CULTIVATION AND ENRICHMENT OF ANAMMOX CULTURE IN A SUBMERGED MEMBRANE BIOREACTOR

Results of a research project comparing membrane fouling rates of PVDF to PTFE membranes

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ABSTRACT

Submerged membrane bioreactors (SMBRs) have been used to cultivate Anammox bacteria in laboratories from start-up cultures to help overcome the slow growth rate associated with these microbes. Membrane fouling is, however, a limitation of SMBRs and a significant amount of research has been conducted to identify the causes of fouling and how best to manage it.

This research project compared the membrane fouling rates of PVDF to PTFE membranes and concluded that the industry standard PVDF membranes performed significantly better than the novel PTFE membranes in Anammox SMBRs. PVDF membranes showed more resistance to membrane fouling with and without backwashing. It also demonstrated a better membrane fouling recovery rate when backwashing was applied.

In addition, it was demonstrated that the PVDF membranes were more resistant to membrane fouling in a start-up Anammox SMBR than PP membranes used in a similar project when no backwashing was applied. The results from this project also demonstrated that both the PVDF and PTFE membranes performed best when backwashed for nine minutes every 90 minutes, compared to other backwash frequencies. Anammox activity was achieved within 80 days in two start-up SMBRs, seeded with anaerobic sludge from an Australian industrial wastewater treatment plant

Keywords: Anammox, wastewater, submerged, membrane, bioreactor, PVDF, PTFE, fouling.

INTRODUCTION

Anammox (ANaerobic AMMonium OXidation) is a fairly new process that has been developed to remove ammonia from wastewater. The Anammox process was first observed in the Netherlands in 1986 with the unexpected drop of ammonia levels in a denitrifying fluidised bed reactor with a constant production of nitrogen gas. Four years later the process was identified as anoxic ammonium oxidation and, in 1995, the first scientific journal articles were released; (Van de Graaf *et al.*, 1995).

The first publications identified the Anammox process as:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$$

Upon further investigation it was discovered that the overall ammonia conversion occurred in two separate processes and by two different groups of bacteria. The first process was performed by ammonia-oxidising bacteria (AOB) to convert a portion of the ammonia to nitrite (1), and the second by the newly discovered Anammox bacteria to convert the nitrite and remaining ammonia to nitrogen gas (2) (Strous *et al.*, 1998):

$$NH_4^+ + 0.75O_2 \rightarrow 0.5NH_4^+ + 0.5NO_2^- + H^+ + 0.5H_2O \quad (1)$$

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (2)$$

Anammox can thus be utilised as an alternative biological ammonia removal process compared to the traditional nitrification/denitrification processes, with significant benefits. Data from the first full-scale plant in the Netherlands showed that the Anammox process removed nitrogen at a rate of up to 2.6 kg N/m3/d with a removal efficiency of up to 95%. It was also revealed that the Anammox process operated at a significantly lower overall operating cost compared to conventional nitrification/denitrification, due to a reduction in plant footprint size (up to 50%), and a reduction of power consumption by up to 60%, due to lower aeration costs. The Anammox process also reduced CO_2 emissions by up to 90%, making it a very 'green' process (Paques, 2008).

Like most biological systems, there are limitations to utilising this new process. All species of Anammox bacteria are strict anaerobes and are inhibited by low pH, high nitrite and high chemical oxygen demand (COD) levels. They also have a doubling time of nearly two weeks, which meant that it took approximately three years to grow the biomass for the first full-scale plant in the Netherlands (Strous, 2006; Tang *et al.*, 2009).

One way to overcome these long start-up periods is by using submerged membrane bioreactors (SMBR). SMBRs in general have many benefits, such as smaller footprint sizes, higher rates of organic matter degradation and better effluent quality (van der Marel *et al.*, 2009). One of the main benefits of SMBRs is that the microbial populations are retained in the system, leading to higher microbial population yields, making it an attractive option for Anammox start-up cultures (Wang *et al.*, 2009).

Various small-scale Anammox SMBR configurations have been operated to demonstrate shorter start-up periods compared to full-scale plants. Two of these achieved 75% nitrogen removal after 80 days (Gong *et al.*, 2007) and 90% nitrogen removal rate after just 60 days from start-up (Wang *et al.*, 2009).

