SEDIMENT MICROBIOLOGY IN
PULP MILL EFFLUENT RECEIVING
ENVIRONMENTS : A LITERATURE REVIEW

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Sediment microbiology in pulp mill effluent receiving environments : a literature review

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Sediment Microbiology Literature Review

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I. INTRODUCTION

Pulp milling operations have been carried out in British Columbia since about 1911, involving continuous waste discharges to aquatic receiving environments. Effluent discharges modify the sedimentation rate in the vicinity - a flocculation reaction resulting in significantly increased deposition occurs with discharges to marine waters (Pocklington and MacGregor, 1973; Stanley et al, 1978). Receiving basin shape and area with characteristics of water volume replacement, turbulence and current activity (hydrodynamics) normally govern sedimentation in a water body. Interacting with effluent volumes these influences determine the dilution and dispersal of effluents in the benthic habitat. Oxygen availability (concentration) is also normally governed by basin hydrodynamics. Effluent and sediment oxygen demands are satisfied according to characteristics of oxygen supply in specific receiving basins.

Sediment accumulation is increased overall in most receiving basins as a result of effluent discharges, and a gradient of effluent components according to specific gravity can be determined with distance from the point of discharge by physical sediment analysis. Thus the more dense and stable complex wood by-products are localized in the vicinity of the discharge, while lighter and more labile materials are accumulated in the deepest parts of receiving basins. Inhibition of sediment communities is observed in response to effluent discharge and generally corresponds with the gradient of physical distribution.

Complex organisms such as bottom-feeding fish and macroinvertebrates are commonly inhibited and may be eliminated from receiving basins due to oxygen and nutrient requirements in interaction with altered activity of sediment microbial populations. Progressive domination by the microbial community results from macroinvertebrate inhibition and tends to maintain conditions preventing re-establishment of these populations. (Stanley et al, 1978; Van der Wal, 1976).

Direct observation of these impacted benthic habitats by SCUBA divers in Nova Scotia (Pocklington and MacGregor, 1973), using submersible craft in British Columbia (Environment Protection Service, since 1975), and with remote-controlled television in Scotland (Stanley et al, 1978) has demonstrated the common reaction of white (colourless) sediment surface mat or veil formation. Experimental studies have shown these mats to be created by intensive activity of the bacterial genus Beggiatoa, which participates directly in cellulose decomposition (Vance et al, 1979) while veils in overlying waters are formed by genus Thiovulum (Jørgensen and Revsbech, 1983). Both genera derive energy from oxidation of hydrogen sulfide (Jørgensen, 1982) and their activities at the sediment surface indicate severe inhibition of decomposition activities performed by the normal microbial community.

This paper will review our understanding of sediment microbial communities in natural conditions and detail the changes and trends which appear in response to pulp effluent discharges.

II. SEDIMENT MICROBIOLOGY IN NATURAL CONDITIONS

A. GENERAL CONSIDERATIONS

Opposing gradients of aerobic and anaerobic conditions are generally observed in naturally forming sediments. Bacterial and other microbial populations tend to stratify and concentrate in these zones in relation to nutritional requirements and physical limits of adaptation. Within the bacterial community, three functional groups are of primary importance in respect to numbers and to consequences of their activities. These include the aerobic heterotrophs - most dense in the surficial oxygenated sediments, the anaerobic heterotroph population - usually concentrated in deeper, permanently anaerobic sediments and sulfide oxidizers, which populate the interface zone between these conditions. Together these groups strive to exploit organic materials and energy resources (electron acceptor compounds - oxygen, nitrate, sulfate and carbonate) before they are carried away to other areas or compressed to a depth in the anaerobic zone where chemical reactions dominate biological activity.

Benthic biological activity alters chemical parameters to such an extent that reduction-oxidation potential, a measureable index of chemical reaction intensity, exhibits a discontinuity in the surface region. The redox potential discontinuity represents a zone in which biological (primarily bacterial) activity modifies chemical concentrations and creates pH, oxygen and sulfide minima of concentrations. The importance of macroorganism activities in surficial sediments in enhancing exposure to oxygen has been noted as controlling the position of the redox potential discontinuity (RPD) (Pearson and Rosenberg, 1976; Stanley, et al, 1978). Vertical migration of this zone affects and reflects the intensity of microbial activity, and has been discussed in physical terms:

> "Increase of protection (e.g., by embayment) reduces (sediment) permeability and brings the RPD closer to the surface over a long-term range. Seasonal changes are due to temperature cycles since higher temperature causes higher position of the RPD, while mixing by winter storms lowers the RPD level, whereas at the same time input of organic matter causes it to rise again."
>
> (Fenchel and Riedl, 1970)

The first three influences affect the whole environment and hence the whole microbial community by modifying oxygen and sulfide concentrations. Upward migration of the RPD due to organic matter input is the result of bacterial activity as it modifies concentrations of oxygen and sulfide.

Climatic influences such as temperature and turbulence are common to all aquatic systems. Seasonal changes in availability of inorganic electron donor compounds, however, vary among aquatic systems. Freshwater systems can regularly exhibit inhibition of the bacterial exploitation of organic resources due to limited supply of oxygen, nitrate and sulfate. Estuarine basins do not in general experience limited oxygen supply but they do exhibit nitrate limitation. Sulfate limitation in estuaries on a seasonal basis is exhibited as a gradient correlated with the degree of freshwater influence. (Parkes et al, 1979). Marine systems can exhibit seasonal nitrate limitation, but high concentrations of sulfate in sea water do not vary significantly (Fenchel and Riedl, 1970). Thus, organic resources can be decomposed through sulfate reduction if oxygen limitation occurs through water column stratification (Stanley, et al, 1978).

