The Feasibility of Mango (*Mangifera indica* L.) Peel as an Alternative Substrate for Butanol Production

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The suitability of dried mango peel as substrate for biobutanol production was investigated. The amount of mango waste generated can be very high; it has been recorded as high as 30% to 50% of the total weight of the fruit. The utilization of this waste is both a necessity and a challenge. Dried mango peel contains 30 ± 2.5% (w/w) of reducing sugars. Fermentation of mango peel extract by *Clostridium acetobutylicum* 2878 yielded 10.5 ± 0.4 g/L of butanol. The fermentation process was completed in seven days. Nutrients such as yeast extract, peptone, and beef extract were tested for supplementation of the mango peel medium. It was observed that nutrient supplementation improved both the rate and butanol production significantly, up to 13.3 ± 1.0 g/L of butanol. Scaling up studies using a bioreactor, with optimized mango peel extract medium and fermentation conditions, further improved the butanol production (15.42 ± 1.3 g/L). To the best of our knowledge, this is the first report on the utilization of mango peel for butanol production.

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INTRODUCTION

Butanol is an important industrial chemical. It is also considered a superior liquid fuel, with the potential to eventually replace gasoline (Durre 2005). Currently, it is produced worldwide at over 1.4 billion gal/year, using the chemical route. The market demand is expected to increase dramatically if butanol can be produced economically from low-cost biomass with high productivity. The economic production of butanol is primarily dependent on three factors: raw material cost, high product titer (achievable by overcoming solvent toxicity), and solvent recovery costs from the formation of mixed solvents and low butanol concentration. For sugars or starches containing feedstock, such as sugar cane or maize, the raw material cost accounts for 40% to 70% of the total butanol production cost. Hence, the development of a butanol production process using renewable energy sources (such as starchy and lignocellulosic crops) has gained considerable interest (Qureshi *et al*. 2010a,b). In this regard, several investigators have studied the raw material issue and tested various renewable feedstocks for the production of ABE (Jang *et al*. 2012).

Mango is processed to a maximum extent, thereby producing a high quantity of solid and liquid wastes: approximately 30% to 50% of the total fruit as waste, of which 15% to 25% is peel waste and 15% to 25% is kernel (Madhukara *et al*. 1993; Maini and Sethi 2000). Utilization of this mango waste is both a necessity and a challenge. If a factory processes five tons of *Totapuri* mangoes per hour, approximately six tons of peel would be available as waste per day, after eight hours of work. Approximately 0.6 to 0.8

million tons of mango peel is generated annually in India. This waste is either used as cattle feed or dumped in open areas, where it adds to environmental pollution. The use of mango peels as a source of pectin and fiber production has been investigated (Ajila *et al.* 2007). Grohmann *et al.* (1996) have reported the ability to create ethanol from orange peels. The suitability of mango peels for biogas and bioethanol production has been investigated (Madhukara *et al.* 1993; Reddy *et al.* 2011). However, reports on the use of mango peels for the production of biobutanol through fermentation processes are scant. The presence of a high amount of reducing sugars in fresh mango peel has prompted us to make an attempt to utilize it as a raw material for use in butanol production, as well as focus on the development of a cheap, mango peel-based medium. To the best of our knowledge this is the first report of its kind regarding ABE production from mango peels.

EXPERIMENTAL

Materials

Mango peel waste was procured from the local mango pulp industry (Vinsari fruit pulp Industries Ltd) Renigunta, Tirupati, Andhra Pradesh, India. *Clostridium acetobutalicum* NCIM 2878 was procured from the National Center for Industrial Microorganisms (NCIM), Pune, India. The enzymes cellulase, amylase, and pectinase were procured from Triton chemicals, in Mysore, India. All the chemicals used in medium preparation were produced in Himedia, India. The analytical chemicals used in the analysis were procured from Merck, India.

Methods

Mango peel hydrolysis

The fresh mango peel was dried, 100 g of the dried mango peel was macerated with a mixer (Panasonic Model: 340), and this mix was subjected to enzymatic digestion at 1% (w/v) for better juice and sugar extraction. The enzyme mix contains pectinase (300 IU/mL), cellulose (150 PFU/mL), and amylase (1000 IU/mL) in 6:3:1 ratio, respectively. The enzyme digestion was conducted at 42 ± 2 °C on rotary shaker at 150 rpm with initial pH 4.5 ± 0.5 for overnight. The juice containing sugar was extracted, with the help of cheesecloth, using the squeezing method.

