ISSN : 0974 - 7451 Volume 7 Issue 1

Trade Science Inc.

Environmental Science
An Indian Journal

Current Research Paper

ESAIJ, 7(1), 2012 [10-18]

Bioethanol production from agro wastes of Paddy

Sabitri Nahak[1], Gayatri Nahak[2], Tapoja Priyadarshani Nayak[3], R.K.Sahu[2*]
[1]Therapuetic Chemical Research Corporation, Bhubaneswar, Odisha, (INDIA)
[2]B.J.B.Autonomous College, Botany Department, Bhubaneswar, Odisha, (INDIA)
[3]Academy of Management and Information Technology, Bhubaneswar, Odisha, (INDIA)
sahurajani.sahu@gmail.com; sabitrinahak62@gmail.com; gayatri.science@gmail.com
Received: 2nd October, 2011 ; Accepted: 2nd November, 2011

ABSTRACT

Biomass materials are used since millennia for meeting myriad human needs including energy. Main sources of biomass energy are trees, crops and animal waste. Bioethanol produced from renewable biomass has received considerable attention in current years. There has been an increasing interest in utilizing alternative sources of energy. Paddy straw and Paddy husk are the two basic raw materials chosen for boiethanol production in present study because of their abundant availability and representative sample of crops stovers and unutilizable agro wastes. Each one of this biomass having more than 60-70% cellulose appeared to be very suitable for presence study in collection with bioethanol production. The pretreatment method followed in the present investigation as review from the literature available with other cellulosic biomass are also applicable to this chosen biomass as the percentage of available cellulose free from lignin seal constitute crystalline cellulose and hemicellulose 68% percentage. The subsequent hydrolysis by both chemical and enzymatic methods following the standard method reported by many workers shows significant result in producing fermentable sugar within reasonable incubation period. However for standardization of the protocols use of model sources of cellulase in view of commercial enzyme needs further investigation. With the limitation of time the procedure follow in the present investigation shows encouraging result ranging from 0.583mg/gm to1.919mg/gm of fermentable sugar produced after 4 days incubation of reaction mixture in standard reaction, in enzymatic cellulolysis.

KEYWORDS

Bioethanol;
Biomass;
Paddy straw;
Paddy husk;
Cellulose.

INTRODUCTION

Bioethanol produced from renewable biomass[1] has received considerable attention in current years. Using ethanol as a gasoline fuel additive as well as transportation fuel helps to alleviate global warming and environmental pollution. Bioethanol fuel is mainly produced by the sugar fermentation process, although it can be manufactured by the chemical process of reacting ethylene with steam. Catalytic hydration of petroleum product (ethylene) produces synthetic ethanol. Current interest in ethanol a product of crops lies on discussion whether

it can be used as a sustainable energy resource which may offer environmental and long-term economic advantages over fossil fuels, like gasoline or diesel.

The major source of ethanol production in Brazil, USA, India and other sugarcane raising countries is sugar molasses route. But Researcher's concern is to exploit the agro wastes like paddy straw and paddy husk contain abundant cellulose for ethanol conversion (Figure-1). The interest in use bioethanol as petrol replacement began in Brazil and USA during 1980s. Ethanol can be produced from any biological material that has sugar, starch and cellulose. Lignocellulose or woody biomass consider as a future alternative for the agricultural products that are currently used as feedstock for bioethanol production[2] because it is more abundant and less expensive than food crops. Furthermore, the use of lignocellulosic biomass consists of three main components i.e. carbohydrate polymers called cellulose and hemicellulose that can be converted to sugars, and a non-fermentable fraction called lignin that can be utilized for the production electricity or heat. Although the decomposition of the material in to fermentable sugars is more complicated, the fermentation, distillation and dehydration process steps are basically identical for bioethanol from either agricultural crops or lignocellulosic biomass. Hydrolysis of lignocellolosic material for ethanol production is proposed by[3].