The benthic environments we are discussing are specifically related to deep basins of aquatic systems. Discounting light penetration in the majority of cases to allow for autotrophic carbon fixation, all sediment microbial populations can exhibit carbon (organic material) inhibition on a seasonal basis. Nitrogen and phosphorus compounds are the other major limiting nutrients due to intensive competition for available supplies.

As all coastal and continental aquatic systems are subject to variable supply of labile (reactive or degradable) organic carbon, bacterial density in benthic sediments and position of the redox potential discontinuity are most often limited by this influence.

B. BACTERIAL FUNCTION GROUPS

1. Aerobic Heterotrophs

These organisms decompose organic compounds for food and require the presence of oxygen to maintain efficient metabolism. The majority derive energy for their activity from oxygen or nitrate reduction, though some types can derive both food and energy from decomposition alone. Thus seasonal inhibition due to limited supply of oxygen and organic material can strongly affect these populations, especially as regeneration of nitrate from the ammonia some groups produce enforces competition for available oxygen with other groups which use it directly, including macroorganisms. Low nitrate concentrations in all aquatic systems tend to maintain a strong seasonal influence on nitrate-dependent bacteria. (Stanley, et al, 1978).

The zonal concentration of aerobic heterotrophs with respect to redox potential occurs in the range of positive values, as a potential of 0 specifically correlates with disappearance of oxygen. Depths in the range of 0-2 centimetres commonly support the highest densities of these organisms in most aquatic systems. Seasonal oxygen limitation may inhibit all activity in the sediments by these groups in specific basins.

Aerobic heterotrophs rapidly exploit any aggregation of labile organic materials which reaches the sediment surface in conditions favouring their activity (Fenchel, 1980). Their importance lies in their primary stabilization of sedimenting material to maintain availability, probably by production of mucilage (Vance, et al, 1979), and their production of labile low molecular weight organic compounds. These by-products lower the sediment pH as they diffuse downwards under water pressure and accumulate by absorption to inorganic materials composing the sediment matrix (Stanley, et al, 1978).

2. Anaerobic Heterotrophs

Fermentative decomposition of organic material is predominant in deeper sediments of all aquatic systems; this is carried out mainly by bacterial populations which do not require oxygen or nitrate directly to maintain efficient metabolism. The production of hydrogen sulfide by organic sulfur and sulfate reducer (i.e. sulfide producer) populations is particularly important in decreasing oxygen concentrations below the sediment surface, hence modifying the physical conditions (i.e. redox potential) which affect the entire biological community. Interacting with the physico-chemical dynamics which govern organic matter and sulfate supply, sulfide producers are concentrated where the redox potential ranges between +100 and -250 millivolts (Jørgensen, 1982). In natural conditions, this zone is commonly limited to depths in the sediment greater than 1 centimetre (Gunnarsson and Rönnow, 1982). The lower limit of sulfide producer activity reflects the increasing importance of methane producer activity, which groups represent the slowest biological decomposition process.

Although a population of methane-producing bacteria is always present and active in sediments, their competitive interactions with sulfide producers have been stressed (Gunnarsson and Rönnow, 1982) and methane producers cannot grow in conditions where redox potential exceeds -200 millivolts (Cappenberg, 1975). Thus the activity of methane producers, which has been shown to be significant in some natural surface sediments (surficial 0-5 centimetres, Gunnarsson and Rönnow, 1982), is dependent upon the activities of sulfur-compound reducer bacteria and the maintenance of anaerobic conditions.

The anaerobic heterotrophs slowly and efficiently degrade organic material over which newer deposits of sediment have built up. Sulfate-reducing bacteria are of primary significant in marine waters due to the rich supply of sulfates in this environment, while in fresh waters the sulfate reservoir may be depleted. Organic sulfur compound reduction along with methane production then become dominant influences in the maintenance of anaerobic conditions. Utilization of the low molecular weight by-products of aerobic heterotrophs is favoured by all anaerobic bacteria. In natural conditions, the sulfate reducers benefit first from these resources due their tolerance of less reducing conditions near the sediment surface. Their intensive activity, however, increases the zone of anaerobic conditions and favours competition with the methane producers.

Anaerobic bacteria carrying out sulfide and methane production are responsible for secondary stabilization of the sediments and slow but complete decomposition of labile organic materials. The production of sulfide promotes maintenance of the anaerobic zone and transfers energy back to aerobic habitats. (Jørgensen, 1982).

3. Sulfide-oxidizing Heterotrophs

The genera Beggiatoa and Thiovulum are particularly important to sediment communities in their role as sulfide oxidizers, for which they have adapted to efficient activity at the oxygen-sulfide interface (redox potential discontinuity). They require both oxygen and sulfide and exploit many organic carbon sources, of which acetate is favoured, though use of carbon dioxide has been suggested (Jørgensen, 1982). Beggiatoa is significant for its direct involvement in cellulose decomposition. The sulfide oxidizers prefer a slightly negative redox potential and are thus associated very closely with the redox potential discontinuity, corresponding to equal concentrations of oxygen and sulfide. They are generally concentrated in the upper centimetres of the sediments. Mat and veil formation at the sediment surface occurs only when penetration (diffusion) of oxygen into the sediments is inhibited by low availability, which can occur as a result of intense sulfide production which will limit the bacteria directly.

