Maulin Shah
Aditi Banerjee *Editors*

Combined Application of Physico-Chemical & Microbiological Processes for Industrial Effluent Treatment Plant

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Preface

With the ever-growing urbanization, industrial progress, and overall development, natural resources are being affected by pollutants and contaminants, which are causing harmful and toxic effects on natural flora and fauna. In this regard, recycling and remediation seem the need of the hour. However, the approach can only be implemented properly with comprehensive knowledge of the available technologies and their expanding nature of application. For sustainable and futuristic development, the industries must integrate the best remediation process in their plans. Chemicals discharged from industries cover the air, water, and soil; and no singular approach or method can deal with the aftermath they cause. Toxic pollutants can be divided into two broad categories: inorganic and organic. Using existent technologies and processes, the development of state-of-the-art and cost-effective solution to address the ground-level problem is the main challenge. In this context, grouping the physical and chemical applications with existent conventional biological treatment enhances the results in shorter time.

Excessive pollutants in water and soil affect the nutrition level in crops and food and pose serious threat to health. Availability of fresh drinking water is scarce today. To be treated efficiently by bioremediation process, pollutants have to be biologically accessible and enzymatically breakable by microorganism. Favorable environmental conditions make the breakdown faster. But, like all other technologies, bioremediation too has its limitation, i.e., recalcitrant molecules. There, the evolution of recycle and remediation method has enabled to use a combination of different and complex technologies and bring them together for better outcome and faster process. Keeping the urgency of application in mind, bioremediation became the integral part of research, academia, government policies, and industrial planning. With improved, practical, and stricter policies, effective development and multidisciplinary research approach are being encouraged for economical remediation process. With every trial, researchers are trying to build more imperative green economical and efficient treatment process for the application. This book elaborately discussed the waste treatment methods and techniques for water and soil conservation, in aerobic or anaerobic ways, with or without microorganism, with practicality and efficiency.

This book will be useful and applicable for the skilled as well as the beginners in this field. The chapters discuss contemporary techniques and technologies, with elaborate methodologies along with their advantages and disadvantages, which make the book globally relevant.

Ankleshwar, India	Maulin Shah
Guwahati, India	Aditi Banerjee

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About the Editors

Maulin P. Shah is Chief Scientist & Head of the Industrial Waste Water Research Lab, Division of Applied and Environmental Microbiology Lab at Enviro Technology Ltd., Ankleshwar, Gujarat, India. His work focuses on the impact of industrial pollution on the microbial diversity of wastewater, and genetically engineering high-impact microbes for the degradation of hazardous materials. He is an active editorial board member in top-rated journals.

Dr. Aditi Banerjee currently holds an Independent Research Scientist position in the Centre for Nanotechnology, IIT Guwahati, supported by the Department of Science and Technology, Government of India. She started her research career as an environmentalist, focusing on treating petrochemical and refinery generated wastewater by biological methods. She has long expertise in designing and solving environmental-related issues concerning soil, water, and air. Her wide experience transcends into interdisciplinary approaches and application of treating and making wealth from waste. She has published more than 15 international research publications and 2 books.

By-Products from Forest Activities as Low-Cost Sorbents for Bioremediation of Effluents and Other Polluted Media

1

Avelino Núñez-Delgado, Esperanza Álvarez-Rodríguez,
María J. Fernández-Sanjurjo, Manuel Arias-Estévez,
Juan Carlos Nóvoa-Muñoz, David Fernández-Calviño,
and Manuel Conde-Cid

Abstract

In this chapter, we review available data on the use of forest waste and by-products to remove pollutants. We focus on by-products from forest industry due to their proved efficacy for some specific contaminants and also due to the economic and social relevance of forest industry, as well as to the overall repercussion of recycling these waste materials when considering carbon storage and cycling at local and global scales. The treatment of both water and soil pollution is covered, also differencing among individual wastes and among specific pollutants, due to the various responses found regarding pollution removal and/or retention for different contaminants by using each by-product and on each environmental compartment.

Keywords

Bio-sorbents · Forest waste · Remediation · Soil pollution · Water pollution

1.1 Introduction

Waste materials and many by-products have been a matter of concern, and recycling them correctly is still a task deserving great efforts. However, they are also increasingly viewed as an economic (or business) opportunity, implying a need of surveillance and care to give real priority to people and the overall environment

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(Núñez-Delgado 2014, 2015a, b, c). From a technical point of view, it has been shown that it is clearly interesting and promising to recycle both raw and modified waste and by-products (Núñez-Delgado et al. 2015a). And among them, those derived from forest activities can be considered of special relevance, mainly in countries where forest industries are of main importance, as is the case of Galicia (NW Spain) and many other geographic areas and countries around the world.

1.2 Data on the Use of Forest Waste and By-Products as Sorbents in Bioremediation

1.2.1 The Situation in Galicia (NW Spain)

Some forest by-products, as wood ash, have been used to facilitate decontamination of sewage sludge and other waste. In certain cases, it was based on appropriate sludge/ash technical mixing (Pousada-Ferradás et al. 2012), thus increasing sorption and decreasing risks linked to leaching of pollutants. Photograph 1.1 shows original (not previously published) scanning electron microscopy (SEM) images of wood ash included in those waste mixtures.

In other cases, sludge and wood ash were combined with other various waste materials, such as mussel shell calcination ash (Seco-Reigosa et al., 2014), giving final mixtures with strong As and Hg retention potential and also reducing risks of soil and water pollution (see Photograph 1.2 for original SEM images of some of these mixtures).

