



ORIGINAL ARTICLE

Extracellular laccase production and its optimization from *Arthrospira maxima* catalyzed decolorization of synthetic dyes



S. Afreen ^{a,1}, R. Anwer ^b, R.K. Singh ^{a,1}, T. Fatma ^{a,*}

^a *Cyanobacterial Biotechnology Laboratory, Department of Biosciences, Jamia Millia Islamia (Central University), New Delhi 110025, India*

^b *Department of Anatomy (Microbiology), College of Medicine, Al-Imam Muhammad Ibn Saud Islamic University, Riyadh, Saudi Arabia*

Received 27 July 2015; revised 25 October 2015; accepted 8 January 2016

Available online 19 January 2016

KEYWORDS

Laccase;
Arthrospira maxima (SAE-25780);
Optimization;
Remazol Brilliant Blue R;
Reactive Blue 4;
Decolorization

Abstract In the present study laccase production potential of a photosynthetic, non nitrogen fixing cyanobacteria *Arthrospira maxima* (SAE-25780) was investigated for their probable use in synthetic dye decolorization which poses environmental pollution problem in aquatic bodies. *A. maxima* (SAE-25780) showed a constitutive production of laccase which increased up to 80% in the presence of inducer guaiacol. The optimal condition for laccase was 30 °C, 10 mM sucrose as a carbon source, 10 mM sodium nitrate as a nitrogen source, and 2 mM copper as metal activator. The partially purified laccase showed 84% and 49% decolorization potential for the two anthroquinonic dyes-Reactive Blue 4 and Remazol Brilliant Blue R, respectively (RBBR) within 96 h without any mediator. Therefore the laccase extracted from *A. maxima* (SAE-25780) can be used efficiently in bioremediation of synthetic dyes from paper, pulp and textile industries.

© 2016 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Textile industry is one of the greatest generators of liquid effluent containing synthetic dyes. Textile dyes are responsible for the deep color of the effluents. The color of water has adverse effects on O₂ evolution by photosynthetic organism as reduced dissolved oxygen causes death of flora and fauna of the habitat. In the current time 10,000 synthetic dyes are frequently utilized in the textile industry representing an annual consumption of around 7 × 10⁵ tonnes worldwide (Karthikeyan et al., 2010). These dyes may be classified based on their chromophores and can be divided into several groups

* Corresponding author. Tel.: +91 11 26981717x3414x3412; fax: +91 11 26980229.

E-mail addresses: tasneemfatma15@gmail.com, fatma_cbl@yahoo.com (T. Fatma).

¹ Tel.: +91 11 26981717x3414x3412; fax: +91 11 26980229.

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

such as azo, anthraquinone, sulfur, indigo, triphenylmethyl and phthalocyanine derivatives. Most of these are stable against light, temperature and biodegradation and therefore accumulate in the environment and form recalcitrant compound (Nozaki et al., 2008) and dyes are mutagenic and carcinogenic to the human population (Rindle and Troll, 1975). Thus industrial effluents with synthetic dyes need some tools and techniques to make them inactive before they are discharged into the environment. Physicochemical methods such as- (coagulation, flocculation, membrane filtration, and activated carbon adsorption) have high operating costs (Robinson et al., 2001). A number of biotechnological approaches have been suggested by recent research as of potential interest toward combating this pollution source in an ecoefficient manner. Biological decolorization has been considered as effective, specific, less energy intensive and environmentally benign, since it results in partial or complete bio-conversion of synthetic dyes to stable nontoxic end products (Kuhad et al., 2004).

Cyanobacteria are ubiquitous in nature but their role in the decolorization and degradation of dyes are scanty. Parik and Madamwar (2005) reported textile dye decolorization using cyanobacteria. However, the cyanobacterial enzymes involved in the decolorization of synthetic dyes have not yet been characterized. Microbial sources may provide stable and reliable source of enzymes, due to the possibility of the production of large quantities of enzyme by them and ease of extraction (Desai and Nityananda, 2011).

Laccases (benzenediol:oxygen oxidoreductases, (EC:1.10.3.2) are a diverse group of multi-copper proteins that oxidize a surprisingly wide variety of organic and inorganic compounds, including diphenols, polyphenols, substituted phenols, diamines and aromatic amines, with concomitant a reduction of molecular oxygen to water (Thurston, 1994). Laccase is a dimeric or tetrameric glycoprotein and usually contains four copper atoms per monomer. To function, laccase depends on Cu atoms distributed among the three binding sites. Due to its low specificity for the reducing substrate, laccases have attracted increasing attention in industrial and environmental fields, such as pulp delignification, dye decolorization, environmental pollutant detoxification, biopolymer modification, and biotransformation (Hou et al., 2004; Leonowicz et al., 2001; Yaropolov et al., 1994). The production of active and large quantities of laccase at a relatively low cost is of major interest. Laccases can be either constitutive or inducible enzymes and their production occurs during secondary metabolism and is subject to complex regulation by nutrients (carbon, nitrogen, inducers, and copper) (Dittmer et al., 1997; Palmieri et al., 2000). These regulators affect the transcription levels of laccase and other genes (Collins and Dobson, 1997; Soares et al., 2001). Aromatic and phenolic compounds have been widely used to elicit enhanced laccase production by different organisms (De Souza et al., 2004; Leonowicz et al., 2001). The presence of inducers increase laccase synthesis by providing contact with compounds that may naturally elicit a stress response and further increase production. Laccases are mostly found in plants and fungi but also in some bacteria (Alexandre and Zhulin, 2000; Claus, 2004). One of the advantages associated with laccases is that they do not require H₂O₂ for substrate oxidation unlike peroxidases and, moreover, they have broad substrate specificity (Saito et al., 2003). Of late, the demand for the removal of synthetic

dyes from industrial waste using laccases is being increased tremendously (Abadull et al., 2000; Zille et al., 2003). Therefore, search for potential laccases to cope with this demand is an important task. Due to phototrophic mode of nutrition, short generation time and easy mass cultivation, cyanobacteria may appear as good candidate for laccase production.

