Recent Research in Science and Technology 2011, 3(9): 88-91
ISSN: 2076-5061
Available Online: http://recent-science.com/

To study the various parameters for bioconversion of glucose to gluconic acid by *Penicillium chrysogenum* in submerged culture

Nilesh K. Purane[1*], Shital K. Sharma[1], Swapnil D. Topre[2], Sreejit S. Panikar[2], Dinesh S. Labade[3]

[1]*Bioprocessing & Herbal Division, Mahatma Gandhi Institute for Rural Industrialization, Wardha 442 001, India*
[2]*Department of Biotechnology, Agnihotri college of science, Wardha 442 001, India*
[3]*Vidya Pratisthan School of Biotechnology, Baramati 413133, India*

Abstract

The present study was focused on optimization of various submerged fermentation parameters as glucose concentration, inoculum density and inoculum age for gluconic Acid (GA) production by *P. chrysogenum* 724. At 100 gL^{-1} glucose concentration, the maximum value of GA concentration (31.16 gL^{-1}) with high productivity (0.33 $gL^{-1}h^{-1}$) was achieved over 96 hours. On other hand, lower productivity (0.24 $gL^{-1}h^{-1}$ & 0.21 $gL^{-1}h^{-1}$) was obtained at higher glucose concentration level (150 gL^{-1} & 200 24 & 48 hours gL^{-1}) respectively. The level of GA production was higher at 10% inoculum density as compared to 5% & 20%, also showed a constant increase of GA production (31.36 gL^{-1}) with high production rate (0.33 $gL^{-1}h^{-1)}$ and selectivity (79.66 %) over 96 hours. The inoculum age with 72 hours was showed stable GA production (31.36 gL^{-1}) with higher productivity (0.33 $gL^{-1}h^{-1}$) in comparison other inoculum age (24 & 48 hours).

Keywords: *P. chrysogenum*, Gluconic acid, Glucose concentration, Inoculum density, Inoculum age

INTRODUCTION

Organic acid constitute as key group among the building block chemicals that can be produced by microbial process. Among all the organic acids, gluconic aicd is multifunctional carbonic acid used as bulk chemical in the food, beverage, textile, leather, pharmaceuticals, construction and other biological industries [1, 2, 3]. Gluconic acid production is simple oxidation process carried out by different mode of reaction. Among these, microbial fermentation has become a viable mode for commercial production. It is produced from glucose through a simple dehydrogenation reaction catalyzed by glucose oxidase in case of fungi and glucose dehydrogenase in case of bacteria [4]. Most studied and widely used GA fermentation process involves filamentous fungus *A. niger*. Beside *A. niger*, other species such as *Penicillium, Gliocadium, Scopulariopsis,* and *Gonatabotrys* have been tested and reviewed by Misson and Meers [5] and Ramchandran et al., [6]. Several bacterial species incluidng *G. oxydans, Z. mobalis, A. methanolicus, P. florescens,* and the species of *Morexella, Tetracoccus, pullularia, Micrococcus, Enterobacter* and *Scopulariopsis* participate in GA production.; this process has been reviewed by Misson and Meers [5] and Ramchandran et al., [6]. The yeast-like strain *Aureobasidium pullulans* has been evaluated for GA production with success [3, 7, 8, 9]. The average demand of GA is about 50,000-60,000 tones and estimated higher cost US $ 1.20-8.50/Kg of GA and its derivatives [6]. The uneconomical carbohydrate substrate and the system-specific requirements in fermentation contribute to the high price of GA and its derivatives. However, its huge market consumption has spurred interest in the development of an effective and economical system for GA production. May et al. first reported the production of gluconic acid by *Penicillium luteum purpurogenum* group and showed their grated ability for GA production [10]. In the present report an attempt was made to demonstrate the role of *Penicillium chrysogenum* fungal species as reservoir of glucose oxidase enzyme and their stability as biocatalysts in biotransformation of glucose to gluconic acid in submerged fermentation conditions. We also suggest a careful analysis of diversity of fungal potential, particularly submerged fermenting conditions may facilitate the strategies for commercial production of gluconic acid.

Received: May 01, 2011; Revised August 11, 2011; Accepted August 19, 2011.

