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Impact of oxygen cut off and starvation conditions on biological activity and physico-chemical properties of activated sludge

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Physico-chemical and biological parameters were monitored both throughout different oxygen cut off and starvation (OCS) times (6 h–72 h) and after the restoration of normal operational conditions. Sludge apparent viscosity and soluble extracellular polymeric substances (EPS) characteristics were measured to determine the activated sludge (AS) properties. Oxygen transfer, biological activity with specific oxygen uptake rate (SOUR) measurements during endogenous/exogenous conditions (without any external substrate/with external substrate consumption) and chemical oxygen demand (COD) removal were measured to assess the AS performances. During the different stress times, AS deflocculated as a decrease of apparent viscosity was observed and microorganisms biodegraded the released EPS to survive. After aeration return, and under endogenous conditions, size exclusion chromatographic fingerprints of soluble EPS were modified and macromolecules probably of type humic-like substances appeared in significant quantities. These new macromolecules presumably acted as biosurfactants. Consequently, the liquid surface tension, as well as the oxygen transfer rate (OTR), decreased. Under exogenous conditions, high biological activity ($SOUR = 11.8 \pm 2.1 \text{ mg}_{\text{O}_2} \cdot \text{g}_{\text{MLVSS}}^{-1} \cdot \text{h}^{-1}$) compensated the decrease of oxygen transfer. Finally, AS biomass maintained a constant COD degradation rate ($15.7 \pm 1.9 \text{ mg}_{\text{O}_2} \cdot \text{g}_{\text{MLVSS}}^{-1} \cdot \text{h}^{-1}$) before and after the disturbances for all times tested. This work demonstrates that AS microorganisms can counteract concomitant oxygen and nutrients shortage when the duration of such a condition does not exceed 72 h. Dissociation of endogenous/exogenous conditions appears to offer an ideal laboratory model to study EPS and biomass activity effects on oxygen transfer.

Keywords: activated sludge; viscosity; extracellular polymeric substances; specific oxygen uptake rate; oxygen transfer

1. Introduction

Disturbances in the biological activity, such as the input of chemical oxygen demand (COD)-rich effluents, oxygen cut off time and starvation period, are the main observed causes of failures in sewage plants. The last two issues are the subject of many publications [1–4]. Oxygen deficiency was shown to lead to a bulking formation and the rapid development of filamentous bacteria [1]. Characteristics of activated sludge (AS) systems are also studied under nutrient starvation conditions. Bacteria are reported to rapidly metabolize a part of the soluble extracellular polymeric substances (EPS): the secreted utilization associated products to maintain endogenous respiration [3,4]. Coello Oviedo *et al.* [5] showed that over the first three days of nutrient shortage important decreases in sludge flocculation capacity, active cells percentage and oxygen uptake rate (OUR) occurred. Few articles were found in the literature on the impact of more drastic conditions such as simultaneous oxygen cut off and starvation (OCS) time on AS characteristics. However, this situation may occur in waste water treatment plants (WWTPs), such as in accidental power failure. Geilvoet *et al.* [6] reported an increase in soluble protein

and polysaccharide contents during 24 hours without oxygen and nutrient as well as a decrease in sludge filterability. Longer periods without air provoke total floc disintegration due to a release of metal cations involved in floc links [7].

To our knowledge, specific oxygen uptake rate (SOUR), oxygen transfer and organic matter biodegradation have not been investigated after the restoration of normal operational conditions, even though these measurements are essential for evaluating CAS and MBR performances after such a problem.

Biodegradation mechanisms depend on dissolved oxygen concentration and, thus, on oxygen transfer from the gas phase to the liquid phase. The presence of oxygen consuming cells and their consumption rate were previously assumed to influence the oxygen transfer [8]. This paper aims at linking biological activity to the volumetric oxygen mass transfer coefficient ($k_{\text{L}a}$) obtained after different aeration cut off times and nutrient depletion. Endogenous and exogenous conditions were studied to represent different oxygen consumption rates. The endogenous condition is reached when no external substrate (e.g. a carbon source) is

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available; bacteria are forced to biodegrade their intracellular carbon as substrate and their respiration is minimal. The exogenous condition is obtained after substrate addition; bacteria use the substrate present to supply their energy demand, and the resulting respiration is at its highest value.

An additional consequence of aeration and nutrient issues previously observed is the change in extracellular polymeric substances (EPS) content [6,9]. EPS are described as a matrix embedding microorganisms and originate from (i) a bacterial active secretion and cell lysis or (ii) an abiotic sorption of molecules present in the effluent. EPS are mainly composed of proteins, polysaccharides and humic-like substances; lipids and nucleic acids are found in lower proportions [10]. The structure of sludge flocs is highly dependent on EPS concentration [11,12]. Indeed, a low level of EPS indicates sludge deflocculation while a high level results in foaming [13] and membrane biofouling for MBRs [14–16]. Therefore, the impact of oxygen cut off and nutrient depletion on EPS macromolecular composition must be understood. In this paper, the apparent viscosity of AS during and after different OCS times was made *in situ*, for example, aeration of AS was taken into account in the apparent viscosity determination, which is rarely the case in AS apparent viscosity measurement [17]. These measurements were completed by soluble EPS quantification and characterization by size exclusion chromatography (SEC) coupled with innovative UV and fluorescence detectors [18]. Furthermore, the soluble EPS compounds that may have an influence of oxygen transfer in terms of surface tension modification [19] were linked to the $k_L a$ values obtained in this paper.

The novelty of this work was to combine in a unique study the determination of AS properties and biological performances during and after drastic conditions. Biological activity under endogenous and exogenous conditions was assessed and correlated with changes in $k_L a$, EPS characteristics, apparent sludge viscosity and COD degradation rate.

2. Materials and methods

2.1. Activated sludge

AS was sampled from a municipal wastewater treatment plant (Aix en Provence, France, 175,000 inhabitant equivalent, $35,000 \text{ m}^3 \cdot \text{d}^{-1}$, organic load $0.12 \text{ kg}_{\text{BOD}_5} \cdot \text{kg}_{\text{MVS}}^{-1} \cdot \text{d}^{-1}$). The initial mixed liquor suspended solids (MLSS) concentration ranged from 3 to $4 \text{ g} \cdot \text{L}^{-1}$ and the amount of mixed liquor volatile suspended solids (MLVSS) ranged from 75 to 80%. Samples were taken from the recirculating loop between the aerated tanks and the secondary clarifiers and then transported to the laboratory with no aeration (30 min). AS was concentrated by gravimetric filtration using paper filter (average pore size around $100 \mu\text{m}$) until a MLSS concentration of $12 \pm 1 \text{ g} \cdot \text{L}^{-1}$ was reached. This high MLSS concentration resulted in higher biological activity. The chosen concentration corresponded to the

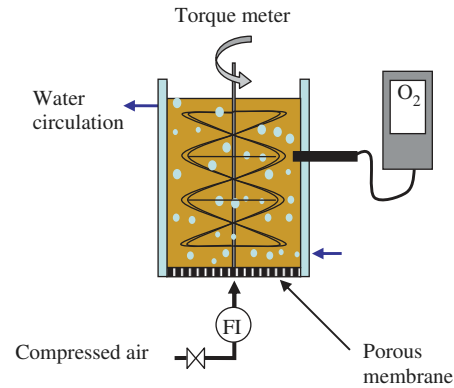


Figure 1. Schematic representation of the experimental device.

high limit of the MLSS concentrations range generally used in MBRs to treat wastewaters and is the minimal range required for accurate measurement of apparent viscosity with the type of apparatus used (section 2.2) [20]. Finally, the AS was aerated during 4 h without substrate addition to ensure endogenous conditions.

