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Gluconic acid production under varying fermentation conditions by *Aspergillus spp.*

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ABSTRACT

The production of gluconic acid with respect to varying substrate concentrations in submerged (SmF), surface (SF) fermentations was analyzed. Under the various fermentation conditions the biomass and specific growth rate varied with different concentrations of glucose. The effects of pH, temperature, incubation time and concentrations of carbon were tested in submerged fermentation process in production of gluconic acid by *Aspergillus spp*. The highest level of gluconic acid was obtained under SmF conditions. In all cases the maximum degree of gluconic acid conversion was observed at on initial substrate concentration of 10gm/100ml. The rate of glucose uptake increased on increasing the initial glucose concentration and glucose utilization was observed to be highest in the SmF process and was comparable with the SSF and SF processes. The maximum rate of cell growth was obtained in all processes at an initial glucose concentration of 10gm. But in comparison to glucose, sucrose is more effective than glucose. The gluconic acid production and change in pH were analyzed at varying time intervals and it was observed that the SmF and SF processes were completed within 5 days of incubation whereas the highest yield was observed after 3rd day of incubation and continued thereafter in the SmF process. The increase in production of gluconic acid corresponds to the increase in cell growth instead of Glucose Oxidase (GOD) activity.

KEYWORDS

Submerged (SmF);
Surface (SF) fermentations;
Gluconic acid;
Glucose oxidase (GOD);
Aspergillus spp.

INTRODUCTION

Gluconic acid (pentahydroxycaproic acid) produced form glucose through a simple dehydrogenation reaction catalysed by glucose oxidase. Oxidation of aldehyde group on C-1 of β-D-glucose to a carboxyl group results in the production of glucono-δ-lactone ($C_6H_{10}O_6$, Figure 1) and hydrogen peroxide. Glucono- δ-lactone is further hydrolysed to gluconic acid either spontaneously or by lactone hydrolysing enzyme, while hydrogen peroxide is decomposed to water and oxygen by peroxidase. The pathway is elaborated in figure 2.

The conversion process could be purely chemical too, but the most commonly involved method is the fer-

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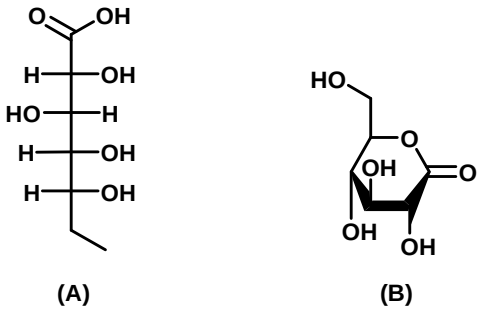

Figure 1 : Formula of gluconic acid (A) and glucono-δ-lactone (B).

mentation process. The enzymatic process could also be conducted, where the conversion takes place in the absence of cells with glucose oxidase and catalase derived from *A. niger.*

Gluconic acid production dates back to 1870 when Hlasiwetz and Habermann discovered gluconic acid[1]. In 1880 Boutroux[2] found for the first time that acetic acid bacteria are capable of producing sugar acid. In 1922 Molliard[3] detected gluconic acid in the *Sterigmatocystis nigra*, now known as *Aspergillus niger*.

