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Stability of Aerobic Granular Biomass Treating the Effluent from A Seafood Industry

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ABSTRACT: The aerobic granular systems represent a good alternative to substitute the conventional activated sludge process in the treatment of industrial effluents due to the lower surface requirements. In this work the effluent from a seafood industry, characterized by a high variability and the presence of residual amounts of coagulant and flocculant reagents, was used to study the development of aerobic granular biomass and its stability. In a first stage with OLRs between 2 and 5 kg $COD_S/m^3 \cdot d$ the development of aerobic granular biomass was promoted with good physical properties: SVI of 35 mL/g TSS, density of 60 g $VSS/L_{granule}$ and average diameter of 2.8 mm. In a second stage the continuous change in the OLR applied from 3 to 13 kg $COD_S/m^3 \cdot d$, to simulate the real conditions of the industry, showed that the removal of organic matter was not affected (90%) but the aerobic granules disintegrated. The maximum OLR treated in the system without granules disintegration was around 4.4 kg $COD_S/m^3 \cdot d$. The nitrogen removal was 30% (for biomass assimilation) and the maximum ammonia removal was around 65% and depending on the solids retention time, the free ammonia concentration and the average granule diameter.

Key words: Aerobic granule, Bioreactor, Industrial wastewater, Nitrogen, Organic matter

INTRODUCTION

The uncontrolled discharges of urban and industrial wastewater without treatment suppose an environmental problem. The choice of the adequate treatment is conditioned by many factors, but generally the capital and operational costs are some of the most important ones. For this reason treatment systems are required not only to be able to eliminate the pollution but also to be economically viable. In this sense aerobic granular systems can be an interesting technology because their surface requirement and sludge production are lower than those of the conventional activated sludge (AS) systems (de Bruin *et al.*, 2004; Campos *et al.*, 2009a).

The low footprint of the aerobic granular technology is related to the good settleability of aerobic granules which allows obtaining high biomass concentrations inside the system to operate at high loading rates and working without the necessity of a secondary settler (Beun *et al.*, 1999). Moreover, due to the stratification of microbial populations inside the granule, the simultaneous removal of organic matter, nitrogen and phosphorus can be achieved in a single unit (de Kreuk *et al.*, 2005). In comparison with the conventional AS the yield of the biomass in aerobic granular systems is lower which would also contributed to the decrease of the operating costs (Campos *et al.*, 2009b).

All these advantages make the aerobic granular technology as a good option to treat industrial wastewaters. However this type of effluents is characterized by high loading rates and a variable composition, which could affect the stability of aerobic granules. Different studies showed that the maximum applicable loading rate in an aerobic granular system is limited and depended on the type of substrate. On Table 1 is presented a summary of different works with aerobic granular biomass for the treatment of industrial and synthetic wastewaters at high organic loading rates (OLR). In the most of the cases when the maximum capacity of the system was reached it led to granules instability. The explanation of this instability differ in the literature, Liu and Liu (2006) attributed it to the overgrowth of filamentous microorganism and Zheng *et al.* (2006) to an intracellular protein hydrolysis and degradation at the anaerobic granule core; Adav *et al.* (2010) demonstrated that under a high OLR the microorganims lost their capability for autoaggregation due to a reduction in the quantity of protein secreted. Which seems clear is that each type of substrate has a maximum OLR that can be treated in the system without to affect the granule stability, but also another

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important point is to know how is the capacity of the system to recover the granulation when an instability episode occurs. The most of the works (Table 1) were performed with a progressive increase in the load treated while the effect of a continuous variation in the load applied was not study, being the interest in some industries due to their mode of operation like the seafood industry, that generates effluents with different composition depending on the product that are processing (Ferjani *et al.*, 2005; Vandanjon *et al.*, 2002). Furthermore the presence of residual concentrations of coagulant and flocculant reagents, normally used in the physical-chemical treatment before the biological one, affects the properties of the biomass forming fluffy and filamentous granules (Val del Río *et al.*, 2012) which can led to granulation instability (Liu and Liu, 2006).

The objective of this work is to study the feasibility of the use of an aerobic granular system to treat an industrial effluent produced in a seafood industry and characterized by a high variability on its organic content and the presence of residual amounts of coagulant and flocculant reagents from the previous physical-chemical treatment. Special attention will be paid to the characteristics of the granular biomass, stability and organic matter and nitrogen removal when the reactor was submitted to suddenly variations in the loading rate treated.

