







Biotechnology in Textile Effluent Treatment (Part 2)

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Dyes have been applied to textile and other substrates for thousands of years, and dyers and their suppliers have continually sought to develop new processes and products that lead to better results or lower costs, in turn translating into commercial gain. Over the last few decades, the environmental impact of those products and processes has become an increasingly large part of the dyer's task. Given the growing emphasis on the environment, it is common to have almost any technical advance in the application of dyes, be it dye, auxiliary, or machine, claimed as environmentally beneficial, however spurious such a claim might be.

The terms pollution and contamination are sometimes used interchangeably in environmental matters to describe the introduction of a substance at a concentration sufficient to be offensive or harmful to human, animal or plant life. The word pollution is more strictly used to describe contamination caused or induced by human activities and is typically measured by reference to predetermined permissible or recommended tolerance limits.

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Anaerobic-aerobic treatment: Decoloration of azo dyes during biological effluent treatment can involve both adsorption to the cell biomass and degradation through azo bond reduction during anaerobic digestion. Generally, bacterial azo dye biodegradation proceeds in two stages. The first stage involves reductive cleavage of the dyes' azo



linkages, resulting in the formation of generally colorless but potentially hazardous aromatic amines. The second stage involves degradation of the aromatic amines. Azo dye reduction usually requires conditions. anaerobic whereas bacterial biodegradation of aromatic amines is an almost exclusively aerobic process (Fig. 3). A wastewater treatment process in which

anaerobic and aerobic conditions are combined is therefore the most logical concept for removing azo dyes from wastewater.

With the objective of complete mineralization, a study involving decoloration of CI Reactive Black 5, a disazo reactive dye using a sequential anaerobic -aerobic system has been conducted. In this experiment the use of partially granulated sludge culture with glucose as co-substrate was found to decolorise the dye up to 98% extend under



anaerobic condition. The released intermediate got minerilized into the aerobic stage as indicated by the lowering of COD. However no color removal was observed aerobically.

-CH-CH-OSO-Na

Figure 4: CI Reactive Black 5

In another study, combination of anaerobic and aerobic operation was done in a Sequencing Batch Reactor (SBR) for biological color removal from simulated textile effluents containing reactive, sulfonated, monoazo and diazo dyes, respectively, Remazol Brilliant Violet 5R and Remazol Black B. The Sequencing Batch Reactor (SBR) technology is well implemented for nitrogen and phosphorus removal, particularly for piggery wastewater treatment due to its good operational flexibility, simple running and compact layout. Different operational parameters were evaluated including the Sludge Retention Time (SRT) and the relative duration of the anaerobic and aerobic phases. Obtain result showed that, 90% of color removal for the violet dye in a 24-h cycle with a Sludge Retention Time (SRT) of 15 days and an aerated reaction phase of 10 h. For the black dye only 75% color removal was achieved with the same operational conditions and no improvement was observed with the increase of the SRT to 20 days. For the violet dye a reduction of the color removal values from 90 to 75% was observed with the increase of the aerated reaction phase from 10 to 12 h. However, this increase did not promote the aerobic biodegradation of the produced aromatic amines. When Abiotic tests were performed with sterilized SBR samples and no color removal was observed in cell-free supernatants. However color removal values of 30 and 12% were observed in the presence of sterilized cells and supernatants with violet and black dye, respectively and could be attributed to the presence of active reducing principles in the sterilized samples.

In another study, the effects of cycle time on the biodegradation of the azo dye remazol brilliant violet 5R (RBV-5R) were investigated in an anaerobic-aerobic sequencing batch reactor (SBR). System performance was determined by monitoring chemical oxygen demand (COD), color, anaerobic enzyme (azo reductase) and aerobic enzyme (catechol

2,3-dioxygenase), and aromatic amine concentration. SBR was operated in three different total cycle times (48 h, 24h and 12 h), fed with a synthetic textile wastewater. In this study, the anaerobic period of SBR was found to allow the reductive decolorization of azo dye and the



aerobic period was found to be effective on further COD removal after the anaerobic period. The percentage reductions in color by the anaerobic stage of the SBR were at 72%, 89% and 86% for the 24-h, 1 2-h and 6-h cycle times, respectively. These results suggest that decreasing anaerobic retention time does not adversely affect total dye removal performance; on the contrary, decreasing anaerobic retention time stimulates

