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The Production of Itaconic Acid from Sweet Potato Peel Using *Aspergillus niger* and *Aspergillus terreus*

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Abstract:

Accumulation of large quantities of agricultural residues results in deterioration of the environment and biomass loss which could be processed to yield value-added products like fuels and a variety of acids. The quest for a solution led to the fermentation of *Ipomoea batatas* using fungi. *Aspergillus niger* (ATCC 16404) and *Aspergillus terreus* (ATCC 20542) were used to ferment sweet potato peel (SPP), an agro-based waste. The physico-chemical analysis of the SPP was carried out. SPP was dried, ground, pre-treated with alkali and steam, re-dried and used as substrate in media containing mineral salt medium and inocula of *A. niger* and *A. terreus.* Fermentation was submerged at pH 5.0; 10% substrate concentration; 10 mL inocula size; temperature 25^0 C for five days with carboxylmethylcellulose (CMC) as control. Optimization experiments were conducted by varying fermentation parameters. Results of physico-chemical analysis revealed carbohydrate 65.9%; sugars 22.60%; protein 5.38%; fibre 3.48%; fat 4.0% ash 4.02% and moisture 8.13%. Itaconic acid yield of 67.67$\pm$1.20 mg mL^{-1} and 70.67$\pm$2.60 mg mL^{-1} produced by *A. niger* and *A. terreus* from SPP respectively. The CMC yielded 3.00 $\pm$0.6 mg mL^{-1}. Results of the optimization experiments showed higher yields of itaconic acid by *A. niger* and *A. terreus* to 112.67$\pm$5.20 mg mL^{-1} and 115.67$\pm$5.30 mg mL^{-1} from SPP respectively at pH 4.0; 10% substrate concentration; 5 mL inocula size on Day-5 of the fermentation. This represented an increase in product yield by both organisms and supports the potential use of this waste for the industrial production of itaconic acid.

Keywords: Itaconic acid, Sweet-potato, *Aspergillus terreus, Aspergillus niger*.

1. Introduction

Large quantities of agricultural and agro-industrial residues that are generated as a result of diverse agricultural and industrial practices represent one of the most important energy-rich resources. Accumulation of this biomass in large quantities every year results not only in deterioration of the environment but in a loss of potentially valuable materials which can be processed to yield a number of value-added by products such as food fuel and variety of chemicals [11]. Large amounts of agricultural wastes consist of polysaccharides which could be converted into valuable fermentable sugars for the production of other organic acids.

Various microorganisms possess the ability to convert carbohydrate to high yield organic acids. However fungi are the most widely used microorganisms in fermentation due to their ability to grow at low pH and moisture content; high tolerance for acids; ability to form hyphae which penetrates substrates and ability to produce various enzymes for the conversion of different wastes to useful products[2].

Organic acids with wide applications in various fields are made from living cells commercially. Organic acids like citric acid, gluconic acid, itaconic acid and lactic acids are manufactured by means of such large-scale bioprocesses, among them itaconic acid is one the most promising [13].

Itaconic acid (IA) is a colourless crystalline carboxylic acid obtained by fermentation of carbohydrates [5]. It has a melting point of 167-168^0C and density of 1.632 [8]. It is an unsaturated dicarboxylic acid in which one carboxyl group is conjugated to the methylene group. The methylene group is able to take part in addition polymerization giving rise to polymers with many free carboxyl groups that confer advantageous properties on the resulting polymer [12]. Itaconic acid with IUPAC name 2-methylenebutanedioic acid and chemical formular $C_5H_6O_4$ is synonymously called 2-Methylenesuccinate, 2-Methylenesuccinic acid, Methylenebutanedioate, Methylenebutanedioic acid, 2 - propene - 1,2 - dicarboxylate, 2 – Propene - 1,2 - dicarboxylic acid, Propylenedicarboxylate, Propylenedicarboxylic acid and itaconate [3].

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Itaconic acid is one of the twelve promising organic acids, can be used for several specialities and so called 'green plastic' [8]. It is used worldwide in the industrial synthesis of resins such as polyesters, plastics, and artificial glass and in the preparation of bioactive compounds in the agriculture, pharmacy, and medicine sectors coatings, and other industrial products, it is stable at acidic, neutral and middle basic conditions at moderate temperatures [20].