Previously various studies have been conducted on purification and decolorization of synthetic dyes from fungal sources but so far, in cyanobacteria only *Phormidium valderianum* BDU 30501 and *Oscillatoria boryana* are reported to have ligninolytic activity and decolorization ability (Palanisami and Uma, 2010). Considering the potential application of cyanobacterial laccase for solving environmental problems, it is essential to define the best conditions for the production of such enzymes. Thus, in the present study laccase production potential of *Arthrospira maxima* (SAE-25780), optimization of culture conditions for its highest yield and synthetic dye (Reactive Blue 4 and Remazol Brilliant Blue R) decolorization ability were investigated.

2. Material and methods

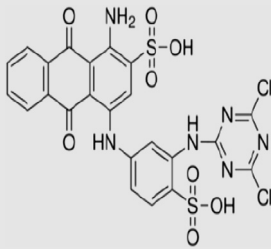
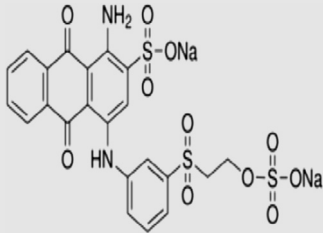
2.1. Chemicals and reagents

All chemicals used were of analytical grade and were obtained from Merck, India. Synthetic Dyes (Reactive Blue 4 and Remazol Brilliant Blue R) were purchased from Sigma-Aldrich Chemical Co, St. Louis, MO, USA. The structure of all dyes is shown in Table 1.

2.2. Culture condition and maintenance

The *A. maxima* (SAE-25780) was procured from the University of Chennai, Tamil Nadu; and was grown in 500 ml Erlenmeyer flask containing 200 ml Zarrouk's medium (1996) containing (g/L) NaCl, 1; CaCl₂, 0.04; NaNO₃, 2.5; FeSO₄, 0.01; EDTA, 0.08; K₂SO₄, 0.08; MgSO₄, 0.2;

Table 1 Structure and maximum visible wavelengths of dyes.

Dye	λ_{\max} (nm)	Structure
Reactive Blue 4	595	
Remazol Brilliant Blue R	592	

NaHCO₃, 16.8; K₂HPO₄, 0.5, Trace metal mix, 1 ml at light intensity 2000 ± 200 lux; photoperiod 12:12 h light: dark; temperature 30 ± 1 °C. The culture was induced with 100 µM guaiacol (dissolved in 50% ethanol) for laccase production. The culture without guaiacol was considered as control. The cultures were swirled twice a day for aeration and mixing of nutrients. Sample were collected by centrifugation at 8000g for 20 min at 4 °C and used for the enzyme assay.

2.3. Assay for laccase activity

Laccase activity was assayed at 25 °C using 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as substrate. The assay mixture contained 2 mM ABTS and 0.1 M glycine HCl buffer, pH 2.5. Oxidation of ABTS was followed by an absorbance increase at 405 nm ($\epsilon = 36,000 \text{ M}^{-1} \text{ cm}^{-1}$). Activity was expressed in units: 1 U as µmol of oxidized product formed in a min. The blank contained all the constituents except the active enzyme.

2.4. Zymogram analysis of laccase on Native-PAGE

Partial purification of laccase was done for zymogram analysis. For this the culture was induced with guaiacol and the supernatant was obtained by centrifugation at 8000 rpm for 20 min. The protein was precipitated with chilled cold acetone at a volume of 1:4 incubated for 1.5 h at -20 °C in a deep freezer and then centrifuged for 30 min at 10,000g. The supernatant was discarded and the pellet was retained. The pellet was air dried to remove any residual acetone and served as the enzyme source.

Native PAGE was carried out at 15 ± 1 °C using tris-glycine as running buffer (pH 8.3) under non-denaturing and non-reducing conditions (Laemmli, 1970). A uniform amount of protein (500 µg, estimated by Lowry et al. (1951)) was loaded to each lane along with native sample buffer (devoid of sodium dodecyl sulfate and *b*-mercaptoethanol). Samples were then electrophoresed at 50 V through the stacking gel (6%) and at 100 V through the resolving gel (10%) using Power Supply-EPS 1001 (Amersham Pharmacia). Laccase activity was visualized in the gel with guaiacol (1 mM) as substrate in 0.5 M sodium citrate buffer (pH 4.5)

2.5. Decolorization of synthetic dyes

For finding out the decolorization potential of partially purified laccase a slightly modified protocol of Couto, 2007) was adopted. The dye solution [Reactive Blue 4 (50 mg/l) and Remazol Brilliant Blue R (50 mg/l)] were incubated with laccase (1.5 U/ml) in 50 mmol/L sodium citrate buffer, pH 6.0 at 30 °C. Samples were taken at 24 h interval and centrifuged at 8000 rpm for 20 min. The decrease of color intensity in supernatant was analyzed spectrophotometrically at $\lambda = 495 \text{ nm}$ for Reactive Blue 4 and at $\lambda = 595$ for Remazol Brilliant Blue R.

