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Granulation during the start-up of a UASB reactor used in the treatment of low strength wastewaters

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A glucose-based wastewater was efficiently degradaded by acidogenic bacteria, with a glucose removal efficiency close to 90%, and although a distinctive granular structure could not be observed, fluffy conglomerates developed in an Upflow Anaerobic Sludge Blanket (UASB) reactor. Subsequently, the pre-acidification of the wastewater promoted the granulation process. An enrichment in methanogenic bacteria was observed on the microscope and was confirmed by an increase in the specific methanogenic activity from 0.1 up to 0.5 kgCOD/kgVSS.day. Such dynamics of microbial communities was also verified through changes in the polysaccharide and protein content, as well as in the electrophoretic mobility of the biomass.

Introduction

The Upflow Anaerobic Sludge Blanket (UASB) reactor has been widely applied for the treatment of many types of wastewaters (Lettinga and Hulshoff Pol, 1992). In general, the operational conditions induce an ecological selection that leads to the formation of microbial aggregates, the so-called 'granules' (Hulshoff Pol *et al.*, 1983; Schmidt and Ahring, 1996). Granules are the best inoculum for the start-up of UASB reactors due to their high specific activity and settleability. However, they are not always available. Consequently, very often the start-up process and the tentative granule formation has to be performed with a inoculum of diffuse sludge collected in conventional anaerobic digesters. Although the polymeric content of fully developed granules was already assessed by several authors (Dolfing *et al.*, 1985; Grotenhuis *et al.*, 1991; Shen *et al.*, 1993) there is a lack of data on the granulation process (operating conditions *vs.* properties of the agregates) during the start-up of a UASB reactor that use diffuse sludge as the inoculum. On the other hand, when treating wastewaters with COD values lower than 1500–2000 mg/l, which are low strength wastewaters in the anaerobic treatment field, the start-up of UASB reactors is difficult and thus much less full-scale reactors are in operation under these conditions than in the case of medium and high strength effluents (Lettinga and Hulshoff Pol, 1992).

The experimental work here described was focused on the treatment of low strength wastewaters and its main purposes were: *i)* to study the start-up operation of a UASB reactor inoculated with non-aggregated biomass, and *ii)* to monitor some physiological and physicochemical characteristics of the biomass, namely specific activity, polysaccharides and proteins as well as the electrophoretic mobility during the granulation process.

Material and methods

Biomass inoculum

The biomass inoculum was collected from a conventional anaerobic digester used in the stabilisation of activated sludge. The inoculum had a volatile suspended solids (VSS) concentration of 8.6 kg VSS/m^3.

Substrate

The UASB reactor was sequentially fed with two substrates, both with a COD value tentatively between 1000–1500 mg/l. In the first period, phase I, the substrate composition was in average 85% of glucose and 15% of volatile fatty acids (VFA). In phase II, the VFA concentration was increased to approximately 80% of the total COD. The VFA solution was a mixture of acetic and propionic acids, 3:1 in a COD base. A macronutrients solution was added to the feed, 2 ml per litre of influent. The composition was the following per litre: NH_4Cl, 174 g; KH_2PO_4, 28.3 g; $(NH_4)_2SO_4$, 28.3 g; $MgCl_2$, 25 g; KCl, 45 g; yeast extract: 3 g. A micronutrients solution was also added, with the following composition per litre: $FeCl_2.6H_2O$, 2 g; H_3BO_3, 0.05 g; $ZnCl_2$, 0.05 g; $CuCl_2.2H_2O$, 0.038 g; $MnCl_2.4H_2O$, 0.5 g; $(NH_4)_6Mo7O_2.4H_2O$, 0.05 g; $AlCl_3$, 0.09 g; $CoCl_2.6H_2O$, 2 g; $NiCl_2.6H_2O$, 0.092 g; $Na_2SeO_3.5H_2O$, 0.164 g; EDTA, 1 g; HCl 12M, 1 ml.

The amount of micronutrients added per litre of feed was 0.03 ml/l, except during the final 15 days, where it was increased to 0.1 ml/l and supplemented with calcium phosphate, 100 mg/l. The media were prepared with tap water and previously neutralised to pH 7.

Reactor

The reactor had a volume of 2 l and was operated at 35°C.