The primary application of itaconic acid is in the polymer industry where it is employed as a co monomer at a level of 1-5 % for certain products. Its derivatives are used in medicine and cosmetic preparation. Itaconic acid can react with acrylic and methacrylic acid or their esters which is widely employing to prepare resins used in emulsion coating, leather coating, coatings for car, refrigerators and other electrical appliances to improve adhesion, colour and weather resistance. In general, though several raw materials can be used glucose, sucrose and xylose are preferred raw materials for itaconic acid fermentation, which are known to be utilized efficiently by most of the *Aspergillus* sp. [13].

The sweet potato (*Ipomoea batatas*) is a dicotyledonous plant that belongs to the family Convolvulaceae. Its large, starchy, sweet-tasting, tuberous roots are an important root vegetable. The young leaves and shoots are sometimes eaten as greens of the approximately 50 genera and more than 1,000 species of Convolvulaceae, *Ipomoea batatas* is the only crop plant of major importance some others are used locally, but many are actually poisonous.

Potatoes are full of starch which is a type of complex carbohydrate, it contains high a carbohydrate content which is actually good as it takes a while for the body to breakdown. All types of potatoes provide complex carbohydrates in the form of starch and fibre [4].

The objectives of this study were to determine the suitability of sweet potato waste as a cellulosic substrate for the fermentative production of itaconic acid; to determine the ability of *Aspergillus niger* and *Aspergillus terreus* to utilize it as substrate and to determine the conditions for optimal production..

2. Material and Methods

2.1. Plant Material and Microorganisms

Sweet potatoes were purchased at Ganmo market in Ilorin, Nigeria. The potatoes were washed to remove dirt; scrapped to remove the peel and air dried at room temperature. The sample was ground after which it was kept in an air-tight dessicator with some bags of silica gel to prevent moisture absorption. The sweet potato peel (SPP) was pretreated using the alkali hydrolysis method of Omojasola and Jilani (2009) Caboxymethylcellulose (CMC) served as control. The test organisms used were *Aspergillus niger* (ATCC 16404) and *Aspergillus terreus* (ATCC 20542). These were collected from Federal Institute of Industrial Research Oshodi, Nigeria (FIIRO) and maintained on Potato Dextrose Agar (PDA). Fungal spore inocula were produced by suspending spores in sterile distilled water and adjusting to approximately $2.6x\ 10^8\ mL^{-1}$ and $3.7x10^8 mL^{-1}$ of *Aspergillus niger* and *Aspergillus terreus* by counting using the improved Neubauer Haemocytometer [17].

2.2. Physico-chemical Analysis of Sweet Potato Peel Waste

The physico-chemical analysis of the ground sweet potato peel (SPP) was determined. The parameters assayed were pH using digital pH meter (Denver Model 20 pH/Conductivity meter); moisture content [1]; ash [1]; crude protein [1]; total sugars [6];crude lipids [16], total carbohydrates and crude fibre [1].

2.3. Fermentation Medium

SPP (10% v/v) was mixed in 100 mL of distilled water in separate flasks. Mary Mandel's Mineral Salts Medium was used for fermentation. The sterilized media were inoculated with $2.6x10^8$ and $3.7x10^8$ spores mL^{-1} of *Aspergillus niger and Aspergillus terreus* separately. Each flask was cultured on a rotary shaker (Gallenkamp, England) at 400 rpm; temperature 29 ± 1^0 C and the samples were assayed for IA at 24 hour intervals with the UV spectrophotometer at 385nm [13].

2.4. Optimization of Itaconic Acid Production

Optimization experiments were conducted to determine the yield efficiency optimal conditions for IA production as a function of variation of fermentation conditions.

Effect of Varying Substrate Concentration

Different concentrations of the waste substrates (SPP) ranging from 2-10% were used in the fermenting media. The pH was held at 5.0; 10% inocula size and temperature 29 ± 1^0 C.

