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BIOLOGICAL FIXED FILM SYSTEMS

Mark W. Fitch and Ellen England

INTRODUCTION

Overview The work reviewed here was published during the catalogue/issue year 2001 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. Similarly, biofilm systems for the treatment of air pollutants is reviewed in the Gaseous Emissions from Wastewater Facilities section of this issue.

Coverage The 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. References related to models of specific processes are included in the section on that technology. The reactor types which 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 (not including the use of membrane filtration in suspended-growth reactors), 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 and Reactor Comparisons A short coverage of small wastewater treatment plants in lower Austria is provided (Franz 2001). Discussion includes information on trickling filters, sequencing batch, activated sludge and constructed wetland processes. Similarly, small-scale wastewater treatment options available in Sweden were discussed and evaluated (Palm et al. 2001). Wastewater treatment options, including biofilm processes, for food processing plants are reviewed (Hougetsu 2001). Self attachment and entrapment to polymer beads is the subject of a review by Cohen (2001). Advantages and disadvantages of both processes are discussed for both gaseous and liquid waste treatment. A review of the relavent literature indicates treatment of high temperature (50 - 60 °C) wastewaters is possible using biological methods (Suvilampi et al. 2001). Although the review focused predominantly on activated sludge processes, high temperature biofilm treatments might also be competitive with mesophilic processes. Another comparison of biofilm processes and activated sludge focused on the treatment of sugar mill wastewater (Polec 2001). Odegaard (2001) discussed the treatment efficiencies of two Norwegian wastewater treatment plants using biofilm reactors in combination with dissolved air flotation. Design and operational recommendations for this process combination are given.

The biodegradation of oxygenates used as gasoline additives was reviewed (Fayolle et al. 2001). Treatment using attached and suspended growth systems is being actively studied and offers promise for removal of the contaminants. Existing treatment methods for removal of micropollutants were described and discussed, with possible optimization factors also examined (Joss and Siegrist 2001).

Jiang et al. (2001) reviewed the removal of heavy metals by EPS and mass transfer within biofilms. A review of the hydrodynamics and performance of bubble column and airlift bioreactors included a hydrodynamic model for an airlift suspension reactor (Kojima 2001). van Nieuwenhuijzen et al (2001) claimed that a combination approach to wastewater treatment using both a biofilm and advanced physicochemical P and N removal may assist in the design of smaller, more efficient, treatment systems.

BIOFILM MEASUREMENT AND CHARACTERIZATION

Biofilms were successfully grown under microgravity conditions aboard a space shuttle flight (McLean et al. 2001). Mature and stable biofilms might be used in the future at space outposts. Under normal gravity conditions, Manz et al (2001) detailed several methods to assess the metabolic potential of biofilm-associated bacteria including the direct viable count, the probe active count assay, the cyanoditolyl tetrazolium chloride reduction, and fluorescence in situ hydbridization combined with microautoradiography (2001b). Components of the biofilm matrix were described by Sutherland (2001b). A review of extracellular electron transfer was provided by Hernandez and Newman (2001). Structures, properties and the relationship of structure to function in biofilm polysaccharides were discussed in a mini-review (Sutherland 2001a). The polysaccharides protection to cells within the biofilm and their interactions were also examined.

A new descriptor for biofilm, "specific number of viable cells" (SN-VC), is introduced and defined as the viable cell number normalized with respect to the surface area covered by the biofilm and with respect to the biomass of the biofilm. To develop the SN-VC, biofilm-imaging techniques including fractal dimension, textural entropy and diffusion distance were used to analyze 4-day-old biofilms of *Pseudomonas aeruginosa*, *P. fluorescens* and *Klebsiella pneumoniae* (Jackson et al. 2001b). Alavi and Belas (2001) examined methods used to analyze surface sensing and swarming behavior associated with biofilm formation.

Bacteria have been shown to have multiple languages for communicating within and between species allowing them to coordinate activities and behave as multi-cellular organisms (Schauder and Bassler Bonnie 2001). The languages of the oligopeptide, the homoserine lactones, and LuxS were described. Miller (2001) reviewed such quorum sensing and communication between cells. A review of surface active agents produced by microorganisms and their influences on their interactions with the environment focused on bioemulsifiers, involved in bacterial pathogenesis, quorum sensing and biofilm formation (Ron and Rosenberg 2001).

Biofilm Adhesion and Attachment Atomic force microscopy was used to quantify the adhesion of *Saccharomyces cerevisiae* cells to a mica surface (Bowen et al. 2001). Physiologically active cells demonstrated different adhesive behavior than gluteraldehyde treated cells, while time of surface contact was determined important to adhesion behavior. Lewis acid-base interactions were found to be important in biofilm adhesion mechanisms (Briandet et al. 2001). The ability of a planktonic micro-organism to adhere to a surface may be influenced by changes in the physico-chemical characteristics of the support related to a biofilm presence. A review of the influence of detachment and shear on biofilm processes was provided by Liu (2001b). Localized growth and detachment mechanisms were elucidated using digital time-lapse microscopy with biofilm flow cells (Stoodley et al. 2001b). Single cell, small clusters of cells, and large aggregates detached, however, the large aggregates contained a

disproportionately high fraction of total detached biomass. *Pseudomonas aeruginosa* PAO1 and anaerobic sulfate-reducing bacteria biofilms of *Desulfovibrio* sp. EX265 grown under varying fluid shear stresses exhibited different yield shear stress (Stoodley et al. 2001a). Biofilm strength appears to be dependent upon cation crosslinking within the extracellular polysaccharide matrix and upon the shear under which the biofilm was grown.

The role of quorum sensing systems were investigated during the early stages of static biofilm formation using fusions to the unstable green fluorescent protein and confocal microscopy (de Kievit et al. 2001). Differences in temporal and spatial gene expression were found. In a study of a Burkholderia cepacia H111 biofilm, it was shown that the guorum-sensing system is not involved in the regulation of initial cell attachment, but rather controls the maturation of the biofilm; swarming motility of B. cepacia is quorum-sensing-regulated, possibly through the control of biosurfactant production (Huber et al. 2001). Complementation of the cepR mutant H111-R with different biosurfactants restored swarming motility; while biofilm formation was not significantly increased, thus suggesting that swarming motility *per se* is not essential for biofilm formation of abiotic surfaces. Pseudomonas putida was found to undergo a global change in gene expression following initial attachment to a surface; sensory processes in addition to quorum sensing are involved in the phenotypic changes associated with attachment (Sauer and Camper Anne 2001). Analysis of protein profiles and mRNA expression pattern analysis helped provide clues to the mechanisms involved. A different pseudomonad, Pseudomonas aureofaciens PA147-2, lost the ability to form biofilm upon the isolation of insertional mutations to the pstC and pstA genes of the phosphate-specific transport (pst) operon (Monds et al. 2001). GacA, of the GacA/GacS two-component regulatory system, in *Pseudomonas aeruginosa* biofilm formation was established as a new and independent regulatory element (Parkins et al. 2001a). When gacA was disrupted in strain PA14, a 10-fold reduction in biofilm formation capacity resulted relative to wild-type PA14. Methods to obtain and analyze bacterial mutants with altered adhesion properties and characterization of cellular functions involved in the first stages of biofilm development were discussed by Thi et al

(2001). Although predominantly medically focused, the described techniques and characterization are applicable to many other biofilm applications.

Methods were presented for characterizing three aspects of extracellular enzyme activity in biofilms: promoter activity of the structural gene, local catalytic activity, and kinetics of collective substrate degradation (Baty et al. 2001). Extracellular enzyme characterization, although still fraught with some technical difficities, helps elucidate biofilm ecology. Although related to clinical applications, it was found that Esp expression in an *E. faecalis* esp-deficient strain promoted primary attachment and biofilm formation on polystyrene and polyvinyl chloride plastic (Toledo-Arana et al. 2001). Results suggest the potential mechanisms and conditions required for attachment to abiotic surfaces.

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Fluorescent Tools and Microscopy A review focused on 2-photon laser scanning microscopy methods for examining biofilms (Neu and Kuhlicke 2001). Sediment samples stained with DAPI and counterstained with SYBR Green II allowed for better identification between bacteria and bacteria-like particles (Griebler et al. 2001). Enumeration of both attached and freely suspended organisms was vastly improved using the new technique. A procedure for determining segmentation thresholds for confocal laser scanning microscopy was developed to further improve the ability to measure biofilm volume and interfacial area without manual intervention (Xavier et al. 2001). The automated procedure was shown to be reproducible and reliable during the examination of two separate biofilm systems.

Application of the environmental scanning electron microscope allowed direct examination of biofilms on clay surfaces (Darkin et al. 2001). The microspatial distribution of bacteria within a biofilm and the extracellular protease activity at the biofilm-substratum interface was elucidated using a new technique (Francoeur et al. 2001). The method, which uses fluorescent molecules bound to cellulose substrata with a lectin, performed similarly to a standard dissolved-substrate assay. Degradation of 1,2-dichloroethane, 3-chloro-4-methylaniline, and p-toluidine occurred in an extractive membrane bioreactor and was measured using two-dimensional scanning fluorometry and artificial neural networks (Wolf et al. 2001).

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Nuclear Magnetic Resonance (NMR) and Magnetic Resonance Imagine (MRI) Humbert (2001) provided an overview of the pulsed field gradient NMR microscopy technique and its potential applications in the field of environmental biotechnology. B-1 gradient NMR offers potential for examining biofilm parameters and characterizing biofilm processes. Bacterial biofilms were studied after receiving 2-¹³C-glycerol using solid-state high resolution NMR (Mayer et al. 2001). Solid-state spectra indicated the ¹³C was mainly incorporated into mannuronate and guluronate and that the biofilm alginate has a high degree of motional freedom. Rather than glycerol, radiolabeled methionine was used to assay acyl-HSL production of bacterial cultures and biofilms (Schaefer et al. 2001). Magnetic resonance tomography and its application to biofilm mass transport analysis was described (Manz et al. 2001a). Three-dimensional magnetic resonance imaging was used to study biofilms in a packed bed reactor with excellent results (Paterson-Beedle et al. 2001). The technique was explained and its advantages and disadvantages discussed. The same technique, three-dimensional magnetic resonance imaging, was used to visualize foam-immobilized Citrobacter (Nott et al. 2001). La^{3+}/Cu^{2+} proved to be a good model system for analysis of this biofilm. Applications and limitations of NMR and MRI techniques to biofilms and bioreactors were discussed by As and Lens (2001). These sophisticated analytical techniques allow nondestructive and noninvasive quantification and visualization of flow, mass transfer, and transport processes over long or short periods of time.

Novel Techniques Green fluorescent protein (GFP) offers the potential for use in studying bacterial biofilms, however, oxygen restriction and low pH may possibly limit its use (Hansen et al. 2001). In a series of experiments, it was shown that densely packed, flow cell-grown biofilms of *S. gordonii* do not develop oxygen gradients inhibitory to GFP fluorescence development, and that the often transient nature of GFP fluorescence in acid-producing bacteria can be overcome in flow cells. Esterase activity was shown to be useful for measuring the metabolic activity of biomass in biofilters and shows potential for studying the performance of biofilters (Kijowska et al. 2001; Merino et al. 2001). The highest biomass activity was found at the bed surface and backwashing was found to significantly influence biomass activity. An assay utilizing synthesized

6

multivalent compounds to alter bacterial chemotaxis was developed to distinguish between swarmer cells and undifferentiated bacteria (Lamanna et al. 2001).

