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the international water association

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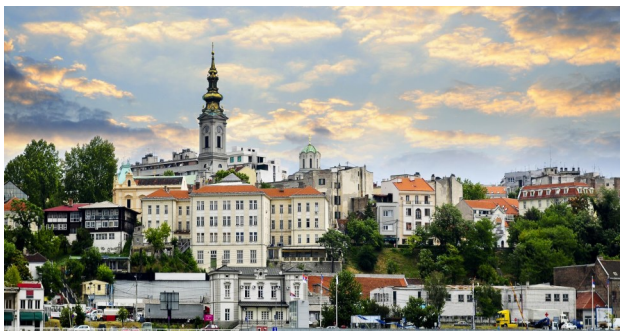

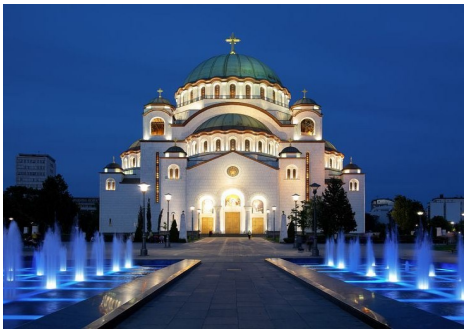

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Biological Treatment of Colored Wastewater by *Streptomyces fulvissimus* CKS 7

A. V. Buntić, M. D. Pavlović, S. S. Šiler-Marinković and S. I. Dimitrijević-Branković *

* Department of Biochemical Engineering and Biotechnology, Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia (E-mail: *abuntic@tmf.bg.ac.rs; marija.pavlovic@tmf.bg.ac.rs; siler@tmf.bg.ac.rs; suzana@tmf.bg.ac.rs*)

Abstract
This study aims to investigate an advanced biological processes these are relayed to biodegradable potential of growing microbial cells for contaminated water treatment. Thus the use of the *Streptomyces fulvissimus* CKS 7 has been evaluated for decolorizing efficiency of a solution containing a cationic triphenylmethane dye, crystal violet (CV). The color reduction was monitored by UV–Vis spectroscopic analysis, through changes in their absorption spectrum and comparing the results, to those of the respective controls. It was found that the CKS 7 performed well and reached up to 100 % of effectiveness. The required process parameters have been apparently mild and include the 10 % inoculum size, reaction temperature of 27-30° C, under shaking conditions, whereas the time course of decolorization has been concentration dependent. A possible mechanism for removing dye from working medium was accomplished in two steps: binding of the dye by bacterial cells on their surface in addition to dye biodegradation by bacterial intracellular enzymes. After one cycle of the complete dye removal, adapted culture was successfully reused for the same purpose. The phytotoxicity analysis revealed that no toxic compounds were present in decolorized medium, indicating that the use of CKS 7 bacteria seems to be promising applicants for contaminated water treatment.

Keywords
Biodegradation; crystal violet dye; *Streptomyces fulvissimus* CKS 7

INTRODUCTION

A frequent appearance of contaminated effluents, spilled in rivers, causes the growing need for development of more effective strategies for environmental pollution control (Kalyani, 2009). Among the most invasive water contaminants, triphenylmethane dyes merit remarkable attention. They belong to the most important group of synthetic colorants and are used extensively in many industrial processes (Bumpus, 1988, Vasdev, 1995, Azmi, 1998, Ayed, 2009). Unfortunately, due to their complex aromatic molecular structures which make them more difficult to degrade (Azmi, 1998, Ayed, 2010), they tend to pass through conventional wastewater treatment systems unaffected. Accordingly, biological processes these include the use of high potential microbial cells and their metabolites for hazardous dye biodegradation to their non-toxic and safe forms have received an increasing interest. It is owing to their cost, effectiveness, ability to produce less sludge, environmental benignity and the possibility to operate under mild conditions (Azmi, 1998, Ayed, 2009). Additionally, the final product, this remained after dye molecule breakdown, may enrich the nutritional value of the waste, as well to be used as soil conditioner or fertilizer. Thus, the environmental damage minimization and the public health protection would be carried on an advanced level.