Effect of Varying pH

The pH of the fermentation media was adjusted to between 2.5-6.5 by the addition of 0.1N HCl and 0.1N NaOH. Substrate concentration and inocula size were held at 10%; temperature 29 ± 1^0 C.

Effect of Varying Time

Itaconic acid production was determined at 24 h intervals for 1-10 days. Substrate concentration and

inocula size were held at 10%; temperature 29±1^0 C; pH 5.0.

Effect of Varying Inocula size

The fermentations were carried out with varying inocula size. The inocula size was varied from 1, 3, 5 and 11ml. Spore suspension containing about 2.6x 10^8 ml and 3.7x10^8ml of *Aspergillus niger* and *Aspergillus terreus* spores per ml suspension were used as inocula for the fermentation process. The pH was held at 5.0; inocula size and substrate concentration 10%; temperature 29±1^0 C.

The fermentation parameters that yielded highest amounts of itaconic acid in the optimization experiments were then combined in a single fermentation in an attempt to further increase yield efficiency.

3. Results and Discussion

3.1. Proximate composition of Sweet Potato Peel

In this study, SPP an agro-based waste generated from *Ipomoea batatas* was used as substrate for itaconic acid production. The composition of SPP was carbohydrate 65.9%; sugars 22.60%; crude protein 5.38%; crude fibre 3.48%; crude fat 4.0% ash 4.02% and moisture 8.13%. The carbohydrate content was high and would be a good carbon source for itaconic acid production. The protein content of 5.38% would also serve as a good nitrogen source for microbial metabolism.

3.2. Fermentation

The pre-optimization fermentation yielded 55.00± 2.2 and 47.33±3.0 g L^{-1} by *A. niger* and *A. terreus* respectively by Day 5 of fermentation (Table 1). This yield exceeded the amount observed by [7] who reported a maximum yield of 38.70 g L^{-1} from *Jatropha curcas* seedcake using *A. terreus.* [13] produced itaconic acid from a variety of substrates ranging from cane molasses, potato peel, banana peel and rice bran using an array of *Aspergillus* spp obtained yields ranging from 4.80-8.10 g L^{-1}. A yield of of 29 g L^{-1} was recorded by [18] from a synthetic medium using *Ustilago maydis.* The differences in substrate and fermenting organisms may account for these differences observed in the yield.

Table 1: The Fermentation of Sweet Potato Peel by Aspergillus niger and Aspergillus terreus for the Production of Itaconic Acid

	Quantity of Itaconic acid produced (g L^{-1})		
	Fermentation Period (Days)		
Test Organism	2	5	10
Aspergillus niger			
Sweet Potato Peel	26.00±2.1^b	55.00±2.2^c	16.00±1.2^a
CMC	7.00±0.8^a	15.67±1.2^b	9.33±0.9^a
Aspergillus terreus			
Sweet Potato Peel	20.00±1.7^b	47.33±3.0^c	15.00±1.7^a
CMC	6.67±0.5^a	10.67±4.3^b	3.00±0.3^a

Fermentation parameters: Substrate concentration 10%; pH 5.4; Inocula size 10ml; Temperature 29±1^{0}C
Values presented are Means ±SD; Values with different superscript are significantly different at p<0.05

The conditions of the fermentation were varied in a series of optimization experiments with a view to increasing the product yield. Generally, changes in culture conditions greatly influence the production ability of a microbial strain. With the variation of fermentation time, IA production increased steadily to a maximum yield of 67.67±1.2 and 70.67±2.6g L^{-1} on Day 5 (Table 2). For substrate concentration, 10% w/v yielded maximum product of 96.0±4.0 and 104.67 ±3.2 g L^{-1} (Table 3); pH 4.0 yielded 96.67±5.7 and 106.67±3.9 g L^{-1} (Table 4); 5 mL inocula size yielded 77.33±1.5 and 88.33±4.3 g L^{-1} (Table 5) by *A. niger* and *A. terreus* respectively. *A. terreus* was more efficient in IA production than *A. niger* and this was observed in almost all the fermentations. *A. terreus* has been observed to be a natural producer of IA and is its strains are reported in production levels up to 80-86 g L^{-1} [14, 9, 22, 23]. *A. niger* is reported to have a high capacity to accumulate the precursor of IA: citric acid. Some researchers theorize that genetic manipulation of *A. niger* by insertion of the *CadA* gene can lead to IA production levels in excess of 135 g L^{-1} [10,23]. Substrate concentration was varied between 2-10%. The results show that IA yield increased with increase in the concentration of substrate. However, attempts to increase the concentration beyond 10% changed the composition of the medium from sloppy to solid. [13]also reported optimum yield from molasses substrate at 10%. The optimal pH for maximum yield of IA was pH 4.0 (Table 4). This pH was found most suitable by [21]

