Leaflet No. 12/2021

The Republic of the Union of Myanmar

Ministry of Natural Resources and Environmental Conservation

Environmental Conservation Department

Dewaxing and Deashing of Sugarcane Trash by solvent extraction and chemical treatment

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December 2021

Content

Dewaxing and Deashing of Sugarcane Trash by Solvent extraction and Chemical Treatment

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Abstract

Sugarcane trash (SCT) is an underused plant residue which usually left on the field. In this study, we focused on developing the methods for de-waxing and de-ashing sugarcane trash. Various solvents (acetone, toluene, methyl isobutyl ketone (MIBK), and ethanol) were used for the de-waxing step with a reflux system at the respective solvent's boiling point for 6 hours retention time. Toluene was found as the most potent solvent in terms of yield. The GC-MS analysis revealed the wax's major chemical components: gamma-sitosterol, stigmasterol, lanosterol, gamma-sitostenone and so on. De-ashing was then applied for removing ash and minerals from the solid residues. Chemical pretreatment process in an aqueous solution with and without supplementation of dilutes acid and alkaline at 80°C for retention time 2 hours with Parr Instrument 0.65L was studied for deashing efficiency. Dilute acid and alkaline were shown for the potential as de-ashing. The element composition of raw sugarcane trash, dewaxed toluene and deashed HCl10% samples were determined by Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES). Chemical compositions of the sugarcane trash and solid residues after the de-waxing and deashing steps were compared, showing efficient removals of wax and ash of the biomass. In de-ashing, HCl 10% is the best for chemical pretreatment process.

Keywords: sugarcane trash, de-waxing, de-ashing, solvent

ကြံပင်အပေါ်ပိုင်း အရွက်အား ဓာတုဗျော်ရည်ဖြင့် ဖယောင်းဆွဲထုတ်ခြင်းနှင့် ဓာတုပစ္စည်း (acid, alkali) ဖြင့် ပြာဖယ်ခြင်း

ဒေါ်မြသန္တာခင်၊ လက်ထောက်ညွှန်ကြားရေးမှူး
ပတ်ဝန်းကျင်ထိန်းသိမ်းရေးဦးစီးဌာန

စာတမ်းအကျဉ်း

ရိတ်သိမ်းပြီး ကြံစိုက်ခင်းများမှ ကျန်ရှိသည့် ကြံပင်များမှ ထွက်ရှိသည့်စွန့်ပစ် အမှိုက်များ (Sugarcane Trash) အား solvents (acetone, toluene, methyl isobutyl ketone (MIBK), နှင့် ethanol) သုံး၍ de–waxing လုပ်ပြီး GC–MS ဖြင့် wax တွင် ပါဝင်သည့် chemical components: gamma–sitosterol, stigmasterol, lanosterol, gamma–sitostenone စသည် ဓါတုပစ္စည်းအစိတ်အပိုင်းများ၏ အသုံးဝင်မှုများကိုလေ့လာ ထားခြင်း ဖြစ်ပါသည်။ ထို့နောက် De–ashing အား Parr Instrument 0.65L စက်ဖြင့် ပြာဖယ်ပြီးနောက် minerals ပါဝင်မှုများကို Inductively Coupled Plasma – Atomic Emission Spectroscopy (ICP–AES) ဖြင့် လေ့လာခဲ့ခြင်းဖြစ်ပါသည်။ De–waxing and deashing လုပ်ငန်းစဉ်များဆောင်ရွက်ပြီးနောက် HCl 10% လုပ်ငန်းစဉ်သည် စွမ်းဆောင်ရည် မြင့်မားလျက်ရှိကြောင်း တွေ့ရှိရပါသည်။

Dewaxing and Deashing of Sugarcane Trash by Solvent extraction and Chemical Treatment

Introduction

Sugarcane trash (SCT) is the plant residue, obtained during harvesting, and it usually left on the field. Open-field burning of SCT is an important source of air pollution. Sugarcane-producing countries increasingly prohibit this habit for reducing negative environmental impact from generation particulate matters[1,2,3]. SCT consists primarily of semi-dried leave and top residues. Collected SCT is used as fuel for generation of heat and electricity in biomass power plants with a heating value of 17.39 MJ/kg and is also considered a promising raw material for biorefinery[4]. Lignocellulose is the fibrous part of plant material consisted of three different types of polymers: cellulose, hemicellulose and lignin. The fractionation process of SCT is a prerequisite step in biorefinery[5]. The separated lignocellulose components can be further converted to products of interest by chemical and biological processes.

In this research, we will explore the potential on dewaxing and deashing of SCT aiming for application as the prior steps before subsequent processing of SCT in integrated biorefinery. De-waxing of SCT helps extract value from the raw material before subsequent processing in biorefinery. This allows the recovery of wax and valuable phytochemicals from SCT. De-ashing is an essential step for removing ash and minerals from lignocellulosic materials. This will lead to alteration of the physical and chemical properties of SCT and the separated lignocellulose-derived fractions for further applications on the conversion of SCT to biofuels, biochemicals, and biomaterials.

Objectives

(1) To study the effects of solvent extraction on de-waxing of SCT and analyze the extracted composition's chemical profiles.
(2) To study the effects of different chemical processes on the de-ashing of SCT and analyze the mineral profiles.

Materials and Methods

1.Materials

(1) Sugarcane Trash (supplied by Mitrphol Sugar Mill)
(2) Cutting mill (HF-170F, MULCHER CHIPPER, Thailand)
(3) Reflux system
(4) Rotary Evaporator (OSB-2100, ELEYA, Japan)
(5) Autoclave (SX-700, TOMY KOGYO, Japan)
(6) High performance liquid chromatography (SIL-20 AC HT**,** Shimadzu, Japan)
(7) Gas chromatography-mass spectrometry (GC-Ms TQ8050, Shimadzu, Japan)
(8) Micro-ED XRF (XRD-6 100/700, Shimadzu, Japan)
(9) ICP-AES (ICP-AES 9820, Shimadzu, Japan)
(10) High pressure batch reactor 0.65L (Parr Instrument, Moline, IL, USA)
(11) Scanning Electron Microscope (SU5000 with EM Wizard, Hitachi, Japan)

2. Chemicals

(1) Ethanol
(2) Acetone
(3) Toluene
(4) Methyl isobutyl ketone (MIBK)
(5) Sulfuric acid
(6) Formic acid
(7) Acetic acid
(8) nitric acid
(9) Phosphoric acid
(10) Hydrochloric acid

- All chemicals and reagents are analytical grade and purchased from major chemical suppliers from Sigma, Merck, and Fluka.

3. Raw material preparation and property determination

3.1 Sugarcane trash preparation

SCT was obtained from the Mitrphol sugar mill (Khon Khan, Thailand). The biomass was physically chopped with a cutting mill (MULCHER CHIPPER) and sieved to the target particles' size range of 1 mm - 0.425 mm. The chemical composition of SCT was determined using National Renewable Energy Laboratory-NREL method [6].

