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Energy



Energy Procedia 52 (2014) 167 - 172

# 2013 Alternative Energy in Developing Countries and Emerging Economies

# Efficacies of various inoculum sources on methane production from agro-industrial wastewaters

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#### Abstract

One key factor in reactor start-up is inoculum seed. Normally, the indigenous seed is used; however, it is not available near the new biogas plant. The exogenous seed from different wastes treatment system should be considered. This study selected inoculum seeds and wastewaters from various agro-industrial wastewaters based on carbohydrate, lipid and protein manufacturers including a concentrated rubber factory (RB), cassava starch factory (CS), palm oil mill factory (PO), swine farm (SW) and soymilk processing factory (SM). A 5 x 5 inoculum seeds and a wastewaters factorial experimental design were investigated in batch operation mode at an organic loading of 2 g COD  $\Gamma^1$  and 37 °C incubation. The results showed that all of the inoculum seeds can be used to degrade the exogenous wastewaters to methane. It was noted that the inoculum seeds can be applied to other sources of wastewater. The efficacy of inoculum seeds to start-up the anaerobic reactor depends on the initial activity and biodegradability of wastewater composition.

© 2014 Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/). Selection and peer-review under responsibility of the Organizing Committee of 2013 AEDCEE *Keywords: Anaerobic digestion; Agro-industrial wastewate; Inoculum seed; Methane; Starter-up* 

## 1. Introduction

Agricultural and agro-industrial wastes are the major sources of feedstock for biogas production in Thailand [1]. Anaerobic digestion or biogas technology is an attractive technology for environmental and renewable energy benefits. The wastes obtained from various agro-industries are different in terms of characteristics such as chemical composition and quantity based on raw materials used in the production process [1, 2]. Various agro-industrial wastewaters are based on carbohydrate, lipid and protein manufacturers. To build up the new biogas plant, one of the most critical steps to be considered is the

reactor start-up. Poor start-up in anaerobic treatment systems can lead to prolonged periods of acclimation and ineffective removal of organic matter. The most important factor for a successful start-up is inoculum seed [3, 4]. Normally, the inoculum seed is taken from the anaerobic treatment that is the same source as the wastewater. Sometimes, finding a source of inoculum seed from a similar type of treated wastewater near the new biogas plant is not possible, which causes expensive transportation expenditures in order to get the indigenous seed. Therefore, exogenous seed from anaerobic reactors treating different wastes is to be considered as a substitute. This study selected inoculum seeds from various agro-industrial wastewaters based on carbohydrate, lipid and protein manufacturers for the evaluation of their efficacies and adaptation period of various inoculum seeds treating different wastewaters in anaerobic digestion.

### 2. Materials and Methods

#### 2.1 Wastewaters and inoculum seeds

Concentrated rubber wastewater (RBw), cassava starch wastewater (CSw), palm oil mill effluent (POw), swine manure (SWw), and soy milk processing wastewater (SMw) were collected from their production process. The characteristics of wastewater were determined in pH, alkalinity (Alk), total volatile acid (TVA), chemical oxygen demand (COD), total solid (TS), volatile solid (VS), oil and grease (O&G), total Kjeldahl nitrogen (TKN) and protein according to the procedures in APHA Standard Methods [5]. The carbohydrate concentration was determined by the difference between 100 and the sum of the percentages of moisture, crude protein, lipid and ash [6].

Five inoculum seeds such as rubber seed (RBs), caasava starch seed (CSs), palm oil seed (Pos), swine (SWs) and soy milk seed (SMs) were collected from the anaerobic treatment system of the above five factories that collected wastewater. The characteristics of inoculum seeds were determined in total suspended solids (TSS) and volatile suspended solids (VSS) in triplicate according to the procedures in APHA Standard Methods [5]. The inoculum seeds were tested for their activity of fermentative bacteria groups by specific glucose utilization (SGU) and methanogen groups by specific methanogenic activity (SMA) [7].

#### 2.2. Experimental set up

Five sources of wastewaters and inoculum seeds were carried out in a 5 x 5 factorial experimental design. Each set of experiments (one starter seed) was conducted with five types of wastewater and the 25 conditions were run in triplicates. The starter seeds from an indigenous source were used as a control. The experiment was done in a 120 ml serum vial with 100 ml working volume containing an organic load of 2 g COD  $\Gamma^1$  and inoculum seed at the F/M ratio of 0.3. The basal medium was used to adjust the working volume. The composition of basal medium was composed of 132 mg  $\Gamma^1$  (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 75.5 mg  $\Gamma^1$  NaH<sub>2</sub>PO<sub>4</sub>.H<sub>2</sub>O, 50 mg  $\Gamma^1$  CaCl<sub>2</sub>.2H<sub>2</sub>O, 90 mg  $\Gamma^1$  MgSO<sub>4</sub>.7H<sub>2</sub>O, 10 mg  $\Gamma^1$  yeast extracts, and 0.3 ml  $\Gamma^1$  trace elements solution [8]. The headspace of the vial was flushed with oxygen-free nitrogen gas to anaerobic conditions and then sealed with a rubber stopper and aluminium cap. Each vial was incubated at 37 °C. The volume of biogas production and gas composition were analysed until the biogas accumulation was constant. The volume of biogas was measured using the water displacement method. The compositions of biogas were measured on a Class-GC 14B gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a thermal conductivity detector and a Porapak-N column (Waters, Milfords, MA).

