Aerobic granular sludge treating anaerobically pretreated brewery wastewater at different loading rates

Alessandro di Biase [a]*, Fabio Santo Corsino[b], Tanner Ryan Devlin [a], Michele Torregrossa[b], Giulio Munz[c], Jan A. Oleszkiewicz [a]

[a] Department of Civil Engineering, University of Manitoba, Winnipeg - Canada, R3T 5V6.

[b] Department of Civil, Environmental, Aerospatial Engineering and Material, University of Palermo, Viale delle Scienze, building 8, 90128, Palermo - Italy.

[b c] Department of Civil and Environmental Engineering, University of Florence, Via S. Marta 3, 50139, Florence - Italy.

*Corresponding author: telephone: +39 3498559906

E-mail address: alessandro.dibiase.fi@gmail.com (Alessandro di Biase)

ABSTRACT

In this study, three different aerobic granular sludge (AGS) reactors fed with anaerobically pre-treated brewery wastewater were studied. The AGS reactors were operated under different conditions including organic loading rates (OLR) between 0.8 and 4.1 kg COD m^{-3} d^{-1}, C:N:P ratios (100:10:1 and 100:6:1) and food to microorganism ratio (F/M) between 0.8±0.6 and 1.2±0.5 and 0.9±0.3 kg-TCOD kg-$VSS^{-1}d^{-1}$.

Stable granulation was achieved within two weeks and the size of the granules increased according to the OLR applied. The results indicated that low C:N:P ratio and F/M were favorable to achieve stable aerobic granules in the long term. The carbon removal rate was load-independent in the range examined (TCOD removal >80%), whereas TN removals were inversely proportional to the OLRs. Overall, a longer aeration reaction time with a lower OLR was beneficial to granular structure which exhibited a more compact and defined architecture. Performance results also indicated that within the other conditions studied microbial community and its complex functionality in nutrient removal was more efficient at operational parameters of OLR of 0.8±0.2 kg-TCOD $m^{-3}d^{-1}$ and F/M ratio of 0.5±0.2 kg-TCOD $VSS^{-1}d^{-1}$. Moreover, the protein to polysaccharide ratio increases as OLR decreases, leading to a more stable granular structure.

KEYWORDS

Aerobic granular sludge, nutrient removal, organic loading rates, compliance, granular stability, brewery wastewater

HIGHLIGHTS:

AGS fed with anaerobically pre-treated brewery wastewater was studied

Rapid granulation occurred and granules size increased with OLR

High organic carbon removal performance were observed independently from the OLR

Low C:N:P ratio and F/M were favorable for granular sludge stability in the long term

Longer cycle duration improved granular sludge stability

1. INTRODUCTION

Food and beverage industries are increasing worldwide, which inevitably has an impact on the environment. Indeed, these industries are increasing resources such as water and energy which lead to more production wastes and wastewater (Olajire, 2012). In the brewery industry, the amount of water consumption depends on the production and cleaning practices, which lead to different beer to wastewater ratios. For instance, in Europe it has been reported that 1L of beer generates on average 2.7L of wastewater, whereas in North America the typical reported value is 3.5 L L^{-1} (Chastain et al., 2011; Donoghue et al., 2012). Brewery wastewater are mainly constituted by sugars, soluble starches, ethanol, fatty acids while very low concentration of heavy metals are generally present (Simate et al., 2011). The organic load of brewery wastewater is generally easily biodegradable with a biochemical oxygen demand (BOD_5) to total chemical oxygen demand (TCOD) ratio of 0.6 – 0.7 (Brito et al., 2007). The typical composition of brewery wastewater is: 2000-6000 mg-COD L^{-1}, 25-80 mg-TN L^{-1}, 10-50 mg-TP L^{-1}, with pH between 4.5 and 12 (Valta et al., 2014). Therefore, brewery wastewaters often lack sufficient amount of nitrogen and phosphorus for microbial communities to achieve the biological activity level required for biological processes, which makes it necessary to supply these nutrients by external sources.

It has been widely demonstrated that anaerobic processes like up-flow anaerobic sludge blanket reactor (UASB), anaerobic membrane bioreactors and anaerobic moving bed biofilm reactors (AMBBR) are reliable and robust technologies that allow achieving high organic carbon removal performances while recovering energy through biogas production (Brito et al., 2007; di Biase et al., 2016, 2017). However, anaerobic bioprocesses generate effluents with not negligible residual pollution that require further treatments before their discharge into the environment (Chastain et al., 2011). Among the aerobic post-treatments, aerobic granular sludge (AGS) is considered a promising technology that could be feasible and competitive in the treatment of several industrial wastewaters because of its low footprint and high nutrients removal performance (Di Trapani et al., 2019; Hamza et al., 2018).

The organic loading rate (OLR) and food to microorganism ratio (F/M) are two of the parameters that most affect the AGS formation and stability in the long-term (Hamza et al., 2018). Several studies demonstrated that AGS enable to operate with OLR higher than conventional technologies. However, there was discrepancy in the literature regarding stability of AGS under high OLR especially in the treatment of industrial wastewater (Adav et al., 2010). Furthermore, there appeared to be a lack of mechanisms to provide for AGS stability under increasing OLR. In addition, due to the peculiarity of the specific industrial wastewater to be treated, the application of design or management criteria derived from the literature and referred to municipal wastewaters leads to not satisfactory results. Consequently, in order to comply with the discharge limits imposed by regulations, it is necessary to establish specific design and management criteria for the specific kind of industrial wastewater.

The AGS has been previously used for the treatment of brewery wastewater. For instance, Stes et al. (2018) investigated the treatment of brewery wastewater with aerobic granular sludge operating at different OLR between 1.2 and 1.5 kg-TCOD m^{-3} d^{-1}, whereas Wang et al. (2007) demonstrated the capability of high-rate degradation as well as simultaneous nitrogen and phosphorous removal on raw brewery wastewater. However, in these studies there is a lack of knowledge about the implementation of operating strategies to improve granular sludge stability preserving their integrity and nutrient removal performances in the long term. Moreover, to the best authors' knowledge, post-treatment of anaerobic pre-treated brewery wastewater with AGS technology has never been assessed so far.

Based on the above, the aim of this study was to determine the feasibility of using AGS as a polishing process after anaerobic pre-treatment of brewery wastewater. More precisely, this work was to evaluate the effects of different operating parameters such as OLR, F/M and COD: N: P ratios on granular sludge development and stability, as well as nutrients removal performances.

2. MATERIAL AND METHODS

2.1 Reactors configuration and operation

Three 4L sequencing batch reactors (SBR) were seeded with conventional activated sludge from the "West End Water Pollution Control Centre" (WEWPCC; Winnipeg, Canada) at total solids of 3.4±0.2 g-TSS L^{-1} and suspend solids 2.7±0.2 g-VSS L^{-1} concentrations. The brewery wastewater collected from Fort Garry brewery, a local brewery in Winnipeg (Manitoba, Canada), was previously anaerobically treated with three 4L anaerobic moving bed biofilm reactors (AMBBR) using media AC920 manufactured from Headworks BIO (Victoria, BC, Canada) with 40% media filling at 8h hydraulic retention time (HRT) in a mesophilic environment (35±1°C). Studies conducted by di Biase et al. (2017) suggested that at this operational conditions, the AMBBR was capable of achieving soluble COD (sCOD) removal greater than 80% without compromising methanogenesis and buffering the pH to neutral levels. The raw brewery wastewater was stored in a cold chamber (4°C) to prevent pre-fermentation of the substrate before anaerobic digestion pre-treatment. The wastewater was prepared by diluting the fermenter underflow (140±10 kg-TCOD m^{-3}) to approximately 1:40±10 times the initial concentration. This resulted in AMBBR influent concentration of 4.0±1.0 kg-sCOD m^{-3} over the 120 days studied, which is close to the average values of typical North American brewery wastewater (Chastain et al., 2011). The measured BOD/COD ratio of the raw brewery wastewater was on average 0.70±0.05 within typical ratio of 0.6 – 0.7 reported in literature (Brito et al., 2007). After AMBBR pre-treatment, the effluent wastewater was sieved through a screen with 0.6 mm porosity and subsequently used as influent of three aerobic granular SBR reactors.

