Nigerian Journal of Fisheries Vol. 19, No. 1; 2022

UTILIZATION OF NATURAL CARBON SOURCES IN BIOFLOCCULATION USING PLASTIC-BIOREACTORS: A TRIAL EXPERIMENT

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ABSTRACT

A three-week trial on the efficacy of local carbon sources in the production of biofloc, and their resulting proximate and mineral compositions were evaluated. Five experimental treatments (labelled A, B, C, D, and E) in triplicates were prepared and maintained with equal volume of inoculum (500ml inoculum and 2.5L tap water) in 5L plastic bioreactors with constant aeration. Groups A, B, C, D, and E received different local carbon sources: white yam, cocoyam, water yam, Elephant ear yam, and glucose respectively. During the biofloc development period, C: N ratio was maintained at 10. The flocs were collected weekly using an improvised Imhoff cone for 10minutes. High floc volume was observed in water yam and cocoyam groups. Floc yield was significantly different between the setups on week 1 (p< 0.0001), week 2 (p<0.0001), and week 3 (p=0.018). The local carbon sources produced floccules that yielded quality nutrients and minerals. The fats, fiber, and carbohydrate compositions were similar between the different species of yam. The total heterotrophic counts (x105cfu/ml) controlled the nitrogenous wastes with total suspended solids level of 111.33 to 311.50mg/L and contributed to better water quality while water yam and cocoyam produced the best biofloc yields.

Keywords: Biofloc, proximate, microbial communities, minerals.

INTRODUCTION

The treatment of wastewater is very significant in aquaculture practice and management (Avnimelech, 2009). Biofloc system was developed to enhance adequate water management (Avnimelech, 2007; Hargreaves, 2013; Hidayah *et al*., 2016; Panigrahi *et al*. 2017; Zhou and Hanson, 2017), treatment, and environmental control (Bossier and Ekasari, 2017) for aquatic animal production (Burford *et al*., 2004). Bioflocs are microbial proteins formed from the utilization of inorganic nitrogenous forms and carbon substrates present in wastewater by heterotrophic microorganisms. Maintenance of a higher C: N ratio stimulates the heterotrophic microbial population (Lima *et al*., 2018; Hamidaghli *et al*., 2019) to mop up ammonia and other nitrogenous wastes, and exploit them by the culture system as growth substrates (Avnimelech, 2009). The more the C: N ratio is maintained through the addition of carbohydrate sources, the better the water quality, and high quality single-cell microbial proteins known as bioflocs are produced (Bossier and Ekasari, 2017). In such conditions, dense microorganisms develop and function both as bioreactors that control water quality and protein food sources (Crab *et al*., 2012). Toxic nitrogen forms are usually immobilized which occur more rapidly in bioflocs, as a result of faster growth rate and microbial production per unit substrate of the heterotrophs compared to the autotrophic nitrifying bacteria. Carbohydrates serve as substrates for supporting biofloc technologies (BFTs) and the consequent production of microbial proteins (Mota *et al*., 2019). Therefore, comparing biofloc technology to standard water treatment technologies is necessary and appears to be more beneficial in aquaculture, with more economical benefits (Lee *et al*., 2017).

Fish diets are mostly enriched with a proportion of protein which results in the high cost of feed. According to Matos *et al*. (2006), proteins consist of nitrogen moiety (16%) in their biochemical structure and as much as, 65% of the protein contained in the fish feed may be lost to the environment. The decomposition of fish feed in water enhances the build-up of total ammonia nitrogen concentration (TAN). Ammonia is toxic to animals (Ballester *et al*., 2018) and effluents from fish farms cause nutrient enrichment of surface waters due to excess nitrogen. To control this, many practices have been adopted some of which are: continuous water replacement systems and re-circulatory aquaculture systems. However, these techniques are capital intensive and non-economical to maintain. To reduce cost, there is ~~a~~ need for an alternative method. The new alternative is, bioflocs technology (BFT) aquaculture practice (Avnimelech, 2007), where ammonia is converted into a beneficial food source that includes minerals and vitamins from microorganisms. Biofloc technology help reduce the feed cost by enriching the pond with floc which fish feast on, and also manages the level of nitrogen (ammonia) in the pond, therefore, reducing the rate of water exchange. Additions of nitrogen compounds such as, fertilizers (urea, nitrate fertilizers, ammonia fertilizers) are beneficial for algae production within the BFTs system (Hai-Hong, 2019).

The carbon sources applied in BFT are usually human and animal-derived by-products that are cheap and locally available (Khatoon *et al*., 2016). Some cheap sources of inorganic carbohydrates such as; acetate (Crab *et al*., 2010), dextrin (Hamidaghli *et al*., 2019), molasses (Sakkaravarthi and Sanka, 2015; Silva *et al*., 2017; Aly *et al*., 2017; Bakhshi *et al*., 2018), glycerol (Crab *et al*., 2010; Dauda *et al*., 2017), and sucrose (Dauda *et al*., 2017) have been utilized to produce viable floc. Likewise, the use of organic or plant-based substrates based on cost and biodegradability have been reported; wheat corn (Liu *et al*., 2014; Caipang *et al*., 2015; Bakhshi *et al*., 2018), rice grain (Zaki *et al*., 2020), sorghum meal (Lopez-Elias *et al*., 2015), wheat bran (Emerenciano *et al*., 2012; Rajkumar*et al*., 2015; Aly *et al*., 2017; Peiro-Alcantar *et al*., 2019), tapioca (Ekasari *et al*., 2019; Rajkumar *et al*., 2015), sweet potatoes flour (Caipang *et al*., 2015), yucca flour (Castro *et al*., 2018), oat bran and amaranth seed (Peiro-Alcantar *et al*., 2019), and cassava meal (Fugimura *et al*., 2015; Silva *et al*., 2017). More so, brewery residues (Fugimura *et al*., 2015), jaggery, and cane sugar (Sakkaravarthi and Sanka, 2015), food wastes such as, bread crumbs and cornmeal (Wakanapol *et al*., 2017) have been effectively utilized as carbohydrates sources to produce bioflocs.

