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Utilization of Pap processing waste in submerged culture of *Aspergillus niger* enriched with poultry dropping extract for citric acid production

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Abstract

Determining the potential of pap processing waste for citric acid production was investigated using *Aspergillus niger* isolated from pap waste. The cost of substrate for citric acid production represents a significant percentage of the total production cost. The aim of this work was therefore, to utilize pap processing waste in submerged culture enriched with poultry dropping extract for citric acid production. The effect of initial pH, inoculums size, poultry dropping extract, pap processing waste concentration and alcohol addition were investigated on citric acid production from pap processing waste. The results show that the initial pH, inoculums size, poultry dropping extract, pap processing waste and methanol had significant effect on citric acid production from pap processing waste ($p < 0.05$). Citric acid concentration increased as the pH increased. A pH value of 6.0 was the optimum for maximum citric acid concentration of 6.5 ± 0.116 g/l after 96 hours of fermentation. Citric acid concentration also increased as the percentage inoculums increased. A 10 % inoculums size was the optimum with the maximum concentration of 8.0 ± 0.231 g/l citric acid. There is also an increase in citric acid concentration as the percentage poultry dropping extract concentrations increased from 5 % to 15 %. A 15 % poultry dropping extract concentrations was the optimum with the maximum concentration of 11.0 ± 0.251 g/l citric acid. As the concentration of the pap processing waste increased up to 20 % the citric acid concentration of 16.0 ± 0.413 g/l was produced after 96 hours of fermentation. A 3 % methanol addition was the optimum with the maximum citric acid concentration of 26.0 ± 0.351 g/l after 96 hours of fermentation. This research work has established the potential of pap processing waste on citric acid production if well harnessed.

1. Introduction

Citric acid is the most produced organic acid measured in tonnage **(Shankar and Sivakumar 2016)**. Naturally, citric acid is produced by metabolic pathways that take place in a living cell via tricarboxylic acid cycle with melting point of 153^{0}C **(Nwoba *et al.*, 2012; Almousa *et al.*, 2018)**. It is the most versatile and widely used organic acid in the field of food, pharmaceutical, beverages, detergents, cosmetics, toiletries and other industries **(Majumder *et al.*, 2010; Shankar and Sivakumar 2016).**
The main reason for constant increase is the large number of applications that can be found for citric, mainly in the food and pharmaceutical industries **(Shankar and Sivakumar 2016; Gruben *et al.*, 2014; weyda *et al.*, 2014; Vander saat *et al.*, 2014; Omojasola *et al.*, 2014)**. To meet rising demand for citric acid in many applications in food and biomedicines, there is need for continued search for more efficient strain from our environment and also cost effective substrate and nitrogen source for citric acid production **(Shankar and Sivakumar 2016; Ezea *et al.*, 2015; Ezea *et al.*, 2021)**. However, the cost of substrate represents a significant percentage of the total production cost.
Cost effect nitrogen source very is important to reduce the cost of citric acid production. Nitrogen consumption leads to pH decrease which is very important point in citric acid fermentation. During citric acid fermentation *Aspergillus niger* metabolism of nitrogen cause a release of protons which lower the pH of the medium **(Papagianni 2007).**
The best solution considering cost effectiveness may be the utilization of the indigenous and cheaper substances as like agricultural waste and by products as substrate for the production of valuable organic acid such as citric acid **(Shankar and Sivakumar 2016)**. And also the utilization of organic nitrogen source such as poultry droppings.
Pap processing waste is a waste generated from corn during pap production. Pap is an African fermented cereal pudding from Nigeria typically made from maize. It is local custard taking by southern part of Nigeria as weaning food or breakfast meal. Within the region, a lot of pap processing wastes are generated during the process of fermentation which can be converted to citric acid. Disposal of this waste product poses considerable environmental problems as they are underutilized. Development of process for value addition and processing of pap processing waste with poultry droppings as organic nitrogen source is a sure way of reducing the cost of citric acid production. This research work was therefore, designed to investigate citric acid production from pap processing waste and develops some optimization strategies to enhance citric acid production.