The SBR were three identical column-type reactors (100 cm height) operated at room temperature (i.e., 19±1 °C). Air was supplied at a flow rate of 2.5 L min^{-1} via three circular ceramic stones laid on the bottom of the reactor at an angle of 120° between each other. This resulted in a superficial up-flow air velocity of 0.5 cm s^{-1} and a dissolved oxygen (DO) concentration of ~7 mg L^{-1}. Two different COD: N: P ratios were investigated in two separate periods. Table 1 reports typical brewery wastewater characterization, discharge into sewer bylaw as a range between three North American areas (i.e., Winnipeg, Manitoba – Canada; Ottawa, Quebec – Canada; and Minnesota bylaw). Table 1 also shows the influent brewery wastewater characterization to AGS after AMBBR treatment. It has been described by Wang et al. (2007) that 100COD : 10N : 1P by weight is required to avoid aerobic heterotrophic growth limitations and the appearance of filamentous bacteria. Typical brewery wastewater characteristics is therefore deficient in nitrogen and phosphorus (Table 1). For these reasons, in the first period studied, nitrogen and phosphorus concentrations were adjusted to have the desired ratio of 100COD: 10N: 1P. During the second period, however, the nutrients in the influent wastewater were not added resulting in a measured COD: N: P ratio of 100: 6: 1. After anaerobic digestion pre-treatment, BOD/COD ratio was measured as ~ 0.5 while pH was 7.1±0.2. The reaction cycle was set with 40 min of up flow anaerobic feeding followed by an aerobic phase with a length different in the two periods, and at the end 2 min of discharge. The number of cycles differed between the two periods (i.e., 100COD : 10N : 1P and 100COD : 6N : 1P) to increase HRT and reduce OLR while maintaining unvaried volume exchange ratio (VER) set at 75, 50, and 25% in the three AGS allowing therefore direct comparison – Table 2. Thus, the first period had 4 cycles per day (6h each) while the second period had 2 cycles per day (12h each). Settling times were also varied to maintain similar hydraulic selective pressure.

Table 1 – Typical brewery wastewater characterization, discharge into sewer bylaw and influent wastewater to AGS (after anaerobic AMBBR) in the two periods operated at different C:N:P ratios.

	Typical brewery wastewater	Discharge into sewer bylaw*	100COD : 10N : 1P	100COD : 6N : 1P
Parameter	**Range**	**Range**	**Value**	**Value**
TCOD (mg L^{-1})	1,000 – 8,000	500 – 600	1,500	1,800
BOD_5 (mg L^{-1})	600 – 5,000	250 – 300	800	720
TN (mg L^{-1})	10 – 30	50 – 100	138	98
TP (mg L^{-1})	10 – 30	10	19	17
pH	4.5 – 12	5.5 – 11	7.1	7.1

* City of Winnipeg (Mantioba, Canada) SEWER BY-LAW NO 92/2010; City of Ottawa BY-LAW NO. 2003-514; Minnesota bylaw (www.metrocouncil.org); D. Lgs. 152/06 (Parte Terza, Allegato 5, Tabella 3) Veneto Province.
TCOD = Total Chemical Oxygen Demand; BOD_5 = Biochemical Oxygen Demand; TP = Total Phosphorous; TN = Total Nitrogen

Table 2 – Operational parameters in the two period (100COD : 10N : 1P and 100COD : 6N : 1P).

	100COD : 10N : 1P			100COD : 6N : 1P		
Operational Parameter	$R_{4.1}$	$R_{2.8}$	$R_{1.5}$	$R_{2.1}$	$R_{1.5}$	$R_{0.8}$
Cycle length (h)	6	6	6	12	12	12
OLR (kg-TCOD m^{-3} d^{-1})	4.1±0.6	2.8±0.4	1.4±0.2	2.1±0.6	1.5±0.4	0.8±0.2
HRT (h)	8	12	24	16	24	48
Q (L/d)	12	8	4	6	4	2
VER (%)	75	50	25	75	5	25
Settling time (min)	4.5	3	1.5	4.5	3	1.5

R_X = X represent the organic loading rate (OLR) of the reactor; VER = Volume Exchange Ratio; HRT = Hydraulic Retention Time

Influent characteristics reported in Table 1 above and operational parameters in Table 2 (i.e., VER and number of cycles) were used to calculated flow (Q; Eq.1), OLR (Eq.2), and F/M ratio (Eq.3).

$$Q\ \left(\frac{L}{d}\right) = VER\ (\%) * \ V_R * n^{\circ}_{cycles}\ \left(\frac{\#}{d}\right) \qquad (Eq.1)$$

Whereas Q is expressed in L d^{-1}, VER is the volume exchange ratio, n°_{cycles} is the number of cycles per day (# d^{-1}).

$$OLR\ \left(\frac{kg-TCOD}{m^3\ d}\right) = \frac{TCOD_{in}\left(\frac{kg-TCOD}{m^3}\right) * Q\ \left(\frac{m^3}{d}\right)}{V_R} \qquad (Eq.2)$$

Whereas, OLR is reported in kg-TCOD $m^{-3}d^{-1}$, $TCOD_{in}$ is the influent concentration of TCOD in kg-TCOD m^{-3}, Q is calculated as in Eq.1 but expressed in m^3 d^{-1}, and V_R is the reactor volume (i.e., 0.004 m^3).

$$\frac{F}{M} ratio \left(\frac{kg - TCOD}{kg - VSS\ d}\right) = \frac{TCOD_{in}\left(\frac{kg - TCOD}{m^3}\right) * Q\ \left(\frac{m^3}{d}\right)}{biomass\ \left(\frac{kg - VSS}{m^3}\right) *\ V_R(m^3)} \qquad (Eq. 3)$$

Whereas F/M ratio is calculated as kg-TCOD kg-$VSS^{-1}d^{-1}$, $TCOD_{in}$ is the influent concentration in kg-TCOD m^{-3}, Q is flow as defined in Eq.1, biomass is the concentration of VSS in the reactor reported in kg-VSS m^{-3}, and V_R is the reactor volume in m^3 (i.e., 0.004 m^3).

2.2 Analytical methods

Physical-chemical analysis of influent and effluent (COD, total nitrogen - TN, total phosphorus - TP, total and volatile suspended solids - TSS and VSS) were performed according to standard methods (APHA, 2012). Biochemical oxygen demand was measured using OxiTop Control BOD measurement system (WTW, Ohio, USA). The nitrogen fraction as total ammonia nitrogen (TAN-N), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N) and orthophosphate phosphorus (PO_4^{3-}-P) were determine by QuickChem 8500 Flow Injection Analyzer (FIA) system. Samples for effluent TSS and VSS were measured three times per week, while total and volatile mixed liquor suspended solids twice a week. Extracellular polymeric substances (EPS) were quantified according to the two-step heating extraction method described by Le-Clech et al. (2006). A sample of mixed liquor (50 mL) was immediately filtered through a 0.22 µm pore-size membrane to extract the unbound EPS. The precipitate was then suspended again with deionized water to its original volume (50 mL) and then placed in a thermal batch at 80±1 °C for 10 min. Afterwards, the sample was centrifuged at 7000 rpm for 10 min at 4±1 °C and the supernatant was filtered through a 0.22 µm pore-size membrane allowing the tightly-bound EPS determination. This research focused on the characterization of tightly-bound EPS in terms of protein and polysaccharides concentrations. The carbohydrates and protein concentrations, therefore, were determined according to the phenol-sulphuric acid methods with glucose as the standard (Dubois et al., 1951) and by the Folin method with bovine albumin serum as the standard (Lowry et al., 1951). Morphological characteristics of the granular sludge were evaluated through stereomicroscope observations (Zeiss, Toronto, ON, CA), while their size distribution was determined by measuring approximately 100 granules through an image analyser software (IM50-Leika).