A technique using a transparent oxygen electrode in modified SnO₂ and image analysis was used to examine biofilm formation and thickness (Cachet et al. 2001). Although used in the study of scale and fouling, the technique might be utilized to analyze bioreactor biofilms. The method of direct biofilm monitoring by a capacitance measurement probe in continuous culture chemostats was described by Jass et al (2001). The increase in capacitance corresponded with increases in biomass. Biofilm optical density and biofilm thickness were measured as a reduction in light intensity and light microscopy, respectively (Bakke et al. 2001). Both measurement techniques were found to be more sensitive and less labor intensive than other commonly used biomass measurement methods.

Photoacoustic sensors were used successfully to monitor changes in biofilm organic mass by Schmid (2001). Fourier transform IR and Raman microspectroscopy were used for identification of clinically important micro-organisms, and study of the nature of biofilm formation with these techniques was suggested (Choo-Smith et al. 2001). Indeed, attenuated total reflection/Fourier transform-infrared spectrometry (ATR/FT-IR) and scanning confocal laser microscopy (SCLM) were used to study the role of alginate and alginate structure in the attachment and growth of *Pseudomonas aeruginosa* on surfaces (Nivens et al. 2001). The importance of alginate O acetylation in *P. aeruginosa* biofilm architecture was reported, and that alginate, although not required for biofilm development, plays a role in the biofilm structure and may act as intercellular material, required for formation of thicker three-dimensional biofilms.

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Real time detection of DNA hybridization within biofilms was accomplished using photocurrent spectroscopy and a quartz crystal microbalance (Lassalle et al. 2001). Sensors were determined to be reusable, offered good sensitivity and reproducibility of the measured signal. Headley et al (2001) demonstrated the use of reversed-phase liquid chromatography with positive-ion electrospray ionization mass spectrometry (LC-MS) along with tandem MS for examining riverine biofilms for N-methylpyrrolidinone. Quinone profiling based on dominant isoprenologue patterns, identified using high-performance liquid chromatography/electrospray/tandem mass spectrometric analysis,

was used for identification and analysis of microbial communities (Lytle et al. 2001). The study results have implications for identifying areas of aerobic metabolism in biofilms. Micro-proton-induced x-ray analysis showed accumulation of Ca, Zn, Pb, and Fe in the fungal mantle and rhizomorph, P, S, K in vascular tissue, and Si and Cl in the endodermis of *Suillus luteusl Pinus sylvestris* mycorrhizas while Cu was identified in the cortex region (Turnau et al. 2001). Difficulties during chemical fixation and elemental imaging were discussed.

Extracellular Polymeric Substances (EPS) A two-part review discussed aspects of the EPS matrix and described its function within biofilms, with the first part focused on structure and ecology (Flemming and Wingender 2001b). The second portion of the review focused on properties of EPS relevant to various applications (Flemming and Wingender 2001a). The same authors offered two further short reviews concerning EPS that focused more on biofilm ecology (Flemming and Wingender 2001c), and on EPS composition, origin, and characterization (Wingender et al. 2001). The factors which may contribute to the structure and stability of complex aggregates of biofilms and flocs were elucidated by Sutherland (2001c). Biofilms grown in a rotating drum biofilm reactor fed with synthetic wastewater (COD 150 mg/L), showed differences in EPS yields, aerobic/anaerobic zones, density, porosity, biomass concentrations and COD concentrations with depth (Zhang and Bishop 2001). Results have implications for biofilm modeling - a priori assumptions of homogenous biofilm structures and metabolic activities need revision to reflect heterogeneities. A film rheometer was used to measure the modulus of elasticity and the yield strength of a *Pseudomonas aeruginosa* biofilm (Koerstgens et al. 2001). Based on the stress-strain curve the biofilm was described as a viscoelastic material demonstrating plastic flow with the EPS forming a temporary network of fluctuating junction points. Calcium ion concentration was found to be a major determinant for the mechanical stability of these *P. aeruginosa* biofilms in another publication (Korstgens et al. 2001). Calcium ions are known to crosslink alginate, an EPS component.

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EPS extraction methods remain the subject of research; in one study, EPS was extracted from activated sludges and microfiltered (Guellil et al. 2001). Bacteria extracted with EPS were inhibited by various substances, protein hydrolysis was found to result from the enzymatic activity of the EPS, and glycolytic activity from the organic colloidal fraction of the wastewater. The effects of incubation time, lectin concentration, fluorescent labeling, carbohydrate inhibition, and lectin interactions during lectin binding analysis were examined in fully hydrated river biofilms (Neu et al. 2001). It was concluded that careful selection is required of the panel of lectins for proper study of chemical heterogeneities within a biofilm. The quantification of EPS within a biofilm was determined as the difference between the volatile solids and the total cell mass (Zhang and Fang 2001). Confocal laser scanning microscopy was used to determine cell volume after fluorescent dye staining of the biofilm.

A dicyclohexy-18-crown-6 ether was used for the extraction of extracellular polymeric substances in biofilm and suspended cultures (Wuertz et al. 2001). 12.2% of Cd and 9.1% of Zn was found in the EPS of a biofilm while 2.9% of Zn was found with the EPS of the suspended culture; it was posited that different binding mechanisms are responsible for EPS sorption in fixed film than in suspended cultures. EPS sorption by different metals, Cu, Pb, and Ni, was studied using the extraction techniques of centrifugation, centrifugation with formaldehyde, EDTA extraction, steam extraction, and were compared with carbohydrate:protein ratios (Jang et al. 2001). Metal sorption was explained using the Freundlich isotherm model while carbohydrate:protein ratios of copper- and lead-exposed biofilm were found to be lower than control ratios.

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The effect of changes in extracellular polymeric substance production was examined in a simulated nitrifying biofilm (Kreft and Wimpenny 2001). Biofilm structure, growth/non-growth of organisms, porosity and clustering were all impacted by changes in EPS production. Acidic polysaccharides were found to influence bioaggregate formation with the addition of D-glucuronic acid enhancing the homocoagulation rate of autotrophic *Nitrosomonas europaea* (Tsuneda et al. 2001). Results suggest the enhancement of nitrifying biofilm formation by the addition of selected EPS.

Nitrifiers Okabe (2001) discussed spatial distributions and activities of nitrifying bacteria as measured by FISH and use of microelectrodes. Several molecular biology techniques, including FISH, visualization of viable bacteria using carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) and PCR based on a method using magnetic beads, were used to collect information on nitrifying biofilms in fluidized beds (Aoi et al.

2001). Visualization of viable bacteria was accomplished, PCR interferences were reduced, and ecological patterns of nitrifiers were elucidated. In situ PCR was used to amplify the amoA gene (partially responsible for encoding ammonia monooxygenase) present in Nitrosomonas europaea cells in a pure culture and biofilms in a nitrifying reactor (Hoshino et al. 2001). Although functional gene expression was not detected in this study, detection of amoA-containing cells near the surface of a biofilm was demonstrated. Two 16S rRNA-directed oligonucleotide probes specific for the phylum and genus Nitrospira, respectively, were developed and evaluated for suitability for FISH during analysis of wastewater treatment plant bioreactor activity (Daims et al. 2001a). Using FISH and other techniques such as fluorescein staining, microautoradiography, and confocal laser scanning microscopy, the Nitrospira-like bacteria in bioreactor samples took up inorganic carbon (as HCO₃⁻ or as CO₂) and pyruvate but not acetate, butyrate, and propionate, suggesting that these bacteria can grow mixotrophically in the presence of pyruvate under aerobic conditions; no uptake by the Nitrospira-like bacteria of any of the carbon sources tested was observed under anoxic or anaerobic conditions. Electric current applied to activated sludge inhibited the metabolism of nitrifying bacteria within activated sludge (>2.5 A/m²) and biofilms (>5 A/m²) immobilized on polypropylene packing (Cao et al. 2001b; Li et al. 2001c). When the current applied was 15 A/m^2 , the nitrification rate decreased by approximately 20%. Electric current < 2.5 A/m^2 had no effect on biofilm nitrification efficiency.

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Genetics and Fluorescent In Situ Hybridization Parkins et al (2001b) detail methods for hybridization-based identification of genes expressed during biofilm growth. The molecular definition of biofilm dependent changes in gene expression and plasmid transfer were explored by Doyle (2001) to assist in the understanding of biofilm properties. The direct contribution of conjugative plasmids to formation of a biofilm was investigated by Ghigo (2001). Natural conjugative plasmids were found to express factors that induced planktonic bacteria to form or enter biofilm communities, which favor the infectious transfer of the plasmid. Changes in genetic and metabolic biodiversity were examined by measuring the concentration of planktonic viable uncharacterized ultramicrobacteria, secretion of extracellular polymeric substances, and biofilm thickness using the single strand conformational polymorphism (SSCP) method, the cloning and sequencing of 16S rRNA gene (16S rDNA) sequences, and the microplate system (Ross et al. 2001). Genetic diversity decreased while metabolic diversity increased after stimulation of biofilm formation on a ceramic surface. Fluorescence in situ hybridization with 16S-rRNA-trageting probes was used to analyze an anaerobic ammonium oxidation culture taken from an RBC and subsequently grown with a low organic carbon content (Egli et al. 2001). Sequence identity indicated *Candidatus Brocadia anammoxidans* or *Candidatus Kuenenia stuttgartiensis* as the likely organisms, yet the culture had higher tolerance to phosphate (20 mM), nitrite (13 mM), and was active at lower cell densities than *Candidatus Brocadia*. Three DNA profiling methods (denaturing gradient gel electrophoresis (DGGE), thermal gradient gel electrophoresis (TGGE), and non-denaturing polyacrylamide gel electrophoresis) were used to assess bacterial strains isolated from a methyl tert-butyl ether (MTBE) degrading compost biofilter (Bruns et al. 2001). The non-denaturing polyacrylamide gel electrophoresis of 300-1500 bp fragments containing 16S/23S ribosomal intergenic transcribed spacer (ITS) regions confirmed the presence of bacterial strain PM1.

