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Comparison of the dynamics, biokinetics and microbial diversity between activated sludge flocs and aerobic granular sludge

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ABSTRACT

This work aimed to compare the dynamics, biokinetics, and microbial diversity between activated sludge flocs (ASF) and aerobic granular sludge (AGS) whose systems were operated under similar experimental conditions in terms of inoculum, feeding, substrate source, etc. Therefore, the kinetic parameters involved in the organic matter removal, nitrification, denitrification, and dephosphatation were determined, as well as the microbial changes were assessed by metagenomics analysis. Regarding the kinetic parameter yield coefficient (Y), values of 0.55 and 0.36 g VSS/g COD were found for ASF and AGS, respectively, showing a higher sludge production in ASF and the importance of feast/famine periods for lowering sludge production in AGS systems. AGS presented a lower sludge production and a higher endogenous consumption rate than ASF. The activity of phosphorus-accumulating bacteria was remarkably higher in AGS. Although both biomasses were aerobic, their kinetic parameters had significant differences.

1. Introduction

The activated sludge (AS) system is still one of the most used biological technologies in wastewater treatment plants (WWTPs), especially in developed countries (van Haandel and van der Lubbe, 2012). In AS, the biological flocs are microbial aggregates composed of different groups of microorganisms, and, usually, high efficiencies of organic matter removal are reached. However, in order to obtain high efficiencies of nitrogen and phosphorus removal, AS-based WWTPs need specific environmental conditions, different solids retention times, etc., which can be achieved by addition of multiple tanks in continuous-flow systems (e.g. UCT process, Bardenpho) or by using sequencing batch reactors (SBRs, intermittent flow), which mixes the aerobic, anoxic and anaerobic periods in a single unit (He et al., 2018).

After the aeration tank in continuous-flow systems, AS systems demand secondary clarifiers or other approaches to separate the biomass from the liquid, which returns to the aeration tank by recirculation pumps. Moreover, operational problems, such as sludge bulking, might sometimes occur, and usual high CAPEX and OPEX are verified, especially when a tertiary treatment is required. Therefore, new solutions were developed in order to overcome these problems. In this context, aerobic granular sludge (AGS) technology appeared, based on various reactions which take place in a spherical biofilm with fast sedimentation, called granules, which are the result of auto aggregation capacity of the biomass in a system with a high selection pressure (Rollemberg et al., 2018).

The aerobic granules are round, dense, compact and have multiples layers (generally aerobic and anoxic), high EPS yield, diameter between 0.2 mm and 5 mm, and settling velocity between 10 and 90 m/h. On the other hand, the activated sludge flocs are irregular in shape, have a single aerobic layer due to the small diameters (< 0.2 mm), low EPS yields, and low settling velocity (< 10 m/h). In terms of costs, the AGS technology presents a 20–25% reduction in operating costs, a 23–40% lower electricity demand and a 50–75% reduction in space requirements (Bengtsson et al., 2019).

In AS systems, the main kinetic parameters used to design and operate WWTPs are the specific growth rate (μ), maximum rate of substrate utilization per unit mass of microorganisms (k), and endogenous decay coefficient (k_d) (Metcalf and Eddy, 2003). However, the reference values of these parameters for the AS may not be applied to the aerobic granular sludge, mainly because: (i) AGS is cultivated in periods of feast and famine, which can affect the growth rate and biomass activity (Krishna and van Loosdrecht, 1999); (ii) in granules with aerobic and anoxic layers and with optimal operating conditions (DO < 4 mg/L), it is possible the occurrence of simultaneous nitrification and denitrification (SND), which reduces the demand for oxygen in the aerobic

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period and changes the rates of oxygen consumption (Rollemberg et al., 2018); and (iii) the high presence of slow-growing bacteria may have an impact on the cellular growth rate of the aerobic granular biomass. For example, denitrifying polyphosphate-accumulating organisms and denitrifying glycogen-accumulating organisms (DPAOs and DGAOs) are 40% less efficient in generating energy, resulting a 20–30% lower cell yield when compared to ordinary heterotrophic denitrifiers (He et al., 2018). Therefore, although both biomasses are aerobic, their kinetic parameters are different (Reino et al., 2016).

Hence, this work aimed to compare the dynamics, biokinetics and microbial diversity between activated sludge flocs (ASF) and AGS whose systems were operated under similar experimental conditions in terms of inoculum, feeding, substrate source, etc. Therefore, the kinetic parameters involved in the organic matter removal, nitrification, denitrification, and dephosphatation were determined, as well as the microbial changes were assessed by metagenomics analysis. As far as the authors are concerned, this is the first report which evaluated simultaneously the main microorganisms involved in organic matter and nutrients removal in both ASF and AGS systems by using respirometric techniques, continuous-flow experiments, and molecular microbiology, bringing important data to the design and operation of AGS systems.

2. Material and methods

2.1. Experimental setup

Two reactors, R1 (continuous-flow activated sludge system) and R2 (aerobic granular sludge), were operated at a room temperature of 28 ± 2 °C and had the same dimensions: a diameter of 100 mm, a height of 1.0 m and a working volume of approximately 7.5 L.

