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Studies on estrone biodegradation potential of cyanobacterial species

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ABSTRACT

The estrone (E1) excreted through faeces and urine by livestock and humans are present in the water bodies. Even at very low concentrations (0.1–1 ng/l) it poses threat to living organisms as it can bioaccumulate through the food chain. It binds to and activate the estrogen receptors and mimics a normal endocrine response which changes the homeostasis of animals and humans and potentially impairs the reproductive ability. It is a major pollutant and is also designated as an endocrine disrupting compound by the World Health Organization. Hence, there is an urgent need to find out ways for their bioremediation. In the present study, 16 different aquatic cyanobacteria species were screened for estrone bioremediation and the experiments were done in three phases. The first two phases included determination of estrone toxicity and its bioremediation in/by cyanobacteria and the third phase included the determination of degradative role of laccase enzyme. Concentration dependent toxic effect of estrone was observed at 50, 100, and 200 ppm estrone but at 20 ppm estrone slight growth promotion was observed. The degradation efficiency ranged between 53.7% and 94.5%. It was highest at 20 ppm estrone in *Spirulina* CPCC-695. In order to find out the role of oxidoreductases in estrone (phenolics) degradation, laccase activity was monitored. Maximum laccase activity (34.22 UL^{-1}) was found in *Spirulina* CPCC-695. Therefore, use of cyanobacteria especially *Spirulina* CPCC-695 could be recommended for degradation of estrone in aquatic bodies.

1. Introduction

Wide range of chemicals that interfere with the normal functioning of the endocrine system of living organisms have been reported from the aquatic environment. These chemicals are classified as endocrine-disrupting chemicals (EDCs) that disrupts the synthesis of specific hormone receptors, antagonizes or mimics the effect of endogenous hormones and obstructs their synthesis and metabolism (Caliman and Gavrilescu, 2009; Mendes, 2002; Roy et al., 2009). EDCs include broad array of chemicals, among which steroid hormones are a class of biologically active compounds that have been classified as Group 1 carcinogens by the World Health Organization (Caserta et al., 2008). One of the steroid hormones, estrogens have been divided into two main groups: natural estrogens (estrone, 17β-estradiol, and estriol) and synthetic estrogens (ethinyloestradiol and diethylstilbestrol) which eventually gets excreted in the urine and faeces of cattles and humans (Combalbert and Hernandez-Raquet, 2010). It has been reported that among all the estrogens, estrone is excreted substantially by females during late pregnancy either in free form or as conjugates of glucuronides or sulfates (Andreolini et al., 1987; Belfroid et al., 1999). Furthermore, it has been also observed that the estrone concentrations in surface water is generally higher than other natural estrogens as these along with the conjugated form of excreted estrone gets converted into the free form by the action of bacteria (Ying et al., 2002; Johnson et al., 2015).

During the wastewater treatment processes, estrone is removed only partially and thus acts as its main entry point in case of aquatic ecosystems (Koh et al., 2007; Manickum and John, 2014). Estrone present in aquatic bodies shows bioaccumulation and bio-magnification through the food chain that interfere with the normal functioning of their endocrine system (Conroy et al., 2007; Jurado et al., 2012; Racz and Goel, 2010). Long-term exposure to estrone-contaminated water has been known to disrupt the sexual development in animals even at very low concentrations (0.1–1 ng/l). Eventually, its increased amount in surface water coincides with the increased incidence of breast cancer and heart disease in women over 50 years (Cui et al., 2006).

To avoid potential menaces posed by estrone in aquatic environments, their removal is significantly important. Research on the different ways for its removal from water has been carried out from all around the globe during the last decades (Bolong et al., 2009; Silva et al., 2012). In recent years, there has been considerable interest in using biological methods for removing this compound from the environment as other methods of degradation including physical and chemical are quite expensive and produces toxic end products (Khanal

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et al., 2006; Liu et al., 2009). Biodegrading microorganisms use estrone as a source of carbon, energy, and nutrients or simply detoxify them through catabolic pathways (Husain and Qayyum, 2013; Kolvenbach et al., 2014; Li et al., 2018; Yu et al., 2013).

Extracellular phenolic oxidases of cyanobacteria viz., laccase have been known to play an important role in phenolic dyes degradation (Afreen et al., 2017; Ansari et al., 2016; Legerská et al., 2016; Murugesan et al., 2007). Thus, in present investigation an attempt has been made to study role of cyanobacteria in degrading another phenolic substance i.e. estrone along with identification of degradative role of laccase (phenol oxidase) enzyme.

2. Materials and methods

2.1. Chemicals

All the chemicals used in this study were of analytical `grade purchased from Sigma (St. Louis, USA). All the buffers and reagents were prepared in Milli.Q.

2.2. Culture maintenance and experimental design

All the 16 cyanobacterial species were procured from different sources including Indian Agricultural Research Institute (IARI) New Delhi, University of Madras, University of Allahabad and Central Food Technological Research Institute (CFTRI), Mysore. All the species were grown in sterilized Zarrouk's medium except *Spirulina* CPCC-695 that was grown in BG +ve medium in 500 ml erlenmeyer flasks at the temperature of 27 ± 2 °C under a 12:12 light:dark photoperiod supplied by cool white fluorescent tubes at 25 µmol photons min^{-1} light intensity. The cells were maintained in the exponential phase by repeated inoculation into the fresh medium before being used in the experiments (De Oliveira et al., 1999; Madkour et al., 2012; Rajasekaran et al., 2015; Zarrouk, 1966).

2.3. Effect of estrone on the growth of species

An estrone stock solution of 1000 ppm was prepared in acetone which was further diluted with Zarrouk's medium and BG +ve medium in case of *Spirulina* CPCC-695 to achieve 20, 50, 100, 200 and 500 ppm estrone concentration. Growth was evaluated after every 24 h for seven days, based on the absorbance at 750 nm by using a UV–Vis spectrophotometer Labomed UVS-2700 (Labomed, INC.) (Zhang et al., 2016).

