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ASSESSMENT OF THE SOURCES AND OF THE ECOLOGICAL RELATIONSHIPS BETWEEN PAPER MILL SLIME FORMING MICROORGANISMS IN A SOUTH AFRICAN PAPER MILL.

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A DISSERTATION PRESENTED TO THE FACULTY OF SCIENCE, UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG, IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

JOHANNESBURG

FEBRUARY, 1985

DECLARATION

I declare that this dissertation is my own *unaided work*. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

Ingrid Anne HARRIS

5th day of April ;1985

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ABSTRACT

Microbiological slime deposits in paper mills were studied to gain a better understanding of their ecology. The four major genera identified and subsequently used for this study were Bacillus, Pseudomonas, Citrobacter and Aeromonas. Raw material sampling showed treated sewage water and broke pulp to be chief sources of inoculation of slime formers into the system. A successive colonization of microorganisms following shutdown and startup of the machine was shown although no interdependance between the microorganisms could be demonstrated. Growth of these microorganisms was shown to be affected by a dithiocarbamate slimicide, Citrobacter being capable of growth at the lower concentrations while growth of all others was totally inhibited.

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1. INTRODUCTION

1.1 Microorganisms and Biofouling - Overview

Varying conditions and nutrients offered by different microbial habitats dictate that those microorganisms better adapted for growth and survival will be prevalent.

In running or flowing water systems the growth and survival of the organisms is primarily dependant on their ability to maintain themselves in such a way that they are not washed out of the system. The most common method is to attach themselves to a solid object (56). This is, in essence, the basis of biofouling (31). By becoming attached they attain a reasonably permanent habitat and ready nutrient supply (11, 39, 49, 68). Early studies by ZoBell (68) showed that not only are nutrients concentrated at solid surfaces, but that the amount of microbial growth increases in proportion to the linear dimensions of the storage vessel. A ready nutrient supply ensures that the organism expends less energy in the search for food and more energy can therefore be channelled into replication.

Attached microbial growths which become viscous and slimy are a problem to a number of industries. Sugar manufacture, food processing and handling, pharmaceutical products, canning factories, velvet fabric plants, chemical plants, water treatment plants and paper mills are all examples which encounter this biofilm problem (2, 10, [illegible] A common element to all of these industries is wate[illegible]erves both as a source of contamination and as [illegible] of dissemination (56).

The generic composition of the biofilm depends to a large extent on the location of the biofilm (12), the type of nutrients, pH, temperature and oxygen tension being factors which govern the composition of the biofilm (7).

As the biofilm increases in size, diversification of genera occurs. The biofilm initially consists of aerobic and facultatively anaerobic organisms (47). Depletion of oxygen in the deeper regions of the biofilm gives rise to anaerobic conditions which promotes colonization and proliferation of obligate anaerobes. With further ageing of the biofilm the flow of nutrients into the base becomes impaired resulting in death and lysis of the deeper lying cells. In this way nutrient is liberated, which diffuses to the outer layers of the biofilm. If gas production occurs at the base this may destabilize the entire biofilm causing it to slough off (67).

1.2 Paper Mills

Paper mills provide a good habitat for growth of microorganisms (58). Temperatures, although sometimes high in preparatory processes, notably pulping and starch "cooking", are generally between 30°C and 40°C, the exception being the production of newsprint where temperatures often reach 60°C. Mills run under slightly acid or alkaline conditions, depending on the process.

At the time of writing all South African paper mills

run under an acid pH of approximately 4,5. This pH favours the growth of fungi and yeasts although certain bacteria can grow at this pH.

The paper mill offers habitats for obligate anaerobes, aerobes and facultative anaerobes (43). Uneven aeration in the process water circulating in the paper machine resulting from machine design leads to the occurrence of small relatively stagnant pockets and dead areas (56). In addition, once anaerobes have initially established themselves, they themselves can, within limits, maintain an anaerobic environment.

Pulp and water are the major raw materials in paper production. Although unpulped wood is not a substrate attacked by many organisms besides those with cellulolytic enzymes, the pulping process, by both mechanical means and acid chemical "cooking action", serves to partially break down the cellulose yielding sugars, starches, cellulose, hemicellulose and various mineral salts, which can be utilized by many microorganisms (58). The nature of the paper making process thus ensures a ready nutrient supply for microbial flora.

