

**OPTIMIZATION OF SECONDARY BIOLOGICAL SYSTEM BY SYMBIOSIS
OF MICRO-ALGAE AND BACTERIA**Harsh Ghaghada¹, Drashti Patel², Kavisha Shah³, Mitul Vaghasia⁴¹Department of Environment science and technology Shroff SR Rotary Institute of Chemical Technology, Vataria²Department of Environment science and technology Shroff SR Rotary Institute of Chemical Technology, Vataria³Department of Environment science and technology Shroff SR Rotary Institute of Chemical Technology, Vataria⁴Department of Environment science and technology Shroff SR Rotary Institute of Chemical Technology, Vataria

Abstract — In the past decades, several models have been developed to describe nutrient removal from wastewater. Most of these models are based on activated sludge systems or photosynthetic organisms, such as algae and plants, which are known to be efficient in nutrients uptake. However, there is a lack of models describing the interactions in combined algal-bacterial systems. This research proposes a model to describe the nutrient removal processes in treating wastewater with an algal-bacterial consortium. “Pollution control is one of the most important factors in preserving a balanced environment”. There are many methods of removing pollution from municipal wastes, ranging from simple municipal primary treatment, to complex biological and chemical primary or secondary or tertiary treatment. All these systems are primarily concentrated on removal of bio logical oxygen demand (BOD) wastes. Even presence of the high ammonical nitrogen, heavy metals like hexavalent chromium, arsenic, nickel, mercury etc. will create problem in secondary treatment. In another manner of expression, the invention concerns a method of pollution control wherein unicellular or we can say similar algae, such algae being a good source of protein, are grown in symbiotic relationship with aerobic bacteria on nitrogen-providing or protein-providing aqueous waste materials such as portions of sewage, cannery wastes, etc., in a way that produces purified water, an algal mass high in protein, and free oxygen, which is liberated to the atmosphere.

Keywords - symbiosis, micro-algae and bacteria, nutrient removal, secondary treatment, biological treatment.

I. INTRODUCTION

Symbiosis is a Close association or relationship between two or more living organisms, where at least one receives some sort of benefit from the relationship.

Conventional treatment of wastewater results in continuous production of waste activated sludge mainly disposed inside the treatment plants premises or land filled. however, these solutions are not feasible on long term due to the necessity to identify new land filling sites and potential contamination risk of ecosystems with pathogens, heavy metals, etc. other alternatives to reuse waste activated sludge are unconsidered or limited especially due to the costs problems. The performances obtained by partial replacement of activated sludge with photosynthetic microalgae species are the result of the trophic cooperation established between bacteria and microalgae. The photoautotrophic microalgae release oxygen through photosynthesis²⁰ which is used by the bacteria, with heterotrophic metabolism, to degrade organic matter. the compounds released from degradation processes are used by the microalgae taxa²¹ support metabolic pathways, conducting to the development of complex biological symbiotic system characterized by bidirectional flows.

II. OBJECTIVE

The main objective of our project is “to provide a robust secondary treatment system for waste water treatment”. For this purpose we will be using the symbiosis of bacteria and algae. Basic conventional system for secondary treatment is activated sludge process. In activated sludge process bacteria are used for treatment.

Developing the novel and Settleable algal-bacterial system with different algal and bacterial compositions to improve the treatment performance and biomass settleability. Selection of high-potential unicellular microalgae species in terms of nutrient removal, biomass productivity and settleability. Investigation of the influence of abiotic and biotic factors on the treatment performance, biomass generation and settleability. Investigation of N and P removal mechanisms. Identification and characterization of the microbial consortium composition in algal-bacterial culture. An object of this process is to provide a process of conversion of organic waste into useful material and also to provide a source of nutrient materials for algae or comparable protein providing materials. Another object of symbiosis by which organic waste containing bacteria and photosynthetic micro-organisms can each contribute economically and affectively to life cycle of the other. Still further object of our process is to provide a process for economically disposing of organic waste and a process for mass culture of photosynthetic micro-organism.

