OPTIMIZATION OF GLUCONIC ACID PRODUCTION USING TERNARY FEEDSTOCKS AND MIXTURE DESIGN METHODOLOGY

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CERTIFICATION

This is to certify that this research project was carried out by OBAZEE FAITH with matriculation number ENG1804651 in the Department of Chemical Engineering, University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

This work is dedicated first to the Almighty God who has kept me up to this moment and also to my Parents – Mr and Mrs Obazee for their ever care, love and support that has seen me through my stay in this University.

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My gratitude goes to Engr. Dr. Andrew Amenaghawon; my supervisor, whose guidance, insight, and assistance was key to the success of this work. I would also like to thank the Head of Chemical Engineering Department, University of Benin – Engr. Dr. (Mrs) E.T. Akhihiero, Prof. F.A Aisien , Prof. Kessington Obahiagbon, Dr. Chris Akhabue, Engr. Prof. S.E. Uwadiae and all other lecturers and staff of the Department of Chemical Engineering for their guidance, mentorship and assistance. Finally my gratitude goes to my family and friends for all the love. May God bless you all.

ABSTRACT

This project is aimed to optimize the production of gluconic acid using ternary feedstocks composed of pineapple peels, watermelon peels, and orange peels. Employing Design Expert software, a D-optimal mixture design was implemented, with *Penicillium chrysogenum* selected as the fermenting microorganism. The methodology involved varying the proportions of pineapple peel, watermelon peel, and orange peels in the fermentation medium according to the experimental design generated by Design Expert. Following fermentation, gluconic acid yield was quantified, and the results were compared with the predictions obtained from the D-optimal mixture design. The analysis of the experimental data demonstrated a close relationship between the predicted gluconic acid yields and the actual values obtained from the fermentation experiments. For instance, in Run 1, the actual gluconic acid yield was 19.7 g/L, whereas the D-optimal predicted value was 20.97 g/L. Similarly, in Run 5, the actual yield was 23.66 g/L, in close agreement with the predicted value of 23.98 g/L. The optimal run, determined based on the highest gluconic acid yield, was Run 8, with an actual yield of 25.63 g/L, closely matching the D-optimal predicted value of 25.46 g/L. This study shows the efficiency of using ternary feedstocks for gluconic acid production and highlights the value of mixture design methodology in optimizing fermentation processes for bioproduct synthesis.

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NOMENCLATURE

1. GA: Gluconic Acid

2. PP: Pineapple Peel

3. WP: Watermelon Peel

4. OP: Orange Peel

5. DMDD-Optimal Mixture Design

6. RSM: Response Surface Methodology

7. HPLC: High-Performance Liquid Chromatography

8. ANOVA: Analysis of Variance

9. SEM: Scanning Electron Microscopy

10. TEM: Transmission Electron Microscopy

11. COD: Chemical Oxygen Demand

12. TOC: Total Organic Carbon

13. LCA: Life Cycle Assessment

14. ROI: Return on Investment

15. IRR: Internal Rate of Return

16. NPV: Net Present Value

17. CCD: Central Composite Design

18. PBD: Plackett-Burman Design

19. DOE: Design of Experiments

20. Yield: Gluconic Acid Yield

21. PCH: Penicillium chrysogenum

22. AD: Actual Data

23. PD: Predicted Data

24. IQR: Interquartile Range

25. ANOVA: Analysis of Variance

26. A. Niger: Aspergillus Niger

27. P. Chryrogenum: Penicillium Chryrogenum

28. SSF: Solid state fermentation

29. SMF: Submerged Fermentation

30. BBD: Box Behken Design

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Gluconic acid is an essential component used in many different industries, including food manufacturing, medicine, cleaning supplies, and more (*Gluconic Acid: Properties, Production Methods and Applications—An Excellent Opportunity for Agro-Industrial by-Products and Waste Bio-Valorization | Request PDF*, n.d.). It is produced by chemical synthesis as well as microbial fermentation, with a strong preference for the former. In the context of submerged fermentation, microorganisms coordinate the conversion of glucose to gluconic acid, producing significant amounts to satisfy industrial requirements. Notably, Aspergillus niger becomes a preferred microbe due to its availability and high productivity in the manufacture of gluconic acid (Baker, 2006).

Gluconic acid, which has the chemical formula C6H12O7, was first discovered in the 19th century. It is a colourless, odourless molecule that dissolves in water (*Gluconic Acid | C6H12O7 | CID 10690 - PubChem*, n.d.). It is a model of a polyhydroxy acid. Because of its adaptability, it is essential in a variety of settings. Gluconic acid improves product stability and quality in the food industry by acting as a chelating agent, preservative, and acidity regulator (*Gluconic Acid - Jungbunzlauer*, n.d.). In a similar vein, cleaning solutions use its chelating characteristics to get rid of tenacious mineral deposits, while pharmaceutical formulations use them for drug delivery systems.

Although chemical synthesis approaches continue to exist, there is a noticeable transition in the production of gluconic acid towards environmentally friendly methods, which is consistent with larger sustainability goals. Nicely addition to being a more environmentally friendly option, microbial fermentation fits nicely with consumers' increasing desire for

natural ingredients. Moreover, plants are involved in the synthesis of gluconic acid, producing it as a metabolic intermediary and potentially providing pathways for biobased manufacturing (Michiels et al., 2023).

In order to ensure effective gluconic acid synthesis, optimisation strategies are essential. Innovative designs, including the central composite and Doehlert matrix, make it easier to adjust operating parameters and maximise yield while lowering resource usage and waste production (Pińkowska et al., 2013) (Almeida et al., 2017). Manufacturers can ensure that gluconic acid is seamlessly integrated into a variety of industrial processes while achieving sustainability goals by carefully optimising their operations to strike a delicate balance between quality, productivity, and environmental responsibility.

Recombinant proteins and a vast array of complex small-molecule compounds with both medicinal and commercial uses can be produced from plants. However, the focus must change from achieving target molecule synthesis to quality, purity, and yield factors that indicate commercial viability when we move past proof-of-concept trials and start looking into the actual prospects of commercial production (Lico et al., 2008). To do this, process optimisation techniques are needed. The best operating conditions are then obtained by using more complex experimental designs, including the Doehlert matrix (DM), central composite designs (CCD), and three-level designs, like the Box-Behnken design (BBD), after the necessary components have been identified. In the multivariate optimisation process (Ferreira et al., 2007), there are two types of variables: factors and responses. The responses make up the dependent variables. Their values are based on the elemental levels, which can be quantitative or qualitative. There are two methods for optimising. Response surfaces can be simultaneously determined and analysed for each response. One can determine a model for a single composite function that considers all three answers in order to obtain a single response surface (Gaitonde et al., 2012).

1.2 PROBLEM STATEMENT

The use of expensive starting materials and energy-intensive processes makes the standard techniques of producing gluconic acid economically challenging. The first hurdle in the synthesis of gluconic acid is the need for expensive reagents to catalyse the oxidation of carbohydrate feedstocks like glucose or starch. The financial strain prevents gluconic acid from being commercialised widely, which forces researchers to look into more affordable and ecologically friendly alternatives. The buildup of agricultural waste worsens environmental deterioration by lowering the quality of the soil and water, filling landfills to capacity, and increasing greenhouse gas emissions. Nonetheless, by using agricultural waste for value-added procedures like the fermentation synthesis of gluconic acid, these environmental issues might be reduced. Yield inhibition is one of the main problems with gluconic acid fermentation, and it frequently results from insufficient substrate conversion and the production of undesirable byproducts. Side effects and the build-up of intermediary chemicals are factors that lead to yield inhibition and impede the effective synthesis of gluconic acid. Solving these problems is essential to improving fermentation procedures and producing gluconic acid in a way that is profitable.

1.3 AIM AND OBJECTIVES OF STUDY

The primary aim of this research project is the Optimization of gluconic acid production from ternary feedstock of pineapple peels, watermelon peels and orange peels. Other specific objectives include;

1. Formulation of ternary feed mixture

2. Production of gluconic acid from ternary feed

3. Modelling the effect of inducers using response surface methodology

4. Modelling the effect of inducers using artificial neural network modelling

5. Optimization of gluconic acid production using genetic algorithm

1.4 SCOPE OF STUDY

This research was carried out at the central research laboratory at the University of Benin, Benin city. The Box Behnken design was used for studying the effect of variations of the factors that affect the fermentation process and production of gluconic acid which include; PH, aeration and temperature. 15g of ternary mixture of locally produced agro-waste materials (pineapple peels, watermelon peels and orange peels) in the ratio 1:1:1 which was obtained via a mixture design and used as the carbon source while *Penicillium chryrogenum* was the main microorganism used for production of gluconic acid in a fermentation broth.

1.5 RELEVANCE OF STUDY

This research holds significance since it has the ability to tackle urgent financial and ecological issues related to gluconic acid manufacturing. The study intends to remove the financial constraints preventing the mainstream commercialization of gluconic acid by investigating substitute, more affordable production techniques. The study helps to mitigate the pollution of the environment caused by waste accumulation by using agricultural waste as a feedstock for fermentation synthesis. Sustainable production practices are also needed by the industry, which is why the study's focus on improving fermentation processes to increase gluconic acid yield and efficiency is appropriate. Through increasing output and process efficiency, the project aims to lower the environmental effect of gluconic acid manufacturing and increase its economic viability.

CHAPTER TWO

LITERATURE REVIEW

2.1 GLUCONIC ACID

Gluconic acid is an organic substance characterized by the molecular formula C6H12O7 and the condensed structural formula HOCH2(CHOH)4CO2H (*D-Gluconic Acid - American Chemical Society*, n.d.). In a neutral aqueous solution, it transforms into the gluconate anion, presenting as a white solid. "Gluconates" are the salts of gluconic acid. Gluconic acid, gluconate salts, and gluconate esters are abundant in nature because they are formed by the oxidation of glucose. Some medications are administered as gluconates.

The chemical structure of gluconic acid is a six-carbon chain with five hydroxyl groups positioned similarly to the open-chained form of glucose and terminating in a carboxylic acid group. 2,3,4,5,6-pentahydroxyhexanoic acid has 16 stereoisomers. The aerobic oxidation of glucose in the presence of the enzyme glucose oxidase produces gluconic acid. The reaction produces gluconolactone and hydrogen peroxide. In water, the lactone hydrolyzes spontaneously to gluconic acid. Gluconic acid is found naturally in foods such as fruit, honey, and wine. It is currently known as an acidity regulator in the food industry (Wong et al., 2008).

