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Biological Fixed-Film Systems

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Biological Fixed-Film Systems

Mark W. Fitch, Jeffrey B. Murphy,
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INTRODUCTION

Overview. The work reviewed here was published during the catalogue/issue year 1998 and described research involving biofilms treating pollutants. This review explicitly excludes research in medical biofilms, dental biofilms, biofilms causing corrosion, and biofilm formation in drinking water treatment and distribution systems. Anaerobic biofilm treatment system research is not reviewed here although a set of references is provided. However, the authors have included coverage of denitrification in traditional biofilm treatment systems or when combined with such systems. Similarly, biofilm systems for the treatment of air pollutants is reviewed elsewhere in this issue and thus a set of references is provided without description.

The roughly 300 references catalogued here are divided on the basis of fundamental research area or reactor type. Fundamental research into biofilms is presented in two sections, characterization/measurement and growth/modeling. When models of specific processes were developed, these references are included in the section on that technology. Reactor types that are covered are trickling filters, rotating biological contactors, fluidized bed biofilters (including airlift bioreactors), submerged bed bioreactors (including moving bed and floating bed bioreactors), biological activated carbon, membrane bioreactors, and immobilized cell bioreactors. Thereafter, innovative reactors which are not easily classified are presented, and an additional section on biofilms on sand, soil, and sediment is given.

Reviews. DeFilippi et al. (1998) provided a review with many references that covers microbiological degradation of wastewater and its application using a fixed-film reactor. Mobile bed biofilm reactors were reviewed, specifically fluidized bed biofilm reactors, airlift biofilm reactors, and moving bed biofilm reactors (Tomaszek and Grabas, 1998). The technical and technological char-

acteristics as well as the efficiency of nitrification and denitrification processes were the main features taken into consideration.

Wilmott et al. (1998) gave a review with a number of references concerning biotechnologies available to remove color from textile effluent. Both the problem and biotechnological solutions were discussed, including adsorption, filtration, precipitation, chemical degradation, photodegradation, biodegradation, biofilters, bioscrubbers, rotating biological contactors, continuously-stirred tank reactors, upflow sludge blanket reactors, and expanded bed bioreactors. Selection of a treatment strategy for a sulfate-rich wastewater depends on the aim of the treatment Lens et al. (1998). This can be (1) removal of organic matter, (2) removal of sulfate, or (3) removal of both.

An on-line collection of biofilm images has been developed (American Society for Microbiology, 1998).

BIOFILM MEASUREMENT AND CHARACTERIZATION

Microelectrodes. Oxygen, sulfide, ammonium, pH, and redox potential microelectrodes were used to explore metabolic parameters as a function of depth in two biofilms, an aerobic oxidation/sulfate reduction biofilm, and an aerobic oxidation/nitrification biofilm (Yu and Bishop, 1998a). Both biofilms had a clearly stratified structure with a well-defined boundary between the aerobic layer and the deeper anoxic zone, but the nitrifying biofilm had a less well defined boundary. Similarly, microelectrodes were used to study the profiles of oxygen, total dissolved sulfide, redox potential, and pH as a function of depth in an aerobic biofilm use to treat azo dye containing wastewater (Yu and Bishop, 1998b). They found that aerobic oxidation only took place near the surface. Sulfite reduction occurred in the deeper anaerobic zone with a sharp decrease in redox potential in a very narrow band of 50 μm near the interface of the aerobic and anaerobic zones supporting the concept of stratification of metabolic processes in biofilms. This group also introduced a new potentiometric sensor electrode for sulfide based on conducting polymer films electrochemically deposited onto an alloy substrate (Atta et al., 1998). The electrode is useful for the measurement of total sulfide in biological environments and can be manufactured in the micron scale.

Mobile microelectrodes of similar scale (tip diameter = 10 mm) and the limiting current technique were used to measure the effective diffusivity of electroactive species of natural and artificial heterogeneous biofilms (Beyenal et al., 1998). The local effective diffusivity was then related to known local biofilm density using an empirical equation. A movable microelectrode was also used to develop a technique to measure the local flow velocity of fluids in biofilms and measure the current resulting from an electrochemical reaction that is a function of mass transport rate (Xia et al., 1998). This electrochemical technique calibrated well against measurements from particle counting and confocal scanning laser microscopy, was more rapid than these techniques, and showed that local velocities were dependent on distance from non-mobile surfaces, void geometry and orientation of voids to the main flow field.

Oxygen microelectrodes were used to measure effective diffusive boundary-layer thickness and local mass-transfer coefficient profiles in biofilm clusters and interstitial voids at velocities of 0.62, 1.53, and 2.60 cm/s (Rasmussen and Lewandowski, 1998). The mass-transfer resistance ranged from little at a thickness of 70 pm to graduated at 350 pm with local mass-transfer coefficients decreased at only 0.62 cm/s flow. The accuracy of values returned by oxygen microelectrodes for biofilms was checked in a system using a polarized graphite felt. The true oxygen flux determined from the polarization current was found to be less than those determined from microelectrode-evaluated oxygen concentrations, apparently due to the effect of the microelectrode on mass transfer as evidenced by the increase in the difference as flow velocities increased (Rasmussen and Lewandowski, 1998).

Biofilm Adhesion. Chen et al. (1998) described a micromanipulation technique developed to directly measure the adhesive strength of biofilms using a T-shaped probe to pull *Pseudomonas fluorescens* biofilms away from the inner surface of a pipe to which they were attached. The adhesive strength between biofilms and the substratum was defined as the work required to remove the biofilms per unit area from the substratum, with adhesive strength found to increase with the fluid velocity in which the biofilms were grown, with a typical value of approximately 0.2 J/m^2. Attachment features that form between a *Pseudomonas* sp. and the Fe(III)-(hydr)oxide minerals hematite and goethite were characterized (Forsythe et al., 1998). Microbial growth curves in Fe-limited growth media indicated that the bacteria were able to obtain Fe from the Fe(III)-(hydr)oxides for use in metabolic processes. Adhesion and biofilm accumulation on stainless steel was studied by comparing the electrostatic characteristics of bacterial cell surfaces with attachment proclivity and biomass accumulation over time between wild type *P. aeruginosa* serotype O6 (possesses A and B band lipopolysaccharide, LPS) and 3 LPS-deficient mutants, viz. A28 (A+B−), R5 (A+B−), and Gt700 (A−B−) (Flemming et al., 1998). Adhesion and biofilm accumulation on both stainless steel and glass were different between strains, following the order R5 > A28 ≫ O6 > Gt700. Adhesion between nitrifying bacteria and mineral carriers in aqueous media was determined from contact angle measurements and thin layer wicking to obtain surface free energy on flat and smooth solid surfaces. The free energy of interaction between nitrifying bacteria and support materials in aqueous medium was calculated and correlated with bacterial adhesion observed in a previous study (Teixeira et al., 1998). A strain of *Hyphomonas,* the genus of primary colonizer of immersed surfaces in marine water, was found to synthesize two adhesion-mediating structures, fimbriae and capsular exopolysaccharide (Quintero et al., 1998).

Fluorescent Tools. A *Pseudomonas putida* isolated from a biofilter treating toluene-containing gas was used to study the interaction of microbial communities by fusing a green fluorescing protein to the promoters for toluene degradation carried by the TOL plasmid (Møller et al., 1998). When grown alone in benzyl alcohol-fed flow cells, one promoter was not activated, while in mixed biofilms of six other strains from the biofilter, both promoters were active, apparently due to *Acinetobacter* forming benzoate from benzyl alcohol. A strain of *Pseudomonas putida* harboring plasmids RK2 and pDLB101 was also exposed to a pure culture biofilm of *Bacillus azotoformans* grown in a rotating annular reactor under three different concentrations of the limiting nutrient, succinate (Beaudoin et al., 1998). Experimental results demonstrated that the broad host range RSF1010 derivative pDLB101 was transferred to and expressed by *B. azotoformans.*

An in situ technique for quantifying bacterial counts in biofilms was developed which relied on the use of fluorescein diacetate (De Rosa et al., 1998). Once hydrolyzed by an exoenzyme produced by most bacteria, fluorescence as a result of adsorption of 490 nm light was found to linearly correlate to bacterial numbers. Systsma et al. (1998) presented a new scanning microscope utilizing two-photon excitation in combination with fluorescence lifetime contrast. In tests of an acridine orange stained biofilm of 100 mm

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depth, fluorescence lifetime imaging was not affected by a decrease of fluorescence intensity. Unspecific staining and a high fluorescence background that hamper the use of epifluorescence enumeration of bacteria when using 4′,6-diamidino-2-phenylindole (DAPI) and polycarbonate filters was relieved with the use of high-affinity nucleic acid dyes such as SYBR Green II and alumina filters (Weinbauer et al., 1998).

The expression of alkaline phosphatase in response to phosphate starvation was studied by frozen sectioning of biofilms and fluorescent labeling (Huang et al., 1998). Alkaline phosphatase expression patterns conformed to the hypothesis that phosphate utilization is governed by local availability of carbon and energy sources. Cells in suspension and in biofilms were demonstrated to be primarily responsible for the hydrolysis of proteins and polysaccharides by using fluorescent protein and saccharide analogs (Confer and Logan, 1998b). The activity of alpha-glucosidase (93%) and 97% of leucine aminopeptidase activity happened in contact with the cells harvested from a trickling filter, and hydrolysis rates were at least five times higher in contact with cells than in cell-free solutions.

