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METABOLIC ENGINEERING OF CITRIC ACID-PRODUCING MICROORGANISMS

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ABSTRACT

The pharmaceutical, food and beverage, beverage, cleaning product, and cosmetics sectors all make extensive use of microbial citric acid, which is a significant organic acid. The filamentous fungus Aspergillus is a workhorse when it comes to the manufacture of citric acid, despite the fact that citric acid may be generated by a variety of other types of microbe. In the current review, special attention has been paid to address the advanced literature of citric acid production. Topics such as microorganisms, substrates, screening methods, various fermentation techniques, various factors affecting citric acid production, and product recovery, as well as numerous biotechnological applications of citric acid are also discussed for simple understanding.

Keywords:*Citric Acid, Genetic Engineering, Metabolism, Microorganism*

INTRODUCTION

Citric acid, also known as CA, is a kind of organic acid that may be found naturally in many different fruits, including limes, lemons, oranges, pineapples, and grapefruits, among others. It is a natural element that helps in cleansing, maintaining energy levels, and promoting good digestion and renal function. These benefits come from the fact that it aids in digesting. It has a flavor that is somewhat acidic and refreshing, and it is used in beverages like soft drinks, juices, and other types of beverages in order to balance off the sweetness. Citric acid, which is used in the food and beverage (F&B) business as a preservative due to the antioxidant capabilities it possesses, or as an acidifier, which improves the tastes and fragrances of fruit juices, ice cream, and marmalades, is one of the most widely used acids. In the pharmaceutical sector, it is used as an antioxidant to preserve vitamins, as an effervescent, as a pH corrector, as a blood preservative, as a supply of iron for the body in the form of iron citrate tablets, as a component in various ointments and cosmetic preparations, and so on. As a foaming agent, it finds application in the chemical sector, where it is utilized for the treatment and softening of textiles. In the field of metallurgy, several metals are often put to use in the citrate form. The detergent industry uses CA as a phosphate alternative since it has less of an effect on eutrophication than phosphate does.

PROPERTIES

The citric acid (CA), also known by its scientific name of 2-hydroxypropane-1,2,3- tricarboxylic acid according to the International Union of Pure and Applied Chemistry (IUPAC), is an intermediary in the citric acid cycle that occurs in the metabolism of all aerobic organisms. CA is a white crystalline powder that is almost odorless and can exist in either its anhydrous or its monohydrate form. CA has no discernible color. Monohydrate CA is only soluble in water, but anhydrous CA is highly soluble in water, freely soluble in ethanol, and sparingly soluble in ether. However, anhydrous CA is more soluble in ether than monohydrate CA is. It has a solid state at room temperature, melts at 153 degrees Celsius, and has a boiling point of 310 degrees Celsius. Above

roughly 175 degrees Celsius, it will begin to breakdown, resulting in the release of carbon dioxide (CO2). As soon as it is dissolved in water, it demonstrates a mild acidity but a strong acid taste. This strong acid taste impacts sweetness and produces a fruity tartness, which is why it is commonly utilized in the food and beverage sector to compliment fruit flavors. The acid demonstrates excellent buffering capacity when combined with citrate, while its excellent metal ions chelating properties add to the list of physicochemical properties that make it ideal for applications in the food, cosmetic, nutraceutical, and pharmaceutical industries. The sheer number of these applications is a testament to the acid's extraordinary versatility.

MICROORGANISMS KNOWN TO PRODUCE CA

As demonstrated in, several kinds of microbes, including bacteria, fungus, and yeast, have been implicated in the biosynthesis of CA, and it is known that these species may generate significant quantities of CA. In addition to bacteria, some yeasts such Candida, Hansenula, Pichia, Debaromyces, Torula, Torulopsis, Kloek era, Saccharomyces, Zygosaccharomyces, and Yarrowia are also capable of producing CA. In spite of the fact that there have been instances of yeast being able to create CA, one disadvantage of yeast fermentation is that it results in the generation of significant quantities of iso-citric acid (isoCA), which is an undesirable by-product. Aspergillus. niger, A. awamori, A. clavatus, A. nidulans, A. fonsecaeus, A. luchensis, A. phoenicus, A. wentii, A. saitoi, A. flavus, Absidia sp., Acremonium sp., Botrytis sp., Eupenicillium sp.

