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Research article

Assessment of holocellulose for the production of bioethanol by conserving *Pinus radiata* cones as renewable feedstock

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ABSTRACT

Renewable and green energy sources are much sought. Bioethanol is an environmentally friendly transportation fuel. Pine cones from *Pinus radiata* were shown to be a potential feedstock for the production of bioethanol. Alkaline (NaOH) pretreatment was carried out to delignify the lignocellulosic material and generate holocellulose (72 wt. % yield). The pretreated biomass was hydrolysed using HCl as catalyst under microwave irradiation and hydrothermal conditions. Microwave irradiation was found to be better than the hydrothermal process. Microwave irradiation accelerated the hydrolysis of biomass (42 wt. % conversion) with the reaction conditions being 3 M HCl and 5 min of irradiation time. Interestingly, even the xylose, which is the major component of the hydrolyzate was found to be metabolized to ethanol using Baker's yeast (*Saccharomyces cerevisiae*) under the experimental conditions. 5.7 g of ethanol could be produced from 100 g of raw pine cones.

1. Introduction

Towards the realization of the objective of practical biorefinery, enormous efforts are being made for converting biomass to biofuels (Baeyens et al., 2015; Fan et al., 2014; Horvath et al., 2008; Kang et al., 2014a, 2014b, 2014c; Kim et al., 2003; Meerbeek et al., 2015; Pulidindi et al., 2014a, b; Victor et al., 2014). The hypothesis tested in the current work is – Can pine cones from *Pinus radiata* be a renewable feedstock for fuel ethanol production? Wood of *P. radiata* is well studied for ethanol production (Reyes et al., 2013; Franco et al., 2011; Fissore et al., 2010; Monrroy et al., 2010; Munoz et al., 2007). Unlike wood chips, the pine cones are a waste and renewable resource from *P. radiata* plantation. The use of pine cones would be a better choice relative to wood for bioethanol production. Currently pine cones are of minor importance relative to the first (sugarcane, corn, wheat, cassava), second (lingo cellulosic biomass) and third (algae) generation bioethanol feedstock which have been extensively investigated (Baeyens et al., 2015). Extensive studies need to be made before pine cones could be economically collected and transformed into a promising biomass source and the present study is an effort in that direction.

Baeyens et al., critically reviewed the challenges involved in improving the state-of-the-art bioethanol production processes. Raw material production cost is an important factor in addition to the improvements in fermentation (very high gravity), micro filtration of the broth, bioethanol distillation (integrated condensers and reboilers), and ethanol dewatering (membrane technology) (Baeyens et al., 2015; Kang et al., 2014c).

Typical challenges in the production of bioethanol include, pretreatment, hydrolysis, ethanol tolerance of the yeast, ethanol yield, and fermentation rate (Pulidindi and Gedanken, 2015). The generation of fermentation inhibitors like acetic acid, hydroxy methyl furfural, furfural, glycol aldehyde and soluble phenolic compounds is also a prominent issue to be solved (Cavka et al., 2011; Sanda et al., 2011; Carter et al., 2011a, b; Raghu et al., 2011; Yong-Jin et al., 2011; Lee et al., 2011; Huang et al., 2011; Zhang et al., 2011; Bellido et al., 2011; Geddes et al., 2011; Sainio et al., 2011; Jayakody et al., 2011). A variety of biomass (cashew apple pulp, coffee pulp waste, switch grass, oil palm empty fruit bunches, seaweeds, bamboo, sugar-cane tops) are being examined as economically viable feedstock for the production of bioethanol (Sindhu et al., 2011; Zhao-Yong et al., 2011; Shenoy et al., 2011;

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Martin and Grossmann, 2011; Han et al., 2011; Yanagisawa et al., 2011a, 2011b). Bioethanol is a renewable and environmentally-friendly (carbon neutral) fuel in contrast to the fast depleting, petroleum-based fuels (Pulidindi et al., 2012; Parmar et al., 2011; Sasaki et al., 2011; Paivi et al., 2011; Dias et al., 2011). Glucose based raw materials like sugarcane juice or molasses (Brazil), corn (US), cassava (China, Nigeria) were extensively used worldwide for bioethanol production as they readily release glucose and facilitate fermentation (Kang et al., 2014a). Cellulosic (second generation) bioethanol could be a promising alternative to first generation bioethanol provided the current bottlenecks (number of operational steps, production of inhibitors, alcohol tolerance and conversion efficiency) are surmounted (Kang et al., 2014b). Intense research is devoted to the exploitation of marine algae for bioethanol (third generation) production (Korzen et al., 2015a, 2015b). Baeyen's have recently reviewed the possibilities and challenges in the third generation bioethanol production (Baeyens et al., 2015).

Biomass (terrestrial or marine) constitutes an abundantly available and renewable feedstock for the production of bioethanol. The objective of the present work is to exploit the pine cones, a sustainable waste material, from the tree, *P. radiata*, as a viable feedstock for the production of bioethanol. Such a lignocellulosic biomass which is available in plenty remained unused for bioethanol production. The feasibility of xylose metabolism to ethanol using *Saccharomyces cerevisiae* (Baker's yeast) was demonstrated.

