Micro-algal based Bioremediation of Ciprofloxacin effluents

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SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the B. Tech. thesis entitled **" Micro-algal based Bioremediation of Ciprofloxacin effluents"** , submitted by Harshita Mahajan (161809) and Kritika Sharma (161842) at Jaypee University of Information Technology, Waknaghat, India, is a bonafide record of his original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

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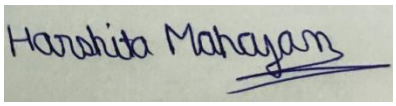
DECLARATION

We hereby declare that the work reported in the B. Tech. thesis entitled **"Micro-algal based Bioremediation of Ciprofloxacin effluents"** submitted at Jaypee University of Information Technology, Waknaghat, India, is an authentic record of our work carried out under the supervision of Dr. Garlapati Vijay

Kumar, Dept. of Biotechnology and Bioinformatics, JUIT, Waknaghat, HP-173234, India. We have not submitted this work elsewhere for any other degree or diploma.



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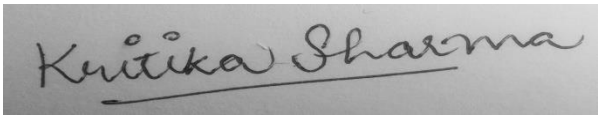

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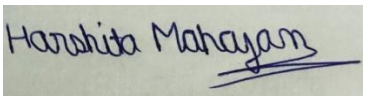

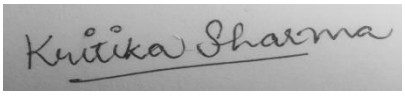

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LIST OF SYMBOLS AND ACRONYMS

°C	Degree Celsius
µg/ml	Microgram per milliliter
mg/ml	Milligram per milliliter
nm	Nano meter
rpm	Revolutions per minute
µl	Micro-liter
BG-11	Blue Green Algae Media
OD	Optical Density
DCW	Dry Cell Weight
µ	Specific Growth rate
n_f	Final biomass
n_i	Initial biomass
t2	Finial time
t1	Initial time
Td	Doubling time

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Abstract

In today world microalgae have a lot of application .They is being used for waste water treatment since the increment in overall sullying with various pharmaceutical contaminants has become a rising overall worry because of their impressive poison levels and related medical problems. With the help of microalgae mediated bioremediation has provide benefit in waste water treatment. Nowadays people are using microalgae mediated bioremediation since they are being better used than conventional methods of treatment for removal of chemicals, heavy metals and pharmaceutical contaminant. Ciprofloxacin is an antibiotic which is being used for the treatment of bacterial infections. Various synthetic detergents, heavy metals, iron are being discharged into the water which is harmful for various animals, plants as well as humans. Earlier different methods were being used for waste water treatment like advance oxidative process. However incomplete mineralization of ciprofloxacin using advance oxidative process has surprisingly delivered increasingly diligent and harmful change. High support cost of advance oxidative procedure likewise confines their use in huge scope applications. These conventional methods are very expensive and are not being used for removal of ciprofloxacin from waste water. Nowadays people are using microalgae for bioremediation. Bioremediation by microalgae is the most vital method being used for the removal of ciprofloxacin from waste water. Various microalgae are being used like *Anabaena ambigua* and other microalgae species. Various factors influence the growth of microalgae like Ph, light intensity temperature and growth conditions. The microalga used is *Anabaena ambigua* for the removal of ciprofloxacin from waste water. The culture *Anabaena ambigua* is first inoculated and grown medium and kept shaker where all the conditions required for the growth are being provided. Then the culture is grown for few days and then we effect of ciprofloxacin on the microalgae and further growth curve and dry cell weight will be calculated to check the results. Microalgae based bioremediation for the removal of ciprofloxacin from waste water using *Anabaena ambigua* is the main idea of this research.

Keywords: Bioremediation, microalgae, *Anabaena ambigua*, pharmaceutical effluents, industrial waste water treatment

CHAPTER 1

INTRODUCTION

These days with fast increment in human populace has prompted mechanical advancement of nations. With increment in mechanical development different manufactured synthetic concoctions overwhelming metals, particles drugs, anti-microbial; pesticides are being released into the water. These contaminants discharged into the water which have different destructive and poisonous impact on the wellbeing of human [1]. This modern wastewater must be treated so as to forestall ecological contamination. In the vast majority of the creating nations numerous individuals are legitimately or in a roundabout way subject to water. The arrival of modern city waste and waste from pharmaceutical enterprises has prompted different natural difficulties for water bodies. The wastewater is wealthy in different unsafe contaminants like nitrogen (N), phosphorous (P), sulfur (S), as nitrate, smelling salts and sulfate prompting eutrophication [2]. The different contaminations in water lead to algal blossom, oxygen consumption additionally prompting the demise of different creatures and human [3]. These days individuals are utilizing anti-microbial for the treatment of different contamination however the broad utilization of anti-microbial for anticipation of sickness , rewarding a disease have prompted visit recognition of anti-infection agents and their corruption item in the earth [4] . The anti-infection agents which are being discharged in nature expand the opposition of microscopic organisms and bargain to the general wellbeing. Anti-toxins are generally being utilized for the treatment of different contaminations yet the opposition of the microscopic organisms to the anti-infection has caused issue in different people [5].

