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PRODUCTION OF CITRIC ACID FROM THE PINEAPPLE WASTE BY USING *ASPERGILLUS NIGER*

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ABSTRACT

Citric acid is one of the most important organic acids and is generally used in different industries. Pineapple's peel which had a harder texture and difficult to be eaten, would always ended up as waste. It is anticipated that discarded fruit as well as the waste material can be utilized for further industrial processes like fermentation, bioactive component extraction, etc. The study was carried out to explore the potential of *Aspergillus niger* for citric acid production from the pineapple waste. In the study the effects of various parameters like temperature, pH, carbon sources and nitrogen sources have also been checked for citric acid production. In the study *A. niger* was isolated from the rotten onion on potato dextrose agar and identified by morphological and cultural characteristics. The *A. niger* isolated was further utilize for the production of citric acid using pineapple waste. Pineapple peels were used as a substrate for the production of citric acid by submerged fermentation method. The production was found to be 2.77 g/lit with 41.56 % of citric acid for peels used as a substrate. The maximum production of citric acid was found to be at 25^{o} C temperature, pH 7 with addition of 1 % sucrose and ammonium chloride at 168 hrs of incubation. The present aspect of utilization of pineapple peels for the production of the citric acid can also be helpful in minimizing pineapple waste disposal.

KEYWORDS: Citric acid, pineapple waste, submerged fermentation, *Aspergillus niger*

INTRODUCTION

The pineapple (*Ananas comosus*) is one of the most important fruits in the world and is the leading edible member of the family *Bromeliaceae*. This fruit juice is the third most preferred worldwide after orange and apple juices (Cabrera *et al.*, 2000). The plant can grow up to a height of 75-150 cm with a spread of 90-120 cm. It is short, having a stout stump with narrow, fibrous and spiny leaves. The plant develops to a cone-shaped juicy and fleshy fruit with crown at the top (Morton, 1987; Tran, 2006). Commercially, it is mainly produced as canned fruits and consumed worldwide (Tran, 2006). Besides, it is also processed as juices, concentrates, and jams. Pineapple slices have also been preserved after freezing (Larrauri *et al.*, 1997). Furthermore, bromelain, the proteolytic enzyme present in the stem of pineapple, is finding wide applications in pharmaceutical and food uses (Hebbar *et al.*, 2008).

Tropical and subtropical fruits processing have considerably higher ratios of by- products than the temperate fruits (Schieber *et al.*, 2001). This is mainly due to selection and elimination of components unsuitable for human consumption. Besides, rough handling of fruits and exposure to adverse environmental conditions during transportation and storage can cause up to 55% of product waste (Nunes *et al.*, 2009). These wastes are usually prone to microbial spoilage thus limiting further exploitation. However, low quality fruits do not fetch market and are left on farms. Besides, during pineapple processing, large amount of unusable waste material are generated (Tanaka *et al.*, 1999). Reports have shown that 40 - 80% of pineapple fruit is discarded as waste having high biological oxygen demand (BOD) and chemical oxygen demand (COD) values (Bankoffi and Han, 1990).

Citric acid monohydrate is widely used as organic acid & pH control agent, flavoring and preservative in food production like as candy, cookies, biscuits, jams, jellies, snacks, instant foods and sauces It is used as acidity regulator and antioxidant in beverage such as alcoholic beverage, carbonated soft drink, syrups, juice drinks, tea & coffee, ice-cream, sports & energy drink. Remove metal oxide from surface of ferrous & non ferrous for operational cleaning of iron & copper oxides. It is used as acidifying agent in many cheese products & as an antioxidant in dairy products. (Kanse *et al.*,2017). Mostly *Aspergillus niger* is used for citric acid production due to its ease of handling (Nadeem *et al.*, 2010), its ability to utilize various substrates such as apple, grape pomace, orange peel, kiwi fruit peel, cotton waste (Hildegard Kiel *et al.,* 1981), okara soy-residue, cane molasses (Ali *et al.,* 2002), bagasse, potato residue, wheat bran, coffee husk (Kareem *et al.,* 2010; Sukesh *et al.,* 2013) because of well developed enzymatic system and also produces more citric acid per time unit. The reasons for choosing *A. niger* over other potential citric acid producing microorganisms is its high citric acid productivity at low pH without secretion of toxic metabolites (Nwoba *et al.,* 2012; Haider, 2014).

