Fungal pretreatment studies on Rice husk and Sugarcane bagasse for biogas production

Allimuthu V[1], *Gopinath LR[1], Sathya P[1] and Bhuvaneswari R[2]

[1]Department of Biotechnology, Vivekanandha College of Arts and Science for Women, Tiruchengode, Namakkal

[2]Department of Zoology, Namakkal Kavignar Ramalingam Government Arts College for Women, Namakkal

* Correspondence: lrgopinathnamakkal@gmail.com

ABSTRACT

Chopped and moist rice husk and sugarcane bagasse were pretreated with *Aspergillus flavus* and *Aspergillus terreus* to produce biogas production. The potential of fungal pretreatment of rice husk and sugarcane bagasse were investigated at different time interval of 0th day, 10th, day, 20th day by determining the biochemical compounds analysis. Analysis of biochemical changes and analysis the voltaile fatty acid production. Estimating the composition and quantity of biogas production. The pretreated rice husk and sugarcane bagasse were used for biogas production in 20 litre capacity biogas digesters. Similarly they also enhanced biogas production with increased methane production in which *Aspergillus flavus* produced 78.41% at and 72.37% by *Aspergillus terreus* at 30th day the control produced only 56.23% and 51.78% respectively. The pretreated rice husk and sugarcane bagasse with Aspergillus flavus and Aspergillus might be more responsible for Aspergillus flavus to produce biogas production.

Keywords

Biogas; Fungal pretreatment; Aspergillus terreus; Aspergillus flavus; rice husk; sugarcane bagasse.

INTRODUCTION

In today's energy demanding life style, need for exploring and exploiting new sources of energy which are renewable as well as eco- friendly is a must. In rural areas of developing countries various cellulosic biomass (cattle dung, agricultural residues, etc) are available in plenty which have a very good potential to cater to the energy demand, especially in the domestic sector [1]. Methane is the main component of natural gas. It is relatively clean burning, colourless, and odourless. Natural generation of biogas is an important part of bio-geochemical carbon cycle. It can be used both rural and urban areas. The gas is used as a fuel substitute for firewood, dung, petrol, agricultural residues, diesel and electricity, depending on the nature of the task and local supply conditions [2]. It said to be ideal in deciding alternative sources of energy for rural people in the sense that is cheap and local in origin and production [3]. Biogas technology is based on the phenomenon that when organic matter containing cellulose is fermented in the absence of air (aerobically) combustible gases but majorly (methane) is formed[4]. This technology represents one of a number of village-scale technologies that are currently enjoying a certain level of patronage among government and aid agencies and that after the technological possibility of more decentralized approaches to development [5].

India is credited for having built the first- ever anaerobic digester, in 1897, when the Matunga Leper Asylum in Bombay (Mumbai) utilised human to generate gas to meet its lighting needs [6]. Biogas production technology has been introduced to Thailand around 1978 for various applications such as the production of energy from organic waste treatments and organic fertilizers, and the improvement of the hygienic condition by reducing pollution [7]. The application for organic fertilizers reduces the need of chemical fertilizers. Organic wastes including domestic, industry, and agriculture waste cane be treated using the biogas production process. The process could potentially reduce plant, animal, and human pathogens. Biogas is also a clean- burning fuel that can reduce the incidence of eye and lung problems [8].

