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Application of Sodium Bi Carbonate for the Production of Sodium Gluconate Through Fermentation Technology

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Abstract- **The present study deals with the production of sodium gluconate by fermentation method. It provides methods for the conversion of glucose into gluconic acid and its derivatives using the *Aspergillus* niger. Due to the presence of sodium bicarbonate in fermentation medium the gluconic acid is converted into to sodium gluconate. Conditions like concentration of substrate, temperature, pH, fermentation period and different sodium sources were optimized during fermentation at shake flask level. The activity observed at 35^0c (pH5.5) after 136 h of incubation. Derivatives of gluconic acid that is, calcium gluconate, sodium gluconate, potassium gluconate, zinc gluconate and copper gluconate were formed by using double displacement and direct methods. The percentage yields of sodium gluconate with sodium bi carbonate as sodium source is by perchloric acid method is 76.9, 78.8% respectively and the sodium elemental percentage yield estimated by AAS method is 7.7 and 7.8% respectively.**

Key words- Aspergillus niger, Soidum gluconate, Fermentation, Sodium bicarbonate.

I. INTRODUCTION

Aspergillus niger is a well known producer of organic acids, enzymes and plant growth regulators, mycotoxins and antibiotics. A.niger is a prodigious exporter of species of homologous proteins and is able to produce certain enzymes in quantities of kg/m3 under right conditions. It is also generally regarded as safe (GRAS) and has long history of usage in the fermentation industry. Gluconic acid is derived from glucose through a simple dehydrogenation reaction. It is multifunctional organic acid used as bulk chemical in the food, feed, pharmaceutical, textile, metallurgy, detergent and construction industries. Glucose oxidase (EC 1.1.3.4., D-glucose: oxygenoxidoreductase) catalyses the oxidation of D-glucose to glucono lactone (C6H10O6) and hydrogen peroxidase using molecular oxygen as the electron acceptor. The worldwide production of gluconic acid is nearly about 87,000 tonnes /year and costs about 1.2.-8.50/kg of gluconic acid (Bussiness Communication Co., inc 2004). There are different approaches such as chemical, biochemical and electrochemical available for its production, but microbial fermentation process using filamentous fungus. A.niger the most widely used (Rohr et al 1996). These systems, although very efficient suffer from general drawbacks of mycelia cultivations, i.e. high viscosity of the medium and problems associated with mixing and aeration.

Glucose is generally used as carbon source for microbial production of gluconic acid. How ever hydrolysates of various raw materials such as agro-industrial waste have also been used as substrate (Kundu and Das 1984). Obtained a high yield of gluconic acid in media containing glucose or starch hydrolysate as the sole carbon source. Glucose is generally used as carbon source for microbial production of gluconic acid. Vassilev et al. (1993) used hydrol (corn starch hydrolysate) as the fermentable sugar to produce gluconic acid by immobilized A.niger. (Rao and Panda, 1994) in their study used Indian cane molasses as a source of glucose.

Sodium gluconate, the principal manufactured form of gluconic acid, is prepared by ion exchange. In the process developed by (Blom et al. 1952), the sodium gluconate from the filtered fermented broth is concentrated to 45% followed by the addition of sodium hydroxide solution raising the pH to 7.5 and drum drying carbon treatment of the hot solution before drying process is practiced for obtaining a refined product.

Sodium gluconate is the sodium salt of gluconic acid produced by fermentation of glucose. It is a white to tan, granular to fine, crystalline powder, very soluble in water. Non corrosive, non-toxic and readily biodegradable 98% after 2 days), sodium gluconate is more and more appreciated as chelating agent. Outstanding property of sodium gluconate is its excellent chelating power, especially in alkaline and concentrated alkaline solutions. It forms stable chelated with calcium, iron, copper, alluminium and other heavy metals, and in this respect, it surpasses all other chelating agents, such as EDTA NTA and related compounds. Aqueous solutions of sodium gluconate are resistant to oxidation and reduction, even at high temperatures. Sodium gluconate is also a highly efficient set retarder and a good plasticizer/water reducer for concrete, motar and gypsum.

Sodium gluconate is a compound with formula NaC6H11O7. It is the sodium salt of gluconic acid. It has the E number E576. Sodium gluconate is widely used in textile dyeing, painting and metal surface water treatment. It is also used as a chelating agent, a steel surface cleaning agent and

as a chelating agent for cement. It is a white colorless powder that is very soluble in water. Sodium gluconate is manufactured from gluconic acid by neutralization with sodium hydroxide. It can be sold as an aqueous solution or a powder crystalline form.