2.1.1 History of Gluconic Acid

Gluconic acid was initially produced in 1870 by Hlasiwetz and Habermann by the chemical oxidation of glucose. Boutroux used glucose fermentation to produce and isolate gluconic acid in 1880. Later, gluconic acid was discovered in filamentous fungi such as Penicillium, Scopulariopsis, Gonatobotrys, and Gliocladium, as well as oxidative bacteria such as strains of Pseudomonas, Gluconobacter (Acetobacter), Moraxella, Micrococcus, Enterobacter, and Zymomonas. Already in the 1940s, considerable quantities of gluconic acid could be obtained

by fermenting A. niger and neutralising the accumulated acid with calcium carbonate (Karaffa & Kubicek, 2021).

2.1.2 Properties of Gluconic Acid

Gluconic acid which is the focus of this research effort, has special physical and chemical features that contribute considerably to its numerous uses and importance in a variety of sectors.

2.1.2.1 Physical Properties

Anhydrous gluconic acid is a white, odourless, crystalline powder. The remarkable part of its physical state is its crystallisation behaviour, which is below 30°C. A monohydrate form, with a unique crystalline structure and a melting point of approximately 85°C, adds complexity to its physical properties. The melting point of gluconic acid ranges from 120-131°C, which is influenced by the formation of intramolecular anhydrides. It is notable for its solubility properties, since it is easily soluble in water, partially soluble in alcohol, and insoluble in ether and other organic solvents. The pH of a 50% gluconic acid solution at 20°C is 1.82, and its density is 1.23 g/cm3 at 25°C (Ramachandran et al., 2017).

2.1.2.2 Chemical Properties

Gluconic acid storage under appropriate circumstances displays fascinating chemical changes. Lactones arise when exposed to a desiccant at ambient temperature or heated beyond 50°C, with pyrolysis happening at temperatures over 200°C. The gluconic acid lactones 1,5-lactone (glucono-d-lactone) and 1,4-lactone (glucono-g-lactone) are in equilibrium with each other and with free acid. The dissociation of the free acid has a complex impact on the equilibrium, with a reported dissociation constant (Ka) of 1.99 × 10^(-4) at 25°C and a pKa of 3.70. The hydrolysis of gluconic acid 1,5-lactone, a white crystalline compound with a melting point of 153°C, affects both pH and specific rotation until

equilibrium is reached. 1,4-lactone, on the other hand, crystallises as fine needles with a melting point of 134-136°C.

Aside from its crystalline properties, gluconic acid is known as a moderate, noncorrosive, and nonvolatile organic acid with high biodegradability and nontoxicity. An interior ester, glucono-d-lactone, gives a sweet flavour followed by a mild acidity. In aqueous solutions, sodium gluconate exhibits high-temperature oxidation and reduction resistance while also acting as an excellent plasticizer and set retarder. Because of certain lactone structures, concentrated gluconic acid solutions have antibacterial effects. Furthermore, at alkaline pH, sodium gluconate outperforms well-known chelators such as EDTA and nitrilotriacetic acid in suppressing bitterness in meals. Gluconic acid is recognised by regulatory organisations and is classified as a generally approved food additive (E574) in European Parliament and Council Directive No. 95/2/EC. The Food and Drug Administration in the United States has given sodium gluconate a GRAS designation (Singh & Kumar, 2007)

2.1.3 Applications of Gluconic Acid

2.1.3.1 Pharmaceutical Industry

Gluconic acid finds wide-ranging applications in the pharmaceutical industry, where it improves medication stability and formulation. Recent studies by the European Parliament and Council Directive No. 95/2/EC, gluconic acid is designated as a generally approved food additive (E574). Sodium gluconate is classified as GRAS (generally recognised as safe) by the US Food and Drug Administration, meaning that its use in food and drug is allowed without restriction. (Ramachandran et al., 2006) and (Mycielska et al., 2019) carried out a research on the most prevalent application of gluconate, which is a salt of gluconic acid. In medicine is as a biologically neutral carrier of K+, Zn2+, Ca2+, Cu2+, and Fe2+ to cure pertinent ion shortages. Moreover, gluconate is combined with other medications, such as

sodium antimony (stibogluconate), which is used to treat leishmaniasis, and chlorohexindine, which is used as an antiseptic in dentistry and surgery.

Studies have shown the medicinal effect of calcium gluconate gel for the treatment of hydrofluoric acid burns (de Capitani et al., 2009). Further research carried out on gluconic acid by (French et al., 2012) showed that Injections of calcium gluconate are used in more severe cases to treat hypocalcemia in hospitalised patients and prevent necrosis of deep tissues.

Quinine gluconate is an intramuscular injection used to treat malaria. It is a salt of quinine and gluconic acid (*Quinine Gluconate | C26H36N2O9 | CID 20841648 - PubChem*, n.d.).

2.1.3.2 Food Industry

As consumer demand for natural and clean-label ingredients rises, gluconic acid's natural origin positions it as a preferred choice for food manufacturers. (*Gluconolactone Market Size, Share & Forecast to 2033*, n.d.)

In the food industry, gluconic acid and its salts have become integral components, revolutionizing food preservation and quality. Research by (*GLUCONIC ACID - Ataman Kimya*, n.d.) has shown that gluconic acid has a unique property in inhibiting the bitterness in foodstuff, it is used as a flavor enhancer to enhance the flavor of food.

More researches done as regards the food industry has also shown that gluconic acid is used as an acidulant and natural preservatives (kirikuma) (https://www.sciencedirect.com/topics/neuroscience/gluconic-acid).This help to curb the growth of spoilage microorganisms and pathogens. Its antimicrobial properties contribute to extending the shelf life of various food products.

In order to increase the durability of fillings and icings, gluconic acid has been added to bakery formulas in recent years. The benefits of gluconic acid in improving the quality and shelf stability of frosting in baked goods are well demonstrated by experiments conducted by (Singh & Kumar, 2007)

Innovative applications of gluconic acid in food packaging materials have been explored, going beyond conventional usage. Research conducted (Zhou et al., 2020) explores the application of gluconic acid in packaging films, highlighting its antibacterial characteristics and potential to increase the shelf life of food goods that are packed.

2.1.3.3 Construction Industry

Based on the studies by (Ye et al., 2021), adding gluconic acid to concrete mixtures increases the material's durability and compressive strength, resulting in stronger, longer-lasting concrete buildings.

In reinforced concrete constructions, gluconic acid functions as a corrosion inhibitor. It combines with metal ions to produce complexes that prolong the life of concrete by preventing the corrosion of steel reinforcement. (Sivasankaran, 2018)

The recent research by (Nafee et al., 2023) explores the synthesis and characterization of gluconic acid with sodium on Ordinary Portland Cement (OPC) and its impact on cement properties. The study found that sodium gluconate is employed to adjust the pH of cement-based grouts, particularly in applications like grouting of post-tensioned cables. This helps in maintaining an optimal pH for the hydration process.

The research by (Napper et al., 2012) discusses the use of gluconic acid to mitigate the alkali-silica interaction, a phenomena that can cause concrete deterioration. The study highlights

how gluconic acid has an additional function in lowering the likelihood of expanding gel formation.

2.1.3.4 Cleaning Industry

The work of (Asemave, 2018) shows in great detail how gluconic acid is used as a chelating agent in cleaning solutions to get rid of scale and mineral deposits on a variety of surfaces, such industrial equipment or coffee makers.

Gluconic acid is used in boiler water treatment formulations in the industrial sector to restrict scale development and prevent corrosion, extending the lifespan and efficiency of boilers. (Kornecki et al., 2020)

Dishwashing detergents contain gluconic acid, a water softener that improves detergent performance because of its ability to increase cleaning effectiveness and decrease spots on dishes and glassware. (“Cleaning Compounds,” 2006)

(Karaffa & Kubicek, 2021) carried research on cleaning products containing gluconic acid. The result gotten from the experiment showed that gluconic acid is effective in rust removal. The chelating properties of gluconic acid help dissolve rust from metal surface, making it useful in rust-stain removal.

2.1.3.5 Cosmetics Industry

Gluconic acid plays a significant role in the cosmetic industry due to its versatile properties and beneficial effects on skin and hair. Its mild acidity, biodegradability, and compatibility with various cosmetic ingredients make it a popular choice for formulators. (S. Li et al., 2023) researched on gluconolactone and found out that it has a high use in the cosmetics industry, Skin cells frequently contain gluconolactone, one of the most popular PHAs (polyhydroxy acids) utilised in cosmetic products. By breaking down the "glue" that holds

dead skin cells together, it works similarly to AHA and BHA acids (such as glycolic, lactic, and salicylic acids). (*GLUCONIC ACID – Ingredient - COSMILE Europe*, n.d.) noted gluconic acid is used to binds metal ions that may have a negative impact on the consistency and/or look of makeup. A research carried out by (Kantikosum et al., 2019), shows that In cosmetics and personal care products, glycolic acid functions as a buffering agent and an exfoliator. Cell shedding is accelerated by glycolic acid. Stated differently, it disrupts the connections that hold skin cells collectively. It has the function of speeding up the natural exfoliation process of dead skin cells by your skin. He also showed that The skin produces more collagen when glycolic acid is present, which keeps your skin supple and firm. In addition, it brightens the dark areas caused by sun damage. The neutralised form of gluconic acid is sodium gluconate, or salt. often employed as a stabilising agent in the cosmetics sector. Monosodium salt, D-gluconic acid, is a synonym. (*Glycolic Acid (Buffering Agent): Cosmetic Ingredient INCI*, n.d.) conducted a research on sodium gluconate and found out that it has beneficial properties to the cosmetics industry. Sodium gluconate is used to stabilise a wide range of cosmetic products, including sunscreen, cosmetics, lotions, shampoos, and conditioners.

2.1.3.6 Textile Industry

The application of gluconic acid to textiles has been shown to have antibacterial qualities, according to recent research by (Parvulescu et al., 2021). With the help of this application, materials with built-in antibacterial properties could be developed, improving cleanliness and lowering odour.

The potential for gluconic acid to lessen the environmental impact of textile production is investigated. In order to encourage more environmentally friendly practice (Yildiz & Karatas, 2018) has conducted research on the integration of gluconic acid into textile processing techniques.

