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International Journal of Current Research
Vol. 6, Issue, 12, pp.10942-10945, December, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

BIOHYDROGEN PRODUCTION BY MICROBIAL COMMUNITIES ISOLATED FROM BIOGAS PLANT SLURRY USING GLUCOSE AS SUBSTRATE

***,[1]Jerry Mechery, [1]Praveen Kumar, C. S., [2]Deepa Nair, K. and [1]Sylas, V. P.**

[1]School of Environmental Sciences, Mahatma Gandhi University, Kottayam, Kerala, India
[2]National Centre for Earth Science Studies, Trivandrum, Kerala, India

ARTICLE INFO

Article History:
Received 21st September, 2014
Received in revised form
04th October, 2014
Accepted 18th November, 2014
Published online 30th December, 2014

Key words:

Biogas slurry,
Biohydrogen,
Facultative bacteria,
Fermentation,
Seed inoculums.

ABSTRACT

Hydrogen energy is the finest choice as it is the only clean carbon neutral fuel which can in the long run substitute the present petroleum based economy. At present commercial production of hydrogen relies on fossil fuels only, so biologically produced hydrogen is becoming the centre of attraction. This study has endeavoured to determine the capability of facultative anaerobic bacteria to produce biohydrogen through anaerobic fermentation. The bacterial seed inoculums were collected from different biogas plant slurry. The pure bacterial colonies were isolated through standard microbiological methods. The anaerobic fermentation was carried out with glucose as substrate in air tight erlenmeyer flask. The identification and characterisation of bacteria were performed by standard methods, which revealed that the strains belonged to *Enterobacter sp., Citrobacter sp., Proteus sp., Providencia sp*.and *Klebsiella sp.* The gas chromatographic results revealed that hydrogen gas evolved from all the samples with maximum production with *Enterobacter sp.* (27.08%) followed by *Citrobacter sp*. (25.81%), *Klebsiella sp.* (18.25%), *Providencia sp.* (7.19%) and least for *Proteus sp.* (0.45%).

INTRODUCTION

Recent research has recognized hydrogen as a promising energy carrier for the future (Bennemann 1998; Ball and Wietschel 2009; Hallenbeck *et al*., 2012). It is a regenerative, environment friendly, carbon neutral fuel with high energy yield (122 kJ/g) producing only water when combusted (Kapdan and Kargi 2006; Balat 2008; Das and Veziroglu 2008; Brown *et al*., 2013). However the conventional methods of hydrogen production are fossil fuel based which are energy intensive, uneconomical and also environmentally not sustainable (Nath and Das 2004; Zuttel 2004). Biological production of hydrogen by dark fermentation has received much attraction as it is a means of clean energy and also minimising waste (Ren *et al.,* 2009; Guo *et al.,* 2010). The present research was an attempt to evaluate the biohydrogen production potential of facultative bacteria isolated from biogas plant slurry by anaerobic fermentation using glucose as substrate.

MATERIALS AND METHODS

a. Bacterial inoculum

The seed inoculum was collected from different biogas plants of MG University campus, Kerala, India, having similar climatic and temperature conditions. These biogas plants were mainly fed with food waste. The raw seed slurry was first filtered through 2mm sieve to remove larger particulate matters, then heated in an oven at 100^0C for about 45 minutes and then cooled (Li and Fang, 2007). The pre-treated slurry was inoculated in sterile nutrient broth incubated at 37^0C under anaerobic condition for 3 days. Afterwards the culture was inoculated on agar plates with same medium, incubated for 24 hrs at 37^0C under anaerobic condition. The single colonies obtained were asceptically picked up and inoculated in enrichment media containing nutrient broth for about 10hrs.

