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Utilization of municipal solid waste for bioethanol production using Trichoderma reesei and Saccharomyces cerevisiae

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A B S T R A C T

In the present study, the utilization of municipal solid waste such as banana peel, paper waste, corn and rice waste was examined for the production of bioethanol. The pre-treatment of the solid waste was done by using different acids such as HNO_3, CH_3COOH, H_2SO_4, HCl with different concentrations and also using ultrasonication method. The conditions of using H_2SO_4 (2%), ultrasonication power (80), temperature (60!) and time (30 min) shows better yields. *Trichoderma reesei* was used for the saccharification of the substrate and reducing sugar yield of 115.5 mg/ml was achieved using the optimized conditions of 40% of biomass concentration, pH of 5, temperature and time of 36! for 96 hrs. The bioethanol production was done through fermentation using *saccharomyces cerevisiae* and highest yield of bioethanol with 21.43 mg/g was achieved. The optimized conditions were concluded from the results of using 4% of the yeast concentration with the temperature and time of 32! for 96 hrs. Thus, the results show that municipal solid waste can be utilize defficiently for the production of bioethanol.

1. Introduction

The production of biofuels from biodegradable municipal solid waste has gained attention in order to overcome the future energy demands in the country. The use of biofuels will help in reducing the greenhouse gas emissions and will also minimize the use of fossil fuels in the market (Passadis et al., 2022). Although sugar and starch-based edible feedstocks have been used for the production of bioethanol, they have been competing in the food cycle and also have some ethical issues (Meng et al., 2021). This led to the use of lignocellulosic biomass, municipal solid waste, macroalgae etc. The municipal solid waste (MSW) like paper, forest and food waste have been considered as the affordable feedstock for bioethanol production due to their low cost and management of waste deposition. The waste to energy technologies provides possible solutions to produce energy from solid waste because of their higher organic content. Normally food waste occurs in various stages like right from production stage to consumption stage. Therefore, the utilization of these solid wastes can be efficiently used for the production of bioethanol (Khraisheh& Li, 2010; Triwahyuni et al., 2015).

Because of having higher octane number and flashpoint, bioethanol seems to be the best alternative biofuel to replace conventional fossil fuels for the production of energy. National biofuels policy – 2019 in India has set the target of blending 20% of ethanol in petrol by 2030 to minimize the price of fossil fuels. Additionally, this will promote the use of greener energies in society in order to protect the environment and reduce the emission of green gas emissions. The production process of bioethanol includes the steps following, (i) pre-treatment of the biomass, (ii) hydrolysis of the biomass using enzyme or microbes to convert them into fermentable sugars, (iii) fermentation of the sugars to produce ethanol and followed by distillation to yield pure bioethanol (Raja Sathendra et al., 2019; Pogaku, 2019; Li et al., 2010).

Pre-treatment of the sample will lead to easy accessibility of the cellulose to the hydrolysing enzyme. Various microorganisms have previously reported on the saccharification of the cellulose to convert it into fermentable sugars (Chauhan et al., 2022; Varjani et al., 2022). Among that *Trichoderma reesei* was found to be most commercially available and efficient one for the saccharification process. The commonly reported strain for yeast fermentation was found to be *saccharomyces cerevisiae* and thus used for the bioethanol production in this study (Jayakumar et al., 2022). Optimization of the process variables is really important to access the efficiency of any process or a reaction. The parameters like the concentration of acids, yeast, biomass used and also the temperature and time of the reaction plays a significant role in the yield production (Singh et al., 2022; Zanivan et al.).

The present research work aims to produce bioethanol by using municipal solid waste as the suitable feedstock. The use of municipal solid waste for bioethanol production will helps in utilizing the solid waste generated in the environment. The use of waste resources as

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feedstocks and commercially available *Trichoderma reesei*will reduce the total cost of bioethanol production (Arnata et al., 2021). The optimized conditions in the production process would sufficiently increase the yield of bioethanol. Thus,the use of municipal solid waste as an energy resource for biofuel production by reducing the total production cost and minimizing pollution in the environment, this solid waste found to be a sustainable feedstock for the production of bioethanol while contributing to a circular bioeconomy (Tsegu et al., 2022).