The biodegradable qualities of gluconic acid are being investigated for use in the textile industry. A study conducted by the (Mehtiö et al., 2016)' evaluates the potential of gluconic acid to reduce the environmental effects of textile auxiliaries.

2.2 PRODUCTION OF GLUCONIC ACID

Gluconic acid, a sugar acid derived from glucose, is an important organic acid with various industrial applications. Its production can be achieved through chemical and biological synthesis methods.

2.2.1 Chemical Synthesis Production of Gluconic Acid

Chemical synthesis of gluconic acid involves the oxidation of glucose, typically using metal catalysts. The most common method is the oxidation of glucose with molecular oxygen in the presence of a metal catalyst, such as copper or manganese. The reaction can be represented as follows:

Glucose + Oxygen $\xrightarrow{\text{Metal Catalyst}}$ Gluconic Acid

This process is widely used in industry due to its efficiency and scalability. However, it may involve the use of toxic metals, which raises environmental concerns. Researchers are continuously exploring alternative catalysts to address these issues (Wong et al., 2008).

In the chemical synthesis of gluconic acid, glucose is converted to gluconic acid using a process known as oxidation. This chemical transformation is an alternative to fermentation processes, which can have operational difficulties.

Catalytic Oxidation Processes

To overcome challenges associated with fermentation, new catalytic oxidation processes have been proposed. These processes involve reacting glucose with molecular oxygen under alkaline conditions in the presence of noble metal catalysts such as platinum or palladium.

2.2.1.1 *Palladium-Bismuth Catalyst (European Patent EP0142725)*

A representative procedure uses a palladium-bismuth catalyst supported on activated charcoal.

Achieves a high glucose conversion of 99.8% and a sodium gluconate yield of 99.5%.

Catalytic activity is less than 1,450 g of product per gram of Pd used per hour of reaction time (*Method for Preparation of Gluconic Acid by Catalytic Oxidation of Glucose*, 1990).

2.2.1.2 *Palladium-Bismuth/Carbon Catalyst (US4843173)*

(*Palladium/Carbon Catalyst Regeneration and Mechanical Application Method,* 2015) describes a process using a palladium-bismuth/carbon catalyst in an aqueous alkali solution.

The catalyst improves activity, selectivity, and durability.

Reaction stops before undesired byproducts contaminate the reaction.

2.2.1.3 *Activated Charcoal Supported Catalyst (US 5,132,452)*

Involves a heterogeneous activated charcoal supported catalyst containing platinum, palladium, and bismuth.

The catalyst is separated by filtration after completion of the reaction and can be reused in several batches (Iwanow et al., 2020).

Physical Parameters for Catalysis

Various physical parameters influence the chemical catalysis of glucose oxidation to gluconic acid:

pH and Temperature: Catalytic oxidation is carried out under alkaline conditions at pH ranging from 8.0 to 11, maintained by adding an alkali metal hydroxide. The reaction temperature typically ranges from 20°C to 80°C, preferably 30-60°C.

Dissolved Oxygen and Pressure**:** Catalytic oxidation is performed with oxygen-containing gases at pressures between standard pressure and 3-10 bars.

Although, the chemical synthesis of gluconic acid has advantages, such as high conversion rates, it also comes with challenges. High energy costs and the need to separate byproducts from the final product are some of the disadvantages.

2.2.2 Microbial Methods of Producing Gluconic acid

Microbial fermentation is a sustainable and economically viable approach for the production of gluconic acid (Ramachandran et al., 2006) microorganisms Involved:

The microbial production of gluconic acid primarily involves bacteria of the genus Gluconobacter and fungi such as Aspergillus niger. Among the Gluconobacter species, Gluconobacter oxydans stands out as the most widely studied and utilized strain due to its robustness, high yield, and efficiency in gluconic acid production. (da Silva et al., 2022) Gluconobacter species are aerobic, Gram-negative bacteria capable of oxidizing glucose to gluconic acid through the activity of the enzyme glucose oxidase (Hommel & Ahnert, 1999).

On the other hand, certain strains of the filamentous fungus Aspergillus niger possess the ability to produce gluconic acid through the oxidation of glucose using glucose oxidase enzymes (Ajala et al., 2017). Although less common than bacterial fermentation, fungal fermentation offers advantages such as ease of cultivation, scalability, and the potential for genetic manipulation to enhance productivity (Lübeck & Lübeck, 2022).

The success of microbial fermentation for gluconic acid production relies heavily on optimizing fermentation conditions to create an environment conducive to microbial growth and metabolic activity. Key parameters include temperature, pH, oxygen availability, agitation, and nutrient composition of the fermentation medium (*Optimization of*

Fermentation Conditions for Gluconic Acid Production by Mutant Aspergillus Niger | Request PDF, n.d.) Several factors influence the efficiency and productivity of microbial gluconic acid fermentation, including substrate concentration, fermentation time, inoculum size, and the presence of inhibitors or co-substrates. Optimization of these factors through experimental design and process engineering is essential to maximize gluconic acid yield and minimize production costs.

2.3 FERMENTATION

Fermentation is a metabolic process that alters the chemical composition of organic materials, through the activity of enzymes. It is widely described as the process of obtaining energy from carbohydrates without the presence of oxygen in biochemistry. More widely, it can refer to any procedure in food production where the action of microbes modifies a food or beverage in a way that is desired. Zymology is the scientific study of fermentation.

Since the Neolithic era, humans have employed fermentation to make food and drink. The use of microorganisms to a large-scale synthesis of chemicals, biofuels, proteins, enzymes, and medicines is referred to as "industrial fermentation."

An essential step in the production of gluconic acid is fermentation, which uses a variety of methods to optimise microbial activity.

2.3.1 Submerged Fermentation

The definition of submerged fermentation, SmF, is the culture of microbes in a nutritional media with an excess of freely flowing water. One method that is frequently used to produce gluconic acid and other biochemicals is submerged fermentation (SmF). Because microorganisms in SmF are cultivated in a liquid medium while submerged, effective nutrient absorption and metabolite synthesis are made possible. The main elements of submerged

fermentation for the synthesis of gluconic acid are examined in this part, along with microbial strains, fermentation conditions, and process optimisation techniques (Kim & Han, 2014).

Through submerged fermentation, a number of microbial strains are able to produce gluconic acid. Because of their strong capacity to produce gluconic acid, certain of these species of fungi—specifically, Aspergillus niger and Penicillium chrysogenum (Lu et al., 2015)—are often employed. Glucose oxidase, an enzyme found in these fungi, catalyses the conversion of glucose to gluconic acid and the subsequent generation of hydrogen peroxide.

For submerged fermentation to maximise gluconic acid output, ideal fermentation conditions are essential. The development of microorganisms and the synthesis of metabolites are greatly influenced by variables including temperature, pH, agitation, aeration, and substrate concentration. A pH range of 2.0 to 6.0 is ideal for the synthesis of gluconic acid, and the fermentation temperature is often kept between 25°C and 35°C (Chen et al., 2009). The amounts of agitation and aeration are adjusted to guarantee adequate mixing and oxygenation of the developing microbial culture.

2.3.2 Solid State Fermentation

Gluconic acid is one of the several biochemicals that are produced by the bioprocess known as solid-state fermentation (SSF) (Ramachandran et al., 2017). Microorganisms in SSF develop on solid substrates in the absence or very close to the lack of freely flowing water, resulting in a heterogeneous environment that is favourable to microbial metabolism. The part discusses about the essential elements of solid-state fermentation for the synthesis of gluconic acid, encompassing the choice of substrate, microbial strains, and techniques for process optimisation.

A number of microbial strains have been investigated for the solid-state fermentation process of gluconic acid synthesis. Aspergillus niger, Penicillium spp., and Rhizopus spp. are examples of fungal species that are frequently used because of their capacity to manufacture

gluconic acid effectively under solid-state conditions (Yafetto, 2022). These fungi are good candidates for gluconic acid production in SSF because they contain the enzyme glucose oxidase, which catalyses the oxidation of glucose to gluconic acid (Bauer et al., 2022).

For solid-state fermentation to produce gluconic acid successfully, the right substrates must be chosen. A wide range of lignocellulosic materials and agricultural wastes have been studied as substrates because of their affordability, availability, and ability to produce gluconic acid. Fruit peels, sugarcane bagasse, wheat bran, and rice bran are examples of common substrates (Yafetto, 2017). These substrates offer an abundant supply of carbohydrates, mostly glucose, which is the building block needed for microbial enzymes to synthesise gluconic acid.

Submerged fermentation is a viable option for large-scale industrial applications because to its exceptional productivity, scalability, and controllability over the process (Sun et al., 2022). Though it has limitations with regard to process control and scale-up, solid-state fermentation has advantages in substrate utilisation, water efficiency, and sustainability (Abdul Manan & Webb, 2017). Process economics, substrate availability, and the particular needs of the intended application are some of the variables that influence the decision between SmF and SSF (Colla et al., 2015).

2.4 FEEDSTOCKS FOR GLUCONIC ACID PRODUCTION

Gluconic acid is synthesised from a variety of feedstocks and is an essential component in many industries. This research delves into four primary categories of feedstocks, each offering unique advantages and considerations.

2.4.1 Sugar Feedstock

Gluconic acid synthesis relies heavily on sugar, which is the main feedstock used in bioprocess engineering. The multi-step enzymatic and microbiological conversion steps

involved in gluconic acid production are explained by (Abd Alsaheb et al., 2022), who explore the complexities of realising sugar's potential. The present study offers a thorough understanding of the intricate relationship among substrate composition, enzyme kinetics, and microbial strains, revealing the fine equilibrium necessary for the highest possible output of gluconic acid. Further research is conducted by (da Silva et al., 2022) on the effects of sugar's intrinsic properties on the gluconic acid production pathway. Going beyond traditional enzymatic methods, this study clarifies the bioprocess engineering factors that affect the generation of gluconic acid. A detailed grasp of the complex linkages regulating the utilisation of sugar feedstock is provided by the study's methodical evaluation of variables like pH, temperature, and substrate concentration.

When it comes to producing gluconic acid, sugar in all of its forms offers advantages as well as drawbacks. There is variation in the enzymatic hydrolysis and microbiological fermentation kinetics due to the fermentability of various sugars, such as fructose, glucose, and sucrose. Gluconic acid production requires optimised sugar feedstock utilisation, which calls for customised techniques due to the dynamic interplay of these components.

