FORMATION AND EVALUATION OF THE FILTERABILITY OF AEROBIC GRANULES IN A GRANULAR SLUDGE MEMBRANE BIOREACTOR

BY

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THESIS

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Abstract

The technologies of aerobic granular sludge sequencing batch reactor (SBR) and membrane bioreactor (MBR) were combined in an attempt to develop an aerobic granular sludge membrane bioreactor (GMBR). The objectives were to determine the mechanisms controlling the formation of granules and granule stability in the GMBR as well as to evaluate the filterability of the granular sludge. The GMBR was operated in parallel with a SBR. In the SBR granulation was achieved after 16 days while no granulation was observed in the GMBR even after 10 weeks of operation. Filterability of the GMBR biomass, which was dominated by flocs, was compared with the filterability of granules cultivated in the SBR. The results showed no significant difference in filterability of granular sludge versus flocular sludge. Also, the clean water flux obtained after filtering flocular sludge was higher than after filtering granular sludge, suggesting that irreversible membrane fouling caused by adsorption of soluble extracellular polymeric substances (sEPS) was more important in the granules than in the flocs. According to the results of this study, granular sludge might not be able to alleviate the membrane fouling problem in MBRs. Furthermore, it could lead to irreversible membrane fouling in a major extent than that produced by conventional flocular sludge.

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Nomenclature

COD	Chemical oxygen demand
DO	Dissolved oxygen
F/M	Food-to-microorganism ratio
GMBR	Granular sludge membrane bioreactor
J_M	Measured flux (L/m ² .h)
J_S	Standardized flux (L/m ² .h)
MBR	Membrane bioreactor
MF	Microfiltration
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
ΔP_M	Measured transmembrane pressure (bar)
ΔP_S	Standard transmembrane pressure (bar)
PVDF	Polyvinylidene fluoride
SBR	Sequencing batch reactor
sEPS	Soluble extracellular polymeric substances
sPN	Soluble proteins
sPS	Soluble polysaccharides
SRT	Solids retention time
SVI ₅	Sludge volumetric index measured after 5 min of settling
SVI ₃₀	Sludge volumetric index measured after 30 min of settling
T_M	Measured temperature (°C)
T_S	Standard temperature (°C)

- TSS Total suspended solids
- UF Ultrafiltration
- VSS Volatile suspended solids

1. Introduction

Development of technically and financially viable wastewater treatment technologies to control surface water pollution is one of the most important environmental challenges of the present and future times.

Membrane bioreactor (MBR) is a wastewater treatment technology that combines the activated sludge process with membranes for the solid–liquid separation of the sludge suspension. MBR offers several advantages over the conventional activated sludge process including a higher biomass concentration, reduced footprint, low sludge production, and better effluent quality (Meng et al., 2008). Nevertheless, its practical application has been limited by its serious membrane fouling problems (Li et al., 2007; Meng et al., 2008; Thanh et al., 2008; Wang et al., 2008) that lead to a rapid decline of the permeation flux and a higher suction pressure requirement, which increases the required energy input. Fouling also leads to a higher frequency of mechanical and chemical cleaning, which can deteriorate the membrane and reduce its life. Overall, membrane fouling results in increased operating costs.

On the other hand, aerobic granular sludge grown in a sequencing batch reactor (SBR) is a wastewater treatment process that is recently becoming attractive due to its compactness, regularity, high biomass retention (Thanh et al., 2008), high metabolic activity, simultaneous organic and nutrient removal ability, excellent granule settleability (de Bruin et al., 2004; Wang et al., 2008; Thanh et al., 2008), and filterability (Wang et al., 2008). Nevertheless, some studies have shown that the aerobic granular sludge technology by itself is not able to meet the effluent standards due to high suspended solids in the effluent (de Bruin et al., 2004; Thanh et al., 2008). Most of the suspended solids can be eliminated by sedimentation or membrane filtration (Thanh et al., 2008). It would be attractive to combine the advantages of membrane separation and aerobic granular sludge resulting in a granular sludge membrane bioreactor (GMBR) (Li et al., 2005; Li et al., 2007; Tay et al., 2007; Wang et al., 2008).

This research aims to develop a GMBR by combining the technologies of MBR and aerobic granular sludge SBR, with the purpose of determining the mechanisms controlling the granule formation and stability in the GMBR as well as evaluating the filterability of the granular sludge. It is hypothesized that the formation of granular sludge can be induced inside a MBR by three mechanisms: first, by aerating with a high superficial air velocity (large shear forces); second, by selectively removing poor-settling biomass by withdrawing excess biomass not while the reactor is mixing but after a short settling period (selective pressure); and third, by pulse feeding the reactor (feast and famine regime).

2. Literature Review

2.1. Membrane Bioreactor

Membrane bioreactor (MBR) is a biological process for the treatment of municipal and industrial wastewaters. MBR combines the use of an activated sludge bioreactor with a membrane module for the solid–liquid separation of the sludge suspension. The bioreactor is an aerated tank containing microbial aggregates, or flocs, that oxidize the organic matter present in the influent wastewater. The flocs are separated from the treated wastewater by means of flat sheet or hollow fiber microfiltration (MF) or ultrafiltration (UF) membranes, and the effluent is discharged into the environment or sent to disinfection for later reuse.

The first MBRs used a cross-flow membrane filtration loop, in which the membrane module was placed outside the bioreactor (Meng et al., 2008). The use of this configuration led to increased energy costs and loss of biological activity due to the destruction of bioflocs as a consequence of high shear forces in the recirculation pipes and pumps (Meng et al., 2008). To overcome these limitations, the submerged MBRs, in which the membrane module is immersed in the bioreactor, were developed (Meng et al., 2008). In submerged MBRs, aeration not only provides oxygen to the biomass and keeps the solids in suspension, but also scours the membrane surface to mitigate membrane fouling (Meng et al., 2008).

The MBR technology offers several advantages over the conventional activated sludge process including a higher biomass concentration, reduced footprint, low sludge production, and better effluent quality (Meng et al., 2008). However, the major problem that limits the application of MBRs is the membrane fouling (Li et al., 2007; Meng et al., 2008; Thanh et al., 2008; Wang et al., 2008) that leads to a rapid decline of the permeation flux and a higher suction pressure requirement, which increases the required energy input. Fouling also leads to a higher frequency of mechanical and chemical cleaning, which can deteriorate the membrane and reduce its life. Overall, membrane fouling results in increased operating costs.

2.2. Aerobic Granular Sludge

Aerobic granular sludge is a biological wastewater treatment technology that is developed in intensely aerated sequencing batch reactors (SBR), where densely packed microbial aggregates with very good settling ability, called granules, consume the organic matter present in the wastewater. At the end of each cycle, a short settling time is provided to separate the granules from the treated wastewater (supernatant), which is removed through the middle port of the reactor and sent to further treatment before being discharged into the environment.

Currently, the precise mechanisms that lead to the granule formation are not well known, but the researches conducted on SBRs have shown that granules can be formed in these environments due to (a) the large shear forces caused by the intensive non-mechanical mixing in the reactor, (b) the short settling time that results in the selection of well-settling biomass (selective pressure), and (c) the feast and famine regime due to the pulse feed of the reactor (Beun et al., 2002; de Bruin et al., 2004; de Kreuk et al., 2005; Tay et al., 2007; Thanh et al., 2008).

The application of the aerobic granular sludge technology is recently becoming attractive due to its compactness, regularity, high biomass retention (Thanh et al., 2008), high metabolic activity, simultaneous organic and nutrient removal ability, excellent granule settleability (de Bruin et al., 2004; Wang et al., 2008; Thanh et al., 2008), and filterability (Wang et al., 2008). Nevertheless, some studies have shown that the aerobic granular sludge technology by itself is not able to meet the effluent standards due to high suspended solids in the effluent (de Bruin et al., 2004).

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al., 2004; Thanh et al., 2008). Most of the suspended solids can be eliminated by sedimentation or membrane filtration (Thanh et al., 2008).

2.3. Granular Sludge Membrane Bioreactor and Granule Filterability

In an effort to complement the benefits of the MBR and the aerobic granular sludge and to mitigate their limitations, recently some researchers have tried to combine these two technologies to create novel granular sludge membrane bioreactors (GMBR) (Li et al., 2005; Li et al., 2007; Tay et al., 2007; Wang et al., 2008).

The approach used by Li et al. (2005), Li et al. (2007), and Wang et al. (2008) to combine the MBR and the aerobic granular sludge technologies was the inoculation of granular sludge previously cultivated in a SBR, into a MF MBR, and the operation of this reactor as a conventional MBR. Using this approach, Wang et al. (2008) observed disaggregation of a significant amount of inoculated granular sludge with particle size larger than 0.9 mm in the GMBR at the beginning of the operation, which they associated with the change of operation mode from SBR to MBR system. Li et al. (2007) also observed a reduction in the average diameter of the granules inside the GMBR during a long time operation (55 days), beginning at 3.0 mm, going down to 2.3 mm at 28 days, and finally approaching to constant at 2.0 mm. Li et al. (2005) examined the membrane permeability of the GMBR and they observed that it was 50% higher than that of a conventional MBR. They also observed that the main membrane foulants in the mixed liquor of the MBR were suspended solids which deposited on the surface of the membrane forming a cake layer. In the case of the GMBR, the main foulants were colloids and solutes adsorbed onto the surface or within the pores of the membrane.