Improvement of strains for CA production

As indicated in Figure 1, the process of improving microorganisms that are significant to industry involves the use of a number of different techniques. These techniques include gene cloning, protoplast fusion, mutations, and recombinant DNA technology. Among them, random mutagenesis and protoplast fusion are the methods that are easiest to perform and most often utilized. In order to enhance the strain, the mutagenic procedures entail both physical and chemical mutagenesis in addition to site-directed mutagenesis. During the process of commercial fermentation, an increase in the production of industrial goods by means of strain enhancement has been taken into consideration. Altering the metabolic processes of the microorganism and then causing mutations in that microbe via either physical or chemical means can also contribute to the improvement of a strain. mutagenic agents Gamma and ultraviolet (UV) radiations are the types of mutagens that are utilized the most frequently among physical mutagens. Diethyl sulfonate, N-methyl-N-nitroso-guanidine, ethidium bromide aziridine, N-nitroso-N-methyl urea, and ethyl methane-sulfate are some of the most prevalent chemical mutagens. Other examples include ethidium bromide aziridine. Genetic modification of A. niger with regard to CA production by protoplast fusion was published. Pontecorvo was the first person to explain the process of strain improvement during the parasexual cycle. Diploids demonstrated increased CA yields in comparison to their parent haploids.

SUBSTRATES AND PRETREATMENT

In fermentation settings, the substrate plays an extremely important role in both lowering costs and achieving optimal yields. As a result, substrate plays a more significant role in terms of both productivity and fermentation output. The purity of the substrate utilized has a direct bearing on increases in yield as well as reductions in the amount of time needed for fermentation. Molasses, sucrose, syrups made from beet or cane sugar, hydrol produced as a by-product during the manufacturing of crystalline glucose, and palm oil are all fermented by Aspergillus niger for the formation of CA. Pretreatment of the substrate that will be utilized in CA fermentation

is necessary in order to remove trace metals. Cane molasses, which are utilized in fermentation, have been shown to include calcium, magnesium, manganese, iron, and zinc, all of which have an impact on the synthesis of CA similar to that of a retardant. It is common practice to apply potassium ferrocyanide to the substrate in order to perform a chemical pretreatment prior to fermentation. This pretreatment can successfully precipitate zinc and iron. In order to bring the concentration of heavy metals down below the necessary limits for formation of CA, enzymatic hydrolysis was also used as a pretreatment process for starch-containing materials including corn, wheat, and potatoes. In the manufacture of CA, agricultural by-products and trash are utilized in order to bring down the overall cost of manufacturing. Coffee husk, rice bran, wheat bran, carrot waste, cassava bagasse, banana peel, vegetable wastes, sugarcane bagasse, tapioca, cheese whey, rice straw, coconut husk, brewery wastes, decaying fruits, corn cob, orange peel, kiwifruit peel, pineapple peel, pomaces of grapes, and apples are some of the agricultural residues that are commonly used.

BIOCHEMICAL ASPECT OF CA PRODUCTION

During the process of glycolysis, pyruvate is generated, which is then oxidized and mixed with coenzyme A to produce carbon dioxide (CO2), acetyl coenzyme A (Acetyl-CoA), and nicotinamide adenine dinucleotide (NAD) plus hydrogen (H) (NADH). After this step, the acetyl-co-A that was produced is then reacted with the oxaloacetate to generate citrate (Figure 2). In addition, the pyruvate that is created as a byproduct of glycolysis is capable of being carboxylated by pyruvate carboxylase (PC), which results in the formation of oxaloacetate. The CA that is generated as a byproduct of the interaction between acetyl-co-A and oxaloacetate is then subjected to a series of reactions that, in addition to producing two molecules of carbon dioxide, also re-generate the four-carbon oxaloacetate. At each stage of the cycle, one molecule of acetic acid is introduced, two molecules of adenosine triphosphate (ATP), and one molecule of carbon dioxide are produced, and one molecule of oxaloacetate is metabolized in order to produce citrate. During the process of CA production, enzymes play a very important role. A. niger uses the pentose phosphate and glycolytic pathways to metabolize glucose and prevent the formation of CA. These pathways serve as a channel. citrate synthase has been identified as the enzyme that is responsible for the reversible catalysis that occurs between acetyl-CoA and oxaloacetate, which is beneficial to the formation of citrate.