Analysis

Chemical Oxygen Demand (COD), Volatile Solids (VS) and Volatile Suspended Solids (VSS) were carried out as indicated in the Standard Methods (1989). Volatile fatty acids (VFA) determination was performed by HPLC, with a Jasco unit. Biomass was examined under ultraviolet at 420 nm and normal light. For scanning electron microscopy (SEM) examination, the sample was fixed in 0.1 M of sodium cacodylate buffer (pH 7.2) during 24 h at room temperature, dehydrated in graded ethanol and air dried, sputter-coated with gold and examined with a Leica Cambridge S360. Total polysaccharides were analysed by the method of the phenol/sulphuric acid (Dubois *et al.*, 1956). The standard was a glucose solution. The absorbance was measured at 490 nm with a spectrophotometer Hitachi U1100. A disruption step was performed by milling of the biomass in a mortar. The protein concentration was measured by the Lowry method using bovine serum albumin as standard. The samples were treated with 2 M HCl to remove sulphide and an equal volume of 2 M NaOH (Grotenhuis *et al.*, 1992). Absorbance was measured at 750 nm. The electrophoretic mobility was measured in a sample directly collected from the reactor with a Zetameter Inc. 3.0+.

Experimental conditions

The volumetric organic loading rate during the UASB operation was step-wise increased up to 3.1 kgCOD/m^3.day by reducing the hydraulic retention time. The operational protocol followed the one proposed by Hulshoff Pol *et al.*, (1983), which is based on: *i)* an ecological selection of the aggregate forming bacteria, mainly focused on the acetotrophic *Methanothrix* spp, and *ii)* an elutriation process to promote the wash-out of dispersed bacteria. In order to attain such objectives, the loading rate was increased when the acetate concentration dropped down to 200–300 mg/l; moreover, during the operation the treated effluent was partly recycled to increase the hydraulic load. The upflow liquid velocity was roughly set at 0.5–1 m/h. The methanogenic activities of inoculum and aggregates were assessed through the measurement of the maximum substrate degradation rate. The substrate in the activity tests was a VFA mixture with a total COD between 2–2.5 g/l, containing 50% acetate, 25% propionate and 25% butyrate. Such tests were performed in a batch reactor at 35°C in an orbital shaker at 150 r.p.m. The solids concentration was 4.4 gVSS/l in the test with the biomass inoculum and 3.0 gVSS/l in the test with the aggregates.

Results

Performance of the UASB reactor

Data on the organic loading rate, overall efficiency, total COD and VFA as COD equivalents (VFA-COD) at the influent and effluent recorded during the present study are presented in Figure 1. As shown, the applied volumetric organic load in the UASB reactor increased from 0.02 up to 3.1 kgCOD/m^3.day, which still is a low value, comparing with others referred to in the literature for soluble wastewaters (Lettinga and Hulshoff Pol, 1992). However, the volumetric load is independent of the biomass hold-up and indeed the specific organic load was increased significantly, up to 1.4 kgCOD/kgVSS.day. An important wash-out of poorly settleable suspended solids occurred, most of it during the first phase of operation. By the end of phase I, the presence of fluffy conglomerates was detected. In the second phase, the niche selection proceeded continuously, while the overall mass of elutriated solids was less than in phase I. At the end of the experimental study, the average granule diameter was approximately 0.5–1 mm, but its apparent structure was more weak than the one observed on granules collected from a full-scale UASB reactor treating a VFA containing wastewater. No clear effect was noticed due to the supplementation of micronutrients and calcium in the final two weeks, suggesting they did not represented a limiting factor during the process. A steady decrease in COD removal down to 30–50% around day 60–80, near the middle of the experimental time, was observed. The efficiency gradually recovered along phase II, reaching a level between 70–80%.

The inoculum and the aggregated biomass had specific methanogenic activities of 0.1 kgCOD/kgVSS.day and 0.5 kgCOD/kgVSS.day, respectively. Filamentous-like bacteria with a morphotype resembling *Methanothrix* spp. (flat ends or septa between the junction of individual cells) were more abundant in a SEM microphotograph taken at day 104 than in a previous examination of the biomass inoculum. However, such aceticlastic bacteria were not the only methanogens present in the reactor. The presence of blue colour fluorescent

