國立臺灣大學生物資源暨農學院森林環境暨資源學系

碩士論文

School of Forestry and Resource Conservation

College of Bioresources and Agriculture

National Taiwan University

Master Thesis

麻竹生產生質乙醇之評估

Evaluation of *Dendrocalamus latiflorus*

as a feedstock for bioethanol

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中華民國 100 年 6 月

June, 2011

國立臺灣大學碩士學位論文
口試委員會審定書

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本論文係 李智遠 君（R98625043）在國立臺灣大學森林環境暨資源學系、所完成之碩士學位論文，於民國 100 年 6 月 21 日承下列考試委員審查通過及口試及格，特此證明

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謝誌

摘要

　　竹林覆蓋台灣 152,300 公頃，約佔台灣總森林面積的 7.2％，因此本研究以麻竹(*Dendrocalamus latiflorus*)為代表，評估竹材作為台灣生質乙醇原料的潛力。竹片經過酸、鹼蒸汽爆碎前處理，酸蒸氣爆碎以 190 ^{o}C 持溫 10 min，鹼蒸氣爆碎以 180，190 及 200°C 持溫 10 至 20 分鐘，進而以相對於試驗材料乾重比例的 2、6、12 % (w/w) 纖維素酶劑量對蒸汽爆碎竹漿進行水解。最高葡萄糖得率：鹼蒸汽爆碎竹漿為 50.4~249.3 mg/g，而酸蒸汽爆碎竹漿為 49.7~209.1 mg/g。此外本研究發現水解效率與試材中的木質素的含量成負相關。本研究並使用菌株 *Saccharomyces cerevisiae* D5A 對上述竹漿進行同時糖化與發酵 (SSF)，條件為：37.5^{o}C、pH 5、yeast extract 10 g/L 及 peptone 20 g/L。經 96 小時後，酸蒸汽爆碎竹漿從原料至乙醇之轉化率為 13.11%，相當於每公斤乾重竹片可產 0.16 L 乙醇；鹼蒸汽爆碎竹漿從原料至酒精之轉化率為 8.8%，相當於每公斤乾重竹片可產 0.11 L 乙醇。以上發酵後之廢渣，經元素分析証實具有相當之熱值。台灣竹林蓄積量估計範圍為 5,020,000~11,640,000 公噸，假設每五年輪伐一次，估計每年約可有 1,328,000 公噸竹材投入生質乙醇生產，以酸蒸汽爆碎法配合與同時糖化與發酵約可產出 220,659 公秉酒精，鹼蒸汽爆碎前處理約可產出 148,162 公秉酒精。本研究結果顯示，以本土竹林蓄積更新生產生質乙醇，具有相當的潛力。

關鍵字：麻竹、生質酒精、蒸汽爆碎、同時糖化與發酵

ABSTRACT

Bamboo covers 152,300 ha in Taiwan, approximately of 7.2 % the total overall forest area. This study evaluated *Dendrocalamus latiflorus* as a feedstock for bioethanols in Taiwan. Acidic and alkaline steam explosion was employed to prepare pulps from *Dendrocalamus latiflorus* chips. Alkali-treated samples were prepared at 180, 190 and 200°C from 10 to 20 min. Acid-treated samples were prepared at 190°C for 10 min. For the saccharification of pretreated bamboo biomass, cellulase formulations were applied with three dosages: 2, 6, 12 percent to dried pulp (w/w). The glucose yields were 50.4 ~ 249.3 mg/g alkali steam-exploded pulps, and 49.7~209.1 mg/g acid steam-exploded pulps. The hydrolysis efficiencies were negatively impacted by lignin contents of pretreated bamboo pulps. Simultaneous saccharification and fermentation (SSF) were also conducted using *Saccharomyces cerevisiae* D5A under 37.5^{o}C and pH 5 with yeast extract 10 g/L and peptone 20 g/L at shake flask level. After 96 hours, the optimal ethanol conversion for acid pretreated pulps was 13.11%, equivalent to 0.16 L ethanol/kg dried chip. And the optimal ethanol conversion for alkaline pretreated pulps exploded was 8.8%, equivalent to 0.11 L/kg dried chip. The post-fermentation residues possessed high heating values, which were verified by elemental analysis. There is estimated 5,020,000~11,640,000 ton storage for in bamboo stands in Taiwan. There could be 1,328,000 ton available as feedstock for bioethanol production annually under 5-year rotation harvesting. SSF could produce 220,659 kL of bioethanols from by acidic steam explosion pulps and 148,162 kL of bioethanols by alkaline steam explosion pretreatment pulps annually. This study demonstrated potentials of the indigenous bamboo storage as a source for bioethanol production.

Keywords: *Dendrocalamus latiflorus*, Bioethanol, Steam explosion, Simultaneous saccharification and fermentation

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Chapter 1 Introduction

Substitute for petroleum-derived fuels now has more importance such as ethanol from biomass, the most abundant renewable resources on earth. The first generation bio-ethanol is made from grain, corn, and the food provisions. They are easy conversing from biomass to fermentable sugars, and have good conversion rate to ethanol. But they may conflicts human food, animal feedstock, and industrial use.

The second generation bio-ethanol is made from lignocellulosic materials, like agricultural wastes (inedible part), wood chips and sawdust. It will not conflict human's food-required, but lignocellulosic materials are not homogeneous as crops. When lignocellulosic materials are enzymatic hydrolyzed, they need pretreatments to make enzyme work easier and more efficiency.

Steam-explosion was used to produce chemithermomechnical pulp. It put woody materials into a sealed container, import high temperature steam to heat and pressurize, and then discharge instant to separate lignocellulosic fibers. Now Steam-explosion is a popular way in lignocellulosic ethanol pretreatments.

The bamboo covers 152,300 ha, roughly of 7.2 % the overall forest area in Taiwan (Taiwan Forestry Bureau, 1995). Bamboo biomass is accumulating daily, but little of them has been used (edible bamboo shoots and inedible part as materials) and most of them are wasted without utilizing. Its accumulation is about 26.1 tons/ha, with annual growth around 13.84 tons/ha under 5 year rotation cutting. Its fast growth and adaptability toward various soil and climate conditions make the bamboo a good candidate for a renewable resource. Bamboo had been conventionally used as the raw materials for producing artefacts, utensils, plywood, fiberboard, and decorated multi-layered panels in Taiwan and many Asian countries. Recently, more attention was

paid for bamboo biomass as biofuel feedstock. Steam-exploded bamboo was employed for methane production (Kobayashi et al., 2004). Japanese bamboo produced 3300 million kg of bamboo are annually accumulating in Japan; most of them are over-matured and left without proper utilization (similarly with Taiwan).

This study evaluated *Dendrocalamus latiflorus* (*Dendrocalamus latiflorus* Munro) as a feedstock for bioethanols in Taiwan. Acidic and alkaline steam exploded bamboo biomass, as well as unbleached and bleached kraft bamboo pulps, were employed as raw materials to investigate the impact of lignin on SSF efficiencies.

Chapter 2   Literature reviews

2.1   Cellulosic ethanol

Some scientist emphasized the accumulation of greenhouse gases in the environment that have already exceeded the "dangerously high" (Schenk et al., 2008) because the utilization of fossil fuels which also accepted as unsustainable fuel due to depleting resources. Alternatives fuels to petroleum-related fuels from renewable resources are considered to diminish the world's dependence of fossil fuels. The most common renewable Alternated-fuel now is "ethanol" which manufactured from corn grain and sugar cane. The global ethanol demands that potential production from the major edible crops, corn, barley, rice, wheat, sorghum, and sugar cane, is limited.

To avoid conflicts between human food use and industrial use of crops, only the wasted crop parts, which is defined as agricultural waste, is considered as feedstock. Lignocellulosic biomass such as crop residues and sugar cane bagasse are included in feedstock for producing bioethanol as well. It is expected that ethanol from edible part will be limits to raw materials supply of ethanol production in the near future, therefore lignocellulosic biomass, the most abundant renewable resources on earth is seen as an attractive feedstock for future supplies of ethanol (Gray et al., 2006).

However, there are also technical and economical barriers to the development of a commercial ethanol processes utilizing biomass. Technologies are being developed that will allow cost-effective conversion of biomass into fuels and byproducts chemicals. These technologies include low-cost pretreatment, highly effective cellulases and hemicellulases and efficient and robust fermentative microorganisms (Gray *et al.,* 2006). Many advances have been made over the past few years that make commercialization more promising.

Extensive research has been focus on conversion of lignocellulosic biomass to

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ethanol in decades, including two processes: hydrolysis of cellulose from the lignocellulosic materials to fermentable reducing sugars, and fermentation of the sugars to ethanol.

The hydrolysis is usually catalyzed by cellulase enzymes which is more specific and environment-friendly, and the fermentation is usually carried out by yeasts or bacteria. The factors that have been identified to affect the hydrolysis of cellulose include porosity (accessible surface area) of the lignocellulosic materials, cellulose crystallinity, and lignin and hemicellulose content (McMillan, 1994).

The presence of lignin and hemicellulose makes the accessibility of cellulase enzymes to cellulose zone more difficult, that reducing the enzyme hydrolysis efficiency. Removal of lignin and hemicellulose, reduction of cellulose crystallinity and increase of porosity in pretreatment processes can significantly improve the hydrolysis (Sun et al., 2001).
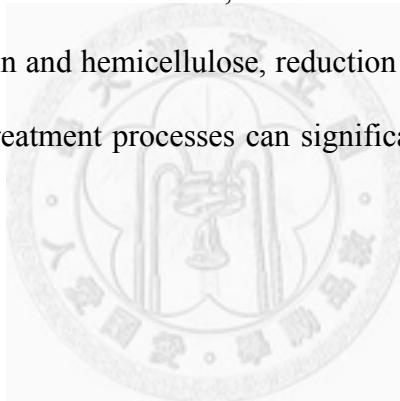
2.2 Steam explosion pretreatments of lignocellulosic materials

Steam explosion is now the most commonly used pretreatment method for lignocellulosic biomass, which was used to made semi-chemical mechanical pulp. In the method, chipped biomass is put in a sealed container with high-pressure saturated steam, and then the pressure is exhausted rapidly, which makes lignocellulosic materials "explosive decompression".

Steam explosion is typically condition at a temperature of 160–260°C (corresponding to saturated steam pressure 0.69–4.83 MPa) for several seconds to a few minutes time-hold before the material is quickly exposed to atmospheric pressure. The process causes hemicellulose degradation and lignin transformation due to high temperature, thus increasing the potential of cellulose hydrolysis (Hendriks and Zeeman, 2009).

Steam explosion pretreatment is good at treating heterogeneous biomass, especially woody plants, form agriculture wastes to softwood and hardwood. Ruiz et al. (2008) used sunflower stalks, a largely and cheap agricultural residue lacking of economic alternatives, were steam explosion pretreated, to optimize pretreatment temperature in the range 180–230°C. After 96 h of enzymatic action, a maximum hydrolysis yield of 72 % was attained in the water-insoluble fiber obtained after pretreatment at 220°C, corresponding to a glucose concentration of 43.7 g / L in hydrolysis media, the maximum yield both cellulose recovery and hydrolysis yield, obtaining 16.7 g of glucose from 100 g of raw material.

As well as hardwood for ethanol feedstock, Cara et al. (2008) produce fuel ethanol from olive tree pruning; raw material is a renewable, low cost, largely available, and lacking of economic alternatives agricultural residue. Olive tree pruning with steam explosion pretreated in the temperature range 190–240°C, with or without previous

soaking by water or sulphuric acid solutions. Both pretreatment temperature and impregnation conditions on sugar and ethanol yields were investigated by enzymatic hydrolysis and simultaneous saccharification and fermentation. Results show that the maximum ethanol yield (7.2 g ethanol/100 g raw material) is obtained from water impregnated, steam pretreated residue at 240°C.

