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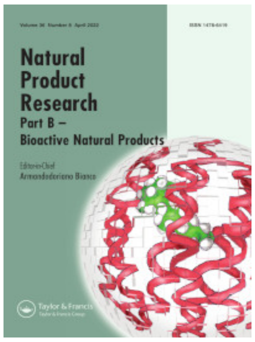

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Production of ethanol, lipid and lactic acid from mixed agrowastes hydrolysate

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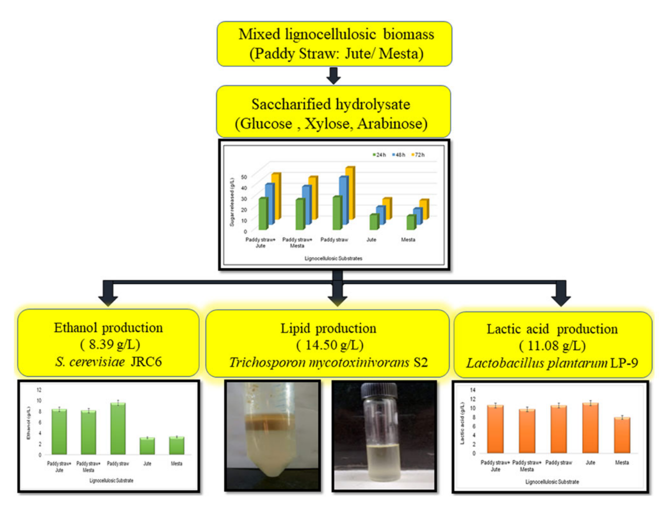
ABSTRACT

To combat the shortage of single agro-residue and overcome the problem of seasonal availability, it is beneficial to use mixture of lignocellulosic biomasses. In the present study, efforts were made to use mixed lignocellulosic biomass for production of bioethanol, along with microbial lipids and lactic acid. Upon enzymatic hydrolysis of mixed biomass at varied proportions it was observed that mixture of paddy straw and jute in the ratio 3:1 resulted in best sugar yield (41.50 g/L) at 10% substrate loading. Ethanolic fermentation of mixed substrate hydrolysate by thermotolerant yeast, *Saccharomyces cerevisiae* JRC6 resulted in 8.39 g/L of ethanol. To maintain sustainability and economic impact, oleaginous yeast (*Trichosporon mycotoxinivorans* S2) and lactic acid bacteria (*Lactobacillus plantarum* LP-9) were used for lipid production (14.5 g/L) and lactic acid production (11.08 g/L), respectively. Therefore, this study explored the potential of mixed lignocellulosic biomass to be exploited for production of various value-added products.



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1. Introduction

India being an agricultural country, produces enormous amount of diverse agricultural residues that must be economically explored for biofuel production without harming the environment (Miskat et al. 2020; Moogi et al. 2020) and thus reducing the dependency on non-renewables as a major energy resource (Deboni et al.2019). These agro-residues are an attractive, renewable resource to develop sustainable biorefineries for biofuels and other value-added products, which in turn enhance the bio-based economy of the world as well as resolve the issue of its disposal (Skaggs et al. 2018). These biofuels not only mitigate climate change by lowering CO_2 emissions, but also reduce dependence on fast depleting petroleum reserves as well as provide socioeconomic benefits for rural communities (Balat 2011).

The seasonal availability of agricultural biomass hampers the year-round supply of any single feedstock type, therefore, to make the biorefineries a profitable and viable option, researchers should try to explore mixed feedstocks for ethanol production or any other commodities. Use of mixed lignocellulosic biomass brings about a significant cost saving and resolve supply issues in comparison to single feedstock. In past, main focus of research was only for second-generation bioethanol production from lignocellulosic biomass using yeast *Saccharomyces* (Cai et al. 2012;Lin et al. 2012; Iqtedar et al. 2015; Yadav et al. 2018). The ability of *Saccharomyces* strains to tolerate high ethanol concentration, organic acids, low pH has made it the most suitable organism of fermentation industry (Albergaria and Arneborg 2016; Tropea et al. 2016; Gervasi et al. 2018b). However, the inability of *Saccharomyces* strains to efficiently utilise pentose sugars is the major drawback in terms of effective fermentation of all the sugars that are present in the biomass hydrolysate. This in turn affects the economics of ethanol production from lignocellulosic biomass. Effective utilisation of both hexose and pentose sugars derived from cellulose and hemicellulose fraction of lignocellulosic biomass is necessary to reduce 25% of the production cost of biomaterials (Hinman et al. 1989).

