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Biomass Deacetylation at Moderate Solid Loading Improves Sugar Recovery and Succinic Acid Production

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Abstract: Biomass deacetylation with alkali prior to dilute acid pretreatment can be a promising approach to reduce the toxicity of the resulting hydrolysates and improve microbial fermentation. In this study, the effect of mild alkaline treatment of oil palm trunk (OPT) biomass on succinic acid production was evaluated. Deacetylation was carried out under different conditions: NaOH loadings (1–5%, *w*/*v*) and reaction times (15–90 min) at 100 °C. Deacetylation using 1% (*w*/*v*) NaOH within 15 min was sufficient to achieve a high acetic acid removal of 5.8 g/L with minimal sugar loss. Deacetylation under this condition resulted in a total sugar concentration of 55.8 g/L (18.0 g/L xylose and 37.8 g/L glucose), which was 37% higher than that of non-deacetylated OPT. Subsequently, succinic acid production using *Actinobacillus succinogenes* was also improved by 42% and 13% in terms of productivity and yield, respectively, at 10% (*w*/*v*) solid loading. This further demonstrated that mild alkaline treatment prior to dilute acid pretreatment is a promising strategy to improve succinic acid production. This study provides a facile approach for reducing the most influential inhibitory effect of acetic acid, and it can be applied to the exploitation of lignocellulosic biomass resources for succinic acid, biofuels, and/or other biochemical co-production in the future.

Keywords: lignocellulosic biomass; oil palm trunk; acetic acid; fermentation; *Actinobacillus succinogenes*

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1. Introduction

Given the increase in worldwide environmental awareness associated with the petrochemical process, there has been a transition in world demand towards producing fuels and chemicals using renewable materials. Lignocellulosic biomass is regarded as a promising potential candidate to replace fossil resources for the production of fuels and chemicals [1]. For effective utilization of biomass, pretreatment is necessary to separate the components of biomass, which include cellulose, hemicellulose, and lignin [2]. Numerous pretreatment methods have been researched, utilizing physical, physicochemical, chemical, and biological techniques, as well as their combinations [3,4]. To date, the most effective pretreatments are physicochemical methods (i.e., steam explosion, wet oxidation, and hydrothermal) and chemical methods (i.e., alkali, dilute acid, deep eutectic solvent, and ionic liquid) [4]. By pretreating biomass effectively, the efficiency of enzymatic hydrolysis and subsequent fermentation will be improved. Dilute acid pretreatment is considered a highly promising approach for the bioconversion of lignocellulosic biomass due to its simplicity, rapidity, and cost-effectiveness [5,6]. During dilute acid pretreatment, hemicellulose depolymerizes to form soluble xylose monomers and oligomers [7]. However, hydrolysates derived from acid pretreatment contain acetic acid caused by cellular oxidation and stress which interferes with enzymatic hydrolysis and inhibits microbial fermentation [8].

Acetic acid, a potent fermentation inhibitor, is formed via cleavage of covalently bonded acetyl groups from hemicellulose backbone during acid pretreatment [7]. The resultant acetic acid concentration in acid hydrolysate can range up to 15 g/L [9], prompting a detoxification step commonly introduced to eliminate acetic acid after acid pretreatment in order to improve microbial fermentation [10]. However, substantial loss of fermentable sugars was reported during detoxification and is therefore not a feasible method [10,11]. Alternatively, acetyl content present in biomass can be removed via deacetylation at mild alkaline conditions and temperatures prior to acid pretreatment [12]. Biomass deacetylation is a simple and effective approach to reduce the toxicity of acid hydrolysate, without the requirement of a conventional detoxification step [8]. This approach has been proven effective in reducing the acetic acid present in acid hydrolysates during bioethanol and succinic acid production from a variety of biomasses including corn stover, sugarcane bagasse, rice straw, hemp, and kenaf [8,12–14].

Oil palm trunk (OPT) is among the most abundant biomasses left to decompose naturally in oil palm plantations for soil enrichment purposes [15,16]. OPT contains a high level of carbohydrate and is ideal for use as a feedstock for biofuels and biochemical industries. It is one of the cheapest biomasses which can be obtained at the cost of USD 7–USD 9 per ton [17]. Nonetheless, the recycling of OPT is less often considered, although OPT is a bioresource that has a high potential for conversion into value-added products [18]. OPT is composed of approximately 31% cellulose, 26% hemicellulose, 25% lignin [17], and 3% acetylated compounds [16] on a dry weight basis. In this study, OPT was utilized to produce an industrially important biochemical, i.e., succinic acid, by *Actinobacillus succinogenes*. Succinic acid was chosen due to its role as an essential precursor with widespread applications in the food, polymer, paints, and pharmaceutical industries [19]. Due to the heterogeneity of the lignocellulosic materials, various operational conditions for practical pretreatment of OPT are required to efficiently use this biomass for the production of succinic acid. This work is the first attempt to explore the applicability of the deacetylation step prior to acid pretreatment for enhancing sugars for subsequent succinic acid production from OPT biomass. Two factors, i.e., alkali loading and reaction time, affecting the deacetylation step were investigated. The influence of deacetylation at different biomass solid loadings during acid pretreatment was also evaluated and reported.

2. Materials and Methods

2.1. Oil Palm Trunk Biomass

The OPT biomass was air-dried at an ambient temperature until the moisture content was <20%. The dried biomass was ground using a laboratory cutting mill (Pulverisette 15, Fritsch GmbH, Idar-Oberstein, Germany) to obtain an average particle size of <10 mm. Thereafter, the ground biomass was sieved through 0.5 mm mesh to remove the powdery tiny particles. The sample was further oven-dried until the moisture content dropped below 10% prior to storage.

2.2. Enzyme and Microorganism

A commercial cellulase Cellic® CTec2 was purchased from Novozyme A/S (Bagsvaerd, Denmark) and used for enzymatic saccharification. The filter paper unit (FPU) of CTec2 activity was assayed as 127 FPU/mL as determined using the standard measurement of cellulase [20]. *A. succinogenes* 130Z was acquired from German Collection of Microorganisms and Cell Cultures (DSMZ, Brunswick, Braunschweig, Germany) and used in all fermentation studies to produce succinic acid.

2.3. Deacetylation

Alkaline deacetylation of OPT biomass was carried out using sodium hydroxide (NaOH), i.e., different loadings, at 0%, 1%, 2%, 3%, 4%, and 5% (*w*/*v*). The process was performed in a water bath at 100 °C for 60 min. The effect of reaction time was then studied by manipulating different reaction times of 15, 30, 60, and 90 min. The raw OPT biomass

without deacetylation (at 0 min reaction time) was used as a control. After the deacetylation process, the treated liquor was collected and analyzed using high-performance liquid chromatography (HPLC). The remaining solid (deacetylated biomass) was washed with water to attain a neutral pH. The deacetylated OPT biomass solid was then dried and used in the next experiments. The schematic description of the process is illustrated in Figure 1.

