PRODUCTION OF REDUCING SUGARS FROM SAGO WASTE VIA SEQUENTIAL IONIC LIQUID DISSOLUTION-SOLID ACID SACCHARIFICATION

LEE KIAT MOON

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Name of Candidate: LEE KIAT MOON (I.C/Passport No: 851031-10-5450)

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ABSTRACT

Lignocellulosic biomass can be saccharified to produce reducing sugars that can be converted into various valuable products. This research aimed to produce reducing sugars from sago waste via sequential ionic liquid dissolution-solid acid saccharification process. The study included determining the best ionic liquid and solid acid catalyst combination, process optimisation, and kinetic study of the process, as well as product separation and catalyst recyclability. The ionic liquids investigated were 1-butyl-3-methylimidazolium chloride ([BMIM]Cl), 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) and 1-ethyl-3-methylimidazolium diethyl phosphate ([EMIM][$(EtO)_2PO_2$]), while the solid acid catalysts investigated were Amberlyst 15 (A15), Amberlite IR120 and Nafion NR50. The study has provided a better understanding of the sequential process.

[BMIM]Cl and A15 combination was the preferred process as it produced the highest reducing sugars yield and with the lowest dissolution energy of 3.04 kJ/g sago waste. The sequential process was optimised by applying central composite design (CCD) of response surface methodology (RSM) to yield 98% reducing sugars at a dissolution condition of 160^{o}C with 1.5% substrate loading in 1.75 h, and a saccharification condition of 130^{o}C with 4% catalyst loading in 0.5 h.

In the kinetic study, the generalised Saeman kinetic model was shown to fit the experimental data and so indicated that saccharification of the prehydrolysates obtained from the ionic liquid dissolution process is first order sugars production-first order sugar degradation reaction. The rate constant for sugars formation (k_1) was significantly higher than the rate constant of degradation (k_2). Empirical equations for k_1 and k_2 that accounted for the interactive effects of temperature and catalyst have been developed. Lower activation energies for sugars production (125.1 kJ mol^{-1}) and sugar degradation

(60.8 kJ mol^{-1}) of sago waste were obtained compared to sulfuric acid-catalysed saccharification of other biomasses. The lower values are attributed to high starch content sago waste.

Reducing sugars formed by the sequential process was successfully separated from the ionic liquid by using an aqueous biphasic system (ABS) containing kosmostropic salt, potassium phosphate (K_3PO_4). Approximately 100% and 60% recovery of reducing sugars and ionic liquid respectively were obtained after three extraction cycles. The saccharification efficiency of A15 dropped to less than 60% after three cycles. However, regeneration with sulfuric acid after each cycle restored the efficiency back to almost 100%. The research findings demonstrated the feasibility of sequential ionic liquid dissolution-solid acid saccharification in producing reducing sugars from sago waste.

ABSTRAK

Biojisim lignoselulosa boleh disakarifikasikan untuk menghasilkan gula penurun yang boleh ditukar kepada pelbagai produk yang berharga. Kajian ini bertujuan untuk menghasilkan gula penurun daripada hampas sagu melalui proses pelarutan cecair ionik berjujukan dengan sakarifikasi asid pepejal. Kajian ini termasuk penentuan cecair ionik dan pemangkin asid pepejal yang serasi, pengoptimuman proses dan kajian kinetik, serta pemisahan produk dan kitar semula pemangkin. Cecair ionik yang telah dikaji ialah 1-butil-3-metilimidazolium klorida ([BMIM]Cl), 1-etil-3-metilimidazolium asetat ([EMIM][OAc]) dan 1-etil-3-metilimidazolium dietil fosfat ([EMIM][$(EtO)_2PO_2$]), manakala pemangkin asid pepejal ialah Amberlyst 15 (A15), Amberlite IR120 dan Nafion NR50. Kajian ini telah memberikan pemahaman yang lebih mendalam daripada proses berjujukan.

Kombinasi [BMIM]Cl dan A15 ialah proses pilihan kerana ia menghasilkan gula penurun yang tertinggi dengan tenaga pelarutan terendah iaitu 3.04 kJ/g hampas sagu. Proses ini dioptimum dengan menggunakan reka bentuk komposit pusat (CCD) metodologi permukaan tindak balas (RSM) untuk menghasilkan 98% gula penurun di mana pelarutan berlaku pada keadaan 160^{o}C dengan 1.5% muatan substrat untuk 1.75 h, dan sakarifikasi pada 130^{o}C dengan 4% muatan pemangkin untuk 0.5 h.

Dalam kajian kinetik, model Saeman kinetik umum ditunjukkan sesuai dengan data eksperimen dan mengesahkan bahawa sakarifikasi daripada pra-hidrolisat yang diperolehi daripada proses pelarutan cecair ionik ialah reaksi peringkat pertama penghasilan gula-reaksi peringkat pertama degradasi gula. Pemalar kadar penghasilan gula (k_1) adalah lebih besar berbanding dengan pemalar kadar degradasi (k_2). Persamaan empirikal untuk k_1 dan k_2 yang mengambilkira kesan interaktif suhu and pemangkin telah berjaya dihasilkan. Tenaga pengaktifan yang lebih rendah untuk penghasilan gula

daripada hampas sagu (125.1 kJ mol^{-1}) dan degradasi gula (60.8 kJ mol^{-1}) telah diperolehi berbanding dengan sakarifikasi asid sulfuric daripada biojisim lain. Nilai-nilai yang lebih rendah adalah disebabkan oleh kandungan kanji yang tinggi dalam hampas sagu.

Gula penurun yang dihasil daripada proses berjujukan telah berjaya diasingkan daripada cecair ionik dengan menggunakan sistem dwifasa akueus (ABS) yang mengandungi garam kosmostropic, kalium fosfat (K_3PO_4). Kira-kira 100% dan 60% pemulihan untuk gula penurun dan cecair ionik masing-masing telah diperolehi selepas tiga kitaran pengekstrakan. Efisiensi sakarifikasi A15 telah turun sehingga kurang daripada 60% selepas tiga kitaran. Walau bagaimanapun, regenerasi dengan menggunakan asid sulfurik selepas setiap kitaran dapat mengembalikan efisiensi sakarifikasi sehingga menghampiri 100%. Keputusan kajian menunjukkan bahawa pelarutan cecair ionik berjujukan dengan sakarifikasi asid pepejal berpotensi dalam penghasilan gula penurun daripada hampas sagu.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

[AMIM]Cl	1-Allyl-3-methylimidazolium chloride
[BMIM][BF_4]	1-Butyl-3-methylimidazolium tetrafluoroborate
[BMIM][CF_3SO_3]	1-Butyl-3-methylimidazolium trifluoromethanesulfonate
[BMIM][PF_6]	1-Butyl-3-methylimidazolium hexafluorophosphate
[BMIM]Cl	1-Butyl-3-methylimidazolium chloride
[EMBy][$(EtO)_2PO_2$]	1-Ethyl-3-methylbutylpyridinium diethyl phosphate
[EMIM][$(EtO)_2PO_2$]	1-Ethyl-3-methylimidazolium diethyl phosphate
[EMIM][DBP]	1-Ethyl-3-methylimidazolium dibutyl phosphate
[EMIM][OAc]	1-Ethyl-3-methylimidazolium acetate
[EMIM]Cl	1-Ethyl-3-methylimidazolium chloride
[EMIM]Gly	1-Ethyl-3-methylimidazolium glycinate
β	Hydrogen bond basicity
β_o	Constant coefficient in second order polynomial equation
β_i	*i*th linear coefficient in second order polynomial equation
β_{ii}	Quadratic coefficient in second order polynomial equation
β_{ij}	*ij*th interaction coefficient in second order polynomial equation
ΔU	Change in internal energy (J)
A	Arrhenius constant (min^{-1})
A15	Amberlyst 15
ABS	Aqueous biphasic system
AIL	Acid insoluble lignin
ANOVA	Analysis of variance
ASL	Acid soluble lignin
BV	Bed volume

CCD	Central composite design
C_p	Specific heat capacity of ionic liquid (J kg^{-1} K^{-1})
C_p, *ion*	Molar heat capacity of the ion in ionic liquid (J mol^{-1} K^{-1})
CRV	Calibration verification standard
DNS	3,5-Dinitrosalicylic acid
DW	Dry weight
E_a	Activation energy (kJ mol^{-1})
EDA	Electron donor-electron acceptor
FT-IR	Fourier transform infrared spectroscopy
H^+	Protons
H_3O^+	Hydronium ion
HMF	Hydroxymethylfurfural
HPLC	High-performance liquid chromatography
k_1	Rate constant for sugars production (min^{-1})
k_2	Rate constant for sugar degradation (min^{-1})
K_3PO_4	Potassium phosphate
KBr	Potassium bromide
m	Mass of sample (g)
$Nb_2O_5 \cdot nH_2O$	Niobic acid
NMR	Nuclear magnetic resonance
NREL	National Renewable Energy Laboratory
P	Concentration of reducing sugars (mg ml^{-1})
P_o	Initial concentration of reducing sugars (mg ml^{-1})
Q	Heat transferred to the system
R	Universal gas constant (J mol^{-1} K^{-1})
RS	Reducing sugars

r_S	Rate of degradation of carbohydrates
r_P	Rate of production of reducing sugars
RSM	Response surface methodology
S	Concentration of carbohydrates (mg ml^{-1})
S_o	Initial concentration of carbohydrates (mg ml^{-1})
SO_3H	Sulfonate
SRS	Sugars recovery standard
t	Reaction time (min)
t_{max}	Time to reach maximum concentration of reducing sugars (min)
T	Absolute temperature (K)
TCI	Total crystallinity index
T_i	Initial absolute temperature (K)
T_f	Final absolute temperature (K)
UV-vis	Ultra violet-visible
V	Volume of sample
w	Weight of sample (g)

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background

The use of non-renewable sources of fossil fuels in manufacturing chemicals can jeopardize the economy, the environment and overall global security. The dependency of this non-renewable energy affects the world economy as fuel price increases when it is in short supply. The use of fossil fuel also has great impacts on the environment, including global warming, air quality deterioration, oil spills, and acid rain. To address the issue, the use of renewable feedstock such as sugars is crucial. Sugars can be produced directly from biomass such as sugarcane, corn or other crops, but there is a concern that such processes may compete with the food supply. Alternatively, sugars can be obtained from the carbohydrates component of lignocellulosic biomass. Lignocellulosic biomass with high proportion of carbohydrates is an advantage for production of sugars. For instance, sago waste, a lignocellulosic biomass contains approximately 90% of carbohydrates, *i.e.* 58% of starch, 23% of cellulose and 9.2% of hemicelluloses (Ozawa *et al.*, 1996), and so is a potential substrate for the production of sugars.

Acid saccharification is frequently used to convert lignocellulosic biomass to sugars in view of its effectiveness in sugars production. However, a few known drawbacks such as the need of expensive corrosion-resistant reactors and major waste disposal problems are commonly associated with acid saccharification (Dadi *et al.*, 2006; Li & Zhao, 2007; Zhang & Zhao, 2009). Also, sugars can be lost due to degradation under severe saccharification conditions (Larsson *et al.*, 1999; Sreenath & Jeffries, 2000; Taherzadeh *et al.*, 1999). It is also energy intensive to separate the products formed by acid saccharification (Marzo *et al.*, 2012).

On the other hand, enzymes can be used to replace mineral acids for saccharification to prevent the use of expensive corrosion-resistant reactors. Since enzyme is specific in its action, no degradation products of sugars would be encountered (Zhang *et al.*, 2006). However, the mild saccharification conditions applied in enzymatic saccharification significantly lowers the saccharification rate. Biomass is often not completely converted (Rinaldi *et al.*, 2010a) as a result of restricted accessibility of the glycosidic bonds in polymers, specifically crystalline cellulose (Sievers *et al.*, 2009). Therefore, physical or chemical pretreatment is required prior to enzymatic saccharification in obtaining high sugars yield. However, the high costs of enzymes and pretreatment process (Aden *et al.*, 2002) have made the application of enzymatic saccharification for large-scale sugars production unfavorable from lignocellulosic biomass.

Besides enzymatic saccharification, the employment of solid acid catalyst can also minimise the drawbacks such as equipment corrosion problems and formation of sugar degraded products (Dwiatmoko *et al.*, 2010; Rinaldi *et al.*, 2010b) as encountered in acid saccharification. Comparatively, a higher saccharification rate and a lower catalyst cost can be achieved by solid acid saccharification than enzymatic saccharification. The solid acid saccharification approach also facilitates catalyst separation and reusability (Rinaldi *et al.*, 2010b).

To achieve an efficient conversion of biomass to sugars, the inherent mass transfer limitation problem between the solid acid catalyst and biomass needs to be overcome. This can be done via biomass dissolution prior to solid acid saccharification (Rinaldi *et al.*, 2010b). Biomass dissolution with the aid of ionic liquid can be considered in view of its reported cellulose dissolution and depolymerisation properties (Rinaldi *et al.*, 2010b). Consequently, saccharification of biomass over the solid acid catalyst would become feasible.

1.2 Problem Statement

Sugars is an important commodity for the production of biochemicals or biofuels. An efficient saccharification method to produce sugars from lignocellulosic biomass is still sought after. Integration of ionic liquid and solid acid catalyst to saccharify lignocellulosic biomass to sugars is gaining attention from the researchers as it can overcome the drawbacks of the conventional saccharification methods. However, sugars yields attained so far from this integrated process is not as promising as the conventional saccharification methods. Approximately 12% of sugars yield was attained for ionic liquid dissolution-solid acid saccharification of *Cryptomeria japonica* (Watanabe, 2010) and 45% yield of sugars was attained when a more readily saccharified substrate, *i.e.* cellobiose was used (Dwiatmoko *et al.*, 2010).

Sugars yield can be further improved by having a better understanding of the integrated process. For instance, the employment of suitable lignocellulosic biomass is essential to achieve high sugars yield. As mentioned earlier, sago waste is a potential feedstock as it contains high amount of carbohydrates that can be converted to sugars. In ionic liquid-solid acid catalyst integrated process, the compatibility of the ionic liquid and solid acid catalyst to give satisfactory saccharification performance is important. Furthermore, there is no report on the process optimisation and the reaction kinetics of the integrated process. Process optimisation is deemed necessary to achieve maximum sugars yield from biomass, while the reaction kinetics is useful for better process manipulation. On the other hand, the chemical cost of the reactants is also one of the concerns of the integrated process. To make the integrated process economically feasible, recovery of the ionic liquid and recyclability of the solid acid catalyst are crucial. Little information is available on these mentioned aspects, *i.e.* compatibility of the ionic liquid and solid acid catalyst, process optimisation and reaction kinetics of the

process, as well as recovery and recyclability of the catalyst. Therefore, they were investigated in this study.

1.3 Research Objectives

This project aimed to produce reducing sugars from sago waste via sequential ionic liquid dissolution-solid acid saccharification. The objectives of the study are:

i. To study the compatibility between ionic liquid and solid acid catalyst for the dissolution and saccharification of sago waste
ii. To optimise the operating conditions for the sequential ionic liquid dissolution-solid acid saccharification process
iii. To determine the kinetic rate constants for reducing sugars production and sugar degradation reactions of the ionic liquid-mediated solid acid saccharification
iv. To separate and recover sugars, ionic liquid and solid acid catalyst used for the conversion of sago waste.

1.4 Outline of research approach

The outlines of research approach are:

i. To characterise the chemical composition of sago waste
ii. To select an effective combination of ionic liquid-solid acid catalyst for sugars production
iii. To screen the influential process variables in both the ionic liquid dissolution and solid acid saccharification reactions

iv. To optimise the ionic liquid dissolution of sago waste and also the solid acid saccharification using central composite experimental design

v. To investigate the effect of temperature and catalyst loading on the production and degradation of sugars rate constants

vi. To separate and recover the ionic liquid and sugars by using aqueous biphasic system

vii. To regenerate and recycle the solid acid catalyst

1.5 Structure of Thesis

This thesis is presented in five (5) chapters and the content of each chapter is described as follows:

Chapter 1: Introduction

This chapter presents the background and problem statement of the research, the objectives of the project, outline of research approach, and the overall structure of the thesis.

Chapter 2: Literature Review

This chapter reviews lignocellulosic biomass, ionic liquid dissolution, solid acid saccharification, and integration of ionic liquid dissolution and solid acid saccharification for an effective conversion of biomass to sugars.

Chapter 3: Materials and Methods

This chapter provides details on methods used to achieve the research objectives in this work. It was prepared according to the objectives of the project.

Chapter 4: Results and Discussion

This chapter discusses the results obtained and they are divided into four sections to address four objectives. In the first section, chemical composition of sago waste is reported and the selection of ionic liquid-solid acid catalyst combination is discussed. The next section presents the findings on screening of influential process variables in ionic liquid dissolution and solid acid saccharification reactions, and also process optimisation of the selected process variables in the sequential ionic liquid dissolution-solid acid saccharification process. Results on kinetic study on the ionic liquid-mediated solid acid saccharifcation process are provided in the third section of this chapter. The recovery of ionic liquid and reducing sugars, as well as reusability of solid acid catalyst are provided in the last section.

Chapter 5: Conclusions and Recommendations

This chapter gives an overall conclusion on the feasibility of the sequential process scheme to produce reducing sugars from sago waste. In addition, the novelties, implications of the study and recommendations for future work are provided.

CHAPTER 2

LITERATURE REVIEW

This chapter reviews on the topics related to lignocellulosic biomass, ionic liquid dissolution and solid acid saccharification. A brief introduction of lignocellulosic biomass and sago waste were provided. Followed by reviewing the factors affecting the dissolution performance of ionic liquid reaction. Besides, methods to recover and recycle ionic liquid were also included. Different types of solid acid catalysts commonly used in solid acid saccharification, and influential variables affecting the saccharification performance were thoroughly reviewed. Lastly, ionic liquid integrated solid acid saccharification process scheme for sugars production were compiled.

2.1 Lignocellulosic Biomass

Lignocellulosic biomass are plant biomass that primary compose of cellulose, hemicelluloses and lignin together with small amounts of pectin, protein, extractives and ash (Jørgensen *et al.*, 2007). The composition of these constituents varies from one biomass to another, and within a single biomass due to age, stage of growth and grow environment (Pérez *et al.*, 2002). Typically, biomass contains of 35-50% cellulose, 20-35% hemicelluloses and 10-25% lignin (Sun & Cheng, 2002). The compositions of three major constituents in commonly available biomass are tabulated in Table 2.1. It can be noticed that in a single biomass, 50-70% of its dry weight is composed of holocellulose, *i.e.* the total polysaccharides composed of cellulose and hemicelluloses. The holocellulose can be converted to sugars as their common building block is made of sugars. Moreover, the use of lignocellulosic biomass in sugars production is more favorable than crops as it does not compete with food supply (Zavrel *et al.*, 2009).

Therefore, the abundantly available lignocellulosic biomass is an inexhaustible source of sugars for significant industrial applications.

Table 2.1: Chemical compositions of commonly available lignocellulosic biomass

Type	Cellulose (% DW)	Hemicellulose (% DW)	Lignin (% DW)	Reference
Corn stover	38.0	26.0	19.0	Lee *et al.* (2007)
Oil palm frond	25.1	24.1	18.5	Tan *et al.* (2010)
Rice husk	53.2	4.6	19.7	Ang *et al.* (2011)
Sago waste [a]	23.0	9.2	3.9	Ozawa *et al.* (1996)
Sugarcane bagasse	41.0	30.1	21.2	Yoon *et al.* (2012)
Switchgrass	36.6	21.1	16.3	Suryawati *et al.* (2009)
Wheat straw	38.0	29.0	15.0	Lee *et al.* (2007)

DW: dry weight
[a] Sago waste also contains 58.0% of starch.

2.1.1 Sago palm and sago waste

Sago palm is a lignocellulosic biomass that is scientifically known as *Metroxylon sagu*. It is a hardy plant that grows well in swampy, acidic peat soils, submerged and saline soils (Flach & Schuiling, 1988; Hisajima, 1994). This species is commercially grown in Asia-Pacific region and South East Asia such as Malaysia, Indonesia, Papua New Guinea, Thailand, Philippines and Vietnam (Singhal *et al.*, 2008). Sago palm is economically important as it contains useful quantities of starch in its pith which serves as primary dietary source to over a million of people (Awg-Adeni *et al.*, 2010).

To maximise the production of starch from sago palm, the palm trees are felled after flowering and before fruiting stage, as starch content declines rapidly after fruiting (Singhal *et al.*, 2008). The trunks are stripped of leaves and cut into length of about 1 m of sago logs. These logs are sent to factory for sago palm processing. The logs are first debarked and the inner portion of the trunk, known as pith contains mainly of starch, has to undergo several processing stages to extract large quantity of starch with good

quality. The pith is rasped, grated into small particles resembling sawdust, kneaded with water and filtered through sieves to extract relatively large starch granules (20-60 μm) (Shipman, 1967). Further purification is carried out on the extracted starch to obtain pure sago starch. During sago starch processing, three major types of by-products are generated, they are bark, fibrous pith residue (commonly known as sago waste) and wastewater. Figure 2.1 shows the schematic flow diagram of sago processing.

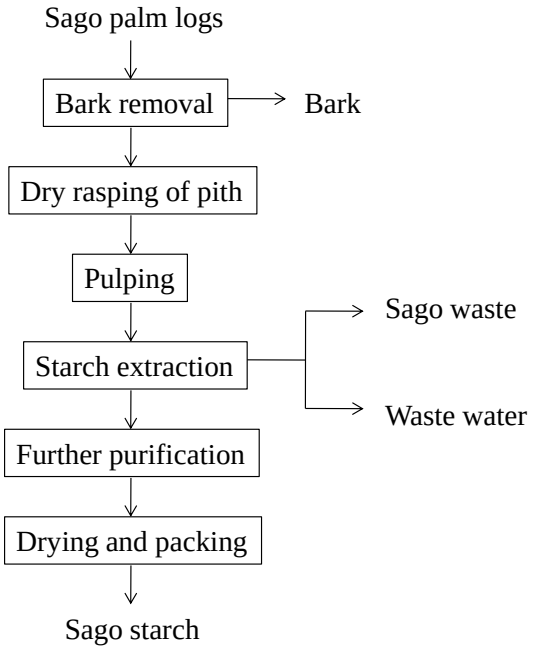

Figure 2.1: Schematic flow diagram of sago processing.

Sago waste contains large amount of cellulosic fibrous materials and it is difficult to be dried due to high moisture and starch content, and has rendered as environmental pollutant. Therefore, it is imperative to utilise this waste to mitigate its effect to the environment. In the past, sago waste was burnt or simply discarded since it had no known significant industrial or commercial uses. Until, it was found that sago waste contained 58% of starch apart from its lignocellulosic materials (Ozawa *et al.*, 1996). This has made sago waste a suitable animal feed, organic fertilizer and it can be used as biosorbent (Quek *et al.*, 1998) and applied to chipboard (Phang *et al.*, 2000) and

enzyme production (Kumaran *et al.*, 1997). In view of its high starch and lignocellulosic materials contents, sago waste can also be a substrate for sugars production.

2.2 Ionic Liquid Dissolution

The utilisation of lignocellulosic biomass in the production of sugars often results in low sugars yield. This is due to major fraction of the sugars molecules are intact within the cellulose and other polysaccharides, hence well protected against chemical processing (Rinaldi *et al.*, 2008). Thus, pretreatment is required to break down the highly-ordered structure of the biomass in order to attain an effective saccharification for production of sugars. Various pretreatment technologies such as physical pretreatment, physicochemical pretreatment, chemical pretreatment and biological pretreatment are available. Physical pretreatment involves the breakdown of lignocellulosic biomass into smaller particles through milling, thereby facilitates the mass transfer during saccharifcation (Chandra *et al.*, 2007). Chemical pretreatment uses chemicals such as acid and alkaline to breakdown the recalcitrant structure of lignocellulosic biomass to assist saccharifcation reaction. Physicochemical pretreatment combines both the physical and chemical techniques to break the structure of the lignocellulosic biomass. While, biological pretreatment involves the use of microorganism to degrade the lignin content of the biomass (Galbe & Zacchi, 2007).

Among the pretreatment technologies, chemical pretreatment is widely explored by researchers and selected to pretreat lignocellulosic biomass. The widely known chemical pretreatments are acid pretreatment, alkaline pretreatment, oxidative pretreatment and organosolv pretreatment. Although they are effective in pretreating lignocellulosic biomass, they have a few significant drawbacks. For instance, acid, alkaline and oxidative pretreatments can saccharify the hemicelluloses and/or cellulose content in the biomass to undesired sugar degraded products (Mosier *et al.*, 2005). All

these pretreatment methods lead to a low production of sugars from lignocellulosic biomass. Furthermore, organosolv pretreatment is difficult to handle as some of the solvents used are highly flammable and explosive (Galbe & Zacchi, 2007).

Therefore, non-conventional chemical pretreatment that employed ionic liquids in dissolving lignocellulosic biomass has been developed (Fort *et al.*, 2007; Haykir *et al.*, 2013; Kilpeläinen *et al.*, 2007; Li *et al.*, 2010a; Li *et al.*, 2009; Sun *et al.*, 2009; Yang *et al.*, 2013; Zavrel *et al.*, 2009). Application of ionic liquids to dissolve lignocellulosic biomass has been attempted attributing to their low volatility, low toxicity, and high thermal stability (Cao *et al.*, 2009; Dadi *et al.*, 2006). Ionic liquids are inorganic salts which composed of both organic nitrogen inorganic cations and inorganic anions (Feng & Chen, 2008). Thus, their properties can be modified by having different combination of cation and anion, so that they can be applied to catalysis, electrolytes, advanced materials and polymer systems (Feng & Chen, 2008; Wang *et al.*, 2011; Zhu *et al.*, 2006).

Ionic liquids are capable of dissolving carbohydrates as they can break the extensive network of hydrogen bonds in cellulose and/or carbohydrates to yield smaller sugar oligomers. The mechanism of cellulose dissolution in ionic liquids involves the formation of electron donor-electron acceptor (EDA) complexes between the ionic liquids, oxygen and hydrogen atoms of the cellulose-OH (Feng & Chen, 2008). As suggested by Kosan *et al.* (2008), this primarily occurs between the hydroxyl groups at C-3 and C-6 positions of neighboured cellulose chains. In EDA complexes, cations and anions of ionic liquids are respectively electron acceptor and electron donor centre while the oxygen atoms and the hydrogen atoms of cellulose are the electron pair donor and electron acceptor. As the result of the interaction, the oxygen and hydrogen atoms from the hydroxyl groups are separated. This disrupts the hydrogen bonds between the

molecular chains of cellulose and subsequently dissolves the cellulose. Figure 2.2 shows the mechanism of cellulose dissolution in ionic liquid.

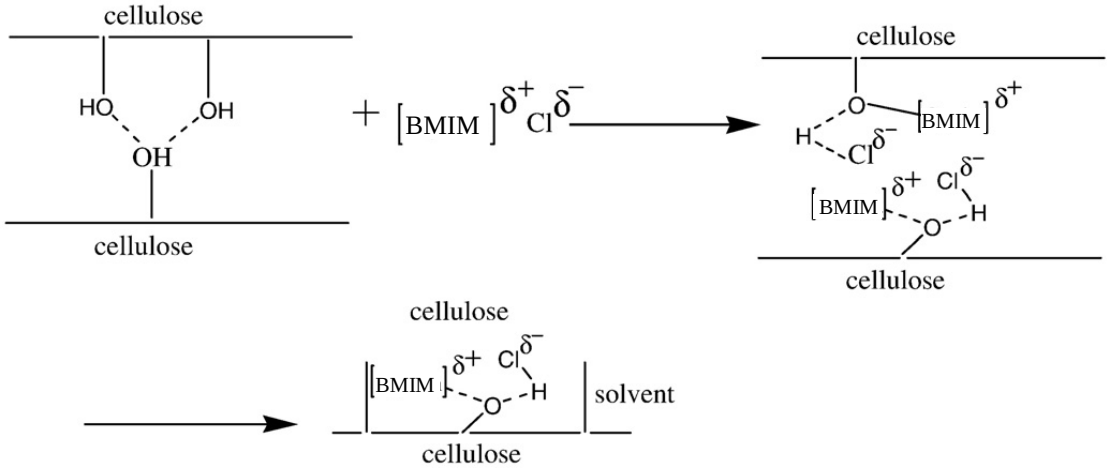

Figure 2.2: Dissolution mechanism of cellulose in 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) (adapted from Feng & Chen (2008)).

As mentioned earlier, to separate the cellulose polymeric chains, ionic liquid only requires to attack the hydroxyl groups at C-3 and C-6 positions of cellulose. However, interaction of C-6 and C-2 hydroxyls creates additional hydrogen bond attack at C-2 hydroxyl group and assists in breaking the interchain hydrogen bonding (Pinkert *et al.*, 2010). Hence, the simultaneous interactions of ionic liquid with C-2, C-3 and neighboured C-6 hydroxyls could facilitate the separation of adjacent cellulose chain. This suggests that an ionic liquid is more likely to attack more than one cellulose hydroxyl groups at once.

