Journal of Advanced Research in Science and Technology
ISSN: 2352-9989

A biotechnological test the production of citric acid by using two strains of *Aspergillus niger* ATCC-16404 and ANSS-B5 in solid-state fermentation by dates

Djafri Kaouthar*, Khemissat Elhayfa; Bergouia Meriem
National Institute of Agricultural Research of Algeria; Experimental station INRAATouggourt BP 17, Ouargla 30200 Algeria

Abstract. The objective of this study is to optimize the production of citric acid by using two strains of *Aspergillus niger* ATCC 16404 and ANSS-B, this strains were cultivated in a medium based on scrap dates flour. In this sense, different culture conditions such as water content, initial pH and nitrogen and phosphorus contents were optimized. The results obtained show the optimum conditions for maximum production of citric acid are:A water content of 70.0%, an initial pH of 2.5, a nitrogen source is yeast extract at a content of 3.0 g / Kg and a content of Potassium phosphate 2.5 g / Kg. Maximum amounts and yields of citric acid of 173.80 - 201.60 g / Kg and 66.84 - 73.31%, were obtained with these optimum conditions. Thus, it can be concluded from the results of the present study that flours obtained from date scrap can serve as a potential substrate for the production of citric acid by A. Niger ATCC 16404 and ANSS-B5 by Solid State Fermentation (SSF).
Key words: Valuation, *Aspergillus Niger*, Citric acid, Date scrap, SSF, Strains

1. Introduction

Agriculture and food industry activities generate large quantities of organic-rich waste, which may constitute new raw materials in several industries. For this purpose,Their valors by biotechnological processes represent a solution for the production of several metabolites, including ethanol, citric acid and enzymes. The production of these metabolites depends on the strain, the composition of the fermentation medium and the culture conditions. The production of dates is evaluated with 790.000 tons among them between 95.000 and 105.000 tons is less appreciated on the market, established by low market value dates and date wastes of Deglet-Nour [1] .cultivar The worldwide production of citric acid is approximately 1.6 million tons a year and it is in increase in day in day[2] .citric acid product par *Aspergillus niger*is one of the examples most commercially. Many microorganismssuchas the mycètes andbacteria can produce the citric acid. Various themycètes, which proved to be good accumulators of the citric acid in their culture media. include the stains of *Awamori, A. niger, A. restrictum, Penicillium, Piriformis,, Trichoderma viride, Mucor* and de *Lipolytica* [3]. Mutant strains could improve the production of citrate compared with the wild origins (stumps). (Sikander and *al.*, 2001) [4]. In addition, there are several processes of production of the citric acid.The process most usually used are submerged fermentation and Solid State Fermentation (SSF).

Fermentationin a solid state (SSF) is anothermethod used for the productionof the citric acid by using the agro-industrial residues. It offerspotential advantages in the treatment of these residues (Brand et *al*., 2002) [5].

* Corresponding author. Djafri K
E-mail:
Address: National Institute of Agricultural Research of Algeria; INRAATouggourt BP 17, Ouargla 30200 Algeria

The SSF is characterized by a development of microorganisms in an environment having a low water activity and the material not soluble as the source of nutrients and as physical support (Pandey and *al.,* on 2000)[6]. During these last years,A. Niger showed a considerable interest in the use of agricultural products and their residues as sources of carbon for the production of the citric acid. Several agro-industrial residues and under products were used for the production of the citric acid by using the SSF (Vandenberghe and *al.,* in 2000) [7].

A reduction of the manufacturing costs of the citric acid can be realized by using less expensive substrates , such as the fruit residues wastes of carrot and orange , the bagasse of the cassava , the peeled coffee, the residues of soybean, the rice and wheat bran (Alben and Erkmen ,2004[8-12].;Hang ,1998.;Pradoand ,2005;Vandenberghe ,1999;Vandenberghe ,2004) these residues are very well adapted to fermentation in a solid state due to their nature 's cellulose and strachy . Lastly the main advantages of the use of fermentation in a solid state (SSF) compared to submerged fermentation are:

1/ the yields are much higher than those in the liquid media .

2/ the space in the tank of fermentation is low compared to the yield of the obtained product,because we use less water and the substratum is concentrated.

3/Operating costs are clearly lower than those of the submerged fermentation.

The objective of this study is to optimize parameters of production of the citric acid produced by two strains of *A. Niger* ATCC 16404 and *A.Niger*ANSS-B5 by using scraps of date of varietyDeglet-Nour. The used process of fermentation is the process Solid State Fermentation (SSF).

2. Methodology

2.1. Plant material

The vegetable material usedis made up by the date wastes produced by the cultivar Deglet-Nour. The latter are dates shrive ledand desiccated before maturity which one calls commonly (H'chiefs).

Figure 01: Deglet nour cultivar wastes

The microorganism implemented is amould of kind *A. niger*. The y are two pure stocks of the collection of laboratory INRAA of Touggourt. These stocks were preservedat4°C in Sabouraud agar in the presence of oxy tetracycline.

- The stain of *AspergillusNigerANSS-B5*, isolated locally from the Saharas oils
- The strain of*AspergillusNigerATCC*16406 commercial strain

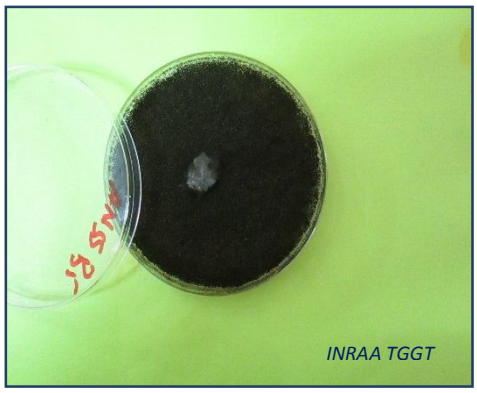

Figure 2. *A. niger* (macroscopic aspect)

Figure 3: *A. niger* (microscopic aspect)

2.2. Method

2.2.1 Preparation of the substrate (flourof date wastes)

To obtain wastes flour, awashing the wastes dates with water distilled in order to eliminate all the undesirable substances, adraining, the nremoving the kernel. After that, a dryingin the drying oven with 50°C during 24h followed byone crushing usin gacrusher 2.0 mm sin diameter.The flouris recovered in kraft paper.

