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Enhancement of Ethanol Production via Hyper Thermal Acid Hydrolysis and Co-Fermentation Using Waste Seaweed from Gwangalli Beach, Busan, Korea

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The waste seaweed from Gwangalli beach, Busan, Korea was utilized as biomass for ethanol production. *Sagassum fulvellum* (brown seaweed, Mojaban in Korean name) comprised 72% of the biomass. The optimal hyper thermal acid hydrolysis conditions were obtained as 8% slurry contents, 138 mM sulfuric acid, and 160°C of treatment temperature for 10 min with a low content of inhibitory compounds. To obtain more monosaccharides, enzymatic saccharification was carried out with Viscozyme L for 48 h. After pretreatment, 34 g/l of monosaccharides were obtained. *Pichia stipitis* and *Pichia angophorae* were selected as optimal co-fermentation yeasts to convert all of the monosaccharides in the hydrolysate to ethanol. Co-fermentation was carried out with various inoculum ratios of *P. stipitis* and *P. angophorae*. The maximum ethanol concentration of 16.0 g/l was produced using *P. stipitis* and *P. angophorae* in a 3:1 inoculum ratio, with an ethanol yield of 0.47 in 72 h. Ethanol fermentation using yeast co-culture may offer an efficient disposal method for waste seaweed while enhancing the utilization of monosaccharides and production of ethanol.

Keywords: Waste seaweed, hyper thermal acid hydrolysis, enzymatic saccharification, co-fermentation

Introduction

Every summer, a massive seaweed bloom, known as the "green tide," occurs in coastal areas around Qingdao, China in the East China Sea [1]. The large quantity of seaweeds formed in this bloom can have a deleterious impact on the local ecosystems, including blocking the growth of benthic seagrasses by increasing light attenuation, as well as altering benthic fauna due to hypoxia resulting from the decay of blooming algae [2]. Floating seaweeds from this bloom drifts northward and may reach the coast of Korea owing to strong winds or typhoons [3]. These large amounts of seaweed accumulate on beaches and in coastal waters, causing significant social and economic losses [4]. However, clean-up and disposal requires large amounts of money and manpower. Therefore, ethanol production using waste seaweed was evaluated as an efficient method for the disposal of waste seaweed.

Pretreament begins the degradation of waste seaweed biomass into monosaccharides through a hydrolysis step that enhances monosaccharide yield from fermentation [5]. Hyper thermal pretreatment is commonly used in the preparation of seaweed hydrolysate for enzymatic saccharification and fermentation [6]. However, dilute acid pretreatment has the major drawback of generating monosaccharide degradation products, such as hydroxymethyl furfural (HMF) and levulinic acid, which are toxic to fermentative yeasts [7]. These inhibitors can retard yeast growth and reduce ethanol production during fermentation. Thus, effective pretreatment is required to obtain a high monosaccharide concentration with low concentration of inhibitors [8]. Therefore, hyper thermal acid hydrolysis was optimized to minimize the degradation of monosaccharides into inhibitors, such as 5-HMF and levulinic acid, by adjusting factors, including the acid dose, temperature, and duration of pretreatment.

Many kinds of yeasts are used in ethanol production, including *Saccharomyces cerevisiae*, *Pichia stipitis*, and *Pichia angophorae*. However, these ethanol producers have very narrow substrate utilization ranges. *Saccharomyces cerevisiae* is widely used for the production of ethanol from glucose, but lacks transhydrogenase and is therefore unable to consume xylose or mannitol [9]. *Pichia stipitis* is one of the most effective xylose-fermenting yeasts. However, its rate of glucose fermentation is lower than that of common glucose-fermenting microorganisms such as *S. cerevisiae* and *Zymomonas mobilis* [10]. *P. angophorae* was reported to produce ethanol from glucose, xylose, and mannitol, but not galactose [11]. Therefore, to achieve efficient conversion of glucose, galactose, xylose, and mannitol to ethanol, co-culture fermentation was carried out in this study.

Ethanol was produced from waste seaweed obtained from Gwangalli, Busan. Hyper thermal acid hydrolysis and enzymatic saccharification were carried out to obtain monosaccharides, followed by co-culture fermentation using various yeast inoculum ratios to optimize the fermentation conditions.

