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Research article

Citric acid production using rotating biodisc reactor (RBR)

Ugur Sidal*[1]

[1] *Manisa Celal Bayar University, Faculty of Arts and Sciences, Department of Biology, 45030, Manisa, Turkey*

Abstract

Citric acid is one of the most remarkable industrial goods fabricated by industrial fermentation using filamentous fungus. When *Aspergillus niger*, a filamentous fungus, is produced under suitable conditions, high amounts of citric acid are obtained. This study aims to explain the citric acid synthesis studied in a biodisc reactor using *A. niger*. Various physiological parameters have been investigated in order to determine the optimum citric acid synthesis in biodisc reactor. Optimum incubation time was found to be as 168 hours in the study. The optimum value of the incubation temperature was determined to be 30°C. The optimum value of the initial pH was found to be 3.8. Optimum citric acid synthesis occurred when the disc rotation speed was 2 rpm. In addition, the optimum value of the initial sugar (sucrose) concentration was determined to be 20%. In a semi-continuous production study with the renewal of the medium after a certain incubation period, it was determined that the citric acid yield increased approximately 3 times compared to the batch system. As a result, the highest value of citric acid was determined as 67.65 g/L.

Keywords: *Aspergillus niger; citric acid; rotating biodisc reactor; semi-continuous production*

1. Introduction

Organic acids are one of the most important groups among organic molecules that include one or more carboxyl categories and are profoundly found in the structure of most living things. Acids including lactic acid, acetate, citrate, isocitrate, α-ketoglutarate, succinate, propionate, butyrate, fumarate, sorbic acid, malic acid and their salts are members of this group (Verhoff, 2003). Among these, citric acid (CA) is one of the most functional tricarboxylic acids among the organic acids of biological origin that find wide use in many industries. Citric acid is an indispensable food additive used in the food industry, food and beverage industry. It is stated that citric acid is added to the composition of many foods as an acidifier, aroma enhancer, preservative, emulsifier, stabilizer and antioxidant in the food industry. The use of citric acid as a food additive has been approved by the Food and Agricultural Organization (FAO) and the World Health Organization (WHO) without any restrictions, and this substance is also included in the Generally Recognized as Safe (GRAS) lists (FAO/WHO, 2002). It is known that the food field uses nearly 70% of the entire citric acid yielded in the world (Sahasrabudhe and Sankpal, 2001; Dhillon et al., 2017). The main feature that makes citric acid more usable than other acidifiers is shown to have low toxicity (Lotfy et al., 2007; Kamzolova et al., 2011).

As reported, citric acid (2-hydroxy-1,2,3-propane tricarboxylic acid) was initially being crystallized from citrus extract by Scheele in 1784 (Mattey, 1992). According to the researchers, it is known that over 90% of citric acid fabrication in the globe is handled by fermentation. In the yield of citric acid by fermentation, the filamentous fungus *Aspergillus niger* and the yeast *Yarrowia lipolytica* are mainly used (Max et al., 2010; Wyrzykowski et al., 2011; Souza et al., 2014; Apelblat, 2016). In recent studies, it has been reported that the yield of citric acid by microorganisms is a complex process involving many metabolic and morphological changes in the cell and is affected by many parameters (Anastassiadis et al., 2002; Kamzolova et al., 2011; Morgunov et al., 2013). It is reported that the type of substrate used in the medium and its initial concentration, mineral content, nitrogen type and nitrogen concentration source

* Corresponding author.
E-mail address: ugur.sidal@bayar.edu.tr (U. Sidal).
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are the effective parameters for production. Besides these; it is stated that some parameters including pH, temperature, oxygen concentration and mixing speed should be kept at optimum values (Soccol et al., 2006; Moeller et al., 2007). Another important parameter is the selection of the strain to be used to increase the yield or the development of new strains. Therefore, from the early 2000s, researchers have focused on obtaining new strains that can produce high levels of citric acid by genetic modifications or mutation (Soccol et al., 2006). It is known that the most important thing in the yield of citric acid is to reduce the process cost by using some natural substrate resources to make the process more economical. The use of some raw materials, industrial residues, wastes and by-products as substrates is quite important in terms of waste removal and process costs (Gonçalves et al., 2009).

