Producing Ethanol from Water Hyacinth through Simultaneous Saccharification and Fermentation with Acclimatized Yeasts

Jun Cheng,* Xin Wang, Rui Huang, Maoling Liu, Cong Yu, and Kefa Cen

The production rate and yield of ethanol was markedly increased when water hyacinth, pretreated with microwave-assisted dilute acid, was fermented with acclimatized yeasts through simultaneous saccharification and fermentation. Water hyacinth hydrolyzate microwaved with 1% (v/v) dilute sulfuric acid was fermented with the acclimatized mixed yeasts *Pichia stipitis* and *Pachysolen tannophilus* at a volume ratio of 1:1. The highest ethanol yield was 0.22 g/g (raw biomass of water hyacinth), which was 76.3% of the theoretical ethanol yield. A maximum ethanol production rate of 0.19 g/(L·h) was obtained after 24 h.

Keywords: Water hyacinth; Ethanol; Microwave; Acclimation; Fermentation

*Contact information: State Key Laboratory of Clean Energy Utilization, Zhejiang University, Hangzhou 310027, China; *Corresponding author: juncheng@zju.edu.cn*

INTRODUCTION

Water hyacinth (*Eichhornia crassipes*), which is among the top 10 most pervasive weeds in the world, grows rapidly at 25 °C to 35 °C. Under favorable environmental conditions, a single plant can reproduce 140 million daughter plants each year, sufficiently covering an area of 1.40 km^2 with fresh biomass weighing 28,000 tons (Zhao *et al.* 2014). Water hyacinth is recognized as one of the fastest growing extant plants. The rapid propagation of water hyacinth pollutes bodies of water, aggravates water eutrophication levels, increases carbon dioxide concentration, reduces oxygen content in water, causes irreversible damage to ecological systems, blocks river-ways, obstructs navigation, and clogs irrigation channels. The spread of water hyacinth interferes with the development of the transportation, tourism, and aquaculture industries. At present, 19 provinces in south China are facing serious problems regarding water hyacinth overflow. Efficiently converting water hyacinth biomass into clean and renewable biofuels, such as bioethanol, hydrogen, and methane, significantly benefits the development of clean and renewable biological energy, emission reduction, and recycling economy (Bayrakci and Koçar 2014; Gunnarsson and Petersen 2007; Mishima *et al*. 2008).

Producing bioethanol from lignocellulosic biomass waste is an extensive process that involves biomass pretreatment, saccharification, fermentation, and product recovery. Biomass pretreatment is necessary because fermentable sugars must be released and made available for fermentation. Fermentation primarily aims to convert reducing sugars into bioethanol through microorganisms such as *Saccharomyces*. Ethanol yield directly determines the potential industrial development of ethanol production from water hyacinth (Abraham and Kurup 1997; Gunnarsson and Petersen 2007; Isarankura-Na-Ayudhya *et al*. 2007; Mishima *et al*. 2008; Mukhopadhyay and Chatterjee 2010; Singh and Bishnoi 2013). Mishima *et al*. (2008) reported an ethanol yield of 0.17 g ethanol/g (pretreated water

hyacinth), through SSF after pretreating powder from the leaves of water hyacinth with 1% (w/v) NaOH at room temperature for 12 h and then with 1% (w/v) H_2O_2 for another 12 h. When water hyacinth was pretreated with 10% (v/v) H_2SO_4 and overlimed with $Ca(OH)_2$, a maximum ethanol yield of 0.19 g/g (pretreated water hyacinth) with a productivity of 0.008 g/L/h was achieved through SSF (Isarankura-Na-Ayudhya *et al*. 2007). When water hyacinth was pretreated with 0.1 N H_2SO_4 and 1% (w/v) NaOH, then fermented by *Saccharomyces cerevisiae* and *Pachysolen tannophilus*, a maximum ethanol yield of 0.21 g ethanol/g (pretreated water hyacinth) was obtained through prefermentation hydrolysis, simultaneous saccharification, and fermentation (SSF) (Mukhopadhyay and Chatterjee 2010). However, the aforementioned ethanol yield was calculated based on the weight of the water hyacinth after pretreatment rather than on its original weight of raw biomass. Because the biomass weight decreased by at least 20% after pretreatment, the aforementioned ethanol yield decreased by at least 20% when it was calculated based on the raw biomass weight of water hyacinth: 0.17 to 0.21g/ g (pretreated water hyacinth)× 80%=0.14 to 0.17 g ethanol/g (raw biomass of water hyacinth, abbr. RBWH).

Ethanol production from the hydrolysate of water hyacinth pretreated with dilute acid by simultaneous saccharification and fermentation has been reported. However, the ethanol yield from water hyacinth was relatively low. Efficient hydrolysis of water hyacinth into fermentable sugars is a key control step in producing clean biofuels by fermentation. High saccharification efficiency of 94.6% was obtained through microwave-assisted dilute acid pretreatment to produce hydrogen and methane in a previous study by the authors (Xia *et al.* 2013). However, whether the hydrolysate obtained from water hyacinth through microwave-assisted dilute acid pretreatment under high pressure can be used by yeasts to produce ethanol has not been reported until now. Whether the hydrolysate can be used to acclimatize yeasts to restrain negative effects of byproducts (*e.g.*, furaldehyde and pyridinol) to enhance ethanol yield from water hyacinth has not been clarified. In this paper, a high ethanol yield, which was 76.3% of the theoretical ethanol yield, was obtained when water hyacinth, pretreated with microwave-assisted dilute acid, was fermented with acclimatized yeasts through simultaneous saccharification and fermentation. The ethanol yield of 0.22 g ethanol/g (raw biomass of water hyacinth) was 30% higher than the reported highest ethanol yield of 0.17 g ethanol /g (raw biomass of water hyacinth).