R1 was continuously aerated (typical configuration of an activated sludge system) by an air compressor (Yuting air pump model ACO-003), resulting in a dissolved oxygen (DO) concentration higher than 5 mg/L. An upward flow was imposed by pumping the influent from the bottom of the reactor, and the hydraulic retention time (HRT) was 12 h.

R2 was operated as a conventional sequencing batch reator (SBR), a recommended configuration in literature for AGS cultivation (Nancharaiah and Reddy, 2018). Its cycle of 6 h was divided in filling (30 min), anaerobic period (90 min), aerobic period (229 min), settling (10 min) and decanting (1 min). The reactor was filled from its bottom, and the effluent was decanted from the half of its working height. The hydraulic retention time (HRT) was also 12 h with a volume exchange ratio of 50%. The reactor was aerated at an airflow rate of 10 L/min by an air compressor (Air Pump–EL – 100 Model), resulting in a superficial gas velocity of 2.0 cm/s and a dissolved oxygen concentration between 2 and 4 mg/L.

2.2. Seed sludge and wastewater

The biomass inoculated in the reactors R1 and R2 was from the aeration tank of an AS wastewater treatment plant located in Fortaleza, Ceará, Brazil. Approximately 3.0 L was introduced into the reactors, resulting in an initial concentration of mixed liquor suspended solids (MLSS) of about 3 g/L. The sludge volume index at 30 min (SVI_{30}) during start-up was 150 mL/g.

The synthetic wastewater used was the same for both systems and contained 800 mg COD/L (acetate), 100 mg N/L (NH_4Cl), 10 mg P/L (KH_2PO_4) and 1 mL/L of a trace elements solution prepared as described elsewhere (Rollemberg et al., 2019).

2.3. Kinetic parameters

Respirometry tests similar to those reported by Zafiriadis et al. (2017) were conducted in order to obtain the following kinetic parameters: microbial activity and substrate utilization of the aerobic bacteria (heterotrophic and autotrophic). The biokinetics of the aerobic microorganisms were estimated according to Chandran and Smets (2000).

A semi-continuous open respirometer (Beluga, Brazil) connected to a software (S32c) was used. In addition, it was necessary to perform batch tests to determine the microbial parameters which require anaerobic or anoxic phases (such as those involved in the phosphorus release, denitrification, etc.). The batch tests were done according to Corsino et al. (2018) and Zafiriadis et al. (2017).

All tests were conducted in triplicate at the same temperature, using 1 L of sludge at a concentration of 2 g VSS/L. The biomass used in the batch tests was collected from both reactors at the end of the operational period (approximately 90 days after the startup), when they were stable in terms of pollutants removal, solids concentration, etc. The same concentration of VSS was used in both tests to ensure equal conditions in terms of food/microorganism (F/M) ratio.

The microbial groups evaluated were divided in four main groups, namely ordinary heterotrophic organisms (OHO), denitrifying organisms (via NO_2^- and NO_3^-), polyphosphate-accumulating organisms with different electron acceptors (via O_2, NO_2^- and NO_3^-) and nitrifying organisms (ammonia-oxidizing bacteria, AOB, and nitrite-oxidizing bacteria, NOB). A summary of the tests is shown in Table 1.

The biokinetics was expressed as the maximum specific oxygen uptake rate (SOUR). The SOUR was calculated by dividing the maximum oxygen uptake rate (dO_2/dt_{max}, mg O_2/L·day), obtained from the linear regression of the respirograms, by the total chemical oxygen demand (tCOD) concentrations, and the DO setpoints were 1 mg/L and 3 mg/L. It is worth mentioning that DO around 2 mg/L is adequate for

Table 1
Configuration of tests performed for the different microbial groups and kinetic parameters obtained.

Microbial Groups	Test	Conditions	Parameters	Substrate and concentration
AOB	Respirometric	Aerobic	OUR, μ, Y	50 mg/L N-NH_4^+
NOB	Respirometric	Aerobic	OUR, μ, Y	50 mg/L N-NO_2^-
DNB	Batch	Anoxic (nitrite as electron acceptor)	OUR	150 mg/L COD (acetate) 40 mg/L N-NO_3^-
		Anoxic (nitrate as electron acceptor)	OUR	150 mg/L COD (acetate) 40 mg/L N-NO_3^-
PAOs	Batch and respirometric	Anaerobic + Aerobic	OUR	300 mg/L COD (acetate) 20 mg/L P-PO_4^{3-}
DPAOs	Batch and respirometric	Anaerobic + Anoxic (nitrite as electron acceptor)	OUR	300 mg/L COD (acetate) 20 mg/L P-PO_4^{3-} 100 mg/L N-NO_3^- (after anaerobic period)
		Anaerobic + Anoxic (nitrate as electron acceptor)	OUR	300 mg/L COD (acetate) 20 mg/L P-PO_4^{3-} 140 mg/L N-NO_3^- (after anaerobic period)
OHO	Respirometric	Aerobic	OUR, μ, Y, K_d	120 mg/L COD (acetate)

All tests were only started after endogenous respiration was verified in the biomass. All tests were performed with VSS concentration of 2 g/L.

