Production of succinic acid from pineapple peel waste

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Research

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Abstract

Background: Succinic acid is a crucial platform chemical for production of various industrially significant compounds. For a sustainable and eco-friendly process, succinic acid synthesis has been shifted towards the fermentative route using renewable biomass substrates. Pineapple consumption and processing generate an immense amount of waste from its non-edible peel portion. As a carbon source, pineapple peel can be valorized for succinic acid bioproduction.

Results: The hydrothermal pretreatment (121°C, 15 min) of pineapple peel waste resulted in the highest sugar release of 35.22 g/L (18 g/L glucose and 17 g/L fructose). The subsequent fermentation of pineapple peel hydrolysate was performed by a natural succinic acid producer, *Actinobacillus succinogenes* TISTR 1994. When the non-detoxified hydrolysate was used as a sole carbon source, 6.21 g/L of succinic acid was produced from 26.16 g/L of sugars. Additional supplementation of 9 g/L mixed nitrogen source enhanced the formation of succinic acid to 9.96 g/L from roughly the same amount of sugar. The current production conditions using mainly

hydrolysate-based medium gave the succinic acid yield of 0.39 g/g sugar suggesting feasibilities for further improvement.

Conclusion: Bio-based succinic acid production was attempted for the first time using the solid pineapple waste as a main starting material. Results demonstrated a proof of concept that the abundant pineapple peel waste can serve as a renewable substrate for a low-cost, value-added bioconversion to succinic acid. Optimization of nutritional composition in hydrolysate is necessary to enhance the yield of succinic acid in future studies.

Keywords: Succinic acid, Fermentation, *Actinobacillus succinogenes*, Pineapple peel waste

Background

Succinic acid ($C_4H_6O_4$) has gained the spotlight over the past decades as a versatile molecule for various industrial applications. In food and beverage industry, succinic acid is used as a flavoring agent, an acidity regulator, and an antimicrobial agent. It also plays a role in pharmaceutical industry in the production of amino acids, vitamins and antibiotics (Saxena et al. 2017). More importantly, the structure of succinic acid serves as a building block for synthesis of commercially valuable chemicals. Via hydrogenation reactions, succinic acid can be converted to γ-butyrolactone (GBL), 1,4-butanediol (BDO) and tetrahydrofuran (THF) (Kang et al. 2015). GBL and THF are widely used as solvents and cleaners for many industrial purposes. BDO has uses in the manufacture of spandex fibers and polyurethanes. More recently, the exploitation of BDO and succinic acid for the production of biodegradable polymer, polybutylene succinate (PBS), has received increasing attention as the eco-friendly plastic alternative. Being the compound of unlimited business potentials, succinic acid was ranked in the top twelve platform chemicals by the U.S. Department of Energy in 2004 (Werpy and Petersen 2004).

Pineapple (*Ananas comosus* L. Merr.) has long been one of the most profitable crops in many countries in the tropical zone. It is either consumed fresh or processed as canned pineapple, pineapple jam, pineapple juice, etc. In whichever form pineapple may be eaten, only the yellow flesh portion is utilized accounting for 40% of the total fruit (Sukruansuwan and Napathorn 2018). This creates about 60% of the waste from the unused peel and core portion per one fruit. Considering the global pineapple production projected to reach 31 million tonnes by 2028, the greater proportion of waste to deal with can be expected in the coming years (FAO 2020). Traditionally, pineapple waste is taken care of by municipality and sent to incineration. Rural people make use of the waste by turning it into compost for gardening. Moreover, the waste is applied as animal feed, and otherwise dumped in landfills, where bacterial decomposition takes place and emits methane, a potent greenhouse gas (Namsree et al. 2012).

The microbial biotransformation of pineapple waste into value-added products has emerged as the superior option to dispose of the waste. First, it generates more income for pineapple processors from converting their production residues into beneficial products. Second, the biosynthetic process in a controlled setting is usually more innocuous with less hazardous byproduct released into the environment. The composition of pineapple wastes includes 17.2% (w/v) α-cellulose, 12.3% (w/v) hemicellulose, and 1.8% (w/v) lignin for the core section, and 22.9% (w/v) α-cellulose, 13.9% (w/v) hemicellulose, and 5.1% (w/v) lignin for the peel section (Sukruansuwan and Napathorn 2018). These lignocellulosic components are subjected to physical, biological, chemical or enzymatic pretreatment to obtain fermentable carbohydrates. Glucose, xylose, fructose, arabinose and galactose are major sugars found in pineapple peel hydrolysate in diverse quantities depending on hydrolysis conditions. They are crucial carbon sources for microbial growth and product formation in the fermentation process. Previous

researches have demonstrated the feasibility of pineapple waste as a starting material for the biosynthesis of industrially significant chemicals, such as citric acid, lactic acid, ethanol, lipid, biohydrogen and polyhydroxybutyrate (Imandi et al. 2008; Abdullah and Sodarto 2007; Soontornchaiboon et al. 2016; Tinoi and Rakariyatham 2016; Cahyari et al. 2018; Sukruansuwan and Napathorn 2018).

In regard to succinic acid, it is conventionally synthesized through chemical reactions from petroleum-derived maleic anhydride as a starting material. This petrochemical approach is not sustainable in the long run due to the gradual scarcity of petroleum as well as the high-cost and pollution-generating procedures (Liu et al. 2008). Accordingly, the manufacture of succinic acid has been geared towards the biological route using microbial cell factories. The advantages of fermentative production of succinic acid comprise utilization of renewable resources and reduction of the air pollutant through carbon dioxide fixation pathway (Willke and Vorlop 2014). The biotechnological production of succinic acid has been explored extensively using various biomass and agro-residues as precursors (e.g., sake lees, cane molasses, corn stover, corncob, bagasse, cassava root) (Yang et al. 2019). However, only few attempts have been made using pineapple waste as the substrate for succinic acid production. Jusoh et al. (2014) acquired liquid pineapple waste from the canned pineapple factory and used the waste as a substrate for succinic acid fermentation. The sugar composition of the liquid waste consisted of 40.23 g/L sucrose, 26.33 g/L glucose and 40.27 g/L fructose. Under aerobic condition, 6.26 g/L of succinic acid was produced by *Escherichia coli* AFP 184 from the liquid pineapple waste. Another study by Ferone et al. (2019) was also conducted on the liquid waste from pineapple juice processing plant. Pineapple juice containing roughly 34 g/L glucose, 12 g/L fructose and 10 g/L sucrose was used as carbon sources for succinic acid fermentation. After 400 h of anaerobic fermentation, 38 g/L

