International Journal of Engineering Inventions
e-ISSN: 2278-7461, p-ISSN: 2319-6491
Volume 3, Issue 3 (October 2013) PP: 60-65

Recycling of agricultural wastes in to bio fuel production: An ecofriendly product

Devendra Pratap Singh[1], Rakesh Kumar Trivedi[2]
[1](Department of Applied Chemistry,Dr. A.I.T.H, Kanpur 208024,. India)
[2](Professor & Head, Department of Oil and Paint Technology H. B.T.l., Kanpur 208002)

ABSTRACT: *Carbohydrate biomass is considered as the future feedstock for bioethanol production because of its low cost and its huge availability. In ancient years ethanol was manufactured by corn and starch, now in these days ethanol is produced by lignocellulosic biomasses and algae in a large excess. Lignocellulose contains carbohydrates units. The major carbohydrate materials found in great quantities to be considered, especially in tropical countries, is wheat bran, sugarcane bagasse and rape straw, which can be easily converted in to ethanol by following pretreatment either by acid or enzyme, hydrolysis and distillation process under feasible conditions. The effects of different pH and temperature with enzymatic saccharification treatment on conversion of these biomasses were studied. The produced glucose was fermented to bioethanol, using Saccharomyces cerevisiae yeast in combination with pentose fermenting enzymes as Pitchia stipititis and the amount of produced bioethanol was measured by gas chromatography. Enzyme treatment at 30ºC and pH 5 is an effective treatment method for converting lignocelluloses to glucose. Up to 23.35% glucose v/v could be achieved after enzyme treatment from bagasse than others. Fermentation of treated lignocelluloses shown that glucose after 3 days fermentation the maximum bioethanol of 19.25% (v/v) by Saccharomyces cerevisiae and 26.75% (v/v) was attained in case of sugarcane bagasse by using Pitchia stipititis in combination with S. cerevisiae. In this paper comparative study of enzymatic treatment and acid treatment is also analyzed. This process is expected to be useful for the bioethanol production from wheat bran, sugarcane bagasse and rape straw as a source of carbohydrate renewable biomass from abundant agricultural by product.*

Keywords: *Biomass, Carbohydrates, Fermentation, Pretreatment, Saccharification.*

I. INTRODUCTION

In the past few years there have been important advances in the field of alternative transportation fuels, primarily bio ethanol and biodiesel. Only biodiesel and bio ethanol are considered in this report due to their similar inherent properties compared to fossil-based fuels, especially auto ignitibility. There is a longer-term potential for other bio fuels such as biobutanol and biogas but little research effort has been seen in either regular or small engines. **Bio ethanol** is an alcohol, made by fermenting any biomass with a high content of carbohydrates through a process similar to beer brewing. Today, bio ethanol is made from starches and sugars. In the future, cellulose and hemicellulose fibrous material will be used. For the production of ethanol as bio fuels, pre-treatment is the initial process to degrade the biomass into carbohydrates, so that it may be converted in to monomers and latter on fermentation of sugars is the valuable process by which ethanol is obtained. Chemical treatment is generally used to remove lignin content of agro residues. Chemical pre-treatment by alkali or acid hydrolysis are common in paper and pulp industries to recover cellulose for paper production. These treatments tend to be expensive hence are not used for bioconversion purposes. Pretreatment with acid or alkali is common chemical method that has the effect of increasing the surface area of the agro-industrial residue due to the swelling and disruption of lignin. Pretreatment is the pre step to release the components of lignocellulosic biomass. Agro- residues consist of lignocelluloses that is compact, partly crystalline structure consisting of linear and crystalline polysaccharides cellulose, branched non cellulosic and non-crystalline hetero polysaccharides (hemicelluloses), and branched (non crystalline) lignin [1]. During the process some of the compounds are formed which inhibits the direct fermentation, so it is necessary to remove them so that an efficient products form. Detoxification of dilute-acid lignocelluloses hydrolysates by treatment with $Ca(OH)_2$ (over liming) efficiently improves the production of fuel ethanol, but is associated with drawbacks like sugar degradation and $CaSO_4$ precipitation. In factorial designed experiments, in which pH and temperature were varied, dilute-acid spruce hydrolysates were treated with $Ca(OH)_2$, NH_4OH or NaOH. The concentrations of sugars and inhibitory compounds were measured before and after the treatments. The ferment ability was examined using the yeast Saccharomyces cerevisiae and compared with reference fermentations of synthetic medium without inhibitors [2].