2.2.1 Factors affecting the dissolution performance of ionic liquid

The dissolution performance of ionic liquid is affected by factors namely type of ionic liquid, type of lignocellulosic biomass, biomass loading, sample particle size, dissolution temperature, reaction time, and moisture content in both the biomass and the

ionic liquid. The effect of each of the process variables was elucidated in the following sub-sections.

2.2.1.1 Type of ionic liquid

The solubility of cellulose in ionic liquid was first reported by Swatloski *et al.* (2002). They demonstrated that the ionic liquid 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) could dissolve 25 wt% of cellulose under microwave irradiation. Other than chloride-based ionic liquids, anions of formate, acetate or alkylphosphonate also possess cellulose dissolution properties (Liebert & Heinze, 2008; Zhao *et al.*, 2009). Table 2.2 lists the dissolution capability of different types of ionic liquids in biomass.

It is found that hydrogen bond basicity (β) value of the ionic liquid is a determining factor for carbohydrate dissolution (Crowhurst *et al.*, 2003; Kilpeläinen *et al.*, 2007; Mäki-Arvela *et al.*, 2010). According to the ^{13}C and $^{35/37}Cl$ NMR relaxation measurements conducted by Remsing *et al.* (2006), the β value is dominated by the nature of the anion. Ionic liquids with higher β values have higher dissolution properties. For instance, 1-allyl-3-methylimidazolium chloride ([AMIM]Cl) and [BMIM]Cl with β values of 0.83 and 0.84 respectively were reported to have higher biomass dissolution compared to 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]) and 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) with lower β values of 0.38 and 0.21 respectively. In another study, ionic liquids with high β values also reported to have greater swelling properties (Brandt *et al.*, 2010). This is because, ionic liquids with high β values are able to weaken the hydrogen bonding interactions by coordinating the hydroxyl groups, leading to swelling of biomass and assist in dissolution.

Table 2.2: Dissolution of lignocellulosic biomass in ionic liquids

Ionic liquid	Hydrogen bond basicity	Biomass	Conditions	Dissolution	Reference
[AMIM]Cl	0.83 [a]	Maple wood flour	80^{o}C, 24 h	>30 g/kg	Lee *et al.* (2009)
		Southern pine powder	80^{o}C, 8 h	8 wt%	Kilpeläinen *et al.* (2007)
		Norway spruce sawdust	80^{o}C, 24 h	5 wt%	Kilpeläinen *et al.* (2007)
		Chestnut chips	90^{o}C, 12 h	Complete dissolution	Zavrel *et al.* (2009)
		Common beech chips	90^{o}C, 12 h	Complete dissolution	Zavrel *et al.* (2009)
		Silver fir chips	90^{o}C, 12 h	Complete dissolution	Zavrel *et al.* (2009)
		Spruce chips	90^{o}C, 12 h	Complete dissolution	Zavrel *et al.* (2009)
		Norway spruce sawdust	110^{o}C, 8 h	8 wt%	Kilpeläinen *et al.* (2007)
		Southern pine TMP	110^{o}C, 8 h	2 wt%	Kilpeläinen *et al.* (2007)
		Norway spruce TMP	130^{o}C, 8 h	7 wt%	Kilpeläinen *et al.* (2007)
		Southern pine TMP	130^{o}C, 8 h	5 wt%	Kilpeläinen *et al.* (2007)
[BMIM]Cl	0.84 [a]	Maple wood flour	80^{o}C, 24 h	>30 g/kg	Lee *et al.* (2009)
		Chestnut chips	90^{o}C, 12 h	Partial dissolution	Zavrel *et al.* (2009)
		Common beech chips	90^{o}C, 12 h	Partial dissolution	Zavrel *et al.* (2009)
		Silver fir chips	90^{o}C, 12 h	Partial dissolution	Zavrel *et al.* (2009)
		Spruce chips	90^{o}C, 12 h	Partial dissolution	Zavrel *et al.* (2009)

Table 2.2, continued

Ionic liquid	Hydrogen bond basicity	Biomass	Conditions	Dissolution	Reference
[BMIM]Cl	0.84 [a]	Wheat straw	100^{o}C, 1 h	Complete dissolution	Li *et al.* (2009)
		Oak	100^{o}C, 24 h, cosolvent DMSO-d_6	57 wt%	Fort *et al.* (2007)
		Eucalyptus	100^{o}C, 24 h, cosolvent DMSO-d_6	65 wt%	Fort *et al.* (2007)
		Poplar	100^{o}C, 24 h, cosolvent DMSO-d_6	68 wt%	Fort *et al.* (2007)
		Pine	100^{o}C, 24 h, cosolvent DMSO-d_6	68 wt%	Fort *et al.* (2007)
		Norway spruce sawdust	110^{o}C, 8 h	8 wt%	Kilpeläinen *et al.* (2007)
		Southern yellow pine, <0.125 mm	110^{o}C, 16 h	52.6%	Sun *et al.* (2009)
		Southern yellow pine, 0.25-0.50 mm	110^{o}C, 16 h	26.0%	Sun *et al.* (2009)
		Spruce wood	130^{o}C, 4.5 h	Complete dissolution	Aaltonen & Jauhiainen (2009)
		Norway spruce TMP	130^{o}C, 8 h	7 wt%	Kilpeläinen *et al.* (2007)
		Southern pine TMP	130^{o}C, 8 h	5 wt%	Kilpeläinen *et al.* (2007)

Table 2.2, continued

Ionic liquid	Hydrogen bond basicity	Biomass	Conditions	Dissolution	Reference
[BMIM]Cl	0.84 [a]	Spruce wood	130°C, 10 h	Complete dissolution	Aaltonen & Jauhiainen (2009)
		Wood chips	130°C, 15 h	Partially soluble	Kilpeläinen *et al.* (2007)
		Spruce wood	130°C, 21 h	Complete dissolution	Aaltonen & Jauhiainen (2009)
[BMIM][BF_4]	0.38 [b]	Maple wood flour	80°C, 24 h	<1 g/kg	Lee *et al.* (2009)
[BMIM][CF_3SO_3]	0.46	Maple wood flour	80°C, 24 h	<1 g/kg	Lee *et al.* (2009)
[BMIM][PF_6]	0.21 [b]	Maple wood flour	80°C, 24 h	<1 g/kg	Lee *et al.* (2009)
[EMBy][$(EtO)_2PO_2$]	nd	Wheat straw	100°C, 1 h	Complete dissolution	Li *et al.* (2009)
[EMIM]Cl	nd	Chestnut chips	90°C, 12 h	Partial dissolution	Zavrel *et al.* (2009)
		Common beech chips	90°C, 12 h	Partial dissolution	Zavrel *et al.* (2009)
		Silver fir chips	90°C, 12 h	Partial dissolution	Zavrel *et al.* (2009)
		Spruce chips	90°C, 12 h	Partial dissolution	Zavrel *et al.* (2009)
[EMIM]Gly	1.20 [c]	Bamboo powder	120°C, 8 h	Complete dissolution	Muhammad *et al.* (2011)
[EMIM][DBP]	nd	Wheat straw	100°C, 1 h	Complete dissolution	Li *et al.* (2009)
[EMIM] [$(EtO)_2PO_2$]	nd	Wheat straw	100°C, 1 h	Complete dissolution	Li *et al.* (2009)

Table 2.2, continued

Ionic liquid	Hydrogen bond basicity	Biomass	Conditions	Dissolution	Reference
[EMIM][OAc]	nd	Maple wood flour	80^{o}C, 24 h	<5 g/kg	Lee *et al.* (2009)
		Chestnut chips	90^{o}C, 12 h	Complete dissolution	Zavrel *et al.* (2009)
		Common beech chips	90^{o}C, 12 h	Complete dissolution	Zavrel *et al.* (2009)
		Silver fir chips	90^{o}C, 12 h	Partial dissolution	Zavrel *et al.* (2009)
		Spruce chips	90^{o}C, 12 h	Complete dissolution	Zavrel *et al.* (2009)
		Wheat straw	100^{o}C, 1 h	Complete dissolution	Li *et al.* (2009)
		Red oak, 0.125-0.250 mm	110^{o}C, 16 h	99.5%	Sun *et al.* (2009)
		Red oak, 0.25-0.50 mm	110^{o}C, 16 h	98.5%	Sun *et al.* (2009)
		Red oak, 0.5-1.0 mm	110^{o}C, 16 h	97.8%	Sun *et al.* (2009)
		Southern yellow pine, <0.125 mm	110^{o}C, 16 h	98.5%	Sun *et al.* (2009)
		Southern yellow pine, 0.125-0.250 mm	110^{o}C, 16 h	98.2%	Sun *et al.* (2009)
		Southern yellow pine, 0.25-0.50 mm	110^{o}C, 16 h	93.5%	Sun *et al.* (2009)
		Southern yellow pine, 0.5-1.0 mm	110^{o}C, 16 h	92.6%	Sun *et al.* (2009)
		Switchgrass	160^{o}C, 3 h	50.7 wt%	Li *et al.* (2010a)

[a] Fukaya *et al.* (2006); [b] Crowhurst *et al.* (2003); [c] Ohno & Fukumoto (2007); [AMIM]: 1-allyl-3-methylimidazolium; [BMIM]: 1-butyl-3-methylimidazolium; [EMBy]: 1-ethyl-3-methylbutylpyridinium; [EMIM]: 1-ethyl-3-methylimidazolium; CF_3SO_3: trifluoromethanesulfonate; Cl: chloride; BF_4: tetrafluoroborate; DBP: dibutyl phosphate; $(EtO)_2PO_2$: diethyl phosphate; Gly: glycinate; OAc: acetate; PF_6: hexafluorophosphate.

The dissolution efficiency of the ionic liquid is also influenced by the size of its cation and anion. Ionic liquids with smaller cations are often more effective in cellulose dissolution as reported by Kosan *et al.* (2008) and Zhang *et al.* (2005) whereby smaller $[AMIM]^+$ ion shows better cellulose dissolution ability than $[EMIM]^+$ and $[BMIM]^+$ ions. As the cation size of the ionic liquid increases, its ability to form hydrogen bonds with cellulose decreases thus affecting the efficiency of dissolution (Zhao *et al.*, 2008). Similarly, a small anion with hydrogen-bond acceptor such as Cl^- is effective in cellulose dissolution but not for large and non-coordinating anions like BF_4^- and PF_6^- (Swatloski *et al.*, 2002). An efficient ionic liquid in dissolution of biomass should therefore contain small size of cation and anion with high β value.

2.2.1.2 Biomass loading

The dissolution performance of lignocellulosic biomass in ionic liquid is also affected by the biomass-to-ionic liquid ratio. More biomass remain undissolved with increased biomass-to-ionic liquid ratios (Xie & Shi, 2006). This is because the contact between the ionic liquid and the biomass is limited at high biomass-to-ionic liquid ratio (Tan *et al.*, 2010). The commonly applied biomass loading was reported in the range of 1% to 10% (w/w).

2.2.1.3 Particle size of biomass

Apart from the low biomass loading, it has been reported that smaller biomass particles gives better dissolution performance in ionic liquid (Muhammad *et al.*, 2011; Sun *et al.*, 2009). According to Sun *et al.* (2009), 52.6% of southern yellow pine with particle size < 0.125 mm was dissolved in [BMIM]Cl compared to only 26.0% for particle size of 0.25 mm to 0.50 mm as shown in Table 2.2. The increase in dissolution performance for biomass with a smaller particle size is due to the increase in surface area for reaction

(Muhammad *et al.*, 2011). In addition, the mechanical grinding process to obtain smaller particle sizes indirectly acts as a pretreatment process by breaking down the internal structure of the biomass, hence subsequent dissolution reaction is enhanced (Sun *et al.*, 2009).

2.2.1.4 Dissolution temperature

The dissolution efficiency of ionic liquid is affected by operational conditions such as temperature, moisture content and time of reaction. Dissolution of lignocellulosic biomass is usually conducted at temperature 80^{o}C to 160^{o}C. Within this temperature range, better biomass dissolution was reported at higher temperature (Xie & Shi, 2006). The enhanced dissolution process at high temperature might have caused by the high energy provided to dissolve the biomass (Muhammad *et al.*, 2011). When dissolution conducted at high temperature, viscosity of the ionic liquid is reduced and this will lead to better mass transfer between the biomass and the ionic liquid (El Seoud *et al.*, 2007; Kuang *et al.*, 2007). Besides, at higher dissolution temperature, the effect of moisture content during the reaction is reduced (Sun *et al.*, 2009) since the presence of water has hampered the dissolution capability of ionic liquid (Cao *et al.*, 2009; Swatloski *et al.*, 2002).

2.2.1.5 Moisture content

The presence of water significantly reduces the dissolution capability of the ionic liquid. As low as 1 wt% of water in [BMIM]Cl is sufficient to impede cellulose dissolution (Swatloski *et al.*, 2002). The presence of water in ionic liquids forms hydrodynamic shells around the ionic liquid molecules making it difficult to have direct interaction with cellulose and thus its cellulose dissolution capability is reduced (Zavrel *et al.*,

2009). Therefore, prior to dissolving the biomass, the residual water in ionic liquid and the lignocellulosic biomass needs to be removed thoroughly.

2.2.1.6 Dissolution time

Ionic liquid dissolution of biomass have been conducted over a period of 1 to 24 h by many researchers (Table 2.2). A long reaction time is useful for biomass dissolution in ionic liquid (Xie & Shi, 2006). However, a contradictory result was reported by Fort *et al.* (2007) whereby the authors discovered that continuing the dissolution process for long period of time did not lead to any increase in dissolution performance but resulted in substantial polymer degradation. The disparity in findings may be attributed to the variation in other operational variables such as type of ionic liquid, type of biomass and dissolution temperature. Therefore, it is important to investigate all the influential process variables and their possible interactive effects for different system when different biomass or ionic liquids are employed.

2.2.2 Costs and impacts of ionic liquids to the environmental

Ionic liquids gain great attention in academic research due to their numerous advantages and wide applications in various areas. Their utilisations in the industries somehow have been limited due to their high costs. Ionic liquids are synthesized laboratory and in view of this, they could only utilise in small-scale and specialized processess. Lately, there is a study of synthesizing ionic liquid via acid-base neutralisation using intensification processing (Chen *et al.*, 2014). It was found that process intensification reduced the production cost of ionic liquids dramatically. The production cost of ionic liquid can be overwhelmed by the raw materials. By manufacturing the raw materials, the raw materials costs could be as little as the conventional organic solvents such as acetone. Process intensification also can lower the energy requirement, which can

further reduce the production cost of ionic liquids. These suggest that ionic liquids may not necessary be expensive, and thus large-scale-ionic liquid-based processes could become a reality.

Ionic liquids are ecological friendly alternatives to the conventional volatile organic solvents due to their high thermal stability and low volatility properties (Cao *et al.*, 2009; Dadi *et al.*, 2006). Despite some clear advantages, some ionic liquids are reported to exhibit toxicity (Docherty & Kulpa, 2005). The toxicity level of ionic liquid reported to be correlated directly with the length of the alkyl chain substituent (Megaw *et al.*, 2015). Many ionic liquids are water soluble, and the high stability of these compounds reflected in its recalcitrant to biodegradation. Thus, absorption of ionic liquid into soils and sediments was reported, and ionic liquids with shorter chains and hydroxyl groups are of higher mobility and expected to potentially contaminate the surface and ground water (Mrozik *et al.*, 2012).

2.2.3 Recovery and recycling of ionic liquid

The recyclability of the ionic liquid is pertinent because of its high costs and its impacts to the environment. Many attempts had been made to recover ionic liquid from the aqueous sugars solution, using aqueous biphasic system (Gutowski *et al.*, 2003) and ion exchange chromatography (Binder & Raines, 2010). Aqueous biphasic systems are widely used for products purification, extraction and enrichment (Abraham *et al.*, 2003; Akama & Sali, 2002; Willauer *et al.*, 2002). A biphasic system based on ionic liquids can be generated with the addition of an aqueous solution containing a kosmotropic anion of phosphate, carbonate or sulfate. Gutowski *et al.* (2003) first reported that the addition of potassium phosphate into an aqueous solution of [BMIM]Cl produced a two-phase system. The upper phase was rich in [BMIM]Cl and the lower phase consisted of potassium phosphate. Different aqueous biphasic systems had later been

generated for different combination of ionic liquids and salts (Bridges *et al.*, 2007; Deng *et al.*, 2007; Deng *et al.*, 2009; Pei *et al.*, 2007; Wu *et al.*, 2008a). Through the formation of two-phase system, hydrophilic ionic liquids were concentrated from the aqueous solutions with recovery of 96.8% of ionic liquid in [AMIM]Cl/salt system (Deng *et al.*, 2007).

Ionic liquids can also be recovered by ion exchange chromatography. Using this technique, a mixture containing both electrolyte and non-electrolyte solutes is separated by passing the mixture through the charged resins (Asher, 1956). To separate the saccharification ionic products containing liquid and sugars using ion exchange chromatography, the electrolyte (ionic liquid) will first be eluted, while the non-electrolyte (sugars) will be retained by the resins and elute in the later stage. The advantage of this method is that the sugar degraded products such as hydroxymethylfurfural (HMF) and furfural can also be separated. The non-polar species of HMF and furfural are absorbed more than sugars and hence eluted at much slower rate. A recovery of more than 95% of ionic liquid was achieved on corn stover hydrolysate by employing [EMIM]-exchanged Dowex 50 resin (Binder & Raines, 2010). The study had also reported to recover 94% and 88% of glucose and xylose respectively.

2.3 Solid Acid Saccharification

The application of ionic liquids on biomass dissolution is encouraging since the solvents can dissolve large quantity of carbohydrates under considerably mild conditions and can be recycled with nearly 100% purity (Heinze *et al.*, 2005). The dissolved carbohydrates can then readily be converted to sugars through saccharification reaction. Solid acid saccharification is one of the preferred methods to convert the carbohydrates to sugars. The employment of solid catalyst in chemical processes is favorable due to its

characteristics such as efficient catalytic activity, high selectivity, long catalyst life spent and catalyst reusability (Guo *et al.*, 2012). Most importantly, solid catalyst eases catalyst separation after reaction. This is essential in chemical industries as separation processes could exceed half of the total equipment investment cost (King, 2000). In view of these advantages, solid catalyst had been employed for approximately 80-85% of the chemical processes (Ertl *et al.*, 2008).

Solid acid catalysts that had been used in catalytic saccharification include inorganic strong Brønsted solid acids such as niobic acid (Nb_2O_5•nH_2O) and H-type zeolites (H-mordenite, H-ZSM5), strong acidic ion exchange resins such as perfluorosulfonated resins (Nafion NR50) and polystyrene-based cation exchange resin (Amberlyst 15), and organic-inorganic hybrid sulfonic mesoporous silicas (Hahn-Hägerdal & Skoog, 1984; Kim *et al.*, 2005; Kitano *et al.*, 2009; Lanzafame *et al.*, 2012; Marzo *et al.*, 2012; Onda *et al.*, 2008; Suganuma *et al.*, 2008; Yamaguchi *et al.*, 2009). The solid acid catalysts mentioned have been applied for saccharification of lignocellulosic biomass, disaccharides, cellulose and starch. The saccharification performances of these solid acid catalysts are listed in Table 2.3.

Table 2.3: Catalytic performance of solid acid catalysts in saccharification

Catalyst	**Catalyst properties**			**Substrate**	**Pretreatment**	**Saccharification conditions**	**Glucose yield (%)**	**Reference**
	Maximum acidity (H_o)	**Surface area ($m^2\ g^{-1}$)**	**Acid density (mmol g^{-1})**					
Nafion NR50	-11 to -13	< 1	0.9	Cellohexaose	-	90^{o}C, 1 h	1	Kitano *et al.* (2009)
Silica-supported Nafion NR50	-11 to -13	344	0.1	Cellohexaose	-	90^{o}C, 1 h	0	Kitano *et al.* (2009)
H-mordenite	-5.6	360	1.5	Cellulose	Milling	150^{o}C, 24 h	6	Onda *et al.* (2008)
				Cellulose	-	190^{o}C, 5 h	18	Lanzafame *et al.*(2012)
				Cellohexaose	-	90^{o}C, 1 h	0	Kitano *et al.* (2009)
Niobic acid	-5.6	128	0.4	Cellohexaose	-	90^{o}C, 1 h	0	Kitano *et al.* (2009)
Amberlyst 15	-2.2	50	4.8	Cellulose	Milling	150^{o}C, 24 h	25	Onda *et al.* (2008)
				Cellulose	-	190^{o}C, 5 h	18	Lanzafame *et al.*(2012)
				Cellohexaose	-	90^{o}C, 1 h	1	Kitano *et al.* (2009)
γ-Alumina	nd	140	0.049	Cellulose	Milling	150^{o}C, 24 h	3	Onda *et al.* (2008)
H-ZSM5	nd	124	0.30	Cellulose	Milling	150^{o}C, 24 h	11	Onda *et al.* (2008)
Silica-supported sulfated zirconia	nd	426	nd	Cellulose	-	190^{o}C, 5 h	5	Lanzafame *et al.*(2012)
Sulfated zirconia	nd	52	1.6	Cellulose	Milling	150^{o}C, 24 h	14	Onda *et al.* (2008)

2.3.1 Factors affecting saccharification performance of solid acid catalyst

The performance of solid acid saccharification is affected by several operational variables such as type of catalyst, catalyst loading, saccharification temperature and duration. The following sub-sections focus on the evaluation of the process variables on the saccharification performance.

2.3.1.1 Type of solid acid catalyst

Solid acid catalysts that have been commonly employed in the saccharification reactions are shown in Table 2.3. Among the solid acid catalysts, Amberlyst 15 gave the best saccharifcation performance. The catalyst also has the lowest maximum acidity (also known as Hammett acidity, H_o), but highest acid density of 4.8 mmol g^{-1}. This excellence activity could be closely linked with the high density of sulfonic groups (SO_3H) that can maintain strong acidity in water, and catalyse the saccharifcation reaction (Kitano *et al.,* 2009). Apart from that, strong acid exchange resins such as Amberlyst 15 also bring many benefits. They could use to remove inhibitors in the hydrolysate (Nilvebrant *et al.,* 2001) and function like a membrane allowing products permeation during the saccharification reaction (Guo *et al.*, 2012). One important note is that the reaction should be avoided at hydrothermal condition to prevent SO_4^{2-} ions to be leached from the catalyst (Onda *et al.*, 2008).

Sulfated zirconia with the second higher acid density gave much lesser glucose yield of 14% compared to 25% by Amberlyst 15 under the same saccharification conditions (Table 2.3). Besides, sulfated zirconia catalyst would undergo decomposition to produce water soluble by-products including carboxylic acids, oligosaccharides and some sugar derivatives during the saccharification reaction (Onda *et al.,* 2008). This suggests that sulfated zirconia is not suitable for saccharification of cellulose or lignocellulosic biomass.

Surface acidic species or specific functional groups available for reaction was improved with supported solid acid catalysts. When sulfated zirconia was supported over mesoporous silica, its surface area had been improved (Table 2.3). It gave glucose yield of 5% at saccharification conditions of 190^{o}C and 5 h. This yield was relatively low compared to the 14% reported by sulfated zirconia. Even though its surface acidic sites increased, the location of the acidic sites could not ease the diffusion of bulky oligomers into the acidic sites for reaction to take place. The acidic sites mostly located inside the channels of the mesoporous silica support. Also, the channels in the silica support had large pore diameter (around 6 mm), thereby failed to limit secondary reactions of glucose and led to the formation of humic-type products and caused a lost in sugars yield (Lanzafame *et al.*, 2012). Similarly, silica-supported Nafion NR50 showed no saccharifcation performance compared to Nafion NR50 that gave 1% glucose yield under the same operating conditions.

In another study, H-ZSM showed higher glucose yield compared to H-mordenite although the latter has a higher acid density (Table 2.3). This suggests that saccharification performance of catalysts does not entirely based on acid density. Other parameters might have affected the glucose yield. Among the H-type zeolites, those with higher Si/Al ratio such as H-ZSM5 (Si/Al = 45) showed higher saccharification activity than zeolites with lower Si/Al ratio such as H-mordenite (Si/Al = 10) (Onda *et al.*, 2008). The relatively higher hydrophobic characteristic exhibited by the high Si/Al ratios zeolites, favored the organic compounds to water and hence resulted in better glucose yield. From Table 2.3, poor saccharification activity of cellulose was detected when γ-alumina was used. This could be attributing to the pore size of catalyst employed, *i.e.* 12.5 nm, which is too small to be reached by the carbohydrates polymers (Rinaldi *et al.*, 2008). The findings further confirmed that the catalyst composition,

porosity and the nature of the support have effects on the catalyst saccharification performance.

2.3.1.2 Catalyst loading

The amount of catalyst used for saccharification was usually within the range of 0.05 – 0.3 g for 0.005 – 2 g of substrate in 1 – 50 ml of water as reaction medium (Kitano *et al.*, 2009; Lanzafame *et al.*, 2012; Onda *et al.*, 2008). Furthermore, the amount of catalyst used in saccharification is dependent on the type of substrate used. Substrate with higher degree of polymerisation or more complex carbohydrates like lignocellulosic biomass or cellulose requires higher loading of catalyst compared to smaller constituent of carbohydrates like cellobiose. This is because, a complex carbohydrates is more recalcitrant to saccharification and in order to have good glucose yield, it required a higher catalyst loading.

It had also been reported that catalytic performance was mildly affected by the substrate to catalyst ratio (Lanzafame *et al.*, 2012). This is due to the solid-solid contact between the substrate and the catalyst. Beyond an optimum value for substrate to catalyst ratio, further increase in the amount of catalyst does not lead to improvement in the contact area but excess catalyst might degrade the glucose units. The optimal cellulose-to-catalyst ratio differs from catalyst to catalysts. The factors that affecting the catalytic performance include the chemical and van der Waals interactions between the substrate and the catalyst, external surface area of the catalyst particles and their morphology (Lanzafame *et al.*, 2012).

2.3.1.3 Saccharification temperature

Solid acid catalyst selected for saccharification should be highly thermal stable as the suitable saccharification temperature range is normally between 100^{o}C to 200^{o}C. It can

be seen from Table 2.3 that temperature has different effect on glucose yield for different catalyst employed. For example, H-mordenite gave the highest glucose yield at 190^{o}C whereas Amberlyst 15 had the highest yield at about 150^{o}C. Both catalysts mentioned yielded negligible amount of glucose at 90^{o}C. Generally, higher saccharification temperature favors saccharification reaction by providing sufficient energy to hydrolyse the glycosidic bonds in the carbohydrates. As a result, higher sugars yield are obtained at higher temperature.

2.3.1.4 Saccharification time

Interactions were observed between the saccharification time and temperature. For example, short saccharification time of 1 h and low saccharification temperature of 90^{o}C resulted in ineffective saccharification reaction with negligible to nearly no glucose yield for all solid acid catalysts employed (Table 2.3). In general, high saccharification performance can be achieved by having a long saccharification time at high temperature.

2.4 Integration of Ionic Liquid and Solid Acid Catalyst in Saccharification

Having had the ionic liquid dissolution and solid acid saccharification discussed, this section focuses on the integration of ionic liquid and solid acid catalyst for an effective saccharification reaction. There are a few process schemes that have been applied to integrate the ionic liquid and solid acid catalyst in saccharification of lignocellulosic biomass or cellulose, as shown in Figure 2.3.

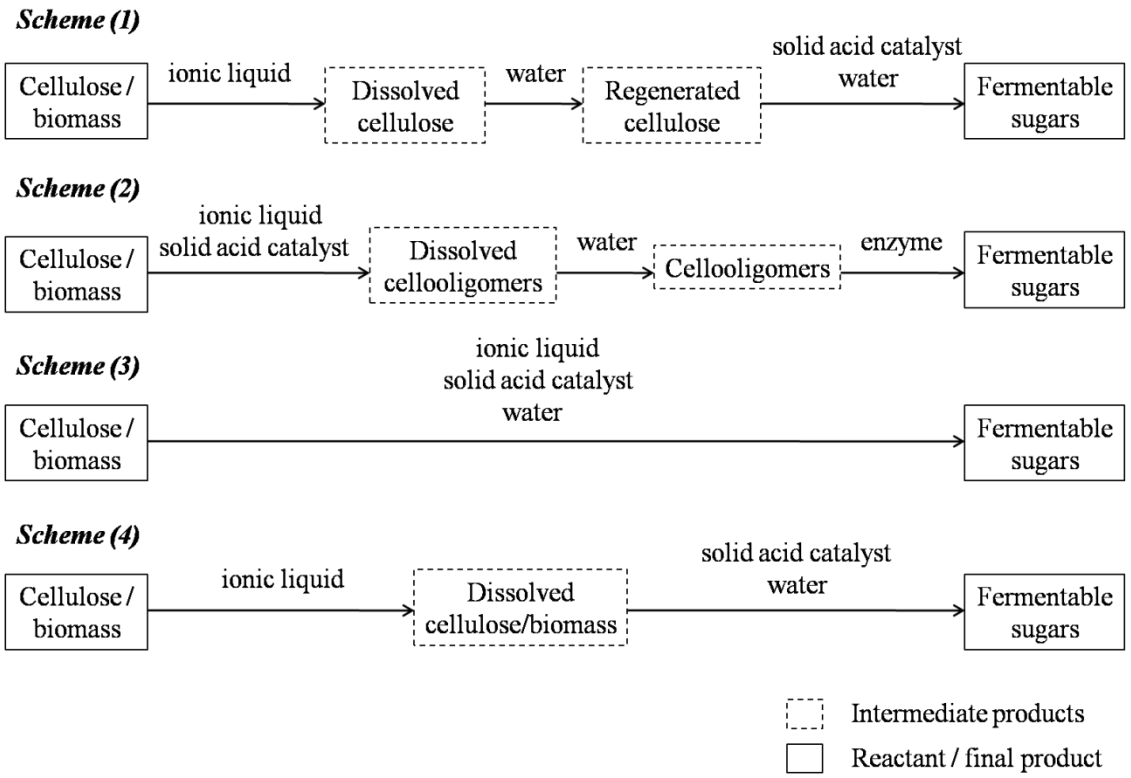

Figure 2.3: Process schemes integrating ionic liquid and solid acid catalyst in saccharification.

