Design and Production of an Algal Biofilter for Industrial Wastewater Treatment

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Received: 2022-07-12 Revised and accepted: 2022-09-25

Abstract

The increasing need for water resources and other factors in reducing these resources, along with the health and environmental problems of wastewater, make it clear that our linear water economy must evolve into a resilient circular water economy, where water is continuously reused and "contaminants" become the feedstocks for other economically valuable processes.

Biofilters as an important emerging technique, which utilizes biological living things as catalysts to harvest valuable components.

Cyanobacteria and microalgae's ability to be mixotrophs provides a competitive advantage against bacteria and fungi to be used in biofilters. Due to the reduction of environmental nutrients, heavy metals, and pathogens, the oxygen productionfor aerobic organisms, and the consumption of carbon dioxide, microalgae play a prominent role in purification processes.

In this research, by examining different types of algal species and immobilization methods, the appropriate species were chosen for biofilters production. The performance of the optimally produced biofilter to reduce the pollution indicators of the industrial effluent was investigated.

The results show the appropriate performance of the biofilter produced with AFC008 and AFC110 species to perfect removal of nitrate and phosphate and 76% COD reduction and 79% reduction of BOD in less than a week (along with aeration pretreatment).

Keywords: Biofilter, Industrial Wastewater, Phycoremediation, Wastewater Treatment, Algae

Introduction

Despite of regional nature, global processes exacerbate water stress. These global-scale influences include climate change, population and economic growth, food production, and trade. Because of these global-scale influences and the importance of the related problem, it is profitable to find a way of evaluating the efficiency of any policies aimed at mitigating water stress at the global level (Wada et al., 2014). So

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our linear water economy must evolve into a resilient circular water economy, where water is continuously reused and "contaminants" become the feedstocks for other economically valuable processes (Mauter and Fiske, 2020).

The environmental and health problems of wastewater, such as the role of nitrates in blue baby disease and methemoglobinemia (Jain et al., 2010), the effect of phosphorus in Eutrophication (Le Moal et al., 2019), the effect of various pathogens in diseases such as typhoid and dysentery, are well known. In contrast, the recent changes in the public perception of wastewater have led to several paradigm shifts: i) Water reuse considers wastewater as a water resource rather than a hazardous waste, ii) Wastewater-based epidemiology considers wastewater as a source of information regarding the overall health of a population through the analysis of specific biomarkers, iii) Circular economy through the implementation of treatment processes aiming to harvest valuable components such as precious metals or produce valuable goods such as biofuel (Villarín and Merel 2020).

According to the Country's Water and Sewage Engineering Company Report (2020), only 2078 million cubic meters of wastewater were collected out of 6647 million cubic meters of produced water. However, assumption of an 80% water-to-wastewater conversion factor, the produced wastewater estimation will be 5318 million cubic meters. Meanwhile, industrial wastewaters with extreme quantitative and qualitative fluctuations, including BOD and COD chemical parameters, harmful and toxic chemicals, and heavy metals are critical. Biochemical oxygen demand (BOD), suspended solids, nutrients (NO_3, NO_2, NH_4, PO_4), coliform bacteria, and toxicity removal are the main goal of perfect wastewater treatment. Common wastewater treatment processes include physical, chemical, and biological treatments. Chemical treatment is often inefficient, and expensive, which leads to toxic waste production due to physical or chemical replacements. Amidst, the Bio-based techniques, biofilters are found to be suitable, sustainable technology, and easy to operate for various contaminants removal in the aquatic environment (Pachaiappan et al., 2022). Biofilters usage is an important emerging technology that utilizes biological living things as catalysts for algae, bacteria, plants, protozoa, viruses, yeast, and mixed microbes (Delhomenie and Heitz, 2005). In simple terms, the biofiltration process can be represented as follows (Bressani-Ribeiro et al., 2,018).

Pollutants + Photosysthetic creatures + Oxygen → Biomass + Water + Carbon dioxide

The biofilter consists of a microbe immobilized on the substrate material. Immobilization of the microbes could be done by natural attachment or artificial immobilization of microbes to the biofilter bed materials (Baltrėnas et al., 2020). The performance of biofilters depends upon the microorganism. They are responsible for the phase transformation and degradation of contamination in input-polluted air or water. Several parameters such as Biological organisms, biofilter bed, Supply of nutrients, pH, operating temperature, moisture contents, and pressure drop determine the efficiency of the biofiltration process (Pachaiappan et al., 2022).

There are several areas of bioremediation as mycoremediation by fungi and phycoremediation by algae depending on the organism or microorganism used. Bacteria as bioremediation agents have more advantages due to their adaptability and rapid growth, higher surface-to-volume ratio, potential ability to horizontally transfer genes of catabolic enzymes, and ease of genetic manipulations (Subashchandrabose, Ramakrishnan et al. 2013). Cyanobacteria and microalgae can live as autotrophs, heterotrophs, or mixotrophs. The ability to be mixotrophs provides a competitive advantage against bacteria and fungi to decompose organic pollutants (Subashchandrabose et al., 2013).

