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Evaluation of Citric Acid Production by *Penicillium* sp. ZE-19 and its Improved UV-7 Strain

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ABSTRACT

The present study was conducted to evaluate citric acid production by a novel *Penicillium* sp. ZE-19 and its UV treated strain *Penicillium* sp. UV-7 using sucrose and fructose based media as sources of carbon and energy. Triplicate fermentations were carried out in shake flasks at 200 rpm and $28\pm 2^\circ\text{C}$ for 144 h and samples were analysed at a twelve hourly interval. The findings indicated that both organisms utilized fructose and sucrose for citric acid and biomass productions. However, both strains produced higher citric acid concentration on sucrose medium than on fructose medium; since higher citric acid concentration (CA; 22.5 ± 0.29 - 23 ± 0.1 g L⁻¹), specific citric acid production rate (q_{ca} ; 0.036-0.038 g/g.h⁻¹) and citric acid yields ($Y_{CA/S}$; 0.32-0.33 g g⁻¹) were obtained from medium containing sucrose after 144 h of incubation and these values were significantly higher ($p < 0.05$) compared to their values under fructose medium. Interestingly, the concentrations of biomass produced by both strains on fructose and sucrose media were significantly not different ($p > 0.05$). Although, the attempt to improve the *Penicillium* sp. ZE-19 gave a negative result, the authors suggest further strain optimization strategies. This study therefore, confirms a novel strain which could be used for possible commercial citric acid bioprocess using sucrose based medium.

Key words: Citric acid, *Penicillium* sp. sucrose, strain UV-7, kinetic parameters

INTRODUCTION

Microorganisms are used in industrial microbiology and biotechnology to create a wide variety of products and to assist in maintaining and improving the environment. These organisms are isolated from nature. As soon as a particular microorganism that forms certain useful product is isolated, the aim exist to improve its yield by changing or improving the growth conditions called process optimization and or through mutation and other genetic means called strain improvement (Prescott *et al.*, 2005; Chalker and Davis, 2010).

Organic acid production is an excellent example of industrial production processes where microorganisms (fungal) are utilized. The success of this fungal bioprocess is ultimately based on rapid and economic conversion of sugars to acid (Magnuson and Lasure, 2004). Further development in the biotechnology field, environmental pressure and the vertical integration of the fermentation and agricultural produce processing industries resulted in much improved economics for organic acid production.

Citric acid ($C_6H_8O_7$, 2-hydroxyl propane -1, 2, 3-tricarboxylic acid), a natural constituent and common metabolite of plants and animals, is the most versatile and widely used organic acid produced in tonnage by fermentation and is the most exploited biochemical product (Kapoor *et al.*, 2004; Yalcin *et al.*, 2010). It is responsible for the tart taste of various fruits e.g., lemons, limes, oranges, pineapples and pears, thus, it is widely used to impart a pleasant, tart flavour to foods and beverages. It also contributes to the production of many beverages and foods as an acidulant, oxidant, emulsifier or preservative (Kamzolova *et al.*, 2005). Commercial applications of citric acid in food, pharmaceutical and biochemical industries cannot be over emphasized.

Citric acid accumulation in culture medium containing sugars and inorganic salts was first demonstrated using *Penicillium* sp. thereafter *Aspergillus* sp. was used and has received an outstanding worldwide preference. Surprisingly, this phenomenal demonstration or productivity using *Penicillium* sp. has been the objective of relatively few or no research programs. This study therefore, took a leap back to the origin by evaluating the production of citric acid from mineral salt medium on shake flasks and the kinetic behaviour of a novel *Penicillium* sp. and its UV irradiated strain.

MATERIALS AND METHODS

Fungi strains and inoculum preparation: *Penicillium* sp. ZE-19 is a stock culture in our laboratory that was isolated from waste water of crude oil exploration company located in Imo State, Nigeria. In a preliminary qualitative study of forty two *Penicillium* strains for citric acid production using the dye method of Kareem *et al.* (2010), strain ZE-19 was selected as the highest citric acid producer. *Penicillium* sp. UV-7 is the improved strain developed from ZE-19 using UV irradiation. The fungi strains were stocked at 4°C on Potato Dextrose Agar (PDA) and subcultured twice a month.

