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RESEARCH ARTICLE

Determination of biofertilizer potentials of bioslurry for possible application in soil fertility

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ABSTRACT

The potential of bioslurry as a biofertilizer was determined. This was examined by screening bioslurry for the presence of organisms known for their biofertilizer abilities; Congo red yeast extract mannitol agar medium was used for *Rhizobium*, Waksman medium No 77 (N-free mannitol agar medium) for *Azotobacter* and Pikoysksya's broth for Phosphobacteria (*Pseudomonas and Bacillus* species). The physical and chemical properties of the bioslurry were also analyzed. Microbiological enumeration of the bioslurry sample revealed that the Total Heterotrophic Bacteria counts ranged from 1.60×10^{10}CFU/ml to 2.05×10^{10}CFU/ml. Result further showed that bioslurry possess bioferilizing organisms; *Rhizobium*, *Azotobacter*, *Pseudomonas* and *Bacillus*, which aids nutrient uptake, nitrogen fixation and solubilizes phosphate. The physicochemical analysis showed that the bioslurry contains adequate concentration of essential macronutrients (Nitrogen, Phosporous Potassium Calcium and Magnesium).These findings indicate that bioslurry can be used as biofertilizer for the purposes of soil fertility and improved crop production.

INTRODUCTION

Biofertilizer is a substance that contains living microorganisms, which when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Vassey, 2003). Microorganisms that are commonly used as bio-fertilizer components include; nitrogen fixers (N-fixer), potassium and phosphorus solubilizers, growth promoting rhizobacteria (PGPRs), endo and ectomycorrhizal fungi, cyanobacteria and other useful microscopic organisms (Itelima*et al.,* 2018). The use of bio-fertilizers leads to improved nutrients and water uptake, plant growth and plant tolerance to abiotic and biotic factors. They enhance the productivity of soil either by fixing atmospheric nitrogen or by solubilizing soil phosphorus or by stimulating plant growth through synthesis of growth promoting substances (Mohammed *et al*., 2009). Hence biofertilizers can be an important component of integrated nutrient management systems for a sustaining agricultural productivity and healthy environment (Adesemoye and Kloepper, 2009). It has the potential to increase sugarcane characteristics and may represent an alternative to soluble fertilizers (Newton *et al.,* 2016). The nutrient content of bioslurry is often more or less the same as that of the manure used as feedstock. However, anaerobic digestion tends to increase the content of immediately available N, in the form of ammonium-N (NH4-N).

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Möller and Müller (2012) reported NH_4-N concentrations in anaerobically digested materials of 45-80% of the total N. Gurung (1997) reported that NH_4 levels in digested farmyard manure were about 2-2.5 times higher than in undigested farmyard manure, while total contents of nitrogen, phosphorus and potassium remained the same after anaerobic digestion. Often, bioslurry from the digester cannot be directly used as a fertilizer and thus needs to be stored. This storage is mainly necessary because fertilizers should only be applied in specific periods of the growing season, while bioslurry is generally produced continuously. There are several systems to store bioslurry, e.g. cylindrical above-ground tank with coverage (e.g. In the Netherlands; de Geest *et al.*, 2013); below ground tank (for example in China: Surendra *et al.*, 2013); concrete slurry store, with or without permeable (weeping) walls (e.g. In UK); uncovered tanks (e.g. In Italy: Menardo *et al.*, 2011); un-lined earth bank slurry lagoon without cover (common in developing countries, e.g. Vietnam: Thu, *et al*., 2012).The 'fertilizer value' or the 'fertilizer replacement value' of a nutrient in an organic product such as bioslurry is generally defined as the percentage of the total amount of that nutrient that is coming available for plant uptake. This can be determined by relating the amount of a nutrient from a regular mineral fertilizer (Bonten *et al.,* 2014) that is required to attain the same yield or uptake of that nutrient as a certain amount of the organic product (e.g. bioslurry). Currently, most agricultural lands are under intensive monoculture system, andreceive heavy application of chemical fertilizers alone. Chemical fertilizers are composed of known quantities of nitrogen, phosphorus and potassium. The use of low cost biofertilizer gain added importance in rainfed agriculture because most farmers cannot afford expensive chemical fertilizers (Bisen *et al*., 2015). The use of chemical fertilizers causes air and ground water pollution as a result of eutrophication of water bodies (Youssef, 2014; Chun-Li *et al*. 2014), slow productivity and deteriorates environmental quality (Rakshit *et al.,* 2015). It also weakens the plant roots, and makes them prone to unwanted diseases. Mfilinge *et al*. (2014) deposited that low crop productivity is a general problem facing most farming systems in Sub- Saharan Africa. One of the most important constraint limiting crop yield in developing nations worldwide and especially among resource–poor farmers, is soil infertility Khosro and Yousef (2012). The present study was designed to examine bioslurry for itsapplication as a biofertilizer, which would help to supplement, if not eliminate the over-dependency on chemical fertilizers.

