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Research Paper

Phycoremediation of Synthetic Dyes in an Aqueous Solution using an Indigenous *Oscillatoria* sp., from Ethiopia

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Abstract

Synthetic dyes usages are inevitable, but they are recalcitrant and toxic, hence need treatment before discharge. Lately, phycoremediation of dyes gained more attention due to its value-added benefits, however, it need more investigation of indigenous species for ease. Therefore, the aim of the study was to isolate indigenous microalgae from Elias Tesfaye textile factory runoff, Addis Ababa. The sample was inoculated to Bold's Basal Medium and incubated for 15 days and the dominant colonies were purified and identified using a microscope. The identified isolate, *Oscillatoria* sp., which belongs to prokaryotic filamentous cyanobacteria was checked for the dye removal efficiency and biomass production using synthetic dyes of malachite green (MG), methylene blue (MB) and Safranin (SF). The axenic cultures of Os*cillatoria* sp., was inoculated (0.2 g/L) with various concentrations of dyes (1-5 mg/L) and incubated under Sunlight for 5 days and analyzed the residual color absorbance and biomass production. A maximum of 93% dye color was removed in MG, followed by MB (66%) and SF (52%). Interestingly, the biomass was increased 3.75 times in MB dye (0.75 g/L), while 3.1 and 1.9 times in MG (0.63 g/L) and SF (0.38 g/L), respectively. It seems that the *Oscillatoria* sp., was decolorizing the dyes by utilizing as nutrients and shown unique potentials on different dyes. Yet, more studies are needed for the exact mechanism of removal and large scale application. This Ethiopian native species of microalgae can be used effectively for the phycoremediation of synthetic dyes locally.

1. Introduction

Synthetic dyes are visible chemical, water-soluble, complex organic compounds, xenobiotic, recalcitrant and are more stable to light, heat, oxidizing agents and difficult to remove from contaminated water (Brahmbhatt and Jasrai, 2016). Nevertheless, the uses of synthetic dyes are inevitable and significantly increase in the various industries (textiles, tannery, paper, printing and dyeing, food and cosmetics) for as coloring agents.

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In fact, over 10,000 different synthetic dyes are used and approximately 0.7 million tons of dyes are synthesized every year worldwide (Andrade and Andrade, 2018) in which 40% (0.28 million) is discharged in effluents annually (Jin et al., 2007). This huge amount of dye pollutants in the effluent causes serious environmental problems. For instance, the dye can affect the photosynthetic activity of aquatic organisms by reducing the sunlight penetration and its chemicals (metals, chloride) can accumulate in the ecosystem and leads to human systems and causes various diseases from mild (allergic, vomiting, nausea, etc.) to serious diseases (cancer, malfunctions of organs, etc.) (Crini and Badot, 2008). Therefore, it is important to safeguard the ecosystem from such contaminants. In order to overcome these problems, many physical and chemical methods are applied such as adsorption, precipitation, photolysis, oxidation and reduction, electrochemical treatment, filtration, coagulation and flocculation (Alaguprathana and Poonkothai, 2017). However, these physicochemical processes are costly, not able to remove completely, release toxic metabolites and generate secondary sludge which may cause secondary pollution (Wang et al., 2019; Zhou et al., 2019). The use of biological systems (bacteria, yeast, fungi, microalgae, and plant) for the removal of synthetic dyes is an attractive alternative to the bioremediation for the effluent containing dyes (Solís et al., 2012). Biological discoloration can occur via absorption, enzymatic degradation or a combination of both (Wu et al., 2012) and has advantages over physiochemical methods mainly in terms of cost, environmental benignity, easy and safe operation–green chemistry concept (Andrade and Andrade, 2018). Among biological systems, microalgae have raised eminence, due to its photoautotrophic nutrition and their potential to remove pollutants, CO_2 mitigation and produce high value-added chemicals, concurrently (Suresh and Benor, 2019; Suresh et al., 2019). Therefore, many researchers isolated and recommend that the microalgae biomass may hold the key to solve dye contamination in aqueous solutions worldwide (Vikrant et al., 2018; Alaguprathana and Poonkothai, 2017; Solís et al., 2012). The environmental problem caused by hazardous dyes is particularly important in Ethiopia because of the high utilization of synthetic dyes in industries particularly leather and textile, where periodic releases of liquid effluent to the water bodies occur without proper treatment (Zinabu et al., 2018). Therefore, need a potential, preferably biosystems to solve the dye pollutants and petite attention has been given to the indigenous microalgae in Ethiopia and its dye decolorization. Given the great diversity of microalgae (two orders of magnitude more than currently known species of 35,000), it is suggested that Ethiopian native microalgae species (naturally modified) are potential candidates for local application with ease (Abate Ayele et al., 2019). Therefore, the objective of this study was to isolate microalgae from local textile factory runoff and its potential in removing synthetic dyes of Malachite Green (MG), Methylene Blue (MB) and Safranin (SF) from aqueous solutions and biomass production. The present study is a part of ongoing efforts to screen efficient native microalgae strains for their phycoremediation of industrial pollutants in Ethiopia.

