The effect of salt on the one stage combined anaerobic-aerobic processes for the treatment of synthetic wastewater containing Reactive Red 195

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Abstract
In this study, the effect of NaCl at different concentrations in the range 0-100 g/l on the performance of a one stage anaerobic-aerobic process for treatment of a synthetic wastewater containing Reactive Red 195 (RR195) was investigated. Preliminary studies suggested that 72 hours was the optimum period for anaerobic reduction of RR195, and the adaption of activated sludge with the dye was done in the one-stage anaerobic-aerobic cycles. At the end of each stage, the degradation of azo dye with 100 mg/l as the initial concentration of dye was examined with the various concentration of NaCl. The results showed a statistical enhancement of the anaerobic decolorization efficiency for NaCl concentration up to 60 g/l. However, for concentrations higher than 60 g/l, adding NaCl had an opposite effect effect and led to a decrease in anaerobic reduction of RR195.

Keywords: Anaerobic-aerobic process, azo dye, decolorization efficiency, anaerobic reduction, sodium chloride

Introduction
Azo dyes are extensively used in various industries, and they are among the main causes of contamination of water resources [1]. Reactive azo dye is one of the important types of these dyes, and they have been widely used in textile industries and dyeing operations [2]. Disposal of colored wastewater leads to significant environmental problems [3]. To enhance the bonding of dyes to textile, notable quantities of chemicals, especially sodium salts, are added to the dye baths [4]. Several physical, chemical, and biological methods have been used in order to remove dyes from the wastewaters. Most physicochemical decolorization methods have a limited usage because of their high operating cost, low efficiency, and production of by-products [5]. Alternatively, the biological treatment techniques are environmentally friendly and do not produce extra pollution, and these methods are cost-effective [6]. The sequential anaerobic-aerobic biological treatment method has been effectively considered as a technique for the treatment of wastewaters containing azo dyes [7]. During the anaerobic phase, azo dye reduction occurs, and the aromatic amines which result from the anaerobic

degradation can potentially biodegrade under aerobic conditions [8]. The treatment of wastewaters that contain azo dye can be accomplished by the anaerobic-aerobic method both using one or two stages [9]. There are several contrasting reports on the effect of NaCl on anaerobic dye reduction. Some studies reported that wastewaters containing a large quantity of sodium chloride could create a negative effect on anaerobic degradation, and it can disrupt the bacterial growth of activated sludge [10]

In this study, the adaption of dye and activated sludge during the anaerobic-aerobic cycles was carried out. Then, by using the adapted activated sludge, the effect of sodium chloride in the range of 20- 100 g/l on the performance of a one-stage anaerobic-aerobic process for the treatment of synthetic wastewater containing Reactive Red 195 (RR195) was investigated.

Experimental

The azo dye was RR195 was obtained from Nasajsabet company. To produce the dye stock in a form which simulated the dyebath wastewaters, hydrolyzing of the dye was accomplished based on the method described previously [9]. Based on the UV-Vis spectral analysis in the range of 400-700 nm, the dye maximum absorption wavelength (λ_{max}) was determined. The calibration curve was drawn by reading the absorptions of solutions containing 20, 40, 60, 80, and 100 mg/l of dye at λ_{max}. According to the calibration curve and average absorbance of samples at λ_{max}, the concentration of the dye was determined. The color removal was calculated by Eq. 1; in which, C_0 is the initial dye concentration, and C_t is the dye concentration of decolorized medium in mg/l.

$$\text{Color removal\%} = [(C_0 - C_t)/C_0]*100 \qquad \text{Eq. 1}$$

In order to get an idea about the optimum time needed for anaerobic phase, experiments were carried out in 100 ml serum bottles with the initial Mixed Liquor Suspended Solid (MLSS) of 1000 mg/l and the inital dye concentration of 50 mg/l. 80 ml of the serum bottles were filled with the nutrient media and RR195. pH was adjusted to 7 with the aid of 1 molar $NaHCO_3$ solution. Each serum bottle was sealed with rubber stoppers and was flushed with the nitrogen gas for approximately 4 minutes. The serum bottles were sealed with the PTFE and aluminum crimps and were incubated in an incubator shaker at 170 rpm and 35℃. This process was continued until the color removal variation of the sample versus time was not notable. In order to analyze the color removal during the anaerobic phase, 4 ml of samples were taken from each serum bottle and were mixed with 4 ml of phosphate buffer (10.68 g/l $NaH_2PO_4.2H_2O$, 5.38 g/l $Na_2HPO_4.H_2O$) including ascorbic acid (200 mg/l). Ascorbic acid was added to the samples in order to inhibit the autoxidation of the dye metabolites [9]. The samples were centrifuged at 4500 rpm for 20 minutes to separate the activated sludge, and samples were analyzed by spectrophotometer.