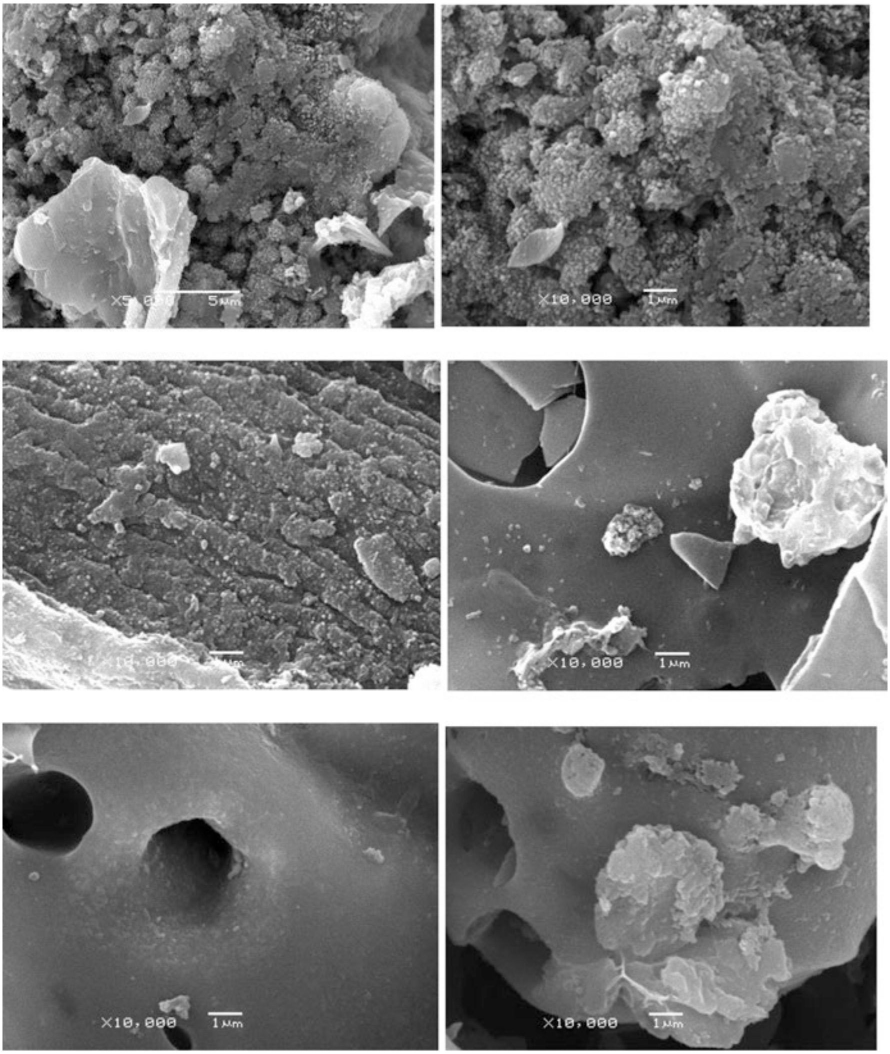
Other treatment alternatives were performed to reach inactivation/retention of abiotic and biotic contaminants through composting, this time including vegetable remains together with sewage sludge and wood ash (Estévez-Schwarz et al. 2009, 2012) (see Photograph 1.3).

Various kinds of forest by-products have been studied to remove different contaminants from polluted media. Photographs 1.4 and 1.5 are original not amplified and SEM images (not previously published) showing two examples of these by-products (pine bark and oak wood ash).

A synthesis of the main results regarding sorption/desorption efficacy is presented in Table 1.1.

Specifically, Seco-Reigosa et al. (2013) found As(V) adsorption higher than 97%, with desorption <1%, using pine wood ash, while lower efficacy was reported for oak wood ash, and it was clearly lower for pine sawdust. Quintáns-Fondo et al. (2017) reported As(V) sorption >87% and <7% desorption for oak ash, while pine bark sorbed <3% and desorbed 100%.

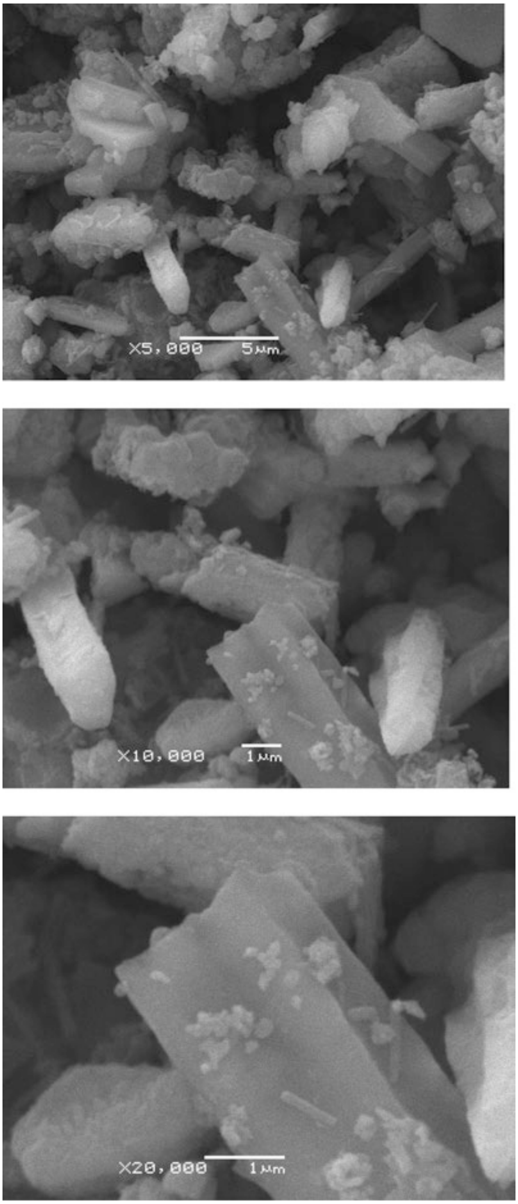
Núñez-Delgado et al. (2015b) studied Cr(VI) retention on pine sawdust and oak wood ash, finding poor results for both materials (sorption 23% and 17%, respectively). Rivas-Pérez et al. (2017) found sorption up to 50% higher for As(V) than for Cr(VI) on oak ash, whereas it was 95% higher for Cr(VI) on pine bark. Regarding the effects of amendments on soil samples, oak ash increased As(V) retention, pine bark increased Cr(VI) sorption, and, in addition, pine bark amendment increased Cr(VI) sorption on pyritic material.



Photograph 1.1 SEM images of wood ash. (Original from authors, not previously published)

Quintáns-Fondo et al. (2016b) studied As(V)/Cr(VI) competitive sorption on pine bark and oak ash, finding more affinity for As(V) sorption in oak ash (sorption up to >84 %), whereas Cr(VI) sorption was clearly higher in pine bark (>90 %). They also found that oak ash could be used to remove high concentrations of As(V), even in the presence of a wide range of Cr(VI) contents, while pine bark could be used to remove high Cr(VI) concentrations.

