Development of a method to enhance granulation in a laboratory batch system

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Abstract

The success and efficiency of the UASB process are dependent on the formation of active granular biomass and since this is a slow process, one of the main problems in the application of the technology remains the long start-up periods. Batch cultures with lactate, glucose or sucrose as substrate, were seeded with anaerobic sludge and incubated in shake waterbaths over a period of 14 d. For all substrates, a drop in pH within the first 2 d was experienced. In the glucose and sucrose units the pH dropped to 6.0 and to below 5.5, respectively within the first 24 h. Thereafter, a continual drop was experienced, eventually resulting in system acidification. With the lactate units, the pH dropped to 6.5 by Day 2, with a subsequent climb until the pH stabilised at around 6.7 to 6.9. The volatile fatty acid (VFA) profiles of all the units showed an increase of acetic and propionic acids, with the latter at the highest concentration during the first 5 d, corresponding to the decrease in pH. An increase in granulation was observed for the glucose (354%) and lactate (559%) units, but no granulation increase was found for the sucrose units. The increase in granule formation indicated that granulation may be enhanced in batch systems over a shorter period and that the granulation process is facilitated by a rapid drop in pH at the start, resulting from the major increase in propionic and acetic acids, followed by a subsequent increase and stabilisation in pH, and an increase followed by a steady decrease in propionic and acetic acid concentrations until the formation stabilised.

Introduction

The upflow anaerobic sludge blanket (UASB) process is one of the most extensively applied anaerobic treatment systems in the world (Lettinga et al., 1997; Weiland and Rozzi, 1991). In this bioreactor design (Lettinga et al., 1980) the biomass retention is promoted by bacterial self-aggregation into dense granules (El-Mamouni et al., 1997) which enhances the performance, since the good settling properties of granules minimise biomass washout and the close cell packing optimises the interspecies exchange of metabolites. Although many explanations have been given, the mechanism of granule formation is still not clear (Schmidt and Ahring, 1993; Slobodkin and Verstraete, 1993).

When the UASB system is seeded only with non-granular anaerobic sludge, it can take several months before a highly effective granular bed can be cultivated. This clearly restricts the general application in countries where granules from operating UASB systems are not readily available, unless the granulation reaction can be induced in other treatment systems. Since the operational efficiency and performance of these systems are mainly dictated by the formation, amount and specific activity of the granules, the rather extended start-up periods (Wentzel et al., 1994) can limit the potential use of these systems. The full potential of the UASB system cannot be exploited until the granule-formation conditions are better defined. In this study a method was developed to enhance granulation in batch systems.

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Received 27 September 2000; accepted in revised form 13 September 2001.

Materials and methods

Experimental set-up

A linear-shake waterbath (Scientific Manufacturing, Paarden Eiland, Cape Town) was used to cultivate biomass in a batch system at 35°C and 150 $r \cdot min^{-1}$. The batch systems consisted of units containing 400 mℓ of each specific sterile growth medium inoculated with 50 mℓ sludge from the anaerobic tank of a local sewage works. Daily, for a period of 14 to 26 d, and after allowing the sludge to settle, 100 mℓ of the units upper volume was removed and replaced with one of the following: Lactate medium (Riedel and Britz, 1993) which consisted of 20.0 $g \cdot \ell^{-1}$ lactate, 5.0 $g \cdot \ell^{-1}$ yeast extract, 2.0 $g \cdot \ell^{-1}$ peptone and 1 $m\ell \cdot \ell^{-1}$ Tween 80; Sucrose medium (Quarmby and Forster, 1995) which consisted of 5.0 $g \cdot \ell^{-1}$ sucrose, 0.5 $g \cdot \ell^{-1}$ yeast extract, 1.0 $g \cdot \ell^{-1}$ urea and 2 $m\ell \cdot \ell^{-1}$ Tween 80; and glucose medium (Lens et al., 1993) which consisted of 5.0 $g \cdot \ell^{-1}$ glucose, 0.5$g \cdot \ell^{-1}$ yeast extract and 1.0 $g \cdot \ell^{-1}$ urea.