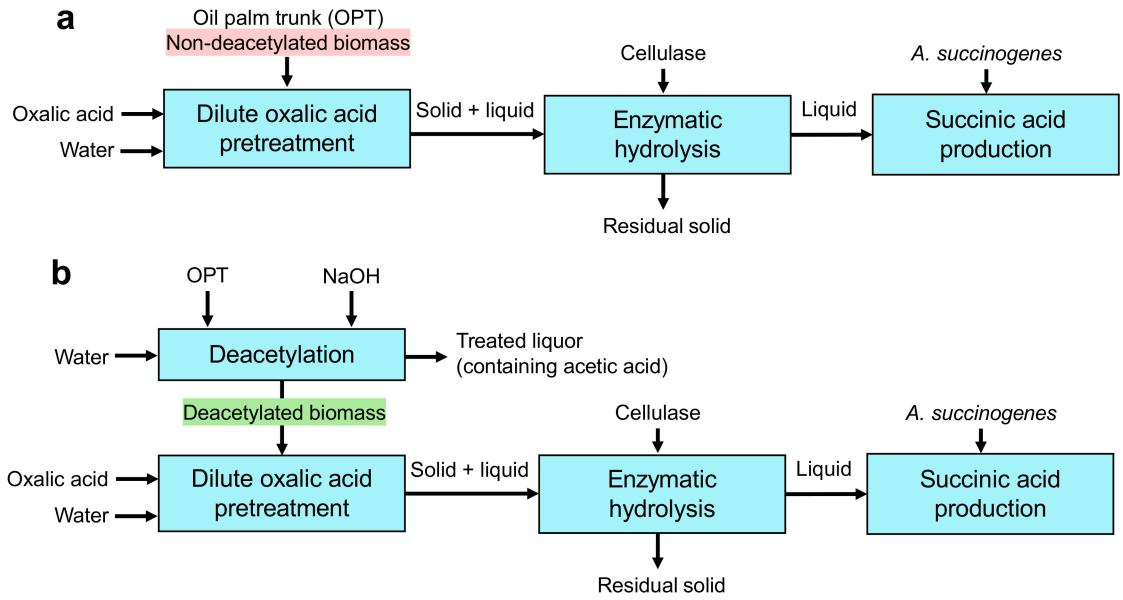

Figure 1. Diagram of the process used to produce succinic acid in this study. Hydrolysate from oil palm trunk (OPT) was produced from two different configurations: (**a**) only dilute oxalic acid pretreatment (DOAP) is applied prior to enzymatic hydrolysis and succinic acid fermentation, and (**b**) deacetylation step is introduced prior to DOAP, followed by enzymatic hydrolysis and succinic acid fermentation.

2.4. Dilute Oxalic Acid Pretreatment (DOAP)

Dilute oxalic acid pretreatment (DOAP) was incorporated to evaluate the effect of optimized deacetylation on the recovery of sugars in the resultant hydrolysate. The OPT biomass (deacetylated and non-deacetylated) at 10% (*w*/*v*) solid loading was immersed in 1% (*w*/*v*) oxalic acid solution and autoclaved at 120 °C. In order to investigate the effect of solid loading, OPT biomass was loaded at 10%, 15%, 20%, 25%, and 30% (*w*/*v*). After pretreatment, the acid hydrolysate was collected for sugars and organic acid analyses using HPLC.

2.5. Enzymatic Hydrolysis of Pretreated Slurries

Prior to enzymatic hydrolysis, the pH of the acid hydrolysate was adjusted to 4.8 ± 0.2 using 5M NaOH. The resulting pretreated slurries were enzymatically hydrolyzed using a commercial cellulase (Cellic® CTec2) at the enzyme loading of 15 FPU/g [21]. The samples were incubated in a rotary incubator shaker (Innova® 40, New Brunswick™, Hamburg, Germany) at 50 °C with an agitation speed of 150 rpm for 72 h. At the end of enzymatic hydrolysis, the liquid fractions were collected for sugar analysis and subsequent fermentation.

2.6. Assessment for Succinic Acid Production

A. succinogenes 130Z was used for succinic acid fermentation using the enzymatically hydrolyzed pretreated OPT hydrolysates. The inoculum was cultivated in Brain Heart Infusion (BHI) medium at 37 °C and 120 rpm for 6 h. The resulting hydrolysates were initially filtered to remove insoluble solid fraction. Then, the hydrolysates were each supplemented with 0.2 g $MgCl_2.6H_2O$, 0.2 g $CaCl_2.2H_2O$, 3.0 g KH_2PO_4, 1.0 g NaCl, and 15.0 g yeast extract per liter of medium [21]. The pH of the medium was adjusted to 6.8 ± 0.2 using 5M NaOH. A 10% (*v*/*v*) seed inoculum was added to the 100 mL serum

bottles containing 50 mL of fermentation medium supplemented with 40.0 g/L $MgCO_3$ to maintain the pH at 6.8. The fermentation was carried out in a rotary incubator shaker at 37 °C and 200 rpm for 48 h.

2.7. Analytical Methods

The chemical compositions of native and pretreated OPT were analyzed according to ASTM D1104-56 (1978), ASTM D1103-60 (1978), and TAPPI T222 om-11 (2011) for holocellulose, α-cellulose, and lignin, respectively. The hemicellulose composition was calculated as the difference in the weights of α-cellulose and holocellulose.

Sugars and acetic acid contents of OPT hydrolysates were quantified using HPLC (Waters 2707, Milford, MA, USA) equipped with a Phenomenex Rezex™ ROA column (300 × 7.8 mm) (Sunnyvale, CA, USA) at 60 °C. Sulphuric acid (H_2SO_4) at 2.5 mM was used as the eluent at a flow rate of 0.6 mL/min. The apparatus was integrated with an auto sampler (Waters 2707, Milford, MA, USA), refractive index detector (Waters 2414, Milford, MA, USA) set at 40 °C, and an isocratic HPLC pump (Waters 1515, Milford, MA, USA).

The weight recovery was determined as the percentage of treated solid material recovered after drying (g) in relation to the initial solid material loaded (g). The succinic acid (product) yield from OPT biomass was calculated as the gram of product per gram of sugar consumed (g/g). The productivity (g/L.h) was calculated based on the tangent to the slope of the product formation curve.

All experiments were performed in triplicates. Data related to enzymatic hydrolysis (total sugars) were analyzed using Minitab®18 (University Park, PA, USA) through analysis of variance (ANOVA), followed by Tukey's multiple comparison test. The level of statistical significance was set at 5 % ($p < 0.05$).

3. Results and Discussion

3.1. Effect of Deacetylation at Different Conditions

Selection of the optimal NaOH loading during biomass deacetylation is essential in ensuring maximum sugar recovery for subsequent fermentation by minimizing loss of sugars [8]. Thus, different NaOH loadings and reaction times were evaluated for deacetylation of OPT biomass aiming at minimizing the loss of both C5 (xylose) and C6 (glucose) sugars (Figure 2). After NaOH treatment, the treated liquid fractions obtained were mainly composed of acetic acid (Figure 2a,b). Without the presence of NaOH (control), no acetic acid was detected in the liquor. At 1% (*w*/*v*) NaOH loading, 7.0 g/L of acetic acid was released, while at the higher NaOH loading of 2–5% (*w*/*v*), the amount released was in the range of 8.2–8.5 g/L (Figure 2a). The amount of acetic acid released from OPT biomass was fairly high for each NaOH loading. In terms of sugars solubilization, no apparent difference in the amount of sugar released (~0.6 g/L xylose and ~0.2 g/L glucose) was observed with increasing the NaOH loading from 1% to 5% (*w*/*v*), suggesting that deacetylation via NaOH treatment is effective in removing the acetyl group while minimizing the released sugars.

Meanwhile, at different reaction times (using 1% NaOH, *w*/*v*), the highest acetic acid released was 7.0 g/L after 60 h treatment, followed by 6.7 g/L after 90 h. A lower concentration (5.8–6.0 g/L) was released at a shorter reaction time of 15 to 30 min (Figure 2b). A longer reaction time tends to cleave additional acetyl groups from the dissolved xylooligomers, resulting in a higher concentration of free acetic acid [3]. In terms of sugar solubilization, the xylose released was in the range of 0.6–0.7 g/L, while glucose accumulation was negligible (<0.1 g/L) at different reaction times from 15 to 90 min.