Water is obtained from different sources, which are often dependant on mill siting. Potable water is not required for paper production and therefore the water used for the process is often effluent providing many organic substrates and minerals. Other nutrients for

microorganisms could enter the process with additives such as starch, clay, rosin, size and alum (58). and different mills make different kinds of paper and board and therefore utilize different additives.

Prior to the actual sheet formation in paper making, wood or wastepaper must be pulped. Following this process water and desirable additives are mixed with the pulp. The function of these various additives is chiefly to obtain certain desirable characteristics such as optical brightness, colour and water retention capabilities. In addition slimicides are also added to prevent excessive slime buildup or to disperse already existing slime accumulations (25, 35).

Slime buildup is most often found at the "wet end" of the machine, which is the area where the mixing of pulp and water takes place, and also the sheet forming wire upon which a thin, even layer of pulp and water are deposited in preparation for the final draining, pressing and drying. However, results of a heavily fouled system often manifest themselves after this stage while the sheet is traversing the series of rolling drums designed to dry, press and treat the paper, as well as the final product (25, 35).

Paper breaks tend to occur while the sheet is being fed over and under the series of moving drums where the sheet is being subjected to stress. Such breaks can be caused

by weak points in the paper created as a result of prior deposition of slime onto the wire during initial sheet formation. Under tension a sufficiently large weak spot is liable to give way resulting in a break. An incomplete jumbo roll (the final roll of paper) has to be repulped and the machine must be rethreaded once the torn paper is removed (4, 30, 42, 43, 61).

In addition to paper breaks caused by slime being deposited onto the sheet, other undesirable qualities such as colour marking and transparent patches or "windows" could be caused by smaller slime deposits on the sheet (43).

1.2.1 Organisms Commonly Encountered in the Mill

Microorganisms were implicated as a causative agent of slimes in paper mills as early as 1931 (4). Much work has since been done by Sanborn (51, 52, 53, 54, 55, 56).

Fungi, yeasts, moulds and bacteria have been reported to contribute to slime buildup in the mill (12, 42, 44, 52, 55, 56). Algae and protozoans, although occasionally associated with slimes, appear to be of little significance (58).

Sanborn (50) has divided the types of slime formers into the following groups:

a)mucoid types

b)fermentation groups

c)other viscous groups
d)spore-forming bacteria
e)filamentous bacteria
f)yeast-like fungi
g)mould or thread fungi

Important genera which contribute to slime formation are Aerobacter (formerly the genus assigned to any organism classified as a member of either Klebsiella, Enterobacter or Serratia), Paracolabactrum (fermenters), Flavobacterium, Xanthomonas, Achromobacter, Pseudomonas and Micrococcus (viscous growth), Streptomyces and the iron bacteria Leptothrix, Sphaerotilus, Gallionella and Beggiatoa (filamentous). Cytospora and Monilia (yeast-like fungi) and Aspergillus, Penicillium, Trichoderma, Cladosporium, Mucor and Thamnidium, all of which are classified as mould or thread-like fungi are also found (55).

The putrefactive bacteria, notably Proteus, Pseudomonas, Alcaligenes, Flavobacterium, Bacillus and Clostridium are also often encountered in the mill or have been reported as important slime formers (55). Apart from the degradation of the various components used in paper making, they could also cause undesirable odours in the paper. The putrefactive bacteria are introduced into the system via putrefying materials and by water, soil and sewage.

Eveleigh and Brewer (17) working on a Canadian paper

mill, reported the slime to be predominantly fungal in nature. The following five genera were dominant: Nectria, Sporotrichum, Geotrichum, Phialophora and Trichosporon. Pseudomonas, Aerobacter, Flavobacterium and Bacillus were commonly encountered bacterial genera, the pseudomonads always outnumbering the other genera.

Work by Nason et.al.(42) showed slimes in an Ontario sulphite pulping mill (sulphite pulping being the predecessor to sulphate pulping now currently widely used in many paper mills) to be predominantly bacterial in nature, particularly in totally submerged areas. Aerobacter, Escherichia, Cellulomonas, Chromobacter, Flavobacterium, Bacillus and Micrococcus featured as prominent slime formers. The large slime capsule elaborated by Aerobacter made it a more troublesome organism than Escherichia which has a comparitively small capsule. This work showed fungi to prefer environments that are moist but not aqueous. Fungi have also been reported to favour aerobic conditions in a report by Coster (12).

