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Production of Bioethanol from Waste Newspaper

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Abstract

Paper, which is one of the largest constituent of Municipal solid waste, has become a severe problem for disposal in developed and developing countries due to the shrinking landfill capacity. It is very important and challenging task in managing the solid waste. Newspaper, which is a cellulosic feed stock, is emerging as an attractive option for the production of bio-ethanol because of lower feedstock costs, higher potential for fossil fuel displacement and also there will be reduction in greenhouse gas emission as compared to production of ethanol from corn. The main objective of the current project is to minimize the newspaper load on municipal solid waste by efficiently utilizing the waste newspaper in the production of bio-ethanol. Experimental studies have been carried out to optimize the pre-treatment process for increasing the efficiency of bacterial hydrolysis, the efficient conversion of cellulose to sugars from cellulose degrading microorganisms and to convert the sugars released to Ethanol by using Fermentation process. Pretreatment, hydrolysis and fermentation are the steps involved in the production of Bioethanol. In the pre-treatment process, the Lignin, Hemicellulose and Cellulose are separated to enhance the hydrolysis process. The optimized condition for the pre-treatment was found to be 1.5% concentration of H₂SO₄ at 121°C and 45 minutes. The bacteria *CytophagaHuchnosonni* was used for hydrolysis process, which helped in converting the cellulose to sugars and was analysed using DinitroSalicilicacid. The reducing sugars were fermented to produce Bioethanol using the Yeast *Saccharomyces Cerevisae* and the yield was estimated using specific gravity method and also by using HPLC.

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1.0 Introduction

Increase in the population over the last century lead to the increase of the energy consumption worldwide. To

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meet the increased energy demand crude oil has been used as the major resource. The global oil production would decline to 5 billion barrels from 25 billion barrels approximately. Due to this unavoidable depletion of the world petroleum resources in the coming years the worldwide interested aroused in seeking an alternative non-petroleum based energy source, (Zhi Sheng Zu et al.). One of the best alternative fuels in order to beat severely the energy crises is from Biofuel. From biologically carbon fixation the energy is derived from Biomass. The various factors like need for increasing energy security and hikes and gaining the scientific and public attention the biomass are driven. The main contents of ethanol are sugar, starch or cellulose. The Bioethanol is one of the environment friendly fuels, the effects on environment is less because the Ethanol contains oxygen. With comparison to the conventional gasoline the blends of E10 resulted in 12-25% less emission of carbon monoxide, (BibiZainsab et al., 2014). The sugarcane and corn are the first generation bio-fuels. Due to vast increase in the ethanol production using these crops they cause immoderate pressure on the global food supply. The second generation biofuels can be produced by means of different sources like waste chicken feathers, cellulosic biomass food and organic waste. The cellulosic biomass, such as agricultural residue and industrial waste are the most abundant and cheap source of renewable energy in the world.

The second generation biofuels may also include the fuels produced from mixed paper waste which is separated from the municipal solid waste, cash crops *Jatropha*, *Honge*, *Cotton*, *Maize* etc. can be utilized to produce bioethanol. The third generation biofuels can be produced from micro-organisms mainly *Algae*. The fourth generation biofuels produced from vegetable oil, biodiesel. The table shows the summary of classification of the biofuels.

In developed and developing countries municipal wastes have become a severe problem during the last century, (Demitrios H et al.). The shrinking of landfill capacity resulted in rising of landfill costs which is mainly due to the waste paper from the municipal waste. Because of the above concern the waste paper is used as cheap source for the production of bioethanol. Due to the shrinking landfill capacity, the tighter environmental control exists on their siting operation, construction, and of the unwillingness of communities to have new landfill sites nearby. The tighter environmental regulations are responsible for the premature closure of existing landfills and higher costs for constructing new ones, (Alya L et al., 2012). Among the various components the municipal solid waste consists of food waste, wood, leaf, garden or yard trimmings, rubber, textile, leather, metals (ferrous metals or Non ferrous metals), glass and major of paper and paper boards. About 35% to 40% by weight of the municipal solid waste is made of the paper.

