Microbial Bioremediation & Biodegradation
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The scientific interest in the fate of environmental pollutants generated by industrial and urban activities does not only refer to the search for ways to favor mitigation or, ideally, the complete elimination of the affected sites. It also provides information on how new environmental microorganisms evolve for first molecular devices to tolerate and then catabolize many of these toxic molecules. Along with resistance to antibiotics, the emergence of new biodegradative routes for new compounds is one of the most outstanding cases of contemporary biological evolution in real time. Understanding the rules of this evolution thus provides new principles for predicting and, if accelerated, biochemical adaptation to the new chemical structures. These phenomena occur in space and time and also at very different scales depending on the nature and size of the pollutants at stake. The impact of pollutants that received considerable attention decades ago is diminishing in many cases due to better industrial procedures along with environmental awareness and growing legal regulations. Unfortunately, the last decade has witnessed the appearance of other types of pollutants (particularly greenhouse gases, plastics, and micropollutants) that threaten not only specific sites but also the functioning of the environment. This state of affairs calls for new bioremediation strategies that take into account the multinational complexity involved in possible interventions far beyond the focus on specific biodegradation pathways. Fortunately, the environmental microbiome and the possibilities to engage it with the tools of Systems and Synthetic Biology are the best resource to face the phenomenal challenge of preserving the biosphere in a good way for future generations. The growing industrialization and urbanization of our societies over the last century has left us a heritage of emissions that, whether they are natural or synthetic molecules, have had an impact on virtually all Earth’s ecosystems. This combination of circumstances has paved the way for the science of biodegradation (that is, understanding—and ultimately refactoring—how microorganisms catabolize otherwise unpleasant environmental chemicals) and the technology of bioremediation (using biological agents to eliminate or at least mitigate pollution at given sites).

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About the Editor

Maulin P. Shah received his Ph.D. (2002–2005) in Environmental Microbiology from Sardar Patel University, Vallabh Vidyanagar, Gujarat. He has served as an Assistant Professor at Godhra, Gujarat University, in 2001. He has edited 25 books in the area of wastewater microbiology.
Abstract
Pulp and papermaking industry is a large consumer of fresh water and also an important source of dark-brown-colored wastewater, generated during various stages of pulping and papermaking activities. The colored wastewater discharged from pulp and paper industry even after secondary treatment remains toxic and complex in nature and retains high amount of lignin, lignin residues, resins, acids, chlorinated phenols, and various persistent organic pollutants (POPs) including the adsorbable organic halides (AOXs; halogenated or organochlorine). The existing various conventional methods along with integrated processes (aerated lagoons and activated sludge plants) cannot efficiently treat pulp and paper industry wastewater due to its complex and recalcitrant nature. Hence, the discharged partially treated/or untreated wastewater are contributing to deteriorating water quality due to increasing biological oxygen demand and chemical oxygen demand and decrease of dissolved oxygen.

In a terrestrial ecosystem, the wastewater irrigated soil showed decrease of moisture content and increase of pH and toxic heavy metals content. To tackle this problem associated with hazardous waste disposal, the existing pulp and paper industry wastewater treatment process needs to be improved with better treatment outcomes. Although, several physicochemical methods are available for the treatment of such wastewater, they are more energy intensive and suffer from residual effect. In addition, they are very expensive, inefficient, and produce a huge amount of toxic sludge which is difficult to handle and also produces volatile organic compounds on burning. To combat these challenges, biological approaches are necessary.
treatment using bacteria, fungi, yeasts, and algae has evolved as a preferred means to treat and reduce the toxic organic compounds loaded in generated pulp and paper industry wastewater.

Keywords
- Adsorbable organic halides
- Persistent organic pollutants
- Phytoremediation
- Chlorinated lignin
- Bleaching

1.1 Introduction

Pulp and paper industry are among the most important industries in the world not only for an economical purpose but also for a social purpose. Besides, it is also one of the major polluting industry discharging a variety of organic and inorganic pollutants such as gaseous, liquid, and solid into the environment (Ali and Sreekrishnan 2001; Lacorte et al. 2003; Singh and Chandra 2019). Consider that manufacture of paper consumes significant quantities of wastewater, as high as 200–350 m³ tonne⁻¹ of paper produced, of which nearly 75% is discharged as wastewater (Nagarathnamma et al. 1999). Currently, there are 759 paper mills operating in India, out of which 30 are wood-based large-scale mills, 150 are agro-based medium-scale mills, and 579 are recycled fiber-based medium and small-scale mills, producing 3.40, 2.42, and 5.10 Mtpa paper, respectively (Rajwar et al. 2017). As per the Ministry of Environment, Forest and Climate Change (MOEF&CC), Government of India, the pulp and paper sector is in the Red Category list of 17 industries having a high pollution potential owing to its serious environmental threat. Most significant sources of pollutants in pulp and paper mills are pulping of raw materials (wood chips), pulp bleaching, and paper coating processes (Bajpai et al. 1993; Nagarathnamma et al. 1999; Yadav et al. 2010; Rocha-Santos et al. 2010; Singh and Chandra 2019). Pulping process results in dissolved forms of lignin and other wood components called black liquor (BL) due to its black color, whereas bleaching produces the mono-aromatic compounds like chlorophenols, catechols, and guaiacols and numerous high-molecular-weight organic compounds like phenols, chlorolignins, chlorophenols, adsorbable organic halides (AOXs), extractable organic halides (EOXs), and plasticizers (Mishra and Thakur 2010; Mishra et al. 2014; Chandra et al. 2011a, b). Besides, bleached effluent is heavily loaded with organic matter, having high suspended solids (SS), color, biological oxygen demand (BOD), chemical oxygen demand (COD), total organic chlorides (TOC), chlorinated resin acids, phenols, dioxins, and furans (Larsson et al. 1988; van Driessel and Christov 2001; Leadbitter 2009; Yadav et al. 2010; Malaviya and Rathore 2007). These parameters have discharge limits, laid down by various environmental regulatory authorities around the globe. The high values of COD in wastewater also indicate the recalcitrance of chemicals that have escaped biodegradation processes (Mahesh et al. 2006; Gommers et al. 2007; Chen et al. 2012a, b, c). These chemicals may be persistent in nature and may cause several problems to animals, plants,
microorganisms and human health (Singh and Chandra 2019). According to the United States Environmental Protection Agency (USEPA), 27% (wt %) of municipal solid waste is composed of paper waste, and about 100 million kg of toxic pollutants are released every year from the paper industries.

Some large-scale pulp and paper mills have recovery boilers to burn much of the BL they produce, generating steam and recovering the cooking chemicals, viz., sodium hydroxide (NaOH) and sodium sulfide (Na₂S₂) which are used to separate lignin from the cellulose fibers of wood chips needed for papermaking (Pokhrel and Viraraghavan 2004; Mishra and Thakur 2010). This chemical reaction and burning of organic materials release a considerable amount of heat energy which is recovered by transferring it through water-filled tubes in walls of the recovery boiler. However, they are inefficient, costly, and produce a huge amount of toxic sludge which is difficult to handle (Thompson et al. 2001; Mishra and Thakur 2010; Qadir and Chhipa 2015). On burning, volatile organic compounds (VOCs) like dioxins and furans are formed which are more toxic than the parental compounds. Small-scale pulp and paper mills often lack such installations due to their high operational costs and do not have satisfactory and adequate wastewater treatment facilities; as a result, unrecovered wastewater amplify the pollutant toxicity and are a cause of serious environmental concern (Oke et al. 2017; Singh and Thakur 2006; Medhi et al. 2011; Ojunga et al. 2010; Tyor et al. 2012). In aquatic system, it blocks the photosynthesis reaction processes and decreases the dissolved oxygen (DO) level which adversely affects the flora and fauna and causes toxicity to aquatic ecosystem (Poole et al. 1977; Leadbitter 2009; Hou et al. 2018), whereas in the contaminated soil, it showed the accumulation of toxic recalcitrant organic pollutants and heavy metals (Kumar and Chopra 2011; Pradhan and Behera 2011; Roy et al. 2008; Medhi et al. 2011). Several pollutants that discharged in pulp and paper mill are also reported as carcinogenic, mutagenic, clastogenic, and endocrine-disrupting in nature (Haq et al. 2017; Mishra et al. 2014). Therefore, it is mandatory for pulp and paper mills to comply with the appropriate standards set by Central Pollution Control Board (CPCB), New Delhi, India. However, potential advanced processes that are used for wastewater treatment discharged from pulp and paper industry includes chemical coagulation, flocculation, precipitation, ion exchange, advanced oxidation processes, ozone treatment, electrochemical degradation, membrane processes (especially reverse osmosis, nanofiltration, and ultrafiltration), photocatalytic degradation, and adsorption on activated carbon, as a means of removing color and turbidity from wastewater (Subramonian et al. 2017; Gonder et al. 2012; Birjandi et al. 2016; Mahesh et al. 2016; Yeber et al. 1999; Stephenson and Duff 1996; Pihlajamäki and Nyström 2002; Mahesh et al. 2006; Rodrigues et al. 2008).

