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Role and variations of supernatant compounds in submerged membrane bioreactor fouling

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Abstract

Many studies have been performed to analyze the influence of extracellular polymeric substances (EPS) in membrane fouling. Most of these works deal with the impact of solid contents in the fouling, and some of them have studied the role of supernatant compounds. The aim of this work was to clarify the role of the different sludge fractions in the context of membrane bioreactor fouling. The laboratory-scale reactor used for experiments consists of a submerged membrane bioreactor for the treatment of synthetic wastewater. For the same organic load (0.4 g COD/g MLSS.d), several samples of sludge were taken off and divided into three fractions (solid contents, soluble and colloids). COD and extractable EPS were quantified (carbohydrates and proteins). Dead-end filtration tests for each fraction were also carried out. According to these experiments, no correlation between EPS concentration in the solid part of the sludge and filtration resistance was found. Instead, a change of the filtration resistance was explained as a function of COD in the supernatant, and more especially as a function of proteins concentration. Indeed, when the value of proteins concentration in the supernatant changes from 30 to 100 mg/l, the value of specific resistance increased by a factor of 10. Finally, the characterization of the supernatant was shown as a key parameter for the MBR operating control. When the COD and proteins concentration in the supernatant remained low, the transmembrane pressure in the reactor remained even lower. Moreover, with the biomass growth rate analysis, our results suggest that the EPS production was linked to growth of microorganisms. The faster the growth, the less EPS production.

Keywords: SMBR membrane fouling; Extracellular polymeric substances; Biomass growth rate; Specific resistance; Sludge retention time

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1. Introduction

Membrane bioreactor (MBR) technology has been used for years in wastewater treatment as a modification of the conventional activated sludge process where the separation of the effluent is facilitated by membrane filtration instead of sedimentation. The advantages of the MBR process are the control of solids, a high effluent quality, good retention of all microorganisms and viruses, maintenance of high biomass concentration, and a real compactness. However, a major limitation in membrane filtration is the significant reduction of permeate flux caused by membrane fouling. Membrane fouling is defined as permeated flux decline due to the retention/accumulation/adsorption of substances within membrane pores and/or onto the membrane surface.

Recent studies have quantified the fouling caused by several fractions of sludge (suspended solid, colloids and solutes). It is surprising to observe a very strong divergence in the fouling phenomenon interpretation: Lee et al. [1] reported that the relative contribution of supernatant to overall membrane fouling was at a maximum value of 37%, whereas Bouhabila et al. [2] reported that the supernatant contributed 76% to the fouling resistance. Wisniewski and Grasmick [3] explained that 50% of the total resistance could be due to soluble compounds.

In spite of the differences of these results, the extracellular polymeric substances (EPS) content of activated sludge is a well known factor controlling membrane fouling [4–6]. A large variety of exocellular polymers can be found in the mixed liquor of sludge resulting from biotreatment of used water. EPS come from the natural secretions of bacteria, cell lysis and hydrolysis products. Among them, proteins and carbohydrates are the most numerous components [7,8]. These EPS are also known to have the most important role in the cohesion of the flocs of the sludge [6,9]. They are responsible for the

attraction between the solid particles. The presence of EPS in mixed liquor is directly correlated to the flocculation state of the sludge. As flocs can be considered as solids in the mixed liquor, their size and electrical surface charge have effects on the filterability of the sludge.

The extent to which EPS production occurs at the expense of biomass synthesis depends partly on the growth rate of organisms and the nutrient status of the growth medium. Many investigators have shown that nitrogen limitation shifts partitioning of the substrate carbon toward EPS production, reducing the yield of cellular biomass [5,10].

The relationship between the EPS and fouling is pointed out in this paper through the establishment of a link between the specific resistance of the sludge, measured in the dead-end mode, and the quantification of EPS, proteins and carbohydrates in the mixed liquor. With this aim of investigation, we have firstly related the soluble EPS to the irreversible fouling of the membrane. Secondly, the effect of the physiological states of activated sludge on the production of EPS was investigated. The objective of reducing the amount of EPS entering the membrane system should be reached by an optimisation of the biological reaction.

2. Materials and methods

2.1. Reactor and membrane

The experimental study was performed using a submerged membrane bioreactor (SMBR) represented in Fig. 1. A U-shaped, hollow-fiber membrane module with an area of 0.3 m² (provided by Polymem, Fourquevaux, France), was immersed in a bioreactor of 10.5 l of working volume. Hollow fibers were made of polysulfone with a pore size of 0.1 μm, and internal/external diameter of 0.4/0.7 mm.

