

**UTILIZATION OF MULTISPECIES MICROALGAL BIOSYSTEM
FOR WASTEWATER REMEDIATION AND
BIOFUEL PRODUCTION**

**A Summary of the Thesis Submitted to the
Babasaheb Bhimrao Ambedkar University, Lucknow in
Fulfilment of Requirement for the Award of Degree of**

Doctor of Philosophy

IN

ENVIRONMENTAL SCIENCE

**BABASAHEB
BHIMRAO
AMBEDKAR
UNIVERSITY**



**• LUCKNOW •
प्रज्ञा शील करुणा
ESTABLISHED 1996**

BY

Dig Vijay Singh

(Enrolment No. -1350/18)

SUPERVISOR

Prof. Rana Pratap Singh

**DEPARTMENT OF ENERGY & ENVIRONMENT
SCHOOL OF ENVIRONMENTAL SCIENCES
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A CENTRAL UNIVERSITY)
VIDYA VIHAR, RAEBARELI ROAD, LUCKNOW-226025
UTTAR PRADESH (INDIA)**

2022

Summary

Extraordinary population expansion is the forefront cause of freshwater, land, energy scarcity along with urbanization, industrial development, enormous waste generation, environmental pollution and ultimately global warming. Water pollution is the serious environmental issues which need to be addressed by developing stringent policies, planning and sustainable approaches. In India, the majority of the treatment facilities for wastewater management are deployed in urban areas but still, huge quantity of wastewater is discharged without any necessary treatment into the environment. The leading global problems require considerable attention to ensure the smooth functioning of life. Due to the incompetent treatment systems, the disposal of wastewater interrupts the functioning of biota and ultimately perturbs the stability of aquatic ecosystem. The inefficient nutrient removal through secondary treatment and huge investment as well as energy required in tertiary treatment, it become important to develop the techniques which can transform the treatment plants from energy consumer to energy producers. Additionally, the concerns related to depleting energy reserves are raising with the growing population. The swift decline in fossil fuel reserves and rising price has increased the focus on renewable fuel that has potential to offset the challenges posed by fossil fuel scarcity. Currently, no standalone techniques have been devised that can address the problem of energy and water scarcity simultaneously. Compared to different physico-chemical methods, microalgae can serve as the important biological organisms that simultaneously meet the targets of wastewater treatment and energy production in sustainable manner. Besides improving the vital attributes of the wastewater, the biomass obtained can be the cheap feedstock for the biofuel production.

Fluctuation of temperature and light intensity within day, day to day and with season is the natural phenomenon. Furthermore, under natural conditions, maintaining axenic and optimum environmental condition is impossible. Thus, screening algal consortia that can grow under different temperature and light intensity conditions can be the appropriate solution for wastewater treatment and biomass production. It is also unclear that whether the defense mechanism stimulated in the form of enzymatic and non enzymatic antioxidants assist in regulating the physiological and metabolic pathways against temperature and light stress. The aim of this study was to screen algal consortia that have ability to grow efficiently in the wastewater with excellent removal efficiency as well as promising potential of lipid production. Therefore, the systematic studies were carried out to examine the effect of municipal wastewater, light intensity and temperature on algal growth, biomass production, lipid accumulation, photosynthetic performance, biochemical profile, stress biomarkers, antioxidants system and eventually biofuel production of algal consortia. The present study will also help to screen the algal consortia adaptable under different light intensity and temperature regimes.

The first objective was to screen the monoalgal species that can adapt under natural environmental conditions. The monoalgal species were selected and their potential to survive under natural conditions was analyzed in terms of growth, pigment content and defense responses. In this study, the algal species *Chlorococcum humicola*, *Tetradismus* sp. and *Oscillatoria* sp. were examined for their tolerance potential against different wastewater concentrations under natural environment. The results revealed that selected microalgae reutilized nutrient resource and removed heavy metals from municipal wastewater. Microalgae *Tetradismus* sp. and *C. humicola* efficiently removed toxic metal pollutants from wastewater than the