MATERIALS & METHODS

A SBR with a total volume of 2.7 L and a working volume of 1.8 L was used. The dimensions of the unit were: height of 480 mm and inner diameter of 85 mm. The H/D ratio was of 5.6. Oxygen was supplied to reactor by means of air spargers to promote the formation of small air bubbles. A set of two peristaltic pumps was used to feed and to discharge the effluent, respectively. The influent was introduced through a port located at the top of the reactor. The effluent was discharged through the sampling port placed at medium height of the column reactor and the exchange volumetric ratio was fixed at 50%. The reactor was operated at room temperature (15-20 ºC) and the dissolved oxygen concentration was between 4 and 8 mg O_2/L. The cycle of operation was of 3 hours distributed as follows: 3 minutes of feeding, 171 minutes of aeration, 3 or 1 minute of settling and 3 or 5 minutes of effluent withdrawal. The hydraulic retention time was kept at 0.25 d. A programmable logic controller (PLC) Siemens model S7-224CPU controlled the actuations of the pumps and valves and the length of every operational period comprising the cycle. The system was fed with the effluent from a seafood industry which was pre-treated in an air floatation unit to remove thick solids and fats by addition of coagulant and flocculant reagents. The wastewater was stored at 4 ºC prior to be fed to the SBR. The industrial wastewater was characterized by a wide variability of its composition due to the different products processed in the plant. Due to this variability the reactor was operated in two different stages (Table 2). The Stage I (days 0-295) corresponded to the start up when the formation of aerobic granular biomass and its evolution were studied, along this period the OLR was maintained between 2 and 5 kg $COD_S/m^3\cdot d$. On Stage II (days 296-330) a study of the aerobic granules stability, to the effluent variability, was performed with an OLR between 3 and 13 kg $COD_S/m^3\cdot d$. The SBR was inoculated with 500 mL of AS from the biological reactor operated in the own seafood industry, characterized by a sludge volumetric index (SVI) of 125 mL/g TSS and a solids concentration of 3.21 g VSS/L. The pH, conductivity, ammonia, nitrate, nitrite, phosphate, total suspended solids (TSS), volatile suspended solids (VSS) and SVI were determined according to the Standard Methods (APHA-AWWA-WPCF, 2005). Chemical Oxygen Demand (COD) was determined by a semi-micro method (Soto *et al.*, 1989); total COD (COD_T) was measured directly in the sample and the soluble COD (COD_S) from the sample filtered through 0.45 µm pore size filters. The protein content was determined by the Folin-Lowry method (Lowry *et al.*, 1951). The morphology and size distribution of the granules were measured regularly by using an Image Analysis procedure (Tijhuis *et al.*, 1994) with a stereomicroscope (Stemi 2000-C, Zeiss). Biomass density, in terms of g VSS per litre of granules, was determined with dextran blue and following the methodology proposed by Beun *et al.* (1999). The biomass yield and the amount of nitrogen assimilated for biomass growth were estimated according to Mosquera-Corral *et al.* (2005).

RESULTS & DISCUSSION

During the first sixteen days of operation the settling time in the reactor was fixed at 3 min which supposed that only biomass with a settling velocity higher than 3.2 m/h was retained in the system. Then it was changed to 1 min to promote a better washout of flocculent biomass with a settling velocity lower than 9.5 m/h. After 21 days of operation the formation of the first aggregates with a filamentous surface (Fig. 1.a) was observed, that presented an average diameter of 1.8 mm, a SVI around 125 mL/g TSS and a density of 15 g $VSS/L_{granule}$ (Fig. 2). Then the diameter of these aggregates progressively increased to 5.4 mm around day 90, while the SVI and density varied slightly. These aggregates were not stable and gradually disappeared to give rise to granular biomass with a smooth surface and compact structure (Fig. 1.b), a lower average diameter (2.8 mm) and good settling properties (SVI of 35 mL/g TSS and density of 60 g $VSS/L_{granule}$) around

Table 1. Performance of some aerobic granular reactors with high OLRs

Type of wastewater	Granulation (days-OLR)	OLR_{max} (kg COD/ m^3 d)	NLR (kg N/ m^3 d)	COD_{rem} (%)	N_{rem} (%)	SVI (mL/g VSS)	D_{feret} (mm)	Granules stability	Ref.
Glucose	21 – 6.0	15.0	-	92	-	31	3.3	entire	Moy et al. (2002)
Acetate	21 – 6.0	9.0	-	97	-	42	4.2	disintegration	Moy et al. (2002)
Sucrose	30 – 6	6.0	-	96	-	50	10.0	disintegration	Zheng et al. (2006)
Acetate	15 – 16.7	21.3	-	95	-	40	4.0	disintegration	Adav et al. (2010)
Dairy products	21 – 1.0[a]	7.0	0.7	90	70	60	3.5	entire	Arrojo et al. (2004)
Dairy plant	105 – 5.9	5.9	0.28	90	80	50	-	filamentous outgrowth	Schwarzenbeck et al. (2005)
Soybean-processing	20 – 6.0	6.0	0.3	98.5	-	26	1.2	entire	Su and Yu (2005)
Winery	40 – 2.7[a]	6.0	0.01	95	-	-	2.0	entire	Lopez-Palau et al. (2009)
Pig farm	10 – 2.2	7.3	0.96	91	-	72	5.2	filamentous /disintegration	Figueroa et al. (2011)
Palm oil mill	110 – 3.0	6.0	-	90	-	21	4.0	disintegration	Gobi et al. (2011)

[a] Granulation with synthetic media

Table 2. Composition of the feeding in the different stages

Stage	Days	COD_T (mg/L)	COD_S (mg/L)	NH_4^+-N (mg/L)	PO_4^--P (mg/L)	TSS (mg/L)	VSS (mg/L)	Conductivity (mS/cm)	pH
IA	0-90	902 ± 239	785 ± 194	74 ± 25	27 ± 5	59 ± 40	54 ± 41	4.0 ± 2.4	6.7 ± 0.3
IB	91-130	1076 ± 250	931 ± 189	112 ± 27	33 ± 7	122 ± 98	109 ± 87	4.6 ± 1.4	6.7 ± 0.6
IC	131-180	476 ± 96	462 ± 88	56 ± 11	16 ± 4	39 ± 11	30 ± 11	1.8 ± 0.3	6.9 ± 0.1
ID	181-296	870 ± 172	785 ± 183	90 ± 22	50 ± 10	63 ± 22	55 ± 20	2.9 ± 0.7	6.9 ± 0.2
IIA	296-303	2538 ± 506	1775 ± 278	253 ± 113	37 ± 5	93 ± 10	80 ± 8	4.7 ± 0.8	6.7 ± 0.1
IIB	304-315	958 ± 114	796 ± 85	96 ± 11	28 ± 6	90 ± 17	80 ± 13	2.4 ± 0.2	6.6 ± 0.1
IIC	316-321	3262 ± 548	2808 ± 562	250 ± 81	42 ± 9	190 ± 34	160 ± 28	6.1 ± 0.1	6.6 ± 0.1
IID	322-330	871 ± 193	662 ± 182	102 ± 19	7 ± 11	48 ± 19	47 ± 15	2.5 ± 0.2	6.7 ± 0.1

