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Production of Gluconic Acid by Some Local Fungi

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Forty-one fungal species belonging to 15 fungal genera isolated from Egyptian soil and sugar cane waste samples were tested for their capacity of producing acidity and gluconic acid. For the tests, the fungi were grown on glucose substrate and culture filtrates were examined using paper chromatography analysis. Most of the tested fungi have a relative wide potentiality for total acid production in their filtrates. Nearly 51% of them showed their ability of producing gluconic acid. *Aspergillus niger* was distinguishable from other species by its capacity to produce substantial amounts of gluconic acid when it was cultivated on a selective medium. The optimized cultural conditions for gluconic acid yields were using submerged culture at 30°C at initial pH 6.0 for 7 days of incubation. Among the various concentrations of substrate used, glucose (14%, w/v) was found to be the most suitable carbon source for maximal gluconic acid during fermentation. Maximum values of fungal biomass (10.02 g/*l*) and gluconic acid (58.46 g/*l*) were obtained when the fungus was grown with 1% peptone as sole nitrogen source. Influence of the concentration of some inorganic salts as well as the rate of aeration on the gluconic acid and biomass production is also described.

KEY WORDS: Gluconic acid production, *Aspergillus niger*, acidity

D-gluconic acid is an important organic acid resulting from the oxidation of D-glucose. The unique property of this acid such as low toxicity, low corrosively and complexing capability with metal ions has enabled its wide application in the food, pharmaceutical, textile, leather and other industries (Singh *et al.*, 1999). Thus, the overall demand of this organic acid has been increased for almost 20 years and, recently it production is amounting to more than 60,000 tons per year and still growing (Singh *et al.*, 1999; El-Enshasy, 2003). Commercially, gluconic acid is produced by three different methods, chemical oxidation of glucose with a hypochlorite solution (Kundu and Das, 1984), electrolytic oxidation of glucose solution containing a known value of bromide (Amberkar *et al.*, 1965), and fermentation process where specific microorganisms are grown in medium containing glucose and other ingredients (Hill and Robinson, 1988; Shah and Kothari, 1983; Lee *et al.*, 1998). The microbial fermentation process offer an attractive techniques for the gluconic acid production to alleviate the problems related to chemical production such as the inevitable side reactions and also to further economize the bioprocess (Velizarov and Beshkov, 1994; Singh *et al.*, 1999).

A wide group of microorganisms, particularly filamentous fungi have the ability for gluconic acid production (Cochrane, 1958; Lockwood, 1975). The production of gluconic acid is mainly done in batch cultivation using several species belonging to the following fungal genera, *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor*, and *Gliocladium* (Lockwood, 1975; Rosenberg *et al.*, 1922; Petruccioli *et al.*, 1994; Singh *et al.*, 2001). Among the different fungal genera, it has been reported that the accumulation of large amounts of the gluconic acid and its salts are restricted to certain species of *Aspergillus*, especially *A. niger* which considered as the most industrially important gluconic acid producer in fermentation industry (Roukas, 2000; Sankpal *et al.*, 2001; Sankpal and Kullkarni, 2002; El-Enshasy, 2003). Because of its industrial importance, many investigators have been worked for optimization as well as overproduction of gluconic acid by improving fungal producers (Vassilve *et al.*, 1993; Sankpal *et al.*, 1999; Singh *et al.*, 2001; Sankpal and Kulkarni, 2002; El-Enshasy, 2003). Also, researchers employed conventional screening protocols to identify and develop potential indigenous fungal strains for commercial exploitation of the process (Singh *et al.*, 1999), despite the abundant availability of commercial gluconic acid.

The main object of the present work is to isolate some local fungal strains from Egyptian soil and waste materials of sugar cane that are capable of producing large amounts of gluconic acid.