2.0 Literature review

Though the earlier combustion powered transportation vehicles were fuelled with ethanol, crude oil derivatives have provided the vast majority of transportation fuels throughout the 20th and 21st centuries. In 2006, global demand for petroleum and other liquid fuels was 85.0 million barrels oil equivalent per day (Mb/d) and this is forecasted to grow to 106.6 Mb/d in 2030, with the growth in transportation fuel use being responsible for 80% of the higher total crude oil use, (Suhail J C et al., 2013). Despite improvements in the energy efficiency standards in many countries & the dampened demand resulting from the global economic recession experience in 2008-09, global crude oil consumption is expected to increase by over 1% annually driven primarily by the growth in demand in India and China, (Bishnu J et al., 2011). However, increasing demand of fossil fuels will likely to cause diminishing of world fuels reserve, which may lead to the scarcity of this type of fuels while also cause the price to increase dramatically. The release of carbon dioxide (CO₂) from vehicle and other industries is one of the largest potential contributors to global warming. Development of alternative energy source such as biofuels becomes important to reduce these problems. The only non-fossil liquid fuel currently of significance on a global scale is biofuels, including bioethanol & biodiesel. Utilization of bioethanol as transportation fuel and as a gasoline supplement has been proved to be more environmentally friendly. Bioethanol is a clean-burning, high octane number fuel that can readily substitute gasoline and its combustion results in significant reductions of toxic emissions such as formaldehyde, benzene and 1-3 butadiene, while blending ethanol with gasoline can increase the octane of the mixture and can reduce carbon monoxide (CO) emissions by 10 ~ 30%, (Sadashivam S M, 2006). When bioethanol is produced from renewable sources such as biomass it can both decrease urban air pollution and reduce the accumulation of carbon dioxide (CO₂), so called green house gases (GHG). Thus, replacement of

gasoline with ethanol, derived from renewable biomass feedstocks that sequester CO₂ during growth, is expected to reduce CO₂ emissions by 90 ~ 100%, (ASTMD ,1993). Besides that, development of biofuels is expected to assure availability of new and renewable energy resources, increase the economic value of forest and also can reduce the poverty and unemployment.

Currently, bioethanol production is focused on sugar crops including sugar cane and sugar beets and also starch crops, including wheat, potatoes and sweet potatoes, which is often based on excess agricultural production and it is generally recognized that this volume is too small in comparison with the anticipated levels of production required for total conversion of transportation fuel markets from gasoline to ethanol. It is also apparent that there is a potential for competition with food production for both the sugar and starch feed-stocks and that prime agricultural lands normally required for producing foodstuffs should not be diverted for fuel production. Therefore, bioconversion of lignocellulosic biomass into bioethanol is very important to be developed since this resource is more economical and is available easily. Biomass resources obtained from lignocellulosic materials such as agricultural and forestry residues, municipal solid waste, and various industrial wastes are still not well utilized, hence often present disposal problems. These residues can be found easily for bioethanol production. Furthermore, woody and herbaceous energy crops can be planted and underutilized land can be employed to support indigenous production of such forms of biomass. Not only it is renewable, these biofuels can also reduce emission of gases which potentially can cause global warming (Priya R M et al. ,2010).

Cellulosic resources are in general very widespread and abundant. Being abundant and outside the human food chain makes cellulosic materials relatively inexpensive feedstocks for bioethanol production. Cellulosic materials are comprised of lignin, hemicellulose, and cellulose and are thus sometimes called Lignocellulosic materials. One of the primary functions of lignin is to provide structural support for the plant. Thus, in general, trees have higher lignin contents than grasses. Unfortunately, lignin which contains no sugars, encloses the cellulose and hemicellulose molecules, making them difficult to reach. Cellulose molecules consist of long chains of glucose molecules as do starch molecules, but have a different structural configuration. These structural characteristics plus the encapsulation by lignin makes cellulosic materials more difficult to hydrolyze than starchy materials. Hemicellulose is also comprised of long chains of sugar molecules but contains, in addition to glucose (a 6-carbon or hexose sugar), contains pentoses (5-carbon sugars). To complicate matters, the exact sugar composition of hemicellulose can vary depending on the type of plant. Since 5-carbon sugars comprise a high percentage of the available sugars, the ability to recover and ferment them into ethanol is important for the efficiency and economics of the process. Recently, special microorganisms have been genetically engineered which can ferment 5-carbon sugars into ethanol with relatively high efficiency, (Hsu A T ,1996).