2.3 Solids retention time estimation

The VER applied (Table 2) and the geometry of the reactors (i.e., discharge valve located at 1L) allowed to have the smallest volume for biomass retention in $R_{4.1}$. The sludge retention time (SRT) was therefore not directly controlled but simply managed by discharging the excess solids settled above the valve at operational C:N:P ratio of 100:10:1. In $R_{2.8}$ and $R_{1.4}$, however, SRT was manually maintained as close as possible to $R_{4.1}$. At nutrient ratio of 100C : 6N: 1P, the SRT was not controlled in any of the reactors. At steady state, the SRT was estimated according to the following equation – Eq.4.

$$SRT = \frac{X_{TSS\ steady\ state}(g\ TSS\ L^{-1}) * V_{reactor}\ (L)}{X_{TSS\ steady\ state}\ (g\ TSS\ L^{-1}) * V_{sample}(L\ d^{-1}) + X_{TSS\ effluent}(g\ TSS\ L^{-1}) * V_{discharge}\ (L\ d^{-1})} \quad \text{(Eq.4)}$$

Where the $X_{TSS\ steady\ state}$ is the average TSS concentration over 20 days of steady state in g TSS L^{-1}, $V_{reactor}$ is the reactor volume (4L), V_{sample} is the volume of samples withdraw per day (L d^{-1}) for analytical measures, $X_{TSS\ effluent}$ is the concentration of solids in the effluent (g-TSS L^{-1}) and $V_{discharge}$ is the volume discharged per day. The granular sludge settles faster than the flocs and suspended biomass, therefore, the mass of solids wasted per day was assumed as floccular, suspended, and slough biomass not contributing to the granular biomass. Equation 4 allowed estimating the total SRT of the system. The granular sludge retention time however was estimated with the same equation (Equation 4) but not considering the volume discharge $V_{discharge}$ in the denominator.

3. RESULTS AND DISCUSSIONS

3.1 Granular sludge development

3.1.1 Mixed liquor and effluent solids

The total mixed liquor and effluent solids profile at different OLR in the two nutrient ratios studied are shown in Figure 1. Figure 1A reports the early stages of operation in which granulation took place at 100C: 10N: 1P. Under these conditions, $R_{4.1}$ achieved an average maximum mixed liquor solids concentration of 3.6±0.3 g-TSS L^{-1} after two weeks from the seeding. In $R_{4.1}$, the solids concentration was maintained by effluent discharge at 1L volume (75% VER). In $R_{2.8}$ and $R_{1.4}$ TSS in mixed liquor concentration were of 3.5±0.2 and 2.0±0.2 g L^{-1}. This favored granulation over free-floating suspended biomass and flocs development. At steady state conditions (i.e., from day 30 to 35), mixed liquor TSS were measured 3.6±0.7, 3.7±0.6, and 2.0±0.2 g-TSS L^{-1} in $R_{4.1}$, $R_{2.8}$, and $R_{1.4}$ respectively. Concentration of mixed liquor solids were found statistically similar (p-value < 0.05) in $R_{4.1}$ and $R_{2.8}$ suggesting that manual discharge of settled solids was capable in maintaining mixed liquor solids at comparable values at F/M ratio of 1.8±0.6 and 1.2±0.5 kg-TCOD kg-VSS^{-1}d^{-1}. However, lower OLR in $R_{1.4}$ did not achieved similar mixed liquor solids concentration at F/M ratio of 0.9±0.3 kg-TCOD kg-VSS^{-1}d^{-1}. At nutrient ratio of 100COD: 6N: 1P and lower OLR (Figure 1B), no manual selective solids wasting was provided to any of the three reactors. The mixed liquor solids concentrations at steady state were 3.1±0.8, 3.0±1.0, 2.0±0.2 g-TSS L^{-1} in $R_{2.1}$, $R_{1.5}$, and $R_{0.8}$ resulting in F/M ratio ranging between 0.5 to 1.0 kg-TCOD kg-VSS^{-1}d^{-1}. Effluent solids reported in Figure 1C (i.e., 100C: 10N: 1P) and 1D (i.e., 100C: 6N: 1P) for the two periods resulted overall comparable independently from OLR. Effluents solids concentrations at steady state were consistently lower than 350 mg-TSS L^{-1} but a few data points. Scaling up AGS process as polishing step after anaerobic digestion may require solids separation technologies after treatment to further reduce effluent concentrations at desired levels imposed by effluent discharge limits (e.g., 350 mg-TSS L^{-1} in city of Winnipeg, Canada, sewer bylaw). Alternatively, an independent selective wasting system could be employed to ensure that lighter mixed liquor fractions of biomass do not contribute to effluent solids concentration (Devlin and Oleszkiewicz, 2018). Selective wasting the lighter fractions also have been shown to improve granular structural morphology and its stability (Henriet et al., 2016; Wilén et al., 2018).