III. METHODOLOGY USED

3.1 Immobilized culture[1]

Cells immobilization is another technique evaluated for wastewater treatment by microalgae. Microalgae living cells are kept in a gel matrix metabolically active for long time[2] This offers a solution for the small size of microalgae cells, since biomass harvesting represents an important part of total production cost[3] These matrices being effective and non-toxic offer an alternative to ion exchange resins which are routinely used as supports. The most common supports used for immobilization are alginate and carrageenan polymers, however, agar, chitosan, acrylamide, polyurethane, polyvinyl are also used. Higher removal rates of P were achieved by co-immobilization of two species of *Chlorella* (*C.vulgaris* and *C.sorokiniana*) with *Azospirillum brasilense* Cd a microalgae growth-promoting bacterium[4] De-Bashan also evaluated *C. vulgaris*, *C. sorokiniana*, and *A.brasilense* strain Cd in immobilized alginate bead remove pollutants of an aerobic activated sludge from municipal wastewater. Results showed removals of 100% NH_4^+ , 15% NO_3^- , and 36%P in 6 days. Reported that *C. vulgaris* and *S. rubescens* were able to remove 95% of the PO_4^{3-} in 2 days, while 94% NH_4^+ removal were achieved in 9 days.

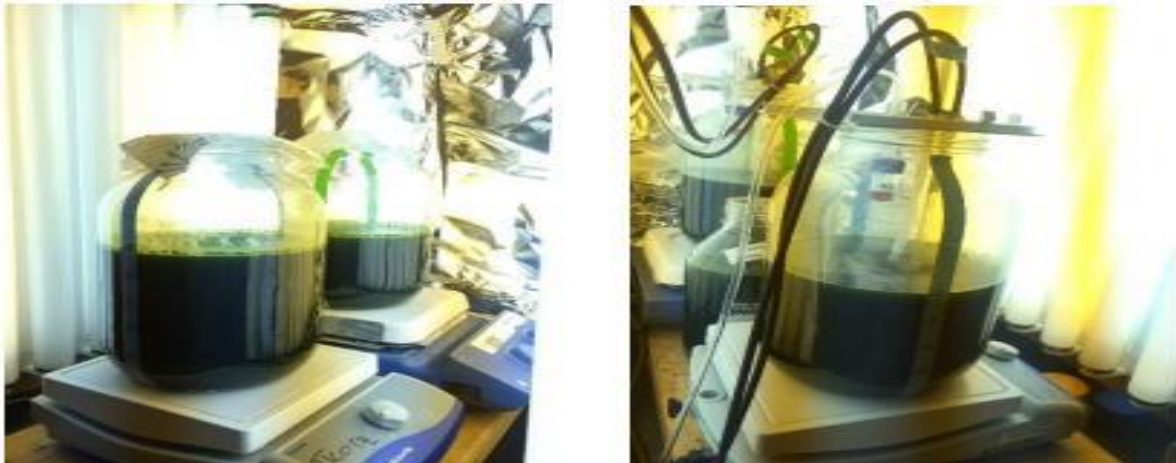


Fig 1. PBR arrangement

3.1.1 Nutrient removal[5]: Removal property of ammonium (NH_4^+), phosphate (PO_4^{3-}), and variations observed in COD and BOD, values in different concentrations of wastewater up to 15 days in batch culture. Ammonium decreased dramatically in the presence of *Chlorella* sp. and the microalgal consortium, whereas *Synechocystis* sp. showed a lower efficiency relative to other treatments, with respect to control. The *Chlorella* sp. will show highest removal efficiency.

3.1.2 Phosphate removal[5]: With respect to the phosphate concentration, within 15 days of culture, *Synechocystis* sp. showed a greater removal than the other treatments. While *Chlorella* sp. showed a removal percentage in very high proportional rate.

3.1.3 COD removal[5]: COD was drastically decreased in all treatments. The *Chlorella* sp. showed an average removal percentage of 64.27%.

3.1.4 BOD removal [5]: Furthermore, as BOD is directly related to the COD, so the percentage reductions were similar in both parameters. The highest percentages of reduction in BOD were observed at 80% concentration by *Synechocystis* sp. With respect to *Chlorella* sp. there was no significant difference in the reduction of BOD between different slurry concentrations.

- I. From this experiment it has been observed that better removal efficiency have been achieved by both consortia that is cyanobacteria (*Synechocystis* sp.) and micro-algae (*Chlorella* sp. *vulgaris*).

3.2 Factor affecting algae growth[6]

1) Abiotic factor—pH, Nutrient concentrations, Ions, salinity Toxicants, Light (quantity, quality), Temperature, O_2 , CO_2 concentrations.

