ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 4 (4): 275-278, 2005
ISSN 1682-296X

Screening of *Aspergillus* for Citric Acid Production from Palm Oil Mill Effluent

Parveen Jamal, Md. Zahangir Alam, M. Ramlan, M. Salleh and Masrina M. Nadzir
Bioenvironmental Engineering Research Unit, Department of Biotechnology Engineering,
Faculty of Engineering, International Islamic University Malaysia,
Jalan Gombak, 53100 Kuala Lumpur, Malaysia

Abstract: In this study, screening of potential microbes, especially *Aspergillus,* for citric acid production from Palm Oil Mill Effluent (POME) is carried out to improve the product yield. The fermentation of the raw material POME for the production of citric acid was conducted by the liquid state fermentation process. A total of ten strains of *Aspergillus* were selected for the screening test of which six strains were isolated from Sewage Treatment Plant Sludge (STP Sludge), purified and identified up to genus level and four strains of *Aspergillus* were from lab stock. All strains were screened under controlled fermentation conditions such as pH range of 2-3, temperature 30°C and agitation 150 rpm, using 1% (w/w) of substrate (POME), 2% (w/w) co-substrate (wheat flour) with inoculum size of 2% (10^6 spore mL^{-1}). These strains were examined in terms of maximum citric acid production, biosolid production (TSS%) and Chemical Oxygen Demand (COD) removal. The strain *Aspergillus* (A103) produced the highest concentration of citric acid (0.28 g L^{-1}), TSS (12.7 g L^{-1}) and COD removal (72%) followed by A1020, A-SS106 and others on 2-4 days of fermentation.

Key words: Biosolids, COD, potential microbes, liquid state fermentation, fermentation

INTRODUCTION

Citric acid is used in variety of applications, from food and beverages to pharmaceuticals and cosmetics. It was first isolated and crystallized from lemon juice by Scheele in 1784 and Currie was the first person that produced commercial citric acid in 1917 using *A. niger* by surface fermentation process[1]. Presently, it is a primary product of *A. niger* metabolism[2] and it is estimated to be produced 500,000 tons annually exclusively by fermentation of *A. niger*[3]. This microorganism is used in the manufacture of citric acid, gluconic acid and the enzyme alpha-galactosidase and has been assessed as acceptable for daily intake by the World Health Organisation[4]. In developed countries, 65% of citric acid consumption is in food and beverages and 20% in household detergents and the estimated global growth rate in demand: 4 to 5% per year[5]. Due to ever increasing demand of this chemical, it is necessary to use inexpensive and readily available raw materials in industrial processes. Fermentation media for citric acid biosynthesis should consist of substrates necessary for the growth of microorganism, primarily the carbon, nitrogen and phosphorus sources. Moreover, water and air can be included as fermentation substrates[6,7]. It is notable here that a variety of raw materials such as molasses[8], starchy materials[9,10] and hydrocarbons[1] were used as substrates for commercial citric acid production. In this study, a new substrate, Palm Oil Mill Effluent (POME) is introduced for the production of citric acid as well as removal of COD. Oil palm is an important source of income to the Malaysian economy. In the year 2002, approximately 11.9 million tones of crude palm oil were produced that amounted to RM 14.79 billion[11]. The process to extract the oil requires significantly large quantity of water for steam sterilizing the palm fruit bunches and clarifying the extracted oil. It is estimated that for 1 tonne of crude palm oil production, 5-7.5 tonnes of water is required and more than 50% of water ended up as POME, which contain 95-96% water, 0.6-0.7% oil, 4-5% total solids, 2-4% suspended solids, pH 4.7, BOD-25000 mg L^{-1}, COD-50000 mg L^{-1} and total nitrogen-750 mg L^{-1} which can, therefore, cause severe pollution of waterways due to oxygen depletion and other related effects[12]. Extremely high content of degradable organic matter, carbohydrate and other nutrients in POME enable it to serve as substrate in the liquid state bioconversion by microorganism. According to study

Corresponding Author: Parveen Jamal, Bioenvironmental Engineering Research Unit, Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, Jalan Gombak, 53100 Kuala Lumpur, Malaysia Tel: +603 2056 4558 Fax: +603 2056 4442

done by Oswal, *et al.*[13], POME treatment by yeast, *Yarrowia lipolytica* showed substantial reduction in COD within 48 h indicating utilization of organic material in POME[13].

