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Conversion of glucose into calcium gluconate and determining the process feasibility for further scaling-up: An optimization approach

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Abstract: The present study investigates the conversion efficacy of glucose into calcium salt of gluconic acid (calcium gluconate) through fermentation by using the fungus *Aspergillus niger (A. niger)*. The study further evaluates the impact of various process parameters and thereby identifies the optimum parameters to maximize the output. The different process parameters evaluated in this study were glucose concentration, pH, temperature, and aeration. After that, the optimized process pre-treatment parameters were used to determine the feasibility of the process for further industrial scale-up. The obtained results indicated enough competency of the optimized parameters to be reproduced at the industrial level. The obtained product was expected to meet the requirement as per the specifications led by various industrial requirements like food and feed, pharmaceutical industry, aquaculture and food industries, etc. The overall scheme of work presented in this study will help to define the techno-economic viability (in terms of energy consumption, environmental concerns, and higher yield) of the process and thereby will benefit various stakeholders.

Introduction

Gluconic acid is a cardinal compound obtained from glucose to produce food additives, detergents textile, leather & textile industries, making plastic materials, and many more (Znad et al., 2004). Previously conducted studies have indicated that the hydrolysis of various biomass specimens has resulted in high gluconic acid yield. However, the industrial production of gluconic acid involves aerobic fermentation of glucose (at very high oxygen concentration) using fungus like *A. niger*. The bio-conversion of glucose into gluconic acid involves elementary dehydrogenation synthesis (Ramachandran et al., 2006). One of the main required substrates for bioconversion of glucose during gluconic acid fermentation is oxygen. Moreover, oxygen is also required for the respiration of mycelium. Earlier studies have reported that around 5 bar pressure of oxygen is required for the high production of gluconic acid. Upon synthesis, gluconic acid is neutralized with calcium carbonate to obtain calcium gluconate (Sankpal and Kulkarni, 2002).

A. niger, synthesizes a wide spectrum of compounds like antibiotics, mycotoxins, enzymes, organic acids, etc. Under optimum conditions, *A. niger* is also a promising exporter of various homologous proteins and other compounds. Gluconic acid has been traditionally utilized over decades and has been reported to be safe (Yang et al., 2017). The enzyme Glucose Oxidase (bearing enzyme commission number 1.1.3.4) promotes the catalysis of oxidation of D-glucose into gluconolactone along with hydrogen peroxidase. The process involves the utilization

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of oxygen as the acceptor of electrons. Previously conducted studies reported that approximately 90000 tonnes of gluconic acid are being generated globally every year, and the cost of the same might reach up to 9 INR/kg. The literature also cited that the calcium salt of gluconic acid contains around 9.3% calcium, bearing molecular formula $C_{12}H_{22}CaO_{14}.H_2O$ and molecular weight of 448.38 (Yadav et al., 2021). Calcium gluconate is mostly used in pharmaceutical industries to treat and prevent calcium deficiency (Ramachandran et al., 2006). Thus, gluconic acid production at an industrial scale holds significant research attention.

Accordingly, the aim of the present study was framed towards the production of the calcium salt of gluconic acid using glucose as the feedstock material. The impact of various process parameters like concentration of glucose, pH, temperature, and aeration towards the production of gluconic acid was evaluated in the study. Subsequently, the optimum levels for each process parameter were defined, and thereby the optimized protocol was evaluated for techno-economic viability at further industrial scale-up. The overall scheme of the study presented in the study will help to promote overall sustainability and thereby will be helpful towards various stakeholders.

Materials and Methods

Materials

The culture for *A. niger* was obtained from Mitushi Biopharma (Gujrat, India). All the required chemicals were obtained from Nice® Chemicals Pvt. Ltd. (Kerala., India). Throughout the experimentation, double distilled water was used, which was prepared in the laboratory using Borosil double-distilled apparatus (Maharastra, India).

Media preparation for spore formation

The media for the formation of spores of *A. niger* (in slant) has been detailed in table 1. The molasses was obtained from local markets and were 50% diluted with double distilled water, and the resulting mixture was pre-sterilized for 30 minutes at 15 lbs pressure and 121ºC. After mixing all the media components, the pH of the media was maintained around 5.6±0.1 and was further sterilized at 15 lbs pressure and 121ºC for 30 minutes before further use.

Table 1. Media composition for spore formation of *A. niger*.

