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Improvement of microalgal-based biological treatment of municipal wastewater by adding $ZnCl_2$ and $FeCl_2$ as trace elements

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Abstract

Microalgal-based biological treatment of municipal wastewater has received great attention due to its simplicity and environmentally friendly features. In this research, $FeCl_2$ and $ZnCl_2$ were used as trace elements (TEs) to improve microalgal growth rate and wastewater treatment ability. The removal of nutrients from real municipal wastewater was monitored during 15 days. According to the results, adding 0.2 and 0.5 mg/L of $FeCl_2$ and $ZnCl_2$ could increase microalgal biomass concentration from 2.2 mg/L (without adding TEs) to 3.24 g/L (45%) and 3.84 g/L (75%), respectively after 12 days of cultivation. The results of nutrient removal revealed that adding TEs could significantly improve microalgal-based biological treatment of municipal wastewater. Based on the treatment results, after 12 days of microalgal cultivation in the wastewater, the final concentrations of COD, PO_4^{3-} , NO_3^- and NH_3 were significantly reduced from 198, 7.24, 2.81 and 35.5 mg/L (without treatment) to 51.12, 0.81, 0.5 and 5.41 mg/L and 22.13, 0.1, 0.25 and 0.00 mg/L by adding 0.5 mg/L of $FeCl_2$ and 0.2 mg/L of $ZnCl_2$, respectively. The final concentrations of the nutrients in the wastewater meet the minimum standard or reuse-water for agriculture purposes.

Key words: Microalgal, Municipal Wastewater, Biological Treatment, $FeCl_2$, $ZnCl_2$



1. Introduction

The increasing of water utilization in urban area due to urbanization, industrialization and population growth has led to generate large amount of municipal wastewater. The discharge of untreated wastewater into the ecosystem results in several environmental problems due to its high pollutants concentrations such as nitrate, phosphate and chemical oxygen demand (COD) [1-3]. So far, several methods have been investigated for municipal wastewater treatment including chemical/physical treatments such as electrochemical or chemical oxidation [4] which can remove high concentration of COD, and biological treatments such as anaerobic sequencing batch reactor [5], constructed wetlands [6] and microalgae cultivation [7]. However, most of the chemical/physical methods are not environmentally friendly and in the case of biological methods, removal efficient in phosphorus is not be satisfactory [8]. Another problem of the conventional wastewater treatment methods is the production of high amount of sludge which significantly limits the application of these methods [9].

Microalgal-based biological treatment of wastewater has received great attention due to its promising application and eco-friendly approach [10-12]. Microalgae can reduce the nutrients such as nitrogen and phosphorus from wastewater by consuming them which promote microalgae biomass growth rate [13]. Several research have investigated the performance of different microalgae strains for treatment of municipal and industrial wastewater. Among them some microalgae species which grow rapidly have shown an excellent performance in nutrient removal from wastewater includes *Chlorella sp.*[14], *Nannochloropsis sp.* [15] and *Scenedesmus sp.* [16]. According to Kumar et al., (2019), *Chlorella sp.* microalgae could remove nitrate and phosphate up to 85% from urban, industrial and agricultural wastewaters [17]. In another attempt, Wang et al. (2017) could achieve a removal rate of 81% of COD and 96% of NH_4^+ - N by cultivation of *Neochloris aquatica CL-M1* microalgae in swine wastewater [18]. Novoveská et al. (2016) investigate the potential of using *scenedesmus dimorphus* microalgae for raw municipal wastewater treatment. They could achieve 75% of total nitrogen, 93% of phosphorus and 84% of COD removal efficiency [19]. However, the treatment time still remain as the main obstacle of the microalgal-based biological wastewater treatment approach.