2. Materials and methods

2.1 Collection of municipal solid waste

The municipal solid waste like banana peel, paper waste, corn and rice waste were collected from nearby markets and washed to remove the dust and impurities present. Then the washed solid wastes were dried in hot air oven at 80! until moisture content was completely removed. The dried waste was grounded into powder and kept in a sealed plastic container to avoid growth of fungus and was further used for bioethanol production.

2.2 Pre-treatment of collected solid waste

The pre-treatment of the collected waste was done by using diluted acids and ultrasonication methods. Different acidssuch as nitric acid (HNO_3), acetic acid (CH_3COOH), sulfuric acid (H_2SO_4) and hydrochloric acid (HCl) were used at different concentrations (1%,2%,3%,4%,5%) to pretreat the waste. The acids used for pretreatment were purchased from Merck life sciences Pvt. Ltd. Mumbai. Ultrasonication method was also studied for the pre-treatment of the waste by varying the Ultrasonication power (50,60,70,80,90), temperature (40!,50!,60!,70!,80!) and time (10 min,20 min,30 min,40 min,50min). The estimation of cellulose content was done using anthrone method and the OD was taken at 630 nm using UV-Vis spectrophotometer. The concentration was calculated by plotting the graph using cellulose as the standard curve (Barampouti et al., 2022).

2.3 Preparation of inoculum

The fungal strain*Trichoderma reesei* (MTCC No 3192) was obtained from Microbial Type Culture Collection and Gene bank, Institute of Microbial Technology, Chandigarh, India. The strain was sub cultured using potato dextrose agar (PDA) containing potato of 200g/l, dextrose 20g/l and agar 15g/l. The sub culture was incubated 30! for 5 days to obtain the colony formation and stored later at 4! for future use. The yeast *Saccharomyces cerevisiae* was purchased from the market.*S. cerevisiae* was grown on YEPD medium containing yeast, peptone and dextrose in a shake flask incubated at a temperature of 30 ° C for 1 day at 150 rpm (Bharathiraja, 2016).

2.4 Optimization of saccharification and glucose estimation

2.4.1 Effect of biomass concentration and pHon saccharification process

The saccharification process was carried out in a shaker conical flask with the*Trichoderma reesei* to convert the cellulose into fermentable sugars. The optimization of the saccharification process was studied by varying substrate biomass concentration with the percentages ranging from 10,20,30,40,50% and the pH varying between 4,4.5,5,5.5,6. After the saccharification process, the yield of the fermentable sugars was estimated using the DNS method. The absorbance was calculated at 575nm using UV-Vis spectrophotometer and graph was plotted against the standard curve and the yield was estimated (Khoshkho et al., 2022).

2.4.2 Effect of time and temperature on saccharification process

The effect of time and temperature on the saccharification was studied by varying the time for 24,48,72,96,120hr and temperature of 32, 34,36,38,40! to increase the yield of fermentable sugars. Also, after the process the estimation of the fermentable sugars was done by using DNS method (Narisetty et al., 2022).

2.5 Optimization of bioethanol production through fermentation

2.5.1 Effect of yeast concentration on fermentation

The production of bioethanol was done through fermentation process using the yeast strain *saccharomyces cerevisiae.* The optimization of the fermentation process was carried out by changing the concentration of the yeast strain with percentage of 1, 2, 3, 4, 5%. The concentration of the strain plays a major role in the fermentation process of the fermentable sugars to convert them into bioethanol(Sathendra et al., 2019).

