African Journal of Microbiology Research Vol. 7(17), pp. 1729-1734, 23 April, 2013
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.1702
ISSN 1996-0808 ©2013 Academic Journals

Full Length Research Paper

Performance evaluation of cyanobacteria removal from water reservoirs by biological method

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Accepted 14 May, 2012

With the rapid growth of urbanization, the discharge of industrial, agricultural and municipal wastewater into water resources is increasing. Cyanobacteria are a dominant component of the phytoplankton that causes problems in water reservoirs. The aim of this study is to evaluate the performance of bacteria in the removal of cyanobacteria algae from water reservoirs. In this study, a biological method with four types of bacteria was applied for algae removal. First of all, species of cyanobacteria were identified, and then their specific medium BG-11 was prepared. The species of bacteria (*Pseudomonas aeroginosa*, *Enterobacter aerogenes*, *Klebsiella oxytoca,* and *Citrobacter freundii*) were used for removing cyanobacteria for a period of 0 to 10 days. Variables such as chlorophyll *a*, nitrate, phosphate, dissolved oxygen, heterotrophic plate count, and algal cell count were measured during the study period. The results of the present study showed that *P. aeroginosa* and *C. freundii* were effective for the removal of chlorophyll *a* in the first five to six days of the study period with removal efficiency of 63.5 and 56.59% respectively. The other parameters such as phosphate, nitrate, and algal cells decreased relatively. This method is relatively efficient and effective for removing algae from water resources and can be used for removing nutrients and cyanobacteria algae from water resources.

Key words: Algae, biological, biotechnology, cyanobacteria, water.

INTRODUCTION

With the rapid growth of urbanization, the discharge of industrial, agricultural, and municipal wastewater into water resources is increasing, causing expansion of eutrophic systems that lead to algal blooms (Rogalus and Watzin, 2008; Ji et al., 2009). Nuisance algal blooms alter the water quality and create aesthetic problems such as water decolorization and deoxygenation, and finally lead to taste and odor (Randolph et al., 2008; Boyer et al., 2009). As a result, destabilization of sediments occur (Boyer et al., 2009). Cyanobacteria are a dominant component of the phytoplankton (Rogalus and Watzin 2008) and oxygenic photoautotrophic microorganisms.

Different physical, chemical, and biological methods are used for the removal of algae and toxins from water reservoirs. Some of the methods that have been used for the removal of algal cells include coagulation and sedimentation (Bernhardt and Clasen, 1991), rapid filtration (Himberg et al., 1989), slow sand filtration, membrane processes (Her et al., 2004; Gijsbertsen-Abrahamse, et al., 2006), dissolved air flotation (Aulenbach et al., 2010), ultrasonic irradiation (Lee et al., 2002; Dehghani and

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Changani, 2006), and oxidation processes (Dehghani and Changani, 2006). In addition, various methods have been used for the removal of soluble toxins from cyanobacteria, such as powder and granular activated carbon (Gayle, 2002), ozone oxidation processes (Rositano et al., 2001; Gayle, 2002), chlorination and chlorine dioxide (Hoffman, 2003), potassium permanganate (Chen and Yeh, 2005), hydrogen peroxide/UV radiation (Tsuji et al., 1995), and membrane processes such as nanofiltration and microfiltration (Her et al., 2004; Gijsbertsen-Abrahamse et al., 2006). In recent years, algalytic bacteria that are able to control algal growth, such as *Staphylococcus* sp., *Bacillus* sp., *Arthrobacter* sp., and *Pseudomonas* sp., have been considered as useful tools for reducing the impact of harmful blooms (Li et al., 2007; Zhao et al., 2005). In a recent study conducted by Wang et al. (2010), they aimed at isolating extracellular algae-lysing compounds from bacteria, which are called algalytic bacteria. It is believed that these bacteria have lysed *Anabaena*, *Nostoc*, *Microcystis*, *Lyngbya*, and *Phormidium* (Peng et al., 2003). The main objective of the present study was to use four bacterial species such as *Pseudomonas aeroginosa*, *Enterobacter aerogenes*, *Klebsiella oxytoca*, and *Citrobacter freundii* for the removal harmful organisms, especially cyanobacteria. Parameters such as chlorophyll *a*, nitrate, phosphate, dissolved oxygen (DO), heterotrophic plate count, and algal cell count were measured for a period of 10 days from water samples.

