Available online at: http://www.ijmtst.com/vol5issue08.html
International Journal for Modern Trends in Science and Technology
ISSN: 2455-3778 :: Volume: 05, Issue No: 08, August 2019

Treatment of Primary and Secondary Domestic Wastewater Effluents by using Microalga *Oscillatoria Sp*

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To Cite this Article
Aravindh S and P Ruban, "Treatment of Primary and Secondary Domestic Wastewater Effluents by using Microalga *Oscillatoria Sp*", *International Journal for Modern Trends in Science and Technology*, Vol. 05, Issue 08, August 2019, pp.: 60-65.

Article Info
Received on 15-July-2019, Revised on 06-August-2019, Accepted on 10-August-2019, Published on 13-August-2019.

ABSTRACT

The scarcity of water all around the world been increasing and has resulted in the development of new water reuse technologies. But, the success of water reuse projects have not been so effective and also with so many disadvantages. Hence Laboratory-scale experiments were performed to develop a procedure for biological treatment of secondary and tertiary wastewater treatment using microalgae. Ten microalgae strains were isolated from Munnakal beach, Kerala and were identifiedthrough microscopic examination. Then, the ability of microalgae to remove nutrients and organic carbon from primary and secondary effluents were investigated. Among the isolated strains, M1 strain alone showed maximum reductions in soluble concentrations total nitrogen, phosphorus, and COD (27%, 91.7%, 88.6% and 25.5. 30.8%), in both for primary and secondary wastewater respectively. The high nutrient removal capacity of the M1(Oscillatoria sp.,) microalgae suggests that these microalgae have the potential to be used for tertiary treatment and treatment of side streams at treatment plants under simple and inexpensive operational conditions. Therefore, a microalgae-based treatment system can reduce or eliminate the need for chemical disinfection methods. The results of this study indicate that microalgae-based treatment systems can be incorporated in treatment plants in a very simple and inexpensive way without the need for providing CO_2, nutrients, heating, pH adjustment, etc.

KEYWORDS: *Microalgae, wastewater treatment, Oscillatoria sp., reduction of N and P.*

I. Introduction

Though the earth is covered by two-thirds of water, most of them are not usable. Due to increase in population and development of industries have decreased the quality of freshwater's all around the world and it leads to freshwater demand in certain areas. A Report from the U.S. geological survey has stated that by the year 2025 there will be a global demand for fresh water(Prerna jain et al., 2019). To overcome this situation, there is a need for wastewater treatment technique to meet the requirement of fresh water for a growing population. Water is an essential liquid required by the entire living organism (plant and animals) to live. They are appeared to be colourless and also odourless, tasteless liquid and are found in the form of lakes, rivers, ponds, rains, etc.,(Griffin,2016) When they are been released into the environment after use by humans (domestic, industries, agriculture) forms wastewater(Thangavel, 2017). Water pollution is one of the major unavoidable environment issues facing all around the world. They contain pollutants like physical, chemical and biological wastes. Every day there is a huge amount of wastewater released due to increasing population and rapid industrialization(Tietenberg, 2016).

Wastewater treatment plants available today are mostly employing conventional wastewater treatment plans to decrease the concentration of pollutants in water and to increase the quality of the used water before it reaches the groundwater or to the other water reservoirs (Pradeep Kumar,2013). This method can be used only for treating municipal wastewater and it cannot be used for treating the toxic wastes that are released by industries. Nowadays, the problems related to the heavy metals, nitrogen and phosphate forms a major environmental issue faced by the society which is released by industrial wastewater (Barakat, 2011). There are several environmental laws has been strictly aroused to overcome these issues. The World Health Organisation has listed out several criteria to limit the nitrogen, phosphorous and heavy metals discharges from domestic and industrial wastewater treatment plants(Nitrogen = 10mgl-1 and Phosphate = 0.1 mgl-1)(Murugan, 2011). The Conventional Removal Techniques such as Nitrogen Removal Technology, Phosphorous Removal Technology and Several Physico-Chemical Technologies (Chemical precipitation, Ion-Exchange, Adsorption, Filtration, Phytoremediation, etc.,) are been used to limit the heavy metal wastes in industrial wastewater (Hemant, 2014).