The inoculum used for citric acid production was prepared following the method of Jamal *et al.* (2005) with slight modification. Fungi isolates grown on PDA dishes at 32°C for five days were transferred into 250 mL Erlenmeyer flasks containing 100 mL of sterile distilled water. After incubation of the flasks on a shaker incubator at 200 rpm for 18-24 h, the cultures were decanted. The supernatants were used as inoculum after measuring its spore concentration of 1.3×10^6 spores mL^{-1} using haemocytometer.

UV irradiation: Conidial suspension of the culture of the fungi isolate was prepared by suspending a five days old PDA slant cultures having profuse conidial growth in 100 mL of sterile deionized distilled water. A sterile wire-loop was gently used to break the conidial clumps. The conidia in the culture was washed twice with 0.1 M phosphate buffer (pH 7.2) and suspended in the same buffer. About 3 mL of the conidial suspension (1.0×10^8 conidia mL^{-1}) in a petri dish without cover was irradiated for 10 min (giving a 95% death rate) with a 30 W UV lamp at a distance of 8 cm. The treated conidia were kept in the dark for 2 h to avoid photo reactivation repair. Thereafter, 0.1 mL of the suspension was plated out on PDA plates and incubated at 32°C for five days.

Fermentation: The fermentation medium used for production of citric acid from sucrose and fructose, respectively contained per litre: 1 g KH_2PO_4 , 0.25 g $MgSO_4 \cdot 7H_2O$, 32.5 g NH_4NO_3 and 150 g sucrose or fructose (Ikram-Ul-Haq *et al.*, 2001; Kamzolova *et al.*, 2011). Citric acid production by shake flask experiments were conducted in 1 L Erlenmeyer flasks containing 150 mL fermentation medium inoculated with 2% (v/v) spore suspension at 30°C in a rotary incubator

shaker at 200 rpm for 144 h at a pH 3.5. All the experiments were carried out in triplicates. Sample aliquots were withdrawn at intervals for citric acid production, biomass concentration and sugar (fructose or sucrose) consumption analysis.

Analytical methods: The samples from the production medium were taken at intervals of 12 h and centrifuged at 10,000 x g for 10 min. The decanted supernatant was separated and used for sugar and citric acid concentration analysis. After washing with distilled water, the residues were dried for dry cell mass determination at 105°C to a constant mass. Citric acid concentration was determined spectrophotometrically at 405 nm using the pyridine-acetic anhydride method (Marier and Boulet, 1958; Kishore *et al.*, 2008). The Dinitrosalicylic acid (DNS) method was used for the analysis of sucrose (Kishore *et al.*, 2008) while fructose was analysed by the adopted Seliwanoff's method as earlier described by Ferreira *et al.* (2010).

Statistical analysis: The results indicate the Mean±Standard Deviation of triplicate values. Statistical analyses were performed with student's t-test. The results were considered to be statistically or significantly different when p values were less than 0.05 (p<0.05) and vice versa.

Growth and citric acid production kinetics: Specific growth rate (μ) for the exponential growth phase was calculated from the plot of natural logarithm of biomass concentration against time. Values of the biomass ($Y_{X/S}$, $Y_{X/F}$) and citric acid production yields ($Y_{CA/S}$, $Y_{CA/F}$) were calculated from the slopes of biomass formation versus sugar consumed and citric acid produced versus sugar consumed, respectively. Specific citric acid production rates (q_{ca}) were calculated according to Eq. 1, where $Y_{CA/X}$ is the citric acid yield on the basis of biomass formed ($g\ gcell^{-1}$):

$$q_{ca} = \mu \cdot Y_{CA/X} \quad (1)$$

Specific sucrose and fructose consumption rates (q_s or q_f) were calculated by Eq. 2 and 3, where $Y_{X/S}$ and $Y_{X/F}$ are the biomass yields on sucrose and fructose, respectively:

$$q_s = \mu / Y_{X/S} \quad (2)$$

$$q_f = \mu / Y_{X/F} \quad (3)$$

Where:

CA/ca = Citric acid

X = Biomass

S/s = Sucrose

F/f = Fructose

RESULTS

A novel *Penicillium* sp. ZE-19 and the improved strain UV-7 were used for the production of citric acid from mineral salt media containing fructose or sucrose as a source of carbon and energy. Comparative analysis on citric acid production and some kinetic parameters were calculated.

