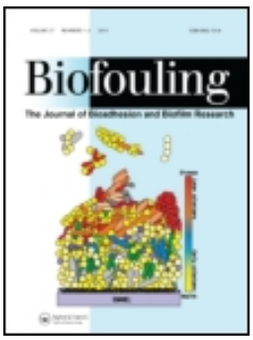

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Biofouling and pollutant removal during long-term operation of an anaerobic membrane bioreactor treating municipal wastewater

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Two different sludge retention times (SRTs) were tested in order to assess the impact on membrane fouling and effluent quality in an anaerobic membrane bioreactor (AnMBR). Two up-flow anaerobic sludge bed (UASB) reactors (1 l volume) coupled to external tubular ultrafiltration membranes (filtration area = 81 cm^2) were operated at a hydraulic retention time of 3 h and two different SRTs (100 and 60 days). The transmembrane pressure (TMP), flux (J) and relevant parameters to assess water quality were measured. Effluents from UASB reactors were filtered for 500 h without intermediate cleaning. The permeate met Mexican standards for wastewater reclamation in both tested conditions. Abrupt and periodical changes in the TMP and J were noticed during the experimental period. A fouling layer collapse and compression hypothesis was set forth in order to explain these changes. An autopsy performed on biofouled membranes indicated that deposited mass was mainly composed of volatile solids (85%) and the rest related to mineral matter, with the presence of inorganic salts containing Ca, Mg, Fe, P and Si. Biomass in the fouling layer was estimated at 0.27% based on the DNA/biomass ratio for the bacterial biofilm. No clear difference in membrane fouling was detected under the two SRTs applied to the systems. However, when operated over 500 h, repetitive sudden TMP and flux changes occurred later in system A (SRT of 100 days) than in system B (SRT of 60 days) suggesting a stronger fouling layer structure in the former.

Keywords: anaerobic; membrane bioreactors; fouling; municipal wastewater; UASB; ultrafiltration

Introduction

Wastewater reclamation concepts in industrial and municipal sectors during the last decade faced a paradigm shift due to membrane bioreactors (MBR) (Fane 2007). Aerobic MBR effluents generally meet stringent regulations for water reclamation around the world (Ben Aim 2007). Research in this area has been focused on aerobic systems in order to determine the influence of operational parameters, such as hydraulic retention time (HRT) sludge retention time (SRT) and organic loading rate (OLR) on membrane fouling and flux decrease. A major concern has been to elucidate the causes of membrane clogging, an unavoidable disadvantage of this technology (Cicek 2003; Pontié et al. 2005).

Anaerobic technology for wastewater treatment has advantages over aerobic processes, especially for energy requirements and sludge management. However, due to the kinetic limitations of anaerobic metabolism, anaerobic effluents rarely meet discharge standards for wastewater reuse. In order to overcome this disadvantage, membranes may be used to polish anaerobic effluent.

Anaerobic membrane bioreactors (AnMBRs) have been applied to wastewater treatment, mainly for industrial or high-strength wastewaters (Cicek 2003; Jeison and van Lier 2006). A potential application for AnMBRs is in the rapidly growing field of municipal wastewater reclamation (MWWR). Several studies have been published dealing with AnMBR and MWWR applications, mostly from developing countries (Wen et al. 1999; Li-Bing et al. 2005a, b; Saddoud et al. 2007; Huang et al. 2008). Nevertheless, there is a lack of experimental data for long-term supracritical filtration of effluents from high-rate anaerobic reactors.

Changes in operational SRT usually affect the molecular weight distribution of dissolved organic solids prevailing in effluents from anaerobic reactors. Barber and Stuckey (1999) reviewed anaerobic reactor operation at different SRTs and reported that molecular weights of soluble organic matter ranged from 0.5 to 200 kDa but, for SRTs > 15 days, high-molecular weight compounds (HMWC) prevailed. Huang et al. (2008), worked with submerged anaerobic membrane reactors operated at different SRTs (30, 60

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and infinite days) and HRTs (10 and 12 h), and concluded that longer SRTs produced higher carbohydrate/protein (C/P) ratios in soluble microbial products (SMP) and lower C/P ratios for extracellular polymeric substances (EPS), a situation that leads to fouling development.

