Fjrs.futa.edu.ng

FUTA Journal of Research in Sciences

ISSN: 2315 – 8239 (Print); E-ISSN: 2489 - 0413

FUTA Journal of Research in Sciences, Vol. 13 (1) 2017:17-24

PURIFICATION OF MUD WATER USING A GREEN FILAMENTOUS ALGAE (*CLADOPHORA* SPECIE)

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ABSTRACT

Water samples were collected from a private concrete pond in Ijiba layout, off Ada Road, Akure, Ondo State, Nigeria. Microbiological analyses were carried out on the water samples using standard methods. Mud water was subjected to purification by *Cladophora* sp. isolated from the pond water. The physiochemical analyses of the mud water before and after purification were carried out using standard methods. The algae species isolated and identified from the pond water were *Oscillatoria* sp., *Chlorella vulgaris, Spirogyra* sp., *Euglena* sp., *Cladophora* sp. *and Oedogonium* sp., *Scenesdesmus quadricauda*, *Ankistrodesmus falcatus*, *Cyclotella meneghiana*, *Cylindrocytis* sp. *Coccomyxa* sp. and *Chlamydomonas* sp. The bacteria isolated and identified from the pond water were *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Proteus* sp., *Enterobacter* sp., *Klebisiella* sp. while *Fungi isolated were Aspergillus fumigatus, A. flavus, Fusarium* sp. *and Geotrichum* sp. The biological oxygen demand (BOD) and chemical oxygen demand (COD) values of the mud water were observed before purification as 61.50 $\pm 0.12^e$ mg/l and 45.63$\pm$ 0.09^d mg/l respectively. The pH of the water was observed to be 7.62, the conductivity was 240 uS/cm, alkalinity was 332.00mg/ml, turbidity was 14.47NTU and hardness was 166.00mg/ml. After purification, the COD and BOD of the mud water was observed to reduce to 3.50 mg/l and 1.23mg/l respectively. The pH of the water increased to 8.07, while the turbidity and hardness reduced significantly to 0.72NTU and 126.0mg/l respectively. The physicochemical properties of the mud water after purification and in particular, the reduction in the values of BOD, COD and increase in pH of the mud water clearly indicates that *Cladophora* sp. was involved in the purification process.

Keywords: Algae, Mud water, Purification, Pond water, *Cladophora* sp.,

INTRODUCTION

When rain falls, surface run off waters find their way to the streets, drainage, ponds and farm land. Some of these waters are mixed with eroded soil and clay from farm land causing the water to be muddy with characteristics brownish color. Muddy water results from activities in the watershed soil runoff from nearby roads, croplands, livestock trails, overgrazed shorelands, and timber harvest areas, all these make water supply to be muddy, thereby contaminating pond and drinking water sources. Muddy and cloudy waters are not only unattractive, but can be harmful to aquatic lives (Helfrich and Newcom 2009). High suspended sediment loads in ponds can result in the following problems: low sunlight penetration; reduced plankton production; low dissolved oxygen production; increased water temperatures; suffocated fish eggs and young; reduced fish food availability; reduced visibility and fish growth; off-flavor in food fish; diminished pond water volume; and unfit for recreational activities and aesthetic benefits. Muddy water makes a pond very unattractive and most swimmers will avoid swimming in such water (William and Norland 2014).

Cladophora sp. is a green filamentous algae, belonging to the member, Ulvophyceae and is thus related to the sea lettuce (*Ulva* sp.) (Xavier *et al*., 2015). The genus *Cladophora* has one of the largest number of species within the macroscopic green algae that do not conjugate (form bridges between cells) but simply branch (Xavier *et al*., 2015). Muddy waters favor blue-green algae and bacterial growth, which can impart a bad flavor to drinking water and food fish. Algae have become significant organisms for biological purification of wastewater since they are able to accumulate plant nutrients, heavy metals, pesticides, organic and inorganic toxic substances and radioactive matters in their cells/bodies without creating secondary pollution. This makes them to be environmentally friendly unlike chemicals such as flocculants and coagulants (Hargreaves, 1999; Wu and Boyd 1990).

Algae have an important role in self-purification of organic pollution in natural waters (Kalesh and Nair 2005). Microalgae offers an interesting step for waste water treatments by providing tertiary and quaternary biotreatment because the microalgae have the ability to utilize inorganic nitrogen and phosphorous for their growth (Abdel-Raouf *et al*., 2012). Algae are also able to accumulate highly toxic substances such as selenium, zinc and arsenic in their cells and/or bodies thus eliminating such substances from aquatic environments. Filamentous algae are a common concern among pond inhabited organisms. They are mostly referred to as pond scum or incorrectly as moss (moss is a different division of plants) while several are cyanobacteria. Depending upon the species, they can resemble mats of wet wool, hair, cotton or slime that are usually green, but can become yellowish, grayish or brownish. Filamentous algae occur naturally in most surface waters (AG news and View 2011).