2.2.2. Preparation of the inoculum

The strain is inoculated into petri dishes containing 10.0 ml of the culture medium of the following composition (El-Holi and *al.*, 2003) [13]: Sucrose, 140 g / L, Agar, 20 g / L, KH_2 PO_4 1.0 g / L, $MgSO_4$, 0.25 g / L, NH_4NO_3, 2.5 g / L, $CuSO_4$, 0.34 mg / L, $ZnSO_4$, 2.3 4mg / L and NH4Fe (SO_4) 2, 0.8 mg / L. After 4days of incubation at 30°C, spores appear on the surface of the mycelia carpet. The yare recovered in sterile distilled water then counted using the cell of Thomas. The fungi suspension is then diluted in order to obtain a concentration at level of 106 to 107 spores/ml [13; 14.]

2.2.3. Real fermentation:

The Fermentation was carried out on a solid medium (Solid State Fermentation) in an incubator. The cultures were carried out in Erlen meyers of 1000 ml of capacity, filled with 250.0 ml of the culture medium. The sterilized media were inoculated with a concentration of 106 spores / ml. The amount of inoculum used is 0.5%. These media were incubated at 30 ° C. for 144 h and under continuous stirring at 200 rpm / min. Then, 3.0 ml of methanol and a few drops of the silicone oil are added in order to avoid the overflow of the foam.

2.2.4. Methods of analysis

2.2.4.1. Analyses of the date flour (Date flour analysis)

The water content is determined by drying 10 g of dates in an oven at 105 ° C. for 18 h. The ash content is determined by incinerating one gram of dates in a muffle furnace at a temperature of 550 ° C. for 3 hours. The acidity was determined by titration of 10.0 ml of the date juice diluted ten times with 0.1 N NaOH and a few drops of phenophthalein as a color indicator. The pH is determined using a previously calibrated pH meter (A.O.A.C, 2003) [15]. Reducing sugars, saccharose and total sugars were determined by the method of Bertrand, brought back by Audigieandal (1984) [16].

The principle of this method consists in making an excess of cupro-alkaline liquor act under well-established conditions, and the cuprous oxide is then separated, and treated with a ferric sulphate. In this case, only the reducing sugars.
In order to determine the total sugars, an acid hydrolysis is first carried out in order to release the aldehyde or ketonic functions. In this way, the saccharose is converted into reducing sugars. Finally, sucrose is determined as follows: (Total Sugars - Reducing Sugars) × (0.95).

2.2.4.2. Fermentation product analyzes.

The determination of the pH is essential for the control of the culture medium, before and during fermentation. The variation of the pH in forms us about the metabolicactivity of mushroom (fungus), the refore about the transformation of sugarsintocitrate.The pH is measured using a previously calibrated pH meter. The biomass quantity is determined by filtration through a What man filter, washing with distilled water and drying to constant weight at 70 c°(Haq and Daud, 1995)[17]. The citric acid and residual sugars were determined by HPLC equipped with a detector UV model SPD-10Avp, column models CTO-10Avp. The eluent used for analysis was 0.01 Nsulphuricacid solution [18]

3. Results and discussions

3.1. Biochemical composition of the wastes flour.

Any process of fermentation is related to the quality of the culture medium used. There fore, the determination of the biochemical composition of the date flour is necessary. The results obtained show that the wastes flour produced by Deglet-Nour (H'chefs) has a high total sugar content of 81.6%, a reducing sugar content of 52.1% and a saccharose content of 27.93%. As regards the pH, the wastes flour has a pH of 4.85. It is a pH favorable for the development of *A.niger*. Concerning the dry matter rate, this last is high either 90.0% and one water content weak or 10.0%. As for rock salt, the results obtained show that the flour contains an a verage ash contentis1.93%. In short, one can say that the date flour contains fermentable sugars (glucose and fructose) directly assimilable by *A.niger*. These results are comparable with those obtained by Açourene and *al.* (2001)[19]; Bin-Shahna and *al.*(1987) [20]. Finally, the biochemical analysis of the date flour makes it is of priority to say that the latter can constitute a medium of choice for the production of citric acid by using *A. niger*.

Table 1. Biochemical composition of the date flour

Constituents	Deglet-nour scrap flour
Moisture (%)	5.0
P^H	4.85
Reducting sugars(%)	52.1
Sugars totals (%)	81.6
Saccharose(%)	27.93
Ashes (%)	1.93

3.2 .Citric acid production.

3.2.1 .Effect of moisture content

The results of the effect of the different water contents (70%, 75%, 80% and 90%) on the yield of citric acid during fermentation with A. Niger ANSS-B5 and ATCC 16404 are reported in Table 2.

Table 2.- Effect of moisture content of the substrate on the different fermentation parameters

Moisture	Strains	**70 %**	75 %	80 %	85 %	90 %
Final pH	ANSS-B5	**2.28**	2.34	2.45	2.50	2.63
	ATCC-16404	**2.30**	2.42	2.50	2.65	2.75
Citric acid quantity (g/Kg)	ANSS-B5	**142.00**	105.60	86.80	61.25	42.60
	ATCC-16404	**136.20**	100.60	71.25	53.36	41.56
Biomass (g/Kg)	ANSS-B5	**8.70**	5.50	4.90	4.22	3.90
	ATCC-16404	**7.42**	4.45	3.62	3.56	3.25
Sugars consumed (g/Kg)	ANSS-B5	**228.0**	181.00	158.44	125.62	95.90
	ATCC-16404	**231.65**	181.00	142.66	118.54	94.36
Yielding (%)	ANSS-B5	**62.28**	58.34	54.78	48.75	44.42
	ATCC-16404	**58.79**	55.58	49.94	45.01	44.04

One of the important factors affecting the citric acid production by SSF is the water content of the solid substrates used because it influences thegrowth and the metabolism of the micro-organism, like the phenomena of transfer mass mainly related to the diffusion of the nutrients and oxygen [21]. Furthermore, it causes swelling of the substrate, which facilitates the penetration of the mycelium [21]. The decrease in water content of 90 to 70% resulted in a decrease in the pH value, a rise of the quantity in biomass, the quantity and the yield in citric acid , The decrease in the water content of the medium is advantageous because the risk of contamination of the culture medium is reduced. However, there is a limit below which *A. niger* cannot produce citric acid. This is probably due to the increase in osmotic pressure at low substrate water contents [22]. Thus, the results obtained show that the final pH values are low in 70% water content is 2.28 - 2.30 and slightly higher in 90% water content, is 2.63 - 2.75. Concerning the quantities of biomass produced, they are high with a water content of 70% are 7.42- 8.70 g/Kg and weak with a water content of 90%are 3.25 - 3.90 g/Kg (table 2). In addition, the best quantities and outputs in citric acid were obtained with a watercontent of 70.0% are 136.20 - 142.00g/Kg and 58.79 - 62.28%, respectively.On the other hand, the smallest quantity and the yield are 42.56 - 52.60 g/kg and 45.10 - 54.89 % were obtained with a 90.0 % moisture content (figure 04).

