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Full Length Research Paper

Production of ethanol from mango (*Mangifera indica* L.) peel by *Saccharomyces cerevisiae* CFTRI101

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Mango fruit processing industries generate two types of waste, including solid waste (peel and stones) and liquid waste (juice and wash water). Utilization of this waste is both a necessity and challenge. This work was aimed to investigate the suitability of dried mango peel for ethanol production. The mango peel contained good amount of reducing sugars up to 40% (w/v). Direct fermentation of mango peel extract gave only 5.13% (w/v) of ethanol. The rate of the fermentation was very slow. Nutrients such as yeast extract, peptone and wheat bran extract were tested for the supplementation of mango peel medium and it was observed that the nutrient supplementation increased the ethanol production significantly up to 7.14% (w/v). The suitability of wheat bran extract (WBE) based medium, which is cheap and abundantly available for mango peel fermentation was also discussed.

Key words: Mango peel, ethanol fermentation, nutrient supplementation, wheat bran extract.

INTRODUCTION

The excessive consumption of fossil fuels, particularly in large urban areas, has greatly contributed to generation of high levels of pollution. There is a need for environmentally sustainable energy sources to find a viable and long-term substitute for liquid petroleum. As a step to solve this problem, the use or addition of biofuels to gasoline, which reduces emission of carbon monoxide and unburned hydrocarbons that form smog, has widely been enforced in recent years (Wyman, 1994). In this regard, India reforms are taken by blending 10 to 15% ethanol in its gasoline usages. Converting a renewable non-fossil carbon, such as organic wastes and biomass consisting of all growing organic matter (plants, grasses, fruit wastes and algae) to fuel would assure a continual energy supply (Wyman, 1996).

The economics of ethanol production by fermentation are significantly influenced by the cost of the raw materials, which accounts for more than half of the production costs (Classen et al., 1999). To achieve a lower production cost, the supply of cheap raw material is thus a necessity. Production of value added products from agro-industrial and food processing wastes is now a focusing area, as it reduces pollution in the environment in addition to energy generation. The annual availability of these wastes amounts to 1.05 billion tons (Anonymous, 2004). The major part of this is mostly discarded and it is the main source for increasing the pollution in environment on occasions and also, the discarding process become a very expensive step due to high transportation costs. Majority of fruit and vegetable wastes available from their processing industries are seasonal and they do not decompose rapidly. The mechanical drying of these wastes (mango peel, citrus peel, pineapple peel and tomato processing wastes) gave opportunity to store the substrate all over the year. The yeast *Saccharomyces cerevisiae* and facultative bacterium *Zymomonas mobilis* are better candidates for industrial alcohol production. *Z. mobilis* possesses advantages over *S. cerevisiae* with

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respect to enthanol productivity and tolerance. However, ethanol is produced commercially by yeast because it ferments glucose to ethanol as a virtually sole product and it is known for its high ethanol tolerance, rapid fermentation rates and insensitivity to temperature and substrate concentration (Linden and Hahn-Hägerdal, 1989).

Mango is processed to a maximum extent, thereby producing high quality of solid and liquid wastes. Solid wastes, stones, stalks, trimmings and fibrous materials are obtained during the preparation of raw material. This contributes about 40 to 50% of total fruit waste out of which, 5 to 10% is pulp waste and 15 to 20% is kernel (Anonymous, 2004; Madhukara et al., 1993; Maini et al., 2000; Pandey et al., 2000). Liquid waste is the waste material that comes out of a factory after washing of fruits, packaging, blanching, cooling and plant and machinery clean up and so on. Utilization of this mango waste is both a necessity and a challenge. If a factory is processing five tons of *Totapuri* mangoes per hour, about six tons of peel would be available as waste per day of 8 h work. Approximately, 0.4 to 0.6 million tons of mango peel is generated annually in India (Anonymous, 2004). This waste is either used as cattle feed or dumped in open areas, where it adds to environmental pollution. The use of mango peel as a source of pectin and fibre production also has been reported (Pandia et al., 2004). Grohmann et al. (1994; 1995; 1996; 1998) previously reported ethanol production from orange peel. Ethanol production from banana (Manikandan et al., 2008) and pineapple peels (Ban-koffi and Han, 1990) were also investigated. Mango peel is difficult to decompose, as it takes a very long time, because of its complex composition. Suitability of mango peel for biogas production was investigated by Madhukara et al. (1993). However, ethanol fermentation from fruit and vegetable wastes like mango peel appears to give better returns. The presence of high amount of reducing sugars in dried and fresh mango peel prompted us to make an attempt to utilize it as a raw material for ethanol production and development of cheap medium. As far as we know, this is the first report of its kind on ethanol production from mango peel.

