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Microbial Gluconic Acid Production: A Value Addition to the Sugar Industry

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ABSTRACT

There is a growing interest for gluconic acid production due to its significant applications in different industries like chemical, pharmaceutical, food, beverage, construction and textile. In the present study, batch scale scale fermentation was carried out using a fungal culture for bio-conversion of glucose to gluconic acid. Two strategies such as $CaCO_3$ and KOH were followed to maintain the pH of the medium to 6.0 during fermentation. Flask scale experiments were carried out using $CaCO_3$ and fermenter scale experiments were carried using KOH as the neutralizing agent. Batch fermentation using $CaCO_3$ resulted maximum 123.98 g/l of calcium gluconate with 0.95 g/g yield and 0.73 g/l/h productivity. As per the literature, 128.5 g/l of maximum calcium gluconate is reported till date under similar operating conditions. Also, NaOH/KOH has been used as the superior. Therefore, further experiments were carried out using KOH as the neutralizing agent. The batch fermenter scale experiments using KOH as the neutralizing agent resulted maximum 274.32 g/l of potassium gluconate with 0.78 g/g yield and 2.29 g/l/h productivity. This efficacy for gluconic acid production using the fungal culture can be commercialised to use surplus sugar of sugar and allied industries. It will allow sugar mills to diversify towards gluconic acid production to improve their profitability.

Keywords: Fungal culture, gluconic acid, batch fermentation, pH control strategies.

INTRODUCTION

Valorization of glucose is a crucial for industry in bio-refinery producing chemicals such as gluconic acid (GOA), as excessive sugar is being produced in India since last 10 years and its diversification will utilise surplus sugar and surplus sugarcane juice for the production of valuable products enhancing the economic returns to the sugarcane industry (Canete-Rodriguez et al. 2016). In this aspect, except ethanol, sugar mills can also diversify towards production of important bio-chemical such as gluconic acid to improve their profitability.

Gluconic acid and its salts are important materials used in pharmaceutical, food, textile, detergent (Anastassiadis, and Morgunov, 2007), leather, photographic and other biological industries. Gluconic acid is a bio-based inexpensive organic compound (market price of 50% aqueous solution is about US$ 300–800/ton) with numerous applications in different fields. In 2017, the global gluconic acid market was more than US$ 50 million, expecting to exceed US$ 80 million by 2024 with more than 120 kilo ton consumption of gluconic acid by industry (Ahuja and Singh 2018).

There are different ways for production of gluconic acid such as chemical, electrochemical, bio-chemical and bio-electrochemical (Isabell et al. 1932, Pfizer et al. 1957, Wilt et al. 1972). Manufacturing gluconic acid by microbial fermentation is most widely acceptable and widely practiced method. Fungus, A. niger and bacteria G. oxydans are widely used micro-organisms

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for fermentation. Gluconic acid can be produced by batch fermentation which has limitations because of substrate inhibition when high amount of substrate is used (Anastassiadis et al. 2007).

The main problem with the process of gluconic acid production is to control the pH during fermentation as it decreases during the process of fermentation and it affects the metabolism of an organism. So, various neutralising agents such as NaOH, KOH (Ramachandran et al. 2008), $CaCO_3$ (Moyer et al. 1940) are being used to tackle the situation. However, at high glucose concentration, calcium gluconate cannot be used as calcium gluconate has limited water solubility (Bankar et al. 2009). Sodium or potassium hydroxide has been considered as the superior alternative to calcium carbonate process, allowing the use of higher glucose concentrations.

The current research explored flask and fermenter-scale gluconic acid production using a fungal culture with use of pH adjustment strategies using either calcium carbonate or potassium hydroxide.

MATERIALS AND METHODS

Micro-organism and maintenance

A fungal species used in this study was purchased from (NCIM) Pune, India. The culture was maintained on potato-dextrose agar (PDA) plates (39 gm in 1000 ml distilled water) by incubating at proper temperature for certain period of incubation before storing at 4°C. Cultures were sub-cultured in every 15 days.

Inoculum preparation and growth conditions

The spores of fungal species grown on PDA plates were harvested in 20 ml sterile distilled water. The spore suspension of 270 µl was inoculated into a 250 ml Erlenmeyer flask containing 50 ml spore germination medium required for the flask scale experiment. The spore suspension of 2.7 ml was inoculated separately into two 2L erlenmeyer flask each containing 500 ml spore germination medium required for the fermenter scale experiment. The spore germination medium has the following composition (in g/L): glucose, 100; KH_2PO_4, 0.50; $(NH_4)_2HPO_4$, 1.80; $MgSO_4$,0.19 and corn steep liquor, 2.10. The media were sterilized at 121 ºC for 15 min. The pH was adjusted at 5.5 with H_2SO_4. The inoculated medium was placed in a shaker incubator (Jeio Tech) at 150 RPM and incubated for 48 h at 30 ºC.