A) Light[6]—As well as for plants, light is the source of energy which conducts photosynthetic processes in algae; in this sense, the intensity, spectral quality, and period to which the algae culture is exposed are very important parameters for well-functioning. Light is the most important factor affecting microalgal photosynthesis kinetics. In general, this is the limiting factor for most microalgal mass culture systems, since with the presence of microalgae suspension, the light penetration is affected because it is absorbed and diffused by these organisms cells.

B) Temperature[6]—Cultured microalgae species can tolerate different temperature ranges, but an intermediate value of 18–20°C is usually employed, although this depends on the culture medium characteristics and species. Adequate cultivation temperature may prompt microalgae growth, however at a high temperature, the synthesis of microalgae biomass can be hindered, due to denaturing of necessary proteins and enzymes, and inhibitory effects on cellular physiology.

C) pH [6]-The reported pH range for most of algae species is between 7 and 9, with the optimum range being 8.2–8.7, although there are some species that can be present in more acid/basic mediums. The pH variation can substantially affect the cellular processes, which prompts disruption and further collapse of the culture.

D) Nutrient[6]-The term ‘nutrient’ is generally defined as a chemical required in relatively large amount for growth, but can be used for any element or compound necessary for algal growth. Nitrogen is a key element regarding the algae structure and activities since it comprises more than 10% of their biomass. In this sense, high ammonium strength wastewaters could be effectively treated as supporting algae growth. However, very high ammonium concentrations can also have negative effects on algae growth and several researchers have reported the concentration from which the ammonium can be considered toxic to many algae species in distinct conditions.

2) **Biotic factors** -Pathogens (bacteria, viruses), competition between cyanobacteria and algae

3) **Operating factors** –Agitation, renewal rate, addition of bicarbonate and harvesting frequency

3.4 Determination of micro-algae growth [5]:

Cell number was determined by cell count with under the light microscope. Cell density was determined using the following equation: $DC = N \cdot 10^4 \cdot FD$

Where N is the average number of cells in relation to quadrants used in 104 is the conversion factor of 0.1 μ L to 1 mL and FD is the dilution factor. The specific growth rate (μ) in an exponential phase was measured using the following equation: $U = \ln(n_2/n_1) / (t_2 - t_1)$ Where N1 and N2 are defined as cell density (cells mL⁻¹) at time t1 and t2, respectively. All data were expressed as a mean and standard error.

3.4.1 Phototrophic growth rate:The formulation of the growth rate of phototrophic organisms was based on following component:

a) Ammonium: substrate for algae growth

b) Light intensity

3.4.2 Phototrophic respiration rate

3.4.3 Assumption must be considered:

Algae growth is based on ammonium as the only nitrogen source;

Phosphorus is not considered a limiting nutrient

Alkalinity is not considered a limiting factor

Microalgae cultivation and bacteria collection: mixed liquor containing nitrifying and heterotrophic bacteria with a consortium of algae, consisting mainly of *Chlorella* sp.

3.5 Result: Micro-algae growth[5]:The growth characteristics of *Chlorella* sp., *Synechocystis* sp. and consortium (*Chlorella* sp. -*Synechocystis* sp.) under the three concentrations of slurry were evaluated up to 15 days. The curves illustrate all phases of characteristic growth of micro algae batch culture. In case of *Chlorella* sp. the lag phase was not apparent in all concentrations. In the exponential phase a significantly increased cell density meanwhile, the specific growth rate μ in 40%, 60% and 80% slurry concentrations was found to be 0.13, 0.16 and 0.17 days⁻¹, respectively. In the stationary phase, fluctuations between 6 and 12 days were observed. The maximum cell density ($1.70 \pm 0.09 \times 10^7$ cells/mL) was achieved at 60% slurry concentration.

3.6 Factor affecting micro-algae growth: Although the growth rate and cell composition of micro algae is directly dependent on the species characteristics, many studies have approached the possibility to improve micro algae growth rate by adjusting the environmental conditions favorable to their growth. The most important factors related to algal growth are nutrient quantity and quality, light intensity, pH, turbulence, salinity, and temperature. The tolerated ranges and optimal values vary according to species and all the factors may be interrelated.

3.7 Advantages:

This methodology include many advantages, including low operational cost, reuse of the nitrogen and phosphorus up taken by algae as a fertilizer and the discharge of oxygenated effluent into water bodies Furthermore, the algae-bacteria synergistic effect has attracted attention and recent research has addressed its contributions to wastewater treatment, providing attractive opportunities for synthetic biotechnology.