This study evaluated the efficacy of different carbon sources in biofloc formation, and their proximate and mineral compositions in a bioreactor.

MATERIALS AND METHODS

Procurement and Processing of the Local Carbon Sources

The natural carbon sources, *Dioscorea rotundata* (white yam), *Dioscorea alata* (water yam), *Colocasia esculentum* (cocoyam), and *Xanthosoma sagittifolium* (elephant ear yam) were obtained from Ogige Market, Enugu, Nigeria. The glucose was purchased from Jochem chemical store in Nsukka, Enugu State. The white yam, cocoyam, water yam, and elephant ear yam were sliced without the skin, washed thoroughly, weighed, and dried in an oven at 20°C. After which the dried flakes were ground into a fine powder, well-labeled, and stored separately in sterile, air-tight containers.

Experimental Design

The experiment was carried out for three (3) weeks in the Department of Zoology and Environmental Biology Laboratory, University of Nigeria, Enugu State, Nigeria. The setup was prepared using five transparent plastic containers in triplicates (17.5 x19 cm) with aerators (TECAL, AP-1500, AIR PUMP 30.0 WATTS, AIR OUTPUT 1500CC/MIN) to enhance daily bio-flocculation.

The plastics were labeled A-E in triplicates and perforated on the lids, through which air tubes were passed. The experimental groups were as follows;

Plastic Bioreactor A: Biofloc developed by White yam (*Dioscorea rotundata*)

Plastic Bioreactor B: Biofloc developed by Cocoyam (*Colocasia esculenta*)

Plastic Bioreactor C: Biofloc developed by Water yam (*Dioscorea alata*)

Plastic Bioreactor D: Biofloc developed by Elephant ear yam (*Xanthosoma sagittifolium*)

Plastic Bioreactor E: Biofloc developed by glucose

Two and a half litres (2.5L) of tap water (with NO^{3-}, 0.91; Na^{+}, 4.7; Cl^{-},7.4; SO_4^{2-}, 15.2; Ca^{2+}, 4.01; Mg^{2+}, 9.72 and total phosphorus, 0.03) was poured into each bioreactor followed by addition of 500ml of natural inoculum~~s~~ enriched with a mixed culture of microorganisms (TAN;, 0.67; pH, 5.6; total dissolved solids, 207mg/L; dissolved oxygen, 5.26mg/L; total phosphorus; 0.3mg/L; NO^{3-},0.87; total suspended solids, 102mg/L), 100g of feed (35% crude protein) and 2.5L autoclaved (121°C) tap water. The C: N ratios of the carbohydrate sources were adjusted following the protocols of Avnimelech (1999) to C: N 10, which consisted of 27.35g, 19.60g, 21.72g, 26.12g, and 44.74g of white yam, cocoyam, water yam, elephant ear yam, and glucose respectively for optimum production of biofloc; as against initial 50g substrates added as a trial before a repeat of the experiment.

Air tubes already connected to an air pump were passed through the perforations created on the lids of the plastics, to help form and maintain bioflocs in the culture system. The plastics were sealed up using cotton wools and masking tape to prevent media contamination by insects like *Drosophila* sp. Subsequently, at every 4-day interval, evaporated water from the bioreactors was simultaneously replaced with tap water to make up for the loss. The flocs volumes were checked weekly, using an improvised Imhoff cone.

The carbon sources were added once every three days based on the concentrations of TAN in the water (>1g) to obtain an optimum C: N ratio for bacteria.

Harvesting of the Flocs and Measurement of Floc Volume

Due to the size of the improvised Imhoff cone, 50ml of heterogeneous aggregates were obtained in triplicates every 7days. A 50ml aliquot of harvested heterogeneous aggregates of suspended particles was put in an improvised Imhoff cone and flocculated for 10minutes. The presence of floccules was premised on the observance of the presence of suspended particles, which was removed by opening the central point of the improvised Imhoff cone and the particles (flocs) harvested in small containers. The gas formation, which could lead to re-suspension of the flocculated particles was avoided as a precaution. The harvested flocs were measured in a 100ml measuring cylinder, to obtain the floc volume recorded in ml. The floc obtained was subjected to proximate analysis, mineral composition, and microbial identification. They were also viewed under a binocular optical microscope (Olympus) (Figure 1). Water samples were regularly taken to monitor the activity of the biofloc formed. Once the biofloc system turned brown, aeration was increased to maintain a high respiration rate. The dissolved oxygen level, total ammonia nitrogen concentration, and total suspended solids were determined according to Nurhatijah, (2016).

