Nigerian Journal of Microbiology, Vol. 25: 2397 – 2402 2011
www.nsmjournal.org

EFFECT OF SUGAR SUPPLEMENTATION ON CITRIC ACID PRODUCTION FROM ORANGE PULP BY ASPERGILLUS NIGER USING SUBMERGED FERMENTATION

DASHEN M. M.[1], S. A. ADO [2], J. B. AMEH[2], J. D. MAWAK[1]
1. Dept. of Microbiology, Faculty of Natural Sciences, University of Jos, P.M.B. 2084 Plateau state. Tel.08035165995. E-mail macvrendashen@yahoo.co.uk
2. Dept. of Microbiology, Faculty of Science, Ahmadu Bello University (ABU) Zaria, Kaduna state.

* Corresponding author.

ABSTRACT
The effect of sugar supplementation on citric acid production by Aspergillus niger from orange pulp using submerged fermentation was studied. Highest citric acid yields were obtained from three and five percent glucose supplemented orange pulp, the citric acid yields obtained were 14.58g/l and 13.72g/l respectively, while orange pulp without glucose supplementation yielded the least amount of citric acid (11.53g/l).

KEYWORDS: Citric acid, sugar supplementation, orange pulp, *Aspergillus niger*, submerged fermentation.

INTRODUCTION
Citric acid is the most important organic acid produced in tonnage by fermentation. 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). Many by-products and residues of the agro-industry can be used in the production of citric acid. A cost reduction in citric acid production can be achieved by using less expensive substrates. The used of agro-industrial residues as support in solid-state fermentation is economically important and minimizes environmental problems (Soccol *et al.*, 2006). The study was aimed at improving citric acid yield from orange pulp using sugar (glucose) supplementation.

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.

Preparation and Proximate Analysis of the Agricultural wastes.
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 and sieved to obtain fine particles. Proximate analysis of the waste 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 determine are

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. Twenty grams of each of the wastes were placed in three sets of two flasks. The first set of two flasks was without sugar supplementation while the second and third sets were supplemented with three and five percent glucose respectively. The fermentation medium consisted of 20g of the waste, 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 in 300ml of distilled water (Lasure *et al.*,2003). 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. Citric acid yield, residual sugar and dry mycelia weight were determined daily.

Analytical method

Citric acid was determined daily by filtering 10ml of the culture medium through Whatman filter paper no. 41. 2-3 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.

Concentration of citric acid in g/ l =

$$\frac{\% \text{ citric acid calculated above} \times 1000}{100}$$

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 in 2.5.1 above using the 3, 5- Dinitrosalycilic acid (DNS) method (Miller, 1959).

RESULTS

Proximate Analysis

The proximate composition of and orange pulp is as follows, 59.03% carbohydrate, 9.47% crude fibre, 5.25% crude protein, 0.70% fat content, 3.76% ash content and 21.79% moisture content. These analysis shows that orange pulp contain 68.50% of the portions (carbohydrate and crude fibre) that can be fermented to citric acid.

Citric Acid Yields

Figures 1, 2 and 3 show citric acid yields, residual sugar concentration and mycelia weight of the *A. niger* isolate without glucose supplementation, with three percent glucose supplementation and with five percent glucose supplementation during submerged fermentation of orange pulp. The highest citric acid yield of 14.58g/l was obtained from orange pulp supplemented with three percent glucose, followed by a citric acid yield of 13.72g/l from orange pulp supplemented with five percent glucose in both cases after 144h (six days) of fermentation. The highest citric acid yield obtained from fermentation from orange pulp without glucose was 11.53g/l after 120h (five days) of fermentation.

With respect to orange pulp without glucose, residual sugar concentration increased steadily from 0.35g/l at day one to 3.60g/l at day five then reduced to 3.20g/l at day seven. However, mycelia weight increased progressively from 3.05g/l at day one to 12.84g/l at day seven.

Both three and five percent glucose supplemented orange pulp show a similar pattern of residual sugar concentration, a decreased from 21.90g/l and 27.40g/l at day one to 0.90g/l and 0.40g/l at day four followed by an increase to 1.10g/l and 0.75g/l at day five and a decreased to 1.35g/l and 1.25g/l at day seven respectively. Mycelia weight also increased progressively in both cases from 2.48g/l at day one to 11.60g/l at day seven with respect to three percent glucose supplemented orange pulp and from 3.11g/l at day one to 11.49g/l at day seven with respect to five percent glucose supplemented orange pulp. There was no significant difference in mean citric acid yields from orange pulp without glucose, with 3% glucose and with 5% glucose (P>0.05).

