# DUAL-STAGE CERAMIC MEMBRANE BIOREACTORS FOR THE TREATMENT OF HIGH-STRENGTH INDUSTRIAL WASTEWATERS

**Report to the**
**WATER RESEARCH COMMISSION**

**by**

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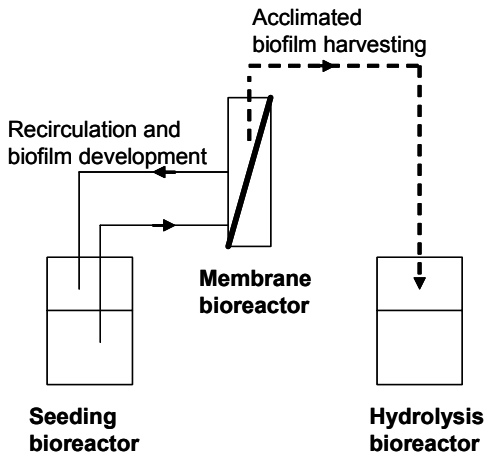
**In memory of Winston Daniel Leukes**

**1969 – 2006**

## EXECUTIVE SUMMARY

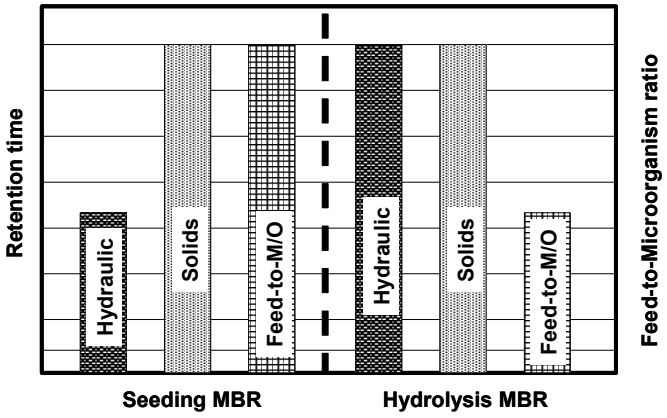
This project focused on the development of a unique operations strategy employing membrane bioreactors for the treatment of wastewaters of industrial origin. The process facilitated a continuous development and acclimation design strategy for generating groups or consortia of microorganisms capable of degrading specific industrial wastewaters. These adapted consortia were then 'harvested' to be used in the continuous operation of 'hydrolysis' reactors. The 'hydrolysis' reactors were operated under similar conditions as conventional wastewater treatment tank facilities, however, the continuous addition of adapted microbial populations 'developed' within the 'seeding' reactor configuration facilitated, firstly, significantly decreased adaptation periods associated with conventional treatment strategies, and, secondly, an inherent robustness facilitated by obviating the requirement for adaptation within the hydrolysis reactor configuration.

The overall project objectives were thus: to design and construct the seeding and hydrolysis membrane bioreactors incorporating an appropriate reticulation system and operating configuration; to analyse the performance of the membrane bioreactor (MBR) seeding reactors for treating industrial origin wastewaters whilst generating stable, adapted microbial consortia; to evaluate the performance of the MBRs via comparison with conventional activated sludge (AS) bioreactors; to monitor population changes within the MBR and AS systems using molecular techniques (phospholipid fatty acid (PLFA) analysis for identifying the relative proportions of the major bacterial groups coupled with ribosomal DNA 'fingerprinting' (16S/18S rDNA) which gives insight into any variation at the specific microbial strain level) for determining the microbial population stability of the seeding reactors; to incorporate the seeding MBR process into the overall dual-stage hybrid MBR process and evaluate the process efficiency of the system. This hybrid process is shown diagrammatically below:



The initial studies involved the design and construction of the seeding MBR for the treatment of Stripped Gas Liquor (SGL) industrial effluent (COD of ± 2000 $mg.L^{-1}$). To facilitate evaluating the performance of the MBR system in terms of process efficiency against a familiar benchmark, a conventional AS system comprising an aerated CSTR-type vessel coupled to an in-series clarifier was operated under similar hydraulic conditions. The seeding MBR was operated for approximately 300 days with an average COD removal of between 70 and 80% maintained after an initial 17 day acclimation period. In contrast, the AS system was operated for a period of 300 days with a maximum COD removal of only 60% observed. Furthermore, fluctuations in COD removal efficiency were minimal in the MBR compared with the AS process. This indicated greater stability of the MBR system in terms of operational performance. The combination of PLFA and 16S/18S rDNA analysis confirmed this by revealing that although the proportions of the major microbial groups during the 300 day operating period were less variable (i.e. more stable) in the MBR than the AS system, 16S rDNA analysis revealed that within those major groupings, greater variation at the bacterial strain level was observed in terms of relative strain abundance. Thus, the MBR generated and maintained a highly stable microbial group structure (with the major group being Gram negative) within the biofilm population that was sufficiently dynamic at the strain level to adapt at an accelerated rate to compensate for hydraulic load variations as well as to simulated pollutant shock loading.

The design of the hybrid seeding-hydrolysis dual-stage MBR system was based on a seeding MBR configuration capable of maintaining a high solids retention time and low hydraulic retention time to ensure a high feed-to-microorganism ratio enabling the selection of microbial consortia capable of degrading any persistent compounds in the wastewater (see diagram below).



Membrane-based biomass retention facilitated the utilisation of this microbial community as a continuous source of inoculum supporting the design of the hydrolysis reactor aimed at operating at conventional high $BOD_5$/COD ratios, i.e. a high hydraulic retention time and high solids retention time to maintain a low feed-to-microorganism ratio.

In terms of long term stability, the operations data obtained from the hybrid dual-stage system over a period of 250 days indicated that whereas a conventional system represented by the seeding bioreactor tank approached a maximum of 40% COD removal, the hydrolysis reactor configuration, supplemented on a continuous basis with acclimated biofilm developed and retained by the seeding MBR, showed a start-up time of less than 10 days to achieve 55-60% COD removal efficiency. Compared with 40 days acclimation for the AS system, this translated into a 75% improvement in acclimation efficiency. This substantiated the initial proposal hypothesis describing the nature and effectiveness of the dual-stage approach to industrial origin wastewater treatment using the MBR configuration in order to enhance the performance and increase the long-term adaptability and stability of the developed and retained microbial populations within the system.

**CONCLUSIONS**

The application of solid-liquid retention membrane bioreactors was shown to be a highly efficient system for the treatment of high-strength industrial effluents containing recalcitrant pollutants.

In comparison to activated sludge systems, the long-term operation of this membrane bioreactor process treating high-strength effluents was characterised by more stable microbial populations significantly less susceptible to deleterious shifts in the community dynamics resulting in enhanced process efficiency due to less process variability.

The dual-stage hybrid membrane bioreactor enabled the intermittent transfer of acclimated retained biofilm developed in a seeding reactor to a hydrolysis reactor. This facilitated significant improvements in the enhancement of microbial population adaptability and performance efficiency as well as drastically decreasing the usual acclimation time necessary by 75% when compared with activated sludge wastewater treatment processes.

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# CHAPTER 1: LITERATURE REVIEW

## 1.1 INTRODUCTION

The concept of the membrane bioreactor (MBR) has developed as a result of combining membrane technology with that of biological reactors. This has led to the development of three generic membrane processes within bioreactor applications, as depicted in Figure 1.1: for solids recycle and separation; for bubbleless aeration of bioreactors; and for the extraction of priority pollutants from industrial waste streams (Brindle & Stephenson, 1996; Visvanathan *et al*., 2000). For the purposes of this literature review, membrane processes aimed at solids recycle and separation will be focussed upon. The combination of membrane technology with biological reactors is fast achieving considerable success for the treatment of domestic and industrial effluent. This is due mainly to the low necessary footprint requirement, reduced sludge production, and the decrease in overall capital costs as a result of the availability of economically competitive membranes and modules. The operational characteristics of MBRs, however, are as diverse as the number of different module configurations in use to date.

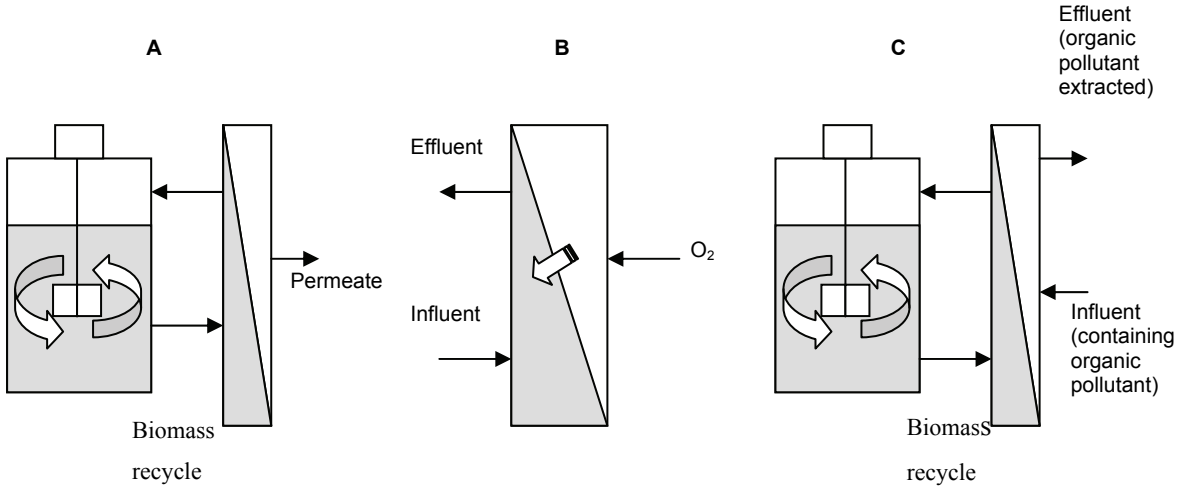



**Figure 1.1: Three main Membrane Bioreactor operating configurations: (A) solid-liquid membrane separation bioreactor; (B) membrane-facilitated aeration; (C) extractive membrane bioreactor (Adapted from Brindle & Stephenson (1996)).**

### 1.1.1 Historical Perspective

The first reported use of coupled membrane / biological wastewater treatment technology was by Smith *et al*. (1969) (cited in: Visvanathan *et al*., 2000) and Hammer and Borchardt (1969). These applications involved the use of ultrafiltration membranes in the separation of activated sludge and dialysis separation of anaerobic sewage sludge, respectively. This ultrafiltration/activated sludge application was subsequently commercialized by Dorr-Oliver in the late 1960s, however, it is only recently that major commercialization of MBR plants is being realized (Visvanathan *et al*., 2000). Although a number of companies are involved in commercial scale solid/liquid separation MBR plants, as of 2001, only four major companies were active in marketing membrane bioreactor systems. Of the major companies, those marketing submerged or immersed hollow fiber MBRs include ZENON Environmental Inc. (Canada), Mitsubishi Rayon Corporation (Japan), and Kubota Corporation (Japan). The fourth company, Suez-Lyonnaise des Eaux/Infilco-Degremont Inc. (France/USA), is involved in the marketing of in-series ceramic-based MBRs (Adham *et al*., 2001; Adham and Gagliardo, 1998). A number of commercial scale MBR plants have been commissioned as well by companies in France (Rhone Poulenc-TechSep), USA (Thetfor Syst, Dorr Oliver), Germany (Grantmij), and South Africa (Weir-Envig, formerly Membratek) (Visvanathan *et al*.,2000). These commercial scale MBR plants are actively treating a diverse range of effluents including domestic, industrial, as well as landfill leachate.

### 1.1.2 Conventional Wastewater Treatment

Conventional wastewater treatment facilities employ aerobic activated sludge (AS) systems for the majority of wastewater treatment applications. These systems are used in conjunction with downstream separation via decantation (Wisniewski *et al*., 1999). The quality, however, of the final effluents generated by conventional activated sludge systems is significantly dependent on the settling characteristics of the sludge as well as the hydrodynamic conditions within the sedimentation/decantation tanks (Visvanathan *et al*., 2000). This necessitates the requirement for large volume tanks enabling relatively long residence times to facilitate adequate liquid/solid separation. Effective operation of activated sludge processes however, requires sedimentation of the aggregated mixed microbial flocs. This process is facilitated by the physico-chemical interactions between the microorganisms, mineral particles, multivalent cations, and microbial exopolysaccharide production enabling the binding of the microorganisms thus resulting in sufficient floc size development to promote settling (Wisniewski *et al*., 1999; Scott *et al*., 1998). The exopolysaccharide-producing microorganisms are, however, usually not the microorganisms responsible for the majority of COD reduction within these processes. As a result, activated sludge plants usually operate at significantly lower efficiencies due to this intrinsic requirement to maintain the

exopolysaccharide-producing populations. Furthermore, the overall biological process may be limited due to diffusional constraints within the flocculated biological mass (Wisniewski *et al*., 1999).

In contrast, MBRs do not have this requirement due to the fact that microfiltration (MF) and ultrafiltration (UF) membranes prevent the loss of biological solids and high molecular weight solutes. As a result, the maintenance of a high biomass concentration within the MBR potentially allows for complete mineralisation of influent organic matter (Brindle and Stephenson, 1996). Furthermore, most wastewater treatment plants experience peak flows of at least twice the normal average flow, with diurnal flow variations being even higher. Compensation of these daily variations in flow can be achieved by construction of large tanks to facilitate flow equalization. This option is, however, impractical both economically and in terms of footprint requirements. The versatility of membrane bioreactor separation systems enables these processes to be designed to cope with the design dry weather flow. The modular nature of MBR systems then compensates for both the minimum plant inflow by shutdown of some modules as well as the maximum plant inflows by raising the permeate flux due to maximum plant inflow periods being usually in the order of a few hours (Günder and Krauth, 1998).

### 1.1.3 Membrane bioreactors

The membrane bioreactor constitutes a combination of biological and physical processes for solid/liquid separation thus combining conventional suspended growth systems with membrane filtration (Jefferson *et al*., 2000; Gander *et al*., 2000). Membrane processes provide reliable, high rate filtration as small footprint units (Davies *et al*., 1998). Furthermore, membrane filtration processes are easily retrofitted to existing wastewater treatment facilities. The associated small footprint requirement of membrane processes is also facilitated by the fact that both primary and final settling tanks are not required for the effective operation of membrane systems (Davies *et al*., 1998). MBRs are most suitable for processes where relatively long residence times are required to achieve sufficiently high biomass concentrations in order to achieve desirable pollutant removal efficiencies.

Typically, mixed liquor suspended solids (MLSS) concentrations as high as 20 000 mg/L can be maintained by MBRs in comparison to 4000 mg/L typical of conventional activated sludge/secondary clarifier systems. This results in significantly lower reactor volume requirements (Günder and Krauth, 1998) with minimum sludge wastage as well as less sludge production from longer sludge retention times (SRT) (Jefferson *et al*., 2000; Gander *et al*., 2000). Furthermore, the operational characteristics of MBRs allows for the complete

uncoupling of the hydraulic retention time (HRT) with that of the SRT (Ragona and Hall, 1998).

### 1.1.4 Membrane Bioreactor Operation

Two main configurations of solid/liquid separation membrane processes are employed in the operation of MBRs. These are defined as immersed (submerged) membrane process operation or in-series membrane separation units operated in the external circuit of the bioreactor.

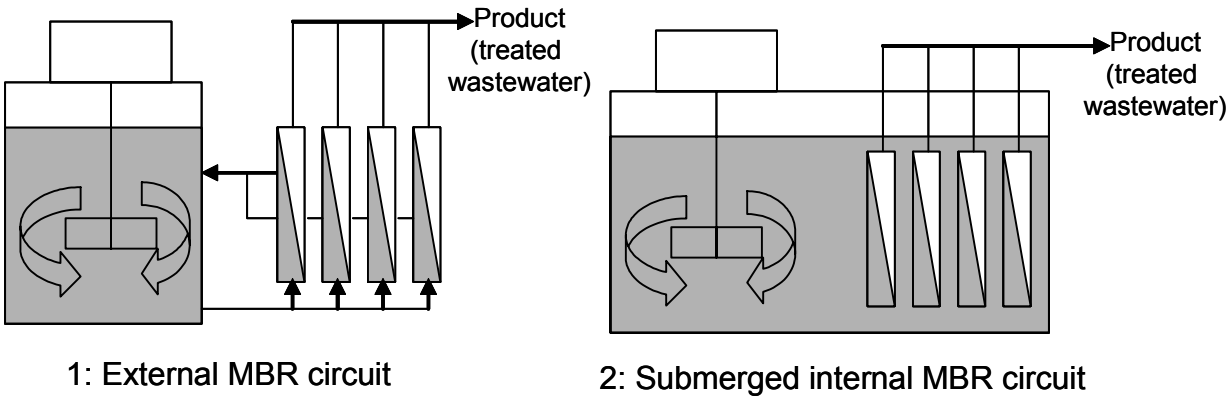



**Figure 1.2. External circuit (1) MBR operation and internal (submerged) MBR operation**

At steady-state conditions, MBRs are capable of greater than 90% removal of influent COD. The production of high quality permeates at high organic loading rates is also associated with significantly greater MLSS concentrations in comparison with conventional biological wastewater treatment systems.

Loading rates of between 0.2 $kgCODm^{-3}.d^{-1}$ for aerobic systems and as high as 19.7 $kgCODm^{-3}.d^{-1}$ for anaerobic systems have been recorded with MLSS concentrations approaching 28.7 and 113.3 $kg/m^3$ for aerobic and anaerobic systems, respectively. Typically this variability is also reflected in the operating conditions associated with MBRs with HRTs ranging from 1.8h for aerobic processes to as much as 240h for anaerobic processes. Of the various membrane types, materials, and configurations used in MBRs, the steady-state operating flux of these systems also varies considerably from 2.1 to 70 $Lm^{-2}h^{-1}$. The various operating characteristics and module configurations described here are referenced and expanded upon in Appendix 1.

A technical summary of a selection of the various membrane processes described within the available literature is given in Appendix 1. The technical summary is aimed at describing in detail the different types of reactor configurations in use along with the various and diverse operating conditions employed during the running of these laboratory and pilot-scale MBRs.

In addition, detailed summations of the various effluents being treated and the average post-treatment results obtained under these different operating conditions are also presented. The aim is therefore to enable a comparative evaluation of project-related configurations and operating conditions that are envisaged, with that of systems described in published literature. The effluent treatment results of similar configurations and operating systems are then to be used as benchmarks by which the project data can be comparatively evaluated.

Two main configurations of solid/liquid separation membrane processes are employed in the operation of MBRs. These are defined as immersed (submerged) membrane process operation or in-series membrane separation units operated in the internal circuit of the bioreactor. At steady-state conditions, MBRs are capable of greater than 90% removal of influent COD. The production of high quality permeates at high organic loading rates is also associated with significantly greater MLSS concentrations in comparison with conventional biological wastewater treatment systems.

Loading rates of between 0.2 kgCOD/$m^3$.d for aerobic systems and as high as 19.7 kgCOD/$m^3$.d for anaerobic systems have been recorded with MLSS concentrations approaching 28.7 and 113.3 kg/$m^3$ for aerobic and anaerobic systems, respectively. Typically this variability is also reflected in the operating conditions associated with MBRs with HRTs ranging from 1.8h for aerobic processes to as much as 240h for anaerobic processes. Of the various membrane types, materials, and configurations used in MBRs, the steady-state operating flux of these systems also varies considerably from 2.1 to 70 L/$m^2$.h.

# CHAPTER 2: CERAMIC MEMBRANE MANUFACTURE

## 2.1 INTRODUCTION

### 2.1.1 Ceramic Membrane Production

Inorganic ceramic membranes are composed of $Al_2O_3$, $ZrO_2$, $TiO_2$, $SiO_2$, or combinations thereof. Both the physical stability in terms of temperature and stress as well as chemical stability in terms of pH extremes and chemical compatibility make inorganic ceramic membranes ideal for many applications when compared with conventional organic-based polymer membranes (Ahn *et al*., 1998). Furthermore, ceramic membranes are self-supporting thus enabling the application of backpulsing techniques for membrane cleaning purposes where reversal of the transmembrane pressure can lead to improvement in overall filtration rate as well as extending cleaning intervals (Sondhi&Bhave, 2001; Sondhi *et al*., 2000).

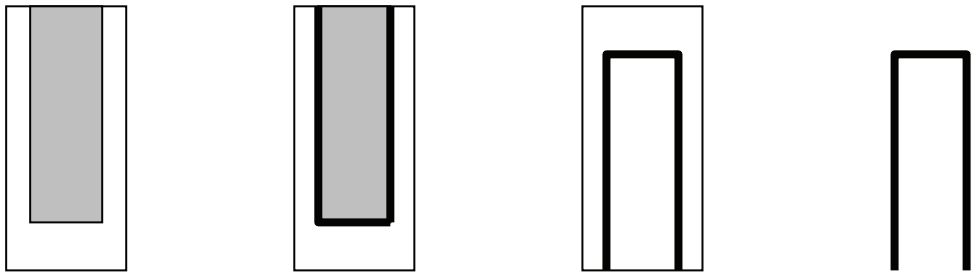
Asymmetric inorganic ceramic membranes are composed of a separating layer bonded to a substratum support. Due to the top coating layer being responsible for the separation characteristics of the membrane, it is necessary for the substratum support layer to be highly permeable, robust, and free from surface defects. In contrast, symmetric inorganic ceramic membranes are homogeneous in cross section differing from asymmetric membranes in that the entire path length of a solute through the membrane wall is subject to the same separation characteristics. The ceramic supports can be manufactured using a variety of techniques including colloidal processing, paste processing, or dry pressing. The subsequent membrane is then sintered at high temperatures to achieve the desired strength, stability, and rigidity (Biesheuwel and Verweij, 1999; Ahn *et al*., 1998).

### 2.1.2 Method of slip-cast moulding

The method of slip cast moulding was used to produce tubular ceramic oxide membrane elements; it is known as a method of template casting. According to this method the ceramic article of required shape is formed from a slip (a water suspension of powdered reagents for ceramic production) by casting into a gypsum mould. The gypsum mould provides for the diffusion of liquid from the slip, whilst maintaining the shape of the solid particles. This method has a number of advantages; these include:

- the ability to produce articles of irregular shapes;
- the ability to produce articles of high porosities;
- low construction and mould expenses; and
- the simplicity of control of the molding process.

A simplified scheme illustrating the process by which tubular ceramic oxide membranes are made using this method is shown in Figure 2.1.



a) Filling with slip b) Setting c) Slip removal d) Drying

**Figure 2.1: Schematic illustration of the making of ceramic articles by the method of slip-cast moulding**

The mould is filled with slip (a) and then left for staying. The gypsum mould absorbs the liquid which is contained in the slip. As a result, the walls of the article will be formed on the walls of the mould (b). The level of liquid, absorbed by the gypsum form, is retained by adding of the slip. After the necessary thickness of walls has been created the retained slip is removed by draining (c). The gypsum mould is left in the inverted position, while it continues to absorb moisture from the material. After the article has contracted it is removed from the mould (d).

In many publications describing the synthesis and results of research into ceramic membrane structure it was noticed that the tubular materials with a two-layer structure appeared to be the most interesting (in terms of applications). The outer support layer is thick and has large pores while the internal (working) layer has pores of less than 1 μm and its thickness is less than 100μm. Slip-cast moulding affords ceramic tubular elements with graded wall thickness, from large pores (5 μm) to small ones (0.1-0.5 μm). In the process of slip-casting the larger particles settle faster than the small ones on the surface of the gypsum mould. The smaller particles concentrate on the internal surface of the tube being formed (surface distant from the gypsum wall). The choice of composition of slip suspension in the given study was made after taking the following factors into consideration:

- information available on the application of the individual and combined oxides of aluminium and zirconium for membrane materials and heterogeneous catalysts,
- zirconium and aluminium oxides have high durability and chemical stability,

- incorporation of sub-micron particles of tetragonal zirconium dioxide provides the maximal durability for the porous body.

## 2.2 MATERIALS AND METHODS

### 2.2.1 Ceramic Membrane Production (RSA patent 97/7639)

#### *2.2.1.1 Zirconium dioxide slip production - stabilisation with yttrium oxide*

Quartz cells were filled with stabilised zirconium dioxide and then placed in an electric oven. The zirconium dioxide had been stabilised by heating at a rate of $200^{o}C \cdot h^{-1}$ until the temperature was as high as $900 \pm 10^{o}C$, for 2 h, and then cooled. The heat-treated zirconium dioxide was ground for 60 h in a ball mill in the presence of steel balls. The ratio of zirconium dioxide:balls:water was 1:2:1. The dispersed zirconium dioxide was washed free of iron impurities with hydrochloric acid. To purify zirconium dioxide the suspension of graded zirconium dioxide was poured into polyethylene vessels and then concentrated hydrochloric acid was added (amounts of 100 g $ZrO_2$ in 30 ml HCl ). The mixture was then stirred for 2 h and left to stand for 24 h, to effect sedimentation. The supernatant was then poured off and the sediment of zirconium dioxide was rinsed with distilled water until the acidity was pH 1.5-2.0. The cleaned suspension of zirconium dioxide was left for 2-3 weeks, during which time excess water was periodically poured off. The dense sediment was then placed in a laboratory glass reactor fitted with a mechanical stirrer. 15-20% distilled water (by mass of zirconium dioxide) was added and the mixture stirred for 6 h. After the slip had been strained through a nylon-6 sieve, with mesh less than 0.1 mm, it was again placed into the reactor and stirred with a mechanical stirrer for 1 h. The strained slip with pH 1.5-2.0 was used for producing the working suspensions for the casting of ceramic tubular elements by the method of slip-cast moulding. The density of the produced slip of $ZrO_2$ was $2.9\text{-}3.1 g \cdot cm^{-3}$.

#### *2.2.1.2 Aluminium oxide slip production*

The corundum cells were filled with aluminium oxide and then placed in an electric oven. They were heated at a rate of $200\text{-}250^{o}C.\ h^{-1}$ until the temperature was $1450 \pm 5^{o}C$. After the aluminium oxide had been left for 2 h at $1450^{o}C$ it was cooled. It was then fired for 6-8 h at a temperature of $1200^{o}C$. The alumina was ground in a ball mill by steel balls. The ratio of aluminium oxide:balls:water was 1:2:1. The milling was continued for 50 h. The alumina was then washed free of iron impurities as had been done for zirconium oxide. The sediment of aluminium oxide was rinsed with distilled water until the acidity was pH 4.0-5.0. The cleaned suspension was then left to stand for 2-3 weeks. During this time the cloudy supernatant was periodically decanted. The dense sediment was then placed in a glass reactor and 15-20% distilled water (by mass of aluminium oxide) was added, and the mixture stirred

for 4-6 h. The slip was then strained through a nylon-6 sieve with a mesh less than 0.1 mm and again placed in the reactor and stirred for 1 h. The strained slip, with a pH of 4.0-4.5, was used for producing the working suspensions for the slip cast moulding. The density of the produced slip of aluminium oxide was 2.4-2.6g·cm$^{-3}$. To produce working suspensions the slip of zirconium dioxide with density 2.9-3.1g·cm$^{-3}$ (10 vol. %) and the slip of aluminium oxide with density 2.4-2.6g·cm$^{-3}$ (90 vol %) were stirred in a laboratory glass reactor for 1h until homogeneous suspensions were formed. The produced suspensions were used for the manufacturing of ceramic tubular elements by the method of slip-cast moulding.

