Detection of phenol constituents employing bioconjugate of Tyr-AuNps mediated by novel isolate *Streptomyces tuirus* DBZ39.

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Abstract

In the present investigation we could report the significant role of bioconjugate of Streptomyces tyrosinase and gold nanoparticles for the rapid detection of phenol constituents in the industrial effluents. The phenol constituents in the effluents of wine, paper and plastic industries were detected by Streptomyces tyrosinase, mushroom tyrosinase and bioconjugate of Streptomyces tyrosinase-gold nanoparticles. Bioconjugate was proved as most efficient for the detection of phenol constituents with highest color intensity and optical density, than mushroom tyrosinase followed by Streptomyces tyrosinase. Streptomyces tyrosinase and gold nanoparticles as bioconjugate could exhibit a much better rapid detection of phenol constituents. Formulation and development of a bioconjugate with both tyrosinase and gold nanoparticles synthesized by the same strain of *Streptomyces tuirus* DBZ39 was an innovative criteria employed for the rapid detection of phenol constituents in the industrial effluents. The stability of bioconjugate of tyrosinase and gold nanoaprticles was found to be stable up to six weeks, when compared to mushroom tyrosinase and gold nanoparticles. The bioconjugate could show a higher rapid detection time and stability because of greater biocompatibility between tyrosinase and gold nanoparticles, in the process of detection of phenol constituents, as both of them have been obtained from the same source. This strategy would be used in future for the development of a powerful biosensor.

Keywords: Streptomyces, Tyrosinase-Gold Nanoparticles, , Phenol Detection

1. Introduction

Widespread contamination of water and soil by phenol has been recognized as an issue of growing importance in recent years, across the world and especially in India. Phenols are toxic contaminants in the wastewater of different industries such as plastics, resins, steels, dyes and organic chemicals [1]. Most of these phenolic compounds are toxic and have been classified as hazardous pollutants, which are included in the list of high priority pollutants by the US Environmental Protection

Agency (EPA) and several other countries [2,3]. Current methods used to remove phenolic compounds from waste water include microbial degradation, adsorption on activated carbon, chemical oxidation, incineration, solvent extraction method, etc [4], failed due to certain disadvantages, such as resistant to treatment and many of these compounds are toxic and are known to be carcinogenic [2]. For these reasons, more attention has been paid for the development of alternative method for detection of phenolic pollutants in water and soil [5]. Environmental sensors are gaining importance because of growing environmental concerns and their ability to provide rapid information on identification of contaminants or toxic compounds in water and soil samples [6]. Biological processes are gaining more importance over physicochemical process, as biological systems are more effective and the end products formed are nontoxic [7].

The streptomyces were well known for tyrosinase [8] and nanoparticles synthesis [9,10]. The stability of streptomyces tyrosinase was well studied in comparison with mushroom tyrosinase where resistant towards organic solvents had 44% and 6% respectively in the presence of 50% ethanol [8]. Nanoparticles offers unique approaches to control wide variety of biological and environmental process which have a successful impact on biology and environment [11,12] because of their novel properties. Novel and green approach method for the synthesis of gold nanoparticles was the use of microbial cell. Among microorganisms, actinomycetes are very potential for the synthesis of gold nanoparticles. Gold nanoparticles have attracted a significant interest because of their remarkable surface Plasmon resonance and non toxicity [13]. Gold nanoparticles have been used as a matrix for enzyme where the bioactivity of macromolecules is retained because of the gold surface permits absorption of protein molecules [12]. The interactions of gold nanoparticles with tyrosinase are useful for their employment as novel bioconjugate in the biocatalysis of phenol constituents. The stability of biosensor is usually considered as one of the key factor considerably hampering the practical applicability of biosensor. The operation stability of biosensor was tested by repetitive measurements during one month [14]. Mandal *et al.* [15] reported that amino acids bind effectively to gold nanoparticles through the amine groups have been used in the immobilization of proteins and enzymes on gold nanoparticles, as means of developing a simple biocatalyst with good reuse characteristics, pH, temperature and stability. Immobilization of enzymes directly on gold nanoparticles in solution yielded excellent catalytic activity of the enzymes and in many cases enhancement in the enzyme thermal stability as well.

Several reports are available for the detection of phenol constituents includes electrochemical method involving enzyme and nanoparticles from different sources [16]. According to the literature there are no reports available for the detection of phenol constituents employing bioconjugate considering the enzyme and nanoparticle synthesized from the same isolate of an organism. Therefore,

in the present investigation we report on the rapid detection of phenols from the bioconjugate of tyrosinase -gold nanoparticles synthesized by Streptomyces.

2. Materials and Methods

2.1.Bioprocess and synthesis. Tyrosinase, gold nanoparticles and bioconjugate of tyrosinase- gold nanoparticles for detection of phenol constituents were synthesized by following the standard procedures.

