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Production of Gluconic Acid from Whey and Black Jaggery by Aspergillus Niger

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Abstract
Gluconic acid is a non-corrosive, non-volatile, non-toxic, mild organic acid. The production of gluconic acid following traditional chemical process involves very high cost which in turn provides an opportunity to explore cost effective fermentation method. Hence in the present investigation the microbial production of gluconic acid has been attempted using a mutant Aspergillusniger through optimization of some physical and chemical parameters. Production parameters like incubation period, volume of innoculum, concentration of black jaggery, Di-Ammonium hydrogen Phosphate (NH4)2HPO4, Pottasium Dihydrogen Orthophosphate(KH2PO4), Magnesium Sulphate (MgSO4.7H2O) have been individually optimized one after one. The maximum concentration of gluconic acid produced is found to be 21.57 g/L with 60 hours of incubation time, 1ml volume of innoculum, 8 % of black jaggery, 0.050 % of Di-Ammonium hydrogen phosphate, 0.075 % of Pottasium Dihydrogen Orthophosphate and0.025 % of Magnesium Sulphate. The produced gluconic acid will find its application in food, pharmaceutical, metallurgy, paper and textile industries.
Keywords: Gluconic acid, Aspergillusniger, Whey, Black jaggery.

Introduction: Gluconic acid is a mild, noncorrosive, nonvolatile, and nontoxic organic acid with diverse industrial applications in pharmaceuticals, food, animal feed, textiles, and leather. It is also used in cement to control setting time and enhance strength and water resistance. Recognized as a food additive (E574) in Europe, it imparts a sour taste to foods. Microbial fermentation is the preferred method for gluconic acid production due to its efficiency and cost-effectiveness. Additionally, whey, a cheese byproduct, requires further processing to avoid disposal issues, with its type influenced by the coagulation method and bacteria used in cheese making.
Objectives: The primary goal is to explore and Production of gluconic acid from whey and black jaggery by Aspergillus niger
Methods: Microbial production of gluconic acid has been attempted using a mutant Aspergillusniger
Results: The production of gluconic acid can be produced from the whey and black jaggery from Aspergillus niger under optimized conditions of fermentation.
ConclusionsThe present study indicates that a relatively good concentration of gluconic acid can be obtained after optimization of certain physical and chemical parameters by the mutant *Aspergillus niger*. However, in the present investigation only limited parameters were examined. An attempt to scale up the process is underway.

Keywords: *Food traceability ,security, CNN, machine Learning, tiny ML*

1. Introduction

Gluconic acid is a mild organic acid that has gained much interest as it has many industrial applications such as in the pharmaceutical, food, animal feed, textile and leather industry. It is also applied as additive in cement to control the setting time and increase strength and water resistance. Gluconic acid can have further applications for the solubilization of phosphate [1] and as cement additive. Gluconic acid is a noncorrosive, nonvolatile, nontoxic mild organic acid. It imparts a refreshing sour taste in many food items. In the European Parliament and Council Directive No. 95/2/EC gluconic acid is listed as a generally permitted food additive (E574).The microbial fermentation processes offer attractive techniques for the gluconic acid production to alleviate the problems related to chemical production such as the inevitable side reactions and also to further economize the bioprocess [2].

Gluconic Acid (GA) is a multifunctional carbonic acid belonging to bulk chemical, with outstanding properties, including extremely low toxicity, very low corrosiveness, and a capability of forming water soluble complexes with different metal ions. Due to these physiological and chemical properties, GA itself and its salts have found extensive demand in construction, chemicals, pharmaceuticals, food, beverage, textile, leather and other industries [3]. There are various approaches available for the production of gluconic acid namely, chemical, electrochemical, biochemical, bioelectrochemical, and photocatalytic approach. Because of some limitation regarding these approaches, fermentation has been proved as efficient and dominant techniques for manufacturing GA [4].