*Corresponding Author

Nilesh K. Purane
Bioprocessing & Herbal Division, Mahatma Gandhi Institute for Rural Industrialization, Wardha 442 001, India

Tel: +91-9960-613765
Email: npurane08@gmail.com

MATERIALS & METHODS

Microorganism

A strain of *P. chrysogenum* 724 (NRRL 811) was provided by NCL Pune. During the study, the stain was maintained on Potato Dextrose Agar at 4-6 ^{0}C and sub-cultured every month.

Harvesting of spores and inoculum preparation

The spore of *P. chrysogenum* from slant were harvested with the help of 0.1% pre-sterilized Tween 80. The spores in inoculum were maintained at 2 × 10^6 and it was inoculated in Erlenmeyer flasks containing spore germination medium with following composition: Glucose 5%, Yeast extract 1%, $CaCO_3$ 0.3%, $NaNO_3$ 0.25%, $MgSO_4$ 0.005%, KH_2PO_4 0.025% (pH 5.5). The inoculated medium was put on orbital shaking incubator 28^{0}C with 150 rpm.

Culture conditions for GA production

The fermentation was carried out in 500ml of Erlenmeyer flasks containing 300 ml fermentation medium with following composition: Glucose 10%, $CaCO_3$ 2.8%, $NaNO_3$ 0.3%, $MgSO_4$ 0.0125%, KH_2PO_4 0.015% (pH 5.5). The fermentation medium was inoculated with 10% inoculum and incubated at 28^{o}C on orbital shaker with 150 rpm up to

96 hours. In these studied, only one parameter was varied at time, keeping other parameter unaltered. When a parameter was showing significant results at particular point, that parameter was regarded as optimum. Toward this purpose three parameters were taken in consideration including glucose concentration (5-20%), inoculum density (5-20%), inoculum age (24-72 hours). The samples were collected after specific interval during fermentation and analysis of samples were carried out.

Determination of dry biomass

Culture fluid was filtered through Whatman No 1 paper. The filtered mycelia were washed with acidified (pH 2.5 with 4M HCl) distilled water to convert the insoluble $CaCO_3$ to soluble $CaCl_2$. The separated mycelia were washed several times with demonized water until the pH of spent wash was 7.0. Then mycelia were dried (75°C) to a constant weight. And its dry weight was determined by subtracting the average predetermined dry weight of Whatman No 1 paper from the combined weight of Whatman No 1 paper along with mycelium [11].

Determination of glucose and GA

The glucose content in filtrate sample was estimated by standard Di-nitro-salicyclic acid (DNSA) method using glucose as standard [12]. The GA concentrations in samples were analyzed as described by Lehman [13].

RESULTS & DISCUSSION

At the industrial level, *Aspergillus niger* is most commonly employed microorganism. *P. chrysogenum* was also significantly producing gluconic acid in submerged culture [14]. Crognale et al., [15] reported different feeding strategies for industrial fed-batch gluconic acid production from *P. variable.* Hence, this strain was selected for present study.

Effect of glucose concentration

Effect of glucose concentration on cell growth & the production of GA were investigated in 500 ml flasks (300 ml fermentation medium) containing 50-200 gL^{-1} of glucose. The course of GA concentrations and productivity, during the GA production by *P. chrysogenum* is depicted in figure 1 and 2.

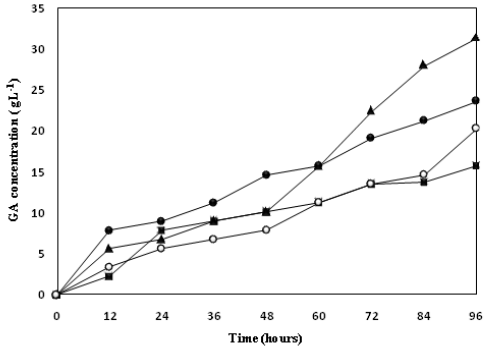

Figure 1. Effect of glucose level as 5% glucose (■), 10 % glucose (▲), 15% glucose (●), 20% glucose (○) on GA concentration by *P. chrysogenum*