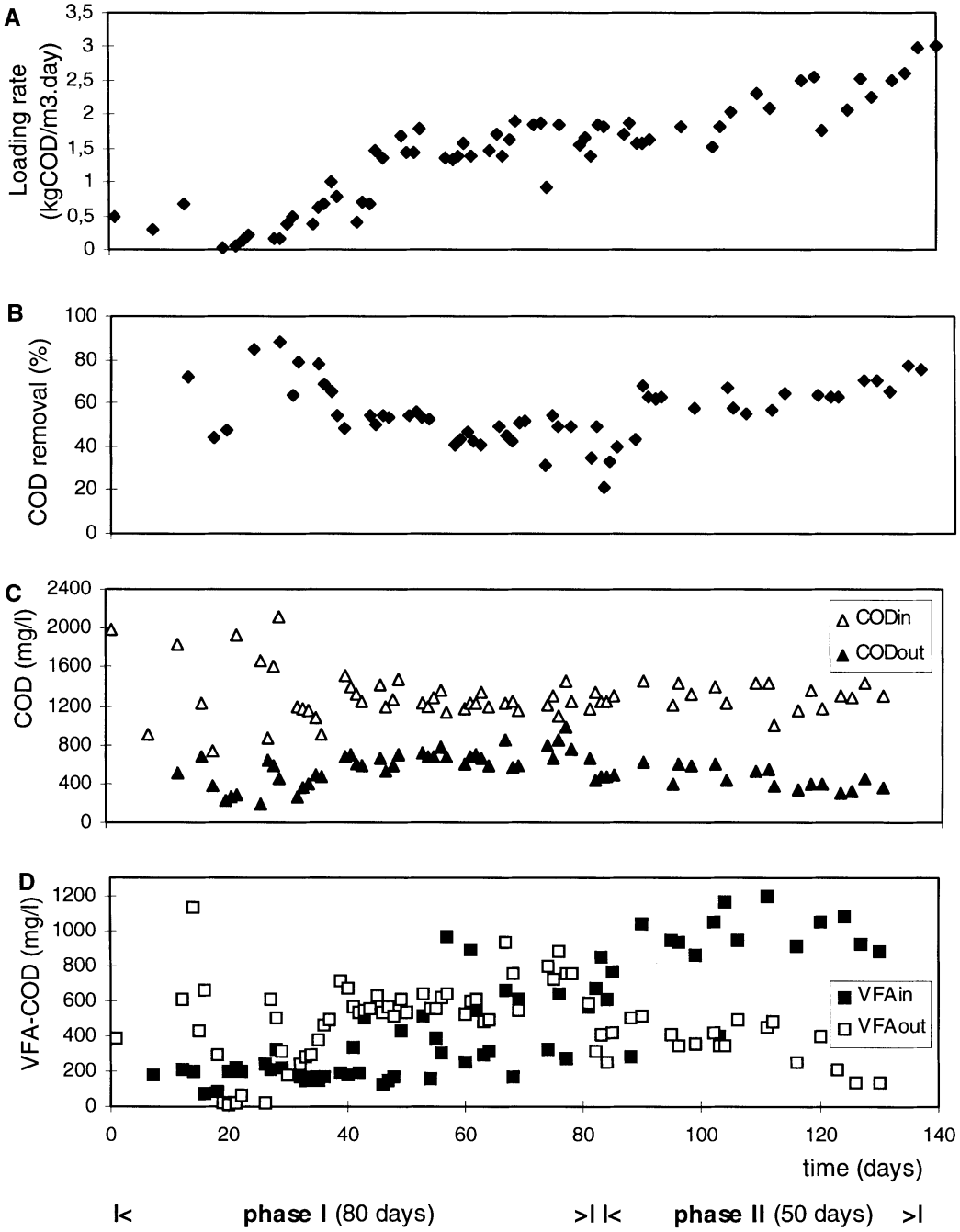

Figure 1 Operation and efficiency of the UASB reactor in a low strength wastewater: A. Organic loading rate (volumetric); B. COD conversion; C. COD concentration; D. VFA-COD concentration (in: influent, out: effluent).

organisms could be also seen in the microscope when the inoculum and crushed aggregates were exposed to the UV light. Most likely, such autofluorescence was related to coenzyme F_{420}, which is considered, in anaerobic environments, as a specific marker of hydrogenotrophic bacteria like, for instance, *Methanosarcina* spp. (Dolfing and Mulder, 1985). Both methanogenic groups are well known in granular structures (Hulshoff Pol *et al.*, 1983; Alibhai and Forster, 1986).

