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Original Research

Remazol Blue Removal and EPS Production by *Pseudomonas aeruginosa* and *Ochrobactrum* sp.

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Abstract

Pseudomonas aeruginosa and *Ochrobactrum* sp. were tested according to EPS production at different pH, temperatures, Remazol Blue concentrations, and incubation periods. Optimum pH was 7 for *P. aeruginosa* and 8 for *Ochrobactrum* sp. according to the highest EPS amount. *P. aeruginosa* produced the highest EPS (456.4 $mg \cdot l^{-1}$) after incubation for 48 h (40ºC) and *Ochrobactrum* sp. gave the highest yield (404.6 $mg \cdot l^{-1}$) after incubation for 72 h (30ºC). *P. aeruginosa* had a low capacity to remove Remazol Blue (12.5%); however, *Ochrobactrum* sp. had a significant potential to remove Remazol Blue yielded as 89.4%.

Keywords: exopolysaccharide, Remazol Blue, bacteria, production, wastewater

Introduction

Wastewaters from industries like textiles, pulp and paper, dyeing, and tanning include toxic coloring substances that need to be treated. Highly colored synthetic dye effluents from textile industries have been released into receiving water and have polluted main water resources. Moreover, dyes may significantly affect photosynthetic activity in aquatic life due to reduced light penetration and may also be toxic to some aquatic life due to the presence of aromatics, metals, and chlorides, etc. in them [1-3]. The processes used to remove dye from wastewaters include biological treatment, coagulation-flocculation, and adsorption on powdered activated carbon, electrochemical processes, ozone treatment, membrane processes, reverse osmosis, nanofiltration, ultrafiltration, and microfiltration [4, 5]. Some dye decolorizing microorganisms that can remove colored pollutants under aerobic or anaerobic conditions have been isolated in previous studies [6-12]. Some studies also mentioned that extracellular polysaccharides (EPS) that produce microorganisms are able to remove dye from polluted wastewaters [12-14].

EPSs play an important role in the aggregation of bacterial cells in flocs, stabilization of a biofilm structure, and formation of a protective barrier. Related to its properties, EPS also has roles like protecting the microorganism from desiccation, osmotic stress, low temperature, and biosorption of toxic pollutants from industrial wastewaters [3, 8, 15-17]. Although EPS is mostly known as a toxic metal chelator [18, 19], it might also be used as a trap for dyestuff because of its properties [11, 13].

While many studies have examined heavy metal removal by EPS-producing microorganisms, little work has been done on dye removal by microorganisms having EPS. The decolorization of dye by *Pseudomonas* spp. [8, 10, 14] and *Ochrobactrum* spp. [10, 20] has been reported before, but the amount of EPS produced by these bacteria and the environmental conditions affecting EPS have not been clarified. In the present study, the effect of different pH levels, temperatures, Remazol Blue concentrations, and incubation periods on EPS production by the bacteria – namely *P. aeruginosa* and *Ochrobactrum* sp. – was examined on media with Remazol Blue. Our major objective was to investigate the correlation between EPS production and dye removal by the bacteria tested in this study.

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Experimental

The bacteria used in this study were *P. aeruginosa* and *Ochrobactrum* sp. All the strains were isolated from tannery effluents (Sepiciler Leather IND, INC). *P. aeruginosa* and *Ochrobactrum* sp. were previously identified by [10].

Remazol Blue dye stock solution obtained from Aytemizler Textile Co. (Turkey) in pure form was prepared by dissolving the powdered dyestuff in distilled water to a concentration of 2% (w/v). Appropriate volumes of the stock dye solution were added to nutrient agar media.