FACTORS AFFECTING CA PRODUCTION

During the process of glycolysis, pyruvate is generated, which is then oxidized and mixed with coenzyme A to produce carbon dioxide (CO2), acetyl coenzyme A (Acetyl-CoA), and nicotinamide adenine dinucleotide (NAD) plus hydrogen (H) (NADH). After this step, the acetyl-co-A that was produced is then reacted with the oxaloacetate to generate citrate (Figure 2). In addition, the pyruvate that is created as a byproduct of glycolysis is capable of being carboxylated by pyruvate carboxylase (PC), which results in the formation of oxaloacetate. The CA that is generated as a byproduct of the interaction between acetyl-co-A and oxaloacetate is then subjected to a series of reactions that, in addition to producing two molecules of carbon dioxide, also re-generate the four-carbon oxaloacetate. At each stage of the cycle, one molecule of acetic acid is introduced, two molecules of adenosine triphosphate (ATP), and one molecule of carbon dioxide are produced, and one molecule of oxaloacetate is metabolized in order to produce citrate. During the process of CA production, enzymes play a very important role. A. niger uses the pentose phosphate and glycolytic pathways to metabolize glucose and prevent the formation of CA. These pathways serve as a channel. citrate synthase has been identified as the

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METABOLIC ENGINEERING OF CA PRODUCTION

Increasing the synthesis of CA has been attempted using a number of different metabolic engineering strategies, which involve making changes to the genes and metabolic pathways. Systems metabolic engineering is an essential method that may be used to design and introduce in A. niger a whole new biochemical pathway in order to increase CA synthesis. Deletion of gene acl1 in A. niger that is responsible for ATP-citrate lyase synthesis and could increase the CA production in A. niger was reported found that with deletion of two cytosolic ATP citrate lyase (ACL) subunits (Acl1 and Acl2), not only CA production in A. niger decreased but also inhibits vegetative growth, pigmentation, conidial germination, and subsequently asexual development. The heterogeneous malate dehydrogenase (mdh2), fumarase (FumR), and fumarate reductase that were inserted into the cytosolic reductive TCA reverse tricarboxylic acid cycle (rTCA) were used to construct the rTCA cycle. It has been shown that the mdh2 overexpressing strain is capable of speeding up the generation of CA at the beginning of the process. Overexpression of cytosolic FumR converted fumarate to malate, which in turn increased the amount of substrate available to the mitochondrial malate-citrate antiporter. This resulted in an increase in CA secretion and productivity. Overexpression of Frds1 converted fumarate to succinate, which is also a potential substrate for the mitochondrial CA antiporter.

OBJEACTIVES

1. The Study Metabolic Engineering of Citric Acid-Producing Microorganisms.
2. The Study Aspergillus Is a Workhorse for The Production of Citric Acid.

RESEARCH METHODOLOGY

Rice husk substrate preparation

The use of distilled water allowed for the removal of any contaminants that were present in the rice husks that needed to be cleaned. After that, the rice husks were placed in an oven set to 55 degrees Celsius for 24 hours in order to dry them out and make the refining process easier. A mechanical pretreatment was carried out, in which dry rice husks were crushed with the assistance of a grinder that had a rotor speed of 1500 rpm and a sieve with a 0.5 mm opening. Lignin was eliminated with the application of a chemical preparation that consisted of 1% NaOH and 1% H2SO4. The technique for the pretreatment was carried out in accordance with the protocols described in Wei's research. Following the addition of rice husk powder and 1% NaOH at a ratio of 1:10, the mixture was heated in a shaker water bath to 85 degrees Celsius for one hour. After the sample had been filtered and rinsed with distilled water until a pH of 7 was achieved, it was placed in an oven preheated to 105 degrees Celsius to be dried. Following the completion of an alkaline pretreatment, the rice husk powder was subjected to an acid pretreatment using 1% H2SO4 at a ratio of 1:2.8 before being heated in an autoclave at 121 degrees Celsius for a period of 75 minutes. In addition, the solid phase was removed from the solution and rinsed with distilled water on many occasions until the pH level approached neutral before the hydrolysis step could begin. After the treatment was completed, enzymatic hydrolysis was done.

During the pretreatment, rice husk slurry was combined with aquadest at a volume ratio of 1:5, with rice husk slurry being the larger component of the mixture. Following the addition of 10% H2SO4 to the mixture on a drop-by-drop basis as a buffer until a pH of 5.4 was achieved, the mixture was baked in an oven set to 100 degrees Celsius for thirty-five minutes. After bringing the mixture down to room temperature, an addition of cellulase enzyme (five milliliters of enzyme for every fifty grams of biomass) was made. The mixture was swirled and kept at 45 degrees Celsius for 24 hours while being agitated at 150 revolutions per minute (rpm). By heating the mixture in an autoclave to 121 degrees Fahrenheit, the enzyme activity was halted. The outcomes of the hydrolysis were included into the fermentation process in a straightforward manner. It took 2 grams of cellulase enzymes, 7.3 milliliters of 2 millimeters hydrogen chloride, 0.02 grams of sodium nitrate, 0.143 milliliters of glacial acetic acid, 1 gram of sodium hydroxide, and 85 milliliters of distilled water to produce 100 milliliters of cellulase enzyme. The manufacture of the cellulase was carried out in accordance with the Megazyme endo-1,4--glucanase (cellulase) test technique (T-CAF1000 08/13).

