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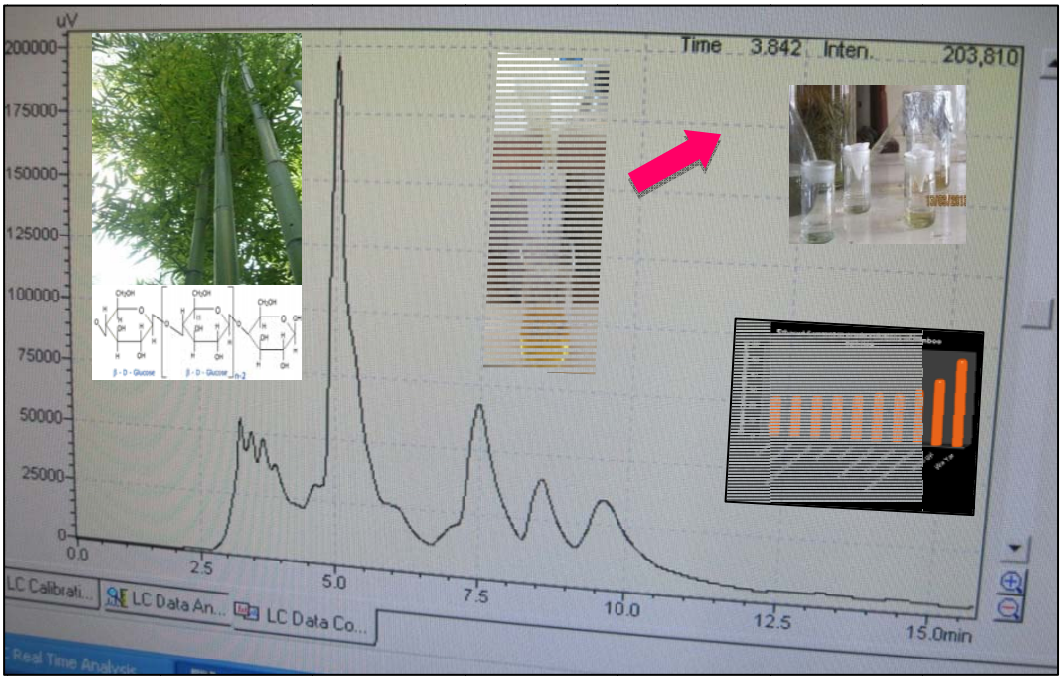
Preliminary Investigation of Ethanol Potential from Fermented Liquid Filtrates of Some Bamboo Species



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ဝါးမျိုးစိတ်အချို့၏ အချဉ်ဖောက်ရာမှရရှိလာသော ပျော်ရည်များမှ အီသနောပါဝင်နိုင်မှု အလားအလာကို ပဏာမစူးစမ်းလေ့လာခြင်း

သီတာချို၊ သုတေသနလက်ထောက်–၂
ခင်မေလွင်၊ သုတေသနအရာရှိ
ခိုင်ဝတ်မှုံ၊ တောအုပ်ကြီး

စာတမ်းအကျဉ်း

မြန်မာနိုင်ငံတွင်ဝါးမှ ဇီဝလောင်စာထုတ်ယူရရှိသော သုတေသနဆောင်ရွက်ချက်များလည်း မတွေ့ရှိရသေးပါ။ ဤသုတေသန ပြုချက်သည် ဝါးမှဇီဝအီသနော ထုတ်ယူနိုင်မှုကို လေ့လာသော ပထမခြေလှမ်းလည်းဖြစ်ပါသည်။ သို့ပါ၍ ဤသုတေသန ပြုချက်တွင် ဝါးမျိုးစိတ် (၁၀) မျိုးကို alkali သုံး၍ pretreatment ပြုလုပ်ပါသည်။ ထို့နောက် enzyme ဖြင့် hydrolysis ပြုလုပ်၍ yeast သုံးကာ အချဉ်ဖောက်ပါသည်။ အချဉ်ဖောက်ရာမှ ရရှိလာသော ဝါးမျိုးစိတ်အသီးသီး၏ ပျော်ရည်များတွင် အီသနော ပါဝင်မှုပြင်းအားကို သိရှိပြီး နှိုင်းယှဉ်နိုင်ရန် HPLC ဖြင့် ခါတ်ခွဲစမ်းသပ်မှု ပြုလုပ်ခဲ့ပါသည်။ဤသုတေသန ပြုချက်တွင် ဝါးမျိုးစိတ် အသီးသီး၏ cellulose ပါဝင်မှုကိုလည်း ခါတ်ခွဲစမ်းသပ်ခဲ့ပါသည်။ ၎င်းစမ်း သပ်ချက်အရ အီသနောထုတ်ရန်အတွက် လိုအပ်သော လောင်စာလက္ခဏာရှိသည့် cellulose fraction သည် ဝါးယားတွင် အခြားဝါးမျိုးစိတ် များထက် ပိုမိုကြွယ်ဝကြောင်း တွေ့ရှိရပါသည်။ HPLC ဖြင့်တိုင်းတာ ရရှိသော Chromatographic profile များကို Ethanol standard ဒြပ်ပေါင်း၏ ကွဲထွက်ချိန်နှင့် နိုင်းယှဉ်သောအခါ ဝါးမျိုးစိတ်(၁၀) မျိုး၏ အချဉ်ဖောက်ရာမှရရှိလာသော ပျော်ရည်များ မှ တိုင်းတာရရှိသော HPLC chromatogram များတွင်ပါဝင်သော ဒြပ်ပေါင်းများ၏ ကွဲထွက်ချိန်သည် Ethanol standard ဒြပ်ပေါင်း၏ ကွဲထွက်ချိန်နှင့် ကိုက်ညီနေကြောင်းတွေ့ရှိရပါသည်။ ထို့အပြင် HPLC ခါတ်ခွဲစမ်းသပ်မှု ရလဒ်အရ အချဉ်ဖောက်ရာမှ ရရှိလာသော ဝါးယား၏ ပျော် ရည်တွင် အီသနောပါဝင်မှု ပြင်းအားသည် အခြားသော အချဉ်ဖောက်ရာမှရရှိလာသော ဝါးမျိုးစိတ်များ၏ ပျော်ရည်များထက် သာလွန်ကြောင်း တွေ့ရှိရပါသည်။ သို့ပါ၍ ဝါးယား သည် alkali pretreatment နှင့် enzymatic hydrolysis ကိုအသုံးပြုကာ ဇီဝအီသနော ထုတ်ရန် အတွက် သင့်လျော်ဆုံးသော ဝါးမျိုးစိတ်ဖြစ်ပါသည်။ ထို့အပြင် ဤပဏာမ စူးစမ်းလေ့လာခြင်းသည် ဝါးမှဇီဝအီသနော ထုတ်ယူမည့် နောက်သုတေသနအတွက် တွန်းအား တစ်ခုလည်း ဖြစ်သည့်အပြင် သင့်လျော်သော guideline များလည်း ပေးနိုင်ပါလိမ့်မည်။

