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The decolorization of synthetic dyes with immobilized *Bacillus* species

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Abstract: Although, synthetic dyes have toxic effects on to environment, are very important for coloration of large number of materials and lead to increasing concerns about color removal worldwide. Immobilization of microorganisms is one of the promising techniques for biological treatment of wastewater with the many advantages for industrial usage. This study aimed to determine the efficiency of immobilized four different *Bacillus* species (*B. cereus, B. megaterium, B. subtilis* and *B. thuringiensis*) on decolorization of dyes (maxilon red, methyl red and doracryl blue) at different Na-alginate concentrations. *B. megaterium* and *B. thuringiensis* were most effective and decolorized the maxilon red 92.63% and 90.74%, respectively, with the Na-alginate concentration of 2.5%. *B. megaterium* was the most effective strain in decolorization of doracryl blue with 75.91% decolorization value and with beads at 10% Na-alginate concentration. *B. megaterium* was the most effective strain in decolorization of methyl red with 64.99% decolorization and the beads were prepared with 10% Na-alginate concentration. Among the four *Bacillus* species *B. megaterium* was the most effective in the decolorization experiments of all three dyes. Results of this study could be used as a reference for the development of effective removal technique for dyes in textile wastewater.

Keywords: *Bacillus* species; Decolorization, Immobilization, Na-alginate, Methyl red, Maxilon red, Doracryl blue

1 Introduction

Synthetic dyes are frequently used in the cosmetics, textile, food, leather, papermaking and pharmaceutical industries due to their high stability, low cost and variety in color (Przystaś et al. 2018). Dyes are categorized in three groups as anionic, cationic and non-ionic dyes. Azo, anthraquinone and phthalocyanine groups of reactive dyes, which are hard to remove under aerobic conditions, are widely used in textile industry. The textile wastewater contains these dyes as pollutant in high concentrations. Removal of azo dyes from aqueous effluents is difficult, as they might be stable when they exposed to light and heat (Saravanan et el. 2013, Al-Fawwaz et al. 2016, Hameed and Ismail 2018, Nath et al. 2019).

In last two decades, the application of immobilization techniques attracted attention for decolorization of wastewater. In a variety of scientific and industrial applications of immobilized microbial enzymes, organelles, and cells have been widely used due to the economic view that could be useful application in research for industry. The immobilization technology is an ecofriendly technique, in which high removal yields can be achieved, compared to physical and chemical processes or procedure that use not immobilized biological materials and reduces the secondary pollutants (Cassidy et al. 1996, Suganya and Revath 2006, Wen et al. 2018).

A wide variety of organisms, including bacteria, fungi, yeast, algae and plants, are capable of decolorizing dyes. The major problem in dye removal is the toxic effect of dyes on microorganisms. Therefore, a good method to increase the concentration of microorganisms and to protect from toxicity is immobilization. The immobilized microorganisms are cost effective by using several times without significant loss of activity (Saratale et al. 2011).

Among the various developed methods for cell immobilizing the encapsulation in calcium alginate beads was used in this study. The Na-alginate that used for immobilization of microorganisms is easy to use, non-toxic to humans and the environment, are legally safe for human use, present in large quantities and an economic option. The major advantage of alginate is that it is not exposed to major changes in the physicochemical state during immobilization and that the gel is transparent and permeable (de-Bashan and Bashan 2010).

This study aimed to decolorize of synthetic dyes (maxilon red, methyl red and doracryl blue) present in industrial wastewaters with different *Bacillus* species (*Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis* and *Bacillus thuringiensis*) by immobilization method with Na-alginate. The effect of Na-alginate concentrations were also determined on decolorization ratio.

2 Materials and Method

2.1 The synthetic dyes

The synthetic dyes MR, the MeR, The DB (is a basic dye) were used in this study and obtained from textile industry in Niğde (Birko Carpet Factory) and Figure 1 shows the chemical structure of the dyes (El-Syed et al. 2014, Vatandoostarani et al. 2017). The stock solution of the dye was prepared by membrane filtration at 10000 mg/L concentration. The maximum absorbans (λmax) of the each dye was determined at 430 nm for MeR, at 515 nm for MR and at 600 nm for DB by UV-Visible spectrophotometer (Jenway).

