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Improvement of Bio-Hydrogen Production and Intensification of Biogas from Kitchen Waste

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ABSTRACT

Background: Hydrogen is widely used feedstock for the production of chemicals, hydrogenation of fats and oils and in reformualation of gasoline etc. Bio-hydrogen is an environmentally friendly alternative automotive fuel that can be used in an internal combustion engine. Due to its environmental merits, the share of bio-hydrogen in the automotive fuel market will grow fast in the next decade. There are several reasons for bio-hydrogen to be considered as a relevant technology by both industrialized and developing countries. **Objective:** Biogas production from starch-rich kitchen waste from the Sathyabama University, Chennai was investigated in a laboratory scale using single-state digester with different combination of slurry made from cow dung (inoculum) and kitchen waste (substrate). **Results**: The maximum efficiency of 32% hkydrogen production in the digester was obtained at pH of 5.0–6.0, temperature of 32 ± 1^{o}C. The amount of the gas produced was calculated by water displacement using a mini-digester and hydrogen concentration was identified by GC method. The organisms were isolated by 16s rRNA method. The gene sequence were submitted to NCBI and accession numbers JF682389- *Bacillus licheniformis* HRNT01 JF683611 - *Bacillus funiculus* HRNT02 were assigned. **Conclusion:** Bacteria were isolated from the cowdung along with starch rich kitchen waste. Hydrogen was produced and analysed using GC.

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INTRODUCTION

Hydrogen is the element of greatest abundance in the universe; however, its production from renewable resources remains a major challenge. Biohydrogen produced from bio-renewable feedstocks by chemical, thermo-chemical, biological, biochemical, and biophotolytical methods. The comparative studies and the effectiveness of kitchen waste, and liquid effluent and mixtures of these feedstock in facultative anaerobic digestion for biogas production. This would provide relatively cheap and reliable fuel source for human consumption. The process would also reduce global warming which arises from major primary pollutants produced by human activities.

MATERIALS AND METHOD

Source of Microorganism:

The kitchen waste along with the effluent (liquid) was collected from the University dumpsites. The wastes were collected in a sterile plastic bags and cans were used for the present investigation. All the samples were maintained at 25^{o}C in the laboratory.

Identification of the Organism:

The organism was cultured which showed pure colonies were isolated from the culture medium was isolated, subcultured and stored at 4^{o}C. DNA was isolated from the organism and the large fragment of the 16S rRNA gene was amplified by PCR using the universal primers BAC-F-(5'-AGA GTTTGA TC(AC) TGG CTC AG-3') BAC-R (5'AAG GAG GTG(AT)TC CA(AG) CC-3') The PCR products were purified using a Wizard PCR Preps DNA Purification System (Promega, USA) according to the manufacturer's instructions. The PCR product after purification is sequenced using a BigDye^{TM} Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) and a model 3100 automatic sequencer (Applied Biosystems, USA). The closest

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known relatives of the new isolates were determined by performing a sequence database search. The sequences of closely related strains were retrieved from GENBANK and the Ribosomal Database Project (RDP) libraries.

Slurry Preparation:

The samples collected were macerated separated by adding 50 ml of sterile distilled water in motor and pestle for about 30 minutes. Care was taken that the slurry did not go into paste.

Construction of the Mini-Digester:

Mini-digester comprises of 6 gas cells. Each cell consists of a reaction vessel (500 ml Bottle) and a well closed gas pipe. The gas pipe is immersed to a liquid which has leveling vessel up to 50 ml volume and the end of tube is connected to the glass pipe which is graded. The gas produced is read on the glass pipe. The temperature of digester is maintained at different temperature according to the compound in composition. During the test the biogas production must be read daily. The volume produced is let out in case of each reading, each day at the beginning of test, later on every two or three days, when the gas formation diminishes (P.Vindis *et al*.,2009)

In each sample

A =kitchen waste (50%) + Cow dung (50%) + Sterile water*
B =kitchen waste (75%) + Cow dung (25%) + Sterile water*
C= kitchen waste (25%) + Cow dung (75%) + Sterile water*
D= kitchen waste (50%) + Cow dung (50%) + effluent*
E= kitchen waste (75%) + Cow dung (25%) + effluent*
F= kitchen waste (25%) + Cow dung (75%) + effluent*
*Sterile water and effluent was used to mix the slurry