A trace element solution (10 $m\ell \cdot \ell^{-1}$) (Nel et al., 1985) was added to each of the media used. To prevent the too rapid acidification of the units, 10.0 $g \cdot \ell^{-1}$ KH_2PO_4 was added to each medium. The pH of all the media was poised at 7.0 using 1M NaOH and the media steam sterilised at 121°C for 15 min.

Analytical procedures

Granule increases were determined by directly counting the number of granules formed over time by using a round flat-bottomed glass container with a graded grid underneath. For each count, a 10 mℓ sample was withdrawn, diluted 10 times with saline water and then counted visually. The granule nuclei at the start of each study were very small, and in combination with a cloudy and very viscous solution, it was difficult to always accurately detect the black

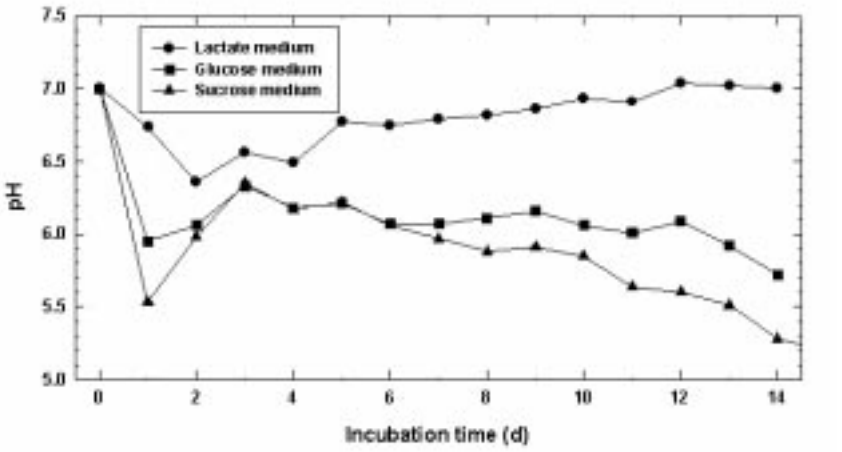

Figure 1
Influence of lactate, sucrose and glucose on system pH over 14 d in batch systems (values are averages of triplicates)

