



**Research Article / Araştırma Makalesi**

**MICROALGAE PRODUCTION WITH MICROALGAL SUBMERGED  
MEMBRANE PHOTO BIOREACTOR (MSMPBR) AND EXAMINING THE  
NUTRIENT REMOVAL YIELD**

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**ABSTRACT**

One of the most important and advanced treatment methods applied to decrease the damages of treated waters to receiving bodies to minimum level is the one applied by using microalgae. With the use of microalgae, the waste waters are treated at further levels and biomass is obtained to use for beneficial purposes. In this study, the nitrogen and phosphorus removal was investigated with the formation of micro-algae that was 3 days mature in terms of sludge retention time in 24-hour hydraulic retention time at 5.5 L Microalgae Submerged Membrane Photo Bioreactor (msMpBR) fed by synthetic wastewater. The solid-liquid separation from the system was made with 0.45 µm hollow fiber membrane. The concentration was started with 156 mg/L suspended solid (SS) in the reactor, and when the stable conditions were achieved, 354 (±18) mg/L was added, and the Chlorophyll-a concentration was increased from 2.44 mg/L to 11.54 (±0.30) mg/L. Maximum removal yields in this study were as follows; COD; >24%; Total nitrogen (TN); 30.30%; NH<sub>4</sub>-N; 50.08%; PO<sub>4</sub>-P; 4.54%. This study has shown that there are low TN and PO<sub>4</sub>-P removal with this system in present conditions. In order to increase nitrogen and phosphorus removal, the conditions of the operations are being changed and optimization works are continuing.

**Keywords:** Waste water, biomass, membrane, microalgae, nutrient.

**1. INTRODUCTION**

Today, although it is estimated that nearly 25% of the energy need of the global energy consumption may be covered with the energy that will be obtained with various methods from microalgae. Micro-algae may be considered as an alternative and renewable energy source instead of petroleum, which is becoming extinct day by day. In addition to this, it is also known that microalgae may be made use of in chemical, medical and food industry as raw material. Many studies were conducted in the past on specific microalgae or mixed culture algae by using broth medium at various quality and amount. In these studies, the living conditions of microalgae were minimized with various methods and the parameters like pH, light intensity, broth medium quality and light/dark cycle, and the biomass character obtained with these parameters were reported. In the light of the data obtained in these studies, it was also investigated how much the biomass, which was obtained in these studies, were suitable for the desired aims [1, 2, 3]. Although several

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broth media are used in biomass production, it is also known that purified or raw industrial and municipal wastewaters are also used [4].

It is known that there are nitrogen and phosphorus in industrial and municipal waste waters at excessive amounts. These waste waters may be purified with biological nitrogen and phosphorus removal systems; however, the removal rates cannot go beyond certain concentrations [5]. These waste waters that are discharged to receiving body cause a eutrophication problem because of the organic compounds and inorganic compounds like nitrogen and phosphorus in them [6]. The eutrophication problem that occurs in the receiving body disrupts the beneficial usage aims. There are certain methods to remove or minimize the eutrophication problem, which has become a global problem all over the world. The most prominent one among these methods is the treatment method in which microalgae are used. With this method, the nutrients that exist in treated or raw wastewaters are removed by microalgae; however, meanwhile, a biomass with a potential to produce bioenergy is obtained. This situation has paved the way to the use of microalgae as biofuel when the fact that petroleum and its derivatives are becoming extinct is considered [7]. Energy may be produced by obtaining methane in anaerobic digesters as well as biodiesel and biomass with various methods [8, 9, 10].

