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Study of Enzymatic Saccharification and Fermentation of Hydrolyzed Mango Wood

to Produce Biofuel for Electrochemical Renewable Energy in Fuel Cell

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Abstract

Glucose based and alcohol based biofuels were produced from pretreated mango tree wood floor by enzymatic saccharification and fermentation. Two major components of plants, starch and cellulose, are both made up of sugars, the cellulose part of a plant is broken down to sugars and subsequently converted to ethanol. The acidic or alkaline pretreatment is found to be very effective to break complex organic polymer of cellulose and hemi-cellulose into simple monomer and removing lignin which is unleachable. The quantity of fuel produced was estimated as the amount oxygen required for its full combustion (COD mg/l). The total content of glucose based fuel formed by enzymatic saccharification is found to be less than the alcoholic based fuel formed by subsequent fermentation. The fuels are converted to pure electrical energy by electrochemical oxidation on a electrolytically developed MnO_2 . This inexpensive electrode outperformed the well known Pt electrocatalytic material and delivered a current density in the range of 5-12 mA/cm².

Keywords : Enzymatic Saccharification; Fermentation; Acid hydrolysis; woody biomass; Biofuel ; Fuel cell ; Electro catalytic material ;

1.Introduction

With fast urbanization, automation, high tech cities, the demand for energy is increasing and the resource of fossil fuel and oil reserve are diminishing. The use of fossil fuels is also creating a huge environmental pollution and greenhouse gas effects on earth. The need of hour is to find more resource of alternative energy: green energy with little or no pollution. Development of fuel cell using the derived fuel from the biomass [1,2] will be the most useful clean energy synthesis from the alternative fuel to meet the global growing demand of pollution free renewable energy. Bioethanol is one of the extensively researched derived fuels [3-7] from woody biomass or agriculture waste that can be fed into the Direct Ethyl alcohol fuel cell. On earth, there is a vast resource of agricultural biomass and forest waste which have solar energy stored that is unutilized. Bio fuels can be generated using theses wastes . The author and his group has cultivated these resources to generate fuel. Hydrogen from algae [1], bioethanol from rice husk [4], bio oil from wood floor [2,3]. Woody biomass contains about 36% cellulose and 12% hemicellulose, with some quantities of lignin (16%) and ash (20%) which complicates their use as lignocellulosic feedstock for conversion to ethanol. Acid hydrolysis of bio mass depolymerizes the fibrous polymeric structures of cellulose and hemicellulose to sugar monomers, consisting of xylose, glucose, arabinose, organic acids [5-7] and lignin which is mostly insoluble in mineral acid [7]. Hemi-cellulose is readily hydrolyzed by dilute acids under moderate conditions, but much more extreme conditions are needed for hydrolysis of cellulose. Both acid and alkali hydrolysis processes have been used at elevated temperature and pressure to depolymerize cellulose and hemicelluloses [7-9]. The acid concentration in the dilute acidhydrolysis process is in the range of 2–5% [7] and in concentrated acid-hydrolysis process, it is in the range of 10– 30%. Strong acid allows complete breakdown of components of biomass, but can result in the production of furfural, an inhibitory byproduct. Lime pretreatment has been studied on various biomass substrates such as corn, wood and municipal wastes [10–13]. Lime offers certain advantages such as: inexpensive, safe to handle and can be recovered easily.

Enzymatic saccharification of the hydrolyzed product will produce sugar monomers that itself can be used as fuel. Further treatment of the product by microorganisms like E-Coli or yeast can act upon sugar monomers to ferment them to ethanol [5-7]. Yield of ethanol production depends on time of incubation, temperature and other interfering organisms, which favor organic acids formation, inhibiting efficiency of yeast [7].

The present work aims at producing such biofuels using pretreated wood floor from mango tree. India has a vast resource of mango tree as mango fruits are heavily grown in this soil and environment. Huge amount of tree waste as tweak, stem, trunk are accumulated as agricultural waste. Two types of fuels were generated, sugar based fuel after Enzymatic saccharification of the hydrolyzed product and alcohol based fuel after fermentation of sugar monomers.

