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EFFECT OF SOME GROWTH FACTORS ON THE PRODUCTION OF CITRIC ACID AND SINGLE CELL PROTEIN (SCP) BY ISOLATED FUNGI

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ABSTRACT

The main goal of this study is to choose the best citric acid producing fungi and single cell protein taken from various plants sources. The best fungi samples were chosen then study of some factors such as incubation period, carbon, hydrogen and hydrogen number affecting their growth and production. Results showed that the best production and growing dynasties were (*Aspergillus niger, A. flavus*). The best acid production reached (18.42,20.81)gm/l respectively on 8th incubation day .Best production of protein content reached (4.02, 6.18)gm/l respectively. During the same incubation period for the same species followed by *Pencillium sp.* Species unlike other species that failed to give tangible amount. Thus *A. flavus* was selected as the best growth and production fungi. Also the results showed that sucrose and Ammonium uniphosphate were the best hydrogen resources respectively at the hydrogen number at (6.0).
Keywords **:** Single Cell Protein (SCP), Citric acid, *Aspergillus niger, A. flavus*.

Introduction

"Citric acid is a carboxylic acid. Its chemical symbol is ($C_6H_8O_7$) found in fruits. It is one of the compounds participating in physiologic oxidation of fat, protein and carbo-hydrates. Isolated firstly by sugar cane fermentation or molasses" (Grapulin *et al.,* 2005). "Its most important commercial product reaching 736.000 tons per year" (Daniel *et al.,* 2006). "It uses as a flavor enhancer in drinks to give taste and flavor, a preservative and food complement. It is used in home hygiene, cosmetics as well as a medical material, it help bones to absorb calicium easly, prevents creation of kidneys stones" (Tsay *et al.*, 2010). "Citric acids produced from microorganisms is widely used than fruits such as fermentation, by bacteria and fungi" (Srchae *et al.,* 2017).

Single cell protein is also produced from microorganism used globally. It can be vastly produced in a short time compared to traditional production methods (Smorimur *et al.,* 2014). It is used to feed fish with high protein needs like Carribeans" (Sharma *et al.,* 2011).

The study aims at selecting the most efficient locally isolated fungi species to produce citric acid and single cell proteins and to study the effect of different circumstances on the growth of these species, its production of acids and protein.

Material and Methods

The fungi used throughout this study. (*Aspergillus niger, A. flavus, Pencillium sp., Fusarum sp.* and *Alternria alternate)*, and identification by phenol and microscopic characters, all strains were grown on incubation (potato dextrose agar) slant at (30) for 7 days and used as inoculums. Fungal isolation were done from infected fruits and vegetables collected from Mosul markets, Iraq.

Standard Media

This media was used to pre fungi pollen and as a media to produce citric acid .it consists of gm/L (140) sugarose, (2.50) $(NH_4)_2CO_3$, KH_2PO_4(0.5), (2.50) $MgSO_4.7H_2O$.as well as the following materials measured in gm/L $CuSO_4H_2O$(0.06), $ZnCl_2$(0.25),$FeCl_3.6H_2O$ (1.3) and set pH at the level of(6.0). Sterilization of the media by autoclave for (20) min.

Preparation of fungi pollination

Fungi pollination was prepared using (5) ml of distilled water to each slant tube within 1 week. Pipes were shaken well manually to separate spores. The resulting liquid was collected with a tight lock.

Zaic Standard Media:

This media was used to grow fungi while experiment. The media consists of the following materials in the following concentrations gm/100ml: (30)$NaNO_3$ (5) $MgSO_4.7H_2O$ (0.1) $FeSO_4.7H_2O$.(5)KCl and added the volume into (100)ml, then added (1) $KHPO_4$ to the concentrated zaic media (100) ml,(5)yeast extract (0.65), sucrose sugar and continued to (1) liter. Choosing the best fungi to produce single cell protein to find the efficiency of isolated fungi used were cultivated in Zaic standard media after dissolving all ingredients in distilled water, set hydrogen number at (6.0). The media was distributed in (48)

ml on of a capacity of (250) ml, purified media polluted using (2) ml of spore resolution with two repeated for each treatment, incubated at a rotating speed of (200)c/min with 30 for 7 days the conical flask were taken randomly. The dry weight of fungi was estimated .The same method used to specify the most efficient fungi to produce citric acid using acid production media.

Determination of the fungal Biomass:

After the prescribed incubation period is over the biomass was estimated by filtering fungi culture using pieces of canvass the residue left aside to estimate citric acid. Fungi cell were collected in a pre-know weight plates were then put in an oven under 50^0 C for 24 hours. Biomass was extracted by the variance between the two masses via a sensitive scale.