day 170 of operation. The biomass concentration in the reactor was between 1 and 2 g VSS/L until day 130 of operation (Stages IA and IB) and the effluent had a high amount of solids, around 0.3-0.7 g VSS/L (Fig. 3). From day 130 of operation the compact aerobic granules predominated inside the reactor and the solids began to accumulate until reaching values of 11.8 g VSS/L. Accordingly, the concentration of solids in the effluent decreased to 0.1 g VSS/L. The food-to-microorganism (F/M) ratio before the granulation process was maintained in values over 1 g COD_s/g VSS·d (Fig. 3), but when the aerobic granules predominated in the system and the solids concentration increased the F/M ratio decreased and was maintained between 0.3-0.6 g COD_s/g VSS·d (Stages IC and ID). On Stage II, due to the increase in the OLR and the reduction in the solids concentration, this ratio reached values between 1 and 2.5 g COD_s/g VSS·d, which coincided with a worsening in the physical properties of the aggregates (Fig. 2). These results are in accordance with other authors who observed that when the F/M ratio was over 1 g COD/g VSS·d the biomass granulation not occur, being the adequate ratio for aerobic granulation around 0.5 g COD/g VSS·d (Yang *et al.*, 2008; Jungles *et al.*, 2011). Aerobic granules with good settling properties were obtained from day 130 and at OLR around 2 kg COD/m^3·d. The time required to obtain the granulation is belonged with the type of substrate used to feed the reactor (Table 1) and the longer times were observed for industrial wastewaters (Schwarzenbeck *et al.*, 2005; Gobi *et al.*, 2011), although it is necessary to be pointed that the definition of mature aerobic granules may vary in the studies listed in Table 1. In the present work the composition of the wastewater varied widely in the different collected batches due to the different products processed in the industry (prawn, squid, hake, etc.) and this affected the evolution of the characteristics of the aerobic granules. Despite that the first aggregates were observed since day 21 of operation, it was from day 130, with a new batch of wastewater and a lower OLR applied (from 4 to 2 kg COD/m^3·d, Fig. 4), when a clear improvement on the settling characteristics of the aerobic granules was observed (Fig. 2).

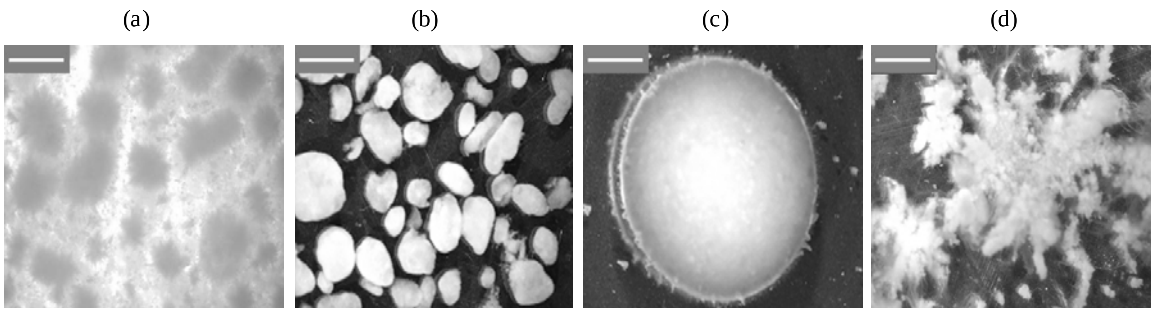

Fig. 1. Images of the granular biomass on day 21 (a), 169 (b), 217 (c) and 263 (d) of operation. The size bar corresponds to 3 mm

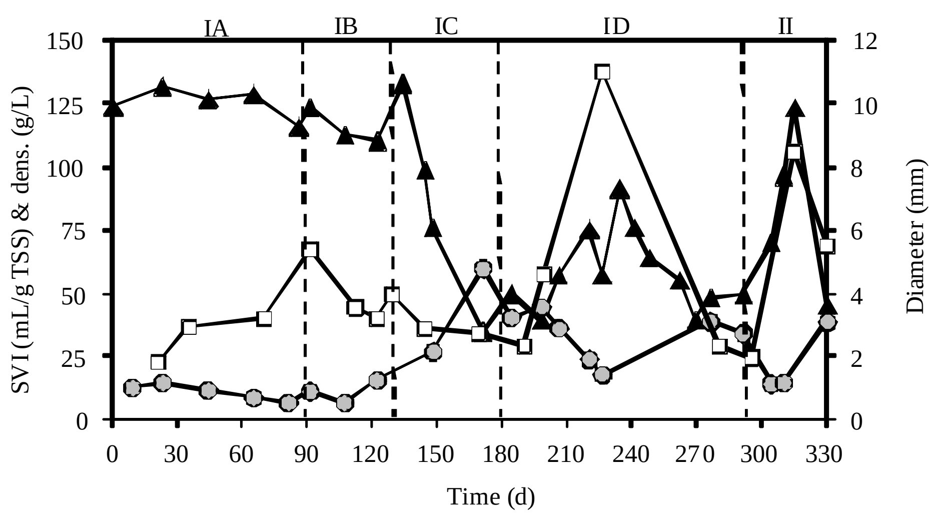

Fig. 2. Evolution of the SVI (mL/g TSS) (Δ), diameter (mm) (□) and density (g VSS/$L_{granules}$) (○) of the granules along the different stages