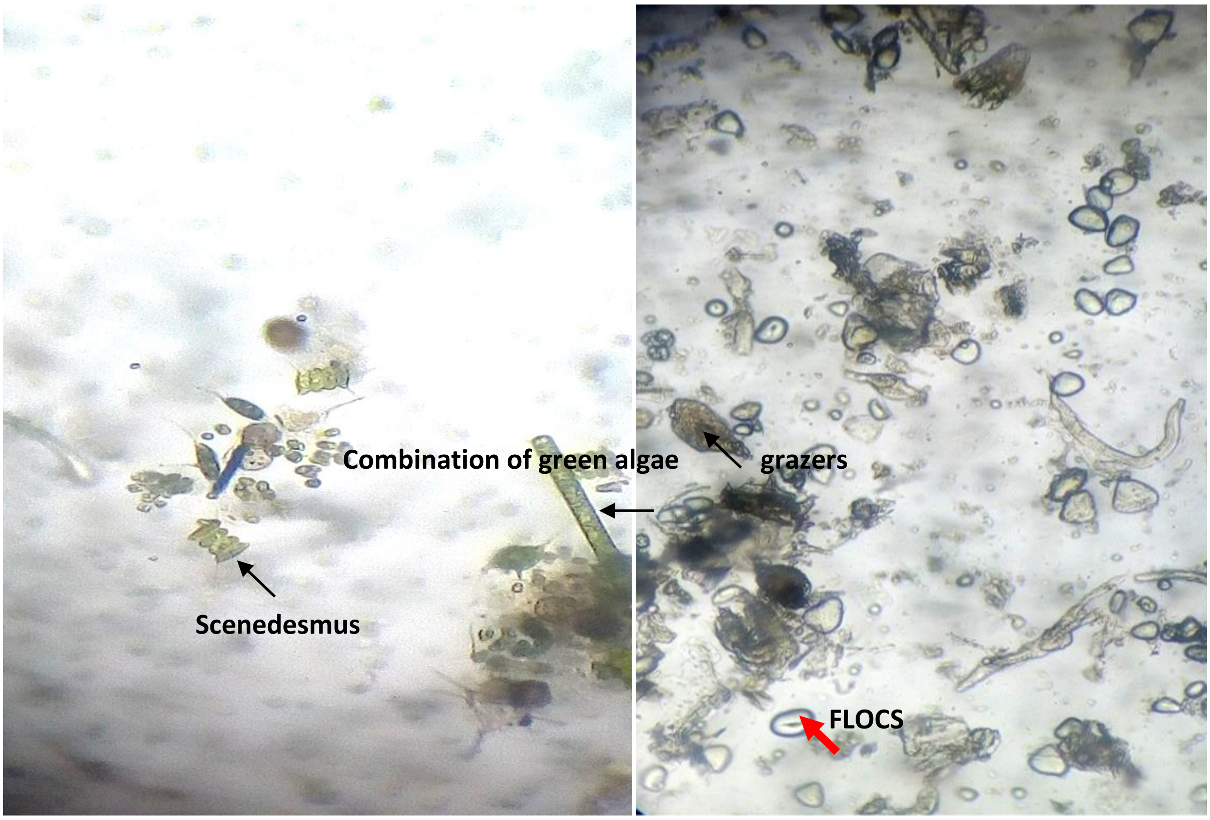


Figure 1: Microscopic views of harvested Biofloc in a biofloc medium showing produced floc (red arrow) and relevant mixed cultures of microorganisms (black arrows).

Proximate Composition of the Harvested Floc

Proximate analysis was evaluated following the protocols of AOAC (1989).

Mineral Composition of the Floc

Mineral compositions (calcium, magnesium, sodium, and potassium) of the harvested flocs were evaluated following the protocols of AOAC (1989).

Determination of Vitamins A, D, and E

Vitamins A, D, and E were analyzed following the protocols of Ranganna (1999).

Identification of Heterotrophic Bacteria

Biofloc samples from each bioreactor were taken and approximately 1-2g flocs (net weight equivalent) were suspended in 10ml sterile saline solution (0.85% NaCl) and peptone water (Merck). After the preparation of ten-fold serial dilution (1ml of sample in 9 ml of diluent), 0.1 ml of the dilution was spread on the surface of sterile Petri dishes containing Tryptone Soy Agar (TSA) and De Mann-Rogosa and Sharpe Agar (MRS) in triplicates. Inoculated plates were incubated at 30ºC for 48h aerobically and anaerobically for TSA and MRS cultures, respectively. Bacterial colonies formed on the incubated plates were counted and calculated against the cultured volume and dilution factor to get the colony-forming units per milliliter (CFU/ml) thus;

$$C = \frac{n}{VD}$$

Where:
C = Concentration in cfu/ml
n = Number of colonies formed on culture plate
V = Volume cultured
D = Dilution factor.

Analysis of Data

Data obtained were analyzed using R packages version 3.6.1(R Core Team, 2019). One-way Analysis of Variance (ANOVA) was used to compare the differences in proximate and mineral compositions of flocs from the different carbon sources. The volume of floc was compared using One-way ANOVA, *ggplot2* (Wickham, 2016) coupled with *ggpubr* package (Kassambara, 2019) were used for plots. The level of significance was tested at $p < 0.05$.

RESULTS

Physicochemical Properties of Biofloc and Control

Dissolved oxygen (DO) in the carbon groups showed little variation throughout the study. On days 1, 7 and 14, there was no significant difference between the DO levels of the groups ($p < 0.05$). Total Suspended solids (TSS) varied throughout the experiment. Baseline TSS values differed significantly from TSS values in the water samples of the different carbon sources ($p < 0.05$); TSS decreased by the 21st day in all the BFT groups compared with their baselines. However, TSS in all the groups attained ~~a~~ similar levels by day 7 except in group D (glucose) which was relatively low. Changes in nitrogen in the BFT groups appeared to be dependent on the medium and duration of the setup (Table 1).