Components	Quantity (gm/lit)
Agar-agar	20.00
Ammonium iron(II) sulfate	1.60
Calcium sulphate	0.25
Copper sulphate	1.00
Glycerin	7.50
Magnesium sulphate	5.00
Molasses	7.50
Potassium Dihydrogen Phosphate	6.00
Sodium chloride	10.00
Yeast extract	5.00

Table 2. Media composition for spore formation of *A. niger*.

Components	Quantity (gm/lit)
Diammonium phosphate	2.00
Glucose	50.00
Magnesium Sulphate	2.50
Potassium Dihydrogen Phosphate	1.00

Media preparation for spore germination

The composition of the media used for the germination of the spores has been listed in table 2. The pH of the resulting media was maintained at 5.6±0.1 and was sterilized at 15 lbs pressure at 121ºC for 15 minutes before use.

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Table 3. Media composition for the production of calcium gluconate.

Components	Quantity (gm/lit)
Calcium carbonate	55.00
Diammonium phosphate	0.90
Glucose	150.00
Magnesium Sulphate	0.15
Potassium Dihydrogen Phosphate	0.20
Urea	0.11

Table 4. Datasheet for monitoring fermentation

Duration (hours)	pH	Dry biomass (g %)	Wet biomass (g %)	Calcium gluconate (%)	$CaCO_3$ (g)	Reducing Sugar (%)
00	5.60±0.10	0.78±0.04	4.68±0.12	0.00	Nil	16.24±0.21
08	3.40±0.08	0.81±0.05	4.84±0.13	2.3±0.11	8.76±0.23	12.68±0.32
24	5.64±0.12	0.77±0.04	2.65±0.07	6.56±0.14	13.4±0.31	2.25±0.12
48	5.69±0.11	0.79±0.06	2.74±0.06	6.74±0.15	Nil	1.98±0.08

Media preparation for commercial calcium gluconate preparation

The media composition for the commercial production of calcium gluconate has been listed in table 3. The pH of the media was maintained at 5.6±0.1 and was sterilized at 15 lbs pressure at 121ºC for 15 minutes before use.

Media preparation for spore formation

The media required to prepare the formation of spores has been listed in table 4. After adding all the components into test tubes, non-adsorbent cotton plugs were inserted and were sterilized at 15 lbs pressure for 15 minutes at 121ºC in an autoclave. After sterilization, the media-containing tubes were kept at rest at the slanting position to prepare the slants at room temperature for 1 day to check the sterility of the slants. After successful inspection, the slants were inoculated with the spores of *A. niger*. In order to maintain the aseptic condition, all these procedures were carried out inside a laminar airflow. Finally, the inoculated slants were incubated at 32±2°C for 2-3days.

A. niger spores harvesting

The spores of *A. niger* from the successful slants were harvested by using 7.5ml 0.1% Tween-80 pre-sterilized solution. The resulting mixture was mixed thoroughly, and the spore concentration was determined using a hemocytometer under the microscope at aseptic conditions. The spores in suspension were maintained within a range of 10^6–10^7 spores per ml. The resulting mixture was inoculated with the spore germination medium as indicated in table 2 and was kept at an orbital shaker and incubator at 300 rpm and 32°C, and was checked at regular intervals to ensure proper aeration.

Typical Experimental Set-up

Defining and adhering to the strict and standard experimental protocol is essential to carry out experimental work. The media for the production of calcium gluconate was prepared as indicated in table 3. The media was sterilized at 15 lbs pressure for 15 minutes at 121°C and was kept at a shaking orbital incubator to check the sterility. After that, the media was inoculated with 100 ml (24 hours old) inoculum for every 1000 ml of production media having spore concentration 10^6–10^7 spores per ml and was incubated at an orbital shaker and incubator at 300 rpm and 32°C for 2 days (48 hours) to determine the kinetics of the formation of calcium gluconate. The parameters listed in table 4 were checked and monitored after regular intervals.