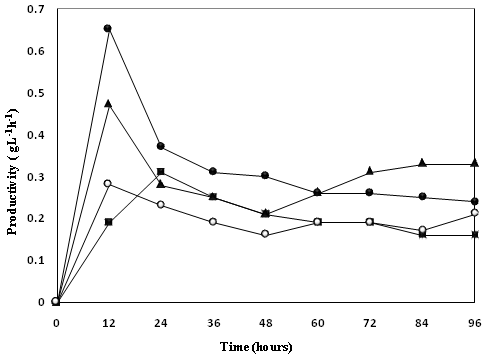

Figure 2. Effect of glucose level as 5% glucose (■), 10 % glucose (▲), 15% glucose (●), and 20% glucose (○) on productivity of GA production by *P. chrysogenum.*

At 50 gL^{-1} glucose concentration, the high productivity (0.19 $gL^{-1}h^{-1}$) with 2.24 gL^{-1} GA production value was observed after 12 hours. This was followed by decreasing productivity with increasing selectivity of *P. chrysogenum* toward GA production. After 96 hours, it showed highest selectivity (97.15%) with final 15.59 gL^{-1} GA production.

At 100 gL^{-1} glucose concentration, the productivity (0.14 $gL^{-1}h^{-1}$) was high at 12 hours, beyond that, rate was decreased but showed proportional increment in selectivity. The maximum value of GA concentration (31.16 gL^{-1}) was achieved after 96 hours. On other hand, lower productivity (0.24 $gL^{-1}h^{-1}$ & 0.21 $gL^{-1}h^{-1}$) was obtained at higher glucose concentration level (150 gL^{-1} & 200 gL^{-1}).

This difference may be originated from the positive influence of higher level of glucose on enzyme activity. It is seemed that culture condition at 100 gL^{-1} glucose concentration promoted cell for GA production with less biomass formation (19.97 gL^{-1}), but higher concentration of glucose (150 gL^{-1} & 200 gL^{-1}) decreased the level of GA production, but showed more biomass level (22.00 gL^{-1} & 20.31 gL^{-1}). So, the 100 gL^{-1} glucose concentration was appropriated for gluconic acid production by *P. chrysogenum.* In addition, the high sugar level also created problem of accumulation of excess sugar proved to be critical for product recovery process.

Effect of inoculum density

In commercial fermentation, high concentration of inoculum is usually used to shorten lag period. In order to examine the effect of mycelium inoculum concentration on length of lag period and production of GA, different density of seed-cultured mycelia were inoculated (5%, 10% & 20%) and cell were cultivated in 100 gL^{-1} sugar level. As anticipated, the length of lag period was shortened, apparently to increase the size. The GA production with different inoculum density is as shown in figure 3 and 4.

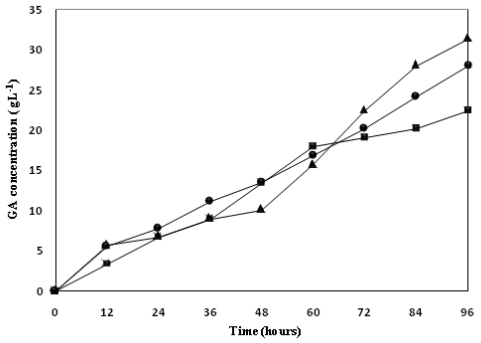

Figure 3. Effect of inoculum density as 5% (■), 10 % (▲) and 20% (●) on GA concentration by *P. chrysogenum.*

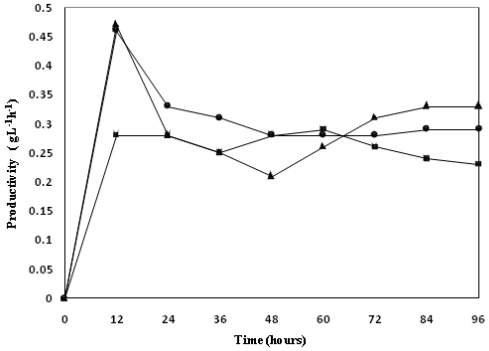

Figure 4. Effect of inoculum density as 5% (■), 10 % (▲) and 20% (●) on productivity of GA production by *P. chrysogenum.*