Fermentation profiles; changes in citric acid concentration, dry biomass and sugar consumption with time for both strains were determined as represented in Fig. 1 and 2(a-b). Overall, sugar content of the media were reduced by the strains during fermentation and the concentration of citric acid and dry biomass produced increased in proportion to the sugar utilization. In the medium

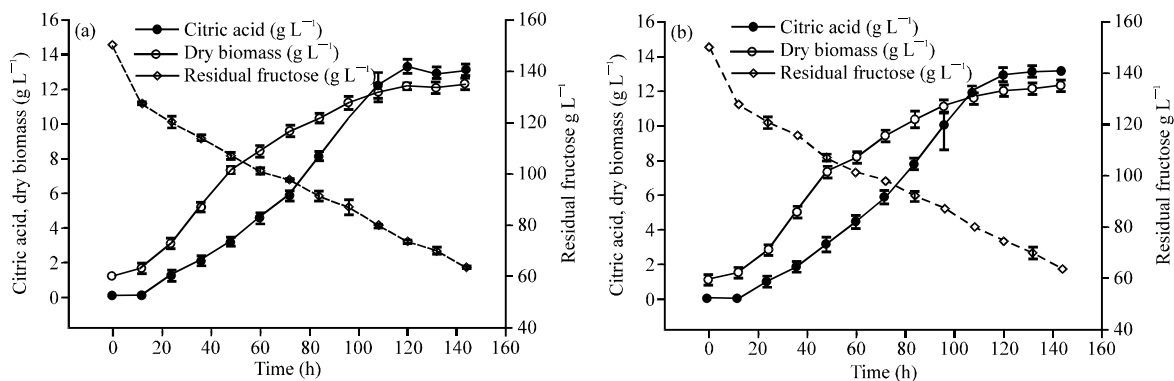


Fig. 1(a-b): Fermentation profiles of citric acid production by *Penicillium* sp. UV-7 (a) and Strain ZE-19 (b) From fructose salt medium, Y-error bars indicate the standard deviation of the triplicates

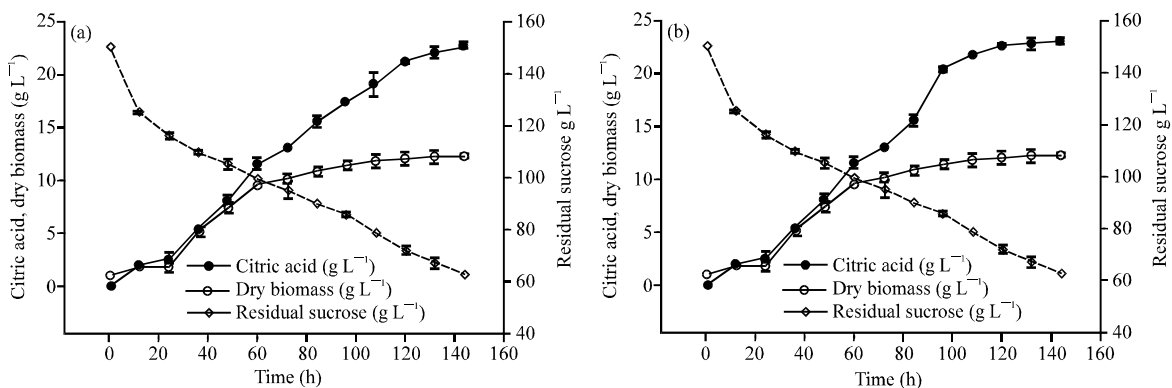


Fig. 2(a-b): Fermentation profile of citric acid production by *Penicillium* sp. ZE-19 (a) and Strain UV-7 (b) From sucrose salt medium, Y-error bars indicate the standard deviation of the triplicates

containing fructose (fructose salt medium), citric acid production started after 12 h of incubation and recorded a maximum of 13.1 and 13.0 g L⁻¹ for strains ZE-19 and UV-7, respectively after 144 h of incubation. Between 0-72 h of incubation, both strains produced 2~fold higher biomass concentration than its citric acid concentration. Citric acid and biomass concentrations increased progressively after 12 h of incubation but remained approximately steady after 120 and 108 h of incubation, respectively. Also, there was a high sugar consumption of 22.4 and 22.8 g L⁻¹ by strains ZE-19 and UV-7, respectively within 12 h of incubation. Subsequently, sugar consumption increased throughout the fermentation period at a concentration range of 3.6-7.2 g L⁻¹ per 12 h.