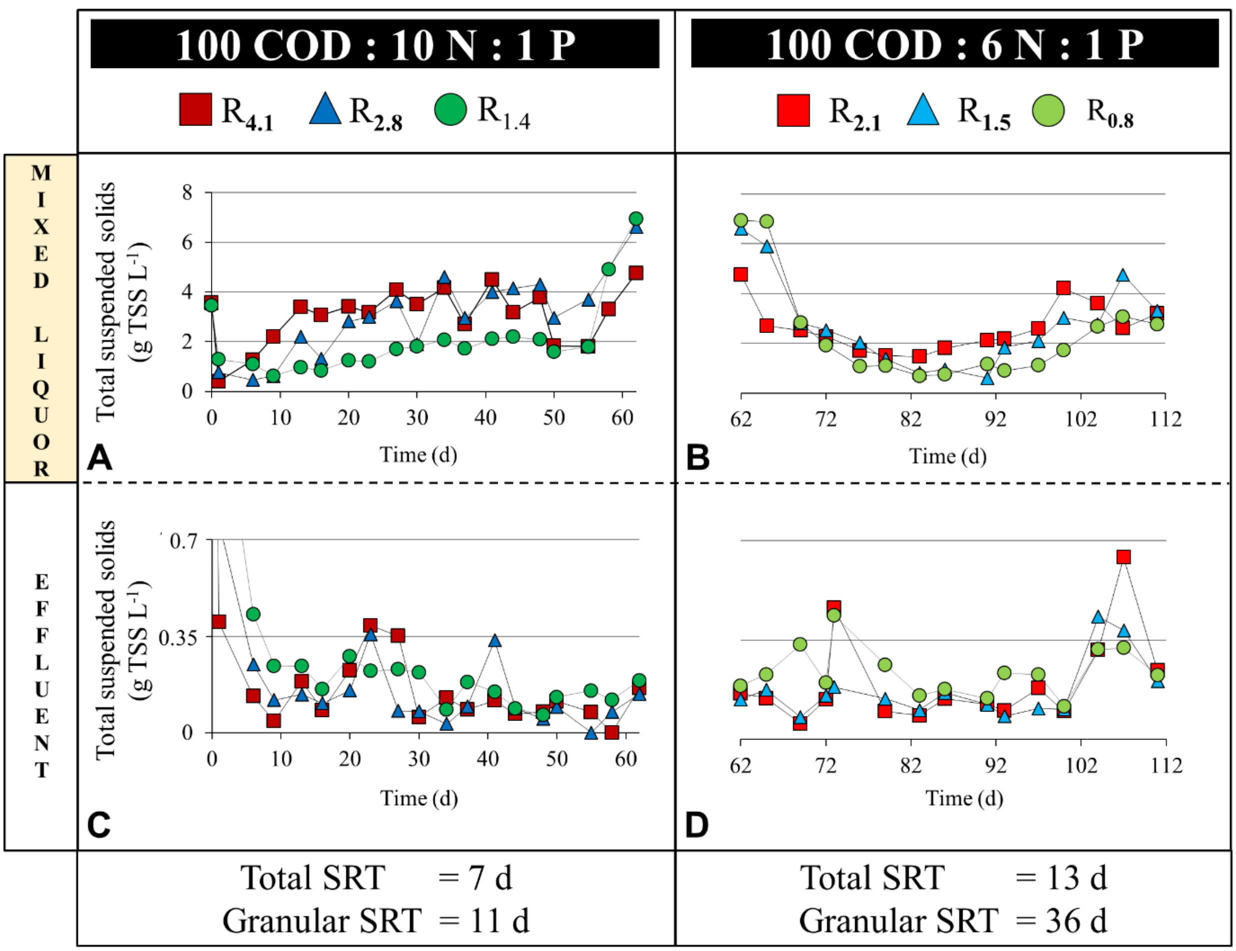

Figure 1 – Mixed liquor suspended solids in the three reactors operated at different OLR (X of the R_X) and COD:N:P ratio of 100: 10: 1 in A and 100: 6: 1 in B; Effluent total suspended solids at different OLR and COD:N:P ratio of 100: 10: 1 in C and 100: 6: 1 in D.

3.1.2 Granular sludge morphology

Figure 2 shows the profile of granular sludge development and the difference in morphological structure during experimental periods as a function of F/M ratio calculated according to Equation 3. Two, 4, and 8 weeks stereomicroscopic imaging are reported.

After one week, the first granular structure precursors were developed. Full granulation was observed after 2 weeks of operation (Figure 2A). Aerobic granules dominated after 4 weeks, when their average sizes reached 1.6±0.2, 1.9±0.3, and 1.2±0.1 mm at F/M ratio of 1.8±0.6, 1.2±0.5, and 0.9±0.3 kg-TCOD kg-VSS^{-1}d^{-1}. As expected, the F/M ratio resulted from the difference in OLR had an impact in granular sludge development. The lower is the OLR and F/M ratio the smaller is the diameter of the granules (Di Bella and Torregrossa, 2013). However, the reactor operated at OLR of 2.8±0.4 kg-TCOD m^{-3}d^{-1} resulting in F/M ratio of 1.2±0.5 kg-TCOD kg-VSS^{-1}d^{-1} did not follow this decrement trend of granular size with OLR due to most likely different organic load and settling time. After seven weeks, the reactors started to accumulate solids on granular surface and in the bulk mixed liquor (Figure 1A and 1B) which is clearly visible in Figure 2A (i.e., 8 weeks). This was caused by changes in operational parameters (i.e., previously, 7d SRT control with 4 cycles a day 6h long) which however did not affected granular sludge stability, settling characteristics and effluent solids concentrations (Stes et al., 2018). Indeed, at the beginning of week 8 the SRT was not controlled and the number of cycles were decreased to 2 per day to further reduce OLR while maintaining unvaried the first period operational parameters (Table 2).

At 100COD: 6N: 1P ratio (Figure 2B), the reactors were receiving approximately half the OLR compared to the previous stage. The longer cycle length and no SRT contributed to extend aerobic oxidation of organics allowing redistribution and recovery of granular structure into a more discrete morphology. This is evident comparing the reactors stereomicroscopic image at 4 weeks to 8 weeks (Figure 2B). Hence, one month was required to re-establish the granular morphology into a more define architecture. The granular sludge became dominant presenting an average diameter of 1.5±0.3, 1.2±0.3, and 1.0±0.1 mm at OLR of 2.1±0.6, 1.5±0.4, and 0.8±0.2 kg-TCOD $m^{-3}d^{-1}$ corresponding to F/M ratio of 1.0±0.3, 0.7±0.3, and 0.5±0.2 kg-TCOD kg-$VSS^{-1}d^{-1}$.

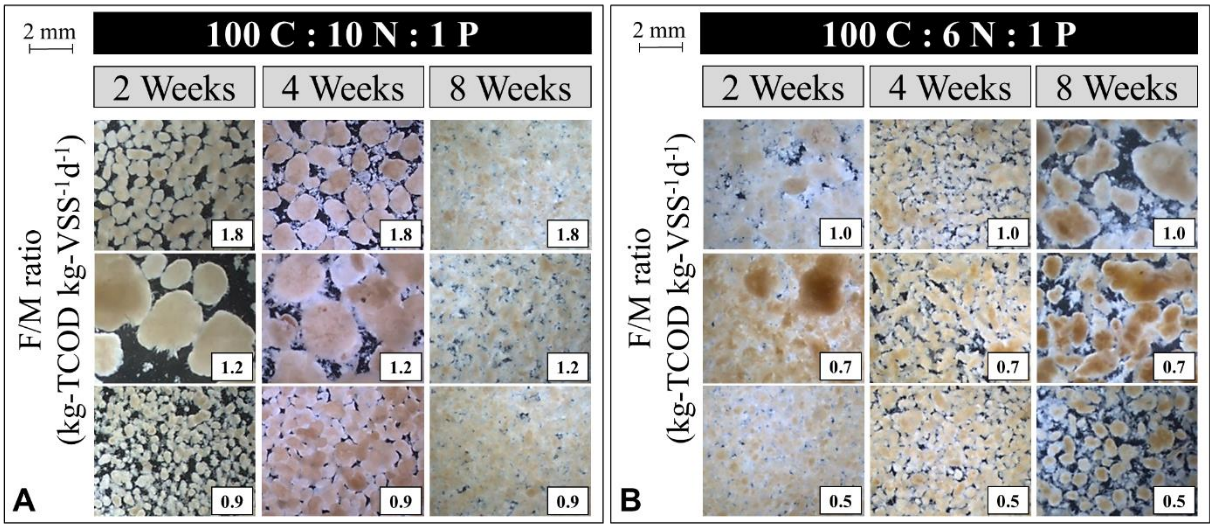

Figure 2 – Weekly stereomicroscope pictures over the two periods (100COD: 10N: 1P in A, and 100 COD: 6N: 1P in B) as a function of the F/M ratio.

In general, during the second period (100COD: 6N: 1P), the difference in OLR and F/M ratio showed an emphasized impact in granular structure and morphology. Indeed, the trend already observed in the first period became more evident as OLR decreased. The size distribution resulted therefore directly dependent on OLR, F/M ratio, and longer cycle length leading to a longer famine period producing more discrete granular architecture (Corsino et al., 2017). The granular structure presented more irregularities at a higher F/M ratio suggesting that the applied loads affected appearance and biomass structural aggregation. Hamza et al. (2018) concluded that high F/M ratio negatively impact granular stability showing overgrown biomass and poor settleability at F/M ratio above 1.5 kg-TCOD kg-$TSSm^{-3}d^{-1}$ leading to breakdown and biomass washout at values above 2.5. The author indicated an F/M ratio ranging from 0.5–1.4 kg-TCOD kg-TSSm^{-3}d^{-1} as optimal in long-term granular stability. Stes et al. (2018) found that granular structure treating brewery wastewater is resistant to operational parameters fluctuation at a lower F/M ratio (i.e., 0.01–0.3 kg-TCOD kg-TSS^{-1}d^{-1}) and pH. During one-step AGS brewery wastewater treatment the authors observed long-term granular stability, preserved performance and settling properties. In the present study, the reason why a change in granular morphology was observed lies in the microbiology of different microorganisms forming granules, EPS production/accumulation, and cycle length. The ordinary heterotrophs (OHO) are fast growing microorganisms that reside on the surface of the granules and therefore mainly drive its structural appearance (Devlin et al., 2017). At high OLR and F/M ratio, the OHO had more substrate availability for growth and more EPS are stored within and on the granular surface, which led to an amorphous superficial architecture. This phenomenon is also driven by the aerobic cycle length and superficial air velocity supplied (Devlin et al., 2017). In fact, the longer the aeration cycle a loss of loosely bound EPS and more consumption of tightly bound EPS will take place, which promotes a discrete granular appearance (Corsino et al., 2016). Therefore, the stereoscopic imagine of granular sludge suggested that the lower OLR and therefore lower F/M ratio presented the most discrete and compact granules which agrees with literature (Di Bella and Torregrossa 2013, Hamnza et al., 2018).