Mushroom Tyrosinase is procured from SIGMA (Sigma SLBB9478V) as standard. Extracellular Streptomyces tyrosinase [17,18] and gold nanoparticles [19-24] were synthesized by following the prescribed protocols. Bioconjugate was prepared employing two important functional components, tyrosinase and gold nanoparticles obtained from *Streptomyces tuirus* DBZ39 by following standard flocculation assay methods [25].

2.2.Detection of phenol constituents. The presence of phenol constituents in the effluents of wine, plastic and paper industries were detected employing tyrosinase, gold nanoparticles and bioconjugate of tyrosinase- gold nanoparticles independently, as per the standard procedures [26,27]. Catechol, pyrogallol and resorcinol were used as standard phenols along with mushroom tyrosinase for comparative account.

Industrial effluents were collected from Indian Ambinence Wineyards Pvt. Ltd. Humnabad, Mysore Paper Mill Ltd., Hallikhed and Mathapati Plastic industry, Gulbarga. 1 ml, 5 ml and 10 ml of effluent samples were treated independently with freshly prepared 0.5 ml of Streptomyces tyrosinase, gold nanoparticles, bioconjugate and commercially obtained mushroom tyrosinase. 1 ml sodium phosphate buffer was added to each of the treated sample. Then the treated samples were incubated at 40 ^{0}C for 2 h. The incubated samples were drawn at 5, 10, 15, 20, 25 and 30 min. and were centrifuged at 10000 rpm for 10 min. A mixture containing 200 µl of 4-AAP (4-Aminoanti pyridine Phosphate) (20.8 mM), 200 µl potassium ferricyanide (83.4 mM) and 750 µl sodium bicarbonate (0.25 M) was added to 50 µl of the supernatant. Visual observation and UV-vis absorption spectra, at 510 nm, of the supernatant of treated samples drawn at every 5 min were recorded. A standard curve of catechol, pyrogallol and resorcinol were prepared by box method to compare and determine the presence of phenol constituent in the effluent samples.

2.3. Stability of bioconjugate for the detection of phenol constituents. Stability of Streptomyces tyrosinase, gold nanoparticles and bioconjugate for the detection of phenol constituents from all the effluents was also carried out. The functional potential of tyrosinase, gold nanoparticles and bioconjugate were assessed from the day of their preparations, at every week for a period of six weeks, employing fresh

samples of effluents. The similar protocol as mentioned earlier for the detection of phenol constituents was followed. Visual observation and UV-vis absorption spectra of the treated samples were recorded at every week.

3. Results and discussion

3.1. Detection of phenol constituents. The detection of phenol constituents present in the effluents of three industries was detected by the bioconjugate of tyrosinase and gold nanaoparticles. Streptomyces tyrosinase, gold nanoparticles and commercial mushroom tyrosinase was examined independently to understand their ability to detect phenol constituents and compare with the bioconjugate. Wells of microtitre plate in 'C', 'Bc', 'St', 'Mt' and 'GNps' series indicates effluent + reagents (C) and effluent + reagents treated with Biocnjugate (Bc), Streptomyces tyrosinase (St), Mushroom tyrosinase (Mt) and gold nanoparticles (GNps) respectively. Change in color of the treated effluents of wine industry was visually observed and recorded (Figure 1) over a period of 30 min. The high intensity of the color was observed at 10 min, 15 min, 20 min and 5 min in the effluent treated with bioconjugate, Streptomyces tyrosinase, Mushroom tyrosinase and gold nanoparticles respectively. UV-vis absorption spectra of the treated effluent of wine industry over a period of 30 min were recorded at 510 nm (Figure 2) for the detection of phenol constituents. Maximum UV-vis absorption spectrum was recorded from the effluent treated with bioconjugate (at 10 min) followed by Streptomyces tyrosinase (at 15 min), Mushroom tyrosinase (at 20 min) and gold nanoparticles (5 min) respectively.

Change of color in the treated effluent of paper industry was recorded (Figure 3) over a period of 30 min. The maximum intensity of the color was recorded at 10 min, 20 min, 25 min and 10 min in the effluent treated with bioconjugate, Streptomyces tyrosinase, Mushroom tyrosinase and gold nanoparticles respectively. UV-vis absorption spectrum of the treated effluent of the paper industry was as recorded in Figure 4. Maximum UV-vis absorption spectrum was recorded from the effluent treated with bioconjugate (at 10 min) followed by Streptomyces tyrosinase (at 20 min), Mushroom tyrosinase (at 25 min) and gold nanoparticles (10 min) respectively. Similar observations were also recorded with plastic industrial effluent. Figure 5 presents the change of color and Figure 6 presents the UV-vis absorption spectra. The more intensity of the color was observed (Figure 5) at 20 min, 20 min, 25 min and 10 min in the effluent treated with bioconjugate, Streptomyces tyrosinase, Mushroom tyrosinase and gold nanoparticles respectively. Figure 6 illustrates the UV-vis absorption spectra of the treated effluent of plastic industry over a period of 30 min. Maximum UV-vis absorption spectra was recorded from the

effluent treated with gold nanoparticles (10 min), bioconjugate and Streptomyces tyrosinase (20 min) followed by Mushroom tyrosinase respectively.