Specific growth rate (µ) can be calculated from the relationship given below which is the slope of the line between natural log of *X* and time (De Oliveira et al., 1999).

$$\ln X = \mu t + lnXo$$

$$\mu = \frac{lnX - lnXo}{t}$$

Where, Xo is the initial absorbance, X is the absorbance at time t, and µ is the specific growth rate.

2.4. Determination of residual estrone in the medium

The estrone degradation potential of cyanobacterial species were measured by quantifying the estrone content in the culture medium after every 24 h. The cultures were centrifuged at 8000 rpm for 20 min and the supernatant was collected and filtered with 0.22 µm pore-size filter. The decrease in estrone concentration was interpreted spectrophotometrically at λ_{335} after every 24 h for seven days. The absorbance recorded was further used to calculate the degradation efficiency of estrone (Zhang et al., 2016).

$$Degradation\ efficiency\,(in\ \%) = \frac{[(Ac - At)]}{Ac} * 100$$

where, A_c is the initial concentration of estrone and A_t is the concentration of estrone at time t.

2.5. Determination of laccase activity

Laccase (EC 1.10.3.2) activity (if any) was determined spectrophotometrically as described earlier with some modifications in all the cyanobacterial strains under study in absence of estrone. The assay was done at pH 4.0 (the optimum pH for laccase of cyanobacteria) with 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) as the substrate. Laccase activity was assessed throughout the growth phase of cyanobacteria i.e. for 20 days. To increase its production, cultures were induced with 100 µM guaiacol. Guaiacol was dissolved in 50% ethanol and filtered with 0.22 µm pore size filter prior to use.

In order to assess the role of laccase in estrone degradation, the cyanobacterial cell (in exponential phase) were exposed to 20 ppm estrone (that showed highest degradation) for seven days. The culture without estrone was considered as control.

$$Laccase\ activity\,(U/L) = \frac{Absorbance\ X\ Total\ volume\ X\ Incubation\,\text{time}}{Sample\,\text{volume}\,X\ Extinction\ coefficient\ of\,\text{ABTS}}$$

The cell cultures were centrifuged at 8000 rpm for 20 min at 4 °C after every 24 h. The supernatant was collected and laccase activity of the cyanobacterial species was determined by performing the enzyme assay. The reaction mixture (in a total volume of 1 ml) contained 100 mM citrate buffer 2 mM ABTS and extracellular culture fluid (Conroy et al.). The reaction was monitored by measuring the absorbance at 420 nm using Labomed UV–VIS spectrophotometer (UVS-2700) after 10 min. The laccase activity was expressed in international units per litre (UL^{-1}), defined as the amount of enzyme needed to produce 1 µmol product min^{-1} at 30 °C. The extinction coefficient (ε) of ABTS was used as 36,000 M^{-1} cm^{-1}. The green colour that develops due to oxidation of ABTS confirmed the presence of laccase (Afreen et al., 2016).

3. Results and discussion

3.1. Relationship between cyanobacterial growth and estrone

Till date, no studies have been done that emphasizes on estrone toxicity in cyanobacteria but in case of dimethyl phthalate (DMP) it has been found that its high concentration (≥ 50 ppm) is detrimental to cyanobacterial species like *Cyanothece* sp. PCC7822, *Synechocystis* sp. PCC6803 and *Synechococcus* sp. PCC7942 (Zhang et al., 2016). For analyzing the toxic effect, cyanobacterial culture were exposed to estrone in the growth medium (0, 20, 50, 100, 200 and 500 ppm). Growth behaviour of these strains were studied for seven days. The cyanobacterial species grew at 20, 50, 100 and 200 ppm estrone (Fig. 1). But at 500 ppm estrone (highest concentration) the cyanobacterial cells died immediately. Beyond 20 ppm estrone, concentration dependent toxic effect of estrone was observed. The specific growth rate of studied cyanobacterial species in presence of estrone showed the maximum values at 20 ppm in *Spirulina* CPCC-695 (µ = 59.35 day^{-1}) and minimum in *Arthrospira platensis* (Behrampur) (µ = 39.94 day^{-1}) reflecting their tolerance and sensitivity respectively (Table 1).

Different cyanobacterial species showed different tolerance to estrone; it may be due to the structural difference of their surface, membrane permeability, enzyme machinery and the area exposed to estrone. A positive correlation was observed between estrone and growth rate at 20 ppm in all the 16 species. Estrone higher than 20 ppm viz., 50, 100 and 200 ppm showed concentration dependent toxicity suggesting that beyond optimal concentration and tolerance level physiological machinery of the organism adopted degeneration and it could not degrade estrone.