Bioethanol from celluloses holds great potential due to the wide availability and relatively low cost of cellulose material[4]. The three main raw materials for ethanol production are sugars (from sugarcane, sugar beet, molasses and fruits), starch (from corn, cassava, potatoes and root crops) and cellulosics. Most of the materials in the first two categories come under food stuffs. This limits their use for ethanol production. So, the interest obviously relies on the abundant cellulosics[5]. Biomass is composed of lignocellulosics. Lignocellulose is a complex of cellulose, hemicellulose and lignin[6]. Biomass on an average consists of 40–60% cellulose, 20–40% hemicellulose and 10–25% lignin. The cellulose and hemicellulose, which typically comprise two-thirds of the dry mass, are polysaccharides that can be hydrolyzed to sugars and eventually to ethanol by fermentation[7]. Cellulose is a linear polymer of glucose. The orientation of the linkage (β-1, 4 linkage) and additional hydrogen bonds make the polymer rigid

Paddy Straw

Paddy Husk

Figure 1 : Photographs of Paddy straw and Paddy husk

and difficult to break. Hemicellulose is a polymer of short highly branched chains of the various sugars. Hemicelluloses are often polymers of pentoses mainly xylose and further arabinose with hexoses such as galactose, glucose and mannose. Lignin is a large complex polymer of phenyl propane and methoxy groups, a non-carbohydrate polyphenolic substance which encrusts the cell walls and cements the cells together. The combination of hemicellulose and lignin provides a protective sheath around the cellulose, which must be modified or removed before efficient cellulose hydrolysis can occur, but the crystalline structure of cellulose makes it

insoluble and resistant. Therefore, pretreatment must be employed which is an important tool for practical cellulose conversion process. Pretreatment is required to alter the structure of cellulosic biomass to make cellulose accessible to the enzymes that convert carbohydrate polymers into fermentable sugars[8, 9].

By far most pretreatments are done through physical or chemical means. In order to achieve higher efficiency some researchers seek to incorporate both effects.

Considering the above aspects, the present study on "Pretreatment of agro-residues for bioethanol production" was conducted with the following objectives.

- Cellulose content of two different biomasses.
- Evaluation of available fermentable sugar and total polysaccharide of Paddy straw and Paddy husk.
- To evaluate combination of physical and chemical pretreatment methods for maximum delignification.
- Study of different pretreatment methods on liberating cellulose for hydrolysis.
- Chemical and enzymatic hydrolysis and conversion of pretreated biomass to fermentable sugar.
- Alcoholic fermentation and estimation of bioethanol production

MATERIALS AND METHODS

Experiment pertaining to the study of bioconversion of Paddy straw and Paddy husk were conducted in biochemistry laboratory of A.M.I.T., Khorda, Odisha, India, during the session 2009_2010. Two agricultural waste materials such as paddy straw and paddy husk were collected locally and were processed. Then 700gm of each sample was taken and dried under sun for better grinding. Both the samples were ground in to powered form and stored very well for further analysis.

Plant materials

1. Paddy straw 2. Paddy husk

Estimation of total carbohydrate

The carbohydrate estimation was done by the method described in[10]. A Standard graph was drawn by plotting concentration of the standard on X-axis vs absorbance on Y-axis. From the graph the carbohydrate present in the sample tube was calculated, by the following formula.

$$\text{Amount of carbohydrate present in 100mg of the sample} = \frac{\text{mg of glucose} \times 100}{\text{Volume of the test sample}}$$

Pretreatment of biomass

For pretreatment of biomass 2 types of methods are used.

*Physical pretreatment

*Chemical pretreatment

Physical pretreatment

In physical pretreatment, soaking the material overnight followed by steam explosion (rising to 120^0C at 15 p.s.i. for 30sec to 1 min) followed by reducing the pressure to normal as early as possible. The another method is without soaking steam flashing is done in a pressure cooker to dry matter in a test tube and treated with steam with high pressure.

Chemical pretreatment

Nitric acid/Acetic acid reagent treatment

1 gm of sample was taken and 3ml. of acetic/nitric acid reagent added in a test tube, the tube was placed in a water bath for 100^0C for 30 min. After 30 min. the sample containing the tube was cooled and the contents were centrifuged for 15 min. at 10,000 rpm. After centrifugation the supernatant was discarded and the residue was washed with distilled water.

Dilute sulphuric acid treatment

3 ml of 1% H_2SO_4 was added with 1 gm of sample in a test tube. The tube containing the sample was placed in a boiling water bath 100^0C for 2 min. then the tube was cooled and the content was centrifuged for 15 min. The supernatant was discarded and the pellet was washed with distilled water.

Cellulolysis (67% H_2SO_4)

After the pretreatment of the sample or algal biomass by physical and chemical treatment, the samples were treated with 10ml of 67% sulphuric acid.