Bacterial Distributions. The presence of sulfate-reducing bacteria (SRB) in aerobic biofilms was found to be high (10^8 SRB/mL determined by in situ rRNA probes) in both oxic and anoxic zones (Santegoeds et al., 1998). *Desulfovibrio* and *Desulfobulbus* were the main SRB present, but diversity and dominant species increased with biofilm age.

Okabe, Kuroda, and Watanabe (1998) analyzed evolutional changes in interior structures of mixed population biofilms grown on domestic wastewater using a cryosectioning technique and also analyzed the transport of particulates to the biofilms using fluorescent microbeads. Microscopic observation of the cryomicrotomy biofilm sections indicated the biofilms were very porous and consisted of intertwined filamentous biomass acting as a framework of the biofilm. Corkidi et al. (1998) developed an automated imaging system to count confluent colonies on petri dishes with 96% accuracy.

The widely used BIOLOG GN system for characterizing microbial communities was compared to ribotype analyses of potato rhizosphere and activated-sludge samples (Smalla et al., 1998). The community profiles derived from BIOLOG largely agreed with the 16S rRNA fragments isolated from the inocula, but enrichment for fast-growing strains was observed. A principle components analysis was used to determine clustering and characterizing the bacterial populations in five wastewater infiltration systems; for high and low loadings, bacterial populations were diverse and narrow, respectively (Pell et al., 1998). Methods for examining mixed species of biofilm communities where the unique interactions between species determine the true properties of the resultant biofilms were developed (Skillman et al., 1998). A study of methods for the disruption of biofilms and flocs to enumerate colony-forming units of different species indicated that a combination of enzymatic and ultrasonic treatment gave the highest yields (Salhani and Uelker-Deffur, 1998). In the biofilms, pretreatment for 2 hours with cellulase followed by 2 minutes of ultrasonic treatment at 50 W gave the highest colony-forming unit counts.

Novel Techniques. Schmitt and Flemming (1998) discussed the use of different Fourier transform infrared spectroscopy (FTIR-spectroscopy) techniques to allow rapid characterization of microbial strains and analytical discrimination between microorganisms, inorganic material or other foulants in a nondestructive manner in an ultrapure or drinking water system as well as on filtration membranes. Plowman et al. (1998) presented expressions that govern integrated optical wavelength methods and described the common experimental configurations used in attenuated total reflection, fluorescence, and Raman applications. These methods were used in a study of adsorbed or surface-bound proteins to polymer and glass waveguides. Deshusses et al. (1998) described the first use of computed axial tomography (CAT) scanning to characterize the structure of packed bed biofilters and biotrickling filters used for waste air treatment. A detailed analysis of CAT scan images demonstrated the heterogeneities of the channels through which air and water—in the case of biotrickling filters—flow.

The results of measuring the mobility of water in intact biofilms with pulsed field gradient nuclear magnetic resonance (PFG-NMR) obtained with several well-defined systems including pure water, agar, and agar-containing inert particles or active bacteria were compared to glucose diffusion coefficients measured with microelectrodes and those calculated utilizing theoretical diffusion models (Beuling et al., 1998). A good correspondence was observed indicating that PFG-NMR should also enable the measurement of diffusion coefficients in heterogeneous biological systems. Truesdail et al. (1998) constructed and operated a simple streaming potential apparatus capable of measuring the zeta potential of granular media, which can be used in the study of a variety of granular materials in biofilms. The overall precision of this instrument was ±10% based on the 95% confidence interval. Biological processes can result in a significant change of zeta potential of water flow, making the zeta potential of effluent less negative than that of raw water (X.H. Zhang et al., 1998). This potential change was combined with a coagulative process using a biofilm in the shape of a cobweb, which proved capable of holding a large number of turbidity-producing particles.

Other. A review elucidated the experimental measurements of effective diffusive permeabilities and effective diffusion coefficients in biofilms (Stewart, 1998). It was proposed that large solutes are effectively excluded from microbial cells, small solutes partition into and diffuse within cells, and ionic solutes are excluded from cells but exhibit increased diffusive permeability due to sorption to the biofilm matrix. The transfer of the TOL plasmid in a flow chamber biofilm community was investigated by scanning confocal laser microscopy, 16S rRNA hybridization, and expression of green fluorescence (Christensen et al., 1998). Transconjugants were found to be always associated with recipient microcolonies, with minimal horizontal transfer. A computer program and flow cell system was developed to perform automated investigations of biofilms using image acquisition and analysis from confocal laser scanning microscopes to generate biofilm information in three dimensions (Kuehn et al., 1998). Joannis et al. (1998) compared three methods for determining the total biofilm amount in biphasic cultures: dry weight by filtration after solvent treatment, optical diameter with a biomass probe, and protein content. The optical density by biomass probe was the most reliable method (repeatability $< 0.5\%$) to quantify total biofilm and a linear relation was verified against dry weight. Another method for studying biofilms used confocal scanning laser microscopy in combination with a wide range of fluorescent probes and markers to determine biofilm depth, bacterial cell area, exopolymer area and algal biomass at various depths and locations (Lawrence et al., 1998). The method proved simple and effective for determining treatment effects such as grazing by invertebrates.

Another comparative study of methods examined the reproducibility and efficiency of five techniques to extract extracellular polymeric substances (EPS) from two biofilms (X. Zhang et al.,

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1998). A washing step was found to be essential in collecting the polymers from biofilms, but none of the five methods, regular centrifugation, EDTA extraction, ultracentrifugation, steaming extraction, or regular centrifugation with formaldehyde, was found to be clearly superior to the others.

The development in and closure of channels and pores by biofilms was investigated by examining breakthrough curves of retained (cadmium) and nonretained (phenol) compounds (Du Plessis et al., 1998). The curves were significantly influenced by microbial growth within the porous media, and thus identified different stages of biofilm development and could be used to predict porosity reduction due to microbial growth.

Stoodley et al. (1998) grew mixed population biofilms consisting of *Pseudomonas aeruginosa, P. fluorescens,* and *Klebsiella pneumoniae* in a flow cell under turbulent conditions with a variable water flow velocity of 18 cm/s (Reynolds number, *Re*, of 1192) to 50 cm/s (*Re* of 3351). After 7 days the biofilms were patchy and consisted of cell clusters and streamers separated by interstitial channels, and the streamers demonstrated an oscillation frequency related to the flow velocity by the Strouhal relationship, suggesting that the oscillations were possibly caused by vortex shedding from the upstream biofilm clusters.

The rates of sorption and distribution coefficients by a mature biofilm representing an estuarine biofilm were determined for tetrabutyltin, p,p-DDT, diclofop-methyl, triallate, lindane, atrazine, and parathion-methyl (Headley et al., 1998). Sorption rate coefficients, in 10^{-4} per minute, were, in the order given above, 180, 8, 70, 110, 230, 370, and 100, and the distribution coefficients, in 10^3 mL/g, were 60, 800, 90, 55, 30, 25, and 60; these coefficients were found to strongly correlate to aqueous solubility of the compounds. Spaeth et al. (1998) investigated the differing sorption properties, preferences, and capacities of EPS cell walls, cell membranes, and cytoplasm with respect to pollutants such as benzene, toluene, and xylene (BTX) or heavy metals such as cadmium and zinc. They found that 60% of BTX was localized in the EPS, while 80% of cadmium and zinc were found in the cellular fraction. The sorption of nonbiodegradable compounds resembling natural organic matter was studied in packed bed bioreactors (Carlson and Silverstein, 1998). As molecular size increased, sorption decreased, and negatively charged molecules sorbed less than neutral molecules.

Roeske et al. (1998) used two tubular plug flow reactors, one operated as continuous flow and the other as a sequencing batch reactor, fed from a full-scale biological wastewater treatment plant to identify spatial gradients and distribution of bacteria using group-specific oligonucleotide probes. In both reactors, beta-Proteobacteria dominated with the cytophaga-flavobacterium group and gram-positive bacteria abundant, and only small amounts of gamma-bacteria detected.

Acuna et al. (1998) evaluated the transfer and elimination of pollutants by a moist packed bed containing immobilized microorganisms and fitted the Gompertz model to the experimental data. Toluene consumption rate measurements in biofilter microcosms could reach equivalent elimination capacities higher than 615 g toluene/m^3 · h. A mathematical model was also developed for determining the oxygen diffusion coefficient and maximum respiration activity in a biofilm for *p*-toluenesulphonic acid degradation by *Comamonas testosteroni* T-2 for multispecies biofilms in a fixed bed biofilm reactor (Khlebnikov et al., 1998b). The oxidation coefficient obtained ($2 \times 10^{-10} - 1.2 \times 10^{-9}$ per m^2·s) was in good agreement with published values. X. Liu et al. (1998) showed that consortia of catalase-positive bacteria consisting of *Pseudomonas aeruginosa, Pseudomonas fluorescens,* and *Klebsiella pneumoniae,* in both the planktonic form and as biofilms, disproportionate hydrogen peroxide into oxygen and water. The biofilm, however, continued to disproportionate the hydrogen peroxide in the presence of the catalase inhibitor, 3-amino-1,2,4-triazole, while the planktonic organisms did not.