3.2 Ultimate Analysis

The ultimate analysis of SCT was analyzed using an elemental analyzer, J-Science, MICRO CORDER JM10 (J-Science, JM10, J-SCIENCE LAB Co., LTD, Japan), BBOT (C=72.6%, H= 6.1%, N=6.5%) ASTM D5373-21 [7] reference standard for carbon, hydrogen, and nitrogen. A carbon/sulfur analyzer, Horiba, Emia-220V2 with high-frequency induction furnace reverence method for sulfur. Oxygen was calculated by difference[8, 9].

3.3 Proximate Analysis

The proximate analysis was used for analyzing the sample on an as-received basis for the percentage of moisture, volatile matters, fixed carbon, and ash contents in biomass with Thermogravimetric Analyzer (TGA), PerkinElmer, Pyris 1 TGA [9, 10]. (referring to Standard ASTME-1755-01) [11].

3.4 Chemical composition analysis

Cellulose, hemicellulose, lignin, and ash composition of biomass were determined using the National Renewable Energy Laboratory-NREL method[6]. Cellulose and hemicellulose were analyzed with High Performance Liquid Chromatography (HPLC, SIL-20 AC HT, SHIMADZU, Japan) equipped with an Aminex HPX-87H column and a reflective index detector. Sulfuric acid (0.005M) was used for the mobile phase. The column temperature was 65°C, flow rate 0.5ml/min, detector – refractive index, run time 20 min. Acid soluble lignin (ASL) was analyzed UV- Visible spectrophotometer (OD240-UV light 249 nm) with run a background of 4% sulfuric acid. Acid insoluble lignin (AIL)

and ash were analyzed the furnace at 575°C for 3 h based on the NREL method [6] and calculated.

According to the procedure, 0.3 g of biomass and 3 ml of 72% H_2SO_4 were put into the bottles and mixed every 15 min by using Vortex for duration time of 2 h. After that, 84 ml of H_2O was added and heated in an autoclave at a temperature of 121°C for 1 h retention time. The mixture was cooled down, and the liquid fraction was taken for HPLC and spectrophotometer analysis. For sugar component analysis, liquid fraction was adjusted for pH with $CaCO_3$ and was centrifuged at 6000 rpm for 5 min. After that, HPLC was used for the analysis of the sugar components in the fraction. For Acid soluble lignin (ASL) composition analysis, the liquid fraction and Blank 4%H_2SO_4 were analyzed by spectrophotometer at the wave length of 249 nm. The solid and liquid fractions were separated by a pore-5 filter and washed with H_2O. For Acid insoluble lignin (AIL), the solid was dried at 105°C and 4 h retention time. After that, the solid was cooled down and weighted. Then the solid was put into the furnace at 575°C for 3 h and cooled down and calculated based on the NREL method. For ash content in biomass, different biomass was analyzed by putting it into the furnace at 575°C for 3 h and cooled down. The ash analysis was based on the NREL method [6] and calculated.

3.5 Protein and fat analysis

The protein test was applied ISO 5983-2:2009. Fat was analyzed with In-house method TE-CH-160 based on AOAC (2019) 920.39 by, Soxhlet extraction with petroleum ether[12].

4. De-waxing

SCT was dewaxed with four different solvents. The chopped and sieved biomass was treated with acetone, toluene, methyl isobutyl ketone (MIBK), or ethanol to choose the suitable solvent for de-waxing. Biomass (15 g) was mixed with 300 ml of the solvent and heated for 6 h in a reflux system. After that, the mixture was cooled down, and the solvent was separated from the extracted oil fraction. The solid pulp was then washed with the respective solvent to get de-waxed biomass and dried at the oven at 105°C for 4 h for subsequent analysis. The pulp yield was calculated based on a dried weight basis. The crude wax was isolated from the solvent fraction by a rotary evaporator. The temperature and duration time depends on the boiling point of each solvent. The boiling points are as followed: acetone (56°C), toluene (110.6°C), methyl isobutyl ketone (MIBK) (116°C), and ethanol (78°C). The crude wax was weighed and collected to calculate the % fat yield to analyze the chemical compositions by using Gas Chromatography - Mass Spectrometry (GC-MS). After de-waxed sugarcane trash with solvents were analyzed for its chemical composition by using the NREL method [6]. The dewaxed SCT using toluene was used for the subsequent deashing experiment.

$$\text{Pulp yield}(\%) = \frac{\text{final Biomass weight}}{\text{initial Biomass weight}} \times 100$$

$$\text{Extractive yield}(\%) = \frac{\text{Extractive weight by solvent}}{\text{Fat weight by AOAC 920.39}} \times 100$$

initial Biomass fat weight = 1.06 g/100g

$$\text{Glucose (recovery)} = \frac{\%\ \text{w/w Glucose}}{\text{Sugar recovery of Glucose}}$$

$$\text{Xylose (recovery)} = \frac{\%\ \text{w/w Xylose}}{\text{Sugar recovery of Xylose}}$$

$$\text{Arabinose (recovery)} = \frac{\%\ \text{w/w Arabinose}}{\text{Sugar recovery of Arabinose}}$$

Sugar recovery of Glucose = 1
Sugar recovery of Xylose = 0.95
Sugar recovery of Arabinose = 1

$$\text{Cellulose (\%)} = \frac{\text{cellulose factor} \times \text{Glucose(recovery)} \times \text{final biomass wt.}}{\text{initial biomass wt.}}$$

$$\text{Hemicellulose(\%)} = \frac{\text{hemi: factor} \times (\text{Xylose(rec:)} + \text{Arabi:(rec:)}) \times \text{final biomass wt.}}{\text{initial biomass wt.}}$$

Cellulose factor = 0.9
Hemicellulose factor = 0.88

$$\text{Cellulose recovery} = \frac{\text{final}}{initial} x100$$

$$\text{Hemicellulose recovery} = \frac{\text{final}}{initial} x100$$

$$\text{Lignin recovery} = \frac{\text{final}}{initial} x100$$

$$\text{Hemicellulose removal} = \frac{\text{(initial-final)}}{initial} x100$$

$$\text{Lignin removal} = \frac{\text{(initial-final)}}{initial} x100$$

$$\text{Ash removal} = \frac{\text{(initial-final)}}{initial} x100$$

$$\text{Lignin} = \text{AIL} + \text{ASL}$$

AIL = Acid insoluble lignin
ASL = Acid soluble lignin
initial = initial biomass weight
final = final biomass weight

$$\%\ \text{Acid soluble lignin} = \frac{UV_{abs} \times \text{Volume of filtrate} \times \text{Dilution(87) x 100}}{\varepsilon \times \text{Weight of biomass sample} \times \text{Path length}}$$

$UV_{absorbance}$ = average of UV-Vis absorbance for a sample at an appropriate wavelength.
ε = The absorptivity coefficient of biomass at a specific wavelength (L/g cm) by 25 L/g cm of Sugarcane Bagasse (Sluiter et al., 2008b)

Pathlength = 0.2767 g (from Beer Law) pathlength of UV-Vis's cell

$$\%\ \text{Acid insoluble lignin} = \frac{\text{(lignin + ash)} - \text{ash}}{\text{Biomass}} \times 100$$

$$\% \text{ Ash} = \frac{\text{After furnace(crucible + sample)wt.} - \text{crucible wt.}}{\text{sample wt.}} \times 100$$

5. Effects of de-ashing with hot water, acid and alkaline

The de-waxed sugarcane trash by toluene was used as the starting material for deashing experiments using hot water, acids, and alkalis.