The SMBR was initially filled with activated sludge from the Toulouse-Ginestous wastewater

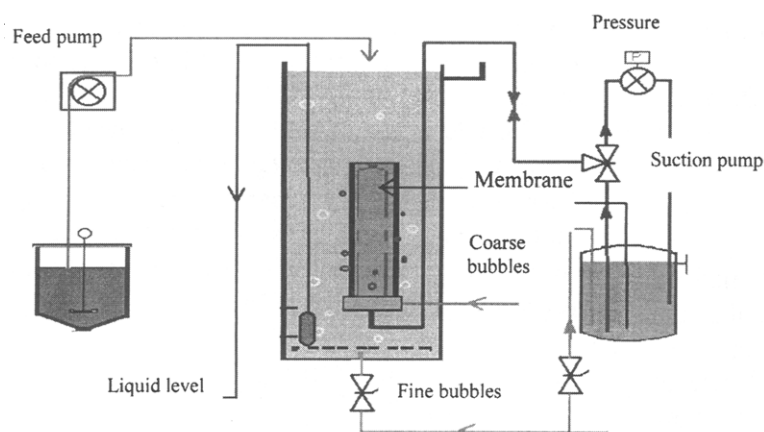


Fig. 1. Scheme of the submerged membrane bioreactor.

plant. A synthetic effluent was then continuously introduced. The synthetic effluent is composed of a cooking sauce (called Viadox, made of meat extract, yeast extract, sugar and soya sauce), complemented with nitrogen salt, and diluted with tap water to reach the composition indicated in Table 1. The fresh water and complemented substrate (Viadox) are introduced separately in the reactor. The fresh water flow rate is controlled by the liquid level in the reactor, whereas the synthetic solution is continuously introduced. Thus, the parameters of hydraulic retention time (HRT) and loading are independent.

The coarse bubbles were injected closed to the fibers providing a tangential liquid movement. The flow was 100 l/m² injected during 1 min every 6 min. These conditions were proposed by Van Kaam [11] to avoid the membrane fouling by reduction of cake formation (reversible fouling), whereas fine bubbles were injected through a perforated membrane at the bottom of the reactor providing mixing and biomass oxygenation.

Filtration was operated in an intermittent sequence of filtration–relaxation–backflushing. The transmembrane pressure (TMP) was continuously monitored as an indicator of membrane fouling. The operation was stopped when the TMP reached 0.6 bar under atmospheric pressure, and a chemical cleaning was applied. Chemical cleaning consists of continuous contact of the

Table 1

Composition of synthetic effluent (in g/l)

COD	82.5
NTK	6.25
N-NH ₄	1.3
P-PO ₄	1.1

Table 2

Operating conditions

Working volume, l	10.5
Filtration flux, l/h m ²	10
Cycle filtration/relaxation, min	5/5
Cycle back flushing, s	30/3600, 1 bar
HRT, h	8 h
Organic loading, g COD/g MLSS.d	0.3–0.4
pH	6.8–7.5

membrane with solutions of 2 M chlorine for 2 h, and 0.1 N NaOH for 24 (Table 2).

2.2. Analytical methods

2.2.1. Solid concentration

Analyses of the mixed liquor suspended solids (MLSS), chemical oxygen demand (COD) for the sludge, supernatant, and permeate were conducted, respectively, using the procedures described in standardized methods:

- MLSS: centrifugation at 5000 rpm for 10 min and drying at 105°C for 24 h.
- COD: micro method COD 420, Odyssey, Hach.

2.2.2. EPS concentration

The EPS quantification was made on the sludge supernatant that had been obtained by centrifugation at 5000 rpm for 20 min, and on the suspended solid. The EPS from the suspended solid were extracted by addition of 2 N NaOH at 4°C for 5 h. The extracted solutions were then centrifuged at 20,000 rpm for 20 min and filtrated on a 0.2 μm membrane.

The total protein and carbohydrate contents were measured to study their influence on sludge fouling ability. Proteins and polysaccharides were analyzed by spectrophotometric methods. The Lowry method was used for protein analysis with bovine serum albumin as a reference [12]. For quantitative analysis of polysaccharides, the anthrone method was used with glucose as a reference [13].

2.2.3. Flocculation of colloids

From a sludge sample, centrifugations (5000 rpm for 20 min) led to obtaining a supernatant that contained colloids and solutes. The colloids were removed by flocculating them with 250 mg/l of $\text{Al}_2(\text{SO}_4)_3$, followed by a second centrifugation (5000 rpm at 20 min).

2.3. Dead-end filtration tests

Dead-end filtration tests were performed to investigate the contribution of various constituents of the sludge for membrane fouling. The filterability of activated sludge is an important indicator for the fouling of membrane bioreactors, and thus filtration index measurements were performed in a special cell. The experimental filtration device was a Sartorius filtration pressured cell, with a working volume of 50 ml,

on a plane organic membrane of cellulose acetate; 47 mm diameter, filtration area 0.17 cm^2 and pore size 0.2 μm .

Considering the filtration resistance as a deposit, the specific resistance was calculated at least once a week to characterize the fouling ability. For a given pressure ΔP , the specific resistance, α , can be calculated using Eq. (1) (dead-end filtration law) [14].

$$\frac{t}{V} = \left(\frac{\mu \alpha C}{2 \Delta P \Omega^2} \right) V + \frac{\mu R_{\text{ini}}}{\Delta P \Omega} \quad (1)$$

where μ is the viscosity of the sludge (Pa.s), C is the biomass concentration (kg/m^3), Ω is the membrane surface (m^2), V is the volume filtrated (m^3), ΔP is transmembrane pressure (Pa); R_{ini} is the membrane initial resistance (m^{-1}) and t is the time (s).