Sulfide oxidizers produce Sulfur (S^o) which is either excreted or stored as granules (Jørgensen, 1982). They also produce low molecular weight organic compounds, most of which compose the mucilaginous matrix in which they live. The primary function of this matrix is to establish boundary layers between the opposing concentrations of oxygen and sulfide, allowing accurate positioning of the organisms at the narrow interface between these compounds and stabilizing the interface itself and thus preventing the spontaneous reaction of sulfide oxidation. (Jørgensen, 1982; Jørgensen and Revsbech, 1983). A secondary function, conservation and concentration of degradative enzymes, has been postulated as favouring efficient exploitation of organic materials. (Vance, et al, 1979).

Sulfide oxidizers contribute to secondary stabilization of sedimented materials and the rapid decomposition of organic material. They benefit directly from the lack of competition in the anaerobic zone for sulfide as an energy source (Jørgensen, 1982) while their activity in controlling oxygen and sulfide concentrations around them is significant in maintaining the stability of aerobic-anaerobic zonation.

C. COMPETITIVE MICROBIAL POPULATIONS

1. Grazing Organisms

A complex grazer community of animals of single-celled (protozoan) and more complex animals compete to exploit the bacterial community as food. Competition has been demonstrated in the areas of physical adaptation or specialization. Opportunistic feeding and resting cell formation by oxygen-requiring organisms contrast with facultative or obligate anaerobic populations which migrate to exploit favourable conditions. Protozoans which exclusively exploit sulfur bacterial concentrations have been described (Fenchel, 1969).

Redox-related distribution has been described for many ciliate populations of intertidal and nearshore habitats (Fenchel, 1969) but very little is known of natural benthic populations. The adaptive tolerances of grazer populations appear to encompass the full range of redox potentials favourable to bacterial metabolism (Fenchel and Riedl, 1970). Grazers are likely to be concentrated in association with dense bacterial populations throughout the sediment column.

> "Production of organic carbon by chemosynthesis starts within the usually aphotic RPD (redox potential discontinuity) layer by sulfur and other chemoautotrophic bacteria, and most probably constitutes the major energy source for Protozoa and most of the higher (animal) forms,... which all have a pronounced maximum of biomass in the vicinity of the RPD layer."
>
> (Fenchel and Riedl, 1970)

Grazer populations form a complex community in relation to the variety and intensity of bacterial activity in natural sediments, and their patterns of distribution largely, but not exclusively, correspond to patterns of physico-chemical gradients. (Fenchel and Riedl, 1970). The bacterial activities in sediments are of primary significance for maintenance or alteration of physico-chemical conditions and grazer populations of micro-organisms need not be involved in direct modification of oxygen availability. Thus the effects of natural grazer communities include the maintenance of variety in microbial populations and increased stabilization of sedimented materials and the physico-chemical conditions favouring grazers themselves. It can be inferred that grazer activities in natural sediments contribute significantly to maintenance of high rates of organic decomposition.

2. Direct Competitor Organisms

While benthic population studies of natural sediments have been few in number and limited to shallow water habitats (e.g. Fenchel, 1969), distinction of deep-basin benthic communities has been attributed to physical influences as indicated by position of the redox potential discontinuity. In shallow waters the position and sharpness of the RPD is predictable on the basis of sediment composition and hydrodynamic energy inputs (Fehchel and Riedl, 1970). The redox gradient occupies a broad depth range due to penetration of oxygen and sediment scouring in the intertidal zone. A significant decrease in vertical scale for position of the RPD in the benthic sediments occurs, for example, in marine systems; "... the RPD rises toward the sediment surface as one approaches deeper or more sheltered sea bottoms." (Fenchel and Riedl, 1970).

Phototrophic populations are significant components of the sediment community in any aquatic system where light penetrates to the sediment surface, with green and purple bacteria making a strong contribution to the exploitation of all trophic niches (Fenchel and Riedl, 1970). Genera of Cyanophytes (blue-green algae), many protozoan phyla, fungi and metazoans (Catenulid and Gnathostomulid worms) have been described which are primarily heterotrophic and are tolerant or desirous of conditions in deep water sediments. These organisms have particularly adapted to compete directly with heterotrophic bacteria for labile organic food resources in aphotic habitats and organisms dependent on the reducing conditions of the "sulfide zone" have been described, though for most metazoan species the importance of dissolved versus particulate organic food requirements is still an open question. (Fenchel and Riedl, 1970).

Many organisms which compete directly with bacteria for organic resources in the sediment will be limited in deep basin sediments by oxygen tolerance, and vertical migration of autotrophs and facultative heterotrophs increases competition for available substrates. This phenomenon is most significant at the sediment-water interface, so that while aerobic heterotrophic bacteria experience the primary effects of competition, a number of secondary consequences for the anaerobic population will also result. This pattern of limitation or bacterial dominance in sediment decomposition processes is considered important for the rapid recycling of nutrients and biomass to higher trophic levels in any aquatic system (Pearson and Rosenberg, 1976). The overall effect of microbial competition results from the contribution to stabilization of the sediment surface and production of low molecular weight compounds. Both activities favour the enrichment of anaerobic populations and contribute to short-term upward migration of the redox potential discontinuity (Fenchel and Riedl, 1970).

