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BIOFLOCCULANT PRODUCTION FROM PLANTAIN (*Musa paradisiaca*) PEELS USING *Aspergillus niger* ISOLATED FROM WATER SEDIMENT

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ABSTRACT

Bioflocculants have gain substantial consideration as potential substitute to chemical flocculating agents because they are biodegradable and produce no toxic effect. This study evaluates *Musa paradisiaca* peels as nutrient source for bioflocculant production. plantain peels were collected from Minna, Niger State, Nigeria. The hemmer milled peels were pulverized to fine powder, sieved and refluxed with NaOH in Erlenmeyer flask containing 1L distilled water and autoclaved at 121°C. The *A. niger* isolated from the water sediment of Bosso dam were grown in plantain peel broth supplemented with different nitrogen and carbon sources to produce the bioflocculant. A suspension of Kaolin (4 g/L) was used as model wastewater for estimating flocculating efficiency of the produced crude bioflocculant. The four (4) fungi namely *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus* and *Trichophyton spp* isolated from the water sediment yielded bioflocculation efficiency of 80.1% ± 1.9, 73.7 ± 5.0%, 72.8 ± 2.1, and 66% ±1.8, prompting the used of *A. niger* for subsequent investigations. Yeast extract (95%) and peptone (91%) were the most preferred nitrogen source while glucose, maltose, fructose and sucrose all show remarkable bioflocculant production when used as supplementary carbon sources (83 to 86.9% flocculation efficiency). The utilization of plantain peels to produces bioflocculant can resourcefully lower the cost of bioflocculant production and offers an alternative strategy of handling environmental pollution by the Plantain peels as well maximizing the use of biomolecules in the peels.

Keywords: Bioflocculant, Water Sediment, Plantain Peels, Aspergillus Niger.

INTRODUCTION

Microbial cells produce natural metabolites which have ability to flocculate many substances, such as cells, and colloidal substances. Flocculants are used in many industrial techniques for treating wastewater (Deng *et al*., 2003), Removal of impurities in consumable water (Li *et al.*, 2009), Wastewater management (Liu *et al*., 2009) and downstream processing. The removal of colloidal substances from wastewater by microorganisms or their product is called bioflocculation. The polymers produced by microbial cells during replication can be used to achieve flocculation and are called microbial flocculants or Bioflocculant (Deng *et al*., 2003). Bioflocculants are produced by many microbial species including *Rhodococcus erythropolis* (Kurane *et al*., 1994), *Brachybacterium sp.* (Nwodo *et al*., 2013), *Cellulomonas sp.* Okoh (Nwodo and Okoh, 2013) and *Bacillus subtilis* F9 (Giri *et al*., 2015).

Flocculant can generally be classified into (a) Natural flocculants such as: (microbial flocculant, chitosan, tanin) (b) Organic flocculants (polyacrylamide derivatives and polyacrylic acid), and (c) Inorganic flocculants (polyaluminum chloride and aluminium sulphate). Moreover, several industries today prefer using the last two category due to their low-cost of production; nevertheless, they have

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negative impact in both environment and human health (Mabinya *et al*., 2011). For example, polyacrylamide are carcinogenic to humans and neurotoxic due to their acrylamide monomer residues (Nwodo *et al*., 2014). Alzheimer's disease developed due to the constituent of polyaluminium (Li *et al*., 2007; Okaiyeto *et al*., 2015). As a result, scientists are concentrating on seeking safer alternative to these chemical flocculants.

Microbial flocculants have received much attention as alternative to the chemical flocculants considering their advantage of not being toxic in nature, lacking secondary pollution and being biodegradable (Cosa *et al*., 2011). Metal ions removal from polluted effluents, humic acid elimination, dye solutions treatment, treatment of wastewater has been achieved by the use of microbial flocculant (Li *et al*., 2000; Salehizadeh and Shojaosadati 2001).

Although bioflocculant have been proved to offer all the aforementioned advantages, elevated cost of production that arise from cost of fermentation substrate and low flocculant production and efficiency have been the major issues hindering their large-scale production for industrial application. Thus, prompting the need to search and screen new microorganisms with enhanced microbial flocculant producing capacity and search for cost-effective fermentation substrates. Optimization of culture conditions for potential new organisms and fermentation substrates could also help to increase bioflocculant production. The present study aimed to grow fungi isolated from a natural flocculating site (water sediment) on plantain peel broth to evaluate the bioflocculant production potential of the fungi.