Preliminary Investigation of Ethanol Potential from Fermented Liquid Filtrates of Some Bamboo Species

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Abstract

No research about bamboo biofuel is available in Myanmar. This research represents an initial stage in the study of bamboo bioethanol. Thus in this research, (10) bamboo species were therefore pretreated by alkali. Then, enzymatic hydrolysis, and yeast based fermentation were also conducted. All liquid filtrates that obtained from fermentation were determined by Reversed-phase high-performance liquid chromatography (RP-HPLC) to compare concentration of ethanol content of each bamboo species. The composition of cellulose in each bamboo species was invested in this study. It was found that Wa ya was more rich in cellulose fraction which is desirable fuel characteristic for ethanol production. By comparing the chromatographic profiles with the retention time of reference ethanol standard, identification of HPLC chromatograms of liquid filtrates of (10) bamboo species that obtained from fermentation were matched with the retention time of ethanol standard. According to the results of HPLC analysis, the liquid filtrate of Wa ya that obtained from fermentation has highest ethanol concentration than that of other bamboo species. Thus Wa ya is the most suitable bamboo species for exploring bioethanol with alkali pretreatment and enzymatic hydrolysis. In addition this preliminary investigation serves as a force for further research in the application of bamboo for bioethanol production and may provide guidelines.

Keywords: Enzymatic hydrolysis, RP-HPLC, Biofuel, Concentration, Investigation

Contents

List of Tables and List of Figures

Preliminary Investigation of Ethanol Potential from Fermented Liquid Filtrates of Some Bamboo Species

1. Introduction

Both bioethanol and biodiesel are termed liquid biofuel. Bioethanol is the majority (~90%) of the biofuel utilized all over the world. It is considered as an ECO-friendly fuel produced from monomeric simple sugar and starch in plants. It is obtained by fermentation from sugars, starches or cellulosic biomass. Lignocellulosic biomass mostly contains 50%-80% (dry basis) carbohydrates that are polymers of 5C (Xylose) and 6C (Glucose) sugar units. Most carbohydrates can be processed either chemically or biologically to yield ethanol. Production of bioethanol from biomass can reduce not only the consumption of crude oil but also environmental pollution. Bioethanol mitigates global warming by recycling carbon dioxide from the atmosphere. Carbon dioxide is absorbed by the plants. Then Oxygen is released back into the atmosphere. Carbon is stored in a plant's cellulose, hemicellulose, starches, sugars, and oils. When bioethanol is combusted due to transportation, it is converted back to carbon dioxide. Ethanol is an oxygenated fuel that contains 35% oxygen, which reduces particulate and NO_x emissions from combustion. When burned, ethanol derived from fermentation produces no net increase in carbon dioxide in the atmosphere. It is an octane enhancing additive and removes free water which can plug fuel lines in cold climates (Lang et al., 2001 b).

Bamboo is mainly composed of the polymeric constituents of cellulose, hemi cellulose and lignin, which chemically and physically associated with each other to form complex structure. Conversions of biomass including bamboo, to ethanol consist of four steps: pretreatment, enzymatic hydrolysis, fermentation and distillation (ethanol purification). Pretreatment is needed to remove the lignin components and to promote the enzymatic digestibility of cellulosic components. In general, pretreatment with alkalis are more effective in lignin solubilization, but they cause less cellulose and hemicellulose hydrolysis and solubilization than acid processes. Alkali can swell cellulose, increase the internal surface of cellulose, and decrease the degree of polymerization and crystallinity, which consequently benefits lignin disruption (Taherzadeh and Karimi 2008). Enzymatic hydrolysis is used to break complex cellulose into monomeric simple sugars. Zheng Y, Pan ZL, Zhang RH, (2009) were also proposed that biomass pretreatment can remove lignin and hemicelluloses, which significantly enhance the hydrolysis of cellulose.

Now, bioethanol has been manufacturing from sugarcane, cassava, sorghum, maize, potato and sweet potato in Myanmar. The present status of ethanol production from sugarcane in Myanmar is large scale conducted by private sector. One of the private companies has installed an alcohol distillation unit, capacity of 11 million gallons anhydrous ethanol per annum and already produced 860,000 gallons of anhydrous ethanol from sugarcane. Private companies are planning cassava and sweet sorghum in large scale for ethanol production (IEEJ: May 2009). But

production of ethanol from bamboo has not been developed yet. Bamboo does not require nutrients and fertilizers that will cause damage to environment and animal habitat. It is fast growth, can easily grown and less affected by weather. It needs little silvicultural management and can prevent soil erosion. As bamboo possesses good characteristics, it is suitable to use as a raw material for bioethanol production. If bioethanol which is manufacture from bamboo is used as fuel in domestic production, Myanmar can get many benefits such as decrease dependence on petroleum based fuels from the abroad, reduce environmental pollution, create jobs in rural areas and etc. Moreover bamboo is the key biomass material for the balance of oxygen and carbon dioxide in the atmosphere. Its carbon dioxide storage per unit area of plantation is four times that of hard wood, and the release of oxygen is 35% higher than that of trees (Southern Metropolis Daily Mark 2012). In addition bamboo has great potential to be used as fuel ethanol production in some countries, such as U.S, Brazil, China, Taiwan, Iran and other countries. As bamboo resources are very abundant, it will be a potential as a bio-energy resource in the future of Myanmar.