From their study Nason et. al. (42) concluded that aerobes were the most important slime formers. In the light of the results obtained from their study however, it would possibly be more appropriate to include the facultative anaerobes as 85% of the organisms cited fall into this category (12, 17, 54, 56).

Direct microscopical staining of slimes from a Californian

paper mill led Beckwith (4) to the conclusion that the slime problem was caused by gram negative bacteria rather than fungi which generally appeared to be sparse and degenerating. Yeasts were only found occasionally.

Ultimately the major microorganisms present in a system would be dependant on the type of mill (12). Groundwood and strawboard systems are particularly rich in many nutrient types while sulphite systems are relatively poor in nutrient. The exact location of the slime in the system would also tend to influence the nature of the organisms in the slime to some degree.

1.3 Bacterial Attachment

The attachment of microorganisms is a common phenomenon in natural environments (13, 19, 40, 68). Slime formers tend to aggregate, often attaching themselves to solid objects (24). To become attached to a surface the microorganism, or group thereof, must not only be capable of anchoring itself but it must either present or be presented to the surface in such a way that attachment can be accomplished (10). Motility is a manifestation of the chemotactic response. A solid surface at which a nutrient concentration exists (see section 1.1) would thus represent an area which would elicit a positive chemotactic response in bacteria. It would therefore be expected to move towards the surface. Attraction of non-motile organisms would be accomplished via a physicochemical mechanism (39).

The initial binding of bacteria to a surface is characteristically a loose, reversible association. The negative and positive forces are thought to counteract one another thus initiating a state of equilibrium (40). The formation of a permanent, irreversible bond follows, attributed largely to the production of extracellular polysaccharide by the organisms (8, 19,40 , 68). The delay between reversible and irreversible bonding is thought to be the time required by the microorganisms to synthesize polysaccharide (68).

1.3.1 Attachment Position

Work by Marshall et. al. (40) on one motile and one non-motile marine rod-shaped organism showed a non-random attachment configuration ng a cine camera distinct patterns were viewed 1)

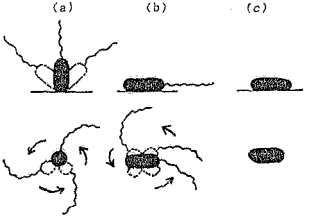


Figure 1 : Diagrammatic representation of the reversible sorption of Pseudomonas R3 to a glass surface (a) and (b) illustrate the rotational movements of motile bacteria in an edge-to-face and a face-to-face manner respectively; (c) face-to-face sorption of non-motile bacteria. (from Marshall, K. C., Stout, R. and Mitchell, R, (1971). J. Gen. Microbiol. 68: pp 337-348)

1.3.2 Factors Influencing Attachment

Interactions between microbial extracellular polymers and solid surfaces are dependant upon the following factors:

a)Temperature - it has been observed that the strength of adhesion may decrease with an increase in temperature (19). Since the optimum growth temperature is often higher than for polysaccharide production, it is likely that at higher temperatures more energy is utilized in the actual replication process rather than the production of secondary metabolites such as extracellular polysaccharide.

b)Electrolyte Concentration - at higher electrolyte concentrations a secondary minimum or "attractive trough" is experienced. This was shown by Marshall et. al. (39) working on Achromobacter. Increased repulsive forces were observed when the electrolyte concentration decreased.

c)Particle Size and Concentration - this factor is a function of the total available surface area. There exists a linear relationship between the optimal polymer concentration and the total surface area (27).

d)Intensity and Time of Agitation - an increased intensity of agitation will decrease the amount of polymer adsorbed, although prolonged agitation times had little effect (27).

e)Polymer Concentration and Configuration - chain length and molecular mass have been found to be important factors (27). Since it is the chains themselves which make contact with the surface, length is an advantage. Lower molecular mass polymers have been found to bind more efficiently to surfaces of opposite charge, possibly by reduction of repulsive forces (27).

f)pH and Ionic Properties of the Solution - factors involving adhesion influenced by pH include surface potential, charge, nature of the double layer, the charge and the charge density of the polymer, the polymer elongation and solubility and the extent of complex formation (19,27).