Percent dye decolorization was calculated according to the formula: $D = 100(A_{\text{ini}} - A_{\text{obs}})/A_{\text{ini}}$, where D is decolorization (in%), A_{ini} , initial absorbance and A_{obs} , observed absorbance.

2.6. Optimization of laccase production

To investigate optimal time for laccase production, the experimental cultures were incubated for 16 days and their laccase activity was measured after every 24 h. The effect of temperature on enzyme production was determined by incubating the inoculated flask at different temperatures (30–60 °C) under control and induced conditions. Similarly 10 mM of glucose, fructose and sucrose were tested individually for the effect of carbon source on laccase yield. Sodium nitrate, ammonium chloride, Urea at a concentration of (10 mM) each was tested as nitrogen source to examine their effect on laccase production. After 72 h of after inoculation copper as copper sulfate (0.1, 0.5, 1.0, 1.5 and 2.0, 2.5, 3.0 mM) was added in the culture medium and its effect on laccase production was determined at regular intervals.

2.7. Statistical analysis

All experiments were carried out in triplicates and the results were expressed as mean ± standard error. Dunnett's multiple range test was applied and differences among the means were analyzed by one way ANOVA using Graph Pad Prism version-6.1 (Graph Pad Software, San Diego, CA, USA).

3. Results and discussions

3.1. Time course study

The main purpose of this study was to examine the laccase production potential of *A. maxima* (SAE-25780). The normal and guaiacol induced cultures (100 µM) were incubated for 16 days and laccase activity was measured at 24 h intervals. Under normal condition maximum laccase secretion started on the 2nd day of incubation and the maximum activity (54.671 U/ml) was achieved on the 10th day suggesting its constitutive production which was also reported by Scheel et al., 2000. From the growth curve study of *A. maxima* (SAE-25780) we found that during lag phase on the 2nd day biomass was (3.08 mg/g) then it increased exponentially during log phase and reached its maximum value (21.42 mg/g) on the 10th day and thereafter stationary phase started which suggested that total laccase activity increased proportionally with the biomass production. In our study we found that under the induced condition the average time production of laccase was decreased and highest activity was measured on the 4th day (56.894 U/ml) (Fig. 1). It was found that there was a 2–4 fold enhancement (ANOVA $p < 0.0001$) in the laccase activity in the presence of inducer guaiacol as compared to control. The activity ranged from (12.114–54.671 U/ml) in control condition to (25.632–56.685 U/ml) in induced condition (Fig. 1). Aromatic and phenolic compounds have been widely used to elicit enhanced laccase production by different organisms and the nature of the compound that induces laccase activity differs greatly with the species (De Souza et al., 2004; Leonowicz et al., 2001). Earlier we have reported the presence of laccase in *Synechocystis* NCCU-370 but its production was quite low under normal condition to (25.37 mU/ml) that peaked on the 7th day (Afreen and Fatma, 2013). *A. maxima* (SAE-25780) is better than *Synechocystis* NCCU-370 in

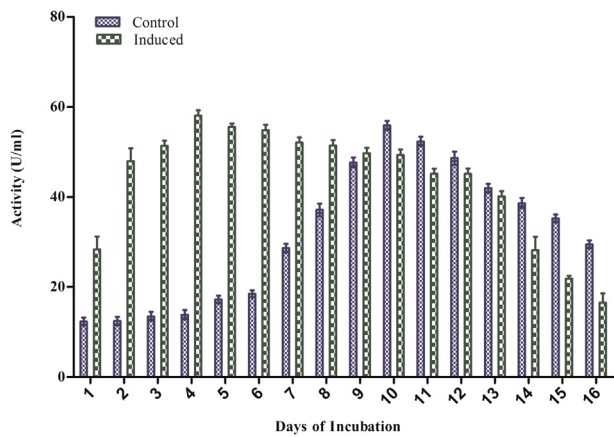


Figure 1 Extracellular laccase production by *Arthrospira maxima* (SAE-25780) under controlled and guaiacol induced condition.

laccase production as it produced (56.894 U/ml) on the 4th day in the presence of guaiacol. It is reported that guaiacol supplementation (1 mM) enhanced laccase production in different fungi eg. *Phlebia* spp., *Pleurotus ostreatus*, *Coriolus hirsutus* (Hou et al., 2004; Koroljova-Skorobog^oko et al., 1998; Patel et al., 2014). Mongkolthananaruk et al. (2012) reported that 8 mM guaiacol acts as a good inducer. It induced laccase production from 33 to 56 mU/l after 48 h of incubation in bacteria viz *Rhodococcus* sp., *Enterobacter* sp., and *Staphylococcus saprophyticus*. It has been reported that marine cyanobacteria *Phormidium tenue* induced laccase production to various folds after the addition of 100 μM of guaiacol, tannic acid, caffeic acid, copper (Palanisami and Uma, 2010).