Thisharmful emerging contaminant can be accumulated through food web, food chain which can induces effect on high tropic level organism such as human beings plants and animal[6]. Various forms of antibiotic are being observed in drinking water, ground waterand waste water. Earlier various conventional methods were being used for wastewater treatment. These methods were designed to remove organic matter and nutrients [7]. The conventional methods have certain drawbacks. The method being used for treatment of waste water is bioremediation by microalgae. Microalgae offer an elective favorable position towards

treatment approach in organic treatment so they can expel supplements and convert them into biomass [8]. Microalgae were formerly being utilized concentrated in treatment of different wastewaters , including palm oil plant gushing, elastic plant wastewater ,sago starch wastewater, material wastewater and waste water containing overwhelming metals .The property of microalgae empowers them to flourish in even extraordinary conditions and has demonstrated to have a significant preferred position in treatment of wastewater [9]. They are broad investigations accessible about the microalgae developmentin waste water. Microalgae are being used for the co- metabolic removal of antibiotics and their toxicity .The microalgae used was *Anabaenaambigua* for the removal of ciprofloxacin and theretoxological effect of ciprofloxacin on *Anabaenaambigua* . The topologicaleffect ciprofloxacin on *Anabaena ambigua* was studied by determing its growth rate and further through cell count [10]

CHAPTER 2

REVIEW OF LITERATURE

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2.1 Problems related to waste water

Clean water is a fundamental source and factor for the protected occupations of individuals. Different engineered synthetics including cleansers, synthetics, anti-infection agents, pesticides have been ceaselessly released into ground water or surface water through horticultural exercises, mechanical squanders [11]. As we probably am aware a considerable lot of the more normally utilized medication bunches like anti-toxins and other comparable pesticides and other miniaturized scale poisons are not required to experience a similar degree of testing for conceivable natural impact. Albeit a large number of these mixes have been recognized in wide assortment of ecological examples including sewage effluents, surface water, groundwater and drinking water so there fixations by and large range from low ppt to ppb levels. The broad utilization of anti-infection agents for malady counteraction, treatment of microbial contaminations, and advancement of creature and plant development has prompted visit environment[12] Due to the fast increment in development of total populace has made a relating request in the expansion of Earths constrained gracefully of freshwater. A few examines in the previous 30 years have indicated pharmaceutical and individual consideration items as developing natural small scale contaminants in light of their broad use in veterinary and human drugs and their expanding event in sea-going condition. A portion of these mixes arrive at squander water treatment plants they are not totally evacuated and these lingering groupings of these synthetic substances are much of the time released in the rewarded effluents. There is an exploration that low convergences of toxins keep on releasing in the earth and can debase surface water, seawater and groundwater and have some negative natural effects [13]. Here and there different medications enter nature at low concentrations primarily as metabolites which are excreted by humans and animals or in effluents and are disposed into wastewater by from various hospital, pharmacies and chemical manufacturing facilities.

It has been seen it is due to the non availability of analytical instruments which can identify low concentrations of those contaminants. Different antibiotics from pharmaceutical industries are released into the waste water. To remove various antibiotics various methods are being used like physical, chemical biological processes and combination. But these methods have not given significant results for the treatment of waste water [14].

2.2 Fungi-based bioremediation

Customary organic treatment produces huge measure of low bacterial biomass. A wide range of types of biomass with higher caliber could essentially change the financial matters of wastewater treatment. Different kinds of high worth biochemical which are produce by business development of parasites under aseptic conditions. Organism was being utilized for bioremediation of waste water treatment. Be that as it may, these days bioremediation by microalgae have demonstrated more prominent potential for future improvement because of condition similarity and cost adequacy [15]. The biomass delivered during parasitic wastewater treatment has conceivably a lot higher methodology than microorganisms actuated ooze. Growth acts in wastewater treatment plant. For bioremediation of wastewater treatment anaerobic microorganisms are utilized. Growth utilized for bioremediation of wastewater because of its reaping productivity. Organism is being utilized for expulsion of pharmaceutical contaminants from squander water [16]. Despite the fact that it is by and large helpful yet these days the use of growths has become a specialized issue. From a candidate perspective a parasitic cell treatment gives some disadvantage since the mycelium could be presented to natural burdens. The immobilization of fungus could allow the use of system repeatedly with different advantages [17]. In the environment as we know fungi are competing with bacteria for nutrients and carbon source .Fungi can grow at lower levels of moisture. Many fungi have adapted to fast grown on wood and other plant debris which is useful in wastewater treatment .Sometimes fungi grow in attached mode which is used for both substrate and support for growth. Earlier fungus was used for bioremediation of waste water treatment but due to its various drawbacks people use microalgae for bioremediation of waste water treatment since they are easy to cultivate and the cost is very less as compared to bioremediation by fungus.

Though fungus has its advantages but it also has some advantages due to which nowadays microalgae is being preferred over fungi. Bioremediation of waste water treatment we use microalgae because of its growing advantages [18].

2.3 Why microalgae is used for wastewater treatment as compared to fungi

As we know fungi are not photosynthetic as compared to microalgae which are photosynthetic. Microalgae are easy to grow and cultivate. They do not require any host whereas for fungi they are difficult to grow and various food resources should be provided to them for their growth [19]. So the cost of producing fungi is relatively more as compared to fungi. Nowadays people use microalgae for bioremediation of waste water treatment as compared to fungi. Microalgae are used due to the capacity for effective photosynthetic uptake of high concentrations of minerals and organics by microalgae [20] .Microalgae is been used for removal of nutrients at low operating costs which is an effective way for bioremediation of wastewater treatment .Microalgae are eukaryotic and prokaryotic microorganism due to which they can be grown easily .Fungi are not photosynthetic as compared to microalgae which are photosynthetic. Microalgae are easy to grow and cultivate. They do not require any host whereas for fungi they are difficult to grow and various food resources should be provided to them for their growth. Due to the growing demand for bioremediation of wastewater treatment researchers are using microalgae due to their properties and they are easy to grow and cultivate. Microalgae based technology has been used has potential advantage for the treatment of wastewaters containing antibiotics The various inorganic nutrients and harmful pharmaceutical from various industries are being exposed to the water which is harmful for humans, plants and animals. Since we have studied earlier we use fungi for wastewaters treatment but due to its various drawbacks we use microalgae for bioremediation of wastewater [21].