The environmental plasmid pQKH6 was transferred conjugatively between strains of Pseudomonas putida within pilot-scale sewage filter beds at mean frequencies 10-fold higher, 8.4 x 10⁻⁴, than previously reported (Ashelford et al. 2001). Results may have implications for wastewater purification. Previously unidentified gene mutations were found in *P. aeruginosa* using Tn5 insertion screening (Vallet et al. 2001). Various structures are thought to function at different biofilm formation stages or in different environments. Also using P. aeruginosa, RpoS expression and rpoS levels, measured using immunoblot analysis, were used as growth indicators of biofilms (Xu et al. 2001a). RpoS expression in a 3-day-old biofilm was found to be three-fold higher than the average expression in stationary phase planktonic culture while *P. aeruginosa* biofilms may show stationary phase characteristics even when cultured in flow conditions. The oxidative stress response and gene expression in *Pseudomonas aeruginosa* were examined using sodA (encoding manganese-cofactored superoxide dismutase (Mn-SOD)) and Mn-SOD as a reporter gene and an endogenous reporter enzyme, respectively (Bollinger et al. 2001). The oxidative stress response and gene expression in this study demonstrated that (1) the nutritional status of the cell must be taken into

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11

account when one is evaluating quorum sensing-based gene expression; (2) in the biofilm mode of growth, quorum sensing may also have negative regulatory functions; (3) quorum sensing-based gene regulation models based on studies with planktonic cells must be modified in order to explain biofilm gene expression behavior; and (4) gene expression in biofilms is dynamic. Prigent-Combaret et al (2001) showed that in Escherichia coll the OmpR234 protein promotes biofilm formation by binding the csgD promoter region and stimulating its transcription, which eventually leads to the development of curli, extracellular structures involved in bacterial adhesion. Results have implications for biofilm formation; the formation of biofilm by E. coli is inhibited by increasing osmolarity in the growth medium. The gene mgtE, Mg²⁺, and possibly Co²⁺ apparently participate in the swarming motility and thus adherence and biofilm formation in Aeromonas hydrophila AH-3 (Merino et al. 2001). Aeromonas hydrophila AH-3 strains carrying mutations in mgtE, which encodes a Mg²⁺ and Co²⁺ transport system, showed a 50% reduction of in vitro adherence to HEp-2 cells, a reduction in swarming in semisolid swarming agar, and decrease in biofilm formation of over 60% in comparison to the wild-type strain

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Other Immobilized and freely suspended *Escherichia coli* cells submitted to cold shock at 4 °C were examined using computing scanning densitometry (Perrot et al. 2001). Specific molecular mechanisms of stress response, shown as changes in the synthesis of 203 proteins, varied between immobilized and suspended cells, suggesting one possible reason for high resistance to stresses. A gold electrode was used to measure biofilm parameters including thickness, elasticity and rigidity under laminar and turbulent conditions (Herbert-Guillou et al. 2001). The research has implications for detection and monitoring of biofilm formation. Redox potential measurements were used to determine microbial activity and the sensitivity to antimicrobials in a biofilm similar to those treating papermill effluent (Holtmann and Sell 2001). Voltages measured were valuable in determining biofilm characteristics. Two ultrasonic methods for removal of biofilms from sediment for enumeration were compared (Mermillod-Blondin et al. 2001). A narrow tip ultrasonic generator required less exposure time to remove biofilms and produced less alteration of the sediment than did an ultrasonic bath. Eight gram-negative cells, differing in shape, size and capsule

and cytoplasmic inclusions, were found present in a consortium using alkylsulfonates, the main contaminant in rubber industry waste (Mogil'naya et al. 2001).

An (2001) described an open channel flow chamber for characterizing biofilm formation. Hydrogen sulfide production and its inhibition was studied in a biofilm containing *Desulfovibrio desulfuricans* and a nonsulfate-reducing bacteria, Pseudomonas fluorescens using microelectrodes (Beyenal and Lewandowski 2001). At velocities < 2 cm/s, H₂S flux from cell clusters was dependent upon the flow velocity while at velocities > 2 cm/s, H_2S production was limited by biofilm metabolic reactions. Using optical, electron, and atomic force microscopy, iron sulfate minerals were found to be formed by the microbiologic activity of a biofilm treating mine waste water (Sasaki and Tazaki 2001). The iron-sulfate mineralization was found to occur during both aerobic and anaerobic conditions. Iron was important in a completely different study; both planktonic cultures and biofilms were studied to determine reasons for differential expression of catalase in cells (Frederick et al. 2001). Results suggest that iron availability, rather than oxygen availability, is a major contributor to catalase expression in biofilms. Acylated homoserine lactone concentration in biofilms was modeled as a function of population growth rate, diffusion and autoinduction rate (Nilsson et al. 2001). The theoretical work suggests that acylated homoserine lactone regulation may be a trade-off between factors that dilute intracellular concentrations and those that increase concentration.

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Hydrophobic and electrostatic properties were found to vary for bacterial strains degrading a substrate while slight differences in zeta potential were measured for isolated strains cultivated in nutrient broth (Martienssen et al. 2001). Results indicate the zeta potential measurements under standardized conditions might be used for surface structure comparison of bacteria. Nitrite accumulation and oxygen availability were suggested by the incomparable Smets and Mueller (2001) as limitations to complete 2,4 - dinitrotoluene biomineralization by *Alcaligenes* JS867. Anticipated results were described using a dual-Monod biokinetic model with the inclusion of a noncompetitive inhibition term for NO₂⁻. Numerous microelectrodes were used to measure oxygen, total dissolved sulfide, oxidation reduction potential, and pH at various depths within an aerobic sulfate-reducing biofilm treating a 160 mg COD/L wastewater

(Yu and Bishop 2001). Using the probes, stratification of microbial processes were confirmed. Deuterated styrene in combination with phospholipid fatty acid analysis proved to be successful for identifying and describing microbiological communities present within a biofilm treating waste gases (Alexandrino et al. 2001). Differences in the patterns of labeled fatty acids present suggested certain organisms present were responsible for the degradation of styrene while others were not. Glass wool was used as a substratum for the study of biofilm, surface influenced planktonic, and planktonic cells (Steyn et al. 2001). Protein patterns were determined to be different in the three *Pseudomonas aeruginosa* cell types. Jirku et al (2001b) demonstrated that biofilms of *Candida maltosa* and *Fusarium proliferatum* had reduced sensitivities to acetone and phenol, compared to freely suspended cells. Results suggests the fungi's potential for use in fixed film wastewater treatment processes.

GROWTH AND MODELING

Bacterial colonization and biofilm architecture and their controlling factors were discussed, as were techniques used to monitor changes in bacterial populations (Geesey 2001).

Mass Transfer Increased turbulence, as measured by the Reynold's number (Re), resulted in improved total ammonia nitrogen removal by a biofilm (Zhu and Chen 2001a). The increase in Re number likely improved the nutrient mass flux to the biofilm.

Kinetic and Structural Models A 1-D biofilm reactor model was used to compare two situations; one rate-limiting substrate with liquid film diffusion and one rate-limiting substrate without liquid film diffusion (Bonomo et al. 2001). Simplified kinetics used within the model were found to cause an overestimate of removal rates and an overestimate of the liquid film layer thickness necessary for high kinetic orders. A biofilm kinetic model was applied to column experiments to help explain clogging in landfill leachate systems (Cooke et al. 2001). COD, Ca⁺² concentrations, and porosity profiles showed reasonable agreement with measured values. A mathematical model was developed by Tiwari and Bowers (2001) that helps to explain biofilm activity and growth in the small pore structure of porous media. Modeling results might be coupled to existing porous media hydrodynamics models. A new model describing biofilm

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14

growth within a packed bed reactor was used to explain pore network structure, pressure drop, and non-uniform growth of the biofilm (Schwarz et al. 2001). A pseudo toxic concentration model was proposed and compared with existing models describing pH inhibition of activated sludge and nitrifying biofilms (Ko et al. 2001).

A Monte Carlo simulation of the growth patterns of *Eschericia coli* on polyetherketone and titanium/glass substrates was used to study biofilm removal by lasers (Richter et al. 2001). The model offers the potential for use in determining growth patterns, as it accounts for both the microorganism aspect ratio and nutrient diffusion over the surface. The individual-based modeling approach of BacSim was compared and contrasted to the more established biomass-based model (Kreft et al. 2001). Because of the same diffusion-reaction processes present in each, the individual-based model and biomass-based model were in agreement concerning the overall growth of the biofilm, however, because of different biomass spreading algorithms, the predicted biofilm shape varied. Results from a quantitative cellular automation biofilm model were compared to those of a differential equation model with good agreement (Pizarro et al. 2001). Cellular automation modeling offers advantages including the ability to stimulate growth of heterogeneous biofilms with irregular boundary conditions. An EPS-biofilm model was developed to more accurately describe biofilm structure and was found to yield results similar to those models that do not consider the EPS fraction (Horn et al. 2001). The self-adaptive Galerkin-h-p-method was used to solve the balance equations developed for the model. A two-dimensional model predicted that bulk nutrient concentrations and external mass transfer resistance have a significant impact on biofilm structure (Hermanowicz 2001). The model included cell growth, cell detachment, and mass transport and was developed to determine potential biofilm morphologies under various conditions. A general model of bacterial assemblages, grown on glass slides and analyzed using denaturing gradient gel electrophoresis (DGGE), suggests the assemblages may not be structured by resource competition or niche-driven patterns, as are macro-organisms (Jackson et al. 2001a). The model and associated experiments were used to describe spatial and temporal patterns found in biofilm structures. A single species biofilm model was presented that allowed steady state solution under some conditions (Pritchett and Dockery 2001).

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Hydraulics The growth of a fluorescently labeled strain of *Sphingomonas* sp. and the development of extracellular polymeric substances was digitally recorded by automated confocal laser scanning microscopy (Kuehn et al. 2001). Results of the imaging were used as input for flow field and solute transport modeling. Erosion and sloughing were modeled in a biofilm system using various hydrodynamic conditions (Picioreanu et al. 2001). Sloughing of the biofilm can be avoided using high liquid shear and low microbial growth rates.

Other Water binding, water retaining and the stability of a biofilm matrix formed within homogenous agarose beads (50 - 500 µm diameter) and porous beads (260 µm diameter) were studied by Strathmann et al (2001). Two artificial EPS matrix models were used to further elucidate results of experimentation. Anthracene and naphthalene were found to be better degraded within a biofilm than within planktonic cultures of lake water (Walczak et al. 2001). Danese et al (2001) used microscopic and macroscopic experiments of E. coli biofilm formation to examine: whether biofilms develop only under certain growth phases and growth conditions, if biofilm formation structure could be altered by changing environmental conditions, and whether any mutations that alter macroscopic biofilm structure also affect biofilm structure at the microscopic level. An annular reactor was used to study nitrifying biofilms (Liu and Tay 2001c). Relative specific growth rates, relative initial ratio between Nitrosomonas and Nitrobacter on the support surface, and the level of free ammonia were found to influence nitrite build-up in a nitrifying biofilm reactor. An improvement was made to an existing mathematical model, and the resulting model was found to accurately predict dynamic transients and explain the mechanism of adsorption by peat in biofilters (McNevin and Barford 2001). Peat offers the potential for use in biological systems where a narrow pH range is required. Physical contact with a support was demonstrated to be sensed by bacteria with a resulting response (Jirku et al. 2001a). Whole cell attachment was found to improve the osmotolerance of Saccharomyces cerevisiae cells.

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TRICKLING FILTERS

A review of the trickling filter solids contact process at four large plants is provided by Parker (2001). Improvements over original plants of twenty years prior include

16

higher organic and solids loadings successfully treated, lower space requirements, with subsequently lower costs of operation. Parker's (1999) review of trickling filter mythology resulted in some discussion: Albertson and Okey (2001) commented, and Pearce (2001) discussed the reliability and cold weather performance of trickling filters and design information such as speed control of the motorized drive and maximum loading requirements for plastic cross flow media. In response, Parker (2001) was given the last word.

Spatzierer (2001) gave an overview of techniques used in small wastewater treatment plants in Austria with emphasis on the activated sludge process and trickling filters. Monitoring results, operator influence and impact, as well as a commentary on future legal requirements for water protection are provided. A sequenced combination of an upflow anaerobic packed bed reactor, 2 anoxic fixed bed reactors and an aerobic trickling filter provided energy savings of up to 80% when compared with more conventional treatment strategies (Risse 2001). At the trickling filter effluent BOD₅ was found to be 3 - 7 mg/L and NH₄-N < 10 mg/L.

Models A bench-scale unit was used to develop and validate a dynamic VOC degradation model for synthetic media trickling filters (Alonso et al. 2001). The model focused on the effects of nitrate concentration and filter backwashing, with periodic backwashing represented by a reduction in biofilm thickness and recalculation of reactor specific surface area.