Biodiesel is made by combining alcohol (usually bio ethanol) with vegetable oil, animal fat, or recycled cooking grease. These materials contain triglycerides and other components depending on type. Some

of the feedstocks are palm oil, coconut oil, canola oil, corn oil, cottonseed oil, flex oil, soy oil, peanut oil, sunflower oil, rapeseed oil and algae. It can be used as an additive to reduce vehicle emissions or in its pure form as a renewable alternative fuel for diesel engines. In the near future, agricultural residues such as corn Stover (the stalks, leaves, and husks of the plant) and wheat straw will also be used. Biodiesel is one of the future fuels for the engines that help to reduce the global warming and other harmful pollutants. Converting vegetable oils into their esters of methyl and ethyl alcohols is known as Bio-diesel. Ester formation is a possible way to overcome almost all the problems associated with vegetable oils. By the process of esterifications the high viscosity of vegetable oils could be brought down to acceptable limits. Esters can be produced from oils and fats by 3 methods. I) Base catalyzed trans-esterifications of oil with alcohol. ii) Direct acid catalyzed esterifications of oil with methanol. iii) Conversion of oil to fatty acids and then to alkyl esters with acid catalysts. The first method is preferred because it is economical. The conversion of vegetable oil (Triglyceride Esters) to methyl esters through Tran's esterifications process, this reduces the molecular weight to one-third, reducing the viscosity by a factor of 8 and increasing the volatility. Vegetable oil is mixed with alcohol, NaOH as catalyst. The mixture is heated and maintained at 650C for one hour, while heating, the solution is stirred continuously with stirrer. Two distinct layers are formed, the lower layer is glycerin and the upper layer is ester. The upper layer is separated with moisture and the ester is removed by using calcium chloride. It is observed that 90% of ester is obtained from vegetable oil. Experimental research has been done on various blends of gasoline and ethanol, and results have shown that 7.5% addition of ethanol was the best suitable for SI engine with reduced CO emission [3]. Biodiesel reduces particulate, carbon monoxide, and sulphur dioxide emissions compared to diesel fuel.

Lignocellulose is an important structural component of woody plants and consists of cellulose, hemicelluloses, and lignin. The cellulose molecules, which are polymers with six carbon sugars linked in long chains, and five carbon sugar chains called hemicelluloses, are reinforced by cross linked organic molecules called lignin. This structure is very difficult for microbes to break down into simple sugars. In order to exploit the structural sugars from plant fibers for bioethanol, the recalcitrance of biomass must be overcome in a way that is cost competitive in current markets. The kind of cost- reducing measures that bio-refineries are working towards are efficient de-polymerization of cellulose and hemicelluloses, efficient fermentation (including pentose and hexose sugars) that can handle inhibitory compounds, optimizing process integration, and a cost efficient use of lignin [18,19]. Table1 shows the composition of different biomass.

Lignocellulosic substrate	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Sugarcane bagasse	45	35	15
Wheat straw	30	50	15
Rice straw	32.1	24	18
Rape straw	33.4	30	17
Rice bran	30.4	22	16
Wheat bran	31.3	23	17.5