2.2. Bioreactor

A 2 L bioreactor was filled with the sampled and concentrated AS (Figure 1). A double helical ribbon impeller (HRI) was added to perform rheological measurements and to homogenize the AS. Both the diameter and height of the impeller were 9.5 cm, while the bioreactor diameter was 12 cm. Sludge temperature was maintained constant at $20 \pm 1^\circ\text{C}$ with water circulating in an external envelope.

Compressed air was injected in a soft porous membrane to ensure the diffusion of fine bubbles through the whole bottom surface. The compressed air pipe was equipped with a flowmeter and a valve to control the air flow rate at $3 \text{ L} \cdot \text{min}^{-1}$. Dissolved oxygen (DO) concentration was measured with a HQ30D oxymeter (Hach Lange) perpendicular to the air flow to avoid air bubble deposition on the probe surface. The bioreactor was aerated until the DO stabilized (equilibrium between endogenous respiration and oxygen dissolution), and next, OCS times from 0 h to 72 h were applied. This limit of 72 h was fixed because many authors had found that main AS characteristics changes occurred within this time range [2,5]. All the experiments were performed in triplicate.

2.3. $k_L a$, SOUR and surface tension measurement

The first set of experiments were aimed at measuring $k_L a$ and the SOUR under endogenous conditions before and after OCS. The second set of experiments was performed under exogenous conditions with glucose addition ($0.4 \text{ g}_{\text{COD}} \cdot \text{g}_{\text{MLVSS}}^{-1}$) after the OCS; measurement of SOUR and $k_L a$ were performed until the substrate was totally consumed (around 2 h).

SOUR and $k_L a$ were measured during different aeration phases. Aeration was first turned off and DO concentrations were recorded every 10 s. OUR was then calculated as the slope of the linear decrease of the DO concentration. To obtain SOUR values, OUR values were divided by the MLVSS concentration of AS. Aeration was turned on when the DO concentration was below $0.5 \text{ mg} \cdot \text{L}^{-1}$, and DO concentrations were recorded every 10 s. The $k_L a$ was then calculated from the classical mass balance of DO in a biological medium (Equation (1)). When the steady state between the microorganism respiration and the oxygen dissolution was reached, the mass balance was defined in Equation (2). Finally, the $k_L a$ value was calculated by combining Equations (1) and (2) and integrating the resulting Equation (3) during the oxygenation phase. A rotation speed of 200 rpm was maintained during $k_L a$ measurement to avoid AS settling and to enable homogeneous bubble distribution.

$$dC/dt = k_L a(C^* - C) - OUR \quad (1)$$

where C^* is the saturation DO concentration and C is the DO concentration.

$$OUR = k_L a(C^* - C_{eq}) \quad (2)$$

where C_{eq} is the steady state equilibrium DO concentration.

$$dC/dt = k_L a(C_{eq} - C) \quad (3)$$

$k_L a$ and surface tension (σ) of the supernatant were also measured. After gravimetric filtration of AS, $k_L a$ of the supernatant was obtained with the sodium sulphite oxidation method [21] and σ values with the ring method using a Nouy tensiometer [22].

2.4. COD measurement

COD degradation rate was measured before and after OCS periods to evaluate the impact of these drastic conditions on biodegradation of organic pollution. Glucose was added to the bioreactor to observe the maximal SOUR ($0.4 \text{ g}_{\text{COD}} \cdot \text{g}_{\text{MLVSS}}^{-1}$), and after 1 min of homogenization, 30 mL of AS were sampled and centrifuged during 15 min at 13,500 rpm. The obtained supernatant was analysed with a COD kit (Aqua Lytic) at 605 nm after 2 h of heating at 150°C . The same protocol was applied 1 h after the glucose addition to calculate the COD degradation rate.

2.5. EPS extraction and characterization

Soluble EPS were quantified and characterized from 30 mL sampled steadily before, during and after the different OCS times using the control method described by Comte *et al.* [23]. The samples were stored in a refrigerator until a temperature of 4°C was reached and were then centrifuged (Sigma 2-16) for 20 min at 4°C and 6800 rpm to extract

soluble EPS. This soft extraction technique was used to collect only the soluble EPS, also called loosely bound EPS because they are more sensible to the structural changes of the sludge and easily removable from bioflocs. The final EPS solutions were stored at -18°C .

As the major components of soluble EPS contents, protein [24] and polysaccharide [25] contents of the soluble EPS were first quantified with colorimetric methods; bovine serum albumin was used as a standard for proteins and glucose was used for polysaccharides. Then, qualitative characterization of the EPS was performed with size exclusion chromatography (SEC). The chromatograph (LA Chrom, Merck Hitachi) was equipped with a pre-packed Superdex 200 column (10/300 GL, Amersham Biosciences), an autosampler (L7200), a quaternary pump (L7100), an interface (L7000), a fluorescence detector (L7485) and a diode array UV detector (L7455). The fluorescence detector wavelengths were set for the detection of tryptophan protein-like compounds (excitation/emission = 221 nm/350 nm) [26,27]. The UV detector was set at a wavelength commonly used for the analysis of proteins, humic-like substances and nucleic acid contents of EPS (280 nm) [28]. EPS samples were filtered with $0.2 \mu\text{m}$ filters (Millipore) and then injected in a $100 \mu\text{L}$ loop.

The total bed volume of the column was determined to be 23.5 mL by using a solution of $0.6 \text{ g} \cdot \text{L}^{-1}$ sodium azide. The column was equilibrated with 0.05 M phosphate buffer at pH 7.0 added with 0.15 M NaCl at a flow rate of $0.4 \text{ mL} \cdot \text{min}^{-1}$.