Materials and Methods

Raw Materials and Composition Analysis

Waste seaweed was obtained from Gwangalli, Busan, Korea, after Typhoon Dolphin hit the area (May 2015). The biomass was dried with sunlight and ground in a hammer mill. The biomass powder was sieved through a 0.35-mm (45-mesh) sieve prior to pretreatment. The biomass composition was determined by the Feed and Foods Nutrition Research Center at Pukyong National University in Busan, Korea, according to the AOAC method [12].

Hyper Thermal Acid Hydrolysis

Individual experiments were carried out to determine the main factors contributing to optimal fermentation. Hyper thermal acid hydrolysis conditions for waste seaweed were optimized by varying the slurry content (4–14%), sulfuric acid concentration (92–276 mM), temperature (120–200°C), and treatment time (5–20 min). For hyper thermal acid hydrolysis, a 50 ml stainless steel batch reactor (working volume of 40 ml) was filled with the various amounts of seaweed slurry and sulfuric acid. The reaction was initiated by raising the reactor temperature in an oil bath that had been preheated for 5 min. The reaction was monitored and adjusted using a PID temperature controller (TC200P; Misung Scientific Co., Ltd., Korea). A magnetic stirrer was placed in the stainless steel batch reactor to maintain efficient contact between the biomass and the acid. Upon completion of the reaction, the reactor was quickly cooled to room temperature in a water bath [13]. The efficiency of hyper thermal acid hydrolysis was calculated using Eq. (1),

$$E_P(\%) = \frac{\Delta S_{mono}}{TC} \times 100 \quad (1)$$

where E_P is the efficiency of pretreatment (%), ΔS_{mono} is the increase in galactose and glucose (g/l) during hydrolysis, and TC is the total carbohydrate (g/l) content of the waste seaweed biomass.

Effects of Enzymatic Saccharification

The waste seaweed hydrolysate was neutralized to pH 5.0 using 5 N NaOH. Saccharification was conducted by the addition 16 units/ml of Celluclast 1.5 L (8.4 units/ml; Novozymes, Denmark), Viscozyme L (1.2 units/ml, Novozymes, and their mixture (1:1 of Celluclast 1.5 L and Viscozyme L). Celluclast 1.5 L contains cellulase activity. Viscozyme L contains xylanase, hemicellulase, and cellulase activities. Saccharification was carried out in 100 ml of seaweed slurry at 45°C with 150 rpm shaking for 48 h. Samples were taken for determination of the monosaccharide and HMF concentrations using high-performance liquid chromatography (HPLC).

Yeast Preparation

Saccharomyces cerevisiae KCCM 1129, *Pichia angophorae* KCTC 1757, and *Pichia stipitis* KCTC7228 were obtained from the Korean Collection for Type Cultures (KCTC) Biological Resource Center (Korea). Yeasts were cultured on yeast peptone dextrose (YPD) agar plates (10 g/l yeast extract, 20 g/l peptone, 20 g/l glucose, and 5 g/l agar). A yeast colony from the agar plate was inoculated into 30 ml of YPD medium (containing 10 g/l yeast extract, 20 g/l peptone and 20 g/l glucose) and cultured at 30°C and 150 rpm for 24 h.

Single Strain and Co-Fermentation

Fermentation was carried out with *S. cerevisiae*, *P. angophorae*, and *P. stipitis* and their mixture to optimize the optimal yeast for fermentation. Erlenmeyer flasks (250 ml) containing 100 ml of waste seaweed hydrolysate were used in the single strain and co-culture fermentation. The inoculation of yeasts was a total of 0.2 grams dry cell weight (g dcw) per liter in the same ratio (1:1). Fermentation was carried out at 30°C and 150 rpm for 144 h under semi-anaerobic condition.

Optimization of Co-Fermentation with Various Ratios of Yeasts

The μ-*S* relationship was determined to evaluate the fermentation efficiency of co-culture of *P. stipitis* and *P. angophorae*. The rate of microbial growth is characterized by the net specific growth rate, defined by Eq. (2),

$$\mu = \frac{1}{X} \times \frac{dX}{dt} \quad (2)$$

where X is the cell biomass concentration (g/l), t is the time (h), and μ is the specific growth rate (h^{-1}) [14].