Yeast and yeast-like organisms responsible for the fermentation constitute a homogeneous and very large group of fungi. About 1500 species have been identified to date, but the taxonomy of yeast is constantly changing with the development of molecular biological methods. In addition, the increasing interest in yeast and yeast taxonomy lead to a raise in the number of species (Corbaci, 2008; Akpinar et al., 2011). One of the most important members of this group is *A. niger*. In 1917, Currie discovered that *A. niger* could produce significant amounts of citric acid in sugar-containing media. Later, it was determined that other Aspergillus species can also produce citric acid. However, studies have indicated that the most suitable mold for the yield of citric acid is *A. niger* (Yalcin et al., 2010). Mainly citric acid obtained from molds including *A. niger* is affected from different conditions including carbon source, substrate, pH, nitrogen amount, temperature, trace elements, thiamine amount, aeration. Another important factor is the fermentation type. It is stated that a great amount of the world's citric acid production is handled by fermentation (Soccol et al., 2006). Industry-related citric acid yield is performed in three distinct methods: surface fermentation (SF), submerged culture fermentation (SmF) and solid state fermentation (SSF), also known as the “koji process”. Today, approximately 80% of citric acid production is done by submerged culture fermentation (SmF) carried out with batch, batch fed or continuous systems (Dhillon et al., 2017).

In this study, a rotating biodisc reactor (RBD) was utilized for citric acid production. The rotating biodisc reactor, which is most commonly used for wastewater treatment, was used for fermentation in this study. Controlling the conditions of environment and thickness of biofilm in rotating biodiscs is easier than biological filters because the liquid phase is more homogeneous and the biofilm is apparent and accessible in the process of the procedure. In addition, ventilation is highly more influential for rotation of biodiscs as a consequence of direct connection of biofilm and air turning process (Kargi and Eker, 2002). Considering these advantages, rotating biodisc reactor was preferred in this study.

2. Materials and methods

2.1. Microorganism and growth conditions

A. niger culture was obtained from Hacettepe University Faculty of Science Biology Department Culture Collection. Cultures were prepared by seeding on Sabouraud Potato dextrose-agar (SPA) media and incubated at 30ºC for 5 days in the incubator. The continuity of the cultures was provided by periodic passages to SPA media. The microorganism was planted in a sterile medium prepared in petri dishes, and spores in black color were formed in the incubator for 7 days at 30ºC. Since the duration of the microorganism's activity in the solid medium is approximately 30 days, the continuity of the culture was ensured by planting in the solid medium and storing it at +4ºC.

2.2. Citric acid production medium

The ingredients of the medium used in citric acid production are as follows; sucrose: 200 g/L, KH_2PO_4: 1 g/L, $MgSO_4.7H_2O$: 0.25 g/L, NH_4NO_3: 0.05 g/L, $CaCl_2$: 3 g/L, $CuSO_4.5H_2O$: $0.06x10^{-3}$ g/L, $ZnSO_4.7H_2O$: $0.25x10^{-3}$ g/L, $FeSO_4.7H_2O$: $1.3x10^{-3}$ g/L. Sucrose/saline solutions were prepared separately and sterilized by adjusted to pH 3.5.

2.3. Determination of citric acid concentration

The concentration of citric acid was determined by the pyridine-acetic anhydride (PAA) assay (Marier and Boulet, 1958). Citric acid analysis was performed at 425 nm wavelength with a Cecil 5000 UV/VIS model spectrophotometer.

2.4. Biodisc reactor and citric acid production

The reactor used in this study is made of glass and 19x25x27 cm in size. 8 pellet discs with 17 cm diameters connected to a shaft are placed in this container (Fig. 1). Disc rotations are provided by a DC-type electric motor connected to the shaft. An exchangeable transformer has been integrated into the system to adjust the rotation speed of the discs. The prepared production medium (total volume 4 L) was filled into the reactor to cover the half surfaces of the discs and the system was placed in the incubator (Sanyo). The microorganism in the stock medium was added to the medium at a ratio of 1: 20 (200 mL culture / 4 L production medium). Under these conditions, production was continued for 7 days. Periodically (12 hours) specimens were taken to detect the overall amount of sugar and citric acid in the biodisc reactor. The growth in the biodiscs was determined by measuring the thickness of the biofilm with a millimeter caliper.