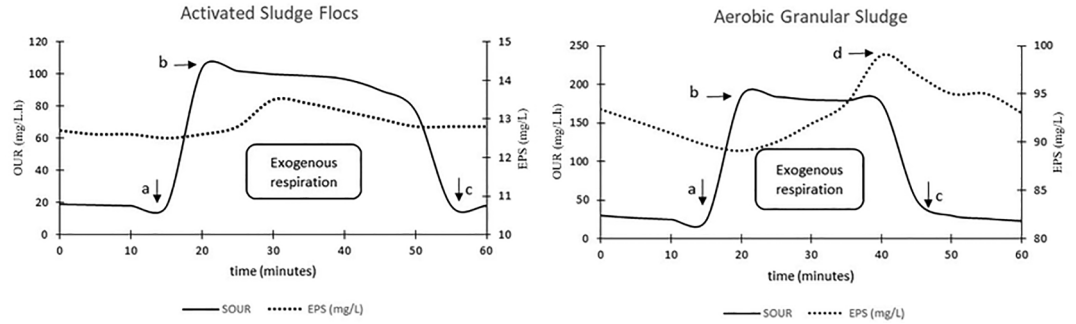

Fig. 1. Specific oxygen uptake rate (SOUR) in the oxidation of organic matter in activated sludge flocs (ASF) and aerobic granular sludge (AGS). a – addition of organic matter (120 mg/L COD – sodium acetate); b – maximum OUR; c – end of exogenous respiration; d – EPS consumption.

the biological processes investigated in the present work (microorganisms involved in carbon and nutrients removal).

The proposed equations for substrate utilization and microbial growth are based on the studies conducted by Liu et al. (2005) and Metcalf and Eddy (2003).

2.4. Analytical methods

COD, pH, NH_4^+-N, NO_3^--N, NO_2^--N, total phosphorus (TP), MLSS, and the sludge volume index at 10 and 30 min (SVI_{10} and SVI_{30}) were determined according to APHA (2012), whereas DO was measured by a YSI 5000 meter. Total inorganic nitrogen (TIN) was regarded as the sum of NH_4^+-N, NO_3^-N, and NO_2^--N (Rollemberg et al., 2019).

The extracellular polymeric substances (EPS) were extracted from 5 mL of mixed liquor under alkaline conditions by addition of 5 mL of NaOH (1 mol L^{-1}) at a high temperature (30 min at 80 °C), followed by ultrasound (5 min at 55 kHz) (Yang et al., 2014). Therefore, the carbohydrate and protein concentrations were determined according to the phenol-sulphuric acid method with glucose as the standard (DuBois et al., 1956) and by the Folin method with bovine serum albumin as the standard (Lowry et al., 1951), respectively. The total EPS content was evaluated as the sum of the soluble microbial products (SMP), loosely bound EPS (LB-EPS), and the tightly bound EPS (TB-EPS) (Rollemberg et al., 2019).

The shape of the activated sludge flocs and aerobic granules were monitored by optical microscopy (Opton) and scanning electron microscopy (SEM) (Inspect S50 – FEI).

2.5. Microbiological analysis

Samples from the mixed liquor were collected at the end of the operational period (approximately 90 days after the startup), and the DNA was extracted using PowerSoil® DNA isolation kit (MoBio Laboratories Inc., USA) according to manufacturer's instructions. All analytical procedure is described elsewhere (Rollemberg et al., 2019).

3. Results and discussion

3.1. Reactors performance

As mentioned before, both systems were fed with the same synthetic wastewater (800 mg COD/L, 100 mg N-NH_4^+/L and 10 mg P-PO_4^{3-}/L). However, quite different removal efficiencies were observed. In R1 (ASF), the biomass was stable in the first week, reaching removal efficiencies of 80 ± 2%, 64 ± 3%, 31 ± 4%, and 12 ± 2% in terms of COD, N-NH_4^+, total nitrogen (TN), and total phosphorus (TP), respectively. These results are in accordance with previous studies on AS systems (with similar wastewater) which were not designed for tertiary treatment (van Haandel and van der Lubbe, 2012).

On the other hand, about three weeks were necessary to form and stabilize the aerobic granules cultivated in R2, reaching removal efficiencies of 92 ± 3%, 90 ± 6%, 87 ± 5%, and 89 ± 4% in terms of COD, N-NH_4^+, TN, and TP, respectively, which are also in agreement with previous works on AGS (He et al., 2018; Long et al., 2016).

In the particle size distribution test, the AGS presented diameters between 1 and 2 mm, while the ASF had diameters close to 0.2 mm.

3.2. Dynamics and biokinetic parameters in the aerobic oxidation of organic matter

The bioactivity of ASF and AGS was quantified based on the specific oxygen uptake rate (SOUR) through respirometry analysis. In the beginning, there was a notable difference of endogenous OUR between the biomasses, i.e. 18 and 25 mg/L·h for ASF and AGS, respectively.

