0273-2289/06/129–132/130–152/$30.00

Biofiltration Methods for the Removal of Phenolic Residues

LUIZ CARLOS MARTINS DAS NEVES,[1] TÁBATA TAEMI MIAZAKI OHARA MIYAMURA,[1] DANTE AUGUSTO MORAES,[1] THEREZA CHRISTINA VESSONI PENNA,[1] AND ATTILIO CONVERTI*,[2]

[1]Departamento de Tecnologia Bioquímico-Farmacêutica, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, SP, Brazil; and [2]Università degli Studi di Genova, Dipartimento di Ingegneria Chimica e di Processo, Genoa, Italy, E-mail: converti@unige.it

Abstract

Industrial effluents from the pharmaceutical industry often contain high concentrations of phenolic compounds. The presence of "anthropogenic" organic compounds in the environment is a serious problem for human health; therefore, it merits special attention by the competent public agencies. Different methods have been proposed in the last two decades for the treatment of this kind of industrial residues, the most important of which are those utilizing absorption columns, vaporization and extraction, and biotechnological methods. Biofiltration is a method for the removal of contaminants present in liquid or gaseous effluents by the use of aerobic microorganisms, which are immobilized on solid or porous supports. Although several bacteria can utilize aromatic compounds as carbon and energy source, only a few of them are able to make this biodegradation effectively and with satisfactory rate. For this reason, more investigation is needed to ensure an efficient control of process parameters as well as to select the suited reactor configuration. The aim of this work is to provide an overview on the main aspects of biofiltration for the treatment of different industrial effluents, with particular concern to those coming from pharmaceutical industry and laboratories for the production of galenicals.

Index Entries: Biofiltration; phenolic residues; bioremediation.

Introduction

Environmental biotechnology is the science that uses biological systems for the destruction of pollutants from industrial effluents or present in the environment. Such technologies show a certain number

*Author to whom all correspondence and reprint requests should be addressed.

of advantages if compared with the traditional chemical and physical methods because of their low cost and impact on the environment *(1,2)*. Through these biotreatments, the organic pollutants are biodegraded to inorganic compounds such as carbon dioxide and water, whereas the traditional methods (vaporization, adsorption, extraction, and so on) imply their simple transfer to other compartments of the environment. The treatment by activated sludge has been a common practice to remove pollutants from industrial liquid effluents for more than 80 yr *(3)*, whereas soil, river, and sea bioremediation goes back only to the 1990s *(4–6)*. The most recent studies on these technologies, dealing with bacteria isolation, their classification and physiological characterization, and molecular analysis of the enzymes responsible for pollutant degradation led to the construction of so-called "super-microorganisms" able to degrade various types of pollutants. They are usually employed as consortia of microorganisms directly isolated from the polluted environment, without any characterization or physiological investigation.

There are two different ways to develop environmental biotechnology studies. According to the former, laboratory-scale studies are focused to the isolation and investigation of the bacterium responsible for the pollutant degradation; although the other has the practical aim of making the treatment method effective and optimizing the related process parameters. A combination of these two approaches is needed to make environmental biotechnology able to overcome the present difficulties associated to the use of these technologies, i.e., the insufficient number of bench-scale studies needed to interpret the phenomena occurring during the treatment and the complexity of systems using more than one microorganism for the effluent treatment.

To obtain satisfactory results with these technologies, it is necessary to get a thorough knowledge of the biodegradation process, to control the process conditions, and to optimize the construction of both the biofilter and the support material *(7,8)*. Cell immobilization is a fundamental step for the success of the process; therefore, the correct choice of the support material is necessary mainly to avoid possible phenomena responsible for high head losses in the bed as well as to increase microbial activity and operative steady-state stability.

The present advances in the isolation, selection, and construction of strains or consortia of various microorganisms, mainly bacteria, have extended the biodegradation processes to the treatment either of anthropogenic or xenobiotic compounds. The basis for the development of an efficient biofiltration process is the capability of microorganisms to adapt themselves to new substrates and the ability of some of them to use xenobiotics.

The isolation of microorganisms able to degrade pollutants is usually done from natural microbial populations by batch cultivations using

enriched media containing high pollutant levels. Alternatively, bacteria with high affinity for the polluting substrate can be isolated by continuous cultivation.

Another interesting technique to isolate microorganisms is the dilution of the contaminated effluent with uncontaminated effluent. Marine oligotrophic bacteria have been successfully isolated by this technique consisting in the dilution of seawater with sterile seawater (containing low concentrations of organic nutrients), and pure cultures might be obtained *(9)*. These isolation protocols promoted the discovery and utilization of new microorganisms exhibiting different genotypes and phenotypes with respect to those obtained by the traditional batch cultivation *(10)*.

When compared with traditional techniques, biological means are generally friendlier for the treatment of such residues, because they are usually cheaper and release fewer byproducts. Among the biological methods, biofiltration has been increasingly proving to be particularly effective for the treatment of organic compounds at low concentrations. It is a technology for the biological removal of contaminants using aerobic microorganisms immobilized in solid and porous supports, which can be used for the destruction either of gaseous or liquid pollutants. Table 1 shows the different types of pollutants removed by biofiltration from effluents of different industrial sectors, and Table 2 indicates the degradability of the main polluting compounds.

The main contaminants which can be removed by biofiltration with excellent yields are butadiene, cresols, ethyl benzene, xylene, trimethylamine, alcohols (butanol, ethanol, methanol, and so on), esters, ethers, ketones, organic acids, methyl mercaptan, and some inorganic acids (hydrogen sulfide, hydrochloric acid, and hydrofluoric acid), among others *(11)*.

Studies have been developing on applications of biofilters for the removal of phenols and chlorophenols *(12)*, toluene *(13,14)*, styrene *(15)*, and benzene *(16)*. In all studies, the effluents under investigation were present either in liquid or in gaseous phase, and the reported results seem to be satisfactory for large-scale application of such a technology for the treatment of these effluents.

Several studies dealt with continuous *(17)* and batch processes *(18,19)*, performed either using fixed- *(15,16)* or fluidized-bed reactors *(18,20)*, hence suggesting that the choice of the process and reactor configuration can be influenced by the residue to be treated and the microorganism employed. The most commonly used microorganisms, belonging to the genera *Pseudomonas* sp., *Acinetobacter* sp., and *Bacillus* sp., are immobilized onto different types of inert supports (spheres of glass, calcium alginate, and resins) *(11)*. New immobilization procedures, employing cheaper materials, such as sugarcane bagasse and other solid residues, could be an interesting alternative to further reduce the operating costs of biofiltration *(16)*.

Table 1
Different Types of Pollutants Removed by Biofiltration From the Effluents of Different Industrial Sectors

Industries	Aliphatics	Aromatics	Oxygenated	Sulfuric	Nitrogenous	Halogenated	Inorganics
Foundries	X	X	X				X
Plastics	X	X	X		X		
Lacquer	X	X	X				
Adhesive		X	X				
Oil and fat	X	X		X		X	
Polyester				X	X		
Friction-linings			X	X	X		X
Glue		X	X				
Composting and waste processing	X	X	X	X	X	X	X
Sewage treatment and sludge drying	X	X	X	X	X	X	X

Table 2
Classification of the Main Polluting Compounds According to Their Biodegradability Using Biofiltration

High	Good	Low
Acetaldehyde, acetone	Acetonitrile, isonitriles	Dichloroethane
Butyric acid	Amides, benzene	Dichloromethane
Hydrochloric acid	Chlorophenols	Dioxane
Hydrofluoric acid	DMS	Carbon disulfide
Hydrogen sulfide	Styrene, hexane	Methane
Ammonia, sulfates	Phenols, toluene	Pentachlorophenol
Butadiene, butanol	Methylisobutylketone	Pentane
Ethanol, methanol	Pyridine	Perchloroethylene
Formaldehyde, cresols	Thiocyanates	Carbon tetrachloride
Ethyl acetate	Thioethers, thiophene	Trichloroethane
Ethyl benzene		Trichloroethylene
Nitrocompounds	Uncertain	
Tetrahydrofurane	Acetylene	Very low
Trimethylamine	Isocyanates	1,1,1-Trichloroethane
Xylenes	Methylmethacrylate	

Biofiltration History

The first reports about the application of biofiltration, going back to 1950s in Germany and 1960s in United States, respectively, demonstrated its ability to remove odors (sulfides, ammonia, and so on). Such biofilters were applied to the control of odors from industrial effluents, plants for waste thermal treatment, activated sludge systems, and so on *(21–23)*. In 1991, the biofilters operating in United States and Canada were less than 50, whereas more than 500 biofilters were present in Germany and the Netherlands. In the same period, this technology had a certain success in Japan where the number of biofilters grew from 50 to 90. Although the biofilters were initially designed for the deodorization of industrial residues, they are growingly employed since the 1980s for the control of pollutant emissions.