#### *2.2.1.3 Slip-cast moulding of ceramic tubular elements*

Gypsum was strained through a 0.5-1.0 mm mesh sieve and then added to water (100 g of gypsum to every 150 ml of water). The mixture was stirred for 1 min and then moulded into forms in steel models, which were lubricated with oil. After 30 min the models were removed and the gypsum forms dried at a temperature lower than 80$^{o}$C, for 48 h.

The slip, which consists of a suspension of zirconium dioxide and aluminium oxide, was placed into gypsum moulds, which had previously been moistened with water. The slip was added until the walls of the tubular elements were formed. The operation took between 10 s and 4 min (depending on the wall thickness). Then the tubes were withdrawn from the gypsum moulds and the moulded tubes were dried at 20$^{o}$C for 2-3 days. The tubes were visually inspected with a looking glass. Only those tubes which had no abscess formation, foreign particulates or chips on either the internal or the outside surfaces were used for further studies. Having been controlled, the tubes were fired. The heating rate was 100-110$^{o}$C·h$^{-1}$. The firing temperature was 1220-1280$^{o}$C. The soak time at firing temperature was 60 min.

## 2.3 RESULTS

### 2.3.1 Ceramic membrane characterisation

#### *2.3.1.1 Chemical composition and characterisation*

According to the data of atomic absorptive analysis of the ceramic material (after dissolving it in hydrofluoric acid) it was found that the chemical composition of the ceramic material generally corresponded to the ratio of the oxides used in the synthesis:

- aluminium oxide $Al_2O_3$ - 70 %
- zirconium dioxide $ZrO_2$ - 29 %
- yttrium oxide $Y_2O_3$ - 1 %

It is expected, however, and is seen in Table 2.1, that the chemical compositions of the internal and external surfaces (30 μm in thickness) are quite different. The internal surface of the tubular ceramic element is enriched with zirconium dioxide, while the external surface contains more aluminium oxide. The observed anisotropy of the composition of the ceramic material illustrates that the sedimentation kinetics of oxides particles on the walls of a gypsum mould are different. This fact is of great importance in the direct synthesis of anisotropic membrane materials.

**Table 2.1. Chemical composition of tubular ceramic material (in volume and in the 30 μm surface layer) according to analytical data of X-ray spectra.**

| Chemical element | Chemical composition, atom. % ( in volume of ceramic material ) | Chemical composition, atom. % (internal surface) | Chemical composition, atom. % (outside surface) |
|---|---|---|---|
| Al | 84.8 | 62.9 | 92.5 |
| Zr | 14.6 | 35.7 | 7.2 |
| Y | 0.6 | 1.4 | 0.3 |

#### *2.3.1.2 Structure of ceramic material - SEM (Scanning electron microscopy)*

According to results of electron microscopy the structure of the porous ceramic material was formed by agglomerates of baked oxide particles with an average diameter of 0.3 μm. Because the method of sample preparation for electron microscopy included the sequential spraying of quite a thick coat of carbon and gold (about 30 nm), the real diameter of the particles was much smaller. Analysis of the structure of the synthesised tubular ceramic material shows that the internal surface of the tubes had a much higher density than the external surface. This fact corresponds to the revealed differences in chemical composition of the internal and external surfaces of ceramic samples. Hence, the method of slip-cast

moulding of ceramic materials creates a gradient of porous structure, from the external to the internal wall of tubular ceramic samples.

#### *2.3.1.3 Pore size determination and distribution*

According to the data of mercury intrusion porosimetry the porosity of the synthesised ceramic material is about 45 %, the specific surface area is 4 $m^2 \cdot g^{-1}$, and the average diameter of the pores is 0.21 μm. This ceramic material has quite a narrow distribution of pore radii in the area of the macropores (Figure 2.2). There are almost no pores with a radius greater than 0.15 μm. The pore size distribution curve has its peak at about r = 0.10 μm. An investigation into ceramic structure by the method of low-temperature nitrogen adsorption revealed that there were a great number of pores with radii of less than 1 nm. These pores were, however, considered to be surface defects, caused by oxides, which were used in the synthesis.

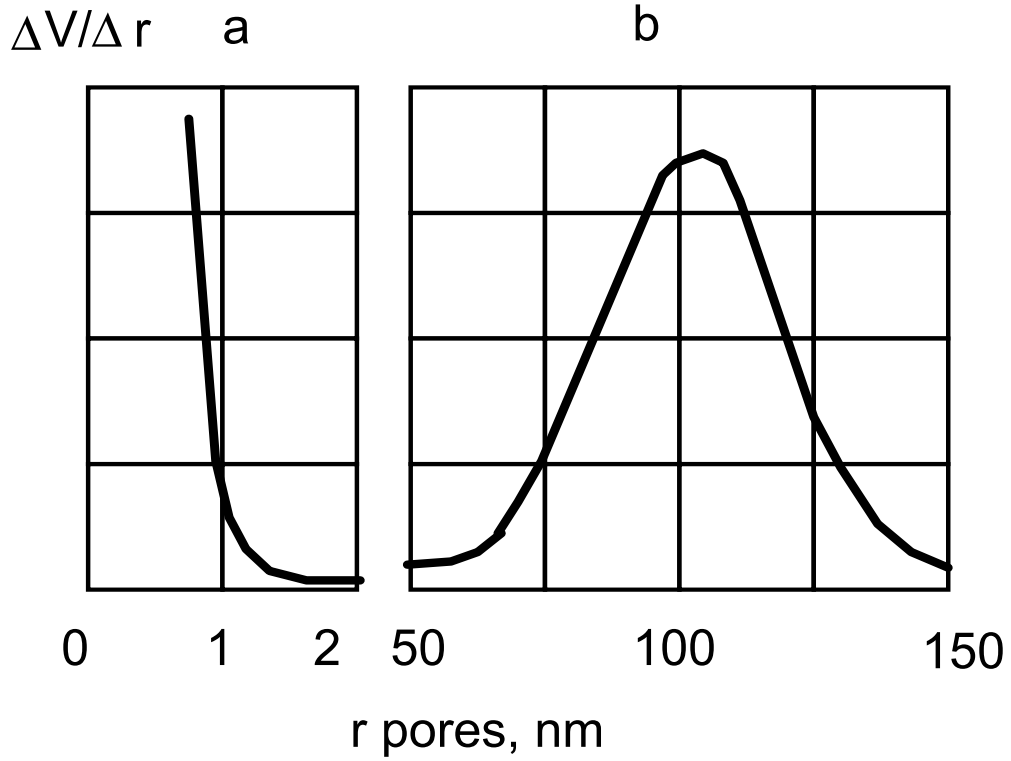



**Figure 2.2: Pore size distributions in a porous ceramic material, determined by: a – the method of low-temperature nitrogen adsorption; b – mercury intrusion porosimetry**

#### *2.3.1.4 Permeability*

Results of a study of the permeability of a porous ceramic material to distilled water revealed a linear plot of productivity (permeability) versus pressure, as seen in Figure 2.3. The slope is about 500 L· $h^{-1}$· $m^{-2}$· mPa. The membranes could withstand pressures up to 1 mPa.

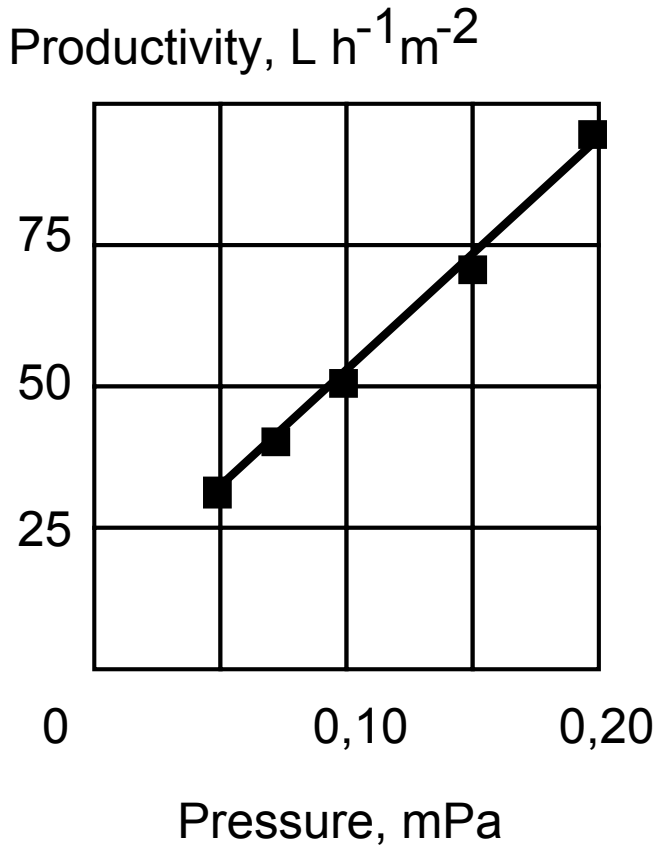



**Figure 2.3: Permeability of a porous ceramic material to distilled water at various pressures**

The study of the permeability of a porous ceramic material with dextran samples of different molecular weights indicated at the possibility of its application for microfiltration. It appears, from Figure 2.5, that this ceramic material can efficiently reject dextran fractions of molecular weights greater than 2000 kD.

#### *2.3.1.5 Chemical stability*

As a criterion for the determination of the chemical stability of ceramic tubular elements, their mass losses after boiling in 1, 3, and 5 N solutions of $H_2SO_4$ and KOH, for different times, were used. It is seen in Figure 2.5 that prolonged boiling of the ceramic material in alkali did not lead to a pronounced mass loss and was proof of the high chemical stability of the given material at a pH range > 10. The ceramic material also had high chemical stability in strong acid solutions (boiling 1N sulphuric acid).

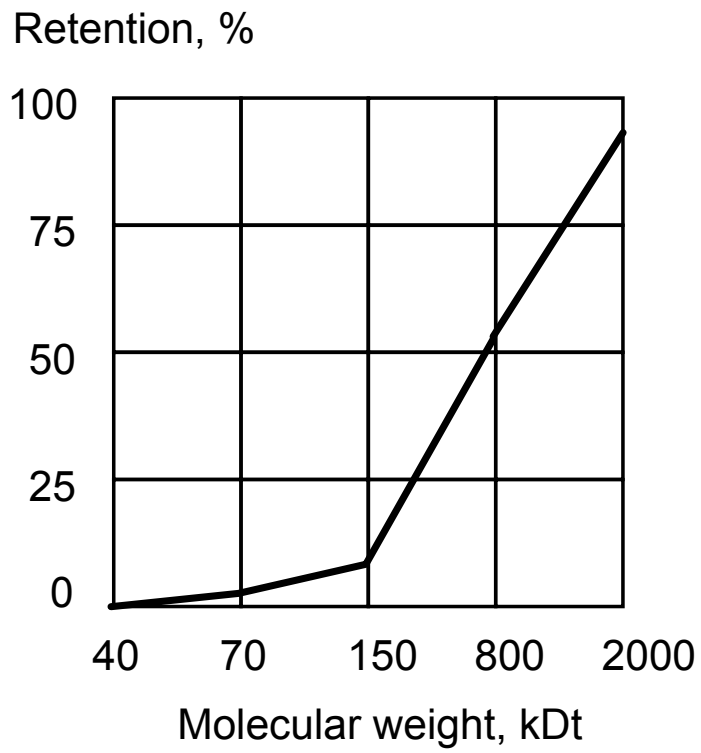



**Figure 2.4: Ability of ceramic material to retain dextrans with different molecular weights**

There was, however, a pronounced mass loss and therefore a loss of chemical stability, when the sulphuric acid concentration was increased from 3N to 5N. Acid or alkaline treatment, without heating, did not lead to any mass loss of the ceramic material (in the concentration ranges studied).

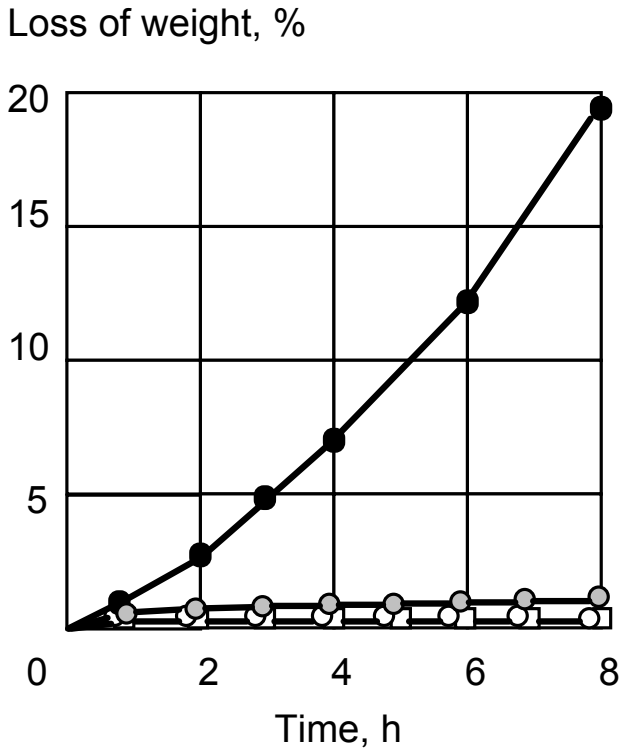



**Figure 2.5: Chemical stability of ceramic material produced from zirconium and aluminium oxides, in boiling solutions of sulphuric acid (hollow circles – 1 N $H_2SO_4$, light circles – 3 N $H_2SO_4$, dark circles – 5 N $H_2SO_4$ ) and potassium hydroxide, squares - 1, 3 or 5 N KOH)**

## 2.4 DISCUSSION AND CONCLUSIONS

On the basis of experimental results, it was evident that the tubular ceramic membranes displayed significantly greater chemical stability than would be expected with organic-based polymeric membranes. Furthermore, the narrow pore size distribution (0.10 μm±0.05 μm) would facilitate even permeation rates (500 $L \cdot h^{-1} \cdot m^{-2} \cdot mPa$) resulting in reproducible operating conditions for the MBR systems. From these results, it was concluded that the synthesised porous ceramic material would display microfiltration properties. This would facilitate higher flux necessary for effective wastewater treatment in terms of volumetric productivity, but would also facilitate biomass retention necessary for effective biofilm maintenance and development.

# CHAPTER 3: SEEDING REACTOR TREATMENT OF STRIPPED GAS LIQUOR EFFLUENT

## 3.1 INTRODUCTION

### 3.1.1 MBR treatment of industrial wastewater

Several studies on MBR, activated sludge and anaerobic digestion systems have shown how the microbial population adapt to waste composition and that shock loading or changes in the operational parameters are inevitably detrimental to the 'remediation active' population (e.g. Eichner *et al*., 1999; La Para *et al*., 2002). This could have increased cost implications for operating a MBR system (Yoon *et al*., 2004). Monitoring the microbial population dynamics is thus essential for optimizing and operating such systems (La Para *et al*., 2002). With industrial effluents in particular, the use of micro- or ultrafiltration-based biomass retention enables the build-up of a waste-specific microbial population. Due to the biomass retention characteristics of membranes these populations do not necessarily include organisms responsible for flocculation as in traditional AS systems (Scott *et al*., 1998). This circumvents the necessity for maintaining microorganisms responsible for producing high levels of exopolysaccharide – as is necessary for floc formation in conventional AS systems. The resultant microbial population within the developed biofilm is therefore composed of a highly 'remediation active' population (Scott *et al*., 1998). Furthermore, the ability of biofilms to support a variety of different populations at various locations within the biofilm enables fixed film processes to be more amenable to the degradation of xenobiotics than the conventional activated sludge process (Bishop, 1997).

### 3.1.2 Analysis and characterization of biofilm-associated microbial communities

The accurate assessment of the viable fraction of microbial populations using culture-dependent techniques such as Biolog® and MIDI is limited by the lack of technology presently available to culture more than 90% of the microorganisms present in most complex communities such as those associated with biofilms and soil (White and Macnaughton, 1997). Utilisation of non-culture dependent molecular based techniques circumvents this problem. As a result, direct assessment of non-culturable viable microorganisms present in complex interactive communities is possible by direct extraction of cellular components such as the nucleic acid and lipid biomembrane fractions (Ibekwe *et al*., 2002; Kehrmeyer, *et al*., 1996). The combination of nucleic acid analysis with lipid biomarker identification and quantification can provide complementary information with each approach substantiating the results of the other.

### 3.1.3 Molecular techniques – DGGE analysis

Community genomic analysis using techniques such as community DNA hybridization, %G+C profiling, restriction digestion and sequence comparisons, amplified ribosomal DNA restriction analysis (ARDRA), terminal restriction fragment length polymorphism (T-RFLP), ribosomal intergenic spacer analysis (RISA), as well as various combinations of these techniques can reveal considerable information regarding the richness, evenness, and composition of the community (Kozdrój and Van Elsas, 2001; Torsvik and Øvreås, 2002). An increasingly popular tool within microbial ecology has been the PCR-based amplification of bacterial 16S rDNA and fungal 18S rDNA fragments combined with electrophoretic separation based on melting (TGGE) or denaturing (DGGE) behaviour. In combination with hybridisation, sequence analysis, community substrate utilisation, PLFA analysis, and various other culture methods, TGGE and DGGE profiling gives a reasonable estimation of the dominant *in situ* microbial community structure (Luxmy *et al*., 2000; Brüggemann *et al*., 2000). Furthermore, 16S and 18S rDNA-based techniques also provide a wealth of information concerning specific microbial population activity and more importantly, community stability (Dabert *et al*., 2002).

### 3.1.4 Molecular techniques – PLFA analysis

The analysis of phospholipid fatty acids is a useful measure of the viable biomass content, microbial community population structure, and the physiological status of the community (Kehrmeyer, *et al*., 1996). Although the analysis of PLFA profiles does not enable the identification of microorganisms at a species or strain level, it does, however, provide a comprehensive description of the microbial community based on the different functional groupings within the PLFA profiles (Ibekwe *et al*., 2002; Ibekwe and Kennedy, 1998). Furthermore, the identification of specific genera is possible by the presence of certain signature fatty acids (for example, *cis*-octadec-8-enoic is associated with Type II methanotrophs). The identification of physiological stress is also possible by monitoring increases in *trans*-mono-unsaturated fatty acids. Through the use of different functional groupings from PLFAs, characterisation of the microbial community structure is possible using a community level approach by evaluating shifts in PLFA profiles. Changes in the PLFA profiles would therefore be indicative of changes or stress responses as well as the *in situ* nutritional/metabolic status within the microbial community (Ibekwe *et al*., 2002; Ibekwe and Kennedy, 1998; Macnaughton *et al*., 1997; Kehrmeyer, *et al*., 1996).

By combining 16S and 18S rDNA-based nucleic acid analysis with lipid biomarker identification and quantification, it is envisaged that stress responses experienced by the membrane-associated biofilm and planktonic tank communities may be evaluated and

correlated with process operational characteristics. Furthermore, as PLFAs are associated with rapid turnover within the cell, immediate stress-related responses to shock or toxic loading effects may be correlated with shifts in the dominant *in situ* microbial community structure determined using 16S/18S rDNA-based DGGE analysis and subsequent sequence identification. Thus, specific MBR operating conditions under which the metabolic status and stability of the membrane- and tank-associated populations is subject to shifting may therefore be identified. The operation of the MBR may therefore be optimized in terms of residence time, crossflow velocity, TMP, and loading rates, where optimal microbial populations associated with the greatest productivity and process efficiency may be maintained.

## 3.2 Membrane Bioreactor Process

### 3.2.1 Membrane bioreactor tank

The membrane bioreactor tank, used in association with the tubular ceramic membrane modules, had a working volume of 60 L. The MBR tank was maintained at 25-27ºC for the duration of the experimental period. The dissolved oxygen concentration was maintained between 4 and 6 mg.L-1 with the pH of the system remaining stable between 7.5-8.5. The system was initially operated for a period of 7-days under batch operating conditions and thereafter operation followed a continuous feed-and-bleed approach for a total period of 20-days. Thereafter, the membrane module component of the membrane bioreactor system was started up. The feed-and-bleed approach entailed the removal of 20 $L.day^{-1}$ mixed liquor thereby maintaining a hydraulic retention time in the tank of 3-days. The removed mixed liquor was replaced daily with 20 L of fresh SGL make-up feed corrected to a C:N:P ratio of 100:10:1 using $KH_2PO_4/K_2HPO_4$ and urea.

#### *3.2.1.1 Tubular ceramic membrane modules*

Each single-membrane reactor module comprises an effective membrane area of 0.0067 $m^2$. The membrane module construct, comprising three in-series modules, have a total membrane area of 0.02 $m^2$ with each MBR assembled (comprising 5 constructs in series) having an effective total membrane area of 0.133 $m^2$ for the membrane bioreactor (refer to Fig. 3.1). Fluid reticulation within the MBR system was achieved using a magnetic drive centrifugal pump (March MFG., Inc.; Ill. USA) with a recirculation flow rate of 150 $L.h^{-1}$ from the MBR tank and a membrane module influent flow rate of 10 $L.h^{-1}$. At this flow rate, the cross flow velocity (V) was 0.035 $m.s^{-1}$ and the transmembrane pressure was <2 kPa.

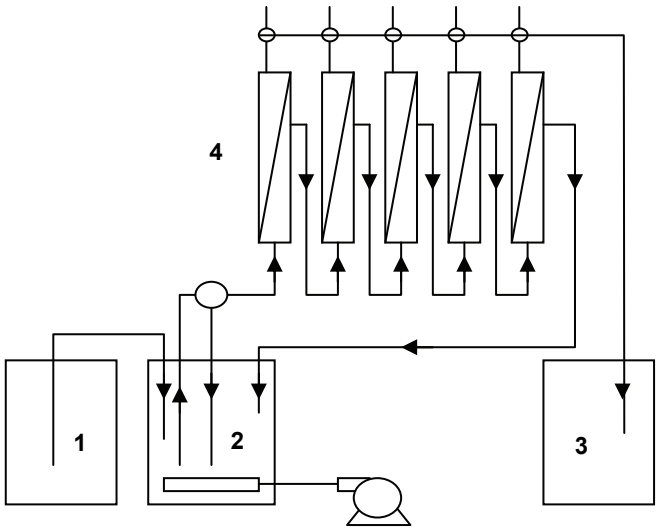



**Figure 3.1: MBR operating configuration: (1) SGL feed tank; (2) MBR recirculation tank; (3) MBR effluent receiving tank; (4) MBR membrane modules**

The hydrodynamics of the membrane module units enabled the maintenance of laminar flow conditions at this cross flow velocity with a resultant Reynolds number (Re) of 350. Periodic displacement of the accumulated biofilm in each membrane module for PLFA and 16S rDNA analysis was achieved by increasing the cross flow velocity to 0.21 $m.s^{-1}$ to induce turbulence within the system (Re=2100) thus facilitating removal of the accumulated biomass.

### 3.2.2 Activated Sludge Process

Activated sludge reactors comprising aerated CSTR-type vessels coupled to in-series clarifiers were used (see Fig. 3.2). The working aerated reactor volume was 3.4 L and the temperature of the reactor was maintained at 20°C in a thermostat-controlled room. Air sparging of the reactor facilitated dissolved oxygen maintenance within the aerated reactor (YSI 55 DO meter, Monitoring & Control Laboratories, Lyndhurst, S.A.). Mixing of the aerated reactor was facilitated using Rushton-type impeller-based agitation. The pH of the reactors was monitored using an YSI 63 pH/salinity probe (Monitoring & Control Laboratories, Lyndhurst, S.A). The SGL feed solution was fed using a peristaltic pump (101U, Watson Marlow) maintaining the hydraulic retention time of the system at 4 days. The sludge purge volume of the system was kept constant resulting in a SRT of 30 days. The airlift sludge return mechanism of the clarifier facilitated sludge recirculation back to the aerated reactor at a return flow rate of 0.18 $L.min^{-1}$. The AS system was initially operated for a period of 7-days under batch operating conditions and thereafter operation followed a continuous feed approach for the duration of the operating period.

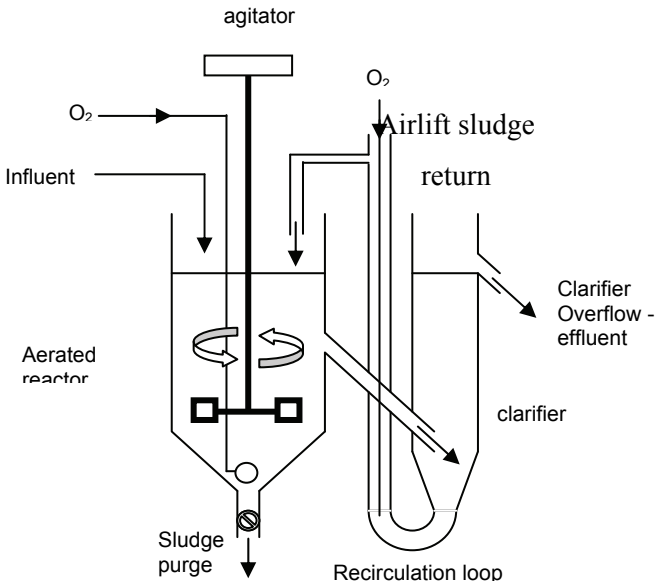



**Figure 3.2: Activated sludge reactor configuration**

### 3.2.3 Effluent

The industrial effluent was obtained from Sasol and is referred to as Stripped Gas Liquor (SGL). SGL is the condensate (gas liquor) that is generated by Sasol's coal gasification process. This condensate then undergoes a sedimentation process for the removal of tar products. Once the removal of the tar products is complete, the condensate is treated via the Phenolsolvan process for the removal of phenolics and then undergoes steam-stripping for the removal of ammonia and to facilitate a decrease in the pH of the condensate. The resultant effluent is then referred to as SGL.

The SGL feed composition, as used in these experiments, is as follows: COD 1500-2500 mg/L; $NH_3$ 550 mg/L; pH 8-8.5; Conductivity 4500 µS/cm; Phenolics 200 mg/l; Fluoride 250 mg/L. $KH_2PO_4/K_2HPO_4$ and urea were added to the both the membrane bioreactor tank and activated sludge feed to achieve a molar C:N:P ratio of 100:10:1. Both the activated sludge and membrane bioreactor tank were seeded with mixed liquor suspended solids (MLSS) obtained from the waste activated sludge plant at Sasol, Secunda. Both reactors were seeded with a 20% (v/v) inoculum consisting of MLSS at 10 000 $mg.L^{-1}$. This mixed culture was then acclimatized to the hydrodynamic and biological conditions by operating both the activated sludge and membrane bioreactor CSTR for a period of 7-days under batch conditions. Thereafter the AS system was operated continuously as described in section 3.2.2 and the MBR tank was operated as described in section 3.2.1.

### 3.2.4 Analytical Methods

Measurement of the dissolved oxygen concentration was performed using an YSI 55 DO meter (Monitoring & Control Laboratories, Lyndhurst, S.A.). Determination of pH was performed using an YSI 63 pH/salinity probe (Monitoring&Control Laboratories, Lyndhurst, S.A). Analysis of the Total and Volatile Suspended Solids was performed according to Standard Methods (APHA, 1985). Chemical Oxygen Demand (COD) analysis was performed using Merck Spectroquant® kits.