No research about bamboo biofuel is available in Myanmar. Thus this preliminary investigation will serve as a force for further research in the application of bamboo for bioethanol production. In this research, (10) bamboo species were therefore pretreated by alkali. Then enzymatic hydrolysis, and yeast based fermentation were also conducted. All liquid filtrates that obtained from fermentation were determined by Reversed-phase high-performance liquid chromatography (RP-HPLC) to compare concentration of ethanol content of each bamboo species. Present research is aimed towards finding one of the bamboo species with maximum ethanol concentration, for the purpose of further research, based on the results of HPLC analyses. In addition, Reversed-phase high-performance liquid chromatography (RP-HPLC) is used as an analytical technique in many chemical laboratories and it can produce higher resolution in the separation of organic compounds.

1.1 Objectives

- To investigate the bamboo species with maximum concentration of ethanol for the purpose of further research.
- To provide a new sustainable energy sources alternative to petroleum-based fuels with the regard of reducing the environmental pollution.
- To share effective ethanol production method to rural people, who are living in bamboo forests vicinity, in order to the improvement of socio economic in rural areas.

1.2 Literature Review

The first generation biofuels are produced from sugarcane and starch rich feed stocks such as corn, potato, etc. But Second-generation biofuels from lignocellulosic materials are not

in competition with food sources and animal feed. Being abundant and outside the human food chain makes cellulosic materials relatively inexpensive feed stocks for ethanol production (P.C. Badger, 2002). Bamboo is the key biomass material for the balance of oxygen and carbon dioxide in the atmosphere. Its carbon dioxide storage per unit area of plantation is four times that of hard wood, and the release of oxygen is 35% higher than that of trees (Southern Metropolis Daily Mark 2012).

Bamboo resources are rich in Myanmar. It belongs to the Graminae family. Myanmar has genera 17 and more than 90 bamboo species which is an important forest resource that occupies almost 50% of the county's land area (U Myint Swe, 2002).The largest commercial applications include shoot production for food, clums for material uses such as construction, industry, transportation, agricultural and as a raw material for pulping. Its uses may be more than 1,500 items (Khin May Lwin, 2006). Its cell wall is composed of the polymeric constituents; cellulose, hemicellulose and lignin. Thus it is sometimes called lignocellulosic materials. Cellulose is the principle component of lignocellulosic biomass and its concentration ranges from 40 to 50% of dry weight. Cellulose is composed of β-D-glucose units linked by β (1 → 4) glycosidic bonds. It is a homopolysaccharide composed of linear, long chains of glucose molecules but have a different structural configuration. The structure of cellulose molecule is shown in figure.

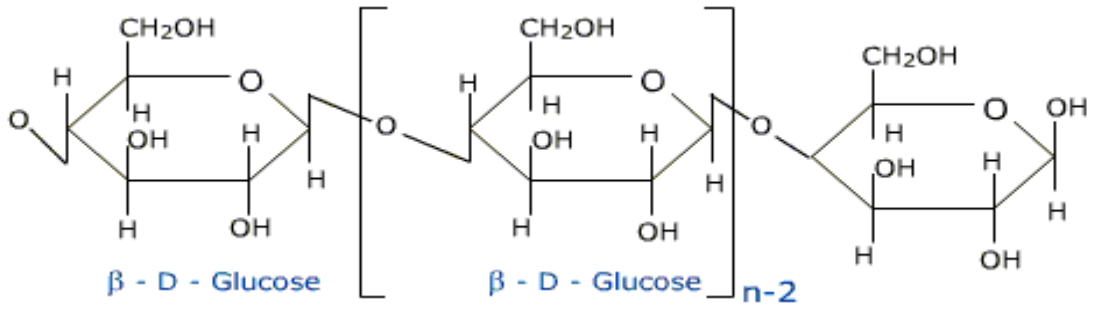


Figure (1) Structure of Cellulose Molecule

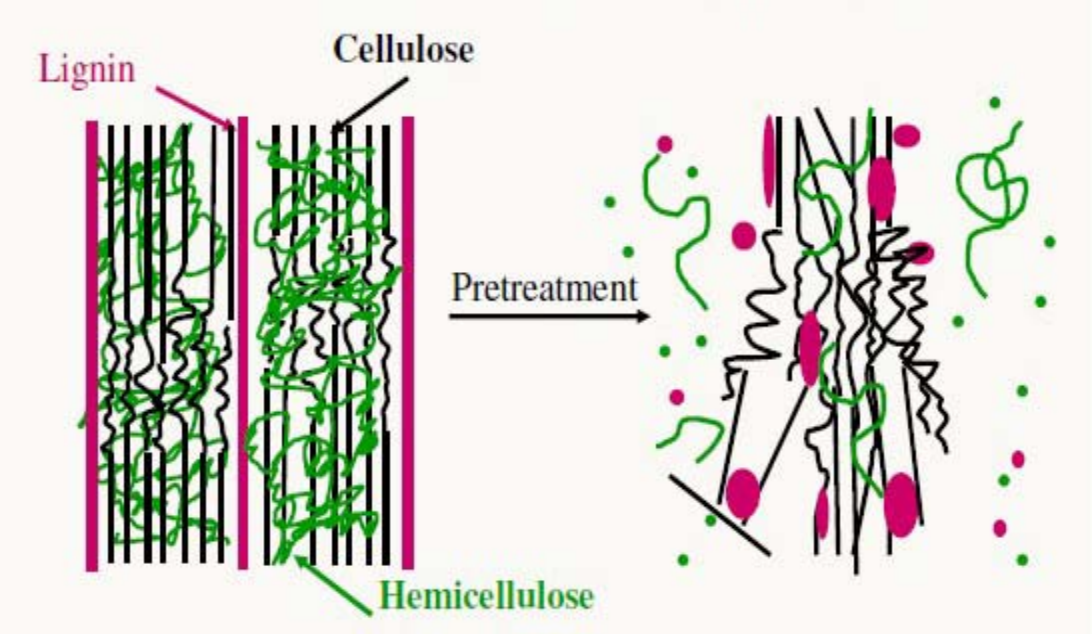
The degree of polymerization and crystallinity of cellulose varies from species to species and this is shown to have a significant impact on hydrolytic process (acidic and enzymatic) (Zhang et al., 2004).

