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PRODUCTION OF CITRIC ACID FROM DIFFERENT AGRICULTURAL WASTE USING *Aspergillus niger*

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ABSTRACT

The demand of citric acid in various industries especially in food industries is growing continuously. Various techniques for production of citric acid in large scale were searched and still continuously studies are going on. However, there are still some very efficient manners by which we can achieve production of citric acid which are ecofriendly too. In this study some agricultural waste / fruit peels such as Banana peels, Rice straw and coconut husk are used as a substrate for production of citric acid by using of *Aspergillus niger*. The above substrates are very commonly available in our surroundings. In this method solid state fermentation has been performed by using *Aspergillus niger* as the inoculums. Satisfactory results obtained by using of simple filtration and recovery methods. The bio production of citric acid by this method will result in very effective means of waste management.

KEYWORDS: Solid State Fermentation, Citric acid, Banana peels, Rice straw and coconut husk

Many organic acids such as citric acid, lactic acid, fumaric acid, itaconic acid and succinic acid etc, are very important microbial product. However, among these citric acid is widely used because of its low toxicity compared with other acidulates (Radwan *et al.*, 2010). Biological name of citric acid is 2-hydro-1,2, and 3-propane tricarboxylic acid. The name citric is derived from latin word citrus. As per the literature the first isolation of citric acid from lemon juice was carried out in 1784 by Carl Scheele (Scheele *et al*., 1784). Citric acid is found in many fruits and vegetables such as orange, banana, pineapple, tomato, lemon etc. citric acid is one of the most organic acid which is used in worldwide with a demand of about $6.0x10^5$ tons per year (Karaffa, and Kubicek, 2003). It was also reported that 70% citric acid is used in commercial purpose in food and 12% in pharmaceuticals industry and 18% citric acid is used in other industry (Haq *et al*., 2001).The fulfillment of the global requirement is a challenge.

It has been known that many microorganisms have the ability to produce citric acid. Some mold and yeast strains are also known producer of citric acid from n-alkanes and carbohydrates (Mattey 1999). However, it is reported in literature some specific fungus are suitable producer of citric acid production especially *Aspergillus niger* (Usami *et al.*, 1977; Steinbock *et al*., 1991). *Aspergillus niger* a haploid fungus, it produces black mass of spore (conidia). It is mostly found in chain form arising from secondary sterigmata. *Aspergillus niger* produces citric acid by mostly utilizing starchy and sugar substrate (Kristiansen, *et al.*, 1978; Lakshmi narayana, *et al*., 1975). It is preferred over other fungus because of its high yielding and high tolerance potential to acid accumulation (Pandey*et al.*, 2013). Many other strain of *Aspergillus niger* are *A. awamori, A. nidulans, A. fonsecaeus, A. wentii, A. flavus etc.* have also exploited for citric acid production (Yokoya, 1992 and Papagianni, 2007). It has been seen that Solid State Fermentation (SSF) has been prefered by many researcher and in many studied for citric acid production (Kareem *et al*., 2010, Ali *et al.*, 2011, Iralapati*et al*., 2014). SSF was first discovered by Cahn in 1935. SSF was first developed in Japan and it is also widely used for the raw materials such as fruit wastes and rice bran. It is reported in literature SSF is one of the simplest and effective methods for production of citric acid utilizing agro-industrial wastes (Pandey*et al.*, 1992a, 1992b; Soccol and Vandenberge, 2003).

Citric acid has many applications and is very useful in many areas such as food industry, pharmaceutical industry and cosmetic industry. Citric acid has also been used as flavoring, enhancer, preservative, antioxidant and emulsifier and chelating agent (Rohr, 1998). Based upon the global need for Citric acid, the vast production of Citric acid is of foremost requirement. At the same time utilization of some of the biological waste products and microorganisms for Citric acid is most promising. It fulfils two broad prospective first one production of Citric Acid & second one is the utilization

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of environmental waste. The waste materials disposed from human activities such as Coconut husk, fruits peels & pulp and sugarcane bagasse etc can be used for citric acid production. It has also been known from the literature that citric acid can be produced from these wastes by utilizing some of the specific microorganisms (Bazalwar, *et al*., 2013). Therefore, our present study is focused on carry out comparative study on production of citric acid from rice straw, banana peels and coconut husk by utilizing the fungus *Aspergillus niger*.