Materials and Methods

Isolation and identification of organisms. Different fungal isolates were obtained from cultivated Egyptian soil samples and waste materials of sugar cane processing from Hawamedia Distilleries Factories. The dilution plate method

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described by Johnson *et al.* (1959) and Czapek Doxs Agar medium (Oxoid, 1982) supplemented with rose bengal (1/15000, w/v) as bacteriostatic agents (Smith and Dawson, 1994) was used for isolation of fungi. For the isolation, plates were incubated at $28 \pm 2^{\circ}C$ for 7 days, and developing fungi were purified and identified by macro-and microscopic characteristics using the following references; Gilman (1957), Barron (1968), Raper and Fennell (1977), Carmichael *et al.* (1980), Domsch *et al.* (1980) and Nelson *et al.* (1983). Isolated fungi were maintained on potato dextrose agar (PDA) slants and incubated at $30^{\circ}C$ for 7 days. The slants were stored at $4^{\circ}C$ and subcultured every month. The spore suspension was prepared by suspending the spores on the slant in 10 *ml* of sterilized distilled water.

Fermentation technique. Gluconic acid fermentation was carried out by submerged fermentation in 250 *ml* cotton wool plugged Erlenmeyer flasks with 50 *ml* of fermentation media: Medium I used for total acidity as well as gluconic acid production, the modified Czapek Dox Broth was of the following composition (g/*l*): glucose 100.0, $NaNO_3$ 3.0, yeast extract 3.0, KH_2PO_4 1.0, $MgSO_4 \cdot 7H_2O$ 0.5, KCl 0.5, and $FeSO_4 \cdot 7H_2O$ 0.01 having pH 6.0. Medium II, Czapek Dox Broth consisted of (g/*l*) sucrose 30.0, $NaNO_3$ 3.0, KH_2PO_4 1.0, $MgSO_4 \cdot 7H_2O$ 0.5, KCl 0.5 and $FeSO_4 \cdot 7H_2O$ 0.01 having pH 6.0. Medium III, Molliard medium (Molliard, 1992) was composed of (g/*l*) glucose 150.0, $(NH_4)_2HPO_4$ 0.388, KH_2PO_4 0.1 and $MgSO_4 \cdot 7H_2O$ 0.15 having pH 6.0. Medium IV (El-Naghy and Megalla, 1975) had the following composition: (g/*l*): glucose 150.0, Peptone 10.0, KH_2PO_4 0.5 and $MgSO_4 \cdot 7H_2O$, 0.5 having pH 6.0. Medium V (El-Ktatney, 1978) contained (g/*l*): glucose 50.0, Peptone 2.0, KH_2PO_4 2.0, and $MgSO_4 \cdot 7H_2O$ having pH 5.5. The pH of each medium was adjusted with 1 M NaOH. The sterilized media were inoculated with 2 *ml* of well-dispersed spore suspension of each tested fungal species (6×10^6 spores/*ml*). The flasks were incubated at $28^{\circ}C$ for up to 8 days using a rotary shaker (New Brunswick Scientific Edison N-J. USA). All the experiments were carried out in duplicate. The nitrogen sources added were yeast extract, peptone, $NaNO_3$, NH_4NO_3, $(NH_4)_2HPO_4$, urea, NH_4Cl and KNO_3.

Dry biomass estimation. The content of each flask was filtered and the mycelial residues were washed with distilled water. These mycelial residues were dried in an oven for 24 h at $90^{\circ}C$ till their weight are to be constant and the dry biomass was calculated in g/*l* of fermentation medium (Singh *et al.*, 1999). The culture broth was pooled and the volume measured. The aliquots were also used for biochemical analysis.

Biochemical analysis.
Assay of total acidity (T.A.): The total acidity of the culture filtrates was determined by titration against standard alkaline solution (Peppler, 1967; El-Ktatney, 1978), using phenolphthalein as an indicator.

Detection of gluconic acid. The gluconic acid produced was determined qualitatively and quantitatively by chromatographic analysis (Koepsell *et al.*, 1952; Singh *et al.*, 1999).

Estimation of glucose. DNSA (Dinitro salicylic acid) method was used for glucose estimation (Tasun *et al.*, 1970). The transmittance was measured at 700 nm using a spectrocolormeter (Carl-Zeiss JENA type). The amount of residual reducing sugar was determined by inference from the standard curve of different known concentrations of glucose.

Statistical analysis. The data were statistically analyzed as a complete randomized block design. The means was compared by analysis of variance (ANOVA) (Hicks, 1983).

