

Biological Treatments of Fish Farm Effluent and its Reuse in the Culture of Nile Tilapia (*Oreochromis niloticus*)

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Abstract

Aquaculture wastewater collected from a catfish farm in Ibadan metropolis was treated with duckweed, *Lemna minor* (Td) for two weeks and thereafter used in the culture of Nile tilapia (*O. niloticus*). The performance of *O. niloticus* raised in *Lemna minor* treated waste water was compared with bacteria-treated waste water, *Bacillus sp.* (Tb) and well water (Tc) as control (untreated). The *Bacillus sp.* was isolated from the catfish wastewater, and was positive to Gram's staining, catalase and glucose fermentation test. Nile tilapia juveniles (n=54) of an average initial weight of 10.43 ± 0.04 g were stocked in triplicates per treatment and fed to satiation twice daily for 8 weeks. There was significant difference ($P < 0.05$) in the quality of waste water in all the treatments. Compared to initial waste water, Td showed a significant reduction in biological oxygen demand, BOD (1.23 ± 0.03 mg/L vs. 36.80 ± 1.89 mg/L), chemical oxygen demand, COD (2.20 ± 0.06 mg/L vs. 58.81 ± 1.89 mg/L), sulphate (0.50 ± 0.06 mg/L vs. 5.53 ± 0.33 mg/L) and phosphate (5.40 ± 0.31 mg/L vs. 18.43 ± 0.78 mg/L) after 2 weeks of treatment. The level of phosphate, BOD, COD, nitrate, and TSS were lowest in Td compared to Tb and Tc ($P < 0.05$). The lowest level of ammonia was obtained in Tc (0.15 ± 0.10 mg/L), compared to Td (0.15 ± 0.10 mg/L) and Tb (0.66 ± 0.28 mg/L). The highest percentage weight gain (WG) of $34.37 \pm 0.60\%$ and the lowest feed conversion ratio (FCR) of 1.59 ± 0.03 were recorded in fish raised in Td ($P < 0.05$). *Oreochromis niloticus* juveniles raised in Td also had the highest specific growth rate (SGR) of $0.23 \pm 0.01\%$ compared to $0.19 \pm 0.00\%$ recorded in fish raised in both Tb and Tc. Fish raised in Tc had the highest survival rate ($100 \pm 0.00\%$) compared to the fish cultured with Tb ($77.80 \pm 2.30\%$) and Td ($72.20 \pm 1.95\%$). The research findings suggest that *Lemna minor* could be used in fish culture with positive effect on water quality and growth performance.

Keywords: Duckweed; *Bacillus sp.*; Wastewater; Fish culture

Introduction

The aquaculture industry is one of the fastest growing agriculture sector globally. With a total production of 66.6 million metric tonnes in 2012, it provides almost half of all fish production for human consumption [1]. However, the long-term sustainability of aquatic environment has raised concerns over the environmental impact of this vital sector, due to its negative impact on aquatic ecology and systems [2,3]. This is because intensification of aquaculture involves the use of highly nutritious feeds and other chemical products, which generate wastes that, in most cases, are difficult to curtail and toxic to aquatic lives [4-6]. Effluent water containing wastes are discharged in all aquaculture systems [7]. The amount of wastes generated from aquaculture practices depends on the culture system characteristics, choice of species, feed quality and management practices [8].

The discharge of wastewater in the form of effluents into aquatic ecosystems could lead to the alterations of the receiving environments. High organic load in aquaculture wastewater can result in the eutrophication of receiving water bodies, which causes a lot of havoc on the biodiversity in aquatic ecosystems [9,10]. Nitrogenous wastes, which are the major component of aquaculture waste, are highly toxic to macro-fauna in the open water body. Stephen and Farris [11,12] reported that an increase in ammonia concentrations could elevate blood ammonia, which is highly toxic to fish. Suspended solids in aquaculture wastes in receiving water bodies cause interstitial clogging and substrate embeddedness [13]. The deposition of solids and sediments could enhance the growth of heterotrophic bacteria and increase the formation of colony-forming units, leading to additional interstitial clogging and deoxygenation [14].

The use of microorganisms to degrade and reduce harmful

wastes in contaminated sites has been reported in several studies. Bio-remediation offers the possibility of rendering harmless various contaminants in wastewater. Different microorganisms, including bacteria, fungi, algae, and plants have been used to decontaminate polluted environments [15-17]. Under controlled conditions, organic wastes are degraded by microbes to levels that are harmless, or below concentration limits [18,19]. Bio-remediation techniques are cheaper than traditional methods such as incineration; and some pollutants can be treated on site, which reduces exposure risks of cleanup personnel as a result of transportation accidents [20]. It also provides an alternative for effective management of wastewater for the purpose of reuse, thereby reducing pressure on limited freshwater resources.

Microbes exist in diverse environmental conditions, which make them useful in waste management. Prescott et al. [21,22] reported that microorganisms, indigenous (native) or extraneous (introduced), are prime agents in any bio-remediation system. Indigenous bacteria are crucial to bio-remediation processes, due to the important role they play in the biogeochemical cycle of nutrients [23]. The potentials for

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bio-remediation have been reported for different organisms, with microbes showing the highest efficiency in several studies [24]. Dead microbial cells are also useful in bio-remediation technologies [25].