This paper had two main aims: (1) to evaluate pollutant removal in a bench-scale AnMBR process used for municipal wastewater treatment, and (2) to evaluate long-term biofouling components in those systems, operated at different biological conditions (SRT 1 = 100 days and SRT 2 = 60 days).

Materials and methods

Experimental set-up

Two identical laboratory-scale systems (A and B) were set up. Each system (Figure 1) was composed of a up-flow anaerobic sludge bed (UASB) reactor (1 l volume, glass column); a balance flow tank (2.5 l volume; acrylic cylindrical structure); a single membrane tube (hydrophobic, 81 cm^2, 40 kDa MWCO) and their respective pumping units, *viz* a Cole-Parmer Masterflex peristaltic pump (7553–30, USA) for a UASB supply and a positive displacement device (Moyno pump series 33201, ½ HP Baldor, USA), for a membrane supply. The membrane tube was taken from a commercial module tubular membrane (Aquatech Memtuf©, Korea). The cross flow velocity was set at 2 m s^{-1}. Transmembrane pressure (TMP), was measured using a pressure transmitter (Cole-Parmer 68001, 0–2100 kPa, USA) coupled to electronic devices for on-line data acquisition commanded by a LabVIEW 7.0 application. A globe valve was put in the discharge zone for optimal control of TMP. The UF permeate was measured in the container under the filtration tube. Long term UFs were conducted at ambient temperature in southern Mexico City (20–25°C). Both UASB reactors were operated for 3 months in an acclimation period before coupling them to their respective membrane units for a further operational period of 103 days, at HRT = 3 h. System A worked at an SRT closer to 100 days (without sludge discharge) and system B at 60 days. The SRT in reactor B was controlled by wasting 5 ml of sludge per day. SRTs were selected to fit typical values for full-scale UASB reactors.

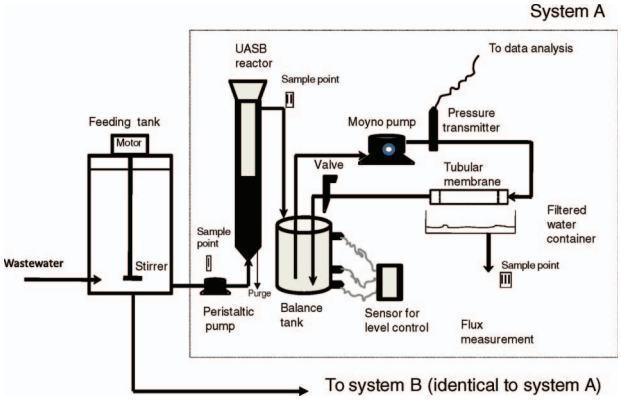

Figure 1. Schematic representation of AnMBR experimental system.

Municipal wastewater and **Ascaris suum** *inoculation*

Wastewater was collected from the inlet line to the Cerro del Agua UNAM wastewater treatment plant. Due to null counts of helminth eggs (HE) in wastewater, artificial addition of *A. suum* ova was used; mature female *A. suum* specimens were obtained from a municipal slaughterhouse for pigs (Tlalnepantla, Mexico). Specimen dissections and further uterus extraction and ovum counting were performed using a modification of the techniques of Capizzi and Schwartzbrod (2001a, b). *A. suum* ova were transferred to NaCl physiological solution. After vortex homogenization for 30 s, 100 μl aliquots were transferred to Duncaster cells for ovum quantification, performed by optical microscopy (Zeigen X52-G7). Ten *A. suum* ova μl^{-1} of suspension were obtained after Milli-Q water dilution. This suspension was stored at 4°C and used to maintain a constant concentration (25 *A. suum ova* l^{-1}), in the AnMBR influent.