Scheme *(1)* was conducted by Kim *et al.* (2010) when ionic liquid was first applied to dissolve the cellulose in the biomass. The dissolved cellulose was regenerated by adding water to give an amorphous structure of regenerated cellulose. In this case, water acted as anti-solvent for ionic liquid and it created competitive hydrogen-bonding to the cellulose microfibrils leading to solubilisation inhibition (Swatloski *et al.*, 2002). The regenerated cellulose was subjected to solid acid saccharification for the production of sugars. This process scheme produced low glucose yield of 16% as shown in Table 2.4. The poor yield is caused by the mass transfer constraint between the regenerated cellulose and the solid acid catalyst (Kim *et al.*, 2010).

Table 2.4: Saccharification performance of different process schemes integrating ionic liquid and solid acid catalyst in saccharification

Process scheme[a]	**Conditions**	**Glucose yield**	**Reference**
Scheme *(1)*	**Ionic liquid pretreatment** 0.2 g microcrystalline cellulose, 60 mmol [BMIM]Cl, 130^{o}C, 2 h. **Solid acid saccharification** Regenerated cellulose, 0.1 g Nafion NR50, 20 ml distilled water, 160^{o}C, 4 h.	16 mol%	Kim *et al.* (2010)
Scheme *(2)*	**Ionic liquid-solid acid depolymerisation** 5 g α-cellulose, 100 g [BMIM]Cl, 1 g Amberlyst 15DRY 100^{o}C, 5 h **Enzymatic saccharification** 20 g of wet cellooligomers, 0.5 ml cellulase (Celluclast, 350 U/g substrate), 100 ml acetate buffer (0.1 M, pH 4.5), 45^{o}C, 4 h	40%	Rinaldi *et al.* (2010a)
Scheme *(3)*	**Ionic liquid-solid acid saccharification** 0.2 g cellobiose, 0.05 mmol [BMIM]Cl, 0.1 g Nafion NR50, 20 ml distilled water, 130^{o}C, 4 h.	44.5%	Dwiatmoko *et al.* (2010)

Table 2.4, continued

Process scheme[a]	Conditions	Glucose yield	Reference
Scheme *(4)*	**Ionic liquid dissolution** 0.1 g cellulose, 2 g [BMIM]Cl, 100^{o}C **Solid acid saccharification** Cellulose/[BMIM]Cl solution, 0.1 g Dowex 50WX8, 53% (w/w) water, 120^{o}C, 2.5 h	27%	Watanabe (2010)
Scheme *(4)*	**Ionic liquid dissolution** 0.1 g *Cryptomeria japonica*, 2 g [BMIM]Cl, 100^{o}C **Solid acid saccharification** *Cryptomeria japonica* /[BMIM]Cl solution, 0.1 g Dowex 50WX8, 53% (w/w) water, 120^{o}C, 3 h	11.5%	Watanabe (2010)

[a] Refer to Figure 2.3 for the corresponding process scheme.

In a different work performed by Rinaldi *et al.* (2010a), enzymatic saccharification was introduced in the final stage of saccharification to achieve a complete saccharification of cellulose as in scheme *(2)* of Figure 2.3. Ionic liquid was applied in the beginning to depolymerise the cellulose into shorter chain of cellooligomers. The depolymerisation rate was enhanced by adding solid acid catalyst during the reaction (Rinaldi *et al.*, 2008). The enhancement of depolymerisation rate is caused by the high preference of solid acid catalyst to cleave the longer cellulose chains. Good depolymerisation would have shortened the duration for enzymatic

saccharification in the later stage. Approximated 4 h was needed to achieve a high cellulose conversion by adopting this scheme compared with 20-50 h in the conventional enzymatic saccharification of the ionic liquid regenerated cellulose (Rinaldi *et al.*, 2010a). Comparing with acid saccharification, scheme *(2)* allowed the depolymerisation reaction to proceed in a more controlled manner. The depolymerisation was controlled by size-specific preference for the cleavage of large chains, with negligible amount of reducing sugars released at the beginning of the reaction. As a consequence, the formation of sugar degraded products was prevented and the separation of the products mixture was relatively simple (Rinaldi *et al.*, 2010a). This integrated process of ionic liquid-solid acid depolymerisation and enzymatic saccharification is evidently efficient.

Despite that scheme *(2)* has high conversion rate, its operation is tedious as water needed to be added during cellooligomers formation to separate them from the reaction mixture to facilitate subsequent enzymatic saccharification. A simple scheme of single-step ionic liquid-solid acid saccharification (scheme *(3)*) was suggested (Dwiatmoko *et al.*, 2010). This scheme enabled both the dissolution and the saccharification reactions to happen simultaneously. However, the dissolution capability of the ionic liquid in this scheme was not as good as that in scheme *(2)*; since water present in the reaction mixture had lowered the performance of ionic liquid (Swatloski *et al.*, 2002), and affected the saccharification of cellulose to sugars.

Scheme *(4)* introduced by Watanabe (2010) involved the creation of homogeneous liquid mixture of carbohydrate rich solution as the first step via biomass or cellulose dissolution in ionic liquid. This was followed by the addition of solid acid catalyst for saccharification. Since water was only added during the saccharification, the activity of ionic liquid in biomass dissolution and depolymerisation was therefore not hampered. The schemes discussed have their pros and cons and the scheme selection

criteria should be based on the researchers' goals. Besides, understanding the mechanism of ionic liquid-mediated solid acid saccharification would assist the scheme selection and it is included in the following section.

2.4.1 Mechanism of ionic liquid-mediated solid acid saccharification

According to Mosier *et al.* (2002), protons are necessary for hydrolysis activity. So, Dwiatmoko and his co-authors reported that ionic liquid induced the release of protons from the catalyst (Dwiatmoko *et al.*, 2010). They found that a correlation between the glucose yield and [BMIM]Cl/[H^+] ratio, in which glucose yield increased with [BMIM]Cl/[H^+] ratio. The glucose yield remained constant at higher ratios of 1 implies that there is a maximum enhancement in hydrolysis activity by ionic liquid, *i.e.* when the ionic liquid has released all the protons available in the catalyst. Another study reported that the induction period during hydrolysis activity of solid acid catalyst (Amberlyst 15) was attributed to the time needed for the proton to diffuse out from the catalyst into the reaction solution (Rinaldi *et al.*, 2010b). These collectively suggest that the hydrolysis activity is a function of the amount of protons released from the catalyst. The release of protons from catalyst is due to ion-exchange at the sulfonate (SO_3H) group (Rinaldi *et al.*, 2010b) as shown in Figure 2.4.

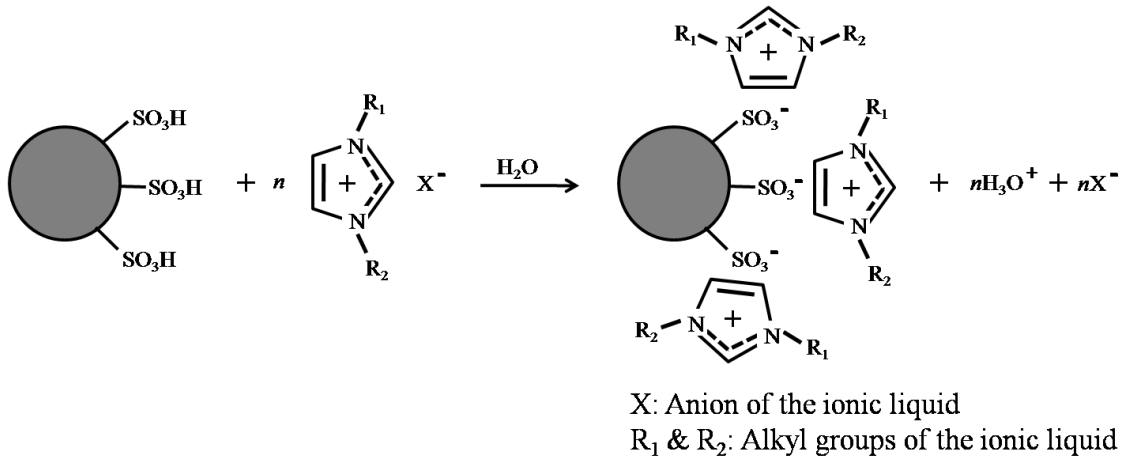

Figure 2.4: Mechanism of ion exchange between ionic liquids cations and protons at the terminal sulfonic group on the acid resin (adapted from Dwiatmoko *et al.* (2010)).

The mechanism of carbohydrate saccharification in ionic liquid-mediated solid acid saccharification would then follow the acid-catalyzed hydrolysis of glycosides as illustrated in Figure 2.5. Three steps are involved in the mechanism. They are (1) protonation of oxygen atom, (2) formation of carbocation, and (3) re-establishment of anomeric center and regeneration of H_3O^+ species for consecutive hydrolysis. Two pathways have been proposed for the protonation of oxygen atom; one involves the protonation of the glycosidic oxygen (pathway 1) and another, the protonation of the pyranic oxygen (pathway 2). Considering the nature of H_3O^+ species and conformational restriction of the cellulosic chains along the glycosidic bond, both oxygen atoms are expected to undergo partial protonation as shown in the squared bracket in Figure 2.5 (Philipp *et al.*, 1979). The protonated oxygen would rearrange to form carbocation through unimolecular reaction (Krässig *et al.*, 2004). Cyclic carbocation and acyclic carbocation are formed for pathway 1 and 2 respectively. Due to the stability of methyl glycosides towards acid hydrolysis, the reaction proceeded through cyclic carbocation (Edward, 1955) as shown in pathway 1. In the final step, the carbocation reacted with water to reestablish the anomeric center and regenerate the H_3O^+ species. Through these steps, a single 1,4-β-glycosidic bond in the cellulose polymeric chain is cleaved to produce two shorter chains of cellooligomers. As the cleaving process continuous, cellulose monomers in the form of sugars such as glucose are obtained.

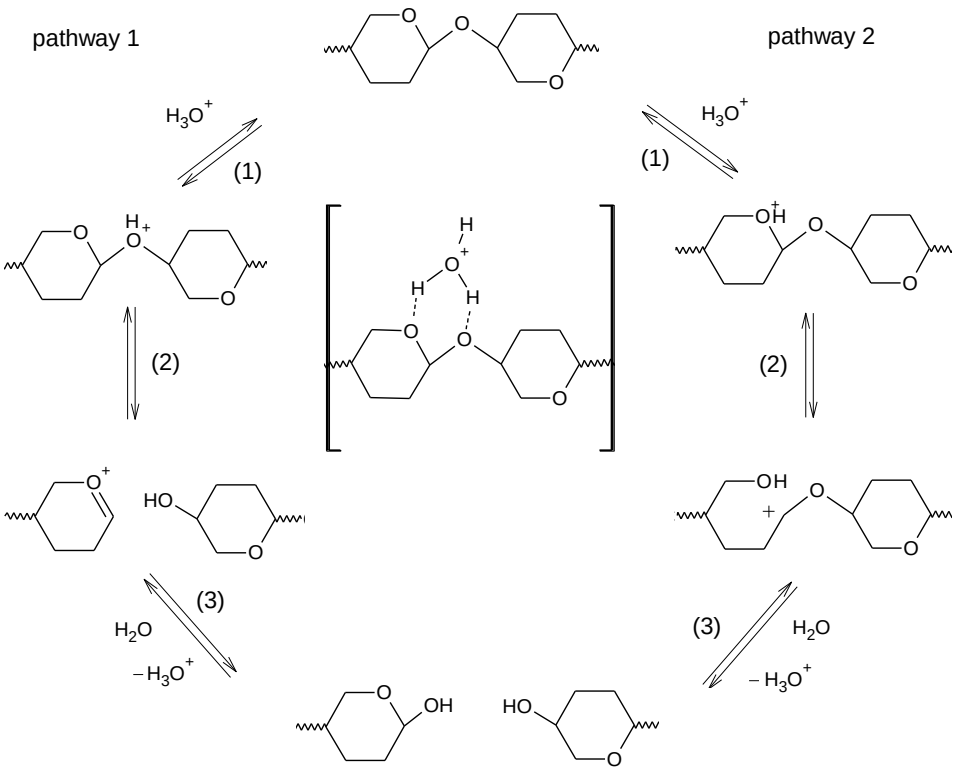

Figure 2.5: Molecular mechanism of acid-catalyzed hydrolysis of glycosidic bonds (adapted from Philipp *et al.* (1979)).

The aim of this work is to produce reducing sugars from lignocellulosic biomass. Having intensively reviewed on works related to the focus of this study, few important aspects need to be considered to achieve high production of reducing sugars. Firstly, the choice of lignocellulosic biomass is important, in which the biomass should contain high amount of carbohydrates. Therefore sago waste with high carbohydrates content is a suitable feedstock for production of reducing sugars in this work. Integrated ionic liquid-solid acid saccharification reported to be effective in providing a more controlled saccharification reaction and at the same time can prevent formation of sugar degraded products. This process also requires shorter saccharification time compared to that of the enzymatic saccharification making it feasible for large scale production. Besides that, selecting a suitable type of ionic liquid and solid acid catalyst is essential and this chapter has provided some useful information on selection of ionic liquid and solid acid catalyst. The factors affecting the dissolution and saccharification

performances were critically reviewed with further investigation on process variables such as process optimisation results in high reducing sugars yield. Mechanism of ionic liquid-mediated solid acid saccharification could provide a basic understanding on the reaction kinetics. This aspect is useful for process manipulation and reactor design when large scale production is concerned. Finally, information on recovery and recycling of the catalyst is helpful to make the overall process more economically feasible.

CHAPTER 3

MATERIALS AND METHODS

3.1 Sample Preparation and Characterisation of Sago Waste

The raw material used in this study was dried sago waste collected from C.L. Nee Sago Industries Sdn. Bhd., Sarawak, Malaysia. The stock was ground and sieved into different size ranges, *i.e.* < 0.25 mm, 0.25-0.5 mm and 0.5-1.0 mm, and stored in a dry cabinet. Sago waste sample was characterised prior to be used in experiments. Starch content in sago waste was determined according to the procedure described by Megazyme in Total Starch Assay Procedure K-TSTA 07/11, while cellulose, hemicelluloses, lignin, ash and moisture content were based on the procedure explained in the National Renewable Energy Laboratory (NREL) Technical Report NREL/TP-510-42618 (Sluiter *et al.*, 2011). Details of each of the procedure were reported under Analytical Methods.

3.2 Sequential Ionic Liquid Dissolution-Solid Acid Saccharification

Sequential ionic liquid dissolution-solid acid saccharification is one of the main focuses of the research. This section describes the general experimental procedures for the sequential reaction. As for the specific operational conditions, they will be included in subsequent sections.

Dissolution of sago waste was performed in a screw cap test tube containing ionic liquid and sago waste. The reaction was initiated by heating the sago waste-ionic liquid mixture in an oil bath (MC, Julabo, Germany). Sample was taken at desired time and the sample taken was cooled to room temperature and evenly mixed with 5 ml of ultrapure water (Arium® 611UF, Sartorius, Germany). Safety precaution for handling the samples in test tube boiled under high temperature of more than 100^{o}C with thermal

insulated gloves is essential. The mixture was then centrifuged (Centrifuge 5810R, Eppendorf, Germany) at 3500 rpm for 20 min to separate the liquid portion (hereafter called prehydrolysate) from the sago waste solid residue.

A portion of approximated 1.5 ml of the prehydrolysate was analysed for its reducing sugars by 3,5-dinitrosalicylic acid (DNS) assay as described in Section 3.7.3 under Analytical Methods. And the remaining prehydrolysate was used for solid acid saccharification. The saccharification was conducted in a screw cap test tube containing 2.5 ml of prehydrolysate and sufficient amount of solid acid catalyst, in the oil bath. Samples were withdrawn throughout the reaction and cooled to room temperature. The catalyst in the sample was allowed to settle while the hydrolysate (liquid product from the saccharification reaction) was collected and measured for reducing sugars concentration using DNS assay. The overview of the sequential ionic liquid dissolution-solid acid saccharification is shown in Figure 3.1. All the experiments were performed in triplicates.

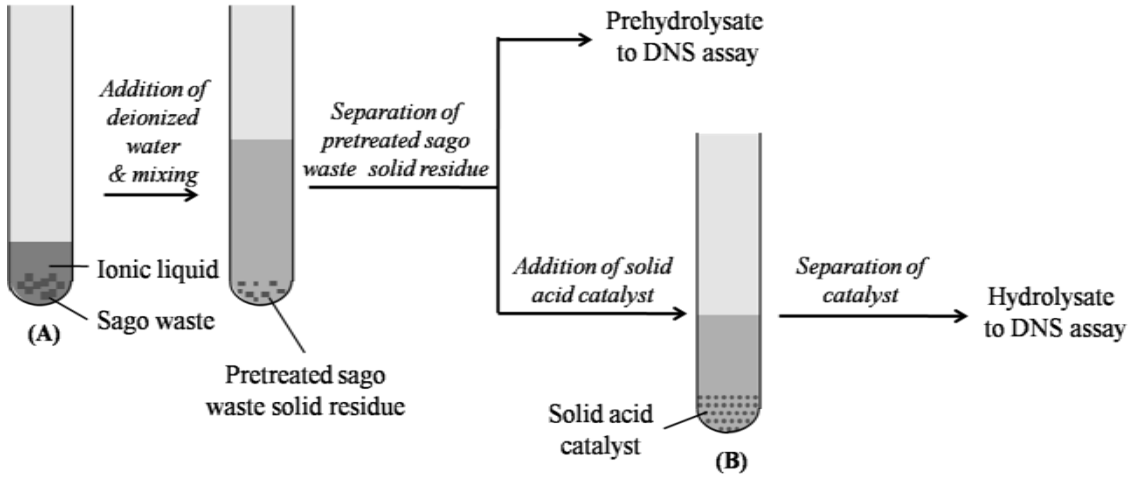

Figure 3.1: Overview of sequential ionic liquid dissolution-solid acid saccharification of sago waste (A) ionic liquid dissolution of sago waste, (B) solid acid saccharification of prehydrolysate.

3.3 Compatibility Study on Ionic Liquid and Solid Acid Catalyst

3.3.1 Selecting suitable combination of ionic liquid and solid acid catalyst

Different types of ionic liquids and solid acid catalysts were applied during dissolution and saccharification reaction respectively to select the most compatible combination of ionic liquid and solid acid catalyst for the sequential reaction. The ionic liquids under investigation were 1-butyl-3-methylimidazolium chloride ([BMIM]Cl, ≥ 98.0%, Merck, Germany), 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc], ≥ 90.0%, Sigma-Aldrich, USA) and 1-ethyl-3-methylimidazolium diethyl phosphate ([EMIM][$(EtO)_2PO_2$], ≥ 98.0%, Sigma-Aldrich, USA) based on their reported strong dissolution properties. The dissolution reaction was conducted according to the procedures mentioned in Section 3.2 with 4% (w/v) of sago waste in 1 ml of ionic liquid, heated in oil bath at 100^{o}C for 3 h and samples were taken periodically.

Three types of solid acid catalysts, *i.e.* Amberlyst 15 (A15, Sigma-Aldrich, USA), Amberlite IR120 (Sigma-Aldrich, USA) and Nafion NR50 (Sigma-Aldrich, USA) were applied during solid acid saccharification in view of their high saccharification activity. Catalyst with loading of 10% (w/v) was added into a test tube containing 2.5 ml of prehydrolysate and the saccharification reaction was conducted in oil bath preset at 120^{o}C for 1.5 h and samples were collected throughout the reaction.

3.3.2 Enzymatic saccharification of ionic liquid pretreated solid residue

The sago waste solid residue from ionic liquid dissolution was washed with ultrapure water and final rinsed with acetate acid-sodium acetate buffer solution (50 mM, pH 4.8) followed by drying in an oven (EU115 TS, Jouan, France) at 60^{o}C for 48 h prior to enzymatic saccharification. Enzymatic saccharification was carried out with *Trichoderma viride* cellulase (Merck, Germany) at a concentration of 30-50 FPU/g

substrate. The mixture was buffered with 1 ml of 50 mM, pH 4.8 acetate acid-sodium acetate buffer solution. The reaction was carried out in a water bath (WB14, Memmert, Germany) maintained at 50^{o}C for 48 h. The supernatant of the enzymatic saccharification was analysed for its reducing sugars content using DNS assay. Mask was worn when handling the enzyme that present in fine powder form as it could irritate the respiratory system.

3.3.3 Computation of energy requirement for ionic liquid dissolution

The energy requirement for dissolution reaction was determined according to the first law of thermodynamics as shown in Equation (3.1):

$$Q = \Delta U = m \int_{T_i}^{T_f} C_p \ \mathrm{dT} \qquad (3.1)$$

where Q is the heat transferred to the system, ΔU is the change in internal energy, m is the mass of ionic liquid, C_p is the specific heat capacity of the ionic liquid, T_i and T_f are the initial and final absolute temperatures respectively.

Molar heat capacities of the ionic liquids are the sum of their cations and anions molar heat capacities where each of which can be determined by using Equation (3.2) (Soriano *et al.*, 2010).

$$C_{p,\,ion}\ (\mathrm{J\ mol^{-1}\ K^{-1}}) = A + B\mathrm{T} + C\mathrm{T}^2 \qquad (3.2)$$

A, B and C are the empirical parameters and T is the absolute temperature.

3.4 Process Optimisation of Sequential Ionic Liquid Dissolution-Solid Acid Saccharification

3.4.1 Screening of influential process variables

In ionic liquid dissolution, operational variables of dissolution time, temperature, substrate loading and its particle size affected the dissolution performance (Mäki-Arvela

et al., 2010). Therefore, these operational variables were screened during the ionic liquid dissolution reaction. The studied ranges of each of the variables are tabulated in Table 3.1.

Table 3.1: Variables investigated in preliminary studies

Variable	Range of study
Ionic liquid dissolution	
Dissolution time (h)	0.25 - 5
Temperature (^{o}C)	100 - 160
Substrate loading (%, w/w)	2 - 6
Substrate particle size (mm)	< 0.25 (fine), 0.25 - 0.5 (medium), 0.5 - 1 (coarse)
Solid acid saccharification	
Saccharification time (h)	0.25 - 5
Temperature (^{o}C)	80 - 120
Catalyst loading (%, w/v)	1 – 10

The process variables on solid acid saccharification were saccharification time, reaction temperature and catalyst loading. Refer to Table 3.1 for the ranges of variables applied. Both the ionic liquid dissolution and solid acid saccharification reactions were conducted based on the procedures mentioned earlier in Section 3.2. The screening was only conducted for the most compatible ionic liquid-solid acid catalyst determined from previous section, *i.e.* [BMIM]Cl-A15.

3.4.2 Experimental design and statistical analysis

Two different sets of central composite design (CCD) of response surface methodology (RSM) were performed for the ionic liquid dissolution and solid acid saccharification reactions in the sequential ionic liquid dissolution-solid acid saccharification. The CCD design matrix was generated with the aid of Design Expert 7.0.0 software (STAT-EASE Inc., Minneapolis, USA).

From the preliminary studies, dissolution time, temperature and substrate loading had affected the ionic liquid dissolution. Therefore, the effects of these independent variables on carbohydrates dissolution capability of the ionic liquid were investigated in the first set of CCD. The ranges for these variables were based on the preliminary study and listed in Table 3.2. The dissolution capability of the ionic liquid was evaluated based on the reducing sugars yield obtained after solid acid saccharification which conducted at a fixed condition, *i.e.* 120^{o}C saccharification temperature with 10% (w/v) catalyst loading for 1.5 h.

In the second set of CCD, the effects of the influential variables during solid acid saccharification, *i.e.* temperature, time and catalyst loading on the reducing sugars yield were investigated. Dissolution was conducted at the optimised condition determined from the first set of CCD, followed by solid acid saccharification at different conditions as outlined in Table 3.2. The saccharification performance was evaluated based on the reducing sugars yield in the hydrolysate.

Table 3.2: Independent variables and their coded and actual levels

Variable	**Coding**	**Unit**	**Level**				
			-1	**-α ***	**0**	**α ***	**1**
1st set of CCD (Ionic liquid dissolution)							
Time	A	h	0.5	1.0	1.5	2.0	2.5
Temperature	B	^{o}C	140	145	150	155	160
Substrate loading	C	% (w/w)	0.5	1.0	1.5	2.0	2.5
2nd set of CCD (Solid acid saccharification)							
Time	D	h	0.5	1.0	1.5	2.0	2.5
Temperature	E	^{o}C	100	110	120	130	140
Catalyst loading	F	% (w/v)	2	4	6	8	10

* α = the distance from the center point was set at 0.5

The experimental response, y (reducing sugars yield) was fitted into a second order polynomial regression model as:

$$y = \beta_o + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{3} \beta_{ij} x_i x_j \qquad (3.3)$$

where x_i and x_j are the independent variables studied, β_o is the constant coefficient, β_i is the i^{th} linear coefficient, β_{ii} is the quadratic coefficient and β_{ij} is the ij^{th} interaction coefficient. The significance of the model was determined by performing analysis of variance (ANOVA). Three-dimensional response surface plots and contour plots generated by the program were used to provide some graphical illustration of the interaction between the operational variables. To evaluate and verify the predicted optimum reducing sugars yield, three experiments were conducted at the suggested optimum conditions. The optimum reducing sugars yield is defined as the reducing sugars yield achieved by integrating both optimum operational conditions of ionic liquid dissolution and solid acid saccharification reactions.

3.5 Kinetic Study of Ionic Liquid-Mediated Solid Acid Saccharification

3.5.1 Experimental design for kinetic study

Sago waste was dissolved in [BMIM]Cl according to the procedure described in Section 3.2 at optimised operational conditions of 160^{o}C, 1.75 h and 1.5% (w/w) substrate loading. Prehydrolysate collected from dissolution reaction was subjected to solid acid saccharification with solid acid catalyst A15. Kinetics of the ionic liquid-mediated solid acid saccharification was investigated by varying the saccharification temperature from 100-140^{o}C, catalyst loading 2-10% (w/v) and reaction time 5-300 min. Hydrolysate collected was analysed for total reducing sugars consisting mainly of glucose and trace amount of arabinose. The amount of reducing sugars in the reaction mixture was determined by using DNS assay.

3.5.2 Hydrolysis kinetic model

The experimental data were fitted into the generalised Saeman model using MATLAB R2009b (MathWorks Inc., USA). The model used was two consecutive pseudo-first order sugars production and sugar degradation reaction as expressed in Equation (3.4):

$$\text{Polymers} \xrightarrow{k_1} \text{Monomers} \xrightarrow{k_2} \text{Decomposition products} \quad (3.4)$$

where polymers and monomers are the carbohydrates and the reducing sugars respectively, while k_1 and k_2 are respectively the rate constants for sugars production and degradation reactions.

For an irreversible first order reaction, the model outlined can be presented in two differential equations as shown in Equation (3.5) and (3.6):

$$r_S = -\mathrm{d}S/\mathrm{d}t = -k_1 S \quad (3.5)$$

$$r_P = \mathrm{d}P/\mathrm{d}t = k_1 S - k_2 P \quad (3.6)$$

where r_S is the rate of degradation of carbohydrates, r_P is the rate of production of reducing sugars, S is the concentration of carbohydrates, P is the concentration of reducing sugars and t is reaction time. By solving the differential equations, concentration of reducing sugars as a function of time was determined using Equation (3.7):

$$P = P_o e^{-k_2 t} + S_o \frac{k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \quad (3.7)$$

where the subscript o indicates their respective initial concentrations.

