annual costs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steam (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sterilization medium</td>
<td>2657.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sterilization main fermenter</td>
<td>190.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sterilization pre-fermenter</td>
<td>9.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sterilization pipe (2X)</td>
<td>21.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (kg steam)</td>
<td>2877.00</td>
<td>22.83/ton</td>
<td>4400.00</td>
</tr>
<tr>
<td>Tap water (m³)</td>
<td>50</td>
<td>1.87/m³</td>
<td>6264.00</td>
</tr>
<tr>
<td>Cooling water (ton)</td>
<td>830.2 + 19.4 = 849.6</td>
<td>0.05/m³</td>
<td>2846.00</td>
</tr>
<tr>
<td>Electricity (kWh)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>stirrer motor</td>
<td>3.0 kWh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>blower (main fermenter)</td>
<td>665.0 kWh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>blower (pre-fermenter)</td>
<td>64.0 kWh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (kWh)</td>
<td>732.0</td>
<td>0.12/kWh</td>
<td>5885.00</td>
</tr>
<tr>
<td>TOTAL (Dfl)</td>
<td></td>
<td></td>
<td>19395.00</td>
</tr>
</tbody>
</table>
### TABLE 9.4

Annual Production Costs:

<table>
<thead>
<tr>
<th>Cost Factors</th>
<th>Dfl</th>
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</thead>
<tbody>
<tr>
<td>Raw materials</td>
<td>100885.00</td>
</tr>
<tr>
<td>Maintenance</td>
<td>925752.00</td>
</tr>
<tr>
<td>Personnel</td>
<td>249600.00</td>
</tr>
<tr>
<td>Depreciation</td>
<td>1851505.00</td>
</tr>
<tr>
<td>Utilities</td>
<td>19395.00</td>
</tr>
<tr>
<td><strong>Total (Dfl)</strong></td>
<td><strong>3147137.00</strong></td>
</tr>
</tbody>
</table>
## NOMENCLATURE

### Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>area</td>
<td>m²</td>
</tr>
<tr>
<td>A</td>
<td>area, constant</td>
<td>m², -</td>
</tr>
<tr>
<td>c, C</td>
<td>concentration per kg or kmol</td>
<td>kg/m³, kmol/m³</td>
</tr>
<tr>
<td>c_p</td>
<td>specific heat capacity</td>
<td>J/(kg.K)</td>
</tr>
<tr>
<td>C_x</td>
<td>concentration biomass</td>
<td>moleDM/m³</td>
</tr>
<tr>
<td>d</td>
<td>thickness</td>
<td>m</td>
</tr>
<tr>
<td>d_p</td>
<td>particle diameter</td>
<td>m</td>
</tr>
<tr>
<td>D</td>
<td>diameter</td>
<td>m</td>
</tr>
<tr>
<td>D_p</td>
<td>dispersion coefficient</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>diffusion coefficient</td>
<td>m²/s</td>
</tr>
<tr>
<td>E</td>
<td>energy</td>
<td>J, N.m</td>
</tr>
<tr>
<td>E_a</td>
<td>activation energy</td>
<td>J, N.m</td>
</tr>
<tr>
<td>g</td>
<td>acceleration due to gravity</td>
<td>m/s²</td>
</tr>
<tr>
<td>G</td>
<td>tension of steel</td>
<td>atm</td>
</tr>
<tr>
<td>h, H</td>
<td>height</td>
<td>m</td>
</tr>
<tr>
<td>ΔH, H</td>
<td>enthalpy</td>
<td>J/kmol</td>
</tr>
<tr>
<td>k</td>
<td>reaction rate constant</td>
<td>-</td>
</tr>
<tr>
<td>k_1</td>
<td>oxygen transfer coeff.</td>
<td>l/s</td>
</tr>
<tr>
<td>k_o</td>
<td>Arrhenius constant</td>
<td>-</td>
</tr>
<tr>
<td>k_o'</td>
<td>Kozeny's constant</td>
<td>-</td>
</tr>
<tr>
<td>l, L</td>
<td>length</td>
<td>m</td>
</tr>
<tr>
<td>m</td>
<td>mass</td>
<td>kg</td>
</tr>
<tr>
<td>m_o</td>
<td>maintenance factor on oxygen</td>
<td>O-moles/moleDM H</td>
</tr>
<tr>
<td>m_s</td>
<td>maintenance factor on substrate</td>
<td>(C-)moles/moleDM h</td>
</tr>
<tr>
<td>n</td>
<td>mole quantity</td>
<td>mole</td>
</tr>
<tr>
<td>N</td>
<td>stirring rate</td>
<td>l/min, l/s</td>
</tr>
<tr>
<td>P</td>
<td>pressure</td>
<td>N/m², Pa, bar</td>
</tr>
<tr>
<td>P</td>
<td>power</td>
<td>W</td>
</tr>
<tr>
<td>Q</td>
<td>heat</td>
<td>J</td>
</tr>
<tr>
<td>r</td>
<td>radius</td>
<td>m</td>
</tr>
<tr>
<td>r_o2</td>
<td>oxygen consumption rate</td>
<td>mole/m³ h</td>
</tr>
<tr>
<td>r_s</td>
<td>rate of substrate consumption</td>
<td>C-mole/m³ h</td>
</tr>
<tr>
<td>r_x</td>
<td>rate of biomass production</td>
<td>mole/m³ h</td>
</tr>
<tr>
<td>R</td>
<td>gas constant</td>
<td>kmol/m³.s</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
<td>Unit</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
<td>------</td>
</tr>
<tr>
<td>S</td>
<td>specific surface area</td>
<td>m²</td>
</tr>
<tr>
<td>t</td>
<td>time, temperature</td>
<td>s, °C</td>
</tr>
<tr>
<td>T</td>
<td>temperature, vessel diameter</td>
<td>K, m</td>
</tr>
<tr>
<td>U</td>
<td>total heat transfer coefficient</td>
<td>W/(m².K)</td>
</tr>
<tr>
<td>v</td>
<td>velocity</td>
<td>m/s</td>
</tr>
<tr>
<td>v_g</td>
<td>sedimentation velocity</td>
<td>m/s</td>
</tr>
<tr>
<td>V</td>
<td>volume</td>
<td>m³</td>
</tr>
<tr>
<td>W</td>
<td>work</td>
<td>J, Nm</td>
</tr>
<tr>
<td>Y_{ox}</td>
<td>yield factor for biomass on oxygen</td>
<td>mole/O-mole</td>
</tr>
<tr>
<td>Y_{sx}</td>
<td>yield factor for biomass on substrate</td>
<td>mole/C-mole</td>
</tr>
</tbody>
</table>

**Greek letters**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>heat transfer coeff.</td>
<td>W/(m².K)</td>
</tr>
<tr>
<td>κ</td>
<td>c_p/c_v</td>
<td></td>
</tr>
<tr>
<td>Y_{i}</td>
<td>generalized degree of reduction</td>
<td>-</td>
</tr>
<tr>
<td>Y_{i}</td>
<td>degree of reduction of compound i</td>
<td>-</td>
</tr>
<tr>
<td>Δ</td>
<td>difference</td>
<td></td>
</tr>
<tr>
<td>ΔT</td>
<td>temperature difference</td>
<td>K</td>
</tr>
<tr>
<td>ε</td>
<td>porosity</td>
<td>-</td>
</tr>
<tr>
<td>η</td>
<td>dynamic viscosity</td>
<td>Pa.s, N.s/m²</td>
</tr>
<tr>
<td>λ</td>
<td>heat conductivity</td>
<td>W/m.K</td>
</tr>
<tr>
<td>μ</td>
<td>specific growth rate</td>
<td>hr⁻¹</td>
</tr>
<tr>
<td>μ_{max}</td>
<td>maximum specific growth rate</td>
<td>hr⁻¹</td>
</tr>
<tr>
<td>ρ</td>
<td>density</td>
<td>kg/m³</td>
</tr>
<tr>
<td>ρ_p</td>
<td>particle density</td>
<td>kg/m³</td>
</tr>
<tr>
<td>ρ_f</td>
<td>fluid density</td>
<td>kg/m³</td>
</tr>
<tr>
<td>ζ</td>
<td>acceleration factor</td>
<td>-</td>
</tr>
<tr>
<td>Σ</td>
<td>centrifuge constant</td>
<td>m²</td>
</tr>
<tr>
<td>ϕ, Φ</td>
<td>flow rate</td>
<td>-</td>
</tr>
<tr>
<td>Φ_H</td>
<td>heat energy</td>
<td>J</td>
</tr>
<tr>
<td>τ</td>
<td>process time</td>
<td>s</td>
</tr>
</tbody>
</table>

78
<table>
<thead>
<tr>
<th>Sub- and Superscripts</th>
</tr>
</thead>
<tbody>
<tr>
<td>c: cold, consumption</td>
</tr>
<tr>
<td>g: gas, gassed</td>
</tr>
<tr>
<td>H: heat</td>
</tr>
<tr>
<td>h: hydraulic</td>
</tr>
<tr>
<td>i: in</td>
</tr>
<tr>
<td>l: liquid</td>
</tr>
<tr>
<td>m: mass, metal, mixing</td>
</tr>
<tr>
<td>max: maximum</td>
</tr>
<tr>
<td>o: out, ungassed</td>
</tr>
<tr>
<td>p: pressure</td>
</tr>
<tr>
<td>s: substrate, superficial</td>
</tr>
<tr>
<td>t: temperature</td>
</tr>
<tr>
<td>u: uptake</td>
</tr>
<tr>
<td>V: volume</td>
</tr>
<tr>
<td>vap: vapor</td>
</tr>
<tr>
<td>w: wall, warm</td>
</tr>
<tr>
<td>x: biomass</td>
</tr>
<tr>
<td>*: saturated</td>
</tr>
</tbody>
</table>
(11) LITERATURE


82


[41] DACE – WEBCI-prijzenboek, 12e druk (1986), uitgave NAP.
APPENDIX 1

VITAMINS

Generally, a vitamin is the sole, or main component of a coenzyme (see tables 1 and 2). Co-enzymes may be regarded as components of an enzyme prosthetic group complex which catalyse chemical reactions. Unless both enzyme and co-enzyme are present no catalysis takes place.

Considerable differences of opinion exist among nutritionists and physicians concerning the adequacy of the average diet in terms of vitamin content. However, nutritional books do provide adequate amounts of all nutrients though most of these stated requirements are concerned with 'adequate' requirements, rather than the optimal amounts which would allow full and total activity. Table 3 gives a reasonable estimate of vitamin requirements [31].

PQQ is a bioorganic compound whose role as a vitamin is under extensive study. PQQ is present in minute quantities in vinegar and some other natural products. Increased craving for vinegar by women in early stages of pregnancy may ultimately unravel the role of PQQ in the human body [4]. The clinical conditions that may arise due to deficiency of PQQ have not been documented though its value in pharmaceutics is worth mentioning.
### General considerations

**Table 1**

Summary of the co-enzyme functions of certain vitamins.

<table>
<thead>
<tr>
<th>Co-enzyme</th>
<th>Group transferred</th>
<th>Vitamin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Hydrogen transferring co-enzyme</td>
<td>Hydrogen</td>
<td>Nicotinamide</td>
</tr>
<tr>
<td>Nicotinamide-adenine dinucleotide (NAD)</td>
<td>Hydrogen</td>
<td>Nicotinamide</td>
</tr>
<tr>
<td>Nicotinamide-adenine dinucleotide phosphate (NADP)</td>
<td>Hydrogen</td>
<td>Nicotinamide</td>
</tr>
<tr>
<td>Nicotinamide mononucleotide</td>
<td>Hydrogen</td>
<td>Riboflavin</td>
</tr>
<tr>
<td>Flavine mononucleotide (FMN)</td>
<td>Hydrogen</td>
<td>Vitamin E</td>
</tr>
<tr>
<td>Flavine-adenine dinucleotide (FAD)</td>
<td>Hydrogen</td>
<td>Vitamin K</td>
</tr>
<tr>
<td>Quinone-hydroquinone</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2 **Group transferring enzyme**

| Co-enzyme A (Co.A) | Acyl groups | Pantothenic acid |
| Tetrahydrofolic acid | Formyl groups | Folic acid |
| Biotin | Carboxyl groups | Biotin |
| Thiamine pyrophosphate | C2 aldehyde groups | Thiamine |
| Pyridoxal phosphate | Amino groups | Pyridoxine |

3 **Co-enzymes of Somcories & Lyoses**

| Pyridoxal phosphate | Decarboxylation | Pyridoxine |
| Thiamine pyrophosphate | Decarboxylation | Thiamine |
| B12 co enzyme | Carboxyl displacement | Cobalamin |

### Table 2

List of the recognized vitamins and their main synonyms (Those in italics are the trivial names that are accepted)

<table>
<thead>
<tr>
<th>Letter designations</th>
<th>Synonyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat soluble</td>
<td>Retinol</td>
</tr>
<tr>
<td>Vitamin A1</td>
<td>Retinoid, antixerophthalmic vitamin</td>
</tr>
<tr>
<td>Vitamin A2</td>
<td>Dehydroretinol</td>
</tr>
<tr>
<td>Vitamin D2</td>
<td>Ergocalciferol</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>Cholecalciferol</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>a, b, g...tocopherol antisterility vitamin</td>
</tr>
<tr>
<td>Vitamin K1</td>
<td>Phylloquinone phytenoadione</td>
</tr>
<tr>
<td>Vitamin K2</td>
<td>Farnoquinone</td>
</tr>
<tr>
<td>Vitamin K3</td>
<td></td>
</tr>
<tr>
<td>Water soluble</td>
<td>Thiamine</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>Thiamine aneurine, antineuritic vitamin</td>
</tr>
<tr>
<td>Vitamin B2</td>
<td>Riboflavin lactoflavin</td>
</tr>
<tr>
<td>Vitamin PP</td>
<td>Nicotinamide niacinamide</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Nicotin acid niacin</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>Pellagra preventive factor</td>
</tr>
<tr>
<td>(as a group)</td>
<td>Pyridoxine comprising pyridoxal pyridoxol pyridoxamine</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>Cobalamin</td>
</tr>
<tr>
<td>(as collective)</td>
<td>Cyanocobalamin</td>
</tr>
<tr>
<td>(pure substance)</td>
<td>Hydroxocobalamin</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>Nitrilcobalamin</td>
</tr>
<tr>
<td>Vitamin B12c</td>
<td>Pantothenic acid</td>
</tr>
<tr>
<td>Vitamin M or B12</td>
<td>Folic acid lactobacillus casei factor</td>
</tr>
<tr>
<td>(as a group)</td>
<td>Pteroylmonoglutamic acid folacine</td>
</tr>
<tr>
<td>(pure substance)</td>
<td>Biotin</td>
</tr>
<tr>
<td>Vitamin H</td>
<td>Ascorbic acid antiscorbutic vitamin</td>
</tr>
<tr>
<td>Vitamin C</td>
<td></td>
</tr>
</tbody>
</table>