MATERIALS AND METHODS

Sample collection and preservation

Water samples containing algae, especially cyanobacteria, were collected from Collage Basin in April, 2010 in a 100 ml glass bottle and taken to the laboratory within one hour of sample collection. For identification of algal cell, a microscope (Zeiss, Germany) with 40x magnification was used. Cyanobacteria algae were identified based on morphological characteristics according to the Standard Methods for Examination of Water and Wastewater (Clescerl et al., 1999; Pinto, et al. 2001).

Pilot and culturing medium

In this study, algae medium was located in a shaking incubator, Innova 4340, that had adjustable temperature and light intensity (Gillor et al., 2003). Culturing medium for cyanobacteria was BG11 (Rippka et al., 1979; Lefebvre et al., 2007; Wang et al., 2010). The pH was adjusted to 7.5 and the medium was autoclaved at 120°C for 15 min. Three hundred milliliter of the medium was poured into a 500-ml flask and water containing algae with dominant cyanobacteria was added to it. The batch cultures were kept at a temperature of 25°C under continuous illumination of 35 µmol photons m^{-2} s^{-1} with cool-white fluorescent light. They were then shaken at the rate of 110 rpm (Baker et al., 2006; Kellmann et al., 2008). When their growth was fixed, phytoplankton were dominated by cyanobacteria and a smaller number of green algae. Four species of bacteria such as *P. aeroginosa* PTCC 1310, *E. aerogenes* PTCC 1221, *K. oxytoca* PTCC 1402, and *C. freundii* PTCC 1600 were cultured on the nutrient agar plate through lyophilized injection for 24 h at 35±0.5°C. Colonies at 10^9 densities were added to the algal medium. Four algal media were inoculated with any of the bacteria. The mixed bacteria were added to other algal media and compared with the control medium for a period of 0 to 10 days. Then parameters such as chlorophyll *a*, nitrate, phosphate, algal cell count, dissolved oxygen, and heterotrophic bacterial counts were compared for different media.

Parameter analysis

The use of chlorophyll *a* to estimate phytoplankton concentration in water resources is one of the most useful measures applied in the field of limnology and oceanography (Gregor and Marsálek, 2004; Kasprzak et al., 2008; Rogalus and Watzin, 2008). Chlorophyll *a* concentration is used as a quick and easy way of measuring phytoplankton biomass (Kamoto, 1966). Most methods used for the measurement of chlorophyll *a* concentrations are based on extraction by organic solvents such as methanol, ethanol, and acetone and determination by spectrophotometery, fluorometery, or chromatography (Gregor and Marsálek, 2004). One milliliter of well-mixed sample was settled for 5 to 10 min on a Sedgwick-rafter chamber and evaluated using an inverted microscope at x100 for the identification of cyanobacteria taxa. The species that were identified include *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nodularia*, *Nostoc*, *Oscillatoria* and *Lyngbya*, and *Spirogyra*. For algal cell count, we used inverted microscope at x400 after sedimentation in Sedgwick-rafter chamber. The most important parameter was chlorophyll *a*. In order to measure chlorophyll *a* concentration, samples were filtered by vacuum pump after collection. The sample was filtered on a Whatman glass fiber (GF/F) filter. The filter was stored in a clean 15 ml centrifuge tube covered with foil to block light and prevent them from freezing by the time of the analysis. The filter was then cut into small pieces and chlorophyll *a* was extracted by 90% acetone. The extract was filtered and 10 ml of it was taken for acidification with 10 µL of 3 M HCL. The absorbance of both extract was measured at 665 and 750 nm and the concentration of chlorophyll *a* determined according to the guideline, 10200H, from the Standard Methods for Examination of Water and Wastewater (Clescerl et al., 1999-2001).

Generally, phosphate and nitrate can be determined by DR 5000. However, in this study, phosphate concentrations were measured using PhosVer 3 reagent and DR 5000 Hack Model. Nitrate concentrations were measured according to the method, 4500 - NO_3^- – B, from Standard Methods for Examination of Water and Wastewater, and were found to be at the upper limit. Pour plate agar method was used for the determination of the number of heterotrophic bacteria. The data obtained were analyzed using SPSS 14 and Microsoft Excel 2007.