Membrane fouling is, however, a limitation of SMBRs and can sometimes be very hard to manage at a large scale, especially in anoxic/anaerobic SMBRs (Feng *et al.*, 2009). Previous research indicated that membrane fouling could be a problem in Anammox

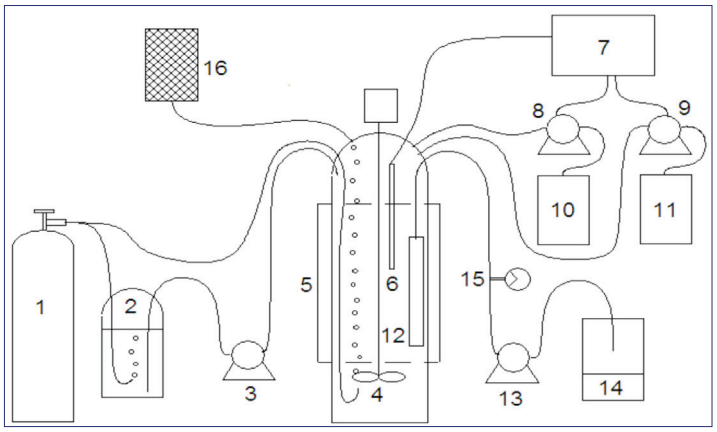

Figure 1. Scheme of Anammox SMBR: (1) Nitrogen Gas, (2) Synthetic Feed Vessel, (3) Feed Pump, (4) Mechanical Stirrer, (5) Thermal Jacket, (6) pH Probe, (7) pH Controller, (8) Acid Pump, (9) Caustic Pump, (10) Acid Solution, (11) Caustic Solution, (12) Membrane Module, (13) Permeate Pump, (14) Permeate Vessel, (15) Vacuum Pressure Gauge, (16) Gas Outlet Filter.

SMBRs without the use of anti-fouling mechanisms such as sparging and backwashing, especially in the start-up phase. Wang *et al.* observed a fouling rate of approximately 2 kPa/day at a constant flux of 0.5 L/m^2/h during the start-up phase of the Anammox reactor, which was marginally lower than normal fouling rates of standard anaerobic membrane bioreactors (Le-Clech *et al.*, 2006; Wang, 2009).

Researchers over the last two decades have investigated how the influent properties, biomass characteristics, operating conditions and membrane characteristics impact on the rate of fouling in aerobic and anaerobic SMBRs (Feng *et al.*, 2009; Meng *et al.*, 2008; Le-Clech *et al.*, 2006). Fouling can be categorised according to its ease of removal and long-term effect on the performance of a membrane. Removable fouling normally refers to the fouling substances and formations, like cake formation, that can be removed through physical cleaning; and irremovable fouling through chemical cleaning. Irreversible fouling refers to the fouling substances that cannot be removed by either (Feng *et al.*, 2009; Le-Clech *et al.*, 2006; Tian *et al.*, 2009).

Fouling of membranes by extracellular polymeric substances (EPS) and soluble microbial products (SMP) from microbial populations in bioreactors is one of the biggest problems facing SMBR technologies for wastewater treatment (Metzger *et al.*, 2007). Membrane characteristics such as pore size, porosity, roughness, surface charge and hydrophobicity also have a significant influence on the fouling rate of membranes in SMBRs. The types of material used have also been identified as an influencing factor on membrane fouling (Meng *et al.*, 2008). The three major types of material that are used in membrane bioreactors are ceramic, metal and polymer.

Polymeric membranes are used in the majority of SMBRs due to their low cost. They can, however, show less resistance to fouling compared to other types of membrane, like metallic and some ceramic membranes, due to the hydrophobic nature of the material (Feng *et al.*, 2009). Membranes can be manufactured from a variety of polymeric materials such as polypropylene (PP), polyvinylidene fluoride (PVDF), polyethylene (PE), polyethersulfone (PES), polyacrylonitrile (PAN), Polyester (PETE), polycarbonate (PCTE) and polytetrafluoroethylene (PTFE) (Meng *et al.*, 2009; Choi *et al.*, 2009). The three most commonly used polymeric membranes in SMBRs are PE, PP and PVDF (Choi *et al.*, 2009; Zhang *et al.*, 2008).

Previous studies suggest that PVDF membranes perform better than most other membranes in both aerobic and anaerobic SMBRs, including PE and PP membranes. It was speculated that PVDF membranes had better overall resistance against organic membrane fouling, lower levels of static absorption of EPS, exhibited better pore blocking resistance and displayed better cake layer removal ability (Yamato *et al.*, 2006; Feng *et al.*, 2009). However, current studies are looking at alternative membranes for SMBRs, with the novel PAN membranes showing signs of increased fouling resistance as compared to PVDF membranes (Tian *et al.*, 2009; Zhang *et al.*, 2008). Very little research has been conducted on selecting the best polymeric membrane for Anammox SMBRs.