Determination of the Citric acid:

Citric acid was assessed using Marrier and Boulwet method (Person *et al,* 2012).One ml of produced residue moved to a test pipe then to it add 1.3 ml of predine indicator .Tubes were shaken fast then 5.7ml of Acetic anhydrate was added put in water bath on 32^0 C for 35 min to improve segment, absorbent was collected on the wave length of 450 nm, with a spectrometer.

Determination of the single cell protein:

Protein amount in dry material in all experiment using Lowry method and derived by Pollak and Schacterle (Woodams, 2010).

Effect of different incubation period on growth and production:

Used many incubation period (5, 6, 7, 8, 9, 10) to note the effect of incubation period on production of citric acid and single cell protein after end.

Effect of different carbon sources to growth and production of citric acid and protein:

Used five carbon sources as (glucose, fructose, sucrose, maltose and starch) .

Effect of different (pH) on the growth and production Citric acid and single cell protein from *A.niger* and after seven day of incubation:

To determine the best hydrogen number for best production of acidic and protein ,this experiment was designed and various hydrogen number were selected (4,5,6,7,8,9).

Effect of different nitrogen sources on growth and production:

Six different nitrogen sources to know their effect on growth and production were selected they were (Ammonium phosphate, Urea, Ammonium chloride, Ammonium sulphate).

Results and Discussion

Diagnosis of fungi isolation: Fungi were isolated from different resources of infected plants (table 1).Fungi were diagnosed depending on appearance using microscope and classification keys (Roukas,2002).Five isolates were isolated: *Aspergillus niger, A.flavous, Pencillium sp., Fusarium sp,, Aternaria alternate.*

Table 1 : Fungi isolated from different resources

No. of isolation	Name of fungi	Source of isolation
1	*Aspergillus niger*	Rotten apple
2	*Aspergillus flavus*	Rotten orange
3	*Pencillium sp.*	Rotten cucumber
4	*Fusarium sp.*	Rotten potato
5	*Aternaria alternate*	Rotten tomato

Comparison of citric acid production, fungi isolation during various incubation periods:

In this experiment various local fungi isolations were grown in the standard medium for citric acid production .The results in table (2) showed that incubation period has an effect on the activity of isolated fungi regarding growth and production and productivity. The variance of period needed to yield a high production of citric acid from the *A. niger* isolation giving (20.81) gm/L after (7) days of incubation. Secondly came *A. flavus* regarding productivity reaching (18.42) gm/L with the same period of incubation. The isolated *Pencillium* sp. Gave the lowest citric acid production (16.12)gm/L after nine days of incubation. The production of (*Alternaria alternate, Fusarium sp.*) isolation was(6.32) and (2.56)gm/L respectively after nine and ten days of incubation .The results showed that the best incubation period to yield highest citric acid production was(7) days and the (*A. niger*) these findings resemble the results reached earlier (Guebel, 2015). Regarding biomasses, there is an increase in biomass with the increase in incubation period .It varied with different isolation (*A. niger, A. flavus*) yielded the highest biomass (19.81,24.03)gm/L respectively at day 8 of incubation while (*Pencillium sp.*) registered (13.24)gm/L in the same incubation period regarding protein content (*A. niger* and *A. flavus*) gave the highest amount reading (6.18,4.02) gm/L respectively. While (*Alternaria alternate*) registered the lowest protein content (2.16) gm/L. Thus (*A. niger*) fungi was adopted in the next experiment and 7^{th} day of incubation to gave highest growth and productivity. "These results resemble what each of (2.13,14) reached". This results are similar with study (Ravindar*et al,*2011). When used "*A.flavus* gave highest production through 7^{th} day,". While (Zayed, 2014). was found the best fungi to production citric acid and (SCP) *A.niger* via 7^{th} day also. But Shuker, *et al.*, 2015 found in *Pencillium sp*. It is best to produced (SCP) via 8^{th}, and *A.niger* gave highest amount of Citric acid in 7^{th} day.

Table 2 : Best fungi to produce growth, citric acid, single cell protein

Fungi isolation	Incubation	biomasses	incubation	Citric acid	Incubation	Single cell protein
A.niger	7	24.03	8	20.81	7	6.10
A.flavus	7	19.81	8	18.42	7	4.02
Pencillium sp	9	13.04	9	16.42	7	3.93
Fusarium sp.	10	12.01	9	10.97	9	2.34
Alternaria alternate	9	14.81	10	7.38	9	2.16

Effect of carbon sources on growth and production of (*A. niger*) at 7th day of incubation:

Results in table(3), figure (1), shown that the best growth and production of citric acid and protein content using glucose (24.23) gm/l for biological mass the best acid production and protein content reached (6.83, 21.65) respectively with sucrose as the carbon source followed by glucose was (17.09) gm/l the results showed a clear variance for these sugars to enhance and support citric acid production from fungi species the reason maybe difficulty of representing them or being disable to produce the needed enzymes to break some sweets like lactose fungus has invertase enzyme to break sucrose into glucose the results resemble the ones reached by(15.16).similar results with (Shuker, 2019). Was gave highest yield from citric acid when used glucose as a carbon source, and also similar results were reported by (Rashid, 2019). for production (SCP) and citric acid by *A.niger.*

Table 3 : Effect of different carbon sources on growth and produce.