The higher endogenous OUR obtained with AGS can be explained by its specific metabolism, as literature reports a higher use of endogenous content as source of electrons in the absence of external organic matter (Yang et al., 2017; Rollemberg et al., 2018). The EPS production and the presence of polyhydroxyalkanoates (PHA)-accumulating microorganisms are important to the formation and maintenance of the granules. These compounds can be used as carbon sources during endogenous respiration, thus promoting a higher OUR. Another hypothesis can be related to the formation of soluble microbial products (SMP), as granules are considered important SMP producers, especially because of the stress caused by the selection pressure (aeration rate and prolonged famine) and due to the high quantity of polysaccharides and proteins in EPS. Some researches even found a relationship between EPS content and SMP production (Xi et al., 2018).

The aerobic oxidation test (respirometric analysis) for organic matter (120 mg COD /L) is shown in Fig. 1. A maximum OUR of 104 mg/L·h was found in ASF, while, in AGS, the value obtained was 185 mg/L·h, indicating a higher biodegradation rate in the aerobic granules. The greater microbial activity (in terms of SOUR) observed in the granules may be related to the cultivation of this biomass (feast-famine conditions induce biomass to rapidly store extracellular biodegradable organic matter), the higher amount of EPS in the AGS, and greater amount of active sludge (Xa) in the aerobic granular biomass (as is known, not every portion of VSS is effectively active sludge).

The variation of EPS content was evaluated during the respirometric tests. An expected high EPS content was found for AGS biomass, which possibly influenced the greater endogenous OUR verified. It is also important to highlight the greater variation (production and consumption) of EPS during the exogenous and endogenous phases in the AGS. At the beginning of the famine period, after the degradation of the organic matter, there is a slightly higher OUR compared to the value found during the endogenous phase, even though no residual COD was available, suggesting EPS consumption (Fig. 1).

Another important observation is the required time for the biomasses to return to the endogenous respiration, taking about 40 min for ASF and 20 min for AGS (Fig. 1), revealing a higher activity of AGS.

As very well described in literature, the use of carbon by heterotrophic bacteria takes place in two pathways: catabolism and anabolism. In catabolism, organic matter is oxidized to obtain energy, which is used to essential cellular processes (movement, respiration etc.) and cell synthesis (growth or anabolism). It is worth mentioning that during aerobic metabolism, although there is DO available in the medium, there are several compounds in the Krebs cycle which can be formed in the catabolic pathway for the cellular maintenance and cell synthesis before organic matter mineralization, thus consuming part of the influent COD without DO consumption (Zafiriadis et al., 2017).

As shown by Marais and Ekama (1976), aerobic condition has the coefficient of cellular yield (Y) of 0.45 g VSS/g $COD_{metabolized}$. Considering the conversion factor (fcv, ratio of volatile solids mass to COD) of 1.5 kg COD of the sludge/kg VSS, it is observed that Y*fcv = 1.5 kg COD of sludge/kg VSS * 0.45 kg VSS/kg $COD_{metabolized}$ = 0.67, i.e. 67% of the organic material is converted into cellular material through anabolism, the other fraction (1 – fcv * Y = 1–0.67 = 0.33) being the organic material catabolized.

In this sense, it was expected that the addition of 120 mg/L of soluble COD would lead to a consumption of approximately 40 mg/L of DO (1/3 of the total soluble COD, as explained before). It is emphasized that the respirometer evaluates the consumption of DO in the catabolic pathway (oxidation).

Comparing the DO consumption during the respirometric tests with the ASF (38.2 mg/L) and AGS (65.8 mg/L) biomasses, a higher consumption was found for AGS. Therefore, approximately 55% of the organic matter present was catabolized in AGS compared to 31.2% found for ASF, revealing the large difference between the biomasses in terms of metabolic pathways. From these results, it is possible to calculate the growth yield coefficient (Y), a parameter which expresses the relationship ΔXv/ΔSmet, in which ΔXv is the generated bacterial mass (volatile sludge mass), and ΔSmet is the metabolized COD mass. It seems that even though AGS is formed by aerobic microorganisms, they have similarity with anaerobic microorganisms in terms of COD flow towards catabolism (Fig. 2) (van Haandel and Lettinga, 1994).

Regarding the kinetic parameter yield coefficient (Y), values of 0.55 and 0.36 g VSS/g COD, were found for ASF and AGS, respectively, showing a much higher sludge production of ASF and the importance of feast/famine periods for lowering sludge production in AGS systems. The Y values found for ASF and AGS are according to the literature (Table 2). Even though a lower yield coefficient (Y) was found for AGS, the value was still higher compared to the range of 0.05 to 0.15 g VSS/g COD reported for anaerobic systems (van Haandel and Lettinga, 1994).

The maximum specific microbial growth rate ($\mu_{máx}$) also presented different values among the biomasses studied, reaching values of 3.5 and 0.9 d^{-1} for ASF and AGS, respectively. The values found are in the range of 2–10 d^{-1} reported for AS flocs (Al-Malack, 2006; Mardani et al., 2011) and 0.5–1.5 d^{-1} for aerobic granules (Liu et al., 2016), therefore justifying the lower sludge production observed in AGS systems.

The decay constant (k_d) was another parameter which presented remarkable differences, reaching values of 0.4 and 0.1 d^{-1} for ASF and AGS, respectively. Such a result might be related to the higher amount of organic material stored and adsorbed on the aerobic granules, thus reducing the decay rate.