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dye removal performance. It is also found that, Most of the COD removal occurred in the anaerobic stage of the reactor for the all operational conditions. In this study, it was found that very small portion of COD removal was achieved in aerobic cycle of the SBR. Total COD removal efficiencies were over 75% for all operational conditions and about 70% of the COD removal was achieved in the first 3h of anaerobic stages. During the decolorization of RBV-5R, two sulfonated aromatic amines (benzene based and naphthalene-based) were formed and detected by HPLC. Aerobic phases of SBR with total cycle times of 48 h, 24h and 12h were able to remove benzene based aromatic amines with removal efficiency of 64%, 92% and 89%, respectively. The results indicated that the best SBR performance in terms of color removal and aromatic amine degradation was achieved from total cycle timeof24 h.



Figure 4: Typical color (A) and COD (B) removal profiles obtained during 24-h cycles of SBR 1 fed with 100 mg l-1 Remazol Brilliant Violet 5R(*) and SBR 3, fed with 30 mg l-1 Remazol Black B(•). The SRT was 15 days and the aeration time was 10 h.







Figure 7: Residual RBV-5R concentration in SBRs with three different total cycle times



Enzymatic Processes

Biodegradation of organic compounds (and maintenance of life sustaining processes) are reliant upon enzymes. There are numerous enzyme systems in bacteria that perform highly specific reactions. These biological reactions hasten and regulate cellular activity such as' energetic and biosynthesis. Enzymes are proteins produced by living organisms that catalyze cellular reactions. These proteins exist within cellular cytoplasm, attached to cellular membranes, and attached to series of kinetic limitations referred to as enzyme inhibition. Extra cellular enzymes are also susceptible to proteases (protein-cleaving enzymes) that may be present in the environment.

Enzymes catalyze chemical reactions involving the substrate(s). In the case of a single substrate, the substrate binds with the enzyme active site, and an enzyme-substrate complex is formed. The substrate is transformed into one or more products, which are then released from the active site. The active site is now free to accept another substrate molecule. In enzymes with more than one substrate, these may bind in a particular order to the active site, before reacting together to produce products.

The concept of using isolated and partly purified enzyme preparations has several advantages over whole cell approaches. The expression of the enzymes involved in dye



degradation is not constant with time but dependent on the growth phase of the population when living organisms are used. This can be circumvented by using isolated enzymes. Instead of maintaining living cultures of micro-organisms at pollution. the site of the production, down streaming and preparation of stabilized biocatalysts or enzyme cocktails provided is off-site by

specialized production technologies. Enzymes are easier to handle than living organisms and can be regarded more as speciality chemicals. Enzymes can be produced on a large scale and may already be applied in crude form in order to keep the costs considerably low. For industrial applications, immobilization of enzymes allows the reuse of the enzyme and thus further reduces the cost for such a process.

Enzymatic treatment has been proposed by many researchers as a potential alternative to conventional methods. Firstly, enzymes are highly selective and can effectively treat even dilute wastes. Secondly, they are enzymes are highly selective and can effectively treat even dilute wastes and their cost could eventually be less than that of other methods if commercially available enzymes are produced in bulk quantities. Moreover, enzymes operate over a broad aromatic concentration range and require low retention times with respect to other treatment methods.



Enzyme-based methods have a minimal impact on ecosystems. They also present some other interesting properties as low energy requirements, easy process control and operation over a wide range of pH, temperature and ionic strength. Furthermore, enzymes are active in the presence of high concentrations of organic solvents in which hydrophobic molecules are soluble. In addition, enzyme-based treatments used alone could be sufficient when the enzymes transform toxic compounds to less harmful products. In this case, complete degradation of the contaminants is therefore not necessary.

Tailor-made enzymes can be optimized independently by exploring induction reagents or using genetic engineering methods. This results in specialized biocatalysts, which may be superior to their naturally evolved counterparts. Efficiency may be increased upon combination with suitable additives and stabilizers and their application in much higher concentrations and in clearly defined quantities is possible unlike with naturally grown systems, which are much more susceptible towards variations. Thus, constant performance may be easier achieved.

