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Full Length Research Paper

Potential use of cyanobacterial species in bioremediation of industrial effluents

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This study investigated the potential degradation of industrial effluents by environmental species of cyanobacteria. Cyanobacterial species isolated from the Pharmaceutical and Textile industries Mandideep, Bhopal were exposed. Isolation and utilization of the locally generated cyanobacterial biomass for remediation of private industrial activities will generate a source of revenue in Bhopal localities. Biodegradation and biosorption capacity of some potential cyanobacterial species: *Oscillatoria* sp., *Synechococcus* sp., *Nodularia* sp., *Nostoc* sp. and *Cyanothece* sp. dominated the effluents and mixed cultures showed varying sensitivity. Contaminant was removed by all the species, either as individuals or mixtures, at both concentrations. The abundance of cyanobacteria in this effluent was due to favorable contents of organic matter, rich calcium and nutrients such as nitrates and phosphates with less dissolved oxygen. Removal efficiencies of the different contaminants were evaluated and compared. Results confirmed the high efficiencies of the investigated species for the removal of the target contaminants which were species and contaminant-dependent. The contaminants removal efficiency (RE) percentage of cyanobacterial species ranged between 69.5 and 99.6% with a maximum of 97.0 to 99.6% at 5 ppm, 83.9% and 99.7% at 10 ppm and maximum between 95.5 and 99.7%. Mixed culture RE percentages ranged between 91.6 and 100% at 5 ppm with a maximum range of 99.3 to 100%, while at 10 ppm, the RE percentage ranged between 90.4 and 100%, with a maximum range of 96.0 to 100%. Results indicate the potential of natural resources as efficient agents for pollution control.

Key word: Cyanobacteria, industrial effluents, bioremediation.

INTRODUCTION

Blue green algae (cyanobacteria) are considered as the most primitive photosynthetic prokaryotes which are supposed to have appeared on this planet during the Precambrian period (Ash and Jenkins, 2006). Possibly, these are first photosynthetic microorganisms which persisted over a period of 2 to 3 billion years, performing an important role in evolution of higher forms. Cyanobacteria are a unique assemblage of organisms which occupy and predominate a vast array of habitats as a result of several general characteristics; some belonging to bacteria and others unique to higher plants (Wilmotte, 1991, Abd Allah, 2006, Haande et al., 2010). Cyanobacteria are very susceptible to sudden physical and chemical alterations of light, salinity, temperature and nutrient composition (Boomiathan, 2005; Semyalo, 2009). The application of cyanobacteria showed immense potential in waste water and industrial effluent treatment, bioremediation of aquatic and terrestrial habitats, chemical industries, biofertilizers, food, feed, fuel, etc. (Cairns and Dickson, 1971). In addition, pH, carbon dioxide, organic matter, alkalinity, nitrates and phosphates are factors important in determining the distribution of cyanobacteria (Podda et al., 2000). However, the study of physical, chemical and biological characteristics of industrial effluents is so vast that each waste water habitat requires a separate study.

Cyanobacteria have a great deal of potential as a source of fine chemicals, as a bio-fertilizer and as a source of renewable fuel (Lem and Glck, 1985). Recently, there has been increasing awareness about using cyanobacteria as bioremediation and pollution control agents, either as wild-type, mutant or genetically engineered forms. In addition, their viability and metabolic activity are not affected by the decrease in the levels of the biode-

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gradable pollutants that they may break down. Blue greens have been shown to be highly effective as accumulators and degraders of different kinds of environmental pollutants, including pesticides (Megharaj et al., 1994), crude oil (Sokhoh et al., 1992; Al-Hasan et al., 1998, 2001), naphthalene (Cerniglia et al., 1980a, b), phenanthrene (Narro et al., 1992), phenol and catechol (Ellis, 1977; Shashirekha et al., 1997) and xenobiotics (Megharaj et al., 1987). Worldwide, cyanobacteria have been used efficiently as a low-cost method for remediating dairy wastewater by converting the dissolved nutrients into biomass (Lincoln et al., 1996) and for biotreatment (removal) of dissolved inorganic nutrients from fish farms (Duma et al., 1998), to allow them to be used as economic and low-maintenance remediation technology for contaminated systems.