3. Results

3.1. Relations between specific resistance of sludge and extractable extra cellular polymers substances concentration

The extent of fouling was known [4] to vary according to the mixed liquor composition in the bioreactor. In fact, membrane fouling is the result of the contact of the membranes with both fractions of the mixed liquor: (1) soluble fraction and (2) suspended solids including biomass and other colloids.

The EPS in the sludge was distinguishable into two classes [6]: the extractable EPS, which envelope the cell, and the suspended exopolymers which are dissolved in solution. Whatever their origin, they were quantified in terms of COD, proteins, polysaccharides and humic substances. In this study, the last parameter was not investigated because of the choice of the synthetic effluent that does not contain humic substances.

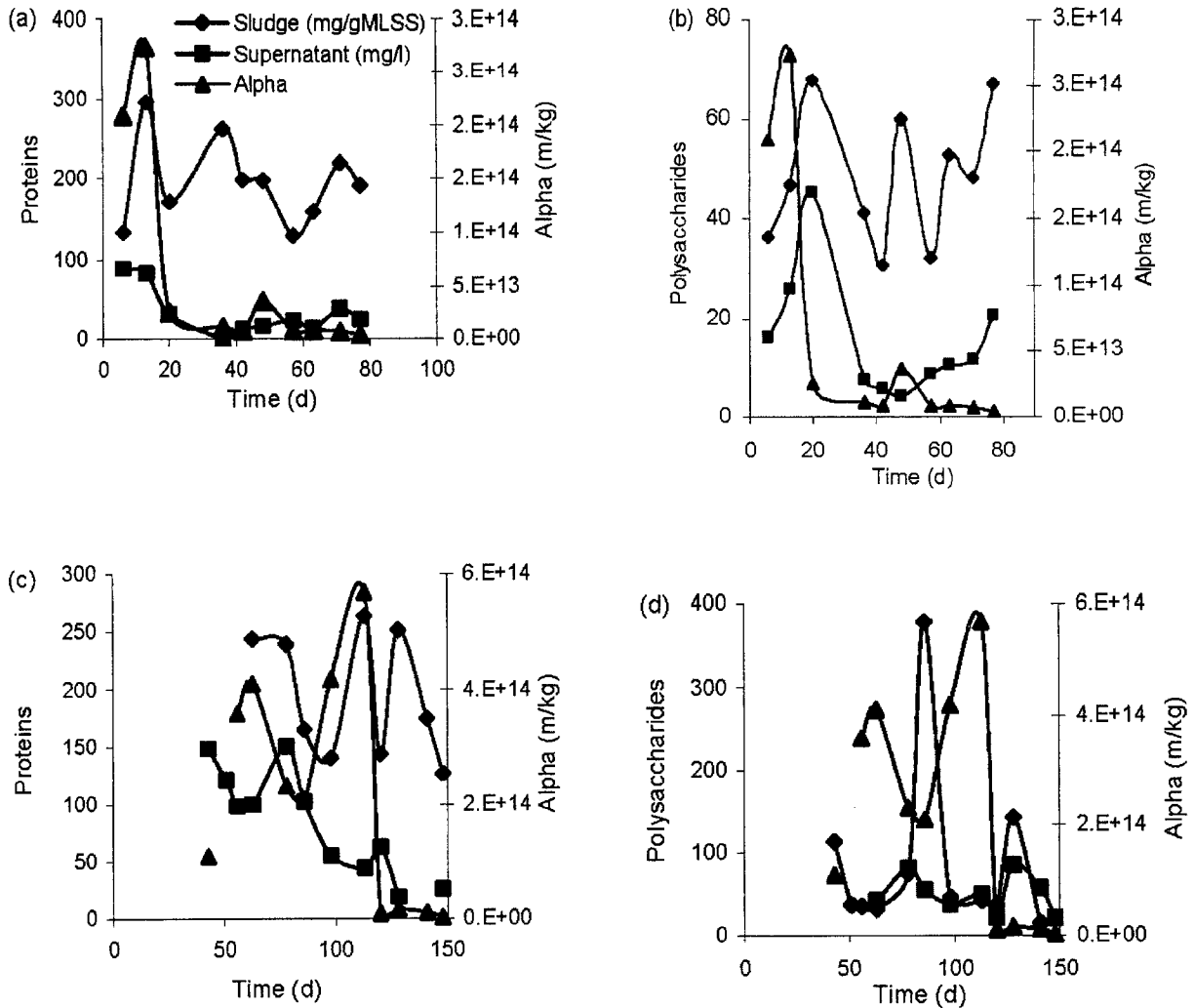


Fig. 2. Variations of the chemical compositions of the sludge and its specific resistance. a, b. Sludge retention time 10 days; carbon loading kg COD/kg MSSL.d 0.4, MLSS 4.7±1.3 (g/l). c, d. Sludge retention time 20–30 days, carbon loading kg COD/kg MSSL.d 0.4, MSSL 7–10 (g/l).

