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Production of Citric Acid from Garri Processing Solid Waste using Submerged Culture of *Aspergillus niger*

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ABSTRACT

Background and Objective: Citric acid is a weak organic acid that occurs naturally in all citrus fruits. It is the largest consumed organic acid and is widely used in beverage, food and pharmaceutical industries. This study, therefore, investigates the efficient utilization of garri processing solid waste for citric acid production using *Aspergillus niger* in submerged culture. **Materials and Methods:** A series of experiments were designed on various fermentation parameters to establish the optimal conditions for citric acid production. **Results:** This study revealed that production parameters such as initial pH, incubation temperature and fermentation period had a profound effect on the amount of citric acid produced. Citric acid concentration, mycelium crude protein and biomass dry weight increased with initial pH of 5.5, time of 120 hrs, incubation temperature of 30°C and shaking condition of 200 rpm. **Conclusion:** The results revealed that the accumulation of biomass and mycelium crude protein has no significant effect on citric acid accumulation on garri processing waste by *Aspergillus niger* in submerged fermentation. The results of this study suggest that garri processing solid waste material could be harnessed for large-scale citric acid production.

KEYWORDS

Citric acid, garri processing solid waste, mycelium crude protein, biomass, submerged culture, *Aspergillus niger*

INTRODUCTION

Citric acid which is also known as 2-hydroxy-propane-1, 2, 3-tricarboxylic acid ($C_6H_8O_7 \cdot H_2O$) is a weak organic acid, with a pH of 0.2, which occurs naturally in all citrus fruits[1]. Citric acid is colourless, odourless and easily soluble in water and alcohol with a pleasant taste[2]. It is solid in its pure form at room temperature and has a melting point of 153°C[3]. Naturally, citric acid is produced by metabolic pathways that take place in a living cell via the tricarboxylic acid cycle[4]. In other words, it exists as an intermediate in the Krebs cycle when carbohydrates are oxidized to carbon (iv) oxide[2]. Citric acid is the largest consumed organic acid and is widely used in beverage, food and pharmaceutical industries[5]. About 1,000,000 metric ton of citric acid is produced globally every year to service the huge demand by food, chemical, cosmetic and beverage industries[6]. Three principal methods are applied for microbial production of citric acid, these are surface culture, submerged culture and solid-state culture[7,8].

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In general, citric acid is commercially produced by submerged microbial fermentation of molasses, the fermentation process using *Aspergillus niger* is still the main source of citric acid production on both laboratory and industrial scales[9] because it is easy to deal with, has a wide range of fermentable materials as well as high production[10-12]. There are a lot of studies that have reported the use of organic wastes such as sugar cane bagasse, beet molasses, banana stalk, apple peels, *et cetera* to produce citric acid[13-15].

The species of organism and the conditions of fermentation are factors that are essential and influential for the high production of citric acid[9]. An appropriate initial pH is critical for the successful fermentation process and varies from strain to strain. It has been established that a low pH inhibits the production of unwanted organic acids such as gluconic acid and oxalic acid[4]. Low pH also competes against bacteria contaminants. The initial pH must be very well defined and optimized depending on the organism used[9]. Garri processing waste is a waste generated in Nigeria from cassava during garri food processing. Currently, the disposal of this waste product poses considerable economic and environmental problems, thereby causing pollution in both homes and garri processing industries. So this research work was aimed at the utilization of this solid waste material for effective citric acid production using *Aspergillus niger*.

MATERIALS AND METHODS

Study area: This study was carried out in the year 2018 at the Industrial Microbiology Laboratory of the Department of Microbiology, University of Nigeria, Nsukka, Nigeria.

Microorganism and culture maintenance: *Aspergillus niger* ATCC 1015 strain was obtained from the Department of Microbiology University of Nigeria, Nsukka, Nigeria. The cultures were maintained on potato dextrose agar (PDA) slants at 4°C and sub-cultured at intervals.

Inoculums preparation: The spores of *Aspergillus niger* were harvested from potato dextrose agar slant using a sterile solution of 0.01% Tween 80. The inoculation wire loop was used to dislodge the spores and to ensure proper mixing of the culture with the Tween 80. A 10 mL of 5×10^7 spores mL^{-1} was counted using the improved Neubauer haemocytometer (Hawksley, England).