Table 1. Effect of pH on glucose content of sugarcane baggase

In recent years, bio fuels producers have achieved significant improvements in crop production and processing efficiencies and today the volume of bio fuels produced in a specific planted area is several times Higher than it used to be. Improved production methods and technologies are expected to increase efficiencies even further. In a simplified way, bio fuels can be sub-divided into two large categories: substitute for diesel (biodiesel) and substitute for petroleum (ethanol). This division is based on the key properties of the two products. On the one hand, biodiesel (which replaces diesel in cars) is produced from oil rich plants (e.g. rapeseed, sunflower, algae, etc.) by mixing the vegetable oil (90%) with methanol (10%) in the process called trans-esterifications. On the other hand, bio ethanol (which replaces petrol in cars) – also known as alcohol - is produced through the fermentation of sugar from cereals (wheat, maize, etc.) or sugary feedstock's (sugarcane, sugar beet). Since biodiesel is derived from vegetable oils or animal fats made up of esters, these vegetable oils are renewable biological sources. It has been reported that they emit substantially lower quantity of harmful pollutants compare to conventional diesel; Researchers also found that comparable engine performance with diesel was achieved at relatively lower emission [4, 5]. The merits of using biodiesel instead of conventional diesel are has comparable energy density, cetane number, heat of vaporization, and stoichiometirc air /fuel ratio [6]. Biodiesel is also non-toxic and rate of biodegradation is much faster than conventional diesel. Green house gases effects were least in case of biodiesel [7, 8].

Use of Bio ethanol in motor engines is beneficial to the environment, because it produce CO_2 from combustion engines which is absorbed by plants these plants provide lignocelluloses, which turns in ethanol as shown in Fig: 1, it is a recycle process which helps to control global warming and air pollutants. Another environmental benefit of ethanol as a fuel is that the emission of carbon monoxide, nitrogen oxides and

hydrocarbons in general is less compared to gasoline [9]. However, ethanol-containing fuels will contribute to an increased emission of formaldehyde and acetaldehyde [10]. Nevertheless, the environmental damage caused by reactive aldehydes is far less than that of poly-nuclear aromatic compounds, which are emitted when gasoline is combusted [9].

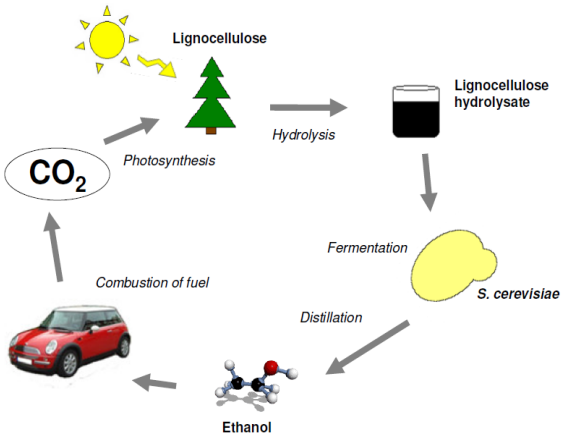

Figure: 1 Production of ethanol from lignocelluloses

The choice of microorganism and strain is very important not only for high sugar utilization, ethanol tolerance, and ethanol producing properties, but also for the robustness and the ability to withstand inhibitors. The selection of microorganism may be influenced by its ability to ferment pentose sugars. The fraction of pentose sugars is generally low in softwood hydrolysates, but if hardwood or agricultural residues are considered, the ability to ferment Pentoses becomes more important. There are several naturally occurring microorganisms that can utilize pentoses. E. coli is a natural pentose fermenter, but it gives low ethanol yields. This problem has been addressed by genetic engineering to increase the ethanol production. *S. cerevisiae* and *Z. mobilis* are excellent ethanol producers, but they are unable to metabolize Pentoses. Recombinant strains that are able to utilize pentose sugars have been developed for both Z. mobilis and S. cerevisiae studied by [11, 12]. However, the genetically engineered pentose-fermenting S. cerevisiae strains have not yet been especially successful; this is due to problems like low pentose fermentation rates, low ethanol yields, and redox imbalances [13].

II. MATERIAL AND METHODS

2.1 **Material:** Wheat bran, sugarcane bagasse, Rice bran and rape straw were used for the treatment process. Before pretreatment these biomasses are reduced in to smaller particles after milling and crushing (particle size <180 µm). Glucose was obtained from hydrolysis of wheat bran, sugarcane bagasse, Rice bran and rape straw. After the hydrolysis hydrolyzed biomass were kept at 18°C until use [14].