Several studies have shown *Bacillus pumifus* to be a good candidate for bio-remediation [26]. Other *Bacillus sp*, including *B. cereusmycoides*, *B. megaterium*, *B. mucosus*, *B. agglomerates*, *B. cartilaginous* could be used for bio-remediation because they possess antagonism, proteolysis and catalytic activity characteristics [27]. In a study by Quiroz and Boyd [28-30], commercially prepared *Bacillus* species mixed with rearing water of channel catfish (*Ictalurus punctatus*) improved production and survival rate of fish. High capacity for bio-remediation of organic sediments has also been reported for *Bacillus sp* such as *Bacillus subtilis*, *B. licheniformes*, *B. cereus*, *B. coagulans* and *Phenibacillus polymyxa*, have been shown capacity for bio-remediation. However, they are present in large amount in sediments.

Phytoremediation has been found to be well suited for use for sites with low concentration of pollutants and which require expensive technology for bio-remediation [31]. The potential of plants to reduce high load of harmful wastes and tolerate harsh environmental conditions has been reported [32]. Duckweeds are small aquatic plants belonging to the family Lemnaceae [33]. They are reported to have a high potential to absorb and remove nutrients in wastewater, such as nitrate, phosphate, calcium, sodium, potassium, magnesium, carbon, and chloride. These nutrients are permanently removed from the system when the plants are harvested. More so, the use of duckweeds in waste treatment has been shown to reduce harmful substances such as total suspended solids (TSS), biochemical oxygen demand (BOD), and chemical oxygen demand (COD) in wastewater significantly, and have reported to tolerate ammonia level as high as 240 mg/L.

Smith and Moelyowati [34] stated that wastewater treatment systems are feasible for developing countries in hot climates to provide low-cost treatment of domestic sewage particularly in rural areas. The sustainability of aquaculture industry would, therefore, depend on the availability of cheap and affordable technology for waste treatment. The full potential of duckweed as a cheap and low cost method of waste treatment, as opposed to high cost technologies, has not been exploited, particularly in Nigeria, which is one of the largest producers of cultured fish species in Africa [1].

Therefore, the study was carried out to evaluate the effectiveness of duckweed and microorganism (*Bacillus sp*) in bio-remediating wastewater from a catfish farm and the effect of the bio-remediated water on the performance of Nile tilapia (*Oreochromis niloticus*).

Materials and Methods

Wastewater sampling and analysis

The bio-remediated aquaculture wastewater used in this study was obtained from a reputable fish farm (SDC Farm, Ibadan, Oyo State, Nigeria, located on coordinate of N7°35'38.69'', E3°85'42.79'') in active fish production in Ibadan metropolis, Nigeria. The sampled fish farm operated a semi-intensive production system. The wastewater were collected at point of discharge between 6.30-7.00 and transported in 25 litres plastic containers immediately to the Department of Aquaculture and Fisheries Management laboratory, University of Ibadan, Nigeria (N7°26'27.98'' E3°54'8.99''). Two litres of aquaculture wastewater were collected in sampling bottles at the point of discharge and were analyzed for the following physicochemical parameters: Dissolved Oxygen (DO), Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), phosphate, sulphate, Total Ammonia Nitrogen

(TAN), nitrate, Total Suspended Solid (TSS), pH and temperature were monitored weekly before the commencement and after the bio-remediation process according to APHA [35] standard procedure.

Microbial remediation

Isolation of the microorganism: The bacteria strain (*Bacillus sp.*) used for the bio-remediation experiment was isolated from the wastewater collected using sterile sampling bottles. Isolation of *Bacillus sp.* was carried out on nutrient agar (NA, Oxoid CM3). The media was prepared by suspending 28.0g of NA in 1 litres of distilled water. The dissolved agar solution was autoclaved at temperature of 121°C for 15 minutes. The medium plates were inoculated by pour plate method with 1 mL aliquot of the diluents pipetted aseptically into labeled sterile Petri dish after serial dilution of the wastewater samples in 9 mL sterilized distilled water from 10⁻⁹ to 10⁻¹⁰ dilution. The inoculated plates were incubated in an incubator at 37°C for 24 hours. Discrete colonies from plate prepared by pour plate methods were sub-cultured into sterile NA agar plates (incubated for 24hrs) without contaminants aseptically by streaking, using a wire loop sterilized with spirit-lamp flame to obtain pure cultures of the isolates. The isolated pure bacteria colonies were characterized using standard morphological and biochemical tests, such as Gram staining, catalase, oxidase and sugar fermentation tests, after 24 hours of incubation as described in Berge's Manual of Bacteriology.

Inoculation of the wastewater with the prepared bacterial inoculums: The inoculum was prepared in nutrient broth and the concentration of the bacterial cells was adjusted to a 10⁵ colony-forming unit using sterile physiological saline to correspond to 0.5 MacFarland standards. Fifteen (15) milliliter of the bacterial inoculums was introduced into 15 litres of wastewater in experimental tanks (0.39 m × 0.28 m × 0.26 m), using a sterile needle and syringe. Mosquito net was used to screen the wastewater treatments to prevent insect infestation. The bio-remediation experiment lasted for two weeks. The water quality parameters were recorded before and after bio-remediation.

Phytoremediation of the wastewater: Duckweed, *Lemna minor*, used for phytoremediating the wastewater were obtained from University of Ibadan Botanical Garden, Nigeria (N7°26'46.59'', E3°54'11.72''). Fifteen (15) litres of aquaculture wastewater sample collected was bio-remediated in plastic tanks (0.39 m × 0.28 m × 0.26 m) with about 49.53 ± 0.25 g (mean wet weight) of fresh duckweed (*Lemna minor*) plants, enough to cover the entire surface of the water with approximately a single layer of fronds to avoid direct contact with sunlight (Al-Nozaily, 2001). This was done to prevent the formation of green algae in the experimental setup. The experimental tanks were arranged outside the laboratory to have adequate access to sunlight. Harvesting and weighing of the plants followed after two weeks of bio-remediation to determine change in the plants biomass. AOAC [36] provided analytical method the proximate compositions of the plant before and after bio-remediation.