2.4 Microalgae –*Anabaena ambigua*

Anabaena ambigua are blue green microalgae class of filamentous cyanobacteria exists as tiny fish. They have harmonious relationship with certain plant, for example, mosquito greenery. Out of four genera of cyanobacteria that produce neurotoxins which is unsafe to neighborhood untamed life [19]. Creation of neurotoxins is gathering to be a contribution to its advantageous relationship shielding plant from brushing pressure.

They have nitrogen fixing capacities. *Anabaena ambigua* has different highlights [22]. The way of life utilized for the development of microalgae is BG-11 medium. *Anabaena ambigua* settlements develop in filamentous bunches of multi cell chain. The cells are round and hollow or barrel formed. The end cells are frequently any longer than mid chains .They are gram negative species , photosynthetic cyanobacteria and are basic to new condition all

through the world[23]. They are heterocyst framing photoautotrophic cyanobacteria and perform oxygenic photosynthesis. They develop in long fibers of vegetative cells. *Anabaena ambigua* replicates by vegetative and biogenetic strategies. The sexual propagation in these species is totally missing [24]. The vegetative strategies by which *Anabaena* recreated are discontinuity. They have a place with the realm Anabaena and are genuine microorganisms. The cyanobacterium *Anabaena* is prokaryotes and is unicellular and are undifferentiated. The way of life is when moved to a medium without smelling salts, 10% of the cells started to heterocyst. *Anabaena ambigua* are anaerobic living being and they live under anaerobic conditions [25]. This specie is equipped for horizontal and bowing developments. These societies are green since they are cyanobacteria and can do photosynthesis, fix nitrogen from the earth and furthermore produce hydrogen .They have shade chlorophyll a just as blue green color phycocyanin. They are developed in free living society and hold their capacity to contaminate their host plant. They have no obvious core. *Anabaena ambigua* societies are developed in lab. *Anabaena ambigua* requires different conditions for development like pH, temperature and light [26]. The medium utilized for the development of *Anabaena ambigua* is Chu 10 medium and BG11 medium .The best development of *Anabaena ambigua* is in BG11 medium where all the supplements accommodated the development of microalgae are being given and it is utilized for the most part for the development of *Anabaena ambigua*. *Anabaena ambigua* species are utilized for nitrogen obsession and structure harmonious relationship with specific plants .and greeneries. They are blue green growth with dab like or Barrel like cells and have huge pores known as heterocyst [27]. They are found as tiny fish in shallow water and on sodden soil. They are both singular and provincial structures they look like firmly related variety *Nostoc* .They structure water sprouts.

They are filamentous phototrophic cyanobacteria. The phones are generally circular and they are at some point barrel shaped and structure a fiber called as trichome. The trichome of *Anabaena* is typically observed by two particular cell types. The first is little, roundabout, photoautotrophic vegetative cell and performs oxygenic photosynthesis and is ordinarily blue green in shading. The second is heterocyst which is bigger, paler progressively homogenous cell produce by *Anabaena* to fix environmental nitrogen. Here and there during the hours of low ecological nitrogen around one cell out of each ten will separate into heterocyst [28]. They are photoautotrophic cyanobacteria. Heterocyst has flexibly neighboring cells with air nitrogen as an end-result of the result of photosynthesis which they no longer can perform.

Anabaena ambigua they are used in Solid State NMR and they are also used because of their nitrogen fixing abilities. They are also used for oxygen free hydrogen production. They are used for bioremediation of wastewater .They have the ability to degrade oil components and other substances .like surfactants herbicides. They are also used as biofertilzers [29] .They are also used as healthy foods. Nowadays *Anabaena ambigua* are mostly used for bioremediation of waste water treatment. Algae is an important component of arid and semi arid ecosystem. *Anabaena ambigua* a blue green algae is now widely being used because of its distinct features. *Anabaena ambigua* has different applications but the nowadays microalgae is mostly used for bioremediation of wastewater [30]

2.5 Ciprofloxacin antibiotic its effect in waste water treatment

Ciprofloxacin is a third era of fluoroquinolones is one of the most generally utilized wide range anti-toxins in people and veterinary meds. Ciprofloxacin is utilized for the treatment of bacterial contaminations. It neutralizes gram negative and gram positive microscopic organisms [31]. It restrains the bacterial DNA by loosening up and copying. It represses the movement of DNA gyrase .Ciprofloxacin is one of the anti-toxin originating from veterinary use which contaminates the water. As ciprofloxacin can go through all the treatments to venture with ciprofloxacin content in wastewater. Ciprofloxacin is one of the anti-infection found in effluents in a few pieces of the world because of its strength in the amphibian condition and its serious use in medical clinic network .As we ciprofloxacin content is uncovered in water

Because of emergency clinic effluents and different pharmaceutical businesses [32].Ciprofloxacin is a second era fluoroquinolones anti-toxin and it is one of the most recommended tranquilize on the planet. Furthermore, it has been found in wastewater at MIC level which could incorporate bacterial obstruction. It is generally been utilized against disease with gram negative microbes .Ciprofloxacin antimicrobial properties are caused because of hindrance of DNA gyrase and topoisomerase 4 which is fundamental for loosening up of overly wound DNA during replication and interpretation in prokaryotes Ineffective evacuation of CIP by ordinary wastewater treatment advances has caused its ceaseless release in the ground water and waste water. The overall convergence of CIP in freshwater biological system has been accounted for to 0.164 mg/l and its most extreme recognized fixations were 6.5mg/l [33]. It environmental impacts are being expanded worldwide and it has been found to hurt a wide scope of microorganisms, for example, green

growth, microscopic organisms and spineless creatures even at low focuses. Different propelled oxidation process has been examined to expel ciprofloxacin from surface water and wastewater [34]. Ciprofloxacin being in its half life for 3-5 hours and is for the most part disposed of through renal discharge thus entering the sea-going framework.

