J. Himalayan Ecol. Sustain. Dev. Vol 18 (2023) **ISSN 0973-7502**

Effect of Acid Concentration on the Reducing Sugar Yield in Seed Kernels of *Mirabilis jalapa* L.

Laldingliani Pachuau[1], D. Paul[1]* and Ghanashyam Bez[2]

[1]Department of Environmental Studies, North-Eastern Hill University, Shillong-793022, India
[2]Department of Chemistry, North-Eastern Hill University, Shillong-793022, India
***Corresponding Author email: pauld97@rediffmail.com.**

ABSTRACT

Mirabilis jalapa (Four O' Clock) is valued for its versatile biological properties which is believed to be relevant for some of the purported medicinal benefits of this plant. This study investigated the optimum acid concentration to maximize the reducing sugar yield in *Mirabilis jalapa* seed kernels for bioethanol production. Reaction time, temperature, and acid concentration are the key parameters affecting sugar release and degradation. The hydrolysis parameters include dilute H_2SO_4 concentrations (1 – 5%, v/v), and reaction time (15 to 45 mins), while the biomass loading and reaction temperature were kept constant at 10% w/v and 121℃ respectively. The maximum reducing sugar yield (1.9 ± 0.04 mg/g) was obtained at a 2% v/v acid concentration hydrolysed for 15 minutes. The reducing sugar yield increased to 1.9 ± 0.04 mg/g from 1.42 ± 0.05 mg/g after pre-treatment, representing a 33% increase in the sugar yield. A low acid concentration and short pre-treatment time were found favourable for enhancing the total sugar yield in the feedstock under investigation.

Keywords: *Mirabilis jalapa, reducing sugars, dilute acid pre-treatment, bioethanol.*

INTRODUCTION

Global energy consumption has steadily increased due to population growth and improved living conditions over the last century (Byadgi and Kalburgi, 2016). The present worldwide consumption of energy scenario includes fossil fuels such as coal, oil, and gas, as well as renewable energy sources such as biofuels, solar, wind, and hydroelectric power (Fanchi, 2023). Global energy demand is predicted to double by 2030, peaking at 80 to 120 million barrels of oil per day (Ávila *et al*., 2023). Crude oil has been the primary energy source to meet the energy demand (Maliha and Abu-Hijleh, 2022). Bioethanol, which is a liquid biofuel has gained traction in recent years as a promising alternative fuel to substitute petroleum and has already been used in the transportation sector (Izmirlioglu and Demirci, 2016) as ethanol blends in an attempt to reduce the use of fossil fuels (Yan *et al.,* 2017). Bioethanol can be produced from sucrose-rich raw materials such as sugar cane and beet containing fermentable sugar. Starch present in grain crops (e.g. Corn and wheat) and tubular crops (cassava, potato, sweet potato) is the major polymer used for bioethanol production (Bušić *et al.,* 2018). Bioethanol produced from such edible feedstocks are called as the first-generation bioethanol. Since the production of bioethanol from edible sources poses a threat to food security, alternative biomass sources from non-edible feedstocks called as the lignocellulosic biomass or the second-generation bioethanol have emerged (Kowalski *et al.,* 2022). With recent advancement in bioethanol production, the third and fourth generation bioethanol have also developed which explored the carbohydrate rich feedstocks such as microalgae and the use of genetically engineered microorganisms allowing the identification of novel organisms and metabolic pathways (Carvalho *et al.,* 2019). The vast carbohydrate

stock could indeed be a sustainable source of feedstock for bioethanol production (Pulidindi *et al.,* 2014).