The impact of different deacetylation conditions on OPT biomass was then evaluated based on weight recoveries from NaOH-treated biomass, as well as their main chemical compositions, i.e., cellulose, hemicellulose, and lignin. All NaOH-treated samples recorded a lower total weight recovery when compared to the control (Figure 2c,d). NaOH affected the composition of OPT components and the subsequent weight by removing the acetyl group, as demonstrated by the subsequent amount of acetic acid released (Figure 2a,b) and

the partially solubilizing hemicellulose and lignin components (Figure 2c,d). As can be seen in Figure 2, lignin was the main fraction affected by NaOH treatment, their removal being favored with increasing NaOH loading (Figure 2c). During the deacetylation step, a higher amount of hemicellulose was retained at a lower NaOH loading (Figure 2c). When deacetylation was at 1% (*w*/*v*), NaOH demonstrated the highest weight recovery of 82.7% (*w*/*w*), as compared to 77.9–81.2% (*w*/*w*) at the higher NaOH loading of 2–5% (*w*/*v*) (Figure 2c). The hemicellulose contents in the recovered biomass deacetylated OPT ranged 14.7–21.9%, with lignin contents ranging from 14.2% to 19.7%.

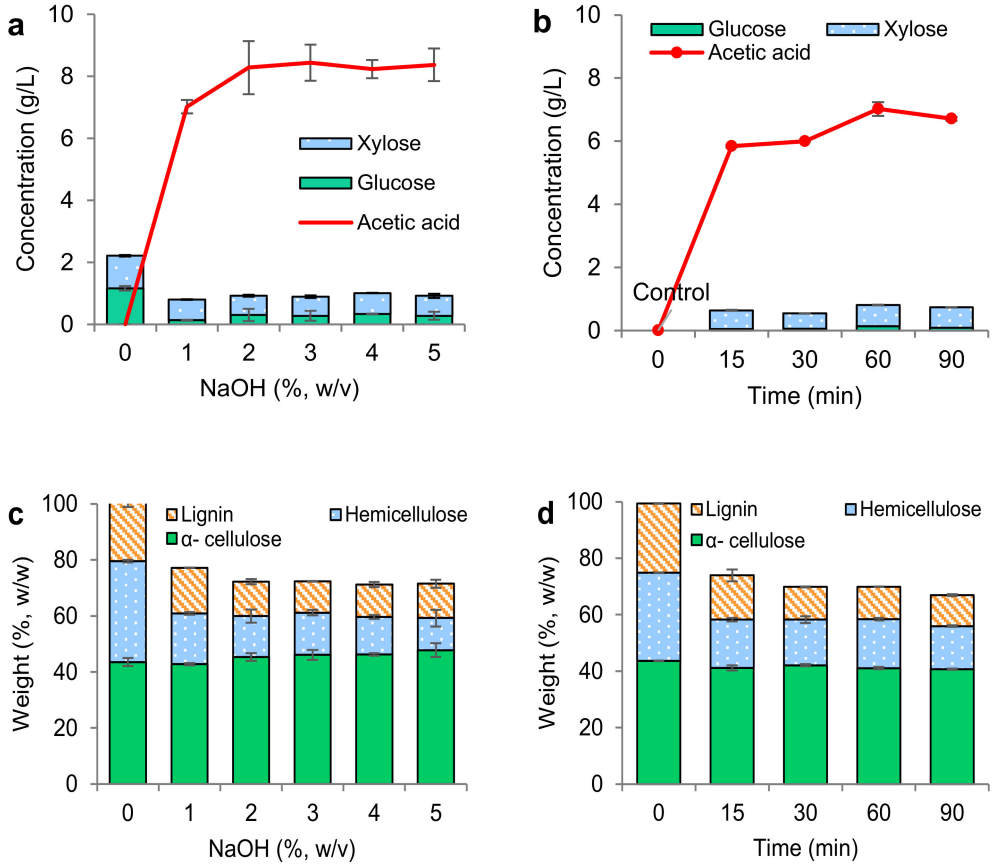

Figure 2. Composition of the soluble and insoluble fractions of OPT biomass on sugars and acetic acid released in the liquor, and the chemical composition after deacetylation (calculated based on weight recovery); (**a**,**c**) effect of different NaOH loadings; (**b**,**d**) effect of different reaction times.

Under the evaluated conditions, the content of celluloses under treatment at various NaOH loadings were unaffected and preserved during the deacetylation, which indicated that celluloses were not easily degraded to monomeric sugars under mild alkaline conditions (Figure 2c). The cellulose contents in the recovered deacetylated OPT biomass ranged 51.7–61.4%. Compositions of cellulose from other biomasses, i.e., corn stover, hemp, and kenaf, were also reported to be unreactive under similar conditions [7,22].

Regarding different reaction times, deacetylation at 15 min showed the highest weight recovery of 81.9% (*w*/*w*), as compared to 77.8–81.1% (*w*/*w*) at longer reaction times of 30–90 min (Figure 2d). The cellulose and hemicellulose contents in the recovered deacetylated OPT biomass at different reaction times ranged 50.1–53.9% and 19.7–21.9%, respectively, while that of lignin ranged 14.1–19.7%. From these trends, it is postulated that deacetylation at different reaction times would not affect the cellulose content in the biomass (Figure 2d). Hence, the objective of the mild alkaline treatment was successfully achieved with acetic acid as the expected main fraction released from OPT biomass. Additionally, the treatment partially removed lignin while preserving most sugars, which is preferred for the subsequent processing steps.

3.2. Effect of Deacetylation on Sugars Recovery Post-DOAP and Enzymatic Hydrolysis

In order to assess the impact of deacetylation on the subsequent steps, DOAP was first performed using the OPT biomass treated at different conditions. The main component of the hydrolysate after DOAP was xylose, as shown in Figure 3a,b. The highest concentration of xylose was exhibited by the control, i.e., in the absence of NaOH, at 13.5 g/L (Figure 3a). A 28% xylose reduction was obtained at 1% (*w*/*v*) NaOH loading, reaching 9.7 g/L due to a significant hemicellulose loss during the deacetylation step. A further increase in NaOH loading (5% *w*/*v*) resulted in another 29% reduction, i.e., only 5.7 g/L of xylose were contained in the acid hydrolysate.

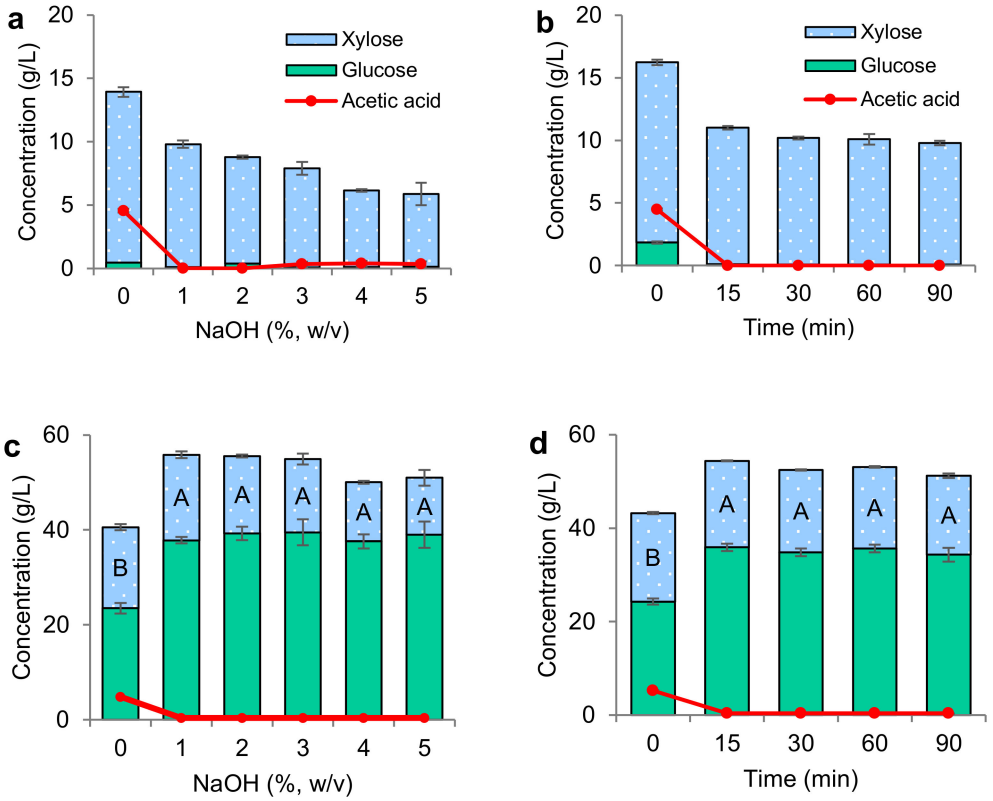

Figure 3. Effect of deacetylation using (**a**) different NaOH loading and (**b**) different reaction times on sugar solubilization and the acetic acid released during dilute oxalic acid pretreatment, and the effect of deacetylation using (**c**) different NaOH loadings and (**d**) different reaction times on the total sugars and acetic acid released during enzymatic hydrolysis. Note: A and B represent the grouping information using the Tukey Method at 95% confidence. Means that do not share the same letter are significantly different; data presented are calculated as means ± standard deviations of the experiments performed in triplicate.