2.2 Dilute Acid pre-treatment: For the comparative result equal amount of biomasses were pretreted in two ways one is acid trement where 2-3% acid (H_2SO_4) was used for the pre-treatment method. In this content acid soaked biomass slurry was autoclaved at 121^0C for 30 minutes. To separate the solid and liquid fraction centrifuge method was used. The dilution and pH was maintained at 5 by adding alkali of centrifuged biomass before fermentation process.

2.3 Enzyme pre-treatment: 150 g of each biomass were suspended in 500 mL H_2O in ratio of 3:10 (w/v) sugarcane bagasse and added of 0.1 mL of α-amylase enzyme. The pH of sample was adjusted at pH 5, 5.5, and 6. The sample was incubated in water bath 100°C for 30 minutes, after that the mixture was applied for second enzymatic treatment (0.2 ml of glucoamylase). Finally, hydrolzsate was pressed through cheese cloth. The amount of reducing sugar in juice was measured.

2.4 Fermentation: The pre-treated samples from 2.2 and 2.3 were carried out for fermentation experiments. The yeast *S. cerevisiae* was used for fermentation (1.5g, 3.0g, and 4.5g). After 3 fermentation days the ethanol content was measured by gas chromatography. All the measurements were duplicated and the data reported are average of two replications. Yeast *S. cerevisiae* was also used with *Pitchia stipititis* for both the fermentation of pentose and hexose. Equal amount of both the yeast and P. Stipititis were taken for the efficient hydrolysis and fermentation of both Pentoses and hexoses sugar present in the hydrolyzed.

III. RESULT AND DISCUSSION

3.1. Effect of enzyme pre-treatment methods on glucose content of sugarcane baggase: For the maximum production of ethanol hydrolyzed biomass (both acid and enzyme pretreated) were fermented at different ph scale, after fermentation the maximum result was obtained at pH 5 for the sugarcane bagasse. From the Table: 2, it is clear that, increasing pH showed reverse effect on glucose concentration in sample. This is expected because of conversion of carbohydrate to glucose [15]. The highest glucose up to 23.35 % glucose on the sugarcane bagasse basis could be obtained on pH 5. Hence ph 5 at 30^0C was found optimum for fermentation; hence it was taken for all the hydrolyzed biomass for fermentation.

pH	Temperature (°C)	Glucose (%)
5	30	23.35
5.5	30	22.80
6	30	22.00
5	40	21.05
5.5	40	19.43
6	40	18.95

Table 2. Effect of pH on glucose content of sugarcane baggase

3.2. Effect of acid pre-treatment methods on glucose content of sugarcane baggase: 150 g of each biomass were suspended in 500 mL dilute acid of different concentration in the ratio of 3:10 (w/v) sugarcane bagasse. The percentage of cellulose converted and the available form of substrate after enzymatic hydrolysis of various concentrations of acid pre-treated corncob samples were calculated based on the amount of sugar released. The results are presented in the Table 3. The results show that the percentage of cellulose conversion increased with the increased concentrations of acid used for pre-treatment and it was also noted that the available form of substrate reduced with increased concentrations of acid. Since maximum sugars were obtained at 3% acid pre-treatment (optimum), hence it was used for further pre-treatment of other biomasses.

Sample	Sample-Acid used for Pre-treatment (%)	Cellulose Conversion (%)	Available Substrate (%)
S1	0.5	11.8	88.2
S2	1.0	12.8	87.2
S3	1.5	13.6	86.4
S4	2.0	14.2	85.8
S5	2.5	15.2	84.8
S6	3.0	16.0	84

Table 3. Effect of acid pretreatment on carbohydrate content of sugarcane baggase

3.3. Fermentation of enzyme treated biomass: Bio ethanol was produced by using *S. cerevisiae* and *S. cerevisiae* in combination with *Pitchia stipititis* at pH 5 and 30^0C. 3.0g of each enzyme were taken which yields maximum ethanol 19.25% by using *S. cerevisiae* while 26.75% was found by using *S. cerevisiae and p. stipititis*, for sugarcane baggase. It can be seen in Figure 2 that pH 5.0 tended to give higher ethanol concentration at 30^0C. Therefore, pH 5.0 was chosen for ethanol fermentation. This was supported by [16].

