Organic acid pretreatment of oil palm trunk biomass for fermentable xylose production

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Abstract. Green alternatives to chemical pretreatment in hydrolysing hemicellulose for efficient biorefineries are very much sought-after. In this study, we aimed to determine the effectiveness of several organic acids in hydrolysing hemicellulose of oil palm trunk biomass (OPTB) to form fermentable xylose. Various organic acids (citric acid, formic acid and oxalic acid) of different concentration (0.5-5.0%) were assessed to find a suitable catalyst in hydrolysing OPTB for reaction time (15-120 min) at a fixed temperature (120°C), aiming for high xylose selectivity. Of these, oxalic acid was shown the most effective in solubilizing hemicellulose from OPTB, producing a maximum xylose yield of ~70% at the optimum catalyst concentration of 5.0% (w/v) for 120 min. The main components in the hemicellulosic hydrolysate were xylose, 16.26±0.30 g/L; glucose, 2.11±0.04 g/L; arabinose, 0.19±0.05 g/L; acetic acid, 5.59±0.07 g/L and trace amount of furfural and hydroxymethylfurfural (HMF). The resulting xylose recovery was comparable to the conventional pretreatment using dilute sulphuric acid, thus provides an alternative approach of biomass pretreatment for process improvement. The fermentability of xylose from the hemicellulosic hydrolysate was further evaluated for succinic acid production.

1. Introduction

The mass production of bio-based chemicals requires huge feedstock input. One such lignocellulosic feedstock i.e. oil palm trunk biomass (OPTB) which is renewable, abundant and low-cost can be a good candidate for bioconversion of industrially important chemicals such as succinic acid. Succinic acid, an aliphatic C4 dicarboxylic acid (butanedioic acid), has been identified as a promising value-added chemical according to the US Department of Energy (DOE) [1] and is one of the most contending biomass-derived chemicals. Succinic acid or its derivatives can be used as a source of food additives, pharmaceuticals, surfactants, detergents, solvents, biodegradable plastics and fuels [2].
Based on 5.81 million ha of the total oil palm planted area in 2017 [3], approximately 21.64 million tonnes (dry weight) of OPTB was generated from 290,500 ha of oil palm plantations. Presently, only a small portion of OPTB are used in plywood manufacturing; the remaining are cut into pieces or left on the ground to be naturally degraded for nutrient recycling [4]. The utilisation of lignocellulosic OPTB for succinic acid production seems promising and could be a practical and profitable way to optimise and diversify its potential utilisation.

Lignocellulosic oil palm biomass consists mainly of cellulose (30-50%), hemicellulose (20-35%) and lignin (15-25%) [5]. Despite its significant potential to serve as a sustainable feedstock for bioconversion, the necessity for pretreatment to disrupt the complex lignocellulose structure in releasing monomeric fermentable sugars coupled with insufficient recovery faced, have demanded further process improvement. For an efficient and economically viable bioconversion, utilisation of hemicellulose, the second most abundant carbohydrate available in lignocellulosic biomass, is anticipated to be essential [6]. However, xylose recoveries from hemicellulosic hydrolysate is often neglected as most microorganisms employed prefer to metabolise hexose and are more susceptible to inhibitors produced during degradation of hemicellulose such as furfural and hydroxymethyl furfural (HMF) [7].

Xylose from lignocellulose is typically hydrolysed using dilute acid pretreatment. Formerly, inorganic acid pretreament using sulphuric acid, phosphoric acid, hydrochloric acid and nitric acid have been extensively studied due to their high catalytic performance and low-cost [4, 8, 9], however, it suffers some disadvantages such as equipment corrosion, low reaction selectivity and inhibitory by-products formation [10]. Organic acids have emerged as a more environmentally sound alternative [11, 12] to overcome these disadvantages. Various organic acids have been examined in pretreating lignocellulosic biomass, with little attention given to OPTB. To date, only acetic acid, citric acid and oxalic acid have been deployed in OPTB producing hydrolysate for subsequent ethanol production [12].

This study mainly focused on assessing three types of organic acid (citric acid, formic acid and oxalic acid) for xylose recovery from OPTB. These organic acids were relatively low in cost. In addition, the fermentability of the deriving hemicellulosic hydrolysate was evaluated for succinic acid production using Actinobacillus succinogenes 130Z considering its capability of metabolising a broad range of sugars, including xylose.

2. Materials and methods

2.1. Oil palm trunk biomass

The OPTB samples were collected from the Malaysian Palm Oil Board (MPOB) Research Station, Pekan Bangi Lama, Selangor, Malaysia. The OPTB was ground using a laboratory stainless steel grinder to obtain an average particle size of less than 10 mm. The raw OPTB consisted of 30.86% cellulose, 25.84% hemicellulose, 24.29% lignin and 2.29% ash [4].