The presence of laccase in cyanobacteria *A. maxima* (SAE-25780) was further confirmed by native Page followed by zymogram analysis. The native PAGE result revealed a single laccase enzyme was detected by activity staining with guaiacol. The enzyme was able to oxidize the substrate (guaiacol) and developed reddish brown color which is due to the oxidative polymerization of guaiacol in the presence of laccase (Fig. 2).

3.2. Effect of temperature on laccase production

Temperature affects the properties of aqueous environment as well as all metabolic processes of the organism including nutrient availability and uptake. During optimization of culture condition for the highest yield of laccase from *A. maxima* it was found that temperature has a profound influence on enzyme activity that was significantly increased (ANOVA $p < 0.0001$) daywise and maximum activity was found on the 4th day at 30 °C under both normal and induced conditions (Figs. 3a and 3b). Results also indicated that, by increasing the incubation temperature above 35 °C, a gradual decrease in laccase production occurred. At 60 °C after the 2nd day the culture started to decay and became pale yellow. It produced only a small amount of enzyme (3.714 U/ml).

A previous study carried in our lab suggested that the optimal temperature for the better growth of this *Spirulina* strain was found to be 27 °C–33 °C (Jetley et al., 2004). Above 40 °C, both the growth and laccase production decreased. This suggested that proper growth of *Spirulina* is necessary for the optimal laccase production.



Figure 2 Confirmation of laccase production by zymogram analysis of *Arthrospira maxima* (SAE-25780) using guaiacol as substrate.

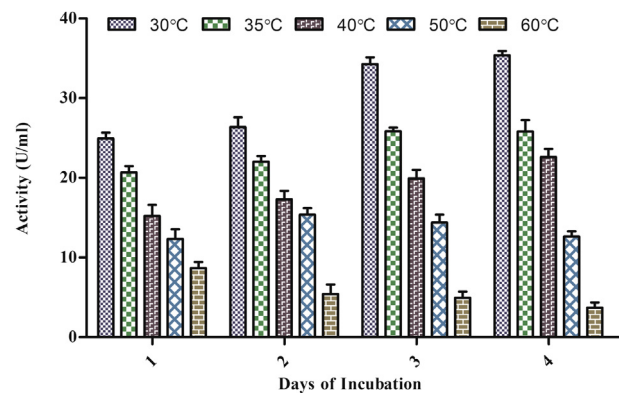


Figure 3a Effect of temperature on the laccase production in spent medium under control condition.

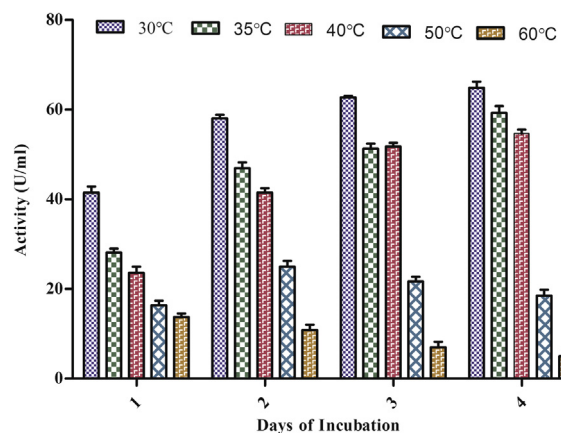


Figure 3b Effect of temperature on the laccase production in spent medium under guaiacol induced condition.

Nakamura et al. (1999) and Zadrazil et al. (1999) have reported maximum laccase activity at 30 °C in *Pleurotus*, *Dichomitus squalens* and *Bjerkendera adusta* at 30 °C. Farnet et al. (2000) and Pointing et al. (2000) have found 25 °C and

50 °C as optimal temperature in *Marasmius quercophilus* strain and *Pycnoporus sanguineus*.

3.3. Effect of carbon on laccase production

The concentration and nature of carbon source used in the medium showed a notable effect on enzyme production (Gawande and Kamat, 2000). Exogenous addition of fructose, glucose, and sucrose as different carbon sources increased the laccase production in *A. maxima* (Fig. 4). In terms of maximum activity during carbon supplementation experiment, sucrose and fructose showed a much higher activity than glucose being 60.914, 60.114 and 37.118 U/ml. Mansur et al. (1997) found a 100 fold increase in the specific laccase activity of basidiomycetes fungi with fructose. But on the 2nd day glucose supplementation showed the highest laccase production in comparison to sucrose and fructose. Early, enzyme enhancing response of glucose may be due to ready and quick utilization by organism (Fig. 4). The order of activity was 52.863, 47.083 and 44.457 U/ml in the presence of glucose, fructose and sucrose. Glucose supplementation also showed increased laccase activity in *C. hirsutus* (Koroljova-Skorobogt'ko et al., 1998) and *Ganoderma* sp. (Revankar et al., 2007).

3.4. Effect of nitrogen on laccase production

Nitrogen is involved in amino acid synthesis which makes up proteins and other value added substances. The effect of nitrogen sources on laccase production by different organisms appears to be greatly controversial (Collins and Dobson, 1997). Nitrogen sufficient media has been reported to enhance ligninolytic enzymes production in many fungi (Kaal et al., 1995) but in *Phanerochaete chrysosporium* laccase was limited by nitrogen sources (Buswell et al., 1992). In the present study the effect of different nitrogen sources viz urea, ammonium chloride, and sodium nitrate on laccase production was determined. All nitrogen sources (10 mM) tested stimulated the laccase production that ranged from 2.5–3.5-fold. Among the tested nitrogen sources on the 4th day maximum enzyme production (47.885 U/ml) was achieved with sodium nitrate as compared to control (13.485 U/ml) (Fig. 5) that may be due to higher growth of the organism (Anwer et al., 2012).