International units for vitamins and their equivalents in pure substance

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>1 i.u. is equivalent to</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*</td>
<td>0-30 microgram crystalline all trans vitamin A alcohol or 0-344 microgram crystalline vitamin A acetate</td>
</tr>
<tr>
<td>D*</td>
<td>0-025 microgram cholecalciferol</td>
</tr>
<tr>
<td>E</td>
<td>1 mg synthetic racemic alpha tocopherol acetate</td>
</tr>
<tr>
<td>B1</td>
<td>0-003 mg thiamine hydrochloride</td>
</tr>
<tr>
<td>C</td>
<td>0-05 mg crystalline L-ascorbic acid</td>
</tr>
</tbody>
</table>

*Only these international units are now used
### Table 3

Daily vitamin allowances for normal adults recommended by various countries

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>GB</th>
<th>Australia</th>
<th>Canada</th>
<th>USA</th>
<th>USSR</th>
<th>Germany</th>
<th>Norway</th>
<th>Switzerland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A, I.U.</td>
<td>5000</td>
<td>2500</td>
<td>3700</td>
<td>5000</td>
<td>4500</td>
<td>5000</td>
<td>2500</td>
<td>5000</td>
</tr>
<tr>
<td>B₁ mg</td>
<td>1.2</td>
<td>1.1</td>
<td>0.9</td>
<td>1.2</td>
<td>3.0</td>
<td>1.7</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>B₂ mg</td>
<td>1.8</td>
<td>1.6</td>
<td>1.4</td>
<td>1.7</td>
<td>3.0</td>
<td>1.8</td>
<td>1.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Nic. acid mg</td>
<td>12.0</td>
<td>18</td>
<td>9</td>
<td>19</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B₆ mg</td>
<td>(1.5-2.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panto. Acid mg</td>
<td>(10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic Acid mg</td>
<td>(0.003-0.005 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C mg</td>
<td>20-30</td>
<td>30</td>
<td>30</td>
<td>70</td>
<td>100</td>
<td>75</td>
<td>30</td>
<td>75</td>
</tr>
<tr>
<td>E mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 12

Recommended dietary allowances in the elderly. Data for various countries.

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>USA</th>
<th>Netherlands</th>
<th>West Germany</th>
<th>Czechoslovakia</th>
<th>Hungary</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₁ i.u.</td>
<td>5000</td>
<td>5000</td>
<td>5000</td>
<td>5000</td>
<td>3100</td>
</tr>
<tr>
<td>B₂ mg</td>
<td>0.8-0.9</td>
<td>0.7-0.9</td>
<td>1.5-1.7</td>
<td>1.0-1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>B₆ mg</td>
<td>1.2-1.3</td>
<td>1.1-1.4</td>
<td>1.8</td>
<td>1.4-1.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Nic. acid mg</td>
<td>13-15</td>
<td>7-9</td>
<td></td>
<td>10-12</td>
<td>16</td>
</tr>
<tr>
<td>C mg</td>
<td>70</td>
<td>50</td>
<td>75</td>
<td>60</td>
<td>65</td>
</tr>
</tbody>
</table>
Appendix 2

Fig. 9.16. Effect of different types of flow (as shown by different PeB values) on the destruction of organisms \( \frac{N}{N_0} \) at different rates of destruction (measured as \( N_r = kL/d \)).
solely by the parabolic velocity distribution of flow in a pipe with no end effects. The final result of Taylor's analysis is

\[
D = \frac{\varepsilon d^3}{192 D_s}
\]

(9)

which, in the dimensionless form of Equation 7, yields

\[
\frac{D}{ud} = \frac{1}{192} \left( \frac{\mu}{\rho D_s} \right) \left( \frac{u}{D_s} \right) = \frac{1}{192} \left( \frac{\mu}{\rho D_s} \right)
\]

(10)

The condition of applicability of Equation 7 is that

\[
28.8 \left( \frac{L}{d} \right) \gg \left( \frac{d_D}{\mu} \right) \left( \frac{\mu}{\rho D_s} \right) = \frac{ud}{D_s}
\]

(11)

This is Equation 8 in dimensionless form. When plotted (Figure 2), Equation 10 predicts that all experimental data should fall on a straight line of slope 1 and intercept 1/192. When Equation 10 is plotted as in Figure 2 a family of curves of slope 1 and intercept (1/192) (\(\mu/\rho D_s\)) should result.

Turbulent Flow. In turbulent flow molecular diffusion should not be expected to contribute significantly to the transport of material, this process being greatly overshadowed by turbulent eddy mixing. Thus, Equation 6 should reduce to

\[
\frac{D}{ud} = \Phi \left[ \left( \frac{d_D}{\mu} \right), \left( \frac{\mu}{\rho D_s} \right) \right]
\]

(12)

and consequently a plot of \(D/ud\) vs. \(d_D/\mu\) should result in a family of curves with \(\mu/\rho D_s\) as a parameter.

In his theoretical analysis of mixing Figure 2. Correlation for both streamline and turbulent flow ranges of \(D/ud\), a longitudinal dispersion number, as a function of Reynolds number

\(a, b, c, \ldots\): Ideal conditions

\(\alpha, \beta, \gamma, \ldots\): Data obtained in pipe with few bends-

\(\alpha, \beta, \gamma, \ldots\): Commercial pipeline

\(\alpha, \beta, \gamma, \ldots\): Mixing between fluids of different physical properties

\(\alpha, \beta, \gamma, \ldots\): Flow in artificially roughened pipe

\(\alpha, \beta, \gamma, \ldots\): Curved pipe

in turbulent flow, Taylor (72) considered the diffusion model to be universally applicable, in which case the longitudinal dispersion coefficient is given by

\[
\frac{D}{ud} = 3.57 \sqrt{f}
\]

(13)

where \(f\) is the Fanning friction factor. The analysis is based on the assumption that Reynolds' analogy holds. According to this assumption the intra-phase transfer of heat, momentum, and matter by a fluid in turbulent flow are analogous. Hence the extent of fluid mixing (transfer of matter) can be calculated by shear stress and pressure drop information (transfer of momentum).

To find \(D\) from Equation 13 the value of \(f\) must be known. Experimentally, \(f\) has been found to be a function of the flow and system variables, or

\[
\Phi = \left[ \left( \frac{d_D}{\mu} \right), \left( \frac{\mu}{\rho D_s} \right) \right]
\]

thus checking Equation 12. A number of analytical expressions of the above relationship are available (15, 16). However, as these are relatively complex compared to a graphical representation, the latter is used. The result of Taylor's analysis is shown in Figure 2 by a curve based on Equation 13 and a value of \(f\) for commercial pipe given in Perry (75). A family of curves for different values of the parameter \(d_D/\mu\) are obtained if the charts for \(f\) presented by Moody are used (74); however, for the present purposes the one curve found in Perry (75) is sufficient.

Discussion

To test the reliability of the predictive equations they should be checked against experiment. For this purpose all published investigations which present sufficient information to allow them to be reanalyzed are considered. Experiments in the streamline flow region, done by Taylor (78) and Fowler and Brown (7), are shown in Figure 3. Taylor's three runs were made by measuring the dispersion of potassium permanganate in water flowing in a tube 0.0504 cm. in diameter. The conditions of Equation 11 were satisfied, and Figure 3 shows that the points are close to the theoretical curve. In plotting the points a problem arose in choosing a value from the molecular diffusion coefficient, \(D_s\), for the potassium permanganate-water system as this varies considerably with concentration (9). The value chosen was that reported at the average potassium permanganate concentration. By contrast to the good agreement found by Taylor, the data of Fowler and Brown are much below the theoretical curve. However, this is not surprising in the condition for applicability of the diffusion model, Equation 11, was far from being satisfied. For the points further away from the curve

\[
28.8 \frac{L}{d} = \frac{1}{220} \left( \frac{d_D}{\mu} \right) \left( \frac{\mu}{\rho D_s} \right)
\]

while for the points closest to the curve

\[
28.8 \frac{L}{d} = \frac{1}{3} \left( \frac{d_D}{\mu} \right) \left( \frac{\mu}{\rho D_s} \right)
\]

As the requirements of Equation 11 are approached the experimental points approach the curve; however, in no case are the conditions of Equation 11 satisfied.

The transition from streamline to turbulent flow is shown by Fowler and Brown to occur at a Reynolds number of about 2300. A marked change in the magnitude of mixing as measured by \(D/ud\) accompanies this transition.
Fig. 10.13. Collection efficiency, $\overline{\eta}$, as affected by superficial velocity of air, $v_4$; PVA filter, equivalent pore size, $d_e = 60-80 \mu$; thickness, $L = 0.5 \text{ cm}$.

Fig. 10.14. Collection efficiency, $\overline{\eta}$, as affected by superficial velocity of air, $v_4$; PVA filter (cont'd).

"Re-entrainment."
Appendix 5

Table 1. Experimental results.

<table>
<thead>
<tr>
<th>Dia of filter</th>
<th>Flow rate of air l/min</th>
<th>Spray volume of spore suspension ml/h</th>
<th>Dia. of Nozzle mm</th>
<th>V&lt;sub&gt;A&lt;/sub&gt; cm/sec</th>
<th>η %</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mmφ (△)</td>
<td>135.0</td>
<td>5.3</td>
<td>4.4</td>
<td>115.0</td>
<td>99.99956</td>
</tr>
<tr>
<td>100 mmφ (○)</td>
<td>86.38</td>
<td>5.7</td>
<td>3.5</td>
<td>73.5</td>
<td>99.99983</td>
</tr>
<tr>
<td>155 mmφ (×)</td>
<td>44.19</td>
<td>2.9</td>
<td>2.5</td>
<td>37.5</td>
<td>99.99984</td>
</tr>
<tr>
<td></td>
<td>22.86</td>
<td>1.5</td>
<td></td>
<td>25.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13.86</td>
<td>0.9</td>
<td></td>
<td>14.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.55</td>
<td>0.66</td>
<td></td>
<td>11.8</td>
<td></td>
</tr>
</tbody>
</table>

V<sub>A</sub> = Superficial air velocity  η = Collection efficiency

実験結果

1. 压力損失 ∆P について

Fig. 2 に示すごとく、空気流の見かけの流速 V<sub>A</sub> = 100 cm/sec 以下では、圧力損失 ∆P はきわめて低い値であり、∆P は V<sub>A</sub> ならびに P. V. A. フィルターの厚さ L に比例する。

2. 液滴の相当濃度について

形成した液滴の相当濃度すなわち一次の図表を各フィルターについて三相粒子変えても捕集効率 η はそれぞれ大体一定した値を示した。したがってこの相当の範囲では、圧力損失のいかんに関係なく捕集効率 η は常に一定である。

3. フィルターの径について

フィルターの経の大きさのいかんにかかわらず、空気流の速度が同じならば捕集効率 η も、略々一定の値になる。したがって Fig. 3 において△印、○印、×印を求める結果、3 種の図は完全に一致する。

4. 空気流速 V<sub>A</sub> と捕集効率 η の関係

\[ \eta = \frac{N_1 - N_3}{N_1} = 1 - \frac{N_1}{N_1} = 1 - e^{-x} = 1 - e^{-x} \]

ただし N<sub>0</sub> : フィルター前の空気中の液滴数, 座/m²
Appendix 6: Heat exchange optimisation program:

two liquid phases.

1000DEFPROCSTARTINVOER
2000PROCSTARTINVOER
3000PROCSTOFEIGENSCHAPPEN_BUIS
4000PROCSTOFEIGENSCHAPPEN_PIJP
5000ETAWAND_PIJP=ETA_PIJP
6500=M_PIJP*(TPJP_UIT-TPIJP_IN)+CP_PIJP
5700PRINT"TBIUS_UIT = ":FNTBIUS_UIT;TBIUS_UIT=FNTBIUS_UIT;INPUT"GEKO
ZEN GEMIDDELDE WAARDEN CORRECT J/N";ANTWOORD$:IF ANTWOOED$="N" OR
ANTWOORD$="" THEN PROCSTOFEIGENSCHAPPEN_BUIS
6000ETAWAND_BUIS=ETA_BUIS
7000PRWAND_PIJP=PA_PIJP
8000PROCSTOFVOLUMEAARBEIDSCHEDELING:
9000PRINT"RESULTATFILE NAAM ALSTUBLIEFT";FILE$
9500SCLASS"SPOOL" "-FILE$
1000PROCUITVOER
1050SPOOL
1100END