### 3.2.5 Denaturing Gradient Gel Electrophoresis (DGGE)

#### *3.2.5.1 Sample collection*

Duplicate samples (50 ml) were collected from the feed, inoculum, membrane bioreactor tank, the membrane bioreactors and the activated sludge reactor. The 50 ml samples were centrifuged (4000xg) for 5 minutes and 35 ml of supernatant carefully discarded. The remaining 15 ml were used as the microbial community samples from which total community DNA were extracted.

#### *3.2.5.2 DNA extraction and purification*

The DNA extraction and purification protocol encompassed physical and chemical lysis in a high-salt extraction buffer followed by phenol/chloroform/isoamyl alcohol, chloroform/isoamyl alcohol extraction and ethanol precipitation (Stewart&Via, 1993). The method is detailed as follows:

One ml of this sample was placed in a weighed 1.5 ml microfuge tube and centrifuged at full speed for 5 minutes. The supernatant was discarded and the pellet weighed. The pellet was resuspended in 100 µl of TE buffer, lysozyme added to a final concentration of 50 µg/ml and incubated for 30 minutes at 37°C. Hot (65°C) 2X CTAB [hexadecyltrimethylammonium bromide] isolation buffer (100 mM Tris pH 8.0, 20 mM EDTA, 2% w/v CTAB, 1.4 M NaCl, 0.2 % v/v β-mercaptoethanol) [β-mercaptoethanol added immediately before use] was added and further incubated at 65°C for a further 30 minutes gently inverting the tubes 3 or 4 times (every 3 minutes). One freeze-thaw step involved 5 minutes on ice then incubation at –85°C for 15 minutes followed by addition of hot (65°C) PVP solution (final concentration of 0.5 %) and incubation 65°C for further 30 minutes inverting the tubes 3 or 4 times (every 3 minutes). This was followed by hot (65°C) buffered phenol:chloroform:isoamyl alcohol (pH 8.0) (25:24:1) extraction for 10 minutes at 65°C (gently inverting the tubes 3 or 4 times (every 3 minutes). The phases were separated by centrifugation and the aqueous phase re-extracted with an equal volume buffered chloroform:isoamyl alcohol (pH 8.0) (24:1) for 10 minutes at room temperature gently inverting the tubes 3 or 4 times. After centrifugation (13,500 rpm for

5 minutes) the DNA in the aqueous phase was precipitated with NaCl, ice cold 95 % Ethanol and incubation at -80°C for at least 1 hour. The precipitating DNA mixture was then immediately centrifuged at 4°C (full speed in microfuge) and the pellet washed with ice cold 70 % ethanol. The DNA was vacuum dried and reconstituted by adding 50 µl $dH_2O$ and incubating at 65°C (1 hour).

#### *3.2.5.3 DNA quantification*

The extracted DNA was quantified spectrophotometrically using 260 nm values and diluted accordingly. The 260/280 nm ratios were used to determine the purity of the DNA (Sambrook *et al*., 1989; Kozdrój and Van Elsas, 2001).

#### *3.2.5.4 Polymerase Chain Reaction (PCR) conditions*

The eubacterial primer combination GM5F and 907R was used for PCR amplification of community DNA (Muyzer *et al*.,1993). The sequence of forward primer (GM5F) was 5'-GCC CGC CGC GCC CCG CGC CCG TCC CGC CGC CCC CGC CCG CCT ACG GGA GGC AGC AG-3' [the GC-clamp is underlined] and the reverse primer (907R) 5'-CCG TCA ATT CCT TTG AGT TT-3'.

PCR amplification was performed in a Hybaid thermal cycler and the conditions for the euabacterial primers consisted of 5 min at 94°C followed by 35 cycles consisting of 30 sec at 94°C, 30 sec at 65°C and 1 min at 72°C. The final cycle was followed by a 4 minute extension at 72°C. The total reaction volume of 25-µl contained 1.0 U of *Taq* DNA polymerase (Super-therm *Taq*, AB Biolabs), 1 X Roche PCR master mix (10 mM Tris–HCl pH 8.0 , 50 mM KCl, 1.5 mM MgCl , each deoxyribonucleotides at a concentration of 200 µM), 4 m MgCl [additional], 25 pmol of each primer, 100 ng of the extracted DNA, 100 ng of BSA overlaid with 25 µl of mineral oil (AB Biolabs). Amplification of PCR products of the proper size was confirmed by electrophoresis through a 1.0 % w/v agarose gel containing ethidium bromide (50 µg/ml) in TAE buffer. Electrophoresis was performed for 45 minutes at 100 V (Sambrook *et al*.,1989). A molecular size marker (1 kb ladder, Roche DNA molecular weight marker XIV) was used as the reference. A gel image analysis system (Syngene Gene Genius BioImager) was used to document and analyse the gels.

#### *3.2.5.5 DGGE analysis*

DGGE analysis was performed using a D-Code universal mutation detection system (BioRad) and electrophoresis reagents from BioRad. PCR products (15 µl) were loaded onto a 6.0% (w/v) polyacrylamide gel cast in 1 x TAE (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH

8.0). The polyacrylamide gels (acrylamide:bisacrylamide, 37.5:1) were made with denaturing gradients ranging from 30 to 60% (100% denaturant contained 7 M urea and 40% formamide). Gels were equilibrated to 60°C before loading the PCR products. Electrophoresis was at 60°C for 16.5 h at 100 V. After electrophoresis, the gels were stained for 20 min with ethidium bromide (50 μg/ml) and destained for 15 minutes in 1X TAE. A gel image analysis system (Syngene Gene Genius BioImager) was used to document the gels. Syngene Gene Tools and Gene Directory software were used to analyse the gels.

Manual statistical analysis using data obtained from the software was also performed estimating species richness & diversity within microbial populations analysed. Since each band is likely to be derived from a single phylogenetically distinct clade/species (Dabert *et al*., 2002; Ampe&Miambi, 2000; Schramm&Amann, 1999), species richness was calculated by total number of bands on the gel. The Shannon-Weaver species diversity index *H,* which is computed according to equation 1:

$$H = -\sum_{i=0}^{n} Pi.\log Pi \qquad (1)$$

where *Pi* is the portion of the total sample occupied by species *i* and *n* is the number of species, was used to estimate species richness (Shannon&Weaver, 1949).

In addition pair-wise similarity indices were compiled and from these dendrograms were derived that graphically demonstrated the relatedness of the various community DGGE profiles.

### 3.2.6 Fatty Acid Methyl Ester (FAME) analysis

#### *3.2.6.1 Sample collection*

Triplicate samples (50 ml) were collected from the feed, inoculum, MBR tank, the membrane bioreactors and the activated sludge reactor. The 50 ml samples were frozen immediately, freeze dried (Dura Dry™, FTS Systems, Inc., N.Y.), and stored at -84ºC until extraction procedures were initiated.

#### *3.2.6.2 Phospholipid extraction*

All glassware used for extraction, fractionation, and analysis was washed with phosphate-free soap, air dried, and heated at 450°C in a muffle furnace for 4 hours prior to use. Total community phospholipid was extracted from 0.05g lyophilized biofilm and planktonic

samples using the modified Bligh and Dyer procedure as described by White and Ringelberg (1998).

#### *3.2.6.3 Phospholipid separation*

The total community lipid extract was subjected to fractionation using salicic acid column chromatography to separate the glyco-, neutral, and polar lipids. The resultant polar lipid fraction was then transesterified using mild alkali methanolysis resulting in the recovery of the PLFA as methyl esters (FAMEs) in hexane.

#### *3.2.6.4 Phospholipid analysis*

The transesterified PLFAs (FAMEs) were separated, quantified, and identified using capillary gas chromatography (GC) with flame ionization detection. The system hardware consisted of a HP 6890 series II chromatograph (Hewlett-Packard, Wilmington, DE, USA) fitted with a 60 m SPB-1 capillary column (0.25 mm I.D.; 0.25 μmfilm). Methylnonadecanonate (C19:0) was used as the internal standard and FAMEs were expressed as equivalent peak responses to the internal standard. Definitive peak identification was facilitated using a HP 6890 series II chromatograph interfaced with a HP 5973 series mass selective detector (Hewlett-Packard, Wilmington, DE, USA).

## 3.3 RESULTS

### 3.3.1 Membrane Bioreactor System

#### *3.3.1.1 Hydraulic performance*

After initial characterization of the membrane modules, the flux of the membrane bioreactor was monitored continuously for a period of ±300 days (7200 hours). After inoculation, the MBR tank system was operated initially as a batch system for 7 days and then for a further period of 13 days under continuous feed-and-bleed conditions as described above. Thereafter, the membrane modules were started up and operated using the mixed liquor in the MBR tank as the influent feed. During this 20-day start-up period prior to the membrane modules being operated, the MLSS in the MBR tank dropped from 2000 $mg.L^{-1}$ to approximately 300 $mg.L^{-1}$. This showed minimal fluctuation over the full 300-day MBR tank operating period.

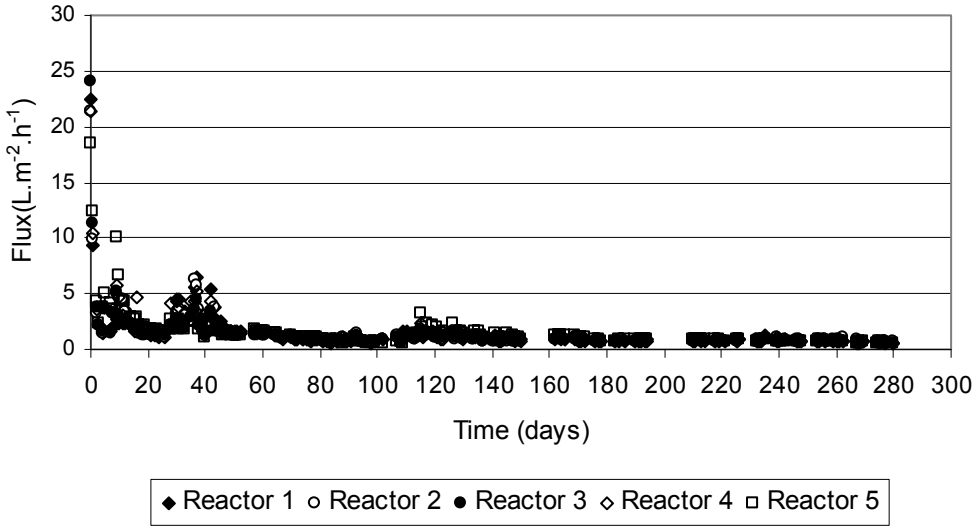



**Figure 3.3: Average flux for the SGL-MBR over a period of 280 days (1700 operating hours). Data is represented as the average flux for the five membrane modules operated in-series**

The main objective of the seeding reactor was the establishment of an acclimated biofilm for maintenance of a constant inoculation regime to enhance the productivity of the hydrolysis reactor. Maintenance of a high flux operating regime was therefore secondary in terms of operational requirements. Due to the nature of the influent feed, rapid flux decline (65% loss in initial flux) was observed within the first 24-hours after start-up of the membrane modules (see Figure 3.3). Over the operating period of 1700 hours, slight flux recovery was observed at days 8-12, 28-37, and 40-43, however, the average steady-state flux was 2 $L.m^{-2}.h^{-1}$. This operating flux enabled the formation of a loose, easily dislodged biofilm that was removed periodically for analysis using low pressure backflushing pulses. A similar biofilm management strategy was envisaged for the coupled seeding and hydrolysis MBR configuration.

### *3.3.1.2 Performance of the MBR and AS systems*

The initial operation of the MBR tank was as a batch process for a period of 7-days after inoculation, and then as a feed-and-bleed approach for a further 13-day period prior to circulation of the MLSS and SGL feed through the ceramic membrane modules. During this acclimation period a maximum of 25 – 35% COD removal was observed (results not shown). Thereafter, during the MBR operating period up to 180-days, an average COD removal of 45% was observed (Refer to Figure 3.5).

In contrast, the MBR membrane module-associated COD removal efficiency increased rapidly from an initial 40% to 80% over the 17-day operating period. Thereafter the removal efficiency stabilized for the following 180-day operating period with an average of 70-80% COD removal observed (refer to Figure 3.3 and Figure 3.4). Thereafter (day 197), additional COD in the form of 100ppm phenol and 100ppm 2,4-dichlorophenol was added to both the AS and MBR feed (see Figure 3.4).

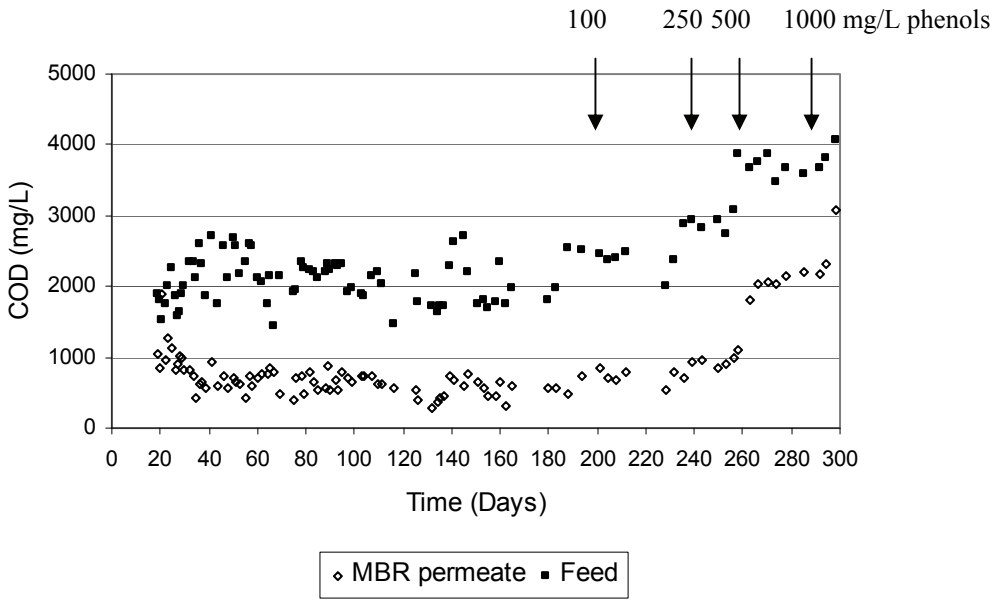



**Figure 3.4: COD removal efficiency of the MBR. Phenol and 2,4-dichlorophenol addition is indicated with arrows and respective concentrations in ppm ($mg.L^{-1}$)**

The phenol and 2,4-dichlorophenol concentration was then increased to 250ppm (Day 235), 500ppm (Day 257), and 1000ppm (Day 293). A comparative evaluation of the MBR process with conventional AS system revealed that during these incremental loading periods, no significant decrease in operating efficiency in terms of COD removal was observed at concentrations of 250ppm (an accumulative concentration of 500ppm aromatic load). Thereafter, from 500 to 1000ppm addition, the average COD removal of 75% for the MBR decreased to 20% (refer to Fig. 3.5) and from 55% for the AS reactor to 20%.

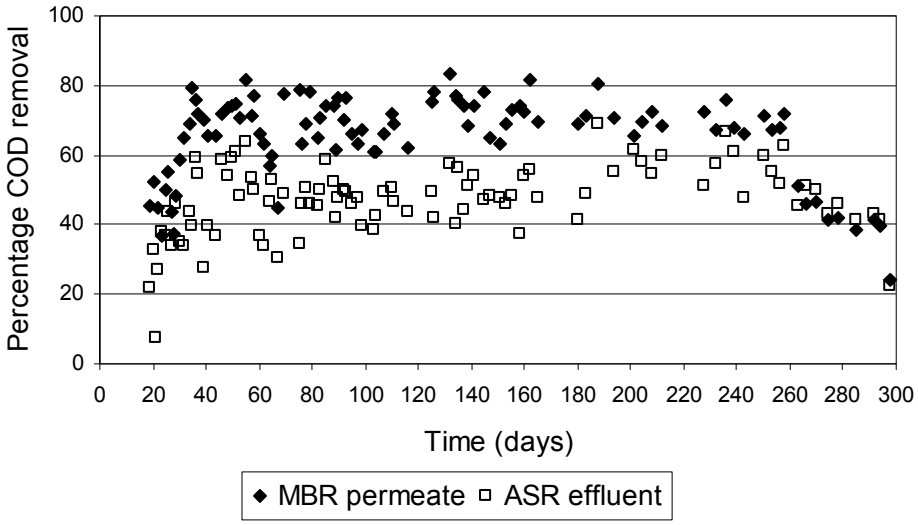



**Figure 3.5: COD removal efficiency of the AS process**

As the design and control of MBR systems is usually based on the operational stability and ability of the MBR system to treat different wastewaters under varying operating conditions (Wen *et al*., 1999), the stability of the MBR system over the initial 256-day operating period (0-250ppm) is observed as an essential component of the seeding reactor. Substrate assimilation may therefore be quantitatively described as the amount of substrate utilized ($r_s$) per unit biomass (X) for both the synthesis of cellular material (P) as well as cellular maintenance energy (E) (Wisniewski *et al*., 1999; Chaize and Huyard, 1991).

### 3.3.2 Community profiling – DGGE

#### *3.3.2.1 Community profile analysis using DGGE*

The DNA profiles of the various MBR- and AS-associated communities after DGGE analysis are indicative of microbial community changes in the MBR tank and the conventional activated sludge reactor (AS) as determined using DGGE analysis from day 0 to day 18 (profiles not shown). Numerical analysis of these DGGE profiles is displayed in dendrogram format in Figure 3.6. The dendrogram was obtained by the neighbour joining method and topology of the tree as well as the branch lengths are informative.

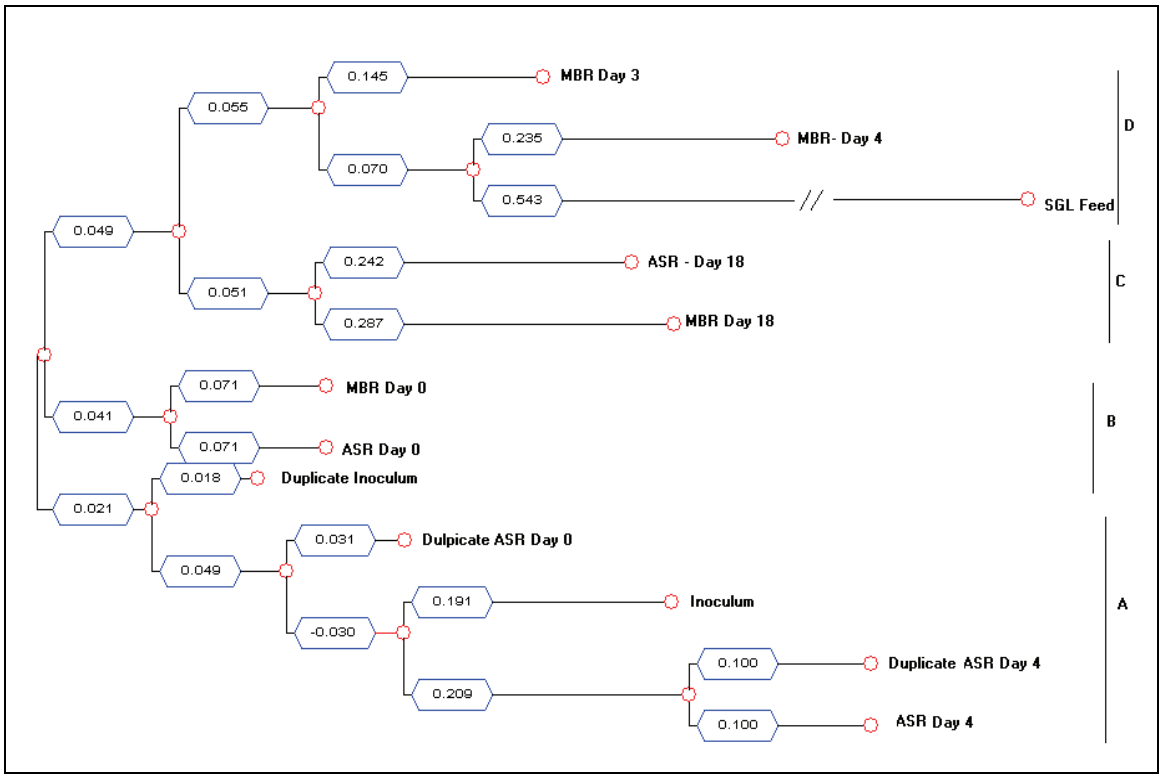



**Figure 3.6: A dendrogram showing relationships between the various samples analysed by DGGE (see explanation below)**

Four different clusters A to D were observed. These clusters are based on track similarities based on band matching data. The topology of the dendrogram showed four major clusters labelled A to D. Samples that were taken and analysed for the first four days of the operation of the reactors were in cluster A and B. The profiles of these samples were very similar. On the other hand, clusters C and D included the samples that showed differences in the DGGE profiles that could be correlated with bacterial population structure. Branch lengths of these neighbour joining dendrograms are also informative and represent similarity between tracks - the shorter the lengths the more similar the lanes. In the first three weeks where the SGL-MBR and SGL-AS processes were analysed and compared, 12 potentially different clades or species (referred to as operational taxonomic units (OTUs)) were observed. The numerical

analysis of these profiles showed graphical (dendrogram) relationships of bacterial communities that were reflected in the visual comparison of the DGGE profiles.

Further analysis of the population profile up to the point during operation when additional COD was added in the form of 100-1000ppm phenol and 2,4-dichlorophenol indicated considerable variation in the population based upon 16S and 18S rDNA analysis. As much as 23 individual operational taxonomic units (OTU's) (determined using DGGE) were observed to exist at various operating periods during the 305-day course of analysis.

Figure 3.7. (A and B) depicts examples of the 16S and 18S DGGE profiles. The 16S rDNA gel examples depicted here show complex banding patterns (melting types or operational taxonomic units).

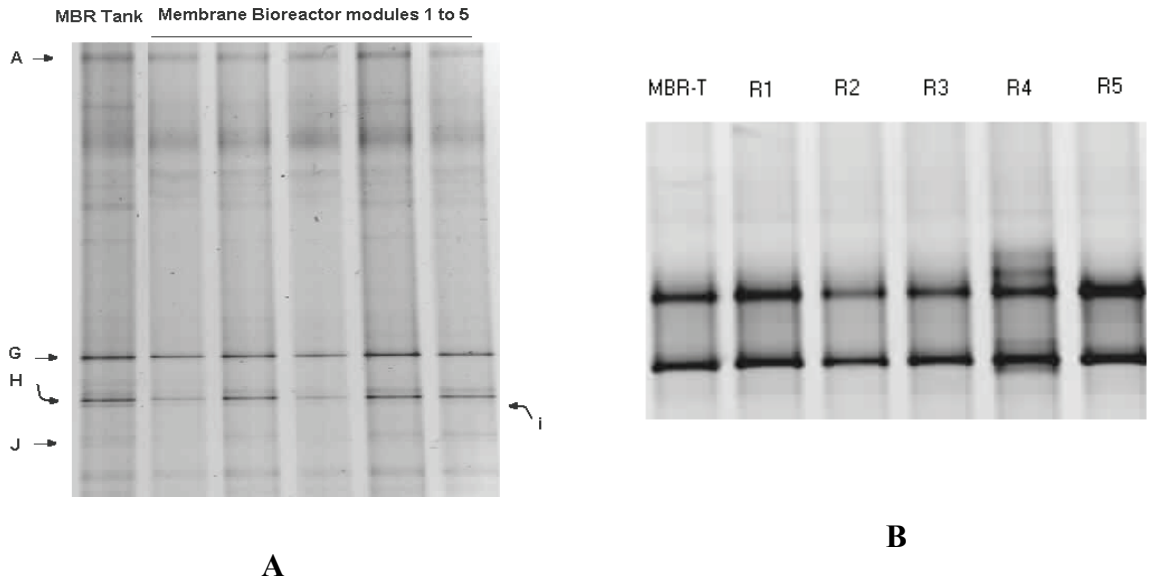



**Figure 3.7: Examples of DGGE profiles of 16S rDNA (A) and 18S rDNA (B) fragments in MBR tank and reactor samples (The letters (A, B, H and J) in Figure 3.6. (A) show the bands that were the marker positions used. A total of 23 operational taxonomic units (OTU's) were analyzed for presence/absence as well as band intensity data. Figure 3.6. (B) represents DGGE profiles of 18S rDNA. The profiles were less complex and diverse than the bacterial 16S profiles with a total of only 6 OTU's resolved and analyzed. The numerical data sets of both 16S and 18S rDNA profiles were further analyzed for OTU diversity using Shannon-Weaver biodiversity index (Figure 3.7.)**

From Figure 3.8. it is clear that there were considerable changes in bacterial OTU diversity in the feed-tank during the initiation of the experiment. The first samples for the reactor modules were analysed 41 days after the system was inoculated, thus the first results for the reactors were on day 41. The dynamics indicated by the 18S rDNA data were less variable but showed an increase during the initial stages of the experiment with subsequent continuous fluctuation

over the course of the experiment. It gradually decreased over the 305 days. On the last day of the experiment (day 305), the 18S diversity was higher in the tank than in the reactors.

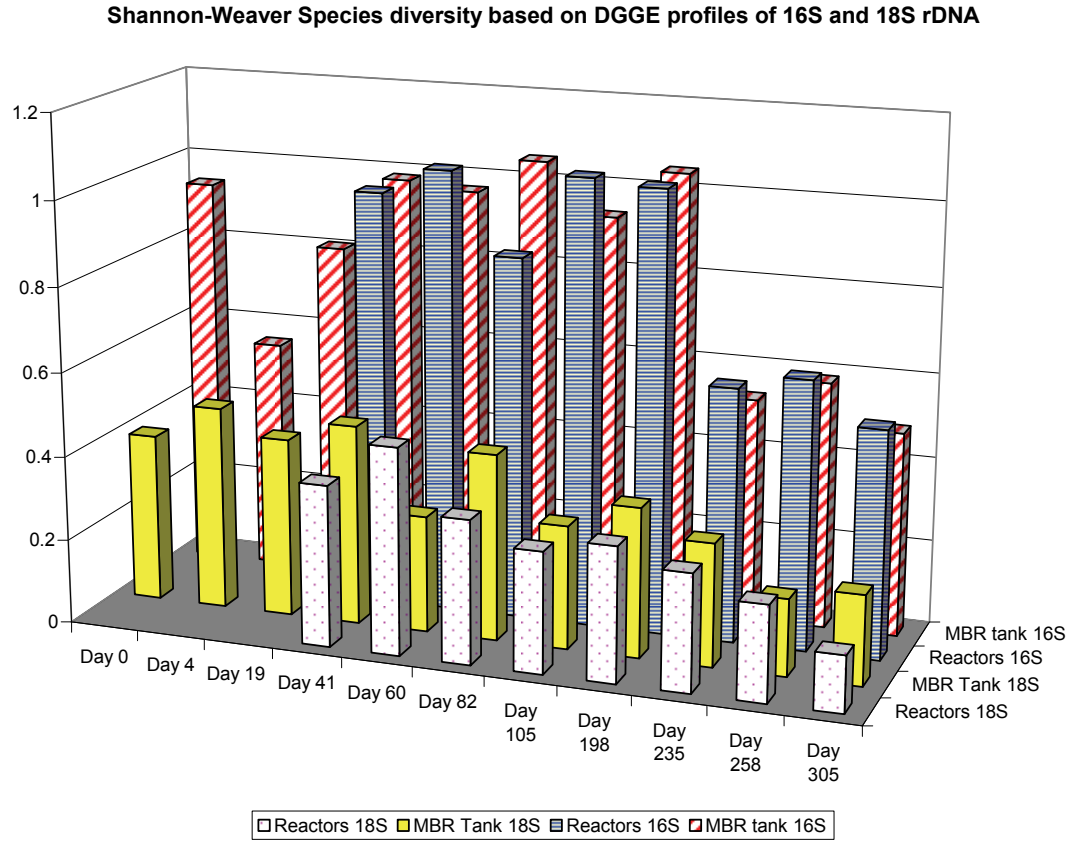



**Figure 3.8: Comparison of community changes between bacterial and fungal OTUs in MBR tank and reactor modules, using the Shannon-Weaver diversity index**

The Shannon-Weaver indices of MBR tanks, based on 16S rDNA data, were also compared to the reactors. It shows that bacterial diversity in the reactors was greater to that of the tanks on days 41, 60, 105, 235 and 305. On day 82 the opposite was observed i.e. the bacterial diversity in the tank was less than the diversity in the reactors. The bacterial diversity after day 198 was also lower in both the compartments of the MBR (reactors and tank) than the diversity between days 41 and day 198.