If algae are used, the environmental trinity is established. It meant wastewater treatment, CO_2 stabilization, and biomass production. Treatment is accomplished under light conditions without oxygen demand, and biologically biomass is produced by carbon stabilization. Phycoremediation has many applications; removal of food from municipal wastewater and streams rich in organic matter. Removal of xenobiotics and food compounds with the help of algal absorbents, treatment of acidic and metal wastes, CO_2 consumption, transformation, and decomposition of xenobiotics. Detection of toxic compounds with the algal biosensors' assistance (Rawat et al., 2011).

Due to the reduction of environmental nutrients, heavy metals, and pathogens, the production of oxygen for the consumption of bacteria and aerobic organisms, and the consumption of carbon dioxide, microalgae played a prominent role in purification processes (Muñoz and Guieysse, 2006).

The heavy metals are removed by various mechanisms such as rapid surface absorption (biosorption) and intracellular absorption depending on metabolism (bioaccumulation).

Several environmental or nutritional factors are effective on the growth of algae and consequently on the efficiency of the treatment process. The pH value determines the solubility of CO_2 in the culture medium, and its high values are responsible for the depletion of NH_4-N (Bouldin et al., 1974) and the deposition of PO_4-P (da Silva Cerozi and Fitzsimmons 2016). Therefore, the mechanisms of carbon concentration and nitrogen and phosphorus removal by microalgae cells are most affected by the pH value. The light intensity required by algae is lower compared to higher plants. Photosynthetically active solar radiation (400 to 700 nm), used as an energy source for algae, is 43-45% of the total incoming radiation. Theoretically, the maximum conversion of this radiation to carbohydrates is 27% (Prajapati et al., 2013). An increase in temperature leads to higher metabolic activities and nutrient absorption, as well as a decrease in the solubility of some nutrients such as CO_2 and NH_4-N (Gonçalves et al., 2017).

The main sources of carbon include atmospheric CO_2 or the output of industrial gases or soluble carbonates. Although carbon is often 60% of the algae's total nutrient requirement, levels higher than optimal level inhibit the algae growth (Goldman et al., 1972, Chen and Xu, 2021).

Microalgae plays a crucial role in the treatment of the third stage of domestic wastewater in large or small ponds which related process can remove phosphorus and nitrogen in a brief period, even about 1 hour. In addition to the basic demand for nitrogen and phosphorus, small amounts of micronutrients such as Na, Mg, Ca, Mn, Zn, Cu, Fe, and Mo are also needed for optimal algae growth.

The proper species was investigated by examining different types of algal species and immobilization methods for biofilters production in this research. In the next stage, the performance of the optimally produced biofilter was examined to reduce the pollution indicators of the industrial effluent.

Materials and methods

Wastewater collection

Exemplification of industrial wastewater was investigated between May and July 2017. The Effluent Analysis is indicated in Table 1. The effect of sterilization on pathogens removal in the growth rate of stabilized microalgae and the bead's decay in 70% and 100% dilutions wastewater were investigated. The growth of free microalgae in non-sterile wastewater was also analyzed as a control in the experiments. Changes in the chemical characteristics of the effluent such as pH and EC were recorded at the end. The samples were cultured in a volume of 100 ml, 150 rpm, and 3000 lux illumination with three replications.

Screening and strain selection

After four days of continuous aeration, the green effluent was cultured on agar plates to purify the samples. In addition to conventional culture media (BG_{11}, N8, BBM, WALN, F26, BG_{110}), 1% cefotaxime antibiotic was used to control the growth of pathogens. Finally, after proving the purity of the species with a light microscope DNA extraction, and final identification were done.

In the first phase four strains out of tens of ones screened, which were cultivated in actual wastewater, were selected and cultivated in four different dilutions of wastewater with similar illumination.

Based on the 8-day growth curves, microalgae with the highest growth in high effluent concentrations were chosen for stabilization experiments. 100 ml samples were cultured at 150 rpm, constant illumination of 3000 lux with three replications. The absorbance was recorded at a wavelength of 680 nm every other day. Cyanobacterial genomic DNA was extracted using a DNA extraction kit (Thermo ScientificK0512, Lithuania) according to the instructions, and the quality of extracted DNA was evaluated by electrophoresis on 0.8% agarose gel. The genus of the extracted samples was the green alga morphologically. Gene duplication was done through 16S rDNA and 18S rDNA sequences to determine the genus accurately. The PCR reaction was performed on the sample's DNA with a thermocycler (Corbet, Australia) and a pair of specific primers in a volume of 50 microliters. To observe the band pattern of the PCR product, one hour of electrophoresis was performed on a 1% (w/v) agarose gel at 70 V. 1Kb molecular size marker (Fermentase GenRuller SM0373) was used to determine the size of PCR products. Photography was taken by GelDoc device (UVP, USA).

After the cultivation of the isolated species isolated from the wastewater, two species, AFC 008 and AFC 111, which had the highest growth rate and resistance in the wastewater, were selected for future experiments.

Immobilization

Alginate was used as a fixation substrate for algae stabilization due to its cheapness, high efficiency, and biodegradability. Variable efficient factors in the preparation of matrices are as follows.

Alginate concentration (2 and 3%), solidification solution 2% ($BaCl_2$, $CaCl_2$), adding some salts ($CaCO_3$ 1%), and using other polymers (PVP, Chitosan and 3-methyl hydroxy cellulose) in combination with alginate or to cover the Beads.