Nitrification Design and operating information for nitrifying plastic media trickling filters was provided from data collected during the study of pilot- and full-scale units (Gebert 2001). The results may be summarized as: biofilm thickness increased during winter months and thinned during summer, macroinvertebrates had no significant impact on performance, homogenous and continuous application of wastewater is critical to performance, and the specific surface area must be limited to prevent packing material channel clogging. Ammonia, nitrite, and nitrate concentrations were correlated to biofilm samples analyzed by fluorescent in situ hybridization (FISH) from a full-scale nitrifying trickling filter (Biesterfeld et al. 2001). Uniform biofilm coverage of the trickling filter was found, however FISH analysis suggested genera other than *Nitrosomonas* were present at various locations in the biofilm. Alpha-glucosidase and peptidase

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activities were measured in the biofilm samples from a nitrifying trickling filter's filtered effluent (Mustafa and Soerensson 2001). The presence of starch, peptone and ammonium resulted in an increased activity of heterotrophs, leading to an inhibition of the nitrifiers, probably via competition for available oxygen *Nitrosomonas* were shown to grow optimally at 15 °C when incubated with 5 mg/L NH₃-N (Bao and Xiang 2001). Nitrite formation was influenced by temperature, however, salinity (1-3%) and pH (7.5-8.5) did not significantly influence ammonia oxidation.

Operational experiences at two plants using a combined high-load activated sludge stage and trickling filter were described and discussed by Schreff and Steinle (2001). The highest N elimination rates were measured during constant maximum hydraulic loads in the sedimentation basin located between the activated sludge and trickling filter processes. Nitrobacteria discharged from trickling filters were found to cause a considerable degree of N oxidation at a municipal sewage treatment plant (Vestner 2001). Simultaneous N oxidation and reduction took place in the anoxic aeration tank. The same group is studying supplementary activated sludge units as upgrades to existing single-stage trickling filter treatment plants for biological nutrient removal (Vestner and Gunthert 2001). 15547531, 2002, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.2175/106143002X140440 by Missouri University Of Science, Wiley Online Library on [11/04/2023]. See the Terms and Conditions (https://anlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Industrial Applications A trickling filter coupled with an activated sludge process was used to remove trichloroethylene from wastewater under various hydraulic retention times (Misra and Gupta 2001). During the acclimation period of 60 days, COD removal varied from 54.6 - 97.5% and TCE removal ranged from 72.6 - 99.9% while after acclimation, a HRT of 28 hours (TF 18 h and ASP 10 h) achieved a 99.99% removal of TCE. At NASA, a trickle bed reactor was deemed superior at laboratory-scale to a batch reactor system for the treatment of highly concentrated methylhydrazine, hydrazine, citric acid and their reaction products (Nwankwoala et al. 2001). The *Achromobacter* sp., *Rhodococcus* B30 and *Rhodococcus* J10, immobilized on coarse sand grains, had rate constants of 0.137 1/d and 0.232 1/d for the removal of methylhydrazine, and hydrazine, respectively, while in batch cultures, rate constants were calculated as 0.046 and 0.079 1/d for methylhydrazine and hydrazine, respectively. Sulfur bacteria were found tolerant of pH variations and provided >70% COD and 98% thiosulfate removal in a pilot-scale trickling filter supplied with 6 kg

18

 $S_2O_3/m^3 \cdot d$ (Risse et al. 2001). Energy consumption during operation was minimal due to natural ventilation providing sufficient oxygen to the biomass. A trickling filter was used to biologically degrade components (anthraquinone, anionic detergent, NaCl) of textile wastewater treated with ozone and advanced oxidation processes (AOPs) (Ledakowicz and Solecka 2001). Microbial kinetic rates were determined and the various AOPs were shown to decrease the overall inhibition of biofilm growth by the wastewater. A cost-benefit analysis led to the recommendation a trickling filter be used for treating high strength BOD wastewater from a squid processing facility (Park et al. 2001a). The trickling filter pre-processing was also coupled with improved housekeeping and management systems to bring down influent BOD values. A high load trickling filter without recirculation was found to remove 75 - 80 % of CODCr, 83 -91% of BOD₅, and 85 - 92% of suspended solids with a hydraulic load of 20 m³/m²·d and loading of 1.0 - 1.3 kg BOD₅/m³·d (Yang et al. 2001b).

The use of a trickling filter in combination with reverse osmosis to recycle process water from a mineral water bottling facility was reported (Hose and Kotowski 2001). A very different kind of mineral water was the focus of treatment for a 7.4 m high pilot-scale trickling filter packed with plastic rings, used to treat cyanide, thiocyanate, copper and zinc from a wastewater (Evangelho et al. 2001). 90% of influent contaminants were removed, and higher recirculation ratios lowered the efficiency of zinc removal. Mine water was treated by two pilot scale trickling filter reactors with two separate high surface area plastic medias and a variety of influent iron concentrations (1.08 - 1.84 mg/L) and flow rates (1 - 12 L/min). With effluent iron concentrations ranging from 0.20 - 1.04 mg/L, a strong correlation was found between influent iron loading and its removal and at Mn loading rates of 0.5 - 0.9 g/d, over 50% was removed (Jarvis and Younger 2001).

Other Wastewater and dump seepage was treated in a trickling filter plant with improvement in treatment capacity noted when lignite coke dust was added (Verch et al. 2001). The coke was reported to serve as an adsorbent and as a substrate for the biofilm. Solids characterization of trickling filter effluent was completed using particle size measurements, image analysis and zeta potential measurements (Schubert and

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Wolfgang Gunthert 2001). 90% of particles are <30 microns and do not readily settle, increasing the turbidity of effluents.

ROTATING BIOLOGICAL CONTACTORS (RBCs)

Fukuda (2001) reviewed wastewater treatment with rotating biological contactors. Biofilm density, thickness, growth yield, and dehydrogenase activity were found to be related to shear exerted in a RBC (Liu and Tay 2001a). Results suggest shear stresses may be interpreted in relation to energy.

Biofilm characterization One group did extensive work on characterizing RBC biofilms, studying a full-scale RBC system for a year to determine the distribution of protozoans and metazoans (Martin-Cereceda et al. 2001d). Efficient removal of BOD₅ and TSS occurred although the abundance and diversity of species varied in the different RBCs. Three previously unidentified species of ciliates (Chaenea stricta, Holosticha mancoidea, Oxytricha lanceolata) were found; the overall protozoa community being found sensitive BOD₅ loading variations (Martin-Cereceda et al. 2001c). Confocal scanning laser microscopy and light microscopy revealed the internal architecture, including the spatial distribution of protozoa (Martin-Cereceda et al. 2001a). Peritrich ciliates were the most abundant group identified with the protozoan and metazoan communities, microbial distribution varied with depth, and the porosity of the exopolymeric matrix changed with depth also. Differences in these extracellular polymeric substances, consisting mostly of proteins, humic substances and polysaccharides, from both the RBCs and activated sludge flocks were identified (Martin-Cereceda et al. 2001b). RBC biofilms had 3.5 times as much protein, twice as much humic substances and polysaccharides and were twice as hydrophobic as the activated sludge flocs.

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Nutrient Removal Teixeira and Oliveria (2001) measured denitrification efficiency using both a completely submerged (100%) and a partially submerged (64.5%) RBC disc. Biofilm activity was found to be dependent upon the degree of hydration, biofilms were >0.6 mm thick, and the completely submerged disc provided higher denitrification efficiencies but had a longer delay in start-up. Residence time distribution experiments using the tracer lithium chloride revealed hydraulic dead volumes (40% for HRT of 0.94

and 2 h) appearing as stagnant eddies within corners of the unit (Teixeira et al. 2001). Dead volumes decreased in the presence of biomass under normal operation with HRT of 2 h giving the minimum amount of dead volume while dispersion numbers increased with increasing HRT for both abiotic and biotic experiments. A three-stage RBC containing a *Thiosphaera pantotropha*-containing biofilm was used for nitrification of contaminated groundwater (Gupta and Gupta 2001). The first stage gave high removal rates of C and NH_4^+ -N (8.7-25.9 g COD/m²•d and 0.81-1.85 g N/m²•d, respectively) for the corresponding loadings of 10.0-32.0 g COD/m²•d and 1.0-3.35 g N/m²•d. A RBC employing a membrane was used to perform solid-liquid separation and ammonia oxidation simultaneously (Kimura et al. 2001). The 87 \Box m biofilm gave a 930 g/m³•h zero-order and 808 1/h first order ammonia oxidation rate. CODCr and NH₃-N removal rates exceeded 93% and 94%, respectively, in an anaerobic - aerobic RBC process with influent CODCr levels of 1049 mg/L and NH₃-N levels of 275 mg/L (Sun et al. 2001).

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Domestic Wastewater Effluent BOD_5 and SS from small treatment plants using RBCs or sequencing batch reactors (SBRs) were compared (Akunna and Shepherd 2001). Both treatment options produced effluents with <10 mg/L BOD_5 and <20 mg/L SS, however, nutrient removal was greater in the SBR plants and effluent quality was more consistent. An RBC employing perforated tubes was used to treat high-strength synthetic wastewaters (Kargi and Eker 2001). Design equations based upon process parameters and kinetic rates for COD removal were determined.

Industrial Wastewater The performance of an RBC and a bubble column were compared and modeled for the degradation of hydrocarbons, with the RBC reported to give reasonable treatment efficiencies (Suzuki et al. 2001). Such hydrocarbon degradation was shown follow zero order kinetics for toluene using a three-stage laboratory-scale RBC (Alemzadeh and Vossoughi 2001). A different three-stage RBC was used to treat metal-contaminated wastewater with removal efficiencies of 73% (Cu), 42% (Zn), 33% (Cd) (Costley and Wallis 2001b). Energy dispersive x-ray spectroscopy indicated that metals accumulated on the surface of the biofilm and COD removal efficiency was found to vary in each of the three reactors, most likely related to differences in biofilm thickness and biomass. Sorption - desorption cycles with the same process showed that subsequent biofilm sorption ability was not affected by acid

wash sorption procedures (Costley and Wallis 2001a). CrO_4^{2-} reduction to Cr(III) was assessed in both batch and RBC experiments (Hatzikioseyian et al. 2001). CrO_4^{2-} biosorption was apparently significant at low pH, and the Cr(VI) was completely reduced to Cr(III), which remains soluble as it was neither biosorbed nor precipitated. An influent concentration of 2800 mg thiocyanate/L was reduced to < 1 mg/L by an RBC (flow rate 30 mL/min, HRT 11.1 h, 20 m² reactor surface) inoculated with two bacterial strains isolated from a gold mine (Stott et al. 2001). Effluent from the reactor was found nontoxic to iron- and sulfide-oxidizing bacteria and could be recycled to a biological oxidation plant.

An RBC's performance was evaluated during the treatment of a synthetic saline wastewater containing molasses, urea, KH₂PO₄ and salt (Dincer and Kargi 2001). As the total disc surface area increased, COD removal efficiency improved, however, as COD loading rate and salt concentrations increased, COD removal efficiency declined. A pilot-scale RBC was used to treat ethanol-containing wastewaters, with plans to treat industrial wastewaters in Vietnam (Dang et al. 2001). A lab-scale RBC was used successfully to treat effluents containing up to 1.5 ppm isothiazolone biocide (Kathon WT) (Laopaiboon et al. 2001). Isothiazolone concentrations above 3 ppm reduced treatment efficiency, 15 ppm concentrations inhibited all microbial activity, while planktonic and biofilm bacteria were found to have different susceptibilities to isothiazolones. Decolorization of paper bleaching plant wastewater by *Coriolus versicolor*, a white-rot fungus, and *Rhizomucor pusillus* strain RM7, a mucoralean fungus, was found to be directly proportional to initial color intensities (Van Driessel and Christov 2001). 53 - 73 % decolorization occurred with a hydraulic retention time of 23 h, and the effluent was found to be essentially nontoxic.