The co-fermentation was initiated with design by the addition of 10 ml of mixed inoculum containing a total of 0.2 g dcw/L of *P.*

angophorae and *P. stipitis* in different ratios (0:4, 1:3, 2:2, 3:1, and 4:0) to produce ethanol from waste seaweed hydrolysate. The seaweed hydrolysates were fermented at 30°C and 200 rpm for 96 h. Samples were taken periodically for monosaccharide content and ethanol production analyses. The yield of ethanol production (Y_{EtOH}) was calculated using Eq. (3),

$$Y_{EtOH}(g/g) = \frac{[EtOH]_{max}}{\Delta S_{PS}} \quad (3)$$

where Y_{EtOH} is the ethanol production yield (g/g), $[EtOH]_{max}$ is the maximum ethanol concentration obtained during fermentation (g/l), and ΔS_{PS} is the total fermentable monosaccharide content increase (g/l) after pretreatment and saccharification [13].

Analytical Method

Glucose, galactose, xylose, mannitol, and ethanol profiles were analyzed using an HPLC sysem (Agilent 1100 Series; Agilent. Inc., USA) equipped with a refractive index detector. The concentrations of these and components in the samples were determined using 5 mM H_2SO_4 at 0.6 ml/min on a Bio-Rad Aminex HPX-87H column (300 × 7.8 mm) at 65°C. The sugar and ethanol concentrations were determined using a standard curve correlating the peak area with concentration.

Statistical Analytical Methods

The statistical significance of the efficiency of pretreatment (E_P) was evaluated by one-way analysis of variance and Duncan's multiple range test ($p < 0.05$) using SPSS software (ver. 23.0; SPSS, USA).

Results and Discussion

Composition of Waste Seaweed from *Gwangalli, Busan*

Waste seaweed obtained from Gwangalli, Busan after Typhoon Dolphin was composed of seven species of red, brown, and green seaweeds. Brown seaweed made up over 95% of the total biomass, and was composed of *Sargassum fulvellum*, *Hizikia fusiformis*, and *Undaria pinnatifida* (Table 1). *Sargassum fulvellum* accounted for 75% of the waste seaweed. All seaweeds were mixed and then used as biomass. The mixed waste seaweeds were composed of carbohydrate, fiber, crude ash, crude lipid, and crude protein; among these, fiber and carbohydrate are the main resources used for ethanol production, and they comprised 48.34% of the waste.

Hyper Thermal Acid Hydrolysis of Waste Seaweed from Gwangalli, Busan, Korea

A successful hydrolysis must produce the maximum monosaccharide concentration from the raw material with minimal inhibitory compounds. To maximize monosaccharide production, the waste seaweed was treated using four operational variables, including slurry content (4–14%), sulfuric acid concentration (92–276 mM), temperature (120–200°C), and treatment time (5–20 min). Fig. 1A shows the effects of various slurry contents evaluated using 184 mM sulfuric acid at 160°C for 10 min. An increase in slurry content from 4% to 14% (w/v) resulted in an increase in the concentration of monosaccharide produced. However, above 8% (w/v), further increases in the slurry content did not have a significant effect on E_P. A previous study also used E_P as the criterion to select the optimal slurry content among various slurry contents. The optimal slurry content was selected with highest E_P. If the E_P value was similar, the optimal condition was selected by considering the inhibitory compounds [13]. Therefore, 8% (w/v) slurry was selected for ethanol production.

Fig. 1B shows the effect of sulfuric acid concentration on monosaccharide production from waste seaweed. Hyper thermal acid hydrolysis was conducted at 8% (w/v) content and 160°C for 10 min. The monosaccharide concentration increased with an increase of the sulfuric acid concentration up to 138 mM. Above 138 mM, the monosaccharide concentration and E_P showed no significant differences between 138 and 184 mM sulfuric acid concentrations; however, a higher acid concentration resulted in an increase in inhibitory compounds, such as levulinic acid, along with a decrease in HMF formation. These results might be due to the formation of levulinic acid from HMF that was previously formed from monosaccharides [15]. Thus, the maximum monosaccharide concentration of 22.73 g/l and E_P of 58%, with low concentrations of inhibitory compounds (0.2 g/l levulinic acid and 0.4 g/l HMF), were obtained by hyper thermal acid hydrolysis with 138 mM sulfuric acid.

Table 1. Species distribution of obtained waste seaweeds from Gwangalli, Busan, Korea.