The approach used by Tay et al. (2007) to combine these two technologies was the introduction of a MF membrane module in a SBR and the operation of this reactor as a

conventional SBR, but discharging the effluent in two fractions: 3/8 or the reactor working volume was removed through membrane filtration during part of the aeration period and 1/8 of the reactor working volume was removed through the middle port of the reactor after the settling period. Through this approximation, they developed a GMBR with stable granules with a mean particle size of 0.7 mm. They compared the filtration characteristics of the mixed liquor of the GMBR with that of a traditional MBR. GMBR mixed liquor showed much better filterability based on continuous reactor operation and batch dead-end UF tests. The main membrane foulants in the mixed liquor of the GMBR were colloids and dissolved molecules, whereas in the MBR were suspended solids. Nevertheless, contribution to membrane fouling by colloids and dissolved molecules was similar in the GMBR and the MBR. Therefore, the much better filtration characteristics of the GMBR mixed liquor was due to the low compressibility of its biomass, which was dominated by aerobic granular sludge.

Thanh et al. (2008) evaluated the membrane fouling potential of the supernatant of an aerobic granular sludge reactor. The results suggested that irreversible adsorption of soluble extracellular polymeric substances (sEPS), which were dominated by soluble polysaccharides (sPS), was the main cause of membrane fouling. According to Thanh et al. (2008), the major soluble foulants are classified as sEPS, which are mainly comprised of soluble polysaccharides (sPS) and soluble protein (sPN); while total sEPS (sPS and sPN) can influence membrane fouling, sPS has been found to be a major membrane foulant. Furthermore, Thanh et al. (2008) reported that the composition of sEPS in the supernatant of the granulation reactor was different compared to a conventional MBR. As indicated by them, researches conducted on conventional MBRs have shown that sPN is usually dominant or equivalent with sPS in the mixed liquor of these MBRs.

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Formation and Evaluation of the Filterability of Aerobic Granules in a Granular Sludge Membrane Bioreactor¹

Abstract

The technologies of aerobic granular sludge sequencing batch reactor (SBR) and membrane bioreactor (MBR) were combined in an attempt to develop an aerobic granular sludge membrane bioreactor (GMBR). The objectives were to determine the mechanisms controlling the formation of granules and granule stability in the GMBR as well as to evaluate the filterability of the granular sludge. The GMBR was operated in parallel with a SBR. In the SBR granulation was achieved after 16 days while no granulation was observed in the GMBR even after 10 weeks of operation. Filterability of the GMBR biomass, which was dominated by flocs, was compared with the filterability of granules cultivated in the SBR. The results showed no significant difference in filterability of granular sludge versus flocular sludge. Also, the clean water flux obtained after filtering flocular sludge was higher than after filtering granular sludge, suggesting that irreversible membrane fouling caused by adsorption of soluble extracellular polymeric substances (sEPS) was more important in the granules than in the flocs. According to the results of this study, granular sludge might not be able to alleviate the membrane fouling problem in MBRs. Furthermore, it could lead to irreversible membrane fouling in a major extent than that produced by conventional flocular sludge.

Key Words

Granular sludge; Flocular sludge; Membrane bioreactor; Sequencing batch reactor; Membrane fouling; Filterability.

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3.1. Introduction

Membrane bioreactor (MBR) is a wastewater treatment technology that combines the activated sludge process with membranes for the solid–liquid separation of the sludge suspension. MBR offers several advantages over the conventional activated sludge process including a higher biomass concentration, reduced footprint, low sludge production, and better effluent quality (Meng et al., 2008). Nevertheless, its practical application has been limited by its serious membrane fouling problems (Li et al., 2007; Meng et al., 2008; Thanh et al., 2008; Wang et al., 2008) that lead to a rapid decline of the permeation flux and a higher suction pressure requirement, which increases the required energy input. Fouling also leads to a higher frequency of mechanical and chemical cleaning, which can deteriorate the membrane and reduce its life. Overall, membrane fouling results in increased operating costs.

On the other hand, aerobic granular sludge grown in a sequencing batch reactor (SBR) is a wastewater treatment process that is recently becoming attractive due to its compactness, regularity, high biomass retention (Thanh et al., 2008), high metabolic activity, simultaneous organic and nutrient removal ability, excellent granule settleability (de Bruin et al., 2004; Wang et al., 2008; Thanh et al., 2008), and filterability (Wang et al., 2008). Nevertheless, some studies have shown that the aerobic granular sludge technology by itself is not able to meet the effluent standards due to high suspended solids in the effluent (de Bruin et al., 2004; Thanh et al., 2008). Most of the suspended solids can be eliminated by sedimentation or membrane filtration (Thanh et al., 2008). It would be attractive to combine the advantages of membrane separation and aerobic granular sludge resulting in a granular sludge membrane bioreactor (GMBR) (Li et al., 2005; Li et al., 2007; Tay et al., 2007; Wang et al., 2008). Li et al. (2005), Li et al. (2007), and Wang et al. (2008) tried to combine these two technologies by inoculating granular sludge previously cultivated in a SBR, into a microfiltration (MF) MBR, and operating this reactor as a conventional MBR. Using this approach, Wang et al. (2008) observed disaggregation of a significant amount of inoculated granular sludge with particle size larger than 0.9 mm in the GMBR at the beginning of the operation, which they associated with the change of operation mode from SBR to MBR system. Li et al. (2007) also observed a reduction in the average diameter of the granules inside the GMBR during a long time operation (55 days), beginning at 3.0 mm, going down to 2.3 mm at 28 days, and finally approaching to constant at 2.0 mm. Li et al. (2005) examined the membrane permeability of the GMBR and they observed that it was 50% higher than that of a conventional MBR. They also observed that the main membrane foulants in the mixed liquor of the MBR were suspended solids which deposited on the surface of the membrane forming a cake layer. In the case of the GMBR, the main foulants were colloids and solutes adsorbed onto the surface or within the pores of the membrane.

Tay et al. (2007) combined the aerobic granular sludge and the MBR technologies by introducing a MF membrane module in a SBR and operating this reactor as a conventional SBR, but discharging the effluent in two fractions: 3/8 of the reactor working volume was removed through membrane filtration during part of the aeration period and 1/8 of the reactor working volume was removed through the middle port of the reactor after the settling period. Through this approximation, they developed a GMBR with stable granules with a mean particle size of 0.7 mm. They compared the filtration characteristics of the mixed liquor of the GMBR with that of a traditional MBR. GMBR mixed liquor showed much better filterability based on continuous reactor operation and batch dead-end ultrafiltration (UF) tests. The main membrane foulants in

the mixed liquor of the GMBR were colloids and dissolved molecules, whereas in the MBR were suspended solids. Nevertheless, contribution to membrane fouling by colloids and dissolved molecules was similar in the GMBR and the MBR. Therefore, the much better filtration characteristics of the GMBR mixed liquor was due to the low compressibility of its biomass, which was dominated by aerobic granular sludge.

Thanh et al. (2008) evaluated the membrane fouling potential of the supernatant of an aerobic granular sludge reactor. The results suggested that irreversible adsorption of soluble extracellular polymeric substances (sEPS), which were dominated by soluble polysaccharides (sPS), was the main cause of membrane fouling. According to Thanh et al. (2008), the major soluble foulants are classified as sEPS, which are mainly comprised of soluble polysaccharides (sPS) and soluble proteins (sPN); while total sEPS (sPS and sPN) can influence membrane fouling, sPS has been found to be a major membrane foulant. Furthermore, Thanh et al. (2008) reported that the composition of sEPS in the supernatant of the granulation reactor was different compared to a conventional MBR. As indicated by them, researches conducted on conventional MBRs have shown that sPN is usually dominant or equivalent with sPS in the mixed liquor of these MBRs.

From the study performed by Tay et al. (2007), it seems that a GMBR could be successfully developed by introducing a membrane module in a SBR and operating this reactor as a conventional SBR, but discharging the effluent in two parts: one through membrane filtration and the other one through settling and supernatant removal, and that with this approach membrane fouling in MBRs might be lessened. Nevertheless, the findings of Thanh et al. (2008) pose the concern that granular sludge could lead to irreversible membrane fouling in a major extent than that produced by the flocular sludge found in conventional MBRs. It would be worthy to verify whether a GMBR can be developed by using a similar approach as Tay et al. (2007) and evaluate the actual filtration characteristics of the granular sludge, to see whether this type of biomass can effectively help to solve the membrane fouling problem in MBRs or not.

Currently, the precise mechanisms that lead to the granule formation are not well known, but the researches conducted on SBRs have shown that granules can be formed in these environments due to (a) the large shear forces caused by the intensive non-mechanical mixing in the reactor, (b) the short settling time that results in the selection of well-settling biomass (selective pressure), and (c) the feast and famine regime due to the pulse feed of the reactor (Beun et al., 2002; de Bruin et al., 2004; de Kreuk et al., 2005; Tay et al., 2007; Thanh et al., 2008).

This research aims to develop a GMBR by combining the technologies of MBR and aerobic granular sludge SBR, with the purpose of determining the mechanisms controlling the granule formation and stability in the GMBR as well as evaluating the filterability of the granular sludge. It is hypothesized that the formation of granular sludge can be induced inside a MBR by three mechanisms: first, by aerating with a high superficial air velocity (large shear forces); second, by selectively removing poor-settling biomass by withdrawing excess biomass not while the reactor is mixing but after a short settling period (selective pressure); and third, by pulse feeding the reactor (feast and famine regime).

3.2. Materials and Methods

3.2.1. Experimental setup and operation conditions.

A MBR with 20 L of working volume was used for the experiments and was operated as a GMBR (Figure 1a). The reactor used submerged flat sheet MF membranes with a pore size of $0.2 \ \mu m$ and a total membrane area of $0.48 \ m^2$. It was operated with 3-h cycles as shown in Figure

2a, where the 30-s settling retained all particles with settling velocity larger than 12 m/h. During the last 20 min of the aeration period, 1 L of water was removed through membrane filtration at a permeate flux of 7.9 L/m².h and 10-min relaxation cycles where the effluent pump was on for 8 min and off for 2 min., and during the 5 min of supernatant removal, 4 L were withdrawn. Coarse air bubbles were supplied for aeration through an air diffuser at the bottom of the reactor. The air was supplied at a flow rate of 19.6 L/min, which provided a superficial air velocity of 80 m/h.