Secondary Polymer was used according to reports on the coating of calcium alginate microcapsules by polycations for various purposes, such as the immunoprotective of carriers in cell transport, enzyme stabilization, and drug release systems. It has been demonstrated that the attachment and stability of alginate-polycation capsules depend on the composition of the alginate gel and the molecular weight, flexibility, and charge density of the polycation (Thumvijit et al., 2013).

Chitosan in concentrations of 1% to 0.1%,

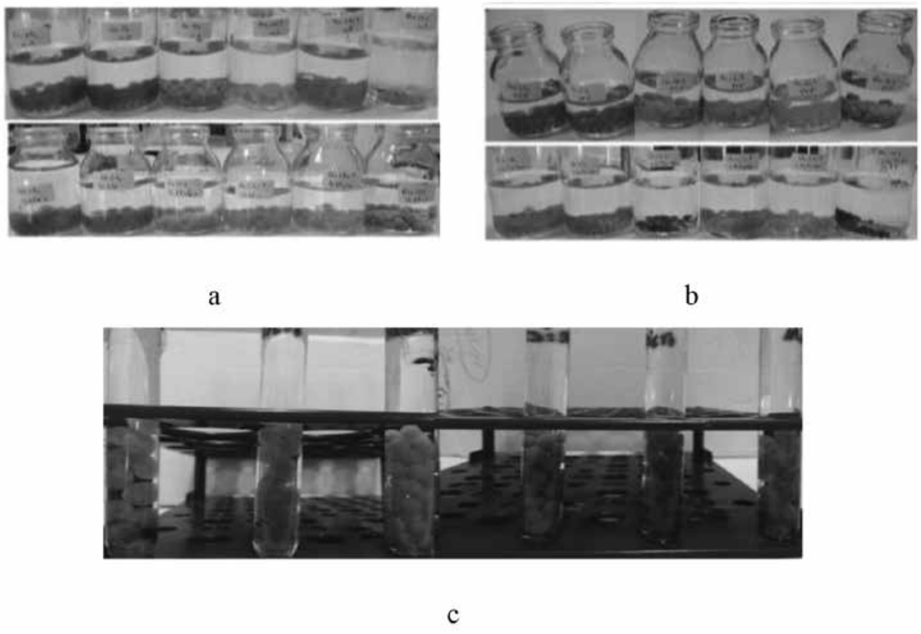

Fig. 1 a, b, c. The appearance and coating of beads

PVP 1%, and hydroxymethyl cellulose 1% in combination with 1% and 2% alginate were added after alginate was dissolved. Then algal biomass was added. The viability of the beads was checked with 48 hours of pretesting in sewage and radiation, and the appropriate substrate was selected. As in all experiments, beads without algae were used as controls. The appearance and coating of beads are showed in Figure1.

Alginate calcium beads are highly unstable when exposed to chelators such as phosphate, lactate, citrate, or non-gel cations such as sodium or manganese ions. Introducing calcium into phosphate and forming calcium phosphate causes disorder in the beads' structure. Therefore, it is necessary to check the stability of formed beads in different concentrations of phosphate buffer. In addition, in some studies, sodium chloride ions have been mentioned as a disintegrating agent. Different phosphate buffer concentrations were used for three days, and a mixture of 1% sodium chloride was used on the shaker for two days to check the disintegration of the beads along with phosphate ion treatments. Beads with a resistant structure were selected as stable patterns after several days of exposure to high phosphate concentrations.

The optical density on the 8th day of cultivation in the wastewater was recorded along with the effluent color and the color and health status of the stabilization structure so that the microalgae selected from this section could be used for forming experiments.

Shaping of stabilization structures

In the first part of the biofilter production, the microalgae stabilization formulation was obtained according to factors such as the algal survivorship and its active metabolism to absorb nutrients and gas exchange, the stability of this structure in the presence of anions and various chemical agents in actual wastewater. Then different immobilization structures, like bead, sheet, sponge, and net, were compared to establish the obtained composition.

Bead

Titration of alginate suspension in Barium Chloride solution produces uniform seeds with the same diameters called beads. The wastewater was treated with 2 to 4% of these beads.

Sheet

A plate or sheet refers to a fixed flat structure that covers a wide area and is used in the industry for purification in pools and bioreactors. Considering the stability of the structure, they should be designed as thinly as possible both in terms of the possibility of food and gas exchanges and economic issues (the amount of alginate).

To manufacture the sheet, two thicknesses of alginate suspension were poured on the bottom of the container. Then Barium Chloride solution was added and kept at room temperature for one day. Two concentrations of selected microalgae were also used in this experiment. In another attempt to prepare the sheet, the solution was poured into hollow molds, and the solidification solution was slowly added. The formed sheet had a uniform and acceptable diameter.

Sponge

Due to the resistant infrastructure of sponges, slices were prepared and immersed in alginate suspension until they were completely saturated. After washing with Sodium Chloride solution and distilled water, they were placed in wastewater (70 and 100%) and distilled water. After one week, the degree of opening of the structure and color change of the effluent was checked.

Net

In this experiment, pieces of plastic mesh plates were used. Four treatments were considered for two selected algae and a consortium of the two algae together with purified Cyanobacteria at the same ratio. After adding alginate-containing algae, the filters were placed in a $BaCl_2$ solution for 4 hours. Then, it was washed with $NaCl_2$ solution and kept in distilled water for an average of 3 hours until the beginning of the treatment.