with *A. flavus* and [7]using *A. terreus* but pH 3.0 by [19] using *U. maydis.*

Table 2: Effect of Varying Fermentation Time on Itaconic Acid Production by *Aspergillus niger* and *Aspergillus terreus* Using Sweet Potato Peel

	Itaconic Acid (g L^{-1})			
	Aspergillus niger		*Aspergillus terreus*	
Fermentation Period (Days)	SPP	CMC	SPP	CMC
1	16.33±1.8^{a}	3.00±1.2^{a}	22.33±1.9^{b}	2.67±1.2^{ab}
2	20.33±1.5^{a}	8.00±1.2^{bc}	32.33±1.5^{c}	8.67±1.5^{cd}
3	26.33±1.5^{b}	16.67±1.2^{e}	49.33±3.0^{d}	13.33±0.9^{ef}
4	38.00±2.1^{c}	22.67±1.8^{f}	63.67±2.3^{e}	21.00±0.6^{g}
5	67.67±1.2^{f}	30.00±0.6^{g}	70.67±2.6^{f}	29.00±1.7^{h}
6	59.00±2.1^{e}	24.00±1.5^{f}	59.33±1.2^{c}	20.00±0.6^{g}
7	48.33±1.5^{d}	14.67±0.9^{de}	50.00±2.1^{d}	15.33±1.5^{f}
8	40.67±2.2^{c}	11.33±0.9^{cd}	33.33±2.4^{c}	10.00±0.6^{de}
9	27.67±1.2^{b}	7.00±0.6^{bc}	21.33±1.4^{b}	5.67±1.8^{bc}
10	17.67±1.5	4.67±1.3^{ab}	15.00±1.7^{a}	1.67±0.6^{a}

SPP: Sweet Potato Peel, CMC: Carboxymethycellulose:Time: 5days, pH: 5.4; Inocula size: 10ml;
Values presented are Means ±SD;Values with different superscript are significantly different at $p<0.05$.

Table 3: Effects of Varying Substrate Concentration on Itaconic Acid Production by*Aspergillus niger* and *Aspergillus terreus* Using Sweet Potato Peel

Waste Substrate	Substrate Concentration (%)					
	0	2	4	6	8	10
Aspergillus niger						
Sweet Potato Peel	0	54.33±2.9^{a}	67.67±1.2^{b}	73.33±3.3^{c}	81.67±2.7^{c}	96.00±4.0^{d}
CMC (Control)		11.00±1.2^{a}	15.00±1.2^{b}	17.33± 1.8^{c}	20.00±2.3^{cd}	23.33±3.3^{d}
Aspergillus terreus						
Sweet Potato Peel	0	56.00±2.1^{a}	65.00±1.5^{b}	81.33±1.5^{c}	90.00±1.7^{d}	104.67±3.2^{d}
CMC (Control)	0	16.33±1.2^{ab}	18.00±1.5^{b}	21.33±0.9^{c}	23.67±1.8^{d}	26.00±2.1^{d}

CMC: Carboxymethycellulose: Time: 5days, pH: 5.4; Inocula size: 10ml; Values presented are Means ±SD;
Values with different superscript are significantly different at $p<0.05$.