However, the beneficial application of cyanobacteria in remediation of contaminated waters, either natural aquatic environments or industrial effluents, is still not optimally manipulated. For example, cyanobacterial species such as *Oscillatoria salina, Plectonema terebrans, Aphanocapsa* sp. and *Synechococcus* sp., developed as mats in aquatic environments, have been successfully used in bioremediation of oil spills in different parts of the world (Raghukumar et al., 2001; Radwan and Al-Hasan, 2001; Cohen, 2002). Bioremediation is considered as an efficient and environmentally safe technology for inexpensive decontamination of polluted systems (Yoo et al., 1995). The main objectives of this study were: (1) to isolate naturally occurring cyanobacterial species from the industrial effluents and (2) to isolate naturally occurring cyanobacterial species with high biodegradation and/or removal capabilities for pollutants.

MATERIALS AND METHODS

Microorganisms

Cyanobacterial species were isolated from the pharmaceutical industries and Textile industries effluents, Mandideep, Bhopal, Madhya Pradesh, India. Effluent discharge in Bhopal River had improper treatment as a result of the severe pollution in the river and field. Micro flora dominates the Bhopal River and small lakes. All species involved in the study were isolated and identified by the laboratory of Phycology, Dr. H. S. Gour University, Sagar. These species included *Synechococcus* sp., *Oscillatoria* sp., *Nostoc* sp. (SH), and *Nodularia* sp. (Desikachary, (1959). They were previously isolated and identified at IGSR (Mansy and El-Bestawy, 2002), Alexandria University.

Description of isolation sites

The geographical location of the Bhopal City lies within North Latitude 23°16' and East Longitude 77°36'. The location of Bhopal falls in the northwestern portion of Madhya Pradesh, India. In the Map of India, Bhopal occupies the central most region of the country. Old Bhopal is situated at the northern part of the city, while the southern part is called the New Bhopal. Textile and pharmaceutical industries are situated in Mandideep which lies in the northern part of the city, 21 km from new Bhopal. The Bhopal city receives heavy industrial untreated wastewater, domestic primary treated waste water, and agricultural wastewater, and suffers much at present, from the intense pollution.

Sampling of industrial effluent

Samples were collected from the Pharmaceutical industries and Textile industries effluents, Mandideep, Bhopal, and Madhya Pradesh, India. Thus, it was expected that their final effluents will contain industrial pollutants such as heavy metals which are not likely to be removed by that kind of treatment. Grab samples representing all wastes entering the plant during 24 h were collected from both industries to avoid the fluctuation in the flow and the strength of the influent.

Media and culture conditions

BG-11 modified medium (SP) was used. This consisted of solutions A and B, containing (in grams): A; $NaHCO_3$ 11.61 g, Na_2CO_3 3.53 g, K_2HPO_4 0.5 g dissolved in 500 ml distilled water and B; $NaNO_3$ 5 g, K_2SO_4 1 g, NaCl 1 g, $MgSO_4.7H_2O$ 0.2 g, $CaCl_2$ 0.04 g, and 1 ml EDTA (0.5 M). Micronutrient solution (CHU no.10) consisted of the following trace metals (in milligrams) dissolved in one liter distilled water: Na_2-EDTA 50 g, H_3BO_3 618 g, $CuSO_4.5H_2O$ 19.6 g, $ZnSO_4.7H_2O$ 44 g, $CaCl_2.6H_2O$ 20 g, $MnCl_2$ 12 g and $Na_2MoO_4.2H2O$ 12.6 g.

