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WASTEWATER TREATMENT BY USING LOCALLY ISOLATED ALGAE SPECIES *COELASTRELLA TERRESTRIS (REISIGL)*

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Abstract

In this study locally isolated microalgae (*Coelastrella terrestris Reisigl*) was used in wastewater treatment to reduce the pollutant parameters, six parameter were studied to determine the efficiency of *C. terrestris* in reducing NO_3, NO_2, NH_4, PO^{-3}_4, BOD, and COD. Dry weight and optical density of microalgae were calculated daily, Samples of wastewater were taken from secondary tank in the Al-Rustumia wastewater treatment station. The samples were diluted 1:1, samples were halved in two parts A sterile and B non-sterile. NO_3 values start with 13.3 ppm , 13.0 respectively, then the removal rate reached after 14 days to 98.9% in sample A, and to 79.2% in sample B. NO_2 values start with 9.6 ppm for the samples A and B respectively, the removal rate reach to78.1% in sample A and to 67.7% in the sample B,NH_4 value start with 4.9 ppm in both samples A and B, the removal rate reach to 89.7% in sample A, and to 91% in sample B. PO^{-3}_4 values start with 2.1 ppm for both samples A,B. the removal rate reach to 100% in both samples. Value of BOD for the both samples start with 85 ppm, then the removal rate reach to 82.3% in sample A and to 87.6% in sample B. COD started with 97 ppm, for the both samples A,B. the removal rate reach to 88.6% in sample A and to 87.6% in sample B. The result show a significant and gradually increase in the biomass and optical density of algae.

Keywords: Al-Rustumiya wastewater treatment plant, Wastewater treatment, Microalgae, Nutrients removal.

Introduction

Water pollution is rising at an alarming rate mainly caused by waste generated through manmade activities including domestic, industrial, and agricultural wastes which are being discharged directly into the water bodies (Agarwal *et al*., 2019). Ideal wastewater treatment include three stages, the primary treatment involves separation of the suspended matter through physical (Damtew *et al*., 2018). Methods such as sedimentation, the secondary treatment uses techniques such as aeration and chemical methods to oxidize the organic matter present in the wastewater. The effluent coming out of the secondary stage carries large amounts of nitrogen and phosphorus, which are discharged to large water bodies (de la *et al*., 1992; Kainthola, 2016). Tertiary treatment processes have many drawbacks like excessive generation of chemical and biological sludge and need of organic substrate for nitrification and de-nitrification processes (Kainthola, 2016). Biological treatment of nitrogen and phosphorus wastewater has been widely studied, and organisms such as bacteria, fungi, and microalgae have been used for this purpose (Shahriari *et al*., 2016). Microalgae have the potential to remove mineral nutrients such as nitrogen and phosphorus from wastewater and therefore received considerable attention in the recent years (Khan & Yoshida 2008). Microalgae require nitrogen and phosphorous as major nutrients besides other micronutrients and produce valuable biomass, which can further be processed for biodiesel production (Singh *et al*., 2017). Many authors revealed the importance of *Coelastrella* spp., because they contain antioxidants and other commercial compounds (Vílchez *et al*., 2011 ; Aburai *et al*., 2013) .The *C.terrestria*l belongs to Coelastroideae (subfamily), Scenedsmaceae (family), Sphaeropleales (order) and Chlorophyceae (class) (Guiry, 2018). Recent studies have reported that many algal species, *Chlamydomonas* (Kong *et al*., 2010), (*Spirulina* Olguin *et al*., 2003), *Scenedesmus* (Xin *et al*., 2010), were used to remove nitrogen, phosphorus and organic matter (biochemical oxygen demand, (BOD) and chemical oxygen demand, (COD) from raw wastewater. Mohammed *et al.* (2016), and Hammad *et al*., (2019), Use microalgae (*Chlorella vulgaris*) in wastewater treatment to reduce the pollutant parameters and to reduce the pollutants of the municipal wastewater, (Dolatabadi and Hosseini, 2016), use *Spirolena platensis* to reduce the value of total dissolved solids (TDS), biological oxygen demand (BOD), chemical oxygen demand (COD), total hardness (TH), calcium hardness (CH), magnesium hardness (MH).