Results and Discussion

Screening of the different fungal isolates for total acid and gluconic acid production. The amount of gluconic acid produced by isolated fungal cultures ranged from 1.00 to 58.46 g/*l*. The dry weight of mycelia was ranging from 2.5 to 10.02 g/*l* (Tables 1 and 2). *A. niger* showed the highest (58.46 g/*l*) production of gluconic acid. Most of the isolates used have been recorded as acid-producers by the aid of compendium of soil fungi (Domsch *et al.*, 1980). In accordance with our screening, several species of *Aspergillus* and *Penicillium* in addition to some Mucorales were described by other investigators as acid producers as well as gluconic acid producers in their filtrates (El-Ktatney, 1978; Rosenberg *et al.*, 1992; Singh *et al.*, 1999, 2001). The screening experiments were extend to determine quantitatively the yields of gluconic acid in the twenty three selected fungi which previously showed positive results after paper chromatography analysis of the culture filtrates of the fungi grown on the modified Czapek Dox Broth.

Other investigators developed a similar system to screen diverse fungi for their metabolic activities and in accordance with our results they considered *A. niger* as a very strong producer of gluconic acid in addition to other known *Aspergillus* spp. and *Penicillium* spp. (Temsah and Olama, 1999). The gluconic acid production by the proposed fungus was also reported in other studies (El-Kataney, 1978; Vassilve *et al.*, 1993; Petruccioli *et al.*, 1994; Singh *et al.*, 1999). Our results revealed that a wide range of variations form low gluconic acid production as well as total acidity in its culture filtrate under the experimental conditions. These preliminary findings justified the selection of gluconic acid producing fungus for further investigation in

Table 1. The potentiality of the tested fungal species to produce total acid (*ml* of N NaOH/*l*) and gluconic acid (as shown by the paper chromatography technique) on submerged culture at 28°C for 7 days

Fungal species	Total acidity (*ml* NaOH/*l* medium)	Gluconic acid*	Dry biomass (g/l medium)
Aspergillus carneus (V. Tiegh.) Bloch.	720	+	5.83
A. flavipes Bain. & Sart.	600	+	5.52
A. flavus Link	980	–	7.81
A. fisheri Wchmer	1000	–	8.24
A. fumigatus Fres.	800	+	6.7
A. nidulans (Eidam) Winter	200	+	5.75
A. niger Van tieghem	3580	+	10.02
A. ochraceous Wilhelm	150	+	9.08
A. stellatus Curzi	250	+	6.15
A. sydowii (Bain. & Sart.) Thom & Chuch	480	+	6.18
A. terreus Thom	1380	–	6.20
A. versicolor (*Vuill.*) *Triaboschi*	220	+	7.45
Chaetomium jodphurence Lodha	ND**	–	3.5
Chaetomium sp.	ND**	–	2.5
Penicillium decumbens Thom	1800	+	8.20
P. frequentans (Westling)	1980	+	8.62
P. janthinellum Biourage	560	+	6.02
P. litacinum Thom	580	+	7.08
P. purpurogenum Stoll	680	+	7.50
P. stoloniferum Thom	805	+	8.68
Alternaria alternata (Fr.) Keissler	80	+	5.3
Alternaria citri	70	–	4.9
Cladosporium cladosporiodes (Fr.) de Vries	60	–	4.95
Cladosporium herbarum (Pres) Link ex. Fr.	ND**	–	4.52
Fusarium moniliforme sheldon	400	+	7.50
F. semitectum Berkely & Rovenel	98	–	5.10
F. solani (Mart.) sacc.	350	–	5.34
Humicola fuscoatra traaen	50	–	4.10
Myrothecium verrucaria (Alb. & Schw.)	480	–	4.8
Paecilomyces fusisporus Sakisena	102	–	6.42
Paecilomyces varioti Bainier	200	–	6.72
Pleospora herbarum (Pers. Ex. Fr.) Rohenh	90	–	4.32
Scopulariopsis brevicalus (Sacc.) Bainier	150	–	4.56
S. candida (Gueguen) Vuill.	195	–	4.69
Trichoderma koningii	300	–	7.50
T. viride Pers	290	–	8.10
Trichoderma sp.	250	–	7.80
Ulocladium atrum Pres	ND**	–	5.91
Mucor circinnoloids (Brain.) Van Tiegh.	1020	+	4.80
M. racemosus Fres	900	+	4.50
Rhizopus oryzae Went & Prinsen Gerrlings	1000	–	4.08

*(+) indicates gluconic acid production, (–) indicates no gluconic acid production.
**(ND) not detected.

order to enhance its productivity of gluconic acid.

