



Citric Acid Production by *Aspergillus niger* and *Trichoderma longibrachiatum* using *Ananas comosus* Waste and *Saccharum officinarum* Bagasse

¹Omojasola, P. F., ¹Okun, H. A., ²Oladoye, C. O., ³Kayode, R. M. O. and ¹Ahmed El-Imam, A.

¹Department of Microbiology, Faculty of Life Sciences, University of Ilorin, P.M.B. 1515 Ilorin, Nigeria.

²Department of Science Laboratory Technology, Federal Polytechnic Offa, Nigeria

³Department of Food Science, Faculty of Agriculture, University of Ilorin

Abstract: *Trichoderma longibrachiatum* and *Aspergillus niger* were used to ferment two agro-based wastes; Pineapple peel (PP) and sugarcane bagasse (SB) as substrates for citric acid production. The wastes were ground, used as carbon source in mineral salts media. Submerged fermentation was conducted at (29±1°C) for five days and citric acid production and residual glucose were evaluated. pH, substrate concentration and inocula size were varied to determine their effect on citric acid production. 0.62 and 0.61 g L⁻¹ of citric acid were produced from PP; while 0.08 and 0.24 g L⁻¹ were produced from SB by *T. longibrachiatum* and *A. niger* respectively on Day 5 of fermentation. Maximum citric acid yield by *A. niger* was 0.61 g L⁻¹ at pH 3; 0.92 g L⁻¹ at 25% substrate concentration and 0.70 g L⁻¹ at 0.5% inocula size on PP and 0.24 g L⁻¹, 0.5 g L⁻¹ and 0.52 g L⁻¹ respectively on SB. Maximum yield with *T. longibrachiatum* was 0.6 g L⁻¹ at pH 3; 0.62 g L⁻¹ at 15% substrate concentration and 1.26 mg mL⁻¹ at 0.5% inocula size on PP and 0.24 g L⁻¹, 0.50 g L⁻¹ and 0.52 g L⁻¹ at pH 3.0, 25% substrate concentration and 1.5% inocula size respectively on SB. Under optimized conditions, *T. longibrachiatum* yielded 0.46 g L⁻¹ citric acid which was significantly higher (P<0.05) than 0.41mg mL⁻¹ produced by *A. niger* on PP. No significant difference (P<0.05) in the yield by both organisms on SB. Both wastes were found to be suitable substrates for commercial citric acid production.

KEYWORDS: Citric acid, *Trichoderma longibrachiatum*, *Aspergillus niger*, pineapple waste, sugarcane bagasse, fermentation

1.0 Introduction

Citric acid also known as tricarboxylic acid is one of the most economically important organic acids with an estimated production of about 1.4 - 1.5 million tonnes per year and production growth of 3.5 - 4.0% annually (Berovic and Legisa, 2007; Amenaghawon and Aisien, 2012; Rao and Reddy, 2013). Citric acid is widely applied industrially as it is low in toxicity and is generally regarded as safe. It is used as an acidulant in beverage production, pH modifier and buffering agent, flavoring agent to impart sour taste in foods and in the production of some dietary supplements, detergents, cosmetics among others (Ikram *et al.* 2002;

Parveen *et al.* 2005; Soccol *et al.*, 2006). It is mainly produced from beet/cane molasses, a by-product of the sugar production process (Gupta, 1994) or simple sugars such as glucose and sucrose (Papagianni, 2007). With an estimated annual production of one million tonnes, it is currently one of the most important products of fermentation (John *et al.*, 2012). In spite of an annual 2-3% increase in the demand for citric acid over the years (Ali *et al.*, 2002), probably accounts for the continuous research into the production of citric acid from cheaper or more readily available alternate substrates such as cheese whey (El-Holi and Al-Delaimy, 2003), palm oil mill effluent (Salihu *et al.*, 2011), cassava peel (Kuforiji *et al.* 2010) among other substrates.

Pineapple (*Ananas comosus* Merr., syns. *A. sativa* Schult.f., *Ananassa sativa* Lindl.,

*Corresponding Author

Tel.: +2348035951352

E-mail: folakejasola@yahoo.co.uk

Bromelia ananas L., *B. comosa* L.) is a multiple fruit found in the tropics. It is of the leading edible member of Family Bromeliaceae and consists of coalesced berries. The plant is a herbaceous perennial which may grow to 1.0 - 1.5m in height (Morton, 1987). Pineapple is commonly called *pina* (spanish); *abacaxi* (portuguese); *ananas* (dutch and french); *ope oyinbo* (yoruba) and *akwuchukwu* (ibo). It contains a high proportion of sugar and water and is thus highly perishable. It is used singly or in combination with other fruits in the production of jams, juices, wines, jellies or eaten fresh. Sugar cane (*Saccharum officinarum* L.) is a tall perennial grass of the tribe Andropogoneae, Family Poaceae, native to the warm temperate and tropical regions of South Asia (Rezende *et al.*, 2011) which has a sap with high sugar content. It is extensively cultivated in the tropics as the major raw material for table and industrial sugar production in the world, and is also consumed raw. Its common names include *ikshu*, *khanda* (Sanskrit); *pundia*, *paunda* (Hindi); *poovan karumbu* (Tamil); *ireke* (yoruba) and *achara* (ibo).