Wastewater treatment and analysis

Industrial wastewater was aerated for two days. After straightening with a cleaning cloth, it was divided into 10 L containers and were transferred to the laboratory. The filters were suspended in the effluent with the help of a string for three weeks at 25 ± 2° C and illuminated at 16: 8 h light/dark, and aerated constantly and slowly.

The levels of BOD, COD, PO_4^{3-}, and NO_3^- were analyzed in the laboratory (RehanAzma Iranian). The primary effluent samples were tested immediately after preparation, after two days of aeration (before treatment), and on days 6, 13, and 21 after treatment with screen filters. In addition, the final sample was sent to this laboratory for microbial analysis. ICP analysis of all the elements of the untreated wastewater was also performed in Aria Chemical Laboratory.

Results

Wastewater effluent

The results of the effluent analysis using ICP-MS and ICP-OEC devices are shown in Table 1. Among heavy metals, chromium showed the highest amount of 1.7 mg/L in the wastewater sample.

Among other metals, the amount of sulfur,

Table 1. Analysis of the main elements of the treatment plant effluent

Element	S[1]	Ca[23]	K[4]	Mg[5]	Si[6]	P[7]	Na[8]	B[9]	Sr[10]
Concentration (ppm)	902	295	82	62	26	73	5.1	1.9	1.8
Element	Cr[11]	Li[12]	Cu[13]	Fe[14]	Al[15]	Zn[16]	Ni[17]	Mn[18]	B[19]
Concentration (ppb)	1700	350	260	215	200	90	60	30	20

[1]Sulfur, [2]Calcium[3], Potassium, [4]Magnesium, [5]Silicon, [6]Phosphor, [7]Sodium, [8]Boron, [9]Strontium
[10]Chromium, [11]lithos, [12]Cuprum[13], Ferrum, [14]Aluminum, [15]Zinc, [16]Nickel, [17]Manganese, [18]Barium

Table 2. Analysis of the acidity and EC of the effluent and Cr and BOD/COD

Test	Unit	Value (1397)	Value (1398)
BOD	ppm	5200	3736
COD	ppm	10000	7950
pH	-	8.1	8.3
TSS	ppm	589	-
Cr	ppb	1700	2300
N	ppm	780	-
EC	mho/cm	27250	-
Total Caliform	ppb	-	< 3

calcium, potassium, and magnesium due to the functional role of acids and bases in leather-making processes is considerable.

The results of the output acidity and EC are shown in Table 2. According to the high BOD/COD, re-sampling was done to re-examine BOD/COD, Cr, and total microbial load.

Isolated microalgae

Several microalgae samples grew in the aerated effluent, mainly belonging to the blue-green algae and green algae. The absorbance of four selected species in four dilutions of 10, 30, 50, and 70% at 680 nm is shown in Figure 2. Finally, After eight days of cultivation of the isolated species in different wastewater dilutions, two species with the highest growth rate were chosen for this study. As it is illustrated in Figure 2, all species had better growth in 10 and 30% dilutions. The genomic DNA band in agarose gel electrophoresis, the specific band obtained from PCR for the 16S rRNA gene, and the 18S rRNA gene are presented in Figure 3 a-c, respectively.

Algal cultivation

Cultivation of the two selected species in 100% and 70% effluent dilutions and sterile and non-sterile conditions indicated the stability and survival of willows in species AFC008 compared to species AFC010. After six days, the chemical characteristics of the effluent, especially the pH were between 7.9 and 8.2 and did not change much.

The plate shape of alginate initially led to the formation of heterogeneous sheets. For this reason, other sections of the sheets were exposed to effluent. Due to the high agitation intensity, dissolution started after one day, and free algae were released into the effluent. An aeration system with a lower stirring speed should be used in future studies.

Contaminants reduction

After two days of aeration, COD and BOD values decreased from 2600 and 1300 to 1700 and 760 mg/L, respectively. After that, by adding algae treatments in the form of two separate species and a combined consortium, COD and BOD decreased by about 55%