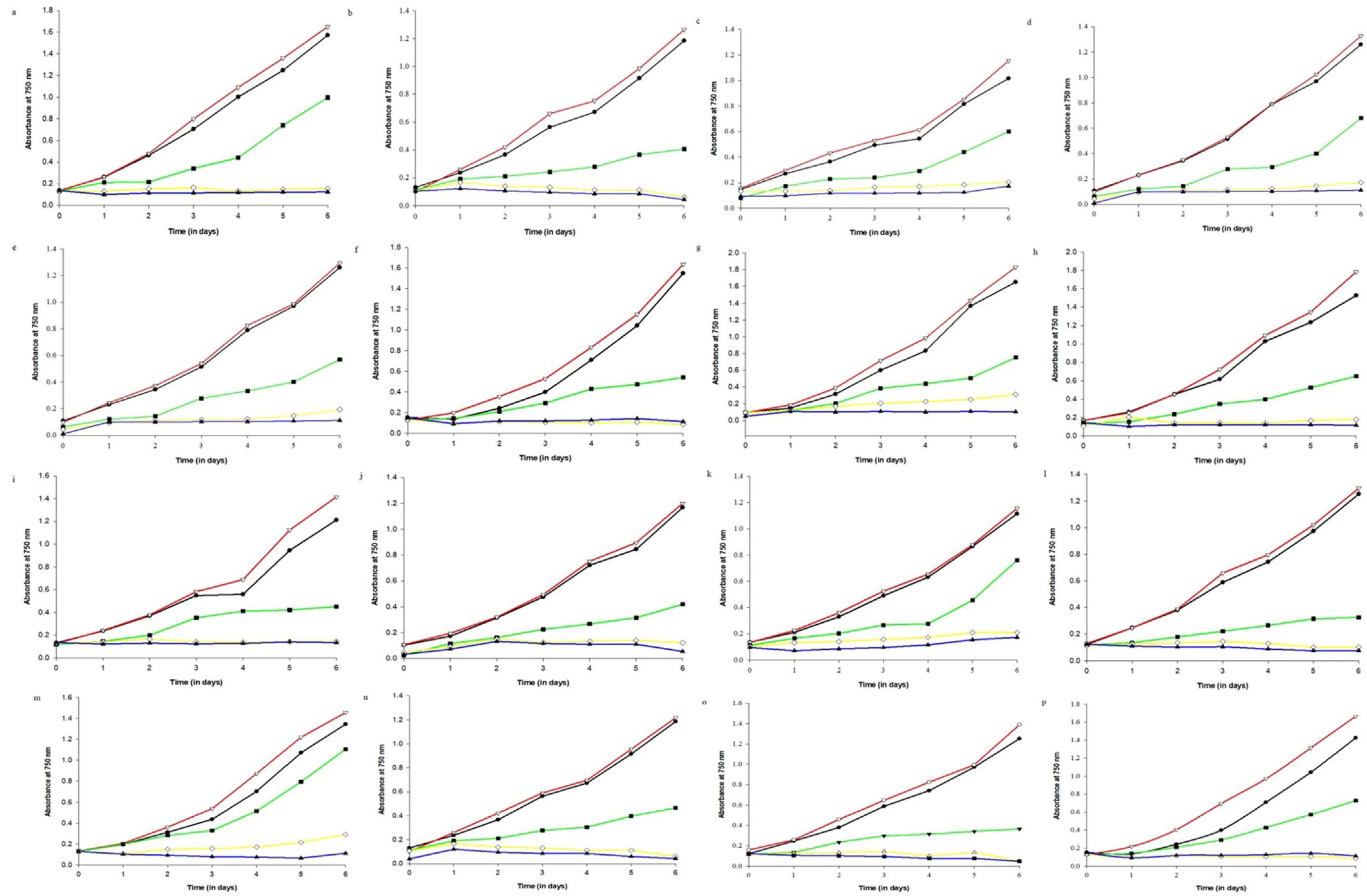

Fig. 1. —●— Control —▽— 20 ppm —■— 50 ppm —◇— 100 ppm —▲— 200 ppm Effect of estrone on the growth of Arthrospira and Spirulina species. (A) Arthrospira platensis (B) Arthrospira platensis (CFTRI) (C) Arthrospira platensis (Behrampur) (D) Arthrospira indica (SOSA-4) (E) Arthrospira maxima (SAE-49–88) (F) Arthrospira indica (Kenya isolated) (G) Arthrospira indica (SAE-84) (H) Arthrospira indica (Lonar-SV) (I) Arthrospira platensis (NEERI) (J) Spirulina platensis (S-5) (K) Spirulina NCCU-477 (L) Spirulina NCCU-479 (M) Spirulina NCCU-483 (N) Spirulina platensis (S-2303) (O) Spirulina platensis (CFTRI, Mysore) (P) Spirulina CPCC-695.

3.2. Estrone biodegradation by cyanobacteria

In this study, the biodegradation propensity of cyanobacteria was studied by exposing cyanobacterial cultures to estrone (20, 50, 100 and 200 ppm). The amount of estrone in the spent medium was measured after every 24 h. The difference in the amount of estrone left indicated the extent of degradation (Fig. 2). Similar estrone degradation trend was observed in all the studied cyanobacterial species. Initially, the degradation was slow but gradually increased with time; and it reached highest on the seventh day of the study. Maximum degradation was observed at 20 ppm in all the species. The degradation of estrone at 20 ppm was highest in *Spirulina* CPCC-695 (94.5%) and least in *Arthrospira platensis* (Behrampur) (53.7%).

Biodegradation has been described as a major removal mechanism

Table 1
Specific growth rate of species under study. All the cells had higher specific growth rate at 20 ppm than control (with no estrone). *Spirulina* CPCC-695 showed fastest growth ($\mu = 59.35\ day^{-1}$) in presence of 20 ppm. Lower concentration of estrone promotes growth whereas higher concentrations of estrone inhibited their growth.

Sr No.	Strains	Control	20 ppm	50 ppm	100 ppm	200 ppm
1	*Arthrospira platensis*	46.43	47.56	42.67	5.96	2.98
2	*Arthrospira platensis (CFTRI)*	46.31	51.14	47.29	8.34	0.53
3	*Arthospira platensis* (Behrampur)	38.66	39.94	28.08	8.45	5.20
4	*Arthospira indica* (SOSA − 4)	46.31	50.82	47.29	8.34	3.53
5	*Arthrospira maxima* (SAE − 49 to 88)	48.95	50.08	42.18	2.65	1.99
6	*Arthrospira indica* (Kenya isolated)	44.53	47.51	40.96	2.52	1.25
7	*Arthrospira indica* (SAE − 84)	44.34	48.53	29.27	1.73	0.15
8	*Arthrospira indica* (Lonar-SV)	48.57	48.51	40.0	9.67	0.29
9	*Arthrospira platensis* (NEERI)	44.05	49.77	33.26	8.42	1.45
10	*Spirulina platensis* (S − 5)	44.05	50.54	33.26	9.42	0.66
11	*Spirulina* NCCU − 477	42.23	43.24	35.30	4.05	1.93
12	*Spirulina* NCCU − 479	49.13	52.33	37.58	9.57	1.89
13	*Spirulina* NCCU − 483	49.13	51.81	47.58	9.57	2.98
14	*Spirulina platensis* (S − 2303)	46.58	46.11	40.43	3.70	1.89
15	*Spirulina platensis* (CFTRI, Mysore)	46.58	43.34	38.43	6.71	0.24
16	*Spirulina* CPCC − 695	58.78	59.35	49.43	9.43	5.27

