A long-chain fatty acid, oleate, as sole substrate in upflow anaerobic sludge bed (UASB) reactor systems

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Abstract

The response of a UASB reactor system to an influent with the salt of a long-chain fatty acid, oleic acid (sodium oleate), as sole substrate was investigated. No pelletisation was obtained, supporting one of the predictions of the biochemical model of Sam-Soon and co-workers on pelletisation that a prerequisite for pelletisation is a high hydrogen partial pressure; long-chain fatty acids can be fermented only under low hydrogen partial pressure. A well-defined sludge bed was formed but of a gelatinous texture. COD removal efficiency was low (≈ 65 per cent). As the mechanisms for gelatinous bed formation are not understood it is recommended that feasibility and pilot-scale studies be undertaken prior to full-scale application of UASB reactor systems for the treatment of wastes with a significant lipid content.

Introduction

In terms of the hypothesis on pelletisation proposed by Sam-Soon *et al.* (1987), substrates that do not generate hydrogen (e.g. acetate) in the anaerobic fermentation process, or, that do generate hydrogen but only under low hydrogen partial pressure (e.g. short and long-chain fatty acids or lipids), will not give rise to the formation of a pelletised sludge in the UASB system.

Literature reports that mixtures of short-chain fatty acids (SCFA), such as acetate and propionate, fed as substrate to UASB systems produced poor quality pellets (De Zeeuw and Lettinga, 1980; Hulshoff Pol *et al.*, 1982; Hulshoff Pol *et al.*, 1984; Wiegant and Lettinga, 1985; Dolfing, 1987) whereas no pellet formation was expected in terms of the hypothesis. However, Sam-Soon *et al.* (1990a) were of the opinion that the poor pellet formation that had been observed was due to a substantial COD fraction of yeast extract in the SCFA influent.

With olive oil processing (lipid containing) wastes no pelletisation was observed, as expected in terms of the hypothesis. A sludge bed was formed, but it was uniform, gelatinous and smooth in consistency with good settleability (Boari *et al.*, 1984). However, olive oil processing wastes are complex and contain in addition to lipids, organics such as polyphenols, sugars, polyalcohols and proteinaceous compounds (Boari *et al.*, 1984) — it is not clear whether the presence of these organics influenced the response observed and thus there is uncertainty whether the observations of Boari *et al.* provide substantive support for the pelletisation hypothesis. To rectify this situation a study was undertaken on the response of a UASB system treating an influent consisting solely of a salt of a defined long-chain fatty acid, sodium oleate.

Biochemical background

Lipid fermentation

Lipids (fats and greases) are first hydrolysed to organic monomers such as long-chain fatty acids (LCFA) and glycerol. Neutral fats are hydrolysed as follows (Hanaki *et al.*, 1981):

$$\begin{array}{l} CH_2OCOR_1 \\ | \\ CHOCOR_2 + 3H_2O \rightarrow \\ | \\ CH_2OCOR_3 \end{array} \quad \begin{array}{l} R_1COOH \\ \\ R_2COOH + \\ \\ R_3COOH \end{array} \quad \begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} \qquad (1)$$

neutral fat → long-chain fatty acids + glycerol

where R_1, R_2 and R_3 are alkyl groups.

The hydrolysis reaction above, to yield free LCFA, is reported to be rapid (Heukelekian and Mueller, 1958). The LCFA are degraded further by "H_2-producing acetogenic bacteria" (Hanaki *et al.*, 1981) via β-oxidation to short-chain fatty acids (SCFA) and hydrogen (H_2); even-carbon numbered LCFA usually are degraded to acetic acid and H_2 and odd-carbon LCFA to acetic acid, propionic acid and H_2 (Jeris and McCarty, 1965; McInerney *et al.*, 1981). Examples are:

Even-numbered carbon chain, palmitic acid:

$$CH_3(CH_2)_{14}COOH + 14H_2O \rightarrow 8CH_3COOH + 14H_2 \qquad (2)$$

Odd-numbered carbon chain, heptanoic acid:

$$CH_3(CH_2)_5COOH + 4H_2O \rightarrow CH_3CH_2COOH + 2CH_3COOH + 4H_2 \qquad (3)$$

Thermodynamically both these reactions are feasible only if the hydrogen partial pressure ($\bar{p}H_2$) is very low ($\bar{p}H_2 < \pm 10^{-4,1}$ atm) - Novak and Carlson (1970) indicated that H_2 generated during the degradation of LCFA inhibits the reaction; Heukelekian and Mueller (1958) reported that LCFA were not degraded during the acid-forming phase where methane was **not** produced; presumably methane formation from hydrogen would reduce the $\bar{p}H_2$ and hence allow degradation of LCFA.