10 g of dewaxed SCT was mixed with 100 ml of water only or in the presence of acids or alkalis and was leached with water or with acid (1% w/w HCl, acetic acid, H_2SO_4, formic acid, H_3PO_4, and HNO_3) and 2.5, 5 and 10% w/w HCl or alkalis (1% w/w NaOH, KOH, Ca $(OH)_2$, and ammonium hydroxide). The reactions were incubated in a Parr reactor 0.65 L at the temperature of 80°C, retention time 2 h, and mixing at 250 rpm. After that, the solid and liquid fractions were separated with 125mmØ Whatman No. 4 filter paper. This solid was dried in the oven at 105°C and 4 h and then cooled down and calculated for the %pulp yield.

The chemical composition of the de-ashed SCT was determined according to the method of the National Renewable Energy Laboratory-NREL method[6] as described above.

6. Analytical methods

6.1 Gas Chromatography - Mass Spectrometry (GC-MS)

The wax composition of SCT extracted by toluene was determined using GC/MS (GC-MS TQ8050, Shimadzu, Japan) according to GC-MS/MS method with a DB-5ms column, length: 30m, thickness: 0.25um, diameter: 0.25mm [4, 13].

6.2 Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES)

The biomass was digested as a liquid to test ICP-AES (ICP-AES 9820, Shimadzu, Japan) using the In-house method based on the ASTM D4951 NCTC method[14, 15]. Firstly, sugarcane trash was digested with the Digestion method for ICP-AES. Ten milligrams of biomass were put into the furnace at the temperature of 500°C for 1 h and cooled down. After that, the biomass was added with 500 µl of nitric acid and heated on a hot plate at 115°C to vaporize and get the solid condition. Then, it was cooled down and put into the furnace at 500°C and 1 h. Finally, it was cooled down and 500 µl of 37% hydrochloric acid and 5 ml of water was added. It was then put into the 10 ml cylinder flask with a 0.2 um syringe filter and mixed. Finally, the digestive biomass was analyzed with ICP-AES.

Results

1. **Ultimate and proximate analysis (on dry basis) of raw sugarcane trash**

Components of the raw material was analyzed by ultimate and proximate analysis. The ultimate analysis demonstrated the higher amount oxygen (53.05%) and carbon (40.51%) **Table 1**. The proximate analysis got the highest volatile matter (71.95%) and the lowest moisture (5.56%) **Table 2**.

Table 1 Ultimate analysis of raw sugarcane trash

Elements	Weight (%)
Hydrogen	5.81
Carbon	40.51
Nitrogen	0.41
Sulfur	0.22
oxygen (by difference)	53.05
Total	**100**

Table 2 Proximate analysis of raw sugarcane trash

Contents	Weight (%)
Moisture	5.56
Volatile matters	71.95
Fixed carbon	12.49
Ash	10.00
Total	**100**

2. Chemical composition of raw sugarcane trash

The raw sugarcane trash composition contained 32.40% cellulose, 23.05% hemicellulose, 26.88% lignin as the major components **Table** 3. Minor components included 2.07% protein, 1.06% fat, and 11.42% ash. The composition of SCT in this study was in a comparable range of sugarcane trash with 32-48% cellulose, 23-32% hemicellulose, 13-24% lignin and 1.7-8% ash [16].

Table 3 Protein, fat, cellulose, hemicellulose, lignin, and ash % in raw sugarcane trash

Components	Results	Unit
Protein	2.07± 0.05	%
Fat	1.06± 0.02	%
Cellulose	32.40 ± 0.44	%
Hemicellulose	23.05 ± 0.68	%
Lignin	26.88 ± 0.98	%
Ash	11.42 ± 0.45	%
Total	**96.94**	**%**

Discussions

Table 4 shows the extractive yield % of raw sugarcane trash using different solvents: ethanol, acetone, toluene, and methyl isobutyl ketone (MIBK). The yield of wax extraction obtained from ethanol, acetone, toluene, and MIBK were 189%, 88%, 98%, and 139% respectively. 98% extractive yield obtained by toluene was selected as the suitable solvent for de-waxing SCT for subsequent experiments.

Table 4 Extractive yield % of raw sugarcane trash using different solvents in reflux system

Solvents	Extractive yield (%)
Ethanol	189
Acetone	88
Toluene	98
MIBK	139

1. **Dewaxed of SCT using different solvents**

Table 5 Pulp & Extractive yield % with Reflux system

Solvents	Pulp yield (%)			Extractive yield (%)		
	Average	SD	Pulp yield (%)	Average	SD	Extractive yield (%)
Ethanol	14.32	0.66	95.47	0.30	0.01	189
Acetone	14.34	0.58	95.60	0.14	0.01	88
Toluene	14.36	0.37	95.73	0.15	0.01	98
MIBK	14.35	0.38	95.66	0.22	0.01	139

Initial weight of SCT is 15 g. Calculation of Extractive yield (%) was based on the 1.06 g/100 g of the Fat composition of SCT.

According to **Table 5**, de-waxing with toluene was the most effective than other solvents. The yield of extracted wax obtained from toluene was around 98% and the color of pure wax was light-yellow, suggesting higher purity. The pulp yield **%** obtained by toluene was also slightly higher than others. The time needed to evaporate toluene from the samples was also shorter due to its lower latent heat of evaporation (351 kJ/kg) compared to acetone (518kJ/kg). These justified the use of toluene as the potent solvent for dewaxing under our experimental conditions. The yield of wax obtained from ethanol and MIBK (methyl isobutyl ketone) solvent obtained were over than 100%. This can be explained by the polarity index of toluene (2.4) which is more comparable to petroleum ether (0.1) and lower than that of other solvents used in this study i.e., MIBK (4.2), acetone (5.1) and ethanol (5.2). The higher polarity of ethanol and MIBK tends to result in extraction of more polar products compared to that obtained with petroleum ether which is mostly non-polar products. The pulp content recovered was >95% using all solvents. Therefore, toluene was selected as the suitable solvent for de-waxing SCT for subsequent experiments.

Table 6 Chemical composition of SCT after de-waxing using different solvents

Sample	Cellulose %			Hemicellulose %			Lignin %			Ash %		
	Average	SD	**recovery**	Average	SD	**recovery**	Average	SD	**recovery**	Average	SD	**removal**
SCT	32.40	0.44	-	23.05	0.68	-	26.88	0.98	-	11.42	0.45	-
Ethanol	31.83	0.27	**98.24**	19.71	0.08	**85.53**	24.33	0.75	**90.53**	9.43	0.36	**17.43**
Acetone	31.03	2.13	**95.79**	19.77	1.34	**85.77**	24.66	0.56	**91.75**	9.98	1.00	**12.59**
Toluene	32.28	0.46	**99.63**	20.07	0.76	**87.09**	26.41	1.79	**98.25**	9.93	0.08	**13.02**
MIBK	31.87	0.29	**98.36**	19.88	0.48	**86.24**	26.04	1.64	**96.87**	10.11	0.15	**11.50**

Table 6, after de-waxing with ethanol, acetone, toluene, and MIBK, the chemical composition of SCT residues was analyzed by the NREL method. Ash removal % is 17.43%, 12.59%, 13.02% and 11.50% respectively. Cellulose, hemicellulose, and lignin recovery % of toluene, which were 99.63%, 87.09%, and 98.25% respectively were higher than those obtained using other solvents. The extractive yield and pulp yield of toluene solvent were 98% and 95.73%. According these results, toluene is the most effective solvent for dewaxing SCT for subsequent experiments.

