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Screening of Bio-ethanol in Water hyacinth

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Abstract

As the search for alternatives to fossil fuel intensifies in this age of modernization and industrialization, fuelled by increasing energy costs, water hyacinth- world's most notorious aquatic weeds, holds a strong promise in the 21st century renewable bio-fuel industry. Bio-ethanol can be produced from inexpensive and abundant lignocellulosic biomass by"2nd generation bio-ethanol processes". Maximum ethanol could be produced from saccharification of water hyacinth by using protein isolated strain. "Eradication through utilization" should be the strategy for controlling water hyacinth through a sustainable biofuel technology.

Key words: *Bio-fuel, Renewable energy, Water hyacinth, Saccharification*

Introduction

Renewable bio-fuels generally involve contemporary carbon fixation, such as those that occur in plants or microalgae through the process of photosynthesis. Other renewable bio-fuels are made through the use or conversion of biomass[1]. The biomass conversion can result in fuel in solid, liquid or gas form. Bio-fuels are solid, liquid or gaseous fuels that are produced from biomass. Bio-fuels can be derived directly from plants, or indirectly from agricultural, commercial, domestic, and/or industrial wastes[2]. This new biomass can also be used directly for bio-fuels. The biomass or organic matter that is converted to bio-fuels may include food crops, dedicated bioenergy crops, agricultural residues, wood/forestry waste and by-products, animal manure and algae[3,4,5].

The primary liquid bio-fuels used in the transport industry on a commercial scale today are ethanol, made from the fermentation of sugary/starchy crops such as sugar cane and corn, and biodiesel, which can be obtained from oil crops such as oil palm and soybeans[6]. Both ethanol and biodiesel can be blended with conventional gasoline and diesel and used as liquid fuels in conventional engines for transportation. Biogas is also a commonly used bio-fuel that is made from the anaerobic fermentation of biomass and used for cooking, heating, and can be used in natural gas vehicles[7-9].

Bio-fuels are most important in the present world's situation because currently, we rely on imported oil to produce gasoline for vehicles. In addition, 93% of our transportation fuels come from petroleum, unlike other energy sectors which can rely on several sources of energy[10]. Bio fuels are promising alternative for liquid type transportation fuel that historically comes from petroleum. Since ethanol is a clean-burning renewable fuel that works in today's vehicle fleet and delivers significant "Green House Gas (GHG)" reductions: an average of more than 66 percent GHG savings over fossil petrol in 2016,

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according to new certified data from European ethanol producers. Bio-fuel is expected to solve the global warming problem by decreasing the carbon dioxide levels in the atmosphere[11]. Bio-ethanol, as a clean, safe and renewable resource, is considered as a potential alternative to fossil fuels. However, it is mainly produced from either starch- or sugar-rich crops that may raise land competition between food production and biomass energy utilization, and can lead to deforestation[12].

The increasing demand of ethanol for various industrial purposes such as alternative source of energy, industrial solvents, cleansing agents and preservatives have necessitated increased production of this alcohol[13]. Ethanol production is usually accomplished by chemical synthesis of petrochemical substrates and microbial conversion of carbohydrates present in agricultural products[14]. Production of ethanol provides several advantages over gasoline like- utilization of abundant and inexpensive renewable resources, reduction in green house gas emissions and toxic substances, macro economic benefits for rural community, social aspect of sustainability and pertaining in national energy security. Some of the important sources for bio-fuels listed here all have potential, and when used in combination could go a long way toward meeting our energy needs in the coming decades[15-16].

Experimental Methods

Collection of Water hyacinth

Water hyacinth [*E. crassipes]*, approximately 50 Kg of the fresh plant was collected from Fewa Lake located in Pokhara-Lekhanath metropolitan city of Kaski, Nepal. The collected Water hyacinth was washed with tap water to remove clay and unwanted materials. Leaves and stalks together with roots of the plant were dried under sunlight for 15 days. The roots of the plant were removed and the remaining parts of the plant were cut into fine pieces. The leaves and petioles were first cut into particle size of about 2-3 cm and then dried. The dried pieces of Water hyacinth were ground in a mixer/grinder thereby reducing the particle size to few mm. The ground sample was then stored in air tight containers at room temperature. The sample thus prepared was subjected to further processing in the P.N. Campus Chemistry laboratory.