2. Materials and Methods

2.1. Microalgae sample collection and identification

The microalgae sample (~100 mL volume) was collected from Elias Tesfaye Textile Factory Runoff (ETTFR), Addis Ababa, and small amount (around 1 mL sample) was inoculated into 100 mL sterile Bold's Basal medium (BBM) and incubated in the lab under Sunlight at 25°C for 15 days with light and dark cycle. The culture flasks were manually shaken twice a day for 5-10 seconds. The microalgae were purified using spread plate assay and then dominant colonies were picked and inoculated into 100 mL BBM and incubated the same as above. The axenic culture was stored in a BBM slant for later use. A purified alga was identified morphologically using a light microscope (Labomed, USA) by a wet slide mount method at 450x magnification. The photomicrographs were taken with a camera via the ocular lens and followed the Van Vuuren et al. (2006) manual to identify the microalgae genera.

2.2. Microalgae dye removal efficiency and biomass production

The dominant filamentous microalgae of *Oscillatoria* sp., (ETTFR) was tested for the dye removal efficiency. The synthetic dyes of MG, MB and SF were prepared (1 g/L stock) in deionized water and

used at five different concentrations (1, 2, 3, 4 and 5 mg/L). Ten days old axenic *Oscillatoria* sp., (ETTFR) culture was taken and rinsed with sterile deionized water and small amounts equal to 2 mg (dry weight) of culture was introduced into the test tubes (10-12 mm diameter) which contains 10 mL of sterile BBM with different concentration of dye and the control was without dye. Inoculated tubes were incubated as mentioned previously. Decolonization of dyes was monitored by taking one mL of sample, centrifuged at 3000 rpm for 5 min and the supernatant used for taking the absorbance (600 nm) in the UV-spectrophotometer (Biochrom, Libra S80PC, UK) at different day intervals (0, 3 and 5 days). The amount of dye removal efficiency (RE) was calculated based on Eq. 1 and the RE was compared with previous reports.

$$RE\ (\%) = \frac{A_i - A_f}{A_i} \times 100 \qquad (1)$$

Where A_i and A_f are initial and final absorbance of the dyes at 600 nm.

Remaining, microalgal biomass was collected after 5 days of growth and filtered using pre-weighed Whatman No.1 filter paper. Then the filter paper was rinsed with distilled water (5 mL) and dried at 60°C in a hot air oven for overnight then weighed for algal biomass production.