Biofuels so produced can be chemically oxidized by combustion to heat energy which in turn is used to rotate the turbine to generate electricity. The energy conversion by this step (limited by Carnot cycle, 2nd law of thermodynamics) is about 25%,. The products of combustion are polluting gases. Whereas in fuel cell, the same biofuel can be electrochemically oxidized in the anode chamber, the electron released at anode goes to external load, fuels such as H₂ is ionized as H^+ , move to cathode chamber through membrane, unite with O_2 from the atmospheric air to pure H_2O . Since the electrochemical oxidation of the fuel directly produces electron or electrical energy, the energy efficiency of conversion is over 80%. Electrochemical oxidation of the biofuel in a fuel cell can deliver clean electrical energy with no pollution and the maximum energy conversion efficiency. The cell potential and current developed from the fuel cell can be used as renewable battery with no recharging required. But an electrocatalytic material is required over which the electrochemical oxidation can take place, releasing electron at a fast rate. Platinum is a good electro-catalytic material for many fuel cell application but due to its high cost, its application is limited to laboratory scale fuel cell and development of non platinum based, low cost, electrodes is a thrust area of research. Paul [4] had developed anodized Aluminum of nano pores, filled up by Pt, Pt-Ru and Pt-Ru-C by electrodeposition. So the quantity of expensive Pt based material used is the minimum. The fuel cell using this electrode (for alcoholic fuel cell), produced a high current of 15-25 mA/cm² and high power density of 10-15 mW/cm². Several oxides and metal alloys have been found to show good electro catalytic properties. Materials such as CoTi [14], PbO₂ [15], TiO₂ [16] and β -MnO₂ [17] show very encouraging results for application in fuel cell electrodes. Paul et al[18-20] produced MnO₂ and MnO₂-nano carbon electrodes by electro synthesis to produce clean energy through a fuel cell, using glucose as fuel. Nano composite metal oxides, ZnO-Al₂O₃ produced by paul et al [21] performed as very good electro catalytic energy material as glucose fuel cell electrode. There are a few works on Ni based alloy electrodes development for fuel cell. A small addition of Al to Ni produced an improved electrode property as cathode in molten carbonate fuel cells (MCFC) [22]. A wide compositional range of Ni–Pd alloy catalysts were prepared by Suresh Kumar for application as anode materials for methanol oxidative fuel cells in alkaline conditions [23].

In the present investigation, electrocatalytic MnO_2 electrode was produced by electrodeposition in $MnSO_4$ bath under controlled parameters of potential, current and electrolyte chemistry. The performance of electrochemical oxidation of above two fuels over this electrode was studied by cyclic voltammetry and polarization studies. The image of the electrode surface was scanned under scanning electron microscope to reveal the morphology of MnO_2 structure.

2.Experimental Methods

2.1 Biofuel Production

The initial raw material is wood of mango tree. Reduction of particle size of mango wood to uniform size of 1-2 mm is done to increase surface/volume ratio. The woody agriculture waste contains some alkali or alkaline earth metal, that may be harmful for electrode material or machinery parts. They are removed by soaking in 0.25-wt % Nitric acid at 80° c for one hour. The physically absorbed moisture in it, is removed by drying at 160° c for one hour.

2.1.1 Pretreatment

Pretreatment of cellulose opens up the structure and removes secondary bonds between glucose chains. Treatment with alkali can removes lignin, thus promoting hydrolysis and improving the glucose recovery from cellulose

2.1.1.1 Acid hydrolysis

Acid pretreatment is used to solubilize the hemicellulose and make the cellulose more accessible to enzymes. Initially, the biomass of 20g and the aqueous sulfuric acid solution of 3%, 5%, and 8% were loaded into a 500-ml stainless-steel container. The stock was heated in an autoclave for one hour at 100° c. After removing the cap of the container, the cellulosic hydrolysate was quantitatively separated by filtration and neutralized by adding NaOH. PH was recorded as 6.9-7. Three samples were made , coded as AE1,AE2 and AE3 (Table-I & II).