A column shaped SBR with 3 L of working volume (Figure 1b) was operated in parallel with the GMBR, with a 3-h cycle as shown in Figure 2b, where the settling period was chosen to ensure retention of particles with a settling velocity larger than 12 m/h. Coarse air bubbles were supplied through aeration stones located at the bottom of the reactor. The air was supplied at a superficial air velocity of 80 m/h (6.2 L/min).

3.2.2. Influent composition.

A synthetic wastewater with sodium acetate as carbon source and a volumetric organic load of 1.6 kg COD/m³.d was used as influent for both reactors. The components of the synthetic wastewater for the GMBR were as follows: 1700 mg/L CH₃COONa·3H₂O (800 mg/L COD), 152.86 mg/L NH₄Cl (40 mg/L N), 7.90 mg/L MgSO₄·7H₂O, 29.80 mg/L KH₂PO₄, 4.40x10⁻³ mg/L MnCl₂·4H₂O, 8.82x10⁻³ mg/L CoSO₄·7H₂O, 1.47x10⁻³ mg/L CuCl₂·2H₂O, 3.52x10⁻³ mg/L NiSO₄·6H₂O, 5.28x10⁻³ mg/L Na₂MoO₄·2H₂O, 7.34x10⁻³ mg/L ZnSO₄·7H₂O, 2.94x10⁻³ mg/L H₃BO₃, 8.96 mg/L CaCl₂·2H₂O, 0.266 mg/L FeCl₃. The influent to the SBR was diluted 50% to achieve the same volumetric organic loading as in the GMBR while the SBR was operated with half the hydraulic retention time of the GMBR. To obtain this synthetic wastewater, a sterile, concentrated nutrient feed containing the above components was diluted with dechlorinated tap water to achieve the desired concentrations. The tap water was previously dechlorinated using a granular activated carbon column.

3.2.3. Seed sludge.

Seed sludge was obtained from a phosphorus removal activated sludge wastewater treatment plant (Urbana & Champaign Sanitary District – Southwest Plant, Champaign, IL) and was used to start the GMBR and the SBR. The concentration of inoculated sludge was 3000 mg/L.

3.2.4. Reactor performance monitoring and analytical methods.

Total suspended solids (TSS) and volatile suspended solids (VSS) were measured in the mixed liquor and the supernatant of both reactors once a week according to the Standard Methods for Examination of Water and Wastewater (APHA, 1998).

Chemical oxygen demand (COD), ammonia (NH_4^+) , nitrite (NO_2^-) , and nitrate (NO_3^-) were measured in the influent and the effluent of both reactors once a week following the Standard Methods (APHA, 1998), and all these samples were filtered using a 0.45 µm cellulose acetate filter.

Dissolved oxygen (DO), pH, and temperature of the mixed liquor of both reactors were measured on-line (WTW Multi 340i, Weilheim, Germany) and recorded using the software LabView (National Instruments, Austin, TX). Suction pressure in the GMBR was measured online in the permeate line (Alicat Scientific, Tucson, AZ) and recorded using LabView as well.

During the earlier development stages, the evolution of the biomass in both reactors was followed using a Zeiss Axioskop optical microscope (Carl Zeiss, Oberkochen, Germany), with a

10× or 5× magnification Plan-Neofluar objective (Carl Zeiss, Oberkochen, Germany). Bright field images were captured every week using an Axiocam MRm camera supported by the software AxioVision (Carl Zeiss, Oberkochen, Germany). For later development stages and larger biomass sizes, a Zeiss Stemi 2000-C stereo light microscope (Carl Zeiss, Oberkochen, Germany) coupled with a Nikon Coolpix 4500 digital camera (Nikon, Tokyo, Japan) was used.

The sludge volumetric index of the mixed liquor of both reactors was evaluated once a week after 30-min sedimentation (SVI_{30}) according to the Standard Methods (APHA, 1998) and after 5-min sedimentation (SVI_5).

Filterability of the mixed liquor of both reactors was tested once a week in batch deadend filtration flux decline experiments using an Amicon stirred cell model 8200 (Millipore, Billerica, MA) mixed at 175 rpm and pressurized to 1.03 bar (15 psi) using nitrogen. Polyvinylidene fluoride (PVDF) UF membranes (30 kDa) provided by GE Osmonics (Minnetonka, MN) were used. The pressure loss across the membrane was 6.90×10⁻² bar (1 psi). The initial clean water flux of the membrane was characterized for approximately 20 min (or until stable flux was obtained) before measuring the sample flux. A new piece of membrane was used for each experiment. The sample flux was measured for approximately 40 min (or until stable flux was obtained). After measuring the sample flux, final clean water flux was characterized for 20 min approximately (or until stable flux was obtained). Clean water flux measurements were performed using NANOpure ultrapure water (Barnstead, Dubuque, IA).

Flux was measured by weighing permeate at fixed time intervals on a top loading balance (Model PB3002-S, Mettler Toledo, Columbus, OH) and the data was collected automatically using the software WinWedge (TAL Technologies, Philadelphia, PA). Feed pressure was monitored with a digital pressure gauge and was recorded manually. Sample temperature was measured at the end of each test. Flux was calculated assuming a permeate density of 1 g/mL and the value measured at actual feed pressure and temperature conditions was standardized to 1.03 bar (15 psi) and 25°C, using the following equation, from Howe et al. (2006):

$$J_{s} = J_{M} \left(\frac{\Delta P_{s}}{\Delta P_{M}} \right) \times 1.024^{(T_{s} - T_{M})}$$
(1)

in which *J* is the flux (L/m².h), ΔP is the transmembrane pressure (feed pressure minus pressure loss across the membrane, bar), *T* is the temperature (°C), and the subscripts *M* and *S* refer to measured and standard conditions, respectively.

The filterability experiments were performed using two types of samples:

- Sample 1: Mixed liquor with total suspended solid concentration of 1000 mg/L. To achieve the desired concentration, dilution was performed using permeate from the GMBR.
- Sample 2: Mixed liquor soluble fraction, obtained by filtering the mixed liquor samples using a Whatman 934-AH glass fiber filter with a nominal pore size of 1.5 µm.

3.3. Results

3.3.1. Biomass characteristics and general reactor performance.

The GMBR and the SBR were started-up with an initial settling time of 6 min to prevent biomass washout. This settling time was gradually reduced in the SBR during the first 4 weeks of operation until the target settling time of 1 min and 40 s was reached. In the GMBR, the settling time was gradually reduced down to 2 min during the first 3 weeks of operation, but then severe biomass washout was observed and it had to be successively increased. At the end of the experiment the settling time was 8 min in this reactor.

A sequence of the evolution of the biomass in both reactors is presented in Figure 3. As it can be seen, the first granules became visible in the SBR at day 16 (Figure 3f). In the GMBR, even after 10 weeks of operation, granulation was not achieved. The mixed liquor remained

dominated by flocular sludge and experienced a bloom of filamentous bacteria at the end of the operation period (Figure 3d). Disaggregation of granules was observed in the SBR following day 44 due to predation by mites from the species *Tyrophagus sp.* (Figures 3g and 3h). On day 55 the SBR was operated under anaerobic conditions for 24 h in an effort to eliminate the predators, but the strategy did not work. After 10 weeks of operation, complete disaggregation of the granules was observed (Figure 3h).

Since the biomass was present as granules in the SBR only for 5 weeks, the experiment was repeated, and both reactors were cleaned and started over with seed sludge from the same wastewater treatment plant and under the same operation conditions. As in the first experiment, the settling time was gradually reduced in the SBR from 6 min to 1 min and 40 s during the first 4 weeks of operation. In the GMBR, due to the poor settling ability of its sludge, the settling time of 6 min could not be reduced and had to be successively increased to prevent biomass washout. At the end of the experiment, the settling time was 22 min in this reactor.

The development of the floc structure in the second experiment is shown in Figure 4. Again, the SBR experienced an infestation with mites that initially prevented the formation of granules. Reactor operation was adjusted aiming at removing these mites from the system by increasing the air flow rate from 6.2 L/min (superficial air velocity of 80 m/h) to 7.4 L/min (superficial air velocity of 95 m/h) (after day 27), and decreasing the settling time from 1 min and 40 s (retention of particles with settling velocity larger than 12 m/h) to 1 min and 20 s (retention of particles with settling velocity larger than 15 m/h) (after day 61). The abundance of mites was reduced in response to these changes of operation, but mites were never completely eliminated from the SBR. Following a reduction of mites, the first granules became visible on day 33 (Figure 4f). The granules continued growing until day 61 (Figure 4g) when they stabilized until the end of the experiment (Figure 4h). In the GMBR, as in the first experiment, the biomass was dominated by flocular sludge and overgrowth of filamentous bacteria was observed at the end of the experiment (Figures 4c and 4d).

Very similar performance was observed in the GMBR and the SBR throughout both experiments. In general, all soluble substrate was consumed within the first hour of the cycle. Also, the treatment efficiency (COD and N removal), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), solids retention time (SRT), food-tomicroorganism ratio (F/M), DO, pH, and temperature, were very similar in both reactors. The main difference was observed in the effluent TSS and VSS, which were higher for the GMBR. The average values of these parameters are presented in Table 1.