Cellulolysis (1% H_2SO_4)

After the pretreatment of the material, the hydrolysis was conducted with 10 ml of 1% H_2SO_4 incubated

for 1hour in room temperature and the total carbohydrate and percentage of was calculated.

Enzymatic hydrolysis

Isolation, purification & assay of cellulose

The extracellular cellulase was extracted from naturally rotting biomass, after isolating the microbial species and culturing in nutrient broth containing cellulose as inducer. The crude enzyme extract was subjected to salting out followed by desalting. The resultant extract was taken as partially purified enzyme complex and assayed. For overall activity assay as described[11]. Accordingly 1 mg of total protein was assumed equivalent approximately 1 FPU activity. The enzyme content was estimated in terms of soluble protein by spectrophotometric method.

Enzymatic cellulolysis

The reaction mixture was prepared by taking 0.5gm of each sample in test tube which was pretreated physically or chemically. The chemical pretreated materials washed very well with distill water to remove acid. To each pretreated test tube 50mM phosphate buffer containing enzyme extract 1 F.P.U per 100mg was added and incubated at 40^0C for 4 days. Then the hydrolysate was centrifuged and the supernatant was collected. Then from supernatant, different concentration of solution was taken in test tubes (0.5-1.5) To each test tube 3ml of DNS reagent was added. The test tubes were heated in boiling water bath for 20 min. 1ml of Rochelle salt solution was added when contents of the tubes still warm. The test tubes were cooled and the intensity of dark red colour was measured at 510 nm.

Preparation of reaction mixture

The reaction mixture was prepared by taking 1gm of sample broth fresh and dry suspended in 50mM phosphate buffer (pH 4.5) to which enzyme extract of 5 FPU per 100mg was added, and incubated at 30^0C for 4 days, up to which the hydrolysate was centrifuged and analyzed for reducing sugar content (DNS method) for subsequent fermentation process.

Estimation of fermentable sugar by DNS methods

The reaction mixture was prepared by taking 2gm of each sample in test tube which were pretreated chemically or physically. The chemically pretreated materials were with distilled water to remove acid. The fermentable sugar was determined by dinitrosalicylic acid (DNS) method[12] with glucose as standard.

Alcoholic fermentation

Both hydrolysate and non hydrolysed material were taken for fermentation. To some pretreated material enzyme was added for hydrolysis. Alcoholic fermentation was done in two phases: Primary and Secondary fermentation. 2gm of sample was pretreated material was washed thoroughly with distilled water. The samples were incubated with 1gm of commercial yeast at 37^0C in a thermostatic shaker in aerobic condition. After 3-4 days the conical flask containing all the materials were subjected to anaerobic incubation for 7 days. The primary alcohol of the content was estimated before and after fermentation. Secondary fermentation was done taking the fermentable of primary fermentation with sucrose for fermentation of existing yeast in the fermentation of another 7 days.

RESULTS AND DISCUSSION

Now-a-days, phasing out of lead from gasoline due to environmental concerns which has promoted markets for alcohol as octane enhancer. In this field ethanol has gained increased importance as engine fuels, since it is easily blended with gasoline. Thus, with the expectation of supply shortfalls in future from non-renewable fossil fuels, the production of fermentatively produced bioethanol from low cost biomass such as cellulosic wastes to meet energy demands is a viable alternative. The alternate biomass tried successfully for bioethanol production are the pineapple, cannery waste[13], starch[14] and carob pods[15]. Lignocellulosic materials like sugarcane bagasse, paddy straw and wheat straw can also be employed. Lignocellulosic is a complex of two polysaccharides (cellulose and hemicellulose) and an aromatic polymer *i.e.*, lignin. To obtain the polysaccharides the lignin must be removed. So, pretreatment methods serve this purpose. In the present study, combination of physical and chemical pretreatment methods was studied.