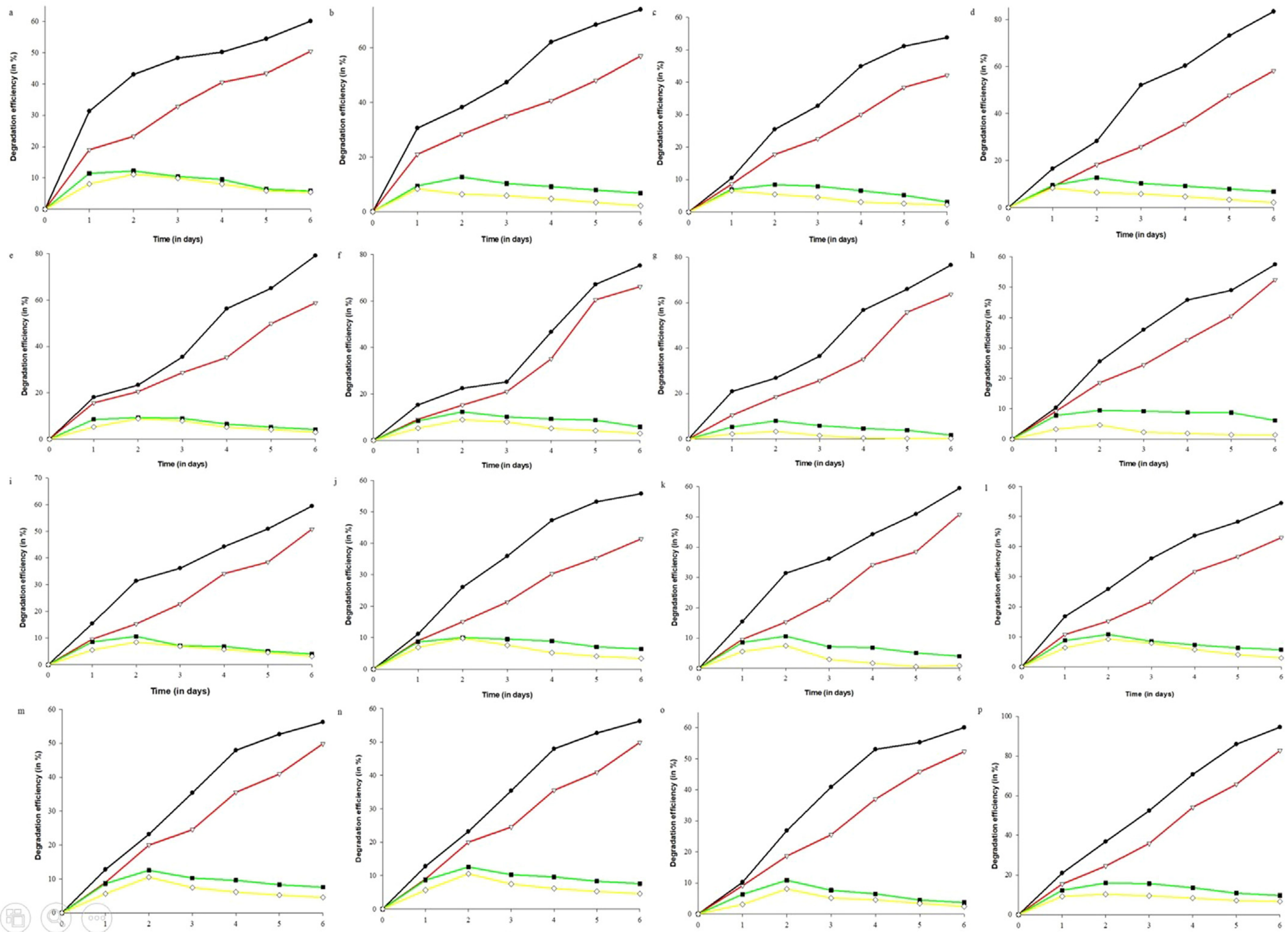

Fig. 2. —●— 20 ppm —▽— 50 ppm —■— 100 ppm —◇— 200 ppm Degradation efficiency (in %) of estrone by Arthrospira and Spirulina species. (A) Arthrospira platensis (B) Arthrospira platensis (CFTRI) (C) Arthrospira platensis (Behrampur) (D) Arthrospira indica (SOSA-4) (E) Arthrospira maxima (SAE-49–88) (F) Arthrospira indica (Kenya isolated) (G) Arthrospira indica (SAE-84) (H) Arthrospira indica (Lonar-SV) (I) Arthrospira platensis (NEERI) (J) Spirulina platensis (S-5) (K) Spirulina NCCU-477 (L) Spirulina NCCU-479 (M) Spirulina NCCU-483 (N) Spirulina platensis (S-2303) (O) Spirulina platensis (CFTRI, Mysore) (P) Spirulina CPCC-695.

Table 2
Estrone degradation efficiency (in %) of cyanobacterial species.

Sr. No	Strains	20 ppm	50 ppm	100 ppm	200 ppm
1	*Arthospira platensis*	60.1	50.4	5.86	5.5
2	*Arthospira platensis (CFTRI)*	74	57	6.5	2.1
3	*Arthospira platensis* (Behrampur)	53.7	42.1	3.1	2.2
4	*Arthospira indica* (SOSA – 4)	83.4	58.1	6.7	2.1
5	*Arthospira maxima* (SAE – 49 to 88)	79.2	58.8	4.2	3.0
6	*Arthospira indica* (Kenya isolated)	75.2	66.1	5.8	3.0
7	*Arthospira indica* (SAE – 84)	76.5	63.7	1.7	0.2
8	*Arthospira indica* (Lonar-SV)	57.4	52.4	6.1	0.4
9	*Arthospira platensis* (NEERI)	59.4	50.8	4.0	3.1
10	*Spirulina platensis* (S – 5)	59.4	50.8	4.1	0.9
11	*Spirulina* NCCU – 477	54.4	42.95	5.6	2.9
12	*Spirulina* NCCU – 479	56.2	49.9	7.6	4.6
13	*Spirulina* NCCU – 483	56.2	49.8	7.5	4.5
14	*Spirulina platensis* (S – 2303)	60.1	52.3	3.7	2.4
15	*Spirulina platensis* (CFTRI, Mysore)	55.7	41.4	6.4	3.4
16	*Spirulina* CPCC – 695	94.5	82.7	9.7	6.6

that affects the fate and transport of estrogenic compounds in natural environments (Yu et al., 2013). Specifically, there is no report on biodegradation of estrone using cyanobacteria but biodegradation of EDCs have been shown by other microorganisms (Blánquez and Guieysse, 2008; Yu et al., 2007; Zhang et al., 2011; Zhao et al., 2018). As suggested by Yu et al. (2007), microorganisms degrade steroidal hormone using two possible degradation mechanisms which are growth-linked and non-growth-linked (cometabolic) (Yu et al., 2007). The degradation results indicated growth linked degradation, suggesting that cyanobacteria utilize estrone as energy and/or carbon source for their growth. *Spirulina*-CPCC-695 showed highest (94.5%) degradation efficiency of estrone (Table 2).

