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Potential in bioethanol production from various ethanol fermenting microorganisms using rice husk as substrate

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Abstract. *Nachaiwieng W, Lumyong S, Pratanapol R, Yoshioka K, Khanongnuch C. 2015. Potential in bioethanol production from various ethanol fermenting microorganisms using rice husk as substrate. Biodiversitas 16: 320-326.* Rice husk was investigated as the potential substrate for bioethanol fermentation. It was collected from five locations in northern Thailand and found that the main component of rice husk approximately 51-54% (w/w) was holocellulose. The sugar composition in rice husk holocellulose was glucose, xylose and arabinose in the ratio 66.68, 27.61 and 5.71%, respectively. Before further fermentation, acid and alkali pretreatment of rice husk were prior investigated and 2% (w/v) NaOH at 130°C for 30 min was proved to be the most suitable pretreatment method without fermenting inhibitors generation. Then, rice husk hydrolysate obtained by enzymatic saccharification with Meicelase enzyme was used as carbon sources for ethanol fermentation in comparison among 11 ethanol fermenting microorganisms including 3 strains of *Saccharomyces cerevisiae*, 3 strains of *Zymomonas mobilis*, 3 strains of *Kluyveromyces marxianus* and 2 strains of pentose sugar fermenting microbes, *Candida shehatae* TISTR 5843 and *Pichia stipitis* BCC 15191. All three strains of *Z. mobilis* exhibited the best ethanol fermentation yield, giving the ethanol yield of 0.48 g g^{-1} available monosaccharides and fermentation profile of each individual genus was also demonstrated. However, some unutilized sugars still remained in rice husk fermenting medium, therefore, conversion to valuable products or optimization of co-culture ethanol fermentation needs to be further investigated.

Keywords: bioethanol, pretreatment, ethanol, fermentation, Meicelase, rice husk

INTRODUCTION

Due to rapid increasing of gasoline price and depletion of readily available oil resource, renewable resources alternative to oil, such as biomass, wind, solar, geothermal and hydroelectric energy have gained increasing attention in recent years. Bioethanol (C_2H_5OH) is widely accepted as an important source for transportation fuel and energy. It reduces CO_2 emission and pollutants when replacing gasoline in modified engine (De Oliveira et al. 2005). Since ethanol produced from edible materials caused high production cost and created ethical problem regarding competitiveness to food supply, bioethanol from lignocellulosic material has gathered keen attention (Nigam and Singh 2011). Although the bioethanol from lignocellulosics could not replace all gasoline, its importance is still widely recognized. Over the past decade, the number of bioethanol plant from lignocellulosic materials have begun to increase (Eisentraut 2010), and its production level, 21 billion gallons from cellulosic feedstock by 2022 was described in law of USA (Nigam and Singh 2011). In addition, as expected by Limayem and Ricke (2012), one billion tons of various lignocellulosic feedstocks and an additional cultivation of high yielding energy crops on Conservation Reserve Program (CRP) lands that are efficiently managed are expected to meet a 30% petroleum-based gasoline displacement in 2030.

As previously reports, ethanol are widely produces from various lignocellulosic residues such as agricultural crops i.e. wheat and paddy straws, corn stover, groundnut shell, sunflower stalks, alfalfa fiber, cotton stalks and agricultural by-products i.e. sugarcane bagasse, corncobs, palm bagasse, barley and sunflower hulls, wheat barn and especially rice husk (Arora et al. 2015). Rice husk is an agricultural waste abundantly available in rice producing countries including Thailand. Thailand was reported to be the Asia 3th rice production as the productivity approximately 4% of world rice's production was gained (Gadde et al. 2009). The annual world rice production amounts to approximately 400 million metric tons, of which more than 10% is husk (Conradt et al. 1992). In addition, rice husk contains around 50% (w/w) of cellulosic component (Wannapeera et al. 2008; Mansaray and Ghaly 1999). Industrial use of rice husk is mostly burning as a source of heat for generation of electricity, which causes environmental problems owing to a large quantity of CO_2 emission (Bharadwaj et al. 2004). Due to the environmental problem and availability as an agricultural residue in Thailand, conversion of rice husk into bioethanol is an important subject.