Similarly, in the medium containing sucrose (sucrose salt medium), citric acid production and sucrose consumption for both strains started within the first 12 h and increased subsequently (Fig. 2). Maximum citric acid concentration of 22.5 and 23.0 g L⁻¹ was produced by strains ZE-19 and UV-7, respectively after 144 h of incubation. The result revealed that biomass concentration for both strains increased steadily from the onset of the fermentation, however, the concentration increased slightly at a proportion range of 0.1-0.8 g L⁻¹ after 60 h of incubation. At the end of the fermentation period of 144 h, strains ZE-19 and UV-7 had consumed approximately 57 and 58% of the initial sucrose concentration used, respectively while the concentration of citric acid was approximately 2~fold higher than the biomass concentration.

Table 1: Comparison of citric acid production between *Penicillium* sp. ZE-19 and its improved (UV-7) strain after growth on two different fermentation media

Fermentation time (h)	Citric acid concentration (g L ⁻¹)			
	Sucrose salt medium ⁱ		Fructose salt medium ^j	
	ZE-19 ^{a,*}	UV-7 ^{a,*}	ZE-19 ^{a,h}	UV-7 ^{a,h}
0	0.0±0.00 ^{a,c}	0.0±0.00 ^{a,c}	0.0±0.00 ^{a,c}	0.0±0.00 ^{a,c}
24	2.5±0.08 ^{a,c}	2.6±0.08 ^{a,c}	1.0±0.16 ^{a,c}	1.2±0.08 ^{a,c}
48	7.9±0.45 ^{a,c}	8.1±0.24 ^{a,c}	3.1±0.37 ^{a,d}	3.1±0.14 ^{a,d}
72	12.5±0.21 ^{a,c}	13.0±0.29 ^{a,c}	5.8±0.29 ^{a,d}	5.8±0.16 ^{a,d}
96	17.4±0.22 ^{a,c}	20.4±0.29 ^{b,c}	10.0±1.41 ^{a,d}	10.2±0.08 ^{a,d}
120	21.2±0.08 ^{a,c}	22.6±0.22 ^{a,c}	12.9±0.54 ^{a,d}	13.2±0.43 ^{a,d}
144	22.5±0.29 ^{a,c}	23.0±0.10 ^{a,c}	13.1±0.00 ^{a,d}	13.0±0.22 ^{a,d}

^{a,b}First subscripts in the same row of data are for comparison between the two strains on the same media, ^{c,d}Second subscripts in the same row of data are for comparison between the same strains on the different media, ^eFirst subscripts on the strains are for overall comparison between the two strains on the same medium, ^{f,h}Second subscripts on the strains are for overall comparison between the same strains on the different media, ^{i,j}Subscripts on media are for overall comparison for both strains between the different media. Different letters indicates statistical difference at p<0.05 and vice versa

Table 2: Kinetic parameters of citric acid production experiment using *Penicillium* sp. ZE-19 and strain UV-7 on sugar (sucrose and fructose) fermentation media

Kinetic parameters	Sucrose salt medium		Fructose salt medium	
	ZE-19	UV-7	ZE-19	UV-7
μ	0.020	0.020	0.020	0.020
$Y_{CA/S}$	0.320	0.330	-	-
$Y_{CA/F}$	-	-	0.200	0.200
$Y_{CA/X}$	1.780	1.920	1.180	1.190
$Y_{X/S}$	0.170	0.160	-	-
$Y_{X/F}$	-	-	0.160	0.160
q_{ea}	0.036	0.038	0.024	0.024
q_s	0.118	0.125	-	-
q_r	-	-	0.125	0.125

Comparative analysis of citric acid production by *Penicillium* sp. ZE-19 and strains UV-7 after growth on the media used are shown in Table 1. Overall, citric acid production by both strains on sucrose salt medium was approximately 2~fold higher than the concentration produced on fructose salt medium throughout the fermentation period, with the difference being statistically significant (p<0.05). Citric acid production by strain UV-7 was significantly higher (p<0.05) than the concentration produced by strain ZE-19 on sucrose salt medium after 96 h of incubation. Interestingly, citric acid concentration between the strains at the end of fermentation (144 h) on sucrose salt medium was statistically not different (p>0.05). Also, throughout the fermentation period using fructose salt medium, citric acid productions by both strains were not statistically or significantly different (p>0.05).