For EPS determination experiments, cultures were inoculated onto Nutrient Broth No. 1 media (Fluka-BioChemika) with agar in petri dishes. The composition of the media was 15 g of peptone, 3 g of yeast extract, 6 g of sodium chloride, 1 g of glucose, and 15 g of agar in 1 l. To determine the optimum pH for the highest EPS production, the pH of the media including 100 $mg \cdot l^{-1}$ Remazol Blue was adjusted to 6, 7, 8, and 9, with 0.1 M NaOH and 0.1 M HCI. The petri dishes were prepared in three replicates and incubated for 48 h at 30ºC (Memmert, Germany). To determine dry cell mass of the bacteria, wet biomass of the bacteria was dried at 100ºC for overnight.

The effects of different temperatures and initial Remazol Blue concentrations on EPS production by the microorganisms were investigated in another series of experiments. Microorganisms were grown on media, including 100, 200, 300, and 400 $mg \cdot l^{-1}$ Remazol Blue at predetermined optimum pH values. *P. aeruginosa* was cultured at pH 7 and *Ochrobactrum* sp. was inoculated into the media at pH 8. Nutrient agar media in petri dishes containing four different Remazol Blue concentrations were incubated at three different temperatures (20, 30, and 40ºC) for 48 h (Memmert, Germany). Experiments were also performed on media without Remazol Blue.

To identify the effect of different incubation periods on EPS production, experiments were performed under three different media conditions. Microorganisms were cultured on media including Remazol Blue at different temperatures that produced the highest EPS. Cultures were grown for different incubation periods, i.e. 48, 72, 96, 120, 144, and 168 h. EPS analysis was performed at the end of these incubation periods.

To determine Remazol Blue removal in liquid media by the microorganisms, cultures were grown in 100 mL of nutrient broth in 250 mL Erlenmeyer flasks and incubated in a rotary shaker (New Brunswick Scientific innova 4230, USA) at 100 rpm. Microorganisms were grown in media in which they produced the highest EPS. *P. aeruginosa* was grown in media including 100 $mg \cdot l^{-1}$ Remazol Blue at pH 7 (40ºC) and *Ochrobactrum* sp. was grown in 100 $mg \cdot l^{-1}$ Remazol Blue at pH 8 (30ºC). Remazol Blue removal was analyzed during the incubation period.

EPS Extraction

EPS isolation was carried out as described by [21], with minor modifications. To isolate EPS from cultivated cultures, colonies were first picked up from the agar surface with a glass rod and suspended in an osmolar solution. The suspensions were boiled at 100ºC for 15 min. After cooling, trichloroacetic acid (TCA) was added to a final concentration of 4% (w/v) to precipitate proteins, followed by centrifugation at 10,000 xg for 30 min at 4ºC. The supernatant was removed and mixed with cold ethanol. The suspension was centrifuged at 10,000 xg for 30 min at 4ºC. The extraction was repeated once. The pellet was removed and dissolved in distilled water. The sugar content of EPS was determined using phenol-H_2SO_4 according to the glucose standard [22].

Dye Analysis

During the incubation period, a 3 ml sample was taken from each flask. Samples were centrifuged to precipitate suspended biomass at 3,421 xg for 10 min. The concentration of Remazol Blue in the supernatant was determined by reading the absorbance at 600 nm. Cell-free Nutrient Broth medium was used as the blank. The percentage removal of Remazol Blue was calculated from the equation:

$$removal\% = \left[\left(Co - Cf\right) \ / \ Co\right] \times 100$$

In the equation, *Co* and *Cf* are initial and final concentrations ($mg \cdot l^{-1}$), respectively. Absorbance measurements and centrifugation were performed using a Shimadzu UV 2001 model spectrophotometer (Japan) and Hettich EBA12 model centrifuge (Germany).

The experiments were set up in a completely randomized design with three replicates. The data were subjected to analysis of variance using Minitab 14 and significant differences among treatment means were compared by descriptive statistics (±SE).

Results and Discussion

The effect of media pH on EPS formation by the bacteria was determined in samples, including 100 $mg \cdot l^{-1}$ initial Remazol Blue at the end of 48 h of incubation (Fig. 1).