2.3 Separately hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF)

Simultaneous saccharification and fermentation (SSF) processes were firstly described by Takagi et al. (1977): it combined enzymatic hydrolysis of cellulose and simultaneous fermentation of the fermentable sugars together to obtain ethanol. In the SSF process, the conditions are nearly the same as in separate hydrolysis and fermentation systems (SHF), just one different is that saccharification and fermentation are performed in the same reactor.

Thus, put the yeast and the cellulolytic enzyme complex together reduces the accumulation and inhibition of sugars in the reactor which increasing ethanol yield and hydrolysis rate with respect to separate hydrolysis and fermentation (Wyman and Hinman, 1990). Another advantage of SSF is that used one fermenter throughout the whole process reducing the facilities costs.
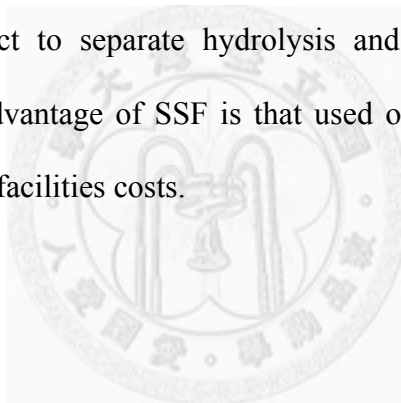
2.4 Simultaneous saccharification and fermentation process (SSF) with *Kluyveromyces marxianus* or *Saccharomyces cerevisiae* D5A

Temperature is an important factor of fermentation conditions. Different strains of yeasts have different tolerances about temperature. Behind its limitation, the higher temperature usually haves the more activities of enzyme (dosage-saving) and higher chemical reaction rate.

Yeast strains of *Kluyveromyces marxianus* have the ability to ferment glucose into ethanol at relatively high incubation temperatures (up to 47°C) (Aderson et al., 1986). At lower incubation temperatures, the activity of yeast cells can be growth better and the final ethanol concentration in the fermentation broth is greater than at a higher temperature, which is common in thermotolerant yeasts, in that a higher temperature lowers the yeast's viability and its tolerance to ethanol. For the reason to choose the optimum conditions, strike balance between the maintenance of cellulase and the yeast's activities during simultaneous saccharification and fermentation (Lark et al., 1997).

And then *K. marxianus* was used to ethanol fermentation of lignocellulosic materials, woody (poplar and eucalyptus) and herbaceous (*Sorghum* sp. bagasse, wheat straw and *Brassica carinata* residue) (Ballesteros et al., 2004). Moreover, it was displaying cellulolytic enzymes by Yanase et al. (2010) which successfully converts a cellulosic β-glucan to ethanol directly at 48°C with a yield of 4.24 g / L from 10 g / L within 12 h. The yield (in grams of ethanol produced per gram of β-glucan consumed) was 0.47 g / g, which corresponds to 92.2 % of the theoretical yield.

There is still another popular yeast strains, *Saccharomyces cerevisiae* D_5A. Mielenz et al. (2009) evaluated Soybean hulls as a resource for production of ethanol with *Saccharomyces cerevisiae* D_5A by the simultaneous saccharification and fermentation (SSF) process without pretreatment. With cellulase, β-glucosidase and

pectinase dosages were determined at 15 % biomass loading, and ethanol concentrations of 25–30 g / L were routinely obtained 3–4 times higher ethanol yield under these conditions corn stover, wheat straw, and switchgrass produced. A new pretreatment technology using dilute ammonium hydroxide was evaluated for ethanol production on sorghum. Approximately, 44 % lignin and 35 % hemicellulose were removed during the process. Hydrolysis of untreated and dilute ammonia pretreated fibers was carried out at 10 % dry solids at an enzyme concentration of 60 FPU Spezyme CP and 64 CBU Novozyme 188 / g glucan. Cellulose digestibility was higher (84 %) for ammonia pretreated sorghum as compared to untreated sorghum (38 %). Fermentations with *Saccharomyces cerevisiae* D_5A resulted in 24 g ethanol /100 g dry biomass for dilute ammonia pretreated sorghum and 9 g ethanol / 100 g dry biomass for untreated sorghum.

Faga et al. (2010) compared five thermotolerant yeast strains *Kluyveromyces marxianus* IMB 1, IMB 2, IMB 3, IMB 4, and IMB 5 at 45°C and *Saccharomyces cerevisiae* D_5A at 37°C with Hydrothermolysis pretreated switchgrass at 200°C for 10 min was used in a simultaneous saccharification and fermentation (SSF) process. SSF was carried out for 7 days using 5, 10, and 15 FPU/g glucan. Fermentation by *K. marxianus* IMB 1, IMB 2, IMB 4, and IMB 5 ceased by 72 h and fermentation by *K. marxianus* IMB 3 ceased by 96 h, while fermentation *S. cerevisiae* D_5A continued for 7 days. At 96 and 120 h, IMB 3 and *S. cerevisiae* D_5A had similar ethanol yields while the other *K. marxianus* strains were lower at a 95 % confidence level. Final ethanol yields for IMB 3, IMB 1, IMB 5 strains were similar to one another, however, ethanol yield for *S. cerevisiae* D_5A (92 % maximum theoretical) was greater than all of the IMB strains except IMB 3 at a 95 % confidence level. Reducing enzyme loading reduced the ethanol yields for both *K. marxianus* IMB 3 and *S. cerevisiae* D_5A.

2.5   Energy estimation of compacting fuel and its high heating value

Mani et al. (2006) estimated the specific energy requirement for compacting corn stover, which was a major crop residue for biomass conversion to produce chemicals and fuels in North America. Corn stover had low bulk density and it had little energy to smash which may be similar to fermented residue. In that study, chopped corn stover samples were compacted in a piston cylinder under three pressure levels (5, 10, 15 MPa) and at three moisture content levels (5 %, 10 %, 15 %) to produce compacting fuel briquettes. The total energy requirement to compress and extrude briquette ranged from 12 to 30 MJ / ton.

The High Heating Value of the compacting fuel (represented its energy content) could be estimated by it ultimate analysis mentioned by Demirbas (1997). Demirbas calculated the biomass fuel HHV content with the formula, High heating values: HHV (MJ / kg) = {33.5 [C] + 142.3 [H] - 15.41 [O] - 14.5 [N] } × 10^{-2}. Sheng and Azevedo (2005) emphasized the carbon content effect of the HHV with the ultimate analysis formula: HHV_{ult} (MJ / kg) = 0.3259 Carbon content (%) + 3.4597. And he emphasized the negative effect of the ash content by the proximate analysis formula: HHV_{prox} (MJ / kg) = 19.914 - 0.2324 Ash (%).

2.6   Energy balance of the ethanol production

Shapouri et al. (2002) belonged to the United States Department of Agriculture, estimated the energy balance of the corn ethanol production in United States. Ethanol production in the United States grew so fast that from just a few million gallons in the mid-1970s to over 1.7 billion gallons in 2001. But not all cases had net energy gain; the energy input in process consumed too much unrenewable petroleum sometimes including growing the corn, harvesting it, transporting it, and distilling it into ethanol. Then considered ethanol as energy output minus energy input, estimated the net energy value (NEV). Production of corn-ethanol is energy efficient, in that it yields 34 percent more energy than it takes to produce it. The estimated NEV of corn ethanol was 21,105 Btu / gal (= 7.45 MJ / kg).

Cardona Alzate and Sanchez Toro (2006) emphasized that lignocellulosic biomass is the most promising feedstock for producing bioethanol due to its global availability and to the energy gain that can be obtained when non-fermentable materials from biomass are used for cogeneration of heat and power. In that work, several process configurations for fuel ethanol production from lignocellulosic biomass were studied through process using Aspen Plus. The flowsheet variants were analyzed from the energy point of view utilizing as comparison criterion the energy consumption needed to produce 1L of anhydrous ethanol. Simultaneous saccharification and co-fermentation process with water recycling showed the best results accounting an energy consumption of 41.96 MJ / L ethanol. A net energy value of 17.65~18.93 MJ / L ethanol (= 22.37~23.99 MJ / kg) was calculated indicating the energy efficiency of the lignocellulosic ethanol.

Chapter 3   Materials and methods

3.1   Objectives

The bamboo covers 152,300 ha, roughly of 7.2 % the overall forest area in Taiwan (Taiwan Forestry Bureau, 1995). Bamboo biomass is daily accumulating, but little of them have been used and most of them are wasted without utilizing which may overmature to useless at 10 years-old. Its fast growth and adaptability toward various soil and climate conditions make the bamboo a good candidate for a renewable resource.

The *Dendrocalamus latiflorus* has the most bamboo stand area and stock volume of bamboo species in Taiwan, have the greater possibility as the sustainable feedstock.

Steam explosion as pretreatment, the most popular way is acid steam explosion. When pulping process, alkali pulping has good work to eliminate lignin which is also barrier to lignocellulosic ethanol. How about use alkali steam explosion way to pretreat *Dendrocalamus latiflorus*? Acid steam explosion also be used as positive control.

Temperature is an important factor of fermentation conditions. Different strains of yeasts have different tolerances about temperature. Behind its limitation, the higher temperature usually haves the more activities of enzyme (dosage-saving and cost-saving) and higher chemical reaction rate. Yeast strains of *Kluyveromyces marxianus* have the ability to ferment glucose into ethanol at relatively high incubation temperatures. And high yield yeast strain, *Saccharomyces cerevisiae* D5A is adopted.

3.2   Experimental material

The *Dendrocalamus latiflorus* sample, approx. 4-year-old *Dendrocalamus latiflorus* culms, was collected from the Experimental Forest of National Taiwan University at April 2009. The stem was chopped into $6 \times 3 \times 1$ cm (length $\times$ width $\times$ thickness), then air dried for a month.

3.3   Steam-explosion

Air-dried sample was soaked into 1.5 % H_2SO_4 solution or 1.5 % NaOH & Na_2SO_3 solution for a week to make chips fully swelling. Steam explosion conditions were: solid/liquid ratio=1:7; temperature and pressure of alkali-treated samples were held at 180, 190 and 200^oC from 10 to 20 min. Acid-treated sampled were held at 190^oC for 10 min. Samples were then washed by tap water on 200 mesh screen until neutral, and then kept in 4 ^oC refrigerator.

3.4   Kraft pulping and bleaching

Dendrocalamus latiflorus air-dried kraft pulp was cooked by M/K digester with wood to liquid ratio = 1/4 (w/v), 25 % sulfidity and 17 % active alkali. H-factor is about 650, with temperature raised at 1.5^oC / min to 160^oC in 90 min, then kept at 160^oC for another 90 min. After washing and screening, then keep in 4^oC refrigerator. Bleached pulps were prepared by a commercial DEDD bleaching sequence, and then washed and kept in 4^oC refrigerator. "D"and "E" stand for chlorine dioxide and alkali extraction stages.

3.5 Enzyme hydrolysis

Acid and alkali steam-exploded *Dendrocalamus latiflorus* pulps (biomass) were hydrolyzed with cellulases complex 50010 and 50013 (containing xylanase) from Novozymes[®]. Three enzyme loadings were equivalent to 1.5, 4.5, 9 IU endoglucanase (CMCase) / mL, 0.15, 0.44, 0.89 IU cellobiohydrolases / mL, and 1.2, 3.5, 7.1 IU xylanase/mL in reaction solutions. Hydrolysis was conducted in a total volume of 200 mL liquid with 0.05 M citrate buffer (pH 5), 2.5 % (w/v) samples in a 250 mL conical flask. The flasks was water bathed at 50^oC, shaken at 100 rpm for 96 h, and the samples were analyzed every 12 h.