In recent years, using trace elements (TEs) such as low dosage of heavy metals for improvement of microalgae growth has been noted by many investigators [20-22]. Hui-Ling et al. in 2012 investigated The impact of sub-lethal concentrations ($0.05, 0.5, 5 \mu\text{mol L}^{-1}$) of the heavy metals copper (Cu), chromium (Cr), zinc (Zn), cadmium (Cd) and lead (Pb) on the growth and photosynthesis of *Chlorella vulgaris* was studied during 96 h exposure experiments [23]. The results showed that the effects of these five metals on the growth of *Chlorella vulgaris* were dependent on both concentration and exposure time. It was found that $5 \mu\text{mol L}^{-1}$ treatments of Cu, Cr, Zn, Cd and Pb significantly inhibited the growth of *Chlorella vulgaris*, and the effect became weaker with an increase in exposure duration. The effect of FeCl_2 on growth and lipid accumulation in marine microalgae *Chlorella vulgaris* were investigated by Liu et al (2208) [24]. Total lipid content in cultures supplemented with



1.2×10^{-5} mol/L FeCl_2 was up to 56.6% biomass by dry weight and was 3–7-fold that in other media supplemented with lower iron concentration. It should be noted that most of the previous studies regarding the impacts of heavy metals as TE on algae were mainly focused on finding the optimum concentration of TEs for maximum biomass production. To the best of our knowledge limited studies have been conducted on the effect of TE treatment on microalgal-based biological wastewater treatment.

In this research, the effect of adding two well-known trace elements (TEs) of ZnCl_2 and FeCl_2 on microalgae cultivation and biological treatment of municipal wastewater were investigated. To do this, TEs with different concentration were added to the culture medium and then microalgae growth rate and the removal rate of chemical oxygen demand (COD), ammonia (NH_3) and phosphate (PO_4^{3-}) were measured for two weeks (14 days). *Sorokiniana P.91* was used as model microalgae.

2. Materials and methods

2-1-Wastewater

The wastewater samples were obtained from real municipal wastewater (RMWW) influent after preliminary settling tank was collected from wastewater treatment plant (WWTP), Sari, Iran. After separation of solids, the wastewater was used as culture medium to evaluate the microalgae capacity for nutrient removal from real municipal wastewater. Before each experiment, wastewater medium was centrifuged at 11000 rpm for 15 min to remove solid particles. The properties of wastewater samples are given in Table 1.

All the chemicals including the heavy metals (e.g. FeCl_2 and ZnCl_2) were provided from Merck Company, Germany and were of analytical grade.

Table 1: Characteristics of the municipal wastewater sample in this study

Parameter	Unit	Value
pH	-	7.5
COD	mg/L	198
TP	mg/L	7.24
NO_3	mg/L	2.9
NH_3	mg/L	35.5

2-1-Microalgae and TE

A new strain of *Chlorella sorokiniana pa.91* which was previously isolated from Gela dairy wastewater treatment plant (Amol, Iran) was selected and used in this study [19]. This strain was recognized and analyzed phylogenetically based on 18S rRNA gene sequence [19]. Because of the possibility of impurities and contamination, three purification phases were performed in plates containing agar and BG-11 culture medium. The microalgae cells were inoculated into several 500 ml Erlenmeyer flasks of BG-11 medium to prepare larger volumes of microalgae cultivation in RMWW. The trace elements (TEs) of ZnCl_2 and FeCl_2 were purchased from Merck Company with laboratory grade.



2-3-Experimental procedure

Microalgae cultivation experiments were performed in a pilot-scale flat-plate photobioreactor (FP-PBR) at the optimum condition to investigate the microalgae capacity for nutrient removal from real municipal wastewater (RMWW). The PBR used in this study was 8 L glass flat-plate photobioreactor (34cm length×10 cm width×40 cm height), equipped with a gas sparger at the bottom of the PBR. On the top of the photobioreactor, three holes were placed to measure CO₂ output. Liquid samples were also collected from a sample point at the side of the photobioreactor daily to determine temperature, biomass concentration, pH and nutrient concentrations. The light intensity was provided by white fluorescent lamp that exposed to back side of photobioreactor with cycle of Light/dark 12/12h. In addition, CO₂ gas (16%) was aerated by the sparger at the rate of 0.6 vvm into the photobioreactor to providing mixing. Various dosages of 0.2, 0.5, 1 and 2 mg/L of FeCl₂ and ZnCl₂ were added into the reactor to investigate the effect of TEs on microalgae growth rate and nutrients removal. During the cultivation period, the concentration of main nutrients from the municipal wastewater influent were determined based on standard methods (Association et al., 1912). 50 mL sample of microalgae medium (RMWW) from the photobioreactor was collected and centrifuged at 3500 rpm for 15 min, then, the supernatant was used for measuring the nutrients concentration.

