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# Submerged fermentation of *Jatropha curcas* seedcake to produce oxalic acid by *Aspergillus niger* and *Aspergillus terreus*

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## Abstract

*Agro-industrial wastes mainly from the agricultural and food industries account for several thousand tonnes of waste yearly. These wastes can be further processed to other value-added products via the activities of microorganisms The potential use of Jatropha seedcake as a carbon source for the production of oxalic acid (OA) was investigated using Aspergillus niger (ATCC 16404) and Aspergillus terreus (ATCC 20542). The JSC was used as substrate in submerged fermentation carried out in separate flasks using Mineral Salts Medium for 10 days. Carboxymethyl cellulose was used as a control. Fermentation conditions were: pH 4.68; substrate concentration 10g; inocula size 2 % and temperature 28±2° C. The fermentation conditions were later varied to optimize OA production. Results showed OA yield of 200 g $L^{-1}$ and 135 g $L^{-1}$ by A. niger and A. terreus respectively on Day 8 of fermentation. Results from the optimized fermentations for A. niger that gave highest OA yields were pH 6; substrate concentration of 4g and inocula size of 5 mL. For A. terreus, they were pH 4, substrate concentration 6g and inocula size 4mL. These results support the potential use of Jatropha seed cake for fermentative production of oxalic acid*.

**Keywords:** Oxalic acid; *Aspergillus niger; Aspergillus terreus; Jatropha curcas;* submerged fermentation

## Introduction

Oxalic acid (OA) also known as ethanedioic acid is a naturally occurring acid that can be found in many plant species, such as orange, rhubarb, spinach, tea, cocoa, nuts, berries and beans (Magnuson and Lasure, 2004). OA is used widely in the hydrometallurgical process and pulp industry due to its chelating properties (Cameselle et al., 1998).Its compounds have widespread industrial applications in several fields such as textiles, tanning, oil refining, catalysts, pharmaceuticals, dyes, explosives, straw bleaching, printing, marble polishing, and metal and cloth cleaning. It is also a very important chemical in petroleum, rare-earth, ink, rust, corrosion inhibitor, and dental adhesive processing (Guru et al., 2000). Typically OA occurs as dihydrate with the formula $H_2C_2O_4.2H_2O$. In its concentrated and pure form, OA is very toxic and needs to be handled with extreme care. However most of its products are in diluted forms and hence do not possess much danger. Currently, OA is mostly synthesized chemically using oxidation of olefins and glycols, fusion of sawdust with NaCl, decomposition of formats followed by treatment with $H_2SO_4$ and oxidation of carbohydrates with $HNO_3$ (Mandal and Banerjee, 2005; Nakata et al., 2010). These methods impact on the environment negatively and are not commercially attractive; therefore it is desirable to develop other environmental friendly processes involving microbial fermentations for OA production.

A variety of fungi, including saprophytic and phytopathogenic species can be used in the synthesis of this acid (Dutton and Evans, 1996). Saprophytic species, such as *Aspergillus niger*, remains the organism of choice for oxalic acid production due to ease of handling, its ability to ferment a variety of cheap raw materials, and high yields (Emeko et al., 2015). The production of oxalic acid by fungi is highly regulated depending much on the factors like carbon and nitrogen sources and the initial medium pH, as well as the culture/broth pH during fermentation (Strasser et al., 1994; Bohlmann et al., 1998; Cameselle et al., 1998; Santoro et al., 1999).

*Jatropha curcas* L. (Physic nut) is a small shrub plant which grows widely in the tropics and subtropics belonging to the family Euphorbiaceae (Belewu and Sam, 2010; Joshi and Khare, 2011). It is perennial, multipurpose and drought resistant, widely distributed in parts of Central and South America, Africa, South East Asia and India (Dahake et al., 2013). *J. curcas* seeds have been widely used for biodiesel production and the resulting seedcake is considered toxic due to its phorbol esters and other anti-nutrient constituents such as trypsin inhibitor, saponin, phytate and lecithin (Makkar et al., 1997;

Rakshit et al., 2008; Abdo and Juamat, 2009; Ncube et al., 2012). *Jatropha curcas* Seedcake (JSC) has been used as substrate in the production of itaconic acid, gibberellic acid, xylanase, cellulose and solid biodiesel (Ncube et al., 2012; El Imam et al,. 2013; Kavalek et al., 2013; Omojasola and Benu, 2016).

Oxalic acid is an important chemical in industries such as laundry, pharmaceutical and hospitals. The isolation and purification of this chemical from waste agricultural materials is very feasible. The utilization of these waste materials will aid in the reduction of toxic compounds discharged into the environment and can supplement the quantity of oxalic acid imported into the country.

The objectives of this study were to determine the suitability of *Jatropha* seed cake (JSC) as a cellulosic substrate for the production of oxalic acid acid; to determine the ability of *Aspergillus niger* and *Aspergillus terreus* to utilize JSC as substrate for oxalic acid production and optimal conditions for the production under laboratory conditions.

