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Biological Treatment of Fish Pond Waste Water by *Coelastrum morum*, a Green Microalgae

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Abstract: *The use of natural remediation methods to remove contaminants from waste water is becoming more popular. One of the aims of waste water treatment is to reduce nutrient such as nitrate and phosphate level in effluent to a protective level of the receiving water body. Microalgae especially green algae have been used for several decades for biotreatment of fish pond waste water, yet the there is limited or no report on use of Coelastrum morum CoC for bio-treatment of wastes water from fish pond. This study is aim at treatment of used/wastes water discharged from fish pond at Agric, Oke Osun area, Osogbo and assessing wastewater treatment plants performance by Coelastrum morum CoC. Freshly discharge fish pond waste water sample (FPWWS) was collected from Agric Oke osun in Osogbo, Osun State, Nigeria and analyzed for the physicochemical parameters such as pH, Total Dissolved Solid (TDS), Electrical conductivity, dissolved oxygen, total Alkalinity, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), ammonia, phosphate and Nitrate by standard methods. 190ml of the sterilized FPWWS was inoculated with 10ml of Coelastrum morum. The inoculated sample was incubated under 2ft cold fluorescence light for two weeks and determines the physicochemical parameters at 7 days interval. The results observed for raw, biological treated and removal efficiency showed: pH (7.66 and 8.85), TDS (283.33 and 95.33mg/l with 66.35%), Electrical conductivity (449.33 and 195.00µS cm^{-1}), Dissolved oxygen(3.57 and 1.13 mg/l with 68.35%), Total Hardness (64.17 and 33.29 mg/l with 48.12%), BOD (274 and 55.36mg/l with 79.80%), COD (420 and 72mg/l with 82.86%), ammonia (31.4 and 4.41 mg/l with 85.96%, phosphate (10.27 and 2.04 with 80.14%) and nitrate (27.11 and 6.57 mg/l with 75.77%). Biological treatment with Coelastrum morum CoC S a potential removal of pollutant and other wastes from the fish pond waste water.*

Keywords: Biological, Treatment, *Coelastrum morum* CoC, Physicochemical, fish pond and waste water,

1. Introduction

Water is essential for all known life forms; still water pollution and the destruction of ecosystems continue to increase. Water contamination is now a major problem in the global context as a consequence of industrialization, globalization, population growth, urbanization and warfare combined with increased wealth and more extravagant lifestyles (UN-Water, 2013). Water that is released after being used by the domestic and industrial sector into the environment is called wastewater (Mohapatra, 2006).

Wastewater is the major contributor to the aquatic ecosystem pollution. The waste contained high inorganic nutrients, mainly ammonia, nitrate, carbon and phosphate. Besides the inorganic nutrients, in the wastewater also contain heavy metals. These heavy metals and the inorganic nutrients may cause harm to the aquatic organisms, including humans, as water is the main fundamental source to human's daily life. The factors that contribute to the water pollutant are domestic wastewater, agricultural runoff, and landfill, industrial effluent and animal waste.

Wastewater is a general term used to represent the water with poor quality that contains more amounts of pollutants like soluble organic, inorganic, insoluble inorganic materials, macro solids, toxins, etc and microbes such as bacteria and protozoa and which must be treated before it is released into another body of water, so that it does not cause further pollution of water.

Fish pond waste water consists of excretory product of fishes and nutrients not totally exhausted by the fishes. The quantity and quality of waste generated and discharged into natural water bodies from fish ponds has recently indicated the need for different strategies to address water quality challenges in the environment. The main cause of poor water quality is waste accumulation through hyper-nitrification resulting from excessive feeding rates and high nutrient dietary composition, both of which are common phenomena in intensive aquaculture systems (Shimeno *et al.*, 1997). High levels of nitrate and phosphate accumulation predispose fish to infestation by parasites and pathogens and also pose a threat to the environment (Jana and Jana, 2003).

Many aquaculture systems generate high amounts of wastewater containing compounds such as suspended solids, total nitrogen and total phosphorus. Today, aquaculture is imperative because fish demand is increasing. However, the load of waste is directly proportional to the fish production. Therefore, it is necessary to develop more intensive fish culture with efficient systems for wastewater treatment. Wastewater treatment is an important measure to reduce the pollutant and other contaminants present in wastewater. A number of physical, chemical and biological methods used in conventional wastewater treatment have been applied in aquaculture systems. There are many aerobic, anaerobic and physicochemical processes that can treat wastewaters to almost any standard of effluent from the simple removal of gross solids to membrane systems that can produce drinking water quality.

Biological treatment involves the use of plants, microorganisms and others naturally occurring materials for removal of nutrients and others pollutants from discharged wastes water. Biological wastewater treatment is neither toxic nor contributes to the existing problems as we have it in the case of others means of waste treatment (chemical treatment). Also, biological treatment is a cost-effective and environment friendly method for treating wastewater. The use of a wide range of microalgae such as *Chlorella*, *Scenedesmus*, *Phormidium*, *Botryococcus*, *Chlamydomonas* and *Spirulina* for treating wastewater has been reported to be efficiency and promising in wastes removal (Pittman *et al*., 2010; Stephens *et al*., 2010).

The potential for microalgae in waste water remediation is however much wider in scope than its current role (De-Bashan and Bashan, 2010). Algae, particularly green unicellular microalgae have been proposed for a long time as a potential renewable fuel source (Oswald and Golueke, 1960). In addition, waste water treatment by microalgae is an eco-friendly process with no secondary pollution as long as the biomass produced is re used and allows efficient nutrient recycling (Godos *et al*., 2003). Despite enormous work reported on use of green algae for removal of wastes from fish pond effluents, there is no report on efficiency of *Coelastrum morum* CoC for waste water removal; hence the present work is targeting the treatment of fish pond waste water using *Coelastrum morum* CoC through laboratory scale.

The objectives of this research work includes are: Analysis of the physicochemical parameters of fish pond waste water, treatment of the waste water with microalgae (*Coelastrum morum*) and to analyze the physicochemical parameters of treated fish pond waste water .

2. Materials and Methods

2.1 Sample Collection

Raw waste water sample was collected from fish pond in Agric oke osun fish farm, Osogbo, Osun-state. The sample was aseptically collected in laboratory clean containers, and then corked before transferred to the laboratory for analysis.

2.2 Isolation and Characterization of *Coelastrum morum* CoC

i. Water sample collection and Isolation

Microalgae water sample was obtained from Osajin fish farm in Akinyele Local Government, Ibadan, Oyo State. Isolation and purification of microalgae was carried out using sterilised Bold's Basal Medium (BMM) (Starr and Zeikus, 1987), as 10 mL of water sample was introduced into 200 mL of the prepared medium. This was followed by incubation under an illumination provided by cold white fluorescent tubes with a light exposure period of 16h:8h light and dark cycles at varying temperature of 25^{o}C to 37^{o}C (Fagade, 1990). The flasks were observed every two days for the presence of microalgae growth using a compound light microscope. Serial dilution was made in Bold's Basal Medium from the flasks showing growth. Isolation was made by inoculation of 50 µL culture solution onto petri plates containing agar-based preparations of Bold's Basal Medium. Incubation of Petri-dishes was done at 27^{o}C under, and illumination was supplied using cold fluorescent tubes for up to two weeks. Culture purity was ensured by plating repeatedly and by regularly observing under a microscope as described by Dayananda *et al*. (2007).

ii. Morphological characterization

This was carried out with the aids of compound light microscope based on morphological characteristics like shape, size, cellular structure i.e unicellular/multicellular, chloroplast, pyrenoid, motility and nucleated.