Aspergillus niger preparation

was revitalized by being grown on sterile Potato Dextrose Agar (PDA) medium for three days while being kept in an incubator at 37 degrees Celsius. At a temperature of 37 degrees Celsius, up to 12 inoculation loops of an A. niger culture were allowed to grow on a medium consisting of 400 milliliters of yeast peptone glycerol (YPG). The length of time spent in incubation was determined by the amount of time that had elapsed since the cells entered the exponential phase of their development, which was determined to be 36 hours with an absorbance of 0.39 at a wavelength of 600 nm and a cell concentration of 27.5 g/mL.

Glucose standard curve preparation

In order to create seven different concentrations of the solution, a glucose stock solution that had an initial concentration of 10 mg/mL was diluted using distilled water. Following the addition of 3 mL of DNS solutions (3.5-dinitrosalicylic acid) and incubation at 100 °C for 5 minutes, a total of 5 mL of each standard solution was incubated at 50 °C for 10 minutes. After this, the temperature was increased to 100 °C. After the combination was allowed to reach room temperature, an absorbance reading was taken using a UV-VIS spectrophotometer at a wavelength of 540 nm. The results were recorded. The data on concentration and absorbance were used to formulate a linear regression equation, which reads as follows: $y = 0.2736x – 0.2829$.

Citric acid standard curve preparation

In order to create eight different concentrations of the solution, the stock solution of citric acid, which had a concentration of 10 mg/mL, was diluted with distilled water. The pyridine-acetic anhydride technique was utilized in the taking of the measurements. After adding a total of 3 mL of each standard solution and homogenizing the mixture using a vortex, 1.3 mL of pyridine was added, and after that, 5.7 mL of anhydrous acetate was added, and the mixture was homogenized once more. After incubating the mixture for thirty minutes at a temperature of 32 degrees Celsius, the desired yellow hue was achieved. The absorbance was determined to be 407 nm when the measurement was taken. The linear regression equation that was utilized in this research was derived from an earlier study that was carried out and can be expressed as $y = 0.0024x - 0.0014$.

Preparation of fermentation media

The working volume that was utilized for this investigation was 2 liters, and its components included NH4NO3 (0.25% weight-per-volume), KH2PO4 (0.1% weight-per-volume), MgSO4 (0.025% weight-per-volume), CuSO4.5H2O (200 ppm), rice husk substrate (22.42% weight-per-volume), sucrose (10% weight-per-volume), and methanol (3% volume-per-volume). The produced fermentation media was sterilized in an autoclave at 121 degrees Celsius for two hours, while the methanol was sterilized by the process of microfiltration using a sterile membrane that had pores measuring 0.1 microns.

Submerged fermentation

In the fermentation medium, an amount of up to 5% v/v of A. niger inoculum was added. The mixture was then agitated at 400 rpm and incubated at 37 degrees Celsius until the optimal pH for the formation of citric acid was about 2.0 0.2. The fermentation procedure was performed in triples, and samples were examined once every twenty-four hours.

Ion exchange chromatography

At the conclusion of the fermentation process, the citric acid was subjected to a process of clarification. After fermentation, the samples were centrifuged at 3000 rpm for 20 minutes to separate the supernatant from the biomass particles, and then the supernatant was collected for further purification. The chromatographic process was carried out in the same manner as the technique carried out by Van den Bergh. In order to achieve equilibrium, a total of 6.94 grams of poly (4-vinyl pyridine) resin was dissolved in a volume of 50 milliliters of the supernatant and agitated for a period of two hours at room temperature. In order to separate the supernatant from the adsorbent, the sample was centrifuged for 15 minutes at a speed of 4500 revolutions per minute. The supernatant was thrown away, and the adsorbent pellet was allowed to dry at 25 degrees Celsius for 12 hours. After adding 50 mL of ethanol with a concentration of 70%, the mixture was agitated at a speed of 400 revolutions per minute for two hours. After that, it was centrifuged at a speed of 4500 revolutions per minute for fifteen minutes in order to separate the eluent from the adsorbent. A rotary evaporator operating at a temperature of sixty degrees Celsius was utilized in the process of separating ethanol from the eluent.