Since isolated enzymes are protein molecules, they do not metabolize dyes like living whole cell organisms do. They only catalyze a specific type of transformation. Mineralization of dyes can therefore never be achieved by only using enzymes. However, enzymatic modification of dyes may often be sufficient at a certain stage in the process. Decolorization may be readily achieved by enzymatic destruction of the chromophoric centre of the dye. Detoxification may already be achieved after enzyme treatment by the transformation of the functional group conferring toxicity.

The major potential for enzyme reactors lies in special treatments of specific partial process streams of relatively constant and known composition. In such cases, biological processes other than defined enzymatic systems may not be applicable at all. For example, the selective biological removal of hydrogen peroxide at high pHs and temperatures from partial process streams within the plant is only possible by using an immobilized catalase enzyme system specifically designed for this purpose. Such a reactor system has al ready been successfully tested in industry.

Although the enzymes used for dye remediation display broad substrate specificities and practically all the different structural 'patterns such as the triphenylmethane, anthraquinonoid, indigoid, and azodyes can be degraded, the molecular structure of the waste dyestuff plays a considerable role on the rate and extent of transformation. Dyes are generally designed to exhibit high stability. They must resist irradiation with UV light, they must survive numerous washing processes and, of course, they have to resist microbial attack while in use on a textile fabric.

Oxidative enzyme remediation: In general, there are two kinds of classes of oxidoreductases that are involved in dye degradation: electron transferring enzymes and hydroxygroup inserting enzymes. Peroxidases and laccases act via electron transfer and yield an oxidized dye species. They are the most important types of enzymes involved in enzymatic dye degradation. Both are secreted by lignolytic fungi.

The second type of oxido-reductive enzymes is the oxygenases. They insert hydroxygroups into a substrate. Depending on the number of hydroxyl groups transferred by the



enzymes they are classified as mono- or dioxygenases. Oxygenases rely on complicated organic cofactors such FAD(H), NAD(P)(H) or cytochrome P450. For efficient regeneration of the catalytic system, living organisms are needed and, thus they are not used for enzyme reactors as such. Oxygenases are not only present in whole cell systems and mixed cultures used for dye degradation, but are ubiquitously found within the cell walls of every living organism. They playa vital role in the breakdown of aromatic ring systems. Upon hydroxylation, the subsequent cleavage of the aromatic ring yields carboxylic acids, which are further metabolized.

Peroxidases (e.g. EC 1.11.1.9) are enzymes that catalyze the transfer of two electrons from a donor molecule to hydrogen peroxide or organic peroxides. The oxidized substrate may be a textile dye. They catalyze a variety of reactions but they all require the presence of peroxides such as hydrogen peroxide (H_2O_2) to activate them. Hydrogen peroxide first oxidizes the enzyme, which in turn oxidizes the substrate.

Peroxidases are a much more diverse group of enzymes than laccases and the structure of the electron donor may limit the choice of peroxidases. Most commonly, manganese peroxidases and lignin peroxidases from ligninolytic fungi are employed in the degradation of textile dyes. The presence of low molecular substances may enhance the performance of peroxidases as well. Thus, the addition of veratryl alcohol was shown to positively influence decolorization of azo and anthraquinone dyes catalyzed by lignin peroxidase. However, this effect may either be attributed to the protection of the enzyme against being inactivated by hydrogen peroxide or to the completion of the oxidationreduction cycle of the lignin peroxidase rather than to redoxmediation.

The catalytic cycle of peroxidases in presence of hydrogen peroxide is shown in Fig. (6). The heme group of the enzyme first reacts with one molecule of hydrogen peroxide to produce compound I. This compound is an oxoiron (IV)-porphyrin radical, or an oxoiron (IV)-protein radical, which is two oxidizing equivalents above the ferric state. During this reaction step, the hydrogen peroxide is reduced to water while the enzyme is oxidized. Compound I then oxidizes the reduced substrate (RH) to give a substrate radical (Re) and is reduced to compound II which contains an oxyferryl centre coordinated to a porphyrin. Both compounds I and II react with the same RH. Therefore, once the reaction between compound I and the RH starts, compound II is produced and simultaneously reacts with a second molecule of RH. As a result, compound II is reduced back to the ferric state, corresponding to the native form of the enzyme, with concomitant oxidation of a second molecule of substrate and production of water. Some hemoproteins such as cytochrome c (Cyt c) and haemoglobin (HMG) are also able to oxidize organic substrates in the presence of hydrogen peroxide, probably by following the same reaction mechanism.