Flask scale gluconic acid production

Batch fermentation for gluconic acid production in shake flask scale was carried out in 500 ml cotton plugged erlenmeyer flasks containing 100 ml of fermentation medium. The fermentation was carried out in the medium with composition (g/L): KH_2PO_4, 0.17; $(NH_4)_2HPO_4$, 0.25; $MgSO_4$, 0.20. Glucose was used as the sole carbon source at different concentrations such as 100 g/L, 150 g/L & 200 g/L. The initial pH was maintained at 6.0 adjusted by H_2SO_4. Further pH was controlled by the addition of $CaCO_3$. The concentrations $CaCO_3$ were calculated from stoichiometry of glucose and gluconic acid. The flask containing fermentation medium was sterilized in an autoclave at 121 ºC for 15 min. The medium in flask was aseptically inoculated 15% inoculum and placed in an incubator shaker at at proper temperature with an agitation speed of 150 rpm.

Fermenter scale gluconic acid production

Effect of RPM on Fermenter batch scale gluconic acid production using $CaCO_3$

Batch fermentation for gluconic acid production in fermenter scale was carried out in 10 L bio-reactor (New Brunswick Scientific Co., U.S.A) with a working volume of 6 L. The composition of fermentation medium was similar as described in section 2.4. The fermenter containing fermentation medium with respective initial glucose concentration (100 and 200 g/l) respectively were sterilized in an autoclave at 121 ºC for 15 min. Media was supplemented with $CaCO_3$ for pH maintenance. After sterilization and cooling of the fermentation medium, fermenter was aseptically inoculated with 15% inoculum. Experiments on fermenter scale were carried out at different agitation of 300 and 500 rpm, with optimized aeration and temperature.

Gluconic acid production using $CaCO_3$ and KOH as a neutralising agent

Batch fermentation for gluconic acid production in fermenter scale was carried out in 10 L bio-reactor (New Brunswick Scientific Co., U.S.A) with a working volume of 6 L. The composition of fermentation medium was similar as described in section 2.4. The fermenter

containing fermentation medium with initial glucose concentration of (200 to 400 g/L) were sterilized in an autoclave at 121 ºC for 15 min. One fermenter media was supplemented with $CaCO_3$ for pH maintenance. After sterilization and cooling of the fermentation medium, fermenter was aseptically inoculated with 15% inoculum. Experiments on fermenter scale were carried out at agitation of 500 rpm, optimized aeration and temperature and pH of the other fermenter was maintained with KOH.

MATERIALS AND METHOD

Samples were withdrawn after 24 h intervals and centrifuged and stored at 4 ºC prior to analysis. Glucose and gluconic acid were analyzed using high-performance liquid chromatography.

RESULTS AND DISCUSSION

Flask scale gluconic acid production

The flask scale gluconic acid production profile at different glucose concentration using fungal culture and $CaCO_3$ as the buffering agent is shown in (Figure 1). Gluconic acid production of 99.67 g/l with yield of 1.0 g/g and productivity of 0.69 g/l/h was achieved with fully utilization of 103.27 g/l of glucose concentration within 6 days of fermentation period. Similarly, in case of 151.45 g/l of initial glucose concentration, maximum 123.98 g/l of calcium gluconate was achieved with 0.95 g/g yield and 0.73 g/l/h productivity through utilization of utilization of 66.48% glucose within 7 days of fermentation period. However, 22.13 g/l of glucose could not be utilize by the fungus and fermentation was stopped due to precipitation of calcium gluconate. There are other neutralizing agents such as NaOH and KOH which can be utilize to control the pH during fermentation and in result, sodium or potassium gluconate is usually formed. Continuous flask-scale gluconic acid production using KOH was not possible due to manual addition process. Therefore, further gluconic acid production at higher glucose concentrations were carried out at fermenter scale to determine the effects of a KOH-based pH control strategy. As the $CaCO_3$ experiment showed failure at 200 g/L initial glucose concentration on flask scale, fermenter scale experiments were carried.

