Andres Novoa Lamprea: Data curation, Investigation, Validation Writing- Original draft preparation Luca Fortunato: Supervision, Writing- Original draft preparation, Writing- Reviewing and Editing. Zahid Ur Rehman: Resources. TorOve Leiknes: Conceptualization and Writing-Reviewing and Editing.

Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:
Figure 1. Representation of the experimental setup

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Evaluating the effect of hydraulic retention time on fouling development and biomass characteristics in an algal membrane photobioreactor treating a secondary wastewater effluent

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Abstract

Coupling algal biomass growth to wastewater treatment is a promising alternative for the simultaneous removal and recovery of nutrients. This study aims to evaluate the effects of the Hydraulic Retention Time (HRT) on the fouling behavior and biomass characteristics of
C. Vulgaris in a Membrane Photobioreactor (MPBR), fed with a secondary synthetic wastewater effluent. The changes in the algal cell characteristics and in their metabolic products were assessed at three different HRTs (12 h, 24 h and 36 h). Experimental results showed that higher loading rates led to a broader Particle Size Distribution (PSD) resulting from looser and less stable algal flocs. In contrast, bigger and homogeneously distributed particles observed at lower loading rates, led to a porous layer with lower fouling rates and organic removal. The presence of smaller particles and dissolved organics resulted in a more compact and less porous layer that increased the removal of small-MW organics.

**Keywords:** Fouling; Microalgae separation; Nutrient removal; Membrane photobioreactor; Chlorella vulgaris;
1. Introduction
The increase in water demand has led to a global upsurge in the wastewater produced and its overall pollution. The removal of nutrients present in wastewater is associated with large demands of land and high capital expenditures. Growing attention towards the environmental impacts of wastewater treatment has increased the demand for less energy-intensive processes, where valuable nutrients are recovered and new value streams are created (Gonçalves et al., 2017; Luo et al., 2017; Xu et al., 2015).
The use of microalgae has been proposed as an energy and cost-efficient alternative to tackle different water and energy challenges (Liu et al., 2020). Wastewater represents a cost-efficient solution to supply nutrients and water for algal growth, as it contains the key chemical components required, i.e. carbon, nitrogen and phosphorous (Kanchanatip et al., 2016). Coupling algal biomass growth to wastewater treatment results in the simultaneous nutrient removal, carbon dioxide capture, and recovery of valuable products (Vo Hoang Nhat et al., 2018). This creates a closed-loop system whereby environmental impacts are minimized whilst achieving a more efficient use of energy and recovering valuable materials (Gonçalves et al., 2016; Liao et al., 2018).
A membrane photobioreactor (MPBR) is a process that couples the culture of photosynthetic microorganisms with a continuous biomass separation using a membrane filtration system (Low et al., 2016); the process has been proven efficient in removing nitrogen and phosphorous from wastewater (Gao et al., 2016; Praveen and Loh, 2016). Nevertheless, membrane fouling and the consequent permeate flux decline challenge it’s large-scale applications. The effect is exacerbated over time as algal cells and their algal organic matter (AOM) accumulate on the membrane surface (Elcik and Cakmakci, 2017). Fouling control is hence crucial for MPBR systems in order to ensure their long-term operation (Guo et al., 2012). It encompasses strategies such as physical and chemical cleanings, that increase the membrane lifespan, in exchange for large demands of energy and chemicals, and interruptions in the permeate production, that affect the process efficiency (Bagheri and Mirbagheri, 2018; Fortunato et al., 2019).
The influence of different factors affecting biofouling and the MPBRs performance are reported elsewhere (Krzeminski et al., 2017). These include membrane characteristics such as pore size, biomass characteristics such as cell size (Eleik and Cakmakçi, 2017), properties
of metabolic products, such as proteins and polysaccharides (Shekhar et al., 2017; Wu et al., 2018), and operating conditions such as the hydraulic retention time (HRT). Operating parameters can generate different impacts on the fouling propensity of an MPBR; changes in the HRT, and subsequently in the food to microorganism ratio (F/M), alter the characteristics of the algal biomass and their metabolic products (Low et al., 2016). The HRT is thereby regarded as a critical parameter affecting both removal efficiency and biomass productivity; higher nutrient removal efficiencies are achieved at longer HRTs in exchange of nutrient limitation and lower biomass productivities. Nonetheless, impacts on the fouling development and control remain unclear, highlighting the importance of understanding this relation in order to improve the process performance and facilitate its widespread application.