3.2 Aerobic granular sludge performance

3.2.1 Carbon, nitrogen, and phosphorus removal

Overall organic carbon, nitrogen, and phosphorus removal rates performance for both 100C: 10N: 1P and 100C: 6N: 1P periods as a function of loading rates are presented in Figure 3.

At different OLR (Figure 3A), a strong correlation with R^2 of 0.99 between removal rates was found with an overall percent removal of 82.5% when the two periods were considered. Specifically, at a nutrient ratio of 100C: 10N: 1P, the removal rates of the reactors operated at different OLR resulted comparable at around 80% TCOD removal. Effluent concentrations were measured as ranging between 0.2 and 0.8 g-TCOD L^{-1} at OLR from 1.4 to 4.1 kg-TCOD m^{-3} d^{-1}. In the second period, by applying a nutrient ratio of 100COD: 6N: 1P and lower OLR (i.e., 4 to 2 cycles per day), the TCOD removal slightly improved by 5% to 85% producing effluents concentration lower than 0.2 g-TCOD L^{-1}. The bylaw in North American brewery industry legislate a maximum of 0.3 g-BOD_5 L^{-1} as dischargeable concentration of organic carbon into sewer (Table 1). The effluent BOD_5 in period 100C: 10N: 1P ranged from 0.06 to 0.24 at 1.4-4.1 kg-TCOD m^{-3} d^{-1} OLR with BOD_5/COD ratio of 0.2-0.3. In the second period 100C: 10N: 1P at OLR 0.8–2.1 kg-TCOD m^{-3} d^{-1}, BOD_5 concentrations were found even lower than 0.2 g-BOD_5 L^{-1}. Hence, all the reactors were able to comply with the organic carbon bylaw at the operational conditions applied.

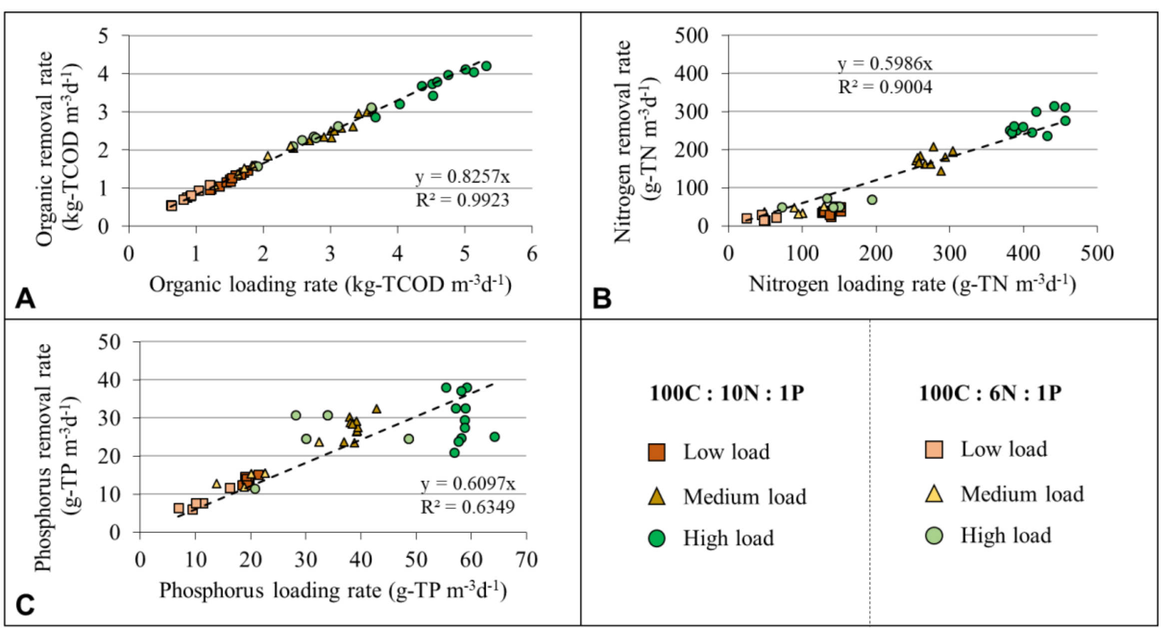

Figure 3 – Total carbon (A), nitrogen (B), and phosphorus (C) removal rates as a function of loading rates.

Nitrogen removal rates as function of loading rates are plotted in Figure 3B resulting in high correlation (i.e., R^2 of 0.90) with overall nitrogen removal of approximately 60% considering both periods. Similar overall removals were found when plotting phosphorus results as a function of loading rate however a weaker correlation of 0.635 R^2 was observed (Figure 3C).

In the first period 100C: 10N: 1P, nitrogen removal raged between 35 to 40% of the influent nitrogen which was adjusted by dosing ammonia to maintain nutrient ratio. A general increasing trend could be observed (Figure 3A and 3B) which shown that the higher is the organic loading rate the higher is the nitrogen removal. This suggested a higher nitrogen demand to support a greater microbial growth and activity which resulted not been limited at the operational conditions imposed. Zhao et al. (2013) demonstrated that heterotrophic removal through assimilation might contribute more to nitrogen removal than autotrophic nitrification at high COD and nitrogen loading rates (NLR). Total nitrogen effluent concentration was found higher than 80 mg-L^{-1} not reliably complying with typical North American discharge into sewer limits (Table 1). Similarly, phosphorus effluent concentration limits of 10 mg-TP L^{-1} were not achieved, with removals around 30% of the TP loaded.

In the second period (i.e., 100C: 6N: 1P) with no nitrogen addition, nitrogen removal increased from approximately 40 to 60% while TP (~ 30%) did not shown an improvement compared to the previous period. The effluent BOD_5 ranged from 50 to 75 mg L^{-1}, TN was measured lower than 40 mg-TN L^{-1} which were found in compliance with the bylaw reported in Table 1. However, TP in effluent samples was greater than 14 mg-TP L^{-1} not meeting the required <10 mg-TP L^{-1}.

Overall, the carbon removal rate was load-independent in the range examined (i.e., 0.8 – 4.1 kg-TCOD $m^{-3}d^{-1}$). The slightly higher removal (~5%) in the second period of experimentation was linked to the doubled aerobic reaction time that gave more time to hydrolyze residual particulate COD for further degradation and therefore achieved a greater removal. Similar independent correlation between loads (from 10 to 61 g-TP m^{-3} d^{-1}) and removals was observed in effluent TP results (Figure 2C). However, TN removal was greater at the lower loadings of 50 – 140 g-TN $m^{-3}d^{-1}$ (i.e., 100COD: 6N : 1P ratio) compared to the higher 140 – 400 g-TN $m^{-3}d^{-1}$ (i.e., 100COD: 10N: 1P ratio). Hence, TN removals were inversely proportional to the OLRs.

3.2.2 Nitrification and free ammonia inhibition

Soluble nitrogen fractionation in effluent as TAN, nitrite, and nitrate allow understanding nitrification process in the two operational periods. Figure 4 shows nitrogen speciation in effluents from both periods.