Until day 180 of operation the granules properties were similar to those obtained by others authors working with industrial wastewater (Arrojo *et al.*, 2004; Schwarzenbeck *et al.*, 2005; Figueroa *et al.*, 2008) and the size was kept between 2 and 3 mm. But from day 180 (Stage ID), coinciding with a new change of the feeding, the granules began to grow disproportionately (Fig. 4.c) reaching on day 226 an average diameter of 11 mm and a few granules a maximum value of 17 mm. This increase in size which took place in only few days could be related to an increase of the residual levels of coagulant-flocculant reagents in the feeding (Guo *et al.*, 2010) due a failure in the mixing system of the pre-treatment unit from the seafood industry. This size increment led to a worsening of the settling properties of the biomass: the SVI increased up to 91 mL/g TSS and the density diminished to 18 g VSS/$L_{granule}$. Toh *et al.* (2003) also observed that the density started to decrease when the size reached a certain limit (4 mm of diameter) and that the bigger granules possessed higher SVI because their packing is less effective in a column than the smaller size ones. In the present work the high size of the granules was observed to produce a bad packing of the aggregates and consequently after settling the biomass could reach the level of the effluent port. For this reason a purge of biomass was performed and a decrease in the VSS concentration inside the reactor was observed on day 200 of operation (Fig. 3).

Around day 240 due to their large size, the granules started to break up into small pieces and subsequently

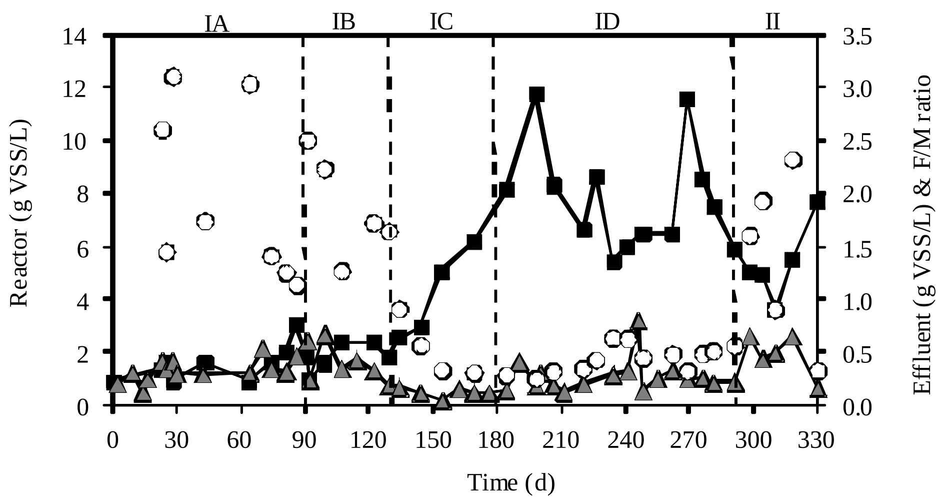

Fig. 3. Concentration of biomass (g VSS/L) inside the reactor (■) and in the effluent (▲) and F/M ratio (g COD_s/g VSS·d) (○) along the different stages

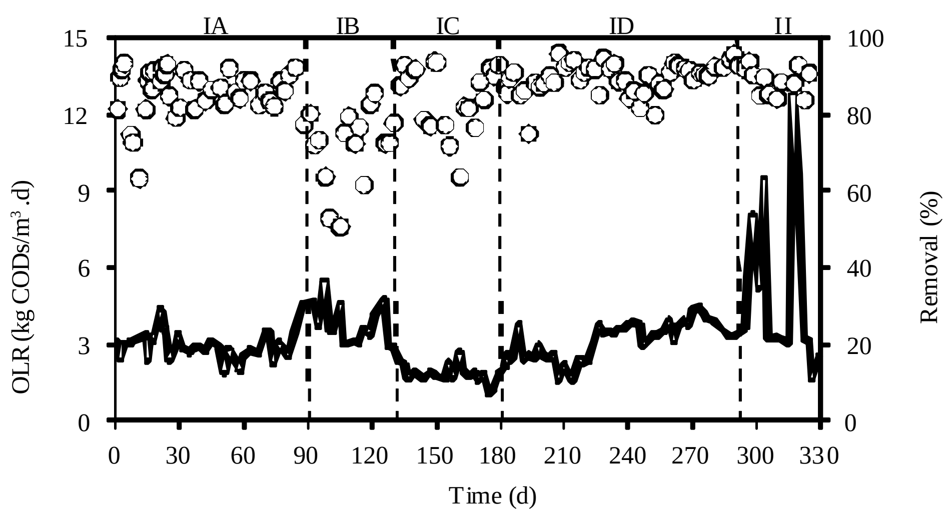

Fig. 4. Profile of OLR (—) and percentage of COD removal (○) on the different stages of operation