There was a huge stage ingested in the decrease of most pharmaceutical medications from squander water treatment plant .Ciprofloxacin has been recorded between fixations 0.7 and 124.5 smaller scale gram/liter. It has been seen that both assimilation and photograph corruption firmly impact ciprofloxacin destiny in sea-going frameworks despite the fact that apparently the prevailing instrument rely on the natural particulate carbon level .Since we have examined the impact of ciprofloxacin on wastewater treatment and its impact on different plant creature and human. Ciprofloxacin is utilized for advancing development in animal cultivating Many natural examinations have demonstrated that ciprofloxacin from European sewage plant to be as high as 186.2 tones .Ciprofloxacin has been created in sea-going condition from different clinic effluents, pharmaceutical ventures and veterinary use Ciprofloxacin present in drinking water sources is of significant worry because of obscure wellbeing impacts of ceaseless low level presentation to anti-infection agents over lifetime . So these days it has been a developing worry for expulsion of anti-toxin from wastewater. Numerous strategies like coagulation sedimentation, biodegradation, chlorination and adsorption was utilized . In the event that ciprofloxacin is available in high focuses in drinking water cause genuine unfavorable impacts .Various strategies have been utilized for expulsion however they were not compelling because of its impediments.

Ciprofloxacin removal from wastewater is important .Many new process like bioremediation of wastewater by microalgae is being used [31] .Ciprofloxacin is used for treatment of various human infection and it is considered an important worldwide .As we know ciprofloxacin is a fluoroquinolones antibiotic and due to its mechanism it is an effective antibiotic .The waste from various hospitals , pharmaceutical industries is being discharged into water and it affect various plants and animal so it is important to treat wastewater .Microalgae used for removal of ciprofloxacin is an effective method as it has shown various effective results. The topological effect of ciprofloxacin is studied using growth curve and dry cell weight. Earlier methods like pressure driven process have been used which include reverse osmosis, nanofiltration, ultra filtration and microfiltration but these methods were not effective and did not give promising results. So bioremediation has risen as a potential innovation to treat effluents. Diverse microorganism is being utilized for the evacuation of

ciprofloxacin .However microalgae are more compelling and give a promising outcome than different organisms. Microalgae have demonstrated compelling outcomes for the expulsion of anti-infection agents from wastewater. Be that as it may, strategies which were utilized require significant expense, high operational and high upkeep cost and because of these techniques they are confined for long haul application. Once in a while deficient mineralization during cutting edge oxidation procedure can create change items that display harmfulness similar to or more noteworthy degree than parent compound. Ciprofloxacin additionally influence the microbial networks in wastewater, stream water and marine water. Now and then the presentation of microbes in nature may add to spreading anti-microbial protection from pathogen [34].

2.6Bioremediation approaches for ciprofloxacin effluents

For the evacuation of ciprofloxacin a few procedures have been decimation, ozonation, illumination, Fenton oxidation and UV/H_2O_2.Most of these procedures are exorbitant, vitality, serious and low productive [35]. Bioremediation has risen as a potential innovation to treat effluents. Diverse microorganism is being utilized for the evacuation of ciprofloxacin. Anyway microalgae are more compelling and give a promising outcome than different microorganisms [36].

2.6.1 Microalgae Bioremediation for removal of ciprofloxacin

Microalgae can be developed and can be utilized for the expulsion of pharmaceutical squanders from squander water. Microalgae bioremediation is the most imperative utilized strategy for the evacuation of ciprofloxacin. Bioremediation of polluted wastewater by mixotrophic green growth is as of late pulling in microbial networks [37]. The miniaturized scale algal organic bioremediation framework is sun based force driven, natural far reaching and reasonable recovery technique. Mixotrophic microalgae are the key markers for checking the water quality and natural harmfulness. Algal intervened evacuation of pharmaceutical by microalgae has given promising outcomes. Mixotrophic microalgae are ideal for expulsion of pharmaceutical waste from squander water because of their capacity to take up supplements and convert them into biomass. Microalgae are of critical significance as they are fit for playing double capacity for bioremediation of wastewater the miniaturized scale algal organic bioremediation framework is sun based force driven, natural exhaustive and supportable recovery technique. Mixotrophic microalgae are the key markers for checking the water quality and natural poisonousness.

Algal intervened expulsion of pharmaceutical by microalgae has given promising outcomes. Mixotrophic microalgae are ideal for expulsion of pharmaceutical waste from squander water because of their capacity to take up supplements and convert them into biomass. Microalgae are vital as they are skilled for playing double capacity for bioremediation of wastewater [38]. Microalgae are the essential maker in amphibian food networks and are the key marker life form for getting to the water quality and ecotoxicity of poisons. Microalgae by their photosynthetic abilities use accessible supplements present in wastewater. Mixotropic microalgae are local species in freshwater principally making due on nitrogen and Phosphorous rich mixes and can adequately take-up natural contamination as carbon hotspot for their development. The expulsion of biochemical oxygen request in the wastewater treatment framework suspended soils, supplements, colliform microbes, and poisonousness are the principle objective for getting purged squander water [40]. Microalgae these days are developing step by step and their expanding interest for the expulsion of anti-toxins from squander water is expanding.