The polysaccharide in form of polymers of monosaccharide such as cellulose and hemicellulose etc. are the

Current Research Paper

TABLE 1 : Effect of Pretreatments on Chemical and Enzymatic Cellulolysis in Paddy straw and Paddy husk

Pretreatments	Sample Wt.(gm)	Chemical Cellulolysis		Enzymatic Cellulolysis	
		Paddy straw	Paddy husk	Paddy straw	Paddy husk
		Fermentable sugar content (mg/gm)	Fermentable sugar content (mg/gm)	Fermentable sugar content (mg/gm)	Fermentable sugar content (mg/gm)
Nitric acid	0.5	92.4	74.8	0.583	0.002
Dil.H_2SO_4	0.5	365.2	118.8	1.919	0.847
Steam Flashing	0.5	61.6	55.0	0.940	0.515
Over Night Soaking	0.5	99.0	79.2	1.245	0.556

TABLE 2 : The Conversion (%) of Fermentable Sugar in Paddy straw and Paddy husk

Sample	Cellulose content (mg/gm)	Types of hydrolysis	% Of conversion			
			T_1	T_2	T_3	T_4
Paddy straw	638	Chemical	14.48	9.65	15.51	57.21
		Enzymatic	0.09	0.14	0.19	0.30
Paddy husk	330	Chemical	22.66	16.66	24	36
		Enzymatic	0.006	0.15	0.16	0.25

T_1 : Nitric acid/acetic acid pretreatment; T_2 : Steam flashing pretreatment; T_3 : Soaking over night pretreatment; T_4 : Dilute sulphuric acid(H_2S0_4) pretreatment

TABLE 3 : Potential Alcohol Value of sample after alcoholic fermentation

Sample	No of pretreatment	P.A value before fermentation	P.A value after fermentation	Difference
Paddy straw	T_1	1.21	1.33	0.12
	T_2	1.16	1.25	0.09
	T_3	1.17	1.29	0.12
	T_4	1.19	1.34	0.15
Paddy husk	T_1	1.01	1.09	0.08
	T_2	1.00	1.02	0.02
	T_3	1.03	1.13	0.10
	T_4	1.06	1.19	0.13

T_1 : Nitric acid/ acetic acid pretreatment; T_2 : Steam flashing pretreatment; T_3 : Soaking over night pretreatment; T_4 : Dilute sulphuric acid(H_2S0_4) pretreatment

basic starting materials for conversion to bio ethanol by using biotechnical tool. The process includes pretreatment of biomass, hydrolysis or cellulolysis of complex saccharide; estimation of hydrolysate for fermentable sugar is also presented in above TABLES. The subsequent fermentation for *in vitro* production of ethanol has been summarized (TABLE-1). The result of chemical cellulolysis by 67% sulphuric acid (H_2SO_4) after nitric acid pretreatment is evaluated on basis of fermentable sugar resulted and available in the hydrolysate. The result indicates cellulose in paddy straw yield more quantity of fermentable sugar/ reducing sugar i.e. 92.4mg/gm, comparatively that available in paddy husk which is 74.8mg/gm[16], observed loss of hemicellulose by subjecting wheat straw to wet oxidation at 170^{0}C. The loss was 50% (from 35% w/w before treatment to 17% w/w/ after treatment)[17]. Recorded hemicellulose content of 12.5% after exposing wheat straw to alkaline treatment followed by alkaline/oxidative treatment[18]. Reported aqueous ammonia pretreatment of corn stover resulted in 40-60% of hemicellulose solubilization during delignification process.

The main objective of the acid pretreatment is the solubilization of the hemicellulosic fraction of the biomass, in order to increase the accessibility of the enzymes in the enzymatic hydrolysis reaction[19]. Inorganic acids like H_2SO_4, HCl and H_3PO_4 have been used for the pretreatment of the lignocellulosic biomass, in order to improve the enzymatic hydrolysis. There may be used both concentrated and diluted inorganic acids. Pretreatment of the biomass with concentrated acids, at ambient temperature, will lead to higher yields of fermentable sugars and to the hydrolysis of both cellulose and hemicelluloses. There are frequently used acids like H_2SO_4 72%, HCl 41% and trifluoroacetic acid 100%.