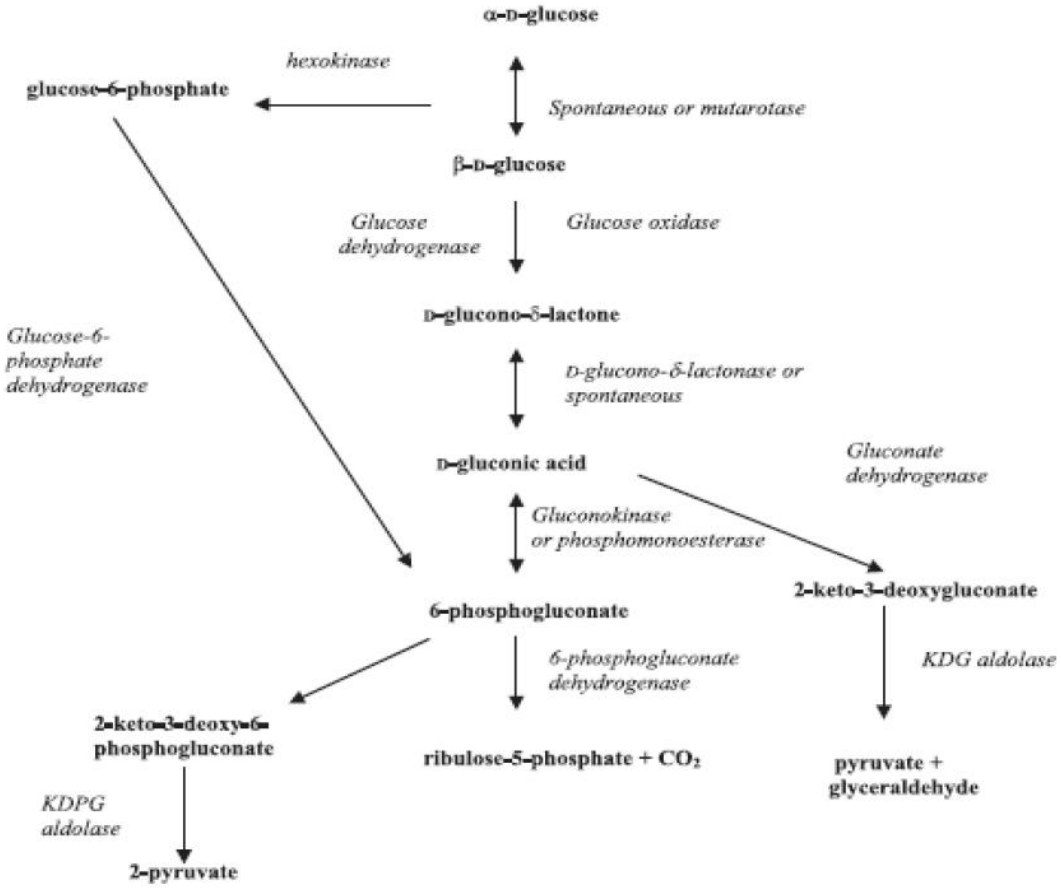

Figure 2 : Gluconate production pathway.

Later, production of gluconic acid was demonstrated in bacterial species such as *Pseudomonas, Gluconobacter, Acetobacter*, and various fungal species. High yields of gluconic acid when it was neutralised by calcium carbonate and the production was found to be highly pH dependent. It was found that with *Penicillium* sp., the pH dependence is not as critical when compared to *A. niger*[4-6]. Gluconic acid production has been extensively studied by May *et al.*[7], Moyer[8], Wells *et al.*[9], and Stubbs *et al.*[10] using *A. niger*.

Gluconic acid is a mild organic acid, which finds applications in the food industry. As stated above, it is a natural constituent in fruit juices and honey and is used in the pickling of foods. Its inner ester, glucono-d-lactone imparts an initially sweet taste which later becomes slightly acidic. It is used in meat and dairy products, particularly in baked goods as a component of leavening agent for preleavened products. It is used as a flavouring agent (for example, in sherbets) and it also finds application in reducing fat absorption in doughnuts and cones. Foodstuffs containing D-glucono-δ-

lactone include bean curd, yoghurt, cottage cheese, bread, confectionery and meat. The reaction involving the conversion of glucose to gluconic acid by filamentous fungi is catalysed by the enzyme glucose oxidase (β-D-glucose: oxygen 1-oxidoreductase, E.C. 1.1.3.4). The enzyme was first isolated from a press juice obtained from *Penicillium glaucum* by Müller[11]. Fungal spores are generally utilized for strain conservation and dissemination. In industrial larga scale fermentation, spores are used as inoculums, which after vegetative growth produces primary and secondary metabolites. However, spores could accomplish a wide range of conversion reactions without being allowed to germinate. Studies showed that spores of *Aspergillus niger* can be utilized as a catalyst in bioconversion of glucose to Gluconic acid[12].