3.6 Analytical methods

Dendrocalamus latiflorus chip and steam exploded samples oven-dried and hand-kneaded, screened to 40-60 mesh before the compositional analyses: ash (TAPPI T211 om-07), acid-insoluble lignin (TAPPI T222 om-06), holo-cellulose and α-cellulose (TAPPI T203 cm-09). Enzymatic hydrolysate and SSF samples were analyzed by high performance liquid chromatography (HPLC) using a ICSep ION-300 column and a RI detector for identifying organic acids, alcohols and mono sugars at 70^oC; mobile phase was 0.0085 N sulfuric acid at flow rate 0.4 mL / min. Error bars indicates the standard deviations from   triplicate experiments.

3.7 Fiber morphology

Fiber morphology measured by Morfi (TECHPAP, 10 rue de Mayencin 38400 Saint Martin d'Hères) at Chung-Hwa Pulp Corporation at Hualien. Put experimental sample into 1 L beaker then dilute with l L RO water, let it well-dispersed to consistency 0.3 % solution. Then put the 1 L beaker with experimental sample solution on the holder of Morfi measuring. Data contains Fines area, Fines length, Fiber distribution, Fiber width and etc.... MorFi define that "Fiber" : 200-10000 μm length and 5-75 μm width; "Fine" element: < 200 μm length and <5 μm width.

3.8 Powder and Fiber X-ray Diffraction

Fiber crystallinity determined with X-ray Diffraction at National Synchrotron Radiation Research Center, X-ray beam number: BL17A1 Powder and Fiber X-ray Diffraction. Fiber sample which was dispersed well to RO water solution then air-dried to 1.5 cm diameter and 1mm thickness paper sample. With silicon standard, each sample exposed 90 second on disc holder. Then calculate fiber crystallinity with Segal method (Segal et al., 1959) after Silicon standard correction.

Segal method crystallinity: Cr% = 1 − (I_{AM} / I_{200})

The I_{200} stand the overall intensity of the peak (crystalline region and amorphous region) at 2θ, then the I_{AM} is the intensity of the peak of amorphous region at 2θ.

3.9 Simultaneous saccharification and fermentation (SSF)

100 mL reaction solution, with 5 % (w / v) steam-exploded pulp, 1 % (w / v) yeast extract and 2 % (w / v) peptone, was subject to 37.5^{o}C and pH 5 in a 250 mL conical flask. The sterilized reaction solution was inoculated with 5% (v / v) *Saccharomyces cerevisiae* D5A or *Kluyveromyces marxianus var. marxianus* culture solutions with optical density at 0.1. The culture solution was prepared by   was cultivated with yeast extract 10 g / L, peptone 20 g / L,   dextrose 20 g / L for 18 h at 100 rpm, 38°C on a rotary shaker. The enzymes were added into reaction solutions with endoglucanase 9 IU / mL, cellobiohydrolases 0.89 IU / mL and xylanase 7 IU / mL at 38°C for 96 h, then the samples were analyzed every 12 h.

SSF by *Kluyveromyces marxianus var. marxianus* has the same condition with the previous; the only difference is culture and cultivated temperature at 42°C for 72 h.
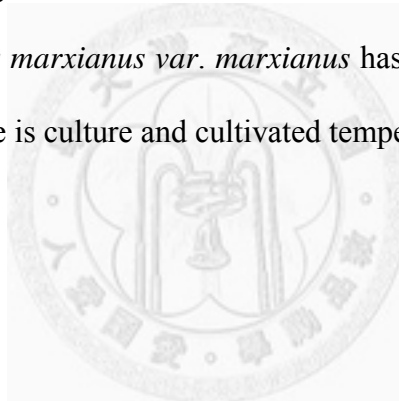
Chapter 4   Results and discussion

4.1   Chemical compositional analysis

Various yields after pretreatment were calculated (on dried bamboo basis): 57 % for acid steam explosion, 58 % (200^oC-10 min) to 83 % (180^oC-10 min) for alkali steam explosion, 45 % for kraft pulping and 42 % for bleached pulp.

Table 1. Chemical compositional analysis of *Dendrocalamus latiflorus* samples. Units are mg / g dried wood.

	Condition	Ash	Lignin	Holocellulose	α-cellulose
Raw material		17±4.36	227.7±39.6	722±66.4	427.7±41.2
Alkali steam-explosion	180^oC-10min	65±11	160±14.1	703±44	480±20.5
	190^oC-05min	54±13	148±21.8	820±81.7	598±69.7
	190^oC-10min	70±18.5	175±31.9	644±98.6	473±117.4
	190^oC-20min	46±8.1	171±44.1	702±35.2	550±49.1
	200^oC-10min	63±8.5	180±29.8	690±22.5	525.5±44.1
Acidic steam-explosion	190^oC-10min	7.6±0.5	339±44.7	526±82.9	260±63.3
Kraft pulp	Kraft pulp	6.4±1.7	25.5±3.6	928±36.8	847±41.2
	Bleached pulp	9.0±2.1	< 2	966±28.9	869±43.5

* Acid steam-exploded: 190^oC held for 10 mins at 1.26 MPa
* Alkali steam-exploded: 180^oC to 200^oC and 0.99 MPa to 1.53 MPa of 5 samples

Compositional analysis of raw material *Dendrocalamus latiflorus* and pretreated samples were listed in Table 1. Great varieties of holocellulose contents were demonstrated for alkali steam exploded pulps. Generally, the lignin contents of alkali steam exploded pulps were lower than the ones of acidic steam exploded pulps. Holocellulose contents were highest for kraft pulp and bleached pulps, their hemi-cellulose and lignin were almost eliminated at pulping process. Acidic conditions might oxidize the cellulosic components and reduced the holocellulose of the pulps (stubborn lignin content was relatively higher). In addition, the greater incomplete closure of compositional analysis for acidic steam exploded pulps, with its greater lignin content, was consistent to the above assumption. Lignin contents of alkali exploded pulps were increased with increasing treatment times. Although alkali would soften lignin in higher temperatures, soften lignin might melt onto cellulose fibrils; and lignin condensation (Kobayashi et al., 2004) would contribute the increasing lignin contents among alkali steam exploded pulps. The higher ash contents of alkali steam exploded pulps might because of the sodium ion oxide.

4.2   Enzyme hydrolysis

Enzyme hydrolysis for pretreated samples was depicted in Figure.1. Enzyme loading was 9 IU endoglucanase / mL, 0.89 IU cellobiohydrolases / mL, and 7.1 IU xylanase / mL in reaction solutions. Conversions for sugar were based on dried weight of samples. 190°C-10 min was chosen for alkali steam-exploded pulp in Figure 1.
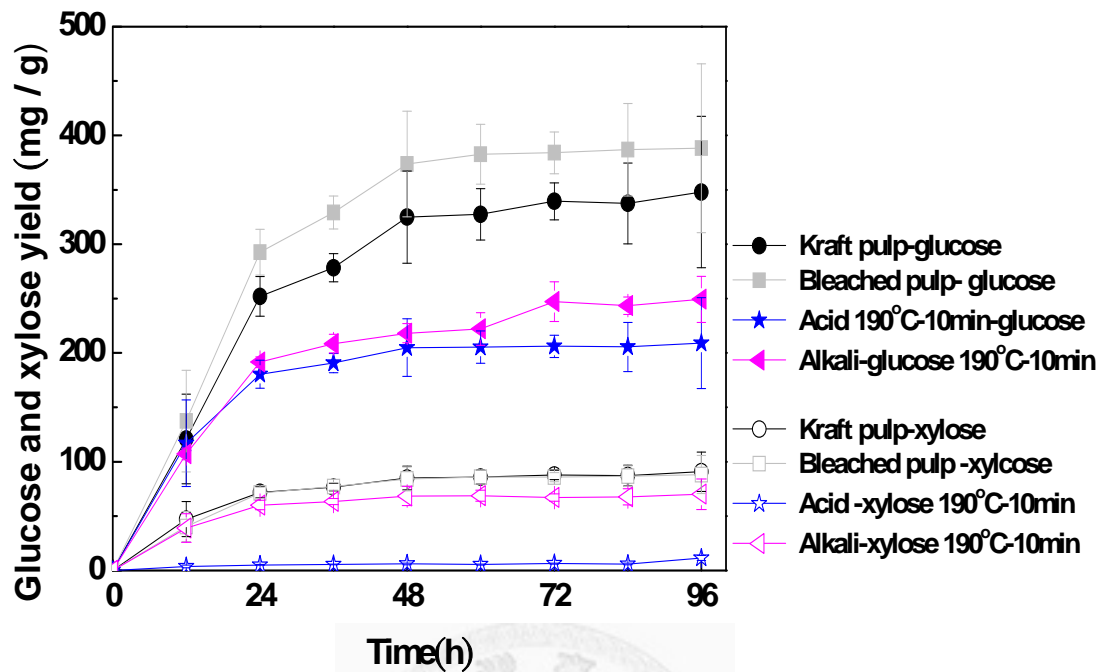
Figure 1. Enzyme hydrolysis to sugar conversion of pretreated samples with respect to time.

Glucose and xylose increased steadily prior to the first 24 h for all pulps, and the saturation was then reached at approx. 48 hours. At 96 h, the bleached pulp showed a optimal yield 388.2 $\pm$ 69 mg / g, kraft pulp 347.8 $\pm$ 77 mg / g, alkali steam-exploded (190°C-10 min) 249.3 $\pm$ 21 mg / g and acid steam-exploded 209.1 $\pm$ 41 mg / g. And the enzyme loading 1.5 and 4.5 IU hydrolysis to samples, were 24.1~ 206.4 mg / g.
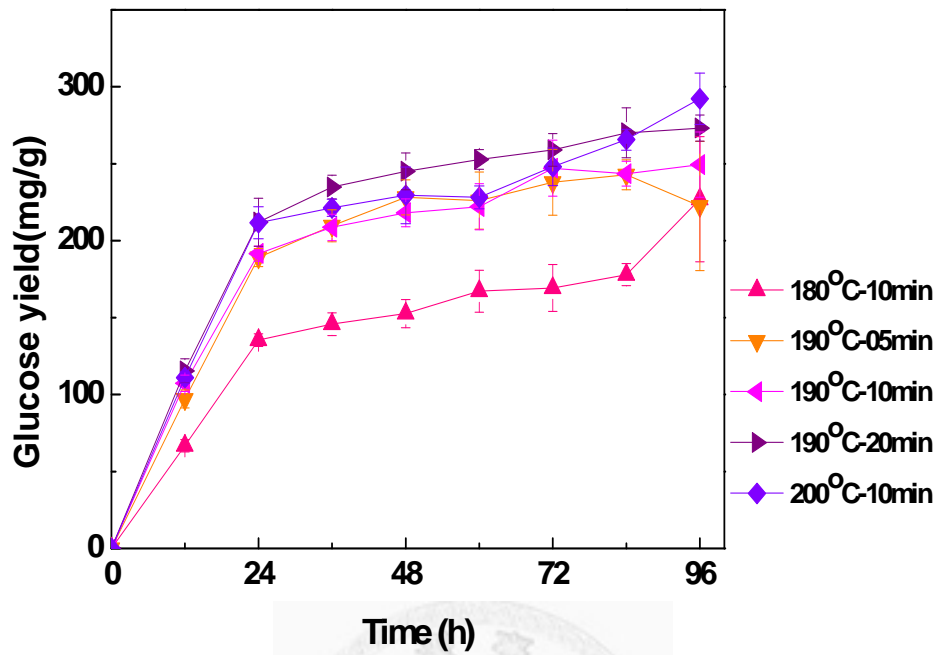
Figure 2. Enzyme hydrolysis to sugar conversion of alkali steam-exploded samples with respect to time.