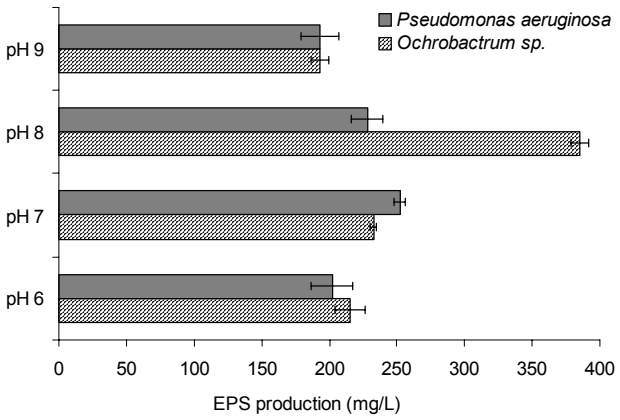

Fig. 1. Effect of different pH levels on EPS production by *P. aeruginosa* and *Ochrobactrum* sp. at 100 $mg \cdot l^{-1}$ Remazol Blue (T: 30ºC).

To find the optimal pH value for the highest EPS production by the bacteria, four different pH values (6, 7, 8, and 9) were tested. EPS production by *P. aeruginosa* increased slightly with an increase in pH from 6 to 7 and, above pH 7, it decreased with an increase in alkalinity. On the other hand, *Ochrobactrum* sp. produced the highest EPS amount in alkaline media. EPS production increased up to pH 8 and decreased at pH 9 for *Ochrobactrum* sp. At the end of this series of experiments, the maximum EPS production by *P. aeruginosa* (252.2 mg·l^{-1}) occurred at pH 7, whereas that of *Ochrobactrum* sp. (385.0 mg·l^{-1}) was at pH 8.

P. aeruginosa and *Ochrobactrum* sp. produced different amounts of EPS at increasing temperatures and Remazol Blue concentrations as shown in Figs. 2 and 3.

EPS formation of *P. aeruginosa* decreased with an increase in Remazol Blue concentration at all Remazol Blue concentrations tested (Fig. 2). At 20ºC the highest EPS amount was 298.6 mg·l^{-1} at 100 mg·l^{-1} Remazol Blue concentration. Above this Remazol Blue concentration at the same temperature, production of EPS was affected negatively and decreased. At 30ºC, the highest EPS production by *P. aeruginosa* was 252.2 mg·l^{-1} at 100 mg·l^{-1} Remazol Blue. Production decreased with an increase in Remazol Blue concentration and therefore EPS formation decreased after 100 mg·l^{-1} Remazol Blue concentration at 30ºC. Under 20ºC and 30ºC conditions, EPS produced by bacteria was not induced from the difference of the temperature from 20 to 30ºC. Although the production was lower at 30ºC than it was at 20ºC, the amount was not as high as the amount at 40ºC. Interestingly, the bacteria were able to tolerate high temperatures, and maximum EPS production occurred at 40ºC in 100 mg·l^{-1} Remazol Blue. Under these conditions the maximum EPS amount for *P. aeruginosa* was 456.4 mg·l^{-1}. At this temperature, production of EPS decreased when Remazol Blue concentration increased as at 20 and 30ºC. The results showed that EPS produced by *P. aeruginosa* could not be induced by the temperatures as 20ºC and 30ºC, but could be induced by the increase of the dye and the temperature as 40ºC.

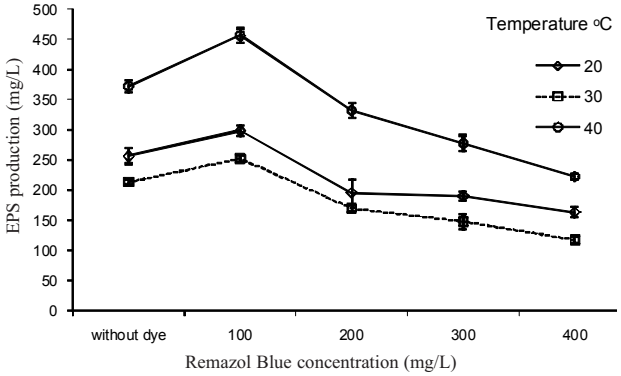

Fig. 2. Effect of different temperatures on EPS production by *P. aeruginosa* at increasing Remazol Blue concentrations.