This study has explored the potential of *Streptomyces fulvissimus* CKS 7, this belongs to the collection of natural forest soil isolates, for decolorizing a solution containing a cationic triphenylmethane dye, crystal violet (CV). The effect of time required for dye degradation and other rate-dependent environmental parameters (agitation, inoculum size, reaction temperature and dye

concentration) were characterized. The phytotoxicity of the products formed after decolorization were evaluated.

MATERIAL AND METHODS

Dyestuffs and other chemicals

Crystal Violet (tris(4-(dimethylamino)phenyl)methylium chloride) dye was purchased from Acros organics Co. (New Yersey, USA), while yeast extract powder and casein hydrolysate for growing medium were obtained from Biolife Italiana (Milano, Italy) and Sigma Aldrich (Steinheim, Germany), respectively.

Microorganism and culture medium

Streptomyces fulvissimus CKS 7 (CKS7) was isolated from forest soil (Kranjska gora, Slovenia). The purified culture was maintained in the ISP1 broth. The CKS7 culture was growing for 48 h in an ISP1 medium which was prepared by 5 g/L of casein hydrolysate and 3 g/L of yeast extract powder. For successful growing, this microorganism required an aeration condition and temperature of 30 °C. Thus, prepared fresh culture of CKS7 was used for all experiments without prior acclimatization.

Biodegradation experiment

Biodegradation experiments were done in the three sets with 100 mL Erlenmeyer flask on a translatory shaker (IKA – KS 4000i control, Staufen, Germany) under the aeration (120 rpm). The CKS7 culture was incubated with 25 mL of the predetermined working dyes solution (1, 3 and 5 mg/L) in the ratio 1:10 (v:v). The effects of time, inoculum size, shaking/aeration and temperature have been examined on the biodegradation process. The aliquot (2 ml) of the culture media was taken after 24 h, centrifuged at 10000 rpm for 10 min and separated from the bacterial cell mass.

Color reduction was monitored by UV–Vis spectroscopic analysis, (using UV–vis spectrophotometer – Ultrospec 3300 pro, Amersham Biosciences, USA), through changes in their absorption spectrum (450-650 nm), and comparing the results, to those of the respective controls. The decolorization percentage is expressed as follows equation (Buntić, 2013):

$$\textit{Decolorization (\%)} = \frac{A_I - A_O}{A_I} \times 100 \quad (1)$$

where A_I is an initial absorbance and A_O is an observed absorbance.

Phytotoxicity study

Phytotoxicity of the CV dye solutions and its biodegradation products produced by CKS7 microorganism were performed in order to assess their toxicity. The samples, before (1-20 mg/L) and after biodegradation process were dissolved in the agar in the ratio 1:4 (v:v) and mixtures were poured into Petri dishes. The study was carried out using *Triticum aestivum* seeds, with pure agar medium as control. The test results were expressed by relative seed germination (%) index (RSGI) and length of shoot and root were recorded after four days (Ayed, 2009, Colón, 2010). All parameters were calculated using three replicates. The percentage of relative seed germination was calculated according to equation:

$$RSGI\ (\%) = \frac{SG_S}{SG_C} \times 100 \quad (2)$$

where *RSGI* is a relative seed germination index, SG_S is seeds germination in samples and SG_C is seeds germination in control.

RESULTS AND DISCUSSIONS

Effect of static and shaking conditions

The effect of shaking/aeration conditions on the biodegradation process was carried out in the range of 0 to 200 rpm on a translatory shaker and results were presented on Figure 1.