Propionic acid generated in the above reaction [Eq. (3)] is converted to acetic acid by acetogenesis, a process which also can take place only at low $\bar{p}H_2$ ($\bar{p}H_2 < \pm 10^{-4,1}$ atm). This reaction is:

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2 \qquad (4)$$

Acetic acid and hydrogen generated in Eqs. (2), (3) and (4) are oxidised to form methane by methanogenesis, as follows:

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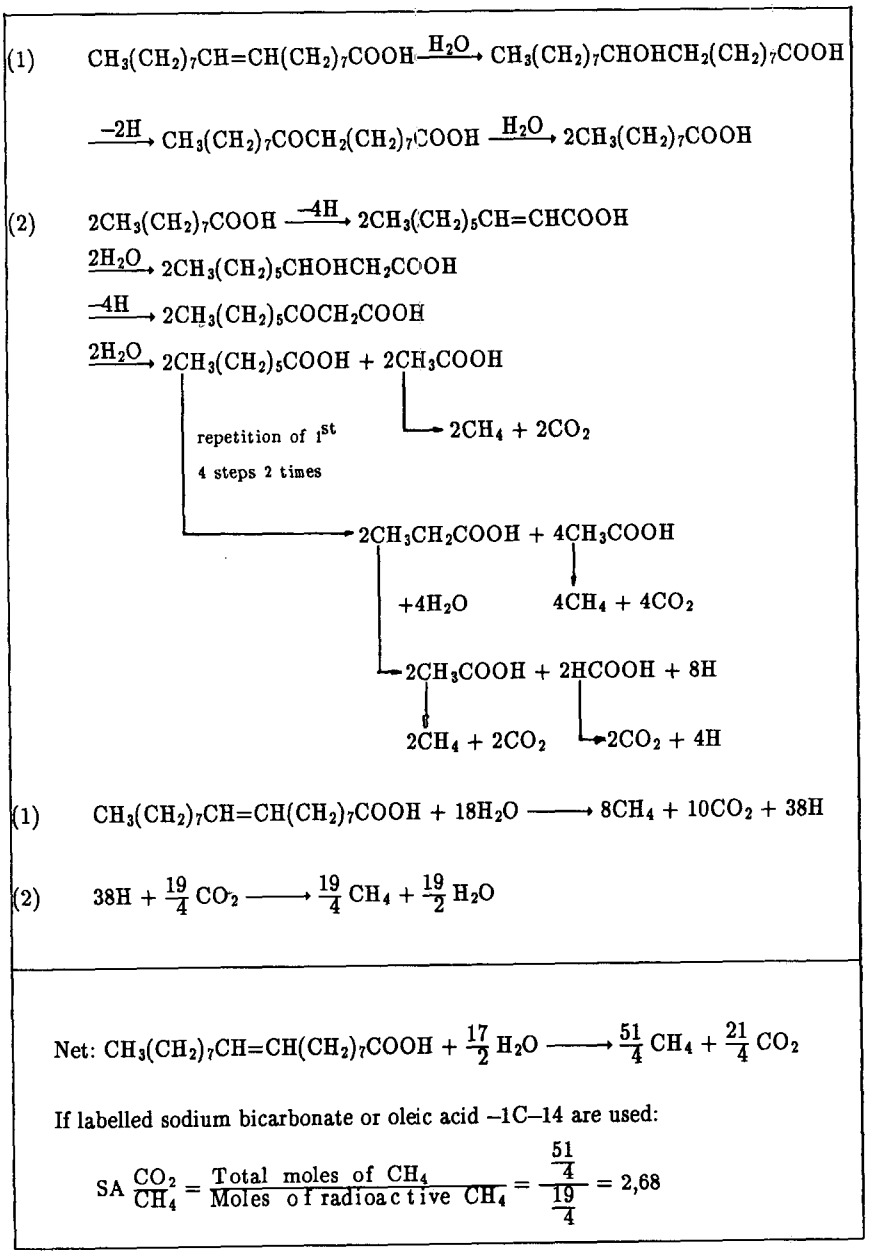