Material and Methods

Wastewater sampling

Wastewater samples were taken from the local wastewater station from Al-Rustumiya wastewater treatment plant in Baghdad, Al-rusafa .Wastewater was collected from effluent of secondary treatment tank. The waste water sample with characteristics (pH 7.3, PO_4 2.1 ppm, NO_3 13 ppm, NO_2 ,9.6 ppm, NH_3, 4.9 ppm, BOD 85 ppm, COD 97 ppm respectively), was filtered using vacuum with Whatman filter paper of (0.45µ,) to remove large particles and indigenous bacteria (Tran *et al*., 2020; Teshome *et al*., 2014). Samples were halved in tow part, one part was autoclaved at 121 ºC for 20 min to kill any microorganisms in the wastewater in order to show the effect of the algae species *Coelastrella terrestris (Reisigl)* on wastewater treatment (A), other part not autoclaved (B) . The dilutions of wastewater were tested as 1:1 with distilled water. *Coelastrella terrestris (Reisigl)* is first time to use in wastewater treatment in Iraq.

Algae Isolation

Algal isolatation were obtained from the Advance Algal Laboratory of the Department of Biology, College of Science for Women at the University of Baghdad, it is first recorded in Iraq by (Al-Rawi *et al*., 2018). BG-11 media were used for algae cultivation which prepared from minerals nutrients. 10 ml of isolated culture were added to a flask containing 100 ml of BG-11 media, then incubated for 14 days at 25 ± ºC at photoperiod of LD: 14:10, then transported to 1000 ml of sterilized and filtered wastewater sample (Mohammed *et al.*, 2016).

NO_3, NO_2, NH_3 , PO^{-3}_4 , BOD, and COD removal

The wastewater measurement for Nitrate (NO_3), nitrite (NO_2), ammonia (NH_3), (PO^{-3}_4), Biochemical Oxygen Demand (BOD), and Chemical Oxygen Demand (COD) were done using the standard method techniques described by (APHA , 2017).

Dry weight (biomass) and optical density of algae

The dry weight was measured after desiccation on pre-weighed filters with a porosity of 0.45 µm. 100 mL of cultures sample were filtered by vacuum and dried in 105-110 ºC (Kopp, 1978; Vitkus *et al.*, 1985). The dry weight calculated daily according to (Fogg, 1965). The result was expressed (mg/l), Algal cell density were determined by Optical Density (OD) measurement by Spectrophotometer at 540 nm every day for 14 days to all experiment (Miyachi *et al.*, 1964). Growth rate (k) as well as doubling time (G) were determined according to (Fogg, 1965).

Result and Discussion

pH changes

The pH of the samples was directly laboratory tested using a pH meter containing electrode placed in a photo bioreactors tube after calibration. After algae cultivation , the results of all treatments show in figure (1) there is a gradual rise in the pH , which ranged between 7.3 -8.8 in sample A and from 7.4-9.0 in sample B, it show a significant increase in the 6th day of treatment, which reached 8.5 in sample A, and 8.1 in sample B. At the final day of treatment the pH reached highest values which reached 8.8 in sample A, and 9.0 in sample B. This rise because of the microalgae have the ability to rise the pH value and convert the pH medium from the neutral to the alkalinek, this is due to photosynthesis process carried out by algae (Noue & Pauw 2008). These result are agree with Sayadi *et al.* (2016), in a study carry out to evaluate the ability of microalgae *S. platensis* and *C. vulgaris* to remove nitrate and phosphate in aqueous solutions, and agree with Mousavi *et al.* (2009), the highest pH value was indicated in the highest growth, and the highest cells density ,and growth of algae was observed in the final days i.e. on the 8th day in a study carried out by Sayadi *et al.* (2016), on the removal efficiency of nitrate, and orthophosphate from wastewater by using several species of microalgae to check out the pH changes during 12 days culturing period the results showed a gradually increased in pH value for the most of the studied species, from the first day to the end of the experimental period which was in concurrence with the results of this study.