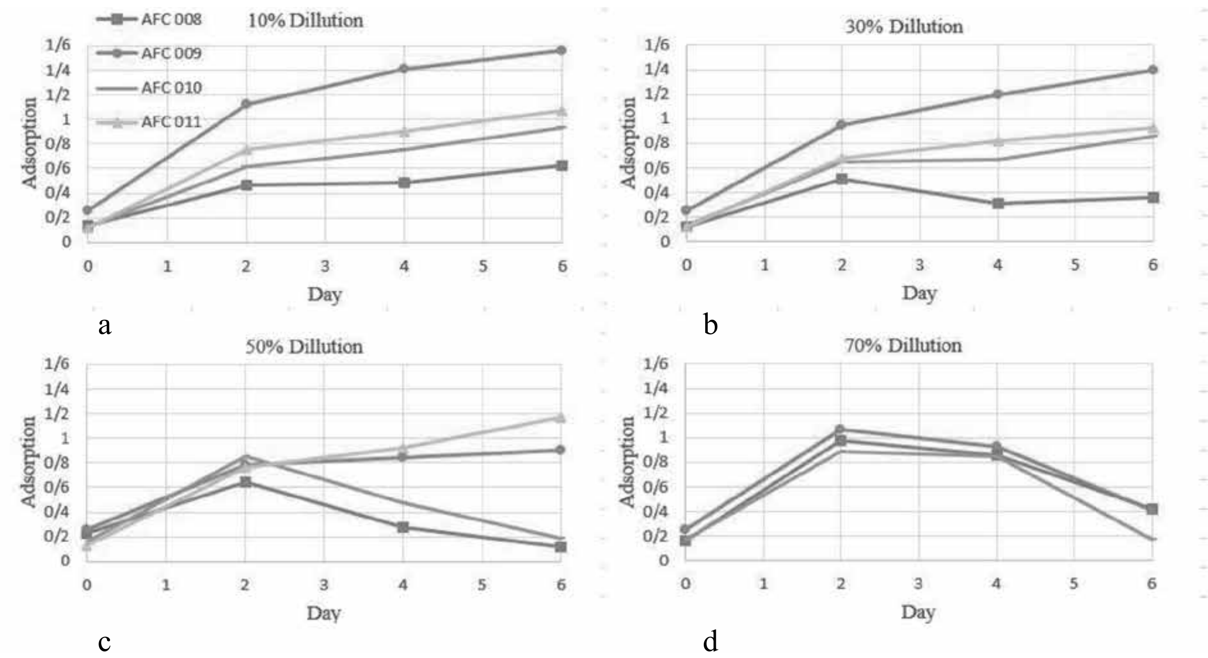

Fig. 2 a, b, c, d. Several samples of microalgae grew in the aerated effluent; the absorbance at 680 nm wavelength for four selected species in different dilutions of 10, 30, 50 and 70%

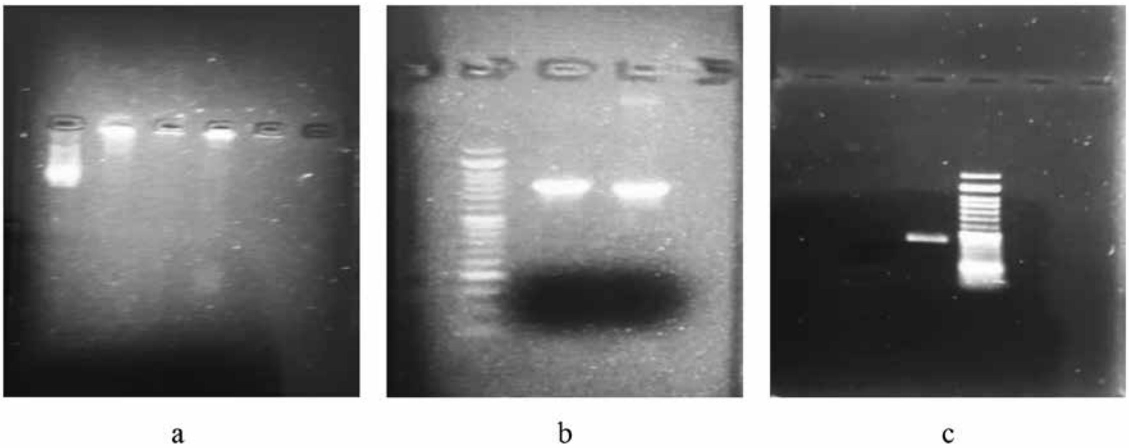

Fig. 3. *(*a) The bands obtained from genomic DNA electrophoresis (Chlorella sp.), (b) specific band obtained for 16S rRNA gene (*Chlorella sorokiniana*), (c) specific band obtained 18S rRNA gene (*Chlorella* sp.)

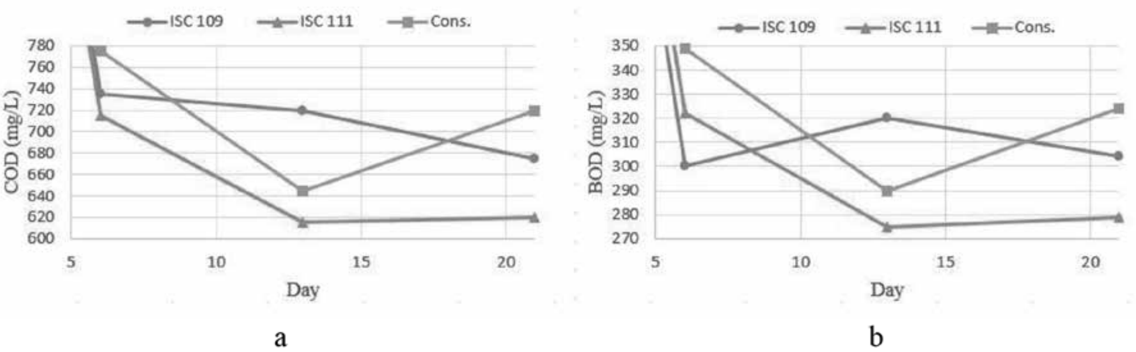

Fig. 4. BOD (a) and COD (b) reduction by algal treatment (initial value: BOD= 760, COD=1700 mg/L)