Table 1: Physicochemical Parameters in Biofloc Medium after Twenty-One Days

Parameter	Groups	Baseline	DAY 7	DAY 14	Day 21
DO (mgL^{-1})	A	5.45 ± 0.09^{a}	5.37 ± 0.18^{a}	5.57 ± 0.18^{a}	4.93 ± 0.18^{a}
	B	5.45 ± 0.09^{a}	5.73 ± 0.38^{a}	5.33 ± 0.09^{a}	4.97 ± 0.26^{a}
	C	5.55 ± 0.09^{a}	5.77 ± 0.15^{a}	5.80 ± 0.31^{a}	5.07 ± 0.03^{ab}
	D	5.55 ± 0.09^{a}	5.53 ± 0.23^{a}	5.40 ± 0.17^{a}	5.30 ± 0.26^{ab}
	E	5.55 ± 0.09^{a}	5.73 ± 0.49^{a}	5.27 ± 0.20^{a}	5.53 ± 0.32^{ab}
TSS (mgL^{-1})	A	311.50±12.99^{a}	222.00±84.01^{a}	156.00±103.7^{a}	166.67 ±38.44^{a}
	B	311.50±12.99^{a}	194.67±9.45^{ab}	294.67±14.71^{a}	220.67 ±40.70^{a}
	C	270.50 ±6.63^{b}	144.67±43.46^{a}	280.67±40.83^{a}	193.33±23.22^{a}
	D	270.50 ± 6.63^{b}	111.33 ± 21.46^{b}	270.67 ± 46.34^{a}	269.33 ± 38.68^{a}
	E	270.50 ± 6.63^{b}	240.00 ±23.86^{ab}	167.43 ± 68.49^{a}	234.67 ± 12.72^{b}
TAN (mgL^{-1})	A	0.22 ± 0.00^{a}	0.23 ± 0.01^{a}	0.23 ± 0.01^{b}	0.24 ± 0.00^{a}
	B	0.22 ± 0.00^{a}	0.24 ± 0.00^{a}	0.27 ± 0.02^{ab}	0.26 ± 0.02^{a}
	C	0.23 ± 0.00^{a}	0.24 ± 0.02^{a}	0.26 ± 0.01^{ab}	0.24 ± 0.00^{a}
	D	0.23 ± 0.00^{a}	0.26 ± 0.02^{a}	0.26 ± 0.03^{ab}	0.26 ± 0.03^{a}
	E	0.23 ± 0.00^{a}	0.26 ± 0.02^{a}	0.31 ± 0.03^{a}	0.23 ± 0.01^{a}

Values as mean ± S.E. Values with different alphabets within a column were significantly different ($p < 0.05$). A (white yam), B (cocoyam), C (water yam), D (elephant ear yam), E (glucose)~~,~~.

Proximate and Mineral Composition of Floc in Carbon Sources

There were changes in proximate (Table 2) and mineral compositions of the flocs produced by the different carbon sources. The fats, fibre, and carbohydrate compositions were similar between the different carbon sources. On the average, the mean for these carbon sources were 0.04 (95% CI = 0.03 – 0.06), 0.55 (95% CI = 0.53 – 0.58), and 10.80 (95% CI = 9.34 – 14.01). Significant differences were observed in the ash and protein values for the flocs from the carbon sources in three weeks. Ash and protein content were highest in the white yam carbon source and lower in glucose, water yam, and elephant ear yam respectively (Table 3).

For the mineral composition, calcium, magnesium, potassium, sodium, vitamin A, vitamin E and vitamin D were determined (Table 4). Calcium, potassium sodium and vitamin A were statistically different ($p < 0.05$) amongst the carbon sources.

Table 2: Proximate Composition of Produced Bioflocs on Three Weeks

Composition	*Dioscorea rotundata*	*Dioscorea alata*	*Colocasia esculenta*	*Xanthosoma sagittifolium*	Glucose	Yield of Setup*
Moisture	64.56 ± 3.94^{b}	72.42 ± 1.06^{a}	70.73 ± 2.16^{a}	72.63 ± 1.21^{a}	71.22 ± 0.77^{a}	70.31 (61.27 – 72.79)
Ash	0.47 ± 0.05^{a}	0.29 ± 0.03^{c}	0.42 ± 0.05^{ab}	0.34 ± 0.11^{bc}	0.29 ± 0.02^{c}	0.36 (0.29 – 0.44)
Fats	0.04 ± 0.02^{a}	0.03 ± 0.01^{a}	0.04 ± 0.01^{a}	0.06 ± 0.00^{a}	0.06 ± 0.01^{a}	0.04 (0.03 – 0.06)
Protein	20.95 ± 1.79^{a}	18.64 ± 1.52^{ab}	20.26 ± 1.27^{ab}	12.76 ± 1.54^{c}	17.37 ± 1.62^{b}	18.00 (16.56 - 20.00)
Fibre	0.58 ± 0.06^{a}	0.55 ± 0.04^{a}	0.55 ± 0.07^{a}	0.54 ± 0.02^{a}	0.54 ± 0.05^{a}	0.55 (0.53 – 0.58)
Carbohydrate	13.40 ± 5.64^{a}	8.06 ± 0.48^{a}	8.34 ± 0.74^{a}	13.67 ± 1.36^{a}	10.52 ± 1.39^{a}	10.80 (8.34 – 14.01)

Values as mean ± standard deviation. Values with different superscript alphabet across a row were significantly different ($p < 0.05$). Yield of setups as mean (95% confidence interval).

Table 3: Proximate Compositions of Local Carbon Sources

Local Carbon	Protein	Carbohydrates	Fat	Ash	Moisture
Dioscorea rotundata (White yam)	2.05	59.66	4.02	3.11	31.2
Dioscorea alata (Water yam)	1.09	57.14	6.84	2.16	32.8
Colocasia esculentum (Common cocoyam)	1.14	47.35	2.08	1.07	48.36
Xanthosoma sagittifolium (Elephant ear yam)	1.21	49.72	3.01	1.24	44.82

Vitamin A, vitamin E and fat contents of the flocs from all the carbon sources were similar. Mean vitamin A, vitamin E and magnesium were 0.34 (95% CI = 0.30 – 0.36), 0.14 (95% CI = 0.12 – 0.14) and 0.36 (95% CI = 0.32 - 039). All the mineral parameters assessed were highest in white yam. Vitamin A content~~s~~ of white yam was significantly higher than the other carbon sources ($p < 0.05$) (Table 4).