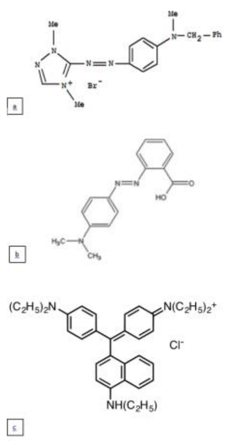

Figure 1. The chemical structure of synthetic dyes a) MR (El-Syed et al. 2014) b) the MeR (Vatandoostarani et al. 2017). c) The DB (is a basic dye)

2.2 Growth conditions for *Bacillus* species

The bacterial strains used in this study, *Bacillus thuringiensis*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium* were obtained from Niğde Ömer Halisdemir University, Biotechnology Department, Turkey. The bacterial cultures were grown in nutrient agar medium containing 5g peptone, 5g NaCl, 3g beef extract in 1L and incubated for 48h at 30°C under static conditions for sporulation. The medium was autoclaved at 121 °C for 15 min and the *Bacillus* species were grown at sterile conditions. The sporulated pure cultures of *Bacillus* species were used for immobilized beads (Amin et al. 2015).

2.3 Preparing of immobilized beads

In this study, four different concentrations of Na-alginate (1%, 2.5%, 5%, and 10%) were tested to determine the optimum Na-alginate concentration. Bacterial cultures were collected from petri dishes as 0.5 g sporulated bacteria and washed with 0.85% NaCl solution. The washed bacterial cultures were used for making Na-alginate beads and were mixed with different concentration Na-alginate solutions. The prepared solutions of bacteria and Na-alginate was dripped in 0.1 M $CaCl_2.2H_2O$ solution with a syringe to obtain the immobilized beads (Fig.2). By replacement of sodium and calcium ions, bacterial cells were trapped in alginate gel. After 30 minutes for hardening, the beads were filtered out and stored in 0.05 M $CaCl_2.2H_2O$ in the refrigerator for further studies. Beads did not prepared at sterile conditions and were washed with 0,85 % NaCl before experimental steps (Moreira Santos et al. 2002, Ogugbue et al. 2012).

Figure 2. The preparation of immobilized beads

2.4 Decolorization experiments

Decolorization studies were conducted in batch conditions at room temperature and the immobilized cells of *Bacillus* species were prepared with four different Na-alginate concentrations. Bio-beads of *Bacillus* species were made with 1%, 2.5%, 5%, 10% Na-alginate concentrations and 10 gram bio-beads transferred into 250 mL ErlenMeyer flasks which were containing 100 mL dye solution at 100 mg/L initial dye concentration. The flasks were kept at room temperature and the samples were taken in every 24 hours for ten days. The maximum absorbance was measured by spectrophotometer (Jenway). The dye decolorization yield was calculated with given formula below (Eq. 1). The C0 indicates that the dye concentration at the beginning and the Cf indicates that the dye concentration at the end of decolorization period (Castro et al. 2017).

$$\text{Decolorization Yield\%} = \frac{Co-Cf}{Co} * 100 \quad \text{(Eq. 1)}$$

3 Results and Discussion

Bioremediation is usage of microorganisms such as bacteria, fungi or algae to reduce, eliminate or treat the pollutants present in soils, sediments, water, and air The dye decolorization capacity of different types of microorganisms is affected by several conditions such as nutrients, pH, temperature and initial dye concentration. (Gül and Dönmez 2013, Gül 2018). The influence of immobilization technique with different type of microbial cells were reported and worked elsewhere before. Immobilized cells is used for wastewater treatment and color removal because of facilitates the separation and recovery of the immobilized bacteria and binding agent, and makes it usable by reducing the total cost also (Ha et al. 2009, Hyde et al. 1991, Zeroual et al. 2001). Therefore, this work focused only on the immobilization technique and the effect of Na-alginate concentration on dye decolorization. This work is about the dye decolorization capacity of different *Bacillus sp.*, the dye concentrations and decolorization yield of immobilized cells. The results were shown with following graphics for synthetic dyes and *Bacillus* species separately (Figures 3a,b,c,d, 4a,b,c,d, 5a,b,c,d).