The values of the specific resistance α were reported vs. operating time in Fig. 2, as well as the EPS concentrations in the flocs (solid part of the sludge) and EPS concentration in the liquid fraction of the sludge. These results were obtained during two series of long experiments under different operating conditions, low suspended solid concentration and low sludge retention time of 10 days (Fig. 2a and b), and higher

solid concentration with sludge retention time of 20 and 30 days (Fig. 2c and d).

3.1.1. Solid part of the sludge

The observation of the curves (Fig. 2b and d) reporting the specific resistance values and the EPS concentrations in the solid part of the sludge leads to the following. The quantity of polysaccharides and proteins in the flocs has no

correlation with the filtration ability of the sludge. The concentration of extractable EPS presents fluctuations with time which are not simultaneous nor coupled with the variations of the specific resistance. Moreover, it is noticeable that the variations of the EPS concentration have no obvious regular evolution; the variations could be increasing as well as decreasing.

3.1.2. Liquid part of the sludge

The observation of the curves (Fig. 2a and c) reporting the specific resistance and the EPS concentrations in the liquid fraction of the sludge leads to the following: The amount of extractable EPS quantified as proteins decreased during the first 20 days of operation. After that, the production remained under values of 30 mg/l. This was observed independently of the sludge retention times.

A good consistency can be seen between the variations of the specific resistance and the variations of the EPS concentrations in liquid. Positive variations in the concentration of the exopolymers were directly simultaneous with the increase of the specific resistance. This increase was due to a greater fouling of the membrane, which was, by the way, attributed to the compounds in the supernatant. Fig. 3a and b emphasize the effect of EPS of the supernatant.

3.2. Variations of the proteins and polysaccharides of the supernatant liquor as a function of sludge retention times

Based on the previous observations, the compounds found in the supernatant are those responsible for the fouling of the membrane. Fig. 3a and b present the evolution of the specific resistance of sludge and the concentration EPS in solution when the treatment is operated at a different sludge retention time 10. These concentrations were related to the suspended solid mass unit to erase the effect of solid concentration.

Fig. 3a presents two phases: the first 30 d correspond to a stabilization period where the biomass collected in the municipal plant has to acclimate to the synthetic effluent. The following comments are more concerned with the experiment performed after this period, in a stabilized mode. During the stable period (from day 30, SRT = 10 days), the concentration of proteins and polysaccharides was less than 5 mg/l /gSS, and the specific resistance between 10^{13} – 10^{12} m/kg.

Fig. 3b presents the concentrations and specific resistance obtained for three phases:

- in stabilized mode, for SRT equal to 20 d (from day 40 to 80)
- in transient mode, for SRT coming from 20–30 d (days 80 to 115)
- in stabilized mode, for SRT equal to 30 d, (from day 115 to 150).

For SRT equal to 20 d (Fig. 3b, all in stabilized mode), the concentration of the proteins and polysaccharides increased to values equal to 10 mg/l/ gSS (compared to SRT = 10 days) and the specific resistance increased to 10^{14} m/kg. For the SRT of 30 d, the specific resistance and the concentration of proteins diminished to a value of 10^{13} m/kg and less than 5 mg/l/gSS, respectively.

From these results it was observed that the major production of compounds responsible for fouling occurred when SRT is equal to 20 d. The explanation is found by considering the sludge activity: for STR equal to 10 d the sludge presents major activity and the substrate is used for the production of biomass; consequently, the excretion of exopolymers is very low. For STR equal to 30 d, the metabolic activity is very low, so that the metabolism of the biomass alone is sufficient to keep it alive.

We showed with the previous graphs that the liquid fraction of the activated sludge contributes markedly to the fouling of membrane, with a preferred link toward the EPS. We also tried to obtain more details on these EPS. Fig. 4 shows the variations observed during 5 months of COD