Whey is the green yellow translucent watery portion of milk remaining after milk coagulation and removal of the curd. It is a byproduct of cheese manufacture and is sometimes regarded as a waste and constitutes a major problematic disposal if not processed further to valuable products. Milk curdles when its acidity rises and the curdling is complete when it reaches its isoelectric point which is pH 4.7. Success in cheese making is dependent on this level of acidity in milk[5]. The differences in the composition of milk used in cheese manufacture influences the composition of the manufactured cheese and whey.

The type of bacteria used in the curdling of the milk also influences the type of cheese and whey produced. Cheese can be classified into two broad groups namely, soft and hard cheese. Hard cheese has most of the protein coagulated, while Soft cheese, a considerable amount of the protein remains behind in the whey .There are two types of whey, sweet and acid whey depending on the coagulation method used. When acid coagulation is used, acid whey is produced, and when enzymatic coagulation is used, sweet whey is produced [6].

Gur (Jaggery) is a natural, traditional sweetener made by the concentration of sugarcane juice and is known all over the world in different local names. It is a traditional unrefined non centrifugal sugar consumed in Asia, Africa, Latin America and the Caribbean. India is the largest producer and consumer of jaggery. Out of total world production, more than 70% is produced in India [7].

In India, of the 300 Mt of sugarcane produced, 53% is processed into white sugar, 36% into jaggery and khandsari, 3% for chewing as cane juice, and 8% as seed cane. Jaggery and khandsari have withstood competition protecting farmers' interests besides meeting ethnic demands. Processes and equipments have been developed for quality solid, liquid and powder jaggery. Liquid jaggery has been commercialized. The organic clarificants developed help to retain jaggery as organic food [8].

India is world's largest producer of sugar and sugarcane. India is world's largest producer of sugar and sugarcane. Sugarcane in India is processed in to sugar, gur and khandsari and undergoes considerable weight reduction during processing. The methods of converting sugarcane and manufacturing sugar, gur and khandsari are different but a great value is added in the manufacturing of these consumable final products. Further it offers employment opportunity to millions of people.

Gur is prepared in all parts of the country. It is also known as Gul, gud, Jaggery, Vellum and Bella. Gur is known to produce heat and give instant energy to a human body. In many parts of India, there is a tradition of serving a glass of water with Gur to welcome the guests. Gur is also used as a cattle feed, in distillery, medicine manufacturing unit, ayurvedic medicines, ayurvedicsura and ayurvedic health tonics [9].

2. Materials And Methodology

Whey and black jaggery was analyzed for their physio-chemical analysis is by AOAC method.

2.1 Production of gluconic acid

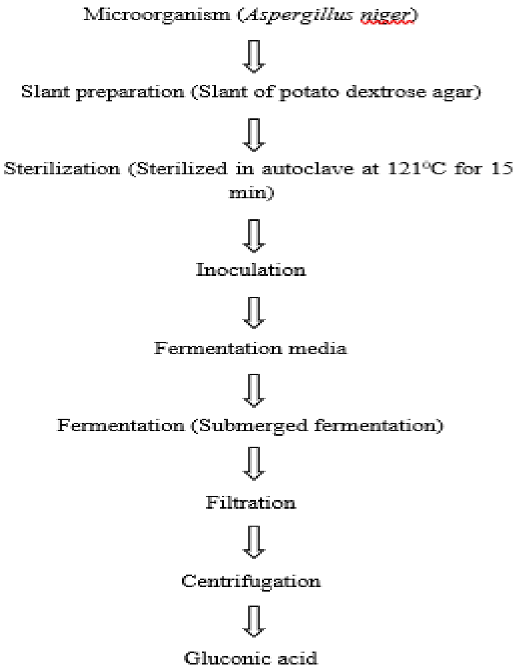

Figure 1: Production of gluconic acid [10]

2.2 Measurement of gluconic acid

Standards and specifications for synthetic additives by Korea Food and Drug Administration [11]

3. Results and Dicussions

3.1 Physicochemical Analysis of whey

Table 1: Proximate Analysis of Whey

Sr. no	Parameter	Experimental values
1.	Moisture (%)	93.25±0.34
2.	Ash (%)	0.92±0.03
3.	Protein(%)	0.58±0.08
4.	Fat (%)	0.30±0.01
5.	Lactose(%)	4.38±0.15
6.	Lactic acid(%)	0.43±0.01
7.	P^H	5 ±0.1

Proximate analysis along with some chemical components of whey was carried out and presented in table 1. From the result it was seen that moisture in the whey was more that is 93.24%. Ash 0.92 %, Protein 0.58%, Fat 0.30%, Lactic acid 0.43%. Most important parameter responsible for further production of gluconic acid is Lactose content in whey which was 4.38% .The results obtained in present investigation are in close agreement with the results obtained by [12].