of succinic acid was produced by *Actinobacillus succinogenes* DSM 22257. From a different starting material, Dessie et al. (2018) investigated the possibility of solid fruit and vegetable wastes as substrates for succinic acid bioproduction. Biological hydrolysis of mixed fruit and vegetable wastes (e.g., pineapple, banana, orange, onion, potato) was performed by *Aspergillus niger* and *Rhizopus oryzae* to release fermentable sugars. The biotransformation of the mixed solid waste hydrolysate (12 g/L glucose and 13.83 g/L fructose) into succinic acid was accomplished by *Actinobacillus succinogenes* NJ113. Under anaerobic carbon dioxide-enriched condition, 27 g/L of succinic acid was achieved within 40 h of fermentation.

In the current study, a divergent source of precursors, solid pineapple waste, specifically the peel portion, was employed for bio-based succinic acid production. The peel waste was collected after pineapple peeling and trimming from a market where pineapple was sold in a ready-to-eat, fresh-cut form. The effect of acid hydrolysis conditions on the release of fermentable sugars from the waste was examined. In fermentation experiments, the effect of nutrient supplementation on succinic acid formation using pineapple waste hydrolysate as a main carbon source was studied. *Actinobacillus succinogenes*, the best-known natural producer of succinic acid, was selected as a workhorse microbe for this purpose to evaluate the efficiency of pineapple peel waste as a starting biomass for succinic acid biosynthesis.

Methods

Preparation of pineapple peel powder

Pineapple peel waste, a remainder after fresh-cut pineapple preparation, was collected from a local market. The fruit peels were briefly washed with tap water to get rid of dirts and contaminants. The peels were roughly chopped up into shorter and thinner pieces in order to

decrease the subsequent dehydration time. The peel pieces were then put in a hot air oven at 70°C for 12 h. The dried peels were ground using a kitchen blender. Finally, the pineapple peel powder was sifted through a 40-mesh sieve to get fine powder particles and stored in a desiccator until use.

Hydrolysis of pineapple peel powder

The peel powder underwent hydrolysis using diluted acid and heat to convert cellulose and hemicellulose into fermentable carbohydrates. The powder was suspended in acid solutions (0, 1.5 and 3% (v/v) sulfuric acid) with a solid:liquid ratio of 1:10 (g/mL). The powder suspension was subjected to heat treatment (105 and 121°C) using autoclave for 15 min. The acid pretreatment conditions were adapted from Tinoi and Rakariyatham (2016) and Sukruansuwan and Napathorn (2018). Afterwards, the whole mixture was centrifuged at 9,000 rpm for 10 min to separate the pineapple peel hydrolysate from the peel residues. The liquid hydrolysate was strained through four layers of cheese cloth to remove light particles. The hydrolysate was pH-adjusted to 7 using 6 M sodium hydroxide and preserved at 4°C prior to use.

Determination of fermentable sugar composition in pineapple peel hydrolysate

The peel hydrolysate was filtered through a 0.45 µm syringe filter. The filtered sample was then analyzed for sugar composition (glucose, xylose and fructose) using the High Performance Liquid Chromatography (HPLC) (Shimadzu, Japan). The instrument is equipped with two LC-20AD pumps, a DGU-20A5 degasser, an SIL-20AC autosampler, a refractive index detector (RID-10A), and an Inertsil NH2 column (5 µm particle size, 250 mm x 4.6 mm, GL Sciences Inc., Japan). The analysis conditions modified from Veena et al. (2018) included 40°C

RID temperature, 40°C column temperature, acetonitrile:water (85:15, v/v) mobile phase filtered through a 0.22 µm filter, 0.5 mL/min flow rate, and 10 µL injection volume.

Preculture conditions

Actinobacillus succinogenes TISTR 1994 was obtained from Thailand Institute of Scientific and Technological Research and preserved as a glycerol stock at -80°C. The stock culture (2% v/v) was transferred into Tryptic Soy Broth (TSB) supplemented with 10 g/L glucose. A 100-mL serum bottle was used for culture cultivation. It was sealed with a rubber septum and an aluminum cap to maintain anaerobic condition. Additionally, carbon dioxide was flushed into the bottle headspace for 5 min. The culture bottle was placed in a shaking incubator at 37°C, 100 rpm for 36-48 h. The grown culture was transferred again to a new medium bottle and incubated at the same conditions for 24 h to get the inoculum for main fermentation.

Succinic acid production from pineapple peel hydrolysate

The medium components for the main fermentation were 0.2x TSB (6 g/L TSB) plus pineaaple peel hydrolysate from each hydrolysis condition. The fermentation was carried out in a serum bottle and maintained in anaerobic condition using a sealed cap and carbon dioxide flushing identical to the preculture. The *A. succinogenes* inoculum from the preculture step (2% v/v) was injected into the fermentation bottle. The culture bottle was placed in a shaking incubator at 37°C, 100 rpm for 36 h. Sampling was performed at 0, 22 and 36 h to measure succinic acid and sugar concentrations over the course of fermentation.

Effect of nutrient supplementation on succinic acid production

The influence of medium composition on succinic acid formation was studied using the best hydrolysate yielding the highest succinic acid in the previous experiment. Five fermentation conditions were tested including (1) TSB supplemented with the same amounts of glucose and fructose as contained in the hydrolysate, (2) hydrolysate only, (3) hydrolysate plus 0.2x TSB, (4) hydrolysate plus 0.2x TSB and 5 g/L yeast extract, and (5) hydrolysate plus 0.2x TSB and 40 g/L magnesium carbonate. All media types were put in serum bottles prepared for anaerobic fermentation in the same manner as the previous experiment. The *A. succinogenes* preculture (2% v/v) was injected into the medium bottle to begin fermentation. The culture bottles were incubated at 37°C, 100 rpm for 36 h. Sampling was performed at 0, 18 and 36 h to quantify succinic acid and sugars during fermentation.