Seaweed	Red seaweed				Brown seaweed			Green seaweed
Species	*Grateloupia elliptica*	*Ahnfeltiopsis flabelliformis*	*Hypnea spinella*	Others	*Sargassum fulvellum*	*Hizikia fusiformis*	*Undaria pinnatifida*	*Ulva pertusa*
Biomass (g)	49.10	12.63	11.03	18.19	4,144.19	1,036.05	360.21	136.12
Ratio (%)	0.8	0.2	0.2	0.3	72	18	6.2	2.3

The results of hyper thermal acid hydrolysis under conditions of 8% (w/v) slurry content and 138 mM sulfuric acid concentration for 10 min with varying temperatures, are shown in Fig. 1C. The monosaccharide concentration increased with an increase in the hydrolysis temperature up to 160°C, and then decreased gradually from 160°C to 200°C. According to Saha *et al*. [7], high temperature improved the yield of hydrolysis compared with low temperature. Our data show that the temperature of hyper thermal acid hydrolysis is important for the hydrolysis of waste seaweed, as the total monosaccharide yield increased by 22.73 g/l at 160°C compared with other temperatures. Therefore, 160°C was selected as the optimal temperature for hyper thermal acid hydrolysis.

As shown in Fig. 1D, the effects of various treatment times were evaluated under conditions of 8% (w/v) slurry, 138 mM sulfuric acid, and 160°C. The monosaccharide concentration increased with an increase in hydrolysis time up to 10 min, and then decreased gradually with a further increase in the time from 10 to 20 min. The inhibitory compounds levulinic acid and HMF were obtained at 0.2 g/l and 0.4 g/l, respectively, in 10 min of hydrolysis. A further increase in hydrolysis time increased the production of inhibitory compounds. Ra *et al*. [13] reported that an extended hydrolysis time or a high temperature could have a negative effect on monosacchride production owing to the degradation of monosaccharides and formation of inhibitory compounds [13]. Thus, 10 min of hyper thermal acid hydrolysis was selected as the optimal hydrolysis time considering the production of both monosaccharides and inhibitory compounds.

On the basis of these results, the optimal hyper thermal acid hydrolysis conditions were determined to be 8% (w/v) slurry content and 138 mM sulfuric acid at 160°C for