Analytical methods

The system had three sample points (I, II and III in Figure 1). The pH, total solids (TS), total volatile solids (TVS) and total fixed solids (TFS), total suspended solids (TSS) volatile suspended solids (VSS) and fixed suspended solids (FSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), and fecal coliform concentration were measured according to North American standards (APHA, AWWA, WEF, 1999). Whatman GF/A (1.6 μm) prefilters were used for suspended solids and dissolved solid determination; the same filtered samples were used for soluble COD. *A. suum* ova were measured as HE by using the procedure described in the official Mexican environmental regulation (NOM-001-SEMARNAT-1996, in SEMARNAT 1997).

After long-term operation (500 h), DNA isolation was performed from both membranes as follows: 2 cm pieces of each fouled membrane were cut into small pieces and then placed in a 2-ml tube containing 0.9% NaCl solution. Abrasive particles taken from a DNA isolation kit (MO BIO for soil, Ultraclean 12–800, CA, USA), were used for membrane/biofilm separation. DNA isolation was completed following the MO BIO

kit directions. Spectroscopy (260 nm in a Biophotometer, Eppendorf, GER) was used to determine the DNA concentration in the fouling layers. Gravimetric tests were conducted to determine the volatile and fixed solids content of the fouling layer using a physiological saline solution as the blank after membrane/biofilm separation.

Fouling layer observation using scanning electron microscopy and microanalysis

The ultrastructure of the biofouling was explored in both tested conditions by using scanning electron microscopy (SEM) (Cambridge/Leica, Stereoscan 440, UK). Microanalysis by Energy Dispersive X-ray microanalysis (EDX) was conducted on biofilm-bearing surfaces (100 μm^2) by using a solid-state Si/Li detector (Oxford, PentaFET, UK) and the data were analyzed with ISIS Link software, version 1.04a.

Results and discussion

Pollutant removal

Table 1 shows the results for long-term operation for both systems. As expected, COD removal was limited (around 50% in both UASB reactors), taking into account that the operational HRT was quite low (3 h) compared to full-scale UASB applications for municipal wastewater treatment, where the HRT is usually 6–8 h. A bold operational HRT was tested in order to obtain lower reactor volume and savings in investment costs and treatment rate on the one hand, and because a combination of a bold HRT and a low nominal molecular weight cut-off membrane (40 kDa) may achieve discharge standards for wastewater reclamation. The UF membrane reduced COD, BOD_5 and TSS concentrations to standard secondary sedimentation effluent levels. The BOD_5 in tested AnMBR effluents was slightly above the maximum levels established for reclamation of public services wastewater (32 and 31 *vs* 30 mg l^{-1}) (NOM-003-SEMARNAT-1997, in SEMARNAT 1998).

A quite low VDS removal in both systems (11% in B and zero in A) was achieved. This indicates that biological removal of soluble substrate in anaerobic reactors such as membranes was very limited. SMPs produced in UASB reactors at the tested conditions were smaller than the nominal membrane pore size and thus they passed freely through the filter.

Table 1. AnMBR pollutant removal in both tested conditions.

	Raw water	UASB effluent	UF permeate	Total removal (%)
System A, SRT = 100 days				
CODt, mg O_2 l^{-1}	646 (103)	300 (69)	104 (12)	87
CODs, mg O_2 l^{-1}	385 (63)	198 (55)	104 (12)	73
BOD_5, mg O_2 l^{-1}	ND	ND	32 (5)	–
TS, mg l^{-1}	681 (76)	612 (76)	317 (124)	50
VTS, mg l^{-1}	277 (43)	225 (23)	158 (49)	43
FTS, mg l^{-1}	404 (38)	387 (66)	159 (110)	60
TSS, mg l^{-1}	140 (18)	77 (17)	<1	100
VSS, mg l^{-1}	120 (13)	69 (15)	<1	100
FSS, mg l^{-1}	20 (7)	8 (3)	<1	100
TDS, mg l^{-1}	541	535	315	41
VDS, mg l^{-1}	157	156	157	0
FDS, mg l^{-1}	384	379	158	63
Helminth eggs, HE l^{-1}	25 (0)	10 (3.5)	0 (0.9)	100
Fecal coliforms, MPN 100 ml^{-1}	10 E 6	ND	0	100
System B, SRT = 60 days				
CODt, mg O_2 l^{-1}	646 (104)	318 (41)	106 (14)	86
CODs, mg O_2 l^{-1}	385 (63)	226 (40)	106 (14)	72
BOD_5, mg O_2 l^{-1}	ND	ND	31 (6)	–
TS, mg l^{-1}	681 (76)	601 (68)	301 (161)	56
VTS, mg l^{-1}	277 (43)	201 (91)	142 (45)	49
FTS, mg l^{-1}	404 (38)	400 (62)	159 (110)	60
SST, mg l^{-1}	140 (18)	68 (13)	<1	100
VSS, mg l^{-1}	120 (13)	58 (11)	<1	100
FSS, mg l^{-1}	20 (7)	10 (3)	<1	100
TDS, mg l^{-1}	541	533	299	45
VDS, mg l^{-1}	157	143	141	11
FDS, mg l^{-1}	384	390	158	59
Helminth eggs, HE l^{-1}	25 (0)	10 (1.7)	0 (0.6)	100
Fecal coliforms, MPN 100 ml^{-1}	10 E 6	ND	0	100

