Appl Microbiol Biotechnol (1993) 39:132–137

Applied
and Microbiology
Biotechnology

Properties of anaerobic granular sludge as affected by yeast extract, cobalt and iron supplements

Chun Fang Shen, Naim Kosaric, Roman Blaszczyk

Department of Chemical and Biochemical Engineering, The University of Western Ontario, London, Ontario, Canada, N6A 5B9

Received: 9 July 1992/Accepted: 16 November 1992

Abstract. The effect of yeast extract (YE), iron (Fe) and cobalt (Co) on anaerobic bacterial granules grown in eight laboratory-scale upflow anaerobic sludge blanket reactors was investigated using a factorial design. The experiment was performed in three periods in which different chemical oxygen demand (COD) loading rates were applied to the reactors. The COD digestion rate and the specific activity of the bacteria were positively affected by supplementation of Fe and Co in the feed under a high COD loading rate. YE had a strong positive effect on the bacterial growth rate, but no significant effect on the specific activity of the bacteria. With Fe supplementation, an excellent COD digestion rate was maintained in the reactors, and addition of YE in the feed was not necessary. Granules with better settling properties were developed under a relatively low bacterial growth rate. With the increase in COD loading rate, the percentage of calcium increased rapidly in the granules from the reactors without YE supplementation. The bacteria grown under high COD loading rate and without an Fe supplement could be Fe-deficient. *Methanothrix*-like rod-shaped bacteria were dominant in the granules from all reactors.

Introduction

In the early 1980s, Lettinga et al. (1980) developed active bacterial granular sludges with excellent settling properties in an upflow anaerobic sludge blanket (UASB) reactor. Since then, many full scale UASB reactors have been operated in the food, beverage and forest industries (de Zeeuw 1987; Lettinga et al. 1991). An active and well settled granular sludge, which stays in the reactor up to a certain upflow velocity, is a prerequisite for UASB reactor operation. Under these conditions a high concentration of biomass in the reactor can be provided and a high digestion rate is maintained.

All microorganisms have an array of mineral requirements for growth. Many metals are involved in important enzymatic activities. Analysis of ten methanogenic strains showed the following order of metal concentration: $Mg \approx Ca > Fe > Zn \geq Ni > Co \approx Mo > Cu > Mn$ (Scherer et al. 1983). The physiological role of iron (Fe) appears to be similar to the one found in aerobic bacteria because of the bacterial redox property. Fe may be involved in energy metabolism as cytochrome and ferredoxin in methylotrophic methanogens, as reported by Kenealy and Zeikus (1982) and Kuhn et al. (1983), and in some enzymes as Fe-sulphur (S) proteins (Takashima and Speece 1990). A large portion of cobalt (Co) has been suggested to be present in corrinoids (vitamin B_{12} derivatives), which are involved in the activity of methyl transferase and co-dehydrogenase (Jarrell and Sprott 1982; Whitman et al. 1982). Nickel (Ni) is a component of methyl coenzyme M reductase (F_{430}) and of hydrogenase and co-dehydrogenase. It has been reported that yeast extract (YE) is a good source of "vitamin B_2 complex" and some mineral salts (Cook 1958). YE is a good micronutrient for bacterial growth. However, the cost of YE as a nutrient on an industrial scale is high, and replacement of YE with other low-cost chemicals is very beneficial in industry.

Inorganic precipitates, such as sulphide and carbonate salts, could serve as supports for adhesion of anaerobic bacteria. Ferrous sulphide has been observed to stick firmly to the sheath of *Methanothrix* sp. By this interaction, ferrous sulphide precipitates might contribute to stabilization of bacterial aggregates within granules (Dubourguier et al. 1985). A positive effect of calcium (Ca) in the influent medium on the pelletization and settling properties of granular sludge was reported by Hulshoff Pol et al. (1983) and Mahoney et al. (1987). The biomass accumulation rate was high and granular sludges settled fast in a Ca-positive reactor. The higher atomic weight ions have a more pronounced effect on the floc density than do lower atomic weight ions, thus increasing the sedimentation rate. A significant correlation between the density and ash content of the granules was reported by Hulshoff Pol et al. (1986). The ash con-

---

Correspondence to: N. Kosaric

tent comes mainly from ferrous sulphide and calcium carbonate or phosphate that are present inside the bacterial granules (Dubourguier et al. 1987). The inorganic precipitates contribute not only to settling characteristics but also to granule stability.