The SEC method is based on the increase in elution volume as hydrodynamic volume of the molecule (assumed as molecule size) is decreasing. The relationship between molecular weight and elution volume in SEC is only relevant for the molecules used during the calibration [29]. Therefore, calibration was made with protein standards that are considered as major components of EPS [28]. The protein standards (Sigma-Aldrich, France) were Ferritin type I horse spleen (440 kDa); immunoglobulin G from human serum (155 kDa); bovine serum albumin (69.3 kDa); cytochrome C from horse heart (13 kDa) and insulin from porcine pancreas (5.8 kDa). For elution volumes ranging from 10 to 19 mL, which correspond to proteins with a molecular weight between 440 kDa (ferritin) and 5.8 kDa (insulin), the calibration curve showed a linear relation between the log of molecular weight (MW) and elution volume (V_e (mL)) (Equation (4)).

$$\log(MW) = -0.21(V_e) + 7.7(r^2 = 0.99) \quad (4)$$

2.6. In situ rheological device and rheological model

The in situ viscosity was measured before and after the OCS periods at air flow rates ranging from 0 to $3 \text{ L} \cdot \text{min}^{-1}$. The bioreactor impeller was connected to a rheometer (Rheomat 115, Contraves). Stirring torque was measured for different

rotation speeds in the range imposed by the rheometer (from 0.2 to 200 rpm). Before the torque measurement, AS was pre-sheared at 200 rpm for 30 s to homogenate the AS suspension and prevent sludge settlement. The rotation speed was then decreased to the desired value and the torque was recorded for 15 s after 15 s of stabilization. The in situ viscosities were calculated with the Metzner-Otto's principle. Calculation and calibration details can be found in the work published by Seyssiecq *et al.* [20]. The stirrer was stopped during the unaerated phase to avoid damages on the rheoreactor and was turned on 10 min before the in situ viscosity measurement.

In the rheological part of this study, the Ostwald power law model has been used (Equation (5)) to represent the viscosity curves of AS.

$$\tau = K\gamma^n \quad (5)$$

The two parameters of this model, that is, the consistency index (K in $\text{Pa}\cdot\text{s}^n$) and flow index (n) have been calculated using a simple linear regression in log-log scale.

3. Results and discussion

3.1. AS characteristics during different OCS times

3.1.1. Evolution of AS in situ apparent viscosity

In situ viscosity curves were presented before stress conditions and after OCS times of 48 h in terms of apparent viscosity as a function of shear rate (Figure 2). The MLSS concentration was $12 \pm 1 \text{ g} \cdot \text{L}^{-1}$ and the air flow was $0 \text{ L} \cdot \text{min}^{-1}$. The viscosity curves obtained for the OCS times of 6 h, 12 h and 24 h were similar to the one without stress conditions. The ones for 48 h and 72 h were similar with each other and different from the one without stress conditions. The apparent viscosity decreased when shear rate rose from 0.07 to 41 s^{-1} for all oxygen cut off and starvation times. The Ostwald parameters calculation indicates a decrease in the shear thinning level of the suspension after 48 h of stress. Indeed, the stress conditions equal or higher than 48 h lead to a decrease of 26% of the consistency index K (from 0.39 to $0.29 \text{ Pa}\cdot\text{s}^n$) while the flow index n increases from 0.17 to 0.2 .

During the first 24 h, the drastic conditions have no effect on AS apparent viscosity. This result is contradictory with those obtained by Coello Oviedo *et al.* [5], which proved that the highest MLSS decrease ($0.8 \pm 0.01 \text{ g} \cdot \text{L}^{-1}$) happened during the first day under starvation conditions. In the case of this study, the bioflocs strength does not seem to be sufficiently modified to induce significant variations of the rheological properties of the sludge. We can hypothesize that, during rheological measurements, under the shear field induced by the HRI (low shearing impeller), flocs size does not change significantly between the initial AS and after 24 h of stress.

After OCS times equal to or greater than 48 h, the apparent viscosity measured at low shear rate showed a weak

decrease as a decrease of K and an increase of n . This result can be explained by the breakage of part of bacterial flocs structure, leading to a release of water molecules previously trapped in the flocs. An increase of the flowing properties of the suspension (decrease in shear thinning properties) is then observed [30].

In the high shear region (above $41 \cdot \text{s}^{-1}$), the apparent viscosity was the same during the different OCS times. The apparent viscosity was only controlled by the mechanical stirring.

The stress performed during 48 h or more has provoked a decrease of 46% of K and an increase of 54% of n after the air return at $3 \text{ L} \cdot \text{min}^{-1}$. The Ostwald parameters calculations indicate that the deflocculation was maximal not during the stress conditions (substrate depletion and oxygen cut off) but as soon as air came back. The in situ viscosity curves at 6 h, 12 h and 24 h were the same as the one without stress conditions and the one at 72 h showed the same trend as the one at 48 h.

The influence of aeration on AS apparent viscosity was shown at low shear rates (from 0.07 to 41 s^{-1}). In this low shear region, the apparent viscosity at an air flow rate of $3 \text{ L} \cdot \text{min}^{-1}$ was lower than the apparent viscosity obtained for non-aerated suspensions. With regard to the structural aspect, complex fluids can be described as concentrated dispersions of structural units, for example flocs in the case of biological sludge. Flocs were defined as groups formed by smaller primary elements [31]. Flocs were called structural units and had a certain size depending on the hydrodynamic stress applied. At low mechanical shear rates, the injection of air bubbles interacted with the configuration of sludge flocs and strongly affected the steady state of the structural AS units.

Microorganisms were under extreme conditions, not being fed or aerated, and then bacterial floc structure was weakened and easily broken by the air bubbles. The apparent viscosity decrease was higher at a flow rate of $3 \text{ L} \cdot \text{min}^{-1}$ than at $0 \text{ L} \cdot \text{min}^{-1}$ as the non-mechanical shear of the air bubbles controlled the AS structure at low shear rate.

One of the objectives of this paper was to evaluate the impact of low and high biological activity on oxygen transfer after OCS conditions. As oxygen transfer is influenced by the apparent viscosity [32], variations due to the air flow rate or the length of the OCS times was eliminated. Therefore, a high shear rate of 84 s^{-1} was applied for the following experiments.

3.1.2. Evolution of the soluble EPS contents

Proteins and polysaccharides were considered to be major components of the EPS present in AS [10]. Their concentrations were measured constantly during 72 h in two AS samples: (i) one with no aeration and (ii) another one aerated with a flow rate of $3 \text{ L} \cdot \text{min}^{-1}$ to detect changes due to OCS conditions (Figure 3(a) and (b)).