3.3.2. Biomass settleability.

The SVI₅ and SVI₃₀ of the GMBR and the SBR mixed liquors throughout experiments 1 and 2 are presented in Figure 5. During the first experiment, the biomass was present as granules in the SBR from day 16 to day 44, with SVI₅ values that varied from 16 to 86 mL/g and very similar values of SVI₃₀ that went from 16 to 83 mL/g, corresponding to a highly compact and fast-settling sludge. For the same period, the GMBR mixed liquor was dominated by flocs with SVI₅ ranging from 128 to 259 mL/g and SVI₃₀ that was in the range of 84 to 175 mL/g, corresponding to a typical good-settling sludge compared to conventional activated sludge, but not to granular sludge. During the second experiment, the biomass was present as granules in the SBR from day 33 to day 77, with SVI₅ varying from 40 to 366 mL/g and identical SVI₃₀ values. This can be explained by the biomass settling very fast but occupying a considerable volume due to the large size that the granules obtained as a consequence of the strong selective pressure applied to the reactor to keep the predators out of it. During the same period, the GMBR mixed liquor was dominated by poor-settling flocular sludge with values of SVI_5 and SVI_{30} ranging from 344 to 1537 mL/g and 157 to 886 mL/g, respectively, as a consequence of the overgrowth of filamentous bacteria experienced in this reactor at the end of the operation period.

3.3.3. Biomass filterability.

The mixed liquor flux measured after filtering 25 L/m² and the final clean water flux determined during experiment 1 are presented in Figure 6a. For the period in which the biomass was present as granules in the SBR (days 16 to 44), there was no significant difference in the filterability of the SBR and the GMBR mixed liquors. The average GMBR mixed liquor flux was $35\% (\pm 9\%)$ of the initial clean water flux. Whereas for the SBR mixed liquor, the average flux was $31\% (\pm 9\%)$ of the initial clean water flux. Nevertheless, the final clean water flux was higher after filtering GMBR mixed liquor (average flux: $86\% (\pm 16\%)$ of initial clean water flux) than after filtering SBR mixed liquor (average flux: $67\% (\pm 14\%)$ of initial clean water flux), suggesting that irreversible adsorption of sEPS to the membrane was more important in the granular sludge than in the flocular sludge.

The mixed liquor flux measured after filtering 25 L/m² and the final clean water flux obtained during experiment 2 are shown in Figure 6b. As in the first experiment, during the time that the biomass was present as granules in the SBR (days 33 to 77), no significant difference in the filterability of the SBR and the GMBR mixed liquors was observed. The average GMBR mixed liquor flux was 30% (\pm 6%) of the initial clean water flux. While for the SBR mixed liquor, the average flux was 33% (\pm 3%) of the initial clean water flux. Also, the final clean water flux was a higher after filtering GMBR mixed liquor (average flux: 56% (\pm 4%) of initial clean water flux) than after filtering SBR mixed liquor (average flux: 47% (\pm 7%) of initial clean water flux), corroborating what was found in the first experiment.

In addition to the flux decline due to mixed liquor, in the second experiment the flux decline due to the soluble fraction was monitored (Figure 7). During the time that the biomass was present as granules in the SBR (days 33 to 77), no significant difference in the filterability of the soluble fraction of the SBR and the GMBR mixed liquors was observed. The average GMBR soluble fraction flux measured after filtering 25 L/m² was 40% (\pm 4%) of the initial clean water flux. Whereas for the SBR soluble fraction, the average flux was 42% (\pm 5%) of the initial clean water flux. However, the final clean water flux was higher after filtering GMBR soluble fraction (average flux: 66% (\pm 4%) of initial clean water flux) than after filtering SBR soluble fraction (average flux: 57% (\pm 3%) of initial clean water flux). These results suggest that although the contribution of the soluble fraction to the overall membrane fouling was similar in the GMBR mixed liquor, dominated by flocular sludge, and the SBR mixed liquor, dominated by granular sludge soluble fraction was more irreversible than the fouling caused by the flocular sludge soluble fraction.

3.4. Discussion

3.4.1. Granule formation and stability in the GMBR.

The formation of granules could not be induced inside the GMBR even though this reactor was operated under very similar conditions than the SBR, with large shear forces provided by aeration at the same superficial air velocity (80 m/h); strong selective pressure induced by the removal of the poor-settling biomass after a short settling time that was selected to keep inside the reactors only particles with settling velocity larger than 12 m/h; and feast and famine regime generated by the pulse fed of the reactor with the same volumetric organic load (1.6 kg COD/m³.d) and the same feast period duration (soluble substrate depletion within the first hour of the cycle). Furthermore, these operation conditions led to an overgrowth of

filamentous bacteria in the GMBR and the corresponding reduction in the settleability of the biomass.

The approach used in this study to form granules in the GMBR was very similar to the one used by Tay et al. (2007), who could develop a GMBR with stable granules with an average diameter of 0.7 mm. The main difference was that Tay et al. (2007) started the reactor without the membrane module inside, discharging 50% of the working volume through the middle port of the reactor after the settling period, and once they obtained granules, they introduced the membrane module and removed only 12.5% of the reactor volume through this mechanism. In this study, the membrane module was always present and 20% of the reactor volume was removed through settling and supernatant withdrawal during the entire experiment. In other words, the selective pressure induced in this study was weaker than the one induced by Tay et al. (2007) at the beginning of the operation, but stronger at the end.

The reasons why granulation was not achieved in the GMBR are not clear.

3.4.2. Filterability of granular sludge versus flocular sludge.

The results of the batch dead-end UF experiments revealed no significant difference in the filterability of granular sludge versus flocular sludge, even though the huge dissimilarities observed in size, structure, and settleability of these two types of biomass. Furthermore, no substantial difference in the contribution of the soluble fraction of granular sludge and flocular sludge to the overall membrane fouling was observed either. This evidences the important role that solutes and colloids play in the membrane fouling process, and that the contribution of these two fractions may be as large for granular sludge as for flocular sludge.

On the contrary, Tay et al. (2007) observed that granular sludge had much better filtration characteristics than flocular sludge based on batch dead-end UF tests. However, the operation

conditions of the granular and flocular reactors used in that study were very different from the ones used in this study (lower food-to-microorganism ratio (F/M = 0.59 kg COD/kg MLSS.d), lower superficial air velocity (72 m/h), settling time that kept particles with a lower settling velocity in the granular reactor (8.9 m/h approximately)) and might have influenced the composition of the suspended, colloidal, and soluble fractions of both granular and flocular sludge, as well as the extent of the membrane fouling caused by these fractions.

In terms of the irreversibility of the membrane fouling, the results or this study showed that the fouling produced by the filtration of granular sludge was less reversible than the one produced by the filtration of flocular sludge, suggesting that the sEPS produced by the granular sludge adsorbed more strongly to the membrane than those produced by the flocular sludge. This is consistent with what Thanh et al. (2008) reported. According to Thanh et al. (2008), while total sEPS, which consist of sPS and sPN, can influence membrane fouling, sPS has been found to be a major membrane foulant. They observed that the composition of sEPS in the supernatant of an aerobic granular sludge reactor was dominated by sPS, and that this composition was different compared to that of a conventional MBR mixed liquor, where sPN is usually dominant or equivalent with sPS. Moreover, they found that the main cause of membrane fouling of the supernatant of the granulation reactor was irreversible adsorption of sEPS, particularly sPS.

The engineering implications of these results are very important and show that granular sludge might not be able to alleviate the membrane fouling problem in MBRs. Moreover, in the case in which granular sludge could be sustained under stable operation inside MBRs, it could lead to irreversible membrane fouling in a major extent than that produced by conventional flocular sludge, which would increase the requirements for chemical cleaning of the membrane,

accelerating membrane deterioration and increasing the operating costs, not only for the use of chemicals, but for a shortened life of the membrane.

3.5. Conclusions

In this study formation of granules could not be induced inside a MBR under large shear forces, selective pressure, and feast and famine regime. The reasons for the observed response are not clear.

Filterability of granular sludge was compared with the filterability of flocular sludge. The results showed no significant difference. However, in terms of irreversibility of the membrane fouling, the results showed that the fouling produced by the filtration of granular sludge was more irreversible than the one produced by the filtration of flocular sludge, suggesting that the soluble extracellular polymeric substances (sEPS) produced by the granular sludge adsorbed more strongly to the membrane than those produced by the flocular sludge.

According to the results of this study, granular sludge might not be able to alleviate the membrane fouling problem in MBRs. Furthermore, it could lead to irreversible membrane fouling in a major extent than that produced by conventional flocular sludge, which would increase the requirements for chemical cleaning of the membrane, accelerating membrane deterioration and increasing the operating costs. Under these conditions, there is no advantage o having a GMBR over a conventional MBR.

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Tables

Table 1. General performance of the GMBR and the SBR during experiments 1 and 2

	Experiment 1		Experiment 2	
Average values of range	GMBR	SBR	GMBR	SBR
COD removal (%)	96.0 (±0.8)	94.4 (±1.9)	95.9 (±0.6)	95.0 (±0.9)
N removal (%)	70.9 (±9.8)	69.4 (±10.1)	69.1 (±11.4)	65.0 (±8.8)
MLSS (mg/L)	1672 (±845)	1583 (±481)	1530 (±591)	1301 (±335)
MLVSS (mg/L)	1504 (±741)	1486 (±443)	1412 (±521)	1231 (±316)
Supernatant TSS (mg/L)	325 (±127)	116 (±34)	400 (±190)	133 (±76)
Supernatant VSS (mg/L)	304 (±123)	112 (±33)	371 (±173)	133 (±77)
SRT (d)	4.9 (±5.9)	3.5 (±0.9)	3.7 (±4.2)	3.1 (±1.8)
F/M (kg COD/kg MLSS.d)	1.2 (±0.7)	1.1 (±0.3)	1.2 (±0.5)	1.3 (±0.4)
DO* (mg/L)	5.2 – 7.9	8.1 – 8.6	4.7 – 7.9	8.5 - 8.8
рН	7.5 – 8.7	7.9 – 8.7	7.6 – 8.6	7.9 – 8.8
Temperature (°C)	20 - 25	21 – 26	21 - 23	21 - 24

*Measured after all soluble substrate was depleted and before settling.