Table 4: Effect of Varying pH on Itaconic Acid Production by *Aspergillus niger* and *Apergillus terreus* Using Sweet Potato Peel

Waste Substrate	pH						
	2.5	3.5	4.0	4.5	5.0	6.0	6.5
Aspergillus niger							
Sweet Potato Peel	48.33±2.9^{e}	56.33±3.7^{ef}	96.67±5.7^{g}	62.00±2.9^{f}	46.00±3.2^{cd}	37.00±1.7^{bc}	31.67±1.8^{b}
CMC (Control)	17.00±2.1^{c}	19.33±2.9^{d}	24.33±3.3^{e}	21.00±2.3^{cd}	15.00±1.8^{d}	13.00±1.5^{b}	11.00±1.2^{a}
Aspergillus terreus							
Sweet Potato Peel	59.0±1.2^{d}	83.00±3.2^{e}	106.67±3.9^{f}	65.0± 3.6^{d}	57.33±2.6 ^{d}	48.00±1.7 ^{c}	40.00±0.6^{b}
CMC (Control)	18.0±1.2^{b}	22.00±2.1^{c}	26.67±4.1^{d}	21.33±1.8^{c}	18.00±2.1^{b}	14.00±1.5^{b}	10.33±1.2^{a}

CMC: Carboxymethycellulose: Time: 5days, pH: 5.4; Inocula size: 10ml; Values presented are Means ±SD;
Values with different superscript are significantly different at $p<0.05$.

Table 5: Effect of Varying Inocula Size on Itaconic Acid Production by *Aspergillus niger* and *Apergillus terreus* Using Sweet Potato Peel

Waste Substrate	Inocula size (ml)			
	1	3	5	11
Aspergillus niger				
Sweet Potato Peel	38.00±1.2^{b}	49.33±0.9^{c}	77.33±1.5^{d}	53.00±1.2^{c}
CMC (Control)	15.00±1.2^{b}	18.00±1.5^{c}	23.33±2.1^{d}	20.67±1.2^{c}
Aspergillus terreus				
Sweet Potato Peel	56.00±2.1^{b}	66.67±2.9^{c}	88.33±4.3^{e}	75.33±2.2^{e}
CMC (Control)	17.67±1.5^{b}	20.00±2.1^{c}	25.33±2.1^{d}	22.00±1.5^{b}

CMC: Carboxymethycellulose: Time: 5days, pH: 5.4; Inocula size: 10ml; Values presented are Means ±SD; Values with different superscript are significantly different at $p<0.05$.

When the conditions of that produced maximum yields of IA were combined in an optimized fermentation (10% w/v substrate concentration, 5mL inocula size, pH 4 for 5 days at 29± 1 ^{0}C), the results showed an increase in yield of IA to 112.67 ±5.2 and 115.67±5.3 g L^{-1} by *A. niger* and *A. terreus* respectively (Table 6). This represents 75.8 and 125.27% increase over the highest yield recorded during the variation of conditions fermentations; and 104.85 and 144.39% increase in the final fermentation which combined all the optimized parameters.

Table 6: Optimized Production of Itaconic Acid by *Aspergillus niger* and*Aspergillus terreus*

	Itaconic Acid (g L^{-1}) / Days	
	2	*5*
Aspergillus niger		
Sweet Potato Peel	57.00±2.1	112.67±5.2
CMC (Control)	15.00±1.2	27.00±1.7
Aspergillus terreus		
Sweet Potato Peel	72.33±4.9	115.67±5.3
CMC (Control)	29.67±1.5	38.33±1.2

Fermentation parameters: substrate concentration:10% ; pH :4.0; time :5 days; Inocula size 5 mL

4. Conclusions

In this study, submerged fermentation was employed the peel of *Ipomoea batatas* an agro-based waste as substrate for the production of itaconic acid using *A. niger* (ATCC 16404) and *A. terreus* (ATCC 20542).

The results show that the peel of *Ipomoea batatas* is a suitable substrate for IA production by both *A.niger* and *A.terreus.* These organisms produced > 100 g L^{-1} of IA from the SPP waste substrate. This yield represents 75.9 and 66.9% increase than the yield from CMC the control substrate. In addition, it is more than the yield of 80-86 g L^{-1} reported by other workers. The potential of this agro-based waste can be explored in the industrial production of itaconic acid.

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