Design of the Single State Digester:

The design of a batch-type consisted of a digester made of glass and covered properly and made air tight. The total internal volume of the vessel is about 1000 ml (Qualigens). The outlet of the vessel is connected with 75mm diameter delivery tube with three segments, two connectors attached at each end of the first and second tube. The end of the third tube is covered with parafilm. All the connections were ensured that there was no leakage. At the end of the first tube a three way connector was attached and one end is attached with a balloon which will monitor the formation of gas in the digester.

Loading of Digester:

After 5 days of analysis in the mini digester, the maximum gas producing mixture was used as feed in the Digester. A wet weight of 50% of kitchen waste and 50% cow dung was introduced into laboratory type digester containing 100 ml of effluent and labeled as D and wet weight of 75% of kitchen waste and 25% cow dung was introduced into laboratory type digester containing 100 ml of effluent E. In digester C a mixture of 50% of kitchen wastes was mixed with 50% of cow dung with 100 ml of water.

Sampling of Digester Content for Microbiological Analysis:

During the incubation period, samples from the digester were taken at 24 hour interval for 72 hours. Bacterial populations were determined.

Enumeration of Microorganism:

The samples from the digester inoculated in the sterile distilled water tubes and serial dilution was carried out within few hours. Appropriate dilutions 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} were selected and replicates were maintained throughout the study. The plates were incubated aerobically for 24hrs at 37^{o}C. The colonies formed were enumerated and subcultured and identified by cultural and biochemical characteristics. The bacterial cultures which were isolated were inoculated in separate test tube and stored in the refrigerator.

Hydrogen Ion Concentration (pH):

The pH values of the digestion mixture were monitored at 24 hours intervals for 120 hours using pH probe

Temperature:

The temperature ranges of the digestion mixtures were determined at 24 hours intervals for 120 hours using thermometer probe.

Data Processing:

During the experiment the date, hour, room temperature, room pressure and volume of formation of biogas were measured.

Measurements were conducted according to DIN 38 414. Biogas production is given in norm litre per kg of volatile solids (NI (kg VS) $^{-1}$), i.e. the volume of biogas production is based on norm conditions: 273 K, and 1013 mbar, because the temperature and pressure in room was variable.

Firstly, it is necessary to lead out the norm volume of produced biogas (V0) due the Equation:

$$\mathbf{Vo} = \mathbf{V}\frac{(\mathbf{Pl} - \mathbf{Pw}).\mathbf{To}}{\mathbf{Po}.\mathbf{T}}$$

Vo – norm volume of biogas; in ml
V - volume of formation of biogas; in ml
Pl – air pressure; in mbar
Pw – steam pressure of water in dependence upon room temperature; in mb
To – norm temperature;
To= 273 K
Po – norm pressure;
Po=1013 mbar
T - room temperature; in K

For each experiment with substrate and inoculum the test protocol was made. Also the norm volume from inoculum was measured and the quota of produced biogas from inoculum was calculated due the Equation:

$$\mathbf{Vis} = \frac{\sum \mathbf{V_{is}} \text{ x } \mathbf{m_{is}}}{\mathbf{m_M}}$$

V_{IS} – volume of formation of biogas from inoculum; in ml
$\sum V_{IS}$ – the sum of formation of biogas from inoculum in experiment; in ml
mIS – the mass of inoculum used in mixture; g
mM – the mass of inoculum used in check sample; in g

The mass of inoculum used in mixture was 385 grams, meanwhile the mass of inoculum used in check sample was 400 grams. The sum of norm volume of the experiment minus volume of formation of biogas from inoculum represents the net gas volume of biogas. The specific biogas production (VS) in norm litre per kg of volatile solids (NI (kg VS)$^{-1}$) was calculated due the Equation:

$$\mathbf{Vs} = \frac{\sum \mathbf{Vn} \text{ x } \mathbf{10^4}}{\mathbf{m} \text{ x } \mathbf{Wt} \text{ x } \mathbf{Wv}}$$

Vs - the specific biogas production (VS) in norm litre per kg of volatile solids (NI (kg VS)$^{-1}$)
$\sum$Vn - the net gas volume of biogas; in ml
m - the mass of our test; in g
Wt - dry matter of the substrate; in %
Wv - the ignition of the dry solids at 550 degree C; in %
The mass of our test is the mass of used substrate (silage) in the experiment. For each experiment the 500 grams of silage was used.