After day 198 the phenol was added to the system starting with 100 ppm at day 198. At day 235 the phenol concentration was increased to 250 ppm, and sequentially thereafter from 500 ppm to 1000ppm after day 258. The decline in species diversity (both bacterial and fungal) was attributed to the addition of increasing concentrations of phenol to the system.

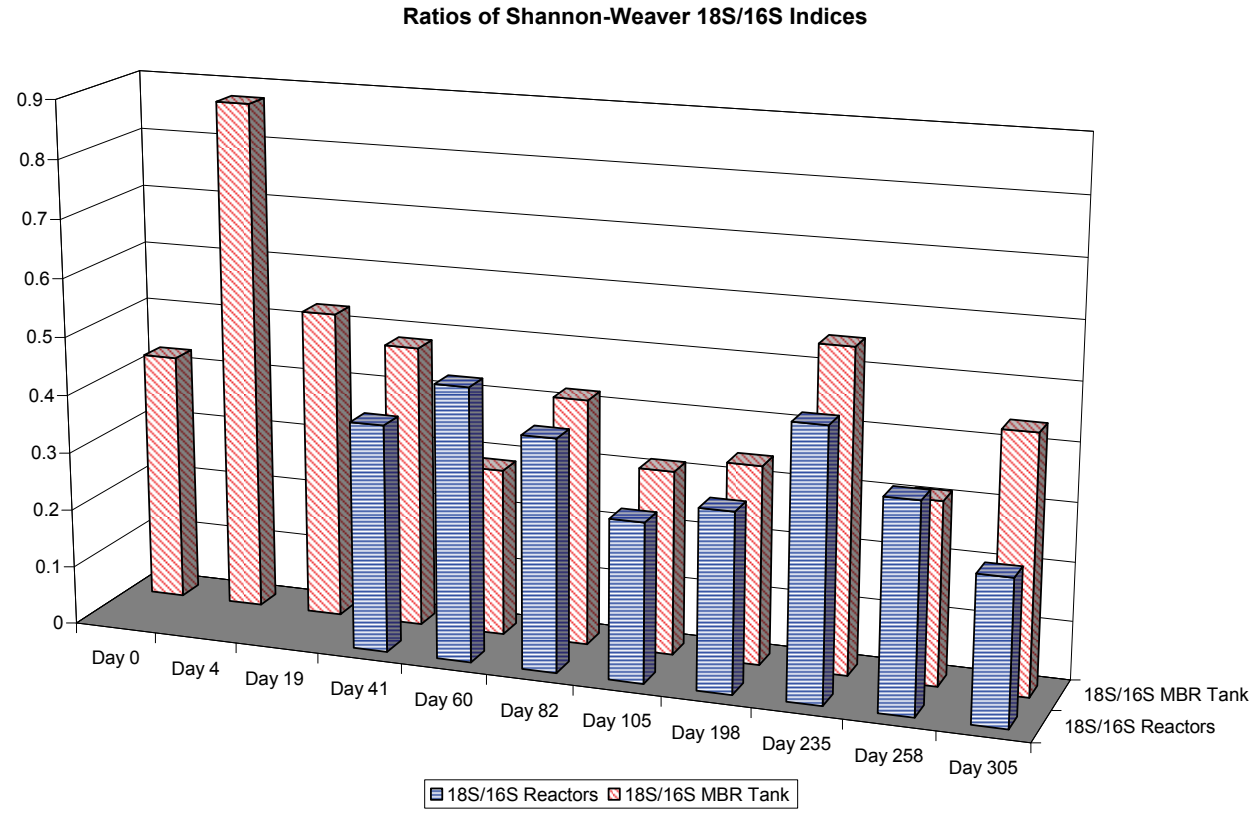



**Figure 3.9: Ratios of Shannon-Weaver 18S/16S indices indicating the ratios of fungal and bacterial species in the tank and membrane reactor compartments of the MBR system**

In the analysis of the fungal/bacterial diversity ratio (Shannon-Weaver 18S/16S, Figure 3.9.) show that this ratio was higher in the reactors than in the MBR tank only on day 60 and day 258. On the other days the ratio was higher in the MBR tank than in the reactors. This trend in fungal/bacterial ratio could be linked to operational efficiency fluctuations described earlier. Day 60 samples were associated with a decline in COD removal efficiency (Figure 3.5). The samples for the other days were taken when the COD removal efficiency was recovering.

The changes in Shannon diversity indices could be related to changes in operational conditions of the system as well as efficiency as measured by the COD removal. The results further showed that ratio of 18S/16S indices could be more clearly linked to these observed changes. The question thus arises whether these ratios would a useful tool for explaining certain events or effects of changes in operational parameters in an MBR system. This has not been tested. Well designed experiments would be necessary to support or disprove this suggestion.

The data presented here demonstrate that the DGGE technique is a powerful tool for resolving more than just the dynamics of the microbial community in biofilms. This observation is supported by previous studies (Luxmy *et al*., 2000; Forney *et al*., 2001). In this study DGGE analysis showed that microbial communities in the two compartments of the MBR system were differently affected by the duration of the experiments as well as differences in operational conditions and shock loading. The DGGE showed that the operational taxonomic units in both the bacterial and fungal components were dynamic over time but that the dynamics are also influenced by changes in operational conditions. Furthermore, species diversity indices may be useful for comparative studies such as those between various MBR and, activated sludge systems.

### 3.3.3 Community profiling – PLFAs

#### *3.3.3.1 PLFA analysis of microbial community structure*

PLFA data for the initial 82 days of MBR operation (refer to Figure 3.10) indicated that the major PLFA group observed consisted of the monounsaturated PLFAs (indicative of Gram-negative bacteria) with the second major grouping being the terminally branched saturated PLFAs (indicative of Gram-positive bacteria). Relative proportions of these two major groupings were also consistent within the MBR over this operating period.

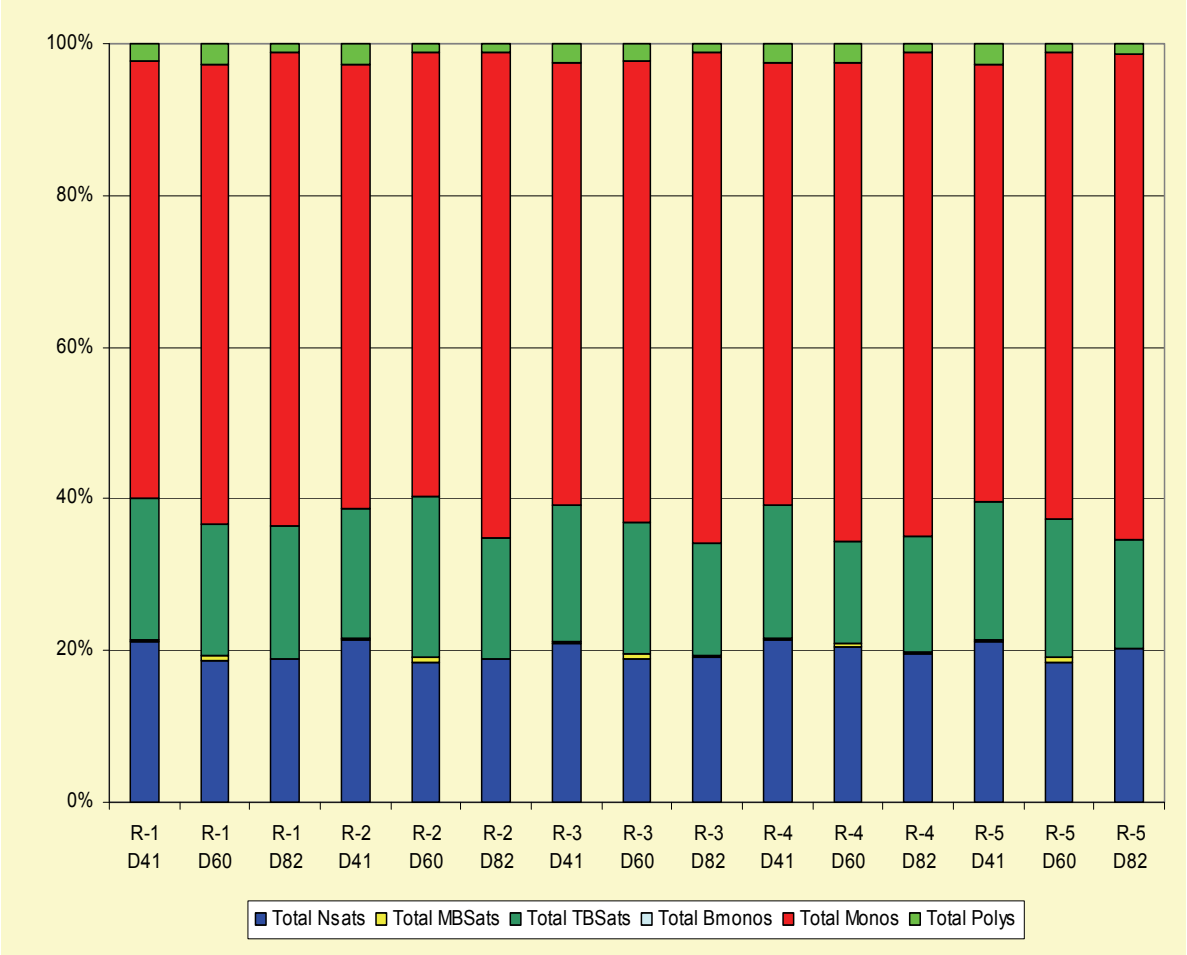



**Figure 3.10: Relative proportion of the major phospholipid fatty acid groups (mol %) within the MBR**

In contrast, relative proportions of these major bacterial groups were less consistent within the first 82 days of operation associated with the AS reactor (see Figure 3.11).

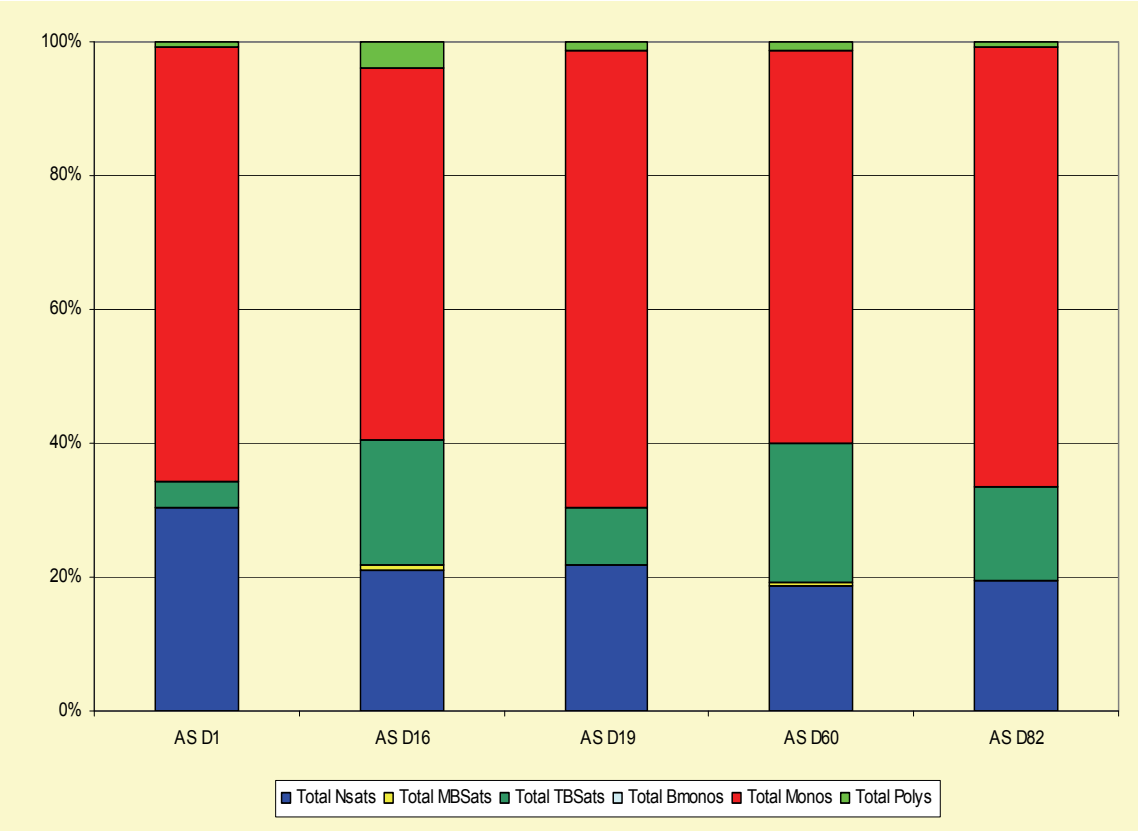



**Figure 3.11: Relative proportion of the major phospholipid fatty acid groups (mol %) within the activated sludge system**

Coupled with the DGGE data, significant insight concerning the impact of COD loading stress on the major microbial population groupings was evident. Furthermore, although the results of the PLFA analysis indicate relatively consistent proportions of both Gram negative and Gram positive groups (with Gram negative comprising the majority in both the AS and MBR systems), the data obtained using DGGE indicate that the relative diversity within these two major groupings is still consistently high. Sequence data based on individual species within the microbial consortia within the MBR and AS systems will enable the identification of specific organisms at a species level. The combination of DGGE, PLFA, and sequence data will enable the identification of the organisms at a species level and therefore conclusions to be reached in terms of whether the frequency of species succession and level of structural diversity is greatest in the Gram negative fraction of the population or the Gram positive fraction. These data may then be correlated with published identifications of similar organisms in other wastewater treatment processes.

## 3.4 DISCUSSION AND CONCLUSIONS

Although the efficiency of the seeding MBR has been shown to be more consistent compared with the conventional AS process, it would, however, be necessary to determine at what point would the decrease in productivity outweigh the overall efficiency of the MBR system in order to ascertain the stability, or lack thereof, of the associated microbial population using DGGE and PLFA analysis. The assessed process efficiency could then be correlated in terms of COD removal coupled with PLFA and 16S/18S rDNA analysis to enable correlations to be made as to any stress response within the microbial biofilm population as a result of changes in the MBR operational characteristics (for example, as increased COD loading by pollutant addition) being implemented within the system. The structural diversity succession within the system could then potentially be correlated back to the overall performance within these systems enabling the identification of which major microbial groupings are contributing to a lesser or greater extent to the overall process efficiency. Addition of these populations to the hydrolysis reactor may then be directly correlated with expected efficiency and process predictability.

# CHAPTER 4: DUAL-STAGE SEEDING / HYDROLYSIS MEMBRANE BIOREACTOR TREATMENT OF STRIPPED GAS LIQUOR EFFLUENT

## 4.1 INTRODUCTION

### 4.1.1 Reactor Configurations

The initial reactor design for the preliminary seeding MBR (SGL-treatment) was based on a feed-and-bleed approach where the operating conditions based on the Biological Oxygen Demand to Chemical Oxygen Demand ($BOD_5$/COD) ratios were facilitated by the bleed ratio of the seeding reactor coupled with the HRT of the solid/liquid separation membrane module. The experimental approach and operating configuration adopted for the seeding membrane bioreactor for the treatment of petrochemical-based SGL has been described in detail in Chapter 3 of this report. These preliminary results enabled the assessment of population shifts within microbial groups and population stability within these groups over extended operating periods to ascertain the applicability of using these populations as a continuous inoculum source for the hydrolysis reactor.

The biodegradability of a compound can be described by the $BOD_5$/COD ratio maintained within the contact phase of the microbial population and the substrate (pollutant) of interest. An easily biodegradable compound such as citric acid has a $BOD_5$/COD ratio of 0.95, while a less easily biodegradable compound such as triethanolamine will have a $BOD_5$/COD ratio of 0.54 (Mochidzuki and Takeuchi, 1999). Compounds highly resistant to biodegradation can even have $BOD_5$/COD ratios as low as 0.1 (Iami *et al*., 1995). By operating the seeding reactor at a low $BOD_5$/COD ratio i.e. a high solids retention time and low hydraulic retention time to ensure a high feed-to-microorganism ratio, the selection of microbial populations capable of degrading the highly persistent compounds will be achieved. The maintenance of the low $BOD_5$/COD ratio will be facilitated by firstly, adopting a high feed flow rate into the seeding reactor, thus maintaining high COD loading rates. Due to the usually slow growth rates of the bacterial population of interest (pollutant degraders) this will result in a desirable low $BOD_5$/COD ratio for pollutant degradation, however, the high loading rate will eventually favour an increase in the predatory population resulting in an undesirable high $BOD_5$/COD ratio as a result of the easily degradable COD fraction being preferentially consumed. Secondly, by operating the coupled MBR at high sludge recirculation rates, the usual associated cell removal in the effluent is avoided due to the cell retention characteristics of the membrane thus facilitating the continued maintenance of the pollutant-degrading population within the seeding reactor. Under these operating conditions, the $BOD_5$/COD ratio will

increase gradually until at steady-state operating conditions the main component of the resident microbial community will constitute the pollutant-degrading fraction.

Utilisation of this microbial community as a continuous source of inoculum for the hydrolysis reactor which will operate at conventional high $BOD_5$/COD ratios i.e. a high hydraulic retention time and high solids retention time to maintain a low feed-to-microorganism ratio, will facilitate continued operation at these ratios at high loading rates but with alleviated conditions of predator-prey interactions within the present microbial consortia. Balancing the sludge bleed ratio with that of the $BOD_5$/COD ratio within the seeding reactor will facilitate the maintenance of the correct operating $BOD_5$/COD ratio conditions for optimal pollutant degradation rates at significantly higher loading rates in comparison with conventional AS systems.

Furthermore, due to the solids retention time (SRT) being independent of hydraulic retention time (HRT), in terms of the biofilm component, the MBR will be characterized by higher biomass concentrations (MLSS) than conventional activated sludge systems. Consequently, higher maintenance energy requirements mean that continuous biosynthesis and cell growth necessitates high oxygen concentrations within the membrane bioreactor (Brindle and Stephenson, 1996; Chaize and Huyard, 1991).

### 4.1.2 Seeding and Hydrolysis Membrane Bioreactor Design

The design of the seeding-hydrolysis hybrid dual-stage MBR system was based on a seeding MBR configuration capable of maintaining a high solids retention time (SRT) and low hydraulic retention time (HRT) to ensure a high feed-to-microorganism ratio enabling the selection of microbial consortia capable of degrading any persistent compounds in the wastewater. Membrane-based biomass retention facilitated the utilisation of this microbial community as a continuous source of inoculum supporting the design of the hydrolysis reactor aimed at operating at conventional high $BOD_5$/COD ratios i.e. a high hydraulic retention time and high solids retention time to maintain a low feed-to-microorganism ratio. The piping and instrumentation diagram (see Figure 4.1 below), shows the combination seeding and hydrolysis MBR system with associated feed, seeding, hydrolysis, and permeate (or product) collection vessels. The operating configuration comprises two membrane bioreactor systems (described below) operating as biomass retention/biofilm development processes for two seeding reactor tank systems. Backflushing of the membrane-associated biofilms facilitated continuous inoculum introduction into a single hydrolysis reactor operated as described above.

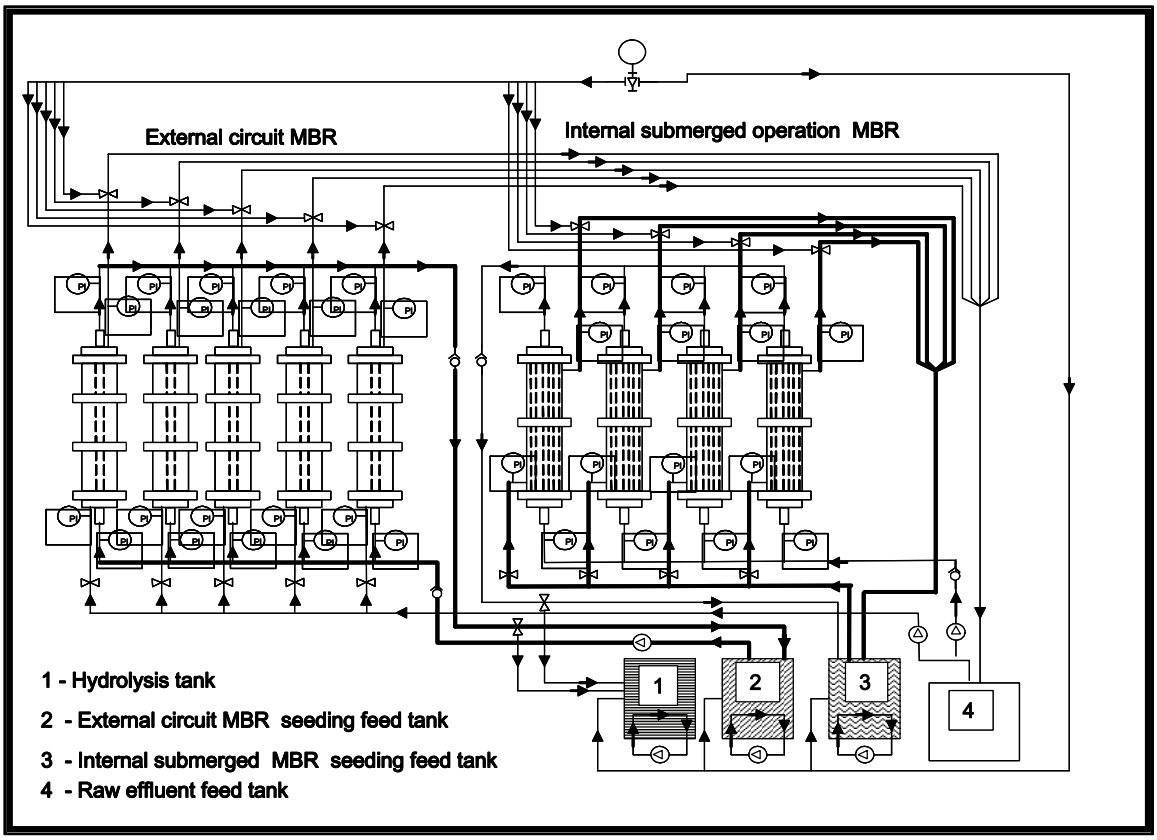



**Figure 4.1: Piping and Instrumentation Diagram of the seeding and hydrolysis reactor**

### 4.1.3 Submerged-type MBR system configuration

Each membrane reactor module comprised an effective membrane area of 0.0265 $m^2$. The membrane module constructs, comprising three separate modules, had a total membrane area of 0.0795 $m^2$ . Fluid reticulation within the MBR system is achieved using a magnetic drive centrifugal pump (March MFG., Inc.; Ill. USA) with a recirculation flow rate of 100 $L.h^{-1}$ from the MBR tank and a membrane module influent flow rate of 100 $L.h^{-1}$. At this flow rate the transmembrane pressure was greater than 10 kPa. Air sparging was implemented during backflush sampling and intermittent operation to maintain aerobic conditions and facilitate biofilm dislodging during hydrolysis tank seeding.

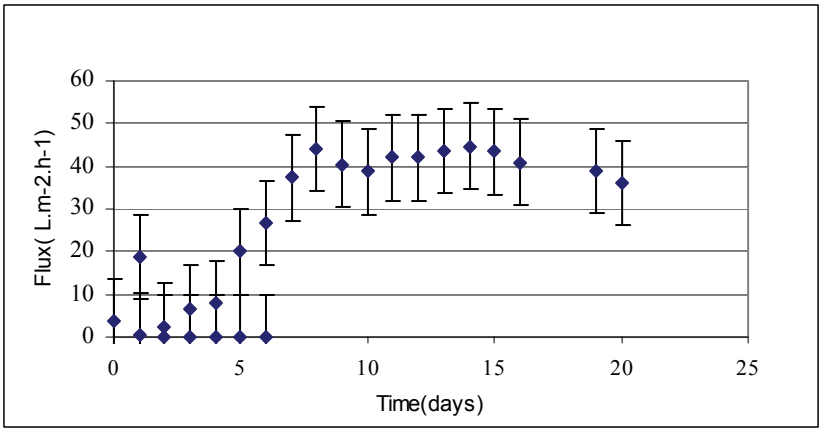



**Figure 4.2: Initial operating flux of submerged-type MBR system**

### 4.1.4 External circuit membrane modules

Each single-membrane reactor module comprised an effective membrane area of 0.0067 $m^2$. The membrane module constructs comprising three in-series modules have a total membrane area of 0.02 $m^2$ with each MBR assembled (comprising 5 constructs in series) having an effective total membrane area of 0.133 $m^2$ for the membrane bioreactor (refer to Fig. 4.1). Fluid reticulation within the MBR system was achieved using a magnetic drive centrifugal pump (March MFG., Inc.; Ill. USA) with a recirculation flow rate of 150 $L.h^{-1}$ from the MBR tank and a membrane module influent flow rate of 10 $L.h^{-1}$. At this flow rate, the cross flow velocity was 0.035 $m.s^{-1}$ and the transmembrane pressure was <2 kPa.

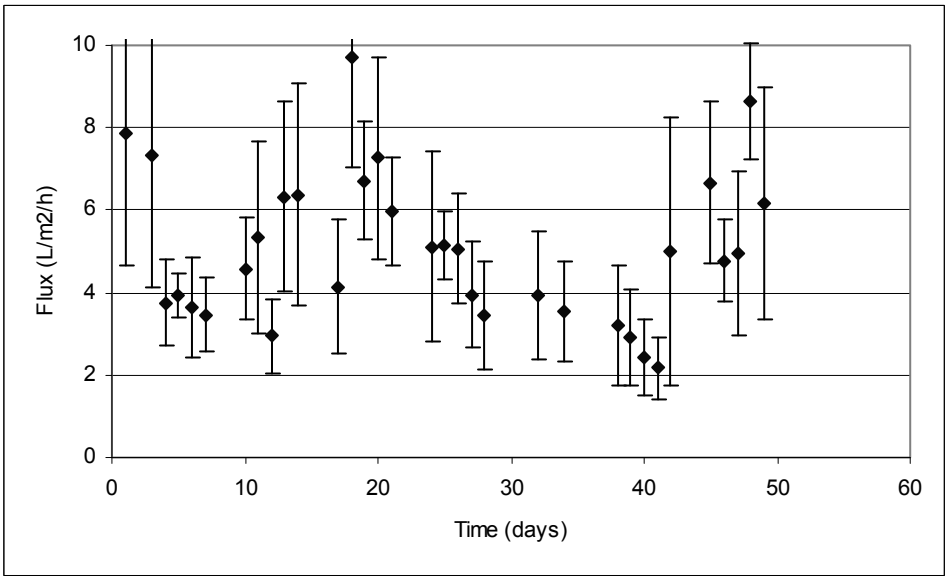



**Figure 4.3: Initial operating flux of external circuit MBR system**

## 4.2 HYDRAULIC PERFORMANCE

After inoculation, the MBR seeding tank system was operated initially as a batch system for 40-days. Thereafter, the membrane module start-up was initiated and these were operated using the mixed liquor in the seeding reactor tanks as the influent feed to initiate biofilm accumulation and microbial consortia acclimation / selection. During initial characterization of the membrane modules, the flux of the membrane bioreactor was monitored continuously to determine variation or stability in flux and the onset of biofilm growth-associated transmembrane pressure (TMP) increase.