Figures



Figure 1. Schematic representation of the GMBR (a) and the SBR (b)



Figure 2. Operation conditions of the GMBR (a) and the SBR (b)



Figure 3. Evolution of the biomass in the GMBR (a – d) and the SBR (e – h) during experiment
1. Image (g) was captured with the Zeiss Stemi 2000-C stereo light microscope. All other images were captured with the Zeiss Axioskop optical microscope



Figure 3 (cont.)



Figure 4. Evolution of the biomass in the GMBR (a – d) and the SBR (e – h) during experiment2. Images (g) and (h) were captured with the Zeiss Stemi 2000-C stereo light microscope. All other images were captured with the Zeiss Axioskop optical microscope



Figure 4 (cont.)


(a)



(b)

Figure 5. SVI_5 and SVI_{30} of the GMBR and the SBR mixed liquors during experiments 1 (a) and 2 (b). "A": start of granules in the SBR. "B": end of granules in the SBR



(a)



(b)

Figure 6. Filterability of the GMBR and the SBR mixed liquors during experiments 1 (a) and 2 (b). "A": start of granules in the SBR. "B": end of granules in the SBR



Figure 7. Filterability of the GMBR and the SBR soluble fractions during experiment 2. "A": start of granules in the SBR. "B": end of granules in the SBR

4. Conclusions

In this study formation of granules could not be induced inside a membrane bioreactor (MBR) under large shear forces, selective pressure, and feast and famine regime. The reasons for the observed response are not clear.

Filterability of granular sludge was compared with the filterability of flocular sludge. The results showed no significant difference. However, in terms of irreversibility of the membrane fouling, the results showed that the fouling produced by the filtration of granular sludge was more irreversible than the one produced by the filtration of flocular sludge, suggesting that the soluble extracellular polymeric substances (sEPS) produced by the granular sludge adsorbed more strongly to the membrane than those produced by the flocular sludge.

According to the results of this study, granular sludge might not be able to alleviate the membrane fouling problem in MBRs. Furthermore, it could lead to irreversible membrane fouling in a major extent than that produced by conventional flocular sludge, which would increase the requirements for chemical cleaning of the membrane, accelerating membrane deterioration and increasing the operating costs. Under these conditions, there is no advantage of having a granular sludge membrane bioreactor (GMBR) over a conventional MBR.

5. Suggestions for Future Research

Only a few studies have been developed on the filterability of granular sludge versus flocular sludge (Li et al., 2005; Tay et al., 2007), and their results contradict the findings of this research, probably because the method used to evaluate filterability was different (Li et al. (2005) evaluated filterability based on continuous microfiltration (MF) reactor operation, whereas in this study batch dead-end ultrafiltration (UF) tests were used) or because the operation conditions of the reactors used in those studies were different from the ones used in this study (Tay et al. (2007) evaluated filterability based on batch dead-end UF tests but their reactors had a lower food-to-microorganism ratio (F/M = 0.59 kg COD/kg MLSS.d), lower superficial air velocity (72 m/h), and settling time that kept particles with a lower settling velocity in the granular reactor (8.9 m/h approximately)), which might have influenced the composition of the suspended, colloidal, and soluble fractions of both granular and flocular sludge, as well as the extent of the membrane fouling caused by these fractions. Further research on the filterability of granular sludge and flocular sludge from reactors operated under similar operation conditions, such as seed sludge, F/M, shear forces, and SRT, must be performed in order to elucidate the actual filtration characteristics of these two types of biomass and the applicability that this could have on solving the membrane fouling problem in membrane bioreactors (MBRs).

Also, although other researchers (Thanh et al., 2008) have measured the content of irreversible foulants in the supernatant of granular reactors, only in this study a direct measurement of the irreversibility of the membrane fouling caused by granular sludge and flocular sludge has been made. Therefore, additional filtration flux decline experiments that measure the final clean water recovery after filtering granular sludge and flocular sludge from

reactors operated under similar operation conditions could help to corroborate the results of this research and verify the extent of the irreversible membrane fouling caused by these two types of biomass.

Finally, in the case that granular sludge could be proved to help solving the membrane fouling problem in MBRs, it would be necessary to perform further research on the formation and stability of aerobic granules in a granular sludge membrane bioreactor (GMBR), operating the system as close as a sequencing batch reactor (SBR) as possible.

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Appendix A: GMBR Performance - Experiment 1

Start-up date: 16-Sep-09

Data	Operation		COD (mg	COD	
Date	time (d)	Influent	Permeate	Supernatant	Removal (%)
23-Sep-09	7	894	13.5	33.9	96.7
30-Sep-09	14	767	13.5	38.7	95.6
7-Oct-09	21	803	17.5	39.0	95.7
14-Oct-09	28	769	17.5	40.7	95.3
21-Oct-09	35	837	14.9	21.9	97.6
28-Oct-09	42	769	15.9	29.1	96.6
4-Nov-09	49	790	16.8	39.5	95.6
11-Nov-09	56	790	20.3	28.4	96.6
18-Nov-09	63	813	33.1	44.8	94.8
24-Nov-09	69	798	12.0	39.5	95.7
				Average	96.0
				Std. Dev.	0.8

Table 2. GMBR performance – Experiment 1 – COD

Table 3. GMBR performance – Experiment 1 – Ammonia

Deta Operation		NH₄⁺-N (mg/L)				
Dale	time (d)	Influent	Permeate	Supernatant		
23-Sep-09	7	50.7	0.5	0.6		
30-Sep-09	14	40.8	1.1	1.1		
7-Oct-09	21	42.3	12.4	11.4		
14-Oct-09	28	42.1	13.2	13.3		
21-Oct-09	35	44.6	15.9	15.8		
28-Oct-09	42	41.6	20.3	20.2		
4-Nov-09	49	41.0	12.5	11.2		
11-Nov-09	56	41.8	5.9	5.6		
18-Nov-09	63	41.1	2.4	2.1		
24-Nov-09	69	37.0	7.3	6.3		

Data	Date Operation		NO ₂ ⁻ -N (mg/L)			
Date	time (d)	Influent	Permeate	Supernatant		
23-Sep-09	7	3.83	0.17	0.72		
30-Sep-09	14	2.37	0.23	0.28		
7-Oct-09	21	3.23	0.10	0.72		
14-Oct-09	28	2.17	0.04	3.12		
21-Oct-09	35	1.91	0.00	0.44		
28-Oct-09	42	3.65	0.21	0.48		
4-Nov-09	49	5.06	1.60	0.92		
11-Nov-09	56	1.90	4.38	4.63		
18-Nov-09	63	1.41	5.62	6.36		
24-Nov-09	69	2.07	0.73	1.99		

Table 4. GMBR performance – Experiment 1 – Nitrite

Table 5. GMBR performance – Experiment 1 – Nitrate

Date Operation		NO ₃ ⁻ -N (mg/L)				
Date	time (d)	Influent	Permeate	Supernatant		
23-Sep-09	7	0.00	6.30	7.08		
30-Sep-09	14	0.00	15.89	16.00		
7-Oct-09	21	0.00	0.41	0.48		
14-Oct-09	28	0.00	0.09	0.04		
21-Oct-09	35	0.00	0.00	0.03		
28-Oct-09	42	0.00	0.02	0.02		
4-Nov-09	49	0.00	0.16	0.03		
11-Nov-09	56	0.00	0.07	0.05		
18-Nov-09	63	0.00	0.08	0.06		
24-Nov-09	69	0.00	0.02	0.06		

Data	Date Operation Total N (mg/L)		g/L)	N Romoval (%)	
Date	time (d)	Influent	Permeate	Supernatant	N Kemoval (70)
23-Sep-09	7	54.53	6.96	8.40	85.1
30-Sep-09	14	43.17	17.22	17.38	59.8
7-Oct-09	21	45.53	12.92	12.60	72.2
14-Oct-09	28	44.27	13.33	16.46	64.2
21-Oct-09	35	46.51	15.90	16.27	65.2
28-Oct-09	42	45.25	20.54	20.70	54.3
4-Nov-09	49	46.06	14.26	12.15	72.7
11-Nov-09	56	43.70	10.35	10.28	76.5
18-Nov-09	63	42.51	8.10	8.52	80.2
24-Nov-09	69	39.07	8.05	8.34	78.8
				Average	70.9
				Std. Dev.	9.8

Table 6. GMBR performance – Experiment 1 – Total N

Table 7. GMBR performance – Experiment 1 – TSS, VSS, SRT, and F/M

	Operation		TSS (mg/L)		SS (mg/L)	SPT	F/M
Date	time (d)	Mixed liquor	Supernatant	Mixed liquor	Supernatant	(d)	(kg COD/kg MLSS.d)
25-Sep-09	9	2750	410	2380	395	4.2	0.6
30-Sep-09	14	1625	355	1405	325	2.9	0.9
7-Oct-09	21	1145	335	1030	315	2.1	1.4
14-Oct-09	28	840	240	785	223	2.2	1.8
21-Oct-09	35	2470	77	2245	73	20.1	0.7
28-Oct-09	42	2995	203	2720	185	9.2	0.5
4-Nov-09	49	1895	272	1700	240	4.4	0.8
11-Nov-09	56	1570	423	1405	383	2.3	1.0
18-Nov-09	63	770	460	710	453	1.0	2.1
24-Nov-09	69	655	478	655	450	0.9	2.4
	Average	1672	325	1504	304	4.9	1.2
	Std. Dev.	845	127	741	123	5.9	0.7