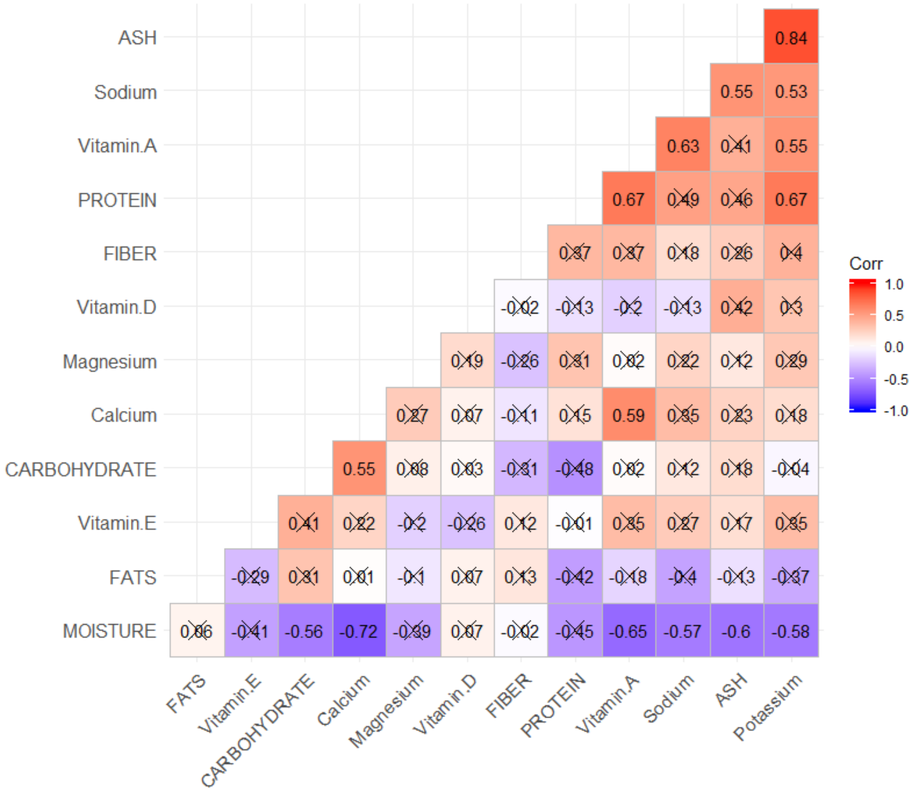
Table 4: Mineral Composition of floc produced by carbon sources on three weeks

Composition	*Dioscorea rotundata*	*Dioscorea* *~~A~~alata*	*Colocasia esculenta*	*Xanthosoma sagittifolium*	Glucose	Yield of Setup*
Calcium	0.52 ± 0.03^{a}	0.45 ± 0.05^{b}	0.42 ± 0.02^{b}	0.45 ± 0.03^{b}	0.47 ± 0.01^{b}	0.46 (0.43 – 0.49)
Magnesium	0.38 ± 0.05^{a}	0.37 ± 0.05^{a}	0.36 ± 0.05^{a}	0.34 ± 0.04^{a}	0.37 ± 0.05^{a}	0.36 (0.32 – 0.39)
Potassium	0.44 ± 0.02^{a}	0.36 ± 0.02^{b}	0.41 ± 0.02^{a}	0.36 ± 0.04^{b}	0.35 ± 0.02^{b}	0.38 (0.35 – 0.42)
Sodium	0.30 ± 0.02^{a}	0.28 ± 0.01^{ab}	0.25 ± 0.02^{bc}	0.23 ± 0.01^{b}	0.23 ± 0.03^{b}	0.26(0.23 – 0.28)
Vitamin A	0.72 ± 0.06^{a}	0.59 ± 0.01^{b}	0.59 ± 0.02^{b}	0.52 ± 0.04^{b}	0.58 ± 0.01^{b}	0.60 (0.57 – 0.61)
Vitamin E	0.38 ± 0.05^{a}	0.32 ± 0.04^{a}	0.34 ± 0.02^{a}	0.35 ± 0.08^{a}	0.30 ± 0.01^{a}	0.34 (0.30 – 0.36)
Vitamin D	0.15 ± 0.02^{a}	0.12 ± 0.01^{a}	0.13 ± 0.01^{a}	0.18 ± 0.11^{a}	0.13 ± 0.01^{a}	0.14(0.12 – 0.14)

Values as mean ± standard deviation. Values with different superscript alphabet across a row were significantly different ($p < 0.05$). Yield of setups as mean (95% confidence interval).

Relationships between Proximate and Mineral Composition of Flocs

There were strong correlations between some of the proximate and mineral compositions of the flocs. Moisture contents of the flocs were significantly and negatively related to the calcium, carbohydrate, ash, sodium, vitamin A and potassium (r > /0.5/, p < 0.05). Potassium was positively and significantly related to ash, sodium, vitamin A and protein contents of the flocs (Figure 1). The positive and negative relationships between potassium and the proximate and other mineral compositions of the flocs are presented in Figure 2.



	FATS	Vitamin.E	CARBOHYDRATE	Calcium	Magnesium	Vitamin.D	FIBER	PROTEIN	Vitamin.A	Sodium	ASH	Potassium
ASH												0.84
Sodium											0.55	0.53
Vitamin.A										0.63	0.41	0.55
PROTEIN									0.67	0.49	0.46	0.67
FIBER								0.37	0.37	0.18	0.26	0.4
Vitamin.D							-0.02	-0.13	-0.2	-0.13	0.42	0.3
Magnesium						0.19	-0.26	0.31	0.02	0.22	0.12	0.29
Calcium					0.27	0.07	-0.11	0.15	0.59	0.35	0.23	0.18
CARBOHYDRATE				0.55	0.08	0.03	-0.31	-0.48	0.02	0.12	0.18	-0.04
Vitamin.E			0.41	0.22	-0.2	-0.26	0.12	-0.01	0.35	0.27	0.17	0.35
FATS		-0.29	0.31	0.01	-0.1	0.07	0.13	-0.42	-0.18	-0.4	-0.13	-0.37
MOISTURE	0.06	-0.41	-0.56	-0.72	-0.39	0.07	-0.02	-0.45	-0.65	-0.57	-0.6	-0.58

Figure 2: Correlation of proximate and mineral composition of floc from carbon sources.