RESULTS

Algal biomass reduction due to inoculation with bacteria

Chlorophyll *a* was used as an indicator for the estimation of the algal biomass (Boyer et al., 2009). Figure 1 illustrates that chlorophyll *a* changed with time in the cyanobacteria medium that was inoculated with bacteria, but did not in the control medium (without bacteria inoculation), during the 0 to 10-day period of the experiment. Chlorophyll *a* reduction was mostly observed in the algal medium that was inoculated with *P. aeroginosa* and *C. freundii*. In this medium, from days five to six, chlorophyll *a* decreased from 156.5 and 161.5 to 57.1 and 70.1 µg/L, respectively. The removal efficiency was about 63.5% and 56.59% respectively.

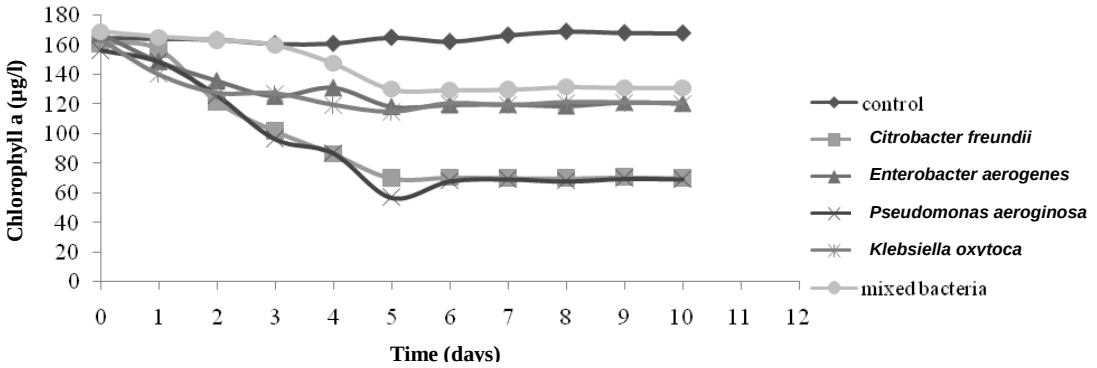

Figure 1. Chlorophyll *a* in cyanobacteria medium inoculated with bacteria and in control medium from days 0 to 10.

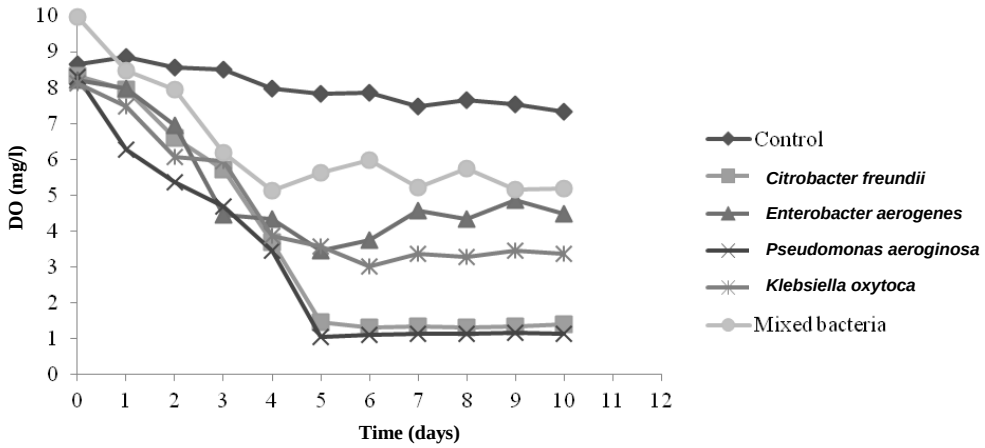

Figure 2. Dissolved oxygen in cyanobacteria medium inoculated with bacteria and in control medium from days 0 to 10.

ANOVA test showed a statistically significant difference between the amounts of chlorophyll *a* in the two media and control medium for *P. aeroginosa* medium ($P < 0.001$ and mean difference = 72.64) and for *C. freundii* ($P < 0.001$ and mean difference = 69.67). The removal efficiencies for the algal media that were inoculated with *K. Oxytoca*, *E. aerogenes,* and mixed bacteria, for the same number of days as stated earlier, were 30.36, 28.25, and 21.29%, respectively.