Advantages and Drawbacks of Biofiltration

Most of the research-work was carried out to demonstrate the economic feasibility of biofiltration methods, particularly that of Jäger and Jager *(24)*. The biofiltration effectiveness is related to the bed and the distribution of the effluent inside the reactor *(25)*. Thistlethwayte et al. *(26)* described a biofiltration method for the purification of air contaminated by industrial residues containing sulfur, ammonia, and alcohols, able to ensure removals from 40 to 100%. Helmer *(27)* utilized a biofilter with humidified earth as a support to extract anaerobic fermentation products

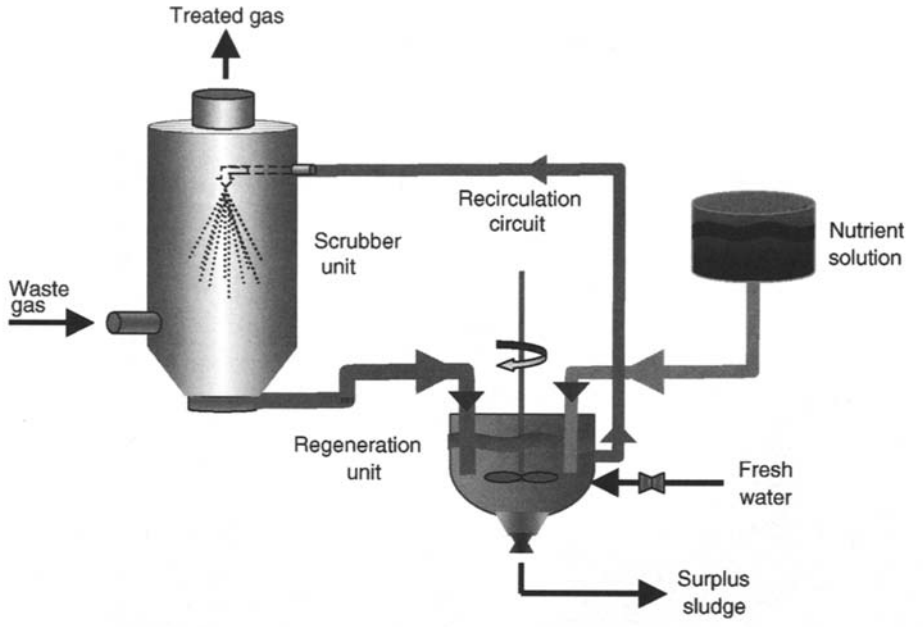

Fig. 1. Schematic setup of a bioscrubber for the removal of gaseous contaminants.

(ammonia, sulfides, alcohols, and aldehydes) from organic materials. Among the main advantages of the use of biofilters, there are the low energy needs because of moderate pressure and temperature, easy control and management, low operating costs, and possibility of performing the process in continuous mode for long time with a very few maintenance and cleaning requirements.

The main advantage of biofiltration is that it does not imply any transfer of the pollutant from one environment compartment to the other, as it occurs with most of the traditional technologies. Through biofiltration, the pollutants are in fact transformed to products belonging to natural cycles without imparting any secondary pollution. On the other hand, the biodegradation of organic compounds by pure microbial cultures can release several toxic intermediates; this problem can become even more serious when using complex microbial consortia as a result of the formation of a wide spectrum of metabolites *(19)*.

Biofiltration and Bioscrubbing

Bioscrubbers (Fig. 1) and biofilters (Figs. 2 and 3) are the main technologies developed for the biological treatment of pollutants.

Bioscrubbing can be defined as a methodology by which microorganisms utilized for the degradation are freely dispersed in the liquid phase (cultivation medium) of the treatment system, whereas they are immobilized in semi-inert supports in biofilters. In conventional bioscrubbers, the scrubber unit consists, in the case of gaseous pollutants, of an absorption

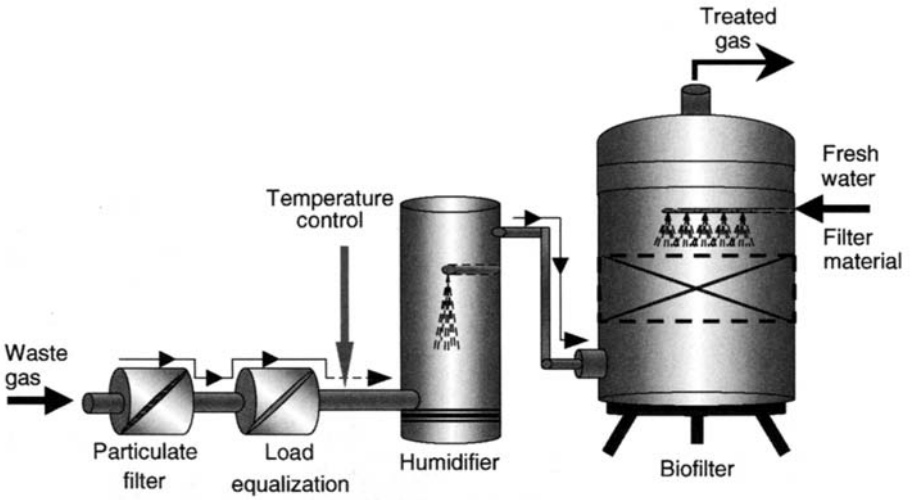

Fig. 2. Schematic setup of a biofilter for the removal of liquid contaminants.

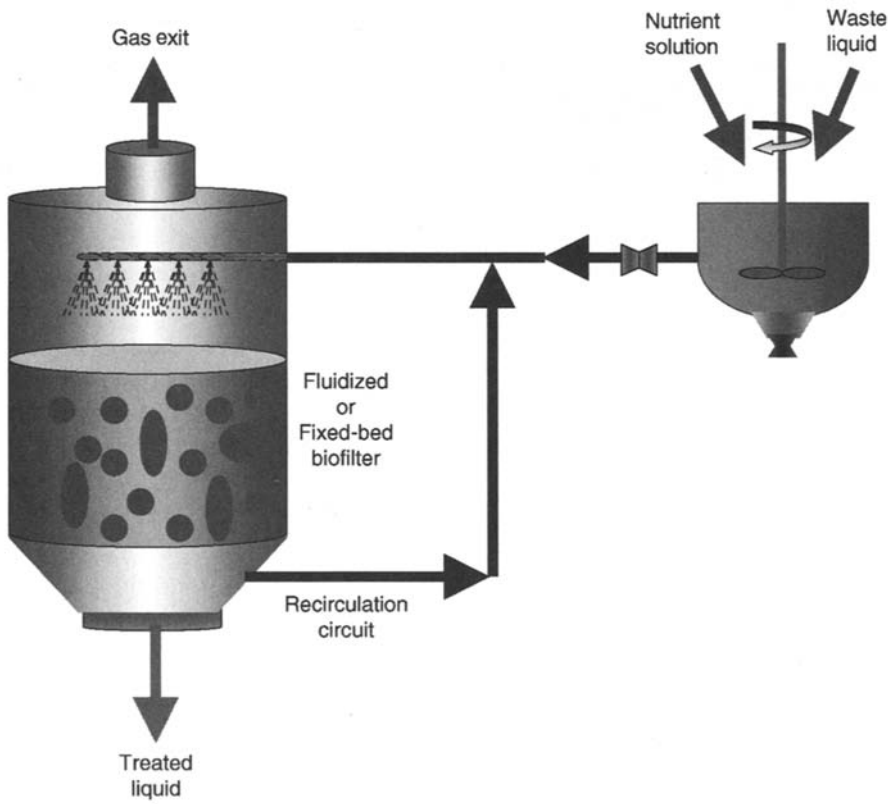

Fig. 3. Schematic setup of a biofilter (biotrickling filter) for the removal of gaseous contaminants.