2. Wax composition from toluene extraction with Gas Chromatography - Mass Spectrometry (GC-MS)

The composition of the wax obtained from toluene extraction were determined by Gas Chromatography - Mass Spectrometry (GC-MS). The results showed various sterols and fatty alcohols as the significant compositions in the wax fraction. Various sterols and fatty alcohols in the wax fraction are potent for application in food supplementation, cosmetic and healthcare industries.

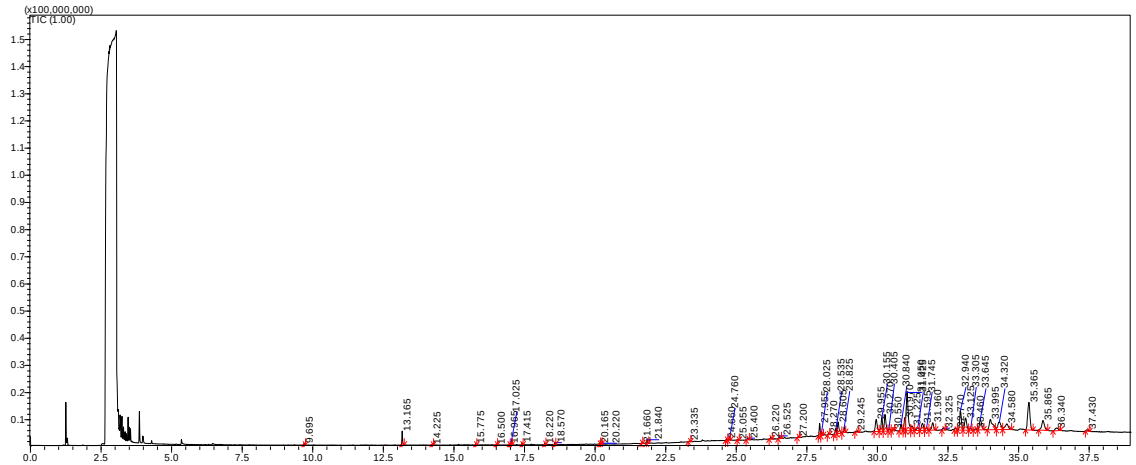

Figure 1 Chromatogram of sample extracted using toluene by GC-MS analysis

Table 7 Potent compounds from chemical composition analysis of the wax fraction from toluene extraction by GC-MS ranking based on the highest % area

No.	Ret. Time	Name of compound	Area %
1	31.051	. gamma. - Sitosterol	12.46
2	35.365	Tris(2,4-di-tert-butylphenyl) phosphate (alcohol)	10.56
3	33.997	D: B-Friedo-B': A'-neogammacer-5-en-3-ol, (3. beta.)-	5.81
4	32.940	. gamma. - Sitostenone	5.70
5	30.269	Stigmasterol	4.85
6	33.125	Undec-10-ynoic acid, hexadecyl ester	4.50
7	35.864	Stigmastane-3,6-dione, (5, alpha.)-	4.46
8	34.318	1-Hexacosanol	4.12
9	33.646	Lanosterol	3.95
10	29.956	Ergost-5-en-3-ol, (3. beta.)-	3.70

Chemical composition analysis of the wax fraction from toluene extraction by GC-MS showed various chemical including .gamma.-Sitosterol ($C_{29}H_{50}O$) – 12.46%, Tris(2,4-di-tert-butylphenyl) phosphate ($C_{42}H_{63}O_4P$) – 10.56%, D:B-Friedo-B':A'-neogamma cer-5-en-3-ol, (3.beta.)- ($C_{30}H_{50}O$) – 5.81%, .gamma.-Sitostenone ($C_{29}H_{48}O$) – 5.70%, Stigmasterol ($C_{29}H_{48}O$) – 4.85%, Undec-10-ynoic acid, hexadecyl ester ($C_{27}H_{50}O_2$) – 4.50%, Stigmastane-3,6-dione, (5,alpha.)- ($C_{29}H_{48}O_2$) – 4.46%, 1-Hexacosanol ($C_{26}H_{54}O$) – 4.12%, Lanosterol ($C_{30}H_{50}O$) – 3.95%, and Ergost-5-en-3-ol, (3.beta.)- ($C_{28}H_{48}O$) – 3.70%. These sterols and fatty alcohols can be used for the various applications such as food supplementation, cosmetic and healthcare industries.

Table 8 Potent compounds and their applications

Name of compound	Applications
. gamma – Sitosterol	- Potent inhibitor of the complement C1 complex. - Potential for diabetic treatment in rats. - Dietary supplementation (reduces intestinal cholesterol absorption, lowering plasma LDL cholesterol concentration in humans) - Various vitamin D analogs could be synthesized simultaneously.
Stigmasterol	Various vitamin D analogs could be synthesized simultaneously.
Lanosterol	- Role as a bacterial metabolite, a human metabolite, a Saccharomyces cerevisiae metabolite, and a mouse metabolite. - Drug product: modified by veterinary, animal, or pet if indicated by source. - Food for human consumption does not include food additives also includes manufacture of food, facilities related to food. - Cosmetics -> Antistatic; Emollient; Hair conditioning; Skin conditioning. - use in eye drops (brand name of **Lanomax)**. - mitigates cytotoxicity in cells, mediated by different

	stress-inducing agents.

3. De-ashing with acid and alkalis processes

De-ashing with acid and alkalis processes was performed in a Parr reactor 0.65 L at a temperature of 80°C, 2 h retention time and 250 rpm. De-ashing with acid and alkalis treatment processes using water leaching or with supplementation of acids (hydrochloric acid, acetic acid, sulfuric acid, formic acid, phosphoric acid and nitric acid) or alkalis (sodium hydroxide, potassium hydroxide, ammonium hydroxide, and calcium hydroxide) were compared for their efficiency on deashing and retention of other components in the solid residues.

3.1 De-ashing with Parr reactor

3.1.1 De-ashing with water leaching

Table 9 Pulp yield (%) for de-ashing of dewaxed SCT with water leaching

Solvent	**Initial wt. (g)**	**Final wt. (g)**	**Pulp yield (%)**
H_2O	10	9.28 ± 0.03	92.80

According to **Table 9**, ten grams of dewaxed SCT in water (solid: liquid = 1:10) was treated at 80°C for, 2 h with mixing at 250 rpm. The pulp yield % was 92.80% using the water leaching method under the experimental conditions.