Sample analysis

During the fermentation process, samples were obtained at regular intervals of one day in order to determine the pH level at which citric acid synthesis is most efficient. At each of the three times, a sample of up to 20 milliliters was obtained.

Aspergillus niger biomass concentration

The absorbance of fermented samples as much as 3 milliliters in volume was measured at 600 nanometers. The fermentation media utilized as blanks were those that did not have any biomass added to them. The absorbance value was plugged into the linear regression equation of the A. niger standard curve that was derived from the earlier research: $y = 0.6117x – 0.4937$.

pH analysis

In order to get the supernatant, the sample was centrifuged for twenty minutes at a speed of three thousand revolutions per minute. A pH meter was used to determine the value of the pH in 3 milliliters of the supernatant.

Reducing sugar concentration

Using the DNS approach, a total of 3 milliliters of the supernatant were examined, and the absorbance values were inserted into the linear regression equation of the glucose standard curve.

Citric acid concentration

The pyridine-acetic anhydride technique was used to conduct an analysis on a total of 3 milliliters (mL) of supernatant and 3 milliliters (mL) of purified samples. The absorbance values were then replaced into the linear regression equation of the citric acid standard curve.

DATA ANALYSIS

Citric acid production

It took 18 days of submerged fermentation by A. niger utilizing rice husk as a substrate to make citric acid. Once it achieved the optimal pH for citric acid synthesis, the fermentation process was considered complete. The fermentation procedure was repeated a total of three times, and the capacity of the working space was raised to two liters. During the fermentation of citric acid, the profile of citric acid concentration and the drop in pH that can be seen in Figure 1 are depicted. On the last day of observation, the citric acid production reached its greatest concentration of 148,639 g/L, as shown by the graph, which indicates that the production of citric acid continued to rise until that point. The pH value fell during the duration of the fermentation process, going from 4.86 on the first day of fermentation to 1.90 on the final day of observation. This change occurred from a starting pH of 4.86.

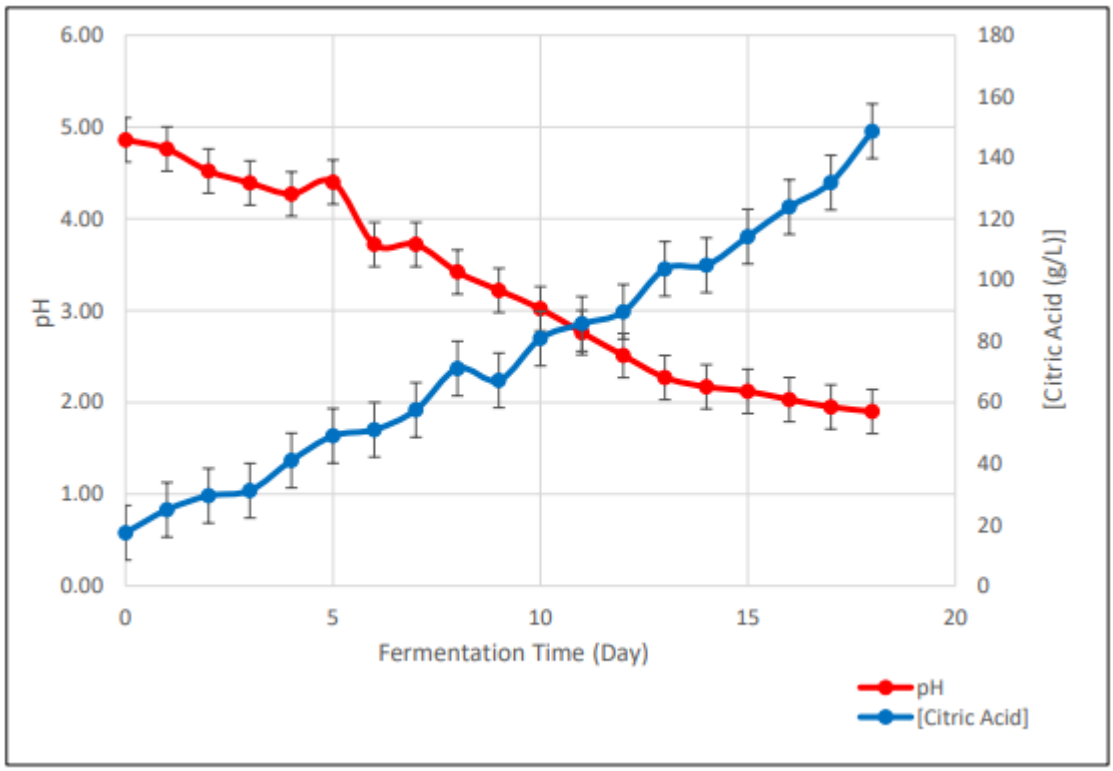

Figure 1. Profile of citric acid concentration and pH value during fermentation.

