academicJournals

Vol. 8(16), pp. 1672-1675, 16 April, 2014
DOI: 10.5897/AJMR2013.6554
Article Number: 03269C744041
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Bioprocessing of sugarcane factory waste to production of Itaconic acid

R. Paranthaman*, S. Kumaravel and K. Singaravadivel

Department of Food Safety and Quality Testing, Indian Institute of Crop Processing Technology, Ministry of food processing Industries, Thanjavur- 613 005, Tamil Nadu, India.

Received 9 December, 2013; Accepted 7 February, 2014

This paper aimed at advances in industrial biotechnology over potential opportunities for economic utilization of agro-industrial residues sugarcane bagasse. Sugarcane bagasse, which is a complex material, is the major by-product of the sugarcane industry. The present work has been taken up with a view of exploring the possibilities of using agriculture by products as a source for the production of itaconic acid from various fungi. *Aspergillus oryzae, Aspergillus niger, Aspergillus flavus and Penicillium* sp., were selected and optimized for itaconic acid production in solid state fermentation using cheaper raw material sugarcane baggasse powder. *A. niger* produced the highest itaconic acid level (8.241 ±1.5 mg/kg) in solid state fermentation at 35°C. Optimum fermentation medium and pH for itaconic acid production were found to be 3.5. This article shows the recent developments on processes and products developed for the value addition of sugarcane bagasse through the biotechnological means.

Key words: Sugarcane bagasse, itaconic acid, fermentation medium.

INTRODUCTION

In the developing and developed countries, year after year there has been an increasing use of organic acids particularly itaconic, gluconic, lactic, fumaric and kojic acids. Itaconic acid ($C_5H_6O_4$) is an unsaturated dicarboxylic acid, crystalline, relatively non-toxic with a melting point of 167 to 168 ºC and density of 1.632 (Milsom and Meers, 1985). The property that makes itaconic acid a uniquely valuable compound is the conjunction of the two carboxyl groups and the methylene group. The methylene group is able to take part in adding polymerization giving rise to polymers with many free carboxyl groups that confer advantageous properties (Mattey, 1992). Itaconic acid is known to be produced on a commercial scale using only *Aspergillus terreus* and *Aspergillus itaconicus* (Milsom and Meers, 1985). In the cane sugar industry, as in many other industries, control and disposal of wastes is of major concern. There are two important reasons for increased attention to these problems: First, the greatest possible recovery, use, and reduction of wastes is necessary for most economical production in small as well as in large plants. Second, protecting the Nation's limited water resources for maximum use is essential to our health and continued economic growth. Thus, wastes which cannot be

*Corresponding author. E-mail: paranthhu@gmail.com. Tel: 91-04362228155.

Table 1. Itaconic acid production by solid state fermentation.

Substrate	Itaconic acid (mg/Kg) of fermented substrate
Aspergillus niger MTCC 281	8.241 ± 1.5
Aspergillus flavus MTCC 277,	5.102 ± 2.0
Aspergillu oryzae MTCC 634	2.307 ± 2.0
Penicillium Chrysogenum MTCC 6795	5.062 ± 2.0

eliminated must be disposed of in a manner which will not impair the usefulness of stream waters for other beneficial purposes. In the present study, attempts were made to develop a potent strain for improved production of itaconic acid. Alternatively, with a view to reducing the substrate costs, cheap and abundantly available substrates such as fruit wastes, which had not been reported earlier and corn starch were used.

MATERIALS AND METHODS

Microorganism and culture conditions

Aspergillus niger MTCC 281 strain *Aspergillus flavus* MTCC 277, *Aspergillu oryzae* MTCC 634 and *Penicillium chrysogenum* MTCC 6795 was used in this study (Yahiro et al., 1995; Dwiarti et al., 2002). The strain was maintained on potato dextrose agar (PDA) slants and sub cultured. The spore suspension was prepared by adding sterile distilled water containing 0.1% Tween 80 to a fully sporulated culture. The spores were dislodged using a sterile inoculation loop.

Production medium

Fifty gram (50 g) of sugarcane bagasse powder was mixed in 100 ml of distill water (Bressler and Braun, 2000), sodium nitrate 3.0 g, dipotassium hydrogen potassium phosphate 1 g, magnesium sulfate 0.8 g, ferrous sulfate 0.01 g, potassium chloride 0.5 g. The pH of the solution was adjusted to desired value using nitric acid at room temperature. This medium was sterilized in an autoclave at 121°C at 15 psi for 20 min (Xu et al., 1989; Nubel and Rakajak, 1964). For shake flask fermentation, a different microbial culture was inoculated to the production medium in a 500 ml shaking flask and cultured on a rotary shaker. The sample was collected at an interval of 24 h (Walinky, 1984). The collected sample was used for the determination of itaconic acid and glucose consumed was estimated. The qualitative analysis of itaconic acid was measured by ultraviolet-visible (UV) spectrophotometer at 210 nm (Kautola et al., 1985; Welter, 2000).

Effect of pH

The pH of the solution was adjusted to desired value by the addition of nitric acid and sodium hydroxide to pH values 2.0, 2.5, 3.0, 3.5 and 4.0.