Domestic or industrial utilization of pineapple flesh and sugar cane sap results in the accumulation of pineapple peel and sugarcane bagasse respectively. These agro-industrial wastes are composed primarily of cellulose, hemicelluloses and lignin which are not easily utilized by most degradative organisms, thus they are indiscriminately disposed of and constitute an environmental nuisance as wastes. For instance, sugarcane bagasse consists of approximately 43-52% fiber of which cellulose makes up roughly 50%, hemi-cellulose 25% and lignin 25% (Pandey *et al.*, 2000). These compounds can however be converted to fermentable sugars by the processes of physical pre-treatment which breaks down the crystalline structure, reduces the particle size and increases the surface to volume ratio; followed by chemical pre-treatment which breaks down the long chains of sugars in cellulose, thus freeing them from the lignin for subsequent fermentation to the desired products.

The most commonly employed organisms are various strains of *Aspergillus niger* (Ali *et al.*, 2002; Alvarez-Vasquez *et al.*, 2000; El-Holi and Al-Delaimy, 2003; Papagianni, 2007; Umar *et*

al., 2013), though *A. nidulans*, *Trichoderma viride*, *Penicillium janthinellum*, *P. restrictum*; and yeasts such as species of *Saccharomyces*, *Zygosaccharomyces*, *Pichia*, *Debaromyces* and *Yarrowia* have also been used (Papagianni, 2007).

The increasing global demand for citric acid is due to its versatility in the pharmaceutical and food industries and its low toxicity when compared to other acidulants (Socol, *et al.*, 2006). The conventional substrates for citric acid production include molasses and starch hydrolysate, however the relatively high costs of these substrates has fuelled the search for low cost alternatives. Cheap raw materials may be the critical factor in profitable citric acid production. Other agro-based wastes that have been used include corn cob, cassava bagasse, rice bran, wheat bran, orange and pineapple peel (Socol, *et al.*, 2006).

This work was carried out to evaluate the potential of two agro-based waste products: pineapple peel and sugarcane bagasse for the production of citric acid using *Aspergillus niger* and *Trichoderma longibrachiatum*. Both fungi are well documented producers of cellulases (Sandhu and Kalra, 1982; Royer and Nakas, 1989; Bahkali, 1995; John *et al.*, 2012; Kareem and Rahman, 2013; Auta *et al.*, 2014). While *A. niger* is well documented in citric acid production, *T. longibrachiatum* is also being introduced as a potential alternative. Attempts were made to vary the different physico-chemical parameters of the fermentation process with the purpose of obtaining the maximum yield of the acid using the selected fungi.

2.0 Materials and methods

2.1 Microorganisms and Waste Substrates

This work used *Trichoderma longibrachiatum* and *Aspergillus niger*, both of which were sourced from the International Institute for Tropical Agriculture (IITA), Ibadan in Oyo State, Nigeria. The organisms were maintained on Potato Dextrose Agar (Lab M) slants at 4°C prior to use. The preparation of the spore suspension was carried out according to the method of Alam (2003). The concentration

of the spore suspension was determined with a Haemocytometer.

Pineapple fruits were purchased from the local market in Ilorin, Kwara State, Nigeria. They were washed to remove dirt and peeled with a knife. The pineapple peel was dried in an oven at 70°C, ground in the mill attachment of a Moulinex blender and stored at 4°C pending use. The sugar cane was peeled with a knife, pounded, and the juice pressed out manually. The bagasse residue was dried in an oven at 70°C, ground in the mill attachment of a Moulinex blender and kept in a plastic container 4°C until used (Ali *et al.*, 1991; Omojasola *et al.*, 2008).

2.2 Fermentation media preparation

Mineral Salts Medium (MSM) was prepared thus: 20g Pineapple waste/sugarcane bagasse, 0.5g (NH₄)₂SO₄, 0.02g MgSO₄, 0.03g KH₃PO₄ were mixed in 100ml of water (Willey *et al.*, 2008) with the wastes serving as the carbon sources. The pH of both media were adjusted to 3.0 with concentrated HCl and sterilized at 15 psi and 121°C for 15 minutes. The media were labelled Mineral Salts Pineapple Peel Medium (MSPPM) and Mineral Salts Sugarcane Bagasse Medium (MSSBM) respectively.

2.3 Fermentation

To 100 ml of sterilized MSPPM and MSSBM in separate 250 ml Erlenmeyer flasks, 1ml of the spore suspension of *A. niger* was introduced while to another set of flasks, same was done with *T. longibrachiatum*. The flasks were incubated at 30°C on a shaker at 150 rpm for two days (Parveen *et al.*, 2005). Samples were taken at 24 hour intervals, filtered with muslin cloth, centrifuged at 2,500 rpm for 15 minutes and the supernatant was used to assay for citric acid and the amount of sugar utilized.

2.4 Effect of Different Fermentation Conditions on Citric Acid Production

i. *Effect of varying pH:* The pH of the fermenting media were adjusted to values from 3.0-5.0 with 0.5 interval by the addition of 0.1N HCl and 0.1N NaOH using a pH meter (Denver

Instrument, model 20 pH/conductivity meter). The fermenting conditions were 1% inocula size, substrate concentration of 20% over a period of five days.

ii. *Effect of varying inocula size:* Various quantities of the spores of the organisms ranging from 0.5-2.5% were employed in the fermentations. The fermentation was conducted at pH 3.0, and substrate concentration of 20% for five days.

iii. *Effect of varying substrate concentration:* Different concentrations of the PP and SB wastes in distilled water ranging from 5-30% were used in the composition of the fermentation media. The fermentation was conducted at pH 3.0, inocula size of 1% over a period of five days.