To facilitate enzymatic or acid hydrolysis, pre-treatment is necessary to delignify and disintegrate the crystalline structure of the cellulose content of lignocellulosic biomass (Singh *et al.*, 2011). Hydrolysis of carbohydrate polymer release monosaccharides and make them more conducive for microbial fermentation. The different types of pre-treatment hydrolysis aim to minimise sugar loss, energy consumption, and enhance enzymatic digestibility. Physical pre-treatment improve bioconversion effectively by reducing crystallinity and substrate particle size generating new surface area, improve enzymatic accessibility, and the overall conversion of biomass into biofuels with minimum generation of toxins (Amin *et al.,* 2017; Li *et al.,* 2022). In order to produce highly digestible solids, acid pre-treatment is usually carried out under high concentrations of acid (Ramadoss and Muthukumar, 2015). However, high acid concentration and high temperature requires high input of energy which results in high cost and also the production of fermentative inhibitors apart from releasing the reducing sugars (Deshavath *et al.,* 2017). According to Deshavath *et al.,* 2017, the key parameters which affects the release of sugars and their degradation is the reaction temperature, time, and acid concentration. When pre-treatment is subjected to higher temperatures (> 160℃), it is shown to have facilitated the formation of inhibitory compounds apart from the sugar formation, which becomes more apparent as temperature increases (Saha and Bothast, 1999). Therefore, dilute acid pre-treatment (DAP) at mild temperatures can be used as a method for improving the pre-treatment efficiency and subsequent enzymatic hydrolysis (Cotana *et al.,* 2014).

Mirabilis jalapa is a perennial herbaceous plant that belongs to the family *Nyctaginaceae* (Walker *et al.,* 2008) and is native to tropical America (Ray *et al.,* 1988). The plant grows abundantly in India (Ray *et al.,* 1988) and is listed among the invasive plant species in the Himalayan region of India (Chandra and Sekar, 2012). The fruits are single-seeded, rugose, and nut ellipsoid in shape (Singh *et al.,* 2012) and turn black upon maturity (Rozina, 2016). It is also a versatile and fast-growing perennial plant with high yield, low-cost of cultivation and grows well in marginalised soils. The seed kernels have low moisture and ash content and are a rich source of starch and carbohydrate making *Mirabilis jalapa* a novel and favourable feedstock suitable for sustainable bioethanol production. The present investigation aims to find the optimum dilute acid concentration to maximize the reducing sugar yield from the feedstock under investigation. The acid concentration plays a crucial role in influencing sugar yield during the hydrolysis process. The hydrolysis process aims to breakdown the complex components of plant biomass into simpler sugars, mainly hexose and pentose sugars, which can then be fermented into bioethanol or other valuable products. Compositional analysis as well as morphological characterization of native and pre-treated samples were examined using Scanning Electron Microscope (SEM).

MATERIALS AND METHOD

Collection of raw materials: The seeds were collected from North Eastern Hill University (NEHU) campus, Shillong. The extracted seed

kernels were dried overnight in hot air oven and grounded to powder form. It was made to pass through 1 mm sieve to obtain uniform texture and then stored for further use.

Chemical composition analysis: The moisture and ash content were analysed by Laboratory Analytical Procedure number 001 (LAP # 001) and number 005 (LAP # 005) respectively from National Renewable Energy Laboratory (NREL) Protocol (Ehrman, 1994). To determine the moisture content, 5 g of the sample was weighed and placed in an oven dried and pre-weighed empty dish and dried overnight at 105℃. The sample was reweighted after cooling in a desiccator. The percentage of moisture was calculated as:

$$Moisture\ (\%) = \frac{W1 - W2}{W1 - W} \times 100$$

Where, *w* = weight of dish with lid; w_1 = weight of dish + sample before drying; w_2 = weight of dish + sample after drying.

The ash content was measured by weighing 5 g of sample placed in oven dried and pre-weighed empty silica crucible. The crucible was placed on a burner under fume hood and the temperature was gradually increased until smoking ceased and the sample becomes charred. The crucible was transferred to a muffle furnace at 575℃ for 4 to 5 hours until white ash was obtained. The furnace was allowed to cool below 100℃ and the sample was reweighted after cooling in a desiccator. The ash percentage was calculated as:

$$Ash\ (\%) = \frac{W2 - W1}{W1 - W}$$

Where, *w* = weight of empty silica crucible; w_1 = weight of crucible + initial weight of sample; w*2* = weight of dish + weight after ash formed.

Estimation of starch: Starch was estimated by Anthrone reagent (Sadasivam and Manickam, 1996). Preparation of standard graph: Stock solution was prepared by dissolving 100 mg of glucose in 100 ml distilled water. The working standard was prepared by taking 10 ml of the stock solution and diluted to 100 ml with distilled water. The standard was prepared by taking (0.2 – 1.0) ml of the working standard and the volume of each tube were made up to 1 ml with distilled water. 4 ml of anthrone reagent was added to each tube and boiled in water bath for eight minutes. after cooling, the intensity of the dark green colour was measured at 630 nm using a UV-VIS spectrophotometer.