RESULT AND DISCUSSION

Sample Collection:

The Cow dung and kitchen waste were collected from the University Mess dumpsite in a sterile plastic bags and cans, respectively were used for the present investigation.

Isolation and Enumeration of Microorganisms:

The dung and the kitchen waste samples were brought to the laboratory and were inoculated in the sterile distilled water tubes and serial dilution was carried out within few hours. Appropriate dilutions 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} were selected and replicates were maintained throughout the study. After 24hrs of incubation the bacterial cultures were enumerated, isolated and inoculated in separate Petri plates and vials and stored in the refrigerator for further analysis.

The reduction of the organisms after the biogas production may be due to the following reasons like substrate reduction, increase of pressure due to the production of biogas and changes happening with the pH and temperature

Identification of Microorganisms:

The microorganism were isolated and indentified as *Bacillus licheniformis* HRNT01, *Bacillus funiculus* HRNT02, *E.coli* HRNT03 and *Enterococcus* sp HRNT04.

The Single State Digester:

The digester was designed in a manner that the about of gas produced can be collected in a balloon which can be send for the GC analysis. B. Mursec *et al*., 2009 has design a digester which was used to analyze the production of biogas. The bottom of the digester is large so that the amount of reaction taking place in effective manner.

Hydrogen Ion Concentration (pH):

The pH values of the digestion mixture were monitored at 24 hours intervals for 120 hours using pH meter and the results were comparable with Adrive, V *et al*., 2000

Temperature:

The temperature ranges of the digestion mixtures were determined at 24 hours intervals for 120 hours using clinical digital thermometer

Table 1: showing the distribution of microorganism in the sample before and after bio-hydrogen production.

S.No	Type of Slurry used	Before Biohydrogen production	After Biohydrogen production
1.	A =kitchen waste (50%) + Cow dung (50%) + Sterile water	4.2×10^8	1.0×10^7
2.	B =kitchen waste (75%) + Cow dung (25%) + Sterile water	2.2×10^7	2.0×10^6
3.	C= kitchen waste (25%) + Cow dung (75%) + Sterile water	3.1×10^8	1.2×10^5
4.	D= kitchen waste (50%) + Cow dung (50%) + effluent	6.8×10^8	4.1×10^7
5.	E= kitchen waste (75%) + Cow dung (25%) + effluent	4.9×10^8	4.0×10^7
6.	F= kitchen waste (25%) + Cow dung (75%) + effluent	1.2×10^6	1.0×10^5

.* Duplicates were used

Table 2: Bio-gas production from different types of slurry.

S.No	Types of slurry	Gas production (in ml)				
		1 day	2 day	3 day	4 day	5 day
1.	A =kitchen waste (50%) + Cow dung (50%) + Sterile water	33	30	28	25	15
2.	B =kitchen waste (75%) + Cow dung (25%) + Sterile water	24	19	16	9	7
3.	C= kitchen waste (25%) + Cow dung (75%) + Sterile water	18	24	16	9	0
4.	D= kitchen waste (50%) + Cow dung (50%) + effluent	45	44	43	28	15
5.	E= kitchen waste (75%) + Cow dung (25%) + effluent	34	32	25	15	10
6.	F= kitchen waste (25%) + Cow dung (75%) + effluent	23	17	15	8	2

The amount of Biogas produced was tabulated above with was referred from Vindis.P *et al*., 2009.

Table 3: showing the changes in pH during the Bio-hydrogen production.