2.5 Optimization experiments

The parameters that yielded highest amounts of citric acid in the earlier fermentations were combined in single optimized fermentations for each organism in attempt to further increase yield efficiency.

2.6 Citric acid assay

The determination of citric acid was carried out according to the pyridine-acetic anhydride method of Marier and Boulet (1958). One millilitre of the culture filtrate with 1.30 ml of pyridine was added to a test tube and swirled briskly prior to the addition of 5.70 ml of acetic anhydride. The test tube was placed in a water bath at 32° C for 30 minutes. The absorbance was measured at 420 nm on a spectrophotometer (CamSpecM105) and the concentration of citric acid produced was estimated by referring to a standard.

2.7 Reducing sugar determination

The reducing sugar content of the medium was determined by the Dinitrosalicylic acid (DNSA) method of Miller (1959). Three millilitres of the DNSA reagent was added to 3 ml of the culture filtrate in a test tube. The mixtures were heated for 5 minutes in a boiling

water bath and then cooled under running tap water at ambient temperature. The absorbance was read at 550 nm and the concentration estimated by referring to a standard curve for glucose.

2.8 Statistical analysis

The mean and standard deviation of the data were obtained. Duncan's Multiple Range Test was used in a post-hoc test to separate the means. The data was also subjected to Analysis of Variance (ANOVA) under completely randomized design (SPSS, 2004).

3.0 Results

The citric acid yield of the initial fermentation of PP was 0.46 and 0.41 g L⁻¹ of citric acid by *T. longibrachiatum* and *A. niger* respectively (Table 1). These yields were significantly higher than 0.13 and 0.10 g L⁻¹ from SB ($p < 0.05$).

The results of the optimization experiments varying time recorded maximum yield of citric acid as 0.62 mg mL⁻¹ by *Trichoderma longibrachiatum* on pineapple peel (PP) was recorded on the second day of fermentation (Fig. 1) this was not significantly different ($p > 0.05$) from the highest yield of 0.61 mg mL⁻¹ citric acid from the same substrate by *A. niger*. However by Day 5 of fermentation, *A. niger* recorded a yield of 0.90 mg mL⁻¹ of citric acid on PP (Figure 1). Citric acid production on PP was higher than on sugarcane bagasse (SB). An initial lag was observed until Day 2, after which citric acid production started to rise by Day 3.

A highest amount of citric acid was produced at pH 3.0 for both *A. niger* and *T. longibrachiatum* for both substrates (Figure 2; Table 2).

When inocula size increased, the citric acid yield also increased up to 1.5% (Figure 3). The citric acid yield from PP remained consistently higher than on SB. Maximum citric acid yield of 1.26 g L⁻¹ and 0.70 g L⁻¹ were obtained on PP with 0.5% inocula size of *T. longibrachiatum* and *A. niger* respectively. With *A. niger*, the highest yield of 0.52 g L⁻¹ was obtained using inocula size of 1.5% on SB (Figure 3). Overall,

T. longibrachiatum produced higher amounts of citric acid than *A. niger* with variation of inocula size. The amount of residual glucose initially rose with increase in inocula size but later declined (Table 3).

When inocula size increased, the citric acid yield also increased up to 1.5% (Figure 3). The citric acid yield from PP remained consistently higher than on SB. Maximum citric acid yield of 1.26 g L⁻¹ and 0.70 g L⁻¹ were obtained on PP with 0.5% inocula size of *T. longibrachiatum* and *A. niger* respectively. With *A. niger*, the highest yield of 0.52 g L⁻¹ was obtained using inocula size of 1.5% on SB (Figure 3).

The effect of varying substrate concentration produced maximum yield on PP and SB at concentrations of 30% and 25% respectively by *T. longibrachiatum* while with *A. niger*, citric acid yield increased with PP and SB concentrations up to 25%. The amount of residual sugars also increased with increasing substrate concentration (Figure 4).

T. longibrachiatum produced higher amounts of citric acid than *A. niger* with variation of inocula size. The amount of residual glucose initially rose with increase in inocula size but later declined (Table 3).

The highest quantity of residual glucose was 1.84 g L⁻¹ obtained from PP fermented using *A. niger* after one day, while the least residual sugar of 0.37 g L⁻¹ was from PP fermented using *T. longibrachiatum* for two days (Table 4). This was significantly higher ($p \leq 0.05$) than the amount of residual glucose produced on other days.

4.0 Discussion

The results of this study reveal that both PP and SB are suitable substrates for the production of citric acid. However, the data suggest that PP is a better substrate than SB in citric acid production. A variety of carbon substrates have been used with success for the production of citric acid. This includes wood apple, beetroot, Indian jujube, pineapple, papaya, guava (Pratik *et al.*, 2013) and cassava bagasse (Kumar and Jain, 2008), carob pod, areca husk, soy residues and coffee husk (Khare *et al.*, 1995; Roukas, 1998; Narayanamurthy *et al.*, 2008; Torrado *et*

Table 1: Citric acid production and residual glucose during fermentation of pineapple peel and sugarcane bagasse by *A. niger* and *T. longibrachiatum*

Test Organism	Substrate	Citric acid (g L ⁻¹)	Residual glucose (g L ⁻¹)
<i>T. longibrachiatum</i>	PP	0.46±0.03 ^c	1.32±0.06 ^b
	SB	0.13±0.01 ^a	1.60±0.02 ^c
<i>A. niger</i>	PP	0.41±0.01 ^b	0.51±0.10 ^a
	SB	0.10±0.00 ^a	1.82±0.03 ^d