1000DEFPROCSTARTINVOER
1010PRINT"INVOERGEVEENS VAN DE INWENDIGE STROOM"
1020INPUT"MASSASTROOM PIJP (KG/S) = ":M_PIJP
1030INPUT"INGANGS TEMPERATUUR (C) = ":TPJP_IN
1035INPUT"UTGAANDE TEMPERATUUR(C) = ":TPJP_UIT
1040INPUT"VERWILINGSFATOR = ":A_PIJP
1050PRINT"INVOERGEVEENS VAN DE UITWENDIGE STROOM"
1060INPUT"MASSASTROOM BUIS (KG/S) = ":M_BUIS
1070INPUT"INGANGS TEMPERATUUR (C) = ":TB UIS_IN
1075INPUT"UTGAANDE TEMPERATUUR(C) = ":TB UIS_UIT
1080INPUT"VERWILINGSFATOR = ":A_BUIS;PRINT'
1100INPUT"GESCHAPTE WARMTEOVERDRACHT (W/M2/K) = ":U;PRINT'
1110ENDPROC
1120
1200DEFPROCSTOFVOLUMEAARBEIDSCHEDELING
1210PRINT"STARTGEVEENS VAN DE WARMTEWEZELKAPPAR"n
1220INPUT"INWENDIGE PIJDIAETER (M) = ":DUI
1230INPUT"INWENDIGE PIJDEDIAMETER (M) = ":DIN
1240INPUT"STEEL (M) = ":S
1250INPUT"SOORT STEEL (ONDIEK/VIERKANT)";S$
1260INPUT"MINIMALE PIJPLENGTHE/ ROMPOOIETER VERHOUDING = ":LODIMIN
1270INPUT"MAXIMALE PIJPLENGTHE/ ROMPOOIETER VERHOUDING = ":LOMAX
1280INPUT"MAXIMALE KEERSCHOTOPENING/ROMPOOIETER = ":BAFFLEOPEN
1290INPUT"BIJHOEDENDE ROMPOOIETER/KEERSCHOT VERHOUDING = ":DIB
1300INPUT"MINIMALE ROMPOOIETER/KEERSCHOT VERHOUDING = ":DIBMIN
1310INPUT"MAXIMALE KEERSCHOTAFSTAND (M) = ":BAFFLEPITC
HMINT
132INPUT"KEERSCHOTDIAMETER (M)" = ":KEERSCHOTDIAMETER
133INPUT"MAXIMALE DURKVAL AAN BUITENKANT PIJPEN (PA)" = ":OPBIJSMAX
135INPUT"MAXIMALE DURKVAL AAN BINNENKANT PIJPEN (PA)" = ":OPPIJPMAX
136ENDPROC
1370
140DEFPROCSTOFEGENSCHAPPEN_BUIG
1410PRINT"FYSISCHE GEGEvens UITWENDIGE FASe"
1420INPUT"GEMIDDELDE DICHTHEID (KG/M3)" = ":RHO_BUIS
1430INPUT"GEMIDDELDE VISCOSITEIT (PA.S)" = ":ETA_BUIS
1440INPUT"GEMIDDELDE WARMTECAPACITEIT (J/K/GG)" = ":CP_BUIS
1450INPUT"GEMIDDELDE WARMTEOVERDRACHT (J/M/K)" = ":LABDA_BUIS
1460INPUT"PRAANDTGL TGL UITWENDIGE FASe" = ":PA_BUIS
1470INPUT"WARMTEOVERDRACHT PIJPMAT. (J/M/K)" = ":LABDAWAND
1480ENDPROC
1490
150DEFPROCSTOFEGENSCHAPPEN_PIJP
1510PRINT"FYSISCHE GEGEvens INWENDIGE FASe"
1520INPUT"GEMIDDELDE DICHTHEID (KG/M3)" = ":RHO_PIJP
1530INPUT"GEMIDDELDE VISCOSITEIT (PA.S)" = ":ETA_PIJP
1540INPUT"GEMIDDELDE WARMTECAPACITEIT (J/K/GG)" = ":CP_PIJP
1550INPUT"GEMIDDELDE WARMTEOVERDRACHT (J/M/K)" = ":LABDA_PIJP
1560INPUT"PRAANDTGL TGL INWENDIGE FASe" = ":PA_PIJP
1570M_PID = ( PID_UIT- PID_IN) * CP_PIJP
1590PRINT"TBUIS_UIT = ":FNTBUIS_UIT;TBUIS_UIT=FNTBUIS_UIT;INPUT"GEKOJEN GEMIDDELDE WAARDEN CORRECT J/N";ANTWOORDS:IF ANTWAAORDS="N" OR ANTWAAORDS="n" THEN PROCSTOFEGENSCHAPPEN_UIT
1580ENDPROC
1590
160DEFPROC SHELLBEPALING
1610PRINT"F = ":FNP
1620PRINT"R = ":FNR
1630INPUT"AANtal GEWENSTE SHELLPASSES = ":NSHELL
1640INPUT"WAARDE VAN CORRECTIEFACTOR F = ":F
1650ENDPROC
1660
170DEFPROC WARMTEWISSELAARROODREKENING
1710AU=FNA
1720HOLDSET=O
1730PROC COLDI OPTIMALISATIE
1740HOLDSET1=FALSE
1760PROC DRUK_MANTEL
1780PROC WARMTE_OVERDRACHT
1790PROC COPPERVLAKTESREKENING
1800ENDPROC
1810
190DEFPROC COLDI OPTIMALISATIE
1910INPUT"ROMPDIAMETER (M)" = ":DI
1930INPUT"AANTAL PASSAGES PER SHELL = ":P PASSAGE
1940PROC COLDI OPTIMALISATIE1
1950ENDPROC
1960
200DEFPROC COLDI OPTIMALISATIE1
2006INPUT"AANTAL PIJPNEN = ":N
2010L=AU/PI/DUIT/NP/NSHELL
2020IF L/DI>LODIMAX THEN INPUT"TE LAND. VOLGENDE ROMPODIAMETER = ":DI:PRINT"AANTAL PIJPPASSAGE IS ":PPASSAGE:PROCOLDI_OPTIMALISATIE1:ENDPROC
2030DPIJP=X_PIJP/RHO_PIJP/PI/A4/DIN/2/NP/PPASSAGE
2040RE_PIJP=RHO_PIJP*CFIJP/DIN/ETA_PIJP
2050PRINT"REYNOLDS = ":RE_PIJP
2060INPUT"WEERSTANDSCOEFFICIENT = ":f
2070DPIJP=FNDR
2080IF DPIJP<DPPIJP_MAX AND HOLDS=O THEN PRINT":INPUT"AANTAL PIJPPASSAGES MAXIMAAL J/N":ANTWOORDS$:IF ANTWOORDS<>"N" AND ANTWOORDS<>"O" THEN HOLDSET=1:ENDPROC
2083IF DPIJP>DPIJP_MAX AND HOLDS=O THEN PPASSAGE=PPASSAGE+2:PROCOLDI_OPTIMALISATIE1:ENDPROC
2085IF HOLDSET=O THEN PRINT":INPUT"AANTAL PIJPPASSAGES MINIMAAL J/N":ANTWOORDS$:IF ANTWOORDS="N" OR ANTWOORDS="O" THEN HOLDSET=1:PRINT"VORIGE WAARDEN NEMEN.":PPASSAGE=PPASSAGE-2:PROCOLDI_OPTIMALISATIE1:ENDPROC
2090IF L/DI>LODIMIN AND HOLDSET=O THEN INPUT"KLEINERE MAXIMALE LENGTE/RROMPODIAMETER = ":LODIMAX:PROCOLDI_OPTIMALISATIE ELSE IF HOLDSET=O PRINT"IES EEN ANDERE W.W. CONFIGURATIE BIJvoorbeeld EXTRA PARALLEL: SUCCESS!":END
2095ENDPROC
2096
2100DEFPROCDRUK_MANTEL
2110PRINT"STEKEN IS ":S$:INPUT"CORRECTE TERM J/N":ANTWOORDS$:IF ANTWOORDS="N" OR ANTWOORDS="O" THEN INPUT"VERDERE TERM ALSTUBLIEFT ; ":S$
2120IF S$="VIERKANT" THEN DE=FNDE_VIERKANTE_STEKEN ELSE DE=FNDE_DRIEHOEK_STEKEN
2140PRINT"REYNOLDS = ":RE_BUIS;". LEES BIJBEHOORENDE FRICITIEFACTOR A F EN VOER IN: "::INPUTf_out
2150KEERSCHOTAANTAL=INT(L/B)
2160DPBUI=NSHELL*f_out*GS.2*DI*(KEERSCHOTAANTAL+1)/2/RHO_BUIS/DE/(ETA_BUIS/ETAWAND_BUIS).14:IF HOLDSET=TRUE THEN ENDPROC
2165PRINT"BIJELUK BEDRAAGT "::DPBUI:"PA."
2170IF DPBUI<DPBUI_MAX THEN IF B>BAFFLEPITCHMIN PRINT"KEERSCHOTAANST AAND TE KLEIN. KIES HOGEER WAARDE KEERSCHOTOPENING":PROCEERINVOER :HOLDSET=TRUE:PROCEERDRUK_MANTEL:ENDPROC
2180IF DPBUI<DPBUI_MAX AND DI<DI_MAX THEN PROCEERINVOER:PROCEERDRUK_MANTEL:ENDPROC
2190IF DPBUI<DPBUI_MAX AND DI=DI_MAX THEN ENDPROC
2200IF DPBUI<DPBUI_MAX AND HOLDSET=FALSE THEN IF DI=DI_MIN PROCEDAMTELSTOP ELSE PROCERERERUG:ENDPROC
2210
2300DEFPROCWARMTE_OVERDRACHT
2310PRINT"WARMTE OVERDRACHTSBEREKENING"  
2320X=1.82*LOG(HE_PIJP)-1.64)  
2330NU_PIJP=XI/B*(HE_PIJP-1000)*PR_PIJP/(1+12.7*SQRT(XI/B)*(PR_PIJP*(2/3)))*[(DI/N)(2/3)]*PR_PIJP/PRAWAND_PIJP)*11
2340 ALFA_PIJP=NU_PIJP*LABDA_PIJP/DIN
2350 PRINT"TJH VOOR RE BUIS VAN "RE_BUIS:" EN BAFFLECUT VAN "BAFFLECUT:	" IS ":" INPUTTJH
2360 ALFA_BUIS=JH*PH_BUIS/(1/3)*(ETA_BUIS/ETAWAND_BUIS)*12*LABDA_BUIS /
2380 DUIT/DIN/(1/ALFA_PIJP+R_PIJP) + DUIT + LN(DUIT/DIN)/2/ETAWAND
+H_BUIS+1/ALFA_BUIS)
2360 DUIT
2370 TPIJP_GEM=TPJP_IN+TPJP_UIT)/2: TBUIS_GEM=(TBUIS_IN+TBUIS_UIT)/2
2380 TWAND=TPJP_GEM+ALFA_BUIS/(DIN/DUIT*ALFA_PIJP+ALFA_BUIS)*(TBUIS_ GEM-TPIJP_GEM)
2390 INPUT"GEMIDDELDE WANDTEMPERATUUR BEDRAAGT "TWAND: "CELSIUS."
2400 INPUT"IS AANPAETING VAN PRAANDT EN VISCOSITEIT VOOR WAND NOODZak
2410 E1J=J/N",ANTWOORDS:IF ANTW0ORDS="J" THEN IF ANTW0ORDS="" THEN N ENDPROC
2420 INPUT"PRAANDTGETAL WANDEMPERATUUR BINNEN PIJP = ":PRANDT_PIJP
2430 INPUT"VISCOSITEIT AAN WAND UITWENDIGE FASE = ":ETAWAND_BUIS
2440 INPUT"VISCOSITEIT AAN Wand INwendige fase = ":ETAWAND_PIJP
2450 PROCWARMTEWISSELAARDOORDREKENING
2460 ENDPROC
2470
2500 DEF=PROCOPPERVLAKTEBEREKENING
2510 ABER=FNA
2520 PROCUPRUIF
2530 IF "Aber"="Aber""Aber"="Aber""Aber"="Aber" THEN PRINT "START MET BERKENDE
2540 TOTAAL WARMTEOVERDRACHTSCOEFFICIENT":PROCWARMTEWISSELAARDOORDREKENING 
2550 ENDPROC
2560 ABER="Aber" "1":L=L*1.1
2570 DPPIJP=FNDDP: IF DPPIJP>DPPIJPMAX THEN PRINT"PIJPDRUK NU ":DPPIJP:" PA.
2580 IS DIT ACCEPTABEL J/N": INPUTTANTWORDS:IF ANTW0ORDS="N" OR ANT
2590 TWOORDS="" THEN PRINT"NEEM BREDERE ROMP OF BREDERE PIJPEN":END
2600 IF (L/DI)>LDMAX THEN PRINT"TE GROTE LENGTE, NEEM KORTEER WARMTE
2610 WISSELAAR":ENDPROC
2620 HOLDSETI=TRUE:PROCDRUK_MANTEL
2630 DIFI DPBUIS>DPBUISSMAX THEN PRINT "TE GROTE Drukval IN SHELL, NEEM 
2640 BREDERE ROMP":ENDPROC
2650 ENDPROC
2660 PROCOPKEERINVORDER
2670 INPUT"KEERSCHOTOPENING = ":BAFFLECUT
2680 BIJEHOORENDE DIB = ":DIB
2690 ENDPROC
2700
2700PROCOPKEERTERUG
2710 INPUT"HOGERE WAARDE KEERSCHOTOPENING NEMEN, LAATSTE KERR DOORREK
2720 E1J=J/N",ANTWOORDS:IF ANTW0ORDS="J" OR ANTW0ORDS="" THEN HOLDSE
2730 TRUE:PROCKEERINVORDER:PROCDRUK_MANTEL:ENDPROC
2740 ENDPROC
2750 PROCOPKEERINVORDER:PROCDRUK_MANTEL:ENDPROC
2760
2770 PROCOPCUIF
2780 IF "PRINT"EINDRESULTATEN":
2790 ONPRINT"TEMPERATUUR UITGANG UITWENDIGE STROOM = ":TBUIS_UIT:" C.
2800 ONPRINT "SEMISCHEL TEMPERATUURVERSCHIL = ":FNT_VERSCHIL:" C."
2740PRINT "TOTALE WARMTEOVERDRACHT = " :U:" W/M²/°K."
2750PRINT "DRUKVAL BINNEN PIJLEN = " :OPPIJP:" PA."
2760PRINT "DRUKVAL BUITEN PIJLEN = " :OPBUIS:" PA."
2770PRINT "WARMTEWISSELAAR DIMENSIES":
2780PRINT "ROMPDIAMETER = " :DI:" M."
2790PRINT "AANTAL SHELLPARKAGES = " :NSHELL
2795PRINT "AANTAL PIJPSTUKKEN = " :NP
2810PRINT "LENGTE PIJLEN = " :L:" M."
2820PRINT "KEERSCHOTOPENING = " :BAFFLECUT
2830PRINT "AANTAL KEERSCHOTTEN = " :KEERSCHOTAANTAL
2840PRINT "TOTAAL WARMTEWISSELEND OPPERVLAK = " :Aber:" M²."
2850ENDPROC
2860
2900DEFPIJP=PIJP*2
2910PRINT "UITWENDIGE DRUK TE GROOT, KIES ANDERE W.W. CONFIGURATIE B
     V. GROTERE PIJPSTEEK, OPSPLITSGING AFVOER, SUCCES!":END
2920ENDPROC
2930DEFPIJPTUBUS_UIT=TBUS_IN-Q/CP_BUIS/M_BUIS
2940DEFPIJTP=TW_IN-TK_IN)/(TW_IN-TK_IN)
2950DEFPIJP=(TW_IN-TW_UIT)/(TW_UIT-TK_IN)
3000DEFPIJP=VERSCHIL IF (TW_IN-TW_UIT)*(TW_UIT-TK_IN) THEN TMAX=(TW_IN
     -TW_UIT), TMIN=(TW_UIT-TK_IN) ELSE TMAX=(TW_UIT-TK_IN), TMIN=(TW_IN
     -TW_UIT)
3020=ABS(TMAX-TMIN)/LN(TMAX/TMIN)
3030DEFPIJP=ABS(Q/U/PIU_VERSCHIL)
3040DEFPIJP=PIU_VIERKANTE_STEEK =4*(S²-PI/4*DUIT²)/PI/BUIT
3050DEFPIJP=PIU_DRIEHOEK_STEEK =4*(.43*S²-PI/8*DUIT²)/.5/PI/BUIT
3060DEFPIJP=PIU_PIJP<2100 THEN PHI=(ETAPIJP/ETAWAND_PIJP)*.25 ELSE
     PHI=(ETAPIJP/ETAWAND_PIJP)*.14
3070=PPASSAGE+NSHELL*(PIL/DIN/PHI+4)*.5*RHO_PIJP*PIJP²
**INVOERGEGEVENS VAN DE INWENDIGE STROOM**