On the human side of biofilm characterization, Sayler et al. (1998) discussed the passage of information from scientists to operators. Complex information on the composition and genetics of cultures treating wastewater may be available in the near future, but systems to allow operators to use such information for decision making have not been significantly developed.

GROWTH AND MODELING

Mass Transfer. Wood and Whitaker (1998) identified three regimes in which the spatially smoothed transport equations take on special forms and determined the domain of validity of the resulting three models. The first is the one-equation model that is valid when the principle of local mass equilibrium is satisfied, the second is the two-equation model that is not constrained by the principle of local mass equilibrium, and the third is a pseudo one-equation model, which occurs when the reaction in the intracellular phase can be treated as instantaneous. A numerical method was developed for the microscale model of the mass transport, and bioreaction results in spatial gradients of nutrients and electron acceptor concentrations in bacterial growth in soil (Dillon et al., 1998). Also, a predictive, two-dimensional mathematical model to describe microbial uptake, diffusion through a biofilm, and mass transfer of volatile organic chemicals (VOCs) from gas to liquid was validated by experimental data collected from operating trickle-bed bioreactors designed for removing sparingly soluble gaseous contaminants (Barton, Zhang, et al., 1998). Operation in regimes in which both mass-transfer and kinetic factors play significant roles and predictive modeling implications were discussed. S.F. Zhang et al. (1998) used 1,1,2-trichloroethane, a nonreacting tracer, to determine the flux and mass-transfer rates of the substrates being degraded, 1-2-dichloroethane and monochlorobenzene for *Xanthobacter autotrophicus* GJ10 and *Pseudomonas* JS150, respectively, in a single tube extractive membrane bioreactor. In JS150 the diffusion coefficients range from twice that of water, at the surface, to 30% of water for biofilms of 1 mm thick and for GJ10 they remained about the same as water.

A transient mathematical model was established to evaluate oxygen diffusivity in non-steady-state biofilms, and was evaluated in a submerged fixed bed biofilm system fed *p*-toluene sulfonate (Khlebnikov et al., 1998a). The oxygen diffusion coefficients calculated from concentration profiles varied with biofilm development and reported values (2×10^{-10} to 1.2×10^{-9} m^2/s) were in good agreement with literature data.

Kinetic Models. A mathematical model for biomass density, spreading, and distribution for diffusion-reaction-microbial growth systems was tested for immobilized cells growing in spherical gel beads where it closely corresponded in each area (Picioreanu et al., 1998a). Samb et al. (1998) developed a mathematical model for estimating maximal oxygen removal rate, saturation constant and internal diffusion coefficient based on a bioreactor consisting of a column divided in separate units packed with expanded clay pellets and used for wastewater treatment.

Riefler et al. (1998) constructed a model and measured biokinetic parameters in a completely mixed attached growth bioreactor subjected to a pulse of substrate by monitoring oxygen consump-

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tion from the bulk liquid. Measured values were compared to the model, and a sensitivity analysis revealed that the dissolved oxygen profiles were sufficiently sensitive to the biokinetic parameters to support parameter estimation if accurate estimates of other model parameters were obtained. The model BacSim, describing bacterial properties including substrate uptake, metabolism, maintenance, cell division and death at the individual cell level, was developed to simulate growth and behavior of bacteria (Kreft et al., 1998). With the aim of making the model easily applicable to various bacteria under different conditions, the model uses as few as eight readily obtainable parameters, which can be randomly varied.

Sommer et al. (1998) also developed a mathematical model based on mass balances of a disc-shaped reactor volume element taking into account residence time and distribution of fluid phases by the axial dispersion model. This model can be used to optimize biological processes in a three-phase, fluidized-bed reactors. Another mathematical model of biofilm development, based on simple local rules for growth and detachment of individual cells, produced several simulations suggesting that the thickness of concentration and hydrodynamic boundary layers as well as biofilm strength to withstand erosion, to a lesser extent, have an important effect on the developing biofilm structure (Hermanowicz, 1998).

A conceptual model that includes reaeration and main aerobic and anaerobic microbial processes in water phase and sewer biofilms emphasizes microbial transformations of heterotrophic biomass and solution and particulate fractions of organic substrate while including sulfate respiration (Vollertsen et al., 1998). This model concept can be used in the design process of sewers taking into account quality aspects. Henneken et al. (1998) studied the microbial mineralization of EDTA in wastewater by a mixed culture with suspended and immobilized cells. A complete set of kinetic parameters was determined that enabled the modeling of EDTA degradation and, related to this, bacterial growth, ammonium release, and maintenance requirement as well as oxygen uptake.

Hydraulics and Structure. A laboratory-scale biofilter packed with peat was used to analyze residence time distribution to verify an axial dispersion model that was developed and solved numerically. The predicted concentration profiles were in very good agreement with the experimental data (Zarook et al., 1998). In an article largely in French, Lakel et al. (1998) reported the modeling of an in-series denitrifying/nitrifying filter pair with recycle with a dispersed-flow model. The model, describing the filters as a dynamic (flow) region interacting with a static (sorptive) phase was validated with operational data at laboratory scale. Picioreanu et al. (1998b) used a hybrid differential-discrete mathematical model to simulate biofilm structures (surface shape, roughness, porosity) as a result of microbial growth in different environmental conditions. Quantitative two- and three-dimensional models were evaluated and then statistical measures were introduced to characterize the complete biofilm structure. A different model based on a pore network was used to predict porosity and permeability changes in a porous medium as a result of the buildup of a biofilm composed of four bacterial species and a nutrient species (Suchomel et al., 1998a and 1998b). The adsorbed species influence the effective radii of the pipes in the network, which affect the porosity and permeability. The authors developed a technique for integrating the coupled system of ordinary and partial differential equations that govern transport of these species in the network.

Treatment Design. Wik and Breitholtz (1998) examined models and simulation methods to optimize the design of plants using biofilm reactors, and developed a model that uses transfer functions describing the fast dynamics of stirred-tank reactors with zero- or first-order reactions inside the biofilm. A dynamic mathematical and numerical model of adsorption and biological degradation of nutrients in an organic peat perfusion column with recycle, containing four dimensionless parameters without biological activity and two with biological activity, was suitable for industrial applications involving biodegradation of nutrients in wastewater, such as biofilters and biotrickling filters where concentrations are dilute and solid surface coverage is low (McNevin and Barford, 1998). The models of Rittmann were built upon to provide a model of biofilm reactors in series of equal volumes (Ojha and Shrivastava, 1998).

From information obtained from a trickle-bed bioreactor, which degraded VOCs, a model was created based on a two-step process: mass transfer in which the VOCs diffuse into the liquid biofilm, and kinetics by which VOCs are degraded by the biofilm (Barton, Hartz, et al., 1998). Modeling results revealed that both kinetic and mass-transfer resistances were significant under typical operating conditions.

Other. Two groups of strains isolated from biofilters for the treatment of waste gases were assigned to the genus *Paracoccus* by phylogenetic and chemotaxonomic methods. Based on these results an amended description for the species *P. solventivorans* was proposed (Lipski et al., 1998). Predation of *Pseudomonas putida* by the small ciliate *Tetrahymena* sp. was studied for attached and suspended bacterial growth (Eisenmann et al., 1998). Predation rates of 1 382 ± 1 029 bacteria/individual·h were observed under attached conditions, with decreased grazing rates in suspended cultures.

Laboratory experiments indicated enzymes responsible for hydrolysis in suspended and fixed film biological wastewater treatment systems are primarily those that remain attached to the cell (Confer and Logan, 1998a). A generalized mechanism for macromolecular degradation was found that features cell-associated hydrolysis followed by the release of hydrolytic fragments, where fragment release was found to be larger for proteins than for carbohydrates.

TRICKLING FILTERS

Pilot Scale. Both biological and chemical removal of manganese were observed in a pilot-scale trickling filter (Gouzinis et al., 1998). Operation as a sequencing batch reactor improved removal of Mn to 94%, and simultaneous operation with ammonia and iron showed some inhibition of Mn removal at ammonia concentrations above 2 mg/L. Another pilot-scale biotrickling filter demonstrated greater than 97% removal of 300 ppm_v styrene over a 175-day period at empty-bed contact times of 2, 1, and 0.67 minutes (Sorial et al., 1998). Backwashing was necessary to control biomass despite attempts to control biomass with nutrient-phosphorus limitations. The secondary treatment of Alexandria, Egypt, wastewater in a pilot-scale trickling filter designed for 1.4 $kg/m^3 \cdot d$ (85 lb $CBOD_5$/d 1 000 cu ft) was sufficient to produce an effluent in accordance with Government of Egypt Law 48 of 1982 (de Barbadillo et al., 1998). The data were fit with a linearized form of the modified Velz equation, giving a treatability coefficient of 0.001 3 to 0.001 4 $(L/m^2 \cdot s)^{0.7}$ with an assumed hydraulic coefficient (n) of 0.7, although at a Spulkraft intensity (SK) of 100, 0.64 was the observed n, and at a daily flushing SK of 300, n was 1.44.