EXPERIMENTAL

Feedstock and Pretreatment

The water hyacinth samples were collected from the Yiwu River in Jinhua City, Zhejiang Province, China. Samples were sun-dried, and the roots were discarded (Carvalho Dos Santos and Lenzi 2000). The sample was dried to a constant weight at 105 °C and then pulverized to less than 200 μm. The lignocellulosic composition of the sample was tested by a Sweden 2010 automatic fiber tester and a 2300 automatic protein tester. The organic composition of the sample was as follows (w/w): 24.15% cellulose, 27.23% hemicellulose, 12.39% lignin, and 22.12% protein.

Biomass powders of water hyacinth (0.5 g to 1.0 g) were mixed with H_2SO_4 or NaOH solution (20 mL to 40 mL) in a 60-mL digestion reactor for pretreatment. The sample was pretreated by the following methods: (1) microwave and lightwave pretreatment at a power ratio of 30:70, having a total power = 700 W, combined with 1%

(w/v) NaOH for 150 s; (2) microwave-assisted 1% (w/v) NaOH pretreatment under high pressure (~0.14 MPa) with 40 g/L substrate concentration at 110 °C for 30 min; and (3) microwave-assisted 1% (v/v) H_2SO_4 pretreatment under high pressure (~0.36 MPa) with 20 g/L to 35 g/L substrate concentration at 140 °C for 15 min. A WG700TL20-K6 domestic microwave oven (Galanz Enterprise Group, China) was used for the microwave- and lightwave-assisted alkali pretreatment. The microwave-assisted dilute acid/alkali pretreatment of the water hyacinth under high pressure was conducted using a WX-4000 microwave digestion system (Shanghai Yiyao, China).

Fermenting Strains

The *S. cerevisiae* used for fermentation was isolated from distiller's yeast. The medium consisted, in g/L, of: yeast extract, 10; peptone, 20; and glucose, 20. *P. stipitis* (CICC1960) and *P. tannophilus* (CICC1770) were purchased from the China Center of Industrial Culture Collection. These yeasts were grown and maintained at 25 °C and 4 °C, respectively. The culture medium was, in g/L, comprised of yeast extract, 3; malt extract, 3; peptone, 5; glucose, 10; and agar, 20.

To acclimatize the culture medium, 10 g of water hyacinth powder and 500 mL 1% (v/v) H_2SO_4 were mixed and placed in a 1-L beaker. The medium was sterilized by autoclaving at 121 °C for 20 min. Thereafter, the beaker was placed at the center of a rotating circular glass plate in a microwave oven for microwave treatment at a power of 700 W for 15 min. After cooling, 1.5 g yeast extract, 1.5 g malt extract, 2.5 g peptone, and 4 g glucose were added into the solution. Deionized water was added to the beaker until the solution volume reached 500 mL. The solution was steam-sterilized at 121 °C for 20 min, and the pH was adjusted to 5.0 with 6 M NaOH. Acclimatization of the *P. stipitis* and *P. tannophilus* were performed in a ZHWY-2102C shake incubator (Shanghai Zhicheng Corp., China) at a controlled temperature of 25 °C. The suspension cultures of yeasts, after acclimatization for four generations, with each generation having been cultured for 48 h, were used as inocula for fermentation.

SSF

SSF is the simultaneous enzyme hydrolysis of cellulose and fermentation of reducing sugars by bacteria or fungi (Stenberg *et al*. 2000). Compared with independent saccharification and fermentation, SSF has a higher potential to improve production and economic efficiency by decreasing by-product inhibition and the number of required reaction tanks (Wyman 1999; Sun and Cheng 2002).

The pH of the hydrolyzate of the pretreated water hyacinth was adjusted to 4.5 to 5.5 using 6 M NaOH or HCl solution. *Trichoderma reesei* cellulase (Shanghai Boao Biotechnology Corp., China) was added to the solution at 5 wt% of the original weight of the untreated water hyacinth biomass. The carboxymethyl cellulose saccharification activity of this cellulose was 15 ± 0.6 IU/mg, which was measured as the initial rate of reducing sugar formation (Mandels and Weber 1969).

The supernatant of the yeast culture was discarded after standing, and a certain proportion (*i.e.*, 6.7%, 10%, 15%, and 20%, v/v) of yeast or mixed yeasts was added to the fermentation bottle. After air was removed by passing N_2 for ~10 min, hydrolysis and fermentation were performed in a thermostatic water bath at 37 °C. The samples were centrifuged at 5000 rpm for 8 min, and the supernatant was sampled to measure ethanol and reducing sugar yields.

Analytical Methods

The physicochemical properties of water hyacinth with microwave-assisted alkali or acid pretreatment under pressure were analyzed on various instruments. The surface morphologies of pretreated water hyacinth were determined on a scanning electron microscope (SEM, JSM-6390A, Japan). The organic compositions of pretreated water hyacinth were measured on a Fourier transform infrared spectrometer (FTIR, Nicolet 5700, America). The crystalline structures of pretreated water hyacinth were detected on an X-ray diffractometer (XRD, Shimadzu XRD-6000, Japan). The thermal properties of pretreated water hyacinth were examined on a thermal gravimetric analyzer (TGA, Mettler TGA/SDTA851e, Switzerland).

Total reducing sugar yields during fermentation were measured according to the dinitrosalicylic acid method (Miller 1959). The concentrations of different reducing sugars during fermentation were measured using a high-performance liquid chromatography (HPLC) system (Waters 2690, Waters Corp., USA) equipped with a 300 mm × 7.8 mm Polyspher CH PB column (Pb2 + type, Merck Corp., Germany). The HPLC system consisted of the following independent instruments: a differential refractive index detector (Waters 2410, Waters Corp., USA), a quaternary gradient pump, a degasser, an autosampler, and a system controller. The carrier liquid was water at a flow rate of 0.4 mL/min. The sugar concentration was evaluated *via* a calibration curve generated from HPLC-grade sugars. The concentration of the bioethanol produced during fermentation was analyzed using a gas chromatography (GC) system (TRACE 2000, Thermo Fisher Scientific, USA). The GC system consisted of a flame ion detector (280 °C; column temperature, 240 °C) and a DB-Waxtre chromatographic column, φ5 mm × 2 m (initial column temperature, 60 °C; maintained for 5 min, heated to a final temperature of 250 °C at a rate of 10 °C/min, and maintained for 6 min). The carrier gas was He with a flow rate of 50 mL/min. The bioethanol concentration was quantified through a calibration curve prepared by injecting different concentrations (varied from 0.1% to 1%, v/v) of ethanol standard liquid.