- Massastrroom pijp (kg/s) = 76.9
- Ingaaande temperatuur (°C) = 726
- Uitgaande temperatuur (°C) = 755
- Vervuilingsfaktor = 78.8E-4

**INVOERGEGEVENS VAN DE UITWENDIGE STROOM**

- Massastrroom buis (kg/s) = 76.529
- Ingaaande temperatuur (°C) = 720
- Vervuilingsfaktor = 78.8E-4

**GESCHATTE WARMTEOVERDRACHT (W/M2/K) = 7450**

**STARTGEGEVENS VAN DE WARMTEWISSELAAR**

- Uitwendige pijpdiameter (m) = 7.25E-2
- Inwendige pijpdiameter (m) = 7.2E-2
- Steek (m) = 7.32E-2
- Soort steek ( driehoek/vierkant)? driehoek
  - Minimale pijplengte/rompdiameter verhouding = 1
  - Maximale pijplengte/rompdiameter verhouding = 10
  - Maximale keerschotopening/rompdiameter = 0.45
  - Bijbehorende rompdiam./keerschot verhouding = 1
  - Minimale rompdiameter/keerschot verhouding = 1
  - Maximale rompdiameter/keerschot verhouding = 5
  - Minimale keerschotafstand (m) = 75E-2
  - Keerschot dikte (m) = 7.2E-3
  - Maximale duikwa aan buitenkant pijpen (Pa) = 750000
  - Maximale duikwa aan binnenkant pijpen (Pa) = 750000

**FYSISCHE GEGEVENS UITWENDIGE FASE**

- Gemiddelde dichtheid (kg/m³) = 7984.7
- Gemiddelde viscositeit (Pa.s) = 7.4885E-4
- Gemiddelde warmtecapaciteit (J/K/kg) = 74181
- Gemiddelde warmteoverdracht (J/M/K) = 7.06489
- Prandtlgetal uitwendige fase = 73.148
- Warmteoverdracht pijpmat. (J/M/K) = 711

**FYSISCHE GEGEVENS INWENDIGE FASE**

- Gemiddelde dichtheid (kg/m³) = 7955.3
- Gemiddelde viscositeit (Pa.s) = 7.3149E-4
- Gemiddelde warmtecapaciteit (J/kg/kg) = 74181
- Gemiddelde warmteoverdracht (J/M/K) = 706763
- Prandtlgetal inwendige fase = 71.947

**TBUIS_UIT = 93.9776382**

**Gekozen gemiddelde waarden correct? J/N? J**

**TBUIS_UIT = 93.9776382**
GEKOZEN GEMIDDELDE WAARDEN CORRECT J/N?

\[ \rho = 0.704548935 \]
\[ \mu = 0.946231884 \]

AANTAL GEWENSTE SHELLPASSES \( = ?2 \)
WAARDE VAN CORRECTIEFACTOR \( F = ?0.75 \)

ROMPDIAMETER \( (M) = ?0.75 \)

AANTAL PASSAGES PER SHELL \( = ?4 \)
AANTAL PIJPEN \( = ?368 \)
REYNOLDS \( = 15162.4271 \)
WEERSTANDSCOEFFICIENT \( = ?0.03528 \)

AANTAL PIJPPASSAGES MAXIMAAL J/N?

AANTAL PIJPEN \( = ?338 \)
REYNOLDS \( = 24762.307 \)
WEERSTANDSCOEFFICIENT \( = ?0.03056 \)

AANTAL PIJPPASSAGES MAXIMAAL J/N?

AANTAL PIJPEN \( = ?298 \)
REYNOLDS \( = 37448.1421 \)
WEERSTANDSCOEFFICIENT \( = ?0.02736 \)

AANTAL PIJPPASSAGES MAXIMAAL J/N?

STEEK IS DRIEHOEK
CORRECTE TERM J/N?

REYNOLDS \( = 2161.9488 \), LEES BIJBEHOORENDE FRICTIEFACTOR AF EN VOER IN: \( ?0.31 \)
BUISDRUK BEDRAAGT 202.007518 PA.
KEERSCHOTOPENING \( = ?25 \)
BIJBEHOORENDE DIB \( = ?2 \)

STEEK IS DRIEHOEK
CORRECTE TERM J/N?

REYNOLDS \( = 4335.48982 \), LEES BIJBEHOORENDE FRICTIEFACTOR AF EN VOER IN: \( ?0.432 \)
BUISDRUK BEDRAAGT 2075.470445 PA.
KEERSCHOTOPENING \( = ?0.15 \)
BIJBEHOORENDE DIB \( = ?5 \)

STEEK IS DRIEHOEK
CORRECTE TERM J/N?

REYNOLDS \( = 10926.6061 \), LEES BIJBEHOORENDE FRICTIEFACTOR AF EN VOER IN: \( ?0.63 \)
BUISDRUK BEDRAAGT 39698.49 PA.

WARMTEOVERDRACHTSBEREKENING
JH VOOR RE BUIS VAN 10926.6061 EN BAFFLECUT VAN 0.15 IS ?70

GEMIDDELDE WANDELTEMPERatuUR BEDRAAGT 74.9889664 CELSIUS.
IS AANPASSING VAN FRANTIL EN VISCOSEITTE VOOR WAN NODDZAELIJK J/N?
PRANDTLGETAL WANDTEMPERATUUR BINNEN PIJP = 2.376
VISCOSITEIT AAN WAND UITWENDIGE FASE = 3.784E-4
VISCOSITEIT AAN WAND INWENDIGE FASE = 3.784E-4
ROMDIAMETER (M) = 0.75
AANTAL PASSAGES PER SHELL = 8
AANTAL PIJPN = 298
REYNOLDS = 37448.1421
WEERSTANDSCOEFFICIENT = 0.02736

AANTAL PIJPPASSAGES MAXIMAAL J/NJ

STEKE IS DRIEHOEK
CORRECTE TERM J/NJ

REYNOLDS = 10926.6061. LEES BIJBEHORENDE FRICvisión Factor AF EN VOER IN: 0.53
DRUK VAL BEDRAAGT 46816.2131 PA.

WARMTEOVERDRACHTSBEREKENING
JH VOOR RE BUIS VAN 10926.6061 EN BaffleCut VAN 0.15 IS 770

GEMIDDELDE WANDTEMPERATUUR BEDRAAGT 74.4831461 CELSIUS.
IS AANPASSING VAN PRANDTL EN VISCOSITEIT VOOR WAND NOODZAKELIJK J/N?

EINDRESULTATEN

TEMPERATUUR UITGANG UITWENDIGE STROOM = 93.9776382 C.
GEMIDDELD TEMPERATUURVERSCHIL = 24.7284016 C.
TOTALE WARMTEOVERDRACHT = 350.168804 W/M²/K.
DRUK VAL BINNEN PIJPN = 31141.9251 PA.
DRUK VAL BUITEN PIJPN = 46816.2131 PA.

WARMTEWISSELAAR DIMENSIES

ROMDIAMETER = 0.75 M.
AANTAL SHELLPASSAGES = 2
AANTAL PIJPPASSAGES = 8
AANTAL PIJPN = 298
LENGTE PIJPN = 4.86072465 M.
KEERSCHOTPENEN = 0.15
AANTAL KEERSCHOTEN = 32
TOTAAL WARMTEWISSELEND OPPERVLAK = 226.738443 M².
Au=227.061114 Aber=226.738443 Au-Aber=0.322670877

STEKE IS DRIEHOEK
CORRECTE TERM J/NJ

REYNOLDS = 10926.6061. LEES BIJBEHORENDE FRICvisión Factor AF EN VOER IN: 0.53
TE GROTE DRUK VAL IN SHELL. NEEM BREDERE ROMP

EINDRESULTATEN
TEMPERATUUR UITGANG UITWENDIGE STROOM = 93.9776382 C.

GEMIDDELD TEMPERATUURVERSCHIL = 24.7284016 C.

TOTALE WARMTEOVERTOEHRAFCT = 360.168804 W/M²²/K.

DRUKVAL BINNEN PIJPEN = 33103.6372 PA.

DRUKVAL BUITEN PIJPEN = 52490.9056 PA.

WARMTEWISSELAAR DIMENSIES

ROMPDIAMETER = 0.75 M.

AANTAL SHELLPASSAGES = 2

AANTAL PIJPPASSAGES = 8

AANTAL PIJPEN = 298

LENGTE PIJPEN = 5.33579712 M.

KEERSCHOTOPENING = 0.15

AANTAL KEERSCHOTEN = 36

TOTAAL WARMTEWISSELEN OPPEVLAK = 226.738443 M²².

Au=227.061114   Aber=226.738443   Au-Aber=0.32267087

STEKEN IS DRIEHOEK
CORRECTE TERM J/N?

REYNOLDS = 10926.6061 . LEES BIJBEHOERDE FRICHTIEFACTOR AF EN VOER IN: ?0.53
TE GROTE DRUKVAL IN SHELL, NEEM BREDERE ROMP
RESULTAATFILE NAAM ALSTUBLIEFT?RESULTATEN

EINDRESULTATEN

TEMPERATUUR UITGANG UITWENDIGE STROOM = 93.9776382 C.

GEMIDDELD TEMPERATUURVERSCHIL = 24.7284016 C.

TOTALE WARMTEOVERTOEHRAFCT = 360.168804 W/M²²/K.

DRUKVAL BINNEN PIJPEN = 33103.6372 PA.

DRUKVAL BUITEN PIJPEN = 52490.9056 PA.

WARMTEWISSELAAR DIMENSIES

ROMPDIAMETER = 0.75 M.

AANTAL SHELLPASSAGES = 2

AANTAL PIJPPASSAGES = 8

AANTAL PIJPEN = 298

LENGTE PIJPEN = 5.33579712 M.

KEERSCHOTOPENING = 0.15

AANTAL KEERSCHOTEN = 36

TOTAAL WARMTEWISSELEN OPPEVLAK = 249.412287 M²².
Biomass grows during fermentation and consumes substrate in the process. The fermenter must be able to supply the biomass with all the required nutrients like minerals, oxygen (in the case of aerobic growth) and substrate. Therefore, it is essential to know the absolute amount of biomass present in the fermenter and also its growth rate. The required calculations to obtain a value for the biomass are presented here.

Three growth regions are distinguished during fermentation:

- **exponential phase**: 
  \[ t = 0 \text{ to } t_1; \quad r_x = \mu_{\text{max}} \frac{C_x}{C_x + m_s C_x} \]
  \[ r_s = l / Y_{sx} \mu_{\text{max}} C_x + m_s C_x \]  \(1\)

- **linear phase**: 
  \[ t = t_1 \text{ to } t_2; \quad r_x = \frac{(C_x,2 - C_x,1)}{(t_2 - t_1)} \]
  \[ r_s = l / Y_{sx} (C_x,2 - C_x,1) / (t_2 - t_1) + m_s C_x \]  \(2\)

- **stationary phase**: 
  \[ t = t_2 \text{ to } t_3; \quad r_x = 0 \]
  \[ r_s = m_s C_x \]  \(3\)

The total amount of substrate consumed, \(n_{s,\text{total}}\), is known. A certain amount of biomass, \(n_{x,\text{total}}\), is produced from the substrate. A precondition is that the final amount of biomass in the main fermentation is eleven times the initial amount, thus:

\[ n_{x,\text{total}} = 11 n_{x,0} \]

The total volume in the fermenter during fermentation is considered constant. In that case:

\[ n_x = C_x V_1 \]

\[ V_1 r_s = - \frac{d n_s}{dt} \]

\[ V_1 r_x = \frac{d n_x}{dt} \]

Equations 1, 2 and 3 must be integrated to calculate the substrate consumption for the individual growth phases and the total substrate consumption during the process. Substrate consumption is a function of the
initial amount of biomass and of the time durations of the phases. The following outline describes the integration of equations 1, 2 and 3.

exponential growth: \( n_x(t) = n_{x,0} e^{\mu_{\text{max}} t} \)

\[
\int_0^s n = \int_0^{t_1} \left( \frac{1}{Y_{sx}} + m_s \right) n_{x,0} e^{\mu_{\text{max}} t} \, dt = \left( \frac{1}{Y_{sx}} + m_s \right) n_{x,0} \left[ e^{\mu_{\text{max}} t_1} - 1 \right]
\]

\[
\Rightarrow n_s,1 = \left( \frac{1}{Y_{sx}} + m_s \right) n_{x,0} \left( e^{\mu_{\text{max}} t_1} - 1 \right)
\]

linear growth: \( t = t_1 \quad n_{x,1} = n_{x,0} e^{\mu_{\text{max}} t_1}; \quad t = t_2 \quad n_{x,2} = 11 n_{x,0} \)

\[
\hat{n}_x = \frac{d n_x}{dt} = \frac{11 n_{x,0} - n_{x,1}}{t_2 - t_1} \Rightarrow \int_{n_{x,1}}^{n_{x,2}} \frac{11 n_{x,0} - n_{x,1}}{t_2 - t_1} \, dt = \int_{n_{x,1}}^{n_{x,2}} \hat{n}_x \left[ \frac{1}{Y_{sx}} + m_s \left( \frac{n_{x,1}}{n_x} + t - t_1 \right) \right] \, dt
\]

\[
\Rightarrow n_s,2 = \hat{n}_x \left[ \frac{n_{x,1}}{Y_{sx}} \left( 1 + m_t t + \left( \frac{1}{8} t_2 - t_1 \right) \right) \frac{t_z}{n_x} \right]
\]

\[
= \hat{n}_x \left( t_2 - t_1 \right) \left( \frac{1}{Y_{sx}} + m_s \frac{n_{x,1}}{n_x} \right) + m_s \left( \frac{1}{8} \left( t_2^2 - t_1^2 \right) - t_1 t_2 + \frac{t_2}{2} \right)
\]

\[
= \hat{n}_x \left( t_2 - t_1 \right) \left( \frac{1}{Y_{sx}} + m_s \frac{n_{x,1}}{n_x} \right) + m_s \left( \frac{1}{8} \left( t_2^2 + t_1^2 \right) - t_1 t_2 \right)
\]

stationary growth: \( t = t_2 \quad n_{x,2} = 11 n_{x,0}; \quad \hat{n}_x = 0; \quad t = t_3 \quad n_{x,3} = n_{x,2} \)

\[
\int_0^{t_3} n = \int_{n_{x,0}}^{n_{x,2}} m_s \, dt = \Rightarrow n_s,3 = m_s \int_{n_{x,0}}^{n_{x,2}} (t_3 - t_z)
\]