an increase of the solids concentration in the effluent until values around 0.8 g VSS/L was observed (Fig. 3). Zheng *et al.* (2006) observed also the disintegration of the aerobic granules when they reached a diameter of 16 mm and then the biomass was washed out with a consequent failure of the reactor. These authors explained that mass transfer limitations and the possible presence of anaerobic biomass inside the granules provoked this phenomenon. In this study the biomass left in the reactor (Fig. 1.d) served as inocula to the formation of new aggregates that started to form since day 270, leading to the increase in the biomass concentration (11.6 g VSS/L) until reached the level of the effluent port, so a new purge was performed in order to avoid the presence of high concentrations of solids in the effluent. The new granules presented on day 280 an average diameter of 2.4 mm, a SVI of 49 mL/g TSS and a density of 39 g $VSS/L_{granule}$. From day 296 to the end of the operation a variable OLR was applied to the system (Stage II) to simulate the real conditions of the wastewater production in the industry and to determine whether the aerobic granular system was capable to maintain stable operational conditions with fluctuating loads. Between days 290 and 295, with an OLR around 3.5 kg $COD_S/m^3\cdot d$, the granules had an average diameter of 2.0 mm, a SVI of 50 mL/g TSS and a density of 34 g $VSS/L_{granule}$. But with the increase of the OLR (9 and 13 kg $COD_S/m^3\cdot d$ around days 300 and 315, respectively) the granules started to grow in size until reaching 9.0 mm, which supposed a worsening in the settling properties (SVI of 124 mL/g TSS and density of 15 g $VSS/L_{granule}$). Again they broke up on day 320. But at the end of the operation, with a lower OLR (around 3 $COD_S/m^3\cdot d$), from the broken granules a new granulation process occurred again producing granules with an average diameter of 5.5 mm, SVI of 46 mL/g TSS and density of 39 g $VSS/L_{granule}$.

Therefore although along the operation the aerobic granular biomass lost its stability twice due to the excessive growth in size of the granules (first by the presence of higher residual levels of coagulant-flocculant reagents and second by the application of high and variable loads), the retained biomass in the system served as nucleus to form new aerobic granules in a few days and with good settling properties. One of the drawbacks observed was the increase in the solids concentration in the effluent when the granules broke, which can suppose a problem in the application of aerobic granular systems to the full scale plant. To avoid this aspect a selective purge can be applied when the granules reached certain size and before their disintegration. Also a filter after the withdrawal could be suitable to diminish the presence of solids in the effluent (Arrojo *et al.*, 2004).

The reactor was operated during 330 days. The OLR fed to the SBR during the first 90 days of operation (Stage IA) was around 3.0 kg $COD_S/m^3\cdot d$ (Fig. 4) with removal efficiency of 85% for COD_S. Then the applied OLR increased up to 4.5-5.0 kg $COD_S/m^3\cdot d$ due to a change in the feeding (Stage IB) and the efficiency of organic matter removal worsened to values of 50%, maybe due to an increase in the slowly or non biodegradable fraction of the organic matter. On day 130 with a new batch of industrial wastewater the OLR applied was of 2.0 kg $COD_S/m^3\cdot d$ (Stage IC) which supposed a reduction in the F/M ratio until values lower than 1 g COD/g VSS·d, the disappearance of flocculent biomass and the prevalence of granular biomass. Once the granules were mature the OLR in the influent was gradually augmented from 2.0 kg $COD_S/m^3\cdot d$ (day 180) to 4.4 kg $COD_S/m^3\cdot d$ (day 270), with a removal efficiency of 90% for COD_S (Stage ID). Results obtained in the present study were in accordance with Figueroa *et al.* (2008) who obtained removal efficiencies of 90-95% for COD_S treating a similar industrial effluent but at lower OLR (1.6 kg COD/$m^3\cdot d$).

In order to determinate if the aerobic granular system was capable of maintaining stable operational conditions with fluctuating loads (Stage II), the OLR was suddenly increased from 3 to 9 kg $COD_S/m^3\cdot d$ on day 296 (Stage IIA) and restored to the previous organic load on day 303 (Stage IIB). Again, the following week (Stage IIC), the organic load was increased up to 13 kg $COD_S/m^3\cdot d$. During this stability test, despite the decrease in the solids concentration and the increase up to 1 g COD/g VSS·d in the F/M ratio (Fig. 3), the removal of COD_S was kept similar to the rest of the operational period with values between 85% and 90%. Thereby the system was capable to maintain the removal efficiency of organic matter even when the industry produced variable effluents. Along the whole operation the NLR fed to the SBR varied between 0.2-0.6 kg NH_4^+-$N/m^3\cdot d$ on Stage I and 0.3-1.5 kg NH_4^+-$N/m^3\cdot d$ on Stage II (Fig. 5).

The removal efficiencies of total nitrogen (TN) and ammonia were also variable, with the maximum values of 30 and 65%, respectively. The estimation of the nitrogen used for growth was 30% of the TN fed, which indicated that the removal of nitrogen in the process was due to the assimilation by biomass and denitrification was negligible. In order to know the limitation in the ammonia removal process along the operation its percentage as a function of the solids retention time (SRT), the free ammonia (FA) concentration and the average granule diameter is

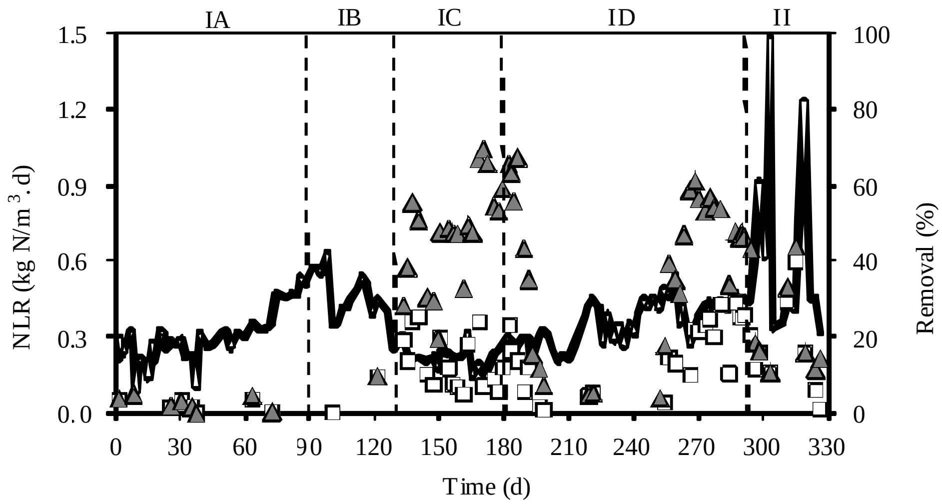

Fig. 5. Profile of NLR (—), percentage of TN removal (□) and NH_4^+ oxidation (▲)