The objective of this study is to evaluate the effects of HRT on the fouling behavior and biomass characteristics of *Chlorella vulgaris* in a submerged membrane photobioreactor (SMPBR) fed with a synthetic secondary wastewater effluent. This was evaluated by varying the HRT of a SMPBR in continuous operation mode, while the changes in the algal cell characteristics were assessed (e.g. particle size distribution, biomass concentration and zeta potential) as well as their metabolic products (e.g. proteins and polysaccharides). The fouling propensity was considered in relation to fluctuations in the transmembrane pressure (TMP) over time, whilst keeping a constant flux by changing the membrane area used at different HRTs, namely, 12 h, 24 h and 36 h.
2. Materials and methods

2.1. Algae inoculum and pre-cultivation

The SMPBRs were inoculated with a strain of the microalgae *Chlorella vulgaris* (UTEX 259), obtained from the Culture Collection of Algae of UTEX (US). The strain was pre-cultivated autotrophically under batch mode in a Modified Bold’s Basal Medium (BBM) purchased from Sigma-Aldrich. It was placed in an incubation chamber, and sodium bicarbonate \((\text{NaHCO}_3)\) was added to the medium as inorganic carbon source \((750 \text{ mg/L})\). More detailed information about the cultivation of the cells is available in literature (Najm et al., 2017).

2.2. Experimental setup

A schematic diagram of the SMPBR is presented in Fig. 1. A customized cylindrical airlift MPBR was designed and fabricated with a surface to volume ratio of 13.7, and total and working volumes of 2.5 and 2.0 L, respectively. Temperature was kept at 25±1°C, and light radiation continuously at a photosynthetic photon flux density (PPFD) of 130 µmol photons/m²/s, provided by using cool white LED lamps (Najm et al., 2017).

An algal seed from a running MPBR with the herein described media characteristics and growing conditions was employed for the experiments. Three different hydraulic retention times (HRT) were used for the experiments: 12, 24 and 36 hours (Low et al., 2016). Under each experimental condition, the reactor was continuously operated for 10 days at a constant flux of 10 L/m².h (LMH). Following an acclimatization period, the initial biomass concentration was adjusted to 800 mg/L prior to the start of the experiments. At the end of each experiment, the algal cells were washed twice with 0.85% NaCl solution and DI water, the reactor was cleaned, and the working volume was recharged with fresh medium prior to the next acclimatization period.

The MPBR was continuously operated using synthetic secondary domestic wastewater with total nitrogen (TN) and dissolved inorganic phosphorous (DIP) concentrations of 65 and 10 mg/L, respectively, having a Redfield ratio of 14:1. Compressed air was the sole source of inorganic carbon, passed through a 0.2 µm filter and bubbled with air diffusers into the reactor \((30 \text{ L/h})\), as this was found sufficient for the algal growth during the acclimatization period. The pH was monitored daily and kept between 7 and 8 using sodium bicarbonate \((\text{NaHCO}_3)\).
The synthetic secondary wastewater contained 230 mg/L of ammonium chloride and 44 mg/L potassium phosphate, the detailed composition of the feed can be found elsewhere (Fortunato et al., 2019; Najm et al., 2017). The wastewater was continuously fed into the reactor using a peristatic pump (Masterflex®, Cole-Parmer, United States. The liquid level in the reactor was maintained constant using a level controller (Figure 1)(Fortunato et al., 2016). The as-grown C. vulgaris cell mixture from the reactor was continuously fed into an automatic filtration unit (Osmo InspectorTM, Convergence, The Netherlands) wherein a hollow fiber module was used to uninterruptedly extract the permeate. The cell suspension was circulated through the membrane filtration unit at a speed of 2 L/h. The permeate was extracted and collected in a pre-weighted tank, whereas the retentate was continuously recirculated back into the MPBR, both operations carried out by pumps incorporated in the filtration unit.