Meanwhile, a low level of acetic acid (~0.3 g/L) was detected in the acid hydrolysates that were deacetylated with 3–5% (*w*/*v*) NaOH, whereas it was not detected in those treated with 1–2% (*w*/*v*) NaOH (Figure 3a). According to Castro et al. [12], xylose concentration was found to be higher in the deacetylated hydrolysate, which indicated that the deacetylation was able to weaken the hemicellulose structure by cleavage of the arabinoxylan–lignin and structural protein linkages, and therefore would make the acetyl ester (hemicellulose) linkages more labile and thus more exposed to degradation. As previously studied, deacetylation leads to higher monomeric xylose yields, higher overall hemicellulose solubilization, and lower xylooligomer yields [3]. However, the obtained results did not indicate the release of soluble monomeric xylose during DOAP (Figure 3a,b). This was likely due to the losses of loosely bound hemicellulose components during the washing step, which was also reflected by the hemicellulose compositions in the treated OPT (Figure 2c,d).

Similarly, Shekiro et al. [23] also reported ~5% of hemicellulosic sugar loss from corn stover hydrolysate after washing.

A similar trend was observed when DOAP at a fixed 1% (*w*/*v*) NaOH loading was performed on OPT at different reaction times (Figure 3b). The results showed a decreasing trend in xylose released as the deacetylation reaction time increased from 15, 30, 60, to 90 min. As compared to the control (0 min), lower xylose contents were measured in the acid hydrolysates of OPT, which indicated a significant loss of hemicellulosic fraction during the washing step. In the non-deacetylated OPT (control), a higher concentration of monomeric xylose of 14.4 g/L was released compared to 9.3–10.5 g/L from those deacetylated samples. The xylose released from the deacetylated sample was the highest at 15 min. At a prolonged DOAP treatment time, the released xylose was slightly reduced by 4% at each specified time interval. In such situations, no acetic acid was detected in any of the deacetylated hydrolysates, compared to 4.5 g/L in the non-deacetylated control.

In the subsequent stage, enzymatic hydrolysis was crucial to determine the enzymatic digestibility of the pretreated OPT by cellulase, as indicated by the glucose released in the hydrolysates. The concentration of glucose released from all the deacetylated OPTs was significantly improved by ~60% ($p < 0.05$) (Figure 3c). Deacetylation using 1% (*w*/*v*) NaOH resulted in the highest concentration of total sugars of 55.8 g/L (18.0 g/L xylose and 37.8 g/L glucose), 37% higher than that of non-deacetylated OPT at 40.6 g/L (17.1 g/L xylose and 23.5 g/L glucose). Insignificant reduction ($p > 0.05$) was attainable when higher NaOH loading (4–5%, *w*/*v*) was used. Deacetylation also improved xylose yields during enzymatic hydrolysis, with an additional ~40% of monomeric xylose released during the process, compared to only ~20% for the control. This finding demonstrated that treating NaOH under mild conditions improves the overall sugars released, as indicated by the significant yield increase ($p < 0.05$) in monomeric xylose and glucose in the enzymatic hydrolysates.

When adopting 1% (*w*/*v*) NaOH at different reaction times, the results demonstrated insignificantly lower glucose yields ($p > 0.05$) with increasing reaction times (Figure 3d). The glucose yield was found to decrease from 36.2 g/L (15 min) to 34.9 g/L (90 min). The total sugars deriving from deacetylation for 15 to 90 min were in the range of 51.7–54.4 g/L. With respect to the inhibitory acetic acid, their concentrations in all the deacetylated hydrolysates were below 0.35 g/L, compared to 5.25 g/L in the non-deacetylated hydrolysate. From this finding, deacetylation at the milder 1% (*w*/*v*) NaOH for a shorter reaction time (15 min) would suffice for the next experiment to achieve satisfactory results.

The effects of different NaOH loadings at the DOAP stage were more pronounced than reaction time. About ~12% xylose reduction was shown at 1% (*w*/*v*) NaOH intervals compared to ~4% at 15 min intervals (Figure 3a,b). This was likely due to higher hemicellulose loss in deacetylation at different NaOH loadings, as clearly shown in Figure 2c compared to Figure 2d at a different reaction time. Similar results were reported for the deacetylation process optimized using response surface methodology (RSM), where the responses were more significantly affected by reaction temperature and NaOH loading than reaction time [23]. As mentioned earlier, NaOH is also consumed in the reaction, rather than simply acting as a catalyst. Thus, the extent of acetic acid removal can be controlled by limiting the amount of NaOH loaded during deacetylation, which helps to reduce the extent of hemicellulose solubilization.

The positive effects of the deacetylation step prior to pretreatment on enzymatic hydrolysis efficiency have been described by many researchers [7,8,12,22]. The highest acetic acid released was 98.8% at 70 °C using 80 mg NaOH/g rice straw for 45 min; concurrently, 42.7% of lignin and 59.4% of ash were removed, with some losses in glucan (1.2%) and hemicellulose (7.7%) of rice straw [12]. Prior to that, lower acetyl removal (75%) was accomplished during the alkaline pretreatment of 48 mg NaOH/g corn stover at 80 °C for 180 h [7]. Accordingly, the impact of deacetylation on chemical composition of corn stover was highly dependent on vegetative variety. Further, their developed process featuring dilute alkaline deacetylation prior to disc refining resulted in xylose yields of 71–77% and glucose yields of 78–84% after enzymatic hydrolysis [22]. More recently, the

yields of glucose and xylose obtained from the deacetylated hemp and kenaf were increased by 14–18%, compared with those from the non-deacetylated biomass [8]. In comparison to the common acetic acid removal method, i.e., detoxification, only 4–15% of acetic acid was removed along with a significant reduction in glucose recovery (33–65.2%) [10,11]. Deacetylation is superior to detoxification in terms of higher acetic acid removal as well as sugar recovery.

In sum, deacetylation significantly improves the enzymatic hydrolysis of DOAP-pretreated OPT biomass. Glucose yields can be improved by up to ~47% on average, while preserving most xylose in the hydrolysate. The significant sugar yield improvement hints at the importance of removing acetyl groups from hemicellulose prior to DOAP and enzymatic hydrolysis. This step represents an advantage prior to DOAP in optimizing acetic acid removal for the overall process improvement.

3.3. Assessment for Succinic Acid Production

In order to assess the effects of deacetylation on succinic acid production, fermentation employing *A. succinogenes* was carried out using both the deacetylated and non-deacetylated OPT hydrolysates (Figure 4). At 48 h, the cells were able to consume 89% of total sugars yielding 16.15 g/L succinic acid in the non-deacetylated hydrolysate (Figure 4a). This was improved by 20% using the deacetylated OPT hydrolysate, involving 94% of total sugars for 19.16 g/L succinic acid. The succinic acid yield and productivity were correspondingly higher in the deacetylated hydrolysates compared to those in the non-deacetylated hydrolysates (0.44 vs. 0.39 g/g and 0.51 vs. 0.36 g/L.h, respectively) (Table 1). After 48 h, the amount of succinic acid was slightly reduced in the deacetylated hydrolysate, but still much higher than that in the non-deacetylated sample, reaching a titer of ~18 g/L in both substrates at 72 h. These results showed that the productivity of succinic acid from OPT hydrolysate was the main parameter negatively affected under a non-deacetylated condition. Low productivity is attributable to an increase in free radicals in cells, additional energy required to expel excessive protons to maintain the intracellular pH homeostasis, and inhibition of intracellular metabolic functions caused by acetic acid in hydrolysates of non-deacetylated biomass [24].