III. RECEIVING ENVIRONMENT MICROBIOLOGY

A. BACTERIAL FUNCTIONAL GROUPS

1. Aerobic Heterotrophs

The oxygen-requiring heterotrophic bacteria may experience direct inhibition in response to the oxygen demand of pulp mill effluents discharged to benthic receiving environments. Oxygen availability may be limited already in bottom waters of any system due to historical and seasonal influences, so that vertical and horizontal distribution of the natural aerobic fermentative decomposers will be modified. A decrease in the vertical range and the capacity for efficient decomposition of organic resources occurs in response to combined water column oxygen loss and sediment oxygen demand. Predominance of inorganic oxygen demand of the sediments has been attributed to sulfide storage in metal sulfide compounds in both lakes (Wang, 1976) and estuaries (Poole, et al, 1977).

Nitrate, the alternative electron acceptor compound for aerobic heterotrophs, may be increased in concentration in the surface sediments in response to pulp effluent discharge. Dispersal of effluents is accompanied by significantly increased sedimentation rates, a phenomenon particularly notable in marine receiving systems. (Carter and MacGregor, 1981; Stanley, et al, 1978; Waldichuk, 1962). Nitrates can be adsorbed to sedimenting materials or transported in solution by advective currents associated with sedimenting materials. The benefits of increased nitrate availability are countered, however, by increased concentration of the reduction product ammonia which through diffusion into the water column will increase oxygen demand and inhibit the aerobic microbial population in general. (Stanley, et al, 1978).

Short-term increase in aerobic heterotroph activity through increased nitrate supply also results in inhibition over the long term in stable conditions. Increased aerobic decomposition of organic materials will produce more low molecular weight compounds as resources for anaerobic populations. Increased sulfide accumulation may result with consequently increased stress on oxygen penetration of the sediments. Organic material decomposition by aerobic bacteria of the oxygenated surface sediments is thus particularly susceptible to modification of oxygen supply which may be directly influenced by effluent discharge.

The composition of pulp effluent discharges includes components favourable and unfavourable to natural aerobic heterotroph populations. Purified cellulose and low molecular weight compounds (e.g. free sugars) are desirable resources. Competition for these materials ensures their rapid and efficient exploitation in favourable conditions. Presence of efficient degrader "guilds" or associated populations has been postulated for all natural freshwater sediments (Mallory and Sayler, 1983), while decreased taxonomic diversity and predominance of specialized populations in marine systems has been characterized as the response to environmental stress. (Hauxhurst, et al, 1981). Limitation of the range of heterotroph activity in natural lake sediments to depths of one millimetre has been determined by autoradiography (Hayes, et al, 1958) and cellulose decomposition in marine waters was found to proceed at significant rates in only the top centimetre of sediments (Vance, et al, 1982).

Larger, more complex organic compounds and inorganic materials are important components of effluent loading for their inhibitory influence on the aerobic heterotrophic bacteria. Lignin and related compounds form a major component of discharges which is significant primarily in contributing to increased sedimentation, as these humic compounds are known to be resistant to microbial decomposition in all environments and the pulping process increases their stability (Tabak, 1983). During sedimentation, complex and inorganic compounds may absorb nutrients from the water column as well as labile organic effluent components, which results in more rapid burial of the sediment surface. This will limit oxygen penetration and favour sulfide accumulation, both factors known to inhibit aerobic heterotrophic metabolism. Increased nutrient supply may thus be associated with conditions unfavourable to aerobic bacterial exploitation, resulting in an overall decrease in the rate of effluent decomposition. Seasonal limitation of oxygen and nutrient supply and temperature-related inhibition will generally increase the significance of effluent stress on aerobic bacteria in all aquatic systems.

2. <u>Anaerobic Heterotrophs</u>

The anaerobic bacterial community of any aquatic receiving basin will benefit directly from the potential oxygen demand associated with effluent discharge. Decreased oxygen penetration of the sediments and decreased activity of aerobic heterotrophs will naturally favour increased vertical range of the anaerobic zone and the associated heterotrophic populations. Sulfur cycle bacteria have exhibited population enrichment in response to pulp effluent discharge to Lake Superior (Rokosh, et al, 1976). Anaerobic cellulose decomposition predominates in the Lower Fox River of Tennessee (Springer and Atalla, 1974). The interactions of sulfate reducing populations, sediment sulfide concentration and anaerobic cellulolytic bacteria govern the rate of cellulose waste decomposition in the River Don estuary. (Parkes, et al, 1979). The overall rate of cellulose degradation in a well-flushed marine receiving basin (i.e. high oxygen availability) was shown to be dominated by aerobic heterotroph activity (Vance, et al, 1982) but numbers of cellulose degraders were correlated with both numbers of anaerobic sulfate reducer bacteria and sulfide concentration in the sediments (Stanley, et al, 1978). The latter marine investigation found trends in change of all bacteriological parameters to correspond with pulp effluent discharge variations.

The direct benefit of pulp effluent discharge to the anaerobic heterotrophic community relates to the phenomenon of increased sedimentation. Simple organic compounds comprise an important component of effluents, particularly if biological treatment is practiced. Treatment of pulp mill effluents is generally practiced for discharges to fresh water in British Columbia (Leach, et al, 1976). Due to the rapid transport process, much of this resource will be carried to the sediments, often supplemented with absorbed or entrained resources from the water column.