## 4.3 OPERATING CONDITIONS

The membrane bioreactor tank, used in association with the tubular ceramic membrane modules, had a working volume of 60 L. The MBR tank temperature varied between 25-26ºC and did not exceed 30ºC for the 250-day operating period. The dissolved oxygen concentration was maintained between 4 and 5 mg.L-1 by using a diffused air sparging, with the pH of the system ranging 7.5-8.7over the same period. The membrane modules were operated at a transmembrane pressure greater than 10 kPa and the system was initially operated for a period of 40-days under batch operating conditions. Thereafter, the membrane module component of the membrane bioreactor system was started up. The feed approach entailed the removal of 30 $L.day^{-1}$ mixed liquor thereby maintaining a hydraulic retention time in the tank of 3-days. The removed mixed liquor was replaced daily with 30 L of fresh SGL make-up feed corrected to a C:N:P ratio of 100:10:1 using KH2PO4/K2HPO4 and urea.

## 4.4 RESULTS

### 4.4.1 Membrane Bioreactor System

Figures 4.4 and 4.5 indicate the initial (30 days) COD removal performance of the external circuit membrane bioreactor modules and the simulated submerged membrane bioreactor modules respectively after the initial batch operation start-up of the seeding tanks (40 days).

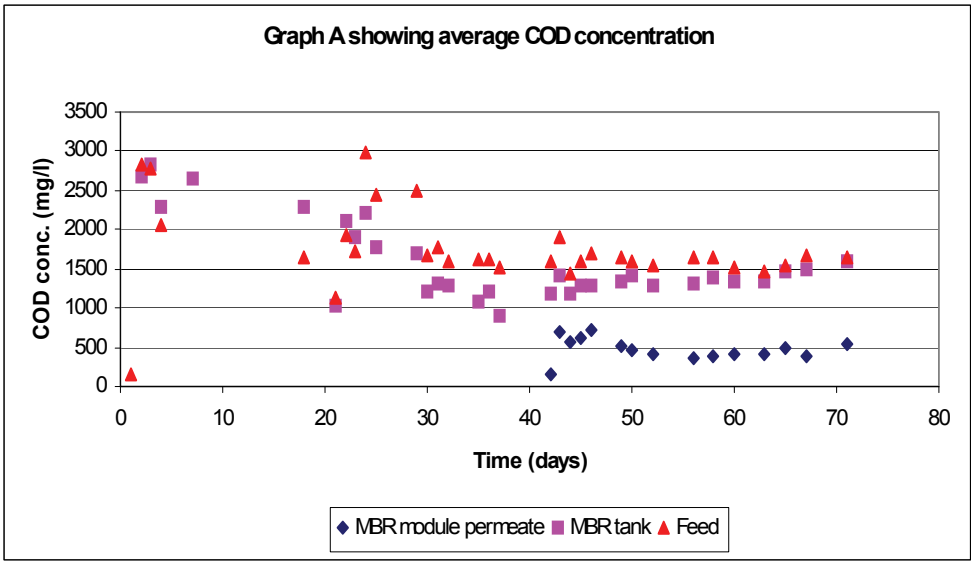



**Figure 4.4: COD removal associated with external circuit MBR system**

Significantly better performance was observed in the external circuit modules and this was attributed to longer residence times of the wastewater within the membrane-associated biofilms due to the lower flux performance of these membranes.

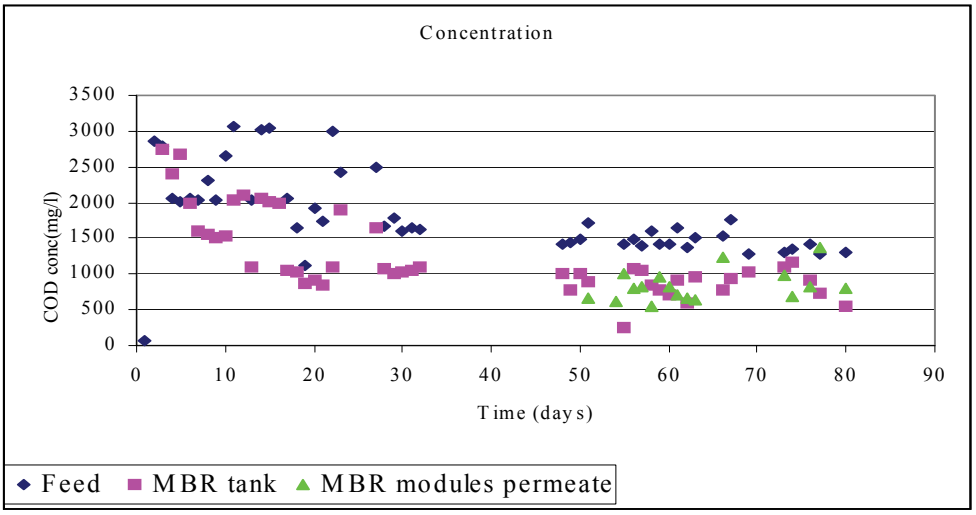



**Figure 4.5: COD removal associated with submerged MBR system**

### 4.4.2 Seeding/Hydrolysis Hybrid Bioreactor System

Initially operated as a batch process for approximately 40-days, the seeding reactor performance peaked at 40% COD removal efficiency with a gradual performance decline to approximately 10-15% COD removal efficiency over a three month operating period. This was attributed to the removal of biomass from the system (retained as biofilm within the membrane bioreactor system). This is typically the same scenario associated with many AS processes where, operated as continuous systems, COD removal efficiencies (for industrial effluents) are typically low and recovery periods are lengthy when no reinoculation or

reseeding procedures are implemented to compensate for washout phenomena associated with slow growing microbial community fractions (from an economic perspective these procedures are more costly in terms of the associated plant downtime than the actual reseeding process itself).

In contrast, the seeding reactor-associated MBR system consistently operated within a 60-80% efficiency range similar to the performance reported in the seeding reactor study summarised in Chapter 3. Figure 4.6 below is a summarised representation of the initial performance of the hybrid dual-stage seeding/MBR/hydrolysis process (first three months of operation).

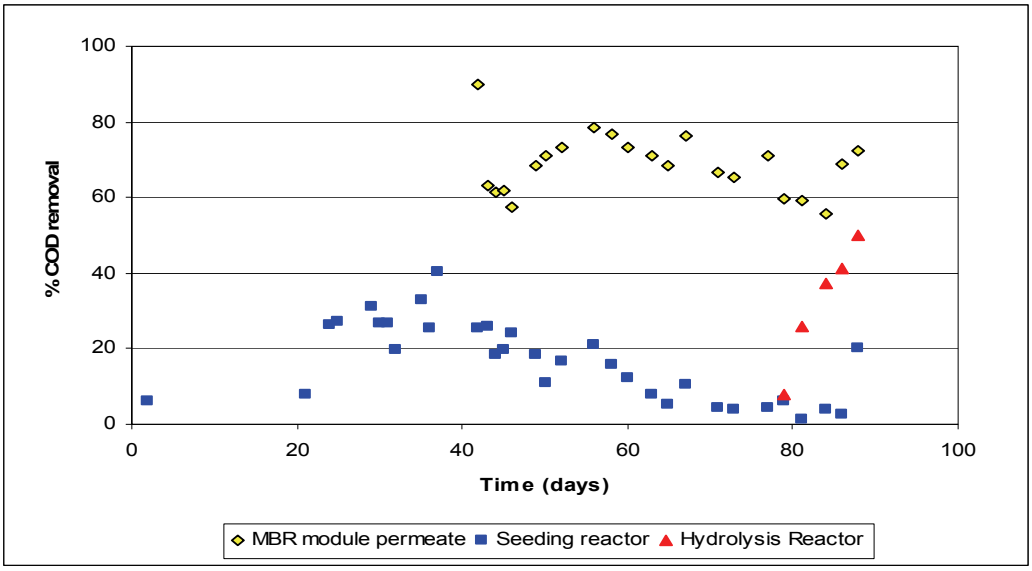



**Figure 4.6: COD removal efficiency of the combined dual-stage system and indicating the initial performance efficiency of the seeding tank system, MBR biofilm retention system and the final hydrolysis process**

After approximately 80 days of operation (40 days as seeding reactor batch operation and 40 days with continuous operation of the membrane bioreactor modules), the hydrolysis reactor start-up was initiated. No acclimation period was initiated (i.e. operation in batch mode) and the initial seeding of the hydrolysis bioreactor was started immediately using backflush transfer of the membrane module-associated biofilm. COD removal efficiency reached 60% within 10 days. In contrast, the seeding reactor reached a maximum COD removal efficiency of 40% after 40 days of operation in batch mode.

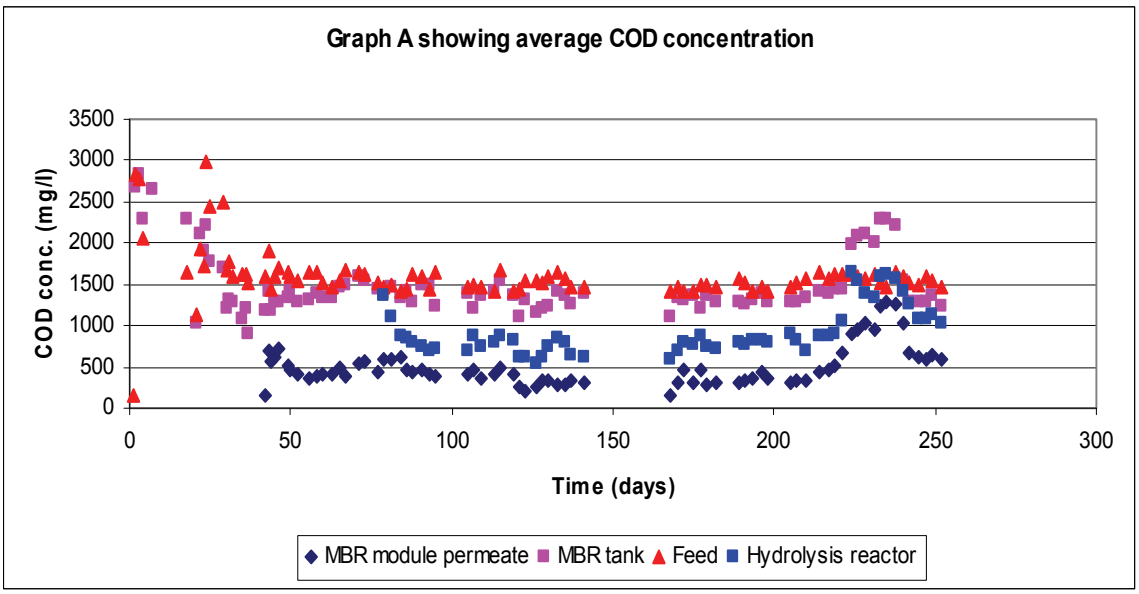



**Figure 4.7: Comparison of the average COD concentrations associated with the unit operations of the combined dual-stage system indicating the overall (250-days) performance efficiency of the seeding tank system, MBR biofilm retention system and the final hydrolysis process**

To ascertain the robustness and recoverability of the hybrid MBR system, an aromatic organic pollutant (phenol) and halogenated aromatic (2,4-dichlorophenol), in combination, were added to the system to simulate high shock loading. In this scenario the simulation facilitated observation of the effect of a high organic load perturbation (as rapidly increased total COD) and as a rapid pollutant load increase. Between days 217 and 238, both MBR systems and the hydrolysis reactor were supplemented with increasing concentrations of phenol and 2,4-dichlorophenol. The effect on total COD concentration increase at the different concentrations of supplemented phenols is indicated in Table 4.1 below.

**Table 4.1: The effect of aromatic compound addition on total COD concentration (range average indicated)**

| DAY | PHENOL + 2,4-DICHLOROPHENOL | COD (mg/l) | | |
|---|---|---|---|---|
| | | MBR | PERMEATE | HYDROLYSIS |
| 217 | 100 ppm | 1000-1500 | 0-500 | 500-1000 |
| 224 | 250 ppm | 2000-2500 | 500-1000 | 1000-1500 |
| 231 | 500 ppm | 2000-2500 | 1000-1500 | 1500-2000 |

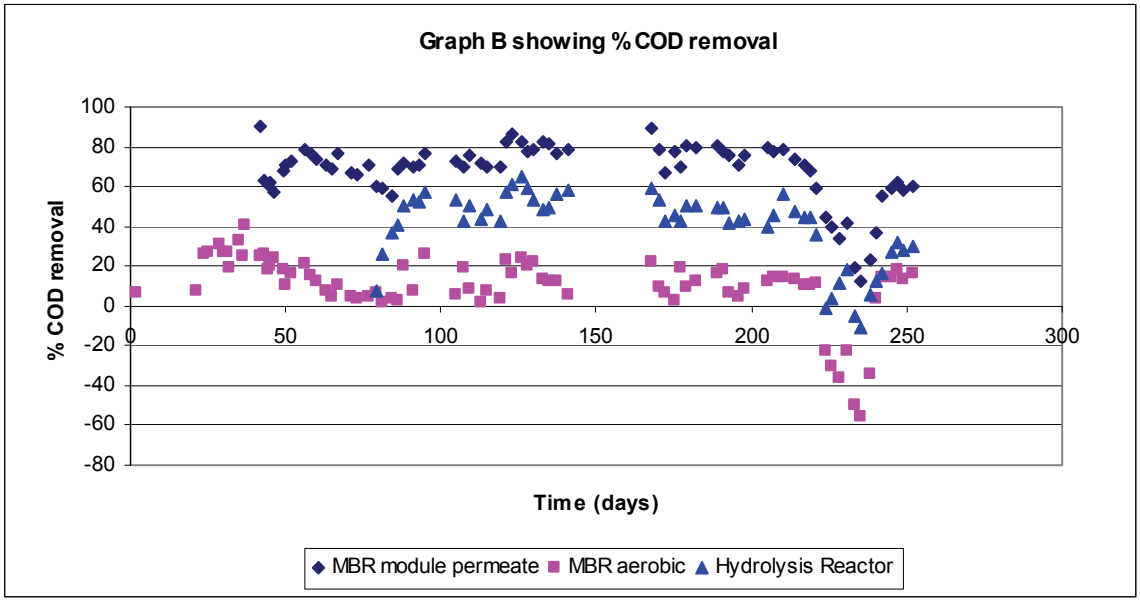



**Figure 4.8: Comparison of the average COD concentrations associated with the unit operations of the combined dual-stage system indicating the overall (250-days) performance efficiency of the seeding tank system, MBR biofilm retention system and the final hydrolysis process**

Following pollutant addition, significant decreases in the operating efficiency (in terms of COD removal) were observed for all systems (see Figure 4.8.). The percentage COD removal associated with the MBR tank initially ranged between 20 % and 40 % - this decreased to 0% with the shock load simulation. Recovery of this system once the pollutant was slowly removed was the slowest when compared with both the hydrolysis tank and the MBR modules. This was expected and confirmed observations of AS systems where washout after shock loading is a common problem. Membrane modules operating efficiency was also severely affected however, rapid recovery was observed once the pollutant was removed from the system. Rapid recovery was also observed within the hydrolysis system. The maximum recovery that this system could potentially achieve was not realised as operations were discontinued after approximately 250 days of operation. At that point in the experiment, the overall performance trend with the hydrolysis reactor was still increasing, as opposed to the

MBR tank system which levelled off and never recovered significantly or reached equivalent performance levels observed within the first 40-days of operation.

## 4.5 DISCUSSION AND CONCLUSIONS

A long-term (250-days) operating process with imposed shock loading treatment using a hybrid system for industrial wastewater treatment comprising a seeding and hydrolysis reactor coupled to intermediate membrane module operation was tested. Continuous inoculum development in the seeding MBR and intermittent continuous transfer of that acclimated retained biofilm to the hydrolysis reactor facilitated significant improvements in the enhancement of population adaptability and performance efficiency as well as drastically decreasing the usual adaptation time necessary (75% faster), associated with the majority of conventional wastewater treatment processes. A 75% improvement in microbial population adaptability translated to accelerated efficiency improvement in terms of COD removal capabilities. Furthermore, and more importantly, the coupling of the hybrid system enabled rapid recovery of a treatment process when perturbed with a simulated shock load. In contrast, systems such as conventional activated sludge processes are not capable of microbial population retention and are thus significantly more susceptible to washout risks associated with either variations in hydraulic load (such as summer/winter influent volume variations) or with shock loading (as rapid COD load elevation or the introduction pollutant compounds).

# CHAPTER 5: TREATMENT OF SYNTHETIC CYANIDE-CONTAINING MINE TAILINGS EFFLUENT

## 5.1 INTRODUCTION

Several wastewater sources were targeted to develop a generic strategy for the dual stage MBR treatment of industrial origin wastewaters. Below is a summation of the preliminary MBR-based results obtained during treatment of simulated mine tailings effluent containing cyanide.

The heap leach process used in the removal of gold from low-grade ore results in spent ore as well as leachate contaminated with residual cyanide (White *et al*., 2000). The toxicity of cyanide is well documented due to its ability to bind to the terminal oxidase of the electron transport chain thus inhibiting respiration (Kao *et al*., 2003). A number of techniques exist for the treatment of these leachates including ion exchange resin contact (Fernando *et al*., 2002) and chemical oxidation mechanisms, including alkaline chlorination, ozonation, and wet-air oxidation (Kao *et al*., 2003). The most common method of treating cyanide-containing effluents is alkaline oxidation (Kao *et al*., 2003; Bose *et al*., 2002); however, with all of these oxidation processes, the chemicals required are both expensive as well as hazardous. For these reasons, the biological treatment of CN is an attractive option in comparison with chemical treatment procedures (Barclay *et al*., 1998: White and Schnabel, 1998; White *et al*, 2000)

## 5.2 MATERIALS AND METHODS

### 5.2.1 Effluent

#### *5.2.1.1 Synthetic cyanide-containing mine tailings effluent*

The synthetic cyanide-containing mine tailings effluent consisted of: KCN (with CN at 100-500 mg.$L^{-1}$ for the AS process and 100-1000 mg.$L^{-1}$) $K_2HPO_4/KH_2PO_4$; as well as a mineral trace element solution containing $FeCl_2.H_2O$; $CaCl_2.6H_2O$; NaCl; $MnCl_2.4H_2O$; $CuCl_2.H_2O$; $ZnSO_4$; $H_3BO_3$; $NiCl_2.6H_2O$; $Na_{3\ m}oO_4$; $Na_2SeO_3.5H_2O$; $NaNO_3$.

#### *5.2.1.2 Bacterial strains and culture conditions*

The consortium used in this study was isolated from a gold mine tailings dump and was kindly donated by BHP-Billiton.

### 5.2.2 Analytical Methods

Measurement of the dissolved oxygen concentration (OUR) was performed using an YSI 55 DO meter (Monitoring & Control Laboratories, Lyndhurst, S.A.). Determination of pH was performed using an YSI 63 pH/salinity probe (Monitoring & Control Laboratories, Lyndhurst, S.A.). Analysis of the Total and Volatile Suspended Solids was performed according to Standard Methods (APHA, 1985). Chemical Oxygen Demand (COD) analysis was performed using Merck Spectroquant® kits. Residual cyanide concentration was determined using Merck Spectroquant® kits.

### 5.2.3 Denaturing Gradient Gel Electrophoresis (DGGE)

PCR and DGGE conditions were as described in section 3.2.5.

### 5.2.4 Fatty Acid Methyl Ester (FAME) analysis

Preparation, extraction, and fractionation conditions for PLFA analysis were as described in section 3.2.6.

### 5.2.5 Membrane Bioreactor System

The tubular ceramic oxide membranes were assembled into membrane module constructs comprising three in-series membranes having a total membrane area of 0.02 $m^2$ with the CN-treatment MBR comprising 4 constructs in-series having an effective total membrane area of 0.08 $m^2$. The membrane modules were operated at a cross flow velocity of 0.1 $m.s^{-1}$ with an associated transmembrane pressure of <2 kPa with synthetic CN-containing mine tailings effluent.

### 5.2.6 Activated Sludge Process

Activated sludge reactors were used as described in section 3.2.2, however, a number of alterations to the standard protocol were necessary due to the nature of the consortium and effluent. In addition to the use of conventional probes as described previously (see section 3.2.2.) pH was continuously monitored and maintained at 10.5 using a peristaltic dosage pump connected to a 0.1 M NaOH feed. Furthermore, due to the relatively slow growth rate associated with CN-degrading consortiums, effluent discharge from the clarifier was further retarded by the addition of polystyrene spheres (O.D. 2 mm). This facilitated the prevention of cell washout enabling the reactor to be operated at dilution rates that were higher than the maximum specific growth rate of the organisms present within the consortium.

## 5.3 RESULTS

### 5.3.1 Membrane Bioreactor Process

#### *5.3.1.1 Hydraulic performance*

The CN-MBR comprised four membrane constructs in-series. Almost immediately after start-up, it was observed that reactors 3 and 4 experienced irreversible fouling with the result that the permeation rate or flux associated with these two reactors effectively dropped to zero. The implication was therefore that the effective membrane area for the CN-MBR was half that of the original surface area (equivalent to 0.04 $m^2$). Continuous monitoring of MBR flux revealed steady state (5 $L.m^{-2}.h^{-1}$) was achieved after approximately 55 days (refer to Fig. 5.1).

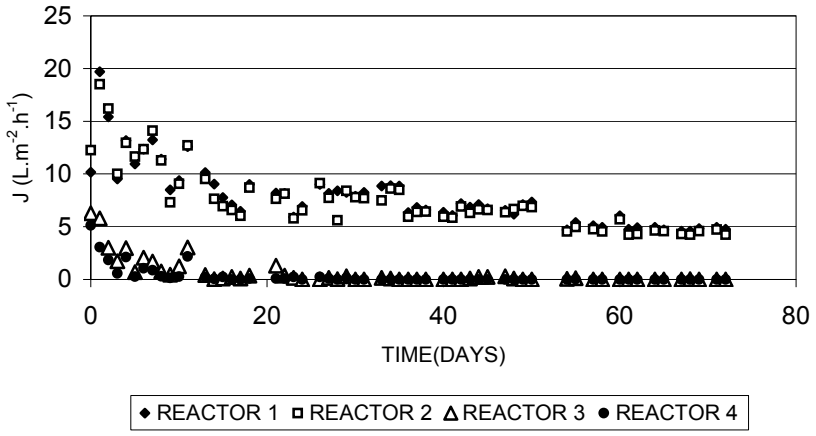



**Figure 5.1: Average flux for the CN-MBR over a period of approximately 75 days**

No attempt was made to clean the membranes associated with reactors 3 and 4 in order to facilitate flux recovery and thereafter the MBR was considered to have an effective membrane operating area of 0.04 $m^2$.

#### *5.3.1.2 Operational characteristics of the CN-MBR*

Maintenance of dissolved oxygen in the CN-MBR was achieved using diffuse air sparging on a continuous basis. An average dissolved oxygen (DO) concentration of 5 $mg.L^{-1}$ was maintained for the duration of the 90-day operating period (refer to Fig. 5.2).

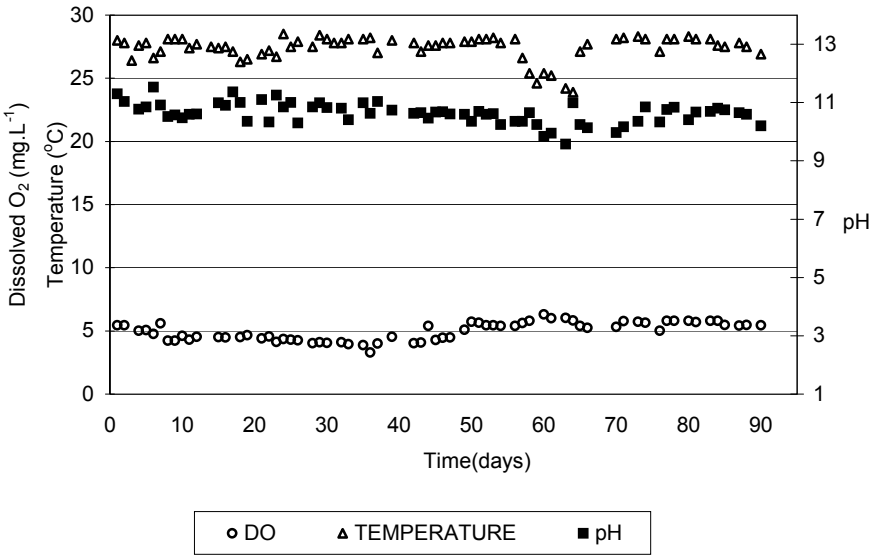



**Figure 5.2: Dissolved oxygen concentration, pH, and temperature associated with the CN-MBR during the initial 90-day operating period**

The temperature of the MBR process was maintained at ±28°C and pH was continuously monitored and maintained at 10.5 using a peristaltic dosage pump connected to a 0.1 M NaOH feed. This was necessary in order to maintain the cyanide feed added in the ionic ($CN^-$) form. Any drop in pH to below the pKa (pH 10.2) for $CN^-$ would have resulted in the hazardous release of the gaseous form of cyanide, HCN.

#### *5.3.1.3 Performance of the CN-MBR*

The CN-MBR was operated for a 90-day period at a hydraulic retention time of 4-days. Over the duration of the operating period the loading rate was increased from 0.06 kgCN.m$^{-3}$.day$^{-1}$ to 0.14 kgCN.m$^{-3}$.day$^{-1}$. During this 90-day operating period the CN removal capacity of the MBR was observed to increase as the CN-loading rate was increased.