Specific microalgae cultures were used in many of the studies conducted on microalgae production and nutrient removal from treated or raw wastewaters for different purposes or for biomass production. The authors of these studies tried to determine the removal rates of phosphorus and nitrogen and its forms, and measure the biomass that reproduced in the system [11, 12, 13]. In these studies, which reported various removal yields, the suspended solid (SS) concentrations did not increase much. The reason for this is the failure in preventing the suspended solid from being not filtered or the excessive amount of SS after the precipitation [14, 9]. To avoid this, and to obtain output water with a good quality, and to increase the SS concentration in the system, new systems have been developed in which membranes are used. However, the number of these studies is too few when compared with the present literature. In some studies conducted with these systems, increases were obtained in the SS concentrations by using membranes with various mesh diameters, and better yields were obtained in terms of nutrient removal. However, the most striking characteristics of these studies are the lack of studies that lasted beyond 45 days, and more important than this, no sludge was removed from the system [5, 15, 16, 17]. Among the studies that were examined in the context of the present study, it was determined that Hu et al. (2015) [18] conducted a study as follows; HRT: 0.5 day and SRT: 25 days; Honda et al. (2012) [19] HRT: 1-2 days SRT: 9-18 and 36 days; and according to this SRT, sludge removal was performed and good results were reported.

In this study, the biomass production and nutrient removal yield were examined by using mixed microalgae culture with 3 days' sludge retention time in 24-hour hydraulic retention time with submerged membrane photo bioreactor from synthetic wastewater.

## 2. MATERIAL AND METHOD

### 2.1. Microalgae and Wastewater Characteristics

The mixed microalgae culture used in the study was collected from the wastewater outlets of the last settling tank of the Kayseri Organized Industrial Zone Wastewater Treatment Plant, and it was filtered and purified from huge particles. Before feeding to the reactor, samples were taken from the mixed microalgae culture, and the species were determined in microscope. In addition, during the reactor operation time, samples were collected at certain times, and species were determined in the microscope to determine the dominant species in the reactor in 3-day sludge retention time.

Synthetic wastewater was used as feeding water. Glucose ( $C_6H_{12}O_6 \cdot H_2O$ ), potassium hydrogen phthalate ( $KH_2PO_4$ ) and ammonium chloride ( $NH_4Cl$ ) were dissolved in fresh tap water

and the feeding water was prepared in this manner. The average values of the feeding water are as follows; COD: 32.89 mg/L ( $\pm 2.3$ ), TN: 18.35 ( $\pm 0.30$ ) NH<sub>4</sub>: 18.01 mg/L ( $\pm 0.38$ ), PO<sub>4</sub>-P: 8.81 mg/L ( $\pm 0.17$ ).

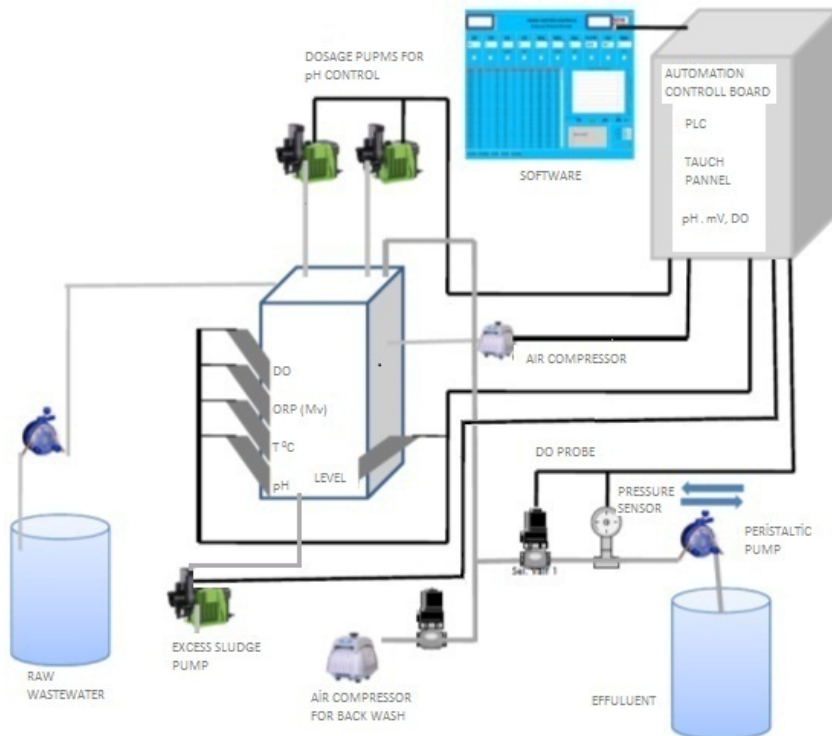
## 2.2. Laboratory-Scale Photobioreactor

