P-092 **Biogas production by Encapsulated digesting bacteria**

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INTRODUCTION AND OBJECTIVES

Biogas is an energy source that is used worldwide as car fuel, or for production of heat and electricity. Biogas consists mainly of methane and carbon dioxide, but may also contain several impurities (Deublein 2008). The anaerobic digestion process and production of methane require a long retention time in the digester, a processs that methane-forming bacteria can easily washout (Gerardi 2003). This problem can be improved by retaining the bacteria inside the digester. Cell immobilization is one of the most attractive methods in maintaining high cell concentration in the reactor and has been extensively studied for e.g. bioethanol production. In addition, immobilizing microbial cells in a high density not only improves the productivity of a bioreactor but also provides many benefits over free cells. Among various cell immobilization methods, encapsulation is the method which is interesting for biogas production. The objective of this work was to study the possibility of encapsulation method for biogas production

MATERIALS AND METHODS

Encapsulation procedure

The digested sludge including the methanogenic bacteria was suspended in 100 mL of 1.3, 2.6 and 5.5%(w/v) of $CaCl_2$ solution containing 2.6% carboxymethylcellulose (CMC) of 0.9 degree of substitution of cellulose structure which was added to increase the viscosity of the first solution in order to form spherical capsules (Geraldi 2003). This solution was dripped into the 0.6% (w/v) sterile sodium alginate solution, containing 0.1% (v/v) Tween 20 to improve the permeability of the capsule membrane. The sodium alginate solution was stirred at 330 rpm during capsule production. After 10 minutes of gelation, the capsules were washed with sterile water for 10 minutes, and then let to harden in 1.3% (w/v) of $CaCl_2$ for 20 minute.

Biogas production

Anaerobic digestion was carried out in a batch digester at 55°C (Hansen 2004). The digesters were serum glass bottles with 118 ml working volume, closed with butyl rubber seals and aluminum caps. For each bottle 15 ml of capsules and 20 ml of basal medium were added together, and then the head space of each bottle was flushed with 80% nitrogen and 20% carbon dioxide gas mix to obtain anaerobic conditions. Gas samples from the headspace of each bottle were withdrawn regularly and analyzed by gas chromatography in order to obtain the methane and carbon dioxide production during the digestion time.

RESULTS AND DISCUSSION

Among several techniques for cell encapsulation, the liquid-droplet-forming, one-step method was used in this experiment (Talebnia 2005). In this method, the methanogenic bacteria was suspended in $CaCl_2$ solution containing carboxymethylcellulose (CMC) This solution was added through an extruder into the sterile sodium alginate solution containing Tween 20, and then let to harden in $CaCl_2$.

Figure 1: Capsules containing digesting bacteria cell

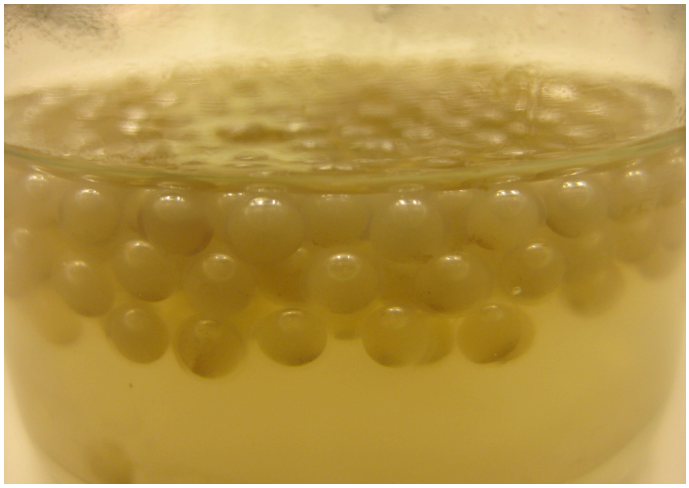

Figure 2: Capsules containing digesting bacteria cell after 1 day of digesting time

The capsules' shapes and diameters were examined. The average diameter of the capsules was 0.43 cm, and they had spherical shapes (Fig.1).