Exploitation of increased organic resources by anaerobic bacteria can be strongly limited due to seasonal supply of the electron acceptor sulfate, and has been demonstrated for pulp discharges to estuaries (Parkes, et al, 1979) and for other types of organic enrichments in marine waters (Gunnarsson and Rönnow, 1982). Sulfate, however, is a normal component of pulp mill effluents and sulfate loads directly discharged may be supplemented by adsorption during the sedimentation process. The general stimulation of anaerobic bacteria and sulfate reducers in particular by pulp mill discharges has been noted for all fresh water receiving environments (Wetzel, 1975). The consequent increase in anaerobic sulfur bacteria density was demonstrated at distances as great as nineteen kilometres from a pulp effluent discharge to Lake Superior (Rokosh, et al, 1977).

Further enhancement of resources for anaerobic heterotrophs and sulfur cycle bacteria in particular is a secondary influence of effluents on the sediment community. Cellulose degradation in conditions favouring activity of surficial aerobic heterotroph populations provides low molecular weight compounds (e.g. lactate and acetate) demonstrated to stimulate sulfate reducer activity in a marine receiving basin (Stanley, et al, 1978) and shallow estuaries (Poole, et al, 1977).

Direct toxicity of the effluent materials may affect sediment bacteria, as postulated by Hofsten and Edberg (1972) and supported by comments regarding difficulties with biological treatment of toxic pulp effluent components (Walden and Howard, 1981). Decomposition of Chlorophenolic compounds was demonstrated to occur in sediments at low temperatures (0-4°C), stimulating aerobic more than anaerobic heterotrophic bacteria at 0°C. (Baker, et al, 1976). In the temperature range 0-20°C., many toxic phenolic compounds appear to degrade spontaneously at rates faster than bacterial decomposition (ibid, 1976), while the common resin acid Dehydroabietate (DHA) exhibits a sediment half-life of twenty one years (Brownlee, et al, 1976).

While direct inhibition of the bacterial community by toxic components of pulp effluents has not been demonstrated, these compounds contribute to the alteration of physical and chemical dynamics through increased rate of burial of the sediment surface.

> "The biodegradability of the organic material entering the sediment and the oxygen regime of the overlying water will determine, to a large extent, the biologically important physical and chemical characteristics of the sediment. ... The organic molecules possess functional reactive sites which allow them to form metal complexes and chelates and these further co-ordinate with the inorganic anions in seawater such as phosphate which can then be reversibly attached and detached under the right redox conditions. This process is particularly important in relation to phosphate regeneration, and the increased phosphate level in the water overlying highly reduced sediments in Sullom Voe (a marine basin in Scotland) is an example of this process.
>
> The inorganic matrix of the sediment will also act as an ion exchange column allowing the exchange of heavy metals or organic molecules both within the sediments and across the sediment/water interface..."
>
> (Stanley, et al, 1978)

Increased sediment formation as a result of pulp effluent discharges thus alters the reservoir capacity for nutrient, energy transfer and metabolic by-product compounds. While this influence will decrease in significance with increasing distance from the discharge in relation to basin hydrodynamics, other factors continue to favour increased density of anaerobic heterotrophs.

Anerobic dominance in sediment microbial communities has been demonstrated for all aquatic basins receiving pulp mill effluents. Even desirable materials such as cellulose may accumulate for six months in turbulent rivers limited only by scouring of the river bed at high flow velocities (Springer and Atalla, 1974). Limitation of cellulose decomposition through the influence of the interdependent factors of temperature and sulfide concentration has been modelled for estuaries by Poole, et al, (1977) and rivers (Springer and Atalla, 1974) and demonstrated in modelling experiments for a marine receiving basin (Pearson, 1980).

Sulfide accumulation has been determined as limiting to the entire sediment community including sulfide-producing bacteria despite unlimited oxygen availability in rivers (Springer and Atalla, 1974) and estuaries (Parkes, et al, 1979). In both studies temperature was found to be the primary influence on activity of the sediment community. Sulfide stress of sediment bacteria is relieved in warming periods even in severely polluted estuaries (Parkes, et al, 1979; Poole, et al, 1977), but the spontaneous increase in oxygen demand within the water column can maintain the inhibition of aerobic cellulose decomposition (Poole, et al, 1977) and consequently limit the enhancement of resources for the anerobic populations in conditions which otherwise favour their activity. Trophic interactions such as sulfide oxidation (energy exchange) and grazing by "herbivores" (decrease in population density) have been demonstrated to relieve sulfide stress in a marine receiving basin (Pearson, 1980; Stanley, et al, 1978).

3. <u>Sulfide-oxidizing Heterotrophs</u>

The requirement for oxygen and limitation by extremes of reduction-oxidation potential favours a limited range of vertical distance for efficient activity by sulfide-oxidizing bacteria (Jørgensen and Revsbech, 1983).