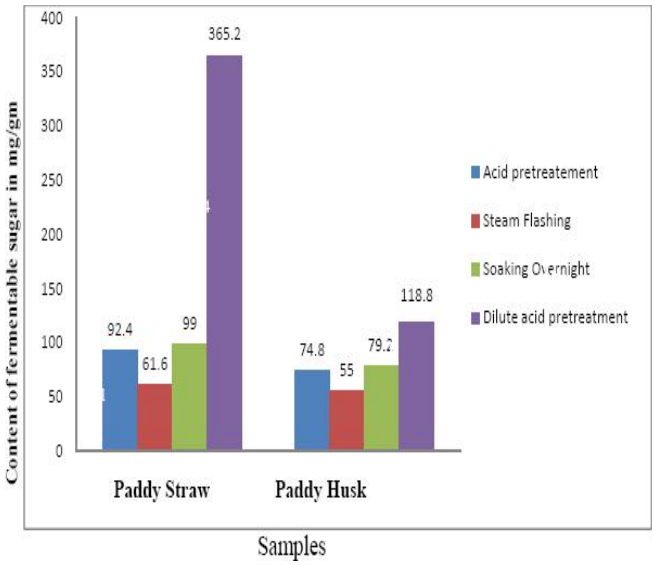

Figure 2 : Comparative Result of Chemical Cellulolysis of Different Biomass

In this case, a necessary step is the recovery of the acid, in order to lower the economic costs of the process[20].

Pretreatment with 1% H_2SO_4 at 120^{0}C for 1 hr show commendable results when hydrolysed with 67% sulphuric acid as the hydrolysate analysis gives 365.2mg/gm and 118.8mg/gm of fermentable sugar of paddy straw and paddy husk respectively (TABLE-1). The increase in cellulose percentage is just relative to the loss of hemicelluloses and lignin. Hemicellulose is soluble in alkali due to hydrolysis and is expected to be less stable during pretreatment due to the branched structure[16]. Cellulose was not affected significantly by lime pretreatment at 22 to 25^{0}C even though corn stover was contacted with alkali for 16 weeks. The degree of crystallinity slightly increased with delignification due to loss of lignin and hemicellulose (amorphous components) and increase of glucan content in the pretreated corn stover[21]. Pretreatment of rice bran with NaOH caused separation of hydrogen bonds of cellulose[22].

Experimental results of effects of physical pretreatments followed by chemical cellulolysis by 67% sulphuric acid in (TABLE-1). The results obtained are compared by graphical representation through bar diagram (Figure-2). It observed that the combination of over night soaking followed by steam flashing show significantly high yield of fermentable sugar compared to steam flashing alone. The results also indicates higher yield of fermentable sugar from pretreated paddy straw

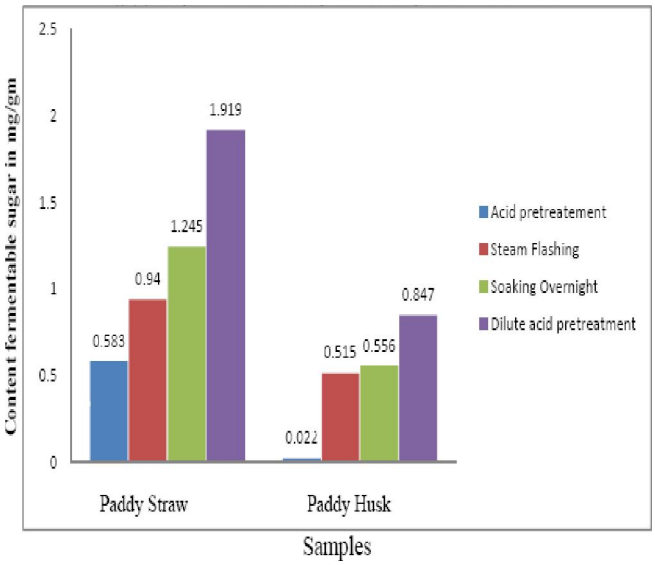

Figure 3 : Comparative Result of Enzymatic Cellulolysis on Different Biomass

compare to paddy husk with which may be related to lower percentage of average cellulose content in paddy husk. The results confirm the finding of[2]. The procedure combines the advantages of alkaline pretreatment and steam explosion. It will lead to an efficient delignification and to the chemical swelling of the lignocellulosics fibers[23]. Use of a combined process (Steam explosion and NaOH 10%) led to a significant increase of the free sugars concentration towards the pretreatment with H_2O_2 1% and NaOH 1%[24].

Results shows hydrolysis after overnight soaking followed by steam flashing makes cellulose more accessible to hydrolysis as the fermentable sugar content on the hydrolysate in paddy straw is 99.0mg/gm against the effect of only steam flashing 61.6mg/gm. Result shows hydrolysis after overnight soaking followed by steam flashing makes cellulose more accessible to hydrolysate in paddy husk is 79.2mg/gm against the effect of only steam flashing 55.0mg/gm.