Figure 2. showed hydrolysis for 5 alkali steam-exploded samples, with other conditions as same as Figure 1. Except for the pulp for 180°C-10 min, the other pulps have similar glucose yields at around 250 mg glucose per gram dried bamboo. Interestingly, the yields of 200°C-10 min pulp were still slightly growing after 48 hours.
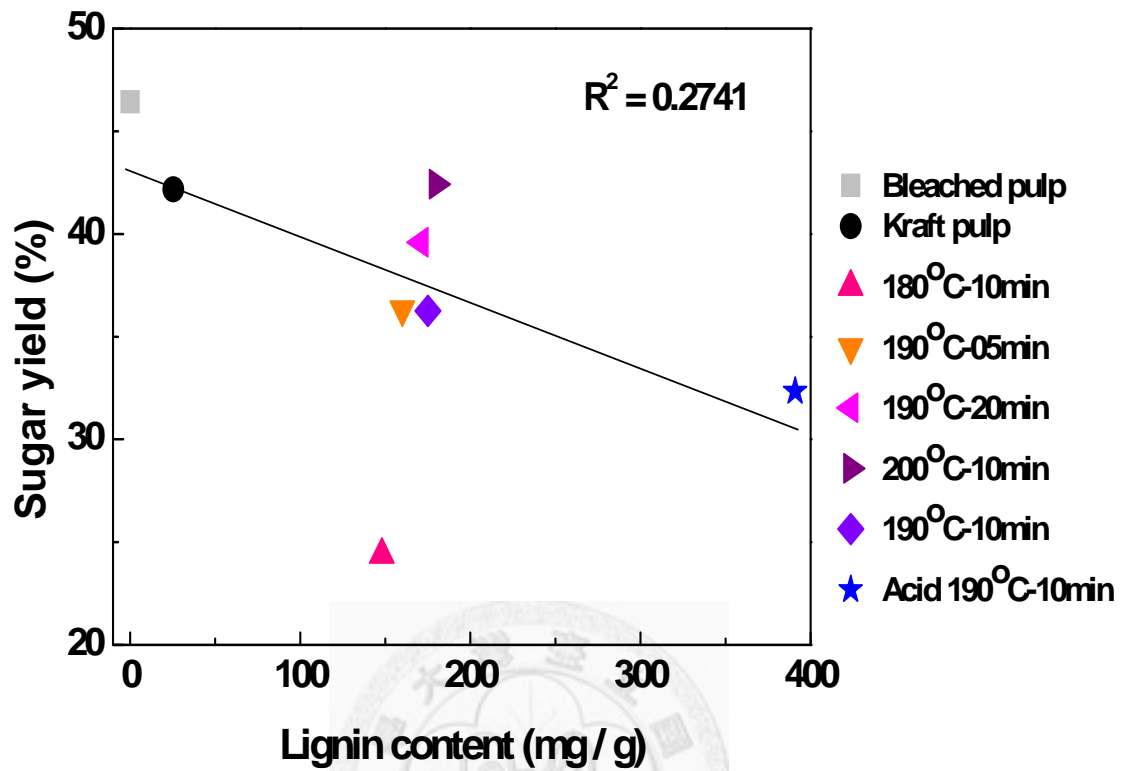
Figure 3(a) Correlation between pulp lignin contents and sugar yield during enzyme hydrolysis.
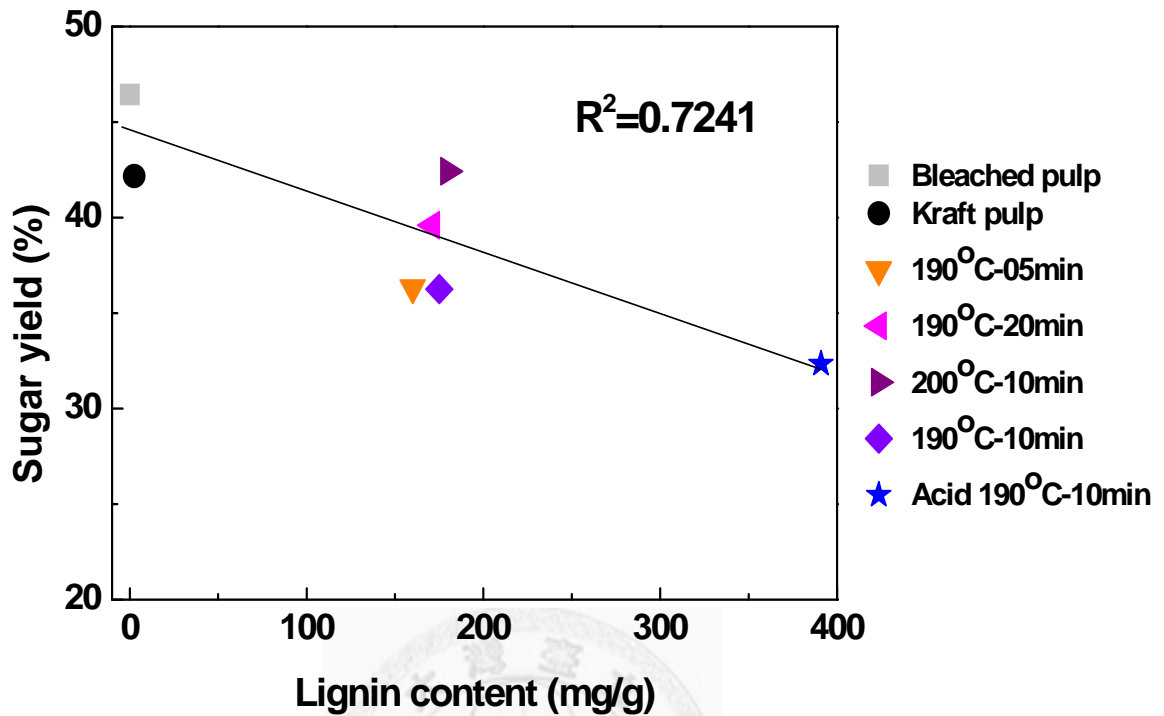
Figure 3(b) Correlation between pulp lignin contents and sugar yield during enzyme hydrolysis.

Effects of lignin contents on sugar yield during enzyme hydrolysis were shown by Figure 3a & 3b. The trend was clearly shown, although the correlation was not clear (Figure 3a shown). If the $180^{o}C$-10 min pulp were removed, the correlation would be more obvious (Figure 3b shown). There was almost no correlation for lignin contents and sugar yield among all alkali steam-exploded samples. The lowest sugar yield of $180^{o}C$-10 min pulp suggested that other factors, like fiber dimensions, might also play important roles during enzyme hydrolysis. Since the mechanical separation of $180^{o}C$-10 min pulp during alkali steam explosion should be the least.

4.3 Fiber morphology

The lowest sugar yield of 180°C-10 min pulp suggested that other factors, like fiber dimensions, might also play important roles during enzyme hydrolysis. As Table 2. shown, kraft and bleached pulp had more fibers, less coarseness, less broken end(%) (maybe long and thin fiber-shaped); Acidic pulp had least fiber, largest coarseness, largest broken end(%) and largest fine content(%) of these pulps; the alkali steam-exploded pulps (180°C-10 min~200°C-10 min) had familiar morphologies, but the 180°C-10 min pulp was slightly outstanding. Compared with other alkali steam-exploded pulps, the 180°C-10 min pulp has little numbers of fibers, bigger coarseness, and more fine content. It might concerned that the 180°C-10 min pulp wasn't enough mechanical separated from chip to fibers during steam-explosion which may cause the lowest sugar yield.

Table 2. Fiber morphology of different pretreated pulps. (Acidic steam-exploded, alkali steam-exploded 180°C-10 min~200°C-10 min, kraft pulp and bleached pulp)

Pulps	Fibers (million/g)	Arithm. length (μm)	Weighted length (μm)	Width (μm)	Coarseness (mg/m)	Microfibrills length rate (%)	Broken end (%)	Fine (%) (in length)	Fine (%) (in area)
Kraft	5.41	1.14	1.54	15.8	0.16	0.17	13.61	36.10	16.47
Bleached	5.61	1.11	1.46	15.6	0.16	0.14	12.27	22.70	5.65
Acidic	1.51	0.57	0.83	23.8	0.97	1.03	44.01	93.80	63.37
Alkaline									
180-10	2.22	1.12	1.49	18.5	0.38	0.24	20.07	51.70	22.84
190-05	3.47	1.30	1.95	20.6	0.21	0.46	17.86	36.30	10.15
190-10	3.82	1.18	1.60	19.1	0.21	0.25	17.45	40.30	14.12
190-20	3.10	1.19	1.57	18.0	0.26	0.24	16.85	35.70	9.45
200-10	3.09	1.15	1.50	18.1	0.28	0.22	14.7	37.80	15.35

* There are not significantly different according to the Kruskal-Wallis test by SPSS (Average of triplicate measurements).

Table 3.   Fiber morphology of different enzyme dosages hydrolysis acidic
steam-exploded pulp.

Dosage(% v/w) - time (h)		Control	2-24	2-96	6-24	6-96	12-24	12-96
Fiber length(μm)		1025±7.95	757±35.2	896±6.14	586±3.15	479±7.28	592±4.14	393±2.01
Fiber width(μm)		23.8±0.09	25.2±1.15	23.8±0.14	26.1±0.15	25.4±0.15	24.5±0.98	33.4±2.0
Fines length(μm)		28.1±2.52	29.3±0.73	29.3±0.21	28.4±0.58	28.7±0.43	28.2±0.56	29.5±0.71
Fines content (%)	in length	93.8±3.28	94.8±5.11	95.2±4.03	95.9±2.77	97.4±1.07	97.5±0.49	97.8±2.23
	in area	63.37±2.91	67.5±1.13	71.41±1.93	73.41±3.96	85.14±3.32	80.79±3.88	78.37±1.85

*Average of triplicate measurements. There are not significantly different according to
the Kruskal-Wallis test by SPSS

The Fiber morphology of different enzyme dosages hydrolysis by time courses of pulps was listed in Table 3. ~ Table 10. They had a trend that more enzyme dosage and more time of hydrolysis contributed the less fiber length, the more fiber width and the more fines contents (Table 4 excepted).

Enzyme may break long fibers into wider and shorter pieces (fines), hydrolysis then to mono sugar. MorFi define that "Fiber": 200-10000 μm length and 5-75 μm width; "Fine" element: < 200 μm length and <5 μm width. When enzyme hydrolyzed, might produce more "fibers" from the larger pulp-fiber (>10000 μm length).

The coarseness (mg / m) of fiber may represent the fiber diameter (Table2), and there were not apparent trance. Interestingly, the acid steam-exploded pulp had largest coarseness, fiber width and shortest fiber length.

Table 4. Fiber Morphology of different enzyme dosages hydrolysis alkali
steam-exploded pulp 180°C-10 min.

Dosage(% v/w) - time (h)	Control	2-24	2-96	6-24	6-96	12-24	12-96
Fiber length(μm)	1750±11.66	1601±13.81	1755±6.47	1742±24.6	1682±21.6	1801±16.78	2074±46.6
Fiber width(μm)	18.5±0.06	17.1±0.11	18.2±0.09	16.6±0.13	17.0±0.03	16.8±0.60	15.6±0.53
Fines length(μm)	32±0.02	31.3±0.13	32.3±0.11	31.9±0.14	31.8±0.08	29.4±0.11	29.8±0.15
Fines content (%) in length	51.7±1.12	62.8±1.38	39.3±1.01	44.8±1.03	45.4±0.89	20.5±0.07	11.3±1.44
Fines content (%) in area	22.84±0.13	36.76±0.16	11.79±0.08	18.01±0.10	14.44±0.03	20.5±0.09	11.3±0.18

*Average of triplicate measurements. There are not significantly different according to
the Kruskal-Wallis test by SPSS

Table 5. Fiber Morphology of different enzyme dosages hydrolysis alkali
steam-exploded pulp 190°C-05 min.