The rapid expansion of fermentation biotechnology over the past 30 years has led to a greater awareness of the usefulness of filamentous fungi for the production of large amount of organic acids, antibiotics, enzymes, hormones and fuel from inexpensive and/or waste ingredients[14]. Fungus for instance *A. niger* has long been known to synthesis organic acid that is gluconic acid[15]. Thus, screening of potential microbes, especially *Aspergillus,* using POME is carried out to improve the product yield. Liquid state bioconversion is used for the fermentation process with controlled process condition that includes pH, inoculum size, substrates, co-substrates, nitrogen concentration, temperature and agitation rate.

MATERIALS AND METHODS

Raw materials-substrate and co-substrate: The substrate used for this study was Palm Oil Mill Effluent (POME). This raw material was collected from oil palm industry (Seri Ulu Langat Palm Oil Mill Sdn.Bhd.), Dengkil, Selangor, Malaysia. Samples were stored at 4°C for further use.

Wheat flour was used as the co-substrate and additional carbon source. The previous study reported that it enhances biodegradation and the enzyme production[16].

Fungal strain and inoculum preparation: Ten strains of *Aspergillus* were selected in order to search the potential microorganism for maximum citric acid production. Six of them (A-SS101, A-SS102, A-SS104, A-SS105, A-SS106 and A-SS107) were selected from isolated strains taken from sewage treatment plant sludge and another four (A103, A1020, A2017 and SC906) from the lab stock. *Aspergillus* from lab stock were cultured on PDA plate and incubated at 32°C for ten days. They were sub cultured once a month and stored at 32°C in incubator.

Inoculum preparation (spore suspension) was done according to the method suggested by Alam[17]. Cultures grown on PDA medium in petri dishes at 32°C for 7 days was transferred into Erlenmeyer flask (250 mL) containing 100 mL of sterile distilled water. It was shaken in a rotary shaker with 150 rev min^{-1} for 24 h. The cultures were filtered and the supernatant was used as inoculum after measuring its strength (spores mL^{-1}) by Haemocytometer. The inoculum was kept in the refrigerator at 4°C for further studies. All flasks, funnel, filter paper, distilled water were sterilized prior to use.

Screening: Screening of strains was done to determine the best producer of citric acid and having high ability to remove organic content under controlled process conditions: 1% (w/w) of substrate (POME), 2% (w/w) co-substrate (wheat flour) and fungal strain with inoculum size of 2% (10^6 spore mL^{-1}) were incorporated in 250 mL flasks. Fermentation was done at pH 3, temperature range between 27-30°C and agitation: 150 rpm. A total of ten strains of *Aspergillus* were screened with harvesting time (sampling): 2, 4, 6 days. The best strain was chosen according to the maximum production of citric acid, bio-solids and removal of COD.

Data analysis: Assay of the citric acid was done according to an improved pyridine-acetic anhydride acid method suggested by Marier and Boulet[18]. Concentration of citric acid was measured at 420 μm with the development of color density. Chemical Oxygen Demand (COD) was determined by using the digestion solution for COD having a range of 0-1500 ppm. Reducing sugar was estimated by dinitrosalicylic acid (DNS) method suggested by Miller[19]. Bio-solid was measured to determine the fungal biomass obtained by following the standard methods of APHA[20].

RESULTS AND DISCUSSION

Strain A103 produced the highest concentration of citric acid (0.276 g L^{-1}) with the decreasing trend of COD value. The production of citric acid within the period of six days fermentation time is shown in Fig. 1. In overall, productions of citric acid have a decreasing trend with the highest production of citric acid (0.28 g L^{-1}) on the second day by A103. Maximum production of citric acid by other strains also occurred on the second day of