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2. Material and methods

2.1 Isolation and screening for citric acid producing fungi

Aspergillus niger was isolated from pap processing waste compost samples collected from a designed pap processing waste compost from different location at Nsukka by using serial dilution method. The dilutions was inoculated on Potato Dextrose Agar (PDA) using spread plate techniques and incubated at 30^{o} C for 5 days. The fungal isolates were screened qualitatively for citric acid production using methods described by **Kareem *et al.* (2010).** A sterilized Czapek dox agar medium (10 ml) incorporated with Bromo-cresol green dye was poured into individual sterile petri plates and allowed to cool at room temperature. Approximately 0.5 ml of the fungal spore suspension was transferred to each of the petri plates. The petri plates were incubated at 30^{o} C for 5 days. The isolate with the widest yellow zone were used for further studies. Fungal isolate was identified using slide culture technique and based on morphological features observed and comparison with reference fungi atlas.

2.2 Inoculum preparation

The inoculum was prepared according the method of **Ezea *et al.* (2021),** the spores of *Aspergillus niger* was 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 5 ml of 5 x10^{7} spores/ml was counted using haemocytometer.

2.3 Pretreatment of pap processing waste

Pap processing waste was obtained from pap processor at Nsukka in Enugu State of Nigeria. The pap processing waste was, sundried, ground and sieved into fine powder (flour) using Muslim cloth. The flour was thermally pretreated to gelatinize the starch by suspending in 100ml of the basal nutrient medium. The sample was pretreated with an autoclave at 121^{0}C for 20 minutes.

2.4 Submerged fermentation of pap processing waste flour

Submerged fermentation was carried out in a modified method **Ezea *et al.* (2021)**, in 250ml foam-plugged Erlenmeyer flask. A 10 g pap processing waste flour was weighed and suspended in 100 ml nutrient medium containing NH_4NO_3, 2 g/l; KH_2PO_4, 0.2 g/l; $ZnSO_4.7H_2O$, 0.01 g/l; $Fe(SO_4)_2.7H_2O$, 0.01 g/l and $MgSo_4.7H_2O$, 0.5 g/l before pretreatment. The sample was inoculated with 5 ml of *Aspergillus niger* spores and incubation at 30^{o} C for 144 hours under rotary incubator shaker (model: VWR International by B. Bran Scientific & Instrument Company England) at 225 rotations per minutes.

2.5 Effect of initial pH on citric acid production from pap processing waste

The effect of initial pH on citric acid production from pap processing waste was carried out by adjusting the pH to 3, 4, 5, 6, 7 and 8 using 0.1M HCl and 0.1M NaOH before Pretreatment.

2.6 Effect of inoculums size on citric acid production from pap processing waste

The effect of inoculums size on citric acid production from pap processing waste was carried out by inoculating different inoculums concentrations from 5 % to 25 % in 100 ml basal nutrient into 250 ml foam-plugged Erlenmeyer flask.

2.7 Preparation of poultry dropping extract as nitrogen source for citric acid production

Poultry droppings were collected from Opi poultry farm at Nsukka in Enugu state. Foreign objects were removed from the sample and the poultry droppings were dried under the sunlight for 5days. The dried poultry droppings was crushed into a fine powder and 20g was weighed using a weighing balance and was soaked in 100 ml of distilled water for 3days. The mixture was filtered using a muslin cloth.

2.8 Effect of poultry dropping extract as organic nitrogen source on citric acid production

After preparation of poultry dropping extract as the organic nitrogen source, 3 % of the extract was added in place inorganic nitrogen source in 100 ml nutrient medium into 250 ml foam-plugged Erlenmeyer flask. Thereafter, different percentages of poultry dropping extract ranging from 1 % to 7 % on citric acid production were determined.

2.9 Effect of pap processing waste concentration on citric acid production

Effect of pap processing concentration on citric acid production was carried by suspending different percentage of pap processing waste ranging from 5 % to 25 % in 100 ml nutrient medium into 250 ml foam-plugged Erlenmeyer flask.

2.10 Effect of alcohol additions on citric acid production from pap processing waste

The effects of methanol addition on citric acid production by *Aspergillus niger* was determined by adding 2 % of absolute methanol in 100 ml nutrient medium into 250 ml foam-plugged Erlenmeyer flask. Thereafter, different percentages of absolute methanol ranging from 1 % to 5 % on citric acid production were determined.

