Journal of Food Technology 3 (4): 535-537, 2005

Additive Effect of Soybean Curd Residue, *Okara*, for Enhancement of Methane Production from Pretreated Woody Waste

[1]Harumi Take, [2]Godliving Mtui, [1]Fumihisa Kobayashi and [1]Yoshitoshi Nakamura
[1]Graduate School of Natural Science and Technology,
Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan
[2]Applied Microbiology Unit, University of Dar es Salaam,
P.O. Box 35060, Dar es Salaam,Tanzania

Abstract: In order to convert woody waste into methane gas efficiently, the pretreatment effect of steam explosion and the additive effect of soybean curd residue, *okara*, were clarified. 180 mL of methane gas was obtained from 1 g of steam-exploded Japanese cedar chips at a steam pressure of 4.51 MPa and a steaming time of 5 min while no methane gas was produced from untreated chips. The addition of *okara* into the chips was attempted and the optimal condition, i.e. C/N of 18, increased the methane gas produced to 315 mL.

Key words: C/N ratio, methane production, woody waste, soybean curd residue, steam explosion

INTRODUTION

Woody waste, i.e. wood chips, wood powder and bark, is a useful source that can be converted into fuel energy such as alcohol and methane[1]. Though polysaccharide (cellulose and hemicellulose) in woody waste is a substrate for methane production, it is difficult to convert the polysaccharide into methane by direct biological methods owing to lignin covering the polysaccharide layers strongly[2]. Recently, steam explosion has been adopted as one of the effective pretreatment methods for degrading and removing lignin from polysaccharide surface[3-6]. The steam-exploded woody waste seems to be converted into methane, but the yield of methane produced might be comparatively low because it has little nitrogen. It is generally known that not only carbon source but also nitrogen source are necessary for the efficient methane fermentation[7]. On the other hand, *okara* is a nitrogen-rich refuse of tofu, i.e. soybean curd. In Japan, processing industries discharge as much as 800,000 tons of *okara* annually. Therefore, addition of *okara* into steam-exploded woody waste followed by fermentation seems to be effective means of increasing methane yield.

MATERIAL AND METHOD

In this study, the pretreatment effect of steam explosion on the methane production from woody waste was examined using Japanese cedar as a sample. After the organic elemental analysis of *okara* and steam-exploded Japanese cedar, the *okara* was added into the steam-exploded Japanese cedar and the mixture was fermented for increasing the yield of methane produced.

Wood chips of Japanese cedar (*Cryptomeria japonica*) were used as a woody waste sample. The length and width of the wood chips were 2-3 and 5-8 cm, respectively. *Okara*, i.e. soybean curd residue, was also used. The apparatus for steam explosion (Japan Chemical Engineering and Machinery Co. Ltd, Osaka, Japan) consisted of a steam generator, a pressurized reactor, a receiver and a condenser with a silencing action[8]. The reactor was insulated to maintain a constant temperature. The capacity of the reactor was 1.2 L, the highest pressure was 6 MPa and the highest temperature was 275. Approximately 100 g of the chipped Japanese cedar was introduced into the reactor and then steam-heated at a steam pressure of 4.51 MPa and a steaming time of 5 min. Total dry weight of steam-exploded Japanese cedar chips and *okara* was adjusted into 1 g and added into a 500 mL conical flask containing 500 mL seed methanogens sludge and then incubated anaerobically for an incubation time of 700 h at 37°C. The percentages of *okara* in *okara* and steam-exploded Japanese cedar chips were set as 0, 9, 23, 50, 67 and 100%. The seed (methanogens sludge, SS 8000 ppm) was obtained by culturing the sludge withdrawn from anaerobic digestion unit at a Kanazawa city wastewater treatment plant in Japan. Evolved gas was passed through a tube into 500 mL cylinder that was submerged in saturated sodium chloride water. Stock gas was collected by a syringe for analysis. The amounts of elements, C, N and H, were determined by using an

Corresponding Author: Yoshitoshi Nakamura, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan, Tel: +81 76 234 4819 Fax: +81 76 234 4829

organic elements analyzer (CHN CORDER MT5, Yamako Co. Ltd., Kyoto, Japan). The gas composition was analysed by using a gas chromatography unit equipped with a thermal conductivity detector (GC-8APT, Shimazu Co. Ltd., Kyoto, Japan). The column was stainless steel (4 m by 0.3 cm) filled with Shincarbon ST (Shimazu Co. Ltd., Kyoto, Japan) and heated to 100°C. Helium gas (50 cm^3 min^{-1}) was a carrier gas.