"The sulfur bacteria living at the O_2-H_2S interface must therefore always compete with the autocatalytic oxidation of sulfide. Both Beggiatoa and Thiovulum species have adapted to this requirement by growing as sheets at the transition between oxygen and sulfide. Beggiatoa cells, which are filamentous and gliding organisms, form thin mats over solid substrates from which H_2S is released. Thiovulum cells, which are very large, spherical bacteria with peritrichous flagella, form fragile veils floating in the water, only loosely attached to the underlying substrate. These typical growth forms demonstrate a highly developed chemotactic behaviour of the bacteria, which enables them to adjust rapidly to the unstable chemical gradients. ... The formation of Thiovulum veils serves the same purpose as the formation of Beggiatoa mats ... The veils are sufficiently rigid to withstand slow movements of the water, and they enable an expansion of the anoxic zone into the overlying water. In this way, Thiovulum veils can outcompete the sediment-bound Beggiatoa filaments, which have sometimes been observed to glide upon the top side of Thiovulum veils."

(Jørgensen and Revsbech, 1983)

Experimental studies of pulp effluent loading in a marine receiving basin demonstrated Beggiatoa surface mat formation under conditions of "excessive cellulose fibre deposition". (Stanley, et al, 1978). Mat and veil formation at any depth in the sediments involves the creation of a boundary layer separating oxygenated and sulfide zones of the habitat. The organisms benefit directly from the stabilization of oxygen and sulfide supply for their metabolic energy requirements, and the entire microbial community benefits in consequence. Significant oxygen demand in overlying waters due to sulfide release from pulp waste accumulations has been noted by Hofsten and Edberg (1972), Poole, et al, (1977) and Parkes, et al, (1979). The extremely efficient oxidation of sulfide in mats or veils will minimize this stress and result in the maintenance of favourable conditions for the sulfide oxidizers.

Aerobic heterotrophs will benefit by sulfide oxidizer activity in several ways. Control of oxygen availability will foster their continued activity in rapid decomposition of complex organic materials. The potential for pH and redox limitation due to accumulation of their reduced low molecular weight by-products is decreased by the exploitation of these materials by the sulfide oxidizers.

Sulfide oxidizers can thus benefit from vertical migration of the redox potential discontinuity in response to pulp effluent discharge. Their dense populations create a strong decrease in pH in the vicinity (Jørgensen and Revsbech, 1983) which assists in the maintenance of high sulfide concentration for their exploitation. Sulfide exploitation in mats or veils will foster its diffusion from deeper sediment strata, resulting in less inhibition of the anaerobic sulfide producers. However, sulfide producer activity is limited by the exploitation of the aerobe by-products by the sulfide oxidizers and by decreased sulfate diffusion into the anaerobic zone due to the presence of the mat or veil boundary.

Limitations on the activity of sulfide oxidizers generally occur through physical influences such as receiving basin hydrodynamics and temperature variation. Experimental modelling of marine sediment response to pulp effluent discharge determined water flow rate and hence oxygen supply to be more significant than high cellulose loading in favouring sulfide accumulation and lowered redox potential (Pearson, 1980). In contrast, seasonal temperature variations determine activity of the sediment bacterial community in estuaries, with winter temperature minima governing all heterotrophic activity and spring warming stimulating sulfide release and thus aerobe inhibition and low oxygen availability.

Receiving basin hydrodynamics generally govern the dispersal and distribution of pulp effluent materials. Inhibition of sulfide oxidizers as well as obligate aerobes can occur in proximity to an effluent discharge as a result of the rapid sedimentation of the components of high specific gravity, as they tend to exert a large oxygen demand and require long and complex processes of decomposition. In these areas the primary exploitation of valuable effluent components is carried out by anaerobes including sulfide producers. Mat formation by Beggiatoa will therefore be restricted to surficial sediment strata where oxygen availability is maintained.

As high sulfide concentration generally accompanies this pattern close to a discharge source, the important activities of aerobes may be very much inhibited. Mat formation then contributes to the vital function of sediment primary stabilization. Such conditions, involving rapid burial of the sediment surface on a continuous basis, do not favour the efficient metabolism of Beggiatoa though their motility favours continued exploitation of the sulfide energy resource. Thiovulum species with their fragile waterborne veils will not be favoured either, especially in marine basins where "boiling" of effluents, due to low specific gravity with respect to the deep waters, occurs following release from the diffuser.

Thus, in general, slow anerobic decomposition of pulp effluent materials predominates in proximity to the point of discharge. These processes can be inhibited by sulfide accumulation due to the increased storage capacity of the sediments, while relief of sulfide stress by Beggiatoa activity is inhibited due to the associated oxygen demand and presence and activity of Thiovulum of incidental occurrence in this physically unstable habitat.

Limitation of sulfide oxidizers to the sediment-water interface is not limited to the vicinity of the source of effluents. Transport processes in a receiving basin favour concentration of low molecular weight effluent components (including cellulose) in the deepest areas of the basin, though excessive discharge can apparently disturb this pattern:

> "Observations made in the field using underwater TV in 1971 showed extensive areas of mat formation over a large part of the deep basin of Loch Eil (marine) following unusually large inputs of pulp fibre."
>
> (Stanley, et al, 1978)

Deep basin areas with low current flows will favour the efficient activity of both Beggiatoa and Thiovulum with Thiovulum favoured when hydrodynamic energy is too low to foster oxygen penetration of the sediment surface or satisfaction of effluent oxygen demand. Where oxygen availability is maintained within the sediments, the formation of a Beggiatoa mat favours aerobic activity above it (i.e. colonization of newly sedimented materials) and anaerobic activity below, as well as contributing directly to cellulose decomposition. The most recent investigation of the well-flushed (high hydrodynamic energy) marine receiving basin Loch Eil, while noting the limitation of measurable cellulose decomposition to the surficial centimetre of the sediments, concludes that the bacterial community achieves relatively complete exploitation of desirable resources such as cellulose discharged to the basin over the long term (Vance, et al, 1982).