Note: SD in parenthesis, ND: not determined.

Low VDS removal is a consequence of either prevailing water temperature (20°C), or HRT that might limit reactor removal capacity. Anaerobic treatment for low-strength wastewater (COD < 500 mg l^{-1}) may not be an efficient process for water temperatures <20°C (Noyola et al. 1988).

The unexpected removal of high fixed dissolved solids (FDS) on both membranes (about 60%) suggests that flocculation and other physical or/and chemical interactions may have occurred at the fouling layer level. Flemming and Wingender (2001a, b) report that the ability of EPS to chelate metals may favor inorganic bio-flocculation within the fouling layer. Additionally, Volesky (2007) noticed that sorption, flocculation and other electrostatic attractions may be performed by the supported biomass and its polymers.

Regarding pathogens and parasites control, a total removal of HE and fecal coliforms was accomplished in both systems, and therefore AnMBR effluents meet Mexican standards. UASB reactors by themselves retained 60% of total HE. After 100 days of operation, sludge samples were taken-off from reactors A and B and revealed concentrations of 27 HE g^{-1} TS and 16 HE g^{-1} TS, respectively.

Membrane fouling

Figure 2 shows permeate flux and TMP *vs* filtration time plots during the first 10 h of operation for both units. TMP was set at 355 kPa to operate upon the critical flux, determined in previous assays (45 l m^{-2} h^{-1}). A quick flux reduction in the first minutes is a typical response for external membrane systems (Amy and Cho 1999; Vera et al. 1996). After 4 h, both systems reached a flux value which remained relatively constant throughout the filtration run. The similar response of both systems suggests that there is no significant relation between fouling rate and SRT, at least under the operating conditions applied in this experiment (SRT of 100 and 60 days) and for the evaluated filtration period. Also, the maximum hourly fouling rates were of the same order of magnitude (System A, 14.5 and System B, 17.7 l m^{-2} h^{-1}). After operation for 10 h, once an apparent steady-state was reached, remnant fluxes on systems A and B were 6.9 and 8.2 l m^{-2} h^{-1}, respectively. These values, compared to J_0 (48.1 l m^{-2} h^{-1}), produced flux reductions of 83 to 86%, respectively. Also, it was observed that after 4–5 h both membranes were almost completely fouled. The sudden fall in flux during the first hours has been related to concentration-polarization (CP) phenomena in aqueous media (Lee et al. 1999) when fouling is basically induced by electrostatic attraction between molecules and the membrane surface. After this point, both curves showed slopes close to zero, which may represent steady-state biofouling.

TMP and flux *vs* time plots for long-term filtration runs, without intermediate cleaning, are shown in Figure 3. It is evident for these experiments that the gradual decreases in permeate flux are abruptly altered after 175 and 140 filtration hours in system A and B, respectively. Likewise, these changes correspond to important increases in TMP. After 100 additional UF hours, unexpectedly, sudden new increases in TMP and flux decreases occurred again in both tested conditions, a situation followed by a steady-state lapse for TMP and flux. This pattern was observed again at the end of the experimental time (almost 450 h) in both experimental conditions. It was determined that the sudden pressure and flux changes registered during long-term runs did not correspond to any abrupt changes in TSS concentration in the UASB effluents; the anaerobic effluent composition was practically constant.