MATERIALS AND METHODS

Sample Collection: The bioslurry sample (5mls) was collected from a spent biogas plant installed behind the Microbiology laboratory, Cross River University of Technology. It was collected through the outlet tap of the digester into a sterilized container, cocked and taken to the laboratory for microbiological and physicochemical analysis.

Enumeration of the Total Heterotrophic Bacterial (THB) Count: The THB counts were enumerated by transferring one milliliter (1ml) of the bioslurry sample onto a prepared nutrient agar plate in triplicatesusing surface method. The inoculated plates were incubatedfor 24 hours at 37^{o}C. After the incubation, the THB counts were enumerated following standard bacteriological procedures (Cheesbrough, 2002). The isolates were stained, characterized and identified (Cheesbrough, 2002).

Screening the Bioslurry for Bacteria used as Biofertilizers: The screening of bioslurry for biofertilizers was effectively carried out using selective media. Congo red yeast extract mannitol agar medium was used to assess the presence of *Rhizobium*. The medium was prepared and 1ml aliquot of the slurry inoculated using spread plate method and incubated at atmospheric temperature for 4 to 5 days. Waksman medium No 77 (N-free mannitol agar medium) was used to examine the presence of *Azotobacter.* Spread plate method was used and incubated at atmospheric temperature for 6 to 7 days. Phosphobacteria (*Pseudomonas and Bacillus* species) were isolated using Pikoysksya's broth after 2 to 3 days incubation at atmospheric temperature.

Determination of Physicochemical Parameters of Bioslurry: The physicochemical parameters were analyzed using standard procedures. Total nitrogen was determined by the micro-kjeldahl digestion method (Jackson, 1962). Available phosphorus was determined by Bray and Kurtz (1945) No. 1 method. The potassium and sodium in the extract were determined by flame photometry while calcium and magnesium were determined by Versenate EDTA titration (Jackson, 1962).

RESULTS

The mean value of triplicate enumeration of the Total Heterotrophic Bacteria associated with the bioslurry revealed high counts ranging from 1.60x10^{10}CFU/ml to 2.05x10^{10} CFU/ml (Table 1). The screening of the bioslurry for biofertilizers using various selective medium revealed the presence of *Rhizobium*, *Azotobacter, Pseudomonas and Bacillus species* (Table 2). The *Rhizobium* colony showed a white translucent, glistering, elevated morphology on a Congo red yeast extract mannitol agar medium, which is a selective media for Rhizobium sp. Selective media, N-free mannitol agar (Waksman Medium No. 77) was used to identify *Azotobacter*spp. The result of the physicochemical p analysis of the bioslurry is displayed on Table 3. It showed the presence of major soil elements (N, P, K) in appreciable quantity, which are essential for enhanced plant growth and yield.

DISCUSSION

The high total heterotrophic bacterial counts observed from the slurry is an indication of high microbial activity during fermentation of waste in a digester. Akubuenyi and Achor (2018) in a related study observed a high total heterotrophic bacterial count from waste in a biogas plant, which decreased over a 28 day period of anaerobic digestion, and was attributed to chances in physicochemical and biochemical characteristics of the digester. Anaerobic digestion of waste in biogas plant has been reported to be mediated by aerobic, facultative anaerobic and anaerobic bacteria (Akubuenyi and Achor, 2018). The result of the biofertilizer screening corroborates the findings of Rodriquez *et al.,* (1996) who identified *Pseudomonas, Azospirillum, Bacillus, Rhizobium, Burkholderia, Arthrobacter, Alcaligenes, Serratia, Enterobacter, Acinetobacter, Flavobacterium and Erwinia*as bacterial species with the capacity to solubilize phosphate. It also agrees with the position of various experimental results that inoculation of *Rhizobium* in different cereal grains increased yield to some extent (Bhattacharjee*et al.,* 2008; Hussain*et al.,* 2009).