Changes in dissolved oxygen

Oxygen changes in the control medium were constant, but in all media that were inoculated with bacteria, the dissolved oxygen decreased after bacteria inoculation. The reduction in dissolved oxygen mostly occurred in the algal media that were inoculated with *P. aeroginosa* and *C. freundii*. In five to six days, dissolved oxygen decreased from 8.62 and 8.85 mg/L to 1.05 and 1.45 mg/L, respectively (Figure 2).

Phosphate and nitrate variations

Figure 3 shows that phosphate varied with time in the cyanobacteria medium that was inoculated with bacteria, but did not in the control medium, from days 0 through 10. Phosphate concentration decreased in the media that were inoculated with *E. aerogenes*, *P. aeroginosa* and *C. freundii*, but no significant changes are observed in other media. From six to seven days, phosphate concentrations in the three media reduced from 2.89, 2.91, and 2.82 mg/L to 1.01 ± 0.11, 1.15 ± 0.19, and 1.67 ± 0.17 mg/L, respectively. The removal efficiencies during these days were equal to 65%, 60.5%, and 40.7%, respectively. ANOVA test showed that *P. aeroginosa* medium ($P < 0.001$ and mean difference = 1.35), *C. freundii* medium ($P < 0.001$ and mean difference = 1.27), and *E. aerogenes* medium ($P < 0.001$ and mean difference = 0.92) are effective for phosphate removal. Muslim et al. suggested that there is a strong correlation between dissolved inorganic phosphate and chlorophyll *a* (Muslim and Jones 2003).

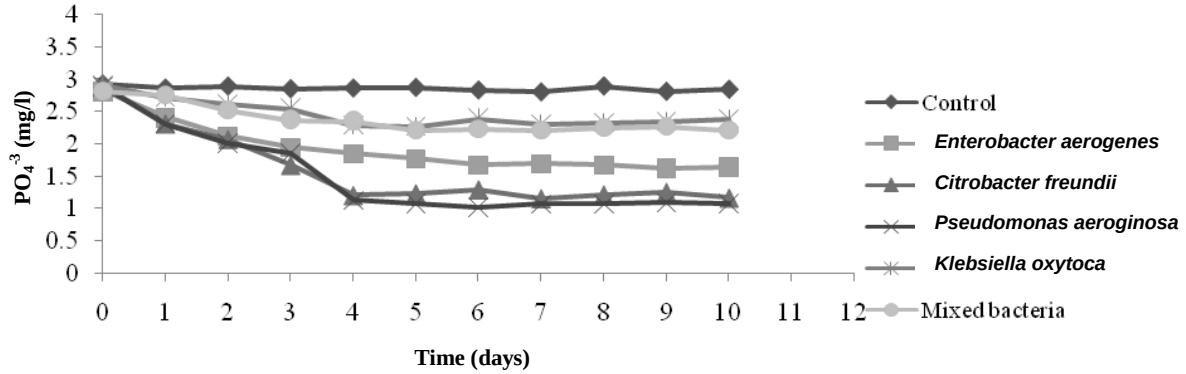

Figure 3. Phosphate in cyanobacteria medium inoculated with bacteria and in control medium from days 0 to 10.

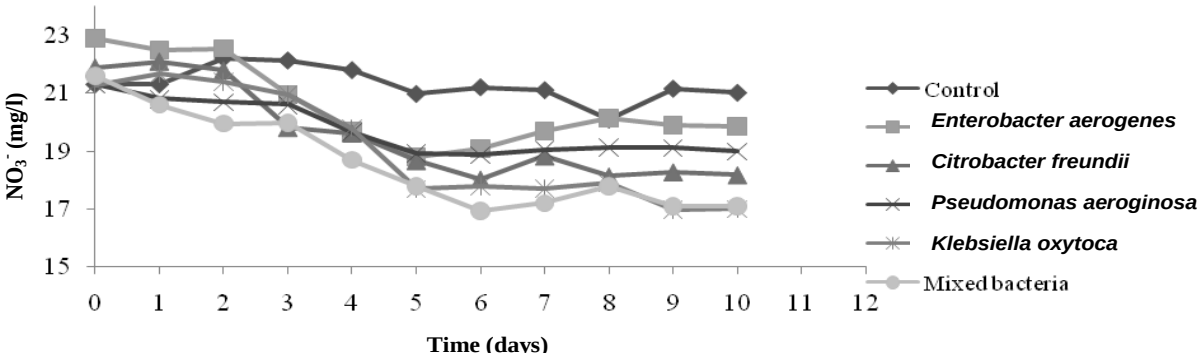

Figure 4. Nitrate in cyanobacteria medium inoculated with bacteria and in control medium from days 0 to 10.

