Dynamics of extracellular polymeric substances in UASB and EGSB reactors treating medium and low concentrated wastewaters

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Abstract In this work the dynamic study of EPS (Extracellular Polymeric Substances) concentration and distribution during the operation of two different reactor configurations (UASB and EGSB) is presented, treating medium (6 g COD/l) and low-concentrated (0.5 g COD/l) wastewater. Medium-concentrated wastewater was supplied for granules maturation as well as for stabilisation of the process. The effect of substrate change on granule characteristics was followed in both reactors. Total concentration of EPS associated to steady operation, was higher in the UASB reactor. The change to a low-concentrated substrate led to an increased difference, promoting a sharp destabilisation of the EGSB reactor, observing an increment in filamentous structures, causing biomass flotation and wash out. Although total concentration of EPS remained almost constant in the UASB reactor, their composition and distribution presented significant differences. The ratio of protein/polysaccharides as well as acidic-polysaccharides/total (neutral + acidic) polysaccharides decreased drastically in the EGSB reactor, while in the UASB reactor, the decrease was not so important and not enough for destabilisation of granule structure. Moreover, polysaccharides distribution seemed to have an important role in granule stability being enough to maintain granule cohesion only in the case of the UASB reactor. These observations point to composition and distribution of EPS rather than their total concentration as key parameters for granule stability and settleability.

Keywords Anaerobic treatment; expanded granular sludge bed (EGSB) reactor; extracellular polymeric substances (EPS); granule; upflow anaerobic sludge blanket (UASB) reactor

Introduction

The Upflow Anaerobic Sludge Blanket (UASB) reactor is a proven technology, which represents the most used world-wide system (Frankin, 2001). The Expanded Granular Sludge Bed (EGSB) reactor is quite a promising version of UASB reactors operated at high superficial upflow velocities, obtained by means of high recycling rates, biogas production and elevated height/diameter ratios. EGSB reactors are gaining more popularity and gradually replacing UASB applications which is most likely due to the EGSB higher loading rates favoured by the hydrodynamics. Although most anaerobic reactors are designed for treatment of medium- to high- strength wastewaters; in recent years growing efforts have been directed towards anaerobic treatment of lower strength industrial and domestic wastewaters (Lettinga et al., 1997). In this sense, UASB and EGSB systems have been applied to treatment of a wide range of very different industrial and domestic wastewaters with chemical oxygen demand (COD) below 2000 mg/l (Kato et al., 1997; Jeison and Chamy, 1999; Rajesh et al., 1999). However, there are a number of problems associated with anaerobic treatment of low-strength wastewaters (Kato et al., 1997).

The performance of UASB and EGSB reactors is based on the granulation phenomenon. Granules consist not only of microorganisms but also of inorganic matter, and vary widely in physical, chemical and microbiological characteristics, depending on seed sludge, chemical composition of wastewaters and sludge bed level in reactors (Schmidt and
Although granulation has been observed in different wastewater types and defined media, some specific wastewater types have been found where granules could not be developed. Moreover, some cases of sudden disintegration without any apparent cause have been reported (Schmidt and Ahring, 1996). Extracellular polymeric substances (EPS) count as one of the factors affecting granulation as they are considered essential for the adhesion between different species present in granules, improving their long-term stability (Forster, 1992; Schmidt and Ahring, 1994); these represent a relevant subject of study during recent years (Quarmby and Forster, 1995; Veiga et al., 1997; El-Mamouni et al., 1998; Batstone and Keller, 2001; Liao et al., 2001; Laspidou and Rittmann, 2002). Several researchers have shown, using microscopic observation, the presence of filamentous substances surrounding cells within and around the structure of anaerobic granules (Forster, 1992; de Beer et al., 1996; Veiga et al., 1997). The EPS production enhancement has been associated to different factors, such as nutrients excess (Wentzel et al., 1994) or deficiency (Veiga et al., 1997; Puñal et al., 2000); addition of external polymers (El-Mamouni et al., 1998) or wastewater type (Batstone and Keller, 2001).

In a previous study performed in our lab (Jeison and Chamy, 1999), the operation of two UASB and EGSB reactors was studied treating different wastewaters. A negative effect, particularly on EGSB granule characteristics, was observed when treating diluted beer (0.5 g COD/l), but no further studies were followed in order to achieve a better understanding of these results. In this work, the dynamic study of EPS (Extracellular Polymeric Substances) concentration (total polysaccharides, acidic polysaccharides (mucopolysaccharides, e.g. uronic acids family), proteins and DNA) and distribution (acidic polysaccharides) during the operation of two different reactor configurations (UASB and EGSB) together with operational parameters is presented. The substrate was, initially, medium COD-concentrated synthetic wastewater for the start up and maturation of granules and, subsequently, the substrate was changed for treating diluted beer.