Romar-Gasalla et al. (2016) studied P retention on pine sawdust and on a mixture including sewage sludge, mussel shell ash, and pine wood ash, with P sorption reaching only around 40% for pine sawdust, whereas clearly better results were



Photograph 1.2 SEM images of waste mixtures including wood ash. (Original from authors, not previously published)

reported for the mixture (70–90% retention). Romar-Gasalla et al. (2018a) found that initial poor P retention on pine sawdust improved with prolonged contact time.

Rivas-Pérez et al. (2015) studied As(V) and P competitive sorption on pine sawdust, and they reported clearly higher affinity for P.

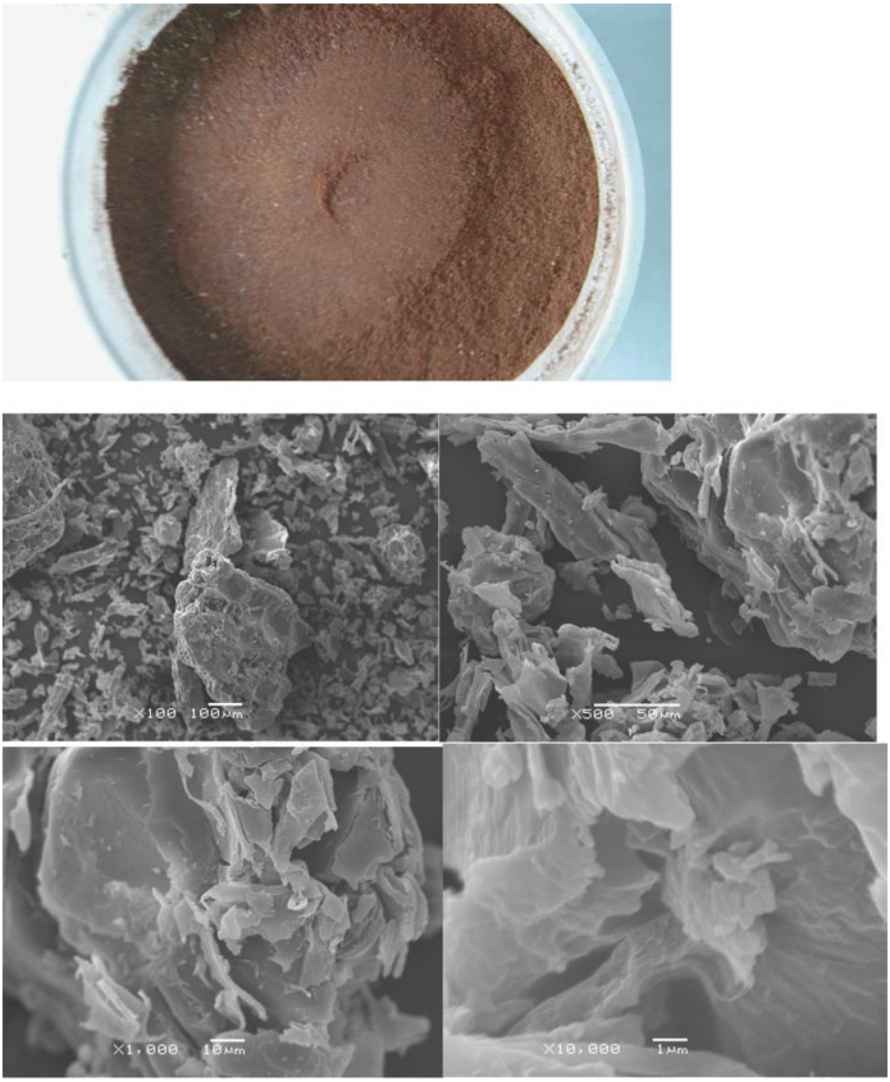
Photograph 1.3 Piles during composting at pilot scale. (Original from authors, not previously published)

Quintáns-Fondo et al. (2016a) studied different forest waste and by-products; one of them was a waste mixture including pine wood ash, which showed >73% sorption capacity for F^-, with poorer results for wood ash alone and for pine sawdust. Quintáns-Fondo et al. (2018) found high F^- sorption on oak wood ash, even at alkaline pH (it was close to 100% at pH < 6, and around 70% at pH 10), while F^- sorption was low on pine sawdust, at any pH. They also reported that competition for sorption sites between As(V) and F^- was almost negligible.

Romar-Gasalla et al. (2018b) found that for pine bark, Cr(VI) sorption was > 97% and desorption <1.5%, while for oak ash, Cr(VI) sorption was < 32%. Regarding F^- retention, sorption was 62–73% and desorption 10–15% in pine bark, whereas in oak ash, sorption was 60–69% and desorption 11–14%. When added to forest and vineyard soils and to pyritic material, both by-products increased F^- sorption and decreased desorption, while pine bark increased Cr(VI) retention on both soils and pyritic material.