Thus, the results show that the optimal moisture content for a maximal production of citric acid is 70.0 %. Similar results were obtained by[7-24-25] (Ashraf ,2004; Assadi, 2002 ; Gopinath,2011); Kareem ,2010; Kumar ,2008; Pintado,1998; Torrado, 2011; Vandenberghe,2000 . However, Hang , 1998 ; Khare,1994; Khosravi,2008; Roukas [8-21-29-30]Have obtained better yields of citric acid with a water content of the substrate varies between 75 and 80%. On the other hand, Narayanamurthy (2008) [31-32-33]; Prado (2005); Shankaranand (1994) obtained better yields of citric acid with a water content between 50 and 60%.

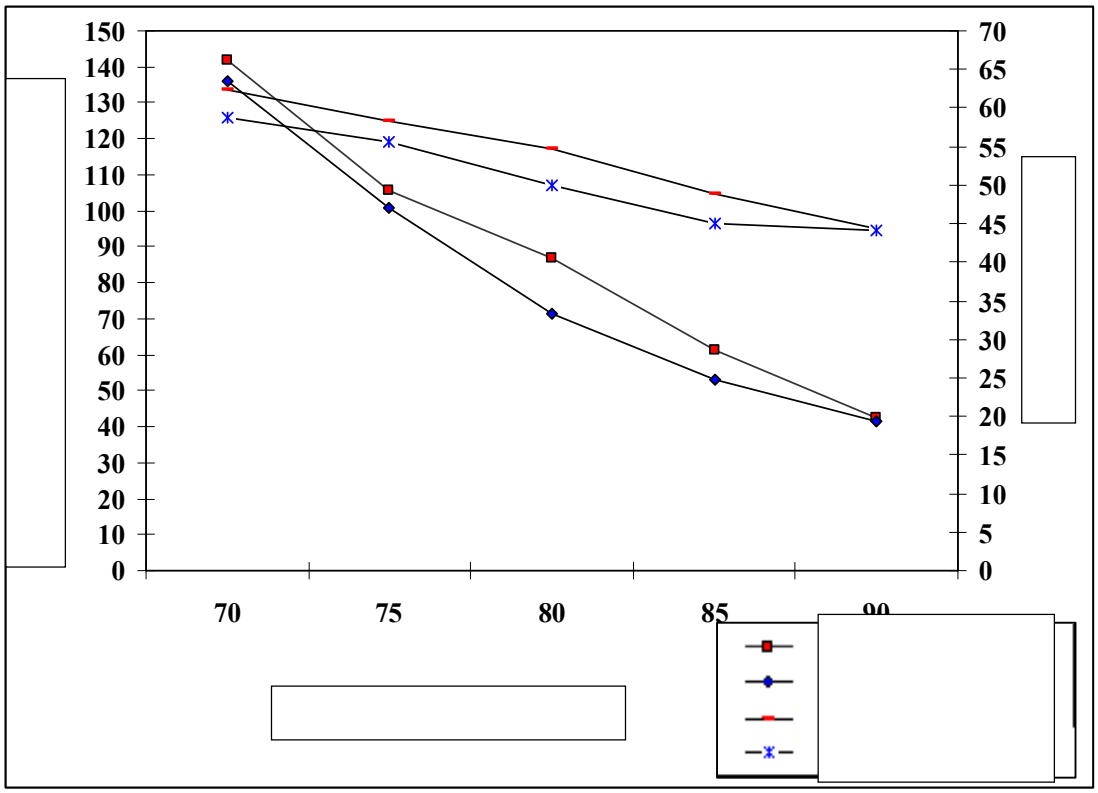

Figure 4. Evolution of the quantity and the yield in citric acid following the moisture content of the substratum

3.2.2.Effect of the initial pH

The production of the citric acid in SSF by *A.niger* is sensitive to the initial pH of the middle of fermentation. Thus, the value of the final pH is slightly higher is 3.20 -3.45 at initial pH 5.0 and bass is 1.69 - 1.96 at initial pH 2.5. More over, a low initial pH inhibits slightly the growth of A. Niger and reduced the risk of contamination of the fermentation by other microorganisms. Furthermore, the quantities of biomass obtained at pH 2.5 and 3.0 vary between 6.82 and 8.60 g/kg. On the other hand, above a pH of 4.0, the quantity in dry biomass increases and the quantities obtained at pH 5.0 vary between 9.60 and 10.22 g/kg (table 3).

Table 3.- Effect of pH on different fermentation parameters.

	***Aspergillus niger* ANSS-B5**					***Aspergillus niger* ATCC16404**				
Initial pH	2.5	3.0	3.5	4.0	5.0	2.5	3.0	3.5	4.0	5.0
Final pH	1.69	2.60	2.28	3.20	3.52	1.96	2.1	2.95	3.30	3.45
Citric acid (g/Kg)	158.40	148.2	142.0	86.4	72.22	153.6	144.80	136.2	96.0	88.75
Biomass (/Kg)	7.80	8.60	8.70	9.54	10.22	6.82	7.00	7.42	9.25	9.60
Sugars consumed (g/Kg)	249.0	242.00	228.0	220.42	225.64	251.44	240.25	231.65	226.34	235.62

Yield in %	63.61	61.23	62.28	39.19	32.00	61.09	60.27	58.79	42.41	37.66

The quantity and yield in citric acid increase as the pH of the medium becomes more acid. Furthermore, a quantity and a best performance in citric acid are 153.60-158.4 g/kg and 61.09-63.61%.respectivety , were obtained with an initial pH of the medium of 2.5(figure 05).