MATERIALS AND METHODS

Strain and medium

Non-amylolytic and ethanol-producing yeast strain *S. cerevisiae* CFTRI 101 was used throughout the experiments; was obtained from CFTRI, Mysore, India. The culture was maintained on MPYD (malt extract 0.3%, peptone 0.5%, yeast extract 0.3% and dextrose 2%) agar (1.5%) slants at 4°C. The inoculum was prepared by inoculating the slant culture into 25 ml of the sterile MPYD liquid medium in 100 ml conical flask and growing it on a rotary shaker (100 rpm) for 48 h. 10% (v/v) inoculum (3×10^4 cells ml^{-1}) was inoculated into 100 ml sterile mango peel extract broth in 250 ml conical flask and was incubated up to 5 days under stationary conditions. All the stated experiments were conducted at pH 5.0 and 30°C.

Mango peel

Mango peel was procured from local mango pulp industry (Vinsari Fruit Pulp Industries Ltd., Renigunta, Tirupati, India). It was dried and milled to a particle size of 40 BS (British Standard) mesh in an apex mill.

Extraction of sugars from mango peel

Mango powder (100 g) was mixed with water (1:3) and left overnight. The liquid containing sugars was extracted with the help of cheesecloth by squeezing. This acted as control. In the case of enzymatic digestion with 1% (v/v) pectinase, Trizyme 50 (Triton Chemicals, Mysore, India) was used for improved results. The extraction medium pH was 5.0 and the temperature was 37°C. The extract was suitably diluted to obtain the desired concentration of sugars (15-17%, w/v), and was supplemented with various nutrients (yeast extract 1%, peptone 1.5%, ammonium phosphate 2% and wheat bran 3%) in order to study the effect of nutrients on fermentation. The unsupplemented medium acted as the control. In the case of wheat bran extract supplementation experiments, mango peel sugars were extracted into the wheat bran extract solution instead of water.

Preparations of wheat bran extract (WBE)

Wheat bran obtained from the local market was used in preparation of the wheat bran extract. Wheat bran (30 g) was boiled with 500 ml of water for 10 min. After cooling, it was filtered and equal volume (500 ml) of the extract was collected by washing the residue and made to 1 L (Shamala and Sreekantiah, 1988).

Analytical methods

Mango peel analysis for the determination of moisture, non-reducing sugars, protein, total soluble solids, cellulose and lignin was carried out according to the methods of Ranganna (1986). Reducing sugar concentration was estimated by Shaffer and Somogyi (1933) method. Ethanol and other metabolites were determined by gas chromatography coupled with flame ionization detector (Antony, 1984). Final cell biomass was estimated by weighing the dried yeast cells after fermentation. All data are shown as the average values and standard deviations from three independent experiments, unless otherwise stated.

RESULTS

Extraction of sugars from dried mango peel

From the aqueous extraction, low amount of sugars were obtained; only 20% (w/v). Mango peel treated with crude pectinase yielded higher levels of solubilisation and reducing sugars (30 ± 5%, w/v) (Tables 1 and 2). The optimum incubation period for solubilisation of the maximum

Table 1. Composition of fresh and dried mango peel used in this study.

Content	Fresh mango peel	Dried mango peel
Moisture	70 ± 5	10 ± 1.2
Total solids	25.6 ± 4.6	70.5 ± 2.7
Reducing sugars	7.0 ± 1.8	30 ± 2.5
Non-reducing sugars	5.9 ± 0.4	4.3 ± 0.5
Protein	3.5 ± 0.5	4.0 ± 0.8
Cellulose and lignin	25.2 ± 2.0	23 ± 1.2

Table 2. Effect of pectinase enzyme (1%, v/v) on sugar extraction from dried mango peel.