column in which they are absorbed and partly oxidized in the liquid phase. After passing through the washing column, the effluent is transferred to a regeneration unit made up of a mechanically mixed reactor, in which the

pollutants are completely degraded. To increase the effectiveness of the treatment, a portion of the effluent is often recycled to the scrubber unit. Biofiltration systems can be subdivided into two distinct processes, namely biofiltration *sensu latu* (Fig. 2) and biotrickling filtration (Fig. 3), both methodologies consisting, in the case of treatment of gaseous effluents, in the prior absorption of the polluting compounds, and their subsequent biological degradation.

Biofilters

Biofiltration systems are more compact than bioscrubbers and are made up of only one treatment unit (column), in which the polluted effluent passes through a biologically active bed containing microorganisms immobilized in the form of a biofilm. Both organic and inorganic pollutants are degraded by the microorganisms that utilize them as carbon and energy sources.

Biotrickling filtration makes use of a bed of inert materials (porous glass, ceramic, or plastic materials). The effluent passes through a column counter-currently with respect to the cultivation medium containing inorganic nutrients. Either the bioscrubbers or the biotrickling filters are mainly used for effluents containing pollutants which present high or medium solubility in water. On the contrary, because of better mass transfer, the biofilters are more suited in the presence of scarcely hydro-soluble pollutants at concentrations lower than 1000 ppm. The main drawback of the use of biofilters is the difficult control of the operating parameters influencing the degradation efficiency, like pH, temperature, and nutrient concentration.

Reactors for the Biofiltration of Liquid Effluents

Biofilters are usually operated continuously and their configuration strongly depends on the characteristics of the substance to be removed from the gaseous or liquid effluent. Bioreactors utilized for this purpose employ the biocatalyst immobilized inside the system and can be classified according to the type of catalyst (cells or enzymes) or the type of mixing system. In general, studies on the biofiltration of pollutants present in industrial effluents employ immobilized cells. The difficult identification, purification, and characterization of the enzymes (or enzyme mixtures) to be used in the treatment make the employment of immobilized enzymes difficult. Effective biofilter utilization in the treatment of effluents requires that it has the ability of treating the largest amount of residues as possible. For this reason, biofilters containing more than one microorganism are often employed whose degradation metabolism implies a large variety of enzymes.

Bioreactors used for biofiltration either of liquid or gaseous effluents must ensure cell immobilization in the support. The bioreactor configurations allowing for such a condition are the so-called fixed-bed *(15,16)* and

fluidized-bed reactors *(18,20)*. The reactor aeration, which is crucial requisite depending on both the residue to be eliminated and the microorganism employed, can be obtained by plant configurations in which the contact between immobilized cells and effluents *(28)* is ensured either by the pressure difference or by the presence of an air flow ("airlift" and bubble column) *(29)*. In addition, the cells can be entrapped between semi-permeable membranes, as it occurs either in plane-membrane bioreactor *(30,31)* or in hollow-fiber bioreactor *(32)*.

The main feature of immobilized-cell systems is the use of some physical structure that compels the cells to keep confined within a specific region of the bioreactor. Among the several advantages of these systems, it should be mentioned the possibility of increasing cell concentration so as to raise the process rate and often the conversion efficiency of the biofilter.

Fixed-Bed Biofilter

Among the existing fixed-bed configurations, the most common is the vertical fixed bed, although there are reports also on the use of horizontal fixed bed and parallel flux bioreactors *(33,34)*. The immobilization takes place usually in this bioreactor configuration by entrapment (hydrophilic gel) or by adsorption (resins) *(34)*. The major drawback of the use of such a bioreactor in the treatment of effluents is the difficult long-term continuous operation, owing to the possible occurrence of excess cell growth in the bed and consequent clogging of the system and formation of preferential channels. These problems, which are responsible for a decrease in system effectiveness with time, can be overcome by the removal of some nutrients that are necessary for cell growth so as to reduce the growth rate as much as possible. In those treatments releasing gases, such as CO_2, the use of the horizontal bed is recommended in order to avoid the increase in pressure drop and the formation of preferential channels in the biofilter.

Some process parameters demonstrated to remarkably influence the performance of microorganisms in the treatment of effluents. Onysko et al. *(35)* investigated the effect of temperature on a mixed microbial culture in fixed bed either in batch or continuous process. Because this parameter was shown to have a strong effect on both cell growth and biodegradation of phenolic compounds, it needs to be optimized so as to ensure, at the same time, a low growth rate in the immobilized-cell reactor as well as a high removal yield of the industrial residue.

Fluidized-Bed Biofilter With Mechanical Mixing

In the biofiltration methods employing fluidized-bed reactors the cells are immobilized onto an expanded bed. These bioreactors consist of vertical columns with circular section in which the particles containing immobilized cells are filled up to about 70% of the working volume and then expanded by introduction of air or some other inert gas from the

bottom, or by partial recycle of the effluent from the column or by mechanical mixing. Such a bioreactor configuration is widely used because it allows overcoming the aforementioned problems often encountered with the fixed bed.

Several studies performed using different bioreactor configurations with immobilized cells demonstrated that the fluidized-bed reactor is a promising biotechnology for the treatment of phenolic compounds; however, satisfactory performance of the process can only be ensured by optimization of the concentrations of phenol, biomass, and substrate in the biofilm and of biofilm thickness (*18,20,36*).

One of the limiting factors in the use of the fluidized bed is the transient phase during the first hours of the process, which can affect the efficiency of a continuous process utilizing this kind of bioreactor. During this phase, the cell growth or substrate consumption can in fact create a kind of inhibition to the pollutants degradation. It has been suggested that adequate removal and growth kinetics (to be established through studies performed with biomass not acclimated to the process conditions) can minimize these problems and increase the final yield of phenolic compounds degradation. For example, Onysko et al. *(36)*, utilizing a fluidized-bed biofilter containing *Pseudomonas putida*, obtained satisfactory removal of phenol at 10°C.

Air-Lift Fluidized-Bed Biofilter

Koch et al. *(37)* were able to aerobically degrade a mixture of 22 phenolic, heterocyclic and aromatic compounds with yields in the range 59–69% by a mixed culture previously isolated from the soil and acclimated to selected conditions in a continuous fluidized-bed reactor using sand and charcoal as supports. Dluhy et al. *(38)*, comparing conventional and air-lift fluidized-bed bioreactors, concluded that both reactor configurations can be successfully used for the treatment of phenolic residues and developed mathematical models to optimize the process.

Reactors for the Biofiltration of Gaseous Effluents

A large number of biofilter configurations are described in the literature for different applications *(39)*. Open biofilters, consisting of simple compost or porous earth beds having depth of about 1 m, are widely utilized in the removal of odors or volatile organic compounds because of their very low cost and simplicity. However, as they are in direct contact with the atmosphere, their effectiveness is affected by the climate, additional disadvantages being the difficulty of monitoring the process and the surface needed for their installation.