Key: PP pineapple peel; SB sugarcane bagasse Fermentation parameters: Time 3 days; Temp 30°C; pH 2.5; Substrate Concentration 1%; Inocula size 1%

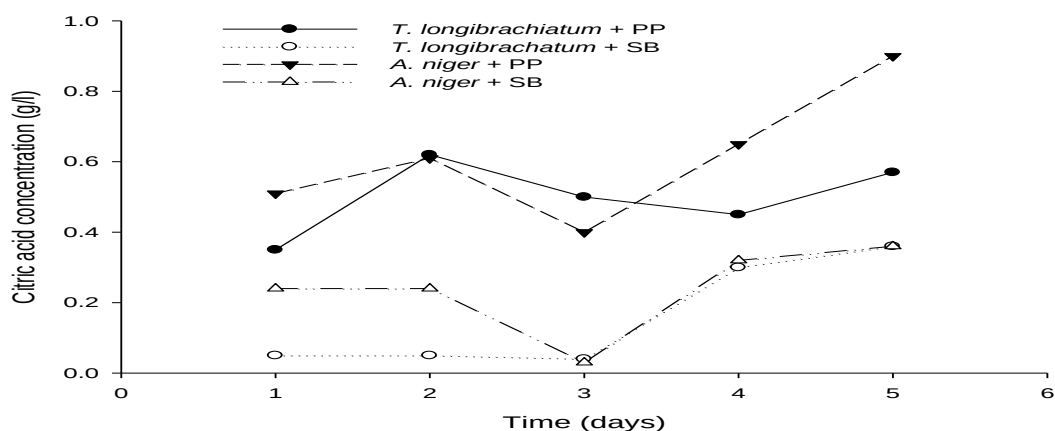


Figure 1: Citric acid from pineapple peel and sugarcane bagasse by *T. longibrachiatum* and *A. niger*

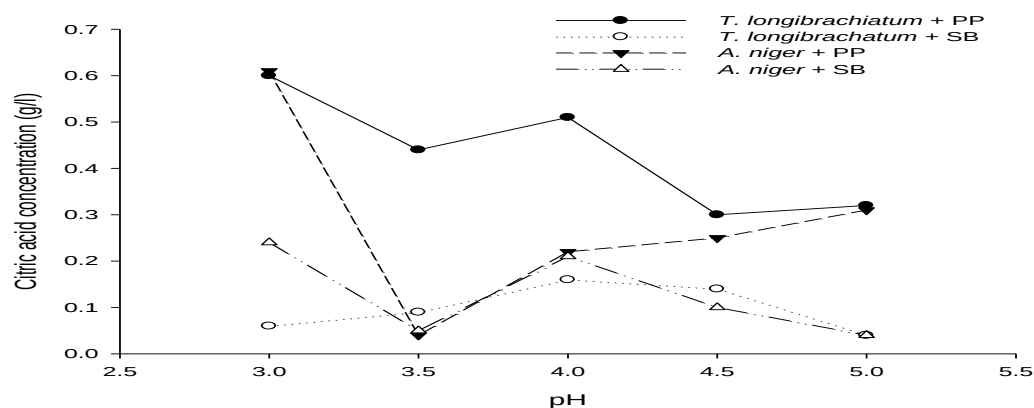


Figure 2: Effect of pH on citric acid production by *T. longibrachiatum* and *A. niger* using pineapple peel and sugarcane bagasse

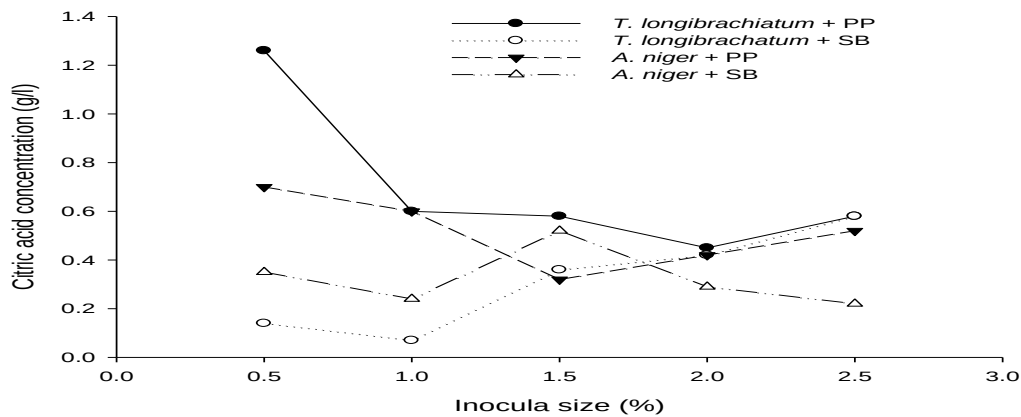


Figure 3: Effect of inocula size on citric acid production by *T. longibrachiatum* and *A. niger* using pineapple peel and sugarcane bagasse