2-3-Analytical methods

The cell dry weight was measured by using the optical density at 680 nm in a UV spectrophotometer (2800 UV/VIS UNICO, China). The biomass concentration was determined by washing and drying the samples of microalgae cells in an oven at 70 °C for 24 h. The relation between OD₆₈₀ and biomass concentration was obtained according to Eq. 1 which was obtained from standard curve, as follows:

$$\text{Biomass concentration} = 1.824 \text{ OD}_{680} - 0.043, (R^2 = 0.9889) \quad (1)$$

The concentration of main nutrients of the municipal wastewater influent such as phosphate (PO⁻³₄), ammonia (NH₃), nitrate (NO₃) and COD were determined based on standard methods (Association et al., 1912) [25]. The removal efficiencies of the nutrients calculated through following equation:

$$\text{Removal efficiency} = \frac{(C_0 - C_t)}{C_0} \times 100 \quad (2)$$

where, C₀ and C_t were the concentrations of the nutrients in the wastewater before and after treatment with TEs, respectively. COD was measured using COD reactor (HACH 45600) and spectrophotometer (2800 UV/VIS UNICO, China) according to 5220 D standard method of American Public Health Association (APHA, 2012). The concentration PO⁻³₄, NH₃ and NO₃ were measured based on the standard methods (Association et al., 1915).

3. Result and discussion

3-1-Effect of TE on microalgae growth

Fig. 1 shows the effect of using different TE dosage on microalgae growth rate. As can be seen from Fig. 1a, adding 0.2 mg/L of FeCl_2 could improve the microalgae growth rate up to 47% (from 2.2 to 3.24 g/L) during 12 days cultivation of microalgae. While in the case of ZnCl_2 , adding 0.5 mg/L of TE results in 76% improvement in biomass concentration (from 2.2 to 3.87 g/L).