3.2 Physicochemical analysis of Black jaggery

Table 2: Proximate analysis of Black jaggery

Sr. No.	Parameter	Experimental values
1.	Moisture (%)	9.26±0.10
2.	Ash (%)	1.74±0.05
3.	Protein (%)	0.40±0.06
4.	Fat (%)	0.15±0.02
5.	Reducing sugar(%)	19.83±0.45
6	Non reducing sugar(%)	63.41±0.52
7.	Water insoluble ash(%)	1.06±0.11
8.	Acid-insoluble ash (%)	0.26±0.11

Proximate analysis along with some chemical components of black jaggery was carried out and presented in table 2. From the result it was seen that the moisture content black jaggery was 9.26%. Ash 1.74%, Protein 0.40%, Fat 0.15%, Water insoluble ash 1.06%, Acid insoluble ash was 0.26% and important parameters responsible for further production of gluconic acid are Reducing and non-Reducing sugar in the black jaggery are 19.83 and 63.18% respectively. The results obtained in the present investigation are in close agreement with the Bureau of Indian Standards (IS 12923:1990).

3.3 Fermentation Technique

3.3.1 Microorganism

The *Aspergillus niger* strain used in this study was obtained from the Department of Microbiology, Shivaji university, Kolhapur and maintained on Potato Dextrose Agar.

3.3.2 Fermentation media

Sporulation medium contained 0.05% $(NH_4)_2HPO_4$, 0.06% KH_2PO_4 and 0.015%$MgSO_4.7H_2O$. Ten different whey media were tested in which the different concentration of Black Jaggery was adjusted. All media were dispensed in 100 ml quantities in 250 ml conical Flask and autoclaved at 121^{0}C for 15 min. The P^H of the medium was maintained at 6.

Vol 45 No. 6
June 2024

3.3.3 Fermentation Process

100 ml of whey along with various concentration of black jaggery were dispensed in 250 ml conical flask was inoculated with 1 ml of spore suspension of *Aspergillusniger* and incubated at 30^{0}C on a rotary shaker(150 rpm) for up to 84 hours. At different time intervals, fermented broth was separated from mycelia by centrifugation process.

3.4 Optimization of production of gluconic acid

3.4.1 Effect of period of incubation

Table 3: Effect of period of incubation

Sr. No.	Period of incubation(hr)	Gluconic acid (g/L)
1	24	15.692
2	36	16.477
3	48	19.419
4	60	21.577
5	72	20.792
6	84	20.400

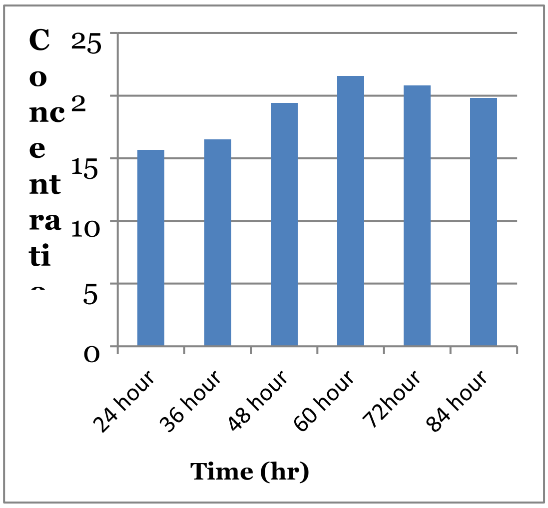

Figure 2: Effect Incubation period

To optimize the period of the incubation for the production of gluconic acid the fermentation broth was incubated for different periods (24-84 hrs), and it was found that at 60°C of incubation the gluconic acid production was maximum that is 21.577 g/L.