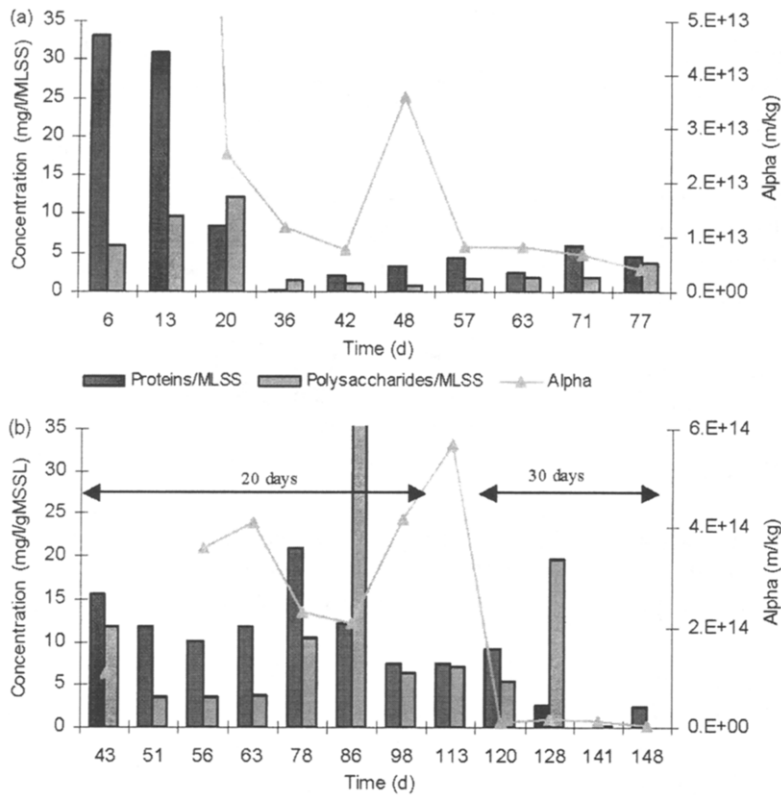


Fig. 3. Variations of the proteins and polysaccharides of the supernatant at different sludge ages. (a) Sludge retention time 10 days, carbon loading kg COD/kg MLSS.d 0.4, MLSS 4.7±1.3 (g/l). (b) Sludge retention time 20–30 days, carbon loading kg COD/kg MLSS.d 0.4, MLSS 7–10 (g/l).

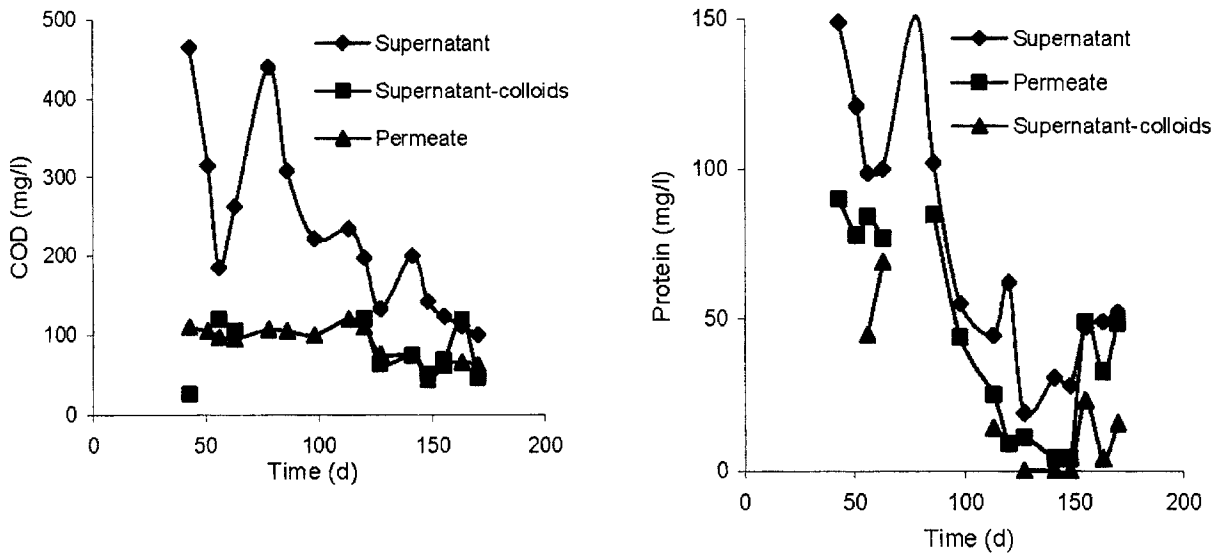


Fig. 4. Comparison of the different sludge fraction compounds. (a) Carbon organic demand. (b) Proteins (idem to polysaccharides). Sludge retention time 20–30 days, carbon loading kg COD/kg MLSS.d 0.4, MLSS 4.7±1.3 (g/l).

concentration in three parts of the liquid involved in the reactor: supernatant of the sludge, the same from which colloids have been eliminated by precipitation (using $\text{Al}_2(\text{SO}_4)_3$ precipitation), and the permeate.

The concentration of COD in the supernatant without colloids is the same as in the permeate (Fig. 4a). This indicates that the compounds that are retained in the membrane are the colloids. The same comparison was made for the polysaccharides and the proteins, before and after precipitation, and the same behaviour was obtained (Fig. 4b). These observations were reported in a different way by Bouhabila et al. [2], who proposed microscopic colloids on a membrane which has filtered water for 60 days from a bioreactor fed with synthetic water. Of all these results, it can be concluded that the colloidal part is the main contributor to fouling of the membrane. Moreover, these colloids are known to have strong affinities with the material of the membrane, which let us suppose that they are mainly responsible for the irreversible part of membrane fouling.

3.3. Performances of the filtration

From the analysis of the biochemical composition of sludge, it was observed that EPS in the supernatant have a great part in membrane fouling. In order to quantify this effect on reactor performances, the TMP vs. time was plotted simultaneously with EPS production (Fig. 5).

The most interesting results are shown in Fig. 5b where a rigorous coupling between TMP vs. time with proteins vs. time was observable. The TMP variations revealed the consequence of fouling. This coupling is then an indication that proteins linked to the sludge play a great part in fouling.