presented on Fig. 6. It was found that the ammonia removed was limited for SRT lower than 4 days, FA concentrations higher than 4 mg N/L and average granule diameters higher than 3 mm. The minimum SRT necessary for the nitrification at the temperature of the process (22 ± 2 ºC) is, according with the literature, around 3-4 days (Salem *et al.*, 2003). Respect to the FA concentration Yang *et al*. (2004) found that the nitrification was completely inhibited at a concentration greater than 10 mg N/L and that the specific oxygen utilization rate of nitrifying bacteria was reduced by a factor 5 and 2.5 as the FA concentration increased from 2.5 to 39.6 mgN/L. De Kreuk *et al.* (2007) studied the effect of the granule size in the nitrogen removal and they observed that when the diameter was larger than 1.4 mm the removal efficiency started to decrease, being the optimal between 1.2 and 1.4 mm. In the present work the lowest granule sizes achieved were around 2 mm that coincides with the maximum ammonia and nitrogen removal efficiencies measured (Fig. 6c). To identify what variable limited the ammonia removal for each stage on Table 3 their values are presented. On Stage IA the ammonia oxidation did not occur and even the concentration in the effluent was higher than in the influent in some days due to the hydrolysis of the proteins present in the fed wastewater, as could be checked measuring their concentration in the influent (between 100 and 300 mg protein/L) and in the effluent of the reactor (between 10 and 50 mg protein/L). The low SRT and the slightly high FA concentration were the responsible of the ammonia removal absence. On Stage IB the SRT, FA concentration and average diameter were unfavourable. On Stage IC, coinciding with the granulation process, the ammonia removal percentage was around 60%. The higher biomass concentration achieved with the aerobic granular biomass (Fig. 3) supposed an increase in the SRT from 2 days (at the end of Stage IB) to 13 days (at the end of Stage IC), which favoured the retention of microorganisms with relatively slow growth rates, such as nitrifying bacteria, and promote the ammonia removal, besides the low FA concentration and average diameter. However the denitrification process was no favoured which resulted in the accumulation of NO_x^- compounds, therefore the TN removal was only around 20-30% on Stage IC (Fig. 5). Then, on Stage ID, as the aerobic granules increased in size the ammonia removal was worsening, the lower specific surface availability became limiting for oxygen transport and thus for the ammonia oxidation process (de Kreuk *et al.*, 2007) which led to a rise of the FA concentration. The ammonia removal process took place again from day 250, probably due to the breaking up of the previous granules and that the bacteria had access to the dissolved oxygen for ammonia oxidation.

On Stage II (stability test) the ammonia oxidation was around 15% when the NLR applied was high (1.5 NH_4^+-N/m^3·d) and around 40% when the NLR was low (0.3 NH_4^+-N/m^3·d), which indicated that the system had not capacity to treat variable NLRs. The decrease in the solids concentration inside the reactor at the beginning of the Stage II that implied a reduction in the SRT and the increase in the average particle size provoked the decrease in the ammonia removal efficiency. The profiles of different compounds concentrations were also analyzed during some operational cycles to determine how the different processes occurred in the system during the three hours of the cycle. As an example the profiles on the operational days 72 (Stage IA) and 290 (Stage ID) are shown on Fig. 7. An important aspect to obtain the