But the fact is that these methods are not effective and they have several disadvantages on their own. Their disadvantages like consuming more amount of energy use of chemicals and also too expensive. Several chemical methods lead to toxic sludge generation (Nachiket,2009). Hence, to overcome these disadvantages there is a need for some new technologies to improve the quality of toxic waste removal efficiency with cost-effective. It can be done by the use of the biological method called bioremediation, the use of biological sources (microorganism, plants, animals, etc) for degrading/remove the wastes was termed to be bioremediation (Niha, 2014). Microalgae are one of the important biological sources which are photosynthetic s like as plants and are unicellular, they consume nitrogen, phosphate and carbon dioxide for their growth by releasing oxygen (Vanmathi, 2017). They also have a high affinity towards the polyvalent metal. So, they can effectively remove the heavy metals, nitrate and phosphate in wastewater by consuming them for growth (Abdel-Raouf, 2012). Therefore, by employing microalgae for bioremediation of wastewater provides potential advantages than the other technologies that are available today. Hence, this investigation was carried out to understand the capability of Microalgae that are isolated from munnakal beach, Kerala, India to treat the primary and secondary wastewater.

II. Methodology

Study Area and Sample Collection

The rocks and Seawater samples were collected from munnakal Beach, Kerala, India Fig.1. In polythene bags and were packed in ice boxes carried through laboratory for a further process(Betsy, 2015).

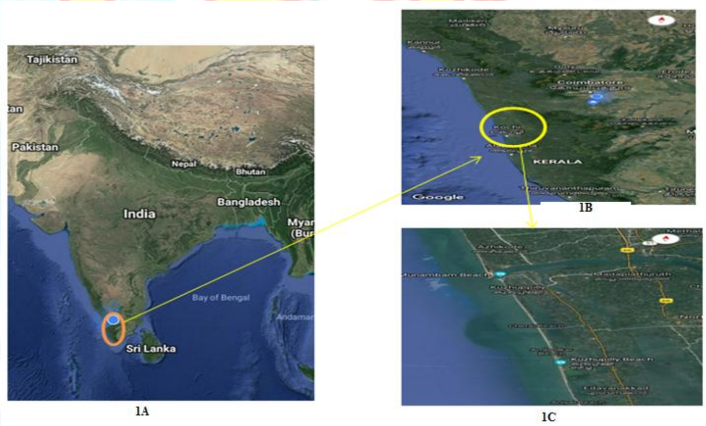

Fig.1 Study Area in satellite view (Munnakal Beach, Kerala. (10.1805°N, 76.1624°E))

Isolation of Microalgae

The Microalgae were isolated from collected samples by using suitable media (ASN III and F/2 Media) and isolated algae were identified morphologically by the use of a Microscope(Andersen, 2005).

Microalgae Cultivation System

Microalgae were cultivated in ASNIII medium in order to help fast and easy growth as well as to maintain an adequate concentration of microalgae biomass that was needed for the experiments. Microalgae strain was cultivated and maintained in ASN III medium at room temperature in the lab under continuous light provided through cool-white fluorescent light (two bulbs of 15 watts and a length of 24 in). The operational parameters for microalgae cultivation are light (1.4-2mWcm^{-2}), Temperature(24 ± 2^{0}C) and Cultivation medium (ASNIII). The medium was seeded with an initial wet weight of 100mg microalgae/L. Every 12 days a volume of 100mL/L was taken from the microalgae suspension and replaced with the new medium to maintain enough nutrients for the algal biomass. Microalgae samples were harvested after 7 to 10 days of cultivation. They were centrifuged at 3000 rpm, 20°C for 15 min. The supernatant was removed and the pellet was washed with distilled water three times to remove salt residues. Then, the wet biomass was used in the wastewater treatment tests at the selected concentrations by suspending them in the wastewater samples(Chen, 2011).