Media for gluconic acid production. To choose the best substrate for acid production, the growth of the selected *A. niger* and its ability of producing gluconic acid was investigated by growing the fungus on some common fermentation media. As shown in Table 3, the formation of gluconic acid was found to be dependent on the chemical composition of culture media used. Both the addition and concentration of glucose and organic nitrogen in media had an effect on good gluconic acid production. *A. niger* gave the optimum yields of gluconic acid and mycelial growth on modified Czapek Dox medium containing sucrose as carbon source. Several workers reported the effects of glucose and organic nitrogen on the formation of gluconic acid by fungi. (El-Naghy and Megalla, 1975; El-Ktatney, 1978; Singh *et al.*, 1999; El-Enshasy, 2003). Thus we selected medium (I) for further study in order to elucidate the optimal conditions for gluconic acid production.

Several conditions for gluconic acid production.
Incubation period: The growth of *A. niger* and its pro-

Table 2. Gluconic acid (g/*l*) produced by the screened fungal species on submerged culture after 7 days at 28°C

Fungal species	Gluconic acid* g/*l* medium	Fungal species	Gluconic acid* g/*l* medium
Aspergillus carneus	12.12	*A. verisocolor*	3.82
A. flavipes	10.20	*Penicillum decumbens*	32.28
A. flavus	15.20	*P. frequentans*	35.08
A. fisheri	20.16	*P. janthinellum*	10.00
A. fumigatus	10.81	*P. lacinum*	10.08
A. fumigatus (group)	10.00	*P. purpurogenum*	14.90
A. nidulans	3.00	*P. stoloniferum*	14.77
A. niger	58.46	*Alternaria alternata*	1.00
A. niger (group)	51.50	*Fusarium moniliforme*	6.00
A. ochraceous	2.10	*Mucor circinelloids*	18.09
A. stellatus	3.08	*M. racemosus*	15.00
A. sydowii	7.65		

As determined by titration technique.

Table 3. The growth of *Aspergillus niger* and its production of gluconic acid in different fermentation media at 30°C for 7 days

Fermentation media*	Dry biomass (g/*l* medium)	Gluconic acid (g/*l*)
Medium I	10.02	58.46
Medium II	8.20	20.14
Medium III	9.85	52.51
Medium IV	10.00	56.95
Medium V	8.50	40.10
Mean ± SD	9.31 ± 0.89	45.63 ± 15.97

*Composition is described in the materials and methods.

Table 4. Influence of fermentation period on gluconic acid production and growth of *A. niger* in submerged culture at 30°C for 12 days and at pH 6.0

Incubation period (days)	Dry biomass (g/*l* medium)	Gluconic acid (g/*l*)*
1	0.93	6.1
2	4.51	28.68
3	7.01	40.56
4	8.71	47.72
5	10.04	52.68
6	10.90	57.10
7	10.02	58.46
8	10.00	58.00
9	9.60	57.00
10	9.20	55.10
11	9.00	52.20
12	8.85	51.00
Mean ± SD	8.23±2.85	47.05±15.51

*Contains: 10% glucose = 150 g/*l* (w/v).

duction of gluconic acid were determined during the incubation period of 12 days. The results in Table 4 showed that the active growth of the fungal mycelia began after the elapse of 2 days of fermentation period and was accompanied by the increasing levels of gluconic acid production. As the growth reached in linear phase the acid yield increased exponentially and the optimum growth attained after 6 days. The maximum gluconic acid yielded up to 58.46 g/*l* after 7 days of fermentation.