Determination of succinic acid content in fermentation experiments

The sample taken from fermentation was centrifuged at 9,000 rpm for 15 min. The supernatant was filtered through a 0.45 µm syringe filter. The filtered sample was then analyzed for succinic acid concentration using the same HPLC (Shimadzu, Japan), but a photodiode array (PDA) detector (SPD-M20A) and an Inertsil ODS-3 column (5 µm particle size, 250 mm x 4.6 mm, GL Sciences Inc., Japan) were employed. The analysis conditions modified from Kuenz et al. (2020) consisted of a D2 lamp on PDA detector, 40°C column temperature, 0.1% metaphosphoric acid mobile phase filtered through a 0.22 µm filter, 0.8 mL/min flow rate, 10 µL injection volume, and a 210-nm wavelength.

Results and discussion

Determination of fermentable sugar composition in pineapple peel hydrolysate

185 The acid and thermal hydrolysis of pineapple peel powder was undertaken in six
186 conditions including water at 105°C, water at 121°C, 1.5% H_2SO_4 at 105°C, 3% H_2SO_4 at
187 105°C, 1.5% H_2SO_4 at 121°C, and 3% H_2SO_4 at 121°C. Each condition was held at the tested
188 temperature for 15 min. After hydrolysis, the hydrolysate (liquid fraction) was separated from
189 the solid residues. The pH of hydrolysate was adjusted to 7. The fermentable sugar content
190 released following hydrolysis was examined using HPLC (Fig. 1). According to the results, the
191 hydrolysis condition leading to the maximal release of fermentable sugars was the treatment with
192 just water at 121°C. Two types of sugar were detected consisting of 18.05 ± 1.63 g/L glucose and
193 17.17 ± 1.57 g/L fructose. Unsurprisingly, the mere water treatment was not sufficient to
194 discharge xylose. The hydrothermal treatment of pineapple waste was formerly carried out by
195 Tinoi and Rakariyatham (2015). Glucose and fructose were only sugars found in the hydrolysate,
196 but the concentrations of the two sugars were higher in the current study. In order to liberate
197 xylose, acid-associated pretreatment was necessary. Acid catalyzes the breakage of
198 hemicellulose polymer resulting in higher yields of its corresponding sugar monomers (Wyman
199 et al. 2005). In our experiments, xylose was present in all acid-treated hydrolysate regardless of
200 the acid concentration. The greatest xylose liberation of 6.69 ± 0.71 and 6.64 ± 0.37 g/L was
201 achieved via pretreatment at 121°C coupled with 1.5% and 3% H_2SO_4, respectively.
202 The summary of reducing sugars and sugar yields derived from each hydrolysis condition
203 was displayed in Table 1. The combined amount of sugars was calculated from xylose, fructose
204 and glucose peaks shown on HPLC chromatogram equipped with a refractive index detector
205 (RID). However, more sugar types such as galactose and arabinose were additionally detected in
206 pineapple peel hydrolysate by Sukruansuwan and Napathorn (2018). One of the reasons for this
207 inconsistency could be due to the difference in HPLC detectors employed in the analysis. In their

study, the evaporative light scattering detector (ELSD) was utilized which is normally perceived to be a superior detector to RID in the aspect of sensitivity and stability (Ganzera and Stuppner, 2005). This suggested that the total sugars existing in the hydrolysate could be more diverse in the reality. Therefore, the values of fermentable sugars shown in Table 1 represented the minimal sugar concentration identifiable in the current analysis condition. Considering the sugar content and yield, the hydrothermal treatment at 121°C was the best condition contributing to 35.22 ± 3.20 g/L of fermentation sugars and 0.27 ± 0.02 g/g of sugar yield from hydrolysis.

Succinic acid production from pineapple peel hydrolysate

Pineapple peel hydrolysate from six pretreatment conditions was employed as the main carbon source for succinic acid biosynthesis by *A. succinogenes*. In all fermentation bottles, 0.2x TSB equal to 4 g/L of nitrogen source (tryptone and soy peptone) was supplemented in addition to the hydrolysate. To achieve anaerobic condition, a serum bottle tightly sealed with a rubber septum and an aluminum cap was used as a fermentation container. Moreover, carbon dioxide was flushed into the bottle headspace for 5 min to ensure a carbon dioxide-saturated gas phase. This gas plays an important role in succinic acid bioproduction. It functions as a co-substrate which directs the metabolic flux of carbon towards carbon dioxide-fixing pathway encouraging the conversion to succinic acid (Zou et al. 2011). The titer of succinic acid was compared among six types of hydrolysate fermentation. Unfortunately, the fermentation of hydrolysates derived from acid-containing pretreatment resulted in low quantities of succinic acid (less than 2 g/L) in all conditions (data not shown). It is possible that toxic byproducts such as furfural, 5-hydroxymethylfurfural (5-HMF) and levulinic acid were generated during hydrolysis more than anticipated. These toxic compounds are products of monosaccharide-degradation reactions

occurred in the presence of acid and heat. They negatively affect sugar yields and microbial growth in the subsequent fermentation step also known as fermentation inhibitors (Wyman et al. 2005). Nevertheless, the amounts of these post-hydrolysis toxic products were not measured in this study. According to Sukruansuwan and Napathorn (2018), analysis of pineapple peel acid-hydrolysate revealed low concentrations of inhibitor compounds (less than 2 g/L). To aim for a reasonable processing cost in light of a large-scale production, detoxification procedures were deliberately skipped for a simplified biosynthetic process of succinic acid. In future work, the content of toxic compounds formed after the biomass hydrolysis step should still be determined to ensure an effective bioconversion of sugars to succinic acid in the following step.

Fig. 2 shows changes in levels of sugars and succinic acid over the course of fermentation of hydrolysates produced from hydrothermal pretreatment. Only fermentation profiles of hydrolysates extracted with water and heat were illustrated here because acid-treated hydrolysates resulted in a small amount of produced succinic acid (less than 2 g/L) as mentioned earlier. Moreover, hydrothermal treatment normally leads to the decreased production of toxic byproducts due to the absence of acid catalyzing the sugar degradation reaction. As expected, the hydrolysate prepared using water at 121°C contributed to the highest titer of succinic acid (8.97 ± 0.61 g/L) in the current fermentation condition. This could be straightforwardly explained by the greater amount of initial sugar components (15.87 ± 1.40 g/L glucose and 14.67 ± 1.35 g/L fructose) in 121°C-pretreated hydrolysate.