Floc Yield of Carbon Sources

The floc yields of the different carbon sources are presented in Figures 4. Floc yields were significantly different between the setups for week 1 (p<0.0001), week 2 (p<0.0001), and week 3 (p = 0.018). Floc volume in week one was least in the elephant ear yam, followed by the glucose. They were both significantly lower compared to the other carbon sources. Floc volume by week 2 was significantly higher in cocoyam and water yam compared to water yam and elephant ear yam. By week 3, the yield was least in glucose and highest in cocoyam and water yam (Figure 4). The floc yields in week 3 were at similar levels.

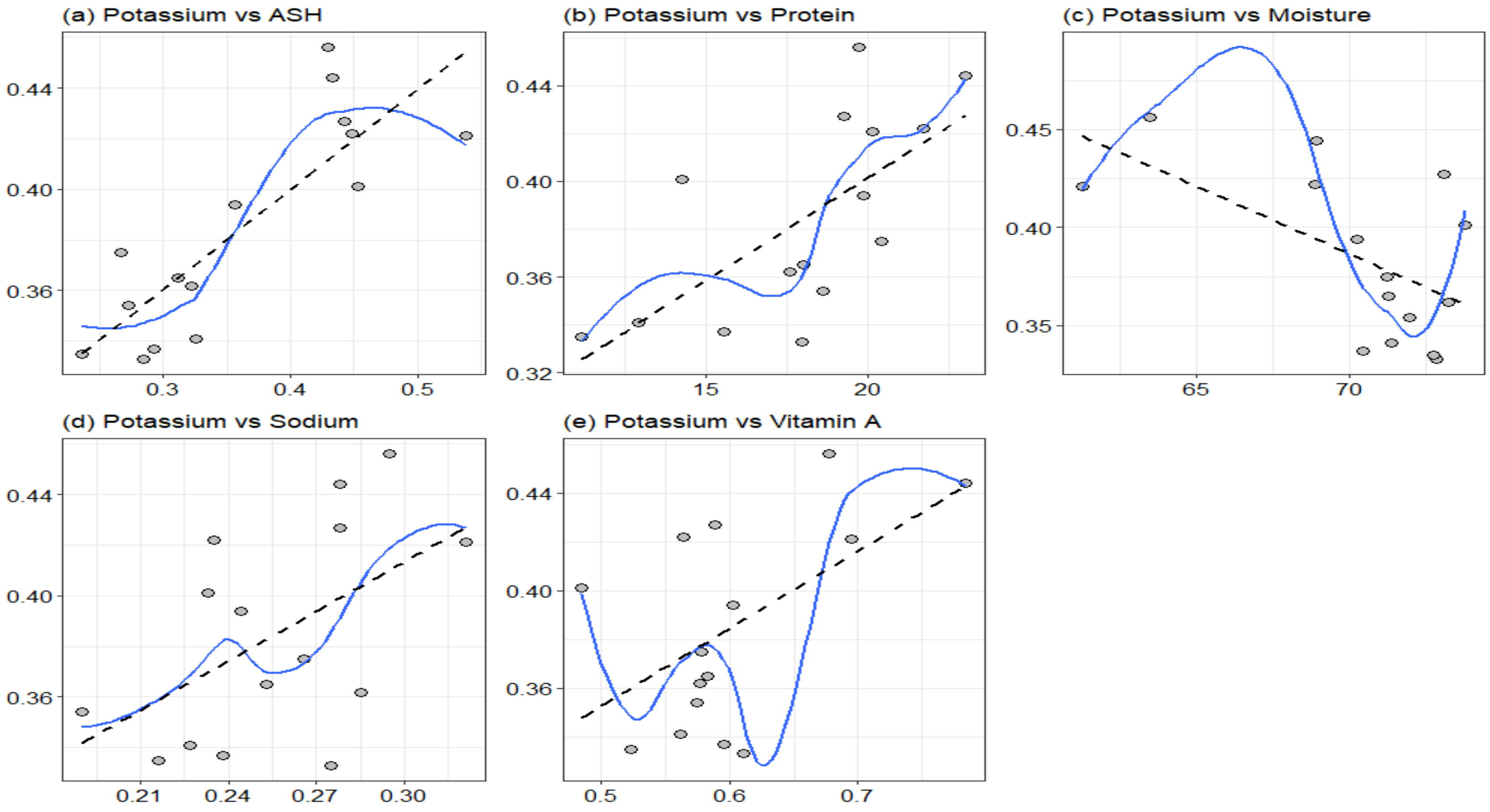


Figure 3: Relationship between potassium and proximate and mineral contents of flocs.