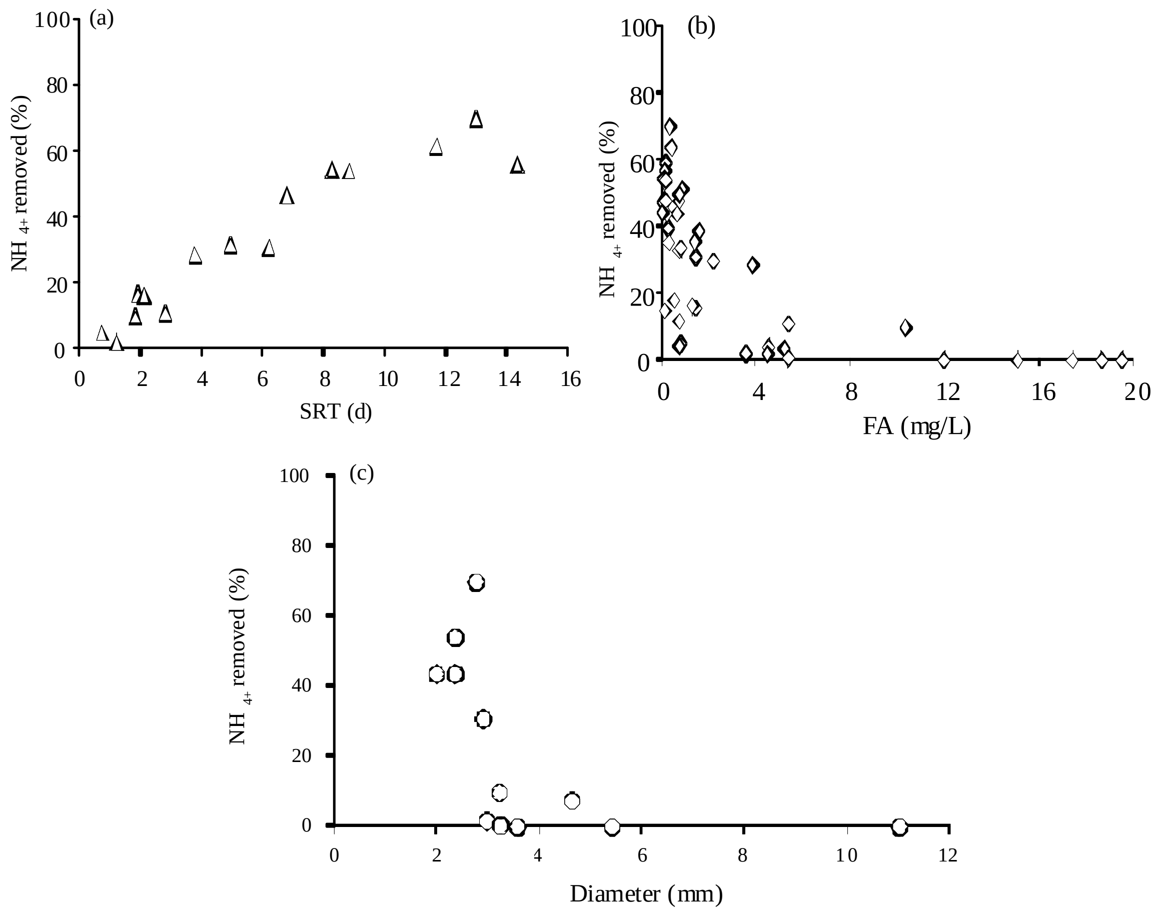

Fig. 6. Relation between NH_4^+ removed and SRT (△), **FA** (◊) and diameter (○)

Table 3. Parameters influencing the ammonia removal

Stage	SRT (d)	FA (mg/L)	Diameter (mm)
IA	1.1 ± 0.4	5.2 ± 2.7	2.7 ± 0.7
IB	1.4 ± 0.7	15.0 ± 5.1	4.0 ± 1.0
IC	3.8 – 13.0	1.1 ± 0.6	2.8 ± 0.1
ID	4.8 – 16.0	0.4 – 9.2	2.4 – 11.0
II	2.2 ± 0.4	0.2 – 5.4	2.0 – 8.5

granular biomass is the fact that the organic matter has to be degraded in the first minutes of the cycle, i.e. the feast period must be short. In this way the proliferation of filamentous micro-organisms is inhibited and the growth of bacteria capable to accumulate the organic substrate is favoured. During the famine period only the bacteria with stored compounds can grow and this strategy promotes the granulation process (Campos *et al.*, 2009a). When the process of granulation did not occur (day 72) the COD_S readily biodegradable took 60 minutes to be removed from the liquid phase (long feast period) and approximately a percentage of 30% of COD_S was not consumed probably due to the non-biodegradable fraction of the treated effluent. During this period of operation, the nitrification and denitrification processes did not occur so the ammonia concentration remained constant along the cycle and nitrite and nitrate did not appear. The dissolved oxygen concentration was around 6.5 mg O_2/L during the feast period and then increased to 7.5-8.0 mg O_2/L until the end of the cycle. On day 290 the biomass was in the form of aerobic granules and the profiles of the liquid compounds along the cycle measurement were different. The COD_S readily biodegradable only took 20 minutes to be eliminated, which implies a short feast period compared