Due to the presence of lignin and hemicellulose in lignocellulosic structure, the access of cellulolytic enzymes

to cellulose for hydrolysis and finally ferment to ethanol is difficult. Therefore, the lignocellulose needed to be delignified by pretreatment processes which various available including physical, physico-chemical, chemical and biological pretreatment (Sun and Cheng 2002) and all having their specific advantages and disadvantages. However, the chemical pretreatment was selected in this study because of no expensive equipment required and their availability. Both acid and alkaline pretreatment are able to increase accessible surface area and alter lignin structure, moreover, alkaline pretreatment has a strong effect to remove lignin which lead to easier access of cellulolytic enzyme to cellulose structure (Mosier et al. 2005).

Until now, various ethanol producing microorganisms have been discovered. *Saccharomyces cerevisiae*, a good brewer yeast, is found to be the most popular and produce high ethanol yield from 6-carbon atom sugars such as glucose (Piškur et al. 2006). In contrast to *S. cerevisiae*, *Zymomonas mobilis* is another candidate which capable of grows and produces high concentration of ethanol from higher initial sugar and more tolerate to ethanol concentration with lower biomass formation (Rogers et al. 1979). However, the processes of ethanol fermentation by both strains described above are favorable working under 30-35ºC which is not suitable for tropical countries and incompatible when using in Simultaneous Saccharification and Fermentation (SSF) process. Therefore, *Kluyveromyces marxianus*, a thermotolerant ethanol fermenting yeast, has been chosen for ethanol fermentation at high temperature condition instead, in particular for SSF (Ballesteros et al. 2004). Not only glucose, various 5-carbon atom sugars such as xylose and arabinose are consisted in hemicelluloses component of lignocellulosic residues. Unfortunately, *S. cerevisiae*, *Z. mobilis* and some strains of *K. marxianus* could not utilize those sugars as their carbon source. Potential strains which capable of fermenting these sugars such as *Pichia stipitis* and *Candida shehatae* are called pentose fermenter. Therefore, to complete and efficient conversion of both 5- and 6- carbon atom sugars, co-culture fermentation between both hexose and pentose fermenter should be carried out, even though few experiments were succeeded (Fu and Peiris 2008; Fu et al. 2009).

This manuscript describes a rice husk compositions and the most suitable chemical pretreatment method for obtaining the highest rice husk sugar yield. Moreover, this is the first report demonstrated the comparison in ethanol fermentation profile of various ethanol fermenting microorganism groups including common yeast, thermotolerant yeast, pentose fermenter yeast and ethanol fermenting bacteria. This could be helpful for further strain selection when using rice husk as substrate for ethanol production.

MATERIALS AND METHODS

Microorganisms

Fermenting microorganisms, *Saccharomyces cerevisiae* TISTR 5088 [S5088], *S. cerevisiae* TISTR 5169 [S5169], *S. cerevisiae* TISTR 5339 [S5339], *Zymomonas mobilis* TISTR 405 [Z405], *Zymomonas mobilis* TISTR 548 [Z548], *Z. mobilis* TISTR 551 [Z551] and *Candida shehatae* TISTR 5843 [C5843] were purchased from Thailand Institute of Scientific and Technological Research (TISTR). *Pichia stipitis* BCC 15191 [P15191], *Kluyveromyces marxianus* BCC 7025 [K7025] and *Kluyveromyces marxianus* BCC 7049 [K7049] were purchased from Biotech Culture Collection (BCC), Thailand. *Kluyveromyces marxianus* CK8 [KCK8] was previously isolated from rotten fruit in Chiang Mai (Am-aiam and Khanongnuch 2015).

Sample preparation

Rice husk samples were randomly collected from Northern provinces of Thailand including Chiang Mai, Chiang Rai, Lamphun, Lampang and Nan without rice variety consideration. Samples were washed thoroughly with tap water and dried at 60^{o}C for 3 days. Dried samples were milled with hammer mill and size screened by sieving through 16 mesh aluminium sieve. All samples were kept in the desiccators until the experiments.