Full Scale. The effects of six trickling filters and four activated-

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sludge wastewater treatment plants on the environment were studied to assess the removal of alkyl ethoxylate sulfates (AESs), alcohol ethoxylates (AEs), and linear alkylbenzene sulfonates (LASs). McAvoy et al. (1998) found the receiving waters immediately below treatment plant outfalls were less than their corresponding biological predicted no-effect concentrations in greater than 98% of the locations under low-flow conditions. Six different trickling filter wastewater treatment plants were monitored for LAS effluent concentration, which ranged from 4 to 460 mg/L, with an average value of 240 mg/L (Holt et al., 1998). The plants gave a removal of 70 to 99% and a simple model was developed to accurately predict the variation in LAS concentrations in the final effluent when measured influent concentrations, flow, and settling tank volumes are known.

Nutrient Removal. Raj (1998) looked at nitrification performance and attachment of biomass on packing media in a trickling filter. A maximum specific surface nitrification rate of approximately 1.21 $g/m^2 \cdot d$ at loading rates of 5, 9, and 13 $m^3/m^2 \cdot d$ was found on packing media in a trickling filter. The maximum specific surface nitrification rate of a synthetic wastewater in a cross-flow medium trickling filter was approximately 1.21 $g/m^2 \cdot d$ (Amal and Murthy, 1998). Consumption and the results of investigating the ammonium conversion along the filter depth by scanning electron microscope were found to be satisfactory. The nitrite oxidation capacity in biofilms was found to be variable and sensitive to environmental disturbances when developing a model for a pilot-scale trickling filter on eel farms that sufficiently predicts ammonium removal (Kamstra et al., 1998). Morgan and Farley (1998) described removals of ammonium, nitrogen, and phosphorus in an upgrade of a wastewater treatment plant (WWTP) in Tasmania from a trickling filter process treating 4.1 ML/d to a hybrid trickling filter/biological nutrient removal (BNL) treatment train with a capacity of 10.4 ML/d treating combined wastewater. The plant is presently undergoing process testing to meet a performance guarantee. Chemical pre-, simultaneous, and post-precipitation for phosphorus removal at trickling filter wastewater treatment plants were all capable of 2 mg/L, while only pre- and post-precipitation options attained 1 mg/L (Pearce, 1998). When attaining 1 mg/L, careful attention was paid to the rapid mixing, subsequent flocculation of the coagulant, and P solubility equilibrium from the primary settling tanks, used for sludge storage.

Raj and Murthy (1998) looked at nitrification performance and attachment of biomass on packing media in a trickling filter. They found a maximum specific surface nitrification rate of approximately 1.21 $g/m^2 \cdot d$ at loading rates of 5, 9, and 13 $m^3/m^2 \cdot d$. Balmer et al. (1998) studied a highly loaded activated-sludge plant in Sweden that had been upgraded using a compact process based on tertiary nitrification in trickling filters and recirculation to a highly loaded activated-sludge unit for denitrification. The necessary volumes were achieved by expanding the plant upwards, thus making it possible to place the trickling filters as part of the area occupied by the former aeration basins, but it was necessary to double the secondary settler capacity.

Media. Nitrification rates in polystyrene bead and trickling media biofilters were compared, with the trickling filter's nitrification rate 7.5 times higher than that in the polystyrene bead filter (Greiner and Timmons, 1998). Shanableh and Hijazi (1998) used a three-phase fixed film biological nutrient removal biofilter system for reuse and discharge of aquaculture water that alternates aeration and nonaeration cycles to allow phosphorus removal in two of three biofilters. The removal of TOC and ammonia improved with cycle duration, but phosphorus removal was limited by available internal organic carbon.

Upgrades. An upgraded trickling filter process in Texas included installation of a new influent structure that screened solids using a heli-sieve (inclined shaft-less screw with screen) (Vivona et al., 1998). Screened wastewater flowed to a splitter box where portions of the flow were sent to the trickling filters and the activated-sludge plant, respectively. The screenings, which were dewatered, compacted, and discharged to a dumpster, optimized the two treatment processes for maximum reliability under the full range of influent conditions year-round. A low-technology and low-cost system for municipal wastewater treatment, which uses a ponding process producing microalgae followed by a trickling filter, suggested that microalgae contribute to biofilm production and organic load reduction in the trickling filter (Shipin et al., 1998). To upgrade a WWTP in Canada, where average monthly wastewater temperatures fall to 9 to 10 °C, a trickling filter/solids contact (TF/SC) treatment scheme was evaluated that took advantage of the low BOD of the WWPCP's chemical primary effluent. In testing over 1 year, the TF/SC process consistently met the target objectives, moving it up from fourth place to a tie with the other first-ranked process, the biological aerated filter (BAF) (Parker et al., 1998).

Other. In laboratory experiments under various load conditions, a slight enhancement of the removal of coliforms and fecal streptococci was indicated by magnetic fields induced either by a direct current passing through the filter or by a coil surrounding the filter column, but not by magnetic particles added to the filter medium (Filipkowska and Krzemieniewski, 1998).

ROTATING BIOLOGICAL CONTACTORS

Design. Rather than design rotating biological contactors (RBCs) on the basis of empirical loading factors, a loading curve derived from steady-state biofilm kinetics was evaluated using literature-derived data (Seagren and Qin, 1998). The loading curve was found to predict organic removal relatively well, but the model descriptions of the data could be improved, possibly with better parameter estimates.

Biofilm Thickness and Loading. Biofilm formation and the significance, effect, and measurement of biofilm thickness on wetted disc surfaces of RBCs, as well as the role thickness of biofilm has on the MLSS/MLVSS ratio, was studied (Venkataraman and Ramanujam, 1998). Increasing substrate loading from 6 to 30 g soluble chemical oxygen demand on an RBC biofilm resulted in an increase in dry mass from 2.5 to 4.7 mg/cm^2 and thickness from 0.5 to 1.6 mm (Park et al., 1998). At high substrate loading rates, the morphology also changed to a filamentous structure.

Respiration and Oxygen Transfer. Okabe, Matsuda, et al. (1998) studied O_2 respiration, H_2S oxidation, and SO_4^{2-} reduction in aerobic RBC biofilms by measuring concentration profiles with microelectrodes for O_2, S^{2-}, and pH. The distribution of sulfate-reducing bacteria, determined by the most probable number (MPN) method and fluorescently labeled 16S rRNA-targeted oligonucleotide probes, corresponded well with the O_2 and S^{2-} concentration gradients measured by the microelectrodes. A thorough study of oxygen transfer in RBCs used propane as a tracer gas to determine the overall mass-transfer coefficient, K_La, of clean discs and the enhancement of K_La by the growth of biofilms (Boumansour and Vasel, 1998). An enhancement factor ranging

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from 1 to 3.6 was observed, and existing models of both mass-transfer and enhancement factors were compared to data.

The toxic effects on respiratory activity of phenol was studied in cultures of phenol-oxidizing bacteria grown in two chemostats at mean cell residence times (MCRT) of 3.8 and 18.7 days, and one culture with RBC at a MCRT of 71 days was modeled using Haldane kinetics (Lee and Cheng, 1998). Cells from the chemostat with 3.8 MCRT had the highest respiratory activity and those from RBC reactor were least active.

Nitrogen Removal. A pilot-scale RBC was used to investigate nitrate-nitrogen removal from groundwater using methanol, ethanol, and acetic acid as carbon sources (Mohseni-Bandpi et al., 1998). The optimum ratios of methanol, ethanol, and acetic acid to nitrate-nitrogen ratios were 2.9, 2.35, and 4.3, respectively, yielding 93, 91, and 98% removal efficiency at a loading rate of 76 $mg/m^2 \cdot h$. Up to 90% loss of inorganic nitrogen without nitrite accumulation in the RBC step in a biological pretreatment of landfill leachate in Mechernich (Germany) was experienced and confirmed through aerobic batch tests without the addition of organic substrate (Helmer and Kunst, 1998).

Other. Biofilm growth in continuous flow, rotating annular biofilm reactors neither helped nor hindered the activity and stability of the trichloroethylene degradative plasmid TOM-31c in selective and nonselective biofilm cultures (Sharp et al., 1998). Perez et al. (1998) studied communities in biofilms of three RBCs in different facilities to characterize, determine abundance and spatial variation, and relate spatial segregation and richness. The 33 to 67 ciliate species were different in group and species composition from activated-sludge communities even though both consisted of mainly peritrichs. Rotating biological contactor biofilm dry mass as a function of disk material measured dry matter ranging from 7 mg/cm^2 for glass particle and diatomite earth support materials, to 9 mg/cm^2 for sand disk, and 10 mg/cm^2 for activated carbon disks (Apilanez et al., 1998). Growth occurred in two phases: a first phase of colonization, of approximately 1 to 2 days, followed by a growth phase with a specific growth rate constant of 1.1–1.3 per day, which indicated that carbon was the best of the tested support materials for biofilm formation.