Table 5. Effect of initial pH on the gluconic acid production and growth of *A. niger* in submerged culture at 30°C for 7 days

pH values	Dry biomass (g/*l* medium)	Gluconic acid (g/*l*)*
2	2.65	7.20
3	4.80	22.92
4	8.08	44.81
5	10.50	57.30
6	10.02	58.46
7	9.01	50.90
8	8.10	43.51
9	7.11	40.00
Mean ± SD	7.53 ± 2.65	40.64 ± 17.56

*Contains: 10% glucose = 150 g/*l* (w/v).

The kinetics of growth and gluconic acid production have been studied by several investigators (El-Naghy and Megalla, 1975; Madhavi *et al.*, 1999; Znad *et al.*, 2004) and their observation unequivocally supports our results. However, some workers found that an incubation period of 6 days was optimal for gluconic acid production by *A. niger* strains (Rao *et al.*, 1993; Singh *et al.*, 1999).

The pH value: The pH value is one of the most critical factors affecting on the fungal growth as well as the formation of organic acids. The profiles gluconic acid and dry mycelial mass with respect to initial pH of the fermentation medium is shown in Table 5. Over 50% yield of gluconic acid was observed in a pH range from 5 to 7. The best yield was 58.46 g/*l* at pH 6.0. However, the acid yield above and below this pH was poor. The suitability of pH ranges 5~6 for both the growth and gluconic acid production was also reported from other fungi (El-Naghy and Megalla, 1975; Singh *et al.*, 1999).

Incubation temperature: Table 6 shows the effect of

Table 6. Effect of temperature on the gluconic acid production and growth of *A. niger* in submerged culture at pH 6.0 for 7 days

Temperature (°C)	Dry biomass (g/*l* medium)	Gluconic acid (g/*l*)*
20	5.82	30.22
25	10.00	50.72
30	10.02	58.46
35	8.01	48.50
40	2.52	18.53
50	–	–
Mean ± SD	7.27 ± 3.17	41.29 ± 16.40

*Contains: 10% glucose = 150 g/*l* (w/v).
–: not detected.

incubation temperature on gluconic acid production (20~50°C). The optimum temperature for efficient fermentation for gluconic acid (58.46 g/*l*) and biomass yield (10.02 g/*l* medium) was 30°C. This temperature was also reported as optimal for maximal gluconic acid production by other authors (Moresi *et al.*, 1991; Subba-Rao *et al.*, 1994). At 45°C, the effect of temperature on the production of gluconic acid was negligible.

Aeration: Fig. 1 shows the effect of increased aeration on the bioconversion of glucose to gluconic acid by *A. niger* in submerged culture. It is apparent from the data that increased gluconic acid production is affected by increasing the aeration and the size of fermentation containers. The optimal gluconic acid yield (62 g/*l*) was obtained in fermentation flask having 2L-capacity of containing medium.

Carbon sources: The results of fermentation using different carbon compounds are summarized in Fig. 2. The data indicated that glucose and fructose were found to be the best carbon followed by sucrose and maltose for gluconic acid production as well as for the growth of the experimental fungus. On the other hand, CMC (carboxy methyl cellulose), lactose, galactose and cellobiose seemed unsuitable for gluconic acid production and mycelial biomass yield. A similar situation has been described for gluconic acid production by other investigators (Rosenberg *et al.*, 1992; Madhavi *et al.*, 1999; Singh *et al.*, 1999). Their observation indicated that D-glucose was the best carbon source not only for gluconic acid production but also for biomass production by different fungal strains.