Rhizobium spp, *Azospirillum* spp and blue-green algae work by fixing atmospheric nitrogen and converting them to organic forms in the soil and root nodules of legumes, thereby making them available to plants in a usable form. Nitrogen fixing biofertilizers are crop specific biofertlilizers (Choudhury and Kennedy, 2004). Mia and Shamsuddin (2010) reported both more crop enhancing and biofertilizer attributes in cereal crops due to rhizobial inoculation. Inoculation with bacterial biofertilizer may reduce the application of fertilizer-N by increasing N uptake by plants (Choudhury and Kennedy, 2004; Mia *et al*., 2007). The bacterial community composition in the rhizosphere is important for the performance of the plant, as bacterial species can have beneficial neutral or harmful relationship with the roots.Result agrees with the position of Schutz *et al*. (2018) that improving crop yield can be achieved through biofertilization, thereby paving way for organic agriculture (Ritika and Uptal 2014). *Rhizobacteria* and *Pseudomonas* spp produce hormones and anti-metabolites which promotes root growth, decomposition of organic matter which help in mineralization of the soil thereby increasing availability of nutrients and improving crop yield (Khosro and Yousef, 2012; Bhattacharyya and Jha, 2012). *Rhizobacteria* inhabit root surfaces and have symbiotic interaction with legume roots (Khosro and Yousef, 2012). They are crop specific plant growth promoting bio-fertilizers. Besides acting as growth promoting agents, biofertilizers provide resistance against pathogens by producing metabolites (Backman and Sikora, 2008). This result is also in line with the findings of Amutha*et al.* (2014), which isolated and mass

Table 1. Total heterotrophic bacterial count of the bioslurry (CFU/ml)

Bioslurry Sample	THB (CFU/ml)
A	$2.05x10^{10}$
B	$1.76x10^{10}$
C	$1.60x10^{10}$

Table 2. Cultural characterization and identification of biofertilizerson selective media

Media	Colony Characteristics	Bacterial organism
Congo red yeast extract mannitol agar	Creamy white, translucent, glistering, elevated circular configuration, regular argin, rod and comparatively small colonies	*Rhizobium* spp
N-free mannitol agar (Waksman medium No. 77)	Milky white, circular configuration, raised, regular margin, rod	*Azotobacter*spp
Pikovskaya's broth	Rod shaped, purple coloured, Gram positive	*Bacillus*spp
	Rod shaped, pink coloured, Gram negative	*Pseudomonas*spp

Table 3. Physicochemical parameters of bioslurry

Parameters	Quantity
pH	6.8
Temperature	31^{o}C
Calcium (Ca)	0.06mg/l
Magnesium (Mg)	0.02mg/l
Nitrogen (N)	0.51mg/l
Phosphorous (P)	1.56mg/l
Potassium (K)	0.79mg/l

produced biofertilizers (*Azotobacter* and *Phosphobacter*). These organisms are known for nitrogen fixing, phosphate solubilizimg, and are known to improve nutrient uptake and soil fertility (Afzal and Asghari, 2008). The beneficial effect of *Azotobacter*inoculation in fruit and vegetable crops was well discussed by Asokan *et al.* (2000).