**Materials and methods**

**Reactors**

Two UASB (Up-flow Anaerobic Sludge Blanket) and EGSB (Expanded Granular Sludge Bed) reactors were set up. The EGSB reactor had a useful volume of 2.55 l (D = 5 cm, L = 130 cm), while the UASB reactor useful volume was 2.66 l (D = 8 cm, L = 55 cm). Reactors were placed in a thermostatised chamber maintained at 37 ± 2°C. Upflow velocities were fixed by adjusting the recycling flow rate. According to the conditions usually found in industrial reactors, upflow velocities were 0.7 and 7 m/h for UASB and EGSB reactors, respectively. The inoculum was poorly granulated biomass from a treatment plant of yeast production wastewater. Its Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) concentrations were 68.3 and 49.9, respectively, and its specific methanogenic activity was 0.33 kg COD/kg VSS·d.

Two different substrates were fed to the reactor. A medium COD-concentrated (5 g COD/l) synthetic wastewater (MCW) was supplied during the start up and maturation of granules. This substrate consisted of a mixture of glucose, yeast extract, ethanol, acetate, propionate, butyrate, N and P sources (C/N/P ratio = 100/5/1) as well as oligoelements and buffer capacity (2 g NaHCO₃/l). Both reactors were fed with this substrate from day 0 to day 200 of operation, achieving an OLR (Organic Loading Rate) of 5 kg COD/m³·d. Diluted beer (C/N/P ratio = 100/1/1 and 0.5 g COD/l) was used as low COD-concentrated wastewater (LCW) from day 200 to day 290 of operation, increasing progressively the influent OLR from 0.5 up to 7 kg COD/m³·d. The diluted-beer represents in a good way an important fraction of wastewater generated in a typical brewery industry.
Analytical methods
Volatile Fatty Acids (VFA) were determined by Gas Chromatography (Shimadzu GC 8A equipped with a Flame Ionisation Detector). Chemical Oxygen Demand (COD), P-orthophosphate, Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) were analysed as proposed by Standard Methods (APHA, 1985). Ammonium N was measured using a selective electrode. Specific Methanogenic Activity (SMA) of biomass was determined following the methodology described by Soto et al. (1993), using a mixture of acetate, propionate and butyrate (2:0.5:0.5) as substrate.

The extraction conditions for EPS (temperature, agitation speed and time) proposed by Schmidt and Ahring (1994) were initially considered and tested, in order to determine the optimal values for this case. Hence, they were further fixed at 65°C, 70 rpm and 4 hours, respectively, using phosphate buffer (3XPBS) as extractive solution. Polysaccharides, uronic acid (acidic polysaccharides), proteins and DNA were determined in the extracted sample. Polysaccharides were assayed by the method of Miller (1959) using glucose as standard. The uronic acid is a replicate unit of acidic polysaccharides (or mucopolysaccharides) (Blumenkrantz and Asboe-Hansen, 1973) and it is a representative substance in biofilms and in bacterial aggregation in general. Uronic acid (acidic carbohydrates) was determined by the m-phenylphenol method using galacturonic acid as standard (Blumenkrantz and Asboe-Hansen, 1973). Proteins were determined by using the method of Lowry et al. (1951) with bovine serum albumin as standard. DNA was measured by fluorescence spectrometry using ethidium bromide as reactive agent and calf thymus DNA as standard (Haugland, 1992).

For the study of acidic polysaccharides distribution, the samples of granules, previously fixed with paraformaldehyde 4%, stained with calcofluor (Fluorescent Brightener 28, Sigma-Aldrich, St Louis, Mo, USA) and sliced with a cryomicrotome (Reichert 343109), were observed by fluorescence microscopy in an Olympus IX70 microscope using a 40×, 0.85 NA Fluo objective. The calcofluor binds to β-1.3 and β-1.4 polysaccharides, which represent the family of uronic acids, e.g. acidic polysaccharides. This technique was previously used by de Beer et al. (1996) to stain EPS in biofilms.

Results and discussion
The operational results obtained in UASB and EGSB reactors during the start up and stabilisation period, treating MCW are presented in Table 1. During the first 100 days, corresponding to start up period treating MCW, the UASB and EGSB reactors achieved similar COD removal capacities, as well as similar conversion efficiencies for methane from removed COD, while specific methanogenic activity (SMA) remained lower in the UASB reactor. This fact could reflect a slower growth and development in the active biomass of the UASB reactor.