**Materials and methods**

*Preparation of Jatropha seed cake (JSC)*

*Jatropha curcas* seeds were collected from the Department of Crop Production, Faculty of Agriculture, University of Ilorin, Kwara State, Nigeria. After drying, 250 g of JSC seeds were then crushed and soaked in 500 mL of petroleum ether for 24 hours at room temperature, in order to extract the oil from the seeds. They were then filtered using a muslin cloth to separate the oil from the seeds. The defatted seeds were then dried in the oven at 170°C for 2 hours. (Mohit et al., 2011)

*Proximate analysis of Jatropha seed cake*

The proximate analysis of the JSC was carried out. The parameters investigated were moisture content, ash, crude protein, total carbohydrate and crude fibre (AOAC, 2000), crude lipids (Parkouda et al., 2008).

*Substrate Pretreatment*

The ground seeds were then dried and pretreated with equal volume of 0.5% $H_2SO_4$ (ratio 1:1 w/v), autoclaved and dried again (Mohit, 2011). The resulting cake was then ground again before use.

*Collection of Microorganisms*

*Aspergillus niger* (ATCC 16404) and *Aspergillus terreus* (ATCC 20542) were obtained from the Federal Institute of Industrial Research Oshodi (FIIRO), Lagos State, Nigeria. The cultures were maintained on potato dextrose agar (PDA) slants and kept at temperature of about 4°C prior to use.

*Preparation of spore suspensions*

For the preparation of suspensions, 10 ml of sterile distilled water was added to 7-day old culture slants of the test fungi separately. The surface of the culture was scratched with sterilized loop and agitated thoroughly on a shaker to suspend the spores. The number of the spores were counted by using the improved Neubauer Haemocytometer (Weber England B.S 748) and adjusted to approximately 2.6 x $10^8$spores $mL^{-1}$ (*A. niger*)and 3.7 x $10^8$ spores $mL^{-1}$ (*A. terreus*). These were used as inocula throughout the study.

*Mineral salt medium for OA production*

Mary Mandels' Mineral Salt medium as described by Jefferies (1996) was used for the fermentation: $NaNO_3$ (1.5 g/L); $KH_2PO4$ (0.5 g/L); $MgSO4.7H_2O$ (0.025 g/L); KCl (0.025 g/L); yeast extract (1.6 g/L). Ten

grams of JSC was added as the carbon source. These were suspended in 1 L of distilled water and sterilized in an autoclave at 121°C for 15 minutes.

*Submerged fermentation*

Two mL of the *A. niger* and *A. terreus* inocula were added separately into the fermentation medium aseptically. Each flask was cultured on a rotary shaker at 28 ±2° C at 400 rpm. Samples were collected at an interval of 24 hours and assayed for OA. The quantitative estimation of oxalic acid was determined using the UV spectrophotometer at 325 nm (Thermo Fisher Scientific. GENESYS 20. Model 4001-4). During fermentation, the pH of the culture was maintained at pH 6.5 ±0.5 using 4 M NaOH. Conditions of the fermentation were: 10% substrate concentration; pH 4.68; inocula size 2 ml; temperature 28 ± 2°C. The fermentation was continued for 10 days and samples were analyzed daily for OA production (Strasser et al., 1994).

*Estimation of OA*

OA was estimated in the fermentation medium using the spectrophotometric method described by Jiang et al. (1996), based on catalytic effect on the redox reaction between dichromate and rhodamine B in $H_2SO_4$ which was measured at wavelength of 325 nm. Ten millilitres was withdrawn from the fermentation medium and filtered using 0.02 µm filter. One milliliter of the filtrate was mixed thoroughly with 100 mL of distilled water and the resulting solution was used for OA analysis. The analysis was done under the condition of 0.05 mol l-1of sulphuric acid, 0.03 mol l-1 potassium dichromate and 3.28 x $10^{-6}$ of rhodamine for 8 min after which the calibration graph of oxalic acid had been obtained.

*Optimization of OA production*

Optimization experiments were carried out to determine yield efficiency optimal conditions for OA production as a function of variation of fermentation conditions. These conditions were later combined in a single fermentation to produce maximum yield of the acid.

i. *Effect of varying substrate concentration*

Different concentrations of JSC ranging from 2 - 10% were used in the fermentation media for the production of OA. The other conditions of the fermentation were maintained at: pH 4.68; inocula size 2 mL; temperature 28 ± 2°C; fermentation time 9 days.

ii. *Effect of varying pH*

The pH of the fermentation media were adjusted to values ranging from 2.5-6.5 with 0.1N NaOH and 0.1N HCl. The pH was determined using pH meter (Denver Model 20 pH/conductivity meter). Other conditions of the fermentation were: 10% substrate concentration; inocula size 2 mL; fermentation time 9 days; temperature 28 ± 2°C.

iii. *Effect of varying time*

The fermentation period was varied from 1-10 days for oxalic acid production. Aliquots of each of the fermenting media were taken for the analysis of acid at 24 hours interval. Other conditions for the fermentation were: 10% substrate concentration; pH 4.68; inocula size 2 mL; temperature 28 ± 2°C.

iv. *Effect of varying inocula size*

The fermentations were carried out with varying inocula size. The inocula sizes were varied from 3-6 mL. One millilitre of inoculum was approximately 2.6 x $10^8$ spores $mL^{-1}$ (*A. niger*) and 3.7 x $10^8$ spores $mL^{-1}$ (*A. terreus*). Other conditions for the fermentation were: 10% substrate concentration; pH 4.68; temperature 28 ± 2°C.