MATERIALS AND METHODS

Isolation and Preparation of Inoculums

Isolation of *Aspergillus niger* was done by using soil sample.Soil was collected from different site. *Aspergillus niger* was isolated with help of serial dilution. Diluted tubes were inoculated on potato dextrose agar media and incubated at 27C under incubator for few days. After 3 days of incubation colonies with spore were observed.

Cotton Blue Staining

by cotton blue staining method colonies are identified as *Aspergillus niger*. Inoculum was maintained on potato dextrose agar slants, for citric acid production

Collection of Substrate

Substrate such as Ripe Banana peels; Rice straw and Coconut husk were procured from local market.

Pre-treatment of Substrate

The collected substrate were over dried at 60^0C for 2hr for dry substrate and used for production of citric acid. Substrate were cut in to small pieces and grind in to the grinder, used for fermentation to produce citric acid.

Preparation of Fermentation Media

Fermentation: Solid substrate fermentation is carried out to produce citric acid

Preparation of Media

The basal medium was prepared by introducing different dry substrate in different concentration (5grm, 10grm and 15grm) into separate 100ml Erlenmeyer flask. The medium was supplemented with nitrogen supplement and by adding of ammonium phosphate, potassium hydrogen phosphate and peptone to the basal medium. The flask was cotton plugged and autoclaved at 151lbs for 15minutes. After cooling at room temperature each medium was inoculated with *Aspergillus niger* dilution suspension and incubated in rotary shaker at 30^0C for different days (7^{th}, 8^{th} and 9^{th} days).

Effect of Variables on Citric Acid Production

Effect of pH was investigated on citric acid production. The range of pH investigated was 7, 8, 9 .and thetemperature was 30^oC . The citric acid production was studied at different fermentation periods such as days 7, 8, and 9. The total titratable acidity was also determined by 0.1N NaOH. The effect of different concentration 5gm, 10gm and 15gm of the substrate was carried out and inoculums size $6.0X10^6$ of the substrate was also studied. All experiments were incubated in a rotary shaking incubator.

Filtration

The medium was diluted with sterile distilled water and then filtered through sterile paper to get filtrate.

Citric Acid Determination

Citric acid was determined titrimentrically by using 0.1 NaOH and phenolphthalein as indicator and calculated according to the formula

$\mathbf{N_1V_1=N_2V_2}$ (N_1= Normality of 0.1NaoH, V_1= Volume of NaOH, N_2= Normality of citric