The computer-controlled Microalgal Submerged Membrane Photo Bioreactor (msMpBR) used in the study was made of Plexiglas material, it was transparent, and its size was 7.1 cm semi-diameter with a 49 cm height. Reactor active volume was 5.5 L. The reactor was established to a room of 10 m<sup>2</sup> which was closed completely to daylight, and white fluorescent lamps were used at approximately 6.000 lux light intensity. The light/dark cycle was adjusted with automatic timer as 12h/12h.

In order for the light source to reach all of the SS in the reactor, and to provide carbon dioxide, non-stop air was pumped to the reactor with an air compressor. The air was given to the reactor with stable debit, and it was observed whether the system produced oxygen or not in light/dark cycle, in other words, whether microalgae survived or not.

The dissolved oxygen, pH and ORP parameters in the reactor were measured and recorded online.

The solid-liquid separation in the system was made by using the 0.45  $\mu\text{m}$  hollow fiber membranes provided from the National Membrane Technologies Research Center (MEM-TEK). The hollow fiber membranes with 20 l/m<sup>2</sup>-sa flux were used. The schematic view of the msMpBR is given in Figure 1.



**Figure 1.** The schematic view of the msMpBR.

### 2.3. Analyses

The SS analyses in the reactor were made according to SM 2540 D [20]; chemical oxygen need was determined according to SM 5220D [20]; total phosphorus analyses were made according to SM 4500-P. D [20]; chlorophyll-a analysis was made according to Becker E. W. (1994) [21]. Total nitrogen analyses were made with LCK138 (Hach-Lange) kit in DR2800 spectrophotometer. NH<sub>4</sub> analyses were made with Merck Brand Test kit in DR2800 spectrophotometer.

### 2.4. Continuous Cultivation in Photo Bioreactor

The peristaltic pump connected to the membranes in the reactor was operated to obtain 8-minute absorption with 4-minute re-wash.

The temperature of the reactor was kept stable at 23°C (±2) with a heater.

1833 ml sludge per day was taken from the 5.5 L reactor in 24-h hydraulic retention time in order to obtain 3-day sludge age. This was removed with the help of a pump within 24 hours. The system was operated for 37 days non-stop.

## 3. RESULT AND DISCUSSION

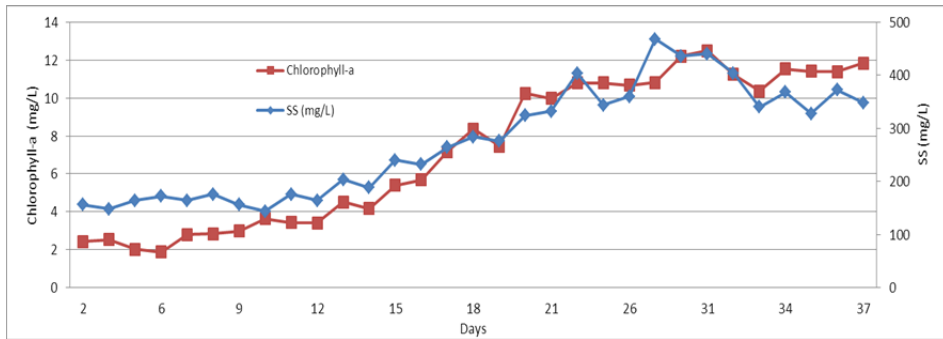
### 3.1. Microalgae Species in Mixed Culture

It was determined that the *Amphora sp.* was dominant in the sample when it was taken into the reactor. After 16 days, it was observed that *Amphora sp.* decreased at a significant level; however, *Chaetophorous sp.* became dominant. At the end of the 26<sup>th</sup> day, it was determined that *Chaetophorous sp.* constituted one half, and *Navicula sp.* constituted the other half. On the last days when stable water quality was obtained, the algae that were observed on the 26<sup>th</sup> day were observed.