2.1.1.2 Lime treatment

Lime pretreatment increases cellulose digestibility and is very effective in lignin removal, exhibiting minor cellulose and hemicellulose solubilization. 20g of the mango wood and aqueous solution of 0.13g, 0.15g, and 0.17g CaO /gm of mango wood were loaded into a500-ml stainless-steel container, The stock was heated in an autoclave for one hour at 100° c. After removing the cap from the container, the cellulosic hydrolysate was quantitatively separated from the pretreated solids by filtration. Three samples were made , coded as. LE1,LE2 and LE3 (Table-I & II).

2.1.2 Enzymatic saccharification to form glucose molecule

Enzymatic hydrolysis of such cellulosic material by cellulase enzymes is the most promising approach to get high product yields. 100mg of a commercial enzyme was dissolved to 200ml pretreated mango wood solution, maintained at 60° c and shaken gently for 4 hours to achieve the maximum sugar recovery.

2.1.3 Fermentation to Alcohol by Yeast

0.1g tryptone and 5g yeast were dissolved to 100ml extract sugar solution .The mixed solution was kept in seal packet in vessel. The fermentation was done at 30° C for two days.

2.1.4 Determination of fuel content

The fuel content in the glucose or alcoholic based fuel derived from woody biomass was estimated by determining the Chemical Oxygen Demand (COD) of the fuel in UV vis spectrophotometer at 600nm wave length.

2.2 Electrode preparation

A sample of 304SS (3X3 cm2) was polished by 2/0 and 3/0 emery paper and was cleaned by acetone, water and dried in hot air. A solution having 0.34M MnSO4 and 0.6M H_2SO_4 was prepared. Electro deposition was done over 304SS in a galvanostatic circuit of current density 150 mA/cm2 at 80° C. The coated sample was rinsed by acetone, Ethanol. and dried in hot air at 70°C.

2.3 Electrochemical Characterization

2.3.1 Cyclic Voltammetry:

Cyclic Voltammetry of electroplated sample was studied in a computer controlled electrochemical system machine (DY 2300 Potentiostat) with three electrode system where graphite was taken as counter electrode and saturated calomel electrode as reference electrode. The electrodeposited MnO_2 sample was taken as working electrode. Potential was scanned from -1V SCE to 1V vs SCE in the biofuels so produced. From the curve, the maximum current which is generated through electrochemical oxidation is determined.

2.3.2 Polarization test:

Potentiodynamic polarization test of electrodeposited MnO_2 sample was also performed in a cell of 3 electrode system in computer controlled Gamry Machine with a scan rate of 1mV/sec and polarization data are plotted in graphics.

3.RESULTS AND DISCUSSIONS

The present works discusses about converting, acid and alkali pretreated, woody biomass to glucose fuel by enzymatic saccharification and subsequently to fuel alcohol by fermentation and the electrochemical oxidation of the produced fuel to pure electrical energy on a electrolytically produced MnO_2 anode. The results of the following effects will be discussed in the subsequent sections.

3.1 Effect of Pretreatment

The goal of the pretreatment process is to remove lignin and hemicellulose, reduce the crystallinity of cellulose, and increase the porosity of the lignocellulosic materials. Pretreatment

must meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by hydrolysis, (2) avoid the degradation or loss of carbohydrate, (3) avoid the formation of byproducts that are inhibitory to the subsequent hydrolysis and fermentation processes, and (4) be cost-effective. Fig.1 schematically illustrates the effect of acidic or alkaline pretreatment to break complex organic polymer of cellulose and hemi-cellulose into simple monomer and removing lignin which is unleachable.