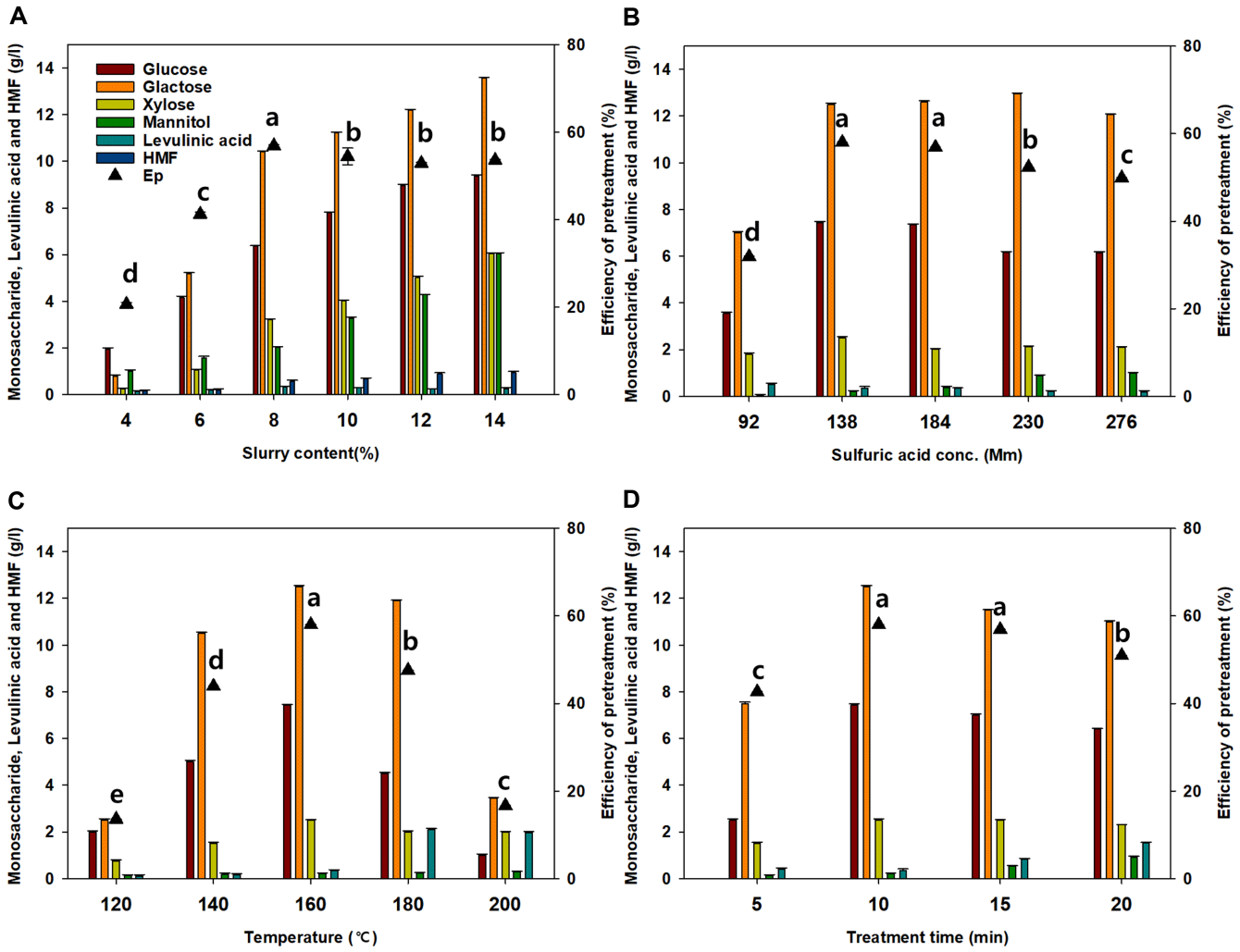

Fig. 1. Optimization of hyper thermal acid hydrolysis with various conditions using waste seaweed.

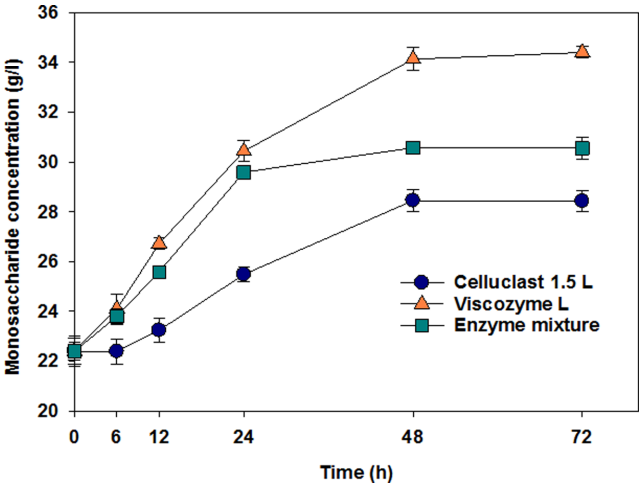

Fig. 2. Effects of various enzymes on the production of monosaccharides from the hyper thermal acid hydrolysis of waste seaweed slurry by enzymatic saccharification at 45°C and 150 rpm for 72 h.

10 min, which exhibited the highest monosaccharide concentration of 22.73 g/l and an E_P of 58%.

Enzymatic Saccharification

After hydrolysis of the waste seaweed, enzymatic saccharification was performed to increase the monosaccharide concentration before fermentation. Enzymatic saccharification was carried out using Celluclast 1.5 L, Viscozyme L, and a mixture of these two enzymes. As shown in Fig. 2, the effect of enzymatic saccharification was determined on the basis of the increase in monosaccharide concentration. Monosaccharides increased for the first 48 h of enzymatic reaction, and did not increase thereafter. Thus, the optimal enzyme treatment time was determined to be 48 h. The maximum monosaccharide concentration of 34 g/l was obtained using Viscozyme L with thermal acid hydrolysate containing 8% waste seaweed. A previous study reported that a mixture of enzymes had a synergistic effect and yielded a higher glucose content than a single enzyme [16]. However, the structural composition of *Sargassum fulvellum* is cellulose (20.35%), hemicellulose (25.73%), holocellulose (46.08%), and mannitol (5.04%) [17]. Therefore, Viscozyme L, a mixture of cellulase, hemicellulase, and xylanase, led to the greatest level of monosaccharide production among the enzymatic treatments. These enzymes degraded cellulose and hemicellulose from the seaweed, forming 34 g/l of monosaccharides including glucose, galactose, and xylose.