Oscillatoria sp. as evident from the improvement in the water quality parameters such as EC, TDS, TS, BOD and DO level of water. The total chlorophyll (82%), protein (37.50%) and carbohydrate content (123.12%) was observed highest in *Tetradesmus* sp. after treatment with different concentration of wastewater while the maximum increase in lipid content (39.81%) was observed with *C. humicola* at 75% wastewater concentration. Furthermore, a compositional alteration showed by FTIR spectroscopy clearly demonstrated that wastewater (100%) enhanced lipid synthesis which was maximum in *C. humicola* and *Tetradesmus* sp. than the *Oscillatoria* sp. As compared to *Oscillatoria* sp., a greater increase in the level of anti-oxidative compounds in *Tetradesmus* sp. and *C. humicola* with simultaneous decline in the oxidative stress markers exhibited tolerant behaviour against the wastewater induced toxicity. In order to counterbalance the wastewater induced toxicity, *Tetradesmus* sp. showed highest proline (4.63 folds), catalase (4.95 folds), superoxide dismutase (1.76 folds) and glutathione reductase (7.00 folds) while ascorbic acid (9.25 folds) and cysteine (4.92 folds) activity was highest in *C. humicola* at different concentrations of wastewater. Similarly, the growth pattern, cellular modification, oxidative stress markers, and defense responses of *Chlorella vulgaris* and *Scenedesmus vacuolatus* were analyzed after treatment with municipal wastewater (25-100%). The results revealed that the nutrients and heavy metal removal was highest at 25% of wastewater concentration while maximum specific growth rate (0.0708 day^{-1}), total chlorophyll ($0.118 \text{ mg g}^{-1} \text{ fw}$), and carbohydrate content ($24.06 \text{ mg L}^{-1} \text{ fw}$) was observed with *Scenedesmus vacuolatus* at 50% concentration of wastewater. Under natural environment, the algal species showed declined pigment and carbohydrate synthesis at 75% and 100% concentration of wastewater. Furthermore, the lesser accumulation of thiobarbituric acid reactive species and hydrogen peroxide while increment in antioxidants activity

may be responsible for imparting tolerance to *Scenedesmus vacuolatus* against different concentrations of municipal wastewater. Under natural conditions, 50% and 100% wastewater concentration was suitable for excellent growth and lipid accumulation in *Scenedesmus vacuolatus*, respectively. Overall, it was observed that *Scenedesmus vacuolatus*, *C. humicola* and *Tetradesmus* sp. were tolerant to different wastewater concentrations and exhibited higher nutrient capturing potential, growth rate and lipid production due to their proficient cellular defense system.

In the 2nd objective, the effort was made to form the algal consortia on the basis of nutrient reutilization, metal detoxification potential, lipid production and antioxidants defense response of monoalgal species under natural environment. Two algal consortia (consortia 1: *Tetradesmus* sp. + *Scenedesmus vacuolatus* and consortia 2: *Chlorococcum humicola* + *Scenedesmus vacuolatus* + *Tetradesmus* sp.) were employed for municipal wastewater treatment under laboratory conditions (light intensity 30 W/m² and temperature: 30° C). The results of remediation efficiency, growth profile and biomass production reflected greater adaptability and tolerance of consortia 1 against different concentrations of municipal wastewater. The photosynthetic parameters such as Fv/Fo, Fv/Fm, and Pi_{abs} were increased by 1.20-2.35 folds in consortia 1 treated with municipal wastewater (25-100%). Additionally, FTIR spectroscopy showed neutral lipid accumulation (1750 cm⁻¹) in consortia 1 after treatment with wastewater (100%). The oxidative stress markers showed considerable decline in consortia 1 as compared to consortia 2. Interestingly, increased antioxidant activity (1.02-4.14 folds) reflected that oxidative damage to consortia 1 was counterbalanced by the strong defense mechanisms. Overall, remediation efficiency, photosynthetic performance and antioxidants activity suggested that the consortia 1

can be used for efficient wastewater treatment and lipid production in sustainable manner.