3. Results and Discussion

3.1. Microalgae isolation and identification

In this study, a dominated and fast-growing native species of *Oscillatoria* (ETTFR) was isolated from Elias Tesfaye textile factory runoff, Addis Ababa (Figure 1, control). The isolated prokaryotic cyanobacterial microalgae of *Oscillatoria* sp., (ETTFR) was dark green and shown long filamentous consisting of a single row of cells and un-branched filaments with a very thin gelatinous sheath. The filaments were similar in shape except the apical cell which is convex at the tip. This ETTFR *Oscillatoria sp.*, was isolated from the dye industry wastewater, therefore it has been naturally adapted to the particular environment. Based on this circumstance, this train could utilize or reduce the synthetic dyes effect and produce more biomass. Previous studies were also shown on the *Oscillatoria* sp., which dominated the industrial effluents of other countries (El-Sheekh et al., 2009) and preferred for the dye remediation (Priya et al., 2011; Saha et al., 2010). Besides, Wilkie et al., (2011) envisaged that the microalgae are the solution for the world problems (pollution, hunger, fuel, disease) and said many studies have been done using commercial strains rather than the native species towards the above problems. Indigenous species are having more advantages over custom strains and genetically modified organisms.

3.2. Synthetic dyes removal and biomass production

Textile wastewater adapted microalgae of *Oscilltoria* sp., (ETTFR) was checked for the removal of synthetic dyes of MG, MB, and SF. The clear evidence of dye removal ability by live *Oscilltoria* sp., (ETTFR) isolate was shown in Figures 1 and 2.

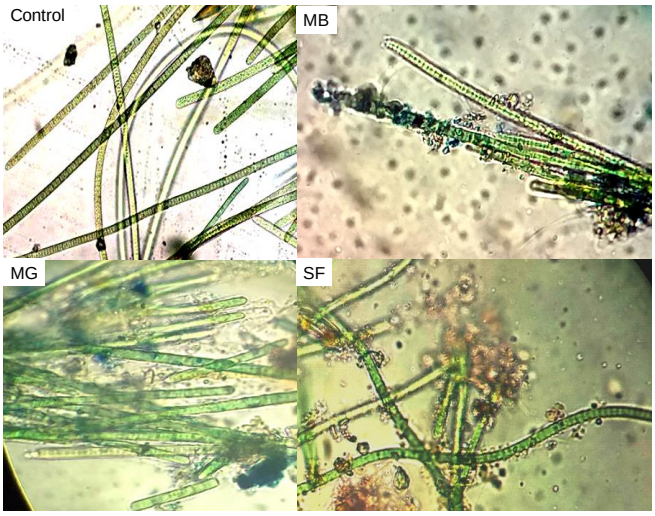

Figure 1: Light microscopic image of *Oscillatoria* sp., (ETTFR) with and without synthetic dye treatment (5 mg/L). Control; no dye, MG; Malachite Green, MB; Methylene Blue and SF; Safranin. The picture was taken on the 5th day of incubation at 450x magnification.

The dye colors were reduced and almost completely removed on the 5th day of incubation in MG and MB dyes, whereas, in SF, the residual color was visible with the naked eye (Figure 2). However, when analyzed using a spectrophotometer, a maximum of 93% of MG color was removed, followed by MB (66%) and SF (52%) on the 5th day of incubation, respectively (Figure 3). It seems that the dyes were first adsorbed and disintegrated as seen on the 5th day of culture (Figure 1). Interestingly, the biomass production was reached at the maximum of 0.75 g/L from 0.2 g/L (inoculums size) in MB dye added, while 0.63 g/L in MG and 0.38 g/L in