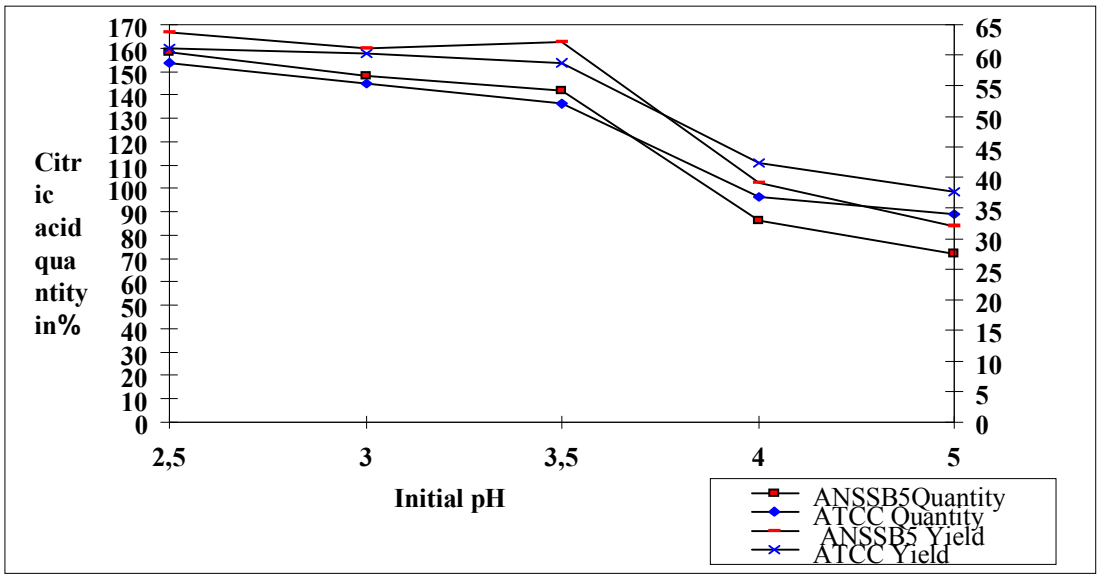

Figure 5.- Evolution of the quantity and the yieldin citric acid following the initial pH

For this purpose, we can say that the optimum initial pH for a maximum production of citric acid is 2.5. These results agree with[12-26- 34] (Darouneh., 2009); (Kareem et *al*., 2010); (Prado.,2004). Which obtained best performance with an initial pH lower than 3.0. According to these authors, a low initial pH below 3.0 inhibits the production of other organic acids such as gluconic acid and oxalic acid**.** In addition, when the pH increase esbeyond 3.5, the production of citric acid decreases gradually and the quantities and the out puts obtained with the pH of 4.0 and 5.0 are about 86.40-96.0 g/Kg, 72.22-88.75 g/Kg and 39.19-42.41 %, 32-37.66 %, respectively. Similar results were got by [4-35] (Al-Obaidi.,1981); (Sikander.,2001). However, (Ashraf.,2004); (Khosravi., 2008); (Narayanamurth, 2008); (Torrado.,2011); (Vandenberghe., 2000) [7-21-23-30-31] obtained high citric acid yields with an initial pH of the culture medium ≥ 4.0. Finally, according to (Kristiansen and Sinclair., 1978) [36], the initial pH has no direct effect on citric acid production. It is possible that pH affects enzymes that are active in the degradation of cellular substances and the permeability of the cell membrane.

3.2.3. Effect of the nitrogen source

Citric acid production is directly influenced by the nitrogen source. According to (Mattey., 1992 [36], the enrichment of the culture medium by the addition of nitrogen is necessary. The results obtained show that the end-fermentation pH values obtained with urea are slightly higher, i.e., 2.60 to 2.87 relative to the other sources of nitrogen, i.e.,1.60 to 1.96 (Table 4).

Table 4. Effect of the nitrogen source on the different fermentation parameters

	***Aspergillus niger* ANSS-B5**				***Aspergillus niger* ATCC16404**			
Nitrogen source	Yeast extract	urea	NH_4NO_3	$(NH_4)_2CO_3$	Yeast extract	urea	NH_4NO_3	$(NH_4)_2CO_3$
Final pH	**1.80**	2.87	1.69	1.88	**1.88**	2.60	1.60	1.96
Citric acid (g / Kg)	**172.82**	80.16	158.4	167.04	**163.52**	70.25	153.6	158.22
Biomass (g / Kg)	**9.46**	11.32	8.83	9.82	**8.54**	10.22	7.42	8.12
Sugars consumed (g / kg)	**269.00**	223.66	249.00	263.22	**263.35**	235.62	251.44	256.44
Yield in (%)	**64.24**	35.84	63.61	63.46	**62.10**	29.81	61.09	61.70

According to several authors, the lowering of the pH(acidification of the culture medium) can be caused by the preferential assimilation of cations NH+ and the accumulation of anions NO3 in the culture medium [14-38-39] As for the quantity of biomass, the latter is highereither10.22-11.32 g/Kg with urea compared to ammonium nitrate i.e.,7.42-8.83g/Kg. In addition, the use of the yeas text ractas nitrogen source gives quantities and out puts in citric acid high are 163.52 - 172.82 g/Kg, and62.10-64.24%, respectively compared to urea are 70.25-80.16 g /Kg and 29.81-35.84 %., respectively (figure06).

