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Pretreatment and Bioprocess Trials in Various Reactor System on Lignocellulosic Biomass for Cellulosic Biomaterials

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ABSTRACT

*Pretreatment on lignocellulosic biomass prior to extraction of biomaterials, degradation of bioproduct, or production of biomaterial/bioproduct/biofuel, crucially influences the intended outcomes. The pretreatment of oil palm fronds (OPF), one of the most abundant agriculture residues in Malaysia, can be conducted based on the need of the methodology, either for small, lab, pilot, or industrial scales. In this article, examples of reactors for the pretreatment for instance microreactor (Bioshake iQ), conical shake flask, and mini-cylindrical reactor scale (fabricated) as well as the monitoring bioreactor (BlueSens Monitoring GmbH) reactor system dedicated for fermentation process using the outcome material from pretreatment process, are presented. All pretreatment trials with ionic liquid (IL) of 1-ethyl-3-methylimidazolium acetate [EMIM]Ac on OPF were conducted with a scaling-up strategy from micro-to-macro to fabricated reactors, monitoring Crystallinity Index (CrI) and Lateral Order Index (*LOI*). Electron beam*

irradiation pretreatment using 1000kGy was also tested in macroscale mode for CrI and LOI. *Effectiveness of approximately 23 to 37% of CrI via microreactor experiments using 50, 70, and 90% v/v of [EMIM]Ac and at a temperature of 99°C was observed. Higher concentration of IL and temperature with nearly insignificance of solid loading of OPF in reaction liquid to the increase of the amorphous level of OPF was reported by macroscale mode in the 570-mL fabricated reactor. A short oxygen uptake rate (OUR) phase was observed in a 500-mL BlueSens shake flask with the real-time monitoring systems for 45-mL working volume, a nearly 10% of the total reactor volume for saccharification-fermentation using Escherichia coli K011 ATCC 55124 on approximately 2.22% w/v pretreated OPF from macroscale mode. Various data examples from these micro-to-macro scales including in a fabricated reactor system mode are crucially needed for further observations prior to pilot or industrial scales, needing a systematic data collection to be simulated and investigated in the future.*

Keywords: lignocellulosic, oil palm frond, electron beam irradiation, ionic liquid, scaling-up, pretreatment

INTRODUCTION

A number of various kinds of biomaterials or biofuel types can be extracted from the various blends of lignocellulosic biomass (LCB) which may become the current trend demand or emergingly developed [1], [2]. There are many types of LCB produced from oil palm industries like oil palm trunks (OPT), empty fruit bunch (EFB), oil palm fronds (OPF), oil palm or pressed fibers (OPFb), and oil palm shells (OPS) which are for instance highly prospective for the production of bioethanol [3]. The oil palm frond is abundantly found in Malaysia in estate plantations [4] and Malaysia is one of the largest producers and exporters of palm oil in the world of 47% from the world supply [3, 5]. In general, the estimated quantity of LCB types produced from oil palm trees was reported by [6] laying out for OPF, EFB, OPFb, OPS, and OPT total mass in 2007 with approximately 47,000, 18,000, 11,000, 4,500, and 11,000 kilotonnes, respectively.

The pretreatment process is a method to deconstruct lignocelluloses into cellulose, hemicellulose, and lignin which are contained in the oil palm

biomass. As reported in the literature about the compositions of these three main biopolymers, cellulose is the main component of almost approximately 40-60%. The reported compositions for OPF, for instance, are approximately 62.3%, 14.8%, 24.2%, and 1.8% for cellulose, lignin, hemicellulose, and extractives, respectively [6].

There are many methods for pretreatments, for instance mechanical [7, 8], chemical, physicochemical [9] pretreatment methods prior to enzymatic hydrolysis to convert the extracted cellulose from the pretreated biomass to biofuel. Few solvents are used in LCB pretreatment ranging from simply water [10], organic or inorganic solvents, to special synthesized-ionic liquid solvent [9, 11, 12]. These pretreatments can be conducted in various reactor systems depending on the volume size of the pretreatment reactions. The volume range follows the scaling up for biofuel strategy starting from the bench scale (small scale, micro to macroscale), pilot scale to industrial scale, in line with the traditional Chemical Process Industry (CPI) processes [13]. The micro and macro scaling definition may also depend on the readily available settings for reaction investigations. In this article, the small-scale trials are ranged from microreactor to microreactor modes for instance by using Bioshake iQ (Quantifoil Instruments GmbH) [9, 11, 12, 14, 15], or 250-mL sized shake flask and fabricated cylindrical reactor system, respectively.

