Eco. Env. & Cons. 27 (August Suppl. Issue) : 2021; pp. (S130-S135)

ISSN 0971–765X

Paddy Straw: A source for Bioethanol production and compost formation

Manish Sakhlecha[1] and Toshan Singh Rathore[2]

[1]Civil Engineering Department, ICFAI University, Kamalghat, Agartala 799 010, Tripura, India
[2]Civil Engineering Department, Shri Shankaracharya Institute of Professional Management and Technology, Sejbahar, Raipur, India

(Received 21 March, 2021; Accepted 24 April, 2021)

ABSTRACT

Agricultural wastes contains lignocellulosic compound that serves as an attractive feed stocks for bioethanol production. It has several characteristics such as high content of cellulose and hemicelluloses that can be readily hydrolysed into fermentable sugars. A simple process (saccharification and fermentation) to produce ethanol from paddy straw was developed in order to establish an efficient bioethanol production. Also, the maximum ethanol concentration and ethanol yield from paddy straw were 69 and 60%, respectively. Nevertheless, the direct saccharification and fermentation shows the potential for lower cost and higher efficiency for bioethanol production. Further, an aerobic composting of spent wash obtained as the distillation waste from the ethanol generation process was performed by mixing it with 6 kg of press mud and 2 kg of bagasse along with total 6 liters of spent wash during the composting period. The samples of composting mass were analyzed for pH, moisture content ,temperature, organic carbon, nitrogen, COD, chlorides and conductivity. The percentage of Nitrogen, Phosphorus and Potassium were 2.42, 0.80 and 4.29 respectively. The values of parameters analyzed were quite comparable to a good quality compost. The utilization of rice straw for bioethanol production requires the production technology to be cost-effective and environmentally sustainable. Biological conversion of straw into fermentable sugars, employing hydrolysing enzymes is at present the most attractive alter-native and requires the involvement of the government by issuing supporting energy policies

Key words *: Bioethanol, Paddy straw, Saccharification and fermentation, Spentwash, Compost*

Introduction

In the current economy bioethanol is the most critical biofuel that can contribute to global biofuel production as an alternative energy for oil fuel, since depletion of fossil fuel, global warming, and reduction of natural resources has been increased concern for sustainability of planet earth. United states of America (USA) is the top producer of ethanol followed by Brazil and European union. India is in sixth position (https://afdc.energy.gov/data). Production of Bioethanol from lignocellulosic biomass is becoming an attractive concern for all over the world (Chandra *et al.,* 2012). However mere ethanol is not just the product to be focused. According to modern top-down approach in the biorefinery concept based on zero-waste generation, an integrated system is required to be established for the use of various biomass fractions and wastes and generation of different products for the market. The study is an attempt to adopt the new top to bottom approach by producing ethanol from rice paddy and utilizing the spent wash generated as post distillation waste for composting.

Lignocellulosic biomass is arising from agricultural, forestry, and industrial waste and paddy straw is one such source of lignocellulosic biomass In India rice is grown in 43.86 million ha, the production level is 104.80 million tones and the productivity is about 2390 kg/ha (Agricultural Statistics at a glance- 2015). Paddy straw is an agricultural wastes caused from rice cropping and is a hopeful feedstock for bioethanol production, because cellulose and hemicellulose content is more than 50% (Juanssilfero *et al.,* 2015). Although many researches are going on bioethanol production from paddy straw, still the production has not been realized because of high cost for collection and preservation from rice field ridge, fine-grinding and dissolution, fermentation system, etc.

Development of effective ethanol production system from abundant biomass such as paddy straw is an urgent necessity for novel energy supply. Moreover the spentwash which is a waste produced after distillation process is rich in plant nutrients, organic carbon and contains negligible toxic substances. However direct disposal to soil and water body is very much harmful. The most promising method of treatment of spentwash is aerobic composting, in which it can be mixed with other agro industrial wastes like pressmud, bagasse, paddy waste etc. In this study spentwash was mixed with pressmud and bagasse to produce compost through aerobic process.

Paddy straw has heterogeneous complex structure in which hard cellulose fiber due to the crystal structure and entangled lignin and hemicellulose is contained (Takano and Hoshino, 2018). In the bioconversion to ethanol from lignocellulose, pre-treatment by physical and/or chemical method is necessary to break the strong structure and obtain fermentable sugars easily by biocatalyst such as cellulase, xylanase, ligninase, etc (Robak and Balkarak, 2020).