Algal media inoculated with mixed bacteria ($P < 0.001$, mean difference = 2.67) and *K. oxytoca* ($P = 0.003$, mean difference = 2.18) were effective for nitrate removal, while no change was observed with the control medium, from days five to six (Figure 4). The removal efficiencies in the two media are 21.57 and 16.85%, respectively.

Heterotrophic plate count (HPC) and counting algae cell

Figure 5 shows that heterotrophic plate count varied with time in the cyanobacteria medium inoculated with bacteria, but did in the control medium, from days 0 to 10. Based on the growth curve, the bacteria were in the lag growth phase at first. Then logarithmic growth phase occurred from days one to four. From days four to six, the bacteria reached fix growth phase; this means growth rate equal to death rate. *P. aeroginosa* and *C. freundii* had more growth than the other species. This study showed that these bacteria were more effective for the removal of chlorophyll *a*, lysing algal cells, phosphate and other microorganisms from the water samples.

Changes in algal cell count

A reduction in algal cell count was mostly observed in the mediums that were inoculated with *P. aeroginosa* (from 312,250 algal cells/ml to 114,200 algal cells/ml) and *C. freundii* (from 325,403 algal cells/ml to 140,220 algal cells/ml), from days five to six. A good relationship ($R = 0.995$, $P < 0.001$) was observed between chlorophyll *a* and algal cell counts (Figure 6).

DISCUSSION

For the effective removal of contaminants, especially algae from water, biological method is most appropriate. So far, extensive methods are used for the removal of cyanobacteria algae and toxins from water reservoirs. Each of these methods has advantages and disadvantages. In this study, four types of bacteria such as *P. aeroginosa*, *C. freundii*, *K. oxytoca*, and *E. aerogenes* were used to remove these algae in laboratory scale. Parameters such as chlorophyll *a*, phosphate, nitrate, DO, HPC, and algal cell count were measured from days 0 to 10. *P. aeroginosa* and *C. freundii* were most effective, with removal efficiencies of 63.5 and 56.59% for chlorophyll *a* from days five to six. Due to the problems created by cyanobacterial toxins that influence human health and wildlife sustainability, scientists were forced to study and isolate some bacteria

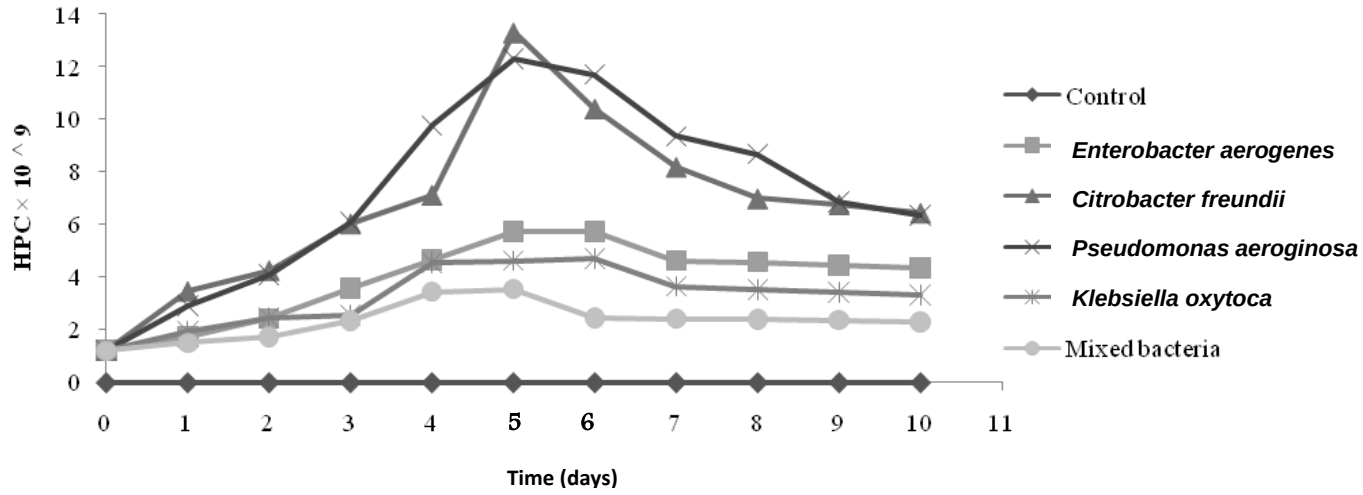

Figure 5. HPC in cyanobacteria medium inoculated with bacteria and in control from days 0 to 10. HPC, Heterotrophic plate count.