Preparation of sample: The sample (0.5 g) was homogenized in hot 80% ethanol to remove the sugars and then centrifuged. After centrifugation, the residue retained was washed repeatedly with hot 80% ethanol till the washings do not give color with anthrone reagent (200 mg anthrone in 100 ml ice-cold 95% sulphuric acid). After drying the residue in a water bath, 5 ml of distilled water and 6.5 ml of 52% perchloric acid were added. It was again centrifuged at 0℃ for 20 mins and the supernatant was saved for subsequent steps. The extraction was repeated with fresh perchloric acid, and the supernatant was pooled after centrifugation and the volume was made up to 100 ml with distilled water. 0.2 ml of the supernatant was pipetted out and the volume was made up to 10 ml with distilled water. 4 ml of anthrone reagent as added to this solution and boiled in water bath for 8 mins. After cooling, the intensity of the dark green colour was measured at 630 nm using a UV-VIS spectrophotometer.

Determination of reducing sugar: The reducing sugar content of the biomass feedstock was determined by the method of Miller, 1959. The

reducing sugar content of native and pretreated feedstock was measured by the 3,5-dinitrosalicylic acid (DNS) method using a UV-VIS spectrophotometer. 3 ml of DNSA reagent to a series of standard using glucose as stock solution (0 – 500 µg) to prepare the standard for calibration curve. Distilled water was used as blank instead of glucose. The contents of the tubes were boiled for 5 minutes and to these, while the tubes are still warm 1 ml of 40% Rochelle Salt was added. After cooling, the colour formed was estimated spectrophotometrically at 540 nm against blank. 1.5 ml of the sample was run in parallel and the concentration of the sugar was estimated using the standard graph. The percentage of reducing sugar was arrived at using Eq. (1):

$$Reducing\ sugar\ yield\ (mg/g) = \frac{Reducing\ sugar\ concentration,\ mg/l}{Biomass\ concentration,\ g/l} \quad \text{..... Eq. (1)}$$

Effect of acid pre-treatment in *Mirabilis jalapa* seed kernel: The optimization of dilute acid pre-treatment (DAP) was determined using 60 ml of varied H_2SO_4 concentrations (1 - 5%, v/v) at a 10% (w/v) solid biomass content and different time periods (15, 30, and 45 mins). Hydrolysis was then carried out in an autoclave at a constant temperature of 121℃. The hydrolysate, after treatment, was separated by filtering the contents using muslin cloth. The pH was analysed using a pH meter and the residue was washed with distilled water till the pH was close to neutral and dried overnight in a hot air oven at 60°C. The 3,5-dinitrosalicylic acid (DNSA) method was used to determine changes in reducing sugar yield in the pretreated samples.

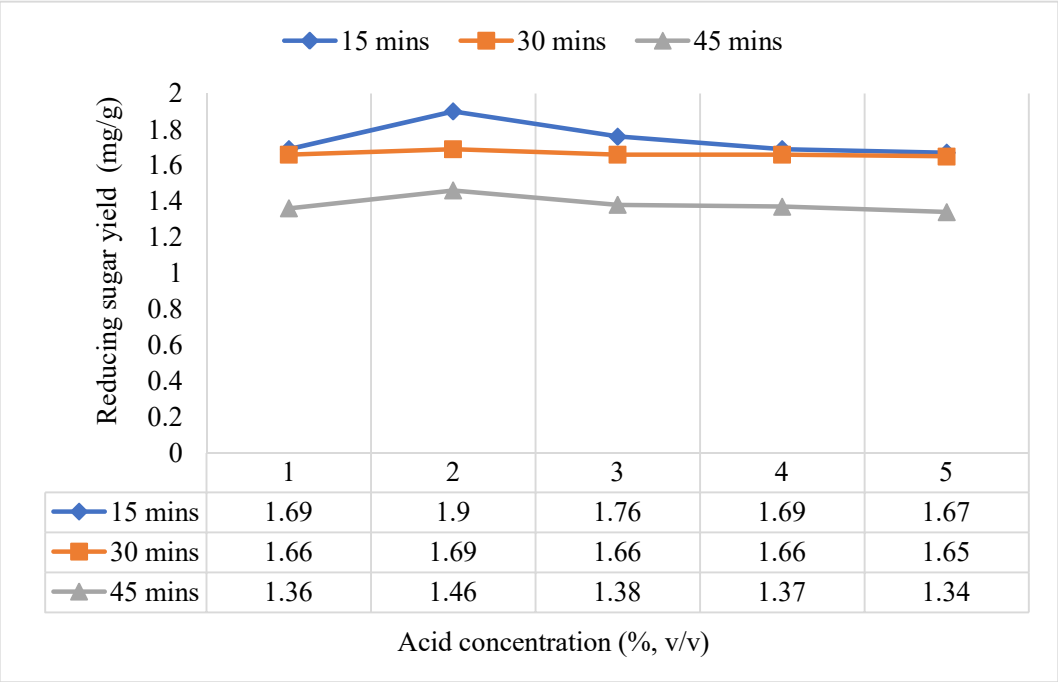
Scanning Electron Microscope (SEM): SEM was performed for morphological characterization of native and pretreated feedstock at a magnification range of ×500. All scanning electron micrographs were acquired on a JSM-3630 (JEOL) at a voltage of 10 kv.