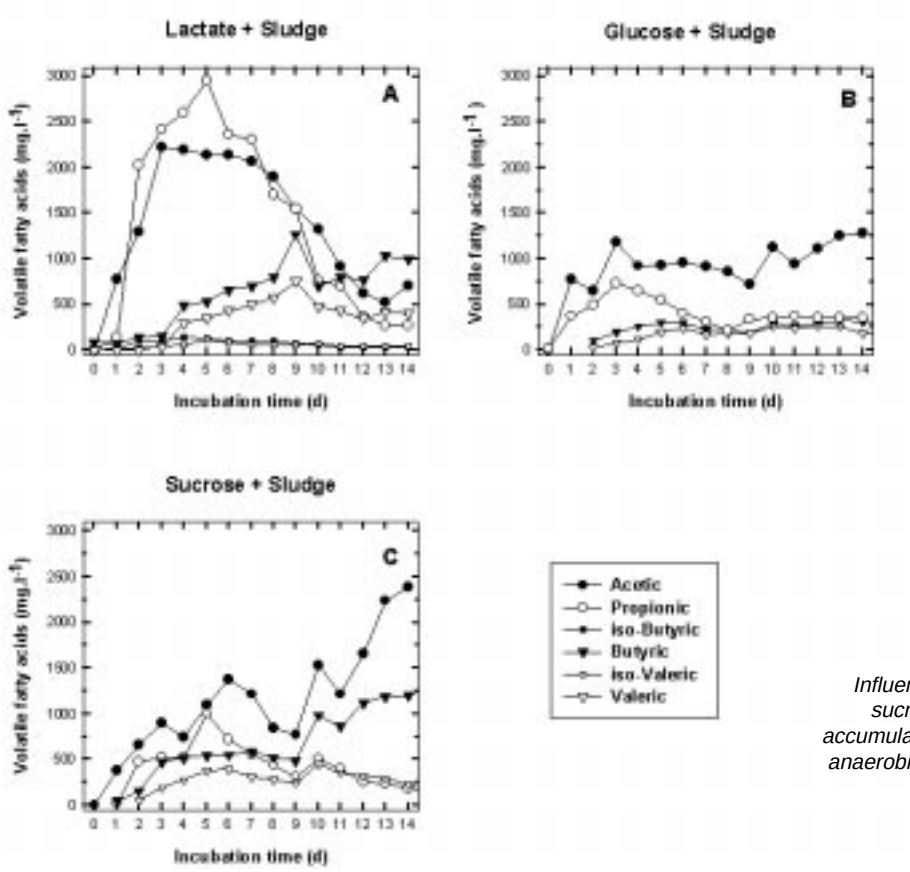

Figure 2
Influence of lactate (A), glucose (B) and sucrose (C) on the volatile fatty acid accumulation in batch systems inoculated with anaerobic sludge and operated over a period of 14 d at 35°C

nuclei.

The following parameters were monitored: pH; total solids (TS); total non-volatile solids (TNVS) and total suspended solids (TSS) (Standard Methods, 1992). Volatile fatty acids (VFAs) were determined using a Varian Model 3700 gas chromatograph equipped with a flame ionisation detector and a bonded phase 007FFAP (Quadrex Corp., New Haven) fused silica capillary column with a 30 m length and diameter of 0.32 mm. The column temperature was initially held at 105°C for 5 min, then increased at a rate of 10°C per min. to 219°C and held for 5 min. The injector temperature was set at 250°C, while the detector was set at 260°C. Nitrogen was used as carrier gas at a flow rate of 2.5 $m\ell \cdot min^{-1}$.

Results and discussion

Since the start-up of a UASB bioreactor is a lengthy and complicated process, it was decided to make use of simple shaking batch systems to simulate anaerobic bioreactor type conditions on a small scale. Vanderhaegen et al. (1992) showed in growth studies with granules in batch cultures that the factor foremost in influencing the build-up in the reactor of granular sludge was the presence of high-energy sugars (Thaveesri et al., 1994). For this study, glucose (Lens et al., 1993), sucrose (Quarmby and Forster, 1995) and lactate (Riedel and Britz, 1993) were selected as carbon sources that have been reported to be granulation enhancers.

It has also been reported in the literature that shear forces may be instrumental in providing cell growth in granules, as these forces gave the granule cells a selective advantage over free suspended cells (Bossier and Verstraete, 1996). Thus, in this study these forces were simulated by incubating the batch systems in linear-shake waterbaths at 150 $r \cdot min^{-1}$. To further put environmental stress conditions on the sludge populations, each day a portion of the

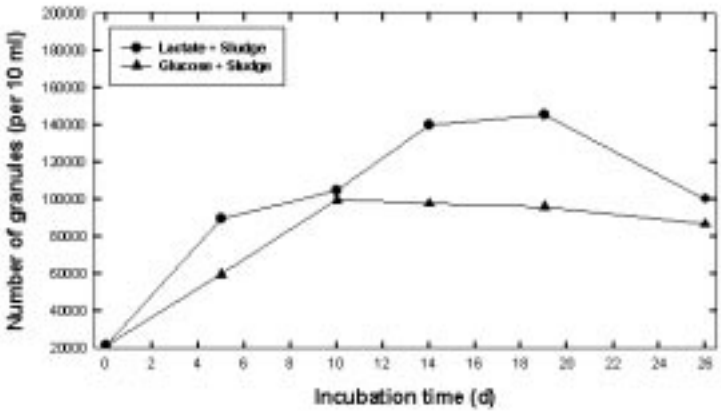

Figure 3
Influence of different carbon sources (lactate and glucose) on granule formation in batch systems inoculated with anaerobic sludge and shaken at 150 r·min^{-1} over a period of 26 d at 35°C