Trace element deficiencies have been reported by a number of researchers. Takashima and Speece (1989), in a series of pH-stat experiments, demonstrated that an acetate utilization rate of 30-40 g/l per day could be attained only after the metal dose reached approx. 1.0, 0.1 and 0.1 mg/l per day for Fe, Ni and Co, respectively. A similarly high (1000% increase) stimulation of the acetate-utilization rate was demonstrated by Hoban and van den Berg (1979) with sporadic weekly additions of 28–280 mg/l of Fe. Oleszkiewicz (1989) used three laboratory-scale UASB reactors for treatment of waste-waters from the food-processing industry, and found that hard granular sludge was maintained only in the reactor with supplements of Fe, Ni and Co, which performed undisturbed through shock chemical oxygen demand (COD) loads of 5–15 kg/m^3 per day at an upflow velocity of 1.5–6.0 m/h. The aim of the present work was to investigate the effect of YE, Co and Fe supplements on the COD digestion rate, specific activity and settling properties of granules formed in UASB reactors.

Materials and methods

Culture methods. Synthetic feed was used in this anaerobic process. Carbon sources in the feed were acetate, propionate and butyrate. The feed was buffered with sodium bicarbonate. Some trace elements required for bacterial growth were also supplied. The pH was adjusted to 6.8±0.2 by sodium hydroxide solution. The feed composition was the same as that used by Kosaric et al. (1991), except for the Ca^{2+} concentration, which was 20 mg/l.

Eight laboratory-scale UASB reactors with a volume of 1.2 l each were used in the experiment. The feed for the reactors was supplied with different amounts of YE, Co and Fe salts. The experimental design is presented in Table 1.

The experiment was carried out in three periods. In the first period (Period 1), the COD loading rate was maintained at 13.8 g COD/l per day for 28 days. At the end of this period, part of the granules in the reactors were removed, and the COD loading rate was gradually increased to 27.7 g COD/l per day within 14 days (Period 2). After Period 2, part of the granules in the reactors were again removed and the COD loading rate was increased rapidly from 13.8 to 39.6 g COD/l per day in 14 days (Period 3, see Fig. 1). At the end of each period, the total suspended solids (TSS) and total volatile suspended solids (VSS) were measured, and the trace element composition in the granules from each reactor was determined.

Table 1. Experimental design

Supplement	Reactor							
	R1	R2	R3	R4	R5	R6	R7	R8
YE	−	+	−	+	−	+	−	+
Fe	−	−	+	+	−	−	+	+
Co	−	−	−	−	+	+	+	+

The concentration of supplied iron (Fe) and cobalt (Co) was 1.0 and 0.1 ppm (in chloride form), respectively, and was independent of chemical oxygen demand (COD) concentration. The concentration of yeast extract (YE) was varied with COD loading rate. According to the factorial design, +1 was used for feed with an individual nutrient, and −1 was used for the feed without addition of that nutrient

Granule sedimentation. The settling properties of granules were evaluated by the fractions of granules exited under certain upflow velocities in a fractionating device (Andras et al. 1989). About 16 ml of granules from each reactor were separated into five fractions under four upflow velocities and at each velocity for 3 min. Each fraction from the fractionating device was collected in a Whatman (1PS) filter paper. TSS in each fraction was determined using the method described by Andras et al. (1989).