iii. Molecular characterization

a. DNA Extraction: The InstaGeneTM Matrix Genomic DNA Isolation kit was used for isolation of genomic DNA of microalgae. The following below procedure was used as required by the kit instruction. The colonies of isolated microalgae were picked and immersed in a microfuge tube contained sterile water of 1mL.At 10,000–12,000 rpm, the supernatant was removed by centrifugation for 1 minute. This was followed by addition of 200 µl of Insta Gene matrix to the pellet and incubation at 56 °C for 15 minutes. After vortexing at high speed for 10 seconds, the tube was place in boiling water bath for 8 minutes at 100 °C. Finally, the content was vortex for 10 seconds and spin at high speed for 2 minutes 10,000–12,000 rpm. In result, 20µl of the supernatant was used per 50 µl PCR reaction.

b. Polymerase Chain Reaction: Amplification of Universal primers gene fragment using MJ ResearchPTC-225 Peltier Thermal Cycler through 18S rRNA ITS Region

c. Primer Details: 1µl of template DNA was added to 20µl of PCR reaction solution. The PCR reaction was performed using 18S-C[a]/18S-D[a]/18S C-2[b]/18S D-2[b] primers (Table 1) with stated conditions : Initial Denaturation at 94ºC"for"2"min"and"then 35 amplification cycles at 90 ºC for 45sec, 50 ºC for 60sec, and 72 ºC for 60sec. Final Extension at 72 ºC "for"10"min. DNA fragments are amplified. Include a positive control (E.coli genomic DNA) and a negative control in the PCR

Table 1:18S rRNA PRIMERS USED FOR PCR REACTION

PRIMER	SEQUENCE
18S rRNA	
18S-C[a]	5'-TGATCCTTCYGCAGGTTCAC-3'
18S-D[a]	5'-ACCTGGTTGATCCTGCCAG-3'
18S C-2[b]	5'-ATTGGAGGGCAAGTCTGGT-3'
18S D-2[b]	5'-ACTAAGAACGGCCATGCAC-3'

d. Purification OF PCR Products: Unincorporated/unattached PCR primers and dNTPs were removed from PCR products using Montage PCR Clean up kit (Millipore). 18S-C[a]/18S-D[a]/18S C-2[b]/18S D-2[b] primers were used for the sequence of PCR product. The sequencing reactions were performed using ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems).

e. Sequencing Protocol: Single-pass sequencing was performed using 18s rRNA universal primers on each template. The fluorescent-labelled fragments were purified from unincorporated terminators using ethanol precipitation. After suspension in distilled water, the samples were subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems).

f. Bioinformatics Protocol: The rRNA sequence was blast using the NCBI blast similarity search tool . The phylogeny analysis of the sequence with the closely related sequence of blast results was performed follow by multiple sequence alignment. The multiple alignments of sequences were carried out using programs MUSCLE 3.7 (Edgar, 2004). The aligned sequences were cured using program G blocks 0.91b. The G blocks were used for elimination of poorly aligned positions and divergent regions (removes alignment noise) (Talavera and Castresana, 2007). Finally, the program PhyML 3.0 a LRT was used for phylogeny analysis and HKY85 as Substitution model. PhyML was shown to be at least as accurate as other existing phylogeny programs using simulated data, while being one order of magnitude faster. PhyML was shown to be at least as accurate as other existing phylogeny programs using simulated data, while being one order of magnitude faster. The program Tree Dyn 198.3 was used for tree rendering. (Dereeper *et al*., 2008).

2.3 Determination of Physicochemical Characteristics of Fish Pond Waste Water Samples

The physico-chemical parameter was analyzed using standard analytical procedure (APHA, 1998). The physico-chemical parameters analyzed include; pH, total dissolved solid, Electrical Conductivity, Dissolved oxygen (DO), Total hardness, chloride biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonium, phosphates and nitrate.

i. *Determination of pH*

This was determined using a pH meter (Toledo, MP220). The water sample was measured into 100 cm^3 beaker and by inserting the pH meter probe after standardization into the beaker the reading of the pH was determined (AOAC, 2006).

ii. *Electrical Conductivity*

Electrical conductivity is the measure of the ability of an aqueous solution to convey an electric current. This was determined by using conductivity meter with the procedure described by Richard (1954).

iii. *Determination of Total Dissolved Solids*

Total Dissolved Solids (TDS) was determined mathematically as a product of conductivity multiplied by a constant value, 0.6 (APHA, 1985).

TDS = Conductivity × 0.6.

iv. *Determination of Dissolved Oxygen (DO)*

Dissolved Oxygen (DO) was determined using the Dissolved Oxygen meter (Model OXi315i), WTW82362. The dissolve oxygen meter was dipped into a sample, allowed to be steady and the result was recorded (APHA, 1985).

v. *Total Hardness*

This was determined by using Titrimetric method based on the fact that ethylene diamine tetra acetic acid and its sodium salts (EDTA) form a chelated solution complex when added to solution of various metal ions.

Reagents

NH_3/ NH_4Cl buffer solution of pH= 10.0; 27g of NH_4Cl was dissolved in 30ml distilled water and thoroughly mixed with 17.5ml of 25% NH_3 solution, this was diluted to 500ml. Standard EDTA titrant 0.01M, standard zinc sulphate heptahydrate 0.02M : 2.88g of the salt dissolved in the 500ml of de ionized distilled water. Ferrochrome Black T indicator and distilled de-ionized water.

Procedure

The EDTA was first of all standardized with standard $ZnSO_4.7H_2O$ solution using ferrochrome black T indicator. 3-4 ml NH_3/NH_4Cl buffer solution was added. The solution turned from wine colour to blue on titration. 50ml of wastewater was transferred into a clean conical flask; 3-4ml buffer solution was added followed by 2 drops of indicator. Standardized EDTA solution was added slowly with continuous stirring until blue colour appeared which indicated the end point.

Calculation: Hardness as mg $CaCO_3$/l = titre value (ml) x 1000/ml of sample.

vi. *Determination of Chloride*

Five drops of a Phenolphthalein indicator solution was added to 50 mL of the sample and neutralized with 0.1 N sulphuric-acid to the colorless side of Phenolphthalein. To this was added one mL of potassium chromate indicator solution before titration with standard silver nitrate solution to the pinkish-yellow end point. A reagent blank titration was carried out in parallel to the sample titration. Chloride concentration was calculated as follows (APHA, 2005):

Chloride, mg/l = {(A – B) (N) (35.45)/V} × 100

Where,

A= Silver nitrate solution, in ml for sample titration;

B=Silver nitrate solution, used for blank titration (in ml);

N= Normality of the silver nitrate solution; and

V= Sample volume in (ml).

vii. *Biochemical Oxygen Demand*

Two 100 ml bottles were obtained with lids and cleaned well. To 25 ml sample was taken in each bottle, 75 ml of distilled was added to the two bottles and were tightly closed. One of the bottles was kept in the incubator for 5 days at 20-22°C. To the other bottle below the surface of the liquid, 10 ml of Manganese sulphate solution and 2 ml of alkali- iodide solution were added using a syringe. By inverting the bottle several times, the bottles were closed and mixed. A clear supernatant was left above, when the precipitate settles, by inverting the bottle, the precipitate was shaken again slowly and 8 ml of conc. H_2SO_4 was added when the setting has produced at least 50 ml supernatant. Through gentle inversion until dissolution is completed, the bottle was closed and mixed. Then, until a pale yellow solution is reached, 100 ml of the sample was titrated with 0.05 M Na2S2O3 solutions; this was followed by addition of 2 ml of freshly prepared starch solution and titration ceased when a blue colour appeared. The procedure was repeated using 100 ml distilled water (blank) and this was repeated for incubated sample after 5 days. The BOD was calculated as follows:

BOD as mg/L = 16(*V*1 - *V*2)

Where:

*V*1 = ml of $Na_2S_2O_3$ used for the sample before incubation;

*V*2 = ml of $Na_2S_2O_3$ used for the sample after incubation.