However, these treatment approaches offer an economic nonviability, limited versatility, operational constraints, partial treatment, and plausible formation of secondary hazardous by-products and also generate a huge amounts of toxic sludge that limit their industrial applicability (Pokhrel and Viraraghavan 2004; Thompson et al. 2001; Zhang et al. 2009). Researchers across the globe have tried to devise innovative methods for achieving maximum reduction in the color, BOD, and COD loadings of pulp and paper mill wastewater (Gommers et al. 2007; Singh and Thakur
The conventional biological treatment methods, such as activated sludge (AS) and aerated lagoons (extended aeration methods), are ineffective in removing color and phenolics and also do not decolorize wastewater very effectively (Lerner et al. 2007; Qadir and Chhipa 2015; Erkan and Engin 2017). However, certain advanced biotechnological treatment methods, such as biodegradation using potent microorganisms, can prove to be effective for further treatment of toxic organic pollutants and decolorization of such wastewater compared to chemical treatment and conventional aerobic–anaerobic treatment, as lesser sludge would be produced, with an additional low-cost benefit (Ragunathan and Swaminathan 2004; Abira et al. 2005; Dias et al. 2005; Chandra and Singh 2012; Chandra and Kumar 2015b, 2017b). The use of microbes for biodegradation of refractory organic compounds is an efficient, relatively cost-effective, and environment-friendly tool for the treatment of industrial wastewater (Kumar et al. 2018; Kumar and Chandra 2018a, b). However, biotechnological methods using fungi, bacteria, and actinomycetes are less effective for complete decolorization and detoxification of pulp and paper industry wastewater (Latorre et al. 2007; Raj et al. 2005; Raj et al. 2014a, b; Singhal and Thakur 2009a, b). Although a plethora of information is available on biological treatment methods for BL, there is an acute shortage of efforts to make the process being implemented effective on a large scale application.

1.2 Pulp and Paper Industry Wastewater Generation and its Characteristics

Paper manufacturing process involves three steps: pulping (also called delignification), bleaching, and finally papermaking. The purpose of pulping is to extract cellulosic content from plant materials obtained from hardwood or softwood trees. Generally, three approaches like mechanical pulping, chemical pulping, and a combination of both mechanical and chemical pulping are known to produce pulp from wood (Sandstrom et al. 1988; Esposito et al. 1991; Martin and Manzanares 1994; Thompson et al. 2001). However, the main drawback of mechanical pulping is yielded low-quality pulps, unsuitable for high-strength fiber products, and high energy requirements (Stephenson and Duff 1996). Mechanical pulping causes less pollution than chemical pulping. The most important delignification (chemical pulping) processes are kraft, sulfite, and soda pulping (Abdelaziz et al. 2016; Becker and Wittmann 2019; Wong 2009). Kraft pulping is a process in which wood chips are cooked in a large pressure vessel called a digester at 155–175 °C in an aqueous solution NaOH and Na2S2, also known as white liquor, to dissolve lignin from cellulose and hemicellulose fibers of the wood chips. The thus formed hydroxide (OH-) and hydrosulfide (HS-) anions crack the aromatic ether bonds within the lignin structure and release low-molecular-weight thiolignin oligomers (Abdelaziz et al. 2016). Sulfite pulping is a process of cooking of wood chips at 140–170 °C in alkaline, a pH neutral or an acidic environment, depending on the added sulfite salt (Abdelaziz et al. 2016; Schutyser et al., 2018). The ether bonds within the lignin structure are thereby hydrolyzed and subsequently sulfonated by the sulfite ions.
(SO$_3$)$^2^-$) in the liquor. Sulfite pulping produces fully water-soluble, highly degraded lignosulfonates with a sulfur content of 4–7 wt% (Abdelaziz et al. 2016; Schutyser et al., 2018; Van den Bosch et al., 2018). Established in 1874, sulfite pulping became the dominant process for wood delignification until kraft pulping was established in the 1930s. Similar to kraft and sulfite pulping, soda pulping involves cooking biomass at 160–170 °C in presence of soda (NaOH) and—optionally—anthraquione, the latter increasing the efficiency by promoting reductive ether bond cleavage (Abdelaziz et al. 2016; Schutyser et al., 2018; Van den Bosch et al., 2018). The wastewater generated at the end of pulping stage called BL is a dark brown in color due to dissolved lignin and its degradation products, hemicelluloses, resins, acids, and phenols (Hermosilla et al. 2015). The BL has high COD, BOD, and TSS (Pokhrel and Viraraghavan 2004). In the pulping process, less than 50% yields are achieved, and the pulp requires further extensive bleaching. During the bleaching process, wood components such as lignin and some carbohydrates are structurally modified, oxidized, degraded and chlorinated (Thompson et al. 2001; Leibbitter 2009; Oke et al. 2017). This is followed by an alkaline extraction phase using high temperature, pH, and consistency, which transforms the oxidized products into a soluble form. In the extraction stage, chlorinated oxidized lignins, not soluble in the acidic chlorination stage, are solubilized and dissolved into the spent liquor. The final bleaching is performed by oxidizing agents: chlorine dioxide and hydrogen peroxide. In India, bleaching is still being done with chlorine. Chlorine dioxide is used by very few mills for viscosity protection in the first bleaching stage (10–15% substitution) and for brightening in the final bleaching stages (Nagarathnamma et al. 1999). The use of chlorine-based bleaching chemicals results in the generation of a large number of toxic chlorinated organic compounds. The wastewater generated at bleaching stage has toxic colored compounds, including chlorophenols, EOXs, AOXs, and a small proportion of extremely toxic DDT, polychlorinated biphenyls (PCBs), and polychlorinated dibenzodioxins (PCDDs) (Savant et al. 2006; Chandra and Kumar 2015b; Lacorte et al. 2003; Rocha-Santos et al. 2010; Singh and Chandra 2019). In addition, chromophoric and highly oxidized polymeric lignin/chlorolignin derivatives are formed giving rise to the characteristic dark color to BL (Fig. 1.1; Nagarathnamma et al. 1999; Esposito et al. 1991; Chedchant et al. 2009; Chandra et al. 2011a, b; Mishra et al. 2014). A large number of pulp and paper mills are reluctant to recycle bleach plant wastewater to the chemical recovery system due to the corrosive nature of chloride ion and the substantial dilution of the chemicals to be recycled. Acid precipitation of lignin is a commonly applied treatment to BL after precipitation of more than 90% of lignin is removed from the solution as solid material. In addition, the precipitated lignin generates large volumes of sludge, which requires further treatment and disposal (Thompson et al. 2001; Pokhrel and Viraraghavan 2004). Nevertheless, the remaining soluble percentage is composed of oxidized and partially degraded lignin (predominantly composed of oligomeric lignin compounds) chlorinated organics responsible for the mutagenicity of the effluent. The high-molecular-weight persistent chlorinated organic compounds along with residual lignin generated during pulp bleaching are the major contributor to effluent color, COD, and chronic toxicity (Ali and Sreekrishnan 2001; Pandey...
et al. 2012; Verma 2008; Maheshwari et al. 2012; Thompson et al. 2001). Finally, the brown/black color effluent generated during pulping and bleaching processes is a complex mixture of hundreds of compounds like lignin, tannin, chlorinated phenol compounds, suspended solids, diterpene alcohols, waxes, fatty acids, resin acids, fatty acids and their degraded products, phenols, dioxins, furans, chlorinated resin acids, chlorinated phenol, chlorinated hydrocarbons, various surfactants, dibenzo-p-dioxins, and dibenzofurans (Fig. 1.1; Ali and Sreekrishnan 2001; Rocha-Santos et al. 2010; Savant et al. 2006; Lacorte et al. 2003). While some of these pollutants are naturally occurring wood extractives (e.g., tannins, resin acids, stilbenes, lignin), others are xenobiotic compounds that are unintentionally generated formed during the process of pulping and papermaking processes (Thompson et al. 2001; Lacorte et al. 2003).