SF, respectively. The maximum biomass was produced up to 3.75 times in MB dye and the least was 1.9 times in SF dye within 5 days. Similarly, Boduroğlu et al. (2014) also found that the microalgae of *Gonium* sp., biomass was almost doubled in the Reactive Blue 220 dye added (25 mg/L) medium in 14 days of incubation with 87.7% removal rate. Both studies showed that the isolated microalgae were degrading the dyes and used as nutrients because the growth was increased as dye color diminished. At the same time, it might be due to adsorption or bioaccumulation fashion and not the degradation phenomenon. However, Figure 1 fairly proved that the disintegration of dyes around the cell wall of the microalgae and the dye color was not dense, whereas it was diluted. It would be an intense color in the case of adsorption or bioaccumulation phenomenon. Moreover, the pellets of the culture did not show any dye color after centrifugation that indicated that the *Oscilltoria* sp., (ETTFR) mineralized the dyes added. Previous studies also supported that the most of the dyes were assimilated as nutrients by degrading using enzymes (Dellamatrice et al., 2017; Solís et al., 2012; Priya et al., 2011; Saha et al., 2010). Indeed, the same genus (*Oscillatoria* sp.,) from the marine source shown 84% removal of the diazo dye, and Acid Black dye in 8 days (Priya et al., 2011) and 52% decolorization of poly R-478 dyes in 7 days of incubation in a medium free of nitrogen (Saha et al., 2010). The dye degrading ability was attributed to the activities of the enzymes such as laccase, polyphenol oxidase, and azoreductase and proved that these dyes were used as a nitrogen source in an oligotrophic environment (Priya et al., 2011; Saha et al., 2010; El-Sheekh et al., 2009). Further studies are needed to explore the exact mechanisms of remediation of dye pollutants by *Oscillatoria* sp., (ETTFR) for efficient use on large scale. Essentially, synthetic dyes are the most common colorant in many industries (textile, paper, leather) and azo dyes contribute to 60–70% of the total dyes produced (Moawad et al., 2003). Dyes can be absorbed or adsorbed or transformed or degraded by a variety of microorganisms for the survival (as nutrients, avoid its toxic effect, increase light utilization) (Vikrant et al., 2018; Solís et al., 2012). Among them, microalgae are preferable as it belongs to oxygen-evolving photoautotrophs and therefore, oxygenate the dye effluent and help to reduce the secondary pollution (Saha et al., 2010).

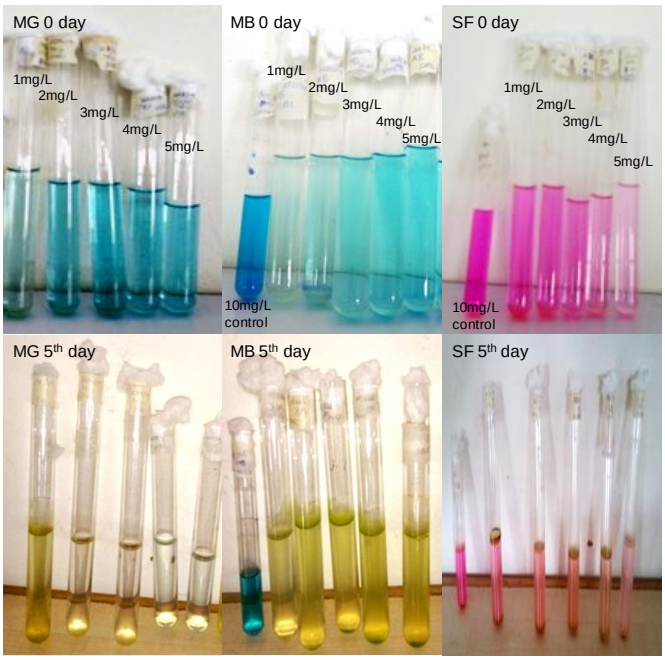

Figure 2: Decolorization synthetic dyes of Malachite Green (MG), Methylene Blue (MB) and Safranin (SF) by filamentous microalgae of *Oscillatoria* sp., in five days of incubation

