Research Article Open Acces

Keywords Bio-remediation; Endocrine disrupting chemicals; Bisphenol-A

Introduction

Scienti c developments, innovations and achievements not only facilitate human life style but invariably pollute the environment di erently. In recent times di erent types of toxic chemicals enter into the insitu environment due to industrial development. It is well known to us that endocrine-system is one of the important and complicated system in our body which can control vital hormonal secretion, can regulate di erent types of major functions in our body, but recently EDC's (endocrine disrupting chemicals) are the most important threat to us, as well as for all living organisms due to blocking the endocrine function in di erent ways and it may come from any other sources like our daily life, it would be a tough challenge for us to restrict their devastating activity using our scienti c concepts [1,2]. Not only EDC's there are several other types of environmental threats having di erent ways of function and e ect on environment, like heavy metal toxicity it is a serious issue for environment in recent time [3]. In another side industrial wastes mainly, toxic industrial dyes also a biggest problems for water toxicity and have several negative e ects on living organisms as well as on environment [4]. In most cases these kinds of threats are from our domestic discharges which are route cause for some of our serious issues as well as for environment. Several techniques are there to restrict these kinds of problems but either most of them are complicated or costly or non e ective, but for this aspect it is the time to nd out some e ective, economical and simplest ways. It is very well known that microbes having massive diversity in several aspects, metabolic activity on several types of chemicals one of them, because they can use those chemicals (toxic /non toxic) for their basic source like C, N, P etc and a er utilization several toxic chemicals lost their toxicity completely [5-7]. Now our most important duty is to use microbial metabolic activity for the degradation of toxic chemicals, so bioremediation will be an important phenomenon in future [8]. Side by side we have to extract the metabolic enzymes from microbes by which they can degrade toxic chemicals and we can use them in several types of industrial and environmental aspects. Some of microbial enzymes are there having wide variety of positive e ect, like laccase [8-10] most of the cases fungus are the major source of these enzyme but some bacteria have this kind of laccase production ability. is laccase is widely used in di erent aspects, not only for industrial development, it has lots of positive ability to make the environment green.

Materials and Methods

Sampling and positive isolates selection

Samples were collected from di erent regions of India like Kanpur, Kharagpur, Burdwan, Howrah, Pondicherry, Madurai, Meghamalai etc. Serially diluted soil samples added into potato dextrose broth in presence of 1% (w/v) benomyl 0.01% (w/v) chloramphenicol, 0.01% (w/v) tetracycline and kept it in room temperature for minimum seven days [11]. All the laccase producers are screened a er benomyl selection on the basis of laccase speci c substrates like guaiacol, tannic acid 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt(ABTS), Syringaldazine and - napthal etc [12-17]. Positivity of the isolates was determined from colour formation in presence of di erent substrates in both liquid, solid medium in presence of organism and using their crude extracts.

Growth determination

All the positive isolates growth kinetics carried out a er taking lyophilized dry biomass weight for the identi cation of fast grower among all positive isolates isolated from di erent parts of India [18,19]. In this comparison, study all positive isolates growth is very signi cant because enzyme production is directly proportional to the growth.

All the decolourization percent determined from the 15th day treated sample using this formula [22-25].

$$\text{Decolourization percentage (\%)} = \frac{\text{(Abs. Initial)-(Abs. Final)}}{\text{(Abs. Initial)}} \times 100$$

Abs. initial=absorbance on 1st day; Abs. nal=absorbance on 15th day

Enzyme activity determination

Crude extracts from all positive isolates was passed through Whatman No: 1 lter paper and 0.22 µm of syringe lter. Enzyme activity determined in presence of guaiacol using spectrophotometer at 470 nm [26] Lowry method followed for protein estimation [27] for enzyme activity determination the formula used [14,28,29].

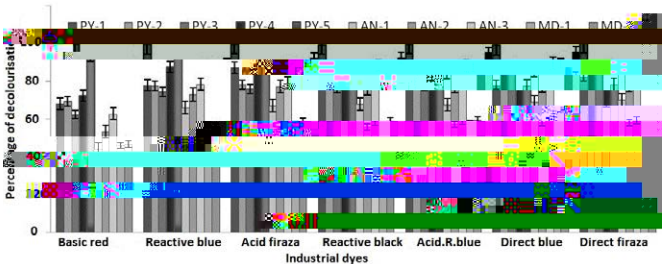

Figure 1: Percentage of decolourisation in different of different positive isolates against industrial dyes.

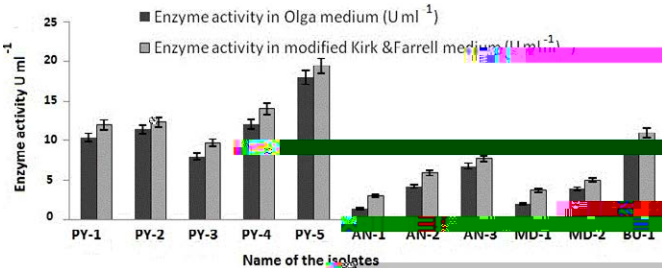

Figure 2: Lactase enzyme activities in different media of different positive isolates.