At the same time, Fig. 5a, during the steady-state phase (from day 40 the specific resistance values are quasi-constant), it can then be deduced that proteins of sludge contribute to fouling in a

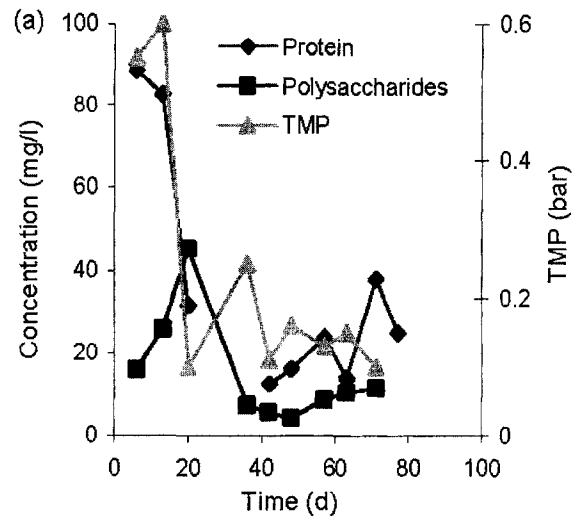


Fig. 5a. Relation between the TMP and the composition of supernatant. Sludge retention time 10 days, carbon loading kg COD/kg MSSL.d 0.4, MLSS 4.7 ± 1.3 (g/l).

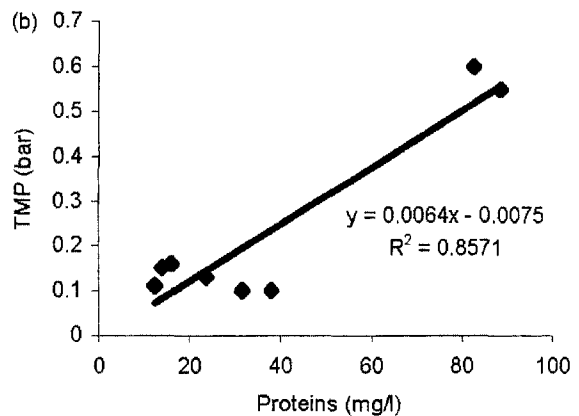


Fig. 5b. Correlation between protein concentration in the supernatant and TMP. Sludge retention time 10 days, carbon loading kg COD/kg MSSL.d 0.4, MLSS 4.7 ± 1.3 (g/l).

form different from usual deposits. They probably play a role in the permeability loss through adsorption phenomena.

3.4. Qualification of bio-physical state of the biomass

From the previous results, it was observed that the excretion of EPS is irregular all along the

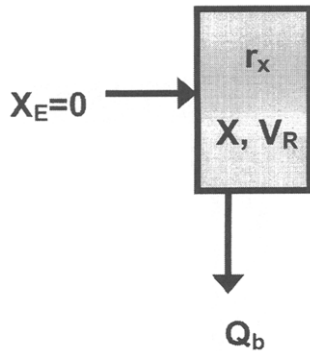


Fig. 6. Suggested conceptual for mass balance on the biomass. Q_b = bleeding flux.

experiments performed. The variations seem to be linked to the physiological state of the biomass, itself linked to the operating phase of the bioreactor (starting/transient phase or stationary phase). In order to characterize these phases, the growth rate of the biomass has been determined, following three steps:

- A mass balance on the biomass was written on the reactor, taking into account the value of the bleeding and ignoring the membrane module that occurred an entrance equivalent to the outlet, as shown in Fig. 6 and Eq. (2).
- The hypothesis was made that the biomass variation is linear on a given range of time [slope k_i , Eq. (3)].
- Finally, after combining both previous equations, an analytical expression of the growth rate is obtained [Eq. (4)].

$$V_r \cdot \frac{dX}{dt} = r_x \cdot V_r - Q_b \quad (2)$$

$$\frac{dx}{dt} = k_i \Rightarrow X = X_i^0 + k_i \cdot (t - t_i^0) \quad (3)$$

for $t_i^0 < t < t_i$

$$r_x = k_i + \frac{Q_{bi}}{V_r} \cdot X_i^0 + \frac{Q_{bi} \cdot k_i}{V_r} \cdot (t - t_i^0) \quad (4)$$

where r_x is the growth rate (g MLSS/l/d), V_r the reactor volume (l), X the biomass concentration (kg/m³), Q_b the bleeding flow rate (m³/d), and k_i is the slope of $X(t)$. Subscript i represents the concerned period.

The values of the growth rates and the corresponding biomass are plotted in Fig. 7.

Fig. 8 presents the evolution of the composition of the supernatant and the values of growth rate as a function of operating time. Fig. 8a concerns the experiment with SRT equal to 10 days. The evolution of the growth rate indicates that the reactor presented a stationary period after the 15th day. During this period, the biomass growth rate increased regularly from 0.16 to 0.20 g/l/d, which is opposite to the evolution of the concentration of proteins which are diminished to half of their initial value during the same time. The concentration of the polysaccharides also diminishes. This decrease, correlated to a stable evolution of the growth rate, is an indication of good functioning of the bioreactor with good flocculation of the sludge.