b. Hydrogen Production Reactor

The experiment was conducted with glucose (Himedia) as the carbon source for anaerobic digestion. The substrate solution was prepared as 1.5% glucose in sterilised distilled water. The fermentation reaction was carried out in 250ml Erlenmeyer flask with screw cap. Each flask contained 75% substrate solution, 20% nutrient medium and 5% bacterial inoculums. The reactors were purged with CO_2 gas for 5 minutes and sealed air tightly. The experiments were conducted in anaerobic condition at 37^0C for 7 days. The nutrient solution consisted of 3.77g/l NH_4CO_3, 0.125g/l K_2HPO_4, 2g/l $NaHCO_3$, 0.005g/l $CuSO_4$, 0.1g/l $MgCl_2$, 0.015g/l $MnSO_4$, 0.025g/l $FeSO_4$ and 0.00125g/l $CoCl_2$ (Fang and Liu, 2002). Each experiment was done in quadruplicate. All the experiments were set at an initial pH of 6.5.

Corresponding author: Jerry Mechery,
School of Environmental Sciences, Mahatma Gandhi University, Kottayam, Kerala, India.

c. Analytical methods

The evolved gas were collected into gas tight syringes from the headspace of the reactor bottles and analysed in Gas Chromatograph (Nucon 5700) equipped with Thermal Conductivity Detector (TCD). Isothermal separation was done in a packed 2m long (PORAPAK Q) 80/100 mesh column. The total concentration of H_2 gas was calculated by the formula, Concentration of H_2 = Area of H_2 peak in sample/ Area of H_2 standard * Concentration of H_2 standard.

d. Biochemical bacterial characterization

The biochemical characterisation was conducted to identify the microbial species those having capability of hydrogen production. The following were the tests done: gram staining, motility, mannitol utilization, oxidase, catalase of IMViC, citrate, H_2S production, urease production (Barrow and Feltham, 1974).

RESULTS AND DISCUSSION

The GC analysis results showed that the evolved gases of all the samples (S1- S5) contained hydrogen and carbon dioxide in percentage level (Table 1).

Table 1. Concentration of gases in GC

Sample	H_2%	CO_2%
S1	25.81	73.22
S2	7.19	60.65
S3	0.45	31.05
S4	27.08	37.71
S5	18.25	29.71

The range of hydrogen content varied from 0.45% in sample S3 to 27% in sample S4 (Fig. 1). The GC chromatogram of samples S1 and S4 are represented in Fig. 2 and 3. The identified bacteria of each sample and their corresponding biochemical reactions are given in Table 2. The result of gas chromatography revealed that the microbial communities in all the samples evolved hydrogen gas by anaerobic fermentation of glucose substrate. CO_2 gas was also found in the samples which could have been produced as a result of the fermentation and also the due to head space purging during the initial stages. Presence of methane was not recorded in any of the sample. This might be the positive effect of heat pre-treatment. The heat pre-treatment was done to eliminate non spore forming hydrogen consuming methanogens and selectively favour the growth of heat resistant spore forming bacteria which are hydrogen producers (Lin *et al.,* 2006; Li and Fang, 2007; Sivaramakrishna *et al*., 2014). The final pH of the solution was found to be decreased in all the samples (S1 – 5.87, S2 – 5.8, S3- 5.74, S4 – 5.78 and S5 – 5.81). The lowered pH indicated the production of acidic compounds during the digestion (Devi and Joseph, 2004).

In the chromatogram (Fig. 2 and 3), the vertical axis represents the time in minutes and horizontal axis represents the area. The peak corresponding to hydrogen was found at 1.46 minutes. The concentration was calculated using the formula described in section 2.c. All the samples contained bacterial strains which were gram negative, rod shaped and motile. Among the different bacteria identified, *Enterobacter sp.* recorded maximum H_2 gas production (27.08%) followed by *Citrobacter sp*. (25.81%), *Klebsiella sp.* (18.25%), *Providencia sp.* (7.19%) and least for *Proteus sp.* (0.45%).

