Renewable Energy from Floral Biomass: The Potential of *Rosa indica* in Biofuel Production

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Abstract
The present study explores the potential of Rosa indica flower waste as a renewable feedstock for bioethanol production. The bioethanol fermentation process was carried out using Saccharomyces cerevisiae. Both chemically pretreated and untreated rose flower waste hydrolysates were employed as primary carbon sources under submerged fermentation conditions. Process optimization was conducted using the One-Variable-at-a-Time (OVAT) approach to enhance bioethanol yield.

The maximum bioethanol concentration of 28.2 g/L was achieved under optimal conditions: 6 g% substrate concentration, 1.5 g% of fructose supplementation, 0.5 mL % corn steep liquor as a nitrogen source, 4 mL % inoculum size, pH 5.5, temperature of 30 °C and an incubation period of 3 days. These results indicate that the optimized fermentation process significantly improves bioethanol production from rose flower waste, highlighting its potential as a sustainable biofuel source.

Keywords: Valorisation, Flower waste biomass, Pretreatment, Bioconversion, Bioethanol.

Introduction

The growing demand for sustainable energy sources has led to increased interest in bioethanol production as an alternative to fossil fuels. Bioethanol, a renewable fuel derived from biomass, offers significant environmental benefits and serves as a promising solution to mitigate greenhouse gas emissions[14]. Among various biomass sources, agricultural waste materials present an attractive opportunity for bioethanol production due to their abundance and potential for conversion into valuable products[3].

In this context, rose flower, a widely cultivated ornamental plant, represents an underutilized biomass resource that holds immense potential for bioethanol production. The valorisation of waste rose flowers not only addresses the environmental challenge of waste management but also contributes to the sustainable production of biofuels. This research aims to explore the feasibility of utilizing waste rose flowers as a feedstock for bioethanol production through fermentation processes[9]. The conversion of lignocellulosic biomass into fermentable sugars involves pretreatment, enzymatic hydrolysis and fermentation by suitable microorganisms. *Saccharomyces cerevisiae*, a widely employed ethanologenic yeast, has been extensively studied for its ability to ferment hexose sugars obtained from the hydrolysis of cellulose and hemicellulose fractions of lignocellulosic biomass[11].

In the classical approach to medium optimization, the one-variable-at-a-time (OVAT) method involves varying a single factor or variable while keeping all other parameters constant. The concentrations of selected medium components are adjusted within a specified range. Due to its simplicity and convenience, OFAT has been widely preferred by researchers for designing medium compositions, particularly in the initial stages of studies across various fields[12]. The study aims to use rose flower waste (RFW) as a zero-cost substrate for bioethanol production and its optimization by OFAT methodology for an elevated bioethanol production.

Material and Methods

Substrate collection and processing: RFW was collected from different temples in Anand, Gujarat. Petals were separated from RFW and were washed by tap water and distilled water in order to achieve dust and debris free substrate. Washed material was sundried until a constant weight was achieved followed by grounding and sieving (200 mm mesh size) the material, which was utilized as biofuel substrate.

Proximate analysis of substrate: The dried RFW was analysed for its compositional content including moisture content and ash content by the standard method of Association of Official Analytical Chemists (AOAC - 1965), reducing sugar by the DNS method, total sugar by the anthrone method, crude nitrogen content by the Kjheldahl method, crude fibre, hemicellulose, cellulose and lignin content by Van soest method[4,8].

Fermentation: Production medium for bioethanol was formulated where hydrolysate of pretreated as well as untreated RFW was utilized as substrate for bioethanol production. Hydrolysates were achieved upon acid (1% H_2SO_4) and alkali (1% NaOH) treatment to dried RFW. 100 ml of total production medium included 5 g% of substrate, 0.3 g% yeast extract, 1 g% peptone and 0.6 g% K_2HPO_4 with 5.5 pH. The active culture of yeast *Saccharomyces cerevisiae* was inoculated (5 mL%) to the sterilized production medium and incubated at 33 °C, partially anaerobic and static condition for 3 days.

Distillation: The fermented production medium was centrifuged at 10,000 rpm in order to separate yeast cell mass. The supernatant was subjected to distillation apparatus. The distillation process was carried out at 78 ℃ where bioethanol was separated from the medium supernatant.

Ethanol estimation and concentration: The distillate was examined for concentration of bioethanol by potassium dichromate ($K_2Cr_2O_7$) method spectrophotometrically at 660 nm[10]. The estimation procedure was followed with modifications and optimization, where the analytical procedure involves the addition of 1 mL distillate aliquot to 25 mL acidified $K_2Cr_2O_7$ reagent. The reaction mixture is thermally equilibrated at 80 °C for 15 minutes in a water bath, followed by cooling to ambient temperature and volumetric adjustment to 50 mL by distilled water. Bioethanol concentration of the distillate was calculated using a standard graph prepared on the basis of absorbance values achieved from the known ethanol concentration.