A summary of the kinetic parameters values related to aerobic organic matter oxidation, which are important to the design and system optimization, are shown in Table 2.

3.3. Dynamics and biokinetic parameters in the nitrification

The respirometric tests conducted to evaluate nitrification provided quite different and interesting results. Based on the nitrification stoichiometry, the amount of nitrogen added (5 mg/L N-NH_4^+) would consume about 22.88 mg/L of O_2 for the complete nitrification (about 4.6 mg O_2/mg N). The oxygen consumption found was 21.1 and 16.3 mg O_2/L for the ASF and AGS, respectively, representing that 92% of ammoniacal nitrogen was completely nitrified in R1 and 70% was completely nitrified in R2.

Considering that the nitrification test was carried out with DO between 1 and 3 mg/L, there was an expectation of complete nitrification without the occurrence of denitrification, as it is known that DO concentrations higher than 1 mg/L inhibit denitrification (Metcalf and Eddy, 2003). However, it was observed that the reason for the lower oxygen demand for nitrification in R2 was the occurrence of denitrification, as the granules had an average size of 2 mm and possibly different layers (aerobic and anoxic). The occurrence of denitrification even with an aerobic condition outside the granule is a mechanism known as SND – simultaneous nitrification and denitrification (He et al., 2018; Long et al., 2016).

The growth rates (μ) of AOB and NOB were higher in ASF than in AGS, i.e. 0.5 d^{-1} and 0.3 d^{-1} for AOB, and 0.6 d^{-1} and 0.2 d^{-1} for NOB. The results show that not only heterotrophic but also nitrifying bacteria have a growth rate reduced in AGS, which also contributes to the lower sludge production found for this system (Table 3). According to Lema and Martinez (2017), partial nitrification can reduce the sludge

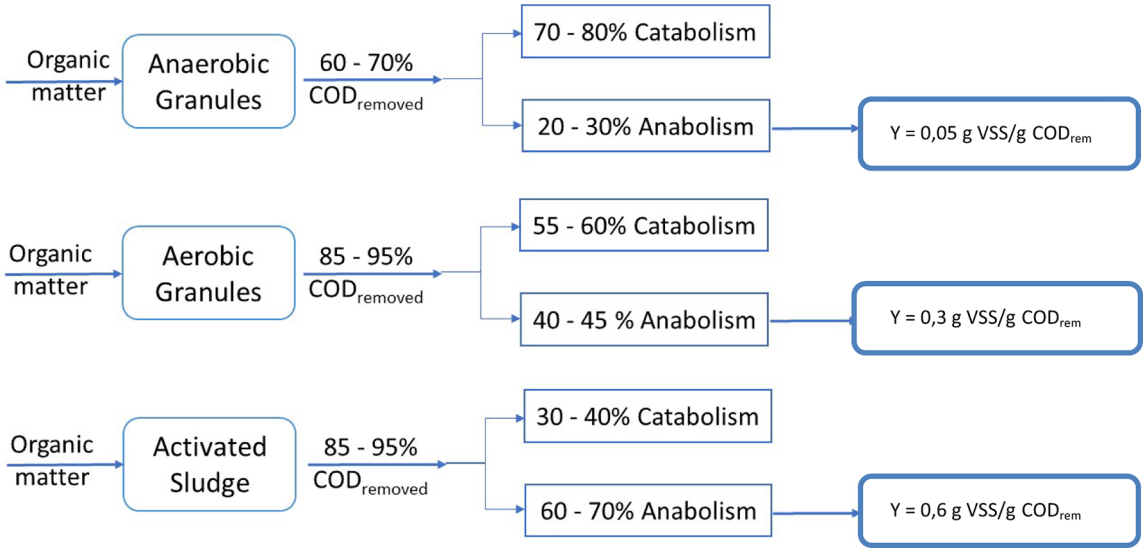

Fig. 2. Comparison of catabolic and anabolic fractions in different biomasses.

Table 2
Parameters related to OHO bacteria in activated sludge floc (ASF) and aerobic granular sludge (AGS).

Symbol	Parameter	Biomass	Substrate	System	Value	Reference
Y_H (kg VSS/kg COD)	Yield coefficient	ASF	Acetate	Lab	0.61	Barneto et al. (2009)
			Glucose	Lab	0.62	Al-Malack (2006)
			Domestic wastewater	Full-scale	0.62	Mardani et al. (2011)
			Acetate	Lab	0.55	This work
		AGS	Acetate	Lab	0.20	Liu et al. (2016)
			Domestic wastewater	Lab	0.33	Awang and Shaaban (2016)
			Domestic wastewater	Full	0.27	Pronk et al. (2015)
			Citrus wastewater	Lab	0.30	Corsino et al. (2018)
			Acetate	Lab	0.36	This work
$\mu_{máx}$ (d^{-1})	Maximum specific microbial growth rate	ASF	Acetate	Lab	6–10	Al-Malack (2006)
			Domestic wastewater	Full-Scale	2–5	Mardani et al. (2011)
			Acetate	Lab	3.5	This work
		AGS	Acetate	Lab	0.5–1.5	Liu et al. (2016)
			Domestic wastewater	Lab	0.4–1.0	Awang and Shaaban (2016)
			Citrus wastewater	Lab	0.6–1.0	Corsino et al. (2018)
			Acetate	Lab	0.9	This work
k_d (d^{-1})	Decay coefficient	ASF	Domestic wastewater	Full-Scale	0.2	Mardani et al. (2011)
			Acetate	Lab	0.3	Al-Malack (2006)
			Acetate	Lab	0.4	This work
		AGS	Glucose	Lab	0.05	Liu et al. (2005)
			Domestic wastewater	Lab	0.1	Awang and Shaaban (2016)
			Acetate	Lab	0.1	This work