Thus, effluents discharged from industries are heavily loaded with organic matter containing 200 organics and 700 kinds of inorganic compounds (Table 1.2; Chandra and Singh 2012; Chandra and Abhishek 2011; Chandra et al. 2011a, b; Haq et al. 2016; Haq et al. 2017; Karrascha et al. 2006). Table 1.1 summarizes the physico-chemical characteristics of different kinds of influent generated during pulp and paper making process in pulp and paper industry. Some of the pollutants notably polychlorinated dibenzodioxins and dibenzofurans (dioxins and furans) are
Table 1.1 Physicochemical characteristics of wastewater discharged from various industries (Chandra and Abhishek 2011; Chandra et al. 2011a, b; Arivoli et al. 2015)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BL</th>
<th>RGPPME</th>
<th>PPME</th>
<th>PPME</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.8 ± 0.2</td>
<td>9.0 ± 0.2</td>
<td>8117.5 ± 185</td>
<td>7.80 ± 0.012</td>
</tr>
<tr>
<td>Color (Pt/co)</td>
<td>3100 ± 22.32</td>
<td>6100 ± 3.5</td>
<td>–</td>
<td>877.29 ± 4.65</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.87 ± 0.06</td>
</tr>
<tr>
<td>BOD (mg L⁻¹)</td>
<td>5100 ± 167.6</td>
<td>7360 ± 153</td>
<td>5850 ± 50.12</td>
<td>230.18 ± 2.75</td>
</tr>
<tr>
<td>COD (mg L⁻¹)</td>
<td>12,245 ± 439.5</td>
<td>18,700 ± 440</td>
<td>16,400 ± 120</td>
<td>981.75 ± 4.29</td>
</tr>
<tr>
<td>TDS (mg L⁻¹)</td>
<td>402.68 ± 53.92</td>
<td>1402 ± 1.5</td>
<td>840 ± 32.45</td>
<td>2129.17 ± 37.16</td>
</tr>
<tr>
<td>TSS (mg L⁻¹)</td>
<td>–</td>
<td>–</td>
<td>100 ± 4.00</td>
<td>1179.17 ± 30.43</td>
</tr>
<tr>
<td>Total phenol (mg L⁻¹)</td>
<td>38.5 ± 2.61</td>
<td>38.5 ± 2.8</td>
<td>1272 ± 30.45</td>
<td>4.93 ± 0.07</td>
</tr>
<tr>
<td>AOX (mg L⁻¹)</td>
<td>4.7 ± 0.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lignin (mg L⁻¹)</td>
<td>–</td>
<td>663 ± 4.23</td>
<td>1000 ± 1.1</td>
<td>614 ± 8.13</td>
</tr>
<tr>
<td>Sulphate (mg L⁻¹)</td>
<td>1762 ± 41.11</td>
<td>1800 ± 14</td>
<td>–</td>
<td>73.67 ± 1.43</td>
</tr>
<tr>
<td>Tannin (mg L⁻¹)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>42.38 ± 0.49</td>
</tr>
<tr>
<td>PCP (mg L⁻¹)</td>
<td>–</td>
<td>–</td>
<td>145.11 ± 4.56</td>
<td>–</td>
</tr>
<tr>
<td>Phosphate (mg L⁻¹)</td>
<td>BDL</td>
<td>BDL</td>
<td>2.65 ± 0.05</td>
<td>–</td>
</tr>
<tr>
<td>K⁺ (mg L⁻¹)</td>
<td>–</td>
<td>12.2 ± 1.33</td>
<td>86.52 ± 2.58</td>
<td>–</td>
</tr>
<tr>
<td>Na⁺ (mg L⁻¹)</td>
<td>–</td>
<td>102 ± 11</td>
<td>136.56 ± 4.56</td>
<td>–</td>
</tr>
<tr>
<td>Cl⁻ (mg L⁻¹)</td>
<td>–</td>
<td>–</td>
<td>31.42 ± 0.86</td>
<td>–</td>
</tr>
<tr>
<td>Nitrate (mg L⁻¹)</td>
<td>–</td>
<td>3 ± 4.5</td>
<td>41.52 ± 3.56</td>
<td>–</td>
</tr>
</tbody>
</table>

**Heavy metals**

| Cd (mg L⁻¹)     | 0.06 ± 0.03 | BDL | 0.2078 ± 0.09 | – |
| Cr (mg L⁻¹)     | 0.255 ± 0.04 | BDL | 0.2020 ± 0.01 | – |
| Cu (mg L⁻¹)     | 0.105 ± 0.05 | 0.105 ± 0.013 | 0.5110 ± 0.10 | – |
| Fe (mg L⁻¹)     | 3.99 ± 0.91 | 3.990 ± 0.47 | 1.203 ± 0.04 | – |
| Ni (mg L⁻¹)     | 2.84 ± 0.06 | 2.840 ± 0.38 | 0.1500 ± 0.02 | – |
| Zn (mg L⁻¹)     | 1.5 ± 0.30 | 1.500 ± 0.17 | 0.3330 ± 0.01 | – |
| Hg (mg L⁻¹)     | –         | – | 0.8750 ± 0.03 | – |
| Pb (mg L⁻¹)     | –         | – | 0.0148 ± 0.00 | – |

BL black liquor, RGPPME rayon grade pulp paper mill effluent, PPME pulp paper mill effluent, BDL below detection limit, Cd cadmium, Cr chromium, Cu copper, Fe iron, Ni nickel, Zn zinc, Hg mercury, Pb lead, BOD biological oxygen demand, COD chemical oxygen demand, TSS total suspended solid, TS total solid, TDS total dissolved solid, TOC total organic carbon, TVS total volatile solids, EC electrical conductivity, PCP pentachlorophenol, K⁺ potassium, Na⁺ sodium, Cl⁻ chloride, AOX adsorbable organic halides

recalcitrant to degradation and tend to persist in nature (Mishra and Thakur 2010). They are thus known as POPs and have been classified as “priority pollutants” by the USEPA as well as the “dirty dozen” group of POPs identified by the United Nations Environment Program. It is well-established that many of these contaminants are
acute and/or chronic toxins (Nestmanna and Lee 1985; Costigan et al. 2012). This has resulted in a growing concern about the potential adverse effects of genotoxicants on aquatic biota and public health through the contamination of drinking water supplies, recreational waters, or edible organic species.

Eventually, pulp is used to produce paper, but the short fibers are not retained within the paper production and are returned to the wastewater (Jenkins et al. 2003). These residual sludge fibers and other materials detrimental to paper production (e.g. filler, ink) are separated from the wastewater by decantation in the clarifier. Then, the sedimentation material is directed to the press where it becomes the sludge. The sludge is called primary sludge when it originates from the production of virgin wood fiber or deinked paper sludge when it is produced by removing inks from postconsumer fiber. The secondary sludge formed after treatment of wastewater by activated sludge process (Fig. 1.2). Paper production generates around 45% of wastewater sludge. The wastewater sludge is enriched with various fiber wood compounds such as lignin, carbohydrate polymers (cellulose and hemicellulose), and other extractives (lipids and others) in addition to some potentially toxic compounds such as chlorinated organics, resin acids, and heavy metals (Raj et al. 2007a). The heavy metals (HMs) in wastewater sludge are of major concern from the ecotoxicological risk perspectives. A variety of odorous compounds generated by secondary treatment units have also been reported, including sulfur compounds, wood-derived terpenes, and organic acids. These compounds contribute to the pungent stack emissions of total reduced sulfur and other compounds from pulp and paper mills (Watson et al. 2003).