Key parameters which influence the gluconic acid production are given below (Rohr et al 1983)::

- Glucose at concentrations between110-250g/L
- Nitrogen and phosphorous sources at a very low concentration(20mM)
- pH value of medium around 4.5 to 6.5.
- very high aeration rate by the application of elevated air pressure(4 bar)

II. MATERIAL AND METHODS

Microorganism and media- A.niger culture was taken form culture library from Prathista industries ltd R&D center and it was transferred into a potato dextrose agar (PDA) slant for 4 days at 30^{o}C for the production of spores which was subsequently used as inoculums for the fermentation. The following medium composition (g/L) Glucose 120; yeast extract 40; KH2PO4 1.0; K2HPO4 1.0; MgSO4 0.5; NaCl 1.5 Na SO4 1.5, at pH 5.5 was used in production of mycelia and sodium gluconate.

The conversion of glucose into gluconic acid and its derivatives can be followed by analysis of the glucose content, analysis of the gluconic acid content, confirmation of gluconic acid, conversion of gluconic acid into a gluconate determination of the percentage yield of metal gluconate.

Micro-organism- The strain of *Aspergillus* niger was grown on potato dextrose agar (PDA) and malt extract agar medium at pH5.5.

Slants preparation- PDA was prepared by dissolving 40.0g of PDA in 1000 ml of distilled water. To make the clear solution, medium was first boiled with constant stirring up to 15-20 min and then poured in the cotton plugged sterilized test tubes.

Sterilization- The medium in the cotton plugged tubes and flasks were sterilized in the auto clave at 121^{o}C and 15lbs/inch for 20 min and the test tubes were placed in slanting positions for 24 hrs after

Inoculation- The slants were inoculated with the fresh strain of *A. niger* with the help of inoculums needle and incubated in the incubator at 37°C for 24 h. After every two weeks, propagation of strain on the fresh medium was continued. The pure and identified colonies of *A. niger* were stored in cold incubator/refrigerator at 4°C.

Fermentation media-Submerged fermentation was used for the production of sodium gluconate from *A. niger* in 250 ml shake flasks. pH was maintained with different sources of sodium salts for the formation of sodium gluconate. Bassically NaOH, sodium carbonate and socium bicornate can be utilized for the production.Sodium hydroxide (Na- 57.5%), sodium carbonate (Na- 43.4%), sodium bicarbonate. (Na -27.3%)

Estimation of sodium gluconate by perchloric acid method- Assay weight accurately about 0.15g of Sodium Gluconate, previously dried, and dissolve in 75 ml of acetic acid, and titrate with 0.1 mol/L perchloric acid solution until the red color of the solution disappears (indicator:10 drops of quinaldine red TS).perform a blank test in the same manner.

1 ml of 0.1 mol/l perchloric acid solution = 21.81 mg of C6H11NaO7

III. ANALYTICAL METHODS

The supernatant was used for sodium gluconate and glucose determination, where as the cell concentration was determine by pellet. The concentration of sodium gluconate was analyzed using AAS and glucose concentration was determined by dinitrosalicylic acid (DNS) method. Glucose dehyrogenase activity was estimated according to the method as described by lamble *et al*., and cell dry weight calculation followed the prabhu *et al* method.

Table 1: fermentation production of sodium gluconate by using sodium bicarbonate as sodium source instead of sodium hydroxide.

Strain	Glucose concentration (%)	Fermentation time in hours	Maximum cell concentration (g)	Maximum sodium gluconate concentration	Percentage of sodium by AAS
A.niger	10	136	32	76.59	7.7
A.niger	15	408	60	78.8	7.8

IV. DISCUSSION

The results revealed sodium gluconate synthesis has been increased until the growth stage of mycelia, where glucose was directly converted to gluconic acid and it salts. Subsequently, glucose concentration was consequently decreased in the culture which indicates that glucose was converted in to gluconic acid during a growth phase. The maximum sodium gluconate percentage of (76.59 and 78.8%) respectively was obtained in between 136-408 hours of fermentation in shake flask with 10 and 15% reducing sugars. (Among the various sodium sources sodium bicarbonate were played important role in sodium gluconate synthesis with maximum biomass production. Our results are in accordance with the results of Mischak(1985) and petruccioli and federici(1993); they reported that 8% glucose concentration enhanced the reaction, while Ray and Banik (1999) reported that 15% glucose concentration was affective. Probably sodium hydroxide can be used in chemical process as is highly alkaline it can be replaced with other sodium salts with low alkaline character. Sodium hydroxide suppress the growth of the culture if close observations is not done. Gluconic acid is readily reacting with sodium salts for sodium gluconate production. Sodium bicarbonate can be dissolved in reaction solution because of great chelating power of sodium gluconate. The reaction rate was proportional to the amount of sodium slats used for this oxidation reaction with glucose monohydrate. This result brought out that the sodium hydroxide can be replaced by sodium bicarbonate in fermentation process.

V. CONCLUSION

The above studies and predicated results have been successfully used to improve sodium gluconate producing fungus, A.niger. The strain was capable of producing higher sodium gluconate and gluconic acid. This simple and strain development method could be useful to development of fermentation process for other industrial production.

Further research work is needed to focus on utilising more glucose concentration for maximum production of sodium gluconate through fermentation.

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