Journal of Applied Sciences

ISSN 1812-5654

ANSInet
an open access publisher
http://ansinet.com

Journal of Applied Sciences 10 (21): 2648-2652, 2010
ISSN 1812-5654

Effect of Particle Size on Production of Citric Acid from Oil Palm Empty Fruit Bunches as New Substrate by Wild *Aspergillus niger*

[1,2]M.N. Bari, [1]M.Z. Alam, [1]S.A. Muyibi, [1]P. Jamal and [1]A.A. Mamun
[1]Bioenvironmental Engineering Research Unit, Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, Gombak, 50728 Kuala Lumpur, Malaysia
[2]Department of Civil Engineering, Rajshahi University of Engineering and Technology, Bangladesh

Abstract: An investigation was carried out to evaluate the effect of different particle size of oil palm Empty Fruit Bunches (EFB) and their distribution as new substrate for the production of citric acid through Solid State Bioconversion (SSB) in laboratory scale by *Aspergillus niger*. The experiment was carried out to observe the productivity of citric acid with different particle size of EFB in 250 mL Erlenmeyer flasks. The result obtained through this study showed that the 0.5 mm down graded particle of EFB gave the highest production of citric acid compared to other particle size. The maximum production of citric acid of 131.3 g kg^{-1}-EFB with the production rate of 16.4 g kg^{-1}-day was obtained from EFB with particle size of 0.5 mm after 8 days of bioconversion. Furthermore, the results of particle size distribution show that the well-graded particle size of EFB produced the highest citric acid compared to uniformly-graded and gap-graded particle.

Key words: Citric acid, oil palm Empty Fruit Bunches (EFB), particle size, solid state bioconversion, *Aspergillus niger*

INTRODUCTION

Citric acid is one of the largest fermentation products which is widely used in the food processing, beverage, cosmetic, pharmaceutical, chemical, textile and electroplating industry (Tran *et al*., 1998) and bioremediation of heavy metal contaminated soil (Kim and Barrington, 2003). The demand of citric acid is increasing from 4-5% every year (Pandey *et al*., 2001; Vandenberghe *et al*., 2000) that can only be met economically by using less expensive and renewable agro industrial residues as raw materials through solid state bioconversion with appropriate microorganism (Tran *et al*., 1998).

The oil palm Empty Fruit Bunches (EFB) as a lignocellulosic residue is produced from oil palm industries, which are abundantly available in Malaysia. The annual production of the EFB 12.4 million tones (Tanaka *et al*., 2006) which shows a great challenge to the solid waste management for its safe, scientific and environment friendly disposal (Bari *et al*., 2009; Suhaimi and Ong, 2001). Lignocellulosic residues are very well adapted to solid-state cultures due to their cellulosic and starchy nature (Soccol and Vandenberghe, 2003). Therefore, oil palm EFB was considered to be a potential alternative renewable raw material for the citric acid production by solid state bioconversion with *Aspergillus niger*.

The investigation on particle size of different lignocellulosic residues has been carried out by different researchers for citric acid production using different strains of *Aspergillus niger* through solid state fermentation. The lignocellulosic residues such as cassava bagasse (Prado *et al*., 2005a, b), sugarcane bagasse (Kumar *et al*., 2003a), carob pod (Roukas, 1999), coffee husk. Shankaranand and Lonsane (1994) have been examined by grinding in different particle size to evaluate the effect on production of citric acid by solid state bioconversion.

From the above discussions, it is clear that the particle size of substrate is an important parameter for the citric acid production from lignocellulosic substances. Therefore, the aim of this study is to evaluate the effect of particle size of oil palm empty fruit bunches towards the enhancement of production of citric acid through solid state bioconversion with wild strain of *Aspergillus niger*.

MATERIALS AND METHODS

Microorganism and inoculum: Wild strain of *Aspergillus niger* IBO-103MNB (IMI396649) was used for this study by selecting through screening test among 26 locally

Corresponding Author: M.Z. Alam, Bioenvironmental Engineering Research Unit, Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, Gombak, 50728 Kuala Lumpur, Malaysia

isolated strains from two citrus fruits viz., lemon and orange. The strains were maintained at 4°C by sub-culturing every month for further use. The inoculum was prepared by washing spores grown on PDA plate after 4 days of culture growth with 25 mL sterilized distilled water and collected in 100 mL Erlenmeyer flask by filtering with Whatman No. 1 filter paper. The spores were counted by haemocytometer to maintain the concentration of inoculum at 3×10^8 spores mL^{-1}.