Apart from ethanol production, these sugars may also be catabolised by oleaginous yeast to produce single cell oil. Many yeast strains co-metabolise xylose, the second most abundant sugar in lignocellulosic biomass, into lipids for biodiesel production. The similarity of the single cell oils (SCOs)/lipids which is produced by oleaginous microorganisms in fatty acid composition with that of vegetable oils, makes SCOs a promising candidate among all other oil sources. Besides, it has been demonstrated that SCOs might also contain medically important poly-unsaturated fatty acids like c-linolenic acid and medically important pigments like astaxanthin and β-carotene (Ahmed et al. 2008; Gervasi et al. 2018a). Specific environmental conditions for the growth of oleaginous microorganisms (yeast and fungi) enable the accumulation of more than 20-70% oils inside the microbial cells. The short life cycle of the microbes, reduced labour demand, convenient scalability are the major advantages of single cell oils (SCOs). Therefore, production of oil from lignocellulosic biomass is also one of the most attractive options for bio-based biorefineries.

Due to the abundance, low price, high polysaccharide content and renewability of the lignocellulosic materials, it is a potent feedstock for commercial production of lactic acid by microbial fermentation (Duff and Murray 1996; Parajo et al. 1996; Wyman

1999; Taniguchi et al. 2005; Sharma et al. 2020). With an average growth rate of $>5\%$ between 2018 and 2023, lactic acid has become one of the most promised emerging bioproducts due to its multivariate applications (Bozell and Petersen 2010; Biddy et al. 2016). The biodegradable and biocompatible properties of lactic acid and its salts (e.g., calcium lactate), polymers (e.g., polylactic acid) and esters (e.g., ethyl lactate) have enabled its wide use in food and beverages (as an acidulant, in preservative and flavouring agents, etc.), medical, pharmaceutical, and food industries (Markit 2018). Therefore, the objective of the present study was to explore the potential of mixed lignocellulosic feedstocks for sustainable production of bioethanol, lipid and lactic acid using combination of jute, mesta and paddy straw.

2. Results and discussion

2.1. Enzymatic hydrolysis of mixed lignocellulosic biomass

The mixed biomass (paddy straw, jute and mesta biomass) used in this study at varying proportions and percentage of substrate loading was analysed for sugar yield after enzymatic hydrolysis with commercial enzymes. Sugar concentrations (g/L) were measured via DNSA method for each varying ratios of biomass and are summarised in Table S1. From the results, it was observed that the total reducing sugar released was more at 10% substrate loading as compared to 5% in all combinations of substrates used. Our results are in agreement with López-Linares et al. (2014), who also observed the increase in sugar yield after enzymatic hydrolysis of rapeseed straw with higher substrate loading. Further, maximum sugar release was obtained in alkali treated paddy straw and jute biomass (3:1) i.e., 41.50 g/L as compared to alkali treated paddy straw and mesta biomass (38.50 g/L) after 72 h of hydrolysis (Table S1). However, the highest sugar release was obtained with paddy straw alone proving the superiority of paddy straw in terms of sugar yield during saccharification, which might be due to structural differences between the biomasses (Figure S1).

But keeping in mind that compared to rice, jute requires very less water and fertilizer; is largely pest-resistant, and its rapid growth spurt ensures that weeds don’t stand a chance, makes jute suitable to be explored for bioethanol production (Sarkar and Wang 2020). Above all, jute is twice more economically viable than paddy because nine quintals of fibre is also produced per ton of biomass. (The Hindu newspaper 2020). Since sugar release form jute alone is less, complementing it with paddy straw is a feasible option to use this less-intensive crop for bioethanol production in biorefineries in a cost-effective and environment-friendly manner.

2.2. Fermentation of sugars obtained from mixed substrate hydrolysate using yeast

Mixed substrate hydrolysate with 10% substrate loading was evaluated for production of ethanol by *Saccharomyces cerevisiae* JRC6. The results revealed that the maximum amount of ethanol i.e., 8.39 g/L was obtained within 72 h of fermentation by the fermentation of paddy straw and jute mixture whereas the mixture of paddy straw and mesta produced 8.11 g/L of ethanol in same amount of time (Figure S2). In past, other researchers also investigated mixed substrate for ethanol production (Table S2). The results of the present study are in agreement with previous studies as listed in the table, indicating the potential of lignocellulosic biomass to produce 4.10 to 10.34 g/L of ethanol from different type of substrates.