The fermentation parameters that produced highest OA yields were then combined in a single fermentation with a view to increase yield efficiency.

*Statistical analysis*

The statistical analysis of the data was done using the Statistical Package for Social Sciences for Windows version 15.0 (SPSS, 2004).

**Results**

The results from this study confirmed that *Jatropha curcas* seed cake is a suitable substrate for OA production by fungal fermentation using *Aspergillus niger* (ATCC 16404) and *Aspergillus terreus* (ATCC 20542).JSC was a better substrate than CMC used as control which gave lower OA yields (Table 1). The results of the proximate composition of the defatted *Jatropha curcas* seed cake analyzed were: protein 29.34±0.02%; carbohydrate 42.09 ±0.02%; moisture 6.15±0.13%; ash content 5.75±0.03%, fat 6.23 ±0.16% and crude fibre 10.42±0.01%. These serve as nutrients, raw materials and carbon source that can be converted into valuable sugar by microorganisms for the production of oxalic acid.

Results of the pre-optimization fermentation of JSC to produce oxalic acid (Table 1) showed the yield peaking on

Day 6 with the production 135.0 ±0.42 g $L^{-1}$ by *A. niger* and 165.0 ±0.51 g $L^{-1}$ by *A. terreus.* Thereafter, the OA yield dropped after Day 7. The OA yields from the JSC substrate were significantly higher ($p \leq 0.05$) than the CMC control by both test organisms (Table 1). In this fermentation, the OA yield of *A. terreus* was higher than *A. niger*. The fermentation was optimized by varying the conditions of the fermentation: substrate concentration 2-10%; pH 2.5-6.5; fermentation time 1-10 days; and inocula size 3—6 mL.

Table 1: Production of oxalic acid by submerged fermentation from *Jatropha* seed cake using *Aspergillus niger* and *Aspergillus terreus*

| , .Fermentation time (Days) | Oxalic Acid (g/L) | | | |
|---|---|---|---|---|
| | *Aspergillus niger* | | *Aspergillus terreus* | |
| | JSC | (CMC) Control | JSC | (CMC) Control |
| 1 | 83.0± 0.01$^{a}$ | 71.0 ±0.50$^{a}$ | 76.0 ±0.50$^{a}$ | 62.0 ±0.20$^{ac}$ |
| 2 | 90.0 ± 0.43$^{b}$ | 76.0 ±0.43$^{ab}$ | 96.0 ±0.43$^{ab}$ | 65.0 ±0.40$^{c}$ |
| 3 | 93.0 ±0.04$^{a}$ | 80.0 ±0.046$^{a}$ | 103.0 ±0.01$^{ac}$ | 85.5 ±0.15$^{b}$ |
| 4 | 99.5 ±0.09$^{a}$ | 91.0 ±0.16$^{a}$ | 119.0 ±0.03$^{a}$ | 95.0 ±0.06$^{ab}$ |
| 5 | 110.0 ±0.01$^{ab}$ | 96.0 ±0.48$^{ab}$ | 122.0 ±0.28$^{ab}$ | 99.5 ±0.40$^{a}$ |
| 6 | 135.0 ±0.42$^{a}$ | 101.0 ±0.45$^{a}$ | 165.0 ±0.51$^{a}$ | 118.0 ±0.45$^{b}$ |
| 7 | 133.0 ±0.43$^{ac}$ | 94.0 ±0.24$^{ab}$ | 133.5 ±0.42$^{a}$ | 108.0 ±0.33$^{ac}$ |
| 8 | 131.0 ±0.50$^{b}$ | 76.0 ±0.44$^{a}$ | 125.0 ±0.04$^{a}$ | 106.0 ±0.25$^{ab}$ |
| 9 | 130.0 ±0.51$^{a}$ | 73.0 ± 0.08$^{ab}$ | 120.0 ±0.37$^{a}$ | 105.0 ±0.03$^{a}$ |

Key: JSC: Jatropha Seed Cake. CMC: Carboxymethylcellulose.

(Substrate Concentration: 10%. pH:4.68. Inoculum size: 2ml; temperature 28±2 $^{o}$C.)