Table 3
Parameters related to nitrifying bacteria in activated sludge floc (ASF) and aerobic granular sludge (AGS).

Symbol	Parameter	Group	Biomass	Value	Reference
μ_{max} (d^{-1})	Maximum growth rate constant	AOB	ASF	0.25–0.75	Chandran et al. (2008)
				0.4–0.8	Beltran (2008).
				0.5	This work
			AGS	0.1	Fang et al. (2009)
				0.3	Reino et al. (2016)
				0.3	This work
		NOB	ASF	0.2–0.9	Grady and Lim (1980)
				1.0	Beltran (2008)
				0.6	This work
			AGS	0.3	Fang et al. (2009)
				0.2	This work
Y (g VSS/g N)	Yield coefficients	AOB	ASF	0.2	Wiesmann (1994)
				0.2	This work
			AGS	0.1	Fang et al. (2009)
				0.1	de Kreuk et al. (2007)
				0.1	This work
		NOB	ASF	0.057	Wiesmann (1994)
				0.05	This work
			AGS	0.03	Fang et al. (2009)
				0.05	Vadivelu et al. (2006)
				0.02	This work

Table 4
Specific uptake rates (mg/L·h) for denitrification in activated sludge floc (ASF) and aerobic granular sludge (AGS).

VSS (g/L)	Donor	Acceptor	Biomass	Value	Reference
2	Acetate	NO_3^-	ASF	98	This work
			AGS	114	
		NO_2^-	ASF	39	
			AGS	73	
	Endogenous (storage material)	NO_3^-	ASF	8	
			AGS	25	
		NO_2^-	ASF	2	
			AGS	16	
1.2	Acetate	NO_3^-	ASF	58	Turk and Mavinic (1987)
		NO_2^-		36	
1.5	Glucose	NO_3^-	ASF	99	Chung and Bae (2002)
		NO_2^-		23	

yield in 33% in comparison with complete nitrification. Moreover, because ASF is cultivated with constant aeration, differently from AGS, which has anaerobic and anoxic periods, nitrifying bacteria growth can be favored in ASF systems (van Haandel and van der Lubbe, 2012). It is worth mentioning that, even though nitrifying bacteria has a low growth rate, sometimes 10-fold slower when compared to heterotrophic microorganisms, they can still contribute to produce excess sludge, especially in effluents rich in ammonia (Mota et al., 2005).

3.4. Dynamics and biokinetic parameters in the denitrification

The specific uptake rates for denitrification via nitrite or nitrate (Table 4) were evaluated in two situations for ASF and AGS biomasses: exogenous denitrification (having acetate as electron donor) and endogenous denitrification (using the intracellular stored material).

It was observed that nitrate consumption rate was similar to oxygen consumption rate (in the presence of organic matter) in both systems (Table 4). However, a reduction in the consumption rate was obtained when nitrite was the electron acceptor, especially in the ASF system.