Dosage(% v / w) - time (h)	Control	2-24	2-96	6-24	6-96	12-24	12-96
Fiber length(μm)	2424±15.85	1976±8.77	1725±15.31	1798±17.1	1841±10.21	1830±17.82	1642±12.99
Fiber width(μm)	20.6±1.03	17.6±1.02	17.5±0.89	16±0.03	17.6±0.08	16.1±0.08	17.3±0.09
Fines length(μm)	42.3±0.36	32.6±0.25	32.9±0.22	32.0±0.11	30.4±0.24	32.7±0.24	25.1±0.19
Fines content (%) in length	36.3±1.01	39.1±1.89	49.1±1.190	47.7±1.30	58.7±0.39	52.3±1.84	65.5±1.33
Fines content (%) in area	10.15±0.09	16.18±0.03	19.22±0.04	22.36±0.01	17.82±0.18	20.61±0.17	28.5±0.18

*Average of triplicate measurements. There are not significantly different according to
the Kruskal-Wallis test by SPSS

Table 4 shows the opposite trance to the other pulps when enzyme hydrolysis
(fiber length extend, less fines). The result may have relatives to the pretreated
condition 180°C-10 min which wasn't enough to fiber well-separated (more possible to
cut pulp into the "fiber definition period").

Table 6. Fiber Morphology of different enzyme dosages hydrolysis alkali steam-exploded pulp 190°C-10 min

Dosage(% v/w) - time (h)	Control	2-24	2-96	6-24	6-96	12-24	12-96
Fiber length(μm)	1900±13.3	1914±13.97	1885±12.44	1960±11.56	1865±12.31	1607±8.96	1704±10.21
Fiber width(μm)	19.1±1.52	17.6±0.35	19.6±0.98	17.2±0.57	17.8±1.01	16.6±0.16	16.0±0.25
Fines length(μm)	42.5±0.19	32.4±0.51	31.7±0.77	30.3±0.69	29.3±0.43	32.4±0.21	28.5±0.11
Fines content (%) in length	40.3±0.94	45.6±0.69	48.8±0.70	45.7±1.06	57.5±1.04	50.8±0.22	68.5±1.19
Fines content (%) in area	14.12±0.09	14.43±0.15	20.76±0.14	13.52±0.11	17.2±0.06	21.03±0.09	28.48±0.19

*Average of triplicate measurements. There are not significantly different according to the Kruskal-Wallis test by SPSS

Table 7. Fiber Morphology of different enzyme dosages hydrolysis alkali steam-exploded pulp 190°C-20 min.

Dosage(% v/w) - time (h)	Control	2-24	2-96	6-24	6-96	12-24	12-96
Fiber length(μm)	1853±22.87	1725±8.83	1603±9.47	1825±6.73	1797±11.35	1735±2.13	1602±9.54
Fiber width(μm)	18.0±0.09	17.2±0.07	17.0±0.11	16.8±0.05	16.5±0.03	15.7±0.08	16.9±0.17
Fines length(μm)	33.0±1.24	35.6±1.50	32.8±0.89	31.0±0.77	29.3±0.24	30.2±0.11	32.3±0.15
Fines content (%) in length	35.7±0.99	37.0±0.81	42.5±0.81	48.3±0.71	53.3±0.91	46.2±0.42	55.5±0.41
Fines content (%) in area	9.45±0.01	9.83±0.03	4.24±0.08	13.2±0.07	17.02±0.04	16.36±0.05	20.2±0.03

*Average of triplicate measurements. There are not significantly different according to the Kruskal-Wallis test by SPSS

Table 8.   Fiber Morphology of different enzyme dosages hydrolysis alkali steam-exploded pulp 200°C-10 min.

Dosage(% v/w) - time (h)		Control	2-24	2-96	6-24	6-96	12-24	12-96
Fiber length(μm)		1740±24.98	1837±10.89	1730±14.37	1729±7.59	1629±11.74	1609±5.35	1397±5.29
Fiber width(μm)		18.1±0.11	17.50.09	18.9±0.03	15.9±0.04	17.2±0.08	16.4±0.07	17.1±0.07
Fines length(μm)		37.1±1.09	35.5±0.89	46.6±0.77	50.0±0.71	47.3±0.49	46.8±0.80	31.3±0.91
Fines content (%)	in length	37.8±0.81	38.2±0.95	46.6±0.61	50.0±0.74	47.8±0.96	46.8±0.74	59.1±0.77
	in area	15.35±0.08	14.06±0.07	14.65±0.07	14.81±0.09	15.24±0.06	16.47±0.07	24.08±0.11

*Average of triplicate measurements. There are not significantly different according to the Kruskal-Wallis test by SPSS

Table5 to Table8 showed similar trend of alkali steam explode pulps, and figured out that the higher temperature and persist time make the shorter fiber length, shorter fiber width, less fines content and less fiber numbers. The stronger pretreated condition may harm the fiber and suggested that condition should not over 190°C-10 min.

Table 9. Fiber Morphology of different enzyme dosages hydrolysis kraft pulp

Dosage(% v/w) - time (h)		Control	2-24	2-96	6-24	6-96	12-24	12-96
Fiber length(μm)		1847±14.35	1618±8.95	1340±9.82	1549±9.23	753±6.13	1349±8.52	1438±8.61
Fiber width(μm)		15.8±0.16	17.4±0.23	18.7±0.33	18.6±0.09	19.3±0.16	18.6±0.07	19.3±0.03
Fines length(μm)		50.1±0.89	47.3±0.91	44.5±1.13	49.6±0.08	36.3±0.33	49.3±0.82	32.6±0.10
Fines content (%)	in length	36.1±1.13	41.4±0.98	34.2±0.55	46.5±0.16	87.2±1.53	50.8±1.30	86.0±0.44
	in area	16.47±0.15	18.09±0.09	12.99±0.11	20.51±0.17	68.5±2.11	24.04±1.05	53.06±1.13

*Average of triplicate measurements. There are not significantly different according to the Kruskal-Wallis test by SPSS

Table 10. Fiber Morphology of different enzyme dosages hydrolysis bleached pulp

Dosage(% v/w) - time (h)		Control	2-24	2-96	6-24	6-96	12-24
Fiber length(μm)		1754±14.43	1305±7.2	691±5.87	1152±7.75	569±3.66	748±6.73
Fiber width(μm)		15.6±0.08	18.5±0.11	20.0±0.06	19.4±0.17	23.9±0.12	20.3±0.03
Fines length(μm)		33.3±1.30	48.9±1.26	46.1±0.13	50.6±0.13	36.0±0.04	37.2±0.09
Fines content (%)	In length	22.7±0.96	52.7±1.03	79.2±1.08	66.4±0.89	96.8±0.06	93.2±0.11
	in area	50.65±0.25	25.15±0.06	57.08±0.19	38.44±0.03	83.4±1.23	80.46±0.13

*Average of triplicate measurements. There are not significantly different according to the Kruskal-Wallis test by SPSS

Table9 and Table10 showed the fiber morphology difference of kraft pulp and bleached pulp when enzyme hydrolysis. Bleaching may harm the fibers but enhance the enzyme works. The condition of bleached pulp 12 dosage(% v/w)- 96 (h), was hydrolysis too complete to MorFi measurement.

4.4　Crystallinity by XRD (Powder and Fiber X-ray Diffraction)

Crystallinity differences of experimental pulps were shown as Table 11. Original *Dendrocalamus latiflorus*'s crystallinity 47.41% then decreased after acidic and alkaline steam explosion treatment, except the 200$^{\mathrm{o}}$C-10 min and the 190$^{\mathrm{o}}$C-10 min were slightly increased.　After kraft pulping and bleaching, crystallinity also increased more.

Table 11. Crystallinity (%) of experimental pulps (acidic steam-exploded 190$^{\mathrm{o}}$C-10 min, alkali steam-exploded 180$^{\mathrm{o}}$C-10 min ~ 200$^{\mathrm{o}}$C-10 min, kraft pulp and bleached pulp, I_{AM} and I_{200} as peak intensity)

Pulps		I_{AM}	I_{200}	Cr (%)
Dendrocalamus latiflorus	chip	2238.343	4255.914	47.41
Alkali	180 $^{\mathrm{o}}$C -10 min	1643.475	930.454	43.38
	190 $^{\mathrm{o}}$C -05 min	1234.533	701.879	43.14
	190 $^{\mathrm{o}}$C -10 min	1478.297	753.820	49.01
	190 $^{\mathrm{o}}$C -20 min	938.867	520.689	44.54
	200 $^{\mathrm{o}}$C -10 min	1378.282	720.038	47.75
Acidic	190 $^{\mathrm{o}}$C -10 min	385.698	221.668	42.52
Kraft pulp	Kraft pulp	6152.968	2531.700	58.85
	Bleached pulp	7528.223	2721.780	63.84

Table 12. Crystallinity (%) of enzyme hydrolysis experimental pulps in 96h. (acidic steam-exploded 190$^{\mathrm{o}}$C-10 min, alkali steam-exploded 190$^{\mathrm{o}}$C-10 min, bamboo kraft pulp and bleached pulp.

Time (h)	Control	24	48	96
Alkali	52.65	38.58	30.64	53.68
Acidic	42.52	50.73	33.87	50.93
Bleached pulp	63.84	63.79	62.23	65.89
Kraft pulp	58.85	62.87	57.52	56.07

Acidic steam-exploded, alkali steam-exploded (190°C-10 min), kraft pulp and bleached pulp were enzyme hydrolysis for 96h, Crystallinity changed in time course shown as Table 12. There is no obviously correlation between crystallinity and enzyme hydrolysis in time course, which were hold identical views with Mansfield et al.(1999) and Lynd et al.(2002). The crystallinity still isn't the hydrolysis key factor and the uncertain of crystallinity measurements may also contribute that.

4.5   Effect of simultaneous saccharification and fermentation (SSF) on ethanol yield
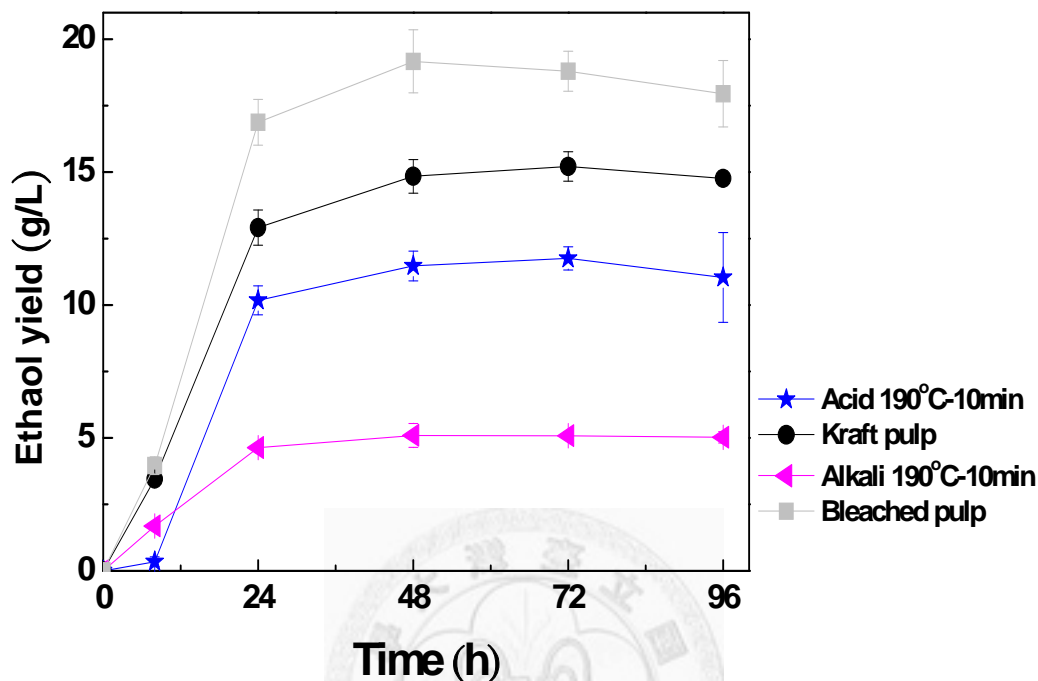
Figure 4. Ethanol yields for acidic, alkali (190°C-10 min) steam-exploded pulp, kraft and bleached pulps by SSF by *Saccharomyces cerevisiae* D$_5$A.