Many different types of antifouling mechanism have been developed to reduce the fouling rate of polymeric and other membranes used in aerobic and anaerobic SMBRs. Some of these include sparging the membrane surfaces with air or other gases to prevent foulants from attaching, and backwashing the membranes with water or cleaning chemicals, such as sodium hypochlorite, to remove foulants from membrane pores and surfaces (Kornboonraksa and Lee, 2009; Aryal *et al.*, 2009). However, very little information is currently available on optimising antifouling techniques for membranes in Anammox bioreactors.

The purpose of this project was to investigate the influence of backwashing on membrane fouling rates in an Anammox SMBR, utilising both industry standard and novel membranes. The project tried to establish the lowest fouling rate of the two membranes by varying both the membrane backwashing amounts and frequencies. It also compared the membrane fouling rates of an industry standard membrane (PVDF) to a novel membrane (PTFE).

METHOD

SUBMERGED MEMBRANE BIOREACTOR

The experimental setup was designed to simulate a typical industrial SMBR wastewater plant. The experiment was performed in duplicate to reduce the risk of accidental loss of Anammox biomass and to verify the data obtained from the bioreactors. Two 7.0L Applikon glass bioreactor vessels with a working volume of 4.0L were utilised (Figure 1). They were covered with black sunblock cloth to minimise algal growth. The bioreactors were fed with synthetic wastewater media (2) via Applikon ADI 1035 peristaltic feed pumps (3).

Nitrogen gas (1) was supplied to the feed vessels to keep them under constant positive pressure and ensure an anaerobic environment. Applikon P100 mechanical stirrers (4) were operated at 100RPM to keep the bioreactor suspension homogeneous, and thermal jackets (5) were utilised to ensure a constant temperature of 32°C. Applikon 1030 pH monitoring and auto correction systems (6–11) were also incorporated to maintain pH levels between 8.0 and 8.5. Permeate was drawn through PVDF and PTFE membrane modules in each bioreactor (12) via a Watson Marlow 701S/R peristaltic pump (13) into a collection vessel (14). The trans-membrane pressures were monitored on Ambit positive pressure gauges (0 to

600kPa) and vacuum pressure gauges (-100 to 0 kPa) (15). All gases produced from the bioreactor exited via 0.45µm air filters (16).

MEMBRANE MODULES

PTFE and PVDF membrane modules were installed in both bioreactors. Memcor hydrophilic hollow fibre PVDF membranes with a surface area of 0.01m², were used. The PVDF membranes had an unknown pore size and an outer diameter (OD) of 1.1 mm. Hydrophobic hollow fibre PTFE membranes with a surface area of 0.01m², with an unknown pore size and an outer diameter (OD) of 1.5mm, were also used for this experiment. These membranes were still under development by the manufacturer and no further information about the membranes could be released at the time of the project.

MEMBRANE MODULES CLEANING

Chemical cleaning was applied to the membrane modules between all experiments. Cleaning was achieved by removing the membrane modules from the bioreactor and gently removing the biofilm layers from the membrane surface. Great care was taken not to touch the membranes by hand or damage them in any way.

Chemical cleaning was performed by backwashing the membrane modules with 10% v/v sodium hypochlorite. The cleaning was performed outside the bioreactor to ensure the microbial-population was not negatively affected by the sodium hypochlorite. The membranes were also backwashed several times with distilled water to ensure all chemicals were removed before reinstalment.

MEMBRANE FOULING RATE

Pressure gauge readings and flow rate measurements were collected at least twice a week. All readings and measurements were completed exactly 20 minutes after the completion of a backwash cycle. The membrane fouling rate was based on the increase in trans-membrane pressure over time. However, because the peristaltic pumps utilised in this project were not true positive displacement pumps, the flow rate decreased over time as the trans-membrane pressure increased. For this reason, the flux (flow rate per unit of membrane area) had to be used. Membrane fouling rate in this experiment was thus calculated as the increase of trans-membrane pressure (kPa) per flux (L/m²/h) per hour. Straight line standard curves were generated to indicate increases of membrane fouling and were used to calculate the membrane fouling rates. The gradients of the standard curves were equal to the membrane fouling rates.

Table 1. Composition of synthetic wastewater feeding media for Anammox SMBRs.