Inoculum preparation and fermentation

The inoculum was prepared following a previously reported protocol (Qureshi *et al.* 2010a). Fermentation was conducted in 125-mL bottles, containing 50 mL of production medium, using mango peel extract (MPE) as a substrate. The headspace was purged with N_2, and $Na_2S \cdot 9H_2O$ (0.02% (w/v)) was added to remove traces of dissolved oxygen. Nutrient supplementation experiments were conducted with supplementations of 0.3% (v/v) yeast extract, peptone, and beef extract, individually. The sterile medium was inoculated with 3% (v/v) seed culture and incubated at 37 °C for 1 week (7 days) with intermittent gentle shaking. For bioreactor studies, a Biotron fermenter (Spectrochem Instruments Pvt Ltd, Hyderabad, India) 3-L volume fermenter with pH, temperature, and DO_2 sensors was used with 1.5 L of MPE medium. All experiments were conducted in an anaerobic chamber (Thermo Anaerobic work station Model 1029) under a N_2 atmosphere.

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Analytical methods

The composition of fresh and dry mango peel was carried out according to AACC methods (2000). Cell growth was determined by measuring the optical density at 660 nm, using a spectrophotometer (Shimadzu, Japan). The amount of reducing sugar was estimated by the dinitrosalicylic acid (DNS) method (Miller 1972). During the fermentation period (168 h), a 1.5-mL sample was taken every 24 h using a syringe, and the resulting sample was centrifuged at 8000 rpm, 4 °C for 25 min. The supernatant was analyzed for butanol by gas chromatography (Hewlett Packard) using a glass column (HP-INNOWax Polyethylene Glycol) and a flame ionization detector, with helium as the carrier gas. The temperature of the detector and injector were maintained at 270 and 230 °C, respectively.

Statistical analysis

All data are the average values of triplicates and standard deviations from three independent experiments, unless otherwise stated.

RESULTS AND DISCUSSION

The enzymatic digestion of mango peel powder resulted in the release of 30 ± 1.8% (w/w) reducing sugars, and the composition of dried mango peel was as shown in Table 1. The sugars released from the MPE are comparable to citrus waste extract (Pourbafrani *et al*. 2010). In the present investigation, the pH and sugar concentration of the MPE was adjusted to 6.5 and 6.0% (w/v), respectively, which are the optimum values for a good yield of ABE solvents. The changes in the growth kinetics and pH level during fermentation are depicted in Fig. 1.

Table 1. Composition of Fresh and Dried Mango Peel

Contents	Fresh Mango peel (%)	Dried Mango peel (%)
Moisture	70.5 ± 5	10 ± 1.2
Total solids	29.6 ± 1.2	73.5 ± 2.7
Reducing sugars	7 ± 1.8	30 ± 2.5
Pectin	3.5 ± 0.5*	4 ± 0.8
Cellulose and Lignin	23.2 ± 2.0*	25 ± 1.2
Hemicellulose	7.4 ± 0.5*	8.1 ± 0.3

* % on dry weight basis

After inoculation, the *clostridial* cells entered into the lag phase for a while to help in adapting to the new environment with the mango peel extract. Then, it was observed that the cells entered into the log phase and exhibited rapid proliferation for about another 15 to 30 h. During this growth phase, the production of acids was initiated, and the production was observed until the middle of the stationary phase (acidogenic phase). This will confirm with change in pH in the initial stage of fermentation. The