RESULTS AND DISCUSSION

The cellulose and hemicellulose in the water hyacinth biomass were assumed to be completely hydrolyzed into hexose (*e.g.*, glucose) and pentose (*e.g.*, xylose), respectively. The water hyacinth contained 24.15% cellulose and 27.23% hemicellulose. The cellulose was completely transformed into glucose [reaction formula: $(C_6H_{10}O_5)_n + nH_2O \rightarrow nC_6H_{12}O_6$), 24.15% × 180/(180 – 18) = 26.83%], and hemicellulose was completely hydrolyzed into xylose [reaction formula: $(C_5H_8O_4)_n + nH_2O \rightarrow nC_5H_{10}O_5$), 27.23% × 150/(150 – 18) = 30.93%] (Hu and Wen 2008). Thus, the theoretical maximum reducing sugar yield of 100 g water hyacinth was 57.76 g (=26.83 + 30.93). The reaction equations of ethanol production from glucose and xylose were described as follows: $C_6H_{12}O_6 \rightarrow C_2H_5OH + 2CO_2$; $3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2$. The theoretical ethanol yield was 0.51 kg for each kilogram of glucose and xylose. Therefore, the theoretical maximum ethanol yield was 29.46 (=57.76 × 0.51) g/100 g RBWH.

Physicochemical Properties of Pretreated Water Hyacinth

The surface morphologies, organic compositions, crystalline structures, and thermal properties of water hyacinth with microwave-assisted alkali or acid pretreatment

under pressure were analyzed on SEM, FTIR, XRD, and TGA instruments. The SEM images of pretreated water hyacinth are shown in Fig.1. The raw biomass of pulverized water hyacinth had many big particles about 40 μm with very smooth and compact surfaces. The particles were covered with a thin film, which might be the wax layer commonly found in herbaceous biomass. The pretreated water hyacinth with microwave-assisted alkali presented many channels with widths about 5 μm on particle surfaces, implying that lignin matrix structure was broken by alkali reactions. The pretreated water hyacinth with microwave-assisted acid generated a lot of irregular fragments about 5 to 10 μm and many pores in particles. This implied that lignocellulose structure was significantly broken, hemicellulose was almost completely hydrolyzed, and most of the crystalline cellulose was fragmented and exposed with increased specific surface area.

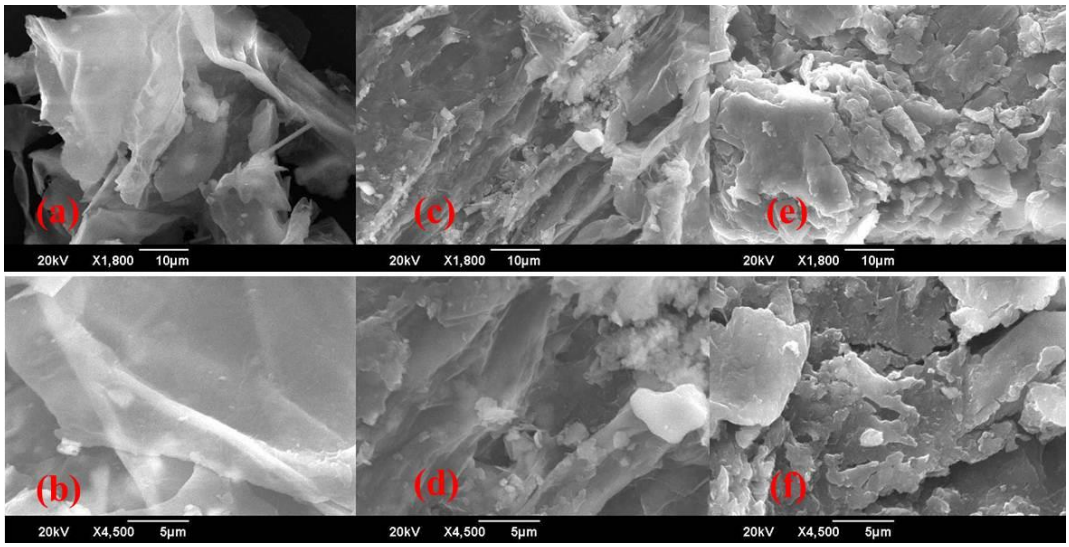


Fig. 1. SEM images of water hyacinth. (a) raw biomass of water hyacinth at 1800×, (b) raw biomass of water hyacinth at 4500×, (c) pretreated water hyacinth with microwave-assisted alkaline at 1800×, (d) pretreated water hyacinth with microwave-assisted alkaline at 4500×, (e) pretreated water hyacinth with microwave-assisted acid at 1800×, (f) pretreated water hyacinth with microwave-assisted acid at 4500×.

The FTIR spectra of pretreated water hyacinth with microwave-assisted alkali or acid are shown in Fig. 2. The peak intensity of 1630 cm^{-1}, which was ascribed to the C=O of acetyl groups in lignin, became weaker after alkali or acid pretreatment, due to the broken lignin matrix. The peak intensity of 1739 cm^{-1}, which reflected the hemicellulose, markedly decreased after acid pretreatment, due to the complete hydrolysis of hemicellulose. The peak intensity of 894 cm^{-1}, which reflected the cellulose, increased after acid pretreatment, because the relative cellulose content increased with removal of amorphous hemicellulose.

