Benha University
Faculty of Science
Botany and Microbiology Department

Studies on The Efficiency of Some Micro-Algae in Wastewater Treatment

A Thesis

Submitted as a partial fulfillment of requirements for the degree of Doctorate philosophy in Microbiology.

By

SARA EID ADLY ALI

Assistant Researcher at Microbiology Department
Soil, Water and Environment Research Institute (SWERI)
Agricultural Research Center

Supervised by

Prof. Dr.
Mohamed Gomaa Battah
Professor of Phycology
Botany Department, Faculty of Science - Benha University.

Prof. Dr.
Fekry M. A. Ghazal
Professor of Microbiology
Soils, Water and Environ. Res. Inst.
Agriculture Research Center.

Prof. Dr.
Azza Ahmed M. Abd El-Aal
Professor of Microbiology
Soils, Water and Environ. Res. Inst.
Agriculture Research Center.

Prof. Dr.
Hamed Mohamed El-Adel
Professor of Phycology
Botany Department, Faculty of Science
Benha University.

2019

ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Studies on the Efficiency of Cyanobacteria on Textile Wastewater Treatment

Fekry M Ghazal[1]*, Mohamed G Battah[2], Azza A Abd EL-Aal[1], Hamed M Eladel[2], and Sara E Adly[1].

[1]Agric. Micrbiol. Res. Dept., Soils, Water & Environ. Res. Inst., Agric. Res. Center, Giza, Egypt.
[2]Botany Dept., Fac. Sci., Benha Univ., Qalubia, Egypt.

ABSTRACT

Releasing of textile dye effluents into general water bodies is a major environmental and health problem. Color removal, in particular, has recently become of major scientific interest, as indicated by the multitude of related research reports. During the past two decades, several physico-chemical decolorization techniques have been reported, few, however, have been accepted by the textile industries. Their lack of implementation has been largely due to high cost, low efficiency and inapplicability to a wide variety of dyes. The ability of microorganisms to carry out dye decolorization has received much attention. Cyanobacteria are considered as an important source for decolorizing dye and textile effluent. In the current study four local cyanobacteria strains ***viz***. *Anabaena fertilissima, Nostoc muscorum, Phormidium fragile and Wollea* sp., were used in the preliminary screening for their ability to grow on and the removal of the red color and the heavy metals, i.e., iron, manganese, boron, lead and arsenic from the crude effluent discharged by EL-Shafie textile factory at EL- Mahalla EL- Kobra East Delta, Gharbia Governorate, Egypt. Results revealed that all tested cyanobacteria strains were able to gradually remove the color of the crude textile effluent in parallel with increasing the incubation periods. Also, *N. muscorum* recorded the highest percentage of color removal percentage followed by *Wollea* sp., *Ph. fragile* and finally *A. fertilissima* after 28 days of incubation. All the tested cyanobacteria strains were able to remove any of Fe, Mn, B, Pb, and As in different degrees. *N. muscorum* was superior in removing all discharged heavy metals compared to the other tested cyanobacteria strains. On the other respect, all tested cyanobacteria strains had slightly raised pH of the discharged textile crude effluent, while they reduced any of EC, COD and BOD compared to the initial values of the discharged textile crude effluent. However, the research of cyanobacteria to remove color in dye wastewaters is still in the early stage, but is a promising alternative of biological treatment.

Keywords: *Anabaena fertilissima, Nostoc muscorum, Phormidium fragile and Wollea* sp. - textile wastewater-physico-chemical properties**,** heavy metals and declorization.

Corresponding author

CONTENTS

SUBJECT	PAGE
List of tables	iii
List of figures	v
List of abbreviations	vii
Introduction	1
Aim of the work	4
Literature review	5
Materials and methods	33
Microalgae Used	33
Textile wastewater effluent (CTWE)	33
Media used	34
Fertilizers Used	36
Propagation and maintenance of cyanobacteria strains used in laboratory experiments	37
Purification of cyanobacteria isolates	38
Identification of cyanobacteria	40
DNA Extraction	40
Bioremediation of textile wastewater effluent (CTWE) by using microalgae strains	48
Greenhouse work	51
Soil analyses	53
Plant analyses	55
Results	59
Laboratory work	59
Characteristics of crude textile wastewater effluent	59
Identification of the cyanobacteria isolates from the crude textile waste effluent	61
Bioremediation of textile wastewater effluent (CTWE) by using microalgae strains	66