Moreover, many studies revealed that algae remove nutrients especially nitrogen and phosphorus, heavy metals, pesticides, organic and inorganic toxins, pathogens from surrounding water by accumulating and/or using them in their cells. A study by Mohammad and Rina (2009) showed that the growth of the green filamentous alga *Cladophora* was limited by both nitrogen and phosphorus. Hence *Cladophora* sp. metabolized the organic and inorganic substances present in the mud water for its growth. There is a symbiotic relationship among bacteria and algae in aquatic ecosystems. Algae support aerobic bacterial oxidation of organic matter by producing oxygen via photosynthesis whilst the released of carbon dioxide and nutrients in aerobic oxidation are used for growth of algal biomass (Tam *et al*., 2001). Algae use nitrogen and phosphorus as they grow thereby reducing the nutrients load of wastewater from a few hours to a few days in wasteswater during water treatment as a result of their bioaccumulation abilities (Craggs *et al*., 1996).

Microalgae have potentials to metabolize heavy metals, toxic organic compounds thereby not leading to secondary pollution when they are applied for waste water treatment rather they produce potential valuable biomass such as sludge which can be used for several purposes (Mahapatra *et al*., 2013). Algae have become significant organisms for biological purification of waste water since they are able to accumulate plant nutrients, organic toxic substances in their cells/ bodies (Kalesh and Nair 2005; Alp *et al.*, 2012). In this study, microorganisms (bacteria, fungi and algae) were isolated from a concrete pond using standard microbiological techniques and the ability of *Cladophora* species to purify mud water was monitored using physicochemical analysis.

MATERIALS AND METHOD

Sample collection

Water samples were collected aseptically from a concrete pond at 10-15 cm depth from water surface using sterile cap bottles. The water samples were transported to the laboratory in an ice packed container for physico-chemical and microbiological analyses. The mud water was collected in sterile plastic bottles from a pot-hole in Obakekere, FUTA.

Media preparation

The growth medium used for isolation of algae was Bristol medium which was prepared according to the recipe given by UTEX (Bristol medium, 2011). Nutrient Agar (NA) and MacConkey agar for bacteria isolation, Potato Dextrose Agar (PDA) for fungi isolation were prepared according to manufacturer prescriptions (Fawole and Oso 2007; Cheesbrough, 2006).

Isolation of microorganisms from the pond water

The isolation of microorganisms from the pond water was carried out as described by Cheesbrough (2006). One milliliter (1ml) of the pond water was transferred to 9ml of sterile water and diluted serially to obtain a dilution of 10^{10} factor. One milliliter (1ml) was pipetted from dilution tube (10^{-3}) into sterile petri dishes. Thereafter, 20ml of each of the media was poured separately onto each of the plates and incubated accordingly.

Identification of Algae and Bacteria

After incubation, the algae were identified using the method of Edward and David (2015). The identification of bacteria was based on morphological characteristics and biochemical tests as described by (Fawole and Oso 2007; Cheesbrough, 2006)

Identification of Fungi

The fungal colonies were sub cultured on Potato dextrose agar (PDA). The isolates were identified based on their morphological and microscopic features. Two drops of cotton-blue-in-lactophenol were placed on clean glass slide and small pieces of mycelium free of medium was removed with sterile inoculating needle and transferred on to the stain on the slide. The mycelium was teased (picked) out with the needles and covered with clean cover slip carefully avoiding air bubbles (Hunter and Bamett 2000).

Determination of total coliform using Most Probable Number (MPN)

The total coliforms were estimated by using the 5-tube most probable number method. Sterile lactose broth of single strength and double strength were used for the presumptive test and samples of 10ml, 1ml and 0.1ml were inoculated into respective dilution tubes containing inverted Durham's tube and incubated at 37^{o}C for 24hrs. At the end of the 24hrs incubation, each tube was examined for the presence of gas, if present, the tube is considered positive. The negative tube were re- incubated for a further 24hr period. After incubation, the tubes again were checked for gas production. Gas production at the end of either 24 or 48 hrs incubation is presumed to be due to the presence of coliforms in the samples. Numbers of positive tubes with acid (yellow coloration) and gas production were matched with the Mecrady's Statistical Table and the most probable number (MPN) of coliforms present in 100ml of each sample was thus determined (Cheesbrough, 2006).

Physicochemical analysis of the mud waste-water

The pH, conductivity, turbidity, COD, BOD etc of the mud before and after purification were analysed according to the method described by Ehiagbonare and Ogunrinde (2010).

Procedure for purification

The mud waste water (250ml) was collected into a conical flask and placed at room temperature. The algae of initial dosage 3g was fed to the flask. The optimum condition necessary for algae growth were applied for the purification techniques in line with method of Karin (2006).