Moreover, during the digesting period, the biogas bubbles were developed inside the capsules (Fig.2). However, the biogas had difficulty in diffusing through the capsules' membrane.

Table 1: The methane production in comparison to the concentration of calcium chloride as a hardening solution

$CaCl_2$ (w/v)	Methane (mL /g VS) Time (Days)					
	0	**1**	**2**	**4**	**6**	**8**
1.3 %	**0**	6.50± 0.08	7.87± 0.52	1.77± 0.47	41.90± 1.05	2.97± 0.23
2.6%	**0**	0.68± 0.45	5.73± 0.84	0.46± 2.11	34.76± 2.36	5.61± 3.44
5.2%	**0**	2.46± 0.0	0.60± 2.80	1.09± 5.87	35.32± 4.36	7.24± 0.10

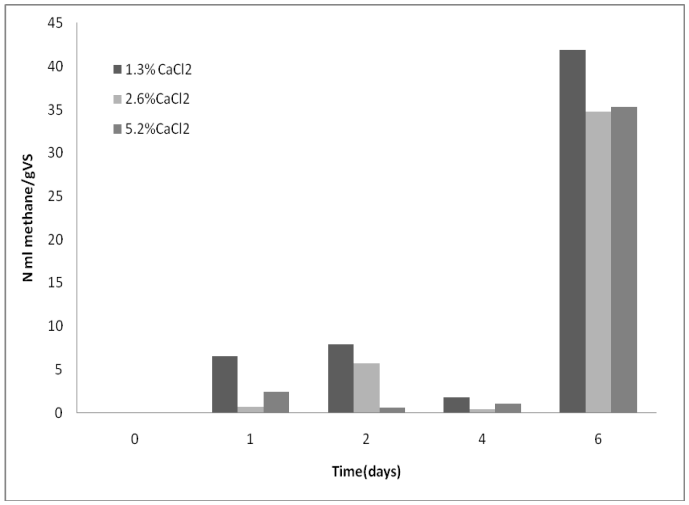

Figure 3: Accumulated methane production from different materials to produce capsules expressed in ml/g

The alteration of the concentration of $CaCl_2$ for encapsulation process is an important factor that should be considered. The purpose is for developing the wall thickness, pore size, surface charge and mechanical strength of the capsules (Park 2000). Improving the permeability of the capsule membrane was also investigated in this work. Anaerobic digestion in batch reactor was performed on the encapsulated anaerobic culture which is produced from different concentration of $CaCl_2$ as a hardening solution mixing with CMC. The digestions were performed at thermophilic conditions for 8 days. The results of digestion are presented in Fig. 3 and Table 1.

The methane production rates of all samples with different concentration of $CaCl_2$ increased continuously during digestion period. Moreover, the result was observed that for all different treatments, the capsules were destroyed by gas pressure which was produced during incubation. It resulted in a sharply increasing methane production because gas was released from capsules quickly. Treatment with addition of 1.3% $CaCl_2$ resulted in the highest methane volume of 41.90 mL /g VS at the 6th day of incubation period followed by treatment with addition of 2.6% $CaCl_2$ and 5.2% $CaCl_2$, respectively. It means that the capsule membranes with 1.3% $CaCl_2$ are more permeable, but less stable than the capsule membrane with 2.6% $CaCl_2$ and 5.2% $CaCl_2$, respectively; thus, gases can pass through easily. Although the anaerobic culture capsule with 1.3% $CaCl_2$ gave higher methane productivity than another, but their membranes were destroyed by gas pressure easier as well. Some destroyed capsules can be observed at the 4th day of digestion.

CONCLUSIONS

Different concentration of $CaCl_2$ for producing capsule membranes has an effect on methane production. All of the results indicate that methane production from encapsulated bacteria was clearly successful and it is possible to develop the encapsulation method for biogas production.

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