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The data presented in table 4 indicated that within 8 hours, the pH of the media decreased from 5.60±0.10 to 3.40±0.08, which in turn indicated that the formation of gluconic acid had been initiated. For neutralization, the parameter. Accordingly, the various process parameters were optimized using one factor at a time to obtain the optimum process parameters finally. The various process parameters which were taken into

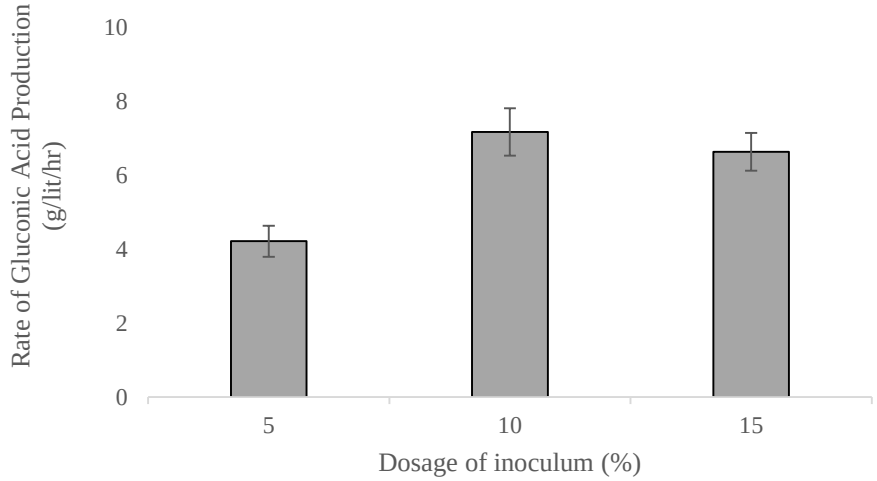

Fig. 1. Impact of dosage of inoculum on gluconic acid production

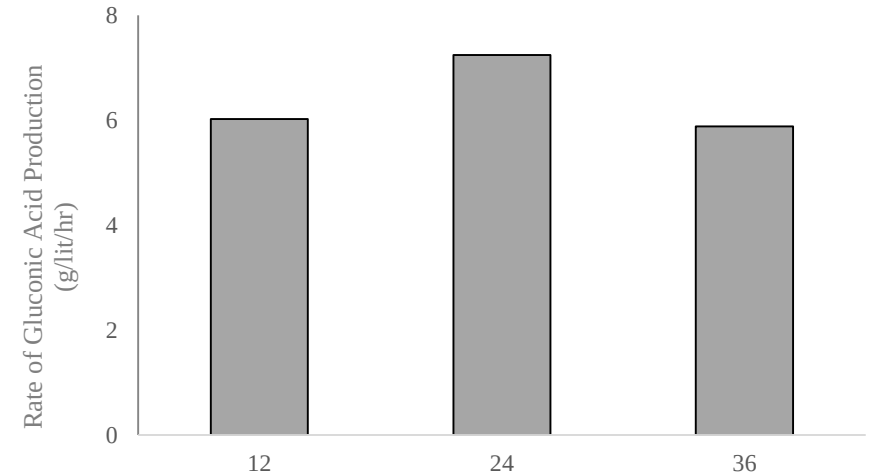

Fig. 2. Impact of inoculum age on gluconic acid production

consideration were pre-sterilized slurry of calcium carbonate was introduced to the broth to continue fermentation towards the optimum level. The fermentation was continued for up to 48 hours. All the experimentations were carried out in triplicates to estimate the associated errors. Accordingly, an ardent need was identified towards optimizing the process parameters of calcium gluconate production after establishing the formation of calcium gluconate.

Results and Discussion

In the present study, the impact of a single factor was evaluated towards the formation of gluconic acid by keeping the other factors invariant. The maximum gluconic acid production for a particular parameter was chosen as the optimum point for that particular process dosage of the inoculum, inoculum age, rate of agitation, pH, temperature, rate of aeration, and glucose concentration.

Impact of dosage of inoculum on the gluconic acid production

The inoculum dosage was varied between 5–15 % at three regular intervals. The resulting gluconic acid production was evaluated at every interval, and the obtained results have been presented in figure 1.

The data obtained from figure 1 indicated that the maximum production rate was obtained from 10% inoculum dose. Accordingly, the inoculum dose of 10% was considered for further studies.

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Impact of inoculum age on the gluconic acid production

To determine the impact of the age of inoculum corresponding to maximum gluconic acid production, the age of inoculum was varied up to 36 hours at three regular intervals. The obtained results have been presented in figure 2. The obtained results indicated that the inoculum age of 24 hours corresponds towards maximum gluconic acid production. Accordingly, the 24 hours inoculum age was considered the optimum level for gluconic acid production.

Impact of the rate of agitation on the gluconic acid production

The agitation rate plays an important role in the industrial production of value-added chemicals (Kumar et al., 2020). Accordingly, to evaluate the impact of the rate of agitation towards gluconic acid production, organized monitoring was conducted. The rate of agitation was varied between 150-250 rpm at three equal levels towards the productivity of the gluconic acid. The obtained results have been showcased in figure 3. The obtained results indicated that 200 rpm has the maximum impact towards the gluconic acid formation and thus it was shortlisted for further study.