There are procedures of wastewater purging utilizing microalgae that can expel supplements from wastewater than customary ordinary techniques. Ciprofloxacin content in nature ranges from ng/L to mg/L. Ciprofloxacin portion in human 45% - 62% is discharged unmetabolized by means of pee and 15-20% through dung [41]. Ciprofloxacin arrive at nature through sewage release from sewage treatment plants filtering from landfills and discharge from pharmaceutical industry. The majority of the past examinations concentrated on the ecotoxological impact of ciprofloxacin on microalgae species without its expulsion. This examination includes for the expulsion of ciprofloxacin at expulsion of ciprofloxacin from microalgae. Bioremediation by microalgae species for the evacuation of ciprofloxacin is being examined. In this examination the ecotoxiological impact of ciprofloxacin on *Anabaena ambigua* and the expulsion energy of ciprofloxacin by *Anabaena ambigua* were assessed [42]. A broad examination was done in regards to upgrade of *Anabaena ambigua* intervened ciprofloxacin evacuation.The topological effect of ciprofloxacin was studied using growth rate and dry cell weight. *Anabaena ambigua* used for the removal of ciprofloxacin is the most vital method used and it is the most effective method being used. Microalgae species are being used for removal of antibiotics from waste water treatment [43].

2.6.2*Anabaenaambigua* for the removal of Ciprofloxacin

Anabaena is a blue green microalgae having a place with the family cyanobacteria .As we have considered that the nearness of anti-microbial on a superficial level have been broadly announced and is become a developing worry because of its topological impact on amphibian species just as the opposition they can remember for some bacterial resist low focuses [44]. As we probably am aware different mixes arrive at the earth through outcomes of various exercises like veterinary medication or farming yet because of their utilization in human medication and the wastefulness of wastewater evacuation of these sorts of contaminants which are not biodegraded [45].Antimicrobial property of compound has prompted different conversation lately because of developing anti-microbial opposition and further increment in the contamination by anti-toxin safe microorganisms. Furthermore, in wastewater treatment it is critical to evacuate anti-infection agents. *Anabaena ambigua* have high nitrogen and phosphorous substance [46]. They are additionally discovered proficient in the expulsion of different contaminants, for example, substantial metals inorganic and natural contaminants from different wastewaters. They can replicate either by heterotrophically or by chemoheterotrophically since we realize bioremediation is viewed as a viable strategy and naturally safe technique innovation [47]. Bioremediation is characterized as the capacity of certain bio atoms or sort of biomass to tie and thought chosen particles. As we probably am aware bioremediation by microorganism is helpful because of activity of microorganisms on toxins in any event, when they are available in exceptionally weaken arrangements and they can be adjusted to extraordinary conditions. *Anabaena ambigua* is utilized for the expulsion of ciprofloxacin from squander water treatment [48] .As we have talked about before microalgae because of its different properties is viewed as significant for the evacuation of anti-toxins. *Anabaena ambigua* are gram negative microorganisms which are fit for performing oxygenic photosynthesis.

It assumes a significant job in development lakes, and in local, facultative or vigorous lakes, and treats little and center scale city wastewater, in view of their amazing capacities to evacuate supplements substantial metals and pathogens [49]. Different microbial procedures like adsorption precipitation intracellular collection decrease and entanglement paralysis a urgent job in determine the destiny and dispersion of ciprofloxacin in the sea-going condition .Cyanobacteria are photoautotrophic prokaryotic microorganisms which represents a capacity to expel pharmaceuticals from wastewater .Sometimes mixotrophic development of microalgae gives higher biomass just as lipid productivities than development under

photoautotrophic conditions .Researches have demonstrated that the phototoxic impact of MIC in different lowered and skimming plants rely upon focus time. The overall Eutrophication of water bodies which is because of exorbitant supplements and toxins which are originating from human exercises increment the convergence of cyan bacterial blossom. As we probably aware *Anabaena ambigua* and other cyanobacteria have higher resistance to anti-toxins than other algal species and high evacuation proficiency for anti-microbial. Green growth has magnificent expulsion capacities with respect to the contaminants [50].

Microalgae are as of now being utilized for evacuation of anti-microbial and have given promising outcomes in the present research. From utilizing initiated muck which was utilized before now microalgae is utilized. Mixotrophic microalgae can switch their digestion among autotrophic and heterotrophic relying upon the accessibility of carbon source and supplements in the general condition which help to make due in extraordinary conditions. Microalgae are utilized and it has more noteworthy favorable circumstances. *Anabaena ambigua* a blue green microalgae we will use for the evacuation of ciprofloxacin [51]. This examination will concentrate on the expulsion of ciprofloxacin from squander water treatment by utilizing microalgae *Anabaena ambigua.*

Objectives of the Research:

The objectives of the research are as follows

- Cultivation and growth kinetics study of *Anabaena ambigua* in BG 11 medium
- Check the toxicity \ tolerance limits of microalgae to different concentrations' of ciprofloxacin
- Characterization and Bioremediation studies of Ciprofloxacin effluents

CHAPTER 3

MATERIALS AND METHODS

==========

3.1 Materials Required

Anabaena ambigua was produced from NCIM, Pune (National Collection of Industrial Microorganism). *Anabaena ambigua* strains were grown in BG-11medium [52].