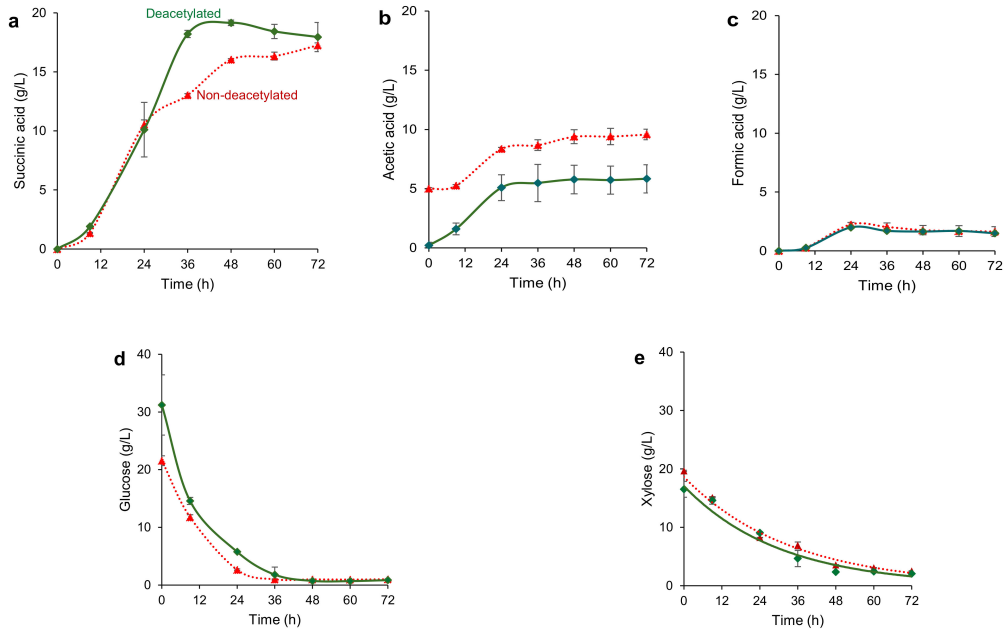

Figure 4. Time course of *A. succinogenes* fermentation for (**a**) succinic acid production, by-products (**b**) acetic acid, (**c**) formic acid formation, (**d**) glucose, and (**e**) xylose consumption from both deacetylated (solid lines) and non-deacetylated (dashed lines) oil palm trunk hydrolysates.

Table 1. Fermentation parameters of succinic acid production by *A. succinogenes* 130Z using acid-pretreated oil palm trunk hydrolysate for 48 h of fermentation.

Hydrolysate	Non-Deacetylated	Deacetylated	Improvement/Reduction (%)
Succinic acid yield (g/g) *	0.39 ± 0.01	0.44 ± 0.01	12.82
Productivity (g/L.h) *	0.36 ± 0.00	0.51 ± 0.01	41.67
Glucose consumption (%)	95.40 ± 0.31	97.67 ± 0.26	2.27
Xylose consumption (%)	81.93 ± 0.76	85.50 ± 3.52	3.57
Total sugar consumption (%)	88.98 ± 0.03	93.56 ± 0.48	4.58
By-product			
Formic acid (g/L)	1.76 ± 0.40	1.64 ± 0.21	−7.32
Acetic acid (g/L)	9.39 ± 0.58	5.78 ± 1.20	−38.45

* calculated at 36 h. Values are reported as means ± standard deviations of the experiments performed in triplicate.

The ratios of succinic/formic acid and succinic/acetic acid are also important indicators for behavioral determination of bacterial metabolism [13]. These ratios increased over time in both the deacetylated and non-deacetylated hydrolysates with the former showing a better performance (9.12 vs. 11.68, 1.71 vs. 3.31, respectively). A higher acetic acid inhibition in the non-acetylated hydrolysate might affect the yield and productivity of succinic acid. Higher accumulation of acetic acid took place, as demonstrated in Figure 4b, up from the initial concentration of 5 g/L presence in the non-deacylated hydrolysate compared to the deacetylated counterpart, from none to ~5 g/L. Meanwhile, about the same amount of formic acid was generated during the course of fermentation (24–72 h) in both the deacetylated and non-deacetylated cultures (Figure 4c).

The trends for glucose and xylose consumption were also similar in both hydrolysates (Figure 4d,e). Almost all the glucoses (>95%) were consumed by the cells while ~12–16% of xylose still remained in both cultures until the end of fermentation (Table 1). In this study, performing the deacetylation step prior to pretreatment successfully reduced acetic acid accumulation and subsequently enhanced succinic acid production. A positive effect of such processes in treating corn stover was also reported [7]. The performance of *A. succinogenes* in the deacetylated corn stover hydrolysate was 42.3% higher than that of non-deacetylated substrates, reaching titers up to 42.8 g/L and yield of 0.74 g/g with an initial sugar of 80 g/L.

The impact of deacetylation on succinic acid production by *A. succinogenes* is summarized in Table 1. All the fermentation parameters were improved when OPT biomass was subjected to deacetylation prior to DOAP, with the greatest succinic acid productivity improvement of 42%. In a nutshell, deacetylation step reduces by-product formation, i.e., 7% and 38% of formic acid and acetic acid, respectively, during *A. succinogenes* fermentation in the culture broth. The reduction in by-products is beneficial in reducing the overall downstream processing cost of succinic acid.

The deacetylated OPT biomass had a lesser degree of accumulation of acetic acid, hence leading to low toxicity in the hydrolysate for subsequent bioprocessing. For example, bioethanol production by *Spathaspora passalidarum* with deacetylated hydrolysate was 16.92 g/L, whereas only 1.3 g/L of bioethanol was obtained with non-deacetylated hydrolysate [9]. Deacetylation provides a 13-fold increase in bioethanol production, showing that alkaline deacetylation prior to dilute acid pretreatment is mandatory in bioethanol production using *S. passalidarum*. Contrarily, this study demonstrated that A. succinogenes could efficiently metabolize lignocellulosic sugars for succinic acid production even without taking the deacetylation step. *A. succinogenes* is robust, resistant to impurities in hydrolysates, and tolerant to high concentrations of inhibitors [21]. Accordingly, this study provides a strategy to reduce hydrolysate toxicity for the further improvement of succinic acid production.

3.4. Effect of Deacetylation at High-Solid DOAP Followed by Enzymatic Hydrolysis

An increase in total sugar recovery from OPT biomass is required for subsequent succinic acid production. Conversion and processing of lignocellulosic biomass at a high solid loading is necessary to reduce both capital and operational expenditures [25]. A high-solid process is defined by no presence of free water/liquid in the slurry or roughly ≥15% (*w*/*v*) [26]. Biomass processing conducted at a high solid loading would be advantageous as it is associated with high targeted products while reducing water consumption. However, a decrease in the sugar conversion rate is inevitable as the substrate concentration increases [27].

In order to determine the appropriate solid loading of OPT biomass for the pretreatment process, its influence on DOAP followed by enzymatic hydrolysis was investigated ranging from 10% to 30% (*w*/*v*), with other pretreatment variables being fixed. After DOAP in the non-deacetylated OPT, the xylose concentrations significantly increased proportionate to the employed solid loading from 13.7 g/L to 36.2 g/L (Figure 5a), and at the same time, glucose was also released at a much lower concentration (ranged 0.4–3.7 g/L). Similarly, the release of acetic acid was proportionally higher to the increased solid loading (Figure 5a). The range of acetic acid released was 4.6–10.9 g/L in the non-deacetylated OPT biomass.