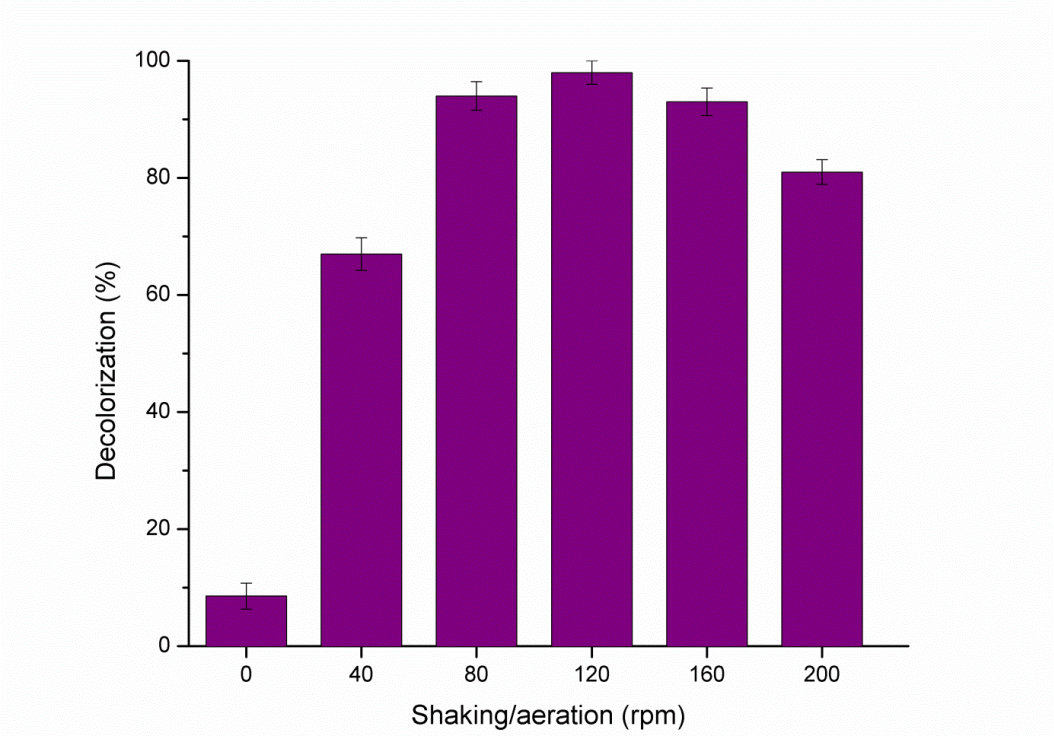

Figure 1. Effect of shaking/aeration on CV biodegradation by CKS7.

It has been observed that the static culture was shown almost no growth in nutrient medium. The static conditions were not appropriate for biodegradation nor the decolorization of CV dye. The percentage of decolorization was very low, just about 10%. Thus, the biodegradation process was very slow, probably due to the poor cell growth. On the other hand, very strong, dynamic conditions were unsuitable, too. During the microbial action, the change in pH was recorded to be negligible in both cases. It confirms the decolorization of dye was due to microbial action. The optimal shaking/aeration conditions were at 120 rpm. The literature reports also show that for successful biodegradation of CV dye and other triphenylmethane dyes by *Staphylococcus epidermidis* was required 150 rpm of shaking condition (Ayed, 2010). Therefore, agitate conditions were adopted to investigate bacterial decolorization in the following experiments Table 1.

Table 1. Factors and levels of single factor experiment.

Factors	Level									
Shaking/aeration (rpm)	0	40	80	120*	160	200				
Temperature (°C)	27	30*	35	40						
Inoculum size (%)	2	5	10*							
Time (h)	1	2	4	6	12	22	24*	27	31	36
Dye concentration (mg/L)	1*	3	5							

*the levels kept constant when other factors were investigated

Effect of inoculum size and temperature on the biodegradation

Effects of inoculum size (2, 5 and 10 %) and temperature (27, 30, 35 and 40 °C) on the biodegradation of CV dye by CKS7 were presented on Figure 2. With the increase of the inoculums size, the percentage of decolorization was increasing, too. Along the entire temperature range investigated, the inoculum size of 10 % has expressed the highest values of decolorization percent. A similar observation was reported during decolorization of Malachite Green (triphenylmethane dye type) by *Kocuria rosea* MTCC 1532 and *Sphingomonas paucimobilis* (Parshetti, 2006, Ayed, 2009).