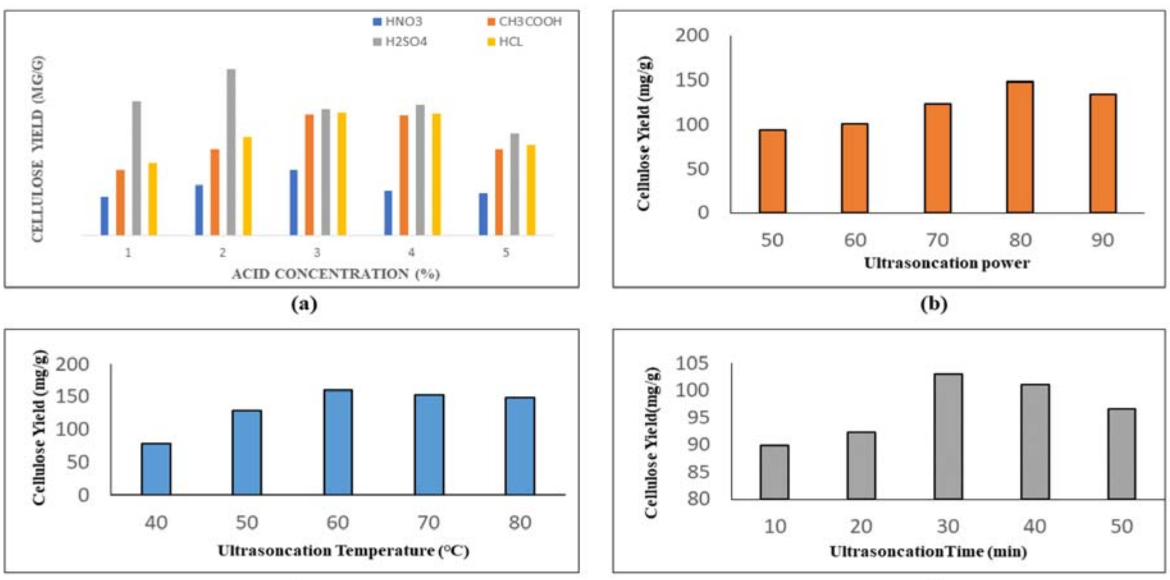
2.5.2 Effect of temperature and time on fermentation

The effect of fermentation temperature and time on the fermentation of the fermentable sgars was studied by differing their temperature between 30, 32, 34, 36, 38, 40! and fermentation time with the intervals of 24, 48, 72, 96, 120hr. After the fermentation process, the bioethanol yield was estimated using the potassium dichromate method. To 1 ml of the extract, an equal volume of potassium dichromate was added. To that 4 ml of H_2SO_4 was added and vortexed. The mixture was used to measure the OD at 660nm using a UV-Vis spectrophotometer and the bioethanol yield was estimated(Moreno et al., 2021).

3. Results and discussion

3.1 Pre-treatment of collected solid waste

The pre-treatment of solid waste was done to increase the higher accessibility of the strain to cellulose. Different acids like HNO_3, CH_3COOH, H_2SO_4, HCl of concentration 1% - 5% was varied and studied [fig. 1 (a)], from that the use of H_2SO_4 with a concentration of 2% showed higher yield of cellulose. The use of ultrasonication showed better results with the variation of ultrasonication power, time and temperature. The ultrasonication power of 80 shows higher yield when compared to others [fig.1 (b)], also the ultrasonication temperature of 60! [fig.1 (c)] and ultrasonication time of 30 min [fig. 1 (d)] showed better yield of cellulose. Therefore, the optimized conditions for pre-treating the sample were found be to be use of H_2SO_4 with 2% and Ultrasonication with power 80, temperature of 60! and time of 30 min with the cellulose yield of 101.5 mg/g. The yield was affected when subjected to higher ultrasonication temperature and time (Gengiah et al., 2020).



(c) Fig.1 Effect of acids on the pre-treatment of solid waste (d)

3.2 Optimization of saccharification and glucose estimation

3.2.1 Effect of biomass concentration and pHon saccharification process

The optimization of the saccharification process to reduce the cellulose to fermentable sugars using *Trichoderma reesei*was studied by varying the parameters like biomass concentration and pH of the reaction. The experiments were conducted in a conical flask and shaken using an orbital shaker. The estimation of the reducing sugar was done using the DNS method. The results showed that the yield increases with the increase in biomass concentration upto 40% and then decreased and this shows the optimal concentration of biomass should be 40% [fig. 2]. The study on pH shows the better yield on pH 5 with the highest yield and later the reducing sugar concentration was found to be decreasing on increasing the pH further. This shows thatpH of 5 was optimal for the saccharification process [fig. 3] (Sivarathnakumar et al., 2019).