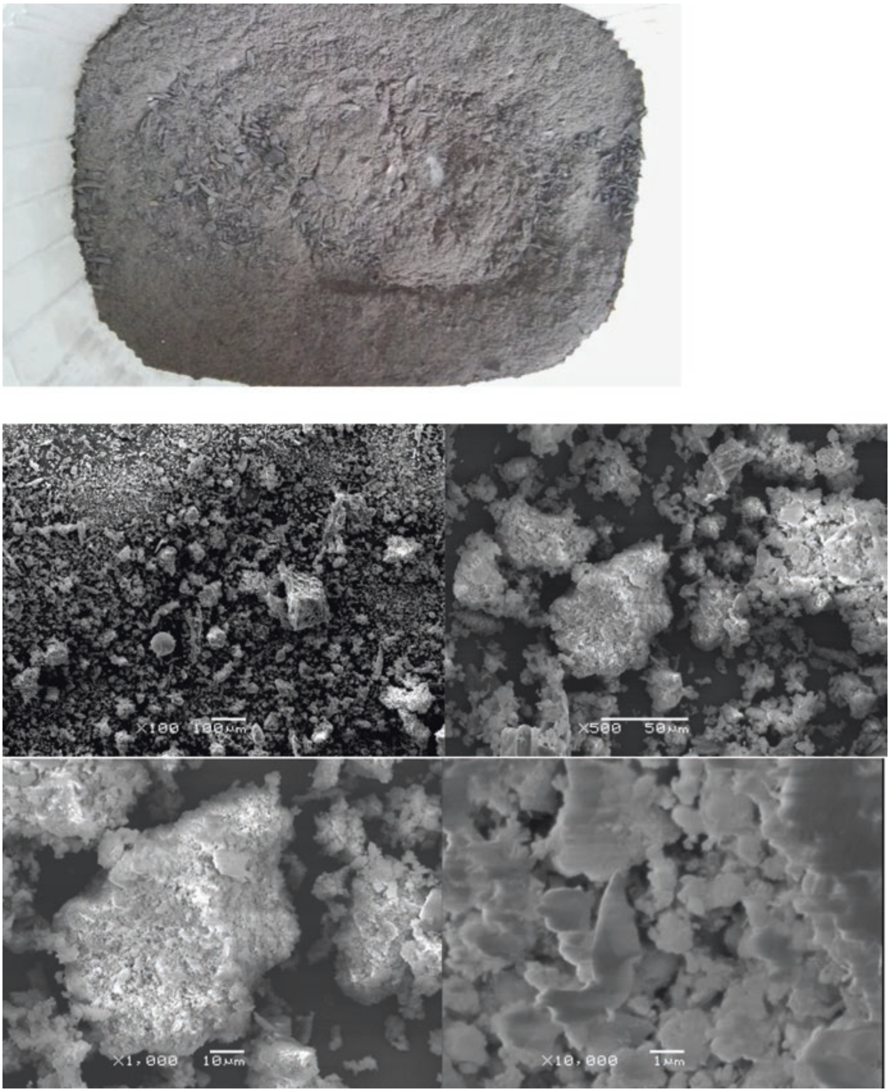
Núñez-Delgado et al. (2017) found that oak ash amendment clearly increased Cd and Pb sorption on soils and pyritic material (reaching near 100%) and decreased desorption, while results were rather low for pine bark amendment.

As regards works that focused specifically on pine bark, Cutillas-Barreiro et al. (2014) found that adsorption was between 98 and 99% for Pb, 83–84% for Cu, 78–84% for Cd, 77–83% for Zn, and 70–75% for Ni, with those differences being



Photograph 1.4 Pine bark: not amplified image and SEM images. (Original from authors, not previously published)

mainly due to different affinity for adsorption sites. In another work, Paradelo et al. (2016) studied the potential of pine bark as adsorbent when heavy metals circulated through it into laboratory columns, finding 100% removal for low metal concentrations (2.5 mM), whereas just 16–43% was retained when the highest metal concentration (25 mM) was assayed. These authors reported a progressive adsorption of the metals entering the column, followed by a significant release when a saturation concentration is reached. Cutillas-Barreiro et al. (2016) studied



Photograph 1.5 Oak wood ash: not amplified image and SEM images. (Original from authors, not previously published)

competitive sorption/desorption for heavy metals in pine bark, with results showing retention in the order Pb>Cu>Zn>Cd>Ni, as well as a strong influence of Pb and Cu on the retention of the other metals. In another work, Cutillas-Barreiro et al. (2017) studied the efficacy of pine bark to control Cu pollution in acid vineyard soils. These authors found that pine bark amendment decreased Cu levels in shoots and roots of grass growing in that environment, mostly due to a significant decrease in the most mobile Cu fractions in the soil. Fernández-Calviño et al. (2017) studied the effects

Table 1.1 Sorption (and desorption) percentages for different pollutants achieved by various authors in Galicia (Spain) using different forest waste and by-products as sorbent materials

Sorbent	Pollutant	% Sorp. (desorp.)	Country	Source
Pine wood ash	As(V)	>97 (<1)	Galicia (Spain)	Seco-Reigosa et al. (2013)
Oak wood ash	As(V)	67–80 (6–8)	Galicia (Spain)	Seco-Reigosa et al. (2013)
Oak wood ash	As(V)	>87 (<7)	Galicia (Spain)	Quintáns-Fondo et al. (2017)
Oak wood ash	Cr(VI)	17	Galicia (Spain)	Núñez-Delgado et al. (2015b)
Oak wood ash	As(V)	>84	Galicia (Spain)	Quintáns-Fondo et al. (2016b)
Oak wood ash	As(V)	91–95	Galicia (Spain)	Rivas-Pérez et al. (2017)
Oak wood ash	Cr(VI)	37–60	Galicia (Spain)	Rivas-Pérez et al. (2017)
Oak wood ash	Cr(VI)	<32	Galicia (Spain)	Romar-Gasalla et al. (2018b)
Oak wood ash	F^-	70–99	Galicia (Spain)	Quintáns-Fondo et al. (2018)
Oak wood ash	F^-	60–69 (11–14)	Galicia (Spain)	Romar-Gasalla et al. (2018b)
Pine sawdust	As(V)	43–60 (32–50)	Galicia (Spain)	Seco-Reigosa et al. (2013)
Pine sawdust	Cr(VI)	23	Galicia (Spain)	Núñez-Delgado et al. (2015b)
Pine sawdust	P	40	Galicia (Spain)	Romar-Gasalla et al. (2016)
Pine bark	Pb	98–99	Galicia (Spain)	Cutillas-Barreiro et al. (2014)
Pine bark	Cu	83–84	Galicia (Spain)	Cutillas-Barreiro et al. (2014)
Pine bark	Cd	78–84	Galicia (Spain)	Cutillas-Barreiro et al. (2014)
Pine bark	Zn	77–83	Galicia (Spain)	Cutillas-Barreiro et al. (2014)
Pine bark	Ni	70–75	Galicia (Spain)	Cutillas-Barreiro et al. (2014)
Pine bark	Cr(VI)	>90	Galicia (Spain)	Quintáns-Fondo et al. (2016b)
Pine bark	As(V)	<3 (100)	Galicia (Spain)	Quintáns-Fondo et al. (2017)
Pine bark	Cr(VI)	95–96	Galicia (Spain)	Rivas-Pérez et al. (2017)
Pine bark	Cr(VI)	> 97 (<1.5)	Galicia (Spain)	Romar-Gasalla et al. (2018b)