Table 1 Operational parameters and their standard deviations during the operation with medium concentrated wastewater (MCW) in UASB and EGSB reactors

<table>
<thead>
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<th>Start up (0–99 days)</th>
<th>Stabilisation (100–200 days)</th>
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<tr>
<td></td>
<td>UASB</td>
<td>EGSB</td>
</tr>
<tr>
<td>% COD removal</td>
<td>90.1 ± 1.4</td>
<td>89.2 ± 1.2</td>
</tr>
<tr>
<td>l CH₄/g COD removed</td>
<td>0.29 ± 0.14</td>
<td>0.30 ± 0.06</td>
</tr>
<tr>
<td>pH</td>
<td>7.2 ± 0.1</td>
<td>7.9 ± 0.1</td>
</tr>
<tr>
<td>Total VFA (g/l)</td>
<td>0.16 ± 0.04</td>
<td>0.18 ± 0.05</td>
</tr>
<tr>
<td>Propionic Acid (g/l)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Washed TSS (g/l)</td>
<td>1.2 ± 0.2</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>Washed VSS (g/l)</td>
<td>0.4 ± 0.1</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>SMA (kg COD/kg VSS d)</td>
<td>0.77(1)</td>
<td>0.85(1)</td>
</tr>
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(1) day 50; (2) day 180. SMA at day 0 (inoculum) was 0.33 kg COD/kg VSS d
During the stabilisation period, an increase of biomass SMA in both systems was observed, together with a decrement and stabilisation of solid concentration (e.g. biomass) washed from both reactors. The performance improvement was, however, more important in the case of the EGSB reactor, whose biomass was 8% more active in terms of methanogenesis than the biomass located in the UASB reactor. It is important to note the higher pH registered in the EGSB reactor compared to that from UASB reactor during the whole period (days 0-200) treating MCW. However, the extra alkalinity added with the feeding, is the same in both reactors, as well as CO₂ produced. Hydraulic behaviour may favour CO₂ transference within the liquid phase, thus, enhancing the buffer capacity in the EGSB reactor.

At day 200 of operation the substrate was changed and diluted-beer as low concentrated wastewater (LCW) was fed into both reactors. The procedure of load increase was the same as before, attaining 5 kg COD/m³.d at day 245 (starting at 0.5 kg COD/m³.d at day 200). In order to study the effect of a load increase on performance and granule characteristics in both reactors, during the next 45 days a further increase of influent OLR up to 7 kg COD/m³.d was performed. Operational parameters corresponding to LCW treatment are presented in Table 2.

When treating LCW, both reactors initially presented similar performances, but when the OLR applied was higher than 3 kg COD/m³.d more significant differences appeared. Average values corresponding to the period 200 to 245 days (OLR from 0.5 to 5 kg COD/m³.d) reflect the worsening of the performance registered in both systems, although the UASB reactor maintained a better COD removal capacity and COD conversion into methane, as well as higher SMA (5%, 6% and 12% more, respectively). Moreover, VFA presented concentrations up to five-fold those determined during operation with MCW, finding in both reactors a low presence of propionic acid, which was not previously detected. It is important to note the increase in solids wash-out observed in both systems, with a VSS major proportion, particularly in the EGSB reactor (56%) when compared to those obtained during operation with MCW (36%). Although the behaviour of pH during this period (days 200–250) still maintained the same pattern as during operation with MCW (days 0–200), pH in the EGSB reactor was only 0.2 units higher (compared to the former 0.7 units) than that in UASB reactor when treating LCW. This issue, together with the increase of biomass (particularly VSS) washed out from the EGSB reactor, may point to the compromise within a narrow range of upflow velocities required to properly operate EGSB systems (Kato and Lettinga, 1997), in order to maintain, at the same time, acceptable levels of biomass retention and stabilisation.

The differences observed between UASB and EGSB reactors became more important when the OLR was increased from 5 up to 7 kg COD/ m³.d (days 245–290). During this