Major Salts		Trace Elements 1		Trace Elements 2	
Distilled Water	1L	Distilled Water	1L	Distilled Water	1L
$(NH_4)_2SO_4$	0.50 g/L	EDTA	5 g/L	EDTA	15 g/L
$NaNO_2$	0.50 g/L	$FeSO_4$	5 g/L	$ZnSO_4.7H_2O$	0.43 g/L
$KHCO_3$	1.25 g/L			$CoCl_2.6H_2O$	0.24 g/L
KH_2PO_4	0.025 g/L			$MnCl_2.4H_2O$	0.99 g/L
$MgSO_4.7H_2O$	0.30 g/L			$CuSO_4.5H_2O$	0.25 g/L
$CaCl_2.2H_2O$	0.20 g/L			$NaMoO_4.2H_2O$	0.22 g/L
Trace elements 1	1.25mL			$NiCl_2.6H_2O$	0.19 g/L
Trace elements 2	1.25mL			$NaSeO_4.10H_2O$	0.21 g/L
				H_3BO_4	0.014 g/L
				NaWO4.2H2O	0.050 g/L

SYNTHETIC WASTEWATER FEEDING MEDIA

A synthetic wastewater medium was used to feed the Anammox bioreactors (Table 1). The composition of the synthetic feed was used by Wang *et al.* (2009), which was based on the original synthetic media of Van de Graaf *et al.* (1995). The concentrations of ammonium sulphate and sodium nitrate in the synthetic feed media were slowly increased over the duration of the project as nitrite and ammonia consumption increased in the SMBRs.

SMBR INOCULATION

Anaerobic sludge from an industrial wastewater treatment digester was used to inoculate the bioreactors. Previous Anammox bacteria have been successfully isolated from wastewater treatment plants (Sànchez-Melsió *et al.*, 2009). The seed originated from anaerobic digesters with low levels of COD and high levels of NH_4. Small red clusters in the seed sludge were also observed under the microscope.

ANAMMOX OBSERVATIONS

The presence of Anammox bacteria in the bioreactors was observed in two ways: by physically observing the biomass in the SMBRs over time under a microscope and by monitoring the ratio of nitrite and ammonia removal from the bioreactors. Samples were observed under an Olympus BH2 light microscope and images were captured with a Canon A410 digital camera every two weeks. Observations of the amount, spread and size of red cell clusters were recorded. General observations of other micro-organisms were also recorded.

The Anammox process utilises roughly equal parts of ammonia and nitrite to drive the reaction towards nitrogen gas and water (Mulder *et al.*, 1995; Van de Graaf *et al.*, 1995). Therefore, equal amounts of ammonia and nitrite removal from the bioreactors would also indicate the presence of Anammox activity. Therefore, water analyses were performed weekly to monitor the consumption of nitrite and ammonia from the bioreactors. Ammonia, nitrite and nitrate analysis were performed on the influent and effluent of both systems.

WATER ANALYSIS

COD (Hach Method 2125925), ammonia (Hach Method 10031), nitrite (Hach Method 8153) and nitrate (Hach Method 8039) analyses were performed with a Hach DR 5000 spectrophotometer, using EPA approved methods. pH measurements were performed on a Hach Senslon 156 analyser and DO measurements on a Hach HQ40D analyser.

RESULTS

BIOREACTOR OPERATING CONDITIONS

An optimum Anammox growth environment was maintained throughout the project with a constant temperature of 32°C, pH between 8 and 8.5 and stirring speed of 100±1 RPM in both SMBRs. Biomass settled sludge volume (SSV) remained between 15% and 20% with hydraulic retention times between 3.0 and 3.5 days.