Photo 1
An example of the granular sludge obtained after the batch granulation enhancement with lactate as carbon source. Granule sizes vary from pinpoint to 2 mm.

batch units' volume was removed and replaced with the sterile, easily degradable substrates so as to simulate anaerobic bioreactor operational parameters under sudden increases of the organic loading rate.

pH profiles

The pH of the units was monitored as an indication of metabolic activity (production of volatile fatty acids) at the start and during the studies. In the experimental batch studies (Fig. 1), where the units (in triplicate) had been inoculated with anaerobic sludge and incubated at 35°C over a period of 14 d, the pH for the Lactate units dropped to about 6.5 within the first 2 d, with a slight increase from there onwards until the pH stabilised at around 6.7 to 6.9 (Fig. 1). This stabilisation persisted with an extended incubation of up to 26 d.

For the sucrose units the pH also dropped within the first 24 h from 7.0 to 5.5 (Fig. 1) and from 7.0 to about 6.0 for the glucose units (Fig. 1). This was expected due to the strong acid formation from the specific carbohydrates in spite of a high buffering capacity with 10.0 g·ℓ^{-1} KH_2PO_4. However, in both cases, from Day 2 to Day 4 the pH increased. Thereafter, a continual drop was experienced until Day 15 of the experiment. When the glucose and sucrose units were incubated for a longer time (up to 26 d), the pH dropped to below 5.0 resulting in acidification and system failure.

Volatile fatty acid profiles

The VFA profiles of the lactate units (average of triplicates) obtained during the study are shown in Fig. 2A. The data showed that an initial sharp increase in acetic and propionic acids was found. During the first 5 d the propionic acid was the highest. However, after Days 6 to 7 both these acids decreased again. By Day 14, the propionic and acetic acid concentrations had stabilised with a corresponding stabilisation of the pH data (Fig. 1). The butyric and valeric acids showed smaller increases. An extended incubation of up to 26 d showed no further major changes in the VFA profiles nor further changes in the pH.

In the case of the VFA profiles of the glucose units (Fig. 2B), the acetic and propionic acid concentrations showed a similar strong increase from the first day and the propionic acid was also found to be the major VFA for the first 5 d. However, a steady increase in the acetic acid concentrations was found and in conjunction with the decrease in pH (Fig. 1) indicated the start of