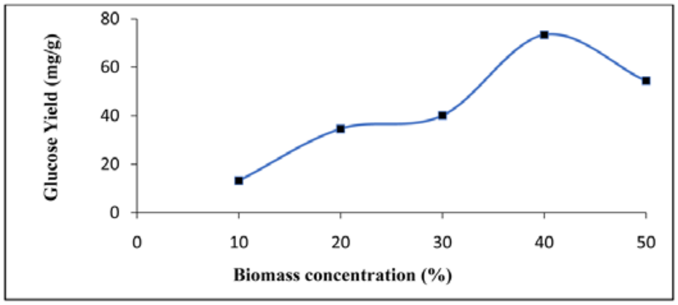


Fig. 2 Effect of biomass concentration (%) on saccharification process

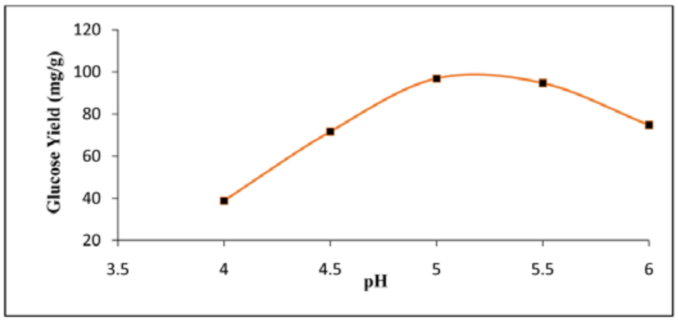


Fig. 3 Effect of pH on saccharification process

3.2.2 Effect of time and temperature on saccharification process

The study on the effect of temperature and time on the saccharification process was studied. The estimation on the fermentable sugars on varying the temperature shows the highest yield at 36![fig. 4] and further increasing the temperature shows no improvement which affects the yield. The study on different time period shows higher yield for the reaction taking place for 96hr. The yield increased gradually and later suddenly decreased on 120 hr [fig. 5] which shows the optimal time for saccharification process was 96 hr. Hence the optimal temperature and time for the process of saccharification was found to be of 36! for 96 hr(Chaudhary et al., 2021).

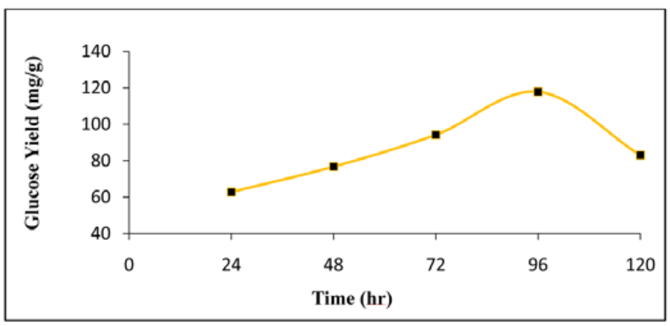


Fig. 4 Effect of time on saccharification process

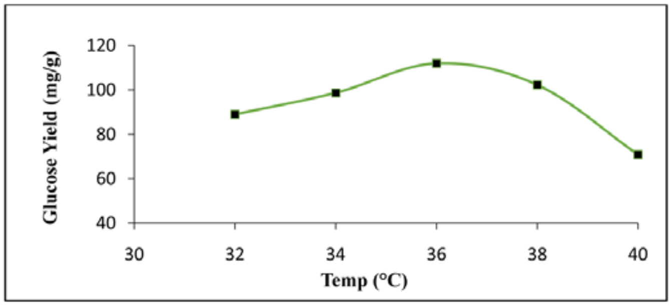


Fig. 5 Effect of temperatureon saccharification process

3.3 Optimization of bioethanol production through fermentation

3.3.1 Effect of yeast concentration on fermentation

The bioethanol production from the fermentable sugars of municipal solid waste was performed through fermentation process using the strain *Saccharomyces cerevisiae.* The yeastconcentration (%) was varied from 1-6% and the results have shown that the yield of bioethanol increases with the increase in yeast concentration up to 4% and decreases on further increasing concentrations (fig. 6). This shows the optimal yeast concentration of 4% will be effective for the efficient production of bioethanol from the fermentable sugars of municipal solid waste (Cherian et al., 2015).

