Effect of Substrate Shift on the Characteristics of Methanogenic Granular Sludge

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Abstract

Physical, chemical and biological changes in the upflow anaerobic sludge blanket (UASB) granules were investigated after the substrate was changed from brewery to methanolic waste. The granular sludge required 5-6 weeks for acclimatization (start-up) and this period accounted for the changes that the granules exhibited. Sharp decrease in settling velocity was accompanied by decrease in ash content and a reciprocal increase in the volatile solids content. The mineral content and the extracellular polymer fraction of the granular sludge reduced to 67% and 74% of the initial value respectively. Hydrogenotrophic and acetoclastic methanogenic groups seemed to be the two predominant methane forming groups in the granular sludge before and after the substrate shift. The results of this study demonstrate that change in substrate bring about physical, chemical and biological changes in the granular methanogenic sludge but may not impede its biological conversion capabilities provided that favorable environmental conditions are maintained.

Key words : substrate shift, methanogenic granular sludge, characteristics, extracellular polymers, minerals

Introduction

Upflow anaerobic sludge blanket (UASB) process has emerged as a leading high rate anaerobic wastewater treatment (AWWT) process and hundreds of full-scale plants are operating worldwide⁽¹⁾. This process employs the principle of bacterial immobilization commonly known as granulation in which distinct biogranules are formed. The formation of biogranules is desirable since it greatly enhances the amount of retained viable biomass thus establishing very long solids retention time (SRT). The mechanism of granulation seems to be, however, not fully understood at present^(2,3,4). A granule seems to be a complex ecosystem with

substrate transfer occurring among different bacterial groups within a granule. Extracellular polymeric (ECP) material and ash content have been reported to be important binding materials⁽⁵⁾. We have recently shown that inert material of silt or clay nature is also an ingredient of the granular sludge (results to be published).

The start-up of a UASB reactor is known to take long time. However, if UASB reactor is seeded with fully developed granular sludge, only few weeks may be required for the start-up. With the increasing practical application of the UASB process especially to the industrial wastes, transfer of granular sludge from one treatment plant as a seed sludge to another UASB plant may not only become common but also

commercial. The full-scale UASB treatment plants of Asahi Brewery Co. Ltd. in Tokyo and Suita, Osaka, for example, were seeded with fully developed granular sludge obtained from the Netherlands. Both of these plants are operating satisfactorily although the granules in the Suita UASB reactor underwent significant physical, chemical and biological changes. It would thus be of great technical interest to examine the changes in the characteristics of granular sludges after they are operated in a different reactor and/or are subjected to waste streams of different chemical nature.

Developing countries are encountering a major water pollution problem due to industrial wastes. UASB process is highly applicable in developing countries and can serve the dual purpose of waste stabilization and energy production simultaneously. Since methanol is the main pollutant in the effluent from the sulfite pulp and paper industry, we have been investigating the treatment feasibility of methanolic waste in UASB reactors.

In the present study, changes in physical, chemical and biological characteristics of granular sludge cultivated on brewery waste were investigated after this sludge was seeded in a reactor employed for the treatment of methanolic waste. The objective of this study was to examine how a granular sludge cultivated under a set of conditions responds to another set of operational and environmental conditions and what changes as a consequence are brought about.

Materials and methods

UASB reactor and source of granular sludge

Stable and fully developed granular sludge was kindly provided by the Asahi Brewery Co. Ltd., Tokyo from the full-scale UASB plant employed for the treatment of its factory's brewery waste. This reactor was operated under mesophilic temperature and neutral pH conditions. Granular sludge from this reactor was used as a seed sludge

in a laboratory UASB reactor that has been fully described previously⁽⁶⁾.

Operational conditions

After substrate shift, the 6.5 l laboratory UASB reactor was operated for four months treating synthetic methanolic wastewater. The reactor temperature and pH were 40°C and 6.0–6.3 respectively. Loading rate was increased in a stepwise manner with maximum methanol · TOC concentration of 8000 mg/l (corresponding to a COD concentration of about 20,000 mg/l) at an HRT of 16 hours.

Substrate and nutrients

Methanol, diluted to the desired concentration with tap water, was used as the sole energy source. N and P were added according to the C:N:P ratio of 350:5:1. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ was continuously added to the feed at a concentration of 0.1 g/l. Trace nutrients were added from the stock solution prepared according to the composition of Okamura et al⁽⁷⁾.