Solutions A and B were sterilized by autoclaving separately at 121°C for 20 min. Micronutrient solution was sterilized by filtration through 0.22-mm polycarbonate membrane to avoid interaction and precipitation of heavy metals. After sterilization, solutions A and B were combined and 1 ml of the micronutrient solution and 1 ml of vitamin B12 (stock solution 15.0×10^{-6} g) were added. Two modifications of BG11 were developed in order to define the optimal conditions required for the enrichment and propagation of cyanobacterial isolates and to enhance the natural biodegradation activity of the purified isolates. The first modification was doubling vitamin B_{12} concentration, which enhanced growth. The second was increasing nitrogen content, based on the fact that the enzyme responsible for dechlorination activities (nitrate reductase) is induced at high nitrogen levels (Kuritz et al., 1997). Optimization of cyanobacterial growth included adjusting the light/dark cycle, with 16/8 h of white light 8 ft 40 W with light intensity (3200 lux), temperature at 25 to 30°C,and shaking (120 rpm) of the cultures, all of which led to enhancement of mass production. Prior to biodegradation bioassays, all cultures were tested for the presence of heterotrophic bacteria microscopically and by plating on bacterial nutrient medium (nutrient agar, Difco, UK) and incubating at 30°C for 1 week. Only axenic cultures, either uni- or multi-algal species, were used in the assays.

Characterization of industrial effluent

Industrial effluent quality parameters included biochemical oxygen demand (BOD), chemical oxygen demand (COD) and inorganic and organic phosphates, (DO). Ammonia, nitrate and magnesium were characterized before and after treatment to determine the effectiveness of the remediation process. All the investigated parameters were determined using the standard techniques described by Clesceri et al. (1999) in the standard methods for the examination of water and industrial effluent.

Remediation bioassay

The selected species were inoculated individually or as mixtures

Table 1. Physio-chemical analysis of industrial effluent.

S/N	Parameter	Industrial effluent	
		Textile	Pharmaceutical
1	Colour	Pale brown	Colourless
2	Temperature (°C)	29	28
3	pH	6.9	7.0
4	BOD	245	282
5	COD	702	669
6	DO	2.2	2.1
7	Ammonia	236	55
8	Nitrite	69	69
9	Nitrate	170	154
8	Inorganic phosphate	23	22
11	Organic phosphate	26	23
12	Calcium	57	77
13	Chloride	1581	1589
14	Magnesium	40	68

Except for pH and temperature, all values are expressed in mg^{-1}.

into 50-ml culture medium and incubated for 1 week until heavy growth was obtained. For each species, as well as for each mixed culture, nine flasks (100 ml of sterilized modified cyanobacterial medium in 250-ml conical flasks) were prepared and sterilized. Each of the nine flasks was inoculated with 5 ml of the 1-week dense individual cyanobacterial suspensions, or with multiple species in the case of mixed cultures, incubated under the previously mentioned conditions and left to reach mid-late log phase of growth (E10 days).

Growth monitoring

Growth was monitored to determine the stimulatory or inhibitory effect of pollutants on the tested cyanobacteria (their resistance or sensitivity) in order to define the most resistant and promising bioremediation species. At each exposure time, triplicates of 5 ml from the three replicates of each sample were aseptically drawn from the control culture (pollutants free) and the six cultures were supplemented with pollutants (5 and 10 ppm). The samples were centrifuged at 6000 rpm for 10 min to harvest cyanobacterial cells. In this case, supernatants were discarded and the chlorophyll a content in the pellets was extracted using the standard acetone extraction method. After extraction, chlorophyll a was determined spectrophotometrically at 750 and 665 nm before and after acidification (0.1N HCl) using a UV spectrophotometer.

Statistical analysis

Values were expressed as mean ± S.D. The statistical analysis was performed using ANOVA followed by Dennett's multiple comparison tests in order to compare more than two groups. All the data were processed with instate version 2.1 software.

RESULTS AND DISCUSSION

Physio-chemical analysis of industrial effluent

The observations of Munawar (1970) and Kannan (2006) suggest that cyanophyceae grow luxuriantly with great variety and abundance in ponds rich in calcium. The present data of the two effluents also showed that calcium is possibly one of the factors (Table 1). Whether it plays its role individually or in combination with other factor, complexes can only be understood by culture studies. Besides calcium, high amounts of oxidizable organic matter, traces of dissolved oxygen, considerable amounts of nitrate and phosphates in all the effluents investigated were probably the factors favoring the growth of cyanobacteria as suggested by Rai and Kumar (1977), Nazneen (1980), Venu et al. (1984), Burch et al., (2001), Murugesan and Sivasubramanian, (2005), Singh and Saxena, (1969). Venkateswarlu (1976) reported that high values of BOD, COD, phosphates and nitrates with very low DO favored the growth of cyanobacteria than any other algae. Their findings were supported by the observations of Jeganathan (2006) and Haande, (2008), in different industry effluents, respectively. In this study also, all the effluents showed a considerable amount of nitrates and phosphates, with increased level of BOD and COD along with very low DO level by Ebtesam (2008) and Larsson et al., (2009).