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 (P.C. Badger, 2002). Its concentration in lignocellulosic biomass is 25 to 35% and it is easily hydrolysable to fermentable sugars (Saha et al., 2007).

Lignin is the third major component of lignocellulosic biomass and its concentration ranges for 20 to 35%. It is a complex polymer of phenyl propane (*p*-coumaryl, coniferyl and

sinapyl alcohol). Lignin acts as cementing agent and an impermeable barrier for enzymatic attack (Howard et al., 2003). Lignin provides plants with the structural support and impermeability they need as well as resistance against microbial attack and oxidative stress. These properties of lignin may be attributed to its amorphous nature, water insolubility and optical inactivity. The later properties also make it tough to degrade it (Fengel and Wegener, 1984).

Production of ethanol from lignocellulosis biomass, including bamboo consists of three major processes, such as pretreatment, hydrolysis, and fermentation. The pretreatment process will break the lignin-hemicellulose-pectin stringent seals around cellulose. Moreover, Zheng Y, Pan ZL, Zhang RH. (2009) found that pretreatment can remove lignin and hemicelluloses, which significantly enhance the hydrolysis of cellulose. It is required to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic structural and chemical composition to facilitate rapid and efficient hydrolysis of carbohydrates to fermentable sugars. As shown in figure (2) the simplified impact of pretreatment on biomass, including bamboo (Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M R.2005).



Figure(2). Simplified impact of pretreatment on biomass modified from Mosier et al.

Based on the application and type of pretreatment catalyst, pretreatment techniques have been divided into physical, physiochemical, chemical, and biological pretreatments. Physical pretreatments such as pyrolysis, uncatalyzed steam explosion, mechanical size reduction, microwave oven and high energy radiation do not use chemical agents. Physiochemical pretreatments typically include steam explosion, liquid hot water (LHW) and steam explosion, and CO_2 explosion. Chemical pretreatments, the most studied pretreatment techniques including catalyzed steam-explosion, acid, alkaline, ammonia fiber/freeze explosion, organosolv, pH-

controlled liquid hot water, and ionic liquids pretreatments. Biological pretreatment employs wood degrading microorganisms such as white-, brown-, soft-rot fungi, and bacteria (Yi Zheng[1], Zhongli Pan[1,2], Ruihong Zhang[1].2009).

Hydrolysis converts the polysaccharides into fermentable monomeric sugars. There are three different processes, namely, acid hydrolysis, enzymatic hydrolysis and thermochemical. The most common is acid hydrolysis. In acid hydrolysis, sulfuric acid, hydrochloric acid, hydrofluoric acid and nitric acid (mineral acids) are widely used for the hydrolysis of lignocellulosic biomass (Ladisch et al., 1983; Wright et al., 1988). However, sulfuric acid is most commonly used since it is usually the least expensive.

Enzymes are naturally occurring plant proteins that cause certain chemical reactions to occur. The enzymatic hydrolysis reaction is carried out by means of enzymes that act as catalysts to break the glycosidic bonds. For enzymatic processes to be effective, some kind of pretreatment process is thus needed to break the crystalline structure of the lignocellulose and remove the lignin to expose the cellulose and hemicellulose molecules. Depending on the biomass material, either physical or chemical pretreatment methods may be used (P.C. Badger, 2002).The factors affecting activity of cellulases include enzyme source and the concentration of enzyme. An effective concentration of enzyme for cellulose hydrolysis has been determined to be 10 to 60 FPU (filter paper units) per gram of dry cellulose or glucan- glucanase- β- D-glucosidase ratio of 1-75-2 IU (Kim et al., 2005). For thermochemical, the first system is actually a hybrid thermochemical and biological system. Biomass materials are first thermochemically gasified and the synthesis gas (a mixture of hydrogen and carbon oxides) bubbled through specially designed fermenters. A microorganism that is capable of converting the synthesis gas is introduced into the fermenters under specific process conditions to cause fermentation to ethanol. The second thermochemical ethanol production process does not use any microorganisms. In this process, biomass materials are first thermochemically gasified and the synthesis gas passed through a reactor containing catalysts, which cause the gas to be converted into ethanol (P.C. Badger, 2002).

Both bacteria and fungi can produce glucanases (cellulases) that hydrolyze of lignocellulosic materials. These microorganisms can be aerobic. Bacteria belonging to genera of *Clostridium, Cellulomonas, Bacillus,Thermomonospora, Ruminococcus, Bacteriodes, Erwinia,Acetovibrio, Microbispora*, and *Streptomyces* are known to produce Cellulase (Bisaria, 1998). Anaerobic bacterial species such as *Clostridium phytofermentans, Clostridium papyrosolvens* produces cellulases with high specific activity (Duff and Murray, 1996; Bisaria, 1998). Most commercial glucanases (cellulases) are produced by *Trichoderma ressei* and β-D-glucosidase is produced from *Aspergillus niger* (Kaur et al., 2007). Fungi known to produce cellulases include *Sclerotium rolfsii, Phanerochaete chrysosporium* and various species of *Trichoderma, Aspergillus, Schizophyllum and Penicillium* (Sternberg, 1976; Fan et al., 1987; Duff and Murray, 1996). Among the fungi, *Trichoderma* species have been extensively studied for cellulase production (Sternberg, 1976).

Once the sugars have been obtained from the cellulosic materials, they are fermented using yeast. The liquid resulting from the fermentation process contains ethanol and water; the water is removed through distillation, similar to the corn-based ethanol process. Finding the most effective and low-cost enzymes for the pretreatment process and organisms for the fermentation process has been one of the main areas of research in development of cellulosic ethanol (Climate Techbook, 2009). The chemical reaction for the fermentation of glucose by yeast to form ethanol is given by:

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$$

(Thomson, 2006).

Researchers at the University of California at Berkeley estimated that on a life-cycle basis, cellulosic ethanol could lower GHG emissions by 90 percent relative to petroleum-based gasoline. Other analyses have shown that cellulosic ethanol produced using certain feedstocks could be carbon-negative, which means that more carbon dioxide (CO2) is removed from the atmosphere than is emitted into the atmosphere over the entire life-cycle of the product. California Low Carbon Fuel Standard found significant life-cycle GHG emission reductions from cellulosic ethanol relative to gasoline (Climate Techbook (2009). Life-cycle GHG Intensity for Cellulosic Ethanol, based on the California GREET Model is shown in table 1.