Table 2. Bacteria identified and biochemical reactions

Sample	Bacteria	Shape	Motility	Mannitol	Gram Stain	Oxidase	Catalase	O/F	Indole	MR	VP	Citrate	H_2S	Urease
S1	*Citrobacter sp.*	Short rod	motile	+	-	-	+	F	-	+	-	+	-	+
S2	*Providencia sp.*	Short rod	motile	+	-	-	+	F	+	+	-	+	-	+
S3	*Proteus sp.*	Short rod	motile	+	-	-	+	F	+	-	+	+	+	+
S4	*Enterobacter sp.*	Short rod	motile	+	-	-	-	F	-	-	+	+	-	+
S5	*Klebsiella sp.*	Short rod	motile	+	-	-	-	F	-	-	+	+	-	+

(O/F – oxidation/ fermentation, MR – Methyl Red, VP –Voges Proskauer)

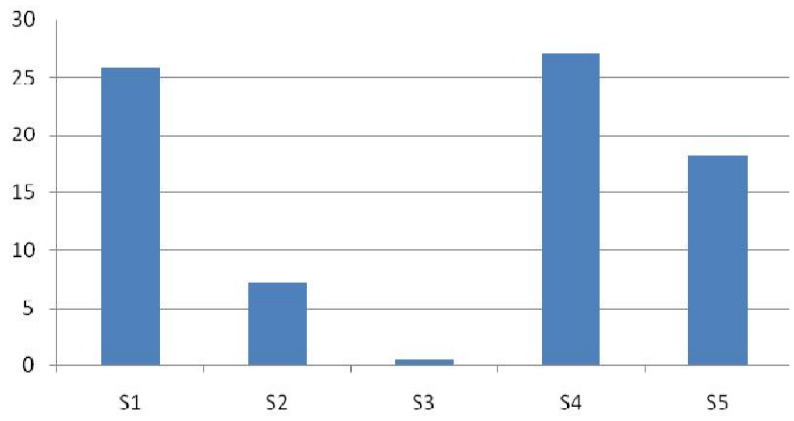

Fig. 1. Hydrogen gas concentration (%) of 5 samples

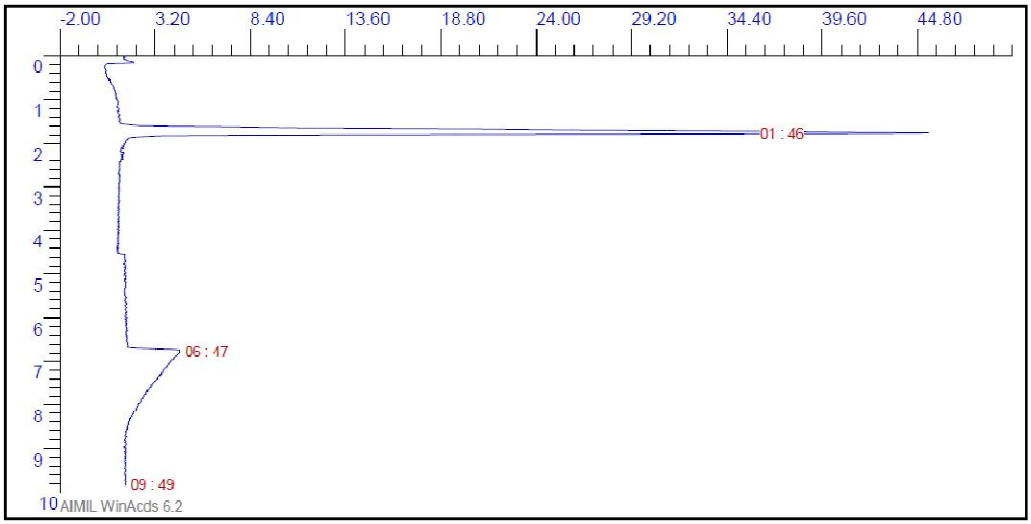

Fig. 2. GC chromatogram of sample S1

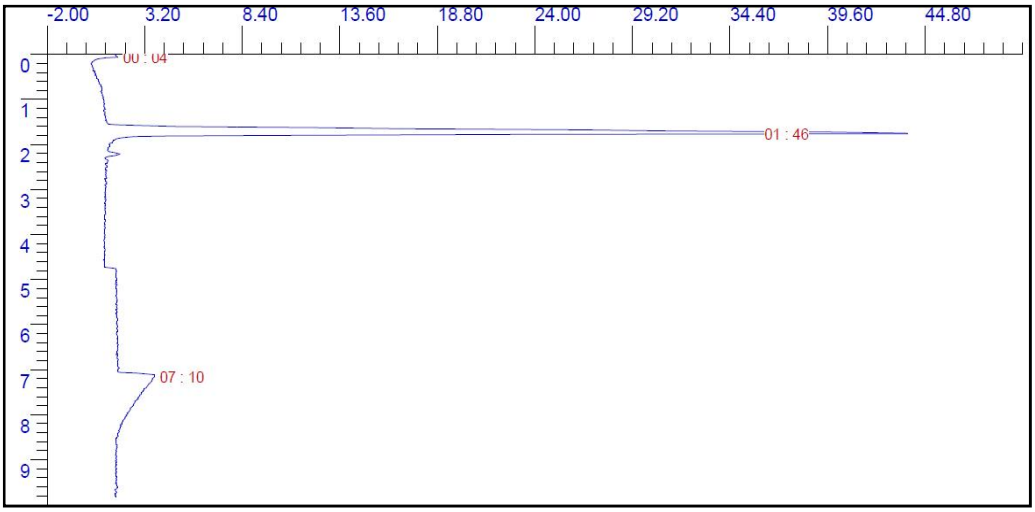

Fig. 3. GC chromatogram of sample S4

Enterobacter sp. is the most common gram negative and facultative anaerobe yielding more amount of hydrogen (Kotay and Das 2006; Chong *et al.,* 2009). The *Enterobacter cloacae* IIT-BT 08 strain produces about 6.0 mol/mol sucrose (Kotay and Das 2006), while *Enterobacter aerogenes* produced only 1.09 mol/mol glycerol with glycerol as substrate (Jo *et al.,* 2008). *Citrobacter amalonaticus* Y-19 strain can yield about 2.49 mol/mol of glucose (Oh *et al.,* 2008) and *Klebsiella oxytoca* yielded about 1 mol/mol glucose (Minnan *et al.,* 2005). While studies related to *Enterobacter sp., Klebsiella sp*. and *Citrobacter* sp. are reported, those related to *Providencia* sp. and *Proteus* sp. are very limited.

Conclusion