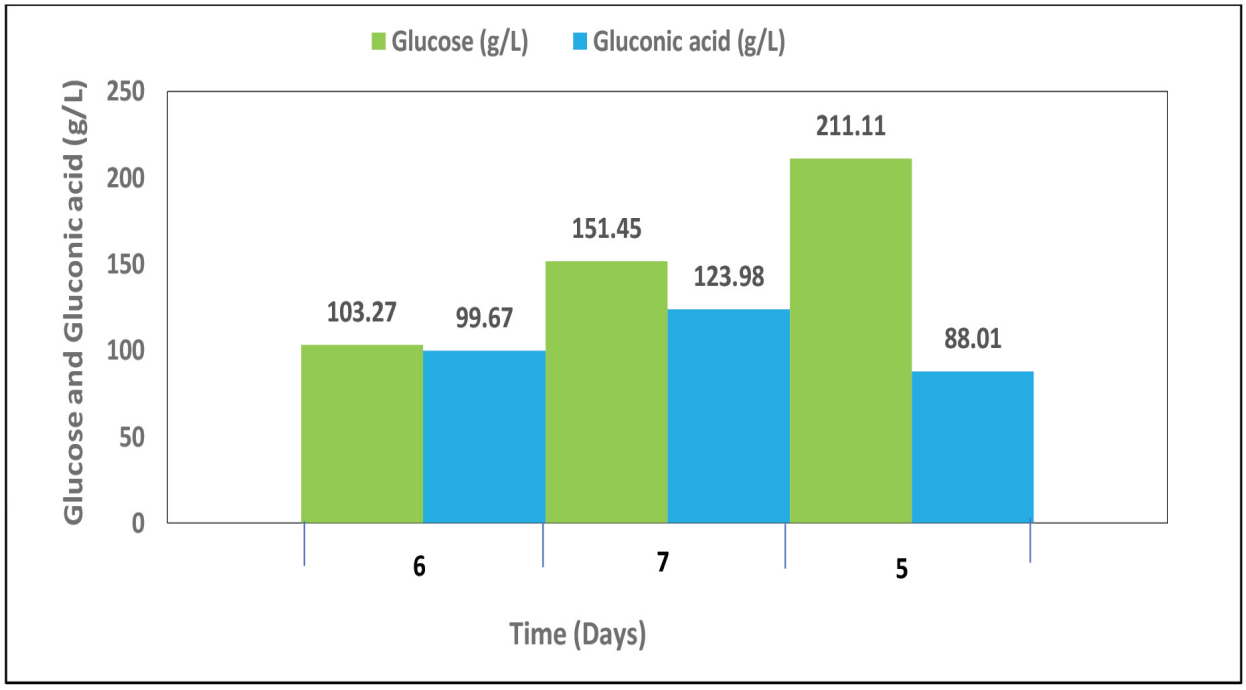

Figure 1 : Fermentation profile of fungal culture for utilization of different glucose concentration for flask scale calcium gluconate production

Fermenter scale gluconic acid production

Effect of RPM on Fermenter batch scale gluconic acid production using $CaCO_3$

Agitation have huge impact on fermentation rate therefore, this aspect was studied on fermenter scale using $CaCO_3$ as a neutralising agent and 100 g/l of initial glucose. According to Hanley agitation at 300 RPM resulted in highest gluconic acid production so, in this study fermentation was carried out at 300 and 500RPM. 99 g/l of titre of gluconic acid was achieved in both cases i.e, at 300 RPM and 500 RPM but in case of 300 RPM, it took 72 hrs to achieve the titre while in case of 500 RPM, it took 48 hrs to achieve the titre as described in (Figure 2). Yield and productivity at 300 RPM was 1.05 g/g and 1.35 g/l/H respectively and at 500 RPM it was 0.86 g/g and 2.06 g/l/H respectively. Decrease in time of fermentation at 500 RPM could be attributed to proper mixing of nutrient and oxygen. As the gluconic acid titre increased at 500 RPM, next experiments were carried out on fermenter scale using 500 RPM agitation speed.