2.1 Feedstock's for lignocellulosic ethanol

Lignocellulosic materials can be derived from wood, grasses, agricultural residues, and waste materials. The table 2.1 shows the contents of cellulose, hemicellulose and lignin for different lignocellulosic materials.

Table 1: Composition of Cellulose, Hemicellulose and Lignin for different Lignocellulosic materials

Lignocellulosic Materials	Cellulose (%)	Hemic-ellulose(%)	Lignin (%)
Hard wood	40-55	24-40	18-25
Softwood stems	45-50	25-35	25-35
Switch grass	45	31.4	12-20
Miscanthus	40	18	25
Coastal Bermuda grass	25	35.7	9-18
Corn stover	35-40	17-35	7-18
Wheat straw	30	50	15
Rice straw	36-47	19-25	10-24
Cotton seed hairs	80-95	5-20	0
Newspaper	40-55	25-40	18-30
White paper	85-99	0	0-15

Sources: (Ye Sen et al, 2002).

3.0 Materials and Methods:

3.1 Collection of substrate:

Newspaper, which was used as a substrate for the production of bioethanol, was collected from the households. The substrate was collected in a dust free and fungus-free state and was dried in sunlight and was made into small pieces and stored in sealed plastic bags.

3.2 Chemical Analysis of the substrate:

Composition of the substrate and its properties were analyzed before pre-treatment. The cellulose content and total carbohydrate in the substrate was estimated by anthrone method, (Praveen K et al., 2009), Moisture content and ash content of the substrate were also estimated using standard methods, (Zheng Y Zhangli et al., 2009).

3.3 Optimization of pretreatment process:

The pre-treatment optimization for the substrate was carried out by using different combination dilute sulphuric acid ranging from 0 to 6% and heating period of 30, 45 and 60 minutes at 121°C and 15lb pressure. 1gm of substrate was added with 10 ml of dilute sulphuric acid (1:10). Cellulose released during this optimization process was analysed by anthrone method, (Zahid Anwar et al., 2011). After the release of maximum amount of cellulose during pre-treatment process, the solution was taken for hydrolysis.

3.4 Hydrolysis of the pretreated substrate:

Maximum cellulose released during the pretreatment was hydrolysed by the isolated cellulose degrading bacteria. The pretreated substrate was washed with distilled water several times to neutralise the acid concentration. The substrate was oven dried till constant weight and the pH was adjusted to 7.0. Pure culture *Cytophagahutchisonni* (CH) (NCIM 2338) was procured from National Collection of Industrial Microorganisms (NCIM), Pune and the isolated organism is taken from Department of Biotechnology, BEC Bagalkot. Comparison study between isolated cellulose degrading bacteria and the pure culture, CH was performed. A 24hr grown inoculum of isolated cellulose degrading bacteria and pure culture, CH were added to the pretreated substrate. Reducing sugars release during substrate hydrolysis were analysed by Dinitrosalicylic Acid (DNS) method every 24hr from zero hour, for both the organisms, (Zahid Anwar et al., 2011). Maximum sugars released during this period were further taken for fermentation to produce bioethanol.

3.5 Fermentation of hydrolysed broth:

Fermentation was carried out using commercially available yeast, *Saccharomyces cerevisiae*. The pH of hydrolysed broth was adjusted to 4.6 and an inoculum of active yeast (in log phase) was added to the hydrolysed broth. The fermentation was carried out at 36°C until maximum sugars are converted into bioethanol. The reducing sugar utilization during fermentation was analysed by DNS method, (Zahid Anwar et al., 2011), and the bioethanol production was analysed by using specific gravity method, (Sahail J et al., 2011).

Calculation for specific gravity:

$$\frac{W2 - W1}{W3 - W1} = \text{Specific Gravity}$$

Where,

W1 = empty weight of specific gravity bottle

W2 = Weight of sample + specific gravity bottle

W3 = Weight of distilled water + specific gravity bottle.