The investigation of alternate sugar feedstocks becomes more important as industry look for more affordable and sustainable alternatives. Researching novel sources and refining techniques are necessary to extract sugars with a high potential for gluconic acid conversion. The complex interplay between process optimisation and sugar feedstock selection becomes a crucial research focus, impacting the synthesis of gluconic acid from an environmental and economic standpoint.

2.4.2 Starch Feedstock

Gluconic acid synthesis has found a compelling use for starch, a complex polysaccharide that is abundant in many plant sources. Starch provides a rich substrate for enzymatic breakdown

and subsequent microbial fermentation. (Pucci et al., 2023) explored the complexities of using starch feedstocks, revealing the mutually beneficial connection between gluconic acid production and enzymatic saccharification. Beyond the traditional use of starch, this research illuminates the mutually beneficial interactions between microbial strains and enzymatic hydrolysis enzymes, highlighting the necessity of an integrated strategy to maximise gluconic acid output.

By concentrating on the optimisation techniques involved in starch hydrolysis, (Zeng et al., 2022) study adds to our understanding of starch feedstock utilisation. The intricacies of enzymatic processes are examined in this study, along with the effects of many parameters like substrate composition, enzyme concentration, and reaction kinetics. The research offers significant insights into optimising gluconic acid production efficiency, a crucial component for industrial scalability, by analysing the complexities of starch hydrolysis.

The variable substrate composition of starch feedstocks, which come from a variety of agricultural sources, makes customised enzymatic techniques necessary for the best saccharification. Because starch-based feedstocks are dynamic, enzymatic conversion rates, fermentation kinetics, and overall process economics must be balanced. This presents both opportunities and challenges. Researchers are currently delving into the intricacies linked to distinct starch feedstocks with the objective of formulating resilient approaches that are adjustable to diverse starch compositions and industrial environments (Sakhuja et al., 2021).

The search for novel sources of starch and the incorporation of cutting-edge enzymatic methods are becoming increasingly important as industries work towards sustainability. Researchers are extending the frontiers of starch feedstock research in gluconic acid synthesis by exploring the use of non-traditional starch-rich biomasses and agricultural leftovers in their pursuit of environmentally benign and economically viable methods.

2.4.3 Fruit wine Feedstock

Fruit wine residues, which are sometimes disregarded and regarded as insignificant byproducts of the winemaking process, take on a key function in the ongoing search of sustainable gluconic acid production. The circular economy's guiding principles—which emphasise resource efficiency and the conversion of waste into useful inputs—align well with this shift towards trash valorization.

The investigation by (Vázquez et al., 2023) is enhanced by their emphasis on maximising the effectiveness of turning fruit wine leftovers into gluconic acid. By helping to fine-tune applications their work ensures high yields and process efficiency. The kinetic analysis carried out in this work offers important insights into the parts of gluconic acid synthesis that are reliant on time, which are essential for accelerating the process.

Fruit wine leftovers provide a number of benefits when used as a feedstock. Because these by-products are inexpensive, there are financial benefits to waste reduction in addition to environmental ones. Fruit wine leftovers' distinct composition adds variety, which affects enzymatic methods and opens the door for designed strategies (Kornecki et al., 2020).

2.4.4 Biomass waste Feedstock

Biomass waste is a rich and diverse source of gluconic acid synthesis. It comes from a variety of sources, including forestry byproducts and agricultural wastes. This recycling of waste materializes the concepts of the circular economy, turning what was formerly seen as an inconvenience into a useful resource. Using biomass waste to produce GA is an example of a strategy that is dedicated to eco-friendly valorization. This approach tackles environmental issues and helps create a more circular and sustainable industrial ecosystem by repurposing waste resources.

2.5 MICRO ORGANISM FOR GLUCONIC ACID PRODUCTION

Microbial fermentation or chemical synthesis are two methods that can be used to produce gluconic acid. Microbial fermentation is a typical process used for producing gluconic acid because it offers numerous benefits, such as sustainability, cost-effectiveness, and the potential for increased yields and purity (Ma et al., 2022). Gluconic acid production can be achieved through various microorganisms, including bacteria and filamentous fungi. Each organism possesses unique characteristics and metabolic pathways influencing their suitability for industrial-scale production.

2.5.1. Gluconic Acid Production using Bacteria

Several bacterial species possess the ability to produce gluconic acid, offering potential avenues for industrial fermentation. However, two (Gluconobacter Oxydans and Gluconobacter Suboxydans) primary strains stand out due to their efficiency and historical significance:

2.5.1.1. Gluconobacter Oxydans

A bacterium belonging to the family Acetobacteraceae. G. Oxydans is an obligate aerobe that uses oxygen as the terminal electron acceptor in its respiratory form of metabolism. Sugary niches such as ripe grapes, apples, dates, garden soil, baker's soil, honeybees, fruit, cider, beer, and wine are ideal for the growth of certain Gluconobacter strains. Gluconobacter strains are non-pathogenic towards man and other animals but are capable of causing bacterial rot of apples and pears accompanied by various shades of browning. *Gluconobacter Oxydans* initiate the incomplete oxidation of alcohols, acids and sugar. Incomplete oxidation leads to almost quantitative yields of the oxidation products making G. oxydans important for industrial purpose. Gluconobacter strains are used industrially to produce D-gluconic acid, 5-keto- and 2-ketogluconic acids from D-glucose; L-sorbose from D-sorbitol; and dihydroxyacetone from glycerol (Gätgens et al., 2007).

Gluconobacter Oxydans Employs the Entner-Doudoroff pathway for glucose oxidation, leading to the preferential production of gluconic acid over other organic acids.

2.5.1.2. Gluconobacter Suboxydans

One species of bacteria in the Gluconobacter genus is *Gluconobacter suboxydans*. It is well-known for its capacity to oxidise a wide range of substrates, especially sugars like fructose and glucose, to create other organic acids and gluconic acid. This bacterium is used in a variety of industrial processes, including as the gluconic acid and vinegar manufacturing. It is significant in biotechnological applications, especially in fermentation processes, due to its oxidative characteristics.

Gluconobacter suboxydans are the preferred microorganisms for the production of gluconic acid because of their highly active respiratory chain, which oxidises a variety of sugars and sugar alcohols (Ameyama et al., 1987). Gluconic acid has beneficial properties like extremely low toxicity, low corrosiveness, and the formation of water-soluble complexes with divalent and trivalent metal ions (Hommel, 2014).

The benefit of using *Gluconobacter suboxydans* is that free-form glucononic acid can be obtained. The reason for this is that bacteria can withstand high acidity levels quite well (Shiraishi et al., 1989).

2.5.2. Gluconic Acid Production using Filamentous Fungi

The ability of filamentous fungus, like *Aspergillus niger*, to ferment and create gluconic acid has been the subject of much research. One important organic acid used in many industries, such as food, medicine, and agriculture, is gluconic acid.

As important industrial cell factories, filamentous fungi are involved in the synthesis of several enzymes and primary and secondary metabolites. Fungi overproduce organic acids in

these metabolites, which has important commercial applications. Due to the fact that wild type fungal strains are accumulating greater quantities, microbial synthesis of organic acids such as citric acid, lactic acid, and gluconic acid has reached commercial status (Narisetty et al., 2023).

Several species from the following fungal genera are used in batch culture to produce gluconic acid: Aspergillus, Penicillium, Fusarium, Mucor, and Gliocladium (Singhania et al., 2010). It has been published that, of the various fungal genera, only a few species of Aspergillus—particularly A. niger, which is regarded as the most significant producer of gluconic acid in the fermentation industry—are capable of accumulating significant amounts of gluconic acid and its salts (Shindia et al., 2006).

2.5.2.1. Aspergillus Niger

Aspergillus niger, a filamentous fungus, produces gluconic acid through fermentation. This process involves the secretion of glucose oxidase, which converts glucose into gluconic acid. pH regulation and optimization of fermentation conditions are essential for maximizing production. Gluconic acid finds diverse industrial applications, making *Aspergillus niger* a valuable organism in biotechnology.

Up to 492 species of Aspergillus, a sizable genus within the Aspergillaceae family, are now included in the National Centre for Biotechnology Information (NCBI) database. Its segment The A. niger aggregation, which consists of eight morphologically identical taxa, is the most complex taxonomic subdivision within the significant niger group of species (Perrone et al., 2011). A. niger can be found everywhere in nature, including on land (Arya et al., 2022), in the ocean (D. H. Li et al., 2016), in the Arctic (Yu et al., 2021), and in space, because of its exceptional adaptability and survival. Beyond that it inhabits a variety of environments found in plants and animals, including lichen (Elissawy et al., 2019), shrimp (Fang et al., 2016), sea

sponges (Hiort et al., 2004), shrubs (Liu et al., 2016), trees (Wang et al., 2019), and herbs (Manganyi et al., 2018). The strain of A. niger grows well in a range of mediums containing diverse carbon sources, such as lactose, glucose, bran, xylan, xylose, sorbitol, and maltose (Toghueo et al., 2018).

Aspergillus niger uses an aerobic fermentation process with a high oxygen requirement to produce glutamic acid. Without the presence of intricate metabolic cell pathways, the biotransformation of glucose to gluconic acid is a straightforward dehydrogenation process (Znad et al., 2004).

2.5.2.2. Penicillium Chrysogenum

A species of fungus in the genus Penicillium is Penicillium chrysogenum, formerly known as Penicillium notatum. It can be found on salted food products and is typical in temperate and subtropical countries (Samson et al., 2010), However, it is primarily found inside, particularly in damp or water-damaged structures (Andersen et al., 2011). P. notatum, P. meleagrinum, and P. cyaneofulvum have all been identified as members of this species complex. (Houbraken et al., 2011). While A. Niger and additional bacteria species can be used for effective fermentation of gluconic acid production, *P. chrysogenum* exhibits a higher potential for utilising its glucose oxidase activity for gluconic acid production and will be applied in large-scale fermentation. These studies examined the performance of the *P. chrysogenum* strain's fermentative gluconic acid production under a variety of fermentation conditions, including substrate concentration, inoculum density, and inoculum age. The results demonstrated the strain's improved performance under optimal conditions, such as 10% glucose concentration, 10% inoculum density, and 72-hour inoculum age (Purane et al., 2011).