Enzymatic hydrolysis was conducted with extra cellular cellulase on the basis of its activity level assayed as per standard procedure in terms of enzyme protein in mg/ml and activity in F.P.U/ml. Extracellular cellulase was isolated from the culture filtrate of the intracellular microorganism grown in presence of cellulose in the media (Figure-5). The protein content was estimated in terms of soluble protein by spectrophotometric method and expressed in mg/ml with BSA as standard (TABLE-1). The result shows hydrolysate contain 1.919mg/gm of fermentable sugar with the best performance in 1% sulphuric acid (H_2SO_4) pretreatment followed by enzymatic hydrolysis. The results obtained are compared by graphical representation through bar diagram (Figure-3).

The result of enzymatic cellulolysis by extra cellular cellulase after acid pretreatment is evaluated by DNS method taking glucose as standard (Figure-3). The result indicates cellulose in paddy straw yield more quantity of fermentable sugar i.e. 1.919mg/gm in dilute acid pretreatment and 0.583mg/gm in nitric acid pretreatment. Where as paddy husk yield 0.847mg/gm in dilute acid pretreatment and very negligible amount of fermentable sugar i.e. 0.002mg/gm in nitric acid pretreatment followed by enzymatic hydrolysis.

The result shows that paddy straw yield 1.245mg/gm of fermentable sugar in case over night soaking fol-

lowed by enzymatic hydrolysis and 0.940mg/gm in steam flashing pretreatment followed by enzymatic hydrolysis. The paddy husk yield 0.556mg/gm and 0.515mg/gm in overnight soaking and steam flashing followed by enzymatic hydrolysis respectively.

The summary of results are given in (TABLE-2) clearly indicates better performance in cellulolysis by chemical methods compare to enzymatic cellulolysis. With similar pretreatment the result exhibited high yield in fermentable sugar in case of paddy straw compare to paddy husk gives better platform for subsequent alcoholic fermentation. Other workers like Sun and Cheng [3] working on wheat Stover sugarcane bagasse show similar findings. The result shows that percentage of conversion is very commendable in chemical cellulolysis both in paddy straw and paddy husk The percentage of conversion to fermentable sugar from the total cellulose content is attractive in case of paddy husk.

The hydrolysate from saccharification reaction was used to determine the ethanol yield. The hydrolysates of paddy straw paddy husk were subjected to alcoholic fermentation by *Saccharomycse cerevisiae* incubating for 7days. The fermentated hydrolysates are analyzed for ethanol production at the end of the fermentation period by hydrometric method taking in to account (Figure-4). P.A. (Potential Alcohol) value of hydrolysate at pre and post fermentation stage. The difference of P.A value representing mg of alcohol produced per ml has been depicted (TABLE-3). The alcohol produced in proportion with the fermentable sugar content of hydrolysate which is highest in case of enzymatic hydrolysis after 1% sulphuric acid pretreatment showing better result. The comparative Figure (Figure-2&3) show higher ethanol yield of 0.15mg/