Scanning electron microscopy (SEM). The washed granules from different reactors were placed in 5-ml vials, which contained 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), at 4° C overnight. The samples were post-fixed in 1% osmium tetroxide in cacodylate buffer for 2 h, dehydrated in a water-ethanol series and then critical-point dried using liquid CO$_2$ in a Denton critical-point dryer. The dried specimens were sputter-coated with gold and examined in a Hitachi S-650 scanning electron microscope.

Metal analysis. Freeze-dried granules were digested with nitric acid and hydrogen peroxide. Digested samples were diluted with distilled water and filtered through a filter paper (Whatman 40). Metal analysis was performed with a PU 9100X atomic absorption spectrometer (Philips scientific).

Suspended solids. TSS and VSS were measured by standard methods (APHA 1985).

COD measurement. COD in the feed and effluent was measured by the method of Knetchel (1977). The sample was digested with potassium dichromate and sulphuric acid. The absorbance was measured at 600 nm with a Varian DMS 90 UV-VIS spectrophotometer.

Results

Reactor performance

In Periods 1 and 2, the COD digestion rate in each reactor was excellent (higher than 94%) and no significant difference between the reactor performances was observed. However, after Period 2, when the COD loading rate was increased rapidly up to 39.6 g COD/l per day, the COD digestion rate in the reactors in which Fe was not supplied decreased. The COD loading rates and digestion rates in each reactor during Period 3 are shown in Figs. 1 and 2.

At the end of the Period 3, the COD digestion rates from R1 to R8 were 57.8, 77.7, 93.9, 96.0, 75.1, 84.6, 93.0 and 96.7%, respectively. The data in Figs. 1 and 2 indicate that the COD digestion rate was significantly affected by the supplement of YE, Fe and Co. These effects have been analysed and are modelled in Eq. 1.

$$\begin{aligned}\%\ \text{COD digestion rate} &= 84.4 + 4.4\cdot \text{YE} + 10.6\cdot \text{Fe} \\ &+ 3.0\cdot \text{Co} - 3.0\cdot \text{YE}\cdot \text{Fe} - 1.1\cdot \text{YE}\cdot \text{Co} - 3.1\cdot \text{Fe}\cdot \text{Co} \\ &+ 1.5\cdot \text{YE}\cdot \text{Fe}\cdot \text{Co} \qquad (1)\end{aligned}$$

Mathematical modelling, as represented by Eq. 1, shows that the main effects of YE, Fe and Co on the COD digestion rate were 4.4, 10.6 and 3.0%, respectively. The interactive effects between YE and Fe, YE and

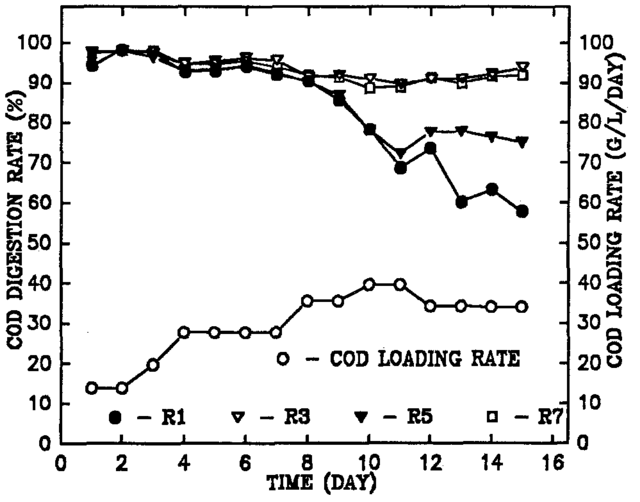

Fig. 1. Chemical oxygen demand (COD) loading rate (O) and COD digestion rate in reactors (●, R1; ▽, R3; ▼, R5; □, R7) without yeast extract (YE) supplementation