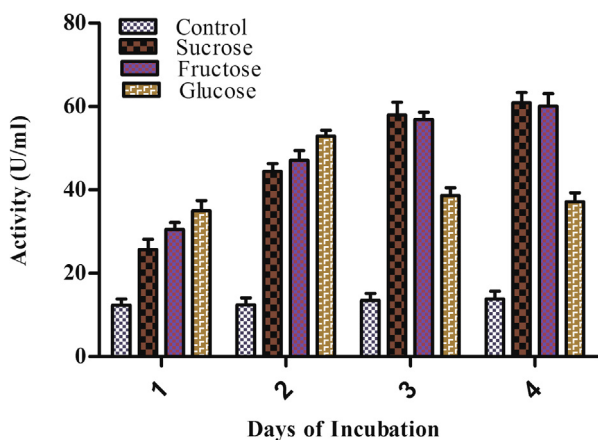


Figure 4 Effect of carbon sources on the laccase production by *Arthrospira maxima* (SAE-25780).

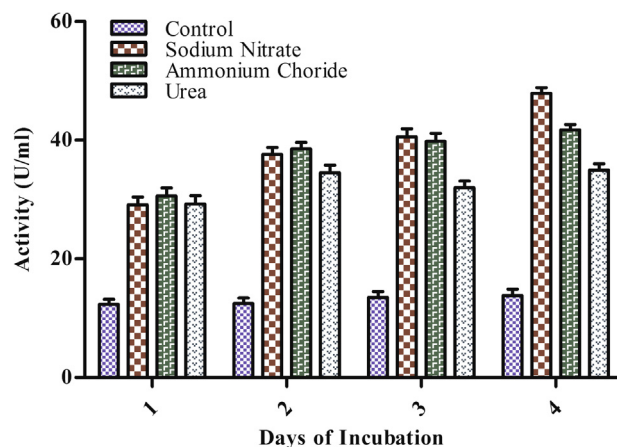


Figure 5 Effect of nitrogen sources on laccase production by *Arthrospira maxima* (SAE-25780).

Shraddha et al. (2011) also found sodium nitrate as the best nitrogen source for laccase production.

Ammonium chloride also significantly increased (ANOVA $p < 0.0001$) laccase activity on the 4th day of incubation. *P. ostreatus* fungi showed the highest laccase activity with ammonium chloride (Stajic et al., 2006). Culture supplemented with urea resulted in least laccase activity (34.971 U/ml) but still it was much higher than control (13.485 U/ml). *Pleurotus sanguineus* also showed contrary higher laccase activity with urea than sodium nitrate (Lee et al., 2006). The order of different nitrogen sources on laccase production was sodium nitrate > ammonium chloride > urea.

3.5. Effect of copper on laccase production

Laccase are multicopper oxidases therefore, copper as micronutrients has key role as metal activators. For optimizing copper concentration for higher laccase production by *A. maxima*, copper as CuSO_4 (0.1–3.0 mM) was added in culture medium on the third day of inoculation and on the 4th day it showed a considerable increase in activity (ANOVA $p < 0.0001$) (Fig. 6). Enhanced laccase yield after the third day copper addition might attribute to the fact that organism was already adapted to the media conditions and exponential growth was starting, leading to enhancement of laccase production by the primary metabolism (Palmieri et al., 2000). On the 4th day lower CuSO_4 concentration (0.1 mM–1.5 mM) does not affect significantly as compared to control. In 2.0 mM CuSO_4 laccase activity increased and was the highest, whereas, above 2 mM CuSO_4 (2.5 and 3.0 mM) its laccase activity decreased. The suppression at higher copper concentration may be due to downfall in the growth of organism due to metal toxicity that results from a low photosynthetic rate (Fathi et al., 2005). Copper has been reported to be a strong laccase inducer in the fungal species *Trametes versicolor* and *P. chrysosporium* (Collins and Dobson, 1997; Dominguez et al., 2007). Hao et al. (2007) also found 2.0 mM of CuSO_4 as optimal concentration of copper in *Pestalotiopsis* sp. but Palmieri et al. (2000) found 150 μM of copper sulfate as the best concentration for laccase activity. The regulation of the synthesis of several laccase isoforms by copper occurs at the level of gene transcription (Galhaup et al., 2002; Palmieri et al., 2000).

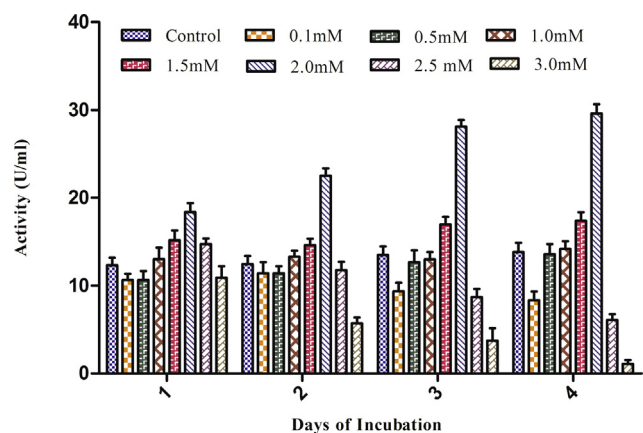


Figure 6 Effect of different concentration of CuSO_4 on the laccase production by *Arthrospira maxima* (SAE-25780).