Most biofilters for the treatment of gaseous effluents are covered, contain mixtures of organic materials as supports, and allow controlling and monitoring some process parameters; nevertheless, they exhibit low operating flexibility. The "Multiple-layer" configuration, which consists

of beds disposed in series, each one containing a different type of microorganism under optimized growth conditions, allows for a more accurate control of the process inside the bed.

Reports on styrene biodegradation are few and very difficult to compare, owing to the different conditions employed *(15,40–42)*. Although the trickling filter is considered to be the most effective configuration *(43,44)*, it can only be used in short operation owing to excess biomass growth *(45)*. "Perlite-packed biofilters" inoculated with fungi able to degrade styrene were shown to be an interesting alternative to the trickling filters because of better ability to face drying out and bed acidification *(40,46)*.

Microorganisms

Main Microorganisms Utilized for the Biofiltration of Liquid Effluents

Table 3 lists the main microorganisms utilized for the removal of the main pollutants. As far as the biofiltration of liquid effluents is concerned, Onysko et al. *(36)* tried to optimize the treatment at 10°C of phenolic residues by *P. putida* Q5 immobilized in fluidized-bed reactor. Sánchez et al. *(47)* proposed effective mathematical models for the biodegradation at 10°C and 25°C of phenolic compounds utilizing the same strain in the presence of the inhibiting effects exerted by the intermediate metabolites secreted in the medium.

P. putida can be used in biological oxidation of chlorophenols, but these compounds exert toxic effects on microorganisms. Kargi and Eker *(48)* developed a new rotating perforated tubes biofilm reactor that was used in continuous mode for removal of 2,4-dichlorophenol (DCP) from synthetic wastewater and toxicity reduction. A special culture of *P. putida* capable of degrading DCP supplemented with activated sludge was used for this purpose resulting in removal efficiencies up to 96%.

Bacillus subtilis has widely been employed in biofilters for the treatment of contaminants in liquid effluents, with particular concern to the degradation of aromatic hydrocarbons and the production of biosurfactants. Christova et al. *(49)* focused on the simultaneous degradation of *n*-hexadecane and naphthalene, whereas Moran et al. *(50)* discussed on the application of the surfactin produced by the strain *B. subtilis* O9 grown on a sucrose-based medium for the biodegradation of industrial residues. The presence of surfactin in the medium was shown to increase the degradation yield of aliphatic and aromatic hydrocarbons from 20.9% to 35.5% and 41%, respectively, its positive effect on the biodegradation being higher when using residues with long lateral aliphatic chains. The quick production of surfactin by *B. subtilis* strains makes this microorganism of particular interest for the treatment of effluents and bioremediation applications.

Feitkenhauer et al. *(51)* investigated the potential of different *B. thermoleovorans* strains to degrade phenol. Growth rates were about four times higher than those of mesophilic microorganisms such as *P. putida*,

Table 3
Main Xenobiotic Compounds and Microorganisms Utilized for Their Degradation

Pollutant	Microorganism
Chlorinated hydrocarbons	
Dichloromethane	*Hyphomicrobium* sp.
Methyl chloride	*Pseudomonas* DM1
	Methylobacter DM111
	Hyphomicrobium sp.
1,2-Dichloroethane	*Xanthobacter* GJ10
Vinyl chloride	*Mycobacterium* L1
Epichlorohydrine	*Pseudomonas* AD1
Chlorobenzene	*Pseudomonas* WR1306
1,2-Dichlorobenzene	*Pseudomonas* GJ 60
1,3-Dichlorobenzene	*Pseudomonas* sp.
1,4-Dichlorobenzene	*Alcaligenes* A175
Aromatics	
Benzene	*Pseudomonas* sp.
	Acinetobacter calcoaceticus RJE74
Toluene	*Pseudomonas* sp.
	P. putida
	Bacillus sp.
Monoalkylbenzenes	*Pseudomonas* sp. NCIB 10643
	P. fluorescens
	P. putida RE204
	Acinetobacter lwoffi
m-, *p*-Xylene	*Pseudomonas* sp.
	Nocardia sp.
o-Xylene	*P. stutzeri*
	Corynebacterium sp. C125
Styrene	*Xanthobacter* sp. 124X
	P. putida
	Pseudomonas sp.
Biphenyls	
Biphenyl	*Pseudomonas* sp. NCIB 10643
	Nocardia sp. NCIB 10503
	P. cruciviae
	P. putida
	P. pseudoalcaligenes
	P. aeruginosa
	Escherichia coli
Others	
Naphthalene	*Pseudomonas* sp.
	P. fluorescens
	P. putida

and the high values (about 2.8/h) were detected at phenol concentrations of 15 mg/L. The thermophilic strain *B. thermoleovorans* sp. A2 was found to be insensitive to hydrodynamic shear stress in stirred bioreactor experiments (despite of possible membrane damage caused by phenol) and flourished at an ionic strength of the medium of 15–60 g of NaCl/L. These exceptional properties make this strain an excellent candidate for technical applications.

Main Microorganisms Utilized for the Biofiltration of Gaseous Effluents

The composition of the microflora present in a biofilter depends on that of the gaseous effluent as well as the ability of some contaminants to be catabolized as nutrients.

Before the continuous operation, the microorganisms present in the biofilter are subject to progressive adaptation to the organic pollutants present in the gaseous stream, which usually lasts a period of 10 d. The microorganisms to be used can be furnished either as isolated pure cultures or as an industrial sludge *(15)*. The degradation of aromatic organic compounds from the pharmaceutical industry, like phenolic residues, needs heterogeneous populations to make the parallel degradation of other pollutants possible and then to accelerate the overall process *(52–54)*. Since heterogeneous populations used in biofilters are natural consortia, they are particularly resistant to the pollutants, thus increasing the overall time of biofilter operation. Regarding the specific degradation of styrene, biofilters inoculated with heterogeneous consortia, although characterized by low conversion yields, are usually preferred to mono-culture biofilters because they are more easily operated and managed *(8,42,44,55–59)*.

Pseudomonas sp. is largely employed in styrene degradation that starts with the oxidation of the vinyl chain. After preliminary formation of styrene oxide, such an intermediate is transformed to phenyl-acetaldehyde and phenyl-acetate, which are subsequently converted to intermediates of the tricarboxylic acid (TCA) cycle through a complex series of reactions and then metabolized. Notwithstanding the incomplete characterization of *Xanthobacter* 124X *(60)* and *Pseudomonas* MST *(61)* with respect to their capability to metabolize aromatic compounds, these microorganisms seem to be good alternative styrene degraders. Jang et al. *(62)* isolated *Pseudomonas* sp. SR-5 as a styrene-degrading bacterium immobilized in organic (peat) and inorganic (ceramic) packing material and evaluated its ability to degrade styrene at different concentrations. The effectiveness of styrene degradation varied according to the packing material utilized; a maximum degradation capacity of 236 g/(m^3·h) was attained with peat, corresponding to 90.4% removal.

P. putida has been used to remove toluene and ethanol present in waste air. Lim et al. *(63)* studied the transient behavior of a hybrid system made up of biofilter and photo-catalytic reactor. The system was inoculated with a pure culture of *Burkholderia cepacia* G4 and *P. putida*, whereas

a photo-catalytic reactor was made up of 15W ultraviolet (UV)-A lamps and annular tubes packed with glass beads coated with TiO_2 solution before calcination. The maximum elimination capacities of toluene and ethanol turned out to be 130 g/(m^3·h) and 230 g/(m^3·h), although in experiments using only a biofilter, they decreased to 40 g/(m^3·h) and 130 g/(m^3·h), respectively. Marek et al. *(64)* evaluated xylene and toluene degradation in waste air using a laboratory-scale biofilter containing immobilized *P. putida*. A decrease in bed pH improved the efficiency of toluene degradation, but the simultaneous degradation of both pollutants required higher pH.