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Other A RBC was part of system used for landfill leachate treatment, anaerobic digestion followed by RBC then flowing to aerobic treatment and ending with a flocculation-sedimentation and adsorption processes (Park et al. 2001b). Treatment efficiency for organic materials approached 98% with 95% of the BOD associated with organic materials of less than 500 molecular weight. Results of a study by Tawfik et al (2001) indicate that a two-stage RBC (HRT 10 h, loading rate 6.4 g COD/m²•d) gives effective post-treatment of effluent from an up-flow anaerobic sludge blanket. *E. coli*

removal was greater than 89% at all HRTs examined while the two-stage RBC system provided a better effluent quality than the one-stage RBC.

FLUIDIZED BED AND AIRLIFT BIOREACTORS

A brief review of types, performance, and internal structure of three phase fluidized beds in wastewater treatment was provided by Wei et al. (2001a). COD removal rates were >85% for a 3-phase fluidized-bed bioreactor with porous polymer carriers (Pan et al. 2001). With a HRT of 2.5-3 h and organic loading of 6 - 7 kg COD/m³•d, biofilms formed steadily on the carrier with highest COD removal rates seen at 15 kg COD/m³•d. KNT[®] polypropylene particles were used as a biomass support in a fluidized bed reactor to treat brewery wastewater (Sokol 2001). 70% removal of COD was achieved in raw wastewater; when amended with nutrients, 90% COD removal was achieved. Magnetic polystyrene particles were also used in a fluidized bed as a biofilm carrier (Yavuz and Celebi 2001). Organic carbon was effectively removed from the wastewater source under constant DO, pH, and temperature conditions.

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Industrial Wastewater Treatment Bohlmann (2001a) compared process stability and effect of peak quinoline loads in a suspended biomass process to two immobilized biofilm units (aquacel and silicone carriers). Immobilized biofilms showed similar growth, stability and activity while completely degrading peak loads better than the biomass suspension. The same reactor showed that shock loads of less than 1 kg/m³ allowed for continuous degradation of quinoline by immobilized *Comamonas acidovorans* (Bohlmann and Bohnet 2001b). Continuous flow experiments by a different group studying a fluidized bed bioreactor treating quinoline-containing wastewater resulted in complete degradation of 100 mg/L quinoline at an HRT of 2.5 h (Han et al. 2001b). A 500 mg/L concentration degraded within 12 h, and reaction kinetics were modeled as a series of zero-order equations.

The effect of dilution rate on phenol degradation in an inverse fluidized bed biofilm reactor was examined by Kryst and Karamanev (2001). Biodegradation rates were affected by the amount of suspended biomass present with the overall process being very stable over the three month operational period. A fluidized bed reactor with anthracite granulate was used to treat flow-through and recirculated car-wash water

(Kuhl et al. 2001). Car-wash products entering the influent gave a COD of 380 - 800 mg/L, with effluent COD values of 1 –2 mg/L reported. Bacteria strains *Acidovorax facilis* B and *Pseudomonas nautica* utilized up to 279 mg acrylonitrile/L as a denitrification substrate were identified in a lab-scale fluidized bed treating acrylonitrile-butadiene-styrene resin-containing wastewater (Wang and Lee 2001).

Nutrient Removal A lab-scale fluidized bed biofilm reactor with granular activated carbon was used to denitrify reverse osmosis brine containing high concentrations of ammonia, chloride, salts, alkalinity, phosphate, sulfate, and trace amounts of heavy metals (Ersever et al. 2001). The acclimation period was short, growth yield high, organisms resilient, while 99% removal of nitrate was achieved under various conditions. Several carriers for use in the Sorption-Denitrification-P-Removal-process (S-DN-P-process) for fluidized bed biofilm reactors were investigated (Brandt and Hegemann 2001). Most materials had similar performances while costs per m³ of carrier material varied significantly. Chalk as a biomass carrier was reported to result in high nitrification rates (Green et al. 2001). A maximal nitrification rate of 1.44 g NH₄+-N/L reactor•d, average cell yield of 0.1 g cells/g N, and specific ammonium oxidation rates ranging from 0.08 - 0.15 mg NH₄⁺-N/mg protein•h were observed. Up to 98% COD removal efficiency was attained during the treatment of a synthetic wastewater containing 180-230 mg COD/L, 25-30 mg NH₄⁺-N/L, 12-13 mg P/L, and micronutrients (Tavares et al. 2001). Both nitrification and denitrification were efficiently accomplished.

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Models A kinetic model of organic degradation in an internal loop fluidized bed bioreactor was developed (Deng et al. 2001). The model was found to accurately predict removal by biofilm attached to the porous polymer carriers over a variety of test conditions. The effects of structural and operating parameters on gas holdup, liquid circulation velocity, volumetric mass transfer of oxygen, and mixing time were examined for an inner loop three-phase fluidized bed reactor (Wei et al. 2001b). Quantitative and semi-quantitative mathematical models were developed for scale-up of the reactor system. In a model developed to describe protein adsorption in a fluidized bed, superficial velocity and particle radius were found to have the largest effects on breakthrough behavior for all conditions while the effect of axial dispersion, film mass transfer and solid diffusion coefficients were less significant (Wright and Glasser 2001). Intraparticle mass transfer effects at high degrees of bed expansion limited the performance of the fluidized bed.

Other A fluidized bed system was used to treat eutrophic lake water with an average influent chlorophyll-a concentration of 90 μ g L (Tanaka et al. 2001). By monitoring dissolved oxygen concentrations and chlorophyll-a, up to 64% removal of algae was accomplished. A lab-scale fluidized bed was used to convert sulfide to elemental sulfur and to produce a sulfur sludge with good settling qualities (Annachhatre and Suktrakoolvait 2001). Oxygen concentration., sulfide loading rate and upflow fluid velocity affected sulfur production. Influent H₂S concentrations of 500 mg/L and COD concentrations of 200 - 800 mg/L were treated with a hybrid fluidized bed bioreactor (Guo et al. 2001). The unit removed 90% of the H₂S, 80% of the COD, and was also found to control odors. Fluidized bed treatment technology transfer from Japanese to Chinese wastewater treatment plants was demonstrated by Takahashi (2001). Treatment of phenol-containing wastewater was successfully accomplished.

SUBMERGED BED BIOFILM REACTORS

Domestic Wastewater Treatment BOD removal from high strength wastewater within an aerobic reactor containing polyvinyl alcohol gel particle packing material improved when temperatures increased from 20 - 50 °C but decreased when temperature was increased fro 50 to 60 °C (Lim et al. 2001). Quinone profiling indicated microbial communities changed as temperatures increased. A four-compartment pilotscale hybrid aerated submerged fixed-film (HASFF) reactor, in which fixed ceramic plates act as biofilm support, was operated using various hydraulic residence times (2, 4, 6, and 8 h) and organic loadings \leq 0.7 g BOD/g MLTVS•d (Al-Sharekh and Hamoda 2001). BOD removal was found to be >94%, COD removal was 66-76%, the effluent mean filtered BOD and filtered COD concentrations were 4.5-7.5 and 70.0-89.6 mg/L respectively, and the units were found to be resistant to organic loadings. Overall operational information for a package plant employing structured-sheet, cross-flow trickling filter media packed around a central draft tube, and operated in anaerobic, aerobic, and in some cases, anoxic mode, showed that anaerobic cells removed 65-85% of influent BOD and process 180 lb BOD/10³ ft³•d while the aerobic submerged 15547531, 2002, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.2175/106143002X140440 by Missouri University Of Science, Wiley Online Library on [11/04/2023]. See the Terms and Conditions (https://anlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

fixed-film cells removed the remaining BOD and NH₃ (McDowell and Hubbell 2001). Two pilot-scale units were operated to compare the performance of an activated sludge process with a fixed-film supplemented activated sludge process for the treatment of monosodium L-glutamate-containing wastewater (Bai et al. 2001). After 45 days of operation both processes gave effluent values of < 700 mg COD/L (>90% removal), < 400 mg NH₃-N/L (>80% removal), and although the activated sludge process showed an efficiency decline at lowered water temperatures, the fixed film system efficiency remained constant.

An optimum hydraulic load of 4-6 m³/m²•h¹, CODCr volumetric load of 10-12 kg CODCr/m³•d, and BOD₅ volumetric load of 5-6 kg BOD₅/m³•d was determined for a tilted-pipe sedimentation-upflow aeration biofilter for the treatment of domestic wastewater (Zheng et al. 2001). Effluent BOD₅ and CODCr were <15 mg/L and <60 mg/L, respectively, with the addition of 6 - 8 mg/L polyaluminum (3%) and 7 - 9 mg/L oil coagulant. A biological aerated filter packed with quartz sand spherical media, treating 1.2 - 1.4 kg sCOD/m³•d did not adequately treat shock loads of 5.1- 7.3 kg sCOD/m³•d, however good performance was again achieved within 60 minutes after the increase in loading (Mendoza-Espinosa and Stephenson 2001). Hydraulic velocity changes (0.7-1.0 m/h to 1.5-2.9 m/h) had only limited effect on removal performance. Biofilm formation and the impact of hydraulic retention time changes were examined for a twostage bioreactor (Qiu et al. 2001). At a 9.8 h HRT and influent CODCr concentrations of 164-310 mg/L, removal rates were as follows: CODCr 88%, SS 85%, and TN 39%.

Moving Bed Bioreactors (MBBR) Both biofilm and suspended bacteria were instrumental in the biodegradation of toluene within an aerobic circulating-bed biofilm reactor (Yu et al. 2001). The suspended biomass, less than 1% of the total biomass, converted <= 30% of the toluene to intermediates; the toluene intermediates were removed predominantly within the biofilm. Greater than 86% COD and sulfide removal from a tannery wastewater was accomplished with a combined air flotation-mixed sludge and biofilm aeration process (Wei 2001). Aeration time was 11.25 h with influent 2.18 kg CODCr/m³•d.

<u>Nitrification</u> Coke and cinder particles outperformed plastics in an airlift loop reactor (Li et al. 2001b). Determination of the optimum lift diameter combined with proper

carrier type shows promise for increasing lift, circulation, and nitrification. The performance of two moving bed systems, one employing polyurethane particles and the other granular activated carbon were compared using a sequencing batch process mode (Loukidou and Zouboulis 2001). Nitrogen, organic matter, color and turbidity were effectively removed using both carriers. A general autotrophic ammonium-removal efficiency of 70% was achieved for a high NH_4^+ -N wastewater with low C:N ratio in a floating bed reactor operated at 28 °C, pH 8.0 and DO of 0.8 - 1.0 mg/L (Yang et al. 2001a).

<u>Denitrification</u> Two types of carriers showed similar response (denitrification capacity, temperature dependency, and COD and nitrate removal) when compared during their use in a full-scale and pilot scale municipal wastewater treatment plant (Maurer et al. 2001). The maximum denitrification rate was 420 g N/m³•d at 10 °C and 730 g N/m³•d at 20 °C, average denitrification rate 240 g N/m³•d at 10 °C, and maximum COD removal rate of 37% with influent to the anoxic zone of 2.2 kg COD/m³•d.

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Phosphorous Results suggest diffusion limitations may seriously impact the ability of a biofilm reactor employing polystyrene balls as a support medium (BIOSTYR) to remove phosphorous from influent waste streams (Falkentoft et al. 2001b). Computer modeling indicated backwash interval, biofilm thickness after backwash, and phase length could impact phosphate diffusion. The lab-scale BIOSTYR's phosphorous and nitrate removal efficiency varied significantly over its 1.5-year operational period despite well defined inputs - perhaps because of microbial population shifts (Falkentoft et al. 2001a). Results from the experiments suggest on-line monitoring and microbial characterizations may optimize this biofilm process for phosphorous and nitrate removal. Nitrification, phosphorous uptake and denitrification was accomplished in the aerobic phase of a moving bed biofilm reactor operated in a sequencing batch mode (Helness and Odegaard 2001). Nitrogen and phosphorous removal was maximized when virtually complete removal of COD was accomplished in the anaerobic phase and complete nitrification occurred in the aerobic phase.