3.1 The Maxilon red decolorization

The beads that prepared with 2.5% Na-alginate concentration and *B. megaterium* were demonstrated the highest removal yield, which was 92.63% at ten days decolorization period. The other *B. megaterium* beads, which were prepared with 1%, 5% and 10% Na-alginate concentrations the dye removals were, determined as 55.99%, 60.72% and 48.94% respectively (Fig.3d).

The *B. thuringiensis* beads demonstrated similar dye removal capacity to the *B. megaterium* with 2.5% Na-alginate beads as 90.74% decolorization. The MR decolorization was determined as 50.75%, 44.74%, and 43.44% for 1%, 5% and 10% Na-alginate sequentially for beads of *B. thuringisensis* (Fig. 3a).

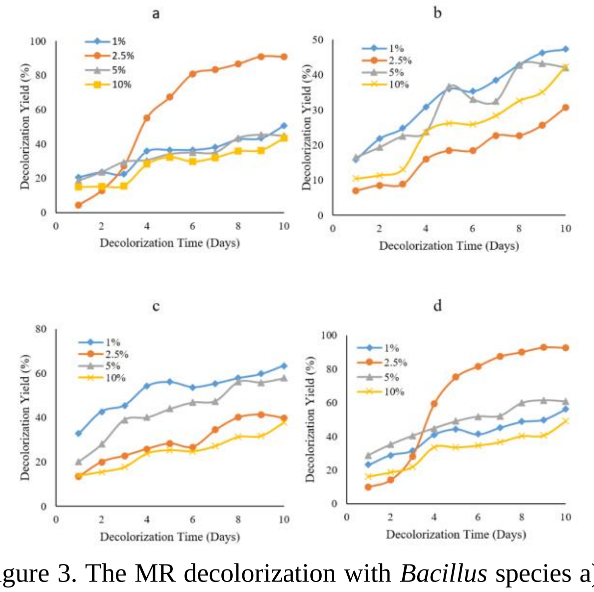

Figure 3. The MR decolorization with *Bacillus* species a) *B. thuringiensis* b) *B. cereus* c) *B. subtilis* d) *B. megaterium*
Blue, orange, grey and yellow lines refer to 1%, 2.5%, 5%, 10% Na-alginate concentration sequentially

B. subtilis was showed at 63,49% ratio that the maximum decolorization with 1% Na-alginate concentration at decolorization period. The beads which were prepared with 5% Na-alginate concentration showed 57.75% removal of MR and the 2.5% and 10% Na-alginate concentration demonstrated at 39.74% and 37.90% dye removal sequentially (Fig. 3c).

The lowest degrees of dye decolorization for MR were determined with the *B. cereus* beads and the maximum MR decolorization with *B. cereus* was determined as 47.25%. The 5% and 10% Na-alginate beads with *B. cereus* were showed 42% decolorization both and the lowest dye decolorization ratio for MR was determined for 2.5% Na-alginate-*B.cereus* beads as 30% removal (Fig. 3b).

The maxilon red decolorization was tested with immobilized *Phanerochaete chrysosporium* (1557) mycelium with kissiris that decolorized at maximum ratio (Karimi et al. 2009). Also electrooxidation and electrocoagulation technique could be used for MR decolorization (El-Sayed et al. 2014). In this work the maximum decolorization ratio was tested as 90% with immobilized *B. thuringisensis* with 2.5% Na-alginate concentration and this results are similar to other decolorization techniques for MR.

3.2. The doracryl blue decolorization

The doracryl blue was decolorized by only the 10% Na-alginate beads for all *Bacillus* species. The decolorization values, which showed at Figure 4, were determined as 10.49%, 26.49%, 25.16% and 75.91% for *B. thuringiensis* (Fig 4a), *B. cereus* (Fig. 4b), *B. subtilis* (Fig. 4c) and *B. megaterium* (Fig. 4d) respectively. The *B. megaterium* beads were the most effective biosorbents for DB. The other beads, which were prepared with 1%, 2.5% and 5% Na-alginate concentrations with all *Bacillus* species, showed approximately 5% dye decolorization.