Culture of fish in bio-remediated wastewater: The University of Ibadan Fish Farm, Ibadan Oyo state, Nigeria (N7°26'27.2472'', E3°53'58.1532''), provided ninety (90) juveniles of Nile tilapia (*Oreochromis niloticus*) of an average weight of 10.44 ± 0.90 g used in the study. The fish were acclimatized in well water (control treatment) for two weeks before being used in the study. Triplicate plastic tanks (0.39 m × 0.28 m × 0.26 m), with six fish per tank were randomly allocated to the treatments consisting of 7 litres of bio-remediated wastewater with duckweed (Td), bacteria (Tb) and well water as the control treatment (Tc) and fed to satiation twice (morning, 8:00 and evening, 16:00) per day. Imported floating feed (ME-2, Skretting, France) was used for the

Treatment	Phosphate (mg/L)	Nitrate (mg/L)	Sulphate (mg/L)	BOD (mg/L)	COD (mg/L)	DO (mg/L)	pH (mg/L)	Temp (oC)	TAN (mg/L)	TSS (mg/L)
RAW	18.43 ± 0.78	9.93 ± 0.36	5.53 ± 0.33	36.80 ± 1.89	58.81±1.89	4.00 ± 0.14	6.83 ± 0.67	24.63 ± 0.22	1.17 ± 0.48	2136.75 ± 332.37
Tc	16.57 ± 0.23	6.48 ± 0.02	3.74 ± 0.17	31.90 ± 0.21	58.17±0.20	4.09 ± 0.06	7.46 ± 0.03	24.38 ± 0.03	0.15 ± 0.10	2015.00 ± 2.89
Tb	16.47 ± 0.03	6.77 ± 0.04	4.17 ± 0.09	27.27 ± 0.09	52.10±0.31	4.81 ± 0.02	7.86 ± 0.08	24.26 ± 0.07	0.66 ± 0.28	2034.00 ± 3.06
Td	5.40 ± 0.31	5.94 ± 0.47	0.50 ± 0.06	1.23 ± 0.03	2.20±0.06	4.45 ± 0.06	8.11 ± 0.21	27.96 ± 0.26	0.26 ± 0.11	1347.33 ± 1.45
Sig-values	0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Note: Mean values on the same column with Sig-values > 0.05 are not significantly different ($\alpha_{0.05}$).

Abbreviations: BOD: Biological Oxygen Demand, COD: Chemical Oxygen Demand, DO: Dissolved Oxygen, Temp: Temperature, TAN: Total Ammonia Nitrogen, TSS: Total Soluble Solids.

Table 1: Mean water quality parameters of raw aquaculture wastewater (RAW); bioremediated wastewater with bacteria (*Bacillus* sp.) (Tb) and duckweed (Td); and non-bioremediated wastewater (Tc) after two weeks.

feeding the fish throughout the experiment duration, which lasted for 8 weeks. Water quality was analysed biweekly for a period of eight weeks.

Growth performance and feed utilization of experimental fish:

Growth performance measured every two weeks throughout the experiment enabled quantification of growth and nutrient utilization parameters. Standardized metre rule and sensitive scale provided measurement for length and fish weight, respectively. Mean weight gain (MWG) and specific growth rate (SGR) were determined from the mean initial and final weight of fish at the end of the experiment (8 weeks), while feed conversion ratio (FCR) was determined from mean data of feed consumed and weight gain. Gross feed conversion efficiency ratio (GFCE) was derived from the reciprocal of feed conversion ratio and expressed in percentage. Protein efficiency ratio (PER) was derived from mean values of weight gain and protein intake (PI), while survival rate, which was calculated from the initial number of fish and mortality after the experiment was terminated.

Data analysis: Data collected were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 20 software. Non-parametric statistics were used in analyzing the data generated. Descriptive statistics was used in estimating the mean and standard deviation while non-parametric analysis of variance (ANOVA) of Kruskal-Wallis and the Mann & Whitney's U tests were used to determine the level of significant difference ($p < 0.05$) observed between groups in the ANOVA analysis. Spearman's Rank Order Correlation analysis was used to establish relationship between nutrient utilization, growth performance and water quality parameters.

Results and Discussion

Water quality parameters of experimental water samples

The result of analysis of water physicochemical parameters indicated that there was significant difference in the raw aquaculture wastewater, duckweed bio-remediated (Td), microbial (*Bacillus* sp.) bio-remediated (Tb) and untreated wastewater (Tc) after two weeks (Table 1). The mean values of phosphate, nitrate, sulphate, TAN, BOD, COD and TSS measured in the raw aquaculture wastewater (RAW) were significantly higher than the values observed in the treatment. These differences could be explained by the ability of bio-remediators (duckweed and microbes) in removing nutrients and other related pollutants in aquaculture wastewater. This is an indication that pollutants in aquaculture wastewater are biodegradable. Similar observation was also made by Martinez-Cordova [37] in a related study. Throughout the period of study, the least DO value was recorded in RAW within the range of 3.91-4.21 mg/L. This lower DO level in the wastewater probably shows high aerobic microbial activities (BOD) and chemical oxidation demand which are indicators of pollution. Lower DO ranged of 3.00 mg/L to 3.20 mg/L was reported by Ling [38] in wastewater drained from shrimp ponds.

