EFFECT OF ADDITION OF AN AMMONIUM SOURCE IN UASB AND EGSB REACTORS WHEN TREATING LOW CONCENTRATED WASTEWATER: GRANULES MORPHOLOGY

***Vivanco E.*[1], *Puñal A.*[1], *Pizarro C.*[1], *Ferreira E. C.*[2], *Alves M. M.*[2] *and Chamy R.*[1*]**

[1]Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Avda. Brasil 2950, Valparaíso, Chile.

[2]Centro de Engenharia Biológica, Universidade do Minho, Braga, P-47100057, Portugal.

[*]e-mail: rchamy@ucv.cl

ABSTRACT

In this work the effect on granules morphology by adding a supplementary nitrogen-ammonium source to UASB and EGSB reactors, treating a low concentrated wastewater is studied. Previously, a complex-synthetic-medium-concentrated-wastewater (C/N ratio, 100:4) was fed for granule development and maturation, achieving similar removal performances (higher than 95 % in terms of COD) in both reactors. A subsequent period of 88 days treating low concentrated wastewater (C/N ratio, 100:0.5) led to the destabilisation of granules properties and operational performance in general in both reactors (COD removal efficiencies of approximately 50 %). Ammonium was subsequently supplemented to the low concentrated wastewater, in order to balance the nutrient content (C/N ratio, 100:10) and to study its effect on destabilised granules. After 28 days operating under these conditions, COD removal efficiencies recovered values of 82 and 90 % in UASB and EGSB, respectively. It was also observed, an improvement in granule appearance as given by microscopic observation.

INTRODUCTION

The granulation phenomenon may represent a crucial issue for the stable performance of anaerobic reactor technologies such as UASB and EGSB. Several factors have been reported as influencing granulation, among which inoculum characteristics, wastewater type, reactor configuration or hydrodynamics are counted. Attempting to understand the causes of granule destabilisation, flotation and wash out from sludge bed reactors, several approaches can be made, being the observation of granule morphology a good and early indicator to detect changes related to granule stability. A helpful tool for this purpose is the use of different microscopy techniques, from optic microscopy, which allows to observe closely the external granule morphology, to scanning electron microscopy, permitting the observation of the different microorganisms morphologies present throughout the granule. This last technique has often been used to find relathionships between the different microorganisms morphologies found and the operational performances observed (Grotenhuis *et al*., 1991; Wu *et al*., 1993; Puñal *et al*., 2000). Furthermore, image analysis has been recently used to describe in a quantitative way the dynamics of aggregates morphology and the performance attained in the corresponding reactors from where these aggregates where sampled (Amaral *et al.* 2004; Araya-Kroff *et al.* 2004).

In previous works, when treating low concentrated wastewater in UASB and EGSB reactors, the deterioration of granules characteristics and reactor performance, was observed to be related to the content and particularly the composition and location of extracellular polymeric substances (EPS) present in granules (Puñal *et al*., 2003), as well as to a decrease in granule density and an increase in granule surface hydrophobicity (Puñal and Chamy, 2004). C/N ratio has been reported as an important factor affecting production and/or excretion of extracellular polymeric substances (EPS) (Thaveesri *et al*., 1994; Wentzel *et al*, 1994; Grootaerd *et al*., 1997; O'Flaherty *et al*., 1997; Puñal *et al*., 2000), which are likely related to biomass attachment and aggregation (Laspidou and Rittmann, 2002). Thus, in this work, the addition of a supplementary nitrogen source as ammonium was considered as a potential strategy for performance recovering in UASB and EGSB reactors treating low concentrated wastewater and presenting deteriorated granule characteristics. Thus, an increase in C/N ratio from 100:0.5 to 100:10 in UASB and EGSB reactors when treating low concentrated wastewater was performed, studying its effect on the operational performance, as well as on granules morphology and settling velocity.

MATERIALS AND METHODS

Reactors. A UASB (Up-flow Anaerobic Sludge Blanket) and EGSB (Expanded Granular Sludge Bed) reactors were set up. Useful volume was 2.66 l (D = 8 cm, L = 55 cm) and 2.55 l (D = 5 cm, L = 130 cm) for UASB and EGSB reactors, respectively. Reactors were inoculated with a poor granulated biomass, with 68.3 g SST/l and 49.9 g SSV/l and 0.77 kg COD/kg VSS·d of specific methanogenic activity (SMA). Effluent recycling was performed in each reactor in order to regulate the liquid upflow velocity to 0.8 and 8 m/h in the UASB and EGSB reactors, respectively.