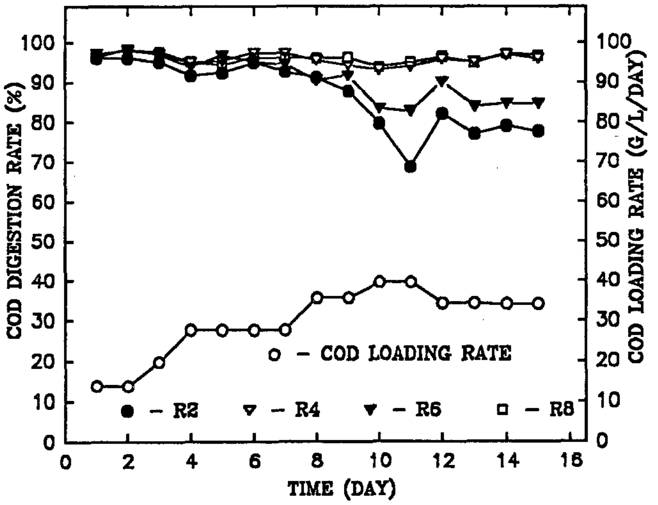

Fig. 2. COD loading rate (O) and COD digestion rate in reactors (●, R2; ▽, R4; ▼, R6; □, R8) with YE supplementation

Co, Fe and Co on COD digestion rate were negative: −3.0, −1.1 and −3.1, respectively. However, from the biological point of view, when the environmental conditions are identical for all reactors, the COD digestion rate is only related to two factors: the amount of bacteria (VSS) and the specific activity of the bacteria. Table 2 shows that the growth rate of bacteria was significantly affected by YE supplementation.

The specific activity of bacteria in the reactors could be affected by trace nutrients (e.g. trace elements), which may be limiting during growth. The specific activity of bacteria (g COD/g VSS per day) was defined as the amount of converted COD divided by total VSS in the reactor, which was 1.63, 1.73, 2.25, 2.03, 1.84, 1.88, 2.17 and 2.06 g COD/g VSS per day for reactors R1 to R8, respectively. The effects of YE, Fe and Co on the specific activity of bacteria are shown in Eq. 2.

$$\begin{aligned}&\text{Specific activity of bacteria} = 1.95 - 0.024 \cdot \mathrm{YE} \\ &+ 0.18 \cdot \mathrm{Fe} + 0.039 \cdot \mathrm{Co} - 0.059 \cdot \mathrm{YE} \cdot \mathrm{Fe} + 0.006 \cdot \mathrm{YE} \cdot \mathrm{Co} \\ &- 0.051 \cdot \mathrm{Fe} \cdot \mathrm{Co} + 0.021 \cdot \mathrm{YE} \cdot \mathrm{Fe} \cdot \mathrm{Co} \qquad (2)\end{aligned}$$

According to this equation, Fe and Co had a positive effect on the activity of bacteria. A weak negative effect was found from the YE. A negative interaction existed between Fe and YE, and Fe and Co.

Settling properties of granular sludge

Fractional granule distribution against upflow velocity for the granules from each reactor at the end of Period 3 is presented in Table 3. The last fraction of the granules in Table 3 corresponded to an upflow velocity higher than the final upflow velocity, because this fraction remained in the fractionating device under the final velocity. The data in Table 3 offer a general profile of granular fractions exited at different upflow velocities. The general settling properties of the granules from different reactors were evaluated by the upflow velocity corresponding to 50% washout of the sludge (V_{50}), which was determined from the cumulative plot. In the cumulative plot, the cumulative percentage of TSS exiting from the fractionating device was plotted against the upflow velocity. The V_{50} gives a clear presentation of granular settling properties from all reactors (Table 3). A typical scanning electron micrograph in Fig. 3 shows that *Methanothrix*-like rod-shaped organisms were supported by inorganic salts.