According to Wang [8] the quality and quantity of waste from aquaculture depend on the culture system characteristics, culture species, feed quality and management practices.

Higher BOD value was recorded in RAW when compared to the untreated wastewater after two weeks and bio-remediated water samples. This is reflection of higher biodegradable organic substances from uneaten feed, fish fecal wastes and metabolites from microbial activities in the discharged wastewater. Similarly observation was also made by Lee [39]. The range of BOD (35.20-39.50 mg/L) recorded in the sampled wastewater was higher than the range of 5.90-18.70 mg/L recorded by Ling [38] in shrimp pond while Babatunde and Woke [40] reported higher BOD of 78.04 mg/L in effluent from fish pond.

Although the mean values of COD in RAW was lower than that of untreated wastewater after two weeks, however there was no significant difference in the COD values. This is an indication that temporal variation does not have significant influence on the amount of dissolved oxygen require to chemically oxidize organic materials in the wastewater as the rate this process occurs naturally is slow. This could be as a result of high organic contamination in aquaculture wastewater. In related studies, Amirkolaie [41,42] and Ogwo and Ogu [43] ascribed high COD in aquaculture wastewater due to the presence of high organic matter.

The pH of the RAW ranged between 6.25-7.75 with an average value indicating slight acidic condition. This is an indication of septic condition of the wastewater resulting from putrefaction of organic matters resulting to production of acidic substances such as humic acids which reduces the pH below 7. Soonnenholzner and Boyd [44] reported that oxidation of sulfide produced from wastewater during microbial decomposition process lead to production of sulfuric acid creating acidic condition which could harm the culture fish species. This result corroborates the observation of Babatunde and Woke [40] in wastewater from fish ponds.

Similar to the observed trend in other parameters, the phosphate, sulphate and nitrogenous pollutants (nitrate and TAN) in the RAW were higher than the mean values in untreated and bio-remediated wastewater. Elevated level of these pollutants in the wastewater may be as a result of leached nutrients from fish feed which are rich in proteinaceous feed components. The phosphate level in the RAW ranged between 17.55 mg/L to 19.45 mg/L which was higher than the phosphate values of 0.11 mg/L and 0.16 mg/L recorded in wastewater from extensive and intensive aquaculture farms by Bowley and Allan [45]. Ganczarzyk [46] and McCasland [47] reported that phosphate levels above 1.00 mg/L could prevent coagulation of wastewater in water treatment system. The mean nitrate level of 9.93 ± 0.36 mg/L recorded in RAW was lower than the value recorded by Babatunde and Woke [40] while TAN of 1.17 ± 0.48 mg/L observed in RAW was higher than 0.03 mg/L recorded Martinez-Cordova [37] in effluent

water from shrimp culture system. Ammonia, nitrate and nitrite are primary forms of nitrogen in inorganic form in wastewater [48]. These inorganic nitrogen forms are indicators of bacterial (such as nitrifiers) contamination [49,50] which could result to anoxic condition while ammonia is oxidized to nitrate [51]. Excess phosphate and nitrogenous pollutant content in wastewater could also result to algal bloom (eutrophication) in the receiving waterbody [52,53].

According to the Federal Ministry of Environment in Nigeria, the permissible pH, BOD, COD, TSS, sulphate, phosphate, nitrate and TAN in effluent wastewater discharge into surface water is 6.00-9.00, 30 mg/L, 80.00 mg/L, 30.00 mg/L, 500.00 mg/L, 5.00 mg/L, 20.00 mg/L and 0.10 mg/L respectively [54-56]. The results of this study showed that the aquaculture wastewater from the sampled fish farm had BOD, phosphate, TSS and TAN above the permissible limits. This could be due to accumulative effect of uneaten fish feed and metabolic wastes from culture fish on the culture water prior discharge from production unit. This result was in line with the observation of Babatunde and Woke [40,41] of wastewater from fish ponds in southwestern Nigeria.

Bio-remediation experiment

Identification of the bacteria candidate use in bio-remediation experiment: The microorganism used for the microbial bio-remediation experiment was tested positive to Gram staining test and appeared rod-like in shape when viewed under microscope. In colony morphology test, the microbe colonies were large with undulating circular margins. Bubbles were produced when the microbes were exposed to hydrogen peroxide indicating the production of oxygen and water due to production of the enzyme catalase. The bacterial was able to ferment fructose completely and maltose partially and other sugars (mannitol, lactose, sucrose, galactose and glucose) were not fermented. The probable micro-organism used in this study was *Bacillus sp* which is in agreement with the observation of Turnbull [57].

Percentage efficiency in reduction and improvement of water quality parameters: Presented in Figures 1 and 2 are percentage reduction of phosphate, nitrate, sulphate, BOD, COD and TSS; and percentage improvement of DO and pH in bio-remediated aquaculture wastewater after two week. The highest percentage reduction of phosphate, sulphate, BOD, COD, nitrate and TSS of 70.70%, 90.96%, 96.66%, 96.26%, 40.18% and 36.94% respectively and highest percentage of improvement of pH (18.74%) were recorded in wastewater bio-remediated with duckweed. Meanwhile, the *Bacillus sp.* bio-remediated wastewater tends to have the highest ammonia reduction percentage of 87.18% and DO improvement of 20.25%. Untreated wastewater had the lowest phosphate, BOD, COD and TSS reduction efficiency.