A synthetic complex-medium-concentrated wastewater (MCW) described elsewhere (Puñal *et al*, 2003) was supplied during granule maturation periods (days 0-41) operating at organic loading rate (OLR) 5 Kg COD/m^{3}d. Diluted beer was used as a low-concentrated substrate (LCW) during the destabilisation (days 42-130) and recuperation (days 131-158) periods, operating from 5 to 15 and 8Kg COD/m^{3}d OLR, respectively. Ammonium was additionally supplemented to the low concentrated wastewater during the last period (days 131-158).

Analytical methods. SMA was determined following the methodology described by Soto *et al*. (1993). Settling velocity was estimated using a graduated cylinder, measuring height variation of sludge *vs*. time. Optical microscopy was performed using a stereomicroscope Olympus BH (40X). Scanning electron microscopy observation was performed as described in Grotenhuis *et al*. (1991) using a JEOL JSM-25-SII at 30 KV.

RESULTS AND DISCUSSION

Prior to the operation treating low concentrated wastewater, a complex-synthetic-medium-concentrated wastewater, presenting a balanced content in nutrients (C/N ratio, 100:4), was supplied during 41 days to reactors UASB and EGSB for granule development and maturation, attaining similar operational performances in both systems (COD removal efficiency higher than 95 %) for an OLR of 5Kg COD/m^{3}d. The granules obtained were sampled and their morphological properties studied for further comparison with granules obtained during the following periods (see Figures 1, 2, 3 and 4). The sample corresponding to day 0 is the biomass used as inoculum and is presented in order to compare it with the biomass developed under different operational conditions. Although both reactors presented similar evolution in terms of COD removal efficiency during granule maturation, the appearance of granules evolved distinctly as can be observed in Figure 1, where the images of optical microscopy observation corresponding to the whole period studied are shown. Some of the granules obtained in the EGSB reactor after the maturation period (day 41) were bigger and presented a smother surface than those sampled from the UASB reactor. However, the more representative fraction of granules in the EGSB were smaller than those sampled from the UASB reactor, as correlated by the lower settling velocities (Figure 2) obtained for EGSB granules and the less homogeneous distribution obtained (Figure 3), when compared to UASB granules. The different hydraulic conditions in both reactors with higher upflow velocity (8 m/h) in EGSB reactor appear to be the more determinant parameter for a less homogeneous distribution (Figure 3) in this system, where the hydraulic regime may lead to a selective wash out by size and density of aggregates. Similar SMA where determined in both reactors (Figure 2), whereas the observation of granules surface by means of SEM shows a more diverse types-morphology of microorganisms in EGSB granules (Figure 4), observing predominantly *Methanosaeta*-like morphology in UASB reactor granules and some *cocci*, *bacilli* and *spirilli* types (together with the *Methanosaeta*-like morphology) in EGSB granules.

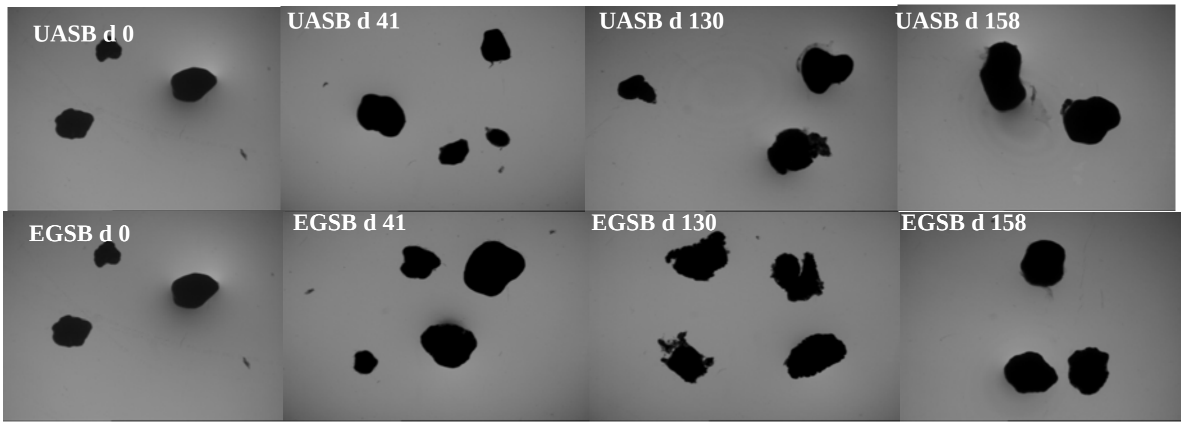

Figure 1. Optic microscopy (x 40) of granules from UASB (U) and EGSB (E) reactors in samples corresponding to days 0 (inoculum), 41 (mature granules), 131 (unstable granules) and 158 (recovered granules).

