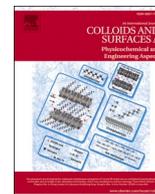




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# Colloids and Surfaces A: Physicochemical and Engineering Aspects

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## Effect of salinity and temperature on the extraction of extracellular polymeric substances from an anaerobic sludge and fouling in submerged hollow fibre membranes

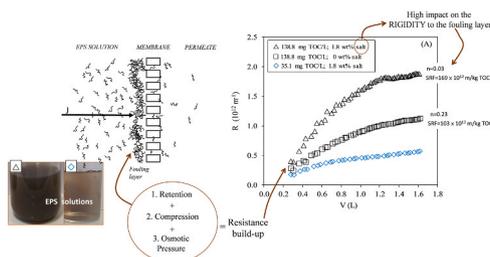
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### HIGHLIGHTS

- Ultrasonication and salt reduction favoured the diffusion of EPS.
- Maximum EPS extraction required over 60 min of ultrasonication with salt-free water.
- Humic substances were the predominant EPS in the side stream AnMBR sludge.
- Salt increased the specific resistance and decreased the gel layer compressibility.
- The retention degree increased with EPS and salt concentration.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

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### ABSTRACT

The results of the characterisation of anaerobic sludge-derived extracellular polymeric substances (EPS) under different extraction conditions and their effects on the fouling in submerged hollow fibre membranes are presented. A wide range of EPS extraction results was obtained depending on the extraction conditions. Proteins, polysaccharides, and humic substances represented 80–99% of the total organic matter extracted, with 54–60% identified as humic substances. Ultrasonication was more effective than vortex agitation, ensuring higher EPS extraction yields in a shorter contact time. The increase in temperature from 30 °C to 50 °C and the decrease in NaCl concentration favoured the EPS diffusion, but this positive temperature effect was negligible for an ultrasonic contact. Linear, quadratic, and combined effects of ultrasonication time and solvent salinity were statistically significant effects for ultrasonication-assisted EPS extraction. The highest extraction yield was achieved using ultrasonication at 30 °C for at least 60 min with salt-free water as the solvent, and an EPS extract with 138.8 mg TOC/L was obtained. Batch ultrafiltration-backwash tests at different permeation rates allowed to determine the specific resistance to filtration of the EPS extracts, which increased from  $103.8 \cdot 10^{12}$  m/kg TOC to  $169.4 \cdot 10^{12}$  m/kg TOC in presence of 1.8 wt% of NaCl. Salinity also provided rigidity to the EPS gel layer, reducing the compressibility index from 0.23 to 0.03.

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| Nomenclature         |   |
|----------------------|---|
| A                    | membrane area   |
| C                    | solute concentration (g/L)  |
| C <sub>NaCl</sub>    | sodium chloride concentration in the solvent for the extraction process (wt%) |
| h                    | parameter of Eq. (2)  |
| HS                   | humic substances  |
| J                    | permeate flux (m <sup>3</sup> /m <sup>2</sup> ·s)                             |
| L <sub>p</sub>       | permeability of the membrane (L/m <sup>2</sup> ·h·bar)                        |
| m                    | mass of solute retained by the membrane (g)                                   |
| n                    | compressibility index   |
| P                    | proteins  |
| PS                   | polysaccharides   |
| r                    | linear regression coefficient   |
| R                    | filtration resistance (m <sup>-1</sup> )                                      |
| R <sub>bw</sub>      | backwash resistance (m <sup>-1</sup> )  |
| R <sub>n</sub>       | normalised resistance (m <sup>-1</sup> )                                      |
| R <sub>0</sub>       | initial resistance (m <sup>-1</sup> )   |
| R <sub>S</sub>       | resistance due to total retention of solute (m <sup>-1</sup> )                |
| R <sup>2</sup>       | determination coefficient   |
| SRF                  | specific resistance to filtration (10 <sup>12</sup> m/kgTOC)                  |
| s                    | parameter of Eq. (2)  |
| t                    | contact time for the extraction process (min)                                 |
| t <sub>min</sub>     | minimum ultrasonication time (min)  |
| T                    | temperature (°C)  |
| TS                   | total solid   |
| TMP                  | transmembrane pressure (Pa)   |
| TOC                  | total organic carbon  |
| thTOC                | theoretical value of total organic carbon by Eq. (20)                         |
| V                    | volume filtered (L)   |
| w <sub>i</sub>       | mass extracted per unit of mass of sludge (g/kg)                              |
| w <sub>0i</sub>      | mass extracted at time 0 (g/kg)   |
| w <sub>∞i</sub>      | ultimate mass extracted (g/kg)  |
| <i>Greek symbols</i> |   |
| α                    | degree of retention (%)   |
| β <sub>0</sub>       | offset term   |
| β <sub>n</sub>       | slope or linear effect  |
| β <sub>nn</sub>      | quadratic effect  |
| β <sub>nn'</sub>     | linear by linear interaction effect   |
| κ                    | adjustment parameter power-law compressibility correlation                    |
| μ                    | viscosity of the permeate (Pa·s)  |

## 1. Introduction

Membrane fouling is still a problem in anaerobic membrane bioreactor (AnMBR) technology. A reduction in the cleaning stages of the bioreactor membrane, longer filtration times and higher quality water as permeate are required to improve the saline wastewater treatment. The water-soluble fraction of extracellular polymeric substances (EPS), which is also known as soluble microbial products (SMP), has high fouling potential in membrane bioreactors (MBR) [1–3]. It is widely known that EPSs are mainly composed of proteins, polysaccharides, humic substances, nucleic acids, and lipids, which can be originated from cell lysis, microbial metabolism, or similar unmetabolized wastewater components [4–6]. EPS production depends on different factors, such as solid retention time (SRT), organic loading rate (OLR), carbon-to-nitrogen ratio (C/N) or shear stress. EPS concentration in the mixed liquor is closely connected to sludge characteristics, such as sludge volume index, flocculation ability, hydrophobicity, surface charge, and viscosity. This dissolved matter (soluble EPS), with high gelling properties, can cause severe fouling by collecting upon the pores and spreading over the surface of the membrane, which will lead to continuous changes in transmembrane pressure (TMP) and in membrane permeability along the wastewater treatment.

Extraction can be employed for the characterization of the organic matter derived from sludge. Different solvents such as water, sodium chloride solution, 2,2',2'',2'''-(Ethane-1,2-diyl)dinitrilo)tetraacetic acid (EDTA), formaldehyde, formaldehyde plus sodium hydroxide, acetone, and ethanol, have already been used in the EPS extraction [3,7–11]. Conventional stirring [10,12], vortex agitation [3,8,9,11,13], and ultrasonication [7,10,13,14] are commonly used to increase the EPS diffusion rate. Although short contact times of between 1 and 2.5 min are generally used, contact times of up to 45 min have also been reported [15,16]. The literature gives variable values for the extraction temperature. Frølund et al. [12] and Liu et al. [10] carried out the extraction of EPS with refrigeration at 4 °C, Morgan et al. [11] at room temperature, Ding et al. [9] at 50 °C, Han et al. [7] at 60 °C, Li et al. [8] at 70 °C and Cosenza et al. [3] at 80 °C. However, a high temperature can lead to a partial denaturation of EPS. It is also reported that different solvents and extraction techniques led to a slightly preferential extraction of different EPS [10,17,18]. Liu et al. [10] analysed the effectiveness of different solvents under different extraction conditions and found that the extraction of humic substances increased by using EDTA as the solvent.

Salinity can also influence membrane fouling [19]. An increase in sodium chloride has been shown to cause a decrease in membrane permeability, which was recovered when the salt is removed [20]. Several researchers have already reported the importance of osmotic pressure in cake-layer filtration caused by the retention of different ions within the matrix of biopolymers in the cake [21–23]. Ionic strength directly affects osmotic pressure and filtration resistance by reducing the cake layer porosity when the ionic concentration increases [22]. The effect of osmotic pressure depends on the salt concentration in the mixed liquor, but it also depends on the compression of the cake layer. When TMP increases, water migrates from the cake and osmotic pressure increases, acting as a resistance to external forces applied to the cake [23]. Although a gel layer is very porous, SMP and colloids in the gel pores provide negatively charged positions to retain counter-ions, thus increasing the filtration resistance of the gel layer even above that of the cake layer [24].

The aim of this study was to evaluate the extraction of EPS from the anaerobic sludge of a pilot scale side-stream AnMBR in order to determine the effect of these soluble polymeric substances on the membrane fouling. The response surface methodology with a Box-Behnken design (BBD) has been employed to examine the combined effect of contact time, temperature, and salinity on the extraction efficiency. The ratios of proteins, polysaccharides and humic substances in the total extracted organic matter were calculated based on the experimental and theoretical TOC values. A kinetic study was also performed under the BBD optimal conditions to obtain the extraction velocity and the minimum contact time. Filtration tests were carried out with a submerged ultra-filtration hollow-fibre to assess the impact of polymeric substances and salinity on membrane fouling. A fouling model has also been proposed to determine the specific resistance to filtration and retention degree of soluble EPS under the same filtration/backwashing cycles of an AnMBR system.

## 2. Experimental

### 2.1. Materials

The sludge used came from a pilot scale side-stream AnMBR plant installed in a snack factory. Total and volatile solid concentration were 26.3 g TS/L and 15.3 g VS/L, respectively. Bovine serum albumin, D

(+)-anhydrous glucose and sulphuric acid were supplied by VWR Chemicals (Germany) and humic acid was supplied by Sigma-Aldrich (USA). Sodium chloride and phenol were purchased from Labkem (Spain) and Riedel-de Haën (Germany), respectively. Folin-Ciocalteu reagent, copper (II) sulphate pentahydrate, potassium antimony (III) tartrate trihydrate and sodium hydroxide were provided by Panreac AppliChem (Spain).