A polyvinylidene fluoride (PVDF) micro filtration hollow fiber membrane module (GE™, USA), pore size 18 nm, was used as the solid/liquid separator in the filtration unit. New membranes were used for each experiment. The membranes were soaked in Milli-Q water for 24 hours, rinsed and operated with DI water at a flux of 10 LMH for 2 hours prior to the experiments.

The water permeability was determined in the laboratory by filtering DI water and measuring the TMP profile. All tests were conducted using a constant flux of 10 LMH and applying customized specific membrane areas of the filtration module to account for the varying HRTs. The specific aeration demand was kept the same for all conditions, normalized to membrane area (SADm) at 0.6 m³/m².h.
2.3. Analytical techniques

The transmembrane pressure (TMP) was continuously monitored and recorded every 10 seconds using a pressure sensor incorporated in the filtration unit.

The biomass concentration was determined by measuring the optical density of the microalgae culture at 680 nm (OD680) with a UV spectrophotometer (DR 2500, Hach®, United States). The calibration curve Eq. (1) between OD680 and the algal dry weight per litter was used to determine the growth trend of *C. vulgaris*.

\[
MLSS \left( \frac{mg}{L} \right) = 0.0025 \ OD_{680} + 0.0602 \quad R^2 = 0.999 
\]

(1)

The particle size distribution (PSD) of the biomass suspension was determined by triplicate measurements using a laser diffraction particle analyzer (Master Sizer Macro TM, Malvern, UK), using a refractive index (n) of *Chlorella* of 1.086, and water as dispersant (n=1.33) at an obscuration of 18%.

The soluble algal organic matter (AOM) was quantified and then characterized in terms of protein and polysaccharide fractions in the algal suspension and permeate. The AOM was extracted and concentrated using the formaldehyde and NaOH method (Liu and Fang, 2002). The polysaccharides content was determined by means of the phenol-sulfuric acid method, using glucose for calibration at a UV absorbance of 485 (Hach, DR 5000, US). The protein concentrations were determined by the modified Lowry method, using a Qubit® Protein Assay Kit (Invitrogen TM, US) with bovine serum albumin (BSA) for calibration (Fr/olund et al., 1995). Polysaccharide and protein measurements were performed on quadruplicate samples.

Total Organic Carbon (TOC) was measured by triplicates using a previously filtered (0.45 µm PES filter, Milipore, USA) volume of 20 mL, and processed in a TOC-L analyzer (Shimadzu TM, Japan), where the TOC is determined by CO₂ infrared absorption at 680°C. Liquid chromatography-Organic Carbon Detection (LC-OCD) (DOC Labor, Germany) was used to characterize the molecular weights of soluble compounds and the relative organic carbon in the mixed liquor supernatant. The samples were previously filtered through a 0.45 µm PES filter (Milipore, USA), and 10 mL of each sample were added into disposable glass vials. The sample volume injection was 2000 µL and the running time was set at 180
minutes. Potassium phosphate monobasic and disodium hydrogen phosphate were used for the mobile phase at a concentration of 2.5 and 1.5 g/L, respectively.

The Zeta-Potential of *C. vulgaris*, was assessed at room temperature by electrophoretic mobility measurements of the algal diluted suspensions according to the Smoluchowski model. The zeta potential was determined using a Zeta Sizer Nano-ZS (Malvern, UK).
3. Results and discussion

3.1. Effect of hydraulic retention time (HRT) on the fouling behavior

The experimental setup was designed to evaluate the impact of different HRTs on the fouling propensity of a SMPBR system operated with a constant flux. The TMP profiles for 10 days of steady operation are presented in Figure 2; during the first 3 days of the experiments, membrane fouling increased more rapidly at lower HRTs. Correspondingly, after 10 days of operation, SMPBR$_{12h}$ presented the highest TMP (11 kPa), followed by SMPBR$_{24h}$ (7.3 kPa) and SMPBR$_{36h}$ (6.1 kPa). The convection rates towards the membrane were kept constant by keeping a constant flux in all the experiments, thus highlighting the significant role of other parameters different than the sole flux in the fouling development. Consequently, the effect of HRT on the algal cell and AOM characteristics was evaluated.