Equation 4 is rewritten to obtain \( t_1 \) as a function of \( n_s,1 \) and \( n_{x,0} \):
The biomass concentration is calculated with $t_1$. Besides a starting value for $n_{x,0}$, values for total time $t_3$ and the duration of the stationary phase can be prescribed:

$$t_{\text{stat}} = t_3 - t_2$$

At the end of one iteration cycle the total calculated substrate consumption is:

$$n_{s,1} + n_{s,2} + n_{s,3}$$

is verified with the total amount of substrate based on the PQQ production. If the deviation between the amounts is too big, a new initial biomass concentration is estimated by:

$$n_{x,0,\text{new}} = n_{x,0,\text{old}} \frac{n_{s,PQQ}}{n_{s,1} + n_{s,2} + n_{s,3}}$$

The iteration is repeated until the deviation between both substrate amounts is small enough. This explanation probably is somewhat disturbing, but a program which calculates several parameters for the main fermentation uses this iteration. Hopefully, if this program is considered, the above is clear to the reader.
**Appendix 8**

10REM "BEREKENING VAN STARTBiomassa IN HOOFDFERMENTOR"
15@%=G0050A
16VDU2
20PROCinvoer
25NxO=Nxstart
30PROCiteratie
35PROCuitvoer
40PROCzuurstofberekeninginvoer
50PROCzuurstofberekening
70PROCcroervermogenberekening
80END
90DEFPROCinvoer
100INPUT "Yield biomassa op substraat = ";Ysx
110INPUT "Maximale groeisnelheid (1/uur) = ";mumax
120INPUT "Substraatverbruik exponentiële groei = ";Ns1
130INPUT "Maintenance biomass op substraat (1/uur) = ";ms
140INPUT "Totale substraatverbruik fermentatie = ";Nstotaal
150INPUT "Totale fermentatietijd (uur) = ";ttotaal
160INPUT "Tijd duur stationaire fase (uur) = ";tstat
170INPUT "Startwaarde van biomassa voor iteratie = ";Nxstart
180ENDPROC
190DEFPROCiteratie
200N1=LN(Ns1*mumax/(mumax/Ysx+ms))/NxO+1)/mumax
210DNxDt1=EXP(mumax*t1):DNxDt1=mumax*Nx1
220DNxO=DNxDt1*((1+Ns0-Nx1)/(t2-t1)):IF DNxO>DNxDt2 THEN DNxO-DNxDt1 ELSE DNxO-DNxDt2
230DNs2=DNxDt2*((1/Ysx+ms/N1)*DNxO/Nx1)+ms*(.59*(t1^2+t2^2)-t1^2)
240DNs3=ms*N11*NxO^tstat
250Nsgeschat=Ns1+Ns2+Ns3:IF Nsgeschat<N THEN INPUT "Gaarne nieuw schatting voor biomassa";NxO
260IFABS(1-Nsgeschat/Nstotaal».001 THEN NxO=NxO*Nstotaal/Nsgeschat
270ENDPROC
280DEFPROCuitvoer
290PRINT "Resultaten van biomassa berekening in Hoofdfemtmentor"/
300PRINT "Tijd duur exponentiële groei = ";t1;" uur."
310PRINT "Beginhoeveelheid biomassa = ";NxO;" mol DM."
320ENDPROC
330DEFPROCzuurstofberekeninginvoer
340INPUT "Reductiegraad substraat = ";gs
350INPUT "Reductiegraad biomassa(NH3 als N-bron) = ";gx
360INPUT "Reactortemperatuur in C = ";temp
370INPUT "Schatting voor volume beslag": Vliquid
380INPUT "Uitputtingsfractie zuurstof van ingaande luchtstroom":uitputting
390INPUT "GESchatte methanolconcentratie in beslag in molen/m^3 = ";Gmethanol
400ENDPROC
410DEFPROCzuurstofberekening
420REM Zuurstofverbruik aan einde exponentiële fase
430DNODt1=.25*(gs/Ysx-gx)*mumax+gs*ms)*N1
440DNODt2=.25*(gs/Ysx-gx)*DNODt2+gs*ms)*N1
450IF DNODt1>DNODt2 THEN DNODtmax=DNODt1 ELSE DNODtmax=DNODt2
Berekening van de zuurstofverbruikstijd voor maximale r0

Zuurstofdebit = DNODtmax = 8,314( temp+273,15)/101325: Luchtdebit = zuurstofdebit/uutputting = 100/21

Percgemiddeld = 21-21/2 uitputting

Cosat: Casat = Percgemiddeld / 2

PRINT "Luchtinrroom met temperatuur van 30C en druk van 1 atm bedraagt": "Luchtdebit = " m^3/uur.

PRINT "Maximale zuurstofverbruik bedraagt ", DNODtmax = " mol/uur."

PRINT "Gewenste KIA bedraagt ", KIA = " s^-1."

PRINT "Zuurstofverbruikstijd tijdens de grootste zuurstofbenoefte is ", "zuurstofverbruikstijd" = " s."

KIA20 = KIA*1,022^10: REM Omrekening van KIA bij 30C naar 20C

tsubstraat = Vliquid*Cmethanol/(ONxDtmax/Ysx+ms*1000*X0)*3600

PRINT "Suistofverbruikstijd bedraagt ", tsubstraat = " s."

PRINT "Zuurstofverbruikstijd bij maximale zuurstofbenoefte bedraagt ", PgV = " m^3."

PROC tabelhoofd

Corr = 1: D = DIAMETER: HR = O/4

PROC optimalisatie: PROCuitvoer1: INPUT "Correctiefactor i.v.m. aeratie 

CORR

350 FOR D = DIAMETER TO T/2 STEP 0,5

360 IF antwoord$ ="J" OR antwoord$ ="j" THEN PROCroervermogenberekening

2200ENDPROC

2260DEFPROCuitvoer1

2270PRINT "HR TAB (15) " HR TAB (25) " N TAB (35) " P ONBEL. " TAB (45) " TM TAB (55) " V TIP TAB (65) " AERATIEGETAL"

2280PRINT "TAB (S) " (M) " TAB (15) " (M) " TAB (25) " (S-1) " TAB (35) " (W/M-3) " TAB (45) " (S) " TAB (55) " (M/S) " TAB (65) "(-)"

2290ENDPROC

2290ENDPROC
Yield biomassa op substraat = 70.67%
Maximale groeisnelheid (1/uur) = 70.5
Substraatreduktie exponentiële groei = 1500
Maintenance biomassa op substraat (1/uur) = 70.05
Totale substraatreduktie fermentatie = 18122.5
Totale fermentatietijd (uur) = 750
Tijdsduur stationaire fase (uur) = 78
Startwaarde van biomassa voor iteratie = 7500

Resultaten van biomassa berekening in Hoofdfermentor

Tijdsduur exponentiële groei = 2.0064 uur.
Beginhoeveelheid biomassa = 545.39 mol DM.

Reductiegraad substraat = 76.
Reductiegraad biomassa NH₃ als N-bron = 74.2
Luchtzuurstofverzadiging voor gebruikte temperatuur = 70.245
Reactorstemperatuur in C = 30
Schenning voor volume beklag = 50.5
Uitputtingsfractie zuurstof van ingaande luchtstroom = 0.2
Schatte methanolconcentratie in beslag in molen/m³ = 0.312

Luchtinstroom met temperatuur van 30°C en druk van 1 atm bedraagt 589.63 m³/uur.

Maximale zuurstofverbruik bedraagt 995.59 mol/uur.

Gewenste KIA bedraagt 7.4508E-2 s⁻¹.
Zuurstofverbruikstijd tijdens de grootste zuurstofbehoefte is 26.843 s.
Substraatreduktiestijd bedraagt 40.232 s.
H/T verhouding bedraagt 1.5
Powernumber bedraagt 12
D/T verhouding bedraagt 0.25716
Viscositeit bedraagt 0.001
Gewenste mengtijd bedraagt 15
Superficiele gassnelheid bedraagt 1.7026E-2 m/s.

Minimaal begast vermogen bij maximale zuurstofbehoefte bedraagt 1413.2 W/m³.

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<th>D (M)</th>
<th>HR (M)</th>
<th>N (S⁻¹)</th>
<th>P (W/M³)</th>
<th>ONBEL. (W/M³)</th>
<th>TM (S)</th>
<th>V TIP (M/S)</th>
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Appendix 9: average oxygen percentage

During fermentation, oxygen from the injected air is being consumed by the organisms. The consumption causes a decrease in the level of oxygen in the air while its bubbles up through the liquid. Oxygen transfer from air into the liquid is related to the oxygen concentration in air. Injected air contains about 21 mol% oxygen. If the amount of oxygen present in the inflowing air is depleted with a fraction $x_g$, then the mole percentage of the outflowing air is lower. The average mole percentage of oxygen in air is estimated by adding the percentages of inflowing and outflowing air and dividing the sum by two.

The composition of air changes during its flow through the broth. The outflowing air is expressed in terms of flow of several components, the components being inflowing air, produced carbon dioxide, water vapor, and consumed oxygen:

$$\phi_{\text{air, out}} = \phi_{\text{air, in}} + \phi_{\text{CO}_2} + \phi_{\text{vap, H}_2\text{O}} - \phi_{O_2}$$

The oxygen percentage in the outflowing air is expressed by:

$$\text{mole\% } O_2_{\text{out}} = \frac{0.21 (1-x_g) \phi_{\text{air, in}}}{\phi_{\text{air, out}} + \phi_{\text{CO}_2} + \phi_{\text{vap, H}_2\text{O}} - \phi_{O_2}}$$

It is assumed that the total molar flows of inflowing and outflowing air are approximately equal. In that case, the average mole percentage of oxygen is:

$$\text{mole\% } O_2 = \frac{\text{mole\% } O_2_{\text{in}} + \text{mole\% } O_2_{\text{out}}}{2} = \frac{21 + 21 (1-x_g)}{2} = 21 (1 - \frac{1}{2} x_g)$$
Appendix 10: Mass change during fermentation

1. Water loss due to vaporization.

The air utilized for broth aeration originates from the surroundings. The pre-conditions for surrounding air are a pressure of 1 bar (unfortunately most of the calculations are based on 1 atm), a temperature of 25 °C, and a humidity of 70%. A humidity of 70% means that the available water vapor estimates a fraction, $H_r$, of 0.7 of its saturated value. It is assumed that humid air of surrounding conditions can be regarded as an ideal gas. In that case pressure and molarity are linearly related to each other by:

$$p \frac{\phi_v}{\phi_m} = RT \Rightarrow \frac{\phi_m}{\phi_v} = \frac{p}{RT} \quad (1)$$

In this case the water vapor flow to the fermenter estimates:

$$\phi_{mol,H_2O}^{25} = 0.7 \frac{P_{H_2O, sat}}{P_{total}} \phi_{mol,air \ in}^{25} = 0.7 \frac{P_{H_2O, sat}}{P_{total}} (\phi_{mol,air \ in}^{dry} + \phi_{mol,H_2O}^{25})$$

$$\Rightarrow \phi_{mol,H_2O}^{25} = \frac{\phi_{mol,air \ in}^{dry} \frac{25}{P_{total}}}{1 - 0.7 \frac{P_{H_2O, sat}}{P_{total}}} \quad (2)$$

If it is assumed that the outflowing air is saturated with water vapor, the water vapor outflow is estimated to be at a temperature of 30 °C and a total pressure of 1.1 atm:

$$\phi_{mol,H_2O}^{30} = \frac{0.7 \frac{P_{H_2O, sat}}{P_{total}} \phi_{mol,air \ out}^{30}}{1 - 0.7 \frac{P_{H_2O, sat}}{P_{total}}} \quad (3)$$

Using equations 1 to 3, it is possible to calculate a value for the amount of water lost during pre- and main fermentation by:
In the following the water loss for both fermenters is calculated.

**Pre-fermenter**

From the pre-fermentation program a dry air flow of 210.5 m³/hr is estimated at 30 °C and 1 atm. The corresponding molar flow is calculated with equation 1:

\[
\dot{n}_{\text{dry}, \text{mol, air in}} = \frac{210.5 \times 101325}{8.314 \times 303.15} = 8463 \text{ mole/hr}
\]

The water vapor pressures at respectively 25 and 30 °C are [30]:

\[
P_{H_2O, \text{sat}}^{25} = 3170 \text{ Pa} \quad ; \quad P_{H_2O, \text{sat}}^{30} = 4243 \text{ Pa}
\]

It is assumed that the inflowing and outflowing dry air have approximately the same molar flow:

\[
\dot{n}_{\text{dry}, \text{mol, air in}} = \dot{n}_{\text{dry}, \text{mol, air out}}
\]

The water vapor in- and outflows are:

\[
\dot{n}_{\text{mol, } H_2O}^{25} = \frac{0.7 \times \frac{3170}{101325} \times 8463}{1 - \frac{3170}{101325}} = 189.5 \text{ mole/hr}
\]

\[
\dot{n}_{\text{mol, } H_2O}^{30} = \frac{4243}{1.1 \times 101325} \times 8463 = 334.9 \text{ mole/hr}
\]

Thus, the water loss during fermentation due to aeration estimates 145.4 mole/hr or 2908 moles in 20 hours time. This is equal to about 52 kg loss during a period of 20 hours. The total air inflow, including water vapor, is 8653 mole/hr or 212 m³/hr.