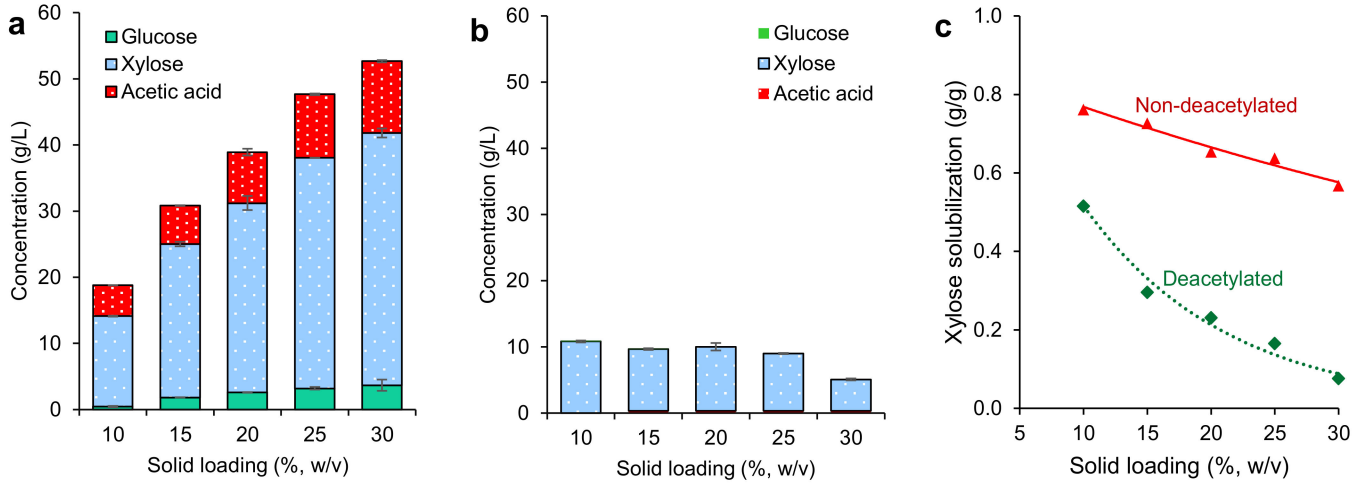

Figure 5. Effect of deacetylation at different solid loadings post-dilute acid pretreatment (**a**) non-deacetylated oil palm trunk (OPT), (**b**) deacetylated OPT, and (**c**) xylose solubilization of non-deacetylated and deacetylated OPT.

For comparison, the deacetylated OPT biomass was also performed during DOAP (Figure 5b). At a mild alkaline treatment employing various solid loadings, about 5.3–6.8 g/L acetic acid was removed from the OPT biomass. Meanwhile, after DOAP, the monomeric xylose released had decreased as solid loading increased from 10.8 g/L to 4.8 g/L at 10% and 30% (*w*/*v*), respectively (Figure 5b). The mild alkaline deacetylated OPT exhibited a very low solubilization of monomeric xylose specifically at high solid loading from 0.52 g/g to 0.08 g/g at 10% and 30% (*w*/*v*), respectively (Figure 5c), as compared to that of non-deacetylated OPT from 0.76 g/g to 0.57 g/g at 10% and 30% (*w*/*v*), respectively.

The findings suggested that the sugars of deacetylated OPT biomass are preserved during DOAP, mostly in the form of oligomeric sugars. At a higher solid loading, longer reaction times or more severe conditions are required to cleave the xylosidic bonds for the release of monomeric xylose. In this study, the reduced dissolution of hemicellulose with increasing the solid loading is acceptable because the oligomeric sugars will be hydrolyzed later during enzymatic hydrolysis.

Consecutively, after enzymatic hydrolysis, the trends for both the non-deacetylated and deacetylated OPT showed similarities. As solids loading increases, the total sugar concentration increases (Figure 6). A very high total of sugars of 107.1 g/L (63.2 g/L glucose and 43.9 g/L xylose) was achieved in the non-deacetylated OPT at 30% (*w*/*v*)

solid loading (Figure 6a) as compared to 81.2 g/L (53.9 g/L glucose and 27.3 g/L xylose) which was attainable employing the deacetylated OPT (Figure 6b). There was no apparent difference in the concentration of glucose in both the non-deacetylated and deacetylated OPT as compared to xylose concentration, suggesting that xylose (hemicellulose) had been partially removed during the deacetylation step as evidenced in Figure 2c.

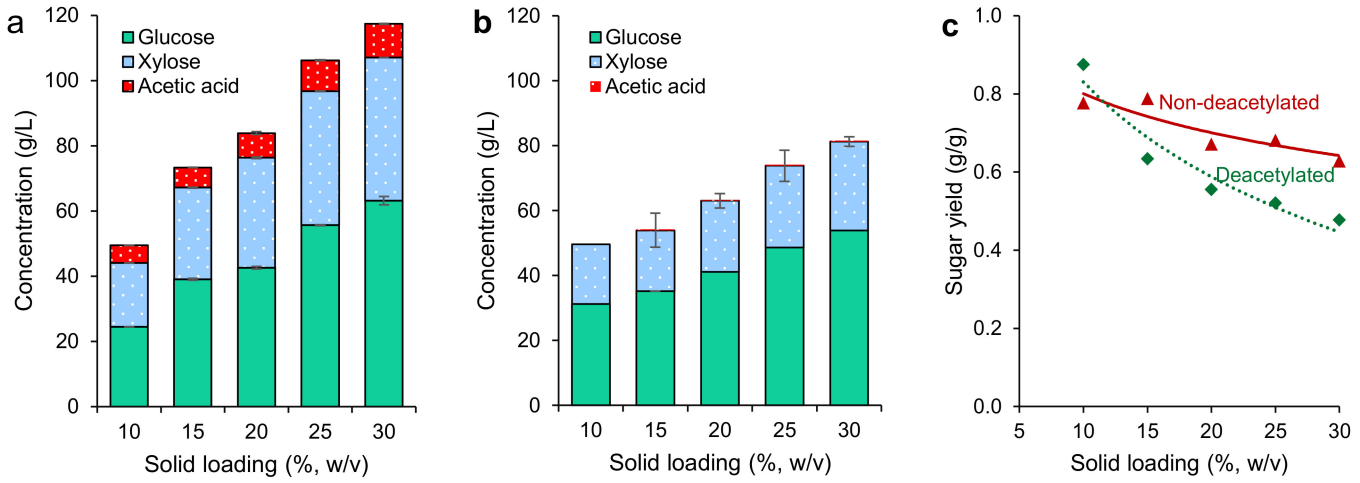

Figure 6. Effect of deacetylation at different solid loadings post-enzymatic hydrolysis (**a**) non-deacetylated oil palm trunk (OPT), (**b**) deacetylated OPT, and (**c**) sugar yield of non-deacetylated and deacetylated OPT.

The relationship between sugar yield and solid loading after enzymatic hydrolysis is shown in Figure 6c. Sugar yield shows a strong negative correlation with increasing solid loading specifically in the deacetylated OPT. It shows a substantial yield drop at different solid loadings in the non-deacetylated OPT. The highest sugar yield (0.88 g/g) was achieved using the deacetylated OPT at 10% (*w*/*v*) solid loading. However, the yield decreased drastically from 0.88 to 0.48 g/g at 10% and 30% (*w*/*v*), respectively. On the other hand, the reduction of sugar yield of the non-deacetylated OPT was less obvious at different solid loadings, decreasing gradually from 0.78 to 0.63 g/g (Figure 6c). The negative trend of sugar yield with solid loading indicates that solid loading is an important parameter for enzymatic hydrolysis and should be seriously determined for optimum results rather than being randomly selected.

It is worth noting that 15% (*w*/*v*) solid loading is often considered as the upper limit for enzymatic hydrolysis of pretreated biomass [28]. This study suggested that the deacetylation is effective in increasing sugar yield at considerably lower solid loadings than 15% (*w*/*v*). It further suggested that increasing the solid loading does not necessarily promote hemicellulose solubilization to affect the DOAP of OPT biomass.