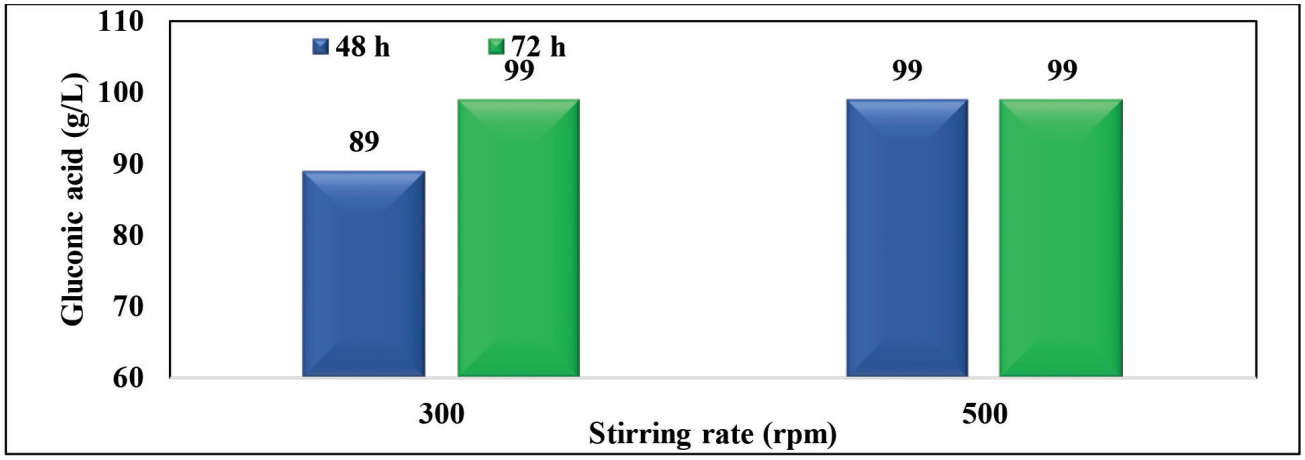

Figure 2 : Effect of agitation speed on fermentation rate using $CaCO_3$ as a neutralising agent

Fermenter scale gluconic acid production using $CaCO_3$ and KOH as a neutralising agent

Fermenter batch scale gluconic acid production was carried out to compare the efficiency of $CaCO_3$ and KOH for pH maintenance. Initial glucose concentration was kept at 200 g/k.72 g/l of titre was achieved in 120 h with yield of 0.83 g/g and productivity of 0.75 g/l/H when $CaCO_3$ was used as neutralising agent. Whereas, 198 g/l of gluconic acid titre was achieved when KOH was used as a neutralising agent as depicted in (Figure 3). The reason could be the same as discussed in 2.4 section. So, all the experiments were carried out using KOH as a neutralising agent.

Batch scale gluconic acid fermentation using KOH at higher glucose concentration

To determine the effect of a pH control strategy with the addition of KOH, batch fermenter scale gluconic acid production was carried out with different glucose concentrations (200-400 g/l) and controlled pH 6.0 using KOH as the neutralising agent at optimum 500 rpm stirring conditions. Glucose was fully utilized within 4 days of fermentation in case of 193.02 and 252.85 g/l of initial glucose concentration. In this fermentation, titre of potassium gluconate was found to be 162.39 and 203.68 g/l. However, in case of 304.65 and 354.39 g/l of initial glucose concentration, fermentation could be completed within 5 days of duration. The titre of potassium gluconate was found to be 233.23 and 274.32 g/l. It was observed that more glucose concentration up to 354.39 g/L could be utilized in batch scale fermentation (Figure 4).

CONCLUSION

The current study revealed the significance of using KOH as the neutralizing agent as the better option rather than using CaCO3 for enhanced gluconic acid production. Further titre of potassium gluconate can be