There has been various research ventured in microscale particularly to allow effective optimisations or screening of the best methods for pretreatment methodology dealing with a variety of chemical components and various engineering parameters. The engineering parameters are for instance shaking frequency, shaking diameter, reaction volume, reactor volume, temperature, and pH. Macroscale may refer particularly to the reactor volume sizes from the range of 50 to 1000 ml of volume. This normally occurs for the enzymatic hydrolysis method in converting the cellulosic biomaterials to sugar content prior to the conversion of sugar to biofuel. Another example of macroscale BlueSens Monitoring GmbH system [16, 17] is a set of macro-scale shake flask monitoring sensor-like toolset to monitor the change of carbon dioxide (CTR) and oxygen transfer rate (OTR) for biological methods. These are crucial monitoring methods and can be part of the assessment strategies for gas-liquid mass transfer phenomenon for instance the out-of-phase phenomenon in a reaction system dealing with biological viscous fluid [18, 19, 20, 21, 22].

The lab-scale method is crucial prior to the pilot-scale or industrial-scale method for bigger size of productions. Lab-scale may range from 1L to 50L, while pilot-scale may start from 50L to 100L. The industrial-scale is defined by the reactor volume of more than 100L and up to 1000L or more for instance dealing with first as the demonstration and then later to commercial sale production [13].

METHODOLOGY

The trials on pretreatment of the LCB from the oil palm industry were conducted with the introduction of ionic liquid: 1-ethyl-3-methylimidazolium acetate ([EMIM]Ac) as the pretreatment solvent to the OPF biomass loading at certain engineering parameters in various reactor systems. The overview of the methodological trials is presented by the flowchart of work as shown in Figure 1.

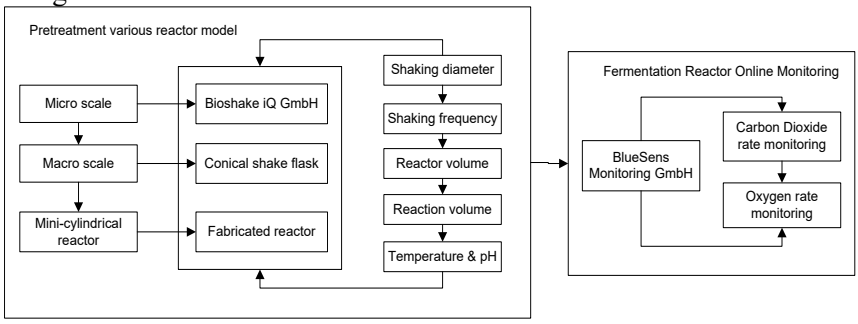

Figure 1: Schematic Flowchart for Various Trials/Reactor Strategies of Pretreatment of Lignocellulosic Biomass and the Fermentation Reactor Online Monitoring System

The pretreatment in microscale started with the dissolution of the OPF biomass loading with a certain concentration of the ionic liquid solvent [EMIM]Ac in Bioshake iQ (Quantifoil Instrument GmbH, Germany) at a shaking frequency of 1800 rpm. The shaking diameter of the setup was at 2.0 mm and the reactor volume was interchangeable according to the chosen reactor size for investigations. 2-mL microtube (centrifuge tube) containing 1.0 mL of reaction volume with OPF solid loading of (5% w/v of mg/µL) was placed in the Bioshake iQ holder with a controlled temperature set by Bioshake iQ at the temperature of within the range of 70 to 99°C. The dissolution time was from one to three hours for a variety of [EMIM]Ac concentrations of from 0 to 100% with an increment of only 10% difference.

Another microscale investigation was conducted in Bioshake iQ (Quantifoil Instrument GmbH, Germany) for the pretreated samples after the physiochemical pretreatment method using irradiation of electron beam. 0.25 gram of pretreated OPF were mixed in 5.0 mL of reaction volume (5% w/v of mg/μL) and preheated at 99°C for four hours with continuous shaking of 800 rpm of shaking frequency with the presence of ionic liquid [EMIM] Ac. The comparative impact of 1800 rpm and 800 rpm to both microscale investigations was assumed negligible in this study.