**Main fermentation**

From the main fermentation program a dry air inflow of 589.6 m³/hr is estimated at 30 °C and 1 atm. This is equal to 23703 mole/hr. Applying equations 2 and 3 the water vapor flows are:
Consequently, the water loss estimates 407.7 mole/hr or 20365 mole during 50 hours. This is equal to a total water loss of 367 kg. The total humid air inflow estimates 24234 mole/hr or 593 m$^3$/hr.

2. Methanol supplementation.

Total methanol supplementation for respectively the pre- and main fermenter are 1063 and 18123 mole/hr respectively; these values are equal to 34.0 and 581 kg.

3. Carbon dioxide production

The total carbon dioxide production is estimated rather simply. Only two carbon sources are present in the process besides carbon dioxide, namely biomass and methanol. It is evident that the difference between ethanol consumption and biomass production is equal to the carbon dioxide production. Thus:

\[ r_{CO_2} = r_{\text{methanol}} - r_{\text{biomass}} \]

If equation 4 is accumulated over the total time the result is equal to the total amount of carbon dioxide that has escaped from the system:

\[ n_{CO_2} = n_{\text{methanol}} - n_{\text{biomass}} \]

pre-fermentation

The total amount of biomass produced is twenty times the value of the initial amount of biomass. Thus, the increase of biomass estimates nineteen times the initial amount. In that case:

\[ n_x = 2.5 \times 19 \times 10.91 = 518 \text{ mole} \]
\[ n_{CO_2} = 1063 - 518 = 545 \text{ moles} \]

or 24.0 kg of carbon dioxide is produced.
During fermentation, totally ten times the initial amount of biomass or 5454 moles is produced. Then,

\[ n_{CO_2} = 18123 - 5454 = 12669 \text{ moles} \]

or 557.4 kg of carbon dioxide is produced.

4. Oxygen consumption

The total oxygen consumption is estimated from the growth kinetics on oxygen. The rate of oxygen consumption for the exponential, linear and stationary phases are expressed by:

**exponential phase:**

\[
r_O = \left[ \frac{1}{\mu_{ox}} \frac{\mu_{\text{max}}}{\mu_{\text{max}}} \right] C_{x,0} e^{\mu_{\text{max}} t} = \int e^{\mu_{\text{max}} t} \]

\[
n_{O_2,1} = V_1 \left( \frac{1}{\mu_{ox}} \frac{\mu_{\text{max}}}{\mu_{\text{max}}} \right) C_{x,0} \int_0^t e^{\mu_{\text{max}} t} \]

\[
= V_1 \left( \frac{1}{\mu_{ox}} \frac{\mu_{\text{max}}}{\mu_{\text{max}}} \right) C_{x,0} \left( e^{\mu_{\text{max}} t} - 1 \right)
\]

**linear phase:**

\[
r_O = \frac{1}{\mu_{ox}} r_x + m_o C_{x} , \text{with } r_x = \frac{C_{x,2} - C_{x,1}}{t_z - t_1}\text{ and } C_{x} = C_{x,1} + r_x (t - t_1)
\]

\[
n_{O_2,2} = V_1 \int_{t_1}^{t_z} \left( \frac{1}{\mu_{ox}} r_x + m_o \{ C_{x,1} + r_x (t - t_1) \} \right) \]

\[
= V_1 \left[ \left( \frac{1}{\mu_{ox}} r_x (t_z - t_1) + m_o \{ C_{x,1} (t_z - t_1) + r_x (\frac{1}{2} [t_z^2 - t_1^2] - t_1 [t_z - t_1]) \} \right) \]

\[
= V_1 \left[ \left( \frac{1}{\mu_{ox}} (t_z - t_1) + m_o \{ \frac{1}{2} (t_z^2 + t_1^2) - t_1 t_z \} \right) r_x + m_o C_{x,1} (t_z - t_1) \right] \]

\[
= V_1 \left[ \left( \frac{1}{\mu_{ox}} (t_z - t_1) + \frac{1}{2} m_o (t_z - t_1)^2 \right) r_x + m_o C_{x,1} (t_z - t_1) \right] \]

\[
= V_1 (t_z - t_1) \left[ \left( \frac{1}{\mu_{ox}} (t_z - t_1) + \frac{1}{2} m_o \right) \right] \left( C_{x,2} - C_{x,1} \right) + m_o C_{x,1}
\]

**stationary phase:**
The totally consumed oxygen is simply calculated by summation of the amounts estimated for the various phases.

Pre-fermentation

During pre-fermentation only the first two phases are of interest. The following parameters are used:

\[
\begin{align*}
\tau_1 &= 2.72 \text{ hr} \\
\tau_2 &= 20 \text{ hrs} \\
V_1 &= 2.5 \text{ m}^3 \\
C_{x,0} &= 10.91 \text{ mole/m}^3 \\
C_{x,1} &= C_{x,0} e^{\frac{\tau_1}{\tau_2}} = 42.5 \text{ mole/m}^3 \\
C_{x,2} &= 218.2 \text{ mole/m}^3 \\
x_{O_2} = \frac{4 \times 0.67}{(6 - 4.2 \times 0.67)} &= 0.84 \\
\mu_{\text{max}} &= 0.5 \text{ hr}^{-1} \\
\end{align*}
\]

During the pre-fermentation a stationary phase does not occur, hence equation 8 is not applicable. The above parameters are used in equations 6 and 7, from which the following answers are calculated for the pre-fermentation:

\[
\begin{align*}
n_{O_2,1} &= 105.7 \text{ mole} \\
n_{O_2,2} &= 944.1 \text{ mole} \\
n_{O_2,\text{total}} &= 1049.8 \text{ mole} \\
m_{O_2,\text{total}} &= 33.6 \text{ kg}
\end{align*}
\]

Main fermentation

During the main fermentation all three growth phases occur. In this case equation 8 is also applicable. The following parameters are used:

\[
\begin{align*}
\tau_1 &= 2.01 \text{ hr} \\
\tau_3 &= 50 \text{ hr} \\
V_1 &= 50 \text{ m}^3 \\
C_{x,0} &= 10.91 \text{ mole/m}^3 \\
C_{x,1} &= C_{x,0} e^{\frac{\tau_1}{\tau_3}} = 29.7 \text{ mole/m}^3 \\
C_{x,2} &= 120.0 \text{ mole/m}^3 \\
x_{O_2} = \frac{4 \times 0.67}{(6 - 4.2 \times 0.67)} &= 0.84
\end{align*}
\]
$$m_o = \frac{1}{4} \times 0.05 \times 6 = 0.075 \text{ hr}^{-1} \quad \mu_{\text{max}} = 0.5 \text{ hr}^{-1}$$

The values calculated from equations 6 to 8 are:

$$n_{O_2,1} = 1261 \text{ mole} \quad \Rightarrow \quad n_{O_2,2} = 16592 \text{ mole}$$

$$n_{O_2,3} = 3600 \text{ mole} \quad \Rightarrow \quad n_{O_2,\text{total}} = 21453 \text{ mole}$$

$$m_{O_2,\text{total}} = 686.5 \text{ kg}$$
Appendix 11: Program for the determination of cooling water flow.

1VDU2
2PRINT"Hooferfementor"
3S$=50060A
4INPUT"GEMIDDELDE TEMPERATUUR WARME FASE IS ";TWARM
5INPUT"HOEVEELHEID OVER TE DRAGEN WARME IS ";Q
6PRINT TAB(5)"T";TAB(20)"DT=U";TAB(35)"R";TAB(50)"MW";TAB(65)"ALFA KW"
7FOR T=20.1 TO 25 STEP 1
8PHI=(3.661*T/(273.1+T))
9H=1-E-3*EXP(-58.2-52*PHI+909*PHI^2-264*PHI^3)
10LABDA=5.607+2/100*T-.795E-5*T^2
11MW=Q/(T-20)/4180;DLN=LN(TWARM-20)/(TWARM-T)
12RE=4*MW/PI/7.06/ETA
13PR=4180*ETA/LABDA
14IF RE<2300 THEN NU=(5.8+(1+.14*SQR(3.54/3.52))^1.19*(RE*PR^0.02/5.83)^(1/12.7*SQR(XI/8)))*(RE-1000)^P
15IF RE>2300 THEN XI=(1.82*log(RE)-1.64)^2:NUIP=XI/8*(RE-1000)^P
16ALFAkW=NU*LABDA/.02
17U=1/(1/3800+1/1000+1/ALFAkW)
18PRINT TAB(1)"DT";TAB(15)"U";TAB(30)"RE";TAB(45)"MW";TAB(60)"ALFAkW"
19NEXT
## Gemiddelde Temperatuur Warme Fase Is 730

### Hoeveelheid Over Te Dragen Warmte Is 7208300

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Appendix 12: heat exchange flows for both fermentations.

The heat flow of air is estimated by:

\[ Q_a = \dot{m}_{\text{air}} \cdot c_{p,\text{air}}^{\text{humid}} \cdot (t_{\text{air in}} - t_{\text{air out}}) \]

The heat capacity, \( c_{p,\text{air}}^{\text{humid}} \), is calculated from [27]:

\[
\frac{c_{p,\text{air}}^{\text{humid}}}{c_{p,\text{air}}^{\text{dry}}} = 1 + 0.622 \left( \frac{t_{\text{air in}}}{t_{\text{H}_2O,\text{sat}}} \right) \]

If surrounding air is considered, then the change in \( c_{p,\text{air}}^{\text{humid}} \) caused by humidity is about 0.01 kJ/kg/K. This result is obtained as follows:

\[
c_{p,\text{air}}^{\text{dry}} = 1.00 \text{ kJ/kg/K} \quad \text{and} \quad P_{\text{total}} = 101.3 \text{ kPa} \]

The pressure ratios for water vapor and total pressure of the air leaving the blowers are approximately equal to the ratio for surrounding air, thus \( c_{p,\text{air}}^{\text{humid}} \) as calculated above can also be used for the inflow situations of air in both fermenters.

The inflow temperatures for the main and pre-fermenter are 73.4 and 55.1 °C respectively; the massflows are 0.193 kg/s and 0.069 kg/s. With these values it is calculated that the heat flows are:

\[
Q_{a,\text{main}} = 0.193 \times 1.01 \times (73.4 - 30) = 8.46 \text{ kW} \approx 8.5 \text{ kW} \\
Q_{a,\text{pre}} = 0.069 \times 1.01 \times (55.1 - 30) = 1.75 \text{ kW} \approx 1.8 \text{ kW} 
\]

The stirring heat \( Q_s \) is straightforwardly calculated applying the parameters found in section 6.3, thus it is unnecessary to give an outline at this juncture.

The maximum production of heat due to oxygen consumption occurs obviously at maximum oxygen consumption rate. The maximum rates for the main and pre-fermentation are, when considered over the total volumes, 0.277 and 0.0197 mole/s respectively. The corresponding heats of reaction are:

\[
Q_{O_2,\text{pre}} = 460 \times 0.0197 = 9.06 \text{ kW} \\
Q_{O_2,\text{main}} = 460 \times 0.277 = 127.4 \text{ kW} 
\]

If heat exchange of the surroundings with the outer wall of the cooling annulus is neglected, then the top and bottom section of the fermenter wall
are responsible for heat loss to the surroundings. The top section has a gas phase on the inside and outside of the wall. The overall heat transfer coefficient is:

\[ U = \left[ \frac{1}{\alpha_{\text{in}}} + \frac{d_w}{\lambda_{\text{wall}}} + \frac{1}{\alpha_{\text{out}}} \right]^{-1} \]

\[ \alpha_{\text{in}} = \alpha_{\text{out}} \approx 10 \text{ W/m}^2/\text{K} \]

\[ d_w = 0.01 \text{ m} \]

\[ \lambda_{\text{wall}} = 11 \text{ W/m/K} \]

\[ U \approx \left[ \frac{1}{10} + \frac{1}{1100} + \frac{1}{10} \right] = 5 \text{ W/m}^2/\text{K} = 0.005 \text{ kW/m}^2/\text{K} \]

The wall area responsible for heat loss at the top section is:

\[ A_{\text{top}} = \frac{1}{4} \pi d_i^2 + (H_{\text{total}} - H_i) \pi d_i \]

main: \[ A_{\text{top}} = \frac{1}{4} \pi (3.52)^2 + (7.28 - 5.83) \pi 3.52 \]

= 25.8 m\(^2\) \(\Rightarrow Q_{w,\text{top}} = -0.005 \times 25.8 \times (30 - 25) = -0.65 \text{ kW}\)

pre: \[ A_{\text{top}} = \frac{1}{4} \pi (1.31)^2 + (2.70 - 1.93) \pi 1.31 \]

= 4.49 m\(^2\) \(\Rightarrow Q_{w,\text{top}} = -0.11 \text{ kW}\)

The bottom section has a liquid phase on the inside of the wall. It is estimated that for the applied stirring rates in the pre- and main fermenter the heat transfer coefficients, \(\alpha_{\text{in}}\), are 7700 and 3800 W/m\(^2\)/K respectively. These values are calculated from equation 6.45, applying the following parameters:

**main fermenter**

- \(\lambda_R = 0.614 \text{ W/m}^2/\text{K}\)
- \(T = 3.50 \text{ m}\)
- \(N = 1.64 \text{ s}^{-1}\)
- \(c_p = 4179 \text{ J/kg/K}\)
- \(D = 1.2 \text{ m}\)
- \(\nu = 8.0 \times 10^{-7} \text{ m}^2/\text{s}\)
- \(\eta = 7.9 \times 10^{-4} \text{ m}^2/\text{s}\)

**pre-fermenter**

- \(\lambda_R = 0.614 \text{ W/m}^2/\text{K}\) (from eq. 6.20)
- \(T = 1.29 \text{ m}\)
- \(N = 5.05 \text{ s}^{-1}\)
- \(c_p = 4179 \text{ J/kg/K}\)
- \(D = 0.55 \text{ m}\)
- \(\nu = 8.0 \times 10^{-7} \text{ m}^2/\text{s}\)
- \(\eta = 7.9 \times 10^{-4} \text{ m}^2/\text{s}\)