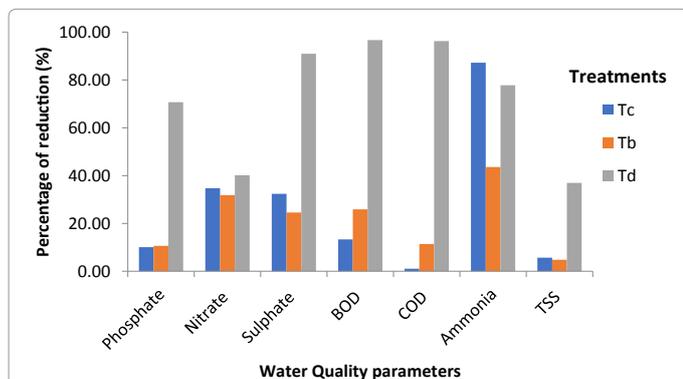


Figure 1: Percentage of reduction of pollutants in non-bioremediated wastewater and bioremediated wastewater with *Bacillus sp.* (Tb) and duckweed (Td) after two weeks of bioremediation.

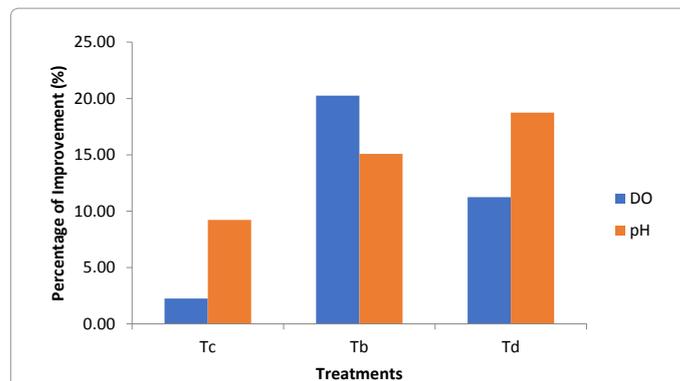


Figure 2: Percentage of improvement of DO and pH in non-bioremediated wastewater and bioremediated wastewater with *Bacillus sp.* (Tb) and duckweed (Td) after two weeks of bioremediation.

Indicative in the results of this study was the change in the water quality parameters of untreated water (Control treatments) after two weeks. Although the Phosphate, nitrate, sulphate, BOD, pH, TAN and TSS in untreated wastewater after two weeks was significantly lower ($P < 0.05$) than the RAW collected from the sampled fish farm. This reduction could be as a result of the biochemical activities of microorganisms and chemical processes associated with the wastewater that have the potential of utilizing pollutants in the wastewater over time. Martinez-Cordova [37] suggested that sedimentation could have resulted to the same result recorded in their study.

The results of the bio-remediation experiment indicated that duckweed had the highest nutrient removal efficiency of phosphate, sulphate, nitrate and ammonia. The high affinity for nutrient uptake in aquaculture wastewater by duckweed was an indication that nutrients uptake improved biomass production of duckweed. Therefore, duckweed has been identified to be an important bio-remediation tool in reducing the nutrient content in aquaculture wastewater before discharge into open environment. Alaerts also demonstrated that the duckweed sewage stabilization pond system achieved 74% and 77% removal of nitrogen and phosphorus respectively. Similar observations were also reported by Körmer and Vermaat [58,59]. Duckweed plants typically contain more phosphorus in its tissue than other floating plants, which makes them suitable for phosphorus removal. High removal efficiency of COD and BOD of the wastewater by duckweed plant after the bio-remediation experiment was similar with the observation of Chaudhary and Sharma [33] and Ugya [60]. This could be due to the ability of the plant to remove organic compounds as well as degradation of organic materials by microbes [61,62]. The reduction efficiency of TSS by duckweed observed in this study was an indication that duckweed has the potential to reduce total suspended solids (TSS) which was similar to the observation of Zirschky and Reed and Ugya [60,61]. Meanwhile, the removal efficiency observed in this study was lower than the values reported by Zirschky and Reed. This could be as a result of high level of total suspended solids in the wastewater which was beyond the capacity of the plant to reduce within the period of study. However, the reduction in TSS shows the efficiency of duckweed in solid removal which was similar with the observation of Ugya [60].

Bacillus sp. exhibited potency in removal of nitrogenous wastes (nitrate and ammonia) and sulphate level in the wastewater. The result is in line with the study of Bhutto and Dahot [63] who reported that some *Bacillus sp.* utilized nitrogen from ammonium nitrate, ammonium sulphate, among other sources, in the production of an enzyme called amylase that is of industrial importance. There was also reduction in

the sulphate and nitrate level of untreated wastewater after two weeks. This could be as a result of biochemical activities of the indigenous microbes within the wastewater which tend to use up the pollutants in the wastewater. This was in agreement with the observation of Sarmila [23] whose study revealed that biological treatments of aquaculture wastewater are carried out by mixed microbial cultures to decompose and remove toxic wastes.

The temperature range measured in the phytoremediated wastewater was within temperature tolerance limit for duckweed growth. Culley [64] reported that the upper temperature tolerance limit for duckweed growth was around 34°C with a slight decrease in growth below 10°C. It was also proved that duckweed survived in outdoor wastewater treatment tanks [65].

The pH level in all the treatments tend to increase after two weeks of bio-remediation but the highest increase in pH was recorded in the duckweed. The high pH value also enhances the process of ammonia volatilization and this means that the duckweed treatment in this case functioned similarly to an algal bio-remediated wastewater pond with major ammonia removal attributed to volatilization [66]. Ammonia volatilization is mainly linked to pH and temperature.