Table (1)- Life-cycle GHG Intensity for Cellulosic Ethanol, based on the California GREET Model.

Fuel	Feedstock	CA GREET GHG (g CO2e/MJ)	GHG Reduction Compared to Gasoline
Cellulosic Ethanol	Farmed Trees	1.60	98.3%
Cellulosic Ethanol	Forest Residues	21.40	77.7%
California Gasoline (incl. 10% ethanol)		95.9	-

Note: These estimates do not include the impact of indirect land use change on GHG emissions.

It has been found that cellulosic ethanol can produce a positive net energy output (Farrell, A. E., R. J. Plevin, et al. 2006). The reduction in green house gas (GHG) emissions from corn ethanol and cellulosic ethanol compared with fossil fuels is drastic. Corn ethanol may reduce overall GHG emissions by about 13%, while that figure is around 88% or greater for cellulosic ethanol (Wanga, M. Q.; Han, J.; Haq, Z.; Tyner, W. E.; Wua, M.; Elgowainy, A. 2011) .As well, cellulosic ethanol can reduce carbon dioxide emissions to nearly zero (Solomon, B. D.; Barnes, J. R.; Halvorson, K. E.2007).

2. Materials and Methods

2.1 Materials

(10) Bamboo species aging with 4 years were used in this study. They were taken from a ITTO project bamboo plantation located in Kawtmu Township, Yangon Region. The stems were cut off to sample size 36 × 1 inches (length × width). They were hit by using a hammer and chopped into tiny pieces to reduce the sample size, and then air dried for two weeks. The moisture content of the tiny pieces bamboo samples were measured in an oven at 105°C ± 2°C for 24 h. The chemical component such as cellulose, in each bamboo species was also determined before the pretreatment at the wood chemistry laboratory.

2.2 Pretreatment (Alkali Pretreatment)

Each of the tiny pieces bamboo samples (50g) on an oven-dry basis were washed with MeOH to remove lipids and treated with a 1% aqueous solution of NaOH (400 mL) at 95 ºC for 1 h(Yasuda *et al.*, 2012). Isolation of lignin-removed holocellulose was performed by centrifugation of the solutions. The solid and liquid fractions were separated by filtration with filter papers. Lignin remained in the alkali solution. Then the solid residues were washed with distilled water to remove the contaminated lignin. Finally they were stored for enzymatic hydrolysis. This alkali pretreatment process results in (i) the removal of all lignin and part of hemicellulose, and (ii) increased reactivity of cellulose in further hydrolysis steps (Hamelinck et al., 2005), especially, enzymatic hydrolysis.

2.3 Enzymatic hydrolysis

Enzymatic hydrolysis was carried out in 250 mL flasks by using a shaker. Each of the bamboo substrate residues 2.5% (w/v) were treated with 0.05 M citrate buffer (pH 4.8), based on previous study (Chun-Han Ko[a.e], Ya-Nang Wang[a], Fang-Chih Chang*[,b], Chih-Yuan Lee[a], Wen-Hua Chen[c],Wen-Song Hwang[c],Yi-Chung Wang[d],2013). About 1.5mg of tetracycline chloride was added to control the growth of microorganisms and prevent consumption of liberated sugars. Cellulase (15 FPU/g substrate) and *β*-glucosidase (15 IU/g substrate) enzymes were loaded into the flasks (Li et al, 2012).

2.4 Fermentation

Firstly, Yeast powder which was made with sticky rice was added into a 250ml conical flask with 50ml of sugar solution. The flask was placed in a water bath at 38C° for 20min.then the mixture was kept at 33C° for another 1.5h (Zhiqiang Li, Zehui Jiang, Benhua Fei,Xing□e Liu, Yan Yu, 2012). Then sugar (5g/L), KH_2PO_4 (2g/L), $MgSO_4$ (1g/L), and $CaCl_2$ (0.25g/L)

were added into the flask. Then this mixture was introduced into the above hydrolyzate for ethanol fermentation. The same procedures were used for each of the remaining bamboo species.

2.5 Analytical Methods

2.5.1 Preparation of standard solution

Ethanol HPLC testing standard was prepared by weighting a certain amount of ethanol into a 10ml volumetric flask and introducing distilled water to the mark. Then diluted solutions were made and filtered with 0.45-μm cellulose acetate membrane filter before injected into HPLC system. Then the solutions were chromatographed to determine the retention time. Standard were prepared freshly and immediately injected to HPLC column.

2.5.2 Preparation of sample solutions

After fermentation the precipitates and supernatant solutions (filtrates) were separated by filtration. Then each of the liquid filtrate that obtained from fermentation was prepared individually by weighting a certain amount into each 10ml volumetric flasks and introducing distilled water to the mark. Then diluted solutions were made and filtered with 0.45-μm cellulose acetate membrane filter. Each of the sample solutions were injected individually into the column in duplicate. The solutions were chromatographed to determine the retention time.

2.5.3 Mobile Phase

About 800ml distilled water were added to a 1000ml reservoir bottle. Then using a pipette 0.2ml 99.7% sulfuric acid was transferred into the 1000ml bottle to make a 0.0045N sulfuric acid stock solution. Next 60ml (6%,v/v) acetonitrile was introduced to that bottle. The bottle was filled to the1000ml mark with distilled water. That solution was used as a mobile phase mixture.

2.5.4 HPLC analysis

Isolation of compounds in all liquid filtrates that obtained from fermentation were analyzed using a Waters Associates chromatographic system equipped with a Shimadzu HPLC model 20A Series, equipped with a prominence degasser (DGU-20 A_3), a pump (LC-20AT), a manual injector (Model), and a reverse phase C18 column (CTO-20A),and UV/VIS detector(SPD-20A) connected in series. The column was operated at 45°C. Isocratic elution at a flow rate of 0.8 mL/min was carried out using a mixture of 0.0045N sulfuric acid and acetonitrile (6%,v/v) as a mobile phase. Peak detection was made using the UV detector set at 214 nm. The amount of the sample injected into the column is 20μl. Peak identification was carried out by

spiking liquid filtrates that obtained from fermentation and pure ethanol standard. Then the retention times of each of the liquid filtrate were compared with that of pure ethanol standard.