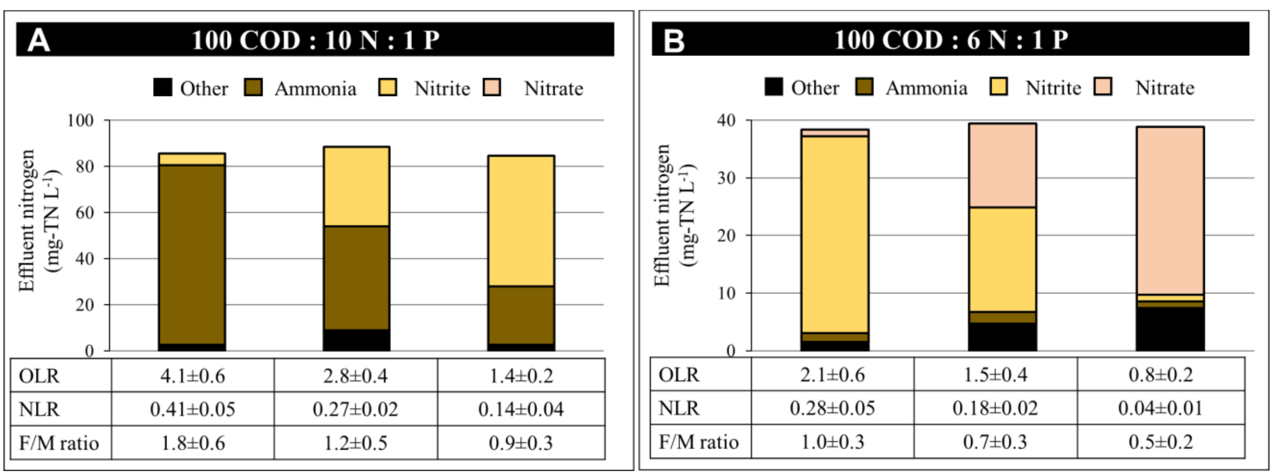

Figure 4 – Effluent total nitrogen concentrations and nitrogen speciation (i.e., TAN, nitrite, nitrate) in steady state conditions as a function of OLR (kg-TCOD $m^{-3}d^{-1}$), NLR (kg-TAN $m^{-3}d^{-1}$), and F/M ratio (kg-TCOD $VSS^{-1}d^{-1}$) at COD:N:P nutrient ration of 100:10:1 in A and 100:6:1 in B.

In the first period 100COD: 10N: 1P (Figure 4A), the influent TAN was converted by ammonia oxidizing bacteria (AOB) to nitrite but no presence of nitrite oxidizing bacteria (NOB) metabolism was observed. Results shown that the nitritation process is inversely proportional to the loading rate and full nitrification was never reached at this ratio and a 6h cycle regime. In the second period 100COD: 6N: 1P (Figure 3B), when the aeration contact time was doubled and external nitrogen was no longer provided reducing NLR, full nitritation was observed only for the higher OLR of 2.1±0.6 kg-TCOD $m^{-3}d^{-1}$, while nitrification increased when OLR decreased to full nitrification at OLR of 0.8±0.2 kg-TCOD $m^{-3}d^{-1}$.

Two factors could be the reasons explaining this phenomenon: free ammonia (FA; NH_3-N) inhibition and OHO interference with AOB and NOB metabolism. Total ammonia nitrogen concentration in influent wastewater were 120±10 and 80±7 mg-TAN L^{-1} resulting in FA concentration of 34±4 and 23±2 mg-NH_3-N L^{-1} at C:N:P nutrient ratio of 100:10:1 and 100:6:1. Nitrogen loading rate were 0.41±0.05, 0.27±0.02, 0.14±0.04 g-TAN $L^{-1}d^{-1}$ in COD100: 10N: 1P and 0.28±0.05, 0.18±0.02, and 0.04±0.01 g-TAN $L^{-1}d^{-1}$ in COD100: 10N: 1P. The reactors' pH were not controlled over the study leading to an alkaline environment after aeration time (i.e., >9 pH), which inhibit AOB and NOB metabolism and their establishment through FA inhibition (Wei et al., 2012). A study conducted by Claros et al. (2013) suggested that optimal pH for AOB is in the range of 7.4–7.8 and inhibitory effects are stronger at higher pH in which FA concentration increases. Research from Anthonisen et al. (1976) have demonstrated that AOB are more resistant to FA than NOB which are sensitive to nitrous acid. The authors defined acute FA inhibitory concentration ranging from 10 to 150 mg-NH_3-N L^{-1} for AOB and from 0.1 to 1 mg-NH_3-N L^{-1} for NOB with initial effects occurring at concentration as low as 0.2–3.0 mg-NH_3-N L^{-1}. Acclimation of nitrifiers to FA is, however, possible over long-term exposure depending on biomass characteristics (e.g, suspended, granular) and NLR. Researches have shown that nitrifying granular sludge could tolerate fluctuation of NLR from 0.7 to 1.8 kg-TAN $m^{-3}d^{-1}$ at 8 pH and aeration length between 2 to 5h at saturation DO (Liu et al., 2008). Other authors have shown in nitrifying AGS complete nitrification to nitrate at NLR of 0.4 g-TAN $L^{-1}d^{-1}$ while 80% of nitratation was achieved at twice the NLR with DO concentration of ~8 mg-O_2 L^{-1} and pH ranging from 7.0 to 8.5 (Vázquez-Padín et al., 2010). In AGS systems designed also for carbon removal, however, the OLR plays an important role in enabling nitrification. Figueroa et al. (2011) reported FA inhibition and oxygen limitation could cause the decrease in nitrogen removal when NLR reaches values of ~1.3 kg-TN $m^{-3}d^{-1}$. Indeed, increasing OLR could decrease granular nitrifying activity caused by reduction of oxygen penetration into the granules depth and its availability for AOB (Chen et al., 2008). The differences in OLR, in the presented study, agrees with the literature. Indeed, microorganisms (i.e, OHO, AOB and NOB) in the granules were developed in a more efficient organization and fully performing at the lowest OLR and NLR of 0.04 ± 0.01 g-TAN L^{-1} – Figure 4B. The OHO have a higher DO affinity compared to nitrifiers and it could be possible that their greater growth and EPS production/storage had hindered AOB establishment within granular structure. In the first period 100C : 10N : 1P, the measured specific tightly-bound EPS on granules were ranging between 160 and 200 mg-TB-EPS VSS^{-1} at OLR from 1.4 to 4.1 kg-TCOD $m^{-3}d^{-1}$ with 1.2- 2 mm granular size. Liu et al. (2015) in their research concluded that particle size between 0.6 and 1.8 mm provide an ideal environment for AOB and NOB establishment with optimal specific oxygen utilization rates (SOUR). The second period 100C : 6N : 1P exhibited a smaller granular size (i.e., 1 to 1.5 mm) and lower EPS production/storage (i.e., 75 to 90 mg-TB-EPS VSS^{-1} at OLR from 0.8 to 2.1 kg-TCOD $m^{-3}d^{-1}$). For these reasons, the lower OLR of 0.8±0.2 kg-TCOD $m^{-3}d^{-1}$ resulted in better stability in granular architecture (see Figure 2) enabling nitrification (Figure 4B).

3.2.3 Extracellular polymeric substances

The difference in EPS composition has been reported to be important for granular structure stability. Liu et al. (2015) reported how the abundancy of protein and carbohydrates significantly affects granular stability. In particular, the sludge hydrophobicity is enhanced by proteins content and a high ratio of protein to polysaccharides promoted cellular aggregation by improving cells adhesion capability. In Figure 5, the protein to polysaccharides ratio at different OLR in steady state condition for both nutrient C:N:P ratios of 100:101 and 100:6:1 is shown. Overall, the protein to polysaccharides ratio decreases as OLR and therefore F/M ratio increases suggesting that at higher values granular structure could be less compact and eventually have lower stability as also described by Hamza et al. (2018).