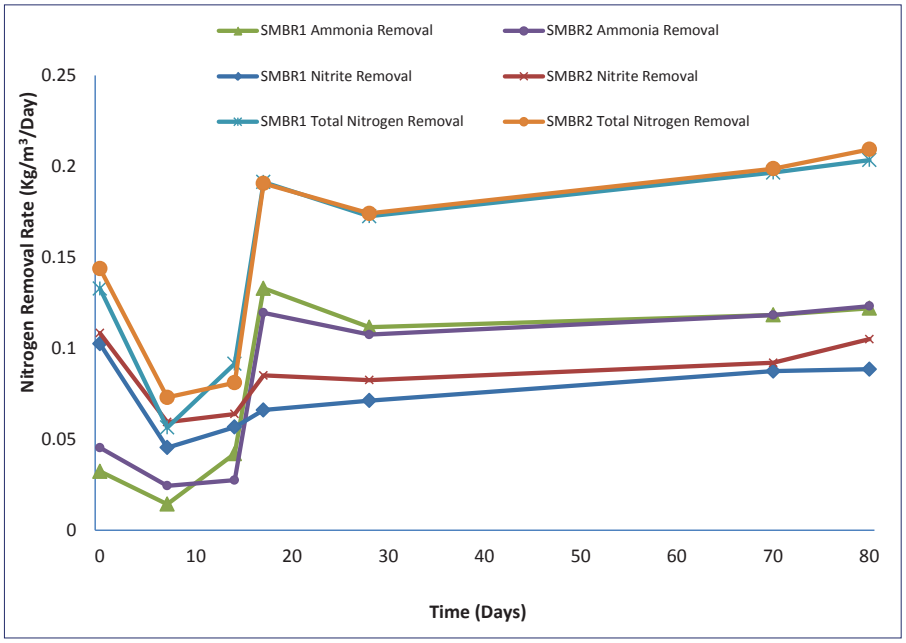

Figure 2. Nitrogen removal rates (kg/m³/day) of Anammox SMBRs.

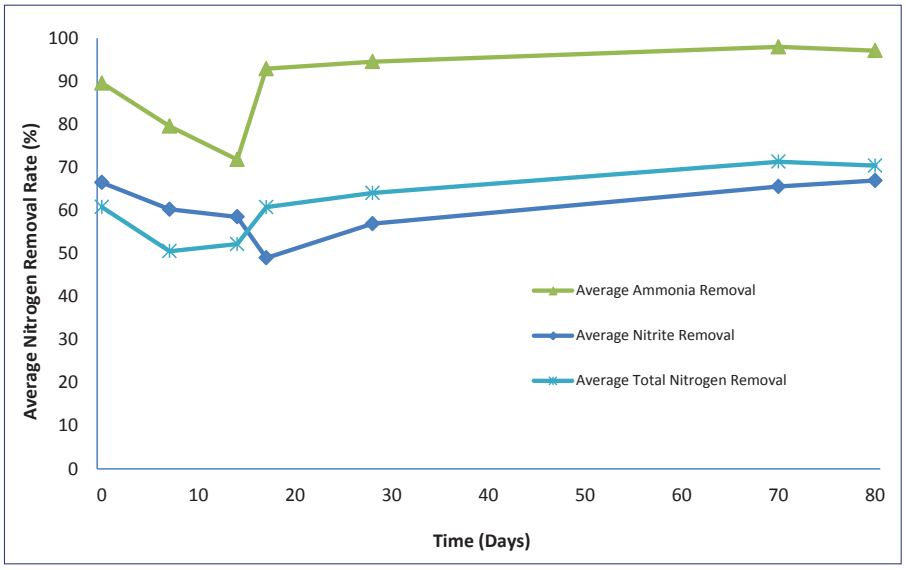

Figure 3. Average nitrogen removal efficiency (%) of Anammox SMBRs.

COD levels in both SMBRs remained between 60 and 140 mg/L for the duration of the project, with Influent COD levels between 40 and 80 mg/L. Effluent COD levels peaked at around 14 days, as anticipated, but then dropped again when 50% of the bioreactor solution in both bioreactors was replaced (Wang *et al.*, 2009).

Effluent ammonia levels remained below 20 mg/L for the duration of the project as the ammonia levels in the feed solution were increased from 20 to 110 mg/L. Effluent nitrite levels peaked around 17 days when nitrite levels were increased in the feed solution, but remained below 70 mg/L. Influent nitrite levels were increased from 80 to 120 mg/L during the project. The nitrate levels in the feed solution remained between 10 and 20 gm/L for the duration of the project and the nitrate levels in the effluent remained between 15 and 30 mg/L.

NITROGEN REMOVAL

Nitrite and ammonia removal rates decreased in the first week and then recovered over the subsequent weeks in both SMBRs (Figure 2). The nitrite removal rate increased fairly steadily over the following weeks, whereas the ammonia removal rate increased sharply after the second week and then stabilised. After 15 days the ammonia removal rate increased above the nitrite removal rate. Both the ammonia and nitrite removal rates remained relatively parallel between days 28 and 80. The gap between the ammonia and nitrite removal rates did, however, appear to reduce towards the end of the experiment, especially in SBMR2.