viii. *Chemical oxygen demand (COD)*
COD analysis was carried out based on Section 5220 of *Standard Methods* (APHA, 1998, 2012) that involves using pre-packaged mercury-free and premixed COD. 5- 150, 20-900 and 100-4,500 mgCOD/L were three types of COD used. A COD reactor was preheated to 150°C before testing. During every test, a 2.5 mL sample was carefully added into one COD vial of ranges 5-150 or 20- 900 mgCOD/L, and 0.5 mL sample were carefully added into one COD vial of range 100 4,500 mgCOD/L. Then, the vial was thoroughly shaken by hand. COD standards and a DW blank were processed exactly the same as the samples. COD vials containing sample, COD standard, and blank, were heated in the COD reactor for 2 h at 150±2°C, and then they were removed from the reactor and placed in a rack until they cooled and any suspended precipitate in the vials settled down. After the outsides of vials were wiped to remove dust, the vials were placed into the Orbeco Hellige MC500 Multi-Parameter Colorimeter one by one, to measure their COD concentrations under a standard curve covering the expected range of sample concentrations. The wavelength of 440, 600, and 600 nm were set for the ranges 5-150, 20-900 and 100-4,500 mgCOD/L, respectively. According to the requirements of the test method for using the COD vials, blanks of the ranges 20-900 and 100-4,500 mgCOD/L were used to set the zero in the colorimeter before sample testing.

ix. *Ammonia nitrogen*
This was carried out using Nessler Method**.** The color formation in the reaction of Nessler Reagent with ammonium ions was aided by the Mineral Stabilizer complexes hardness in the sample and the Polyvinyl Alcohol Dispersing Agent. A Proportion between the ammonia concentration lead to a yellow color formation. Test results were measured at 425 nm. A blank prepared from deionized water treated and measured equally as the sample.

x. *Determination of Phosphate*

0.5ml of ammonium molybdate and 2 drops of stannous chloride were added to 25ml of the sample and mixed by swirling. The intensity was measured using a spectrophotometer (21D) at 690 nm after appearance of blue coloration (APHA, 1998).

The concentration of the phosphate was calculated

Phosphate (mg/l) = A - B X C

Where; A = Absorbance of sample;

B = Absorbance of blank sample,

C = Volume of standard phosphate

xi. *Determination of Nitrate*

One level spoonful (~ 1.5 ml) of nitratest powder (containing zinc dust 60% and barium sulphate 40%) and one nitratest tablet (ammonium chloride) were added to a test tube filled with sample to 20ml mark and was shaken for a minute. The tube was allowed to stand for a minute and was inverted 3-4 times to aid flocculation and was allowed to stand for two minutes to ensure complete settlement. The clear solution was dispersed into 10 ml mark and one nitricol tablet (Sulfanilic acid, acting as the aromatic amine), was added, crushed, and mixed to dissolve, then it was allowed to stand for 10 minutes for color development and readings were taken on the Photometer (Wagtech) at 570 nm wave length.

2.4 Experimental Procedure for conventional treatment of Fish pond wastewater

Below method was employed to determine the effect of *Coelastrum morum* CoC (microalgae) in treatment of fish pond wastewater

Raw Fish Pond Wastewater + *Coelastrum morun* CoC

The experiment were conducted in triplicates and incubated under the same condition in 250 mL Erlenmeyer flask for period of 14 days.

2.5 Inoculation and Sampling

10 mL of exponential growing of *Coelastrum morum* CoC was inoculated into three 250 mL Erlenmeyer flask containing 190ml of Sterilized fish pond waste water sample. Sample was taken for physicochemical analysis at interval of 7 days after inoculation for two weeks.

2.6 Statistical Analysis

Data obtained were subjected to appropriate statistical analysis.

3. Result and Discussion

3.1 Morphological and molecular identification of microalgae

Colonies of Isolate CoC are cubical and small in size. Each cell has a single, parietal chloroplast with a single pyrenoid. The cell walls appear smooth. No flagellated stages are present and they are planktonic (Table 2). The analyses of their 18S rRNA and a blast search of NCBI gene data base revealed that *Coelastrum morum* CoC was closely related to *Coelastrum morum* strain SAG217-5 (Table 3). Cells were wide, spherical and small (Isolate OSK). The walls thickened with age. Chloroplast had a hollow sphere with a lateral pore and single pyrenoid. They are non-motile, planktonic and bi-nucleated (Table 2). Isolate OSK was 99% similar to *Coelastrum morum* gene for, strain: MBIC10280 (Table 3).

Table 2: Morphological Characteristics of *Coelastrum morum* CoC

Morphological characters	Isolate
Shape	Cubical
Size	Small
Unicellular/Multicellular	Unicellular
Planktonic/Benthic	Planktonic
Chloroplast	Parietal
Pyrenoid	Single
Motility	Non-motile
Nucleated	Uninucleated

Table 3: Molecular Identification of *Coelastrum morum* CoC based on partial 18SrRNA gene sequence analysis

Isolate Code	Identity	Identity from BLAST	Percentage similarity (%)
CoC	*Coelastrum sp*	*Coelastrum morum* SAG217-5 (AB 183580.1)	99

3.2 Physicochemical Properties of Wastes and Treated Fish Pond Water

The pH values recorded for raw fish pond waste water increased gradually from 7.66 to 8.85 (Figure 1). The pH of the fish pond waste water is 7.66, which is neutral basic, after 7 days of bio-treatment, the pH increased to 8.14 and 8.88 after 7 and 14 days of treatment with *Coelastrum morum* CiA. The pH values obtained in this study were within the range of optimum pH levels for anaerobic digestion (Speece, 1996) and were within the World health Organisation (WHO) tolerance limits of 6.0 to 9.0 for the discharged of wastewater into aquatic environment (Akan *et al*., 2010). The anaerobic degradation of organic compounds releases ammonia, which react with carbon dioxide produced during the anaerobic process, resulting in ammonium bicarbonate, which contributes to the increase in pH values.

Total Dissolved Solid (TDS) recorded for raw fish pond waste water is 283.33 mg/L (Figure 2a). TDS values obtained were generally within 1000 mg/l the upper limit set by WHO (WHO, 2011). The value later reduced to 164.67 and 95.33 mg/L with removal efficiencies of 41.88 and 66.35% respectively after 7 and 14 days of treatment with *Coelastrum morum* CiA (Figure 2b). The electrical conductivity and total dissolved solid exhibited similar trend in both abattoir effluents, this is as a result of the linear relationship that exist between the two parameters (Radojevic, 1999). Chemical Oxygen Demand (COD) is considered as the amount of oxygen consumed by the chemical breakdown of organic and inorganic matter.

The Electrical conductivity of raw fish pond waste water is 449.33µScm^{-1} (Figure 3). There was a slight decrease in Electrical conductivity (360.67 and 195µScm^{-1}) after 7 and 14 days of bio-treatment (Figure 3).

The dissolved oxygen (DO) in the fish pond waste water is below undetectable concentrations before the end of the first 14 days interval. This observed change is due to the nature of the experimental setup and also as a result of increase in the microorganisms' activities which used up the available dissolved oxygen. The dissolved oxygen was on decreased from the onset with value of 3.57 mg/L to 1.86 mg/L after 7 days and 1.13 was recorded at the end of 14 days (Figure 4a). The highest reduction efficiency (68.35%) was recorded after 14 days which is different from 41.88% observed after 7 days of treatment (Figure 4b). This was as a result of activities and higher number of microorganisms during that period.

The value recorded for Total hardness in raw fish pond waste water is 64.17 mg/L (Figure 5a). This value is within the required standard for discharge into the environment. However, after period of treatment for 7 and 14 days respectively, 60.44 and 33.29 mg/L were observed (Figure 4.5a) at 5.81 and 48.12 % reduction efficiencies (Figure 5b).