Many researches have performed physical and chemical processing for pretreatment of lignocelluloses (Binod *et al.,* 2010). The physical treatment includes steaming (Wang *et al.,* 2011), steam explosion (Liu *et al.,* 2014; Sasaki *et al.,* 2012), grinding and milling (Jin and Chen 2006), and irradiation (Marx *et al.,* 2014). The chemical treatment includes alkali (Gáspár *et al.,* 2007), acid (Nair *et al.,* 2017), and ammonia treatment (Kim and Lee 2007). Especially, among the chemical treatments, alkali treatments using NaOH, KOH, CaOH, and Na_2CO_3 are more effective than other chemical treatments, because it is able to break the ester bonds between lignin, hemicellulose, and cellulose, so that lignin and a part of hemicellulose are concurrently removed (Gáspár *et al.,* 2007; Khaleghian *et al.,* 2015). Moreover, the pretreatment was able to reduce excessive degradation to furfural, 5-HMF, and vanillin more than acid treatment. These reactions result in cellulose fiber swelling and improve of cellulase contact to the fiber.

After pretreatment, biomass requires enzymatic hydrolysis on bioethanol production process. There are many kinds of hydrolysis enzyme reagent worldwide and any pretreated biomass demand the own reagents suitable for effective hydrolysis. However, few researches have performed both selection of the reagents and determination of the mixing ratio statistically, whereas hydrolysis conditions have been mentioned by statistical optimization method (Singh and Bishnoi, 2012). The selection of appropriate cellulase is very difficult because of structural difference of each biomass and difference of enzymatic activities in commercial cellulase reagents. According to Lu *et al*. (2003) Cellulase activity depends on strain of microorganism, fermentation, different substrate used, pH and temperature. As the substrate and the enzyme to be used for this study was fixed, we optimized the values of pH and temperature for maximum enzymatic activity.

Materials and Methods

Study area

The paddy straw used in this study was obtained from a commercial farm at Kendri Village, Abhanpur block. The pressmud and bagasse were collected from a sugar mill at Kawardha distruct of Chattisgarh state, India. The cellulase Cellic CTec2 used in this study for hydrolysis was kindly provided by Novozymes, Denmark. The enzyme activity was 123.6 6 4.9 FPU/ml, which was measured according to the methods recommended by the National Renewable Energy Laboratory (NREL), USA

A Pretreatment of Rice Straw Using alkali

Paddy straw was soaked in 1 M NaOH solution at the concentration of 80 g-dry straw/l-NaOH(aq) overnight at room temperature and then thermally treated by autoclave at 121 °C for 1 h (Janker-Obermeier *et al.,* 2012). The treated material was neutralized by 1 M HCl solution after cooling and then washed thoroughly by water several times. The washed material was dried in oven at 50 °C until completely dried and then powdered using a pulverizer (BLENDER 7011 HB).

B Enzymatic hydrolysis of paddy straw

Hydrolysis reactions were performed in 100 mL bottles containing 100 g/l of the pretreated paddy straw suspended in 25 ml of 0.1 M sodium acetate buffer (pH 5.5) or culture medium. Reaction was started by addition of the filter-sterilized enzyme (cellulase) at the total protein concentration of 2 g/l. The pretreated rice straw suspension was incubated at 28 °C and 120 rpm for 96 h. Hydrolysates were obtained by removal of solid fraction by filtration and its sugar concentration was analyzed by HPLC system.

C Simultaneous saccharification and fermentation (SSF)

SSF experiments were performed in 100 mL bottle containing 100 g/L pretreated suspension with dry yeast concentration 0.1% and 0.25% (w/v) and the pH was adjusted to 5, 5.5 and 6. Then shaken at 120 rpm in an incubator at three different tempertaures (23, 28 and 33 °C) for 96 h. The cultures were filtered and filterate were distillated. The remaining slurry also known as spentwash was used for composting.

Analysis of compost

The press mud and bagasse were mixed in proportion 3:1. The quantity of press mud taken for experiment was 6 kilograms and that of bagasse was 2 kilogram. The spent wash was added to this mixture till the moisture content is approximately 65 %. Table 1 shows the physiochemical properties of pressmud and bagasse used in the study. Table 2 shows the general composition of spentwash (Kaul and Nandi, 1999; Sarangi *et al.*, 2005). The seeding was done with the help of Farm Yard Manure (FYM) and Cow dung. The aeration was carried out manually by overturning the contents for 45 days at the interval of one day. The spent wash absorbed without leaching is 6 liters within 45 days. The spent wash loading was done on the 1st, 5th, 11th and 24th day. Characteristics of compost such as Ph, percentage of ash, moisture, volatile matter, organic carbon, nitrogen, phosphorus and potassium were measured. Also, the carbon:nitrogen and COD, were analyzed in the compost obtained.