MATERIALS AND METHODS

Sample Collection and Preparation

The nutrient source for microbial fermentation; Plantain peels (*Musa paradisiaca*) were collected in clean polythene bags from main market in Minna, Niger State, Nigeria. Moisture content of the samples were removed by air drying at room temperature. Wire mesh of 80-100 pores were used to sieve hemmer milled peels to a fine powder, the sieved plantain peels were weighed and refluxed with NaOH in Erlenmeyer flask containing 1L distilled water and filtered through a muslin cloth and autoclaved at 121°C for 15min. The plantain peel broth was characterized for carbohydrate, crude fiber, ash, protein, fat and trace elements all using standard procedures.

Isolation of the Bioflocculant Producing Fungi

The bioflocculant producing fungi were isolated from the water sediment of Bosso dam in Minna Niger state. The water sediment from the Bosso river in Minna was collected into a clean and sterilized bottle and immediately transported to the laboratory under ice pack. The sediment was further diluted with sterile water. A wire loop was dipped into the diluted sediment sample and aseptically and gently rubbed on the middle of different SDA plates; The plates were incubated at 28°C ± 2ºC for 3 – 5days. The resulting fungal growth with distinct colours and morphologies were subcultured onto fresh SDA plates for pure isolate and incubated for 3-5days. The isolated colonies were preserved on agar slant for further use. Each of the colonies were characterized morphologically and microscopically and compared with kown taxa using fungal atlas.

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Preparation and Standardization of the Inoculum

Each of the fungal isolate was sub-cultured on SDA from a stock culture obtained from slant agar. A loop of each of the fungus was introduced on to the middle of SDA plates following aseptic techniques. The SDA plates was carefully incubated at room temperature for 5 days for the fungi to grow and form mature spores. Subsequently 2 mLs of 1% Tween 80 was slightly added to the five days grown culture plates. The spores were then scrapped into a sterile beaker with the aid of a hockey stick. The spores were separated from the Tween 80 by means of centrifugation. The upper supernatant was poured out, and the spores collected as pellets. The spore concentrations were measured using haemocytometer counting. Using a microscope, the number of spores in every grid of the haemocytometer was counted and multiply by the grid volume to give a total spore per mL. The Fungal spores were poured into sterilized deionized water to give a preliminary spore count per mL.

Production of the Bioflocculant

The bioflocculant was produced by growing the fungi in a plantain peel broth supplemented with glucose as the fermentation medium. To inoculate the production broth, an appropriate number of spores was used as inoculum to ensure a significant number of spores. The spores were inoculated into the screening and production medium (pH 7) at inoculum volume of 4% and incubated for 72 h at temperature of 25°C, agitated intermittently following the optimized conditions stated in Mohammed and Dagang, 2019. Subsequently, the 72h culture broth was dispensed into 50mL centrifuge tubes and spined for biomass removal. The microbial flocculant rich culture supernatant was collected into sterile glass beaker and used as the crude microbial flocculant. The bioflocculant production was compared with cell growth over 0 – 168 hours of incubation. The effects of addition of different nitrogen sources and carbon sources to the plantain peel broth were also examined in terms of efficiency of bioflocculant produced.

Determination of Flocculation Efficiency

Microbial flocculant efficiency was obtained in accordance with the methods established by More *et al*. (2015) and Xia *et al*. (2018). A suspension of Kaolin (4 g/L) was used as model wastewater for estimating flocculating efficiency of the produced crude bioflocculant. Kaolin clay suspension (100 mL), 3 mL of 1% $CaCl_2$ and 2 mL of the culture supernatant were mixed into a conical flask. The solution was shaked for 60s, and then transferred into a 100 mL graduated cylinder for sedimentation for 5 min at room temperature. The optical density (OD) of the upper clarified water was measured at 550 nm with a spectrophotometer and flocculating activity was calculated in percentage using the following formula.

$$FA = [(A-B/A)] \times 100\%$$

where FA = Flocculating activity, A and B are optical densities of control (in which sterilized Plantain peels broth was used in place of the microbial flocculant) and sample measured at 550 nm.