Duckweed plant was more effective in bio-remediation of aquaculture wastewater than *Bacillus sp.* This could be as a result of combined effects of plant uptake and bacteria (endophytic and rhizospheric bacteria) associated with duckweed in phytoremediation process compare with the microbial remediation where only bacteria are involved in the bio-remediation of the wastewater. Similar result was observed by El-Kheir [67-69] and Farrell [70] in bio-remediation of wastewater. Presence of such bacteria in plants leads to more efficient phytoremediation activity, and reduces the need for additional fertilization [71-74].

It should be noted from this that bio-remediation of aquaculture wastewater does not result to complete removal of pollutants in the wastewater. However, bio-remediation system has expressed a great potential in treatment of aquaculture wastewater by reducing the level of nutrient and solid pollutants in the wastewater. Martinez-Cordova [37] study on bio-remediation of effluent from shrimp culture system also supports this observation.

Proximate analysis and biomass yield of duckweed: The results of proximate composition of the duckweed before and after bio-remediation are presented in Table 2. Significant difference was recorded in the crude protein, crude fibre and carbohydrate composition of the duckweed samples before and after bio-remediation. The crude protein value of the duckweed increased from 17.65% to 18.47%; ether extract, from 4.41% to 4.46%; ash, from 13.24% to 13.28%; crude fibre, from 16.18% to 19.11%; while the level of carbohydrate of the plant declined from 48.53% to 44.59%. The average wet of duckweed increased from 49.53g to 98.92 after bio-remediation.

Proximate composition (%)	Before Bioremediation	After Bioremediation	Sig-value
Crude Protein	17.65 ± 0.19	18.47 ± 0.09	<0.05
Ether Extract	4.41 ± 0.92	4.46 ± 0.13	>0.05
Ash	13.24 ± 0.04	13.28 ± 0.07	>0.05
Crude Fibre	16.18 ± 0.12	19.11 ± 0.10	<0.05
Carbohydrates	48.53 ± 0.16	44.59 ± 0.17	<0.05

Note: Mean values on the same row with Sig-values >0.05 are not significantly different ($\alpha_{0.05}$).

Table 2: Proximate composition of duckweed (% Dry matter) before and after bioremediation of aquaculture waste water for two weeks.

Water Parameters	Treatments			Sig.-values
	Tc	Tb	Td	
Phosphate (mg/L)	0.38 ± 0.05	15.25 ± 0.67	7.23 ± 0.40	0.00
Nitrate (mg/L)	1.28 ± 0.04	7.07 ± 0.46	5.84 ± 0.42	0.00
Sulphate (mg/L)	0.33 ± 0.06	5.10 ± 0.34	0.53 ± 0.03	0.00
BOD (mg/L)	3.52 ± 0.39	27.48 ± 0.33	2.77 ± 0.45	0.00
COD (mg/L)	32.13 ± 1.75	71.47 ± 1.09	12.33 ± 0.33	0.00
DO (mg/L)	3.60 ± 0.30	4.03 ± 0.10	4.82 ± 0.28	0.00
pH	6.66 ± 0.18	8.18 ± 0.30	8.54 ± 0.27	0.00
Temperature (°C)	27.71 ± 0.15	26.14 ± 1.02	26.25 ± 0.71	0.03
TAN (mg/L)	0.06 ± 0.03	0.66 ± 0.24	0.26 ± 0.09	0.00
TSS (mg/L)	23.39 ± 0.88	2142.19 ± 61.88	1405.08 ± 13.21	0.00

Note: Mean values on the same row with Sig.-value >0.05 are not significantly different ($\alpha_{0.05}$).

Table 3: Water quality parameters of bioremediated aquaculture wastewater with *Bacillus sp.* (Tb) and duckweed (Td) and well water use in production from the sampled fish farm (Tc) used in culturing the experimental fish.

The crude protein of the duckweed sample showed an increase after two weeks of bio-remediation. This observation corroborates the report of Ansal [75]. Nelson [76] inferred that through transformation, absorbed ammonia is converted to plant protein, which may be utilized for growth; resulting in an increase in biomass yield of duckweed as observe in this study. This may partly explain the reduction in ammonia level of duckweed-treated water and a significant increase in crude protein in the plant. The crude protein value recorded in the duckweed samples prior bio-remediation was similar to the value reported by Solomon and Okomoda [77]. The increase in biomass yield is in line with the work of Edward [78] who observed that pond water with less than 3 mg/L TKN and 0.3 mg/L total phosphate (TP) did not support normal growth of *Lemna perpusilla* and *Spirodela polyrrhiza*. The limiting factor in waters for Lemnaceae growth is mainly phosphorus [79]. In this study, the high value of phosphate in wastewater prior to exposure may indicate the sufficiency in the water for uptake, resulting in a reduction post-exposure (Table 1). Several factors inhibit duckweed growth rates. Growth rate decreases due to overcrowding as biomass accumulates to the point that fronds start overlapping each other [29,53,64] and decline in nutrient level in the wastewater [80].

Environmental condition of experimental fish in bio-remediated wastewater: Significant difference was recorded in the water quality parameters of bio-remediated aquaculture wastewater with duckweed and *Bacillus sp.* and the control treatment (well water used in production from sampled fish farm) as shown in Table 3. The highest mean values of phosphate, nitrate, sulphate, BOD, COD, pH, TAN and TSS were recorded in *Bacillus sp.* bio-remediated wastewater used in culturing Nile tilapia while the highest mean pH value was recorded in duckweed bio-remediated wastewater used in culturing Nile tilapia. However, the lowest mean concentrations of phosphate, nitrate, sulphate, DO, pH, TAN and TSS were observed in the control treatment (well water used in production from the sampled fish farm). Higher physicochemical parameters measured in the bio-remediated (recycled) wastewater used in culturing Nile tilapia could be as a result of incomplete removal of pollutants in the water which is furthered exacerbated by feed used during the feeding trial experiment. This result contradicts the observation of Martinez-Cordova [37] in a related study.