(continued)

Table 1.1 (continued)

Sorbent	Pollutant	% Sorp. (desorp.)	Country	Source
Pine bark	F^-	62–73 (10–15)	Galicia (Spain)	Romar-Gasalla et al. (2018b)
Pine bark	Cr(VI)	99	Galicia (Spain)	Paradelo et al. (2017)
Waste mixture	P	70–90	Galicia (Spain)	Romar-Gasalla et al. (2016)
Waste mixture	F^-	>73	Galicia (Spain)	Quintáns-Fondo et al. (2016a)

of both pine bark amendment and ageing in mine soils, finding a decrease in the soluble fractions and an increase in less mobile fractions for Cu and Pb, as well as a decrease in metal release. Paradelo et al. (2017) showed that pine bark has very high retention potential for dichromate (sorption close to 100% and less than 2% desorption), but very low retention capacity for phosphate, arsenate, or fluoride, with different mechanisms governing sorption of the various anionic pollutants. Fernández-Calviño et al. (2018) studied the effect on microbial function due to pine bark amendment on Cu-polluted mine soils, finding that pine bark increased fungal growth throughout the 2-year period of the study. In addition, the use of 1:1 pine-bark/mussel-shell mixtures favored growth for both bacteria and fungi.

1.2.2 Overall Situation on a Global Basis and Future Perspectives

Considering data from different countries around the world, just 26 results were provided when searching for “Forest by-products AND Remediation” on Web of Science (WOS) (Clarivate Analytics), while searching for “Forest Waste AND Remediation,” the number of results was 790. For the latter, WOS tools show that 37–53 papers were published yearly, considering from year 2010 to 2015, then reaching a maximum of 98 papers during 2016, decreasing to 95 in 2017, and to 66 in 2018. By the end of March 2019, 5 new papers were published on the matter. Regarding countries, 141 papers were from the USA, 95 from China, 58 from Spain, 57 from India, 41 from Italy, 40 from Canada, 40 from Germany, 38 from Australia, 34 from Brazil, and 32 from Poland.

However, when searching for “Forest Waste AND Remediation” on Google Scholar (GS), a higher score of 66,400 results was provided. Even when searching for “Forest by-products AND Remediation” on GS, the number of results was 9810, clearly higher than that from WOS. To view this last number in more detail, 25 results were found till 1980, 90 results between 1981 and 1990, 700 results between 1991 and 2000, 1010 results between 2001 and 2005, and 1360 results between 2006 and 2009. Considering the same years as in the report from WOS, GS found 433 results in 2010, 500 results in 2011, 551 in 2012, 612 in 2013, 689 in 2014, 720 in 2015, 796 in 2016, 806 in 2017, 815 in 2018, and 212 in 2019 (till the end of March), which means that, contrary to WOS, GS did not find a decreasing trend for any interval or specific year.

In previous reviews, Chen et al. (2011) reported a great potential for plant residues (including forest waste) for polycyclic aromatic hydrocarbon removal from wastewater. In a more general manner, these authors commented that raw and modified plant residues are a promising choice for organic pollutant treatment due to their low cost, environmental compatibility, high sorption capability, and ubiquity in the environment.

Saka et al. (2012) indicated that the use of forest (and agricultural) waste adsorbents to remove heavy metals (and specifically Pb^{2+}) from wastewaters has clearly grown, mainly due to the fact of being inexpensive, biodegradable, abundant, and efficient, making them very attractive compared to activated carbon.

Bilal et al. (2013) indicated that forest (and agriculture) waste bio-adsorbents showed high potential for Cu removal, which was due to remarkable adsorption capacities, low cost, and availability, as well as to their mono- to multilayer adsorption behavior. These authors found the following Cu adsorption sequence for different bio-sorbents (including forest by-products): activated carbon > algal > bacterial > agriculture and forest > fungal > yeast biomass. Regarding mechanisms, Bilal et al. (2013) commented that ion exchange and complexation are the two most important processes affecting metal uptake by bio-adsorbents. Finally, they indicated that the major challenges for bio-adsorption are appropriate reuse and chemical modifications of bio-adsorbents.

In a review paper dealing with plant-based biomass (including forest waste), Jain et al. (2016) indicated that these materials remove efficiently diverse toxic metals from water/wastewater. These authors also indicate the need of pilot studies at industrial scale, as well as the development of efficient and economical bio-sorbents for wastewater treatment with commercial level properties.

Focusing on reutilization of sorbents, Reddy et al. (2017) indicated that emerging post-sorption technologies are allowing adsorption products to subsequently be used as fertilizers, in catalysis, in carbonaceous metal nanoparticle synthesis, as feed additives, and as biologically active compounds.

Pyrzynska (2019) reviewed the use of low-cost adsorbents (including forest waste) for Cd removal. This author indicated that although modified bio-sorbents showed better adsorption capacities than those unmodified, these procedures increased cost, reducing their advantages compared to conventional adsorbents and also raising serious environmental questions on eventual generation of toxic wastes. The author indicated that comparing techno-economically low-cost adsorbents and activated carbon, higher effectiveness was found for green low-cost materials, taking into account cost of synthesis and regeneration. Pyrzynska (2019) also commented on the lack of results at pilot plant scale, which should be solved to promote large-scale use of these low-cost adsorbents.

1.3 Conclusions and Future Trends

In this book chapter, we reviewed works carried out in Galicia (NW Spain) regarding the use of forest waste and by-products to remove contaminants from polluted solid and liquid environments, notably soil and water. Most works focused on heavy metals/metalloids, while others dealt with P and F^-. This chapter put together data from a number of papers, considering both sorbents and pollutants, showing which sorbents are effective (and in which extent) for the removal of different contaminants. Considering the matter globally, searching tools for academic and research works find a huge amount of results, and these tools show differing results. In one case, the conclusion would be that the number of research papers is decreasing since 2016, while another tool shows a continued progressive increase. In any case, it is clear that the scientific community and the society as a whole are concerned about effective recycling of forest waste, and most actors agree on the convenience of promoting research and use of raw (or in certain cases modified) forest by-products to fight environmental pollution affecting soil and water bodies. Recently, an increasing interest is focusing on the efficient reuse of already utilized sorbents, which also deserves more future research.