Collection and pretreatment of empty fruit bunches: Sample of Empty Fruit Bunches (EFB) was collected from Seri Ulu Langat palm oil mill in Dengkil, Selangor, Malaysia and preserved in cold room at 4°C to avoid the unwanted bio-degradation by any microorganisms. The EFB samples were washed vigorously with tap water to ensure the removal of all unwanted impurities followed by drying at 105°C for 24 h. EFB fiber was split out manually and then ground by cutting mill to obtain the particle size of 0.5 mm down graded, 0.5 to 1 mm and 1 to 3 mm. Ground EFB was dried at 60°C for 48 h to get constant dry weight for experimental study (Kim and Barrington, 2003; Kumar *et al.*, 2003b). The effect of different particle size was evaluated on the basis of production of citric acid.

Fermentation media: Twenty gram of media containing 5 g (25% w/w) of major substrate-treated and non-treated EFB (particle size = 0.5 mm) with 1 g (5% w/w) of sucrose and 1 mL (5% v/w) of mineral solution obtained with modification from Erikson *et al.* (1980) containing $NH_4H_2PO_4$ (2 g L^{-1}), KH_2PO_4 (0.6 g L^{-1}), K_2HPO_4 (0.4 g L^{-1}), $MgSO_4.7H_2O$ (0.5 g L^{-1}), $CaCl_2.2H_2O$ (74 mg L^{-1}), Ferric acid citrate (12 mg L^{-1}), $ZnSO_4.7H_2O$ (6.6 mg L^{-1}), $MnSO_4$ (5 mg L^{-1}), $CuSO_4.5H_2O$ (1 mg L^{-1}).

Experiment for solid state bioconversion: The experiment for solid state bioconversion was carried out in 250 mL Erlenmeyer flask with initial moisture content of 70% by weight adjusted with mineral solution, distilled water and inoculum. The sample was inoculated with 5% spore suspension of 3×10^8 spores mL^{-1} (inoculum) after autoclaving at 121°C for 15 min. The initial pH of the substrate was recorded 5.5 but was not adjusted during the experimental runs. Bioconversion was carried out for 2, 4, 6, 8 and 10 days by incubating at 32°C. All SSB experiments were conducted in triplicates.

Extraction and analysis of citric acid: Citric acid was extracted from fermented substrate by adding 50 mL distilled water and shaking for 1 h at 150 rpm at room temperature (28±1°C) (Tran *et al.*, 1998). The supernatant was collected by filtering with Whatman No. 1 filter paper and immediately analyzed to determine the content of citric acid and remaining sugar. The concentration of citric acid in extraction was determined by using spectrophotometer at 420 nm using pyridine-acetic anhydride method as suggested by Marrier and Boulet (1958).

RESULTS AND DISCUSSION

Effect of particle size of EFB on citric acid production: The experimental results of five different particle size of EFB are presented in Fig. 1 to evaluate their effect in terms of maximum production of citric acid using lignocellulosic material (EFB) as new substrate by solid state bioconversion. The effect of particle size on citric acid production was clearly observed during the bioconversion period. Figure 1, it is observed that EFB with particle size of 0.5 mm gave higher production of citric acid during the bioconversion period compared to EFB of 0.25, 1, 2 and 3 mm down graded particle size. The maximum production of citric acid from EFB with particle size of 0.5 mm was 131.3 g kg^{-1} of dry EFB with the production rate of 14.6 g kg^{-1}-day obtained after 8 days of bioconversion. However, production of citric acid of 124.9 g kg^{-1} of dry EFB obtained after 6 days of bioconversion with the production rate of 20.8 g kg^{-1}-day from the same article size of EFB. The lowest citric acid production was obtained from EFB of 3 mm particle size. Citric acid production from EFB with particle size of 0.25 mm was very close to 1 mm particle size until 8 days of bioconversion.

Higher production of citric acid from smaller particle size was presumably due to easily availability of substrate to the microbe and the substrate with 0.5 mm particle size exhibited optimum porosity that can provide better heat

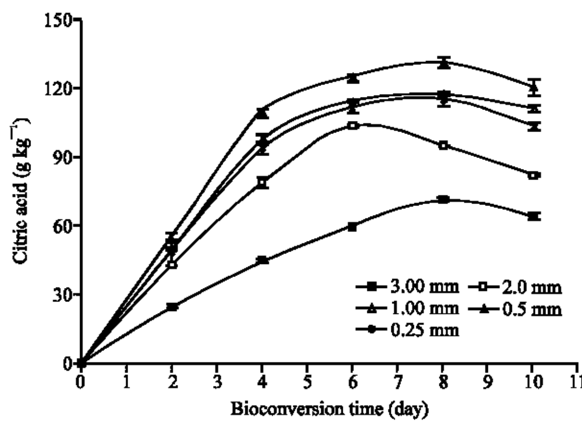

Fig. 1: Effect of particle size of EFB on production of citric acid

Table 1: Consumption of sucrose during the bioconversion

Particle size (mm)	Consumed sucrose ($g\ kg^{-1}$-EFB)					
	Initial	2	4	6	8	10
3	200	35.2	83.3	105.7	125.7	139.6
2	200	51.2	94.9	116.3	129.0	141.5
1	200	55.5	117.4	123.6	130.6	146.7
0.5	200	68.9	121.3	147.8	168.0	171.7
0.25	200	56.5	102.3	117.6	140.3	146.1