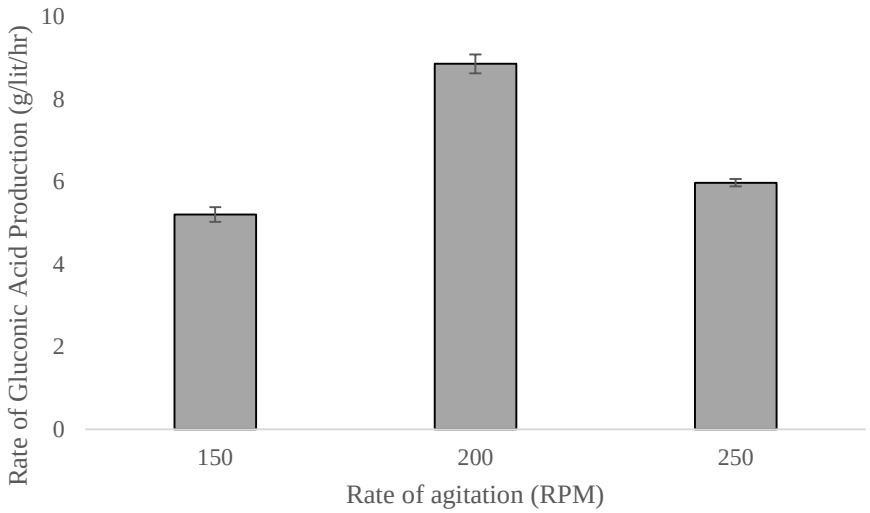

Fig. 3. Impact of the rate of agitation on gluconic acid production

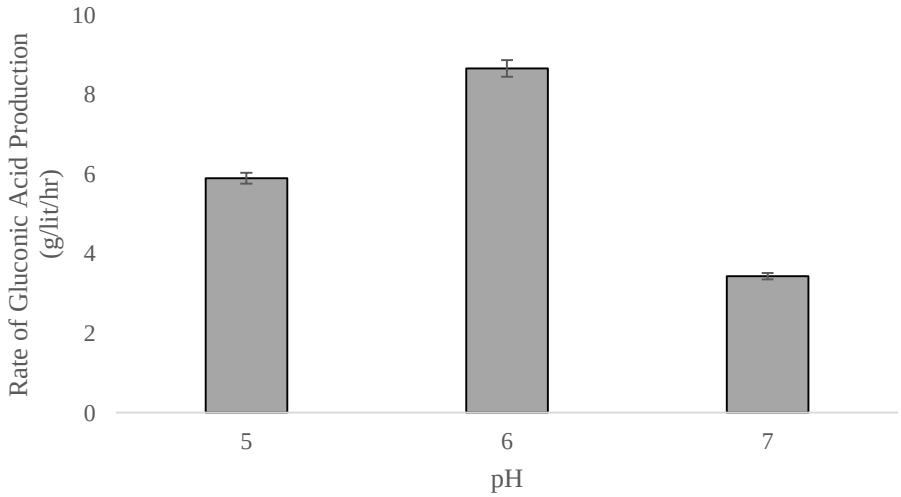

Fig. 4. Impact of pH on gluconic acid production

Impact of pH on the gluconic acid production

The impact of the production media pH on the productivity rate of gluconic acid was monitored within the pH range of 5-7 at three regular intervals. The obtained results have been presented in figure 3. It was evident from figure 4, that the highest gluconic acid productivity was noted for pH 6. Accordingly, this pH level (pH 6) was considered the optimum level and shortlisted for further optimized scaling up the protocol of gluconic acid production.