The advantages emerge from the ability of microalgal taxa to perform photosynthetic process and achievement of nutrients exchange between microalgae and bacteria cells.

Advantages resulted from microalgae use in wastewater treatment process are represented by: good removal of coliform bacterial, contribution to greenhouse gas (GHG) mitigation through direct uptake of carbon dioxide (CO₂) from the atmosphere or flue gases from heavy industries.

The pH adjustment for flocculation has the advantage that it could minimize changes in the culture medium compared with chemically-induced flocculation.

3.7 Other conventional method and its disadvantage.

1) Symbiotic algae bacterial wastewater treatment: effect of food to microorganism ratio and HRT on the process performance [7, 8, 9].

Method: The project “Waste water treatment with symbiotic algae-bacteria-biomass” introduced a symbiosis between micro algae (*Chlorella vulgaris* sp Hamburg) and activated sludge bacteria to improve the performance of waste stabilization ponds. The effect of some operation parameters on the biomass characteristics has also been explored. Low organic loads lead to more stable flocs observed that an increase in the organic load caused an increase in the floc size.

Disadvantage: 1. Crucial step in the activated sludge process, bio flocculation still remains a not fully understood aspect. The floc structure has been characterized as a net of exocellular polymeric substances (EPS) where bacteria, bacteria colonies, inorganic particles are enmeshed
2. Total organic carbon (TOC) removal efficiency was not significantly influenced by F/M ratios, while total nitrogen (TN) removal efficiency evidenced significant differences.
3. Results showed that algae growth in the biomass was significantly influenced by HRT changes; significantly higher Chlorine concentrations in the biomass were reached for larger HRT, while no significant change was noticed for F/M variations.

2) Settleable algal-bacterial culture for municipal waste water treatment [10, 11]

Method: Flotation is a harvest method to make micro algae cells to float on the surface of the medium with dispersed micro-air bubbles and removed as scum. A commonly used flotation method in waste water treatment for algae removal is dissolved air flotation (DAF) In this system, an air compressor is used to supply fine bubbles to supersaturate flotation water. The micro algae cells are adhered by bubbles resulting in floating to the surface. Some flocculants are added to improve the harvest efficiency.

Disadvantage: It is energy-intensive and causes downstream processing of algae. An alternative method is to use surfactants to replace a compressor and saturator to create small bubbles for the algal cells to adhere. And the removal efficiency is dependent on the efficiency of the surfactants adsorbed at the bubble interface.

IV. RESULTS AND CONCLUSION

The nitrogen removal was successfully achieved by nitrification-denitrification processes from high ammonia strength waste water with addition of carbon source for the second process. The proposed model is a useful tool to determine the different interactions inside a PBR. The simulations performed for the nitrogen species and DO variations during one cycle present satisfactory results. The variations of biomass concentration and light intensity factor over the cycle are reliable and in accordance to the expectations. The model presented relatively good fitting for nitrogen removal from both real and artificial waste-water.

Wastewater is a low-cost source of nutrients for microalgae cultivation, while microalgae cultivation represents a sustainable system able to remove undesirable compounds from wastewater. In this review, we describe advancements reported in phytoremediation of wastewater. We focus on removal mechanisms of inorganic and organic contaminants, consortium interactions, different cultivation systems, and biomass applications.

The result of the study concluded that the microalgae growth - promoting bacteria (MGPB), starvation and dilution of the wastewater are the different ways used to reduce different pollutant parameter or enhance the contaminant removal and nutrient removal rate. Wastewater is a low-cost source of nutrients for microalgae cultivation, while microalgae cultivation represents a sustainable system able to remove undesirable compounds from wastewater. We focus on removal mechanisms of inorganic and organic contaminants, consortium interactions, different cultivation systems, and biomass applications. There was reduction observed in various parameters. pH reduction upto 7.5% was observed similarly in COD-62.37%, BOD-70%, NH₄- 85%, TDS- 75.215 reduction was observed.

High value can be achieved from studies that focus on cultivation of local species and microalgae consortia, and inexpensive pre-treatment. The sample of effluent was analyzed for pH, TDS, COD, BOD and Ammonical nitrogen and every month we analyzed various parameters, when analyzed each and every day on every run for 5 days.

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