Penicillium chrysogenum may be grown in submerged fermentation systems using a range of carbon sources, such as industrial byproducts like orange peel, pineapple, and watermelon peel, or glucose from agricultural waste. Its versatility highlights the possibility of using it as a flexible biocatalyst for the synthesis of gluconic acid, providing chances to value agricultural surplus and organic waste streams.

2.6. FACTORS AFFECTING MICROBIAL PRODUCTION OF GLUCONIC ACID

Numerous elements that impact the effectiveness, productivity, and resilience of the fermentation process also have an impact on the microbial synthesis of gluconic acid. Comprehending these variables is essential to maximising fermentation conditions and raising the output of gluconic acid synthesis. The quantity of the substrate, pH, temperature, availability of oxygen, choice of microbial strain, and duration of fermentation are some of the major variables that affect the formation of gluconic acid by microorganisms (Lu et al., 2015)

2.6.1 Substrate Concentration:

- Substrate concentration, particularly glucose, plays a significant role in microbial gluconic acid production.

- Higher substrate concentrations can initially increase gluconic acid productivity, as more glucose is available for microbial metabolism.

- However, beyond a certain threshold, substrate inhibition may occur, leading to decreased productivity and accumulation of inhibitory byproducts.

- Optimal substrate concentration varies depending on the specific microbial strain and fermentation conditions and requires careful optimization through experimental trials (Singh et al., 2003).

2.6.2 pH:

- pH is a critical factor that influences microbial growth, enzyme activity, and product formation during gluconic acid fermentation.

- Gluconobacter species prefer acidic conditions for optimal growth and gluconic acid production, with an optimal pH range typically between 4 to 6.

- Maintaining the pH within the desired range throughout the fermentation process is essential to promote microbial activity and maximize gluconic acid yield.

- pH control strategies, such as the addition of buffering agents or automated pH regulation systems, may be employed to maintain optimal conditions (Dai et al., 2023).

2.6.3 **Temperature:**

- Temperature profoundly affects microbial metabolism, enzymatic activity, and the overall kinetics of gluconic acid fermentation.

- Gluconobacter species thrive at moderate temperatures, with optimal growth and productivity observed within the range of 25°C to 30°C.

- Temperature control is crucial to ensure stable fermentation conditions and prevent thermal stress on the microbial culture.

- Deviations from the optimal temperature range can negatively impact microbial performance and reduce gluconic acid yield (Sriphochanart et al., 2023).

2.6.4 Oxygen Availability:

- Oxygen is an essential substrate for aerobic microbial metabolism during gluconic acid fermentation.

- Adequate oxygen supply is necessary to support the activity of glucose oxidase enzymes and promote efficient conversion of glucose to gluconic acid.

- Stirring or agitation of the fermentation medium facilitates oxygen transfer and prevents oxygen limitation, particularly in high-density fermentation cultures.

- Oxygenation strategies, such as sparging or aeration, may be employed to ensure optimal oxygen availability throughout the fermentation process (Moresi et al., 1991).

2.6.5 Microbial Strain Selection:

- The choice of microbial strain significantly impacts gluconic acid production, as different strains exhibit varying metabolic capabilities, substrate preferences, and productivities.

- Gluconobacter species, particularly Gluconobacter oxydans, are commonly used for gluconic acid fermentation due to their high yield and efficiency.

- Strain selection should consider factors such as substrate specificity, tolerance to fermentation conditions, and genetic stability to ensure consistent and reliable production performance (Ma et al., 2022).

2.6.5 **Fermentation Time:**

- Fermentation time is a critical parameter that influences the extent of gluconic acid production and the overall process efficiency.

- Prolonged fermentation durations may be necessary to achieve higher gluconic acid yields, as microbial growth and metabolic activity continue over time.

- However, excessive fermentation times can lead to substrate depletion, accumulation of inhibitory byproducts, or reduced microbial viability, resulting in decreased productivity.

- Optimization of fermentation time involves balancing the trade-offs between maximizing product yield and minimizing process duration to meet production goals (*(PDF) Kinetic Study of Gluconic Acid Batch Fermentation by Aspergillus Niger*, n.d.).

2.7. RECOVERY OF GLUCONIC ACID

The recovery of gluconic acid from fermentation broth is an important step in the production process to obtain a purified product suitable for various industrial applications. Different techniques are employed for recovery, each offering unique advantages in terms of efficiency, purity, and scalability. Here, three common methods for the recovery of gluconic acid are explored: crystallization, ion-exchange resin, and membrane-based separation (Ma et al., 2022).

2.7.1. Crystallization

Crystallization is a widely used technique for the purification and concentration of gluconic acid from fermentation broth (Choi et al., 2022). The process relies on the controlled precipitation of gluconic acid crystals from a supersaturated solution. Key aspects of crystallization for gluconic acid recovery include:

- Supersaturation: Achieving a supersaturated solution by concentrating the fermentation broth through evaporation or cooling.

- Seeding: Introducing seed crystals into the supersaturated solution to initiate crystal formation and promote uniform growth.

- Temperature Control: Maintaining precise temperature conditions to control the rate of crystallization and ensure the formation of high-quality crystals.

- Separation and Drying: Separating the crystalline product from the mother liquor using filtration or centrifugation, followed by drying to remove residual moisture and obtain solid gluconic acid crystals.

Crystallization offers advantages such as high purity, scalability, and compatibility with continuous operation. However, it requires careful control of process parameters and may be sensitive to impurities and process conditions.

2.7.2. Ion-Exchange Resin

Ion-exchange resin chromatography is another commonly employed method for the purification of gluconic acid from fermentation broth. This technique relies on the selective adsorption of gluconic acid onto ion-exchange resins, allowing for the separation of gluconic acid from other components based on differences in charge and affinity (Gluszcz et al., 2004). Key aspects of ion-exchange resin chromatography for gluconic acid recovery include:

- Resin Selection: Choosing an appropriate ion-exchange resin with functional groups that exhibit affinity for gluconic acid molecules.

- Column Packing: Packing a chromatography column with the ion-exchange resin and establishing optimal operating conditions for efficient adsorption and elution of gluconic acid.

- Elution: Washing the resin-bound gluconic acid with elution buffer to release the adsorbed molecules from the resin matrix.

- Regeneration: Regenerating the ion-exchange resin for reuse by washing with regeneration solution to remove adsorbed impurities and restore resin capacity.

Ion-exchange resin chromatography offers high selectivity, resolution, and reproducibility, making it suitable for the purification of gluconic acid to high levels of purity. However, it

may require significant capital investment and operating costs associated with resin regeneration and disposal.

2.7.3. Membrane-Based Separation

Membrane-based separation processes, such as ultrafiltration and nanofiltration, offer an alternative approach for the recovery of gluconic acid from fermentation broth (Banerjee et al., 2018). These processes utilize semipermeable membranes to selectively separate gluconic acid from other components based on differences in molecular size, charge, and solubility. Key aspects of membrane-based separation for gluconic acid recovery include:

- Membrane Selection: Choosing membranes with appropriate pore sizes and molecular weight cutoffs to selectively retain gluconic acid while allowing passage of smaller molecules.

- Pressure and Flow Control: Applying pressure across the membrane to drive solvent and solute transport, and controlling the flow rate to optimize separation efficiency.

- Concentration: Concentrating the gluconic acid solution by removing water through the membrane, resulting in a more concentrated product.

Membrane-based separation offers advantages such as continuous operation, low energy consumption, and minimal chemical usage. However, it may be sensitive to fouling and require pre-treatment of the fermentation broth to remove particulates and colloidal matter.

2.8 OPTIMIZATION METHOD

The optimization method used for this research, the production of gluconic acid using ternary feedstocks is the mixture design methodology

2.8.2 Mixture Design Methodology:

Mixture design methodology provides a systematic approach to optimizing feedstock composition for gluconic acid production. By varying the proportions of pineapple peel, watermelon peel, and orange peels in the fermentation medium, researchers can explore a wide range of combinations. Response surface methodology aids in developing predictive models to understand the relationship between feedstock composition and gluconic acid yield.

2.8.3 Experimental Design:

Central composite design or simplex lattice design facilitates systematic exploration of the ternary feedstock space. Each experimental run involves fermenting the mixture of fruit peels with suitable microbial strains capable of gluconic acid production. Key parameters such as fermentation time, temperature, pH, and inoculum size are carefully controlled and monitored.

2.8.4 Optimization of Process Parameters:

Statistical analysis of experimental data enables the identification of optimal feedstock compositions that maximize gluconic acid yield. Response surface methodology aids in pinpointing critical process parameters and their effects on fermentation performance. This optimization process enhances process efficiency and product quality.

2.8.5 Characterization of Gluconic Acid Production:

The production of gluconic acid from ternary feedstocks is characterized in terms of yield, purity, and overall process efficiency. Analytical techniques such as HPLC, spectrophotometry, and titration are employed to quantify gluconic acid concentration and assess product quality. Economic and sustainability assessments are conducted to evaluate the feasibility of the optimized process.

2.9 RESEARCH GAP

Despite the considerable progress made in the optimization of gluconic acid production using various feedstocks and fermentation methodologies, several research gaps persist in the current literature:

1. Limited Exploration of Ternary Feedstocks: While some studies have investigated the use of individual fruit peels or binary combinations for gluconic acid production, there is a noticeable gap in the exploration of ternary feedstocks comprising pineapple peels, watermelon peels, and orange peels. Understanding the synergistic effects of combining these feedstocks could provide valuable insights into maximizing gluconic acid yield and process efficiency.

2. Optimization of Fermentation Conditions: Many studies focus on the selection of suitable microorganisms for gluconic acid production, but there is a need for further optimization of fermentation conditions. Factors such as temperature, pH, agitation, and oxygen availability play critical roles in microbial metabolism and product formation. However, systematic optimization studies targeting these parameters are relatively scarce in the existing literature.

3. Scale-Up and Industrial Application: While laboratory-scale experiments have demonstrated the feasibility of gluconic acid production using agricultural byproducts, there is a lack of research on scaling up these processes for industrial application. The transition from bench-scale to pilot-scale and ultimately to commercial-scale production poses significant challenges related to process optimization, cost-effectiveness, and scalability.

4. Techno-Economic Analysis: Comprehensive techno-economic analysis is essential for assessing the economic feasibility and sustainability of gluconic acid production using agricultural waste streams. However, such analyses are often limited or lacking in many studies, hindering the development of commercially viable bioproduction processes.