The enormous complexity and diversity of life presents a great challenge to scientists attempting to reveal its chemical basis. Proteins and other biopolymer regulate and perform biological function by binding to ligands. Accordingly, discovering and characterizing the natural ligands of biopolymers is crucial in understanding biological processes [28]. This concept of advances in bioconjugation motivated the development of a suitable bioconjugates, aiming at the environmental applications. Gas Chromatography (GC), Flame ionization, Electron Capture detection (ECD) or Mass Spectroscopy Detection (MS), High Performance Liquid Chromatography (HPLC), Colorimetry and Capillary Electrophoresis (CE) in combination with ultra violet detection (UV) are not uncommon methods [29], based on physicochemical principles, followed for the detection of environmental pollutants and especially phenol constituents. Several biosensors, based on elcetrochemical principle, including particular functional components are the most advanced and attracting tools for the detection of variety pollutants. Specific biomolecule, especially enzymes are the critical components for the detection of analytes because of their catalytic specificity and accuracy.

The most important criteria used in the present investigation were the development of bioconjugate using tyrosinase and gold nanoparticles of microbial origin. Further, a novel approach of this investigation was the use of both functional components from a single source, i.e., *Streptomyces tuirus* DBZ39. Both the components obtained from a single organism would naturally reveal a greater biocompatibility for the development of an efficient bioconjugate. Earlier literature [30,31] reveals the use of enzymes and nanoparticles obtained from different sources, either chemical or biological.

In the present study, tyrosinase, gold nanoparticles and bioconjugate of both were examined independently to detect the phenol constituents from the effluents of wine, paper and plastic industries. Mushroom tyrosinase also was used for comparative account. A considerable difference in the detection of phenol constituents by all the four independently, in terms of UV-vis absorbance at 510 nm was recorded. It was interesting to note that change of color with higher intensity was achieved within 10 min indicating the detection of phenol constituents from the effluent of wine, paper and plastic industry. The intensity of color developed during the reaction period was reported [32] to be proportional to the quantity of phenol constituents present in the sample. Thus, it reveals that more quantity of phenol was present in effluent of wine industry followed by paper and plastic industries. Further, in-depth studies

are essential to understand the detection and determination of the exact concentration of phenol constituents present in the effluents.

3.2.Stability/ Efficacy of bioconjugate. Similar to the observations made earlier employing microtitre plate, the efficacy of bioconjugate for the detection of phenol constituents from the industrial effluents was examined over a period of six weeks. The efficacy in terms of stability of bioconjugate over a period of six weeks for the detection of phenol constituents from the effluent of wine industry is as presented in Figure 7. Based on the intensity of the color, the bioconjugate was found to be stable upto 5^{th} weeks followed by Streptomyces tyrosinase and Mushroom tyrosinase upto 4^{th} week and gold nanoparticles upto 2^{nd} week. Figure 8 illustrates UV-vis spectrophotometric analysis of stability pattern of bioconjugate and others over a period of six weeks for the detection of phenol constituents from the effluent of wine industry. Maximum absorption spectra of the effluent treated with bioconjugate was maintained upto 5 weeks followed by Streptomyces tyrosinase and Mushroom tyrosinase upto 4 weeks and gold nanoparticles for only 2 weeks. Figure 9 represents the stability pattern of bioconjugate and others over a period of six weeks for the detection of phenol constituents from the effluent of paper industry. The maximum stability based on the intensity of color was observed till 5 weeks in bioconjugate treated effluent followed by Streptomyces tyrosinase and mushroom tyrosinase treated effluent (4 weeks) and gold nanoparticles (2 weeks). The similar trend of the stability pattern of bioconjugate and others was analyzed (Figure 10) in terms of UV-vis absorption spectra. Bioconjugate found to be more stable (5 weeks) followed by Streptomyces tyrosinase and Mushroom tyrosinase (4 weeks) and gold nanoparticles (2 weeks) respectively. Figure 11 and Figure 12 illustrates stability pattern of bioconjugate and others in terms of intensity of color and UV-vis absorption spectra respectively. The trend of stability of bioconjugate and others treated plastic industrial effluent was similar to wine and paper industrial effluent. The stability pattern of bioconjugate and others in terms of intensity of color was not quite distinct. However, the stability pattern was quite distinct in terms of UV-vis absorption spectra over a period of six weeks.