The XRD patterns of pretreated water hyacinth with microwave-assisted alkali or acid are shown in Fig. 3. Such data makes it possible to determine the crystallinity changes. The crystalline structure of cellulose was presented as a big peak at 2θ of 22 to 23°. The crystallinity index (CrI) decreased from 16.0 to 3.4 after alkali pretreatment, due to the decreased crystallinity of cellulose with broken lignin matrix. However, the CrI increased from 16.0 to 30.0 after acid pretreatment, because removal of amorphous hemicellulose resulted in an increased relative content of crystalline cellulose.

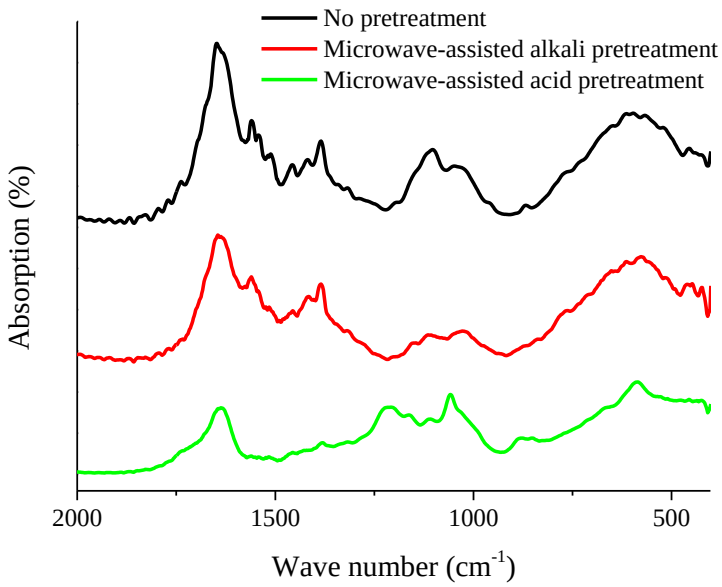


Fig. 2. FTIR spectra of pretreated water hyacinth with microwave-assisted alkali or acid

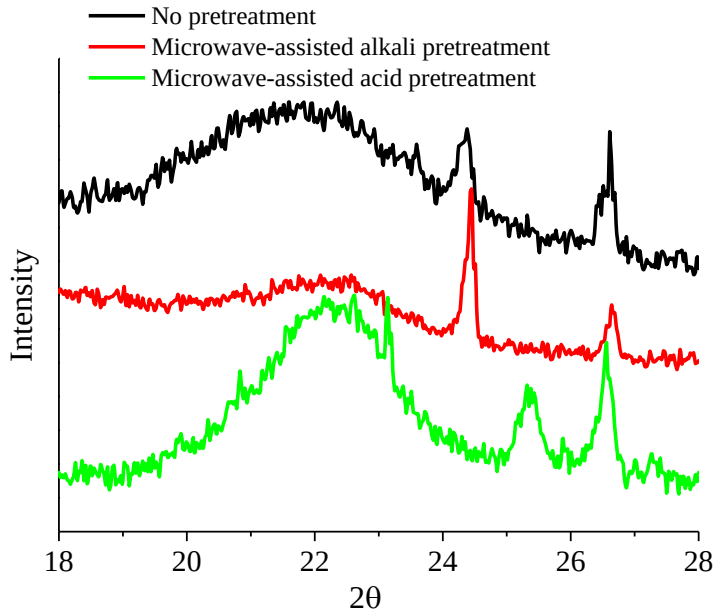


Fig. 3. XRD patterns of pretreated water hyacinth with microwave-assisted alkali or acid

The TGA analysis of pretreated water hyacinth with microwave-assisted alkali or acid is shown in Fig. 4. The peak temperature of differential thermal gravity (DTG) decreased from 324 °C to 285 °C with alkali pretreatment, due to the broken lignin matrix. The DTG peak temperature further decreased to 143 °C with a decreased DTG peak value by 33%, due to the broken lignocellulose structure, completely hydrolyzed hemicellulose and fragmented cellulose crystals.

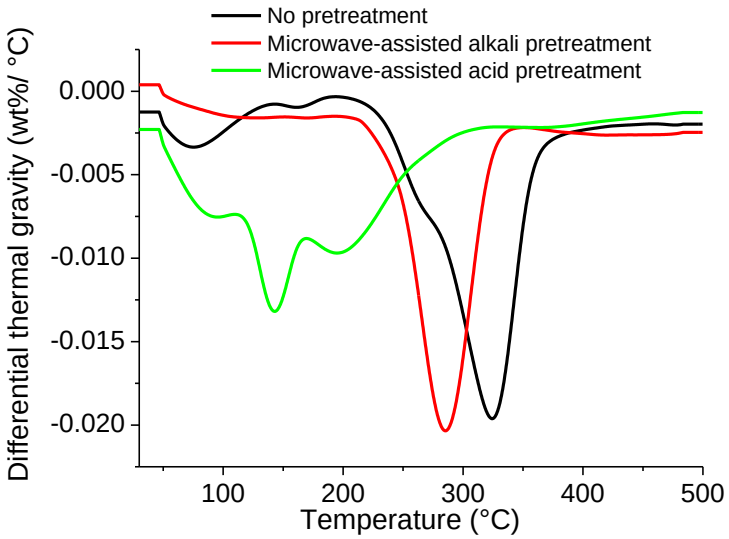


Fig. 4. TGA analysis of pretreated water hyacinth with microwave-assisted alkali or acid

Ethanol Production after Microwave- and Lightwave-assisted Alkali Pretreatment by SSF