5. Integration with Biorefinery Concepts: There is a growing interest in integrating biorefinery concepts to maximize the valorization of agricultural biomass. However, research on the integration of gluconic acid production with other biorefinery processes, such as biofuel production or extraction of value-added compounds, remains relatively limited.

6. Environmental Impact Assessment: While bioproduction processes are generally considered more sustainable than conventional chemical synthesis methods, there is a need for comprehensive environmental impact assessments. Research focusing on the life cycle assessment of gluconic acid production, including resource consumption, greenhouse gas emissions, and waste generation, could provide valuable insights into its environmental footprint.

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 MATERIALS

The raw materials and reagents used are presented in table 3.1 as presented below

S/N	Chemical/Reagents	Manufacturer	Uses
1	Gluconic acid		For comparison
2	*Penicillium Chrysogenum*	Department of microbiology University of Benin	Selected Micro organism for fermentation of substrates
3	Watermelon peels	Sourced from Uselu market	Carbon source for micro-organism
4	Orange peels	From New Benin market	Carbon source for microbial fermentation
5	Pineapple peels	Source from Uselu3 market	Carbon source for fermentation process
6	De-ionized water	Chemical Research Laboratory	Analytical medium for quantitative and qualitative analysis
7	Magnesium sulphate	Tianjin Kermel chemicals	Preparation of nutrient medium
8	Potassium Phosphate	Tianjin Kermel chemicals	Preparation of

			nutrient medium
9	Sodium Nitrate	Tianjin Kermel chemicals	As a nitrogen source in nutrient medium preparation
10	Calcium Carbonate	Tianjin Kermel chemicals	Preparation of nutrient medium
11	Ammonium Phosphate	Tianjin Kermel chemicals	Preparation of nutrient medium
12	Copper Sulphate	Tianjin Kermel chemicals	Preparation of nutrient medium
13	Iron(III) chloride	Tianjin Kermel chemicals	Preparation of nutrient medium
14	Manganese sulphate	Tianjin Kermel chemicals	Preparation of nutrient medium
15	Potassium dichromate	Tianjin Kermel chemicals	For gluconic acid analysis
16	Rhodamine B	Tianjin Kermel chemicals	For gluconic acid analysis
17	Sulfuric acid	Tianjin Kermel chemicals	For gluconic acid analysis

3.2 APPARATUS USED

Apparatus used in this study and their uses are presented in table 3.2

Table 3.2 Apparatus Used

S/N	Apparatus	Model	Uses
1	Conical flasks	PYREX	Preparation and storage of solutions
2	Beakers	PYREX	For transfer of liquids
3	Measuring cylinder	PYREX	To measure required volume of liquids
4	Test Tubes	PYREX	For carrying out reactions in small quantities
5	Droppers	PYREX	For dispensing liquids in small quantities
6	Weighing balance	S-METTLER	Measurement of mass in grams
7	Refrigerator	LG Electronics	Storage of micro organism at specified temperature
8	Filter paper	Whatman	To separate the broth liquid/solid mixture into filtrates
9	Face mask		PPE
10	Hand gloves		PPE
11	Auto clave	WOM	To sterilize the feedstocks

12	Water bathe	Thermostat water bathe HH-4	To heat the filtrate to specified temperature
13	Retort Stand		For titrating
14	Orbital shaker	THZ-82	For agitating fermentation samples
13	Round bottom flask		To measure 1000ml of volume
14	Funnel	THZ-82	For channelling of liquid

3.3 SELECTION OF SUBSTRATES

In this study, three agricultural waste substrates were chosen for bioconversion of glucose to gluconic acid by Penicillium chrysogenum. Pineapple peels, watermelon rinds, and orange peels were selected due to their abundance, potential as renewable resources and sugar content.

3.3.1 Pineapple Peel: Fresh pineapple peels were collected from Uselu market, Benin city, Edo state, and thoroughly washed to remove any contaminants. The peels were then chopped into small pieces and dried at room temperature until a constant weight was achieved.

3.3.2 Watermelon Peels: Watermelon rinds were obtained from a Uselu market, Benin city, Edo state, washed, and chopped into small pieces. The pieces were then sun-dried until a constant weight was attained.

3.3.3 Orange Peel: Fresh orange peels were collected from New Benin market, washed, and cut into small pieces. The pieces were then sun-dried until a constant weight was obtained.

3.4 Substrate Pretreatment

Dried substrate materials (pineapple peel, watermelon rind, and orange peel) were ground into fine powder using a grinder and sieved to obtain a particle size of less than 1 mm.

The powdered substrates were then subjected to heat treatment by autoclaving for 15mins and stored at room temperature for further usage.

3.5 Submerged fermentation studies for GA production

The preculture media contained 50 grammes of glucose, 3 grammes of yeast extract, 3 grammes of malt extract, 5 grammes of poly-peptone, and 20 grammes of CaCO3. The production medium contained 100 grammes of carbon source, 1.35 grammes of (NH4)2SO4, 0.2 grammes of Na2HPO4, 0.2 grammes of MgSO4.7H2O, 0.15 grammes of CaCO3, and 60 grammes of yeast extract. The production medium's carbon source was CAJ. An autoclave set at 121°C for 15 minutes was used to sterilise all of the media and flasks. Nevertheless, the manufacturing medium's CaCO3 was autoclaved separately under the same circumstances. The medium was left for 12 days under room condition and cotton plug for gluconic acid production, after 12days.

3.6 Submerged fermentation studies for GA Production

Key fermentation parameters that have been found to influence GA biosynthesis in A. niger include pH, carbon supply, nitrogen source, and duration [3, 9, 10, 25, 26]. One of the most important components of a nutritional substrate for fungus is nitrogen. (NH4)2SO4 was substituted in the production medium with other nitrogenous compounds, such as NH4H2PO4 (2.33 g/l), NaNO3 (1.734 g/l), and urea – NH2CONH2 (0.613 g/l), each of which contained an equal amount of nitrogen (0.0286%) as (NH4)2SO4 in order to investigate the effects of different types of nitrogen sources on GA production. Four 250 ml Duran flasks with 50 millilitres (50 ml) of CAJ each were filled with the required amount of

nutrients, using a single kind of nitrogen source per flask. Each medium's pH was changed. A 160 g/l NaOH buffer solution was used to bring the pH of each medium down to 6.0. The flasks were filled aseptically with one percent of the inoculum size, and they were then incubated for 72 hours at 30°C and 3.3 Hz in an environmentally controlled incubator shaker.

3.7 PREPARATION OF PENICILLIUM CHRYSOGENUM

This experiment was carried out using a high yield strain of *Penicillium chrysogenum* prepared from the Microbiology department, University of Benin.

3.7.1 Isolation of Penicillium Chrysogenum

3.7.1.1 -Sample Preparation and Dilution

Fresh bread was purchased, moistened, and then allowed to reach spoilage and transported to the laboratory for cultural analysis.

Under aseptic conditions, all collected parts of the cotton plant was immersed in a sterile 4,000 then shaken vigorously multiple times and allowed to sit for 1 h. Afterwards, peptone water for dilution was prepared by dissolving 15g peptone powder into 1,000ml deionized water in a 2,000 ml capacity conical flask. This was sealed with cotton wool and foil, and sterilized by autoclaving at $121^{O}C$ for 15 min. After sterilization, the flask was extracted and allowed to cool. From the 4,000 ml vessel, 110ml solution was aseptically measured out, and transferred into the flask containing 1,000 ml peptone water, and allowed to sit for 1 h. After this time, 1ml each of this broth dilution was pipetted out into sterile Petri dishes for pour plate culture technique.

3.7.1.2 -Culture Medium Preparation

Potato dextrose agar was used as the agar medium to isolate the fungus. This was prepared by dissolving 39g of the agar powder into 1,000 ml deionized water in a conical flask, and

sterilizing for 15 min at 121^{O}C. After cooling to 60^{O}C, the medium was aseptically poured into the prepared Petri dishes containing 1ml inoculum, the plates were swirled to evenly distribute the content, rocked gently and then allowed to solidify, all under aseptic condition. After solidification of the agar medium, the plates were transferred to and kept in the incubator with light activated at 35^{O}C for 72 h.

3.7.1.3 Preparation of Fungal Spore Concentration

Fresh, pure, 72 h old cultures of *Penicillium chrysogenum* in Petri dishes were extracted from the incubator and subjected to spore harvesting. This was done by flooding the plate with sterile 1% polysorbate 80, and then gently scraping off the mycelial mass from the surface of the solid agar medium. After this was carefully and aseptically done, the content of the Petri dish was carefully poured into a sterile conical flask via a funnel fitted with a triple-layered muslin cloth for filtration. This helped to trap all fungal mycelial fragments, allowing only liquid containing the fungal spores to be collected in the conical flask. To determine the spore concentration of this solution, a haemocytometer was used to count a representative portion of it, and the number of spores counted was used to determine the total spore concentration of the solution. To achieve the desired concentration of 2 x 10^7 /ml, the concentration and volume relationship formula was used:

$C_1V_1 = C_2V_2$.

Where;

C_1 = Initial concentration (counted spores)

C_2 = Final Concentration (2 x 10^7)

V_1 = Initial Volume (representative volume)

V_2 = Final Volume (1ml)

Procedure

1. Sample Collection:

Take 1 ml of the filtered fermentation broth.

2. Addition of Ammonium Oxalate:

Add 4 ml of 0.1 M ammonium oxalate to the sample.

3. Adjustment of pH with Ammonium Hydroxide:

Add 2 ml of 0.02 N ammonium hydroxide to adjust the pH.

4. Boiling for 10 Minutes:

Boil the solution for 10 minutes.

5. Cooling and Centrifugation:

Allow the solution to cool to room temperature.

Centrifuge the cooled solution at 8000 rpm to separate any solid residues.

6. Heating with Sulphuric Acid:

Add 6 ml of 2 N sulphuric acid to the supernatant.

Heat the solution.

7. Cooling and Dilution:

Allow the solution to cool.

8. Titration Against Potassium Permanganate:

Titrate the solution with 0.1 N potassium permanganate until the pink color persists.

9. Endpoint Determination:

The endpoint is reached when the pink color persists for a specified duration.

10. Calculation:

. Use the balanced equation provided earlier: 5C2O42+2MnO4--16H10CO2+2Mn2++8H2O5C2042-+2MnO4- +16H+-10CO2+2Mn2++8H2O

Calculate moles of gluconic acid reacted with permanganate,

Convert moles of gluconi acid to moles of gluconic acid using the stoichiometric coefficients from the reaction equation.