3. Results and Discussion

3.1 The composition of cellulose in each bamboo species

Cellulose based bamboo materials are polysaccharide and these can be broken down into 5 or 6-carbon sugars. These sugars can be further broken down. When fermented these sugars convert into the fuel ethanol source. In this study, the chemical component such as cellulose, in each bamboo species was also determined before the pretreatment. The results are shown in the following table.

Table (2)- The composition of cellulose in each bamboo species

No	Name	Scientific Name	Cellulose Content (%)
1.	Tin wa	*Cephaloctachyum pergracile*	42.35
2.	Htiyo wa	*Thyrsostachys siamensis*	44.92
3.	Wa bo myet san gel	*Dendrocalamus hamiltonii*	46.03
4.	Wa net	*Bambusa vulgaris*	47.24
5.	Wa bo	*Dendrocalamus brandisii*	48.21
6.	Kyathaung wa	*Bambusa polymopha*	48.27
7.	Tabindaing wa	*Bambusa longispiculata*	48.81
8.	Hmyin wa	*Dendrocalamus strictus*	49.82
9.	Wa bo gyi	*Dendrocalamus giganteus*	55.40
10.	Wa yar	*Oxytenenthera nigricilita*	58.03

According to the results shown in the table, Wa ya and Wa bo gyi contain high percentage of cellulose, which served as the main resource in bioethanol production. Moreover they are rich in cellulose which is desirable fuel characteristics for ethanol production. Thus the maximum yield of ethanol could be provided from Wa ya and Wa bo gyi bamboo species depend on the pretreatment and hydrolysis methods. In addition the cellulose molecules are composed of long chains of sugar molecules. Hydrolysis (Saccharification) breaks down the hydrogen bonds in the cellulose chains into simple sugar components, such as pentoses and hexoses. These sugars can be fermented into bioethanol.

3.2 Identification of the crude solutions that obtained from fermentation by HPLC

Although the composition of each of the liquid filtrate of bamboo species that obtained from fermentation are complex, ethanol compound was identical by retention time comparison.

The figure (3) shows the HPLC chromatogram of the standard ethanol which was used for identification of each of the liquid filtrate of bamboo species that obtained from fermentation.

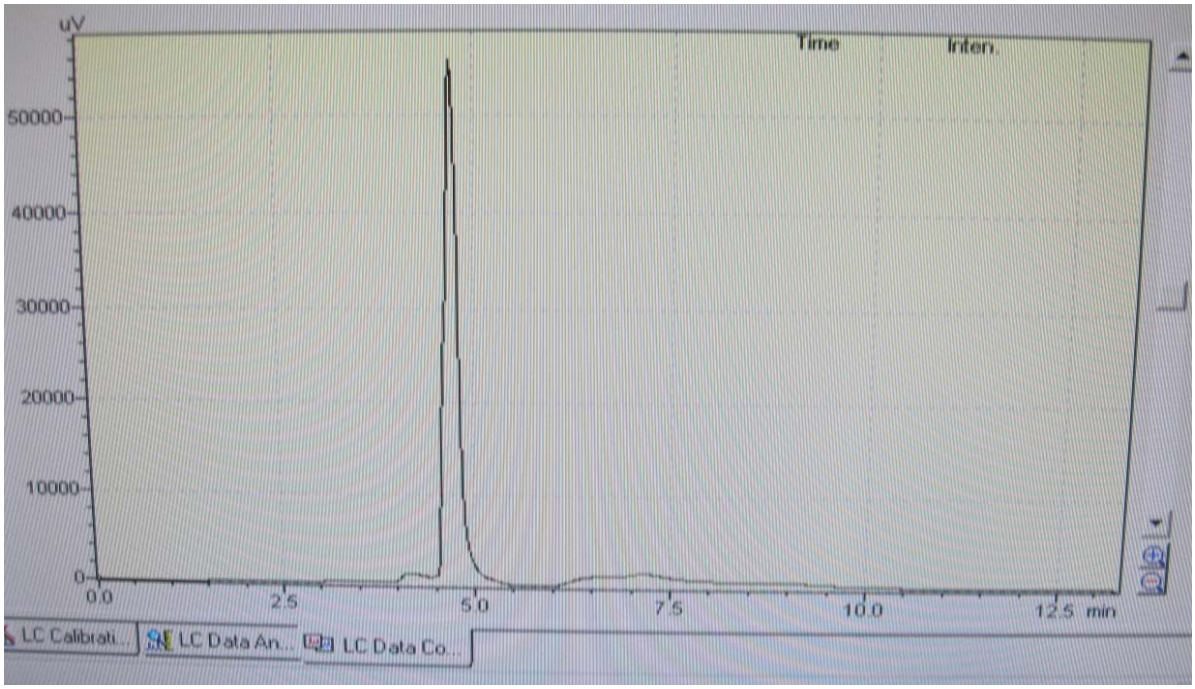


Figure (3) HPLC chromatogram of the standard ethanol

The figures (4), (5) and (6) show the HPLC chromatograms of liquid filtrates of Tin wa, Wa bo gyi and Wa yar that obtained from fermentation.