Bacillus spp, *Pseudomonas* spp and *Aspergillus*spp are phosphate solubilizing biofertilizers. They work by solubilizing the insoluble forms of phosphate in the soil, so that plants can use them. Phosphorus in the soil occurs mostly as insoluble phosphate which cannot be absorbed by plants (Gupta, 2004). However, these organisms possess the ability to convert these insoluble phosphates to their soluble forms. They accomplish this by secreting organic acids which lower the pH of the soil and cause the dissolution of bound forms of phosphate, thereby making them available to plants (Gupta, 2004). *Bacillus* spp possess the ability to solubilize silicates by producing organic acids which cause the decomposition of silicates and helps in the removal of metal ions thereby making them available to plants. Biofertilizers also enhances plant tolerance to environmental stress by acting as bi-protectants (Yang *et al*., 2009). *Pseudomonas aeruginosa* has been shown to withstand biotic and abiotic stress. *Pseudomonas putida* RS-198 enhanced germination rate and several growth parameters including plant height, fresh weight and dry weight of cotton under conditions of alkaline and high salt via increasing the rate of uptake of k^+, Mg^{2+} and Ca^{2+} and by decreasing the absorption of Na^+. Application of *Pseudomonas* spp to basal plants under water stress improves their anti-oxidant and photosynthetic pigment content. *Pseudomonas* spp was found to have positive effect on the seedling growth and seed germination under water stress (Liddycoat *et al.,* 2009).

This result on physicochemical parameters indicates that bioslurry contains adequate physical and chemical elements that can support soil fertility and crop production. The pH of 6.8 and temperature of 31^{o}C are adequate for soil fertility. Nitrogen, Phosphorous, Potassium, Calcium and Magnesium are all essential macronutrients necessary for plant growth.

Nitrogen, phosphorus, and potassium are primary macronutrients; they are needed by plants in large quantities while Calcium and magnesium are secondary macronutrients, needed in small amounts for crop production. Nitrogen functions in plants include there major role; the formation of amino acids- the building blocks of protein, are essential for plant cell division which is vital for plant growth and are directly involved in photosynthesis. It is a necessary component of vitamins, aids in production and use of carbohydrates and affects energy reactions in the plant. Nitrogen often comes from fertilizer application and from the air. In a related study, Islam (2009) reported that Nitrogen contents in composted bio-slurry of both cow dung and poultry manure in Bangladesh were higher than in non-composted manure, whereas concentrations of most other nutrients were not higher. It might be that this additional Nitrogen originates from the biomass which has been added to the bio-slurry for composting. Bonten *et al.* (2014) reported that nutrients in bioslurry, especially nitrogen, are more readily available then in manure, leading to a larger short term fertilization effect. Phosphorus is involved in photosynthesis, respiration, energy storage and transfer, cell division, and enlargement. It promotes early root formation and growth, improves quality of fruits, vegetables, and grains. It is vital to seed formation and helps plants survive harsh winter conditions. It increases water-use efficiency and hastens maturity. Phosphorus often comes from fertilizer, bone meal, and superphosphate. Analysis has shown that it forms a substantial component of seeds and fruits. Excess phosphorus is not good as plants cannot properly assimilate it because most phosphorus is in inorganic form, leading to early maturation and low crop yield in plants (Scalenghe *et al.,* 2012). Potassium is absorbed by plants in larger amounts than any other mineral element except nitrogen and, in some cases, calcium. It helps in the building of protein, photosynthesis, fruit quality and reduction of diseases. It activates enzymes and controls their reaction rates. Potassium is supplied to plants by soil minerals, organic materials, and fertilizer. Calcium is an essential part of plant cell wall structure, provides for normal transport and retention of other elements as well as strength in the plant.

It is also thought to counteract the effect of alkali salts and organic acids within a plant. Sources of calcium are dolomitic lime, gypsum, and superphosphate. Magnesium is part of the chlorophyll in all green plants and essential for photosynthesis. It also helps activate many plant enzymes needed for growth. Soil minerals, organic material, fertilizers and dolomitic limestone are sources of magnesium for plants. The presence of these macronutrients at adequate amounts makes bio-slurry a good soil conditioner and a fertilizer for crop production.

Conclusion

Bioslurry can be applied as biofertilizer because they contain the microorganisms, which when applied to seed, plant surfaces, or soil, are able to fix nitrogen, solubilize potassium and phosphorus and promote growth of rhizobacteria, endo and ectomycorrhizal fungi, cyanobacteria and other useful microscopic organisms.This study also showed that bioslurry contains adequate physical and chemical elements that can support soil fertility and crop production.

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