At day 42 the substrate was shifted to a semi-synthetic substrate, prepared by diluting beer, was fed to both reactors. This substrate represents in a good way an important fraction of wastewater generated in a typical brewery industry and presents a low rate of nitrogen to carbon content (100:0.5). UASB and EGSB reactors were operated treating this substrate during 88 days, increasing progressively the applied OLR from 5 up to 15 Kg COD/m^{3}d. A progressive deterioration in the operational performance was observed, particularly during the last ten days when an OLR of 15 Kg COD/m^{3}d was applied, attaining COD removal efficiencies of only 50 %. In Figure 1, the deterioration of granule morphology after 88 days treating low concentrated wastewater can be clearly observed (day 130), particularly in EGSB reactor granules. A relative higher decrease in the representative settling velocity was as well observed for EGSB granules (Figure 2), although the distribution of settling velocities was more deteriorated in the case of UASB reactor (Figure 3). This seems to be straight related to the hydraulic regime, which allows, in the case of UASB reactor, more diverse (in size and density) granules to remain within the system, while in the EGSB reactor a new normal distribution of settling velocities has been achieved (Figure 3). In Figure 2 the deterioration of biomass SMA in both reactors after the period treating low concentrated wastewater can be observed. SEM images corresponding to core and surface of granules from UASB and EGSB reactors are presented in Figure 4, where different morphologies can be observed. Thus, while on UASB and EGSB surface the predominant morphology corresponds to *Methanosaeta*-like organisms, in EGSB granules some *spirilli* can be observed as well, together with EPS-like structures, which could be here related to the disintegration of granules observed in Figure 1. Regarding granules core, the same morphologies as on the surface are observed in EGSB granules, whereas in UASB granules *cocci* and *bacilli* are the predominant morphologies, indicating a shift in microbial population given by substrate change.

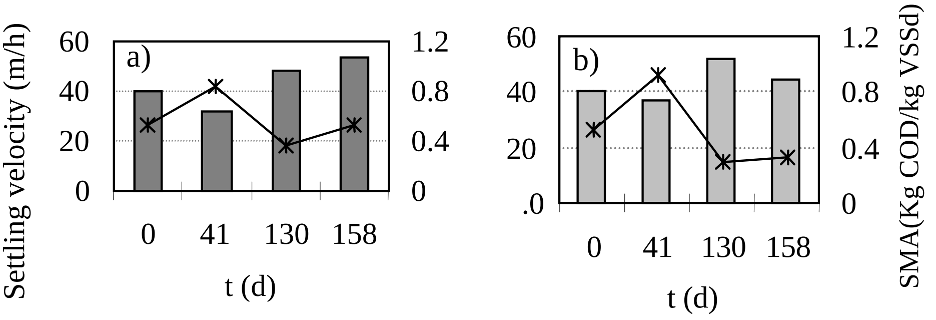

Figure 2. Average settling velocity (▨)and specific methanogenic activity (—*—)of granules from reactors **a**) EGSB and **b**) UASB corresponding to inoculum (day 0), mature granules (day 41), unstable granules (day 130) and recovered granules (day 158) after addition of a supplementary ammonium source.

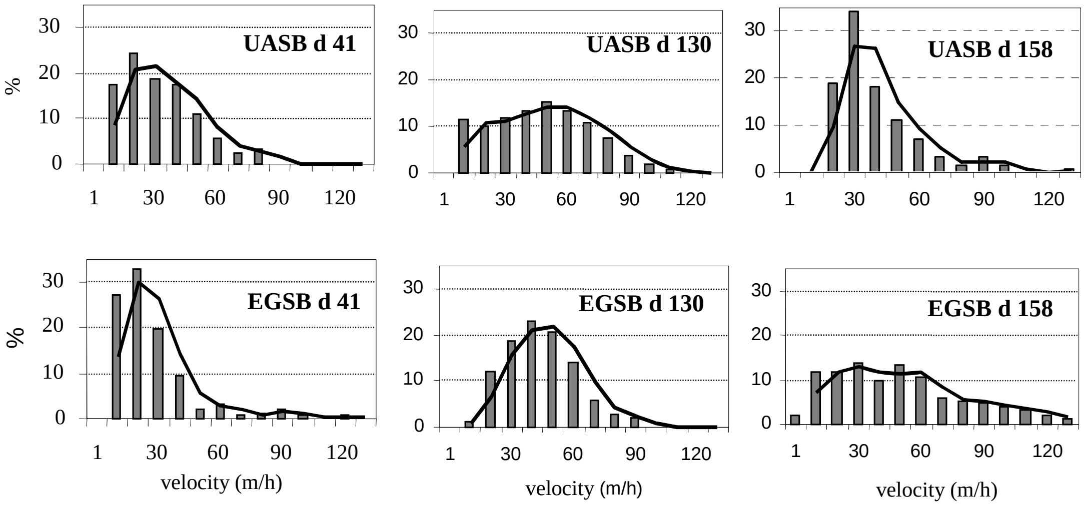

Figure 3. Percentile distribution of settling velocity from granules from UASB and EGSB reactors in samples corresponding to days 41 (mature granules), 131 (unstable granules) and 158 (recovered granules).