RESULTS AND DISCUSSIONS

Determination of Mixed Fungal Growth Obtained from the Water Sediment of Bosso Dam

Inoculation of water sediments sample from Bosso dam on the Sabouraud dextrous agar (SDA) plate, resulted in fungal growth with different colours and morphologies as displayed in Figure 1. The fungal growths colours ranged from vaguely milk colour, greyish to white and

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slightly black colours. The morphologies of the growths were mixtures of smooth walled, velvet with very rough and irregular edges. Upon the morphologic scrutiny, the fungal growths with dissimilar colours and morphologies were each aseptically re-inoculated on a fresh SDA plate to obtain pure growth.

Figure 1: Mixed fungal growths obtained from the water sediment of Bosso dam

Determination of Pure Fungal Growths Obtained from Water Sediment of Bosso Dam

Four pure fungal isolates were obtained from the mixed culture as depicted in Table 2. The isolates were identified by comparing their morphological characteristics and microscopic features with those of known taxa using mycological atlas. The isolates included *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus* and *Trichophyton spp*. One of the isolates appeared greyish and smooth walled with filamentous septate hyphae and green conidia (*Aspergillus fumigatus*). Another one had Yellowish-green on the upper surface, septate and hyaline hyphae, soft velvet surface like shapes with smooth and rough edges (*Aspergillus flavus*), One isolate was greyish to white at 24 to 48 hours that later turned black, filamentous hyphae, smooth surface and conidia (*Aspergillus niger*). Other pure isolates had upper side colours to be pink and under purple, septate hyphae, velvet surface and Smooth wall macro and microconidia (Trichophyton species). In the subsequent experiments, each of these fungal isolates were screened for bioflocculant secretion using a screening medium before growing them on the production broth (plantain peel broth). The pure isolates were also preserved on the slant SDA bottles for further characterization and future use

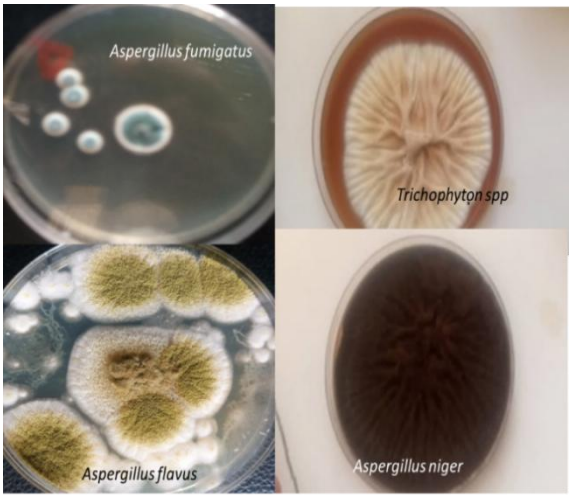

Figure 2: pure fungal isolates obtained from the water sediment of Bosso dam

Production of Bioflocculant by the Fungal Isolates

The bioflocculant secretion by growing each of the fungal isolates on the screening medium as determined through the flocculation rate of the culture supernatant on a model wastewater (Kaolin clay suspension) is shown in table 2. *A. niger* had the highest flocculation rate of 80 ± 1.9 % followed by the culture supernatant of *Aspergilus fumigatus* which had flocculation rate of 73.7 ± 5%. *A. flavus* recorded flocculation rate of 72.8 ± 5%. The *Trichophyton* spp had the least flocculation rate of 66 ± 1.8%.

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Table 1: Morphological and cultural characteristics of the fungi isolated from from the water sediment of Bosso dam

Sample	Colour	Hyphae	Surface	Conidia	Inference
Isolate 1	Initially white then black	Filamented septate	Smooth walled	Smooth conidia	*Aspergillus niger*
Isolate 2	Greyish near apex	Filamentous Septate	Smooth walled	Green conidia with spikes	*Aspergillus fumigatus*
Isolate 3	Yellowish- green	Threadlike Septate and hyaline	Soft velvet	Rough and colourless conidiospores	*Aspergillus flavus*
Isolate 4	Upper side pink and under purple	septate	velvet	Smooth wall macro and microconidia	*Trichophyton spp*

Table 2: Bioflocculant production by the isolated fungal growth on the screening medium for 72 hours.