Kinetic parameters for citric acid production for *Penicillium* sp. ZE-19 and strain UV-7 using sugar based (sucrose and fructose) mineral media at the end of 144 h of fermentation were calculated (Table 2). Specific growth rate (μ) of the strain on the media used was 0.02 h⁻¹. Citric acid yield (based on sugar consumption) of 0.32 and 0.33 g g⁻¹ was observed for ZE-19 and UV-7 on sucrose salt medium, respectively while both gave a yield of 0.2 g g⁻¹ on fructose salt medium.

Based on biomass formed, citric acid yield for ZE-19 and UV-7 on sucrose salt medium was 1.78 and 1.92 g g⁻¹, respectively while on fructose salt medium was 1.18 and 1.19 g g⁻¹, respectively. Biomass yield for both strains on the two different media used, ranged between 0.16-0.17 g g⁻¹. The highest specific citric acid production rate of approximately 0.04 g g⁻¹ h was calculated for the parent and improved strains using sucrose salt medium. Finally, both strains showed no difference in the specific sugar (fructose or sucrose) consumption rates.

DISCUSSION

This research investigated and confirmed growth and citric acid production by *Penicillium* sp. ZE-19 and its improved strain UV-7. The findings indicated that both organisms utilized fructose and sucrose salt media as a source of carbon and nitrogen to support both citric acid and biomass production. Citric acid production is approximately in a direct relationship with respect to the consumption of sugar and the formation of biomass. This implies that citric acid production is growth associated. These findings are in alliance with the report of Kareem *et al.* (2010); Nadeem *et al.* (2010) and Amenaghawon and Aisien (2012). In their researches on the optimization of citric acid production using *Aspergillus niger*, they recorded a similar relationship. The yield coefficients of citric acid by both strains on sucrose ($Y_{CA/S}$) medium were higher compared to fructose medium ($Y_{CA/F}$). Estimation of yield coefficient is important because the cost of the fermentation medium, particularly the carbon source, can be a significant proportion of the overall production cost (Waites *et al.*, 2008). Attempt to optimize the production of citric acid by exposing the parent strain ZE-19 to UV radiation (Strain UV-7) did not yield any positive result as the concentration of citric acid on all the media were not significantly different. This contradicts the reports of Mazhar *et al.* (2003) and Anyanwu and Okerentugba (2013) who, respectively achieved 15-22 and 13% improvement of citric acid production by UV irradiation of *Aspergillus* isolates. However, UV-irradiated DNA is unstable and could recover through photo reactivation or mutation repair mechanisms (Ikram-Ul-Haq *et al.*, 2001; Prescott *et al.*, 2005).

However, both strains produced higher citric acid concentration on sucrose medium than on fructose medium; since higher citric acid concentration, specific citric acid production rate (q_{ca}) and citric acid yields ($Y_{CA/S}$) were obtained from sucrose medium. Sucrose is a disaccharide composed of glucose and fructose monomers and this work revealed that sucrose was a better carbon source for production of citric acid than the monosaccharide; fructose. This is substantiated by the reports of other investigators (Ikram-Ul-Haq *et al.*, 2001; Ali *et al.*, 2002; Kishore *et al.*, 2008; Anyanwu and Okerentugba, 2013). Also, it has been reported that hexokinases contribute to the degradation of hexoses such as fructose and glucose (Kubicek, 1998; Yalcin *et al.*, 2009a, b), thus, the strains have active hydrolytic enzyme for breakdown of sucrose and hexokinases for degradation of its monomers. Nevertheless, sucrose is a major component of most agricultural products and waste e.g., cane and beet molasses, brewery waste and fruit waste. These are relatively cheap and readily available, therefore are promising substrates for citric acid production processes and scale-up.