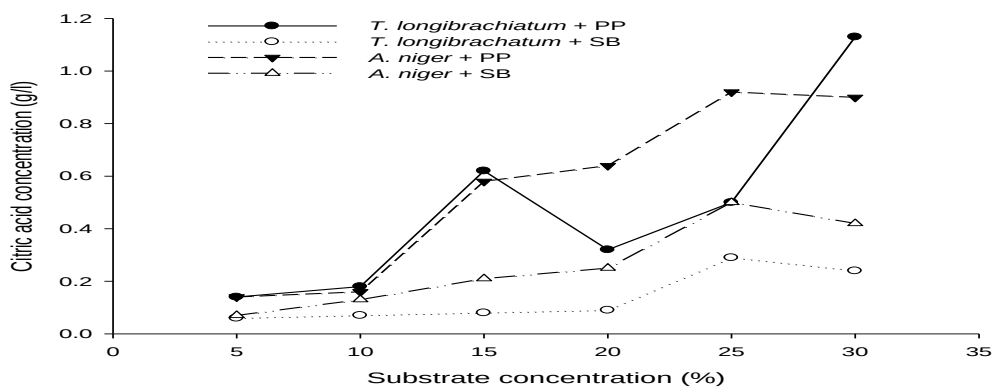


Figure 4: Effect of substrate concentration on citric acid production by *T. longibrachiatum* and *A. niger* using pineapple peel and sugarcane bagasse

Table 2: Effect of varying pH on residual glucose during fermentation of pineapple peel and sugarcane bagasse by *A. niger* and *T. longibrachiatum*

Test Organism	Substrate	pH				
		3.0	3.5	4.0	4.5	5.0
<i>T. longibrachiatum</i>	PP	0.40±0.00 ^d	0.38±0.00 ^d	1.41±0.01 ^e	0.28±0.00 ^b	0.14±0.00 ^a
	SB	0.62±0.00 ^d	0.70±0.00 ^e	0.38±0.00 ^c	0.27±0.01 ^b	0.24±0.00 ^a
<i>A. niger</i>	PP	1.42±0.014 ^e	0.95±0.007 ^d	0.32±0.00 ^a	0.70±0.00 ^b	0.93±0.00 ^c
	SB	0.52±0.62 ^c	0.48±0.00 ^b	0.63±0.014 ^e	0.55±0.014 ^d	0.36±0.00 ^a

Key: PP pineapple peel; SB sugarcane bagasse

Table 3: Effect of varying inocula size on residual glucose during fermentation of pineapple peel and sugarcane bagasse by *A. niger* and *T. longibrachiatum*

Test Organism	Substrate	Inocula size (%)				
		0.5	1.0	1.5	2.0	2.5
<i>T. longibrachiatum</i>	PP	0.35±0.01 ^c	0.38±0.00 ^d	0.62±0.00 ^e	0.22±0.00 ^a	0.31±0.01 ^b
	SB	0.48±0.00 ^a	0.74±0.00 ^c	0.66±0.00 ^b	0.74±0.00 ^c	0.74±0.00 ^c
<i>A. niger</i>	PP	1.40±0.00 ^c	1.52±0.00 ^e	0.82±0.00 ^b	1.48±0.00 ^d	0.35±0.01 ^a
	SB	0.72±0.00 ^b	0.72±0.00 ^b	0.64±0.00 ^a	0.76±0.00 ^c	1.84±0.00 ^d

Key: PP pineapple peel; SB sugarcane bagasse

Table 4: Effect of varying substrate concentration on residual glucose during fermentation of pineapple peel and sugarcane bagasse by *A. niger* and *T. longibrachiatum*

Test Organism	Substrate	Substrate concentration (%)					
		5	10	15	20	25	30
<i>T. longibrachiatum</i>	PP	0.20±0.00 ^a	1.00±0.00 ^b	0.71±0.04 ^c	1.02±0.00 ^c	1.91±0.01 ^e	1.13±0.01 ^d
	SB	1.56±0.00 ^f	0.30±0.00 ^a	0.34±0.00 ^b	0.74±0.00 ^c	0.92±0.00 ^d	1.03±0.01 ^e
<i>A. niger</i>	PP	0.33±0.01 ^a	1.34±0.00 ^b	1.77±0.1 ^d	1.82±0.00 ^e	1.53±0.01 ^c	2.22±0.00 ^f
	SB	0.20±0.00 ^a	0.40±0.00 ^b	0.56±0.00 ^c	0.74±0.00 ^d	0.88±0.00 ^e	2.38±0.00 ^f

Key: PP pineapple peel; SB sugarcane bagasse

al., 2011). These studies suggest that substrates containing maltose, sucrose, glucose mannose and sucrose (in that order) were suitable carbon sources capable of giving high yields of citric acid (Xu *et al.*, 1989). The sugars found in pineapple are glucose, lactose and sucrose (Okonkwo *et al.*, 2012), while those in sugarcane are mainly sucrose, with amounts of glucose and fructose (Wheals *et al.*, 1999; Zabel *et al.*, 2014). Optimal yields have been observed at sugar concentrations of 10% w/v with the exception of glucose (7.5%w/v). The residual glucose levels from this study support this observation (Table 1).

A. niger is reported to be the very productive in citric acid production, this is attributed to its highly synthetic nature and ability to utilize many substrates for growth efficiently (Soccol *et al.*, 2006; Kareem and Rahman, 2013; Munshi *et al.*, 2013). This was confirmed in Figure 1 where *A. niger* produced higher amounts of citric acid than *T. longibrachiatum* on PP. However, *T. longibrachiatum* produced higher amounts of citric acid than *A. niger* at pH 4.0 (Figure 2), 2.5% inocula size (Figure 3) and 25% substrate

concentration (Figure 4). *Trichoderma* spp. that have been used for citric acid production are *T. viride*, and *T. harzianum* (Kalaimahan and Tapobrata, 1995; Torrado *et al.*, 2011). *Trichoderma* spp. are reported to produce cellulolytic and chitinolytic enzymes (Kalaimahan and Tapobrata, 1995; Kovacs *et al.*, 2004; Omojasola *et al.*, 2008; Omojasola and Jilani, 2009). These enzymes aid the conversion of insoluble complex carbohydrates such as cellulose and lignin which are major constituents of many agro-based wastes to fermentable sugars.