Apparatus and Chemicals used

Weighing balance (Systronics) ,pH meter (Systronics)Thermometer (range 0^oC to 100^oC), Burner and Oven, Grinding mill, Temperature controlled water bath, Rubber cork, Connecting tubes.

Sodium hydroxide (NaOH), Sulfuric acid (H_2SO_4), Urea (NH_2CONH_2), Yeast, Calcium chloride ($CaCl_2$), Magnesium sulphate ($MgSO_4$), Fumeric acid (COOH-CH=CHCOOH).

Pretreatment

Pretreatment with Acid

Pretreatment was carried out in Erlenmeyer flasks (250 ml) by mixing 3g of the dried water hyacinth with different acids i.e. HCl/H_2SO_4 /HCOOH(2% v/v) . The mixture was autoclaved at 121 ^oC, 15 lbs for 15 min and further cooled down to room temperature. The hydrolysate was filtered using Whatman filter paper No. 1 to remove the unhydrolysed material. The filtrate was collected and analyzed for the reducing sugar content by using DNS test.

Pretreatment with Alkali

Pretreatment was carried out in Erlenmeyer flasks (250 ml) by mixing 30g of the dried water hyacinth with sodium hydroxide i.e. NaOH (3%v/v). The mixture was autoclaved at 90 °C, 15 lbs for 15 min and further cooled down to room temperature. The hydrolysate was filtered using Whatman filter paper No. 1 to remove the unhydrolysed material. The filtrate was collected and analyzed for the reducing sugar content by using DNS test (Sun, Y., et al., 2002, Zhang, Q., et al., 2008).).

Pretreatment with H_2SO_4 at different Concentrations

Pre-treatment was carried out in Erlenmeyer flasks (250 ml) by mixing 30g of the dried water hyacinth with H_2SO_4 treatment at different concentrations ie. 1%, 2%, 3% and 4% (v/v) to obtain a concentration of H_2SO_4 that yielded maximum sugar. The mixture was autoclaved at 121 °C, 15 lbs for 15 min and further cooled down to room temperature. The hydrolysate was filtered using Whatman filter paper No. 1 to remove the unhydrolysed material. The filtrate was collected and analyzed for the reducing sugar content by using DNS test.

Enzymatic Hydrolysis

The solid residue after pretreatment was collected by filtration and washed extensively with distilled water until neutral pH. Subsequently, this pretreated water hyacinth was dried in the oven at 70^oC to maintain a constant weight to be used as the substrate for enzymatic hydrolysis. Cellulase dosage, hydrolysis temperature and time were selected as three factors for single factor experiment. The reducing sugars in hydrolysates were detected to determine an optimum enzymatic hydrolysis process. After that the mixture was autoclaved at 121^oC for 20 min, and 0.05g cellulase with 0.05g $CaCl_2$ were added to the pretreated sample to hydrolyze. Meanwhile, yeast inoculum was added into pretreated sample according to the experimental design along with 2.0 g/L yeast extract, 0.2 g/L $(NH_4)_2HPO_4$ and 0.02 g/L $MgSO_4$.

Saccharification

For better yield of reducing sugars, the hydrolysate was exposed to enzymatic saccharification. It was carried out in reaction mixture containing treated biomass, Mandel media and enzymes cellulase (103.75 U/g) and xylanase (650.18 U/g) dissolved in citrate buffer. The Mandel's medium was prepared using the following composition (g/L) 10.0 g; urea, 0.3; peptone, 0.75; yeast extract, 0.25; $(NH_4)2SO_4$, 1.4; KH_2PO_4, 2.0; $CaCl_2$, 0.3; $MgSO_4.7H_2O$, 0.3 and trace elements (mg/l): $FeSO_4.7H_2O$, 5; $MnSO_4$. $4H_2O$, 1.6; $ZnSO_4.7H_2O$, 1.4 and $CoCl_2.6H_2O$, 20.0 (Mandels et al., 1976). The reaction mixture was incubated on a rotatory shaker adjusted to 50^oC and 75 rpm. The samples were withdrawn at intervals of 12 h up to 60 h for reducing sugar and xylose content determination. After complete saccharification, the reaction mixture was heated slightly for deactivation of the enzymes. Finally, the saccharified biomass was filtered using filter paper and the hydrolysate was subjected to fermentation.