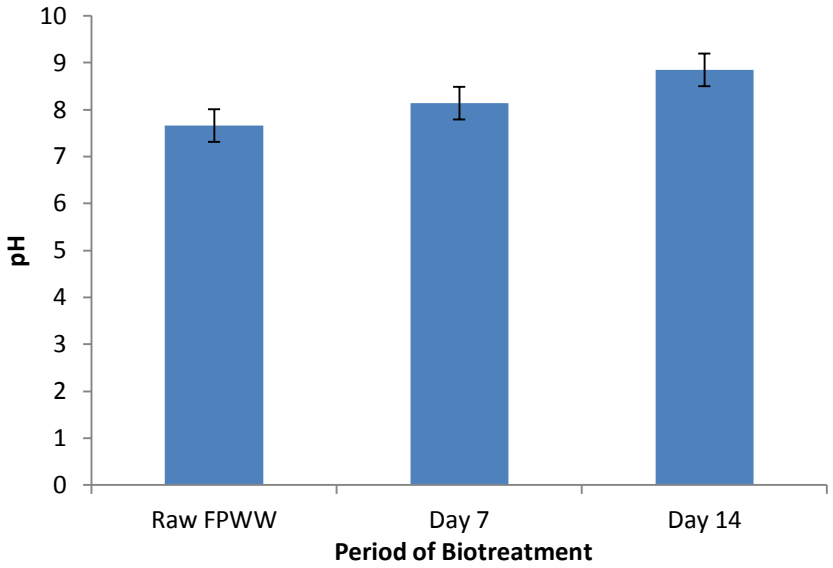

Figure 1: pH of the Bio treated Raw Fish Pond Wastewater with *Coelastrum morum CiA* after 7th and 14th day of treatment

Note: Raw FPWW= Raw Fish Pond Wastewater

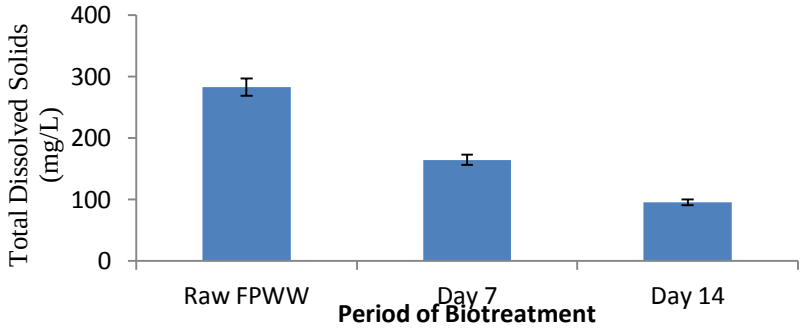

Figure 2a: Total Dissolved Solids of the Bio treated Raw Fish Pond Wastewater with *Coelastrum morum CiA* after 7th and 14th day of treatment

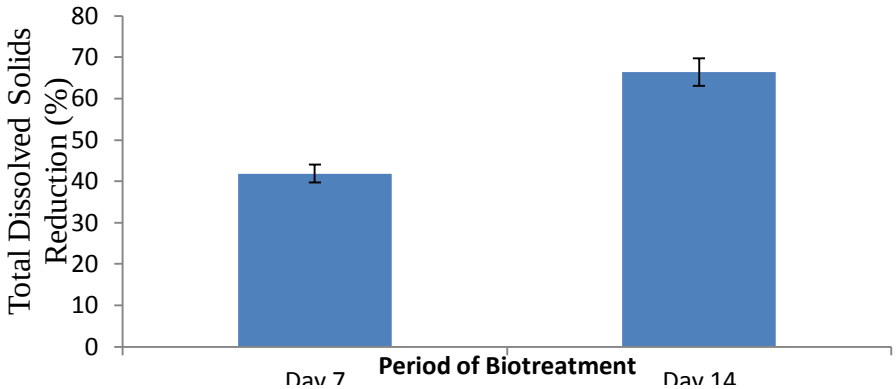

Figure 2b: Total Dissolved Solids removal efficiencies of *Coelastrum morum* after 7 and 14 days of treatment.

Note: Raw FPWW= Raw Fish Pond Wastewater

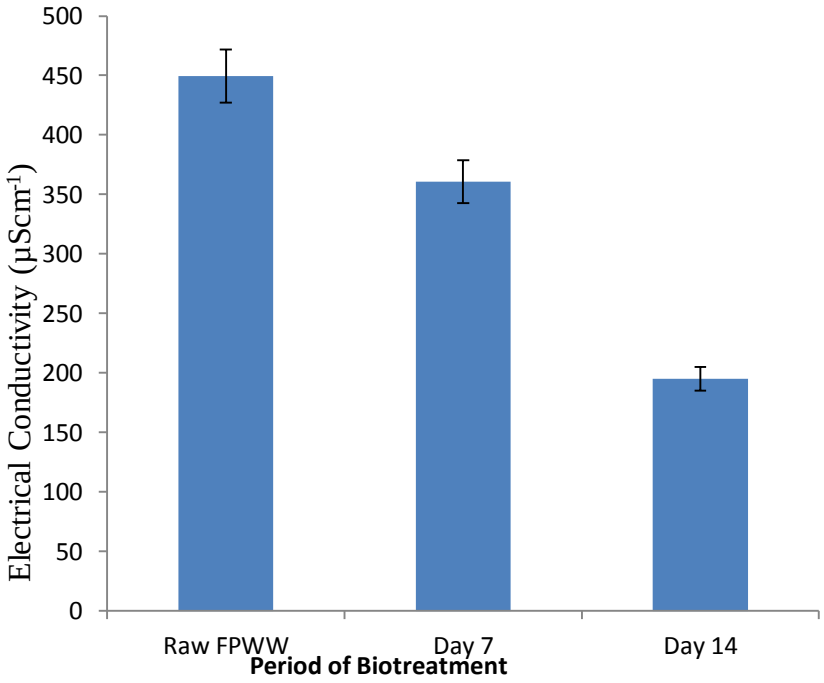

Figure 4.3: Electrical Conductivity of the Bio treated Raw Fish Pond Wastewater with *Coelastrum morum CiA* after 7th and 14th day of treatment

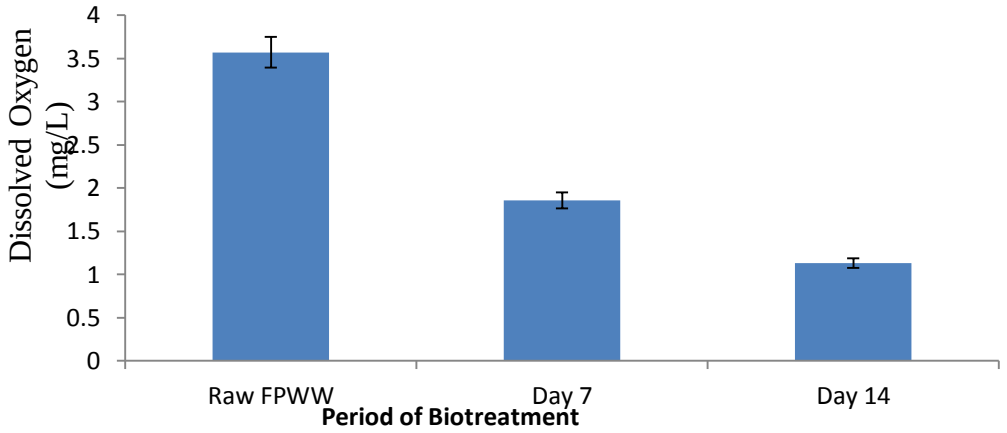

Figure 4a: Dissolved oxygen of the Bio treated Raw Fish Pond Wastewater with *Coelastrum morum CiA* after 7th and 14th day of treatment

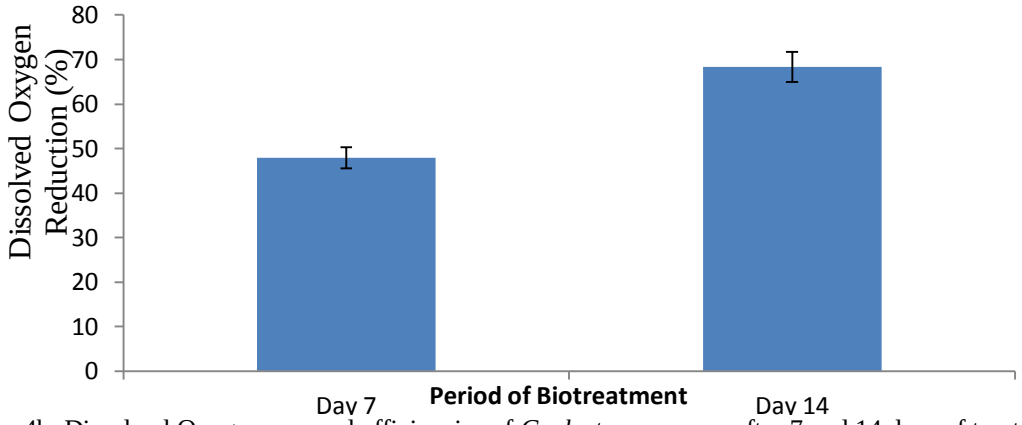

Figure 4b: Dissolved Oxygen removal efficiencies of *Coelastrum morum* after 7 and 14 days of treatment.