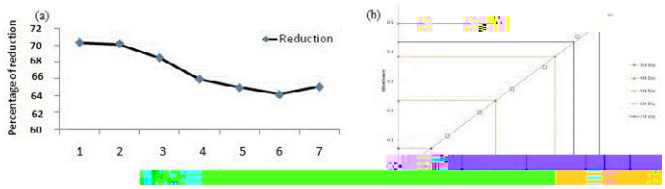

Figure 3(a&b): (a) Percentage difference in reduction, (b) cadmium removal.

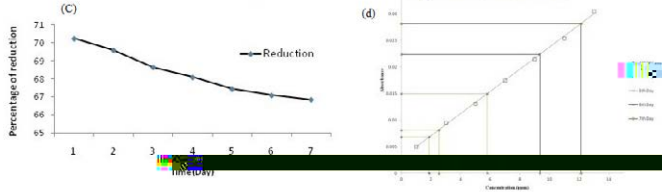

Figure 3(c&d): (c) Percent difference in reduction, (d) chromium removal.

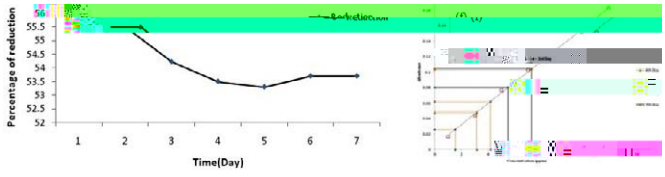

Figure 3(e&f): (e) Percent difference in reduction, (f) lead removal.

Decolourisation percentage (%)

Decolourisation percentage was calculated from eenth day treated sample and it shown maximum decolourisation, a er eenth day change in decolourisation percentage was almost constant. Fi eenth day decolourisation percentage data has given in Figure 1.

Enzyme activity

Enzyme activity of all positive isolates grown in some minimal salt medium like Olga and modi ed Kirk and Farrell medium was used to determined from the crude extract, in both medium strain PY-5 shown greater enzyme activity when compare to others isolates , also the modi ed Kirk and Farrell medium shown more enzyme production than Olga medium. e comparison of enzyme activity level has given in Figure 2.

Heavy metal removal and functional group activation

AAS data clearly reveals that the isolate PY-5 removes heavy metals more e ciently with respect to other positive isolates, a er seven day PY-5 removes 12.34 ppm, 9.4 ppm, 12.13 ppm, 7.23 ppm of cadmium, nickel, chromium, lead respectively. During measurement through AAS all the four heavy metal treated samples diluted four times and simultaneously the viability was determined in presence of alamar blue because viability is indirectly related with heavy metal removal by absorption and adsorption. Adsorption is common for dead and alive cells but absorption is possible through channel or carrier protein and it is possible when cells are alive. e percent di erence in reduction of alamar-blue in case of PY-5 presence of cadmium showed 65.10% of di erences in reductions so remaining 34.90% of cells are inhibited or dead. e heavy metal removal, percent di erence in reduction of alamar blue due to variable viability of the di erent positive isolates and their comparison has given in Figures 3a-3h respectively. Cadmium, chromium, nickel and lead treated biomass samples examined for the functional group activity determination with the help of FT-IR. From the FT-IR peak it is very clear that in presence of cadmium the hydroxyl group peaks became sharp to broad respect to control because of direc contact with the cadmium and hydroxyl groups from rst day onwards, also same kind of trend happened in presence of chromium but in presence of chromium the peak broadening in seventh day respect to rst day showed a signi cant peak broadening respect to control, it is