Time (h)	Reducing sugar (%, w/v)	
	Pectinase non-treated	Pectinase treated
5	5.8 ± 0.5	10 ± 0.63
10	10 ± 0.65	15.6 ± 2.3
15	15.5 ± 1.5	21 ± 1.6
20	18 ± 1.3	24.3 ± 1.3
25	20 ± 1.5	30 ± 5.5

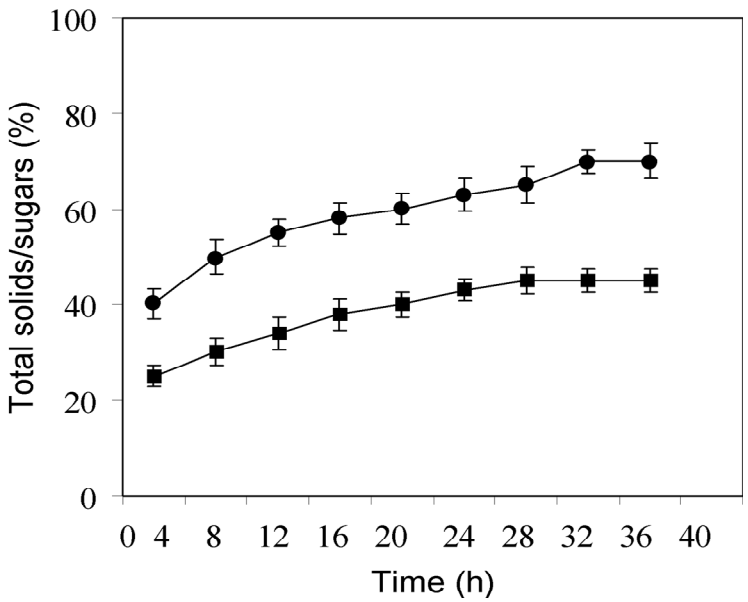

Figure 1. Total percentage of water soluble solids and sugars in the dried mango peel extract after pectinase (1%, v/v) treatment. –●–, total water soluble solids; –■–, soluble sugars.

sugars was found to be 24 h (Figure 1). The results also indicated a relatively low inhibition of hydrolytic enzymes (amylases and cellulases) by the sugars released from the mango peel. Another significant observation made during this study was decrease in the initial pH from 5 to 4.5 at the end.

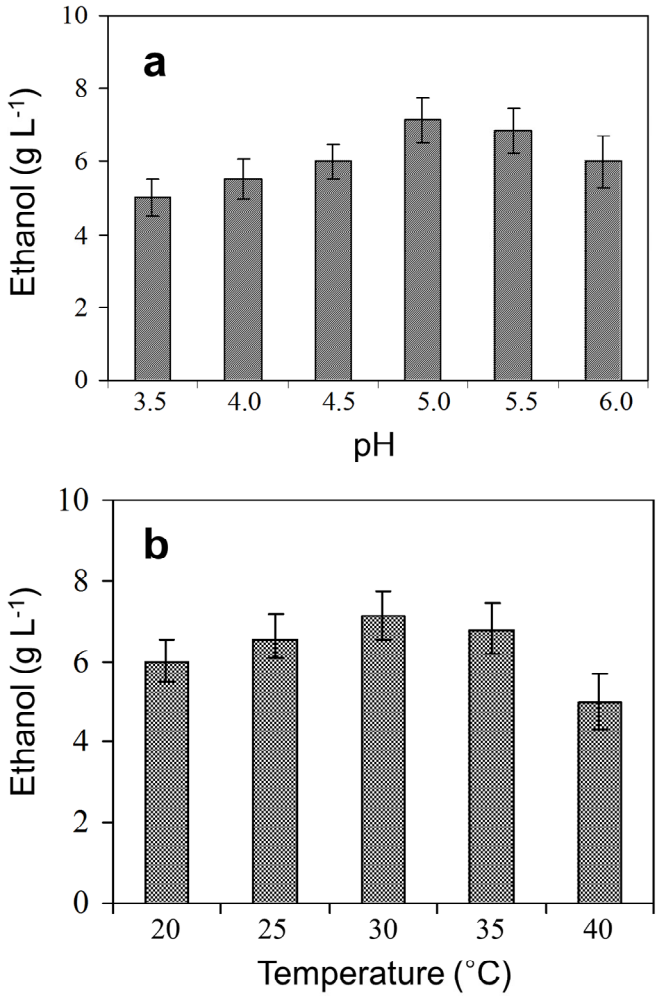

Figure 2. Effect of (a) pH and (b) temperature on ethanol fermentation from the dried mango peel extract.

Effect of pH, temperature and nutrients on the dried mango peel fermentation

The levels of reducing sugars were adjusted to 15% (w/v) with the dilution and the required nutrients were supplemented for fermentation. The direct fermentation of mango peel extract gave 5.14% (w/v) ethanol. The results of optimizing the culture conditions such as pH and temperature indicated that, the changes in pH and temperature could affect the final ethanol concentration. The final ethanol concentration at different pH and temperature experiments (Figure 2a, b) showed that 30°C and pH 4.5 were optimum for ethanol production from mango peel extract.