S. cerevisiae primarily consumed hexose, particularly glucose, as its substrate for fermentation [Fig. 5(a)]. During the hydrolysis of water hyacinth, cellulose was initially converted into cellobiose, and then into glucose. This process was catalyzed by the consumption of glucose by yeast and thus was accomplished rapidly. Cellobiose was detected during the initial stage of hydrolysis and fermentation, but it disappeared after 12 h. Glucose was not detected throughout the process. Hemicellulose is a heterogeneous polymer composed of several types of monosaccharides, including mannose, xylose, galactose, and arabinose. As shown in Fig. 5(a), the concentration of mannose dramatically increased, whereas those of xylose and galactose only slightly increased during SSF. This phenomenon could be ascribed to the gradual hydrolysis of hemicellulose to mannose, xylose, and galactose, whereas *S. cerevisiae* barely consumed pentose. A maximum ethanol yield of 5.39 g/L, equivalent to 0.063 g/g RBWH, was achieved at 72 h. This value was 21.72% of the theoretical ethanol yield. Decreased bioethanol production was observed after 72 h, suggesting that there was depletion of monosaccharides in the fermentation broth. During fermentation, simple sugars were rapidly consumed by the yeast and residual sugars were not sufficient to maintain yeast growth. As a result, the produced bioethanol acted as a carbon source in the fermentation broth and was consumed as the original simple sugar. However, the oxidation and conversion of ethanol also resulted in decreased ethanol yield, which provides an explanation for the decrease in ethanol concentration (Wasungu and Simard 1982; Harun and Danquah 2011).

As shown in Fig. 5(b), *P. tannophilus* primarily consumed glucose. This yeast also consumed galactose effectively during fermentation. Glucose, galactose, and cellobiose were not detected throughout the fermentation of *P. tannophilus*, but cellobiose was detected during the first 12 h of the fermentation of *S. cerevisiae*. This finding suggested that *P. tannophilus* was more effective than *S. cerevisiae* in terms of glucose consumption. *P. tannophilus* constantly and effectively consumed glucose and galactose decomposed from lactose, leading to the gradual decrease in lactose concentration. A maximum ethanol

yield of 6.09 g/L, equivalent to 0.069 g/g RBWH, was achieved at 96 h. This value was 23.79% of the theoretical ethanol yield.

The variation tendency of reducing sugar and ethanol yields resulting from the use of mixed yeasts, traditional *S. cerevisiae* and *P. tannophilus*, is shown in Fig. 5(c). Mixed yeasts initially consumed glucose and galactose. Lactose yield was increased during the initial 12 h of the process and then decreased until the lactose was completely consumed after 96 h. This phenomenon was attributed to the gradual decomposition of lactose into glucose and galactose for further fermentation after the latter were completely consumed by the yeasts. Correspondingly, ethanol yield initially increased rapidly and then gradually increased with extended fermentation time from 36 h to 48 h. Ethanol production began to increase rapidly again after lactose was completely decomposed into glucose and galactose. A maximum ethanol yield of 5.473 g/L, equivalent to 0.063 g/g RBWH, was observed at 96 h. This value was 21.72% of the theoretical ethanol yield.

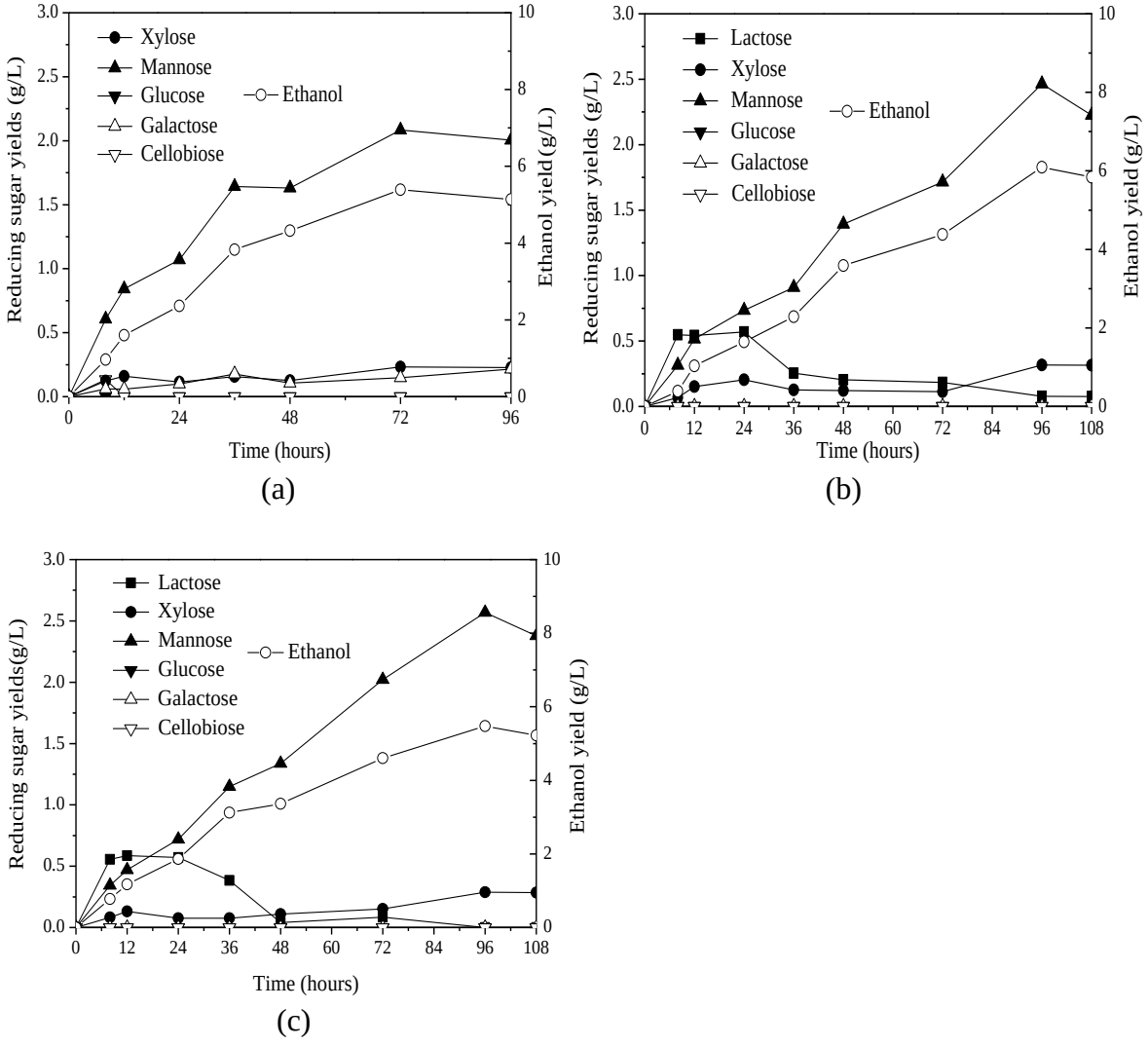
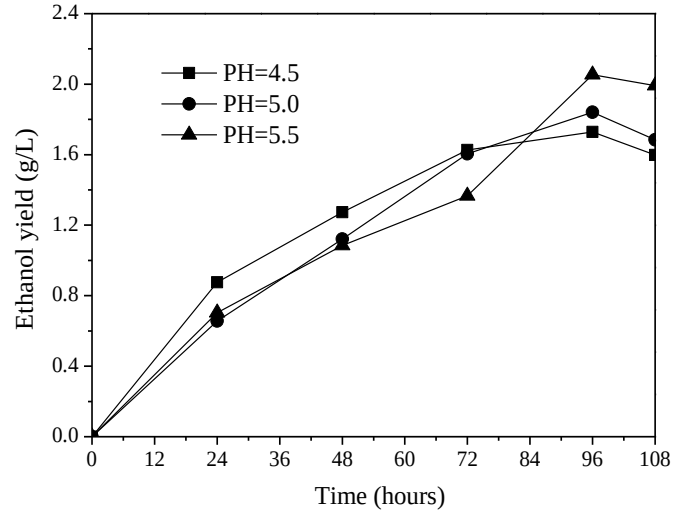