Substrate and pretreatments: Garri processing waste was obtained from garri processing site at Nsukka in the Enugu State of Nigeria. The waste was sundried, ground and sieved into flour using Muslim cloth. The flour was thermally pretreated to gelatinize the starch by suspending 10 g into 100 mL of distilled water. The sample was sterilized with an autoclave at 121°C for 15 min.

Submerged fermentation: Submerged fermentation was carried out using a 250 mL foam-plugged Erlenmeyer flask. About 10 g of the flour was weighed using DENVER Instrument (Model: MXX-123 USA) and suspended in 100 mL nutrient medium containing NH_4NO_3, 2, KH_2PO_4, 0.2, $ZnSO_4{\cdot}7H_2O$, 0.01, $Fe(SO_4)_2{\cdot}7H_2O$, 0.01 and $MgSo_4{\cdot}7H_2O$, 0.5 g L^{-1} before pretreatment. The sample was inoculated with 10 mL of *Aspergillus niger* spores and incubation at 30°C.

Initial Ph: The effect of initial pH on citric acid production was carried out by adjusting the pH to 3.5, 4.5, 5.5, 6.5, 7.5 and 8.5 using 0.1 M HCl and 0.1 M NaOH before pretreatment.

Incubation temperature: The effect of incubation temperature on citric acid production by *Aspergillus niger* was carried out by incubating under the following temperature: 20, 25, 30, 35 and 40°C.

Shaking condition: The effect of the shaking condition on citric acid production was carried out by incubating the flasks in the rotary incubator shaker (model: VWR International by B. Bran Scientific and Instrument Company England) at 100, 125, 200, 225, 300 and 325 revolutions per minute (rpm).

Sugar supplementation: About 100 mL of the waste nutrient medium was supplemented with 3, 4, 5 and 6 g of glucose and incubated under the rotary incubator shaker (model: VWR International by B. Bran Scientific and Instrument Company England).

Analytical techniques: Citric acid was estimated using the pyridine acetic anhydride method[16]. About 1 mL of diluted culture filtrate along with 1.30 mL of pyridine was added to the test tube and swirled briskly and then 5.70 mL of acetic anhydride was added to the test tube. The test tube was placed in a water bath at 32°C for 30 min. The absorbance was measured on a Spectrophotometer (722S B. Bran Scientific and Instrument Company, England) at 405 nm against the blank and the citric acids of the samples were estimated with the reference standard. To determine biomass, the whole fungal culture was filtered with sterile filter paper (Whatman no 1 filter paper) and dried to a constant weight at 105°C. Crude protein was estimated by the method of Lowry *et al.*[17]. ThepH of the sample was determined using a digital pH meter (DENVER Instrument, Model: UB-10058245 Ultra BASIC, USA).

Statistical analysis: Data obtained were subjected to Analysis of Variance (ANOVA) and the means were separated using the Least Significant Difference (LSD) method.

RESULTS

Time course for citric acid production: Figure 1 shows the time course for citric acid production. Citric acid production increased with time up to 120 hrs, accumulating 17.0 g L^{-1} citric acid, 0.291 g L^{-1} crude protein and 12.50 g L^{-1} dry biomass.

Effect of initial pH: The effect of initial pH was shown in Fig. 2 where pH 5.5 was discovered to be the optimum for citric acid production. At pH 5.5, 21.0 g L^{-1} citric acid, 0.341 g L^{-1} crude protein and 13.22 g L^{-1} dry Biomass were produced. Further increase in pH did not lead to more accumulation of citric acid.

Effect of incubation temperature: Incubation temperature has an effect on *Aspergillus niger* for citric acid production (Fig. 3). Incubation at 30°C was the optimum for citric acid production. At 30°C, 26 g L^{-1} citric acid, 0.395 g L^{-1} crude protein, and13.45 g L^{-1} dry biomass was produced while 25°C was the optimum for crude protein and biomass production, which were 0.392 and 14 g L^{-1}, respectively. An increase in temperature up to 40°C did not enhance citric acid production. An increase in temperature also led to a reduction of crude mycelium protein and biomass.