Siegrist et al. (1998) found that increasing partial pressures of oxygen and ammonium concentration favor nitrogen removal over ammonium oxidation in the rich leachate of a hazardous waste landfill. The process used included pretreatment, which reduced dissolved organic carbon to less than 20 mg/L yielding nitrification rates of 3 to 4 g NH_4-$N/m^2 \cdot d$ at a pH of 7 to 7.3 in the first two of three RBC compartments.

FLUIDIZED BED AND AIRLIFT BIOREACTORS

Biological Nutrient Removal. Much of the research in the area of fluidized and airlift bioreactors focused on nitrogen removal. An innovative reactor scheme tied an airlift reactor to nitrify a wastewater, which then fed a suspension of denitrifiers (van Benthum et al., 1998). With a load of 5 kg NH_3-$N/m^3 \cdot d$, a 75% removal of total nitrogen was observed at a COD/N-ratio of 3 g/g. Nitrogen was removed from landfill leachate at pilot scale using an aerobic/anoxic sequence of suspended biofilm reactors (U. Welander et al., 1998). The highest rate of nitrification, 24 g N/m^3 reactor·h at 16 °C, was observed with the highest surface area media (390 m^2/m^3), and with methanol as a C source a denitrification rate of 55 g $N/m^3 \cdot h$ was measured.

Furtado et al. (1998) determined that a hydraulic retention time (HRT) of 8 to 10 hours was enough to reduce ammonia-nitrogen concentration to levels below permitted legal limits (5 mg NH_3-N/L) for a petroleum refinery effluent treated in an airlift bioreactor using activated carbon particles as a biofilm support. The reactor nitrifying performance was maximized at 85% removal of ammonia-nitrogen, for a HRT of 10 hours. The rate of denitrification of a synthetic wastewater in two nitrogen-sparged airlift bioreactors reached 10.5 $kg/m^3 \cdot d$ with a sludge loading rate of 3 g N/g biomass·d (Yu et al., 1998). With sand as a carrier, one reactor with two concentric draft tubes rapidly developed an easily detachable biofilm, while the other reactor, which used a three-phase separator at the top, steadily developed a 25 g/L biofilm. Skjolstrup (1998) used two identical fluidized bed biofilters that, after biofilter maturation, gave stable concentrations of total ammonia-nitrogen, nitrite, and nitrate within the system. Oxygen consumption ranged from 56 to 64% due to nitrifying activity, which produced 68% of the total system nitrate.

For both nitrogen and phosphate removal, Roske et al. (1998) examined the performance and the composition of the microbial community in two bench-scale reactors. One reactor was a fluidized-bed biofilm reactor for nitrification arranged as the ultimate treatment step subsequent to BOD and biological P elimination, the other one a series of anaerobic, anoxic, and aerobic tanks. The performance of both reactors was high, and despite the quite different mode of operation, the microbial composition in terms of primary taxonomic groups did not display significant differences.

Biofilm Carriers. Materials leading to low surface free energies of interaction and with few negative surface charges favored bacterial adhesion and the formation of stable biofilms in external loop airlift reactors (Teixeira and Oliveira, 1998). A mineral support material achieved denitrification volumetric removals of 0.6 kg NO_x-N removed/m^3 of total reactor volume·d in pilot-plant trials of the Mixazur® (Chudoba et al., 1998). The results were confirmed at full scale, and an optimal soluble COD to NO_x-N ratio of 5 to 7 was maintained by installing the reactor as a preaeration process.

A granular activated carbon carrier fluidized bed bioreactor was confirmed as an environmentally acceptable technology for the destruction of aqueous-phase dichloromethane (Flanagan, 1998). After an acclimation period, dichloromethane was used as the sole source of carbon and energy, resulting in a 40 $kg/m^3 \cdot d$ biodegradation rate with detectable dichloromethane in the process effluent. Imai et al. (1998) studied the effects of preozonation of a biological activated carbon fluidized bed in the removal of refractory landfill leachate organics. This resulted in enhanced adsorbability and removal of nonabsorbable organics, and steady-state dissolved organic carbon removal was enhanced from 42 to 57% at a hydraulic retention time of 24 hours.

Sand, volcanite, and diatomaceous earth were compared as biocarriers in a study of chlorophenol degradation at temperatures ranging from 4 to 16 °C (Melin et al., 1998). Volcanite promoted the greatest biomass formation and sand the least. Kinetic parameters for the studied compounds were reported as varying from 0.24×10^{-3} to 31×10^{-3} mg/mgVS·h for V_{max} and from 0 to 7.1 mg/L for K_m, with a 10 °C decrease in temperature reducing rates by at least seven-fold.

Other. An experimental study compared mixing and phase hold-ups in classical and injected three-phase fluidized beds (Buffiere et al., 1998). Injected biofilters had less gas hold-up and differed in the degree of axial mixing. Hirata et al. (1998) also evaluated three-phase fluidized bed biofilm performance as a zero-order reaction. The removal rate was proportional to the specific surface area of the biofilm, with degradation coefficients of 1.25 ×

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10^{-2} kg-phenol/m^2 biofilm·d, and it was found that at a specific biofilm surface area per volumetric phenol loading rate of >80 m^2/kg phenol·d almost 100% of phenol could be removed. A new approach for the determination of liquid–solid mass-transfer coefficients and biofilm reaction rate was based on the analysis of oxygen consumption and a biological oxygen-monitoring system (Nicolella et al., 1998). In a laboratory-scale biofilm airlift suspension reactor the liquid–solid mass-transfer coefficient measured for biofilm-coated particles was found to be smaller (by a factor varying from 5 to 25%) than the values reported for rigid particles.

Sommer et al. (1998) developed a mathematical model based on mass balances of a disk-shaped reactor volume element, taking into account residence time and distribution of fluid phases by the axial dispersion model. This model can be used to optimize biological processes in a three-phase, fluidized-bed reactor. A different model was developed as a new approach to evaluate substrate consumption rate, average biofilm density and active thickness of a spherical bioparticle in a completely mixed fluidized bed system (Tanyolac and Beyenal, 1998). A reasonable correlation was observed between the model prediction and experimental results for substrate consumption rate and average biofilm density for thin and fully active biofilms. Kwok et al. (1998) also studied the influence of process conditions on density, surface shape, thickness and biomass yield on a biofilm in a biofilm airlift suspension for loading rates varying from 5 to 20 kg COD/m^3 and basalt concentrations of 60 and 250 g/L. They observed patchy and heterogeneous biofilms with pores and protuberances for both high and low detachment forces, but with the right balance, smooth, dense and stable biofilms can be obtained.

The TNT precursors 2,4- and 2,6-dinitrotoluene (DNT) were simultaneously degraded by a mixed culture in a fluidized-bed biofilm reactor (Lendenmann et al., 1998): 40 mg/L 2,4-DNT and 10 mg/L 2,6-DNT were removed, 98 and 94%, respectively, at surface loading rates of 36 to 600 mg DNT/m^2·d. An aerobic biological fluidized bed removing ethylene and propylene glycols could achieve 97% TOC removal and sustained TOC removal of 87% with single pulse loadings with a 10-fold increase in pulse magnitude and pulse durations as long as 7 hours (Shieh et al., 1998).

SUBMERGED BED BIOFILM REACTORS

Denitrification. Bourrel et al. (1998) modeled a fixed-bed denitrification bioreactor. Simulation results showed that discrepancies between the solution of the identified model and available on-site measurements were quite small for such a complex process. A proprietary up-flow anoxic biofilm reactor followed by a nitrifying step demonstrated a 70% N-removal efficiency without added carbon and an 85% removal efficiency with methanol addition during a 1-year study (Ninassi et al., 1998). The denitrification stage achieved a removal rate of 1 to 1.2 kg NO_3-N/m^3 of reactor·d with a recycle ratio of 2.5. Hydrolyzed sludge and solid organic waste were compared to ethanol and acetic acid as feed stocks for a denitrifying packed bed bioreactor. A maximum denitrification rate of 2.5 kg NO_3-N/m^3·d was achieved with ethanol, sludge, and solid waste; however, the COD/N loading required for maximal activity varied among the feedstocks from 4.5 g COD/g NO_3-N for ethanol to 10 g/g for sludge and solid waste (Æsøy et al., 1998).