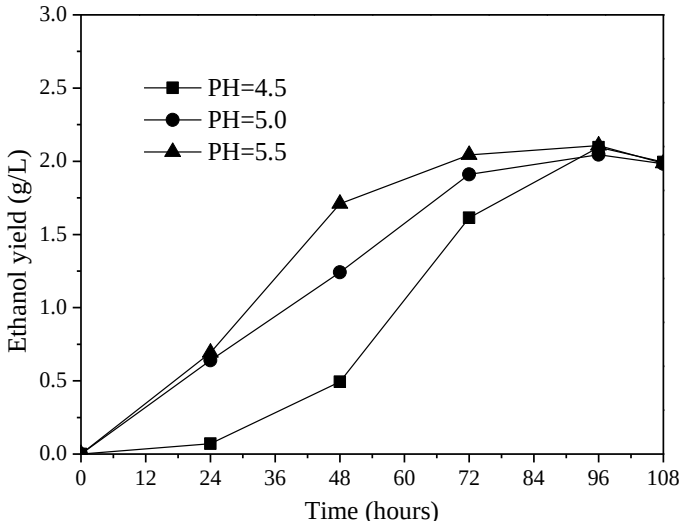
Fig. 5. Ethanol and reducing sugar yield of water hyacinth pretreated with microwave and lightwave-assisted alkali through a simultaneous saccharification and fermentation process with yeasts: (a) *S. cerevisiae*, (b) *P. tannophilus*, and (c) mixed *S. cerevisiae* and *P. tannophilus*. The ratio of microwave power to lightwave power was 3:7.

Ethanol Production after Microwave-assisted Alkali Pretreatment under High Pressure by SSF

As shown in Fig. 6, the effects of pH on the ethanol production of water hyacinth that had been pretreated with microwave and alkali under high pressure were investigated. In Fig. 6(a), a low initial pH of the fermentation broth is shown to result in high ethanol yield for *S. cerevisiae* during the beginning of fermentation. This finding was attributed to the high activity exhibited by *S. cerevisiae* at pH 4.5. However, the ethanol production rate at pH 5.5 was greater than those at pH 5.0 and 4.5 after 72 h. As a result of the organic acids produced during fermentation, the initial pH of 5.5 declined to the optimum pH for yeast, whereas the initial pH of 4.5 and 5.0 were decreased to restrain yeast activity. A maximum ethanol yield of 2 g/L, equivalent to 0.10 g/g RBWH, was observed at 96 h when the substrate concentration was 20 g/L and pH was 5.5. This ethanol yield was 34.48% of the theoretical yield.



(a)



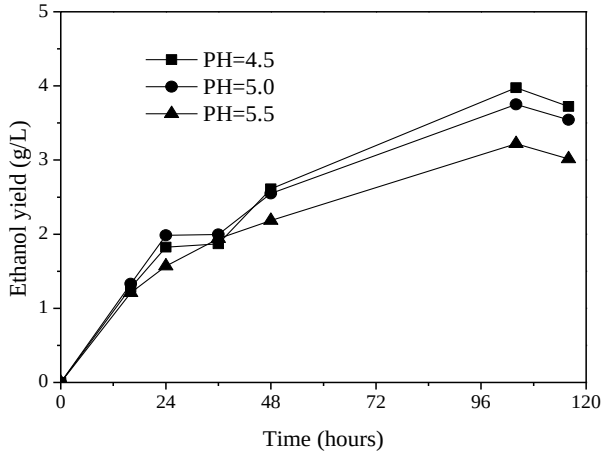
(b)

Fig. 6. Effects of pH on the ethanol yield of water hyacinth pretreated with microwave and alkali under high pressure through a simultaneous saccharification and fermentation process with yeasts: (a) *S. cerevisiae* and (b) *P. stipitis*