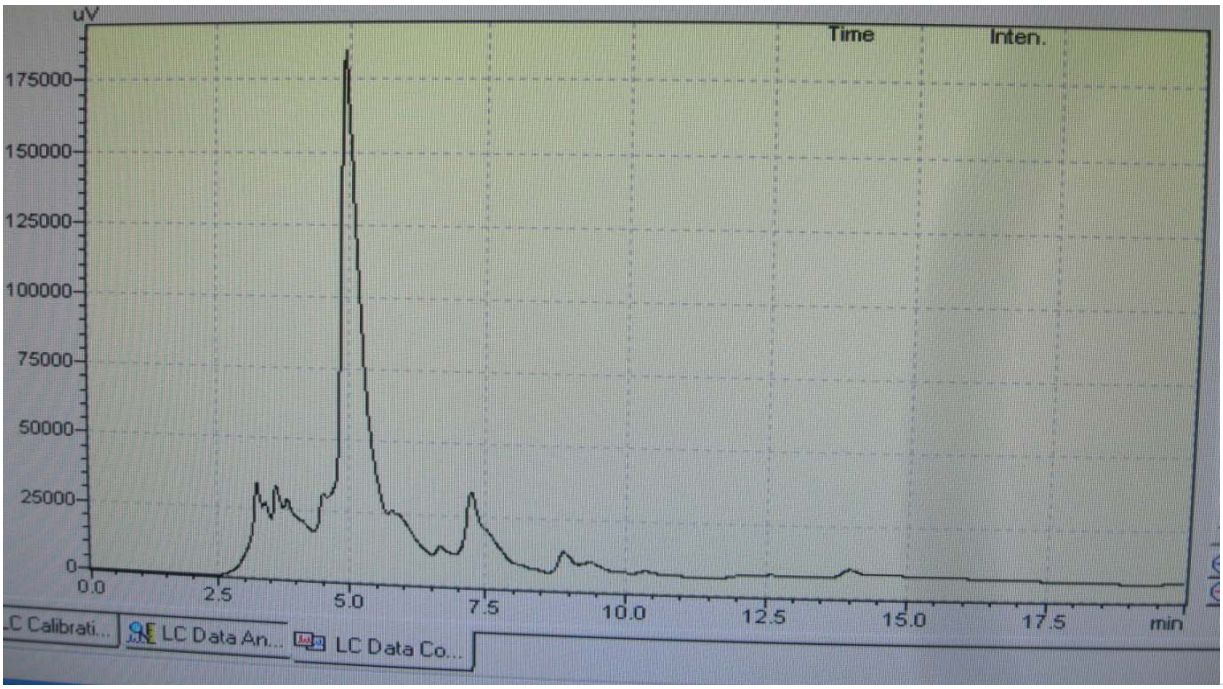


Figure (4) HPLC chromatograms of the liquid filtrate of Tin wa

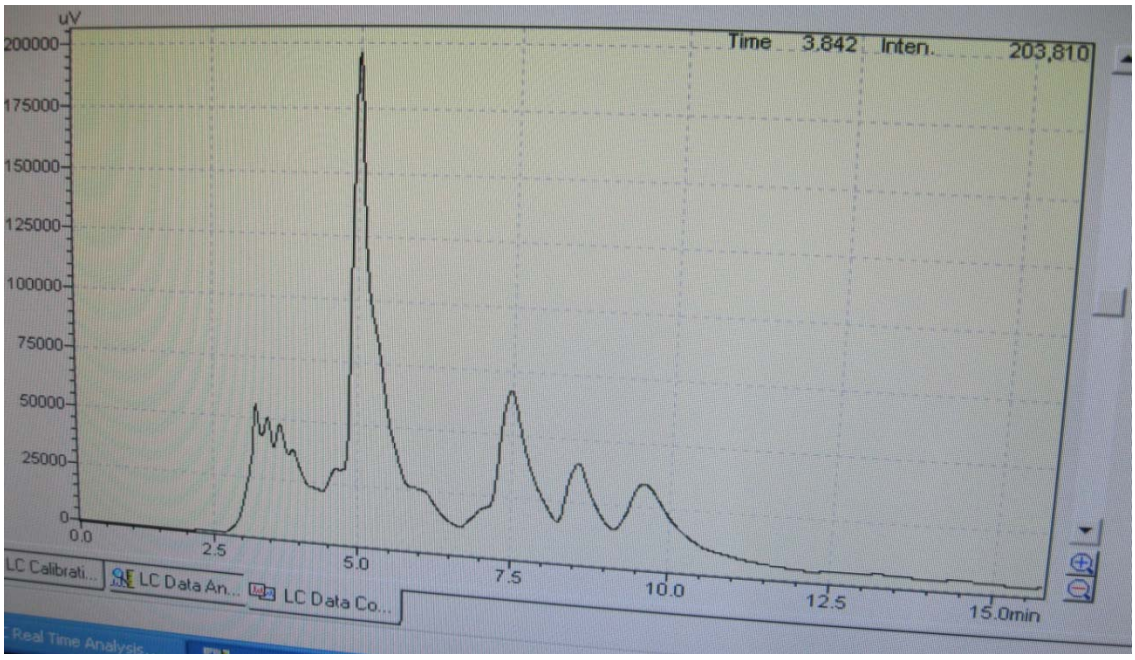


Figure (5) HPLC chromatograms of the liquid filtrate of Wa bo gyi

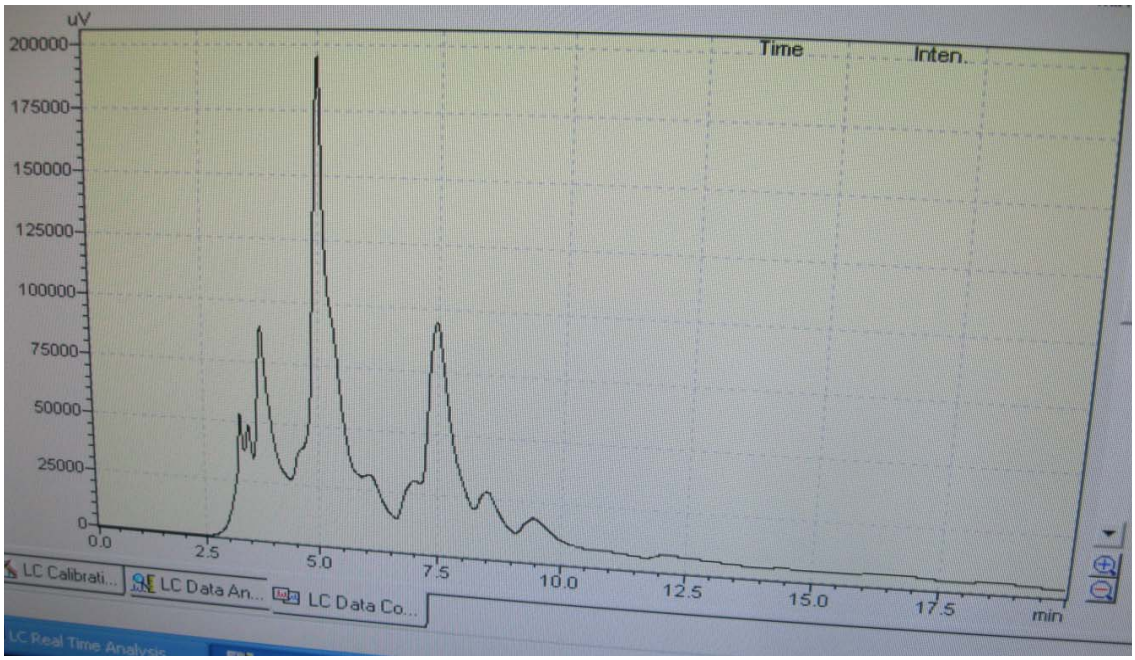


Figure (6) HPLC chromatogram of the liquid filtrate of Wa yar

By comparing the chromatographic profiles with the retention time of reference ethanol standard, preliminary identification of liquid filtrates of (10) bamboo species were matched with the retention time of ethanol standard. In addition many unidentified compounds can be found in the HPLC chromatograms of liquid filtrates of (10) bamboo species.