2.11 Analytical techniques

Citric acid was estimated using pyridine acetic anhydride method as reported by marrier and **Boulet (1958)**. A 1 ml of diluted culture filtrate along with 1.30 ml of pyridine was added in the test tube and swirled briskly. Then 5.70 ml of acetic anhydride was added in the test tube. The test tube was placed in a water bath at 32^{o} C for 30 min. The absorbance was measured on a Spectrophotometer 722S B. Bran Scientific and Instrument Company, England at 420 nm against the blank and the citric acids of the samples were estimated with reference standard. The pH of the sample was determined using digital pH meter (DENVER Instrument, Model: UB- 10058245 ultraBASIC USA).

2.12 Statistical analysis

Data obtained were subjected to one- way analysis of variance (ANOVA) and the means were separated using the least significant difference.

3. Results and Discussion

3.1 Effect of initial pH on citric acid production from pap processing waste

Figure 1 shows the effect of initial pH of pap processing waste flour on citric acid production. Citric acid concentration increased as the pH increased from 3.0 to 6.0. A pH value of 6.0 was the optimum for maximum citric acid concentration of 6.5 ± 0.116 g/l after 96 hours of fermentation. Citric acid concentration decreased from pH 7.0 to pH 8.0 (fig 1). This is in agreement with **Lingappa *et al.* (2007)** who reported 5.5 as the optimum for maximum citric acid concentration after 72 hours. **Dallal and Hamid (2021)** reported pH 3 and 4 as the optimum for maximum citric acid production during optimization of citric acid production by *Aspergillus niger*. This research work is also in agreement with **Ezea *et al.* (2021)** who reported similar trend during biological production of citric acid in submerged culture of Aspergillus niger using cassava pulp wastes. These results suggested that different strain of *Aspergillus niger* have different pH requirement depending on the strain and substrate used.

3.2 Effect of inoculum size on citric acid production from pap processing waste flour

The effect of different inoculums size on citric acid production from pap processing waste flour was investigated. Citric acid concentration increased as the percentage inoculums increased from 5 % to 10 %. A 10 % inoculums size was the optimum with the maximum concentration of 8.0 ± 0.231 g/l citric acid (fig 2). There was a decreased on citric acid concentration from 15 % to 25 % inoculums sizes. This research work is in accordance with **Auta *et al.* (2014).** In their report citric acid concentration increased as the inoculums size increases during production of citric acid by *Aspergillus niger*. **Ali *et al.* (2003)** reported the same trend during vegetative inoculums on submerged citric acid fermentation by *Aspergillus niger*. This suggested that inoculums development is an important step during citric acid production. Each strain of *Aspergillus niger* has its own inoculums size for efficient citric acid accumulation.

3.3 Effect of poultry dropping extract on citric acid production from pap processing waste

The effect of poultry dropping extract on citric acid production from pap processing waste flour was investigated. Citric acid concentration increased as time increased up 96 hours. Pap processing waste flour supplemented with poultry dropping extract as nitrogen source had the maximum citric acid concentration of 10.0 ± 0.232 g/l than pap processing waste flour without poultry dropping extract with maximum citric acid concentration of 6.0 ± 0.132 g/l under the same culture conditions (fig 3). The effect of poultry dropping extract concentrations on citric acid production from pap processing waste flour was investigated. Citric acid concentration increased as the percentage poultry dropping extract concentrations increased from 5 % to 15 %. A 15 % poultry dropping extract concentrations was the optimum with the maximum concentration of 11.0 ± 0.251 g/l citric acid after 96 hours of fermentation (fig 4). There was a decreased on citric acid concentration from 20 % to 25 % poultry dropping extract concentrations. Poultry dropping extract increased the concentration of citric acid in the pap processing waste. Although there is scanty information on the use of poultry dropping extract for citric acid production. The extract from poultry droppings has enough organic nitrogen. Most of nitrogen approximately 60 – 70 % excreted in poultry manure is in the form of uric acid and urea; organic nitrogen, ammonium, nitrate and nitrite are significantly correlated with the amount of nitrogen mineralized from poultry manure **(Nahm, 2007).** Nitrogen affects both the growth of microorganism and the production of citric acid. ***Ganne et al.* (2008)** reported an enhanced citric acid production with ammonium nitrate and sodium nitrate as nitrogen source during production of citric acid by *Aspergillus niger* MTCC 282 in submerged fermentation. **Ezea *et al.* (2015)** reported the same trend using ammonium nitrate during biological production of citric acid in solid state culture of *Aspergillus niger*. The concentration of nitrogen source in the growth media has a considerable influence on citric acid production. Citric acid concentration increased as the nitrogen concentration increases. ***Ganne et al.* (2008)** reported the same trend during production of citric acid by *Aspergillus niger* MTCC 282 in submerged fermentation.