The results of varying substrate concentration showed highest OA yield of 122.0 ± 0.002 g $L^{-1}$ at 4% substrate concentration on Day 6 for *A. niger* (Fig. 1a) and 200 ±0.005 g $L^{-1}$ at substrate concentration 6% also on Day 6 for *A. terreus* (Fig. 1b). The yields of the JSC substrate were significantly higher ($p \leq 0.05$) than the CMC control by both test fungi. The results of the varying pH gave the highest amounts of OA of 200 ±0.32 g $L^{-1}$ by *A. niger* on Day 6 at pH 6.0 (Fig. 2a) and 198 ±0.62 g $L^{-1}$ by *A. terreus* also on Day 6 but at pH 4.0 (Fig. 2b).

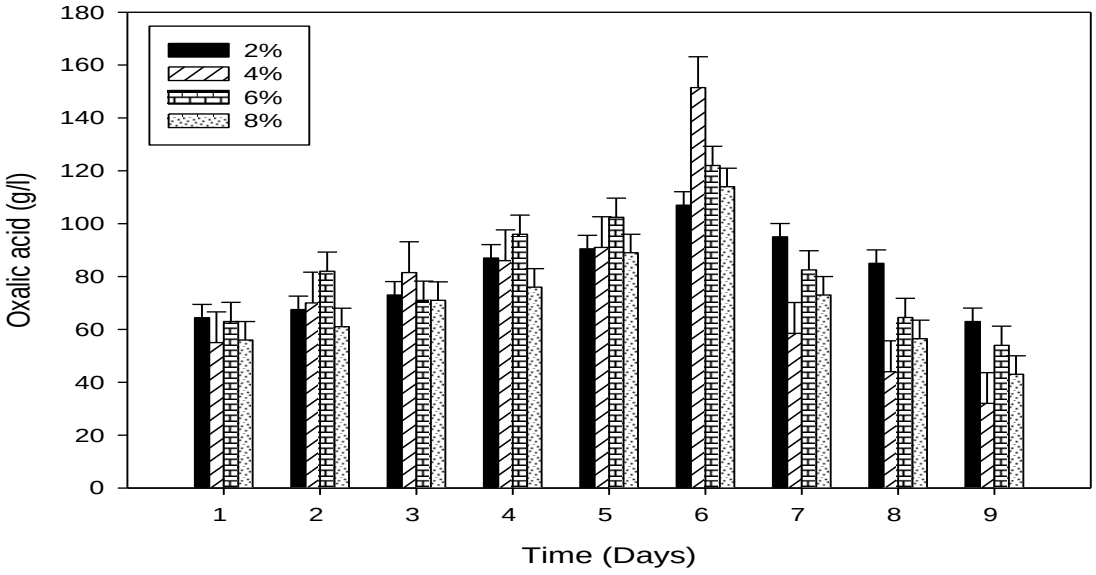


Fig 1a: Effect of Varying Substrate Concentration on Oxalic Acid Production by *Aspergillus niger* using *Jatropha curcas* Seed cake

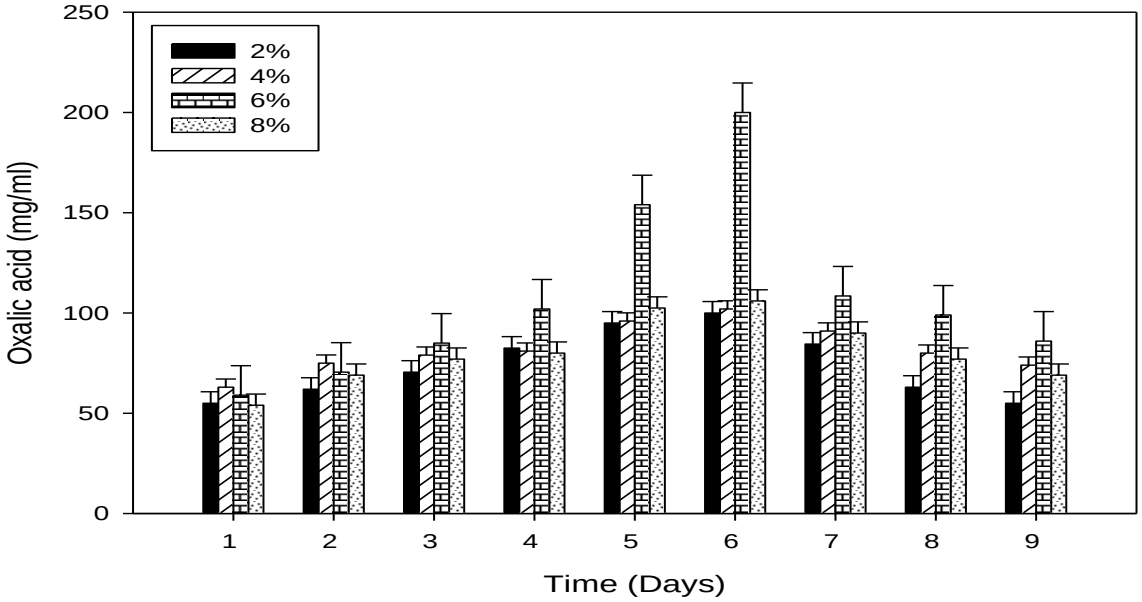


Fig 1b: Effect of Varying substrate concentration on oxalic acid production by *Aspergillus terreus* using *Jatropha curcas* seed cake.