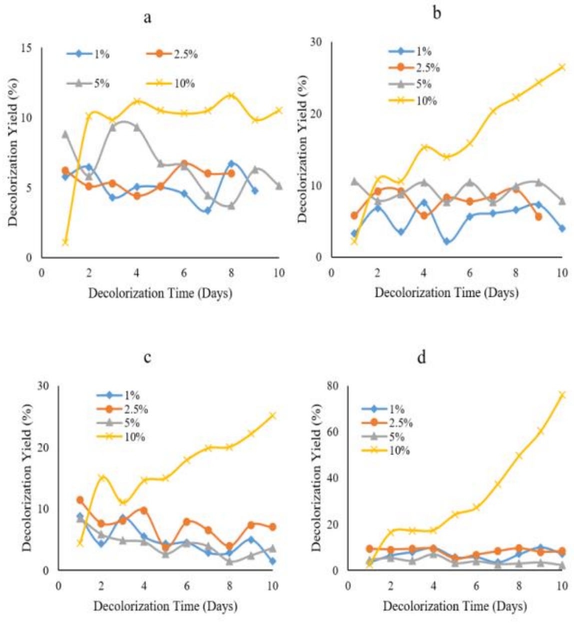

Figure 4. The DB decolorization with *Bacillus* species a) *B. thuringiensis* b) *B. cereus* c) *B. subtilis* d) *B. megaterium*
Blue, orange, grey and yellow lines refer to 1%, 2.5%, 5%, 10% Na-alginate concentration sequentially

Decolorization ratio of methylene blue with immobilized *B. subtilis* was determined as 80% and highest removal capacity of immobilized *Desmodesmus* sp. were determined for 5 mg/L initial methylene blue concentration. Moreover, *B. megaterium* was showed 90% Reactive Blue decolorization yield (Upendar et al. 2016, Erdem et al. 2019). In this work the immobilization of different *Bacillus* species were showed higher removal efficiency for DB at 10% Na-alginate concentration.

3.3 The methyl red decolorization

The decolorization of MeR by *B. megaterium* beads were more effective than the other *Bacillus* species. The 5% and 10% Na-alginate concentration were demonstrated 64,59% and 64,99% dye removal degree at decolorization period and the decolorization ratio of 1% and 2.5% Na-alginate concentrations were determined as 50.86% and 41.37% (Fig 5d).

The *B. subtilis* decolorized MeR at 40% approximately at 1%, 2.5% and 5% Na-alginate concentration. However, the beads that were prepared with 10% Na-alginate and *B. subtilis* and *B. cereus* removed the 54,84% and 54.23% of MeR from the decolorization solution (Fig. 5c).

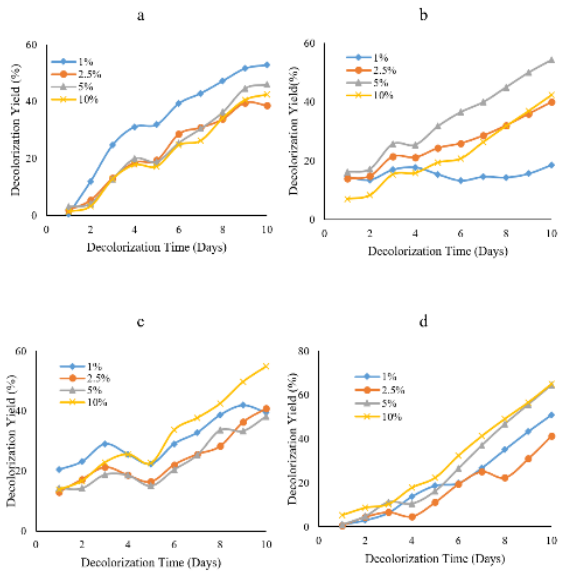

Figure 5. The MeR decolorization with *Bacillus* species a) *B. thuringiensis* b) *B. cereus* c) *B. subtilis* d) *B. megaterium* Blue, orange, grey and yellow lines refer to 1%, 2.5%, 5%, 10% Na-alginate concentration sequentially

The *B. thuringiensis* and 1% Na-alginate beads demonstrated similar dye removal capacity to the *B. subtilis* and *B. cereus* beads as 52.80% decolorization. The MeR decolorization was determined as 38.56%, 45.92%, 42.46% for 2.5%, 5% and 10% Na-alginate concentration sequentially for beads of *B. thuringisensis* (Fig. 5a,b,c).