The maximum citric acid yield in this study was 1.26 g L⁻¹ by *T. longibrachiatum* on PP (Fig. 3). This compares favorably the yields of other workers. Ikram *et al.* (2002) reported citric acid yields of 106.5g L⁻¹ by *A. niger* using a cane molasses medium. Kuforiji *et al.* (2010) reported 55.4% and 57.6% citric acid yield from pineapple waste using *A. niger* NRRL 328 and 567 respectively.

The optimal yield of citric acid preceded by a 2 day lag (Figure 1) is similar to the report of Mahamud and Gomes (2012) who observed a

72-hour lag by *Trichoderma* sp. in FPase activity followed by a sharp increase in enzyme synthesis. This lag phase was however not observed with PP. It can be inferred that with sugarcane bagasse there was an initial lag period of two days whereby the organism may have been synthesising the cellulases and hemicellulases required to hydrolyse the bagasse. Over time, *A. niger* yielded more citric acid than *T. longibrachiatum*, particularly with PP. In both cases, the residual glucose declined with time with the exception of *A. niger* fermentation of PP which showed an increase in residual glucose by the next day (Table 1). This is probably due to a faster conversion of glucose to citric acid than conversion of cellulosic material to glucose. Rao and Reddy (2013) obtained their highest citric acid yield at 72 hours after which a gradual decline was observed.

Effect of pH on citric acid production showed that *T. longibrachiatum* fermentation had the most residual glucose at initial pH 4 from PP, while *A. niger* fermentation had the highest residual glucose at pH 3.0 from PP (Table 2). This trend may be because other compounds such as oxalic and gluconic acid rather than citric acid accumulate at higher pH values. Both *A. niger* and *T. longibrachiatum* possess high tolerance to low pH (Torrado *et al.*, 2011; Singh *et al.*, 2014). This contrasts with the results of Ali (2002) who observed that a decrease in initial pH of fermentation caused reduction in citric acid production and Afifi (2011) who reported an optimum pH of 5.0 for citric acid production from potato substrate. On SB however, citric acid production increased up to pH 4.5. The same amounts of citric acid were produced by both organisms on PP, while on SB *A. niger* had a higher yield (Figure 2) overall than *T. longibrachiatum*. Rao and Reddy (2013) recorded optimum yield of 72.0 g kg⁻¹ citric acid from oat bran at pH 4.

When the inocula size was increased, citric acid yield increased up to 2.5%, except for *A. niger* on SB that dropped after 1.5% (Figure 3). Inocula concentrations in the range of 10³ - 10⁸ spores per g of substrate are usually employed for citric acid production (Roukas, 1998). Increase in inocula size is known to reduce the lag phase in the growth medium (Torrado *et al.*,

2011). The citric acid yield from PP remained consistently higher than on SB. Overall, *T. longibrachiatum* produced higher amounts of citric acid than *A. niger* with variation of inocula size. The amount of residual glucose initially rose with increases in inocula size but later declined (Table 3). High levels of inocula encourages clumping and crowding of cells which can decrease the hydrolytic enzyme production, sugar consumption for biomass reproduction and ultimately reduction in citric acid production (Srivastava *et al.*, 1987; Yadegary *et al.*, 2013; Auta *et al.*, 2014) and is similar to the findings of Omojasola and Jilani (2008) and Auta *et al.* (2014).

The effect of substrate concentration on citric acid production show optimal yields at 25% (Figure 4). The effective sugar concentration for industrial fermentations is between 14 - 22% (Yadegary *et al.*, 2013). Omojasola and Jilani (2009) reported optimum glucose production from *T. longibrachiatum* at 30% substrate concentration. Umar *et al.* (2013) reported maximum yield of 68.7 g L⁻¹ citric acid from sugarcane molasses on Day 6 at 24% substrate concentration also using *A. niger*. However, it is in contrast to the work of Auta *et al.* (2014) who reported optimal yield of 0.83 g L⁻¹ at 2% substrate concentration. The presence of some sugars such as galactose and high lipid contents if present in the substrate can inhibit citric acid production (Auta *et al.* 2014), while methanol and copper ions can enhance yield (Kareem and Rahman, 2013).

In conclusion, this study revealed that spent pineapple peel and sugarcane bagasse both agro-based wastes have potential uses as substrates in the production of citric acid by *Trichoderma longibrachiatum* and *Aspergillus niger*. In addition, while *A. niger* is reported to be the organism of choice in citric acid production, *T. longibrachiatum* also consistently produced appreciable yield of citric acid and residual glucose. These wastes may also still be employed as animal feed after the extraction of the citric acid from the culture and destroying the microorganisms, as fermentation is known to decrease the concentration of anti-nutrients in such wastes.