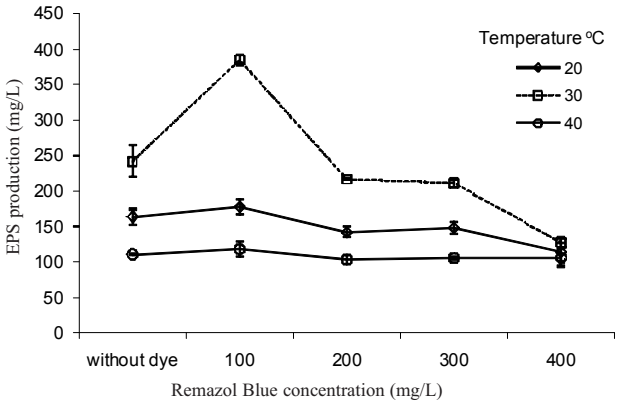

Fig. 3. Effect of different temperatures on EPS production by *Ochrobactrum* sp. at increasing Remazol Blue concentrations.

The EPS production level by *Ochrobactrum* sp. at different temperatures and Remazol Blue concentrations is shown in Fig. 3. At 20ºC and 40ºC, bacteria produced slightly more EPS in 100 mg·l^{-1} Remazol Blue compared to media without Remazol Blue. At this Remazol Blue concentration, the highest production levels were 177.6 mg·l^{-1} (20ºC) and 118.1 mg·l^{-1} (40ºC) while in media with no dye the amounts were 163.7 mg·l^{-1} and 110.6 mg·l^{-1}, respectively. At 200 mg·l^{-1} Remazol Blue concentration, EPS production was 142.0 mg·l^{-1} at 20ºC and 103.3 mg·l^{-1} at 40ºC. In 400 mg·l^{-1} Remazol Blue, production decreased to 114.4 mg·l^{-1} at 20ºC and underwent a small rise (105.9 mg·l^{-1}) at 40ºC. On the other hand, bacteria produced a high amount of EPS at 30ºC compared to at 20 and 40ºC. At 30ºC, the highest EPS production was observed in 100 mg·l^{-1} Remazol Blue (385.0 mg·l^{-1}). Above 100 mg·l^{-1} Remazol Blue, the production of EPS decreased significantly at this temperature. The highest EPS production levels were 216.7 mg·l^{-1}, 211.1 mg·l^{-1}, and 127.4 mg·l^{-1} at 200 mg·l^{-1}, 300 mg·l^{-1}, and 400 mg·l^{-1} Remazol Blue concentrations, respectively.

At the end of these series of experiments, it was found that the two microorganisms used in this study had a distinct tolerance to different temperatures in producing EPS. According to this, *P. aeruginosa* produced the highest amount at 40ºC, while *Ochrobactrum* sp. did so at 30ºC.

The increase in stress conditions like Remazol Blue and temperature significantly affected EPS formation by *P. aeruginosa* and *Ochrobactrum* sp. The two bacteria tested in the study produced more EPS on media with 100 mg·l^{-1} Remazol Blue than in the absence of this pollutant. Similar results were obtained for *P. aeruginosa* and *Ochrobactrum* sp. when these bacteria were exposed to another pollutant like Cr(VI) [23]. According to that study, in response to different Cr(VI) concentrations (50-300 mg·l^{-1}) the two bacteria produced higher amounts of EPS compared to media without Cr(VI). *P. aeruginosa* produced the highest amount of EPS (522.2 mg·l^{-1}) at 20ºC on media, including 50 mg·l^{-1} Cr(VI), while *Ochrobactrum* sp. gave the highest yield (430.5 mg·l^{-1}) at 30ºC on media with 150 mg·l^{-1} Cr(VI). Another study performed with *P. aeruginosa* yielded similar results when the bacterium was exposed to a pollutant like Cu(II) ions [24]. In that study, at 100 mg·l^{-1} Cu(II) bacteria produced more EPS compared to media without Cu(II).