Microorganisms can also be used to treat the lignocelluloses and enhance enzymatic hydrolysis. The applied microorganisms usually degrade lignin and hemicellulose but very little part of cellulose, since cellulose is more resistance than the other parts of lignocelluloses to the biological attack. Several fungi, e.g. brown-, white- and soft-rot fungi, have been used for this purpose. White-rot fungi are among the most effective microorganisms for biological pretreatment of lignocelluloses. Taniguchi [9] group in 2005 has evaluated biological pretreatment of rice straw using four white-rot fungi (*Phanerochaete chrysosporium, Trametes versicolor, Ceriporiopsis subvermispora,* and *Pleurotus ostreatus*) on the basis of quantitative and structural changes in the components of the pretreated rice straw as well as susceptibility to enzymatic hydrolysis. Pretreatment with *P. ostreatus* resulted in selective degradation of the lignin rather than the holocellulose component, and increased the susceptibility of rice straw to enzymatic hydrolysis. Some bacteria can also be used for biological pretreatment of lignocellulosic materials. Kurakake *et al*. [10] studied the biological pretreatment of office paper with two bacterial strains, *Sphingomonas paucimobilis* and *Bacillus circulans,* for enzymatic hydrolysis. Biological pretreatment with the combined strains improved the enzymatic hydrolysis of office paper from municipal wastes. Under optimum conditions, the sugar recovery was enhanced up to 94% for office paper. Biological treatments with microorganisms or enzymes are also investigated to improve digestion in biogas production. The biological pretreatment might be used not only for lignin removal, but also for biological removal of specific components such as antimicrobial substances. Solid-state fermentation of orange peels by fungal strains of *Sporotrichum, Aspergillus, Fusarium and Penicillum* enhanced the availability of feed constituents and reduced the level of the antimicrobial substances .In a similar work, cultivation of white-rot fungi was used to detoxify olive mill wastewater and improve its digestion [11]. Low energy requirement, no chemical requirement, and mild environmental conditions are the main advantages of biological pretreatment. However, the treatment rate is very low in most biological pretreatment processes.

Sugar Cane Bagasse- the residue left after juice extraction is a waste available in abundance worldwide. About 1.34Gt of sugarcane was produced globally in 1999, which equates to approximately 375MT of Bagasse, 50% of which is typically burned. India is the second largest producer of sugarcane next to Brazil with a production of 300MT of sugarcane in 1999-2000. Rice husk (RH) is one of the by-products obtained during milling of rice. This surrounds the paddy grain. It is reported that approximately 0.23 tons of rice husk (rice hull) is formed from every ton of rice produced .World rice production is approximately 645 million tons.

Materials and methods

Fungal samples *Aspergillus flavus* and *Aspergillus terreus* were collected from Doctor's Diagnostic centre, Trichy and were directly brought to the laboratory. Samples were then sub cultured on Czapek-dox agar medium and the same was preserved in Czapek-dox broth. For the collection of substrates, two tanks were sterilized with hot water and then collected rice husk from Salem.

Fungal preparation:

1 litre of Czapek-dox broth was prepared for both *Aspergillus flavus* and *Aspergillus terreus*. Followed by a disc of Czapek-dox agar with respective fungi were taken from the plates and was inoculated in to the flask containing Czapek-dox broth for fungal preparation. Fungal inoculated broths were then incubated at 28^0 C for 7 days at 180rpm.

The samples collected from pretreatment tank was directly brought to the analysis of organic compounds such as the presence of Total Solids, Ash content, Voltaile solids, Total organic carbon, Cellulose, Hemi cellulose, lignin, silica and Total Sugar were analysed after the treatment of 20days. Standard methods of AOAC (Association of analytical communities) 2000 were used for the determination of Total solids, Voltaile solids, ash content, cellulose and hemicelluloses, lignin and silica. Total sugar was estimated using Phenol- sulphuric acid method using glucose as a standard [11]. Total Carbon was determined by Walkely Black method described by Page et al 1982 [12].

The biogas production was studied through incorporating 1 liters of 7days culture into 20 liters of in mini biogas unit. Gas produce were collected at different intervals 15 days and different gases (Methane, Carbon di-oxide, hydrogen sulphide and other gases) were analyzed by GC MS.

Results

Analysis of biochemical compounds of rice husk treated with fungal species showed higher in volatile solids 84.38 % followed by organic carbon 48.4% and cellulose 36.67% initially and lowest concentration was observed in lignin 8.86%. Analysis of biochemical compounds of sugar bagasse treated with fungal species showed higher in total solids 95.27 % followed by volatile solid 91.39% and organic carbon 46.53% initially and lowest concentration was observed in ash 8.89%. Analysis of volatile fatty acids like acetic acid, propionic acid and butynic acid in biogas units showed increased at 15th day and decreased gradually further. Analysis of propionic acid showed highest concentration on 15th day in *Aspergillus flavus* pretreated rice husk biogas tanks 549.69 mg/l. Analysis of butynic acid showed highest concentration on 15th day in *Aspergillus flavus* treated rice husk biogas tanks 966.87mg/l. Comparatively among the rice husk and sugarcane bagasse biogas tanks the sugarcane bagasse biogas tanks showed higher VFA concentration. Among the three VFA in sugarcane bagasse biogas tanks, the acetic acid concentration was recorded higher in tank pretreated with *Aspergillus flavus* during 15th day 1123.24 mg/l.

