



Prediction of bio-methane potential and two-stage anaerobic digestion of starfish



Dong-Hoon Kim^a, Jaehwan Cha^d, Mo-Kwon Lee^a, Hyun-Woo Kim^b, Mi-Sun Kim^{a,c,*}

^a Clean Fuel Department, Korea Institute of Energy Research, 102 Gajeong-ro, Yuseong-gu, Daejeon 305-343, Republic of Korea

^b Green Technology Center, Korea Institute of Industrial Technology, Yugok-dong 889-1, Jung-gu, Ulsan 681-310, Republic of Korea

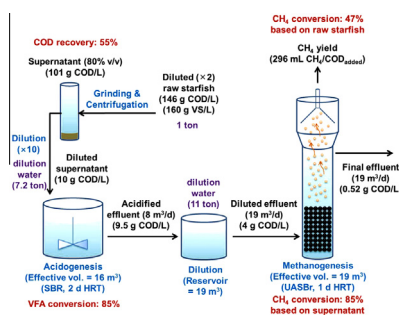
^c Division of Renewable Energy Engineering, University of Science and Technology, 217 Gajeong-ro, Yuseong-gu, Daejeon 305-350, Republic of Korea

^d Hanwha Engineering & Construction, Research Institute of Technology, Sinseong-dong 6, Yuseong-gu, Daejeon 305-804, Republic of Korea

HIGHLIGHTS

- ▶ Biological CH₄ potential (BMP) of starfish.
- ▶ The maximum CH₄ yield of 334 mL CH₄/g COD was estimated using RSM.
- ▶ Two-stage (acidogenic SBR + methanogenic UASBr) fermentation system.
- ▶ Conversion of 44% of organic content in whole starfish to CH₄.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Available online 28 February 2013

Keywords:

Starfish
Biological methane potential (BMP)
Response surface methodology (RSM)
Two-stage fermentation
Up-flow anaerobic sludge blanket reactor (UASBr)

ABSTRACT

The present work reports the first ever evaluation of the biological CH₄ potential (BMP) of starfish, classified as invasive species. Since starfish contain a large amount of inorganic matter, only the supernatant obtained through grinding and centrifugation was used for BMP test. By applying response surface methodology, the individual and interactive effects of three parameters, inoculum/substrate ratios, substrate concentrations, and buffer capacities on CH₄ production were investigated, and the maximum CH₄ yield of 334 mL CH₄/g COD was estimated. In addition, continuous CH₄ production was attempted using a two-stage (acidogenic sequencing batch reactor + methanogenic up-flow anaerobic sludge blanket reactor (UASBr)) fermentation process. Acidification efficiency was maximized at 2 days of hydraulic retention time with valerate, butyrate, and acetate as main acids, and these were converted to CH₄ with showing 296 mL CH₄/g COD_{added}. Overall, the two-stage fermentation process could convert 44% of organic content in whole starfish to CH₄.

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1. Introduction

According to Encyclopedia Britannica, about 1600 species of starfish are living around the world ocean. Starfish are well distributed in many countries and are classified as invasive species (Ward and Andrew, 1995). They have no natural predators or competitors,

which leads to a rapid increase of population and can alter the native ecosystems, eating large amounts of shellfishes such as oysters, abalone, mussels, and scallops. Many countries have developed various methods to remove them and control their propagation. The sea star mop, which removes starfish from the sea bottom by entangling them in cotton bundles, is a typical method. Other measures including manual capture, underwater fences, and manual injection of chemical reagents have also been adopted (Barkhouse et al., 2007).

Although starfish can be removed from the sea bottom, the disposal process entails great expense. Therefore, many researchers

* Corresponding author at: Clean Fuel Department, Korea Institute of Energy Research, 102 Gajeong-ro, Yuseong-gu, Daejeon 305-343, Republic of Korea. Tel.: +82 42 860 3707; fax: +82 42 860 3739.

E-mail address: bmmskim@kier.re.kr (M.-S. Kim).

have considered possible uses of collected starfish. Since starfish possess a remarkable ability for regeneration, they have been studied in terms of extracting biologically active substances for medical purposes (Gorshkov et al., 1998; Prokofeva et al., 2003). Starfish also can be used as an ingredient for animal feed and a nutrient amendment for composting (Line, 1994).

Anaerobic digestion (AD) is a biological process in which organic matter is degraded and converted to clean biogas, methane (CH_4), under an anaerobic condition. It can be an attractive option, both as a disposal route and as a source of alternative energy (Fdez-Guelfo et al., 2011). In the last decade, considerable efforts have been directed toward introducing AD for treating terrestrial waste biomass, whereas less attention has been paid to marine biomass (Yokoyama et al., 2008). However, given that the ocean possess the capability of fixing more than 50% of CO_2 produced in the world, there have recently been several attempts to convert marine biomass, especially seaweed, to biogas by AD (Chung et al., 2011; Gurung et al., 2012).

The present work reports the first ever evaluation of the biological CH_4 potential (BMP) of starfish. As BMP is affected by various factors such as inoculum/substrate (I/S) ratio, substrate concentration, and buffer capacity, the response surface methodology (RSM) was employed to design the experimental sets statistically and determine the maximum BMP of starfish (Raposo et al., 2008, 2011; Xing et al., 2008). In addition, continuous CH_4 production from starfish was attempted using a two-stage fermentation process, consisting of an acidogenic fermenter followed by a methanogenic fermenter. The two-stage fermentation process has been applied to many kinds of organic solid wastes (Jagadabhi et al., 2011; Ueno et al., 2007). To improve CH_4 recovery from starfish, operational parameters such as hydraulic retention time (HRT) and substrate concentration were varied in the acidogenic fermenter and methanogenic fermenter, respectively.