MATERIAL AND METHODS

Microorganism

The samples of *Aspergillus spp.* was isolated from fertile garden soil, by plating method using Sabouraud Dextrose Agar without antibiotic.

Growth media

For isolation of *Aspergillus spp.* Sabouraud Dextrose Agar medium containing Dextrose 40.0g, peptic digest of animal tissue 5.0g, Pancreatic digest of casein 5.0g, agar15.0g and distilled water (1000.0ml) was used.

Production medium

Production medium was composed of Sugars-as carbon source (Glucose, Sucrose, Lactose) 10.0 g, $MgSO_4.7H_2O$ 0.015 g, KH_2PO_4 0.02 g, Na_2HPO_4 0.04g and distilled water 100.0ml. The pH was adjusted to 6.0 and the media were sterilized in an auto clave for 15 min. at 121^{0}C. The media were inoculated with measured amount of spore suspension of *Aspergillus spp.* and then incubated at 30^{0}C in an orbital shaker set at 200-220 rpm. Because aeration is necessary for better production of Gluconic acid. At the end of fermentation, the cell mass was disrupted to extract the intracellular enzyme and centrifuged. The filtrate was analysed for decrease in sugar concentration by DNSA method and Ca-gluconate production. And the supernatant was assayed for glucose oxidase activity.

Enzyme assay

An enzyme assay mixture was prepared by adding 0.5 ml of glucose solution, 1.0ml of crude enzyme preparation, 2.4ml of o-dianisidine (for color development), 0.1 ml of peroxidase. The reaction mixture was incubated at 30^{0}C for 30min. The reaction was stopped by adding 2.5ml of concentrated HCL into the mixture. The absorbance was measured at 550nm. And the activity was measured in terms of µmol units/min/ml of enzyme. One unit of glucose oxidase activity is expressed as the amount of enzyme which converts 1.0 µg of glucose into gluconic acid per 30min. at 30^{0}C.

Optimization of culture conditions

The factors such as pH, temperature, substrate concentration, various sources of carbon, that influence the production of gluconic acid as well as glucose oxidase were optimized by varying parameters on at a time. The experiments were conducted in 250ml Erlenmeyer flasks containing production medium. After sterilization by autoclaving the flasks were cooled and inoculated with culture and maintained under various operational conditions separately such as pH (5.0, 6.0, 7.0, 8.0), temperature (30, 37, 40^{0}C), incubation period (48,72,96,120h), carbon sources (glucose, sucrose, lactose), various inoculums concentrations. After each time interval, the culture filtrate was assayed in duplicate samples for glucose oxidase activity.

Figure 3 : Sterile production medium for gluconic acid production.

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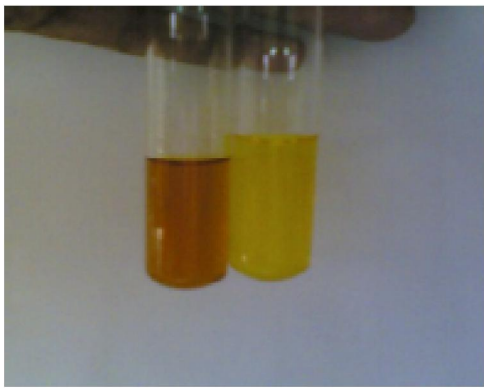

Figure 4 : DNSA result.

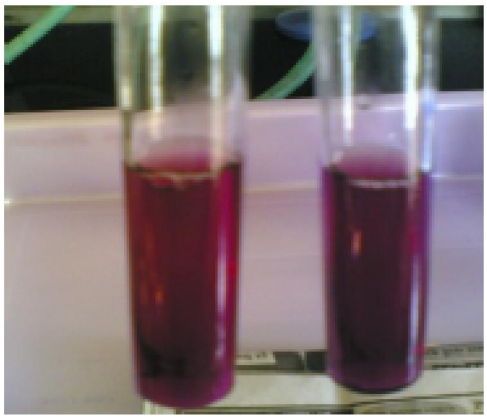

Figure 5 : Detection of enzyme activity.