Analysis of rice husk composition and sugar components

The ground rice husk samples from all 5 sources were subjected to composition analyses including holocellulose, hemicellulose, lignin, ash, protein, lipid and soluble carbohydrate according to the protocols of acid chlorite method (Browning 1963), TAPPI T203 om-88 (TAPPI, 1992), TAPPI T222 om-88 (TAPPI, 1988), TAPPI T211 om-85 (TAPPI, 1985), Kjeldahl method (Conklin-Brittain et al. 1999), Soxhlet extractor method (Wren and Mitchell 1959) and phenol sulfuric method (Dubois et al. 1956), respectively.

For sugar component analysis, approximately 0.5 g of rice husk was hydrolyzed with 8 ml of 72% (w/w) sulfuric acid, gentle mixed and incubated at 30^{o}C for 1 h. The sample was then diluted with 300 ml of distilled water to adjust a final concentration of sulfuric acid to be 2% (w/w). The solution was then autoclaved at 121^{o}C for 30 min and neutralized by addition of $Ba(OH)_2$. A clear supernatant was obtained by centrifugation at 4290 x g for 20 min and subjected to quantitative analysis of neutral sugars by High Performance Liquid Chromatography (HPLC) Shimadzu LC 20A system (Shimadzu Corp., Kyoto, Japan) equipped with Aminex HPX-87P (300 mm×7.8 mm) (BioRad, USA) using deionized distilled water as an eluent at a flow rate of 0.3 ml min^{-1} and the column temperature, 58^{o}C. Glucoheptose was used as an internal standard. Detection was carried out with a Fluorescent Detector (FLD) at 420 nm by post reaction with a mixture of arginine and boric acid (1:3, v/v) at 150^{o}C.

Pretreatment of rice husk samples

Dried rice husk sample was pretreated by 0.5, 1.0, 1.5 and 2.0 % (w/v) of diluted sulfuric acid and sodium hydroxide solution with ratio 1:10. Pretreatment process was carried out in autoclave machine at 130^{o}C for 30 min and allowed to cool down overnight. Solid fractions were

obtained by filtration through Whatman No.1 filter paper and then washed by excess volume of tap water for neutralization. Pretreated rice husk sample was dried at 60°C for 2 days before enzymatic saccharification.

Enzymatic saccharification of pretreated rice husk

Approximately 0.2 g of chemical pretreated rice husk samples were hydrolyzed by 40 FPU g^{-1} substrate of commercial cellulolytic enzyme "Meicelase" (510 FPU g^{-1} enzyme powder, from *Trichoderma viride*, Meiji Seika Company, Tokyo, Japan) dissolved in sodium succinate buffer (pH 4.8), in the presence of 0.1% sodium azide. A saccharification was carried out in a shaking incubator at 45°C, 150 rpm for 48 h and the reducing sugars liberated were finally quantified by dinitrosalicylic acid (DNS) method (Miller 1959).

Rice husk fermenting medium preparation

Rice husk hydrolysate was prior prepared by hydrolyzing the 2.0% (w/v) NaOH pretreated rice husk sample with Meicelase (40 FPU g^{-1} of substrate) at 45°C for 48 h as previously described without sodium azide added. The liquid portion was obtained by filtered through Whatman No.1 filter paper and used as a carbon source for fermenting medium preparation. The sugar concentration was adjusted to approximately 20 g L^{-1} by rotary evaporator. The concentrates were supplemented with yeast extract, $(NH_4)_2HPO_4$ and $MgSO_4.7H_2O$ at the final concentration of 3.0, 0.25 and 0.025 g L^{-1}, respectively (Gupta et al. 2009), and sterilization by autoclaving at 121°C for 15 min.