Note: Raw FPWW= Raw Fish Pond Wastewater

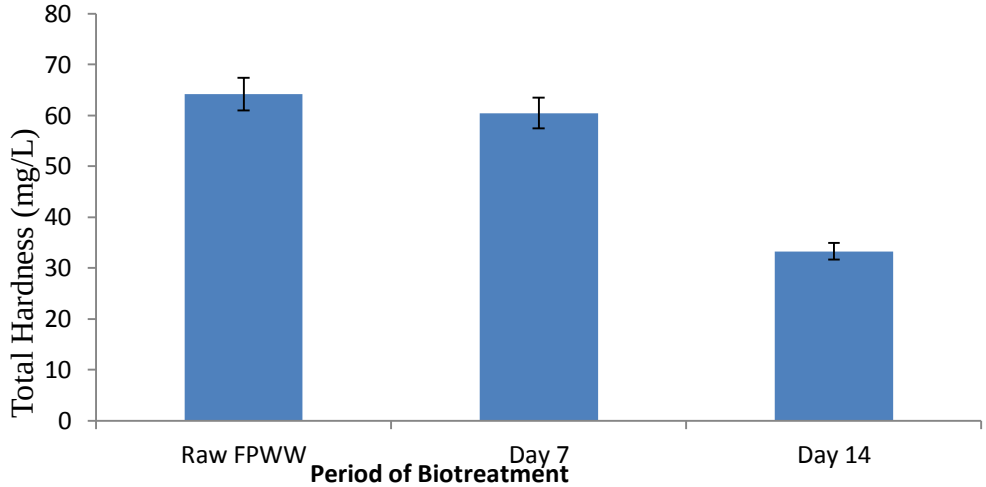

Figure 5a: Total Hardness of the Bio treated Raw Fish Pond Wastewater with *Coelastrum morum CiA* after 7th and 14th day of treatment

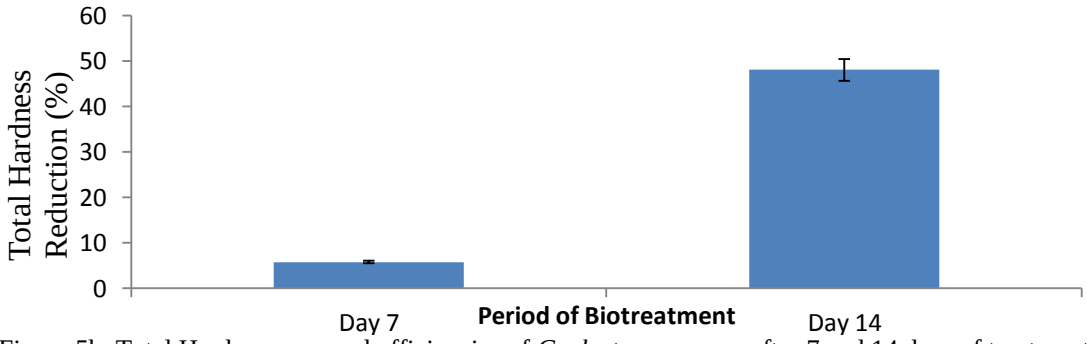

Figure 5b: Total Hardness removal efficiencies of *Coelastrum morum* after 7 and 14 days of treatment.

Note: Raw FPWW= Raw Fish Pond Wastewater

The concentration of Chloride in raw fish pond waste water is 6.62 mg/L. This value later decreased (4.17 and 1.69 mg/L) after 7 and 14 days treatment with *Coelastrum morum* CoC Figure 6a. The percentage of Chloride reduction efficiencies observed after 7 and 14 days are 37.01 and 74.47 % respectively Figure 6b

Biochemical Oxygen Demand (BOD) recorded at raw fish pond waste water is found to be lower (93.33 and 55.36 mg/L) after 7 and 14 days of bio-treatment compare with 274 mg/L obtained for raw fish pond waste water (Figure 7a). They had reduction efficiencies of 65.94 and 79.80 % after 7 and 14 days of biotreatment with *Coelastrum morum* CoC (Figure 7b). High degradation rate at the week two(day=14) could possibly be as a result of the acclimatization of the microorganisms to the prevailing conditions High organic material presents in fish pond waste water are an indication of higher BOD and COD. This is in conformity with the finding of del Pozo *et al*. (2003). This fact had a great influence on the rest of the parameters and the nature of the wastewaters. Some information on the wastewater biodegradability can be gained comparing different measures, example, BOD and COD where a high ratio of BOD to COD shows a relatively high biodegradability whereas a low ratio indicates that the wastewater is more slowly biodegraded (Vollertsen and Hvitved-Jacobsen, 2002).

The COD observed in this study showed that raw fish pond waste water was reduced to 229.67 and 72 mg/L respectively from initial raw waste water value of 420 mg/L after 7 and 14 days treatment with *Chlorella vulgaris* ChA (Figure 8a) at removal efficiencies of 38.98 and 96.80 % respectively (Figure 8b). The rate of reduction of COD raw fish pond waste water of confirms the effectiveness of degradation process to reduce the pollutant load contained in the wastewater.

The value recorded for ammonia in this study is 31.4 mg/L for raw fish pond waste water, however there was a decrease in result obtained after treatment for period of 14 days, with 15.03 and 4.41 mg/L at day 7 and 14 respectively (Figure 9a) as well as 52.83 and 85.96% reduction efficiencies (Figure 9b).

Phosphate and nitrate are among the prominent compounds in fish pond waste water. Relatively higher rate of phosphate decrease (6.72 and 2.04 mg/L Figure 10a) with reduction efficiencies of (52.83 and 80.14 % Figure 10b at day 7 and 14 respectively) was recorded in phosphate concentration after bio-treatment against value observed for raw fish pond waste water 10.27 mg/L. High phosphate levels will result in the eutrophication of the river. Blood is also the major contributor to the nitrogen content while phosphorus originates from stomach contents in the effluent.

The results obtained in this study showed significant reduction of nitrate in raw fish pond waste water after bio-treatment for period of 14 days with 18.07 and 6.57 mg/L at day 7 and 14 against 27 mg/L recorded for raw fish pond waste water (Figure 11a). The higher percentage reduction efficiencies were recorded after 7 and 14 days of bio-treatment (33.35 and 75.77%) respectively (Figure 11b).