2.3. Lipid production from mixed substrate hydrolysate

Mixed substrate hydrolysate when analysed by HPLC, revealed the presence of glucose, xylose and arabinose (Figure S3). Since *S. cerevisiae* JRC6 is a hexose utilising yeast, therefore, only glucose is utilised during ethanolic fermentation process whereas pentose sugar remained unutilized in the hydrolysate even after fermentation (Figure S4). Therefore, efforts were also made to produce lipids by using *Trichosporon mycotoxinivorans* S2 which can utilize both hexose and pentose sugars. The ability of this strain to achieve high productivity even in the presence of inhibitors also makes it a potential candidate for commercial exploitation (Sagia et al. 2020). The results showed that *T. mycotoxinivorans* S2 utilized both the sugars and produced 14.5 g/L of lipid from the saccharified hydrolysate within 7 days of batch fermentation. The fatty acid profile of lipid was also analysed by GC (Table S3). Lipid produced in this study is in accordance with the previous studies as compiled in Table S4. The most common fatty acids of this oleaginous yeasts are palmitic acid (C16:0) and oleic acid (C18:1), resembling closely those of rapeseed and sunflower oil, which are commonly used as feedstock for biodiesel production.

2.4. Lactic acid production from alkali pretreated mixed biomass

The sugar rich mixed biomass hydrolysate (Figure S5) was evaluated for lactic acid production using *L. plantarum* LP-9. Since hydrolysate lacks growth factors, addition of nutrients to a culture broth during batch fermentation increased the lactic acid production and productivity (Abdel-Rahman et al. 2011). pH-controlled batch fermentation significantly improves lactic acid production, yield, and productivity by different LAB strains, e.g., *Lb. delbrueckii* (Tashiro et al. 2011). On the basis of HPLC results, it was observed that jute biomass yielded maximum 11.08 g/L lactic acid within 72 h of fermentation in comparison with another biomass (Figure S6). Mixture of paddy straw and jute biomass produced 10.58 g/L, mixture of paddy straw and mesta biomass produced 9.72 g/L whereas paddy straw, jute and mesta individually produced 10.54 g/L, 11.08 g/L, 7.92 g/L lactic acid, respectively (Figure S7).

Table S5 summarizes the lactic acid yield from previous reports from lignocellulosic biomass which revealed that the yield of lactic acid in present study is comparable with the previous studies and can be further improved by optimising the fermentation conditions.

3. Experimental

3.1. Raw materials and chemicals

All details about raw materials used in the study are provided in supplementary material.

3.2. Enzymes

Information about enzymes used is provided in the supplementary material.

3.3. Microorganisms

The thermotolerant yeast *Saccharomyces cerevisiae* JRC6 (accession number KX668410), *Trichosporon mycotoxinivorans* S2 (accession number MK752668) and *Lactobacillus plantarum* LP-9 (accession number MT008062), procured from Division of Microbiology, IARI, New Delhi for the fermentation experiments. The procedure for culture maintenance and further experimental work is provided in the supplementary material.

3.4. Saccharification of pretreated mixed biomass (paddy straw: jute/mesta)

The detailed description is provided in the supplementary material.

3.5. Alcoholic fermentation of saccharified hydrolysate

The saccharified hydrolysate of alkali pretreated mixed biomass (Paddy straw: Jute/Mesta) was used for production of ethanol using thermotolerant *S. cerevisiae* JRC6 through submerged fermentation. Further details for alcoholic fermentation are provided in the supplementary material.

3.6. Utilisation of saccharified hydrolysate for lipid production

The detailed description for lipid production is provided in the supplementary material.

3.7. Lipid extraction and quantification

The detailed description is provided in the supplementary material.

3.8. Fermentation using lactic acid bacteria **Lactobacillus plantarum** LP-9

Lactic acid fermentation is described in the supplementary material.

3.9. Quantification of sugars, ethanol, and lactic acid by HPLC

The detailed description is provided in the supplementary material.

4. Conclusions

The potential of lignocellulosic residues (paddy straw, jute and mesta) for enhanced saccharification efficiency and production of ethanol along with other value-added products was explored through cascading of various approaches in a biorefinery mode. In the present study, the effects on sugar yield were investigated for different proportions of paddy straw, jute and mesta biomass. The highest sugar yield was obtained at 10% w/v substrate loading of the mixed biomass in the ratio 3:1 through enzymatic hydrolysis. The resultant sugar rich saccharified hydrolysate of mixed biomass was explored for production of various biorefinery product viz., bioethanol, lipid, and lactic acid.