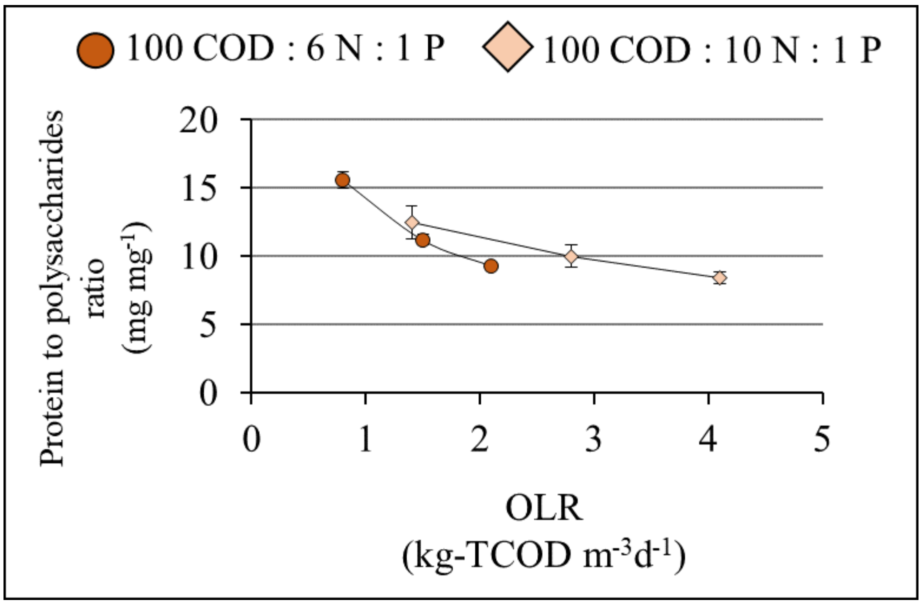

Figure 5 – Protein to polysaccharides ratio trend at different OLR at steady state conditions in the two periods at C:N:P nutrient ratio of 100:10:1 and 100:6N:1

The protein fraction resulted more abundant than carbohydrates, ranging between 8 to 15 as protein to polysaccharide ratio. The highest value was achieved when OLR was decreased by increasing cycle length to 12h from 6h and decreasing number of cycles to 2 from 4 per day at nutrient ratio of 100COD: 6N: 1P increasing therefore organic carbon oxidation potential. Research have shown that an extended aeration period leads to granular famine stage which increases granular stability (Corsino et al., 2017). Adav et al. (2010) studied a one-step AGS treating high strength synthetic wastewater at OLR between 9 to 21 kg-TCOD $m^{-3}d^{-1}$. This research led the authors to the conclusion that EPS protein content is an indicator of granular stability.

Indeed, protein production decreases as OLR increasing leading to weakening granular structure and eventually causing their breakdown. Therefore, a longer aeration reaction time with a lower OLR is qualitatively beneficial to granular structure which exhibited a more compact and defined architecture compared to the other operational conditions evaluated (Figure 2). Performance results also indicated that within the other conditions studied microbial community and its complex functionality in nutrient removal is more efficient at operational parameters of OLR of 0.8±0.2 kg-TCOD $m^{-3}d^{-1}$ resulting in F/M ratio of 0.5±0.2 kg-TCOD $VSS^{-1}d^{-1}$.

3.3 Design considerations

The solids retention time was measured according to Equation 4. Total SRT of 7d comprehensive of granular and suspended solids was estimated for the 100C: 10N: 1P nutrient ratio while the granules SRT was calculated as 11d (Figure 1). In the second period with C:N:P ratio of 100:6:1, the total SRT was estimated 13d while the granular SRT was calculated been three time the total (i.e., 36d). According to BioWin 5.3 (Environsim, 2019), at room temperature (20°C), the minimum SRT of OHO is 0.4d, phosphorus accumulating organisms (PAO) 1.2d, AOB 1.4 d, and NOB 1.9d. In theory, the reactors had sufficient SRT to retain and favor the growth of the entire microbial population leading to carbon, nitrogen, and phosphorus removal. However, full nitrification was inhibited in all conditions studied but at NLR of 0.04 ± 0.01 g-TAN L^{-1}, OLR of 0.8±0.2 kg-TCOD $m^{-3}d^{-1}$ resulting in F/M ratio of 0.5±0.2 kg-TCOD $VSS^{-1}d^{-1}$ (Figure 4B). However, simultaneous nitrification-denitrification and biological phosphorus removal (Bio-P) did not occur at any of the conditions applied. Kinetic tests showed that the readily biodegradable COD (rb-COD) was removed within a maximum of 2h after aeration by OHO. The scarcity of rb-COD together with the lack of anoxic conditions did not provide a suitable environment for simultaneous nitrification-denitrification. The presence of oxygen bound compounds (nitrite and nitrate; Figure 4) at the end of the cycle had a critical effect on the anaerobic feeding period, which became anoxic which negatively affected PAO establishment. Thus, feeding time impacted not only Bio-P but also denitrification. Hydrolysis and further fermentation of the brewery substrate to consistent volatile fatty acids (VFA) production likely required more than the chosen 40 min feeding time. Also, after anaerobic pre-treatment most of the rb-COD is depleted by digestion and therefore more time is required to ferment recalcitrant particulate COD. Thus, without rb-COD (i.e., VFA as acetate), the PAO are not able to grow and be part of the microbial granular structure. A longer feeding time would enable denitrification and increase acetate production via fermentation ensuring anaerobic feeding conditions for Bio-P (Stes et al., 2019). Also, selectively remove microorganisms by wasting the lighter solids fraction through shorter settling time, SRT control, or implementation of a dedicated selective waste system has shown to enhance Bio-P and reduce effluent solids concentration (Henriet et al., 2016).

4. CONCLUSIONS

Granulation was observed after one week of operations. After two weeks, a define granular structure was developed and granulation was completed in all the reactors independently from the OLR and F/M. The stability of the AGS was strongly affected by the operating conditions. Indeed, at high C:N:P ratio and OLRs (or F/M) irregular and weak granules developed, whereas, in contrast, low C:N:P ratio and OLRs (or F/M) were favorable to achieve stable aerobic granules in the long term. Overall, a longer aeration reaction time with a lower OLR was beneficial to the granules structure, which exhibited a clear predominance of proteins in the EPS matrix. In general, the ideal operating conditions were: OLR lower than 0.8±0.2 kg-TCOD $m^{-3}d^{-1}$, F/M ratio lower than 0.5±0.2 kg-TCOD $VSS^{-1}d^{-1}$and C: N: P of 100:6:1.

The carbon removal rate was higher than 80% in all the reactors and it was load-independent. In contrast, TN removal was inversely proportional to the OLRs.

ACKNOWLEDGMENTS

The authors acknowledge the Marie Curie program (project Carbala IRSES 2011 – 295176).

REFERENCE

Adav, S.S., Lee, D.-J., Lai, J.-Y., 2010. Potential cause of aerobic granular sludge breakdown at high organic loading rates. Appl. Microbiol. Biotechnol. 85, 1601–1610. doi:10.1007/s00253-009-2317-9

Anthonisen, A., Loehr, R., Prakasam, T., Srinath, E., 1976. Inhibition of Nitrification by Ammonia and Nitrous Acid. J. Water Pollut. Control Fed. 48, 835–852. doi:10.1017/CBO9781107415324.004

APHA, 2012. Standard Methods - Examination of water and wastewater. American Public Health Association, American Water Works Association, Water Environment Federation.

Brito, A.G., Peixoto, J., Oliveira, J.M., Oliveira, J. a, Costa, C., Nogueira, R., Rodrigues, A., 2007. Brewery and winery wastewater treatment: some focal points of design and operation, Utilization of By-Products and Treatment of Waste in the Food Industry.