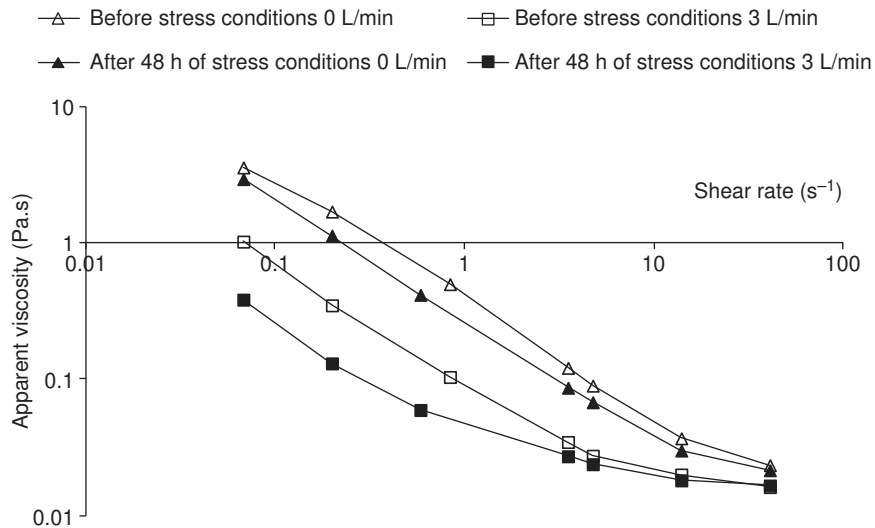


Figure 2. In situ viscosity curves at air flow rate of $0 \text{ L}\cdot\text{min}^{-1}$ and $3 \text{ L}\cdot\text{min}^{-1}$ before stress conditions and after 48 h oxygen cut off and starvation.

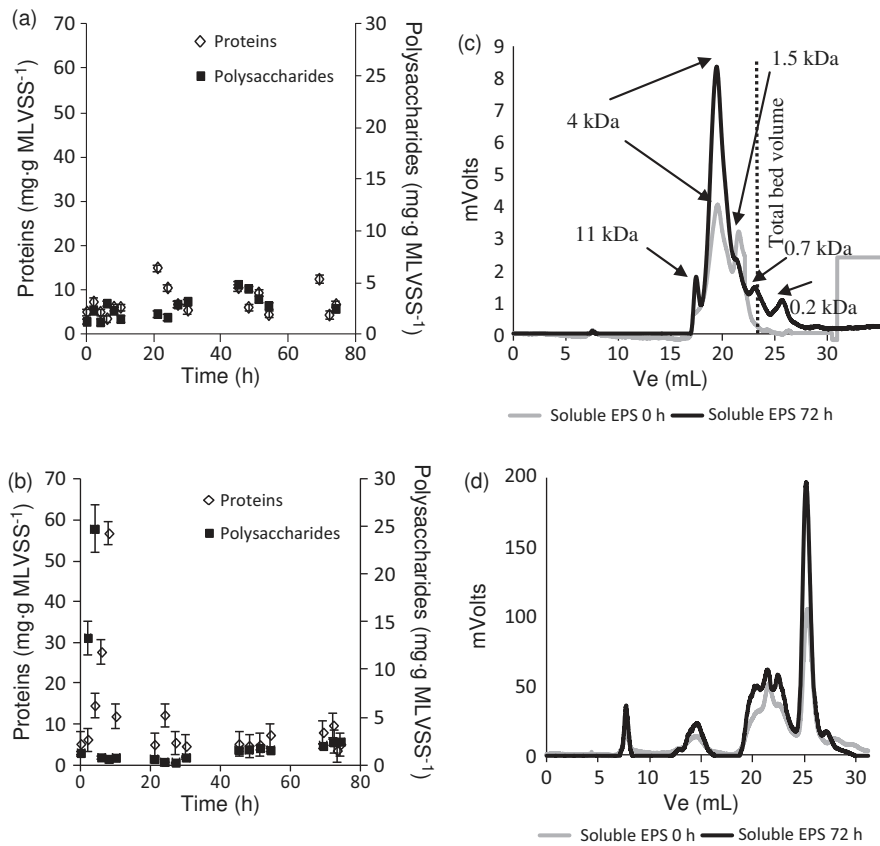


Figure 3. Protein and polysaccharide contents of soluble EPS extracted from activated sludge (a) under aeration and (b) during 72 h of oxygen cut off and starvation. Chromatograms of soluble EPS extracted from activated sludge before and after a 72 h air cut off and starvation at (c) 280 nm and (d) tryptophan-type detection wavelength.

Under aerated conditions and no nutrient (Figure 3(a)), the protein and polysaccharide contents of soluble EPS remained stable at $10.0 \pm 2.5 \text{ mg} \cdot \text{g}_{\text{MLVSS}}^{-1}$ and $2.5 \pm 1.3 \text{ mg} \cdot \text{g}_{\text{MLVSS}}^{-1}$, respectively. These values were in the same

range as the concentrations measured in a similar experiment performed with AS from a full scale MBR during 24 h [6] and other studies performed with an identical extraction protocol [23].

Under unaerated conditions and no nutrient (Figure 3(b)), both protein and polysaccharide contents of soluble EPS increased during the first six hours, followed by a rapid drop until EPS quantities were similar to those obtained under aeration. When the aeration was turned on, protein and polysaccharide contents of soluble EPS remained stable at the values observed prior to the 72 h oxygen cut off. When bacteria consumed substrate they directly formed bound EPS in proportion to substrate utilization. As no substrate was available during these six hours, bacteria could hydrolyse these bound EPS, which afterwards were found soluble in the liquid medium. Thus, after six hours the peak of hydrolysed bound EPS, now called soluble EPS, was measured. These soluble EPS were biodegraded by the microorganisms. Literature is not unanimous on EPS evolution during this kind of stress condition. Geilvoet *et al.* [6] showed an increase in the protein and polysaccharide soluble contents extracted from a full scale MBR during 24 h of anoxic and depletion conditions. The same EPS evolution was previously observed under 12 days of anaerobic condition, where changes in EPS compositions and quantities were measured [33]. Protein and polysaccharide contents of soluble EPS showed the same increase after two or three days, and then the fast decrease observed until the experiment ended was attributed to enzymatic hydrolysis of EPS and biodegradation by anaerobic microorganisms [34]. Finally, the background concentration measured here might correspond to slowly biodegradable EPS. This hypothesis would correspond to the one argued by Lapsidou and Rittman [35], who proposed that certain parts of EPS are attached to inert biomass resulting from the process of endogenous decay of active biomass. The authors consider that these EPS have a hydrolysis rate really slower than the EPS bound to active biomass.

Colorimetric methods were not sufficient to demonstrate a possible influence of prolonged OCS periods on EPS characteristics. SEC was thus applied for a qualitative characterization of EPS regarding their apparent molecular size distribution. Fingerprints of soluble EPS at time equal to zero (when the aeration was turned off) and after the 72 h cut off were determined at 280 nm (observation of conjugated macromolecules such as proteins, humic-like substances and nucleic acid) and at wavelengths used in fluorescence to detect tryptophan protein-like substances ($Ex/Em = 221\text{ nm}/350\text{ nm}$) (Figure 3(c) and (d)). Two methods were applied to compare the chromatograms: (i) the peak number and absorbance and (ii) the apparent MW distribution of soluble EPS.