The macroscale trials were conducted in a shake flask on an ionic liquid pretreated sample from the microscale method. 10% of the total reactor volume shake flask was used as the working volume. Saccharification and fermentation process dealing with simultaneous hydrolysis producing sugar from cellulose and later towards the conversion of sugar to biofuel, namely as the one-pot configuration [23]. Certain microorganisms namely *Escherichia coli* K011 *(E. coli)* ATCC 55124, an ethanologenic bacteria was used to ferment carbon sources for instance cellulosic biomaterials to ethanol. Shake flask enzymatic hydrolysis was conducted at 37°C, at pH 7, and a shaking frequency of 250 rpm for 12 hours, on 45-mL working volume. BlueSens monitoring system GmbH (BlueSens), was later used with a chosen 500-mL shake flask with the monitoring systems for 45-mL reaction working volume (giving approximately 10% from the total reactor volume). Inoculum introduction was conducted and introduced to a specific media [24] in order to allow saccharification to occur. In BlueSens, the online monitoring system, recorded oxygen transfer rate (OTR) and carbon dioxide transfer rate (CTR) were collected, in an engineering parameter of 37°C and at 250 rpm for 24 hours.

An extended macroscale pretreatment on OPF was investigated in a mini-cylindrical reactor (fabrication). The pretreatment methodology by using ionic liquid solvent was conducted in a fabricated reactor design with a reactor volume of approximately 570 mL. The fabricated reactor has a working pressure of a maximum of 150 psi and a working temperature range of 0 to 130°C. The working volume used was approximately 12% of the total reactor volume. An OPF biomass loading of 10% (w/v) with a 40% (v/v) of [EMIM]Ac was investigated for three hours of reaction temperature of 110°C.

For each of the research, trials pretreated biomass was analysed for crystallinity index by using X-Ray Diffraction equipment (Rigaku Rint 2500, Japan). The crystallinity index (*CrI*) was calculated based on the calculations of the parameter from X-Ray diffraction measured plots, as shown in Equation 1. Lateral Order Index (LOI) was also recorded using Equation 2, applying the results obtained on the pretreated biomass from Fourier Transform Infrared (FTIR, Perkin Elmer, USA).

$$CrI = \left(I_{002} - I_{\text{am}}\right) \times 100 \tag{1}$$

$$LOI = \frac{A_{1430}}{A_{898}} \tag{2}$$

$$\%\,\text{of effectiveness} = \frac{CrI_{\text{untreated}} - CrI_{\text{treated}}}{CrI_{\text{untreated}}} \tag{3}$$

where *CrI* is the crystallinity index [-] and LOI is the lateral order index [-]. The other quantities such as I_{002} is the intensity of crystalline 002 peak at $2\theta \approx 22.5°$, I_{am} is the intensity of diffraction of the non-crystalline material taken at $2\theta \approx 18.5°$ in the valley between the peaks 002 and 101, A_{1430} and A_{898} are the absorption from FTIR at wavelength of 1430 nm and 898 nm, respectively, and $CrI_{untreated}$ and $CrI_{treated}$ are the index of raw LCB and treated LCB, accordingly.

RESULTS AND DISCUSSION

The Crystallinity Index (*CrI*) and Lateral Order Index (LOI) are the used parameters to indicate the effectiveness of the microscale pretreatment of LCB. *CrI* and LOI values are higher for the untreated OPF biomass and become lower as pretreatment becomes more effective. For the untreated OPF, the intensity counts for the I_{am} is way lower than the I_{002}. I_{am} indicates the intensity of the amorphous level of cellulosic OPF which is initially very low due to the existing high crystalline structure in raw LCB (untreated). The pretreatment allows the increase of the amorphous level of the cellulose content of the overall LCB, reducing the crystalline structure total percentage. Table 1 tabulates the *CrI* and LOI for the chosen examples of OPF pretreatment trials with [EMIM]Ac (v/v) percentage concentration of 70% and 90%, at an operating temperature of 99°C and operating time of three hours. The *CrI* and LOI values for the raw untreated OPF are 0.47 and

1.10. It is shown that the effectiveness calculated based on *CrI* is 23.4% and 36.2% with the effect of [EMIM]Ac percentage concentration (v/v) of 70% and 90%, respectively. This indicates that with a higher ionic liquid volume percentage, the amorphous level of the cellulose contents becomes higher.