The time required to reach a maximum concentration of reducing sugars, t_{max} was calculated using Equation (3.8). This equation was derived by setting the derivation of Saeman model (Equation (3.7)) to zero for maximum concentration of reducing sugars.

$$t_{max} = \frac{\ln(k_2/k_1)}{k_2 - k_1} \quad (3.8)$$

The dependence of the rate constants, k of saccharification reaction on temperature can be represented by Arrhenius equation, as shown:

$$k = \mathrm{A}e^{-\mathrm{E_a}/\mathrm{RT}} \tag{3.9}$$

where A is the Arrhenius constant (also known as frequency factor), E_a is the activation energy, R is universal gas constant (8.314 J mol^{-1} K^{-1}) and T is the absolute temperature. Assuming that E_a is a constant, integrating Equation (3.9) obtained:

$$\ln k = \ln \mathrm{A} - \mathrm{E_a}/\mathrm{RT} \tag{3.10}$$

ln *k* versus 1/T was plotted and E_a and A were calculated respectively from the gradient and the intercept of the straight line obtained.

While for acid concentration, its effect on rate constants, *k* at constant temperature was expressed as:

$$k = W[\mathrm{H}^+]^{\mathrm{m}} \tag{3.11}$$

where *W* is a constant, $[H^+]$ is acid concentration and m is the gradient of line in logarithm *k* against logarithm $[H^+]$ plot. The rate constant was expanded to Equation (3.12) to include the effect of temperature and acid concentration simultaneously.

$$k = W'[\mathrm{H}^+]^m e^{-\mathrm{E_a}/\mathrm{RT}} \tag{3.12}$$

W' is a new constant to correlate the rate constant to the interactive effect of temperature and acid concentration. The kinetic parameters of the model were determined using MATLAB R2009b.

3.6 Product Separation and Catalysts Recovery

3.6.1 Regeneration and reusability of solid acid catalyst

Solid acid catalyst, A15 was regenerated according to the procedure suggested by Rohm and Haas Company, USA. Spent A15 was collected and loaded into a glass tube with a perforated end to create a catalyst bed. A15 was regenerated by flushing the catalyst bed

with sufficient amount of ultrapure water to remove any chemical residue and the bed was allowed to settle and the water was drained. It was followed by pumping 10% (v/v) sulfuric acid solution at a rate of 4 bed volumes (BVs) per hour until a total of 1.5 BVs of sulfuric acid had passed through the catalyst bed. The bed was rinsed with ultrapure water to remove excess sulfuric acid from the catalyst. Rinsing was carried out at the same flow rate as the regeneration flow rate until approximate 1 or 2 BVs of ultrapure water had passed through the bed. The rinsing flow rate was then increased to 12 BVs/h and continued until the effluent was above pH 4. After drainage of the water, catalyst was placed in the oven at 105^{o}C overnight for dehydration. The regenerated A15 was used for the solid acid saccharification. The catalytic performance of the regenerated A15 was determined based on the reducing sugars yield determined from DNS assay. The schematic diagram for the catalyst regeneration process is shown in Figure 3.2.

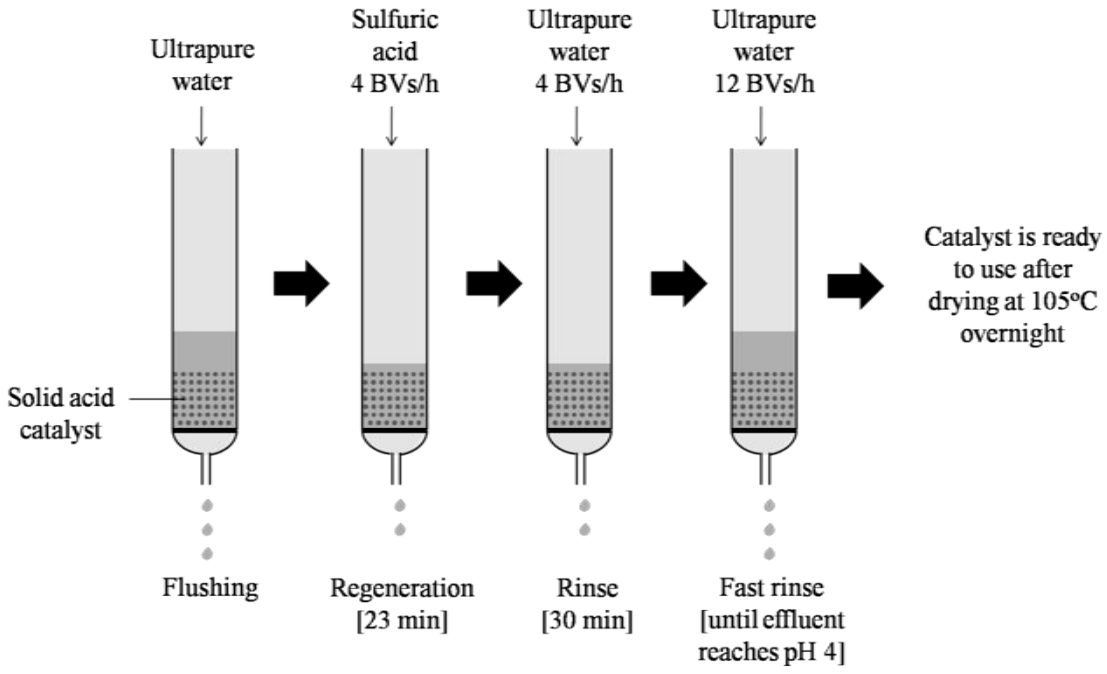

Figure 3.2: Experimental schematic diagram for catalyst regeneration process.

3.6.2 Separation and recovery of reducing sugars and ionic liquid via aqueous biphasic system (ABS)

To separate and recover ionic liquid and reducing sugars produced from ionic liquid-mediated solid acid saccharification, an ABS was used (Gutowski *et al.*, 2003). ABS are usually formed as a result of mutual incompatibility of one or two polymers with another salt that is above a certain concentration (Li *et al.*, 2010b). Hence, it was developed by adding a known amount of 80% (w/w) potassium phosphate (K_3PO_4) salt solution into the hydrolysate containing ionic liquid, reducing sugars and water. Cloud-point method was used to determine the amount of K_3PO_4 required to create a two-phase system. In this method, K_3PO_4 was added dropwise into the hydrolysate in a separating funnel. For each drop of K_3PO_4 added, the separating funnel was shaken and the resulting solution was examined for turbidity as the mixture would turn from clear to turbid prior to form two phases. If the solution remained clear, another portion of K_3PO_4 was added into the mixture until a turbid mixture was obtained. At this point, a two-phase would form and the amount of K_3PO_4 added was noted. The two phases formed were separated and their volumes were measured. The liquid-liquid extraction process involving ABS was repeated for a known portion of lower phase with K_3PO_4 solution. In all the extraction steps, samples from both upper and lower phases were withdrawn and diluted with ultrapure water. The diluted samples were subjected to DNS assay (Section 3.7.3) and HPLC analysis (Section 3.7.6). The schematic diagram for the ABS is shown in Figure 3.3.

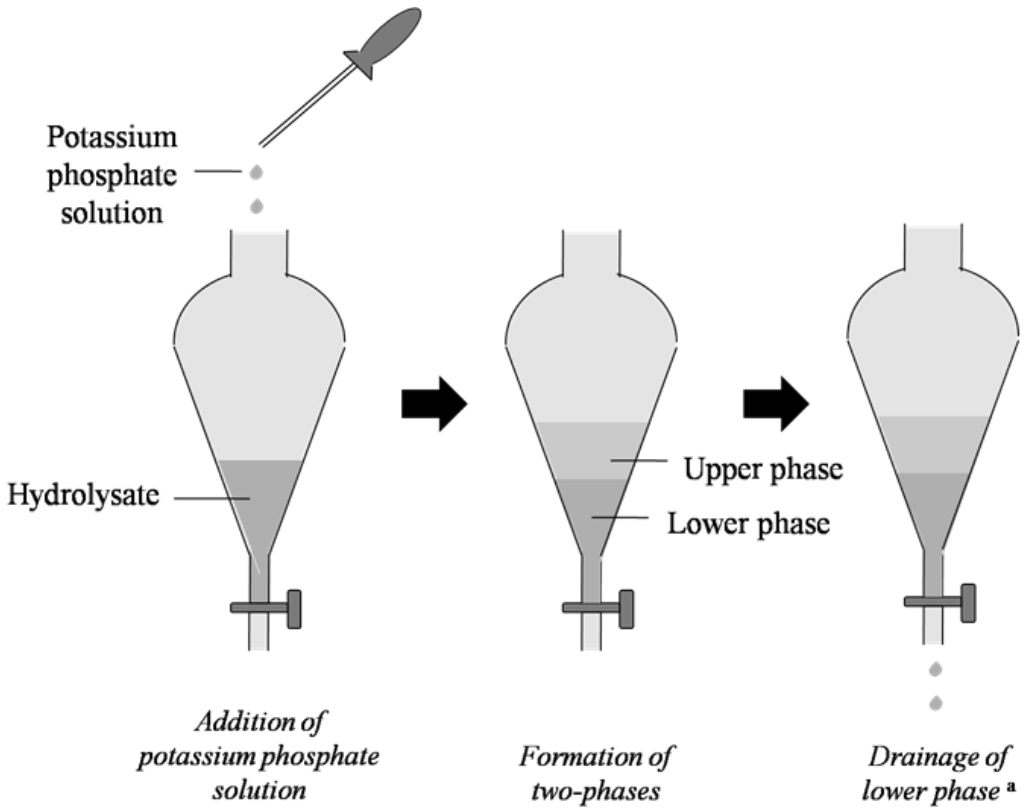

Figure 3.3: Experimental schematic diagram for aqueous biphasic system (ABS).

The extraction efficiency of ionic liquid, $\%E([\mathrm{BMIM}]\mathrm{Cl})_i$ and reducing sugars, $\%E(\mathrm{RS})_i$ in a particular extraction step, *i* were calculated according to Equation (3.13) and (3.14), respectively:

$$\%E([\mathrm{BMIM}]\mathrm{Cl})_i = \frac{[[\mathrm{BMIM}]\mathrm{Cl}]_{Ui} \times V_{Ui}}{[[\mathrm{BMIM}]\mathrm{Cl}]_{Ui} \times V_{Ui} + [[\mathrm{BMIM}]\mathrm{Cl}]_{Li} \times V_{Li}} \times 100 \qquad (3.13)$$

$$\%E(\mathrm{RS})_i = \frac{[\mathrm{RS}]_{Li} \times V_{Li}}{[\mathrm{RS}]_{Ui} \times V_{Ui} + [RS]_{Li} \times V_{Li}} \times 100 \qquad (3.14)$$

where [[BMIM]Cl] and [RS] refer to the concentrations of ionic liquid and reducing sugars respectively, subscript *U* and *L* indicate the upper and lower phases in the ABS, and *V* is the volume of the particular phase. The total extraction efficiency of ionic liquid, %*E*([BMIM]Cl) and reducing sugars, %*E*(RS) were respectively expressed in Equation (3.15) and (3.16):

$$\%E([\mathrm{BMIM}]\mathrm{Cl}) = \frac{\sum_{i=1}^{n}\left\{[[\mathrm{BMIM}]\mathrm{Cl}]_{Ui} \times V_{Ui}\right\}}{m_{[\mathrm{BMIM}]\mathrm{Cl}}} \times 100 \qquad (3.15)$$

$$\%E(\mathrm{RS}) = \frac{\sum_{i=1}^{n}\{[\mathrm{RS}]_{Li} \times V_{Li}\}}{m_{\mathrm{RS}}} \times 100 \qquad (3.16)$$

where n is the total number of extractions, $m_{[BMIM]Cl}$ and m_{RS} are the total mass of [BMIM]Cl and total mass of reducing sugars in the hydrolysate prior to ABS.

3.7 Analytical Methods

3.7.1 Starch determination

The starch content of sago waste was determined using the Total Starch Assay Procedure provided by Megazyme. Approximately 100 mg of sago waste with particle size of 0.5 mm was added into a test tube. The sample was wetted with 0.2 ml of ethanol (80%, v/v) and then the test tube was put on a vortex mixer (VTX-3000L, LMS, Japan) to aid substrate dispersion. After that, the test tube was filled with 2 ml of 2 M potassium hydroxide for approximately 20 min in an ice/water bath with stirring using a magnetic stirrer. Next, 8 ml of sodium acetate buffer (1.2 M, pH 3.8) together with 0.1 ml of thermostable α-amylase and 0.1 ml of amyloglucosidase were added to the test tube and mixed well prior placing the test tube in a water bath at 50^{o}C. The test tube was incubated for 30 min with intermittent mixing. The content of the test tube was diluted with ultrapure water to a 100 ml volumetric flask and mixed well. An aliquot of the solution was centrifuged at 3000 rpm for 10 min while the remaining solution was filtered to collect the solid residue of destarched sago waste. The solid residue was washed with adequate amount of ultrapure water followed by drying at 60^{o}C in an oven for 48 h prior to cellulose, hemicelluloses, lignin and ash determination.

Having had the aliquot centrifuged, 0.1 ml of the clear aliquot was transferred to a new test tube for glucose analysis with 3 ml of glucose determination reagent (GOPOD reagent). Separately, $_{D}$-glucose controls and reagent blanks were prepared. $_{D}$-Glucose controls consisted of 0.1 ml of 1 mg/ml $_{D}$-glucose standard solution and 3 ml of GOPOD reagent, while reagent blank consisted of 0.1 ml of ultrapure water and 3 ml of GOPOD reagent. The sample, control and blank were incubated at 50^{o}C for 20 min. The

absorbance of the sample and $_{D}$-glucose control were read at 510 nm against the reagent blank using a UV-vis spectrophotometer (PRIM, Secomam, France). Percentage of starch content was determined using equation below:

$$\text{Starch}, \% = \Delta A \times \frac{F}{w} \times V \times 0.9 \qquad (3.17)$$

where ΔA is the absorbance read against the reagent blank, F is the conversion of absorbance to μg of glucose, *i.e.* 100 μg $_{D}$-glucose/absorbance for 100 μg $_{D}$-glucose, *V* is the final volume of the sample, *i.e.* 100 ml, and *w* is the weight of substrate in mg. All the experiments were performed in duplicates.

3.7.2 Cellulose, hemicelluloses, lignin and ash determination

Cellulose, hemicelluloses, lignin and ash content were determined according to the National Renewable Energy Laboratory Technical Report TP-510-42618 (Sluiter *et al.*, 2011). Approximately 300 mg of destarched sago waste was measured into a conical flask, followed by the addition of 3 ml of 72% sulfuric acid. The sample was thoroughly stirred with a glass rod to ensure all substrate particles were wetted with sulfuric acid. The conical flask was placed in a water bath set at 30^{o}C and incubated for 60 min. The sample was stirred every five to ten minutes to make sure even acid to particle contact for uniform hydrolysis. Upon completion of hydrolysis, the conical flask was removed from the water bath and the acid was diluted to a 4% concentration by adding 84 ml of ultrapure water.

Separately, a set of sugars recovery standards (SRS) were prepared to correct for losses due to degradation of sugars during dilute acid hydrolysis. The SRS were the sugars monomers found in sago waste, *i.e.* $_{D}$-glucose and $_{L}$-arabinose. Each of the SRS was prepared to a concentration closely resembled the concentration of sugar in the destarched sago waste. The hydrolysed sago waste sample and SRS were autoclaved (Hiclave HVE-50, Hirayama, Japan) at 121^{o}C for 1 h. All the samples were gradually

cooled to room temperature prior to vacuum filtration through a pre-weighed filtering crucible. The liquid sample, known as hydrolysate was transferred to a sample storage bottle for carbohydrates and acid soluble lignin determination, while the solid residue was washed several times with ultrapure water prior to acid insoluble lignin and ash determination.

The acid insoluble residue contained in the filtering crucible was dried at 105^{o}C until a constant weight was achieved. The sample together with the crucible were then placed in a furnace (KL15/11, Thermconcept, Germany) at 575^{o}C and ashed until constant weight was achieved. Percentage of ash in sago waste, % Ash was determined as:

$$\% \text{ Ash} = \frac{w_{\text{crucible +ash}} - w_{\text{crucible}}}{w_{\text{destarch sago waste}}} \times 100 \qquad (3.18)$$

where $w_{\text{crucible+ash}}$ is the total weight of filtering crucible and ash, w_{crucible} is the weight of empty filtering crucible, and $w_{\text{destarched sago waste}}$ is the weight of destarched sago waste used for analysis. And the percentage of acid insoluble lignin, % AIL was calculated as:

$$\% \text{ AIL} = \frac{w_{\text{crucible +AIR}} - w_{\text{crucible +ash}}}{w_{\text{destarch sago waste}}} \times 100 \qquad (3.19)$$

where $w_{\text{crucible+AIR}}$ is the total weight of filtering crucible and acid insoluble residue.

A portion of the hydrolysate (approximately 20 ml) obtained earlier in acid hydrolysis of destarched sago waste was subjected to absorbance reading by using UV-vis spectrophotometer. The reading was taken at wavelength corresponded to maximum absorbance reading, *i.e.* 236 nm. The equation used to calculate the percentage of acid soluble lignin, % ASL was:

$$\% \text{ ASL} = \frac{\text{UV}_{\text{abs}} \times V_{\text{filtrate}} \times \text{dilution}}{\varepsilon \times w_{\text{destarch sago waste}}} \times 100 \qquad (3.20)$$

where UV_{abs} is the average UV-vis absorbance for the sample at 236 nm, V_{filtrate} is the volume of the hydrolysis liquor, *i.e.* 86.73 ml, and ε is the absorptivity of sago waste,

i.e. 30 L g^{-1} cm^{-1}. Total amount of lignin in sago waste was determined by summing % AIL and % ASL.

Cellulose and hemicelluloses content in destarched sago waste can be determined by measuring the sugars monomers in the hydrolysate obtained from acid hydrolysis of destarched sago waste. The hydrolysate remained from acid soluble lignin determination was neutralised to pH 5 - 6 using calcium carbonate powder. The sample was allowed to settle and the supernatant was decanted and filtered using 0.2 μm nylon stringy into the high-performance liquid chromatography (HPLC) vial. A series of calibration standards containing $_{D}$-glucose and $_{L}$-arabinose were prepared for concentration ranges from 0.1 - 3.0 mg/ml. The calibration curves for all sugars were validated by preparing an independent calibration verification standard (CVS) at concentration that falls within the calibration curve. The SRS, CVS and sample were analysed by HPLC (Waters, USA). The operating conditions for HPLC were provided in Section 3.7.6.

Percent of cellulose in sago waste was determined by measuring the percentage of glucose in sago waste while the remaining sugars monomers detected were used to calculate the hemicelluloses content. The calculations steps involved are:

i. Concentration of a sugar in the hydrolysed sample after correction for loss on 4% hydrolysis, C_x

$$C_x = \frac{C_{\text{HPLC}} \times \text{dilution factor}}{C_{\text{SRS determined by HPLC}} / C_{\text{SRS}}} \qquad (3.21)$$

where C_{HPLC} is the concentration of a particular sugar in destarched sago waste determined by HPLC (mg/ml), $C_{\text{SRS determined by HPLC}}$ is the concentration of sugar in SRS after hydrolysis as determined by HPLC (mg/ml), and C_{SRS} is the known concentration of sugar before hydrolysis (mg/ml).

ii. Percentage of each sugar in destarched sago waste, % Sugar

$$\% \text{ Sugar} = \frac{C_x \times \text{anhydro correction} \times V_{\text{filtrate}} \times \frac{1\text{ g}}{1000\text{ mg}}}{w_{\text{destarched sago waste}}} \times 100 \qquad (3.22)$$

where anhydro correction is the correction used to convert the concentration of corresponding monomer sugars to polymeric sugars (0.88 for arabinose and 0.9 for glucose), and V_{filtrate} is the volume of filtrate, *i.e.* 86.73 ml.

3.7.3 3,5-Dinitrosalicylic acid (DNS) assay

The amount of reducing sugars in the sample can be determined by performing DNS assay according to the protocol suggested by Miller (1959). DNS reagent consists of 1% (w/v) 3,5-dinitrosalicylic acid, 0.2% (w/v) phenol, 1% (w/v) sodium hydroxide and 0.05% (w/v) sodium sulfite. The reagent was first prepared without the sodium sulfite and stored in a dark bottle at 4^{o}C. Suitable amount of sodium sulfite was added into the reagent prior assay. Separately, 40% (w/v) of Rochelle salt (potassium sodium tartarate) solution was prepared. Fume hook was used when handling the highly vaporised phenol.

The DNS test was conducted by adding 3 ml of DNS reagent into 1.5 ml of the sample in a test tube. The mixture was boiled for 5 minutes in a vigorously boiling water bath containing sufficient water to cover the reaction mixture in the test tube. Upon completion, sample was cooled with tap water and simultaneously 1 ml of 40% Rochelle salt solution was added to the reaction mixture to stabilise the colour formed. The procedures were repeated for 1.5 ml of ionic liquid solution with 3 ml of DNS reagent, to serve as blank. Exactly 0.2 ml of the coloured sample was diluted with 2.5 ml of ultrapure water in the spectrophotometer cuvette. The mixture was mixed well and subjected to absorbance measurement using UV-vis spectrophotometer at wavelength, λ of 540 nm. The reducing sugars concentrations were then determined from the glucose standard curve.

The concentration of reducing sugars (RS) (mg/ml) in the hydrolysate was calculated using Equation (3.23):

$$\text{Concentration of RS } \left(\frac{\text{mg}}{\text{ml}}\right) = \frac{\text{Absorbance}}{\text{Gradient of the standard curve}} \times \text{dilution} \qquad (3.23)$$

and the reducing sugars yield was calculated using Equation (3.24):

$$\text{Reducing sugars yield} = \frac{w_{RS}}{1.11(w_s + w_c) + 1.14 w_h} \times 100\% \qquad (3.24)$$

where w_{RS} represents the weight of reducing sugars, w_s, w_c and w_h represent the weight of starch, cellulose and hemicelluloses in sago waste, respectively. The values 1.11 and 1.14 are the multiplication factors that convert the respective carbohydrate to its equivalent sugar (Sluiter *et al.*, 2011).

3.7.4 Fourier transform infrared spectroscopy (FT-IR) analysis

FT-IR spectrophotometry was used to examine the crystallinity of sago waste. The sample for analysis was prepared according to potassium bromide (KBr) pellet procedure for solid sample. The spectra were measured with a resolution of 1 cm^{-1} over the range of 400 cm^{-1} to 4000 cm^{-1} using Fourier transform infrared spectrometer (Spectrum RX1, Perkin Elmer, USA) The crystallinity of the biomass was then determined by measuring its two infrared ratios using Equation (3.25) (Nelson & O'Connor, 1964):

$$\text{Total crystallinity index (TCI)} = \alpha_{1378} / \alpha_{2900} \qquad (3.25)$$

3.7.5 Measurement of protons (H^+) concentration

Concentration of H^+ in hydrolysate was determined by measuring the pH of the solution based on Equation (3.26).

$$\text{pH} = -\log_{10} [H^+] \qquad (3.26)$$

pH of the reaction solution was measured at room temperature using a pH meter (827 pH lab, Metrohm, Switzerland). Prior to each use, the pH meter was calibrated at three points using pH 4.00, pH 7.00 and pH 9.00 buffer solution.

3.7.6 High-performance liquid chromatography (HPLC) analysis

Approximately 1 ml of liquid sample was transferred to a HPLC vial using a 0.2 μm nylon stringy filter. The samples were analysed using Hi-Plex H column equipped with an appropriate guard column from Agilent Technologies. The conditions used for the analysis were:

Injection volume: 10 μl

Mobile phase: Ultrapure water, 0.2 μm filtered and degassed.

Flow rate: 0.6 ml/min

Column temperature: 65^{o}C

Detector: refractive index

Run time: 30 min

Sago waste contains large amount of glucose and little amount of arabinose. The sugars have retention time of approximately 10.4 min and 11.8 min, respectively. Therefore, running time of 30 min was sufficient to elute all the sugars.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter is divided into four main sections to cover four research objectives. First section discussed on the chemical composition of sago waste and selection of suitable combination of ionic liquid and solid acid catalyst for the sequential ionic liquid dissolution-solid acid saccharification process. A sub-section of enzymatic saccharification of the ionic liquid pretreated solid residues is included. The second section discusses the process optimisation of the sequential process. This section comprises the screening results of influential process variables in the dissolution and the saccharification processes, statistical and process analysis, model validation and comparison study. The results on kinetic study of ionic liquid-mediated solid acid saccharification are discussed in the third section. This includes the effects of temperature and catalyst loading on the rate of reducing sugars production and degradation, and the determination of kinetic rate constants. Results on the reusability of solid acid catalyst and the recovery of ionic liquid and reducing sugars are also elaborated in the end of this chapter.

4.1 Compatibility Study on Ionic Liquid and Solid Acid Catalyst

A process scheme involving the sequential ionic liquid dissolution-solid acid saccharification was developed to maximise the production of reducing sugars from sago waste. In order to study the performance of the sequential process, chemical compositions of sago waste were first characterised. The best combination of ionic liquid and solid acid catalyst was determined. The selection of ionic liquid and solid acid catalyst was based on the reducing sugars yield, energy consumption, chemicals

costs and their physical stability. Results on enzymatic saccharification of the ionic liquid pretreated sago waste solid residues are discussed at the end of this section.

4.1.1 Characterisation of sago waste

The chemical compositions of sago waste are tabulated in Table 4.1. Approximately 90% of carbohydrates (*i.e.* starch, cellulose and hemicellulose) were being detected in the sago waste. These carbohydrates are important in the production of reducing sugars since they are makeup of sugar monomers. The chemical compositions of sago waste in this study are different compared to other literature values. Ozawa *et al.* (1996) reported 58% of starch, 23% of cellulose, 9.2% of hemicellulose and 3.9% of lignin in their sago waste sample. Each single component varies between studies, but the amount of carbohydrates almost the same at 90%. The variation in the components depends on the source of sago waste, sago plantation, cultivation and starch extraction processes. To avoid discrepancy in results, the sago waste sample used throughout this work was from the same source and from the same batch of collection as mentioned in methodology. Higher carbohydrates content in sago waste of this study compared to other lignocellulosic biomass (approximately 50 to 70% of carbohydrates) presented in the literature review suggests that the sago waste is beneficial for sugars production.

Table 4.1: Chemical compositions of sago waste

Component	**Dry weight percent (%, w/w)**
Starch	36.5 ± 1.2
Cellulose	40.0 ± 2.2
Hemicellulose	13.1 ± 0.1
Lignin	4.5 ± 0.1
Ash	2.0 ± 0.1
Moisture	5.6 ± 0.1

4.1.2 Selection of ionic liquid and solid acid catalyst

The effect of ionic liquid and solid acid catalyst on the performance of the sequential process in saccharification of sago waste was evaluated. In this study, [BMIM]Cl, [EMIM][OAc] and [EMIM][$(EtO)_2PO_2$] were used to dissolve the sago waste. They were selected because of their good dissolution capabilities. The dissolution capability predicted by the Kamlet-Taft beta (β) parameter for both [BMIM]Cl and [EMIM][OAc] were 0.84 (Fukaya *et al.*, 2006) and 1.1 (Doherty *et al.*, 2010) respectively indicating they possessed good dissolution properties. On the other hand, [EMIM][$(EtO)_2PO_2$] was selected in this study due to its low viscosity reported which would impart minimal mass and phase transfer limitations to give better dissolution performance (Li *et al.*, 2009; Mora-Pale *et al.*, 2011). The reducing sugars obtained by applying different types of ionic liquids in dissolution of sago waste are illustrated in Figure 4.1.

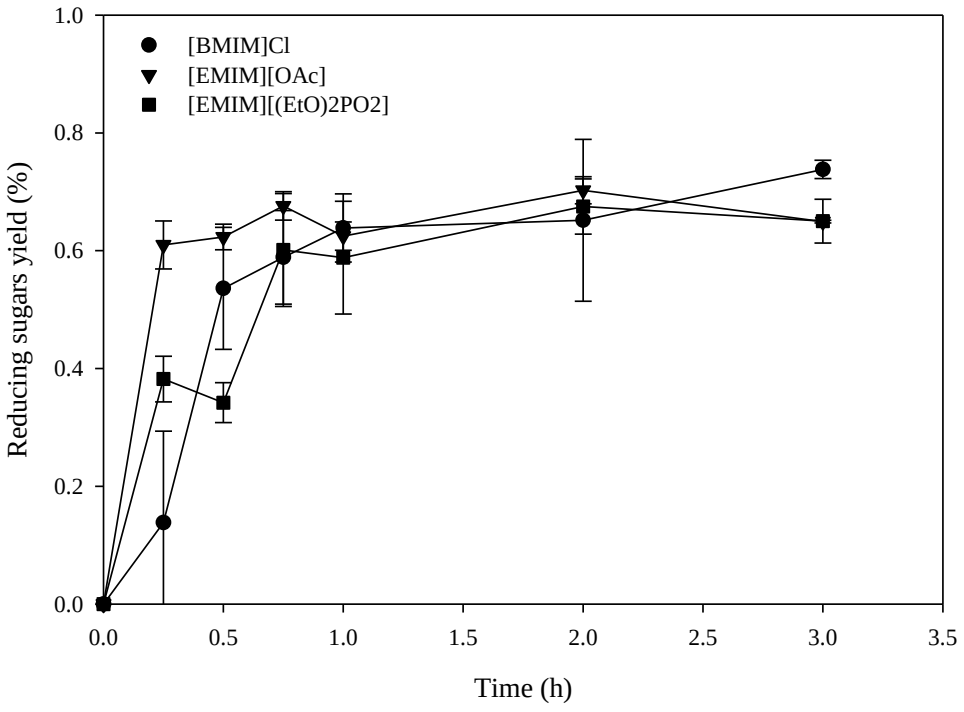

Figure 4.1: Reducing sugars yields in the sago waste prehydrolysates by different ionic liquid dissolution reactions. *Reaction conditions:* 4% (w/v) sago waste-ionic liquid mixture at 100^{o}C, 3 h.