3.3. Identification of degradative enzyme (laccase)

Prior to analyze the degradative role of laccase, extracellular laccase activity (if any) without estrone exposure was determined in both induced and uniduced culture of all the test cyanobacterial species. It was observed that the laccase production enhanced in presence of guaiacol. In case of induced culture laccase activity peaked on the 4th–5th day whereas in case of uninduced ones, the highest production was recorded on 9th–10th day (Fig. 3).

In order to correlate the role of laccase in estrone degradation,

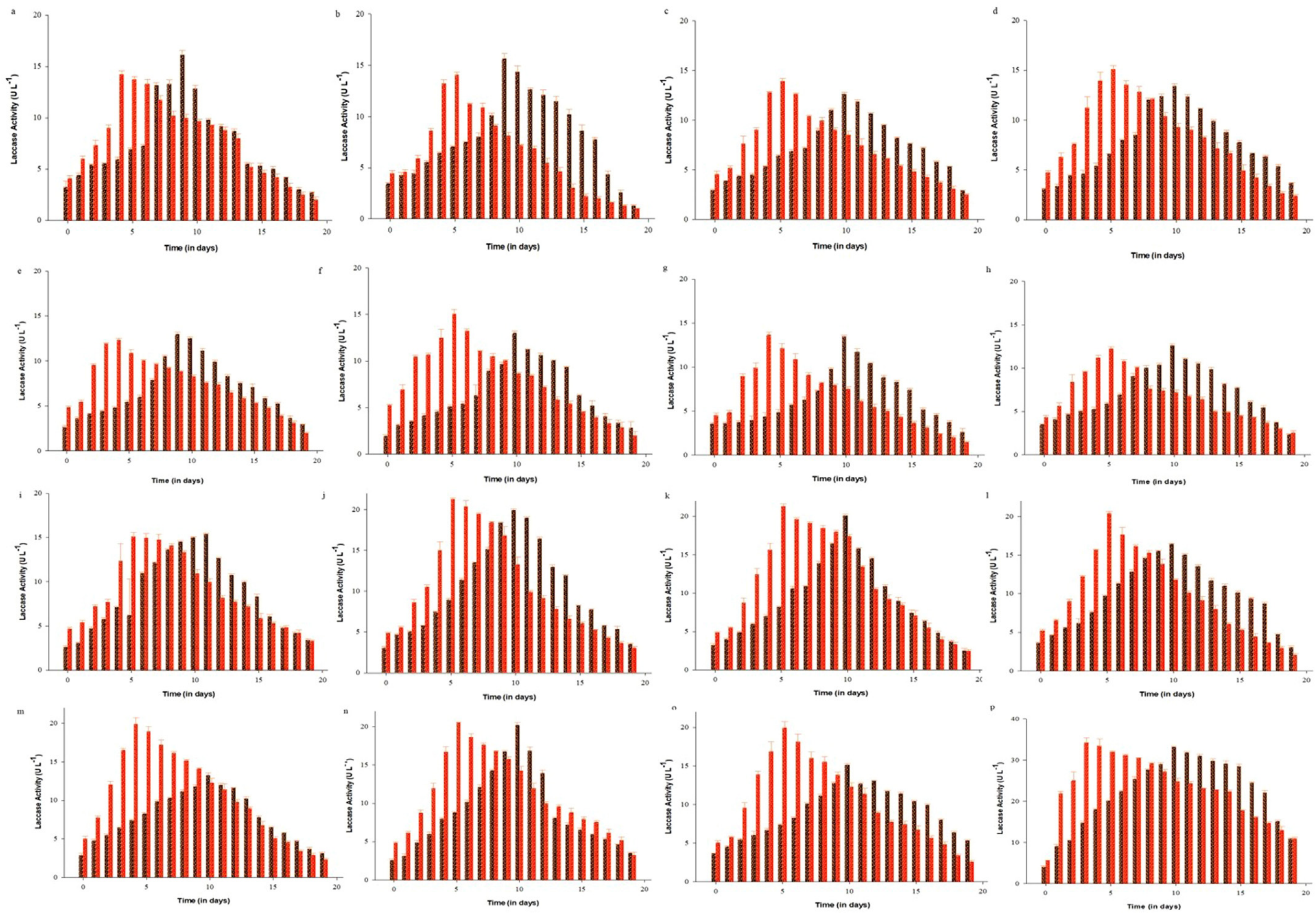

Fig. 3. Control Induced Laccase activity. (A) Arthrospira platensis (B) Arthrospira platensis (CFTRI) (C) Arthrospira platensis (Behrampur) (D) Arthrospira indica (SOSA-4) (E) Arthrospira maxima (SAE-49–88) (F) Arthrospira indica (Kenya isolated) (G) Arthrospira indica (SAE-84) (H) Arthrospira indica (Lonar-SV) (I) Arthrospira platensis (NEERI) (J) Spirulina platensis (S-5) (K) Spirulina NCCU-477 (L) Spirulina NCCU-479 (M) Spirulina NCCU-483 (N) Spirulina platensis (S-2303) (O) Spirulina platensis (CFTRI, Mysore) (P) Spirulina CPCC-695. The laccase activity get enhanced in presence of the guaiacol as compared to control suggesting that it induces production of laccase in these species.

laccase activity was assessed in cyanobacterial culture exposed to estrone (20 ppm) at which the highest degradation was observed. It was found that *Spirulina* CPCC-695 showed the maximum laccase activity (34.22 UL^{-1}) (Fig. 4). This may be due to specific inherent morphological ad physiological characteristics of *Spirulina* CPCC-695 which facilitate efficient estrone degradation. Moreover, cyanobacterial species possess a strong antioxidant defence system that provide them tolerance to phenolic pollutants like estrone (Yasin et al., 2018a, 2018b).

