

# 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.

## 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·min<sup>-1</sup>. 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·l<sup>-1</sup> lactate, 5.0 g·l<sup>-1</sup> yeast extract, 2.0 g·l<sup>-1</sup> peptone and 1 ml·l<sup>-1</sup> Tween 80; Sucrose medium (Quarmby and Forster, 1995) which consisted of 5.0 g·l<sup>-1</sup> sucrose, 0.5 g·l<sup>-1</sup> yeast extract, 1.0 g·l<sup>-1</sup> urea and 2 ml·l<sup>-1</sup> Tween 80; and glucose medium (Lens et al., 1993) which consisted of 5.0 g·l<sup>-1</sup> glucose, 0.5 g·l<sup>-1</sup> yeast extract and 1.0 g·l<sup>-1</sup> urea.

A trace element solution (10 ml·l<sup>-1</sup>) (Nel et al., 1985) was added to each of the media used. To prevent the too rapid acidification of the units, 10.0 g·l<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub> 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

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