## 2.2. Extraction tests

Extraction of EPS from the anaerobic sludge was carried out using mixtures of 0.5 wt% of total solids in the solvent. Distilled water and NaCl aqueous solutions were used as the solvent. Contact between the sludge/solvent phases was performed using vortex agitation (VORTEX MVOR-03-SBS) or by ultrasonication (Ultrasons-H Selecta). EPS analysis was carried out on the supernatant obtained by centrifugation (Eppendorf centrifuge, Centrifuge 5804) at 4500 rpm for 5 min. Total organic carbon (TOC) was measured using a TOC-VCSN (Shimadzu) analyser. Total concentration of proteins (P), polysaccharides (PS) and humic substances (HS) were determined according to the Bradford method [25], Dubois method [26] and Lowry's modified method [27], respectively. Standards for calibration were dissolved in distilled water with 0, 0.9 and 1.8 wt% of sodium chloride. Spectrophotometric readings were taken using a Hitachi U-2000 UV/vis spectrophotometer. A determination coefficient higher than 0.99 was obtained for all calibration curves. Extracts were analysed in triplicate under identical conditions. The experimental extraction results were expressed in units of mass of P, PS, HS, and TOC extracted per unit of mass of sludge as total solid ( $w_i$  for  $i = P, PS, HS$  and TOC, g/kg TS). A Statgraphics Centurion 18 software package (Statistical graph Co., Rockville, MD, USA) was used for statistical processing of the experimental data.

Initial extraction tests were performed using distilled water and NaCl solutions at 30–50 °C with 5–10 min of ultrasonication or vortex agitation. Significant differences between EPS extracts were determined by analysis of variance (ANOVA) using the general linear model procedure. An alpha level of 95% ( $p < 0.05$ ) was used to determine significance.

In a second stage, the individual and combined effect of ultrasonication time ( $t = x_1$ ), NaCl concentration ( $C_{NaCl} = x_2$ ), and temperature ( $T = x_3$ ) on EPS extraction was investigated. Fifteen ultrasonication-assisted extraction tests were performed using a Box-Behnken design (BBD) by combining three levels of each independent factor ( $x_n$ ). The factor levels were coded as  $-1$  (0 min of ultrasonication, 0 wt% NaCl, 30 °C), 0 or central point (30 min of ultrasonication, 0.9 wt% NaCl, 40 °C), and  $+1$  (60 min of ultrasonication, 1.8 wt% NaCl, 50 °C). The mass fractions  $w_P$ ,  $w_{PS}$ ,  $w_{HS}$  and  $w_{TOC}$  were selected as response. The results were analysed using the determination coefficient ( $R^2$ ), the ANOVA analysis and the response and Pareto plots. A non-linear regression method was employed to fit the second-order polynomial (Eq. (1)) to the experimental data of each response and to identify the relevant model terms [28]. Considering all the linear terms, square terms, and linear-by-linear interaction items, the quadratic response model can be expressed as [28]:

$$w_i = \beta_0 + \sum \beta_n \cdot x_n + \sum \beta_{nn} \cdot x_n^2 + \sum \beta_{nn'} \cdot x_n \cdot x_{n'} \quad (1)$$

where,  $\beta_0$  is the offset term,  $\beta_n$  is the linear effect,  $\beta_{nn}$  is the quadratic effect,  $\beta_{nn'}$  is the linear by linear interaction effect between the independent factors  $x_n$  and  $x_{n'}$ , and subscript  $i$  refers to the groups of compounds ( $i = P, PS, HS$  and TOC).

A kinetic study was also carried out, under the selected optimal extraction conditions from BBD results, in order to determine and compare the extraction rate of P, PS and HS. Samples of the EPS extract were taken every 10 min until reaching a constant concentration of P, PS and HS, from which the ultimate extracted mass per gram of sludge,  $w_{\infty i}$ , was calculated. Samples were also taken at 3 h and 6.5 h in order to corroborate the value of  $w_{\infty i}$ . The following  $s$ -order kinetic model was employed to fit the experimental kinetic data [29,30]:

$$\frac{dw_i}{dt} = h_i (w_{\infty i} - w_i)^s \quad (2)$$

where  $w_i$  is the mass fraction of each solute extracted at a given time  $t$ ,  $s$  is the kinetic order of the equation, and  $h_i$  is the extraction rate constant for P, PS or HS. The boundary conditions were from  $t = 0$  to  $t$  and from  $w_{0i}$  to  $w_i$ , and the value of  $w_{0i}$  was the mass of P, PS, and HS extracted at time 0, without application of ultrasonication.

The integrated form of Eq. (2) for  $s = 0, 1$  and  $2$  is shown in Eqs. (3)–(5), respectively, which were used to estimate  $w_i$  during the extraction process.

$$w_i = w_{0i} + h_i \cdot t \quad (3)$$

$$w_i = w_{\infty i} - (w_{\infty i} - w_{0i}) \cdot e^{-h_i \cdot t} \quad (4)$$

$$w_i = w_{\infty i} - \frac{w_{\infty i} - w_{0i}}{1 + h_i \cdot t \cdot (w_{\infty i} - w_{0i})} \quad (5)$$

Eqs. (6) and (7) show the linearized form of Eqs. (4) and (5), respectively.

$$\ln(w_{\infty i} - w_i) = \ln(w_{\infty i} - w_{0i}) - h_i \cdot t \quad (6)$$

$$\frac{t}{w_i - w_{0i}} = \frac{1}{h_i \cdot (w_{\infty i} - w_{0i})^2} + \frac{1}{w_{\infty i} - w_{0i}} \cdot t \quad (7)$$

Eqs. (3), (6) and (7) were employed to fit the experimental kinetic data and  $h_i$  was calculated for each group of EPS. The average absolute relative deviation (AARD) calculations according to Eq. (8), the root-mean square error (RMSE) calculations according to Eq. (9)  $R^2$  were used to assess the goodness of fit of these kinetic models.

$$AARD = \frac{100}{N} \cdot \sum_{j=1}^N \frac{|w_{i(\text{exp})} - w_{i(\text{cal})}|_j}{w_{i(\text{exp})}} \quad (8)$$

$$RMSE = \sqrt{\frac{\sum_{j=1}^N (w_{i(\text{exp})} - w_{i(\text{cal})})_j^2}{N}} \quad (9)$$

where  $N$  is the number of experimental data, and  $w_{i(\text{exp})}$  and  $w_{i(\text{cal})}$  are the mass of each solute obtained experimentally and estimated by the model, respectively. Eqs. (3)–(5) were employed to determine the values of  $w_{i(\text{cal})}$ .

## 2.3. Ultrafiltration tests

The degree of retention ( $\alpha$ ), the specific resistance to filtration (SRF), and the compressibility index of the EPS gel layer ( $n$ ) were determined. For that purpose, two extracts were selected, the ones with the highest and lowest EPS concentration. An additional test was carried out in which 1.8 wt% salt was added to the extract with the highest EPS concentration in order to assess the contribution of salinity to the fouling process.

Fouling capacity was determined using batch dead-end tests lasting 20 h with total recirculation of the permeate, using the experimental setup shown in Fig. 1. A PVDF hollow-fibre ultrafiltration membrane (Micronet R, Porous Fibers) was used with a nominal pore size of 0.03  $\mu\text{m}$ , a length of 0.92 m, a diameter of 2.4 mm and an effective filtration area of 0.00694  $\text{m}^2$ , rolled up on a support 6 cm in diameter. The membrane was connected at each end to a variable-speed reversible peristaltic pump (Watson Marlow 520U). The membrane with 0.8 L of extract was placed in a hermetically sealed 1 L stirred tank. A permeate vessel was placed inside the tank to store a small fraction of the permeate stream for backwashing. TMP and temperature were monitored using electronic probes PN2069 (IFM Electronics) and TR2432 (IFM Electronics). The filtration flux was determined from the pump calibration, the speed of which was controlled by a PLC. After each

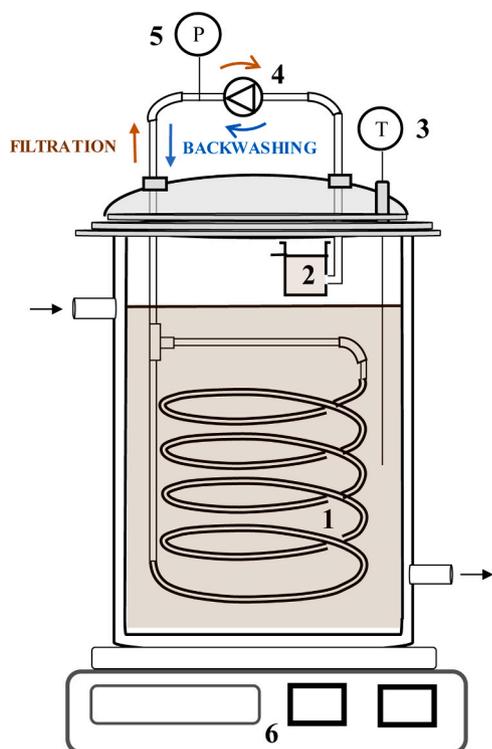


Fig. 1. Schematic diagram of experimental setup. 1. Membrane module inside a jacketed tank filled with an EPS extract; 2. Permeate vessel; 3. Temperature sensor; 4. Reversible pump; 5. Pressure probe; 6. Magnetic stirrer.

filtration test, the membrane was rinsed with tap water, and a chemical cleaning was carried out using a 500 mg/L sodium hypochlorite solution for 2 h. This cleaning allowed to recover the initial water permeability of the membrane,  $1456 \pm 42 \text{ L}/(\text{m}^2 \cdot \text{h} \cdot \text{bar})$ .

Since the materials deposited on the membrane are highly deformable, resistance to filtration is affected by TMP and therefore by the flux rate. To correct that effect, resistance was determined at six different fluxes,  $J$ , and using filtration/backwashing cycles carried out on a continuous basis, as shown by Fig. 2. The flux series did not follow an upward or downward pattern, to avoid hysteresis effects. Filtration fluxes were initially set at 42, 52, 68, 84, 102, and 118  $\text{L}/\text{m}^2 \cdot \text{h}$ . TMP was kept between 50 mbar and 250 mbar in all filtration tests. Fluxes were automatically reduced when TMP exceeded 250 mbar. Thus, membrane has filtered at least twice the volume of EPS solution contained in the tank at the end of each test, which represents a minimum of  $231 \text{ L}/\text{m}^2$  of membrane.