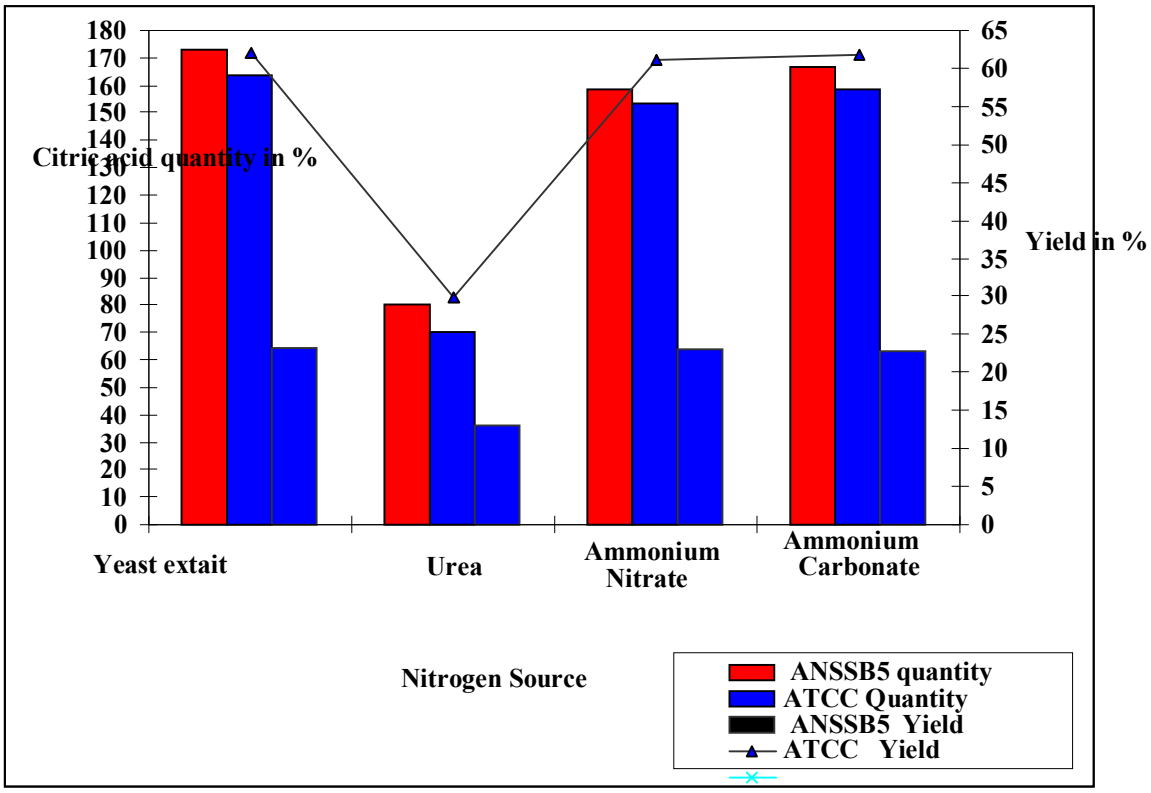

Figure 6. Evolution of the quantity and yield of citric acid according to the nitrogen source

Thus, the yeast extract is the best nitrogen source. These results are consistent with those reported by[28] (Pintado et *al*., 1998). On the other hand, Abou-zeid.,1983; Alben.,2004; EL-Abyad.,1992; Kang.,1989; Kareem.,2010; Shankaranand Lonsane.,1994 [8-26-33-40-41-42] recommends the use of nitrate or ammonium sulphate as nitrogen sources for the production of citric acid in SSF.

3.2.4. Effect of the content of yeast extract

Got the results show that the value of the final pH decreas esslightly with the increase in the content of yeast extract of the medium.This, it passes from2.00-2.30 in the absence of the yeast extract at1.45-1.55 with a content of yeast extract of 4.0g/Kg (table05).

Table 5. Effect of Yeast Extract Content on the Different Fermentation Parameters

	Aspergillus niger ANSS-B5					*Aspergillus niger* ATCC16404				
Yeast extract (g / Kg)	0	1.0	2.0	3.0	4.0	0	1.0	2.0	3.0	4.0
Final pH	2.30	1.96	1.80	1.70	1.45	2.10	2.00	1.88	1.80	1.55
Citric acid (g / Kg)	61.36	111.36	172.82	201.6	198.45	51.45	115.22	163.22	173.8	165.33
Biomass(g / Kg)	3.49	4.68	9.46	13.82	16.28	3.38	3.84	7.54	11.42	15.46
Sugars consumed (g / kg)	152.25	245.54	269.0	275.0	278.0	148.0	240.45	263.35	260.0	252.22
Yield in%	40.30	45.35	64.24	73.31	71.38	34.76	47.91	61.97	66.84	65.54

Quantity in biomass increases as the quantity in extract of yeast added in the middle of fermentation becomes more important and the maximal quantities are 15.46 - 16.28 g / kg were content in yeast extract is 4.0 g / kg yeast. Similar results were obtained by Papagianni and *al*. (2005 [14]). In addition, in absence the yeast extract, small content and yield of citric acid are obtained, namely 51.45 - 61.36 g / Kg and 34.76 - 40.30%, respectively. On the other hand, high yeast extract contents 3.0 and 4.0 g / Kg give high amounts and yields in citric acid . Optimum results were obtained with the yeast extract content of3.0 g / Kg, namely 173.80 - 201.60 g / Kg and 66.84 - 73.31% (Figure 7).

These results agree with those obtained by[26-28 ;35] Al Obaidi.,1981; Kareem .,2010; Pintado, 1998. According to Al-Obaidi , 1981; Kareem , 2010 [26-35]. A content above 3.0 g / kg has as a consequence, caused the disturbance of the fungal growth and the citric acid product. Besides, Kang.,1989; Kristiansen .,1978 ; Shankaran and *al*., 1994 [32-35-41] were obtained maximal quantities in citric acid by adding in the middle of a culture a content in nitrate or sulfate of ammonium varying between 1.0 and 2.0 g / kg.

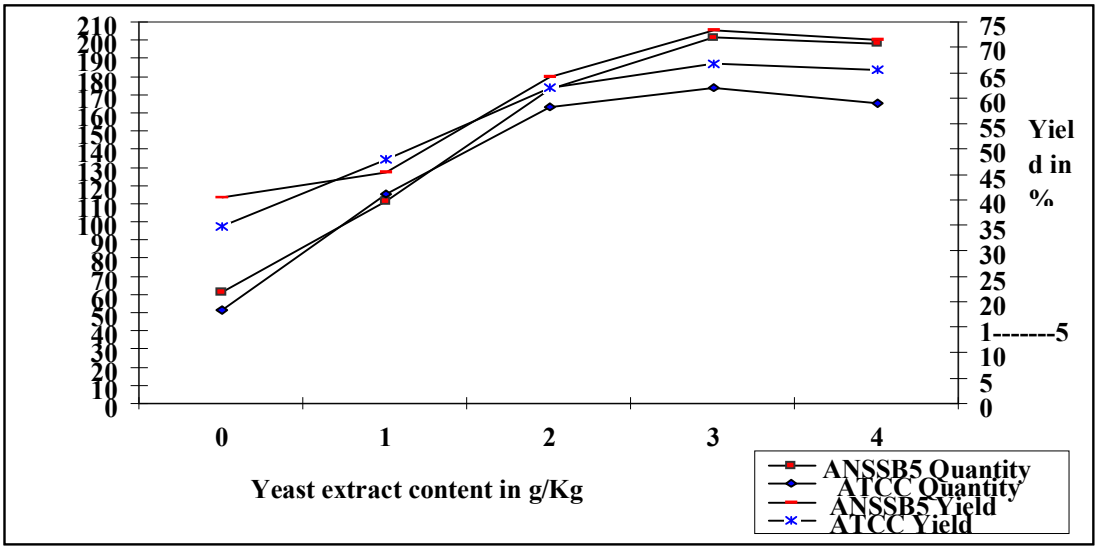

Figure 7.- Evolution of the quantity and the yieldin citric acid following the content of yeast extract