Different methods of fermentation have been employed by different workers for producing citric acid such as solid-state fermentation, submerged fermentation and surface fermentation (Crolla and Kennedy 2001). Cost reduction in citric acid production can be achieved by using several cheap agricultural wastes such as apple and grape pomace, orange peel, kiwi fruit peel, cotton waste, okarasoy - residue and cane molasses (Kiel *et al.,* 1981; Hang and Woodams, 1986; 1987; Khare. *et al.,* 1995; Haq *et al.,*2004). So, the objective of this study was to use of pineapple peel as a cheap constituents of medium which is readily available in large amount for the production of citric acid by *A. niger*.

MATERIAL AND METHODS

Collection of Sample

Pineapple peels were collected from the Juice Centers of Akola City. These peels were washed with the water and treated with the 70% of alcohol. After washing treated peels were dried and grind into the powder form.

Isolation of *Aspergillus niger*

Isolation of *Aspergillus niger* was done by using onion peel. Firstly the Potato Dextrose Agar was prepared and sterilized in the autoclave at 121^0 C for 15 min. After autoclaving the agar was poured into the plates and allowed to solidify. The suspensions was prepared by taking the onion peels with black growth in sterilized distilled water and poured on potato dextrose agar plates and incubated at room temperature for 3 to 7 days. After incubation period the growth was observed on the potato dextrose agar plates. The pure culture was maintained on PDA slants at 4^o C forfurther work.

Production of Citric Acid

The peels powder (10gm) was added in 250 ml Erlenmeyer flasks. The basal medium was prepared by adding the pineapple peels as a carbon source and mineral salts (chemicals) in the Erlenmeyer flasks. The composition of the chemicals are as follows, $MgSO_4{\cdot}7H_2O$ - 0.15 g/lit, $CaCl_2$ - 0.015 g/lit, $ZnSO_4{\cdot}7H_2O$ - 0.002 g/lit, $MnSO_4{\cdot}H_2O$ - 0.006 g/lit, $FeCl_3{\cdot}6H_2O$ – 0.015 g/lit, (Priscilla and Gnaneel, 2020).

After the basal media was prepared it was autoclaved at 121°C for 15 minutes. After autoclaving the flask was cooled at room temperature, each flask was inoculated with the spore's suspensions of *Aspergillus niger* and incubated at room temperature in a rotary shaking incubator for 7 days. After interval of 24 hours the 10 ml of fermentation medium was removed of and check for the citric acid production by the titration method. Effect of temperature, pH, carbon sources and nitrogen sources were also studied.

Determination of citric acid by titration method

In order to determine the citric acid produced after fermentation, 0.1 N NaOH was titrated against the 10ml sample using phenolphthalein as an indicator till appearance of pink colour. After the titration the reading was noted and percentage of citric acid production was calculated by the following formula (Meehan *et al.,* 2019).

$$\text{Normality of Citric acid} = \frac{[\text{Normality of NaOH x NaOH volume}]}{\text{Volume of sample}}$$

$$\text{Citric acid concentration in \%} = \frac{[\text{Citric acid normality x 100 x 96}]}{\text{Volume of sample}}$$

$$\text{Citric acid concentration in g/lit} = \frac{[\text{Titre} \times 100 \times 10 \times 0.0064]}{\text{Volume of sample}}$$

Effect of temperature on citric acid production

Effect of temperature on citric acid production was studied. The basal medium was prepared by adding the peels powder and chemicals. Then it was autoclaved it at 121^oC for 15 min and cool it at the room temperature. The medium was inoculated by spore suspension of *A. niger* and incubate it at temperature 25^oC, 37^oC and 40^oC respectively in a rotary shaking incubator for 7 days. After interval of 24 hours the 10 ml of fermentation medium was removed of and checked for the citric acid production by the titration method. After the titration the reading was noted and percentage of citric acid production was calculated.

Effect of pH on citric acid production

Effect of pH on citric acid production was studied. The basal medium was prepared by adding the peels powder and chemicals. The pH of the medium was maintained at pH 5, 6, 7 and 8 respectively by adding the 0.1 N NaOH and 0.1 N H_2SO_4 solutions in it. It was autoclaved it at 121^0 C for 15 min and cooled it at the room temperature. Then inoculated the medium by spore suspension of

A. niger and incubated at the room temperature in a rotary shaking incubator for 7 days. After interval of 24 hours the 10 ml of fermentation medium was removed of and check for the citric acid production by the titration method. After the titration the reading was noted and percentage of citric acid production was calculated.