OVAT optimization: 'One Variable at a Time' (OVAT) assesses the impact of one element by altering one component at a time rather than many simultaneously. The elements to be assessed for its impact on bioethanol production are: carbon source (glucose, galactose, maltose, fructose, sucrose, sorbose), nitrogen source [yeast extract (YE), peptone, malt extract (ME), corn steep liquor (CSL), soybean meal (SM), ammonium sulphate (AS), potassium nitrate (KNO_3)], substrate (RFW) at different concentrations (5 g% to 10 g%) and inoculum size (2 mL% to 10 mL%). The physical factors to be taken into consideration were pH (3.5 to 6.5), temperature (25 ℃ to 50 ℃) and incubation time (1, 2, 3, 4, 5, 6 days).

Results and Discussion

Substrate collection and its proximate analysis: In present study, the rose flowers were collected, separated, dried and utilized as substrate for bioethanol production. The dried RFW subjected to its proximate analysis resulted into 46.4 % of moisture content, 3.1 % of ash content, 3.5 % of reducing sugar, 8.3 % of total carbohydrates, 2.67 % of crude nitrogen content, 12.98% of crude fibre, 8.83 % of hemicellulose content and 12.2 % of cellulose content. In a similar study, the compositional analysis of rose petals resulted into 63 % of holocellulose, 34.5 % of cellulose, 18.1 % of lignin and 4.7% of ash[9].

Fermentation and Estimation: Bioethanol from untreated and pretreated substrate (RFW) was carried out followed by distillation and estimation by potassium dichromate method. Bioethanol production of 0.27 g/L, 1.8 g/L and 15 g/L was estimated from alkali hydrolysate, acid hydrolysate and untreated RFW respectively. Thus, giving higher bioethanol production, untreated substrate (RFW) was utilized for further optimization process.

OVAT Optimization

Effect of Substrate concentration: The substrate concentration is a crucial parameter that profoundly influences bioethanol production. In the present study, RFW was employed as a substrate for bioethanol production. The substrate concentration range investigated spanned from 4 g% to 10 g%. It was observed that a substrate concentration of 6 g% yielded bioethanol production of 17.3 g/L (Fig. 1). Substrate concentration of 30 g/L derived from indigenous *Ulva prolifera* biomass was optimal for bioethanol production by *Saccharomyces cerevisiae* NFCCI1248[2].

Effect of carbon source: Glucose, galactose, maltose, fructose, sucrose and sorbose were screened as supplemented carbon sources (1 g%) for enhanced bioethanol production (Fig. 2). Carbon source showing maximum bioethanol production was chosen for its optimum concentration. Fructose at 1.5 g% of supplementation showed maximum bioethanol production of 22.14 g/L (Fig. 3) followed by glucose giving 18.06 g/L at its concentration of 1 g%, while galactose and sorbose were found to yield the lowest, 13.9 g/L and 15.2 g/L of bioethanol production. For production of bioethanol, trehalose sugar resulted in optimum sugar supplementation for bioethanol production by *E. coli* DH5α in M9 media[1].

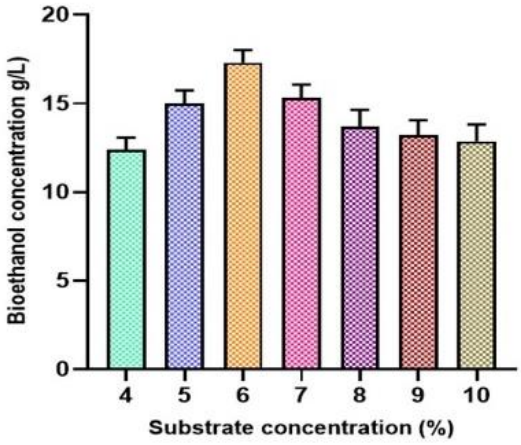

Figure 1: Effect of substrate concentration on bioethanol production by *Saccharomyces cerevisiae*

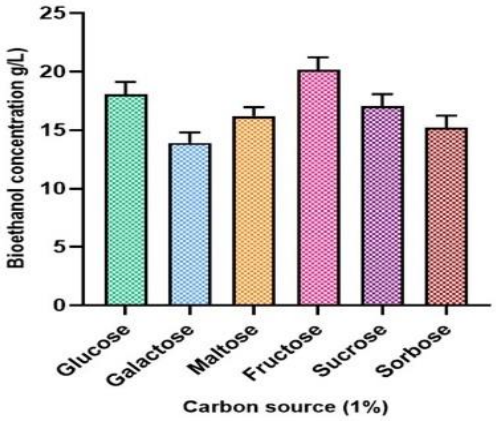

Figure 2: Effect of different carbon sources on bioethanol production by *Saccharomyces cerevisiae*