3. 2.5. Effect of the phosphorus content

The presence of phosphorus in the fermentation medium has a significant effect on the yield of citric acid. In addition, potassium phosphate is considered by many authors to be the most appropriate source of phosphorus (Chen., 1994; Kubicek., 1979; Pintado., 1993 ; Pintado., 1998) 43-44-45]. On the other hand, phosphorus is known to be essential for the growth and metabolism of *A. niger* (Shankaran and Lonsane, 1994). More over, the submerged culture requires quantities out of nitrogen and phosphorus weaker compared to the culture in SSF. To this end, Shankaranand and Lonsane (1994) [33] argue that the SSF culture leads to a decrease in the rate of nutrient diffusion, especially nitrogen and phosphorus, which occurs under conditions of low water activity. Consequently, strains with high nitrogen and phosphorus requirements appear to be disadvantaged due to the restriction of access to these nutrients in the fermentation medium. The results obtained show that the final pH for all the fermentation media and the different phosphorus contents varied between 1.8 and 2.2. As regards the quantity of biomass produced, the latter increases with the increase in the potassium phosphate content to reach maxima of 12.25 - 13.82 g / Kg at 3.0 g / Kg (Table 06).

Table 6. Effect of phosphorus content on the different fermentation parameters

	***Aspergillus niger* ANSS-B5**					***Aspergillus niger* ATCC16404**				
K_2HPO_4 (g/Kg)	0	1.0	2.0	2.5	3.0	0	1.0	2.0	2.5	3.0
Final pH	1.96	1.92	1.8	1.70	2.2	1.97	1.92	1.88	1.8	2.1
Citric acid (g / Kg)	105.62	134.42	172.82	201.60	190.08	124.87	134.44	163.20	173.80	165.22
Biomass(g / Kg)	7.51	8.22	9.46	13.82	14.25	7.84	7.68	8.42	11.42	12.25
Sugars consumed	244.00	247.00	269.00	275.0	265.2	228.00	234.00	252.28	260.0	257.54

(g / kg)										
Yield %	43.28	54.42	64.24	73.31	71.67	54.76	57.45	64.69	66.84	64.15

As for the production of the citric acid, the got results show that the latter increases with ther is ein the content potassium phosphate of the medium to reach a maximum production with a content of 2.5g/Kg. Thus, the quantities and yields of citric acid obtained with this cont ent are 172.82 173.80 g / Kg and 366.84 - 73.31%. Below 2.5 g/ Kg, no improvement was recorded (Figure 8)

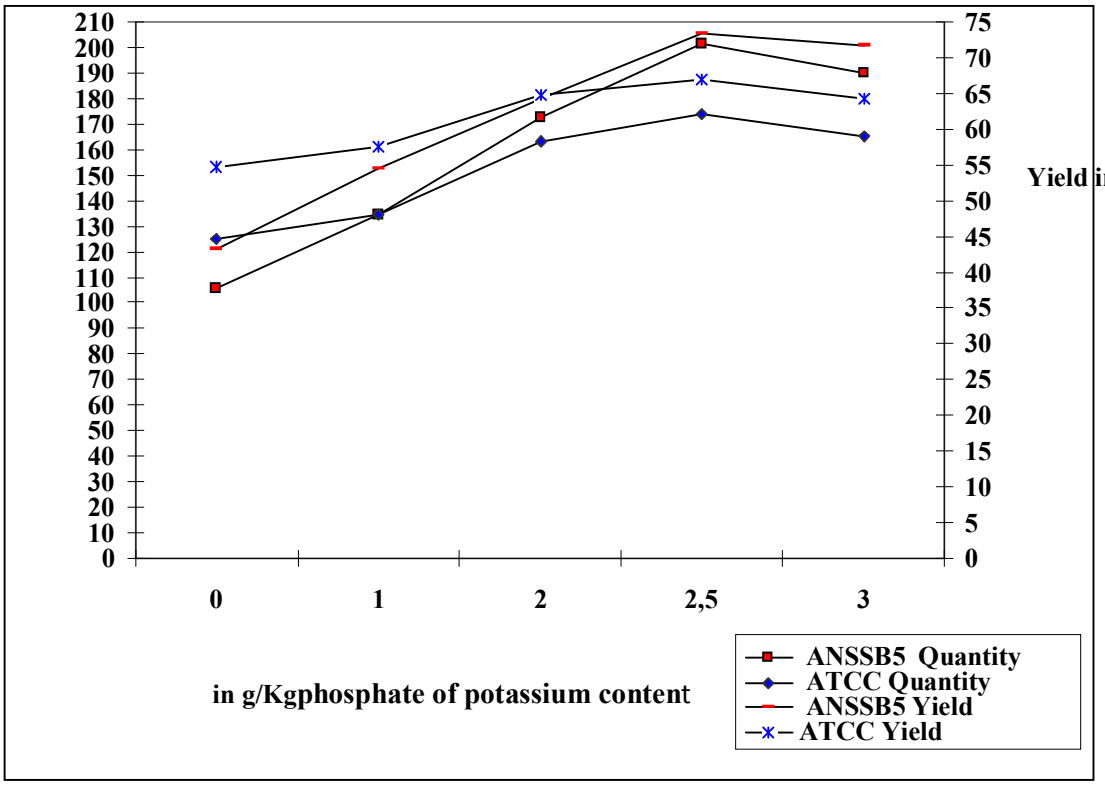

Figure 8.- Evolution of the quantity and yield of citric acid according to the phosphate of potassium content.