Biological hydrogen production by facultative anaerobic bacteria isolated from biogas plant slurry through fermentation was demonstrated in this study. Glucose was used as the carbon substrate for the bacterial digestion. The study revealed the potential of bacterium obtained from biogas plant slurry in producing hydrogen. Five bacteria namely, *Enterobacter sp.*, *Citrobacter sp*., *Klebsiella sp., Providencia sp.* and *Proteus sp.* were identified. Among them, *Enterobacter sp.* was found to produce higher hydrogen production (27.08%) and lowest production recorded for *Proteus sp.* (0.45%).The application of anaerobic microbes for the production of biohydrogen has wide research potential and has high significance in tropical countries for energy security and waste management.

Acknowledgement

The author, JM, is thankful to Kerala State Council for Science Technology and Environment (KSCSTE), Govt. of Kerala, India for research fellowship and the authors are thankful to University Grants Commission (UGC), New Delhi, India [F.41/2012 (SR)] for the partial financial support.

REFERENCES

Balat, M. 2008. Potential importance of hydrogen as a future solution to environmental and transportation problems. *Int. J. Hydrogen Energy.,* 33(15): 4013-29.

Ball, M, and Wietschel, M. 2009. The future of hydrogen – opportunities and challenges. *Int. J. Hydrogen Energy.,* 34: 615 – 27.

Barrow, G.I. and Feltham, R.K.A. 1974. Cowan and Steel's Manual for the identification of Medical bacteria (3rd edn), Cambridge University Press

Benemann, R. 1998. The technology of biohydrogen, International Conference on Biological Hydrogen Prodution, Waikoloa, Hawaii, USA.