Biomass formation is one of the fundamental yardsticks in the characterization of microbial growth. Interestingly, the concentrations of biomass produced by both strains on fructose and sucrose media were significantly not different. Therefore, it indicates that citric acid production is not synonymous with biomass production since the concentration of citric acid by both strains on the different media varied. Nonetheless, it can be suggested that there is a variation in metabolic pathway during the consumption of the sugars used and production of citric acid.

Sugar (sucrose and fructose) concentration decreased throughout the fermentation period. The decrease in sugar concentration observed, is the result of sugar consumption by the organisms. This report is in agreement with those of Alben and Erkmén (2004); Baei *et al.* (2008) and Amenaghawon and Aisien (2012) during their production of citric acid from undersized semolina, apple pomace and corn starch hydrolysate, respectively, using *Aspergillus niger*. This shows that the cells were still viable and it indicates a link between carbon storage, citric acid and biomass production (Papagianni, 2007; Anyanwu and Okerentugba, 2013). The biomass yield (Y_{XS} and Y_{XF}) relates the quantity of biomass produced per gram of substrate utilized, thus it is a parameter for sugar consumption. The higher the yield, the greater the percentage of the original substrate converted into microbial biomass (Waites *et al.*, 2008). The biomass yield reported, however, was higher compared to Amenaghawon and Aisien (2012) who reported a biomass yield of 0.0711 g g⁻¹.

CONCLUSION

It can be concluded from the results reported in this study, that *Penicillium* sp. ZE-19 and *Penicillium* sp. UV-7 are potential organisms for citric acid production. It should be noted that *Penicillium* was the first organism used for microbial production of this organic acid but the discovery of *Aspergillus niger* as a higher producer greatly stalled further research using *Penicillium*. Nevertheless, strain improvement and selection is pivotal for citric acid production process; a panacea for the sustenance and advancement of citric acid industry. The study showed better citric acid production capabilities of both organisms by the consumption of sucrose as source of carbon and energy. This may also be adapted for further studies considering the utilization of some agricultural cum cheaper sources of sucrose. The kinetic data presented, will enable the optimization of citric acid production conditions towards a possible scale-up process. Although, the attempt to improve the *Penicillium* strain gave a negative result, the authors suggest further strain optimization strategies. This study therefore, confirms a novel strain which could be used for possible commercial citric acid bioprocess.

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REFERENCES

- Alben, E. and O. Erkmén, 2004. Production of citric acid from a new substrate, undersized semolina by *Aspergillus niger*. Food Technol. Biotechnol., 42: 19-22.
- Ali, S., Ikram-UL-Haq and J. Iqbal, 2002. Citric acid fermentation by a UV-treated mutant of *Aspergillus niger*. Pak. J. Bot., 34: 125-128.
- Amenaghawon, N.A. and F.A. Aisien, 2012. Modelling and simulation of citric acid production from corn starch Hydrolysate using *Aspergillus niger*. Environ. Nat. Resour. Res., 2: 73-85.
- Anyanwu, V.E. and P.O. Okerentugba, 2013. Citric acid production by wild and UV-treated strains of *Aspergillus niger* on two different mineral salt media. Int. J. Applied Sci. Biotechnol., 1: 145-153.
- Baei, M.S., M. Mahmoudi and H. Yunesi, 2008. A kinetic model for citric acid production from apple pomace by *Aspergillus niger*. Afr. J. Biotechnol., 7: 3487-3489.
- Chalker, J.M. and B.G. Davis, 2010. Chemical mutagenesis: Selective post-expression interconversion of protein amino acid residues. Curr. Opin. Chem. Biol., 14: 781-789.