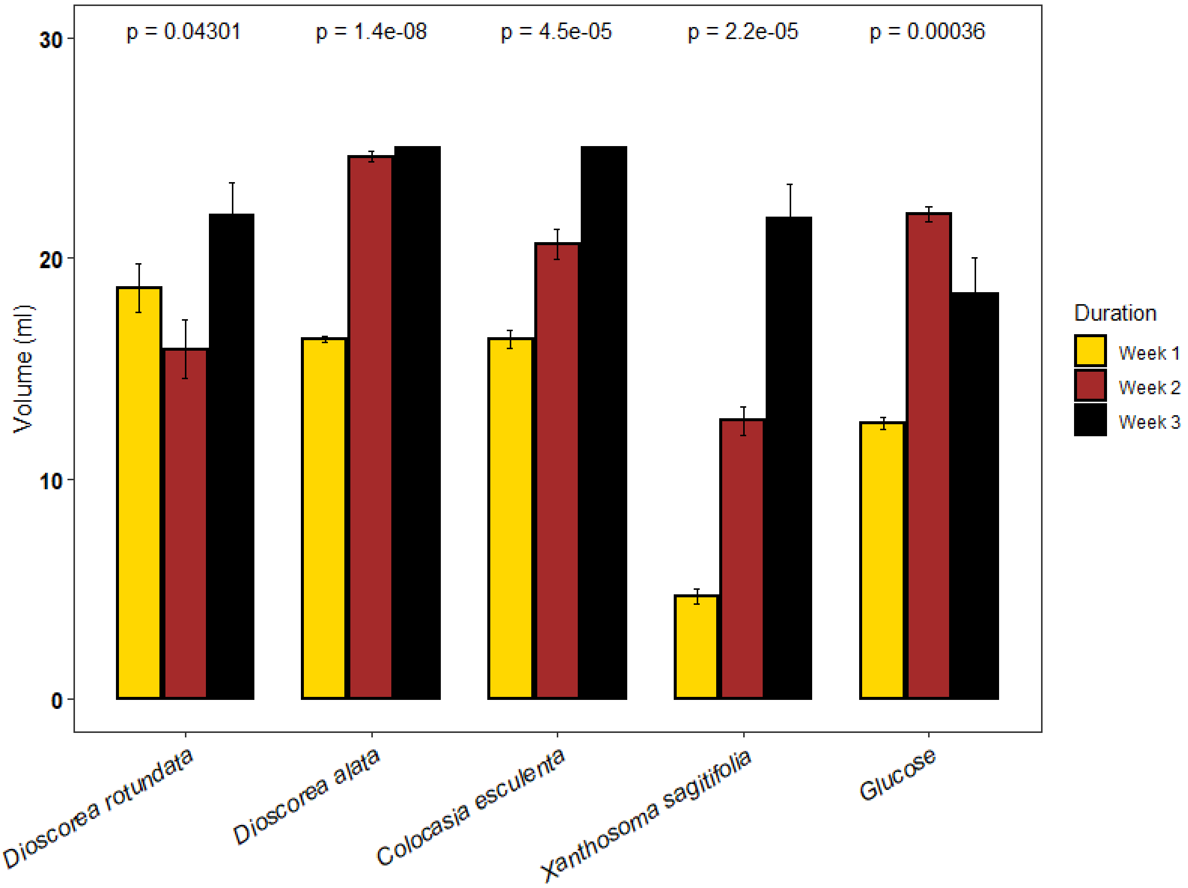


Figure 4: Floc yield per carbon source at different weeks

Yields were generally higher by weeks 2 and 3 compared to week 1. The differences were significant in all the setups (Figure 3). Yield generally increased as the duration of setup increased, except for glucose, though, yields in week 3 were higher compared to week 2.

Bacteria Counts in the different BFT Media

Total heterotrophic bacteria count (THBC) in the baseline was similar in all the BFT groups; hence, no significant difference existed between the groups for THBC ($p < 0.05$). However, significant differences existed in the heterotrophic bacteria count from day 14 to 21 in all the groups. The total bacteria count from the different groups at the end of 21 days is presented in Figure 5. Bacteria counts from the bioflocs produced by white yam, cocoyam and glucose were the highest by day 21.

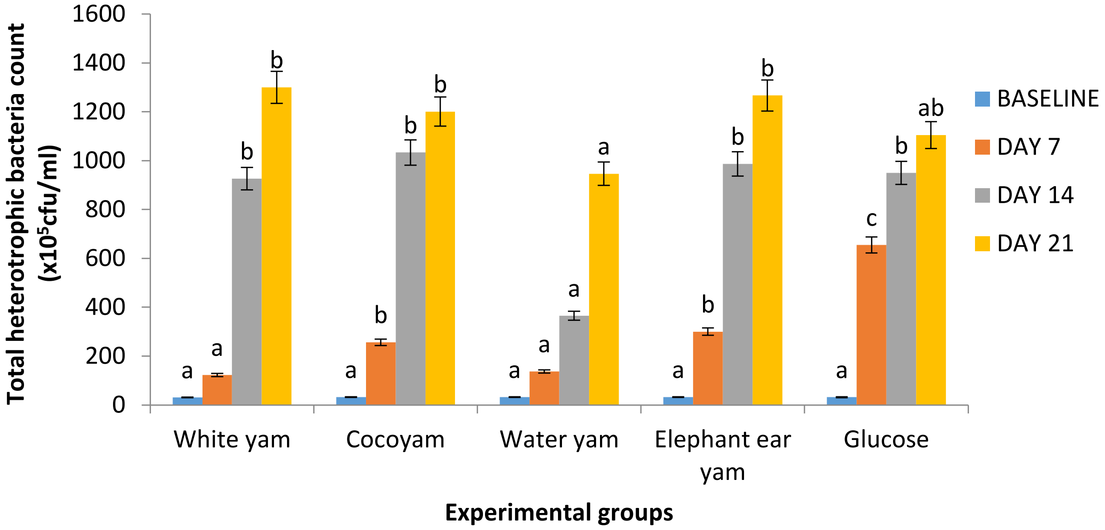


Figure 5: Total bacteria count in harvested biofloc produced by different carbon sources

DISCUSSION

It is highly essential to minimize the waste of water and improve its re-use in aquaculture systems by adopting bio-flocculation technology as a sustainable measure for maintaining water quality, through the production of low-cost single-cell microbial proteins that serve as feed for aquatic organisms (Crab *et al*, 2012). Several reports (Ballester *et al.*, 2018; Bakhshi *et al.*, 2018; Hamidaghli *et al.*, 2019; Zaki *et al.*, 2020) on the use of *in-situ* produced microbial aggregates as a good source of minerals in micro quantities by aquatic organisms have been documented, and it depends on the species used, feeding traits, floc size and floc density (Avnimelech, 2009).