The activated sludge as the microbial biomass was collected from the aerobic unit of Qeytariye wastewater treatment plant in Tehran. The activated sludge was aerated 30 days with an air pump and a porous diffuser through the 4-liter tank at room temperature and cultivated using a nutrient media in a sequencing batch mode.

After sufficient growth of the activated sludge (30 days), the adaption process of the activated sludge and dye was started. This process contained one-stage anaerobic-aerobic cycles. In each cycle, the serum bottles were kept in anaerobic and aerobic phases for 72 and 24 hours, respectively. In anaerobic phase, the experiment was carried out in 350 ml serum bottles (containing 280 ml of activated sludge, nutrient media, and dye) at the 4000 mg/l MLSS and dye with an initial concentration of 50 mg/l. The contents of serum bottles were transferred to Erlenmeyer for the aerobic phase. The samples were transferred to an incubator shaker at 170

rpm and 35℃. The anaerobic-aerobic cycles were continued till the changes in decolorisation efficiencies were not notable for dye with an initial concentration of 50 mg/l. Then, the initial concentration of dye was increased to 100 mg/l, and again, the anaerobic-aerobic adaption cycles were continued as described before, till a relative constant efficiency was reached.
To investigate the effect of salt on the anaerobic- aerobic degradation of RR195, the anaerobic phase was carried out using the serum bottle technique developed by Owen et al [11]. 100 ml serum bottles were used, and 80 ml of each serum bottle were filled with activated sludge, dye, media, and NaCl. The initial dye concentration of all the serum bottles was 100 mg/l, and the samples containing 20, 40, 60, 80, and 100 g/l salt were prepared. The initial pH was adjusted to 7 by one molar $NaHCO_3$ solution. The serum bottles were kept in an incubator shaker at 170 rpm and 35℃ for 72 h. The aerobic stage procedure was conducted like the method which has been explained by Bonakdarpour et al [9]. Whereas, in this study, the 250 ml Erlenmeyer flasks were placed in an incubator shaker at 170 rpm and 35℃ and for 24 h. During the aerobic phase, several Erlenmeyers were filled with distilled water and were put in the shaker to prevent evaporation. At the end of each phase, the samples were analyzed via the spectrophotometer.
MLSS of activated sludge was measured according to the Standard Methods (APHA 2540D).

Results and discussion

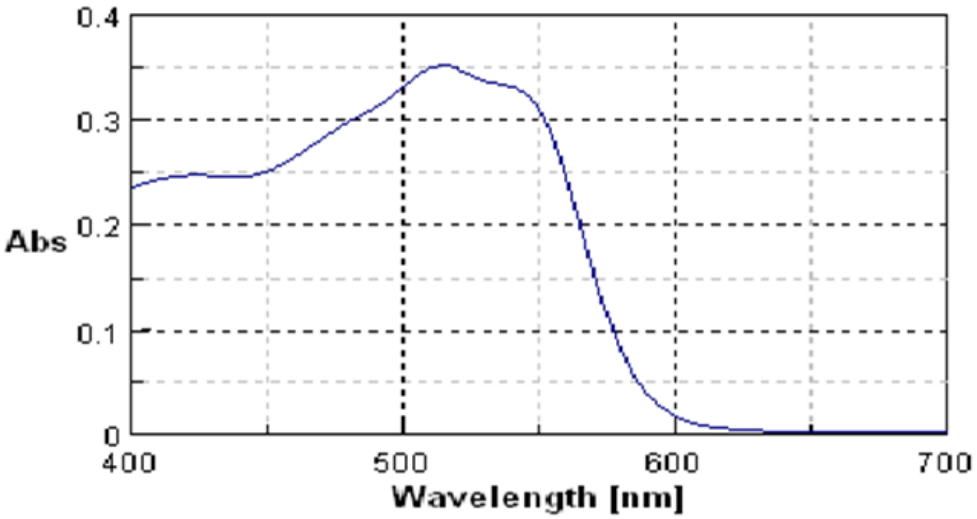

Fig 1. UV-Vis absorbance spectra of RR195 in the range of 400-700 nm wavelength

Fig 1. The concentration of RR195 vs. dye absorbance

Based on Fig 1, λ_{max} is equal to 515 nm. The decolorization efficiency during the anaerobic degradation phase was investigated versus time, and the results are shown in Fig 2. According to Fig 2, the decolorization efficiency reached 76.38% and 79.38% after 72 h and 135 h, respectively. As shown in Fig 3, the majority of dye was removed during the initial hours. 72 h is considered as the optimum time for anaerobic phase since the changes in decolorization efficiency was not notable after 72 h.