The data in the figure were statistically analysed by using the analysis of variance (ANOVA). The ANOVA results (Appendix A1 - A3) showed that the results were reliable as there were no significant differences between batches at 95% confidence level. However, it was found that the dissolution duration affected the reducing sugars yield significantly. When different ionic liquids were applied to dissolve the sago waste, it can be observed that the reducing sugars yields for all the prehydrolysates increased slowly in the first hour of heating before reaching plateau. The reducing sugars yields in all the prehydrolysates were less than 1% as expected since the ionic liquids applied were aimed to dissolve the biomass rather than to produce sugars. Ionic liquids demonstrated the depolymerisation capability in breaking down carbohydrate polymers into smaller sugar oligomers and consequently into corresponding sugar monomers.

Depolymerisation was further confirmed when higher reducing sugars yields were detected in the hydrolysates (Figure 4.2). Furthermore, solid acid catalyst can only saccharify the prehydrolysate that contains sugar oligomers to reducing sugars in the hydrolysate. As such, the reducing sugars yield in [BMIM]Cl hydrolysate increased to approximately 60% compared to less than 1% in its prehydrolysate. The relatively high amount of reducing sugars in the hydrolysates indirectly indicates a high amount of sugar oligomers in the prehydrolysates. Thus, it signifies the good depolymerisation capability of the ionic liquids.

According to Mosier *et al.* (2002), protons play an important role in saccharification. The ability of solid acid catalysts to saccharify the sugar oligomers to sugar monomers was facilitated by the presence of protons in the reaction mixture. The mechanism is shown in Figure 2.4 whereby protons released from the catalyst into the reaction mixture through ion exchange at the catalyst sulfonic groups (SO_3H) with alkylimidazolium cations of the ionic liquid. The release of protons could be verified

from pH measurements of the reaction aliquots. This is because the pH of the aliquots containing pure [BMIM]Cl solution would drop from 5 to ≤ 1 with the addition of solid acid catalyst.

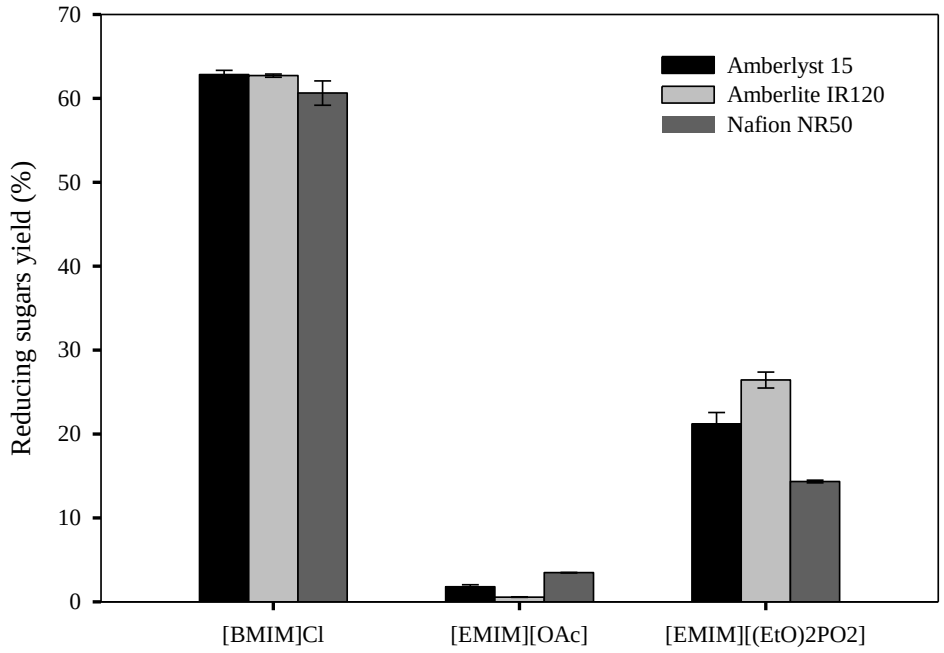

Figure 4.2: Reducing sugars yields in the hydrolysates for different combination of ionic liquids and solid acid catalysts in the sequential ionic liquid dissolution-solid acid saccharification. *Reaction conditions:* dissolution at 100^{o}C, 3 h with 4% (w/v) sago waste-ionic liquid mixture; saccharification at 120^{o}C, 1.5 h with 10% (w/v) catalyst loading.

In Figure 4.2, the reducing sugars yield in [BMIM]Cl hydrolysates was at least three times higher than those in the [EMIM][$(EtO)_2PO_2$] and [EMIM][OAc], regardless of the type of solid acid catalyst used in the saccharification. The ANOVA result presented in Appendix A4 showed that different type of dissolution reaction significantly affect the reducing sugars yield. The huge differences might be attributed to the degree of compatibility between the ionic liquid and the solid acid catalyst. Unlike [BMIM]Cl, both the [EMIM][OAc] and [EMIM][$(EtO)_2PO_2$] ionic liquids contain weakly basic anions of acetate and phosphonic respectively. These basic anions

can be easily protonated to acetic acid and phosphonic acid by solid acid catalyst (Rinaldi *et al.*, 2010b) leading to a considerably reduction in protons available for saccharification. Therefore, less reducing sugars were detected in [EMIM][OAc] and [EMIM][$(EtO)_2PO_2$] hydrolysates as compared to [BMIM]Cl hydrolysates.

Apart from producing a higher amount of reducing sugars, [BMIM]Cl consumed lowest amount of energy compared to [EMIM][OAc] and [EMIM][$(EtO)_2PO_2$] in the dissolution of sago waste. [BMIM]Cl required only 3.04 kJ/g sago waste compared to 3.72 kJ/g sago waste and 3.87 kJ/g sago waste by [EMIM][OAc] and [EMIM][$(EtO)_2PO_2$] as shown in Table 4.2. Comparing the dissolution energy required by the conventional pretreatment method, ionic liquid dissolution employed required relatively low energy compared to that of the dilute sulfuric acid pretreatment. Under the same operating conditions, 1% (w/w) sulfuric acid pretreatment required as much as 18.5 kJ/g sago waste. The energy differences could be huge if large amount of biomass were to be dissolved. Another added advantage of [BMIM]Cl is that it was the cheapest among the ionic liquids investigated. For 100 g of ionic liquid, [BMIM]Cl costs about RM 560 compared to RM 1500 and RM 1800 for [EMIM][OAc] and [EMIM][$(EtO)_2PO_2$], respectively (Sigma-Aldrich, Malaysia). The findings strongly support the suitability of [BMIM]Cl in dissolution of sago waste and hence the ionic liquid was employed for sequential scheme study of this research.

Table 4.2: Energy requirement in ionic liquid dissolution. *Reaction conditions:* 4% (w/v) sago waste-ionic liquid mixture at 100^{o}C

Ionic liquid	**Energy requirement (kJ/g sago waste)**
[BMIM]Cl	3.04
[EMIM][OAc]	3.72
[EMIM][$(EtO)_2PO_2$]	3.87

Different solid acid catalysts such as A15, Amberlite IR120 and Nafion NR50 were incorporated with ionic liquid on saccharification of sago waste. [BMIM]Cl hydrolysates obtained from all three solid acid saccharifications produced nearly the same amount of reducing sugars. Despite their comparable saccharification performances, the solid acid catalysts vary in physical properties and prices. Amberlite IR120 is most susceptible to physical attributions such as continuous wetting and drying due to its gel structure (Kunin *et al.*, 1962). On the other hand, A15 and Nafion NR50 are physically more stable and can withstand long period of heating up to 120-140^{o}C and 280^{o}C, respectively (Harmer & Sun, 2001). Although Nafion NR50 has better thermal tolerance, it is more expensive, at RM 61.00 per gram compared to A15 that costs RM 5.80 per gram. The costs of the catalysts were from Sigma-Aldrich, Malaysia. In view of that, A15 was used to maximise the production of reducing sugars from the ionic liquid dissolved sago waste subsequently.

4.1.3 Enzymatic saccharification of the pretreated solid residue

A maximum reducing sugars yield of 62% was obtained from saccharification of sago waste through the combined actions of [BMIM]Cl and A15. Higher reducing sugars yield is expected as some sago waste which remain undissolved might contain carbohydrates that would be converted to reducing sugars. To validate the hypothesis, enzymatic saccharification was conducted on the ionic liquid pretreated sago waste. The reducing sugars yields from enzymatic saccharification on the solid residues of the ionic liquid pretreated sago waste is shown in Figure 4.3.

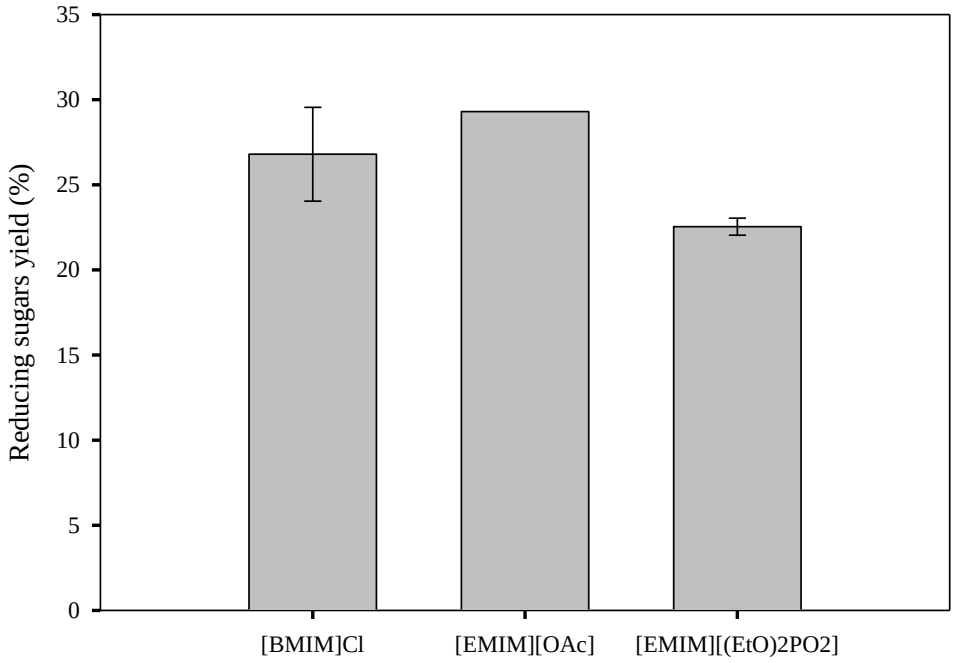

Figure 4.3: Reducing sugars yields from enzymatic saccharification of ionic liquid pretreated sago waste solid residues. *Reaction conditions:* 30-50 FPU/ g *Trichoderma viride* cellulose at 50^{o}C, 48 h in 1 ml of 50 mM, pH 4 acetate acid-sodium acetate buffer solution.

The figure shows that additional 20% to 30% of reducing sugars was produced from the sago waste via enzymatic saccharification. This validated the earlier hypothesis on the carbohydrates remained in the ionic liquid pretreated solid residues. The high reducing sugars yields from enzymatic saccharifications signifies the possible utilisation of ionic liquid pretreated solid residues, to produce maximum amount of reducing sugars from sago waste.

From enzymatic saccharification of the pretreated solid residues, the highest reducing sugars yield of 29% was obtained from [EMIM][OAc] pretreated solid residue, followed by [BMIM]Cl and [EMIM][$(EtO)_2PO_2$] pretreated solid residues respectively at 27% and 23%. This shows that pretreatment using different type of ionic liquid had very little effect on the reducing sugars yield (Appendix A5). Since the performance of enzymatic saccharification reported to be affected by both crystallinity

(Li *et al.*, 2009) and lignin content (Lee *et al.*, 2009; Wu *et al.*, 2011) of the pretreated biomass, both crystallinity indices and lignin contents of the pretreated sago waste solid residues were determined and the results are presented in Table 4.3. The crystallinity indices of all the pretreated solid residues were lowered compared to the untreated sago waste. Furthermore, the pretreated solid residues showed very little differences in crystallinity index, which explains the insignificant difference in reducing sugars yields among the pretreated solid residues.

Table 4.3: Total crystallinity index (TCI) and lignin content of the untreated sago waste and pretreated sago waste solid residues. *Reaction conditions:* dissolution at 100^{o}C, 3 h with 4% (w/v) sago waste-ionic liquid mixture; enzymatic saccharification at 50^{o}C, 48 h with 30-50 FPU/ g *Trichoderma viride* cellulose in 1 ml of 50 mM, pH 4 acetate acid-sodium acetate buffer solution

Sago waste sample	**Total crystallinity index (TCI)**	**Lignin content [a] (mg)**
Untreated sago waste	1.06	13.5 ± 0.4
[BMIM]Cl pretreated solid residue	0.99	12.0 ± 1.5
[EMIM][OAc] pretreated solid residue	1.02	9.0 ± 0.6
[EMIM][$(EtO)_2PO_2$] pretreated solid residue	0.98	9.9 ± 0.9

[a] The lignin contents in all the samples were measured per 300 mg sago waste samples and reported in absolute values for comparison purpose.

Effective delignification of biomass can improve sugars yield (Lee *et al.*, 2009; Wu *et al.*, 2011). Delignification is important otherwise enzyme would irreversible adsorb onto lignin to lower the enzyme activity and also hinder the recycling of enzyme, leading to a lower reducing sugars yield from biomass. From Table 4.3, it can be observed that [EMIM][OAc] pretreated solid residue gave the lowest lignin content which was consistent with the finding of the highest reducing sugars yield showed by the [EMIM][OAc] pretreated solid residue.

4.2 Process Optimisation of Sequential Ionic Liquid Dissolution-Solid Acid Saccharification

As previously discussed, ionic liquid pretreated sago waste solid residues were enzymatically saccharified to enhance the production of reducing sugars. However, biological processes involving enzymes often incur high enzymes cost and require long reaction time. Thus in this section, ionic liquid dissolution reaction was optimised to dissolve the sago waste to facilitate the subsequent optimisation of solid acid saccharification reaction in order to produce maximum amount of reducing sugars. Prior to the process optimisation, potential process variables affecting the production of reducing sugars were screened, and their ranges suitable for optimisation studies were selected.

4.2.1 Screening of influential process variables in ionic liquid dissolution

The process variables that may influence the ionic liquid dissolution of biomass are reaction temperature, dissolution duration, substrate loading and its particle size. Figure 4.4 shows the effect of dissolution temperature on the reducing sugars yield from sago waste. It can be observed that at dissolution temperatures between 100^{o}C to 130^{o}C, the reducing sugars yields obtained were around 60%. When dissolution temperature was increased to 160^{o}C, a drastic increase of reducing sugars yield to 87% was detected. ANOVA result showed that the dissolution temperature significantly affects the reducing sugars yield under 95% confidence level (Appendix A6). This finding implies that the dissolution temperature is crucial to be considered in process optimisation study. Therefore, temperature range of 140^{o}C to 160^{o}C was investigated in the optimisation study.

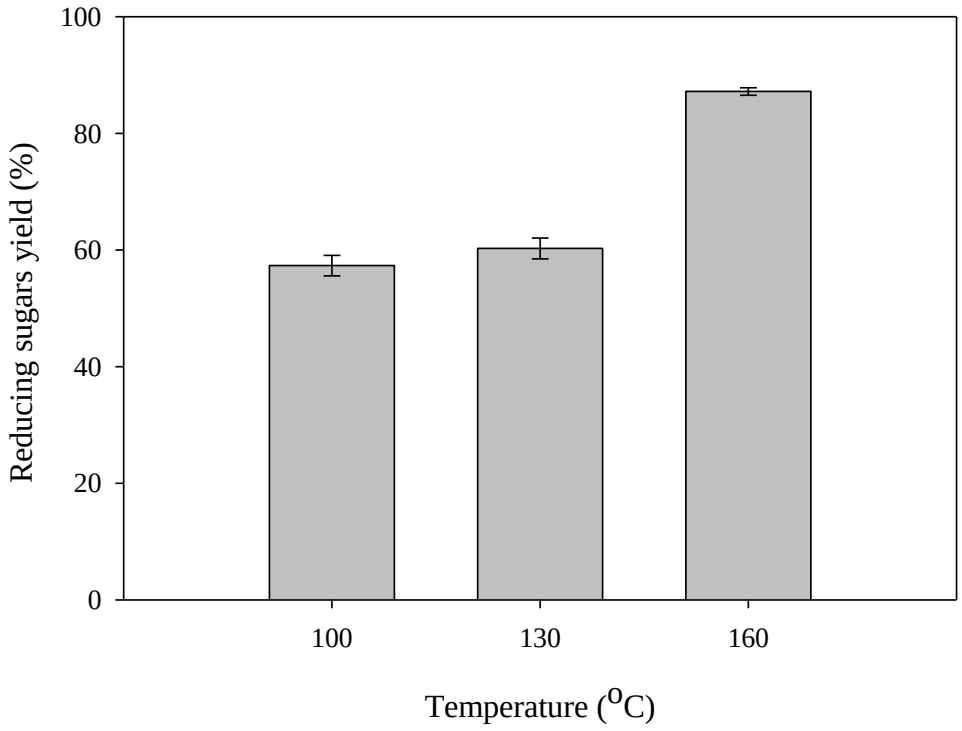

Figure 4.4: Effect of dissolution temperature on reducing sugars yield. *Reaction conditions:* dissolution at 1 h for 4% (w/v) substrate loading with particle size 250 μm – 500 μm; saccharification at 120°C, 1.5 h with 10% (w/v) catalyst loading.

The effect of dissolution time on the reducing sugars yield is presented in Figure 4.5. The figure shows that the reducing sugars yield increased during the first hour of heating and decreased thereafter. Since the yield was significantly affected by the dissolution time, refer to Appendix A7, this process variable was considered in process optimisation with range set between 0.5 h to 2.5 h. Screening result obtained for substrate loading is presented in Figure 4.6. Within the studied range, there was a linear drop in the reducing sugars yield when substrate loading increased from 2% to 6%. A 10% reduction in reducing sugars yield was observed for every 2% increment in substrate loading. From the statistical study conducted, substrate loading had significant impact on reducing sugars yield during the dissolution reaction as shown in Appendix A8. Since the reducing sugars yield decreased with an increase in substrate loading, low range of substrate loading between 0.5% to 2.5% was investigated in the subsequent process optimisation.

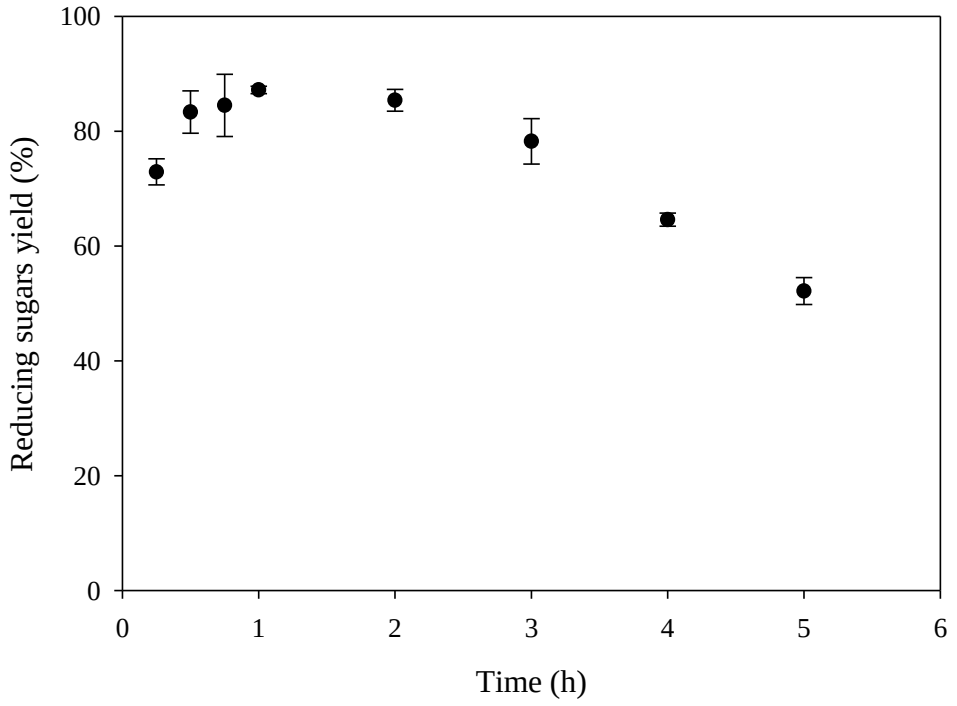

Figure 4.5: Effect of dissolution time on reducing sugars yield. *Reaction conditions:* dissolution at 160°C for 4% (w/v) substrate loading with particle size of 250 μm – 500 μm; saccharification at 120°C, 1.5 h with 10% (w/v) catalyst loading.

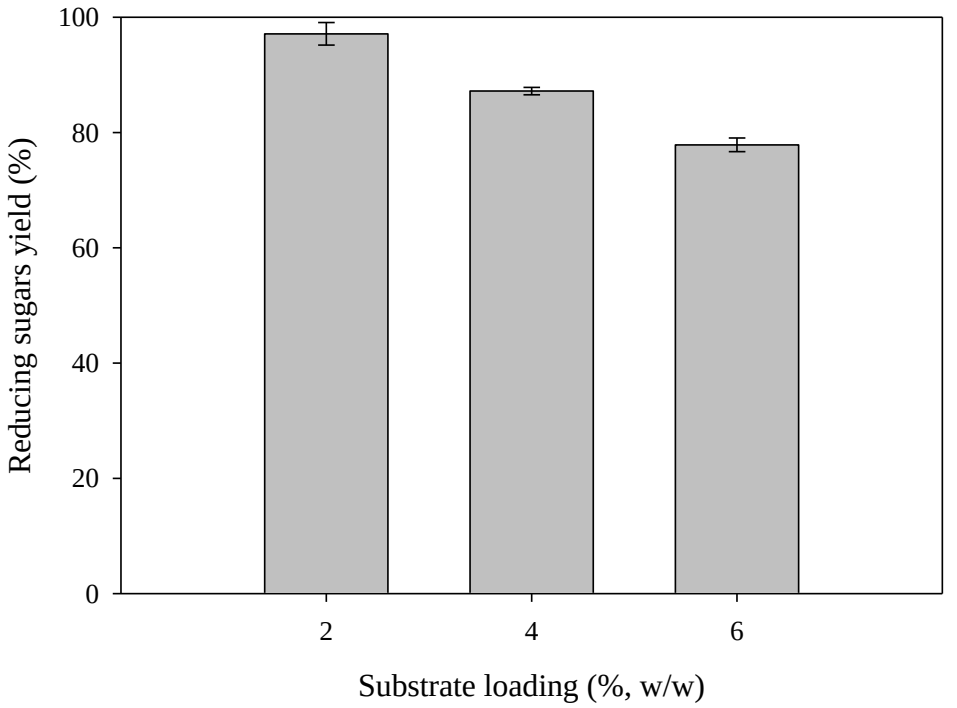

Figure 4.6: Effect of substrate loading on reducing sugars yield. *Reaction conditions:* dissolution at 160°C, 1 h with sago waste of particle size of 250 μm – 500 μm; saccharification at 120°C, 1.5 h with 10% (w/v) catalyst loading.

On the contrary, substrate particle size had negligible effect on the reducing sugars yield (Figure 4.7 & Appendix A9). Similar finding was reported by Sun *et al.* (2009) where red oak of particle size 0.125 mm to 1 mm gave similar ionic liquid dissolution performance under the same operating conditions. The slight variations in reducing sugars yields might have caused by the degree of diffusion of ionic liquid into the substrate. The dissociated ions diffuse from ionic liquid into the substrate and then dissolve the carbohydrates through hydrogen bonds disruption (Cao *et al.*, 2009). Theoretically, smaller size substrate provides larger surface area for ionic liquid ions to diffuse, disrupt and dissolve the biomass and results in higher reducing sugars yield. However, the difference in reducing sugars yields in this study was insignificant for all the particle sizes investigated. Hence, particle size would not be included in process optimisation study. The preliminary study confirmed the three process variables, *i.e.* dissolution temperature, reaction time and substrate loading, with respective ranges of 140-160^{o}C, 0.5-2.5 h and 0.5-2.5% were suitable for the ionic liquid dissolution optimisation study.

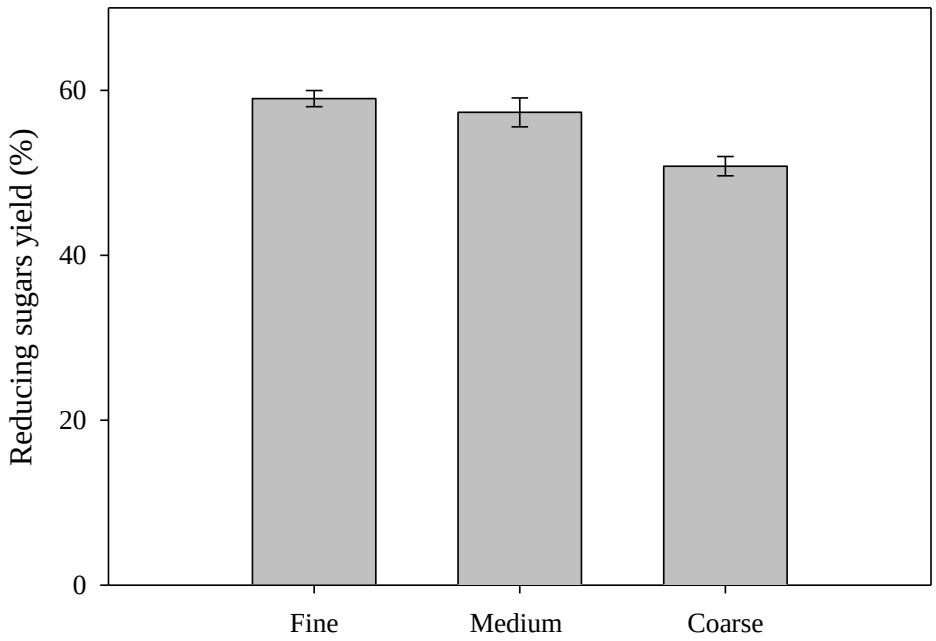

Figure 4.7: Effect of substrate particle size on reducing sugars yield. *Reaction conditions:* dissolution at 100^{o}C, 1 h with 4% (w/v) substrate loading; saccharification at 120^{o}C, 1.5 h with 10% (w/v) catalyst loading.

4.2.2 Screening of influential process variables in solid acid saccharification

The process variables screened for solid acid saccharification were saccharification temperature, saccharification time and catalyst loading. The effect of saccharification temperature on reducing sugars yield from saccharification of ionic liquid pretreated sago waste is shown in Figure 4.8. An increase in approximately 40% of reducing sugars when temperature was raised from 80^{o}C to 100^{o}C. As temperature adjusted to 120^{o}C, only 15% increment in yield was noted. This indicates that the temperature plays a crucial role in sugars production. ANOVA result further confirmed that the saccharification temperature significantly affects the reducing sugars yield (Appendix A10). Hence, saccharification with temperature range between 100^{o}C and 140^{o}C was further optimised in the subsequent study.