Overall, from this study, the water yam, common cocoyam, white yam, and elephant ear yam stimulated the development of microbial biomass and high level of proximate and mineral compositions which aligned with the increased volume of bioflocs produced by cocoyam and water yam by week 3. Different carbon sources can affect the nutritional properties of the bioflocs produced (Ekasari *et al.*, 2014). Several organic carbon sources have favoured the growth of specific bacteria, algae, and protozoa, (Mota *et al.*, 2019) and influenced the microbial composition of the biofloc (Crab, 2010). From this study white yam and cocoyam augmented the nutritional compositions of the flocs produced and simultaneously controlled the water quality while improving the microbiota population. Liu *et al.* (2014), reported that, floccules produced by corn flour and wheat adequately controlled the water quality in the system used for freshwater Tilapia culture. While, the four local carbon sources white yam, cocoyam, water yam, and elephant ear yam served as good sources of bio-flocculating agents in this small-scale production, they also enhanced the reduction of nitrogenous wastes produced. Higher biofloc composition triggered the increase in TSS, and reduced Total ammonium nitrogen (TAN) and dissolved oxygen. Water yam and cocoyam had more yields as substrates compared to white yam in the production of bioflocs from the results of this study. The significant volume of floccules produced by water yam and cocoyam was attributed to enhanced utilization of the starch substrates and other nitrogenous derivatives by heterotrophic bacteria thus, converting them for biomass yield leading to subsequent single-cell protein production as indicated (Ballester *et al.*, (2018). The composition of flocs can differ considerably depending on the carbon source or substrate used to grow the flocs (Crab, 2012) which was in line with the findings of this study.

Microscopic examination of biofloc samples assisted in providing a clearer understanding of the compositions and applications of bioflocs obtained in this study. The presence of green algae mainly chrysophytes such as, *Spirogyra*, *Scenedesmus*, and *Closterium lanceolatum* intensified the breakdown of hazardous nutrients into non-toxic substances in the study. Similar findings were reported by Avnimelech (2009), Crab *et al.* (2010), and Hidayah *et al.* (2016), of the presence of phytoplankton as primary producers, as well as, zooplanktons in studies that aided natural bioremediation in the aquaculture system.

The total heterotrophic microbial population obtained from the flocs utilized the organic compounds as carbon sources. Bacteria counts from the bioflocs produced by white yam and cocoyam were the highest as well as, that of the glucose by day 21. Dauda *et al.* (2017), reported increased floc volume and bacilli count produced by sucrose and glucose (Ekasari *et al*., 2014; Caipang *et al*., 2015) which controlled water quality and produced beneficial microbial protein. These were the case for the glucose group (used as a baseline) in the findings of this study.

It is essential to note that, organic carbon sources are less expensive and non-toxic with good congruity with any fish culture system. From this study, water yam and cocoyam produced significant biofloc yields, with enhanced nutritional properties. Bioflocs produced in this study by utilizing these local carbon sources showed adequate lipid, protein, ash, and carbohydrate contents such that, they could serve as aquaculture feed. The main attributes associated with the choice of local carbonaceous substrates for bioflocculation included: availability, biodegradability, low costs, and solubility; which the carbon substrates used for this study possessed. High levels of nutrient-rich and beneficial biofloc yields were produced by water yam and cocoyam when compared with white yam and glucose in this study. Some researchers have reported microbial aggregates produced by jaggery, ameliorating adequate essential minerals, rich in sodium, magnesium, calcium, iron, potassium, and zinc (Ballester *et al*., 2018), and microbial flocs producing adequate vitamins A, C, D, E, and K (Crab *et al*., 2012). Essential minerals such as, magnesium, calcium, potassium~~,~~ and sodium were adequately produced by microbial flocs in this study, which were in agreement with the findings of Ballester *et al*. (2018).

Vitamin A, D, and E produced by the biofloc using the different carbon sources in this study agreed with the reports of Crab *et al*.~~,~~ (2012), who asserted that, increased vitamin A, C, E, K, and D levels in bioflocs as beneficial for the growth and survival of aquaculture species.

Biofloc technology (BFT) can be utilized in both freshwater and seawater systems, to control water quality and to produce additional feed sources *in-situ* in aquaculture production. With this, production costs will decline considerably since food represents 40-50% of the total production cost in aquaculture systems.

CONCLUSION

The adoption of biofloc technology as a wastewater treatment technology has gained relevance as a practice in aquaculture. Its development has promoted the utilization of local carbon sources in producing viable floccules. As in this study, high nutrient levels were common in the local carbon sources; white yam, water yam, cocoyam, and elephant ear yam, all of which can be utilized as good bio-flocculating agents to enhance sustainable aquaculture production. There is a need to empower the small-scale fish farmers in developing countries who have little or no knowledge of biofloc technology (BFT) on how to integrate, utilize, and optimize the floc nutritional characteristics in aquaculture. More research and inputs are required for adopting better local carbon sources to improve/enhance BFT and the floc nutritional compositions and aquaculture outputs.

Acknowledgment

The authors are thankful to the Head of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Enugu State, Nigeria for providing the necessary facilities for the completion of this research.

Data Availability

Research Data is not shared

CONFLICT OF INTEREST

The authors declare no competing interests exist

ETHICS STATEMENT

The manuscript does not need ethical approval

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