2. Experimental

Pine cones fallen from the trees were collected from the Eli Cohen park near Bar Ilan University, Israel. Various experimental stages involved in the conversion of pine cones to bioethanol include delignification, hydrolysis of holocellulose and fermentation of carbohydrate monomers to ethanol. Pine cones were subjected to grinding in a mechanical blender and sieved with a USA standard testing sieve to a mesh size of 250 μm. The powder material obtained was subjected to delignification. Typical delignification process consists of treating 10 g of ground pine cones with 100 mL of 0.125 M NaOH for 1 h at 70 °C under stirring in a water bath (Han et al., 2011). The contents were then filtered through a filter paper (Whatman® 150 mmΦ) and washed with excess water.

The hydrolysis of pretreated biomass was carried out in the presence of HCl as catalyst. Two methods, namely, microwave irradiation as well as hydrothermal treatment in a polytetrafluoroethylene (Teflon) lined stainless steel autoclave, were used for the hydrolysis of biomass. Microwave irradiation was carried out in two devices: a domestic as well as commercial (MARS, CEM) microwave ovens. The domestic microwave oven (DMWO) operates at 2.45 GHz in a batch mode under atmospheric pressure. The output of the domestic microwave reactor was 1100 W. The microwave oven was modified so as to have provision for a distillation column passing through the MW oven (for enhanced safety of operation) as well as a stirring facility during the reaction (Pulidindi et al., 2014a, b). The temperature attained by the reaction batch (90 °C) upon microwave irradiation is measured using a pyrometer (Fluke, 65 Infrared Thermometer). Use of commercial (MARS, CEM) microwave oven permitted the precise control as well as variation of temperature of saccharification. Typical hydrolysis of pretreated biomass on domestic microwave oven involves irradiating 0.5 g biomass with 10 mL of HCl (1, 3 and 5 M) for 5 min. So as to obtain significant amount of the fermentable sugars to be converted to ethanol, the hydrolysis process was also scaled up to 2.6 g of raw pine cones.

Typical hydrolysis of pretreated biomass on commercial microwave oven involves irradiating 0.25 g of the sample with 5 mL of 3 M HCl for 10 min at 80, 100 and 120 °C. The devices used for the process of saccharification are depicted in Fig. 1.

In addition to using microwave irradiation for the hydrolysis reaction, conventional hydrothermal method has also been employed. Typical hydrolysis of delignified biomass in an autoclave involves subjecting 0.5 g of the sample with 10 mL of HCl (1, 3 and 5 M) to hydrothermal treatment for 60 min at 120 °C.

The unreacted solid mass was separated from the hydrolyzate by filtration through a filter paper (Whatman® 150 mmΦ). The filtrate was analyzed by ^{13}C NMR for fermentable sugars. The residue was dried in an air oven at 100 °C overnight. The wt. % conversion of holocellulose was calculated from the difference in the weight of the initial and final amounts (Pulidindi et al., 2014a, b). Fermentation of the hydrolyzate was carried out using *S. cerevisiae* (Baker's yeast procured from super market) in an incubator at 37 °C and at 150 rpm shaking. Typical fermentation process comprised of placing the 60 mL hydrolyzate (obtained from the hydrolysis of 1.85 g pretreated biomass) in a 250 mL Erlenmeyer flask. The hydrolyzate was neutralized with NaOH solution. To the neutral hydrolyzate 1.2 g Baker's yeast was added. The contents were incubated at 37 °C. Aliquot of sample from the fermentation broth was collected at 18^{th} h. The duration of fermentation was arbitrarily chosen. Qualitative analysis of the sugars in the hydrolyzate and ethanol in the fermentation broth was carried out using ^{13}C and ^{1}H NMR respectively. D_2O was used as the solvent. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DPX 300. Quantification of fermentable sugars in the hydrolyzate and ethanol in the fermentation broth was carried out using HPLC analysis using a strong cation-exchange column (Aminex HPX-87H, 300 × 7.8 mm).

3. Results and discussion

3.1. Pretreatment of pine cones

High cost (~33% of the total cost) of pretreatment of biomass is one of the stumbling blocks for the commercialization of 2^{nd} generation bioethanol (Baeyens et al., 2015). Kang et al., made a detailed review of the biomass pretreatment methods that have been developed so far. Alkaline (dil. NaOH) pretreatment is one of the practically viable methods yielding high fermentable sugars with low investment, among several physical (steam explosion, ultrasound, microwave and gamma ray irradiation) and chemical (acid, alkali, salt, wet oxidation) methods (Kang et al., 2014b).

The alkaline (NaOH, 0.125 M) pretreatment was effective in the delignification of the pine cones. From the original 10 g of pine cones 72 wt. % holocellulose was generated.

The XRD patterns of raw (original pine cone power before delignification), alkali treated (pine cone powder after delignification) and the residual biomass (the unreacted holocellulose upon acid hydrolysis under microwave irradiation) were shown in Fig. 2.