Fermentation

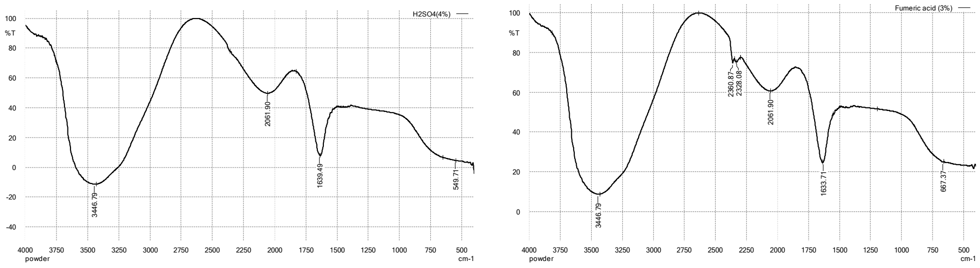
For fermentation, the yeast Pichia stipitis, Candida shehatae and Saccharomyces cerevisiae were used. The fully cultured yeast was transferred into autoclaved broth medium.Broth medium was prepared with the following composition (g/L) 50 mL: D-xylose, 50; Glucose, 5; Yeast extract, 3; Malt extract, 3; Peptone, 5; pH, 5.0. They were cultured for about 20 h in shaker incubator. The broths were then centrifuged at high speed for 10-15 min. The autoclaved yeast was added to sterilized hydrolysate obtained after saccharification. Samples from each flask were collected and estimated for yield of ethanol at a time interval of 1 h, 2 h and thereafter at a regular interval of 2 h up to when the yield of ethanol became almost stagnant. Determination of different sugar contents was done by DNS test (Nigam JN, 2002).

Results and discussion

In the present study, bio-fuel ethanol was obtained by the saccharification and fermentation of Water hyacinth which is abundantly and cheaply available renewable cellulosic substrate. Based on our experimental findings, the whole plant of Water hyacinth possesses high cellulose content. Therefore, full use of the whole plant was done to extract bio-fuel. Bio-fuel production from cellulosic materials greatly depends on the disruption of its complex lignocellulosic structure. Hence a suitable and effective pretreatment method should be selected for different cellulosic substrates. Various kinds of pretreatment methods, including acid, alkali, with different concentration were performed in our experiments. However, acid pretreatment was the most effective method for bio-ethanol production from water hyacinth compared to the other pretreatment methods in this study. The possible mechanism involves dissolution of hemicellulose by loosening of the structure of raw material, which makes acid pretreatment an important method for the production of reducing sugars.

FTIR Analysis

The common used region for infrared absorption spectroscopy is 4000 ~ 400 cm^{-1} because the absorption radiation of most organic compounds and inorganic ions is within this region.



(a) (b)

Figure 1: *FTIR of the sample ethanol pretreated with (a) 4%* H_2SO_4 *(b) 3% Fumeric acid (NAST, Kathmandu)*

From the abbreviated table of group frequencies for organic compound (wade, Jr., L.G. Organic chemistry,5th ed. pearson education lnc,2013),the frequency range between 3200-3600 cm^{-1} is found to be the hydrogen bonded alcohols, phenols whose intensity lies to be variable. From the graph, the frequency 3446.79 cm^{-1} lies in between the range 3250-3500, which confirms the presence of hydrogen bonded alcohols, phenols. Also the frequency 1639.49 cm^{-1} shows that the presence of alkenes whose intensity is variable and lies within the range (1610-1680) cm^{-1}.

Conclusions

As a conventional invasive weed, water hyacinth proved its feasibility for bio-ethanol energy production. Lignocellulose-to-ethanol bioconversion holds great potential as the substrate is abundant and relatively of low cost. However, the integration of low cost pretreatments with advanced ethanol-producing microorganisms may play a crucial role in lowering the cost of biomass bioconversion processes. Sulphuric acid gave best results for the yield of sugars as compared to other.

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