The bottom areas are:

\[ A_{\text{bottom}} = \frac{1}{4} \pi d_i^2 \]

main: \(= 9.7 \text{ m}^2\)

pre: \(= 1.34 \text{ m}^2\)
and the heat losses through the bottom section are:

main: \( Q_{\text{w,bot}} = -0.49 \text{ kW} \)  \hspace{2cm} pre: \( Q_{\text{w,bot}} = -0.07 \text{ kW} \)

\[ \Rightarrow Q_{\text{w,main}} = -0.65 - 0.49 \approx -1.1 \text{ kW} \]  \hspace{2cm} \[ Q_{\text{w,pre}} = -0.11 - 0.07 = -0.17 \text{ kW} \]

The enthalpy of vaporization of water at 30 °C is:

\[ \Delta H_{\text{vap},H_2O}^{24} = 2425 \text{ kJ/kg} \]

Applying the values of water vaporization rates, which are estimated in appendix 10, to equation 6.43 result in the heat losses due to water vaporization; the results are given in section 6.3.
Appendix 13: methanol loss due to vaporization [38,39]

The loss of methanol during the first two hours of the main fermentation, due to evaporation, is considered in detail in this appendix. The initial methanol concentration is 48 kg of methanol in 50 m$^3$ medium; this is approximately equal to a weight fraction of 1x10$^{-3}$ or a mole fraction $x$ of 5.4x10$^{-4}$. Considering a gas-liquid equilibrium of a methanol-water mixture with the same fractions, in literature values are presented of the gas phase fractions of methanol and water for various temperatures. The gas phase fraction of a certain component of a two component system is estimated by:

$$y_1 = \frac{\alpha_{1z} x_1}{1 + \alpha_{1z} x_1 x_2} \quad \text{with} \quad \alpha_{1z} = \frac{x_1 p_1^*}{y_2 p_2} \quad (1)$$

Subscript 1 is used for methanol and subscript 2 for water. The partial pressures of the pure components are estimated from the Antoine equation:

$$10 \log p_i^* = A_i - \frac{B_i}{t + C_i} \quad \text{with} \quad p_i^* \text{ in mm Hg} \quad (2)$$

The parameters for both the components are:

<table>
<thead>
<tr>
<th>Component</th>
<th>Methanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8.08097</td>
<td>8.07131</td>
</tr>
<tr>
<td>B</td>
<td>1582.271</td>
<td>1730.630</td>
</tr>
<tr>
<td>C</td>
<td>239.726</td>
<td>233.426</td>
</tr>
</tbody>
</table>

Equation 2 is solved for a temperature of 30 °C and the result modified to give the partial pressure for methanol, which is 164 mmHg or 0.218 bar. The partial pressure for water has already been mentioned and found from other sources and estimates 0.042 bar for the above temperature.

Subsequently the activity coefficients must be known; from them, together with the partial pressures, the relative volality $\alpha_{1z}$ is calculated. For instance, the third order equation of Van Laar can be applied to estimate the activity coefficients:

$$\ln y_i = \frac{A_{i1} x_j^2}{\left[ x_i \frac{A_{i1}}{A_{ji}} + x_j \right]^2} \quad (3)$$
Unfortunately, it was not possible to find equilibria data at 30 °C, but there are equilibria data available for 25 and 35 °C. For these temperatures the following parameters are applicable.

<table>
<thead>
<tr>
<th></th>
<th>25 °C</th>
<th>35 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>A_{1z}</td>
<td>0.5565</td>
<td>0.7070</td>
</tr>
<tr>
<td>A_{z1}</td>
<td>0.5745</td>
<td>0.4496</td>
</tr>
<tr>
<td>x_{1}</td>
<td>5.4×10^{-4}</td>
<td></td>
</tr>
<tr>
<td>x_{2}</td>
<td>0.999</td>
<td></td>
</tr>
</tbody>
</table>

Applying the above values in equation 3 and calculating $y_{1}$ from the answers, the following activity coefficients are estimated:

<table>
<thead>
<tr>
<th></th>
<th>25 °C</th>
<th>35 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>y_{1}</td>
<td>1.74</td>
<td>2.02</td>
</tr>
<tr>
<td>y_{2}</td>
<td>≈ 1</td>
<td>≈ 1</td>
</tr>
</tbody>
</table>

The above values for pressure and activity coefficient are applied to estimate the relative volatility at 25 and 35 °C; the relative volatilities are 9.28 and 10.16 respectively. Eventually, application of the relative volatilities to equation 1 results in values for the methanol fractions in the gas phase. The results are:

\[
\begin{align*}
  t &= 25 \degree C \\
  t &= 35 \degree C \\
  y_{1} &= 4.98\times10^{-3} \\
  y_{1} &= 5.46\times10^{-3}
\end{align*}
\]

Interpolation of the above values for a temperature of 30 °C gives an estimation for $y_{1}$ of $5.22\times10^{-3}$. The corresponding partial pressure is:

\[
p_{1} = y_{1} p_{1} = 114 \text{ Pa}
\]

The total pressure of outflowing air is about 1.1 bar. The gas phase fraction of methanol is estimated as follows:

\[
y_{\text{methanol,air}} = \frac{p_{1}}{p_{\text{air}}} = \frac{114}{110000} ≈ 1.03\times10^{-3}
\]

The molar air flow estimates 24233 mole/hr; during two hours the maximum loss of methanol is approximately:
The actual methanol concentration in the medium is on an average lower, consequently the methanol loss is probably not as high as calculated above. During the remaining 48 hours of the fermentation the methanol concentration in the medium is approximately a hundred times lower; the methanol loss due to evaporation is, thus, safely assumed to be negligible.
Appendix 14: difference between molar in- and outflow of air

To estimate the difference between the in- and outflow of water the moment of maximal oxygen consumption rate is considered more deeply. At that particular moment the total oxygen consumption rate estimates 995.6 moles/hr. The carbondioxide flow is estimated from the substraction between the substrate consumption rate and biomass production rate:

\[ V_1 r_{x}^{\text{max}} = v_1 \mu_{\text{max}} C_{x,1} = 50.5 \times 0.5 \times 10.908 \times e^{0.5 \times 2.01} = 751 \text{ mole/hr} \]

\[ V_1 r_s = \left( \frac{1}{0.67} \times 0.5 + 0.05 \right) \times 1502 = 1196 \text{ mole/hr} \] (eq. 1 of app. 1)

\[ \Rightarrow \dot{\Phi}_{\text{CO}_2} = V_1 r_s - V_1 r_x = 1196 - 751 = 445 \text{ mole/hr} \]

The water loss due to evaporation is 408 mole/hr.

The difference between in- and outflow of are is estimated by:

\[ \Delta \Phi_{\text{mol,dry air}} = \dot{\Phi}_{\text{O}_2} - \dot{\Phi}_{\text{CO}_2} = 996 - 445 = 551 \text{ mole/hr}. \]

Obviously, this is still an approximation. The water loss is estimated under the assumption that the dry air in- and outflow are equal. The latter is not true, which means a slight difference in the water loss for the considered point of fermentation. One must keep in mind that an extreme situation is treated in this appendix. For other periods of fermentation the molar difference between in- and outflow of dry air is not as significant as during the maximum growth rate period.
Appendix 15

10REM BEREKENING VAN METHANOLVERBRUIK, ROERSNELHEID EN LUCHTTOEVOER IN
ENTFERMENTOR
15@%=G0050A
16VDU2
20PROCinvoer
22VDU2
25PROCzuurstofberekeninginvoer
26VDU3
30PROChoofd
35VDU3
140END
200DEFPROCinvoer
210INPUT"Yield biomass op substraat =":Ysx
220INPUT"Maximale groeisnelheid (1/uur) =":mumax
230INPUT"Startbiomassaconcentratie bedraagt(mol/m3) =":Cx0
240INPUT"Maintenance biomass op substraat (1/uur) =":ms
260INPUT"Totale fermentatietijd (uur) =":ttotaal
270INPUT"Schattin g tijdssduur maximale fase (uur) =":t1
280INPUT"Verhouding eindbiomassa/startbiomassa =":Xverhouding
290ENDPROC
3000DEFPROChoofd
310CX1=CX0*EXP( MUMAX*T1)
320N1=(1/Ysx*MUMAX+M S)/(EXP(MUMAX*T1)-1)*CX0/MUMAX
340DCXDT1=MUMAX*CX1*DCXDT2/(VERHOUDING*CX0-CX1)/(TOTAAL-T1):IF DCXDT1
>DCXDT2 THEN DCXDTMAX=DCXDT1:CRMAX=CX1 ELSE DCXDTMAX=DCXDT2:CRMAX=C
X0*VERHOUDING
350N2=DCXDT2*(((TOTAAL-T1)*Ysx+MS*CX1/DCXDT2+MS*0.5*(TOTAAL-T1)^2
-T1*TOTAAL))
353PROCzuurstofberekening
355PRINT"Tijdsduur van exponentiele groei is ";T1;" uur."
360PRINT"Aantal molen/m3 substraat bedraagt ";N1+N2
370PRINT"Aantal molen/m3 substraat t1 = ";N1
380PRINT"Aantal molen/m3 substraat mu = ";N2
420PROCroervermogenberekening
430ENDPROC
700DEFPROCzuurstofberekeninginvoer
710PRINT"".
720INPUT"Reductiegroei substraat =":gs
730INPUT"Reductiegroei biomassa(NH3 als N bron) =":gx
740INPUT"Luchtzuurstofverzuiming voor gebruikte temperatuur = ";COSAT
750INPUT"Reactor temperatuur in C = ";TTEMP
760INPUT"Schatt in g voor volume beslag";VLIQUID
770INPUT"Uitputtingsfractie zuurstof van ingaande luchtstroom";UZUTPUT
780INPUT"Geschatte methanolconcentratie in beslag in molen/m^3 =":CMET
790ENDPROC
800DEFPROCzuurstofberekening
810REM Zuurstofverbruik aan einde exponentiele fase
820DCODT1=.25*((GS/YSX-GX)*MUMAX*GS*MS)*CX1
830REM Zuurstofverbruik aan einde tweede groeifase
840DCODT2=.25*((GS/YSX-GX)*DCODT2*GS*MS*VERHOUDING*CX0)
850IF ABS(1-DCODT1/DCODT2)>0.01 THEN T1=T1*SQR( DCODT2/DCODT1):PROChoofd
ELSE DCODTMAX=DCODT1:VDU2
860REM Berekening van de zuurstofverbruikstijd voor maximale r0
870ZUURSTOFDEBIEIT=DCODTMAX*8.314*(TEMP+273.15)/101325*VLIEQUID:LUUTNDEnte=ZUURSTOFDEBIEIT/UZUTPUTTING*100/21
890OBERGEMIDDELDE=21-0.21/2*UZUTPUTTING
900COSAT=COSAT*OBERGEMIDDELDE/21
910PRINT"Luchtinstroom met temperatuur van 30C en druk van 1 atm bedr
aagt ":Luchtdoebietin; m^3/uur."

920PROC KL1Aberekening
925PRINT "Maximale zuurstofverbruik bedraagt ";D DCODtmax°Vliquid; mol/u ur."
930PRINT "Gewenste KIA bedraagt ":KIA; s^−1."
940PRINT "Zuurstofverbruikstijd tijdens de grootste zuurstofbehoefte is ":zuurstofverbruikstijd; s."
950KIA20=KIA°1.022−10:REM Omrekening van KIA bij 30C naar 20C
960tsubstraat=Cmethanol/(DCxDtmax/Ys+xms°Crmax)°3600
970PRINT "Substraatverbruikstijd bedraagt ";tsubstraat; s."
980ENDPROC

1100DEF PROC KL1Aberekening
1110K1A=DCODtmax/(1/3°Cosat1)/3600:zuurstofverbruikstijd=2/KIA:REM Zuurstofoverdrachtstijd 2×kleiner dan zuurstofverbruikstijd
1120ENDPROC

2000DEF PROC Croervermogenberekening
2010INPUT "H/T verhouding bedraagt ";HT,"Powernummer bedraagt ";NP,"D/T verhoeking bedraagt ";DT,"Viscositeit bedraagt ";ETA
2020INPUT "Gewenste mengtijd bedraagt ";tm
2050T=(Vliquid/.25/PI/HT)°(1/3)
2060DIAMETER=T°DT
2070vs=Luchtdoebietin/.25/PI/T°2/3600:PRINT "Superficiële gassnelheid bedraagt ";vs; m/s."
2080PGV=(KIA20/2.7e−2×vs−.57)°(1/.43):PRINT "Minimaal begast vermogen bij maximale zuurstofbehoefte bedraagt ";PGV; "W/m^3."
2090PROC tabelhoofd
2096Corr=1:O=DIAMETER:HR=D/4
2100PROC optimalisatie:PROC uitvoer1: INPUT "Correctiefactor i.v.m. aeratie ";Corr
2109FOR D=DIAMETER TO T/2 STEP .05
2110PROC optimalisatie
2119NEXT
2120PROC uitvoer1
2130INPUT "Berekening met aangepaste onbeluchte mengtijd J/N";antwoord$:
2139IF antwoord$="J" OR antwoord$="j" THEN PROC croervermogenberekening
2140ENDPROC

2160DEF PROC optimalisatie
2170N=.6/tm°(T/D)°3°HT/(NP°HR°2/D°2)°(1/3)
2180P=NP°1000×N°3°D°5
2190RE=NP°2×PI°D°2×1000/ETA:IF RE<1E5 THEN PRINT "RE = ";RE:END PROC
2200ENDPROC

2210DEF PROC tabelhoofd
2220PRINT "'
2230PRINTTAB(5)"D"TAB(15)"HR"TAB(25)"N"TAB(35)"P ONBEL."TAB(45)"TM"TAB(55)"V TIP"TAB(65)"AERATIEGETAL"
2240PRINTTAB(5)"(M)"TAB(15)"(M)"TAB(25)"(S−1)"TAB(35)"(W/M^3)"TAB(45)"(S)"TAB(55)"(M/S)"TAB(65)"(-)"
2250ENDPROC
2260DEF PROC uitvoer1
2270AE=Luchtdoebietin/3600/N/D°3
2280PRINTTAB(5);D TAB(15);HR TAB(25);N TAB(35);P/50.S×Corr TAB(45);tm/Corr TAB(65);PI°N°D TAB(65);AE
2290ENDPROC
Yield biomassa op substraat = ?0.6?
Maximale groeisnelheid (1/uur) = ?0.5
Startbiomassaconcentratie bedraagt (mol/m3) = ?10.908
Maintenance biomassa op substraat (1/uur) = ?0.05
Totale fermentatietijd (uur) = ?20
Schatting tijdsduur maximale fase (uur) = ?2
Verhouding eindbiomassa/startbiomassa = ?20

Reductiegraad substraat = ?6
Reductiegraad biomassa (NH3 als N-bron) = ?4.2
Luchtzuurstofverzadiging voor gebruikte temperatuur = ?0.245
Reactortemperatuur in C = ?30
Schatting voor volume beslag = ?2.5
Uitputtingssnelheid zuurstof van ingaande luchtstroom = ?0.04
Geschatte methanolconcentratie in beslag in molen/m3 = ?0.312

Luchtinstroom met temperatuur van 30°C en druk van 1 atm bedraagt 210.81 m3/uur.
Maximale zuurstofverbruik bedraagt 71.09 mol/uur.
Gewenste nLA bedraagt 9.6741E-2 s^-1.
Zuurstofverbruikstijd tijdens de grootste zuurstofbehoefte is 20.674 s.
Substraatverbruikstijd bedraagt 33.206 s.
Tijdsduur van exponentiële groei is 2.7191 uur.
Aantal molen/m3 substraat bedraagt 425.09
Aantal molen/m3 substraat t1 = 50.279
Aantal molen/m3 substraat mu = 374.81
H/T verhouding bedraagt ?1.5
Powernummer bedraagt ?12
D/T verhouding bedraagt ?0.2334559
Viscositeit bedraagt ?0.001
Gewenste mengtijd bedraagt ?2.45
Superficiële gassnelheid bedraagt 4.508E-2 m/s.
Minimaal begast vermogen bij maximale zuurstofbehoefte bedraagt 713.38 W/m3.