Effect of nutrient supplementation in hydrolysate on succinic acid production

The best pineapple peel hydrolysate for succinic acid production was acquired from the hydrothermal method at 121°C. This hydrolysate comprised the highest fermentable sugars

leading to the highest succinic acid titer from the previous experiment. Consequently, the 121°C-pretreated hydrolysate was selected as a fermentation medium base which was also a main carbon source for the next experiment. To investigate the effect of nutrient availability on succinic acid biosynthesis, different levels and types of nutrients in a fermentation medium were compared. Five scenarios of nutrient availability were designed for the fermentation including (1) TSB supplemented with the same amounts of glucose and fructose as contained in the hydrolysate, (2) hydrolysate only, (3) hydrolysate plus 0.2x TSB, (4) hydrolysate plus 0.2x TSB and 5 g/L yeast extract, and (5) hydrolysate plus 0.2x TSB and 40 g/L magnesium carbonate.

Fig. 3 displays the concentrations of sugars and succinic acid over the course of fermentation with varying nutrients. As expected, the highest succinic acid content of 14.59 ± 0.23 g/L was achieved by *A. succinogenes* fermentation in the complex medium, TSB. In this rich medium condition, a sufficient nitrogen source (20 g/L of tryptone and soy peptone) was supplied. In view of carbon source, TSB already had 2.5 g/L of glucose in its ingredients so more glucose was added to match the glucose amount in the hydrolysate (13.3 g/L). Likewise, fructose was supplemented to match the fructose content in the hydrolysate (12.6 g/L). The TSB condition was tested to see how a nutritious medium rich in nitrogen source affected the formation of succinic acid as compared with a hydrolysate medium. Although it is a widely-used medium in a laboratory setting, it is not a cost-effective option for a large-scale production. Agricultural byproducts, such as corn steep liquor, soybean meal and pharmamedia, which are substantially cheaper may be applied as nitrogen sources for a low-cost manufacture of succinic acid. In the fermentation of mere hydrolysate, 6.21 ± 0.35 g/L of succinic acid was produced despite the lowest nutritional level (no added nitrogen source). As the nitrogen source increased, succinic acid production was improved. 7.60 ± 0.30 g/L and 9.96 ± 0.55 g/L of succinic acid

were gained in the hydrolysates supplemented with 0.2x TSB (4 g/L nitrogen source) and 0.2x TSB plus 5 g/L yeast extract (9 g/L of combined nitrogen sources), respectively. Apart from carbon and nitrogen sources, magnesium carbonate ($MgCO_3$) has been shown to play a significant role in promoting succinic acid biosynthesis (Zou et al. 2011; Zhang et al. 2012; Zhu et al. 2012). Magnesium carbonate assists in regulating the pH of fermentation, providing magnesium ions for boosting the activity of phosphoenolpyruvate carboxykinase, a key enzyme in the carboxylation pathway towards succinic acid synthesis, and serving as a carbon dioxide donor which could replace gaseous carbon dioxide when it was fully supplied (Zhang et al. 2009; Zou et al. 2011; Zhang et al. 2012). In this study, a single dose of magnesium carbonate at 40 g/L was supplemented to the hydrolysate containing 4 g/L nitrogen source. 8.56 ± 0.74 g/L of succinic acid titer was obtained which was slightly enhanced from the hydrolysate without magnesium carbonate. Still, the improvement in succinic acid production from magnesium carbonate addition was not as great as that from yeast extract addition. Zhang et al. (2012) mentioned that the influence of magnesium carbonate on succinic acid concentration was not apparent when the glucose level was relatively low. This was seemingly the case in the present study where the initial sugars were rather low (around 25 g/L). In nutrient-restricted condition, the boost of yeast extract could be more significant as it directly provides nutrients such as amino acids and various B vitamins aiding in microbial growth (Zhu et al. 2012).

Table 2 summarizes the concentrations of fermentable sugars and succinic acid as well as the yields of succinic acid obtained from each culture condition. The highest yield of succinic acid (0.56 ± 0.03 g/g) was attained in the complex medium (TSB) fermentation. While the sole hydrolysate condition led to the lowest yield of succinic acid (0.24 ± 0.01 g/g), it acted as a bare minimum of succinic acid from pineapple peel hydrolysate fermentation. This information would

be useful for further enhancement of succinic acid formation from pineapple peel waste. As shown in this study, the yield of succinic acid could be increased with additional nutrients in yeast extract (0.39 ± 0.03 g/g). In future work, inexpensive substrates from agricultural residues should be applied to adjust the nutritional composition of hydrolysate medium in order to improve the amount of succinic acid and keep the raw material cost as low as possible for a large-scale production. Various conditions of fermentation media should be attempted to find the most crucial elements promoting succinic acid production. Considering the theoretical yield of succinic acid of 1.12 g/g sugar (Nghiem et al. 2017), there is a lot of room for improvement for succinic acid biosynthesis from pineapple peel waste.

Conclusions

To our knowledge, this is the first study that the solid pineapple peel waste was solely used as the starting material for succinic acid production. The best hydrolysis conditions for pineapple peel waste were the hydrothermal treatment at 121°C resulting in the highest sugar release of 35.22 g/L. When 4 g/L of nitrogen source was added to the non-detoxified pineapple peel hydrolysate, 8.97 g/L of succinic acid was produced by *A. succinogenes* from 30.55 g/L of starting sugars. Succinic acid biosynthesis was enhanced with further supplementation of nutrients (5 g/L of yeast extract). 9.96 g/L of succinic acid was achieved in the presence of 25.77 g/L of fermentable sugars. Addition of 40 g/L magnesium carbonate as a pH controller and a carbon dioxide donor did not contribute to more succinic acid than nutrient supplementation in tested conditions. The highest yield of succinic acid was 0.39 g/g obtained from the hydrolysate with 4 g/L of combined tryptone/soy peptone and 5 g/L yeast extract. Results suggested a large room for improvement and indicated the plausibility of solid pineapple peel waste as a starting

biomass for production of succinic acid. Optimization of nutrients in hydrolysate is required to enhance the yield of bio-based succinic acid.

Authors' contributions

PP and CH designed the experiments; PP performed the research experiments; CH helped in manuscript preparation; PP analyzed the data; PP wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analyzed during this study are included in this article.