and mass transfer (Kumar *et al*., 2003a). On the other hand, larger particle sizes of EFB reduced substrate availability to the microbe though it increased the mass and heat transfer with higher inter particular spaces. Similarly, lowest particle size of 0.25 mm was more compacted compare to other sized that caused inadequate heat and mass transfer.

The obtained maximum production of citric acid from particle size of 0.5 mm was higher than the citric acid production of 121 $g\ kg^{-1}$ from sugarcane bagasse with particle size of 1.2-1.6 mm obtained by Kumar *et al.* (2003a) and lower than the citric acid production of 176±4 $g\ kg^{-1}$ from carob pod with particle size of 0.5 mm obtained by Roukas (1999). Therefore, the highest productions of citric acid in laboratory scale from EFB with particle size of 0.5 mm are considerably comparable with the production of citric acid from other lignocellulosic substrates reported by different researchers.

Consumption of sucrose by *Aspergillus niger* IBO-103 MNB grown on different particle sizes of EFB during the bioconversion is presented in Table 1. The result shows that the highest consumption of 171.7 $g\ kg^{-1}$ of dry EFB was taken place during the bioconversion of EFB with particle size of 0.5 mm down grade. The result also depicted that the consumption of sucrose was gradually less for higher and for lower particle size compared to 0.5 mm. These phenomena could be explained by the fungal growth during the bioconversion. The maximum consumption is meaning that maximum utilization of sucrose to citric acid through bioconversion.

The comparison of fungal growth in terms of protein content shows that the highest growth was accomplished on 0.5 mm particle size of EFB (Fig. 2). On the other hand, lowest growth was found on particle size of 3 mm. The highest protein content was found of 38 $g\ kg^{-1}$ of dry EFB after 6 days of bioconversion of 0.5 mm particle size of EFB. This result indicated that the particle size of 0.5 mm is the best for the growth among the particle size studied.

The evolution of pH for all ranges of particle sizes followed same trend and differences of pH values were not found among the experiments of different particle sizes. Figure 3 shows that the pH values sharply dropped to around 2.65 after 2 days of bioconversion and almost remain constant at around 2.3 from 4 days to the end of

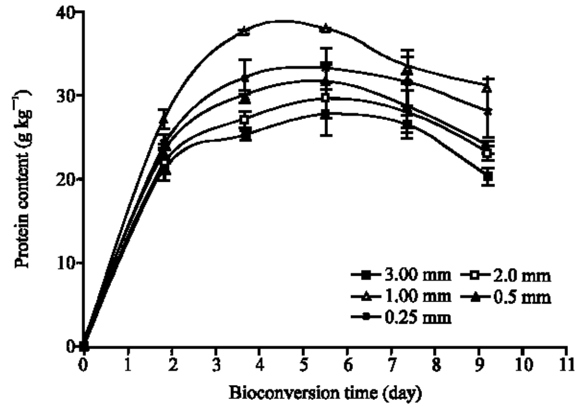

Fig. 2: Growth of fungi on EFB with different particle sizes

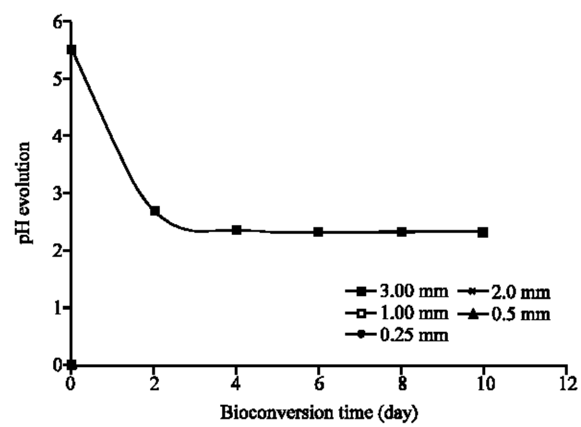

Fig. 3: Evolution of pH during bioconversion of EFB of different particle sizes

bioconversion. However, the lowest pH was observed for particle size of 0.5 mm though the difference was very less.