Considering the first method at 280 nm (Figure 3(c)), soluble EPS extracted at time zero were characterized by two peaks (at a V_e of 19.5 mL and 21.5 mL), while four peaks between a V_e of 17.5 and 26 mL were detected after the 72 h oxygen cut off. The first peak obtained at 0 h eluted at the same volume as the second peak observed at 72 h ($V_e = 19.5\text{ mL}$) with a difference in absorbance. Therefore,

the same molecules are present before and after the oxygen cut off periods with an increase in terms of quantity. The second peak at 0 h ($V_e = 21.5\text{ mL}$) was not detected at 72 h. The corresponding molecules were probably biodegraded. This hypothesis was confirmed by the occurrence of new peaks at 72 h ($V_e = 17.5; 23; 26\text{ mL}$) which correspond to the new class of molecules size in the AS soluble EPS. As no nutrients with proteins or humic-like substances were introduced in the medium during the experiment, the new peaks were certainly due to biological metabolism, which induces also an AS flocs structural change, as was highlighted by the evolution of AS viscosity curves (Figure 2).

Considering now the detection at wavelengths specific to 'protein-like' substances (Figure 3(d)), the peaks number was the same at 0 h and 72 h; only relative absorbances of the peaks were affected by the oxygen cut off period. Therefore, a type of molecules other than proteins could be responsible for the appearance of new peaks and the disappearance of other peaks on the chromatograms obtained at 280 nm. Regarding molecules detected at a wavelength of 280 nm and the more abundant molecules present in the EPS, we can assume that the macromolecules involved in the chromatograms' shape of Figure 3(c) are humic-like substances. The general increase in absorbance indicated an EPS release during the 72 h of stress conditions (Figure 3(d)). This hypothesis seems to be in accordance with the results obtained by Wu and Lee [4]. They found that humic acids were highly released after a long term starvation in a MBR. Garnier *et al.* [36] demonstrated that EPS extracted from AS of WWTPs operated under traditional conditions were quite similar and only drastic conditions can change the EPS fingerprints. In conclusion, a prolonged lack of oxygenation and starvation conditions affected the EPS fingerprints as three new peaks appeared and one disappeared, as compared with constant aeration. Humic-like substances seem mainly released and bacteria probably changed their metabolism to accommodate these long stressful conditions.

The second method to interpret the chromatograms consisted of estimating the apparent MW of the EPS molecules (Figure 3(c)). At 0 h, EPS molecules ($V_e = 19.5$ and 21.5 mL) possessed apparent MW values less than 5 kDa. After 72 h of oxygen cut off, peaks that appeared at 17.5 mL and 23 mL corresponded to apparent MW of 11 kDa and 0.7 kDa respectively. The peak at 26 mL appeared after the total bed volume (23.5 mL). EPS molecules might interact (ionic and/or hydrophobic interactions) with the column packing. These interactions might delay the elution volume of soluble EPS aromatic components leading to an artefactual apparent MW under-estimation (0.2 kDa). The EPS fingerprints obtained from the aerated AS (0 h) revealed similarities in terms of elution volume and apparent MW, as compared with chromatograms of EPS extracted from other AS and analysed at 280 nm [36]. However, the number of peaks was lower in this paper (2–4) than in other studies (6–8) [28,37]. A cation exchange resin was used in

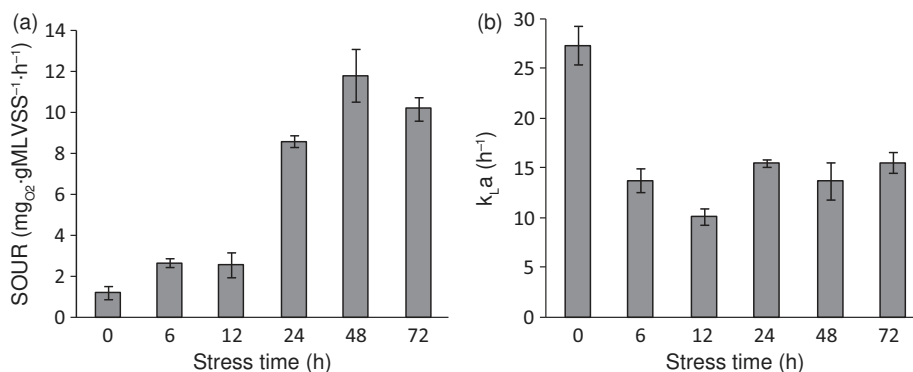


Figure 4. Endogenous values of (a) SOUR and (b) $k_{L}a$ before (time = 0 h) and after a defined oxygen cut off and starvation.

Villain *et al.* [37] to extract both soluble and bound EPS contents while a centrifugation of 4000 g was applied in this work. Therefore, reliable comparison of EPS contents obtained from different studies is difficult.

3.2. Endogenous conditions: biomass characteristics after the oxygen returns

Under endogenous conditions, SOUR and $k_{L}a$ were measured prior to and after the different oxygen cut off and nutrient depletion times (Figure 4). Before these drastic conditions, SOUR measurements were stable within 0.90 ± 0.02 and $1.60 \pm 0.03 \text{ mg}_{\text{O}_2} \cdot \text{g}_{\text{MLVSS}}^{-1} \cdot \text{h}^{-1}$; these values were previously obtained for AS under endogenous conditions [38]. After 24 h and longer OCS times, the SOUR increased significantly. Bacteria dealt with stressful situations by increasing their respiration.

Under unfavourable external conditions, links that maintain floc structure were shown to be strongly weakened and cellular material as EPS was released in the medium [39]. EPS were identified as biodegradable by their own producers (biofilm microorganisms) and by other microorganisms (from AS) when they are starved [34]. As bacteria were not fed during the oxygen cut off times applied in this work, biodegradation of their own EPS was a possible explanation for the SOUR increase; this explanation is supported by a greater SOUR increase after the longest oxygen cut off and starvation times (48 h and 72 h). Rheological measurements and EPS characterization by SEC confirmed this hypothesis. For the longest oxygen cut off time, AS floc structure changed, and soluble EPS chromatograms revealed a release of organic biodegradable matter assumed to be humic-like substances.