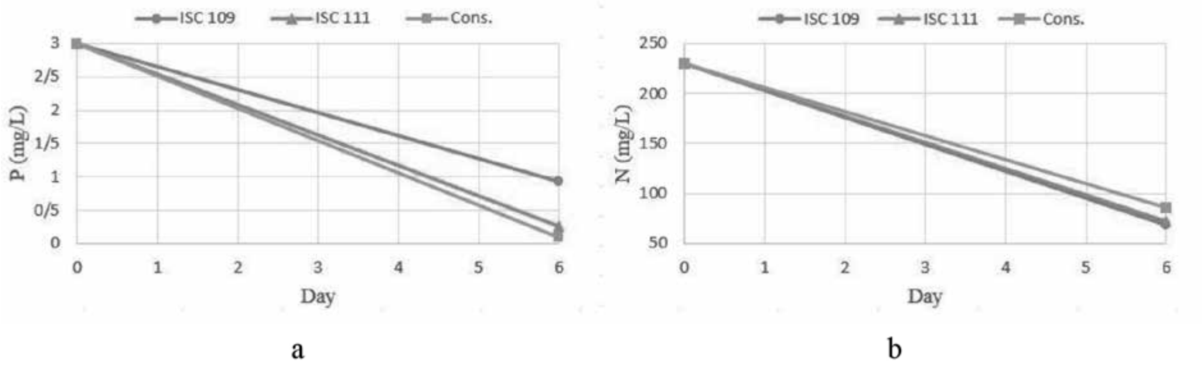

Fig. 5 a, b. Nitrate and Phosphate reduction by algal treatment. (initial value: NO_3= 230, PO_4=3.00 mg/L)

during the first six days. The other values up to day 21 have changed according to Figure 4. The highest reduction of COD and BOD (about 68%) was achieved by the strain SC 111 on the 13th day.

Due to the relationship between BOD and COD with nitrate and phosphate reduction, and their slow decline after the sixth day, phosphate and nitrate values were measured on the sixth day. However, phosphate and nitrate values increased two days after initial aeration from 2.55 and 190 to 3 and 230 mg/L, respectively. The separation of some sediments, dissolution of phosphates in particles, and release of nitrate reserves in wastewater sediments may be the reasons for this increase. The highest reduction of phosphate and nitrate was achieved by consortium and ISC109, respectively. The reduction values are shown in Figure 5.

According to the report of Rehan Azma laboratory, the amount of total and Fecal coliform in the effluent was lower than the standard release limit. The pH and EC levels in the effluent sample after two days of aeration were 8.1 and 5050 mg, respectively, which did not change significantly during the treatment with algae.

Algae AFC 010 was separated from the filter in the first days and grew abundantly in the effluent. The consortium sample, which contained AFC 010 algae, was released in the wastewater a few days after sample 2. After two weeks, other algae were released into the environment and grew both on the biofilter and in the water environment. Therefore, according to the process of reducing nutrients, the time of one week is optimal.

Discussion

Among more than 1000 algal taxa (240 genera, 725 species) that have treatment potential, the most widely used include *Euglena*, *Oscillatoria*, *Chlamydomonas*, *Scenedesmus*, *Chlorella*, *Nitzschia*, *Navicula*, *Stigeoclonium* (Abdel-Raouf et al., 2012).

The use of native microalgae is suggested in conditions where the climate changes in the region and the possibility of competition is high.

Cultivation of several algal species with special metabolic abilities such as mixotrophy, the ability to bind to specific metals, the tendency to use a specific form of nitrogen compounds and eutrophy improves the total treatment capacity when exposed to fluctuating effluent in flows. For example, the superiority of freshwater (*Botryococcus braunii*, *Chlorella saccharophila*) and saltwater (*Dunaliella tertiolecta*, *Pleurochrysis carterae*) microalgae, without symbiotic bacteria, for carpet factory wastewater treatment has been reported (Sriram and Seenivasan, 2012). Also, the problems of monoculture in the presence of various organic and inorganic compounds, as well as toxic substances and microbial communities are mentioned (Fouilland, 2012). In this study, algal consortium cultivation in immobilized conditions is as effective as single cultivation in reducing wastewater pollutants within one week.

In this research, the appropriate ability to grow and absorb nutrients and some heavy elements by different species of *Chlorella* microalgae are in line with the successful results of other studies in removing or absorbing a wide range of pollutants. Effective biosorbents for the absorption of heavy metals using algal biomass have been produced on an industrial scale, of which ALGASORB and AER are examples. Bioabsorption in brown and green algae has been considered due to the presence of alginate in their cell walls, which have functional groups such as carboxylic acids (Mahmoud and Mohamed, 2017) (guluronic and mannuronic acid) as metal binding sites through electrostatic forces and complexation (Blanes et al., 2011). The results of removing copper, nickel, and cadmium metals from drinking water with the immobilized structure of chlorella in alginate (Petrovič and Simonič, 2016) and the higher tendency of alginate beads for chromium compared to lead and copper (Sriram and Seenivasan, 2012) are examples of these studies.