Analytical procedures

Extracellular polymeric (ECP) material was extracted from the granular sludge using autoclaving technique⁽⁸⁾. Protein content of the ECP material was detected by the method of Lowry et al⁽⁹⁾, whereas carbohydrate content was determined by phenol-sulfuric acid method⁽¹⁰⁾ using glucose as standard. Nucleic acids were determined using an ultraviolet technique⁽¹¹⁾.

For the determination of mineral composition of the granular sludge, a known amount was evaporated to dryness on a steam bath and ignited at 450°C in a muffle furnace. The ash was dissolved in concentrated nitric acid and warm water, filtered through 0.45 μm membrane filter and diluted to a known volume. The mineral content of the effluent from laboratory UASB reactor was determined after filtering it through a membrane filter. Portions of filtrate (effluent and digested ash) were used for the determination

Fig. 1 General appearance of granules at (a) 0-days; (b) 63-days (higher sampling port); (c) 63-days (lower sampling port); and (d) 120-days of operational period. Seed granules are black in colour whereas slime layer of brownish colour around the granules could be observed after 120-days of operational period. Breakage of granules is evident from (c).

of mineral content and were analyzed on a Hitachi Atomic Absorption Spectrophotometer Model 208 (Hitachi Co., Japan).

Enumeration of acetate- and H_2 -utilizing methanogens was carried out by the most probable number (MPN) technique. Scanning electron microscopy (SEM) was performed according to the techniques elaborated previously⁽⁶⁾. All other analyses were performed according to Standard Methods⁽¹²⁾.

RESULTS

Changes in physical characteristics

The average size of the granules did not show an appreciable change. It only reduced from 2.6mm to 2.31mm after 120 days of operational

period. On the other hand, settling velocity manifested a sharp decline from 4.19 cm/s to 2.54 cm/s. This decrease may be attributed to the decrease in ash content that reduced from 20.6 to 13.2 %. A slime layer was observed to be coated around some of the granules that might be responsible for the increase in the volatile solids (VS) content of the granules. These changes occurred during the first five to six weeks, after which no appreciable change was observed.

Photograph of Fig.1a shows granular sludge before the start of the experiments, i.e., prior to the change of substrate. These granules were black in colour. After the substrate shift, some breakage of the granules was observed. In the early stages of acclimation, i.e., first 35 days or so, breakage of granules was observed in the

Fig.2 Relative changes in the ECP content of the granular sludges before and after the change of substrate.

Fig. 3 Relative changes in the mineral content of the granular sludges before and after the substrate shift.

bottom part of the reactor. There seemed to be no significant breakage at the top part of the granular bed. However, after about 40 days of operation, the phenomenon seemed to be reversed. The maximum breakage was visualized in the granular sludge sample obtained from top part of the granular bed as depicted in Fig.1b in which small pieces detached from the granules can be seen. Granular sludge withdrawn from lower ports did not relatively contain broken particles as seen in Fig.1c. This trend was noted until the end of 120-days of operational period. Nevertheless, after the end of experimental period, most of the seeded granules were intact and stable and are shown photographically in Fig.1(d). The colour of the granules did not change appreciably except some of the granules that were coated with a slime layer of brownish colour. A thick blanket of sludge consisting of flocs, pieces of broken granules and floating granules was developed at the top of granular sludge bed. Washout of

granules was observed throughout the course of the experiments.

Changes in chemical characteristics

Extracellular polymeric material (ECP)

The ECP fraction of the granular sludge decreased to 74% of the initial value during the experimental period. The relative changes in the protein, carbohydrate and nucleic acid content of the ECP material before and after substrate shift are illustrated in Fig.2. This figure indicates that carbohydrate and nucleic acid content increased by 5.9% and 12% respectively but the protein content, which was the major ingredient of the ECP material, decreased sharply by 37.1% reducing overall ECP material from 17.4% to 12.9% of total suspended solids (TSS). Similarly, the protein to carbohydrate ratio also declined from 7.8 to 4.6.

Fig. 4 Time course of (a) calcium and (b) iron content in the effluent relative to the feed concentration.