Experimental design

All the experiments in this study were conducted in Erlenmeyer flasks under well-known conditions. In all experiments, the reactors were incubated at room temperature (24 ± 2°C) in the laboratory. The reactors were provided with continuous light through 40 fluorescent cool white light, (two bulbs of 15 watts and 24 in.) at 10 cm behind the reactor, with an intensity of 1.4-2mW cm^{-2}. Also, an equal and constant aeration rate was provided for all samples and reactors through flowmeters. Liquid samples were taken daily for direct analyses of the concentration of soluble COD, BOD, dissolved total P, and dissolved TN, NH3-N and NO3-N. The temperature was measured by a thermometer. The experimental periods were between 5 to 7 days and each test sample had three replicates. Wastewater and microalgae culture was maintained in suspension by providing a continuous mixing of 100 rpm using a magnetic stirrer. The effect of the evaporation was taken into consideration by calculating the daily amount of wastewater that was lost. The experiments were completed without the addition of any chemicals or adjustment of environmental conditions. Wastewater in the reactor was seeded with known initial concentrations of microalgae according to the purpose of the experiment.

III. RESULTS

Characteristics of wastewaters

Wastewaters were collected from two different sources such as effluent of primary and secondary treatment. All wastewaters were filtered using glass microfiber filters (Whatman No 1) to remove large particles and indigenous bacteria.

Table 1. Characteristic of primary and secondary wastewater

Sample	COD(mg/l)	pH	TDS(mg/l)	Nitrate(mg/l)	Ammonia-nitrogen (mg/l)	Total Nitrogen (mg/l)	Phosphorous(mg/l)
Primary effluents	69	7.8	854.2	0.41	26	-	4
Secondary effluents	49	7.3	585.7	-	-	52	3.2

Secondary effluent is a relatively clear liquid, it has less solids compared to primary effluent. Primary effluent has very high pathogen content and solids concentration compared to secondary effluent. To simulate realistic conditions, wastewater samples were used directly in the experiments without filtration, autoclaving, or additional processes. Also, there was no nutrient addition to wastewater samples.

Isolation and Identification of Microalgae

Ten Microalgae species were isolated from the collected samples and were named as (M1-M10) and were also morphologically identified as *Oscillatoria sp., Phormidium sp. Lyngbya sp. Leptolyngbya spNavicula sp., Anabaena sp.Planktothrix sp Geitlerinema spTetrasporidium sp. Pseudanabena sp.,* respectively (Fig.2).

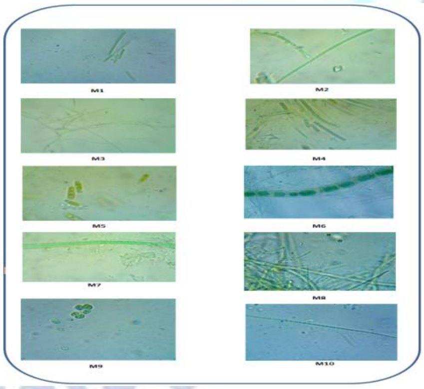

Fig.2 Microalgae isolated from collected samples

Microalgae growth

Microalgae growth were analysed by measuring total solids (TS), and absorbance of the biomass daily. Figure 4 illustrate the microalgae growth in terms of TS, and ABS for both primary and secondary effluent, respectively. In primary and secondary effluent, M1 Strain was able to adapt and grow while other strains (M2-M10) were found to lack in growth. Microalgae growth was very slow due to the new environmental conditions to which microalgae had to adapt. After that, microalgae biomass started to increase until the fifth day of incubation when the growth started to be in a stationary phase.

When ABS was used to monitor the microalgae growth rate, a rapid increase could be seen from the beginning of the experiment. In terms of absorbance, the mean growth rate for the first 4 days was 0.26 d^{-1} while the mean growth rate after the lag phase was 0.27 d^{-1}. These values were lower than the growth rate of 0.91 d^{-1} when microalgae were grown in ASN III. Even though wastewater provides a good medium, it is not the optimal medium and there is competition from other microorganisms.