3.2. Effects on the biomass properties

3.2.1. MLSS concentration and F/M ratio

The algal biomass concentration for the three operating conditions tested are presented in Figure 3a. The MLSS differed significantly amongst the experiments; SMPBR$_{24h}$ presented the highest concentration (1,700 mg/L), followed by SMPBR$_{12h}$ (1,418 mg/L) and SMPBR$_{36h}$ (817 mg/L). Higher loading rates at a lower HRT favored the logarithmic growth of *C. vulgaris* in SMPBR$_{24h}$ and SMPBR$_{12h}$, resulting in a steady growth, and thereby in higher biomass concentrations. In SMPBR$_{12h}$, the MLSS growth rates decreased after 6 days of operation, whereupon the MLSS in SMPBR$_{24h}$ became more prominent, reaching a value of 1700 mg/L after 10 days of operation. A stronger tendency of aggregation onto the glass reactor and recirculation line surfaces was observed in SMPBR$_{12h}$, creating a light shading effect and lower biomass productivity in spite of operating at a higher loading rate than that of SMPBR$_{24h}$. This effect is elsewhere reported for *Chlorella vulgaris* in an Osmotic Membrane Photobioreactor, and for MLSS concentrations greater than 1 g/L in a MPBR (Bilad et al., 2014; Praveen and Loh, 2016).
Higher MLSS concentrations exacerbate the fouling development by increasing the cake layer formation and adsorption rates on the membrane surface (Kanchanatip et al., 2016). Nonetheless, this parameter alone has been found to be insufficient as a fouling indicator due to the complex interactions between algal cells and AOM, and thereby the development of a more compact and nonporous cake layer varies depending on different operating conditions (Javadi et al., 2014). Correspondingly, faster fouling rates observed in SMPBR\textsubscript{12h} did not correlate with higher MLSS concentrations, whereas similar fouling rates (i.e. TMP profiles) were observed in SMPBR\textsubscript{36h} and SMPBR\textsubscript{24h}, in spite of presenting the lowest and highest MLSS concentrations, respectively.

Other parameters such as the food to microorganism ratio (F/M), EPS protein to polysaccharides ratio, and shear rates have been found to correlate with fouling severity better than the biomass concentration alone (Javadi et al., 2014). The F/M ratio is used as a parameter to quantify the amount of nutrients provided to microorganism and thus represents the potential for biomass growth. The F/M ratios for all the experimental conditions are presented in Figure 3b. Higher F/M ratios in SMPBR\textsubscript{12h} correlated positively to the fouling severity, while similarities in the F/M ratios for SMPBR\textsubscript{24h} and SMPBR\textsubscript{36h} corresponded to comparable fouling rates.

Linear regressions between TMP and, both MLSS and F/M ratio, strongly correlated in the cases of SMPBR\textsubscript{12h} ($R^2 = 0.96$ and $R^2 = 0.93$) and SMPBR\textsubscript{24h} ($R^2 = 0.98$ and $R^2 = 0.94$). Lower loading rates in SMPBR\textsubscript{36h}, showed no correlation with the biomass concentration ($R^2 = 0.02$ and $R^2 = -0.03$), suggesting the unsuitability of using these parameters as fouling indicators at low nutrient concentrations and stationary growth. The strong correlation between cell concentration and membrane fouling index during logarithmic growth has been reported to fade during the stationary growth phase, as it was observed in SMPBR\textsubscript{36h}.