Determination of fermenting inhibitor in rice husk fermenting medium

Fermenting inhibitors which may generate during pretreatment process are very important factors for ethanol yield and need to be investigated before further fermentation. Furfural, 5-hydroxy methyl furfural (5-HMF) and acetic acid were determined by HPLC (Shimadzu LC 20A system) equipped with Aminex HPX-87H column (300mm×7.8mm with guard cartridge). Separation was carried out at 35°C using 8 mM sulfuric acid as a mobile phase with flow rate 0.6 mL min^{-1}. Peaks were detected by Photo Diode Array (PDA) detector. Lignin degradation products, vanillin, syringaldehyde and 4-hydroxy-benzaldehyde, were also analyzed by HPLC (Shimadzu LC 20A system) equipped with Imtakt Unison UK-Phenyl (150×4.6 mm, 3 m) without guard cartridge. Separation was performed at 40°C using gradient between 10mM ammonium acetate buffer and acetonitrile as mobile phase with flow rate 1 mL min^{-1} and peaks were also detected by PDA detector.

Comparison of fermentative ability of various microorganisms

The comparative fermentation experiment by fermenting microorganisms were preliminary carried out with 3 strains of common ethanol fermenting yeast *S. cerevisiae*, 3 strains of thermotolerant ethanol fermenting yeast *K. marxianus*, 3 strains of ethanol fermenting bacteria *Z. mobilis* and 2 strains of pentose fermenter, *C. shehatae* and *P. stipitis*, by inoculated 5% (v/v) of inoculum (10^8 CFU) into 125 mL-Duran bottle containing of 50 ml of rice husk fermenting medium. The fermentation was carried out at 30°C, except for *K. marxianus* which carried out at 45°C for 96 h, and the samples were collected 12-h interval. Ethanol concentration was analyzed by gas chromatography (GC-17A; Shimadzu, Tokyo, Japan) using a flame ionization detector and stainless steel column with 15.0 m in length, 0.53 mm of diameter and 0.5 µm of film thickness. The column, injection and detector temperature were maintained at 40°C, 230°C and 250°C, respectively. Nitrogen was used as carrier gas at a flow rate of 30 mL min^{-1}. Reducing sugar was determined by DNS method. Concentration of glucose and xylose were measured by Autokit Glucose (Wako Chemicals, Osaka, Japan) and D-xylose assay kit (Megazyme, Wicklow, Ireland), respectively. Cell density was measured by spectrophotometer at wavelength 600 nm.

RESULTS AND DISCUSSION

Rice husk composition analysis

There were slightly significant differences in compositions among 5 rice husk samples at p-value < 0.05. Variation of rice husk component could occur due to the varieties of paddy sown, watering, geographical conditions, fertilizer used, climate, soil chemistry, age of paddy and growth conditions (Foo and Hameed 2009). Holocellulose, alpha cellulose, hemicelluloses and lignin content were 51-54%, 20-25%, 28-32% and 28-30% (w/w), respectively

Table 1. Compositions of 5 different sources rice husk samples collected from Northern Thailand

Samples*	Holo cellulose (%)	Alpha Cellulose (%)	Hemi Cellulose (%)	Lignin (%)	Ash (%)	Soluble Carbohydrate (%)	Lipid (%)	Protein (%)
CM	52.86^{ab**}	20.86^{a}	32.00^{a}	30.01^{a}	18.73^{a}	1.63^{a}	0.07^{a}	2.34^{a}
CR	51.28^{a}	22.26^{b}	29.02^{c}	28.92^{b}	20.21^{b}	2.04^{b}	0.10^{a}	2.55^{a}
LPo	54.14^{b}	25.50^{c}	28.64^{d}	30.30^{a}	18.79^{a}	1.47^{a}	0.25^{b}	2.49^{a}
LPa	52.73^{ab}	20.87^{a}	31.86^{a}	28.81^{b}	18.67^{a}	1.53^{a}	0.09^{a}	2.47^{a}
NN	51.16^{a}	20.17^{a}	30.99^{b}	29.72^{ab}	19.03^{a}	1.71^{a}	0.12^{a}	2.36^{a}

Note: * CM, CR, LPo, LPa and NN were rice husk sample collected from Chiang Mai, Chiang Rai, Lamphun, Lampang and Nan Provinces. ** Superscript letters represented a significant at $p < 0.05$