### 3.2. Microalgae Production

The reactor was started with 156 mg/L SS concentration, and increases were observed after the acclimation days, which are given in Figure 2. After nearly 20 days, the SS concentration in the system reached 200 mg/L. The basic factor here is the fact that there were no SS that fled out from the reactor from the hollow fiber membranes that had 0.45 µm mesh. When the studies conducted previously were examined in the literature, it was observed that this value was acceptable [17, 18]. In the last stages of the study, the SS concentration was determined as 354 mg/l (±18). When the results were compared with the conventional reactors, it is clear that the membrane had contributions to the increase in the SS concentration [22, 23].

The Chlorophyll-a concentration was initially 2.44 mg/L, and it increased at a rate of 30% at the end of the 10<sup>th</sup> day; and in stable stage, it increased at a rate of 4.73-fold and reached 11.54 mg/L (±0.30) in average. This value is a low value when compared with the value obtained by Gao et al. (2015), which was reported as 24.5-28.6 mg/L [17].



**Figure 2.** The SS and chlorophyll-a concentration change in the reactor.

The pH of the reactor was between 6.7-7.9; and for this, no additional chemicals were added. The dissolved oxygen concentration was between 6-7 mg/L, and the ORP varied between 140-190 mV.

### 3.3. Nutrient Removal

The nutrient influent and effluent concentrations in the reactor were evaluated in terms of system yield. The average composition of the raw wastewater fed and treated is given in Table 1. When a general evaluation is made, it is observed that maximum removal yield in total nitrogen was 30.30%; and this rate was 4.54% in PO<sub>4</sub>-P. Singh and Thomas (2012) conducted a study and reported 35-75% yield in inorganic nitrogen forms; and 60% in PO<sub>4</sub>; Gao et al. (2014) [15] reported 60% yield in TN; and more than 75% in TP; Gao et al. (2015) [17] reported 64.9% yield in total inorganic nitrogen, and 85.2% in PO<sub>4</sub>-P. When compared with these results, the removal yields are lower in our system. 15% of the total nitrogen was converted into ammoniac (NH<sub>3</sub>-N) in the study conducted by Gao et al. (2014) [15] because of the increase in the pH, and this amount joins the air with ventilation. In addition, it is known that nitrogen is removed according to the algae cell assimilation instead of nitrification processes in aerobic conditions by converting into nitrite and then into nitrate, which is also the case in biological treatment processes. Similarly, it was also reported that phosphorus was removed when microalgae were taken into their bodies.

It was determined that the COD removal in the reactor was more than 24%. When the fact that microalgae use CO<sub>2</sub> as carbon source is considered, it appears that this value is acceptable.

**Table 1.** Removal yields and influent and effluent concentrations in the reactor

Parameter	Influent	Effluent	Efficiency (%)
COD (mg/L)	32.89 (±2.3)	< 25	> 24
TN (mg/L)	18.35 (±0.30)	12.80 (±0.80)	30.30
NH <sub>4</sub> -N (mg/L)	18.01 (±0.38)	8.99 (±0.90)	50.08
PO <sub>4</sub> -P (mg/L)	8.81 (±0.17)	8.41 (±0.06)	4.54

### 4. CONCLUSION

In the light of the present findings, the use of 0.45 μm hollow fiber membrane in the reactor for the purpose of solid-liquid separation is extremely interesting in terms of preventing the SS

being not filtered in the outlet water, and in terms of the increase in the concentration of the microalgae in the reactor. In addition, by using mixed microalgae culture instead of specific algae species, the decrease in the total nitrogen and phosphorus concentrations in synthetic wastewater in 3-day sludge retention time with 24-hour hydraulic retention time cast a light on the studies. Studies were conducted in the past to optimize this in a level that may be compared with TN and PO<sub>4</sub>-P removal in classical nutrient removal methods. The aim is to obtain non-problematic discharge of the waste waters with the nutrients in them at non-harmful rates (Total phosphorus <2 mg/L and Total nitrogen <10 mg/L). This was not achieved in 24-day HRT and 3-day SRT. In order to increase nitrogen and phosphorus removal, the conditions of the operations are being changed and optimization works are continuing.

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