Date	Operation time (d)	MLSS (mg/L)	V _T (mL)	V₅ (mL)	V ₃₀ (mL)	SVI₅ (mL/g)	SVI ₃₀ (mL/g)
25-Sep-09	9	2750	495	185	110	136	81
2-Oct-09	16	1580	470	190	130	256	175
9-Oct-09	23	1110	540	155	98	259	163
16-Oct-09	30	1130	555	80	70	128	112
23-Oct-09	37	3070	915	375	235	133	84
30-Oct-09	44	2635	1000	640	280	243	106
6-Nov-09	51	2095	970	440	210	217	103
13-Nov-09	58	1025	990	800	110	788	108
20-Nov-09	65	600	980	940	215	1599	366
24-Nov-09	69	655	990	960	295	1480	455

Table 8. GMBR performance – Experiment 1 – SVI

Table 9. GMBR performance - Experiment 1 - Filterability

			Standardized flux (fraction of initial clean wa					
Date	Operation time (d)	Sample TSS (mg/L)	Mixed liquor (after filtering 25 L/m ²)	Clean water after mixed liquor	Soluble fraction (after filtering 25 L/m ²)	Clean water after soluble fraction		
25-Sep-09	9	990	0.31	0.60				
02-Oct-09	16	973	0.32	0.77				
9-Oct-09	23	968	0.31	0.91				
16-Oct-09	30	1130	0.25	0.70				
23-Oct-09	37	1240	0.37	0.81				
30-Oct-09	44	885	0.48	1.10				
6-Nov-09	51	1106	0.27	0.60	0.30	0.74		
13-Nov-09	58	652	0.24	0.51				
20-Nov-09	65	600	0.33	0.52				
24-Nov-09	69	655	0.28	0.57				



Date: 18-Sep-09 / Operation time (d): 2



Date: 21-Sep-09 / Operation time (d): 5



Figure 8. GMBR performance – Experiment 1 – Microscopy

Date: 25-Sep-09 / Operation time (d): 9





Date: 9-Oct-09 / Operation time (d): 23



Figure 8 (cont.)

Date: 15-Oct-09 / Operation time (d): 29





Date: 30-Oct-09 / Operation time (d): 44



Figure 8 (cont.)

Date: 5-Nov-09 / Operation time (d): 50



Date: 12-Nov-09 / Operation time (d): 57



Date: 20-Nov-09 / Operation time (d): 65



Figure 8 (cont.)



Figure 8 (cont.)

Appendix B: GMBR Performance – Experiment 2

Start-up date: 27-Jan-10

Data	Operation		COD (mg	/L)	COD
Date	time (d)	Influent	Permeate	Supernatant	Removal (%)
3-Feb-10	7	842	15.2	35.2	96.3
10-Feb-10	14	730	12.3	32.4	96.1
17-Feb-10	21	846	9.4	31.8	96.8
24-Feb-10	28	734	21.6	37.4	95.3
3-Mar-10	35	792	18.7	47.6	94.7
10-Mar-10	42	766	21.9	36.0	95.7
16-Mar-10	48	763	23.6	33.9	95.8
25-Mar-10	57	766	14.9	31.8	96.3
30-Mar-10	62	742	20.8	35.1	95.7
7-Apr-10	70	839	16.1	30.8	96.7
13-Apr-10	76	791	15.9	36.8	95.9
				Average	95.9
				Std. Dev.	0.6

Table 10. GMBR performance – Experiment 2 – COD

Table 11. GMBR performance – Experiment 2 – COD profile

Date	7-Apr-10
Operation time (d)	70
Cycle time (min)	Mixed liquor soluble COD (mg/L)
5	246.0
15	202.0
30	138.0
45	81.0
60	33.7
75	26.1
90	28.4
105	28.8
120	34.9
135	29.3
150	34.7
180	30.8

Data	Operation	NH₄⁺-N (mg/L)				
Dale	time (d)	Influent	Permeate	Supernatant		
3-Feb-10	7	44.7	1.6	0.3		
10-Feb-10	14	38.8	2.2	1.4		
17-Feb-10	21	42.9	10.8	10.9		
24-Feb-10	28	38.0	2.9	2.0		
3-Mar-10	35	40.9	1.1	0.9		
10-Mar-10	42	38.7	0.0	0.0		
16-Mar-10	48	40.5	0.8	0.3		
25-Mar-10	57	38.7	9.7	9.1		
30-Mar-10	62	36.9	14.8	15.0		
7-Apr-10	70	43.1	18.5	18.3		
13-Apr-10	76	40.3	15.6	14.7		

Table 12. GMBR performance - Experiment 2 - Ammonia

Table 13. GMBR performance – Experiment 2 – Nitrite

Data	Operation	NO ₂ -N (mg/L)			
Date	time (d)	Influent	Permeate	Supernatant	
3-Feb-10	7	6.57	0.87	6.59	
10-Feb-10	14	3.04	1.27	5.49	
17-Feb-10	21	2.07	1.19	5.33	
24-Feb-10	28	6.18	11.92	10.67	
3-Mar-10	35	4.41	8.89	12.75	
10-Mar-10	42	7.71	6.50	9.26	
16-Mar-10	48	2.19	7.89	4.88	
25-Mar-10	57	4.73	0.00	0.69	
30-Mar-10	62	0.87	0.23	3.52	
7-Apr-10	70	15.88	0.09	3.32	
13-Apr-10	76	5.09	0.04	7.42	

Data	Operation	NO ₃ ⁻ -N (mg/L)				
Date	time (d)	Influent	Permeate	Supernatant		
3-Feb-10	7	0.00	3.14	3.62		
10-Feb-10	14	0.00	10.77	11.40		
17-Feb-10	21	0.00	0.53	0.49		
24-Feb-10	28	0.00	0.18	0.20		
3-Mar-10	35	0.00	0.24	0.14		
10-Mar-10	42	0.03	0.45	0.09		
16-Mar-10	48	0.03	0.46	0.12		
25-Mar-10	57	0.04	0.13	0.07		
30-Mar-10	62	0.03	0.10	0.03		
7-Apr-10	70	0.03	0.02	0.02		
13-Apr-10	76	0.03	0.03	0.02		

Table 14. GMBR performance – Experiment 2 – Nitrate

Table 15. GMBR performance – Experiment 2 – Total N

Data	Operation		Total N (m	N Romoval (%)	
Date	time (d)	Influent	Permeate	Supernatant	N Removal (76)
3-Feb-10	7	51.27	5.61	10.51	81.4
10-Feb-10	14	41.84	14.25	18.29	58.2
17-Feb-10	21	44.97	12.53	16.72	64.7
24-Feb-10	28	44.18	15.00	12.87	69.9
3-Mar-10	35	45.31	10.23	13.80	71.1
10-Mar-10	42	46.44	6.95	9.35	80.9
16-Mar-10	48	42.73	9.14	5.30	85.8
25-Mar-10	57	43.47	9.83	9.86	77.3
30-Mar-10	62	37.79	15.13	18.55	52.7
7-Apr-10	70	59.00	18.61	21.64	64.3
13-Apr-10	76	45.42	15.67	22.14	54.1
				Average	69.1
				Std. Dev.	11.4

	Operation	TSS (mg/L)		VS	SS (mg/L)	SPT	F/M
Date	time (d)	Mixed liquor	Supernatant	Mixed liquor	Supernatant	(d)	(kg COD/kg MLSS.d)
3-Feb-10	7	2030	463	1820	410	2.7	0.8
10-Feb-10	14	1220	165	1135	165	4.6	1.2
17-Feb-10	21	1405	373	1295	333	2.4	1.2
24-Feb-10	28	2085	230	1905	208	5.7	0.7
3-Mar-10	35	2480	100	2240	98	15.5	0.6
10-Mar-10	42	2210	755	2040	695	1.8	0.7
16-Mar-10	48	1135	455	1110	443	1.6	1.3
25-Mar-10	57	1610	528	1475	470	1.9	1.0
30-Mar-10	62	1065	403	1000	385	1.7	1.4
7-Apr-10	70	725	583	695	550	0.8	2.3
13-Apr-10	76	870	345	820	330	1.6	1.8
	Average	1530	400	1412	371	3.7	1.2
	Std. Dev.	591	190	521	173	4.2	0.5

Table 16. GMBR performance – Experiment 2 – TSS, VSS, SRT, and F/M

Table 17. GMBR performance – Experiment 2 – SVI

Date	Operation time (d)	MLSS (mg/L)	V _T (mL)	V₅ (mL)	V ₃₀ (mL)	SVI₅ (mL/g)	SVI ₃₀ (mL/g)
5-Feb-10	9	1600	980	700	285	446	182
12-Feb-10	16	1530	965	890	435	603	295
19-Feb-10	23	1540	980	850	360	563	239
26-Feb-10	30	2460	250	240	160	390	260
5-Mar-10	37	2775	985	940	430	344	157
12-Mar-10	44	1730	975	850	315	504	187
17-Mar-10	49	985	980	955	320	989	332
26-Mar-10	58	1565	985	965	415	626	269
31-Mar-10	63	900	980	970	475	1100	539
9-Apr-10	72	865	980	965	575	1138	678
14-Apr-10	77	648	985	980	565	1537	886

			Standardized flux (fraction of initial clean water flux)						
Date	Operation time (d)	Sample TSS (mg/L)	Mixed liquor (after filtering 25 L/m ²)	Clean water after mixed liquor	Soluble fraction (after filtering 25 L/m ²)	Clean water after soluble fraction			
5-Feb-10	9	787	0.27	0.57	0.39	0.87			
12-Feb-10	16	1255	0.28	0.73	0.26	0.56			
19-Feb-10	23	1096	0.16	0.60	0.25	0.54			
26-Feb-10	30	1181	0.17	0.47	0.30	0.65			
5-Mar-10	37	1121	0.20	0.54	0.36	0.64			
12-Mar-10	44	782	0.28	0.58	0.41	0.71			
17-Mar-10	49	867	0.28	0.53	0.40	0.59			
26-Mar-10	58	970	0.34	0.52	0.48	0.71			
31-Mar-10	63	900	0.37	0.63	0.38	0.65			
9-Apr-10	72	865	0.29	0.55	0.39	0.67			
14-Apr-10	77	648	0.34	0.54	0.36	0.62			

Table 18. GMBR performance – Experiment 2 – Filterability





Date: 1-Feb-10 / Operation time (d): 5



Figure 9. GMBR performance – Experiment 2 – Microscopy

Date: 4-Feb-10 / Operation time (d): 8





Date: 15-Feb-10 / Operation time (d): 19



Figure 9 (cont.)