At the end of the experimental period the nitrite to ammonia removal rates were 0.73:1 in SMBR1 and 0.85:1 in SMBR2. An average ammonia removal efficiency of 89% was achieved with a maximum of 98% at 70 days (Figure 3). Similarly, an average nitrite removal efficiency of 61% was achieved with a maximum of 67% at the end of the experimental period.

Total nitrogen removal rates reduced after the first week and then increased sharply between weeks two and three. The total nitrogen removal rates then stabilised, with a steady increase for the remainder of the experimental period. An average total nitrogen removal efficiency of 61% was achieved with a maximum of 71% at 70 days.

BIOMASS OBSERVATIONS

The seed biomass consisted predominantly of dense dark brown and black microbial colonies and micro-granules. Single red cells and small colonies could also be observed, although they were few in number and dispersed throughout the biomass (Figure 4). After 11 days there were fewer dense dark-brown and black microbial colonies and micro-granules in the SMBR biomass. The red cells appeared more frequent and more clusters were observed.

Very few dark-brown microbial colonies could be detected after 25 days. No black micro-granules were visible any longer. A large number of light brown colonies could be seen and it appeared that they started to form large flocs. Red clusters could now also be seen in abundance. After 39 days a large number of hazel-brown clusters and flocs could be observed. There were virtually no dark-brown or black micro-organisms left in the biomass. Many large red colonies of micro-organisms could also be observed. They also appeared to be producing an abundant amount of an orange substance that looked like dense clouds around the red clusters. Smaller red cells were also observed to be budding off from the larger red cells in the colonies. The further biomass observations remained fairly unchanged for the duration of the project.

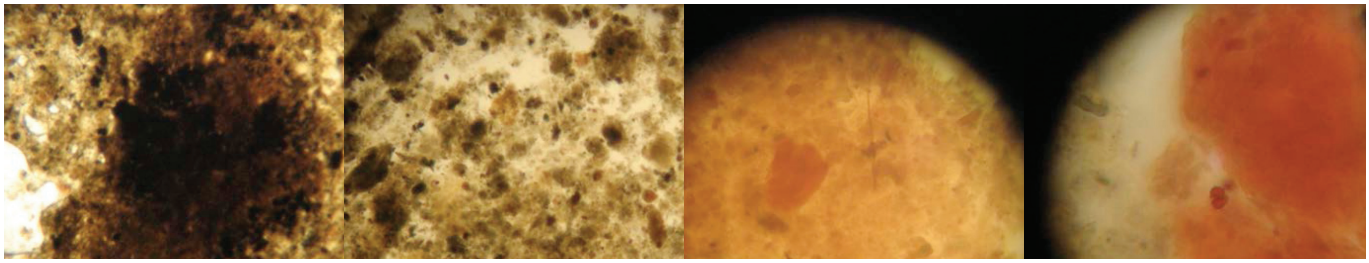

Figure 4. Optical images of biomass in Anammox SMBR (light microscope at 1000x). Photos were captured (from left to right): upon seeding; at 11 days; at 25 days; and at 39 days.

MEMBRANE FOULING

Very similar fouling rates were observed between the same types of membranes in both SMBRs throughout the project. The PVDF membranes displayed a 95% lower fouling rate than the PTFE membranes when no backwashing was applied (Figure 5). PVDF membranes produced a membrane fouling rate of 1.27kPa/ L/m^{2}h /day and PTFE membranes a fouling rate of 23.21kPa/ L/m^{2}h /day. Both membranes did, however, produce lower membrane fouling rates when backwashing was applied. PVDF membranes displayed an average fouling reduction rate of 72% and PTFE membranes a 73% reduction.

Significant differences in the fouling rates of PVDF membranes were produced by varying the backwash amounts and frequencies (Table 2): nine-minute backwashes every 90 minutes produced the lowest membrane fouling rate of 0.29kPa/ (L/m^{2}h)/day and the application of three-minute backwashes every 30 minutes produced the highest membrane fouling rate of 0.5kPa/ (L/m^{2}h) day, with a difference of 31%.

Variation of backwash amounts and frequencies had less of an impact on the PTFE membranes, but still resulted in a 19% difference of membrane fouling rates. Similarly to the PVDF results, the application of nine-minute backwashes every 90 minutes produced the lowest membrane fouling rate of 5.8kPa/(L/m^{2}h)/ day and three-minute backwashes every 30 minutes produced the highest membrane fouling rate of 7.17kPa/ (L/m^{2}h)/day.