Biomass (150g)	Sugar (%)	Ethanol (%) by	
		S.cerevisiae	S.cerevisiae & P. stipititis
Sugarcane baggase	23.35	19.25	26.75
Wheat Bran	20.74	17.47	21.17
Rape Straw	22.75	18.09	25.48
Rice bran	21.03	17.95	24.28

Table 4. Production of Ethanol % (v/v) at 30^0C and pH 5 of enzyme treated biomass.

Production of bio ethanol in Table 2 shows that pH 5 at temperature 60°C had higher quantities of bio ethanol produced and bio ethanol content of 19.25%, 17.47%, 18.09%, 17.95% (v/v) was recorded maximum for bagasse, wheat bran rape straw and rice bran. This confirms that the higher sugar content in the juice, the more bio ethanol can be produce which was also found similar according to the [17]. It was observed that the sugar content was directly proportional to the quantities of the ethanol collected. Therefore, the higher the sugar content, the more the ethanol can be produced [17]. By using *Pitchia stipititis* in combination with *S. cerevisiae* maximum ethanol was found 26.75%, 21.17%, 25.48% and 24.28% for sugarcane bagasse, wheat bran rape straw and rice bran. The high yield of ethanol production is due to the conversion of pentose sugar by *P. stipititis*, because *S. cerevisiae* is only able to ferment hexose sugar as shown in fig: 2.

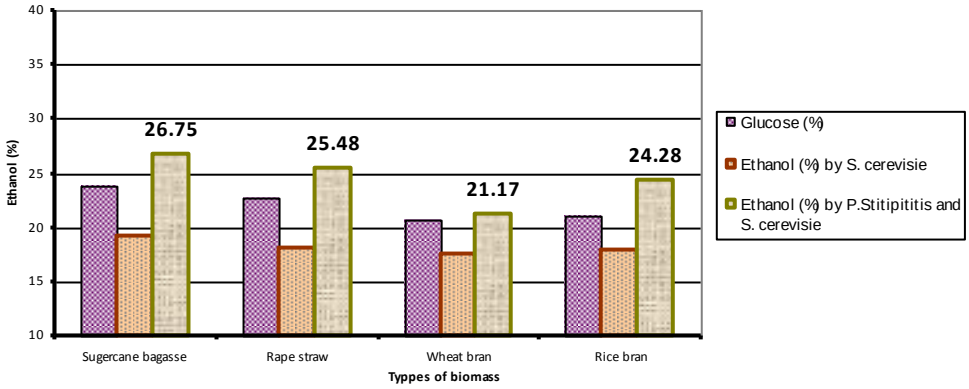

Fig. 2 Production of Ethanol % (v/v) at 30^0C and pH 5from enzyme treated biomass

3.4. Fermentation of acid treated biomass: The feasibility of bio-alcohol production of cellulosic wastes especially sugarcane baggase was made in this study. From the previous studies it was noted that increased acid concentration used for the pretreatment purpose enhanced the sugar release during enzymatic hydrolysis. Fermentation studies also proved that increased acid concentration yielded more amount of alcohol. Alcohol content of about 25.38% (Table 5) was detected in the 72 hours of fermentation broth containing the hydrolysed sample pretreated with 3% sulphuric acid. This study proved that sugars released by means of enzymatic hydrolysis of pretreated bagasse are of fermentable category and alcohol can be produced efficiently using a suitable fermenting strain *Saccharomyces cerevisiae.* Table-5 represents the total Ethanol Production from the kinds of agricultural wastes. The comparative study of production of ethanol from acid treated and enzyme treated biomasses are presented in Fig. 3. Maximum ethanol was found by acid treatment than enzymatic treatment because in acid treatment both Pentoses and hexoses sugar was present.

SN.	Types of biomass samples	Ethanol %(v/v) by	
		S.cerevisiae	S.cerevisiae & P. stipititis
1	Sugarcane baggase	24.25	35.38
2	Wheat Bran	21.47	31.25
3	Rape Straw	23.95	34.37
4	Rice bran	23.05	33.98

Table 5. Production of Ethanol % (v/v) at 30^0C and pH 5 of acid treated biomass.