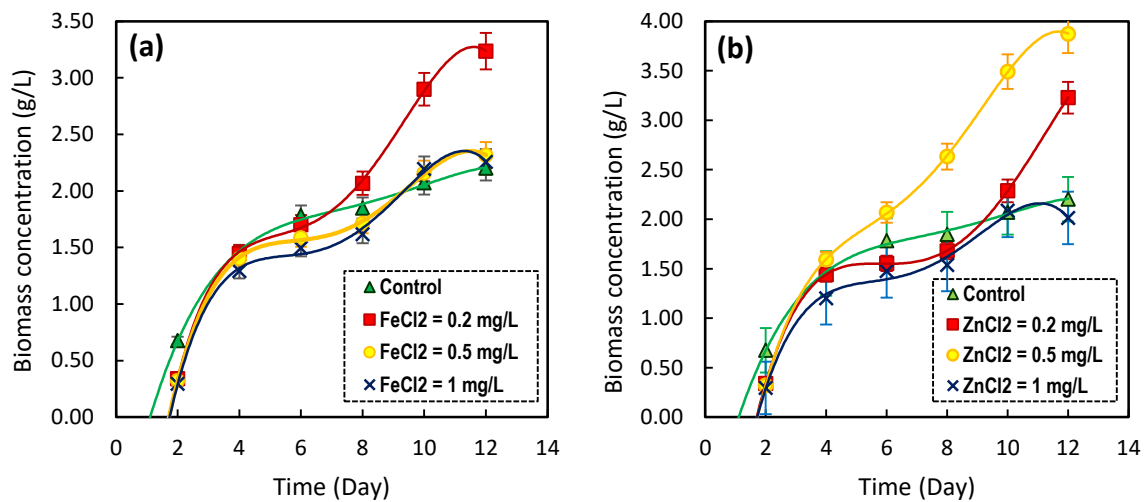


Figure1: Effect of TE dosage of microalgae growth rate

The maximum biomass achieved after adding different dosages of FeCl_2 and ZnCl_2 to the culture medium were shown in Fig. 2. The results revealed that ZnCl_2 has better effect in comparison with FeCl_2 as maximum biomass concentration of 3.24 and 3.87 g/L of biomass were achieved by adding 0.2 and 0.5 mg/l of FeCl_2 and ZnCl_2 , respectively

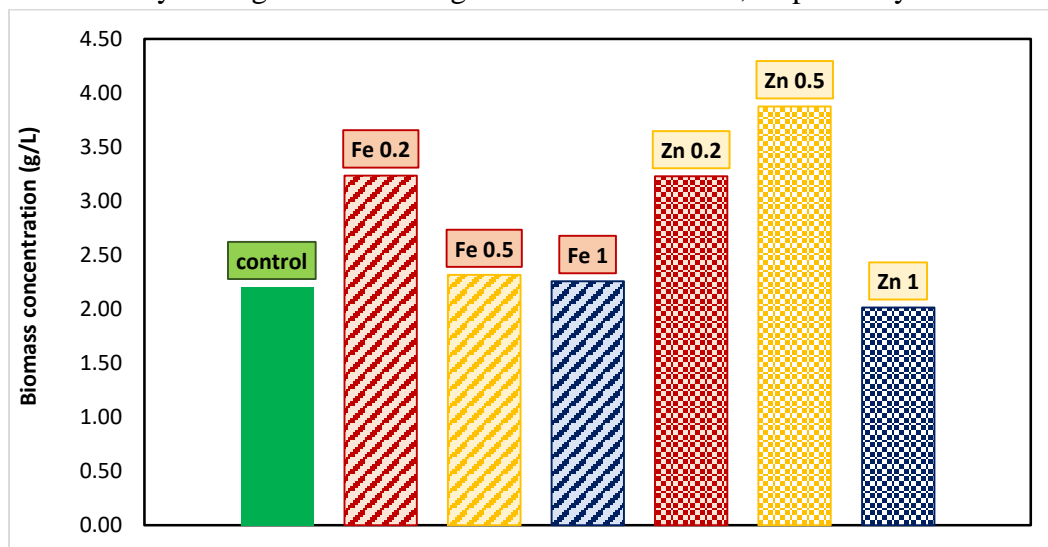


Figure 2: Effect of TE dosage of maximum biomass concentration after 12 days

3-2- Microalgal-based biological treatment of wastewater

Figure 3 demonstrates the effect of adding TEs of FeCl_2 and ZnCl_2 at their optimal dosages of 0.2 and 0.5 mg/L, respectively on nutrients removal. According to the results, microalgae cultivation in the municipal wastewater without adding TEs could reduce the concentration of COD, PO_4^{3-} , NO_3 and NH_3 in the waste water from 198, 7.24, 2.9 and 35.5 mg/L to 87.86, 2.5, 1.1 and 15.01 mg/L, respectively after 12 days. However, the final concentrations of the nutrients in the wastewater could not meet the minimum standard of the re-use water quality for agriculture purposes. It was observed that adding TEs of FeCl_2 and ZnCl_2 could significantly improve the removal efficiencies of nutrient by microalgae. The results revealed that adding 0.5 mg/L of ZnCl_2 .