DISCUSSION

BIOREACTOR OPERATING CONDITIONS

All operating conditions of both SMBRs remained fairly stable during the experimental period, which would have aided in the possible Anammox growth observed in this project. The pH levels remained between 8 and 8.5 as recommended by previous research for optimal Anammox growth (Tang *et al.*, 2009). This pH level was also maintained to minimise the risk of acidifying bacteria colonising the SMBRs in the first two weeks when higher COD levels were present. The lower stirrer speed of 100rpm, as suggested by Trigo *et al.* (2006), could also have contributed to Anammox bacteria forming clusters and larger granules observed under the microscope (Figure 4). The temperature levels close to the optimal of 35˚C would also have aided in Anammox growth (Cema *et al.*, 2004).

Stable biomass measurements observed throughout the project suggested that there were no drastic shifts in the micro-populations and that a favourable environment was also being created for microbial growth. It was also an indication that the potential population shift to an Anammox culture would have happened gradually. A slight decrease in SSV during the first two weeks could have been due to heterotrophic organisms dying off, as the feeding media contained no organic carbon energy. An increase in COD during the first two weeks would further support this and has been commonly observed by other researchers (Trigo *et al.*, 2006; Wang *et al.*, 2009).

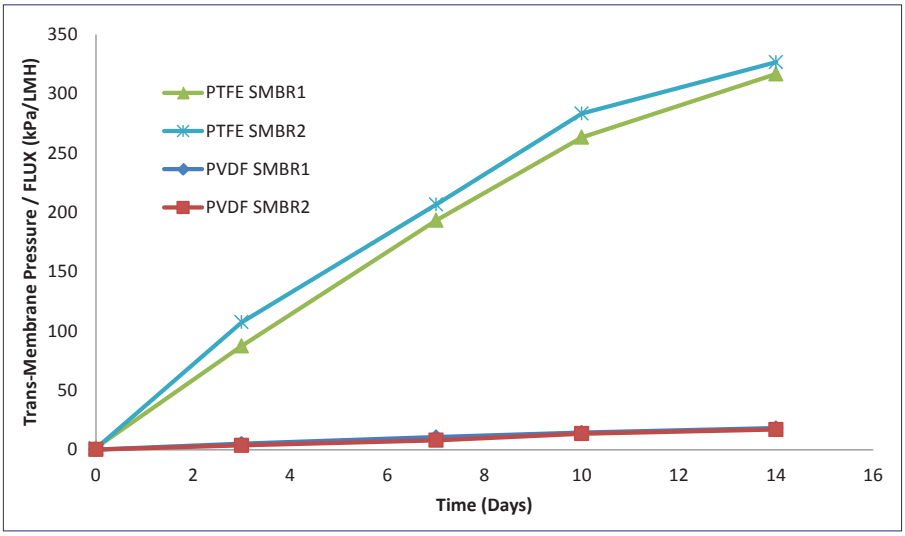

Figure 5. Average fouling rates of PVDF and PTFE membranes, expressed as an increase of Trans-Membrane Pressure (kPa) per FLUX (L/m^{2}h) per day in Anammox SMBRs.

Table 2. Average fouling rates of PVDF and PTFE membranes with and without backwashing, expressed as an increase of Trans-Membrane Pressure (kPa) per FLUX (L/m2h) per day.

Backwash Duration (Minutes)	Backwash Frequency (Minutes)	PVDF Membrane Fouling Rate kPa / (L/m^{2}h) / day	PTFE Membrane Fouling Rate kPa / (L/m^{2}h) / day
None	None	1.2673	23.207
3	30	0.4192	7.1665
6	60	0.3697	6.1736
9	90	0.2889	5.8117
12	120	0.3636	6.1583

NITROGEN REMOVAL

The total nitrogen removal rate increased steadily during the project and achieved a final removal rate of 0.209 kg /m³/day at the end of the project. It is fairly low compared to the first Anammox plant in the Netherlands with a total nitrogen removal rate of 2.6 kg/m³/day. The first plant did, however, operate for more than eight years and contained mature Anammox granules (Paques, 2008).

It can, however, be predicted that the total nitrogen removal rate would have continued to rise by observing the slope of the trend in Figure 2, especially if the ammonia and nitrite concentrations in the feed water would have been increased and more Anammox biomass would have developed. The ammonia removal efficiency of 97–98% seen towards the end of the project also suggests that the SMBRs operated at maximum efficiency (Figure 3).