(Table 1). Rice husk sample (LPo) was selected for further study due to its highest holocellulose content. The holocellulose content (>50% w/w) of rice husk was comparable to previous studies (Saha, Badal C. and Cotta 2008; Banerjee et al. 2009; Hsieh et al. 2009). The ash content was 18-20%, interestingly 95% of ash in rice husk is silicon (Della et al. 2002) and use for silicon-based materials after bioethanol production is also attractive. The neutral sugar composition in rice husk was glucose, xylose and arabinose in ratio 66.68 ± 0.97, 27.61 ± 1.06 and 5.71 ± 0.17%, respectively. This result was similar to previous result which was analyzed the neutral sugar, glucose, xylose and arabinose, from rice husk in ratio 61.62, 34.23 and 4.15%, respectively (Nabarlatz et al. 2007). These results indicated that cellulose and xylan are the major polysaccharides in rice husk and arabinan is also present in the sample but only in trace amount.

To optimize a chemical pretreatment process, the ability of enzymatic saccharification after pretreatment was crucial in the evaluation of the best pretreatment process for rice husk. Sulfuric acid and sodium hydroxide were widely used as pretreatment substances as referred to the previous reports (Saha, Badal C et al. 2005; Singh et al. 2011). Concerning to environmental friendly policy and the high quantity of chemical usage in rice husk pretreatment with sulfuric acid and sodium hydroxide, the maximum concentration of each pretreatment substance was assigned at maximum of 2.0% with implementation by conventional autoclave. The highest yield of liberated sugars from enzymatic saccharification based on 2.0% sodium hydroxide pretreatment process was 37.35 ± 2.11% (w/w) of rice husk dry weight and significantly different at $p < 0.05$ from pretreatment by the other concentrations of sodium hydroxide (Figure 1). In contrast, the highest yield of liberated sugars from enzymatic saccharification after pretreatment by 0.5% sulfuric acid was only 0.96 ± 0.41% of rice husk weight. These indicated that acid pretreatment was not suitable for rice husk pretreatment and hemicellulose might be lost during pretreatment process with the formation of a toxic hydroxymethylfurfural (Lee et al. 1999).

Moreover, even though, both acid and alkaline pretreatment could increase accessible surface area and alter lignin structure, but lignin was only removed in case of alkaline pretreatment (Mosier et al. 2005) especially sodium hydroxide pretreatment on rice husk residue (Nikzad et al. 2015) which allow cellulolytic enzyme to penetrate and hydrolyze cellulose structure, lead to high amount of sugar released. Therefore, alkaline pretreatment process based on 2.0% sodium hydroxide was selected to be the most appropriate chemical pretreatment for rice husk in this experiment. In addition, after pretreatment rice husk by 2% (w/v) NaOH solution, glucose, xylose and arabinose contents were non-significantly decreased, the total sugar loss during pretreatment process was calculated as 6.04% (Table 2) without any detection of fermenting inhibitors such as furfural, 5-hydroxy methyl furfural (5-HMF), acetic acid, lignin degradation products, vanillin, syringaldehyde and 4-hydroxybenzaldehyde. Because of low removal of hemicellulose and cellulose structure by alkaline pretreatment (Mosier et al. 2005; Ang et al. 2013), there was slightly sugar released during pretreatment process as presented in this study. Low sugar released and no sugar degradation during pretreatment is key factors for effective pretreatment method as described by Yang and Wyman (2008). As the result, the alkaline pretreatment was chosen in this study for further ethanol fermentation without concerning of fermenting inhibitor and sugar loss during pretreatment. Furthermore, our results were compatible with previous studies, alkaline pretreatment with 120°C was sufficient to increase the digestibility of low lignin containing lignocellulosic biomass (Kaar and Holtzapple 2000) without any fermenting inhibitors generation (Chang et al. 2001). Even though liquid fractions from various kinds of biomass from acid hydrolysis method are widely used for ethanol production, however, time consuming and complicated detoxification processes are needed because of the presence of various fermenting inhibitors (Larsson et al. 1999).