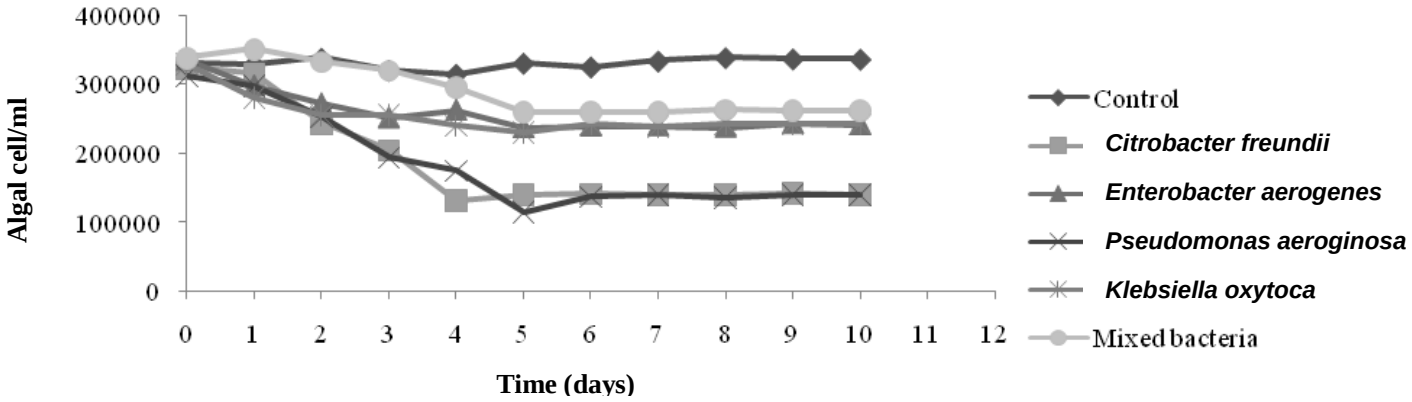

Figure 6. Changes in algal cell count in cyanobacteria medium inoculated with bacteria and in control medium from days 0 to 10.

known as algalytic bacteria (Guan et al., 2008; Wang et al., 2010). Such bacteria that are able to inhibit the algal growth and lyse its cell are called algalytic bacteria (such as *Staphylococcus* sp., *Bacillus* sp., *Arthrobacter* sp., and *Pseudomonas* sp.) (Peng et al., 2003; Zhao et al., 2005). *P. aeroginosa* has the ability to remove algae and microcystins (Choi et al., 2005; Roth et al., 2008; Ji et al., 2009; Ren et al., 2010). To possess lysis characteristics, a certain initial bacteria concentration is needed. At a higher bacteria concentration, the removal rate is higher (Liu et al., 2007). According to study conducted by (Ji et al., 2009), biological method has a high ability to remove algae and microcystins. The removal efficiency for chlorophyll *a* was 62.8%.

The removal efficiency for phosphate by *P. aeroginosa* and *C. freundii* was 65 and 60.5%, respectively; these bacteria can be used for removing phosphate from aqueous solutions with algae. A good correlation was observed between chlorophyll *a* and algal cell counts (Gregor et al., 2007). On the whole, biological method is relatively efficient and effective for removing algae from water resources.

In addition, this method, when compared with physical and chemical methods, has advantages. Biological method has advantages such as being economically and affordable, producing low sludge, low energy consumption, removal of other unwanted substances, and being a modern method.

ACKNOWLEDGEMENTS

This study was conducted in the microbiology laboratory of the School of Public Health, Tehran University of Medical Sciences. The authors would like to thank the faculty staff of the Department of Environmental Health Engineering, especially Mr. Mousavi, Miss. Ghani, and the staff of the chemistry laboratory. This work as a part of thesis project was supported by the grants from the Iran National Science Foundation (INSF).

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