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APPLICATION OF CELL IMMOBILIZATION TO ANAEROBIC PROCESS

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Introduction

Immobilization of microorganisms in spherical gel beads has become attracted interest and much research activity in the past few years. Immobilized cells offer several potential advantages over free cells for wastewater treatment. These include the retention of biomass, the protection of cells from inhibitory substances and selective use of particular bacteria. Recently, research on immobilization of microorganisms in wastewater treatment has been initiated for activated sludge, nitrification, anaerobic digestion and toxic substances degradation[1)-2)]. In anaerobic digestion, methanogenic bacteria are sensitive to unstable environment accosiated with fluctuation of influent quility, constituent concentration and toxic sudstances. Immobilization of anaerobic bacteria is one of promising methods to handle these problems. The most common polymers used for immobilizing bacteria are alginate and carragenan. But these polymers could be degraded by anaerobic bacteria. Polyvinyl alcohol (PVA)-boric acid method[1)] offers preferable characteristics of immobilized beads; including strong gel strength and non-biodegradability.

Materials and Methods

The scheme of preparation of immobilized anaerobic bacteria is shown in Figure 1. The anaerobic bacteria were cultivated using CSTR with continuous feeding at 20°C for 1 month in order to get active bacteria and get rid of innert material.Digested sludge obtained from a wasetwater treatment plant was used as initial seed sludge. The medium contained acetic acid 5250 mg/l; NH_4HCO_3 2 g/l; K_2HPO_4 0.3 g/l; $NaHCO_3$ 2 g/l and yeast extract 0.05 g/l mixed with tap water. The microorganisms were concentrated by using flat-type membrane filtration. The immobilization was performed under anaerobic condition at 20°C using PVA-boric acid method. One portion of concentrated cells was mixed thoroughly with one portion of PVA solution (final concentration 8% PVA). This mixture was pressed through a syringe as droplets into saturated boric acid leading to spherical beads. In order to complete gelation inside the beads, the beads were kept stirred in saturated boric acid for 24 hr. Then the beads were washed with tap water.

Reseach by Tanaka[2)] showed that 8% of PVA solution is suitable for immobilizing anaerobic bacteria. The saturated boric acid has low pH of 4. In order to investigate the effect of pH of saturated boric acid to the immobilized anaerobic bacteria, 4 sets of different pH namely, boric acid without pH adjustment, pH 5.7, 5.9 and 6.0 (adjusted pH by adding Na_2CO_3) were conducted in immobilization. The immobilized cells beads were put into 50 ml vial bottles

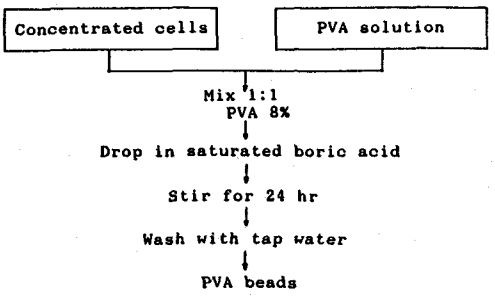

Figure 1 Preparation of immobilized cells

Table 1 The formation and stability of PVA immobilized beads

Boric acid pH	Bead formation	Bead strength	Bead stability	
			37 C	room temp.
Sat. boric acid pH 4	form	++++	+++	++++
Adjust pH with NaOH				
pH 5.0	form	+++	++	+++
pH 6-6.5	form	++	-	+
pH 6.7-7	not form	-	-	-
Adjust pH with Na_2CO_3				
pH 5.0	form	+++	++	+++
pH 6-6.5	form	++	-	++
pH 6.7-7	not form	-	-	-
Carragenan	form (in KCl)	++++	++++	++++
Alginate	Form (in $CaCl_2$)	++++	++++	++++

containing acetic acid 1050 mg/l with nutrients as substrate. Then the vials were flushed with N_2/CO_2 (70/30) gas and incubated at 20°C.