The supplementation of nutrients significantly increased the ethanol concentration and fermentation rate. Yeast extract alone and combination with peptone supplementation attributed to the ethanol formation very rapidly and formed 7.0 and 7.14% (w/v) ethanol, respectively, instead of 5.14% (w/v) from unsupplemented media at the end of the fermentation. The data on fermentation of mango peel extract with nutrient supplementation are presented in Table 3. The mango peel extract with wheat bran extract medium significantly increased the yeast growth and the ethanol formation when compared with the peel extract alone (Table 3). The wheat bran extract increased the ethanol concentration from 51.4 to 67.5 g l^{-1} (Figure 3). We also tried the fresh mango peel, which contained low levels of reducing sugars (7 to 10%, w/v) because of its high amount of water (90%, v/v). Experiments with the fresh mango peel extract without supplementation of nutrients was also done, but it gave low ethanol productivity (3%, w/v).

For cell viability when compared with the control medium,

Table 3. Periodical analysis of ethanol and final cell mass during fermentation of mango peel extract supplemented with various nutrients[a].

Supplement	24 h			48 h			72 h			
	Ethanol concentration (g L^{-1})	Theoretical ethanol yield (%)	Volumetric productivity (g L^{-1} h^{-1})	Ethanol concentration (g L^{-1})	Theoretical ethanol yield (%)	Volumetric productivity (g L^{-1} h^{-1})	Ethanol concentration (g L^{-1})	Theoretical ethanol yield (%)	Volumetric productivity (g L^{-1} h^{-1})	Biomass (g L^{-1})
Control	23	30.7	0.95	45.6	60.0	0.93	51.3	68.4	0.71	5.2
Peptone	30	40	1.25	55.4	73.3	1.15	68.8	91.7	0.95	6.3
Yeast extract	33.2	44.3	1.38	60.5	80.7	1.26	70.0	93.7	0.92	6.6
Ammonium phosphate	25.4	33.6	1.05	46.7	62.3	0.97	53.2	70.9	0.73	5.1
Peptone + yeast extract	34.5	46.0	1.43	62.2	82.9	1.29	71.4	95.2	0.99	6.8
Wheat bran extract	30.8	41.1	1.28	53.9	71.9	1.12	67.5	90	0.93	6.2

[a]The values presented in the table are mean values of three independent experiments.

the supplemented medium had high cell count at the end of the fermentation (data not shown). The stated results were supported by the increase of final biomass in the nutrient supplemented mango peel extract medium (Table 2).

DISCUSSION

The dried mango peel contained high amount of reducing sugars (up to 45%, w/v) and the results are in accordance with those of previous reports (Anonymous, 2004; Madhukara et al., 1993). In the case of aqueous extraction, the sugar content was very low. The reason for the low content could be due to the presence of pectin, which held the sugar molecules and could not be released with simple water extraction. The presence of other enzymes like amylase and cellulase in the crude pectinase, may aid to increase sugar concentration by hydrolyzing the respectable substances (Grohmann et al., 1995). The significant pH drop during the enzymatic hydrolysis of mango peel is undoubtedly caused by the release of D-galacturonic acid from pectin. The pK_a value of D-galacturonic acid is 3.51 (Filippov et al., 1978) and the pH values of peeled hydrolyzates appeared be to stabilized in the range of 3.3 and 3.5. The low yields of ethanol from dried mango peel increase the cost of production. Increase in the ethanol production up to 7 to 7.5% (w/v) as in general industrial output from molasses can economize the process. The development of cheap medium for fruit waste fermentation to ethanol also required low-cost ethanol production.

To improve the concentration of ethanol, the fermentation medium was supplemented with variety of nutrients like yeast extract, peptone and ammonium phosphate to overcome the nutritional deficiency. The result presented in Table 3 clearly indicates that, in the case of supplementation of nutrients, not only the rate of ethanol synthesis but also the final ethanol concentration increased significantly. The combination of yeast extract and peptone gave the maximum improvement in rate of ethanol synthesis as well as final concentration in the medium. The ethanol production as well as viable cell count was significantly increased up to 50 and 20%, respectively, in the mango peel extract with yeast extract and peptone supplementation. However, the ammonium phosphate supplemented one did not stimulate the ethanol production. Similar results were also obtained in the case of ethanol fermentation from orange peel by genetically modified *Escherichia coli*, which can utilize the glucose, galactose and galcturonic acid for ethanol product (Grohmann et al., 1994,