References

- Afifi, M. M. (2011). Naturally Occurring microorganisms of industrial waste for citric acid production by solid state fermentation. *Journal of Environmental Science and Technology* 4:377-386.
- Alam, M. Z. and Fakhru'l-Razi, A. (2003). Enhanced settleability and dewaterability of fungal treated domestic wastewater sludge by liquid state bioconversion process. *Water Research* 37:1118-1124.
- Ali, S., Haq, I. U. and Iqbal, J. (2002). The role of Mn^{++} ions for high and consistent yield of citric acid in recycling fed-batch bioreactor system and its novelty on kinetic basis. *Electronic Journal of Biotechnology* 5(2): Retrieved from http://www.scielo.cl/scielo.php?pid=S0717-34582002000300010&script=sci_arttext Accessed on 17/6/ 2012.
- Ali, S., Sayed, A., Sarker R. T. and Alam, R. (1991). Factors affecting cellulase production by *Aspergillus terreus*. *World Journal of Microbiology and Biotechnology* 7:62-66.
- Alvarez-Vasquez F, González-Alcón C. and Torres, N. V. (2000). Metabolism of citric acid production by *Aspergillus niger*: model definition, steady-state analysis and constrained optimization of citric acid production rate. *Biotechnology and Bioengineering* 70(1): 82-108.
- Amenaghawon, N. A. and Aisien, F. A. (2012). Modelling and simulation of citric acid production from corn starch hydrolysate using *Aspergillus niger*. *Environment and Natural Resources Research* 2(1): 73-85.
- Auta, H. S., Abidoeye, K. T., Tahir, H., Ibrahim, A. D. and Aransiola, S. A. (2014). Citric acid production by *Aspergillus niger* cultivated on *Parkia biglobosa* fruit pulp. *International Scholarly Research Notices*. vol 2014: ID 762021 8pp.
- Bahkali, A.H. (1995). Production of cellulase, xylanase and polygalacturonase by *Verticillium tricorpus* on different substrates. *Bioresources Technology* 51(2):171-174.
- Berovic, M. and Legisa, M. (2007). Citric acid production. *Biotechnology Annual Review* 13:303-343.
- El-Holi, M. A. and Al-Delaimy, K. S. (2003). Citric acid production from Whey with sugars and additives by *Aspergillus niger*. *African Journal of Biotechnology* 2(10):356-359.
- Gupta, S. (1994). Continuous production of citric acid from sugarcane molasses using a combination of submerged immobilized and surface stabilized cultures of *Aspergillus niger* KCU520. *Biotechnology Letters* 16:599-604.
- Ikram, H., Sikander, A., Qadder, M. A., Javed, I. (2002). Citric acid fermentation by mutant strain of *Aspergillus niger* GCMC-7 using molasses based medium. *Electronic Journal of Biotechnology* 5(2): [doi: 10.2225/vol5-issue2-fulltext-2].
- John, S. K., Ali, M. N., Umakumar, G. and Tabassum, H. (2012). Studies on Inductive effect of Methanol on Production of Citric acid from Waste Cellulosic Substrates using locally isolated *Aspergillus niger* and MTCC *Aspergillus niger* strains. *International Journal of Engineering Science and Technology* 4(2): 431-441.
- Kalaimahan, T. and Tapobrata, P. (1995). Application of Response Surface Methodology to Evaluate the Influence of Temperature and Initial pH on the Production of β -1,3-glucanase and Carboxymethylcellulase from *Trichoderma harzianum*. *Enzyme and Microbial Technology* 17(12): 1043-1049.
- Kareem, S. O. and Rahman, R. A. (2013). Utilization of banana peels for citric acid production by *Aspergillus niger*. *Agriculture and Biology Journal of North America* 4(4): 384-387.
- Khare, S. K., Jha, K. and Gandhi, A. P. (1995). Citric acid production from Okara (Soy Residue) by solid state fermentation. *Bioresource Technology* 54(3): 323-325.
- Kovacs, K., Szakacs, G., Pusztahelyi, S. and Pandey, A. (2004). Production of chitinolytic enzymes in solid state fermentation. *Applied Biochemistry and Biotechnology* 118(1-3): 189-204.
- Kuforiji, O. O., Kuboye, A. O. and Odunfa, S. A. (2010). Solid state fermentation process for citric acid production using corn and cassava wastes. *Advances in Food Sciences* 32(2):71-74.
- Kumar, A. and Jain, V. K. (2008). Solid state fermentation studies of citric acid production. *African Journal of Biotechnology* 7(5): 644-650.
- Mahamud, M. R. and Gomes, D. J. (2012). Enzymatic saccharification of sugarcane bagasse by the crude enzyme from indigenous fungi. *Journal of Scientific Research*. 4(1):227-238.
- Marier, J. M. and Boulet, M. (1958). Direct determination of citric acid in milk with an improved pyridine acetic anhydride method. *Journal of Dairy Science* 4: 1683-1692.
- Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry* 31:426-428.
- Morton, J. F. (1987). Pineapple In: *Fruits of Warm Climates*. Miami, FL. p 18-28. Retrieved 18-7-2015.
- Munshi, M. K., Huque, R., Khatun, A., Hossain, M. F., Rahman, M. and Khalil, M. L. (2013). Effect of biomass and sugar in citric acid production by *Aspergillus niger* using molasses and Jackfruit as