Analysis of propionic acid showed highest concentration on 15th day in *Aspergillus flavus* pretreated rice sugarcane bagasse tanks 639.44 mg/l. Analysis of butynic acid showed highest concentration on 15th day in *Aspergillus flavus* treated sugarcane bagasse biogas tanks 1009.29mg/l. In biogas production showed better methane evolution with *Aspergillus flavus* pretreated biogas tank with 78.41% followed by *Aspergillus terreus* pretreated biogas unit with 72.37% whereas control tanks showed 56.23% and 51.78% respectively (Figure.1).Among the different gases methane concentration was higher followed by carbon dioxide and other gases and least was H_2S.

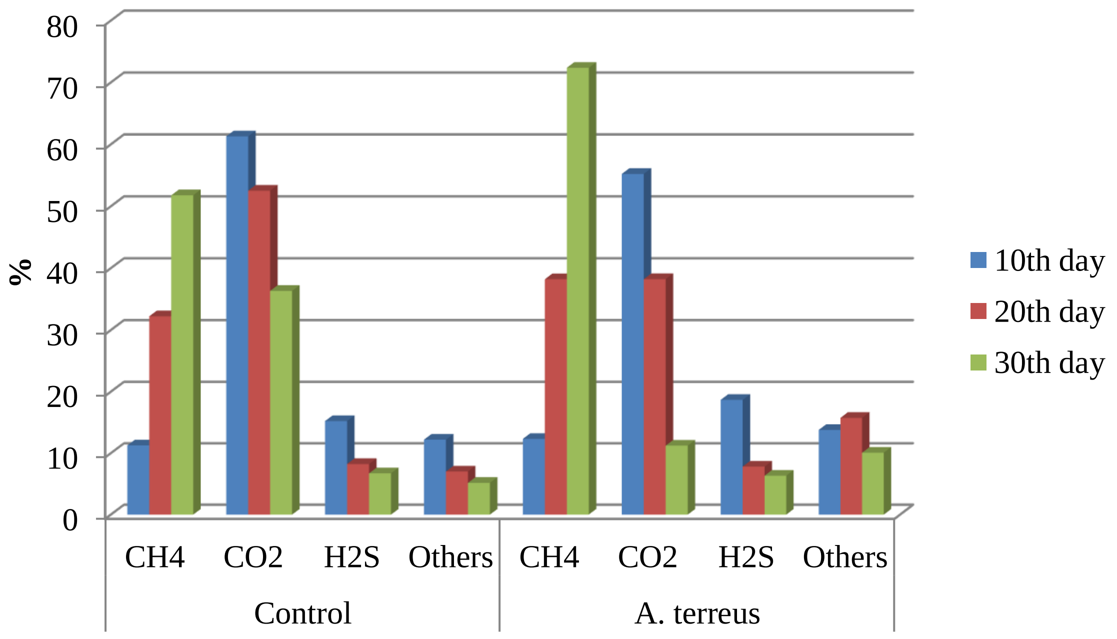
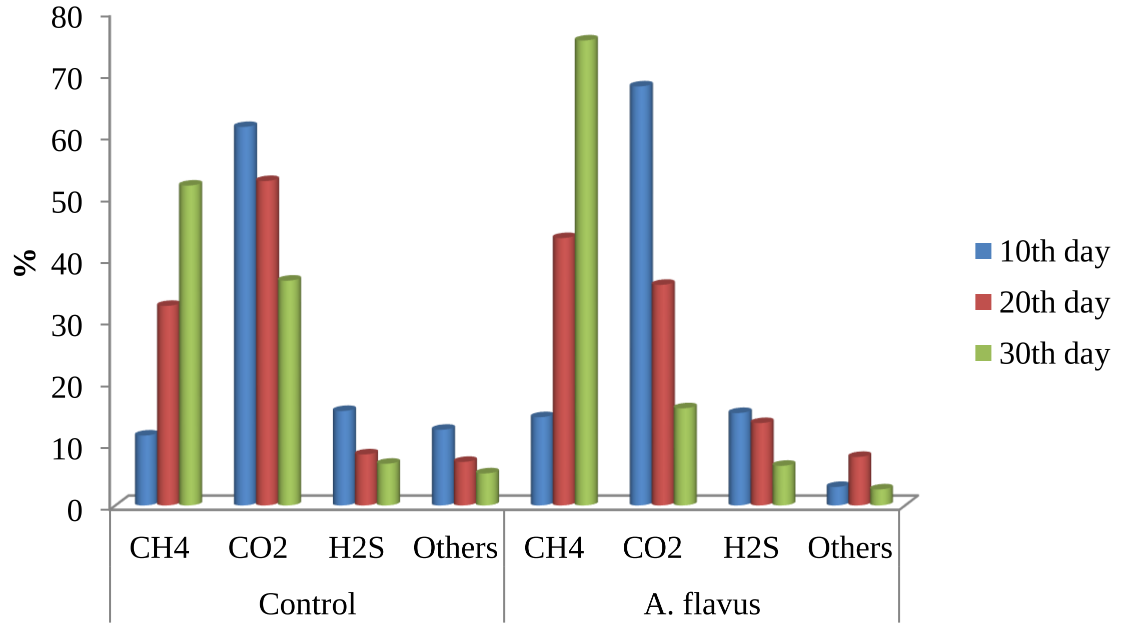