3.1.1 Composition of BG-11 medium:

The medium was prepared for 500 ml by adding all macro element and the microelement in necessary concentration required for the formation of BG-11 medium. Then 100 ml medium was poured in five Erlenmeyer flaks

Table 3.1 BG-11 Media Composition

Macro elements Nutrients	Concentrations(g\l)
$NaNO_3$	1.5
$K_2HPO_4.3H_2O$	0.045
$MgSO_4.7H_2O$	0.036
$CaCl_2.2H_2O$	0.006
Citric Acid	0.001
EDTA	0.02
Ferric Amonium Citrate	0.006
Microelement Nutrients	
H_3BO_3	2.860
$MnCl_2.4H_2O$	1.810
$ZnSO_4.7H_2O$	0.222
$Na_2MoO_4.2H_2O$	0.390
$CuSO_4.5H_2O$	0.079
$Co(No_3)_2.6H_2O$	0.0434

3.2 Sub-culturing of *Anabaena ambigua*

Anabaena ambigua culture was inoculated in the Erlenmeyer flask having BG-11 medium and proper growth conditions were provided for the growth of *Anabaena ambigua* [53].

3.3 Microalgae growth conditions

Microalgae for its growth required BG-11 media for 30 days in dark: light (12:12) conditions in 1500 lux light and100 rpm shaker incubator. They require photosynthetic light 12 hours a day. The temperature should be between 25^{o}Cand lower than 16^{o}Cwill slow down growth and higher i.e. 35^{o}C will be lethal. The pH should be between 8.2-8.7[54].All the conditions required for the growth are provided in the incubator shaker.Now after the growth of microalgae we will see the growth curve of *Anabaena ambigua* and then growth curve of *Anabaena ambigua*

3.4 Microscopic Examination of *Anabaena ambigua*

The *Anabaena ambigua* culture was observed under microscope using the microscope Olympus.

3.5 Growth Measurements

3.5.1 Dry Cell Weight

Dry cell weight was taken by first taking the weight of the empty eppendorf then the sample was taken in 1 ml centrifuge tube then centrifugation was done at5000 rpm for 5 mins for different days .After centrifugation supernatant discarded and pellet was oven dry overnight in desiccators for 1 to3 hours .Then again take cell weight[55].

3.5.2 OD at 680 nm

The *Anabaena ambigua* growth was studied by taking the OD at 680 nm in UV-VIS spectrophotometer. Microalgae growth curve was observed for 31 day

3.6 Ciprofloxacin toxicity

250mg of ciprofloxacin was taken and it was disintegrated in 10 ml refined water and afterward at various focuses, *Anabaena ambigua* was developed to check the poisonousness of ciprofloxacin on *Anabaena ambigua* fixation like 2mg/l, 4 mg/l, 6mg/l, 8mg/l and10 mg/l

[57]. At that point to check the poisonousness of ciprofloxacin on microalgae development bend and DCW was taken for 31 days.

3.7 Specific growth rate, Divisions per day and Doubling time calculations

The specific growth rate, divisions per time and doubling time were calculated from the standard graph of growth curve using dry cell weight [58] .

Specific Growth Rate (μ)

$$\mu = \frac{\ln(nf/ni)}{t2-t1}$$

Divisions per day = $\frac{\mu}{ln2}$

Doubling time (T_d)

$$T_d = \frac{1}{divisions\ per\ day}$$

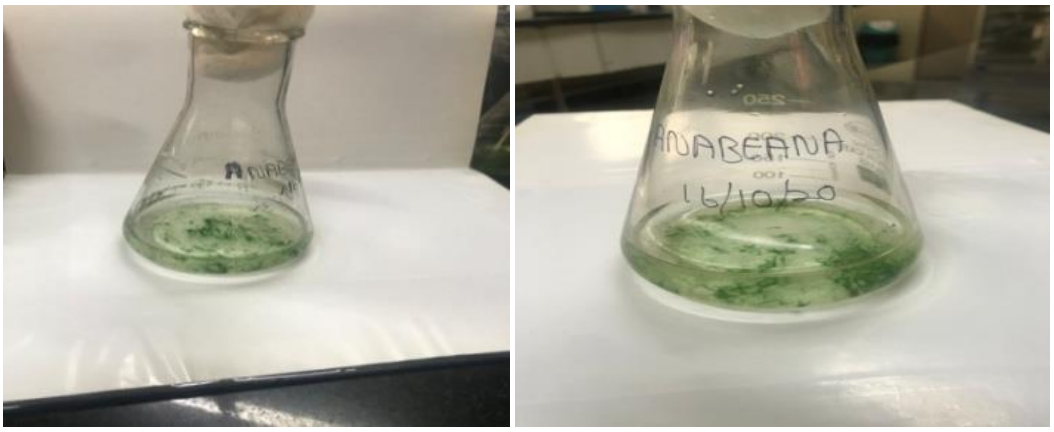
CHAPTER 4

RESULTS AND DISCUSSION

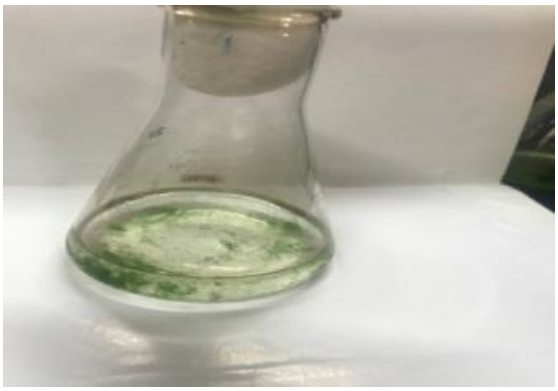
==========

4.1 Sub-culturing of *Anabaena ambigua*

Sub culturing the growth of *Anabaena ambigua* is been done as we can see in Flask1, Flask2 and Flask 3