Filtration resistance ( $R, \text{m}^{-1}$ ) has been determined by the resistance-in-series model (Eq. (10)) using the experimental TPM (Pa) at the beginning of the filtration stage for each flux  $J$  (see Fig. 2).

$$R = \frac{\text{TMP}}{J \cdot \mu} \tag{10}$$

where  $\mu$  is the permeate viscosity (Pa·s), which depends on the temperature,  $T$  ( $^{\circ}\text{C}$ ), in accordance with the equation:

$$\mu = \frac{0.479}{(T + 42.5)^{1.5}} \tag{11}$$

Logarithmic regression of  $R$  vs TMP was used to fit the resistance of the six preceding filtration cycles to the well-known power-law expression (Eq. (12)) and the compressibility index,  $n$ , was calculated [31–33].

$$R = \kappa(\text{TMP})^n \tag{12}$$

Parameters  $\kappa$  and  $n$  have been used to calculate the normalised resistance,  $R_n$ , at a reference pressure ( $\text{TMP}_{\text{ref}}$ ) of 100 mbar:

$$R_n = R \left( \frac{\text{TMP}_{\text{ref}}}{\text{TMP}} \right)^n \tag{13}$$

After correcting the effect of compressibility, SRF of the materials deposited on the membrane can be considered constant at the reference

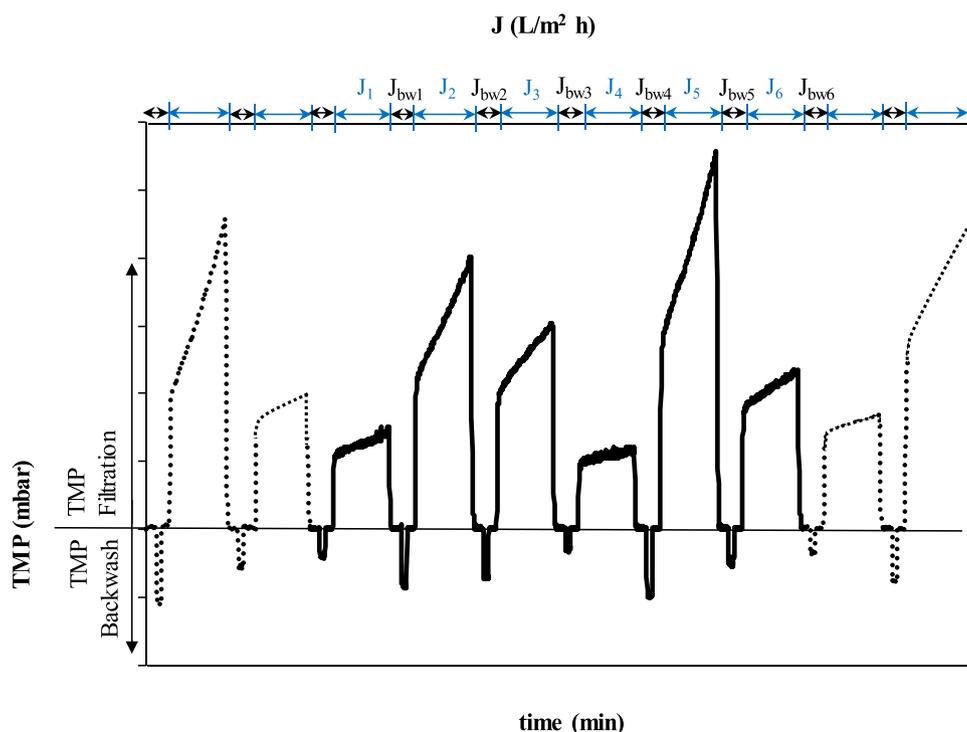


Fig. 2. Characteristic pressure profile (TMP) during the ultrafiltration tests.  $J$  and  $J_{\text{bw}}$  are the filtration flux and the backwashing flux, respectively.

pressure. Assuming that  $\alpha$  is independent of the membrane fouling level, the increase in resistance per unit of filtered volume (Eq. (14)) must be proportional to the EPS concentration,  $C$ , and the decrease in solute concentration can be expressed by Eq. (15) [33].

$$\frac{dR_n}{dV} = \frac{SRF \cdot dm/A}{dV} = SRF \cdot \frac{\alpha \cdot C \cdot dV}{A \cdot dV} = \frac{SRF}{A} \alpha \cdot C \quad (14)$$

$$-dC = \frac{dm}{V_t} = \frac{\alpha \cdot C \cdot dV}{V_t} \quad (15)$$

The concentration of EPS as a function of filtered volume is obtained by integrating Eq. (15) from the initial state, when the concentration is  $C_0$ , and after filtering a volume  $V$ :

$$C = C_0 \cdot e^{-\frac{\alpha V}{V_t}} \quad (16)$$

Combining Eqs. (14) and (16) and integrating with the boundary conditions from  $V = 0$  and  $R_0$  to  $V$  and  $R$ , the resistance can be expressed as:

$$R_n = R_0 + \frac{SRF \cdot V_t \cdot C_0}{A} \cdot (1 - e^{-\alpha V/V_t}) \quad (17)$$

where,  $m$  is the mass of solute retained by the membrane,  $A$  is the membrane area,  $V$  is the volume filtered over time,  $V_t$  is the total volume of solution in the filtration tank,  $0.8$  L and  $R_0$  is the resistance at the beginning of the test or the unrecovered resistance after the chemical cleaning.

The product of SRF multiplied by the total mass of solute per unit of area,  $V_t \cdot C_0/A$ , represents the resistance due to total solute retention,  $R_S$ :

$$R_S = SRF \cdot \frac{V_t \cdot C_0}{A} \quad (18)$$

Finally, Eq. (19) shows the linearized form of Eq. (17), which was employed to obtain  $\alpha$  and SRF from the variation of  $R_n$  with the filtered volume. To minimise bias, robust regressions were carried out using Huber's method, using 1.345 as tuning parameter to obtain the regression parameters with a confidence level of 95%.

$$\ln(R_0 + R_S - R_n) = \ln R_S - \frac{\alpha}{V_t} \cdot V \quad (19)$$

### 3. Results and discussion

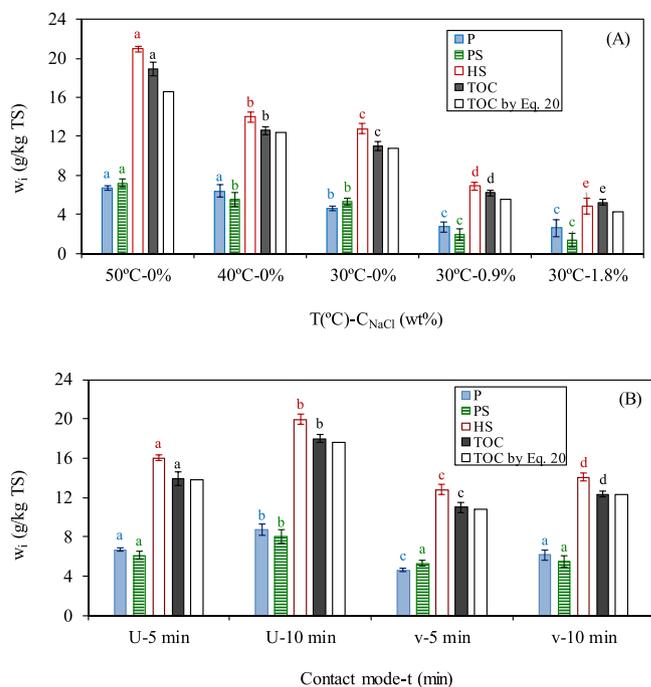
#### 3.1. EPS extraction from anaerobic sludge

##### 3.1.1. Effect of the extraction conditions

The results of EPS extraction as a function of temperature, salinity, contact time and contact mode, are shown in Fig. 3, 4, and Table 1.

As can be seen from Fig. 3A, the overall extraction of organic matter ( $w_{TOC}$ ) increased using salt-free water as the solvent and 50 °C. A significant increase in EPS extraction from about 2–7 g/kg TS for P and PS and from 4 to 21 g/kg TS for HS was obtained when the temperature increased from 30 °C to 50 °C and salinity decreased from 1.8 to 0 wt%. As the salinity was  $\geq 0.9$  wt%, the increase in liquid density and the effects of plasmolysis led to a decrease in the diffusion rate of the bound EPS from the floc to the liquid phase. It is interesting to highlight that HS has resulted to be the main type of EPS released from the anaerobic sludge, both in the presence and absence of salt.

Effects of contact time and contact mode can be compared on the basis of the data shown in Fig. 3B. It is observed that an ultrasonic contact increased the rate of EPS diffusion during extraction, obtaining sludge extracts with higher amounts of P, PS and HS at both contact times. It is also noted that 10 min for vortex agitation only allows a partial extraction of the loosely bound EPS and, consequently, longer contact times must be applied. This means that the ultrasonic contact is more effective than the classical vortex contact, ensuring a better EPS extraction yield and a reduction of the extraction time.



**Fig. 3.** Experimental mass extracted of proteins (P), polysaccharides (PS), humic substances (HS) and TOC from sludge. (A) Extractions with water at 30–50 °C or NaCl solutions at 30 °C using 5 min of vortex agitation. (B) Extractions with water at 30 °C using 5 and 10 min of vortex agitation (v) or ultrasonication (U). Different letters in the same solute indicate significant differences (ANOVA,  $p < 0.05$ ) in the mass extracted of this solute.