Figure 1. Biogas production in biogas unites with A. terreus treated risce husk samples and control

Figure 2. Biogas production in biogas units with A. flavus treated rice husk samples and control



DISCUSSION

Rice husk showed higher volatile solids where as sugarcane bagasse showed high total solids this is basically due to higher lignin content. This higher lignin content may result in slower degradation of organic compound [13,14]. Organic carbon is also higher in sugarcane bagasse as result of increased lignin concentration which plays an important role in sequestering atmosphere carbon di oxide [15]. Ash content was low in sugarcane bagasse than the rice husk due to higher hemicelluloses and cellulose concentration in rice husk.

Initial decrease in hemicelluloses content in pretreatment in both the substrates by both fungus for first ten days might be the result of breakdown or hydrolysis of hemicellulose into fermentable sugars [16]. This observation clearly indicates that the fungus has active hemicellulases during 10 days of its growth cycle and active cellulases after 10 days period. Initially hemicelluloses and then cellulose was utilized for growth and multiplication of fungus [17]. As a result volatile solids, total organic carbon, cellulose, lignin, and total sugar showed increasing trend in all the pretreatments. However, this increase and decrease of biochemical compounds were higher with *A. flavus* pretreated samples and also in sugarcane bagasse substrate. This is due to active growing nature of A. flavus and higher volatile solids in sugarcane bagasse.

The present study, volatile fatty acids acetic acid, propionic acid and butyric acid were estimated showed high level of acetic acid followed by butyric acid, propionic acid and valeric acid in all the biogas tanks treated with the acidogens. All these fatty acids were increasing up to 20th day and decreased thereafter. Among the two pretreated substrates *A. flavus* treated sugarcane bagasse showed high level of volatile fatty acid followed by *A. flavus* treated rice husk substrates in spite of higher concentration of lignin content in sugarcane bagasse this may be due to presence of higher sugar content and pretreatment with fungal species. Previous study carried out on biogas production from rice husk showed that the waste has the potential to generate biogas but with low cumulative gas yields and slow onset of gas flammability as a result of the presence of lignin in the cell wall of the plant bringing about slower rate of dehydration and hydrolysis with consequent acidic environment [18].The present study used both the substrates with higher lignin concentration.

The gas production revealed increased (21.63%) concentration of methane was found in *A. flavus* pretreated sugarcane bagasse fed biogas unit among all biogas units. Among the two pretreated substrates *A. terreus A. flavus* pretreatments showed increased methane yield. Similar results reported by Ofofuele [19] that least cumulative gas production was observed in rice husk.

Similar results were also observed by Jalc et al., [16] in 1998, who reported maximum loss in hemicelluloses content in white rot fungus treated wheat straw. *Pleurotus ostreatus* has been found to be the most promising fungus in increasing cellulose content and decreasing Klason lignin of rice straw [9].