Stability of biosensor, mainly involving the functional operation of bioconjugate is usually considered as one of the key factors, greatly hampering the practical applicability for the detection of environmental pollutants. This operational stability of the bioconjugate is under the influence of ambient conditions. However, longer sustainablilty of bioconjugate depends on the functional components of bioconjugate and their ability of interface and interactions. If, biocompitability of both functional components is greater, then the functional stability of the bioconjugate could be more. In this direction, a bioconjugate developed employing tyrosinase and gold nanoparticles obtained from *Streptomyces tuirus* DBZ39 was assessed for its efficacy over a period of time for the detection of phenol constituents

from the effluents of wine, paper and plastic industries. The efficacy of bioconjugate was found to be stable over a period of six weeks whereas, the efficacy of tyrosinase and gold nanoparticles were stable over a period of four weeks and two weeks respectively. There are several reports indicating the detection of phenol constituents by tyrosinase [14, 26, 27] and gold nanoparticles [32-34]. However, the efficacy of tyrosinase and gold nanoparticles enhances to a greater extent, only on combination and development of a bioconjugate of them. It is more interesting to note that the efficacy of the bioconjugate would be much greater, if both functional components are derived from a single organism. The present investigation discloses a rare physiological potential of the organism *Streptomyces tuirus* DBZ39 which was known to synthesize both tyrosinase and gold nanoparticles. This significant finding may be explored further for the detection of phenol constituents with a greater practical applicability.

4. CONCLUSIONS

Bioconjugate, tyrosinase and gold nanoparticles produced by Streptomyces were evaluated for the detection of phenol constituents from effluents of wine, paper and plastic industries. Commercially available mushroom tyrosinase was also used for a comparative account. . Gold nanoparticles, bioconjugate, Streptomyces tyrosinase and mushroom tyrosinase showed detection of phenol constituents, within 5, 10, 15 and 20 min respectively. However, with regard to stability, bioconjugate was found to be stable up to six weeks, when compared to Streptomyces tyrosinase, mushroom tyrosinase and gold nanoparticles which were stable up to five weeks, four weeks and two weeks respectively. Efficient detection of phenol constituents by the bioconjugate, with regard to detection time and stability, may be attributed to the biocompatibility of gold nanoparticles and tyrosinase, as both of them were synthesized by a single organism. Detection of phenol constituents by bioconjugate reveals higher concentration of phenols in the effluent of wine industry followed by paper and plastic industries.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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Figure 1- Change of color intensity over a period for the detection of phenol constituents in the effluent of wine industry

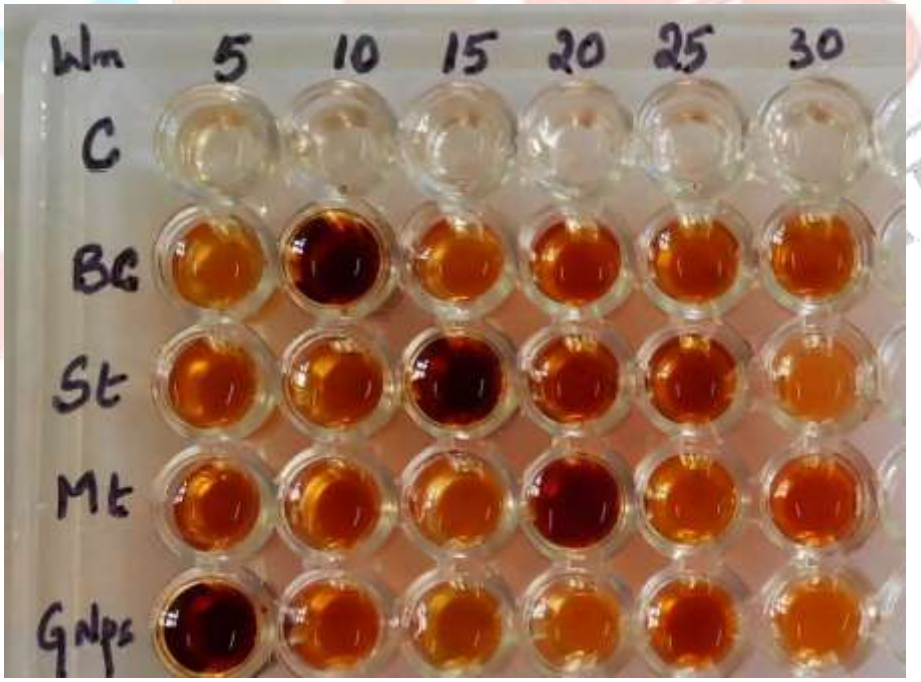

Figure 2- UV-vis absorption spectra of the treated effluent of wine industry over a period for the detection of phenol constituents