As regards contaminants to be treated, the authors of this chapter consider that forest by-products could be very interesting as active materials for the retention/removal of antibiotics and other emerging pollutants from waters, sewage sludge, or soils receiving the spreading of polluted waters and/or sludge.

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2

Exopolysaccharides: Production and Application in Industrial Wastewater Treatment

Ratna Trivedi

Abstract

In recent decades, polysaccharide molecules of plant, bacterial, and fungal origin have been extensively researched due to their multi-functionality. Various kinds of polysaccharides produced by plants, viz., cellulose, pectin, and starch; algae, viz., agar, alginate, and carrageenan; and bacteria, viz., alginate, dextran, gellan, pullulan, and xanthan gum), are commonly used as food additives for their gelling, stabilizing, or thickening properties (Sutherland IW. Microbiology-SGM 147:3–9, 2001a). Both prokaryotes and eukaryotes are able to produce exopolysaccharides (EPSs); prokaryotes such as eubacteria and archaebacteria and eukaryotes such as phytoplankton, fungi, and algae have now got more research attention (Houghton J, Quarmby J, Stephenson T. Water Sci Technol 44(2):373–379, 2001). Bacteria carry the EPS as their metabolic products which accumulate on the cell surface (Kumar AS, Mody K, Jha B. J Basic Microbiol 47:103–117, 2007a). The composition of EPS has a variety of organic and inorganic substances which have structural variable like either homopolysaccharides such as dextran, mutan, and levan or heteropolysaccharides. Application of various nano material, prepared as bionanoparticles, helpful to treat industrial waste water.

Keywords

Exopolysaccharide · Prokaryotic polysaccharide · Eukaryotic polysaccharide · Bionanopaticles · Industrial waste water treatment

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2.1 Introduction

In recent decades, polysaccharide molecules of plant, bacterial, and fungal origin have been extensively researched due to their multi-functionality. Various kinds of polysaccharides produced by plants, viz., cellulose, pectin, and starch; algae, viz., agar, alginate, and carrageenan; and bacteria, viz., alginate, dextran, gellan, pullulan, and xanthan gum), are commonly used as food additives for their gelling, stabilizing, or thickening properties (Sutherland 2001a). Both prokaryotes and eukaryotes are able to produce exopolysaccharides (EPSs); prokaryotes such as eubacteria and archaebacteria and eukaryotes such as phytoplankton, fungi, and algae have now got more research attention (Houghton et al. 2001). Bacteria carry the EPS as their metabolic products which accumulate on the cell surface (Kumar et al. 2007a). The composition of EPS has a variety of organic and inorganic substances which have structural variable like either homopolysaccharides such as dextran, mutan, and levan or heteropolysaccharides. Application of various nano material, prepared as bionanoparticles, helpful to treat industrial waste water.

Perhaps, biomolecules from plants now have captured the commercial market for their ease of availability and cost-effective purification process. But renewability, stable cost, and constant and reproducible physicochemical properties of the microbial polysaccharides have provided them an edge over the macromolecules of plant origin, although only few of them have been commercialized so far (Poli et al. 2009; Reichhardt and Cegelski 2014). Exopolysaccharides (EPS) are polymers excreted by some microorganisms as a protective barrier against harmful conditions. Many microbial EPS, such as xanthan or gellan gums, isolated from terrestrial sources are being successfully exploited in several industries. Indeed, EPS can be used in a wide range of biotechnological applications, such as thickening agents, stabilizers, and texturizers in the food industry, flocculating agents in the wastewater treatment industry, or anti-aging molecules in the cosmetics industry.

EPSs have a significant influence on the physicochemical properties of microbial aggregates, including structure, surface charge, flocculation, settling properties, dewatering properties, and adsorption ability. EPSs bind with cells through complex interactions to form a vast net-like structure with plenty of water that protects cells against dewatering (Sutherland 2001a) and the harm of toxic substances. Part of EPS can serve as carbon or energy sources in conditions of nutrient shortage (Banik et al. 2007; Manjamadha and Muthukumar 2016). They also accelerate the formation of microbial aggregates through binding cells closely (Lepek and D'Antuono 2005). Thus, the in-depth study of EPS is a matter of great interest not only in terms of improving our comprehension of biological wastewater treatment, but also improving the efficiency of such treatment through the optimization of operational parameters.

Bacterial cells are capable to produce two different types of extracellular polysaccharides which bind either to the bacterial cell surface tightly, defined as capsular polysaccharides (CPS), or they can be excreted into the extracellular surrounding environment, described as exopolysaccharides (EPS) which can be lightly attached to the bacterial cells or totally detached from the bacteria. A large and growing body of literature has labelled these two types of extracellular polysaccharides with the

Table 2.1 Microbial EPS and its structure

Bacteria	EPS	Chemical structure
Pseudomonas aeruginosa, *Pseudomonas putida*	Alginate	β-1,4-linked mannuronic and α-1,4-linked guluronic acids
Acetobacter xylinum	Bacterial cellulose	β-1,4-linked glucose
Sphingomonas paucimobilis	Gellan	β-d-glucuronic acid-1,4-β-d-glucose-1,4-β-l rhamnose
Leuconostoc mesenteroides	Dextran	α-1,6-linked glucose; some 1,2-, 1,3-, or 1,4-bonds are also present in some dextrans
Alcaligenes faecalis	Curdlan	β-1,3-glucan
Xanthomonas campestris	Xanthan	Linear β-1,4-glucan backbone with β-mannose-1,4-β-glucuronic acid-1,2-α-mannose trisaccharide side Chain
Bacillus polymyxa	Levan	β-2,6-fructan

term exopolysaccharides (EPS) (Broadbent et al. 2003). The main examples of the applications of microbial exopolysaccharides are listed in Table 2.1.