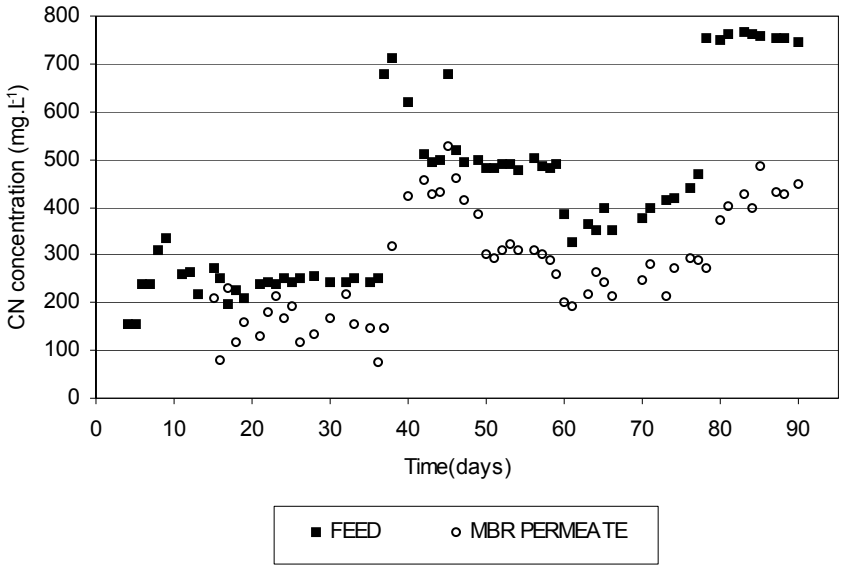



**Figure 5.3: CN removal efficiency of the CN-MBR process**

The MBR was operated at a loading rate of 0.14 kgCN.m$^{-3}$.day$^{-1}$, however, steady state operation was not attained. Rapid decreases in the CN removal capacity of the MBR was not observed as the loading rate was increased (refer to Fig. 5.3). In comparison, varying the loading rate in the CN-AS system was characterized by significant variation in removal capacity as the system acclimated to the increase in CN concentration (refer to Fig. 5.4). It was therefore evident that the MBR process in comparison with the conventional AS system exhibited a significantly higher degree of robustness and stability under conditions of shock loading and/or load increases.

#### *5.3.1.4 Community Profiling of the CN-degrading MBR*

Yields from the DNA isolation procedure were poor with the average $A_{260:280}$ ratio being below 1.0 which indicated that the DNA quality (Turner *et al*., 2001) was extremely poor and not of sufficient quality to facilitate PCR analysis (this was confirmed with several attempts being made to PCR amplify the DNA extracts). Community profiling was therefore not possible for the biofilm-associated microbial populations associated with the CN-degrading MBRs. As a result, community profiling was only possible for the activated sludge reactor associated populations.

### 5.3.2 Conventional Activated Sludge System

To facilitate evaluating the performance of the CN-MBR system in terms of process efficiency, a conventional AS system was operated under similar conditions as described in section 3.2.7. The CN concentration was varied from 100-500 $mg.L^{-1}$ at a hydraulic retention time (HRT) of 5-days. Thereafter, the HRT was decreased to 3-days, 24-hours, and finally, 8-hours over a period of 170 days.

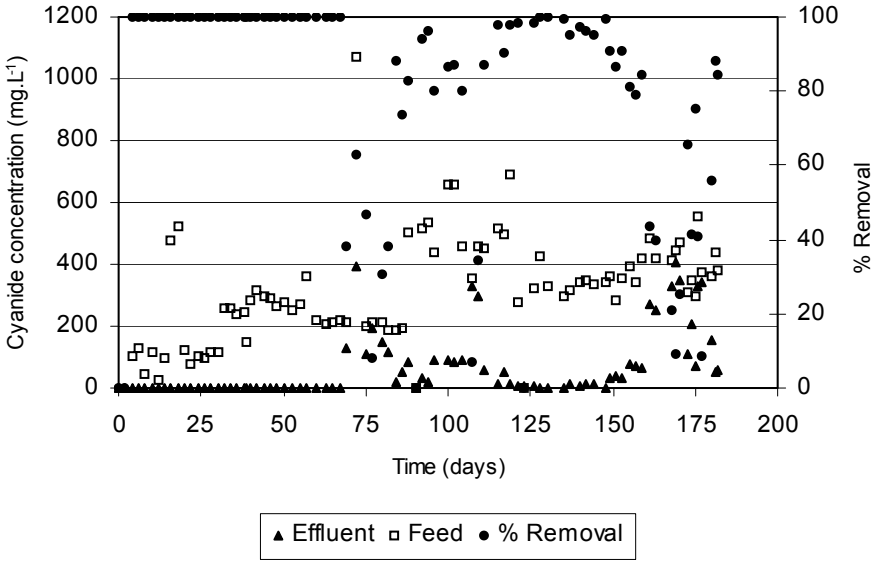



**Figure 5.4: CN removal efficiency of the CN-AS process**

This decrease in HRT corresponded to a maximum loading rate of 0.2 $kg.m^{-3}.h^{-1}$. Thereafter the performance of the CN-AS process in terms of CN removal decreased. Significant variations in the dynamic population structure were observed at each CN loading rate variation. These population shifts were determined using 16S rDNA based techniques.

## 5.4 Community Profiling – DGGE

### 5.4.1 Total community DNA extraction and purity

Yields from the DNA isolation procedure described above were approximately 4 µg. The average $A_{260:280}$ ratio was 1.2 which indicates generally that the quality DNA (Turner *et al.*, 2001) was not optimum but of sufficient quality for PCR analysis.

### 5.4.2 Community profile analysis using DGGE

The image in Figure 5.5 illustrates the 16S rDNA profiles of the various communities after DGGE. This image indicates microbial community changes in the ASR monitored for different hydraulic retention times (HRT) as well as comparisons between sessile aerobic, anaerobic and planktonic population fractions sampled at specific points in the CN-degradation process.

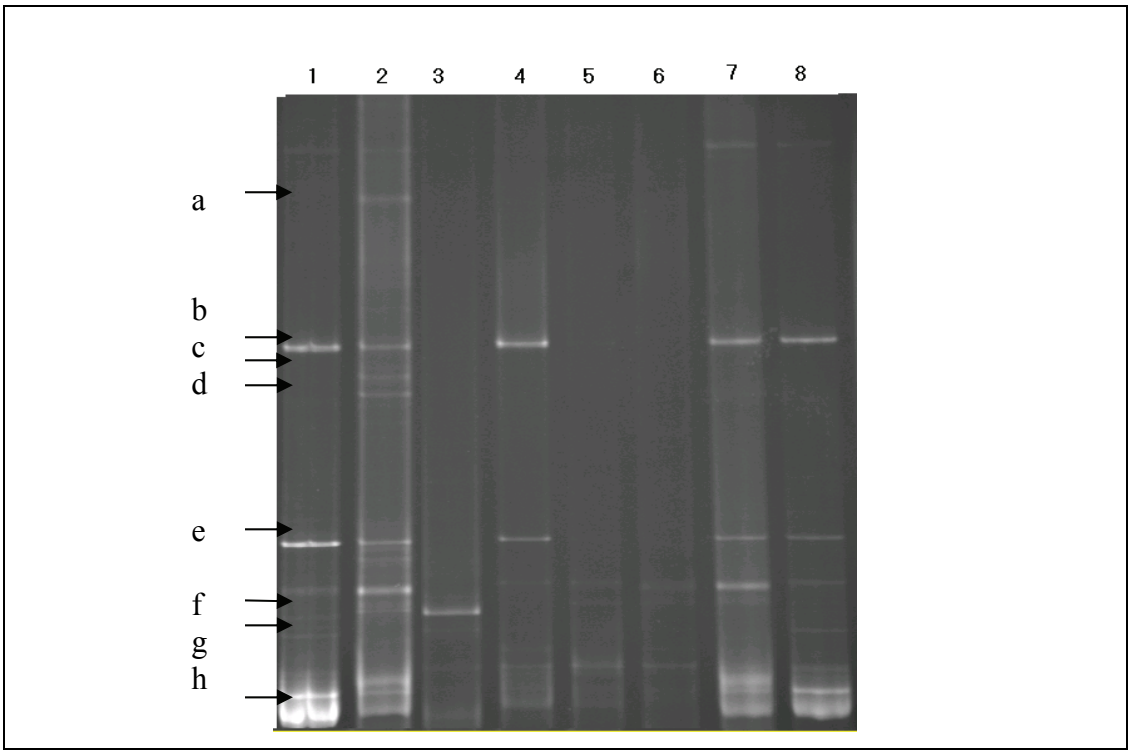



**Figure 5.5: DGGE analysis of PCR amplified 16S ribosomal DNA fragments showing bacterial community dynamics of a cyanide degrading bacterial consortium in an activated sludge reactor.** ***Lanes 1 & 2: planktonic bacterial community structure at 5 day HRT and 1 day HRT respectively. Lanes 7 & 8: Duplicates of lanes 2 & 1 respectively; Lane 3 to 6: bacterial community structure at 8 hour HRT. Lane 3: Planktonic bacteria in aerobic tank, (4) Planktonic bacteria in anaerobic tank. Lanes 5 & 6: Sessile bacterial community structure on polystyrene beads. The arrows indicate the areas of the gel where noticeable band (species) variation occurred.***

The profiles in lanes 1 to 6 show similarities and differences, (based on presence/absence) which are based on changes in bacterial community structure. Lane 1 represented the sample taken at 5 and 1 day HRT and lanes 8 and 7 contained duplicate samples of these. Lanes 3 to 6 represented samples taken when the HRT was reduced to 8 hours. Banding profile changes (presence/absence, intensity changes) in this gel can be ascribed to dynamics within the bacterial community caused by changes in operational parameters of the activated sludge reactor. The most dramatic change was indicated by arrows in Figure 5.5 (positions a, to h). Numerical analysis of these DGGE profiles is displayed as dendrogram in Figure 5.6. The dendrogram was obtained by the neighbour joining method and topology of the tree as well as the branch lengths are informative.

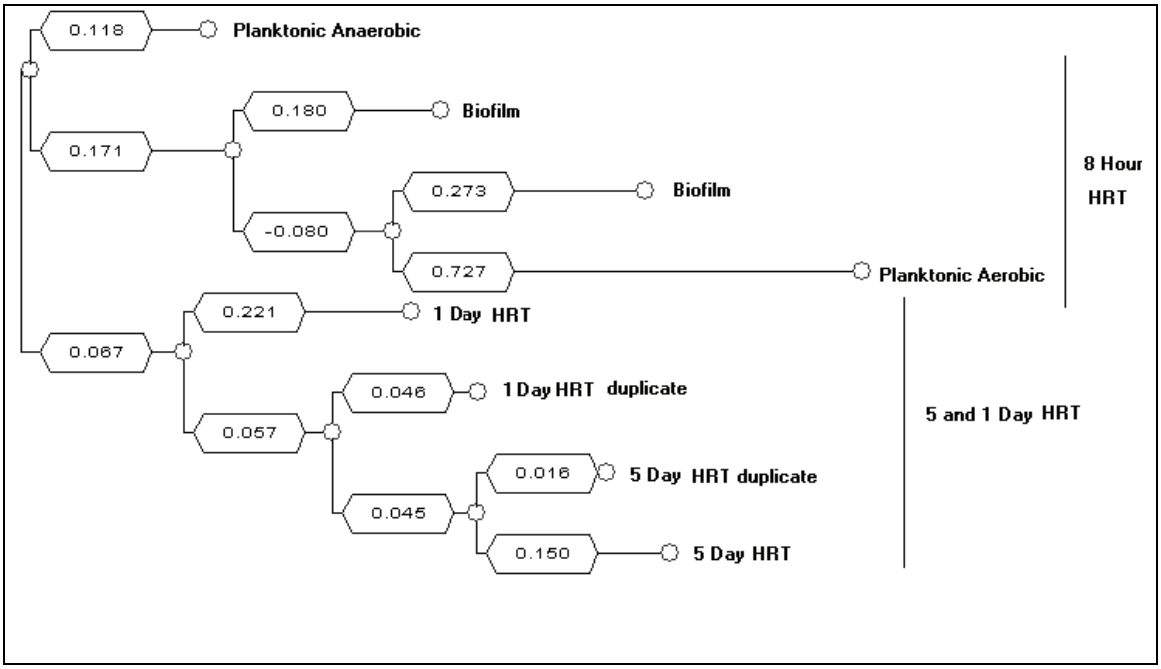



**Figure 5.6: A dendrogram showing relationships between the various CN-AS samples analysed using DGGE.** ***Two clusters were observed. These clusters are based on track similarities and band matching data. The dendrogram was calculated using GeneTools software***

The topology of the dendrogram showed four major clusters with definitive clustering at 5 and 1 day HRT and secondly at 8 hr HRT. Profiles of the samples and their duplicates clustered in 5 and 1 Day HRT were similar. However, the profile of 1 Day HRT sample was different from that of the 5 Day HRT sample. This is reflected in the dendrogram topology and branch lengths. On the other hand, the profiles of the samples contained in 8 hour HRT cluster were extremely different showing a change and decrease in species diversity. This is also reflected in the topology of the dendrogram.

Figure 5.7 illustrates the Shannon-Weaver species diversity indices (*H*) taking various different aspects into account. An overall *H*, and *H* values for various operational conditions were calculated using the image in Figure 5.5. The *H* indices in Figure 5.7 are indicative of the dynamics of the bacterial community. Species diversity was the greatest at 1 day HRT and the lowest at 8 hr HRT.

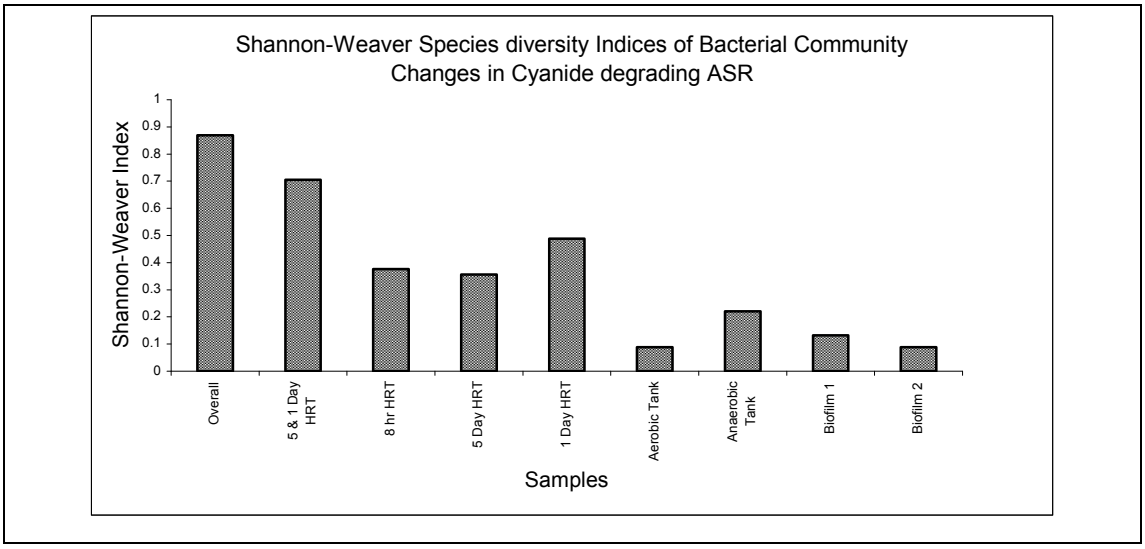



**Figure 5.7: Shannon-Weaver species diversity indices of the bacterial community changes in cyanide degrading ASRs as indicated by DGGE analysis.** ***The Overall value is the Shannon-Weaver index of all the samples analysed, including the duplicates. Shannon-Weaver indices indicated at 5 & 1 Day HRT and 8 hr HRT takes into account all the samples analysed at the indicated HRTs. Shannon-Weaver diversity index of the individual samples analysed are also shown (5 Day HRT to Biofilm 2)***

Shannon indices may be informative when characterizing the operational parameters and may be correlated with system performance data. Manual/visual inspection of the image and numerical analysis by software provided similar results. DGGE profiles in the image (Figure 5.5) that were similar formed clusters (Figure 5.7). Presently only the present/absence data were considered to illustrate how the methods are used. Band intensity data was not included in this study but would further strengthen the analysis. These results can be confirmed using supplementary manual and statistical analysis, however, this was beyond the scope of the present study.

## 5.5 DISCUSSION AND CONCLUSIONS

Unsuccessful PCR-amplification of the microbial population within the MBR-associated biofilms limited the scope of this study significantly. The analysis of comparative data was necessary to effectively compare the MBR and AS systems conclusively with respect to population changes. This was especially relevant in light of the robust nature of the MBR system in response to changes in hydraulic retention times and loading rates. At every instance where increased loading rates challenged the system, no significant variations in CN removal capacity were observed. This was indirectly indicative of the population stability within the biofilm consortia. Reasons for the lack of success with DNA amplification were inconclusively attributed to high localised CN concentrations within the biofilm detrimentally

affecting the extraction process, however, successful amplification of the AS-associated rDNA was achieved and in these samples CN was also present.

With no basis to do a thorough population and community structure analysis, the next stage of developing the system into the dual-stage hydrolysis process was discontinued. However, the preliminary results obtained during the study effectively confirmed that the MBR system was significantly more stable in terms of operational efficiency and recovery when challenged with various loading rate scenarios than was the conventional activated sludge process. Based on the results reported in Chapters 3 and 4, where SGL effluent was analysed, it can be assumed that similar effects were also occurring in the CN-degradation process and that enhanced population stability would have been observed were the thorough population structure analysis possible.

# CHAPTER 6: TREATMENT OF DISTILLARY EFFLUENT

## 6.1 INTRODUCTION

Another wastewater source targeted in order to develop a generic strategy for the dual stage MBR treatment of industrial origin wastewaters was distillery and winery effluent. Below is a summation of the preliminary MBR-based results obtained during the aerobic/anaerobic treatment of a distillery effluent.

Anaerobic digestion is widely used in many applications, such as treatment of sludge and wastewater, in different configurations and modes of operation. However, the treatment efficiencies of these digesters are sensitive to parameters like wastewater composition, especially the concentration of various ions and the presence of toxic compounds such as polyphenols and phenols. An improvement in digestion efficiency can be brought about by either modifying the digester design or incorporating appropriate advanced operating techniques. A distillery wastewater can be defined as the aqueous by-product from the distillation of ethanol following fermentation of carbohydrates. Phenolic toxicants are a broad group of compounds that share at least one phenolic group, while polyphenols are compounds with more than one phenolic group in their molecules. Distillery wastewater consists primarily of organic acids with high soluble chemical oxygen demand ($COD_S$), typically from 20 to 150 g/L [5, 3]. Several generated distillery wastewaters in South Africa typically contain COD concentrations as high as 20 000– 30 000 mg/L at pH values as low as between 3 and 4.

Distillery wastewater is therefore toxic with a large fraction of components being recalcitrant in nature and is therefore not suitable for discharge to the environment. Consequently the pretreatment or treatment of these wastewaters is very costly. In this study anaerobic digestion coupled to a submerged MBR was investigated for removal of polyphenols and phenols from untreated and fungal pre-treated distillery wastewater.

## 6.2 SUBMERGED MEMBRANE BIOREACTOR CONFIGURATION

The dual stage submerged membrane bioreactor was set up in a laboratory at room temperature as in Figure 6.1. Reactor A was operated as a feed to Reactor B. Reactor B was a 10 L digester with a submerged membrane module. During filtration, permeate was collected in Reactor C and fed to Reactor D every 48 hours. The feed in Reactor A was distilled water for the first 2 days of operation then consisted of 30 % (v/v) of raw distillery effluent adjusted from pH 4 to a methanogenic pH range between 6.5 and 8, with 1000 mg/L of $CaCO_3$, 1000 mg/L of $K_2HPO_4$ and 50 mg/L of $Fe(NO_3)_3$ for the next 10 days. After 12 days of operation

the pH of the feed was adjusted with 8000 mg/L of $CaCO_3$, 4000 mg/L of $K_2HPO_4$ and 50 mg/L of $Fe(NO_3)_3$). The 10 L digester (Reactor B) was operated at a high feed-to-biomass ratio at 12 hours of hydraulic retention time (HRT). Reactor B was seeded with 10 % (v/v) of methanogenic sludge, fed with 30 % (v/v) distillery effluent (containing 1000 mg/lL of $CaCO_3$, 1000 mg/L $K_2HPO_4$ and 50 mg/L of $Fe(NO_3)_3$), every 12 hours and operated at 30 d mean cell residence time (MCRT).The purpose of this bioreactor was to place selective pressure on the microbes so that only those microorganisms that degrade phenols and polyphenols survived. The ultrafiltration membrane module was submerged in Reactor B was made of ceramic membranes (Figure 1.1). Ceramic membranes of 0.2 micron pore size, used to construct this module were chosen for their excellent chemical compatibility, durability, surface charge maintenance and ability to be operated dry (for air backflush). The microbes selected for in Reactor B were used to inoculate reactor D. Permeate from filtration collected in reactor C was used to feed the second reactor, a hydrolysis tank. The second bioreactor, hydrolysis reactor of 10 L was operated like a typical membrane bioreactor i.e. a low feed-to-biomass ratio, operated at 48 hr hydraulic retention time (HRT) and 30 day mean cell residence time (MCRT). During feeding, the feed was pumped from Reactor A to Reactor B using pump E and at the same time contents of Reactor B were filtered by the submerged membrane into Reactor C. Pump F was used to feed Reactor D every 48 hours with outlet I opened such that the processed effluent can overflow to the drain. At 22 days of operation Pump H was opened to inoculate Reactor D with sludge from Reactor B. Samples were collected every 48 hours from reactors A, B, C and D. Parameters like pH, total polyphenols and volatile fatty acid concentration were measured. Analytic test kits from Merck were used to determine concentrations of soluble COD, total nitrogen, nitrates, ammonia, total phosphorus and orthophosphate.

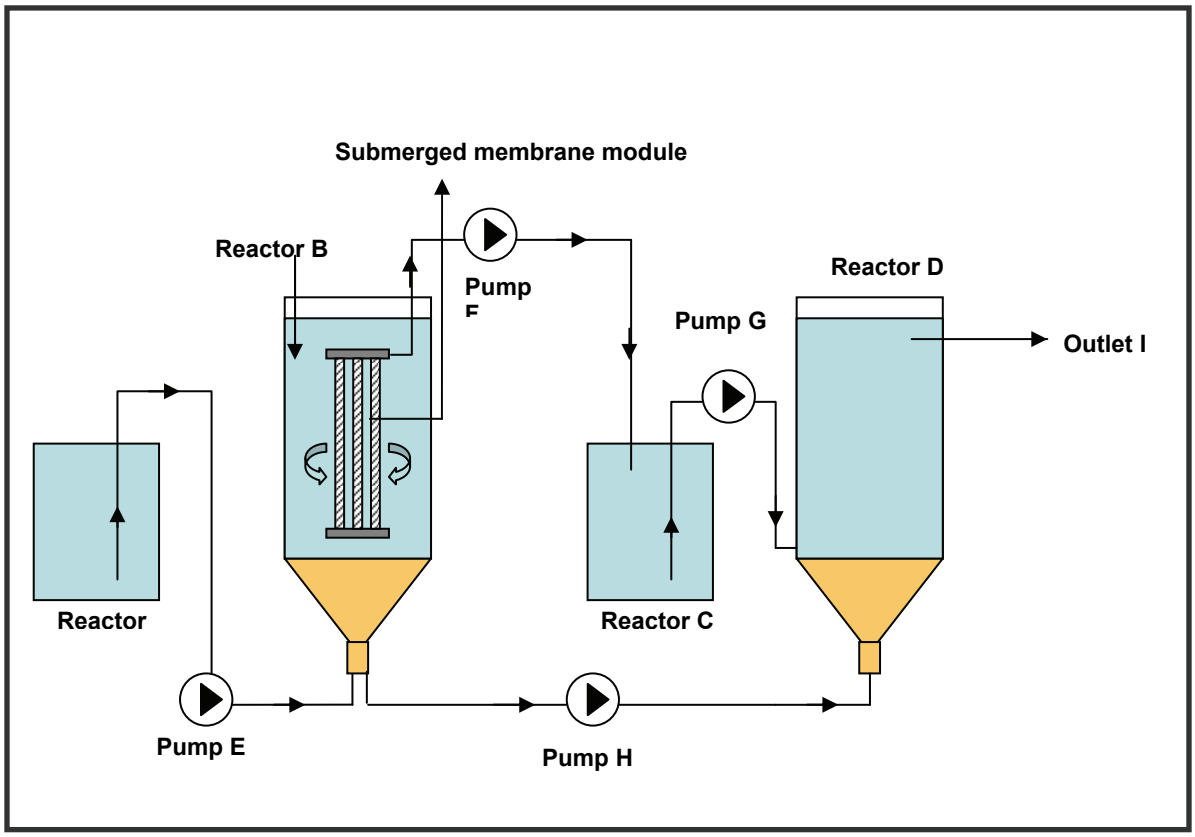



**Figure 6.1: A diagram showing anaerobic and aerobic dual stage submerged membrane bioreactors operated as a semi-batch process**

## 6.3 METHODS

Biodegradability and toxicity of the raw distillery effluent was performed on the following effluent concentrations 0%, 5%, 10%, 20% and 30 % (v/v) using serum bottle assays (Owen, *et al*., 1979). Serum bottles had 100 ml working volume where a control had 0% (v/v) raw distillery effluent; 10% (v/v) of methanogenic sludge with 1000 mg/L of $CaCO_3$, 1000 mg/L of $K_2HPO_4$ and 50 mg/L; and 10% (v/v) of nutrient broth. The test samples had raw distillery effluent at 5%, 10%, 20% and 30 % (v/v) concentrations and the rest was the same as in the control. All samples were sealed using Wheaton al cap natural rubber septum purchased from Sigma-Aldrich Ltd, with a syringe for gas collection inserted onto the cap. Samples were mixed and incubated at 37°C and stirred at 100 rpm using a shaking Incubator (Labcon). The experiment was allowed to run until gas production was constant, in this case 21 days was optimum for gas production. Gas production was monitored every 12 hours and gas volumes were recorded. Soluble COD was monitored before and after incubation using Merck analytical kit (APHA, *et al*., 1998). The performance of a dual stage submerged membrane bioreactor was monitored over a period of 30 days by following various methods stated below. The pH for samples from Reactor A, B, C and D was measured using a Cyberscan

2500 pH meter. Total polyphenol concentration was measured from samples taken from all reactors using the Folin-Ciocalteu method (Waterman and Mole, 1994). For these samples, a microscale adapted version of Folin-Ciocalteu method was performed in triplicate (Arnous, *et al*., 2001). In 1.5 ml Eppendorf tubes, 790 µl of distilled water, 10 µl of sample from each reactor and 50 µl of Folin-Ciocalteu reagent were added and vortexed. Samples were incubated for 1 minute at room temperature, 150 µl of 20 % $Na_2CO_3$ was added and vortexed. Samples were incubated at room temperature in the dark for 120 min. After incubation 300 µl was aliquoted from each sample to a microtiter plate and absorbance was read at 750 nm in KC Junior PowerwaveX (Biotek Instruments). Total polyphenol concentration was calculated from a calibration curve, using gallic acid as a standard and results were expressed as mg/L gallic acid equivalents (GAE). A calibration curve using phenol as a standard was also prepared and total polyphenols were also expressed as mg/L phenol equivalents.

Volatile fatty acid concentration was also monitored in all reactors following a titration method (Kapp, 1984).This titration method was founded on a principle suggested by McGhee (1968). Samples were filtered through 0.45 µm nylon membrane filters. Each filtrate of 20 ml was placed in a titration vessel. Initial pH was measured using a Cyberscan 2500 pH meter. The sample was titrated with 0.05 M of $H_2SO_4$ until pH 5 was reached and the volume added was recorded. The same sample was titrated with 0.05 M of $H_2SO_4$ from pH 5 until pH 4 and volume used for titration was recorded. Volatile fatty acid concentration was calculated and expressed as the amount of acetic acid present in each sample.

$$\text{VFA (mg/L acetic acid)} = \frac{60 \times \text{volume (ml) of 0.05 M, } H_2SO_4 \text{ used from pH 5 - 4}}{0.06588}$$

Analytical test kits from Merck were used to determine concentrations of soluble COD, total nitrogen, nitrates, ammonia, total phosphorus and orthophosphate (APHA, 1998).