Sequencing Batch Biofilm Reactors Joshi (2001) compared the sequencing batch reactor and sequencing batch biofilm reactor technologies. Stable nitrification and

biological phosphorous removal were reported in a 17 m³ sequencing batch biofilm reactor (Arnz et al. 2001). Anaerobic fermentation was rate-limiting and NO⁻³ loads created during backwashing reduced P removal. For the same reactors, a method for anaerobic hydrolysis rates of particulate organic matter was developed and identification of the predominant micro-organisms present was accomplished (Arnz and Wilderer 2001). Using flow cytometry, confocal laser scanning microscopy, and automated image analysis, particle density in the carrier biofilm, basic biofilm layer, biofilm flocs and sessile ciliates was determined for a sequencing batch biofilm reactor with a mature biofilm (Eisenmann et al. 2001). 58% of particles were found attached to the packing material, 15% with suspended flocs, and 10% ingested by the sessile ciliates. O₂, NO₂⁻, and NO₃⁻ profiles were measured and bacterial populations identified within a phosphate-removing biofilm in a sequencing batch biofilm reactor (Gieseke et al. 2001). During the aeration period, nitrification was O₂ limited and had a delayed onset, while fluorescent in-situ hybridization revealed three distinct and spatially separated NH₃ -oxidizing populations were found.

3-methylpyridine (3MEP) was removed from a wastewater stream using ozonation followed by a sequencing batch biofilm reactor (Carini et al. 2001b). The process was initially mass-transfer limited, and the ozone largely reacted with non-stable byproducts or was decomposed. A model of the ozonation process was presented in a separate paper (Carini et al. 2001a). Reaction rates of 16 mg P-nitrophenol (PNP) consumed/L•h, 14 mg p-aminophenol (PAP) formed/L•h, and 20 mg PAP mineralized/L•h were measured in a pilot-scale biofilter packed with volcanic stone and inoculated with activated sludge (Melgoza and Bultron 2001). A combined anaerobic/aerobic process (8 h anaerobic: 3.5 h aerobic) was used during the 230 day operational period to treat an influent concentration of 25 mg PNP/L. A similar two-step anaerobic and aerobic biofilm process was used to treat the azo dye disperse blue 79 with 95% decolorization in the anaerobic process and 65% degradation in the aerobic process (Cruz and Bultron 2001). Residual dye contained within the anaerobic stage effluent was found to inhibit the aerobic process while the presence of a co-substrate within the anaerobic process did not inhibit biodegradation unless dye concentrations were > 48 mg/L.

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Nitrification A review of compact biofilm technologies for high N wastewaters concluded that the direct conversion of ammonia into elementary N by compact biofilms is both cost effective and efficient (Seyfried et al. 2001). Hippen et al (2001a) discussed operational results from six years of operation of industrial and pilot-scale plants using biofilm systems for nitrification. The same group discussed the choice of a biofilm process for the Mechernich treatment plant because of its ability to achieve an old sludge age (Hippen et al. 2001b). De-ammonification stage maintenance was the impetus for change to another system variant from a more traditional system. Stable NO₂⁻ accumulation in the effluent of a completely stirred biofilm reactor fed 250 mg NH_4^+ -N/L was found when DO was < 0.5 mg/L (Bernet et al. 2001). Transient increases in DO caused complete conversion of NH₃ to NO₃⁻ to occur. Potential inhibition of nitrifiers by chloramphenicol and oxytetracycline hydrochloride, antibiotics found in cattle farm and pharmaceutical industry wastewaters, was studied in a 1-L fermentor where nitrifiers existed both in a biofilm and suspension (Campos et al. 2001). At concentrations of 10 - 250 mg/L, chloramphenicol had virtually no effect on biofilm stability or nitrification, while biofilm sloughing was observed at 10 mg/L of oxytetracycline. A synthetic fertilizer industry wastewater (500-2500 mg NH4⁺-N/L) was treated effectively using an upflow aerated biofilter (Chandravathanam and Murthy 2001). A maximum ammonium removal efficiency of 81% was observed for a loading of 0.5 kg NH₄⁺-N/m³•d with lower removal efficiencies at higher loadings, most likely attributed to ammonium or nitrite inhibition.

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Xiao et al (2001) showed that a biofilm process with elastic packing and micropore aerator removed 64 - 95% of NH_4^+ -N at 20 - 27 °C and 40 - 63% at 7 - 12 °C. The reactor was operated at an HRT of 1.4 h, gas:water ratio of 0.5:1 and a DO of 7 - 10 mg/L. An ammonium removal rate of 1100 g N/m²•d was measured in a gravelcontaining vertical bed reactor (Lahav et al. 2001). The nitrification rate was limited by the oxygen transfer rate at all hydraulic loading rates in the 500 L reactor. An ammonium oxidation rate of 1.8 kg NH_4^+/m^3 •d and greater than 90% nitrification efficiency was demonstrated in a bench-scale upflow biological aerated filter (BAF) packed with a polyurethane-based porous medium (Han et al. 2001a). During the biological nitrification of wastewater, significant nitrogen loss was observed in the BAF

29

under oxygen-limited conditions when organic carbon was not provided for denitrification. An up-flow BAF pilot plant for tertiary treatment was operated for two years with the following results: low water temperatures do not impact the process significantly, the optimum rise rate was 8.5 m/h, 50% of the post-secondary TSS and BOD can be removed, and the industrial effluent has not caused degradation of the support material (Payraudeau et al. 2001). Results from a full-scale plant (8257 L/s) showed the same results as the pilot-scale plant.

For wastewater of a different sort, a 6 m³ nitrifying biofilter and associated denitrification process was used to treat recirculating seawater in a black tiger shrimp rearing tank (Menasveta et al. 2001). Three trials, varying the media, inoculant, and carbon source were reviewed for their treatment effectiveness, with a methanol carbon source and crushed oyster shell found to give the best performance. Filter media characteristics rather than the flow scheme was found to be more important for determining biofilter efficiency in a series of packed bed reactors (Yang et al. 2001c). Two filters in series, the first with a cross-link structure media and high bed porosity and the second with rough surfaces was found to have the highest efficiency when treating aquaculture pond water. A C:N ratio of 1 or 2 resulted in a 70% reduction of ammonia nitrogen within a lab-scale seven stage recirculating system (Zhu and Chen 2001b). Removal of organic matter results in improved nitrification rates in these aquaculture recirculating systems. Greater removal percentages were observed during treatment of petrochemical wastewater by a combination biofilm A/O process (Wang et al. 2001b). COD, NH₃-N, and Total N removals were found to be 81%, 94% and 57.3%, respectively, with an 8 h HRT.

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Biological activities were investigated in submerged biofilters operating in both an upflow and a downflow configuration (Chiou et al. 2001). In the upflow biofilter, COD removal occurred mostly between 0-20 cm and the effect of hydraulic residence time on nitrifying autotrophs was significant while in the downflow filter, COD removal occurred in the submerged zone and the maximum nitrifying autotroph activity occurred with a hydraulic residence time of 6 h. The same group examined the effect of recycle ratio on total nitrogen removal and the effluent nitrogen form in a submerged bed bioreactor (Chiou and Ouyang 2001). Maximum total nitrogen removal was measured at a recycle

30

ratio of 2.5 while at low recycle ratios, flow conditions were more stable and effluent concentrations of NH₃-N were lower. Also using a sequencing batch biofilm reactor under automatic control, Cho et al. (2001) treated a high strength organic nitrogen-containing wastewater. Kinetics within the anaerobic and aerobic reaction stages may be modeled using the Michaelis-Menten equation and ORP was identified as a major control parameter for the sequencing nitrogen removal process.

Biomass characteristics and nitrification capacity was examined in a circulating floating bed reactor operated with a 1 h hydraulic retention time and influent wastewater concentration of 50 mg N-NH₄⁺/L (Fernandez et al. 2001). Ammonia removal ranged from 90 - 97%, the volumetric nitrification rate was calculated as $1.1 \text{ kg N-NH}_4^+/\text{m}^3 \cdot \text{d}$, the specific oxidation rate for ammonia was 0.57, 1.42 and 1.97 g N-NH₄⁺/g-protein•d and the nitrite specific oxidation rate was 1.21, 2.84 and 4.59 g N-NO₂/g-protein•d at temperatures of 10, 20 and 30 °C. Powdered kaolin particles were added to an airlift reactor system to improve reactor performance (Vieira et al. 2001). Clay particles, added to the tertiary nitrification system, were incorporated into the biofilm pellets and nitrate production decreased when particle concentrations increased. A comparison study of polypropylene plastic chips and polyethylene bocks for removal of ammonia from aquaculture wastewater showed no significant difference for the different medias, however, plastic chips were less expensive (Ridha and Cruz 2001).

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A variety of techniques including comparative sequence analysis, fluorescent in situ hybridization, digital image analysis, competitive PCR, confocal laser scanning microscopy, and microautoradiology were used to examine nitrifier diversity and population structure of a biofilm in a sequencing batch biofilm bioreactor treating wastewater with high ammonia and salt concentrations (Daims et al. 2001b). A high diversity of ammonia- and nitrite-oxidizers were observed, novel species, and microchannels and cavities were identified within microcolonies of *Nitrospira*-like bacteria.

Denitrification 100% denitrification efficiency was achieved with a volumetric loading rate of 2.84 kg NO_3 -N/m³/d and empty bed contact time of 6.76 h in a reactor employing simultaneous heterotrophic and sulfur-utilizing autotrophic denitrification (Lee et al. 2000). Clogging occurred at short HRTs while the carbon source was found to impact

the fraction of NO₃-N removed by heterotrophic denitrification. Two media types, Ringlace and Kaldnes, were evaluated during use in a prefermenter system for production of short chain volatile fatty acids used in enhanced biological phosphorous removal (Mavinic et al. 2001). For a design evaluated and with a hydraulic residence time of 60 minutes, Ringlace media was found superior, providing 11 and 5.5 mg/L of short chain volatile fatty acids (as acetic acid) with screened raw wastewater and primary effluent, respectively. A critical volumetric loading rate of 9 kg NO₃-N/m³-d was found for two lab-scale submerged bed reactors treating high concentrations of nitrate, 50 - 3000 mg NO₃-N/L (Oh et al. 2001). Effluent NOx concentrations fluctuated greatly above the critical loading rate and reactor performance was related to biomass concentration on the plastic Pall ring bed material. Real time aeration of a pilot-scale biological aerated filter (BAF) delivered effluents with <20 mg N/L using 50% less air than conventional BAF processes (Puznava et al. 2001). Real time aeration delivers the constant low dissolved oxygen levels (0.5 - 3 mg/L) required for simultaneous nitrification and denitrification within the biofilm.