Fungal Isolates	OD 1	OD 2	OD 3	Mean OD	Bioflocculation (%)	std (%)
A. niger	0.189	0.177	0.214	0.193	80.1	1.9
A. fumigatus	0.291	0.201	0.283	0.258	73.7	5.0
A. flavus	0.247	0.268	0.289	0.268	72.8	2.1
Trichophyton spp	0.326	0.358	0.327	0.337	66.0	1.8

Std stands for standard deviation of the triplicate data

Effect of Incubation Time on Bioflocculant Production

The effect of incubation time on bioflocculant production is depicted in figure 3 below. The bioflocculation efficiency rise steadily from 24th hour (61.7%) to 72nd hour (82.7%) after which it became almost steady till about 120th The bioflocculation efficiency then declined to lowest of 57.7% at 168th hour. The bioflocculation efficiency was correlated to the accumulated biomass during incubation periods examined until after the 144th hour when the accumulated biomass continued to rise in spite of the declined bioflocculation rate. The conformity of the bioflocculation efficiency to the biomass is an indication that the bioflocculant production of the *Aspergillus niger* grown on plantain peel broth in this study is growth dependent.

The composition of the production broth, the microbial strain along with other factors generally play important part in growth phases of microorganisms and the corresponding biosynthesis of value-added products especially in a controlled experiment. Previous studies (Okaiyeto et al. 2016. Mohammed and Dagang, 2019) have indicated that biosynthesis of bioflocculant by microorganisms may be growth dependent, growth independent or growth synonymous. The non bioflocculant production at the beginning of incubation, likewise the low production prior to 24th hour is attributable to the lag phase at which the microorganism was still acclimatizing to the new medium. The declined production observed at 144th to 168th hour as indicated by the bioflocculation efficiency could be attributed to exhausted fermentable nutrient in the plantain peel broth used as production medium, diminution of the available oxygen to the fungi and accrual of the poisonous wastes within the

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fermentation broth (Mohammed and Dagang, 2019).

The ability of the exoenzymes to cause deflocculation by means of depolymerization of the bioflocculant into digestible monomers after exhaustion of primary nutrient source in the production medium to be used by the fermenting organism for nutrient and energy was used to explained the decline production despite increase in biomass (Aljuboori *et al.,* 2013).

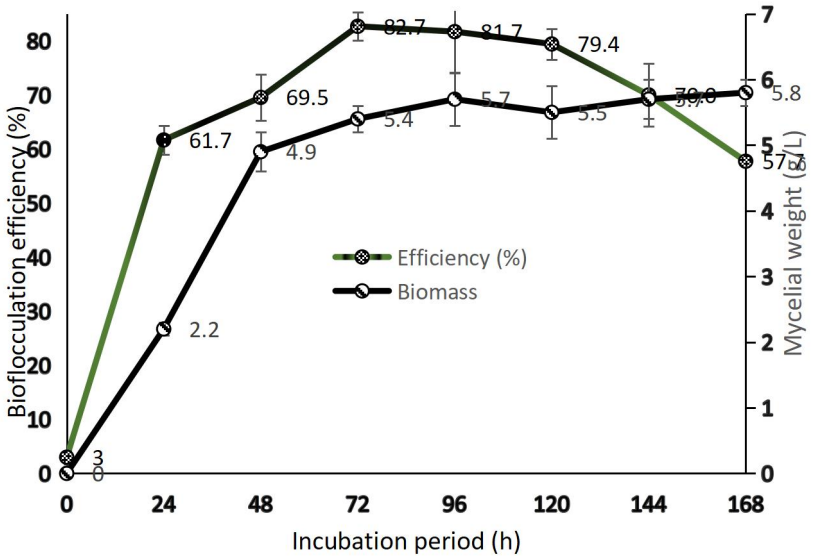

Figure 3: Effect of incubation time on bioflocculant production and mycelial weight. The error bars are standard deviation of the triplicate data