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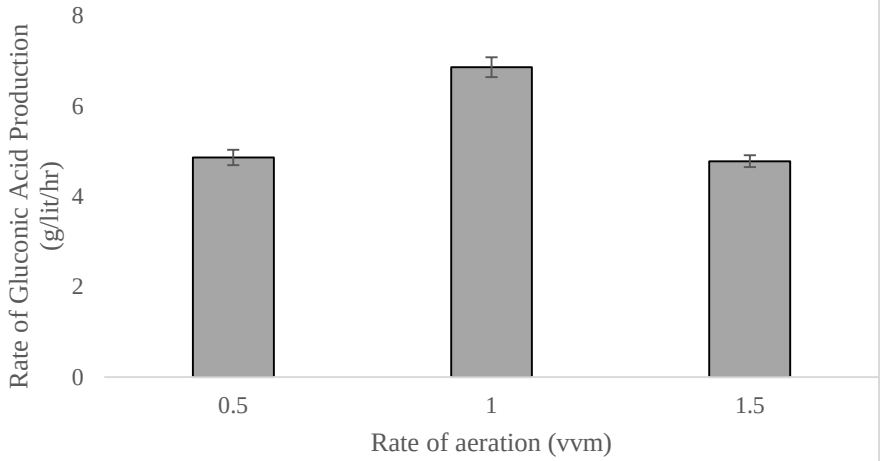

Fig. 6. Impact of aeration on gluconic acid production

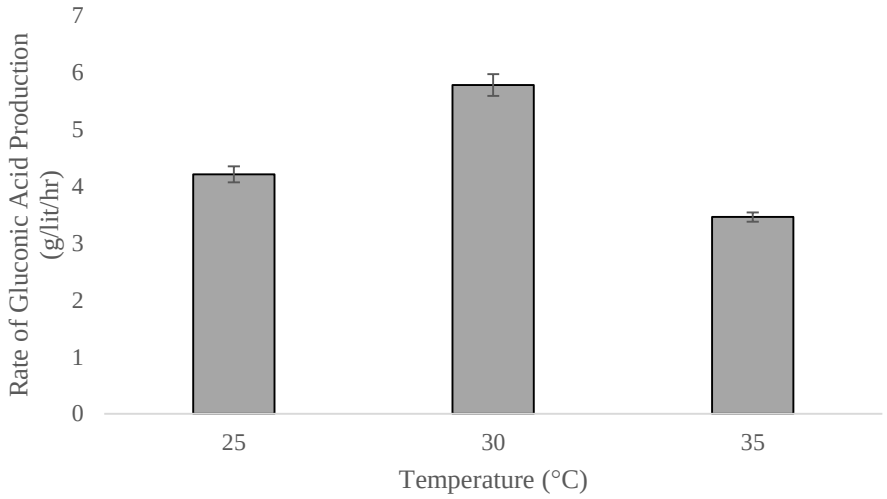

Fig. 5. Impact of temperature on gluconic acid production

Impact of temperature on the gluconic acid production

The literature suggested that temperature has played a vital role in a chemical reaction. Accordingly, the efficacy of temperature towards gluconic acid production was monitored and presented in figure 5. The temperature range was varied at three regular intervals within the range of 25-30°C. The obtained results identified that the highest gluconic acid production was registered at 30°C among the defined range considered in this study. Thus, the temperature for the optimized protocol for gluconic acid production was fixed at 30°C.

Impact of aeration on the gluconic acid production

Aeration is an important parameter for any industrial production of value-added products (Cao et al., 2020). Accordingly, the effect of aeration on gluconic acid production was checked within the range of 0.5-1.5 vvm at three regular intervals. The results obtained are presented in figure 6. It is evident from the obtained results that the highest productivity of gluconic acid was registered at 1 vvm. Accordingly, the aeration rate of 1vvm was registered as one of the key inputs of the optimized process parameters for maximizing gluconic acid production at the industrial level.

Impact of glucose concentration on the gluconic acid production

As indicated in previously conducted studies, the concentration of glucose plays a vital role in forming gluconic acid (Wenkin et al., 2002). Accordingly, the glucose concentration's efficacy on the gluconic acid's productivity was monitored within the range of 5-15% at three regular intervals. The results obtained from the following experimentations have been presented in figure. 7. The obtained results indicated that the highest production rate of gluconic acid was registered for 15% glucose concentration. Accordingly, 15% glucose concentration was considered as one of the input

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parameters of the optimized protocol for the further scale-up study.

The sequential step-wise optimization of the various process parameters (defined in the course of the study) resulted in the development of the optimized protocol for maximizing the productivity of calcium gluconate. The optimized protocol would be cost-effective, require less energy consumption, and be sustainable in turn.

Conclusion

The proposed scheme of the study indicates that glucose is a potential substrate for the production of gluconic acid, which can be converted into calcium gluconate with literally no technical difficulties and can be produced commercially at an industrial scale. The sustainable procedure for gluconic acid production is the ardent need of the hour since gluconic acid has multidimensional application and high demand in various industries like pharmaceuticals, poultry, etc. to name a few. The proposed study will be thus beneficial to various stakeholders.