Biodegradation of industrial effluent

The indigenous cyanobacteria of both industrial effluents were found to be superior degraders or removers of pollutants. They all exhibited highly efficient degradation or removal ability at the two investigated concentrations. Growth and biodegradation capabilities were affected by microbial species, their native environment, pollutants concentration, exposure (contact) time and application as individual or mixed cultures. Although it was thought that

Table 2. Biodegradation of industrial effluents by the selected cyanobacterial species isolated from industrial effluents.

Algal species	Exposure time (days)	5 ppm		10 ppm	
		Residual concentration [b]	Removal efficiency (%)	Residual concentration	Removal efficiency (%)
Synechococcus	2	0.27516±0.009	94.5	0.10453±0.001	98.9
	4	0.07285±0.010	98.5	0.16576±0.030	98.3
	7	0.10452±0.000	97.9	1.04531±0.000	89.5
Oscillatoria	2	0.10127±0.037	97.0	0.45964±0.060	95.4
	4	0.17351±0.060	96.5	0.18448±0.020	98.2
	7	1.02337±0.012	79.5	0.17631±0.032	98.2
Nostoc	2	0.63587±0.248	87.3	0.40527±0.070	95.9
	4	0.09893±0.024	97.0	0.10920±0.009	98.9
	7	0.25431±0.010	94.9	0.28913±0.030	97.1
Nodularia	2	0.25006±0.01	97.3	0.71390±0.076	92.9
	4	0.06424±0.006	97.4	0.44455±0.020	95.6
	7	2.88182±0.000	76.3	1.60840±0.000	83.9
Cyanothece	2	0.43619±0.000	91.3	0.03402±0.000	99.7
	4	0.01993±0.000	99.6	0.02618±0.000	99.7
	7	1.52105±0.000	69.5	0.11879±0.001	98.8

Recovery, 90%. [b] Mean standard error.

A

Figure A. *Oscillatoria* sp.

increasing pollutants concentration up to 10 ppm would suppress the metabolic activities of all species, they exhibited excellent removal capabilities even at this high level. 10 ppm pollutants stimulated the capabilities of these species more efficiently than 5 ppm, leading to a much higher RE percentage at 10 ppm in almost all cases. Removal was attributed to either biodegradation, or bioaccumulation, or both, with different ratios. Unlike the effect of pollutant on cyanobacterial growth, which was positively correlated with the contact time, RE percentage by almost all the investigated species achieved high levels within the first 2 days. Removal efficiency (RE) percentages were increased to reach their maximum in most cases on the 4th day, after which they declined slightly up to the 7th day (Table 2, Figures 1 and 2). This could be attributed to acclimatization of the wild species and appearance of mutants, which reach maximum RE percentage up to the 4th day. Mixed culture (RE) percentages ranged between 91.6 and 100% at 5 ppm with a maximum of 99.3 to 100%, while at 10 ppm, the (RE) percentage ranged between 90.4 and 100% with a maximum of 96.0 and 100% (Table 3).

There was difference in the rate of pollutants removal from the medium. Culture 3 removed 99.8 and 99.3% of the applied 5 and 10 ppm pollutants (Figures 3 and 4), respectively, in only 2 days, while the other two cultures (1 and 2) removed between 96 and 99.5% of both concentrations in the same time, which is considered a very important advantage in that it shortened the remediation time. This was confirmed by culture 1, which showed the highest sensitivity towards pollutants, with complete arrest of growth, while achieving the highest biodegradation and removal (100%) after only 4 days at both concentrations.