Moreover all liquid filtrates that obtained from fermentation were analyzed by using HPLC to know the ethanol concentration of each filtrate. The analytical results are shown in table (3).

Table (3)- Ethanol Concentrations of liquid filtrates of some bamboo species

No	Name	Scientific Name	Ethanol Concentration (mg/ml)
1.	Tin wa	*Cephaloctachyum pergracile*	25.2
2.	Hti yo wa	*Thyrsostachys siamensis*	26.2
3.	Wa bo myet san gye	*Dendrocalamus hamiltonii*	27.06
4.	Wa net	*Bambusa vulgaris*	27.73
5.	Wa bo	*Dendrocalamus brandisii*	28.99

6.	Kyat thaung wa	*Bambusa polymopha*	30.87
7.	Ta bin daing	*Bambusa longispiculata*	31.08
8.	Hmin wa (branches)	*Dendrocalamus strictus*	35.65
9.	Wa bo gyi	*Dendrocalamus giganteus*	43.13
10.	Wa yar	*Oxytenenthera nigricilita*	57.09

The results of HPLC analysis showed that various concentrations of ethanol are content in liquid filtrates of (10) bamboo species that obtained from fermentations. But the liquid filtrate of Wa ya that obtained from fermentation has highest ethanol concentration than other bamboo species.

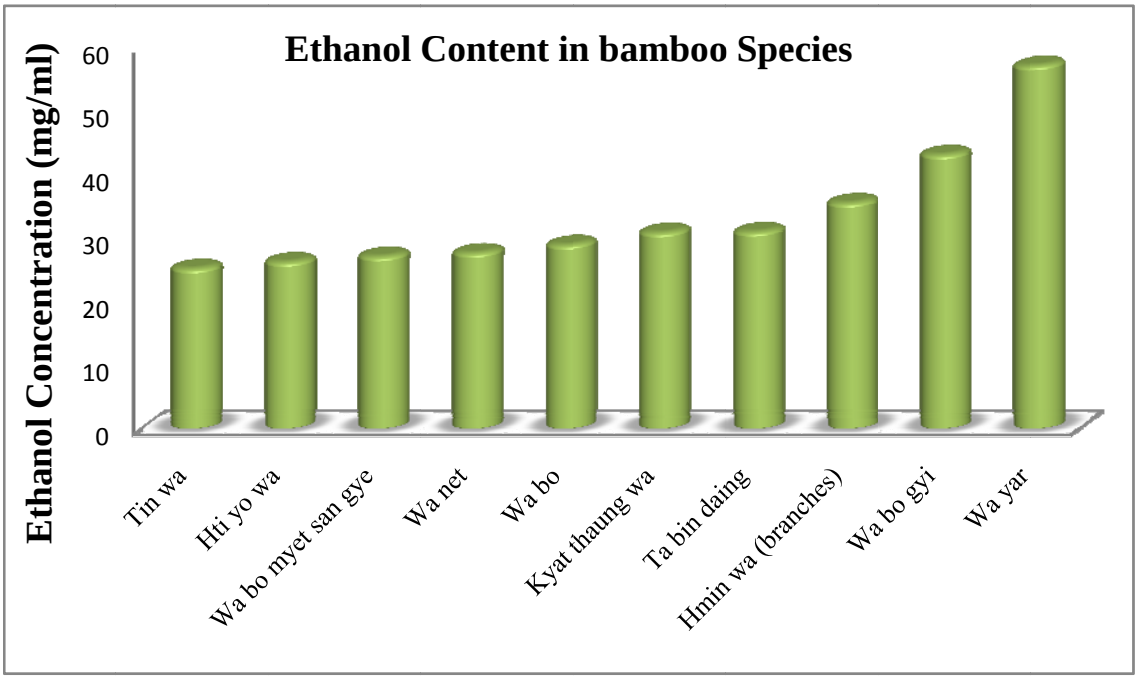


Figure (7) Ethanol Content in liquid filtrates of bamboo Species

According to the bar graph, as illustrated in figure (7), the results indicate that the liquid filtrate of Wa ya that obtained from fermentation contains maximum concentration of ethanol than those of other bamboo species. Thus Wa ya is the most suitable bamboo species for exploring bioethanol. In addition both pretreatment and hydrolysis processes influence the yield of ethanol.

4. Conclusions

Bamboo resources are abundant and widespread in Myanmar. Most of them are not in competition with food sources and animal feed. Although the utilization of bamboo may be more than 1,500 items in Myanmar, the use of bamboo as fuel source for bioethanol has not been developed yet. This research represents an initial stage in the study of bamboo bioethanol. The composition of cellulose in each bamboo species was invested in this study. It was found that Wa ya was more rich in cellulose fraction which is desirable fuel characteristic for ethanol production.

By comparing the chromatographic profiles with the retention time of reference ethanol standard, identification of HPLC chromatograms of liquid filtrates of (10) bamboo species that obtained from fermentation were matched with the retention time of ethanol standard. According to the results of HPLC analysis, various concentrations of ethanol are content in liquid filtrates of (10) bamboo species that obtained from fermentation. But the liquid filtrate of Wa ya that obtained from fermentation has highest ethanol concentration than that of other bamboo species. Thus Wa ya is the most suitable bamboo species for exploring bioethanol with alkali pretreatment and enzymatic hydrolysis. In addition this preliminary investigation serves as a force for further research in the application of bamboo for bioethanol production and may provide guidelines.

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