According to Max and colleagues, the ideal pH range for the synthesis of citric acid is 2.0 with a margin of error of 0.2. In this particular investigation, the rate of decreasing pH was 0.0588 per day, whereas the generation rate of citric acid was 0.1067 grams per day. Based on these findings, it is possible to make an educated guess that the concentration of citric acid can still rise even if the fermentation is allowed to go until it reaches a pH of 1.8. According to Show et al., in the fermentation medium, there is a variation in pH during the generation of citric acid due to the metabolic activity of A. niger. This is because organic acids such as citric acid, gluconic acid, and oxalic acid will be formed. Additionally, the pH of the medium will fluctuate as a result. Because it is tied to the development phase of A. niger, namely the germination phase and the production phase, the pH of the fermentation medium is a crucial component in the production of citric acid. This is due to the fact that it is a factor in the creation of citric acid. The germination phase begins when the pH is about 5, and the spores are in this phase. A lower pH is the consequence of spores in the germination phase releasing protons (H+) after they have absorbed nitrogen from trace elements in the form of ammonium nitrate (NH4NO3). This process causes the pH to fall. At a pH of around 2.0 0.2, the morphology of A. niger is most conducive to the production of citric acid, which is characterized by the creation of tiny aggregates that are composed of short filaments.

When the pH level is just right, the generation of citric acid will prevent the development of by-products such gluconic acid and oxalic acid. According to this research, the rate of citric acid synthesis in the optimal pH range of 1.8-2.2 was 0.1977 g/day, but the rate of citric acid production in the higher pH range of 2.2-4.8 was only 0.0735 g/day. The ideal pH range for citric acid production is between 1.8 and 2.2. The possibility of contamination by other bacteria was reduced to a negligible level, which made the process of purifying citric acid significantly easier. Kusuma demonstrated that pH had an effect on the shape of A. niger as well as its productivity to manufacture citric acid. At a pH of 2.0 0.2, the shape of the tiny aggregates and the short filaments was related with an enhanced generation of citric acid.

The research conducted on the production of citric acid from the solid waste of the Brem industry and the production of citric acid from the cultivation of liquid media suggests that at a pH value of 1.6, the morphology will develop abnormally (by forming hyphae bulbs), and the production of citric acid will decrease significantly. This is the conclusion that can be drawn from the research. An rise in concentration was shown by the creation of citric acid during the duration of the fermentation process. According to the findings of this research, the carbon source that A. niger requires in order to produce citric acid through the fermentation process may apparently be provided by a rice husk substrate that has a concentration of 22.42% weight-per-volume.

The rice husk has the highest levels of carbohydrates in the form of cellulose and hemicellulose, which are the carbon source for A. niger. The amounts of cellulose and hemicellulose estimated to be present in the fermentation medium after the rice husk had been pretreated were around 43% and 24% respectively. During the process of hydrolysis, a cellulase enzyme treatment was carried out. This treatment's primary objective was to hydrolyze cellulose into reducing sugar monomers in the form of glucose. The concentration of citric acid generated was 148,639 g/L, and the concentration of reducing sugar that was created was calculated to be 11%. The microorganisms that produced the citric acid utilised the reducing sugar as a carbon source. Endo-beta-1,4-glucanase is an enzyme that helps break down cellulose into smaller oligosaccharides with free ends. This is necessary for the process of breaking down cellulose and hemicellulose into glucose, which involves a combination of the activities of three other types of enzymes. Glucose is produced when cellulose and hemicellulose are broken down into glucose. In addition, the enzyme exo-beta-1, 4-glucanase is responsible for the release of cellobiose disaccharide units from reducing and non-reducing sugars in the oligosaccharide chain,

and the enzyme beta-glucosidase was responsible for the hydrolysis of cellobiose into glucose. Both enzymes were utilized in the process.