3.6. Decolorization of synthetic dyes

The dye decolorization ability of the partially purified laccase of *A. maxima* (SAE-25780) was assayed regularly against anthraquinonic synthetic dyes Reactive Blue 4 and RBBR (Fig. 7). From the results we found that the decolorization rate of Reactive Blue 4 (50 mg/l) was 84% and 49% for RBBR (50 mg/l) by laccase enzyme within 96 h of incubation. Both the dyes were not decolorized at the same extent that may be due to the difference of the redox potentials and the suitability of their steric structure with the active site of the enzyme (Tavares et al., 2008). The extent of decolorization activity depends on the source of the enzyme and the chemical structure of the dye (Abadull et al., 2000; Couto, 2007). It was reported that laccase hardly decolorized RBBR which might be due to the absence of mediator as suggested by Hamid et al. (2012). According to them crude laccase enzyme requires mediator like – 1-hydroxybenzotriazole (HBT) for efficient dye decolorization. The decolorization rate of Reactive dyes was increased from 3% to 70% by the addition of HBT as the mediator (Claus, 2004). Yemendzhiev et al. (2009) have also observed efficient decolorization of Reactive Blue 4 (50 mg/l) by fungus *T. versicolor*. Parik and Madamwar (2005) reported that the cyanobacteria *Phormidium ceylanicum*, *Gloeocapsa*

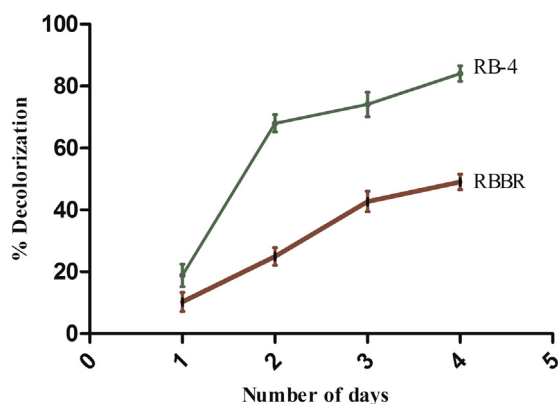


Figure 7 Decolorization of anthraquinonic textile dyes Reactive Blue 4 and RBBR by *Arthrospira maxima* (SAE-25780) laccase.

pleurocapsoides and *Chroococcus minutes* showed 70–90% decolorization of dye (50 mg/l) after 26 days of incubation. However in our study *A. maxima* laccase showed a rapid decolorization of Reactive Blue 4 (84%) and of RBBR (49%) without any mediator within 4 days.

4. Conclusion

The results of this work disclose the potential of cyanobacteria *A. maxima* (SAE-25780) to enhance laccases production. It showed a constitutive production of laccase which increased up to 80% in the presence of inducer (guaiacol). Under normal condition laccase production was the highest on the 10th day but in the presence of inducer (guaiacol) the duration for maximum production of laccase was reduced to 4th day that will reduce cost production during its industrial application and the optimal condition for laccase production was 30 °C, 10 mM sucrose as a carbon source, 10 mM sodium nitrate as a nitrogen source, 2 mM copper as metal activator. The synthetic dyes viz RB4 & RBBR were efficiently decolorized 84% and 49%, respectively within four days of incubation with extracellular laccase. To the best of our knowledge this is the first report on laccase production from cyanobacteria [*A. maxima* (SAE-25780)] that has anthraquinonic dye (Reactive Blue 4 & RBBR) decolorization potential for the bioremediation of synthetic dyes from paper, pulp and textile industries. More efforts are needed to increase the laccase yield from *A. maxima* (SAE-25780) in order to make it more economical.

Acknowledgements

The authors are thankful to University of Chennai, Tamil Nadu for providing the test strains and University Grant Commission India, for providing financial support.

References

- Abadull, E., Robra, K.H., Gübitz, G.M., Silva, L.M., Cavaco, P.A., 2000. Enzymatic decolorization of textile dyeing effluents. *Text. Res. J.* 70, 409–414.
- Afreen, S., Fatma, T., 2013. Laccase production and simultaneous decolorization of synthetic dyes by cyanobacteria. *Int. J. Innovative Res. Sci. Eng. Technol.* 2, 3563–3568.
- Alexandre, G., Zhulin, I.B., 2000. Laccases are widespread in bacteria. *Trends Biotechnol.* 18, 41–42.
- Anwer, R., Khurshed, S., Fatma, T., 2012. Detection of immunooactive insulin in *Spirulina*. *J. Appl. Phycol.* 24, 583–591.
- Buswell, J.A., Arora, D.K., Rai, B., Mukerji, K.G., 1992. Fungal degradation of lignin. In: Kundsen (Ed.), *Handbook of Applied Mycology Soil and Plants*. Marcel Dekker, New York, pp. 425–480.
- Claus, H., 2004. Laccases: structure, reactions, distribution. *Micron* 35, 93–96.
- Collins, P.J., Dobson, A.D.W., 1997. Regulation of laccase gene transcription in *Trametes versicolor*. *Appl. Environ. Microbiol.* 63, 3444–3450.
- Couto, S.R., 2007. Decolouration of industrial azo dyes by crude laccase from *Trametes hirsute*. *J. Hazard. Mat.* 148, 768–770.
- De Souza, C.G.M., Tychanowicz, G.K., De Souza, D.F., Peralta, R. M., 2004. Production of laccase isoforms by *Pleurotus pulmonarius* in response to presence of phenolic and aromatic compounds. *J. Basic Microbiol.* 3, 129–136.