The biological treatment of odorous sulfur-containing compounds was reported by Geng et al. *(65)*, who successfully isolated a dimethylsulfide (DMS)-degrading bacterium from activated sludge, using the enrichment isolation technique. The isolate was able to metabolize DMS as well as hydrogen sulfide. Batch tests demonstrated that over half of DMS could be removed by the isolate in 3 h when the initial DMS amount was approx 0.6–1.5 g. Nearly complete removal of H_2S by the isolate was obtained by a continuous test in a 2-L gas-bubbling bottle. Nevertheless, Oyarzun et al. *(66)* evaluated the use of *Thiobacillus thioparus* for treating a gaseous stream containing high concentrations of H_2S. The biofilter reached an efficiency of almost 100% when fed with 0.5 g/m^3 of the pollutant or less.

Finally, although *Rhodococcus rhodochrous* NCIMB 13259 is the best known microorganism able to degrade styrene *(67–69)*, only a few reports described its application to biofiltration of gaseous effluents *(15)*.

Biofiltration for the Removal of Phenolic Residues From the Pharmaceutical Industry

Phenolic compounds find application in different sectors of chemical, pharmaceutical, and petrochemical industry, being employed as antimicrobial agents (disinfectants) as well as to preserve paints, leather, and some textiles. Because of their widespread use, phenolic residues are often present in industrial effluents in toxic levels, which can give rise to serious environment problems; when contained in industrial effluents without previous treatment or in the absence of adequate treatment, they can in fact contaminate soil and subsoil, being even able to reach groundwater tables and river heads.

The control of emissions of toxic organic compounds to the atmosphere has become a critical and expensive challenge for industry, being necessary to face the new requirements in terms of environmental quality and policy. With special regard to phenolic compounds, which are abundantly released by the pharmaceutical industry, the present legislation of many countries imposes maximum concentration thresholds for these pollutants, owing to their potential hazardous effects on the humans and the environment. For liquid effluents, the maximum threshold values depend, in Brazil, on the water class of the receiving body. Class 1 does refer

to water utilized for domestic use without prior treatment, class 2 to water to be conventionally treated before domestic use, irrigation and recreation, class 3 to water addressed to domestic use or in places where there is need of fauna and flora preservation, and class 4 to water that can be utilized for domestic use after significant treatment, shipping, industrial use, irrigation, and other uses requiring less quality standards. Although water belonging to class 1 has to be absolutely free of phenolic residues, for the others the concentration thresholds vary from 0.001 mg/L (classes 2 and 3) to 1 mg/L (class 4) *(70)*. To solve the environmental and human health problems potentially associated to the widespread presence of highly toxic residues in the environment, the United Nations published, through the *World Health Organization for the International Programme* and the *Environmental Health Criteria*, the maximum limits for the exposition to phenolic residues in the air recommended either in the working place or in the environment. For example, a maximum level of 19 mg/m^3 is allowed in Germany, USA, Japan, and Australia in the working place, whereas Russia and Czech Republic established a maximum daily threshold in the environment of 0.001 mg/m^3 *(71)*.

Removal of Phenolic Compounds From Liquid Effluents

The phenolic compounds are pollutants commonly present in concentrations from 5 to 500 mg/L in effluents from refineries, chemical plants for the production of explosives, resins, pesticides, textiles, and pharmaceuticals *(71)*. To describe phenol biodegradation, Haldane *(72)* related the microbial growth to substrate concentration taking into account the possible inhibition exerted by high substrate levels:

$$\mu = \frac{\mu_m \times S}{K_s + S + \left(\frac{S^2}{K_i}\right)}$$

being μ_m the maximum specific growth rate, K_S the saturation constant of Monod equation and K_i the inhibition constant. Several authors demonstrated that cell growth on phenol depends on phenol concentration, temperature, pH, and the way of inoculum preparation *(73,74)*. As a general rule, the cultivations exhibited a prolonged lag phase *(75)*.

Using this equation, Monteiro et al. *(19)* estimated the kinetic parameters of batch degradation of phenol at 26°C and pH 6.8 by *P. putida* DSM 548, using phenol as the only carbon source at concentrations in the range 1–100 mg/L (μ_m = 0.463/h, K_S = 6.19 mg/L and K_i = 54.1 mg/L). The maximum specific growth rate was higher for pure rather than for mixed culture, hence evidencing the likely influence of secondary metabolites on microbial growth and phenol degradation.

Most research-work has been performed on the biodegradation of phenolic compounds using different microorganisms either in pure or mixed cultures, among which are *Pseudomonas* sp. *(76–79)*, *Alcaligenes* sp.

(80,81), *Rhodococcus* sp. *(82,83)*, and *Cryptococcus* sp. *(84)*. In addition, aerobic cultivations were shown to be more effective than the anaerobic ones *(85)*.

Recent works have been developed using the microorganisms in a cometabolism system, in which phenol biodegradation is realized simultaneously with the main metabolic route for substrate uptake. Dupasquier et al. *(86)* demonstrated the viability of the biodegradation of methyl tertiary butyl ether (MTBE) vapors by cometabolism with pentane using a pentane-oxidizing strain of *P. aeruginosa*. As a cosubstrate, MTBE was degraded during the pentane uptake by the cells. The experimental data of pentane and MTBE removal efficiencies compared satisfactorily with the theoretical predictions of the model under steady-state conditions.

The biodegradation of phenolic compounds can also be carried out using mixed cultures. An example of this application is provided by activated sludge, a heterogeneous mixture of unidentified aerobic microorganisms able to oxidize the pollutants to carbon dioxide. Otherwise, mixed cultures of identified microorganisms can be used. For example, Oh et al. *(87)* utilized with success a mixed culture of *P. putida*, *Flavobacterium* sp., and *Acinetobacter* sp., whereas Wiesel et al. *(88)* employed a mixture of five different microorganisms for the treatment of polycyclic aromatic hydrocarbons.

By biochemical tests and molecular biological analysis using 16S ribotyping, Andretta et al. *(89)* identified a 4,5,6-trichloroguaiacol (TCG)-degrading strain of *B. subtilis*. Biodegradation occurred in a mineral salts' medium only when the inoculum was made up of cells in the stationary phase of growth and was accelerated by an additional carbon source, such as glucose, sucrose, glycerol, or molasses. An additional nitrogen source (like ammonium sulfate) did not affect the rate of 4,5,6-TCG removal. No plasmids were detected in the bacterial cells. It was demonstrated that 4,5,6-TCG is not degraded by cometabolism and that the gene encoding this characteristic is probably located on the chromosome. The lack of requirement for additional nitrogen source, the ability to enhance biodegradation by addition of cheap carbon sources such as molasses, and the fact that the trait is likely to be stable as it is encoded on the cell chromosome, are all characteristics that make this organism attractive for treatment of wastes and environments polluted with organochlorinated compounds.

González et al. *(90)*, comparing the biodegradation of phenolic compounds from industrial effluents by a combined bioscrubbing (with mechanical mixing) and biofiltration (fixed-bed reactor [FBR]) process, demonstrated the significance of the reactor configuration either in reducing the treatment costs or improving its yield. Although the use of mechanical mixing allows for easy control and residence time adjustment, it can stress the culture, thus affecting the process and requiring a long time for culture recovery. By the use of immobilized and acclimated

cells under steady-state conditions, biofilters minimize this deleterious effect by promoting a quicker recovery of the system. According to Holladay et al. *(91)*, the main drawback of the use of fixed-bed biofilters, with cells immobilized on inert supports, would be the natural growth of biomass, which reduces the reactor operating time. Beg and Hassan *(92)* overcame this disadvantage increasing the energy requirements of endogenous respiration.