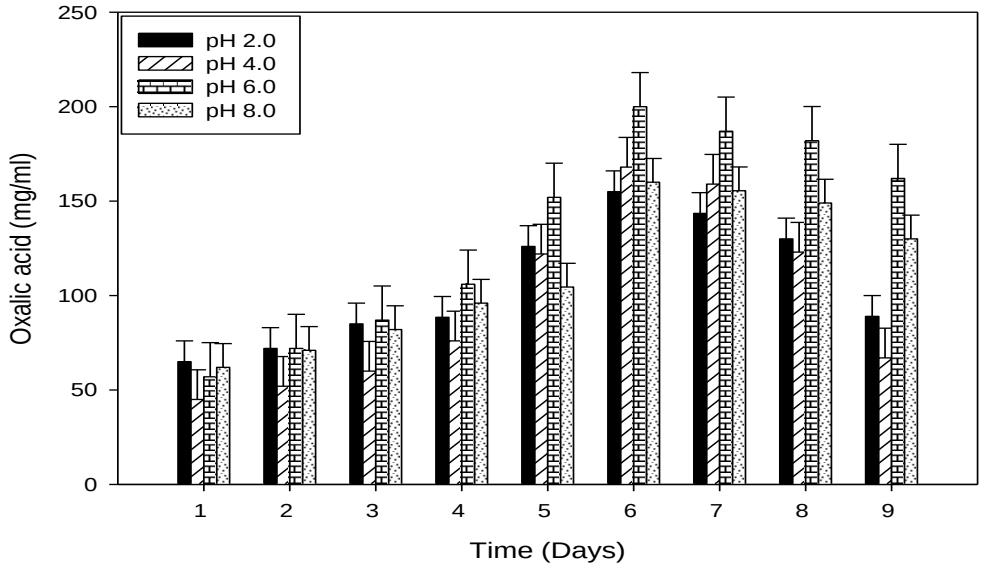


Fig 2a: Effect of Varying pH on Oxalic Acid Production by *Aspergillus niger* using *Jatropha curcas* Seed cake.

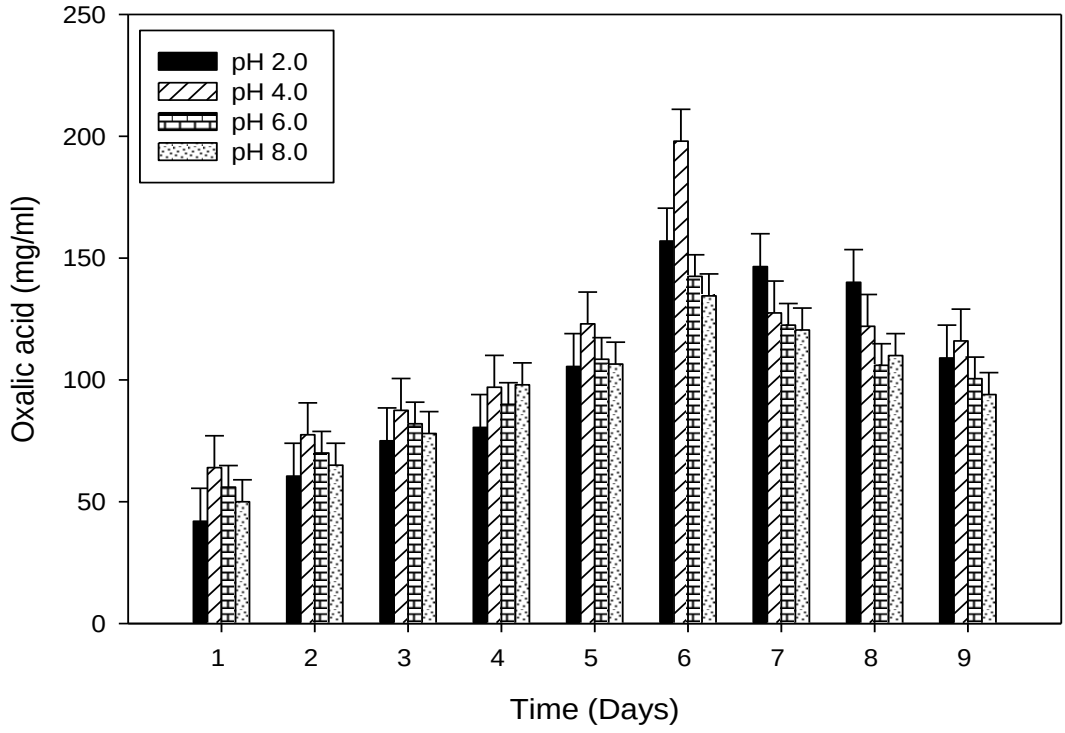


Fig 2b: Effect of varying pH on oxalic acid production by *Aspergillus terreus* using *Jatropha curcas* seed cake.

