Applied Energy 87 (2010) 2352–2355

Contents lists available at ScienceDirect

Applied Energy

journal homepage: www.elsevier.com/locate/apenergy

Comparative study of bio-ethanol production from mahula (*Madhuca latifolia* L.) flowers by *Saccharomyces cerevisiae* and *Zymomonas mobilis*

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ARTICLE INFO

Article history:
Received 6 August 2009
Received in revised form 22 October 2009
Accepted 13 November 2009
Available online 28 December 2009

Keywords:
Bio-ethanol
Fermentation
Madhuca latifolia
Saccharomyces cerevisiae
Zymomonas mobilis

ABSTRACT

Mahula (*Madhuca latifolia* L.) flower is a suitable alternative cheaper carbohydrate source for production of bio-ethanol. Recent production of bio-ethanol by microbial fermentation as an alternative energy source has renewed research interest because of the increase in the fuel price. *Saccharomyces cerevisiae* (yeast) and *Zymomonas mobilis* (bacteria) are two most widely used microorganisms for ethanol production. In this study, experiments were carried out to compare the potential of the yeast *S. cerevisiae* (CTCRI strain) with the bacterium *Z. mobilis* (MTCC 92) for ethanol fermentation from mahula flowers. The ethanol production after 96 h fermentation was 149 and 122.9 g kg^{-1} flowers using free cells of *S. cerevisiae* and *Z. mobilis*, respectively. The *S. cerevisiae* strain showed 21.2% more final ethanol production in comparison to Z. *mobilis*. Ethanol yield (*Yx/s*), volumetric product productivity (*Qp*), sugar to ethanol conversion rate (%) and microbial biomass concentration (*X*) obtained by *S. cerevisiae* were found to be 5.2%, 21.1%, 5.27% and 134% higher than *Z. mobilis*, respectively after 96 h of fermentation.

1. Introduction

The natural energy resources such as fossil fuels (petroleum and coal) are being utilized at a rapid rate and these fossil fuel resources would last only for few more years [1]. From various alternative energy resources, bio-ethanol is the most promising resource because of its biological and renewable origins, normally derived from energy crops such as maize, sugarcane, cassava, sweet potato, mahula flower and by-products of agriculture and forestry [2]. Biofuels offer a number of environmental, social, and economic advantages, lower emission of harmful pollutants and good fuel properties for vehicles [3].

Current ethanol production processes using crops such as sugar cane and corn are well-established. However, utilization of cheaper substrates such as lignocelluloses and other renewable biomasses would make bio-ethanol (ethanol from biomass) more competitive than fossil fuels [4]. But ironically, due to the inherent complexities in processing and utilizing these lignocellulosic and starchy biomass, the cost of production increases significantly [1], thereby leading to a growing interest worldwide to find alternative feed stocks for bio-ethanol production [5]. In this context, mahula (*Madhuca latifolia* L.) flowers provide a great premise as an alternative bio-resource for production of ethanol through fermentation [6–8]. Mahula is a forest tree found in the tropical rain forests of Asian and Australian continents. This tree species, however, has been domesticated by tribal people in India and Pakistan for use as food (flower), feed (leaves and flower), wood (timber) and beverages (flower) locally called 'mahuli'.

Traditionally, the yeast, *Saccharomyces cerevisiae* has been used all over the world as the major ethanol producing microorganism. In our earlier studies, *S. cerevisiae* strain CTCRI was used as free and immobilized cells for production of ethanol from mahula flowers in submerged fermentation [6,8]. Likewise, Mohanty et al. [7] reported bio-ethanol production from mahula flowers by solid-state fermentation using *S. cerevisiae*. In recent years, however, research is focused on processes involving the gram-negative anaerobic bacterium, *Zymomonas mobilis*, because of several better fermentation attributes such as it converts glucose almost stoichiometrically to ethanol and CO_2, grows more rapidly and demonstrates highest ethanol productivity during continuous fermentation [9]. *Zymomonas* spp. grow anaerobically and unlike yeasts do not require the controlled addition of oxygen to maintain viability at high cell concentrations [10,11]. Further the ethanol tolerance of *Zymomonas* spp. is comparable with strains of *S. cerevisiae* [12] and these produce less by-products [13]. Considering the above, this study was carried out to compare the performance of yeast, *S. cerevisiae* (CTCRI strain) with bacterium *Z. mobilis* (MTCC 92) on ethanol production from mahula flowers in submerged fermentation. Further, the growth and fermentation kinetics of *S. cerevisiae* and *Z. mobilis* cells during fermentation are compared.

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0306-2619/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.apenergy.2009.11.018

2. Materials and methods

2.1. Mahula flowers

Fresh mahula flowers were collected from the forests of the Keonjhar District of Orissa, India, during March–April, 2007. The flowers were brought to the Microbiology Laboratory of CTCRI, washed in tap water to get rid of dust and other debris and sun-dried in the open for 7 days to reduce the moisture content to 16–18.6%. The sun-dried flowers collected from various locations were mixed thoroughly before being used for ethanol fermentation. The flowers have the following compositions (expressed in g $100\,g^{-1}$ dry weight basis): moisture, 24–25.85; starch, 0.94–0.95; total sugar (glucose, fructose, fructose, sucrose and maltose), 36–38; crude protein, 6–7; crude fiber, 10.0–12.5; total ash, 1.6–2.0; undetermined solids, 10.6–13.7; and pH 4.5–4.8.