In conventional membrane photobioreactors (MBRs), EPS production by the microorganisms is reported to be influenced by the F/M ratio, wherein high values induce a higher excretion of EPS and increases the TMP during operation (Fortunato et al., 2018). In contrast, lower F/M ratios are known to lead to slower growth rates, higher rates of endogenous metabolism and cell lysis, and lower EPS concentrations, resulting in lower fouling rates (Wu et al., 2013). Results in this study highlight similarities in the fouling behavior of MBRs and MPBRs under a logarithmic growth phase, as strong correlations between biomass concentration and membrane fouling were found in SMPBR\textsubscript{12h} and
SMPBR<sub>24h</sub>. The fading correlations in SMPBR<sub>36h</sub> indicate the significance of other foulants than the biomass alone in the fouling development during the stationary/death phase, such as cell debris and Algal Organic Matter (AOM) (Luo et al., 2017). The influence of AOM in membrane fouling is further discussed in Section 3.3.

### 3.2.2. Particle size distribution

The particle size distribution (PSD) of the foulants and the membrane pore size have a strong influence on the fouling mechanism. Particles and solutes with a size larger than that of the membrane pores are retained and accumulate on the membrane surface due to size exclusion, whereupon a cake layer is formed. In contrast, particles with a size smaller than that of the membrane pores lead to pore blocking or pore narrowing, causing a more severe and irreversible fouling (Elcik and Cakmakci, 2017). The size of the particles in the reactor were overall larger than the membrane nominal pore size (0.02 µm), indicating that cake layer formation was the major contributor to the filtration resistance, as reported elsewhere (Elcik and Cakmakci, 2017; Wu et al., 2013).

Shorter HRTs led to broader and less stable distributions. SMPBR<sub>12h</sub> presented the most heterogenous VPSD (modes at 1.5, 3.2, 10.8, and 42.5 µm). It was followed by SMPBR<sub>24h</sub> (modes at 3.2, 17.2 and 44.8 µm) and SMPBR<sub>36h</sub> (3.2 and 44.8 µm), denoting increasingly homogeneous distributions at longer HRTs. In the NPSD, SMPBR<sub>12h</sub> presented a bimodal distribution at 1.5 and 3.5 µm, differing from SMPBR<sub>24h</sub> and SMPBR<sub>36h</sub>, both with unimodal distributions at 3.9 µm.

Higher loading rates led to the formation of a small number of larger and less stable flocs, prone to create a more heterogeneous PSD as they break up and release AOM in the solution (Figures 5a and 5b). It is worth noting that higher loading rates in SMPBR<sub>12h</sub> led to the presence of a second mode in the NPSD (1.5 µm), with a size smaller than that of the algal cells, originating from the aggregation of AOM and cell debris in the reactor (Medina and Neis, 2007).

Previous studies have investigated the changes in the floc size and stability in response to variations in the loading rates (Low et al., 2016). It was reported that, in symbiotic flocs of sludge and *Chlorella vulgaris* sp., a more compact arrangement of the EPS matrix, and therefore more stable and resistant particles, were found at lower F/M ratios. It contrast, a less compact EPS physical arrangement at higher loading rates was reported to increase the
propensity of floc breakage, hence, affecting the PSD in the reactor (Medina and Neis, 2007).
Smaller particles in SMPBR$_{12h}$ resulted in a lower cake porosity and subsequently a higher specific cake resistance (Bourcier et al., 2016; Marbelia et al., 2014). It has been suggested that the main parameter impacting the specific cake resistance and compressibility is the PSD. A broad PSD leads to a cake made up of uneven layers with different particle sizes, wherein the smaller ones are more prone to move from one layer to another as the filtration pressure increases. Results from this study indicate that broader size distributions and a larger fraction of smaller particles precede a more severe fouling (Bourcier et al., 2016).