Table 2. Initial (VSS_0) and final total volatile suspended solids (VSS_f), and increased VSS (ΔVSS) in each reactor in each period

Reactor	Period 1			Period 2			Period 3		
	VSS_0	VSS_f	ΔVSS	VSS_0	VSS_f	ΔVSS	VSS_0	VSS_f	ΔVSS
R1	12.6	17.4	4.8	10.2	15.3	5.1	10.9	14.5	3.6
R2	12.6	21.5	8.9	11.7	17.9	6.2	11.5	18.4	6.9
R3	12.6	17.9	5.3	10.5	15.4	4.9	10.5	17.1	6.6
R4	12.6	18.9	6.3	10.5	16.4	5.9	10.3	19.4	9.1
R5	12.6	16.7	4.1	10.0	14.6	4.6	10.5	16.6	6.1
R6	12.6	20.1	7.5	10.7	16.0	5.3	10.6	18.4	7.8
R7	12.6	18.3	5.7	10.5	14.8	4.3	10.4	17.4	7.0
R8	12.6	20.9	8.3	11.1	16.7	5.6	10.3	19.2	8.9

VSS is given in grams

Table 3. Fractionation of granules[a]

Vu (m/h)	% TSS washout							
	R1	R2	R3	R4	R5	R6	R7	R8
13.7	20.0	24.5	24.4	27.1	27.0	21.4	21.9	24.3
32.0	16.1	27.2	19.6	24.5	21.6	19.5	17.8	19.1
53.4	17.1	26.4	17.9	30.0	20.1	27.1	21.5	29.3
74.5	19.1	18.0	17.7	16.7	20.1	24.1	20.9	20.7
74.5+	27.7	3.9	20.5	1.7	11.2	7.9	17.8	6.6
V_{50}	50.0	31.0	39.5	31.0	33.5	39.0	42.5	37.0

Vu, water upflow velocity; TSS, total suspended solids; 74.5+, last granule fraction can be washed out by upflow velocities up 74.5 m/h; V_{50}, upflow velocity corresponding to 50% washout of the sludge

[a] According to the method of Andras et al. (1989)

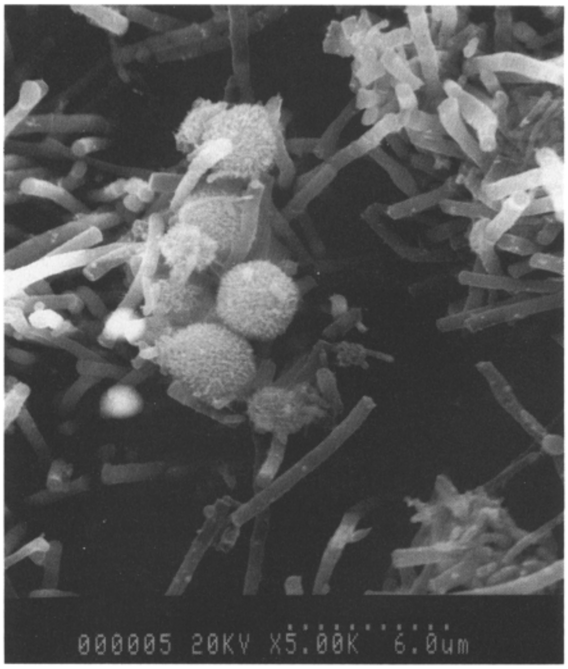

Fig. 3. Scanning electron micrograph indicating *Methanothrix*-like rod-shaped microorganisms supported by inorganic salts

Trace elements

The concentration of some trace elements in the granules from the reactors at the end of each period is presented in Table 4. It is shown that the concentration of Ni, Co and Fe in the granules decreased throughout the whole study, and the concentration of Ca increased steadily. This was particularly the case for the granules in the reactors to which YE was not supplied and during Period 3. The concentration of trace elements in the granules having different settling properties from reactor 7, which was supplied with Fe and Co, is presented in Table 5.