Effect of particle size distribution: Inter particular spaces or porosity of substrate that depends on the particle size distribution is one of the parameters that govern the mass and heat transfer during the bioconversion of substrate. The 0.5 mm down graded EFB particle was evaluated with three particle size distributions, such as well graded, uniformly graded and gap graded. The particle size distribution is presented in Fig. 4 where particle sizes are plotted along the x-axis in logarithmic scale against the percent finer along the y-axis in normal scale.

Well-graded sample is composed of different percentages of all particle sizes that forms an S-shaped curve. In case of uniformly-graded sample, all the particle sizes participate in same percentage while gap-graded sample dose not represents all sizes of particles. In this

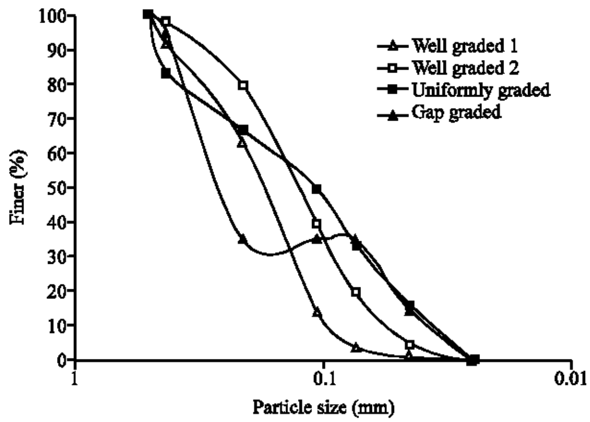

Fig. 4: Particle size distribution in different samples

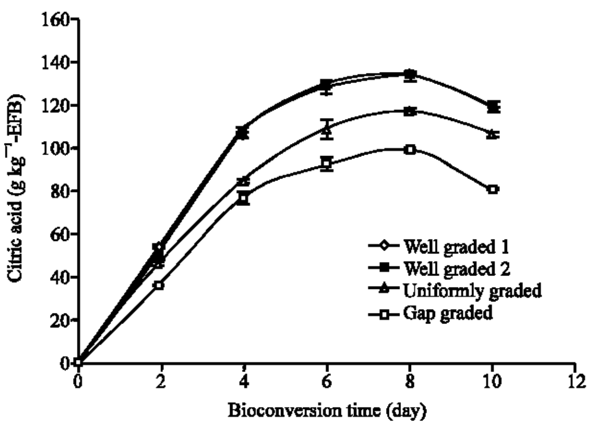

Fig. 5: Production of citric acid with different graded particle sizes

study, representative samples of particle sizes of 0.212 and 0.106 mm were absent from the total sample. In this case, 95% of total sample was finer than 0.425 mm and then 35% was finer than 0.075 mm which is obviously finer than 0.212 and 0.106 mm. Four samples of EFB particle were tested as well-graded-1, well-graded-2, uniformly-graded and gap-graded. Well-graded-1 particle size distribution was obtained naturally in ground EFB sample. Well-graded-2 was prepared by adding at certain percentage of different finer particles to evaluate the difference. Similarly, uniformly-graded and gap-graded samples were prepared with certain composition.

Figure 5 shows that the highest production for all the particle size distribution was obtained after 8 days of bioconversion. The well-graded-1 and well-graded-2 gave the higher production of citric acid compared to well-graded and gap-graded samples. The productions of citric acid between the well-graded samples were almost same through well-graded-2 sample showed slightly better result with the production of 134.3 g kg^{-1}-EFB while, 133.2 g kg^{-1}-EFB was obtained from well-graded-1 sample. This slight variation might be due the variation of substrate accessibility to the microbes as well as heat and mass transfer.

Figure 4 shows that well-graded-2 sample was prepared with higher percentage of smaller particles that provided the higher substrate accessibility. This phenomenon might enhance the production. However, higher percentage of smaller particles reduced the porosity of the substrate that hampered the heat and mass transfer as well as production of citric acid. On the other hand, well-graded-1 sample was composed of comparatively lower percentage of smaller particle that might provide slightly different situation than well-graded-2 sample for bioconversion.

The productions of citric acid of well-graded samples were higher than uniformly-graded and gap-graded samples might be due to the same reasons i.e., well-graded samples were able to provide optimum substrate accessibility as well as heat and mass transfer conditions. The heat and mass transfer ability of uniformly-graded and gap-graded samples might be better than well-graded samples but substrate accessibility was definitely less. Similar reasons were also applicable to justify the difference of production between uniformly-graded and gap-graded samples.