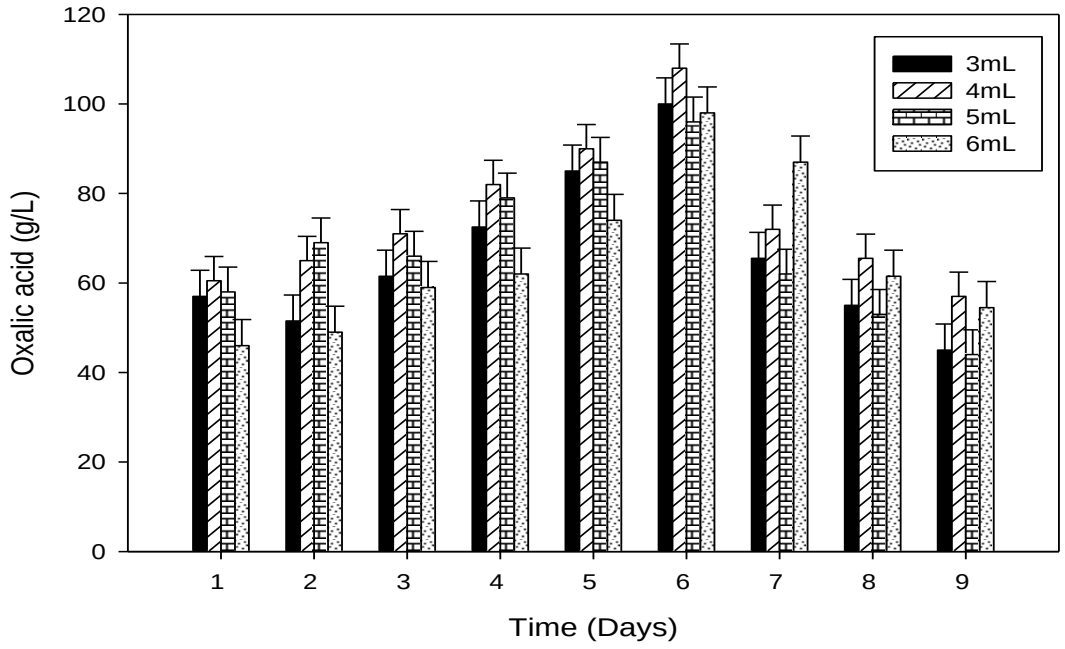


Fig 3a: Effect of Varying Inoculum size on Oxalic Acid Production by *Aspergillus niger* using *Jatropha curcas* Seed cake

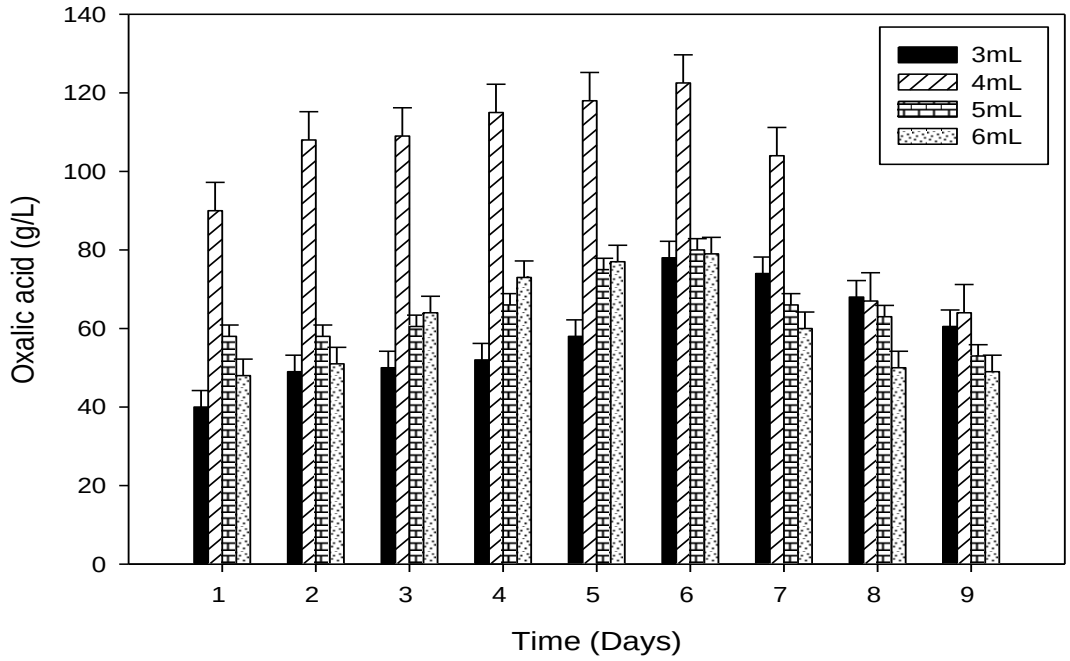


Fig 3b: Effects of Varying Inoculum size on Oxalic Acid Production by *Aspergillus terreus* using *Jatropha curcas* Seed cake.