Table 4. Concentration of trace elements in granules

Sample	Ni	Co	Fe	Ca
Seeding granules	1.43	0.46	7.93	6.17
At end of Period 1				
R1	0.82	0.70	3.86	7.33
R2	0.78	0.39	3.60	4.57
R3	0.80	0.39	5.59	5.01
R4	0.73	0.38	5.56	6.69
R5	0.82	0.70	4.07	7.15
R6	0.80	0.74	4.14	6.72
R7	0.86	0.76	5.85	6.09
R8	0.76	0.64	5.59	6.48
At end of Period 2				
R1	0.75	0.34	3.52	11.90
R2	0.69	0.27	2.93	5.22
R3	0.77	0.34	5.23	10.50
R4	0.60	0.23	4.41	5.66
R5	0.64	0.59	2.99	5.09
R6	0.69	0.61	2.90	5.01
R7	0.68	0.57	4.23	9.28
R8	0.60	0.52	4.27	5.36
At end of Period 3				
R1	0.55	0.14	1.59	19.70
R2	0.48	0.13	1.58	9.50
R3	0.45	0.11	2.96	15.80
R4	0.55	0.13	3.04	5.79
R5	0.57	0.46	1.59	9.42
R6	0.55	0.43	1.54	7.42
R7	0.46	0.39	2.87	17.10
R8	0.44	0.39	3.02	6.47

The concentration of metals is in mg/(g dried solids); Ni, nickel; Ca, calcium

Table 5. Concentration of trace elements in granules with different settling ability

Fraction	Upflow velocity (m/h)	Trace elements (mg/g)			
		Ni	Co	Fe	Ca
1	13.7	0.55	0.53	2.85	12.5
2	32.0	0.55	0.52	3.89	14.5
3	53.4	0.42	0.33	2.77	15.1
4	74.5	0.36	0.27	2.01	18.8
5	74.5+	0.37	0.27	1.70	23.4

Feed for the granules from this reactor (R7) was supplied with Co and Fe

Discussion

Equations 1 and 2 indicate that the bacteria grown in some reactors under a high COD loading rate may be Fe and/or Co deficient. Optimum concentrations of Fe and Co for different methanogenic bacteria were reported to vary from 0.28 to 50 mg/l, and from 0.015 to 5 mg/l, respectively (Takashima and Speece 1990). When the feed was not supplied with Fe and Co, the concentration of Fe and Co as impurities in the chemicals used was about 0.09 and 0.02 mg/l respectively in the feed with a COD concentration of 4200 mg/l. When the COD loading rate reached 39.6 g COD/l per day in Period 3, the Fe content in the granules without Fe supplementation was down from 0.8% to 0.16%. In the pure methanogenic culture, in which the Fe concentration was determined by inductively coupled plasma emission spectrometry, the average Fe concentration was 0.11–0.15% (Scherer et al. 1983). This is close to the above-mentioned drop in Fe to 0.16%. On the other hand, not all Fe in the granules is used by bacteria for their metabolism, and some Fe appears as an inorganic precipitate. Therefore, by comparing the Fe concentration in pure methanogenic culture and in the granules, and the Fe status in the granules, it is suggested that the bacteria grown in the reactors without the Fe and Co supplementation may be Fe and/or Co deficient. When bacteria are under Fe and/or Co deficiency, their specific activities are lower than that of bacteria grown under normal conditions (Goodwin et al. 1990). The low specific activity of bacteria grown in reactors deficient in Fe and/or Co may also support the conclusion that these bacteria were Fe and/or Co deficient. The COD digestion rate in the reactors without Fe supplementation decreased rapidly when the COD loading rate reached 35.6 g COD/l per day (Figs. 1 and 2). The COD digestion rate was not recovered after the COD loading rate decreased. The growth rates of bacteria in these reactors were also low in Period 3.