3.4.2 Effect of Volume of inoculum

Table 4: Effect of inoculum level

Sr. No.	Volume of inoculum (ml)	Gluconic acid (g/L)
1	0.5	20.00
2	1	20.792
3	1.5	19.812
4	2	17.654
5	2.5	16.477
6	3	15.692

To optimize the volume of inoculum for the production of gluconic acid, different level of inoculum were tested (0.5-3 ml) and it was found that inoculation of 1 ml spore suspension of *Aspergillusniger* gives the maximum concentration of gluconic acid that is 20.792 g/L.

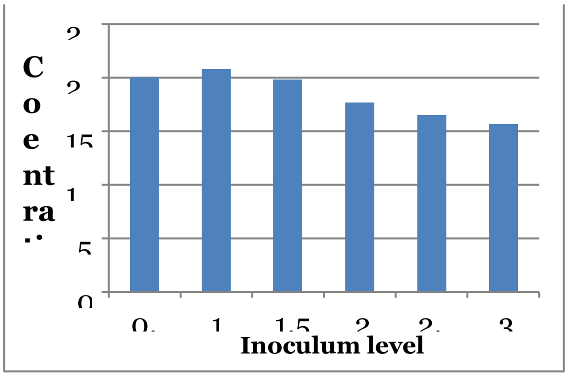

Figure 3: Effect of volume of Inoculum

3.4.3 Optimization of concentration of black jaggery

Table 5: Effect of concentration of black jaggery on production of gluconic acid

Sr. No.	Concentration of black jaggery (%)	Gluconic acid (g/L)
1	2	9.611
2	4	11.181
3	6	14.712
4	7	17.850
5	8	21.381
6	9	20.400
7	10	19.229
8	12	17.065

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Vol 45 No. 6
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9	14	16.281
10	16	15.300

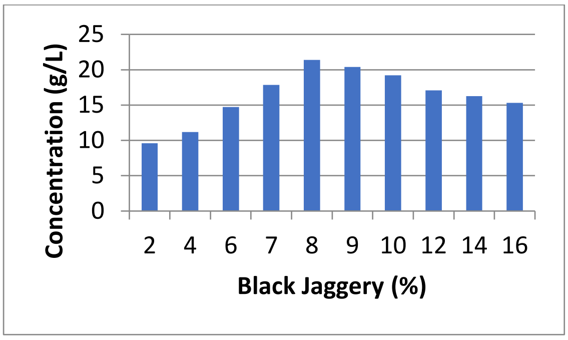

Figure 4: Effect of different concentrations of black jaggery on production of gluconic acid

Above fermentation behavior was shown by *Aspergillusniger* because of the varying concentrations of black jaggery, as the increase in the concentration of black jaggery above optimum limit *Aspergillusniger* reduces the activity of production of gluconic acid. As the increase in sucrose concentration, invertase enzyme activity will decreased by *Aspergillusniger* because of which results in a lower gluconic acid production.

3.4.4 Optimization of Di-Ammonium hydrogen Phosphate $(NH_4)_2HPO_4$

Table 6: Effect of concentration of $(NH_4)_2HPO_4$ on production of gluconic acid

Sr. No.	Concentration of $(NH4)_2HPO_4$	Gluconic acid (g/L)
1	0	18.439
2	0.025	20.400
3	0.050	20.989
4	0.075	20.596
5	0.1	18.831
6	0.125	18.046
7	0.150	17.458

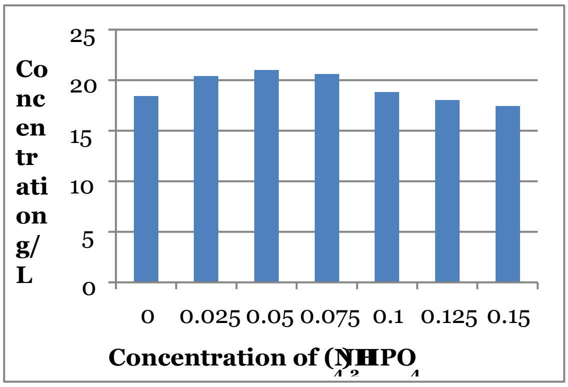

Figure 5: Effect of concentration of $(NH_4)_2HPO_4$

To optimize the concentration of DiAmmonium hydrogen Phosphate (NH4)2HPO4 for the production of gluconic acid different concentrations of Di-Ammonium hydrogen phosphate were tested (0-0.15%) and it was found that0.050 % of Di-Ammonium hydrogen Phosphate gives the maximum concentration of gluconic acid 20.989 g/L.