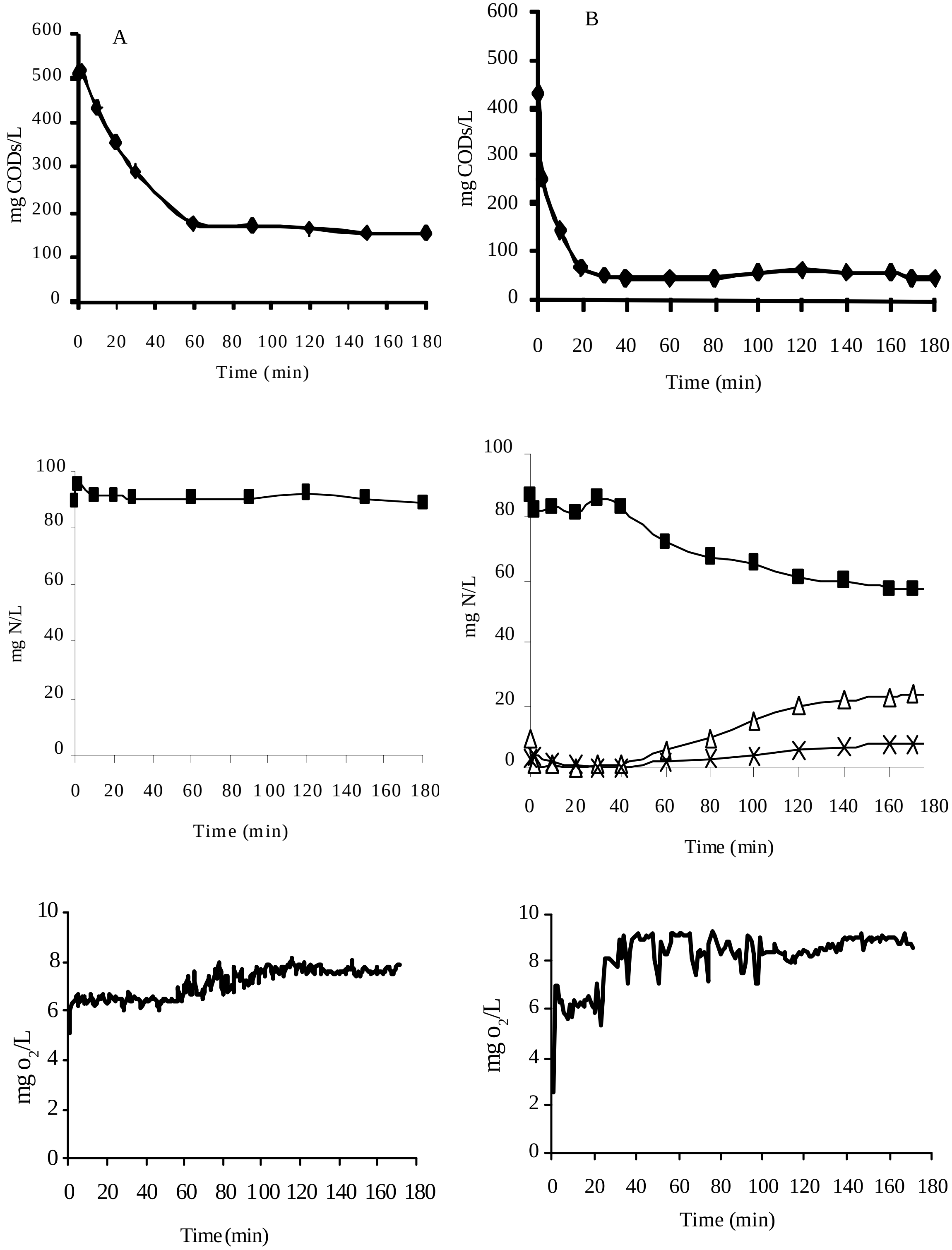

Fig. 7. Concentration profiles during an operational cycle of the SBR on day 72 (A) and 290 (B). Concentrations of soluble COD_s (♦), NH_4^+-N (■), NO_2^--N (△), NO_3^--N (×) and dissolved oxygen (-)

to the flocculent biomass, moreover the non-biodegradable fraction of the COD_S was lower (12%), therefore the reactor effluent presented better quality. The nitrification process occurred and ammonia was oxidised to nitrite, and nitrite to nitrate, during the aerobic period immediately after the disappearance of the biodegradable organic matter from the liquid phase. Part of the nitrite and nitrate accumulated at the end of the cycle were consumed via denitrification during the first minutes of the next cycle. The dissolved oxygen concentration was in the first minutes (feast period) around 6.3 mg O_2/L, and during the rest of the cycle (famine period) near the saturation value (8-9 mg O_2/ L). Aerobic granular systems are characterized by a lower biomass production compared to conventional AS systems and this fact is related to the higher sludge age achieved with aerobic granular biomass (Campos *et al.*, 2009b). In this study the yield of micro-organisms (*Y*) expressed in terms of gram of biomass produced per gram of COD consumed (g VSS/g COD) was calculated and represented as a function of the SRT (Fig. 8). When the flocculent biomass was dominant inside the reactor (days 0-130) the estimated yield was between 0.45 and 0.65 g VSS/g $COD_{removed}$, which corresponded with SRT values around 1-2 days. These results are comparable with those from conventional AS systems with typical growth yields of 0.4-0.6 g VSS/ g $COD_{removed}$ (Droste 1996). Since granulation process occurred the yield decreased down to 0.30 g VSS/g $COD_{removed}$ for SRT values higher than 4 days, except when the granular biomass experimented the process of high growth and breakage (between days 246 and 280) with a yield of 0.39 g VSS/g $COD_{removed}$ for a SRT of 15 days. Therefore the growth yield corresponding to aerobic granules was 54% lower than that obtained when the SBR contained flocculent biomass. Other authors obtained similar values of growth yield (between 0.2 and 0.33 g VSS/g $COD_{removed}$) for aerobic granular system (de Kreuk *et al.*, 2005; Figueroa *et al.*, 2011).

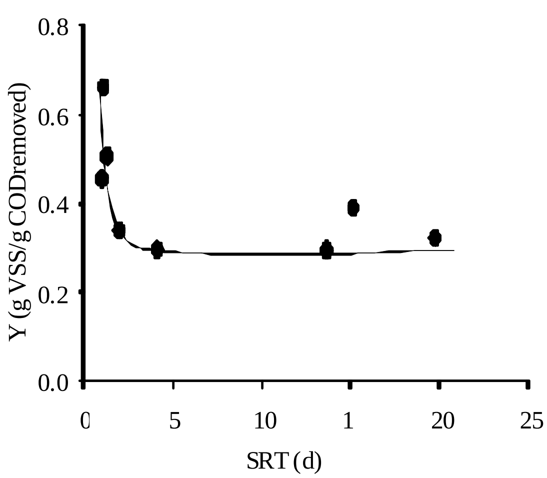

Fig. 8. Yield of micro-organisms (Y) as a function of the SRT (d)

CONCLUSION

The formation of aerobic granular biomass with good settling characteristics (SVI of 35 mL/g TSS and density of 60 g VSS/$L_{granule}$) was achieved in a SBR treating an industrial wastewater coming from a seafood industry with a previous physical-chemical treatment. The reactor treated OLRs between 2 and 13 kg $COD_S/m^3 \cdot d$ with a removal efficiency around 90%. The granulation process took place since day 130 of operation at an OLR of 2.0 kg $COD_S/m^3 \cdot d$ and the granules disintegrated for OLRs higher than 4.4 kg $COD_S/m^3 \cdot d$. The TN removal was due to biomass assimilation and with values around 30%. The ammonia removal was not constant along the full operation and depended on the SRT, FA concentration and average diameter of the granules. The maximum percentage of ammonia removal reached was 65% for a NLR of 0.3 kg NH_4^+-N/$m^3 \cdot d$. During the operation of the SBR with stable aerobic granular biomass and SRT higher than 4 days obtained biomass growth yield was 54% smaller than the operation of the SBR with flocculent biomass and SRT around 1 day.

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