Results and Discussion

Due to methanogenic bacteria are sensitive to low pH environment, gel formation of PVA in various pH and neutral pH of saturated boric acid were conducted. Table 1 shows the gel formation, strength and stability of PVA beads. The PVA beads that formed gel in saturated boric acid with pH higher than 5.0 had low gel strength and low stability when incubated at 37°C. As the results, 20°C incubation was selected for further study.

Figure 2 shows methane gas production of PVA immobilized beads and free cells in batch experiments. Each set of batch experiments contained same amount of microorganisms. The immobilized cells required longer period of incubation before the start of active methane production due to the inhibitory effects by boric acid and oxygen during transfering of the beads. The lower pH of boric acid (pH 4) has stronger effect to the activity of methanogenic bacteria than higher pH of boric acid. The immobilization of anaerobic bacteria by alginate and carragenan was also conducted, mehtanogenic activity could recover in a few days after immobilization. These polymers are not suitable for immobilizing anaerobic bacteria due to their easily biodegraded carbohydrate structure.

After the batch experiment, all the beads of each set were transfered into 500 ml bottle. The reactors were operated in the mode of intermittent feeding with continuous stirring at 20° C. Due to the increasing of ambient temperature, the operating temperature was increased to 25°C and 30°C at 45 and 60 operating period respectively. Continuous experiments were started with 20 days of HRT and HRT was changed to be 16.7 from 60 days of operating period. Figure 4 shows methane gas production of PVA beads. The PVA beads that formed gel in saturated boric acid at pH of 6.0 and 5.9 were too soft and broken by magnetic stirrer in 10 days and 45 days respectively. Higher methane gas production and percentage of methane conversion from acetic acid were observed form the PVA beads that formed gel in saturated boric acid without adjusting pH. These beads could operate for 100 days before the breakage of the beads with 70-90 % of methane conversion from the substrate.

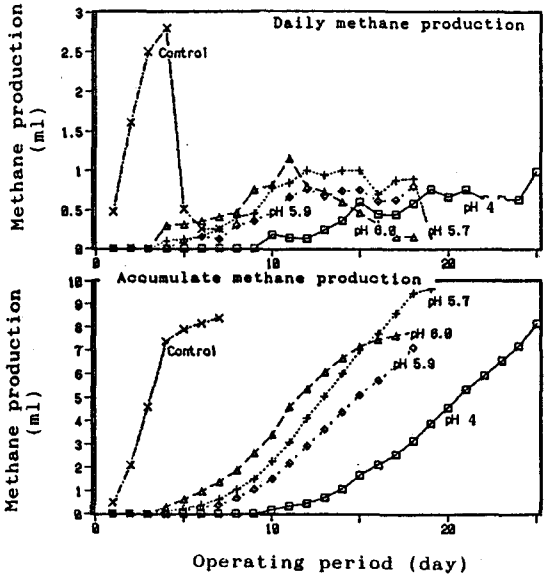

Figure 2 Methane gas production of PVA immobilized cells

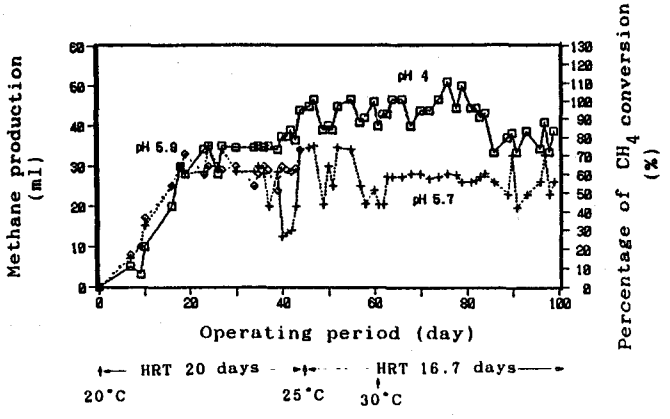

Figure 3 Methane production of PVA immobilized cells in continuous experiment

References

1) Hashimoto, S. et al. (1987) Immobilization of Activated Sludge by PVA-Boric Acid Method. Biotech. Bioeng., vol 30, 52-59

2) Tanaka, S. (1987) Proc. 21st Annual conference of JSWPR., 271 (in Japanese)