3.4 Effect of different pap processing waste flour concentration on citric acid production

Figure 5 shows the effect of different concentrations of pap processing waste flour on citric acid production by *Aspergillus niger*. Citric acid concentration increased as the concentration of the pap processing waste increased up to 20 % with maximum concentration of 16.0 ± 0.413 g/l citric acid after 96 hours of fermentation. This result is in agreement with **Ganne *et al.* (2008)** who reported an increase in citric acid concentration when the carbon source concentration was increased during production of citric acid by *Aspergillus niger* MTCC 282 in submerged fermentation. **Ezea *et al.* (2015)** reported citric acid production in a solid state culture of *Aspergillus niger* using some starchy substrate; rice grain, maize and cassava flour. **Kudzai *et al.* (2016)** reported potato and rice starch extract during citric acid production by *Aspergillus niger* using different substrates. Carbon source and its concentration had a great influence on citric acid production depending on the nature and type carbon used for fermentation.

3.5 Effect of methanol addition on citric acid production from pap processing waste

The effect of methanol addition on citric acid production from pap processing waste flour was investigated. Citric acid concentration increased as time increased up 96 hours. Pap processing waste flour supplemented with methanol had the maximum citric acid concentration of 20.0 ± 0.256 g/l than pap processing waste flour without methanol with maximum citric acid concentration of 16.0 ± 0.162 g/l under the same culture conditions (fig 6). The effect of different concentrations of methanol on citric acid production from pap processing waste flour was also investigated. Citric acid concentration increased as the percentage methanol concentrations increased from 1 % to 5 %. A 3 % methanol concentration was the optimum with the maximum concentration of 26.0 ± 0.351 g/l citric acid after 96 hours of fermentation (fig 7). There was a decreased on citric acid concentration from 4 % to 5 % methanol concentrations. This result is in agreement with **Ganne *et al.* (2008)** who reported increase in citric acid concentration with addition of 3 % methanol in a sucrose based medium during production of citric acid by *Aspergillus niger* MTCC 282 in submerged fermentation. **Ezea *et al.* (2015)** reported enhanced in citric acid production in a solid state culture of *Aspergillus niger* using some starchy substrate with 2 % absolute methanol addition. These results suggested that methanol addition enhanced citric acid production differently depending on the substrate and strain of *Aspergillus niger* used.