Statistical analysis: All experiments were carried out in triplicates and expressed as mean values ± standard deviation (SD).

RESULTS AND DISCUSSION

Biomass composition analysis: The composition analysis of *Mirabilis jalapa* seed kernels was: Moisture (%), 5.33 ± 0.88; ash (%), 2.7 ± 0.08; starch (%), 32.89 ± 0.001. Based on the reducing sugar yield by DNS method, the reducing sugar yield of native sample was 1.37 ± 0.16 mg/g.

Effect of dilute acid pre-treatment on seeds kernels of *Mirabilis jalapa*: The biomass feedstock was pre-treated with different concentrations of dilute acids to obtain the optimal pre-treatment condition to achieve maximum reducing sugar yield. The acid pre-treatment was performed at low concentrations of sulphuric acid (1-5%, v/v) at a relatively lower temperature of 121℃ for 15 – 45 minutes in order to minimize the formation of inhibitory compounds. Pre-treatment with dilute sulphuric acid with low concentration of acid (> 4%) was reported to be more effective and cost efficient (Srivastava *et al.,* 2007). The effect of acid concentration and reaction time on reducing sugar released after pre-treatment is shown in Fig. 1.



	1	2	3	4	5
15 mins	1.69	1.9	1.76	1.69	1.67
30 mins	1.66	1.69	1.66	1.66	1.65
45 mins	1.36	1.46	1.38	1.37	1.34

Fig. 1. Reducing sugar yield (mg/g of biomass) by using dilute acid concentrations at different time intervals

With biomass loading of 10%, the maximum reducing sugar yield (1.90 mg/g ± 0.04) was obtained at 2% (v/v) H_2SO_4 concentration hydrolysed for 15 mins. From the figures and experimental data, it is clear that further increase in acid concentration (> 2.0%, v/v) and pre-treatment time resulted in the decrease in sugar yield. At 5% (v/v), the lowest sugar yield was acquired in all the three different sets of pre-treatment time as shown in Fig. 3. This decrease in sugar yield could be due to the increase in production of interferences with increasing acid concentration and time period. At 30 mins, no significant changes were observed although after 45 mins of hydrolysis, the sugar yield significantly reduced resulting in overall lowest sugar yield. Prolongation of reaction duration and high acid concentration frequently resulted in the conversion of monomeric sugars to inhibitory compounds such as furfural and 5-hydroxyl methyl furfural (5-HMF) from pentose and hexose sugars respectively (Qing *et al.,* 2017). These are potentially toxic compounds that hinder microbial growth and result in low ethanol yield during fermentation (Deshavath *et al.,* 2017). Comparable results were observed by Mannivan, (2014) on the aquatic weed, *Eichhornia crassipes* by performing dilute acid hydrolysis with H_2SO_4. The reducing sugar yield before pre-treatment was 1.42 ± 0.05 mg/g which increased to 1.90 ± 0.04 mg/g, so there was 33% increase in the sugar yield after pre-treatment as shown in Fig. 2.