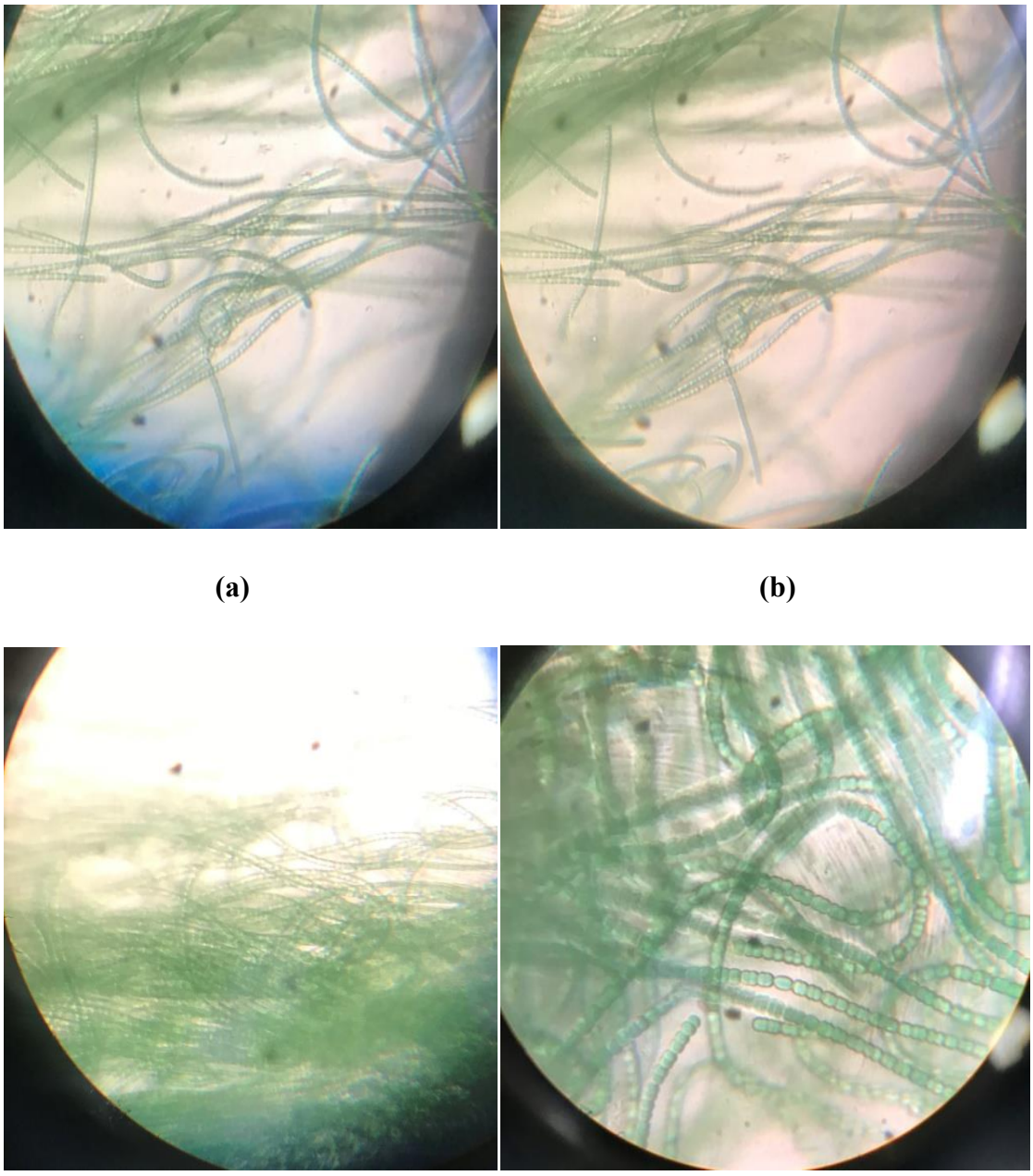
Flask1Flask 2



Flask 3

Fig. 4.1 showed the growth of *Anabaena ambigua* in the BG-11 medium

4. 2 Microscopic view of *Anabaena ambigua*



(a) **(b)**

(c) **(d)**

Fig. 4.2 Microscopic examination of *Anabaena ambigua* in (a) and (b) (microscopic view at 40X) and (c) Cluster view and (d) (microscopic view at 100X)

The above fig 4.2 constitute microscopic view of *Anabaena ambigua* we can notice it is green filamentous algae and further has two types of cells the vegetative cells along with oxidative cells .Through the fig personally able to catch glimpse of clusters with *Anabaena ambigua* at 40X and 100X [59].

4. 3 Growth Studies of *Anabaena ambigua*

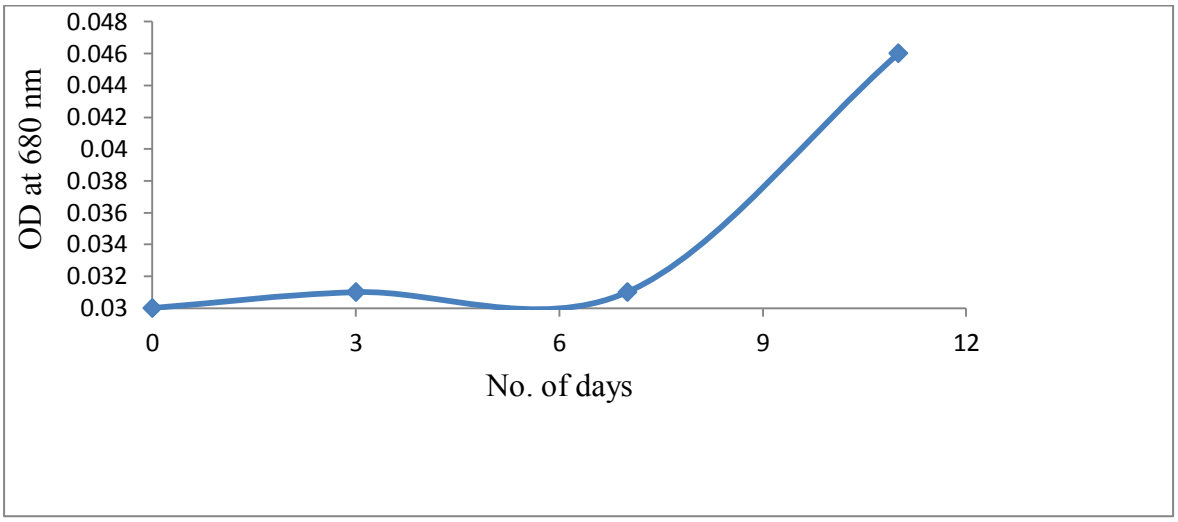


Fig 4.3*Anabaena ambigua* growth studies by taking its Optical density at 680 nm.