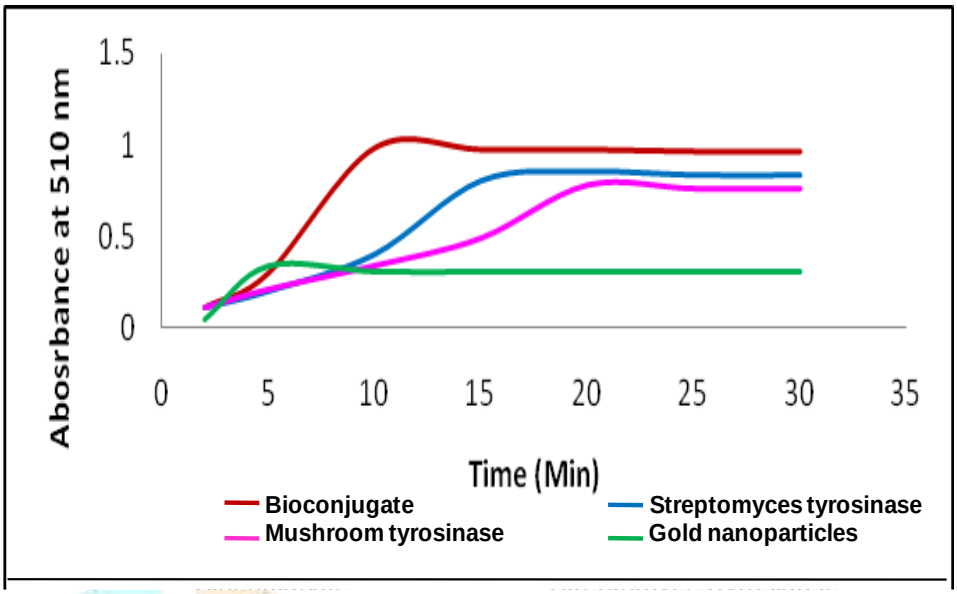

Figure 3- Change of color intensity over a period for the detection of phenol constituents in the effluent of paper industry

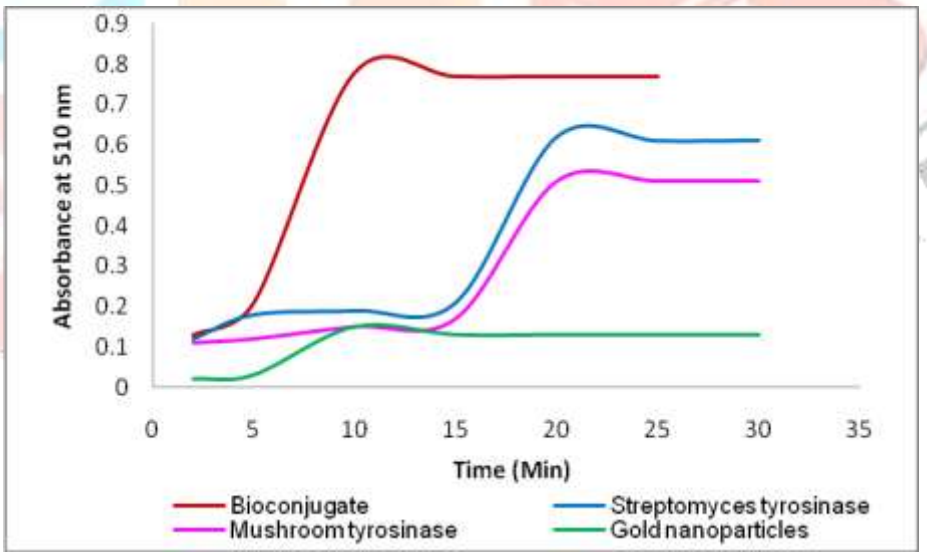

Figure 4 - UV-vis absorption spectra of the treated effluent of paper industry over a period for the detection of phenol constituents

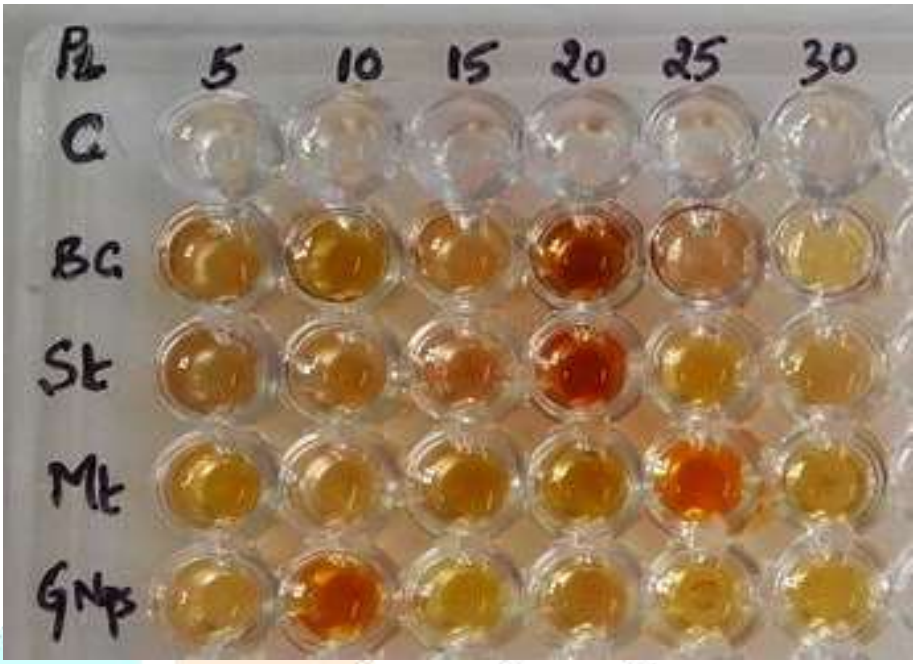

Figure 5 : Change of color intensity over a period for the detection of phenol constituents in the effluent of plastic industry