Brown, JR., Rinaldo, P., Brutoco., James, JD and Cusumano, A. 2013. Hydrogen Revolution: Growth, Jobs, Security and Sustainability. In:Freedom from mid – east oil, USA: World Business Academy: 403-38.

Chonga, M., Sabaratnamb, V., Shiraic, Y. and Hassana, MA. 2009. Biohydrogen production from biomass and industrial wastes by dark fermentation. *Int. J. of Hydrogen Energy.,* 34: 3277–3287.

Das, D. and Veziroglu, T.N. 2008. Advances in biological hydrogen production processes. *Int J Hydrogen Energy.,* 33: 6046-57.

Devi, G. and Joseph, K. 2004. Solid Phase Anaerobic Digestion of Municipal Solid Waste. *Journal of IAEM.,* 31:147 – 152.

Fang, H.H.P. and Liu, H. 2002. Effect of pH on hydrogen production from glucose by a mixed culture. *Bioresource. Technol.,* 82:87–93.

Guo, X.M., Trably, E., Latrille, E., Carre`re, H. and Steyer, J.P. 2010. Hydrogen production from agricultural waste by dark fermentation: a review. *Int. J. Hydrogen Energy.,* 35(19): 10660-73.

Hallenbeck, PC., Abo-Hashesh, M. and Ghosh, D. 2012. Strategies for improving biological hydrogen production. *Bioresource Technology.*, 110: 1-9.

Jo, JH., Lee, D.S., Park, D., Choe, W.S. and Park, J.M. 2008. Optimization of key process variables for enhanced hydrogen production by *Enterobacter aerogenes* using statistical methods. *Bioresour. Technol.,* 99:2061-6.

Kapdan, I.K. and Kargi, F. 2006. Bio-hydrogen production from waste materials. *Enzyme and Microbial Technology*, 38: 569–82.

Kotay, S.M. and Das, D. 2006. Feasibility of biohydrogen production from sewage sludge using defined microbial consortium. W*HEC* 16. 13-16 June– Lyon France

Kumar, N. and Das, D. 2000. Enhancement of hydrogen production by *Enterobacter cloacae* IIT-BT 08. *Process Biochem., 35*:589–93.

Li, C. and Fang, H.H.P. 2007. Fermentation hydrogen production from wastewater and solid wastes by mixed cultures. *Critical Reviews in Environ Science and Technol.,* 37: 1-39.

Lin, C.Y., Hung, C.H., Chen, C.H., Chung, W.T. and Chen, L.H. 2006. Effects of initial pH on fermentative hydrogen production from xylose using natural mixed cultures. *Process Biochemistry,* 41: 1383-1390.

Minnan, L., Jinli, H., Xiaobin, W., Huijuan, X., Jinzao, C., Chuannan, L. *et al.* 2005. Isolation and characterization of a high H2-producing strain *Klebsialle oxytoca* HP1 from a hot spring. *Res. Microbiol.,* 156:76–81.

Nath, K. and Das, D. 2004. Biohydrogen production as a potential energy source - Present state of art. *JSIR,* 63: 729-38.

Oh, YK., Kim, HJ., Park, S., Kim, M.S. and Ryu, D.D.Y. 2008. Metabolic-flux analysis of hydrogen production pathway in *Citrobacter amalonaticus* Y19. *Int J Hydrogen Energy*, 33:1471-82.

Ren, N., Wang, A., Cao, G., Xu, J. and Gao, L. 2009. Bioconversion of lignocellulosic biomass to hydrogen: potential and challenges. *Biotechnol. Adv.,* 27: 1051-60.

Sivaramakrishna, D., Sreekanth, D., Sivaramakrishnan, M., Kumar, B.S., Himabindu, V. and Narasu, M.L. 2014. Effect of system optimizing conditions on biohydrogen production from herbal wastewater by slaughterhouse sludge. *Int. J. of hydrogen Energy*, 39: 7526-7533.

Zuttel, A. 2004. Hydrogen storage methods. *Naturwissensch aften.,* 91: 157–72.

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