Based on the empirical formulae of BSA ( $C_{40}H_{62}O_{12}N_{10}$ ), glucose ( $C_6H_{12}O_6$ ), and humic acid ( $C_6H_9NO_6$ ) used as standards for the analysed compounds, the mass of organic carbon was determined per unit mass of each compound as 0.55 g C/g P, 0.40 g C/g PS, and 0.48 g C/g HS, and the theoretical TOC associated with the three types of compounds,  $w_{thTOC}$ , is shown in Eq. (20).

$$w_{thTOC} = 0.55 \cdot w_P + 0.40 \cdot w_{PS} + 0.48 \cdot w_{HS} \quad (20)$$

The comparison of theoretical and experimental TOC results (Fig. 3) shows that the sum of the three groups of compounds analysed represents between 85% and 99% of the organic matter extracted, depending on extraction conditions. It can be concluded that P values represented between 19% and 30% of total organic matter, PS values were the lowest, between 15% and 24%, and HS was the most abundant EPS, between 54% and 60%. It should be noted that the anaerobic sludge used came from a side-stream AnMBR plant and sludge has been exposed to high shear stress to keep a crossflow rate over the membrane surface of 2.0–2.5 m/s. Dvořák et al. [34] also determined, using a sludge from a submerged MBR process, that the fraction of HS was slightly greater than P, PS, and DNA contents, 35% vs. 24%, 29% and 12%, respectively. However, Chen et al. [35] using granular sludge from a submerged AnMBR, obtained a lower proportion of HS, 22%. Other reported studies only considered P and PS as the predominant EPS, without taking into account the amount of HS. Substantial differences in the ratio of P and PS from anaerobic sludge have been reported. Ding et al. [9] found a P content of 87.8% and a PS content of 12.2% for sludge from a mesophilic submerged AnMBR. Vincent et al. [36] reported a slightly lesser P content, 74%, in the sludge from a side-stream AnMBR, but Luna et al. [37] obtained that PS was the most prevalent EPS, 80.3%, in a submerged AnMBR.

The effects of temperature, salinity, and contact time on EPS extraction for an ultrasonication-assisted extraction were examined using response surface methodology. The results of the experimental-design test in Table 1 show that PS was the least abundant EPS in all liquid extracts,

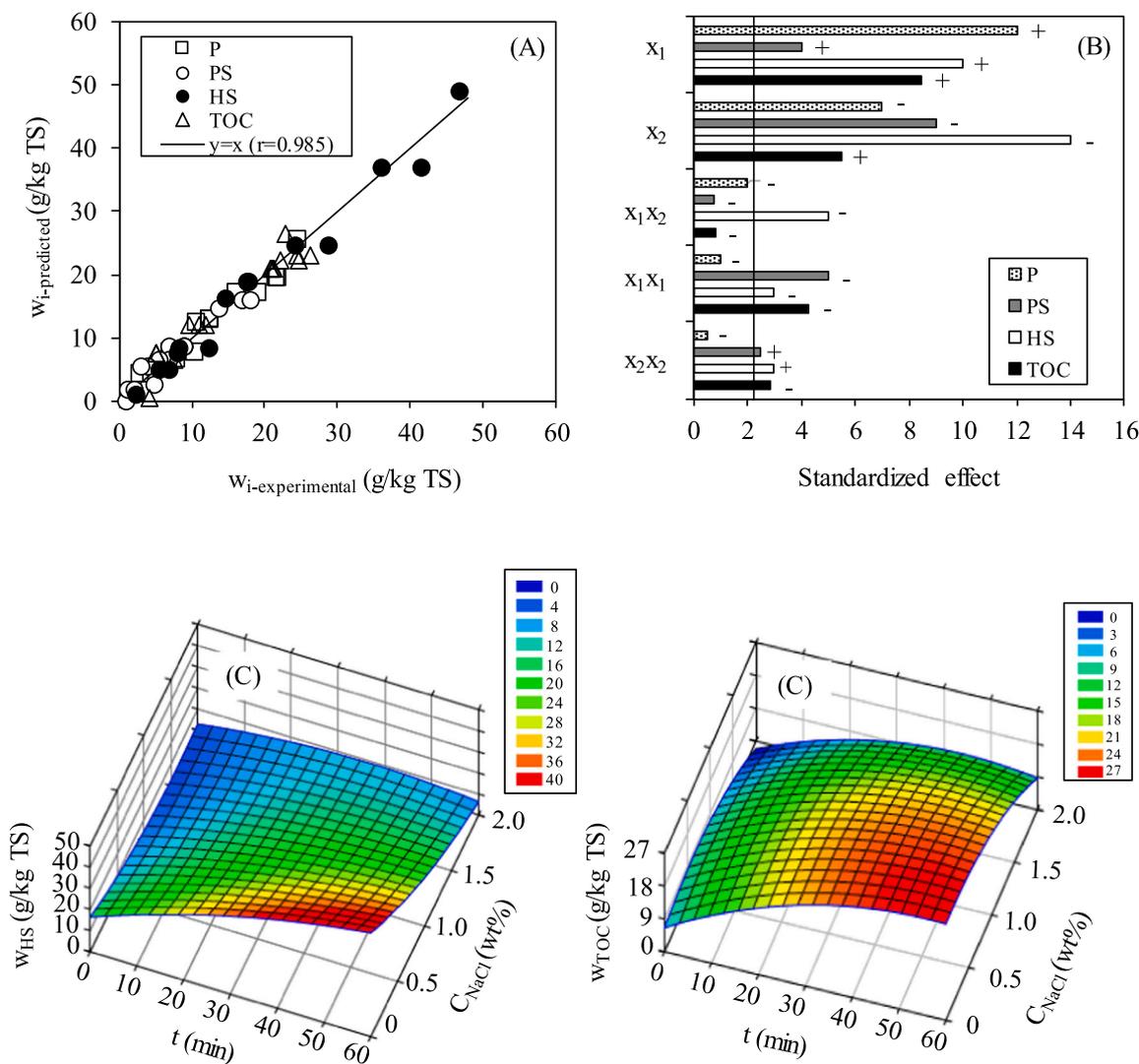


Fig. 4. The statistical treatment of the experimental data in Table 1. (A) The predicted extraction value by Eqs. (21)–(24) compared to the experimental extraction value. (B) Results of the Pareto analysis for effects  $t$  ( $x_1$ ) and  $C_{NaCl}$  ( $x_2$ ). (C) The response surface diagram for HS and TOC extraction obtained using the Box–Behnken design.

Table 1

Experimental values of the mass of proteins (P), polysaccharides (PS), humic substances (HS), and TOC per mass unit of sludge for the experimental-design test in ultrasonication-assisted extraction.

| Run | $t = x_1$ (min) | $C_{NaCl} = x_2$ (wt%) | $T = x_3$ (°C) | $w_P$ (g/kg) | $w_{PS}$ (g/kg) | $w_{HS}$ (g/kg) | $w_{TOC}$ (g/kg) |
|-----|-----------------|------------------------|----------------|--------------|-----------------|-----------------|------------------|
| 1   | 30              | 0.9                    | 40             | 12.4 ± 2.4   | 9.0 ± 0.2       | 17.8 ± 0.8      | 21.2 ± 0.2       |
| 2   | 60              | 0.9                    | 30             | 21.8 ± 2.6   | 6.9 ± 1.2       | 24.3 ± 2.1      | 24.5 ± 0.4       |
| 3   | 60              | 0.9                    | 50             | 21.4 ± 1.2   | 5.5 ± 2.9       | 28.9 ± 3.1      | 34.3 ± 0.1       |
| 4   | 60              | 1.8                    | 40             | 10.6 ± 1.7   | 4.8 ± 1.6       | 8.1 ± 2.4       | 11.1 ± 0.1       |
| 5   | 30              | 1.8                    | 50             | 7.0 ± 2.2    | 3.1 ± 1.0       | 12.4 ± 1.8      | 9.6 ± 0.1        |
| 6   | 0               | 0.9                    | 30             | 1.9 ± 1.1    | 1.3 ± 0.2       | 5.5 ± 2.4       | 5.1 ± 0.3        |
| 7   | 0               | 0.0                    | 40             | 3.8 ± 0.9    | 3.8 ± 2.1       | 14.7 ± 1.3      | 7.7 ± 0.1        |
| 8   | 30              | 0.9                    | 40             | 12.4 ± 3.1   | 8.9 ± 0.5       | 17.6 ± 1.1      | 20.9 ± 0.2       |
| 9   | 0               | 1.8                    | 40             | 1.8 ± 1.4    | 0.9 ± 0.2       | 2.3 ± 1.2       | 4.2 ± 0.1        |
| 10  | 60              | 0.0                    | 40             | 24.4 ± 1.2   | 13.7 ± 1.6      | 46.8 ± 1.4      | 20.8 ± 0.2       |
| 11  | 0               | 0.9                    | 50             | 2.4 ± 1.9    | 2.1 ± 1.1       | 6.9 ± 1.0       | 5.0 ± 0.1        |
| 12  | 30              | 1.8                    | 30             | 10.4 ± 2.1   | 3.9 ± 0.7       | 8.2 ± 1.0       | 11.9 ± 0.3       |
| 13  | 30              | 0.0                    | 30             | 14.1 ± 2.3   | 17.0 ± 0.2      | 36.0 ± 1.7      | 24.7 ± 0.2       |
| 14  | 30              | 0.0                    | 50             | 19.1 ± 1.5   | 18.0 ± 2.4      | 41.5 ± 1.8      | 22.1 ± 0.3       |
| 15  | 30              | 0.9                    | 40             | 12.4 ± 2.1   | 9.0 ± 0.8       | 17.7 ± 1.0      | 21.1 ± 0.3       |

representing between 10% and 25% of the overall polymeric substances extracted. The comparison of the experimental TOC results in Table 1 with the theoretical TOC values by Eq. (20) shows that P, PS and HS account for more than 80% of the total organic matter extracted, which agrees with results depicted in Fig. 3. The statistical treatment of the experimental data in Table 1 from the BBD (Eq. (1)) gave rise to the following second-order polynomials:

$$w_P = 6.73269000 + 0.38439100 \cdot t - 2.02030000 \cdot C_{NaCl} - 0.07222220 \cdot t \cdot C_{NaCl} - 0.00106624 \cdot t^2 - 0.56742600 \cdot C_{NaCl}^2 \quad (R^2 = 0.96) \quad (21)$$

$$w_{PS} = 8.45212000 + 0.40321800 \cdot t - 9.85662000 \cdot C_{NaCl} - 0.02722220 \cdot t \cdot C_{NaCl} - 0.00493974 \cdot t^2 + 2.62868000 \cdot C_{NaCl}^2 \quad (R^2 = 0.94) \quad (22)$$

$$w_{HS} = 16.27170000 + 0.82364400 \cdot t - 16.58960000 \cdot C_{NaCl} - 0.24388900 \cdot t \cdot C_{NaCl} - 0.00461004 \cdot t^2 + 4.94563000 \cdot C_{NaCl}^2 \quad (R^2 = 0.97) \quad (23)$$

$$w_{TOC} = 6.57192000 + 0.7116600 \cdot t + 5.27756000 \cdot C_{NaCl} - 0.07592590 \cdot t \cdot C_{NaCl} - 0.00636795 \cdot t^2 - 4.79155000 \cdot C_{NaCl}^2 \quad (R^2 = 0.93) \quad (24)$$