Thus,the optimal phosphorus content for a maximum production of citric acidi about 2.5g/Kg. Similarly Kang.,1989;Kareem.,2010;Kubicek.,1978;Pintado.,1998;Shankaranand.,1994[26-28-33-42-45] showed that the accumulation of the citric acid is higher when the quantity out of phosphorus of the culture medium is from 2.0 to 2.5g/Kg. On the other hand, Darouneh et *al.* (2009) [34] obtained a maximum yield of citric acid on a molasses fermentation medium by adding 5.0 g / kg of potassium phosphate. However, ahigh quantity≥2.5g/Lout of phosphorus supports the growth of *A. niger* audetriment of the accumulation of the citric acid (Asadstall., 2003) [47]. The same result was obtained in this study, i.e., a biomass quantity 12.25 - 14.25 g / Kg and a citric acid production 165.22 - 190.08 g / Kg with a potassium phosphate content 3.0g / Kg.

4. Conclusion

In conclusion, we can say that the production of citric acid by A. Niger on Deglet-Nour scrap medium using the SSF as a type of fermentation is promising. Thus, date scrap can be useful like low cost substrate for the production of citric acid. More over, the results obtained show that the enrichment of

the fermentation medium by the addition of nutrients allows an improvement in the yields of citric acid. On the other hand, a 70 % moisture content is more successful for a maximal citric acid product. Besides, the fixation of the initial pH at 2.5 allowed a very clear improvement of the yields in citric acid.In the same way, the optimum results were obtained with yeast extract content 3.0 g/Kg and in potassium phosphate2.5 g/Kg.

In summary, the optimum conditions for a maximum citric acid product are as follows: 70% moisture content, an initial pH 2.5 and yeast extract contents 3.0 g / Kg and potassium phosphate of 2.5 g / kg. The maximum yields of citric acid between two competing strains are 201.60 g / Kg with A. Niger ANSSB5 and 173.80 g / Kg with *A. niger* ATCC 16404 were obtained with these optimum conditions. The pulp of dates can be considered as a very economical substrate for citric acid products by *A. niger*. This medium is very profitable economical, especially for countries with a very important date production, such as Algeria. Finally, citric acid is one of the most important products of fungal metabolism produced on an industrial scale by submerged fermentation and SSF with *A. niger* strains. To this end, we believe that this work opens up promising avenues that can contribute to the production of citric acid in Algeria by using date rejects and dates of low market value produced locally.

Bibliographical references

[1] Anonyme .(2015). Statistiques agricoles, superficies et productions. Ed., Direction des Statistiques Agricoles et des Systèmes d'Information du Ministère de l'Agriculture et du Développement Rural (M.A/D.S.A.S.I),Série B, Alger,64p.

[2] Berovic, M; Legisa, M. (2007), Citric acid production. Biotechnol. Ann. Rev., 13, pp. 303-343.

[3] Arzumanov, T.E.,. Shishkanva, N.V. et Finogenova, T.V. (2000).Biosynthesis of citric acid by Yarrowialipolytica repeat-batch culture on ethanol. Appl. Microbiol. Biotechnol., 53 (5), pp. 525-529.

[4] Al-Obaidi, Z. S. et Berry, D. R. (1981). Production of citric acid from date syrup. Date Palm J., 1 (1), pp. 79-98.

[5] Brand, D., Pandey, A., Rodriguez-Leon, J.A., Roussos, S., Brand, I. and Soccol, C.R. (2002). Relation Between coffee husk caffeine degradation and respiration of *Aspergillus sp*. in solid state fermentation. Appl. Biochem. Biotechnol., 102, pp. 169-178.

[6] Pandey, A., Soccol, C.R;Nigam, P; Soccol, V.T; Mohan R. (2000). Biotechnological potential of agro-industrial residues. II. Cassava bagasse. Biores. Technol. 74, pp. 81-87.

[7].Vandenberghe, L.P.S. (2000). Développement d'un procédé pour la production d'acide citrique par fermentation en milieu solide à partir de résidus de l'agro-industrie du manioc. ThèseDoctorat. Université de Technologie de Compiegne, 205 p.

[8] Alben, E., and Erkmen, O. (2004). Production of Citric Acid from Semolina, Food Technol. Biotechnol. 42 (1), pp. 19-22.

[10] Hang, Y.D., Woodams, E.E. (1998). Production of citric acid from corncobs by *Aspergillusniger*. Biores. Technol., 65, pp. 251-253.

[11] Vandenberghe L.P.S., Soccol C.R. Pandey A. and Lebeault J.M., (1999). Solid-state fermentation for synthesis of citric acid by *Aspergillusniger*. Biores. Technol., 29, pp. 129-135.

[12] Prado., F.C., Vandenberghe L.P.S., Lisboa, C. Paca, J., Pandey, A., Soccol, C.R. (2004). Relation between Citric Acid Production and Respiration Rate of *Aspergillusniger* in Solid-State Fermentation. Eng. Life Sci., 21(3), pp 123-142.

[13] El-Holi, M.A., and Al-Delaimy, K. (2003). Citric acid production from whey with sugars and additives by *Aspergillusniger*. African J. Biotechnol., 2, pp. 356-359.