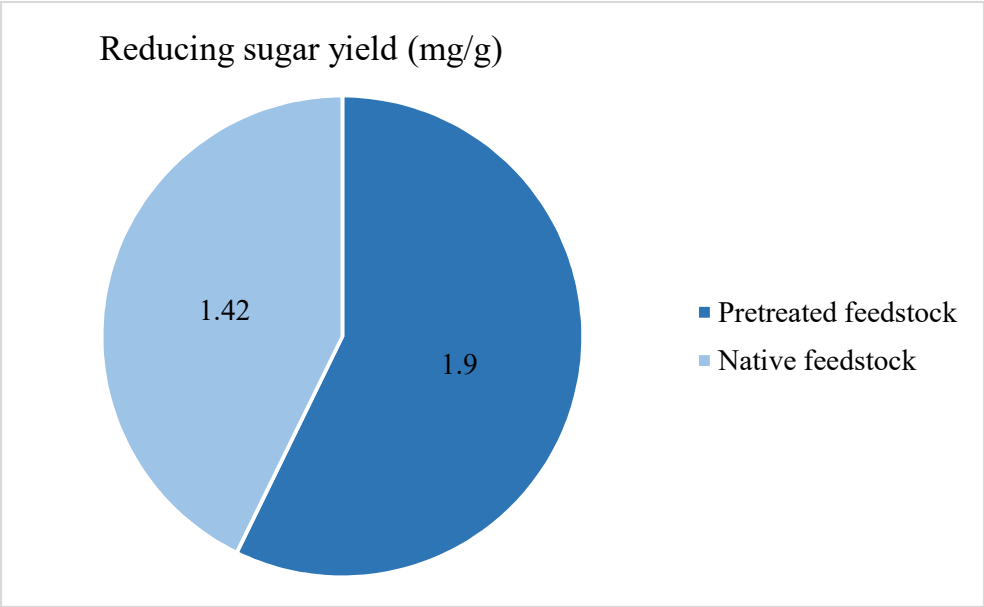


Fig. 2. Comparison of the Reducing sugar yield (mg/g) of native and pretreated feedstock

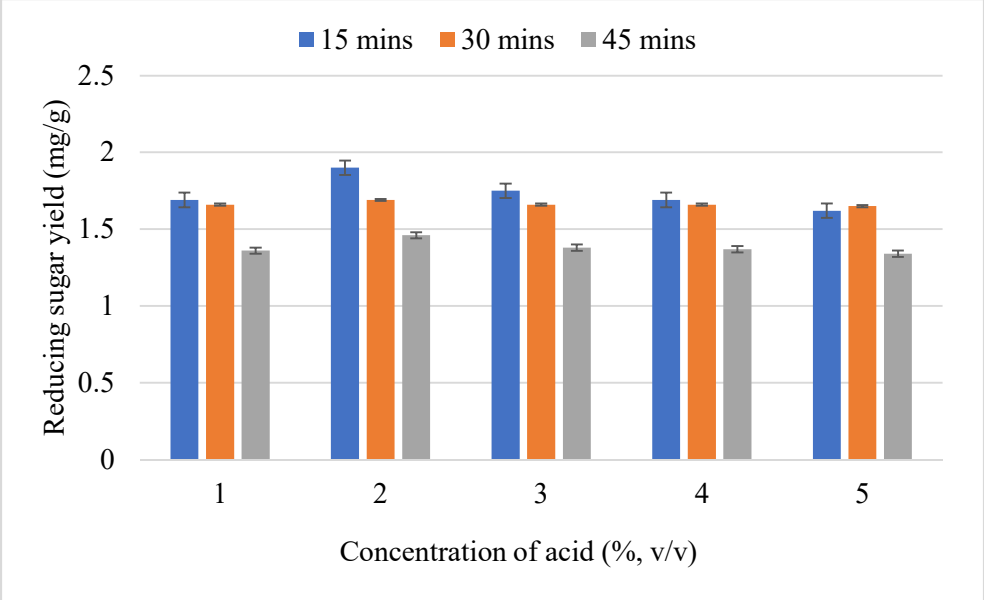
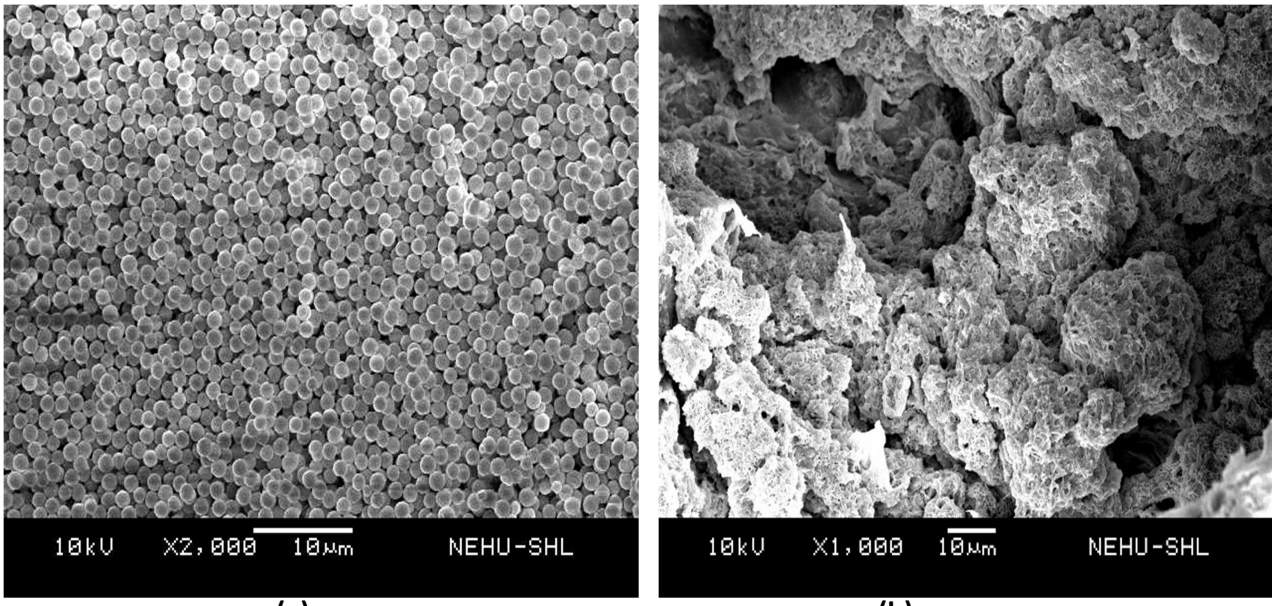