D  HR  N  P ONBEL.  TM  V TIP  AERATIEGETAL
(M)  (M)  (S^-1)  (W/M^3)  (S)  (M/S)  (-)
0.3   7.5001E-2 31.777 18528  2.45 29.949 6.8155E-2
0.35  7.5001E-2 31.777 9264.2  4.9 29.949 6.8155E-2
0.4   0.1    13.406  2931.3  4.9 16.846 6.8155E-2
0.45  0.125  9.4154  1830.0  4.9 13.311 6.8155E-2
0.5   0.125  6.8638  1200.7  4.9 10.782 6.8155E-2
0.55  0.1375 5.1569  820.06 4.9  8.9105 6.8155E-2
0.6   0.15   3.9721  579.02  4.9  7.4873 6.8155E-2

Correctiefactor i.v.m. aeratie = ?0.5
Berekening met aangepaste onbeluchte mengtijd J/N?N
Appendix 16: cost evaluation for economical heat recovery

An economical evaluation has been performed to predict the most attractive configuration of the heat recovery section. To realize such an evaluation energy, apparatus, and maintenance costs must be considered. Initially a cost evaluation has been performed under the assumption that the heat recovery section is only applied for the PQQ production. A treatise of this evaluation is presented at this point.

Energy costs are extremely influenced by political and economical developments. The difficulty to predict market price changes are illustrated by the various scenario proposals for market price developments in the near future. For example, T. Acosta [40] has predicted a slight increase of oil prices until 1992, and a major increase in prices after this period. The predictions are based on oil production capacities and fuel demands in the world.

The gas price in Holland has increased by 20% between the first quarter of 1987 and 1988. Contrarily, a Dutch governmental report has been published this year, in which a gas price of Dfl 0.28 for the year 1995 is predicted, the gas price still being coupled to the heavy oil prices (information from Krachtwerktuigen, Amersfoort).

The steam costs are estimated by [35]:

\[
\text{steam costs/ton} = \text{Dfl. 5.00} + 2.8 \frac{\text{gasprice}}{\text{GJ}}
\]

It is possible to take price developments into account by applying price rise fractions for several years

\[
\text{steam costs/ton for year } a = \text{Dfl. 5.00} + 2.8 \frac{\text{gasprice}_{1988}}{\text{GJ}} \prod_{i=1}^{a} (1 + r_i)
\]

Besides steam costs also cooling water costs needed for pre cooling the fermenters (necessary to cool the hot sterile medium) can be considered; this is not done in this appendix, because during the calculations the cooling water costs were not relevant, as compared to the other costs.

The maintenance costs are estimated to be 5% of the initial apparatus costs annually. Heat exchanger costs consist of the buying price and installation costs. The fraction of the buying price for the installation costs is estimated from table III-45 of the volume II of [35] to be 0.45. The total costs over a depreciation period of ten years is calculated from:

\[
\text{total heat exchanger costs} = \text{buying price} \times (1.45 + 10 \times 0.05)
\]

The buying prices are estimated from [41].
It is somewhat tedious to present all the calculations at this point for various energy price developments, when the sterilization unit is used solely for the PQQ installation. The program used to execute these calculations is inserted in this appendix. The important message is that energy prices must increase more than 15% annually to make a heat exchanger profitable. Therefore it is decided to apply the sterilization unit for at least fifteen processes with about the same demands for sterile medium as for the PQQ process.

In the following table data for heat exchangers near the optimal configuration are presented. The most unattractive situation for energy saving has been chosen for, in which price increases do not occur.

<table>
<thead>
<tr>
<th>$\Delta T$</th>
<th>h.e. area</th>
<th>buying price</th>
<th>total after steam costs</th>
<th>total 10 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>m²</td>
<td>Dfl. install + main</td>
<td>annually Dfl.</td>
<td>Dfl.</td>
</tr>
<tr>
<td>50</td>
<td>104</td>
<td>132900</td>
<td>259155</td>
<td>108557</td>
</tr>
<tr>
<td>60</td>
<td>2 x 68</td>
<td>196200</td>
<td>382590</td>
<td>89522</td>
</tr>
<tr>
<td>70</td>
<td>2 x 125</td>
<td>296500</td>
<td>578175</td>
<td>69922</td>
</tr>
<tr>
<td>80</td>
<td>4 x 92.5</td>
<td>488000</td>
<td>951600</td>
<td>50699</td>
</tr>
</tbody>
</table>

Heat exchange with a temperature recovery of 70 °C for the hot phase is economically most attractive. If slight price increases are to be expected (a very realistic view), better heat efficiency than proposed in this outline might be a good choice.
58%=620209
10PRINT"KOSTENBEREKENING VOOR VERSCHILLENDE ENERGIE-EFFICIENCY"
20PRINT"INPUT"KOSTEN VAN DE WARMTEWISSELAAR":WWKOSTEN
30INPUT"STOOMVERBRUIK PER JAAR":STOOMVERBRUIK
40INPUT"KOELWATERVERBRUIK PER JAAR":KWVERBRUIK
50INPUT"HUIDIG PRIJSNIVEAU GAS PER GJ"GASPRIJS
60INPUT"KOELWATERPRIJS PER M3":KWPRIJS
70DEF FRACTIE(10)
80DIM FRACTIEDUUR(10)
100INPUT"AANTAL JAREN VOOR DOORREKENING":TIDSDUUR
110INPUT"HOEVEEL VERSCHILLENDE PRIJSSTIJGING FRACTIES VOOR GAS";FRACTIE_TOT
300FOR TELLER=1 TO FRACTIE_TOT
310PRINT"STIJGINGSFRACTIE ":TELLER;" OP JAARBASIS IS ":;INPUTFRACTIE(TELLER)
320INPUT"AANTAL JAREN VOOR DEZE FRACTIE";FRACTIEDUUR(TELLER)
330NEXT TELLER
400=STOOMKOSTEN
500=KWKOSTEN*TIDSDUUR*KWVERBRUIK*KWPRIJS
600=OSTENTOTAAL=WWKOSTEN+KWKOSTEN+STOOMKOSTEN
700PRINT"TOTALE KOSTEN OVER ":TIDSDUUR:" JAAR BEDRAAGT ";KOSTENTOTAAL
750GOTO100
1000FPROCSTOOMKOSTEN
1005STOOMKOSTEN=O
1006VERMEERDERINGSFAKTOR=1/(1+FRACTIE(1))
1010FOR FRACTIEVOLGORDE=1 TO FRACTIE_TOT
1020FOR FRACTIETIJD=1 TO FRACTIEDUUR(FRACTIEVOLGORDE)
1030VERMEERDERINGSFAKTOR=VERMEERDERINGSFAKTOR*(1+FRACTIE(FRACTIEVOLGORDE))
1040TARIJF=6+2.8*GASPRIJS*VERMEERDERINGSFAKTOR
1050STOOMKOSTEN=STOOMKOSTEN+STOOMVERBRUIK*TARIJF
1055PRINT"FAKTOR";VERMEERDERINGSFAKTOR."TARIJF";TARIJF."STOOMKOSTEN":STOOMKOSTEN
1060NEXT FRACTIETIJD
1070NEXT FRACTIEVOLGORDE
1080ENDPROC
**KOSTENBEKOMING VOOR VERSCHILLENDE ENERGIE-EFFICIENCY**

KOSTEN VAN DE WARMTEWISSELAAR?282590
STOOMVERBRUIK PER JAAR?3424?
KOELWATERVERBRUIK PER JAAR?
HUIDIG PRIJSNIVEAU GAS PER GJ? .85
KOELWATERPRIJS PER M3?
AANTAL JAREN VOOR DOREKENING?10
HOEVEEL VERSCHILLENDE PRIJSSTIJGING FRACTIES VOOR GAS?!
STIJGINGSFRACTIE 1.00 OP JAARBASIS IS ?0
AANTAL JAREN VOOR DEZE FRACTIE?10

<table>
<thead>
<tr>
<th>FAKTOR 1.00</th>
<th>TARIEF 26.14</th>
<th>STOOKOOSTEN 89521.66</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAKTOR 1.00</td>
<td>TARIEF 26.14</td>
<td>STOOKOOSTEN 179043.32</td>
</tr>
<tr>
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<td>TARIEF 26.14</td>
<td>STOOKOOSTEN 268564.92</td>
</tr>
<tr>
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<td>TARIEF 26.14</td>
<td>STOOKOOSTEN 358086.63</td>
</tr>
<tr>
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<td>TARIEF 26.14</td>
<td>STOOKOOSTEN 447608.29</td>
</tr>
<tr>
<td>FAKTOR 1.00</td>
<td>TARIEF 26.14</td>
<td>STOOKOOSTEN 537129.95</td>
</tr>
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<td>TARIEF 26.14</td>
<td>STOOKOOSTEN 626651.61</td>
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<td>STOOKOOSTEN 716173.26</td>
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<td>STOOKOOSTEN 805694.92</td>
</tr>
<tr>
<td>FAKTOR 1.00</td>
<td>TARIEF 26.14</td>
<td>STOOKOOSTEN 895215.58</td>
</tr>
</tbody>
</table>

**TOTALE KOSTEN OVER 10.00 JAAR BEDRAAGT 1277805.58**
### Appendix 17

Calculation of IRR.

10 FOR I=0.09 TO 0.12 STEP 0.01
15 SUM = -18515045 - 360000: REM INVESTMENT AND WORKING CAPITAL IN YEAR 0, THUS NEGATIVE
17 SUM = SUM
20 FOR N%=1 TO 9
30 SUM = SUM + 3147137 / (1 + I) ^ N%: REM 3147137 IS YEARLY CASH FLOW
40 NEXT
50 SUM = SUM + (3147137 - 360000) / (1 + I) ^ 10: REM WORKING CAPITAL BACK
55 PRINT "TAB(5)"."I",TAB(10).TAB(30)".SUM".TAB(40)SUM
60 NEXT

<table>
<thead>
<tr>
<th>I</th>
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-977108.65
Ion exchange calculations:

Calculations mentioned in the ion exchange section are as follows:

114 litres of methanol/water (1:1, v/v) mixture is required for the elution of PQQ from the ion exchange. From [30] it can be calculated that the mixture contains 60 kg of water and 47.4 kg of methanol. It is assumed that sodium chloride dissolves only in the water fraction of the mixture; thus, 3.51 kg NaCl is needed to attain a 1N solution.

It is assumed that 99% of PQQ adsorbs to Amberlyst A 21. The amount of sodium chloride that binds to PQQ is, therefore, equivalent to the quantity of PQQ present on the ion exchanger. The amount of PQQ is calculated to be 13.58 eq. Thus, the amount of NaCl that binds to PQQ is:

\[
13.58 \times 0.0585 = 0.795 \text{ kg NaCl.}
\]

The following reaction involves the protonation of PQQ and reducing the acidity of water to pH 2.0. For this highly concentrated HCl is used. Amount of pure HCl required for the reaction is calculated as follows:

(i) \[
13.58 \times 0.0365 = 0.496 \text{ kg HCl}
\]
Thus, 0.496 kg of HCl is required to protonate PQQ.

(ii) Amount of HCl needed to reduce the pH of 60 litres water to 2.0 is:

\[
60 \times 10^{-2} \times 0.0365 = 0.0219 \text{ kg HCl}
\]

Total amount of pure HCl required is, thus, 0.518 kg.

6M HCl is used for the reaction. The density of 6M HCl is 1.098 and solubility of HCl in water is 20%. Thus, 2.36 litres of 6M HCl contains 0.518 kg of pure HCl.

\[
\begin{array}{c}
\text{HCl + water} : \\
\text{HCl utilized} : \\
\text{water remaining} :
\end{array}
\begin{array}{c}
2.595 \text{ kg} \\
0.518 \text{ kg} \\
2.077 \text{ kg}
\end{array}
\]

2.077 kg water contains, thus, 0.0219 kg HCl.
In the next unit, the remaining HCl is neutralized with 6M NaOH resulting in the formation of water and sodium chloride.

\[ \text{HCl} + \text{NaOH} \quad \longrightarrow \quad \text{NaCl} + \text{H}_2\text{O} \]

In this reaction the amount of water formed is 0.0108 kg and amount of sodium chloride formed is 0.0351 kg. 0.098 kg water, containing 0.024 kg NaOH, remains over.

The total amount of extra water that comes into the system is:

\[ 2.077 + 0.0108 + 0.098 = 2.186 \text{ kg water.} \]

However, it is assumed that 0.3 kg of water adsorbs to PQQ (equivalent to 1/5th of the weight of PQQ). Thus, amount of extra water accumulated in the system is 1.886 kg. This amount of water must be purged in order to come back to the original amount of 60 kg.

1.886 kg of water is purged; along with this 0.1077 kg of NaCl and 0.144 kg of methanol is lost. On balancing, it can be calculated that after each processing 0.09 kg of sodium chloride and 1.7 kg of methanol must be added to the elution mixture.