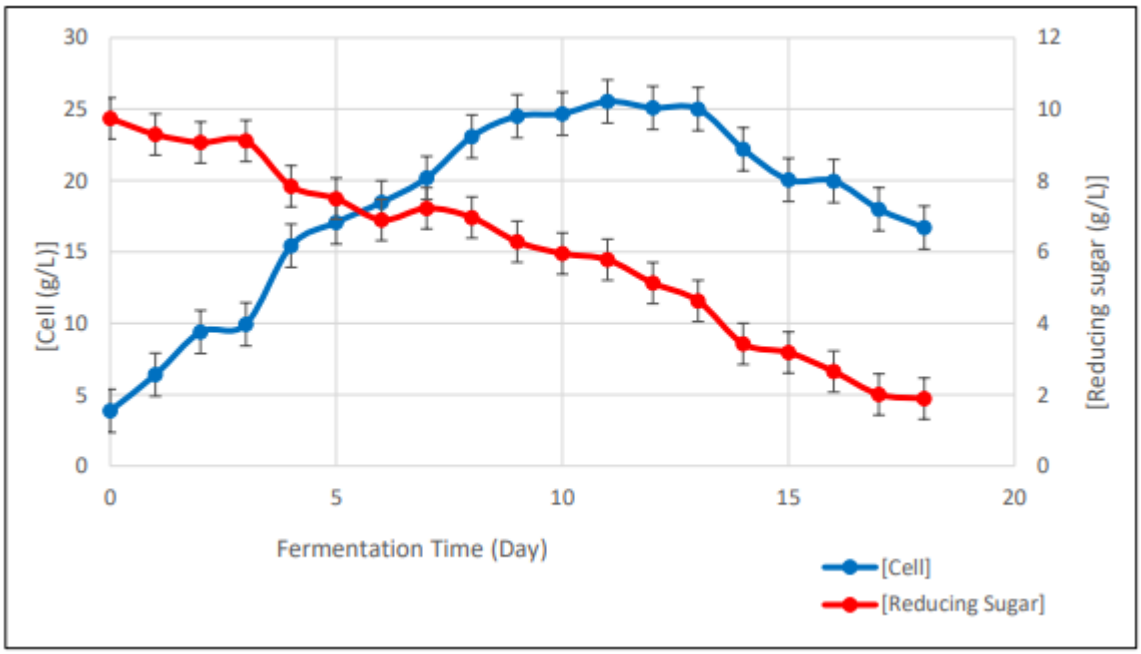

Figure 2. Growth curve of Aspergillus niger and concentration of the remaining reducing sugar.

The profile of cell concentration and the residual concentration of reducing sugars that was determined during the fermentation duration is depicted in Figure 2. The starting inoculum concentration was set at 5% volume to volume (v/v). According to the A. niger growth curve, the exponential phase occurred somewhere between day 0 and day 11 of the experiment. To this point, there was a discernible rise in the total number of cells as the fermentation process progressed further. It was determined that the culture had entered the stationary phase when the number of cells remained unchanged from day 11 to day 13. Because the hydrolysis of rice husks produced a relatively low reducing sugar content, roughly 11%, sucrose was added at a concentration of 10% weight-per-volume in this investigation. Because of this, additional carbon sources were required in order to increase the amount of reducing sugar present in the medium in which the fermentation was taking place. Because monosaccharide is a type of sugar that has short carbon chains and therefore cannot be converted back into simpler sugars, the addition of sucrose can generate more pyruvic acid than that of monosaccharide carbon sources. This is because monosaccharide is a carbon source.

Because A. niger possesses an invertase enzyme that is active at a pH ranging from around 4.0-5.5, sucrose was also added to the mixture. This was done because A. niger is capable of quickly hydrolyzing sucrose into glucose and fructose. In this particular scenario, the sugar that is produced as a byproduct of the hydrolysis will first be turned into fructose-6-phosphate, and then it will be converted into pyruvic acid. In order to identify the optimal concentration of addition of carbon sources in the manufacture of citric acid using A. niger and employing fruit waste as a substrate, Subramaniyan carried out a study to find the optimal concentration. Additional carbon sources were sucrose, which was added at concentrations of 5%, 10%, and 15% weight-per-volume (w/v). According to the findings, the addition of 15% w/v sucrose led to the production of the greatest quantity of by-products, which came out to 13.6% w/v. Comparatively, the addition of 10% w/v sucrose led to the generation of 9.2% w/v by-products, and the addition of 5% w/v sucrose led to the generation of 12.8% w/v by-products.