2.2. Microorganisms and culture conditions

Z. mobilis MTCC 92 was procured from the Institute of Microbial Technology, Chandigarh, for this investigation. *Z. mobilis* and *S. cerevisiae* (CTCRI strain) was earlier used in our laboratory for ethanol fermentation [7,8] maintained on *Z. mobilis* specific medium (ZSM) [(g l^{-1}): glucose, 100; yeast extract, 2; urea, 1; KH_2PO_4, 1; $MgSO_4{\cdot}7H_2O$, 0.5; agar, 15; pH 6.5] and the yeast (*S. cerevisiae*) was maintained on malt extract–yeast extract–glucose–peptone (MYGP) medium [(g l^{-1}): malt extract, 3; yeast extract, 5; peptone, 5; glucose, 20; agar, 15; pH 5.5]. Both the cultures were stored at 4 ± 0.5 °C for further use.

2.3. Preparation of starter culture

The starter cultures were prepared in 100 ml of the respective growth medium (as mentioned above) were sterilized (at 121° C for 20 min) in 250 ml Erlenmeyer flasks. The flasks were inoculated with a loopful of the microbial cultures (*Z. mobilis* or *S. cerevisiae*) and were incubated at 30 °C for 24 h under stationary conditions.

2.4. Fermentation medium

The mahula flowers were grinded (flower:water ratio, 1:5) in a mixer-cum-grinder (TTK Prestige Ltd., Bangalore, India) to make slurry. The slurry was cooked by steaming at 120–122° C for 60–80 min. After cooling $(NH_4)_2SO_4$ was added to the slurry (as nitrogen source at the concentration of 1 g l^{-1}) and pH of the medium was adjusted to 5.5 and 6.5 for subsequent inoculation of the yeast and bacterial culture, respectively. Then the slurry was inoculated with 10% starter culture (dry weight basis). The flakes ($n = 3$) were incubated for 96 h at the room temperature (30 ± 2° C).

2.5. Analytical methods

At 24 h intervals, fermented broths (in triplicate) were removed and the contents were analyzed for total sugar and ethanol. The ethanol content of the fermented broth was determined by measuring the specific gravity of the distillate according to the procedure described by Amerine and Ough [14]. In this procedure, the weight of a certain volume of an alcohol distillate was compared to the weight of exactly the same volume of distilled water. The ratio of the weights of the two (alcohol:water) gave the specific gravity of the distillate [14]. The total sugar was assayed by the Anthrone method [15]. The pH was measured using a pH meter (Systronics, Ahmadabad, India) fitted with a glass electrode. Fermentation kinetics were calculated using the formulae by Bailey and Ollis [16].

2.6. Population count

Yeast and bacterial populations in the fermented mash were calculated by serially diluting the substrate (fermented mahula slurry) in distilled water and plating suitable dilution (10^6–10^8) on Petri plates (18 mm × 150 mm) containing either MYGP (for *S. cerevisiae*) and ZSM (for *Z. Mobilis*) medium. Data were given as mean of three replicates.

2.7. Statistical analysis

The data of ethanol production using *S. cerevisiae* and *Z. mobilis* were analyzed using one way ANOVA. Where significant difference in ANOVA ($p < 0.05$) was detected by the Fisher's Least Significance Difference (LSD) multiple comparison test was applied to compare the factor level difference. The analysis was performed using MSTAT-C (version 2.0, Michigan State University, MI, USA).

3. Results and discussion

The main fermentable sugar components of the mahula flowers were reported to be glucose and fructose [6,8]. In this study, mahula flowers (100 g) after cleaning were blended with water in the ratio of 1:5 so as to dilute the bulkiness of the mash before steaming and subsequent fermentation (submerged) by either yeast, *S. cerevisiae* or bacterium, *Z. mobilis*. The comparison of sugar utilization and ethanol production profile by these two microbial strains are given in Fig. 1.

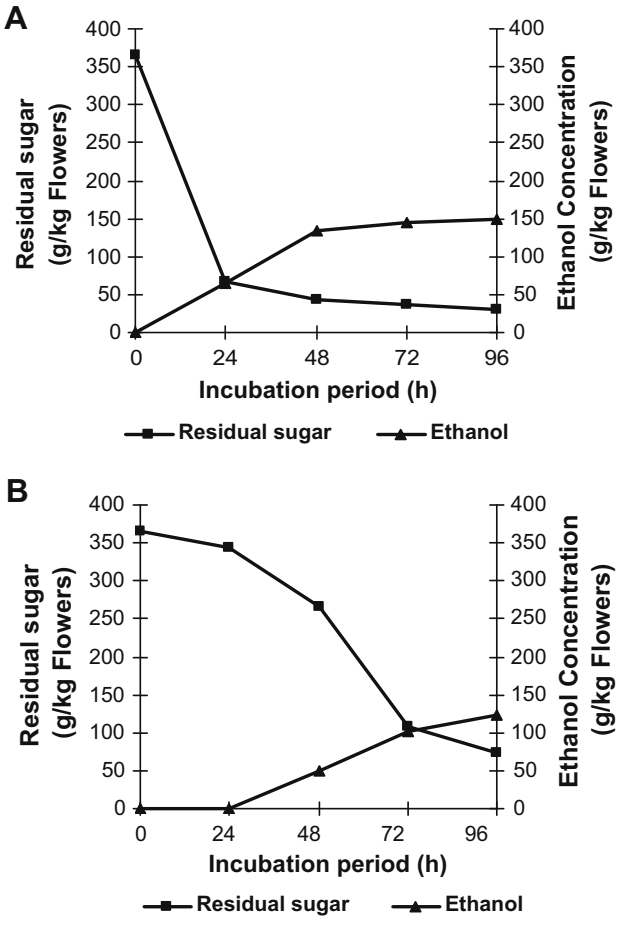

Fig. 1. Comparison of ethanol production between free cells of *S. cerevisiae* (A) and *Z. mobilis* (B).