Two types of packing material (hollow or solid ball) were investigated for use as a biofilm carrier within a shallow open channel (Hsu et al. 2001a). The channel with hollow ball packing material had higher nitrogen conversion rates and contained 3.5 times the amount of biomass as the channel with solid ball packing. Flow velocity was found to impact nitrification and denitrification rates in this biofilm channel, which was used to treat activated sludge process waters (Hsu et al. 2001b). Nitrification and denitrification were found to occur concurrently with trapped suspended solids in the biofilm forming an anoxic environment for denitrification. When operated in pilot-scale to treat municipal wastewater, the BIOFIX sorption/denitrification process was found unsuccessful (Temmink et al. 2001). Uptake of the influent COD into the biofilm was low (34%), 9-21% of influent ammonia was contained within the biofilm and later released to the effluent, while nitrification was inhibited by a high concentration (50 - 60 mg/L) of suspended solids. An upflow sulfur packed-bed reactor containing *Thiobacillus denitrificans* was used to treat synthetic wastewater at various influent flow rates, nitrate concentrations and sulfur particle sizes (Koenig and Liu 2001). A half-

order kinetic model was found to describe autotrophic denitrification rates, and that such rates were an order of magnitude lower than for heterotrophic denitrification.

Phosphorous Removal Nitrogen and phosphorous were successfully removed from wastewater using a packed bed column subjected to alternating aeration/non-aeration periods (Altinbas 2001). 71% of influent nitrogen and 74% of influent phosphorous were removed with effluent concentrations of TKN, Tot -P, NH₄ and NO₃ measured as 3.8, 3, 1, and 2.5 mg/L, respectively. A combined activated sludge/biofilm process was used to removal phosphorous under varying COD/TP ratios (You et al. 2001). Results indicated that when COD/TP ratios exceeded 30, the removal efficiencies of COD, TN and TP were 98%, 76% and 100%, respectively, with TP removal decreasing when COD/TP ratios were less than 30. Phosphorous removal was evaluated at differing hydraulic loads and anaerobic/aerobic cycle time ratios in a sequential batch biofilm reactor comparison with a suspended growth reactor under the same conditions was also performed (Chlou et al. 2001). In the biofilm reactor, polyphosphate accumulating organism activity was closely related to poly(hydroxyalkonate) accumulation and the most efficient cycle time was ratio was identified as 1:2 (An/Ox). A combination precoagulation and aerobic biofilm process with HRT of 4 h provided complete nitrification (Tsuno et al. 2001). The pilot scale plant provided stable and low concentrations of N and P (2 mg N/L and 1 mg P/L) in the effluent when treating municipal sewage.

Various carrier materials, biofilm characteristics, and extrapolymeric substance quantities were investigated in another sequencing batch biofilm reactor used for simultaneous nitrogen and phosphorous removal (Choi et al. 2001). Findings included that thinner biofilms promoted nitrification and phosphorus removal while thicker biofilms enhanced denitrification and reduced phosphorus removal and suspended solids removal correlated well with the biofilm EPS content. Identification and microbial functions of phosphorous removing organisms within a submerged bed reactor showed the dominant bacterial species to be a Pseudomonad (Li et al. 2001a).

Industrial Wastewater Treatment Acetone concentration was monitored as an indicator of 2-propanol degradation in a 1.9-L three-phase fixed bed bioreactor containing solvent tolerant bacterial cells immobilized onto porous glass cylinders (Bustard et al. 2001). A maximum acetone generation rate of 1.3 g/L•h was observed

during a fourth addition of 2-propanol, while the maximum acetone biodegradation rate of 0.38 g/L•h was observed during initial 2-propanol addition. A combined activated sludge biofilm process was improved when HRTs reached 8 h and sludge age 5 d (Li et al. 2001e). Less sludge bulking and less sludge quantity were formed during the combined process when compared to the conventional activated sludge process. 71% of influent phenol and 60% of starch were effectively degraded in a bubble column reactor using pumice stone support (Viswanathan et al. 2001). Near complete degradation of Aroclor 1242, by both a bioreactor augmented with *Rhodococcus* sp. M5 and a non-bioaugmented reactor with natural granular sludge, was seen in a granular biofilm reactor with limited aeration (Tartakovsky et al. 2001). The specific dechlorination rate was calculated as 1.43 mg PCB/g volatile suspended solids•d with only 16-19% of the influent Aroclor recovered from the reactor biomass and effluent.

A pilot-scale plant was erected on-site to treat mine water overflow high in metals and low in pH (Sandstrom and Mattsson 2001). The reactor, filled with a plastic substrate and operating with a ferrous concentration of 3.5 g/L, pH 1.8, flow rate of 330 L/h at 35°C, showed a ferrous iron oxidation rate of 750 mg/L•h. Fe and Mn were found to be simultaneously removed in a biofilter bed (Zhang et al. 2001). Fe⁺² was found to have a significant effect on bacteria and the make-up of the biological community. Driven by requirements for cost reduction for pollution abatement, PVC conduit supporting a biofilm was used to treat copper-containing wastewaters (Qureshi et al. 2001). Scanning electron microscopy with energy-dispersive x-ray microanalysis was then used to analyze the biofilm. A packed bed reactor used to remove ionic mercury from chloralkali wastewater were found to be sensitive to shear forces from air bubbles but was not affected over time by increases to influent mercury concentrations (up to 7.6 mg/L), increased volumetric load, or increases in temperature (up to 41 °C) (von Canstein et al. 2001). The reactors, packed with 80 cm³ of lava chips, were operated for 16 months at 160 mL/h. 15547531, 2002, 7, Downloaded from https://onlinelibrary.wiley.com/doi/10.2175/106143002X140440 by Missouri University Of Science, Wiley Online Library on [11/04/2023]. See the Terms and Conditions (https://anlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Other A feedback control law was developed to provide a sustained, optimal input profile for a fixed-bed bioreactor (Benthack et al. 2001). Although changes in substrate inlet concentrations and biomass growth rate occurred, treatment efficiency was continually kept high using a feedback loop. Perchlorate reduction rates in packed bed

reactors were found to obey first order kinetics, providing a stable design parameter for engineers (Logan 2001). A review of packed bed treatment of perchlorate - contaminated water revealed removals from 0.0007 - 20 mg/L•min.

Three type of bioreactors (aerated submerged biological filter, airlift biological contact oxidation reactor, direct micropore aerated biological reactor) were used to remove algae and results compared (Wu and Wang 2001). The most important algae removal mechanisms were identified as bioflocculation, adsorption, detachment and sedimentation while the aerated submerged biological filter provided the best removal (70% of influent algae).

The effect of pH, temperature, particle size, bacterial support material, and air distribution were examined for a packed bed reactor used for ferrous iron biooxidation (Mazuelos et al. 2001). The reactor, that has potential for use in treating acid mine drainage, produced a maximum of 11.1 g/L•h ferric iron while the air diffuser type was found to be critical for enhanced performance of the system. In a reactor for metal reduction instead of oxidation, *Pseudomonas putida* DMP-1, and *Escherichia coli* ATCC 33456, provided virtually complete Cr(VI) reduction and phenol removal in a reactor fed 5-21 mg Cr(VI)/L•d and 840-3350 mg phenol/L•d (Nkhalambayausi-Chirwa and Wang 2001). The process was found to be resilient to Cr(VI) toxicity, recovering rapidly from overloaded conditions.

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BIOLOGICAL GRANULAR ACTIVATED CARBON (BAC)

When compared to a conventional and a biofilm sequencing batch reactor, a powdered activated carbon reactor was found to be more efficient for treatment of filature wastewater (Xu et al. 2001b). Granular activated carbon, contained within a sequencing batch biofilter, efficiently (97%) removed a mixture (600 mg/L) of phenol, 4-chlorophenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol (Buitron et al. 2001). A second sequencing batch bioreactor, using an automated time-optimal control strategy, removed 99% of the phenolic mixture. A similar reactor operated for 6 months under continuous flow, a feed concentration of 20 - 50 mg/L 4-chorophenol (4-CP), and a hydraulic residence time of 17 min achieved 4-CP removal efficiencies of 69 - 100% (Buitron et al. 2001; Carvalho et al. 2001). Lower performance periods were attributed

to clogging and channeling within the column. Aldehyde removal on granular activated carbon biofilters was predicted well using rate constants derived from a batch recycle attached growth reactor while the removal of biodegradable dissolved organic carbon was underpredicted (Digiano et al. 2001). It was speculated that biokinetic modeling might improve as more sophisticated techniques were employed to measure the concentration and activity of attached biomass and the biodegradability of natural organic matter fractions. A moving bed reactor with biological activated carbon, coupled with pre-ozonation, was used to treat acrylonitrile butadiene styrene-containing wastewater (Lin et al. 2001). A COD removal efficiency of 85-95% and 70-90% was obtained with organic loadings of 3.2-6.3 kg COD/m³·d and 0.6-1.6 kg COD/m³·d (6 h HRT) as secondary and advanced treatment system, respectively.

In a study focused not on recalcitrant organics but rather metals, 50 - 100% of Cr(VI), and 20 - 100% of Cd(II) were removed from solutions containing 4 - 11 mg metal/L at a hydraulic residence time of 1.2 min by a biofilm of Arthrobacter viscosus (Quintelas and Tavares 2001). Uptakes of chromium and cadmium were calculated as 8.5 mg Cr/g carbon and 4.2 mg Cd/g carbon. Associated with some metals processing is cyanide; 22 isolated strains from cyanidation tailings and 4 collection strains were tested for their ability to tolerate cyanide complexes, with the isolated strain UASLP-Micro. Ecol.Z-18 and the collection strain Bacillus cereus reported as the best degraders of cyanide (Quintelas and Tavares 2001). However, when allowed to grow on activated carbon as a biofilm, their metabolism was evidently modified and an increase in pH occurred. 22 isolated strains from cyanidation tailings and 4 collection strains were tested for their ability to tolerate cyanide complexes; the isolated strain UASLP-Micro. Ecol.Z-18 and the collection strain Bacillus cereus were the best degraders of cyanide (Razo et al. 2001). However, when allowed to grow on activated carbon as a biofilm, their metabolism was evidently modified and an increase in pH occurred.

MEMBRANE BIOREACTORS

Peters (2001) reviewed membrane processes in wastewater treatment and comments on the future potential use of membrane processes. *Desulfovibrio*

36

desulfuricans, immobilized on a Pd-Ag alloy membrane, was used to remove and recover metals from leachates prepared from scrap automotive catalysts (Yong et al. 2001). 80% of platinum group metals were recovered with a residence time of 15 min.

Oxygen Delivery A computer model was used to examine oxygen and substrate use within hollow fiber membrane systems and impermeable solid support systems of similar geometry (Semmens and Essila 2001). Oxygen transfer performance was found to be mediated by the thickness of the biofilms, and aeration of the external wastewater around the membranes was suggested for process improvement and limitation of biofilm growth. Hollow fiber membranes were used to treat a high salinity wastewater from food pickling plants (Anon 2001). The aerobic process, employing *Bacillus* removed 99.9% of influent BOD with lower facility capitol and maintenance costs than a comparable activated sludge process.

IMMOBILIZED CELL BIOREACTORS

A review of processes using immobilized biomass showed that treatment was possible even with low hydraulic retention times and without the requirement for nutrient supplementation (Hartmann 2001). Immobilized biofilms were found in use at plants treating wastewater from the microelectronics, food, and photochemical industries. The effects of the carrier concentration, the number of freeze-thaw cycles during preparation, the membrane diffusion coefficient, immobilized-cell activity, and operational stability of a polyvinyl alcohol (PVA) membrane containing nitrifying and denitrifying bacteria were investigated (Cao et al. 2001a). Results indicate a polyvinyl alcohol membrane prepared at PVA concentrations of 13% and 15% (wt./wt.) and 5 freeze-thaw cycles possessed higher strength and higher immobilized cell activity while the immobilized-cell membrane remained stable for a 2 month operational period. Mineralization of 2,4,6-trichlorophenol (2,4,6-TCP) was accomplished by organisms immobilized in kappa -carrageenan/gelatin [2% (w/w) of each polymer] gel beads under both anaerobic and air-limited conditions (Gardin et al. 2001). Results showed that the gel did not influence the activity of the granules, the anaerobic and aerobic communities could be easily co-immobilized in gel beads and cultivated in a reactor, and the mineralization of 2,4,6-TCP and its degradation intermediates could be obtained under

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air-limited conditions if the culture parameters were strictly controlled. A dual system of anaerobic digestion coupled with an aerobic packed bed reactor was used to treat effluent from olive mills (Bertin et al. 2001). Two packed reactors based on initially immobilized cells, one with polyurethane foam and the other with silica beads, rapidly and completely removed nine monocyclic aromatic acids in a synthetic olive mill wastewater.