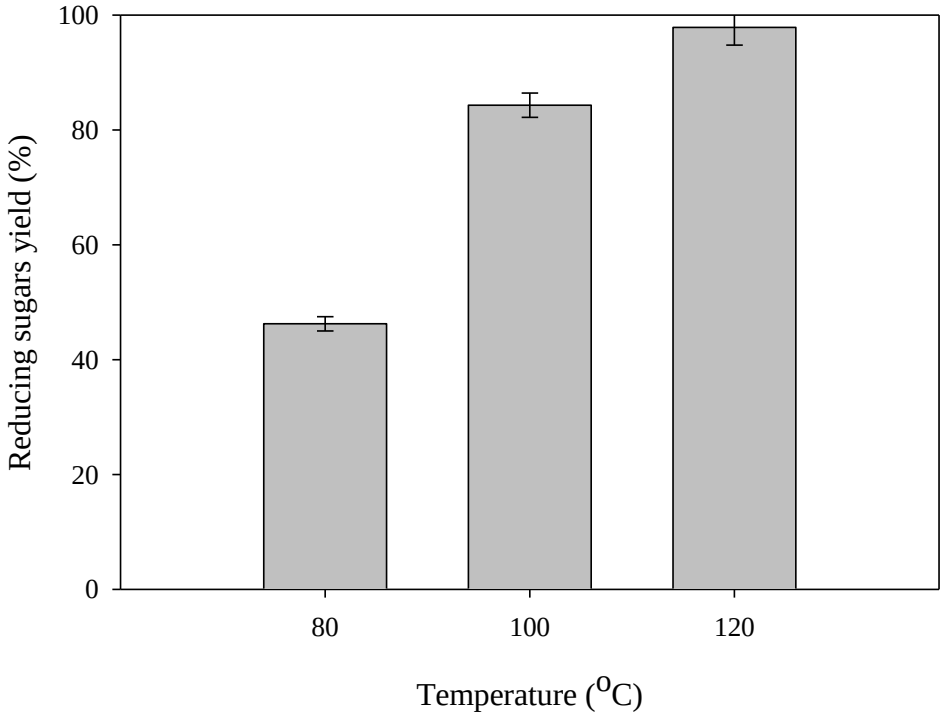

Figure 4.8: Effect of saccharification temperature on reducing sugars yield. *Reaction conditions:* dissolution at 160^{o}C, 1.75 h with 1.5% (w/v) substrate loading; saccharification at 1 h with 10% (w/v) catalyst loading.

Reducing sugars yield for different saccharification time is shown in Figure 4.9. The reducing sugars yield gradually increased from 51.6% to 93.5% as saccharification time increased from 15 min to 2 h. The yield was then remained constant around 94% for another 3 h heating. This implies that reducing sugars yield had not been enhanced after 2 h saccharification. Also, too short a saccharification period was not favorable to produce reducing sugars from sago waste. At 95% confidence level, saccharification time significantly affects the reducing sugars yield (Appendix A11). So, a time range of 0.5 h to 2.5 h was set to study the effect of saccharification time to maximise the reducing sugars production.

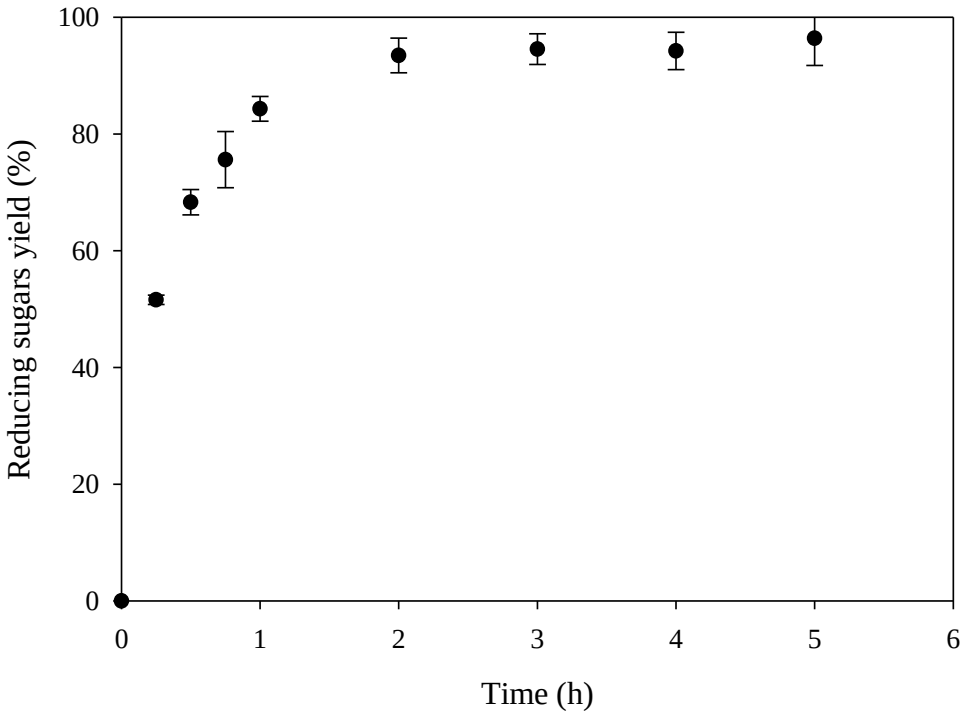

Figure 4.9: Effect of saccharification time on reducing sugars yield. *Reaction conditions:* dissolution at 160^{o}C, 1.75 h with 1.5% (w/v) substrate loading; saccharification at 100^{o}C with 10% (w/v) catalyst loading.

Compared to that of temperature and time, the effect of catalyst loading on reducing sugars yield was less significant (Figure 4.10 & Appendix A12). A difference of 12% in reducing sugars yield was observed between 1% and 5% catalyst loading.

Further increase of 3% reducing sugars yield was obtained when catalyst loading was raised to 10%. Although high catalyst loading favored the production of reducing sugars, the yield increment might not be sufficient for reducing sugars production at large scale. To investigate the effect of catalyst loading on reducing sugars production more closely, the studied range was set between 2% to 10% to optimise the reducing sugars yield.

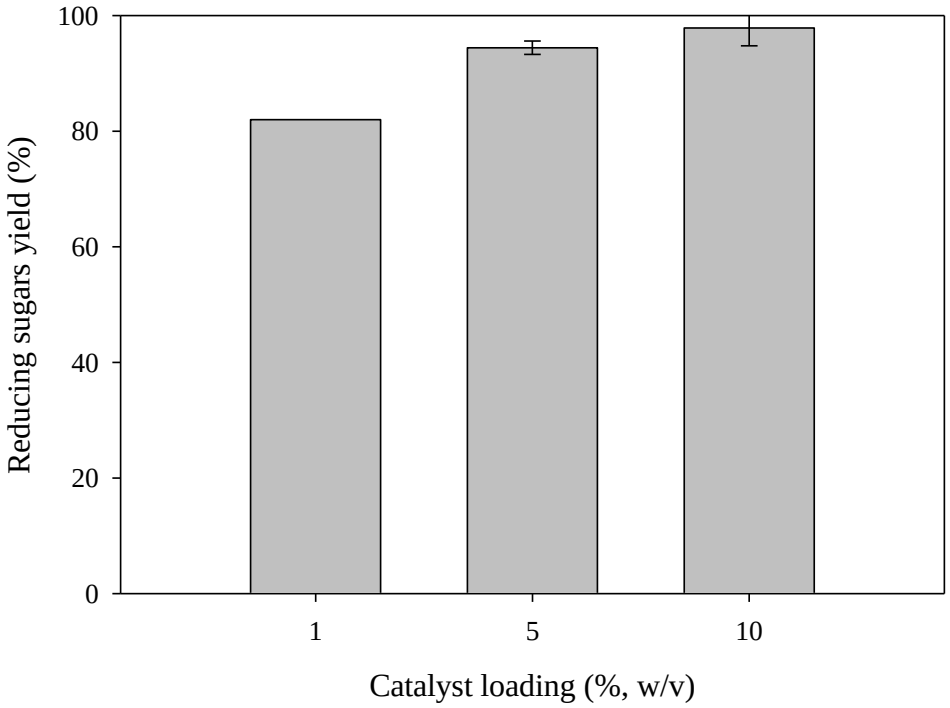

Figure 4.10: Effect of catalyst loading on reducing sugars yield. *Reaction conditions:* dissolution at 160^{o}C, 1.75 h with 1.5% (w/v) substrate loading; saccharification at 120^{o}C, 1 h.

4.2.3 Process optimisation

From the screening studies conducted, three significant variables were respectively determined for dissolution and saccharification reaction. In the dissolution reaction, effect of temperature, time and substrate loading on the reducing sugars yield were investigated; whereas temperature, time and catalyst loading were examined in the saccharification reaction.

4.2.3.1 Statistical analysis

The experimental design matrix and the response, *i.e.* reducing sugars yield for both ionic liquid dissolution and solid acid saccharification reaction are presented in Table 4.4 and Table 4.5 respectively.

Table 4.4: Experimental design matrix with their corresponding responses for ionic liquid dissolution

Run	**Type**	**Experimental variables**			**Response**
		A: Time (h)	**B: Temperature (^{o}C)**	**C: Substrate loading (%, w/w)**	**Reducing sugars yield (%)**
1	Factorial	2.5 (+1)	140 (–1)	0.5 (–1)	58.6
2	Factorial	0.5 (–1)	160 (+1)	0.5 (–1)	61.7
3	Factorial	0.5 (–1)	140 (–1)	2.5 (+1)	64.1
4	Axial	1.5 (0)	150 (0)	2.0 (+0.5)	83.4
5	Factorial	0.5 (–1)	140 (–1)	0.5 (–1)	49.0
6	Centre	1.5 (0)	150 (0)	1.5 (0)	75.5
7	Axial	1.5 (0)	145 (–0.5)	1.5 (0)	73.1
8	Centre	1.5 (0)	150 (0)	1.5 (0)	76.9
9	Factorial	2.5 (+1)	160 (+1)	2.5 (+1)	95.7
10	Axial	1.0 (–0.5)	150 (0)	1.5 (0)	74.7
11	Axial	2.0 (+0.5)	150 (0)	1.5 (0)	80.8
12	Factorial	2.5 (+1)	160 (+1)	0.5 (–1)	71.9
13	Factorial	0.5 (–1)	160 (+1)	2.5 (+1)	79.5
14	Axial	1.5 (0)	150 (0)	1.0 (–0.5)	75.4
15	Centre	1.5 (0)	150 (0)	1.5 (0)	82.6
16	Centre	1.5 (0)	150 (0)	1.5 (0)	81.4
17	Centre	1.5 (0)	150 (0)	1.5 (0)	86.0
18	Factorial	2.5 (+1)	140 (–1)	2.5 (+1)	75.0
19	Centre	1.5 (0)	150 (0)	1.5 (0)	76.7
20	Axial	1.5 (0)	155 (+0.5)	1.5 (0)	93.3

Table 4.5: Experimental design matrix with their corresponding responses for solid acid saccharification

Run	Type	Experimental variables			Response
		D: Time (h)	E: Temperature (^{o}C)	F: Catalyst loading (%, w/v)	Reducing sugars yield (%)
1	Axial	1.5 (0)	120 (0)	4 (–0.5)	92.7
2	Axial	1.5 (0)	110 (–0.5)	6 (0)	94.1
3	Factorial	2.5 (+1)	140 (+1)	10 (+1)	64.7
4	Factorial	0.5 (–1)	140 (+1)	2 (–1)	94.2
5	Axial	1.5 (0)	130 (+0.5)	6 (0)	95.1
6	Centre	1.5 (0)	120 (0)	6 (0)	96.4
7	Centre	1.5 (0)	120 (0)	6 (0)	99.5
8	Factorial	2.5 (+1)	140 (+1)	2 (–1)	84.8
9	Axial	1.0 (–0.5)	120 (0)	6 (0)	98.5
10	Centre	1.5 (0)	120 (0)	6 (0)	98.4
11	Factorial	2.5 (+1)	100 (–1)	10 (+1)	93.1
12	Axial	1.5 (0)	120 (0)	8 (+0.5)	96.7
13	Axial	2.0 (+0.5)	120 (0)	6 (0)	97.3
14	Centre	1.5 (0)	120 (0)	6 (0)	97.0
15	Factorial	0.5 (–1)	100 (–1)	2 (–1)	47.2
16	Centre	1.5 (0)	120 (0)	6 (0)	92.9
17	Factorial	0.5 (–1)	140 (+1)	10 (+1)	89.5
18	Centre	1.5 (0)	120 (0)	6 (0)	97.3
19	Factorial	2.5 (+1)	100 (–1)	2 (–1)	74.2
20	Factorial	0.5 (–1)	100 (–1)	10 (+1)	69.8

The data points obtained from both sets of CCDs were fitted well into the quadratic polynomial equations. The models are expressed in terms of coded factors as shown in Equation (4.1) (for ionic liquid dissolution) and Equation (4.2) (for solid acid saccharification).

$$\text{RS yield (\%)} = 80.41 + 5.88A + 8.50B + 9.05C + 0.75AB + 0.91AC + 1.27BC - 13.04A^2 + 8.70B^2 - 6.57C^2 \quad (4.1)$$

A, B and C represent dissolution time (h), dissolution temperature (^{o}C) and substrate loading (%, w/w) respectively.

$$\text{RS yield (\%)} = 97.17 + 1.82D + 5.81E + 2.19F - 10.56DE - 2.37DF - 8.28EF - 10.32E^2 - 9.67F^2 \quad (4.2)$$

where D, E and F represent saccharification time (h), saccharification temperature (^{o}C) and catalyst loading (%, w/v) respectively. The significance of the individual terms and their interactions on the response, *i.e.* reducing sugars yield were determined from ANOVA. All the terms were significant with p-values less than 0.05 except the quadratic term of saccharification time. The insignificant term was removed from the model to improve the regression model and optimisation results.

The significance of the developed models was also determined from ANOVA and presented in Table 4.6 and Table 4.7 for dissolution reaction and saccharification reaction respectively. The p-values of equal or less than 0.0001 obtained from both the quadratic models imply that the models were significant. The insignificant lack of fit (p-value > 0.05) further emphasizes the good representation of the models in the reactions. Besides, the agreement between the experimental data and the model predicted values was confirmed by the high R-squared values of 0.9297 and 0.9830 for dissolution and saccharification reaction respectively. Also, both the dissolution and the saccharification reactions had adequate precision values greater than 4 further suggest that the models can be used within the design space defined by the CCD.

Table 4.6: ANOVA for response surface quadratic model of ionic liquid dissolution

Source	**Sum of squares**	**Degree of freedom**	**Mean square**	**F value**	**p-value (Prob. > F)**
Model	2187.11	9	243.01	14.68	0.0001
Residual	165.49	10	16.55		
Lack of fit	80.02	5	16.00	0.94	0.5279
Pure error	85.46	5	17.09		
Total	2352.60	19			
R-squared	0.9297				
Adj. R-squared	0.8664				
Pred. R-squared	0.7193				
Adeq. Precision	16.289				

Table 4.7: ANOVA for response surface quadratic model of solid acid saccharification

Source	**Sum of squares**	**Degree of freedom**	**Mean square**	**F value**	**p-value (Prob. > F)**
Model	3618.49	8	452.31	79.27	< 0.0001
Residual	62.76	11	5.71		
Lack of fit	37.51	6	6.25	1.24	0.4166
Pure error	25.25	5	5.05		
Total	3681.25	19			
R-squared	0.9830				
Adj. R-squared	0.9706				
Pred. R-squared	0.7536				
Adeq. Precision	32.402				

The fitness of the models can also be visualised graphically in diagnostics plots of predicted reducing sugars yield versus actual reducing sugars yield as shown in Figure 4.11. The small deviation between the values signifies that the models developed are good representations of the real system. Both the numerical and graphical statistical

tests demonstrated the reliability of the quadratic models for predicting the response of both dissolution and saccharification reactions.

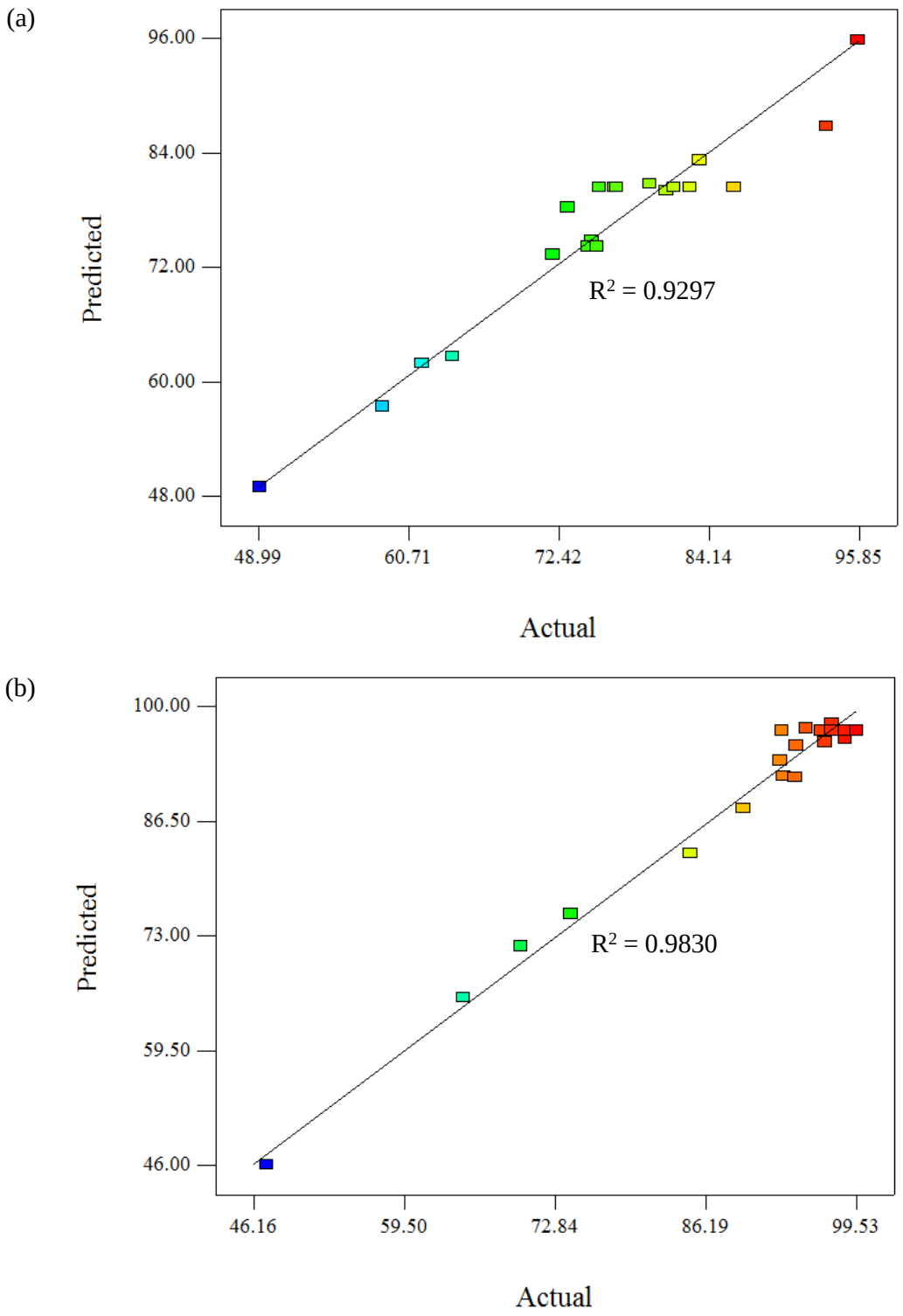

Figure 4.11: Predicted value versus actual value plot for reducing sugars yield, (a) ionic liquid dissolution; and (b) solid acid saccharification.

4.2.3.2 Process analysis

During the sequential ionic liquid dissolution-solid acid saccharification, sago waste was dissolved in [BMIM]Cl to produce a sugar oligomers-rich prehydrolysate, the prehydrolysate was later saccharified into reducing sugars with the aid of solid acid catalyst. The process variables for both the reactions play important roles in reducing sugars production and will be discussed in more detail in the following sections.

a. Ionic liquid dissolution

The effects of the dissolution variables, *i.e.* temperature, time and substrate loading on the reducing sugars yield were analysed using RSM. The interactions between any two of the variables are illustrated by three-dimensional response surface plot and contour plot as shown in Figure 4.12.

The effect of temperature and time on dissolution capability of ionic liquid was evaluated based on reducing sugars yield after solid acid saccharification of the prehydrolysate at a set of predefined conditions as illustrated in Figure 4.12(a). The reducing sugars yield followed an increasing trend for the investigated temperature range of 140^{o}C to 160^{o}C. Positive effects demonstrated by both of its independent and quadratic terms toward the response had contributed to this observation (Equation 4.1). The positive effects on the response gave increment in reducing sugars yield whereas negative effects gave reduction in reducing sugars yield. The increase in reducing sugars yield with temperature is corresponded to the decrease in viscosity of [BMIM]Cl. At lower viscosity, mass transfer between the ionic liquid and sago waste is improved. Thus, higher temperature allows [BMIM]Cl to diffuse easily into the sago waste and assists in swelling and subsequently dissolves the biomass (El Seoud *et al.*, 2007; Kuang *et al.*, 2007).

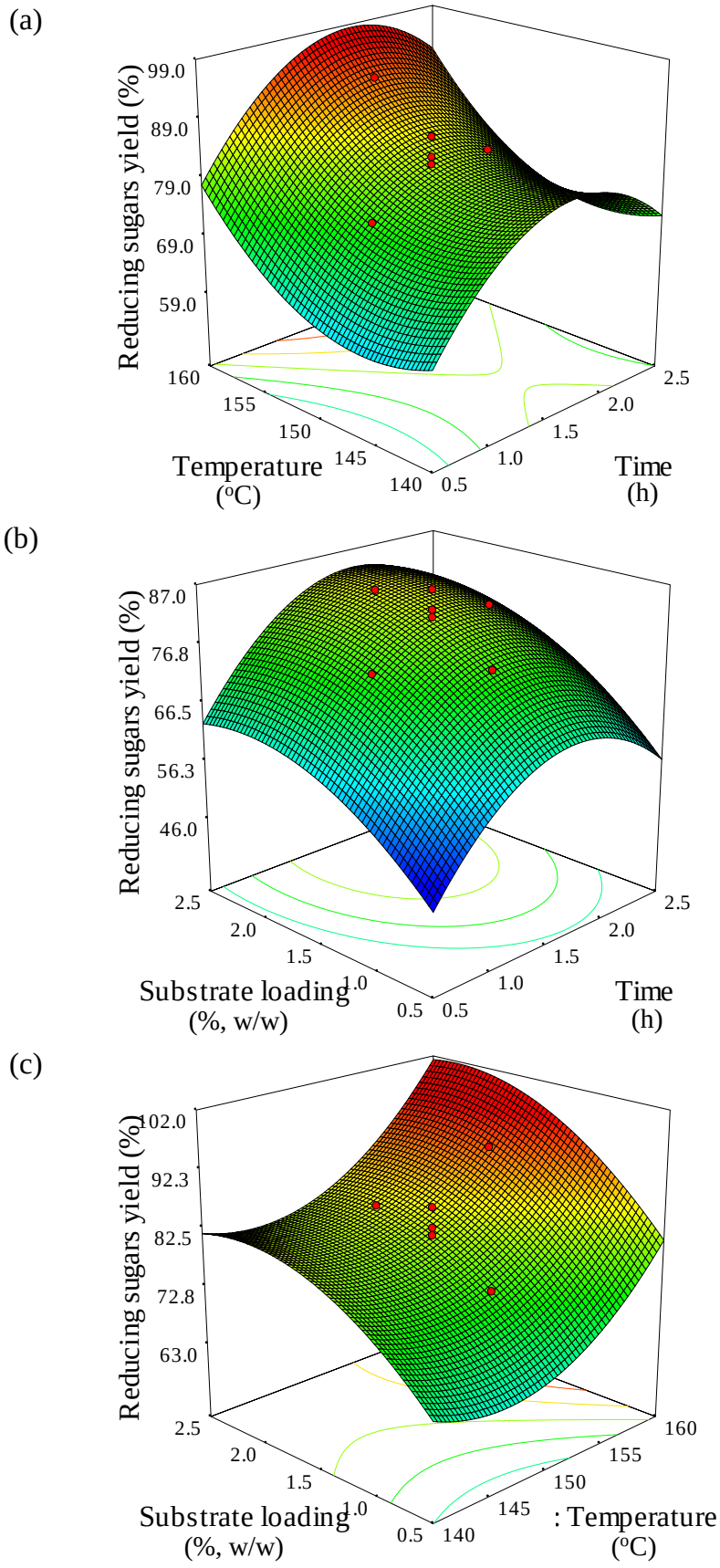

Figure 4.12: Response surface plot of the ionic liquid dissolution variables (a) time-temperature; (b) time-substrate loading; and (c) temperature-substrate loading for reducing sugars yield.

With regards to the effect exerted by dissolution time, the reducing sugars yield increased as the time increased to around the mid-range, *i.e.* 1.5 h to 1.75 h. Beyond the time range, a reduction in reducing sugars yield was observed (Figure 4.12(a) and (b)). The observation was in accordance with the combination of the positive and negative terms of its respective independent and quadratic expression. During the initial stage of the dissolution reaction, the reactant (sago waste) reacts with fresh [BMIM]Cl has allowed the carbohydrates to be readily dissolved in the ionic liquid. This is mainly due to the highest amount of available chloride anions of the ionic liquid for carbohydrates hydrogen bonds disruption. As reaction proceeded, most of the chloride anions have participated in the dissolution reaction and resulted in a constant reducing sugars yield. Another possible explanation for the reducing sugars trend observed is the increased viscosity of the reaction mixture whereby the ionic liquid is concentrated with dissolved carbohydrates as the dissolution progressed (Mäki-Arvela *et al.*, 2010). At 1.75 h, dissolution had reached its saturation point, *i.e.* the ionic liquid was concentrated with dissolved carbohydrates. Thus, extensive depolymerisation and formation of degraded products of reducing sugars occurred beyond 1.75 h. This observation concords with the reported findings (Li & Zhao, 2007; Malihan *et al.*, 2012) that long heating time leads to sugar degradation.

Figure 4.12(b) and (c) showed that the reducing sugars yield increased almost linearly in the beginning, however, once the substrate loading was greater than 1.5%, the reducing sugars yield remained almost constant. Theoretically, a higher substrate loading gives a higher reducing sugars yield as higher amount of hydrolysable compounds are present. Nonetheless, as the substrate loading increased beyond a certain limit such as 1.5% in this study, it has little effect on the production of reducing sugars which could be explained by the limited contact between the ionic liquid and the biomass. To achieve higher reducing sugars yield, mixing can be incorporated to create

more frequent collision between the biomass particles and the ionic liquid to promote carbohydrate dissolution. Besides, heat transfer within the system can also be enhanced with mixing. As agreed by Tan *et al.* (2010), mixing is a contributing factor for better production of reducing sugars in saccharification reaction.

The findings obtained so far in the study suggest that by combining high dissolution temperature, moderate dissolution time and substrate loading, high reducing sugars yield can be achieved. From the point prediction feature shown in the Design Expert software, dissolution condition of 160^{o}C, 1.75 h and 1.5% of substrate loading was the most favorable condition. Sago waste was dissolved at the mentioned condition and subsequently subjected to solid acid saccharification to maximise the production of reducing sugars.

b. Solid acid saccharification

Response surface plots together with their corresponding contour plots of the saccharification variables are presented in Figure 4.13. These plots describe the effects of saccharification variables on the production of reducing sugars.

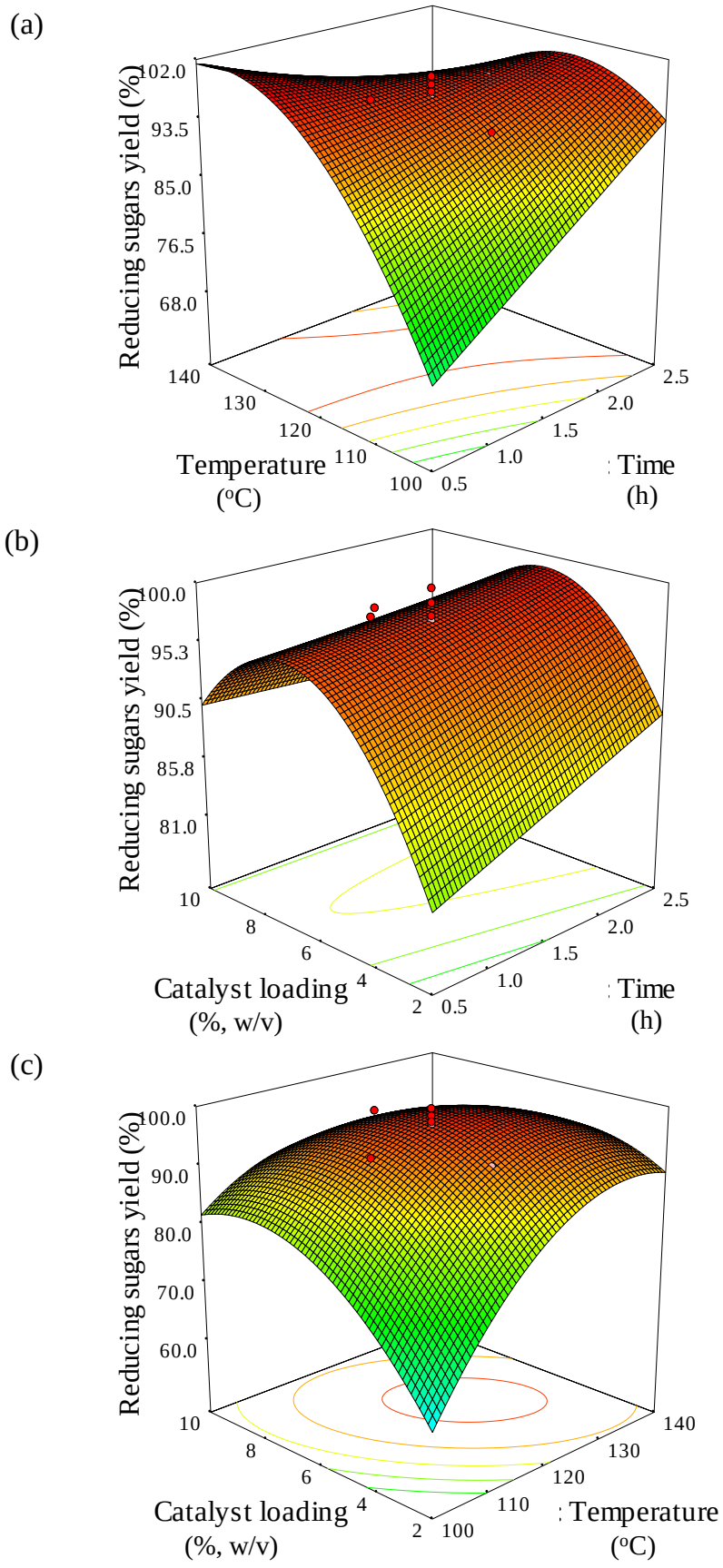

Figure 4.13: Response surface plot of the solid acid saccharification variables (a) time-temperature; (b) time-catalyst loading; and (c) temperature-catalyst loading for the reducing sugars yield.