Table 1: Crystallinity Index (*CrI*) Trial Data Results on Oil Palm Frond (OPF) in Microscale in Bioshake iQ for a Three-Hour Operating Time

Specification	Value	*CrI* [-]	LOI (after three hours)	Effectiveness on *CrI* [-]
Temperature [°C]	99	0.36, 0.30	0.58, 0.26	23.4, 36.2
[EMIM]Ac (v/v) [%]	70, 90			
Temperature [°C]	99	0.50 CrI_o = 0.68	0.53 LOI_o = 0.65	26.5
[EMIM]Ac (v/v) [%], Irradiation dose [kGy]	50, 1000			

Notes: [EMIM]Ac = 1-ethyl-3-methylimidazolium acetate

The preliminary microscale investigations of the pretreatment of the LCB, OPF residues, using Bioshake iQ (QInstrument GmbH) resulted in extended investigations in a fabricated cylindrical reactor for the macroscale pretreatment of the LCB, as shown as in Figure 2A and B. Figure 2A, the 570-mL sized-cylindrical reactor with an approximately 100-mm diameter and 72-mm height (excluding reactor thickness) was designed with a water jacket, a mounted impeller motor, and a replaceable reactor baffle. The operating temperature of 0 to 130°C and a maximum pressure of 150 psi, respectively, with circulating tap water as a cooling system, were designed. The mounting was attached by using ¼ inch National Pipe Thread (NPT) to the base of the top reactor.

Examples of results for the Crystallinity Index (*CrI*) from a macroscale pretreatment of OPF in the fabricated 570-mL reactor are tabulated in Table 2. It is indicated that at a slightly higher operating temperature of 110°C in comparison to 90°C, as well as a higher [EMIM]Ac volume percentage from 40% to 60%, the *CrI* values are shown to decrease only 3.3% for 5% (w/v) OPF loading in reaction volume containing [EMIM]Ac. However,

a higher OPF biomass loading of 15% (w/v) even at a higher temperature and higher [EMIM]Ac volume concentration, does not give so much significant effect to the *CrI*. It was predicted that somehow with higher temperature and higher solvent volume concentration, would increase the amorphous levels of the cellulosic content in OPF after pretreatment. Table 1 also indicates the pretreatment effectiveness for the pretreatment using 50% (v/v) of [EMIM]Ac in Bioshake iQ for the pretreated electron beam irradiation of 1000 kGy at the temperature of 99°C, which was observed to have an approximately 3% higher of effectiveness value against 70% (v/v) [EMIM]Ac at 99°C for three hours.

Finally, the macroscale trials on the washed pretreated OPF from microscale and macroscale fabricated reactor systems were conducted by using BlueSens. The CTR and OTR indicator example of results are shown in Figure 3. BlueSens monitors the concentration of oxygen and carbon dioxide content in the 250-mL BlueSens shake flask throughout the 24-hour experiments.

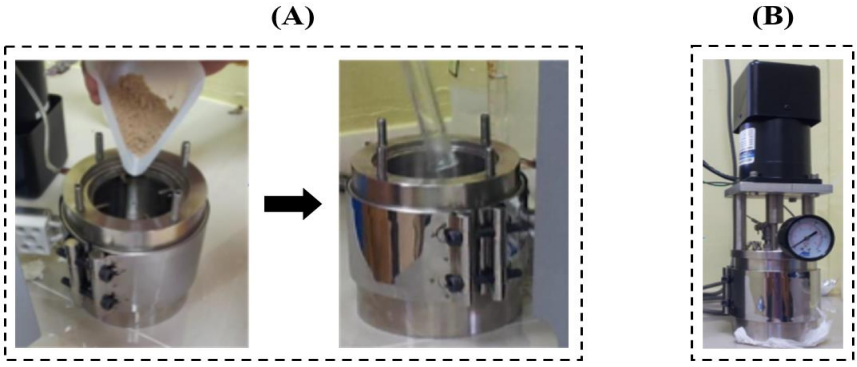

Figure 2: Fabrication Details (A & B) on the Macroscale Cylindrical Reactor for Pretreatment Method (Source by Author)

Table 2: Crystallinity Index (*CrI*) Trial Data Results on Oil Palm Frond (OPF) in Macroscale in Fabricated Cylindrical Reactor for a Three-Hour Operating Time

Specification	Value [-]	(w/v) [-]	*CrI* (after three hours) [-]
Temperature [°C]	110	5, 15	0.59, 0.57
[EMIM]Ac [%]	60		
Temperature [°C]	90	5, 15	0.61, 0.54
[EMIM]Ac [%]	40		