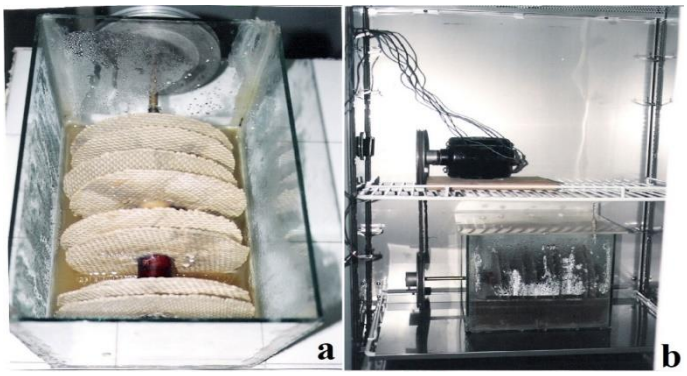

Fig. 1. (a) Top view of rotating biodisc reactor, (b) Side view of rotating biodisc reactor.

2.5. Analysis of the effect of disc rotation speed on growth and citric acid synthesis

To analyze the impact of disc rotation speed on growth and citric acid synthesis, the disc rotation speeds were adjusted to 1, 2, 3, 4 and 5 cycles/min by an exchangeable transformer.

3. Results and discussion

3.1. Reproduction and citric acid synthesis in A. niger depending on time in biodisc reactor

The production of citric acid and increase rate of the growth of *A. niger* strain at the initial sucrose concentration of 20% are shown in Table 1. As indicated in Table 1, the highest growth and citric acid production amount were obtained at 168 hours. Results are given as the average of three replicates.

3.2. The effect of temperature on reproduction and citric acid production with A. niger in biodisc reactor

As given in Table 2, the optimum incubation temperature both for production of citric acid and for the growth rate of *A. niger* strain was found to be 30°C. Results are given as the average of three replicates.

3.3. The effect of preliminary incubation pH on reproduction and citric acid production with A. niger in biodisc reactor

As seen in Table 3, the optimum initial incubation pH for both citric acid production and the growth rate of *A. niger* strain was found to be 3.8. Results are given as the average of three replicates.

3.4. The effect of initial sucrose concentration on reproduction and citric acid production with A. niger in biodisc reactor

As seen in Table 4, the optimum initial sucrose concentration for both citric acid production and the growth rate of *A. niger* strain was obtained at 20% g/L. Results are given as the average of three replicates.

3.5. The effect of disc rotation speed on reproduction and citric acid production with A. niger in biodisc reactor

As seen in Table 5, the optimum disc rotation speed for both citric acid production and the growth rate of *A. niger* strain was found to be 2 cycles/min. Results are given as the average of three replicates.

3.6. Citric acid production with semi-continuous production system

In semi-continuous production analysis performed by renewing the medium after a certain incubation period (1.5 mL of medium per minute addition), the citric acid yield increased approximately 3 times compared to the batch system. As a result of the analyzes, the highest value of citric acid was determined as 67.65 g/L (Table 6).

Table 1
Reproduction and citric acid synthesis in *A. niger* depending on time in biodisc reactor.

Time (Hour)	24	48	72	96	120	144	168	192
Citric acid (g/L)	0.69±0.578	2.04±0.079	3.19±0.07	4.54±0.02	6.4±0.003	11.52±0.055	17.14±0.026	16.87±0.017
Biofilm thickness (mm)	0.8	1.4	2	3.6	5.8	7.6	9.4	9.6

Table 2
The effect of temperature on reproduction and citric acid production with *A. niger* in biodisc reactor.