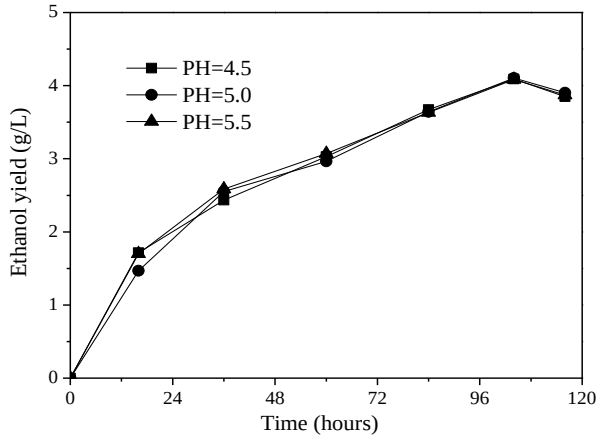
The results of ethanol production by *P. stipitis* under different initial pH conditions are shown in Fig. 6(b). Given the acidification of the organic acids generated during fermentation, the ethanol yield was low at pH 4.5 before 48 h. The ethanol yield at 72 h was increased from 1.61 to 2.04 g/L when the initial pH increased from 4.5 to 5.5. However, initial pH had nearly no influence on the final ethanol yield at 96 h. The final ethanol yields obtained in this study were nearly equal. Ethanol yield reached 2.1 g/L (0.1 g/g RBWH) when fermentation proceeded for 96 h. This value was equivalent to 34.48% of the theoretical ethanol yield.

Ethanol Production after Microwave-assisted Acid Pretreatment under High Pressure by SSF

As reported in a previous study (Xia *et al.* 2013), the weight percentages of residual solid biomass, hemicelluloses, cellulose, and lignin after pretreatment of water hyacinth were 37.5%, 6.2%, 71.7%, and 86.75%, respectively. The yields of total reducing sugar, arabinose, galactose, glucose, and xylose in the pretreated liquor were 18.79, 5.25, 3.81, 3.12, and 2.81 g/100g RBWH, respectively. The effects of pH on the ethanol production of water hyacinth pretreated with microwave and acid under high pressure were investigated, as shown in Fig. 7.



(a)



(b)

Fig. 7. Effects of pH on the ethanol yield of water hyacinth pretreated with microwave and acid under high pressure through a simultaneous saccharification and fermentation process with yeasts: (a) *S. cerevisiae* and (b) *P. stipitis*

According to Fig. 7(a), a low initial pH of fermentation resulted in a high ethanol yield. *S. cerevisiae* exhibited the highest activity when pH was 4.5. The saccharification efficiency of water hyacinth after pretreatment with microwave and acid under high pressure exceeded 90%. Thus, the reducing sugars were converted more effectively into ethanol. Consequently, by-products such as organic acids were correspondingly decreased, thus reducing acidification of the fermentation broth. Ethanol yield reached 4 g/L (0.132 g/g RBWH) when fermentation proceeded for 104 h. This value was equivalent to 34.48% of the theoretical ethanol yield.

The results of ethanol production by *P. stipitis* under different initial pH conditions are shown in Fig. 7(b). The ethanol yield was slightly affected by initial pH, i.e. *P. stipitis* maintained high activity when pH ranged from 4.5 to 5.5. A maximum ethanol yield of 4.1 g/L (0.137 g/g RBWH) was obtained at 104 h. This value was equivalent to 47.24% of the theoretical ethanol yield.

In summary, the ethanol yield was higher in the water hyacinth pretreated with microwave and acid under high pressure compared with the water hyacinth pretreated with microwave and alkali under high pressure. The reducing sugar yield of 48.28 g/100g RBWH with microwave assisted acid pre-treatment and cellulase hydrolysis was much higher than that of 28.13 g/100g RBWH with microwave assisted alkali pre-treatment and cellulase hydrolysis. Therefore, the hydrolysis efficiency of water hyacinth with microwave assisted acid pre-treatment was much more effective than that with microwave assisted alkali pre-treatment. This acid pretreatment broke the compact structure of lignocellulose, which resulted in the effective adsorption of the exposed cellulose by cellulase to produce glucose. Hydrolysis saccharification efficiency and ethanol yield were significantly improved by microwave-assisted acid pretreatment under high pressure.

Ethanol Production by Acclimatized Yeast after Microwave-assisted Acid Pretreatment under High Pressure by SSF

The ethanol production of water hyacinth was examined using acclimatized yeasts of *P. stipitis* and *P. tannophilus* after microwave-assisted dilute sulfuric acid pretreatment. As shown in Fig. 8, a maximum ethanol production of 0.216 g/g RBWH was obtained when SSF proceeded for 84 h. This value was equivalent to 74.4% of the theoretical ethanol yield.

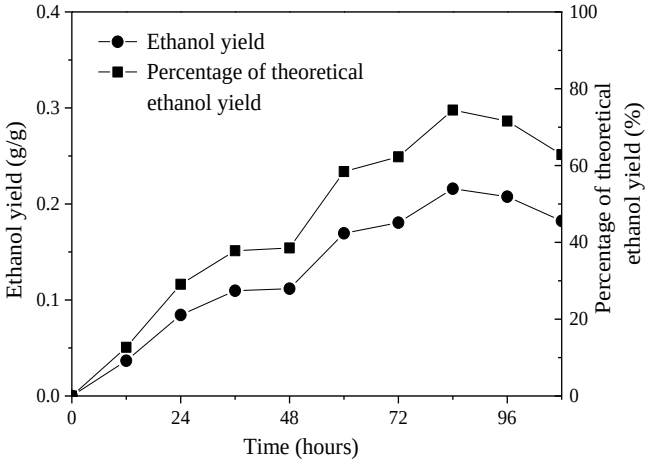
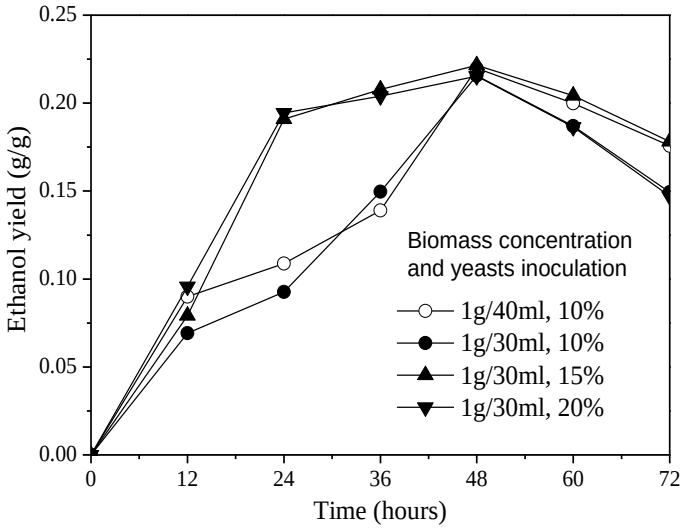