González et al. *(90)* confirmed that phenol and 4-chlorophenol are degraded with no appreciable difference by *P. testosteroni* CPW301 through the same metabolic pathway and that the degradation rate is affected by the concentration of the latter substrate in the cultivation medium. Appreciable loss of microbial activity was observed only after several operations of immobilized-biomass recycling, and the rate of continuous degradation and dilution rates applied were higher in FBR rather than in mechanically mixed bioscrubber (regarded as a continuous stirred tank reactor [CSTR]). The use of a coculture of *P. solanacearum* TCP114 and *P. testosteroni* CPW301 made the simultaneous treatment of 2,4,6-trichlorophenol, phenol, and 4-chlorophenol possible. Although the microorganisms were not affected by the presence of phenol in the medium, the rate of 2,4,6-trichlorophenol degradation was increased by 4-clorophenol, but the rate decreased and inhibition took place when phenol concentration exceeded the level of toxicity to the cultures. In the absence of *P. solanacearum* TCP114, phenol and 4-chlorophenol degradations were affected even in the presence of 2,4,6-trichlorophenol. These results demonstrate that the simultaneous treatment of different compounds requires a better knowledge of the toxic compounds and a deep investigation on their reciprocal interference on cell metabolism. Also the type of reactor and biotreatment can strongly affect the degradation of industrial residues, as it was demonstrated by the higher effectiveness of CSTR with respect to FBR.

Promising results were also obtained by the use of fluidized-bed biofilters for the treatment of phenolic compounds from pharmaceutical industry effluents. González et al. *(90,93)* investigated the biodegradation of phenolic compounds either by free or immobilized cells in continuous or batch operation, the best results having been obtained using the fluidized-bed reactor with immobilized-cells (biofiltration).

A way to remarkably increase the effectiveness of industrial effluents biodegradation is the previous adaptation of the microorganism to the toxic compound *(94)*.

Removal of Phenolic Compounds From Gaseous Effluents

As is well known, biofiltration of pollutants in gaseous phase implies the simultaneous transfer of the pollutant from the gaseous phase to the liquid phase by absorption and its biodegradation in the liquid phase thanks to the contact with the immobilized cells.

Biofiltration is increasingly applied worldwide in the treatment of these gaseous effluents *(8,11,56,95)* because of its low operating costs and high removal efficiency. Zilli et al. *(15)*, who investigated the continuous removal of toluene and styrene in synthetic air streams by means of biofilters, obtained for the former pollutant a maximum elimination capacity of 275 mg/(m^3·h), i.e., a value about 10% higher than those previously reported *(96,97)*. The increased capacity was likely owing to the heterogeneous microflora developed in the biofilter, which seems to confirm the better performances of these systems with respect to those using pure cultures. At low air flowrates, the pollutant was almost completely removed from the air stream, and there was a linear relationship between the rates of pollutant removal and gaseous effluent feeding.

Biofiltration and Simultaneous Production of Surfactants

Biosurfactants are, generally, glycolipids, lipopeptides, protein-polysaccharides complex, phospholipids, fatty acids, and some neutral lipids *(98)*. Because of their wide market and large number of applications *(99)*, the surfactants constitute an important class of chemicals.

They are amphiphilic molecules composed by a hydrophilic polar portion and a hydrophobic apolar portion. This property confers them the ability of reducing the superficial and the interfacial tensions, thus forming emulsions so that the hydrocarbons can be dispersed in water or vice versa *(100)*. As most of the common surfactants are produced chemically from oil derivatives, the production of biosurfactants for cleaning and removal of oily residues is gaining increasing interest in the field of environmental biotechnology.

The biosurfactants are a class of surfacing molecules obtained by microbial cultivations able to reduce superficial and interfacial tensions either in water solutions or in mixtures of hydrocarbons. Several interesting properties of these substances, which spontaneously form during cell growth *(101)*, were extensively investigated *(102–104)*. The world consumption of surfactants has been continuously increasing in the last decades *(99)*, and the petrochemical industry accounts for about one half of its whole utilization.

The biosurfactant surfactin has the potential to aid in the recovery of subsurface organic contaminants (environmental remediation) or crude oils (oil recovery). However, high medium and purification costs limit its use in these high-volume applications *(105)*.

They attracted attention as hydrocarbon dissolution agents for the first time in the late 1960s, and their applications have been greatly extended in the past five decades as an improved alternative to chemical surfactants as they are biodegradable *(106)*.

The numerous advantages of biosurfactants, such as mild production conditions, low toxicity, high biodegradability, and environmental

compatibility have prompted applications not only in the food, cosmetic, and pharmaceutical industries but also in environmental protection and energy-saving technology *(107,108)*.

It seems that their action is related to the consumption of hydrocarbons *(101)*, so they are mainly produced by microorganisms able to degrade hydrocarbons. The production of biosurfactants by *B. subtilis* ATCC 6633 was investigated using commercial sugar, sugarcane juice and cane molasses, glycerol, mannitol, soybean oil, among others *(109)*. The results showed that the best carbon source was commercial sugar because of minimum surface tension.

Additional advantages with respect to chemical surfactants are higher formation of foam, selectivity and specific activity at high temperature, pH, and salinity *(108)*.

Several lipopeptide surfactants have the most potent antibiotic activity and have been a subject of several studies on the discovery of new antibiotics, including surfactin of *B. subtilis (110)*.

Among the various studies on the production of biosurfactants, those dealing with the production of surfactin and iturin A by *B. subtilis* are of particular interest, as this microorganism can also be used for the biodegradation of different phenolic compounds *(98)*.

Concluding Remarks

Besides its traditional application for the control of odors, biofiltration has become a successful technology for the treatment of pollutants contaminating either gaseous or liquid effluents. During the last two decades, thanks to the remarkable progress in the fields of microbiology and process technology, biofiltration has been gaining the interest of various industrial sectors and is increasingly applied. The feasibility of this technique for the treatment of different types of pollutants contained in industrial effluents has been demonstrated, with particular concern to the biodegradation of phenolic compounds. The removal yield is often higher than 90%, mainly in the presence of effluents contaminated by alcohols, ketones, ethers, aldehydes, and volatile aromatic compounds. As far as the phenolic residues are concerned, there are reports on the reduction of their concentration in the effluent from 50 to 1 mg/L, which is a level suitable for the main environmental legislations worldwide. The main advantages of the use of this technology are the low costs, the low energy and space requirements, moderate temperature, pH and pressure conditions, and high compactness.

The applicability of this technology depends on the availability of adequate strains or complex of microorganisms able to perform the biodegradation of one or more pollutants, so as to make it feasible and economically competitive with the traditional physical-chemical methods. More research-work is needed to ensure a more effective control of the

operating parameters as well as the selection of the suited reactor configuration, so as to promote the use of biofilters for the treatment of effluents from the pharmaceutical industry contaminated by phenolic compounds and then to reduce their concentrations below the emission limits imposed in Brazil.