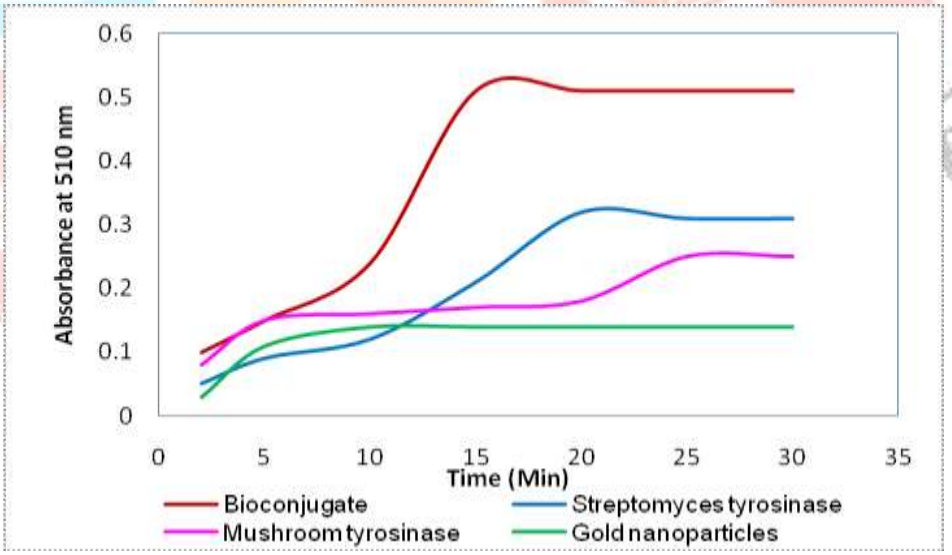

Figure 6: UV-vis absorption spectra of the treated effluent of plastic industry over a period for the detection of phenol constituents

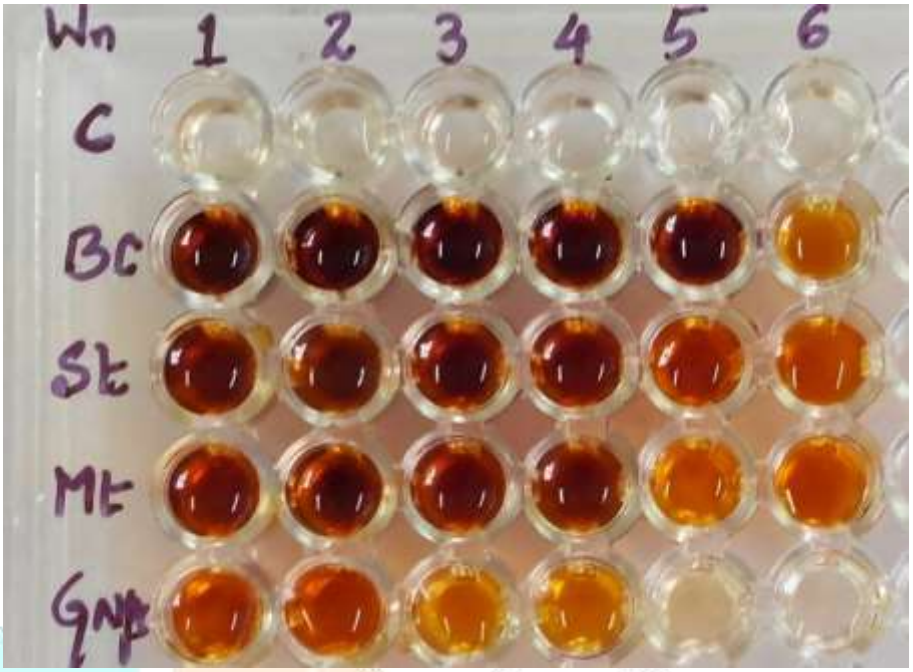

Figure 7: Stability pattern of bioconjugate over a period for the detection of phenol constituents from the effluent of wine industry