The single effect of increasing temperature on EPS production by *P. aeruginosa* and *Ochrobactrum* sp. in media without Remazol Blue is shown in Fig. 4.

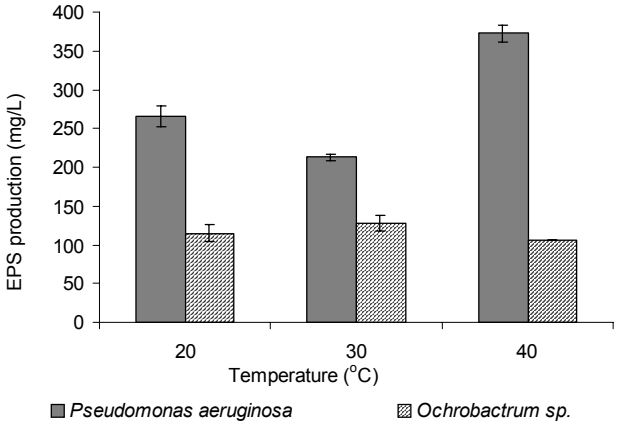

Fig. 4. Comparison of EPS production of *P. aeruginosa* (pH 7) and *Ochrobactrum* sp. (pH 8) at different temperatures in media without Remazol Blue.

EPS production by *P. aeruginosa* underwent a small drop when the temperature increased from 20ºC to 30ºC. However, the highest EPS production occurred at 40ºC (372.4 mg·l^{-1}). This bacterium was able to tolerate all the temperatures tested in the study, and therefore EPS produced by *P. aeruginosa* increased when the temperature increased. Interestingly, different results were obtained when the bacterium was exposed to another pollutant like Cr(VI) on media with less peptone and without glucose and sodium chloride [23]. According to that study, the bacterium was affected negatively by an increase in temperature and could not grow at high temperatures. Therefore, EPS formation was also affected negatively. In the present study, *P. aeruginosa* tolerated high temperatures according to the rich content of the media. This microorganism produced the highest amount of EPS at 40ºC. It was also previously shown that glucose had a positive effect on EPS production [25]. Another study also indicated that at high carbon concentrations (60 g·l^{-1}) the yield of EPS increased [26]. Another microorganism producing much more EPS under high temperature conditions also was reported [27].

On the other hand, *Ochrobactrum* sp. was affected negatively by an increasing temperature and produced low amounts of EPS. The highest production was observed at 30ºC (127.4 mg·l^{-1}). The maximum EPS levels were 114.4 mg·l^{-1} and 105.9 mg·l^{-1} at 20ºC and 40ºC, respectively. Similar results were obtained for *Ochrobactrum* sp. on a different medium with less peptone, without glucose and sodium chloride when the bacterium was exposed to another pollutant [23].

When we compared EPS formation by *P. aeruginosa* and *Ochrobactrum* sp., *P. aeruginosa* had a distinctive potential for producing EPS at high temperatures.

To determine the effect of the incubation period on EPS formation, bacteria were grown in media in which they produced the highest amount of EPS. For this purpose, *P. aeruginosa* (pH 7, 40ºC) and *Ochrobactrum* sp. (pH 8, 30ºC) were grown in 100 mg·l^{-1} Remazol Blue. EPS analysis was performed at the end of the incubation periods, i.e. 48, 72, 96, 120, 144, and 168 h. To determine Remazol Blue removal by *P. aeruginosa* and *Ochrobactrum* sp., microorganisms were inoculated in nutrient broth. The experimental conditions were the same as those used to determine the effect of an incubation period on EPS production. Remazol Blue analysis was performed at the end of the incubation periods tested in the study.