Consent for publication

All authors have read and approved to submit the paper to Bioresources and Bioprocessing.

Ethics approval and consent to participate

Not applicable.

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Fig. 1 Concentrations of glucose, xylose and fructose in pineapple peel hydrolysate derived from different hydrolysis conditions (W105: water at 105°C, W121: water at 121°C, 1.5S-105: 1.5% H_2SO_4 at 105°C, 3S-105: 3% H_2SO_4 at 105°C, 1.5S-121: 1.5% H_2SO_4 at 121°C, 3S-121: 3% H_2SO_4 at 121°C). The error bars represent the standard deviations (n = 3).

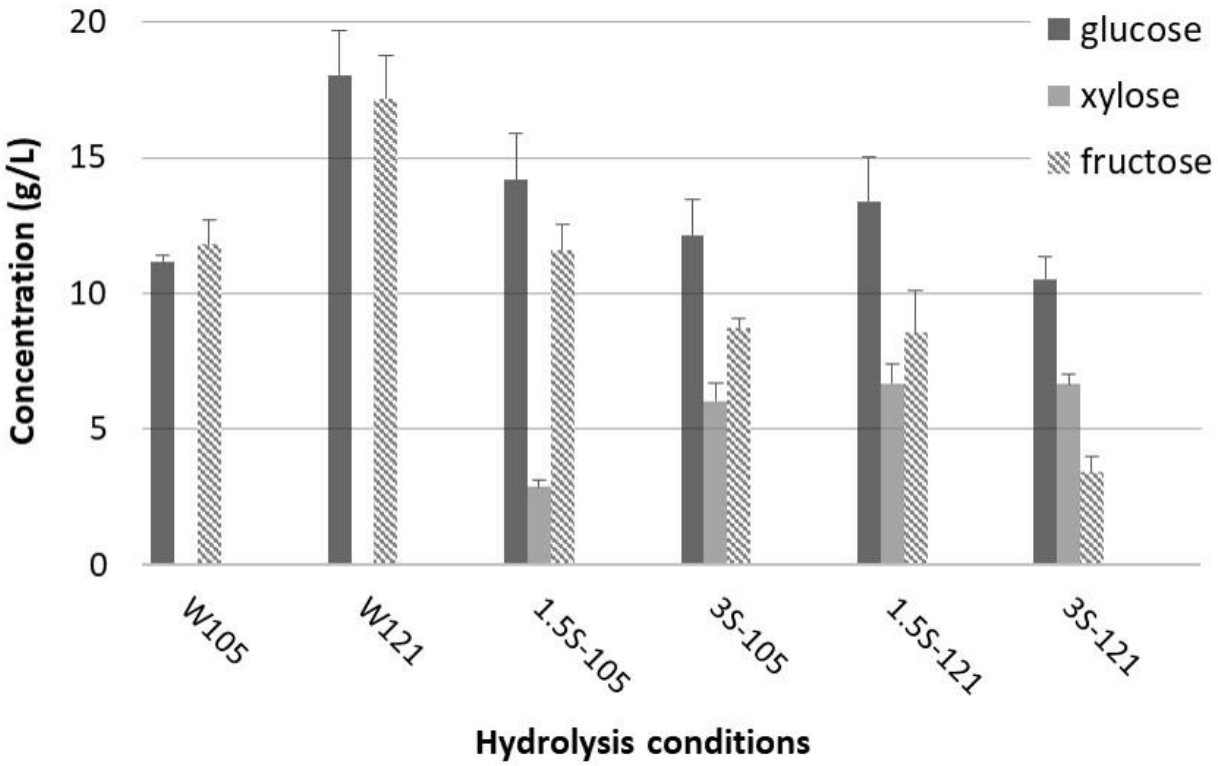

Table 1 Concentrations and yields of fermentable sugars derived from different hydrolysis conditions. Results are expressed as means ± standard deviations from three replicates. Numbers with different superscripts in the same column are statistically different ($p \leq 0.05$).

Hydrolysis conditions	**Fermentable sugars (g/L)** (glucose+xylose+fructose)	**Yield (g/g)** (fermentable sugars/ pineapple peel powder)
water, 105°C	22.94 ± 1.17^{bc}	0.18 ± 0.01^{bc}
water, 121°C	35.22 ± 3.20^{a}	0.27 ± 0.02^{a}
1.5% H_2SO_4, 105°C	28.68 ± 2.88^{ab}	0.22 ± 0.02^{ab}
3% H_2SO_4, 105°C	26.91 ± 2.31^{bc}	0.20 ± 0.01^{bc}
1.5% H_2SO_4, 121°C	28.65 ± 3.89^{ab}	0.22 ± 0.04^{ab}
3% H_2SO_4, 121°C	20.59 ± 1.78^{c}	0.15 ± 0.01^{c}

Fig. 2 Concentrations of glucose, fructose and succinic acid over the course of fermentation using hydrothermal-pretreated hydrolysates at 105°C and 121°C. The error bars represent the standard deviations (n = 3).