When Ammonium was supplemented to the low concentrated wastewater a progressive recuperation in terms of operational performance was observed, attaining after 28 days (day 158) COD removal efficiencies of 82 and 90 % in UASB and EGSB reactors. This correlates to granule appearance, showed in Figure 1, where more compact granules can be observed in both reactors. The effect of Ammonium supplementation on settling velocity distribution is, however, clearly different in each system, obtaining higher representative settling velocities in the EGSB reactor after 28 days of operation (Figure 2) and slightly lower values in UASB reactor granules. The distribution of this parameter points out to the formation of new granule cores and a granule compaction in both systems, although the effects of this granule compaction appear different in each system. Thus, in the case of UASB reactor, new small granules are formed, together with bigger granules presenting in much cases lower densities and thus lower settling velocities; whereas in EGSB reactor, the recovering of more compact granules, together with the formation of new ones, likely allows different sizes and densities to remain in the EGSB reactor, in spite of the high upflow velocities set in this system. A longer period seems to be required for the recuperation of a normal distribution for settling velocity in EGSB reactor, although the high upflow velocities should favour this point, when compared to the UASB system. This observation appears to be confirmed by the fact that the operational performance recuperation was not accompanied by SMA values recovery and microbial populations are still changing under the effect of the new operational conditions applied. A closer observation of microorganisms morphologies associated to this new stage shows the occurrence of new filamentous-structures within granules from both systems, particularly in EGSB, with the presence of some *cocci* and *Methanosaeta*-like organisms in UASB granules. The surface of granules shows a crossed filamentous structure in UASB granules, corresponding to a reinforced granule structure; while in EGSB granules *Methanosaeta*-like organisms in a early stage of aggregation and some *cocci* are observed, likely corresponding to a rather early stage of granule formation.

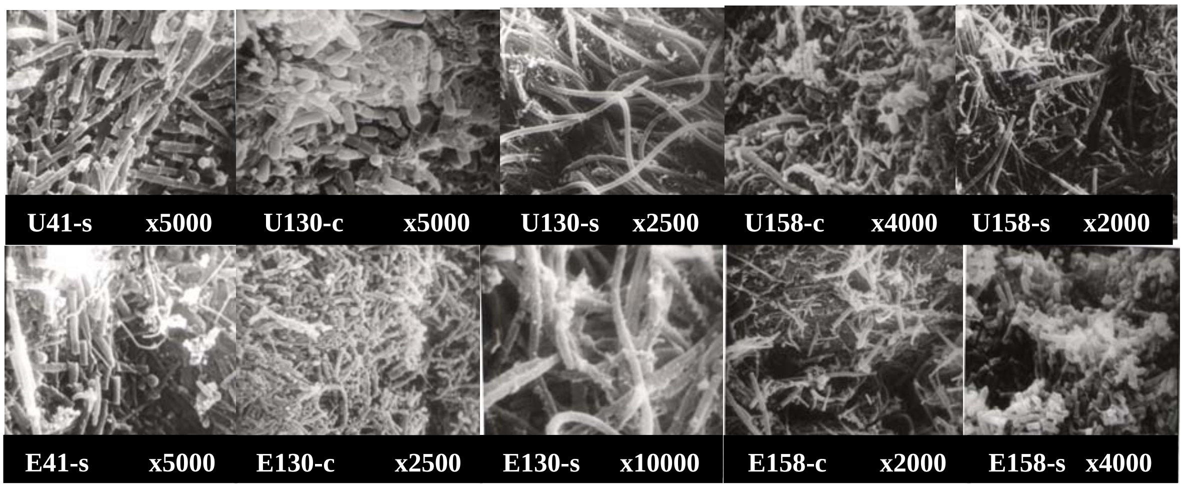

Figure 4. Scanning electron microscopy (SEM) of granules. The notation specifies the reactors UASB (U) and EGSB (E); the days of operation 41, 130 and 158, corresponding to mature granules, unstable granules and recovered granules, respectively, the area from granules (core-c or surface-s) where photos were taken and the augmentation factor.

CONCLUSSIONS

The deteriorated operational performance of two UASB and EGSB reactors treating low concentrated wastewater could be recovered by addition of supplementary ammonium. This strategy allowed as well the recuperation of proper granule morphologic characteristics.

In terms of morphology, the addition of ammonium prompted the compaction of granules as well as the formation of new nuclei for granule formation, observing that a longer period may be required to achieve higher SMA and a normal distribution for settling velocities of granules.

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