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Bio-hythane production from cassava residue by two-stage fermentative process with recirculation

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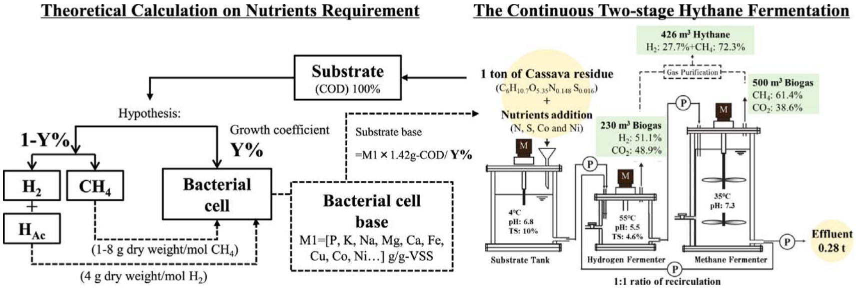
GRAPHICAL ABSTRACT



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ABSTRACT

The two-stage hythane fermentation of cassava residue low in protein, rich in iron, and deficient in nickel and cobalt, resulted in failure after long-term operation, showing a radical decrease in methane production along with an increase in volatile fatty acids (VFAs) accumulation in the second stage. Based on the gap between theoretical demand and existing content of nutrients, the effect of their additions on hythane fermentation was validated in the repeated batch experiment and continuous experiment. The proliferation of hydrolysis bacteria, acidogens, and hydrogen producing bacteria and methanogens was guaranteed by sufficient N (0.7 g/L), S (30 mg/L), Ni (1.0 mg/L), and Co (1.0 mg/L), and the metabolism of a sustainable hythane fermentation was recovered. In this optimal nutrient combination of above trace elements, the highest hythane yield (426 m^3 hythane with 27.7% of hydrogen from 1 ton of cassava residue) was obtained.

1. Introduction

Hythane (a mixture of methane and hydrogen) is considered as a bridge energy carrier in the “decarbonization” between natural gas (consisting mainly of methane) and the final goal of hydrogen. Because it can markedly reduce exhaust emissions of hydrocarbon compounds and drastically improve the efficiency of conventional spark- or compression-ignition engines (Muradov, 2014; Porpatham et al., 2007), the development of hythane fermentation method appears inevitable as the direction for future energy emerges.

Theoretically, the highest hydrogen yield is restricted to 4 mol-H_2/mol-Hexose and the COD removal efficiency gets bogged down at 33.3%, accompanied by extremely unpleasant odor from the accumulation of VFAs in the one-stage hydrogen dark fermentation (Khanal, 2011). On the other hand, the hydrolysis of lignocellulosic biomass has been considered as the rate-limiting step for methane production

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(Noike et al., 1985; Tong et al., 1990; Fey and Conrad, 2003). The phase separation of hydrolysis and hydrogen fermentation from methanogenesis in different reaction environments has been proposed as a strategy to improve overall process performances, in terms of stability, degradation efficiencies and overall energy recovery from biomass (Luo et al., 2011). Wang et al. estimated that 5.78% of the influent COD of food waste was converted to hydrogen in the first stage, and 82.2% of COD was converted to methane in the second stage (Wang and Zhao, 2009). The two-stage hythane fermentation had the ability to enhance 8%–43% of the total energy recovery compared with the one-stage methane fermentation, when the lignocellulosic materials were used as substrates (Nathao et al., 2013; Nielsen et al., 2004; Schievano et al., 2014). Recently, the several successful operations of two-stage hythane fermentation with the recirculation (pumping a part of the methanogenic sludge back into the first stage hydrogen reactor) were reported, which could maintain appropriate condition in the first stage with little or no addition of alkaline and NH_4^+ (Lee et al., 2010), and dilute the concentration of substrate without adding any extra water (Ohba et al., 2005). Consequently, recirculation contributed to the improvement of carbohydrate degradation and the increasing of biogas production (Kobayashi et al., 2012). However, the improper recirculation ratio could upset the balance in the first stage, since certain amount of hydrogenotrophic methanogens and homoacetogens was sent back to the first stage, as hydrogen consumers, with the hydrolysis bacteria and hydrogen producing bacteria (HPB) at the same time. Thus, it is necessary to investigate the effect of operational modes (hydraulic retention times (HRTs) and recirculation) on the practical efficiency of two-stage hythane fermentation.