Table 1. Composition of Pressmud and Bagasse.

Composition	Pressmud	Baggasse
Nitrogen (%)	1.8	0.55
Phosphorus (P_2O_5) (%)	0.9	0.08
Potassium (K_2O) (%)	2.1	0.11
Calcium (CaO) (%)	4.5	0.95
Magnesium (Mg) (%)	1.4	0.55
Organic Carbon (%)	28.5	30.5
pH	6.0-7.0	4.84

Table 2. Physico-chemical characteristics of spentwash

Parameter/composition	Raw Spent wash
Color	Dark brown
pH	4–4.5
Turbidity	High
Ec (dS/m)	<15
BOD (mg/l)	45000-60000
COD (mg/l)	80000-120000
Suspended solids (mg/l)	10000
Total dissolved solids(mg/l)	100000
Total Nitrogen (mg/l)	1000-2200
Phosphorus (mg/l)	400
Sulphate (mg/l)	2100-2300
Chloride (mg/l)	5800-7600
Sodium (mg/l)	400-500
Potassium (mg/l)	1100
Magnesium (mg/l)	1500-2000
Calcium (mg/l)	7500-8200

Source: (Kaul and Nandi, 1999; Sarangi *et al.*, 2005)

Results and Discussion

Bioethanol-The paddy straw contains 37-40% cellulose, 23-25% hemicellulose, 7-9% lignin and 17-19% ash, measured according to Sun *et al.* (1996). The first step in using paddy straw for bioethanol production is size reduction through milling and sieving process. Milling and size reduction were applied prior to enzymatic hydrolysis. This step can improve susceptibility to enzymatic hydrolysis by reducing the size of the materials and degree of crystallinity of lignocelluloses, which improves enzymatic degradation of paddy straw toward ethanol. Size reduction enhanced the susceptibility of substrate to enzymatic hydrolysis. By applied the smallest straw particles in bioethanol production will increase the amount of glucose release for the hydrolysis process compared to untreated substrate.

The pH and temperature of the fermentation media has an important role on fermentation process due to their effect on microbial growth, rate of fermentation reaction, and the by product formation. Therefore, maintenance of pH and temperature are of great importance in fermentation reaction. In our study, maximum bioethanol production was achieved at pH 5.5 (Table 3) and temperature 28 ºC (Table 4). Bioethanol fermentation media were incubated at 28 ºC with agitation at 120 rpm for several sampling times (12, 24, 48, 72, 96 h) (Fig. 1). The highest ethanol yield of 60% was achieved by SSF of the whole pretreated slurry with 0.25% yeast. The faster rate of bioethanol production during SSF by yeast was obtained within the first 48h. The experiments lasted for 4 days. However, the bioethanol concentrations decrease sharply after 2 days. It gives general conclusion that we need around 2 days to reach the maximum bioethanol concentration under anaerobic conditions. In addition, it was found that the physical observation of the paddy straw biomass has merge in second day (48h) of fermentation and indicating the enzyme works suitable in saccharification process. Figure 2 shows the positive test for qualitative bioethanol estimation along with control.

Table 3. Bioethanol yield (%) at different temperatures

SN	Temperature	Bioethanol yield (%)
1	23 ºC	40
2	28 ºC	60
3	33 ºC	45

Table 4. Bioethanol yield (%) at different temperatures

SN	Temperature	Bioethanol yield (%)
1	23 ºC	40
2	28 ºC	60
3	33 ºC	45

Further, the pH of the compost was measured. Some characteristics of compost were measured such as percentage of ash, moisture, volatile matter, organic carbon, nitrogen, phosphorus and potassium. Also, the carbon : nitrogen, COD, calcium and magnesium were analyzed in the compost obtained.

Compost

The final compost was obtained after the aerobic decomposition of raw compost for 45 days. Table 5 gives the parameters of raw compost and final compost.

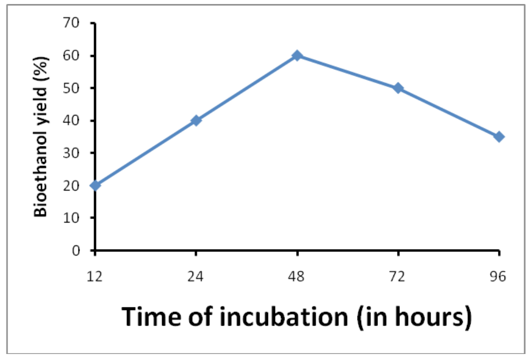

Fig. 1. Bioethanol yield (%) at different time of incubation of whole treated slurry.