The results of varying the inoculum size showed highest yield of OA of 108.0± 0.07 g $L^{-1}$ by *A. niger* (Fig. 3a) and 122.0 ±0.15 g $L^{-1}$ by *A. terreus* (Fig. 3b) with inoculum size of 4 ml on Day 6.

All the conditions that yielded the highest amounts of OA in the optimization fermentations were combined in a single fermentation. This optimized fermentation produced a OA yield of 135.0 ±0.42g $L^{-1}$on Day 6 at a pH 6, substrate concentration of 4% and inocula size of 4 mL with *A.niger*, while with *A. terreus* a yielded of 165.0 g $L^{-1}$ on Days 6 at a pH 4, substrate concentration of 6% and an inocula size of 4 mL (Table 2).

Table 2: Optimized production of oxalic acid from defatted *Jatropha curcas* seed cake using *Aspergillus niger* and *Aspergillus terreus*

| Fermentation time | Oxalic Acid (g $L^{-1}$) | | | |
|---|---|---|---|---|
| | *Aspergillus niger* | | *Aspergillus terreus* | |
| (Days) | JSC | (CMC) Control | JSC | (CMC) Control |
| 1 | 69.5 ± 0.06$^{a}$ | 46 ± 0.13$^{a}$ | 56.5± 0.14$^{a}$ | 31 ± 0.19$^{a}$ |
| 2 | 87 ± 0.84$^{a}$ | 52 ±0.39$^{a}$ | 62 ± 0.29$^{a}$ | 35 ± 0.34$^{a}$ |
| 3 | 99 ± 0.08$^{c}$ | 58 ± 0.14$^{a}$ | 75 ± 0.36$^{a}$ | 64 ± 0.40$^{a}$ |
| 4 | 100 ± 0.46$^{b}$ | 62 ± 0.39$^{a}$ | 86 ± 0.13$^{a}$ | 77 ± 0.16$^{a}$ |
| 5 | 105 ± 0.38$^{ab}$ | 75 ± 0.27$^{b}$ | 92 ± 0.21$^{a}$ | 79 ±0.32$^{a}$ |
| 6 | 124 ± 0.57$^{a}$ | 79 ± 0.09$^{ab}$ | 102± 0.20$^{a}$ | 84± 0.29$^{a}$ |
| 7 | 154 ± 0.25$^{a}$ | 147 ± 0.16$^{b}$ | 123± 0.15$^{a}$ | 92.5±0.07$^{ac}$ |
| 8 | 200± 0.56$^{a}$ | 156 ± 0.13$^{ac}$ | 135 ± 0.32$^{a}$ | 118 ± 0.09$^{ab}$ |
| 9 | 187 ± 0.67$^{a}$ | 143 ± 0.37$^{c}$ | 122± 0.17$^{a}$ | 113 ± 0.33$^{a}$ |
| 10 | 165± 0.18$^{ab}$ | 140 ± 0.19$^{ab}$ | 117± 0.15$^{a}$ | 108 ± 0.09$^{ab}$ |

Fermentation parameters *A. niger*: pH 6.0; inocula size 5ml; substrate concentration 6g; temperature 28±2°C
*A. terreus*: pH 4.0; inocula size 4ml; substrate concentration 4g; temperature 28±2°C

**Discussion**

The proximate composition of the defatted JSC substrate reported in this study was comparable to the amounts reported by other workers(Belewu and Sam, 2010; Saetae and Suntornsuk, 2010; Inekwe et al., 2012; Phengnuam and Suntornsuk, 2013). Subtle deviations may be due to differences in seed variety and oil extraction methods. The C/N ratio will serve for good growth of the fermenting microorganisms; which is one of the factors regulating OA production (Mandal and Banerjee, 2006). In addition, *Aspergillus* being cellulolytic in nature (Dashtban et al., 2009) was able to metabolize the JSC, a cellulosic waste without difficulty.

Results from the pre-optimized fermentation to produce OA gave the highest yields on Day 6; 165 g $L^{-1}$ and 135 g $L^{-1}$ by *A. niger* and *A. terreus* respectively (Table 1). This confirms the suitability of JSC as a good substrate for OA production. Other substrates that have been used to produce OA include: cashew apple juice (Betiku et al., 2016); sugar (Van der Merbel et al., 1994); glucose (Mandal and Banerjee, 2006), whey (Bohlmann et al., 1998), beet molasses (Podgorski and Lesniak, 2003), sweet potato starch hydrolysate (Betiku et al., 2014), corncob (Mai et al., 2016)and glycerol derived from biodiesel waste (Andre et al., 2010). This OA yield was higher than 122.68 g $L^{-1}$ on Cashew apple juice reported by Betiku et al. (2016) and 74.75 g $L^{-1}$ on a synthetic medium with lactose as carbon source reported by Mandal and Banerjee (2006).