$R^2$  coefficients were higher than 0.9, despite the difficulty of sampling such a heterogeneous material as this sludge to obtain only 0.5 wt % TS for each EPS extraction test. The predicted results by Eqs. (21)–(24) were in right agreement with experimental values as can be observed in Fig. 4A. Linear regression coefficients ( $r$ ) above 0.9 and a slope value of 1 were obtained for each EPS group and TOC. No significant temperature effect was found (ANOVA,  $p > 0.05$ ) from BBD analysis, which seems to disagree with the above extraction results for vortex contact. This result may be due to the fact that the increase in operating temperature from 30 to 50 °C is of the order of the temperature gradient generated in the liquid phase during sonication by the cavitation effect. It has already been reported that each collapsing bubble acts as a hotspot, generating energy to increase the local temperature up to 5000 K [38]. Therefore, the temperature profile in the liquid phase can be assumed very heterogeneous, despite thermostatic control during the extraction. These extraction models (Eqs. (21)–(24)) show that the extracted EPS mass from the sludge depends on the linear, quadratic, and combined effect of ultrasound time and of solvent salinity. In addition, Pareto plot (Fig. 4B) justifies that an increase in contact time and a decrease in salinity caused a positive effect on P, PS and HS extractions. From the linear terms of HS, it can be observed that HS extraction is the most negatively affected by the salt presence in the solvent. Finally, the effects  $t^2$ ,  $(t \cdot C_{NaCl})$ , and  $C_{NaCl}^2$  were also significant effects for HS extraction, as can be seen in Fig. 4B.

The three-dimensional response surface diagram for HS and TOC is shown in Fig. 4C. It can be observed that both TOC extraction and HS extraction began to increase when the extraction time increased and salt concentration decreased. As the ultrasound time continues to increase ( $x_1$ ) in low salinity solvents ( $x_2$ ), the extraction yield of TOC and HS reached a maximum at a certain level. As shown in Fig. 4C, extraction degree of TOC and HS reached a maximum value at an ultrasound time higher than 45 min for a salt concentration lower than 0.5 wt%.

**Table 2**

Parameters of each kinetic model used to fit the extraction results shown in Fig. 4 and the values of the initial extracted mass at  $t = 0$  min ( $w_{0i}$ ) and of the ultimate extracted mass at  $t \geq t_{\infty i}$  ( $w_{\infty i}$ ) for proteins (P), polysaccharides (PS), humic substances (HS). AARD and RMSE were calculated by Eqs. (8) and (9).

| Parameters   | i = P      | i = PS     | i = HS     |
|--|------------|------------|------------|
| $w_{0i}$ (g/kg TS)                                     | 4.0 ± 0.5  | 5.8 ± 0.6  | 11.4 ± 0.7 |
| $w_{\infty i}$ (g/kg TS)                               | 23.8 ± 0.4 | 22.0 ± 0.2 | 54.2 ± 0.2 |
| $h_i$ (Eq. (3), g/kg ST·min)                           | 0.325      | 0.278      | 0.761      |
| $w_{0i}$ (by Eq. (3), g/kg TS)                         | 5.211      | 5.937      | 11.361     |
| $R^2$ (Eq. (3))  | 0.990      | 0.992      | 0.994      |
| AARD (Eq. (3))   | 7.50       | 3.66       | 2.59       |
| RMSE (Eq. (3))   | 0.67       | 0.48       | 0.64       |
| $t_{\infty}$ (Eq. (3), min)                            | 57.5       | 57.8       | 56.3       |
| $h_i$ (Eq. (6), min <sup>-1</sup> )                    | 0.036      | 0.035      | 0.037      |
| $(w_{\infty i} - w_{0i})$ (Eq. (6), g/kg TS)           | 19.955     | 16.198     | 42.796     |
| $h_i (w_{\infty i} - w_{0i})$ (Eq. (6), g/kg ST·min)   | 0.718      | 0.567      | 1.583      |
| $R^2$ (Eq. (6))  | 0.949      | 0.892      | 0.793      |
| AARD (Eq. (4))   | 7.86       | 11.00      | 13.97      |
| RMSE (Eq. (4))   | 1.32       | 1.61       | 5.02       |
| $t_{\infty}$ (Eq. (4), min)                            | 83.1       | 79.6       | 101.5      |
| $h_i$ (Eq. (7), kg ST/g·min)                           | 0.00015    | –          | –          |
| $(w_{\infty i} - w_{0i})$ (Eq. (7), g/kg TS)           | 57.803     | –          | –          |
| $h_i (w_{\infty i} - w_{0i})^2$ (Eq. (7), g/kg ST·min) | 0.501      | –          | –          |
| $R^2$ (Eq. (7))  | 0.991      | 0.641      | 0.300      |
| AARD (Eq. (5))   | 0.59       | –          | –          |
| RMSE (Eq. (5))   | 0.05       | –          | –          |
| $t_{\infty}$ (Eq. (5), min)                            | 9720       | –          | –          |

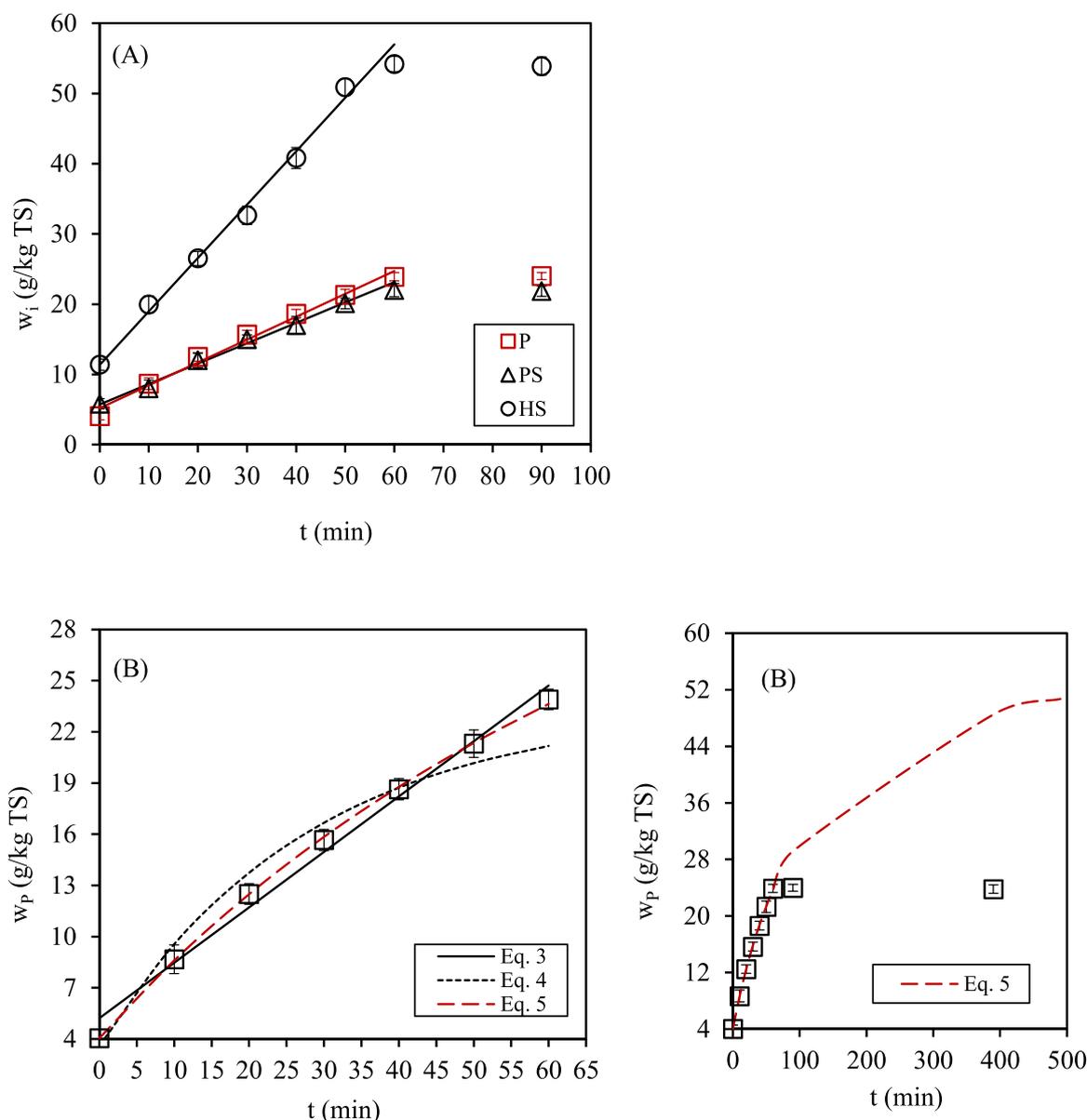


Fig. 5. The effect of ultrasonication time on the mass extracted ( $w$ ) of proteins (P), polysaccharides (PS) and humic substances (HS) using water at 30 °C as the solvent. (A) Experimental data (symbols) and the predicted data by Eq. (3) (line). (B) Comparison of different kinetic models (Eqs. (3)–(5)) for P extraction.