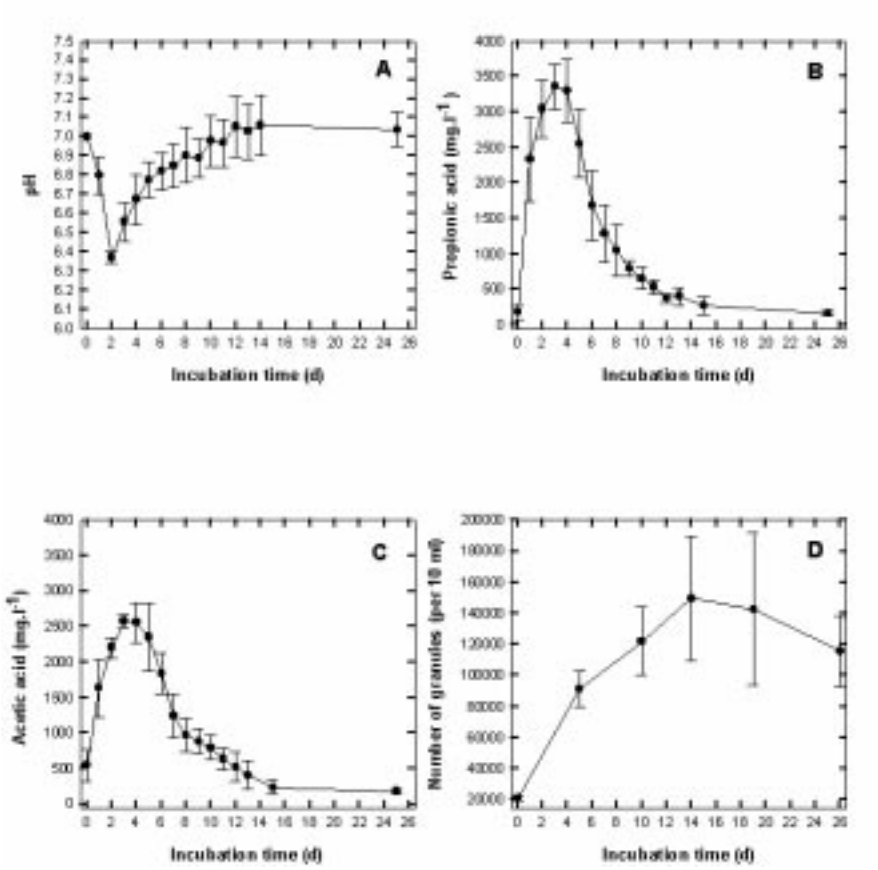

Figure 4
pH changes (A), variation in volatile fatty acid accumulation (B and C) and granule formation (D) in 10 lactate batch units inoculated with different batches of anaerobic sludge and shaken at 150 r·min^{-1} over a period of 26 d at 35°C. The standard deviation was used as the error-bar length

instability in the glucose units. With an extended incubation of 26 d, it was found that the glucose units totally acidified with the pH dropping to below 5.0.

In the case of the sucrose units (Fig. 2C), a similar but faster acidification took place with most of the VFAs showing increases which corresponded with the decrease in pH.

From the VFA concentrations formed in the different batch units, the highest amounts were produced in the lactate units (Fig 2A) where large concentrations of propionic and acetic acid were formed (2 000 to 3 000 mg·ℓ^{-1}) within the first 4 to 5 d of incubation.

In all the cases, after 6 d incubation in the linear-shake waterbaths at 35°C, a visual increase in viscosity, possibly due to the formation of ECP (not determined), was evident. The higher concentration of propionic to acetic acid formed during this time is typical of the growth and metabolic profiles of, for example, members of the genus *Propionibacterium* (Riedel and Britz, 1993). However, this needs further investigation. It can be argued that the growth conditions and the use of lactate as a carbon source probably led to a more competitive environment for lactate, utilising propionic-acid-producing bacteria that are known to secrete extracellular polymers (ECPs). The studies with lactate as carbon source were repeated on 10 separate occasions over a four-month period and very similar VFA and pH profiles were obtained suggesting that, when using lactate as carbon source, a repeatable metabolic pathway is obtained. It is thus possible that a sudden increase in a readily degradable substrate leads to a shift in the population dynamics, giving propionic acid producers a competitive advantage, leading to the formation of ECP.

Granule formation

The influence of the lactate and glucose on sludge granulation is given in Fig. 3. In the lactate and glucose units, the formation of very small granules (sizes varying from pinpoint to 2 mm) were found by Day 14 with the lactate granules being darker in colour (Photo I). In addition, it was observed that in comparison to the original sludge inoculum, a clear fast-separating granular sludge layer was evident at the end of the incubation period. For the lactate units, an increase in granule number of 559% was found. Fewer granules were formed in the glucose units and these were, in contrast to those from the lactate units, a brownish colour and bigger. The granules grown on a glucose medium showed an increase of 354% over the first 10 d. Thereafter, a slight decrease was found over the rest of the incubation period. In the sucrose units no granular sludge enhancement was found even though the viscosity of the medium increased visibly. The size of the granules present in the lactate and glucose units varied from pinpoint to 2 mm in size. The pinpoint-sized granules represented ca. 80% and the 1 to 2 mm size granules ca. 20% of the total granules present per 10 mℓ of granular sludge sample, respectively.