The mechanism of metal absorption in microalgae includes fast surface and Intracellular absorption and prolonged absorption inside the cytoplasm dependent

Table 2. Main binding groups in brown algae

Binding chemical group	Ligand atom	Biopolymer
Carboxyl	Oxygen	Alginic acid
Thiol	Sulphur	Amino acids
Sulfonate	Sulphur	Sulfate polysaccharides, fucoidan
Amine	Nitrogen	Amino acids, peptidoglycan
Amide	Nitrogen	Amino acids

on intracellular metabolism (Kim, 2011). The carboxyl groups of cell wall polysaccharides play a key role in the absorption of heavy metals by algae or cyanobacteria. Other functional groups like sulfonate and amino have a lesser role in this field (Olguín, 2003). Functional groups in the algal absorption of brown algae are listed in Table 3.

Immobilized culture systems have been introduced as an alternative to suspended cell culture systems to overcome the problems of expensive and time-consuming harvesting methods (Aslan and Kapdan, 2006; He and Xue, 2010; Pires et al., 2013). Natural immobilization is based on the inherent ability of cells to attach to a specific surface. Artificial or active immobilization technique includes adsorption, entrapment in liquid-liquid emulsion, sequester in a semi-permeable membrane, covalent attachment, and entrapment in polymers (He and Xue, 2010). The current methods to stabilize microalgae are cell entrapment in a polymer matrix, usually alginate and carrageenan, and cell adhesion and biofilm formation on a solid surface (Eroglu et al., 2015).

Al-Rub et al. (2004) reported that the immobilization of *Chlorella* algae increased the absorption of Nickel from the environment. Lau et al. (1997) studied the growth of *C. vulgaris* in the carrageenan alginate matrix and showed that it does not decrease. They also demonstrated that the immobilization of the cells causes more chlorophyll production than the free cells. However, some reports have mentioned the higher potential of metal absorption in free algae compared to immobilized algae. Changing the wall structure during immobilization and lack of access to a part of the wall surface caused by gelling materials may be barriers to absorption (Sarada et al., 2006). Some studies have compared the increase in biomass efficiency and pigment and lipid content with the high cost of the immobilization matrix (Christenson and Sims, 2011).

De-Bashan et al. (2004) reported using the *C. vulgaris* fixation method in beads alginate matrix. *C. vulgaris* removed 93% of NH_3-N within eight days in synthetic wastewater treatment (De-Bashan and Bashan, 2010). According to this research, algae immobilization can effectively remove nutrients from wastewater. The nutrients are first absorbed on the surface of the matrix, then penetrate the matrix, and are continuously absorbed by the cells (Tam and Wong, 2000).

In this research, an alginate bed was used to immobilize algae cells that facilitate algal harvesting, and better control the effluent current changes. Based on this, immobilized microalgae in undiluted wastewater increased compared to free forms that could not survive in diluted wastewater by 70%. We also tried to increase economic efficiency by using the minimum amount of alginate. However, in this study, the cyanobacterial forms of *S. aeruginosus* and *L. fragilis* could not show stable immobilization structures, and the alginate structures were lost after a while.

Due to the many reports of the high

potential of alginate and the properties of this algal metabolite, this material was used in this research. The results showed that continuous shaking of alginate, especially in high volumes, could create a uniform suspension. Also, at least one hour of heating in the temperature range of 70-80° C has been confirmed as the main factor of polymerization resistance in other studies (Sarada et al., 2006). Because of using different polymers, the beads created in this research had a diameter of 1 to 4 mm with the external and internal application of chitosan, respectively. Their desirable size is listed between 0.7 and 1.5 mm depending on the size of commercial resins to remove metal ions (Sarada et al., 2006).

The bead's surface is one of the most significant absorption factors. For example, the exit of carbon dioxide gases and the development of more pores in the bead's structure lead to an increased cadmium absorption capacity (McGinn et al., 2012). In this study, it has been tried to create thin and resistant plates to increase access levels. Double ions such as Calcium and Barium play an influential role in the consistency and strength of alginate-based structures. According to some studies, the morphology of Calcium and Barium alginate beads has shown the same appearance and behavior in the environment (Ibáñez and Umetsu, 2002). In this study, the Barium influence on the alginate strength and algae structures was confirmed.

Ibáñez and Umetsu (2002) reported that the stability of alginate-chitosan capsules depends on the amount of chitosan binding to them (Ibáñez and Umetsu, 2002). In the one-stage production of the beads, the alginate solution is headed directly into the chitosan solution. All the chitosan was deployed as a thin layer on the surface. These beads are much weaker than the wills made in two steps in chitosan and calcium chloride solutions. In 2-stage production, the connection is 100 more than 1 stage production (Thumvijit et al., 2013). Chitosan's absorption capacity is reported to be 5 to 6 times compared to chitin. That is mainly due to amino T-distillation groups, hydrophilic properties of hydroxyl factor groups, and the flexible structure of the polymer chain. This study also showed the presence of chitosan in the alginate composition when used as an internal application. Although it was effective in increasing the stability of the immobilization systems, algae biomass was destroyed.

Gradual changes in the environment acidity affect the solubility of ammonium or phosphorus in the medium, and its high levels lead to ammonium deficiency or phosphate deposition. Acidity stimulates between 9 and 11 phosphorus deposits in the form of calcium phosphate (Tam and Wong, 2000). The structure of the beads in the pH range of 5 to 9 is stable. This structure does not show very high resistance at pH above nine and less than three. Alginate beads begin to break at pH 11, and treatment with intense acid solutions resulted in the loss of stabilized biomass (Thumvijit et al., 2013).