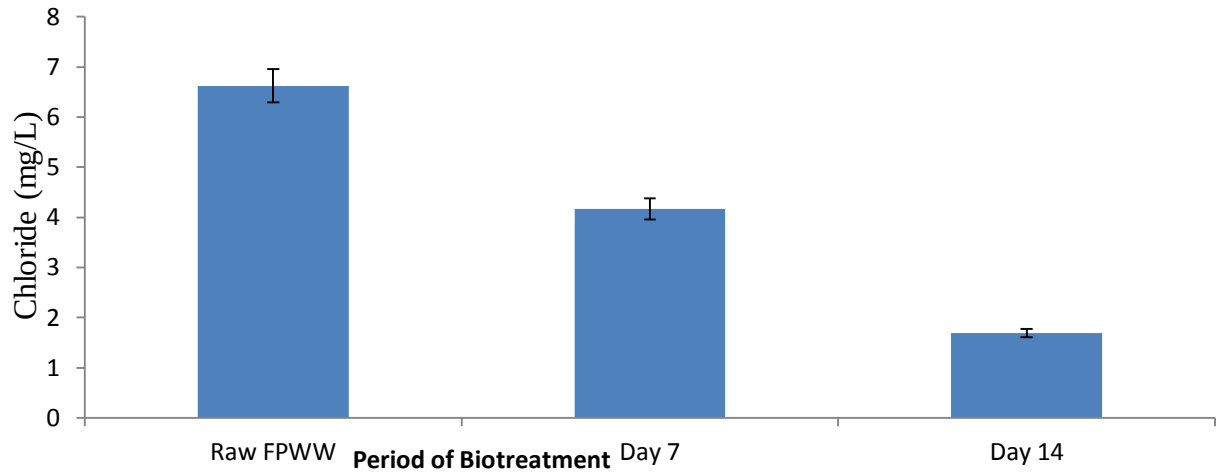

Figure 4.6a: Chloride of the Bio treated Raw Fish Pond Wastewater with *Coelastrum morum CiA* after 7th and 14th day of treatment

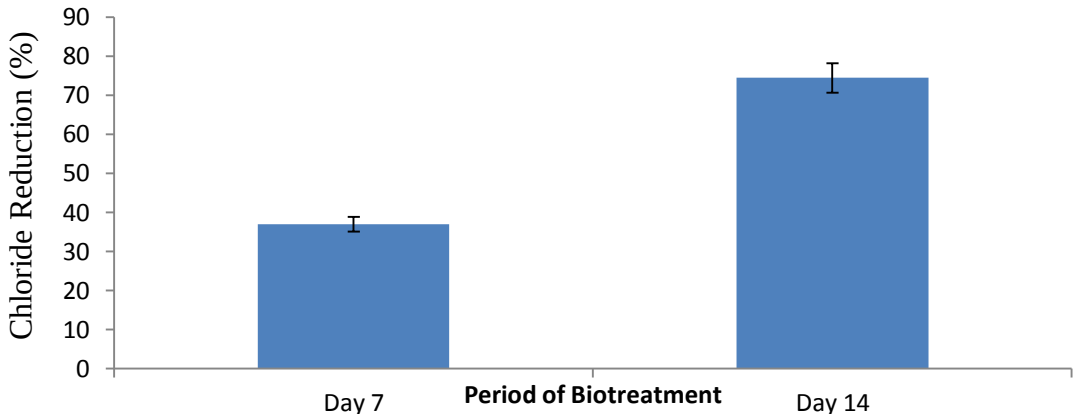

Figure 6b: Chloride removal efficiencies of *Coelastrum morum* after 7 and 14 days of treatment.

Note: Raw FPWW= Raw Fish Pond Wastewater

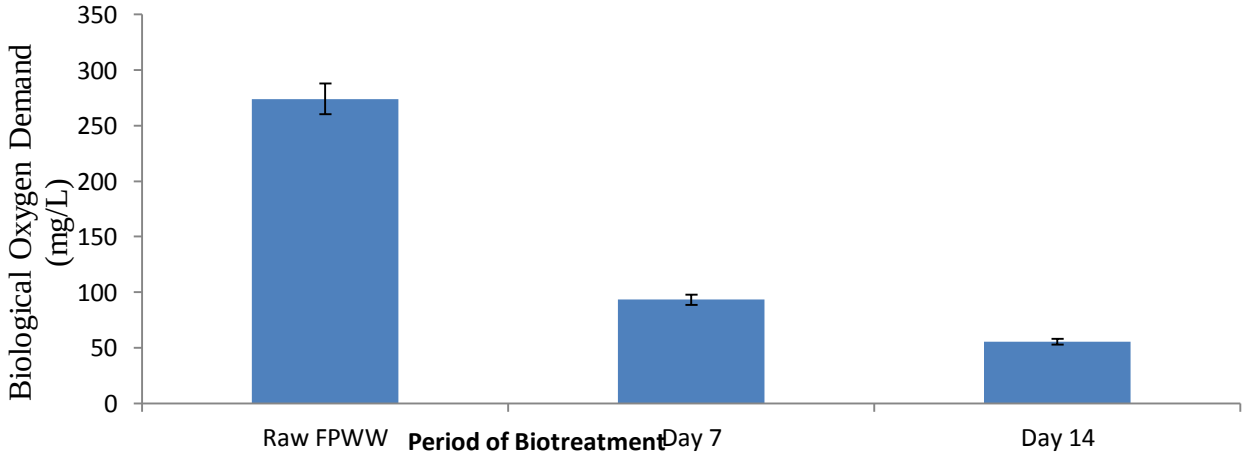

Figure 7a: Biological Oxygen Demand of the Bio treated Raw Fish Pond Wastewater with *Coelastrum morum CiA* after 7th and 14th day of treatment

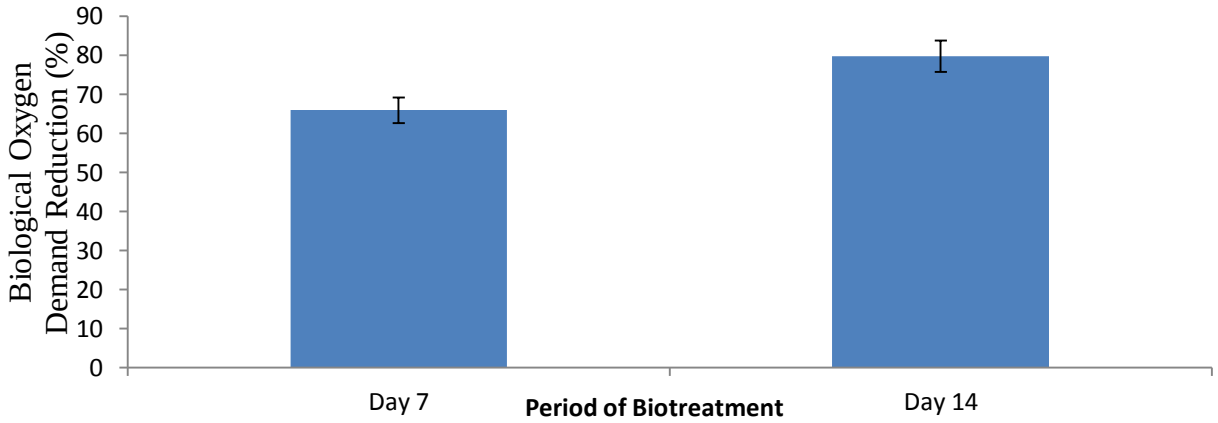

Figure 7b: Biological Oxygen Demand removal efficiencies of *Coelastrum morum* after 7 and 14 days of treatment.