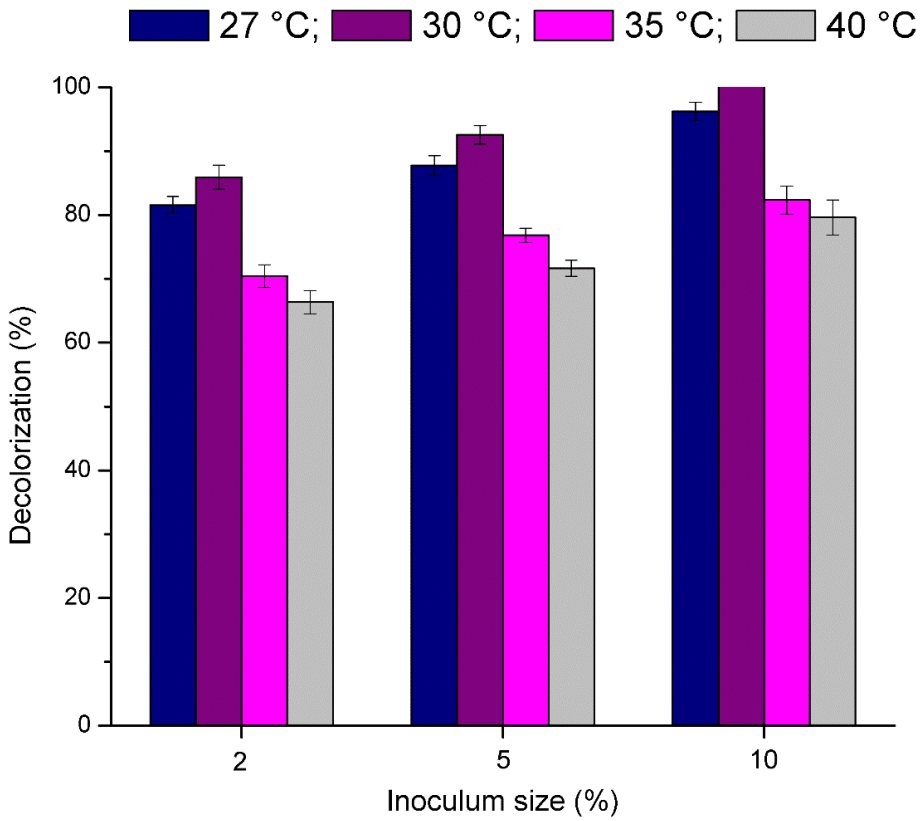

Figure 2. Effect of inoculum size and temperature on CV biodegradation by CKS7.

Regarding to the temperature influence, the 100 % success of decolorization was obtained at 30 °C. Additionally, all results at 27 °C were satisfactory as well. They ranged between 80-96 % during 24 h of the biodegradation, while the percentage of decolorization was lower at 35 and 40 °C, respectively. As for the other investigations of biodegradation of CV dye and other triphenylmethane dyes by different microbial cultures, the most common optimal temperature ranged from 25 to 30 °C (Shedbalkar, 2008, Ayed, 2010). However, in some cases it was 37 °C (An, 2002), 40 °C (Hadibarata, 2012) and 50-60 °C (Yang, 2009) depending on the used strains. From the aspect of application in water treatment, the most acceptable and eco-friendly approach is using lower temperature for dye biodegradation purposes. The achievements of an appropriate and maintaining the constant temperature is one of the most expensive items in the whole process. The optimal temperature of 27-30 °C in our cases, actually presents a benefit. Without cooling, the treated water can be directly discharged in the stream.