Glucose concentration: The concentration of carbon sources plays an important role on the conversion of glucose into gluconic acid. Thus, different glucose concentrations (2~30%, w/v) were used to investigate the effect of carbon level on this fermentation reaction (Fig. 3). It was observed that the better conversion in relatively dilute medium containing 10~20% glucose. Beyond this concentration, gluconic acid production decreased while the biomass yield remained relatively constant. These results

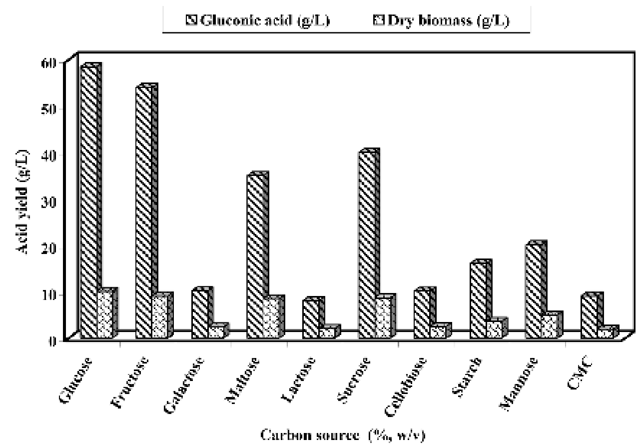


Fig. 2. Effect of different carbon sources (10% w/v), CMC and starch (5% w/v) on the gluconic acid production and biomass of *A. niger* at pH 6.0 and 30°C for 7 days in submerged culture.

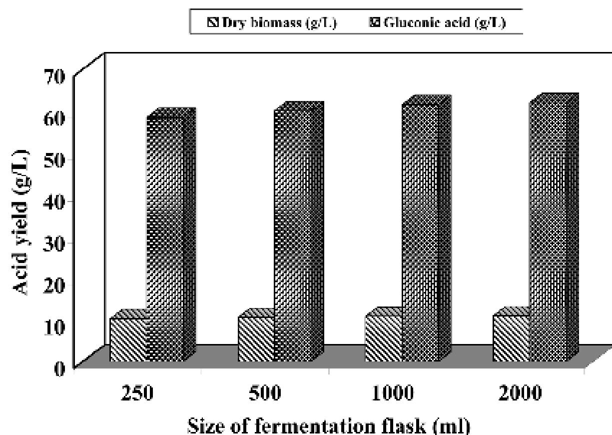


Fig. 1. Effect of aeration on the gluconic acid production and biomass of *A. niger* in submerged culture at 30°C for 7 days.

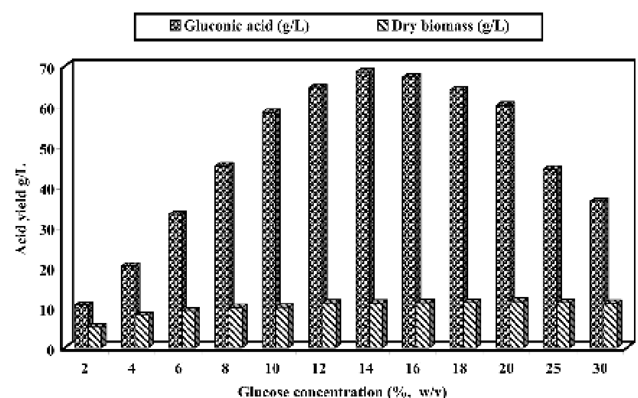
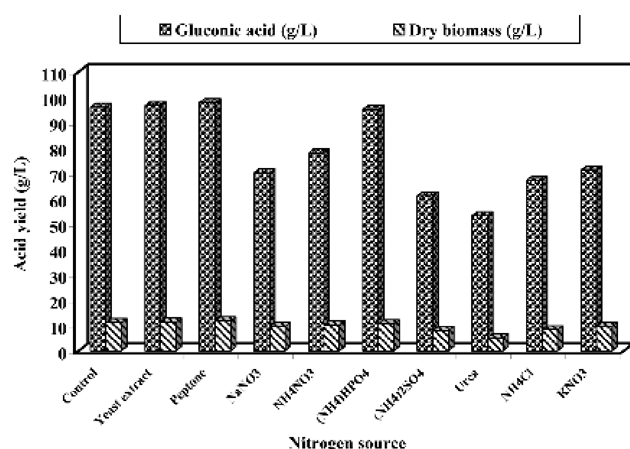


Fig. 3. Effect of different glucose concentrations (%w/v) on gluconic acid production and biomass of *A. niger* in submerged culture at pH 6.0 and 30°C for 7 days.

coincided with those previously reported for several fungal species and indicated that the gluconic acid production was dependent on substrate concentration (El-Naghy and Megalla, 1975; Moresi *et al.*, 1992; Singh *et al.*, 1999; Sankpal and Kulkarni, 2002). Thus, the enhancement in the gluconic acid production can be attributed to the induction of glucose oxidase at increased glucose level in fermented medium (Ptruccioli and Federica, 1993; El-Enshasy, 2003).