S.No	Types of slurry	Hours				
		24	48	72	96	120
1.	D= kitchen waste (50%) + Cow dung (50%) + effluent	5.6	5.4	5.5	5.8	5.7
2.	E= kitchen waste (75%) + Cow dung (25%) + effluent	5.7	5.8	5.0	6.1	6.2
3.	A =kitchen waste (50%) + Cow dung (50%) + Sterile water	6.3	6.2	6.1	5.8	5.6

Table 4: showing the changes in Temperature during the Bio-hydrogen production.

S.No	Types of slurry	Temperature (in ^oC)				
		24	48	72	96	120
1.	D= kitchen waste (50%) + Cow dung (50%) + effluent	32.7	33.2	31.8	32.4	32.5
2.	E= kitchen waste (75%) + Cow dung (25%) + effluent	31.5	32.6	32.8	33.1	33.8
3.	A =kitchen waste (50%) + Cow dung (50%) + Sterile water	32.6	33.4	33.1	33.6	32.4

Table 5: Total Biogas produced was converted in NI kg $VS^{-1.}$

S.No	Types of slurry	Amount of Biogas produced in(NI kg VS^{-1})
1.	D= kitchen waste (50%) + Cow dung (50%) + effluent	293
2.	E= kitchen waste (75%) + Cow dung (25%) + effluent	189
3.	A =kitchen waste (50%) + Cow dung (50%) + Sterile water	124

GC analysis was performed for the samples which had a very high production of biogas:

D= kitchen waste (50%) + Cow dung (50%) + effluent - 32% pure hydrogen

E= kitchen waste (75%) + Cow dung (25%) + effluent – 5% of hydrogen with Carbon-di-oxide

A =kitchen waste (50%) + Cow dung (50%) + Sterile water- 2% of hydrogen with Carbon-di-oxide

It was found the when kitchen waste and cow dung in equal concentration with effluent showed maximum productivity of biogas and in GC analysis show around 32 % of Hydrogen gas without any mixing of Carbon di oxide.

Physico-chemical parameters tend to affect the Hydrogen biogenesis in the digester. In D= kitchen waste (50%) + Cow dung (50%) + effluent, the pH of the digester varied in the ratio of 5.6, 5.4, 5.5, 5.8, 5.7 respectively. The initial decrease in pH values in and in E= kitchen waste (75%) + Cow dung (25%) + effluent from 5.7 to 5.8 of the substrates was probably due to the conversion of products of hydrolysis such as monosaccharide, glycerol and amino acids into volatile fatty acids. But as digestion progressed and these acids were converted to biogas, the pH rose towards neutral point to favor acidogenesis.

Demirer, G.N (1998) also reported similar observation. The average temperature of the digester was 32.2^{o}C throughout the period of the study. Temperatures in this range initiate increases in metabolic activities of mesophilic bacterial population, which invariably initiates gas production. This temperature range is well tolerated by aerobic bacteria for maximal biogas production. The initial high bacterial load of the slurries may be due to the fact that large populations of aerobic and facultative anaerobic organisms are usually involved in the hydrolysis stage and acidogenic phase of biogenesis whereas only strict or obligate anaerobes are involved in the methanogenesis stage. This suggests that starchy waste bio-hydrogen may have a potential for economically viable waste treatment technology through aerobic digestion. Therefore, in view of the indigestibility of kitchen waste and cow dung with their relatively high capacity for generating biogas, the use of waste of these kitchen waste as sources of fuel gas for household cooking, heating and lighting deserves greater exploitation, and the study of the agronomy, physiology and biogasification of these wastes demands further investigation.

16s rRNA ANALYSIS - Accession Numbers:

After the isolation and biochemical identification of organism the 16srRNA was performed and the sequence was submitted in NCBI

The nucleotide sequence data reported in this paper have been submitted to the GenBank, EMBL and DDBJ databases and ssigned the following accession numbers:

JF682389 - *Bacillus licheniformis* HRNT01

JF683611 - Bacillus funiculus HRNT02

Vasudevan P.Ajithkumar *et al*., 2002, A novel filamentous *Bacillus funiculus*, reported that produces endospores and spore-like resting cells; the latter outgrow by budding. Phylogenetic analysis based on 16S rDNA gene sequences reported in the same paper speculated on the proposal of a novel species for this isolate. Likewise *Bacillus funiculus* was isolated and confirmed by 16s r RNA

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