It is known that naturally occurring cellulose (from biomass) exists in a mixture of triclinic (I_α) and monoclinic ((I_β) phases with the I_β phase being the dominant one. The peaks typical of cellulose were indexed (Park et al., 2010). The relative intensities of I_{002} and I_{101} peaks did not change upon delignification, meaning that the cellulose component remain unaffected during alkali pretreatment.

On the contrary, the XRD pattern of the residual biomass after hydrolysis reaction, the I_{002} and I_{101} appear more or less equally intense. In general, the I_{002} peak is attributed to amorphous cellulose. Decrease in I_{002} and increase in I_{101} peak intensities in the residue are expected upon hydrolysis. Generally during acid hydrolysis, the amorphous cellulose is preferentially converted relative to the crystalline fraction. Thus the value of I_{002}/I_{101} close to unity indicates that the residual unreacted component largely comprise of the crystalline fraction of the biomass. Other minor aspects of the XRD patterns include the appearance of additional

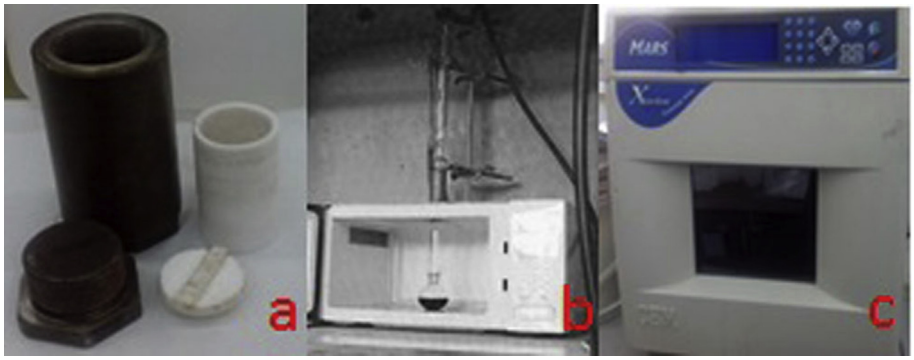

Fig. 1. Teflon lined stainless steel autoclave, (b) modified domestic microwave oven and (c) commercial microwave oven, MARS, CEM.

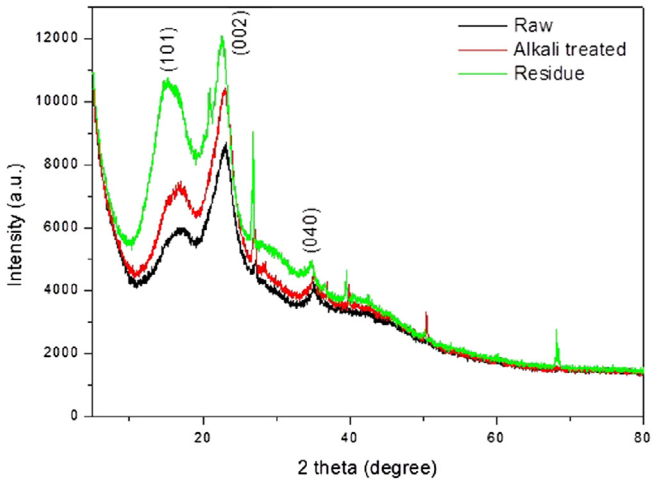

Fig. 2. XRD of raw, alkali pretreated and residual (unreacted during hydrolysis) biomass.

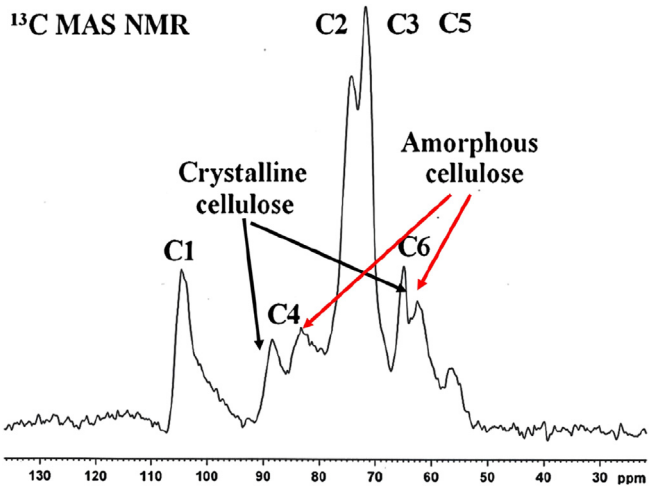

Fig. 3. ^{13}C MAS NMR spectrum of holocellulose from pine cones.

sharp lines (at 16.9, 36.9, 40.1, 50.4 and 68°) for the alkali pretreated and residual biomass which could be due to the oxide species of Na bound to the cellulose component as a result of alkali (NaOH) pretreatment. The exact nature of the oxide species is out of the scope of this study.

In addition to XRD, ^{13}C MAS (magic angle spinning) NMR was also employed to analyze the structure of the holocellulose obtained from pine cones after alkali treatment. The NMR peak pattern of the pretreated biomass is typical of Avicel PH-101 (Park et al., 2010) and microcrystalline cellulose (Earl and VanderHart, 1980). The peaks were assigned to respective carbon nuclei of cellulose and the regions of amorphous and crystalline cellulose were indicated in the spectrum (Fig. 3).

