Bioethanol Production from Apple Pomace left after Juice Extraction

D Chatanta, C Attri, K Gopal, M Devi, G Gupta, T Bhalla

Citation

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Abstract

The production of ethanol from low cost lignocellulosic materials such as crop waste and horticulture waste has considerable promise as a future source of liquid transport fuel. In the present study the pomace left after juice extraction was collected from HPMC, Parwanoo, H.P and used as a substrate for bioethanol production. Different microbial strains viz. Saccharomyces cerevisiae MTCC 173 (ethanol production), Aspergillus foetidus MTCC 151 (pectinase) and Fusarium oxysporum MTCC 1755 (cellulase) were used individually as well as in consortia for ethanol production from apple pomace in solid state fermentation (SSF) systems. With S. cerevisiae MTCC 173 (1% inoculum) 8.44% (v/w) ethanol was recovered after 72h of incubation at 30°C with Bucchi rotary vacuum evaporator and sugar concentration decreased to 0.25% and on the other hand, with co-cultures i.e. S. cerevisiae MTCC 173, A. Foetidus MTCC 151, F. oxysporum MTCC 1755 the ethanol increased to 16.09% (v/w) and sugar concentration further decreased to 0.15% after 72 h incubation at 30°C.

INTRODUCTION

Bioethanol production from sugarcane was started in Brazil and the United States in the early 1970 (Classen et al. 1999). Feasibility of lignocellulosic materials for ethanol production has been explored around the world depending upon availability (Shindo and Tachibana 2006). At present, the fermentation of sugars to ethanol is the best established process for conversion of biomass to energy (Classen et al. 1999). Bioethanol is currently commercially produced from raw materials such as sugar cane, sugar beet or starch from cereals (Gable et al. 2005). The polysaccharides, cellulose and hemicellulose, are degraded slowly by enzymes as their structure is compact and stringent (Chandrakant and Bisaria, 1998). The cellulose cannot be enzymatically hydrolysed to glucose without physical and chemical pretreatment. High concentration of cellobiose and glucose inhibits the activity of cellulase enzymes and reduces the efficiency of the saccharification. One of the methods used to decrease this inhibition is to ferment the reduced sugars along their release (Gnansounou and Dauriat 2005). Simultaneous saccharification and fermentation (SSF) involves the enzymatic hydrolysis of cellulose to glucose and the conversion of fermentable sugars to ethanol in the same vessel (Eklund and Zacchi 1995).

SSF of lignocellulosics leading to the production of biofuels, animal feed, human food and chemicals is economical and should be practiced in developing countries (Sharma et al. 2006). Apple pomace is the residue left after juice extraction and constitutes about 25-35% of the weight of fresh fruit (Smock and Neubert 1956). It contains a large amount of water and sugar, a small amount of protein, and has a low pH. More than 500 food processing plants in the United States produce a total of about 1.3 million metric tons of apple pomace per years. Two main biologiocal fermentation processes could be applied to apple pomace for energy recovery: ethanol alcohol production and biogas generated via anaerobic digestion. Hang et al (1982) have documented the potential for ethanol production from fresh wet pomace and this represents a 20% of energy recovery from the total energy in pomace (Jewell and Cummings 1984). In the present study waste apple pomace left after juice extraction which is generally dumped is used as a substrate for ethanol production in solid state fermentation (SSF) system.

MATERIALS AND METHODS

SAMPLE AND MICROORGANISM

Waste apple pomace was obtained from fruit processing unit of HPMC at Parwanoo (Himachal Pradesh), India. S. cerevisiae MTCC 173 (ethanol production), A. foetidus MTCC 117 (pectinase producer) and F. oxysporum MTCC 1755 (cellulose producer) were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute

of Microbial Technology, Chandigarh, India and maintained as per the supplier instructions at 30oC.