Temperature (ºC)	20	25	30	35	40
Citric acid (g/L)	12.24±0.025	14.69±0.003	17.14± 0.026	13.71±0.005	13.17±0.003
Biofilm thickness (mm)	6.7	8.0	9.4	7.5	7.4

Table 3
The effect of preliminary incubation pH on reproduction and citric acid production with *A. niger* in biodisc reactor.

Initial pH	3.2	3.5	3.8	4.1	4.4
Citric acid (g/L)	14.69±0.001	15.28±0.026	17.14±0.026	12.73±0.005	12.24±0.025
Biofilm thickness (mm)	8.1	8.4	9.4	7.0	6.7

Table 4
The effect of preliminary sucrose concentration on reproduction and citric acid production with *A. niger* in biodisc reactor (here, lower case used for biodisc reactor, but upper case used in the above title).

Initial Sucrose Concentration (g/ L)	14	16	18	20	22
Citric acid (g/L)	15.34±0.01	15.78±0.9	16.23±0.028	17.14±0.026	16.69±0.015
Biofilm thickness (mm)	8.4	8.6	8.9	9.4	9.4

Table 5
The effect of disc rotation speed on reproduction and citric acid production with *A. niger* in biodisc reactor.

Disc Rotation Speed cycles/min	1	2	3	4	5
Citric acid (g/L)	16.29±0.577	17.14±0.026	15.43±0.003	14.57±0.05	12.68±0.05
Biofilm thickness (mm)	8.9	9.4	8.5	8.0	7.0

Table 6
The effect of preliminary incubation pH on reproduction and citric acid production with *A. niger* in biodisc reactor.

Time (day)	7	9	11	13	15	17	19	21
Citric acid (g/L)	17.14±0.026	24.51±0.577	34.08±0.002	42.85±0.079	51.42±0.055	58.92±0.052	67.65±0.017	67.56±0.001
Biofilm thickness (mm)	9.4	9.4	9.5	9.6	9.7	9.8	9.9	10.0

This study consists of two stages. The first stage is for the optimization of the bioreactor working in batch order with sucrose, while the second stage is the production of citric acid with a semi-continuous system in optimized conditions obtained in the first stage. The optimum incubation time for the highest amount of citric acid production rely on the production conditions, the species and the fermentation process. The ratio of citric acid biosynthesis was examined (Table 1) and a maximal profit of citric acid (17.14 ± 0.026 g/L) was obtained 168 hours after inoculation. The production started following a one-day lag period in citric acid fermentation with *A. niger* and achieved its maximal rate at the beginning of stationary period. Biofilm thickness was measured as 9.4 mm. Prolonged incubation did not further increase the citric acid production. In similar studies, the researchers reported the optimum incubation time as between 144-240 hours (Currie, 1917; Wieczorek and Brauer, 1998; Demirel, 2003; Peksel, 2003; Max et al., 2010; Apelblat, 2016). Rajoka et al. (1998) reported that they achieved a product formation rate of 0.0506 g/L/ hour. This result is lower than our presented results.