3.2. Hydrolysis of holocellulose – hydrothermal method

The conventional hydrothermal method for the hydrolysis reaction was carried out in a stainless steel autoclave placed in an air oven. Conversion values of 32, 40 and 42 wt. % were observed with 1, 3 and 5 M HCl concentrations. 3 M HCl is the optimum concentration of HCl. ^{13}C NMR spectra of the hydrolyzate at different concentrations of HCl are shown in (Fig. 4).

As expected glucose (C6 sugar) and xylose (C5 sugar) are observed in the hydrolyzate. The cellulose and hemicellulose fractions of the holocellulose yield glucose and xylose respectively upon hydrolysis (Jacobsen and Wyman, 2000). Even though the ^{13}C NMR spectra appears complex with a cluster of peaks, we assign the ^{13}C NMR peaks (92.2 (C1α), 72.8 (C2α), 74.0 (C3α), 71.4 (C4α), 60.9 (C5α) and 96.6 (C1β), 75.8 (C2β), 75.8 (C3β), 69.2 (C4β), 65.1 (C5β)) to xylose and the peaks at (60.6 (C6), 75.8 (C5), 69.5 (C4), 74.2 (C3), 72.7 (C2), 92.0 (C1α), 95.3 (C1β)) to glucose.

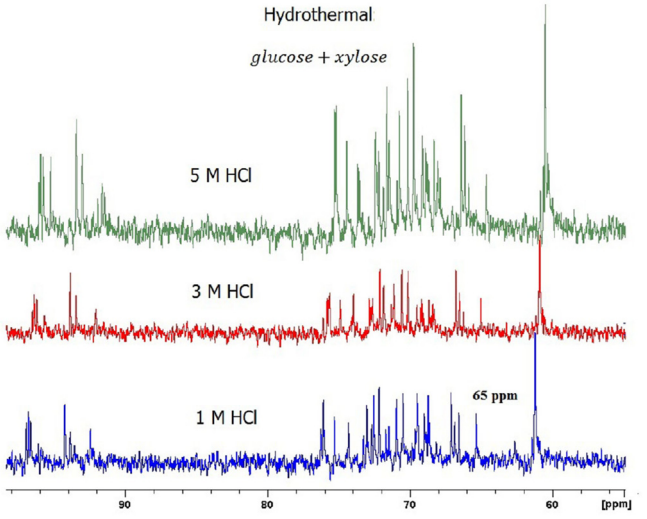

Fig. 4. ^{13}C NMR spectra of the hydrolyzate from hydrothermal hydrolysis of holocellulose.

Thus we could identify the presence of the sugars in the complex spectrum with ease and authenticity. Interestingly the ^{13}C NMR spectra of both glucose and xylose appear exactly alike except the additional peak at 65 ppm typical of C5 of α-D xylose. Thus the hydrolyzate from the holocellulose of pine cones is a complex mixture of α and β isomers of glucose and xylose. The highest amount of conversion of holocellulose under optimal hydrothermal conditions (120 °C, 1 h, 3 M HCl) is 40 wt. %.

3.3. Hydrolysis of holocellulose – microwave irradiation method

Microwave (0.3–300 GHz) irradiation accelerates the hydrolysis of biomass. The biomass structure is degraded by the localized heating. Enormous heat is generated within the material upon absorption of microwave by the components (water, carbohydrates) of biomass. As a result of the disruption of biomass structure, carbohydrate components become accessible to catalytic species leading to the acceleration of the hydrolysis process. Contrary to conventional heating, during microwave irradiation, interaction takes place between the electromagnetic field and the biomass resulting in rapid and volumetric heating. Such a heating causes explosive effect on biomass particles and the cellulosic structure is altered at the nano regime (Pulidindi and Gedanken, 2015).

Microwave irradiation (in domestic oven) of the holocellulose was carried out for 5 min. Holocellulose conversion values of 38, 42 and 44 wt. % respectively were obtained with 1, 3 and 5 M HCl, respectively. The ^{13}C NMR spectra of the hydrolyzate obtained at different acid concentrations were shown in Fig. S1.

Irrespective of the concentration of acid, the product selectivity remained the same (the products being only glucose and xylose). No dehydration product (hydroxyl methyl furfural, HMF) of glucose is observed. HMF was known to inhibit the fermentation of sugars in the hydrolyzate to ethanol. Absence of HMF is advantageous from the viewpoints of absence of subsequent decomposition products of HMF (namely, levulinic and formic acids) and also prevention of inhibition of fermentation kinetics.

To have a precise control over the reaction temperature and also to carry out the hydrolysis process at different temperatures, the reaction was carried out in a commercial microwave oven at 80, 100 and 120 °C with 3 M HCl for 10 min. Conversion values of 32, 36 and 46 wt. % were obtained at 80, 100 and 120 °C respectively. The product selectivity remained the same in the temperature range of 80–120 °C. Only glucose and xylose were observed in the hydrolyzate (Fig. S2). No HMF, levulinic acid and formic acid are noticed indicating the selective conversion of holocellulose to glucose and xylose under microwave irradiation with the optimum reaction conditions being 10 min of irradiation at 120 °C with 3 M HCl.