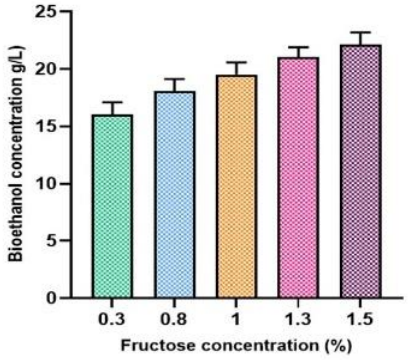

Figure 3: Effect of fructose concentration on bioethanol production by *Saccharomyces cerevisiae*

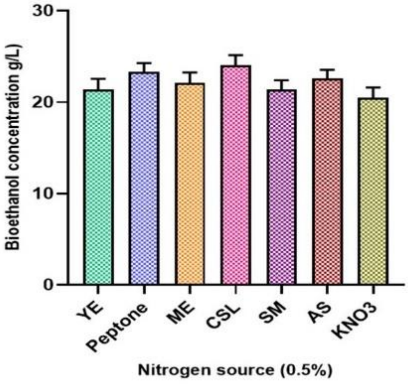

Figure 4: Effect of different nitrogen sources on bioethanol production by *Saccharomyces cerevisiae*

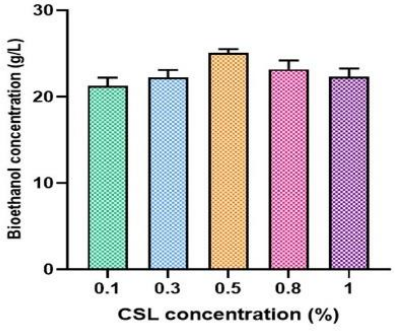

Figure 5: Effect of CSL concentration on bioethanol production by *Saccharomyces cerevisiae*

Effect of nitrogen source: For biosynthesis to occur, nitrogen molecule supplementation is essential. The availability of added nitrogen in the medium can be restricted to reduce biosynthesis[6]. Supplementation of nitrogen compounds is of fundamental importance for biosynthesis. Biosynthesis can be decreased by limiting the availability of nitrogen supplements in the medium. Various organic (YE, peptone, ME, CSL, SM) and inorganic (AS and KNO_3) nitrogen sources were assessed for its effect on bioethanol production (Fig. 4). Corn steep liquor was found to best nitrogen source and later was assessed for its optimum concentration. Corn steep liquor at 0.5 mL% gave maximum bioethanol production of 25.09 g/L (Fig. 5). Soyabean meal was found to be best nitrogen source to enhance bioethanol production (9.21 g/L) by *Pichia stipitis* NCIM 3499 and using rice straw as substrate[5].

Effect of Inoculum size: The yeast inoculum size significantly affects the bioethanol productivity by regulating the rate of substrate consumption in the broth[16]. Optimum inoculum size required for substrate utilization, fermentation and bioethanol production was carried out using varying inoculum sizes (2 %, 4%, 6%, 8 % and 10% v/v). 4 mL% inoculum size was found to be optimum leading to 25.6 g/L of bioethanol production (Fig. 6). 2 mL% and 6 mL% of inoculum size were determined to be optimum for bioethanol production by *Saccharomyces cerevisiae H058* and *Saccharomyces cerevisiae var. ellipsoideus,* from food waste hydrolysates and enzymatic hydrolysate of sunflower hulls respectively[11,15].

Effect of pH: In the current study, different pH (3.5, 4.0, 4.5, 5.0, 5.5, 6, 6.5) were taken into consideration in order to conclude optimum pH for fermentation and bioethanol production. pH 5.5 was found to be optimum pH achieving 26.6 g/L of bioethanol production (Fig. 7). Reduction in bioethanol production was observed with increase in pH where minimum bioethanol production of 10.3 g/L was obtained at pH 3.5. pH 5 and pH 4 were found to be optimum for bioethanol production of 25.9 g/L and 21.77 g/L from rice straw and enzyme treated rice straw as substrate by *Saccharomyces cerevisiae HAU* and *Saccharomyces cerevisiae OR* respectively[5,7].