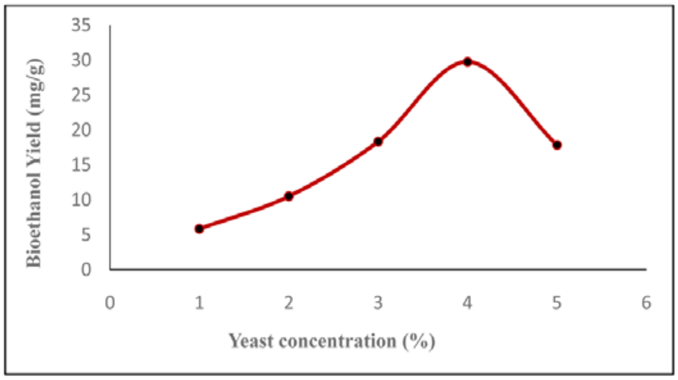


Fig. 6 Effect of yeast concentration on fermentation

3.3.2 Effect of time and temperature on fermentation

The effect of time and temperature on the fermentation reaction are used to optimize the bioethanol production process. The results have shown that the use of the reaction temperature of 32°C has given highest yield of bioethanol when compared to other temperatures (fig. 7). The reaction time of 96 hr shows better yield and further increase in time shows no improvement (fig. 8) in the yield. Thus, the optimal temperature and time for fermentation process was found to be 32°C for 96 hr. The bioethanolyield was estimated by taking the OD at 660nm using the UV-Vis spectrophotometer and the highest yield of bioethanol using the optimized conditions was found to be 21.43 mg/g (Kazemi Shariat Panahi et al.,2022).

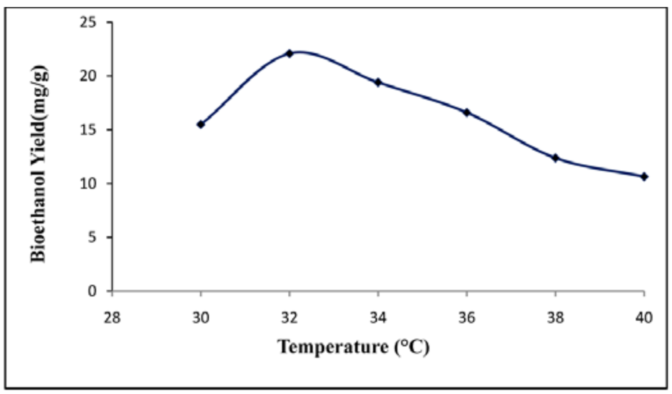


Fig. 7 Effect of temperature on fermentation

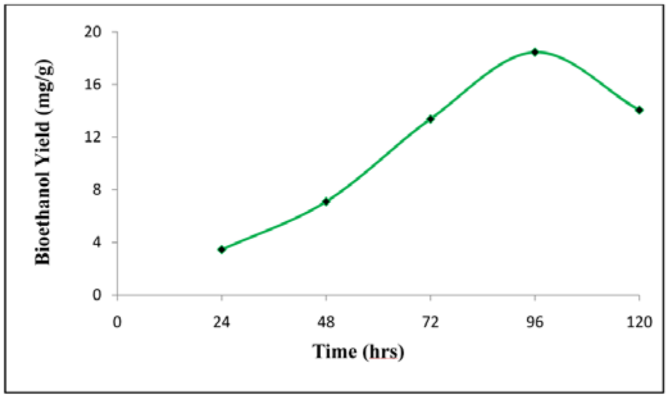


Fig. 8 Effect of time on fermentation

Conclusion

Bioethanol production from municipal solid waste was carried out by using fermentation process. The pre-treatment of the substrate using H_2SO_4 and ultrasonication power of 80, ultrasonication temperature and time of 60! for 30 min showed better results. *Trichoderma reesei*was used for the saccharification process and optimized with the conditions of 40% biomass concentration, pH 5, 36! of temperature and 96 hrs of time. Fermentation using *Saccharomyces cerevisiae* was done by optimizing the parameters with 4% yeast concentration, reaction temperature of 32 ° C and time of 96 hrs. The bioethanol yield was estimated using the potassium dichromate method, and the highest yield was found to be 21.3 mg/g. Therefore, the results concluded that municipal solid waste is a suitable and efficient feedstock for the production ofbioethanol in a sustainable way.

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