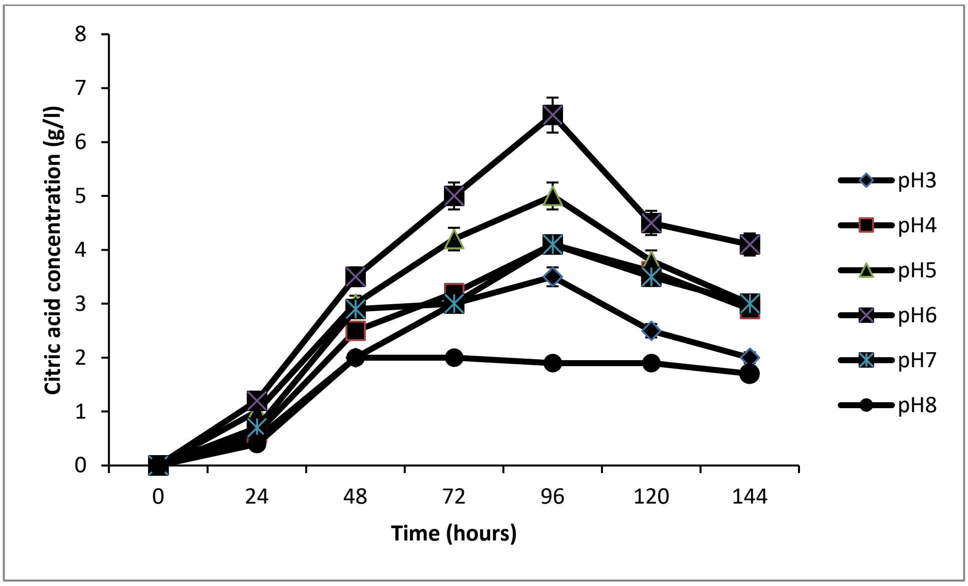

Figure 1 Effect of initial pH on citric acid production from pap processing waste

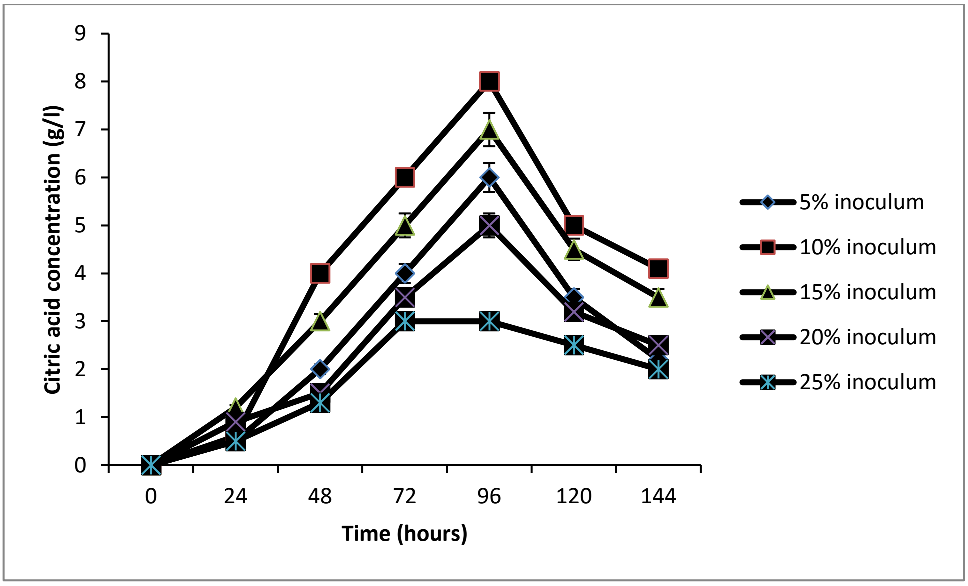

Figure 2 Effect of inoculums size on citric acid production from pap processing waste

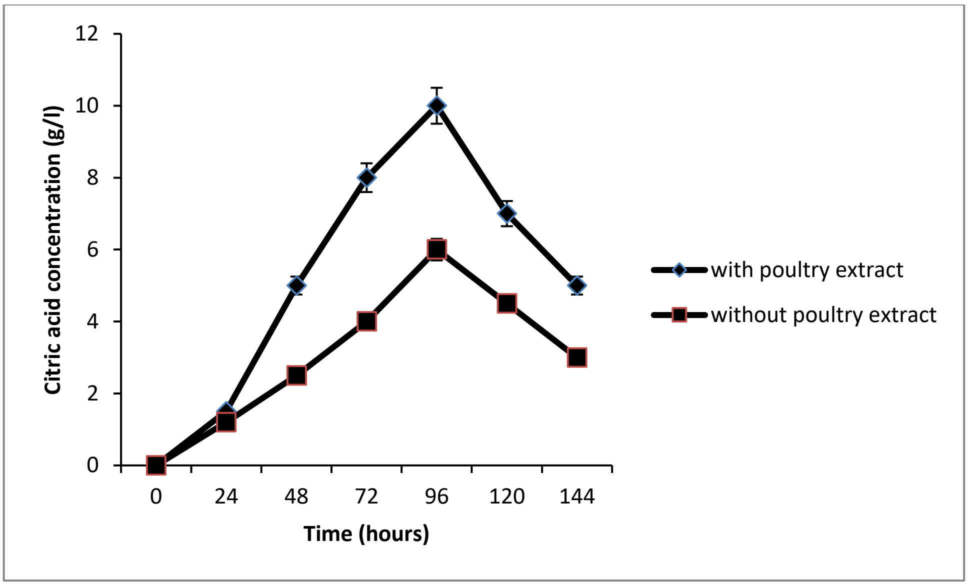

Figure 3 Effect of poultry dropping extract on citric acid production from pap processing waste