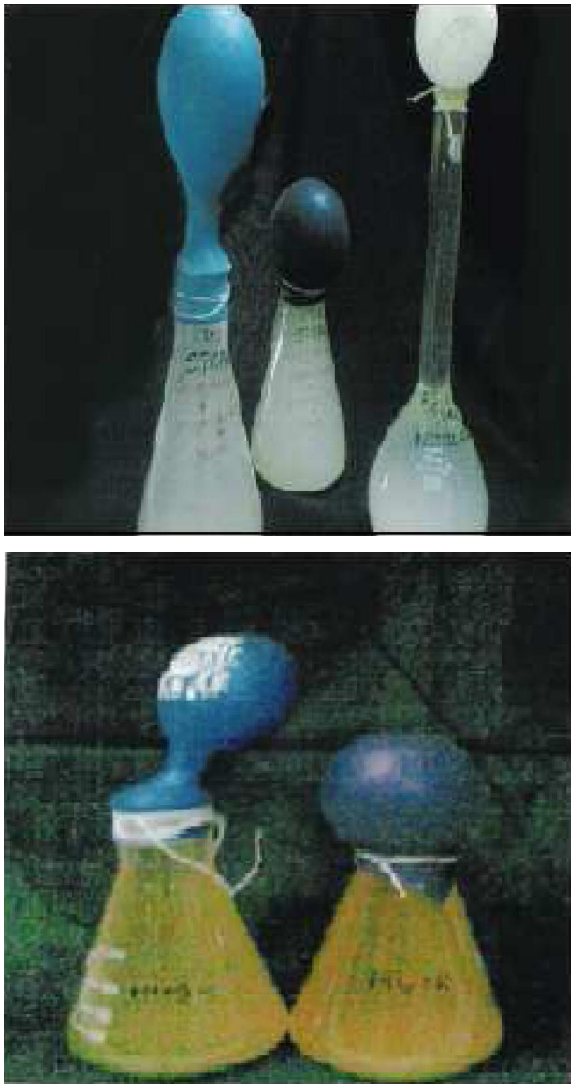

Figure 4 : Production of Ethanol and Co_2 during Fermentation

Figure 5 : Isolation of Bacteria Containing Cellulase by Streak Plate Method

ml in case of paddy straw and 0.13mg/ml in case of paddy husk under similar pretreatment condition. Doelle and Greenfield[25] reported ethanol concentration of 95.5g per l by *Zymomonas mobilis* from 200g per l sucrose within 24 to 30 h of period. *Zymomonas* has higher sugar uptake and ethanol yield and known to divert the less of sugars to its biomass production compared to other yeasts[26, 27]. Observed ethanol production of 28g per l by *Zymomonas mobilis* ZM4, when the hydrolysate of wheat stillage was supplemented with 5g per l yeast extract and 40g per l glucose with residual xylose of 2.6g per l. Zayed and Meyer[28] recorded the ethanol of 11.8g per l from 27g per l reducing sugars derived from 50g per l alkali delignified wheat straw inoculated with *Pachysolen tannophilus*. *Candida shehatae* CBS5813 produced 6.6g per l ethanol and *Pichia stipitis* CBS5773 5.9g per l ethanol from the fermentation medium supplemented with 2% xylose at 25^0C for 10 days[29]. *Candida shehatae* showed greater ethanol production than *Pichia stipitis* due to increased uptake of xylose, glucose, mannose and galactose[30]. *Candida shehatae* assimilated glucose and xylose faster than did *Pichia stipitis*[31]. The sunflower seed hull hydrolysate when inoculated with *Pichia stipitis* NRRLY-7124 at 30^0C pH 6 produced ethanol of 9.66g per l[32].

CONCLUSION

The results obtained as shown in the preceding TABLE clearly indicates the biomass taken under study are quite suitable for bioethanol conversion having sizable quantity of available cellulose for necessary exploitation. The percentage of conversion is related to period of incubation. However the protocol used for different pretreatments followed by hydrolysis needs standardization for quantitative yield of fermentable sugar. Necessary kinetics study of both hydrolysis and fermentation reaction needs further investigation to ascertain the concentration of reactant, temperature, pH and period of incubation etc. Moreover the factors such as inhibitors if any released during pretreatment have to be further investigated so as to give a complete comment on effective and productive conversion. There is no dispute and ambiguity in using paddy straw and paddy husk for commercial production of bioethanol unlike utilization of the other cellulosic biomass. Biofuel research being at its infancy selection of suitable biomass, standardization of pretreatment protocol, efficient use of cellulase for hydrolysis and fermentation process needs through research before drawing any conclusion on recommendation of particular biomass go for bioethhanol production. However selection of starting materials for the process needs the criteria of their local availability sustainable supply through out the year play important role for a meaningful and adaptive research.

ACKNOWLEDGMENT

The authors are thankful to N.K. Mohapatra faculty of Academy of Management and Information Technology, Bhubaneswar, Odisha for his constant supervision and to H.O.D of Biotechnology, O.U.A.T, Bhubaneswar, Odisha, India for providing proper facilities for research work.

REFERENCES

[1] K.L.Kadam, L.H.Forrest, W.A.Jacobson; Biomass.Bioenergy, **8**, 369-389 **(2000)**.

[2] A.Aden, M.Ruth, K.Ibsen, J.Jechura, K.Neeves, J.Sheehan, B.Wallace; Lignocellulosic Biomass, Ethanol Process Design and Economic Utilizing Co-Current., Dilute Acid Hydrolysis and Enzymatic Hydrolysis, National Renewable Energy Lab, 1617 Cole Boulevard Golden, Colorado 80401-3393 **(2002)**.

[3] Y.Sun, J.Cheng; Biores.Technol., **83**, 1-11 **(2002)**.

[4] P.C.Badger; 'Ethanol from Cellulose-A General Review', In : Trends in New Crops and New Uses, Ed. J.Janik, A.Whipky, ASHS Press, Alexandra, USA, 17-21 **(2002)**.