Date: 22-Feb-10 / Operation time (d): 26







Figure 9 (cont.)

Date: 15-Mar-10 / Operation time (d): 47



Date: 24-Mar-10 / Operation time (d): 56





Date: 29-Mar-10 / Operation time (d): 61



Figure 9 (cont.)

Date: 5-Apr-10 / Operation time (d): 68 100 µm a





Figure 9 (cont.)

Appendix C: SBR Performance - Experiment 1

Start-up date: 16-Sep-09

Data	Operation	CO	D (mg/L)	COD
Date	time (d)	Influent	Supernatant	Removal (%)
23-Sep-09	7	468.0	29.7	93.6
30-Sep-09	14	439.5	19.8	95.5
7-Oct-09	21	378.0	26.1	93.1
14-Oct-09	28	406.0	24.7	93.9
21-Oct-09	35	400.5	26.9	93.3
28-Oct-09	42	411.5	24.6	94.0
4-Nov-09	49	420.0	15.0	96.4
11-Nov-09	56	397.0	9.9	97.5
18-Nov-09	63	376.0	34.1	90.9
24-Nov-09	69	387.5	15.2	96.1
			Average	94.4
			Std. Dev.	1.9

Table 19. SBR performance – Experiment 1 - COD

Table 20. SBR performance - Experiment 1 - Ammonia

Data	Operation	NH₄⁺-N (mg/L)		
Date	time (d)	Influent	Supernatant	
23-Sep-09	7	26.1	0.7	
30-Sep-09	14	24.9	2.8	
7-Oct-09	21	20.8	0.4	
14-Oct-09	28	21.7	8.0	
21-Oct-09	35	21.2	9.0	
28-Oct-09	42	23.5	7.7	
4-Nov-09	49	22.1	5.0	
11-Nov-09	56	21.5	4.9	
18-Nov-09	63	18.2	6.4	
24-Nov-09	69	19.6	7.5	

Data	Operation	NO ₂	-N (mg/L)
Date	time (d)	Influent	Supernatant
23-Sep-09	7	0.57	0.04
30-Sep-09	14	0.84	0.93
7-Oct-09	21	3.95	1.86
14-Oct-09	28	6.54	0.62
21-Oct-09	35	1.07	0.59
28-Oct-09	42	6.09	0.52
4-Nov-09	49	7.60	0.05
11-Nov-09	56	8.26	0.02
18-Nov-09	63	2.32	0.48
24-Nov-09	69	3.88	0.54

Table 21. SBR performance – Experiment 1 – Nitrite

Table 22. SBR performance – Experiment 1 – Nitrate

Date	Operation	NO ₃	-N (mg/L)
Date	time (d)	Influent	Supernatant
23-Sep-09	7	0.02	7.92
30-Sep-09	14	0.02	7.74
7-Oct-09	21	0.00	2.84
14-Oct-09	28	0.00	0.60
21-Oct-09	35	0.00	0.42
28-Oct-09	42	0.00	0.24
4-Nov-09	49	0.01	0.06
11-Nov-09	56	0.00	0.02
18-Nov-09	63	0.00	0.04
24-Nov-09	69	0.00	0.03

Dete	Operation	Tota	l N (mg/L)	N Removal
Date	time (d)	Influent	Supernatant	(%)
23-Sep-09	7	26.69	8.66	67.5
30-Sep-09	14	25.76	11.47	55.5
7-Oct-09	21	24.75	5.10	79.4
14-Oct-09	28	28.24	9.22	67.3
21-Oct-09	35	22.27	10.02	55.0
28-Oct-09	42	29.59	8.47	71.4
4-Nov-09	49	29.71	5.11	82.8
11-Nov-09	56	29.76	4.95	83.4
18-Nov-09	63	20.52	6.92	66.3
24-Nov-09	69	23.48	8.07	65.6
			Average	69.4
			Std. Dev.	10.1

Table 23. SBR performance – Experiment 1 – Total N

Table 24. SBR performance – Experiment 1 –TSS, VSS, SRT, and F/M

	Operation		TSS (mg/L)		VSS (mg/L)		F/M
Date	time (d)	Mixed liquor	Supernatant	Mixed liquor	Supernatant	(d)	(kg COD/kg MLSS.d)
25-Sep-09	9	2135	100	1965	97	5.3	0.9
30-Sep-09	14	1320	85	1265	85	3.9	1.3
7-Oct-09	21	1435	142	1335	137	2.5	1.1
14-Oct-09	28	1760	100	1645	93	4.4	0.9
21-Oct-09	35	2430	160	2280	155	3.8	0.7
28-Oct-09	42	1925	162	1810	153	3.0	0.9
4-Nov-09	49	1460	117	1370	115	3.1	1.2
11-Nov-09	56	845	75	785	72	2.8	1.9
18-Nov-09	63	1420	145	1340	145	2.4	1.1
24-Nov-09	69	1095	73	1060	73	3.7	1.4
	Average	1583	116	1486	112	3.5	1.1
	Std. Dev.	481	34	443	33	0.9	0.3

Date	Operation time (d)	MLSS (mg/L)	V _T (mL)	V₅ (mL)	V ₃₀ (mL)	SVI₅ (mL/g)	SVI ₃₀ (mL/g)
25-Sep-09	9	2135	490	90	80	86	76
2-Oct-09	16	1630	475	60	60	77	77
9-Oct-09	23	1515	540	70	68	86	83
16-Oct-09	30	1630	965	55	55	35	35
23-Oct-09	37	2080	930	30	30	16	16
30-Oct-09	44	1165	1000	30	35	26	30
6-Nov-09	51	1255	1000	45	45	36	36
13-Nov-09	58	1445	1000	115	105	80	73
20-Nov-09	65	840	995	45	45	54	54
24-Nov-09	69	1095	995	30	30	28	28

Table 25. SBR performance – Experiment 1 – SVI

Table 26. SBR performance - Experiment 1 - Filterability

			Standardized flux (fraction of initial clean water flux)			
Date	Operation time (d)	Sample TSS (mg/L)	Mixed liquor (after filtering 25 L/m ²)	Clean water after mixed liquor	Soluble fraction (after filtering 25 L/m ²)	Clean water after soluble fraction
25-Sep-09	9	991	0.33	0.58		
2-Oct-09	16	1232	0.34	0.68		
9-Oct-09	23	1054	0.21	0.58		
16-Oct-09	30	926	0.24	0.52		
23-Oct-09	37	857	0.34	0.65		
30-Oct-09	44	606	0.43	0.90		
6-Nov-09	51	858	0.29	0.50	0.36	0.61
13-Nov-09	58	1445	0.46	0.81		
20-Nov-09	65	591	0.33	0.56		
24-Nov-09	69	986	0.34	0.61		



Date: 18-Sep-09 / Operation time (d): 2



Date: 21-Sep-09 / Operation time (d): 5



Figure 10. SBR performance – Experiment 1 – Microscopy





Date: 9-Oct-09 / Operation time (d): 23



Figure 10 (cont.)

Date: 15-Oct-09 / Operation time (d): 29





Date: 23-Oct-09 / Operation time (d): 37



Date: 30-Oct-09 / Operation time (d): 44





Figure 10 (cont.)

Date: 5-Nov-09 / Operation time (d): 50





Date: 20-Nov-09 / Operation time (d): 65



Figure 10 (cont.)