1.3.3 Modes of Attachment and Floc Formation

Generally microbial cells carry a net negative charge which would tend to repel one cell from another. This force is however screened to a large extent by ions in the separating medium. Secondly, Van Der Waal's forces are attractive and function over larger distances. Therefore, the only repulsion between any two cells would be experienced once cells reached a distance equal to the secondary minimum (see fig. 2) (5).

The elaboration of extracellular polysaccharides by some fungi, algae and bacteria (10, 22,63) serves to bind the cells together in a mass but keep them spatially apart (13,33). Slime aggregates are thus formed.

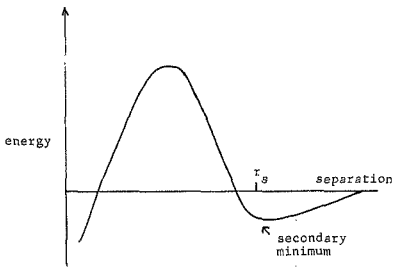


Figure 2 : Energy due to electrostatic and electrodynamic forces plotted against the separation between the two cells. (from Bell, G. I. (1978). Science 12: pp 618-627.).

Extracellular polysaccharides are those which exist free in the medium as opposed to a capsule which remains bound to the cell. Extracellular polysaccharides have been found to be dominantly carbohydrate, the exact chemical composition varying from species to species and in some cases with change in environment (13, 22, 66).

The polysaccharides exist as a mat of fibres radiating outwards which may link with fibres produced by other cells or trap floating debri. Fungal hyphae also serve as a network to which flocs could adhere. Extracellular polysaccharides facilitate both cell to cell and cell to surface adherance (22). Binding must therefore be non-specific. Production of extracellular polysaccharide is particularly prevalent in the stationary phase (13).

Examples of microorganisms which produce extracellular polysaccharides include Xanthomonas campestris (65), Bacillus circulans, Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa (8), Streptococcus mutans (1), Anabaena flos-aquae, Geotrichia echinulata and Oscillatoria rubescens (38).

Bacterial fimbriae are also thought to induce flocs (45). The major difference between the extracellular polysaccharides and fimbriae is their composition - polysaccharide in the case of the former and protein in the case of the latter. Work by Duguid (16) showed bacteria to possess proteinaceous fibrils which adsorb onto cellulose fibrils. Opperman and Wolfson (45) showed 83% of paper mill slimes to harbour at least one fimbriated species.

Fimbriae have also been related to the adhesive properties of a Klebsiella strain, a genus frequently associated with slimes (9).

The production of stalks and holdfasts is a fairly common method of bacterial attachment (19, 68). The ability to lead a sessile existence is advantageous in that it does not necessarily involve the production of slime and is therefore energy efficient. Gallionella is an example of a stalked bacterium (47).

It has been suggested that certain bacteria are capable of more efficient colonization. Work on oral Streptococcus

mutans (24) has shown that a prerequisite for firm attachment of this organism to the tooth surface is the de novo synthesis of extracellular polysaccharide from sucrose. Prior to sufficient polysaccharide being produced, the organisms are weakly and reversibly bound to the tooth surface.

Although the oral surface is more complicated than a concrete wall or metal vessel in terms of specific character and antigenicity, it is also possible that certain slime formers are better adapted to initiation of colonization of a solid surface. This could be a manifestation of the different extracellular polysaccharides. The phenomenon of microbial succession could be partly due to this factor (10, 49).

The concrete or metal walls in a paper machine provide a large surface area for attachment. The roughness of finish of a concrete wall would provide an immense colonizable surface area. Some protection from shear force is also afforded as a function of the uneven surface (10). The slime mass itself provides the individual organism with some degree of protection from the shear force.

1.3.4 Advantages and Disadvantages of Communal and Solitary Existence

Life in a floc holds a number of advantages for the individual organism. Flocs facilitate attachment to solid surfaces which improves the individual's chances of survival (27, 68). The extracellular polysaccharide fibres can function as

a food reservoir (11). Since many nutrients are positively charged, the negatively charged fibres would bind, maintain and possibly even concentrate these nutrients (13, 22, 24). In addition, where byproducts of the metabolism of one organism are useful to another, efficient transfer of nutrients is accomplished by the mass. Symbiotic relationships are thus promoted.

The polysaccharide capsule also offers resistance to phagocytosis, amoebic attack and bacteriophage endotoxins and aggressins (66).