Table 7 Chemical composition of solid residues after de-ashing with water leaching method

Sample	**Cellulose %**			**Hemicellulose %**			**Lignin %**			**Ash %**		
	Average	SD	**recovery**	Average	SD	**recovery**	Average	SD	**recovery**	Average	SD	**removal**
SCT	32.40	0.44	-	23.05	0.68	-	26.88	0.98	-	11.42	0.45	-
H_2O	27.87	0.38	**86.03**	20.94	0.56	**90.84**	24.90	2.18	**92.64**	7.27	0.36	**36.36**

After de-ashing with the water leaching process, the solid SCT residue was analyzed the NREL method. Ash removal % was 36.36% and cellulose, hemicellulose, and lignin recovery % were 86.03%, 90.84% and 92.64%, respectively.

3.1.2 De-ashing with acid leaching

Table 11. Pulp yield (%) for de-ashing of dewaxed SCT with acid leaching

Solvent	**Initial wt. (g)**	**Final wt. (g)**	**Pulp yield (%)**
H_2O	10	9.28 ± 0.03	92.80
HCl	10	8.86 ± 0.01	88.60
Acetic	10	9.02 ± 0.02	90.20
H_2SO_4	10	8.35 ± 0.03	83.50
Formic	10	9.06 ± 0.02	90.60

H_3PO_4	10	8.62 ± 0.05	86.20
HNO_3	10	8.39 ± 0.02	83.90

According with **Table 11**, ten grams of dewaxed SCT in the total solution volume of 100 ml was treated with 1% w/w hydrochloric acid (HCl), acetic acid (CH_3COOH), sulfuric acid (H_2SO_4), formic acid (HCOOH), phosphoric acid (H_3PO_4), and nitric acid (HNO_3) in the Parr reactor at the temperature of 80°C with a retention time of 2 h and mixing at 250 rpm. The pulp yield % of HCl, acetic acid, H_2SO_4, formic acid, H_3PO_4, and HNO_3 were 88.60%, 90.20%, 83.50%, 90.60%, 86.20%, and 83.90% respectively. The pulp yield % of H_2O was 92.80% The pulp yield obtained was lower than that obtained using the water leaching method.

Table 12 Chemical composition of solid SCT after de-ashing with acid leaching method with 1% w/w acid supplementation

Sample	**Cellulose %**			**Hemicellulose %**			**Lignin %**			**Ash %**		
	Average	SD	**recovery**	Average	SD	**recovery**	Average	SD	**recovery**	Average	SD	**removal**
SCT	32.40	0.44	-	23.05	0.68	-	26.88	0.98	-	11.42	0.45	-
H_2O	27.87	0.38	**86.03**	20.94	0.56	**90.84**	24.90	2.18	**92.64**	7.27	0.36	**36.36**
HCl	25.78	0.44	**79.58**	17.96	0.20	**77.93**	20.08	2.11	**74.72**	6.77	0.38	**40.75**
Acetic	27.67	0.69	**85.40**	19.30	0.14	**83.75**	18.24	0.16	**67.87**	7.33	0.42	**35.77**
H_2SO_4	26.08	0.18	**80.50**	15.97	0.07	**69.29**	16.30	0.21	**60.65**	7.28	0.17	**36.28**
Formic	27.85	0.01	**85.96**	18.87	0.32	**81.88**	19.00	0.22	**70.69**	7.44	0.33	**34.87**
H_3PO_4	26.83	0.32	**82.80**	16.81	0.09	**72.92**	16.42	0.57	**61.07**	7.77	0.05	**31.98**
HNO_3	25.62	0.67	**79.06**	16.41	0.42	**71.18**	16.14	0.39	**60.06**	8.13	0.46	**28.82**

After de-ashing with the acid leaching process, the chemical composition of solid de-ashed SCT was analyzed by the NREL method. According to **Table 12** and **Figure 2** ash removal % of HCl, acetic acid, H_2SO_4, formic acid, H_3PO_4, and HNO_3 are 40.75%, 35.77%, 36.28%, 34.87%, 31.98%, and 28.82 % respectively. Ash removal % of HCl 1% is significantly higher than other acid leaching. However, the recoveries of cellulose, hemicellulose, and lignin were lower compared to the use of water leaching. The use of organic acids resulted in higher recoveries of these components in most cases compared to the use of strong acid. Supplementation of HCl resulted in 79.58% recovery of cellulose which was relatively lower than that of other strong acids except HNO_3 but allowed higher recoveries of hemicellulose and lignin compared to other strong acids.

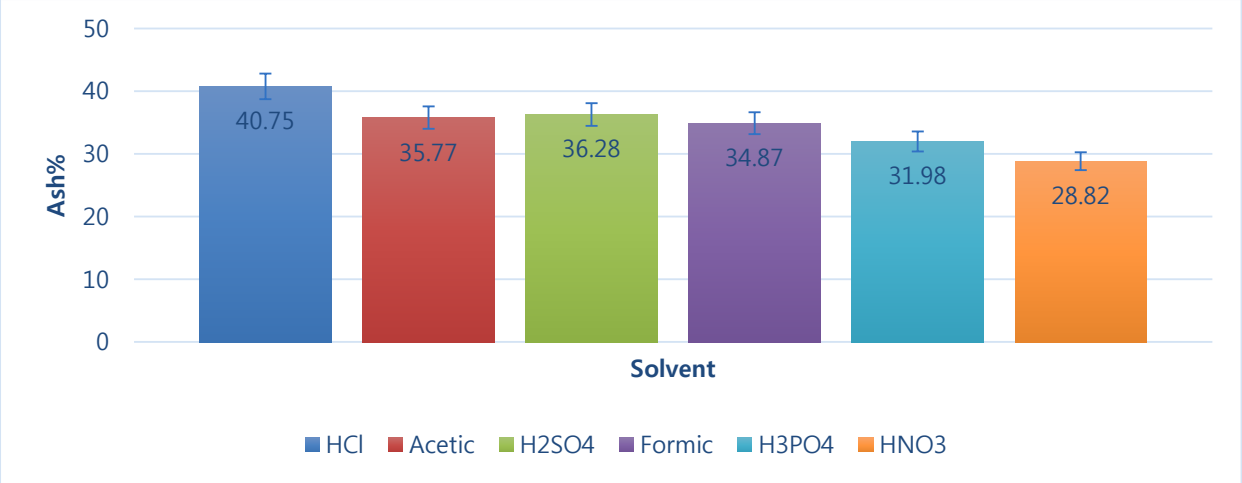

Figure 2 De-ashing of de-waxed SCT with acid leaching in Parr reactor

3.1.3 Comparison of de-ashing efficiency using water leaching and acid leaching with HCl at different concentrations

Table 8 Pulp yield (%)

Solvent	Initial wt. (g)	Final wt. (g)	Pulp yield (%)
H_2O	10	9.28 ± 0.03	92.80
HCl 1%	10	8.86 ± 0.01	88.60
HCl 2.5%	10	8.85 ± 0.03	88.50
HCl 5%	10	8.70 ± 0.06	87.00
HCl 10%	10	8.68 ± 0.06	86.80

Ten grams of dewaxed SCT in the total reaction of 100 ml was leached with H_2O, 1%, 2.5%, 5% and 10% of hydrochloric acid in the Parr reactor at the temperature of 80°C with a retention time of 2 h and mixing at 250 rpm. The pulp yield of H_2O, 1%, 2.5%, 5%, and 10% HCl are 92.80%, 88.60%, 88.50%, 87.00%, and 86.80% respectively **Table 13**. Supplementation of HCl resulted lower pulp yield compared to water leaching. The increasing acid concentration was found to result in only a slight decreasing in the pulp yield.