1.3 Distribution and Structural Components of Lignin

Lignin is a major component of lignocellulosic biomass, and processed in enormous amounts in the pulp and paper industry worldwide. It is a complex heteropolymer, of para-hydroxyphenyl propane units linked together via a variety of ether and C–C bonds. Lignin is basically formed by the random coupling of radical species arising
from the peroxidase-mediated dehydrogenation of three cinnamyl alcohol derivatives: p-coumaryl, coniferyl, and sinapyl alcohols. The corresponding phenylpropanoid units in the lignin polymer (known as lignin polymer units) are denoted as p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units, respectively, based on the methoxy substitution on the aromatic rings (Fig. 1.3). The content of these three immediate biosynthetic precursor alcohols varies not only in different plant species but also in the different tissues of the same plant. In gymnosperms, the primary lignin precursors are the two monolignol conifery and p-coumaryl alcohols, while in angiosperms, sinapyl alcohol is also present (Garg and Modi 1999). It is closely associated with cellulose and covalently attached to hemicelluloses. The ether and C–C linkages present in lignin are not susceptible to hydrolytic attack, and therefore, lignin is highly resistant to breakdown (Bugg et al. 2011). Approximately 50–80% of all interunit bonds are β-O-4 ether bonds. In addition, subunits are connected by α-O-4 linkages, β-5 linkages, β-β linkages, 5–5 linkages, and biphenyl and diaryl ether structures. The double bond, conjugated with the aromatic ring, quinone methides, and quinone groups, is responsible for the color of their solution (van Driessell and Christov 2001). These chemicals are responsible for the dark color and toxicity of the wastewater discharged from the pulp and paper industry (Fig. 1.3). Lignin present in wood is converted to thio-lignin and alkali lignin in the kraft pulping. Chlorophenols from the pulp bleaching process are found both in free and bound forms in dissolved organic matter and particles; high- and low-molecular-weight chlorinated compounds are produced by complex reactions between chlorine and lignin in the wood pulp. Under natural conditions, these compounds are slowly degraded to various chlorinated phenolics which may be methylated under aerobic conditions. The low-molecular-weight phenolics and their methylated counterparts (which are more lipophilic) cause toxicity and
bioaccumulate in fish. The dark brown color not only is aesthetically unacceptable but also could inhibit the process of photosynthesis in natural aquatic environments due to the barrier effect of sunlight. To minimize the impact of effluents on the environment, several treatment technologies have been employed, although little is known on their efficiency to eliminate the toxicity attributed to the presence of organic compounds.

1.4 Environmental Fate of Pulp and Paper Industry Wastewater

The wastewater discharged from pulp and paper industry remains toxic and complex due to retaining of high color, BOD, COD, TDS, TSS, and also consisting of potentially toxic chlorinated compounds even after conventional secondary wastewater treatment processes (Raj and Chandra 2004; Emeka et al. 2011; Mishra et al. 2013; Wu et al. 2005). Due to high pollution load and color-contributing substances, pulp and paper wastewater poses a serious aquatic and soil pollution (Fig. 1.4). In aquatic ecosystem, the dense brown color of this wastewater inhibits the natural process of photosynthesis due to reduced penetration of solar radiation and decreases the dissolved oxygen level, which adversely affects flora and fauna and causes toxicity (Hall et al. 2009; Ojunga et al. 2010; Hewitt et al. 2008; Swamy et al. 2011; Ali and Sreekrishnan 2001). The toxicity assessment of pulp and paper wastewater on fish reproductive system has been reported by various workers (Parks et al. 2001; Orlando et al. 2002; Oakes et al. 2005; Wartman et al. 2009; Orrego et al. 2011; Martel et al. 2017; Hou et al. 2018). The short-term exposure of pulp paper mill wastewater to the flora and fauna of aquatic and terrestrial ecosystem has been observed by Verma (2008). Pathan et al. (2009) also showed the behavioral changes in freshwater fish Rasbora daniconius exposed to paper mill wastewater and further higher concentration created an adverse effect on fish. Tyor et al. (2012) also tested the toxicity of pulp and paper industry wastewater by using the Daphnia test model. Similarly, Pandey et al. (2012) showed the comparison of fish toxicity and Microtox toxicity of luminescence bacteria due to bleach plant effluent released from agro- and wood-based pulp and paper mills and also showed the impact of pulp paper mill wastewater on survival and hatchability of Cyprinus carpio. The result showed that paper mill effluent treated eggs hatch susceptibility and adverse effect and development stages are badly affected and effluent showed ultimately lethal effect. The color-causing organic compounds have also been implicated in the appearance of algal blooms (Dileká et al. 1999). The physicochemical properties of river water were analyzed by Emeka et al. (2011); Lacorte et al. (2003) attempted an overview of organic compounds that contribute to the toxicity of pulp and paper industry wastewater. Presence of organic compounds in the wastewater has contributed to deterioration of water quality due to the mixing of organic compounds in the recipient ecosystem, i.e., aquatic and terrestrial ecosystem. The effect of different pollutants present in pulp paper mill wastewater in long-term study at a multi-tropic level in aquatic communities receiving water bodies in the United States
has been also evaluated. The study has shown the toxic effect on fish macrovertebrate, phytoplankton, and other flora and fauna (Hall et al. 2009). The primary reproductive effects in fish due to being exposed to pulp and paper wastewater were reported by Hewitt et al. (2008). The toxic effect of pulp and paper mill wastewater on phytoplankton and macroinvertebrates in River Nzoia, Kenya was studied by Ojunga et al. (2010). This study has concluded that the wastewater produce changes in both physicochemical parameters of the receiving water and contribute to nutrient loading, especially phosphorus and nitrate, on the deteriorating water quality and

Fig. 1.4 Environmental impact of secondary treated wastewater discharged from pulp and paper industry. (a–c) A view of the contaminated site showing aquatic pollution due to discharging of colored complex wastewater. (d, e) Irrigation of agricultural field through discharged effluent affecting the crop as well as soil microflora and texture
eutrophication eliminates some taxa of both phytoplankton and macroinvertebrates, whereas others such as *Microcystis* sp. and *Chironomus* sp. appear to thrive in contaminated environment due to their tolerance to changing water quality. The genetic disturbance by pulp paper mill wastewater on large mouth bass (*Micropterus salmoides*) was reported by Denslow et al. (2004).

In a terrestrial ecosystem, the wastewater irrigated soil showed the decrease of moisture content and increase of pH as well as accumulation of heavy metals, i.e., Zn, Cu, Cd, Cr, and Pb in soil. The studies revealed that mill effluent has a deleterious effect on seed germination and growth parameter of rice and mustard and pea. It also has been noted that the effluent concentration above 50% was found inhibitory for plant growth parameter. Accumulation of contaminants into the terrestrial ecosystem is due to gradual percolation of contaminants which in turn changes the soil texture (Roy et al. 2008; Pradhan and Behera 2011; Kumar and Chopra 2011). In many developing countries, farmers irrigate their crop plants with water bodies which might be severely exposed to industrial effluents. This leads to risks of bioaccumulation of toxicants in the food chain. Thus, it is important to treat the industrial effluents before their final discharge. This continuous practice of irrigation of agricultural field through discharged effluent affecting the crop as well as soil texture (Medhi et al. 2008, 2011; Devkumari and Selvaseelan 2008). Pathan et al. (2009) reported that the toxicity of paper mill wastewater to fish *Rasbora daniconius* and its LC50 values were assessed for different concentration of effluent for 24–96 h exposure periods. In addition, the impact of paper mill wastewater on the survival and hatchability of eggs of *Cyprinus carpio* was reported by Tyor et al. (2012). However, the health hazards of polluted underground water due to pulp paper mill effluent in the vicinity of the pulp paper industry are not known so far.