Comparison of ethanol fermentation of rice husk sugar by various fermenting microorganisms

According to previous study of fermentation, all fermenting strains could grow and ferment in the presence of succinic acid but some microorganisms could not grow in the presence of acetic and citric acid (Nachaiwieng et al. 2015). Therefore, sodium succinate buffer was used in saccharification process to avoid some inhibitors from acid mentioned above. With this buffer and under a static condition, all fermenting strains could grow well except for *K. marxianus*. This strain need more oxygen to grow and ferment, thus 150 rpm shaking condition was then applied for this strain as previously described by Limtong et al. (2007). Furthermore, an anaerobic bacteria *Z. mobilis*, which could not grow well under aerobic condition needed a sterilized liquid paraffin to make a layer for absorbing oxygen (Li et al. 2000; Yoshida et al. 1970). When the fermentation was terminated, the ethanol yield obtained from the same genus was similar. The highest ethanol yield was obtained from *Z. mobilis* with 0.48 g g^{-1} available monosaccharides or 94.12% of theoretical yield (Table 3). *Z. mobilis* is found to be the highest ethanol yield producing strain because of less biomass is produced and a

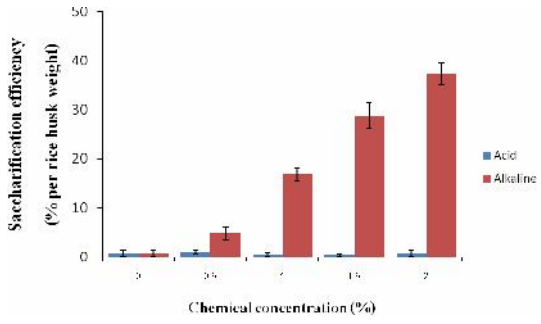

Figure 1. Percentage of liberated sugars per rice husk weight from enzymatic saccharification after acid and alkaline pretreatment of rice husk

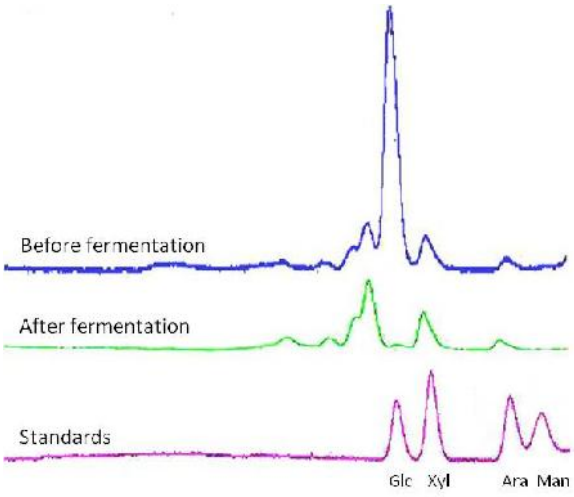

Figure 2. HPLC based sugar analysis of rice husk hydrolysate, before and after ethanol fermentation by hexose fermenting strain (Glc, Xyl, Ara and Man were glucose, xylose, arabinose and mannose, respectively)

Table 2. The amount of rice husk monosaccharide (g L^{-1}) loss after pretreatment rice husk by 2% (w/v) NaOH at 130°C for 30 min

Monosaccharides	Untreated rice husk (g L^{-1})	2% (w/v) NaOH pretreated rice husk (g L^{-1})
Glucose	$0.356^{a} \pm 0.03$	$0.347^{a} \pm 0.04$
Xylose	$0.085^{a} \pm 0.01$	$0.074^{a} \pm 0.01$
Arabinose	$0.039^{a} \pm 0.01$	$0.030^{a} \pm 0.01$

Note: Significant at $p < 0.05$

Table 3. Ethanol yield production from comparative fermentation by various fermenting microorganisms on each microorganism optimum fermenting condition