Single-Strain Fermentation and Co-Fermentation for Optimization of Co-Fermentation Yeasts

Prior to co-culture fermentation, a yeast selection procedure was carried out to evaluate the optimal combination of co-culture of yeasts. As shown in Table 2, single-strain fermentation and co-fermentation were carried out with waste seaweed hydrolysate by *S. cerevisiae*, *P. stipitis*, and *P. angophorae* for 96 h.

S. cerivisiae consumed only glucose and galactose and produced 8.9 g/l of ethanol during 144 h. *P. stipitis* produced the maximum ethanol of 12.7 g/l after 96 h using glucose, galactose, and xylose. *P. angophorae* could utilize only glucose and mannitol and produced 8.0 g/l of ethanol during 96 h. However, the three yeasts could not totally utilize the monosaccharides. Therefore, co-fermentation is needed to ferment various monosaccharides efficiently.

Among the single-strain fermentations, *P. stipitis* was the best yeast, using glucose, galactose, and xylose, whereas *S. cerivisiae* could consume glucose and galactose and *P. angophorae* could utilize glucose and mannitol. Thus, *P. stipitis* was identified as a potential candidate for efficient fermentation because of its ability to utilize glucose, galactose, and xylose rapidly [18].

Combinations of *S. cerevisiae-P. stipitis*, *S. cerevisiae-P. angophorae*, and *P. stipitis-P. angophorae* were carried out to enhance the ethanol production from waste seaweed hydrolysate. As shown in Table 2, the co-culture fermentations by *S. cerevisiae-P. stipitis* and *S. cerevisiae-P. angophorae*

Table 2. Single-strain fermentation and co-culture fermentation using waste seaweed hydrolysate.

Strains	Remained monosaccharide (g/l)				Ethanol (g/l)	Fermentation time (h)	Y_{EtOH}
	Glucose	Galactose	Xylose	Mannitol			
S. cerevisiae	0	3.0	8.2	2.5	8.90	144	0.23
P. stipitis	0	2.4	2.1	2.5	12.70	96	0.37
P. angophorae	0	8.3	8.2	0	7.95	96	0.23
S. cerevisiae -P. stipitis	0	2.5	5.2	2.5	12.20	72	0.35
S. cerevisiae -P. angophorae	0	3.7	8.2	1.2	10.45	72	0.30
P. stipitis -P. angophorae	0	0	0	0	15.74	72	0.45

produced 12.20 and 10.45 g/l of ethanol, respectively. Both fermentations could not utilize all of the monosaccharides and produced less ethanol than single-strain fermentation by *P. stipitis.* Thus, an optimal combination was needed to ferment all of the monosaccharides from waste seaweed hydrolysate.

Co-culture fermentations by *S. cerevisiae-P. stipitis* and *S. cerevisiae-P. angophorae* showed no improvement in ethanol concentration compared with single-strain fermentation by *P. stipitis*. However, the fermentation time was decreased from 144 to 72 h. A previous study also reported that co-culture fermentation by *S. cerevisiae* and *P. stipitis* did not show better results than single-strain fermentation by *P. stipitis*e, except for the fermentation time from 72 to 60 h [19]. These results were mainly from their fermentable monosaccharides. *S. cerevisiae* and *P. angophorae* could not utilize xylose. Thus, 8.2 g/l of xylose remained, with a low ethanol concentration of 10.45 g/l. The other limitation of co-culture fermentation was oxygen, which is required for *P. angophorae* to assimilate xylose and mannitol; however, oxygen reduces the *S. cerevisiae* fermentation yield [19].