3.2 Glucose and Alcoholic fuels production from mango tree woody biomass

Fig 2 exhibits samples of glucose based fuel derived from mango tree wood floor by enzymatic saccharification. The wood floor was pretreated by acid or alkali. Fig 3 shows similar sample photos when the sugar fuel was subsequently fermentated to produce alcoholic fuel. It is to be observed that the alcoholic fuel is of much darker in color than those of glucose fuel samples, indicating higher percentage of fuel content.

The quantity of fuel converted by enzymatic saccharification or fermentation was determined by determining the Chemical Oxygen Demand (COD) of the fuel in UV vis spectrophotometer. The COD indicates the quantity of oxygen needed stoichiometrically to completely oxidize the fuel produced. Hence, the higher the fuel produced , more will be value of COD. Fig4 illustrates the fuel content in glucose fuel and alcoholic fuel , expressed as COD from mango wood floor pretreated with acid , subsequently converted to sugar by enzymatic saccharification and then alcohol by fermentation. The first thing to be noted is that the amount of alcoholic fuel (red shed) conversion is much higher than that for glucose based fuel (blue shed). Two major components of plants, starch and cellulose, are both made up of sugars, the cellulose part of a plant is broken down to sugars and subsequently converted to ethanol.

One mole of glucose is converted into two moles of ethanol and two moles of carbon dioxide:

$$C_{12}H_{22}O_{11}+H_2O + \text{invertase} \rightarrow 2 C_6H_{12}O_6 \qquad (1)$$

$$C_6H_{12}O_6 + Zymase \rightarrow 2C_2H_5OH + 2CO_2 \qquad (2)$$

The second stage is the fermentation process to convert glucose into ethanol and CO_2 . The yeasts mainly metabolizes the glucose and fructose to form alcohol.

It is also seen that increase in acid concentration (sample AE1 to AE3) increases the fuel content. Acid pretreatment is effective to solubilize the cellulose, hemicelluloses and make them more accessible to enzymes. Similar results are shown in fig.5 when lime is used as pretreatment instead of acid. It is also seen here that a good amount of fuel either in sugar form or alcoholic have been converted. Lime pretreatment is one of the promising technologies for grassy feed stocks. Lime pretreatment increases cellulose digestibility and are very effective in lignin removal, exhibiting minor cellulose and hemicellulose solubilization as compared to acid processes. They are more effective on agricultural residues than on woody materials. Thus both the acidic and alkaline are found to be very effective in high conversion of woody biomass into fuels : sugar or alcoholic. There is about 50-60% conversion to fuels.

3.3 Electrochemical Oxidation of derived Sugar Fuel to Energy

The current produced from the oxidation of the sugar fuel so produced on the developed MnO_2 fuel cell electrode is studied by cyclic voltammetry curve. The performance of the electrode is compared with that of Pt electrode, commonly known electrocatalytic material. Fig 6 depicts a comparison of cyclic voltammetry curves of Pt and MnO_2 for three acid pretreatment samples of sugar fuel. It is seen that for both the electrodes the cyclic voltammetry curves show an increase in current with potential scan, with a current max at a certain potential where the rate of oxidation or electron transfer is the highest. It is interesting to find that the fuel produced by enzymatic saccharification of pretreated mango tree wood gives rise to electrical current on anode. The maximum current is higher on MnO_2 electrode than that on Pt. So MnO_2 has come out as better electrocatalytic material than Pt for electro oxidation of this sugar based biofuel. Similar curves for sugar based fuel with alkali pretreatment are shown in fig 7. As far as inexpensive MnO_2 electrode is concerned, the maximum current produced is more for the fuel pretreated with acid than the fuel pretreated with alkali.

3.4 Electrochemical Oxidation of derived Alcoholic Fuel to Energy

Similar curves of cyclic voltammetry for alcoholic fuel produced by fermentation are shown in fig 8 and 9 for wood floor pretreated with acid and alkali respectively. It is seen that the electrical current produced on MnO_2 electrode is very high in the range of 0.004 to 0.05 A/cm². So a fuel cell fabricated with 100 cm² surface area of MnO_2 electrode can deliver a current 400 mA to 5 A using a biofuel derived from waste such as mango tree wood floor. With such encouraging results, similar investigation with other waste product of woody biomass is expected to produce good renewable energy resource.