Where _Beggiatoa_ is inhibited from activity within or atop the sediments due to low oxygen availability, cellulose and other valuable pulp effluent components are degraded only by slow anaerobic processes. Such activity can be favoured through veil formation by _Thiovulum_. Sedimentation and current turbulence can disrupt these fragile boundaries, but such perturbations are rare in stagnant basins favouring veil formation. Relief of sulfide stress by _Thiovulum_ activity generally occurs only in seriously polluted conditions and does not favour aerobic activity in the sediments, but, as previously noted, the sulfide producers will usually be limited in their capacity to renew the high sulfide concentrations by other influences. While _Thiovulum_ veil formation can be a transient phenomenon, it may relieve sulfide stress on anaerobic cellulose degrader populations and other anaerobic activities, and thus contribute to the maintenance of processes which minimize organic material accumulation over the long-term in pulp mill discharge receiving environments.

B. COMPETITIVE MICROBIAL POPULATIONS

1. Grazing Organisms

Conditions which favour effluent decomposition by sediment bacteria will generally favour trophic interactions with microbial populations which exploit the bacteria as a food resource. In contrast to natural conditions favouring the maintenance of complex communities of macroinvertebrates and other animals which compete to exploit bacterial grazer populations, pulp effluent discharges tend to inhibit complex organisms and stress community diversity and density towards a few opportunist species tolerant of the altered physical and microbiological characteristics of the sediment habitat. Such changes take place even beneath an oxygenated water column, but are accentuated by stagnation (Pearson, 1980). Loss of burrowing organisms in particular can be correlated with effluent-stimulated expansion of the anaerobic zone and sulfide accumulation (Stanley, et al, 1978), which reinforces this trend and decreases the availability of oxygen below the surface sediment strata which would be normally increased by macro-organism activities. Decreased activity by aerobic heterotrophic bacteria results, and a limitation of aerobic grazer activity and population density in response to sulfide accumulation correlates with the decrease of this food resource in effluent-stressed marine sediments (Pearson, 1980).

Rapid exploitation of organic materials by aerobic bacteria and rapid changes in grazer population density reflect their opportunist habit and limited adaptability. Rapid build-up of ciliate protozoan populations and maintenance of high population densities were observed in experimental marine studies where high water flow maintained oxygen availability at the sediment surface despite high cellulose loading (Pearson, 1980). While the lag period of grazer population increase occurs over a period of several days, the bloom of ciliates rapidly reduce bacterial density (Fenchel, 1980) and limit the accumulation of aerobic heterotroph byproducts, thus limiting the resource which stimulates anaerobic heterotrophs and limiting rapid consumption of available oxygen.

Stabilization of aerobic heterotrophic bacterial population density at levels which do not contribute to continued expansion of anaerobic conditions is a result of aerobic microbial grazer activity. This is reflected in maintained diversity and efficiency of the bacterial populations and a high rate of cellulose decomposition. This pattern correlates with the observation that fibres are degraded more rapidly in sediments with high fibre accumulations than in areas containing incidental amounts of fibre (Pearson, 1981).

Field studies of marine sediments receiving pulp mill effluents support the similarity between deep - and shallow - water grazer communities (Wyatt and Pearson, 1982). A high proportion of ciliate species, identified from shallow sulfide-containing environments by Fenchel (1969), and high population densities of these organisms were considered a consequence of increased organic input. In contrast to shallow habitats where metazoan species dominate grazer communities in the flocculent surface stratum ciliate populations were observed to dominate this zone in deep basin sediments.

> "... in general, diversity increased with increasing organic enrichment, i.e., the reverse of the trends observed in the response of metazoan populations to enrichment."
>
> (Wyatt and Pearson, 1982)

Anaerobic grazer populations will benefit from the results of aerobic grazer activity in limiting the expansion of the anaerobic zone and the associated potential for sulfide stress. The maintenance of high aerobic heterotroph diversity under favourable conditions provides the greatest variety of degraded organic materials for anaerobic heterotroph exploitation, and by extension the greatest variety of food organisms for anaerobic grazers.

Adaptive tolerances of anaerobic grazers are little known. While many species appear tolerant of high sulfide and methane concentrations, ammonia has been demonstrated as toxic for most ciliate species studied (Fenchel and Riedl, 1970). Ammonia was found to be less toxic at low pH values, and a marked region of low pH is associated with the redox discontinuity (RPD) (Fenchel, 1969). This results from sulfide oxidizer activity and is particularly notable across veil or mat boundaries (Jørgensen and Revsbech, 1983). In the anaerobic zone underlying the RPD, ammonia concentration increases rapidly (Fenchel, 1969), which suggests that anaerobic grazers may be limited in vertical range of distribution by this factor (Fenchel and Riedl, 1970).

Mat or veil formation by sulfide oxidizers at or near the sediment surface will limit anaerobic grazing organisms to activity in the vicinity as the pH minimum region and the RPD are compressed. Inhibition of grazers on this basis will limit their effective maintenance of bacterial diversity and control of sulfide producer populations, resulting in decreased rate of effluent decomposition and greater potential for sulfide stress. Elimination of anaerobic grazer organisms could be anticipated in unfavourable conditions created by anaerobic bacterial dominance of sediment chemistry, for example,
to the water column (Stanley, et al, 1978) when bottom water anoxia favours positioning of the RPD above the sediment surface.