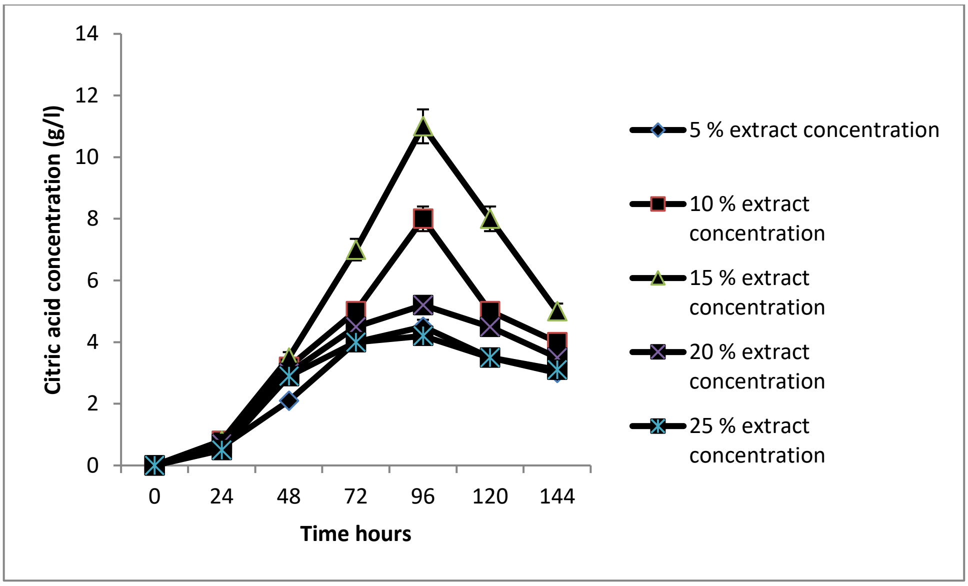

Figure 4 Effect of poultry dropping extract concentration on citric acid production from pap processing waste