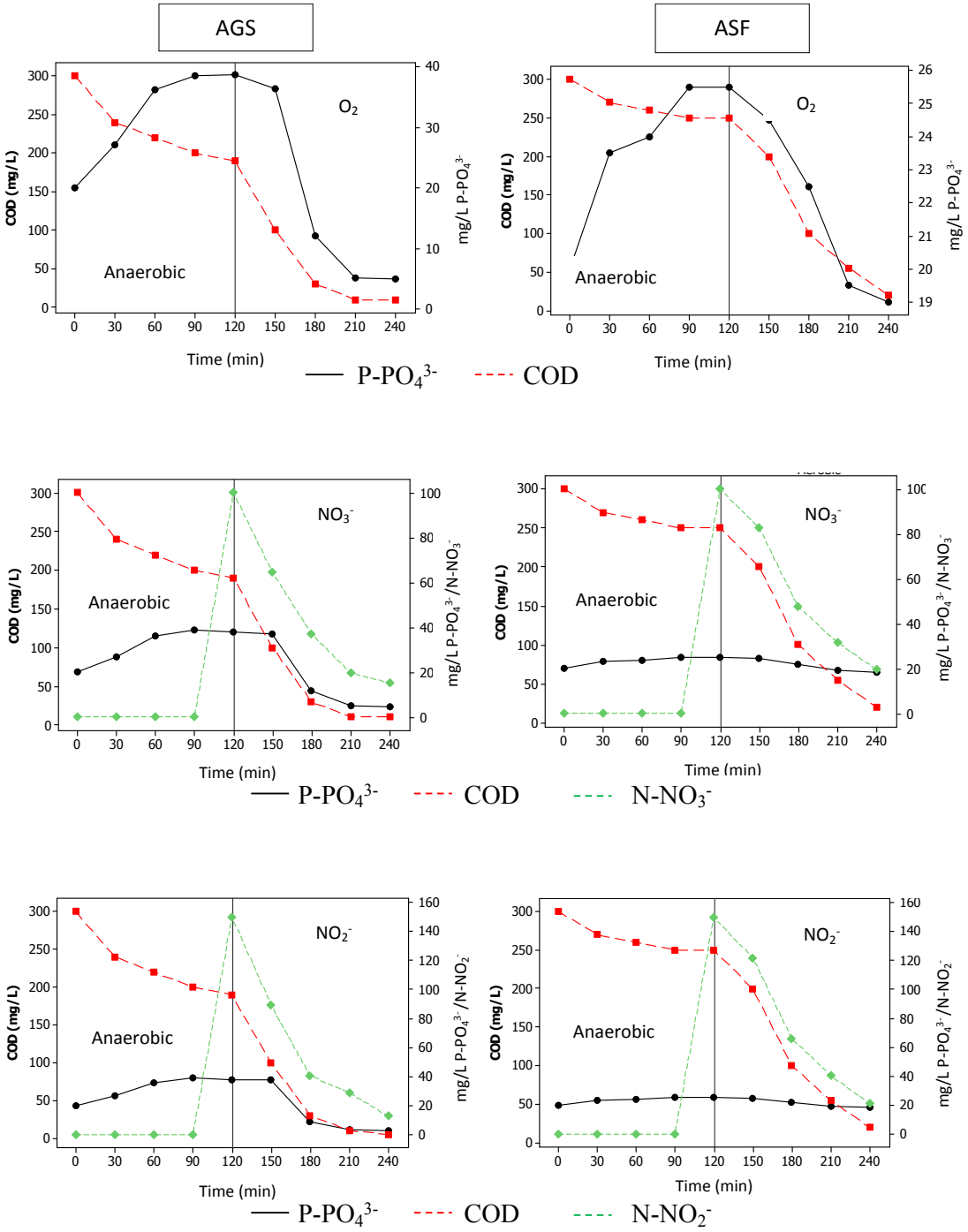

Fig. 3. Dynamics and biokinetic parameters in the phosphorus removal in activated sludge flocs (ASF) and aerobic granular sludge (AGS) with different electron acceptors.

This result was expected because the energy produced upon O_2 reduction ($\Delta G^\circ = -402$ KJ/per reaction) is close to that produced during nitrate (NO_3^-) reduction ($\Delta G^\circ = -398$ KJ/per reaction), resulting in similar consumption rates. Nitrite (NO_2^-) reduction has a lower energy ($\Delta G^\circ = -355$ KJ/per reaction) and demands only 3 electrons per ion, while nitrate needs 5 electrons per ion, justifying the low consumption rates. For both cases, it is also important to consider the affinity of the microbial community to the compounds.

Regarding endogenous denitrification (absence of extracellular organic matter), the differences in the denitrification rates were even higher. While, in ASF, the values were 8 mg/L·h (nitrate) and 2 mg/L·h (nitrite), in AGS, they were 25 mg/L·h (nitrate) and 16 mg/L·h (nitrite). In this context, some studies have shown high endogenous denitrification rates in AGS systems using EPS and SMP as electron donors, especially during the famine period (Yang et al., 2017; Rollemberg et al., 2019).

3.5. Kinetic parameters of polyphosphate-accumulating organisms

The kinetic parameters of phosphorus removal for ASF and AGS were determined with different electron acceptors (oxygen, nitrite and nitrate). The reason to use nitrite and nitrate instead of O_2 is due to recent research which shows the existence of microorganisms (DPAOS) which perform simultaneously denitrification and dephosphatation (He

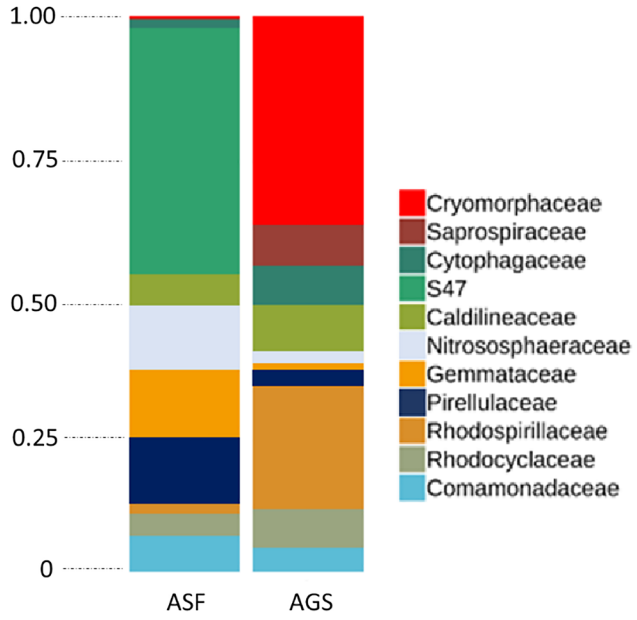

Fig. 4. Taxonomic affiliation at family level of the activated sludge flocs (ASF) and aerobic granular sludge (AGS).

et al., 2018).