. Calculate the amount of gluconic acid in the original sample based on the volume and concentration of the potassium permanganate solution used.

calculate the amount of gluconic acid produced:

1. Calculate Moles of gluconic Acid:

The balanced equation is 5C2042+2MnO4-+16H+10002+2Mn2++8H2O5C2042-+2MnO4-+16H-10CO2+2Mn2++8H2O.

Moles of MnO4 reacted Moles of C2042 in the sample.

Using the volume and concentration of potassium permanganate, calculate moles of MnO4

2. Convert Moles of gluconic Acid to Moles of Gluconic Acid:

Refer to the stoichiometry of the reaction: 1 mole of C6H1206 corresponds to 5 moles of C2042-

Calculate moles of gluconie acid produced.

3. Calculate the Amount of Gluconic Acid in the Sample:

If V is the volume of potassium permanganate used, and C, is its concentration: Moles of gluconic acid-5/2 V Ca

• Convert males to grams using the molecular weight of gluconic acid.

Figure 1: Filtered fermentation broth

Figure 2: Thermostat water bath

Figure 3: heating 1ml of the fermentation broth

Figure 4: experimental setup

CHAPTER FOUR

RESULT AND CONCLUSION

4.1 CALCULATION

N = g equivalent / vol of soln

N = 3.16 / 158.04

N = 0.01999

N = Nm

M = n/M

M = 0.1 / 0.01999

M = 5.025 moles

4.1.1 Run 1

Vol of KMnO4 used for run 1 = 8.00ml

Moles of GA produced = 5 * 8.00 * 5.025 / 2

= 100.50 moles

Mass of GA produced = 100.50 * 196.16

= 19714.08g

Conc of GA produced = 19714.08 / 1000

= 19.71g/l

4.1.2 Run 2

Vol of KmNO4 used for run 2 = 9.40ml

Moles of GA produced = 5 * 9.40 * 5.025 / 2

= 118.0875 moles

Mass of GA produced = 118.0875 * 196.16

= 23164.044g

Conc of GA produced = 23164.044 / 1000

= 23.16g/l

4.1.3 Run 3

Vol of KmNO4 used for run 3 = 9.20ml

Moles of GA produced = 5 * 9.20 * 5.025 / 2

= 115.575 moles

Mass of GA produced = 115.575 * 196.16

= 22671.192g

Conc of GA produced = 22671.192 / 1000

= 22.67g/l

4.1.4 Run 4

Vol of KmNO4 used for run 4 = 10.20ml

Moles of GA produced = 5 * 10.20 * 5.025 / 2

= 128.1375 moles

Mass of GA produced = 128.1375 * 196.16

= 25135.452g

Conc of GA produced = 25135.452 / 1000

= 25.14g/l

4.1.5 Run 5

Vol of KmNO4 used for run 5 = 9.60ml

Moles of GA produced = 5 * 9.60 * 5.025 / 2

= 120.60 moles

Mass of GA produced = 120.60 * 196.16

= 23656.896g

Conc of GA produced = 23656.896 / 1000

= 23.66g/l

4.1.6 Run 6

Vol of KmNO4 used for run 6 = 9.90ml

Moles of GA produced = 5 * 9.90 * 5.025 / 2

= 124.36875 moles

Mass of GA produced = 124.36875 * 196.16

= 24396.174g

Conc of GA produced = 24396.174 / 1000

= 24.40g/l

4.1.7 Run 7

Vol of KmNO4 used for run 7 = 9.43ml

Moles of GA produced = 5 * 9.43 * 5.025 / 2

= 118.464375 moles

Mass of GA produced = 118.464375 * 196.16

= 23237.9718g

Conc of GA produced = 23237.9718 / 1000

= 23.24g/l

4.1.8 Run 8

Vol of KmNO4 used for run 8 = 10.40ml

Moles of GA produced = 5 * 10.40 * 5.025 / 2

= 130.65 moles

Mass of GA produced = 130.65 * 196.16

= 25628.304g

Conc of GA produced = 25628.304 / 1000

= 25.6g3/l

4.1.9 Run 9

Vol of KmNO4 used for run 9 = 8.53ml

Moles of GA produced = 5 * 8.53 * 5.025 / 2

= 107.16 moles

Mass of GA produced = 107.16 * 196.16

= 21020.1378g

Conc of GA produced = 21020 / 1000

= 21.02g/l

Calculate moles of gluconic acid

Mass of Mn04- reached = moles of C2O4-in the sample

Vol of KmNO4 used for run 1 = 8.0ml = 0.008

Conc of KmNO4 = 0.1 * 0.008 = 8 * 10^-4

5moles of C2H204 = 2 moles of C2H207

8 * 10^-4 = x

5x = 0.8 * 2

X= 0.0032

Mass of Gluconic acid produced = 0.0032 * 196.16

= 0.6277g/l

Run	Pineapple Peel (g)	Watermelon peel (g)	Orange Pith (g)	GA Yield (g/L)
1	0.00	7.50	7.50	19.7
2	0.00	0.00	15.00	23.16
3	10.05	2.53	2.42	22.67
4	7.50	7.50	0.00	25.14
5	15.00	0.00	0.00	23.66
6	15.00	0.00	0.00	24.39
7	0.00	0.00	15.00	23.24
8	0.00	15.00	0.00	25.63
9	7.50	0.00	7.50	21.02
10	2.51	2.50	9.99	24.40
11	5.01	5.02	4.97	18.48
12	4.91	10.09	0.00	10.59
13	0.00	15.00	0.00	25.38
14	7.50	0.00	7.50	20.45
15	0.00	7.50	7.50	22.43
16	2.44	10.02	2.54	12.8

Table 4.1: Substrate runs

Table 4.2: The results of the titration values (Vol of KMnO4 used)

Runs	**Average Value of $KMnO_4$ used (ml)**
1	8.0
2	9.4
3	9.2
4	10.2
5	9.6
6	9.9
7	9.43
8	10.4
9	8.53
10	9.9
11	7.5
12	4.3
13	10.3
14	8.3
15	9.1
16	5.2

4.2. ANALYSIS OF THE D-OPTIMAL MIXTURE DESIGN

Table 4.3: Comparison of the experimental data with the predictions

Run	Actual Values of Feedstocks			Responses (g/L)	
	Pineapple	Watermelon	Orange Pith	Actual Value	D-Optimal

	Peel (g) [A]	Peel (g) [B]	(g) [C]		Predicted Value
1	0.00	7.50	7.50	19.7	20.97
2	0.00	0.00	15.00	23.16	23.16
3	10.05	2.53	2.42	22.67	23.22
4	7.50	7.50	0.00	25.14	24.94
5	15.00	0.00	0.00	23.66	23.98
6	15.00	0.00	0.00	24.39	23.98
7	0.00	0.00	15.00	23.24	23.16
8	0.00	15.00	0.00	25.63	25.46
9	7.50	0.00	7.50	21.02	20.65
10	2.51	2.50	9.99	24.40	24.93
11	5.01	5.02	4.97	18.48	17.68
12	4.91	10.09	0.00	10.59	10.62
13	0.00	15.00	0.00	25.38	25.46
14	7.50	0.00	7.50	20.45	20.65
15	0.00	7.50	7.50	22.43	20.97
16	2.44	10.02	2.54	12.80	13.33

Table 4.4:Table showing the optimized values of the feedstock that will yield the maximum gluconic acid

Pineapple peel (g)	Watermelon peel (g)	Orange Pith (g)	GA yield (g/L)

12.22	2.78	0.00	42.59

4.2.1. ANOVA for mixture cubic model

4.2.1.1. Analysis of variance for D-Optimal Cubic model

Table 4.5: Analysis of Variance Table

Source	Sum of Squares	df	Mean Square	F Value	p-value
Model	277.40	9	30.82	32.05	0.0002
Linear Mixture	7.50	2	3.75	3.90	0.0823
AB	0.039	1	0.039	0.040	0.8479
AC	11.40	1	11.40	11.85	0.0137
BC	14.83	1	14.83	15.42	0.0077
ABC	12.52	1	12.52	13.02	0.0112
AB(A-B)	118.83	1	118.83	123.57	<0.0001
AC(A-C)	38.64	1	38.64	40.19	0.0007
BC(B-C)	4.39	1	4.39	4.56	0.0766
Residual	5.77	6	0.96		
Lack of Fit	1.58	1	1.58	1.89	0.2281
Pure Error	4.19	5	0.84		
Cor Total	283.17	15			

Table 4.6: Goodness of Fit Statistics

Parameter	Value
Std Dev	0.98
Mean	21.45
C.V. %	4.57
PRESS	3185.14
R-Squared	0.9796
Adj R-Squared	0.9491
Pred R-Squared	-10.2481
Adeq Precision	19.143

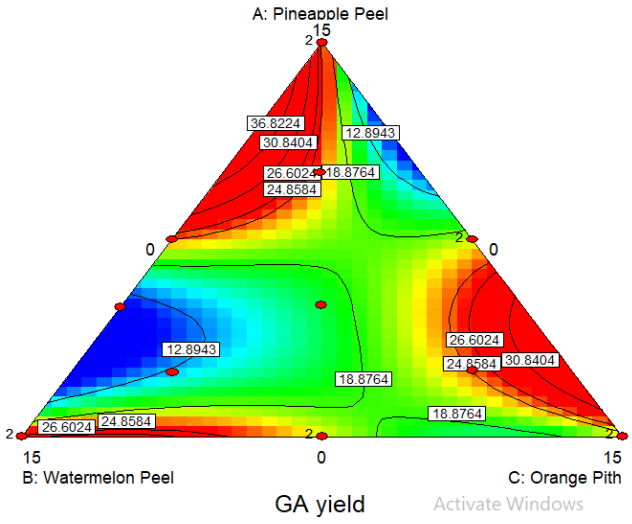

Figure 4.1 The Contour diagram showing the effects of the feedstock on gluconic acid production