Effect of dye concentration and time on the biodegradation

The time course of decolorization and the effect of the dye concentration on the biodegradation were presented on Figure 3 with UV–Vis spectra of CV dye reduction. *Streptomyces fulvissimus* CKS 7 was decolorized completely the initial CV solution of 1 mg/L, 2 mg/L and 3 mg/L during 6 h, 22 h and 36 h, respectively. Therefore, the time course of decolorization has been shown to be apparently concentration dependent. Peaks observed at 584 nm at initial time (0 h) decreased without any shift in λ max up to complete decolorization of the medium. After the specified time, they disappeared completely. In comparison with Ayed (2010) investigation, time of biodegradation of triphenylmethane dyes by *Staphylococcus epidermidi* was reduced by half. However, they were used different microbial culture, but slightly lower the initial concentration from 1 mg/L (Ayed, 2010).

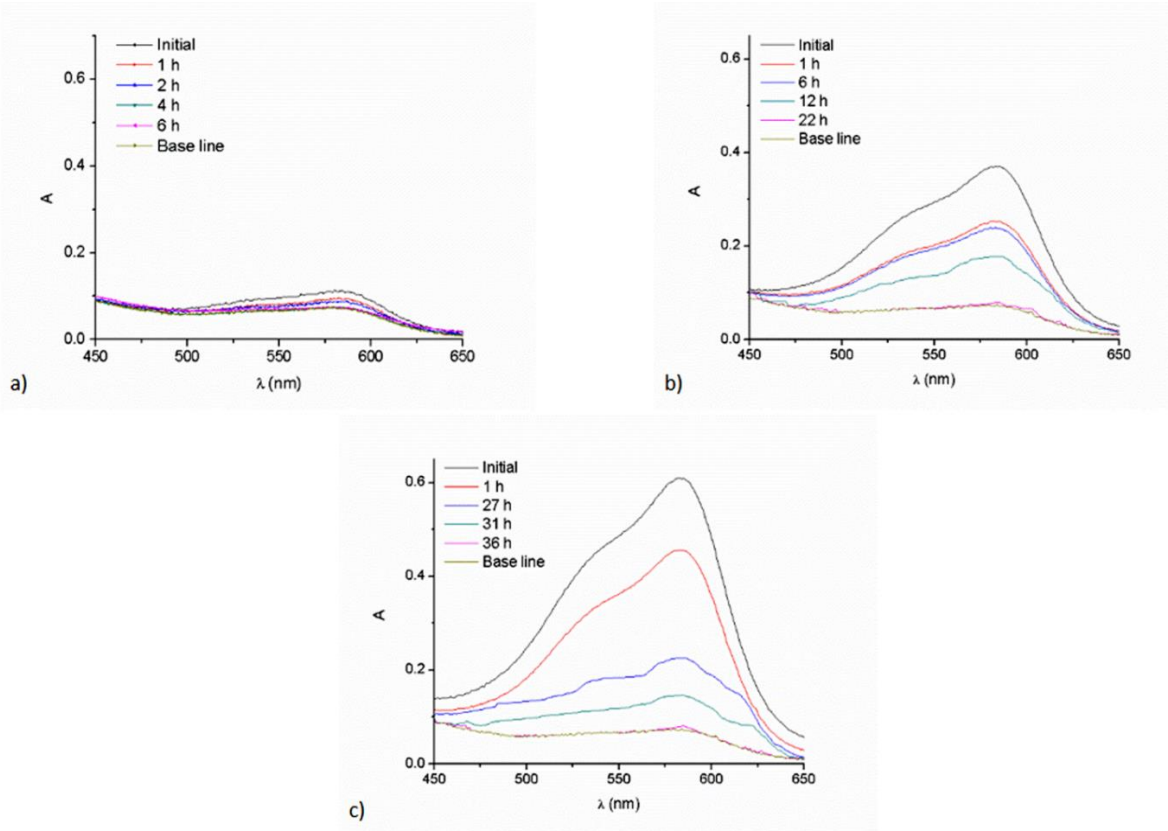

Figure 3. UV–Vis spectrum of CV dye reduction rate, according to dye concentration: a) 1 mg/L, b) 3 mg/L, c) 5 mg/L