The characteristics of concentrated rubber wastewater (RBw), cassava starch wastewater (CSw), palm oil mill effluent (POw), swine manure (SWw) and soymilk processing wastewater (SMw) are shown in Table 1. Most of the wastewaters had an acidic pH except swine manure was in neutral. Four of them (RBw, CSw, POw and SWw) were high strength wastewaters having more than 15,000 mg l<sup>-1</sup> TCOD. The composition of RBs was mainly constituted by proteins. The composition of CSs was mainly constituted by carbohydrate. The palm oil mill effluent (POs) contained high concentration of COD and the composition was constituted of oil and grease and carbohydrates. The swine manure (SWs) contained very high concentration of COD, TKN and the composition of SWs was indicated by protein and carbohydrate. The composition of SMs was mainly constituted by proteins and carbohydrates.

Parameter	Unit	RBw	CSw	POw	SWw*	SMw
pН	-	4.58	4.58	4.26	7.24	4.06
Alkalinity	mg/L	1,100	400	1,100	13,000	400
TVA	mg/L	3,460	1,100	6117	11,000	100
TCOD	mg/L	17,200	25,200	86,200	181,100	7,300
SCOD	mg/L	16,900	14,200	45,000	19,100	3,000
TKN	mg/L	1,800	400	1,100	6,500	300
TS	g/L	16.40	19.37	60.05	188.20	4.67
VS	g/L	13.82	16.91	50.24	146.9	4.09
Protein	g/L	10.98	2.30	6.78	40.47	1.55
Carbohydrate	g/L	2.84	13.97	29.89	81.30	1.73
Oil & grease	g/L	0	0.63	13.56	16.10	0.82

Table 1. The characteristics of various types of wastewaters

\* Unit of swine manure (SW): per kg fresh

The microbial characteristic and activity of rubber seed (RBs), cassava starch seed (CSs), palm oil seed (POs), swine (SWs) and soy milk seed (SMs) are shown in Table 2. The CSs and SWs have high %TSS. The SGU of five inoculum seeds were in the range of 180-380 mg COD  $g^{-1}$  VSS d<sup>-1</sup>. The highest SMA was found in CSs 130 mg COD-CH<sub>4</sub>  $g^{-1}$  VSS d<sup>-1</sup> followed by POs, SWs, SMs and RBs.

Table 2. The microbial	activity of	inoculum seeds
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Inoculum seeds	VSS (%TSS)	SGU (mg COD g <sup>-1</sup> VSS d <sup>-1</sup> )	SMA (mg COD-CH <sub>4</sub> g <sup>-1</sup> VSS d <sup>-1</sup> )
Rubber seed (RBs)	65	183.6	23.8
Cassava starch seed (CSs)	89	256.1	130.9
Palm oil seed (Pos)	76	294.8	88.7
Swine (SWs)	83	378.0	79.0
Soy milk seed (SMs)	62	338.4	32.5

To find the efficacies of various sources of inoculum seeds on methane production from agroindustrial wastewaters, 25 experiments were carried out and the experimental results are shown in Fig. 1 (a-e) and Fig. 2. The first set of the experiment was conducted with rubber seeds (RBs). Fig. 1a shows the cumulative methane production of RBs during 35 days of incubation. The values of methane yields were 284, 283, 280, 266 and 236 ml CH<sub>4</sub> g<sup>-1</sup> COD added for RBsCSw, RBsPOw, RBsSMw, RBsRBw and RBsSWw, respectively. The methane production rates of RBsRBw, RBsPOw, RBsSMw, RBsCSw and RBsSWw (Fig. 2) were 5.91, 5.37, 5.03, 3.92 and 3.01 ml CH<sub>4</sub> g<sup>-1</sup> VSS d<sup>-1</sup> in 17, 30, 20, 30 and 20 days, respectively.