Removal of Heavy metals	66
Decolorization	70
Effect of the textile wastewater effluent on microalgae growth	73
Effect of microalgae treatment on some physic-chemical characters of the crude textile wastewater effluent	75
• Effect on pH & EC	75
• Effect on COD & BOD	76
Identification of cyanobacterial strains used in greenhouse work	81
A- Morphological Features of *Nostoc* sp.	81
B-Molecular and Phylogenetic Analysis	83
Greenhouse work	88
The properties of irrigation waters applied for faba bean plants cultivation	88
Physical and chemical properties of the greenhouse soil	89
Effect of microalgae treated crude wastewater effluent on faba bean plants after 60 days from sowing	89
Effect of the treated textile wastewater effluent on the soil available N, P and K	95
Effect of the treated textile wastewater effluent on the rhizosphere soil biological activity of faba bean plants	96
Discussion	98
Summary	115
Conclusion	118
References	119
Arabic Summary	1

List of Tables

TABLE NO.	TITLE	PAGE NO.
1	Oligonucleotide primers sequences - Source: Metabion (Germany).	41
2	Preparation of PCR Master Mix according to Emerald Amp GT PCR mastermix (Takara) Code No. RR310A kit.	44
3	Cycling conditions of the different primers during cPCR.	45
4	Preparation of master mix using Big dye Terminator V3.1 cycle sequencing kit.	47
5	Initial chemical properties of the textile wastewater.	59
6	Initial physical properties of the textile wastewater effluent.	60
7	Initial heavy metals content of the textile wastewater effluent.	60
8	Removal of heavy metals from the textile wastewater effluent by using different microalgae strains after 4 weeks of incubation.	67
9	Decolorization of dye color of the textile factory effluent discharged from EL-Shafie Garments Factory measured by absorbance (Nano meter ʎ 600) as affected by the presence of different microalgae strains at different incubation periods.	70
10	Dry weight of microalgae strains as affected by the textile wastewater different incubation periods.	74
11	pH and EC of the textile wastewater effluent as affected by the presence of different microalgae strains after 4 weeks incubation period.	76

12	Removal of COD and BOD values from the textile wastewater effluent by using different microalgae strains after 4 weeks incubation period.	78
13	Data of divergence and percentage identity of nucleotide sequence variations between 23 different cyanobacterial strains and the screened Strain_A and Strain_B, based on 16S rDNA gene.	87
14	Physicochemical analysis for irrigation waters applied for faba bean plants cultivation (a, b).	88
15	Some physical and chemical properties of the experimental soil	89
16	Effect of microalgae treated wastewater and untreated wastewater on growth Characters of faba bean plants after 60 days from sowing.	90
17	Effect of treated wastewater effluent on N, P and K contents of faba bean plants after 60 days.	91
18	Effect of treated wastewater effluent on total carbohydrate, crude protein and pigments content of faba bean plants after 60 days.	95
19	Effect of treated wastewater effluent on soil available N, P and K after 60 days.	96
20	Effect of treated wastewater effluent on the biological activity of the soil rhizosphere of faba bean plants after 60 days.	97

List of Figures

FIGURE NO.	TITLE	PAGE NO.
1& 2	Removal of heavy metals from the textile wastewater effluent by using cyanobacteria strains.	68
3	Removal of heavy metals from the textile wastewater effluent by using isolated cyanobacteria strains.	69
4	Removal of heavy metals from the textile wastewater effluent by using green algae strains.	69
5	Color removal from the textile wastewater effluent by different cyanobacteria strains at different incubation periods.	71
6	Color removal from the textile wastewater effluent by different isolated cyanobacteria strains at different incubation periods.	71
7	Color removal from the textile wastewater effluent by different green algae strains at different incubation periods.	72
8	Color removal from the textile wastewater effluent by different microalgae strains after 4 weeks	72
9	Dry weight of microalgae strains grown in the textile wastewater effluent.	74
10	Dry weight of microalgae strains grown in the textile wastewater effluent as compared with control media after 4 weeks.	75
11	Reduction percentages of COD and BOD of the textile wastewater effluent as affected by cyanobacteria strains after 4 weeks incubation.	79

12	Effect of different isolated cyanobacteria strains on both COD and BOD of the textile wastewater effluent after 4 weeks incubation.	79
13	Reduction percentages of COD and BOD of the textile wastewater effluent as affected by green algae strains after 4 weeks incubation.	80
14	Photomicrograph of *Nostoc* sp. (strain A) showing vegetative cells of curved filaments and several intercalary heterocysts	82
15	Photomicrograph of *Nostoc* sp. (strain B) showing the vegetative cells of straight filaments and small number of intercalary heterocysts	82
16	Photomicrograph showing the agarose gel electrophoresis of the DNA fragments obtained from PCR. The band expected size is between 100 bp to 1500 bp. Arrowhead shows the expected size, 1485 bp.	83
17	Phylogenetic tree showing the relationships among the screened Strain_A and Strain_B, and the most similar sequences retrieved from NCBI nucleotide database, based on the 16S rDNA gene (neighbor joining method, Kimura 2-parameter nucleotide substitution model, 1000 rounds of bootstrap resampling).	85