Conclusion

The slow degradation of pollutants, either chemically or

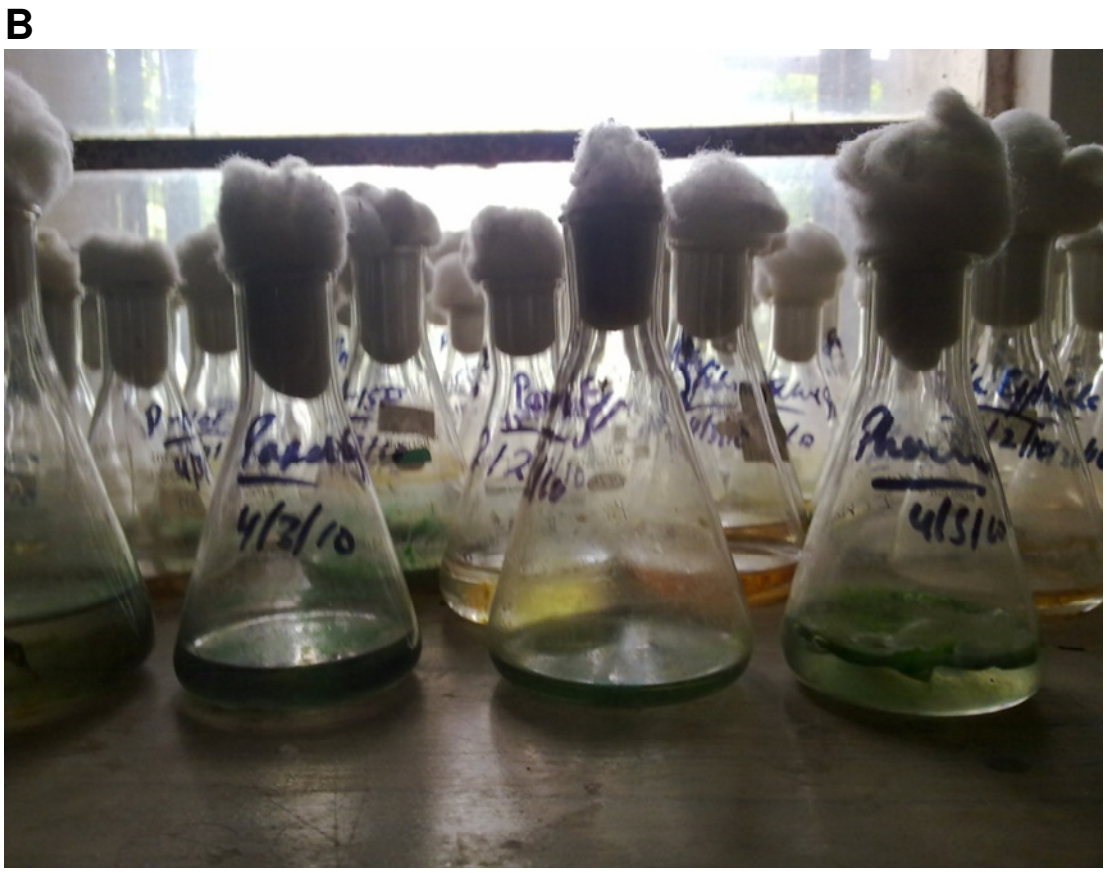

Figure B. *Synechococcus* sp., *Nostoc* sp., *Nodularia* sp. and *Cyanothece* sp. Selected Cyanobacterial species biodegrade the industrial effluent.