The degradation involves the hydroxylation of phenol ring to form catechol that then undergoes ortho and meta cleavage pathways and the ring structure opens as elucidated in the algae *O.danica*. These cleavage pathways involve mono and dioxygenase enzymes that use oxygen that finally break down phenolic compounds to pyruvate and carbon dioxide via phenol degradation pathway (Semple and Cain, 1995). Further, Lika and Papadakis (2009) has hypothesized that the degradation of phenolic contaminants require oxygen (Lika and Papadakis, 2009). Cyanobacterial laccase (an oxidoreductase) are reported to play an important role in degradation of phenolic dyes and other phenolics (Bollag et al., 1988; Legerská et al., 2016; Zhao et al., 2018). Hence, we proposed that it is also responsible for estrone degradation and eventually it was observed that maximum laccase activity was observed in *Spirulina*-CPCC-695 at 20 ppm estrone.

4. Conclusion

Estrone is one of the female estrogen that enters the environment through excreta of living organisms. Moreover, the spreading of cattle and poultry waste on agricultural land increases the risk of groundwater contamination through estrone (Ying et al., 2002). It also persists in the environment and has been detected at up to 3.8×10^{-3} parts per billion (ppb) in treated wastewater, and up to 1.0×10^{-2} ppb in waters downstream of waste water treatment plants (WWTPs) (Jeannot et al., 2002; Lee et al., 2011). Thus, it has become a concern for aquatic wildlife and humans as well, due to its potential to disrupt the endocrine and reproductive systems (Jobling and Tyler, 2003). Microorganisms have been known to play an important role in the degradation of natural estrogens. Consequently, many estrogen-degrading bacteria have been isolated from waste water, soil or animal wastes (Yu et al., 2007; Zhang et al., 2011). It may be concluded from the present study that aquatic estrone contaminants can be bioremediated through cyanobacteria and it had thus open a new arena in the field of biodegradation of endocrine disrupting compounds.

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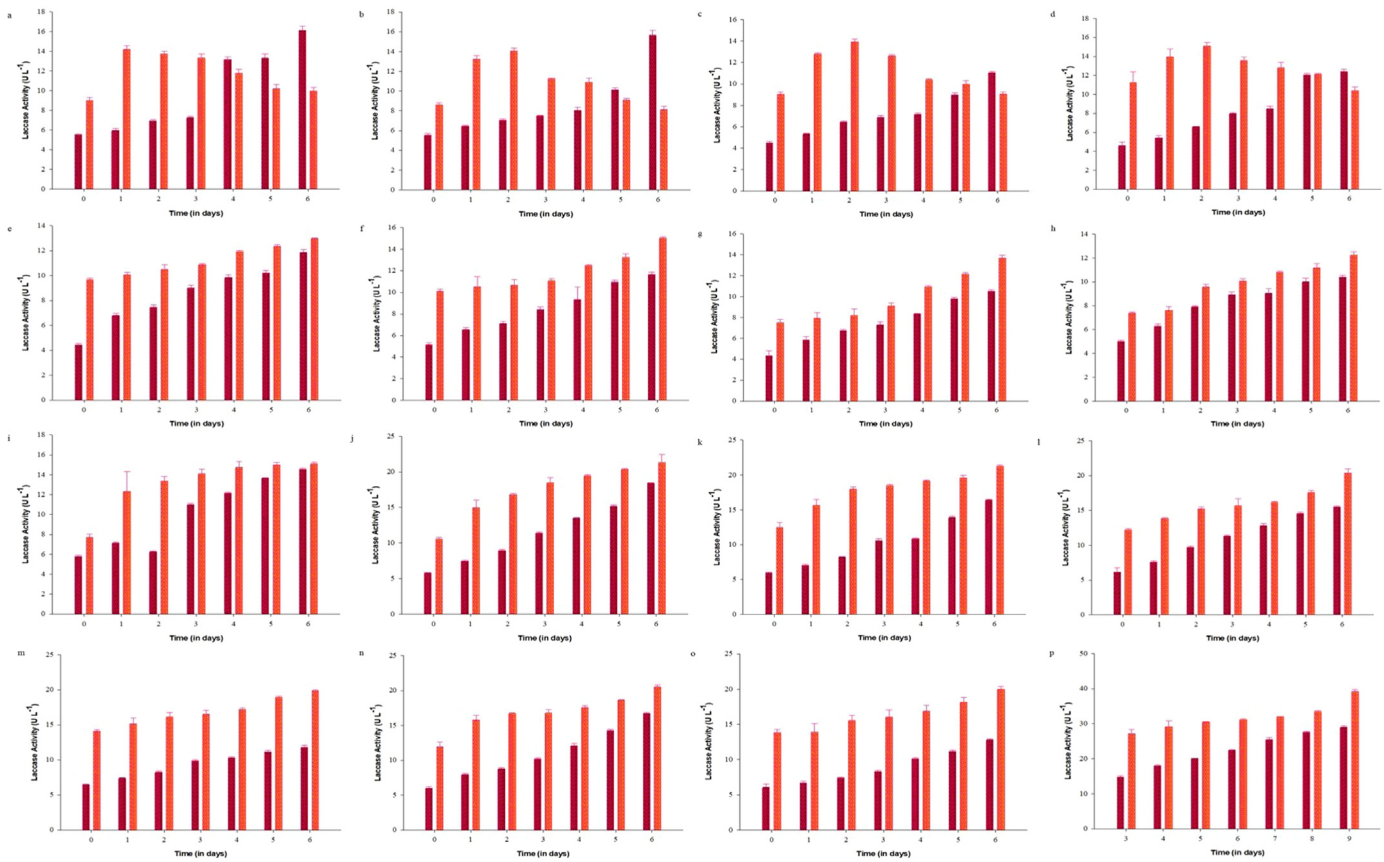