To limit carbon addition costs required for denitrification in a Biostyr® pilot column, a controller was installed that based methanol dose on the influent and effluent nitrate concentrations (Puznava et al., 1998a). The control strategy decreased methanol usage by 20%. The same group used a pilot-scale Biostyr column filled with polystyrene beads to control carbon addition to denitrified waste water (Puznava et al., 1998b). Methane was added based on the inlet/outlet concentration of nitrate, minimizing methanol addition (up to 20% less consumption), thus optimizing operation costs. The wastewater from a fish rearing tank was denitrified for reuse in a packed-bed reactor with added ethanol as a carbon source. A treatment capacity of 2.4 kg NO_3-N/m^3 was observed, and backwash of the medium was required every 3 days (Sauthier et al., 1998). Formaldehyde was used as a carbon source for a denitrifying anoxic filter also fed urea (Garrido et al., 1998). Above 300 mg/L formaldehyde inhibited urea hydrolysis, and nitrous oxide accumulated when formaldehyde loading was increased from 1.5 to 6 g/L·d. Reducing the period required to characterize the performance of a biofilm reactor from steady state to quasi steady state gave approximately equivalent results while speeding the tests on the reactor (Flere and Zhang, 1998). The reactor examined was a sulfur/limestone autotrophic denitrification biofilm reactor, which was found to foul more rapidly with a real groundwater than a synthetic groundwater. In a study of the possible retrofit of a treatment plant to add denitrification, an upflow reactor filled with floating media demonstrated 5 mg NO_x-N at a velocity of 250 m/d (Kawai et al., 1998). Methanol addition was necessary, but even during low-temperature periods (13.7 to 17.2 °C), more than 90% of NO_x-N in secondary-treated water was removed at a NO_x-N volumetric load of 3.0 to 3.25 kg-N/m^3·d.

Odegaard et al. (1998) conducted experiments using a floating filter based on upflow filtration through a randomly packed filter-bed consisting of plastic biofilm carriers typically used in the Kaldnes moving bed biofilm process. Due to the high porosity of the filter bed, head loss was low, resulting in long filter runs and high sludge accumulation capacities. Suspended solids removal efficiency was 75 to 85% at a filtration rate of 5 to 15 m/h, making this concept a very interesting one for primary treatment. A deep bed filter for denitrification was studied for ability to remove nitrate from wastewater entering Chesapeake Bay beyond levels currently reached at the Blue Plains denitrification demonstration facility (Bailey et al., 1998).

Nitrification. Yun et al. (1998) developed a bench-scale airlift submerged biofilm reactor to test the possibility of nitrification of the final effluent discharged from a wastewater treatment process of a steel-making plant with an aim of reusing it as irrigation water. At an aeration rate of 4 m^3/min and the hydraulic retention time of 4 hours, the nitrification efficiency was as high as 92% and the nitrification rate was 34 mg NH_3-N/m^3 bed·h. In submerged aerated biological filters, after 10 days the biofilm activity became stable, an ammonia removal rate of 2 g NH_3/m^3·h was observed from a synthetic saline wastewater (50 g/L NaCl), and the attached biomass reached a maximum value of 172 μg/cm^2 (Rosa et al., 1998). When the system was operated continuously, with a hydraulic retention time of 15 hours, the removal efficiency observed was 94% in the absence of NaCl and 48% in the presence of 50 g/L NaCl. Zeolite was used for ion-exchange removal of ammonium and then taken off line for in-place regeneration by a biofilm growing on the zeolite (Lahav and Green, 1998). A high biomass, 10 g VSS/L of reactor, was established with concomitant removal of 95+% of 40 mg ammonium/L at a 2-minute retention time and little need for regenerant.

Domestic Wastewater Treatment. In a moving bed biofilm reactor/solids contact reaeration (MBBR/SCR), treating municipal

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wastewater at 3 days mean cell residence time (MCRT) in the SCR stage, a final effluent with a 5-day BOD_5 of less than 10 mg/L was achieved at an organic load on the MBBR of 15 g $BOD_5/m^2 \cdot d$ (Rusten, McCoy, et al., 1998). With the same MCRT, a final effluent of less than 15 mg BOD_5/L was achieved at an organic load on the MBBR of 20 g $BOD_5/m^2 \cdot d$. Submerged aerated biofilters can be considered a viable alternative for posttreatment of effluent rather than upflow anaerobic sludge blanket (UASB) reactors treating domestic wastewater (Goncalves et al., 1998). An UASB reactor with a hydraulic detention time of 6 hours achieved approximately the same results as a submerged aerated biofilter with granular media and a theta of less than 3.3 m (11 ft). The performance of five full-scale Biostyr installations in Denmark and three pilot-scale Biostyr installations in the United States was described (Holbrook et al., 1998). The ability of moving bed bioreactors, in which biomass is attached to small plastic elements that move freely in the reactor, to treat small wastewater flows was reported by Rusten and Neu (1998). As a result, a tentative design table for on-site treatment of septic tank effluent from 5 to 1 000 population equivalents was presented. A semiempirical model for the biological oxidation in the aerated submerged fixed film process has been formulated, and rate constants were calculated based on statistical analysis of a pilot plant using the four-stage aerated submerged fixed film process (Hamoda and Al-Ghusain, 1998).

Industrial Treatment. Coke plant effluent, high in ammonia and refractory organics, was treated in an anaerobic–anoxic–oxic biofilm system. Removal of NH_3-N at an influent concentration of 3.1 mg/L was 98.8%, and removal of COD at an influent concentration of 114 mg/L was 92.4% at a hydraulic residence time of 31.6 hours (M. Zhang et al., 1998). Anaerobes were responsible for transformation of high-molecular-weight organics to more biodegradable compounds, with naphthol, naphtholnitrile, methylimidazole, benzofuran, benzoquinoline, and anthrylnitrile completely removed from the effluent, which contained phathalic ester, alkylated pyridines, and quinolines. Tseng et al. (1998) found that a submerged biofilter could be used as a part of a recirculating system for *Penaeus monodon* production, removing pathogens from seawater. Rusten, Siljudalen, et al. (1998) described a biological pretreatment plant for poultry-processing wastewater using an aerated equalization tank followed by two high-rate moving bed biofilm reactors in series. With a specific biofilm surface area of 250 m^2/m^3 and a total volumetric organic load of 30 to 45 kg $COD/m^3 \cdot d$ on the first reactor, the removal of filtered COD was as high as 80% and 90 to 95% removal was observed using both reactors.

Hu, Fujie, et al. (1998) investigated the effects of coexistence of biodegradable substrates and microbial concentration on the acclimation of microbes to acrylonitrile in an aerobic submerged biofilter. The acclimation of microbial film to acrylonitrile was promoted by a higher microbial concentration in the biofilter and by the coexistence of glucose and peptone in the influent. The upper limit of acrylonitrile loading to the biofilter for the ultimate degradation, that is, complete mineralization, of the influent acrylonitrile was approximately 2.0 to 2.2 $kg/m^3 \cdot d$. The same group found that the acclimation of microbes to acrylonitrile in an aerobic submerged biofilter was accelerated by increased initial microbial concentrations and the coexistence of glucose and peptone in the influent (Hu, Nozawa, et al., 1998). Also, a change in the quinone profiles of microbial films suggested that *Brevibacterium sp.* *Pseudomonas aeruginosa* and *Corynebacterium sp.* contributed to the degradation of acrylonitrile.

Hydraulics. Deront et al. (1998) checked the possibility of following the biomass growth by pressure drop measurement in an aerated cocurrent upflow fixed-bed bioreactor. Under operating conditions with biomass, it was demonstrated that column clogging and the operating time between washing cycles can be predicted depending on the volumetric organic load for a given total organic carbon inlet concentration. Seguret et al. (1998) found a full-scale submerged upflow biofilter had 30 to 40% spatial difference in near residence time with a range of dispersion lengths of 60 to 100 mm when operated at 3.7 and 6.5 to 6.7 m/h. They found that this heterogeneity would have no effect on water quality and limited effect in the case of sudden changes in effluent substrate concentration.

Other. Tests were conducted by Hodkinson and Williams (1998) in a full-scale model reactor, with and without support media, to determine the factors affecting aeration in a submerged aerated filter containing high-voidage random-packed plastic media, and to assist in the design and sizing of submerged aerated filter wastewater treatment systems.

An alternate anaerobic/oxic sequencing batch submerged biofilm reactor was characterized and modeled for phosphate removal (Wang et al., 1998). Nuclear magnetic resonance was used to show that a conventional anaerobic release/aerobic uptake as polyphosphate method was at work.

Simultaneous Cr(VI) reduction and phenol degradation was observed in a bench-scale, fixed-film bioreactor using a coculture of phenol-degrading *Pseudomonas putida* DMP-1 and the Cr(VI) reducer, *Escherichia coli* ATCC 33456 (Chirwa and Wang, 1998). Near complete Cr(VI) reduction and phenol degradation was observed during steady-state operation at loadings of 5 to 26 mg Cr(VI)/L·d and 800 to 3 200 mg Phenol/L·d, with an optimum oxygen-uptake rate of 29.4 to 37.2 mg O_2/g cells·d.

BIOLOGICAL GRANULAR ACTIVATED CARBON

The regeneration of activity of biological granular activated carbon (BAC) in an off-line reactor was found to be impractical in a laboratory-scale study of a phenol-treating system (Ivancev-Tumbas et al., 1998). Repeated bioregeneration revealed that older carbon had a decreased capacity for adsorption, and an inhibitory effect of substituted phenols was observed. Niquette et al. (1998) evaluated BAC filters before, during, and after shutdown, with the concentrations of ammonia, nitrite, nitrate, dissolved organic carbon, and dissolved oxygen found to quickly drop to <2 mg/L within the BAC filters during the first 2 hours of filter shutdown. Additionally the fixed bacterial biomass densities, measured at several depths in the filter after a 24-hour shutdown, declined, but backwashing at the end of shutdown eliminated these negative effects and restored the biodegradation performance of the BAC filters (Ahmad and Amirtharajah, 1998). During backwash, monitoring the detachment of biological and nonbiological particles showed that maximum turbidity occurred close to zero time, whereas maximum bacterial detachment occurred later.