- substrates. American Journal of Food and Nutrition 1(1):1-6.
- Narayanamurthy, G., Ramachandra, Y. L., Rai, S. P., Ganapathy, P. S. S., Kavitha, B. T. and Manohara, Y. N. (2008). Comparative studies on submerged, liquid surface and solid state fermentation for citric acid production by *Aspergillus niger* RCNM17. Asian Journal of Microbiology Biotechnology and Environmental Sciences 10: 361-364.
- Okonkwo, S. I., Ogbunike, R. U. and Uyo, B. K. (2012). Elucidation of sugar in edible fruit - pineapple (*Ananas cosmosus*). Research Journal of Chemical Sciences 2(1): 24-29.
- Omojasola, P. F., Jilani, O. P. and Ibiyemi, S. A. (2008). Cellulase production by some fungi cultured on pineapple wastes. Nature and Science 6(2): 64-72.
- Omojasola, P. F. and Jilani, O. P. (2009). Cellulase production by *Trichoderma longi*, *Aspergillus niger* and *Saccharomyces cerevisiae* cultured on waste materials from orange. Pakistan Journal of Biological Sciences 11 (20): 2382-2388.
- Pandey, A., Soccol, C. R., Nigam, P. and Soccol, V. T. (2000). Biotechnological potential of agro-industrial residues. I: sugarcane bagasse. Bioresource Technology 74: 69-80.
- Papagianni, M. (2007). Advances in citric acid fermentation by *Aspergillus niger*: Biochemical aspects, membrane transport and modeling. Biotechnology Advances 25(3): 244-263.
- Parveen, J., Zahangir M., Alam, M., Ramlan, M. Salleh, M., Masrina M. N. (2005). Screening of *Aspergillus niger* for citric acid production from palm oil mill effluent. Biotechnology 4(4): 275-278
- Pratik, B., Ashok, V. G., Harshal, M. S. and Pranita, A. G. (2013). Production and optimization of citric acid by *Aspergillus niger* using fruit pulp waste. International Journal of Current Microbiology and Applied Sciences 2(10): 347-352.
- Rao, P. R and Reddy, M. K. (2013). Production of citric acid by *Aspergillus niger* using Oat Bran as substrate. International Journal of Chemistry and Chemical Engineering 3(3):181-190.
- Rezende, C. A., de Lima, M. A., Maziero, P., deAzevedo, W. G. and Polikarpor, I. (2011). Chemical and morphological characterization of sugarcane bagasse submitted to a delignification process for enhanced digestibility. Biotechnology for Biofuels 4:54-72.
- Roukas, T. (1998). Citric acid production from Carob Pod by solid state fermentation. Enzyme and Microbial Technology 24(1/2): 54-59.
- Royer, J. C. and Nakas, J. P. (1989). Xylanase production by *Trichoderma longibrachiatum*. Enzyme and Microbial Technology 11(7): 405-410.
- Salihu, A., Zahangir, A., AbdulKarim, I. M. and Salleh, H. M. (2011). Suitability of using palm oil mill effluent as a medium for lipase production. African Journal of Biotechnology 10 (11): 2044-2052.
- Sandhu, D. K. and Kalra, M. K. (1982). Production of cellulase, xylanase and pectinase by *Trichoderma longibrachiatum* Transactions of the British Mycological Society 79(3):409-413.
- Singh, A., Shahid, M., Srivastava, M., Pandey, S., Sharma, A. and Kumar, V. (2014). Optimal physical parameters for growth of *Trichoderma* spp. at varying pH, temperature and agitation. Virology and Mycology 3(1): 1000127
- Soccol, C. R., Vandenberghe, L. P. S., Rodrigues, C. and Pandey, A. (2006). New perspectives for citric acid production and application. Food Technology and Biotechnology 44:141-149.
- SPSS (2004). Statistical Analysis for Social Sciences. www.spss.com
- Srivastava, S. K., Gopalkrishnan, K. S. and Ramachandran, K. B. (1987). The production of β -glucosidase in shake-flasks by *Aspergillus wentii*. Journal of Fermentation Technology 65:95-99.
- Torrado, A. M., Cortes, S., Salgado, J. M., Max, B., Rodriguez, N., Bibbins, B. P., Converti, A. and Dominguez, J. M. (2011). Citric acid production orange peel wastes by solid state fermentation. Brazilian Journal of Microbiology 42(1): 394-409.
- Umar, F., Mohammad, F., Faqir, M. A., Zafar, H., Kashif, A. and Ejaz, A. (2013). Citric acid production from sugarcane molasses by *Aspergillus niger* under different fermentation conditions and substrate levels. International Journal of Agriculture and Applied Sciences 5(1):8-16.
- Wheals, A. E., Basso, L. C., Alves, D. M. G., Amorin, H. V. (1999). Fuel ethanol after 25 years. Trends in Biotechnology 17(12): 482-487.

- Willey, J. M., Sherwood, L. M. and Woolverton, C. J. (2008). Microbiology. McGraw-Hill Pub. International. ISBN 978-0-07-110231-5.
- Xu, D. B., Madrid, C. P., Rohr, M., Kubicek, C. (1989). The influence of type and concentration of the carbon source on production of citric acid by *Aspergillus niger*. Applied Microbiology and Biotechnology 30: 553-558.
- Yadegary, M., Hamidi, A. Alavi, S. A., Khodaverdi, E., Yadegary, H., Sattari, S., Bagherpour, G., Yagaghi, E. (2013). Citric acid production from sugarcane bagasse through solid state fermentation using *Aspergillus niger* mold and optimization of citric acid production by Taguchi Method. Jundishapur Journal of Microbiology 6(9):e7625
- Zabed, H., Golam, F., Sahu, J. N. S., Azirun, M. S., Hashim, R. and Boyce, A. N. (2014). Bioethanol production from fermentable sugar juice. The Scientific World Journal 2014: Article ID957102 11pp [dx.doi.org/10.1155/2014/957102].