3.4. Fermentation of hydrolyzate – ethanol production

To produce significant amount of fermentable sugars from the pretreated biomass, the hydrolysis batch was scaled up from 0.5 to 1.85 g pretreated biomass in a domestic microwave oven. Conversion value of 36 wt. % of fermentable sugars (glucose and xylose) was obtained in 5 min of irradiation with 3 M HCl.

The ^{13}C NMR spectrum of the hydrolyzate thus obtained is depicted in Fig. 5. ^{13}C NMR revealed peaks at 17.2 and 58.0 ppm corresponding to $-CH_3$ and $-CH_2O$ fragments of ethanol (Fig. 5).

The spectral features (product distribution) were similar to that obtained with 0.5 g batch. So as to produce ethanol from the fermentable sugars obtained from the neutral hydrolyzate, fermentation reaction was carried out with the addition of 1.2 g Baker's yeast to the hydrolyzate. After 18 h of the fermentation, an

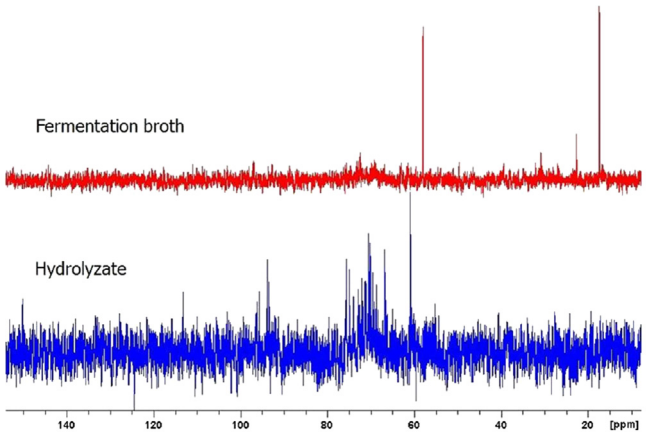

Fig. 5. ^{13}C NMR spectra of the hydrolyzate from 1.85 g holocellulose hydrolyzate and aliquot of sample from the fermentation broth (at 18th h).

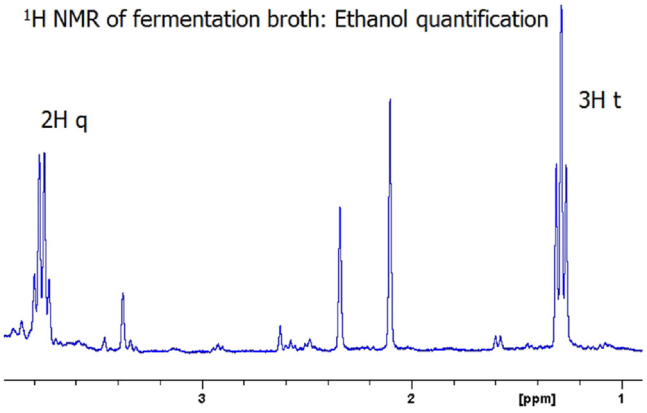

Fig. 6. ^{1}H NMR of the aliquot of sample from the fermentation broth at 18th h.

aliquot of the sample from the fermentation broth was taken and analyzed by ^{13}C NMR (Fig. 5) and ^{1}H NMR (Fig 6).

Ethanol (3H, t at 1.3 ppm and 2H, q at 3.8 ppm) is also identified through ^{1}H NMR (Fig. 6). It can therefore be concluded that both ^{13}C NMR and ^{1}H NMR showed the formation of ethanol from the fermentable sugars (glucose and xylose). It was interesting to note that, almost no trace of fermentable sugars was observed in the ^{13}C NMR spectrum of the aliquot of sample from the fermentation broth at 18th h (Fig. 5). This indicates that in addition to glucose, xylose (generated from hemicellulose) from pine cones could also be metabolized by the Baker's yeast strain.

Quantitative estimation of sugars in the hydrolyzate and unreacted monomer carbohydrates and the ethanol formed in the fermentation broth was carried out using HPLC. Interestingly, the hydrolyzate from pine cone holocellulose comprised of xylose (27.8 mM) and glucose (4.5 mM). Upon fermentation, the xylose (0.62 mM) and glucose (1.7 mM) levels were significantly reduced resulting in the formation of ethanol (41.2 mM) as depicted pictorially in Fig. 7. This corresponds to an ethanol yield value of 5.7 wt. % from raw pine cones.