Laccases (EC 1.10.3.2) are multicopper proteins belonging to the family of blue-oxidases enzymes, which can oxidize a variety of aromatic compounds with concomitant reduction of oxygen to water. These enzymes also have broad substrate specificity. Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) catalyze the removal of one hydrogen atom from the hydroxyl group of ortho- and parasubstituted mono- and polyphenolic substances and from aromatic amines by one-electron abstraction. Thereby, free radicals are formed which are capable of undergoing further degradation

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or coupling reactions, demethylation or quinone formation. In contrast to other types of enzymes, such as hydrolytic enzymes like cellulases or lipases, laccases exhibit very broad substrate specificities. By using additional low molecular compounds such as ABTS, HBT and TEMPO which act as redox mediators, polyoxometalates or osmiumbased redox polymers, their substrate specificity can further be expanded. Redox mediating compounds are also secreted by: lignolytic fungi in order to assist the extra cellular digestion process. Laccases contain active copper centers. Hence, traces of copper may be introduced into the effluent upon excessive addition of laccase.



Figure 9: Catalytic cycle of heme-containing peroxidases in the presence of hydrogen peroxide and halide ions

In a study perform on an immobilized laccase from Trametes modesta by Andreas Kandelbauer, Oliver Maute, Rudolf W. Kessler, Angelika Erlacher &Georg M. Gubitz, the decolorization of numerous dyes of different structural patterns were illustrated. An enzyme-reactor is constructed which allowing the online monitoring of both reactions in solution and on the carrier surface. The reactor set-up was able to efficiently degrade a number of commercial textile dyes, among them Indigo Carmine, the anthrachinoid dyes Lanaset Blue 2R and Terasil Pink 2GLA, and the azo dyes Diamond Black PV and Diamond Fast Brown. However, many industrially important dyes like various Lanasol and Dianix dyes were resistant to treatment with our enzyme reactor. Both adsorption to the carrier material and enzymatic oxidation contributed to dye decolorization by immobilized laccase. The better performance of the immobilized enzyme compared to the free laccase is attributed to the additional contribution of unspecific adsorption.

The control experiments with -aluminum oxide coated with enzymatically inactive protein showed that the dyes do not vary significantly in their adsorption behaviour.



Differences in overall decolorization thus are attributable to variations in substrate specificity of the enzyme. On the other hand, limited accessibility to the immobilized enzyme does not seem to playa role since decolorization by the free enzyme was never better than with the immobilized enzyme. Decolorization data for the immobilized and the free laccases are consistent for Lanaset Blue 2R, Diamond Fast Brown, Diamond Black PV, Indigo Carmine, Crystal Violet, Ponceau Red, and the hydroxy-substituted model azodyes and illustrate that decolorization is achieved via biochemical degradation. However, degradation experiments with free laccase showed also that color removal of dyes such as Terasil Pink 2GLA, Tartrazine, or Solophenyl Blue GL was predominantly due to unspecific adsorption onto the carrier. The complete decolorization of Lanasol Yellow 4G wasb solely caused by adsorption; no biodegradation with free laccase enzyme occurred at all.



Figure 10: Structures of the model dyes used in this study

With the immobilized laccase, for neither the model azodyes nor the commercial dyes could distinct structural requirements for decolorization be identified. However, kinetic measurements of the decolorization of model azodyes with the free laccase showed that electron donating methyl groups facilitated oxidation of the parent molecule. The difference in reactivity between methyl and hydroxyl substituted model azodyes may be due to the general affinity of laccases towards phenolic compounds. The redox potential of the model azodye may be favourably affected by the presence of hydroxy groups. Other electronegative substituents in the same positions of the benzene ring like chloro or fluoro did not significantly support laccase catalyzed oxidation of the molecule; in the case of the nitro group, decolorization was not observed at all. Although pronounced substrate specificities were observed, the chosen structural motif of the model dyes used in this study seems to be no preferred substrate of the Trametes modesta laccase, as evident from comparison with the decolorization rates of equivalent amounts of Diamond Black PV, Diamond Fast Brown, and Indigo Carmine with the free laccase. With these commercial dyes the higher extent of overall decolorization by the immobilized enzyme is in agreement with their high reaction rate when subjected to free laccase.