It can be seen from Figure 4.13(a) that the reducing sugars yield increased with time at low saccharification temperature. For instance, reducing sugars yield increased from 68.7% to 93.4% at 100^{o}C when saccharification of the prehydrolysate was increased from 0.5 h to 2.5 h. During the previous ionic liquid dissolution reaction, carbohydrates were dissolved in [BMIM]Cl and sugar oligomers-rich prehydrolysate was produced. These sugars oligomers were cleaved in a randomised order during the solid acid saccharification reaction (Vanoye *et al.*, 2009). In view of this, more shorter chains of sugar oligomers might have produced during the initial stage of saccharification. And over a period of time, these shorter chains of sugar oligomers could have been accumulated and probability of cleaving at the end chain increased and thus more reducing sugars was produced.

When saccharification was conducted at higher temperature, shorter time was needed to produce higher amount of reducing sugars. In this study, 97% reducing sugars was produced when saccharification was performed at 140^{o}C and 1 h, whereas at lower saccharification temperature of 100^{o}C, the highest reducing sugars yield was 93.4% over a saccharification period of 2.5 h. It is obvious that at higher temperature, more energy has been provided for saccharification, and shorter saccharification time is thus needed to produce a higher amount of reducing sugars. Despite that temperature can accelerate the saccharification reaction, prolong heating at high temperature would lead to the formation of sugar degraded products and resulted in a decline in reducing sugars yield. It can be seen from Figure 4.13(a) that reducing sugars yield had reduced to 92.7% when saccharification was extended to 1.5 h at 140^{o}C and further decreased to 83.9% at the end of 2.5 h period reaction. The interaction between the saccharification temperature and time formed a stationary ridge with constant high reducing sugars yield in the time-temperature three-dimensional response surface plot as illustrated in Figure 4.13(a).

The effect of saccharification time and catalyst loading on the reducing sugars yield is depicted in Figure 4.13(b). High reducing sugars yield was obtained at catalyst loading of approximately 4% to 6% irrespective of saccharification time. According to Mosier *et al.* (2002), protons are required for saccharification to take place. As catalyst loading increases, the amount of protons available for reaction increase and so is the reducing sugars. However, the justification is not applicable to catalyst loading higher than 6% in the current work. When large amount of catalyst is used, the degree of bond cleavage increased which has triggered extensive depolymerisation leading to the production of sugar degraded products (Rinaldi *et al.*, 2010b).

The interaction between the saccharification temperature and catalyst loading at constant time of 1.5 h is presented in Figure 4.13(c). A clear peak was observed at the mid-range of saccharification temperature and catalyst loading. As discussed earlier, moderate temperature and catalyst loading favored a high reducing sugars yield. Low temperature and catalyst loading were ineffective in saccharification, while high temperature and catalyst loading degraded the sugars products. Therefore, saccharification conditions of 130^{o}C, 0.5 h and catalyst loading of 4% were the most suitable condition to produce the highest amount of reducing sugars.

4.2.3.3 Model validation

In the sequential ionic liquid dissolution-solid acid saccharification reaction, an optimum reducing sugars yield of 98.3% was predicted using the quadratic model at dissolution conditions of 160^{o}C, 1.75 h and 1.5% substrate loading; and saccharification conditions of 130^{o}C, 0.5 h and 4% catalyst loading. The model validation results are listed in Table 4.8. An average reducing sugars yield of 97.7% was obtained, which was consistent with the predicted value. This suggests that the experimental data fitted the model well.

Table 4.8: Verification of experiments at optimum conditions for sequential ionic liquid dissolution-solid acid saccharification reaction

	Response (Reducing sugars yield, %)
Experimental value	
First replicate	99.3
Second replicate	96.8
Third replicate	97.1
Average	97.7 ± 1.4
Predicted value by statistical model	98.3
Error	0.6

4.2.4 Comparison of sequential ionic liquid dissolution-solid acid saccharification reaction with other saccharification processes

The performance of the sequential ionic liquid dissolution-solid acid saccharification reaction on sago waste was compared with those from four different saccharification process schemes. They are: (1) single step reaction of ionic liquid dissolution-solid acid saccharification (Dwiatmoko *et al.*, 2010; Rinaldi *et al.*, 2010b; Watanabe, 2010), (2) solid acid saccharification without ionic liquid (Dwiatmoko *et al.*, 2010), (3) acid saccharification with ionic liquid (Li *et al.*, 2008), and (4) ionic liquid pretreatment followed by enzymatic saccharification (Dadi *et al.*, 2006).

The reducing sugars yield of 98.3% obtained in the current work is comparatively higher than those reported single step ionic liquid dissolution-solid acid saccharification reaction. A 28% yield was obtained from the cellulose dissolution and saccharification with [BMIM]Cl and A15 in a single step reaction (Rinaldi *et al.*, 2010b). Dwiatmoko *et al.* (2010) reported a yield of 44.5% when cellobiose was saccharified in the mixture of [BMIM]Cl and Nafion NR50, and a 25.6% yield was obtained by Watanabe (2010) employing *Cryptomeria japonica* wood in saccharification with [BMIM]Cl and Dowex 50WX8. Unlike that of sequential ionic liquid dissolution-solid acid saccharification, water was added into the

carbohydrates/ionic liquid/solid acid catalyst mixture during single step reaction to promote the mass transfer between the reaction mixtures. The added water hampers the dissolution capability of the ionic liquid (Cao *et al.*, 2009; Swatloski *et al.*, 2002) and results in poor saccharification performance. Besides, different substrates used in the processes could contribute to the different reducing sugars yield obtained.

In another process scheme involving only solid acid saccharification of biomass, a glucose yield of 20% was reported when cellobiose was saccharified over Nafion NR50 with distilled water as reaction medium (Dwiatmoko *et al.*, 2010). Without dissolving the biomass with ionic liquid, a homogeneous reaction system is difficult to be generated. Under the circumstance, the physical barriers such as morphology, surface area and crystallinity of the biomass would hinder the saccharification reaction.

The conventional acid and enzymatic saccharifications were also included in the performance evaluation. In acid saccharification, 81% of reducing sugars was obtained when pine wood was saccharified in the presence of [BMIM]Cl and hydrochloric acid (Li *et al.*, 2008). This yield is slightly lower than the yield obtained in the current work. Considering the requirement of corrosion resistant equipments and the handling of acid waste disposal problem during acid saccharification, solid acid saccharification is preferred for the production of reducing sugars. Moreover, solid acid catalyst employed in the saccharification can be regenerated and reused.

The sequential process in this study was further compared with enzymatic saccharification of ionic liquid pretreated biomass. A 90% of cellulose conversion to glucose had been reported when ionic liquid pretreated cellulose was enzymatically saccharified (Dadi *et al.*, 2006). Although both the sequential and enzymatic saccharification processes are effective in biomass conversion to sugars, the sequential process is more effective in terms of conversion rate. While enzymatic saccharification requires 2 to 3 days to produce reducing sugars, a much shorter saccharification period

of only 2 to 3 h is needed for the sequential process. Also, the employment of solid acid catalyst in saccharification incurs lower catalyst costs compared to that of enzyme used in the enzymatic saccharification. The results of the comparison studies collectively substantiate the feasibility of the sequential ionic liquid dissolution-solid acid saccharification for the production of reducing sugars from sago waste.

4.3 Kinetic Study of Ionic Liquid-Mediated Solid Acid Saccharification

In this study, Saeman model was used to represent solid acid saccharification of carbohydrates in the sago waste that had been dissolved in the ionic liquid. The model was designed for hydrolysis of cellulose from Douglas fir using dilute sulfuric acid based on a first order random chain scission of cellulose followed by a first order glucose degradation (Saeman, 1945). It was later applied to hydrolysis of other carbohydrates, including starch (Barnali *et al.*, 2008) and hemicellulose (Lu & Mosier, 2008). Therefore, the generalised model, *i.e.* two consecutive first order carbohydrates hydrolysis and sugar degradation reaction, as expressed in Equation (3.4) was applied in this study to examine the kinetics of solid acid saccharification of carbohydrates dissolved in ionic liquid. Fitness of the experimental data to Saeman model is shown in Figure 4.14. The well fitted data suggests that solid acid saccharification of carbohydrates in sago waste that dissolved in ionic liquid is a first order sugars production-first order sugar degradation reaction.

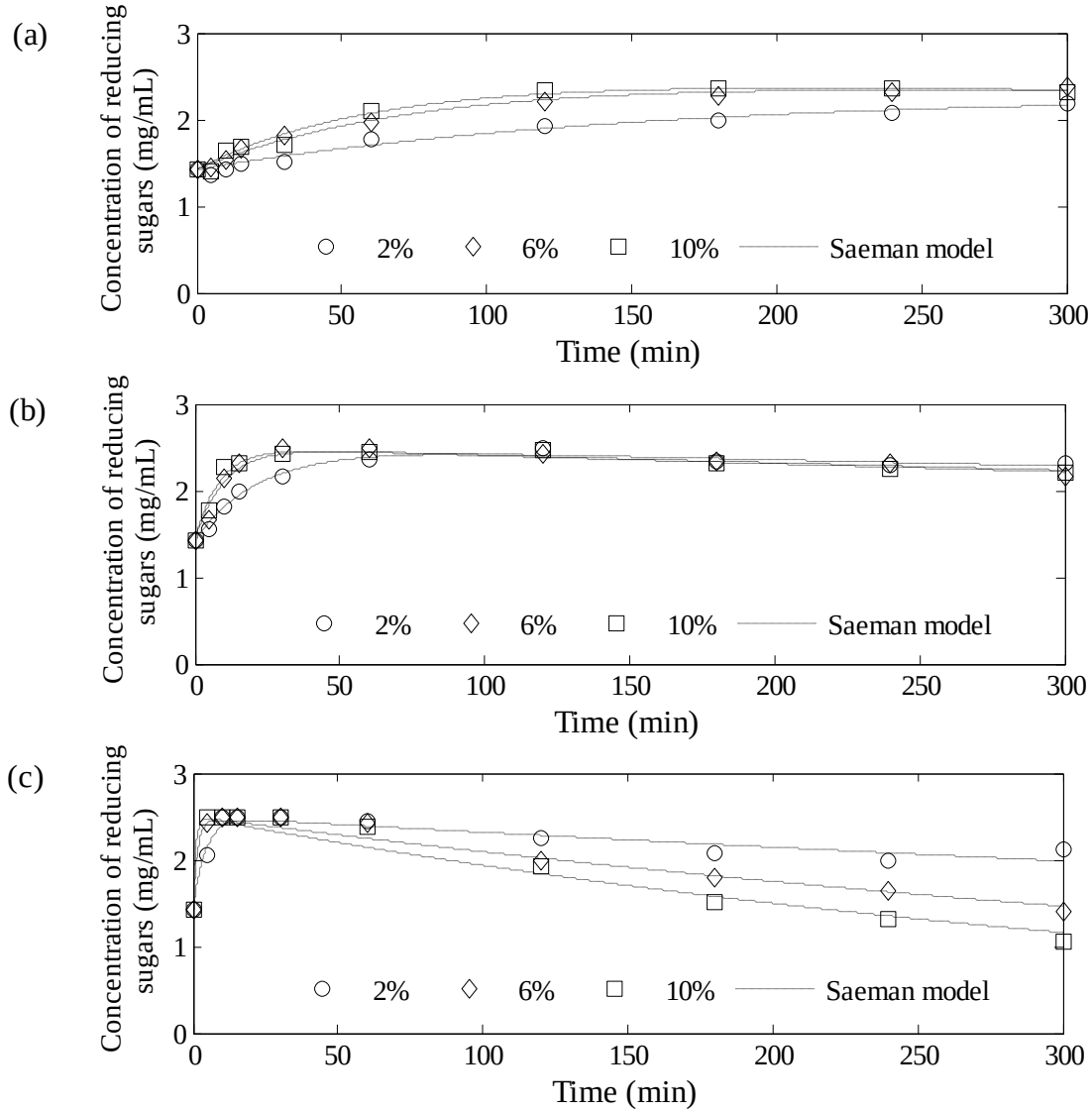

Figure 4.14: Concentration of reducing sugars at different temperature and catalyst loading (a) 100°C, (b) 120°C, (c) 140°C. *Reaction conditions:* dissolution at 160°C, 1.75 h with 1.5% (w/v) substrate loading.

4.3.1 Effects of temperature and catalyst loading on the rate of production and degradation of reducing sugars

The concentrations of reducing sugars obtained during the course of hydrolysis reaction at different temperatures and catalyst loadings are shown in Figure 4.14. Under all the experimental conditions investigated, concentration of reducing sugars increased during the initial stage of the reaction. This suggests that there was a net production of

reducing sugars during the initial stage as the rate of reducing sugars production was greater than the rate of degradation. At 100^{o}C, the concentration of reducing sugars remained almost constant when saccharification reaction was conducted for more than 180 min at higher catalyst loadings of 6% and 10%. This indicates that the production and degradation of reducing sugars were proceeded at the same rate.

In the present work, the saccharification reaction was conducted in batch. Thus, the amount of carbohydrates decreased with the progression of hydrolysis reaction as the carbohydrates was being converted gradually into reducing sugars. Also, the production rate of reducing sugars was reduced proportionally to the carbohydrates concentration in the sugars production reaction. However, further reduction of reducing sugars might have occurred due to probable sugar degradation reaction. Hence, rate of degradation had increased. This explains the trend observed at 120^{o}C and 140^{o}C as shown in Figure 4.14(b) and (c) respectively.

The findings showed that different temperatures and catalyst loadings gave different profiles of reducing sugars concentration. This clearly suggests that the rate of production of reducing sugars from the dissolved carbohydrates in ionic liquid, as well as the rate of degradation of sugars were greatly affected by the reaction temperature and catalyst loading. Both of the rates accelerated with temperature and catalyst loading. The effect of temperature can be explained by the endothermic reaction of the hydrolysis process, whereby high temperature favors the reaction (Jin *et al.*, 2011). Meanwhile, the increase in catalyst loading increased the amount of protons available for hydrolysis. To elucidate the effects of temperature and catalyst loading on the reaction rates, a detailed kinetic study was presented in the next sections.

4.3.1.1 Determination of kinetic rate constants of reducing sugars production and degradation

As discussed earlier, the hydrolysis reaction employed the Saeman model. However, solid acid saccharification of dissolved carbohydrates was expected to have different values in their kinetic parameters. The kinetic constants, k_1 and k_2 in Equation (3.7) were determined by fitting the experimental data to the equation with the aid of MATLAB. The values obtained for all the operational conditions are tabulated in Table 4.9. The rate constants of sugars production, k_1 were higher than the rate constants of sugar degradation, k_2 implies that the operational conditions favored the reducing sugars production over sugar degradation.

Table 4.9: Rate constants for reducing sugars production and degradation

Temperature (^oC)	Catalyst loading (%, w/v)	k_1 (min^{-1})	k_2 (min^{-1})	k_1/k_2
100	2	0.005445	0.000221	24.6
	6	0.013185	0.000224	58.9
	10	0.016327	0.000228	71.6
120	2	0.043886	0.000312	140.7
	6	0.098681	0.000379	260.4
	10	0.117770	0.000405	290.8
140	2	0.231038	0.000771	299.7
	6	0.594673	0.001787	332.8
	10	1.047426	0.002562	408.8

k_1: rate constant of reducing sugars production reaction; k_2: rate constant of sugar degradation reaction.

Table 4.9 shows that the higher the reaction temperature and acid concentration, the higher were the kinetic rate constants. This observation is as explained previously, *i.e.* attributes to the endothermic reaction and the high availability of protons for promoting the hydrolysis reaction. The rate constant for sugars production, k_1 increased

more with the increase in temperature than the increase in catalyst loading. An increase of 20^{o}C from either 100^{o}C to 120 ^{o}C or 120 ^{o}C to 140 ^{o}C at any catalyst loading, a five-fold to nine-fold increment in k_1 values were obtained. Nevertheless, an increment of approximately two-fold was observed when catalyst loading increased from 2% to 6% or 6% to 10% at all the temperatures investigated. Hence, elevation of the operational temperature is a better option to enhance the production of reducing sugars.

To evaluate the efficiency of different operational conditions in saccharification, the selectivity factors, *i.e.* the ratio of rate constant of reducing sugars production to rate constant of degradation (k_1/k_2) were determined and are presented in Table 4.9. The selectivity factor increased with the increase in severity of operational condition giving lowest value of 24.6 at 100^{o}C, 2% catalyst loading and highest value of 408.8 at 140^{o}C, 10% catalyst loading. The hydrolysis conducted at 140^{o}C and 10% catalyst loading achieved maximum concentration of reducing sugars at the shortest time. However, it did not give the highest concentration at the end of the reaction as shown in Figure 4.14. This is because the rate of reaction is affected by both the rate constant and the concentration of reactant. As previously discussed, no reducing sugars was produced when all the carbohydrates had been completely utilised whereas the degradation of sugars continued to take place. A high selectivity factor though promoted the reducing sugars production, the reaction time should be kept within the maximum production of reducing sugars to avoid degradation. Table 4.10 listed the maximum time required to achieve the highest concentration of reducing sugars (t_{max}) at different reaction conditions. The values of t_{max} can serve as guidelines for specific set of operational conditions in achieving maximum reducing sugars concentration.

Table 4.10: Time required to achieve maximum concentration of reducing sugars at various operating conditions

Temperature (^oC)	Catalyst loading (%, w/v)	Time to achieve maximum concentration of reducing sugars, t_{max} (min)	Concentration of reducing sugars (mg/ml)
100	2	613	2.18
	6	314	2.33
	10	265	2.35
120	2	114	2.41
	6	57	2.45
	10	48	2.45
140	2	25	2.45
	6	10	2.46
	10	6	2.46

4.3.2 Determination of kinetic parameters in the rate constants

Generally, Arrhenius equation as expressed in Equation (3.9) was used to study the effect of temperature on rate constants. To determine the kinetic parameters such as Arrhenius constants and activation energies of the reactions in this study, natural logarithmic of rate constant (ln k) versus reciprocal of absolute temperature (1/T) were plotted as shown in Figure 4.15. From the figure, activation energy and Arrhenius constant for both the sugars production and the sugar degradation reactions were determined and presented in Table 4.11.

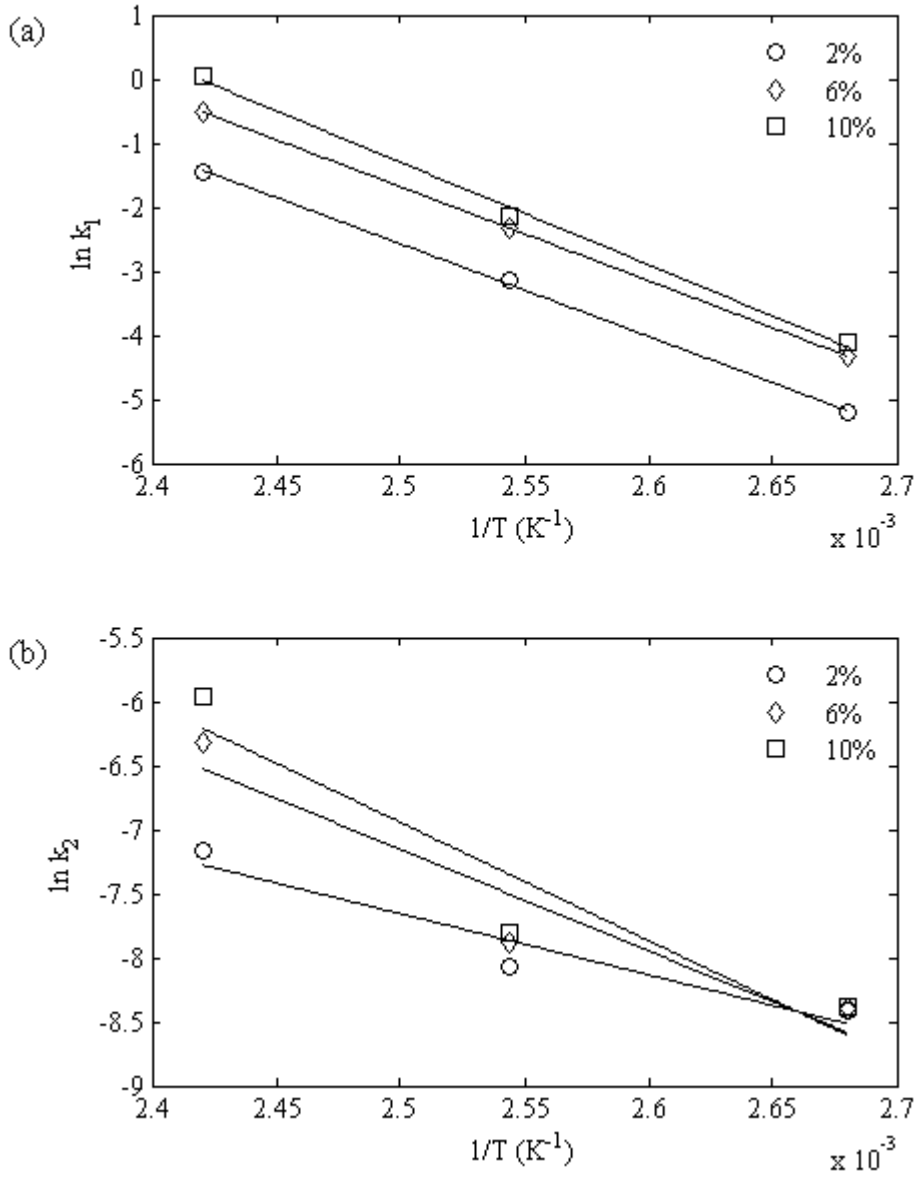

Figure 4.15: Arrhenius plots at various catalyst loadings for (a) rate constant of reducing sugars production, k_1; and (b) rate constant of sugar degradation, k_2.

Table 4.11: Activation energies and Arrhenius constants for reducing sugars production and sugar degradation at different catalyst loadings

Catalyst loading (%, w/v)	Sugars production reaction			Sugar degradation reaction		
	Arrhenius constant, A (s^{-1})	Activation energy, E_a (kJ mol^{-1})	R^2	Arrhenius constant, A (s^{-1})	Activation energy, E_a (kJ mol^{-1})	R^2
2	3.82×10^{14}	120.2	0.9987	7.23×10	39.7	0.9223
4	1.62×10^{15}	122.1	1.0000	3.18×10^{5}	65.9	0.9087
6	6.57×10^{16}	133.1	0.9966	1.02×10^{7}	76.8	0.8988

The activation energy for sugars production of the current work was close to that reported by Kumoro *et al.* (2008) for sago waste hydrolysis with dilute sulfuric acid. When compared to other researchers' works employing different biomass, sago waste employed in this study had a lower activation energy to produce reducing sugars. Dilute acid hydrolysis of corn stover, cellulose and Douglas fir were reported to have activation energy of 171.6 kJ mol^{-1} (Bhandari *et al.*, 1984), 151.5 kJ mol^{-1} (Girisuta *et al.*, 2007) and 179.5 kJ mol^{-1} (Saeman, 1945). The differences could be attributed to the variation in structural and chemical compositions of lignocellulosic biomass employed (Canettieri *et al.*, 2007; Ranganathan *et al.*, 1985). In sago waste, approximately 40% of its total carbohydrates is starch and the remaining content comprised of cellulose and hemicelluloses. Moreover, the branched polymer of starch is more readily to be hydrolysed compared to the densely packed cellulose. Thus, starchy lignocellulosic biomass like sago waste is more susceptible to hydrolysis reaction and requires less activation energy for sugars production.

Similarly, lower activation energy was needed to trigger sugar degradation compared to other activation energies reported for sugar degradation. For instance, Saeman (1945) reported an activation energy of 139.9 kJ mol^{-1} for sugar degradation and 152.2 kJ mol^{-1} was reported by Girisuta *et al.* (2007). Despite that the undesirable

lower activation energy of sugar degradation, the rate constant between 0.0002 min^{-1} and 0.0026 min^{-1} in this study indicates a slow sugar degrading rate. It was estimated that the sugars produced would take 270 min (4.5 h) to 3136 min (52 h) to degrade into undesirable sugars products as compared to 53 min reported in Girisuta's work (Girisuta *et al.*, 2007). The slow sugar degradation rate in this study suggests that the problem of sugar degradation at high temperature encountered in sulfuric acid saccharification can probably be resolved by employing solid acid saccharification on ionic liquid dissolved carbohydrates. With such employment not only it could improve the production of sugars from biomass, but also assist the subsequent fermentation as the inhibitory effect on microorganism by degraded sugar products is minimised (Delgenes *et al.*, 1996; Kootstra *et al.*, 2009; Larsson *et al.*, 1999; Sreenath & Jeffries, 2000; Taherzadeh *et al.*, 1999).

To investigate the effect of catalyst loading on the rate constant, different catalyst loadings were applied in the saccharification reaction. As mentioned earlier in Section 4.1.2, ionic liquid induced the release of H^+ from the SO_3H functional groups of A15 acid catalyst. Since the mobile H^+ ions released into the hydrolysate are the ones responsible for hydrolysis reaction, the effect of catalyst loading on the rate constant was investigated based on the concentration of mobile H^+ ions in the hydrolysate. The concentration of H^+ ions released was determined by measuring the pH of the hydrolysate, which can be used to calculate the amount of H^+ ions in the hydrolysate and the percentage of H^+ ions released into the hydrolysate as shown in Table 4.12. The pH measurement was performed at room temperature with the assumption of negligible temperature influence.

Table 4.12: Amount and percentage of protons (H^+) released from A15 at various catalyst loadings

Catalyst loading (%, w/v)	Amount of acid sites [a] (mmol)	pH of hydrolysate	Amount of H^+ ions in hydrolysate (mmol)	Percentage of H^+ released [b] (%)
2	0.23	1.240	0.14	62.6
6	0.69	0.815	0.38	55.5
10	1.15	0.605	0.62	54.0

[a] Concentration of acid sites in Amberlyst 15 = 4.6 mmol/g
[b] Percentage of H^+ released = Amount of H^+ in hydrolysate / Amount of acid sites × 100

From the table, it shows that the amount of H^+ ions released was lower than the number of acid sites present in the catalyst. This implies that not all the H^+ bonded to the A15 acid catalyst were being released into the hydrolysate. The percentage of H^+ ions released into the hydrolysate decreased as the catalyst loading increased. This could be due to insufficient amount of ionic liquid in the hydrolysate to induce all the H^+ ions to be released from the acid sites at high catalyst loading. Since solid acid saccharification involves mobile H^+ ions present in the hydrolysate, concentration of H^+ ions in hydrolysate was considered in investigating the effect of catalyst loading on kinetic rate constants.

In Figure 4.16, logarithm k versus logarithm $[H^+]$ plot was used to correlate the acid concentration to rate constants of sugars production and sugar degradation. The kinetic parameter, m, that describes the effect of H^+ concentration towards rate constant was determined from the gradient of the graph (Equation (3.11)) and the values obtained at various operating conditions are tabulated in Table 4.13. All the m values were less than 2, which are comparable with those reported values in the literatures (Lu & Mosier, 2008; Saeman, 1945). The m values for k_2 of this study were lower compared to the corresponding value of k_1 indicating the investigated catalyst loadings did not

affect the sugar degradation behavior to a kinetically significant level as compared to the sugars production reaction.