Optimising the response of the experiment design for ultrasonication-assisted EPS extraction shows that maximum extraction occurs when using salt-free water as the solvent and a minimum contact time of 58.0 min ( $=t_{\infty}$ ), with a predicted ultimate value of  $w_{\infty P} = 23 \pm 2$  g/kg TS,  $w_{\infty PS} = 19 \pm 5$  g/kg TS,  $w_{\infty HS} = 54 \pm 9$  g/kg TS and  $w_{\infty TOC} = 27 \pm 5$  g/kg TS.

### 3.1.2. Kinetics of EPS extraction

Based on the optimum response from the experiment-design test, and to analyse the extraction rate of each group of polymeric substances, a kinetic study was performed by ultrasonication-assisted extraction, using water at 30 °C. To describe this extraction process, zero-order (Eq. (3)), first-order (Eq. (4)) and second order (Eq. (5)) kinetic models were fitted the experimental data. The parameters for each model and statistical results are presented in Table 2 and Fig. 5.

Fig. 5A shows that initially, the mass extracted for each group of EPS increases quasi-linearly with ultrasound time, reaching its ultimate value at about 60 min. HS was the majority component in the extract

over time. No increase in P, PS and HS extraction was observed from 60 min to the final time of 6.5 h. A value of  $w_{\infty}$  (Table 2) close to 20 g/kg TS was obtained for P and PS, which was lower than the value of 54 g/kg TS for HS, and all of them similar to those predicted by BBD. In addition, Table 2 and Fig. 5A show the mass of P, PS, and HS extracted at  $t = 0$  min,  $w_{0i}$ , which can be considered the loosely-bound EPS extracted from the outer layer of the flocs. This result indicates that the loosely-bound fraction was considerably larger for HS, 12%, than for P and PS, 4–5%.

The model that best fitted the experimental data of PS and HS extractions was the zero-order model. In the case of P, the second-order model fitted even better than the zero-order model, as can be seen in Fig. 5B and Table 2. Similar estimated values of the protein extraction rate using the second-order and zero-order models were obtained, 0.5 and 0.3 g of protein/min per kg of total solid, respectively. However, this second-order model predicts a final protein extraction of 61.8 g/kg of TS after 162 h of ultrasonication, which does not correspond to the experimental value of  $w_{\infty P} = 23.8 \pm 0.4$  g/kg of TS (or  $23 \pm 2$  g/kg of

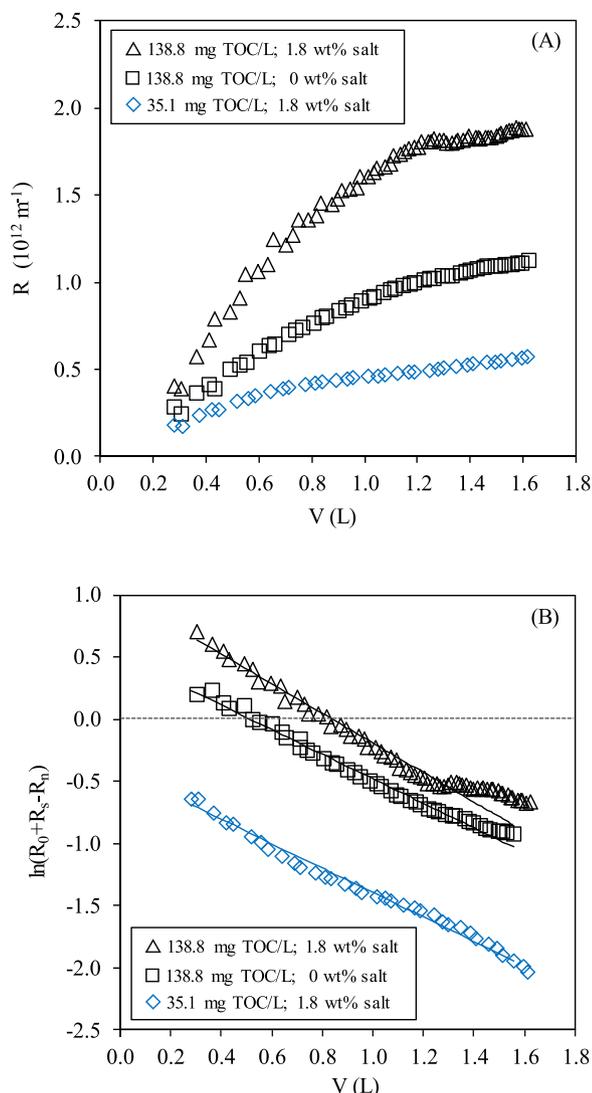


Fig. 6. Evolution of membrane fouling with different EPS extracts relative to the filtered volume based on (A) resistances to filtration, and (B) based on the linearized fouling model (Eq. (19)).

Table 3

Permeability, retention degree, resistance, compressibility index and model parameters (Eq. (19)) for the three EPS extracts.

| $C_{NaCl}$<br>(wt%) | TOC<br>(mg/<br>L) | $L_p^a$ (L/<br>$m^2 \cdot h \cdot bar$ ) | $R^b$<br>( $10^{12}$<br>$m^{-1}$ ) | $n^b$ | $\alpha$<br>(%) | $\alpha$ (by<br>Eq. (19),<br>%) | SRF (by<br>Eqs. (19),<br>$10^{12}$ m/<br>kgTOC) |
|---------------------|-------------------|--|------------------------------------|-------|-----------------|---------------------------------|---|
| 0                   | 138.8             | 1407 – 323                               | 1.11                               | 0.23  | 73              | 79                              | 103.8   |
| 1.8                 | 138.8             | 1436 – 191                               | 1.88                               | 0.03  | 88              | 95                              | 169.4   |
| 1.8                 | 35.1              | 1495 – 630                               | 0.57                               | 0.04  | 69              | 79                              | 161.1   |

<sup>a</sup> Initial and final permeability.

<sup>b</sup> Resistance to filtration, and compressibility index after filtering 1.6 L of solution.

TS from BBD), as can be observed in Fig. 5B. Based on this result and  $R^2$  coefficient, it can be assumed that the zero-order model best describes the experimental kinetic data of P extraction.

The results in Table 2 show that the extraction rate ( $h_i$ ) of the zero-order model and the ultimate extraction ( $w_{\infty i}$ ) were higher for HS than for P and PS compounds. Using Eq. (3) and  $w_{\infty i}$  values, it was determined that the minimum ultrasonication time ( $t_{\infty}$ ) for the maximum extraction of each group of compounds with distilled water was ranged

from 56 to 58 min, and the final EPS extract presented 138.8, 96.7, 89.0 and 219.5 mg/L of TOC, P, PS and HS, respectively. This result of  $t_{\infty}$  is consistent with the optimal BBD response, which predicted an optimal contact time of 58 min

### 3.2. Filtration test of EPS solutions

#### 3.2.1. Fouling rate, SRF and degree of retention

An assessment of the fouling capacity of a submerged hollow-fibre membrane was performed using three solutions: (a) the extract of the highest EPS concentration, 138.8 mg TOC/L, obtained with distilled water and 60 min of ultrasonication, (b) the same extract but with salt added, 138.8 mg TOC/L and 1.8 wt% salt, and (c) the extract of the lowest EPS concentration, 35.1 mg TOC/L, obtained with 1.8 wt% of salt without ultrasonication.

Fig. 6A shows evolution of resistance versus the filtered volume of each solution. The concentrated EPS solution with salt fouled the membrane faster than the concentrated unsalted EPS solution, which in turn fouled faster than the dilute EPS solution. It was observed that the solutions with 138.8 mg TOC/L raised resistance to  $1.11 \cdot 10^{12}$  and  $1.88 \cdot 10^{12} m^{-1}$ , whereas resistance rose to only  $0.57 \cdot 10^{12} m^{-1}$  with the 35.1 mg TOC/L solution (Table 3). The membrane permeability decreased from  $1436 L/(m^2 \cdot h \cdot bar)$  to  $191 L/(m^2 \cdot h \cdot bar)$  and  $323 L/(m^2 \cdot h \cdot bar)$  when it was fouled with the concentrated EPS solutions in presence and absence of salt, respectively. The permeability decrease was less pronounced when the membrane fouled with the diluted EPS solution, keeping a level of  $630 L/(m^2 \cdot h \cdot bar)$ , despite the salt content was the same. These results are consistent with the reported results of the effect of salinity on the fouling of aerobic membrane bioreactors. Guo et al. [39] studied the behaviour of three MBR treating synthetic municipal wastewater without salt and with 0.75% and 1.5% of salt, observing that TMP reached values of 70, 170, and 220 mbar, respectively, after 4 days at a filtration flux of  $12.5 L/m^2 \cdot h$ . Jang et al. [40] determined that after 20 days at a flux of  $3.5 L/m^2 \cdot h$ , salinity halved the time required to reach a reference TMP of 270 mbar, which decreases from 132 h using unsalted wastewater to 60 h using wastewater with 20 g/L of salt.

The contribution of salt to membrane fouling was also studied using EPS-free saline solutions (0–1.8 wt% NaCl in distilled water). It was found that the increase in filtration resistance for a 1.8 wt% NaCl solution was only  $0.16 \cdot 10^{12} m^{-1}$ . This increase can be considered negligible compared to that observed when salt is added to a high-concentration EPS extract, which confirms that the effect of salt on membrane fouling is related to EPS fouling.

The resistance to backwashing increased slightly from  $0.25 \pm 0.1 \cdot 10^{12} m^{-1}$  to  $0.29 \pm 0.1 \cdot 10^{12} m^{-1}$  for the dilute EPS solution with salt and for the concentrated unsalted EPS solution. However, the resistance to backwashing increased appreciably until  $0.38 \cdot 10^{12} m^{-1}$  for the concentrated EPS solution with salt, which means that the increase in internal resistance is also produced by the combined effect of EPS and salt.