With regards to the $k_{L}a$ measurement, the initial values obtained under aeration were between 25.9 ± 1.4 and $30.6 \pm 2.5 \text{ h}^{-1}$. After the different OCS times, an identical decrease (10.1 – 15.5 h^{-1}) indicated that the release of EPS macromolecules (humic-like substances and proteins) highlighted by the SEC analysis might change the liquid composition and thus the oxygen transfer. Many organic

substances found in AS such as proteins [19] and humic-like substances [40] can adsorb on gas–liquid interfaces and may have surfactant activity. Surfactants are defined as molecules with hydrophilic and hydrophobic moieties that decrease the surface tension (σ) between two surfaces (e.g. air bubbles and the liquid phase). As the surface tension is lowered, bubbles can get bigger, and consequently the interfacial area between bubbles and the liquid phase decreases, inducing a lower oxygen transfer.

The hypothesis of change in the liquid phase composition responsible for the $k_{L}a$ decrease was tested by measuring $k_{L}a$ and σ of the AS supernatant before and after 24 h of OCS (Table 1). Decreases of 14.8 h^{-1} and $5.5 \text{ mN} \cdot \text{m}^{-1}$ for $k_{L}a$ and σ respectively were measured in the AS supernatant after the stress conditions. The same analysis performed with tap water resulted in higher values than with the AS supernatant for both $k_{L}a$ and σ . Therefore, the supernatant obtained after the OCS periods contained organic molecules that reduced the interfacial tension between the air bubbles and the liquid phase. The bubble size increased and thus the specific surface area decreased, resulting in a lower oxygen transfer.

3.3. Exogenous conditions: biomass characteristics after the oxygen returns

Exogenous conditions were reproduced with glucose addition ($0.4 \text{ g}_{\text{COD}} \cdot \text{g}_{\text{MLVSS}}^{-1}$) before and after the stress times. As soon as the glucose was injected, SOUR and $k_{L}a$ were regularly measured (Figure 5). The SOUR and $k_{L}a$ increased to their highest values as soon as the glucose was added and then decreased to the same values that were obtained under endogenous conditions. Curves determined for oxygen cut off and starvation times less than 12 h were similar to those with aerated AS. Biomass supported such stress times without any change in their biological activity. After OCS times equal to or greater than 24 h, the first SOUR and $k_{L}a$ values measured after the glucose injection were higher than those obtained with aerated AS and shorter oxygen cut off times; these values were $11.8 \pm 2.1 \text{ mg}_{\text{O}_2} \cdot \text{g}_{\text{MLVSS}}^{-1} \cdot \text{h}^{-1}$

Table 1. Evolutions before and after 24 h of oxygen cut off: (i) SOUR and $k_{L,a}$ of activated sludge; (ii) $k_{L,a}$ and surface tension of the supernatant; (iii) $k_{L,a}$ and surface tension of tap water.

	Before 24 h			After 24 h		
	SOUR ($\text{mg}_{\text{O}_2} \cdot \text{g}_{\text{MLVSS}}^{-1} \cdot \text{h}^{-1}$)	$k_{L,a}$ (h^{-1})	σ ($\text{mN} \cdot \text{m}^{-1}$)	SOUR ($\text{mg}_{\text{O}_2} \cdot \text{g}_{\text{MLVSS}}^{-1} \cdot \text{h}^{-1}$)	$k_{L,a}$ (h^{-1})	σ ($\text{mN} \cdot \text{m}^{-1}$)
AS with supernatant	1.21 ± 0.3	28.1 ± 1.9	—	8.61 ± 0.3	18 ± 0.4	—
Supernatant	—	100.1 ± 19.1	46.7 ± 0.9	—	85.3 ± 3.4	41.2 ± 1.7
Tap water	—	104.4 ± 15.3	72.5 ± 1.3	—	—	—

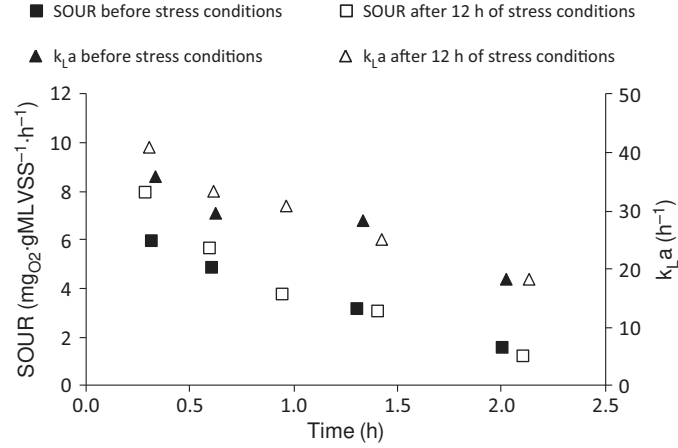


Figure 5. Evolution of SOUR and $k_{L,a}$ before and after an oxygen cut off and starvation time of 12 h.

and $62.3 \pm 13 \text{ h}^{-1}$, respectively. These longer oxygen cut off times seemed to have stressed the microorganisms. The SOUR can be expressed as the sum of two oxygen consumptions: (i) one for biomass synthesis and (ii) one for biomass maintenance. If microorganisms accelerated their SOUR after OCS times longer than 24 h, the biomass probably suffered from structural damages. Ning *et al.* [41] also confirmed an increase in SOUR after 18 h of nitrogen nutrient deficiency in AS when nitrogen substrate was injected.

The highest SOUR measured after stress times longer than 24 h was not correlated with the COD degradation rate. A relatively constant elimination rate of $15.7 \pm 1.9 \text{ mg}_{\text{O}_2} \cdot \text{g}_{\text{MLVSS}}^{-1} \cdot \text{h}^{-1}$ was obtained after the different OCS times. Therefore, bacterial respiration was more impacted by stressful conditions than organic matter degradation. Maurines-Carboneill *et al.* [2] showed that after eight days of anaerobic conditions, most of the bacteria contained in AS fed with Viadox were still alive with constant relative proportion. When a pure culture of the principal AS strains was studied, a lower level of living bacteria was measured. Therefore, maintenance of basic metabolism could be explained by synergies between the different bacterial species present in AS. During the different OCS times reported in this paper, no substrate was added, and thus, bacteria might have consumed their own EPS to survive.

The same trend was observed for both SOUR and $k_{L,a}$ data obtained during the different experiments performed before or after the stress conditions. Compared with the results obtained without AS, the oxygen consuming cells seemed to influence the oxygen transfer and may be responsible for its enhancement. In biological media, $k_{L,a}$ and SOUR can be combined in two steps: the oxygen mass transfer to the liquid phase and the subsequent biochemical reaction in the cells (SOUR) [8]. If the biochemical reaction is faster than the mass transfer, the concentration profile of the absorbed gas may be affected by the biochemical reaction. Then, the absorption rate into the liquid phase may be enhanced as the oxygen is consumed while it diffuses into the liquid. Some studies on oxygen transfer characteristics in fermentation reported an enhancement of oxygen transfer rate due to microorganism consumption. The $k_{L,a}$ increased with higher SOUR during culture of *Xanthomonas campestris* [42]. In addition, $k_{L,a}$ and SOUR were maximal during the log phase of the growth of *Escherichia coli* [43,44]. They affirmed that the SOUR and $k_{L,a}$ increased when the biomass concentration and production increased as well as the substrate consumption. All of these results suggested an effect of biological activity (growth and substrate consumption) on oxygen transfer. However, the studies found in the literature were based on pure culture, and the innovative part of this work was to

confirm the relationship between SOUR and $k_L a$ in a complex mixture of bacteria such as AS, with additional effects of stress phases.