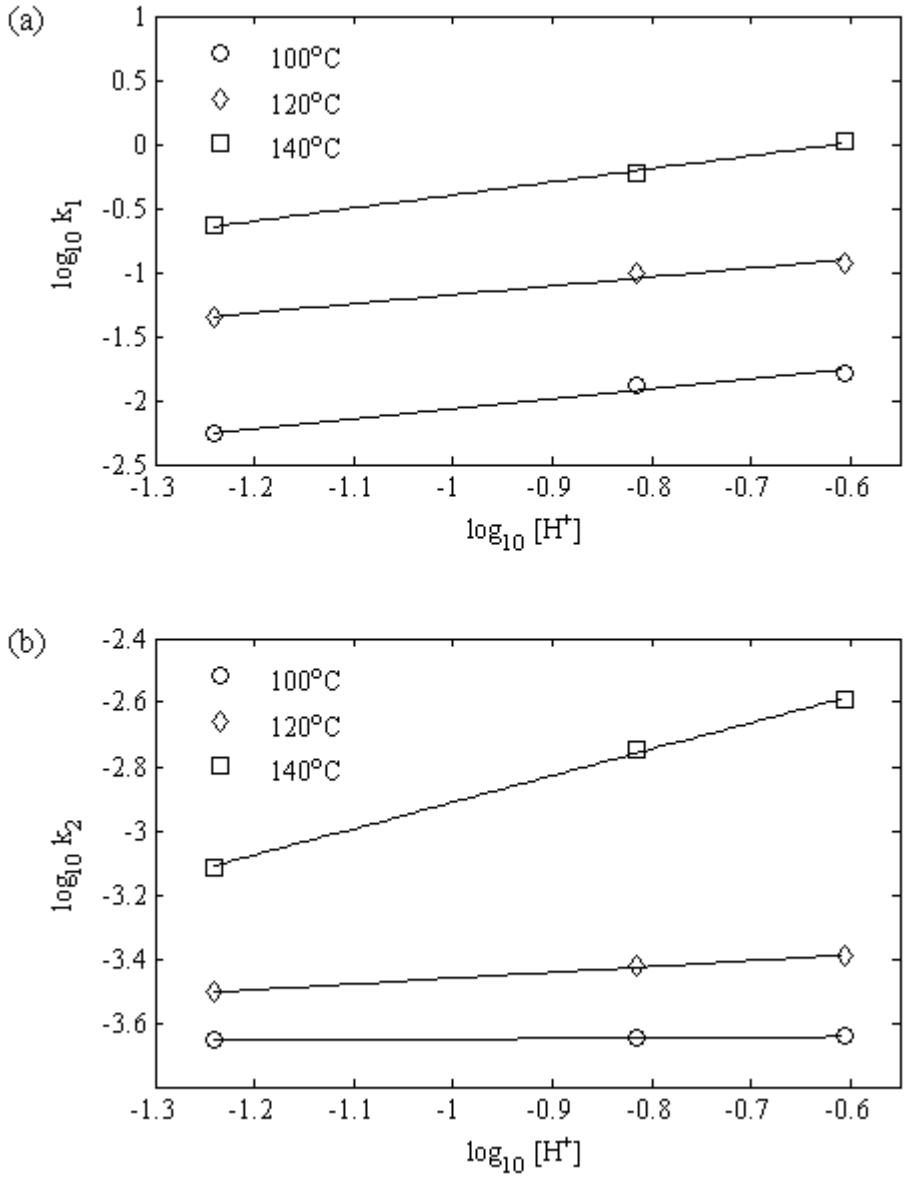

Figure 4.16: Logarithmic plots of rate constant versus $[H]^+$ concentration at various temperatures for (a) rate constant of reducing sugars production, k_1; and (b) rate constant of sugar degradation, k_2.

Table 4.13: The m values for rate constants of sugars production and rate constants of sugar degradation at different temperatures

Temperature	Sugars production reaction		Sugar degradation reaction	
(^{o}C)	m	R^2	m	R^2
100	0.7732	0.9789	0.0202	0.9284
120	0.6973	0.9741	0.1814	0.9931
140	1.0239	0.9976	0.8267	0.9989
Average	0.8315		0.3428	

4.3.3 Empirical equations for rate constants of reducing sugars production and degradation

There were interactive effects between temperature and catalyst loading as discussed previously. Thus their individual effect was incorporated in a single empirical equation to represent the rate constants of sugars production and degradation. Average values of the activation energies (Table 4.11) and m (Table 4.13) were used in expressing the empirical equation. And the new correlation constant, *W'* (Equation (3.12)) of the empirical equation was determined using MATLAB. In this study, the rate constant of reducing sugars production, k_1 and rate constant of sugar degradation, k_2 are respectively expressed in Equation (4.3) and (4.4).

$$k_1 = 1.88 \times 10^{16} e^{-125.1/\mathrm{RT}} [\mathrm{H}^+]^{0.83} \quad (4.3)$$

$$k_2 = 1.24 \times 10^{5} e^{-60.8/\mathrm{RT}} [\mathrm{H}^+]^{0.34} \quad (4.4)$$

where R is the universal gas constant expressed in kJ mol^{-1} K^{-1}, T is absolute saccharification temperature, and [H^+] is the concentration of protons available for saccharification. These equations can be used to predict the reaction rate within the range of study for temperature and catalyst loading to facilitate the design of reactor.

4.4 Product Separation and Catalysts Recovery

The sequential ionic liquid dissolution-solid acid saccharification process were proven to be effective in producing reducing sugars from sago waste. The feasibility of the process can be enhanced if the ionic liquid and the solid acid catalyst were recovered and recycled. In this section, separation and recovery of the ionic liquid and reducing sugars, as well as recyclability of solid acid catalyst were evaluated.

4.4.1 Reusability of solid acid catalyst

To recycle the solid acid catalyst, spent A15 was first regenerated with sulfuric acid. Figure 4.17 compares the saccharification performance of the regenerated A15 and non-regenerated A15. The evaluation criterion was based on the reducing sugars yield in the hydrolysate of solid acid saccharification. It can be seen from the figure that the saccharification performance of regenerated A15 was almost the same as fresh A15 having it recycled for three times. However, the catalytic performance of non-regenerated A15 declined after every saccharification cycle. This finding agreed with the result reported by Rinaldi and his co-authors (Rinaldi *et al.*, 2010b). The regeneration process applied in this study was able to regain the saccharification performance of A15. This is done through the mechanism involving the ionic liquid cations that attach to the SO_3^- groups which is displaced by the H^+ ions from the sulfuric acid to retrieve the sulfonic groups. In short, the solid acid catalyst can be recycled many times as long as it is regenerated with sulfuric acid as the ionic liquid cations and the protons at the sulfonic group is exchangeable to regain the functional group of A15 in each regeneration step.

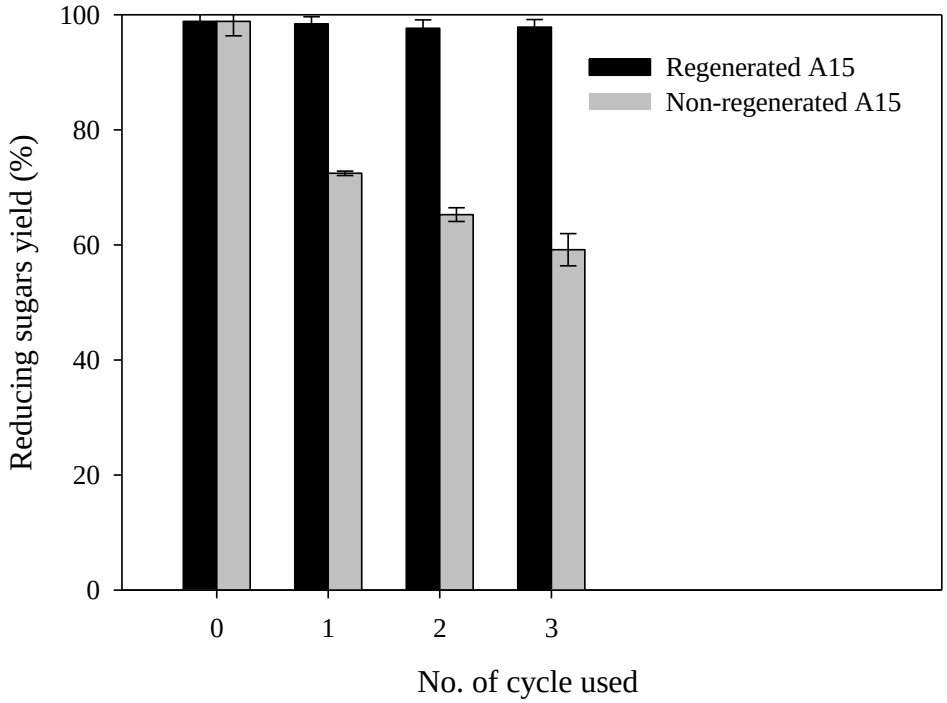

Figure 4.17: Reducing sugars yield from sago waste by regenerated A15 and non-regenerated A15. *Reaction conditions:* dissolution at 160^{o}C, 1.75 h with 1.5% (w/v) substrate loading; saccharification at 130^{o}C, 0.5 h with 4% (w/v) catalyst loading.

4.4.2 Separation and recovery of ionic liquid and reducing sugars

Ionic liquid and reducing sugars in the hydrolysate can be separated using aqueous biphasic system (ABS). The system involves short processing time, consumed low energy, and it is environmental friendly, and also relatively reliable in scaling up (Hatti-Kaul, 2000). Therefore, ABS is an economical, efficient downstream-processing method. This system could be formed by adding a kosmostropic salt such as potassium phosphate (K_3PO_4), potassium hydrogen phosphate (K_2HPO_4) and potassium carbonate (K_2CO_3) into aqueous ionic liquid solution. Anions of the kosmostropic salt have a stronger interaction with water molecules than those between water molecules, causing a phase separation to occur.

In this study, 80% (w/w) K_3PO_4 solution was used to create an ABS to separate the ionic liquid and reducing sugars from the hydrolysate. K_3PO_4 was selected in view

of its good phase separation ability. In the ABS system, ionic liquid was concentrated in the upper phase while reducing sugars gathered at the lower phase of the ABS. The separation performance of the ABS is presented in Table 4.14.

Table 4.14: Efficiency of ionic liquid and reducing sugars recovery in aqueous biphasic system

No. of extraction, *i*	**[BMIM]Cl recovery**		**Reducing sugars recovery**	
	In *i* extraction step (%E_i)	**Total (%*E*)**	**In *i* extraction step (%E_i)**	**Total (%*E*)**
1	39.6 ± 1.1	39.6	94.3 ± 0.1	94.3
2	16.1 ± 1.8	49.3	96.9 ± 0.2	99.8
3	20.5 ± 4.2	59.7	-	99.8

Total of three liquid-liquid extractions involving ABS had been applied to recover the ionic liquid and reducing sugars from the hydrolysate. In the first extraction step, 39.6% of [BMIM]Cl was recovered (Table 4.14). The ionic liquid recovery efficiency obtained was lower compared to those reported in the literatures, ranging between 60% and 75% (Wu *et al.*, 2008b). Apart from the salt used in the ABS, type of ionic liquid would influence the separation performance. This could have contributed to the difference in ionic liquid recovery efficiencies in different studies. Besides, the concentrations of ionic liquid and salt also play important roles in the ionic liquid recovery efficiency of ABS. In the reported literature, various concentrations of ionic liquid solutions were used, indicating that the salt solution concentration was manipulated according to the targeted concentration of ionic liquid. However, the ionic liquid solution used in this study was resulted from the dissolution-saccharification reaction at fixed concentration, thus a fixed concentration of potassium phosphate solution was used. Due to the constraint of applying different concentrations of potassium phosphate solution to the system, the recovery efficiency of [BMIM]Cl was

not high. Thus, a multiple step ABS was attempted to enhance the recovery of [BMIM]Cl. After three extraction steps, 59.7% [BMIM]Cl was obtained and the ionic liquid recovery efficiency is comparable to those reported work (Wu *et al.*, 2008b).

Besides ionic liquid, a good recovery of reducing sugars was also obtained from the ABS. As high as 94.3% of reducing sugars was recovered at the bottom phase of the ABS after the first extraction step. The total reducing sugars recovery was 99.8% in the ABS applied in this study. This signifies the adoption of multiple step ABS to separate and recover the [BMIM]Cl and reducing sugars from the hydrolysate is viable.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

A sequential ionic liquid dissolution-solid acid saccharification process scheme employed in this study produced reducing sugars from the sago waste effectively. This process is also feasible as the ionic liquid and solid acid catalyst used can be recovered and recycled to minimise the chemical cost. The conclusions of the findings are as follow:

i. The high content of carbohydrates in sago waste as determined from the characterisation study showed that sago waste is a good feedstock for reducing sugars production. More than 60% of reducing sugars yield was obtained via sequential scheme of ionic liquid dissolution-solid acid saccharification. The effectiveness of the scheme is attributed to the compatibility of the ionic liquid, 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) with the solid acid catalyst, Amberlyst 15 (A15). This combination also involved the lowest dissolution energy of 3.04 kJ/g sago waste compared to other ionic liquids. The combination also had the lowest chemicals costs compared to other ionic liquids and solid acid catalysts investigated in this study.

ii. The sequential ionic liquid dissolution-solid acid saccharification gave optimum reducing sugars yield of 98.3% at dissolution conditions of 160^{o}C, 1.75 h and 1.5% substrate loading, and saccharification conditions of 130^{o}C, 0.5 h and 4% catalyst loading. The yield obtained in this work was 55% to

74% higher than that reported for the single step reaction of ionic liquid dissolution-solid acid saccharification, 80% higher than the solid acid saccharification, and 18% and 8% higher than the conventional acid and enzymatic saccharification, respectively. The sequential process was modeled into quadratic polynomial equations with good reducing sugars yield prediction as the error between the predicted and experimental values was only 0.6%.

iii. Kinetic study of the ionic liquid-mediated solid acid saccharification confirmed that the reaction is a first order reducing sugars production-first order sugar degradation reaction. The hydrolysis reaction for reducing sugars production was favored over the competing sugar degradation reaction, in which the rate constants for sugars production were more than 25 times higher than the rate constants for sugar degradation reaction. Higher saccharification temperature and catalyst loading resulted in higher reactions rates. Comparatively, temperature had greater effect in both of sugars production and degradation rates than catalyst loading.

iv. A three-step aqueous biphasic system employed in this study successfully recovered 60% of [BMIM]Cl and nearly 100% of reducing sugars. Meanwhile, the regenerated A15 restored its saccharification efficiency back to almost 100% for each cycle.

5.2 Novelties and Implications of Study

This work has provided several insights into reducing sugars production via sequential ionic liquid dissolution-solid acid saccharification of sago waste. The novelties and implications of the study are as highlighted:

i. This is the first comprehensive study that reports the use of sago waste as feedstock for reducing sugars production. The reducing sugars produced can be a useful feedstock for biochemicals and biofuels production. Moreover, utilisation of sago waste in reducing sugars production also mitigates waste disposal problem created by sago waste.

ii. This work reports a novel process that uses ionic liquid and solid acid catalyst to convert sago waste to reducing sugars. The comprehensive study via comparing the suitability of various combinations of ionic liquid and solid acid catalyst in saccharification is most helpful which enables the selection of compatible combination of ionic liquid and solid acid catalyst to produce reducing sugars from lignocellulosic biomass effectively.

iii. Process optimisation and kinetic study of the sequential ionic liquid dissolution-solid acid saccharification to produce reducing sugars from sago waste have not been reported elsewhere. These results are useful for process optimisation and also reactor design for large scale production of reducing sugars from sago waste.

iv. This study provides means of product recovery and recyclability to improve the feasibility of the process scheme. The current work reports a novel idea

of simultaneous recovery of ionic liquid and reducing sugars in a multistep ABS. The multistep ABS was effective for reducing sugars and ionic liquid recoveries. Regenerated solid acid catalyst can be recycled several times with good saccharification performance in each cycle used.

5.3 Recommendations for Future Work

There are potential areas related to the production of reducing sugars via sequential ionic liquid dissolution-solid acid saccharification that can be explored further. The recommendations are:

i. The sequential ionic liquid dissolution-solid acid saccharification can be scaled up to produce reducing sugars at a larger scale for industrial applications.

ii. Mass transfer and heat transfer during ionic liquid dissolution and solid acid saccharification are worth investigating to improve the reaction rate.

iii. To test the applicability of the process scheme for other lignocellulosic biomass such as sugarcane baggase and corn stover in reducing sugars production.

iv. The sequential ionic liquid dissolution-solid acid saccharification in this study involved two separated reactions, and the employment of two catalysts. Exploration on a single catalyst with capability of dissolving and saccharifying lignocellulosic biomass to reducing sugars is worth investigating.

v. Detailed economic analysis can be performed to examine the feasibility of the sequential ionic liquid dissolution-solid acid saccharification in the production of reducing sugars from sago waste.

vi. Further investigation on enhancing the ionic liquid recovery is worth conducted to make the overall process more economically feasible.

LIST OF PUBLICATIONS AND PAPERS PRESENTED

Technical Papers

1. Lee, K.M., Ngoh, G.C., Chua, A.S.M., Yoon, L.W., Ang, T.N. & Lee, M.G. (2014). Comparison study of different ionic liquid pretreatments in maximizing total reducing sugars recovery. *BioResources*, 9(1), 1552-1564. (ISI cited publication, Tier 1 in the category of Material Science, Paper & Wood with impact factor of 1.549).
2. Lee, K.M., Ngoh, G.C. & Chua, A.S.M. (2013). Process optimization and performance evaluation on sequential ionic liquid dissolution-solid acid saccharification of sago waste. *Bioresource Technology*, 130, 1-7. (ISI cited publication, Tier 1 in the category of Energy & Fuels with impact factor of 5.039).
3. Ang, T.N., Yoon, L.W., Lee, K.M., Ngoh, G.C., Chua, A.S.M. & Lee, M.G. (2011). Efficiency of ionic liquids in the dissolution of rice husk. *BioResources*, 6(4), 4790-4800. (ISI cited publication, Tier 1 in the category of Material Science, Paper & Wood with impact factor of 1.549).

Conference Papers (Proceedings)

1. Lee, K.M., Ngoh, G.C. & Chua, A.S.M. (2014). *Solid acid saccharification of ionic liquid dissolved sago waste for the production of reducing sugars. Proceeding of the Regional Conference on Chemical Engineering 2014*, Yogyakarta, 2nd – 3rd December.
2. Lee, K.M., Ngoh, G.C. & Chua, A.S.M. (2013). *Sago waste dissolution using ionic liquid 1-butyl-3-methylimidazolium chloride for reducing sugars production. Proceeding of the 6th Regional Conference on Chemical Engineering*, Century Park Hotel, Manila, Philippines, 2nd – 3rd December.

3. Lee, K.M., Ngoh, G.C. & Chua, A.S.M. (2010). *Incorporation of ionic liquid pretreatment and solid acid catalyst in hydrolysing lignocellulosic biomass. Proceedings of the 13th Asia Pacific Confederation of Chemical Engineering Congress 2010*, Howard International House, Taiwan, 5th – 8th October.
4. Ngoh, G.C., Ang, T.N., Yoon, L.W., Lee, K.M. & Chua, A.S.M. (2013). *Assessment of the effect of ionic liquid pretreatments on various lignocellulosic biomasses. Proceedings of the 6th Regional Conference on Chemical Engineering*, Century Park Hotel, Manila, Philippines, 2nd – 3rd December.
5. Ang, T.N., Yoon, L.W., Lee, K.M., Ngoh, G.C., Chua, A.S.M. & Lee, M.G. (2010). *Application of ionic liquids in the regeneration rice husk cellulose. Proceedings of the 13th Asia Pacific Confederation of Chemical Engineering Congress 2010*, Howard International House, Taiwan, 5th – 8th October.

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APPENDIX

A1: Analysis of variance (ANOVA) on the reducing sugars yield in [BMIM]Cl prehydrolysate

Table A 1: ANOVA on the reducing sugars yield in the [BMIM]Cl prehydrolysate

Source of variation	Sum of square (SS)	Degree of freedom (DF)	Mean square (MS)	F value	95% confidence level
Duration	0.6744	5	0.1349	16.8311	Significant
Batch	0.0472	2	0.0236	2.9473	Not significant
Error	0.0801	10	0.0080		
Total	0.8017	17			

At 95% confidence level,

$F_{0.05,5,10} = 3.33$

$F_{0.05,2,10} = 4.10$

Dissolution duration has a significant effect on reducing sugars yield in the [BMIM]Cl prehydrolysate but batch-to-batch difference is insignificant.

A2: Analysis of variance (ANOVA) on the reducing sugars yield in [EMIM][OAc] prehydrolysate

Table A 2: ANOVA on the reducing sugars yield in the [EMIM][OAc] prehydrolysate

Source of variation	**Sum of square (SS)**	**Degree of freedom (DF)**	**Mean square (MS)**	**F value**	**95% confidence level**
Duration	0.0191	5	0.0038	6.3040	Significant
Batch	0.0016	2	0.0008	1.3048	Not significant
Error	0.0061	10	0.0006		
Total	0.0268	17			

At 95% confidence level,

$F_{0.05,5,10} = 3.33$

$F_{0.05,2,10} = 4.10$

Dissolution duration has a significant effect on reducing sugars yield in the [EMIM][OAc] prehydrolysate but batch-to-batch difference is insignificant.

A3: Analysis of variance (ANOVA) on the reducing sugars yield in [EMIM][$(EtO)_2PO_2$] prehydrolysate

Table A 3: ANOVA on the reducing sugars yield in the [EMIM][$(EtO)_2PO_2$] prehydrolysate

Source of variation	**Sum of square (SS)**	**Degree of freedom (DF)**	**Mean square (MS)**	**F value**	**95% confidence level**
Duration	0.3018	5	0.0604	14.7740	Significant
Batch	0.0083	2	0.0042	1.0221	Not significant
Error	0.0409	10	0.0041		
Total	0.3510	17			

At 95% confidence level,

$F_{0.05,5,10} = 3.33$

$F_{0.05,2,10} = 4.10$

Dissolution duration has a significant effect on reducing sugars yield in the [EMIM][$(EtO)_2PO_2$] prehydrolysate but batch-to-batch difference is insignificant.

A4: Analysis of variance (ANOVA) on the reducing sugars yield in hydrolysate

Table A 4: ANOVA on the reducing sugars yield in the hydrolysate

Source of variation	**Sum of square (SS)**	**Degree of freedom (DF)**	**Mean square (MS)**	**F value**	**95% confidence level**
Dissolution	11364.25	2	5682.1230	9154.38	Significant
Saccharification	43.2474	2	21.6237	34.8376	Significant
Interaction	117.8678	4	29.4700	47.4737	Significant
Error	5.5863	9	0.6207		
Total	11530.95	17			

At 95% confidence level,

$F_{0.05,2,9} = 4.26$

$F_{0.05,4,9} = 3.63$

The main effects of ionic liquid and solid acid catalyst have significant effect on reducing sugars yield in the hydrolysate. There is also a significant interaction between the ionic liquid and solid acid catalyst.

A5: Analysis of variance (ANOVA) on the reducing sugars yield from enzymatic saccharification of ionic liquid pretreated sago waste solid residues

Table A 5: ANOVA on the reducing sugars yield from enzymatic saccharification of ionic liquid pretreated sago waste solid residues

Source of variation	**Sum of square (SS)**	**Degree of freedom (DF)**	**Mean square (MS)**	**F value**	**95% confidence level**
Treatment	46.7929	2	23.3964	12.3008	Not significant
Batch	3.7444	1	3.7444	1.9687	Not significant
Error	3.8041	2	1.9020		
Total	54.3414	5			

At 95% confidence level,

$F_{0.05,2,2} = 19.00$

$F_{0.05,1,2} = 18.51$

Ionic liquid pretreatment has not significant effect on reducing sugars yield in the enzymatic saccharification of the sago waste pretreated solid residues. Batch-to-batch difference is insignificant during reducing sugars production.

A6: Analysis of variance (ANOVA) on the reducing sugars yield at different dissolution temperature

Table A 6: ANOVA on the reducing sugars yield at different dissolution temperature

Source of variation	**Sum of square (SS)**	**Degree of freedom (DF)**	**Mean square (MS)**	**F value**	**95% confidence level**
Temperature	1074.2560	2	537.1278	86.3000	Significant
Batch	0.0929	1	0.0929	0.0149	Not significant
Error	12.4479	2	6.2240		
Total	1086.7960	5			

At 95% confidence level,

$F_{0.05,2,2} = 19.00$

$F_{0.05,1,2} = 18.51$

Dissolution temperature has significant effect on reducing sugars yield but batch-to-batch difference is insignificant.

A7: Analysis of variance (ANOVA) on the reducing sugars yield at different dissolution time

Table A 7: ANOVA on the reducing sugars yield at different dissolution time

Source of variation	**Sum of square (SS)**	**Degree of freedom (DF)**	**Mean square (MS)**	**F value**	**95% confidence level**
Time	2103.8260	7	300.5466	30.3335	Significant
Batch	5.2952	1	5.2952	0.5344	Not significant
Error	69.3566	7	9.9081		
Total	2178.4780	15			

At 95% confidence level,

$F_{0.05,7,7} = 3.79$

$F_{0.05,1,7} = 5.59$

Dissolution time has significant effect on reducing sugars yield but batch-to-batch difference is insignificant.

A8: Analysis of variance (ANOVA) on the reducing sugars yield at different substrate loading during the dissolution reaction

Table A 8: ANOVA on the reducing sugars yield at different substrate loading

Source of variation	**Sum of square (SS)**	**Degree of freedom (DF)**	**Mean square (MS)**	**F value**	**95% confidence level**
Substrate loading	370.8852	2	185.4426	74.1766	Significant
Batch	0.6600	1	0.6600	0.2640	Not significant
Error	5.0000	2	2.5000		
Total	376.5453	5			

At 95% confidence level,

$F_{0.05,2,2} = 19.00$

$F_{0.05,1,2} = 18.51$

Substrate loading has significant effect on reducing sugars yield during the dissolution reaction but batch-to-batch difference is insignificant.

A9: Analysis of variance (ANOVA) on the reducing sugars yield at different substrate particle size during the dissolution reaction

Table A 9: ANOVA on the reducing sugars yield at different substrate particle size

Source of variation	**Sum of square (SS)**	**Degree of freedom (DF)**	**Mean square (MS)**	**F value**	**95% confidence level**
Particle size	76.7167	2	38.3584	13.1170	Not significant
Batch	2.2448	1	2.2448	0.7676	Not significant
Error	5.8486	2	2.9243		
Total	84.8102	5			

At 95% confidence level,

$F_{0.05,2,2} = 19.00$

$F_{0.05,1,2} = 18.51$

Substrate particle size has no significant effect on reducing sugars yield during the dissolution reaction. Batch-to-batch difference is insignificant during reducing sugars production.

A10: Analysis of variance (ANOVA) on the reducing sugars yield at different saccharification temperature

Table A 10: ANOVA on the reducing sugars yield at different saccharification temperature

Source of variation	**Sum of square (SS)**	**Degree of freedom (DF)**	**Mean square (MS)**	**F value**	**95% confidence level**
Temperature	2862.3780	2	1431.1890	1699.8350	Significant
Batch	13.7241	1	13.7241	16.3003	Not significant
Error	1.6839	2	0.8420		
Total	2877.7860	5			

At 95% confidence level,

$F_{0.05,2,2} = 19.00$

$F_{0.05,1,2} = 18.51$

Saccharification temperature has significant effect on reducing sugars yield but batch-to-batch difference is insignificant.

A11: Analysis of variance (ANOVA) on the reducing sugars yield at different saccharification time

Table A 11: ANOVA on the reducing sugars yield at different saccharification time

Source of variation	**Sum of square (SS)**	**Degree of freedom (DF)**	**Mean square (MS)**	**F value**	**95% confidence level**
Time	3604.7150	7	514.9593	168.1763	Significant
Batch	59.1142	1	59.1142	19.3056	Significant
Error	21.4341	7	3.0620		
Total	3685.2630	15			

At 95% confidence level,

$F_{0.05,7,7} = 3.79$

$F_{0.05,1,7} = 5.59$

Saccharification time has significant effect on reducing sugars yield. Batch-to-batch difference is significant during the production of reducing sugars.

A12: Analysis of variance (ANOVA) on the reducing sugars yield at different catalyst loading during the saccharification reaction

Table A 12: ANOVA on the reducing sugars yield at different catalyst loading

Source of variation	**Sum of square (SS)**	**Degree of freedom (DF)**	**Mean square (MS)**	**F value**	**95% confidence level**
Catalyst loading	278.5093	2	139.2547	29.0960	Significant
Batch	1.2125	1	1.2125	0.2533	Not significant
Error	9.5721	2	4.7860		
Total	289.2939	5			

At 95% confidence level,

$F_{0.05,2,2} = 19.00$

$F_{0.05,1,2} = 18.51$

Catalyst loading has significant effect on reducing sugars yield during the saccharification reaction but batch-to-batch difference is insignificant.