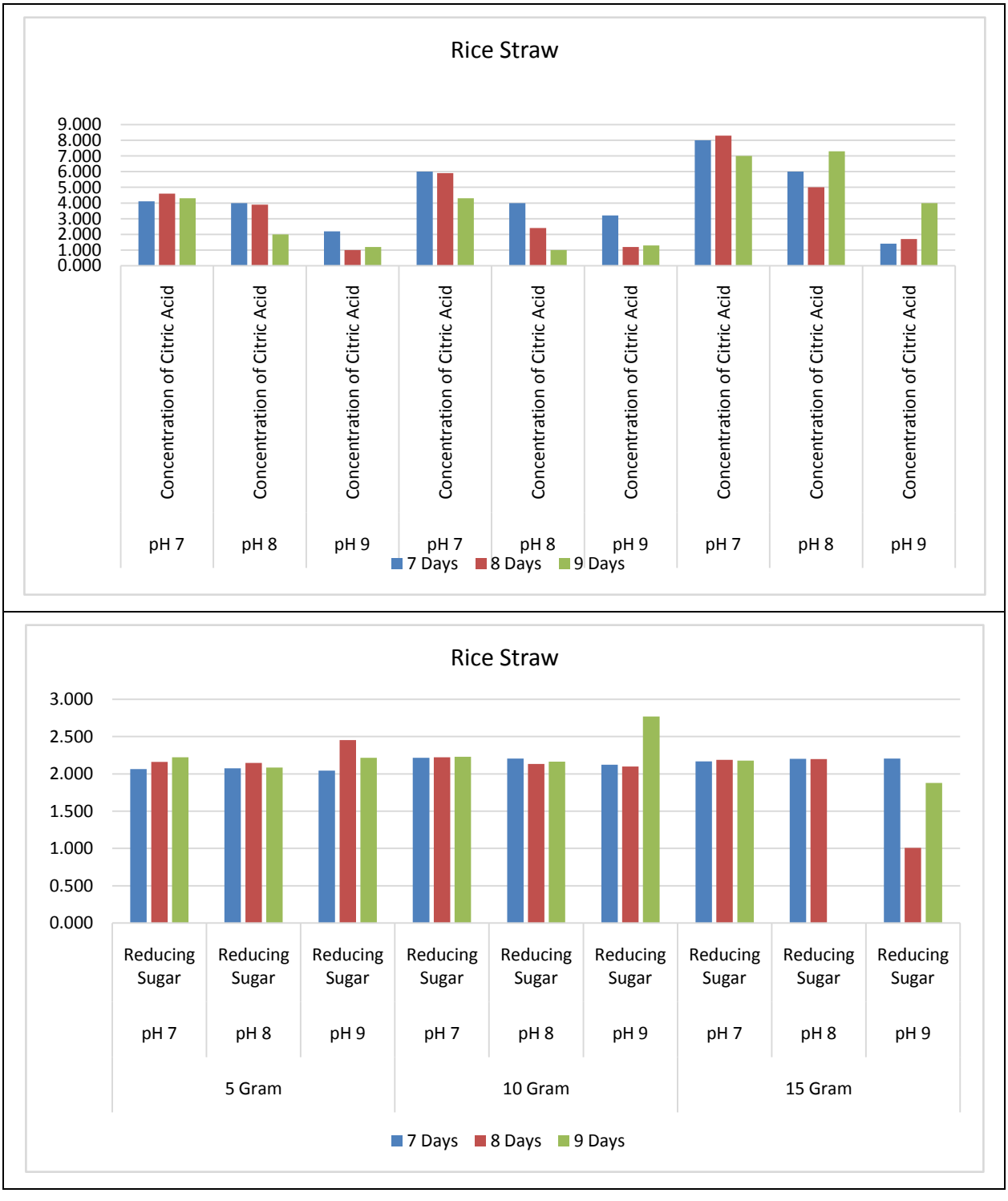
Acid, V_2= Volume of citric acid)

Estimation of reducing sugar

Reducing sugar was estimated by phenol sulphuric acid method.

RESULTS


Banana Peels
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Concentration of Citric Acid
pH 7
pH 8
pH 9
5 Gram
10 Gram
15 Gram
7 Days
8 Days
9 Days
Banana Peels
2.500
2.000
1.500
1.000
0.500
0.000
Reducing Sugar
pH 7
pH 8
pH 9
5 Gram
10 Gram
15 Gram
7 Days
8 Days
9 Days


Rice Straw
9.000
8.000
7.000
6.000
5.000
4.000
3.000
2.000
1.000
0.000
Concentration of Citric Acid
pH 7
pH 8
pH 9
7 Days
8 Days
9 Days
Rice Straw
3.000
2.500
2.000
1.500
1.000
0.500
0.000
Reducing Sugar
5 Gram
10 Gram
15 Gram

The utilization of Coconut husk, Banana peels and Rice straw as substrate for citric acid production by *Aspergillus niger* was evaluated and the impact of initial pH on citric acid production was explored. It has been concluded that, increase in pH brought about decrease in citric acid production. Maximum yield of was obtained at pH 7. It has also been shown that any further increase in pH results in decrease in citric acid production.

The substrate concentration optimization for citric acid accumulation by the isolates was studied and the result indicated that the maximum yield of the citric acid production was obtained at 15gm of the substrate concentration. The results for different substrate levels

and their rate of citric acid and reducing sugar yields are shown in table.

The quantity of citric acid produced varies with the fermentation conditions. To determine the effect of fermentation period, fermentation was carried out for various time periods (7–9 days). In the current experiment, the rate of citric acid biosynthesis is shown in table. Production of citric acid increases with the increase in the fermentation time. The maximum yield of citric acid produced was nine (9) days after fermentation. The optimal time of incubation for maximum citric acid production varies with the fermentation conditions. The rate of citric acid biosynthesis was studied and the maximum yield of citric acid was after 7 days of fermentation

CONCLUSION

The study has shown that *Aspergillus niger* can produce citric acid by using and coconut husk, banana peels and rice straw as potential materials. By utilizing above materials which is commonly available, production of citric acid in large scale is possible.

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