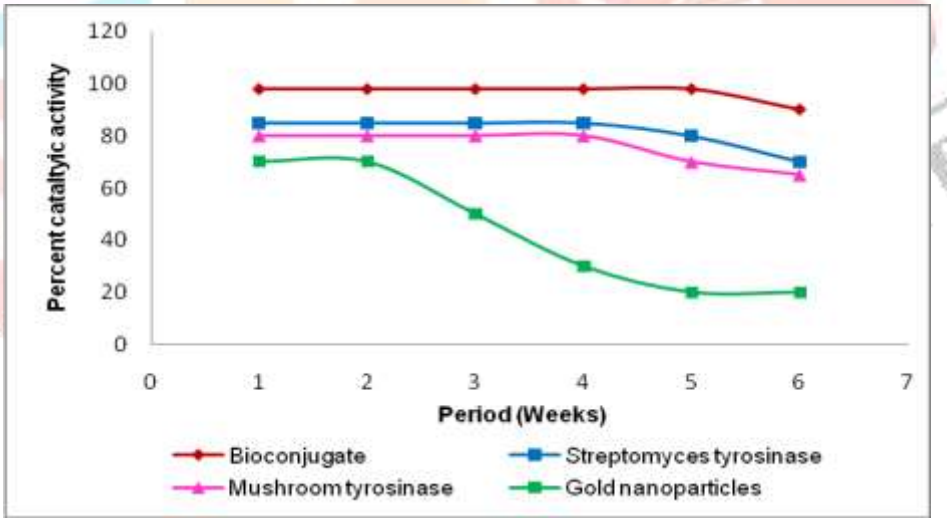

Figure 8: UV-vis spectrophotometric analysis of stability pattern of bioconjugate over a period for the detection of phenol constituents from the effluent of wine industry

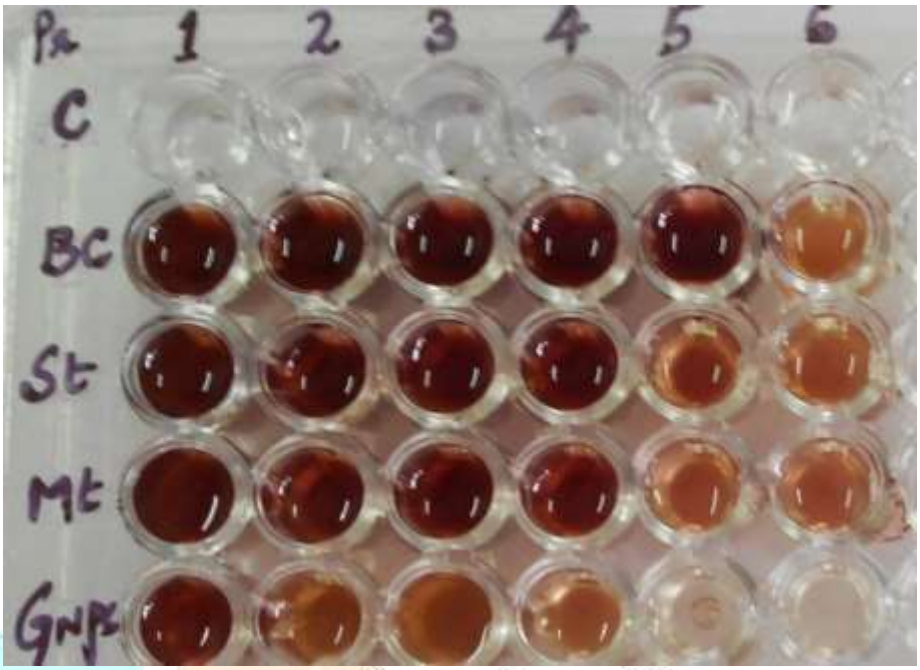

Figure 9: Stability pattern of bioconjugate over a period for the detection of phenol constituents from the effluent of paper industry

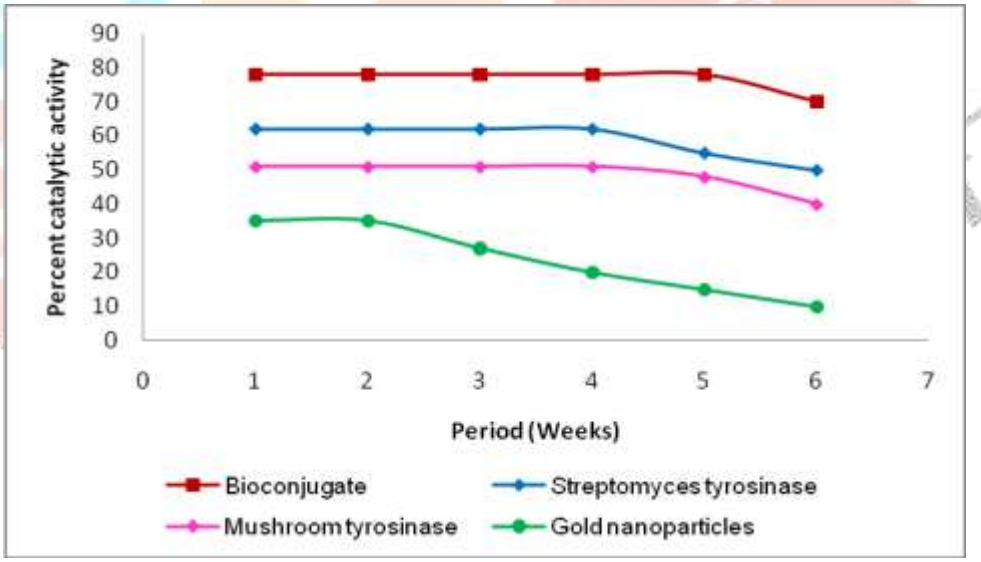

Figure 10: UV-vis spectrophotometric analysis of stability pattern of bioconjugate over a period for the detection of phenol constituents from the effluent of paper industry