Note: Raw FPWW= Raw Fish Pond Wastewater

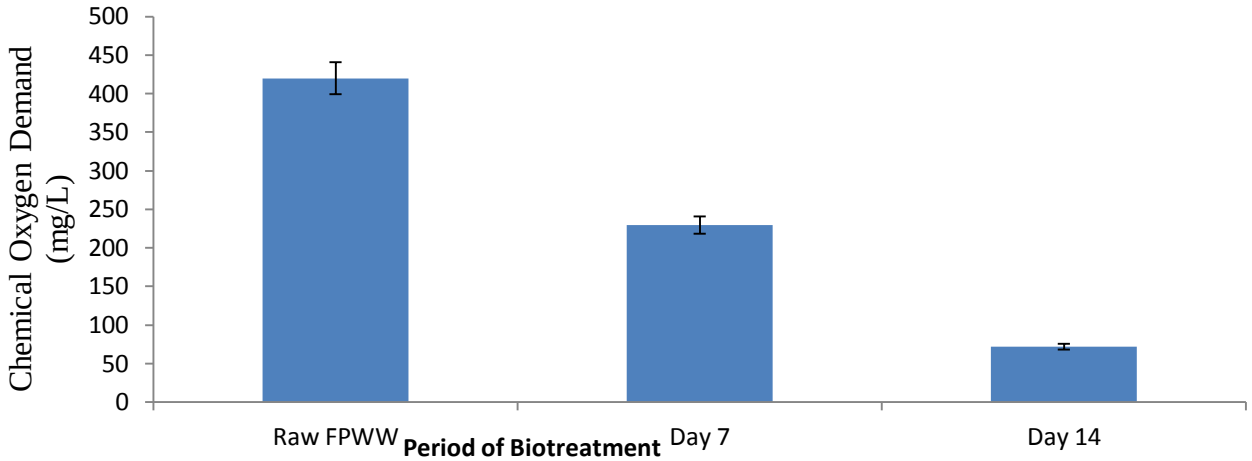

Figure 8a: Chemical Oxygen Demand of the Bio treated Raw Fish Pond Wastewater with *Coelastrum morum CiA* after 7th and 14th day of treatment

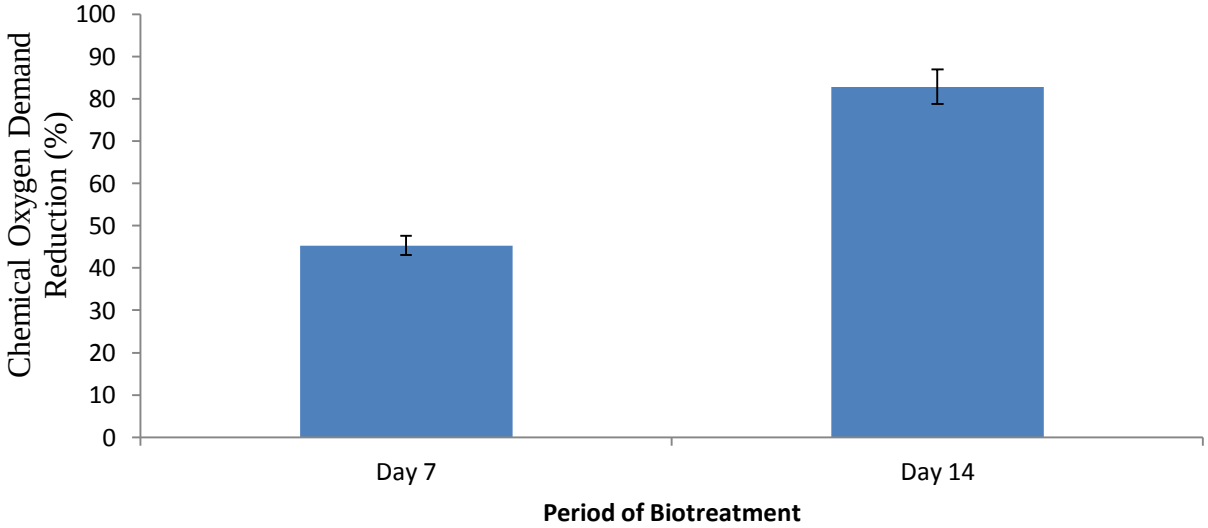

Figure 8b: Chemical Oxygen Demand removal efficiencies of *Coelastrum morum* after 7 and 14 days of treatment.

Note: Raw FPWW= Raw Fish Pond Wastewater

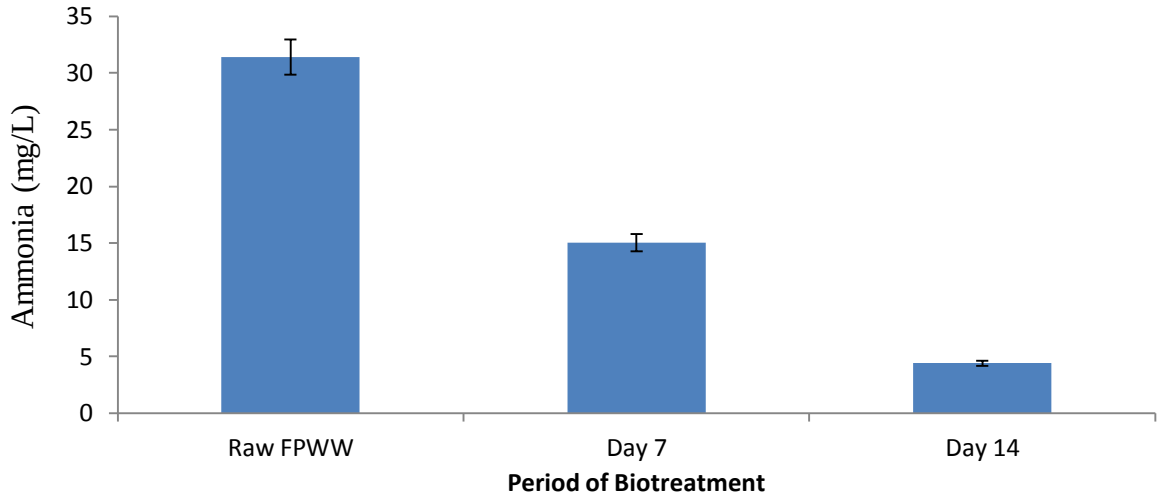

Figure 9a: Ammonia of the Bio treated Raw Fish Pond Wastewater with *Coelastrum morum CiA* after 7th and 14th day of treatment

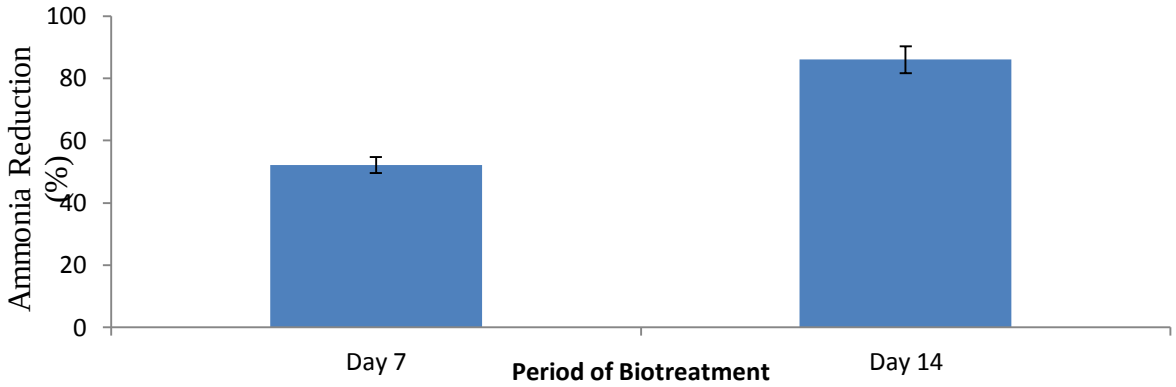

Figure 9b: Ammonia removal efficiencies of *Coelastrum morum* after 7 and 14 days of treatment.