After this period (after the 40th day), the growth rate presented positive and negative variations, and the concentration of proteins seemed to present variations in the same way. The concentration of polysaccharides diminished, and then it presented a slight tendency to weakly increase. Nevertheless, after the period of stabilization, the variations, of the concentration in proteins as well as carbohydrates, present values around 5 mg/l/g MLSS, which are low values.

Fig. 8b concerns the experiment with SRT equal to 20 and 30 d: in the period where STR is equal to 20 d, the micro-organism concentration varied between 7–11 g/l. Nevertheless, in spite of this variation, the growth rate was not modified with respect to the previous conditions of growth. The growth rate stayed between a value of 0.17–0.19. It is important to emphasize that the production of proteins almost doubled to values superior than 10 mg/l. The COD was analyzed with the objective to quantify the totality of

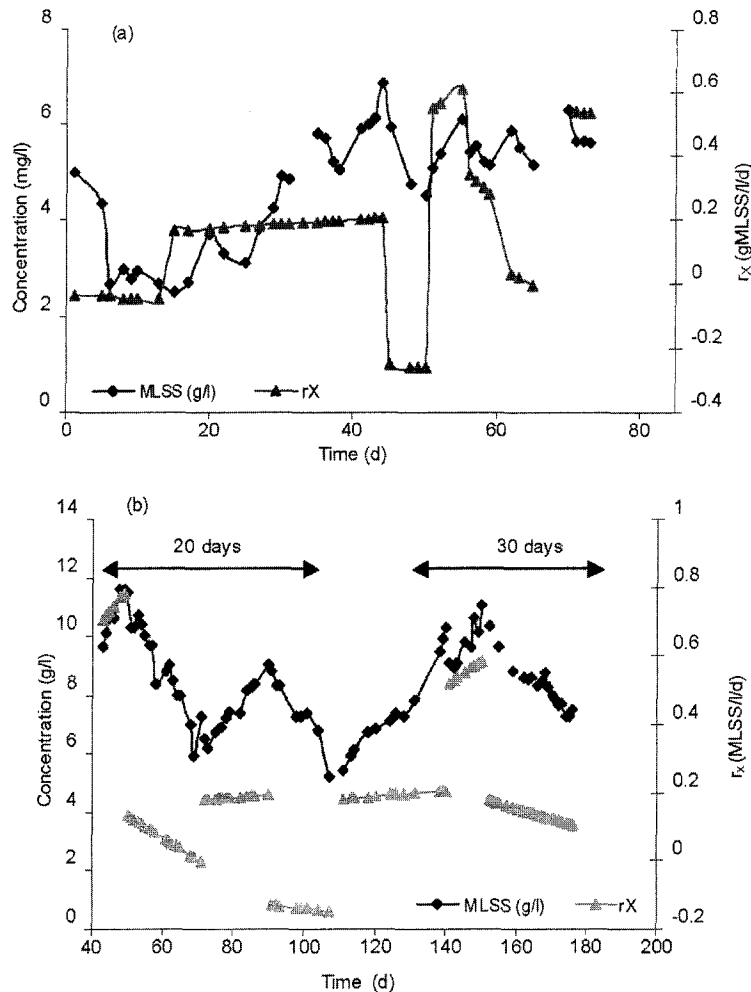


Fig. 7. Relation between the growth rate and biomass concentration. (a) Sludge retention time 10 days, carbon loading kg COD/kgMSSL.d 0.4, MLSS 4.7 ± 1.3 (g/l). (b) Sludge retention time 20–30 days, carbon loading kg COD/kg MSSL.d 0.4, MLSS 7–10 (g/l).

exopolymers expressed as COD. As for the proteins, the concentration of COD obtained in the supernatant was significant, between 20–60 mg/l. With respect to the production of polysaccharides, the COD production does not increase as much as the proteins; the concentration was a little greater than 5 g/l, sometime values of around 50 mg/l were obtained.

After that, the period from 90 to 110 d, was a stage of transition—a SRT of 20 d to a SRT equal to 30 d. During this stage of transition, there were problems of biomass lost due to leakage of the membrane. For this reason the growth rate decreased until negative values were obtained.

After 120 d, a SRT of 30 d was reached. This modification of the operating conditions of the bioreactor led to a modification of the growth conditions; in spite of this change, the growth rate remained between 0.18 and 0.2 g/l/d. The influence of the SRT change was observed in the EPS concentration, which decreased from 20 to 30 d; this tendency was also observed on the COD. It is important to emphasize how quick COD variations are simultaneous with proteins between days 120 to 130, and quite stable COD values corresponded to almost stationary ones for proteins and polysaccharides.