The temperature of the fermentation mixture is known as one of the significant issues that have a limiting effect on fungal citric acid production. In the present study, it was found that the best temperature for citric acid fermentation was 30 ºC (Table 2). When ambient temperature is low, it certainly had no positive impact on citric acid production, as well as on the enzymatic activity. However, the citric acid biosynthesis decreased when the production temperature increased above 30 ºC. This may have occurred as a result of the accumulation of by-products, including oxalic acid. Various researchers made use of 30ºC as the production temperature in different studies and gained higher level of amounts of the concrete yield (Vergano et al., 1996; Arzumanov et al., 2000; Ramesh and Kalaiselvam, 2011). However, as the reported values are proportional to the fermentation time, it is observed that the values obtained with the strain used in this survey are close to the values obtained in other researches, and some values are even lower. It is very important to maintain a proper pH for the desired level of citric acid production. The effect of various initial pHs (3.2 - 4.4) on citric acid production in the medium was investigated and maximum yield was calculated with the initial pH of 3.8 (Table 3). Lowering pH values resulted in a decline in citric acid production amount, because low pH may inhibit mycelium growth. This finding is in agreement with the result reported by Pessoa et al. (1982). In addition, during the fermentation process with *Aspegillus niger* in the production of citric acid, the pH of the medium is of great importance and nitrogen metabolism causes proton release that lowers the pH (Amenaghawon et al., 2013). The pH of a culture can change as a response to microbiological metabolism. The most remarkable cause for this change is the discharge of organic acids including citric acid that will lead a drop in pH. Fluctuations in pH are mostly dependent on microorganisms. The origin of the substances and the manufacturing procedures also affects the pH kinetics. Therefore, the initial pH is to be defined elaborately and made efficiently use of depending on the microorganism, substrate and production technique. A higher initial pH was also an inhibitory factor for *A. niger* growth and citric acid production. *A. niger* has an invertase enzyme active at low pH values which utilizes sucrose, rather than glucose, as the main substrate for citric acid production. Due to its low molecular weight, sucrose is simply transferred by intracellular enzymes in microbial cells for hydrolysis (Kareem et al., 2010; Padvi and Pawar, 2011). Kudzai et al. (2016) found that the medium supplemented with sucrose produces more citric acid than the glucose medium stating that sucrose is the main substrate for citric acid production. In addition, it has been pointed out that the concentration, as well as the type of sugar, play an important role in citric acid production. The maximum level of production is generally occurs between 14-22% sugar concentrations and no citric acid production has been reported in environments containing less than 2.5% sugar. We investigated the effect of initial sucrose concentration (14-22 g/L) on growth and citric acid production and had the maximum results at 20% sucrose concentration (Table 4). Increased sugar concentration in the medium causes a decrease in citric acid produced by *A. niger*. This may be attributed to the fact that high levels of sugar concentrations are caused by an excessive growth of mycelium leading to an increase in viscosity in the environment which reduces the citric acid production in the fermentation medium. On the other hand, it has been reported that low sugar levels cause low citric acid production because of oxalic acid accumulation in the culture medium (Prasad et al., 2013).

Taking the advantages of bioreactor systems into account, rotating biodiscs are mostly utilized in biological treatment. However, one of today's economic principles is to reduce raw material costs and increase the production. This is the specificity of our work towards this purpose, because such a method was rarely encountered in previous studies on production of citric acid. For this reason, citric acid production with this type of reactor becomes important due to its rarity, as well as its numerous advantages. A similar study on obtaining different products was reported by Sidal and Taskin-Ozkale (2003). From this point on, the effect of disc rotation speed on citric acid synthesis was investigated and it was observed that the results obtained at 2 cycles / minute were maximum (Table 5). Similar results were reported by Guven (1995). As can be seen from the results of our study, biodiscs rotation caused by direct contact of the biofilm with the ventilation in the return process is very important. Because, the contact time of the biofilm with air increases the yield. In a semi-continuous production study with the renewal of the medium (1.5 mL of medium per minute) after 7 days of incubation under optimized conditions, it was determined that the yield of citric acid increased approximately 3 times compared to the batch system. After 21 days of incubation, the highest value of citric acid was found to be 67.65 g/L on the 19th day (Table 6). As an important finding, incubation time longer than 19 days did not cause an increase in citric acid production.

4. Conclusion

Our results show that this type of reactor can be used for citric acid production. However, studies that increase productivity as to raise the amount of citric acid to more suitable levels on an industrial scale may increase the possibility of using this process in the future.

Conflict of interest: The author declares that he has no conflict of interests.

Informed consent: The author declares that this manuscript did not involve human or animal participants and informed consent was not collected.

References

Akpinar, O., Ucar, B. F., & Yalcin, H. T. (2011). Screening and regulation of alkaline extracellular protease and ribonuclease production of *Yarrowia lipolytica* strains isolated and identified from different cheeses in Turkey. *Annals of Microbiology*, *61*(4), 907-915.

