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COMPARATIVE STUDIES ON CITRIC ACID FERMENTATION BY PARENT AND MUTANT ASPERGILLUS NIGER FROM SOME AGRICULTURAL WASTES USING SUBMERGED FERMENTATION

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ABSTRACT

Citric acid fermentation by parent and mutant Aspergillus niger from some agro-industrial wastes (wheat bran, rice bran and orange pulp) was studied. The study involved the improvement of a cellulase producing A. niger isolate by exposure to ultraviolet irradiation for 45 minutes using Phillips germicidal lamp, the selection of mutant based on the method of Kubicek et al. (1986) and citric acid fermentation from the aforementioned agro-industrial wastes by the selected mutant and the parent isolate using submerged fermentation. The results obtained show that submerged fermentation of orange pulp by mutant A. niger produced the highest yield of 14.70g/l. Other yields obtained in descending order are, 13.78g/l obtained from fermentation of wheat bran by mutant A. niger, 12.39g/l obtained from fermentation of orange pulp by parent, 11.85g/l obtained from fermentation of rice bran by mutant A. niger, 9.27g/l obtained from fermentation of wheat bran by parent A. niger and 9.08g/l obtained from fermentation of rice bran by parent A. niger. The average length of fermentation that gave the highest citric acid yield was 144h (six days). In all cases the mutant A. niger produced more citric acid than the parent A. niger. However, more citric acid was produced from orange pulp by parent A. niger than from rice bran by mutant A. Niger.

***Keywords:** Citric Acid, Aspergillus Niger, Parent, Mutant, Submerged Fermentation, Wheat Bran, Rice Bran and Orange Pulp.*

INTRODUCTION

Citric acid is the most important organic acid produced in tonnage by fermentation. (Sharifzadeh *et al.*, 2008). Citric acid is a versatile and innocuous alimentary additive. It is accepted world wide as GRAS (generally recognized as safe), approved by the Joint FAO/WHO Expert Committee on Food Additives (Schuster *et al.*, 2002). Global production of citric acid in 2004 was about 1.4 million tonnes estimated by Business Communities Company (BCC) in a recent study of fermentation chemicals markets. Mutant strains of *Aspergillus niger* are employed in commercial citric acid production. (Lofty *et al.*, 2007). Citric acid is widely used to impart a pleasant taste, tart flavour to foods and beverages. It is also used in the manufacture of detergents, pharmaceuticals, cosmetics and toiletries. A variety of agro-industrial residues and by-products such as cassava bagasse, coffee husk, wheat bran, apple pomace, pineapple waste, grape pomace, citrus waste *etc* have been invested with solid state fermentation techniques for their potential to be used as substrates for citric acid production (Soccol *et al.*, 2006, Papagianni, 2007, Barrinton and Kim, 2008). However, there is a dearth of literature information on the use of submerged fermentation for the production of citric acid from most agro-industrial residues including the wheat bran, rice bran and orange pulp used in this study. This study was aimed at contributing to ongoing research on finding alternative and cheaper sources of raw materials for citric acid production other than the conventional molasses.

MATERIALS AND METHODS

Organism and culture maintenance

A cellulase producing *A. niger* isolate obtained from the Microbiology department, Ahmadu Bello University, Zaria, Kaduna state, was used for this work. This isolate was further improved by exposure to ultraviolet irradiation for 45 minutes using Phillips germicidal lamp. The mutants were selected based on the method of Kubicek *et al.* (1986). Their identification was based on the assumption that mutants lacking citrate control of phosphofructokinase would grow faster on a sucrose medium containing citrate. The diluted mutagenized spore suspensions were plated on solid medium containing 20% (w/v) sucrose and 5% (w/v) citrate as carbon sources. Other constituents of the medium include 0.93g ammonium nitrate (NH_4NO_3). 0.045g potassium dihydrogen phosphate (KH_2PO_4). 0.66g magnessium sulphate heptahydrate ($MgSO_4.7H_2O$) and 3% methanol (Lasure *et al.*,2003). The pH of the medium was adjusted to 3.5 with 1M HCl.

The fastest-growing colonies were considered to be less affected in carbohydrate breakdown by citrate and thus selected for further work.

Preparation and Proximate Analysis of the Agricultural wastes

The wheat bran used in this work was supplied by Ideal Flour Mills, Kaduna. The rice bran was obtained from a small scale Rice Milling Industry in Pilgani, Langtang North Local Government Area of Plateau state. The orange pulp was obtained by expressing the juice between the palms of the hands. The pulp was removed, washed, sun-dried and ground using pestle and mortar. All the wastes were sieved to obtain fine particles.

Proximate analysis of the wastes was carried-out at the Biochemistry Laboratory, Industrial Chemicals Research Unit of the National Research Institute for Chemical Technology (NARICT), Zaria, Kaduna state. The parameters determined were percentage carbohydrate, protein, lipids, crude fibre, ash and moisture.

Inoculum Preparation

The spores of the isolates were harvested from slant bottles of 4-6 days old cultures by washing with sterile distilled water containing 0.8% Tween 80(Poolyoxyethylene- sorbitanmonooleate) and enumerated using a haemocytometer (Lasure *et al.*, 2003).

Fermentation Technique

All fermentations were carried-out in 500ml flasks. The fermentation medium for the submerged fermentation (SMF) consisted of 20g of each waste, 4% glucose, 0.93g ammonium nitrate (NH_4NO_3), 0.045g potassium dihydrogen phosphate (KH_2PO_4), 0.66g magnessium sulphate heptahydrate ($MgSO_4.7H_2O$) and 3% methanol (Lasure *et al.*,2003).