Effect of temperature

The production media for itaconic acid was optimized at four different temperatures The temperatures are 25, 30, 35, 40 and 45ºC. Best yield of itaconic acid was studied in these five temperatures.

High-performance liquid chromatography (HPLC) analysis

Itaconic acid was analysed using a HPLC, Shimadzu Corp., Kyoto, Japan (Md. Rafi et al., 2012) consisting of a LC-10ATVp pump, SCL 10A system controller and a variable Shimadzu SPD-10 A Vp UV VIS detector and a loop injector with a loop size of 20 µl. The peak area was calculated with a CLASSVP software. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250 × 4.6 mm i.d., particle size 5 µm, Luna 5µ C-18; phenomenex, Torrance, CA, USA) at 25°C. The mobile solvent 60% Acetonitrile had a significant effect on the resolution of compounds. Detection wavelength was 280 nm. Itaconic acid was used as internal and external standards. Itaconic acid present in sample were identified by comparing chromatographic peaks with the retention time (Rt) of individual standards and further confirmed by co-injection with isolated standards. The amount of each phenolic acid is expressed as milligram per Kilogram of fresh weight unless if not confirmed.

RESULTS AND DISCUSSION

In this study sugarcane bagasse powder shows maximum yield 8.241 ±1.5 after 120 h. (Table 1.). It was concluded that the nature of the substrate, incubation time, temperature and agitation, affect the production of taconic acid on sugarcane bagasse powder. The itaconic acid fermentation process works optimally under phosphate-limited growth conditions (Nubel and Rakajak, 1964). Once the fungal biomass is established preferably after inoculation from spores, the phosphate level should be kept rather low to prevent growth. Although *A. niger* is now the mostly frequently used commercial producer of itaconic acid, other microorganisms that are not as sensitive to a particular conditions (example, substrate impurities) or which have a more favorable product composition have been found. Among the filamentous fungi, some ustilaginales species also produce itaconic acid.

Effect of pH on fermentation medium

pH is one of the most important parameters that affect the production of itaconic acid by fermentation. Fermentable medium with adjusted pH of 2.5, 3.0, 3.5, 4.0 and 4.5 were observed over a period of 15 days and maximum yield was obtained at all pH levels on Day 10 of fermentation with pH 4 yielding the highest itaconic acid concentration of 7.522±0.30 mg/kg of fermented substrate (Table 2). This is close to the findings of Rao et al. (2007) and Chandragiri and Sastry (2011) who reported the highest production of itaconic acid at pH 3.5 and 3, respectively.

Effect of Incubation temperature

Incubation temperature is one of the parameters that

Table 2. Effect of pH on fermentation medium.

pH	Itaconic acid (mg/kg) of fermented substrate
2.5	3.976 ± 0.50
3.0	6.782 ± 0.20
3.5	7.522 ± 0.30
4.0	5.023 ± 0.25
4.5	3.976 ± 0.50

Table 3. Effect of incubation temperature on fermentation medium.

Temperature (°C)	Itaconic acid (mg/kg) of fermented substrate
25	2.814 ± 0.40
30	6.291 ± 0.15
35	8.017 ± 0.50
40	5.138 ± 0.10
45	3.462 ± 0.50

Table 4. Validation data.

	SPD10Avp (280 nm)			
Name*	Retention time	Area	Height	Concentration (mg/kg)
Itaconic acid	5.000	7090	3910	1.19

affect the production of itaconic acid by solid state fermentation. Fermentable medium was incubated with 25, 30, 35, 40 and 45°C observed over a period of 15 days and maximum yield was obtained at all temperature levels on Day 10 of fermentation with temperature 35°C yielding the highest itaconic acid concentration of 8.017±0.50 mg/kg of fermented substrate (Table 3).

High-performance liquid chromatography (HPLC) determination of itaconic acid

The HPLC result showed based on the Retention time (Rt), Itaconic acid (Rt-5.000) content in sugarcane baggase fermented with *A. niger* was found to be 1.19 mg/kg given in Table 4 and Figure 1 the obtained value was compared with standard.

Conclusions

It can be concluded that bioconversion of bagasse could be economically advantageous in some cases, for example, for the production of enzymes, amino acids and drugs. Such processes require only small quantities of bagasse, which would not be difficult to obtain from the sugar factories. Sugarcane baggase powder has been shown to be suitable for the fermentative production of itaconic acid. Hence, bioprocesses which need large quantities of bagasse could eventually be considered only if surplus bagasse availability were ensured to meet such demands.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENT

This work was supported by Indian Institute of Crop Processing Technology, Pudukottai road, Thanjavur, Tamilnadu. The authors would like to specially thank K. Alagusundaram, Director, Indian Institute of Crop Processing Technology, Ministry of Food Processing Industries, Government of India, Thanjavur for Providing all the facilities and support used to carry out the work.