Nitrogen sources: On an equivalent nitrogen basis, the mixture of $NaNO_3$ and yeast extract was replaced by $NaNO_3$, peptone, yeast extract, NH_4NO_3, $(NH_4)_2HPO_4$, $(NH_4)_2SO_4$, KNO_3, NH_4C, and urea (Fig. 4). The results revealed that peptone was the best source for gluconic acid and biomass production (97.8 and 11.7 g/*l*, respectively) followed by yeast extract, control (mixture of $NaNO_3$ and yeast extract), $(NH_4)_2HPO_4$, and NH_4NO_3. Urea was the least favorable nitrogen source. Other investigators have confirmed the selective induction of nitrogenous compounds on gluconic acid production and growth of several fungi (El-Neghy and Megalla, 1975; Singh *et al.*, 1999).



Each medium contains 14% (w/v) glucose as carbon source and control is mixture of 0.3% $NaNO_3$ and 1% yeast extract.

Fig. 4. Effect of different nitrogenous compounds on gluconic acid production and biomass of *A. niger* in submerged culture at pH 6.0 and 30°C for 7 days.

The results of the quantitative effect of peptone on both parameters are presented in Fig. 5. It could be seen that maximum gluconic acid and biomass yields (i.e. 99.80 and 12.01 g/*l*, respectively) was obtained at 1.0% peptone concentration in the basal medium containing 14.0% (w/v) glucose. Generally, gluconic acid production decreased above or below this peptone level. However, mycelial biomass depended on peptone level and increased with the increase of peptone concentration in bioprocess. El-Naghy and Magalla (1975) and El-Ktatney (1978) have been found that 1.0% peptone gave the optimum gluconic acid production by *P. puberulum* and *A. carneus*.

Inorganic salts: In order to enhance the rate of conversion of glucose to gluconic acid, the influence of several inorganic salts constituting the fermented medium were investigated (Fig. 6). Addition of $MgSO_4 \cdot 7H_2O$ up to concentration of 0.025% showed stimulatory effect on gluconic acid production i.e. 100.24 g/*l*. High concentrations decreased the formation of gluconic acid, while the highest mycelial dry weight was obtained at 0.05% $MgSO_4 \cdot 7H_2O$ i.e. 12.01 g/*l*. On the other hand, the addition of KH_2PO_4 up to 0.05% induced gluconic acid yield from 57.5 to 71.78% and then decreased, with high concentrations of this salt (Fig. 7). But, the dry biomass remained constant at high concentration of KH_2PO_4. These observations coincided with those previously mentioned by other investigators (El-Naghy and Megalla, 1975; Singh *et al.*, 1999) who found that the addition of 0.05% KH_2PO_4 increased gluconic acid production by *A.niger* ORS-4. Also, El-Ktatney (1978) reported that the mycelial dry weight of *A. carneus* increased with more additional concentration of KH_2PO_4 but gluconic acid decreased at the higher concentrations. The potassium chloride free medium

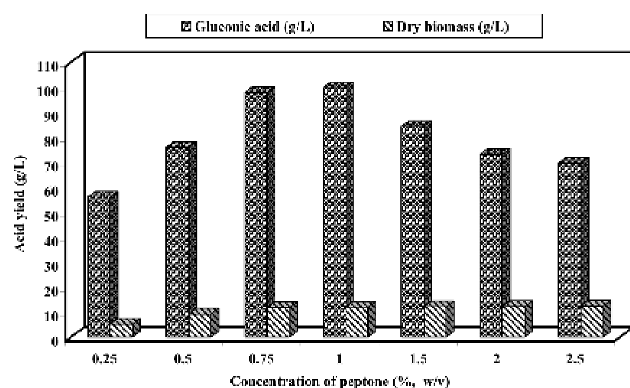


Fig. 5. Effect of different concentrations of peptone on gluconic acid production and biomass of *A. niger* in submerged culture (14% glucose) at pH 4.0 and 30°C for 7 days.