<table>
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<th>Table 2</th>
<th>Operational parameters and their standard deviations during the operation with low concentrated wastewater (LCW) in UASB and EGSB reactors</th>
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<tr>
<td>Day 200-day 245</td>
<td></td>
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<tr>
<td><strong>UASB</strong></td>
<td><strong>EGSB</strong></td>
</tr>
<tr>
<td>% COD removal</td>
<td>83.9 ± 8.2</td>
</tr>
<tr>
<td>l CH₄/g COD removed</td>
<td>0.28 ± 0.11</td>
</tr>
<tr>
<td>pH</td>
<td>7.3 ± 0.1</td>
</tr>
<tr>
<td>Total VFA (g/l)</td>
<td>0.42 ± 0.08</td>
</tr>
<tr>
<td>Propionic Acid (g/l)</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Washed TSS (g/l)</td>
<td>1.2 ± 0.7</td>
</tr>
<tr>
<td>Washed VSS (g/l)</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>SMA (kg COD/kg VSS d)</td>
<td>1.23(1)</td>
</tr>
</tbody>
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(1) day 235; (2) day 280. SMA (Specific Methanogenic Activity)
period, the UASB reactor maintained an acceptable performance, with some signs of adaptation to the LCW, while clear signals of destabilisation appeared in the EGSB reactor (Table 2). In fact, when compared to values registered during the start up phase with LCW, the UASB reactor presented better conversion of removed COD into methane, a higher index of VFA degradation, with similar values for propionate, and a recovering of biomass SMA. Other parameters, such as the decrease in COD removal in the liquid phase or pH, or the increase in solids washed from the UASB reactor, show that this operation could still be improved. On the contrary, the EGSB reactor did not present any signs of adaptation nor recovery, registering a drastic decrease in COD removal efficiency down to 40%, in the capacity to convert COD into methane (0.16 l CH₄/g COD removed), or in biomass SMA (0.82 kg COD/kg VSS.d). Moreover, the sharp decrease of pH to a value of 5.6 indicates the shut down of the EGSB reactor, with the consequent increase of VFA, particularly, propionic acid, as well as the biomass washed from the reactor, mainly VSS (83%).

EPS content extracted from granules located in UASB and EGSB reactors were followed during the maturation period and until the end of the operation when treating MCW and during the whole period when the reactors were fed with LCW. Results obtained from the EPS analysis extracted from the granules are presented in Figure 1. When observing EPS total content in the granules (Figure 1a), the results fit to those obtained from operational performance. A concentration around 150 mg total EPS/g VSS was attained in granules from both reactors, during the stable period, treating MCW at 5 kg COD/m³.d (days 100–200). Major compounds in EPS were protein followed by polysaccharides, which agrees with other authors reported results when they studied EPS in granules from different types of processes and treating different wastewaters (Fukuzaki et al., 1995; Veiga et al., 1997; Batstone and Keller, 2001; Liao et al., 2001). Polysaccharides/total EPS ratio of 20% obtained during the operation also agrees, as well, with the results found by those authors (Fukuzaki et al., 1995; Veiga et al., 1997; Batstone and Keller, 2001). Acidic polysaccharides concentration (e.g. mucopolysaccharides), was particularly high within the EPS of granules from both reactors during the operation treating MCW, representing 100% of total polysaccharides determined in the stable granules. The most relevant difference was that granules from the UASB reactor presented a 33% higher concentration in acidic polysaccharides. However, this fact did not affect the stability of granules, or the performance of UASB or EGSB reactors, as observed from results presented in Table 1, pointing to a relatively wide range of concentration of acidic polysaccharides where granule stability is possible.

The substrate change had a different effect on EPS content evolution in each reactor. Total content of EPS presented a sudden increase in both reactors, as a consequence of metabolic stress, promoting cell lysis and liberation of intracellular products, which lasted from 10 to 15 days. After day 220 the concentration of total EPS followed different patterns of evolution in the UASB and EGSB reactors. In the UASB reactor concentration remained almost constant until the end of the operation, presenting values even higher than those detected when treating MCW. The total EPS content in EGSB granules presented a progressive decrease up to the end of the operation. Present protein in EPS, which had been the major compound during previous operation, presented, as well, a sharp increase in both reactors immediately after substrate shift, which reflects in fact the peak detected in total EPS. After this increase, protein content showed a progressive decrease, down to very low values in the EGSB granules. On the other hand, total polysaccharide concentration increased progressively from 30 mg/g VSS up to 80 and 60 mg/g VSS in UASB and EGSB reactors, respectively. Batstone and Keller (2001) have reported better granule properties at an intermediate protein:total-polysaccharide ratio of 3.4, when treating cannery wastewater, while worse granule qualities were reported at a ratio of 9.6, when treating slaughterhouse
wastewater. Other authors have observed a worsening of aggregation properties associated to a decrease of protein:total-polysaccharide ratio in granules (Liao et al., 2001). In this work, best results were observed at ratios between 3.5–4.0, with a worsening of aggregation at decreasing ratios, detected solely in the EGSB reactor.