YE is considered to be a source of organic micronutrients in the medium: it provides vitamins, especially the vitamin B_2 complex. When YE was supplied in the reactors, the bacterial growth rate was high and more bacteria were maintained in the reactors (Table 2), and the COD digestion rate was high. Therefore, YE showed a positive effect on the COD digestion rate. The specific activity of bacteria in this experiment was mainly affected by the availability of trace metals, as mentioned previously. The concentration of trace elements in YE is very low (60 mg/kg for Fe and 8 mg/kg for Co). Supplementation of YE in the feed did not increase the trace elements significantly, and therefore its effect on the specific activity of bacteria was not significant. The results of reactor performance and specific activity of bacteria indicate that a YE supplement can improve the COD digestion rate in the reactors, even though it does not show a positive effect on the activity of bacteria. The key function of YE is that it enhances the bacterial growth rate in the reactor.

The negative interactive effect between YE and Fe, and Fe and Co on the COD digestion rate indicates that the effects of the above three factors can be replaced with each other up to certain levels. For example, when Co and Fe were supplied together in the feed, the effect on the COD digestion rate from Co was replaced by Fe almost completely. The effect of Co and the interactive effect with other elements or YE on the bacterial activity seem to be complex, and more investigation is required.

The settling properties of granules were significantly affected by the inorganic precipitates inside the granules, as mentioned before. The percentage of the inorganic precipitates in the granules depends on the bacterial growth rate and their formation rate in the reactor system. Moreover, the bacterial growth rate is controlled by the COD loading rate and the availability of micronutrients, while the formation rate of inorganic precipitates is mainly related to the concentrations of Ca and Fe in the feed (other conditions were similar in the reactors). In this experiment, the concentration of Fe in the feed was constant (1 ppm) at all times, but the Ca concentration varied with the COD loading rate. With the increase in COD loading rate, the Ca in the feed increased and the formation rate of Ca precipitates was high. At a high COD loading rate, the bacterial growth rate also increased but variably, depending on the micronutrients in the feed, as shown in Table 2. In the reactor with a higher bacterial growth rate, the percentage of inorganic precipitates was relatively low. Therefore, the percentage of inorganic precipitates inside the granules was affected by the supplement of micronutrients. This conclusion is supported by data in Tables 2 and 4 and further data (not presented here). Tables 3, 4 and 5 show that the granules with high settling velocities contained more inorganic precipitates. A good correlation between the density and ash content of the granules ($r^2 = 0.943$) was reported by Hulshoff Pol et al. (1986). A good correlation ($r^2 = 0.684$) between the V_{50} and ash percentage of the granules from different reactors was also obtained in this experiment.

The amount of Ni, Co and Fe in the granules from the reactors at the end of each period shows that the concentration of these metals in the granules decreased steadily. This trend became more significant when the COD loading rate was increased in Periods 2 and 3. This may explain why inorganic nutrients, such as Ni, Co and Fe, were limited under a high COD loading rate. The bacteria grown under these conditions did not obtain enough minerals for their growth, therefore their concentrations in the granules were down. On the other hand, as the Ca concentration was high in the feed under a high COD loading rate, more Ca precipitates were formed inside the granules, and the concentration of Fe and Co in the granules was therefore relatively lower. The concentration of Co and Fe in the granules with different settling velocities from reactor 7 decreased with increasing concentration of Ca (Table 5). This is due to more Ca precipitates in the granules with higher settling velocities, therefore the concentration of other elements was low.

References

Andras E, Kennedy KJ, Richardson DA (1989) Test for characterizing settleability of anaerobic sludge. Environ Technol Lett 10:463-470

APHA (1985) Standard method for the examination of water and wastewater, 16th edn. American Public Health Association, Washington, D.C.