Figure 10 (cont.)
Appendix D: SBR Performance – Experiment 2

Start-up date: 27-Jan-10

Dete	Operation	COI	D (mg/L)	COD
Dale	time (d)	Influent	Supernatant	Removal (%)
3-Feb-10	7	384	21.2	94.5
10-Feb-10	14	371	22.3	94.0
17-Feb-10	21	392	14.9	96.2
24-Feb-10	28	375	18.8	95.0
3-Mar-10	35	373	17.3	95.4
10-Mar-10	42	383	24.3	93.7
16-Mar-10	48	385	16.3	95.8
25-Mar-10	57	405	20.2	95.0
30-Mar-10	62	386	20.9	94.6
7-Apr-10	70	379	14.2	96.3
13-Apr-10	76	391	22.4	94.3
			Average	95.0
			Std. Dev.	0.9

Table 27. SBR p	performance –	Experiment 2 –	COD
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Table 28. SBR performance – Experiment 2 – COD profile

Date	7-Apr-10
Operation time (d)	70
Cycle time (min)	Mixed liquor soluble COD (mg/L)
3	212.0
15	174.0
30	121.0
45	77.0
60	41.5
75	17.1
90	15.1
105	15.5
120	17.6
135	13.7
150	19.3
165	13.1
180	14.2

Data	Operation	NH_4^+	-N (mg/L)
Date	time (d)	Influent	Supernatant
3-Feb-10	7	20.4	0.7
10-Feb-10	14	20.2	0.0
17-Feb-10	21	20.6	4.2
24-Feb-10	28	20.2	10.1
3-Mar-10	35	20.4	10.5
10-Mar-10	42	20.0	10.1
16-Mar-10	48	20.2	8.8
25-Mar-10	57	20.6	7.0
30-Mar-10	62	19.1	7.7
7-Apr-10	70	19.5	6.8
13-Apr-10	76	19.0	7.4

Table 29. SBR performance - Experiment 2 - Ammonia

Table 30. SBR performance – Experiment 2 – Nitrite

Data	Operation	NO ₂	-N (mg/L)
Date	time (d)	Influent	Supernatant
3-Feb-10	7	8.29	0.57
10-Feb-10	14	5.52	3.56
17-Feb-10	21	2.56	1.82
24-Feb-10	28	15.67	1.05
3-Mar-10	35	7.66	1.62
10-Mar-10	42	10.51	5.15
16-Mar-10	48	4.48	0.02
25-Mar-10	57	2.20	0.24
30-Mar-10	62	4.24	2.56
7-Apr-10	70	7.39	0.77
13-Apr-10	76	13.92	0.43

Data	Operation	NO ₃	-N (mg/L)
Date	time (d)	Influent	Supernatant
3-Feb-10	7	0.00	5.68
10-Feb-10	14	0.00	7.55
17-Feb-10	21	0.00	0.77
24-Feb-10	28	0.01	0.13
3-Mar-10	35	0.02	0.03
10-Mar-10	42	0.03	0.01
16-Mar-10	48	0.04	0.03
25-Mar-10	57	0.04	0.03
30-Mar-10	62	0.03	0.02
7-Apr-10	70	0.04	0.01
13-Apr-10	76	0.04	0.06

Table 31. SBR performance – Experiment 2 – Nitrate

Table 32. SBR performance – Experiment 2 – Total N

Data	Operation	Tota	I N (mg/L)	N Romoval (%)
Date	time (d)	Influent	Supernatant	N Kelloval (70)
3-Feb-10	7	28.69	6.95	75.8
10-Feb-10	14	25.72	11.11	56.8
17-Feb-10	21	23.16	6.79	70.7
24-Feb-10	28	35.88	11.28	68.6
3-Mar-10	35	28.08	12.15	56.8
10-Mar-10	42	30.54	15.27	50.0
16-Mar-10	48	24.72	8.85	64.2
25-Mar-10	57	22.83	7.26	68.2
30-Mar-10	62	23.37	10.28	56.0
7-Apr-10	70	26.93	7.58	71.8
13-Apr-10	76	32.96	7.89	76.1
			Average	65.0
			Std. Dev.	8.8

	Oneration	TS	S (mg/L)	VS	SS (mg/L)	SDT	F/M
Date	time (d)	Mixed liquor	Supernatant	Mixed liquor	Supernatant	(d)	(kg COD/kg MLSS.d)
3-Feb-10	7	1475	110	1350	108	3.4	1.0
10-Feb-10	14	1640	82	1555	82	5.0	0.9
17-Feb-10	21	1800	65	1720	65	6.9	0.9
24-Feb-10	28	1640	255	1540	255	1.6	0.9
3-Mar-10	35	890	110	890	110	2.0	1.7
10-Mar-10	42	1380	81	1350	79	4.3	1.1
16-Mar-10	48	1225	157	1155	157	2.0	1.3
25-Mar-10	57	1160	293	1120	293	1.0	1.4
30-Mar-10	62	1392	113	1286	110	3.1	1.1
7-Apr-10	70	908	57	839	57	4.0	1.7
13-Apr-10	76	801	143	739	143	1.4	1.9
	Average	1301	133	1231	133	3.1	1.3
	Std. Dev.	335	76	316	77	1.8	0.4

Table 33. SBR performance – Experiment 2 - TSS, VSS, SRT, and F/M

Table 34. SBR performance – Experiment 2 – SVI

Date	Operation time (d)	MLSS (mg/L)	V _T (mL)	V₅ (mL)	V ₃₀ (mL)	SVI₅ (mL/g)	SVI ₃₀ (mL/g)
5-Feb-10	9	1535	990	290	195	191	128
12-Feb-10	16	1335	965	285	195	221	151
19-Feb-10	23	1740	980	305	200	179	117
26-Feb-10	30	1330	236	16	16	51	51
5-Mar-10	37	995	252	10	10	40	40
12-Mar-10	44	1200	245	23	22	78	75
17-Mar-10	49	940	248	13	13	56	56
26-Mar-10	58	742	239	29	29	164	164
31-Mar-10	63	1488	250	54	57	145	153
9-Apr-10	72	865	600	190	190	366	366
14-Apr-10	77	1336	610	135	130	166	160

			Standardiz	ed flux (fractior	n of initial clean	water flux)
Date	Operation time (d)	Sample TSS (mg/L)	Mixed liquor (after filtering 25 L/m ²)	Clean water after mixed liquor	Soluble fraction (after filtering 25 L/m ²)	Clean water after soluble fraction
5-Feb-10	9	1044	0.29	0.69	0.35	0.84
12-Feb-10	16	812	0.38	1.04	0.43	0.75
19-Feb-10	23	967	0.16	0.62	0.33	0.68
26-Feb-10	30	995	0.20	0.55	0.43	0.68
5-Mar-10	37	995	0.29	0.52	0.35	0.56
12-Mar-10	44	869	0.32	0.56	0.37	0.56
17-Mar-10	49	767	0.34	0.45	0.44	0.58
26-Mar-10	58	641	0.37	0.44	0.46	0.62
31-Mar-10	63	1071	0.33	0.45	0.43	0.60
9-Apr-10	72	865	0.37	0.51	0.46	0.55
14-Apr-10	77	1336	0.31	0.34	0.46	0.54

Table 35. SBR performance – Experiment 2 – Filterability



Date: 29-Jan-10 / Operation time (d): 2



Date: 1-Feb-10 / Operation time (d): 5



Figure 11. SBR performance – Experiment 2 – Microscopy

Date: 4-Feb-10 / Operation time (d): 8







Figure 11 (cont.)



Date: 1-Mar-10 / Operation time (d): 33



Date: 8-Mar-10 / Operation time (d): 40



Figure 11 (cont.)

Date: 15-Mar-10 / Operation time (d): 47





Date: 24-Mar-10 / Operation time (d): 56



Date: 29-Mar-10 / Operation time (d): 61



Figure 11 (cont.)

Date: 5-Apr-10 / Operation time (d): 68



Figure 11 (cont.)

Appendix E: List of Samples Stored for Potential Subsequent Analysis

During Experiment 2, biomass samples from the GMBR and the SBR were taken every week and stored for potential subsequent analysis. Two 2-mL centrifuge tubes were filled with mixed liquor from each reactor, centrifuged at 10000 rpm for 5 min, and stored at -80°C in the freezer located in 4217 - NCEL. The samples were stored inside a box identified as "Ana Duque GMBR/SBR Samples", and were labeled indicating the name of the reactor and the date in which they were taken as follows:

Name of sample	Date of sampling	Operation time (d)
SS 01/26/10	26-Jan-10	0 (seed sludge)
SS 01/26/10	26-Jan-10	0 (seed sludge)
GMBR 02/01/10	1-Feb-10	5
GMBR 02/01/10	1-Feb-10	5
SBR 02/01/10	1-Feb-10	5
SBR 02/01/10	1-Feb-10	5
GMBR 02/08/10	8-Feb-10	12
GMBR 02/08/10	8-Feb-10	12
SBR 02/08/10	8-Feb-10	12
SBR 02/08/10	8-Feb-10	12
GMBR 02/15/10	15-Feb-10	19
GMBR 02/15/10	15-Feb-10	19
SBR 02/15/10	15-Feb-10	19
SBR 02/15/10	15-Feb-10	19
GMBR 02/22/10	22-Feb-10	26
GMBR 02/22/10	22-Feb-10	26
SBR 02/22/10	22-Feb-10	26
SBR 02/22/10	22-Feb-10	26
GMBR 03/01/10	1-Mar-10	33
GMBR 03/01/10	1-Mar-10	33
SBR 03/01/10	1-Mar-10	33
SBR 03/01/10	1-Mar-10	33
GMBR 03/08/10	8-Mar-10	40
GMBR 03/08/10	8-Mar-10	40
SBR 03/08/10	8-Mar-10	40
SBR 03/08/10	8-Mar-10	40
GMBR 03/15/10	15-Mar-10	47
GMBR 03/15/10	15-Mar-10	47
SBR 03/15/10	15-Mar-10	47
SBR 03/15/10	15-Mar-10	47

Table 36. List of samples stored for potential subsequent analysis – Experiment 2

1 able 50 (cont.)

Name of sample	Date of sampling	Operation time (d)
GMBR 03/24/10	24-Mar-10	56
GMBR 03/24/10	24-Mar-10	56
SBR 03/24/10	24-Mar-10	56
SBR 03/24/10	24-Mar-10	56
GMBR 03/29/10	29-Mar-10	61
GMBR 03/29/10	29-Mar-10	61
SBR 03/29/10	29-Mar-10	61
SBR 03/29/10	29-Mar-10	61
GMBR 04/05/10	5-Apr-10	68
GMBR 04/05/10	5-Apr-10	68
SBR 04/05/10	5-Apr-10	68
SBR 04/05/10	5-Apr-10	68
GMBR 04/12/10	12-Apr-10	75
GMBR 04/12/10	12-Apr-10	75
SBR 04/12/10	12-Apr-10	75
SBR 04/12/10	12-Apr-10	75