Growth performance and nutrient utilization: There was a significant difference in mean weight gain of Nile tilapia juveniles raised in bio-remediated aquaculture wastewater with duckweed and

Parameters	Treatments			Sig-values
	Tc	Td	Tb	
Mean initial weight(g)	10.44 ± 0.01	10.42 ± 0.02	10.43 ± 0.01	0.47
Mean final weight(g)	13.29 ± 0.04	13.39 ± 0.01	14.01 ± 0.05	0.00
Mean weight gain (g)	2.85 ± 0.03	3.58 ± 0.06	2.97 ± 0.03	0.00
Mean daily weight gain (g/day)	0.92 ± 0.02	1.15 ± 0.03	0.95 ± 0.03	0.00
Percentage weight gain (%)	27.33 ± 0.31	34.37 ± 0.60	28.46 ± 0.04	0.00
Specific growth rate (%)	0.19 ± 0.00	0.23 ± 0.01	0.19 ± 0.05	0.00
Condition Factor	0.07 ± 0.01	1.36 ± 0.03	0.08 ± 0.01	0.00
Mean feed intake (g/fish)	6.92 ± 0.09	6.13 ± 0.01	5.71 ± 0.10	0.00
Food conversion ratio	2.42 ± 0.02	1.59 ± 0.03	2.06 ± 0.06	0.00
Gross Feed conversion efficiency ratio	41.27 ± 0.26	62.90 ± 1.19	48.57 ± 1.42	0.00
Protein intake (g)	41.51 ± 0.02	27.57 ± 0.03	36.73 ± 0.03	0.00
Protein efficiency ratio	0.07 ± 0.00	0.13 ± 0.00	0.08 ± 0.00	0.00
Survival rate (%)	100.00 ± 0.00	72.20 ± 1.95	77.80 ± 2.30	0.00

Note: Mean values on the same row with Sig.-value >0.05 are not significantly different ($\alpha_{0.05}$).

Table 4: Growth performance and nutrient utilization of Nile tilapia (*Oreochromis niloticus*) cultured in bio-remediated aquaculture wastewater with *Bacillus sp.* (Tb) and duckweed (Td) and well water use in production from the sampled fish farm (Tc).

Bacillus sp. and the control treatment (well water used in production from sampled fish farm) as presented in Table 4. The mean weight gain, mean daily gain, specific growth rate, percentage weight gain, Gross Feed conversion efficiency ratio and condition factor recorded in Nile tilapia cultured in duckweed bio-remediated aquaculture wastewater were significantly higher than the mean values recorded in Nile tilapia raised in wastewater bio-remediated with *Bacillus sp.* and well water. With respect to FCR, fish cultured in bio-remediated wastewater had FCR values (1.59 ± 0.03 and 2.06 ± 0.06 in duckweed and *Bacillus sp.* treatments respectively) which were significantly lower than FCR of 2.42 ± 0.02 recorded in fish cultured in the control treatment (well water). Base on the results of this study, it can be hypothesized that bio-remediated aquaculture wastewater tend to be more productive in terms of growth and nutrient utilization of culture fish than non-bio-remediated water. This may be attributed to the presence of beneficial microfloral and fauna colonizing the bio-remediated wastewater due to its richness in supporting nutrients as well as improved water quality. Therefore, higher efficiency in conversion of feed to biomass observed in fish cultured in bio-remediated aquaculture wastewater implies better economic returns from utilization of bio-remediated wastewater in aquaculture production system. In a similar context, Martinez-Cordova [37] observed better productive response of shrimp reared in bio-remediated effluents than untreated wastewater. Juarez [13] also