Cook AH (1958) The chemistry and biology of yeasts. Academic Press, New York, p 569

Dubourguier HC, Prensier G, Samain E, Albagnac G (1985) Granular methanogenic sludge. In: Palz W, Coobs J, Hall DO (eds) Energy from biomass. Elsevier, Amsterdam, pp 542-546

Dubourguier HC, Buisson MN, Tissier JP, Prensier G, Albagnac G (1987) Structural characteristics and metabolic activities of granular methanogenic sludge on a mixed defined substrate. In: Lettinga G, Zehnder AJB, Grotenhuis JTC, Hulshoff Pol LW (eds) Granular anaerobic sludge; microbiology and technology. Proceedings of the GASMAT workshop, 25-27 October, 1987, Lunteren, Netherlands, Pudoc, Wageningen, pp 78-86

Goodwin JAS, Wase DAJ, Foster CF (1990) Effects of nutrient limitation on the anaerobic upflow sludge blanket reactor. Enzyme Microb Technol 12:877-884

Hoban DJ, Berg L van den (1979) Effect of iron on conversion of acetic acid to methane during methanogenic fermentation. J Appl Bacteriol 47:153-159

Hulshoff Pol LW, Zeeuw WJ de, Velzeboer CTM, Lettinga G (1983) Granulation in UASB-reactors. Water Sci Technol 15:291-304

Hulshoff Pol LW, Worp JJM van de, Lettinga G, Beverloo WA (1986) Physical characterization of anaerobic granular sludge. In: Proceedings of the NVA/EWPCA Conference. Anaerobic treatment. A grown-up technology, 15-19 September 1986, Aquatech '86, Amsterdam, pp 89-101

Jarrell KF, Sprott GD (1982) Nickel transport in *Methanobacterium bryantii.* J Bacteriol 151:1195-1203

Kenealy WR, Zeikus JG (1982) Characterization and function of phosphoenolpyruvate carboxylase in *Methanobacterium thermoautotrophicum.* FEMS Microbiol Lett 14:7-10

Knetchel RJ (1977) A more economical method for the determination of chemical oxygen demand. Water Waste Eng 14:25-27

Kosaric N, Blaszczyk R, Orphan L, Lynch C, Maat D (1991) Hydraulic impact on granules in UASB reactors. Water Pollut Res J Can 26:453-474

Kuhn W, Fiebig K, Hippe H, Mah RA, Huser BA, Gottschalk G (1983) Distribution of cytochromes in methanogenic bacteria. FEMS Microbiol Lett 20:407-410

Lettinga G, Velsen AFM van, Hobma SW, Zeeuw W de, Klapwijk A (1980) Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. Biotechnol Bioeng 17:699-734

Lettinga G, Field JA, Sierra-Alvarez R, Lier JB van, Rintala J (1991) Future perspectives for the anaerobic treatment of forest industry wastewaters. Water Sci Technol 24:91-102

Mahoney EM, Varangu LK, Cairns WL, Kosaric N, Murray RGE (1987) The effect of calcium on microbial aggregation during UASB reactor start-up. Water Sci Technol 19:249-260

Oleszkiewicz JA (1989) Granulation in anaerobic sludge bed reactors treating food industry wastes. Biol Wastes 27:217-235

Scherer P, Lippert H, Wolff G (1983) Composition of the major elements and trace elements of 10 methanogenic bacteria determined by inductively coupled plasma emission spectrometry. Biol Trace Elem Res 5:149-163

Takashima M, Speece RE (1989) Mineral nutrient requirements for high-rate methane fermentation of acetate at low SRT. J Water Pollut Control Fed 61:1645-1650

Takashima M, Speece RE (1990) Mineral requirements for methane fermentation. Crit Rev Environ Control 19:465-479

Whitman WB, Ankwanda E, Wolfe RS (1982) Nutrition and carbon metabolisms in *Methanosarcina voltae.* J Bacteriol 149:852-863

Zeeuw W de (1987) Granular sludge in UASB reactors. In: Lettinga G, Zehnder AJB, Grotenhuis JTC, Hulshoff Pol LW (eds) Granular anaerobic sludge; microbiology and technology. Proceedings of the GASMAT workshop, 25-27 October 1987, Lunteren, Netherlands, Pudoc, Wageningen, pp 132-145