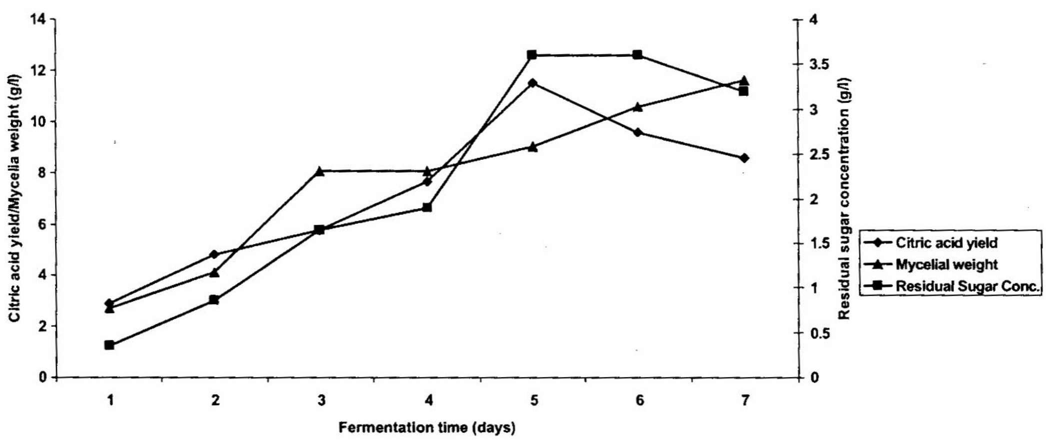

Figure 1:Citric acid yield, residual sugar concentration & mycelia weight of *A. niger* during submerged fermentation of orange pulp without glucose

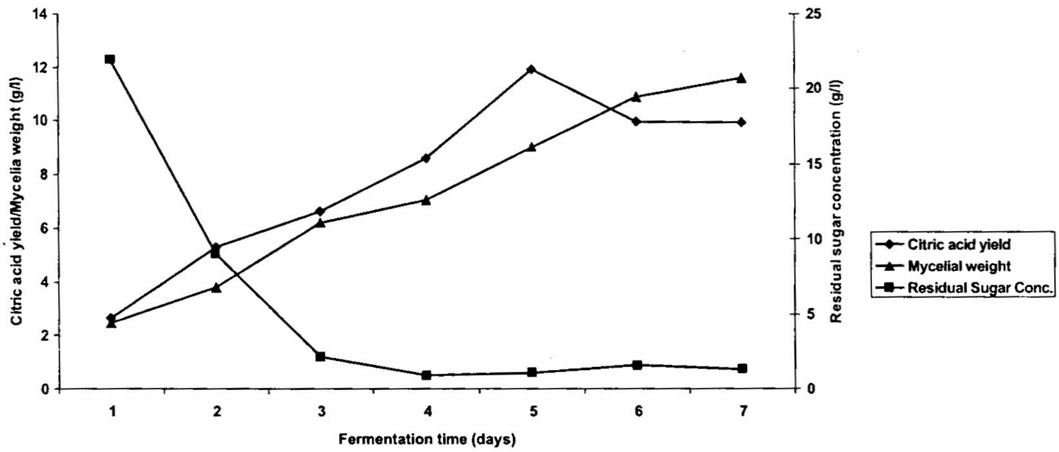

Figure 2:Citric acid yield, residual sugar concentration & mycelia weight of *A. niger* during submerged fermentation of orange pulp with 3% glucose

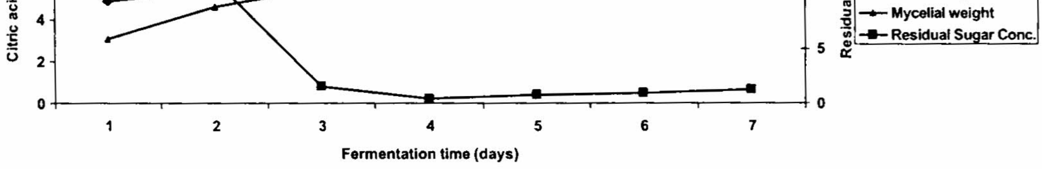

Figure 2:Citric acid yield, residual sugar concentration & mycelia weight of *A. niger* during submerged fermentation of orange pulp with 5% glucose

DISCUSSION

Both 3% and 5% glucose supplemented rice bran gave higher citric acid yields than unsupplemented rice bran, this agrees with the findings of Kiel *et al.*, (1981) on the possible use of cotton wastes as a carbon source for citric acid production by *Aspergillus niger* in which no citric acid was produced when *Aspergillus niger* was grown on cotton waste as a sole carbon source but when supplemented with 1%, 2%, 3%, 5%, 7%, 10% and 14% w/v sucrose, citric acid yield of 35mM, 59mM, 63mM, 81mM, 66mM, 84mM and 87mM respectively was obtained. However, there is a dearth of information on citric acid fermentation from orange pulp by submerged fermentation.

The yields obtained from 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). Other studies gave higher citric acid yields than this study; Hang et al., 1987 reported maximum citric acid yield of 100g/kg from dry Kiwi fruit peel and 56g/kg from dry grape pomace for various *A. niger* strains in solid state fermentation. Buzzini *et al.* (1993) obtained maximum citric acid yield of 67.60g/l from rape grape must in batch culture (Ikenebomeh and Oshoma, 2002).

CONCLUSION

Sugar supplementation was found to improve citric acid production by *Aspergillus niger* from orange pulp under condition of submerged fermentation. Increasing the level of sugar supplementation may further increase the yield from the waste studied.

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ACKNOWLEDGEMENT

The authors wish to acknowledge the Faculty of Natural Sciences Research Grant Committee of the University of Jos, Nigeria for sponsoring this research work.