The effect of an incubation period on EPS production and Remazol Blue removal by *P. aeruginosa* is shown in Fig. 5. *P. aeruginosa* produced less EPS with an increase in incubation period. This bacterium produced the highest EPS as 456.4 mg·l^{-1} (68.5 mg dry weight·g^{-1}) after incubation for 48 h. Production was 147.8 mg·l^{-1} after incubation for 168 h. Although the maximum EPS amount occurred after incubation for 48 h, removal of Remazol Blue was the lowest (3.6%). Remazol Blue was removed with a maximum yield of 12.5% after 168 h of incubation. These data indicated that EPS produced by *P. aeruginosa* had a low potential to remove Remazol Blue from the media. Although bacteria produced fewer amounts of EPS with an increase in incubation period, dye removal was continued by bacteria without using an EPS mechanism.

The effect of incubation period on EPS production and Remazol Blue removal by *Ochrobactrum* sp. is summarized in Fig. 6. EPS production by the bacterium increased with increasing incubation period from 48 h to 72 h. The amount of EPS was 385.0 mg·l^{-1} at the end of 48 h.

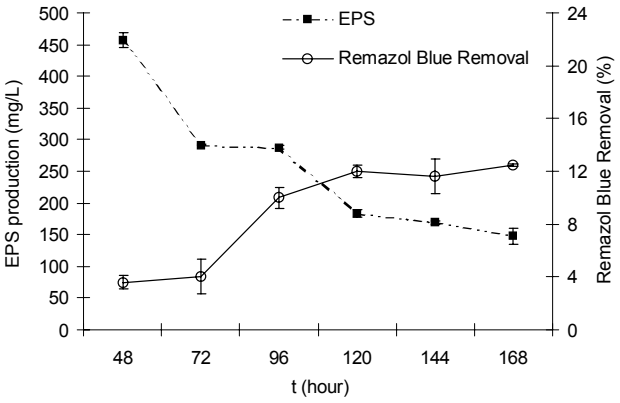

Fig. 5. Effect of incubation period on EPS production and Remazol Blue removal of *P. aeruginosa*.

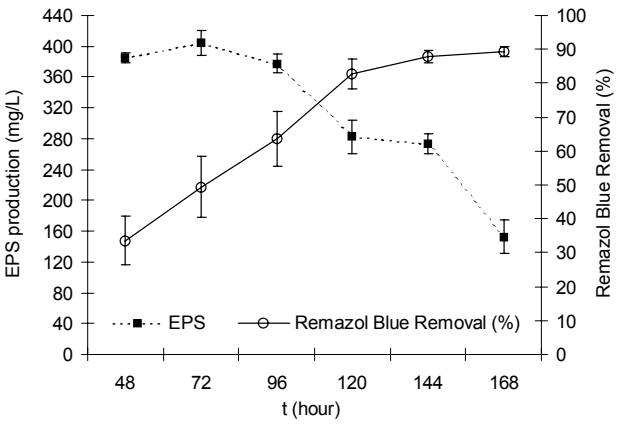

Fig. 6. Effect of incubation period on EPS production and Remazol Blue removal of *Ochrobactrum* sp.

After this incubation period, removal of Remazol Blue was 33.6%. The highest EPS production was obtained after incubation for 72 h as 404.6 mg·l^{-1} (60.7 mg dry weight·g^{-1}). Remazol Blue removal also increased to 49.4% after this incubation period. At the end of 96 h, EPS production by *Ochrobactrum* sp. underwent a small decrease, but the amount was still high (377.4 mg·l^{-1}). After this incubation period, according to the high amount of EPS, bacteria removed Remazol Blue with a yield of 63.6%. When the incubation period increased to 120 h and 144 h, production of EPS decreased to 282.6 mg·l^{-1} and 273.9 mg·l^{-1}, respectively. The removal of Remazol Blue was 82-87% after these incubation periods. At the end of 168 h, EPS production by this bacterium was 152.2 mg·l^{-1}. Remazol Blue was removed with a yield of 89.4% after this incubation period. In contrast to *P. aeruginosa*, it was found that the EPS produced by *Ochrobactrum* sp. had a potential to remove the toxic dye from the media.