3.2.3. Zeta potential
The Zeta Potential measures the charge in the diffusive layer developed at an interface; high zeta potential values (negative or positive) are characteristic in electrically stable particles, whereas particles with a low zeta potential present a higher tendency to aggregate or to adhere to a surface. Negatively charged surfaces were observed, presenting SMPBR$_{12h}$ a slightly lower zeta potential (-25.8 ± 3.5 mV), followed by SMPBR$_{24h}$ (-29.3 ± 1.9 mV) and SMPBR$_{36h}$ (-29.1 ± 3.9 mV). These findings are in agreement with previously reported values ranging between -20 and -30 mV (Bin Liu et al., 2017; Ozkan and Berberoglu, 2013a; Procházková et al., 2012).
The lower Zeta Potential values in SMPBR$_{12h}$ indicate weaker electrostatic repulsive forces, and thereby a slightly higher tendency for attachment and aggregation, as reflected in the fouling severity. The smaller particles in SMPBR$_{12h}$ experienced lower electrostatic repulsive forces and thus, a higher tendency for adhesion onto the membrane surface, as the attractive Van der Waals interactions overcome the size-dependent repulsive forces (Ozkan and Berberoglu, 2013b).
However, it is reported elsewhere that the cell-membrane repulsive electrostatic interactions are solely significant at the beginning of the filtration cycle. Thereafter, foulants such as AOM deposit on the membrane surface, the negatively charged sites are covered, and the repulsive forces are overcome by attractive van der Waals and acid-base interactions, as described by the extended Derjaguin–Landau–Verwey–Overbeek (XDLVO) model (Hadjoudja et al., 2010; Ozkan and Berberoglu, 2013b). Furthermore, in continuous operation mode, the XDLVO forces are overcome by convective and hydrodynamic forces.
Our results support the hypothesis that hydrodynamic and convective forces strongly weakened the effect of the colloidal forces on the membrane fouling, as closely comparable Zeta Potential magnitudes were found across the experiments (Ozkan and Berberoglu, 2013a; Procházková et al., 2012).

3.3. Effects on the Algal Organic Matter (AOM)

3.3.1. Total Organic Carbon (TOC)

The average mixed liquor and permeate TOC concentrations are presented in Figure 4. The experimental data revealed substantial differences across the experiments; SMPBR\textsubscript{36h} presented a significantly lower TOC concentration in comparison with SMPBR\textsubscript{24h} and SMPBR\textsubscript{12h}; the latter showed the highest TOC across the experiments. The lowest TOC removal efficiency was observed in SMPBR\textsubscript{36h} (48%), while SMPBR\textsubscript{12h} exhibited the highest efficiency (94%) (Table 1). The discrepancies across the experiments can be mainly attributed to differences in the cake porosity and removal capacity, and to the molecular weight distribution of the organics present in the MLSS. The higher TOC removal in SMPBR\textsubscript{12h} was positively correlated to higher fouling rates, as higher nutrients loading rates led to higher levels of AOM, and to a more heterogeneous PSD. This resulted in a thick and dense cake layer, responsible for a higher AOM removal. The trade-off between membrane permeability and organics removal is reported elsewhere, as cake layer retention is claimed to be the main removal mechanism of low-molecular weight organics (Zhang et al., 2016).

3.3.2. LC-OCD

The soluble AOM, as determined in the LC-OCD chromatograms, in the mixed liquor and permeate is presented in Table 2. Based molecular weight, the analysis classifies the AOM in four categories: 1) high molecular weight (MW) biopolymers (>20,000 Da); 2) humic-like substances (500-20,000 Da); 3) medium molecular weight components such as building blocks (350-500 Da); and low-MW neutrals (<350 Da).

AOM is heterogeneous in size distribution; smaller molecules penetrate into membrane pores and cause pore blocking, whereas larger particles accumulate on the membrane surface and increase the cake layer resistance (Ozkan and Berberoglu, 2013b). In the mixed liquor,
SMPBR_{12h} presented significantly higher concentrations of medium and low-MW organic components, whereas a larger fraction of high-MW biopolymers was observed in SMPBR_{24h}. A greater proportion of low-MW substances was observed in SMPBR_{36h}, possibly resulting from a prolonged contact time between the algal cells and the organics that enhanced the biological breakdown of large molecules into smaller organics (Low et al., 2016).

On the permeate side, SMPBR_{12h} presented a major peak in OCD corresponding to the retention time of the humic-like substances. In contrast, SMPBR_{24h} and SMPBR_{36h} showed two main peaks in correspondence of humic-like substances and building blocks. The SMPBR_{36h} presented the highest concentration of building blocks in the permeate.