Amenaghawon, N. A., Areguamen, S. O., Agbroko, N. T., Ogbeide, S. E., & Okieimen, C. O. (2013). Modelling and statistical optimisation of acid production from solid state fermentation of sugar cane bagasse using *Aspergillus niger*. *International Journal of Sciences*, *2*, 56-62.

Anastassiadis, S., Aivaidis, A., & Wandrey C. (2002). Citric acid production by *Candida* strains under intracellular nitrogen limitation. *Applied Microbiology and Biotechnology*, *60*, 81-87.

Apelblat, A. (2016). Representation of volumetric and compressibility properties in binary and ternary system. *Journal of Solution Chemistry*, *45*, 1530-1547.

Arzumanov, T. E., Shishkanova, N. V., & Finogenova, T. V. (2000). Biosynthesis of citric acid by *Yarrowia lipolytica* repeat-batch culture on ethanol. *Applied Microbiology and Biotechnology*, *53*(5), 525-529.

Currie, J. N. (1917). The citric acid fermentation of *Aspergillus niger*. *Journal of Biological Chemistry*, *31*,15-37.

Corbaci, C. (2008). The effect of sodium on the growth of *Debaryomyces hansenii* strains at high temperature and extreme pH, Master Thesis, (pp.1-186). Ege University, Institute of Science, Turkey.

Demirel, G. (2003). Citric acid production using immobilized *Aspergillus niger* and investigation of various effects on citric acid production, Master Thesis, (pp. 1-56). Gazi University Institute of Science and Technology, Turkey.

Dhillon, G. S., Lea Rosine, G. M., Kaur, S., Hedge, K., Brar, S. K., Drogui, P., & Verma, M. (2017). Novel biomaterials from citric acid fermentation as biosorbents for removal of metals from waste chromated copper arsenate wood leachate. *International Biodeterioration and Biodegradation*, *119*,147-154.

FAO/WHO (2002) Food and Agriculture Organization of the United Nations and World Health Organization. Human Vitamin and Mineral Requirements, Rome. https://www.fao.org/3/y2809e/y2809e.pdf, Last Accessed on March 13, 2022.

Gonçalves, C., Lopes, M., Ferreira, P. J., & Belo, I. (2009). Biological treatment of olive mill wastewater by non-conventional yeasts. *Bioresource Technology*, *100*, 3759-3763.

Guven, M. **(**1995). Citric acid production using *Aspergillus niger* (Fürsan) in Biodisc Reactor, Doctoral Dissertation, (pp.1-85). Hacettepe University, Institute of Science and Technology, Turkey.

Kamzolova, S. V., Lunina, J. N., & Morgunov, I. G. (2011). Biochemistry of citric acid production from rapeseed oil by *Yarrowia lipolytica* yeast,.*Journal of American Oil Chemistry Society*, *88*, 1965-1976.

Kareem, S. O., Akpan, I., & Alebiowu, O. O. (2010). Production of citric acid by *Aspergillus niger* using pineapple waste. *Malaysian Journal of Microbiology*, *6*(2), 161-165.

Kargi, F., & Eker, S. (2002). Comparison of performances of rotating perforated tubes and rotating biodiscs biofilm reactors for wastewater treatment. *Process Biochemistry*, *37*, 1201-1206.

Kudzai, C. T., Ajay, K. & Ambika, P. (2016). Citric acid production by *Aspergillus niger* using different substrates. *Malaysian Journal of Microbiology*, 12(3), 199-204.

Lotfy, W. A., Ghanem, K. M., & El-Helow, E. R. (2007). Citric acid production by a novel *Aspergillus niger* isolate: II. Optimization of process parameters through statistical experimental designs. *Bioresource Technology*, *98*, 3470-3477.

Marier, J. R., & Boulet M. (1958). Direct determination of citric acid in milk with an improved pyridine-acetic anhydride method. *Jornal of Dairy Science*, *4*, 1683-1692.

Mattey, M. (1992). The production of organic acids. *Critical Reviews Biotechnology*, *12*(1/2), 87-132.

Max, B., Salgado, J. M., Rodriguez, N., Cortes, S., Converti, A., & Dominguez, J. M. (2010). Biotechnological production of citric acid. *Brazilian Journal of Microbiology*, *41*, 862-875.