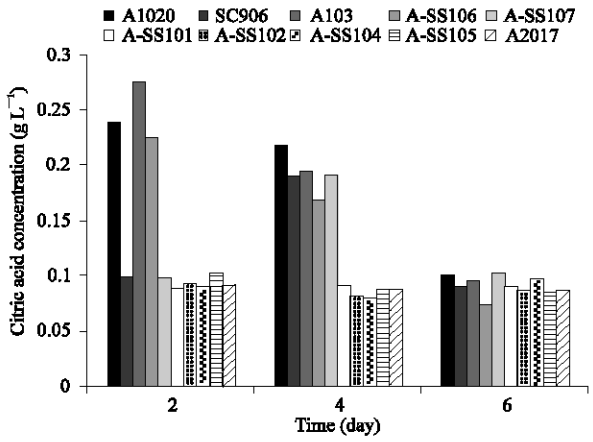

Fig. 1: Production of citric acid

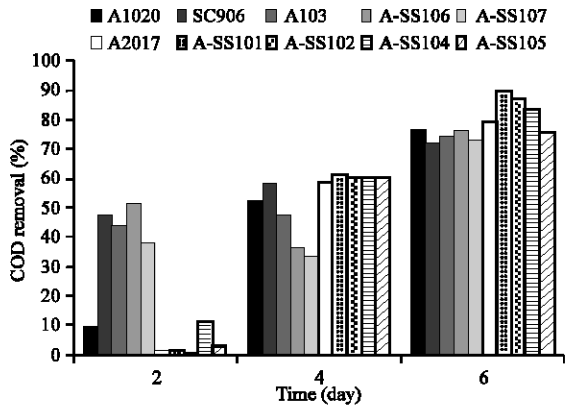

Fig. 2: Removal of COD by *Aspergillus* from POME during citric acid production

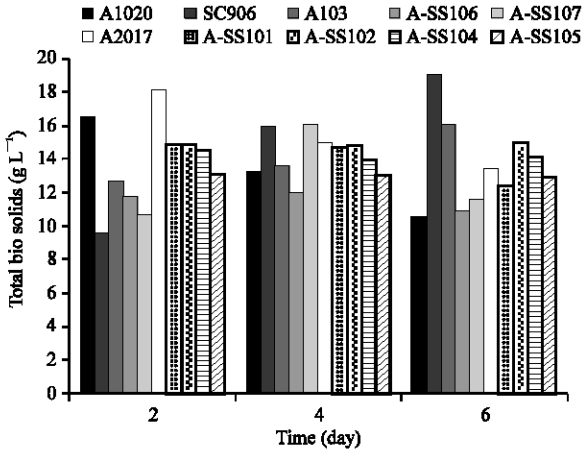

Fig. 3: Total bio-solids accumulation during the fungal treatment of POME

fermentation except for SC906, A-SS107 and A2017, which produced the highest on the fourth day. A-SS102, showed the highest production on the sixth day. However, the increment in product concentration compared to the second day of fermentation is very little with only 6.875 mg L^{-1} increase. Strains with heavy sporulation (A103, A1020 and A-SS106) showed high citric acid production, which is in agreement with the findings of previous research[21]. The amount of citric acid produced using POME is very little compared with conventional substrates such as sucrose or glucose may be due to presence of trace metal, which is known to affect citric acid fermentation negatively[22].

It is clear from Fig. 2 that the percentage removals of COD have an increasing trend with the highest percentage removal on the sixth day of fermentation. The highest COD percentage removal was by A-SS101 (89.58%) followed closely by A-SS102 (87.02%) and A-SS104 (83.46%). However, none of them were selected as potential strain for further study because they did not comply with the major concern that is high yield of citric acid. COD removal by strain A103 on the second day of fermentation is high (43.82%) compared to A-SS101 (1.52%), A-SS102 (0.19%), A-SS104 (11.12%), A-SS105 (2.76%), A2017 (1.47%) and A1020 (9.6%). The COD removal by A103 increased up to 74.05% by the last day of fermentation.

Total bio-solid is measured to determine the fungal biomass obtained, due to assimilation of suspended and soluble substances by microbes. Figure 3 indicates the highest reductions of bio-solids for SC906, A103, A-SS106, A-SS107, A-SS104 and A-SS105 on the second day of fermentation. Nevertheless, an increment in bio-solids was observed for the subsequent days except for A2017, A1020 and A-SS101 where bio-solids value decreased significantly on the sixth day of fermentation.

Based on the ability to produced high citric acid concentration and high COD removal, A103 can be selected as the potential strain for optimization study. The percentage removal of COD by A103 also increases with time indicating the suitability of A103 not only in producing valuable product but also in treatment of wastewater. Since Malaysia is one of the major palm oil producers in the world, this method will be beneficial in providing a more effective way in managing palm industry waste, development of an environmental friendly process for the production of citric acid and contributing to the country's economy.