The initial concentration of pollutants can be one of the main factors in the output of work and standards, especially when there is a metal composition in the environment. In one study in the stabilized yeast in alginate, as a result of chromium concentration (VI) from 200 to 1000 mg/l, chromium absorption percentage showed a significant decrease (Mahmoud and Mohamed, 2017). The difference between algae growth at different wastewater concentrations confirms that in this study.

The presence of multiple metals leads to competing in active positions and attracts different absorption of a single element. The ability to interfere with other elements is also different. For example, 1.5 mM of iron reduces the cadmium's uptake by 24%, but the same cadmium concentration causes a 45% reduction in iron absorption by *Sargassum sp*. (Sarada et al., 2006). Alginate-containing alginates almost completely absorb Zinc and nickel solutions, but when the zinc was added to the nickel solution, both absorption was reduced by free and immobilized algae. Competition on the same algae connection stations has led to a reduction in absorption (Mihranyan, 2011). Similar results are observed on zinc and cadmium (Bootsma et al., 2004). The same inhibitory effect was observed in the process of growth and absorption of algae in this study.

Algae have been used either in a single form or in different algal and bacterial consortia to remove nutrients and reduce BOD/COD. There are several advantages to using microalgae for removing nutrients including nitrogen and phosphorus absorbed by microalgae can be recycled as biofertilizers from microalgae biomass, Production of bioenergy, food, animal feed, and medicines from algal biomass (Aslan and Kapdan, 2006; Renuka et al., 2013).

According to the results of this research, microalgae *Chlorella* sp. among algal species is generally used in basic research for wastewater treatment for its potential for high growth rate, ability to survive in wastewater environment, short reproduction time, and effective removal of nutrients (Pittman et al., 2011; He et al., 2013). It has been reported that *Chlorella vulgaris* can remove 55-88% of nitrogen and 12-100% of phosphorus from municipal wastewater (Ruiz-Marin et al., 2010). The nutrient removal percentages obtained in the research are comparable with similar cases: González et al. (1997) reported 55% phosphorus uptake from industrial, agricultural wastewater of total phosphorus concentration by *C. vulgaris* and *Scenedesmus dimorphus* (González et al., 1997). When *C. vulgaris* is fixed together with *Azospirillum brasilense* for two days, it removes 91% of ammonium (from 3.2 mg/L) (De-Bashan et al., 2002). Liang et al. (2013) reported 78% nitrogen removal (from 20 mg/L) after six days using co-culture of microalgae *C. vulgaris* and bacteria *Bacillus licheniformis* (Liang et al., 2013). The mechanisms related to the removal of carbon, nitrogen, and phosphate using microalgae are discussed by Gonçalves et al. (2017).

Alone, microalgae are not able to remove nutrients efficiently from wastewater. Growth of Microalgae growth promoting bacteria, starvation, and effluent dilution are different pathways that increase the rate of nutrient removal (Sriram and Seenivasan, 2012). Co-cultivation of activated sludge with algae and higher removal of nutrients (Su et al., 2012), use of plant growth-promoting bacteria alongside algae (De-Bashan and Bashan, 2010), and simultaneous stabilization of *Chlorella vulgaris* species with *Azospirillum brasilense* (McGinn et al., 2012) are all examples of multiple cultures.

The cellular mechanisms of nitrogen removal are as follows. Firstly, the enzymes involved in the nitrogen metabolism of microalgae like as glutamate dehydrogenase and glutamine synthetase increase. Secondly, PGPB can produce growth hormones to enhance microalgae growth.

Starvation also plays a significant role with a synergistic effect on phosphorus absorption from wastewater (De-Bashan and Bashan, 2010). The same integrated cultivation systems have also had efficient removing BOD and COD. Increasing the efficiency of BOD removal in the co-culture system of algae and activated sludge (Vasseur et al., 2012) and removing 80% of COD using the algae-bacteria system (Hoffmann, 1998) are examples of these studies.

The fine performance of AFC 008 and AFC 010 species in reducing BOD/COD like nitrate and phosphate absorbing in less than a week is confirmed with several similar studies. *Chlorella* can treat wood-based pulp industrial wastewater and remove 58% COD, 84% color, and 80% absorbable organic xenobiotics (AOX) (Tarlan et al., 2002). Mixed culture of *Chlorella* vulgaris and removal of 88% of COD from the initial concentration (250 mg/L) in 190 hours (Travieso et al., 1996), removal with the efficiency of 61%, 76.6% and 28% for COD, nitrogen, and phosphorus respectively, by *C. vulgaris* in the treatment of diluted ethanol and citric acid produced in industrial wastewater (Valderrama et al., 2002), reduction of 84% BOD and 89% COD using *C. vulgaris* and *Scenedesmus* sp. in a batch system (Hammouda et al., 1995); reduction of 89% BOD and 88% COD using *C. vulgaris* in 48 hours (Azeez, 2010).

Based on the results of this research, it is possible to reduce 80% of BOD and COD using a microalgae system within a week. Nevertheless, giving more time without effective change in pollutant reduction will cause algae destruction and release.

Acknowledgments

This research was done with the support of Research Institute of Applied Sciences, ACECR, in the molecular identification segment.

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