Parameters	WG	FI	FCR	GFCE	M	K	PO ₄ ³⁻	NO ₃ ⁻	SO ₄ ²⁻	BOD	COD	DO	pH	Temp	TAN	TSS	
WG	R	1															
	Sig.	.															
FI	R	0.279	1														
	Sig.	0.159	.														
FCR	R	-.641**	.498**	1													
	Sig.	0	0.008	.													
GFCE	R	.685**	-.457*	-.973**	1												
	Sig.	0	0.017	0	.												
M	R	-.476*	.529**	.923**	-.884**	1											
	Sig.	0.012	0.005	0	0	.											
K	R	.771**	-0.175	-.807**	.873**	-.686**	1										
	Sig.	0	0.382	0	0	0	.										
PO ₄ ³⁻	R	-.704**	0.129	.808**	-.818**	.780**	-.697**	1									
	Sig.	0	0.52	0	0	0	0	.									
NO ₃ ⁻	R	-.807**	-.434*	.489**	-.457*	.435*	-.579**	.510**	1								
	Sig.	0	0.024	0.01	0.017	0.023	0.002	0.007	.								
SO ₄ ²⁻	R	-.670**	-.428*	.384*	-0.355	.494**	-.441*	.537**	.866**	1							
	Sig.	0	0.026	0.048	0.069	0.009	0.021	0.004	0	.							
BOD	R	-.498**	.455*	.878**	-.850**	.945**	-.694**	.735**	.468*	.507**	1						
	Sig.	0.008	0.017	0	0	0	0	0	0.014	0.007	.						
COD	R	-.500**	.452*	.877**	-.851**	.943**	-.696**	.736**	.469*	.504**	1.000**	1					
	Sig.	0.008	0.018	0	0	0	0	0	0.014	0.007	0	.					
DO	R	-0.095	-.904**	-.526**	.554**	-.436*	0.325	-0.223	.436*	.537**	-.403*	-.405*	1				
	Sig.	0.639	0	0.005	0.003	0.023	0.099	0.263	0.023	0.004	0.037	0.036	.				
pH	R	.559**	-.493**	-.858**	.884**	-.783**	.868**	-.651**	-0.324	-0.29	-.704**	-.703**	.537**	1			
	Sig.	0.002	0.009	0	0	0	0	0	0.099	0.143	0	0	0.004	.			
Temp	R	.846**	.444*	-.468*	.498**	-.403*	.661**	-.605**	-.864**	-.825**	-0.341	-0.34	-.390*	.500**	1		
	Sig.	0	0.02	0.014	0.008	0.037	0	0.001	0	0	0.082	0.082	0.044	0.008	.		
TAN	R	-0.342	-.786**	-0.259	0.297	-0.307	0.15	-0.151	.630**	.490**	-0.245	-0.244	.797**	.475*	-.406*	1	
	Sig.	0.081	0	0.191	0.132	0.12	0.456	0.453	0	0.009	0.218	0.22	0	0.012	0.035	.	
TSS	R	-.720**	-.442*	.431*	-.405*	.494**	-.447*	.587**	-.916**	.950**	.553**	.554**	.488**	-0.207	-.776**	.581**	1
	Sig.	0	0.021	0.025	0.036	0.009	0.019	0.001	0	0	0.003	0.003	0.01	0.3	0	0.001	.

Note: **Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed);

Abbreviations: WG: Weight Gain; FI: Feed Intake; FCR: Feed Conversion Ratio; GFCE: Gross Feed Conversion Efficiency Ratio; PO₄³⁻: Phosphate; NO₃⁻: Nitrate; SO₄²⁻: Sulphate; BOD: Biological Oxygen Demand; COD: Chemical Oxygen Demand; DO: Dissolved Oxygen; Temp: Temperature; TAN: Total Ammonia Nitrogen; TSS: Total Suspended Solid.

Table 5: Spearman correlation matrix indicating the relationship between the culture water quality and growth parameters of Nile Tilapia.

considered lower FCR to be a profitable value for commercial purposes in fish production.

Effect of physicochemical parameters of bio-remediated wastewater on growth performance and nutrient utilization of Nile tilapia: Regarding the influence of physicochemical characteristics of culture water on growth and nutrient utilization performance of Nile Tilapia, the correlation matrix (Table 5) indicates that phosphate, nitrate, sulphate, BOD, COD and TSS had a significant strong negative influence on the weight gained of the experimental fish while nitrate, sulphate, TAN and TSS tend to exhibit a strong significant negative influence on the feed intake of the culture fish. This is an indication that excessive of the enlisted water quality variables above (indicators of pollution) as a result of indiscriminate discharge of aquaculture wastewater in aquatic ecosystem will hamper productivity of fish in the affected waterbody. This therefore necessitates the need for treatment (bio-remediation) of aquaculture wastewater before discharge. In line with Redner and Stickney, [30] and El-Sherif and El-Feky [68] observed nitrate (nitrogenous pollutant) has a negative influence on the survival rate of the experimental fish. It depresses feed intake and growth at concentrations as low as 0.1 mg/L [68]. The relatively higher level of ammonia recorded for bacteria-treated water (0.66 mg/L) may partly explain the lower growth performance compared to duckweed. However, the recorded value of ammonia in duckweed bio-remediated wastewater was higher than the control treatment which had the highest fish survival rate. The lowest survival rate recorded for duckweed compared to bacteria and control treatments may stem from elevated level of pH and temperature. High pH could increase the toxicity of ammonia lower dissolved oxygen level which reduces fish survival [74]. This may be due to species differences in optimum levels for fish growth. The optimum concentration of ammonia for Nile tilapia was estimated to be below 0.05 mg/L [68]. This may suggest that 0.26 mg/L ammonia did not affect growth performance; survival rate could be reduced following prolong exposure. The correlation analysis of water quality and growth performance established a strong negative linear relationship between nitrogenous compounds (TAN and nitrate) and growth as well as feed intake of the culture fish. Frias-Espericueta and Ray also observed that exposure of penaeid shrimps to high concentrations of nitrogenous compounds and suspended solids have negative impact on their growth and food intake.

Conclusion

It was evident from all the results obtained from the experiment that duckweed is highly effective in the removal of high toxic organic waste components of wastewater discharge from catfish farm. The technology involved in the use of duckweed is very simple and at the lower cost when compare to the use of *Bacillus sp.* in bio-remediation of waste water, which require high level of expertise in identification, isolation, mass production and application. The use of duckweed in bio-remediation was effective in reducing high phosphate, sulphate, ammonia, nitrate, biological oxygen demand and chemical oxygen demand in aquaculture wastewater. The reuse of Duckweed-treated wastewater is suitable for fish culture without affecting growth performance, and dissolved oxygen level as most polluting substances were reduced significantly. *Bacillus sp.* was only effective in removal of sulphate, ammonia and nitrate in the wastewater. Future research effort should investigate long term growth studies under different culture conditions and fish species to assess the use of duckweed as an effective approach to sustain aquaculture development.

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