In the 3rd objective, suitability of municipal wastewater as growth media for consortia was analyzed by examining the phyco-remediation potential, growth profile, biomass production and photosynthetic efficiency of consortia 1 and 2 under light intensities of 20 W/m² and 40 W/m². By the end of experiments under laboratory condition, significant decline of 40-90% in different physico-chemical attributes such as EC, TDS, BOD, nitrogen, phosphorus and heavy metals was observed with consortia 1 and 2 under light intensity of 40 W/m². Additionally, abundance of lipid, protein, and carbohydrates showed by FTIR spectroscopy in treated consortia 1 and 2 exhibits different functional groups for bio-adsorption of metal from wastewater. Higher light intensity (40 W/m²) induced neutral lipid accumulation in consortia 1 was reflected by the absorption peak at 1750 cm⁻¹ from FTIR spectra. Furthermore, the maximum total chlorophyll content and photosynthetic performance revealed that light intensity of 20 W/m² and 40 W/m² increased the functioning of PS II in consortia 1 than consortia 2. Simultaneously, the elevated ascorbic acid, proline, cysteine, superoxide dismutase, catalase and glutathione reductase activity may offset the damage posed by wastewater and light intensity. The results also exhibited that fatty acid responsible for good quality biofuel production were in abundant percentage in consortia 1 and 2 treated with wastewater (100%) under light intensity of 20 W/m² and 40 W/m². Overall, consortia 1 were competent to adapt under different wastewater concentrations and light intensities as evident by the increment in growth, photosynthetic performance, antioxidant activity and fatty acid composition while consortia 2 showed promising potential at 40 W/m².

Under natural conditions, the lack of control on temperature is major limitation for large scale cultivation of microalgae. Therefore, it is very imperative to examine the effects of different temperature regimes on remediation efficiency, growth, lipids, antioxidant activity and biofuel production in algal consortia. The results exhibited that temperature regime of 40° C enhanced the nutrient and metal removal efficiency of selected consortia with maximum in consortia 1. Highest photosynthetic performance and lipid accumulation indicated efficient tolerance and adaptability of consortia 1 at 20° C and 40° C. Further, the fatty acid profile revealed the highest percentage of SFA in consortia 1 (43.70%) and consortia 2 (46.99%) treated with wastewater (100%) at temperature regimes of 40° C. Furthermore, the maximum increment in TBARS (7.32 folds) and H₂O₂ (5.95 folds) was observed in consortia 2 at 40° C and 20° C, respectively. Antioxidants like ascorbic acid (3.93 folds), SOD (4.50 folds) and GR (1.10 folds) activity was also observed to increase in consortia 1 with wastewater concentration gradient at 20° C than 40° C. Overall, the results suggested that consortia 1 showed high photosynthetic efficiency, lipid production, antioxidant activity and dominance of fatty acid at 20° C and 40° C. Thus, consortia 1 can serve as the flexible system for wastewater treatment and bio-energy production under different temperature regimes.

The operating conditions in the laboratory and natural environment are totally different therefore; the selected algal consortia were examined for tolerance and adaptability under natural conditions. The results showed that consortia 2 was efficient in recovering nitrate-nitrogen (82.92%) and phosphorus (70.47%) from municipal wastewater. Further, the results exhibited highest metal removal efficiency in case of consortia 2 treated with municipal wastewater (100%). The excellent remediation efficiency of consortia 1 and 2 was also evident from the FTIR spectra.