When the substrate was changed, a very strong and rapid decrease in acidic polysaccharides concentration was observed in both reactors. However, its concentration was 30 to 70% higher in granules from the UASB reactor for the last 50 days of operation. In the granules, it is the acidic polysaccharides, together with polypeptides that mediate the attachment between bacterial cells by intermolecular cross-linkage via polyvalent cations (e.g. calcium; Fukuzaki et al., 1995). De Beer et al. (1996) found stable granules from UASB reactors presenting concentrations of acidic polysaccharides of 1 to 1.6 mg/g VSS, while those having floc consistency contained below 1 mg of acidic polysaccharides/g VSS. In this work, granules from the EGSB reactor contained between 2–3 mg acidic polysaccharides/g VSS at the end of the operation, which seems to be a limiting value for the consistency of granules, as well as for their activity, as was observed in the operational performances shown in Table 2. The hydraulic regime in the EGSB reactor could particularly condition the lower threshold found for acidic polysaccharides, when compared to the results obtained by de Beer et al. (1996) studying UASB granules.

DNA concentration presents a very different pattern between reactors when the substrate was shifted at day 200 from MCW to LCW. DNA sharp increase in EGSB granules

![Figure 1](https://iwaponline.com/wst/article-pdf/48/6/41/423508/41.pdf)
could be the result of dead and/or lysis biomass. But the method used to determine DNA did not allow us to establish the difference. A weaker bacterial wall, as a result of operational stress, or cell disruption by extraction method, could also be the origin of this important increase. However, no parallel increment was detected in other EPSs in EGSB or UASB granules. Moreover, the ratio DNA/protein in EGSB granules is 1.0 at the end of the operation, which indicates a complete loss of protein and polypeptides in general from EPS of EGSB granules. These facts indicated the weaker consistency of granules and cells from the EGSB as compared to the UASB reactor, when the same extraction procedure was applied.

Figure 2 shows the distribution of extracellular acidic polysaccharides. Two representative samples were chosen for each reactor: one from day 177 (mature granules and high efficiency in both reactors) and the other from day 284 (84 days after substrate change). The fluorescence intensity of representative zones of the section related to a non-stained standard is presented in the figure, as well as a surface cut of each sample.

At day 177 of operation (Figure 2a), an accumulation of polysaccharides was observed in a narrow area on the surface of the UASB granules, while the centre was much less fluorescent. This profile corresponds to a stable granule, which presented good aggregation and settling properties and agrees with what de Beer et al. (1996) observed in stable granules from UASB reactors treating either a mixture of glucose and VFA or lactate. Granules from the EGSB reactor presented an homogeneous distribution of acidic polysaccharides during the first operational phase, observing the highest concentration inside the granule. De Beer et al. (1996) presented similar results for flocs fed with lactose. Acidic polysaccharides distribution showed that, when treating MCW, 45% of the fluorescence was located in the outer shell in UASB granules, while high fluorescence location was moved inside the granule for those coming from the EGSB reactor. However, as the total distribution remained mainly homogeneous for EGSB granules, their stability, and therefore the performance of the system, was not affected.

Profiles observed at day 284 substantially changed for granules obtained from both reactors (Figure 2b), particularly in the EGSB reactor. A lightly fluorescent outer layer appeared in the UASB and EGSB granules, representing 16 and 26% of total fluorescence detected, respectively. Moreover, while granules from the UASB reactor maintained an homogeneous distribution inside the granule and a continuous surface layer concentrating 50% of fluorescence, EGSB granules presented a desegregated structure, with a discontinuous layer of acidic polysaccharides, reflecting the weak consistency of granules.

Conclusions
When diluted-beer was used as substrate, the EGSB reactor performance presented a progressive worsening until shut down at the end of the study period (40% COD removal efficiency and pH 5.6). However, the UASB reactor could adapt to the new substrate,
maintaining stability and obtaining acceptable performances. A deeper study of granules was performed in order to clarify the causes of destabilisation, finding out that performances could correlate well to granule characteristics during different operational periods.

Total granule EPS content in the UASB reactor was higher during the whole operation. However, this parameter seems not to be significant, as the highest concentrations were obtained in the UASB reactor when granules presented worse aggregation properties. EPS major components during stable operation were proteins and, to a lower extent, total polysaccharides. An important decrease in (protein:total-polysaccharide) ratio in the EGSB reactor was observed associated to deterioration of granule properties. Acidic polysaccharides decrease in both reactors when they were fed with diluted−beer may explain the worsening of aggregation properties in both UASB and EGSB reactors. The study of polysaccharide distribution showed a higher accumulation of extracellular acidic polysaccharides on the surface of granules with good aggregation properties, although it was also evident that granule properties required for a good performance, differ from UASB to EGSB reactor.

Acknowledgements
The support from CONICYT (Chile) through the project FONDECYT-3020026 are gratefully acknowledged.

References