Also the ethanol yield was estimated using High Performance Liquid Chromatography (HPLC). The instrument used was “SHIMADZU-C-10AVP”. Ethanol was analyzed by using HPLC with cation exchanger SugarPak column (C₁₈). Acetone-Nitrate and water (80:20) was used as a mobile phase at a flow rate of 1mL/min. The injection volume was 5µl and the column temperature was maintained at 90°C. All the samples were filtered through a 0.45µm filter before subjected to HPLC analysis. The evaluate was detected by a refractive index detector at 50°C.

4.0 Results and Discussions:

4.1 Chemical analysis of the substrate:

The chemical analysis of the substrate showed that the substrate consisted of 45% cellulose. Ash content and moisture content were found to be 5.17% and 6% respectively.

4.2 Optimization of pretreatment of the substrate:

In pre-treatment with dilute sulphuric acid, the structure of the cellulosic biomass will be altered to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars rapidly and with greater yield.

In the present study, pre-treatment optimization of substrate was carried out at various concentrations of dilute sulphuric acid (0 to 6%) and at varying heating periods (30, 45 and 60 minutes). Acetic acid furfural and other inhibitors of yeast metabolism are usually present in the pre-treated sample. Hence to remove these inhibitors pre-treated substrate was washed with distilled water for several times. The graphical representation of the results is given in Figure 1. From the results obtained it is observed that the maximum recovery of cellulose was 55% with dilute sulphuric acid concentration of 1.5% and a heating period of 45min at 121°C. In the literature also it has been reported that dilute sulphuric acid with concentration below 4% has been used to make the pre-treatment process inexpensive and effective, (Ajeetkumar S P et al. ,2014).

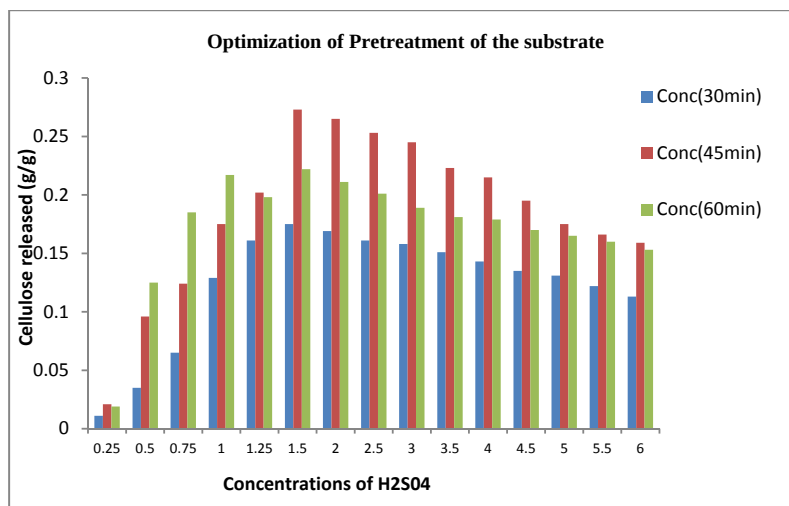


Fig. 1 : Pretreatment optimization of substrate

4.3 Hydrolysis of the pretreated substrate:

In the hydrolysis process the cellulose present in the substrate is converted into ethanol using the cellulose degrading organism after the pre-treatment process. In the present study bacteria were used for hydrolysis instead of enzymes, since the enzymes are expensive and in turn increase the cost of bioethanol production. Hydrolysis was

carried out at neutral pH . A comparative study for hydrolysis between the pure culture and isolated culture of bacteria was carried out. The pure culture *Cytophagahutchisonni* (CH) (NCIM 2338) was procured from National Collection of Industrial Microorganisms (NCIM), Pune and the isolated organism, WG3, was taken from Department of Biotechnology, BEC Bagalkot. The graphical representation of the results obtained is shown in Figure 2.