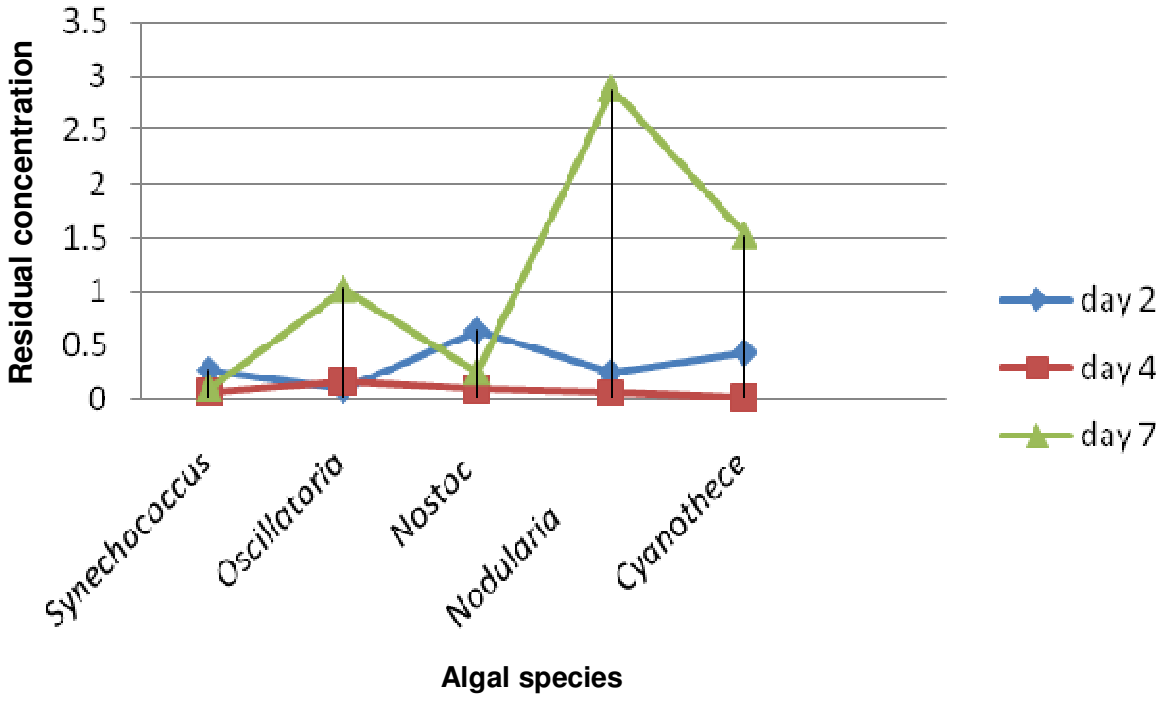

Figure 1. Algal species and their residual concentration in 5 ppm.

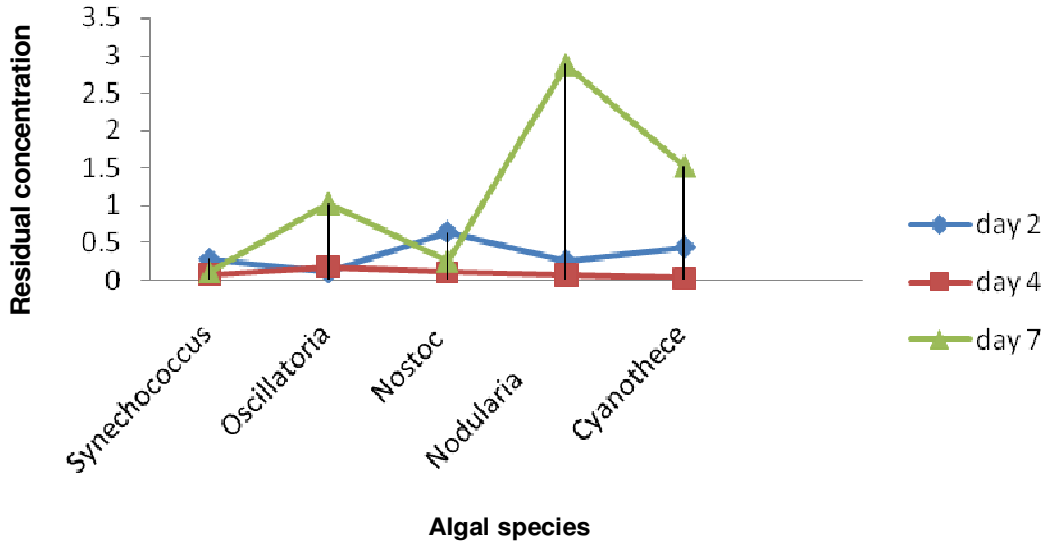

Figure 2. Algal species and their residual concentration in 10 ppm.

Table 3. Biodegradation of industrial effluents by mixed cultures of the selected cyanobacterial species.