RESULTS AND DISCUSSIONS

Figure. 1 shows the time courses of total gas evolved and methane gas evolved in the methane fermentation of steam-exploded Japanese cedar chips and untreated chips. The pH of reaction mixture, i.e. methanogens sludge and sample was about 7 (data not shown). The amounts of total gas evolved and methane gas evolved increased with the increase of incubation time reaching their maximum values of 305 and 180 mL, respectively, at an incubation time of 400 h. On the other hand, no gas evolved was detected in the methane fermentation of untreated chips. It was found that the steam explosion was a very effective pretreatment method for the enhancement of methane production from the Japanese cedar chips. According to Buswell's theory[9], about 420 mL of methane gas is produced from 1 g of polysaccharide such as cellulose and hemicellulose. Since 1 g of dry softwood contains about 0.7 g of polysaccharide[10], the methane yield of holocellulose in the steam-exploded Japanese cedar chips corresponded to approximately 0.61: i.e. ratio of the methane gas produced, (180 mL) to the theoretical value, (294 mL).

Table 1 shows the amounts of C, N, H and others in sample to the dry weight of sample, i.e. steam-exploded Japanese cedar chips and *okara*. Though little difference of carbon content between the steam-exploded Japanese cedar chips and the *okara* was observed, the nitrogen content ratio of *okara* was about 20 times larger than that of the steam-exploded Japanese cedar chips. The C/N ratio of steam-exploded wood chips was very high as 257, while the C/N ratio of *okara* was 12. The reason why the methane yield of steam-exploded Japanese cedar chips was low (Fig.1) seems to be that the steam-exploded Japanese cedar chips have little nitrogen source, i.e. 0.22%. On the other hand, since the *okara* is not a wood-based waste but a soybean-processing waste, it has much nitrogen source. The composition of *okara* is 26% protein, 19% lipid, 51% polysaccharide and 4% ash[11]. Therefore, in order to increase methane yield, it is necessary to add the *okara* into the steam-exploded Japanese cedar chips prior to fermentation process.

Table 1: Organic elements analysis of steam-exploded Japanese cedar chips and *okara*

Samples	C (%)	N (%)	H (%)	Other (%)	C/N ratio(-)
Steam-exploded wood chip	56.49	0.22	5.92	37.37	257
Okara	46.79	4.06	7.01	42.14	12

Table 2: Amounts of total gas and methane gas evolved in the methane fermentation of sample containing steam-exploded Japanese cedar chips and *okara*

Samples Steam-expoloded wood chip (g)	Okara (g)	C/N ratio (-)	Total gas evolved (mL)	Methane gas evolved (mL)
1	0	257	305	180
0.91	0.09	98	310	196
0.77	0.23	49	320	205
0.50	0.50	24	420	280
0.33	0.67	18	480	315
0	1	12	411	271

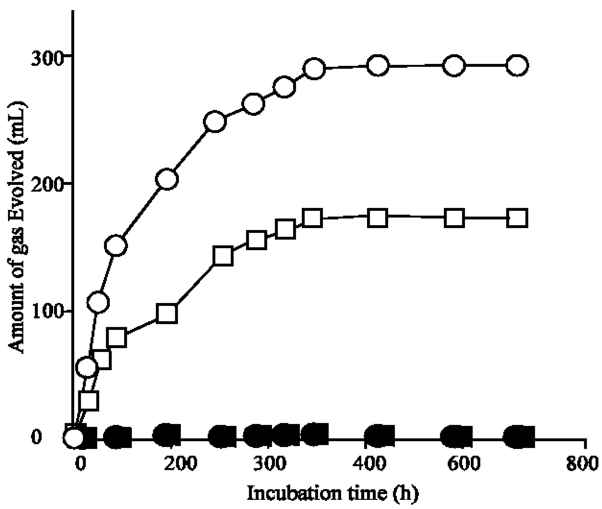

Fig. 1: Methane fermentation of steam-exploded Japanese cedar chips and untreated chips. Symbols, total gas evolved of steam-exploded Japanese cedar chips, methane gas evolved of steam-exploded Japanese cedar chips; total gas evolved of untreated chips methane gas evolved of untreated chips.