Chastain, C., Crandall, S., Del Grande, D., Flores, T., Gilliland, M., Horwitz, L., Opela, C., Swersey, C., Utz, M., 2011. Water and Wastewater : Treatment / Volume Reduction Manual 1–47.

Chen, Y., Jiang, W., Liang, D.T., Tay, J.H., 2008. Biodegradation and kinetics of aerobic granules under high organic loading rates in sequencing batch reactor. Appl. Microbiol. Biotechnol. 79, 301–308. doi:10.1007/s00253-008-1421-6

Claros J.J., Jiménez E., Aguado D., Ferrer J., Seco A., S.J., 2013. Effect of pH and HNO2 concentration on the activity of ammonia-oxidizing bacteria in a partial nitritation reactor. Water Sci. Technol. 11, 2578–2594.

Corsino, S.F., Capodici, M., Torregrossa, M., Viviani, G., 2016. Fate of aerobic granular sludge in the long-term: The role of EPSs on the clogging of granular sludge porosity. J. Environ. Manage. 183, 541–550. doi:10.1016/j.jenvman.2016.09.004

Corsino, S.F., di Biase, A., Devlin, T.R., Munz, G., Torregrossa, M., Oleszkiewicz, J.A., 2017. Effect of extended famine conditions on aerobic granular sludge stability in the treatment of brewery wastewater. Bioresour. Technol. 226. doi:10.1016/j.biortech.2016.12.026

Devlin, T.R., di Biase, A., Kowalski, M., Oleszkiewicz, J.A., 2017. Granulation of activated sludge under low hydrodynamic shear and different wastewater characteristics. Bioresour. Technol. 224, 229–235. doi:10.1016/j.biortech.2016.11.005

Devlin, T.R., Oleszkiewicz, J.A., 2018. Cultivation of aerobic granular sludge in continuous flow under various selective pressure. Bioresour. Technol. 253, 281–287. doi:10.1016/j.biortech.2018.01.056

Di Bella, G., Torregrossa, M., 2013. Simultaneous nitrogen and organic carbon removal in aerobic granular sludge reactors operated with high dissolved oxygen concentration. Bioresour. Technol. 142, 706–713. doi:10.1016/j.biortech.2013.05.060

di Biase, A., Devlin, T.R., Kowalski, M.S., Oleszkiewicz, J.A., 2017. Performance and design considerations for an anaerobic moving bed biofilm reactor treating brewery wastewater:

Impact of surface area loading rate and temperature. J. Environ. Manage. 1–7. doi:10.1016/j.jenvman.2017.05.093

Di Trapani, D., Corsino, S.F., Torregrossa, M., Viviani, G., 2019. Treatment of high strength industrial wastewater with membrane bioreactors for water reuse: Effect of pre-treatment with aerobic granular sludge on system performance and fouling tendency. J. Water Process Eng. 31, 100859. doi:10.1016/j.jwpe.2019.100859

Donoghue, C., Jackson, G., Koop, J.H., Heuven, A.J.M., 2012. The Environmental Performance of the European Brewing Sector.

Dubois, M., Gilles, K., Hamilton, J.K., Rebers, P.A., Smith, F., 1951. A colorimetric method for the determination of sugars. Nature. doi:10.1038/168167a0

Figueroa, M., Val Del Río, A., Campos, J.L., Mosquera-Corral, A., Méndez, R., 2011. Treatment of high loaded swine slurry in an aerobic granular reactor. Water Sci. Technol. 63, 1808–1814. doi:10.2166/wst.2011.381

Hamza, R.A., Sheng, Z., Iorhemen, O.T., Zaghloul, M.S., Tay, J.H., 2018. Impact of food-to-microorganisms ratio on the stability of aerobic granular sludge treating high-strength organic wastewater. Water Res. 147, 287–298. doi:10.1016/j.watres.2018.09.061

Henriet, O., Meunier, C., Henry, P., Mahillon, J., 2016. Improving phosphorus removal in aerobic granular sludge processes through selective microbial management. Bioresour. Technol. 211, 298–306. doi:10.1016/j.biortech.2016.03.099

Le-Clech, P., Chen, V., Fane, T.A.G., 2006. Fouling in membrane bioreactors used in wastewater treatment. J. Memb. Sci. 284, 17–53. doi:10.1016/j.memsci.2006.08.019

Liu, Y., Kang, X., Li, X., Yuan, Y., 2015. Performance of aerobic granular sludge in a sequencing batch bioreactor for slaughterhouse wastewater treatment. Bioresour. Technol.

190, 487–91. doi:10.1016/j.biortech.2015.03.008

Liu, Y.Q., Wu, W.W., Tay, J.H., Wang, J.L., 2008. Formation and long-term stability of nitrifying granules in a sequencing batch reactor. Bioresour. Technol. 99, 3919–3922. doi:10.1016/j.biortech.2007.07.041

Lowry, O.H., Randall, R.J., Lewis, A., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem 193, 265–275. doi:10.1016/0304-3894(92)87011-4

Olajire, A. a., 2012. The brewing industry and environmental challenges. J. Clean. Prod. 1–21. doi:10.1016/j.jclepro.2012.03.003

Simate, G.S., Cluett, J., Iyuke, S.E., Musapatika, E.T., Ndlovu, S., Walubita, L.F., Alvarez, A.E., 2011. The treatment of brewery wastewater for reuse: State of the art. Desalination 273, 235–247. doi:10.1016/j.desal.2011.02.035

Stes, H., Aerts, S., Caluwe, M., D'Aes, J., De Vleesschauwer, F., Dobbeleers, T., De Langhe, P., Kiekens, F., Dries, J., 2019. Influence of mixed feeding rate in a conventional SBR on biological P-removal and granule stability while treating different industrial effluents. Water Sci. Technol. 79, 645–655. doi:10.2166/wst.2019.081

Stes, H., Aerts, S., Caluwé, M., Dobbeleers, T., Wuyts, S., Kiekens, F., D'Aes, J., De Langhe, P., Dries, J., 2018. Formation of aerobic granular sludge and the influence of the pH on sludge characteristics in a SBR fed with brewery/bottling plant wastewater. Water Sci. Technol. 77, 2253–2264. doi:10.2166/wst.2018.132

Valta, K., Kosanovic, T., Malamis, D., Moustakas, K., Loizidou, M., 2014. Overview of water usage and wastewater management in the food and beverage industry. Desalin. Water Treat. 53, 3335–3347. doi:10.1080/19443994.2014.934100

Vázquez-Padín, J.R., Figueroa, M., Campos, J.L., Mosquera-Corral, a., Méndez, R., 2010.

Nitrifying granular systems: A suitable technology to obtain stable partial nitrification at room temperature. Sep. Purif. Technol. 74, 178–186. doi:10.1016/j.seppur.2010.06.003

Wang, S.-G., Liu, X.-W., Gong, W.-X., Gao, B.-Y., Zhang, D.-H., Yu, H.-Q., 2007. Aerobic granulation with brewery wastewater in a sequencing batch reactor. Bioresour. Technol. 98, 2142–7. doi:10.1016/j.biortech.2006.08.018

Wei, Y., Ji, M., Li, R., Qin, F., 2012. Organic and nitrogen removal from landfill leachate in aerobic granular sludge sequencing batch reactors. Waste Manag. 32, 448–455. doi:10.1016/j.wasman.2011.10.008

Wilén, B.M., Liébana, R., Persson, F., Modin, O., Hermansson, M., 2018. The mechanisms of granulation of activated sludge in wastewater treatment, its optimization, and impact on effluent quality. Appl. Microbiol. Biotechnol. 102, 5005–5020. doi:10.1007/s00253-018-8990-9

Zhao, Y., Huang, J., Zhao, H., Yang, H., 2013. Microbial community and N removal of aerobic granular sludge at high COD and N loading rates. Bioresour. Technol. 143, 439–446. doi:10.1016/j.biortech.2013.06.020