The amount of ethanol was found to be 0.1485 g from 1.85 g holocellulose (8.03 wt. %). It should be noted that 2.59 g original pine cones yield 1.85 g holocellulose. Thus 0.1485 g ethanol could be produced under optimal reaction conditions from 2.59 g pine cones. Rezic et al., have reported an ethanol yield of 10 wt. % from the sugar beet pulp (Rezic et al., 2013). De-oiled rice bran was reported to yield 10.5–11.4 % ethanol (Beliya et al., 2013). Switch grass

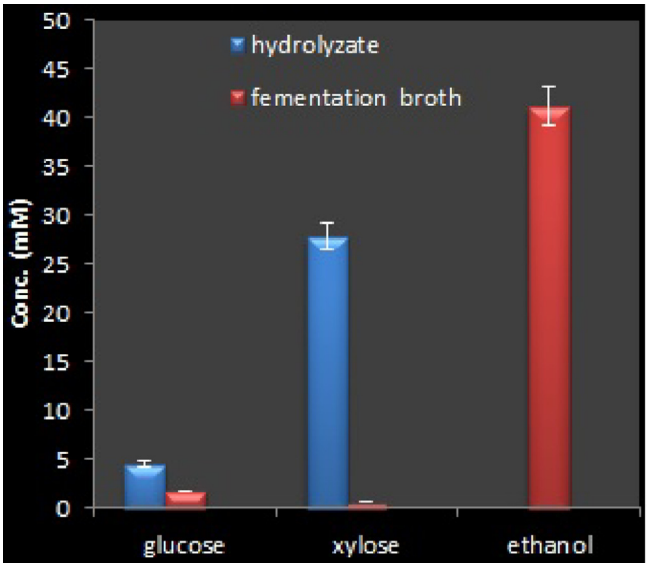

Fig. 7. HPLC analysis of the aliquot from the pine cone hydrolyzate, and the fermentation broth at 18th h (replicate no. n = 3, error bars indicate standard deviation, SD).

germplasms yielded 0.082 g ethanol/g of raw biomass (Yang et al., 2009). Thus the yield of ethanol from pine cones was similar to the yield obtained from other feedstock like sugar beet pulp, de-oiled rice barn and switch grass. Moreover, the feasibility of the xylose metabolism to ethanol using Baker's yeast is demonstrated in the specific instance of *P. radiata* cones as biomass feedstock.

4. Conclusion

Pine cones which are usually a waste were utilized for the production of bioethanol. Dilute alkali treatment was found to be effective for the removal of lignin from the pine cones. Microwave irradiation accelerated the acid hydrolysis of holocellulose to fermentable sugars (xylose and glucose) relative to the conventional hydrothermal process. Pine cones from *P. radiata* form a potential and sustainable feedstock for ethanol production with 5.7 wt. % yield of ethanol.

Compliance with ethical standards

All the authors of the manuscript disclose that they do not have any potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jenvman.2015.07.038.

References

Baeyens, J., Kang, Q., Appels, L., Dewil, R., Lv, Y., Tan, T., 2015. Challenges and opportunities in improving the production of bioethanol. Prog. Energy Combust. Sci. 47, 60–88.

Beliya, E., Tiwari, S., Jadhav, S.K., Tiwari, K.L., 2013. De-oiled rice bran as a source of bioethanol. Energy. Explor. Exploit. 31 (5), 771–782.

Bellido, C., Bolado, S., Coca, M., Lucas, S., Gonzalez-Benito, G., Garcia-Cubero, M.T., 2011. Effect of inhibitors formed during wheat straw pretreatment on ethanol fermentation by *Pichia stipitis*. Bioresour. Technol. 102, 10868–10874.

Carter, B., Gilcrease, P.C., Menkhaus, T.J., 2011a. Removal and recovery of furfural, 5-hydroxymethylfurfural, and acetic acid from aqueous solutions using a soluble polyelectrolyte. Biotechnol. Bioeng. 108, 2046–2052.

Carter, B., Squillace, P., Gilcrease, P.C., Menkhaus, T.J., 2011b. Detoxification of a lignocellulosic biomass slurry by soluble polyelectrolyte adsorption for improved fermentation efficiency. Biotechnol. Bioeng. 108, 2053–2060.

Cavka, A., Alriksson, B., Ahnlund, M., Jonsson, L.J., 2011. Effect of sulfur oxyanions on lignocellulose-derived fermentation inhibitors. Biotechnol. Bioeng. 108, 2592–2599.

Dias, M.O.S., Cunha, M.P., Jesus, C.D.F., Maciel, R.F., Bonomi, A., 2011. Second generation ethanol in Brazil: can it compete with electricity production? Bioresour. Technol. 102, 8964–8971.

Earl, W.L., VanderHart, D.L., 1980. High resolution, magic angle sample spinning ^{13}C NMR of solid cellulose I. J. Am. Chem. Soc. 102, 3251–3252.

Fan, S., Jian, L.Q., Chaia, C.H., Fang, Z., Zakaria, S., Kah-Leong, C., 2014. High yield production of sugars from deproteinated palm kernel cake under microwave irradiation dilute sulphuric acid hydrolysis. Bioresour. Technol. 153, 69–78.

Fissore, A., Carrasco, L., Reyes, P., Rodriguez, J., Freer, J., Mendonca, R.T., 2010. Evaluation of a combine brown rot decay-chemical delignification process as a pretreatment for bioethanol production from *Pinus radiata* wood chips. J. Ind. Microbiol. Biotechnol. 37, 893–900.

Franco, H., Mendonca, R.T., Marcato, R.D., Duran, N., Freer, J., Baez, J., 2011. Dilute acid pretreatment of *Pinus radiata* for bioethanol production using immobilized *Saccharomyces cerevisiae* IR29 in a simultaneous saccharification and fermentation process. J. Chil. Chem. Soc. 56, 901–906.