Ethanol yields for acid, alkali steam-exploded pulp, kraft and bleached pulps by SSF were shown by Figure 4. As expected, the ethanol yields of bleached and kraft pulps were higher than the values of acid and alkaline (190°C-10 min) steam exploded pulps. Although the lignin content of alkaline 190°C-10 min pulp was lower than the value of acid steam exploded pulps, melted lignin in alkali conditions might further hamper enzyme hydrolysis and fermentation.
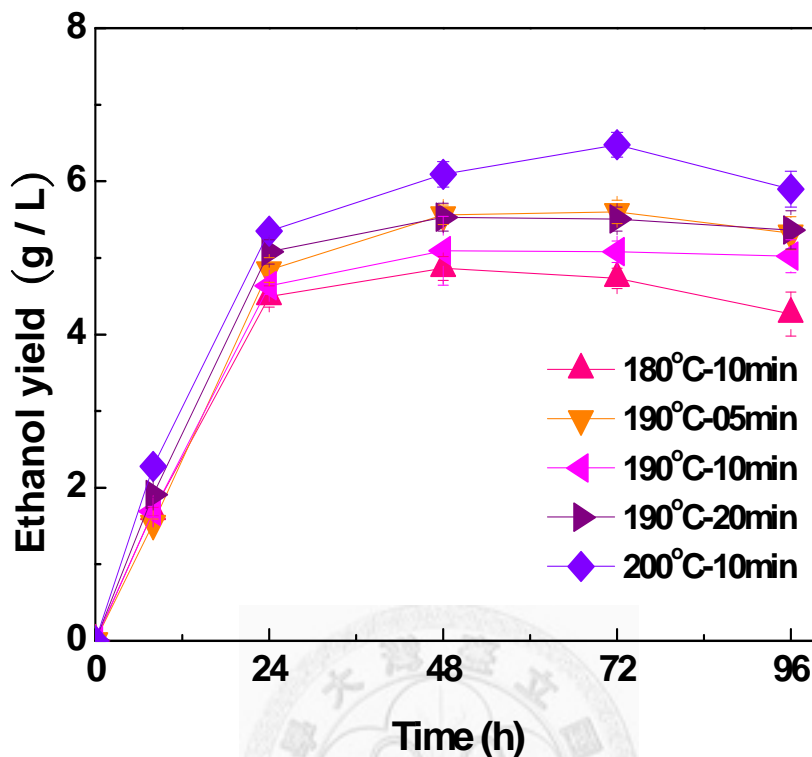
Figure 5. Ethanol yields for five alkali steam-exploded pulps by SSF by *Saccharomyces cerevisiae* D_5A.

Ethanol yields for five alkali steam-exploded pulps by SSF were shown by Figure 5. The yields increased steadily for the first 24 hours; then the optimal yields were reached after the next 48 hours. The yield from 200 °C-10 min pulp was the most, and the yield from 180 °C-10 min pulp was the least. With the respect of results from Table 1, lignin contents were not the deciding factor for the above observation.
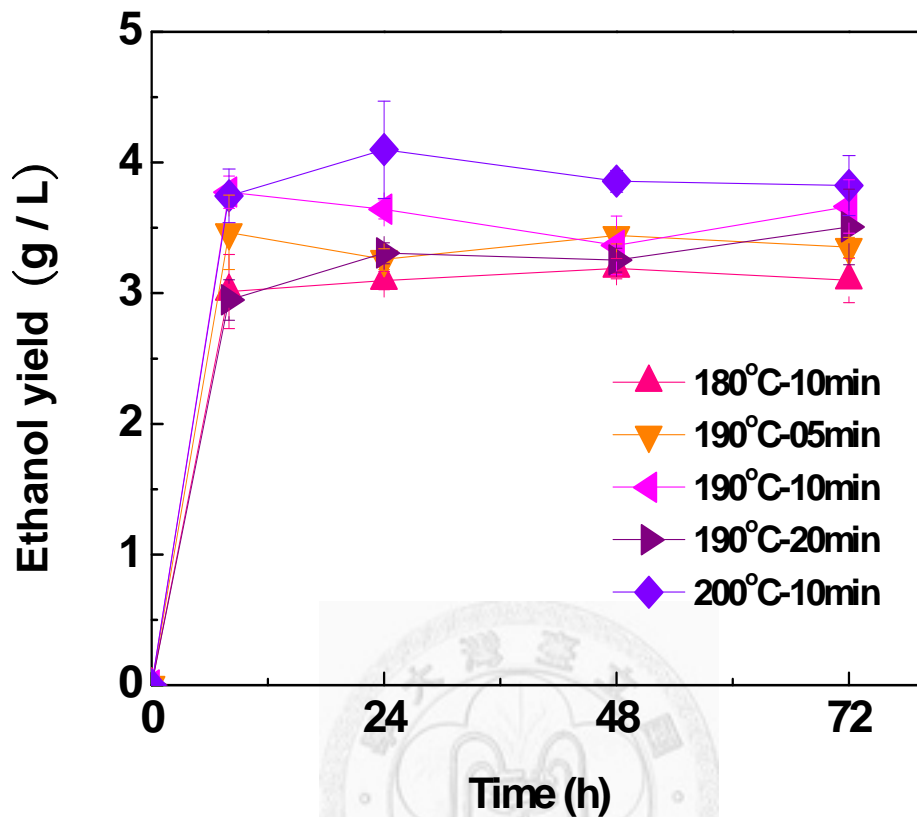
Figure 6. Ethanol yields for five alkali steam-exploded pulps by SSF by *Kluyveromyces marxianus var*. *marxianus*.

Ethanol yields for five alkali steam-exploded pulps by SSF by *Kluyveromyces marxianus var*. *marxianus*. were shown by Figure 6. The yields increased for the first 12 hours; then the optimal yields were reached after the next 24 hours. The yield from 200 ^{o}C-10 min pulp was the most, and the yield from 180 ^{o}C-10 min pulp was the least. The most yield from 200 ^{o}C-10 min pulp by *K. marxianus var*. *marxianus*. was 81.95 mg / g O.D. pulp, about 4.1 g / L which was only 63% ethanol yield than which by *S. cerevisiae* D_5A.

Chapter 5　Estimated feedstock volume and ethanol production energy balance

5.1　Estimated the bamboo annually growth in Taiwan

Table 13 and Table 14 showed biomass stock of bamboo in one hectare and bamboo area in Taiwan. Suppose that the top3 bamboo species stock volume in Taiwan as all bamboos represented. With optimistic estimated, multiplied Table 13. bamboo stock ton per ha by Table 14. the bamboo area (ha) in Taiwan. There is 5,020,000~11,640,000 ton optimistic stock volume in bamboo stands in Taiwan.

Table 13. Number, volume, biomass stock and carbon sequestration of bamboo culms in one hectare.　(Wang, 2004 and 2006)

Top3 bamboo species in Taiwan	Number (No./ha)	Volume (m³/ha)	Biomass stock (ton/ha)	Carbon sequestration (ton/ha)
Phyllostachys pubescens	7420	66.7	46.6	22.6
Phyllostachys makinoi	18000	45.5	33.6	16.3
Dendrocalamus latiflorus	9085	137.5	77.9	37.7

Table 14. The bamboo area and number of bamboo culms in one hectare in Taiwan. (Taiwan Forestry Bureau, 2001)

	10^4(No./ha)	Area(ha)		10^4(No./ha)	Area(ha)
Taipei	111,90	10113	Yunlin	2,784	7,033
Keelung	1,115	717	Chiayi	4,746	21,800
Taoyuan	9,711	6519	Tainan	5,721	16,074
Hsinchu	14,136	9591	Kaohsiung	4,836	15,428
Miaoli	22,790	15334	Pingtung	267	918
Taichung	3,836	5912	Ilan	4,037	2,648
Changhua	756	1171	Hualien	14,033	8,598
Nantou	6,972	22310	Taitung	39,595	5,359

It generally takes about 3~4 years for bamboo growth to utilization. If rotation was set as 5 year, its annual growth would be 1,000,000~2,320,000 ton (with the average of 1,660,000 ton). There could be 1,328,000 ton available as feedstock for bioethanol production annually, with the deduction of underground portions and loss during processing (Wang, 2004). As Table 15. shown, 174,100 tons of bioethanols could be produced annually by acidic steam explosion (total conversion 13.11 %) and by bleached pulp 211,900 tons (total conversion 15.96 %). The ethanol conversion in this study could be compared with the olive pruning (hardwood) by Cara et al. (2008), 7.2 % ethanol conversion from raw material without 5-C sugar co-fermentation.

Table 15. Annual ethanol production from *Dendrocalamus latiflorus* by SSF.

Pulps		Raw material to ethanol (%)	Estimate yield (ton / Yr)
Alkali	180°C-10 min	8.21	109,000
	190°C-05 min	8.80	116,900
	190°C-10 min	7.60	100,900
	190°C-20 min	6.93	92,000
	200°C-10 min	7.54	100,100
Acid	190°C-10 min	13.11	174,100
Kraft pulp	Kraft pulp	13.50	179,300
	Bleached pulp	15.96	211,900

*Alkali steam explosion condition from 180°C-10 min to 200°C-10 min
*Estimated bamboo growth 1,328,000 ton annually as feedstock

5.2　　Steam explosion ethanol energy consumption estimation

Energy consumption analyses for production of fuel ethanol from lignocellulosic biomass steam explosion processing were followed by the process of Institute of Nuclear Energy Research (2011).

Table 16. Energy consumption estimation for ethanol by steam explosion and SSF

Energy Use (MJ / L ethanol)	References	Acid	Alkali
Pretreatment	**18.68**	**23.68**	**34.44**
Electricity	2.56	3.20	4.65
Steam	16.00	20.00	29.09
Sulphuric Acid Production	0.12	0.15	0.22
Alkali Production	0.27	0.34	0.49
SSF	**7.14**	**8.93**	**12.99**
Electricity	5.61	7.01	10.20
Enzyme	1.47	1.84	2.68
Yeast	0.06	0.08	0.12
Product Recovery	**8.67**	**10.84**	**15.77**
Steam	8.2	10.25	14.91
Cooling Water	0.26	0.33	0.48
Wastewater Treatment	0.17	0.21	0.31
Dehydrate	0.04	0.05	0.07
Total Energy Use	36.17	44.35	64.51

The Institute of Nuclear Energy Research (2011) produced 1 L ethanol from feedstock rice hull 5.0 kg (0.2 L / kg conversion). In this study, produced 1 kg ethanol from acidic steam explosion pretreatment need 6.25 kg *Dendrocalamus latiflorus* chip (0.16 L / kg conversion); produced 1 kg ethanol from alkali steam explosion (190°C-05 min) pretreatment need 9.09 kg *Dendrocalamus latiflorus* chip (0.11 L / kg conversion). Energy consumption estimation for ethanol by steam explosion and SSF, expressed by MJ per liter ethanol, were listed in Table 16. (Ethanol density: 0.789 g/ cm^3)

5.3   Estimated energy consumption of ethanol from kraft pulp

Kraft pulping and bleaching energy consumption estimation was calculated from Chung-Hwa Pulp Corporation factory 2009 statistics:

CHP's bleaching pulp consumption   Electricity 334 kWh / ton pulp (O.D.)