The overhead fig considers the graph which exhibit growth of microalgae and we can perceive that the cells of *Anabaena ambigua* are multiplying in the culture media and they are in the exponential phase of growth together with microalgae are multiplying [60].

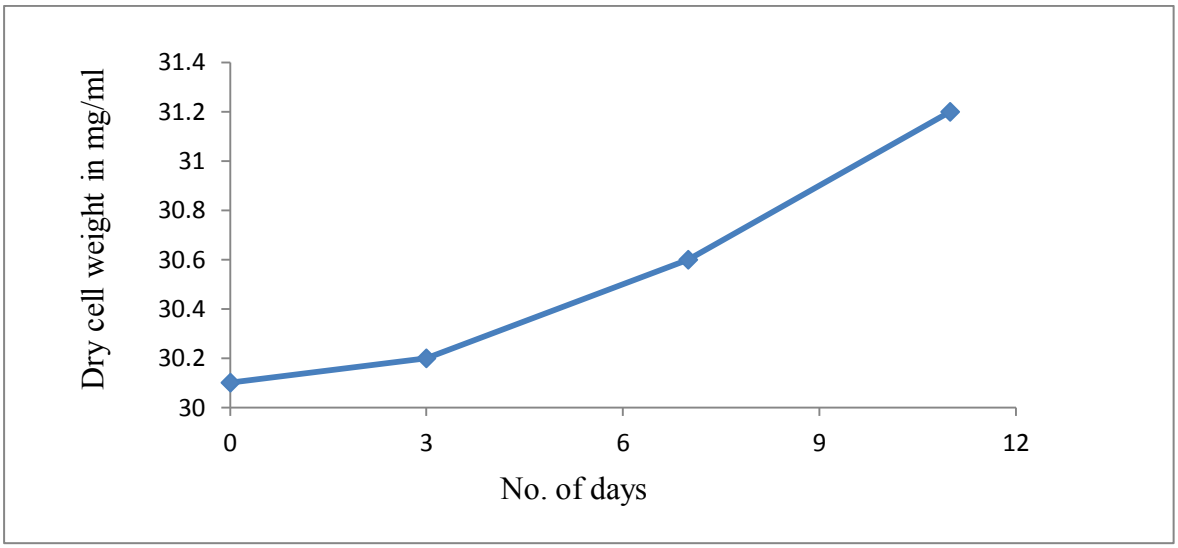


Fig. 4.4 Dry cell weight studies of *Anabaena ambigua* BG-11 medium

The above graph shows that cells are growing on the grounds of dry cell weight and they are in log phase or exponential phase of. The *Anabaena ambigua* grow slow in its initial days as there time of growth period increases the higher the growth. The *Anabaena ambigua* enter the log phase after 6th day of its growth and it's doubling time also increased [61].

4.4 Calculation of specific growth rate, divisions per day and doubling time of *Anabaena ambigua*

Table 4.1 Specific growth rate, divisions per day and doubling time of *Anabaena ambigua*

Anabaena ambigua growth on basis of Dry cell weight (mg/ml)	**Sp**ecific Growth Rate μ (d^{-1})	**D**ivisions per day (d^{-1})	Doubling Time T_d(d)
31.1	**0**.001299	0.004315	231.74

In the above table 4.1 we calculated the specific growth rate which is 0.00129 $d^{-1,}$ divisions per day is 0.004315 d^{-1} and the doubling time is 231.74 d of *Anabaena ambigua* and we can see that that micro alga is growing in its biomass [62].

4.15 Determination of Ciprofloxacin Toxicity

When ciprofloxacin toxicity was calculated using concentration like 2mg/l , 4mg/l ,6mg/l ,8mg/l and 10mg/l .It was observed that the growth of *Anabaena ambig*ua was maximum in 2mg/l and there was very less growth in 8 mg/l and 10 mg/l concentration so we reduced the concentration and the maximum growth was in 2 mg/l [63] .It shows that this concentration of ciprofloxacin is not toxic for *Anabaena ambigua.*

CHAPTER 5

Conclusion and Future Prospects

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5.1 Conclusions

- From the above results we can see that the sub culturing of *Anabaena ambigua* is done the growth of microalgae has come and from the microscopic structure we can see the structure of the filamentous microalgae i.e. *Anabaena ambigua*.
- We can assume since the growth of microalgae has come work can be done further. From the growth curve and DCW we can see that the cells have grown .
- The doubling time , specific growth rate and division per day can be calculated .

5.2 Future Prospects

- To perform the growth curve of the microalgae *Anabaena ambigua*
- To check the toxicity/ tolerance limits of microalgae to different concentration of Ciprofloxacin.
- Characterization and microalgae-based Bioremediation studies of Ciprofloxacin effluents

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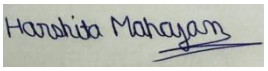

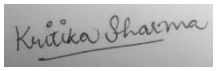

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