Figure 1
Possible pathways for oleic acid fermentation with propionic and acetic acids as intermediates (after Weng and Jeris, 1976)

For hydrogen (mediated by hydrogenotrophic methanogens):

$$4H_2 + H_2CO_3 \rightarrow CH_4 + 3H_2O \quad (5)$$

and for acetic acid (mediated by acetoclastic methanogens):

$$CH_3COOH + H_2O \rightarrow CH_4 + H_2CO_3 \quad (6)$$

Details of the thermodynamics and other aspects relating to Eq. (4), (5) and (6) have been given by Sam-Soon *et al.* (1990a).

Substrate selection

The limiting rate in the fermentation of a lipid to methane is in the conversion of LCFA to SCFA (Hanaki *et al.*, 1981). Hence, in studying the response of a UASB system to a lipid substrate, one needs only to use the monomer LCFA as the influent substrate.

Of the LCFA, sodium oleate was selected as an appropriate substrate for four reasons:

- It is more soluble than other LCFA at room temperature.
- It is an unsaturated fatty acid with an even-numbered carbon chain (C = 18); being even-numbered acetic acid will be the predominant SCFA produced.
- Its degradation rate is reported to be faster than any of the saturated LCFA (Vishwanathan *et al.*, 1962).
- Its fermentation pathway has been described (Weng and Jeris, 1976).

Oleic acid fermentation pathway

Organisms take up oleic acid in the undissociated form. Conse

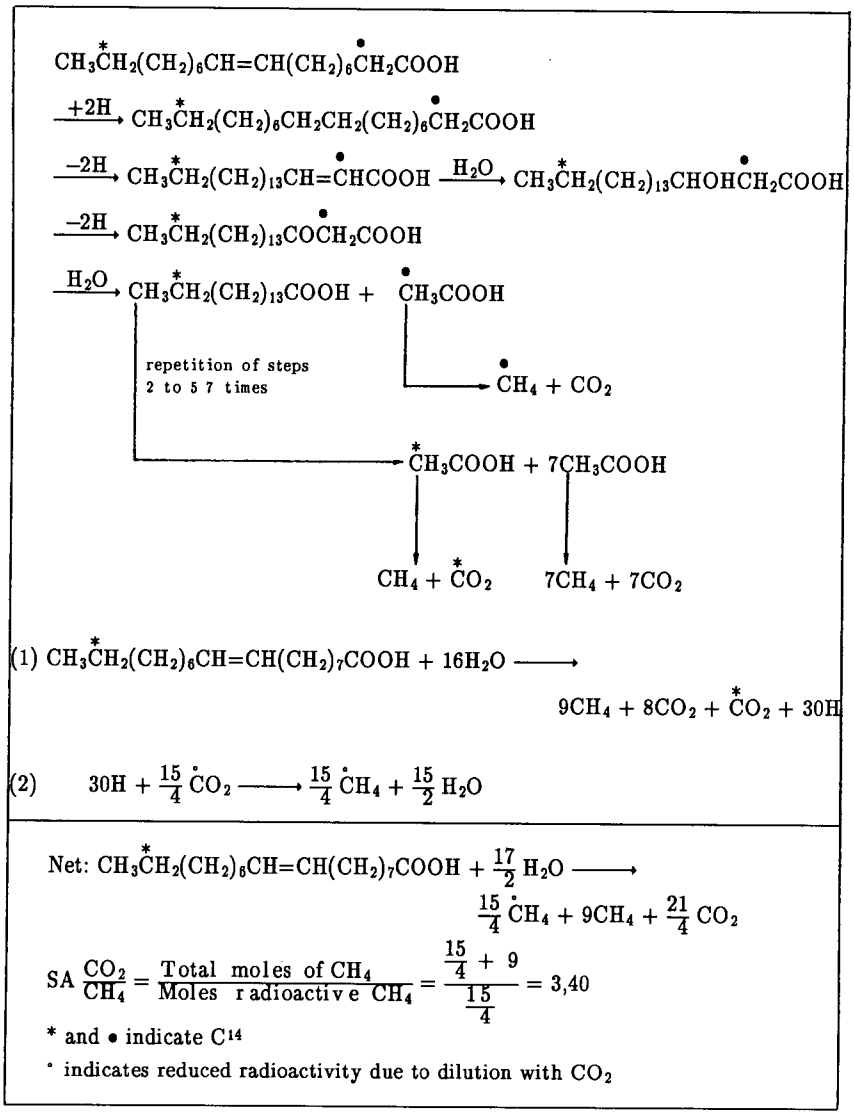

Figure 2
Possible pathways for oleic acid fermentation with acetic acid only as intermediate (after Weng and Jeris, 1976)