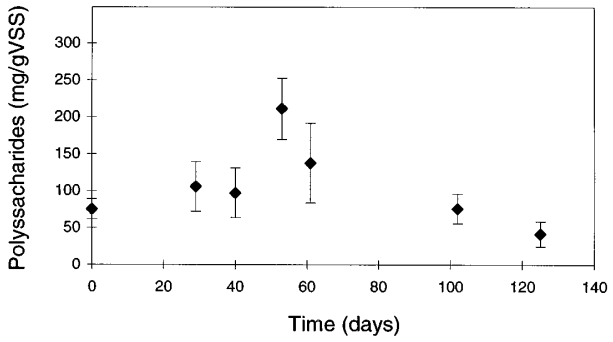

Figure 2 Polysaccharide concentration during operation.

Table 1 Protein concentration during UASB operation

	Proteins (mg/gVSS)
Biomass inoculum (day 0)	37.0 ± 11.3
Biomass at day 102	37.1 ± 13.7
Biomass at day 125	23.2 ± 13.5

Polysaccharides, proteins and electrophoretic mobility

The polysaccharides and proteins measured in the biomass during the operation time of the UASB reactor are presented in Figure 2 and Table 1, respectively. Both data are presented with an analysis of the propagation of random errors. Table 2 presents a measurement of the electrophoretic mobility of the inoculum and of the biomass aggregates at day 103 (middle of phase II), along with the surface charge of selected methanogens.

Discussion

Some conclusions about the microbial populations present in the UASB reactor may be drawn from the results presented in Figure 1. During phase I, the non-VFA-COD removal efficiency attained roughly 90%, indicating the presence of an abundant acidogenic population degrading remarkably well the glucose. The high concentration of VFA in the effluent reveals some limitations in the food chain, namely regarding the methanogenic group. In phase II, a better VFA removal was noticed. Such progressive enrichment in slow-growing methanogens was confirmed by the activity tests results and by the observations at the microscope. Regarding the increase in the biological rate of reaction, this is one of the positive results expected from the granulation process, as reviewed by Schmidt and Ahring (1996). Taking into account the system recovery along phase II, a further increase in the performance of the UASB reactor might be expected if the experimental time were extended. The final observations performed during the experiment also suggest that the aggregates were still developing towards their climax.

The results displayed on Figure 2 suggest a relationship between the polysaccharides fraction and the substrate composition supplied to the UASB reactor. The polysaccharide increase during phase I is considered to be the result of the acidogenic population dynamics. This trophic group combines its high growth rate and yield with an abundant excretion of polysaccharides, as observed with scanning and transmission microscopy (Harada *et al.*, 1988). The significant development of acidogenic bacteria causes the colour to change from dark to yellowish, observed after the start with the unacidified wastewater feeding. The presence of a bulking sludge like this one is also reported by Méndez-Pampin *et al.*, (1986), in the start-up of a UASB fed on a similar wastewater. When the carbon source was shifted in phase II (reducing glucose and increasing VFA concentration) there was a decline in polysaccharide concentration. As mentioned previously, the granulation process seemed to be promoted then, an increase in the consistency and settleability of the aggregates being noticed, probably due to the progressive methanogenic enrichment and some reduction of the acidogenic population. A somehow similar phenomenon was observed during studies focused on the stability of existing granules facing changes in the feed composition (Yang and Anderson, 1993; Alphenaar, 1994). In both studies, the granules when fed with a non-acidified sucrose substrate decreased their strength and a fluffy attached acidogenic layer covering the original granular seed was noticed. On the other hand, Alphenaar (1994), also reported an increase in the density of granular biomass when their feed changed

Table 2 Electrophoretic mobility measured in the different types of biomass

	pH	Electrophoretic mobility ($\mu.V^{-1}.s^{-1}$)	Specific conductance (μmhos/cm)	Reference
Biomass (day 0)	7.0	–2.77 ± 0.13	297	This study
Biomass (day 103)	6.9	–2.09 ± 0.16	245	This study
Methanothrix sohengenii	7	–0.32 ± 0.15	–	Grotenhuis et al. (1992)
Methanosarcina barkeri	7	–2.40 ± 0.15	–	Grotenhuis et al. (1992)

from sucrose to a VFA mixture, observing then a wash-out of light biomass. Regarding the protein values presented in Table 1, the few analysis hinder a full evidence of the evolution pattern and the analysis of uncertainty considering all steps of the measurement process advise some precaution in their evaluation. In spite of that, it appeared that a protein content decrease occurred during phase II. Considering the whole experiment, the average ratio of proteins to polysaccharides had only a small increase, from 5:1 up to 6:1.