2. Methods

2.1. Inoculum and feedstock preparation

The sludge obtained from the anaerobic digester at a municipal wastewater treatment plant (Daejeon, South Korea) was used as an inoculum for the BMP test and acidogenic fermenter. The pH, alkalinity, volatile suspended solids (VSS) concentration and total solids concentration (TSS) were 7.4, 2.8 g CaCO_3/L , 22 g VSS/L and 31 g TSS/L, respectively. Prior to use, the inoculum was acclimated under mesophilic condition (35 °C) until biogas production was no longer observed. The granulated sludge obtained from a full-scale anaerobic plant treating brewery wastewater was used as a seeding source for the CH_4 -producing up-flow anaerobic sludge blanket reactor (UASBr). The pH, alkalinity, and the concentrations of VSS and TSS were 7.5, 6.7 g CaCO_3/L , 102.6 g VSS/L and 157.3 g TSS/L, respectively.

Starfish were collected from the South sea in South Korea and stored at -30°C , and then thawed at room temperature prior to use. Since starfish contain a large amount of inorganic matter, they (raw starfish) were diluted with tap water two times (w/w) for the ease grinding. The comminuted starfish were centrifuged at 6000 rpm for 10 min using a centrifugal separator (Supra 22 k, Hanil, South Korea), and the supernatant was used as a feedstock. Table 1 summarizes the general characteristics of starfish and the supernatant. The comminuted starfish contained the organic compounds as 146.2 g chemical oxygen demand (COD)/L, while the COD concentration in the supernatant separated after centrifugation was 101.4 g COD/L. Since the supernatant accounted for 80% of the total volume, 55% of COD in the starfish was recovered to the supernatant through centrifugation.

Table 1

Characteristics of starfish and the supernatant after grinding and centrifugation.

Items	Units	Starfish	Supernatant ^a
Total solid	g TS/L	393.0	ND ^b
Volatile solid	g VS/L	160.0	ND ^b
Total COD	g COD/L	146.2	101.4 (± 7.9)
Carbohydrate	g/L	10.0	1.2
Total nitrogen	g N/L	ND ^b	5.7 $\pm$ 0.3
pH	–	ND ^b	6.5

^a Supernatant: solution separated after grinding and centrifuging starfish.

^b ND: not determined.

2.2. Batch tests for determining BMP of starfish

BMP of the starfish was determined in batch tests using a 250 mL serum bottle (effective volume of 100 mL). After placing designated amounts of inoculum, the supernatant of centrifuged starfish, and buffer medium (40 g NaHCO_3/L) to the serum bottle, N_2 gas was purged for 5 min to establish an anaerobic condition. No other nutrients or medium were supplemented. The bottles were incubated at 35 °C using a water bath with agitation at 200 rpm using a shaking incubator. A central composite design (CCD), generated by Design-Expert software (Star-Ease Inc., USA), was employed to determine the optimum CH_4 production condition and to ascertain the interactive effects of three major parameters. As shown in Table 2, 16 experimental sets for the BMP test were designed according to CCD at various I/S ratios (0.5–3.0, g VSS/g VS), substrate concentrations (2.0–8.0 g COD/L), and buffer capacities (0.0–5.0 g NaHCO_3/L). The experimental results obtained from the BMP tests were further followed a procedure of RSM, which is a collection of mathematical and statistical techniques that are useful for process optimization (Montgomery, 2012). The second-order polynomial model was used for better approximation. The p -value was used to check the significance of the model term: the smaller the p -value, the more significant is the corresponding coefficient. The significance meant that there was only a small chance that a wrong prediction would occur because of experimental errors or other noise factors. Response surfaces obtained from the model were presented graphically using contour plots, where the CH_4 yield was plotted versus the level of two independent variables, and a further optimization analysis was performed to locate the optimum CH_4 yield according to the combination of independent variables.

2.3. Continuous CH_4 production from starfish

On the basis of the results from the BMP tests, the feasibility of continuous CH_4 production from starfish was investigated. First, continuous CH_4 production from starfish supernatant was performed using only a UASBr (working volume 3.5 L; 690 mm high by 65 mm ID). One-third of the reactor was filled with inoculum, and N_2 gas was purged for 5 min to make an anaerobic condition. UASBr was first fed by glucose as a substrate for 12 days, and subsequently the substrate was changed to the starfish supernatant.

In the two-stage fermentation process, acid fermentation of the starfish supernatant was conducted using a completely-stirred sequencing batch reactor (SBR) (working volume 5.0 L; 325 mm high by 120 mm ID). One-third of the reactor was filled with the anaerobic digester sludge, and N_2 gas was purged for 5 min to establish an anaerobic condition. The SBR was operated according to the following cyclic steps: feed, reaction, settling, and discharge. A batch cycle took 24 h in total with a reaction time of 21 h and a settling time of 3 h. By changing the discharged volume, the SBR was operated at various HRTs in the range of 2–5 days to find out the optimal operation conditions. The acidified effluent from

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