The application of these structural features has great demand in the natural components of bacterial EPS to gain a special interest in recent years in chemistry, medicine, pharmaceuticals, and especially the food industry (Jin et al. 2004). Several functions are attributed to the bacterial EPS such as protecting bacterial cells from desiccation and the environment, antibiotics, phagocytosis, and phage attacks, and they are also believed to play a role in biofilm formation (Kumar et al. 2007b). Besides the role of EPS at the single cell level, they are widely used in the food industry as viscosifying, stabilizing, and emulsifying agents because of their unique physicochemical properties related to their structures (Dhayalan et al. 2017). Dextran, xanthan, gellan, pullulan, yeast glucan, and bacterial alginate are some examples of microbial EPS used in the food industry for decades to improve the physicochemical properties of food formulations (Sutherland 1998). There are many other examples of the technological applications of EPS in food or non-food industries. Recently, EPS also gained special interest after the recent reports showing that EPS may stimulate and modulate the immune system and they may play a role as antitumor, antiviral, anti-inflammatory, and antioxidant agents (Lepek and D'Antuono 2005; Liu et al. 2007).

2.2 Types of EPS

Microbial EPSs are classified into two groups according to their chemical composition, as either homopolysaccharides or heteropolysaccharides, and show a wide diversity in their structures. Homopolysaccharides are composed of the same sugar submits in their repeating unit structure with different linkages (Mikkelsen and Keiding 2002; Mu and Yu 2006). Homopolysaccharides can be part of the capsule layer of Gram-positive and Gram-negative bacteria such as oral *Streptococcus* spp. and *Escherichia* spp.; they can also be secreted to the environment as a slime material like the bacterial cellulose produced by *Acetobacter* spp. One of the most important examples of the microbial homopolysaccharides is dextran produced by *Leuconostoc mesenteroides* which has been found to have a wide range of applications in medicine (Kanmani et al. 2011). Several lactic acid bacteria (LAB) also produce homopolymeric EPS which will be discussed later in this section. Heteropolysaccharides are made of repeating units which are composed of two or more types of sugar subunits, substituted sugars, and other organic and inorganic molecules (Broadbent et al. 2003; Cheng et al. 2011). A general example of microbial heteropolysaccharide is peptidoglycan, which is the main component of bacterial cell walls and is composed of the repeating units of N-acetylglucosamine and N-acetylmuramic acid residues.

The polysaccharides produced by microorganisms can be classified into three main groups according to their location in the cell: (1) cytosolic polysaccharides, which provide a carbon and energy source for the cell; (2) polysaccharides that make up the cell wall, including peptidoglycans, teichoid acids, and lipopolysaccharides; and (3) polysaccharides that are exuded into the extracellular environment in the form of capsules or biofilm known as EPSs (Freitas et al. 2012; Kalogiannis et al. 2003). Exocellular polysaccharides constantly diffused into the cell culture medium are easily isolated, while cell wall and intercellular polysaccharides are more difficult to separate from cell biomass. The intracellular biopolymers are few and have very limited use. The external cellular structure capsule has covalent bonds and cohesive layers while slime is completely excreted into the environment. Extracellular polymers are polysaccharides in more than 95% cases. Unattached EPS production is especially valuable for biotechnological industry as there is no need for costly procedures for removing cell debris.

2.3 Composition and Structure

Carbohydrates and proteins are usually found to be the major components of EPS. Humic substances may also be a key component of the EPS in sludge in biological wastewater treatment reactors, accounting for approximately 20% of the total amount. In addition, lipids, nucleic acids, uronic acids, and some inorganic components have also been found in EPS from various matrixes. Their fractions in EPS depended strongly upon the extraction methods and the sludge origins. The content and compositions of the EPS extracted from various microbial aggregates are reported to be heterogeneous (Sutherland 2001a). The variation in the compositions of the extracted EPS is attributed to many factors, such as culture, growth phase, process parameter, bioreactor type, extraction method, and analytical tool used (Fig. 2.1).

Different numbers of chemical structures of bacterial EPSs are available; in general, they are heteropolysaccharides possessing three or four different monomers organized in groups of a set of 10 or less to give the repeating units. The molecular weight of these polymers is between 1×10^5 and 3×10^5 Da and they are linear too. In addition to monosaccharides, they could possess substituents such as acetate, pyruvate, succinate, phosphate, and sulfate; the presence of uronic acids or ketal-linked pyruvate resulted in polyanionic chains. The most frequent monosaccharides found in EPSs are hexoses,

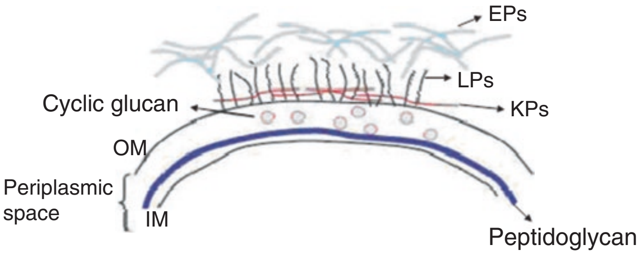

Fig. 2.1 Schematic representation of bacterial surface polysaccharides. *EPS* exopolysaccharides, *KPS* exopolysaccharides attached to the bacterial surface, *LPS* lipopolysaccharides, *IM* cell internal membrane, *OM* cell outer membrane (Lazaridou et al. 2002)

pentoses, uronic acids, and amino sugars which bind each other by 1,4-β or 1,3-β linkages in the strong rigidity polymer structure while the 1,2-α- or 1,6-α linkages are generally present in the flexible polysaccharides (Freitas et al. 2009).

The position in EPS structures is very important to explain the physicochemical and biological properties of these biopolymers and to characterize and in some case to predict the biotechnological applications of the EPS-producer microorganisms or of their bioproducts. Numerous chemical and physical techniques are used to determine the primary structure of EPSs such as chemical degradation and derivatization, in association with chromatographic methods together with mass spectrometry analysis; some are used to determine the sugar composition, their absolute configuration, and the presence and the position of possible substituents (Pan and Mei 2010). With the help of rheological properties of these polymers, the primary conformation can be easily detected. Moreover, secondary configuration regularly takes the form of aggregated helices, and the presence and the absence of specific acyl groups, for example, O-acetyl or O-succinyl esters or pyruvate ketals, can influence the formation of ordered helical aggregates.