2. Direct Competitor Organisms

Under conditions of rapid burial of the sediment surface associated with pulp effluent discharge, mobility (motility) is a distinct advantage. Most direct competitors with bacteria for organic resources are adapted to this lifestyle due to the natural variability of resources. Ascomycetes and some fungi, however, have been observed to be inhibited in marine pulp effluent receiving basins, and this was suggested as resulting from rapid burial as a continuing stress (Churchland and McLaren, 1972).

In conditions favouring diversity of microbial activity, particularly continuous oxygen supply, direct competitors contribute to the maintenance of high rates of effluent decomposition in both aerobic and anaerobic zones. Photosynthetic organisms are generally inhibited but high diversity and density of heterotrophic blue-green algae has been described for aphotic sediments where the redox potential discontinuity approaches the sediment-water interface (Fenchel and Riedl, 1970).

Anaerobic populations of direct competitors for bacterial resources such as low molecular weight organic compounds will generally be favoured by pulp effluent discharge though they are likely to be limited in vertical range of distribution by ammonia concentration as noted for grazer populations (Fenchel and Riedl, 1980). Strongly developed layering of anaerobic metazoan populations in shallow water sulfide zones (Fenchel, 1969; Fenchel and Riedl, 1970) may be disturbed by expansion of the anaerobic zone and sulfide accumulation in unfavourable conditions. Inhibition of these populations in receiving basin sediments releases the anaerobic heterotrophic bacteria from substrate competition, thus favouring sulfide accumulation and autoinhibition of the sulfide-producing bacteria.

In receiving environments where pulp effluent discharges result in unfavourable conditions for diverse bacterial zonation and activity, stress to microorganisms which compete directly with anaerobic bacteria for these resources can be expected. Inhibition of these populations will likely parallel inhibition of the anaerobic bacteria, resulting in particularly slow effluent decomposition and probable accumulation of organic material for very long periods.

IV. SUMMARY

Co-ordination of the information available from studies of natural sediment community dynamics with results of investigations of sediment microbial communities in pulp mill effluent receiving environments indicates that, in general:

- Anaerobic degradation of effluent organic components has been recognized as a long-term process in relation to rapid accumulation of these materials.

- Surficial oxygenated sediment strata support higher diversity of microbial groups and activities in both fresh and marine waters.

- Maintenance of oxygen availability at the sediment-water interface is desirable for the sediment community and promotes vital primary degradation and sediment stabilization process.

- Facultative and obligate aerobic cellulose-degrading bacteria are present in surface sediments under natural conditions. These populations will rapidly proliferate to exploit supplements of cellulose and related compounds. Rapid degradation can be maintained in favourable conditions, and can be maintained despite developing oxygen limitation in the habitat.

- Interactions with other microbial flora and fauna and macrofauna are significant in maintaining maximal bacterial activity and hence maximal effluent decomposition in favourable conditions. Microfauna increase in population density and diversity in response to bacterial enrichment and its consequences, while macrofauna are inhibited by physical and chemical alterations of the habitat in response to pulp effluent loading in all aquatic systems.

- Pulp effluent discharges perturb both the sediment habitat and the microbial community directly. The community will respond to counteract the stresses imposed by these inputs, but lag periods are common and adaptive tolerances and responses can be overwhelmed.

- Bacterial species of the Cytophagales group as well as various Pseudomonads are considered vital in their contribution to high rates of cellulose degradation. Their activities are favoured in aerobic conditions. When these forms are inhibited, the related genus Beggiatoa is favoured in surface sediments. Beggiatoa is an opportunist species, and modifies the habitat by mat formation to maintain its preferred physico-chemical conditions.

- Sediments deeper than approximately four centimetres are predominantly anaerobic in natural conditions. The obligately anaerobic bacteria which reduce sulfur compounds (particularly the sulfate-reducing genus Desulfovibrio in estuaries and marine waters) actively maintain the stability of anaerobic conditions in interaction with specialized grazer and competitor populations.

- A particular though poorly understood population of anaerobic cellulolytic bacteria carry out slow but complete decomposition of accumulated cellulose in stable conditions in all aquatic habitats. Activity of these populations can govern the decomposition rate of pulp mill effluents in freshwater, estuarine and marine receiving basins under conditions unfavourable to aerobic bacterial activity in surficial sediments.

- Detoxification of incapacitated sediments is carried out by sulfide-oxidizing bacteria of genera Thiovulum, which forms veils, and Beggiatoa, previously noted for cellulolysis, which forms boundary mats at the sediment surface. While Beggiatoa is limited for efficient activity to the sediments, Thiovulum veils can form in the overlying water.

Fundamental research of sediment microbial populations and their interactions continue and favour our better interpretation of changes observed in response to pulp mill effluent discharge. The significance and value for monitoring strategies of phenomena such as redox potential discontinuity migration and its measurement, surface mat formation by Beggiatoa and alterations in the population balance of the various bacterial functional groups is much better understood due to recent studies. Long-term monitoring is required in order to evaluate the significance of observed trends in m crobial community response to pulp effluent discharges for the overall trophic web dynamics in receiving basins and adjacent regions of all aquatic environments.

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