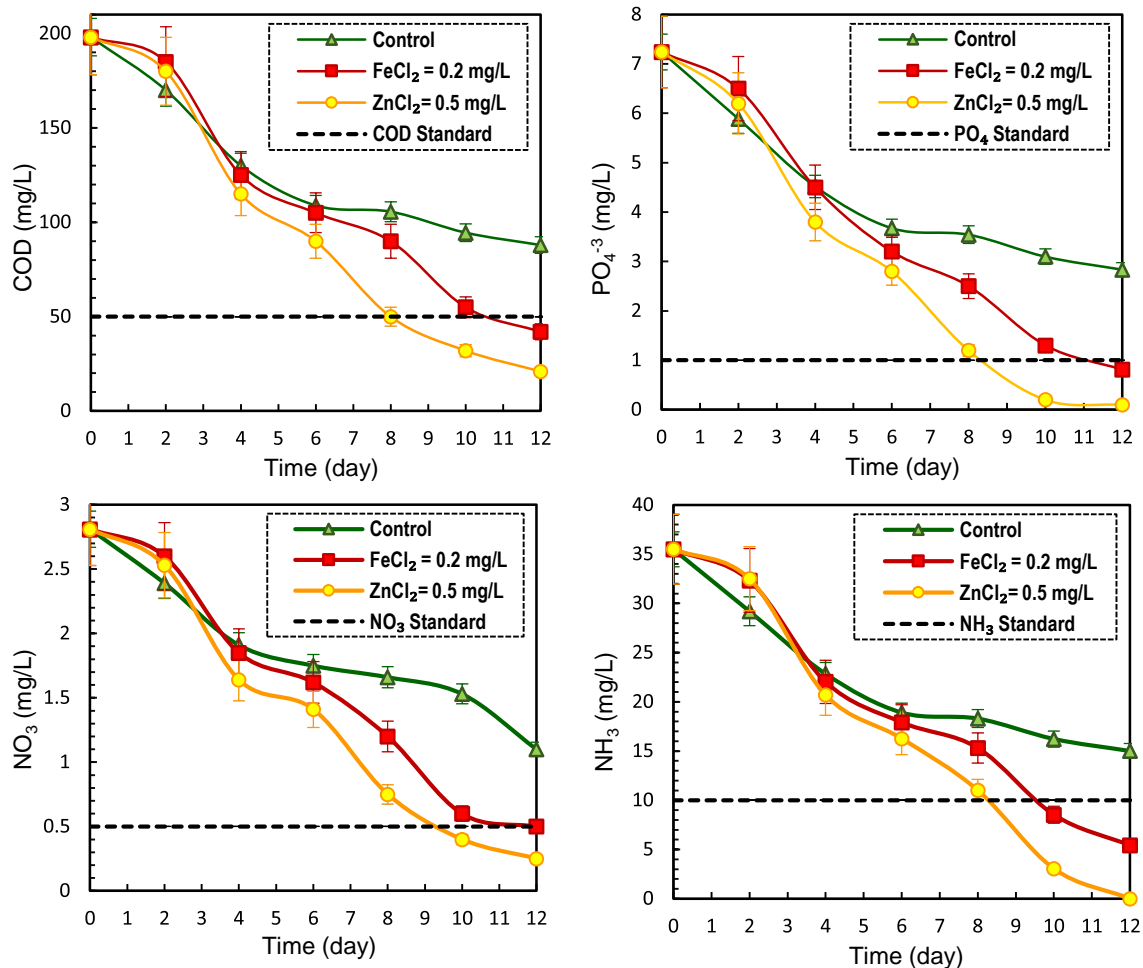


Figure3: Effect of TE dosage of microalge growth rate



4. Conclusions

The main objective of this research was to evaluate the effect of adding trace elements of FeCl₂ and ZnCl₂ on microalge growth rate and wastewater treatment ability. According to the results, adding 0.2 and 0.5 mg/L of FeCl₂ and ZnCl₂ could improve microalge biomass up to 45% and 75%, respectively after 12 days cultivation. The results of nutrients removal revealed that adding TEs could significantly improve microalgal-based biological treatment of municipal wastewater. Based on the treatment results, after 12 days of microalge cultivation in the wastewater the final concentrations of the nutrients in the wastewater met the minimum stand or reuse-water for agriculture purposes.

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