These compounds, mostly complex aromatic in nature, also impart heavy toxicity to the aquatic systems, thus entering the food chain. Many researchers have reported that the mixing or direct entry of pulp paper mill effluent into the recipient ecosystem (aquatic and terrestrial ecosystem) is responsible for potential health hazards as mill wastewater mixing consequently increases the organic or inorganic compounds, i.e., enhancing or supporting the growth of numerous *total coliform*, *fetal coliform*, *Klebsiella* spp., *E. coli*, *Enterobacter* spp., *Klebsiella* spp., *Enterobacter* spp., *Salmonella*, *Vibrio cholerae*, *Shigella* spp., *Citrobacter* sp., etc. (Huntley et al. 1766; Clark et al. 1992; Liss and Allen 1992; Megraw and Farkas 1993; Gauthier and Archibald 2001; Chandra et al. 2006). Beauchamp et al. (2006) investigated the thermotolerant coliform population of one paper mill effluent and two paper mill sludges and wood chips screening rejects using chromogenic media. Large numbers of thermotolerant coliforms, i.e., 7,000,000 MPN g−1 sludge (dry weight; d.w.), were found in combined sludges. From this first series of isolations, bacteria were purified on the MacConkey medium and identified as *Citrobacter freundii*, *Enterobacter* sp., *E. sakazakii*, *E. cloacae*, *Escherichia coli*, *K. pneumoniae*, *K. pneumoniae* subsp. *rhinoscleromatis*, *K. pneumoniae* subsp. *ozaenae*, *K. pneumoniae* subsp. *pneumoniae*, *Pantoea* sp., *Raoultella terrigena*, and
R. planticola. Second, the presence of thermotolerant coliforms was measured at more than 3700–6000 MPN g\(^{-1}\) (d.w.) sludge, whereas E. coli was detected from 730 to more than 3300 MPN g\(^{-1}\) (d.w.) sludge. The presence of thermotolerant coliform bacteria and E. coli was sometimes detected from wood chips screening rejects in large quantities. Also, indigenous E. coli were able to multiply into the combined sludge, and inoculated E. coli isolates were often able to multiply in wood chips and combined sludge media. This study points out that the coliform bacteria are introduced by the wood chips in the wastewater, where they can survive through the primary clarifier and regrow in combined sludges. Furthermore, Emeka et al. (2011) reported the variation in physicochemical dynamics due to the impact of paper mill wastewater that discharge into the Owerrinta River, Eastern Nigeria. Long et al. in 2012 from the United States have reported the characterizing paper mill wastewater using indicators and source-tracking methods. This study examined potential public health implications of E. coli in a Wisconsin river that receives paper mill wastewater upstream of a public beach. Furthermore, the effects of solid wood waste discharge on the physicochemical and microbial identification of the Warri River were reported by Idise et al. in 2012 from Nigeria. Lee et al. (2012) carried out some significant work where they have conducted a survey and reported the effect on the skin and health of children living in upstream and downstream villages from a pulp and paper mill. This study has reported that the ill effect on children who drank water directly from the river was compared with those who never did. River water analysis has shown physicochemical variation within the acceptable range except for fecal coliform (6 MPN/100 mL). Moreover, Lee et al. (2012) surveyed and observed that the pulp and paper mill wastewater has created health-related problems to the downstream population of the river.

### 1.5 Biological Treatment Methods of Pulp and Paper Industry Wastewater

Pulp and paper industry is a very water-intensive industry in terms of freshwater use. Currently, the increasing needs to reduce water consumption and to satisfy tightened discharge standards in stringent environmental regulations have forced pulp and paper industries to treat their effluent for safe disposal in environment using advanced treatment processes. Most wastewater treatment processes (WWTPs) use aerobic and/or anaerobic biological processes to remove organic contaminants in wastewaters (Singh and Thakur 2006). The commonly available biological treatment methods adopted in the pulp and paper industry to lower the pollution load indices like BOD and COD include anaerobic lagoon, stabilization pond, aerated lagoon, activated sludge process, or its modification depending on the local conditions. Aerobic processes are preferably used in most pulp and paper mills because of their ease of operation as well as the relatively low capital and operating costs.
1.5.1 Aerobic Treatment Process

Among aerobic technologies, AS and aerated lagoons are commonly used wastewater treatment approach applied in pulp and paper industry (Fig. 1.5; van Ginkel et al. 1999; Erkan and Engin 2017; Pokhrel and Viraraghavan 2004; Lerner et al. 2007). Despite the widespread usage, these technologies still suffers from instability, high sludge production, and high operating cost. The most important operational difficulty associated with activated sludge is the separation of sludge from the clarified wastewater. Implications of conventional AS process used for pulp and paper industry wastewater with modification to a low sludge production (LSP) process have been studied for treating (Talat Mahmood et al. 2006). The LSP system produced 36% less sludge than the base case system, while both systems removed 96% BOD, 73% COD, and 56% AOXs from a bleach kraft mill wastewater. The LSP system required approximately 25% higher aeration than the conventional activated sludge system. The LSP sludge settled much better than the conventional activated sludge and had superior dewatering properties. This could lead to settling and dewatering chemical cost savings. The odorous compound released from pulp and paper mill wastewater and their reduction were also investigated by Watson et al. (2003). They reported that the AS may be helpful for reduction of odorous gases. However, the AS and aerated lagoons are not able to effectively mitigate the pollution load of pulp and paper mill wastewater. Because the microorganisms present in the conventional activated sludge system are not effective in degrading compounds like lignin, therefore, complete treatment of such wastes remains elusive.

Fig. 1.5 A view of the aerated activated sludge treatment of pulp and paper industry wastewater
1.5.1.1 Bioaugmentation/Biostimulation Process for Efficient Treatment of Pulp Paper Effluent

Bacterial Bioaugmentation/Biostimulation
In the recent past, biotechnological approaches for the remediation of contaminated environment have gained worldwide attention (Chandra et al. 2018a, b, c, d, e; Kumar and Chandra 2020a, b; Perestelo et al. 1989; Morii et al. 1995; Thakur 2004). Bioremediation is considered a cost-effective and environment-friendly technology with great potential to remove target compounds from contaminated sites or for treatment of wastewater (Malaviya and Rathore 2007; Raj et al. 2014a, b; Kumar et al. 2018; Kumar and Chandra 2020a, b; Chandra and Kumar 2017a, b). It is a set of techniques that improve the degradation capacity of contaminated areas (Chandra and Kumar 2015b). They use bioaugmentation strategy (introduction of specific degradable strains or consortia of microorganisms) (Yu and Mohn 2002; Yadav et al. 2016) or biostimulation strategy (introduction of nutrients, inducers, and oxygen) (Chandra et al. 2018a). Bioaugmentation is the introduction of a group of natural or genetically engineered microorganisms to decontaminate soil and water (Chen et al. 2012b). Comparing with the common biotreatment process, the inoculated indigenous or allochthonous microbial strains can enhance the biodegradation of target pollutants, serving to strengthen or complement the metabolic capabilities of the indigenous microbial community (Mishra et al. 2014). An important factor for successful bioaugmentation is the selection of potential bacteria that can not only degrade contaminants but can also adapt to an adverse environment, usually higher toxicity of the contaminated area (Dudášová et al. 2014). The major bacterial species successfully used in bioaugmentation and biostimulation processes for kraft lignin degradation and decolorization of pulp and paper industry wastewater are *Paenibacillus* sp., *Aneurinibacillus aneurinilyticus*, *Bacillus* sp. (Raj et al. 2007a, b, 2014a), *Serratia marcescens*, *Citrobacter* sp., *Klebsiella pneumonia* (Raj et al. 2007a, b; Chandra and Abhishek 2011), *Pseudomonas*, *Bacillus*, *Pannonibacter*, *Ochrobactrum* (2011), *Bacillus megaterium*, *Pseudomonas aeruginosa* (Tiku et al. 2010), *Novosphingobium* sp., *Aeromonas fomicans* (Gupta et al. 2001), *Pseudomonas fluorescens* (Chauhan and Thakur 2002), *Comamonas* sp. B-9 (Chen et al. 2012a) *Pseudomonas*, *Ancylobacter*, and *Methylobacterium* (Keharia and Madamwar 2003). Yu and Mohn (2002) successfully used the *Zoogloea resiniphila* DhA-35 in the bioaugmentation treatment of resin acid containing pulp and paper mill wastewater. Similarly, Muttray et al. (2001) used *Pseudomonas abietaniphila* BKME-9 for the treatment of resin acid in the pulp and paper mill wastewater. Chauhan and Thakur (2002) treated pulp and paper mill wastewater in a fixed-film bioreactor by *P. fluorescens* and noted reductions of 45% lignin, 75% color, 79% COD, and 66% phenol within 15 days of incubation. Removal of organochlorine from bleached Kraft pulp and paper mill wastewater by dehalogenating indigenously grown *Pseudomonas*, *Ancylobacter*, and *Methylobacterium* strains was reported by Fulthorpe and Allen (1995). Keharia and Madamwar (2003) compared the degradation potential of *Pseudomonas*, *Ancylobacter*, and *Methylobacterium* strains for organochlorine from bleached
kraft pulp and paper mill wastewater. They observed that *Ancylobacter* showed the broad substrate range but could significantly reduce the AOXs from softwood wastewater only, whereas *Methylobacterium* with limited substrate range was capable of degrading AOXs from both hardwood and softwood effluents. Singhal and Thakur (2009a, b) reported the decolorization and detoxification of pulp paper mill wastewater under un-optimized and optimized conditions by *Cryptococcus* sp. This bacterial isolate reduced the 27% color and 24% lignin content of the wastewater in 15 days under un-optimized conditions. However, enhanced reduction in color (50–53%) and lignin (35–40%) was noted to occur after optimum treatment conditions were reached during the 24 h incubation: pH 5.0, temperature 35–40 °C, shaking speed 125 rpm, dextrose 1.0% w/v, tryptone 0.1% w/v, and inoculum size 7.5% v/v. Recently, *Halomonas* sp. and *Bacillus* sp. have been used for BL degradation and decolorization at high pollution load (Yang et al. 2008).