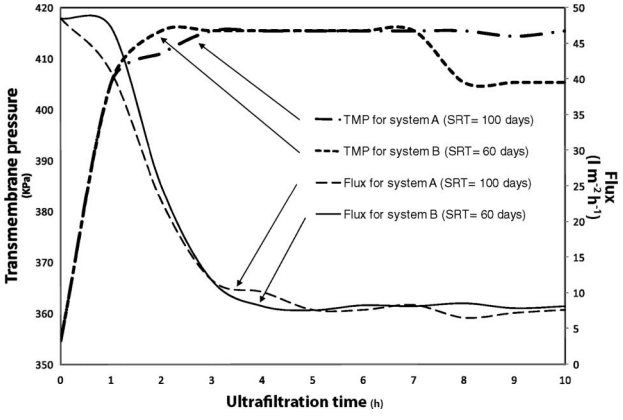

Figure 2. Flux and TMP *vs t* during early ultrafiltration for systems A and B.

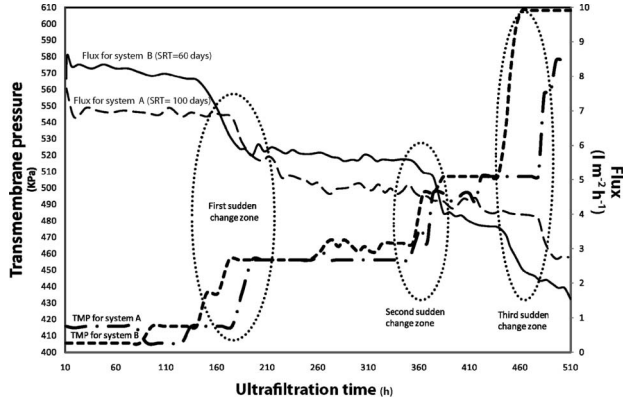

Figure 3. Flux and TMP *vs t* during long-term ultrafiltration for systems A and B.

Figure 4 shows details of the ultrastructure of the fouling layers after long-term filtration runs. An apparent multilayer fouling film can be seen. By associating evidence between sudden changes in PTM and J (Figure 3), with the information provided by micrographs (Figure 4), a hypothesis for fouling layer collapse-compression is proposed. This hypothesis is based on the supposition that fouling layers grow continuously until a point when the internal structure (assumed to be a complex and multi-channel network, created by deposition and biomineralization) collapses, and the filtration process becomes more efficient. As a consequence, particles that previously flowed freely may be retained, producing major fouling and increases in local flux. Local flux increases also provoke increases in solid accumulation along the membrane leading to a sudden collapse of the fouling layer structure and further to compression, with a sharp increase in filtration resistance. This phenomenon results in an abrupt increase in the TMP and a flux decrease, followed by a more stable period during which a new fouling layer formation process begins.

The fouling layer structure assumed in the hypothesis would be composed of a random accumulation of biopolymers (EPS, SMP), biomineralized compounds, cellular biomass and other precipitated materials. It is well known that EPS are involved in AnMBR fouling; they have been identified as an important factor in fouling layer build-up in UASB, coupled to microfiltration reactors (ie Cho and Fane 2002). EPS is also capable of flocculating inorganic materials under specific conditions (Volesky 2007), which would modify, by electrostatic interactions, the fouling layer structure. Reyes-Grajeda et al. (2002) noticed that a small egg shell protein, Ovocleidin-17 (142 amino acid residues, 17 kDa) was capable of precipitating calcium salts *in vitro*. This protein could concentrate electric charges in its C-type lectine-like domain and therefore favoring ordered and well-oriented calcium carbonate crystallization.