2.2. Organic acid pretreatment

The OPTB at 10% (w/v) solid loading was pretreated at 120°C using three types of organic acid (citric acid, formic acid and oxalic acid) at different concentration (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0%, w/v) and reaction time (15, 30, 60, 90 and 120 min). Pretreatment of OPTB (10%, w/v) using sulphuric acid at different concentration (0.25, 0.5, 1.0, 1.5, 2.0 and 2.5%, v/v) was also performed using previously optimised conditions (120°C, 60 min) [4] for comparison purposes.

2.3. Fermentability of hemicellulosic hydrolysate

Actinobacillus succinogenes 130Z (DSMZ, Brunswick, Germany) was purchased and used for pretreated OPTB fermentation to produce succinic acid. The inoculum was cultivated in Brain Heart Infusion (BHI) medium at 37°C and 150 rpm. The OPTB pretreated with oxalic acid and sulphuric acid (from 2.2) were
filtered to remove the insoluble solid fraction. The resulting OPTB hemicellulosic hydrolysates were each supplemented with 0.2 g MgCl$_2$.6H$_2$O, 0.2 g CaCl$_2$.2H$_2$O, 3.0 g KH$_2$PO$_4$, 1.0 g NaCl, 15.0 g yeast extract and 40.0 g MgCO$_3$ per litre of medium. The pH of the medium was adjusted to 6.8±0.2 using 5M NaOH. Fermentation was carried out in a 100-mL serum bottle by adding 10% (v/v) inoculum to a working volume of 50 mL and the culture was incubated at 37°C, 200 rpm for 60 h.

2.4. Analyses

Sugars and organic acid contents of OPTB hydrolysates were determined using high performance liquid chromatography (HPLC) (Waters 2707) equipped with Phenomenex Rezex™ ROA column (300 mm × 7.8 mm) (Sunnyvale, USA) as follows: temperature, 60°C; mobile phase, 2.5 mM H$_2$SO$_4$; flow rate, 0.6 mL/min. The apparatus was integrated with an auto sampler (Waters 2707, MA, USA), refractive index detector (Waters 2414, MA, USA) set at 40°C and an isocratic HPLC pump (Waters 1515, MA, USA).

3. Results and discussion

3.1. Organic acid pretreatment of OPTB

The OPTB was pretreated at 120°C using citric acid, formic acid and oxalic acid at different concentration (0.5-5.0%, w/v) and reaction time (15-120 min) to find a suitable catalyst in hydrolysing OPTB, aiming at high xylose recovery. For pretreatment using oxalic acid, xylose yield increased steadily starting from 0.5 to 3.0% (w/v) acid concentration for 120 min reaction time (Figure 1A). The xylose yield also increased with increasing reaction time, over a period of 120 min using 5.0% (w/v) oxalic acid (Figure 1B). In this study, oxalic acid was found most effective in solubilizing hemicellulose from OPTB, producing a maximum xylose yield of ~70% at optimum conditions of 5.0% (w/v) oxalic acid for 120 min. On the other hand, citric acid and formic acid were not efficient, yielding only 3.44 ± 0.08 g/L and 5.33 ± 0.05 g/L under the same conditions, respectively. Based on the findings, it is postulated that higher concentration is required for the acids to effectively hydrolyse hemicellulose from OPTB. The results also demonstrated that a more severe condition could enhance dissolution of OPTB components during acid pretreatment [13].

The oxalic acid-pretreated OPTB hemicellulosic hydrolysate contained xylose, 16.26 ± 0.30 g/L; glucose, 2.11 ± 0.04 g/L; arabinose, 0.19 ± 0.05 g/L and trace amount of furfural (3 mg/L) and HMF (22 mg/L). The resulting xylose recovery was 13.7-fold higher compared to that without organic acid pretreatment. In order to evaluate the performance of oxalic acid pretreatment, the conventional dilute sulphuric acid pretreatment employing previously optimised conditions was performed. Figure 1C shows that 1.0% (v/v) sulphuric acid gave the highest xylose yield of 73.7% which was comparable to the pretreatment using oxalic acid. The sulphuric acid-pretreated OPTB hemicellulosic hydrolysate contained xylose, 17.24 ± 0.17 g/L; glucose, 2.46 ± 0.05 g/L and trace amount of furfural (2 mg/L) and HMF (17 mg/L).