In a study performed on laccase from the fungus Rhizoctonia praticola, Bollag et al. demonstrated the ability of the enzyme to detoxify some of the phenolic compounds tested. Detoxification of a particular phenol appeared to be dependent on the ability of



the enzyme to transform the compound, as demonstrated by the disappearance of the parent phenol. However, the reaction products were not identified. Bollag et al. concluded that the ability of laccase to detoxify a solution containing phenols appears to be function of the particular compound being treated, the source of the enzyme and other environmental factors.

In another study, an enzymatic cocktail made of laccase plus additional peroxidases with corresponding co-factors such as hydrogen peroxide and a redox mediator was used for the decolorization of process wastewater, containing mainly three reactive azo dyes, from a textile dyeing and printing company. Result shows that under optimal conditions, the extent of decolorization was 91, 78 and 17%, for the Reactive Black 5, Reactive Red 158 and Reactive Yellow 27, respectively. The pilot scale reactor was installed at the outlet prior to confluence with the printing process wastewater, thereby making it possible to decolorize about 85% of the total volume of water discharged. Although the pH was well adjusted for optimal enzymatic performance, the temperature fluctuated somewhat with a mean of 42.1°C and standard deviation of ± 5.91 °C. Even within the optimal temperature range (about 45°C), significant variation was seen in the extent of color removal. This reflects the complexity of the industrial environment in which production routines vary considerably as do the concentration of auxiliary compounds. Nevertheless, to the best of our knowledge, this is the first report of an enzymatic process for the decolorization of textile wastewater implemented in an industrial environment. Furthermore, technical parameters relating to washing fastness and the color difference show the potential of the bio-process for reuse of textile wastewater. Although the variability in the extent of decolorization cannot be overlooked, on average the decolorization achieved was within the consent limits established by legislation. Due to the enormous diversity of dyes currently in use, it is unlikely that a single universal solution will be found for wastewater decolorization. In any case, the new possibilities that have been developed for enzyme overproduction suggest that enzymatic decolorization may be a viable option. The overall benefits of the process may include not only a reduction in water costs but also energy savings and reduction in sludge volume, among others, all of which will contribute toward the development of a sustainable textile dyeing process.

The major advantage in using laccases lies in that they just require molecular oxygen as a co-substrate. Such treatment systems therefore only require sufficient aeration of the system and are therefore relatively simple. Peroxidases require hydrogen peroxide as a co-substrate in order to oxidize the dye molecules and catalyze degradation. Thus, here additional chemical load is required in order to gain catalytic transformation. Consequently, in the past decade, the focus on designing enzyme reactors for dye decolorization has been on laccase systems. Laccases decolorize a wide range of industrial dyes.

One major problem with enzyme reactors is currently their limited lifetime under harsh conditions. Especially when real-life effluent streams are approached, enzyme reactors seem currently no useful solution for end of pipe operations. Here, whole cell populations with mixed cultures will always perform much more reliably.



The lifetime and resistance of enzyme reactors can possibly significantly be enhanced by applying enzymes from other sources. Currently, practically all laccases used for dye remediation are derived from white-rot fungi. Despite promising efficiency in dye decolorization in general, with these enzymes, one is typically restricted to reaction conditions which correspond to the natural environment of the fungi in which they are formed. Thus, no decolorization activities at all are observed at values higher than 7 and temperatures well above 45°C.

Reductive enzymes: Unlike the oxidative enzymes laccase and peroxidase, the application of reductases or oxidases requires cofactors like Nicotinamide adenine dinucleotide NAD(H), Nicotinamide adenine dinucleotide phosphate NADP(H), or FAD(H), which are extremely expensive compounds. Enzyme remediation systems based upon such enzymes is therefore economically not feasible and industrially very difficult to implement. Most decolorizations in connection with reductive enzymes usually take place in whole cell applications. Bacterial degradation of azo dyes may be attributed to unspecific reduction of dyes or the action of azo reductase activity.