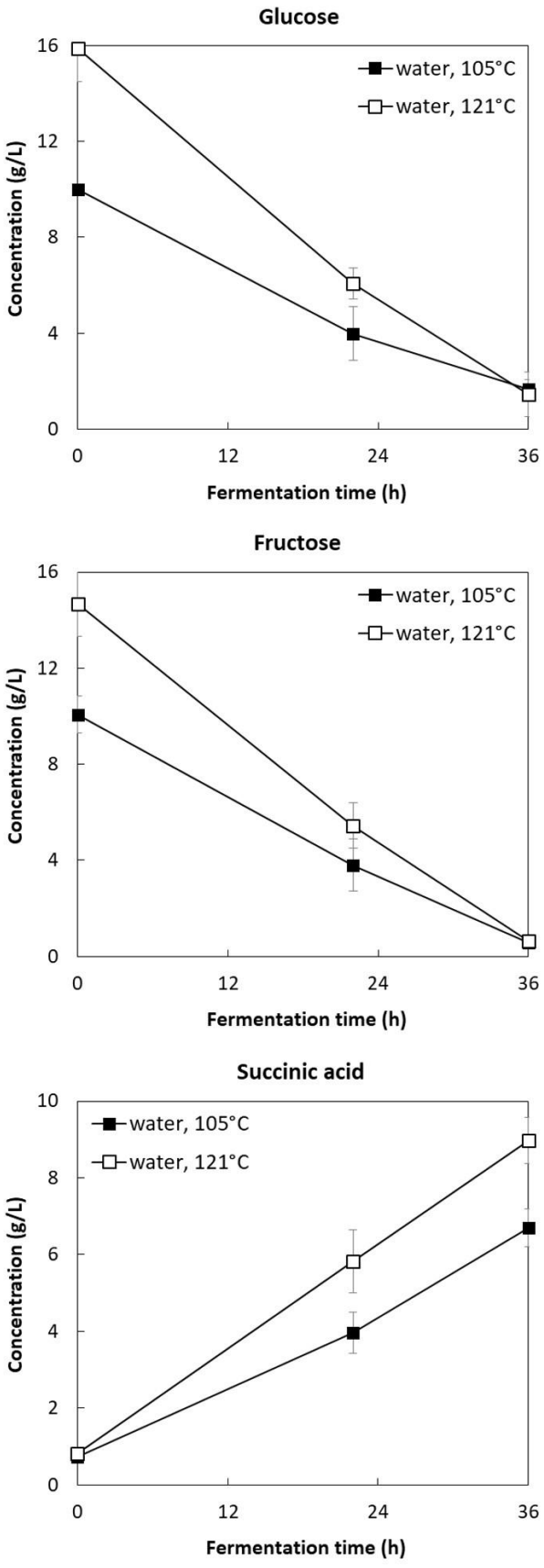

Fig. 3 Concentrations of glucose, fructose and succinic acid over the course of fermentation using hydrothermal (121°C)-pretreated hydrolysate in the presence of varied nutritional content (TSB: Tryptic Soy Broth, hyd: hydrolysate, 0.2x TSB: 6 g/L Tryptic Soy Broth, YE: 5 g/L yeast extract, MC: 40 g/L magnesium carbonate). The error bars represent the standard deviations (n = 3).

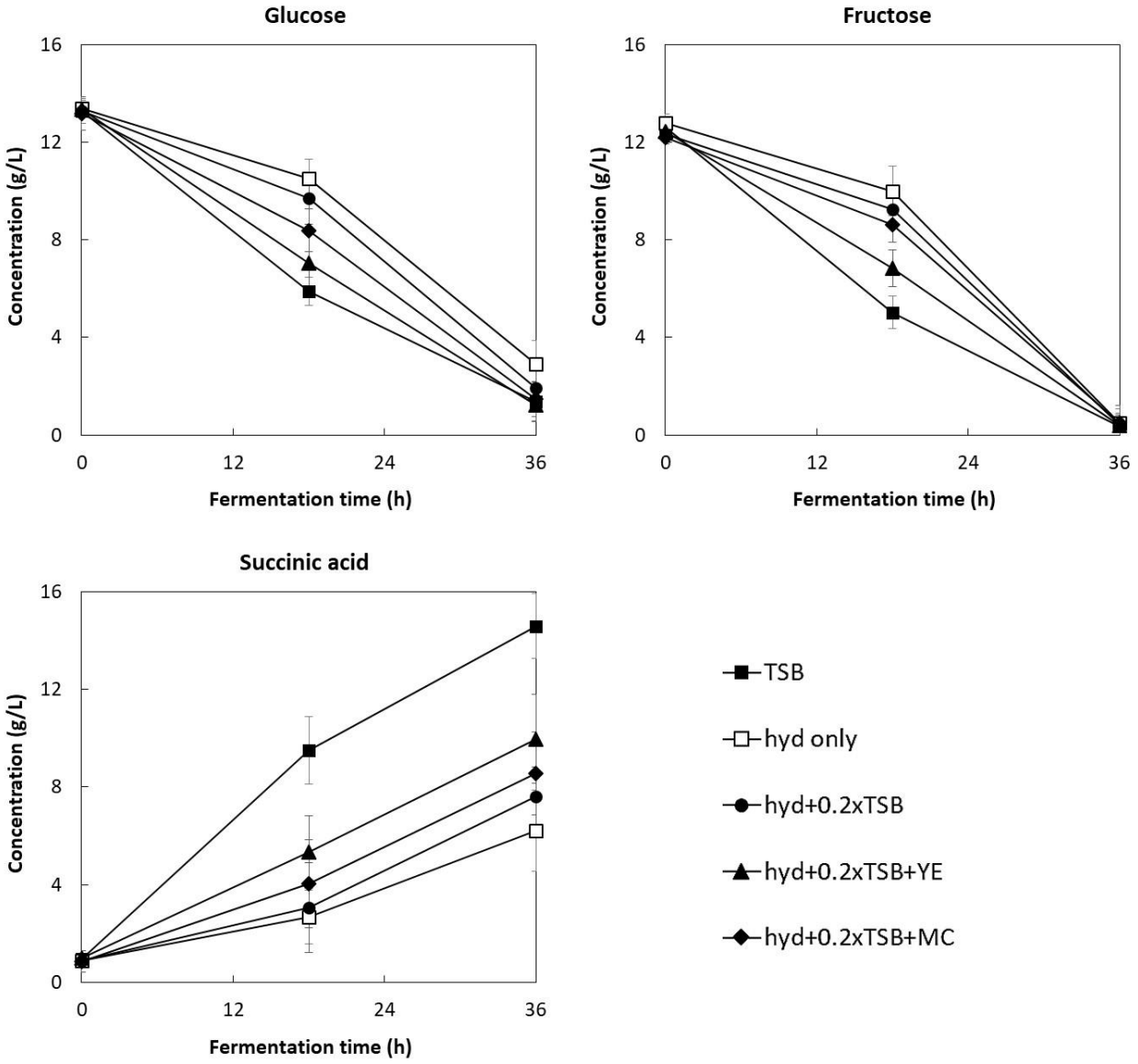

Table 2 Concentrations of fermentable sugars and succinic acid, and yields of succinic acid derived from hydrothermal (121°C)-pretreated hydrolysate fermentation in the presence of varied nutritional content (0.2x TSB: 6 g/L Tryptic Soy Broth, YE: 5 g/L yeast extract, MC: 40 g/L magnesium carbonate). Results are expressed as means ± standard deviations from three replicates. Numbers with different superscripts in the same column are statistically different ($p \leq 0.05$). A column with an ns superscript means that all numbers in that column are not significantly different ($p > 0.05$).