Notes: [EMIM]Ac = 1-ethyl-3-methylimidazolium acetate

Figure 3A indicates that as the oxygen concentration is lowered, the carbon dioxide concentration increases. This is due to the increasing growth of *Escherichia coli* K011 *(E. coli)* ATCC 55124 during the fermentation in the fermentation media in BlueSens online monitoring shake flask. As the cell growth increases and the cell replicates, the oxygen consumption for E. coli also increases. This occurs starting at the sixth hour of the fermentation, two hours after the inoculum introduction to the fermentation media in BlueSens. The carbon dioxide emission rate (CER) and oxygen uptake rate (OUR) indicated also started to be exponentially observed starting at the sixth hour. The exponential growth remained for approximately two hours prior to the stationary growth for as long as six hours before it reached the death rate region from the 14th hour onwards. The short exponential region is expected due to the low pretreated OPF loading being fermented in a specific *E. coli* fermentation media [24].

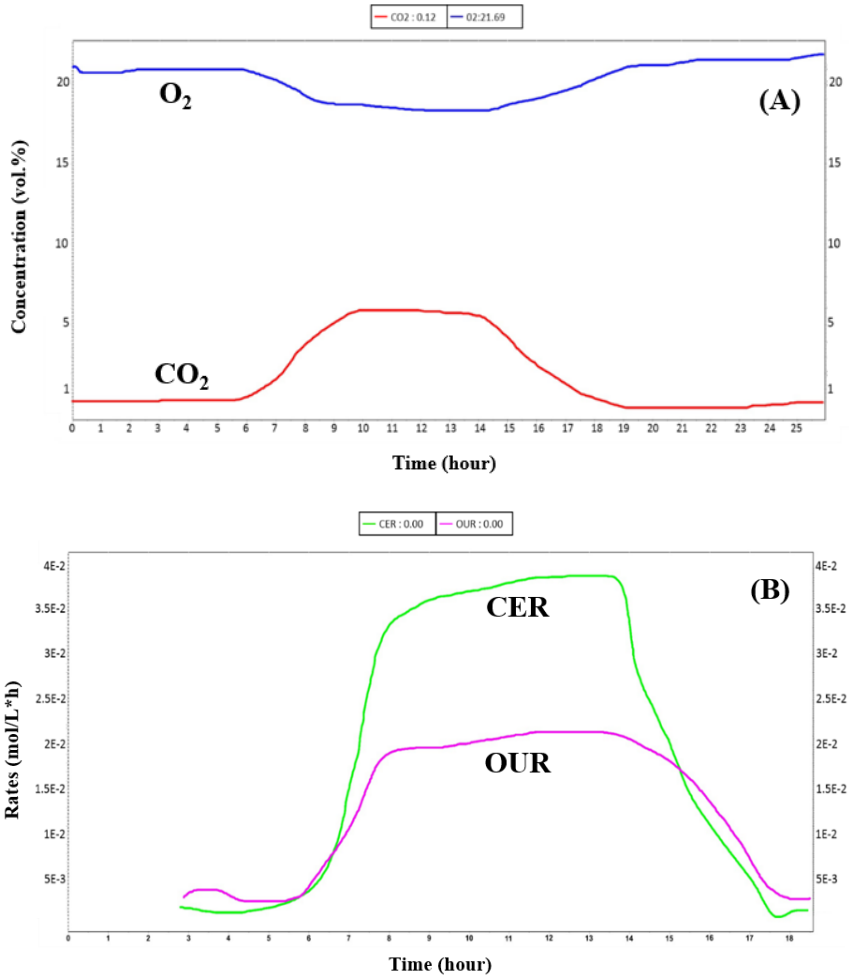

Figure 3A and 3B: Concentration of Carbon Dioxide (Red) and Oxygen (Blue) (in 3A) as well as Carbon Dioxide Emission Rate (Green: CER) with Oxygen Uptake Rate (Pink: OUR) (in 3B) of the Fermentation Activity of the Reaction Fermentation Enriched with Washed Pretreated OPF, Respectively

CONCLUSION

The microscale to macroscale pretreatment reactor system is the crucial step towards better strategies during upscaling for biofuel for instance on OPF. The microscale is mainly significant for various optimisation trials with a variety of engineering parameters allowing a better overview of the effectiveness of the solvent pretreatment via analytical crystallinity index values, particularly dealing with ionic liquid which maybe still expensive and yet to be cost-effective for current industrial-scale expectations. The BlueSens online monitoring steps ensure the reliability of fermentation data collections dealing with cell biomass recording in line with the gas-liquid mass transfer occurrences. Towards better strategies of the pretreatment of LCB, technological advancement of various reactor systems is highly recommended for biofuel investigations.

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