Microorganisms	Ethanol yield (g g^{-1} available mono-saccharides)*	Ethanol yield (g g^{-1} available sugar)
S. cerevisiae TISTR 5088	0.45	0.27
S. cerevisiae TISTR 5169	0.45	0.27
S. cerevisiae TISTR 5339	0.45	0.26
Z. mobilis TISTR 405	0.48	0.29
Z. mobilis TISTR 548	0.48	0.28
Z. mobilis TISTR 551	0.48	0.28
K. marxianus BCC 7025	0.43	0.24
K. marxianus BCC 7049	0.42	0.24
K. marxianus CK8	0.42	0.25
C. shehatae TISTR 5843	0.40	0.24
P. stipitis BCC 15191	0.42	0.25

Note: *Hydrolyzed rice husk sugar contained unidentified oligomers and could not utilize by microorganisms

higher metabolic rate of glucose is maintained through its special Entner-Doudoroff pathway (Bai et al. 2008). Ethanol yield produced by *Z. mobilis* strains in this study are corresponding to previous reports that ethanol yield from *Z. mobilis* ATCC 10988 and *Z. mobilis* ATCC 31821 at 0.472 and 0.468 g g^{-1} available glucose, respectively (Rogers et al. 1982; Tao et al. 2005). Other fermenting strains, *S. cerevisiae*, *K. marxianus*, *P. stipitis* and *C. shehatae* TISTR 5843, produced ethanol 0.45, 0.42-0.43, 0.42 and 0.40 g g^{-1} available monosaccharides, respectively. Interestingly, ethanol yield obtained from all *S. cerevisiae* strains in this study were significantly higher than 0.41 g g^{-1} obtained from *S. cerevisiae* ITV-01 (Ortiz - Muniz et al. 2010) and also higher than those strains mentioned by Hahn-Hägerdal et al. (2006) from various source of agricultural hydrolysate fermenting media. Moreover, comparing ethanol yield from rice husk hydrolysate, ethanol yield obtained from this study is better than 0.43 g g^{-1} sugar obtained using commercial *S. cerevisiae* as fermenter yeast (Dagnino et al. 2013). As previous results, various strains used in this study, especially *Z. mobilis* and *S. cerevisiae*, seemed to be suitable strains for producing ethanol from rice husk hydrolysate although the fermentation process has not yet optimized in this study. In addition, possible highest ethanol yield from rice husk in this study was 0.26-0.28 g g^{-1} of dry rice husk which is potential substrate when compared to barley straw, corn stover, oat straw, rice straw, sorghum straw, wheat straw and bagasse which gave an ethanol yield at 0.26-0.31 g g^{-1} of dry biomass (Kim and Dale 2004). However, this ethanol yield was calculated from rice husk holocellulose and maximum ethanol yield (0.51 g g^{-1} sugar), therefore, further study of optimization on fermentation process and co-culture fermentation between hexose and pentose fermenter should be carried out to reach to expecting target of ethanol yield.

As presented in Figure 3, the fermentation profile was unique in each genus. Highest ethanol yield from *S. cerevisiae, Z. mobilis* and pentose fermenter was obtained after 24 h and continued constant. In contrast, the highest ethanol yield from *K. marxianus* was obtained after 12 h and gradually decreased as similar due to high temperature used in fermentation process (Teixeira and Vicente 2013) as presented in previous study (Signori et al. 2014). Interestingly, there were some sugars remaining after fermentation by all microorganisms and were detected by HPLC as xylose, arabinose and unidentified oligomers (Figure 2) while all of glucose was utilized within 24 h. Except for *C. shehatae* TISTR 5843 and *P. stipitis* BCC 15191 which could utilized both glucose and a little amount of xylose (Figure 3). Unfortunately, even both strains could utilize both glucose and xylose but ethanol yield obtained from these strains were lower than others. Therefore, a fermentation of rice husk medium with high potential ethanol producing strain and the conversion of remaining pentose and its oligomer to high valued substances such as xylooligosaccharide or xylitol, or optimize a co-culture ethanol fermentation between pentose and hexose fermenter have to be further investigated.