The level of GA production (5.56 gL^{-1}) was higher at 10% inoculum density, also showed significant productivity (0.47 $gL^{-1}h^{-1}$) after 12 hours, then followed by constant increase of GA production (31.36 gL^{-1}) with high production rate (0.33 $gL^{-1}h^{-1)}$ and selectivity (79.66 %) was observed. While in other two inoculum level, GA production rate was higher but later showed declined level of productivity. At 5% inoculum level, low GA concentration (22.42 gL^{-1}) was obtained after 96 hours. This may be because of low biomass availability to glucose to bring out the GA formation. It also showed efficient biomass formation (22.30 gL^{-1}) as compared to 10% inoculum level (19.97 gL^{-1}), because less inoculum level may require more residence time during fermentation to acquired efficiency for GA production. However, high level of biomass accumulation (56.71 gL^{-1}) may reduce the oxygen circulation during fermentation and retard the GA formation activity of fungus at 20% inoculum level.

Effect of inoculum age

The fungus shows their glucose oxidase activity after lag period of growth during fermentation. So, inoculum age takes importance for submerged fermentation condition. In case of *P. chrysogenum*, different levels of inoculum were employed and studied their effect on GA concentration during fermentation process (Figure 5 and 6).

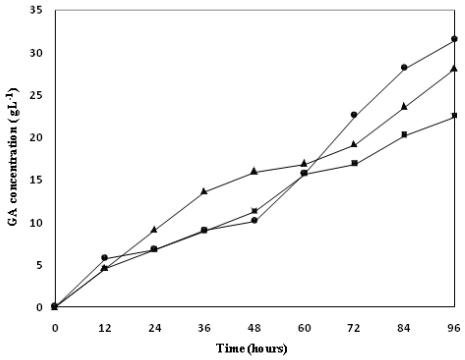

Figure 5. Effect of inoculum age as 24 hours (■), 48 hours (▲) and 72 hours (●) on GA concentration by *P. chrysogenum.*

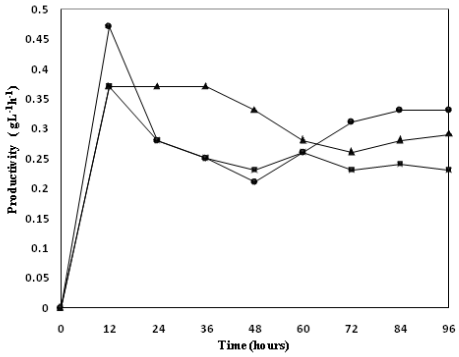

Figure 6. Effect of inoculum age as 24 hours (■), 48 hours (▲) and 72 hours (●) on productivity of GA production by *P. chrysogenum.*

At 24 hours inoculum age level, lower GA concentration (4.48 gL^{-1}) and productivity (0.37 $gL^{-1}h^{-1}$) was observed after 12 hours. Later, productivity of *P. chrysogenum* was increased. The final GA production was obtained as 22.42$gL^{-1}h^{-1}$ with 0.23 $gL^{-1}h^{-1}$ productivity. At 48 hours inoculum age, results showed the constant production rate (0.37 $gL^{-1}h^{-1}$) for GA production with increment in selectivity after 36 hours during fermentation. Finally 28.03 gL^{-1} GA was produced with 0.29 $gL^{-1}h^{-1}$ productivity.

At 72 hours inoculum age, initially the production rate (0.47 $gL^{-1}h^{-1}$) of GA during fermentation was more in comparison of other inoculum age levels. This production proportionally increased, maximum up 31.36 gL^{-1} GA concentration with 0.33 $gL^{-1}h^{-1}$ productivity.

Among these all inoculums, 72 hours inoculum was showed stable GA production with higher productivity. However, remaining two low age inoculum showed less potential for GA production under submerged fermentation. In addition, these inoculum showed decreasing order of production rate because they required more residence time accumulation of glucose oxidase activity for GA production.