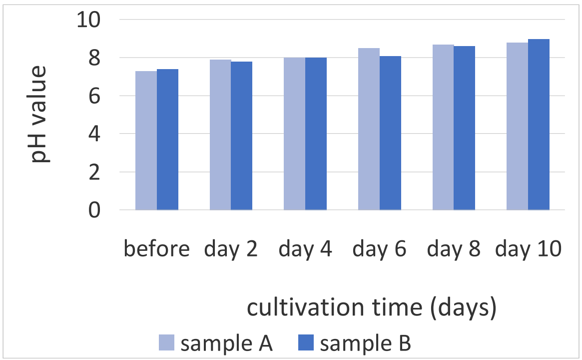


Fig. 1 : pH profile during wastewater treatment. by *Coelastrella terrestris (Reisigl)* during cultivation time .

Removal of Nutrients (NO_3, NO_2, NH_3, PO^{-3}_4)

Values of NO_3 were measured for the two samples (A, B) as shown in figure (2, a). NO_3 level start with 13.3 ppm, 13.0 respectively for the samples A and B. Sample A show significant change start from the 6th day, in the 6th day the removal rate reach to 48%, and then reach to 78.9% at the 10th day. Where sample B the removal rate was 46% in the 6th day, and then reached to79.2% at the 10th day. Were values of NO_2 figure(2,b) for the two samples (A,B) start with 9.6 ppm for the samples A and B. Sample A show significant change start from the 4th day ,in the 4th day it reached to 32.2%, and to 78.1% at the 10th day. Where is sample B was 21.8% in the 4th day, and reached to 67.7% at the 10th day. As show in figure (2, c). Values of NH_3 start with 4.9 ppm in both samples A and B. in sample (A) The results show a significant change start in the 6th day of treatment, were the removal rate was 69.3% and then reached to 89.7% in the 10th . Sample B start the change in the 6th day to reach to 61.2%, and removal rate reach to 91%, in the last day. Values of PO^{-3}_4 start with 2.1 ppm for both samples A, B. In sample (A) it can be notice that the change in PO^{-3}_4 concentration reached 56.6% in the 4th day and end with 0.00 (100%) in the 8th and 10th day. while sample (B) show significant change in the 6th day of removal, where the removal rate reached 89.3% and attained 100% in the 10th day of treatment as show in figure (2, d).

Coelastrella terrestris (Reisigl) promote increasing the losing in NO_2 NO_3 NH_3 and PO^{-3}_4 values of the wastewater, and this could be attributed to the increasing of algal growth rate and that because of the good activity of photosynthetic .These result are agree with Singh (2017). which found that *C. vulgaris* have the ability to remove nitrogen and phosphorous in the wastewater, and agree with Choi & Lee (2012), which observed that increasing the concentration of *C. vulgaris* in the wastewater caused an apparent increase of removal rates in total nitrogen (TN), total phosphorus (TP), NH_3 and PO_4, the result also agree with a study conducting by Sayadi *et al.* (2016), which observe the ability of *C. vulgaris* and *S. platensis* in the removal of nitrate and phosphate ions influenced by different nitrate and phosphate concentrations ,and agree with a study conducting by Shawky *et al.* (2015), that demonstrated the use of microalga *Scenedesmus quadricauda* is capable of reducing ammonia and phosphate from water resources .