Fig. 5 Relative changes in the most probable number of H₂- and acetate-utilizing methanogens.

Mineral content

The mineral content of the granules decreased to 67% of the original value. Relative changes in the mineral content of the ash of the granules are demonstrated in Fig. 3. Except magnesium (Mg), all other major mineral ingredients

manifested a decrease after the substrate shift. The reason for increase in the Mg content is due to the fact that 0.1 g/l of MgSO₄·7H₂O was constantly added in the feed throughout the study period. The decrease in the mineral quantity may partly be attributed to the breakage of granules causing the minerals to flow out of the reactor. This can be seen from the sample graphs of Fig. 4a and Fig. 4b in which the effluent calcium (Ca) and iron (Fe) concentration is explained relative to the feed Ca and Fe concentrations respectively. High concentrations of Ca and Fe in the effluent can be noted during the initial 50 days or so which implies that the decrease in the mineral content took place during the first few weeks of acclimatization.

Changes in biological characteristics

The number of viable methanogens before and after the substrate change is depicted in Fig. 5. There was slight increase in the number of hydrogenotrophic and acetoclastic methanogens after the substrate shift. This may partly be due to the slight increase in the volatile solids content of the granular sludge. However, the enumeration

Fig. 6 Scanning electron micrographs of the surface of the granules (a) before and (b) after the change of substrate. Seed granules are predominated by filamentous type of microorganisms while taper ended short rods identical to the genus of *Methanobrevibacter* are seen in abundance along with filaments after the change of substrate.

results suggested that both these groups were respectively predominant in the granular sludge before and after the change of substrate. Fig. 6a and b show the scanning electron micrographs of the surface of the granule before and after substrate shift respectively. Filamentous types of microorganisms are abundant in Fig. 6a along with rods. However, in Fig. 6b, tapered ended short rods are seen in abundance besides the filamentous and rod type microorganisms.

Discussion

The results of this study demonstrate that the granular sludge exhibited physical, chemical and biological changes after the shifting of substrate. Notable changes were the decrease in settling velocity, ash content, mineral content and ECP fraction; slight increase in the H_2 -utilizing and acetate utilizing methanogens; and the alteration of the biological morphology at the surface of the granules. These changes seemed to have occurred

during the first 5-6 weeks that may be regarded as the acclimatizing period or the start-up period. The changes might have been brought by the change in the microbial species in the granules. However, the alteration of the characteristics of granules did not affect the overall capability to utilize the new substrate.

Although the granular sludge manifested significant physical and chemical changes, the exact mechanism of these changes was not fully understood. Sharp decrease in the protein content of ECP matter may have occurred due to its degradation by the microbial population. Since micro-organic nutrients such as vitamins were not supplied in the feed, proteins may have been degraded into amino acids and vitamins that were subsequently consumed by microorganisms for their growth. Thus proteins may have acted as a source of micro-organic nutrients. The decrease in minerals (except Mg) may be attributed to the decrease in ash content of the granular sludge. Fig. 4a and b indicate that there was washout of

minerals during the start-up period of the reactor. This washout was clearly explained by the decrease in ash content during that period. Brewery wastewater is a complex wastewater rich in minerals and carbonates thus providing favorable conditions for the formation of CaCO_3 , MgCO_3 , etc., precipitates which may have acted as nuclei for bacterial aggregation. However, in the case of synthetic methanolic waste, only limited minerals were supplied in the feed and it is anticipated that the already formed precipitates of CaCO_3 , MgCO_3 , FeCO_3 , etc., may have dissolved due to change in cultural conditions during the initial period resulting in the washout of minerals as observed in Fig. 4a and b. The biological diversity seems to be a result of the breakdown of methanol to methane via two routes, i.e., through volatile fatty acids and acetate formation. The quantitative extent of involvement of each of these routes is currently not known but this investigation is being carried out at our laboratory.

The SEM observations suggest that a granule is a mixed consortium composed of diverse biological groups. Thus the groups that find relatively more favorable environments will pre-dominate. This is an additional property of granular sludge for its application to different waste streams. Thus a granular sludge developed in a set of operational and environmental conditions may successfully be used for the start-up of reactors treating different kind of wastewater. This property of granular sludge of containing diverse biological groups may play an important role in the development and transfer of high-rate anaerobic wastewater treatment technology.

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