Algal species	Exposure time (days)	5 ppm		10ppm	
		Residual concentration $_b$	Removal efficiency (%)$_a$	Residual concentration$_b$	Removal efficiency (%)
Culture 1	2	0.02401±0.014	99.5	0.39910±0.001	95.9
	4	0.00±0.000	100.0	0.01429±0.000	99.9
	7	0.00±0.000	100.0	0.27041±0.001	97.2
Culture 2	2	0.15492±0.002	97.1	0.39469±0.012	96.0
	4	0.02277±0.000	99.3	0.42359±0.003	95.9
	7	0.31783±0.001	91.6	0.85918±0.001	90.4
Culture 3	2	0.00803±0.001	99.8	0.06542±0.001	99.3
	4	0.00313±0.000	99.9	0.00±0.000	100.0
	7	0.04767±0.003	99.0	0.83123±0.004	91.7

$_a$Recovery 90%. $_b$Mean standard error.

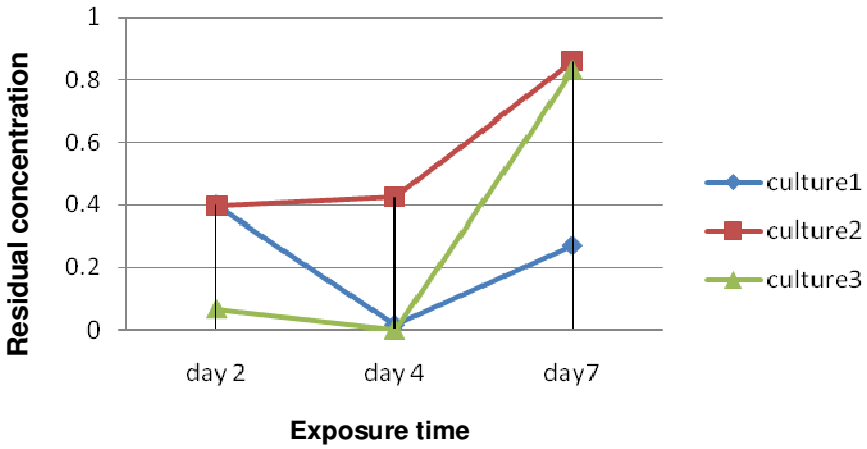

Figure 3. Mixed cultures of the selected cyanobacterial species and there residual concentration in 5ppm.

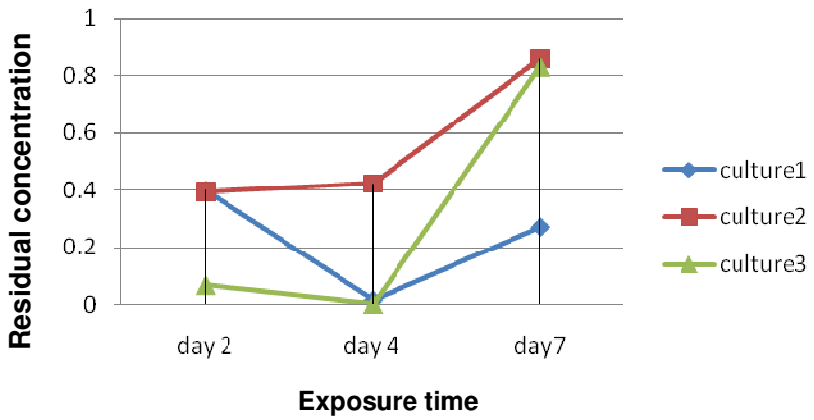

Figure 4. Mixed cultures of the selected cyanobacterial species and their residual concentration in 10 ppm.

biologically, of all the tested species proved to be efficient in degrading the pollutant at a very fast rate, as well as showing high resistance against its toxicity. These findings are important regarding the practical use of such species in large-scale bio treatment of contaminated effluents, where RE, coupled with the fast flow rate, are considered as the main parameters from the feasibility and economic point of view. Therefore, the cyanobacteria species investigated in this study are highly recommended for beneficial bioremediation applications for *in-situ* and off-site removal of pollutants. The most promising species should help in the optimization of the self-purification and remediation of polluted and contaminated effluents before discharging into surface aquatic systems, providing a low-cost and naturally renewable technology

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