Table 14 Chemical composition of dewaxed SCT after de-ashing using water and acid leaching with different HCl concentrations

Sample	Cellulose %			Hemicellulose %			Lignin %			Ash %		
	Average	SD	**recovery**	Average	SD	**recovery**	Average	SD	**recovery**	Average	SD	**removal**
SCT	32.40	0.44	-	23.05	0.68	-	26.88	0.98	-	11.42	0.45	-
H_2O	27.87	0.38	**86.03**	20.94	0.56	**90.84**	24.90	2.18	**92.64**	7.27	0.36	**36.36**
HCl 1%	25.78	0.44	**79.58**	17.96	0.20	**77.93**	20.08	2.11	**74.72**	6.77	0.38	**40.75**
HCl 2.5%	25.05	1.72	**77.33**	17.51	0.68	**75.95**	19.20	1.59	**71.43**	6.45	0.27	**43.54**
HCl 5%	24.23	2.82	**74.79**	16.65	1.97	**72.25**	18.89	0.93	**70.29**	6.12	0.20	**46.40**
HCl 10%	23.53	2.76	**72.62**	16.78	0.17	**72.80**	18.85	0.56	**70.12**	5.57	0.43	**51.25**

After de-ashing with the acid leaching process, the chemical composition of solid de-ashed SCT was analyzed by the NREL method. According to **Table 14** and **Figure 3**, the ash removal % of H_2O, 1%, 2.5%, 5% and 10% HCl were 36.36%, 40.75%, 43.54%, 46.40%, and 51.25 % respectively. This showed an increasing trend of % de-ashing with increasing HCl concentration while the decreasing trends of cellulose, hemicellulose, and lignin were observed.

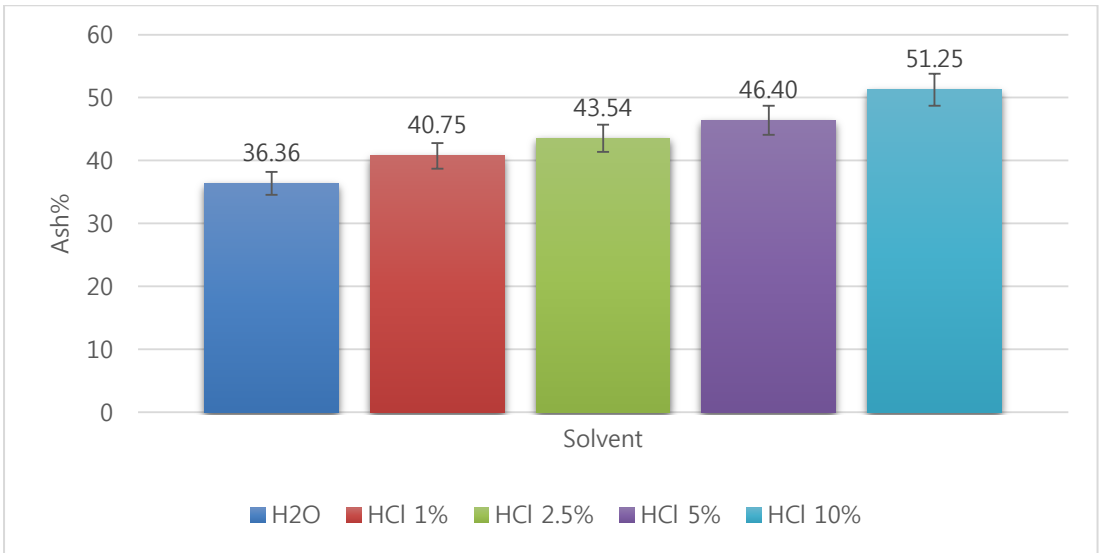

Figure 3 De-ashing of dewaxed SCT with water and acid leaching

3.1.4 De-ashing with alkaline leaching

Table 9 Pulp yield (%) for de-ashing of dewaxed SCT with alkaline leaching

Solvent	Initial wt.(g)	Final wt.(g)	Pulp yield (%)
NaOH	10	8.60 ± 0.07	86.00
KOH	10	8.68 ± 0.01	86.80
$(NH_4)_2OH_3$	10	8.69 ± 0.01	86.90
Ca $(OH)_2$	10	8.77 ± 0.04	87.70

Ten grams of dewaxed SCT in the total aqueous solution volume of 100 ml was leached with 1% w/w of sodium hydroxide (NaOH), potassium hydroxide (KOH), ammonium hydroxide $(NH_4)_2OH_3$ and calcium hydroxide (Ca $(OH)_2$) in the Parr reactor at the temperature of 80°C with a retention time of 2 h with mixing at 250 rpm. The pulp yield % obtained using NaOH, KOH, $(NH_4)_2OH_3$, and Ca $(OH)_2$ were 86.00%, 86.80%, 86.90%, and 87.70% respectively **Table 15**. The pulp yield was lower than that obtained with water leaching and was in the same range compared to that of the acid leaching process.

Table 10 Chemical composition of dewaxed SCT after de-ashing with alkali leaching method using 1% w/w alkali

Sample	Cellulose %			Hemicellulose %			Lignin %			Ash %		
	Average	SD	**recovery**	Average	SD	**recovery**	Average	SD	**recovery**	Average	SD	**removal**
SCT	32.40	0.44	-	23.05	0.68	-	26.88	0.98	-	11.42	0.45	-
NaOH	26.87	1.42	**82.93**	17.49	2.34	**75.90**	22.72	0.70	**84.51**	6.88	0.00	**39.72**
KOH	28.64	0.48	**88.39**	20.05	0.31	**87.00**	24.11	2.36	**89.68**	6.89	0.16	**39.68**
$(NH_4)_2OH_3$	28.82	0.56	**88.96**	19.87	0.31	**86.20**	19.92	1.79	**74.11**	6.83	0.15	**40.20**
Ca $(OH)_2$	29.20	0.95	**90.11**	20.70	0.54	**89.81**	21.46	1.86	**79.84**	6.79	0.51	**40.56**

After de-ashing with the alkali leaching process, the chemical composition of solid de-ashed SCT was analyzed by the NREL method. According to **Table 16**, the ash removal % of NaOH, KOH, $(NH_4)_2OH_3$, and Ca $(OH)_2$ are 39.72%, 39.68%, 40.20%, and 40.56% respectively. This was higher than water leaching and slightly high compared to that obtained using the acid leaching process and the same catalyst concentration. However, remarkable losses of cellulose and hemicellulose were observed with NaOH while substantial loss of lignin was found when $(NH_4)_2OH_3$ and Ca $(OH)_2$ were used. On the other hand, the recoveries of the lignocellulose polysaccharides and lignin from the alkali leaching process tended to be higher compared to that of the acid leaching process.