Effect of carbon sources on citric acid production

To study the impact of different carbon sources on citric acid. The basal media were supplemented with 1 % of glucose, sucrose and mannitol respectively in the medium along with peels and chemicals. It was autoclaved at 121^0 C for 15 min and cooled at the room temperature, then inoculated by spore suspension of *A. niger* and incubated at the room temperature in a rotary shaking incubator for 7 days. After interval of 24 hours the 10 ml of fermentation medium was removed of and check for the citric acid production by the titration method. After the titration the reading was noted and percentage of citric acid production was calculated.

Effect of nitrogen sources on citric acid production

The effect of various nitrogen sources on the fermentation was studied by incorporating the 1 % of ammonium sulfate, ammonium chloride and urea in addition to peels and basal medium. It was then autoclaved at 121^0 C for 15 min and cooled at the room temperature, then inoculated by spore suspension of *A. niger* and incubated at the room temperature in a rotary shaking incubator for 7 days. After interval of 24 hours the 10 ml of fermentation medium was removed of and check for the citric acid production by the titration method. After the titration the reading was noted and percentage of citric acid production was calculated.

Recovery of citric acid

After completion of the fermentation process, the fermented broth was filtered for the separation of pellet of fungal culture. Lime was added to the fermentation broth to allow the precipitation of citric acid in the form of calcium citrate. After that, the precipitate was treated with dilute sulphuric acid (H_2SO_4) to obtain the precipitated solution and then filtered. The solution obtained was then evaporated for getting purified citric acid in crystal form (Cholke *et al.*, 2019).

RESUTLS AND DISCUSSION

In the study *A. niger* was isolated from the rotten onion on potato dextrose agar and identified by morphological and cultural characteristics. The *A. niger* isolated was further utilized for the production of citric acid using pineapple waste. Pineapple peels were used as a substrate for the production of citric acid by submerged fermentation method. The production was found to be 2.77 g/lit with 41.56 % of citric acid for peels used as a substrate.

In the study the effects of various parameters like temperature, pH, carbon sources and nitrogen sources have also been checked for citric acid production. The citric acid production on a various temperature 25°C, 30°C and 40°C and its effects on it were analyzed. It was found that the maximum citric acid production was achieved at 25°C yielding 2.04 g/lit of citric acid after 24 hrs while it was 3.64 g/lit after 72 hrs, 3.71 g/lit after 120 hrs, 3.77g/lit after 168 hrs. At 37°C the yield of citric acid was 1.47 g/lit, 1.81 g/lit, 2.77 g/lit, and 2.88 g/lit, after 24 hrs, 72 hrs, 120 hrs and 168 hrs respectively. While at 40° C the citric acid production was less which was 1.53 g/lit, 1.98 g/lit, 2.56 g/lit, 2.75 g/lit, at the time interval of 24 hrs, 72 hrs, 120 hrs and 168 hrs respectively. The citric acid production on a various pH 5, 6, 7 and 8 and its effects on it was also analyzed. It was found that the maximum citric acid production was achieved at pH 7 yielding 1.92 g/lit of citric acid after 24 hrs while it was 2.49 g/lit after 72 hrs, 3.71 g/lit after 120 hrs, 4.03 g/lit after 168 hrs. At pH 5 the yield of citric acid was less which were 1.47 g/lit, 1.72 g/lit, 2.04 g/lit, and 2.17 g/lit, after 24 hrs, 72 hrs, 120 hrs and 168 hrs respectively. At pH 6 the yield of citric acid was 1.60 g/lit, 2.36 g/lit, 2.75 g/lit and 3.07 g/lit, after 24 hrs, 72 hrs, 120 hrs and 168 hrs respectively. While at pH 8 the yield of citric acid was 1.66 g/lit, 1.98 g/lit, 2.36 g/lit and 2.62 g/lit after 24 hrs, 72 hrs, 120 hrs and 168 hrs respectively. The effect of various carbon sources glucose, sucrose and mannitol on citric acid production was also checked. It was found that the maximum citric acid production was achieved at sucrose yielding 2.36 g/lit of citric acid after 24 hrs while it was 3.26 g/lit after 72 hrs, 4.09 g/lit after 120 hrs and 4.22 g/lit after 168 hrs. At mannitol the yield of citric acid was 3.26 g/lit, 3.58 g/lit, 3.90 g/lit and 3.96 g/lit, after 24 hrs, 72 hrs, 120 hrs and 168 hrs respectively. While at glucose the yield of citric acid was less which was 2.88 g/lit, 3.13 g/lit, 3.39.g/lit and 3.52 g/lit, after 24 hrs, 72 hrs, 120 hrs and 168 hrs respectively. The effect of various nitrogen sources such as ammonium chloride, ammonium sulfate and urea and its effects on it were analyzed. It was found that the maximum citric acid production was achieved at ammonium chloride yielding 4.48 g/lit of citric acid after 24 hrs while it was 5.10 g/lit after 72 hrs, 5.50 g/lit after 120 hrs and 5.60 g/lit after 168 hrs. At ammonium sulfate the yield of citric acid was 4.30 g/lit, 4.80 g/lit, 5.24 g/lit and 5.37 g/lit after 24 hrs, 72 hrs, 120 hrs and 168 hrs respectively. While at urea the yield of citric acid was less which were 1.60 g/lit, 2.30 g/lit, 2.88 g/lit and 3.00 g/lit after 24 hrs, 72 hrs, 120 hrs and 168 hrs respectively.