Conclusion

The present study was able identify that fungal pretreatment of high lignin concentrated substrate could be useful in anaerobic decomposition and increase the methane concentration. Among rice husk and sugarcane bagasse pretreatments with fungal species sugarcane bagasse was able to produce more methane. Among the fungal species *A. flavus* was able to increase methane production in both the substrates.

Reference:

[1] Kashyap DR, Dadhich KS and Sharma SK (2003) Biomethanation under psychrophilic conditions: a Review. *Bioresour Technol,* 87: 147-153.

[2] Madu C and Sodeinde OA (2001) Relevance of biomass in the sustainable energy development in Nigeria. In proc. National Engineering Conference and Annual General Meeting of the Nigerian Society of Engineers. 220-227.

[3] Tanbawal AD (2002) "A Comparative Study of Biogas Production from Camel, Cattle, and Horse Dungs". Paper presented at NASEF O2. Kaduna, Nigeria.

[4] Ghosh S, Henry MP, Sajjad A, Mensinger MC and Arora JL (2000) Pilot- scale gasification of municipal solid wastes by high-rate and two-phase anaerobic digestion (TPAD). *Water Sci Technol,* 41: 101-110.

[5] Fernado CEC and Dangogo SM (1986) Investigation of some parameters which affect the performance of biogas plants. Nigerian J Solar Energy, 5:141-147.

[6] Khanal SK (2008) Anaerobic biotechechnology for bioenergy production: principles and Applications. Wiley- Blackwell, Ames.

[7] Bhurniratana S, Tanticharoen M, Putamayothin N and Supajunya N, Biogas technology in Thailand. In Bidin R, Chong CN and Wang CW (Eds). Proceedings of the second ASEAN Workshop on Biogas technology Applied to the management and utilization of Food waste materials. Kuala Terengganum, Malaysia, 104-110, 1982.

[8] Stuckey DC, Biogas: A global perspective. In EL-Halwagi MM (Ed). Biogas technology: Transfer and diffusion. New York: Elsevier Applied Science, 18-44, 1984.

[9] Taniguchi M, Suzuki H, Watanabe D, Sakai K, Hoshino K and Tanaka T (2005) Evaluation of pre-treatment with Pleurotus ostreatus for enzymatic hydrolysis of rice straw. J Biosci Bioeng, 100: 637-43.

[10] Kurakake M, Ide N and Komaki T (2007) Biological pre-treatment with two bacterial strains for enzymatic hydrolysis of office paper, Curr Microbiol, 54: 424–428

[11] Dhouib A, Ellouz M, Aloui E and Sayadi S (2006) Effect of bioaugmentation of activated sludge with white- rot fungi on olive mill wastewater detoxification, Lett Appl Microbial, 42: 405-411.

[12] Page AL, Miller RH and Keeney DR (Eds) Methods of Soil Analysis: Part 2, Chemical and Microbiological Properties. Agronomy Series No 9, American Society of Agronomy, Madison, WI. 1982.

[13] Adler E (1911) Lignin chemistry- past present and future, Wood Sci Technol. 11, 169-218.

[14] Higuchi T (1990) Lignin Biochemistry : Biosynthesis and Biodegradation. Wood Sci Technol. 24, 23-63.

[15] Hooker TD and Compton JA (2003) Forest ecosystem carbon and nitrogen accumulation during the first century after agricultural Abandonment Ecol Appl, 13: 299-313.

[16] Jalc D, Nerud F and Siroka P (1998) The Effectiveness of Biological Treatment of Wheat Straw by White-Rot Fungi. Folia Microbiol, 43(6): 687-689.

[17] Mehta P (1990) Bhopal tragedy's health effects, a review of methyl isocyanate toxicity. JAMA, 264: 2781-2787.

[18] Uzodinma EO, Ofoefule AU, Eze Jl and Onwuka ND (2007) Biogas production from blends of agro- industrial wastes. Trends Appl Sci Res, 2(6): 554-558.

[19] Ofoefule AU (2011) Investigation of the biogas production potentials of Bambara nut chaff (Vigna Subterranea), Advances in Applied Science Research, 2(2): 55-61.