- Ferreira, N.R., M.I.M. Sarquis, C.N. Alves and A.S. Santos, 2010. Biotransformation of sucrose into 5-hydroxy-2-hydroxymethyl- α -pirone by *Aspergillus flavus*. Anais Academia Brasileira Ciencias, 82: 569-576.
- Ikram-Ul-Haq, S. Ali, H. Ashraf, W.A. Butt, M.A. Qadeer, K. Shafiq and J. Iqbal, 2001. Effect of mineral nutrient on the production of citric acid by *Aspergillus niger* UV-6, using sucrose salt media. Pak. J. Bot., 33: 535-540.
- Jamal, P., M.Z. Alam, M.R.M. Salleh and M.M. Nadzir, 2005. Screening of *Aspergillus* for citric acid production from palm oil mill effluent. Biotechnology, 4: 275-278.
- Kamzolova, S.V., A.R. Fatykhova, E.G. Dedyukhina, S.G. Anastassiadis, N.P. Golovchenko and I.G. Morgunov, 2011. Citric acid production by yeast grown on glycerol-containing waste from biodiesel industry. Food Technol. Biotechnol., 49: 65-74.
- Kamzolova, S.V., I.G. Morgunov, A. Aurich, O.A. Perevoznikova, N.V. Shishkanova, U. Stottmeister and T.V. Finogenova, 2005. Lipase secretion and citric acid production in *Yarrowia lipolytica* yeast grown on animal and vegetable fat. Food Technol. Biotechnol., 43: 113-122.
- Kapoor, K.K., K. Chaudhary and P. Tauro, 2004. Citric Acid. In: Prescott and Dunn's Industrial Microbiology, Reed, G. (Ed.). 4th Edn., CBS Publishers Ltd., New Delhi, India, pp: 709-739.
- Kareem, S.O., I. Akpan and O.O. Alebiowu, 2010. Production of citric acid by *Aspergillus niger* using pineapple waste. Malaysian J. Microbiol., 6: 161-165.
- Kishore, K.A., M.P. Kumar, V.R. Krishna and G.V. Reddy, 2008. Optimization of process variables of citric acid production using *Aspergillus niger* in a batch fermentor. Eng. Lett., 16: 572-577.
- Kubicek, C.P., 1998. The role of sugar uptake and channelling for citric acid accumulation by *Aspergillus niger*. Food Technol. Biotechnol., 36: 173-175.
- Magnuson, J.K. and L.L. Lasure, 2004. Organic Acid Production by Filamentous Fungi. In: Advances in Fungal Biotechnology for Industry Agriculture and Medicine, Tkacz, J.S. and L. Lange (Eds.). Kluwer Academic, Plenum Publishers, New York, pp: 307-340.
- Marier, J.R. and M. Boulet, 1958. Direct determination of citric acid in milk with an improved pyridine-acetic anhydride method. J. Dairy Sci., 4: 1683-1692.
- Mazhar, R., A. Rehman, S. Ali and Ikram-Ul-Haq, 2003. Kinetics of citrate over-production by *Anasergillus niger* mut ant rabt-10. Pak. J. Bot., 35: 547-552.
- Nadeem, A., Q. Syed, S. Baig, M. Irfan and M. Nadeem, 2010. Enhanced production of citric acid by *Aspergillus niger* M-101 using lower alcohols. Turkish J. Biochem., 35: 7-13.
- Papagianni, M., 2007. Advances in citric acid fermentation by *Aspergillus niger*: Biochemical aspects, membrane transport and modeling. Biotechnol. Adv., 25: 244-263.
- Prescott, M.L., J.P. Harley and D.A. Klein, 2005. Microbiology. 6th Edn., McGraw-Hill, New York, pp: 239-245.
- Waites, M.J., N.L. Morgan, J.S. Rockey and G. Higton, 2008. Industrial Microbiology: An Introduction. Blackwell Publishing, Oxford, pp: 155-158.
- Yalcin, S.K., M.T. Bozdemir and Z.Y. Ozbas, 2009a. A comparative study on citric acid production kinetics of two *Yarrowia lipolytica* strains in two different media. Indian J. Biotechnol., 8: 408-417.
- Yalcin, S.K., M.T. Bozdemir and Z.Y. Ozbas, 2009b. Utilization of whey and grape must for citric acid production by two *Yarrowia lipolytica* strains. Food Biotechnol., 23: 266-283.
- Yalcin, S.K., M.T. Bozdemir and Z.Y. Ozbas, 2010. Citric acid Production by Yeast: Fermentation Conditions, Process Optimization and Strain Improvement. In: Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, Mendez-Vilas, A. (Ed.). Formatex, Turkey, pp: 1374-1382.