Effluent treatment using fungi

The employment of microorganisms to decon-taminate polluted places by bioremediation has increased in the last years. Fungi from the basidiomycete group, known as wood white-rot fungi, have been indicated as capable of degrading several pollutants of diverse structures by a complex ligninolytic enzymatic system. Several dyes have been studied and the ability of white-rot fungi to decolorize them indicates that these organisms can be used for waste water treatment in dye-employing industries. Doralice S.L. Balan et. al attempt for decoloration of indigo dye by ligninolytic basidiomycete fungi. It was found that decolorization started in a few hours and after 4 days the removal of dye by Phellinus gilvus culture was in 100%, by Pleurotus sajor-caju 94%, by Pycnoporus sanguineus 91 % and by Phanerochaete chrysosporium 75%. In another study, I. Nilsson and his co-worker decolorized Reactive Blue 4 (a blue anthraquinone dye) or Reactive Red 2 (a red azo dye) using batch and continuous reactors inoculated with white-rot fungi and it was found 70% decoloration was possible by this system.



Figure 11: The bottle to the left contains sample of untreated textile wastewater while the bottle to the right contains a sample of the outlet from the reactor inoculated with P.Flabellatus



Future trends

Although enzyme remediation of dyestuff successfully removes its colour, a potentially harmful organic load may remain in the process waters. Thus, an interesting future perspective in the application of laccases for the treatment of process waters containing waste dyestuffs is the coupling of phenolic dye fragments rather than their oxidative breakdown. If such polymerized fragments were of sufficiently increased molecular weight, they could readily be removed by a subsequent filtration step (laccase-assisted dye precipitation). Consequently, future research activities should focus on optimization of the reaction conditions in order to achieve maximum oxidative coupling of primarily formed dye fragments.

Exploring the potential of different or newly discovered organisms such as various species of extremophiles or bacteria will lead to more powerful organismic and enzymatic treatment systems. Genetic methods will be of increasing interest in designing and tailoring new super enzymes with expanded potential not only in effluent treatment but in all fields of industrial microbiology. They will also help to optimize fermentation technology in order to further reduce the costs and increase availability of enzymes.

Application of the present knowledge about the relationships between the chemical structure and its influence on biodegradability could possibly lead to the design of novel dyes that are perfectly stable while in use on the textile but that provide sufficient affinity towards bioremediation systems to be completely biodegradable.

Intelligent combination of different treatment techniques will provide more powerful integrated tools. For instance, the adsorption of dyestuff on living material paired with subsequent enzymatic degradation is not new, but is a very good illustration of the concept for using the advantages and avoiding the disadvantages of two different methods. Another example is the combination of laccase systems with ultrasound in order to enhance the biodegradation process. The use of peroxidases depends on the availability of hydrogen peroxide as a second substrate. Here, a combination of enzyme remediation with conventional chemical regeneration processes relying on a combination of UV and hydrogen peroxide could prove beneficial in the future. In both cases, the enzymes have to be protected against too high an energy input since they easily may be destroyed. Therefore, sequential application of the different techniques may provide an industrially feasible solution. Physicochemical pre-treatment of waste loads in order to facilitate subsequent biological treatments is presently well established.

Conclusion

In seeking to achieve environmental responsibility in dye application there is no single solution since there is no single definition of what is "green", or environmentally responsible. Even in a rare case where a dyeing operation is planned from first principles with environmental responsibility as a main goal, the best approach might be widely debated. More realistically, existing operations can be made 'greener' in many ways, with the different approaches each tending to answer a particular perceived impact. Local circumstances will often dictate which path is the preferred one.



Biological decolorization of wastewater is a very active area of investigation and research in these areas continues to be active and innovative. In comparison to the chemical treatment, biological processes avoid consumption of high quantities of additional chemicals and energy, it has the ability to produce less sludge & it is cost effectiveness. Biological treatment either aerobic or anaerobic, is generally consider to be the most effective means of removing wastes from wastewater enriched in organic constituents.

A large number of enzymes from a variety of deferent plants and microorganisms have been reported to play an important role in an array of waste treatment applications. Enzymes can act on specific recalcitrant pollutants to remove them by precipitation or transformation to other innocuous products. They also can change the characteristics of a given waste to render it more amenable to treatment or aid in bioconverting waste material to value-added products. Enzymes seem to have a promising future. However, before the full potential of enzymes is realized, a number of issues still must be addressed including identification and characterization of reaction by-products, disposal of reaction residues and reduction of enzymatic treatment costs. Also, further research is needed to determine which enzyme is best suited in a particular situation and to optimize the enzymatic process as a whole.

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