Note: Raw FPWW= Raw Fish Pond Wastewater

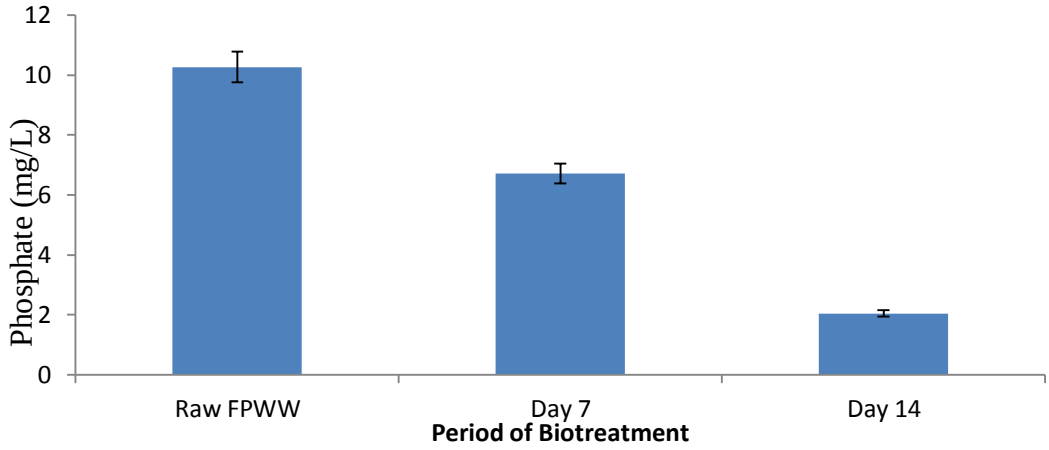

Figure 10a: Phosphate of the Bio treated Raw Fish Pond Wastewater with *Coelastrum morum* CoC after 7th and 14th day of treatment

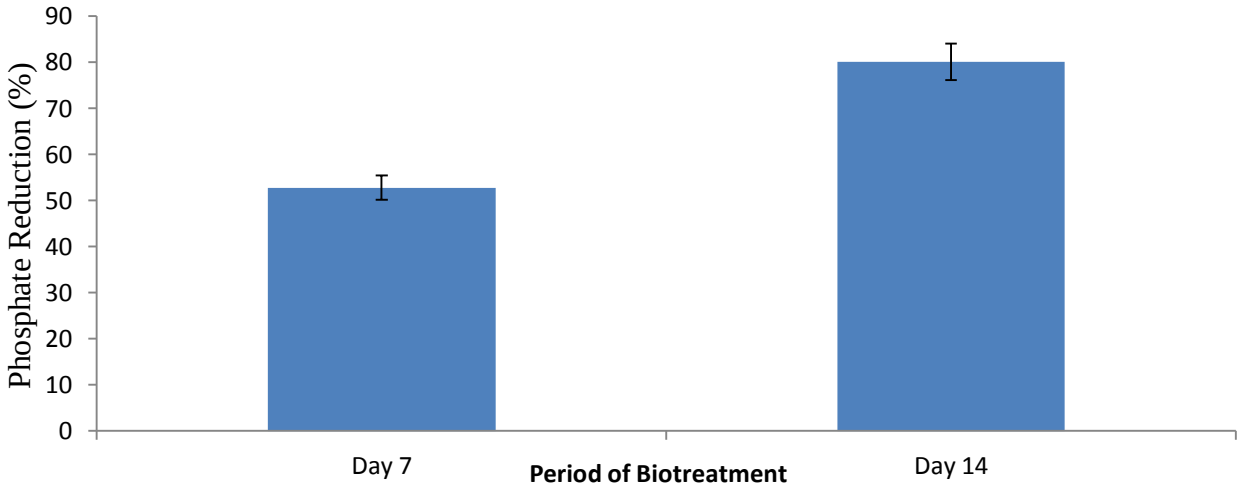

Figure 10b: Phosphate removal efficiencies of *Coelastrum morum* CoC after 7 and 14 days of treatment.
Note: Raw FPWW= Raw Fish Pond Wastewater

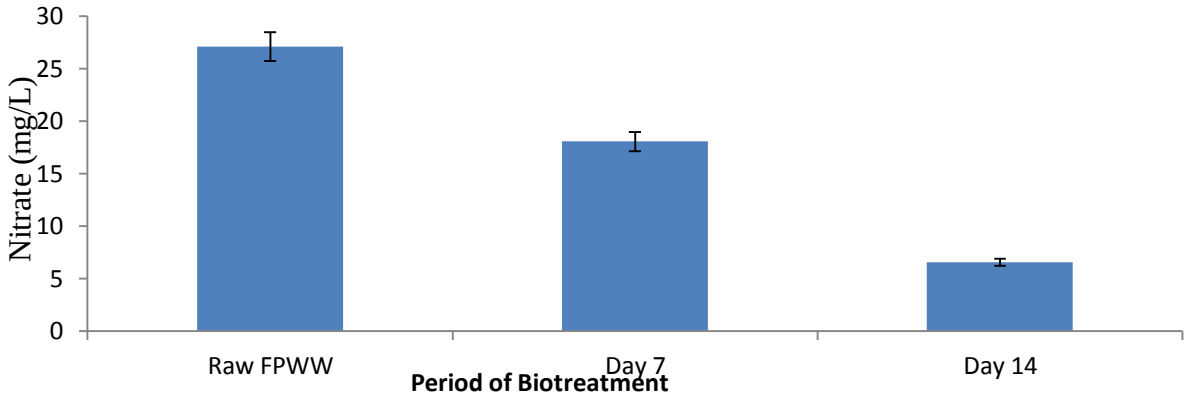

Figure 11a: Nitrate of the Bio treated Raw Fish Pond Wastewater with *Coelastrum morum* CoC after 7th and 14th day of treatment

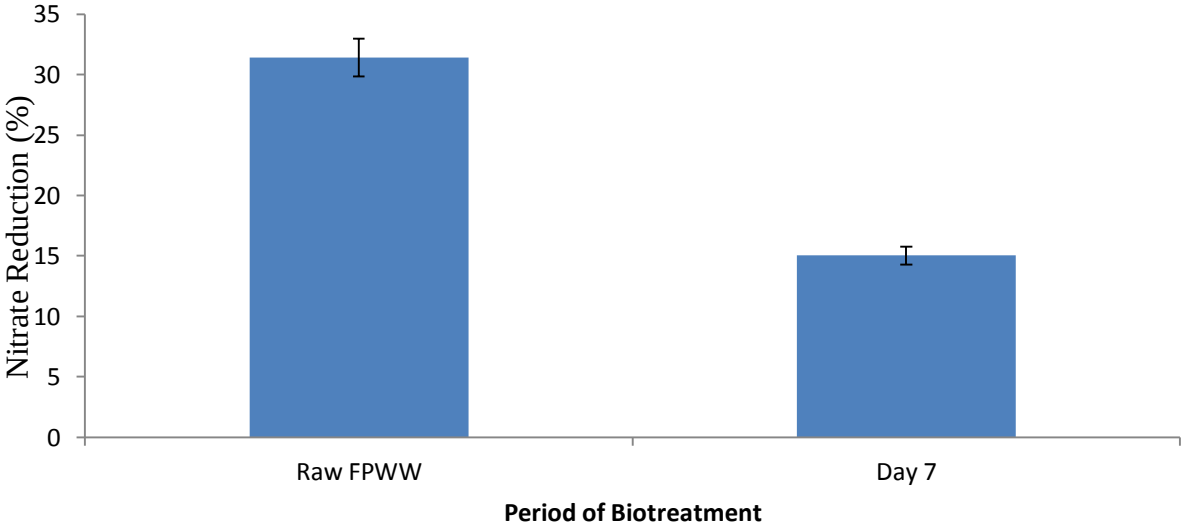

Figure 11b: Nitrate removal efficiencies of *Coelastrum morum* CoC after 7 and 14 days of treatment.

Note: Raw FPWW= Raw Fish Pond Wastewater

4. Conclusion

The results showed that *Coelastrum morum* CoC was effective in removal of wastes; nutrients from raw wastewater from fish pond and it imply that it could promote good algal growth at the same time because the nutrients will serve as source of carbon and energy. *Coelastrum morum* CoA exhibited appreciable removal capacities of nutrients (ammonium-nitrogen, nitrate-nitrogen, phosphorus), BOD, COD. Therefore it is clear that the treatment approach using *Coelastrum morum* CiA offers a low-cost, efficient and environmentally friendly technology for the treatment of fish pond waste water.

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