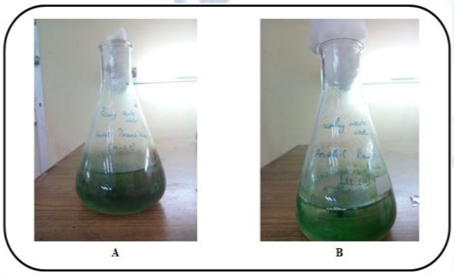

Fig.3 M1 growth in effluents A) Primary and B) Secondary effluent

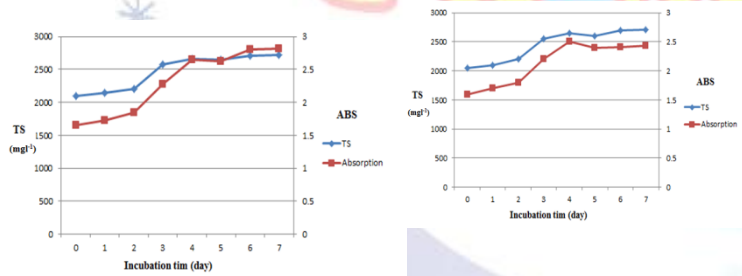

Fig.4 Total Solid and Absorbance(680nm) of A) Primary and B) Secondary effluent for M1 Strain

Change in pH

During the experiment period, there is an increase in pH levels due to microalgae growth as there is photoautotrophic growth; It also due to CO_2assimilation by microalgae(Borowitzka, 1998). Figure 5 illustrates the changes in pH levels during the incubation period for the primary effluent and secondary effluent. pH levels increased from 7.8 to 8.1 for primary effluent and from 7.3 to 7.82 for the secondary during the 7 days of the experiment. Increased pH has many impacts on wastewater treatment. It leads to precipitation of phosphorus through the formation of hydroxyapatite(Sivasankaran et al., 2019), which helps to improve the flocculation of particles and also sanitize the growth of pathogenic organisms as well.

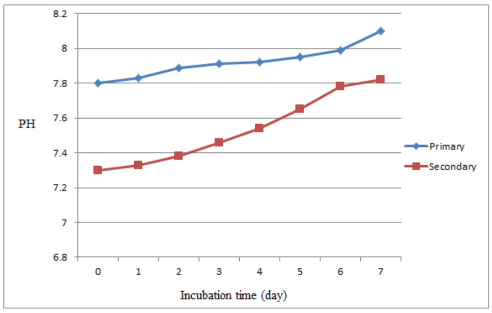

Fig.5 PH variation of Primary and Secondary effluent at incubation day of M1Strain

Removal of dissolved total nitrogen

The ability of Microalgae to uptake inorganic form of nitrogen directly leads to a decrease in nitrogen concentration which was monitored for 7 days. The variation in the dissolved TN concentration for secondary effluent is described in Figure 4.5. Hence, there is no inorganic form of nitrogen in the primary effluent, there is no variation in the primary effluent. TN concentration decreased starting from the first day until the end of the experiment period for secondary effluent. There was some fluctuation in nitrogen removal for the secondary effluent. The increase that was observed in nitrogen on days 5 and 6 for secondary effluent may be due to die-off and decomposition of some of the bacteria and microalgae since no additional nutrient and carbon source was provided during the experiments.

TN reduction for the secondary effluent after 7 days was 27%. TN was likely mainly removed through assimilation by microalgae and other organisms. However, part of the TN may be removed through volatilization due to elevated pH levels occurred as a result of microalgae activity. Regardless, the reduction in total nitrogen for the secondary effluent can be very beneficial for the treatment process.

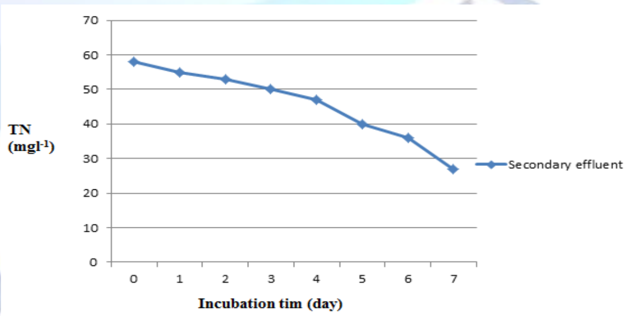

Fig.6 Total Nitrogen concentration of Secondary effluent at incubation day of M1Strain

Removal of dissolved phosphorus

Microalgae take phosphorous in an inorganic form which is essential for their growth. P removal achieved during the experiments is illustrated in Figure 6. Dissolved phosphorus was efficiently reduced for both the primary and secondary effluent by 91.7% and 88.6%, respectively. The most important form of phosphorus for microalgae is orthophosphate PO_4^{-3} which is utilized directly by microalgae. Microalgae cannot utilize other forms of phosphorus until they are hydrolyzed by extracellular enzymes to PO_4^{-3}(Ojaswi Ghadge et al., 2014). High level of pH associated with microalgae treatment can result in phosphorus precipitation. Thus, phosphorus can be removed not only due to microalgae uptake but also due to precipitation. The results also showed that the per cent removal of phosphorus was higher than the per cent removal of nitrogen.