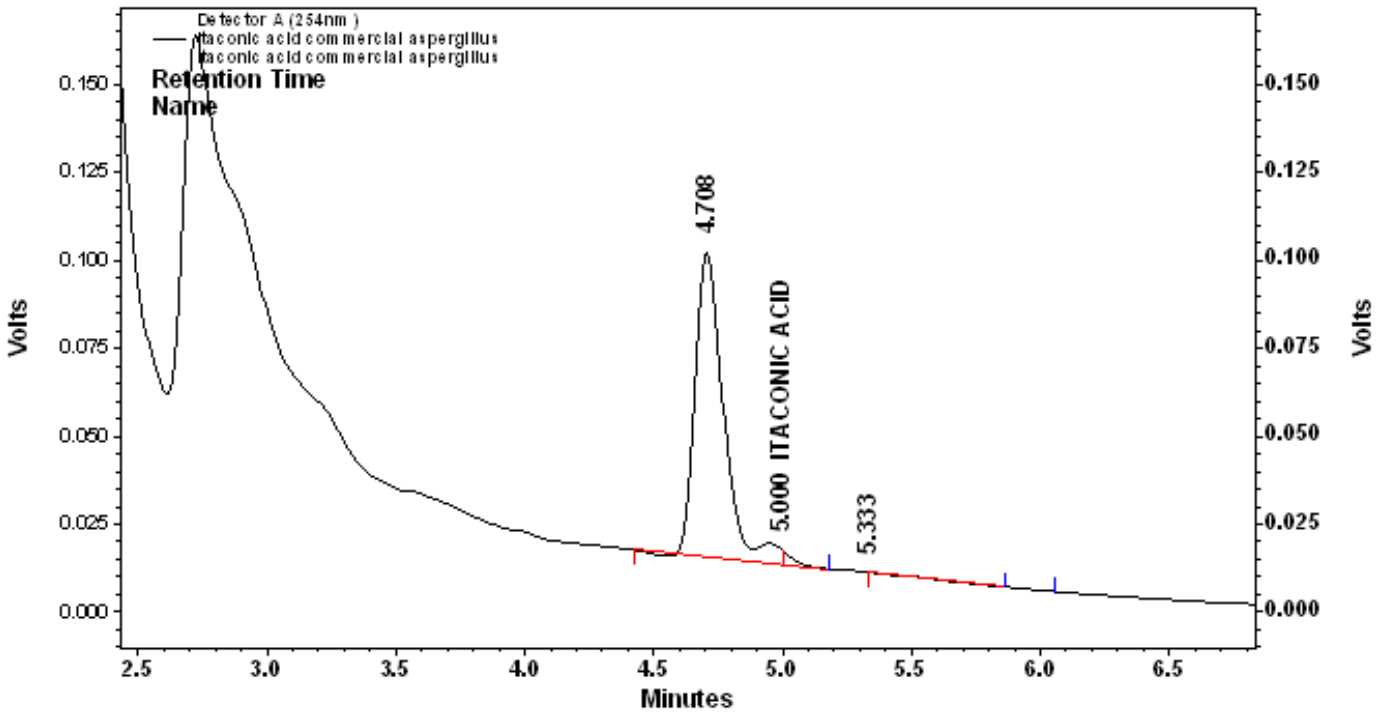

Figure 1. HPLC Chromatogram of itaconic acid in *Aspergillus niger* fermented sugarcane baggase.

REFERENCES

Bressler E, Braun S (2000). Conversion of citric acid to itaconic acid in novel liquid membrane bioreactor. J. Chem. Technol. Biotechnol 75: 66-72.

Chandragiri R, Sastry RC (2011). Synthesis of itaconic acid using *Ustilago maydis.* Can. J. Chem. Eng. Technol. 2(7):128-135.

Mattey M (1992). The production of organic acids. Crit. Rev. Biotechnol. 12 (1/2):87-132.

Md. Rafi M, Hanumanthu MG, Rizwana S, Venkateswarlu K, Rao DM (2012). Effect of different physico-chemical parameters on fermentative production of itaconic acid by Ustilago maydis. J. Microbiol. Biotech. Res. 2(5):794-800.

Milsom PE, Meers JL (1985). Gluconic and itaconic acids. In: Moo-Young, M. (Ed.), Comprehensive Biotechnology, vol. 3. Pergamon Press, Oxford. pp. 672-700.

Nubel RC, Rakajak ED (1964). US - Patent 3 044 941 (to Pfizer): Process for production itaconic acid.

Rao DM, Jaheer Hussain SMD, Rangadu PV, Subramanyam K, Sivarama Krishna G, Swamy AVN (2007). Fermentative production of itaconic acid by *Apergillus terreus* using Jatropha seed cake. Afr. J. Biotechnol. 6(18):2140-2142.

Walinky SW (1984). US - Patent 4 485 223 (to Pfizer): (Meth) acrylic acid/ itaconic acid biosynthesis by *Aspergillus terreus* .

Welter K (2000). Biotechnische Production von Itaconsaure ausnachwachseneden Rohstoffen mit immobilisierten Zellen. PhD thesis, Technical University of Brauschweig.

Xu DB, Madrid CP, Rohr M, Kubicek CP (1989). The influence of type of concentration of the carbon source on production of citric acid by *Aspergillus niger*. Appl. Microbiol. Biotechnol. 30:553-558.