quently, in describing its anaerobic fermentation one may commence from the undissociated form. For experimental application, however, oleic acid *per se* (HOl) is not the most suitable form because it is insoluble at normal room temperature. The salt sodium oleate (NaOl) is soluble. Oleic acid and its salts form a weak acid-base system in aqueous solution:

On addition to water NaOl will dissociate:

$$NaOl \rightarrow Na^+ + Ol^- \quad (7)$$

A fraction, depending on the pH, reforms as HOl:

$$Ol^- + H^+ \rightarrow HOl \quad (8)$$

This fraction provides the undissociated form for uptake by the organisms.

The undissociated oleic acid is taken up by H_2-producing acetogenic bacteria and degraded via β-oxidation to SCFA and hydrogen. Despite the fact that oleic acid has an even-numbered carbon chain, Weng and Jeris (1976) found that acetic acid only or acetic plus propionic acids can be produced during β-oxidation of oleic acid. However, propionic acid usually is produced in trace quantities only. Weng and Jeris set out two biochemical pathways for the β-oxidation to explain the respective processes (Figs. 1 and 2 respectively). In both pathways the reactions are thermodynamically unfavourable unless the $\bar{p}H_2$ is maintained at an extremely low level. Any propionic acid that may be formed is subsequently converted to acetic acid and hydrogen, according to Eq. (4). Hydrogen and acetic acid are converted to methane, according to Eqs. (5) and (6) respectively. These reactions also are given in Figs. 1 and 2, together with equations summarising the complete fermentation of oleic acid to methane and carbon dioxide, i.e.:

$$CH_3(CH_2)_7CH=CH(CH_2)_7COOH + \frac{17}{2}H_2O \rightarrow \frac{51}{4}CH_4 + \frac{21}{4}CO_2 \quad (9)$$

Experimental

The behaviour of a UASB system with sodium oleate as substrate was investigated at 30°C. A 9 ℓ UASB reactor (for reactor configuration see Fig. 3) was seeded with 3 ℓ of pelletised sludge obtained from a UASB reactor at 30°C which had been fed with a glucose substrate (COD concentration ≈ 2 500 mg/ℓ; flow rate = 30 ℓ/d). To acclimatise the seeding sludge, a mixture consisting of glucose (COD ≈ 2 000 mg/ℓ) and sodium oleate (COD ≈ 500 mg/ℓ) with total COD 2 500 mg/ℓ was fed at a flow rate of 15 ℓ/d (i.e. organic loading = 4,17 kgCOD/m³ reactor volume.d). The feed was supplemented with trace elements and essential nutrients (detailed composition is given by Sam-Soon *et al.*, 1987); the nutrient NH_3-N was supplemented in excess, 68 mgN/ℓ. (One of the prerequisites for pellet formation is an excess of NH_3-N, Sam-Soon *et al.*, 1990b). For pH control, the influent was buffered by addition of 100g $NaHCO_3$ per 15 ℓ of feed. The feed was made up with warm tap water to ensure that the oleate dissolved completely. The feed bucket contents were continually stirred and kept at room temperature (20°C). Normally the feed would be kept at about 5°C to reduce degradation action in the feed bucket, but this could not be done with oleate as it would solidify at such a low temperature.

Over a period of about 5 months the oleate concentration in the feed was increased in steps of 500 mgCOD/ℓ, and simultaneously the glucose concentration was decreased by 500 mgCOD/ℓ, until the feed consisted only of sodium oleate (2 500 mgCOD/ℓ). A step change in feed composition was effected only after the overall COD removal had shown a stable response for five consecutive days.