                                  Fuel oil 159.5L / ton pulp (O.D.)

Fuel oil per L 9200 kcal

159.5 (L) * 9200(kcal / L) = 1467396.81 kcal

Converse to MJ (energy consumption)

Fuel oil 159.5L / ton pulp (O.D.) = 6143 MJ / ton pulp (O.D.)

Electricity 334 kWh / ton pulp (O.D.) = 1202MJ / ton pulp (O.D.)

Total energy consumption (Kraft pulping): 1202 + 6143 = 7345MJ / ton pulp (O.D.)

Chung-Hwa Pulp didn't separate kraft pulping part and bleaching, so multiple kraft / bleached pulp energy consumption ratio:

Kraft pulp Electricity = 1202MJ / ton pulp (O.D.) * 8.5 / 9.75 = 1047 MJ

Kraft pulp Fuel oil   = 6143 MJ / ton pulp (O.D.) * 445 / 565 = 4838 MJ

Total energy consumption (Bleached pulping): 1047 +4838 = 5885MJ / ton pulp (O.D.)

Kraft pulping and bleached pulping energy consumption estimation：

Kraft pulping: 5885 MJ / ton pulp (O.D.)

Bleached pulping: 7345 MJ / ton pulp (O.D.)

Pulping energy consumption multiple SSF conversion (from pulp to ethanol):

Kraft pulping:

5885 MJ / ton pulp (O.D.) / 30% = 19.62 MJ / kg EtOH =15.48 MJ / L EtOH

Bleached pulping:

7345 MJ / ton pulp (O.D.) / 38% = 19.33 MJ / kg EtOH=15.25 MJ / L EtOH

In this study, produced 1 L ethanol by kraft pulp pretreatment need 5.85 kg *Dendrocalamus latiflorus* chip (0.17 L / kg conversion); produced 1 kg ethanol by bleached pulp pretreatment need 5.0 kg *Dendrocalamus latiflorus* chip (0.20 L / kg conversion). Energy consumption estimation for ethanol by kraft pulp or bleached pulp through SSF, expressed by MJ per L ethanol, was listed in Table 17. (Ethanol density: 0.789 g/ cm^3)

Table 17. Energy consumption estimation for ethanol by kraft pulp or bleached pulp

Energy Use (MJ / L ethanol)	Kraft pulp	Bleached pulp
Pulping	**15.48**	**15.25**
SSF	**8.40**	**7.14**
Electricity	6.60	5.61
Enzyme	1.73	1.47
Yeast	0.07	0.06
Product Recovery	**10.20**	**8.67**
Steam	9.65	8.2
Cooling Water	0.31	0.26
Wastewater Treatment	0.20	0.17
Dehydrate	0.05	0.04
Total Energy Use	**34.08**	**31.06**

5.4　Estimated energy use of compacting the fermented residue

Experimental pulps would separate liquor (ethanol contained) from fermented solid residue after simultaneous saccharification and fermentation. The residue could still be used as solid-compacting fuel after compacting. Experimental pulps yield (per kg *Dendrocalamus latiflorus* chip) after pretreatment, SSF and its ash content as Table 18. shown. HHV (High Heating Value) shown as Table 19. which calculated from the ultimate analysis of samples by the following formula: HHV, HHV_{ult} and HHV_{prox} (Sheng et al., 2005；Demirbas, 1997). The carbon content was the decisive factor of HHV, after SSF the carbon content of residues increased and HHV increased except the pulp 200°C-10 min was decreased.
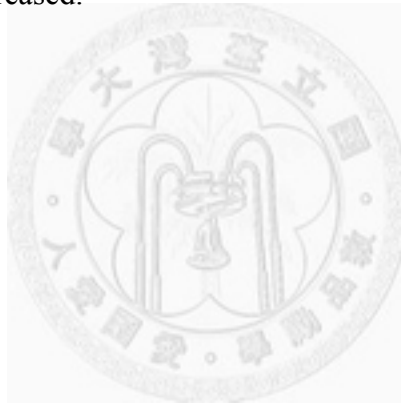
Table 18. Yield of pulps after pretreatment, SSF and its ash content.

From 1 kg *Dendrocalamus latiflorus*	Pretreatment yield (g)	Ash(g)	SSF *(g)	SSF -Ash(g)
Alkali				
180°C -10 min	830	17	586.62	92.6
190°C-05 min	800	65	681.05	77.0
190°C-10 min	760	54	572.96	99.8
190°C-20 min	630	70	400.42	66.0
200°C-10 min	580	46	375.78	89.1
Acid explo.	570	76	152.76	110.0
Kraft pulp	450	6.4	151.42	9.3
Bleached pulp	420	9.0	54.205	12.8

*Add 200g yeast extract and 400g peptone when SSF

Formula:

High heating values: HHV (MJ / kg) =

$\{33.5[C] + 142.3[H] - 15.41[O] - 14.5[N]\} \times 10^{-2}$

Ultimate analysis formula: HHV_{ult} (MJ / kg) =0.3259 Carbon content (%) + 3.4597

Proximate analysis formula: HHV_{prox} (MJ / kg) =19.914 -0.2324 Ash (%).

*(Sheng et al., 2005；Demirbas, 1997)

Table 19. Ultimate analysis of samples and its HHV (MJ / kg) calculated from Sheng et al., 2005； Demirbas, 1997.

Samples	C (%)	H (%)	N (%)	O (%)	Ash (%)	HHV (MJ / kg)	HHV$_{ult}$	HHV$_{prox}$
Dendrocalamus latiflorus	46.91	6.46	0.39	44.05	1.70	18.06	18.75	19.52
Acidic	54.56	5.83	0.44	31.09	7.6	21.71	21.24	18.15
AFN	60.02	5.50	1.22	21.77	11.0	24.40	23.02	17.36
AFW	61.68	5.41	0.93	15.69	15.8	25.80	23.56	16.24
Kraft pulp	42.46	6.49	0.10	49.81	0.64	15.77	17.30	19.77
KFN	44.34	6.61	5.08	42.55	0.93	16.97	17.91	19.70
KFW	45.00	6.44	0.57	45.57	1.93	17.13	18.12	19.47
Bleached pulp	42.18	6.51	0.10	49.81	0.90	15.70	17.21	19.70
BFN	43.94	6.90	7.51	39.88	1.28	17.30	17.78	19.62
BFW	43.98	6.87	0.31	45.68	2.66	17.43	17.79	19.30
1810	43.16	6.07	0.27	43.52	6.50	16.34	17.52	18.40
1810FN	44.44	6.23	2.67	36.91	9.26	17.67	17.94	17.76
1810FW	45.53	6.37	0.93	26.43	20.26	20.10	18.30	15.21
1905	43.64	6.14	0.27	44.06	5.40	16.53	17.68	18.66
1905FN	43.20	6.15	2.85	39.60	7.70	16.71	17.54	18.12
1905FW	45.76	6.38	0.84	36.30	10.23	18.69	18.37	17.54
1910	43.61	6.21	0.30	42.39	7.00	16.86	17.67	18.29
1910FN	44.09	6.24	2.06	37.14	9.98	17.63	17.83	17.59
1910FW	45.51	6.43	0.95	26.87	19.75	20.12	18.29	15.32
1920	44.32	6.26	0.30	44.02	4.60	16.93	17.90	18.84
1920FN	44.19	6.25	1.70	40.77	6.60	17.17	17.86	18.38
1920FW	43.99	6.33	0.30	36.17	12.72	18.13	17.79	16.96
2010	45.60	6.43	1.02	40.16	6.30	18.08	18.32	18.45
2010FN	44.79	6.33	1.88	37.61	8.91	17.94	18.06	17.84
2010FW	45.69	6.55	0.73	29.02	17.53	20.04	18.35	15.84

*FN: pulps after SSF without washed, FW: pulps after SSF with washed；
O (%) calculated from 99.5% minus C (%), H (%), N (%) and Ash (%).

Table 20. Energy consumption of the compacting process from 1 kg material. (MJ / kg)

Pulps	Dehydrate(kg)	Consumption energy (MJ)	Compacted residue (kg)	Consumption energy (MJ)
Alkali				
180°C -10 min	0.18	0.41	0.59	0.014
190°C-05 min	0.21	0.47	0.68	0.016
190°C-10 min	0.17	0.38	0.57	0.013
190°C-20 min	0.12	0.27	0.40	0.009
200°C-10 min	0.12	0.27	0.38	0.009
Acidic explo.	0.05	0.11	0.15	0.004
Kraft pulp	0.05	0.11	0.15	0.004
Bleached pulp	0.02	0.05	0.05	0.001

*SSF-residue moisture content was 33% after squeezing then dehydrate to 15% before compacting, and the vaporization heat 2.26 (MJ / kg). Compacted process energy consumption 23.5 (kJ / kg) from Mani et al. (2006).

Table 20. listed the energy consumption (MJ / kg) in the SSF residue compacting process from 1 kg *Dendrocalamus latiflorus* material. SSF-residue moisture content was about 33% after squeezing, and then dehydrated to 15 % before compacting. The dehydrated energy consumption calculated as vaporization heat of water, 2.26 (MJ / kg). Acidic steam explosion for example, SSF-residue oven-dried weight 0.15 kg, moisture content 33%, the wet residue weight 0.1995 kg, eliminated 23 % moisture content from the wet residue, 0.05 kg water consumed energy 0.11 MJ.   Energy consumption in the compacting process followed Mani et al. (2006), 23.47 MJ per ton (0.0235 MJ / kg).

5.5   Energy and mass balance

Dendrocalamus latiflorus chip was pretreated to pulps through ethanol-production process and residue compacted, then ethanol and compacting fuel produced.   The energy content (HHV) and mass difference from ethanol production process were listed in Table 21. *Dendrocalamus latiflorus* chip HHV (18.06 MJ / kg), then steam explosion, kraft and bleached pulps were about 6.59~14.46MJ left.   Acidic steam explosion pulp (21.71 MJ / kg) for example, 0.57 kg pulp from per kg *Dendrocalamus latiflorus* chip, produced ethanol 0.131 kg (HHV: 26.8 MJ / kg) and 0.15 kg compacting fuel (24.4 MJ / kg), 7.17MJ energy in total.

Table 21. The HHV and mass layout from ethanol production process.
(*Dendrocalamus latiflorus* chip HHV: 18.06 MJ / kg)

From 1 kg *Dendrocalamus latiflorus*	Pretreated yield (kg)	HHV (MJ / kg)	Energy (MJ)	Compacted after SSF(kg)	HHV (MJ / kg)	Energy (MJ)	EtOH yield(kg)	Energy (MJ)
Alkali								
180°C -10 min	0.83	16.34	13.56	0.59	17.94	10.58	0.082	2.20
190°C-05 min	0.80	18.08	14.46	0.68	16.71	11.36	0.088	2.36
190°C-10 min	0.76	16.86	12.81	0.57	17.63	10.05	0.076	2.04
190°C-20 min	0.63	16.93	10.67	0.40	17.17	6.87	0.069	1.85
200°C-10 min	0.58	16.53	9.59	0.38	17.67	6.71	0.075	2.01
Acid explo.	0.57	21.71	12.37	0.15	24.40	3.66	0.131	3.51
Kraft pulp	0.45	15.77	7.10	0.15	15.77	2.37	0.135	3.62
Bleached pulp	0.42	15.70	6.59	0.05	15.70	0.79	0.160	4.29

* Ethanol HHV 26.8 MJ / kg from Thomas and Ramon (2000).