Co-culture of *P. stipitis-P. angophorae* fermented all of the monosaccharides and showed higher ethanol concentration than *S. cerevisiae-P. stipitis* and *S. cerevisiae-P. angophorae.* Many of the parameters showed improvements. The maximum ethanol and yield were 13.7 g/l and 0.40 for 72 h. These high values resulted from the consumption of glucose, galactose, and xylose by *P. stipitis* and utilization of glucose and mannitol by *P. angophorae.* Because, co-culture fermentation

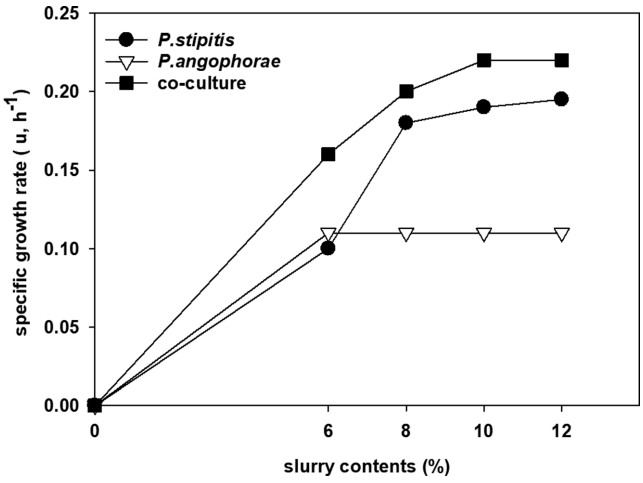

Fig. 3. Relationship between the specific growth rate (μ) and various total monosaccharide (glucose, galactose, mannitol, and xylose) concentrations (*S*) in waste seaweed hydrolysate, for single and mixed cultures of *Pichia stipitis* and *Pichia angophorae*.

by *P. stipitis* and *P. angophorae* can convert glucose, galactose, xylose, and mannitol to ethanol under the semi-anaerobic condition, they were selected as the optimal combination of yeasts for co-culture fermentation.

The combination of *P. stipitis-P. angophorae* produced the maximum ethanol concentration. Rizzi *et al.* [20] reported that at decreasing specific oxygen uptake rate, the electron transfer system is not able to oxidize the NADH produced during respiration and assimilation of xylose and mannitol. Fermentation of sugar alcohols by yeasts requires a supply of oxygen. In a previous study, the bacterium *Zymobacter palmae* could produce ethanol from mannitol in seaweed extract if a small amount of oxygen is provided [21]. However, an excessive aeration supply was also not suitable for the process, causing a reduction in the ethanol production. Thus, the aeration condition is of great importance to achieve high xylose and mannitol conversion to ethanol.

Ethanol Production Using Various Yeast Ratios

Co-culture fermentation was carried out with *P. stipitis* utilizing glucose, galactose, and xylose and *P. angophorae* utilizing glucose, xylose, and mannitol to produce ethanol from the four monosaccharides present in waste seaweed hydrolysate. However, previous research has found that one strain may be inhibited by the other strain and die after a few days of co-culture [22]. Therefore, competition between the species was analyzed using the μ-*S* relationship to evaluate the possibility of co-fermentation with the two yeasts in the same medium (Fig. 3). The outcome of competition between two species for the same growth-limiting substrate in an open system is determined by the relationship between the specific growth rate and limiting substrate concentration [14]. The two strains showed semi-competitive consumption on the glucose. However, they showed no competition in ethanol fermentation with galactose, xylose, and mannitol (Fig. 4). Therefore, the two strains could maintain cell densities at the beginning of the fermentation and converted various monosaccharides to high concentrations of ethanol by consuming all

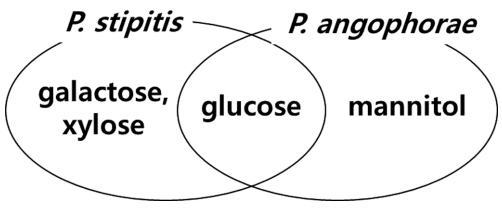

Fig. 4. Substrate utilization by the two yeast strains *Pichia stipitis* and *Pichia angophorae*.