Initially, when the feed consisted of glucose (2 000 mgCOD/ℓ) and oleate (500 mgCOD/ℓ), 100g $NaHCO_3$ per 15 ℓ of feed were required to maintain the pH around 7,2 in the liquid above the sludge bed. As the oleate fraction in the feed increased so the pH in the reactor tended upwards and the mass of $NaHCO_3$ added had to be decreased to maintain the pH around 7,2. By the time the feed consisted of 100 per cent oleate, the mass of $NaHCO_3$ added had been reduced to less than one quarter of the initial mass added. However, even with this low alkalinity supplementation, the pH continued to increase to above 7,7. This behaviour was due to the interaction of a number of factors:

> Dissociation of the sodium oleate, and the subsequent uptake of the substrate by the organisms in the undissociated oleic acid form; this increases the alkalinity and decreases the acidity [Eqs. (7) and (8)] which would tend to raise the pH. In changing from glucose to oleate as substrate the magnitudes of generation of acetic and propionic acids and CO_2 changed; all these influence alkalinity, acidity and thus pH. It is likely that the dominant effect was due to the reduced generation of acetic and propionic acids, causing corresponding reductions in acidity generation and alkalinity loss.

It is the compounded effect of all these factors that gave rise to the observation that, as the sodium oleate fraction increased, addition of $NaHCO_3$ alkalinity could be decreased. However, it was decided that the $NaHCO_3$ mass addition should not be reduced below 25g $NaHCO_3$ per 15 ℓ influent, to ensure that CO_2 limitation (for methanogenesis from H_2) could not arise. Accordingly, the minimum $NaHCO_3$ addition was fixed at 25 g/15 ℓ influent and the pH was controlled by addition of a strong acid, HCl. With 100 per cent oleate in the feed, addition of 100 mℓ 1% (v/v) HCl to 15 ℓ of feed was sufficient to keep the pH of the reactor at approximately 7,3.

When the feed consisted of 100 per cent oleate, once steady state had been established, profiles of COD, TKN, free and saline ammonia, and hence by difference organic nitrogen (orgN), propionic and acetic acids (HPr and HAc) and pH were taken up the bed, as follows: Samples were drawn from the sampling ports, from the top (port No. 11) down, and immediately filtered through 0,45 μm filter paper; COD, TKN, NH_3-N and orgN of the filtrate were measured according to Standard Methods (1985); HPr and HAc were measured using a Packard 417 gas chromatograph fitted with a 6 ft x 1/8" GP 10% SP-1200 (1% H_3PO_4) on 89/100 Chromasorb WAW glass column. The pH was measured by drawing samples from the UASB reactor directly into a sealed chamber with a fitted pH probe.

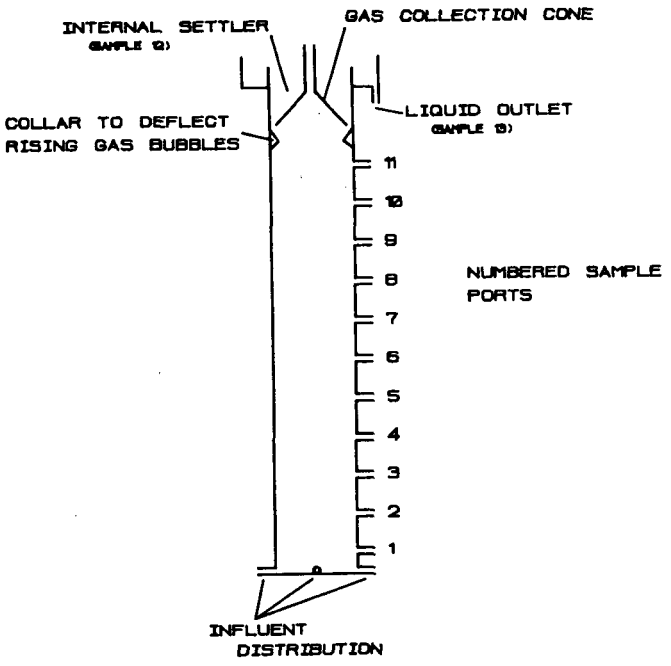

Figure 3
Schematic diagram of the laboratory-scale UASB reactor (internal diameter = 100 mm, height to port No. 11 = 950 mm) showing the numbered sampling ports.