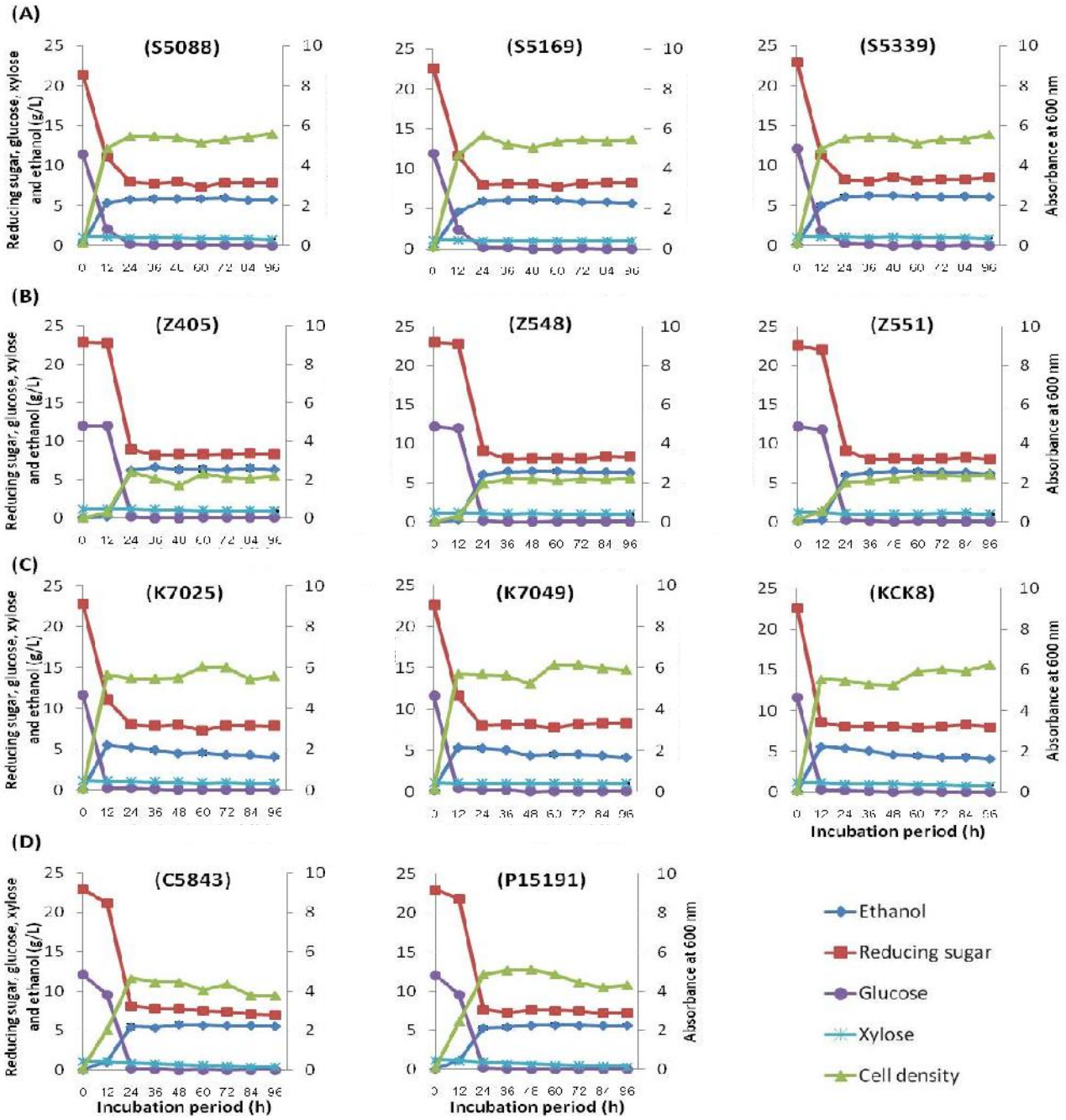

Figure 3. Comparative study of ethanol fermentation from various fermenting microorganism strains, *S. cerevisiae* (A); *Z. mobilis* (B); *K. marxianus* (C) and Pentose sugar fermenter (D)

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