Atrazine-containing river water was ozonated and fed to granular activated carbon columns inoculated with *Rhodococcus rhodochrous* strain SL1. During 467 days of operation, bed life was increased by 39 operational days over noninoculated columns, but the effect of inoculation decreased after 200 to 350 days (Jones et al., 1998). Vahala, Moramarco, et al. (1998) also studied ozonation and BAC, examining filter performance with nutrient addition using chemical pretreated and ozonized lake water treated in three parallel pilot-scale biofilters with the addition of phosphorus, a mixture of inorganic nutrients, or no nutrients. Bacterial growth

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was limited by phosphorus, but the increased bacteria could not attach themselves during the relatively short acclimatization period. The same group also examined upgrading an existing postozonization plant with two-step granular activated carbon (GAC) filtration for assimilable organic carbon (AOC) removal using chemically pretreated humic lake water at 2–14 °C (Vahala, Ala-Peijari, et al., 1998). A biofilter removed 51 and 72% of AOC-phosphate and AOC-NO_x, and the GAC adsorber contributed to <10% of the overall AOC reduction. The effect of particle separation on performance of O_3-BAC based on analyses of the fate of organic substances in the process showed that particulate matter separation significantly decreased the loading of adsorbable and nonbiodegradable dissolved organic carbon fraction onto BAC, and therefore such treatment should extend the service life of BAC (Nishijima and Okada, 1998).

The addition of powdered activated carbon (PAC) to activated sludge was found to produce particles which were denser and caused less decrease in flux through an ultrafilter for retention of the solids (Kim et al., 1998). The PAC particles exhibited both smaller diameters and also less extracellular polymeric substance content than activated-sludge flocs.

Mathematical spreadsheet models were constructed to estimate COD removal efficiency of BAC filters with different loading rates, DO, pH, nutrient requirements, and populations of microorganisms (Scholz and Martin, 1998). Procedures were suggested to control biofilm growth and to use bioindicators to predict total organic carbon (TOC) and COD removal efficiencies, using the observed strong positive correlation between the abundance of some protozoa in the liquid phase of the BAC bed and COD concentration in the effluent.

Scott and Karanjkar (1998) presented studies showing the benefits and effects of a matrix of microorganisms developed on GAC in enhancing metal uptake and degradation from solution. The conditions under which the biofilm developed, including pH and temperature were studied in terms of subsequent influence on metal biosorption.

MEMBRANE BIOREACTORS

A synthetic wastewater containing 2,4,6-trinitrophenol as the sole carbon source was treated in a hollow-fiber membrane biofilm reactor in which pure oxygen was fed from the membranes to the biofilm attached to the membrane outer surface (Jimenez et al., 1998). Brindle et al.'s (1998) laboratory-scale membrane aeration bioreactor was operated with a nitrifying biofilm attached to the surface of oxygen-permeable hollow fibers containing pure oxygen. High rates of nitrogen removal (98%) and nitrification (83%) at a nitrogen loading rate of 1.2 kg NH_4-N/m^3·d were observed, with a specific nitrification rate of 5.4 g NH_4-N/m^2·d and a nitrogen removal rate of 6.6 g NH_4-N/m^2·d.

Chung et al. (1998) studied the feasibility of polysulfone membranes to partially immobilize *Pseudomonas* and evaluate the inhibitory effect of phenol on immobilized *Pseudomonas* by monitoring their growths in partially immobilized cell and free-suspension systems. A pilot-scale extractive membrane bioreactor was demonstrated to remove monochlorobenzene, giving a removal of 98 to 99% at a flow of 500 L/h (Livingston et al., 1998).

Kimura et al. (1998) investigated membrane filtration and simultaneous oxidation of ammonia by a rotating membrane disk module. The filtration resistance due to the accumulated cake on the membrane was found to be dominant compared to the resistance due to the micropore plugging or irreversible adherence.

IMMOBILIZED CELL BIOREACTORS

Karamanev and Samson (1998) showed that using an immobilized soil biofilm reactor, an extremely high volumetric pentachlorophenol degradation rate was obtained, with the biofilm reaching a much higher density than has been generally reported. Essentially, all pentachlorophenol was degraded within the biofilm with little effects from changes in temperature, pH changes, and water soluble concentration.

BIOFILMS ON SAND, SOIL, AND SEDIMENT

Soil. A microcosm enrichment approach with six soil samples yielded 177 isolates capable of biphenyl degradation (Wagner-Döbler et al., 1998): 137 of the isolates were found to belong to the species *Rhodococcus opacus* by several typing techniques.

Sand. During 1 to 2 months of operation, the hydraulic conductivity in small sand columns fed synthetic groundwater contaminated with decane and naphthalene or with propylene glycol fell two- to three-fold (Bielefeldt et al., 1998). The final biomass distribution in the columns was even and did not correlate to hydraulic conductivity changes. Q. Liu et al.'s (1998) two bench-scale sand columns were inoculated with lipolytic microorganisms to colonize the sand with an active biofilm, and two uninoculated columns were used as controls. Uninoculated columns were shown to lag behind inoculated columns by approximately 100 days in COD removal from wastewater enriched with butterfat and a detergent.

Stone. Volcanic rock, a porous medium, was packed in a sequencing batch biofilter for the treatment of a synthetic mixture of chlorophenols (60 to 400 mg/L) used as a toxic wastewater model (phenol, 4-chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol) (Buitron and Ortiz, 1998). This mixture of pollutants was eliminated with an efficiency > 93% as total organic carbon and 99% as total phenols, with a maximum applied load of 3.6 kg COD/m^3·d. The role of sequestration of hydrophobic compounds in nanoporous solids, however, was found to be dominant in studies with phenanthrene. Phenanthrene sorbed in silica and diatomaceous beads with 2 to 15-nm pores was extensively mineralized, but polystyrene beads with 5- and 300 to 400-nm pores resulted in <7% mineralization (Nam and Alexander, 1998).

Wetlands. The bacterial community in the open water and reed epiphytes of a shallow, turbid, mesotrophic lake was characterized, with 296 strains described in the open water but a more limited community present on the submerged reed surfaces (Borsodi et al., 1998). Coryneforms were apparently dominant on the reeds, and lower suspended solids in the water were associated with higher species diversity, which was the opposite of the diversity and suspended solids relationship found in the open water.

Riverine. In an attempt to evaluate surfactant degradation in riverine biofilms, a laboratory-scale system was developed by Lee et al. (1998). On the basis of bacterial densities, surfactant biodegradation, and metabolic capacities, the microcosm appeared to be an adequate model of riverine biofilms formed on slate disks. The entrance of nitrate to groundwater from the river Elbe was influenced by seasonal, temperature-dependent denitrification in river bed sediment (Grischek et al., 1998). Organic matter in the water and in the solid substrate of the river bed supplied carbon for denitrification, resulting in calculated rates of 0.04 mg-N/L·d in the field, one-tenth or less the rates observed in the laboratory. The observation of elevated nitrite concentrations in sediments of the Lahn river on a diurnal basis during the summer led to studies of

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temperature effects on nitrifying biofilms (von der Wiesche and Metzel, 1998). Laboratory studies showed that in the range of 16 to 22 °C there exists a greater activity of *Nitrobacter* than *Nitrosomonas* in these biofilms, resulting in a transient buildup of nitrite.

INNOVATIVE REACTORS

Nitrogen Removal. Total nitrogen removal rates of 65 to 80% with an effluent below 12 mgNO_3-N/L was realized with a nitrifying circulating bed bioreactor coupled to a denitrifying fixed floating bed (Lazarova et al., 1998). The C/N ratio was the key operational parameter, with values above 5 resulting in nitrogen breakthrough, but nitrification was governed by the dissolved oxygen concentration and redox potential, with a maximum of 0.8 kgNH_4-N/m^3·d removed. Woodbury et al. (1998) report the results of a study to investigate the performance of fixed-film, two-stage reversible biodenitrification reactors operated at very short detention times for the removal of nitrates from contaminated groundwater. The results demonstrate that these systems imparted lower concentrations of organisms and suspended solids into the treated effluent than traditional single-stage systems while maintaining higher levels of nitrate removal rates at hydraulic retention time (HRT) values as low as 30 minutes. Cyanide was consumed and ammonia was evolved in a sequencing batch biofilm reactor using silicone rubber tubing as an attachment medium (White and Schnabel, 1998). In a 24-hour cycle, cyanide was removed from 20 to less than 0.5 mg/L, and ammonia was simultaneously removed by glucose addition (10 mol NH_3-N removed/mol glucose).