CONCLUSIONS

The result obtained in this study indicated that the production of citric acid increased with the decrease of particle size of EFB up to a certain level. The maximum citric acid production was 133-134 g kg^{-1}-EFB obtained from well-graded of 0.5 mm down graded particle size of EFB after 8 days of bioconversion with the production rate of 16.62-16.75 g kg^{-1}-day. However, the highest production rate of citric acid was 21.6 g kg^{-1}-day obtained by producing 129.6 g kg^{-1}-EFB after 6 days of bioconversion from the same particle size of EFB. Therefore, the well-graded of 0.5 mm down graded particle size of EFB might be the effective for further studies.

ACKNOWLEDGMENTS

The authors are grateful to the Department of Biotechnology Engineering, International Islamic University Malaysia for their support and to the Ministry of Science, Technology and Innovation (MOSTI), Malaysia, for financing the research project (No. 02-01-08-SF0050) under eScience research grant.

REFERENCES

Bari, M.N., M.Z. Alam, S.A. Muyibi, P. Jamal and A.A. Mamun, 2009. Improvement of production of citric acid from oil palm empty fruit bunches: Optimization of media by statistical experimental designs. Bioresour. Technol., 100: 3113-3120.

Erikson, K.E., A. Grunewald and L. Vallander, 1980. Studies of growth condition in wood for three white-rot fungi and their cellulose less mutants. Biotechnol. Bioeng, 27: 363-376.

Kim, J.W. and S.F. Barrington, 2003. Optimal glucose level for citric acid production by *Aspergillus niger* NRL 567 for soil heavy metal bioremediation. The Canadian Society for Engineering in Agricultural, Food and Biological Systems.

Kumar, D., V.K. Jain, G. Shanker and A. Srivastava, 2003a. Citric acid production by solid state fermentation using sugar cane bagasse. Process Biochem., 38: 1731-1738.

Kumar, D., V.K. Jain, G. Shanker and A. Srivastava, 2003b. Utilization of fruit waste for citric acid production by solid state fermentation. Process Biochem., 38: 1725-1729.

Marrier, J.R. and M. Boulet, 1958. Direct determination of citric acid in milk with an improved pyridine-acetic anhydride method. J. Dairy Sci., 41: 1683-1692.

Pandey, A., C.R. Soccol, J.A. Rodriguez-Leon and P. Nigam, 2001. Production of Organic Acids by Solid-State Fermentation. In: Solid-State Fermentation in Biotechnology-Fundamentals and Applications. Asiatech Publishers Inc., New Delhi, India, pp: 113-126.

Prado, F.C., L.P.S. Vandenberghe and C.R. Soccol, 2005a. Relation between citric acid production by solid-state fermentation from cassava bagasse and respiration of *Aspergillus niger* LPB21 in semi-pilot scale. Brazilian Arch. Biol. Technol. Int. J., 48: 29-36.

Prado, F.C., L.P.S. Vandenberghe, A.L. Woiciechowski, L.A. Rodrigues Leon and C.R. Soccol, 2005b. Citric acid production by solid state fermentation on a semi pilot scale using different percentages of treated cassava bagasse. Braz. J. Chem. Eng., 22: 547-555.

Roukas, T., 1999. Citric acid production from carob pod by solid-state fermentation. Enzyme Microb. Technol., 24: 54-59.

Shankaranand, V.S. and B.K. Lonsane, 1994. Coffee husk: An inexpensive substrate for production of citric acid by *Aspergillus niger* in a solid-state fermentation system. W. J. Microbiol. Biotechnol., 10: 165-168.

Soccol, C.R. and L.P.S. Vandenberghe, 2003. Overview of applied solid-state fermentation in Brazil. Biochem. Eng. J., 13: 205-218.

Suhaimi, M. and H.K. Ong, 2001. Composting empty fruit bunches of oil palm. The Food and Fertilizer Technology Centre (FFTC), 1st November 2001. http://www.agnet.org/library/eb/505a/.

Tanaka, R., M. Yutaka and K. Akihiko, 2006. Utilization of oil palm empty fruit bunches as solid materials. Biomass WS 2006.

Tran, C.T., L.I. Sly and D.A. Mitchell, 1998. Selection of a strain of Aspergillus for the production of citric acid from pineapple waste in solid-state fermentation. W. J. Microbiol. Biotechnol., 14: 399-404.

Vandenberghe, L.P.S., C.R. Soccol, A. Pandey and J.M. Lebeault, 2000. Cassava bagasse, an alternative substrate for citric acid production in solid-state fermentation. Proceedings of the 11th International Biotechnology Symposium and Exhibition, Sept. 3-8, Berlin, pp: 153-155.