3.5 Polarization study

Electrochemical oxidation on electrode polarizes the anode due to activation polarization and concentration polarization. Hence there is overvoltage as given by the following equation.

Ecell, net = Ecell -
$$(\eta_{Act} + \eta_{Conc} + \eta_R)$$
 (3)

Where

 η_{Act} = Activation overvoltage,

 $\eta_{conc} = Concentration overvoltage,$

 η_R = overvoltage due to electrolyte resistance

If the over voltage is more the cell potential in the Fuel cell is less and the current given by following equation is also less .

$$I = Ecell, net / R_{Load}$$
(4)

Thus, it is necessary to perform potentiodynamic polarization of the electrode in the different biofuel produced as discussed above. The potentiodynamic polarization on Pt and MnO₂ electrode are shown in figure 10 and 11 respectively in glucose based biofuel with acid treatment and lime treatment. If the curve shift to the right, it means that the overvoltage is less and the current is high and the electrode has high electro catalytic property for the oxidation of the fuel. It is seen that while on Pt electrode there is not much noticeable difference of the curves, on MnO₂ electrode there is huge shift in the curve to the right for AE3 and AE1 treatments. It indicates that the overvoltage on MnO₂ electrode is less and the fuel cell fabricated with this electrode with the sugar fuel so produced is likely to give a good steady state current. Figure nos. 12 and 13 illustrate similar curves on Pt and MnO₂ respectively in alcoholic fuel produced from wood floor. The results here are also encouraging with the curves for MnO₂ electrode shifting to the right. This indicates lower overvoltage and higher current as illustrated in equations 3 and 4 above. Table-III shows the computed cell current on Pt and MnO_2 electrode substrate for electro oxidation of the sugar and alcohol based fuels produced from wood floor. It is seen that the developed material MnO₂, which is inexpensive compared to Pt, outperforms the Pt electrode, giving much more current on electro oxidation of either of the two fuels for any pretreatment.

3.6 SEM and FESEM images of MnO₂ Electrode

The morphology of MnO_2 electrode under SEM (Scanning Electron Microscope) and EFSEM (Field Emission Scanning Electron Microscope) is shown figs 14a and 14b respectively. The morphology of the surface clearly indicates number of fines globules distributed. There is a recess in between globules that enhances the effective area in 3D several times more than the geometrical area in2D. Hence more surface is available for electrochemical oxidation of the biofuel on MnO_2 which gives an increase in current. compared to Pt.. This accounts for higher current obtained on MnO_2 surface than on Pt for electrochemical oxidation of glucose based and alcohol based biofuel as obtained in cyclic voltammetry and polarization studies.

4.Conclusion

Mango tree wood floor can be converted to biofuel either glucose or alcohol based by pretreatment with acid or alkali and then enzymatic saccharification and fermentation. It is seen that acid pretreatment is better than the lime pretreatment in term of percentage (%) of fuel extracted. The fuel conversion expressed as COD for complete combustion is much higher after fermentation than after enzymatic saccharification. Electrochemical oxidation studies of the fuel on Pt and the developed MnO_2 electrode show a good amount of current is generated , indicating stored energy of woody biomass can be converted to pure electrical energy. The performance of MnO_2 electrode as fuel cell electrode is much better than that of Pt.