Substrate concentration was varied between 2-8%. The results showed highest OA yield of 122.0 ± 0.002 g $L^{-1}$ at 4% substrate concentration on Day 6 for *A. niger* (Fig. 1a) and 200 ±0.005 g $L^{-1}$ at substrate

concentration 6% also on Day 6 for *A. terreus* (Fig. 1b). Nitrogen is reported to be one of the most essential nutrients for fungal fermentation (Betiku et al., 2016) and a ten-fold increase in nitrogen content of the medium resulted in a concomitant increase in OA yield (Strasser et al., 1994; Ruijter et al., 1999). The protein content of the JSC substrate used for the fermentation was 29.34±0.02% which provided adequate nitrogen for the fermentation. It was observed that further increase in the substrate concentration to 8% led to a change in the consistency to the medium to sloppy and a drop in OA yield. This may be due to the sloppy nature of the medium which made aeration of the medium more difficult.

Production of oxalic acid has been reported to be influenced by medium pH and fermentable sugars (Strasser et al., 1994; Bohlmann et al., 1998) Therefore, the effect of pH variation (range 2-8) was investigated and results showed that OA yield increased steadily with increase in pH up to pH 6 during the initial 6 days of fermentation. The highest OA yield by *Aspergillus niger* was at pH 6 on Day 6 of fermentation with OA yield of 200g $L^{-1}$ (Fig. 2a), while *Aspergillus terreus* yielded 198 ±0.62 g $L^{-1}$ also on Day 6 but at pH 4.0 (Fig. 2b). It has been reported that in *A. niger*, fermentation medium pH of 4 causes the induction of the oxaloacetate hydrolase enzyme which is responsible for OA production (Kubicek et al., 1988). Other workers also reported that pH of 6 is important for OA accumulation (Bohlmann et al., 1998; Mandal and Banerjee, 2005; Aghaie et al., 2009). This largely is confirmed by the work of Mandal and Banerjee (2006), who reported maximum oxalic acid production on day 7 at pH 6; Adesina et al. (2014) who reported highest OA oxalic acid production at pH 6.2 with yield of 149.0 g $L^{-1}$. This also confirms the already established findings that oxalic acid has been reported to be optimal in the pH range of 5-8 (Cleland and Johnson, 1956; Lenz et al., 1976).

The inoculum size which gave the highest OA yield was 4 mL on Day 6 yielding 108.0± 0.07 g $L^{-1}$ by *A. niger* (Fig. 3a) and 122.0 ±0.15 g $L^{-1}$ by *A. terreus* (Fig. 3b). Fungal type and density are important factors in fungal fermentation (Papagianni, 2004). Organic acid production may be restricted if there is overcrowding leading to competition for nutrients and depletion of oxygen due to high microbial numbers. This may be responsible for the decreased OA yield observed with higher inoculum amounts.

A variety of fungi have been used to fermentatively produce oxalic acid including *A. niger* (Ruijter et al., 1999; Mandal and Banerjee, 2006; Betiku et al., 2016), *A. brasiliensis*, *G. weberianum*, *Streptomyces* (Liaud et al.,2014). However, *A. niger* seems to be the most studied possibly due to its ability to acidify its environment through extracellular processes. Therefore *A. niger* is reported to be an efficient OA producer (Strasser et al., 1994; Ruijter et al., 1999). This is supported by the overall OA yield in this study which records higher production of OA by *A. niger* than *A. terreus*.

The results of the optimized fermentation gave OA yields of 200±0.56 g $L^{-1}$ and 135±0.32 g $L^{-1}$ by *A. niger* and *A. terreus* respectively (Table 2). In this fermentation, highest OA yields were recorded on Day 8 rather than Day 6 of previous pre-optimized fermentations carried out in this study. Also, *A. niger* produced higher OA yields than *A. terreus* as was observed in the initial fermentation. In addition, while there was a 48% increase in OA yield by *A. niger*, there was a 18% drop in OA yield by *A. terreus*. This is thought to be due to the difference in the physiological conditions brought about by the variation of fermentation parameters in the optimization experiments carried out earlier.

**Conclusion**

This study sought to establish the suitability of JSC as a substrate for the production of OA by *A. niger* ATCC 16404 and *A. terreus* ATCC 20542. Appreciable amounts of OA were produced exceeding amounts recorded previously by other workers. The yield by *A. niger* was further increased by 48% through the manipulation of fermentation parameters. It can therefore be concluded that experimental strain of *A. niger* ATCC 16404 and *A. terreus* ATCC 20542 could be used for producing oxalic acid on a large scale. As the use of JSC as a bio-fuel source is on the increase, the seed cake residue will be available for the production of OA. This will help prevent environmental pollution and at the same time provide cheap substrate for the production process.

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