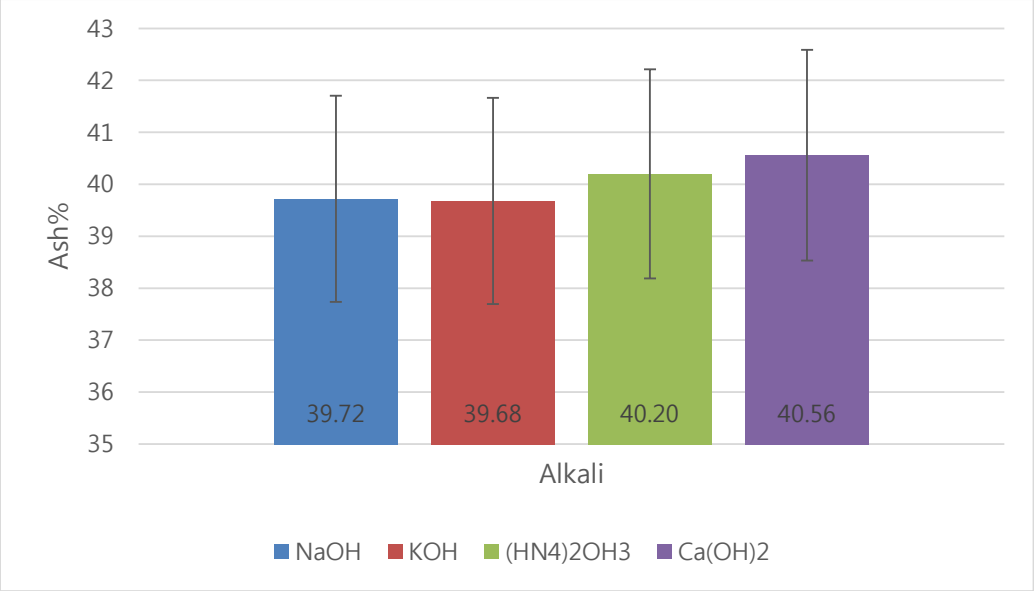

Figure 4 De-ashing of dewaxed SCT with alkaline leaching using Parr reactor

Table 11 Comparison of pulp yield%, cellulose, hemicellulose, and lignin recovery % and ash removal % using 1% of water, acids, and alkalis leaching

Sample	Pulp yield %	Cellulose recovery %	Hemicellulose recovery %	Lignin recovery %	Ash removal %
H_2O	**92.80**	86.03	**90.84**	92.64	36.36
HCl	88.60	79.58	77.93	74.72	**40.75**
Acetic	90.20	85.40	83.75	67.87	35.77
H_2SO_4	83.50	80.50	69.29	60.65	36.28
Formic	90.60	85.96	81.88	70.69	34.87
H_3PO_4	86.20	82.80	72.92	61.07	31.98
HNO_3	83.90	79.06	71.18	60.06	28.82
NaOH	86.00	82.93	75.90	84.51	39.72
KOH	86.80	88.39	87.00	89.68	39.68
$(NH_4)_2OH_3$	86.90	88.96	86.20	74.11	40.20
Ca $(OH)_2$	87.70	90.11	89.81	79.84	40.56

Ten grams of dewaxed SCT in the total reaction of 100 ml was leached with H_2O, 1% w/w of acids, and alkalis in the Parr reactor at the temperature of 80°C with a reaction time of 2 h and mixing at 250 rpm. **Table 17** shows the pulp yield%, cellulose, hemicellulose, and lignin recovery % and ash removal % using 1% w/w of water, acids (HCl, acetic acid, H_2SO_4, formic acid, H_3PO_4, HNO_3) and alkalis (NaOH, KOH, $(NH_4)_2OH_3$, Ca $(OH)_2$) leaching. In short, the pulp yield of H_2O was the highest of the acids and alkalis leaching methods. The cellulose recovery% of H_2O is a little higher than acids leaching. Moreover, the hemicellulose and lignin recovery% of H_2O are significantly higher than not only acids but also alkalis leaching. Ash removal % of HCl 1% was higher than others water, acids and alkalis leaching.

3.1.5 De-ashing with acid and alkaline leaching

Table 18 Pulp yield (%) from de-ashing of dewaxed SCT with different concentrations of acetic and NaOH

Solvents	**Initial wt.(g)**	**Final wt.(g)**	**Pulp yield (%)**
Acetic 1%	10	9.02 ± 0.02	90.20
Acetic 2%	10	9.01 ± 0.01	90.10
Acetic 4%	10	8.90 ± 0.02	89.00
NaOH 1%	10	8.70 ± 0.07	87.00
NaOH2%	10	8.67 ± 0.03	86.70
NaOH4%	10	8.63 ± 0.01	86.30

Ten grams of dewaxed SCT in the total aqueous solution volume of 100 ml was leached with 1%, 2%, and 4% of acetic acid (CH_3COOH), and sodium hydroxide (NaOH) in the Parr reactor 0.65 L at the temperature of 80°C with a retention time of 2 h with mixing at 250 rpm. The pulp yield % of 1%, 2%, and 4% acetic acid and NaOH are 90.20%, 90.10%, 89.00%, 87.00%, 86.70%, and 86.30% respectively **Table 18**. I The increasing acid and alkali concentrations tended to result in slightly decreasing pulp yield while lower pulp yield was observed using NaOH compared to acetic acid.

Table 12 De-ashing with acetic and NaOH leaching NREL method

Sample	**Cellulose %**			**Hemicellulose %**			**Lignin %**			**Ash %**		
	Average	SD	**recovery**	Average	SD	**recovery**	Average	SD	**recovery**	Average	SD	**removal**
SCT	32.40	0.44	-	23.05	0.68	-	26.88	0.98	-	11.42	0.45	-
Acetic 1%	27.67	0.69	**85.40**	19.30	0.14	**83.75**	18.24	0.16	**67.87**	7.33	0.42	**35.77**
Acetic 2%	26.29	0.20	**81.13**	18.60	1.43	**80.70**	18.14	0.51	**67.47**	7.32	0.04	**35.86**
Acetic 4%	25.16	0.72	**77.67**	18.55	0.04	**80.49**	18.10	1.70	**67.35**	6.98	0.97	**38.88**
NaOH 1%	26.87	1.42	**82.93**	17.49	2.34	**75.90**	22.72	0.70	**84.51**	6.88	0.00	**39.72**
NaOH 2%	25.20	0.06	**77.78**	17.42	0.10	**75.58**	18.24	2.76	**67.84**	7.12	0.26	**37.64**
NaOH 4%	24.35	0.12	**75.14**	17.36	0.27	**75.31**	15.34	0.26	**57.07**	7.35	0.54	**35.60**

After de-ashing with acetic and NaOH leaching process, the chemical composition of the de-ashed SCT was analyzed by the NREL method. According to **Table 19** and **Figure 5**, ash removal % of 1%, 2%, and 4% acetic acid and NaOH were 35.77%, 35.86%, 38.88%,

39.72%, 37.64%, and 35.60% respectively. Increasing acetic acid concentration tended to result in increasing ash removal efficiency; however, with lower recoveries of cellulose, hemicellulose, and lignin. In contrast, an opposite trend on de-ashing efficiency was observed with increasing concentration of NaOH, also with decreasing trends on cellulose, hemicellulose, lignin recoveries and ash removal.