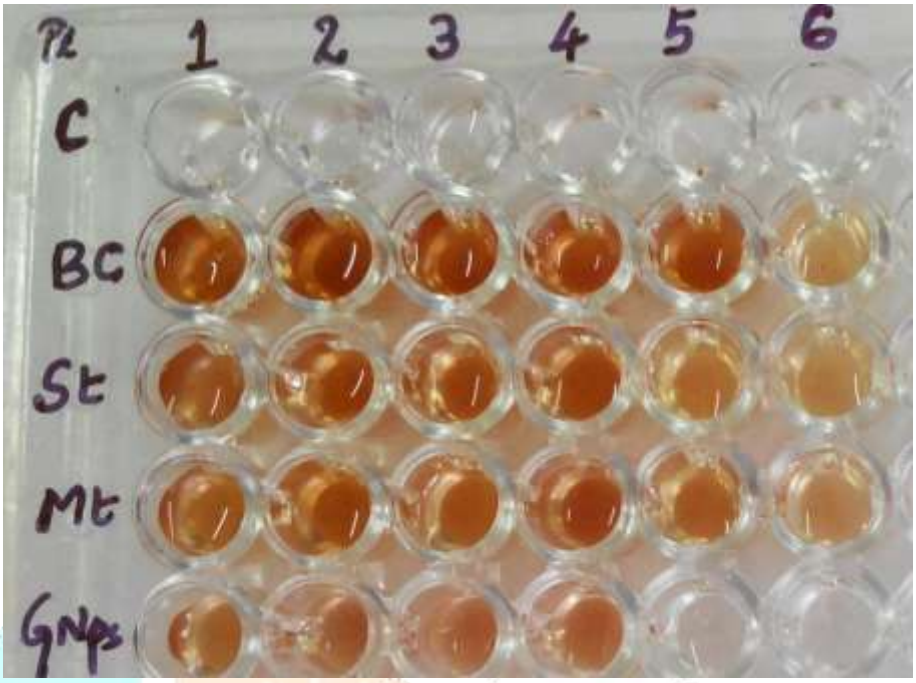

Figure 11: Stability pattern of bioconjugate over a period for the detection of phenol constituents from the effluent of plastic industry

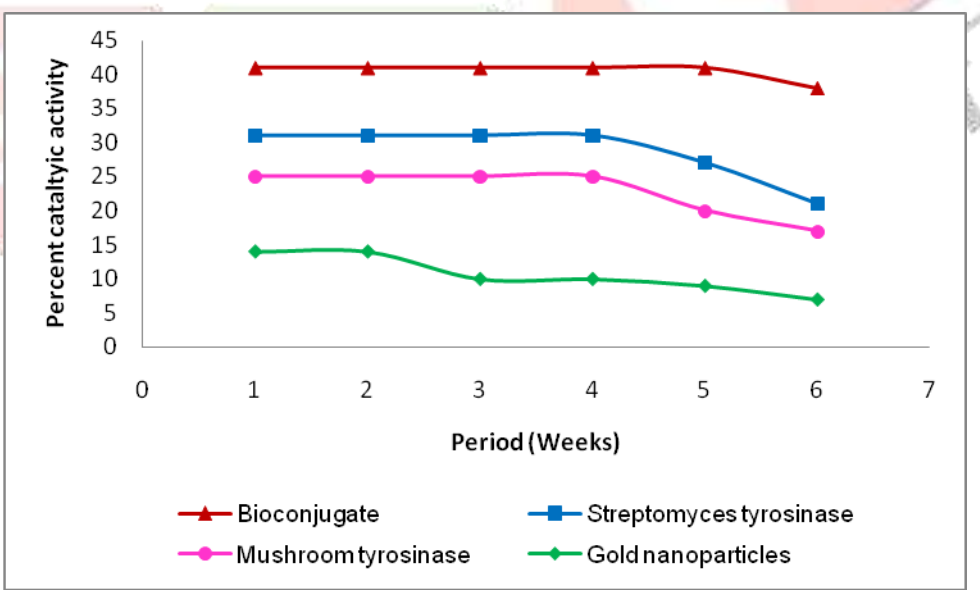

Figure 12: UV-vis spectrophotometric analysis of stability pattern of bioconjugate over a period for the detection of phenol constituents from the effluent of plastic industry