[14] Papagianni, M.,Wayman, W., Mattey, M. (2005). Fate and Role of Ammonium Ions during Fermentation of Citric Acid by *Aspergillusniger*.,Applied Environ. Microb., 71(11), pp. 7178-7186.
[15] AOAC .(2003). Official methods of analysis of AOAC International. 17th edition. 2nd revision. Gaithersburg, MD, USA, Association of Analytical Communities, 220p.
[16]Audigie C.I., Figarella J., Zonszani F.(1984). Manipulationofbiochemicalanalyzes.Ed. Doin editors, Paris, France, 211p.
[17] Haq, P.B.,Daoud, D.A. (1995). Process of mycelial dry weight calculation for citric acid. Biotechnol., 9, pp. 31-35.
[18] Crolla, A., Kennedy, K.J. (2001). Optimization of citric acid production from Candida lipolytica Y-1095 using n-paraffin. Biotechnol., 89, pp.27-40.
[19] Acourene S., Belguedj M., Tama M., Taleb B.(2001). Characterization, evaluation and identification of date quality of date palm cultivars cultivated in the region of Biskra. Rev. Rech. Agr. 8:19-39.
[20] Bin-Shahna S., Qassim Y.A; Al-Qahri M. (1987). The chemical composition of various types of dates in the P.D.R.Y. Date Palm J., 5(2), pp.143-195.
[21] Torrado, A.M., Cortes, S., Slagado, J.M., Noelia-Rodriguez, B.M., Bibbins, B.P., Converti, A., and Dominguez, J.M. (2011). Citric acid production from orange peel wastes by solid-state fermentation. Brazilian J.Microb., 42(1), pp. 16-20.
[22] Roukas, T. (2000). Citric and gluconic acid production from fig by *Aspergillusniger* using solid state fermentation. J. Indus. Microb. Biotechnol., 25, pp 298-304.
[23] Ashraf, H., Asad-ur-Rehman, A. and Ikram-ul-Haq, H., (2004). Effect of Alcohols on the production of citric Acid by Aspergillusniger using solid state fermentation. J. Food Technol., 2(1), pp.01-03
[24] Assadi, M.M. and Nikkhah, M., (2002). Production of citric ccid from date pulp by Solid State Fermentation. J. Agric. Sci. Technol. 4, pp. 119-125.
[25] Gopinath, S.M., Puttaiah, E.T. and Narasimha-Murthy, T.P. (2011). Citric acid production by *Aspergillusniger*etGP12, etGP18 on solid state fermentation and effect of initial temperature on yield. Intern. J. Pharma Bio-Sci., 2(4), pp. 250-256
[26] Kareem, S.O.,Akpan, I. and Alebiowu, O.O., (2010). Production of citric acid by *Aspergillusniger*using pineapple waste., Malaysian J. Microbiol.,6(2), pp. 161-165.
[27] Kumar, A;Jain, V.K., (2008). Solid state fermentation studies of citric acid production., African J. Biotechnol., 7 (5), pp. 644-650.
[28] Pintado, J., Torrado, A., González, M. P., Murado, M. A. (1998). Optimization of nutrient concentration for citric acid production by solid-state culture of *Aspergillusniger* on polyurethane foams. Enzy. Microb. Technol., 23 (2), pp. 149-156
[29] Khare, S.K.,Jha, K; Gandhi, A.P. (1994). Citricacidproduction from Okara (soy-residue) by solid-statefermentation. Biores. Technol., 54(3), pp.323-325.
[30] Khosravi, D.K., Zoghi, A.A., Sayed, A., and Fatemi, Sayed, S. A., (2008). Application of PlackettBurman design for citric acid production from pretreated and ntreated wheat straw. Iranian J. Chem. Chem. Eng., 27(1), pp. 91-104.
[31] Narayanamurthy, G., Ramachandra, Y.L. (2008). Area husk: in inexpensive substrate for citric acid production by *Aspergillusniger* under solid state fermentation. Indian J. Biotechnol., 7, pp. 99-102.
[32] Prado,F.C., Vandenberghe,L.P.S., Woiciechowski, A.L., Rodrigues-Leon, J.A.C., Soccol, R. (2005).Citric acid production by solid-state fermentation on a semi-pilot scale using different percentages of treated cassava bagasse. Brazilian J. Chem. Eng., 22(4), pp 74-85.

[33]Shankaranand, V.S. Lonsane, B.K., (1994). Coffee husk: An inexpensive substrate for production of citric acid by Aspergillusniger in a solid-state fermentation. World J. Microbiol. Biotechnol. 10, pp.165-168

[34] Darouneh, E., Alavil, A., Vosoughi, M., Arjmand, M., Seifkordi, A; Rajabi, R., (2009). Citric acid production: Surface culture versus submerged culture.. African J. Microb. Resea., 3(9) pp. 541-545.

[35] Al-Obaidi, Z. S. et Berry, D. R. (1981). Production of citric acid from date syrup. Date Palm J., 1 (1), pp. 79-98.

[36] Kristiansen, B.,Sinclair. C.G. (1978). Production of citric acid in batch culture. Biotechnol. Bioengin., pp.1711-1722.

[37] Mattey, M. (1992).The production of organic acids. Critic. Rev.Biotechnol., **12**, pp. 87-132.

[38] Kubicek, C. P; Rohr, M. (1986). Citric acid fermentation, Crit. Rev. Biotechnol., 3, pp. 331-373.

[39]Papagianni, M. (2007). Advances in citric acid fermentation by *Aspergillusniger* : Biochemical aspects, membrane transport and modeling., Biotechnol. Adv., 25, pp. 244-263.

[40] Abou-Zeid, A.; Abou-Zeid, A.0.; Baghlaf, J.A.; Khan, A. and Saleh, S.M. (1983). Utilization of date seeds and cheese whey in production of citric acid by *Candida Lyplitica*. Agricultural wastes, pp. 131-141.

[41] El-Abyad, M.S., Hamissa, F.A; and Gad, A.S. (1992). Treatment of beet molasses for citric acid production by a potent strain of *Aspergillusniger van tighem.* Biores. Technol., 39, pp. 191-197.

[42] Kang, S.K., Park, H.H., Lee, J.H., Lee, Y.S., Kwon, I.B., Sung, N.K. (1989). Citric acid fermentation from mandarin orange peel by *Aspergillusniger*. SanopMisaengmulHakhoechi, 17, pp. 510-518.

[43] Chen, H.C.(1994). Response-surface methodology for optimizing citric acid fermentation by *Aspergillusfoetidus*. Process Biochem., 29, pp. 399-405

[44] Kubicek, C.P., Zehentgruber, O. and Rohr, M. (1979). An indirect method for the studying the fine control of citric acid fermentation by *Aspergillusniger*. Eur. J. Appl. Microbiol. Biotechnol., 5, pp. 263-271.

[45] Kubicek, C. P;Rohr, M. (1978). The role of trycarboxylic acid cycle in citric acid accumulation by *Aspergillusniger*. Eur. J. Appl. Microbiol. Biotechnol., 5, pp. 263-271.

[46] Pintado .J., Murado M. A; Gonzalez M. P; Miron J. ; Pastrana L. (1993). Joint effect of nitrogen and phosphorus concentrations on citric acid production by different strains of *Aspergillusniger*grown on an effluent. Biotechnol. Lett., 15, pp. 1157-1162.

[47] Asad, U.R.;Sikander, A;Ikram, U.H.(2003). Phosphate Limitation for enhanced citric acid fermentation using Aspergillusniger mutant UV-M9 on semi-pilot scale. Pakistan J. Biol. Sci., 6 (14), pp. 1247-1249.