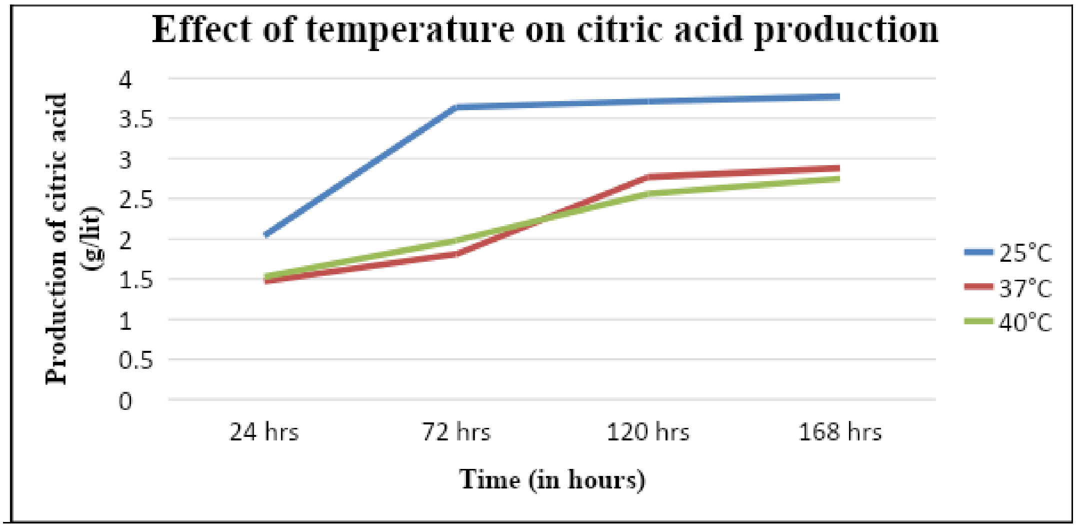

Fig 1: Effect of temperature on citric acid production.

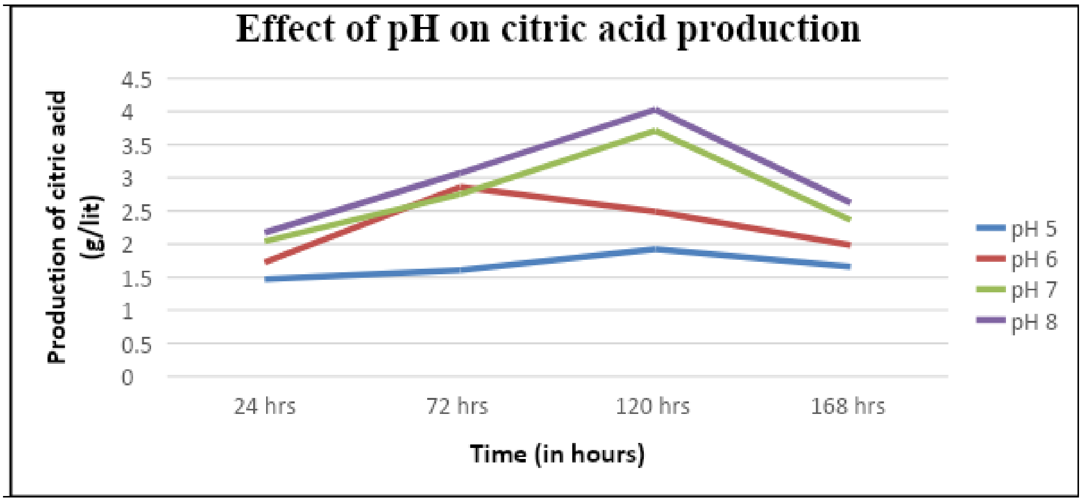

Fig 2: Effect of pH on citric acid production.