Results

Sludge bed characteristics

As the fraction of oleate in the feed increased, the pellets in the sludge bed progressively disintegrated. However, no pellet debris discharged to the liquid above the sludge bed; the pellets and the debris appeared to be encapsulated by a gelatinous mass that accumulated in the sludge bed. By the time the feed consisted of 100 per cent oleate, the sludge had changed completely to a uniform gelatinous mass, off-white in colour. Microscopic examination of the mass, however, indicated that pellet debris still was present but this did not show up in the visual appearance of the sludge. Over the course of the experiment (about 6 months) the bed volume of the sludge decreased; with 100 per cent oleate feed, the bed volume stabilised at about 2,4 ℓ from an initial bed volume of 3 ℓ. The density of the sludge was low (about 15 000 mgVSS/ℓ) compared to that observed in a pelletised bed (about 35 000 mgVSS/ℓ). However, the sludge mass was well defined with a compact appearance and did not seem to be disturbed by the shear action of escaping gas bubbles — the bubbles travelled up the bed and left a well defined trail

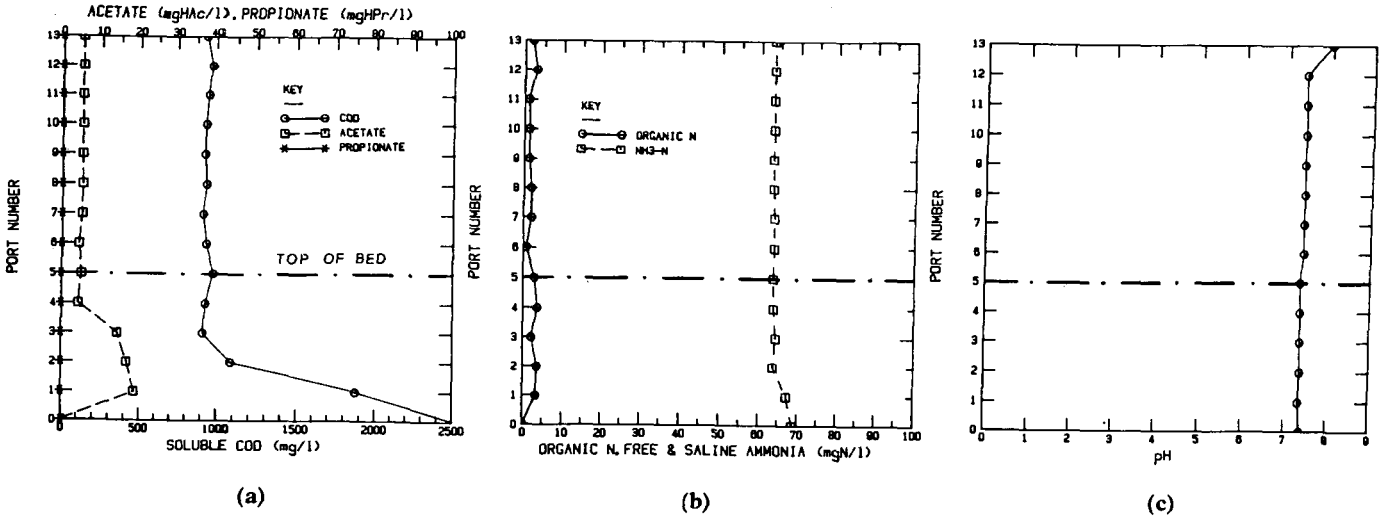

Figure 4
Concentration and pH profiles observed in a single UASB system with sodium oleate substrate (influent COD concentration = 2 518 mg/ℓ, flow rate = 15 ℓ/d)

which slowly closed up. The sludge mass did not shed fines so that no sludge blanket formed above the bed.

System response

As the oleate fraction increased in the feed, the overall COD removal decreased, and when the feed consisted of 100 per cent oleate (2 500 mgCOD/ℓ) at a loading rate of 4,17 kgCOD/m³ reactor volume.d, the effluent COD stabilised at 875 mgCOD/ℓ (COD removal 65 per cent). At this loading rate NH_3-N removal was 5,2 mgN/ℓ; effluent soluble organic nitrogen 1,9 mgN/ℓ; pH 7,40; effluent acetate 13 mgHAc/ℓ; effluent propionate zero.

Bed profiles

Concentration profiles of soluble COD, SCFA, NH_3-N, organic nitrogen and pH along the line of flow in the reactor are shown in Fig. 4 (a), (b) and (c). The profiles exhibit:

- No distinct zones of behaviour (c.f. profiles with glucose as substrate, Sam-Soon *et al.*, 1990a).
- No propionic and very low acetic acid concentration, maximum 19 mgHAc/ℓ.
- Very low NH_3-N removal, 5,2 mgN/ℓ.
- A relatively low percentage COD removal, 65 per cent.
- Virtually no pH change in the sludge bed.