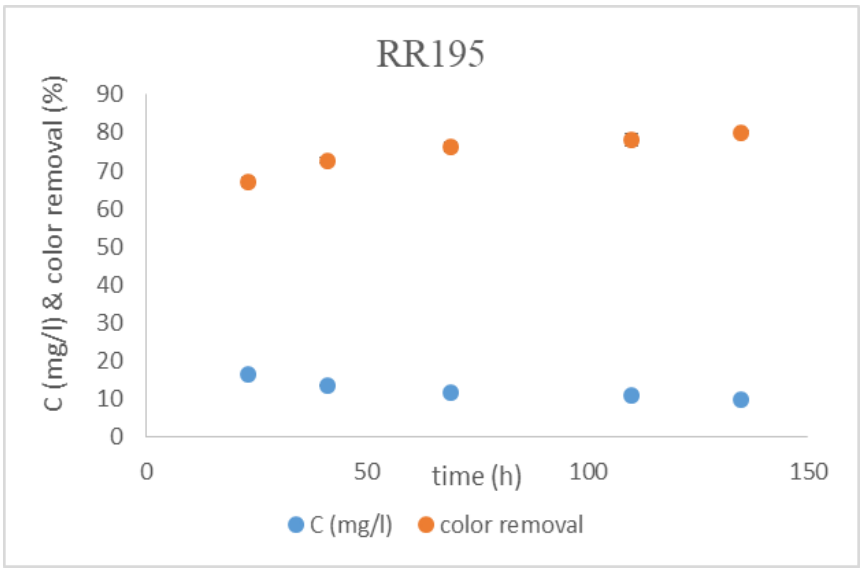

Fig 2. Decolorization efficiency vs. time (Initial RR195 concentration= 50 mg/l, MLSS= 1000 mg/l, Incubator shaker: 170 rpm and 35°C)

The results of the anaerobic-aerobic adaption of the activated sludge and RR195 are shown in Fig 3. The adaption process started with 50 mg/l of dye and continued for 23 days. During this period, the decolorization efficiency of both anaerobic and aerobic stages reduced. One possible reason can be that the contents of serum bottles were exposed to air during the aerobic phase and autooxidation of the dye metabolites occurred. Thus, at the beginning of the next cycle, the initial dye concentration was not exactly equal to 50 mg/l. Furthermore, the activated sludge was not adapted to the continuous anaerobic-aerobic cycles, and this incompatibility led to a decrease in the color removal efficiency. Based on Fig 3, changes in the color removal efficiencies between cycles 6 and 7 were not significant. From cycle 8, the adaption with 100 mg/l of the initial dye was performed. According to Fig 3, the decolorization efficiencies in both phases of a cycle increased. All the adaption procedure continued for 43 days, and 90% of color removal was observed at the end of the final anaerobic phase.

The decolorization efficiency obtained during the anaerobic-aerobic process, when NaCl was added, is presented in Fig 4. According to Fig 4, the main part of color removal happened during the anaerobic phase, and the aerobic stage had a negligible effect on the decolorization of RR195. The analysis indicates that a significant increase in the anaerobic and aerobic degradation efficiency stems from the addition of 20 mg/l of NaCl. Furthermore, the experiments showed that an increase of NaCl concentration in the range of 0-60 g/l led to an enhancement in the color removal in both anaerobic and aerobic stages. Vice versa, further increases in NaCl concentration up to the 100 g/l decreased the decolorization efficiency.