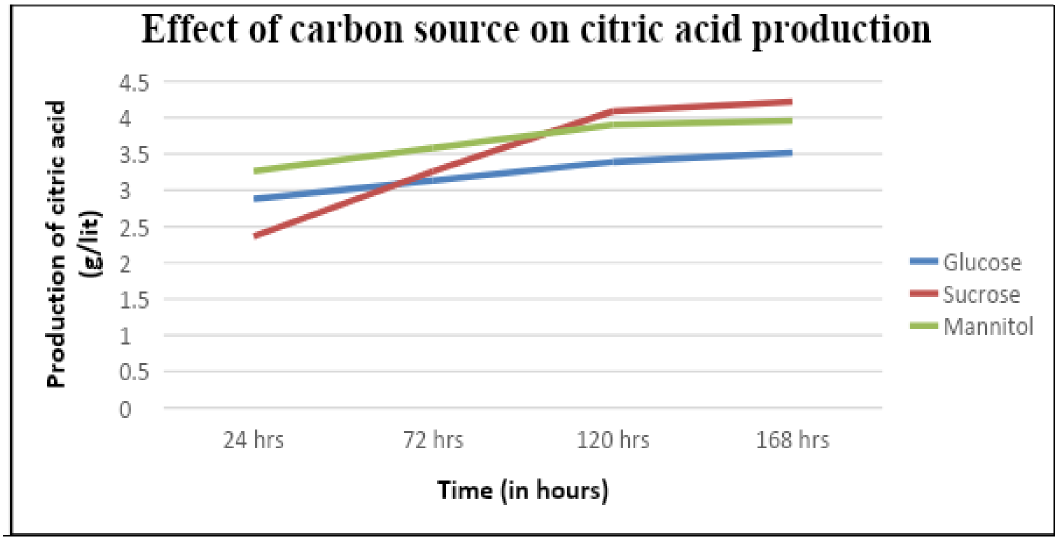

Fig 3: Effect of carbon sources on citric acid production.

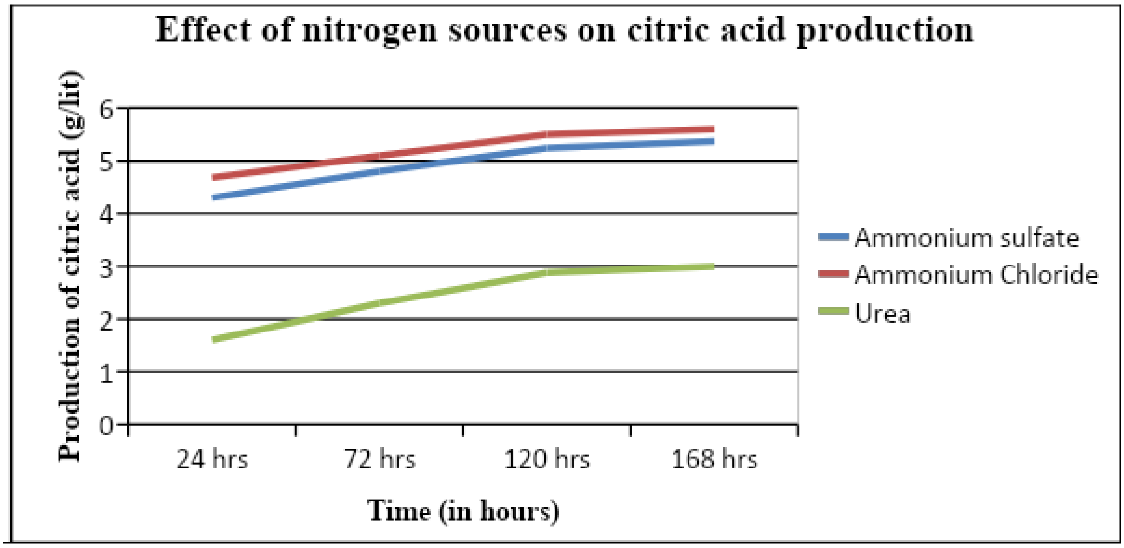

Fig 4: Effect of nitrogen sources on citric acid production.