When it comes to how bacteria removed the applied dye, it was related to bacterial metabolism. Although blue color was observed at the beginning of the incubation period (in 2 days of the incubation period) in the biomass and the occurred EPS, this blue color was not observed in these parts at the end of the incubation period. Therefore, this observation proved that Remazol Blue was removed by the two bacteria used in the study.

Conclusions

For highest EPS production the optimum pH level was 7 for *P. aeruginosa* and 8 for *Ochrobactrum* sp. EPS production by the microorganisms was highly affected by the increasing temperatures, Remazol Blue concentrations, and incubation periods. The two bacteria tested in the present study produced the highest EPS on media with 100 mg·l^{-1} Remazol Blue. The highest yield (456.4 mg·l^{-1}) with *P. aeruginosa* was achieved at 40ºC after incubation for 48 h, while with *Ochrobactrum* sp. (404.6 mg·l^{-1}) it was at 30ºC after incubation for 72 h.

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References

1. SIRIANUNTAPIBOON S., SRISORNSAK P. Removal of disperse dyes from textile wastewater using bio-sludge. Biores. Technol. **98**, 1057, **2007**.
2. CLARKE E.A., ANLIKER R. Organic dyes and Pigments. In: Hand-book of environmental chemistry, anthropogenic compounds part A, Springer vol. **3**. New York, pp. 181-215, **1980**.
3. AKSU Z. Application of biosorption for the removal of organic pollutants: a review, Process Biochem. **40**, 997, **2005**.
4. ALLEGRE, C., MOULIN P., MAISSEU M., CHARBIT F. Treatment and reuse of reactive dyeing effluents. J. Memb. Sci. **269**, 15, **2006**.
5. CRINI G. Non-conventional low-cost adsorbents for dye removal: A review. Biores. Technol. **97**, 1061, **2006**.
6. NIGAM P., BANAT I.M., SINGH D., MARCHANT R. Microbial process for the decolorization of textile effluents containing azo, diazo and reactive dyes. Process Biochem. **31**, 435, **1995**.
7. DÖNMEZ G. Bioaccumulation of the reactive textile dyes by *Candida tropicalis* growing in molasses medium. Enzyme. Microbial. Technol. **30**, 363, **2002**.
8. KHEHRA M.S., SAINI H.S., SHARMA D.K., CHADHA B.S., CHIMNI S.S. Biodegradation of azo dye C.I. Acid Red 88 by an anoxic-aerobic sequential bioreactor. Dyes. Pigment. **70**, 1, **2006**.
9. SADETTİN S., DÖNMEZ G. Simultaneous bioaccumulation of reactive dye and chromium(VI) by using thermophil *Phormidium* sp. Enzyme Microbial. Technol. **41**, 175, **2007**.
10. KILIÇ N.K., NIELSEN J.L., YÜCE M., DÖNMEZ G. Characterization of a simple bacterial consortium for effective treatment of wastewaters with reactive dyes and Cr(VI), Chemosphere **67**, 826, **2007**.
11. CASAS N., BLANQUEZ P., GABARRAELL X., VICENT T., CAMINAL G., SARA M. Degradation of orange G by laccase: fungal versus enzymatic process. Environ. Technol. **28**, 1103, **2007**.
12. LODATO A., ALFIERI F., OLIVIERI G., DI DONATO A., MARZOCCHELLA A., SALATINO P. Azo-dye conversion by means of *Pseudomonas* sp. OX1. Enzyme. Microbial. Technol. **41**, 646, **2007**.
13. SHAH V., GARG N., MADAMWAR D. An integrated process of textile dye removal and hydrogen evolution using cyanobacterium, *Phormidium valderianum*. W.J. Microbial. Biotechnol. **17**, 499, **1999**.
14. CHEN, B. Understanding decolorization characteristics of reactive azo dyes by *Pseudomonas luteola*: toxicity and kinetics. Process Biochem. **38**, 437, **2002**.
15. ZHANG D., WANG J., PAN X. Cadmium sorption by EPSs produced by anaerobic sludge under sulfate-reducing conditions. J. Hazard. Mater. **B138**, 589, **2006**.
16. HUGENHOLTZ J., LOOIJESTEIJIN E., STARRENBURG M., DIJKEMA C. Analysis of sugar metabolism in an EPS producing *Lactococcus lactis* by ^{31}P NMR. J. Biotechnol. **77**, 17, **2000**.
17. CZACZYK K., MYSZKA K. Biosynthesis of Extracellular Polymeric Substances (EPS) and Its Role in Microbial Biofilm Formation. Pol. J. Environ. Stud. **16**, (6), 799, **2007**.
18. IYER A., MODY K., JHA B. Accumulation of hexavalent chromium by an exopolysaccharide producing marine *Enterobacter cloaceae*. Marine Poll. Bull. **49**, 974, **2004**.
19. OZDEMIR G., OZTURK T., CEYHAN N., ISLER R., COSAR T. Heavy metal biosorption by biomass of *Ochrobactrum antropi* producing exopolysaccharide in activated sludge. Biores. Technol. **90**, 71, **2003**.
20. WACKEROW-KOUZOVA N.D. *Ochrobactrum intermedium* ANKI, a nitrogen-fixing bacterium able to decolorize azobenzene. Appl. Biochem. Microbiol. **43**, 403, **2007**.
21. CÉRANTOLA S., BOUNÉRY J., SEGONDS C., MARTY N., MONTROZİE H. Exopolysaccharide production by mucoid and non-mucoid strains of *Burkholderia cepacia*. FEMS Microbiol. Lett. **185**, 243, **2000**.
22. DUBOIS M., GILLES K.A., HAMILTON J.K., REBERS P.A., SMITH F. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**, 350, **1956**.