3.4.5 Optimization of concentration of Pottasium Dihydrogen Orthophosphate (KH_2PO_4)

Table 7: Effect of concentration of (KH_2PO_4) on production of gluconic acid

Sr. No.	Concentration of KH_2PO_4	Gluconic acid (g/L)
1	0	19.419
2	0.025	20.008
3	0.050	20.596
4	0.075	21.185
5	0.1	19.812
6	0.125	18.495
7	0.150	16.869

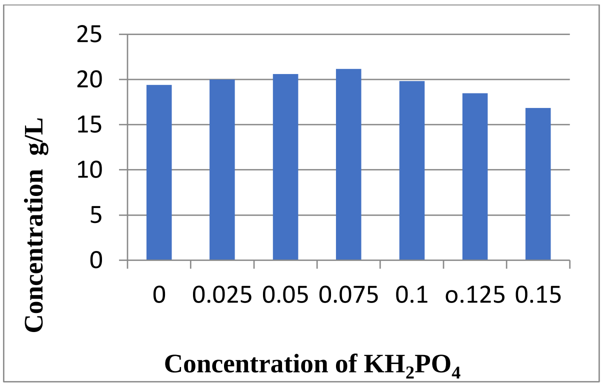

Figure 6: Effect of concentration of (KH_2PO_4)

To optimize the concentration of Pottasium Dihydrogen Orthophosphate (KH_2PO_4) for the production of gluconic acid different concentrations of Pottasium Dihydrogen Orthophosphate were tested (0-0.15%) and it was found that0.075 % of Pottasium Dihydrogen Orthophosphate gives the maximum concentration of gluconic acid 20.989 g/L.

3.4.6 Optimization of concentration of Magnesium Sulphate ($MgSO_4.7H_2O$)

Table 8: Effect of concentration of Magnesium Sulphate ($MgSO_4.7H_2O$)

Sr. No.	Concentration of $MgSO_4.7H_2O$	Gluconic acid (g/L)
1	0	17.065
2	0.015	19.419
3	0.025	21.577
4	0.050	20.596
5	0.075	19.616
6	0.1	19.223
7	o.125	18.046
8	0.150	16.477

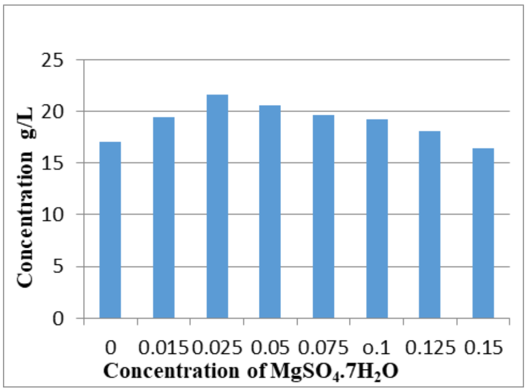

Figure 7: Effect of concentration of $MgSO_4.7H_2O$

To optimize the concentration of magnesium sulphate ($MgSO_4.7H_2O$) for the production of gluconic acid different concentrations of magnesium sulphate were tested (0-0.15%) and it was found that0.025 % of magnesium sulphate gives the maximum concentration of gluconic acid that is 21.577 g/L.

4. Conclusion

The present study indicates that a relatively good concentration of gluconic acid can be obtained after optimization of certain physical and chemical parameters by the mutant *Aspergillus niger*. However, in the present investigation only limited parameters were examined .An attempt to scale up the process is underway.

5. ACKNOWLEDGEMENT

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