PRESENCE OF ANAMMOX BACTERIA

Visual observations strongly indicated an increase of Anammox biomass in the SMBRs (Figure 4). It could not be concluded that the majority of red cells observed were Anammox bacteria, but the probability was very high taking into consideration the type of environment created in the SMBRs and the ratio of nitrite and ammonia removal rates. The orange biofilm appearance and the small red cells budding off the larger red cells also strongly pointed towards the possibility of Anammox bacteria. These particular characteristics were established very soon after the discovery of Anammox bacteria (Van de Graaf *et al.*, 1995).

The ratio of nitrite to ammonia removal during this project suggested a fairly good chance of Anammox activity. A nitrite to ammonia removal ratio of 0.73:1 in SMBR1 and 0.85:1 in SMBR2 was achieved towards the end of the project. This indicated that almost equal parts of nitrite and ammonia could have been converted to nitrogen gas and water as per the original Anammox findings (Mulder *et al.*, 1995; Van de Graaf *et al.*, 1995).

The higher rate of ammonia removal, compared to the nitrite removal, was most probably due to the presence of ammonia-oxidising bacteria (AOB) in the SMBRs. The AOBs could have utilised oxygen diffused into the bioreactors to oxidise ammonia, even though the experiment was set up to create an anaerobic environment. The presence of AOBs in Anammox bioreactors has been documented by other researchers (Tang *et al.*, 2009; Trigo *et al.*, 2006; Wang *et al.*, 2009). Healthy colonies of light brown bacteria were also observed under the microscope, which indicated that another group of bacteria was residing in the SMBRs (Figure 4).

A further characterisation of the microbial culture by a molecular mechanism, such as PCR or FISH analysis, would have given additional conformation of the presence of an Anammox culture in addition to the morphological characteristics and nitrogen utilisation pattern. While such techniques were not utilised in this work, they are currently being developed for future studies.

MEMBRANE PERFORMANCES

This project revealed that PVDF membranes performed better than PTFE membranes in Anammox SBMRs, with or without backwashing (Table 2). Hydrophobicity of the membrane surfaces could have played a significant role in the membrane fouling resistance, with PVDF being a hydrophilic membrane and PTFE being hydrophobic. Other studies in general anaerobic SMBRs have presented similar conclusions (Feng *et al.*, 2009; Meng *et al.*, 2008).

However, further studies on the composition and quantify of extracellular polymeric substances and soluble microbial products in Anammox SMBRs will need to be conducted to further support this theory. As expected, the project demonstrated that both the PVDF and PTFE membranes displayed a great increase in membrane fouling resistance when backwashed. This was consistent with other membrane fouling studies performed on both aerobic and anaerobic SMBRs (Meng *et al.*, 2009).

The project also showed that an increase in backwash duration resulted in better membrane performance for both the PVDF and PTFE membranes, until the intervals between the backwashes became too long. The link between the increased performance and the longer backwash period could have contributed to higher backwash pressures being achieved. The higher pressures inside the hollow fibre membranes could have resulted in a greater quantity of foulants being removed from both inside the membrane pores and attached to the membrane surfaces (Meng *et al.*, 2008). The drop in membrane performance when backwashed for 12 minutes every 120 minutes could have contributed to irreversible fouling. Previous studies have concluded that long periods between backwashes can cause more irreversible fouling by extracellular polymeric substances and soluble microbial products (Feng *et al.*, 2009).

Overall, both PVDF and PTFE membranes performed the best when backwashed for nine minutes every 90 minutes in an Anammox SMBR. The results suggested that this frequency and duration of backwashing was a good balance between achieving high backwash pressures and preventing excessive, irreversible fouling.

This project also suggested that PVDF membranes were more resistant to membrane fouling than the PP membrane used by Wang *et al.*, under similar conditions (2009). The PVDF membranes displayed 50% more membrane fouling resistance compared to the PP membranes when no backwashing was applied.

CONCLUSION

It was concluded that Anammox activity was achieved within 80 days in two start-up submerged membrane bioreactors, seeded with anaerobic sludge from an industrial wastewater treatment digester. This project also concluded that PVDF membranes showed significantly greater resistance to membrane fouling compared to PTFE membranes in Anammox SMBRs, with and without backwashing.

It was also demonstrated that the PVDF membranes were more resistant to membrane fouling in a start-up Anammox SMBR compared to PP membranes used in similar studies when no backwashing was applied. The results from this project also demonstrated that both the PVDF and PTFE membranes performed best when backwashed for nine minutes at 90-minute intervals, compared to other backwashing frequencies.

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