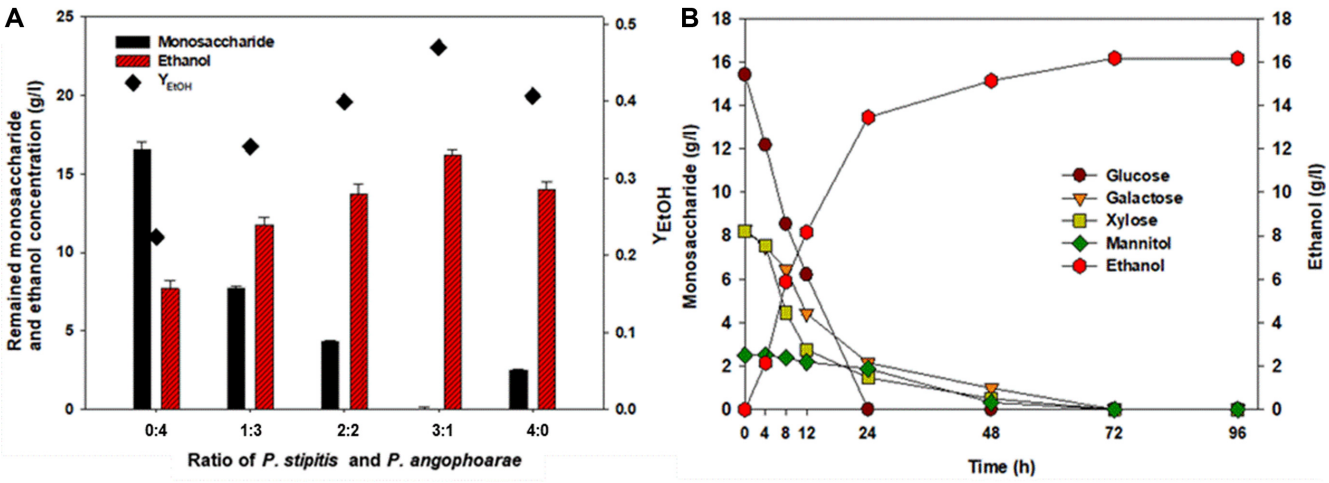

Fig. 5. Ethanol production from hydrolysate of waste seaweed with co-culture fermentation using (**A**) various yeast inoculum ratios of *P. stipitis* and *P. angophorae* for 72 h, and (**B**) inoculation with 3:1 ratio of *P. stipitis* and *P. angophorae*.

monosaccharides.

Thus, *P. stipitis* and *P. angophorae* were able to co-exist and co-ferment in waste seaweed hydrolysate that included glucose, galactose, xylose, and mannitol (Fig. 5). Co-culture fermentation was carried out with various ratios of inoculum. The *P. sipitis* and *P. angophorae* inoculum concentrations were optimized for maximal ethanol production, with a consistent total initial yeast concentration of 0.2 g/l. Ethanol production is shown in Fig. 5A with the remaining monosaccharide and ethanol concentrations and ethanol yield. Fermentation using a yeast ratio of 0:4 *P. stipitis* and *P. angophorae* had the highest remaining monosaccharide concentration, producing 8.5 g/l of ethanol with Y_{EtOH} of 0.31, because *P. angophorae* could not degrade galactose. Monosaccharides remained at 7.7, 4.3, and 2.5 g/l, respectively, with ratios of 1:3, 2:2, and 4:0, and ethanol production was 11.7, 13.7, and 14.0 g/l. A 3:1 ratio of *P. stipitis* to *P. angophorae* consumed 34 g/l monosaccharides and produced 16 g/l of ethanol with an ethanol yield of 0.47 over 72 h (Fig. 5B). These findings are similar to those of Hanly and Michael [23], who used *S. cerevisiae* and *E. coli* ZSC 113 co-fermentation to produce ethanol from glucose and xylose mixtures. The optimal ratio of *S. cerevisiae* to *E. coli* ZSC 113 in the inoculum was the same as the ratio of glucose and xylose that they consumed. Moreover, many previous studies have undertaken co-fermentation of monosaccharides by two different cultures [19, 25, 26]. Chandel *et al.* [26] reported a maximum ethanol yield of 0.48 from *Saccharum spontaneum* hydrolysates using co-culture fermentation with *S. cerevisiae* VS_3 and *P. stipitis* NCIM 3498. This study indicates that co-culture fermentation by *P. stipitis* and *P. angophoarae* at the optimal ratio (3:1) resulted in maximum conversion of sugars present in waste seaweed hydrolysate and produced 16 g/l of ethanol with an ethanol yield of 0.47, close to the maximum theoretical yield of 0.51. Therefore, co-fermentation with an optimized yeast ratio enhanced ethanol production from waste seaweed hydrolysate as a result of efficient conversion of fermentable monosaccharides. This process can lead to economic benefits by reducing the budget required for the clean-up of waste seaweed pollution generated by typhoons.

Acknowledgments

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