3.5. Effect of Solid Loading on Succinic Acid Production

The deployed deacetylation step has a pronounced effect on succinic acid production (Figure 7). The deacetylated OPT biomass showed a higher succinic acid titer of 18.8 g/L, i.e., 13% and 42% higher in yield and productivity, respectively, than that of non-deacetylated OPT (Table 2). To explore the effect of deacetylation at different solid loadings on succinic acid production, fermentation of the resultant hydrolysates was performed. The relationship between succinic acid yield and solid loading for a 72 h fermentation from both the deacetylated and non-deacetylated hydrolysates is shown in Figure 7.

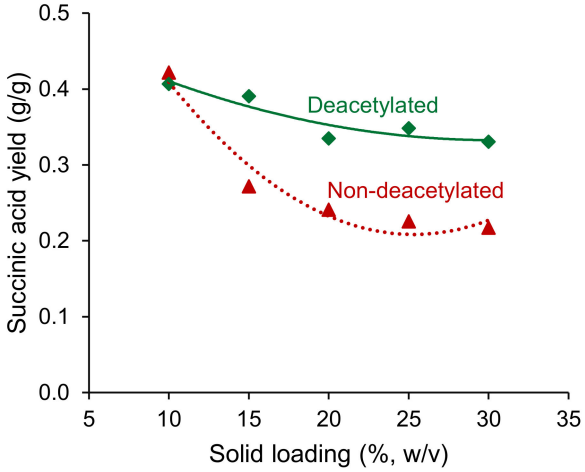

Figure 7. Succinic acid yield throughout 72 h fermentation from both deacetylated (solid lines) and non-deacetylated hydrolysates (dashed lines) at different oil palm trunk solid loadings.

Table 2. Fermentation parameters of succinic acid production by *A. succinogenes* 130Z for 72 h using acid-pretreated oil palm trunk hydrolysate.

Solid Loading (%, *w*/*v*)	Succinic Acid (g/L)	Glucose Consumption (%)	Xylose Consumption (%)	Total Sugar Consumption (%)	By-Product	
					Formic Acid (g/L)	Acetic Acid (g/L)
Non-deacetylated						
10	17.25 ± 0.21	95.86 ± 0.24	87.11 ± 0.52	91.96	1.64 ± 0.42	9.58 ± 0.45
15	14.91 ± 0.71	75.14 ± 5.80	90.88 ± 3.47	81.73	7.08 ± 0.30	11.10 ± 0.03
20	13.09 ± 0.23	61.83 ± 3.41	83.05 ± 2.23	71.22	6.69 ± 0.22	12.15 ± 0.14
25	13.72 ± 0.08	52.28 ± 1.77	77.12 ± 1.19	62.84	5.96 ± 0.07	14.23 ± 0.46
30	14.26 ± 0.06	51.08 ± 1.14	75.95 ± 1.16	61.28	5.65 ± 0.16	14.73 ± 0.72
Deacetylated						
10	18.83 ± 1.24	97.22 ± 0.01	88.88 ± 4.68	94.07	1.49 ± 0.01	5.83 ± 1.19
15	17.85 ± 0.60	83.71 ± 2.96	86.76 ± 1.09	84.77	9.12 ± 0.07	6.25 ± 0.09
20	16.64 ± 0.55	74.67 ± 5.21	86.89 ± 1.98	78.91	7.58 ± 0.16	5.06 ± 0.04
25	18.56 ± 1.09	65.96 ± 2.64	84.23 ± 1.54	72.19	9.14 ± 0.20	6.31 ± 0.17
30	17.40 ± 0.17	57.07 ± 0.74	80.12 ± 0.17	64.81	8.13 ± 0.16	5.69 ± 0.29

Values are reported as means ± standard deviations of the experiments performed in triplicate.

The succinic acid yield was negatively correlated with increasing the solid loading, more so for the non-deacetylated OPT. It showed a drastic drop (~35%) in the succinic acid yield from 0.42 g/g to 0.27 g/g using 10% (*w*/*v*) to 15% (*w*/*v*) solid loading in the non-deacetylated hydrolysate (Figure 7). Beyond 15% (*w*/*v*), the succinic acid yield dropped slowly from 0.27 to 0.22 g/g at 30% (*w*/*v*) solid loading. The highest attainable succinic acid yield was ~0.42 g/g for both the deacetylated and non-deacetylated OPT at 10% (*w*/*v*) solid loading. In the deacetylated hydrolysate, a much higher succinic acid yield could be obtained, as seen with the moderate decrease (~20%) to 0.33 g/g at the higher loading of 30% (*w*/*v*).

The fermentation was almost accomplished within 72 h as indicated by the complete sugar consumption when the OPT solid loading was 10% (*w*/*v*) (Table 2). At high solid levels, the sugars were not fully consumed by the cells, as evidenced by the remaining total sugars available in the culture. This might be attributed to insufficient time for the cells to metabolize these high-loaded sugars. However, an excessively long fermentation time would ultimately reduce productivity, and thus is undesirable. For high solid loadings beyond 10% (*w*/*v*) of non-deacetylated OPT, the succinic acid titers dropped to 13.1–14.9 g/L compared to 17.3 g/L at 10% (*w*/*v*) during the 72 h fermentation period (Table 2). At the high solid loading of 30% (*w*/*v*), the succinic acid titer (14.3 g/L) corresponded to succinic acid yields of 0.22 g/g (Figure 7), representing a ~50% drop from those at 10% (*w*/*v*). This result indicates

that solid loading is a critical element for enzymatic hydrolysis and fermentation, and thus must be properly considered for overall process cost-effectiveness.

In view of the aforementioned findings, it is thus proposed that the best conditions for biomass deacetylation are as follows: moderate solid loading of 10–15% (*w*/*v*), and deacetylation using 1% (*w*/*v*) NaOH at 100 °C for 15 min for further experiments utilizing OPT biomass for succinic acid production.

4. Conclusions

Optimized mild alkaline treatment (deacetylation) using 1% *w*/*v* NaOH for 15 min at 100 °C proved to be an efficient method for the removal of acetyl content from OPT biomass. This step removed approximately 95% of acetic acid and increased glucose yield by up to 47%, while preserving most of the xylose in the hydrolysate. Additionally, the productivity and yield of succinic acid produced by *A. succinogenes* increased by 42% and 13%, respectively, when using 10% (*w*/*v*) solid loading of deacetylated OPT biomass compared to non-deacetylated OPT biomass. This study provides a simple and efficient approach for reducing the inhibitory effect of the dominant by-product, acetic acid, which is deemed helpful for industrial symbiosis, with special focus on lignocellulose-based succinic acid, biofuels, and other biochemicals.