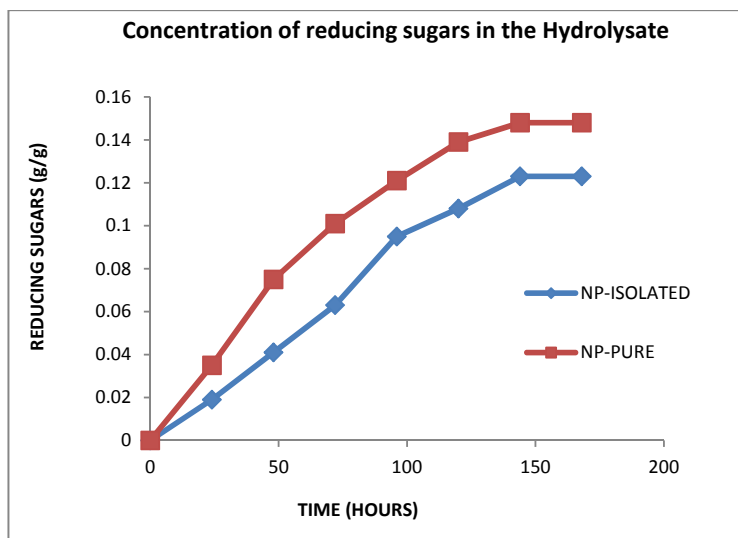


Fig. 2. Hydrolysis of pretreated substrate

The results showed that maximum cellulose degradation occurred with the pure culture *Cytophagahutchisonni*, as compared to the isolated organism. The pure culture organism *Cytophaga hutchisonni* released 0.148g of reducing sugars per gram of substrate as compared to the 0.123g from isolated culture. Conversion was observed within 24 h of inoculation. On 5th day maximum conversion was recorded (Figure 4.2) and after that there was no significant increase in the concentration of reducing sugars.

Similar research work carried out for cellulose hydrolysis by fungi *Tricoderma reesei* resulted in cellulose yield of 0.184g per gram of rice husk substrate, (Ajeetkumar S P et al., 2014). In another study, the fungi *Phanerochaete chrysosporium* and *Aspergillus awamori*, were used for the hydrolysis of the substrates rice straw, bagasse and wheat straw. The cellulose released with these substrates were 62.7 mg, 73.7 mg and 52.4 mg per gram of substrate respectively for rice straw, bagasse and wheat straw, (Sahail J et al. , 2011). In another work, the yield of reducing sugars by Hydrolysis of rice husk and groundnut hulls by isolated fungi, *Aspergillus Niger*, from soil sample, were 200mg per gram of groundnut hull substrate and 185mg of per gram of rice husk substrate, (Srivastava et al.). Similarly, Biological hydrolysis of the pre-treated substrates by various enzymes are widely studied. One such research showed 30.46 mg of reducing sugars released per gram of sugarcane bagasse substrate by using invertase enzyme, (Mir Naiman et al., 2011). In the back ground of these findings, it can be observed that the cellulose degrading bacteria *CytophagaHutchinsonni* used in the present work is more effective in converting the cellulose into reducing sugars.

4.4 Fermentation of the hydrolysed broth:

Fermentation of sugars released during hydrolysis of substrates was carried out by yeast *Saccharomyces cerevisiae* at pH 4.6 and 34°C temperature, to convert into bioethanol. Fresh inoculum of yeast (5% v/v) was added to the hydrolysed broth. Fermentation was carried out for six days with fermented samples being collected every twenty four hours for analysis of reducing sugar by DNS method for substrate utilization. Estimation of bioethanol produced was carried out by specific gravity method and HPLC method.

The graphical representation of the results of utilization of sugars during fermentation and the percentage of bioethanol produced using specific gravity method are shown in Figure 3 and 4 respectively.