Fig. 8. Ethanol yield of water hyacinth pretreated with microwave and acid under high pressure through a simultaneous saccharification and fermentation process with acclimatized yeasts

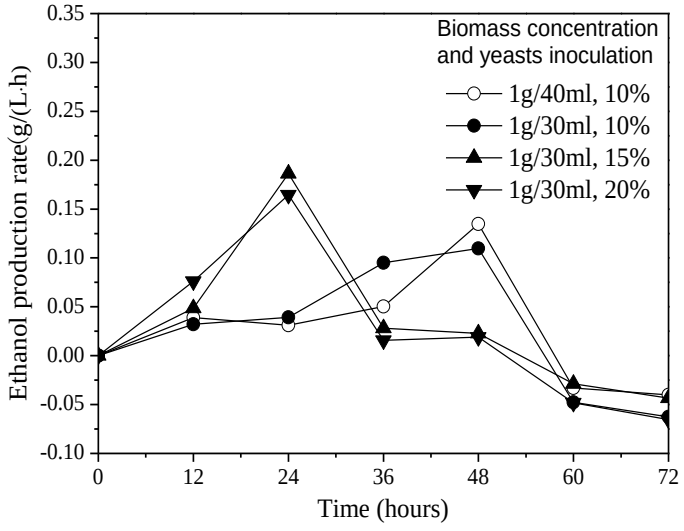
Given that the yeasts acclimatized by the water hyacinth hydrolysate had better adaptability to water hyacinth, the efficiency of ethanol production in water hyacinth that used acclimatized yeasts was significantly improved. To increase the ethanol yield of water hyacinth further, harmful byproducts should be removed from the water hyacinth hydrolysate microwaved with dilute acid.

Improving Ethanol Yield and Rate by Changing Feedstock Biomass Concentration and Acclimatized Yeast Inoculation Amount after Microwave-assisted Acid Pretreatment under High Pressure by SSF

Different feedstock biomass concentrations and acclimatized yeast inoculation amounts were investigated using *P. stipitis* and *P. tannophilus* at a ratio of 1:1 (v/v). The results are shown in Fig. 9.



(a)



(b)

Fig. 9. Effects of the feedstock biomass concentration and acclimatized yeast inoculation amount on ethanol production yield (a) and rate (b) of water hyacinth pretreated with microwave and acid under high pressure

As illustrated in Fig. 9(a), ethanol yield was slightly higher during the initial stage, with a lower solid content treated with microwave and dilute acid under high pressure when the inoculation amounts were equal. This result was attributed to the improved saccharification efficiency of water hyacinth when the substrate concentration was low. However, with the process of enzyme hydrolysis and fermentation, the ethanol yields were nearly equivalent in the end. With an increased inoculation amount, the ethanol yield was slightly increased during the initial 24 h when the feedstock concentrations of pretreatments were the same. An inoculation amount of 15% exhibited the highest bioethanol yield, whereas a higher inoculation amount of 20% resulted in a lower ethanol concentration after 24 h.

Although high yeast concentrations are expected to produce high amounts of bioethanol during fermentation, high inoculation concentrations decreased bioethanol production because of the use of ethanol as a carbon source and the toxicity of high ethanol concentrations toward yeast cells. A maximum ethanol yield of 0.22 g/g RBWH was achieved at 48 h when feedstock biomass concentration was 1 g WH/30 mL dilute sulfuric acid (v/v) and the inoculation amount of yeast was 15%. The ethanol yield of 0.22g ethanol/g RBWH was 30% higher than the reported highest ethanol yield of 0.17 g ethanol /g RBWH (Mukhopadhyay and Chatterjee 2010). This ethanol yield was 76.3% of the theoretical yield. Ethanol yield decreased after 48 h because the monosaccharide was consumed rapidly by the yeast during fermentation and ethanol was used as a carbon source when the monosaccharide was consumed completely.

As shown in Fig. 9 (b), maximum ethanol production rate was achieved at 48 h when the inoculation amount was 10%. The peak time of the ethanol production rate advanced to 24 h when the inoculation amount was increased to 15% and 20%. The maximum ethanol production rate was 0.19 g/(L·h) at 24 h when pretreatment was performed using 1 g RBWH powder/30 mL 1% (v/v) dilute sulfuric acid and the inoculation amount was 15% (v/v).

CONCLUSIONS

1. Microwave-assisted dilute acid pretreatment and the use of the acclimatized mixed yeasts *P. stipitis* and *P. tannophilus* markedly improved the ethanol production rate and yield of water hyacinth through SSF.

2. Acid pretreatment was superior to alkali pretreatment for ethanol production from water hyacinth through simultaneous saccharification and fermentation.

3. The highest ethanol yield was 0.22 g/g RBWH, which was 76.3% of the theoretical ethanol yield of water hyacinth. The maximum ethanol production rate was 0.19 g/(L·h) at 24 h.

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