Effects of Nitrogen Supplementation on the Bioflocculant Production

Figure 4 shows the effect of Nitrogen supplement on the bioflocculation efficiency of the *A. niger* bioflocculant after 72 hours of growth in plantain peel media containing $(NH_4)2SO_4$, $NaNO_3$, peptone, yeast extract, urea and glutamic acid. Yeast extract and peptone were effectively utilized by *A. niger* yielding 91% and 95% bioflocculation efficiency respectively. The highest efficiency recorded with the peptone in this study agrees with the findings of Aljuboori *et al.,* 2013 who reported peptone to be most effective nitrogen source for bioflocculant IH-7 produced from *A. flavus*. Supplementing the plantain peel broth with urea and glutamic acid yield about 85% bioflocculation each while $(NH_4)2SO_4$ and $NaNO_3$ yielded about 80%, a bit lower than production without any nitrogen supplementation.

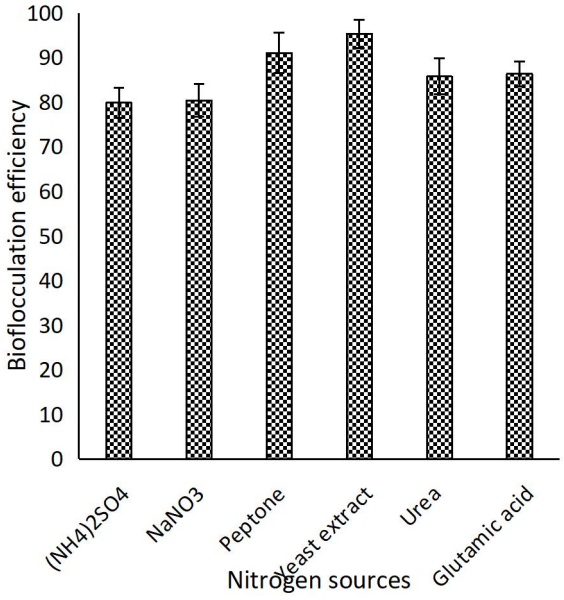

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Figure 4: The effect of nitrogen source supplementation of plantain peel broth on bioflocculant production. The error bars are standard deviation of the triplicate data

Effects of Carbon Supplementation on the Bioflocculant Production

Figure 5 shows the effect of Carbon supplementation on the bioflocculation efficiency of the *A. niger* bioflocculant after 72 hours of growth in plantain peel media containing glucose, maltose, fructose, galactose, sucrose and lactose. sucrose and fructose yielded about 86% bioflocculation each, glucose and maltose yielded about 83% bioflocculation each while galactose and lactose recorded about 77% each.

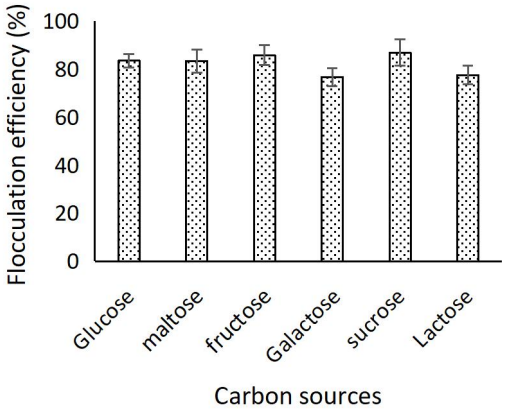

Figure 5: The effect of carbon source supplementation of plantain peel broth on bioflocculant production. The error bars are standard deviation of the triplicate data

CONCLUSION

The findings of the present study show the potential of plantain peel broth as a fermentable medium for bioflocculant production using fungi isolated from a natural waster sediment. Four fungi namely *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus* and *Trichophyton spp* isolated from the water sediment yield flocculation efficiency ranging from 66 to 80.1% . Yeast extract and peptone were the most preferred nitrogen sources (95% and 91% flocculation efficiency) while glucose, maltose, fructose and sucrose all show remarkable bioflocculant production when used as carbon sources (83 to 86.9 flocculation efficiency) for *A nige*r growth on the plantain peel. Overall, this study demonstrates the utilization of plantain peel as a cost-effective fermentation substrate for bioflocculant production

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