Microbial decolorization mechanism

Either adsorption on the microbial biomass or biodegradation of the dyes by the cells are two possible ways of decolorization of the dye solution. In our case, it was observed that at the beginning (in the first 1 hour) of the decolorization process of CV dye solution, the CKS7 cells were intensely colored, while the residual solution was almost decolorized. During the process, in the next 4 to 5 hours, the intensity of the coloration cells was greatly decreased, wherein at the end of the process both microbial cells and working solution were remained colorless. Therefore, a possible mechanism for removing of the dye from working medium was accomplished probably in two steps: binding (adsorption) of dye by bacterial cells on their surface in addition to biodegradation of the dye by bacterial intracellular enzymes. An (2002) had similar observations for decolorization of triphenylmethane dyes by *Citrobacter sp*. These relate for concentration of 1 mg/L. With the increase of CV dye in solutions, described cycles are repeated. It should be noted

that during the decolorization process, the number of CKS7 cells was increased, and decolorization has become more faster over time. Also, UV–Vis spectra of CV dye reduction were showed no new peaks formation along the whole spectrum (220-650 nm). It is evidently that CKS7 was completely metabolized the dye by using it for its growth.

The possibility of the microorganism reusing

Given the complete decolorization after one cycle, repeated use of CKS7 was investigated. The adapted culture was replaced in other CV dye solution (1 mg/L) in the ratio 1:10 (v:v). After 6 hours of incubation time (the time required in the first cycle) the success in second cycle was the 95 % of decolorization. Thus, the acclimated culture needed more time for complete decolorization of the CV dye solution.

Phytotoxicity study

Untreated dyeing effluents from the non-ferrous industries may cause the serious environmental and health hazards after disposal in water bodies. Also, this contaminated water can reach the agricultural land and has a direct impact on fertility of soil. So in this regards, the assessment of the dye and its extracted metabolites phytotoxicity after degradation becomes the mandatory. The relative sensitivities towards the CV dye and its degradation products in relation to *Triticum aestivum* seeds are presented in Table 2.

Phytotoxicity study showed a good germination rate (100 % of the RSGI) as well as significant growth in the shoot and root length in all the produced metabolite samples after biodegradation, as compared to dye samples. All results in Table 2 indicate that no phytotoxic compounds were present in decolorized medium.

Table 2. Phytotoxicity comparison of CV dye and its produced metabolites.

Parameters studied	*Triticum aestivum* Dye / Produced metabolites			
	Control agar medium	1 mg/L	3 mg/L	5 mg/L
RSGI (%)	100	90 / 100	85 / 100	75 / 95
Shoot (cm)	3.17±0.18	2.23±0.54 / 3.38±0.28	1.73±0.61 / 3.11±0.13	1.22±0.43 / 3.22±0.37
Root (cm)	1.18±0.33	0.89±0.43 / 1.22±0.36	0.69±0.45 / 1.25±0.62	0.51±0.71 / 1.3±0.42

CONCLUSION

This study has investigated the potential of *Streptomyces fulvissimus* CKS 7 growing cells to degrade crystal violet dye from water solutions. The results have shown that a single microorganism can tolerate and break down the complex aromatic molecular structure of the dye into non-toxic compounds. The optimal process conditions were found to be mild, under the ambient temperature (27-30 °C), 10 % of the inoculum size and agitation rate of 120 rpm, whereas the time course of decolorization has been revealed to be concentration dependent.

Considering all achieved, the use of the biodegradative potential of CKS 7 bacteria seems to be promising, viable purpose for contaminated water treatment.

ACKNOWLEDGEMENT

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