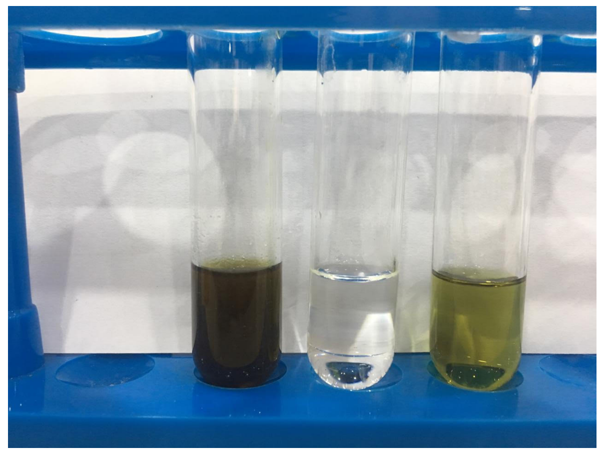

Fig. 2. Bioethanol estimation by Jones test (Left: sample and right: Control)

Initial acidic pH (5.3) of composting mixture was changed to alkaline (7.7) within first four days and again became acidic (5.7 to 6.6) at 8th day of composting. Within further two days it increased above neutral and remained constant (7.2) up to the end of composting. The reduction in soluble COD during composting process was observed in three stages. First stage of 4 days of accelerated reaction, second stage of readjustment for four days and final stage of stabilization after 8th day of composting. The soluble COD reduction was 88 to 89 percent within 30 days. The initial temperature was 27 to 29 °C (atmospheric) which was increased to maximum of 50 to 54 °C within first five days of composting. Thereafter, it decreased to 27 to 25 °C (atmospheric) within further 3 to 4 days and remained constant till the end of the 30 days. The optimum moisture required was 61 to 64 percent

Table 5. Different parameters of raw and final compost.

Parameters	Before mixing of raw materials	After composting for 45 days
pH (Saturated)	5.3	7.2
Ash%	21	30
Volatile Matter %	81	74.2
O.C.%	29.3	23.8
N%	2.0	2.42
P%	0.71	0.80
K%	4.11	4.29
C/N	19.8	12.2
COD (mg/l)	3723	1206

throughout the composting process. Oxygen consumption in the initial stage of 3 days was much more and then slowed down up to eighth day of composting. After 8 days the oxygen consumption rate was very slow till the end of the reaction. The time required to complete stabilization and formation of most stable compost prepared from spentwash and pressmud with 3:1 proportion was observed to be 45 days when optimum conditions were maintained. The decline of C/N ratio is an indication of completion of composting process. Initial C/N ratio was 19.8 while it was 12.2 on the 30th day. The initial COD of leachate was 3723 mg/l but it was only 1206 mg/l at the end of composting. The nitrogen was increasing while organic carbon gets decreased with time and at end C/N was near to 10, which indicated the completion of composting process.

Conclusion

In this study, although a high ethanol yield of 60% was achieved with SSF of the whole pretreated slurry, the highest ethanol concentration in the fermented broth was only 69%, which makes distillation economically unfeasible. Thus, it is necessary to enhance the ethanol concentration by optimizing SSF to increase ethanol yield further, converting xylose to ethanol, and increasing solid load further during pretreatment using an improved reactor.

The utilization of rice straw for bioethanol production requires the production technology to be cost-effective and environmentally sustainable. Biological conversion of straw into fermentable sugars, employing hydrolyzing enzymes is at present the most attractive alter-native and requires the involvement of the government by issuing supporting energy policies. Different approaches in both process engineering and strain engineering have to be carried out to circumvent the difûculties of xylose and glucose co-fermentation and to improve the system efciency.

It can also be concluded from the research that the composting of spent wash from bio-still process requires 45 to 50 days. The compost prepared will have high nutrient value and it will meet the requirement of organic manure which is urgently needed to reduce the use of chemical fertilizers.

Acknowledgment

This research publication is owing to a research project funded by Chattisgarh Swami Vivekananda University. We are extremely thankful to the university for providing us an opportunity to conduct this research project and for giving financial support.

References

Binod, P., Sindhu, R., Singhania, R.R., Vikram, S., Devi, L., Nagalakshmi, S., Kurien, N., Sukumaran, R.K. and Pandey, A. 2010. Bioethanol production from rice straw: an overview. *Bioresour Technol.* 101 (13) : 4767–4774.