Singh et al. (2011) reported bioremediation of pulp and paper mill wastewater by a tannic acid-degrading bacterium *Enterobacter* sp. Prior to the bioremediation of wastewater, authors optimized various parameters, viz., inoculum size, agitation, temperature, and treatment duration by using Qualitek-4 software. In batch culture experiment, the reduction of lignin up to 73% and color up to 82% along with COD and BOD with 16 h retention time was observed. Mishra and Thakur (2010) isolated *Bacillus* sp. from pulp and paper mill sludge and used this isolate in degradation and decolorization of BL. They noted that maximum color was removed at pH 8, temperature 35 °C, shaking speed 200 rpm, sucrose 2.5%, and inoculum size 5% (w/v) within 48 h from 10% BL. However, after optimization of various nutritional and environmental parameters by using the Taguchi approach, twofold increase in the removal of color and lignin from 25–69% and 28–53%, respectively, was noted. This study indicated the significance of Taguchi’s approach in decolorization and delignification of lignin in pulp and paper mill wastewater. Chandra and Abhishek (2011) studied the decolorization of BL in axenic and mixed condition by isolated bacterial strains, i.e., *Citrobacter freundii* and *Citrobacter* sp., and characterized their metabolites. Under mixed culture condition, the aerobic treatment could reduce 79% AOX, 79% color, 82% COD, and 60% lignin after 144 h of the incubation period. It was also observed that mixed bacterial culture produced the optimum level of peroxidase enzyme compared to axenic bacterial strain. The comparative GC–MS analysis of control and degraded BL revealed that along with lignin fragment, some chlorophenolic compounds, 2,4,6-trichlorophenol, 2,3,4,5-tetrachlorophenol, and pentachlorophenol, were detected in BL degraded by axenic culture, whereas these chlorophenolic compounds were completely absent in BL degraded by mixed bacterial culture (Table 1.2). Similarly, the decolorization of BL by a potential bacterial consortium consisting of *S. marcescens*, *Citrobacter* sp., and *Klebsiella pneumoniae* under optimized environmental and nutritional conditions has been reported by Chandra et al. (2011a). The study has shown that bacterial growth and BL degradation were associated with ligninolytic enzyme production and numerous metabolites were also detected in bacterial degraded BL (Table 1.2; Chandra et al. 2011a, b). The pulp and paper mill wastewater decolorization and detoxification by using the different inoculums ratio in mixed bacterial culture have been evaluated at laboratory scale (Chandra et al. 2011b). This study deals with the degradation and
detoxification of pulp paper mill wastewater by three bacterial strains, i.e., *S. marcescens*, *S. liquefaciens*, and *Bacillus cereus* in different ratios, and found that two ratios, 4:1:1 and 1:4:1, were effective for the degradation of pulp and paper mill wastewater. These ratios reduced the various pollution parameters from pulp and paper mill wastewater. HPLC and GC–MS analysis also showed that the mixed bacterial culture in 4:1:1 ratio degraded 95% of lignin and 98% of chlorophenols, and several other related compounds, whereas ratio 1:4:1 reduced lignin and chlorophenols up to 84% and 58%, respectively, after 7 days of incubation (Table 1.2). Chandra and Singh (2012) also studied the decolorization and detoxification of rayon grade pulp paper mill wastewater in different nutritional as well as environmental parameters by a developed bacterial consortium comprising *S. marcescens*, *Citrobacter* sp., and *K. pneumoniae* strains. The degradation study result showed that the ligninolytic activities were found to be growth associated and the developed bacterial consortium was efficient for the reduction of color, BOD, and COD up to 85%, 74%, and 83%, respectively. The GC–MS analysis also showed that most of the compounds detected in untreated wastewater were diminished after bacterial treatment, while formic acid hydrazide, 4-cyclohexane-1,2-dicarboxylic acid, carbamic acid, 1,2-benzenedicarboxylic acid, and erythro pentanoic acid were found as new metabolites. Simultaneously, Chandra and Singh (2012) also reported the decolorization and detoxification of rayon grade (RG) pulp paper mill effluent by mixed bacterial culture comprising *Pseudochrobactrum glaciale*, *Providencia rettgeri*, and *Pantoea* sp. The results showed that mixed culture effectively reduced color, COD, and BOD up to 96.02%, 91%, and 92.59%, respectively, from pulp paper mill effluent within 216 h of the incubation period. During degradation and decolorization, maximum enzyme activity for lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase was recorded at 48, 72, and 144 h of the incubation period, respectively. Further, GC–MS analysis revealed that majority of the compounds present in the untreated sample were completely removed and only a few metabolites were generated after bacterial treatment (Table 1.2). A mammalian cell line-based toxicological evaluation of pulp and paper mill BL biodegraded in a soil microcosm by indigenous alkalotolerant *Bacillus* sp. was reported by Mishra et al. (2014). GC–MS analysis performed after biodegradation showed the formation of simpler compounds like *p*-hydroxyhydrocinnamic acid, homovanillic acid methyl ester, and 3,5-dimethoxy-*p*-coumaric alcohol. The methyltetrazolium assay for cytotoxicity, 7-ethoxyresorufin-O-deethylase assay for dioxin-like behavior, and alkaline comet assay for genotoxicity evaluation were carried out with the human hepatocarcinoma cell line HuH-7 before and after bacterial treatment. The result revealed that bioremediation for 15 days reduced toxicity, as shown by a 139-fold increase in BL LC₅₀ value, a 343-fold reduction in benzo(a)pyrene equivalent value, and a fivefold reduction in the olive tail moment. Similarly, Haq et al. (2016, 2017) evaluated the bioremediation potentiality of ligninolytic enzyme producing *S. liquefaciens* for detoxification of wastewater discharged from pulp and paper industry after secondary treatment and characterized their metabolic products. The bacterium *S. liquefaciens* effectively reduced color, lignin, COD, and phenol of real
<table>
<thead>
<tr>
<th>Effluent</th>
<th>S. no.</th>
<th>Name of identified compound</th>
<th>UW</th>
<th>BTW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rayon grade pulp paper mill wastewater</td>
<td>1.</td>
<td>4-Isopropoxy-butyric acid</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2.</td>
<td>3,7,11,15,18-pentaaoxa-2,19-disilaneicosane</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3.</td>
<td>Butane-1-ol</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4.</td>
<td>Propane</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5.</td>
<td>2-methyl-2,4-dimethoxy butane</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>6.</td>
<td>4,5-octanediol,3,6-dimethyl</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>7.</td>
<td>Diphenylthiocarbazide</td>
<td>+</td>
<td>-</td>
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<tr>
<td></td>
<td>8.</td>
<td>Propane,1-(1-Ethoxyethoxy)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>9.</td>
<td>Cyclohexanecarboxylic acid</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>10.</td>
<td>6-Oxabicyclo9,3,1,0,0hexan-3-one</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>11.</td>
<td>Pyrrolo(1,2A)pyrazine-1,4-dione, hexahydro</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>12.</td>
<td>Pyrrolo(1,2A)pyrazine-1,4-dione, hexahydro</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>13.</td>
<td>Trichloroacetyl isocyanate</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>14.</td>
<td>1-Phenyl-1-nonyne</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>15.</td>
<td>Tetradecanoic acid</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>16.</td>
<td>6-Chlorohexanoic acid</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>17.</td>
<td>2,5-Piperazinedione,3,6-bis(2-methyl propyl)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>18.</td>
<td>Pyrrolo(1,4-dione,hexahydro-3-(phenyl methyl)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>19.</td>
<td>1-Chloro Octadecane</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20.</td>
<td>1,2 Benzene carboxylic acid</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>21.</td>
<td>4,8-Dimethyl undecane</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>22.</td>
<td>3-Trifluoroacetoxypentadecane</td>
<td>+</td>
<td>-</td>
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<tr>
<td></td>
<td>23.</td>
<td>Benzeneacetic acid,3-tetradecyl ester</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>24.</td>
<td>Cyclo-(L-leucyl-l-phenylalanyl)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>25.</td>
<td>Butanoic acid</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Black liquor</td>
<td>1.</td>
<td>Propanoic acid</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2.</td>
<td>Acetic acid</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3.</td>
<td>Butanoic acid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4.</td>
<td>Benzoic acid</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>5.</td>
<td>2,4,6 trichloro phenol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6.</td>
<td>2,3,4,5 tetrachloro phenol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>7.</td>
<td>Tetradecanoic acid</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>8.</td>
<td>Pentachlorophenol</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>9.</td>
<td>Dibutyl phthalate</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>10.</td>
<td>Hexadecanoic acid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>11.</td>
<td>Octadecanoic acid</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>12.</td>
<td>Bis(2-ethylhexyl) phthalate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Black liquor</td>
<td>1.</td>
<td>Propanoic acid</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2.</td>
<td>Formic acid hydrazide</td>
<td>-</td>
<td></td>
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<tr>
<td></td>
<td>3.</td>
<td>4-Cyclohexane-1,2-dicarboxylic acid</td>
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(continued)
<table>
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<tr>
<th>Effluent</th>
<th>S. no.</th>
<th>Name of identified compound</th>
<th>UW</th>
<th>BTW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.</td>
<td>1,2 Butanediol</td>
<td>+</td>
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<tr>
<td></td>
<td>5.</td>
<td>Carboxylic acid</td>
<td>−</td>
<td></td>
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<tr>
<td></td>
<td>6.</td>
<td>3-Cyclohexane 1-methanol</td>
<td>+</td>
<td>−</td>
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<tr>
<td></td>
<td>7.</td>
<td>2-Methoxy phenol (Guaiacol)</td>
<td>+</td>
<td>−</td>
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<tr>
<td></td>
<td>8.</td>
<td>4-Methyl benzaldehyde</td>
<td>+</td>
<td>−</td>
</tr>
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<td></td>
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<td>Benzoic acid</td>
<td>+</td>
<td>−</td>
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<td></td>
<td>10.</td>
<td>Benzene acetic acid</td>
<td>+</td>
<td>−</td>
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<td>11.</td>
<td>Benzylemalonic acid</td>
<td>+</td>
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<td>3-Hydroxy-4-methoxymandelic acid</td>
<td>+</td>
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<td>13.</td>
<td>Butylated hydroxytoluene</td>
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<tr>
<td></td>
<td>14.</td>
<td>2,4-Bis (1,1-diyethyl)-phenol</td>
<td>+</td>
<td>−</td>
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<tr>
<td></td>
<td>15.</td>
<td>Heptadecanoic acid</td>
<td>+</td>
<td>−</td>
</tr>
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<td></td>
<td>16.</td>
<td>2-Methoxy propanoyl chloride</td>
<td>+</td>
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<td>17.</td>
<td>4-Hydroxy-3,5-dimethoxy benzaldehyde</td>
<td>+</td>
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<td>18.</td>
<td>Tetradecanoic acid</td>
<td>+</td>
<td>−</td>
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<td></td>
<td>19.</td>
<td>1,2-benzendedicarboxylic acid</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>20.</td>
<td>Dibutyle phthalate</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>21.</td>
<td>Erythropentanoic acid</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>22.</td>
<td>Ricinoleic acid</td>
<td>+</td>
<td>−</td>
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<tr>
<td></td>
<td>23.</td>
<td>Phthalate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>24.</td>
<td>Cholesterol trimethylsilyl ether</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>25.</td>
<td>1,1-(1,2-ethanediyl) bis[4-methoxy] benzene</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>26.</td>
<td>2,4-Bis (1-phenylethyl)-pheno</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>27.</td>
<td>Bis (2-ethylhexyl) phthalate</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulp and paper mill wastewater</td>
<td>1.</td>
<td>Propanoic acid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2.</td>
<td>Benzeneacetonitrile</td>
<td>−</td>
<td>−</td>
</tr>
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<td>3.</td>
<td>Pyridine</td>
<td>−</td>
<td>−</td>
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<tr>
<td></td>
<td>4.</td>
<td>Phosphoric acid</td>
<td>+</td>
<td>−</td>
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<tr>
<td></td>
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<td>1[(Formyl)oxymethyl]benzene</td>
<td>−</td>
<td>+</td>
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<tr>
<td></td>
<td>6.</td>
<td>(+)-5-Hydroxy-6-(1-hydroxyethyl)-2,7-dimethoxynaphthoquinone</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>7.</td>
<td>l-(+)-Tartaric acid, bis(trimethyl silyl) ether, bis(trimethyl silyl)ester</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.</td>
<td>3-Octadecene, (E)-</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>9.</td>
<td>Uric acid</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>10.</td>
<td>D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-O, methyloxime</td>
<td>+</td>
<td>−</td>
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<tr>
<td></td>
<td>11.</td>
<td>(2 R,3 S)-2-[(E)-2-(Ethoxycarbonyl)ethenyl]-2, 3-dimethylaziridine</td>
<td>−</td>
<td>+</td>
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<tr>
<td></td>
<td>12.</td>
<td>Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>13.</td>
<td>1,4-Diazio-2,5-dioxo-3-isobutyl bicyclo (4.3.0) nonane</td>
<td>−</td>
<td>+</td>
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<td></td>
<td>14.</td>
<td>1-Octadecene</td>
<td>+</td>
<td>−</td>
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</tbody>
</table>