The development of the new superior industrial fermentation process for the submerged fermentation of GA has accomplished the highest expectation of an expertise researcher in the field of industrial applied microbiology and biotechnology, extending the frontiers of known fungal capabilities and showing the importance of the deep understanding of traditional and industrial microbiology in biotechnological science. Although *A. niger* and other microbial

species employed for efficient gluconic acid production fermentation, *P. chrysogenum* has greater potential to utilized their glucose oxidase activity for gluconic acid production and it will used for large scale fermentation. In these studies, different fermentation conditions including substrate concentration, inoculum density and inoculum age were comparatively examined in order to study the performance of the fermentative gluconic acid production by the strain of *P. chrysogenum* and it showed their improved performance in fermentation at optimised condition as 10% glucose concentration, 10% inoculum density and 72 hours inoculum age. Although further experimentation is still needed, *P. chrysogenum* has feasible potential for GA production under submerged fermentation condition.

ACKNOWLEDGEMENT

We take this opportunity to express our sincere thanks and gratitude Dr. A.K. Agnihotri sir for their valuable guidance and help. Finally we acknowledge with deep appreciation, the indispensable help, encouragement and moral support received our colleague and dear ones.

REFERENCES

[1] Hustede, H., H. J. Haberstroch and E. Schinzig. 1989. Gluconic acid. In*:* ULLMANN'S encyclopedia of industrial chemistry. VCH-Weinheim, 12:449–456.

[2] Znad, H., J. Markos and V. Bales. 2004. Production of gluconic acid from glucose by *Aspergillus niger*: Growth and non growth conditions. Process Biochem*.* 39:1341–1345.

[3] Anastassiadis, S., A. Aivasidis and C. Wandrey. 2003. Continuous gluconic acid production by isolated yeast-like mould strains of *Aureobasidium pullulan.* Appl. Microbiol. Biotechnol. 61:10–117.

[4] Singh, O., V. and R. Kumar. 2007. Biotechnological production of gluconic acid: future implications. Appl. Microbiol. Biotechnol. 75:713–722.

[5] Milson, P., E. and J. L. Meers. 1985. Gluconic acid, itaconic acid. In: Blanch HW, Drew S, Wang DIC (eds) Comprehensive biotechnology. Pergamon, Oxford, 3, pp. 681–700.

[6] Ramachandran, S., P. Fontanille. A. Pandey and C. Larroche. 2006. Gluconic acid: properties, applications and microbial production, a review. Food Technol. Biotechnol. 44:185–195.

[7] Anastassiadis, S., A. Aivasidis, C. Wandrey and H. J. Rehm. 2005. Process optimization of continuous gluconic acid fermentation by isolated yeast-like strains of *Aureobasidium pullulans.* Biotechnol. Bioeng. 91:494–501.

[8] Anastassiadis, S. and H. J. Rehm. 2006a. Continuous gluconic acid production by the yeast-like *Aureobasidium pullulans* in a cascading operation of two bioreactors. Appl. Microbiol. Biotechnol. 73:541– 548.

[9] Anastassiadis, S. and H. J. Rehm. 2006b. Continuous gluconic acid production by *Aureobasidium pullulans* with and without biomass retention. Electron. J. Biotechnol*,* 9:494–504.

[10] May, O., E., H. T. Herrick, G. Thom and N. B. Church. 1927. The production of gluconic acid by *Penicillium luteum purpurogenum* Group. I. J. Biol. Chem. 75: 417-422.

[11] Singh, O., V., N. Kapur and R. P. Singh. 2005. Evaluation of agro-food by- products for gluconic acid fermentation by *Aspergillus niger* ORS-4.410. World J. Microbiol. Biotechnol. 21:519–524.

[12] Miller, G., L. 1959. Use of Dinitrosalicylic acid reagent for determination of Reducing Sugar. Anal. Chem. 31:426–428.

[13] Lehmann J., K. 1985. Biotechnology. In: Comparative Tests for Fermentations, (Rehm HJ, Reeds G, eds), VCH, Germany, pp. 620–624.

[14] Ambekar, G., R., S. B. Thadani and V. M. Doctor. 1965. Production of calcium gluconate by *Penicillium chrysogenum* in submerged culture. J. Appl. Microbiol. 13(5):713-719.

[15] Crognale, S., M. Petruccioli, M. Fenice and F. Federici. 2008. Fed-batch gluconic acid production from *Penicillium variable* P16 under different feeding strategies. Enzyme and technol. 42:445-449.