Effect of shaking conditions: Figure 4 shows that shaking conditions enhanced citric acid production. About 200 rpm was the optimum for citric acid production, accumulating 32.0 g L^{-1} citric acid, 0.397 g L^{-1} crude protein and 15.21 g L^{-1} dry biomass. The control has the least citric acid concentration, highest crude protein and biomass accumulation.

In Fig. 5, the substrate was supplemented with 3-6%. About 5% supplement was the optimum, producing 34.0 g L^{-1} citric acid, 0.399 g L^{-1} crude protein and 14.0 g L^{-1} biomass. Citric acid concentration increased significantly ($p<0.05$) when compared with the control. Supplementation with sugar under shaking conditions produced the highest amount of citric acid, without a significant increase in the amount of biomass and crude protein ($p<0.05$).

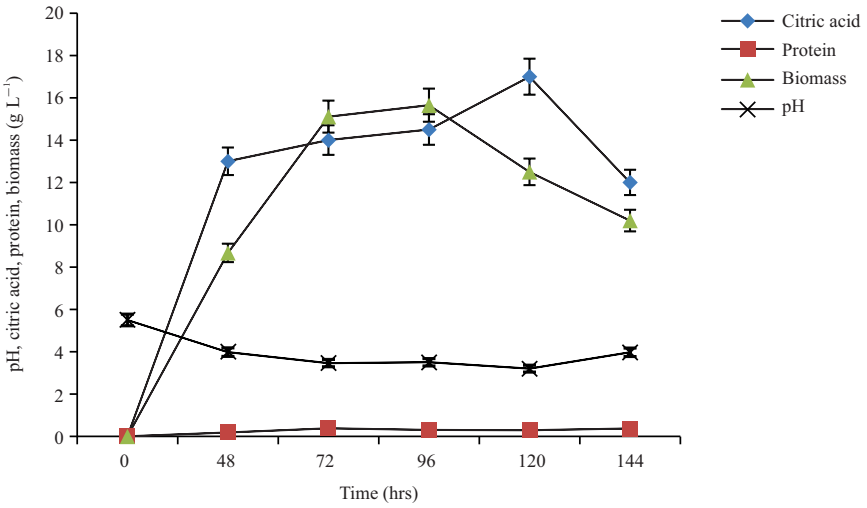

Fig. 1: Time course for citric acid production

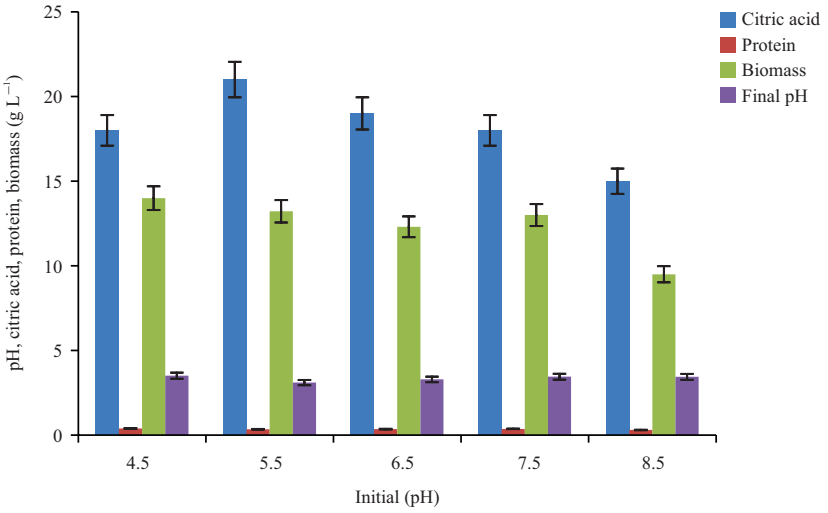

Fig. 2: Effect of Initial pH on citric acid production

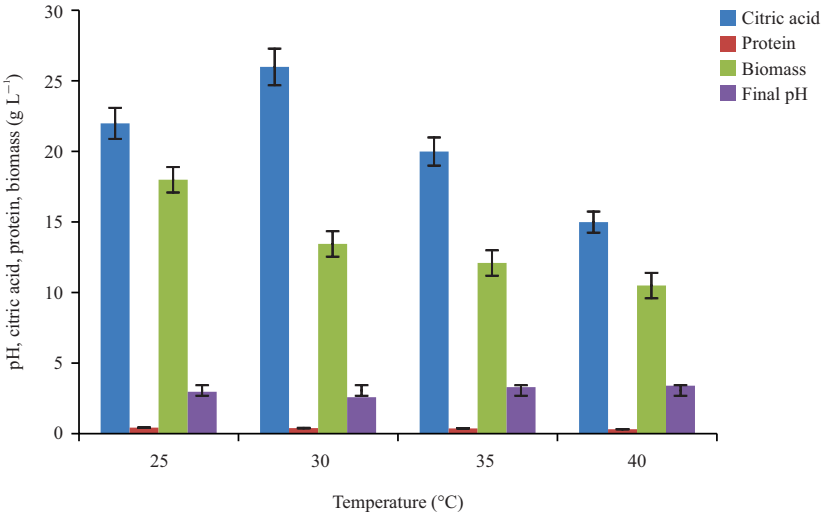

Fig. 3: Effect of incubation temperature on citric acid production

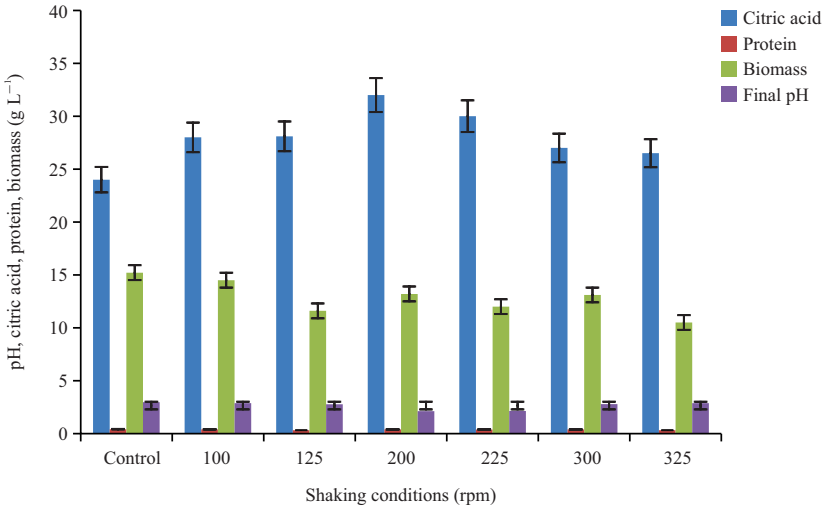

Fig. 4: Effect of shaking conditions on citric acid production

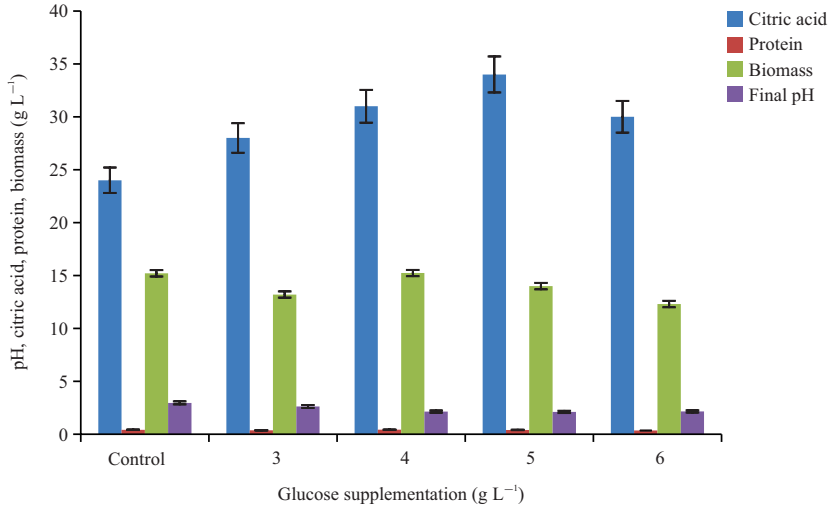

Fig. 5: Effect of sugar supplementation on shaking condition for citric acid production