2.4 Production of EPS

2.4.1 Conditions for Microbial Production of EPS

Fermentation is a very versatile technique for producing value-added products such as microbial EPS production. All fermentation conditions like medium composition, pH, temperature, aeration, as well as mode of operation are known to have a high impact upon the viability and economics of the bioprocess; their optimization is compulsory in designing a commercial bioprocess (Wilen et al. 2003). Moreover, structural features and related physicochemical and rheological properties of the EPS are largely determined according to the metabolic requirements of the microorganism and also by conditions of the fermentation; therefore, each microbial system should be optimized individually by avoiding generalizations. Fermentation process involves conventional methods like batch, fed-batch, and continuous modes of operations, but in this production, drastic changes in rheological properties of the microbial culture, for example, highly viscous and non-Newtonian broth, may result in great problems in mixing, heat transfer, and oxygen supply (Palaniraj and Jayaraman 2011).

These are the challenges encountered in the microbial production of pullulan and xanthan, but are not encountered in the production of low-viscosity polymers such as levan or in high-temperature processes where thermophiles are utilized as microbial producers. Media for fermentation utilizes almost one-third of the opposed to production costs, and particularly the carbon source which is used as the fermentation feedstock has the greatest cost. Irrespective of whether the production is either at laboratory scale or large industrial scale, media with a high carbon to nitrogen ratio are used owing to the carbon-and energy-intensive nature of the process. Intended to biopolymers with high economic values, consistency in product quality relative to production yield becomes vital, which in turn needs chemically distinct medium conditions. In addition to that, recent efforts are mainly devoted to maintain both product quality and yield by using cost-effective and environmentally friendly production methods that employ inexpensive fermentation substrates (Rijnaarts et al. 1999). In consequence, a wide variety of industrial and agricultural waste and by-products are used in many industrial fermentation products like nutrients such as molasses and syrups, wastewater of olive oil pill, whey of cheese, pomade of various vegetables and fruits, pulp and kernels, lignocellulosic biomass like hall rice and bran, sawdust, and fibers (Poli et al. 2013). Utilization of such complex feedstocks requires intensive research activities for the development of feasible pretreatment, fermentation, and downstream processing techniques. From these, syrups and molasses have long been used for microbial production of various polysaccharides such as xanthan, dextran, pullulan, gellan, and levan due to their high sucrose and other nutrient contents, low cost and ready availability, and ease of storage (Morgan et al. 1990; Kalogiannis et al. 2003; Liu et al. 2004; Poli et al. 2013). Another promising resource is carbon dioxide since it is abundant, renewable, nontoxic, and non-flammable. Microalgae due to their high-CO_2 fixation capacity and fast growth served as established resources in next-generation biofuel technology. Moreover, use of microalgae in EPS production is a quite recent subject with only limited number of reports; however, controlled cultivation systems provide high yield production and also required photobioreactors that are very much expensive and energy intensive when compared with open systems (De Vuyst and Degeest 1999).

Subsequently in the microbial fermentation, the EPS is separated from the culture broth by first eliminating the cellular biomass via centrifugation or filtration. Then, the polymer in clarified medium is precipitated by using a suitable organic solvent like ethanol, acetone, or methanol. The polymer pellet can be dried by lyophilization or heat treatment of obtained crude polymer powders. For higher levels of purity, the pellet is dissolved in suitable solvent (usually water) and then either subjected to additional round of solvent precipitation or dialysis or a combination of these followed by a final drying step (Wingender et al. 1999).

2.4.2 Gene, Genetics, and Gene Expression of Regulation of Microbial Polysaccharide

The production of microbial EPS is usually not confined to just one type of EPS but a mixture of various polymers, each being expressed and produced by a certain gene cluster. Usually, the convenience of the precursors encoded by these genes has a high impact on the yield and structure of the EPS produced by the cell (Parolis et al. 1996).

As a systematic approach for biosynthetic pathways of EPS, microbial genome sequence is considered as a starting point, and from this point of view, next-generation sequencing (NGS) technologies play a vital role by enabling high-throughput genomic data at very high speed with a relatively low cost. Such approaches either aim to elucidate biosynthesis mechanisms, to improve the microbial productivity via strain improvement strategies, or to modify physicochemical and/or rheological properties of the biopolymer by changing its composition, length, or degree of branching (Toksoy Öner 2013).

Current research helps to analysis of a genus *Bifidobacterium* revealed high variability in both the number and organization of the EPS biosynthetic gene by comparative genomics, which in turn suggested that these genes were most probably acquired by horizontal transfer (Gauri et al. 2009). Comparisons of six genomes of *Crocosphaera watsonii* strains were studied with production of high-level EPS, where high-level EPS producers were found to retain the EPS biosynthesis gene clusters (Bench et al. 2013). So from the above study, the location of these gene clusters is usually on the chromosome, whereas in most LAB as well, these genes are found to be confined to plasmids.

Sequence data of a taxonomically close species could also be used for systems-based studies while the whole genome sequence of the EPS-producer microorganism is not available. Construction of metabolic model for levan production by halophilic strain *Halomonas* spp., where first, the available whole genome sequence of a taxonomically close microorganism, *Chromohalobacter salexigens* After it was used (Ateş et al. 2013), as a model for recruitatin and adoptation to the producer strain via integration of the available biochemical, physiological, and phenotypic features of *Halomonas* spp. With metabolic system analysis of this generic metabolic model, significant improvement in levan yields was obtained (Ateş et al. 2011).

EPS Application in Wastewater Treatment

EPSs cover the surface or fill in the interior of cells of microbial aggregates during wastewater treatment of vessels. Li and Ganczarczyk (1990) noted that in the interior of activated sludge flocs with amorphous-phase surrounding cells, the presence of plenty of EPS was observed (Li and Ganczarczyk 1990). This recommends that substrate must pass through the EPS layer for proper biochemical processes to the cells. The substrate efficiency is affected by many factors, viz., mass transfer, pores in granular sludge, and chemical nature of EPS (Liao et al. 2001; Vanhaverbeke et al. 2003). Normally, water has high component diffusion coefficients compared