Table 2 shows the methane fermentation results of sample containing steam-exploded Japanese cedar chips and *okara*. Total dry weight of steam-exploded Japanese cedar chips and *okara* was adjusted to 1 g. Both the total gas and the methane gas evolved varied significantly with the C/N ratio. Though 1 g each of steam-exploded Japanese cedar chips and *okara* produced 180 and 271 mL of methane gas, respectively a larger amount of methane gas, i.e. 315 mL, was obtained from 0.33 g of steam-exploded Japanese cedar chips and 0.67 g of *okara*, i.e. C/N ratio of 18. This result was due to the fact that in the low C/N ratio, the growth of methanogens were restricted by the lack of nitrogen source while in the high C/N ratio, they were inhibited by ammonia produced from large amounts of nitrogen source. A comparatively large amount methane gas is produced from only *okara* medium, i.e. C/N of 12, but the effective methane production of woody waste required the addition of nitrogen-rich material such as *okara*. This result also suggests that the addition of material with very high C/N ratio, i.e. animal manure, might be more effective for the methane production of woody waste and this method can be also easily applied to various plant wastes such as wheat straw, bagasse and sweet sorghum. Future research will be focused on the more efficient methane gas production by mixing the different substrates and determining the optimal C/N ratio.

CONCLUSIONS

This study presented a novel method for increasing the yield of methane gas produced from woody waste. The steam explosion was used for the delignification of woody waste and the large amount of methane gas was produced from steam-exploded woody waste while no methane gas was produced from untreated woody waste. Furthermore in order to increase the yield of methane gas the soybean residue, *okara* was added and the additive effect was evaluated. As a result, some valuable findings, i.e. 1) the effectiveness of steam explosion as a pretreatment or methane fermentation of woody waste; 2) the additive effect of *okara* for increasing the yield of methane gas obtained from woody waste; 3) the maximum C/N ratio for maximizing the amount of methane gas produced, were obtained in this work.

ACKNOWLEDGEMENTS

This study was supported in part by a Grant-in-Aid for Scientific Research (B) (1) (No. 15360483) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

1. Klass, D.L., 1998. Biomass for Renewable Energy. Fuels and Chemicals, Academic Press, San Diego, CA, USA.
2. Saddler, J.N., 1993. Bioconversion of Forest and Agricultural Plant Residues. CAB International Press, Wallingford, UK.
3. Excoffier, G., E. Toussaint and V.R. Vignon, 1991. Saccharification of steam-exploded poplar wood. Biotechnol. Bioeng., 38: 1308-1317.
4. Nakamura, Y., T. Sawada and E. Inoue, 2001. Enhanced ethanol production from enzymatically treated steam-exploded rice straw using extractive fermentation. J. Chem. Technol. Biotechnol., 76: 879-884.
5. Sawada, T. and Y. Nakamura, 2001. Low energy steam explosion treatment of plant biomass. J. Chem. Technol. Biotechnol., 76: 139-146.
6. Vallander, L. and K-EL. Eriksson, 1991. Enzymatic hydrolysis of lignocellulosic materials: II. Experimental investigations of theoretical hydrolysis. Biotechnol. Bioeng., 38: 139-144.
7. Yata, M., H. Kawaguchi and K. Sasaki, 2001. Bioconversion of Wastes, Chijinshokan Press, Tokyo, Japan.
8. Nakamura, Y., 2004. Total effective utilization of unutilized plant biomass by using various conversion methods. Recent Res. Develop. Biotechnol. Bioeng., 6: 31-42.
9. Buswell, A.M. and F. W. Jr. Sollo, 1948. The mechanism of methane fermentation. J. Am. Chem. Soc., 70: 1778-1780.
10. Kennedy, J.F., G.O. Phillips and P.A. Williams Wood 1979. Processing and Utilization, Ellis Horwood Press, Chichester, UK.
11. Yamaguchi, M., 1992. Japanese Food Analysis. Ishiyaku Press, Tokyo, Japan.