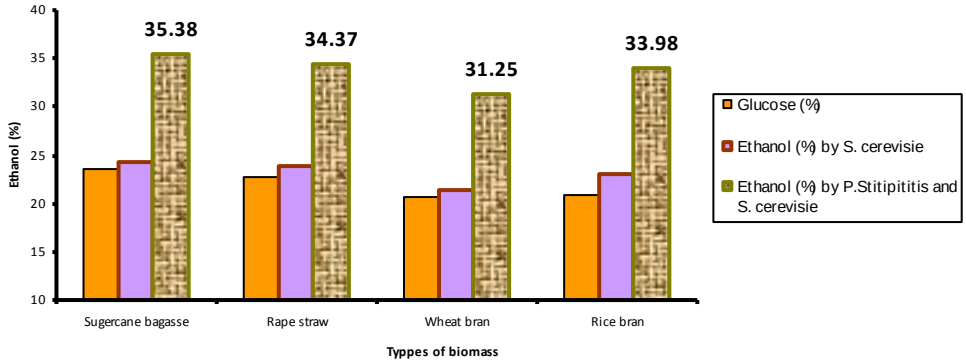

Fig. 3 Production of Ethanol % (v/v) at 30^0C and pH 5from Acid treated biomass

It is clear from the fig.2 and fig.3 that acid treated biomass yields maximum ethanol but there may be chance of formation of inhibitors so that experiments were done very carefully. The maximum ethanol was counted as 35.38% for sugarcane bagasse by fermentation with *S.cerevisiae & P. stipititis* of acid treated, while in case of enzyme treated biomass it was 26.75%.

IV. CONCLUSION

If bio fuels continue their rapid growth around the globe, the impact on the agricultural sector can be significant. Increased jobs and economic development for rural areas in both industrialised and developing countries is one possibility, if governments put the appropriate policies in place and enforce them. The more involved farmers are in the production, processing, and use of bio fuels, the more likely they are to benefit from them. It can be concluded that it is possible to successively use sugarcane bagasse, wheat bran and rape straw for bioethanol. Enzyme treatment at 30ºC and pH 5 is an effective treatment method for converting biomass to glucose. Up to 23.35% glucose v/v could be achieved after enzyme treatment. Fermentation of enzyme treated biomasses shown that glucose after 3 days fermentation the maximum bioethanol of 19.25% (v/v) was attained by *S. cerevisiae* and 26.75% by *S.cerevisiae* & P. *stipititis*, while Fermentation of acid treated biomasses shown that reducing sugar after 3 days fermentation the maximum bioethanol of 24.25% (v/v) was attained by *S. cerevisiae* and 35.38% by *S.cerevisiae* & P. *stipititis*. Production of bioethanol has been considered to be one of economical way for the utilization of rice bran. So the manufacture of glucose could easily be undertaken as an additional source of income.

Bio fuels can play a significant role in the context of a broader transformation of the transportation sector but alone they will not solve all of the world's transportation-related energy problems. To achieve their full potential in providing security of supply, environmental and social benefits, bio fuels need to represent an increasing share of total transport fuel compared to oil. While it is recognised that bio fuels have the capacity to reduce greenhouse gas emissions compared to fossil fuels, their production and use are not entirely without environmental implications. Depending on the crop type and other factors, carbon emissions are not always lower than for traditional fuels. Production of biofuel from biomasses has recycle process to keep the environment green and safe from the reduction of harmful gases.

REFERENCES

[1.] Glasser WG, Kaar WE, Jain RK, Sealey JE (2000) Isolation options for no cellulosic hetero polysaccharides. Cellulose 7: 299.317.

[2.] Alriksson, B., Sjöde, A., Nilvebrant, N.-O., Jönsson, L.J. (2006), Optimal conditions for alkaline detoxification of dilute-acid lignocellulose hydrolysates. Appl. Biochem. Biotechnol., 130, 599-611.

[3.] Hakan Bayraktar. (2005). "Experimental and theoretical investigation of using gasoline–ethanol blends in spark-ignition engines", Renewable Energy Vol. 30 pp1733–1747.