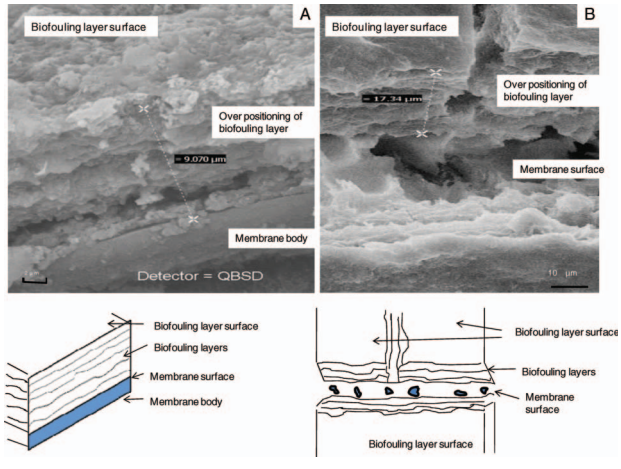

Figure 4. SEM micrographs of membrane cuts. (A) Fouling layer over-positioning transversal view on SRT = 100 days (3500 ×). (B) Fouling layer over-positioning on SRT = 60 days (1000 ×).

Supramolecular chemistry (SC) (hydrogen bonds, van der Waals forces, polymer–crystal interactions) has been proposed as an important factor for fouling layer development (Hamilton 2003; Wei et al. 2007, Kim et al. 2008). According to Flemming and Wingender (2001a), the EPS of mature biofilm can act as a barrier that prevents transport of higher size molecules. Typical polysaccharides associated with biofilms have a relatively high molecular mass ($0.5–2.0 \times 10^6$) and usually increase biofilm viscosity, acting as a barrier that prevents mass transport (Szoboszlai et al. 2009). SC may enhance the internal network connectivity within the fouling layer by cooperative interactions between EPS, cells, mineral salts and diverse colloidal materials, all of which are susceptible to the formation of clusters or 'centers ', in agreement with the Hermanowicz (2008) model for a sudden TMP rise.

EDX identified Ca, Fe, Na, Mg, P and Si as fouling layer components in both systems. This evidence suggests that during long-term UF of UASB effluent, biological mineralization may produce biominerals by organic–inorganic interactions that would enhance the mechanical properties of biofouling. Following this assumption, inorganic compounds coming from the UASB effluent could be susceptible to precipitation at specific sites within the EPS matrix and this could act as heterogeneous epitaxic sites for further inorganic crystallization. In agreement with this assumption, biological mineralization has been documented in other biological systems and it is a common and well accepted process (Silyn-Roberts and Sharp 1986; Mann 2001; Reyes-Grajeda et al. 2002; Villarreal-Ramirez et al. 2009). For the specific case of membrane fouling, EPS, SMP and low solubility inorganic material may produce inorganic crystals or amorphous precipitants, being part of a complex matrix of macromolecules that contributes to the formation of the skeleton on mature fouling layers.

Table 2 shows the long-term run analyses. It is possible to observe that the experimental elapsed time for sudden changes in TMP and flux was always shorter in system B than in system A for all the three events identified in Figure 3. This leads to the assumption that a more rigid structure was developed in the system A fouling layer (SRT 100 days) than in system B (SRT 60 days). Biopolymer and cation associations may produce mineralized-EPS building blocks (MEBB), with properties associated to their

Table 2. Elapsed time for sudden changes in TMP and flux as a function of SRT.

Parameter	First event		Second event		Third event	
	SRT 60 days	SRT 100 days	SRT 60 days	SRT 100 days	SRT 60 days	SRT 100 days
Time[a] for sudden TMP shifts (h)	140	175	354	362	438	478
Time[a] for sudden flux shifts (h)	140	175	350	354	438	472

[a]Determined from Figure 3 considering the point where the sudden change started.

Table 3. Fouling layer composition after long-term operation (500 h).