Carbon Source	Biomass gm/L	Citric acid gm/L	(SCP) gm/L
Lactose	18.98	16.98	4.02
Frictose	19.87	18.01	4.85
Glugose	24.32	17.09	5.63
Sucrose	20.75	21.65	6.83
Starch	17.14	18.58	4.79

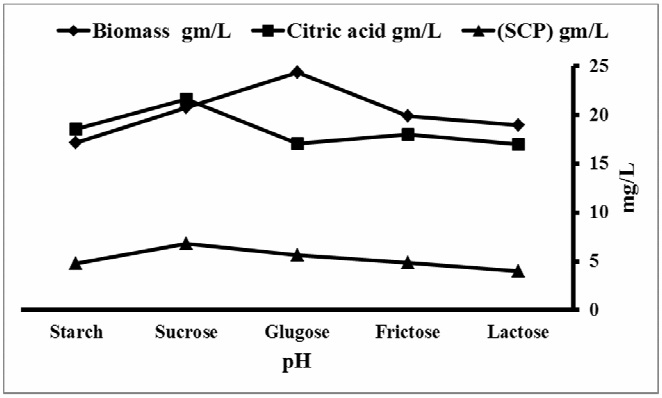

Fig. 1 : Effect of different carbon sources on growth and produce

Effect of different nitrogen sources on growth and production in 7th day:

The results in table (4) ,figure,(2) shows the high produce from growth, (SCP) and citric acid was (25.08,22.14,7.03) gm/L respectively compare with other nitrogen sources. While the Ammonium chloride gave lowest production (13.00, 2.32) mg/L, but the effect of urea to biomss was (20.25)mg/L. The Stuied similar result for the production and growth by *Pencillium sp*. (Singh *et al.,* 2016; Ettler *et al.,* 2015).

Table 4 : Effect of different nitrogen sources on growth and production

Nitrogen sources	Dry weight gm/L	Protein gm/L	Citric acid gm/L
0.0	20.01	19.72	2.41
Urea	20.25	19.85	2.46
Ammunium chloride	21.14	13.00	2.32
Ammunium phosphate	25.08	22.14	7.03
Ammunium sulphates	23.31	20.43	5.62
Spargen	21.67	20.76	5.28

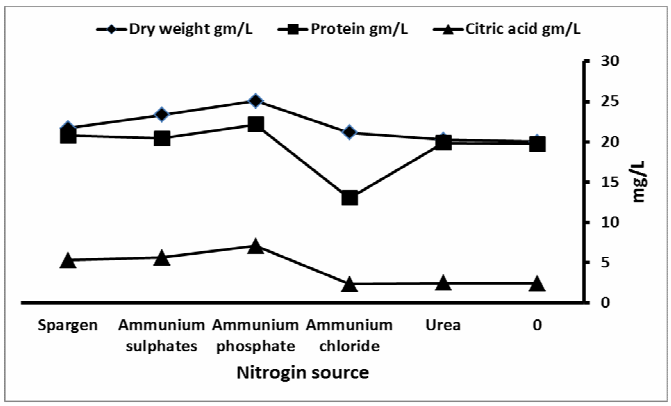

Fig. 2 : Effect of different nitrogen sources on growth and production.

Effect of different (pH) on growth and production:

The results in table (5) , figure (3). Showed that different hydrogen number had an effect on growth and production .In low pH yield was small. Production in pH (4) gave (17.12, 4.92, 18.63) mg/L .While the number increase growth and production noticeably until it reached its peak at the hydrogen number (6.0) giving highest citric acid production of (23.08)gm/L as well as protein content (7.72) gm/L. But as the hydrogen number increases growth and production decreases. This may be due to acidity depletion of nutrients media. Similar results have been reported in literature (Ali *et al.,* 2014). when was used (pH6) gave best growth and production to citric acid by *A. niger*, while M. (Ziino *et al.,* 2014) used *Alternaria alternate* at (pH 7).