## 6.4 RESULTS

Results of biodegradability and toxicity assays on raw distillery effluent at differing concentrations are shown in Table 6.1. The control which had 0% of test compound produced on average 15 ml of gas and had a soluble COD reduction from 3780 mg/L to 1560 mg/L with COD removal efficiency of 66.7 %. When 5% of a raw effluent was added as a biodegradability test compound 13 ml of gas was produced and soluble COD removal efficiency was also decreased to 55.9 %. There was a dramatic decrease in the volume of gas produced when the concentration of raw distillery effluent was increased from 10% to 30% (v/v) and also a decrease in COD removal efficiency. These results suggested that biodegradability of raw distillery effluent decreases once its concentration is increased.

Results also suggest that raw distillery effluent is toxic at higher concentrations thus having inhibitory effects on the methanogenic activity.

**Table 6.1: Results of the volume of gas produced in serum bottle assays and soluble COD concentrations when biodegradability and toxicity of a raw distillery effluent was investigated.**

| Sample | Average volume (ml) of gas produced | Average soluble COD concentration (mg/L) before assay | Average soluble COD concentration (mg/L) after assay | COD removal efficiency (%) |
|---|---|---|---|---|
| **Control** | 15 | 3780 | 1260 | 66.7 |
| **5% distillery effluent** | 13 | 3705 | 1635 | 58.73 |
| **10% distillery effluent** | 5 | 4305 | 1965 | 54.44 |
| **15% distillery effluent** | 1.5 | 4020 | 1890 | 52.99 |
| **20% distillery effluent** | 2 | 6270 | 3960 | 36.84 |
| **30% distillery effluent** | 1 | 4620 | 2385 | 48.4 |

The pH values of Reactor A, B, C and D (Figure 6.1.) over a period of 30 days during the operation of anaerobic and aerobic dual-stage submerged bioreactors are shown in Table 6.2. The pH in all reactors from Day 0 to Day 2 was lower than the methanogenic pH range that is normally between 6.5 and 8. Low pH was due to the fact that microorganisms were starved for the first two days by being fed with distilled water. Raw distillery effluent adjusted with calcium carbonate and dipotassium hydrogen phosphate was fed to the membrane bioreactors from Day 4. The pH of membrane bioreactors did not improve still until it was further adjusted after Day 12. Distilled water that was fed to membrane bioreactors caused further dilution of the effluent thus lowering the pH.

**Table 6.2: pH values for Reactors A, B, C and D over a period of 30 days during the operation of anaerobic and aerobic dual-stage submerged bioreactors. $pH^1$= Reactor A (first balancing tank for feed) $pH^2$ = Reactor B (seeding tank) $pH^3$ = Reactor C (second balancing tank) $pH^4$ = Reactor D (hydrolysis tank).**

| Day | $pH^1$ | $pH^2$ | $pH^3$ | $pH^4$ |
|---|---|---|---|---|
| 0 | 4.38 | 5.13 | 4.58 | 5.19 |
| 2 | 5.61 | 5.83 | 5.89 | 5.88 |
| 4 | 5.27 | 6.17 | 6.24 | 6.38 |
| 6 | 5.63 | 5.68 | 5.81 | 5.84 |
| 8 | 5.68 | 5.44 | 5.95 | 5.65 |
| 10 | 5.71 | 5.64 | 5.63 | 5.68 |
| 12 | 7.53 | 6.2 | 6.18 | 5.98 |
| 14 | 7.2 | 6.37 | 6.9 | 5.85 |
| 16 | 7.09 | 6.7 | 6.79 | 7.15 |
| 18 | 7.3 | 6.83 | 6.69 | 7.03 |
| 20 | 7.26 | 6.67 | 7.38 | 6.48 |
| 22 | 7 | 6.8 | 6.97 | 6.77 |
| 24 | 7.52 | 7.08 | 6.99 | 6.96 |
| 26 | 7.37 | 7.07 | 7 | 6.99 |
| 28 | 7.4 | 7.11 | 6.98 | 7.03 |
| 30 | 7.49 | 7.03 | 6.99 | 7 |

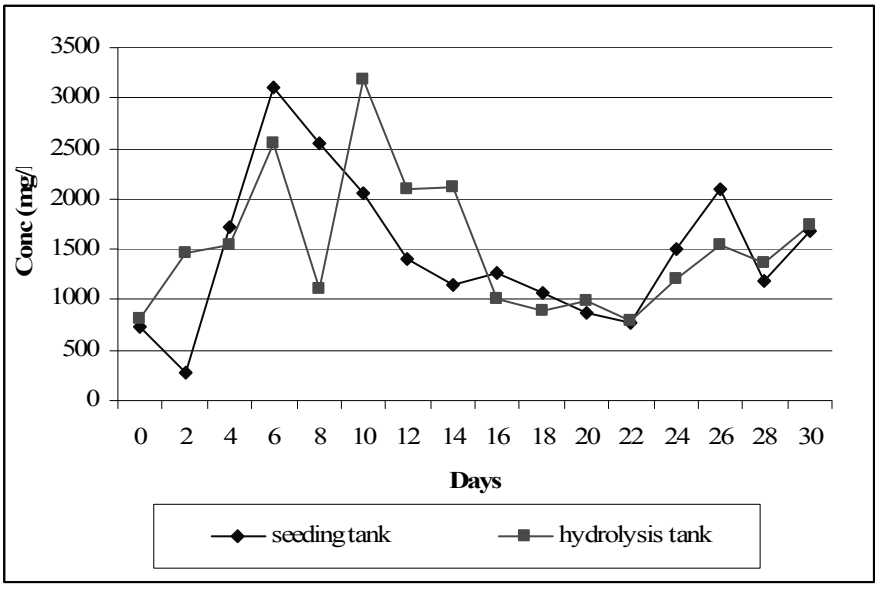



**Figure 6.2: Volatile fatty acid profile on the seeding tank (Reactor B) and hydrolysis tank (Reactor D) during the operation of anaerobic and aerobic dual-stage submerged bioreactors**

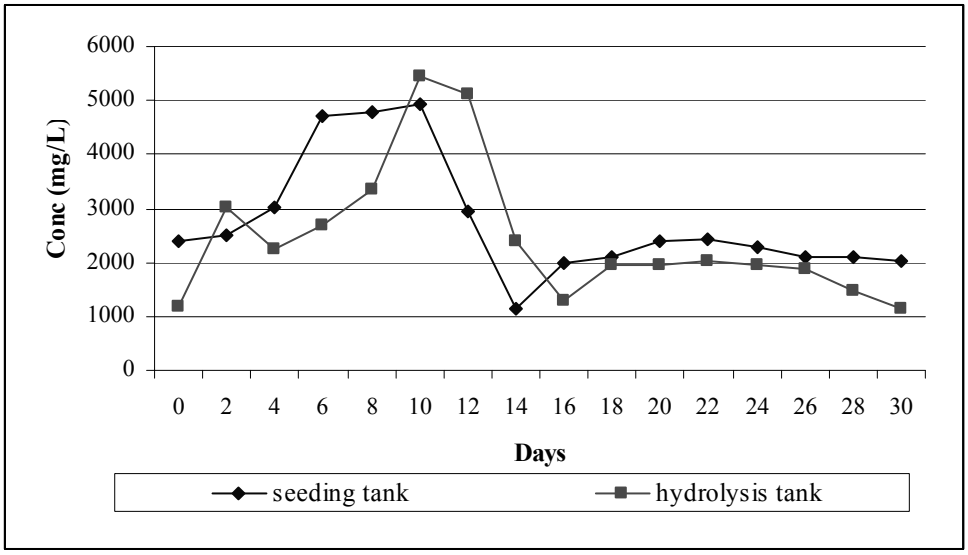



**Figure 6.3: Soluble COD concentration profile over a 30 day period, on the seeding tank (Reactor B) and hydrolysis tank (Reactor D) during the operation of anaerobic and aerobic dual-stage submerged bioreactors**

Degradation of some readily available compounds in the raw effluent led to accumulation of volatile fatty acids that also contributed to further lowering of pH. When the pH in all reactors was increased by the addition of 8000 mg/L of $CaCO_3$ and 4000 mg/L of $K_2HPO_4$ there was improvement in overall performance of the membrane bioreactors. After this adjustment the pH in all reactors stabilized. In Figure 6.2 it is shown that initially the VFA concentration in both membrane bioreactors was above 500 mg/L for the first two days but increased dramatically upon addition of a raw distillery effluent. VFA profiles in the hydrolysis tank suggest efficient anaerobic digestion of effluent as there was an observed accumulation of VFAs that was followed by a dramatic decrease. VFA concentration profiles in Figure 6.2 also indicated a steady decrease in the seeding tank suggesting that although this reactor was most efficient in retaining biomass and nutrients of higher molecular weight, most VFAs were filtered through to Reactor C. Higher VFA concentrations were due to the efficiency of the seeding tank in retaining higher molecular weight compounds that were degraded resulting in an increased VFA content in Reactors C and D. From Day 14 to Day 30, pH stability within the methanogenic range was achieved even though the VFA content was greater than 300 mg/L. It can therefore be concluded that pH stability led to better soluble COD removal as shown in Figure 6.3.

**Table 6.3: Total polyphenol concentration expressed as mg/l gallic acid equivalents**

| Day | Reactor A (mg/L) | Reactor B (mg/L) | Reactor C (mg/L) | Reactor D (mg/L) |
|---|---|---|---|---|
| 0 | 3.951613 | 2.150538 | 3.44086 | 3.27957 |
| 2 | 6.801075 | 2.352151 | 2.043011 | 2.056452 |
| 4 | 4.717742 | 2.33871 | 1.599462 | 2.284946 |
| 6 | 3.991935 | 0.443548 | 2.889785 | 3.333333 |
| 8 | 3.830645 | 9.180108 | 3.629032 | 3.185484 |
| 10 | 7.016129 | 4.435484 | 4.193548 | 4.36828 |
| 12 | 2.701613 | 3.682796 | 3.938172 | 2.244624 |
| 14 | 1.491935 | 1.827957 | 1.384409 | 1.814516 |
| 16 | 2.298387 | 1.948925 | 1.08871 | 1.182796 |
| 18 | 2.365591 | 1.935484 | 1.693548 | 1.142473 |
| 20 | 2.33871 | 1.747312 | 1.747312 | 1.612903 |
| 22 | 2.446237 | 2.5 | 2.231183 | 2.204301 |
| 24 | 2.217742 | 2.513441 | 2.728495 | 2.02957 |
| 26 | 2.300754 | 2.570005 | 2.500931 | 1.80002 |
| 28 | 2.377970 | 2.400111 | 2.674000 | 1.63320 |
| 30 | 2.263489 | 2.000775 | 2.711126 | 1.58764 |

**Table 6.4: Total polyphenol concentration expressed as mg/l phenol equivalents**

| Day | Reactor A (mg/L) | Reactor B (mg/L) | Reactor C (mg/L) | Reactor D (mg/L) |
|---|---|---|---|---|
| 0 | 125.5798 | 377.7374 | 113.604 | 82.00111 |
| 2 | 154.1887 | 347.1325 | 72.68659 | 65.70069 |
| 4 | 92.01423 | 427.304 | 66.36601 | 59.71278 |
| 6 | 95.64024 | 462.5661 | 89.65233 | 60.04544 |
| 8 | 96.30557 | 503.1508 | 115.9326 | 110.2774 |
| 10 | 148.8661 | 459.9048 | 114.9346 | 104.6221 |
| 12 | 59.71278 | 336.4873 | 81.66845 | 83.83075 |
| 14 | 32.26819 | 33.9315 | 27.27826 | 29.93955 |
| 16 | 28.94157 | 31.93552 | 39.91941 | 24.61697 |
| 18 | 31.2702 | 32.26819 | 39.58674 | 25.94761 |
| 20 | 28.94157 | 36.92545 | 37.92344 | 23.28632 |
| 22 | 30.27222 | 43.24602 | 48.23595 | 44.24401 |
| 24 | 36.59279 | 46.57264 | 44.24401 | 44.57667 |
| 26 | 35.00722 | 48.07462 | 44.63218 | 42.45670 |
| 28 | 38.00954 | 47.00432 | 45.86400 | 37.00970 |
| 30 | 38.88766 | 45.99765 | 44.97222 | 32.00365 |

In Figure 6.3 it is shown that COD removal efficiency improved after Day 10 further confirming methanogenic activity. Polyphenol removal was also achieved (see Table 6.3 and 6.4). In the seeding tank, total nitrogen was high between Days 4 and 12 but decreased thereafter from Day 14 to Day 30, suggesting improved nitrogen uptake by the microbial population (Figure 6.4). Figure 6.5 and 6.6 further confirm use of nitrogen by microorganisms in the form of nitrate or ammonia.

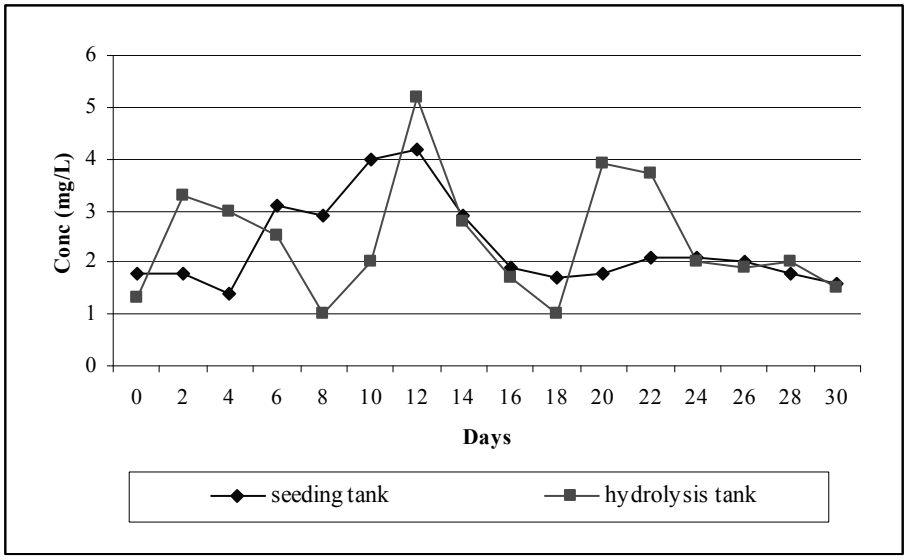



**Figure 6.4: Total nitrogen profile over a 30 day period, on the seeding tank (Reactor B) and hydrolysis tank (Reactor D) during the operation of anaerobic and aerobic dual-stage submerged bioreactors**

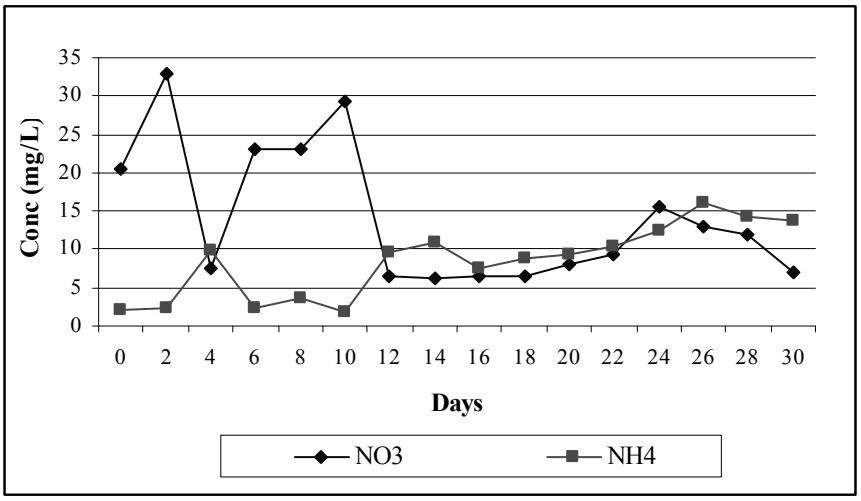



**Figure 6.5: Nitrate and ammonia concentration profiles over a 30-day period in the seeding tank (Reactor B)**

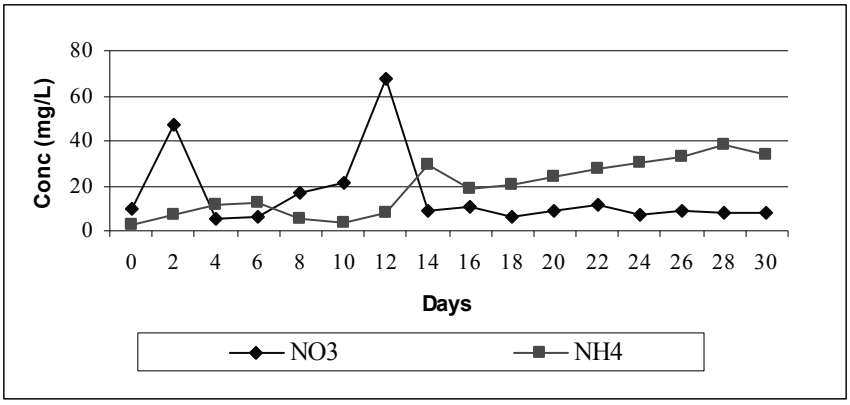



**Figure 6.6: Nitrate and ammonia concentration profiles over a 30 day period in the hydrolysis tank (Reactor D) during the operation of the anaerobic and aerobic dual-stage submerged bioreactors**

In the seeding tank (Figure 6.5) on Day 4 there was a dramatic decrease in nitrates and an increase in ammonia concentration suggesting conversion of nitrate to ammonia. Not all of nitrogen in the form of nitrates was converted to ammonia, suggesting two forms of denitrification, namely, assimilate nitrate reduction, where nitrate is converted to ammonia for cell synthesis, and dissimilatory nitrate reduction or biological denitrification where nitrate is used as an electron acceptor for the oxidation of organic and inorganic electron donors.

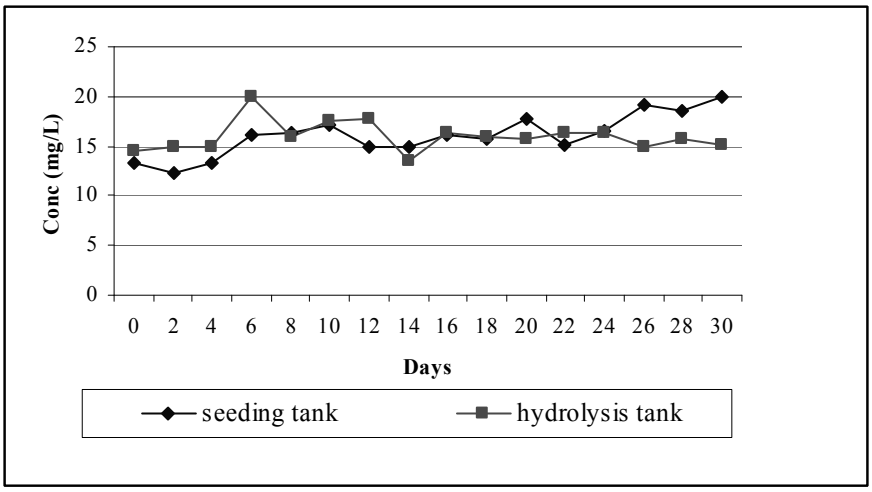



**Figure 6.7: Total phosphorus concentration profile over a 30 day period, on the seeding tank (Reactor B) and hydrolysis tank (Reactor D) during the operation of anaerobic and aerobic dual-stage submerged bioreactors**

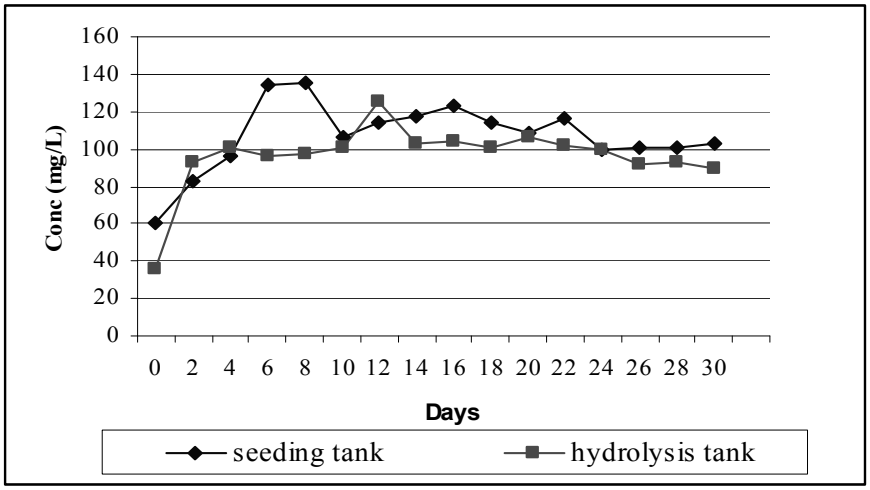



**Figure 6.8: Total ortho-phosphate concentration profile over a 30-day period, on the seeding tank (Reactor B) and hydrolysis tank (Reactor D) during the operation of anaerobic and aerobic dual-stage submerged bioreactors**

Figure 6.7 and 6.8 show high level of phosphorous especially as orthophosphate and this could be attributed to supplementation with dipotassium hydrogen phosphate. After treatment of raw distillery effluent with these membrane reactors the effluent will need to be processed further to remove phosphorus using phosphorus accumulating microorganisms, in order to prevent eutrophication. Formation of oxidation products during anaerobic digestion of distillery effluent, still poses a question as to the efficiency of both aerobic and anaerobic processes.

## 6.5 DISCUSSION AND CONCLUSIONS

Anaerobic treatment of effluents occurs at significantly slower rates than aerobic processes and as a consequence the completed data analysis of the reported process was not available at the time of this report submission. These results, as well as the microbial population dynamics data, will be reported as part of a doctoral submission to Rhodes University.

# CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

## 7.1 CONCLUSIONS

- The application of solid-liquid retention membrane bioreactors was shown to be a highly efficient system for the treatment of high-strength industrial effluents containing recalcitrant pollutants.

- In comparison to activated sludge systems, the long-term operation of this membrane bioreactor process treating high-strength effluents was characterised by more stable microbial populations significantly less susceptible to deleterious shifts in the community dynamics resulting in enhanced process efficiency due to less process variability.

- The dual-stage hybrid membrane bioreactor enabled the intermittent transfer of acclimated retained biofilm developed in a seeding reactor to a hydrolysis reactor. This facilitated significant improvements in the enhancement of microbial population adaptability and performance efficiency as well as drastically decreasing the usual acclimation time necessary by 75% when compared with activated sludge wastewater treatment processes.

- Preliminary results obtained for a cyanide-based effluent confirmed that the MBR system was more stable in terms of operational efficiency and recovery when subjected to various loading rate challenges than was the conventional activated sludge process.

- Preliminary results obtained for the treatment of a distillery effluent were inconclusive in terms of the reported data represented here. The reason for this is that the process is part of a doctoral study that is still undergoing completion at the time of this report submission. Anaerobic-type systems are usually longer term systems when compared with aerobic processes and the process performance data will be reported in the doctoral submission along with microbial population dynamics analysis similar to that reported in Chapters 3 and 4. Preliminary results have indicated that process improvements were evident due to the incorporation of the hybrid MBR operation during the treatment of this effluent type.

- The advantages of modularity associated with commercial membrane-based systems are also applicable to the dual-stage hybrid membrane bioreactor reported here. The significant advantage is therefore that this process design can be retrofitted to existing infrastructure with relatively low capital expenditure. Because the reticulation and process flow control of this system is relatively simple insofar as hardware requirements are concerned, automated control of the process would be significantly less complex and relatively inexpensive compared with the current SCADA systems presently considered when new activated sludge processes and associated infrastructure are designed.

- Coupled with the advantages of this modular, small footprint, easily retro-fitted system, the potential process intensification facilitated by this system design in the treatment of a variety of high strength industrial effluents, and potentially domestic-origin wastewaters as well, cannot be understated. Significant improvements to the current wastewater treatment options available with minimal infrastructure changes can potentially be implemented using this process design.

## 7.2 RECOMMENDATIONS

- The significant improvements in operational efficiency of the dual-stage hybrid membrane bioreactor process, coupled with the improved adaptation time, recovery and overall robustness, signify that this process would have major impact on the overall process efficiency of existing activated sludge processes and possibly several other conventional wastewater treatment processes. However, further development is required to take the system to a fully-operational pilot plant in order to scale up to commercial systems.

- Protection of the intellectual property associated with this process design would be first and foremost prior to developing the system further. It is highly recommended that this system be tested at the pilot scale level as a retrofit process intensification of an existing wastewater treatment facility. Scalability of membrane bioreactor systems due to the modularity of commercially available units is well documented and therefore poses less economic risk because huge infrastructure investment is minimized. The portability of modular units is also advantageous and this advantage is exploitable especially if several effluent source treatment facilities are targeted for further assessment of this process at the pilot scale.