Culture conditions	**Fermentable sugars^{ns} (g/L)** (glucose+fructose)	**Succinic acid (g/L)**	**Yield (g/g)** (succinic acid/ fermentable sugars)
TSB (for comparison)	25.88 ± 0.91	14.59 ± 0.23^{a}	0.56 ± 0.03^{a}
hydrolysate only	26.16 ± 0.69	6.21 ± 0.35^{d}	0.24 ± 0.01^{d}
hydrolysate + 0.2x TSB	25.62 ± 0.86	7.60 ± 0.30^{c}	0.29 ± 0.02^{cd}
hydrolysate + 0.2x TSB + YE	25.77 ± 0.62	9.96 ± 0.55^{b}	0.39 ± 0.03^{b}
hydrolysate + 0.2x TSB + MC	25.36 ± 0.66	8.56 ± 0.74^{c}	0.34 ± 0.04^{bc}

Figures

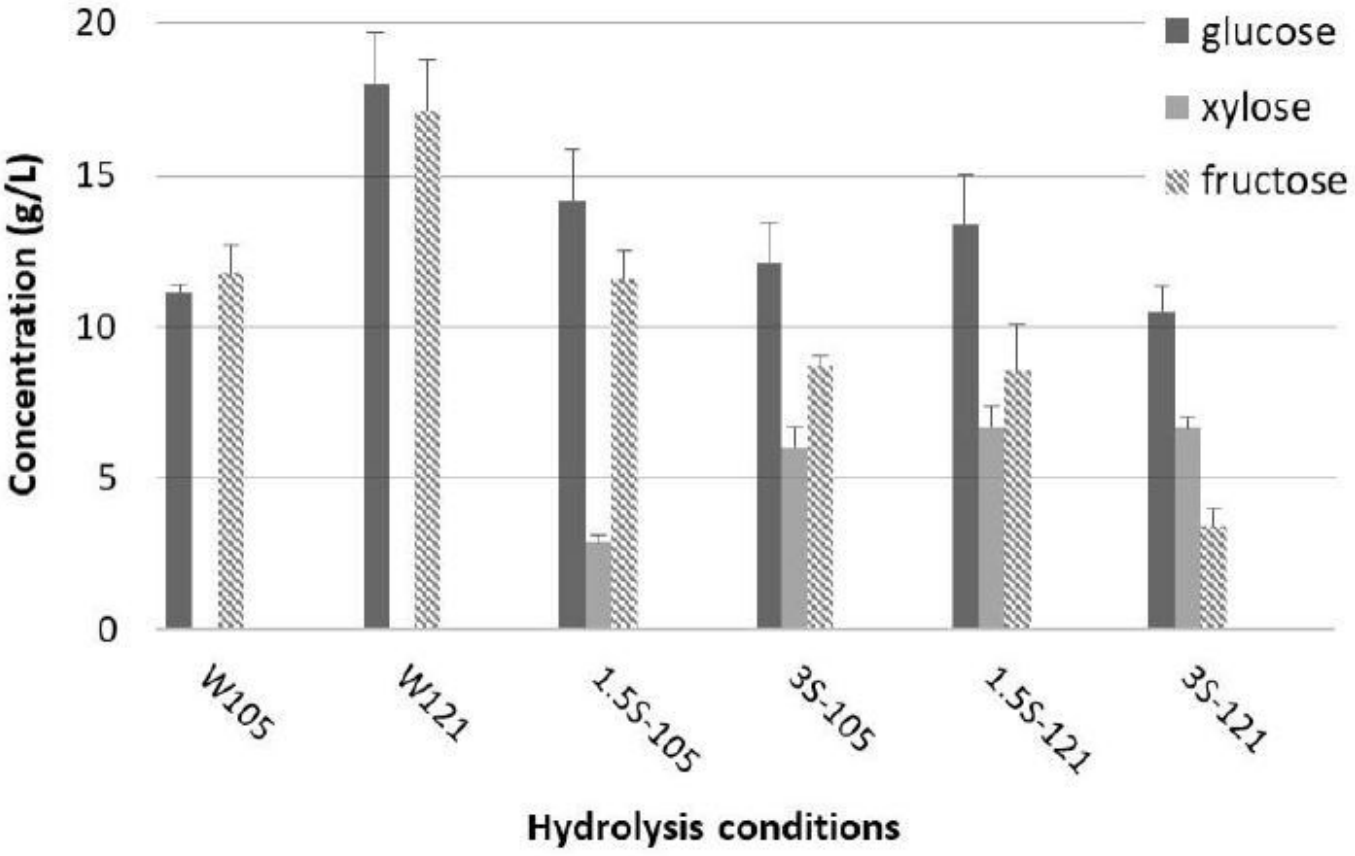

Figure 1

Concentrations of glucose, xylose and fructose in pineapple peel hydrolysate derived from different hydrolysis conditions (W105: water at 105°C, W121: water at 121°C, 1.5S-105: 1.5% H2SO4 at 105°C, 3S-105: 3% H2SO4 at 105°C, 1.5S-121: 1.5% H2SO4 at 121°C, 3S-121: 3% H2SO4 at 121°C). The error bars represent the standard deviations (n = 3).