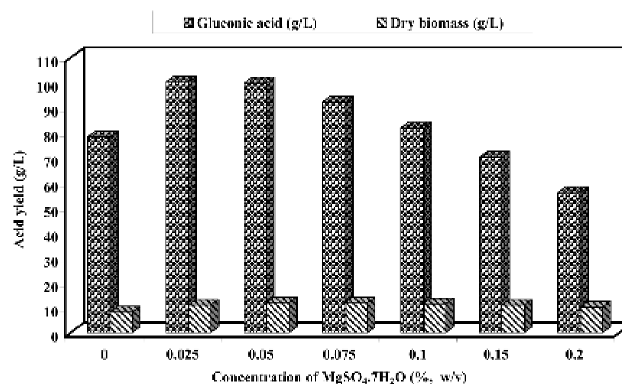


Fig. 6. Effect of different concentrations of $MgSO_4 \cdot 7H_2O$ on gluconic acid production and mycelial dry biomass of *A. niger* in submerged culture (14% glucose and 1% peptone) at pH 6.0 and 30°C for 7 days.

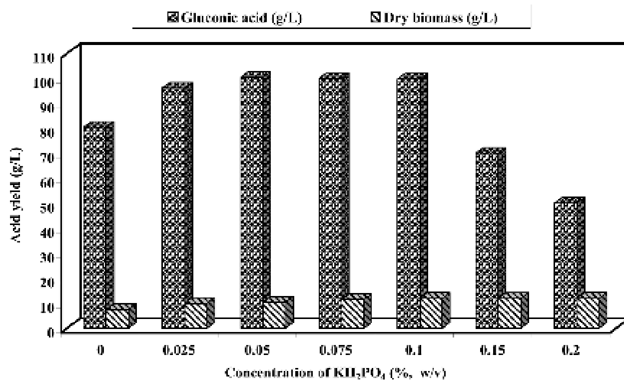


Fig. 7. Effect of different concentrations of KH_2PO_4 on gluconic acid production and mycelial dry biomass of *A. niger* in submerged culture (14% glucose and 1% peptone) at pH 6.0 and 30°C for 7 days.

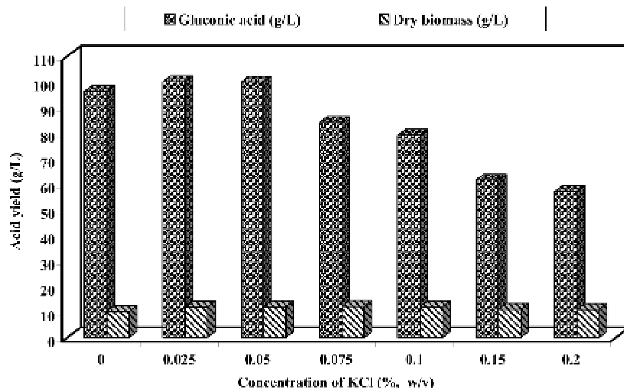


Fig. 8. Effect of different concentrations of KCl on gluconic acid production and mycelial dry biomass of *A. niger* in submerged culture (14% glucose and 1% peptone) at pH 6.0 and 30°C for 7 days.

did not have significant influences on the gluconic acid production as well as fungal dry biomass (Fig. 8). The highest gluconic acid production was obtained with 0.025% and decreased thereafter. In general, Singh *et al.* (1999) reported that the concentrations of inorganic salts greater than 0.1% with an initial pH 6.5 was not beneficial for gluconic acid production by *A. niger* ORS-4.

In summary, the data obtained in this study indicated that a good yield of the desired products depended on the presence of appropriate physical conditions and nutritional requirements during fermentation process. Thus, it can be concluded from this report that experimental strain of *A. niger* could be employed for producing gluconic acid on a large scale as grown on the optimization bioprocess. Further work is in progress for improving producing strain as well as economizing the process of production.

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