Figures and Tables

Table- I

Amount of Sugar converted from wood expressed in Chemical Oxygen Demand (COD)

Sample Code	Pretreatment	Enzymatic Saccharification	COD mg/l
	Acidic at 100 C, 90 mins time H ₂ SO ₄ %		
AE1	3.0	Enzyme added: 5mg per gm of hydrolyzed product,	58
AE2	5.0		73
AE3	8.0	temperature 60C,	99
	Alkaline at 100C , 90 min CaO added per gm biomass	shaken for 4 hours	
LE1	0.13		29
LE2	0.15		39
LE3	0.17		78

Table-II

Amount of Alcohol converted from wood expressed in Chemical Oxygen Demand (COD)

Sample	Pretreatment	Enzymatic	Fermentation	COD mg/l
Code		Saccharification		
	Acidic at 100 C, 90 mins time			
	H_2SO_4 %			
AE1	3.0	Enzyme added:	Yeast added:	235
AE2	5.0	5mg per gm of hydrolyzed	5mg/gm of sugar	331
AE3	8.0	product,	produced	516
	Alkaline at 100C, 90 min	temperature 60C,	Temperature 25 C	
	CaO added per gm biomass	shaken for 4 hours	Time: 12 hours	
LE1	0.13			268
LE2	0.15			371
LE3	0.17			492

Table-III

Sample Code	Glucose Based Fuel		Alcohol Based Fuel	
	Pt	MnO ₂	Pt	MnO ₂
	I(mA/cm ²)	I(mA/cm ²)	I(mA/cm ²)	I(mA/cm ²)
AE1	1.3333	5.4225	1.2964	5.4310
AE2	1.2293	12.72339	1.32287	12.855
AE3	1.42012	5.6745	1.3200	5.2415
LE1	1.4698	5.8331	1.49267	5.7636
LE2	1.52323	5.79957	1.40087	5.6584
LE3	1.4363	6.9927	1.3327	11.5184

<u>Computed from polarization data the cell current I (mA/cm²) on Pt and MnO₂ electrode surface for electrochemical oxidation of the derived fuels from mango tree wood floor</u>



Fig.1 Schematically illustrates the effect of pretreatment by acid hydrolysis or alkaline lime treatment.



Fig.2 Samples of Glucose based fuel derived from pretreated mango wood floor



Fig.3 Samples of Alcoholic fuel derived from pretreated mango wood floor



Fig 4 Amount of fuel expressed as COD obtained as sugar and Alcohol from Mango tree wood floor by acid pretreatment



Fig 5 Amount of fuel expressed as COD obtained as sugar and Alcohol from Mango tree wood floor by alkali pretreatment



Fig 6 Comparison of cyclic voltammetry curves of Pt and MnO2 electrodes, for electro oxidation of sugar derived from Mango tree by acidic pretreatment and Enzymatic Saccharification



Fig 7 Comparison of cyclic voltammetry curves of Pt and MnO2 electrodes, for electro oxidation of sugar derived from Mango tree by alkaline pretreatment and Enzymatic Saccharification



Fig 8 Comparison of cyclic voltammetry curves of Pt and MnO2 electrodes, for electro oxidation of alcohol derived from Mango tree by acidic pretreatment, Enzymatic Saccharification and Fermentation



Fig 9 Comparison of cyclic voltammetry curves of Pt and MnO2 electrodes, for electro oxidation of alcohol derived from Mango tree by alkaline pretreatment, Enzymatic Saccharification and Fermentation



Fig 10 Comparison of Polarization curves of Pt and MnO2 electrodes, in sugar solution derived from Mango tree by acidic pretreatment and Enzymatic Saccharification



Fig 11 Comparison of Polarization curves of Pt and MnO2 electrodes, in sugar solution derived from Mango tree by alkali pretreatment and Enzymatic Saccharification



Fig 12 Comparison of Polarization curves of Pt and MnO2 electrodes, for electro oxidation of alcohol derived from Mango tree by acidic pretreatment, Enzymatic Saccharification and Fermentation



Fig 13 Comparison of Polarization curves of Pt and MnO2 electrodes, for electro oxidation of alcohol derived from Mango tree by alkaline pretreatment, Enzymatic Saccharification and Fermentation



(a)SEM

(b) FESEM

Fig 14 Images of MnO2 coating on 304 stainless steel plate under Electron Microscopy

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