DISCUSSION

Citric acid production increased gradually during the fermentation period and attained its maximum value at 120 hrs after inoculation. Further increase in fermentation time did not enhance additional citric acid, biomass and crude protein production. This is in agreement with previous studies[3,18,19] that reported that biomass generation was increased with fermentation time. The incubation period beyond the optimum time of 120 hrs did not show much enhancement in citric acid production. This might be due to the depletion of starch and sugar in the fermentation medium and the inhibitory effect of a high concentration of citric acid which led to decay in the enzyme responsible for citric acid production and equally reduced the biomass and crude protein.

The initial pH of the substrate is a very critical factor that affects the performance of microorganisms in citric acid production in submerged culture. The production of citric acid depends on the maintenance of the initial pH of the medium. At pH 5.5 the highest amount of citric acid was accumulated while the highest amounts of crude protein and biomass were accumulated at pH 4.5. Further increase in pH did not lead to more accumulation of citric acid. This is in agreement with Aboyeji *et al.*[20], who reported a pH of 6.5 to be the optimum for citric acid production from pretreating crude date syrups by *Aspergillus niger*. During isolation of citric acid-producing fungi and optimization of citric acid production

by selected isolates, Dhandayuthapani *et al.*[15], reported a pH of 5.5 as the optimum pH for citric acid production. Maintenance of a favourable pH is very essential for greater mycelia, protein content and citric acid production by *A. niger*[21]. A similar result was reported on the biological production of citric acid in solid-state cultures of *A. niger* using sugar cane, beet molasses, cassava and cornflour, rice grain and banana peels, etc.[14-16]. This would seem to suggest that the initial pH did not directly influence the citric acid production mechanism but rather affected the enzymes which were active in metabolizing starch and making the cell membrane more permeable to waste nutrient medium.

Incubation temperature has an effect on *A. niger* for citric acid production. Incubation at 30°C was the optimum for citric acid production while 25°C was the optimum for crude protein and biomass production. This result suggests that an increase in biomass and crude protein to some extent does not necessarily increase the amount of citric acid produced. An increase in temperature up to 40°C did not enhance citric acid, crude protein and biomass production. An increase in temperature also led to the reduction of crude mycelium protein and biomass. This result is in line with Kareem *et al.*[21], who reported a temperature of 30°C to be the best for citric acid production by *A. niger* using pineapple waste. This result is not in agreement with that obtained by Karthikeyan and Sivakumar[22], who recorded 28°C as the optimum temperature for citric acid production by *A. niger* using banana peels as a substrate.

Shaking condition is very important for proper agitation and mass transfer. About 200 rpm was the optimum for citric acid production. The control has the least citric acid concentration and highest crude protein and biomass accumulation. This suggests that an increase in crude protein and biomass formation by *A. niger* to some extent does not necessarily enhance citric acid production. This result is in agreement with the findings of Ayodele *et al.*[18], who reported that citric acid production increased from 170-230 rpm in a stirrer fermenter using *A. niger*. Hamdy[23] reported that increasing the agitation rate up to 250 rpm increased the production of citric acid by *A. niger*.

A five percent glucose supplement was the optimum for citric acid production. Supplementation with sugar under shaking conditions produced the highest amount of citric acid, without a significant increase in the amount of biomass and crude protein. This is in agreement with Amenaghawon and Aisien[24], who reported that supplementation with glucose/sucrose enhanced citric acid production. This might be a result of the presence of sugar before the actual growth of *A. nige*r in the medium.

CONCLUSION

In conclusion, the current result revealed some important aspects of citric acid production, biomass and mycelium crude protein accumulation from garri processing waste by *Aspergillus niger*. The optimum conditions were temperature of 30°C, pH 5.5, shaking at 200 rpm and glucose supplement of 5%. This result suggests that the accumulation of biomass and mycelium crude protein has no significant effect on the citric acid production from garri processing waste by *A. niger* in submerged fermentation. This study has demonstrated that garri processing waste generated in Nigeria could be efficiently converted to citric acid using *A. niger*.

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