- Desai, S.S., Nityananda, C., 2011. Microbial laccases and their applications: a review. *Asian J. Biotechnol.* 2, 98–124.
- Dittmer, J.K., Patel, N.J., Dhawale, S.W., Dhawale, S.S., 1997. Production of multiple laccase isoforms by *Phanerochaete chrysosporium* grown under nutrient sufficiency. *FEMS Microbiol.* 149, 65–70.
- Dominguez, A., Gomez, J., Lorenzo, M., Sanroman, A., 2007. Enhanced production of laccase activity by *Trametes versicolor* immobilized into alginate beads by the addition of different inducers. *World J. Microbiol. Biotechnol.* 23, 367–373.
- Farnet, A.M., Criquet, S., Tagger, S., Gil, G., LePetit, J., 2000. Purification, partial, characterization, and reactivity with aromatic compounds of two laccases from *Marasmiusquercophilus* strain 17. *Can. J. Microbiol.* 46, 189–194.
- Fathi, A.A., Zaki, F.T., Ibraheim, H.A., 2005. Response of tolerant and wild type strains of *Chlorella vulgaris* to copper with special references to copper uptake system. *Protistology* 4, 73–78.
- Galhau, C., Goller, S., Peterbauer, C.K., Strauss, J., Haltrich, D., 2002. Characterization of the major laccase isoenzyme from *Trametes pubescens* and regulation of its synthesis by metal ions. *Microbiology* 148, 2159–2169.
- Gawande, P.V., Kamat, M.Y., 2000. Production of xylanases by immobilized *Aspergillus* sp using lignocellulosic waste. *World J. Microbiol. Biotechnol.* 16, 111–112.
- Hamid, F., Moezzi, A., Khozani, A.M., Mahmoudjanlou, Y., Ameri, A., Niknejad, F., Faramarzi, M.A., 2012. Synthetic dye decolorization by three sources of fungal laccase. *Iranian J. Environ. Health Sci. Eng.* 9, 27. <http://dx.doi.org/10.1186/1735-2746-9-27>.
- Hao, J., Song, F., Huang, F., Yang, C., Zhang, Z., Zheng, Y., Tian, X., 2007. Production of laccase by a newly isolated deuteromycetes fungus *Pestalotiopsis* sp. and its decolorization of azo dye. *J. Ind. Microbiol. Biotechnol.* 34, 233–240.
- Hou, H., Zhou, J., Wang, J., Du, C., Yan, B., 2004. Enhancement of laccase production by *Pleurotus ostreatus* and its use. *Process Biochem.* 39, 1415–1419.
- Jetley, U.K., Choudhary, M., Fatma, T., 2004. The impact of physical stresses on the growth of cyanobacterium *Spirulina platensis*-S5. *J. Environ. Sci. Eng.* 46, 303–311.
- Kaal, E.E.J., Field, J.A., Joyce, T.W., 1995. Increasing ligninolytic enzyme activities in several white-rot basidiomycetes by nitrogen sufficient media. *Bioresour. Technol.* 53, 133–139.
- Karthikayan, K., Nanthakumar, K., Shanthi, K., Lakshmanaperumalsamy, P., 2010. Response surface methodology for optimization of culture conditions for dye decolorization by a fungus, *Aspergillus niger* HM11 isolated from dye affected soil. *Iranian J. Microbiol.* 2, 213–222.
- Koroljova-Skorobogt'ko, O.V., Stepanova, E.V., Gavrilova, V.P., Morozova, O.V., Lubimova, N.V., Dzchafarova, A.N., Jaropolov, A.J., Makower, A., 1998. Purification and characterization of the constitutive form of laccase from the basidiomycete *Coriolus hirsutus* and effect of inducers on laccase synthesis. *Biotechnol. Appl. Biochem.* 28, 47–54.
- Kuhad, R.C., Sood, N., Tripathi, K.K., Singh, A., Ward, O.P., 2004. Developments in microbial methods for the treatments of dye effluents. *Adv. Appl. Microbiol.* 56, 185–213.
- Laemmli, U., 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature* 227, 680–685.
- Lee, Y., Park, C., Lee, B., Han, E., Kim, T., 2006. Effect of nutrients on the production of extracellular enzymes for decolorization of reactive blue 19 and reactive black 5. *J. Microbiol. Biotechnol.* 16, 226–231.
- Leonowicz, A., Cho, N.S., Luterek, J., Wilkolazka, A., Wojtas Wasilewska, M., Matuszewska, A., Hofrich-ter, M., Wesenberg, D., Rogalski, J., 2001. Fungal laccase: properties and activity on lignin. *J. Basic Microbiol.* 41, 185–227.
- Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randal, R.J., 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193, 265–275.
- Mansur, M., Suárez, T., Fernández-Larrea, J.B., Brizuela, M.A., González, A.E., 1997. Identification of a laccase gene family in the new lignin-degrading basidiomycetes CECT 20197. *Appl. Environ. Microbiol.* 63, 2637–2646.