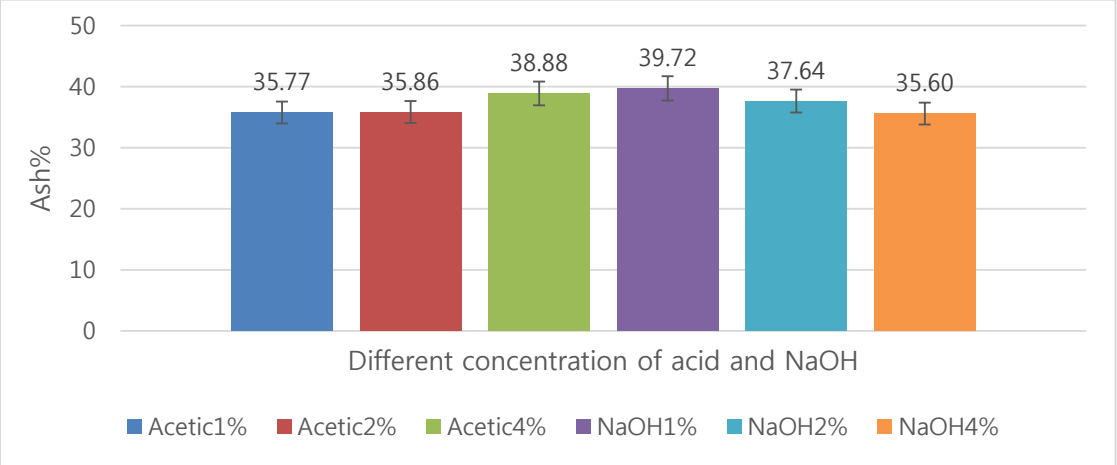

Figure 5 De-ashing with 1%, 2% and 4% of acetic acid and NaOH leaching ash removal %

4. Inductively Couple Plasma Atomic Emission Spectroscopy (ICP-AES) results for biomass

Table 13. ICP-AES of raw sugarcane trash, dewaxed toluene, and deashed HCl 10%

Elements	Results (mg/L)			Results (%)		
	Raw SCT	Dewaxed Toluene	Deashed HCl 10%	Raw SCT	Dewaxed Toluene	Deashed HCl 10%
Aluminum (Al)	0.180	0.246	0.742	0.73	1.33	5.66
Calcium (Ca)	4.930	4.090	5.600	20.14	22.14	42.74
Iron (Fe)	0.004	0.344	0.449	0.02	1.86	3.43
Magnesium (Mg)	1.380	1.150	1.190	5.64	6.22	9.09
Potassium (K)	9.250	6.340	1.930	37.78	34.32	14.73
Silicon (Si)	0.498	0.426	0.488	2.03	2.31	3.72
Sodium (Na)	3.310	2.290	0.868	13.52	12.40	6.63
Phosphorus (P)	1.380	0.548	1.030	5.64	2.97	7.86
Sulfur (S)	3.550	3.040	0.803	14.50	16.45	6.13
Total	**24.482**	**18.474**	**13.100**	**100**	**100**	**99.99**

Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES), revealed that potassium (K), calcium (Ca), sulfur (S), and sodium (Na) were the major elements in the raw sugarcane trash and dewaxed toluene sample. In deashed HCl 10%, calcium (Ca) % is significantly higher than the raw sugarcane trash and dewaxed toluene and silicon (Si) and phosphorus (P) % are a little more than others also. Calcium is a nutrient element. In deashed HCl 10% included potassium (K) 14.73% and Phosphorus (P) 7.86%.

Conclusion

In this study, toluene was the most effective solvent under the experimental conditions for the dewaxing step for recovery of wax extracted from sugarcane trash[17]. Sugarcane trash is a waste product obtained by sugarcane. It is more widely available and less

costly than other wax sources. The use of toluene has also been reported as an efficient solvent for the extraction of wax from other biomass, such as press mud[18], sugar beet[19], bagasse, press cake[20] and sugarcane peels. Pure toluene is an excellent solvent that also has a strong solubilizing capability for waxes[21].

The pulp yield of toluene is higher than others. The extractive yield of toluene was 98% and cellulose, hemicellulose, and lignin recovery of toluene, which were 99.63%, 87.09% and 98.25% respectively that were higher than those obtained using other solvents. Wax form extracted toluene was found very high potent compounds% namely, gamma. -Sitosterol, Stigmasterol, Lanosterol, and Tris(2,4-di-tert-butylphenyl) phosphate (alcohol). The potent compounds from toluene extraction can be widely used for food supplementation, cometic, and industries. For this reason, toluene is the most effective solvent for dewaxing of SCT for subsequent experiments.

This extracted wax may be used in a variety of fields and has market potential at, national and global levels. The presence of several classes of compounds in sugarcane trash wax was discovered in this experiment, include alkane, ester, alcohol, fatty acids, etc. Phytochemicals identified in the wax fraction have been reported for various applications such as dietary supplements, cosmetics and health products.

In de-ashing, acids were shown to have slightly higher efficiency for the ash removal step compared to alkalis. In this experiment, acid and alkali treatment processes utilized (solid: liquid = 1:10) and 1% w/w water leaching or with supplementation of acids (HCl, acetic, formic, H_2SO_4, H_3PO_4, HNO_3) or alkali (NaOH, KOH, Ca $(OH)_2$, $(NH_4)_2OH_3$) and different concentration of 2.5, 5 and 10% of HCl, 1, 2 and 4% of acetic, HCl, formic, H_2SO_4, KOH, NaOH, $(NH_4)_2OH_3$, Ca $(OH)_2$), 0.5% of NaOH and 8% of H_2SO_4 in the Parr reactor with mixing at 250 rpm, 80°C for 2 h retention time. According to the results, HCl 10% was shown the ash removal 51.25% as the most effective catalyst for deashing, although with marked losses in recoveries of the polysaccharide and lignin fractions. The use of dilute acid on the deashing of biomass has been reported for other agricultural wastes, such as SCT and sugarcane bagasse[22]. The leaching process under acidic conditions depend on the at high temperatures and retention time [16]. In this experiment, effective de-waxing and deashing conditions for SCT were reported. These steps can be implemented as an initial step in the processing of SCT before fractionating the biomass and transforming the separated components to biorefinery-valued products.

Acknowledgements

First and foremost, I would like to express my sincere gratitude and respect to supervisor and co-supervisor Assoc. Prof. Dr. Penjit Srinophakun at Kasetsart University and Dr. Verawat Champreda Director of the Biorefinery and Bioproduct Technology Research Group.

I would like to thank Graduate Program Scholarship of The Graduate School, Kasetsart University, and Thailand Advanced Institute of Science and Technology and Tokyo Institute of Technology (TAIST- Tokyo Tech) program under the National Science and Technology Development Agency (NSTDA) for the financial support during the period of my master's study in Thailand.

Special thanks also to Environmental Conservation Department, Ministry of Natural Resources and Environmental Conservation, Myanmar for allowing me to study. I would like to thank my beloved parents, siblings, relatives and friends who are my source of inspiration, crucial encouragement, passion and achievements to complete my studies for Master of Sustainable Energy and Resources Engineering Program at Kasetsart University, Thailand.

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