The genus of *Oscillatoria* is a non-heterocystous filamentous cyanobacterium and cannot fix atmospheric nitrogen, therefore, it needs an external nitrogen source for their growth. Therefore, it uses an external source of organic compounds such as synthetic dyes, which initially adsorb to the cell wall of the microalgae and finally degrades and consume the released inorganic nutrients. Besides, the efficiency of decolorizing is highly influenced by the structure of the dye and the species of algae (Omar, 2008). Both living and non-living algae can be used in the phytoremediation of dyes from wastewater with the availability of the biomass (Lim et al., 2010; Sivarajasekar et al., 2009). It was well proved that the dead microalgae can remove dyes in a short period of time (<1 h) with an ion-exchange or columbic interaction between the algae surface and the dye properties. In case of live algae, for instance, in this study, *Oscillatoria* sp., (ETTFR) took 5 days to remove at the maximum of 93% in MG dye at 5 mg/L concentration, while Priya et al., (2011) found 8 days to remove 84% in 100 mg/L concentration of azo dye and El-Sheekh et al. (2009) observed 81% removal of

Methyl red (20 mg/L) in 5 days. This clearly indicats that more time is needed for the high concentration of dye pollutants using live biomass, but it is opposite to dead biomass that required less time irrespective of dye concentrations (Solís et al., 2012). It is good to mention that less concentration is preferred in live biomass than dead biomass due to the toxicity of dyes otherwise diluted effluent is favored. In support of this phenomenon, Boduroğlu et al. (2014) found that the higher removal rate at lower concentrations of synthetic dye using microalgae of *Gonium* sp., and the biomass was affected negatively by the increasing pollutant concentrations, as the decreases in removal rate. On the other hand, more biomass was preferred for more concentrated dye effluent to achieve more removal rates (Table 1, Boduroğlu et al., 2014). In the case of microalga biosorbents, the pH of the environment played an important role than the dye concentration. For instance, low pH was preferred for the dead biomass that improves its adsorption between dye and biosorbents greatly (Srinivasan and Viraraghavan, 2010), whereas in live biomass, high pH was preferred which supports the growth of microalgae eventually mineralization of dyes occur and used as nutrients (Boduroğlu et al., 2014).

Different microalgae species have different potential of decolonization efficiency of synthetic dyes as observed from previous studies (Table 1). Attractively,

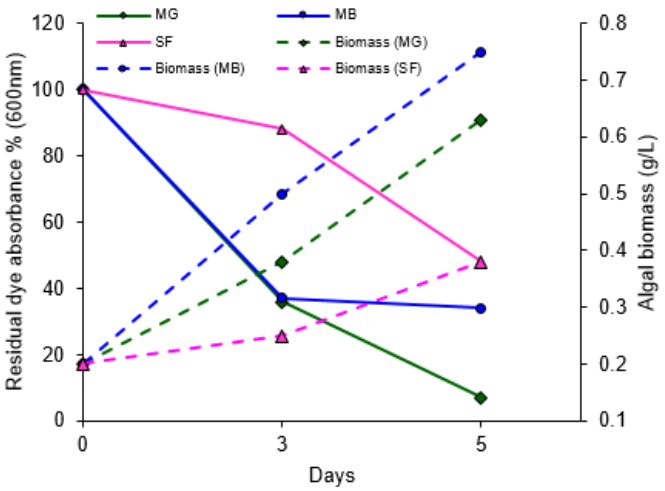

Figure 3: Residual dye absorbance percentage and the biomass production of *Oscillatoria* sp., in Malachite Green (MG), Methylene Blue (MB) and Safranin dye added aqueous solution for five days of incubation with 5mg/L dye concentration.