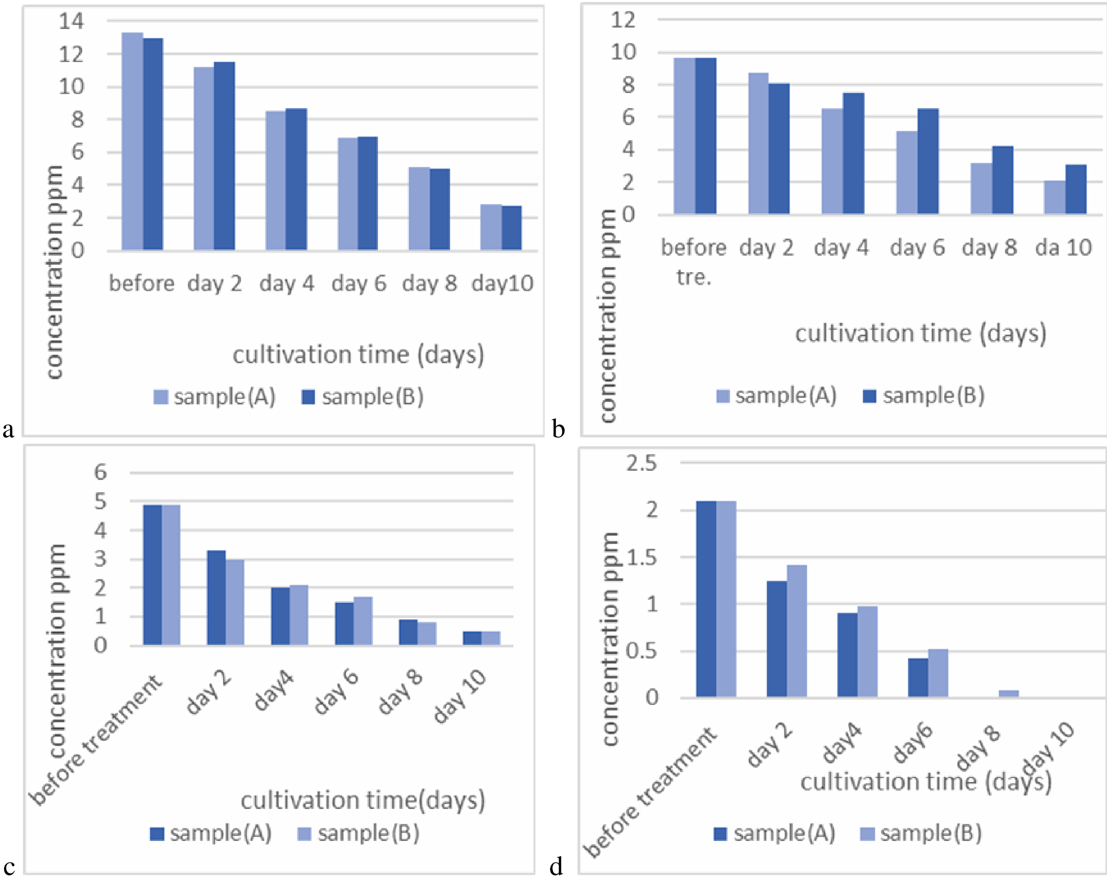


Fig. 2 : Removal of (a) NO_3, (b) NO_2, (c) NH_3, (d) PO_4, in two sample A and B by *Coelastrella terrestris (Reisigl)* during cultivation time.

Removal of BOD and COD

Values of BOD were measured for the two samples (A,B) as shown in Figure (2,a) each 5 days. The value for the both samples start with 85 ppm, Sample (A) show a decrease in it concentration after first five days which reach to 49.4%, in the last day of treatment the removal rate attain 82.3% . Where sample (B) show significant change reached to 38.8% at the first five days and continue to decrease in the concentration to the final day and the removal rate was 78.8%. figure (2,a). Values of COD were measured for the two samples (A,B) as shown in Figure (2,b) each 5 days. COD started with 97 ppm, for the both samples A, B. In samples A it show a significant change in the 5^{th} day of removal which reach to 40.2%, then the removal rate attain 74.2% and reach to 88.6% in the final day. Were in sample B, the removal rate was 52.5% and continue to increase to 69% and reached 87.6% in the final day of treatment.

Results showed that amount concentration of biological oxygen demand and chemical oxygen demand were significantly decreasing and this is agree with Choi & Lee (2012), which observed that increasing the content of *C. vulgaris* in the wastewater caused an apparent increase of removal rates in biological oxygen demand (BOD), chemical oxygen demand (COD), and its agree with Mohammed *et al.*, (2016), which observe increasing the losing in both BOD and COD values in a study use locally isolated microalgae (*Chlorella vulgaris Bejerinck*) in wastewater treatment to reduce the pollutant parameters. Also these result are agree with a study on two microalgae *chlorella* & *Scenedesmus* were used in purification of wastewater which show a significant decrease in the value of COD and BOD, Al-Hilo, (2007).