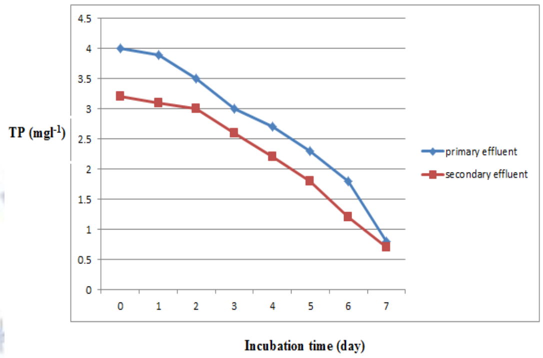

Fig.7 Total Phosphate concentration of Primary and Secondary effluent at incubation day of M1 strain

Removal of COD

sCOD concentration reduction was also monitored during microalgae treatment. The reduction in sCOD, however, was not as high as the reduction in the nutrients particularly in the secondary effluent (Figure 7). The sCOD reduction for the primary and secondary effluent during the 7 days experiment period was 25.5 and 30.8%, respectively. Figure 7 illustrates the reduction in sCOD concentration in the primary and secondary effluent. On day 2, an increase in sCOD was observed likely due to the die-off of some bacteria and microalgaeduring their adaptation to the new conditions. As a result, microalgae have the potential to improve COD removal and hence can effectively contribute to wastewater treatment not only for the removal of nutrients but also for the removal of organic matter

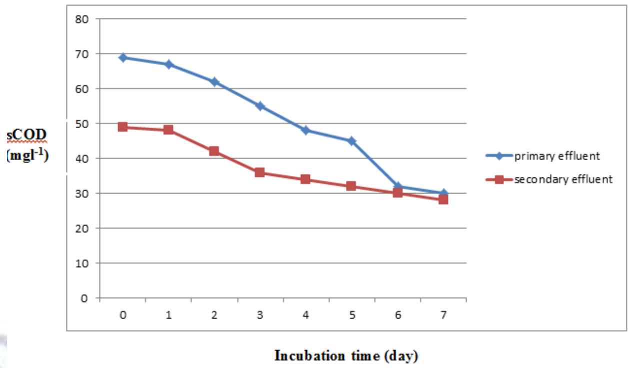

Fig.8 sCOD concentration of Primary and Secondary effluent at incubation day of M1Strain

IV. CONCLUSIONS

In this study, the potential of microalgae to improve conventional wastewater treatment was investigated. Primary and Secondary effluent was treated with microalgae for 7 days and M2-M10 Strains were lacked to grow while M1 Strain found to grow and was successful in removing nutrients, sCOD and TC from wastewater. During this investigation, M1Strain (*Oscillatoriasp.*,) removed 25.5 and 30.8% of the sCOD from the primary and secondary effluent, respectively. In addition, dissolved TN was reduced by 27% for the secondary effluent, respectively. Dissolved phosphorus reduction for both the primary and secondary effluent was 91.7 and 88.6%, respectively. The high nutrient removal capacity of this M1 microalgae suggests that microalgae have the potential to be used for tertiary treatment and treatment of side streams at treatment plants under simple and inexpensive operational conditions. Therefore, a microalgae-based treatment system can reduce or eliminate the need for chemical disinfection methods.

The results of this study indicate that microalgae-based wastewater treatment systems can be combined with advanced treatment plants in a very simple and inexpensive way without the need for high energy, chemicals, nutrients, heating, pH adjustment, etc. By the use of microalgae will not only improve the overall wastewater treatment but would also generate biomass that has high energy value.

Thus, further research should be done on M1strain like phylogenetic analysis and also to better utilize this advantage of microalgae and reduce the overall disadvantages of wastewater treatment systems.

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