For bioethanol production, *S. cerevisiae* JRC6 was used for fermentation which produced highest ethanol (9.55 g/L) from paddy straw while mixture of paddy straw and jute biomass generated comparable yield of 8.39 g/L. One major drawback of the yeast used for ethanolic fermentation is that it ferments only hexose sugar. Therefore, oleaginous yeast *T. mycotoxinivorans* S2 was explored yielding fair quantity of lipid (14.5 g/L). In this process, efficient xylose utilisation offers complete conversion of all available sugars in lignocellulosic biomass. Further improvements in the fermentation process or the yeast strain via metabolic engineering may improve the lipid yield from lignocellulosic biomass. To explore the unlimited pathways of lignocellulosic biorefinery, lactic acid (11.08 g/L) was also produced from jute biomass individually and 10.58 g/L lactic acid was produced from the mixture of paddy straw and jute using *Lactobacillus plantarum* LP-9. By extrapolating the yield obtained in this study one can obtain 83.90 g of ethanol along with 76.58 g of lipid or 110.80 g of lactic acid from 1 kg mixture of paddy straw and jute (3:1 ratio) biomass.

Our present research validated the hypothesis that mixed biomass may be exploited for multiple fermented by-products to get better economic returns. Regarding biorefinery operations, use of renewable biomass will greatly reduce environmental impacts. Moreover, use of mixed biomass is important to expand the utilisation of lignocellulosic wastes and combat the fluctuation in feedstock supply of certain bio-residues.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Abdel-Rahman MA, Tashiro Y, Sonomoto K. 2011. Lactic acid production from lignocellulose-derived sugars using lactic acid bacteria: overview and limits. J Biotechnol. 156(4):286–301.

Ahmed SU, Singh SK, Pandey A, Kanjilal S, Prasad RBN. 2008. Fatty acid profiling during microbial lipid production under varying pO2 and impeller tip speeds. Appl Biochem Biotechnol. 151(2-3):599–609.

Albergaria H, Arneborg N. 2016. Dominance of *Saccharomyces cerevisiae* in alcoholic fermentation processes: role of physiological fitness and microbial interactions. Appl Microbiol Biotechnol. 100(5):2035–2046. doi: 10.1007/s00253-015-7255-0.

Balat M. 2011. Production of bioethanol from lignocellulosic materials via the biochemical pathway: a review. Energy Convers Manage. 52(2):858–875.

Biddy MJ, Scarlata C, Kinchin C. 2016. Chemicals from biomass: a market assessment of bioproducts with near-term potential. doi: https://doi.org/10.2172/1244312

Bozell JJ, Petersen GR. 2010. Technology development for the production of biobased products from biorefinery carbohydrates -the US Department of Energy's "Top 10" revisited. Green Chem. 12(4):539.

Cai Z, Zhang B, Li Y. 2012. Engineering *Saccharomyces cerevisiae* for efficient anaerobic xylose fermentation: reflections and perspectives. Biotechnol J. 7(1):34–46.

Deboni TL, Simioni FJ, Brand MA, Lopes GP. 2019. Evolution of the quality of forest biomass for energy generation in a cogeneration plant. Renew Energy. 135:1291–1302.

Duff SJB, Murray WD. 1996. Bioconversion of forest products industry waste cellulosics to fuel ethanol: A review. Bioresour Technol. 55(1):1–33.

Gervasi T, Pellizzeri V, Benameur Q, Gervasi C, Santini A, Cicero N, Dugo G. 2018a. Valorization of raw materials from agricultural industry for astaxanthin and β-carotene production by *Xanthophyllomyces dendrorhous*. Nat Prod Res. 32(13):1554–1561. 10.1080/14786419.2017.1385024.

Gervasi T, Pellizzeri V, Calabrese G, Di Bella G, Cicero N, Dugo G. 2018b. Production of single cell protein (SCP) from food and agricultural waste by using *Saccharomyces cerevisiae*. Nat Prod Res. 32(6):648–653. 10.1080/14786419.2017.1332617.

Hinman N, Wright J, Hogland W, Wyman C. 1989. Xylose fermentation, an economic analysis. Appl Biochem Biotechnol. 20-21(1):391–401.