Production medium for citric acid production

Estimation of citric acid by titration method

DISCUSSION

Citric acid production has been shown to be viable with many cheap agricultural raw materials (Pawar and pawar, 2014). In the present study also it was achieved by using a pineapple peels as a source and *A. niger* as an inoculum. It was found that 41.56 % (2.77 g/lit) production was observed which make a pineapple peels as a important source for citric acid. Subramaniyan *et al.*, also get 6.29 % of production from pineapple peels and *A. niger*. In the present study the citric acid was produced from the pineapple peels using *A. niger*. The production was determined by titration method. The influence of various physical and chemical parameters was also checked like effect of temperature, pH, carbon and nitrogen sources.

The effect of temperature on the production of citric acid was studied (Fig 1). It was found that at 25^o C maximum yield of citric acid was obtained which was 3.77 g/lit above this temperature 37^o C and 40^o C the citric acid production was decreased, This is similar with the study of Cholke *et al.*, (2019) who also reported the maximum production of citric acid (1.06 g/lit) and further increase in the temperature lowers the production. Chergui (2021) reported 30^o C as optimum temperature for maximum citric acid production. Sarkar *et al.*, (2017) also found 30^o C as a best temperature for maximum citric acid production and supported that 40^o C the citric acid production was less. The decrease in the citric acid production might be due to denaturation of the enzymes present in the *A. niger*. The pH also play important role in production of citric acid and in view of this the effect of pH on the production was also studied (Fig 2). It was noted that at pH 7 maximum production of citric acid 4.03 g/lit (60.48 %) was obtained. Above and below this pH the citric acid production was decreased. Our results are in concordance with the other studies who reported maximum citric acid production at acidic pH (Ayeni *et al.*, 2019, Vendenberghe *et al.*, 1999, Papaginni 2007). Cholke *et al.*, (2019) reported the maximum production of citric acid at pH 8. The citric acid production by *A. niger* from pineapple peels with different carbon sources such as glucose, sucrose and mannitol was shown in (Fig 3). In the present study it was found that supplementation of sugar increases the production of citric acid, it was observed from the results that the sucrose addition increases the production of citric acid than glucose and mannitol. This is in agreement with other studies as Sarkar *et al.*, (2017) also reported the same results. Drysdale and Mckay, 1995; Xu *et al.*, (1989) has also suggested that sucrose is the traditional commercial substrate for the production of the citric acid, although the other sugars like glucose also used for the production. This is may be due to relative low molecular weight of the sucrose and that's why it can be readily transported in microbial cells for hydrolysis by intercellular enzymes. Kareem *et al.*, (2010) also showed that sucrose increases citric acid production more than glucose in their work with pineapple waste substrate. Nitrogen has a profound effect on citric acid production as it is not only important for metabolic rates in the cells but is also a basic part of cell proteins and was shown to induce pellet formation in filamentous fungi (Ali *et al.*, 2002). Nitrogen has been reported to be an important factor in fermentation processes due to an increase in C/N ratio (Kareem and Rahman, 2011; Patil and Patil, 2014). The present study shows that the supplementation of media with 1 % of ammonium chloride and ammonium sulfate increases the production significantly, addition of ammonium chloride gives maximum yield at 84.48 % (5.60 g/lit) and ammonium sulfate gives the 80.64 % (5.37 g/lit) while addition of the urea slightly increases the production but not as significantly as the other two sources. The findings of present study are comparable to others as Kudzai *et al.*, (2015) also reported increase in the production of citric acid to 5.02 g/lit at 168 hrs of incubation. Kareem *et al.*, (2010); Subramaniyan *et al.*, (2019); Sarkar *et al.*, (2017) also reported the increase in production of citric acid by ammonium sulfate.

CONCLUSION

It was concluded from the study that the pineapple peels were the prominent source for the production of citric acid. The maximum production of citric acid was found to be at 25^o C temperature, pH 7 with addition of 1 % sucrose and ammonium chloride at 168 hrs of incubation. This aspect of utilization of the pineapple peels for the production of the citric acid is found not only promising for producing useful product from the waste but will also be helpful in minimizing pineapple waste disposal, cost management and pollution related problems to the society.

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