[5] Y.Lin, S.Tanaka; Applied Microbiology and Biotechnology, **69**, 627-642 **(2006)**.

[6] W.B.Betts, R.K.Dart, A.S.Ball, S.L.Pedlar; 'Biosynthesis and Structure of Lignocellulose' In : Betts (Eds.) Biodegradation: Natural and Synthetic Materials, Springer-Verlag, Berlin, Germany, 139-155 **(1991)**.

[7] C.N.Hamelinck, G.V.Hooijdonk, A.P.C.Faaij; Biomass and Bioenergy, **28**, 384-410 **(2005)**.

[8] L.R.Lynd; Annual Review of Energy and Environment, **21**, 403-465 **(1996)**.
[9] D.Lee, A.H.C.Yu, K.K.Y.Wong, J.R.Saddler; Applied Biochemistry and Biotechnology, **45**, 407-415 **(1994)**.
[10] S.Sadasivam, A.Manickam; 'Biochemical Methods', New Age International Pvt.Ltd., New Delhi, 270 **(2008)**.
[11] T.K.Ghose; Pure and AppL.Chem., **59**, 257-268 **(1987)**.
[12] G.L.Miller; Anal.Chem., **31**, 426-428 **(1953)**.
[13] J.N.Nigam; Journal of Biotechnology, **72**, 197-222 **(1999)**.
[14] G.Verma, P.Nigam, D.Singh, K.Choudhary; Bioresource Technology, **72**, 261-266 **(2000)**.
[15] S.G.Marakis, G.S.Marakis; Journal of Food Science and Technology, **33**, 108-111 **(1996)**.
[16] A.B.Bjerre, A.B.Oleesen, T.Fernquist, A.Ploger, A.S.Schmidt; Biotechnology and Bioengineering, **49**, 568-577 **(1996)**.
[17] N.Curreli, M.Agelli, B.Pisu, A.Rescigno, E.Sanjust, A.Rinaldi; Process Biochemistry, **37**, 937-941 **(2002)**.
[18] T.H.Kim, Y.Y.Lee; Bioresource Technology, **96(18)**, 2007-2013 **(2005)**.
[19] P.Alvira, E.Tomas-Pejo, M.Ballesteros, M.J.Negro; Bioresource Technology, **101**, 4851-4861 **(2010)**.
[20] F.M.Girio, C.Fonseca, F.Carvalheiro, L.C.Duarte, Marques, S.R.Bogel-Lucasik; Bioresource Technology, **101**, 4775-4800 **(2010)**.
[21] T.H.Kim, M.T.Holtzapple; Bioresource Technology, **97**, 583-591 **(2006)**.
[22] S.B.Kodali, R.Pogaku; Electronic Journal of Environmental, Agricultural and Food Chemistry, **5**, 1253-1264 **(2006)**.
[23] X.Zhao, K.Cheng, D.Liu; Appl.Microbiol. Biotechnol., **82**, 815-827 **(2009)**.
[24] H.Chen, W.Qiu; Biotechnology Advances, **28**, 556-562 **(2010)**.
[25] H.W.Doelle, P.F.Greenfield; Applied Microbiology and Biotechnology, **22**, 405- 410 **(1985)**.
[26] P.Gunasekaran, K.Chandraraj; Current Science, **77**, 56-68 **(1999)**.
[27] L.Davis, Y.Jeon, C.Svenson, P.Rogers, J.Pearce, P.Peiris; Biomass and Bioenergy, **29**, 49-59 **(2005)**.
[28] G.Zayed, O.Meyer; Applied Microbiology and Biotechnology, **45**, 551-555 **(1996)**.
[29] Toivola, D.Yarrow, E.Vanden Bosch, J.P.Van Dijken, W.A.Scheffers; Applied and Environmental Microbiology, **47**, 1221-1223 **(1984)**.
[30] H.K.Sreenath, R.G.Koegal, A.B.Moldes, T.W.Jeffries, R.J.Straub; Process Biochemistry, **36**, 1199-1204 **(2001)**.
[31] H.K.Sreenath, T.W.Jeffries; Bioresource Technology, **72**, 253-260 **(2000)**.
[32] M.Telliokur, N.Eken Saracoglu; Turkish Journal of Engineering and Environmental Science, **30**, 317-322 **(2006)**.