3.4. Overall influence of OCS periods on AS

Structural modification was measured after OCS times equal to or greater than 48 h. This structural modification was more visible as AS was aerated since air bubbles were easily able to break weak floc structure. A smaller modification without any impact on sludge viscosity was observed after 6 h without oxygen, as protein and polysaccharide contents of soluble EPS increased. Enzymatic hydrolysis and/or anaerobic degradation were assumed to be responsible for this soluble EPS increase. EPS fingerprints supported the metabolic hypothesis since three groups of molecules (assumed to be humic-like substances) appeared and one disappeared. Thus, the stress conditions tested impacted the composition of the AS liquid phase.

As a consequence of the liquid composition change, the oxygen transfer ($k_L a$) decreased when bacteria were in endogenous conditions after aeration was turned on. This decrease was attributed to the surfactant capacity of EPS organic compounds such as proteins and humic-like substances that induced a decrease in the gas-liquid oxygen transfer. The second effect of the stress conditions under endogenous conditions was higher biomass respiration when aeration was turned on. The biomass was able to adapt its metabolism after stress situation.

The effect of stress conditions under exogenous conditions appeared for periods above 24 h. Indeed, higher bacterial respiration was measured as soon as easily biodegradable substrate was added and the air flow was turned on. In other words, the oxygen amount used to face the oxygen deficiency was higher than under undisturbed conditions. However, the same amount of COD was degraded. The experiments performed under exogenous conditions also showed that the oxygen transfer increased with higher biological activity. In contrast to endogenous conditions, the presence of surfactant EPS in the liquid phase did not result in a decrease of the $k_L a$ as biological activity was higher and thus oxygen consumption was as well.

4. Conclusion

AS was shown to possess the metabolism to highly support and adapt itself to OCS until 72 h. SEC analysis and SOUR values prove that bacteria are able to degrade their own EPS to survive this stress. After a prolonged unaerated phase, in endogenous conditions, EPS highlighted surfactant abilities as humic-like substances were released in the liquid phase after oxygen deficiencies and resulted in a decrease of surface tension leading to a drop of gas-liquid oxygen transfer. In exogenous conditions, as the oxygen consumption by AS is faster than the oxygen mass transfer in the supernatant,

the oxygen transfer in the liquid phase is enhanced. In conclusion, this paper demonstrated that aeration and starvation issues in WWTP for lower than 72 h are harmless as bacteria maintained a constant COD degradation rate before or after these drastic conditions. The stress conditions (e.g. starvation and unaerated phases) applied on AS and the dissociation of endogenous/exogenous activities appear to offer an ideal laboratory model to study biomass metabolism and the subsequent effects of EPS and microbial activity on oxygen transfer.

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References

- [1] G. Gaval and J.J. Pernelle, *Impact of the repetition of oxygen deficiencies on the filamentous bacteria proliferation in activated sludge*, Water Res. 37 (2003), pp. 1991–2000.
- [2] C. Maurines-Carboneill, L. Morin, J.J. Pernelle, N. Derlet, G. Sachon, and G. Leblon, *Reviviscence of aerobic chemoheterotrophic bacteria in an activated sludge pilot plant after a prolonged absence of oxygen*, Water Res. 32 (1998), pp. 2211–2219.
- [3] N.J. Horan and P. Shanmugan, *Effects of starvation and nutrient depletion on the settling properties of activated sludge*, Water Res. 20 (1986), pp. 661–666.
- [4] S.C. Wu and C.M. Lee, *Correlation between fouling propensity of soluble extracellular polymeric substances and sludge metabolic activity altered by different starvation conditions*, Bioresour. Technol. 102 (2011), pp. 5375–5380.
- [5] M.D. Coello Oviedo, J.A. Lopez-Ramirez, D. Sales Marquez, and J.M. Quiroga Alonso, *Evolution of an activated sludge system under starvation conditions*, Chem. Eng. J. 94 (2003), pp. 139–146.
- [6] S. Geilvoet, J. Van der Graaf, and A. Van Nieuwenhuijzen, *MBR activated sludge filterability alteration in stress circumstances*, ICOM Workshop, Honolulu, 2008.
- [7] H. Rasmussen, J.H. Bruus, K. Keiding, and P.H. Nielsen, *Observations on dewaterability and physical, chemical and microbiological changes in anaerobically stored activated sludge from a nutrient removal plant*, Water Res. 28 (1994), pp. 417–425.
- [8] F. Garcia-Ochoa, E. Gomez, V.E. Santos, and J.C. Merchuk, *Oxygen uptake rate in microbial processes: An overview*, Biochem. Eng. J. 49 (2010), pp. 289–307.
- [9] D.T. Sponza, *Extracellular polymer substances and physicochemical properties of flocs in steady and unsteady-state activated sludge systems*, Process Biochem. 37 (2002), pp. 983–998.
- [10] J. Wingender, T.R. Neu, and H.C. Flemming, *Microbial Extracellular Polymeric Substances: Characterization, Structure and Function*, Springer, Berlin, 1999.
- [11] F. Ye, G. Peng, and Y. Li, *Influences of influent carbon source on extracellular polymeric substances (EPS) and physicochemical properties of activated sludge*, Chemosphere 84 (2011), pp. 1250–1255.
- [12] G. Peng, F. Ye, and Y. Li, *Investigation of extracellular polymeric substances (EPS) and physicochemical properties of activated sludge from different municipal and industrial*