Electrolytic Nitrogen Removal. The removal of nitrate was a strong function of applied current in a biofilm-electrode reactor (Feleke et al., 1998). The biofilm grew on the cathode, and a current of 5 mA apparently gave optimal removal. A pair of electrolytic reactors for denitrification built in sand columns was operated with a mixed culture seed and as a sterile control (Hayes et al., 1998). The observed removal in the sterile control was 20% of 20 mg/L NO_3-N, but was 49% in the biological-electrolytic reactor. Long-term performance of a denitrifying biofilm-electrode reactor showed that current intensities of 20 to 25 mA resulted in the lowest effluent nitrate concentration, with 85% removal observed (Islam and Suidan, 1998). At higher currents, nitrification was apparently decreased as a result of charge induced repulsion and hydrogen inhibition.

Sagberg et al. (1998) presented the overall mass balances for water, total nitrogen and organic carbon in a WWTP consisting of chemical preprecipitation followed by upstream biofilm nitrification and denitrification filters and side stream removal of ammonia by air stripping from sludge filtrate water after digestion of sludge. This new process was compact, with a total water retention time of fewer than 4 hours, achieving 74% nitrogen and 96% phosphorus removal.

Recalcitrant Organics. Cox et al. (1998) studied the biofiltration of trichloroethylene (TCE) using *Pseudomonas putida* strains F1 and TVA8. TVA8 is a genetically engineered bioluminescent reporter bacterium for induction of the toluene dioxygenase operon. Trichloroethylene removal in excess of 95% was achieved when toluene was fed intermittently to the biofilter compared to approximately 30% removal when toluene was fed continuously. Darlington et al. (1998) studied a biofilter composed of a scrubber, a hydroponic planting system, and an aquatic system with green plants, which maintained air quality within part of a modern office building by removing trichloroethylene and toluene. The system was challenged for 4 weeks with three common indoor organic pollutants and removed 10% of trichloroethylene and 50% of toluene in a single pass. Alvarez et al. (1998) used benzoate to enhance degradation of benzene, toluene, and *o*-xylene in flow-through aquifer columns, with substantial increases in degradation rates observed.

Mixed liquor biofilms supplemented with three *Sphingomonas* isolates were able to degrade the azo dyes Acid Orange 7 and Acid Alizarin Violet N (Tepper et al., 1998). Although 92% of the Acid Orange dye was initially removed after supplementation, only a 23% average removal of the other azo dye was observed. Tan et al. (1998) described integration of anaerobic and aerobic conditions in a single bioreactor for the complete mineralization of azo dyes. Azo dye reduction rates by two different granular sludges were determined in batch assays with various concentrations of oxygen in the headspace, and the rate of dye reduction was highly correlated with the oxygen-consuming activity of the sludge.

The ability of a moving bed bioreactor to treat refinery wastewater was shown at pilot scale to remove 80% of soluble BOD (Johnson et al., 1998). A field-scale biofilter successfully removed 2 000 mg/L acaricide coumaphos from 15 000-L batches to 10 mg/L over 14 days at 25 to 29 °C (Mulbry et al., 1998). In subsequent experiments, effluent concentrations were reduced to 1 mg/L with the addition of vitamin supplements. Eweis et al. (1998) inoculated a pilot-scale biofilter with a MTBE-degrading culture fed toluene at a concentration of approximately 8 mg/L (8 ppm_v) initially, then 25 and 70 mg/L (25 and 70 ppm_v). The biofilter acclimated quickly to toluene, achieving greater than 90% removal within the first 6 hours, and the presence of toluene at 8 and 25 mg/L did not seem to influence MTBE degradation.

Three-Stage Aerated Filters. The performance and recovery of a three-stage biological aerated filter fed with a mechanically pretreated municipal wastewater was studied at pilot scale (Chernicharo and Machado, 1998). The recovery of normal operation after the period of overloading was almost immediate and demonstrated the importance of maintaining the specific biomass populations in separate reactors to reduce competition among different bacterial species and optimize growth. Chou and Wu (1998) used two sets of three-stages-in-series biofilters, packed with pig manure and coconut-based media. They found that the coconut-based media had higher dimethylformamide-*n* removal, greater than 90% were obtained with loading <50 g dimethylformamide/m^3·h and gas retention time > 23 seconds.

Reactors Using Unusual Media. Pussemier et al. (1998) examined the effectiveness of biofilters constructed using plastic containers (45 L) filled with a mixture of straw, peat, and arable soil and equipped with a drain pipe for the collection of percolating water and in some cases with a granular activated carbon filter cartridge. These biofilters were loaded twice a year with pesticides wastes in concentrations of 0.1 g/L for each pesticide, and the authors observed that the leaching of pesticides was dependent on rainfall and season. A bioretention facility consisting of a porous soil covered with a thin layer of mulch with a stand of various grasses, shrubs, and small trees was used to treat stormwater (Davis et al., 1998). A synthetic stormwater feed was found to have removals of metals, greater than 95%; ammonia, 79 to 92%; TKN, 52 to 68%; and little nitrate removal, 0 to 20%.

Gunnarsson et al. (1998) described the suspended carrier biofilm process developed to overcome conventional biofilm processes problems with clogging at high loads. A removal of 90% of soluble COD in pulp and paper wastewater of low or intermediate strength and 40 kg COD/m^3 · d in highly loaded processes was observed.

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Kramanev et al. (1998) proposed "soil immobilization" based on the entrapment of soil particles showing microbial activity in degrading the target pollutant into a solid membrane with a large pore size distribution. The performance of such a system was tested by developing a microbial system for the mineralization of pentachlorophenol with a volumetric efficiency one to three orders of magnitude higher than reported literature values.

Other. Basnakova et al. (1998) studied how hydrogen uranyl phosphate (HUO_2PO_4:HUP), deposited enzymically on *Citrobacter* N14 cells immobilized as biofilm on ceramic Raschig rings in a flow-through column removed Ni quantitatively from a dilute aqueous solution in the form of Ni uranyl phosphate via intercalative ion exchange. No U release occurred during selective desorption of Ni, proving the integrity of the biofilm within the column (Basnakova et al., 1998).

Various backwashing strategies and hydraulic transients were evaluated to determine how they affected performance of biofilters in terms of effluent quality and head loss (Ahmad et al., 1998). Compared with air-scoured filters, water-washed filters produced lower initial peaks during ripening and similar effluent AOC concentrations.

Skjelhaugen and Donantoni (1998) compared different techniques of treating raw cattle slurry to destroy pathogens before spreading including using aerobic biotreatment. The biotreatment reduced hydrogen sulfide emissions from 2000 to 300 mg/L (2000 to 300 ppm) and reduced thermotolerant coliform bacteria (TCB) from 10^4 to 10^2 TCB/g during a 7-day treatment.

The performance of two full-scale suspended carrier biofilm processes within the Swedish pulp and paper industry was investigated (T. Welander et al., 1998). Without any preceding primary clarifier, and primary, secondary, and chemical sludge separated in one and the same clarifier, a removal of 70 to 80% of soluble COD at a treatment time of 3 hours was observed. Werker and Hall (1998) used spectra of microbial fatty acid methyl esters to assess the influence of pH on the biofilm and suspended biomass populations within bench-scale moving bed bioreactors treating bleached kraft mill effluent. Experimental data for total organic carbon removal suggested that the more recalcitrant fraction of the influent was consumed by the slower growing, high solids retention time biofilm fraction of the biomass.

Approximately 80% of COD removal was achieved by a downflow aerated packed bed reactor followed by an inclined tube settler for intermittently flowing waste waters resulting in 3 to 26 weeks starvation (Yukselen, 1998). Polprasert et al. (1998) assessed the performance of free water surface (FWS) constructed wetlands located in the tropics in the removal of organic matter. A kinetic model incorporating the activities of bacteria and biofilms, dispersion number, and hydraulic retention time was used.

Carlson et al. (1998) conducted pilot-scale biofiltration experiments to determine how empty bed contact time and hydraulic loading rate affected the removal of biodegradable organic matter. The biodegradable organic matter formed during ozonization limited the removal of dissolved organic carbon during biofiltration with two exceptions: non-steady-state organic matter loading and inadequate contact time.

BIOFILTRATION AND BIOTRICKLING FILTERS

A number of papers were published on biotrickling filters and biofiltration. Although not reviewed here, they are listed under a separate heading in the reference section. Additional citations, review, and comments are contained in the Gaseous Emissions from Wastewater Facilities section of this issue.

ANAEROBIC BIOFILM SYSTEMS

A number of papers were published on anaerobic biofilms. Although not reviewed here, they are listed under a separate heading in the reference section. Additional citations, review, and comments are contained in the Anaerobic Processes section of this issue.

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Anaerobic Processes

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INTRODUCTION

The 1998 review of anaerobic processes provides information on both fundamental and applied research performed in this area during the year. The review is organized into a number of sections providing information on microbiology, biotransformation of toxic and recalcitrant compounds, toxicity, development of models, reactor treatment systems, municipal solid waste treatment, and new methods for testing of anaerobic processes. The past year has seen increased research activity in the area of biotransformation of toxic compounds, as well as in the use of existing and novel reactor systems for anaerobic treatment of a variety of industrial wastewaters. An increased emphasis on applied research was observed.

MICROBIOLOGY

Strain Isolation. The study of the complete genome sequence of the methanogenic archaeon *Methanococcus jannaschi* revealed

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