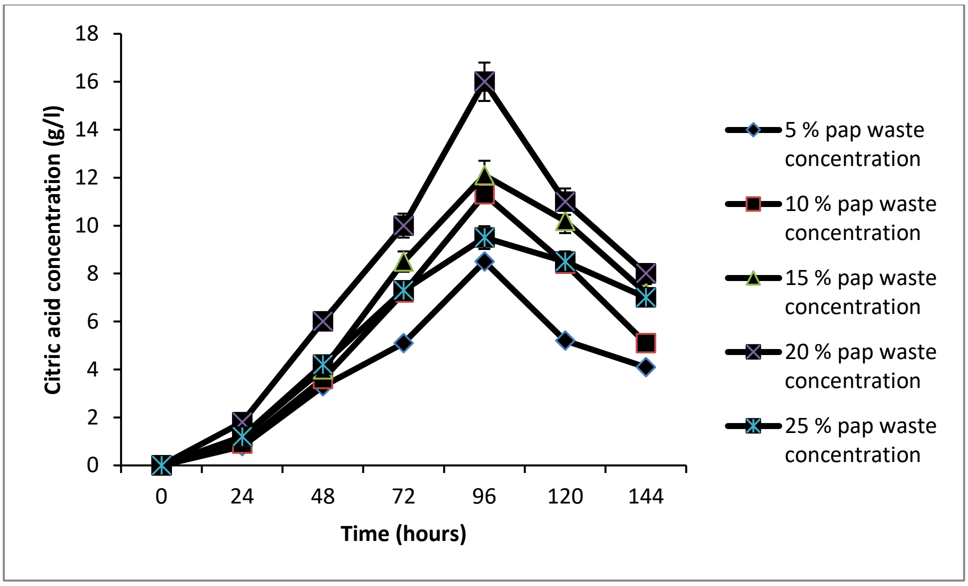

Figure 5 Effect of pap processing waste concentration on citric acid production

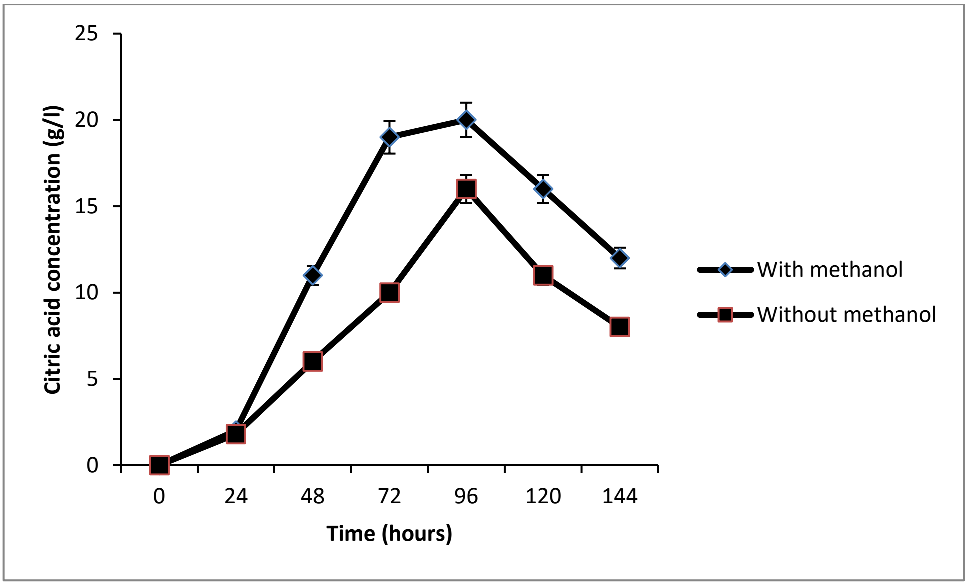

Figure 6 Effect of methanol addition on citric acid production from pap processing waste

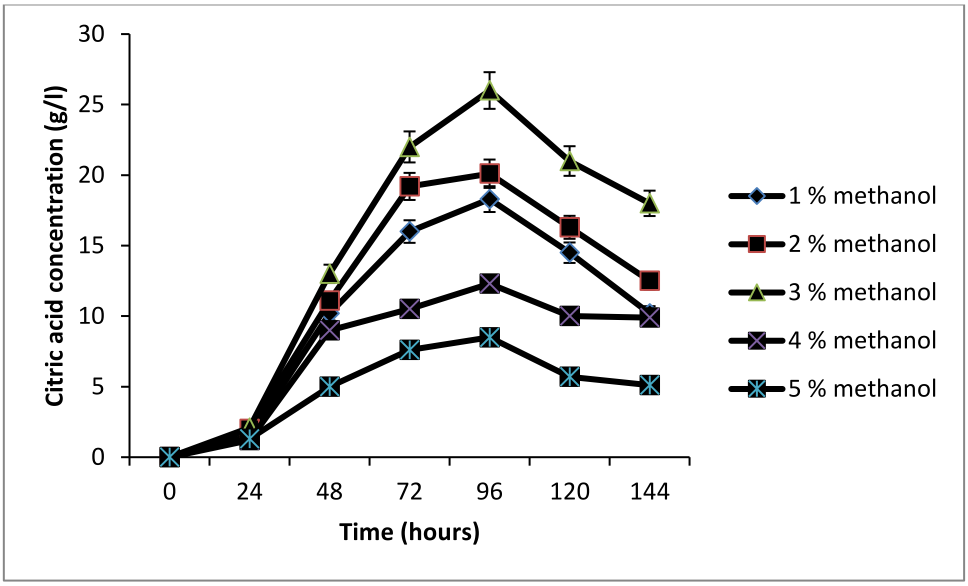

Figure 7 Effect of different concentration of methanol on citric acid production from pap processing waste

4. Conclusion

This study showed that pap processing waste has the potential to be used as substrate in the production of citric acid by *Aspergillus niger* in a submerged culture. However, pap processing waste concentration, initial pH of pap processing waste, inoculums size, poultry dropping extract and methanol addition had significant effect on citric acid production ($p< 0.05$).

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Conflict of Interest

There is no conflict of interest in this work

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