(continued)
<table>
<thead>
<tr>
<th>Effluent</th>
<th>S. no.</th>
<th>Name of identified compound</th>
<th>UW</th>
<th>BTW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15.</td>
<td>Hexadecanoic acid</td>
<td>+</td>
<td>—</td>
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<td></td>
<td>16.</td>
<td>1-Monolinoleoyl glycerol trimethyl silyl ether</td>
<td>+</td>
<td>—</td>
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<td></td>
<td>17.</td>
<td>1-Heneicosanol</td>
<td>+</td>
<td>—</td>
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<tr>
<td></td>
<td>18.</td>
<td>Octadecanoic acid</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>19.</td>
<td>Tetracosanic acid</td>
<td>+</td>
<td>—</td>
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<td></td>
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<td>A-D-Galactopyranoside, methyl 2,3-bis-o-(trimethyl silyl)-, cyclic methylbronate</td>
<td>+</td>
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<td></td>
<td>21.</td>
<td>2′,4′-6′-Trinitro-5′-phenyl-1,1′:3′,1″-terphenyl</td>
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<td>22.</td>
<td>N,N′-Dicyclohexyl-1-cyano-7-pyrrolidinylperylen-3,4:9,10-tetraacyxlic acid</td>
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<td>Pulp paper mill wastewater</td>
<td>1.</td>
<td>1-O-Pentadecylglycerol</td>
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<td>Glycerol</td>
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<td>L-Glutamic acid</td>
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<td>4.</td>
<td>Iron,tricarbonyl(N-(phenyl-2-yridinylmethylene) benzenamine)</td>
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<td>5.</td>
<td>Butanal</td>
<td>−</td>
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<td></td>
<td>6.</td>
<td>Hexanedioic acid</td>
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<td></td>
<td>7.</td>
<td>D-galactofuranose</td>
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<td></td>
<td>8.</td>
<td>D-Fructose</td>
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<td>+</td>
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<td>D-Glucose</td>
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<td>D-Gluconic acid</td>
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<td></td>
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<td>D-Mannitol</td>
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<td>−</td>
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<td>12.</td>
<td>Glucopyranose</td>
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<td>−</td>
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<td>1,6,8-trihydroxy-2-isopropyl-3-methoxy-9,10-anthraquinone</td>
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<td>14.</td>
<td>2,4-dimethoxyphenyl</td>
<td>+</td>
<td>−</td>
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<td>2,6-Dinitro-4,40-di-tert-butylbiphenyl</td>
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<td>16.</td>
<td>Diethyl 3,4-dihydro-2-nethyl-phosphonate</td>
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<td></td>
<td>17.</td>
<td>4,6-dimethoxy-2,3-dimethyl</td>
<td>+</td>
<td>−</td>
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<tr>
<td></td>
<td>18.</td>
<td>2,4,6-trinitro-5-phenyl</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Pulp paper mill wastewater</td>
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<td>2-Ethoxyethoxy-Trimethylsilane</td>
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<td>Propylene carbonate</td>
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<td></td>
<td>3.</td>
<td>Butanoic acid,2-oxo (acid)</td>
<td>−</td>
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<td>4.</td>
<td>Methanedianmine,N,N,N,N-tetramethyl</td>
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<td>5.</td>
<td>2-Ethoxyethoxy-trimethylsilane</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>6.</td>
<td>Butane,2Ethoxy-</td>
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<td>+</td>
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<td></td>
<td>7.</td>
<td>Diphenylthiocarbazide</td>
<td>−</td>
<td>+</td>
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<td></td>
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<td>1-(2,4-Diethoxy-phenyl)Ethanone</td>
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<td>9.</td>
<td>1,4-Dimethoxy-2-Phenylbutane(phenol)</td>
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<td></td>
<td>10.</td>
<td>Oxalic acid,Cyclobutyl heptadecylester (cyclo)</td>
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<td></td>
<td>11.</td>
<td>8-Pentadecaneone(ketone)</td>
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<td>−</td>
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</table>
wastewater after 144 h of treatment at 30 °C, pH 7.6, and 120 rpm. Further, the bacterium-treated effluent was evaluated for residual toxicity assessment by alkaline single-cell (comet) gel electrophoresis (SCGE) assay using *Saccharomyces cerevisiae* MTCC 36 as a model organism. The toxicity reduction to treated effluent was found up to 49.4%. They also characterized the major metabolic products during bacterial treatment of pulp paper mill wastewater as shown in Table 1.2. Tiku et al. (2010) also reported the holistic bioremediation of pulp mill wastewater using three autochthonous bacteria strains, *P. aeruginosa*, and *B. megaterium*, to reduce the BOD and COD level of such wastewater up to permissible level, i.e., 30 mg L\(^{-1}\) and 250 mg L\(^{-1}\), respectively, within a retention time of 24 h in batch culture. However, the continuous mode of treatment may further decrease the retention time. A concomitant reduction in TDS, AOXs, and the color was also observed. The bacterial degradation of lignin is limited compared to fungi.