Statistical analysis of data

Data obtained were subjected to one way analysis of variance (ANOVA) and Duncan's New Multiple Range Test at 95% confidence level using SPSS 16.0 version. Differences were considered significant at $P \leq 0.05$.

RESULTS

Several algae were obtained from the pond water as shown in (Table 1). They were identified in the microbiology laboratory FUTA. *Cladophora* sp. was chosen for the purification technique because it is dominant in the aquatic medium examined. All the five in three set tubes turned yellow with gas production after 24 and 48 hrs of incubation. This is an indication of the presence of coliform in the pond water examined. The colonial, morphological and biochemical characteristics of bacterial isolates are shown in table 2. Six bacterial species were isolated from the pond sample. They were *Pseudomonasa aeruginosa, Escherichia coli, Staphylococcus aureus, Proteus sp.,Enterobacter sp. and Klebisiella sp.* while Table 3 shows the morphological characteristics of fungi isolated from the pond water. The isolates are *Aspergillus fumigatus, A. flavus, Fusarium sp and Geotrichum sp*. The physicochemical analysis of the mud water before purification is represented in Table 4. The COD and BOD of the mud water were 61.5 and 45.63mg/l respectively. The pH of the water was observed to be 7.62, the conductivity was observed to be 240 uS/cm, alkalinity was 332.00mg/ml, turbidity was 14.47NTU and hardness was observed to be 166.00mg/ml. After purification, the COD and BOD of the mud water reduced to 3.50 and 1.23mg/l respectively. The pH of the water increased to 8.07, while the turbidity and hardness reduced significantly to 0.72NTU and 126.0mg/l respectively as shown in Table 4. The mud water was observed to be clearer after the purification technique.

Table 1: Identity of algae isolated from the pond water

Algae	Morphological characteristics
Oscillatoria sp.	It is blue greenish in color, usually free floating, cylindrical or sometimes slightly tapering, unbranched filaments in aquatic environments.
Spirogyra sp.	It is common green algae which feel like wet, soapy hair, bright green in color often found free floating in static water near the surface or in masses in the sediment
Euglena sp.	They are green and sometimes red. They occasionally form green or red powdery films on the surface of ponds or dams. The surface color can change from red to green in few hours. Euglena is free swimming in ponds and lakes and is also found in mud rich in organic matter.
Chlorella sp.	A small grass-green plant which usually stores starch
Cladophora sp.	They can form dense mats in static water or long, rope-like strands in flowing water. Its filaments consists of series of cells being joined end to end giving a thread-like appearance
Oedogonium sp.	A free floating green filament usually occurs attached at the lower end by means of a basal cell, the rhizoidal cell expanded into a flattened disc with outgrowths.

Table 2: Colonial, morphological and biochemical characteristics of bacteria isolated from the pond water

Tests	Isolate 01	Isolate 02	Isolate 03	Isolate 04	Isolate 05	Isolate 06
Colony Shape	Abundant, thin, white, growth with medium turning green	Moist, glistening growth	Opaque, golden growth	Blue/grey, spreading growth	Smooth round colonies	Large Mucoid colonies
Cell shape	Rod	Rod	Cocci	Rod	Rod	Rod
Gram's rxn	-	-	+	-	-	-
Urease	-	-	-	+	-	-
Citrate	-	-	-	-	+	+
Motility	-	+	-	+	+	-
Catalase	+	+	+	+	+	+
Coagulase	-	_	+	-	-	-
Oxidase	+	_	-	-	-	-
Indole	-	+	-	-	-	-
Probable microbes	*Pseudomonas aeruginosa*	*Eschericha coli*	*Staphylococus aureus*	*Proteus* sp.	*Enterobacter* sp.	*Klebisiella* sp.

Table 3: Morphological characteristics of fungal isolates found in the pond water

Fungal isolates	Morphological characteristics
Aspergillus fumigatus	Conidia heads are long, globose to prolate Conidiophores hyaline vesicle ovate
Fusarium sp	Hyphae are septate and hyaline.phailades are cylindrical, conidiophores are medium length
Geotrichum sp	Hyphae are hyaline, septate, branched and breakup into chains of hyaline, smooth, one-celled, subglobose to cylindrical arthroconidia
Aspergillus flavus	Colonies are olive to lime green with a cream reverse.hyphae are septate and hyaline. Conidiophores coarsely roughened and uncolored. Vesicles are globose, metulate are covering the entire vesicle in biserate species.