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References

1. Shen, X.; Sun, R. Recent advances in lignocellulose prior-fractionation for biomaterials, biochemicals, and bioenergy. *Carbohydr. Polym.* **2021**, *261*, 117884. [CrossRef]
2. Mamilla, J.L.; Novak, U.; Grilc, M.; Likozar, B. Natural deep eutectic solvents (DES) for fractionation of waste lignocellulosic biomass and its cascade conversion to value-added bio-based chemicals. *Biomass. Bioenerg.* **2019**, *120*, 417–425. [CrossRef]
3. Olugbemide, A.D.; Oberlintner, A.; Novak, U.; Likozar, B. Lignocellulosic corn stover biomass pre-treatment by deep eutectic solvents (DES) for biomethane production process by bioresource anaerobic digestion. *Sustainability* **2021**, *13*, 10504. [CrossRef]
4. Haq, I.u.; Qaisar, K.; Nawaz, A.; Akram, F.; Mukhtar, H.; Xu, Y.; Mumtaz, M.W.; Rashid, U.; Ghani, W.A.W.A.K.; Choong, T.S.Y. Advances in valorization of lignocellulosic biomass towards energy generation. *Catalysts* **2021**, *11*, 309. [CrossRef]
5. Hans, M.; Pellegrini, V.O.A.; Filgueiras, J.G.; de Azevedo, E.R.; Guimaraes, F.E.C.; Chandel, A.K.; Polikarpov, I.; Chadha, B.S.; Kumar, S. Optimization of dilute acid pretreatment for enhanced release of fermentable sugars from sugarcane bagasse and validation by biophysical characterization. *BioEnergy Res.* **2022**, 1–21. [CrossRef]
6. Kim, S.J.; Kim, T.H.; Oh, K.K. Deacetylation followed by fractionation of yellow poplar sawdust for the production of toxicity-reduced hemicellulosic sugar for ethanol fermentation. *Energies* **2018**, *11*, 404. [CrossRef]
7. Chen, X.; Shekiro, J.; Franden, M.A.; Wang, W.; Zhang, M.; Kuhn, E.; Johnson, D.K.; Tucker, M.P. The impacts of deacetylation prior to dilute acid pretreatment on the bioethanol process. *Biotechnol. Biofuels.* **2012**, *5*, 8. [CrossRef] [PubMed]
8. Ju, Y.; Kim, I.J.; Kim, S.; Olawuyi, I.F.; Kim, K.M.; Kim, S.R. Deacetylation kinetics of promising energy crops, hemp and kenaf, for cellulosic ethanol production. *GCB Bioenerg.* **2022**, *14*, 1150–1161. [CrossRef]
9. Palmqvist, E.; Hahn-Hägerdal, B. Fermentation of lignocellulosic hydrolysates. I: Inhibition and detoxification. *Bioresour. Technol.* **2000**, *74*, 17–24. [CrossRef]
10. Manaf, S.F.A.; Jahim, J.M.; Harun, S.; Luthfi, A.A.I. Fractionation of oil palm fronds (OPF) hemicellulose using dilute nitric acid for fermentative production of xylitol. *Ind. Crops Prod.* **2018**, *115*, 6–15. [CrossRef]

11. Shah, S.S.M.; Luthfi, A.A.I.; Low, K.O.; Harun, S.; Manaf, S.F.A.; Illias, R.M.; Jahim, J.M. Preparation of kenaf stem hemicellulosic hydrolysate and its fermentability in microbial production of xylitol by *Escherichia coli* BL21. *Sci. Rep.* **2019**, *9*, 4080. [CrossRef] [PubMed]
12. de Assis Castro, R.C.; Fonseca, B.G.; dos Santos, H.T.L.; Ferreira, I.S.; Mussatto, S.I.; Roberto, I.C. Alkaline deacetylation as a strategy to improve sugars recovery and ethanol production from rice straw hemicellulose and cellulose. *Ind. Crops Prod.* **2017**, *106*, 65–73. [CrossRef]
13. Salvachúa, D.; Mohagheghi, A.; Smith, H.; Bradfield, M.F.A.; Nicol, W.; Black, B.A.; Biddy, M.J.; Dowe, N.; Beckham, G.T. Succinic acid production on xylose-enriched biorefinery streams by *Actinobacillus succinogenes* in batch fermentation. *Biotechnol. Biofuels* **2016**, *9*, 28. [CrossRef] [PubMed]
14. Lima, C.S.; Neitzel, T.; de Oliveira Pereira, I.; Rabelo, S.C.; Ienczak, J.L.; Roberto, I.C.; Rocha, G.J. Effect of the sugarcane bagasse deacetylation in the pentoses fermentation process. *BioEnergy Res.* **2021**, *14*, 1171–1183. [CrossRef]
15. Hariz, H.B.; Zaidi, S.A.S.; Luthfi, A.A.I.; Bukhari, N.A.; Sajab, M.S.; Markom, M.; Harun, S.; Tan, J.P.; Ding, G.-T.; Abdul, P.M. Succinic Acid Production from Oil Palm Biomass: A Prospective Plastic Pollution Solution. *Fermentation* **2023**, *9*, 46. [CrossRef]
16. Pulingam, T.; Lakshmanan, M.; Chuah, J.-A.; Surendran, A.; Zainab-L, I.; Foroozandeh, P.; Uke, A.; Kosugi, A.; Sudesh, K. Oil palm trunk waste: Environmental impacts and management strategies. *Ind. Crops Prod.* **2022**, *189*, 115827. [CrossRef]
17. Bukhari, N.A.; Jahim, J.M.; Loh, S.K.; Nasrin, A.B.; Luthfi, A.A.I. Response surface optimisation of enzymatically hydrolysed and dilute acid pretreated oil palm trunk bagasse for succinic acid production. *BioResources* **2019**, *14*, 1679–1693. [CrossRef]
18. Ishak, H.; Yoshida, H.; Muda, N.A.; Ismail, M.H.S.; Izhar, S. Rapid processing of abandoned oil palm trunks into sugars and organic acids by sub-critical water. *Processes* **2019**, *7*, 593. [CrossRef]
19. Narisetty, V.; Okibe, M.C.; Amulya, K.; Jokodola, E.O.; Coulon, F.; Tyagi, V.K.; Lens, P.N.; Parameswaran, B.; Kumar, V. Technological advancements in valorization of second generation (2G) feedstocks for bio-based succinic acid production. *Bioresour. Technol.* **2022**, *360*, 127513. [CrossRef]
20. Adney, B.; Baker, J. *Measurement of Cellulase Activities. NREL/TP-510-42628*; National Renewable Energy Laboratory: Golden, CO, USA, 2008.
21. Bukhari, N.A.; Loh, S.K.; Luthfi, A.A.I.; Abdul, P.M.; Nasrin, A.B.; Harun, S.; Jahim, J.M. Whole slurry saccharification of mild oxalic acid-pretreated oil palm trunk biomass improves succinic acid production. *Ind. Crops Prod.* **2021**, *171*, 113854. [CrossRef]
22. Chen, X.; Shekiro, J.; Pschorn, T.; Sabourin, M.; Tao, L.; Elander, R.; Park, S.; Jennings, E.; Nelson, R.; Trass, O. A highly efficient dilute alkali deacetylation and mechanical (disc) refining process for the conversion of renewable biomass to lower cost sugars. *Biotechnol. Biofuels* **2014**, *7*, 1–12. [CrossRef]
23. Shekiro, J.; Chen, X.; Smith, H.; Tucker, M.P. Development and characterization of a high-solids deacetylation process. *Sustain. Chem. Process.* **2016**, *4*, 1–10. [CrossRef]
24. Giannattasio, S.; Guaragnella, N.; Ždralević, M.; Marra, E. Molecular mechanisms of *Saccharomyces cerevisiae* stress adaptation and programmed cell death in response to acetic acid. *Front. Microbiol.* **2013**, *4*, 33. [CrossRef] [PubMed]
25. Agrawal, R.; Bhadana, B.; Mathur, A.; Kumar, R.; Gupta, R.P.; Satlewal, A. Improved enzymatic hydrolysis of pilot scale pretreated rice straw at high total solids loading. *Front. Energy Res.* **2018**, *6*, 115. [CrossRef]
26. Modenbach, A.A.; Nokes, S.E. Enzymatic hydrolysis of biomass at high-solids loadings—A review. *Biomass. Bioenerg.* **2013**, *56*, 526–544. [CrossRef]
27. Zhao, J.; Xu, Y.; Wang, W.; Griffin, J.; Wang, D. High Ethanol concentration (77 g/L) of industrial hemp biomass achieved through optimizing the relationship between ethanol yield/concentration and solid loading. *ACS Omega* **2020**, *5*, 21913–21921. [CrossRef]
28. Rosgaard, L.; Andric, P.; Dam-Johansen, K.; Pedersen, S.; Meyer, A.S. Effects of substrate loading on enzymatic hydrolysis and viscosity of pretreated barley straw. *Appl. Biochem. Biotechnol.* **2007**, *143*, 27–40. [CrossRef]