ETHANOL PRODUCTION

Ethanol production was carried out under solid state fermentation system (SSF). SSF of apple pomace was carried out at different temperature (25 oC, 30 oC, 40oC) and compared with fermentation in synthetic medium (yeast extract 3g, peptone 10 g, dextrose 20 g per litre, pH 6.5). For all experiments 100 g of apple pomace (67% moisture) was taken in 1000 ml round bottom flasks and to this 1% (v/w) of inoculum was added and allowed to ferment at 30oC for 48h. Apple pomace was fermented under similar conditions for ethanol production with three combinations of microorganisms viz., i) S. cerevisae ii) A. foetidus + F. oxysporum and iii) S. cerevisae+ A. foetidus + F. oxysporum at 30oC up to 96 h.

RECOVERY AND ETHANOL AND ANALYSIS

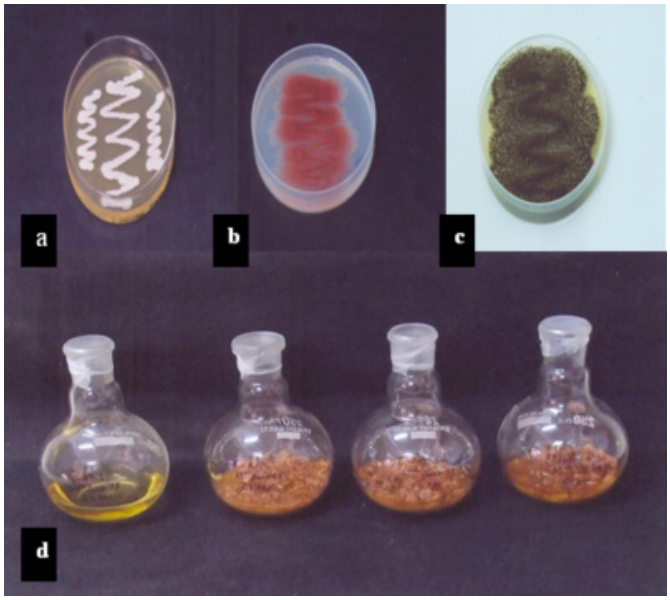
Ethanol produced was recovered using Buchi rotary vaccum evaporator at 78 oC. The distillate collected was analyzed for ethanol and residual sugar. Ethanol was estimated using Caputi et al (1968) method. To 10 µl of distillate add 25 ml of potassium dichromate (325 ml of conc. H_2SO_4 + 34 g potassium dichromate final volume made to 1 litre with distilled water) solution and final volume made to 50 ml with distilled water. All flasks incubated at 60oC for 20 min and absorbance read at 660 nm. Residual sugar was determined with DNS method (Miller 1959) using glucose (10-100 µg/ml) as a standard.

RESULTS AND DISCUSSION

Waste apple pomace obtained from the fruit processing unit of HPMC contained 76% moisture and the same is maintained in the pomace before its fermentation. S. cerevisiae MTCC 173 (ethanol production), A. foetidus MTCC 117 (pectinase producer) and F. oxysporum MTCC 1755 (cellulose producer) were obtained from MTCC, Institute of Microbial Technology, Chandigarh, India and were maintained respectively on YEPD, Czapek yeast extract and Potato dextrose agar at pH 6.5 and 30oC (Fig. 1).

Figure 1

Figure 1: Plate culture of microorganisms used for solid state fermentation (SSF) of waste apple pomace a) b) c) and d) fermentation in synthetic medium and with apple pomace.



Temperature had a profound influence on the rate of alcoholic fermentation of waste apple pomace. Ethanol production was carried out at different temperature to verify the best temperature for ethanol production with waste apple pomace (Fig. 2). The result showed 30°C as best temperature for ethanol production as compared with 25°C and 40°C. Similar results were observed earlier by Hang et al (1982) with apple pomace with out juice extraction.