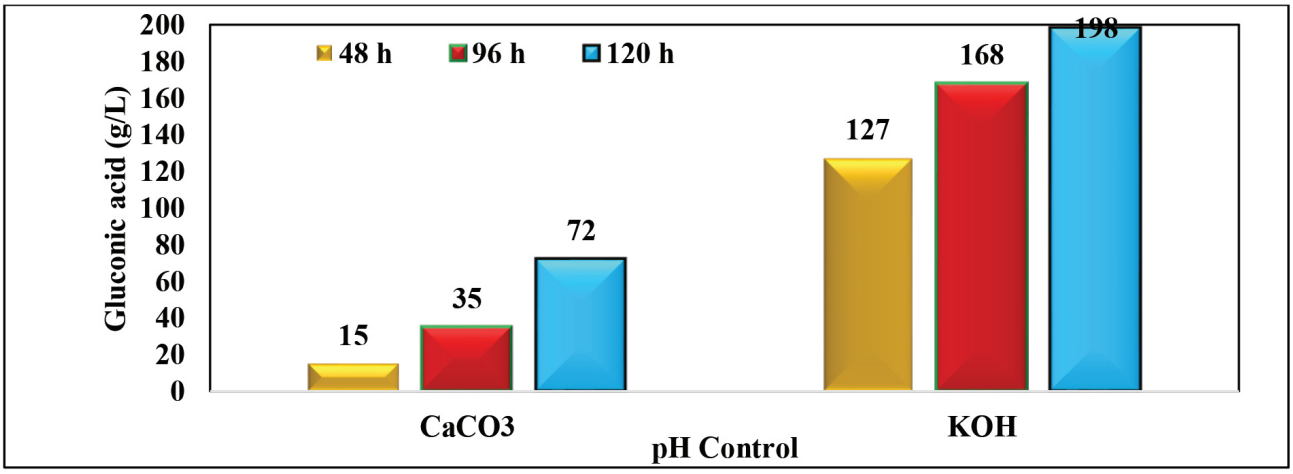

Figure 3 : Comparative analysis of $CaCO_3$ and KOH as a neutralising agent

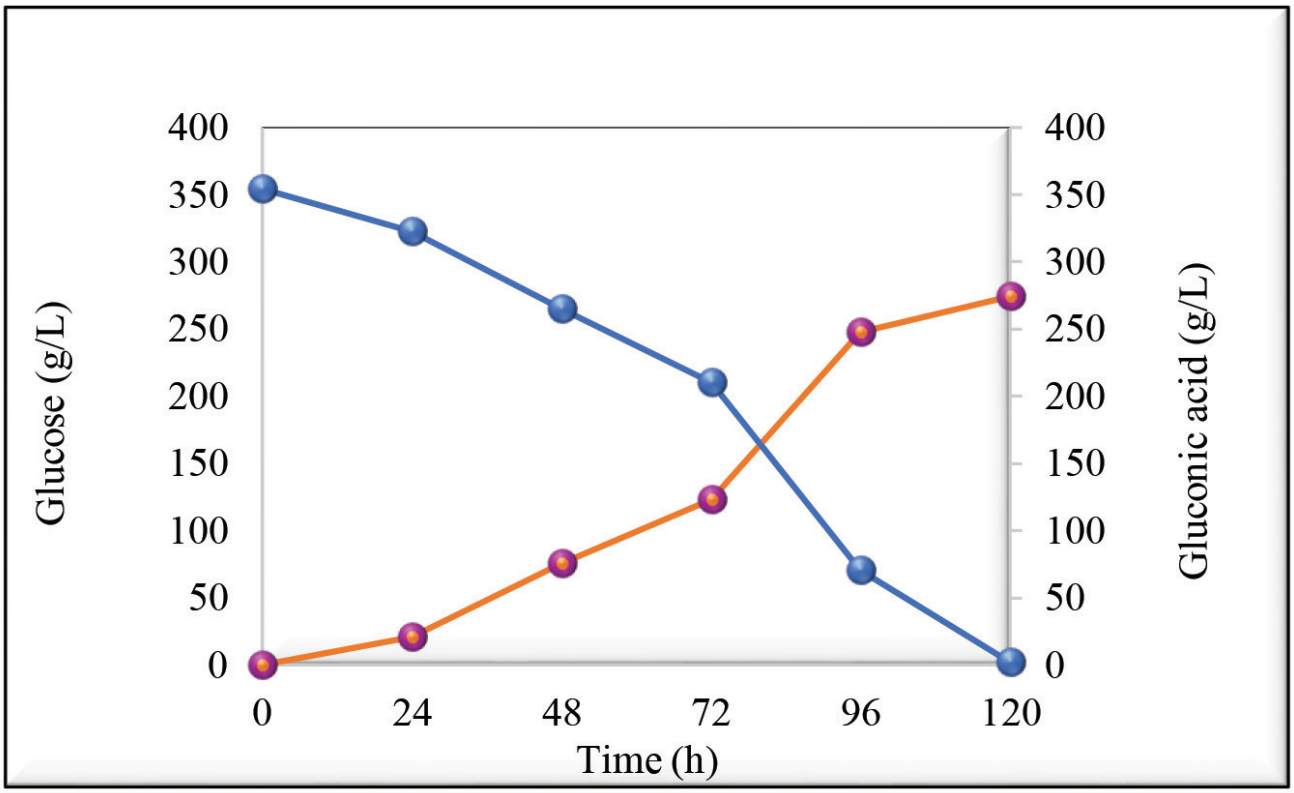

Figure 4 : Batch scale gluconic acid production using KOH as the neutralizing agent

enhanced using high concentration of glucose through fed-batch operation at higher glucose concentration in near future. The development and implementation of this technology can be promoted to deal with surplus sugar and sugarcane juice for the production of valuable gluconic acid in sugar and allied industries.

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