The growth conditions and the use of lactate as carbon source probably led to a more acceptable environment for the propionic-acid-producing bacteria. The data, with standard error bars, from 10 separate repetitions during a four-month period, of the Lactate units inoculated with different batches of anaerobic sludge are illustrated in Fig. 4. From these it can be seen that even with different batches of anaerobic sludge, characteristic acetic and propionic acid and pH profiles were obtained suggesting that, when using lactate as carbon source, a repeatable metabolic pathway is obtained. These data confirm the fact that a sudden increase in a readily degradable substrate leads to a shift in the population dynamics, probably giving the lactate utilising-propionic acid producers a competitive advantage (Riedel and Britz, 1993), leading to the formation of ECP which could serve as an alternative hydrogen sink mechanism.

Data from the study showed that metabolic activity and

granulation were very dependent on the type of anaerobic sludge used. The sludge obtained from the local sewage works was found to differ from week to week. The age of the sludge also had an effect on granule formation. It was found that if a fresher sample was used, acid production and granulation occurred much more speedily, than when an older sample was used. However, besides the age and texture of the sludge, it was extremely difficult to grade the raw sludge.

The granule formation data for the anaerobic sludge inoculated units showed, in most cases, a decrease in numbers from Day 15 onwards. At first this was considered to be a disintegration of the granules, but when the granulation was followed under image analysis systems, it was found that the decrease in numbers was an aggregation of smaller granules into larger granules.

The granulation data showed that a need exists for a method to standardise the raw anaerobic sludge that is to be used as inoculum for batch granule cultivation. Some researchers have used volatile solids (VS) and total solids (TS) of the inoculum as a means of standardisation (Ahring & Schmidt, 1992). The problem with these determinations is that the number of granules cannot be determined in this way, but rather the solids content of the total biomass of the inoculum. In this study it was also found that TS and VS determinations at the end of the incubation time do not give an indication of granule enhancement in terms of numbers of granules. By determining the TS and VS of each unit after the 14 d incubation period, the TS and VS of the total biomass of the unit is being determined, be it granules, some unutilised carbons in the growth medium or even loose microbial cells.

Conclusions

The aim of this study was to determine if granulation could be enhanced in batch culture units inoculated with raw anaerobic sludge. From the fatty acid profiles formed in the different batch units, the most active VFA production system was clearly found in the lactate units. Higher concentrations of propionic and acetic acids were formed within the first 4 to 5 d of operation and the higher percentage of propionic to acetic acid formed during this time was typical of the growth and metabolic profiles of members of the propionic-acid-producing bacteria like the *Propionibacterium* and *Veillonella* (Riedel and Britz, 1993; Slobodkin and Verstraete, 1993). The growth conditions and the use of lactate as carbon source probably led to a more acceptable environment for these lactate-utilising bacteria but this will have to be confirmed. The data showed that even with different batches of anaerobic sludge, characteristic acetic and propionic acid and pH profiles were obtained and that, when using lactate as a carbon source, a repeatable metabolic pathway could be obtained. It is also known that propionate producers are effective ECP and aggregate formers (Mulder et al., 1989; Riedel and Britz, 1993; Slobodkin and Verstraete, 1993) and the formation of extracellular polysaccharides may serve as an alternative hydrogen sink mechanism for the propionate producers (Quarmby and Forster, 1995; Vanderhaegen et al., 1992). The results obtained and the increase in the formation of granules indicated that granulation could be enhanced in batch systems, and that a drop in pH at the start appears to facilitate the process.

In the simulated UASB shake batch studies, with only a daily 'draw and fill' environmental stress, it was found that the reactions were in most cases in agreement with some of the UASB system responses after overloading with easily degradable carbon sources (Britz et al., 2000; Myburg and Britz, 1993). These include a rapid drop in pH, followed by an increase in VFAs, especially propionic acid (Slobodkin and Verstraete, 1993) and then an increase in the number of countable granules. The increase in granule numbers (even though only based on visual observations) indicated that granulation could be enhanced in batch systems.

One factor that was found to be extremely important was the condition (type, age and concentration) of the inoculum sludge. It was also clear from the studies that a more reliable granule counting method must be developed.

Acknowledgements

The financial support of the Water Research Commission and the University of Stellenbosch is gratefully acknowledged.

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