- Mongkolthananaruk, W., Tongbopit, S., Bhoonontong, A., 2012. Independent behaviour of bacterial laccases to inducers and metal ions during production and activity. *Afr. J. Biotechnol.* 11, 9391–9398.
- Nakamura, Y., Sangusis, M.G., Sawada, T., Kuwahara, M., 1999. Lignin degradind enzyme production by *Bjerkendera adusta* immobilized on polyurethane foam. *J. Biosci. Bioeng.* 88, 41–47.
- Nozaki, K., Beh, H.C., Mizuno, M., Isobe, T., Shiroishi, M., 2008. Screening and investigation of dye decolorization activities of basidiomycetes. *J. Biosci. Bioeng.* 105, 69–72.
- Palanisami, S., Uma, L., 2010. Laccase and polyphenol oxidase activities of marine cyanobacteria: a study with Poly R 478 decolourization. *World J. Microbiol. Biotechnol.* 26, 63–69.
- Palmieri, G., Giardiana, P., Bianco, C., Fontanella, B., Sannia, G., 2000. Copper induction of laccase isoenzymes in the ligninolytic fungus *Pleurotus ostreatus*. *Appl. Environ. Microbiol.* 66, 920–924.
- Parik, A., Madamwar, D., 2005. Textile dyes decolorization using cyanobacteria. *Biotechnol. Lett.* 27, 323–326.
- Patel, H., Gupte, S., Gahlout, M., Gupte, A., 2014. A purification and characterization of an extracellular laccase from solid-state culture of *Pleurotus ostreatus* HP-1. *3 Biotech.* 4, 77–84.
- Pointing, S.B., Jones, E.B.G., Vrijmoed, L.L.P., 2000. Optimization of laccase production by *Pycnoporus sanguineus* in submerged liquid culture. *Mycologia* 92, 139–144.
- Revankar, M., Desai, S., Lele, K.M., 2007. Solid-state fermentation for enhanced production of laccase using indigenously isolated *Ganoderma* sp. *Appl. Biochem. Biotechnol.* 143, 16–26.
- Rindle, E., Troll, W.J., 1975. Metabolic reduction of benzidine azo dyes to benzidine in the rhesus monkey. *J. Natl. Cancer Inst.* 55, 181–185.
- Robinson, T., McMullan, G., Marchant, R., Nigam, P., 2001. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. *Bioresour. Technol.* 77, 247–255.
- Saito, T., Hong, P., Kato, K., Okazaki, M., Inagaki, H., Maeda, S., Yokogawa, Y., 2003. Purification and characterization of an extracellular laccase of a fungus (family Chaetomiaceae) isolated from soil. *Enzyme Microb. Technol.* 33, 520–526.
- Scheel, T., Hofer, M., Ludwig, S., Holker, U., 2000. Differential expression of manganese peroxidase and laccase in white-rot fungi in the presence of manganese or aromatic compounds. *Appl. Microbiol. Biotechnol.* 54, 686–691.
- Shraddha, R.S., Sehgal, S., Kamthania, M., Kumar, A., 2011. Laccase: microbial sources, production, purification and potential biotechnological applications. *Enzyme Res.* <http://dx.doi.org/10.4061/2011/217861>.
- Soares, G.M.B., Pessoa-de Amorim, M.T., Costa-Ferreira, M., 2001. Use of laccase together with redox mediators to decolorize Remazol Brilliant Blue R. *J. Biotechnol.* 89, 123–129.
- Stajic, M., Persky, L., Friesem, D., Hadar, Y., Wasser, S.P., Nevo, E., 2006. Effect of different carbon and nitrogen sources on laccase and peroxidases production by selected *Pleurotus* species. *Enzyme Microbiol. Technol.* 38, 65–73.
- Tavares, A.P.M., Cristóvão, R.O., Loureiro, J.M., Boaventura, R.A. R., Macedo, E.A., 2008. Optimisation of reactive textile dyes degradation by laccase–mediator system. *J. Chem. Technol. Biotechnol.* 83, 1609–1615.
- Thurston, C.F., 1994. The structure and function of fungal laccases. *Microbiology* 140, 19–26.

- Yaropolov, A.I., Skorobogatko, O.V., Vartanov, S.S., Varfolomeyev, S.D., 1994. Laccase: properties, catalytic mechanism, and applicability. *Appl. Biochem. Biotechnol.* 49, 257–280.
- Yemendzhiev, H., Alexieva, Z., Krastanov, A., 2009. Decolorization of synthetic dye reactive blue 4 by mycelia culture of white-rot fungi *Trametes versicolor*. *Biotechnol. Biotechnol.* 23, 1337–1339.
- Zadrazil, F., Gonser, A., Lang, E., 1999. Influence of incubation temperature on the secretion of extracellular ligninolytic enzymes of *Pleurotus* sp. and *Dichomitus squalens* into oil. In: *Proc Conf Enz Env. Granada, Spain Ecology and Applications*, pp. 12–16.
- Zille, A., Tzanov, T., Gübitz, G.M., Cavaco-Paulo, M., 2003. Immobilized laccase for decolourization of reactive black dyeing effluent. *Biotechnol. Lett.* 25, 1473–1477.