Submerged fermentation (Shake flask)

All the wastes were subjected to submerged fermentation in shake flask. The spore inoculum used was 10^7 spores per ml. Agitation rate and length of fermentation used were 450 rpm (on orbital shaker) and 168h (7 days) respectively. Potassium ferrocyanide at a concentration of 0.66g was added to the fermentation medium to reduce the deleterious effect of trace metals on citric acid yield while refined vegetable oil was added as an antifoam agent. The fermentation medium consisted of 300ml of distilled water. Citric acid yield, residual sugar and dry mycelia weight were determined daily as described by Al- Delaimy and El-Holi, 2003.

Analytical method

Citric acid was determined daily by filtering 10ml of the culture medium through Whatman filter paper no. 41. Two to three drops of phenolphthalein as indicator was added and the filtrate was titrated against 0.1M NaOH and calculated as % according to the following formula (British Pharmacopea, 1973; Al- Delaimy and El-Holi, 2003):

$$\% \text{ Citric acid} = \frac{192.13 \times M_{NaOH} \times V_{NaOH}}{\text{Weight of substrate}}$$

Where,

192.13 = molar mass of citric acid.

M_{NaOH} = molarity of NaOH

V_{NaOH} = volume of NaOH consumed during titration.

Dry mycelia weight was determined as described by Al – Delaimy and El-Holi, (2003), Haq *et al.*, (2003).

Residual sugar was determined from the filtrate obtained above using the 3, 5- Dinitrosalycilic acid (DNS) method (Miller, 1959).

RESULTS

Proximate analysis

Table 1 shows result of the proximate analysis obtained from the wastes. Orange pulp (68. 50%) and rice bran (62.99%) have the highest combined carbohydrate and fibre content compared to wheat bran (62.65%).

Table 1: Proximate analysis of wheat bran, rice bran and orange pulp

Wastes	% moisture content	% ash content	% fat content	% crude protein	% crude fibre	% carbohydrate
Wheat bran	9.87	18.30	3.80	5.38	27.72	34.93
Rice bran	14.44	4.83	1.30	16.44	9.33	53.66
Orange pulp	21.79	3.76	0.70	5.25	9.47	59.03

Citric acid yields by parent and mutant *A. niger* from the three substrates

Figure 1 shows comparison of citric acid yield from wheat bran, rice bran and orange pulp by parent and mutant *A. niger* during submerged fermentation. The average citric acid yields in descending order is 14.70g/l obtained from fermentation of orange pulp by mutant, 13.78g/l obtained from fermentation of wheat bran by mutant, 12.39g/l obtained from fermentation of orange pulp by parent, 11.85g/l obtained from fermentation of rice bran by mutant, 9.27g/l obtained from fermentation of wheat bran by parent and 9.08g/l obtained from fermentation of rice bran by parent. The average length of fermentation that gave the highest citric acid yield was 144h (six days) in all cases.

DISCUSSION

There was significant difference in the mean citric acid yield from all the agricultural wastes between the parent and mutant *A. niger,* with mutant strain showing improved citric acid yields ($P<0.05$). More citric acid was obtained from the submerged fermentation of orange pulp by parent *A. niger* than the from rice bran by mutant *A. niger.* This may be because orange pulp has the highest quantity (68.50%) of the portions (carbohydrate and crude fibre) that can be fermented to citric acid while wheat bran contain 62.65% and rice bran contain 62.99% (Table 1).

There is a dearth of information on citric acid production by submerged fermentation from the agricultural wastes used in this work. However, Ikenebomeh and Oshoma (2002), reported a yield of 3.65g/l from submerged fermentation of rice bran using mutant *A. niger*. The yields obtained from the submerged fermentation of the three wastes studied in this work is higher than that reported for two agro-industrial wastes, a citric acid yield of 0.050g/l from undersized semolina (a wheat waste) was reported by Erkman and Alben (2004) and a yield of 2.86g/l from waste bread was reported by Lodhi *et al* (2001).

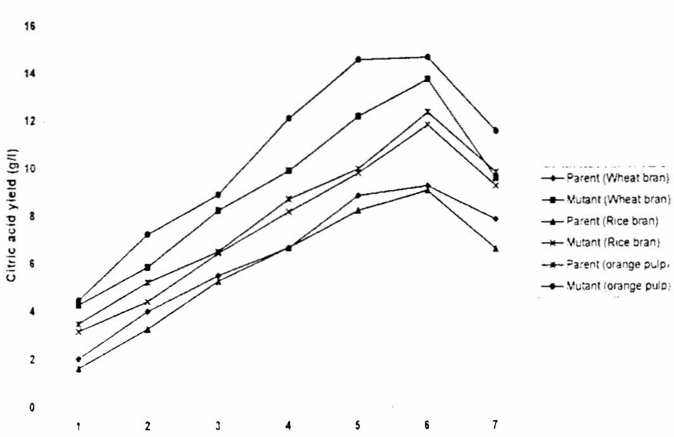

Figure 4 15: Comparison of citric acid yield from wheat bran, rice bran and orange pulp by parent (CP3) and mutant *A. niger* (M45) during submerged fermentation

CONCLUSION

In all cases the mutant *A. niger* produced more citric acid than the parent *A. niger*. However, more citric acid was produced from orange pulp by parent *A. niger* than from rice bran by mutant *A. Niger*. With further strain improvement studies the *Aspergillus niger* strain used in this work has the potential for used on a larger scale for citric acid production by submerged fermentation of the three agricultural wastes.

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