Fig. 4. Control 20 ppm Laccase activity in presence of etsrone. (A) *Arthrospira platensis* (B) *Arthrospira platensis* (CFTRI) (C) *Arthrospira platensis* (Behrampur) (D) *Arthrospira indica* (SOSA-4) (E) *Arthrospira maxima* (SAE-49–88) (F) *Arthrospira indica* (Kenya isolated) (G) *Arthrospira indica* (SAE-84) (H) *Arthrospira indica* (Lonar-SV) (I) *Arthrospira platensis* (NEERI) (J) *Spirulina platensis* (S-5) (K) *Spirulina* NCCU-477 (L) *Spirulina* NCCU-479 (M) *Spirulina* NCCU-483 (N) *Spirulina platensis* (S-2303) (O) *Spirulina platensis* (CFTRI, Mysore) (P) *Spirulina* CPCC-695. The lowest concentration of estrone (20 ppm) showed best results in growth and degradation experiments. Hence, it was selected for performing laccase activity assay. The activity of laccase got enhanced as compared to control suggesting that it is helping in degradation of estrone.

to Culture Collection Centres of India like IARI, University of Madras, University of Allahabad and Central Food Technological Research Institutes (CFTRI), Mysore for providing the cyanobacteria species.

References

Afreen, S., Anwer, R., Singh, R.K., Fatma, T., 2016. Extracellular laccase production and its optimization from Arthrospira maxima catalyzed decolorization of synthetic dyes. Saudi J. Biol. Sci.

Afreen, S., Shamsi, T.N., Baig, M.A., Ahmad, N., Fatima, S., Qureshi, M.I., Hassan, M.I., Fatma, T., 2017. A novel multicopper oxidase (laccase) from cyanobacteria: purification, characterization with potential in the decolorization of anthraquinonic dye. PLoS One 12, e0175144.

Andreolini, F., Borra, C., Caccamo, F., Di Corcia, A., Samperi, R., 1987. Estrogen conjugates in late-pregnancy fluids: extraction and group separation by a graphitized carbon black cartridge and quantification by high-performance liquid chromatography. Anal. Chem. 59, 1720–1725.

Ansari, M.K.A., Khatib, U.M., Owens, G., Fatma, T., 2016. Evaluation of methyl red tolerant cyanobacteria for simultaneous laccase production and dye decolorization. Int J. Waste Resour. 6, 2.

Belfroid, A.C., Van der Horst, A., Vethaak, A.D., Schafer, A.J., Rijs, G.B., Wegener, J., Cofino, W.P., 1999. Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands. Sci. Total Environ. 225, 101–108.

Blánquez, Paqui, Guieysse, Benoit, 2008. Continuous biodegradation of 17β-estradiol and 17α-ethynylestradiol by Trametes versicolor. J. Hazard. Mater. 150, 459–462.

Bollag, J.M., Shuttleworth, K.L., Anderson, D.H., 1988. Laccase-mediated detoxification of phenolic compounds. Appl. Environ. Microbiol. 54, 3086–3091.

Bolong, Nurmin, Ismail, A.F., Salim, Mohd Razman, Matsuura, T., 2009. A review of the effects of emerging contaminants in wastewater and options for their removal. Desalination 239, 229–246.

Caliman, Florentina Anca, Gavrilescu, Maria, 2009. Pharmaceuticals, personal care products and endocrine disrupting agents in the environment–a review. Clean—Soil, Air, Water 37, 277–303.

Caserta, D., Maranghi, L., Mantovani, A., Marci, R., Maranghi, F., Moscarini, M., 2008. Impact of endocrine disruptor chemicals in gynaecology. Hum. Reprod. Update 14, 59–72.

Combalbert, S., Hernandez-Raquet, G., 2010. Occurrence, fate, and biodegradation of estrogens in sewage and manure. Appl. Microbiol. Biotechnol. 86, 1671–1692.

Conroy, O., Saez, A.E., Quanrud, D., Ela, W., Arnold, R.G., 2007. Changes in estrogen/anti-estrogen activities in ponded secondary effluent. Sci. Total Environ. 382, 311–323.

Cui, C.W., Ji, S.L., Ren, H.Y., 2006. Determination of steroid estrogens in wastewater treatment plant of a controceptives producing factory. Environ. Monit. Assess. 121, 409–419.

De Oliveira, MACL, Monteiro, M.P.C., Robbs, P.G., Leite, S.G.F., 1999. Growth and chemical composition of Spirulina maxima and Spirulina platensis biomass at different temperatures. Aquac. Int. 7, 261–275.

Husain, Q., Qayyum, S., 2013. Biological and enzymatic treatment of bisphenol A and other endocrine disrupting compounds: a review. Crit. Rev. Biotechnol. 33, 260–292.

Jeannot, R., Sabik, H., Sauvard, E., Dagnac, T., Dohrendorf, K., 2002. Determination of endocrine-disrupting compounds in environmental samples using gas and liquid chromatography with mass spectrometry. J. Chromatogr. A 974, 143–159.

Jobling, Susan, Tyler, Charles R., 2003. Endocrine disruption in wild freshwater fish. Pure Appl. Chem. 75, 2219–2234.

Johnson, D.R., Helbling, D.E., Lee, T.K., Park, J., Fenner, K., Kohler, H.P., Ackermann, M., 2015. Association of biodiversity with the rates of micropollutant biotransformations among full-scale wastewater treatment plant communities. Appl. Environ. Microbiol 81, 666–675.

Jurado, A., Vazquez-Sune, E., Carrera, J., Lopez de Alda, M., Pujades, E., Barcelo, D., 2012. Emerging organic contaminants in groundwater in Spain: a review of sources, recent occurrence and fate in a European context. Sci. Total Environ. 440, 82–94.

Khanal, Kumar, Samir, Xie, Bin, Thompson, Michael L., Sung, Shihwu, Ong, Say-Kee, Leeuwen, J. Van, 2006. Fate, transport, and biodegradation of natural estrogens in the environment and engineered systems. Environ. Sci. Technol. 40, 6537–6546.