23. KILIÇ N.K., DÖNMEZ G. Environmental conditions affecting exopolysaccharide production by *Pseudomonas aeruginosa*, *Micrococcus* sp., and *Ochrobactrum* sp. J. Hazard. Mater. **154**, 1019, **2008**.
24. KAZY S.K., SAR P., SINGH S.P., SEN A.K., D'SOUZA S.F. Extracellular polysaccharide of a copper-sensitive and a copper-resistant *Pseudomonas aeruginosa* strain: synthesis, chemical nature and copper binding. W. J. Microbial. Biotechnol. **18**, 583, **2002**.
25. VELASCO S., ÅRSKÖLD E., PAESE M., GRAGE H., IRASTORZA A., RÅDSTRÖM P., VAN NIEL E.W.J. Environmental factors influencing growth of and exopolysaccharide formation by *Pedicoccus parvulus* 2.6. Int. J. Food Microbiol. **111**, 252, **2006**.
26. LEE W.Y., PARK Y., AHN J.K., KA K.H., PARK S.Y. Factors influencing the production of endopolysaccharide and exopolysaccharide from *Ganoderma applanatum*. Enzyme. Microbial. Technol. **40**, 249, **2007**.
27. NANDAL K., SEHRAWAT A.R., YADAV A.S., VASHISHAT R.K., BOORA K.S. High temperature-induced changes in exopolysaccharides, lipopolysaccharides and protein profile of heat-resistant mutants of *Rhizobium* sp. (Cajanus). Microbiol. Res., **160**, 367, **2005**.