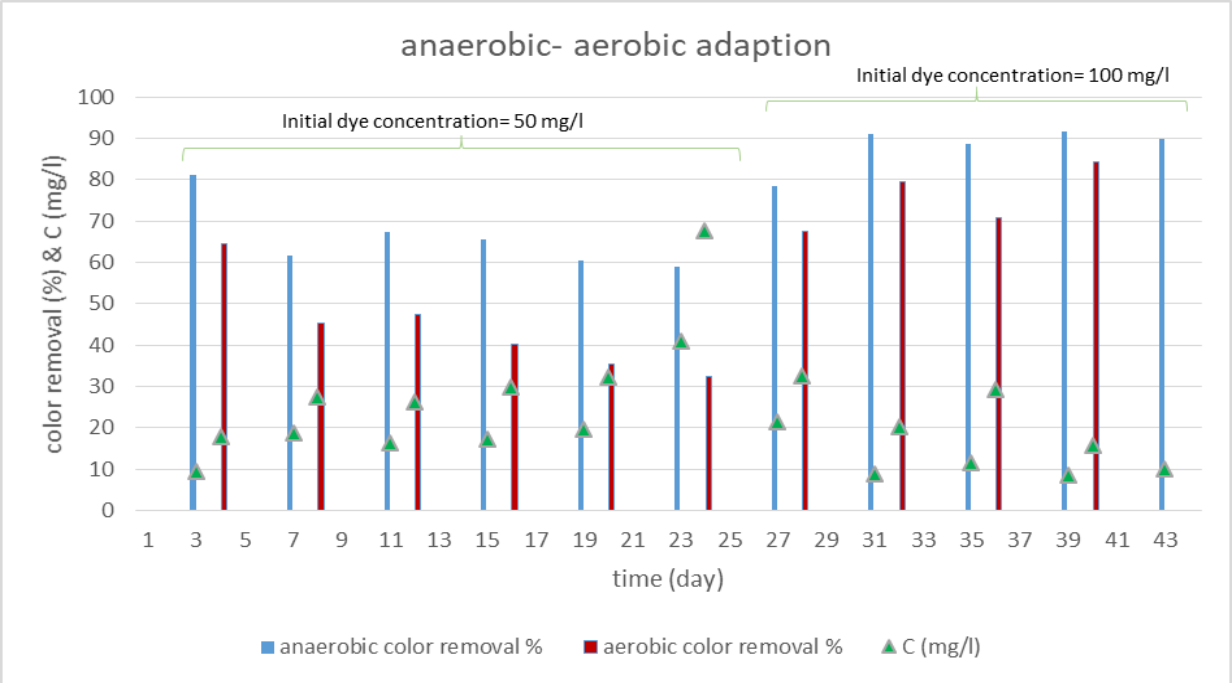

Fig 3. Decolorization efficiency vs. time during the anaerobic-aerobic adaption (Initial dye concentration: 50 and 100 mg/l, MLSS= 4000 mg/l, Incubator shaker: 170 rpm and 35°C)

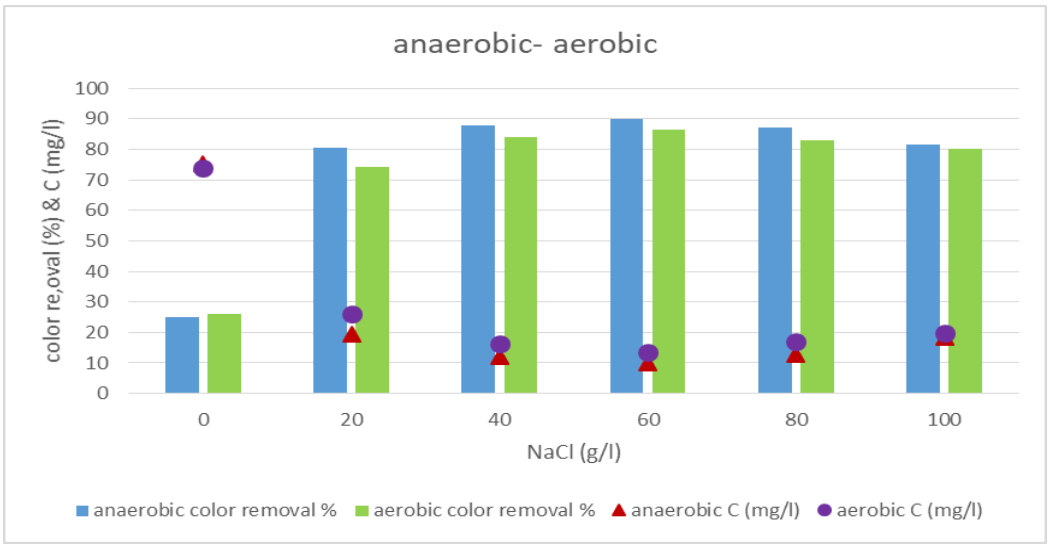

Fig 4. Decolorization efficiency vs. time during the anaerobic-aerobic treatment of samples with various concentration of sodium chloride (Initial dye concentration: 100 mg/l, MLSS= 4000 mg/l, Incubator shaker: 170 rpm and 35°C)

Conclusions

Based on the results from this project, 72 h was obtained as the optimum time for anaerobic decolorization of RR195. Moreover, during the anaerobic-aerobic adaption with the initial dye concentration of 50 mg/l, the decolorization efficiency decreased in both anaerobic and aerobic phases. Changes between the fifth cycle and sixth cycle were not notable, and thus, 60% was chosen as the final efficiency. By continuing the adaption process with the 100 mg/l

of initial dye concentration, the color removal efficiency increased till it reached an almost constant decolorization amountefficiency of 90%.
By adding NaCl up to the 60 g/l to the serum bottles, the decolorization efficiency increased during the one stage anaerobic-aerobic treatment of RR195. Then, by further increases in NaCl, the decolorization efficiency reduced. As a result, 60 g/l of NaCl was selected as the optimum salt concentration for this process.

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