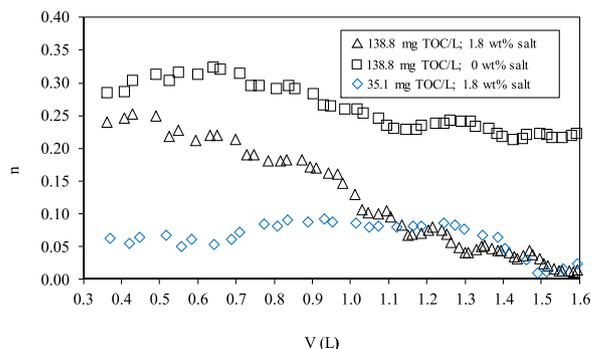


Fig. 7. Evolution of the compressibility index ( $n$ ) relative to the filtered volume for the filtration tests shown in Fig. 6.

The good agreement between the experimental resistance data (symbols) and Eq. (19) (straight line) can be observed in Fig. 6B. The results of SRF and  $\alpha$  by Eq. (19), and of experimental  $\alpha$  are shown in Table 3. It is worth highlighting that, SRF was quite similar for the salted extract with low and high EPS concentration (161 and  $169 \cdot 10^{12}$  m/kg TOC, respectively); however, its value decreased about 30% in the filtration of the unsalted extract with high EPS concentration. Those values of SRF are of the same order as those obtained by Hong et al. [24],  $193 \cdot 10^{12}$  m/kg in a dead-end stirred cell with a flat sheet membrane. However, it should be noted that they used a biomass suspension with a concentration of 4 g/L, which means that the SRF of the cake layer was only slightly higher than the SRF obtained in this work for the gel bio-layer formed by the EPS solutions. Lin et al. [23] determined the cake resistance under the same conditions in a flat-sheet membrane submerged MBR, before and after replacing the supernatant by water, observing that cake resistance decreased from  $6.0 \cdot 10^{12}$  m<sup>-1</sup> to  $0.85 \cdot 10^{12}$  m<sup>-1</sup>, which demonstrates that the biopolymers retained by the cake contribute more to filtration resistance than the particulate matter. Hemmelmann et al. [19] determined a SRF of  $270 \cdot 10^{12}$  m/kg in an AnMBR for saline wastewater treatment, probably due to the formation of a dense and compact cake layer of individual cells growing under saline conditions. Zhang et al. [22] explained that the osmotic pressure is the main contribution to the filtration resistance and the negative electric charge of functional groups in EPS justifies the retention of counter-ions in the cake matrix. It is also observed that the experimental retention of the organic matter was similar to the retention predicted by Eq. (19), and the slight differences can be attributed to the fact that about 10–20% of the total organic matter in the extracts was not EPS. In addition, the EPS retention was notably higher,  $\alpha = 95\%$ , for the saline extract with high EPS concentration than for the other two extracts,  $\alpha = 79\%$ . Thus, it can be concluded that the increase in SRF and  $\alpha$  is produced by the combined effect of EPS and salt.

The results of the EPS retention degree, 79–95%, were higher than those obtained for dissolved P, PS and HS when the anaerobic sludge was directly filtered in a submerged hollow fibre membrane module, which ranged from 27% to 70% [41]. However, it must be taken into account that the molecular weight of the soluble microbial products is smaller than those of loosely bound EPS extracted from the anaerobic sludge [42]. In addition, bio-flocs can have a protective effect from colloidal fouling [43], but with a lower retention capacity of SMP, even when the bulk anaerobic sludge was filtered.

### 3.2.2. Compressibility index

Fig. 7 shows the evolution of the compressibility index versus the filtered volume of the EPS extracts. The index  $n$  of the gel layer formed during filtration was clearly higher for the salt-free solution with high EPS concentration, 138.8 mg TOC/L, than for the other two solutions, and its value slowly decreased from 0.29 to 0.23. Conversely,  $n$  decreased notably during filtration of the solution with the same EPS concentration but with 1.8 wt% of salt, reaching a final value of 0.03, which, in practice, means that the gel layer was incompressible at the end of the test. It should be taken into account that a deformable gel layer with a compressibility index of 0.25 undergoes an immediate decrease in permeability of 21% when TMP increases only from 100 to 250 mbar. These results indicate that the compressibility depends on the elasticity of the gel layer macromolecules, as well as on salinity. The increase in salinity has provided rigidity to the gel layer of EPS as a result of the increase in osmotic pressure that opposes interstitial water migration involved in the compression of gel layer [23]. Finally, the saline solution with low EPS concentration, 35.1 mg TOC/L, showed a low value of  $n$  over time,  $0.06 \pm 0.03$ , probably due to the formation of a thin gel bio-layer on the membrane surface which was barely affected by the compression forces.

## 4. Conclusions

The contact time for extracting EPS from the AnMBR sludge must be longer than the times normally used, and 60 min was the required time for the ultrasonication-assisted extraction. Temperatures between 30 °C and 50 °C do not significantly affect the EPS extraction for ultrasonic contact, whilst contact time and the absence of salt in the solvent favour EPS recovery. Over 97% of the total organic matter extracted from the sludge of the side-stream AnMBR matches proteins, polysaccharides, and humic substances. Humic substances were the main fraction of EPS extracted from AnMBR sludge, with values between 54% and 60% of TOC.

Experimental results of the dead-end filtrations reveal that soluble EPS and salinity play an important role in the hydraulic fouling of the hollow fibre membranes. A kinetic fouling model allowed to determine the specific resistance to filtration, retention degree and the compressibility index of the gel bio-layer formed by EPS extracted from anaerobic sludge in the presence and absence of salt. The retention degree of EPS was higher than 79%, reaching 95% for the highest EPS and salt concentrations. The EPS extract with a concentration of 138.8 mg TOC/L and 1.8 wt% salt causes faster fouling than that due to EPS and to salt separately. 1.8 wt% salt increases the specific resistance to filtration of EPS from  $103.8 \cdot 10^{12}$  to  $165.3 \cdot 10^{12}$  m/kg TOC and provides rigidity to the EPS bio-layer as a consequence of the increase in osmotic pressure that opposes the compression. This hydraulically irreversible fouling is a chemically reversible fouling and the permeability of the hollow-fibre membrane is restored by chemical cleaning.

### CRedit authorship contribution statement

**Raquel Martínez:** Investigation, Data curation, Writing – original draft. **Maria Olga Ruiz:** Conceptualization, Methodology, Supervision, Writing - review & editing. **Ana García:** Investigation, Writing – original draft. **Cipriano Ramos:** Investigation, Visualization, Writing – original draft. **Victorino Diez:** Project administration, Funding acquisition, Supervision, Writing -review & editing.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### References

- [1] C. Wisniewski, A. Grasmic, Flocc size distribution in a membrane bioreactor and consequences for membrane fouling, *Colloids Surf. A Physicochem. Eng. Asp.* 138 (2–3) (1998) 403–411, [https://doi.org/10.1016/S0927-7757\(96\)03898-8](https://doi.org/10.1016/S0927-7757(96)03898-8).
- [2] A. Drews, Membrane fouling in membrane bioreactors-characterisation, contradictions, cause and cures, *J. Memb. Sci.* 363 (2010) 1–28, <https://doi.org/10.1016/j.memsci.2010.06.046>.
- [3] A. Cosenza, G. Di Bella, G. Mannina, M. Torregrossa, The role of EPS in fouling and foaming phenomena for a membrane bioreactor, *Bioresour. Technol.* 147 (2013) 184–192, <https://doi.org/10.1016/j.biortech.2013.08.026>.
- [4] H. Lin, M. Zhang, F. Wang, F. Meng, B.Q. Liao, H. Hong, J. Chen, W. Gao, A critical review of extracellular polymeric substances (EPSs) in membrane bioreactors: characteristics, roles in membrane fouling and control strategies, *J. Memb. Sci.* 460 (2014) 110–125, <https://doi.org/10.1016/j.memsci.2014.02.034>.