Geddes, C.C., Nieves, I.U., Ingram, L.O., 2011. Advances in ethanol production. Curr. Opin. Biotechnol. 22, 312–319.

Han, M., Kim, Y., Kim, S.W., Choi, G., 2011. High efficiency bioethanol production from OPEFB using pilot pretreatment reactor. J. Chem. Technol. Biotechnol. 86, 1527–1534.

Horvath, I.T., Mehdi, H., Fabos, V., Boda, L., Mika, L.T., 2008. γ-Valerolactone – a sustainable liquid for energy and carbon-based chemicals. Green. Chem. 10, 238–242.

Huang, H., Guo, X., Li, D., Liu, M., Wu, J., Ren, H., 2011. Identification of crucial yeast inhibitors in bio-ethanol and improvement of fermentation at high pH and high total solids. Bioresour. Technol. 102, 7486–7493.

Jacobsen, S.E., Wyman, C.E., 2000. Cellulose and hemicellulose hydrolysis models for application to current and novel pretreatment processes. Appl. Biochem. Biotechnol. 84–86, 81–96.

Jayakody, L.N., Hayashi, N., Kitagaki, H., 2011. Identification of glycolaldehyde as the key inhibitor of bioethanol fermentation by yeast and genome-wide analysis of its toxicity. Biotechnol. Lett. 33, 285–292.

Kang, Q., Appels, L., Baeyens, J., Dewil, R., Tan, T., 2014a. Energy efficient production of cassava-based bio-ethanol, of cassava-based bio-ethanol. Adv. Biosci. Biotechnol. 5 (12), 925–939.

Kang, Q., Appels, L., Tan, T., Dewil, R., 2014b. Bioethanol from lignocellulosic biomass: current findings determine research priorities. Sci. World J. 298153.

Kang, Q., Huybrechts, J., Bruggen, B.V., Baeyens, J., Tan, T., Dewil, R., 2014c. Hydrophilic membranes to replace molecular sieves in dewatering the bioethanol/water azeotropic mixture. Sep. Purif. Technol. 136, 144–149.

Korzen, L., Pulidindi, I.N., Israel, A., Abelson, A., Gedanken, A., 2015a. Single step production of bioethanol from the seaweed *Ulva rigida* using sonication. RSC Adv. 5, 16223–16229.

Korzen, L., Pulidindi, I.N., Israel, A., Abelson, A., Gedanken, A., 2015b. Marine integrated culture of carbohydrate rich *Ulva rigida* for enhanced production of bioethanol. RSC Adv. 5, 59251–59256.

Kim, T.H., Choi, C.H., Oh, K.K., 2003. Bioconversion of sawdust into ethanol using dilute sulphuric acid-assisted continuous twin screw-driven reactor pretreatment and fed-batch simultaneous saccharification and fermentation. Bioresour. Technol. 130, 306–313.

Lee, H., Cho, D.H., Kim, Y.H., Shin, S.J., Kim, S.B., Han, S.O., Lee, J., Kim, S.W., Park, C., 2011. Tolerance of *Saccharomyces cerevisiae* K35 to lignocellulose-derived inhibitory compounds. Biotechnol. Bioprocess Eng. 16, 755–760.

Martin, M., Grossmann, I.E., 2011. Energy optimization of bioethanol production via gasification of switchgrass. AIChE J. 57, 3408–3428.

Meerbeek, K.V., Appels, L., Dewil, R., Calmeyn, A., Lemmens, P., Muys, B., Hermy, M., 2015. Biomass Invasive Plant Species as a Potential Feedstock Bioenergy Production, vol. 9, pp. 273–282.

Monrroy, M., Ibanez, J., Melin, V., Baeza, J., Mendonca, R.T., Contreras, D., Freer, J., 2010. Bioorganosolv pretreatments of *Pinus radiata* by a brown rot fungus (*Gloephyllum trabeum*) and ethanolysis. Enzyme Microb. Technol. 47, 11–16.

Munoz, C., Mendonca, R., Baeza, J., Berlin, A., Saddler, J., Freer, J., 2007. Bioethanol production from bioorganosolv pulps of *Pinus radiata* and *Acacia dealbata*. J. Chem. Technol. Biotechnol. 82, 767–774.

Paivi, Y., Johan, F.C., Mohammad, T.J., 2011. Ethanol production at elevated temperatures using encapsulation of yeast. J. Biotechnol. 156, 22–29.

Park, S., Baker, J.O., Himmel, M.E., Parilla, P.A., Johnson, D.K., 2010. Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. Biotechnol. biofuels 3, 10.

Parmar, A., Singh, N.K., Pandey, A., Gnansounou, E., Madamwar, D., 2011.