Iqtedar M, Nadeem M, Naeem H, Abdullah R, Naz S, Syed QUA, Kaleem A. 2015. Bioconversion potential of *Trichoderma viride* HN1 cellulase for a lignocellulosic biomass *Saccharum spontaneum*. Nat Prod Res. 29(11):1012–1019. 10.1080/14786419.2014.971320.

Lin Y, Zhang W, Li C, Sakakibara K, Tanaka S, Kong H. 2012. Factors affecting ethanol fermentation using *Saccharomyces cerevisiae* BY4742. Biomass Bioenergy. 47:395–401. 10.1016/j.biombioe.2012.09.019.

López-Linares JC, Romero I, Cara C, Ruiz E, Moya M, Castro E. 2014. Bioethanol production from rapeseed straw at high solids loading with different process configurations. Fuel. 122:112–118.

Markit IHS. 2018. Chemical economics handbook – lactic acid, its salts and esters. IHS Markit, S P Global, London,UK

Miskat MI, Ahmed A, Chowdhury H, Chowdhury T, Chowdhury P, Sait SM, Park Y-K. 2020. Assessing the theoretical prospects of bioethanol production as a biofuel from agricultural residues in Bangladesh: a review. Sustainability. 12(20):8583.

Moogi S, Jae J, Kannapu HPR, Ahmed A, Park ED, Park Y. 2020. Enhancement of aromatics from catalytic pyrolysis of yellow poplar: Role of hydrogen and methane decomposition. Bioresour Technol. 315:123835.

Parajo JC, Alonso JL, Santos V. 1996. Lactic acid from wood. Process Biochem. 31(3):271–280.

Sagia S, Sharma A, Singh S, Chaturvedi S, Nain PKS, Nain L. 2020. Single cell oil production by a novel yeast *Trichosporon mycotoxinivorans* for complete and ecofriendly valorization of paddy straw. Electron J Biotechnol. 44:60–68.

Sarkar JK, Wang Q. 2020. Characterization of pyrolysis products and kinetic analysis of waste jute stick biomass. Processes. 8(7):837.

Sharma A, Pranaw K, Singh S, Khare SK, Chandel AK, Nain PKS, Nain L. 2020. Efficient two-step lactic acid production from cassava biomass using thermostable enzyme cocktail and lactic acid bacteria: insights from hydrolysis optimization and proteomics analysis. 3 Biotech. 10(9): 409.

Skaggs RL, Coleman AM, Seiple TE, Milbrandt AR. 2018. Waste-to-energy biofuel production potential for selected feedstocks in the conterminous United States. Renew Sustain Energy Rev. 82:2640–2651. 10.1016/j.rser.2017.09.107.

Taniguchi M, Hoshina K, Tanabe S, Higuchi Y, Sakai K, Ohtsubo S, Hoshino K, Tanaka T. 2005. Production of l-lactic acid by simultaneous saccharification and fermentation using unsterilized defatted rice bran as a carbon source and nutrient component. FSTR. 11(4):400–406.

Tashiro Y, Kaneko W, Sun Y, Shibata K, Inokuma K, Zendo T, Sonomoto K. 2011. Continuous d-lactic acid production by a novel thermotolerant *Lactobacillus delbrueckii* subsp lactis QU 41. Appl Microbiol Biotechnol. 89(6):1741–1750.

The Hindu Newspaper. 2020. https://www.thehindu.com/society/the-versatile-jute-could-be-the-fibre-of-the-future-but-the-industry-needs-reforms/article33191655.ece.

Tropea A, Wilson D, Cicero N, Potortì AG, La Torre GL, Dugo G, Richardson D, Waldron KW. 2016. Development of minimal fermentation media supplementation for ethanol production using two *Saccharomyces cerevisiae* strains. Nat Prod Res. 30(9):1009–1016. 10.1080/14786419.2015.1095748.

Wyman CE. 1999. Biomass ethanol: technical progress, opportunities, and commercial challenges. Annu Rev Energy Environ. 24(1):189–226.

Yadav P, Maharjan J, Korpole S, Prasad GS, Sahni G, Bhattarai T, Sreerama L. 2018. Production, purification, and characterization of thermostable alkaline xylanase from *Anoxybacillus kamchatkensis* NASTPD13. Front Bioeng Biotechnol. 6:65. 10.3389/fbioe.2018.00065.