As can be observed in Table 2, the surface charge of the biomass presented a slight trend towards electroneutrality. Alibhai and Forster (1986), also report a more negative zeta potential on non-aggregated biomass (–30 mV) than on some granules (between –14.2 and –24.2 mV). In theory, this evolution reveals a reduction of the electrostatic repulsion barrier, which favours adhesion. Such change on the biomass surface charge could be related to a reduction in the coating with extracellular polymers (Rouxhet and Mozes, 1990). However, an enrichment in *Methanothrix sohengenii* could also play a role in that reduction because this specie has the less negative surface charge among several anaerobic bacteria (Grotenhuis *et al.*, 1992).

Conclusions

The results obtained in this study point out to the feasibility of UASB systems for the treatment of low strength soluble wastewaters with COD values lower than 1500 mg/l. The development of a sludge bed with a fairly loose structure was observed during phase I, when the feed was mainly glucose. However, the increase in the VFA concentration at phase II represented a turning point towards granule formation. An enrichment in methanogenic biomass was observed on the microscope and indeed the final aggregates displayed a higher methanogenic activity than the inoculum.

The experimental results also indicate that polysaccharide and protein content of the biomass had a dynamic pattern directly related to the ecological structure and the dominant trophic community. The polysaccharides increased during the feeding with glucose based wastewater and decreased during the feeding with methanogenic substrates, while a reduction of the protein concentration of the biomass was suggested. Finally, a slight trend to a decrease in the electrophoretic mobility during granulation was also detected, indicating a reduction in the surface charge of the biomass structures that may favour the aggregation process.

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References

American Public Health Association (1989). *Standard Methods for the Examination of Water and Wastewater*. 17th ed., APHA, AWWA, WPCF. Washington DC, USA.

Alibhai, K.R.K. and Forster C.F. (1986). *Enzyme Microb. Technol.*, **8**, 601–606.

Alphenaar, A. (1994). *PhD Thesis*. University of Wageningen, Wageningen, The Netherlands.

Dolfing, J., Griffioen, A., Neerven, A.R.W. and Zevenhuizen, L.P. (1985). *Can. J. Microb.*, **31**, 744–750.

Dolfing, J. and Mulder, J.W. (1985). *Appl. Env. Microbiol.* **49**, 1142–1145.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956). *Anal. Chem.*, **28**, 350–356.

Grotenhuis, J., Smith, M., Lammeren, A., Stams, A. and Zehnder, A. (1991). *Appl. Microb. Biotech.*, **36**, 115–119.

Grotenhuis, J., Plugge, C.M., Stams, A. and Zehnder, A. (1992). *Appl. Microb. Biotech.*, **58**, 1054–1056.

Harada, H., Endo G., Tohya, Y. and Momonoi K. (1989). In: *Proceedings of the 5th Int. Symposium on Anaerobic Digestion*, A. Tilche and A. Rozzi (Eds.), pp. 1011–1020, Monduzzi Editore, Bologna, Italy.

Hulshoff Pol, L.W., Zeew, W., Velzeboer, C.T. and Lettinga, G. (1983). *Wat. Sci. Tech.*, **15**, 8, 291–304.

Lettinga, G. and Hulshoff Pol, L.W. (1992). *Wat. Sci. Tech.*, **24**, 8, 87–108.

Mendez-Pampin, R.J., Sierra Alvarez, R. and Hulshoff Pol, L.W. (1986). In: *Anaerobic Treatment – a Grown-up Technology*, NVA – EWPCA (Eds.), pp. 698–702. Industrial Presentations BV, Schiedam, The Netherlands.

Rouxhet, P.G. and Mozes, N. (1990). *Wat. Sci. Tech.*, **22**, 1/2, 1–16.

Schmidt, J.E. and Ahring, B.K. (1996). *Biotech. Bioeng.*, **49**, 229–246.

Shen, C.D., Kosaric, N. and Blaszczyk, R. (1993). *Water Res.*, **27**, 1, 25–33.

Yang, G. and Anderson, G.K. (1993). *J. Environ. Eng.*, **119**, 5, 958–977.

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