Figure 6 : Ca- gluconate estimation

RESULTS AND DISCUSSION

Production medium inoculated with *Aspergillus spp*. spore suspension and incubated for 120 h, exhibited the gluconic acid production of 3.084gm% (of Ca-gluconate) at pH 7.00 and37 ^{0}C.

Among the carbon sources, sucrose was the best source to enhance gluconic acid as well as enzyme production of.

When using lactose as the carbon source shows the merely the same results as with glucose.

The general optimal condition for gluconic acid production is as follows (*1*):

- Glucose at concentrations between 110–250 g/L
- Nitrogen and phosphorus sources at a very low concentration (20 mM)
- pH value of medium around 4.5 to 6.5
- Very high aeration rate by the application of elevated air pressure (4 bar).

The media optimization is the important aspect to be considered in development of fermentation process. Among physical parameters pH of the growth medium plays very important role by inducing morphological changes in microbes and in gluconic acid production

TABLE 1 : Effect of glucose on production of gluconic acid at pH 7.00, 37^{0}C

Time (Hours)	Glucose Concentration (gm%)	pH	Weight of biomass (gm)	Ca-gluconate (gm)/100ml	Activity (µmol units / minute / ml of Enzyme.)
0	5.91	7.0(Initial)	-	-	-
48	5.31	6.8	5.744	2.636592	17.3328
72	5.31	6.8	5.480	3.084992	11.5552
96	5.21	6.8	6.908	2.860792	17.3328
120	4.91	6.6	7.802	3.084992	14.444

TABLE 2 : Effect of sucrose on production of gluconic acid at pH 7.00, 37^{0}C

Time (Hours)	Glucose Concentration (gm%)	pH	Weight of biomass (gm)	Ca-gluconate (gm)/100ml	Activity (µmol units / minute / ml of Enzyme.)
0	6.60	7.0 (Initial)	-	-	-
48	6.24	7.00	7.170	3.869692	90.9972
72	5.44	7.05	9.3565	4.486242	115.552
96	4.89	6.45	11.0415	4.957062	140.1068
120	3.94	6.05	10.329	5.439092	176.2168

TABLE 3 : Effect of lactose on production of gluconic acid at pH 7.00, 37^{0}C

Time (Hours)	Glucose Concentration (gm%)	pH	Weight of biomass (gm)	Ca-gluconate (gm)/100ml	Activity (µmol units / minute / ml of Enzyme.)
0	3.62	7.00(initial)	-	-	-
48	3.42	7.4	3.993	2.636592	4.3332
72	3.42	7.2	4.491	2.636592	7.222
96	3.22	7.3	4.972	2.636592	12.9996
120	3.32	7.2	4.858	2.860792	14.444.

with respect to enzyme secretion. The pH change observed during fermentation also affects product stability in the medium[13].

Earlier studies also support the optimum pH range 7.0 for the growth of *Aspergillus* strain and enzyme production[14,15] which supports the work.

Short incubation period offers potential for in expensive production of gluconic acid. In present study the GOD Activity increased steadily and reached maximum at 96h of incubation.

CONCLUSION

Although the production of gluconic acid is a simple oxidation process that can be carried out by electrochemical, biochemical or bioelectrochemical methods, production by fermentation process involving fungi and bacteria is well established commercially. Considerable progress has been made in understanding the mechanism of fermentation process by different microorganisms, and highly efficient production process.

From the results which were obtained it is clear that sucrose as the carbon source is very much important in gluconic acid production at 37^{0}C for pH 7.00 and glucose at higher concentration gives the same results.

Glucose and Sucrose can be easilyobtaine from various sources such as fruits, sugarcane etc. So the cost of gluconic acid production can be lowered down by using these alternatives. And by simply adding $CaCO_3$ pH of production medium can be maintained. This will add an advantage to the process.

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