In addition to creating the fewest by-products, the addition of 10% sucrose by weight resulted in the production of the maximum concentration of citric acid (69.2 g/L) when the working capacity was 500 mL. These investigations demonstrate that it is necessary to pay attention to the concentration of carbon sources in the fermentation medium.

In the event that the concentration of carbon sources is insufficient, the metabolic process will result in an increase in the creation of side products in the form of oxalic acid and gluconic acid, which will lead to a reduction in the amount of citric acid that is produced. If the concentration of the carbon source is too high, it will produce an increase in the viscosity of the fermentation medium owing to the overgrowth of mycelium. This, in turn, will result in an increase in the production of by-products and a reduction in the production of citric acid. It is known that the sucrose concentration of 10% w/v is the optimal concentration of carbon source for the manufacture of citric acid because the by-product that is created is low, and an increase in the working volume employed can enhance the amount of citric acid that is produced. These findings were achieved, and as a consequence, the sucrose concentration was determined to be 10% w/v.

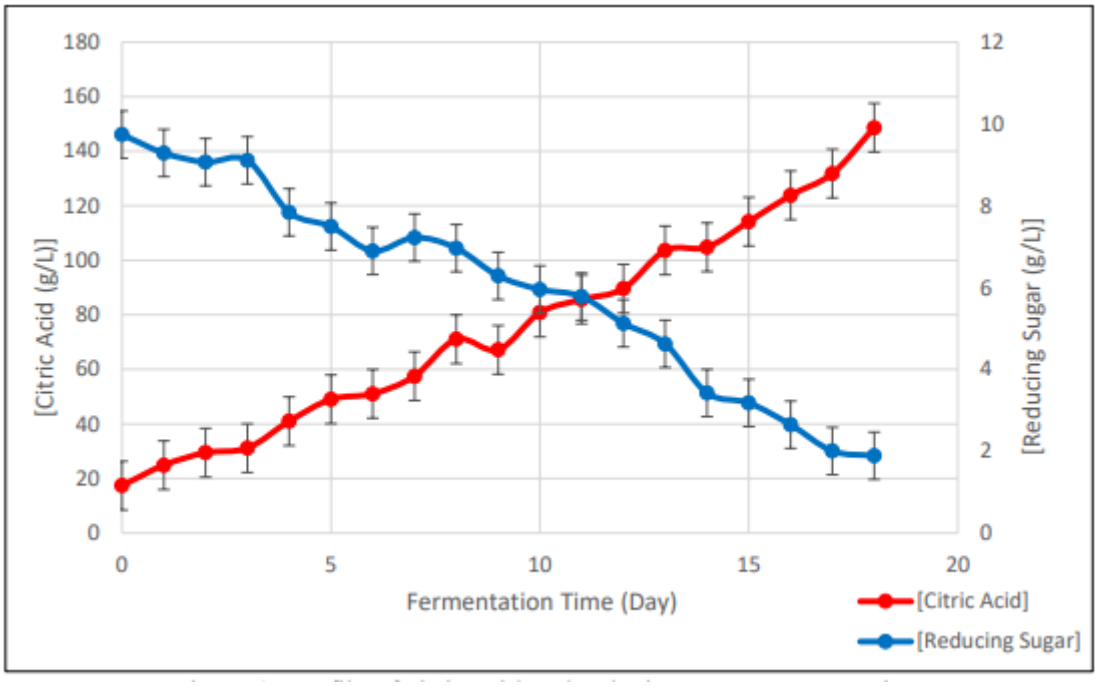

Figure 3. Profile of citric acid and reducing sugar concentration.

CONCLUSION

The widespread nature of CA may largely be attributed to the breadth of its applicability across a variety of industrial fields. There is an urgent need for a cost-effective industrial production process in order to meet the rising demand for CA on a global scale. This process must be highly complex and sensitive, and it must depend on a number of different parameters. Some of these parameters include choices of microorganism, raw materials used, types of fermentation technique employed, designing of appropriate bioreactors with precise control over process parameters, biochemical pathways, factors affecting CA production, quantification techniques, recovery techniques, and strains. Not only can the utilization of agricultural wastes for the manufacture of CA help alleviate the problem of waste disposal, but it may also save important foreign exchange by lowering the amount

of CA that is imported from other countries. The use of cutting-edge technology and the engineering of metabolic pathways are two potential solutions to the challenges posed by key parameters that arise during fermentation; nonetheless, major research efforts are still required.

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