The most commonly found by-product deriving from pretreatment under acidic conditions is acetic acid, which mainly form during hydrolysis of acetyl groups of hemicellulose [14]. Higher concentration of acetic acid was released during oxalic acid and sulphuric acid pretreatments compared to those from citric acid and formic acid pretreatments. The acetic acid concentration reached its maximum of 5.67 ± 0.05 g/L which was slightly higher than that released from oxalic acid pretreatment (5.59 ± 0.07 g/L) (Figure 2). The acetyl groups on the side chain of hemicellulose are responsible for this as they will mostly be hydrolysed to acetic acid under severe conditions, in this case sulphuric acid which is a strong mineral acid [15].
Figure 1. Xylose yield (%) from different acid pretreatment of oil palm trunk biomass (A) effect of organic acid concentration at 120°C for 120 min; (B) effect of reaction time at 120°C using 5.0% (w/v) organic acid concentration and (C) effect of sulphuric acid concentration at 120°C for 60 min.

Figure 2. Acetic acid released from different acid pretreatments of oil palm trunk biomass.
3.2. Fermentability of hemicellulosic hydrolysate

Microbial fermentation of pentose sugars (e.g. xylose, arabinose) is not abundantly performed by most of the microorganisms as compared to hexose sugars. A limited number of microorganisms can convert hemicelluloses or the monomers into targeted products with a satisfactory yield and productivity. The fermentability of hemicellulosic hydrolysate of oxalic acid-pretreated OPTB was carried out to study the performance of *A. succinogenes* 130Z in producing succinic acid. This performance was compared to that using dilute sulphuric acid-pretreated OPTB. The results (Figure 3) ascertained that *A. succinogenes* 130Z was capable of utilising the released xylose for succinic acid production at satisfactory yield even without taking any detoxification step. However, the consumption of xylose was incomplete for both the medium as 22.5% (Figure 3A) and 30.9% (Figure 3B) of xylose were still remained in the culture until the end of fermentation. This might be due to some inhibitory effect caused by high total acid concentration in prolonged culture at 36-60 h. Corona-Gonzalez et al. [16] described that when total acids reach 20 g/L, sugars consumption rates decrease and microbial growth ceases.

The fermentability - succinic acid titer, its yield and productivity - of oxalic acid-pretreated OPTB hydrolysate was slightly higher than that by sulphuric acid (6.27 vs. 5.32 g/L, 0.54 vs. 0.45 g/g and 0.17 vs. 0.15 g/L/h, respectively) (Table 1). The ratios of succinic/acetic acid and succinic/formic acid are also important indicators for bacterial metabolism [17]. The ratios increased over time in both the oxalic acid- and sulphuric acid-pretreated hydrolysates with the former showing a better performance (0.96 vs. 0.79, 5.86 vs. 5.42, respectively). A high sulphur content found in the sulphuric acid-pretreated hydrolysate along with a higher acetic acid inhibition in the fermentation substrate might affect the rate of succinic acid production. Deacetylation could be done prior to pretreatment to remove the acetyl component of the OPTB.

![Figure 3](image)

**Figure 3.** Fermentation profiles of *A. succinogenes* 130Z grown on (A) oxalic acid- and (B) sulphuric acid-pretreated oil palm trunk biomass hydrolysate in serum flasks at 37°C, 200 rpm for 60 h.
Table 1. Fermentation parameters of succinic acid production using acid-pretreated oil palm trunk biomass hemicellulosic hydrolysate by *A. succinogenes* 130Z.

| Parameter                  | Pretreatment | Oxalic acid | Sulphuric acid |
|----------------------------|--------------|-------------|---------------|
| Succinic acid titer (g/L)  | 6.27 ± 0.14  | 5.32 ± 0.83 |
| Succinic acid yield (g/g)  | 0.54 ± 0.02  | 0.45 ± 0.04 |
| Productivity (g/L/h)       | 0.17 ± 0.00  | 0.15 ± 0.03 |
| Sugars consumption (%)     | 69.93 ± 2.00 | 62.50 ± 5.29|
| Succinic/acetic acid       | 0.96         | 0.79        |
| Succinic/formic acid       | 5.86         | 5.42        |

4. Conclusion

This study successfully demonstrated the potential use of oxalic acid as a catalyst in OPTB pretreatment. The resulting hydrolysis of OPTB hemicellulose achieved a maximum xylose recovery of ~70% (16.3 g/100 g OPTB) under the optimised conditions. The utilisation of xylose from hemicellulosic hydrolysate in addition to glucose (through enzymatic hydrolysis) as a fermentation substrate, would improve the overall bioconversion of OPTB into succinic acid. Further work should focus on maximising succinic acid production by getting rid of acetic acid formed during pretreatment.

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