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# APPENDIX 1: MEMBRANE PROCESS TECHNICAL SUMMARY

| | **Scott *et al.*, 1998**<br>**Laboratory scale** | **Davies *et al.*, 1998**<br>**Pilot scale** | **Engelhardt *et al.*, 1998**<br>**Pilot scale** | **Côté *et al.*, 1998**<br>**Pilot scale** | **Nagaoka *et al.*, 1998**<br>**Laboratory scale** |
|---|---|---|---|---|---|
| **Effluent** | Dairy industry waste stream | Domestic / Municipal wastewater | Domestic / Municipal wastewater | Domestic / Municipal wastewater | Synthetic wastewater |
| **Effluent pre-treatment** | DAF fat trap | 0 mm rotating disc screen | 1.5 mm Self-cleaning sieve | 0 mm Mesh | - |
| **Active Reactor Volume** | 10 L | 15.5 $m^3$ | - | 0 $m^3$ (0.35 $m^3$ + 0.65 $m^3$) | 2 x 0.028 $m^3$ |
| **Membrane pore size** | 0.2 \| 0.35 \| 0.8 \| 1.2 µm | 0.4 µm | - | 200 000Da | 0.2 µm |
| **Membrane surface area** | 0.06 $m^2$ | 160 $m^2$ | 27.8 $m^2$ | 12 $m^2$ | 0.273 $m^2$ |
| **Membrane module** | **In-series**<br>Tube-in-shell 0.6 mx0.02 m | **Immersed**<br>Kubota membrane system | **Immersed**<br>ZENON membrane system | **Immersed**<br>ZENON membrane system | **Immersed**<br>Plate and frame |
| **Membrane material** | Ceramic MF | Polyolefin MF | ZeeWeed hollow fibers | ZeeWeed hollow fibers | Polysulphone MF |
| **Permeate flux** | - | 20 L/$m^2$/h | - | - | - |
| **Crossflow velocity** | 160 l/h | - | - | - | - |
| **Permeability recovery** | - | - | - | - | - |
| **Pressure conditions (TMP)** | 0.5 Bar | - | - | - | - |
| **Module aeration** | 0.25-0.45 L /min Membrane facilitated | 142 $m^3$/h | - | - | 36 L/min |
| **Biological reactor** | - | - | - | - | - |
| **HRT** | 24h | 4.5h | - | 4.8h \| 6.5h \| 9.2h | - |
| **SRT** | - | 45d | - | - | - |
| **Temperature (control)** | 25°C (yes) | - | - | - | - |
| **Hydraulic delivery** | Pump | Pump | Negative pressure pump | - | - |
| **Membrane cleaning** | - | Bubble scouring | Air/water scouring; backwash | Air agitation backwash | Air scouring<br>Mechanical - sponging |
| **Cleaning interval** | - | - | periodic | periodic | - |
| **F/M ratio (kg $BOD_5$/kgMLSS.d)** | 4.78 | - | | - | - |
| **Sludge load (kg COD/kg MLSS)** | - | - | 0.4 – 0.6 | - | - |
| **Influent concentration** | COD 9600 mg/L<br>BOD 5300 mg/L | TSS 418(±283)mg/L<br>MLSS 16000 mg/L | COD 800 mg/L<br>TKN 35 mg/L | - | 1) 1.5g TOC/Ld<br>2) 0.5g TOC/Ld |
| **Effluent concentration reduction** | $BOD_5$ 45±5%<br>COD 55±5%<br>SS 99±0.7% | COD 61±20 mg/L 87%<br>BOD 4 mg/L 98% | COD <20 mg/L<br>$NH_4$-N <0 mg/L | - | - |
| **Sludge yield (kg sludge/kg BOD removed)** | - | 0.26<br>(typical AS value 0.48) | - | 0.46 kgDM/kgBOD removed<br>(or 0.2 kgDM/kgCOD removed) | - |
| **Hydraulic loading rate (COD/$m^3$/d)** | - | - | - | 1.2 \| 1.7 \| 2.3 | - |
| **Mass loading kgCOD/kgMLSS/d** | - | - | - | 0.07 \| 0.08 \| 0.09 | - |

| | **Günder & Krauth, 1998**<br>**Pilot scale** | **Jefferson *et al*., 2000**<br>**Pilot scale** | **Gander *et al*., 2000**<br>**Pilot scale** | **Ueda *et al*., 1996**<br>**Pilot scale** |
|---|---|---|---|---|
| Effluent | Domestic / Municipal wastewater | Grey water | Domestic / Municipal wastewater | Domestic / Municipal wastewater |
| **Effluent pre-treatment** | 3 mm screen | - | - | 0 mm bar screen |
| **Active Reactor Volume** | 6.9 $m^3$ and 9 $m^3$ | 0.035 $m^3$ | 0.035 $m^2$ | 21.4 $m^3$ |
| **Membrane pore size** | Plate 0.4 μm ; Hollow fiber 0.2 μm | 0.4 μm | PS 0.4 μm \| NWPP 5 μm \| GFPP 0.5 μm | 0.1 μm |
| Membrane surface area | Plate module 80 $m^2$ Hollow fibers 83.4 $m^2$ | 2 x 0.24 $m^2$ | 3 x 0.24 $m^2$ | 4 $m^2$ |
| **Membrane module** | Immersed<br>Kubota plate module<br>ZENON membrane system | Immersed<br>Plate-and-frame | Immersed<br>Plate-and-frame | Immersed<br>Hollow fiber |
| **Membrane material** | Hollow fibers ZW150 | Polysulphone | Polysulphone (PS), Polypropylene (NWPP)<br>Polypropylene (GFPP) | Polyethylene<br>Mitsubishi Rayon Co. Ltd. |
| **Permeate flux** | Plate 18 L/$m^2$h<br>Hollow fiber 17 L/$m^2$h | 28 L/$m^2$h-8 L/$m^2$h<br>(over 76d operation)<br>sub critical flux | PS 45 – 7 L/$m^2$h<br>NWPP 162 – 5 L/$m^2$h<br>GFPP 0 L/$m^2$h | 0.29 m/d |
| **Crossflow velocity** | - | - | - | |
| **Permeability** | Plate 200 L/$m^2$hbar<br>Hollow fibers 60 L/$m^2$hbar | 466 - 133 L/$m^2$hbar<br>(over 76d operation) | - | |
| **Pressure conditions (TMP)** | Plate 8 kPa<br>Hollow fiber 25 kPa | - | - | |
| **Module aeration** | Plate 80 $m^3$/h<br>Hollow fiber 78 $m^3$/h | 20 L/min | 20 L/min - 5 L/min | 0.7 $m^3$/min |
| **Biological reactor** | pumps | - | - | - |
| **HRT** | 5-6h | 12h | - | 13-16h |
| **SRT** | 15-25d | - | Sludge completely retained | |
| **Temperature (control)** | 12-20°C (no) | - | - | 14-27°C (no) |
| **Hydraulic delivery** | - | - | - | Suction pump |
| **Membrane cleaning** | ZeeWeed backwash 20-50 kPa<br>Air/water scouring | - | Mechanical<br>tap water / sponge | |
| **Cleaning interval** | Continuous aeration | - | - | |
| **F/M ratio (kg $BOD_5$/kgMLSS.d)** | - | Independent of F/M ratio | PS 0.08 ; NWPP 0.04 | |
| **Sludge load (kg COD/kg MLSS)** | - | - | - | |
| **Influent concentration** | COD 200-300 mg/L<br>$BOD_5$ 100-150 mg/L | - | - | COD 71±49 mg/L ; BOD 133±69 mg/L<br>SS 132±69 mg/L |
| **Effluent concentration reduction** | COD <20 mg/L<br>SS <1 mg/L | - | $BOD_5$ PS 97.4% NWPP 96.9%<br>COD PS 92.4% NWPP 93.3% | COD 5 mg/L ; BOD 1 mg/L<br>SS <1 mg/L |
| **Sludge yield**<br>**(kg sludge/kg BOD removed)** | - | - | - | |
| **Hydraulic loading rate**<br>**(COD/$m^3$/d)** | - | 0.005-0.11 kgBOD/$m^3$d | PS 0.2695 kgBOD/$m^3$d<br>NWPP 0.1155 kgBOD/ $m^3$d | 0.156-0.372 kgBOD/ $m^3$d |
| **Mass loading kgCOD/kgMLSS/d** | - | - | - | |

| | **Winnen *et al.*, 1996<br>Pilot scale** | **Ragona & Hall, 1998<br>Laboratory scale** | **Muller *et al.*, 1995<br>Pilot scale** | **Jefferson *et al.*, 2001<br>Pilot scale** |
|---|---|---|---|---|
| Effluent | Synthetic municipal wastewater | Synthetic newsprint mill whitewater | Domestic / municipal wastewater | Grey & Black waters |
| **Effluent pre-treatment** | - | 4 mm mesh screen | 0.1 mm screen; pre-settled (1h) | - |
| **Active Reactor Volume** | 40 L | 10 L (Y=0.975; 0.95)<br>5 L (Y=0.983; 0.90) | 613 L<br>Divided into four compartments | 0.066 $m^3$ |
| **Membrane pore size** | 300 kDa | 500Å | In-series<br>2 x ($UF_1$-MF) 73 x [2 m x 5.2 mm] 2.30 $m^2$ 1-2 m/s<br>$UF_1$- $UF_1$-MF-$UF_2$ 7 x [2 m x 14 mm] 0.63 $m^2$ 4 m/s<br>$UF_1$-MF 7 x [3 m x 14 mm] 0.95 $m^2$ 5 m/s<br>$UF_1$ (polysulphone) MWCO 50 kDa (dextrans)<br>$UF_2$ (acrylic) MWCO 800 kDa (dextrans)<br>MF (polyvinilydene chloride) MWCO 0.1 μm | 0.4 μm |
| Membrane surface area | 0.08 $m^2$ | 0.0055 $m^2$ | | 2 x 0.24 $m^2$ |
| **Membrane module** | In-series<br>Tube-in-shell 0.86 m TECH-SEP KERASEP | In-series<br>Tube-in-shell I.D. 7 mm | | Immersed<br>Plate-and-frame |
| **Membrane material** | Ceramic (K01-X) | Ceramic (T1-70 Membralox) | | Polysulphone |
| **Crossflow velocity** | 20 L/h | 4 m/s (PWF) | | |
| **Permeate flux** | - | 1300 (PWF) - 550 L/$m^2$h | 0.17 $m^3/m^2$h decreased over 120d | - |
| **Permeability** | - | - | - | |
| **Pressure conditions (TMP)** | - | 13.8 kPa (PWF) | 2 x ($UF_1$-MF) 0.35; 0.15 mPa<br>$UF_1$- $UF_1$-MF-$UF_2$ 0.5; 0.4; 0.3; 0.2 mPa<br>$UF_1$-MF 0.3; 0.2 mPa | - |
| **Module aeration** | - | Aquarium pump | Diffused air system | 20 L/min |
| **Biological reactor** | - | - | **Comprehensive comparison with conventional AS system** | **Comparison with Biological Aerated Filter (BAF)** |
| **HRT** | 6h | 12h | 6-8h (0-220d) → 10-15h | 12h |
| **SRT** | 30d | 20d | Sludge completely retained ∴ > 3500d | - |
| **Temperature (control)** | 20°C (Yes – heat exchanger) | 55°C (Yes - water jackets) | 20-30°C (No) | - |
| **Hydraulic delivery** | Pump system | Progressive cavity pumps | Pump system | - |
| **Membrane cleaning** | Alkaline/acid 60-80°C | 250ppm $HOCl_3$ – 20 min; 20% NaOH; 10% $HNO_3$ | 10% Ultrasil ; 0.5-1% $HOCl_3$ | - |
| **Cleaning interval** | 4-5h once per week | After each experimental run | Periodic after 120d; membrane replacement 237d | - |
| **F/M ratio (kg $BOD_5$/kgMLSS.d)** | - | - | - | Independent of F/M ratio |
| **Sludge load (kg COD/kg MLSS)** | - | - | - | - |
| **Influent concentration** | COD 898±164 mg/L | COD 5520 mg/L<br>TSS 460 mg/L ; TDS 4740 mg/L | Total Organic Carbon 13-17.5 mM | Grey COD 9.6±7.4 mg/L<br>Grey/Black COD 10.63±.5.5 mg/L<br>Black COD 15.5±7.5 |
| **Effluent concentration reduction** | COD 3.1 mg/L 99% | TSS/TDS 25-45%<br>COD 45-55% | 90% | - |
| **Sludge yield<br>(kg sludge/kg BOD removed)** | 0.32 kgTSS/kg$COD_{removed}$ | - | 50g MLSS/L (271-330d) | - |
| **Hydraulic loading rate<br>(COD/$m^3$/d)** | - | - | 0.9-2.0 COD/L.d | 0.14-0.18 kgCOD/$m^3$.d |
| **Mass loading kgCOD/kgMLSS.d** | 0.129 kgCOD/kgVSS.d | - | 0.09gCOD/gMLSS.d | - |

| | **Wagner *et al.*, 2000** | **Fan *et al.*, 2000** | **Parameshwaran *et al.*, 2001** | **Scott *et al.*, 1998** |
|---|---|---|---|---|
| **Effluent** | Domestic / municipal wastewater | Domestic / municipal wastewater | High strength brewery wastewater | Food industry waste stream (Dairy) |
| **Effluent pre-treatment** | Primary settling | 0.5 mm screen / pre-filter | UASB; aerobic SBR; MF system in series; 1 mm pre strainer | Dissolved air flotation (fat trap) |
| **Active Reactor Volume** | 220 L | 1.5 $m^3$ | | 10 L |
| **Membrane pore size** | 0.2 µm | 0.02 µm | 0.2 µm | 1.2 – 0.2 µm (0.35) |
| **Membrane surface area** | 0.63 $m^2$ | 1.4 $m^2$ | 1 $m^2$ | 0.06 $m^2$ |
| **Membrane module** | 5-plate module | Tube in shell | 3000 x (650µm x 430µm x 40 cm) | Tube in shell |
| **Membrane material** | Cellulose MF | Ceramic UF | Hollow fibre UF polypropylene | Ceramic MF |
| **Permeate flux** | 14.5 L/$m^2$h) | 141-150 L/h | 45-90; then 35 | |
| **Crossflow velocity** | - | 3 –4 m/s | | 160 L/h |
| **Permeability recovery** | - | 87% | | |
| **Pressure conditions (TMP)** | Atmospheric | 0.6-0.8 bar | 600 kPa | 0.5 bar TMP |
| **Module aeration** | 4 $m^3$/h | | | 0.25-0.45 L/min |
| **Biological reactor** | Nitrification | | | |
| **HRT** | 24 h | 15 h; then 7.5 | | 24 h |
| **SRT** | - | 20 days, (10, 5) | | -- |
| **Temperature (temp. control)** | 20°C (yes) | 30°C (yes) | | 25°C (yes) |
| **Hydraulic delivery** | Pump | | Peristaltic pump | Pump (not specified) |
| **Membrane cleaning** | -- | Alkaline, $H_2O$, Nitric acid | Patented gas pulse backwash | Air sparging; later membrane |
| **Cleaning interval** | | 5-10d | | |
| **F/M Ratio** | 0.05-0.2 | | | 4.78 |
| **Sludge load** kg COD/kg MLSS | 0.04 to 0.2 | 3g/l MLSS | | |
| **Influent []** | COD 60000<br>BOD 36000 | | | COD 9600 mg/L<br>BOD 5300 mg/L |
| **Effluent [] reduction** | -- | | | 95% |
| **Hydraulic loading rate (COD/$m^3$/d)** | -- | | | |
| **Mass loading kgCOD/kgMLSS/d** | -- | | | |

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| | **Lubbecke *et al.*, 1995** | **Ohmori *et al.*, 2000**<br>**Application scale** | **Seo *et al.*, 2000**<br>**Semi-pilot scale** | **Ng *et al.*, 2000**<br>**Lab. Scale** |
|---|---|---|---|---|
| **Effluent** | | Domestic wastewater | Domestic wastewater | Synthetic effluent |
| **Effluent pre-treatment** | | Sedimentation; pre-filtration | -- | -- |
| **Active Reactor Volume** | | - | 0.27 $m^3$ (0.09 $m^3$ + 0.18 $m^3$) | 20 L |
| **Membrane pore size** | 0.01 µm | 0.3 µm - 0.4 µm (PF)<br>0.1 µm (HF) | 0.1 µm | 0.4 µm |
| **Membrane surface area** | | PFM 6.4 $m^2$ – 8.0 $m^2$<br>HF 8.0 $m^2$ | 4.0 $m^2$ | 2 x 0.1 $m^2$ |
| **Membrane module** | In-series<br>Tube-in-shell | **Immersed**<br>Plate and frame ; Hollow fiber | Immersed<br>UF HF | Immersed<br>MF SMBR SBR |
| **Membrane material** | Crossflow UF Polysulfone | Polyethylene | Polyethylene | Polyolefin |
| **Permeate flux** (l/$m^2$/h) | (PWF 300) | 0.45-0.375 $m^3$/$m^2$/d (PFM) | 0.1 $m^3$/$m^2$ | -- |

| | | | | |
|---|---|---|---|---|
| | | 0.24 $m^3/m^2/d$ (HF) | | |
| **Crossflow velocity** | 3.5-6.6 m/s | - | | -- |
| **Permeability recovery** | | - | | -- |
| **Pressure conditions (TMP)** | | - | 10 kPa (suction) | 50 kPa suction |
| **Module aeration** | 1500 $L/h^{-1}$ | - | 5-20 L/min | -- |
| **Biological reactor** | | Nitrification/denitrification | -- | |
| **HRT** | 7.6-24 h | - | 16-19 h | Batch mode 6 h |
| **SRT** | 1.5-8 days | - | 25 days | Batch mode 6 h |
| **Temperature (temp. control)** | 27°C (yes) | Seasonal variance | < 40°C; (16-20°C) | -- |
| **Hydraulic delivery** | | Pump | Pump | Pump |
| **Membrane cleaning** | | Air; NaOH | | Water spray |
| **Cleaning interval** | | 6 months | Air; NaOH | -- |
| **F/M Ratio** | | - | -- | 0.5/d based on COD |
| **Sludge load** kg COD/kg MLSS | 0.5-1.5 | - | | |
| **Influent []** | 8500-17600 mg/L COD<br>6800-14100 mg/L BOD | COD 95 mg/L<br>BOD 144 mg/L | COD 283 mg/L<br>BOD 98.3 mg/L | |
| **Effluent [] reduction** | | 90-95% | COD 95.6-96.2%<br>BOD 98.3% | COD 98.5% |
| **Hydraulic loading rate ($COD/m^3/d$)** | | - | Phase 1 (0.7)<br>Phase 2 (0.6) | |
| **Mass loading kgCOD/kgMLSS/d** | | - | MLSS 2300-3600 mg/L | |

| | **Yoon *et al.*, 2000**<br>**Lab. Scale** | **Luxmy *et al.*, 2000 (pilot)**<br>**(molecular methods)** | **Rosenberger *et al.*, 2000**<br>Pilot scale | **Rosenberger *et al.*, 2000**<br>**Lab scale** |
|---|---|---|---|---|
| **Effluent** | Municipal wastewater | Domestic Wastewater | Municipal wastewater | High strength molasses |
| **Effluent pre-treatment** | - | - | - | - |
| **Active Reactor Volume** | - | - | 2600-3900 | 23 |
| **Membrane pore size** | 0.1 µm | 0.1 µm | 0.2 µm | 0.05 to 0.2 µm |
| **Membrane surface area** | 0.5 $m^2$ | 4 $m^2$; 0.2 $m^2$ | 14 $m^2$ | 0.06-0.17 $m^2$ |
| **Membrane module** | In-series<br>Hollow fiber TIS SMBR | Immersed<br>Hollow fiber SMBR | Immersed<br>ZENON membrane system | In-series<br>MemBrain/Stork |
| **Membrane material** | Polyethylene | - | - | - |
| **Permeate flux** (l/$m^2$/h) | 40 l/$m^2$/hr | 0.15 m/d; 0.05 | - | - |
| **Crossflow velocity** | - | - | - | - |
| **Permeability recovery** | - | - | - | - |
| **Pressure conditions (TMP)** | - | - | - | - |
| **Module aeration** | - | - | - | - |
| **Biological reactor** | - | - | - | - |
| **HRT** | 3-6 h (anoxic); 5-10 h (oxic) | 1.5 days | 10-16 h | 121-24 h |
| **SRT** | - | - | - | - |
| **Temperature (temp. control)** | 10-28°C (no) | - | - | - |
| **Hydraulic delivery** | Peristaltic pump | - | - | - |
| **Membrane cleaning** | - | - | - | - |
| **Cleaning interval** | - | - | - | - |
| **F/M Ratio** | - | 0.07 kgCOD/kgMLSS.d | - | - |
| **Sludge load** kg COD/kg MLSS | 10000-15000 | - | 0.07 MLSS | 0.07 MLSS |
| **Influent []** | COD 250; BOD 177.3 | - | COD 750 | COD 3900 |
| **Effluent [] reduction** | 99% | - | 1.3 | 4.6 |
| **Hydraulic loading rate (COD/$m^3$/d)** | - | - | - | - |
| **Mass loading kgCOD/kgMLSS/d** | - | - | - | - |

| | **Ogosh *et al*., 2000**<br>**Pilot scale** | **Ogosh *et al*., 2000**<br>**Pilot scale** | **Murakami *et al*., 2000**<br>**Pilot scale** | |
|---|---|---|---|---|
| **Effluent** | Municipal wastewater | Municipal wastewater | Municipal wastewater | |
| **Effluent pre-treatment** | Fine screen | Fine screen | 1 mm mesh | |
| **Active Reactor Volume** | 50 $m^3$ | 50 $m^3$ | - | |
| **Membrane pore size** | 0.4 µm | 0.1 µm | 0.2 µm | |
| **Membrane surface area** | 178-225 $m^2$ | 312 $m^2$ | 3 $m^2$ | |
| **Membrane module** | Immersed<br>Multiplate MF | Immersed<br>Hollow fiber MF | Immersed<br>Hollow fiber MF | |
| **Membrane material** | Polyolefin | Polyethylene | Polypropylene | |
| **Permeate flux** ($l/m^2/h$) | - | - | 1.2-1.44 $m^3/m^2/d$ | |
| **Crossflow velocity** | - | - | - | |
| **Permeability recovery** | - | - | - | |
| **Pressure conditions (TMP)** | - | - | - | |
| **Module aeration** | - | - | - | |
| **Biological reactor** | - | - | - | |
| **HRT** | 24 hr | 24 hr | - | |
| **SRT** | > 400 days | > 400 days | - | |
| **Temperature (temp. control)** | - | Gravitation filtration | - | |
| **Hydraulic delivery** | - | - | Pump | |
| **Membrane cleaning** | - | - | Air backwash | |
| **Cleaning interval** | - | - | 30 min | |
| **F/M Ratio** | - | - | - | |
| **Sludge load** kg COD/kg MLSS | - | - | - | |
| **Influent []** | - | - | - | |
| **Effluent [] reduction** | COD > 80%; BOD 90% | COD > 80%; BOD 90% | > 90% COD, BOD | |
| **Hydraulic loading rate ($COD/m^3/d$)** | - | - | - | |
| **Mass loading kgCOD/kgMLSS/d** | - | - | - | |

| | **Scott *et al.*, 1996 Laboratory scale** | **Grimberg *et al.*, 2000 Laboratory scale- monoculture** | **Yamamoto *et al.*, 1989 Laboratory scale** | **Canales *et al.*, 1994 Pilot scale - monoculture** | **Chaize & Huyard, 1991 Laboratory scale** |
|---|---|---|---|---|---|
| Effluent | Domestic/municipal wastewater | Trinitrophenol-containing synthetic wastewater | Synthetic wastewater | Synthetic domestic wastewater | Domestic/municipal wastewater |
| **Effluent pre-treatment** | - | - | - | - | - |
| **Active Reactor Volume** | 10 L | 0.574 $m^3$ | 0.5, 1.2, 5 L | 6 L | 4.5 L working volume<br>8 L total volume |
| **Membrane pore size** | 0.2 μm, 0.35 μm, 0.8 μm | - | 0.1 μm | 200 kDa | 50 kDa |
| Membrane surface area | 0.06 $m^2$ | - | 0.3 $m^2$ | 0.16 $m^2$ | 0.42 $m^2$ |
| **Membrane module** | In-series<br>Tube-in-shell 0.6 m x 0.02 m O.D. | In-series<br>Tube-in-shell 0.325 m x 0.015 m I.D. | Immersed<br>Mitsubishi Rayon | In-series<br>Tube-in-shell | In-series<br>Plate-and-frame |
| **Membrane material** | Ceramic | Mitsubishi Rayon Hollow fiber<br>Polymeric 0.325 m x 281.5 μm O.D | Polyethylene<br>Hollow fibers | Carbosep-SFEC<br>Mineral membrane | Polysulphone GR51PP<br>Cellulose ETNA |
| **Permeate flux** | 40 L/$m^2$h (0.2 μm) | | 2.56x$10^{-6}$ $m^3$/$m^2$.s | - | - |
| **Crossflow velocity** | 1.7-5.0 L/min | Recycle flow rate 190 L/d | - | - | 1.5 m/s |
| **Permeability recovery** | - | - | - | - | - |
| **Pressure conditions (TMP)** | <0.3 bar | | 27-66 kPa | - | 1-2 bar |
| **Module aeration** | 0.15-0.35 L/min; 0.5 bar; sparger or membrane | Lumen supply 2psig >1.76 mg/L $O_2$ | 0.5-1.8 L/min | 30% of air saturation | 80 L/h |
| **Biological reactor** | - | - | Semi-continuous | *Pseudomonas fluorescens* | - |
| **HRT** | 24h | 14h | 0.19-4.8h | 1-2h | 8-2h |
| **SRT** | - | - | - | 10h | 50-100d |
| **Temperature (control)** | 25°C (Yes) | - | 23-28°C | 29°C | 20°C |
| **Hydraulic delivery** | - | - | - | - | pump |
| **Membrane cleaning** | - | - | - | - | - |
| **Cleaning interval** | - | - | - | - | - |
| **F/M ratio (kg $BOD_5$/kgMLSS.d)** | - | - | - | - | 0.06-0.1 kgCOD/kgSS.d |
| **Sludge load (kg COD/kg MLSS)** | - | - | 10-11 kgMLSS/L | - | - |
| **Influent concentration** | - | - | COD 263-311 mg/L<br>TOC 57-142 mg/L | 1gCOD/L | SS 200-400 mg/L COD 250-550 mg/L TOC35-80 mg/L |
| **Effluent concentration reduction** | $BOD_5$ 117%<br>COD 128% | 14 fiber module 99.1%<br>30 fiber module 83.2%<br>54 fiber module 85.4% | COD 1-13 mg/L<br>TOC 1-5 mg/L | 95% | - |
| **Sludge yield (kg sludge/kg BOD removed)** | - | 14 fiber module 16660 mg/L<br>30 fiber module 11240 mg/L<br>54 fiber module 6996 mg/L | 7-16 kgMLSS/$m^3$ | - | - |
| **Hydraulic loading rate (COD/$m^3$/d)** | - | - | 1.5 kgCOD/$m^3$.d | 20 kgCOD/$m^3$.d | 0.45-1.5 kgCOD/$m^3$.d |

| | | | | | |
|---|---|---|---|---|---|
| **Mass loading kgCOD/kgMLSS/d** | - | Trinitrophenol 40 mg/d | - | - | - |
| **$O_2$ consumption** | $k_L a$ 0.377 – 0.453 L/m | - | - | - | 0.14 $kgO_2$/kgSS.d |
| | **Scott & Smith, 1997** | | | | |
| Effluent | Dairy industry waste stream | | | | |
| **Effluent pre-treatment** | DAF fat trap | | | | |
| **Active Reactor Volume** | 10 L | | | | |
| **Membrane pore size** | 0.2 μm | | | | |
| Membrane surface area | 0.06 $m^2$ | | | | |
| **Membrane module** | In-series<br>Tube-in-shell 0.6 m x 0.02 m O.D. | | | | |
| **Membrane material** | Ceramic | | | | |
| **Permeate flux** | 24 $L/m^2h$ | | | | |
| **Crossflow velocity** | Re-circulation rate 160 L/h | | | | |
| **Permeability recovery** | | | | | |
| **Pressure conditions (TMP)** | 0.1 bar | | | | |
| **Module aeration** | 2.5 L/min | | | | |
| **Biological reactor** | - | | | | |
| **HRT** | 24h | | | | |
| **SRT** | - | | | | |
| **Temperature (control)** | 25°C (yes) | | | | |
| **Hydraulic delivery** | - | | | | |
| **Membrane cleaning** | Air backflush | | | | |
| **Cleaning interval** | - | | | | |
| **F/M ratio (kg $BOD_5$/kgMLSS.d)** | - | | | | |
| **$BOD_5$:N:P ratio** | 1000:1:5 | | | | |
| **Sludge load (kg COD/kg MLSS)** | - | | | | |
| **Influent concentration** | COD 13300 mg/L<br>$BOD_5$ 6500 mg/L | | | | |
| **Effluent concentration reduction** | COD 95%<br>$BOD_5$ 95% | | | | |
| **Sludge yield (kg sludge/kg BOD removed)** | - | | | | |
| **Hydraulic loading rate (COD/$m^3$/d)** | - | | | | |
| **Mass loading kgCOD/kgMLSS/d** | - | | | | |
| **$O_2$ consumption** | - | | | | |