The UF membrane efficiently removed the biopolymers present in solution, and thereby a removal efficiency of biopolymers above 90% was observed across all the experiments (Table 2); high-MW compounds accumulated on the membrane surface and became the main cake layer constituent. Nevertheless, the fouling rates in SMPBR_{24h} did not directly correlate with the biopolymer concentration in the reactor, as it presented milder fouling rates than SMPBR_{12h} in spite of having higher biopolymer concentrations. The results indicate that a homogeneous MW distribution towards larger compounds resulted in a more porous and less compact layer responsible for a lower hydraulic resistance and better permeation.

A higher removal of smaller organic substances (e.g. building blocks, low-MW neutrals and LMW acids) was observed in SMPBR_{12h}; it was correlated with a more severe fouling, as the compact and non-porous cake layer enhanced the removal of low-MW organics (Table 2). In fact, the deposition of smaller particles and organics in the cake layer and membrane surface exacerbates the hydraulic resistance, as they strongly attach to the inner pore structure of the membrane and cake layer by adsorption and irreversible plugging (Babel and Takizawa, 2010; Zhang et al., 2013). Correspondingly, a lower removal of building blocks and low-MW compounds observed in SMPBR_{24h} and SMPBR_{36h} was correlated to a lower fouling severity.
3.3.3. Proteins and polysaccharides

The soluble AOM released by algae into the culture medium contains high concentrations of proteins and polysaccharides. These compounds present a wide range of molecular weights and chemical compositions that vary significantly depending on the algal characteristics, growth phase, and availability of nutrients. Over time, the AOM tends to deposit on the membrane surface due to its low back-transport velocity and strong interactions. The AOM released by the algae creates linkages between cells, diminishing the algal dispersion by filling void spaces and thereby increasing the resistance to water flow. As the algal biomass releases AOM and the TMP increases, the cake layer becomes less porous and is compressed. In the absence of AOM, algal cells are more dispersed and thereby a cake layer with more void spaces (i.e. higher porosity, less hydraulic resistance) is built (Hadjoudja et al., 2010; Kanchanatip et al., 2016).

The concentrations of proteins and polysaccharides in the mixed liquor and permeate are presented in Figure 5. Higher loading rates resulted in less stable algal aggregates with a higher propensity for breaking up, thereby increasing the AOM concentration in the solution. The results are consistent with previous studies wherein, higher F/M rations were found to induce a higher surplus of substrate and energy and to increase higher excretion of AOM whereas, lower F/M ratios were related to a lower production of AOM and milder fouling (Bilad et al., 2014; Medina and Neis, 2007).

The results indicate that polysaccharides were the main constituents of the AOM, although remarkably greater differences were found across the protein concentrations. The AOM concentrations of proteins and carbohydrates are shown in Table 3. The amount of AOM released by *C. vulgaris* was affected by the different loading rates; SMPBR<sub>12h</sub> presented the largest AOM concentrations in the mixed liquor. The AOM in SMPBR<sub>12h</sub> was mostly composed of polysaccharides, although, presenting a AOM<sub>POL</sub>/AOM<sub>P</sub> ratio (2.3), similar to SMPBR<sub>36h</sub> (1.9) and lower compared to SMPBR<sub>36h</sub> (4.9).

Significantly higher ratio of polysaccharides respect to the proteins observed for SMPBR<sub>36h</sub>, indicate that the polysaccharides had a stronger impact on membrane fouling at longer HRTs. As reported in the literature, the AOM from stationary growth phase contains greater amounts of polysaccharides responsible of membrane fouling and flux decline (Zhang et al., 2016).
The results presented in this study suggest that proteins played a significant role in the fouling resistance of SMPBR\textsubscript{12h}, in spite of representing only a small fraction of the overall AOM produced. In fact, as reported in the literature, the effect of soluble proteins in the cake layer resistance increases at high F/M ratios (Medina and Neis, 2007).
4. CONCLUSIONS
Higher loading rates in SMPBR_{12h} led to a larger AOM production and a broader PSD. Smaller particles resulted in a dense cake layer, whereby a more severe fouling and a higher removal of small-MW organics were observed. Larger particles and large-MW organics in SMPBR_{24h} resulted in a porous biomass layer, moderate fouling and a relatively stable flux during the period of operation. Milder fouling rates in SMPBR_{36h}, caused by lower biomass layer resistance, were associated to lower TOC removal and to the increase of high-MW compounds into the permeate, highlighting the existing trade-offs between removal and fouling.