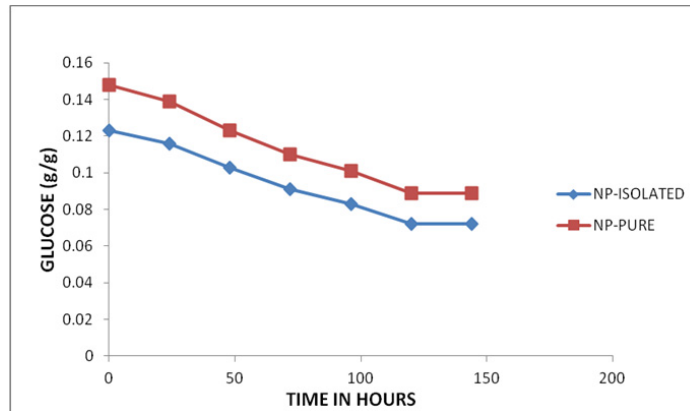


Fig. 3.Utilization of reducing sugars during fermentation

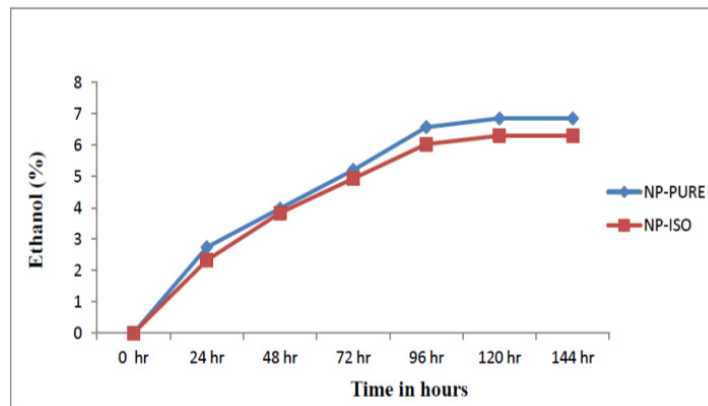


Fig. 4.Percent of bioethanol produced using Specific gravity method

The maximum percent of bioethanol produced as estimated from specific gravity method was 6.849 % v/v (from pure culture organism,) and 6.031 % v/v (from isolated culture). The Ethanol yield was also estimated using HPLC at the interval of every 24 hours. The yields as estimated from HPLC were found to be 6.91% from the pure culture and 6.12% from the isolated culture organisms.

It may be noted that fermentation process would be economical if both the pentose and hexose sugars in the hydrolysate are converted to bioethanol. *Saccharomyces Cerevisiae* can ferment only hexose sugars into ethanol. In one of the studies on fermentation using *Saccharomyces cerevisiae* for various substrates namely, groundnut hull, and rice husk, the yields of 0.142g per gram of groundnut oil and 0.108g per gram of rice husk were reported, (Srivastava et al.). In another study, the yield of 0.218g per gram of cellulosic waste mixture (office paper, newspaper and cardboard in 1:1:1 ratio) was reported, (Iuliana L et al. ,2010). Similarly, the sugarcane leaf litter as feedstock produced 0.130mg/L (alkaline pre-treated) and 0.335mg/L (acid pre-treated) of ethanol. For substrate bagasse pith hydrolysed by cellulase enzyme the bioethanol produced was 7.7% (v/v), Iuliana L (2000)".

5.0 Conclusions

The present work deals with the studies on production of bioethanol from waste news paper which is one of the largest constituent of Municipal solid waste. Experiments were carried for Pretreatment of the substrate, Hydrolysis and Fermentation of the hydrolysate. The optimized condition for the pretreatment was found to be 1.5% concentration of H_2SO_4 at $121^\circ C$ and 45 minutes of contact time. The maximum amount of cellulose recovered under these optimum parameters was 55%. The comparative study of hydrolysis using Bacteria *CytophagaHutchinsonii* purchased from NCRM, Pune and the isolated organism from Department of Biotechnology BEC, Bagalkot was carried out. From the results it was observed that 0.148g of reducing sugar was obtained per gram of the substrate from *CytophagaHutchinsonii* and 0.123g of reducing sugar was obtained from the isolated organism. Yeast *Saccharomyces Cerevisiae* was used to ferment the reducing sugar into bioethanol. The ethanol yield as estimated using specific gravity method was 6.849% (v/v) with the organism *CytophagaHutchinsonii* and from isolated organism, the yield was observed to be 6.031% (v/v). Ethanol was also estimated using HPLC and the yields obtained were 6.91% and 6.12% respectively for *CytophagaHutchinsonii* and isolated organism. Based on these results, it can be concluded that the biological hydrolysis of cellulose by *CytophagaHutchinsonii* bacteria was more efficient compared with the isolated bacteria.

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