Cyanobacteria and microalgae: a positive prospect for biofuels. Bioresour. Technol. 102, 10163–10172.
Pulidindi, I.N., Gedanken, A., 2015. Employing novel techniques (microwave and sonochemistry) in the synthesis of biodiesel and bioethanol (Chapter 6). In: Fang, Zhen, Fan, Liang-shih, Grace, John R., Ni, Yonghao, Scott, Norman R., Smith Jr., Richard L. (Eds.), Springer Book Series - Production of Biofuels and Chemicals: Ultrasound, pp. 159–188.
Pulidindi, I.N., Gedanken, A., Schwarz, R., Sendersky, E., 2012. Mild sonication accelerates ethanol production by yeast fermentation. Energy Fuels 26, 2352–2356.
Pulidindi, I.N., Hakim, M.R., Mayer, P., Gedanken, A., 2014a. Isosaccharinic acid mediated Fine chemicals production from cellulose. J. Fundam. Renew. Energy Appl. 4, 143. http://dx.doi.org/10.4172/20904541.1000143.
Pulidindi, I.N., Kimchi, B.B., Gedanken, A., 2014b. Selective chemical reduction of carbon dioxide to formate using microwave irradiation. J. CO_2 Util. 7, 19–22.
Raghu, N.G., Saurav, D., Liu, Y.J., Snyder, S.W., Menkhaus, T.J., 2011. Removal of enzymatic and fermentation inhibitory compounds from biomass slurries for enhanced biorefinery process efficiencies. Bioresour. Technol. 102, 7850–7859.
Reyes, P., Mendonca, R.T., Aguayo, M.G., Rodriguez, J., Vega, B., Fardim, P., 2013. Extraction and characterization of hemicelluloses from *Pinus radiata* and its feasibility or bioethanol production. Revista Arvore. Vicosa-MG 37, 175–180.
Rezic, T., Oros, D., Markovic, I., Kracher, D., Ludwig, R., Santek, B., 2013. Integrated hydrolyzation and fermentation of sugar beet pulp to bioethanol. J. Microbiol. Biotechnol. 23 (9), 1244–1252.
Sainio, T., Turku, I., Heinoneu, J., 2011. Adsorptive removal of fermentation inhibitors from concentrated acid hydrolyzates of lignocellulosic biomass. Bioresour. Technol. 102, 6048–6057.
Sanda, T., Hasunuma, T., Matsuda, F., Konda, A., 2011. Repeated-batch fermentation of lignocellulosic hydrolysate to ethanol using a hybrid *Saccharomyces cerevisiae* strain metabolically engineered for tolerance to acetic and formic acids. Bioresour. Technol. 102, 7917–7924.
Sasaki, C., Takada, R., Watanabe, T., Honda, Y., Karita, S., Nakamura, Y., Watanabe, T., 2011. Surface carbohydrate analysis and bioethanol production of sugarcane bagasse pretreated with the white rot fungus, *Ceriporiopsis subvermispora* and microwave hydrothermolysis. Bioresour. Technol. 102, 9942–9946.
Shenoy, D., Pai, A., Vikas, P.K., Neeraja, H.S., Deeksha, J.S., Nayak, C., Vaman, R.C., 2011. A study on bioethanol production from cashew apple pulp and coffee pulp waste. Biomass Bioenergy 35, 4107–4111.
Sindhu, R., Kuttiraja, M., Binod, P., Usha, J.K.K., Sukumaran, R.K., Pandey, A., 2011. Dilute acid pretreatment and enzymatic saccharification of sugarcane tops for bioethanol production. Bioresour. Technol. 102, 10915–10921.
Victor, A., Pulidindi, I.N., Gedanken, A., 2014. Levulinic acid production from *Cicer arietinum*, cotton, *Pinus radiata* and sugarcane bagasse. RSC Adv. 4, 44706–447011.
Yang, Y., Sharma-Shivappa, R., Burns, J.C., Cheng, J.J., 2009. Dilute acid pretreatment of oven-dried switchgrass germplasms for bioethanol production. Energy Fuels 23, 3759–3766.
Yanagisawa, M., Nakamura, K., Ariga, O., Nakasaki, K., 2011a. Production of high concentrations of bioethanol from seaweeds that contain easily hydrolyzable polysaccharides. Process Biochem. 46, 2111–2116.
Yanagisawa, M., Ojima, T., Nakasaki, K., 2011b. Bioethanol from sea lettuce with the use of crude enzymes derived from waste. J. Mater Cycles Waste Manage. 13, 321–326.
Yong-Jin, K., An-Zhou, M., Qian, L., Feng, W., Guo-Qiang, Z., Chun-Zhao, L., 2011. Effect of lignocellulosic inhibitory compounds on growth and ethanol fermentation of newly-isolated thermotolerant *Issatchenkia orientalis*. Bioresour. Technol. 102, 8099–8104.
Zhang, K., Agrawal, M., Harper, J., Chen, R., Koros, W.J., 2011. Removal of the fermentation inhibitor, furfural, using activated carbon in cellulosic-ethanol production. Ind. Eng. Chem. Res. 50, 14055–14060.
Zhao-Yong, S., Yue-Qin, T., Tomohiro, I., Tomohiro, S., Kenji, K., 2011. Production of fuel ethanol from bamboo by concentrated sulfuric acid hydrolysis followed by continuous ethanol fermentation. Bioresour. Technol. 102, 10929–10935.