ACKNOWLEDGMENT

The authors are grateful to the Research Center, International Islamic University Malaysia for extending a substantial grant for the project.

REFERENCES

1. Grewal, H.S. and A.L. Karla, 1995. Fungal production of citric acid. Biotechnol. Adv., 13: 209-234.
2. Legiša, M. and M. Gradišnik-Grapulin, 1995. Sudden substrate dilution induces a higher rate of citric acid production by *Aspergillus niger*. Applied Environ. Microbiol., 61: 2732-2737.
3. Jianlong, W. and L. Ping, 1996. Comparison of citric acid production by *Aspergillus Niger* immobilized in gels and cryogels of polyacrylamide. J. Industrial Microbiol. Biotechnol., 16: 351-353.
4. Wikipedia, 2004. http://www.all-science-fair-projects.com

5. Tidco, 2004. Citric Acid. www.tidco.com
6. Sing, S.P., U.N. Verma, M. Kishor and H.K. Samdani, 1998. Effect of medium concentration on citric acid production by submerged fermentation. Orient J. Chem., 14: 133-135.
7. Haq, I., S. Khurshid, S. Ali, H. Ashraf, M.A. Qadeer and Rajoka, 2001. M.I. Mutation of *Aspergillus niger* strain for enhanced citric acid production by black-strap molasses. World J. Microbiol. Biotechnol., 17: 35-37.
8. Ali, S., Ikram-ul-Haq, M.A. Qadeer and J. Iqbal, 2002. Production of citric acid by *Aspergillus niger* using cane molasses in a stirred fermentor. Electronic J. Biotechnol., 5 : 258-271.
9. Ikram-Ul-Haq, S. Ali and J. Iqbal, 2003. Direct production of citric acid from raw starch by *Aspergillus niger*. Process Biochemistry, 38: 921-924.
10. Alben, E. and O. Erkmen, 2004. Production of citric acid from a new substrate, undersized semolina, by *Aspergillus niger*. Food Technol. Biotechnol., 42: 19-22.
11. Anonymous, 2004. Malaysian Palm Oil Promotion Council Home Page (MPOPC). Retrieved March 21, 2004, from http://www.mpopc.org.my.
12. Ahmad, A.L., S. Ismail and S. Bhatia, 2003. Water recycling from Palm Oil Mill Effluent (POME) using membrane technology. Desalination, 157: 87-95.
13. Oswal, N., P.M. Sarma, S.S. Zinjarde and A. Pant, 2002. Palm oil mill effluent treatment by a tropical marine yeast. Bioresource Technol., 85: 35-37.
14. Smith, J.E., D.R. Berry and B. Kristiansen, 1980. Fungal Biotechnology. Academic Press, London, pp: 308.
15. Anonymous,1999. Fermentation (industrial), Production of Organic Acid. In Academic Press Encyclopedia of Food Microbiology. Retrieved July 9, 2004, from http://www.apress.gvpi.net/apfmicro/lpext.dll/
16. Krishna, C., 1996. Microbial enzyme production utilizing banana wastes. Ph.D Thesis, Cochin University of Science and Technology, India, 1996.
17. Alam, M.Z., A. Fakhru'l-Razi and A.H. Molla, 2003. Optimization of liquid state bioconversion process for microbial treatment of domestic wastewater sludge. J. Environ. Eng. Sci., 2: 299-306.
18. Marier, J.R. and M. Boulet, 1958. Direct determination of citric acid in milk with an improved pyridine-acetic anhydride method. J. Dairy Sci., 41: 1683-92.
19. Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 31: 426-428.
20. Anonymous, 1989. Standard Methods for the Examination of Water and Wastewater. 17th Edn., American Public Health Association (APHA), Washington, DC.
21. Röhr, M., P.J. Stadler, W.O. Salzbrunn and C.P. Kubicek, 1979. An improved method for characterization of citrate production by conidia of *Aspergillus niger*. Biotechnol. Lett., 1: 281-286.
22. Ghulam, N.Q., N.G. Chand, K.C. Surya, L.C. Charanjit, T. Michael and O. Ulfert, 1990. Pilot scale citric acid production with *Aspergillus niger* under several conditions. J. Fermentation and Bioeng., 69: 72-74.