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Optimization and production of citric acid from cassava using *Aspergillus niger*

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ABSTRACT

Citric acid ,an industrial organic acid produced by Aspergillus niger from cassava peel with different carbon and nitrogen sources. Glucose enriched medium showed high yield of citric acid. Ammonium chloride and ammonium persulphate reduced the production of citric acid. The maximum yield of citric acid was observed in ammonium dihydrogen phosphate. The optimum yield of citric acid from cassava peel using aspergillus niger was found in 20 days of fermentation with pH3 and room temperature.

INTRODUCTION

Citric acid is a weak organic acid found in citrus fruits. It is a good, natural preservative and is also used to add an acidic (sour) taste to foods and soft drinks. It is important as an intermediate in the citric acid cycle and therefore occurs in the metabolism of almost all living things. It also serves as an environmentally benign cleaning agent and acts as an antioxidant. In 1917, the American food chemist James Currie discovered that certain strains of the mold *Aspergillus niger* could be efficient citric acid producer. citric acid is a white crystalline powder. It can exist either in an *anhydrous* (water-free) form, or as a monohydrate that contains one water molecule for every molecule of citric acid[1].

Citric Acid Solution reacts with alkaline substances to generate heat. Aqueous solutions have corrosive effects on metals including carbon steels, 304 stainless steel, copper, aluminium and zinc alloys[2]. This may result in the formation of hydrogen to produce explosive mixtures. These solutions also decompose cement-based products and attack some plastics such as nylon, polycarbonates, and polyamides.

Citric acid is used in animal feeds to form soluble, easily digestible chelates of essential metal nutrients, enhance response to antibiotics, enhance flavor to increase food uptake, to control gastric pH and improve the efficiency of the feed. In liquid detergents, sodium citrate is used as a builder, to increase the effectiveness of the surfactants, due to its high solubility and bio-degradability. Metal sulphates are dissolved in water and citric acid followed by neutralization with ammonia. The soluble citrate chelate promotes the transfer of the metal nutrient into the plant's root or leaf system.Ctric acid is important for growth of the plants. *Asp niger* strains are used in industrial preparation of citric acid(E330) and Gluconic acid(E574) and have been assesed as acceptable for daily intake by World Health Organization. *A niger* i normally a haploid fungus producing white septate hypha which is profusely branched. It produces black mass of conidia, which are found in chain arising from the secondary sterigmata[3]. Citric acid is mainly produced by a fungus *Aspergilus niger* by utilizing starchy and sugar substrates [4]

Citric acid is added to hair care formulations to adjust the pH, act as a buffer and chelate metal ions to prevent discoloration and decomposition. It is used in liquid preparations to enhance fruit flavors and to impart a desirable tart taste that helps mask medicinal flavors. It helps maintain stability of the active ingredients by buffering aqueous solutions, sequestering trace metals, and assisting in the dispersion of suspensions[,5].

Citric acid in syrups, elixirs, of suspensions and solutions is considered a well established market.The largest use of citric acid in the pharmaceutical industry is for the effervescent effect it produces when combine with bicarbonates or carbonates in antacids[6]. The maximum amount of starch was found in pulp 30.6%.Therefore cassava is used as substrate for ethanol and citric acid production[7].Citric acid is recognized as safe for use in food by all major national and international food regulatory agencies .It is naturally present in almost all forms of life, and excess citric acid is readily metabolized and eliminated from the body. The main objective of this study was to produce citric acid from waste using *Aspergillus niger* and its optimization with different carbon and nitrogen sources.

MATERIALS AND METHODS

Aspergillus niger was extracted and cultured in potato dextrose agar media. Cassava was washed with tap water for several times. It was sliced thinly and dried in dryer at 50°C. The substrate was powdered by using a grinding machine. Dried powder of cassava was hydrolyzed separately in 300 ml solution of 0.05 N HCl and autoclaved at 121°C temperature, 15 Ibs pressure for 20 minutes. The hydrolyzed materials were then filtered through thin cloth and collected the filterate[8].

The basal fermentation media enriched with cassava. Its proximate composition was determined. Different carbon sources of sucrose, glucose, maltose and nitrogen sources of ammonium chloride,potassium dihydrogen phosphate ammonium persulphate and ammonium e were used for its optimization.. Surface liquid culture fermentation process was carried out in a 500 ml Erlemyer flask containing 100 ml media. Each flask was inoculated with the given spore suspension and incubated at 300ºC for up to 20 days[9] .

Different carbon and nitrogen enriched media was carried out after three days of incubation. These were filtered into a clean beaker with the help of whatmann no.1 filter paper.1 M NaOH was prepared and taken in burette.The filtrate was titrated against NaOH with phenolphthalein as indicator till pink colour appears. The readings were noted and amount of citric acid produced was calculated.

RESULTS AND DISCUSSION

The production of citric acid from cassava peel using *Aspergillus niger* and its maximum yield through optimization. Pandey et.al reported that Cassava waste disposal in the environment can cause serious environmental pollution due to its high organic material and biodegradability[5,10]. For optimum pH, media with different pH were titrated and the optimum pH range was found to be within 1-3 where the amount of citric acid produced was estimated to be 0.096 %.. The production of citric acid was found to be higher in glucose enriched medium.Husseiny reported that15% of sucrose enriched medium yield maximum production of citric acid.[11]. The suitable carbon source was found to be as glucose were the yield of citric acid in the glucose containing media was estimated to be 0.0768%.(Table 1).

Table 1: Citric acid production from cassava peel using different carbon sources

Sl.no.	Volume of cassava filtrate (ml)	Carbon source	Volume of naoh added (ml)	Normality of citric acid (m)	Weight of citric acid in 1000 ml G/lt	Weight of citric acid in 100 ml %
1	50	Glucose	0.6	0.012	0.768	0.0768
2	50	Sucrose	0.4	0.008	0.512	0.0512
3	50	Maltose	0.4	0.008	0.412	0.0412

In case of nitrogen source, NH_4Cl was found to be showed lower contribution for the production of citric acid produced was estimated to be 0.058% The medium contained ammonium nitrate and sodium nitrate did not show any change in citric acid production but ammonium dihydrogen phosphate yield more amount of citric acid. Many of them explained that ammonium ions play an important role in regulation of citric acid cycle.

Art (1987) reported that the higher concentration of intracellular ammonium ions could lead to prevent the citrate activity which inhibit the phosphofructokinase activity[12]. The substrates of urea,yeast and ammonium dihydrogen phosphate also showed high production of citric acid (Xie and West . The high concentration of phosphate led to decrease in fixation of carbondioxide which in favor the formation of certain sugars and acids.[13]

Table 2: Citric acid production from cassava peel using different nitrogen sources

Sl.no.	Volume of cassava filtrate (ml)	Nitrogen source	Volume of naoh added (ml)	Normality of citric acid (m)	Weight of citric acid in 1000 ml G/lt	Weight of citric acid in 100 ml %
1	50	NH_4Cl	0.7	0.014	0.058	0.058
2	50	APS	0.7	0.014	0.896	0.0896
3	50	$NH_4H_2PO_4$	0.9	0.018	1.152	0.1152
4	50	NH_4NO_3	0.2	0.01	0.64	0.064

In case of temperature, the amount of citric acid produced was maximum (0.064%) at room temperature.The maximum yield of 0 .1152% was obtained in optimized media after incubating for 5 days at room temperature.

CONCLUSION

The *Aspergillus niger* strain was grown on potato dextrose agar and was used for the production of citric acid. The optimization of cultural conditions was done for high and consistent yield of citric acid. The amount of citric acid produced in optimized media was found to be the maximum.

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