Figure 2

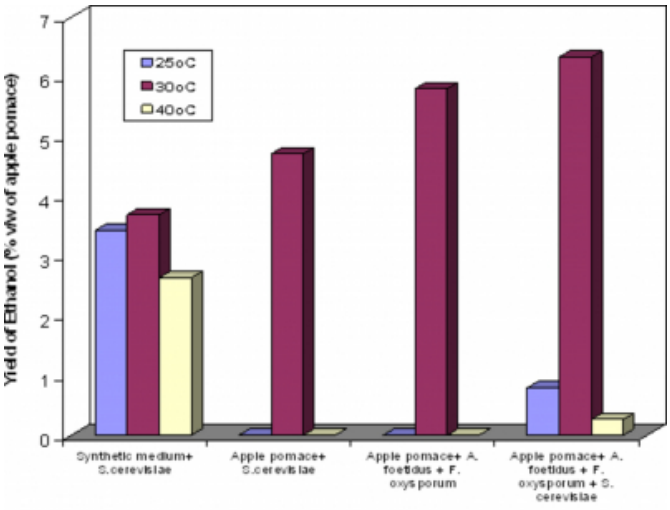


Figure 2. Effect of fermentation temperature on production of ethanol after 24 h in synthetic medium and with apple pomace in solid state fermentation (SSF) using different combinations of cultures.

Preliminary studies have revealed that the apple pomace

fermented by the yeast (S. cerevisiae) at 30°C for 4 days (Fig. 3) contained only a small amount of ethanol (3.43% v/w). It was thus necessary to inoculate the apple pomace with consortia of microorganisms to accelerate the fermentation process. After the inoculation of apple pomace with combination of A. foetidus and F. oxysporum (Fig. 4) and yeast (S. cerevisiae) plus A. foetidus and F. oxysporum (Fig. 5) the sugar present in the apple pomace is further released with simultaneous saccharification by A. foetidus (pectinase) and F. oxysporum (cellulase) and subsequent fermentation with S. cerevisiae and converted into ethanol. Simultaneous saccharification and fermentation involves the enzymatic hydrolysis of lignocellulose to glucose and the conversion of fermentable sugars to ethanol in the same vessel (Eklund and Zacchi, 1995).

Figure 3

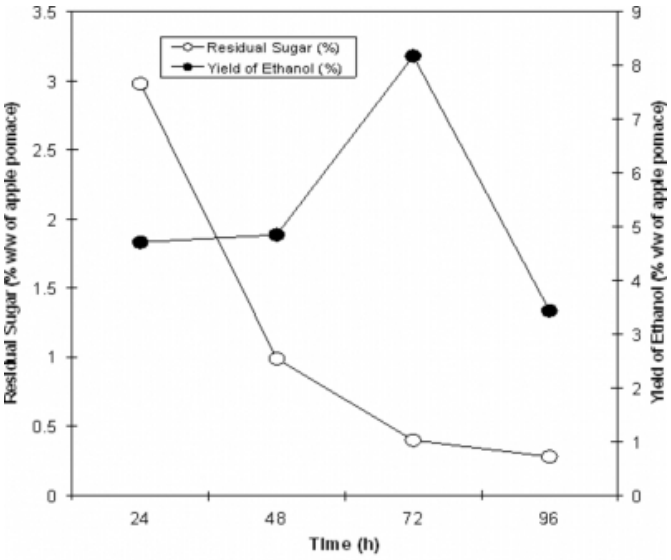


Figure 3. Time course of ethanol production from apple pomace + *S. cerevisiae* at 30°C with Intitial residual sugar concentration of 3.21%(w/w of apple pomace).