Table 5 : Effect of pH on growth and production

pH	Biomass gm/L	Single cell protein mg/L	Citric acid gm/L
4	17.12	4.92	18.63
5	21.01	5.40	21.49
6	23.08	7.72	23.02
7	20.93	6.81	20.43
8	17.28	4.57	20.01
9	12.03	3.26	18.92

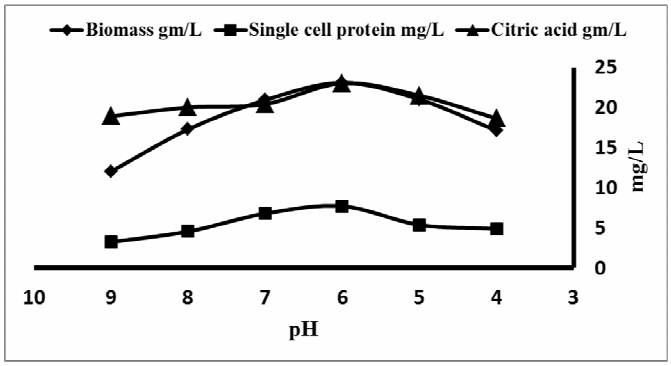

Fig. 3 : Effect of pH on growth and production.

References

Ali, S. and Qader, M.A. (2014). Production of citric acid and single cell protein by *Aspergillus niger* using cane molasses in a stirred fermentor. *J.Food Sci*., 53: 76-89.

Daniel, G.V.; Darias, N.L. and Torres, Z. (2006). Optimization of the citric acid production by *Aspergillus niger* through a metabolic flux balance model. *J. of Biotechnol*, 7: 412-420.

Ettler, P.; Martin, K. and Seligy, I. (2015). Fungal production of citric acid from sugars present in wood hemicelluloses using *Aspergillus niger* and *Saccharomycopsis lipolytica. Biotechnology J.,* 54: 78-99.

Grapulin, M.G. and Legisa (2005). Comparison of specific metabolic characteristics playing a role in citric acid from brewery waste by surface fermentation using *A.niger*. *J. Food Sci*., 52: 127-137.

Guebel, D.V. (2015). Optimization of citric acid production by *A.fluvus* through ametabolic flux balabce model. *J. Crit. Rev. Biotechnol.* 23: 78-84.

Person, D. (2012). Effect of the composition of the sporulation medium on citric acid production by *Aspergillus niger*. *J. Bacteriology*, 53: 78-89.

Rashid, S.I. and Shuker, W.H. (2019). Isolation and identify the antibiotic from the local isolates of the *Streptomyces antibiotics* and test their antifungal activity against plant pathogens. Journal of Global pharma technology. India.

Ravindar, R. and Rao, L.V. (2011). Studies on production of single cell protein by *Aspergillus sp.* In solid state fermentation of rice bran*. J. Biology Technol*. 87: 55-67.

Roukas, T. (2002). Citric acid production using immobilized of *Aspergillus niger*. *J. Food Technol. Biotechnol*. 76: 34-53.

Sharma, C.B. and Nidhee, C. (2011). Production of citric acid and single cell protein from *pencillium sp. National Academy Science Letters*, 54: 78-99.

Shuker, H.W.; Shimal Y.A. and Safaa, I.R. (2015). Citric acid production from a local isolate of the fungus *Aspergillus niger* by standard medium. *J. Food and Microbial,* 87: 68-79.

Shuker, W.H. (2019). Study the effect of some growth factors on the production of L-Asparaginase enzyme by fungal isolate *Aspergillus flavus*. *Journal of Global pharma Technology*.India. 11: 196-201.

Singh, A. and Abidi, A.B. (2016). Single Cell Protein production by Aspergillus niger and its evaluation. *J. of Folia microbiologica.* 32: 45-58.

Smorimura, K. and Nakagawa, K.M. (2014). Production of fungal protein from *Aspergillus niger* by molasses. *Journal of industrial microbiology*, 44: 432-446.

Srchae, E. and Hwang, J. (2017). Single cell proteins from fungi and yeast. *Applied Mycology and Biotechnology,* 78: 54-65.

Tsay, Y.D. and Woodams, E.E. (2010). Microbial production of citric acid by solid state fermentation of Kiwi fruit peel. *J. Food Sci.* 54: 100-110.

Woodams, E.E. (2010). Microbial production of citric acid by solid state fermentation of molasses. *J. Bioengin*. 76: 98-121.

Zayed, G. (2014). Single cell protine production from beet molasses by *Sacharoycess cervisae*. *J. Cultured Dairy Products*, 98: 58-66.

Ziino, M. and Curto, R.B.L. (2014). Citric acid production from okara by solid state fermentation. *J.of Bioresource Technology*, 32: 54-66.