The results further showed that total chlorophyll, carbohydrate and protein content was decreased significantly in consortia 1 treated with municipal wastewater than control. However, an increase of 4.01 and 1.17 folds in lipid content was observed in treated algal consortia 1 and 2 in comparison to their respective control. The enhancement in lipid accumulation was also confirmed through FTIR spectroscopy. The results exhibited the improved photosynthetic performance as evident from increased F_v/F_o , F_v/F_m and P_i_{abs} value in consortia 2. The results further depicted the maximum increase in TBARS (1.56 folds) and H_2O_2 content (1.53 folds) in wastewater treated consortia 1. In case of antioxidants, the maximum increase in ascorbic acid (2.43 folds), proline (3.34 folds) and cysteine (1.29 folds) was observed in consortia 2 while highest SOD (1.75 folds), catalase (2.64 folds), and GR (1.19 folds) activity was observed in consortia 1. The increased activity of antioxidants in selected algal consortia may assist to maintain the cellular balance by scavenging hydroxyl, superoxide and peroxide radicals instigated by wastewater and unfavorable conditions under natural environment. Overall, it can be concluded that due to remarkable nutrient recovery, metal removal and photosynthetic performance, consortia 2 could be exploited for wastewater remediation under natural environment.

Overall, algal consortia 1 showed excellent growth rate, biomass production, lipid yield, photosynthetic performance and biofuel production after treatment with wastewater under laboratory conditions while consortia 2 showed proficient ability under natural conditions. Therefore, wastewater treatment system utilizing microalgae consortia can serve as the futurists approach to deal with water pollution and energy scarcity simultaneously. This work provided the experimental evidence that consortia of different microalgae can be the practical approach to treat wastewater and augment biofuel production in economical and eco-friendly manner. Thus, the synergistic

association of different microalgae may be the neoteric paradigm with multilevel benefits such as resource reutilization, heavy metal detoxification, and excellent defense mechanisms along with suitable fatty acid composition for biodiesel production.

Main outcome of the study:

- Microalgae *Scenedesmus vacuolatus*, *Tetradesmus* sp. and *Chlorococcum humicola* was more competent in removal of dissolved solids, organic matter, nutrients and metal pollutants from wastewater as evident from the improvement in the water quality parameters.
- Monoalgal species and their consortia were efficient to recover the nutrient resource and detoxify metals at 25% and 50% as compared to 75% and 100% wastewater concentration.
- Wastewater concentration of 50% improved growth, total chlorophyll and carbohydrate synthesis in *Scenedesmus vacuolatus*, *Tetradesmus* sp. and *Chlorococcum humicola*.
- Synergistic interactions and the perfect utilization of nutrient resource from wastewater stimulated the prolific growth of algal consortia 1 and 2.
- Maximum nutrients and metal removal along with efficient growth and biomass production reflected greater adaptability of consortia 1 than consortia 2.
- Algal consortia 1 was promising one to withstand different light intensities and temperature regimes as compared to consortia 2.
- Different municipal wastewater concentrations, light intensities and temperature regimes improved the photosynthetic performance of consortia 1.

- Under natural conditions, excellent photosynthetic performance confirmed that consortia 2 could serve as the promising bio-system to adapt in municipal wastewater.
- Lipid accumulation potential of treated consortia 1 was higher than consortia 2 under laboratory as well natural conditions.
- Under different light intensities and temperature regimes, FTIR spectroscopy confirmed wastewater induced TAG accumulation in algal consortia 1.
- Algal consortia 1 and 2 showed excellent performance at higher light intensity (40 W/m²) in terms of growth, biomass production, photosynthetic efficiency and antioxidants activity.
- Increment in antioxidant activity in consortia 1 at 40 W/m² confirmed that oxidative stress was attenuated by the amplified activity of non-enzymatic and enzymatic antioxidants.
- Antioxidants like ascorbic acid, superoxide dismutase and glutathione reductase activity amplified in consortia 1 treated with wastewater concentrations at 20° C.
- FTIR spectroscopy showed that consortia 1 was enriched with different enzymatic antioxidants as well as phenolic compounds that could be responsible for imparting tolerance against wastewater (100%) and temperature stress (40° C).
- Light intensity (40 W/m²) and temperature (40° C) enhanced the percentage of SFA in consortia 1 and 2.
- Palmitic acid was the dominant fatty acid in wastewater (100%) treated consortia 1 and 2 at different light intensity and temperature regimes.
- Promising remediation potential, photosynthetic performance and antioxidants activity reflected that consortia 1 and 2 could be exploited for wastewater treatment under both laboratory as well as natural conditions.