Cassava (*Manihot esculenta*) as one of the world's fastest expanding crops is widely planted in tropical areas and used as food, animal feed and industrial materials. The total production of cassava has continued to rise in the last two decades, mainly due to the development of industrial manufacture on starch and ethanol (Food and Agriculture Organization of the United Nations, 2016). However, 40%–90% of fresh cassava roots were converted to residue in the industrial processing (Edama et al., 2014), and this kind of residue is extremely high in carbohydrate which requires urgent treatment. In recent years, agro-industrial cassava residue, including processing wastewater (Intanoo et al., 2014), cassava stillage and cassava excess sludge (Luo et al., 2010) has gained prominence along with the rapid development of cassava starch processing manufacture. However, because of its low protein concentration, there are certain limits to the practical utilization of cassava residue. To compensate for this, some researchers have considered co-digestion with pig manure (Ren et al., 2014) or adding ammonium-nitrogen to optimize the C/N ratio. A sufficient N-source has been required to guarantee microflora proliferation and the metabolism of fermentation. It has also been shown that the addition of trace metals has a significant effect on hydrogen and methane fermentation (Qiang et al., 2012, 2013; Lin and Lay, 2005).

In this study, both the continuous operation of two-stage hythane fermentation with recirculation and the repeated batch experiment were carried out in order to (i) demonstrate the stability of long-term continuous hythane production from cassava residue using recirculation, (ii) clarify the effect of HRTs on hydrogen and methane production, and (iii) investigate the effect of nitrogen, nickel, cobalt and sulfur supplements.

2. Materials and methods

2.1. Inoculum and substrate

The anaerobic mixed microflora as seed sludge for both hydrogen and methane fermentation was obtained from a mesophilic sewage sludge digester at Sendai municipal sewage treatment plant in Japan. In the beginning, inoculum for hydrogen fermenter was adjusted pH from 7.3 into 5.5 by feeding 2 mol/L HCl solution without heat-pretreatment, due to that the hydrogen consuming bacteria can be effectively inhibited by low pH and thermophilic operational temperature.

Table 1
The characteristics of cassava residue slurry (substrate).

Constituent		AVER.	STDEVA.
pH		6.26	0.014
TS (g/L)		100	5.53
VS (g/L)		91.9	5.41
TSS (g/L)		91.2	7.36
VSS (g/L)		84.1	6.92
TCOD (g/L)		136	7.58
SCOD (g/L)		14.5	1.28
T-Carbohydrate (g/L)		50.6	4.87
S-Carbohydrate (g/L)		5.48	0.902
VFA (g/L)	H_{Ac}	0.334	0.233
	H_{Pr}	0.069	0.033
	Iso-H_{Bu}	0.033	0.027
	n-H_{Bu}	0.258	0.220
	Iso-H_{Va}	0.055	0.022
	n-H_{Va}	0.032	0.036
Ethanol (g/L)		0.049	0.040
Lactic acid (g/L)		4.26	0.936
Protein (g/L)		4.98	1.80

Cassava residue was collected from a starch processing plant in Guangxi Zhuang Autonomous Region, China. The cassava residue was sun dried (16.5% water content), powdered in an electronic blender and screened through a 1 mm size mesh sieve, then mixed with tap water to prepare a 10% TS slurry substrate. The characteristics of the substrate is shown in Table 1.

2.2. Continuous system setup and process operation

The experimental apparatus consisted of one substrate tank and two continuously stirred-tank reactors (CSTRs). The substrate tank for storing the cassava residue slurry was kept at 4 °C by the cooling system. The first CSTR as hydrogen fermenter (R1) was operated at 55 °C by water circulating through water jacket with a 3 L effective working volume, and the second CSTR as methane fermenter (R2) was operated at 35 °C with a 12 L effective working volume. The R1 was fed with cassava residue slurry from the substrate tank, and the homogenized R1 effluent was pumped into the subsequent R2. A portion of the methanogenic sludge in R2 was returned into R1 with a 1:1 recirculation ratio by roller pumps, resulting in that the total HRT of system is twice as the sum of HRT of R1 and that of R2. The total HRT was set from 200 days as start-up run, and decreased in steps to 30 days as shown in Fig. 1.

2.3. Repeated batch experiment

A series of repeated-batch experiments was conducted in sixteen serum bottles (120 ml with 60 ml of effective working volume) under different trace-elements (TEs) supplementations to investigate the effect of the nutrient requirement on methane fermentation. In accordance with the measuring values of elements in cassava residue and the results from the theoretical calculation, the design of repeated batch experiment is shown in Table 2. the TE single or combined stock solutions of cobalt (1 mg/L), nickel (1 mg/L) and sulfur (30 mg/L) were mixed with the seed sludge at a volume ratio of 1:11 (5 mL: 55 mL) in serum bottles, and the seed sludge for batch experiment was taken from the continuous reactor under its steady state. The bottles were flushed with N_2 gas for 2 min to provide anaerobic conditions, then closed with natural rubber stoppers and aluminous screw-caps before they were set in a mesophilic (35 °C) shaking water bath.

The experiment was repeated for four batches. In the first run, to