Figure 4

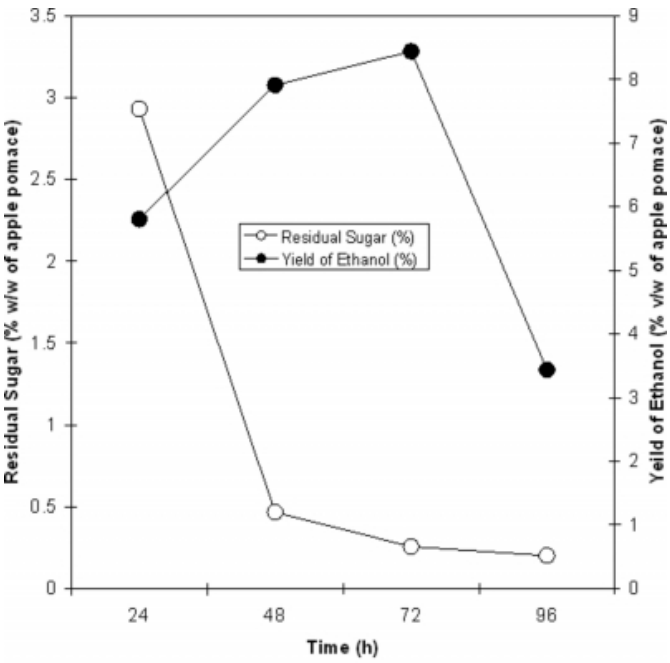


Figure 4. Time course of ethanol production from apple pomace with *A. foetidus + F. oxysporum* at 30°C with Intitial residual sugar concentration of 3.21%(w/w of apple pomace).

Figure 5

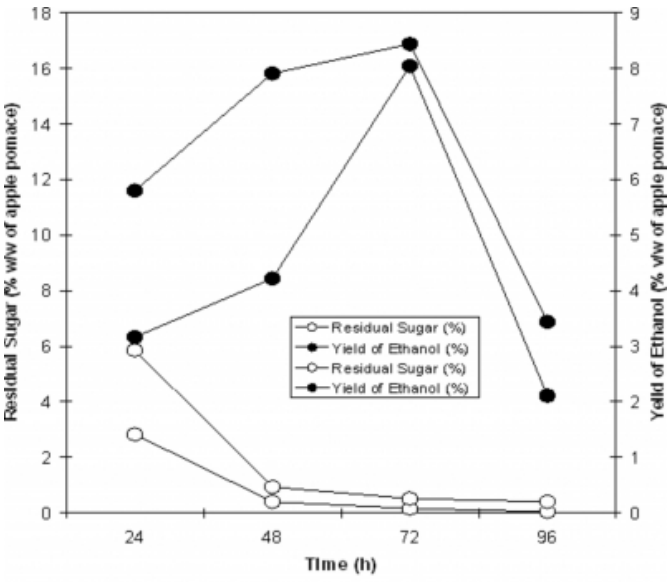


Figure 5. Time course of ethanol production from apple pomace with *A. foetidus + F. oxysporum* at 30°C with Intitial residual sugar concentration of 3.21% (w/w of apple pomace).

The initial sugar concentration present in waste pomace after juice extraction was 3.21 % (w/w). During the fermentation process the sugar level decreased at different time intervals up to 96 h however the maximum ethanol production was observed at 72 h after this there was a decrease in ethanol production at 96 h of incubation in all combinations (Fig.

3-5). This could be due to inhibition of enzyme activity by high concentrations of cellobiose and glucose during saccharification. One of the method used to decrease this inhibition is to ferment the reduced sugar along their release (Gnansounou and Dauriat 2005).The amount of ethanol produced varied apparently dependent upon the initial amount of sugar concentration of the apple pomace fermented.

CONCLUSION

A solid state fermentation process has been reported for the production of ethanol from apple pomace using consortia of cultures viz., S. cerevisiae, A. foetidus and F. oxysporum. This process yielded as high as 16.09 % (v/w of apple pomace) ethanol from fermented apple pomace with a residual sugar of 0.15 % (w/w of apple pomace). The present study indicates that the alcoholic fermentation of apple pomace might be an efficient method for alleviating waste disposal with the concomitant production of ethanol. The economical potential of this solid state fermentation process remain to be assessed.

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Author Information

Dinesh Kumar Chatanta, Ph.D.
Department of Biotechnology, Shoolini Institute of Life Sciences and Business Management (SILB)

Chandrika Attri, M.Sc.
Department of Biotechnology, Shoolini Institute of Life Sciences and Business Management (SILB)

Krishan Gopal, M.Sc.
Department of Biotechnology, Shoolini Institute of Life Sciences and Business Management (SILB)

Mamta Devi, M.Sc.
Department of Biotechnology, Shoolini Institute of Life Sciences and Business Management (SILB)

Gauri Gupta, M.Sc.
Department of Biotechnology, Shoolini Institute of Life Sciences and Business Management (SILB)

Tek Chand Bhalla, Ph.D.
Department of Biotechnology, Himachal Pradesh University