Fig. 3. Reducing sugar yield (mg/g) using different dilutions of acids

Result of SEM: The structural changes that occurred in the seed sample as a result of pre-treatment with dilute H_2SO_4 were studied. Fig. (4) shows the morphology of native (a) native and (b) pre-treated seed kernels of *Mirabilis jalapa*. The rigid structure of lignocellulosic biomass is due the protective sheath of hemicellulose and lignin cellulose that encloses the cellulose making most of the cellulose inaccessible to enzyme and microbe attack (Waldron and Faulds, 2007). The native sample showed a uniform arrangement of

granules indicating a rigid and densely packed structure, whereas the pre-treated JFS had some discernible changes specifically the appearance of pores and flaky surface. The acid pre-treatment hydrolysed most of these hemicellulose to its monomeric sugars by destroying the polymeric bonds and disrupting the lignin structure enhancing cellulose availability and degradability to fermentable sugars improving the sugar yield (Keskin *et al.,* 2019).



(a) **(b)**

Fig. 4. SEM image of (a) native, and (b) pretreated feedstock using dilute sulphuric acid

CONCLUSION

Based on the obtained results, it was observed that the optimum conditions for acid pre-treatment of seed kernels of *Mirabilis jalapa* were: 2% (v/v) H_2SO_4 and hydrolysis time of 15 mins at 121℃. It can be concluded that lower acid concentration and shorter time period were found favourable for producing higher reducing sugar yield. With the application of proper pre-treatment technology and the removal of interfering radicals, the present work has the potential for production of bioethanol and upscaling which is an important mandate towards mitigating the ill effects of fossil fuel combustion.

ACKNOWLEDGEMENTS

The authors are grateful to NEHU Non – Net Fellowship and the Indian Council of Social Science Research (ICSSR), New Delhi and for providing financial support necessary to carry out this research work. A heartfelt gratitude goes to the reviewers for improving the quality of the paper.