Effect of temperature: Temperature effects on the generation of bioethanol were methodically studied at different temperatures including 25 °C, 30 °C, 35 °C, 40 °C, 45 °C and 50 °C. The temperature of 30 °C was found to be optimum and elevated level of bioethanol production of 27.3 g/L by *Saccharomyces cerevisiae* (Fig. 8). Notably, 30 °C was found to be the ideal temperature for bioethanol production of 25.30 g/L by *Saccharomyces cerevisiae HAU* using rice straw as substrate[5]. Bioethanol production from spent seaweed biomass resulted in bioethanol production of 47.5 g/L at 30 °C as the optimal temperature[13].

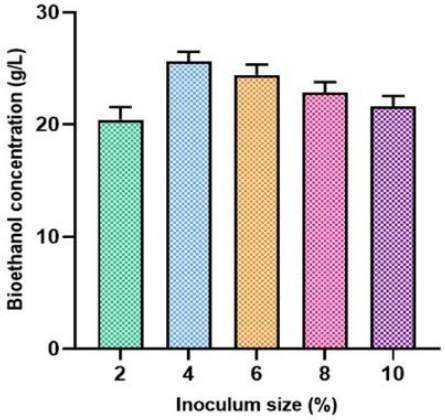

Figure 6: Effect of inoculum size on bioethanol production by *Saccharomyces cerevisiae*

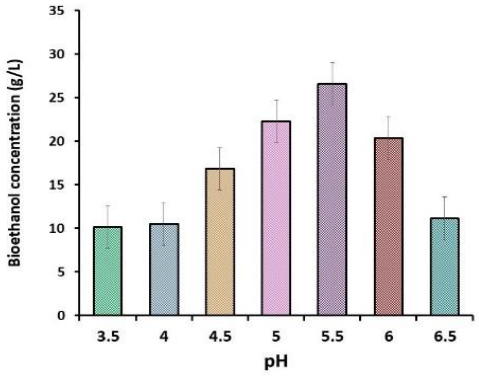

Figure 7: Effect of various pH on bioethanol production by *Saccharomyces cerevisiae*

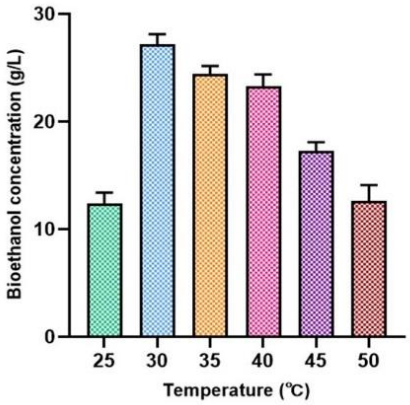

Figure 8: Effect of temperature on bioethanol production by *Saccharomyces cerevisiae*

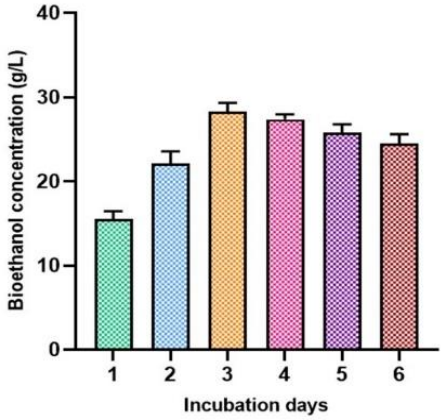

Figure 9: Effect of incubation days on bioethanol production *Saccharomyces cerevisiae*

Effect of Incubation time: To determine the ideal incubation period for optimum bioethanol production, the impact of incubation duration was evaluated. A set of experiments was incubated for varying durations, ranging from 1 to 6 days. The optimum incubation time was 3 days where 28.2 g/L of bioethanol was produced at temperature 30℃ (Fig. 9). 72 h of incubation was found to be the optimum incubation period for bioethanol production of 20 g/L from pretreated rice straw by *Saccharomyces cerevisiae*[7].

Conclusion

The present study investigated the utilization of rose flower waste (RFW) as a substrate for bioethanol production using the yeast strain *Saccharomyces cerevisiae*. Bioethanol synthesis was carried out through submerged fermentation, employing both acid- and alkali-pretreated hydrolysates of rose flower waste, as well as untreated rose flower waste, as primary carbon sources. The optimization of various media components and physical parameters was conducted using the one-factor-at-a-time (OFAT) approach. The highest bioethanol concentration, 28.2 g/L, was achieved under optimal fermentation conditions which included a substrate concentration of 60 g/L, supplementation with 1.5 g% fructose as an additional carbon source, 0.5 mL% corn steep liquor (CSL) as a nitrogen source, an inoculum size of 4 mL%, a pH of 5.5, an incubation temperature of 30 °C and a fermentation period of 3 days.

This study underscores the viability of rose flower waste as a sustainable and economical feedstock for bioethanol production. By optimizing fermentation conditions, bioethanol yield can be significantly improved, supporting renewable energy advancements and efficient waste utilization.

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