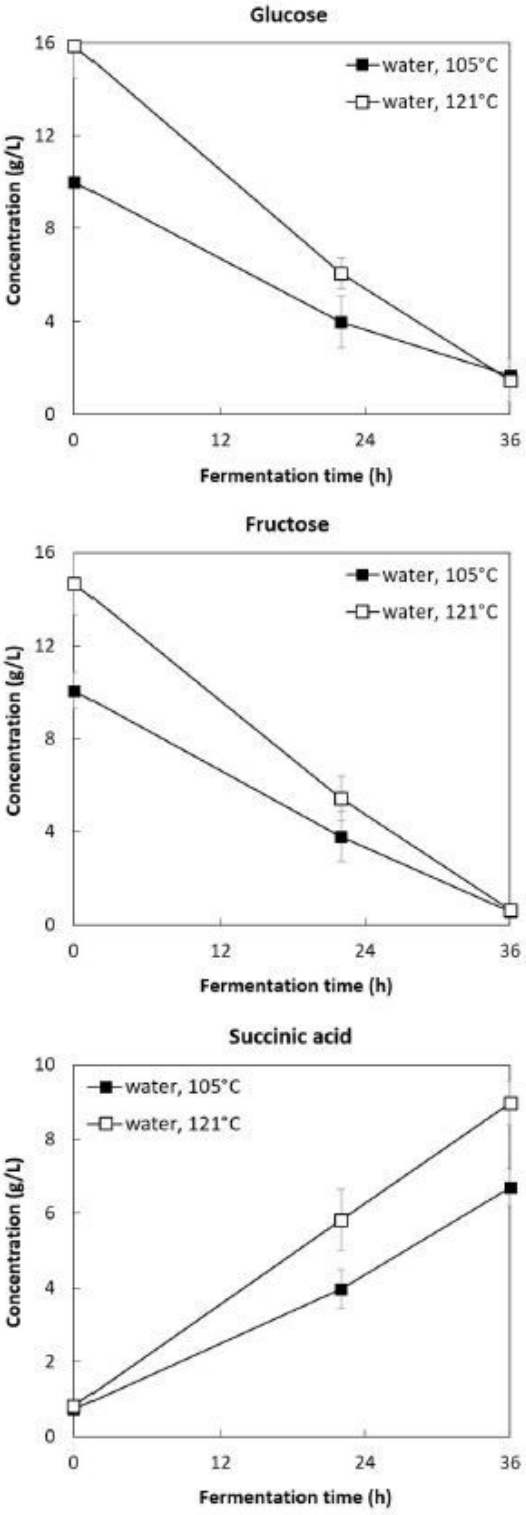

Figure 2

Concentrations of glucose, fructose and succinic acid over the course of fermentation using hydrothermal-pretreated hydrolysates at 105°C and 121°C. The error bars represent the standard deviations (n = 3).

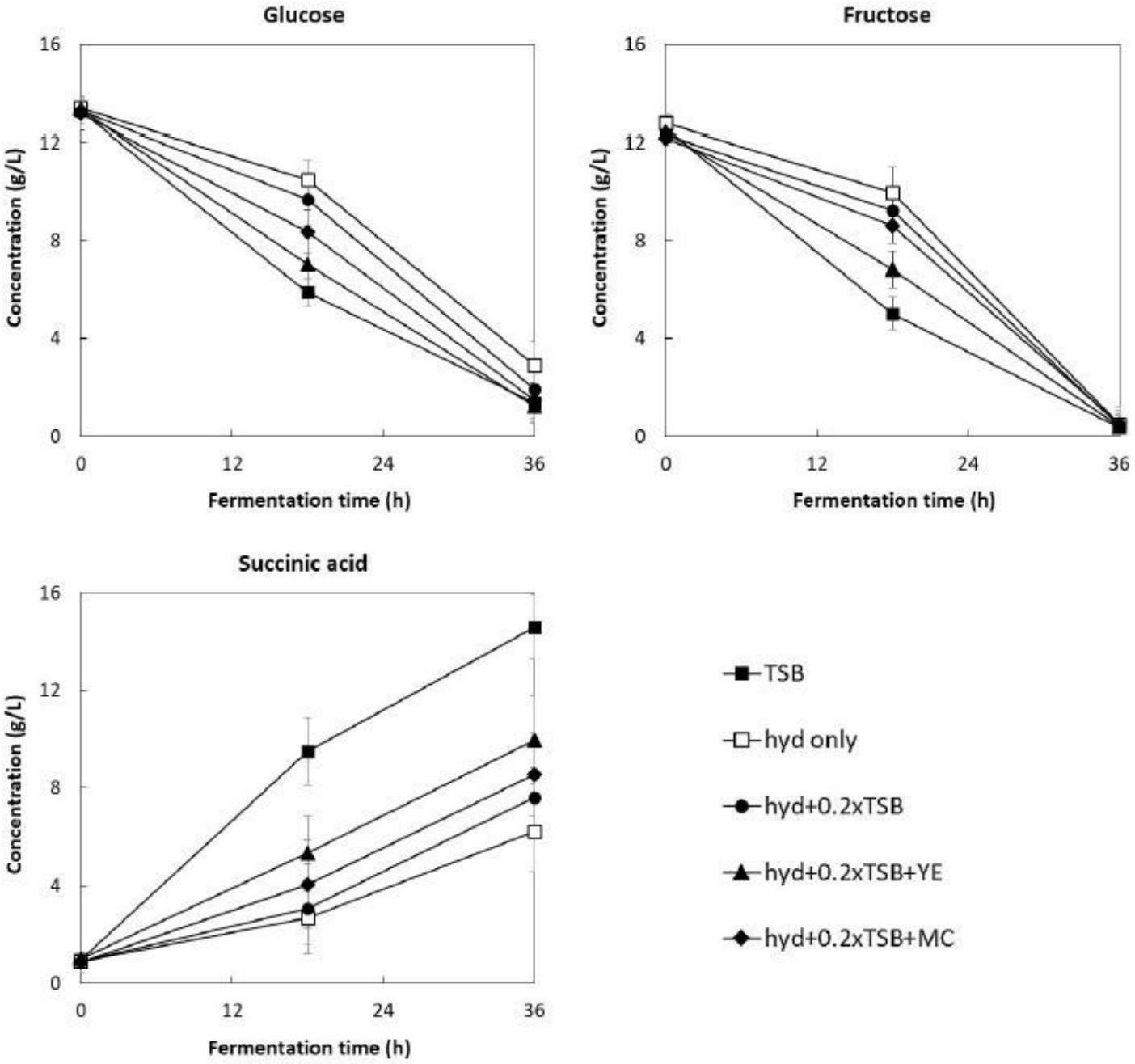

Figure 3

Concentrations of glucose, fructose and succinic acid over the course of fermentation using hydrothermal (121°C)-pretreated hydrolysate in the presence of varied nutritional content (TSB: Tryptic Soy Broth, hyd: hydrolysate, 0.2x TSB: 6 g/L Tryptic Soy Broth, YE: 5 g/L yeast extract, MC: 40 g/L magnesium carbonate). The error bars represent the standard deviations (n = 3).

Supplementary Files

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