	Total solids, mg cm^{-2} (%)	Volatile solids, mg cm^{-2} (%)	Fixed (inorganic) solids, mg cm^{-2} (%)	Cell mass estimation, mg cm^{-2} (%)
System A (SRT = 100 days)	34.31 (100)	30.20 (88)	4.11 (12)	0.091 (0.265)
System B (SRT = 60 days)	35.76 (100)	31.39 (85)	4.36 (15)	0.095 (0.265)

molecular length and to the prevailing electrostatic conditions. The longer times between the sudden changes observed in Figure 3 for system A would indicate that their associated MEBB may have developed a more robust and stronger fouling layer structure than that observed in the other tested condition.

The structural differences between fouling layers obtained for tested SRT (60 days and 100 days) could be related to molecular weight distribution of the SMP or EPS. Aquino et al. (2006) noticed that for submerged AnMBR operation in treating municipal wastewater, HMWC prevailed in higher SRT. A higher viscosity in the membrane boundary can also be expected as a consequence (Szoboszlai et al. 2009). Therefore, for long-term operation, HMWC may achieve a better metal chelating effect due to their polyelectrolyte behavior (as observed for common bacterial polysaccharides such as chitin, alginate and chitosan) as well as to the presence of ampholytes (ie peptides and proteins) according evidences provided by Zhang et al. 2008. Thus, either metal deposition or random mineralization on specific sites along HMWC in the fouling layer, can be favored.

Membrane autopsy

After long-term runs (500 h), the DNA associated with both fouling layers was isolated, measured and used for indirect biomass estimation of the tested biofouling. A similar concentration of DNA in both membranes was found, *viz* 5.488 μg DNA cm^{-2} for system A and 5.722 μg DNA cm^{-2} for system B. Flemming et al. (2000) presented a relationship between DNA and biomass (for *Pseudomonas putida* biofilms): 1 μg of DNA relates to 16.6 μg of biomass. Ninety one μg of cellular biomass cm^{-2} for system A, and 95 μg of cellular biomass cm^{-2} for system B were noticed.

Table 3 shows the analysis of volatile and fixed (ash) solids in the fouling layers for systems A and B. In both fouled membranes, similar increases of material were noticed, but the organic fraction was the main component of fouling layers ($\sim$85% volatile and 15% fixed solids). The amount of microbial biomass per cm^2 calculated above represents just 0.3% of the total organic matter measured in both fouling layers, as Table 3 shows. Therefore, the fouling layer developed on both systems was mainly comprised of cell-free organic substances, inorganic salts and cellular biomass.

Identification of minerals such as Ca, Mg, Si, P and Fe suggests the presence of inorganic salts such as carbonates, hydroxides, phosphates as well as silicates. As already discussed, biologically induced mineralization may be involved in the consolidation of structure during fouling layer build-up and could enhance its mechanical properties. As the layer strengthens, its removal would become more difficult and this would require a more intensive use of chemical cleaning or the development of alternative strategies to effectively accomplish this operation.

Conclusions

Both evaluated AnMBR systems produced a clear effluent free of suspended solids, with a BOD_5 and COD closer to 30 and 100 mg l^{-1}, respectively. In addition, the system accomplished total removal of fecal coliforms and HE in compliance with the Mexican regulation for wastewater reclamation.

After 500 h of UF of UASB effluent, the evaluated biofouling composition on a dry weight basis, was almost 85% organic and 15% inorganic matter. Just 0.3% of the organic matter was related to cell biomass;

Fe, Mg, Ca, P, Na and Si were also identified within the fouling layer by EDX.

Sudden changes in the TMP and flux observed during long-term UF runs may be explained by a fouling layer collapse and compression hypothesis for cross-flow membrane ultrafiltration. This hypothesis is based on the supposition that a fouling layer grows in a continuous way until a point when its internal structure (comprised of cell-free organics, biomineralized compounds, inorganics and cellular biomass) collapses due to the growing local pressure applied over it and the consequent compression provokes sudden increases in TMP and reductions in the flux and filtration capacity in a repetitive pattern. In-depth research is necessary to demonstrate this hypothesis.

The influence of SRT (60 and 100 days) on short term filtration behavior and effluent quality was not noticed. However, for the 500 h filtration runs, system A (SRT of 100 days) showed longer times for sudden changes to occur in the TMP and flux, suggesting a stronger fouling layer structure.

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