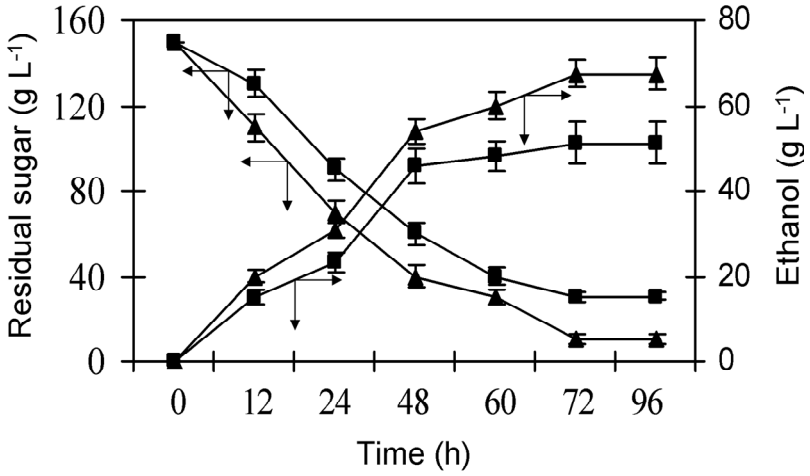

Figure 3. Effect of wheat bran extract on the conversion of mango peel to ethanol fermentation. –▲–, mango peel extract medium supplemented with wheat bran extract; –■–, control (unsupplemented medium).

1996). It is possible to produce high ethanol concentrations by extending exponential growth phase of yeast to longer periods and soluble sugar concen-tration as in the case of beer production (Kirsop, 1978; Casey and Magnus, 1984). It is expected that the nutrient supplementation would overcome nutritional deficiencies of yeast and allow them to stay longer in growth phase. In order to verify whether the amount of nutrients and their mode of feeding influenced the alcoholic fermentation by *S. cerevisiae,* experiments were conducted in batch fermentation with various amounts of nutrients and different feeding strategies.

Compared with initial total supplementation, exponential feeding strategy improved the performance of the fermentation process and the ethanol tolerance of the yeast. In a recent study reported by Reddy and Reddy (2005), the nutrients and polyphenols rich horse gram flour improved the ethanol formation in very high gravity fermentation. The suitability of wheat bran extract as a medium for ethanol production from mango peel investigations successfully replaced the costly medium components and developed a novel wheat bran extract (WBE) medium which could provide a cheap source of amino acids and other nutrients (Shamala and Sreekantiah, 1988). Reddy and Basappa (1996) also successfully replaced the nutrients with wheat bran extract in the direct fermentation of starch to ethanol by *Endomycopsis fibuligera* and *Z. mobilis.* However, supplementation with yeast extract and peptone was superior to that of wheat bran extract. This showed that wheat bran extract has limited amount of nutrients when compared with yeast extract or peptone. Further optimization studies on the supplementation of WBE are to be made to make the process economically viable. The initial fermentability of the unsupplemented peel extracts by *S. cerevisiae* was extremely poor because of insufficient growth nutrients in the peel medium. The fermentation of mango peel extract was stimulated by supplementation with low amounts of yeast extract and peptone. The wheat bran extract though stimulated not only the rate of fermentation and also the final concentration of ethanol, but it was not as good as the yeast extract and peptone. However, higher concentrations of WBE is needed to be supplemented to make the mango peel extract fermentation process more economical since the supplementation with yeast extract and peptone is obviously more expensive. Given the promise of the proposed WBE based medium for ethanol fermentation, it should be tested beyond the bench scale. Generally, the production concentration in commercial ethanol production plants is between 7.5 and 10% (w/v). Based on previous reports, the ethanol production concentration was 4.02% (w/v) in citrus peel waste, 3.5% (w/v) in grape fruit peel and 4.2% (w/v) in pineapple peel (Ban-koffi and Han, 1990; Nishio et al., 1980; Wilkins et al., 2007a, b).

In this study, mango peel was proved as one of the novel and potential raw material for ethanol production. Ethanol production from mango peel requires supplementation of nutrients because of its low nutrient availability. Supplementation of yeast extract, peptone and optimization of fermentation conditions enhanced the fermentation rate and final ethanol concentration. WBE supplementation showed comparable improvement in all terms with the very expensive, yeast extract and peptone. Further optimization studies on peel hydrolysis using commercial enzymes and optimization of WBE supple-

mentation will make the process economically viable and it should be tested beyond the bench scale.

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