Table 1: Comparison of synthetic dyes removal potential using live microalgae

Synthetic dyes	Microalgae (live)	Algae Inoculated	Dye concentration	Incubation	Removal %	Reference
Malachite green	*Oscillatoria* sp.,	0.2 g/L	5 mg/L	5 days	93%	Present study
Methylene blue	*Oscillatoria* sp.,	0.2 g/L	5 mg/L	5 days	66%	Present study
Safranin	*Oscillatoria* sp.,	0.2 g/L	5 mg/L	5 days	52%	Present study
Malachite green	*Chlamydomonas* sp.,	0.75 g/L	10 mg/L	4 h	99%	Al-Fawwaz and Jacon 2011
Methylene blue	*Chlorella* sp.,	0.75 g/L	10 mg/L	4 h	99%	Al-Fawwaz and Jacon 2011
Malachite green	*Cosmarium* sp.,	4.5 x 10^6 cell/mL	10 mg/L	7 h	85%	Daneshvar et al., 2007
Malachite green	*Haematococcus* sp.,	1 g/L	100 mg/L	no data	67%	Liu et al., 2018
Methyl red	*Oscillatoria* sp.,	1.3 x 10^5 cell/L	20 mg/L	5 days	81%	El-Sheekh et al., 2009
Orange II	*Oscillatoria* sp.,	1.3 x 10^5 cell/L	20 mg/L	5 days	34%	El-Sheekh et al., 2009
G-red	*Oscillatoria* sp.,	1.3 x 10^5 cell/L	20 mg/L	5 days	10%	El-Sheekh et al., 2009
Acid Black I	*Oscillatoria* sp.,	0.5 g/L	100 mg/L	8 days	84%	Priya et al., 2011
Poly R-478	*Oscillatoria sp.*,	500 µL/4.5 mL	75 mg/L	7 days	52%	Saha et al., 2010
Reactive blue 220	*Gonium sp.*,	0.21 g/L	25 mg/L	14 days	88%	Boduroğlu et al., 2014
Reactive blue 220	*Gonium sp.*,	0.53 g/L	25 mg/L	14 days	97%	Boduroğlu et al., 2014
Black color	Microalgae mixture	10% inoculum	no data	5 days	40%	Tadele Assefa Aragaw and Abraham Asmare 2018
Aniline blue	*Chlorella* sp.,	0.5 g/L	25 mg/L	11 days	58%	Arteagaa et al., 2018

the non-filamentous microalgae of *Chlamydomonas* and *Chlorella* sp., have shown a more efficient in removing dyes (Al-Fawwaz and Jacon, 2011) than filamentous microalgae (El-Sheekh et al., 2009). It might be due to the surface area and the growth rate of the algae which are more and high in non-filamentous microalgae. When using the live biomass for the phycoremedation of dyes, it is important to mention that incubation time is also a critical factor that varies according to the species. I addition to that, Wilkie et al., (2011) suggested that indigenous species are potential candidates for bioprospecting because native species have an advantage over type culture and genetically engineered organisms. Isolating native microalgae species with desirable properties gives a robust biological platform for phycoprospecting. Native strains come equipped with millions of years of adaptation to the local biotic and abiotic stress (naturally engineered species). Therefore, the locally adapted potential microalgae isolates are preferred for the large scale application in bioremediation of dyes. Here, presented the *Oscillatoria* sp., from ETTFR which is a potential microalga for phycoremedation of dyes in geographic conditions of Ethiopia.

4. Conclusion

The discharge of synthetic dyes into the environment has become a matter of grave concern. In this study, a dominant, filamentous, prokaryotic cyanobacterium of *Oscillatoria* sp., (ETTFR) was isolated from Elias Tesfaye textile factory runoff, Addis Ababa and has shown considerable decolorization of synthetic dyes in live biomass. The maximum of 93% decolorization potential of the native *Oscillatoria* sp., was observed in synthetic dyes of malachite green, followed by methylene blue (66%) and safranin (52%) within 5 days of incubation at 5 mg dye/L concentration. The microalgae biomass production was also reached a significant amount at the maximum of 3.75 times more in methylene blue dye (0.75 g/L), followed by 3.1 times in malachite green (0.63 g/L) and the least was 1.9 times in safranin dye (0.38 g/L) added medium. The *Oscillatoria sp.*, shown typical decolorization efficiency for different dyes and it appeared that this native microalga utilized dye as a nutrient. Much work is still needed in order to understand the exact mechanism of degradation and the enzyme systems involved and large scale studies. This Ethiopian native microalga of *Oscillatoria* sp., (ETTFR) is potential candidates for phycoremediation of synthetic dyes at the local application with ease.

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