Moeller, L., Strehlitz, B., Aurıch, A., Zehnsdorf, A. & Bley, T., (2007). Optimization of citric acid production from glucose by *Yarrowia lipolytica. Engineering in Life Sciences*, *7*(5), 504-511.

Morgunov, I., Kamzolova, S., & Lunina, J. (2013). The citric acid production from raw glycerol by *Yarrowia lipolytica* yeast and its regulation. *Applied Microbiology Biotechnology*, *97*, 7387-7397.

Padvi, M. A., & Pawar, S. V. (2011). Factors affecting production of citric acid. *International Referred Research Journal*, *3*, 37-38.

Peksel, A., Tokatman, K. A., & Arısan Ataç, İ. (2003). Investigation of the effects of some polyols on yield during citric acid fermentation by *Aspergıllus niger*. *Journal of Yıldız Technical University, 4*, 1-6.

Pessoa, D. F., Dıasde, C., & Angela, C. (1982). Production of citric acid by *Aspergillus niger*. *Revista de Microbiologia*, *13*(2), 225-229.

Prasad, M. P. D., Sridevi, V., Surendra N. V., Reddy, O. V. S., & Harsha, N. (2013). Studies on fermentative production of citric acid by *Aspergillus niger* isolate using sorghum malt and it's optimization. *International Journal of Innovative Research in Science, Engineering and Technology*, *2*, 2961-2968.

Rajoka, M. I., Ahmad, M. N., Shahıd, R., Latıf, F., & Parvez, S. (1998). Citric acid production from sugar-cane molasses by cultures of *Aspergillus niger*. *Biologia*, *44*(1), 241-253.

Ramesh, T., & Kalaiselvam, M. (2011). An experimental study on citric acid production by *Aspergillus niger* using *Gelidiella acerosa* as a substrate. *Indian Journal of Microbiology*, *51*(3), 289-293.

Sahasrabudhe, N. A., & Sankpal, N. V. (2001). Production of organic acids and metabolites of fungi for food industry. In: Arora D. K., Berka R. M., Singh (eds) *Applied Mycology and Biotechnology* (pp. 387-425). Elsevier Amsterdam.

Sidal, U., & Taskin-Ozkale, E. (2003). Rhamnolipid production from olive oil mill effluent (OOME) using the rotating biodisc reactor. *Biologia, Bratislava*, *58*(6),1083-1088.

Soccol, C.R., Vandenberghe, L. P. S., Rodrigues, C., & Pandey, A. (2006). New perspectives for citric acid production and application, *Food Technology and Biotechnology*, *44*(2), 141-149.

Souza, K., Schwan, R., & Dias, D. (2014). Lipid and citric acid production by wild yeasts grown in glycerol. *Journal of Microbiology and Biotechnology*, *24*, 497-506.

Vergano, M. G., Femandez, N., Sorıa, M. A., & Kerber, M. S. (1996). Influence of inoculum preparation on citric acid preparation by *Aspergillus niger*. *Journal of Biotechnology*, *12*(6), 655-656.

Verhoff, F. H. (2003*).* Citric acid. In: Elvers B., Hawkins S., Harrer R., Pikart-Müller M. (eds) *Ullmann's Encyclopedia of Industrial Chemistry* (pp. 8:525-531). Germany.

Yalcin, S. K., Bozdemir, M. T., & Ozbas, Z. Y. (2010). Citric acid production by yeasts: Fermentation conditions, process optimization and strain improvement, *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, *9*, 1374-1382.

Wieczorek, S., & Brauer, H. (1998). Continuous production of citric acid with recirculation of the fermentation broth after product recovery. *Bioprocess Engineering*, *18*(1), 1-6.

Wyrzykowski, D., Hebanowska, E., Nowak-Wiczk, G., Makowski, M., & Chmurzyński, L. (2011). Thermal behaviour of citric acid and isomeric aconitic acids. *Journal of Thermal Analysis and Calorimetry*, *104*(2), 731-735.

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