- wastewater treatment plants, *Environ. Technol.* 1 (2011), pp. 1–7.
- [13] G. Di Bella, M. Torregrossa, and G. Viviani, *The role of EPS concentration in MBR foaming: Analysis of a submerged pilot plant*, *Bioresour. Technol.* 102 (2011), pp. 1628–1635.
- [14] J. Cho, K.G. Song, and K.N. Ahn, *The activated sludge and microbial substances influences on membrane fouling in submerged membrane bioreactor: Unstirred batch cell test*, *Desalination* 183 (2005), pp. 425–429.
- [15] A. Drews, C.H. Lee, and M. Kraume, *Membrane fouling—a review on the role of EPS*, *Desalination* 200 (2006), pp. 186–188.
- [16] P. Le-Clech, V. Chen, and T.A.G. Fane, *Fouling in membrane bioreactors used in wastewater treatment*, *J. Membrane Sci.* 284 (2006), pp. 17–53.
- [17] E. Barbot, I. Seyssiecq, N. Roche, and B. Marrot, *Inhibition of activated sludge respiration by sodium azide addition: Effect on rheology and oxygen transfer*, *Chem. Eng. J.* 163 (2010), pp. 230–235.
- [18] B.N. Tsai, C.H. Chang, and D.J. Lee, *Fractionation of soluble microbial products (SMP) and soluble extracellular polymeric substances (EPS) from wastewater sludge*, *Environ. Technol.* 29 (2008), pp. 1127–1138.
- [19] G.T. Tsao, *Simultaneous gas–liquid interfacial oxygen absorption and biochemical oxidation*, *Biotechnol. Bioeng.* (1968), pp. 765–785.
- [20] I. Seyssiecq, B. Marrot, D. Djerroud, and N. Roche, *In situ triphasic rheological characterisation of activated sludge, in an aerated bioreactor*, *Chem. Eng. J.* 142 (2008), pp. 40–47.
- [21] F. Garcia-Ochoa and E. Gomez, *Bioreactor scale-up and oxygen transfer rate in microbial processes: An overview*, *Biotechnol. Advances*, 27 (2009), pp. 153–176.
- [22] F. Garcia-Ochoa and E. Gomez, *Prediction of gas–liquid mass transfer coefficient in sparged stirred tank bioreactors*, *Biotechnol. Bioeng.* 92 (2005), pp. 761–772.
- [23] S. Comte, G. Guibaud, and M. Baudu, *Relations between extraction protocols for activated sludge extracellular polymeric substances (EPS) and EPS complexation properties: Part I. Comparison of the efficiency of eight EPS extraction methods*, *Enzyme Microb. Technol.* 38 (2006), pp. 237–245.
- [24] O. Lowry, N. Rosebrough, A. Fan, and R. Randell, *Protein measurement with the folin phenol reagent*, *J. Biol. Chem.* 193 (1951), pp. 265–275.
- [25] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Colorimetric method for determination of sugars and related substances*, *Anal. Chem.* 28 (1956), pp. 350–356.
- [26] W. Chen, P. Wersterhoff, J.A. Leenheer, and K. Booksh, *Fluorescence Excitation-Emission Matrix regional integration to quantify spectra for dissolved organic matter*, *Environ. Sci. Technol.* 37 (2003), pp. 5701–5710.
- [27] T. Liu, Z.L. Chen, W.Z. Yu, and S.J. You, *Characterization of organic membrane foulants in a submerged membrane bioreactor with pre-ozonation using three-dimensional excitation-emission matrix fluorescence spectroscopy*, *Water Res.* 45 (2011), pp. 2111–2121.
- [28] T. Görner, P. de Donato, M.H. Ameal, E. Montarges-Pelletier, and B.S. Lartiges, *Activated sludge exopolymers: Separation and identification using size exclusion chromatography and infrared micro-spectroscopy*, *Water Res.* 37 (2003), pp. 2388–2393.
- [29] B.O. Frølund and K. Keiding, *Implementation of an HPLC polystyrene divinylbenzene column for separation of activated sludge exopolymers*, *Appl. Microbiol. Technol.* 41 (1994), pp. 708–716.
- [30] M. Mori, J. Isaac, I. Seyssiecq, and N. Roche, *Effect of measuring geometries and of exocellular polymeric substances on the rheological behavior of sewage sludge*, *Chem. Eng. Res. Design* 86 (2008), pp. 554–559.
- [31] D. Quemada, *Rheological modelling of complex fluids: I. The concept of effective volume fraction revisited*, *Eur. Phys. J. A.* (1997), pp. 119–127.
- [32] E. Germain and T. Stephenson, *Biomass characteristics, aeration and oxygen transfer in membrane bioreactors: Their interrelations explained by a review of aerobic biological processes*, *Environ. Sci. Biotechnol.* 4 (2005), pp. 223–233.
- [33] P.H. Nielsen, A. Jahn, and R. Palmgren, *Conceptual model for production and composition of exopolymers in biofilms*, *Water Sci. Technol.* 36 (1997), pp. 11–19.
- [34] X. Zhang and P.L. Bishop, *Biodegradability of biofilm extracellular polymeric substances*, *Chemosphere* 50 (2003), pp. 63–69.
- [35] C.S. Lapidou and B.E. Rittmann, *A unified theory for extracellular polymeric substances, soluble microbial products, and active and inert biomass*, *Water Res.* 36 (2002), pp. 2711–2720.
- [36] C. Garnier, T. Görner, B.S. Lartiges, S. Abdelouhab, and P. de Donato, *Characterization of activated sludge exopolymers from various origins: A combined size-exclusion chromatography and infrared microscopy study*, *Water Res.* 39 (2005), pp. 3044–3054.
- [37] M. Villain, S. Simon, I. Bourven, and G. Guibaud, *The use of a new mobile phase, with no multivalent cation binding properties, to differentiate extracellular polymeric substances (EPS), by size exclusion chromatography (SEC), from biomass used for wastewater treatment*, *Process Biochem.* 45 (2010), pp. 1415–1421.
- [38] L. Clouzot, N. Roche, and B. Marrot, *Effect of membrane bioreactor configurations on sludge structure and microbial activity*, *Bioresour. Technol.* 102 (2011), pp. 975–981.
- [39] G.P. Sheng, H.Q. Yu, and X.Y. Li, *Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review*, *Biotechnol. Adv.* 28 (2010), pp. 882–894.
- [40] H. Hasar, S. Xia, C.H. Ahn, and B.E. Rittmann, *Simultaneous removal of organic matter and nitrogen compounds by an aerobic/anoxic membrane biofilm reactor*, *Water Res.* 42 (2008), pp. 4109–4116.
- [41] Z. Ning, G.G. Patry, and H. Spanjers, *Identification and quantification of nitrogen nutrient deficiency in the activated sludge process using respirometry*, *Water Res.* 34 (2000), pp. 3345–3354.
- [42] O. Vashitz, M. Stheintuch, and S. Ulitzur, *Mass transfer studies using cloned-luminous strain of Xanthomonas campestris*, *Biotechnol. Bioeng.* 34 (1989), pp. 671–680.
- [43] P. Çalik, P. Yilgör, P. Ayhan, and A.S. Demir, *Oxygen transfer effects on recombinant benzaldehyde lyase production*, *Chem. Eng. Sci.* 59 (2004), pp. 5075–5083.
- [44] P. Çalik, P. Yilgör, and A.S. Demir, *Influence of controlled-pH and uncontrolled-pH operations on recombinant benzaldehyde lyase production by Escherichia coli*, *Enzyme Microb. Technol.* 38 (2006), pp. 617–627.