Extensive studies have been performed to determine the role of the diverse groups of bacteria in the decolorization of different textile dyes. Similar to that of free cells, studies were also carried out using immobilized beads. A similar study was observed with the Reactive Yellow 17 and Reactive Blue 36 dyes of immobilized *B. licheniformis* strain. Cells immobilized with sodium alginate showed maximum color removal compared to free cells (Suganya and Revath 2006). In a study in which methylene blue, which is widely used in cotton and silk, was selected as a model dye, the biological removal of MB using *Bacillus subtilis* immobilized with Ca-alginate was tested in both batch and continuous contactors. More than 90% removal has been achieved during kinetic study in batch contactor (Upendar et al. 2016). Decolorization of the Maxilon red dye by *Phanerochaete chrysosporium* fungus immobilized in the trickle-bed reactor (TBR) using basal nitrogen-limited growth medium showed a color removal of approximately 94% in 4-5 days (Karimi et al. 2009).

Table 1. The summarized decolorization yields of *Bacillus* species

	MR		DB		MeR	
	Na-alg. conc. (%)	Decol. Yield (%)	Na-alg. conc. (%)	Decol. Yield (%)	Na-alg. conc. (%)	Decol. Yield (%)
B. thuringiensis	2.5	90.74	10	10.49	1	52.8
B. cereus	1	47.25	10	26.49	5	54.23
B:subtilis	1	63.49	10	25.16	10	54.84
B. megaterium	2.5	92.63	10	75.91	10	64.99

The results mentioned above in this work summarized in the Table 1, that were showed *B. thuringiensis*, and *B. megaterium* beads, which were prepared with 2.5% Na-alginate concentration, could be able to remove almost 90% of dye at decolorization solution. The Na-alginate-*Bacillus* beads were not effective to remove the DB, only 10% Na-alginate beads were decolorized the DB at ten days decolorization period. In addition, the MeR decolorization was approximately 50% with all *Bacillus* species. Besides, not only the 10% Na-alginate - *B. megaterium* beads were showed the maximum dye decolorization effect on the DB with 75% and MeR with 65% but also the 2.5% Na-alginate - *B. megaterium* beads removed the 92% of MR at the same decolorization period.

Bacillus sp. are effective microorganisms for removal of different dyes or textile effluent. Although, different microorganisms are useful for dye decolorization such as *Corynebacterium sp.* or *Pseudomonas sp.* (Gül 2018). *Bacillus sp.* was used for removal of Acid Red 2, Acid Orange 7, Remazol Black B and Congo Red and the removal rate were found as 90-100% (Jaiswal and Gomashe 2017, Shah et al. 2013). However, the *Bacillus subtilis* was removed the textile effluent at 63% decolorization rate (Sivaraj et al. 2011).

Bacterial decolorization is inexpensive and ecofriendly method and immobilization is very efficient method. In general, immobilized cells are more tolerant to factors such as temperature, pH and the presence of inhibitory compounds.

Na-alginate is non-toxic and suitable for use in gelation, so that immobilization with Na-alginate is an acceptable matrix material and is satisfactory for use with microorganisms (Sriamornsak and Nunthanid 1998, Tallur et al. 2009). The results showed that the decolorization of synthetic dyes and immobilized *Bacillus* species cells would be used for decolorization of different dyes at textile wastewater. The *Bacillus* species could be used for biodegradation as immobilized cells promisingly.

4 Conclusion

Many researchers have studied the environmental effects of synthetic dyes and their removal and this study aimed to determine the dye decolorization capacity of the immobilized *Bacillus* sp. It was understand from the results above mentioned that the *B. megaterium* was more effective than the other *Bacillus* species that were used ın the study. The *B. subtilis, B. cereus* and *B. thuringiensis* beads were affect by Na-Alginate concentrations and higher Na-alginate concentrations reduced the dye decolorization ratio. However the bio-beads that were made with *B. megaterium* and 10% Na-alginate concentration showed higher decolorization yields and they were decolorized the MR at 92%, DB at 75% and MeR at 65% at ten day decolorization period. In the light of these results, *B. megaterium* should be use for MR, MeR or DB decolorization as immobilized beads.

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