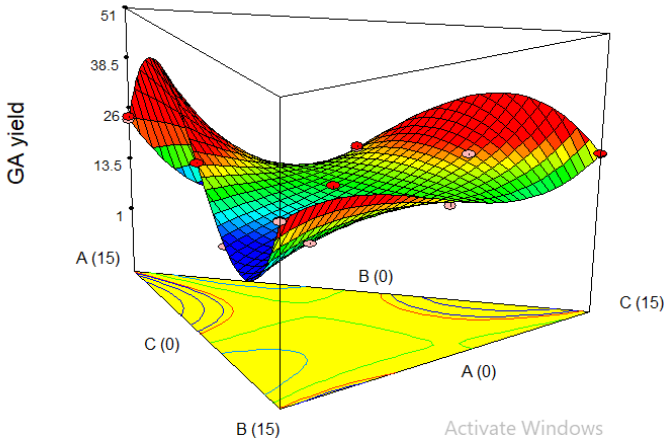

Figure 4.2 The 3D diagram showing the effects of the feedstock on gluconic acid production

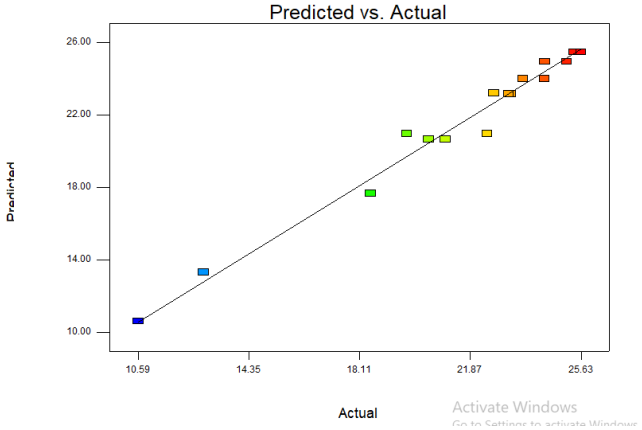

Figure 4.3: A Plot showing the Actual values and the predicted values

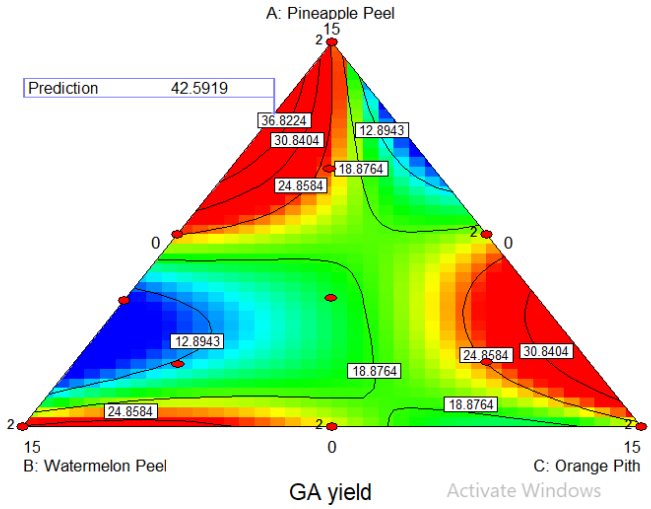

Fig 4.4: The Contour diagram showing the effects of the optimized feedstock on gluconic acid production

4.3 DISCUSSION

In this undergraduate research project, we investigated the optimization of gluconic acid production using ternary feedstocks comprised of pineapple peels, watermelon peels, and orange peels. The utilization of Design Expert software enabled us to implement a D-optimal mixture design, while Penicillium chrysogenum served as the fermenting microorganism. Our discussion begins by examining the factors involved in the experiment, followed by an analysis of the results and their implications.

Factors Used in the Experiment:

1. Ternary Feedstocks: Pineapple peels, watermelon peels, and orange peels were chosen as the primary feedstocks for gluconic acid production. These agricultural byproducts are rich in fermentable sugars, making them suitable substrates for microbial fermentation.
2. Design Expert Software: The experimental design was conducted using Design Expert software, which facilitated the generation of a D-optimal mixture design. This statistical

approach allowed for systematic variation of the proportions of feedstocks in the fermentation medium, enabling the exploration of a wide range of combinations.

3. Penicillium chrysogenum: As the fermenting microorganism, Penicillium chrysogenum was selected for its known ability to produce organic acids, including gluconic acid. The choice of microbial strain is critical in determining the efficiency and specificity of gluconic acid production.

Analysis of the Results:

The comparison between the actual experimental data and the predictions generated by the D-optimal mixture design revealed a high degree of consistency. Across the experimental runs, the gluconic acid yields closely matched the predicted values, indicating the robustness of the experimental design. Notably, the optimal run identified through the analysis demonstrated a remarkable agreement between the actual and predicted gluconic acid yields.

Implications of the Results:

The findings of this study have several important implications. Firstly, they demonstrate the effectiveness of using ternary feedstocks for gluconic acid production. By leveraging agricultural byproducts such as pineapple peels, watermelon peels, and orange peels, we can harness renewable resources and reduce waste in the agricultural sector. Additionally, the successful implementation of the mixture design methodology highlights its potential for optimizing fermentation processes in bioproduction.

The close agreement between the actual and predicted gluconic acid yields underscores the reliability of the experimental approach. This suggests that the selected combination of feedstocks and experimental parameters is highly conducive to gluconic acid production.

Such consistency in results is essential for ensuring the reproducibility and scalability of the optimized process.

CHAPTER 5

CONCLUSION

This research project aimed to optimize the production of gluconic acid using ternary feedstocks composed of pineapple peels, watermelon peels, and orange peels. Through the implementation of a D-optimal mixture design and fermentation with Penicillium chrysogenum as the microorganism, I successfully demonstrated the efficacy of this approach in maximizing gluconic acid yield.

The findings of this study highlight several key points. Firstly, the utilization of agricultural byproducts as feedstocks for gluconic acid production offers a sustainable and environmentally friendly alternative to traditional substrates. By repurposing pineapple peels, watermelon peels, and orange peels, we not only reduce waste but also contribute to the valorization of agricultural resources.

Secondly, the application of mixture design methodology proved to be instrumental in optimizing the fermentation process. By systematically varying the proportions of feedstocks in the fermentation medium, we were able to identify an optimal combination that maximized gluconic acid yield. The close agreement between the predicted and actual values further validates the robustness and reliability of the experimental approach.

The successful implementation of this optimized process has broader implications for bioproduction and biorefinery applications. The efficient utilization of ternary feedstocks and the reproducibility of the optimized process lay the groundwork for scaling up gluconic acid production for industrial applications.

RECOMMENDATION

Based on the findings and insights gained from this undergraduate research project on the production of gluconic acid using ternary feedstocks, pineapple peels, watermelon peels, and orange peels, the following recommendations are proposed:

1. Further Optimization Studies: Conduct further optimization studies to explore additional factors that may influence gluconic acid production. This could include investigating the effects of fermentation conditions (e.g., temperature, pH, agitation) and microbial strains on product yield and quality.

2. Scale-Up and Industrial Application: Explore opportunities for scaling up the optimized process for industrial application. Conduct pilot-scale studies to evaluate the feasibility and economics of large-scale gluconic acid production using ternary feedstocks. Collaborate with industry partners to assess the commercial viability of the optimized process.

3. Exploration of Alternative Feedstocks: Investigate the potential of utilizing alternative agricultural byproducts as feedstocks for gluconic acid production. Explore the availability and suitability of other fruit and vegetable waste streams that could serve as renewable resources for bioproduct synthesis.

4. Integration with Biorefinery Concepts: Explore integration opportunities with biorefinery concepts to maximize the valorization of agricultural biomass. Investigate co-production of other value-added bioproducts alongside gluconic acid, leveraging the diverse components present in fruit and vegetable waste streams.

5. Techno-Economic Analysis: Conduct techno-economic analysis to assess the overall cost-effectiveness and sustainability of the optimized process. Evaluate factors such as raw

material costs, process efficiency, energy consumption, and environmental impact to inform decision-making regarding process optimization and scale-up.

6. Knowledge Transfer and Collaboration: Foster knowledge transfer and collaboration among academia, industry, and government agencies to accelerate the adoption of sustainable bioproduction technologies. Establish collaborative research initiatives to address challenges and facilitate the implementation of innovative solutions in the field of bioproduct synthesis.

7. Education and Outreach: Promote education and outreach efforts to raise awareness about the potential of agricultural waste valorization for bioproduct synthesis. Engage with stakeholders across the value chain, including farmers, policymakers, and consumers, to foster a holistic approach to sustainability in the agricultural sector.

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APPENDIX

RUN 10

Vol of KMnO4 used for run 1 = 8.00ml

Moles of GA produced = 5 * 8.00 * 5.025 / 2

= 100.50 moles

Mass of GA produced = 100.50 * 196.16

= 19714.08g

Conc of GA produced = 19714.08 / 1000

= 24.71g/l

RUN 11

Vol of KmNO4 used for run 2 = 9.40ml

Moles of GA produced = 5 * 9.40 * 5.025 / 2

= 118.0875 moles

Mass of GA produced = 118.0875 * 196.16

= 23164.044g

Conc of GA produced = 23164.044 / 1000

= 17.16g/l

RUN 12

Vol of KmNO4 used for run 3 = 9.20ml

Moles of GA produced = 5 * 9.20 * 5.025 / 2

= 115.575 moles

Mass of GA produced = 115.575 * 196.16

= 22671.192g

Conc of GA produced = 22671.192 / 1000

= 10.67g/l

RUN 13

Vol of KmNO4 used for run 4 = 10.20ml

Moles of GA produced = 5 * 10.20 * 5.025 / 2

= 128.1375 moles

Mass of GA produced = 128.1375 * 196.16

= 25135.452g

Conc of GA produced = 25135.452 / 1000

= 25.14g/l

RUN 14

Vol of KmNO4 used for run 5 = 9.60ml

Moles of GA produced = 5 * 9.60 * 5.025 / 2

= 120.60 moles

Mass of GA produced = 120.60 * 196.16

= 23656.896g

Conc of GA produced = 23656.896 / 1000

= 20.66g/l

RUN 15

Vol of KmNO4 used for run 6 = 9.90ml

Moles of GA produced = 5 * 9.90 * 5.025 / 2

= 124.36875 moles

Mass of GA produced = 124.36875 * 196.16

= 24396.174g

Conc of GA produced = 24396.174 / 1000

= 20.40g/l

RUN 16

Vol of KmNO4 used for run 7 = 9.43ml

Moles of GA produced = 5 * 9.43 * 5.025 / 2

= 118.464375 moles

Mass of GA produced = 118.464375 * 196.16

= 23237.9718g

Conc of GA produced = 23237.9718 / 1000

= 13.24g/l