Table 22. Estimated ethanol production energy from chip to ethanol

Energy Use (MJ / kg ethanol)	Acid	Alkali	Kraft	Bleached
Pretreatment or Pulping	**30.01**	**43.65**	**19.62**	**19.33**
Electricity	4.06	5.89	-	-
Steam	25.35	36.87	-	-
Sulphuric Acid Production	0.19	0.28	-	-
Alkali Production	0.43	0.62	-	
SSF	**11.32**	**16.46**	**10.65**	**9.05**
Electricity	8.88	12.93	8.37	7.11
Enzyme	2.33	3.40	2.19	1.86
Yeast	0.10	0.15	0.09	0.08
Product Recovery	**13.74**	**19.99**	**12.93**	**10.99**
Steam	12.99	18.90	12.23	10.39
Cooling Water	0.42	0.61	0.39	0.33
Wastewater Treatment	0.27	0.39	0.25	0.22
Dehydrate	0.06	0.09	0.06	0.05
Total Energy Use	**55.07**	**80.10**	**43.2**	**39.37**

If 1 kg ethanol produced as energy output 26.8 MJ without fermented residue utilization, the steam explosion pulps and kraft pulps was all net energy negative. As Table 22. shown, the 4 kinds of pulp had different energy consumption per kg ethanol.

Table 23. Estimated Ethanol Production and compacted Energy use from *Dendrocalamus latiflorus* chip (MJ per kg O.D. chip)

Energy Use (MJ / kg O.D. chip)	Acidic	Alkali	Kraft Pulp	Bleached Pulp
Ethanol conversion (L / kg)	0.16	0.11	0.17	0.20
Pretreatment or Pulping	**4.80**	**4.80**	**3.87**	**3.92**
Electricity	0.65	0.65	-	-
Steam	4.06	4.06	-	-
Sulphuric Acid Production	0.03	0.03	-	-
Alkali Production	0.07	0.07	-	-
SSF	**1.81**	**1.81**	**2.13**	**1.81**
Electricity	1.42	1.42	1.67	1.42
Enzyme	0.37	0.37	0.44	0.37
Yeast	0.02	0.02	0.02	0.02
Product Recovery	**2.20**	**2.20**	**2.59**	**2.20**
Steam	2.08	2.08	2.45	2.08
Cooling Water	0.07	0.07	0.08	0.07
Wastewater Treatment	0.04	0.04	0.05	0.04
Dehydrate	0.01	0.01	0.01	0.01
Residue dehydrated	**0.11**	**0.47**	**0.11**	**0.05**
Compacted	**0.004**	**0.016**	**0.004**	**0.001**
Total Energy Use	**8.92**	**9.30**	**8.70**	**7.98**

*190°C-05 min represented the alkali steam explo. pulp.

Estimated ethanol production and compacted energy use (MJ / kg O.D. chip) from *Dendrocalamus latiflorus* chip shown as Table 23. (combined of Table 22. and Table 20.). Acidic steam explosion pulp for example, total energy consumed 8.92 MJ (as Table 23. shown) and total energy output 7.17 MJ (as Table 20. shown), net energy gain -1.75 MJ per kg O.D. *Dendrocalamus latiflorus* chip feed in. The net energy gain was alkali 4.42 MJ per kg chip, kraft pulp -2.71 MJ per kg chip and bleached pulp 2.90 MJ per kg chip. As NEV preferred, the alkali steam explosion 4.42 MJ (=50.22 MJ / kg ethanol) was only energy gain which was shown in Figure 7. The *Dendrocalamus*

latiflorus ethanol's NEV might roughly compare with the corn ethanol by Shapouri et al. (2002) 7.45 MJ / kg ethanol and the lignocellulosic ethanol by Cardona Alzate and Sanchez Toro (2006) 22.37~23.99 MJ / kg ethanol.

Energy Use (MJ)	Input	Energy Production (MJ)	Output
Pretreatment	4.80	Ethanol	2.36
SSF	1.81	Compacting fuel	11.36
Ethanol Recovery	2.20		
Residue dehydrated	0.47		
Compacted	0.02		
Total Energy	9.3		13.72

1.0kg
Ma bamboo chip
HHV: 18.06 MJ / kg

4.80 MJ
Steam explosion

0.80kg
Alkali steam explosion pulp
HHV:18.08 MJ / kg

SSF

1.81 MJ

Residue dehydrated   0.47 MJ
Compacted   0.02MJ

Ethanol Recovery 2.20 MJ

0.088 kg
Ethanol
HHV: 26.8 MJ / kg

0.68 kg
Compacting fuel
HHV: 16.71 MJ / kg
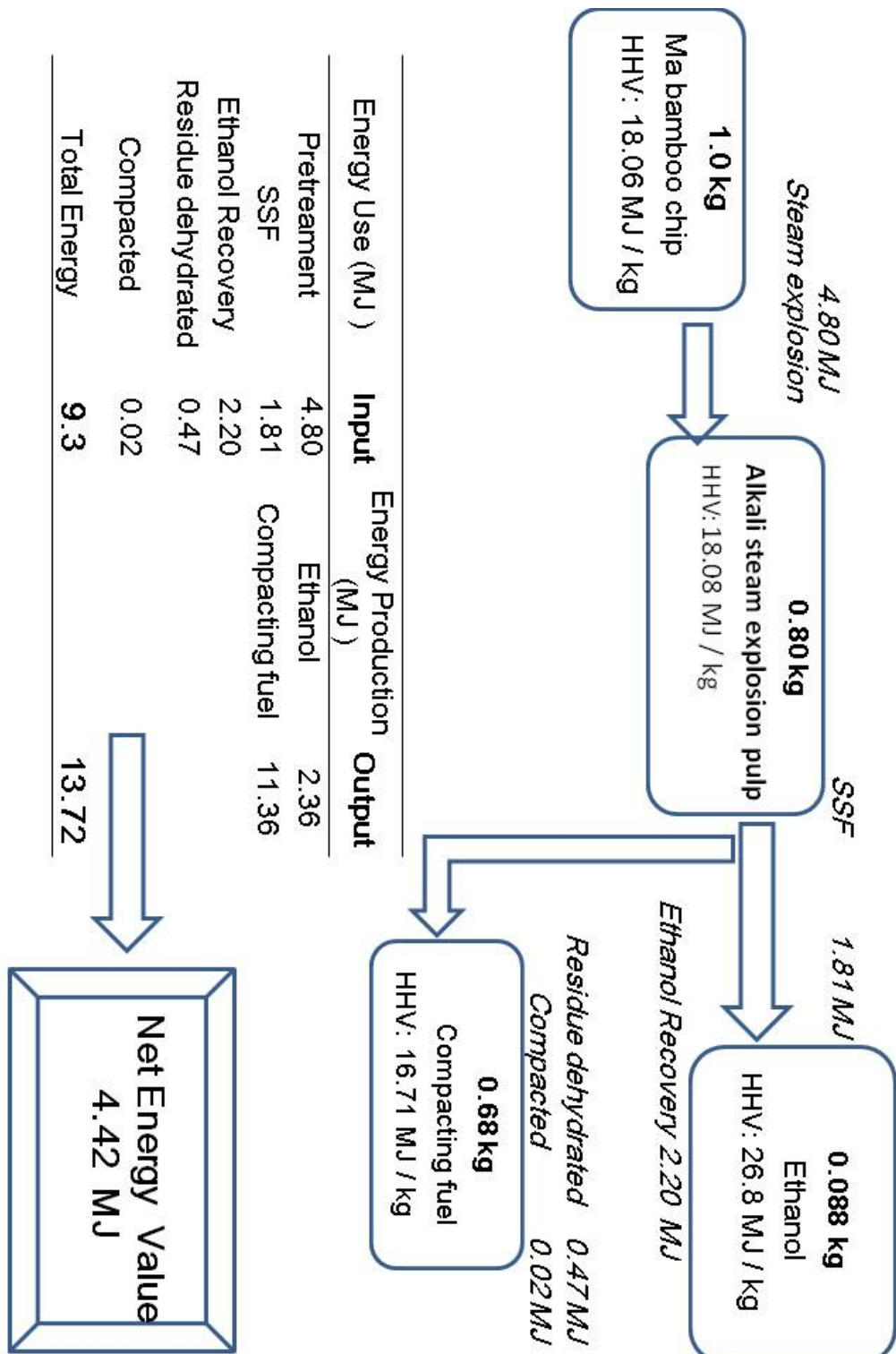
Net Energy Value
4.42 MJ

Figure 7. Flowsheet of energy value by alkali steam explosion pulp

Chapter 6   Concluding remark

Within international trends, the government had policy implementation with the ethanol-gasoline fuel. In 2009, government had set up petrol station supplied ethanol - gasoline E3 in Taipei and Kaohsiung. And there would be petrol station supplied ethanol - gasoline E3 in Taiwan in 2011.

According to the energy statistical annual report (Bureau of Energy, 2010), there were 9,795,000 kiloliter unleaded gasoline consumption for automobile. There was about 235,080 ton ethanol required as E3 additive. In this study, the bamboo optimistic annually growth was 1,328,000 available as feedstock; As much as 174,100 tons of bioethanols could be produced annually by acidic steam explosion (met 74% need as E3 additive), by alkali steam explosion ($190^{o}C$-05 min) 116,900 tons (met 50% need as E3 additive) and by bleached pulp 211,900 tons (met 90% need as E3 additive)

In this study, the conversion efficiency can be further improved if the process can combine 5-carbon sugar co-fermentation and be improved by other measures. Acid steam explosion pretreatment is more efficient way to produce ethanol but only the alkali steam explosion way has net energy value gain. Bamboos' mechanical strength may affect fiber separation in pretreatment which be concerned for pretreatment condition setting.

Chapter 7   Conclusions

The optimal glucose yields were the bleached pulp showed a optimal yield 388.2 ± 69 mg/g, kraft pulp 347.8 ± 77 mg/g, alkali steam-exploded (190°C-10min) 249.3 ± 21 mg/g and acid steam-exploded 209.1±41 mg/g. The hydrolysis efficiencies were negatively impacted by lignin contents of pretreated *Dendrocalamus latiflorus* biomaterials.

The lowest sugar yield of 180°C-10 min pulp suggested that other factors, like fiber dimensions, might also play important roles during enzyme hydrolysis. Acidic pulp had least fiber, largest coarseness, largest broken end(%) and largest fine content(%) of these pulps. Compared with other alkali steam-exploded pulps, the 180°C-10 min pulp has little numbers of fibers, bigger coarseness, and more fine content. It might concerned that the 180°C-10 min pulp wasn't enough mechanical separated from chip to fibers during steam-explosion which may cause the lowest sugar yield. The stronger pretreated condition may harm the fiber and suggested that condition should not over 190°C-10 min.

Simultaneous saccharification and fermentation (SSF) were also conducted using *Saccharomyces cerevisiae* D5A under 37.5^{o}C and pH 5 with yeast extract 10 g / L and peptone 20 g / L at shake flask level. After 96 hours, the optimal ethanol conversion for acid pretreated pulps was 13.11%, equivalent to 0.16 L ethanol / kg dried chip. And the optimal ethanol conversion for alkaline pretreated pulps exploded was 8.8%, equivalent to 0.11 L/kg dried chip.

Acidic steam explosion pulp, kraft pulp and bleached pulp was energy negative. As NEV preferred, the alkali steam explosion 4.42 MJ / kg chip (=50.22 MJ / kg ethanol) was only the only energy gain.

Biomass derived by photosynthesis has strong potentials from bioethanol production. As much as 174,100 tons of bioethanols could be produced annually by acidic steam explosion. The present study demonstrated bamboo in Taiwan was a source couldn't be ignored for bioethanol production.

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