REFERENCES

Amin, F. R., Khalid, H., Zhang, H., Rahman, S., Zhang, R., Liu, G. and Chen, C. 2017. Pretreatment methods of lignocellulosic biomass for anaerobic digestion. *AMB Express*, **7**(1): 72. https://doi.org/10.1186/S13568-017-0375-4

Ávila, J., Silva, J., Pessoa, F. and Pereira, P. 2023. Hydrogen Production via SMR with Carbon Capture: A Bibliometric Analysis. *Journal of Bioengineering, Technologies and Health.* https://doi.org/10.34178/jbth.v5i4.260

Bušić, A., Mardetko, N., Kundas, S., Morzak, G., Belskaya, H., Šantek, M. I., Komes, D., Novak, S. and Šantek, B. 2018. Bioethanol Production from Renewable Raw Materials and Its Separation and Purification: A Review. *Food Technology and Biotechnology,* **56**(3): 289. https://doi.org/10.17113/FTB.56.03.18.5546

Byadgi, S. A. and Kalburgi, P. B. 2016. Production of Bioethanol from Waste Newspaper. *Procedia Environmental Sciences*, **35:** 555–562. https://doi.org/10.1016/J.PROENV.2016.07.040

Cardona, C. A. and Sánchez, Ó. J. (2007). Fuel ethanol production: Process design trends and integration opportunities. *Bioresource Technology*, **98**(12): 2415–2457. https://doi.org/10.1016/J.BIORTECH.2007.01.002

Chandra, K. and Sekar. 2012. Invasive Alien Plants of Indian Himalayan Region—Diversity and Implication. *American Journal of Plant Sciences*, **2012**(02): 177–184. https://doi.org/10.4236/AJPS.2012.32021

Cotana, F., Cavalaglio, G., Gelosia, M., Nicolini, A., Coccia, V., and Petrozzi, A. (2014). Production of bioethanol in a second generation prototype from pine wood chips. *Energy Procedia*, **45:** 42–51. https://doi.org/10.1016/J.EGYPRO.2014.01.006

de Carvalho, L. M., Borelli, G., Camargo, A. P., de Assis, M. A., de Ferraz, S. M. F., Fiamenghi, M. B., José, J., Mofatto, L. S., Nagamatsu, S. T., Persinoti, G. F., Silva, N. V., Vasconcelos, A. A., Pereira, G. A. G., and Carazzolle, M. F. (2019). Bioinformatics applied to biotechnology: A review towards bioenergy research. *Biomass and Bioenergy*, **123**: 195–224. https://doi.org/10.1016/J.BIOMBIOE.2019.02.016

Deshavath, N. N., Mohan, M., Veeranki, V. D., Goud, V. V., Pinnamaneni, S. R. and Benarjee, T. 2017. Dilute acid pretreatment of sorghum biomass to maximize the hemicellulose hydrolysis with minimized levels of fermentative inhibitors for bioethanol production. *3 Biotech*, **7**(2). https://doi.org/10.1007/S13205-017-0752-3

Ehrman, T. 1994. Method for determination of total solids in biomass. Laboratory analytical procedure no. 001. Golden, CO: National Renewable Energy Laboratory.

Izmirlioglu, G. and Demirci, A. 2016. Improved simultaneous saccharification and fermentation of bioethanol from industrial potato waste with co-cultures of Aspergillus niger and Saccharomyces cerevisiae by medium optimization. *Fuel*, **185:** 684–691. https://doi.org/10.1016/J.FUEL.2016.08.035

Keskin, T., Nalakath Abubackar, H., Arslan, K. and Azbar, N. 2019. Biohydrogen Production From Solid Wastes. *Biomass, Biofuels, Biochemicals: Biohydrogen, Second Edition*, 321–346. https://doi.org/10.1016/B978-0-444-64203-5.00012-5

Kowalski, Z., Kulczycka, J., Verhé, R., Desender, L., De Clercq, G., Makara, A., Generowicz, N. and Harazin, P. 2022. Second-generation biofuel production from the organic fraction of municipal solid waste. *Frontiers in Energy Research*, *10*, 1258. https://doi.org/10.3389/FENRG.2022.919415/BIBTEX

Kumar, G., Kim, S. H., Lay, C. H. and Ponnusamy, V. K. 2020. Recent developments on alternative fuels, energy and environment for sustainability. *Bioresource Technology*, **317**: 124010. https://doi.org/10.1016/J.BIORTECH.2020.124010

Li, Y., Zhang, Z., Jing, Y., Liu, T., Fan, X., Zhu, S., Jiang, D., Lu, C., Zhang, H., Yue, J., Zhang, Y., Yao, Z., Petracchini, F., Li, D. and Zhang, Q. 2022. Forecasting of reducing sugar yield from corncob after ultrafine grinding pretreatment based on GM(1,N) method and evaluation of biohydrogen production potential. *Bioresource Technology*, **348**: 126836. https://doi.org/10.1016/J.BIORTECH.2022.126836