Koh, Y.K., Chiu, T.Y., Boobis, A., Cartmell, E., Lester, J.N., Scrimshaw, M.D., 2007. Determination of steroid estrogens in wastewater by high performance liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1173, 81–87.

Kolvenbach, B.A., Helbling, D.E., Kohler, H.P., Corvini, P.F., 2014. Emerging chemicals and the evolution of biodegradation capacities and pathways in bacteria. Curr. Opin.

Biotechnol. 27, 8–14.
Lee, Kathy E, Susan K Langer, Larry B Barber, Jeff H Writer, Mark L Ferrey, Heiko L Schoenfuss, Edward T Furlong, William T Foreman, James L Gray & Rhiannon C ReVello. 2011. Endocrine active chemicals, pharmaceuticals, and other chemicals of concern in surface water, wastewater-treatment plant effluent, and bed sediment, and biological characteristics in selected streams, Minnesota—design, methods, and data, 2009. US Geological Survey Data Series 575.54.
Legerská, Barbora, Chmelová, Daniela, Ondrejovič, Miroslav, 2016. Degradation of synthetic dyes by laccases–a mini-review. Nova Biotechnol. Chim. 15, 90–106.
Li, Mingtang, Zhao, Xingmin, Zhang, Xiufang, Wu, Di, Leng, Su, 2018. Biodegradation of 17β-estradiol by Bacterial Co-culture Isolated from Manure. Sci. Rep. 8, 3787.
Lika, K., Papadakis, I.A., 2009. Modeling the biodegradation of phenolic compounds by microalgae. J. Sea Res. 62, 135–146.
Liu, Z.H., Kanjo, Y., Mizutani, S., 2009. Removal mechanisms for endocrine disrupting compounds (EDCs) in wastewater treatment - physical means, biodegradation, and chemical advanced oxidation: a review. Sci. Total Environ. 407, 731–748.
Madkour, Fadel, Fedekar, Kamil, Abd El-Wahab, Nasr, Hoda Shafik, 2012. Production and nutritive value of Spirulina platensis in reduced cost media. Egypt. J. Aquat. Res. 38, 51–57.
Manickum, T., John, W., 2014. Occurrence, fate and environmental risk assessment of endocrine disrupting compounds at the wastewater treatment works in Pietermaritzburg (South Africa). Sci. Total Environ. 468–469, 584–597.
Mendes, J.J. Amaral, 2002. The endocrine disrupters: a major medical challenge. Food Chem. Toxicol. 40, 781–788.
Murugesan, Kumarasamy, Nam, In-Hyun, Kim, Young-Mo, Chang, Yoon-Seok, 2007. Decolorization of reactive dyes by a thermostable laccase produced by Ganoderma lucidum in solid state culture. Enzym. Microb. Technol. 40, 1662–1672.
Racz, LeeAnn, Goel, Ramesh K., 2010. Fate and removal of estrogens in municipal wastewater. J. Environ. Monit. 12, 58–70.
Rajasekaran, Chandrasekaran, Ajeesh, C.P. Mohammed, Balaji, Sundaramoorthy, Shalini, Mohan, Ramamoorthy, S.I.V.A., Ranjan, D.A.S., Fulzele, Devanand P., Kalaivani, Thiagarajan, 2015. Effect of modified Zarrouk's medium on growth of different Spirulina strains. Walailak J. Sci. Technol. (WJST) 13, 67–75.
Roy, J.R., Chakraborty, S., Chakraborty, T.R., 2009. Estrogen-like endocrine disrupting chemicals affecting puberty in humans—a review. Med. Sci. Monit. 15, RA137–RA145.
Semple, K.T., Cain, R.B., 1995. Metabolism of phenols by Ochromonas danica. FEMS Microbiol. Lett. 133, 253–257.
Silva, C.P., Otero, M., Esteves, V., 2012. Processes for the elimination of estrogenic steroid hormones from water: a review. Environ. Pollut. 165, 38–58.
Yasin, D., Zafaryab, M., Ansari, S., Ahmad, N., Khan, N.F., Zaki, A., Rizvi, M.M.A., Fatma, T., 2018a. Evaluation of antioxidant and anti-proliferative efficacy of Nostoc muscorum NCCU-442. biocatalysis and agricultural biotechnology. Nat. Prod. J. 8, 1.
Yasin, D., Zafaryab, M., Ansari, S., Ahmad, N., Khan, N.F., Zaki, A., Rizvi, M.M.A., Fatma, T., 2018b. Evaluation of antioxidant and anti-proliferative efficacy of Nostoc muscorum NCCU-442. Biocatal. Agric. Biotechnol.
Ying, Guang-Guo, Rai, S. Kookana, Ru, Ying-Jun, 2002. Occurrence and fate of hormone steroids in the environment. Environ. Int. 28, 545–551.
Yu, C.P., Deeb, R.A., Chu, K.H., 2013. Microbial degradation of steroidal estrogens. Chemosphere 91, 1225–1235.
Yu, C.P., Roh, H., Chu, K.H., 2007. 17beta-estradiol-degrading bacteria isolated from activated sludge. Environ. Sci. Technol. 41, 486–492.
Zarrouk, Claude, 1966. Contribution a l'etude d'une Cyanophycee. Influence de Divers Facteurs Physiques et Chimiques sur la croissance et la photosynthese de *Spirulina maxima* mixima (Thesis). University of Paris, France.
Zhang, Tingdi, Xiong, Guangming, Maser, Edmund, 2011. Characterization of the steroid degrading bacterium S19-1 from the Baltic Sea at Kiel, Germany. Chem.-Biol. Interact. 191, 83–88.
Zhang, X., Liu, L., Zhang, S., Pan, Y., Li, J., Pan, H., Xu, S., Luo, F., 2016. Biodegradation of dimethyl phthalate by freshwater unicellular cyanobacteria. Biomed. Res. Int. 2016, 5178697.
Zhao, Lin, Wu, Qi, Ma, Aijin, 2018. Biodegradation of Phenolic Contaminants: Current Status and Perspectives. Paper presented to the IOP Conference Series: Earth and Environmental Science.