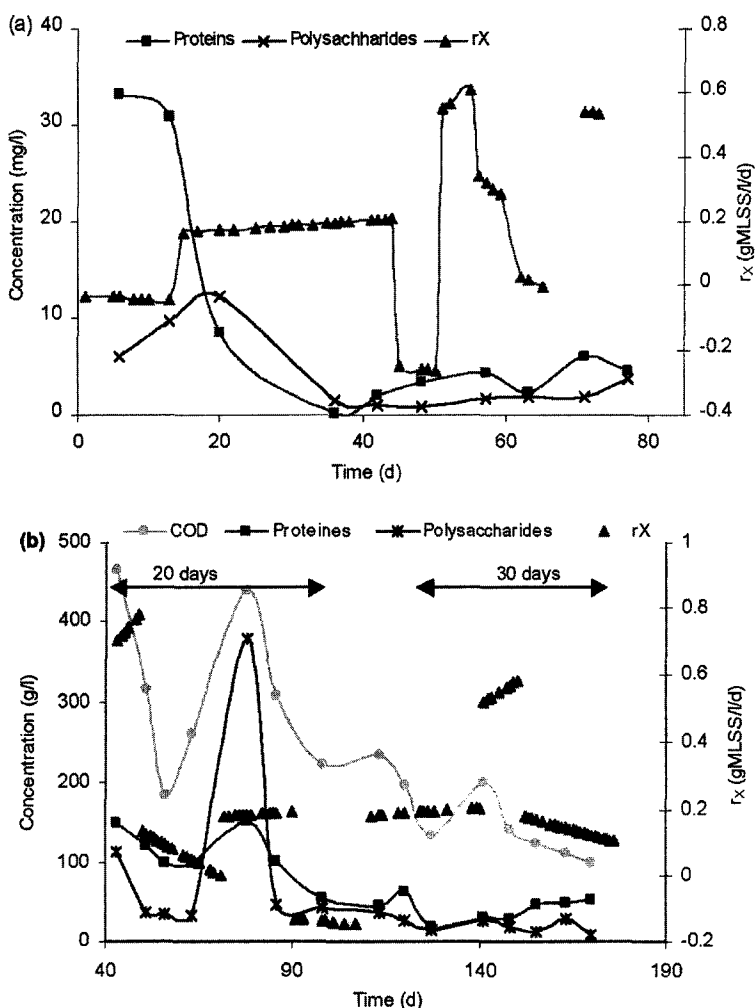


Fig. 8. Relation between the growth rate and the compounds of the supernatant. (a) Sludge retention time 10 days, carbon loading kg COD/kg MSSL.d 0.4, MLSS 4.7 ± 1.3 (g/l). (b) Sludge retention time 20–30 days, carbon loading kg COD/kg MSSL.d 0.4, MLSS 7–10 (g/l).

An explanation of this behaviour can be found in the biomass growth rate observation: In this last period the biomass is probably in nutrient deficit, as its growth rate is decreasing. In fact the biomass concentration was increased from 8 to 10 g/l whereas the feed was not changes, which lead to the nutrient deficit. This hypothesis is confirmed by the observation of Robinson et al. [10] who showed that EPS production is inversely related to growth rate.

4. Conclusions

From the correlation between the values of sludge specific resistance and EPS concentration

in the liquid fractions, we concluded that these compounds play a main role in the fouling of the membrane. Moreover, the specific resistance increases with the concentration of proteins in the supernatant, whatever the operating conditions. We also showed that EPS in the flocs, that is to say, in the solid part of the sludge, have no effect on the specific resistance variations where this has been studied.

In the liquid fraction of the sludge, colloids were identified as soluble compounds that the membrane retains during the different cycles of filtration. This confirms the previous results that show how the soluble EPS are responsible for the membrane fouling. Finally, this evaluation of the

specific resistance through experiments performed on a separated device with samples of sludge, coupled with the knowledge of the EPS contents in the liquid fraction, indicates that this liquid fraction of the sludge has to be carefully examined in the bioreactor.

As the composition of the liquid fraction depends on the operating conditions of the biodegradation in the reactor, these conditions were investigated with regard to liquid composition: the production of soluble EPS depends on the SRT. In a range of SRT from 10–30 d, the greatest EPS production was found at 20 d. Because the range where SRT was investigated is not so large, it was preferred to collect information about the physiological state of the biomass through the calculation of its growth rate. During the periods where the operating conditions (load and bleach) allowed for stable growth, the growth rate is independent of the SRT, and the amount of EPS decreased, probably due to adequate consumption of energy for biomass production.

Variations of EPS were mostly observed during periods where the metabolism is unbalanced; then cell energy lost led to EPS excretion. Most of the time, variations in the main operating conditions, load or bleach, caused a metabolism breakage/breakdown. Moreover, the variation of EPS contents was proportional to the rate changes, although the magnitude is not the same.

Although these results have been obtained from the treatment of synthetic water, they show the sensitivity of the parameters of the bioreaction and their consequences on the filtration performances. In order to optimize the membrane bioreactor functioning and to diminish fouling, it is necessary to adapt the biological parameters to obtain a liquid part of the sludge as free as possible of EPS. These conditions will be tested soon for the treatment of domestic water.

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