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decrease and increase in the pH level during the exponential and stationary phase resulted in a "V-shaped" graph (Fig. 1). Buildup of these acids causes the inhibition of cell division, as well as the commencement of solvent production and cell differentiation (Parekh *et al.* 1998; Ezeji and Blaschek 2008; Ranjan *et al*. 2013).
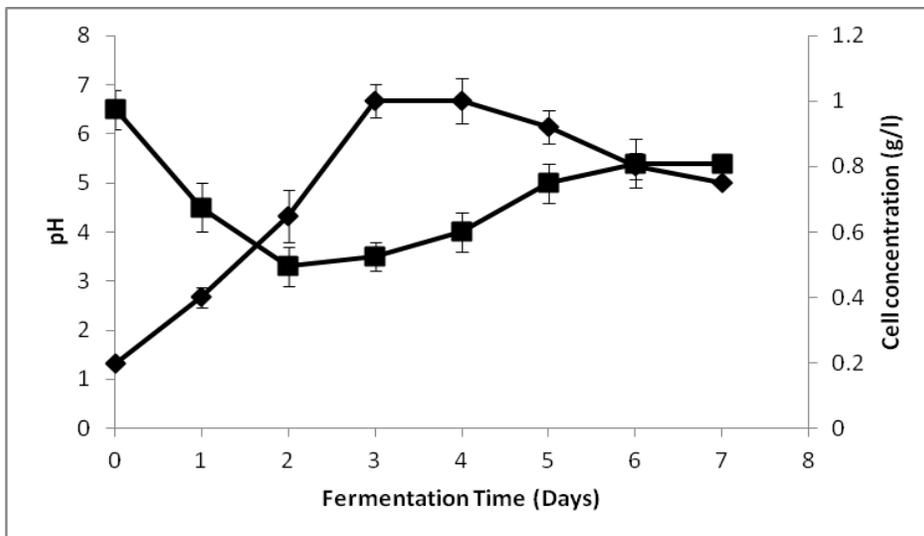

Fig. 1. Growth profile of *C. acetobutylicum* NCIM 2878 during the fermentation of mango peel extract

The sugar utilization profile of *C. acetobutylicum* NCIM 2878 during MPE fermentation is shown in Fig. 2. The utilization of sugars became rapid after the lag phase and remained constant. Furthermore, an insignificant drop in sugar utilization was observed after 120 h of inoculation. Mango peel extract fermentation yielded 10.5 ± 0.4 g/L of butanol. At the end of seven days of fermentation, 18 g/L of reducing sugars remained as residual sugars (Fig. 2).
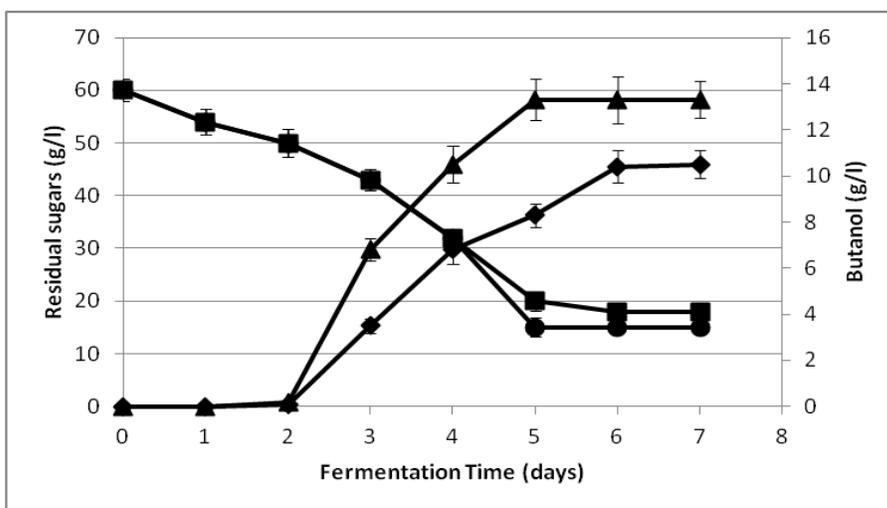

Fig. 2. Profile of Butanol production and sugar utilization pattern of mango peel extract fermentation by *C. acetobutylicum* NCIM 2878 with (▲butanol and ● residual sugar) and without (♦ butanol and ■ residual sugar) nutrient-supplementation

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Butanol production was observed on the 3rd day of fermentation, ranging from the initial 1.8 g/L to the final production level of 10.5 ± 0.4 g/L. At the end of the fermentation, a good amount of sugars were left unutilized. During the fermentation process, microorganisms have a utilization rate of 62%, and more amounts of sugar are unutilized as well (around 20 g/L). The results are comparable with previous investigations that used different substrates for direct fermentation (Qureshi *et al*. 2010a; Ranjan *et al*. 2013).