The second set of the experiment was conducted with cassava starch seeds (CSs). Fig. 1b shows the cumulative methane productions of CSs during 20 days of incubation. The five wastewaters are comparable; the difference in methane production of swine wastewater was lower than other wastewaters. The values of methane yields were 318, 293, 289, 280 and 124 ml  $CH_4$  g<sup>-1</sup> COD added for CSsSMw, CSsPOw, CSsCSw, CSsRBw and CSsSWw, respectively. The methane production rates of CSsRBw, CSsPOw, CSsSMw, CSsCSw and CSsSWw (Fig. 2) were 12.37, 7.36, 6.38, 6.21 and 3.37 ml  $CH_4$  g<sup>-1</sup> VSS d<sup>-1</sup> in 7, 14, 20, 14 and 20 days, respectively.

The third set of the experiment was conducted with palm oil seeds (POs). Fig. 1c shows the cumulative methane production of Pos during 35 days of incubation. The five wastewaters are comparable; the difference in methane production of cassava starch wastewater was lower than other wastewater in the first 14 days. The values of methane yields obtained were 282, 279, 275, 271 and 179 ml CH<sub>4</sub> g<sup>-1</sup> COD added for POsPOw, POsSMw, POsRBw, POsCSw and POsSWw, respectively. The methane production rates of POsRBw, POsPOw, POsSMw, , POsCSw and POsSWw were 8.95, 4.77, 4.35, 4.13 and 2.38 ml CH<sub>4</sub> g<sup>-1</sup> VSS d<sup>-1</sup> in 9, 17, 17, 22 and 22 days, respectively (Fig. 2).

The fourth set of the experiment was conducted with swine seed (SWs). Fig. 1d shows the cumulative methane production of swine seed (SWs) during 42 days of incubation. The values of methane yields obtained were 314, 290, 280, 272 and 266 ml  $CH_4$  g<sup>-1</sup> COD added for SWsPOw, SWsCSw,SWsSWw, SWsSMw and SWsRBw, respectively. The methane production rates (Fig, 2) of SWsRBw, SWsCSs, SWsPOw, SWsSMw and SWsSWw were 8.32, 3.86, 3.74, 2.69 and 2.65 ml  $CH_4$  g<sup>-1</sup> VSS d<sup>-1</sup> in 8, 24, 24, 24 and 30 days, respectively.

The fifth set of the experiment was conducted with soy milk seeds (SMs). Fig. 1e shows the cumulative methane production of soy milk seeds (SMs) during 35 days of incubation. The values of methane yields obtained were 295, 289, 283, 283 and 212 ml CH<sub>4</sub> g<sup>-1</sup> COD added for SMsPOw, SMsSMw, SMsCSw, SMsRBw and SMsSWw, respectively. The methane production rates of SMsRBw, SMsSMs, SMsCSw, SMsPOw and SMsSWw (Fig. 2) were 7.08, 6.94, 4.78, 4.51 and 1.71 ml CH<sub>4</sub> g<sup>-1</sup> VSS d<sup>-1</sup> in 14,14, 22, 22 and 22 days, respectively.

The exogenous seeds (RBs, CSs, POs and SMs) in the digestion of swine manure (SWs) obtained lower methane yields compared to the indigenous seed (SWs). It might be due to the fact that swine manure is primarily composed of cellulose, hemicelluloses and lignin, among which lignin is the least degradable material in anaerobic digestion. Swine manure containing higher lignocellulose content will limit the degradation [9]. This study explored the influence of using exogenous inoculum seeds on the digestion of different wastewaters containing various organic waste compositions. The study of Elbeshbishy, et al. [10] found that two different anaerobic inoculum sources, digested sludge from a municipal wastewater treatment plant and a digester treating the organic fraction of municipal solid wastes can digest food waste. In addition, the inoculum source and test F/M conditions play a significant role in methane potential.



Fig. 1. The cumulative methane production of five starter seeds (s) treated with five agro-industrial wastewaters (w); (a) rubber (RBs), (b) cassava starch seed (CSs), (c) palm oil seed (POs), (d) swine seed (SWs) and (e) soy milk seed (SMs).



Fig. 2. The methane production rate of five starter seeds (s) treated with five agro-industrial wastewaters (w).

#### 3. Conclusions

The results of this study highlighted the importance of the starter seeds applied to other sources of wastewater. Various inoculum seeds from the anaerobic treatment of agro-industrial wastewaters based on carbohydrate, lipid and protein manufacturers can be used as the starter seeds for other wastewater types. Therefore, the exogenous seed from anaerobic reactors treating different wastes can be considered as substitute indigenous seed. The efficacy of inoculum seed to start up the anaerobic reactor depends on the initial microbial activity of acidogens and methanogens as well as its biodegradability on organic types in wastewater.

#### Acknowledgements

This work was supported by the Excellent Center of Waste Utilization and Management (ECoWaste) and the School of Bioresources and Technology, King Mongkut's University of Technology Thonburi.

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