Maliha, A. and Abu-Hijleh, B. 2022. A review on the current status and post-pandemic prospects of third-generation biofuels. *Energy Systems*: 1–32. https://doi.org/10.1007/S12667-022-00514-7/FIGURES/13

Mannivan, A. 2014. Bioethanol production from aquatic weed water hyacinth (*Eichhornia crassipes*) by yeast fermentation. Doctoral disseration, Karunya University.

Miller, G. L. 1959. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*, **31**(3): 426–428. https://doi.org/10.1021/AC60147A030/ASSET/AC60147A030.FP.PNG_V03

Pulidindi, I. N., Kimchi, B. B., & Gedanken, A. (2014). Can cellulose be a sustainable feedstock for bioethanol production? *Renewable Energy*, 71, 77–80. https://doi.org/10.1016/J.RENENE.2014.05.032

Qing, Q., Guo, Q., Zhou, L., Gao, X., Lu, X. and Zhang, Y. 2017. Comparison of alkaline and acid pretreatments for enzymatic hydrolysis of soybean hull and soybean straw to produce fermentable sugars. *Industrial Crops and Products*, **109**: 391–397. https://doi.org/10.1016/J.INDCROP.2017.08.051

Ramadoss, G. and Muthukumar, K. 2015. Influence of dual salt on the pretreatment of sugarcane bagasse with hydrogen peroxide for bioethanol production. *Chemical Engineering Journal*, **260**: 178–187. https://doi.org/10.1016/J.CEJ.2014.08.006

Ray, B., Ghosal, P. K., Thakur, S. and Majumdar, S. G. 1988. Structural studies of a neutral polysaccharide from the root bulb of Mirabilis jalapa. *Carbohydrate Research*, **176**(2): 324–328. https://doi.org/10.1016/0008-6215(88)80145-9

Rozina, R. 2016. Pharmacological and biological activities of Mirabilis jalapa L. *International Journal of Pharmacological Research*. https://doi.org/10.7439/IJPR.V6I5.2725

Sadasivam, S. (1996). Biochemical methods. New age international.

Saha, B. C. and Bothast, R. J. 1999. Pretreatment and enzymatic saccharification of corn fiber. *Applied Biochemistry and Biotechnology*, **76**(2): 65–77. https://doi.org/10.1385/ABAB:76:2:65

Singh, L. K., Chaudhary, G. and Kataria, R. 2011. Production of reducing sugars from lignocellulosic biomass for fuel ethanol production. *Selected paper for National conference on water, energy, and biodiversity with special reference to North East region* (209-221)

Singh, M., Akash, G. and Kalia, A. N. 2012. Mirabilis jalapa-A review. *International Journal of Pharmaceutical, Medical and Applied Sciences*, **1**(3): 22-32

Srivastava, A. K., Agrawal, P., and Rahiman, A. (2007). Delignification of Rice Husk and Production of Bioethanol. *International Journal of Innovative Research in Science, Engineering and Technology* (An ISO, 3297, 2319–8753). www.ijirset.com

Waldron, K. W. and Faulds, C. B. 2007. Cell Wall Polysaccharides: Composition and Structure. *Comprehensive Glycoscience: From Chemistry to Systems Biology*, **1–4**: 181–201. https://doi.org/10.1016/B978-044451967-2/00005-2

Walker, C. I. B., Trevisan, G., Rossato, M. F., Franciscato, C., Pereira, M. E., Ferreira, J. and Manfron, M. P. 2008. Antinociceptive activity of Mirabilis jalapa in mice. *Journal of Ethnopharmacology*, **120**(2): 169–175. https://doi.org/10.1016/J.JEP.2008.08.002

Yan, X., Wang, Z., Zhang, K., Si, M., Liu, M., Chai, L., Liu, X. and Shi, Y. 2017. Bacteria-enhanced dilute acid pretreatment of lignocellulosic biomass. *Bioresource Technology*, **245**: 419–425. https://doi.org/10.1016/J.BIORTECH.2017.08.037

Fanchi, J. 2023. Energy in the 21st Century. https://doi.org/10.5860/choice.49-2693