("E-supplementary data for this work can be found in e-version of this paper online")

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References


HRT changes impacted the particle size distribution and the AOM characteristics
Proteins were found to play a more significant role at shorter HRTs
Heterogeneous PSD in SMPBR_{12h} led to higher fouling and hydraulic resistance
Greater PSD and MW organics in SMPBR_{24h} led to moderate fouling
SMPBR_{36h} showed lowest fouling propensity and lower organic removal in the permeate

Table 1. Comparative TOC concentration in day 10

<table>
<thead>
<tr>
<th>HRT</th>
<th>Mixed liquor</th>
<th>Permeate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TOC (mg/L)</td>
<td>TOC/MLSS (mg/mg)</td>
</tr>
<tr>
<td>12 h</td>
<td>15.7</td>
<td>0.0130</td>
</tr>
<tr>
<td>24 h</td>
<td>11.1</td>
<td>0.0067</td>
</tr>
<tr>
<td>36 h</td>
<td>8.8</td>
<td>0.0011</td>
</tr>
</tbody>
</table>

Table 2. LC-OCD characterization of AOM in feed and permeate after 10 days of operation

<table>
<thead>
<tr>
<th>HRT 12 H</th>
<th>DOC (mg/L)</th>
<th>Biopolymers (mg/L)</th>
<th>Building Block (mg/L)</th>
<th>LMW-Neutrals (mg/L)</th>
<th>LMW-Acids (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant</td>
<td>25.0</td>
<td>7.8</td>
<td>9.8</td>
<td>1.5</td>
<td>5.8</td>
</tr>
<tr>
<td>Permeate</td>
<td>4.7</td>
<td>n.q.</td>
<td>2.3</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Removal</td>
<td>81%</td>
<td>100%</td>
<td>77%</td>
<td>93%</td>
<td>99%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HRT 24 H</th>
<th>DOC (mg/L)</th>
<th>Biopolymers (mg/L)</th>
<th>Building Block (mg/L)</th>
<th>LMW-Neutrals (mg/L)</th>
<th>LMW-Acids (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant</td>
<td>19.5</td>
<td>12.1</td>
<td>4.7</td>
<td>0.7</td>
<td>2.3</td>
</tr>
<tr>
<td>Permeate</td>
<td>5.3</td>
<td>0.2</td>
<td>2.8</td>
<td>0.7</td>
<td>0.8</td>
</tr>
</tbody>
</table>
### Table 3. AOM concentrations after 10 days of operation

<table>
<thead>
<tr>
<th>HRT</th>
<th>Proteins (mg/L)</th>
<th>Polysaccharides (mg/L)</th>
<th>$\text{AOM}_{\text{POL}}/\text{AOM}_P$ ratio</th>
<th>MLSS (mg/L)</th>
<th>Protein/MLSS (mg/mg)</th>
<th>Polysaccharides / MLSS (mg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 h</td>
<td>5.5</td>
<td>12.8</td>
<td>2.3</td>
<td>1418</td>
<td>0.0039</td>
<td>0.0090</td>
</tr>
<tr>
<td>24 h</td>
<td>4.7</td>
<td>8.9</td>
<td>1.9</td>
<td>1675</td>
<td>0.0028</td>
<td>0.0053</td>
</tr>
<tr>
<td>36 h</td>
<td>1.4</td>
<td>6.8</td>
<td>4.9</td>
<td>817</td>
<td>0.0012</td>
<td>0.0083</td>
</tr>
</tbody>
</table>