The incomplete utilization of reducing sugars, in the case of direct fermentation, could be attributed to the insufficient availability of nutrients (nitrogen and other micro nutrient source), as well as the presence of functional compounds (4066 mg GAE equivalent polyphenols/grm dry weight) in MPE that inhibit microbial growth (Masibo and He 2008). To confirm the effect of nutrients on solvent production, this research supplemented the MPE medium with three different nutrients (yeast extract, peptone, and beef extract). The nutrient supplementation experiments confirmed their potential role in solvent production. For the three nutrients tested, the beef extract supplementation produced more solvents (data not presented). Hence the beef extract (BE) was selected for further experiments. The supplementation of beef extract (0.3%) enhanced the butanol production from 10.5±0.4 g/L to 13.3 g/L, and total solvent production also rose from 15.13±0.4 g/L to 18.14±1.0 g/L (data not presented). It was observed that the supplemented medium significantly decreased the total fermentation time (7 days to 5 days). Figure 2 displays the butanol production kinetics of *C. acetobutylicum* NCIM 2878 during MPE fermentation over the course of five days. Nutrient-supplemented media utilized maximum sugars during the conversion, whereas unsupplemented MPE did not.

Using MPE, the butanol production process was validated by conducting fermentation using lab scale (3-L) fermenter with optimized conditions. It was observed that fermenter studies, with optimized conditions and nutrient supplementation (BE), further improved the butanol production, from 13.3 g/L to 15.42±1.2 g/L. Additionally, the total solvent production increased from 16.13±0.4 g/L to 20.40±1.3 g/L. It was also observed that the butanol portion was increased when compared with the flask culture (Fig. 3).
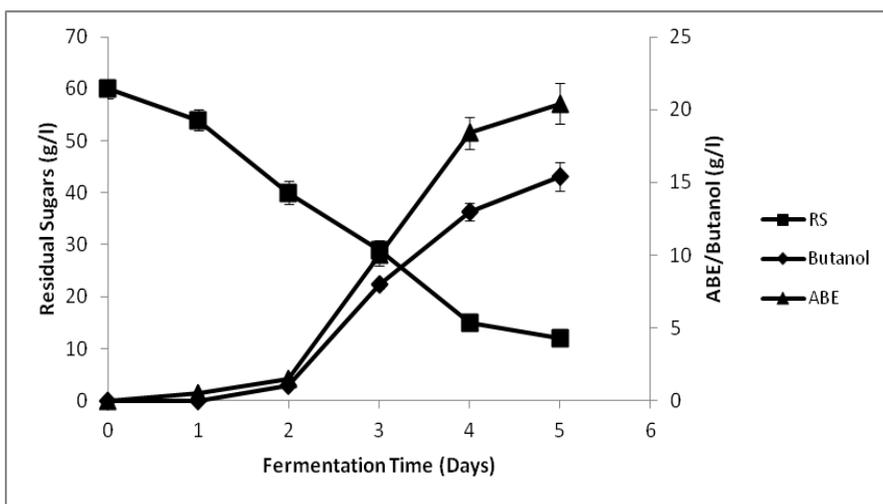

Fig. 3. Profile of ABE (acetone, butanol, and ethanol) production and sugar utilization pattern of *C. acetobutylicum* NCIM 2878 during the bioreactor fermentation

The results shown in Fig. 3 are in accordance with butanol production from barley and rice straws (Qureshi *et al*. 2010a; Ranjan *et al*. 2013). The present study suggests that solvent production could be increased through medium optimization and the use of optimal fermentation conditions. However, further process improvement is required to enhance the overall butanol yield.

CONCLUSIONS

1. From the results, it can be concluded that the enzymatic digestion of mango peel mix released a suitable amount of reducing sugars. Direct fermentation of mango peel extract resulted in low butanol production. However, the supplementation and optimized bioreactor studies produced very good amount of butanol.

2. The beef extract was studied showed the highest yield of butanol/ABE in comparison to other two nutrients tested. Hence, its supplementation produced, a good amount (20.40 g/L) of ABE. This high production was also because of the solvent tolerance of the microorganism, *C. acetobutylicum* NCIM 2878.

3. These results clearly demonstrate the suitability of mango peel extract as an economically feasible alternate substrate for use in biofuel production. The abundant availability of the substrate will enable it to function as a potential feedstock during the fermentation processes for butanol production.

ACKNOWLEDGMENTS

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