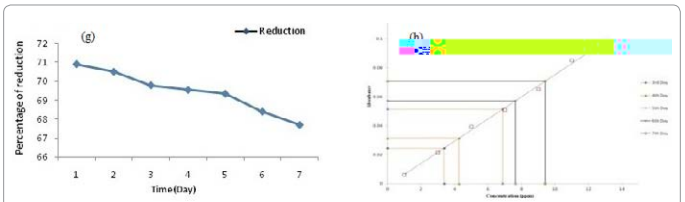

Figure 3(g&h): J 3HUFHQW GLIIHUHQFH LQ UHGXFWLRQ

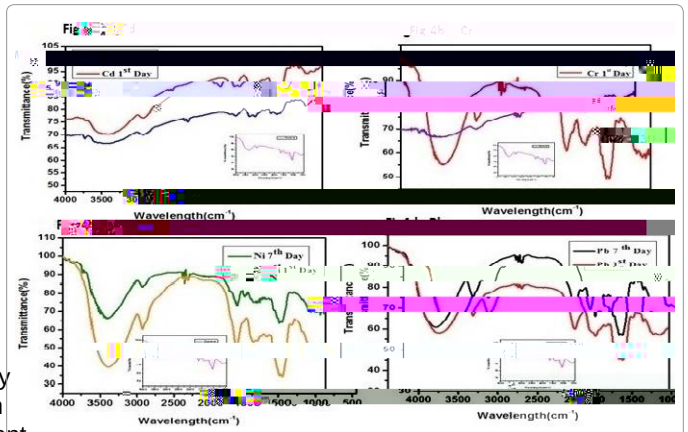

Figure 4(a-d):) 7 , 5 VSHFWUD RI KHDY\ PHWDO WUHD

Citation: Amutha C, Abhijit M (2015) Screening and Isolation of Laccase Producers, Determination of Optimal Condition for Growth, Laccase Production and Choose the Best Strain.

inter-related, decolourization depends on laccase activity, laccase production dirrectly proportional to growth and growth maximum means higher laccase production and enzyme activity will be more. In next study all the positive organisms grew in presence of heavy metals like Cr, Cd, Ni, Pb to detect their removal having wide range of toxicity and their toxicity is very common in all over India. From the comparative study , PY-5 showed removal (adsorption and absorption) of Cd, Ni, Cr, Pb are 12.34, 9.40, 12.13, 7.23 ppm respectively and these concentration will be four times more because during AAS study all the samples diluted four times,from this comparison and data, it showed that the removal of heavy metal in case of PY-5 is more than other positive strains and these amount of removal is more e ective for environment because those removal concentrations are higher than those speci c heavy metal's environmental toxicity level. Along with heavy metal removal the viability testing is more important because removal can be possible through absorption and adsorption,adsorption is common for both dead and alive cells but absorption possible when the cells are alive. In heavy metal removal study, viability is related to absorption , these study showed in presence of Cd the percent reduction change of alamar blue gradualy reducing and from seventh day it was stabilised and increased, it indicates that decreasing order of percent reduction change due to decreasing of cellular activity and it is indirrectly proportional to either cell death or inhibition of cellular function, from seventh day exposure percent reduction change increase means activity again increased and in presence of Cd a er some days of adaptation PY-5 survived and increased its growth. Same kind of incidents happened in case of lead, the percent reduction change from begining decreased but from seventh day onwards the percent reduction change became constant means a er some exposure this strain also adapted and survived, but in case of Ni and Cr ,decrease of the percent reduction change lowered from h day onwards, it

18. 0HL-)*0 0RXWRQ -: 9HUZHLM 3(0HOHWLDGLV - $QDO\VLV RI JURZWK FKDUDFWHULVWLFV RI ¿ODPHQWRXV IXQJL LQ GLIIHUHQW QXWULHQW PHGLD -RXUQDO RI Clinical Microbiology 39: 478-484.

19. 0HOJDU $VVLV)9' 5RFKD /&')DQWL 6& 6HWWH /' HW DO *URZWK FXUYHV RI ¿ODPHQWRXV IXQJL IRU XWLOL]DWLRQ LQ ELR FDWDO\WLF UHGXFWLRQ RI F\FORKH[DQRQHV *OREDO -RXUQDO IRU 6FLHQFH)URQWLHU 5HVHDUFK

20. Christiane A, Steeve M, JeanBosco ST, Kor NM, Brama I, et al. (2013) Biod HJUDGDWLRQ RI 5HDFWLYH %OXH Pycnoporus sanguineus 2UDQJH * E\ Strain Isolated in Gabon. Journal of Bioremediation and Biodegradation 4: 7.

21. 0DMROD 2JEHDJX 2 2ORNH - %RUXDK +' $GHWXQML & %RUGRORL $ ([WUDFWLRQ DQG SXUL¿FDWLRQ RI H[WUDFHOOXODU ODFFDVH IURP ZLOG PXWDQWV DQG K\EULG VWUDLQV of two white-rot fungus and its applications in decolourization and ligninolysis. -RXUQDO RI 0LFURELRORJ\ %LRWHFKQRORJ\ DQG)RRG VFLHQFH

22. $EDGXOOD (7]DQRY 7 &RVWD 5REUD 6 3DXOR .+ HW DO 'HFRORUL]DWLRQ DQG 'HWR[L¿FDWLRQ RI 7H[WLOH 'HV Trametes hirsuta Z LWK D /DFFDVH IURP Applied and Environmental Microbiology 66: 3357-3362.

23. .XPDU 9 9 .LUXSKD '6 3HUL\DUDPDQ 3 6LYDQHVDQ 6 6FUHHQLQJ and induction of laccase activity in fungal species and its application in dye GHFRORUL]DWLRQ $IULFDQ -RXUQDO RI 0LFURELRORJ\ 5HVHDUFK

24. 5DMHQGUD 6 .UUWKLN 6 6XQGDUDP 6. <DVRGKD . 8PDPDKHVZDUL .