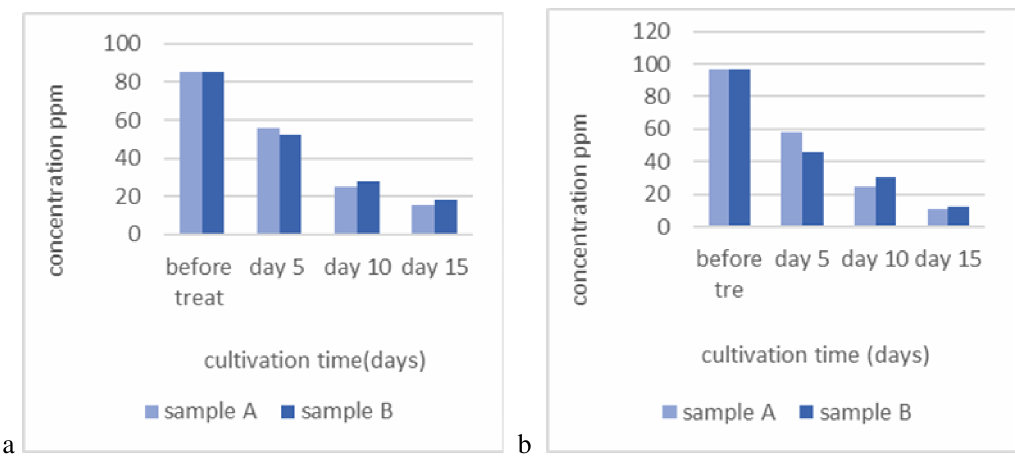
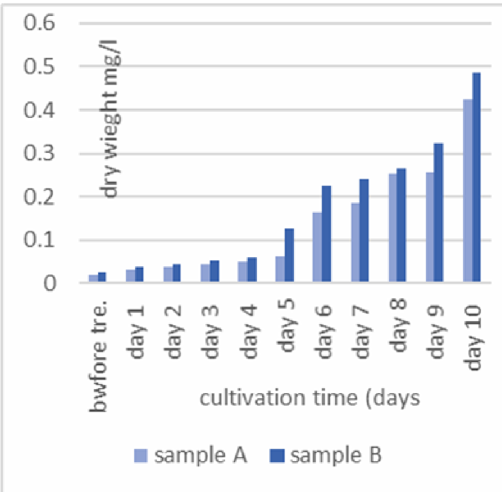


Fig. 2 : Removal of BOD (a),and COD (b) in two sample A and B by *Coelastrella terrestris (Reisigl)* during cultivation time.

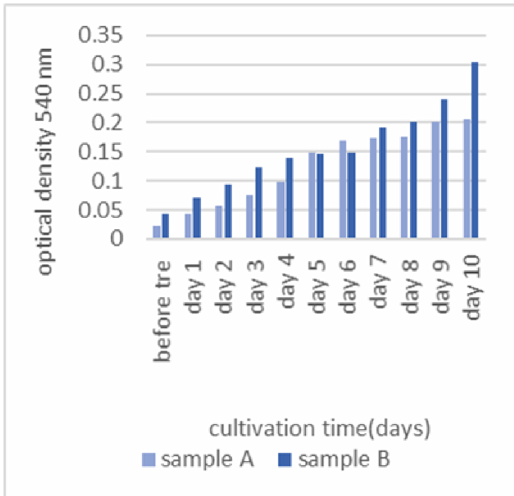
Dry weight and Optical Density of Algae

The result show a gradual increase in the biomass of the algae which leads to increase in the dry weight of algae , it can be notice from figure (3, a) a significant increase in the algae biomass in the 4th day from treatment for sample A and B , the biomass increased continuously and gradually to the 10th day of treatment. The result also show a gradual increase in the optical density of the algae which lead to increase in the density of algae, it can be can notice from figure (3,b) a significant increase in the algae density in the first and second day from treatment for sample A and B , the density increased continuously and gradually to the 10th day of treatment. The result show significant and gradually increase in the biomass and density of algae, which was attributed to cell aggregation or colony formation through long-term batch culture operation. Hu, (2014).

This result is agree with Lu, (2017). Which found significant linear correlation between cell density, optical density, and dry weight in his study on four species of diatom algae. Fatemeh & Mohsen (2016). In her study on *C.vulgaris* found that Optical density (OD) indicated that the best growth of *C. vulgaris* in outdoor condition was obtained in 650 lux and also it increased with increasing amount of luminance.



a



b

Fig. 3a : Dry weight (biomass),(b) Optical density (540 nm) in two sample A and B by *Coelastrella terrestris (Reisigl)* during cultivation time.

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