- [5] F. Meng, S.R. Chae, A. Drews, M. Kraume, H.S. Shin, F. Yang, Recent advances in membrane bioreactors (MBRs): Membrane fouling and membrane material, *Water Res.* 43 (2009) 1489–1512, <https://doi.org/10.1016/j.watres.2008.12.044>.
- [6] R. Campo, M. Capodici, G. Di Bella, M. Torregrossa, The role of EPS in the foaming and fouling for a MBR operated in intermittent aeration conditions, *Biochem. Eng. J.* 118 (2017) 41–52, <https://doi.org/10.1016/j.bej.2016.11.012>.
- [7] X. Han, Z. Wang, C. Zhu, Z. Wu, Effect of ultrasonic power density on extracting loosely bound and tightly bound extracellular polymeric substances, *Desalination* 329 (2013) 35–40, <https://doi.org/10.1016/j.desal.2013.09.002>.
- [8] X.Y. Li, S.F. Yang, Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge, *Water Res.* 41 (2007) 1022–1030, <https://doi.org/10.1016/j.watres.2006.06.037>.
- [9] Y. Ding, Y. Tian, Z. Li, W. Zuo, J. Zhang, A comprehensive study into fouling properties of extracellular polymeric substance (EPS) extracted from bulk sludge and cake sludge in a mesophilic anaerobic membrane bioreactor, *Bioresour. Technol.* 192 (2015) 105–114, <https://doi.org/10.1016/j.biortech.2015.05.067>.
- [10] H. Liu, H.H.P. Fang, Extraction of extracellular polymeric substances (EPS) of sludges, *J. Biotechnol.* 95 (2002) 249–256, [https://doi.org/10.1016/S0168-1656\(02\)00025-1](https://doi.org/10.1016/S0168-1656(02)00025-1).
- [11] J.W. Morgan, C.F. Forster, L. Evison, A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges, *Water Res.* 24 (1990) 743–750, [https://doi.org/10.1016/0043-1354\(90\)90030-A](https://doi.org/10.1016/0043-1354(90)90030-A).
- [12] B. Frølund, R. Palmgren, K. Keiding, P.H. Nielsen, Extraction of extracellular polymers from activated sludge using a cation exchange resin, *Water Res* 30 (1996) 1749–1758, [https://doi.org/10.1016/0043-1354\(95\)00323-1](https://doi.org/10.1016/0043-1354(95)00323-1).
- [13] A.G. Geyik, F. Çeçen, Production of protein- and carbohydrate-EPS in activated sludge reactors operated at different carbon to nitrogen ratios, *J. Chem. Technol. Biotechnol.* 91 (2016) 522–531, <https://doi.org/10.1002/jctb.4608>.
- [14] H. Wang, H. Deng, L. Ma, L. Ge, The effect of carbon source on extracellular polymeric substances production and its influence on sludge floc properties, *J. Chem. Technol. Biotechnol.* 89 (2014) 516–521, <https://doi.org/10.1002/jctb.4147>.
- [15] A. Sweity, W. Ying, M.S. Ali-Shtayeh, F. Yang, A. Bick, G. Oron, M. Herzberg, Relation between EPS adherence, viscoelastic properties, and MBR operation: biofouling study with QCM-D, *Water Res* 45 (2011) 6430–6440, <https://doi.org/10.1016/j.watres.2011.09.038>.
- [16] L. Sun, L. Chen, W.Z. Guo, T. Ye, Y. Yang, Extraction of extracellular polymeric substances in activated sludge using sequential extraction, *J. Chem. Technol. Biotechnol.* 90 (2015) 1448–1454, <https://doi.org/10.1002/jctb.4449>.
- [17] P. D'Abzac, F. Bordas, E. Van Hullebusch, P.N.L. Lens, G. Guibaud, Extraction of extracellular polymeric substances (EPS) from anaerobic granular sludges: comparison of chemical and physical extraction protocols, *Appl. Microbiol. Biotechnol.* 85 (2010) 1589–1599, <https://doi.org/10.1007/s00253-009-2288-x>.
- [18] J. Wingender, T.R. Neu, H.-C. Flemming, What are bacterial extracellular polymeric substances?, in: *Microbial Extracellular Polymeric Substances* Springer, Berlin, Heidelberg, 1999, pp. 1–19, [https://doi.org/10.1007/978-3-642-60147-7\\_1](https://doi.org/10.1007/978-3-642-60147-7_1).
- [19] A. Hemmelmann, A. Torres, C. Vergara, L. Azocar, D. Jeison, Application of anaerobic membrane bioreactors for the treatment of protein-containing wastewaters under saline conditions, *J. Chem. Technol. Biotechnol.* 88 (2013) 658–663, <https://doi.org/10.1002/jctb.3882>.
- [20] E. Reid, X. Liu, S.J. Judd, Effect of high salinity on activated sludge characteristics and membrane permeability in an immersed membrane bioreactor, *J. Memb. Sci.* 283 (2006) 164–171, <https://doi.org/10.1016/j.memsci.2006.06.021>.
- [21] J. Chen, M. Zhang, A. Wang, H. Lin, H. Hong, X. Lu, Osmotic pressure effect on membrane fouling in a submerged anaerobic membrane bioreactor and its experimental verification, *Bioresour. Technol.* 125 (2012) 97–101, <https://doi.org/10.1016/j.biortech.2012.08.038>.
- [22] M. Zhang, W. Peng, J. Chen, Y. He, L. Ding, A. Wang, H. Lin, H. Hong, Y. Zhang, H. Yu, A new insight into membrane fouling mechanism in submerged membrane bioreactor: osmotic pressure during cake layer filtration, *Water Res.* 47 (2013) 2777–2786, <https://doi.org/10.1016/j.watres.2013.02.041>.
- [23] H. Lin, M. Zhang, F. Wang, Y. He, J. Chen, H. Hong, A. Wang, H. Yu, Experimental evidence for osmotic pressure-induced fouling in a membrane bioreactor, *Bioresour. Technol.* 158 (2014) 119–126, <https://doi.org/10.1016/j.biortech.2014.02.018>.
- [24] H. Hong, M. Zhang, Y. He, J. Chen, H. Lin, Fouling mechanisms of gel layer in a submerged membrane bioreactor, *Bioresour. Technol.* 166 (2014) 295–302, <https://doi.org/10.1016/j.biortech.2014.05.063>.
- [25] K. Grintzalis, C.D. Georgiou, Y.-J. Schneider, An accurate and sensitive Coomassie Brilliant Blue G-250-based assay for protein determination, *Anal. Biochem.* 480 (2015) 28–30, <https://doi.org/10.1016/j.ab.2015.03.024>.
- [26] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, *Anal. Chem.* 28 (1956) 350–356, <https://doi.org/10.1021/ac60111a017>.
- [27] B. Frølund, T. Griebe, P.H. Nielsen, Enzymatic activity in the activated-sludge floc matrix, *Appl. Microbiol. Biotechnol.* 43 (1995) 755–761, <https://doi.org/10.1007/BF00164784>.
- [28] D.C. Montgomery, *Design and Analysis of Experiments*, fifth ed., 2017.
- [29] D.T. Veličković, D.M. Milenović, M.S. Ristić, V.B. Veljković, Kinetics of ultrasonic extraction of extractive substances from garden (*Salvia officinalis* L.) and glutinous (*Salvia glutinosa* L.) sage, *Ultrason. Sonochem.* 13 (2006) 150–156, <https://doi.org/10.1016/j.ultsonch.2005.02.002>.
- [30] J.L.A. Dagostin, D. Carpiné, M.L. Corazza, Extraction of soybean oil using ethanol and mixtures with alkyl esters (biodiesel) as co-solvent: kinetics and thermodynamics, *Ind. Crops Prod.* 74 (2015) 69–75, <https://doi.org/10.1016/j.indcrop.2015.04.054>.
- [31] G.T. Vladisavljević, S.K. Milonjić, V.L.J. Pavasović, Flux decline and gel resistance in unstirred ultrafiltration of aluminium hydrous oxide sols, *J. Colloid Interface Sci.* 176 (1995) 491–494, <https://doi.org/10.1006/jcis.1995.9941>.
- [32] Y. Lee, M.M. Clark, Modeling of flux decline during crossflow ultrafiltration of colloidal suspensions, *J. Memb. Sci.* 149 (1998) 181–202, [https://doi.org/10.1016/S0376-7388\(98\)00177-X](https://doi.org/10.1016/S0376-7388(98)00177-X).
- [33] R.S. Juang, H.L. Chen, Y.S. Chen, Membrane fouling and resistance analysis in dead-end ultrafiltration of *Bacillus subtilis* fermentation broths, *Sep. Purif. Technol.* 63 (2008) 531–538, <https://doi.org/10.1016/j.seppur.2008.06.011>.
- [34] L. Dvořák, M. Gómez, M. Dvořáková, I. Růžicková, J. Wanner, The impact of different operating conditions on membrane fouling and EPS production, *Bioresour. Technol.* 102 (2011) 6870–6875, <https://doi.org/10.1016/j.biortech.2011.04.061>.
- [35] C. Chen, W. Guo, H.H. Ngo, S.W. Chang, D. Duc Nguyen, P. Dan Nguyen, X.T. Bui, Y. Wu, Impact of reactor configurations on the performance of a granular anaerobic membrane bioreactor for municipal wastewater treatment, *Int. Biodeterior. Biodegrad.* 121 (2017) 131–138, <https://doi.org/10.1016/j.ibiod.2017.03.021>.
- [36] N. Martin Vincent, J. Tong, D. Yu, J. Zhang, Y. Wei, Membrane fouling characteristics of a side-stream tubular anaerobic membrane bioreactor (AnMBR) treating domestic wastewater, *Processes* 6 (2018) 50, <https://doi.org/10.3390/pr6050050>.
- [37] H.J. Luna, B.E.L. Baêta, S.F. Aquino, M.S.R. Susa, EPS and SMP dynamics at different heights of a submerged anaerobic membrane bioreactor (SABMR), *Process Biochem.* 49 (2014) 2241–2248, <https://doi.org/10.1016/j.procbio.2014.09.013>.
- [38] P. Chowdhury, T. Viraraghavan, Sonochemical degradation of chlorinated organic compounds, phenolic compounds and organic dyes - a review, *Sci. Total Environ.* 407 (2009) 2474–2492, <https://doi.org/10.1016/j.scitotenv.2008.12.031>.
- [39] X. Guo, Y. Miao, B. Wu, L. Ye, H. Yu, S. Liu, X. Xiang Zhang, Correlation between microbial community structure and biofouling as determined by analysis of microbial community dynamics, *Bioresour. Technol.* 197 (2015) 99–105, <https://doi.org/10.1016/j.biortech.2015.08.049>.
- [40] D. Jang, Y. Hwang, H. Shin, W. Lee, Effects of salinity on the characteristics of biomass and membrane fouling in membrane bioreactors, *Bioresour. Technol.* 141 (2013) 50–56, <https://doi.org/10.1016/j.biortech.2013.02.062>.
- [41] R. Martínez, M.O. Ruiz, C. Ramos, J.M. Cámara, V. Diez, Comparison of external and submerged membranes used in anaerobic membrane bioreactors: fouling related issues and biological activity, 1–9. *Biochem. Eng. J.* 159 (2020), 107558 <https://doi.org/10.1016/j.bej.2020.107558>.
- [42] P.-N. Hong, C. Taing, P.-T. Phan, R. Honda, Polarity-molecular weight profile of extracellular polymeric substances in a membrane bioreactor: comparison between bulk sludge and cake layers, *J. Water Environ. Tech.* 16 (1) (2018) 40–53, <https://doi.org/10.2965/jwet.17-020>.
- [43] B. Wu, T. Kitade, T.H. Chong, T. Uemura, A.G. Fane, Role of initially formed cake layers on limiting membrane fouling in membrane bioreactors, *Bioresour. Technol.* 118 (2012) 589–593, <https://doi.org/10.1016/j.biortech.2012.05.016>.