Chandra, R., Takeuchi, H., Hasegawa, T. and Kumar, R. 2012. Improving biodegradability and biogas production of wheat straw substrates using sodium hydroxide and hydrothermal pretreatments. *Energy* 43 : 273–282.

Gáspár, M., Kálmán, G. and Réczey, K. 2007. Corn fber as a raw material for hemicellulose and ethanol production. *Process Biochem*. 42 : 1135–1139.

Janker-Obermeier, I., Sieber, V., Faulstich, M. and Schieder, D. 2012. Solubilization of hemicellulose and lignin from wheat straw through microwave-assisted alkali treatment. *Ind Crops Prod.* 39 : 198–203.

Jin, S. and Chen, H. 2006. Superfne grinding of steam-exploded rice straw and its enzymatic hydrolysis. *Biochem Eng J.* 30 : 225–230.

Juanssilfero, A.B., Djohan, A.C., Purnawan, A. and Yopi, 2015. Bioethanol Production from Indica IR.64 Rice Straw Biomass by Direct Saccharification and Fermentation. *International Journal on Advanced Science Engineering and Information Technology*. 5 : 2-6.

Kaul, S.N. and Nandy, T. 1999. *Waste management idistilleries*. NEERI Technical Report. National Environmental Engineering Research Institute – Council of Scientific and Industrial Research, Department of Science and Technology, Govt. of India, Nagpur, India

Khaleghian, H., Karimi, K. and Behzad, T. 2015. Ethanol production from rice straw by sodium carbonate pretreatment and Mucorhiemalis fermentation. *Ind Crops Prod*. 76 : 1079–1085.

Kim, T.H. and Lee, Y.Y. 2007. Pretreatment of corn stover by soaking in aqueous ammonia at moderate temperatures. *Appl Biochem Biotechnol.* 137-140 : 81-92.

Lu, W., Li, D. and Wu, Y. 2003. Influence of water activity and temperature on xylanase biosynthesis in pilotscale solidstate fermentation by *Aspergillus sulphurous*. *Enzyme Microb. Technol*. 32 : 305-311.

Liu, Z.H., Qin, L., Zhu, J.Q., Li, B. Z. and Yuan, Y. J. 2014. Simultaneous saccharifcation and fermentation of steam-exploded corn stover at high glucan loading and high temperature. *Biotechnol Biofuels.* 7 : 167.

Marx, S., Ndaba, B., Chiyanzu, I. and Schabort, C. 2014. Fuel ethanol production from sweet sorghum bagasse using microwave irradiation. *Biomass Bioenergy.* 65 : 145–150.

Nair, R.B., Kalif, M., Ferreira, J.A., Taherzadeh, M.J. and Lennartsson, P.R. 2017. Mild temperature dilute acid pretreatment for integration of frst and second generation ethanol processes. *Bioresour Technol.* 245 : 145–151.

Sarangi, B.K., Golait, A., Kanungo, S., Chakrabarti, T., Ghosh, T.K., Chakrabarti, T. and Tripathi, G. 2005. Activated biocom- posting of sugarmill pressmud and partially treated distillery spentwash – processed to quality compost for sustainable agriculture. *Biotechnology in Environment Management*.

Sasaki, C., Sumimoto, K., Asada, C. and Nakamura, Y. 2012. Direct hydrolysis of cellulose to glucose using ultra-high temperature and pressure steam explosion. *Carbohydr Polym.* 89 : 298–301.

Singh, A. and Bishnoi, N.R. 2012. Enzymatic hydrolysis optimization of microwave alkali pretreated wheat straw and ethanol production by yeast. *Bioresour Technol*. 108 : 94–101.

Sun, R., Lawther, J.M. and Banks, W.B. 1996. Fractional and structural characterization of wheat straw hemicelluloses. *Carbohydrate Polymer.* 29 : 325-331.

Takano, M. and Hoshino, K. 2018. Bioethanol production from rice straw by simultaneous saccharifcation and fermentation with statistical optimized cellulase cocktail and fermenting fungus. *Bioresour. Bioprocess.* 5 : 16-28.

Wang, L., Yang, M., Fan, X., Zhu, X., Xu, T. and Yuan, Q. 2011. An environmentally friendly and efcient method for xylitol bioconversion with high-temperature steaming corncob hydrolysate by adapted *Candida tropicalis*. *Process Biochem*. 46 : 1619-1626. (Kaul and Nandi, 1999; Sarangi *et al.,* 2005)