Discussion

In this study, with 100 per cent oleate in the feed, the acetic and propionic acid bed profiles (Fig. 4a) show that only acetic acid was generated; this would indicate that the metabolic pathway producing acetic acid only was the predominant one. This metabolic pathway operates only under conditions of low pH_2. (Even if propionic acid would have been generated this would not have been indicative of a high pH_2 — the production of SCFA such as propionic and butyric acids during the fermentation of odd-carbon number LCFA also takes place only under low pH_2 conditions). Accepting that pH_2 was low, one of the requirements for pelletisation was not fulfilled. Non-pellet forming behaviour is confirmed by lack of pellet production; the low uptake of NH_3-N; and the low production of organic nitrogen in the bed — in terms of the hypothesis on pelletisation the NH_3-N uptake and organic nitrogen production should have been high if polypeptide/pellet formation had taken place. Thus, in this study, the predictions of the biochemical model for pelletisation of Sam-Soon *et al.* (1987), that pelletisation is not to be expected with lipids as influent, is supported.

An interesting feature in the treatment of oleate in the UASB system is that a well-defined sludge bed was formed, but of a gelatinous texture. This type of bed and its texture appears to be very similar to that obtained in a UASB system treating an olive oil processing waste water (Boari *et al.*, 1984). Boari *et al.* noted that the sludge bed condition was very poised; if disturbed by mechanical agitation the integrity of the bed was destroyed and the bed was lost in the effluent. This behaviour was not investigated in our study, but it is one worth noting should a UASB system be contemplated for the treatment of lipid wastes.

In this study, the causes for the formation of the gelatinous sludge bed are left unresolved. The causes for gelatinous sludge bed formation are distinctly different from those for pelletised bed formation and merit research both from theoretical and practical points of view. One possibility that merits enquiry is that the gel formation may be a physical phase change, from soluble oleic acid to a gel type aggregation of oleic acid micelles, i.e. a physical phenomenon perhaps mediated biologically.

From the theoretical predictions of the biochemical model for pelletisation and from observations in this study, treatment of liquid wastes having an appreciable lipid concentration (for example from dairies and abattoirs) may give rise to beds with both pelletised and gelatinuous material present. Whether these can co-exist, and whether the system will operate effectively (with regard to COD removal and stable bed formation), cannot be predicted *ab initio* and will need to be established experimentally. For the present, a UASB system to treat such wastes should not be contemplated without feasibility and pilot-scale studies on the particular waste.

Conclusions

- In an experimental UASB system with a long-chain fatty acid,

oleate, as the only substrate source, no biopellet formation was obtained. In terms of the hypothesis of Sam-Soon *et al.* (1987) this was not unexpected. The hypothesis proposes that excess amino acid production (which is discharged to the surrounding liquid as organic nitrogen and polypeptide) arises from a disequilibrium created in the hydrogenotroph *M.* Strain *AZ* (which cannot synthesise the amino acid cysteine). Should an environment with a high hydrogen partial pressure be present, with excess ammonium nitrogen and a deficiency of cysteine, cell synthesis is limited to the supply of cysteine. The other amino acids are produced in excess by the organism and equilibrium is re-established by discharging these as soluble organic nitrogen and polypeptides. However, anaerobic breakdown of long-chain to short-chain fatty acids can take place only under very low hydrogen partial pressures and hence there is little stimulation of the *M.* Strain *AZ* species to produce amino acids (and production of these is unlikely to be in excess of the cysteine supply from death of other organisms). Accordingly, little or no excess amino acids (as, say, polypeptides) would be discharged and pellet formation is not expected. Product formation up the bed supports this conclusion: There was a low ammonium nitrogen uptake, i.e. little nitrogen removal for amino acid formation and a low soluble organic nitrogen discharge to the surrounding liquid, i.e. little evidence pointing to excess extracellular polypeptide formation.

- The sludge bed formed with oleate as substrate was well defined but of a gelatinous texture similar to that obtained in a UASB system treating an olive oil processing waste water (Boari *et al.*, 1984). Clearly the causes for the gelatinous sludge bed formation are very different from those giving rise to biopellet bed formation and merit detailed enquiry.

Acknowledgements

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