Table 4: Physicochemical properties of the mud water before and after Purification technique

Parameters	Means Before Purification	Means After Purification
pH	7.62±0.12[b]	8.07±0.01[d]
Conductivity (uS/cm)	2.40±1.2[i]	4.17±1.16[i]
Alkalinty	3.32±0.12[j]	1.92±0.06[h]
Turbidity (NTU)	14.47±0.09[c]	0.72±0.01[ab]
COD	61.5±0.12[e]	3.50±0.06[c]
TSS (Mg/l)	0.18±0.01[a]	0.07±0.00[a]
TDS (Mg/l)	0.23±0.01[a]	0.15±0.01[a]
TS (Mg/l)	0.43±0.00[a]	0.24±0.01[a]
BOD (Mg/l)	45.63±0.09[d]	1.23±0.01[ab]
DO (Mg/l)	1.10±0.06[a]	2.04±0.03[b]
Hardness	1.66±1.16[g]	1.26±1.16[g]
Nitrate	0.04±0.01[a]	0.07±0.01[a]
Phosphate	1.64±0.01[f]	13.45±0.22[e]
Sulphate (Mg/l)	2.32±0.01[h]	23.20±0.01[f]

DISCUSSION

This study revealed the presence of several algal species in the pond water. The species isolated were *Oscillatoria* sp, *Spirogyra*, *Euglena* sp, *Chlorella* sp, *Cladophora*, and *Oedogonium* sp. These are referred to as pond scum and they form greenish mats upon the water surface. This agreed with the report of Miriam and Nicole (2013), who reported on field guide to algae and other scums in the ponds. The presence of coliform in the pond water is an indication of fecal contamination. This also signifies the presence of pathogenic microorganisms in the water (Nester *et al.*, 2007).

The microbial analysis revealed the presence of *Pseudomonasa aeruginosa, Escherichia coli, Staphylococcus aureus, Proteus sp., Enterobacter sp., Klebisiella sp.* in the pond water. It was observed that gram negative bacteria constitute the most genera bacteria isolated from the pond water.This may be as a result of high contamination from human interference. This agreed with the work of Panneerselvam and Arumugam (2012) who also isolated and identified gram negative bacteria from the lake water. The identities of bacteria obtained in this study conform with the identities of bacteria (*Pseudomonas aeruginosa*, *Enterobacter sp*, *Escherichia coli*, *Proteus sp.)* reported by Saha *et al* (2012) who worked on bacteriological and physicochemical properties of pond water. Fungi species isolated from the pond water were *Aspergillus fumigatus, Fusarium* spp, *Geotrichum* sp and *Aspergillus flavus*.

The physicochemical analysis of the mud water revealed that the pH of the mud increaed from 7.62 to 8.07 after purification. This findings agreed with Krishnan and Neera (2013) who also carried out studies on waste water treatment by algae. Previous studies had shown that low pH is toxic to fish and other aquatic lives (Baker and Schofield 1982). The value recorded for the turbidity was higher than the WHO standard of 5NTU (WHO, 2004). But reduced significantly after purification with the filamentous algae.

Dissolved oxygen (DO) is an important factor used for water quality control. Dissolved oxygen concentration in unpolluted water normally range between 8 and 10mg/l, concentration below 5mg/L adversely affect aquatic life (Rao, 2005). The dissolved oxygen concentration of the mud water was below the 5mg/L, but after purification, the value was observed to increase. The decrease in DO might be due to the fact that the water was cloudy which prevented light penetration required for photosynthesis (Brian, 2017). The increase in DO might be due to metabolic activity of the algae (Olukunle and Oyewumi 2017). This notetable observation in the dissolved oxygen is in agreement with the findings of Sharma and Khan (2013). who reported increase in DO during bioremediation of sewage using selective algae.

The biological oxygen demand (BOD) and chemical oxygen demand (COD) tests are a measure of relative oxygen depletion effect of a waste contamination. Both were adopted has a measure of pollution effect. The BOD test measures the oxygen demand of biodegradable pollutants whereas the COD test measures the oxygen demand of oxidizable pollutants. The COD is a determinant of the level of organic matter and carbon of the waste water. A significant reduction was observed in the COD and BOD after purification with the *Cladophora* spp. This reduction agreed with the findings of Krishnan and Neera (2013), who reported decrease in these parameters during a waste water treatment by algae.

CONCLUSION AND RECOMENDATION

The natural purification of mud water with filmentous green algae offer an opportunity for reducing the environmental impact of various pollutants and they are found to be environmentally friendly. The filamentous green algae used, (*Cladophora* sp.) was found to be efficient in the mud water purification but took longer time to be achieved. Therefore, futher study could be carried out on the dosage of algae that would be introduced to the purification tank which could lead to increased rate of purification.

ACKNOWLEDGEMENTS

We appreciate the Federal University of Technology, Akure for the bench space provided for the research work. We also appreciate Miss Bukola Alade and the Technical crew of the Department of Microbiology for their support and assistance during the study.

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