References

1. Edgington, S. M. (1994), *Biotechnology* **12,** 1338–1342.
2. Head, I. M. (1998), *Microbiology* **144,** 599–608.
3. Eckenfelder, W. W. and Musterman, J. L. (1994), *Water Sci. Technol.* **29(9),** 79–88.
4. Liu, S. and Suflita, J. M. (1993), *Trends Biotechnol.* **11,** 344–352.
5. Bragg, J. R., Prince, R. C., Harner, K. J., and Altas, R. M. (1994), *Nature* **368,** 413–418.
6. Swannell, R. P. J., Lee, K., and McDonagh, M. (1996), *Microbiol. Rev.* **60,** 342–365.
7. Ottengraf, S. P. P., van den Oever, A. H. C., and Kempenaars, F. J. C. M. (1984), In: *Innovations in Biotechnology.* Houwink, E. H. and van der Meer R. R., eds., Elsevier, Amsterdam, pp. 157–167.
8. Ottengraf, S. P. P., Meesters, J. J. P., van den Oever, A. H. C., and Rozema, H. R. (1986), *Bioproc. Eng.* **1,** 61–69.
9. Button, D. K., Schut, F., Quang, P., Martin, R. M., and Robertson, B. (1993), *Environ. Microbiol.* **59,** 881–891.
10. Watanabe, K. and Baker, P. W. (2000), *J. Biosci. Bioeng.* **89,** 1–11.
11. Zilli, M. and Converti, A. (1999), In: *The Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis and Bioseparation.* Flickinger, M. C. and Drew, S. W., eds., Wiley, New York, pp. 305–319.
12. Kim, J. H., Oh, K. K., Lee, S. T., and Kim, S. W. (2002), *Proc. Biochem.* **37,** 1367–1373.
13. Zilli, M., Del Borghi, A., and Converti, A. (2000), *Appl. Microbiol. Biotechnol.* **54,** 248–254.
14. Abu Hamed, T., Bayraktar, E., Mehmetoğlu, Ü., and Mehmetoğlu, T. (2004), *Biochem. Eng. J.* **19,** 137–146.
15. Zilli, M., Palazzi, E., Sene, L., Converti, A., and Del Borghi, M. (2001), *Proc. Biochem.* **37,** 423–429.
16. Sene, L., Converti, A., Felipe, M. G. A., and Zilli, M. (2002), *Biores. Technol.* **83,** 153–157.
17. Moharikar, A. and Purohit, H. (2003), *Int. Biodeter. Biodegr.* **52,** 255–260.
18. Vinod, A. V. and Reddy, G. V. (2005), *Biochem. Eng. J.* **24,** 1–10.
19. Monteiro, Á. A. M. G., Boaventura, R. A. R., and Rodrigues, A. E. (2000), *Biochem. Eng. J.* **6,** 45–49.
20. Clarke, K. L., Pugsley, T., and Hill, T. A. (2005), *Chem. Eng. Sci.* **60,** 6909–6918.
21. Prokop, W. H. and Bohn, H. L. (1985), *J. Air Pollut. Control Assoc.* **35,** 1332–1338.
22. Kampbell, D. H., Wilson, J. T., Read, H. W., and Stocksdale, J. (1987), *J. Air Pollut. Control Assoc.* **37,** 1236–1240.
23. Alfani, F., Cantarella, L., Gallifuoco, A., and Cantarella, M. (1990), *Acqua-Aria* **10,** 877–884.
24. Jäger, B. and Jager, J. (1978), *Müll und Abfall* **2,** 48–54.
25. Hartmann, H. (1977), *Stuttg. Ver. Siedlungswasserwirstsch* **59,** 3–19.
26. Thistlethwayte, B., Hardwick, B., and Goleb, E. E. (1973), *Chimie Ind.* **106,** 795–801.
27. Helmer, R. (1974), *Ges. Ing.* **94,** 21–30.
28. Chen, K. C., Lin, W. H., and Liu, Y. C. (2002), *Enzyme Microbial Technol.* **31,** 490–497.
29. Chiangchun, Q., Hanchang, S., Yongming, Z., and Yi, Q. (2003), *Proc. Biochem.* **38,** 1545–1551.
30. Tsai, H. H., Ravindran, V., and Pirbazari, M. (2005), *Chem. Eng. Sci.* **60,** 5620–5636.
31. Luke, A. K. and Burton, S. G. (2001), *Enzyme Microbial Technol.* **29,** 348–356.
32. Kim, D. J. and Kim, H. (2005), *Proc. Biochem.* **40,** 2015–2020.
33. Zaiat, M., Cabral, A. K. A., and Foresti, E. (1996), *Wat. Res.* **30,** 2435–2439.

34. Schmidell, W. and Facciotti, M. C. R. (2001), In: *Biotecnologia Industrial*. Schmidell, W., Lima, U. A. L., Aquarone, E., and Borzani, W., eds., Edgard Blücher, São Paulo, pp. 179–192.
35. Onysko, K. A., Budman, H. M., and Robinson, C. W. (2000), *Biotechnol. Bioeng.* **70,** 291–299.
36. Onysko, K. A., Robinson, C. W., and Budman, H. M. (2002). *Can. J. Chem. Eng.* **80,** 239–252.
37. Koch, B., Ostermann, M., Hoke, H., and Hempel, D. C. (1991), *Wat. Res.* **25,** 1–8.
38. Dluhy, M., Sefcik, J., and Bales, V. (1994), *Comput. Chem. Eng.* **18,** S725–S729.
39. Swanson, W. J. and Loeher, R. C. (1997), *J. Environ. Eng.* **123,** 538–546.
40. Cox, H. H. J., Houtman, J. H. M., Doddema, H. J., and Harder, W. (1993), *Biotechnol. Lett.* **15,** 737–742.
41. Cox, H. H. J., Houtman, J. H. M., Doddema, H. J., and Harder, W. (1993), *Appl. Microbiol. Biotechnol.* **39,** 372–376.
42. Arnold, M., Reittu, A., von Wright, A., Martikainen, P. J., and Suihko, M. L. (1997), *Appl. Microbiol. Biotechnol.* **48,** 738–744.
43. Sorial, G. A., Smith, F. L., Suidan, M. T., Pandit, A., Biswas, P., and Brenner, R. C. (1998), *Wat. Res.* **32,** 1593–1603.
44. Kim, D., Cai, Z. L., and Sorial, G. A. (2005), *J. Air Waste Manag. Assoc.* **55,** 200–209.
45. Togna, A. P. and Frisch, S. (1993), 86th Meeting of the Air and Waste Management Association, Denver, CO, 14–18 June 1993.
46. Cox, H. H. J., Moerman, R. E., van Baalen, S., and van Gheiningen, W. N. M. (1997), *Biotechnol. Bioeng.* **53,** 259–266.
47. Sánchez, J. L. G., Kamp, B., Onysko, K. A., Budman, H., and Robinson, C. W. (1998), *Biotechnol. Bioeng.* **60,** 560–567.
48. Kargi, F. and Eker, S. (2005), *Proc. Biochem.* **40,** 2105–2111.
49. Christova, N., Tuleva, B., and Nikolova-Damyanova, B. (2004), *J. Biosci.* **59,** 205–208.
50. Moran, A. C., Olivera, N., Commendatore, M., Esteves, J. L., and Sineriz, F. (2000), *Biodegradation* **11,** 65–71.
51. Feitkenhauer, H., Schnicke, S., Muller, R., and Markl, H. (2001), *Appl. Microbiol. Biotechnol.* **57,** 744–750.
52. Oldenhuis, R., Vink, R. L. J. M., Janssen, D. B., and Witholt, B. (1989), *Appl. Environ. Microbiol.* **55,** 2819–2826.
53. Oldenhuis, R., Kuijk, L., Lammers, A., Jannsen, D. B., and Witholt, B. (1989), *Appl. Environ. Microbiol.* **30,** 211–217.
54. Ergas, S. J., Kinney, K., Fuller, M. E., and Scow, K. M. (1994), *Biotechnol. Bioeng.* **44,** 1048–1054.
55. Janssen, D. B., Grobben, G., Hoekstra, R., Oldenhuis, R., and Whitolt, B. (1988), *Appl. Microbiol. Biotechnol.* **29,** 392–399.
56. Ottengraf, S. P. P. (1986), In: *Biotechnology*. Rehm, H. J. and Reed, G., eds., VCH, Weinheim, pp. 425–452.
57. van der Werf, M. J., Swarts, H. J., and de Bont, J. A. M. (1999), *Appl. Environ. Microbiol.* **65,** 2092–2102.
58. Arand, M., Hallberg, B. M., Zou, J. Y., et al. (2003), *EMBO J.* **22,** 2583–2592.
59. Sabo, F., Motz, U., and Fischer, K. (1993), 86th Meeting of the Air and Waste Management Association, Denver, CO, 14–18 June 1993.
60. Hartmans, S., Smits, J. P., van der Werf, M. J., Volkering, F., and De Bont, J. A. M. (1989), *Appl. Environ. Microbiol.* **55,** 2850–2855.
61. Dijk, J. A., Stams, A. J. M., Schraa, G., Ballerstedt, H., de Bont, J. A. M., and Gerritse, J. (2003), *Appl. Microbiol. Biotech.* **63,** 68–74.
62. Jang, J. H., Hirai, M., and Shoda, M. (2004), *Appl. Microb. Biotechnol.* **65,** 349–355.
63. Lim, K. H., Park, S. W., Lee, E. J., and Hong, S. H. (2005), *Korean J. Chem. Eng.* **22,** 70–79.
64. Marek, J., Paca, J., Halecky, M., Koutsky, B., Sobotka, M., and Keshavarz T. (2001), *Folia Microb.* **46,** 205–209.
65. Geng, A. L., Chen, X. G., Gould, W. D., et al. (2004), *Wat. Sci. Technol.* **50(4),** 291–297.