AGS had better results in terms of luxury uptake of phosphorus with all electron acceptors tested (Fig. 3), especially using nitrite. DPAOs have a metabolism which is favored when nitrite is the electron acceptor during the competition with DGAOs and a lower sludge production compared to DOHO (Bassin et al., 2012).

The mean phosphorus release (anaerobic period) in ASF and AGS were 3.7 and 12.5 mg/L·h, respectively, therefore showing a 3-fold increase in the phosphorus release in AGS. The luxury uptake of phosphorus in the presence of oxygen was 5 and 31.3 mg/L·h in ASF and AGS, respectively.

When nitrite and nitrate were used, almost no luxury uptake of phosphorus in ASF was observed, suggesting low concentration or absence of DPAOs. On the other hand, AGS had a higher luxury uptake rate capacity than ASF when alternative acceptors were used, especially nitrite (Fig. 3). These results show that AGS is more capable to remove phosphorus, mainly due to the capacity of the cultivated biomass to accumulate PHA (Nancharaiah and Reddy, 2018).

Another interesting finding was the phosphorus concentration in the sludge, with values close to 0.05 and 1.4 mg P/mg VSS for ASF and AGS, respectively, indicating that the aerobic granules presented almost 30-times more phosphorus in the sludge.

The results regarding phosphorus removal showed that, although AGS systems have lower sludge production, they can still remove more phosphorus than ASF. The explanation for this greater removal can be attributed to the high presence of PAOs in the AGS. The typical phosphorus content found in activated sludge treatment with heterotrophic bacteria is 1.5 to 2.0% (of dry weight), in some cases up to 2.5%. However, in PAOs-rich sludge, a higher fraction of phosphorus (20–30% of dry weight) is observed due to the ability of these bacteria to accumulate phosphorus (Metcalf and Eddy, 2003).

3.6. Microbiology

Even though the systems had the same inoculum, the relative abundance of family was completely different (Fig. 4) in AGS and ASF, showing that granules formation favors the growth of certain groups of microorganisms and disappearance of others.

Fig. 4 shows that the most dominant groups of microorganisms in ASF and AGS are S47 and Cryomorphaceae, respectively. The S47 family is a large group of heterotrophic bacteria, while the Cryomorphaceae is a family of bacteria in the order Flavobacteriales. The Cryomorphaceae is related to fermentative microbial groups (EPS and PHA producers) and denitrifying bacteria group (McIlroy et al., 2017). These results justify the higher sludge production in R1 and higher EPS production and denitrification achieved in R2.

Comamonadaceae and Rhodocyclaceae were found in both systems. However, an increased abundance of Comamonadaceae and Rhodocyclaceae were observed in AGS and ASF, respectively. Most of the species in these groups are related to ordinary heterotrophic organism (OHO) and ordinary heterotrophic denitrifying organism (OHDO), i.e. bacteria without capacity to store PHA (Willems, 2014).

The Rhodospirillaceae was more abundant in AGS than in ASF. Species of this family are related with DGAOs, therefore they have an important role in denitrification through the competition with OHDO for substrate. Additionally, DGAOs have lower growth rate compared to OHDO, which leads to a lower sludge production (Weissbrodt et al., 2013). The high abundance of Rhodocyclaceae in ASF and of Rhodospirillaceae in AGS shows that denitrification in the systems took place in different pathways with the participation of several microorganisms with different growth rates, which may have contributed to the lower Y values found in AGS.

Pirellulaceae, Gemmataceae, and Nitrososphaeraceae families are reported to be related with nitrification (Connan et al., 2017). All of them were present in a higher abundance in ASF compared to AGS, and actually Gemmataceae was absent in AGS. As observed in kinetic parameters, ASF had higher nitrification rates, which can be related to the greater abundance of these families.

Cytophagaceae and Saprospiraceae families are related to phosphorus storage and EPS production (Begum and Batista, 2013), besides some genus can make dephosphatation with nitrite and nitrate as the electron acceptors (simultaneous dephosphatation and denitrification). Both families were found in high abundances in the AGS. On the other hand, only a small presence of Cytophagaceae and absence of Saprospiraceae were found in ASF.

These results justify the better phosphorus removal in AGS. The absence of Saprospiraceae in ASF may justify the lack of phosphorus luxury uptake using nitrite and nitrate as electron acceptors, as many species of this family are DPAOs (Ouyang et al., 2017).

4. Conclusion

Although the systems were inoculated with the same sludge and fed with the same substrate, significant differences were observed between the biokinetic parameters of ASF and AGS in terms of organic matter and nutrients removal. AGS presented a lower sludge production and a higher endogenous consumption rate than ASF, although the nitrification rates were higher in ASF. The activity of phosphorus-accumulating bacteria (PAOs and DPAOs) was remarkably higher in AGS. Remarkable differences in terms of microbial diversity were found, possibly due to the different selection pressures imposed, which influenced the size, EPS content, and activity of the granules.

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