[4.] Alfuso S., Auriemma M., Police G. and. Prati M. V, (1993), "The effect of methyl-ester of rapeseed oil on combustion and emissions of DI diesel engines", SAE Paper 93-2801.

[5.] Peterson C. and Reece D., (1995), "Emissions characteristics of ethyl and methyl ester of rapeseed oil compared with low sulphur diesel control fuel in a chassis dynamometer test of a pickup truck", Transactions of ASAE, Vol. 39**,** No.(3), pp.805-816.

[6.] Agarwal A. K. (2007) "Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines." Progress in Energy and Combustion Science Vol.33, pp 233–271.

[7.] Murugesan A, Umarani C, Subtamanian R, Nedunchezhian N, (2009). "Bio-diesel as an alternate fuel for diesel engines – a review", Renew Sustain Energy Rev; Vol.13 No. (3), pp 653-62.

[8.] Alat M. Balat H. A. (2008). "Critical review of bio-diesel as a vehicular fuel", Energy Converse Manage; Vol.49 No. (10), pp 2727-41.

[9.] Hsieh, W.-D., Chen, R.-H., Wu, T.-L., Lin, T.-H. (2002), Engine performance and pollutant emission of an SI engine using ethanol-gasoline blended fuels, Atmos. Enviro., 36, 403-410.

[10.] Leong, S.H., Muttamara, S., Laortanakul, P. (2002), Applicability of gasoline containing ethanol as Thailand's alternative fuel to curb toxic VOC pollutants from automobile emission. Atmos. Enviro., 36, 3495-3503.

[11.] Aristidou, A., Penttilä, M. (2000), Metabolic engineering applications to renewable resource utilization. Curr. Opin. Biotechnol., 11, 187-198.

[12.] Hahn-Hägerdal, B., Wahlbom, C.F., Gardonyi, M., Van Zyl, W.H., Cordero Otero, R.R., Jönsson, L.J. (2001), Metabolic engineering of Saccharomyces cerevisiae for xylose utilization. Adv. Biochem. Eng. Biotechnol., 73, 53-84.

[13.] Sonderegger, M., Jeppsson, M., Hahn-Hägerdal, B., Sauer, U. (2004). The molecular basis for anaerobic growth of Saccharomyces cerevisiae on xylose investigated by global gene expression and metabolic flux analysis. Appl. Environ. Microbiol., 70, 2307-2317.

[14.] Khongsay, N.; Laopaiboon, L.; and Laopaiboon. 2010. Growth and Batch Fermentation of *Saccharomyces cerevisiae* on Sweet Sorghum Stem Juice Under Normal and Very High Gravity Conditions, Biotechnoogy, 2010, ISSN 1682- 296X © 2010 Asian Network for Scientific Information.

[15.] Yoswantana, N. Phuriphipat, P. Treyawutthiwat. P. 2009. Bioethanol Production from Rice Straw. International Conference on Science Technology and Innovation for Sustainable Well-Being (STISWB).

[16.] Thuesombat, P., Thanonkeo, P., Laopaiboon, L., Laopaiboon , P., Yunchalard ,S., Kaewkannetra, P. and Thanonkeo, S., 2007, The Batch Ethanol Fermentation of Jerusalem Artichoke Using *Saccharomyces cerevisiae*, KMITL Sci. Tech. J. 7, S2, 93-96.

[17.] Yamba, F.D. Wamukwamba, C.K. Matsika, E., and Sangiso, M. 2007. Investigation into the Production and Use of Bioethanol from Sweet Sorghum as an Alternative Fuel. Department of Mechanical Engineering, School of Engineering, University of Zambia, Lusaka.

[18.] Galbe, M., Sassner, P., Wingren, A., Zacchi, G. 1, s.l.: *Applied Biochemistry and Biotechnology*, 2007, Vol. 124. 1101-1117. Process Engineering Economics of Bioethanol Production.

[19.] Hahn-Hagerdal, B., Galbe, M., Gorwa-Grauslund M.F., Liden, G., Zacchi, G. 12, and s.l.: *TRENDS in Biotechnology*, 2006, Vol. 24. Bio-ethanol – The fuel of tomorrow from the residues of today.