BIOFILMS ON SAND, SOIL, AND SEDIMENTS

Sand/Soil Structural variables, extracellular enzymatic activities, photosynthetic activity, and microbial community respiration were measured for epilitich and epipammic biofilms to determine and explain organic matter degradation (Romani and Sabater 2001). Degradation of cellulosic and hemicellulosic molecules were greater on sand although the biofilm biomass and cell size were lower than on tiles. A microbial biofilm from a shallow aquifer was found to degrade MTBE under oxic conditions in the laboratory (Landmeyer et al. 2001). MTBE was also degraded under natural conditions; an influent MTBE concentration of >1 mg/L was reduced to <1 µg/L. Sandy loam soil-packed columns inoculated with *Rhizobium meliloti* A-025 removed atrazine and nitrate under saturated and unsaturated conditions (Mehmannavaz et al. 2001). The results suggest an effective method for controlling agricultural pollutant run-off. Wang et al. (Wang et al. 2001a) looked at the adsorption of p-chlorophenol by kaolin clay and biofilms. At 25 °C and pH 6.1, bacterial cells and EPS adsorbed more p-chlorophenol than kaolin, while kaolin with a biofilm coating adsorbed more than that without biofilm coating.

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Several studies focused on various metals; biosorption capacities for cesium, technetium, uranium and nickel were determined and followed-up with a column experiment for cesium removal (Andres et al. 2001). The effect of different microbial strains, pH and medium composition were elucidated. A similar study of metals removal in a sand filter as a polishing treatment for wastewaters containing Cd, Zn, Cu, Pb, Hg, Ni, and Co, also showed removed COD and nitrates (Diels et al. 2001). When desired, the metal-containing biomass was separated from the sand by an airlift within the filter bed; heavy metals concentrations were > 10% of the sludge dry weight. Arsenic was successfully removed from mining wastewater using sulfate reducing bacteria adhered to forest litter (Isabel et al. 2001). Data collected using Pb L-alpha fluorescence profiles show that metal oxides hematite (alpha $-Fe_2O_3$) or corundum (alpha $-Al_2O_3$) are the dominant sink for Pb(II) at sub-micromolar concentrations while at Pb concentrations greater than 10^{-6} M, Pb uptake by biofilms of *Burkholderia cepacia* occurred (Templeton et al. 2001). Although attached bacteria and adsorbed organic matter is thought to interfere with sorption processes on metal oxide surfaces, this study showed that the formation of a monolayer biofilm did not.

Wetlands Silyn-Roberts et al. (2001) examined the ammonia-oxidizing group *Nitrosomonas* in a wetland treating dairy effluent . In situ hybridization and PCR results showed that proportions of the nitrifiers were <1%, were greatest in the second trench of the subsurface wetland, were susceptible to low temperatures, and single cells, not dense clusters, were present in the biofilms.

Riverine/Sediment Prochnow et al. (2001) attempted to correlate the biologic status of the microbial mat with the erodibility of sediments using measurements of dissolved and particulate organic carbon. Such measurements may be useful in understanding attachment of biofilms to sand, soil and sediments within packed columns. An artificial substrate that forms a fluorescent precipitate in conjunction with the nucleic acid stain DAPI was used to enumerate extracellular phosphatase expression by bacteria in photosynthetic biofilms exposed to various photosynthetic activities and phosphorus supplies (Espeland and Wetzel 2001). Results suggest that the amount of extracellular organic carbon released within the biofilm matrix during photosynthesis indirectly affected bacterial phosphatase synthesis. Microbial ecology and methane concentrations within a biofilm from a sewage outlet were studied using a microscale biosensor for methane (Damgaard et al. 2001). Aerobic respiration consumed much of the methane produced within the biofilm, nitrate addition inhibited methanogenesis in the upper layer of the biofilm while sulfate addition inhibited methanogenesis throughout the depth of the biofilm. In river water the aromatic amines aniline and 2-nitraniline were successfully degraded in a biofilter at differing rates (Boernick et al. 2001). First order kinetics were found to apply to these riverine experiments and might be used for estimation of untested amine degradation rates. In a study of pharmaceuticals, clorfibric

acid was not degraded by river biofilms whereas metabolites of ibuprofen were degraded during a 21 day experimental period (Winkler et al. 2001). Adsorption and abiotic losses were not significant in the biofilm reactors. *Mycobacterium* was found to be more important in a biofilm removing toluene from a contaminated stream than Xanthobacter (Tay et al. 2001). Populations in the contaminated reach were determined using quantitative PCR and found to undergo seasonal variation. In a saline environment, Acinetobacter calcoaceticus was identified as the predominant hydrocarbon-degrading micro-organism in a biofilm attached to gravel particles (Radwan and Al-Hasan 2001). The biofilm coated gravel was used to clean 5 successive challenges of oily seawater with reported success. Lead adsorption to laboratory surrogates, amorphous Fe oxide, biogenic Mn oxide produced by a Mn(II) oxidizing bacterium, AI oxide, the common green alga *Chlorella vulgaris*, and *Leptothrix* discophora SS-1 cells, was demonstrated under varying pH conditions (Wilson et al. 2001). Adsorption was found to be consistent with model results with Fe and Mn oxides contributing up to 90% of lead adsorption on the lake biofilms. Both living and dry cells of the freshwater cyanobacterium *Gloeothece magna* showed biosorption of metals (Mohamed 2001). Adsorption of Cd²⁺ and Mn²⁺ to living cells and dry cells, was dependent on the metal concentrations, fitted the Freundlich adsorption isotherm with dry cells having higher binding capacity for both Cd²⁺ and Mn²⁺ than living cells.

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INNOVATIVE SYSTEMS

Nutrient Removal A combined upflow anaerobic sludge blanket (UASB) and upflow biological aerated filter (UBAF) were used for seven months to treat an industrial wastewater with 10.4 g COD/L and 790 mg TKN/L (Lacalle et al. 2001). 98% of influent organic matter and 91% of ammonia were removed when operated with hydraulic retention times of 3.3 d (UASB) and 1.3 d (UBAF) and recycle ratio of 6.7. A denitrification capacity of 47 g NO₃-N/m•h was determined for a combined heterotrophy-electrode-biofilm reactor with an influent of 40 mg NO₃-N/L (Fan et al. 2001). Nitrate was removed predominantly within the heterotrophic denitrification portion of the reactor. Biofilms, established on bioblocks contained within a lab-scale sedimentation basin, were used to treat suspended solids-containing effluent from a

fish tank (Lekang et al. 2001). The process, known as biological lamella sedimentation, removed 43% of total phosphorous and total nitrogen from the influent. An anaerobic acidification-anoxic-aerobic (A1-A2-O) biofilm process was compared to an anoxic-aerobic (A/O) biofilm process for treatment of coke plant wastewater (Li et al. 2001d). The A1-A2-O biofilm process provided both higher N removal rates and greater reduction in toxicity of the mercury chloride-containing wastewater.

Recalcitrant Organics A multifed upflow filter, operated anoxically or anaerobically (37 °C, HRT 0.3 - 1 d), coupled with an aerobic biofilm airlift reactor successfully treated a wastewater containing 1.5 g/L formaldehyde and 0.46 g/L urea (Garrido et al. 2001). Complete conversion of urea occurred under anaerobic conditions although formaldehyde concentrations greater than 0.05 g/L caused a decrease in urea hydrolysis efficiency, and the COD:N ratios required for complete nitrite and nitrate denitrification with formaldehyde were estimated at 2.1 and 3.5 kg-COD:kg-N, respectively.

Advanced Oxidation Based upon information provided by two case studies, ozonation followed by a fixed bed biofilm process provided adequate treatment for a landfill leachate (Baig and Liechti 2001). COD, chlorides and nitrogen were removed from wood processing wastewater by a combination of activated sludge and nitrifying biofilter, but failed to meet goals (Athanasopoulos 2001). Therefore, electrolysis, ozonation, and H_2O_2/UV , were studied as polishing processes.

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Reactors Using Unusual Media Injection of fresh water, saturated with dissolved oxygen, into a concrete-lined cast iron pipe transporting reclaimed wastewater improved the pipe effluent quality; nitrite removal was complete at the end of the 61 km pipe while nitrate concentrations ranged from 0.4 - 0.5 mg/L (Delgado et al. 2001). For a COD:NO_x--N ratio higher than 5, a first order nitrification rate resulted k(20) = 0.079 1/h for a NO_x--N concentration range of 0.8 - 4.4 mg/L. An RBC-like system using cubic biofilm support modules made of crossflow corrugated plastic sheets or honeycombed plastic repetitively submerged and removed from carbonaceous wastewater, was reported to offer reduced reactor volumes, energy savings, lowered solids production and improved solids removal capability (Rodgers and Burke 2001). The laboratory-scale system removed 7.2 - 7.6 kg COD/m³•d, comparing favorably with other

conventional treatment systems. Modified surface-active polyurethane carriers that varied in size and shape were developed for fluidized bed, fixed bed, and trickling filters (Pascik 2001). The carriers were used and evaluated during aerobic and anaerobic degradation of organo-chlorine compounds and during nitrification processes. New packing materials for the Toha System were investigated including granite, polystyrene, brick pieces, paper and wheat straw (Palma et al. 2001). The paper and wheat straw proved to be the best substitute for the sawdust contained within the original two-process system.

Other Sulfide removal rates of 82 -100% and elemental recovery rates of 75-95% were achieved in a continuous-flow photoreactor with the green sulfur bacterium *Chlorobium thiosulfatophilum* attached to Tygon[®] tubes (Henshaw and Zhu 2001). The reactor was operated with sulfide loading rates of 111 - 328 mg/h•L with a maximum sustainable sulfide loading rate of 286 mg/h•L. Using combinatorial protein engineering and phage display methods, Ni²⁺-binding *Staphylococci* were generated (Wernerus et al. 2001). Results demonstrate the potential for tailor-making metal-binding biosorbent for biofilters.

Removal of starch, cellulose and polyvinyl alcohol was accomplished by different mechanisms within a combined system of a thermophilic upflow anaerobic sludge blanket reactor and aerobic moving bed biofilm reactor (Ji et al. 2001). Starch-COD was degraded equally in the two reactors, 98% removal cellulose-COD occurred by microbial entrapment and sedimentation of fibers while PVA was only minimally degraded by the combined reactors.

BIOFILTRATION AND BIOTRICKLING FILTERS

A number of papers were published on biotrickling filters and biofiltration. Although not reviewed here, 127 papers are listed under a separate heading at the end of References. Additional citations, review, and comments are contained in the Gaseous Emissions from Wastewater Facilities section of this issue. and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons

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ANAEROBIC BIOFILM SYSTEMS

A number of papers were published on anaerobic biofilms. Although not reviewed here, 29 papers are listed under a separate heading at the end of References. Additional citations, review, and comments are contained in the Anaerobic Processes section of this issue.

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ANAEROBIC BIOFILMS

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