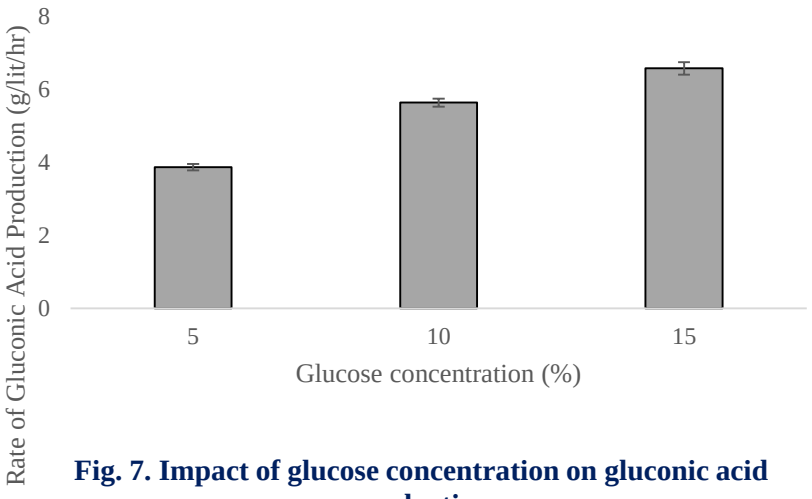

Fig. 7. Impact of glucose concentration on gluconic acid production

Application of the optimized protocol for scaling up the gluconic acid production at an industrial scale (200 litres fermenter)

The optimized protocol for increasing the productivity of the gluconic acid obtained in the earlier section of the study was further applied for scale-up at an industrial scale fermenter of 200 lit capacity. The study was conducted to define the reproducibility of the protocol at an industrial scale, identify any difficulties during the process of scaling up, and finally define the techno-economic viability of the entire process. Accordingly, to evaluate the points mentioned above and establish the reproducibility, three trials of gluconic acid production were conducted (each of 100 litres), and the obtained results have been presented in table 5.

The data presented in table 5 indicated that 15% glucose concentration was reduced to 0.12-0.14 % within 27-28 hours and 28-30°C, 197-199 rpm, 0.94-0.96 vvm and at a pressure of 0.48-0.51. Accordingly, the production of calcium gluconate was almost quantitative and in line with the optimization indicated in the earlier section of the study. Thus, the proposed scheme of the study showed no technical laggings in context to the scaling up at the industrial level. Accordingly, the overall study was found to be sustainable for various stakeholders.

Conflict of Interest

No potential conflict of interest was reported for the conducted study.

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References

Cao, K., Zhi, R., & Zhang, G. (2020). Photosynthetic bacteria wastewater treatment with the production of value-added products: a review. *Bioresource Technology*. 299:122648.

Kumar, M., Sun, Y., Rathour, R., Pandey, A., Thakur, I.S., & Tsang, D. C. (2020). Algae as potential feedstock for the production of biofuels and value-added products: Opportunities and challenges. *Science of the Total Environment*. 716: 137116.

Ramachandran, S., Fontanille, P., Pandey, A., & Larroche, C. (2006). Gluconic acid: properties, applications and microbial production. *Food Technology & Biotechnology*. 44:185-196.

Sankpal, N. V., & Kulkarni, B. D. (2002). Optimization of fermentation conditions for gluconic acid production using *Aspergillus niger* immobilized on cellulose microfibrils. *Pro.Biochem*. 37: 1343-1350.

Wenkin, M., Ruiz, P., Delmon, B., & Devillers, M. (2002). The role of bismuth as promoter in Pd–Bi catalysts for the selective oxidation of glucose to gluconate. *Journal of Molecular Catalysis A: Chemical.* 180: 141-159.

Yang, L., Lübeck, M., & Lübeck, P. S. (2017). Aspergillus as a versatile cell factory for organic acid production. *Fungal Biology Reviews*. 31: 33-49.

Yadav, A. K., Choudhary, V., Gour, N., & Yadav, K. R. (2021). Optimisation of Glucose Fermentation for the Production of Calcium Gluconate in Terms of an Industrial Perspective. *International Journal of Innovative Science and Research Technology*. 6: 526-530.

Znad, H., Markoš, J., & Baleš, V. (2004). Production of gluconic acid from glucose by *Aspergillus niger*: growth and non-growth conditions. *Process Biochemistry*. 39: 1341-1345.

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