66. Oyarzun, P., Arancibia, F., Canales, C., and Aroca, G. E. (2003), *Proc. Biochem.* **39,** 165–170.
67. Warhust, A. M. and Fewson, C. A. (1994), *J. Appl. Bacteriol.* **77,** 597–606.
68. Warhust, A. M. and Fewson, C. A. (1994), *Crit. Rev. Biotechnol.* **14,** 29–73.
69. Warhust, A. M., Clarke, K. F., Hill, R. A., Holt, R. A., and Fewson, C. A. (1994), *Appl. Environ. Microbiol.* **60,** 1137–1145.
70. ANVISA—Agência Nacional de Vigilância Sanitária, RDC n210, DE 04/08/2003, Regulamento Técnico das Boas Prácticas de Fabricação de Medicamentos, D.O.U.—Diário Oficial da União; Poder Executivo, Brazilia, 14 August 2003.
71. WHO, Phenol Health and Safety Guide—Environmental Health Criteria 161: Phenol, Published by the World Health Organization for the International Programme on Chemical Safety, UNEP, ILO, WHO. http://www.inchem.org/documents/hsg/hsg/hsg88_e.htm.
72. Haldane, J. B. S (1965), In: *Enzymes.* MIT Press, Cambridge, MA, p. 84.
73. D'Adamo, P. D., Rozich, A. F., and Gaudy, A. F. (1984), *Biotechnol. Bioeng.* **26,** 397–402.
74. Hill, A. and Robinson, C. W. (1975), *Biotechnol. Bioeng.* **17,** 1599–1615.
75. Yang, R. D. and Humphrey, A. E. (1975), *Biotechnol. Bioeng,* **17,** 1211–1235.
76. Dapaah, S. Y. and Hill, G. A. (1992), *Biotechnol. Bioeng.* **40,** 1353–1358.
77. Hinteregger, C., Leitner, R., Loidl, M., Ferschi, A., and Streichsbier, F. (1992), *Appl. Microbiol. Biotechnol.* **37,** 252–259.
78. Chitra, S., Sekaran, G., Padmavathi, S., and Chandrakasan, G. J. (1995), *Gen. Appl. Microbiol.* **41,** 229–237.
79. Spigno, G., Zilli, M., and Nicolella, C. (2004), *Biochem. Eng. J.* **19,** 267–275.
80. Hill, G. A., Milne, B. J., and Nawrocki, P. A. (1996), *Appl. Microbiol. Biotechnol.* **46,** 163–168.
81. Valenzuela, J., Bumann, U., Céspedes, R., Padilla, L., and González, B. (1997), *Appl. Environ. Microbiol.* **63,** 227–232.
82. Apajalahti, J. H. A. and Salkinoja-Salomen, M. S. (1986), *Appl. Microbiol. Biotechnol.* **25,** 62–67.
83. Oh, J. S. and Han, Y. H. J. (1997), *Kor. J. Appl. Microbiol. Biotechnol.* **25,** 459–463.
84. Morsen, A. and Rehm, H. J. (1987), *Appl. Microbiol. Biotechnol.* **26,** 283–288.
85. Kim, J. H., Oh, K. K., Lee, S. T., and Kim, S. W. (2002), *Proc. Biochem.* **37,** 1367–1373.
86. Dupasquier, D., Revaii, S., and Auria, R. (2002), *Environ. Sci. Technol.* **36,** 247–253.
87. Oh, H. M., Ku, Y. H., Ahn, K. H., Jang, K. Y., Kho, Y. H., and Kwon, G. S. (1995), *Korean J. Appl. Microbiol. Technol.* **23,** 755–762.
88. Wiesel, I., Wubker, S. M., and Rehm, H. J. (1993), *Appl. Microbiol. Biotechnol.* **39,** 110–116.
89. Andretta, C. W. S., Rosa, R. M., Tondo, E. C., Gaylarde, C. C., and Henriques, J. A. P. (2004), *Chemosphere* **55,** 631–639.
90. González, G., Herrera, G., García, M. T., and Peña, M. (2001), *Biores. Technol.* **76,** 245–251.
91. Holladay, D. W., Hancher, C. W., Scott, C. D., and Chilcote, D. D. (1978), *J. Wat. Pollut. Control Fed.* **50,** 2573–2588.
92. Beg, S. A. and Hassan, M. M. (1985), *Chem. Eng. J.* **30,** 1–8.
93. González, G. and Herrera, G. (1995), *Acta Microbiol. Polonica* **44,** 285–296.
94. Zilli, M., Converti, A., Lodi, A., Del Borghi, M., and Ferraiolo, G. (1993), *Biotechnol. Bioeng.* **41,** 693–699.
95. Mpanias, C. J. and Baltzis, B. C. (1998), *Biotechnol. Bioeng.* **59,** 328–343.
96. Morales, M., Revah, S., and Auria, R. (1998), *Biotechnol. Bioeng.* **60,** 483–491.
97. Acuña, M. E., Pérez, F., Auria, R., and Revah, S. (1999), *Biotechnol. Bioeng.* **63,** 175–184.
98. Ahimou, F., Jacques, P., and Deleu, M. (2000), *Enzyme Microbial Technol.* **27,** 749–754.
99. Deleu, M. and Paquot, M. (2004), *C. R. Chimie* **7,** 641–646.
100. Desai, J. D. and Banat, I. M. (1997), *Microbiol. Mol. Biol. Rev.* **61,** 47–64.
101. Banat, I. M. (1994), *Biores. Technol.* **51,** 1–12.
102. Cooper, D. G. (1986), *Microbiol. Sci.* **3,** 145–149.

103. Rosenberg, E. (1986), *Crit. Rev. Biotechnol.* **3,** 109–132.
104. Haferburg, D., Hommel, R., Claus, R., and Kleber, H. P. (1986), *Adv. Biochem. Eng. Biotechnol.* **33,** 53–93.
105. Noah, K. S., Fox, S. L., Bruhn, D. F., Thompson, D. N., and Bala, G. A. (2002), *Appl. Biochem. Biotechnol.* **98,** 803–813.
106. Cameotra, S. S., Hommel, R., Claus, R., and Kleber, H. P. (2004), *Curr. Opin. Microbiol.* **7,** 262–266.
107. Banat, I. M., Makkar, R. S., and Cameotra, S. S. (2000), *Appl. Microbiol. Biotechnol.* **53,** 495–508.
108. Cameotra, S. S. and Makkar, R. S. (1998), *Appl. Microbiol. Biotechnol.* **50,** 520–529.
109. Reis, F. A. S. L., Servulo, F. C., and de França, F. P. (2004), *Appl. Biochem. Biotechnol.* **113–116,** 899–912.
110. Peypoux, F., Bonmatin, J. M., and Wallach, J. (1999). *Appl. Microbiol. Biotechnol.* **51,** 553–563.