### Fungal Bioaugmentation/Biostimulation

Fungi are the only microorganisms studied extensively for the degradation and decolorization of lignin and its related monomers (Hofrichter 2002). The use of fungi has a great potential for tertiary treatment and removal of residual organic compounds in wastewater discharged from pulp and paper industries (Wu et al. 2005; Apiwattanapiwat et al. 2006; Da Re and Papinutti 2011; Rajwar et al. 2017). White-rot fungi, such as *Phanerochaete chrysosporium* (Zouari et al. 2002; Mittar et al. 1992; Wu et al. 2005), *Trametes* (*Coriolus*) *versicolor*

#### Table 1.2 (continued)

<table>
<thead>
<tr>
<th>Effluent</th>
<th>S. no.</th>
<th>Name of identified compound</th>
<th>UW</th>
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</tr>
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<td>treated effluent</td>
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<tr>
<td>BTE</td>
<td>bacterial treated effluent</td>
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<td>12.</td>
<td>1,2Benzenedicarboxylic acid,Bis (2-Methylpropyl) Ester</td>
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<tr>
<td>13.</td>
<td>1-Phenyl-1-nonyne(surfactant)</td>
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<tr>
<td>14.</td>
<td>Sulphurous acid,Octadecyl 2-Propylester</td>
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<td>−</td>
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<tr>
<td>15.</td>
<td>Benzene,1,3-Bis(1-methylbenzyl)</td>
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<td>16.</td>
<td>3-Ethenyl-6-Dimethylnitrometheneinobenzonitrile</td>
<td>−</td>
<td>+</td>
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<tr>
<td>17.</td>
<td>N-(3-Bromo-1-Methoxybenzyl)-1H-Indol-2-Y/lmethyl)-2-Methoxybenzyl</td>
<td>−</td>
<td>+</td>
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<tr>
<td>18.</td>
<td>Proponoic acid,2-(Benzyolaminos)-333 Trifluoro-2-[(Trifluoromethyl)phenyl]amino-ethyl</td>
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<td>+</td>
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<td>20.</td>
<td>2-Propanoic acid,3(4-Methylphenyl), Ethylester</td>
<td>−</td>
<td>+</td>
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<td>21.</td>
<td>2-Propanoic acid,3-(MethylPhenyl), Ethylester</td>
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<td>+</td>
<td></td>
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<tr>
<td>22.</td>
<td>Phthalicacid,Dodecyl 2-Ethylexylester</td>
<td>−</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

+ present, − absent, UE untreated effluent, BTE bacterial treated effluent
P. radiata (Lankinen et al. 1991; Hatakka 2001), Marulius tremellosus (Lankinen et al. 1991), Rhizomucor pusillus (van Driessel and Christov 2001), Lentinus edodes (Esposito et al. 1991; Wu et al. 2005), Pleurotus spp., P. sajor-caju, P. platypus, P. citrinopileatus (Ragunathan and Swaminathan 2004), Steccherinum sp. (Da Re and Papinutti 2011), Datronia sp. (Chedchant et al. 2009), and Trichaptum (Apiwattanapiwat et al. 2006), have been reported to be effective in reducing the various pollution parameters of pulp and paper industry wastewater. Decolorization and detoxification of extraction-stage effluent from chlorine bleaching of kraft pulp by Rhizopus oryzae have been investigated by Nagarathnamma and Bajpai (1999).

Table 1.3 shows the analytical results for the effluent sample. A total of 37 standards of chlorophenols and chloroaldehydes were run, and 13 types of chlorophenols and three types of chloroaldehydes were found in the extraction-stage effluent (Table 1.3). R. oryzae was found to decolorize, dechlorinate, and detoxify bleach plant effluent at lower co-substrate concentrations. With glucose at 1 g L\(^{-1}\), this fungus removed 92–95% color, 50% COD, 72% AOXs, and 37% EOXs in 24 h at pH of 3–5 and temperatures of 25–45 °C, although the fungus removed up to 78% of the color without added co-substrate.

Bioremediation of pulp and paper industry wastewater by a novel fungal consortium, comprising two basidiomycetous fungi (Merulius aureus syn. Phlebia sp. and an unidentified genus) and a deuteromycetous fungus (Fusarium sambucinum Fuckel MTCC 3788), isolated from pulp and paper mill wastewater-affected soils in immobilized condition was assessed by Malaviya and Rathore (2007). First, these fungus isolates were immobilized on nylon mesh, and the developed consortium was further used for the treatment of pulp and paper mill wastewater in a continuously aerated benchtop bioreactor. The treatment resulted in the reduction of lignin, color, and COD of the wastewater in the order of 79.0%, 78.6%, and 89.4% in 4-day incubation period. A major part of reductions in lignin, color, and COD of the wastewater occurred within the first 24 h of the treatment, which was also characterized by a steep decline in the pH of the wastewater. Singhal and Thakur (2009a) evaluated the efficiency of the biological treatment process for the decolorization and detoxification of pulp and paper mill wastewater for its safe disposal in the environment. In this study, they used Emericella nidulans var. nidulans for the treatment process. The process parameters for optimum decolorization of pulp and paper wastewater were optimized by the Taguchi approach. Decolorization of wastewater was improved by 31% with reduction in 66.66% color and 37% lignin after treatment by E. nidulans var. nidulans in batch culture. Variation in pH from 6.0 to 5.0 had the most significant effect on decolorization (71%), while variation in temperature from 30 to 35 °C had no effect on the process. Later, treated effluent was evaluated for genotoxicity by alkaline single-cell gel electrophoresis assay using Saccharomyces cerevisiae MTCC 36 as a model organism, indicating a 60% reduction in toxicity. Rocha-Santos et al. (2010) also evaluated the effects of a tertiary treatment by fungi (Pleurotus sajor-caju, T. versicolor, P. chrysosporium, and R. oryzae) on individual organic compounds of a Eucalyptus globulus bleached