Finally, the mass affords some degree of protection against dessication (22, 66). This is an important consideration in areas where water levels might fluctuate.

Life in close proximity increases competition, but since microorganisms have the choice whether or not to aggregate, aggregated existence must offer some overriding advantage. This might well occur in nutrient poor environments where a competitive relationship is at least an improvement on a solitary existence with an extremely low nutrient concentration.

In rapidly flowing waters, the possibility of cells being dislodged and washed away is greater for a large mass than for an individual organism. This is an important consideration in rivers with erratic flow rates. Water flow rates in paper mills tend to be fairly constant.

1.3.5 Chemical Composition of the Cell Wall Polysaccharide

The are five major groups of bacterial extracellular homopolysaccharide. Thes re cellulose, hyaluronic acid, starch, dextran and levan, the latter two groups being found exclusively in bacteria (66). The individual carbohydrate residues such as carboxyl groups and amino groups determine the environmentally important properties of the polysaccharide. Thus, the presence of many uronic acid substitutions would create an acid polysaccharide (22).

Chemical side groups of the heteropolysaccharides have been found to be partially responsible for conferring the antigenic specificity on certain microorganisms. The relationship to which in most cases, however, unclear (66).

To date specific antigenicity as a direct function of an exopolysaccharide has been reported for a number of gram negative bacteria, notably the Enterobacteriaceae. These organisms possess two main types of antigentic polysaccharide, namely the somatic (O) antigen and the capsular (K) antigen. Whether the gram positive bacteria possess polysaccharides with such specificity is as yet unknown (66).

1.4 Microbial Ecology and Relationships

In any ecological microniche a delicate balance exists between the constituent populations (62). Relationships range from total dependance to total independance. In all cases a change in any outside factor creates

instability which results in either minor readjustment of the community, or in the extreme, collapse and complete change in community character - i.e.,recolonization by other species.

In any habitat one would expect to find an assortment of r and k strategists, the r-strategists being those which, in the face of adverse conditions adapt to withstand the impending death and the k-strategists being those which are eliminated and recolonize a new habitat (36). These terms are analogous to the autochthonous and allochthonous populations respectively. The spore-formers Bacillus and Clostridium, as a result of their tendency to produce spores in the face of adverse conditions, are k-strategists. Spores are an adaptation for survival, not growth. Escherichia coli is an example of both an r and a k strategist (36).

In any habitat where focal points of microbial growth occur such as a paper mill, closer relationships would be expected to be formed between the long term survivors -i.e.,the r-strategists. While it is possibly incorrect to classify the k-strategists as transients, their lack of permanence in comparison with the r-strategists does not serve to make them attractive prospects for long-term dependant relationships. However, because the k-strategists are present more often than not, relationships between k-strategist and k-strategist and k-strategist and r-strategist might be expected, though the relationships

would be expected to be of a relatively loose nature to facilitate the survival of the individual species. A closely dependant relationship could result in periodic death of not one but two species, thereby nullifying any benefit derived from such an association.

The associated growth of microorganisms has been classified as follows (3):

a)synergistic
b)mutualistic symbiotic
c)antagonistic symbiotic
d)commensalistic

In nature numerous examples of each of the above relationships exist. As such, it is likely that such relationships would be important in the development and succession of paper mill slime.

Work by Eveleigh and Brewer (18) has shown that selected slime formers did not grow in a liquid basal medium unless supplemented with thiamine, biotin or calcium pantothenate or a combination thereof. These organisms include *Flavobacterium lactis* (thiamine and biotin requirement), Brevibacterium sp (thiamine and biotin requirement) and Phialophora fastigiata, Cephalosporium sp and Trichsporon pullulans all of which had a requirement for biotin.

Biotin, thiamine and calcium pantothenate are byproducts of bacterial metabolism. This could therefore be an

indication of a symbiotic relationship between these organisms and the producers of these growth substrates.

Aerobacter aerogenes has been found growing in intimate association with Pullularia pullulans and Oospora sp where stable growth of these organisms as well as rapid proliferation results. Cladosporium has also been reported to grow in close association with certain bacteria (51).

An example of two bacteria living in close association is that of Aerobacter aerogenes and Brevibacterium brunneum. Together these organisms form a "lumpy, oyster-like slime" (51).

A more complex situation involves three different fungi, Monilia candida, Pullularia pullulans and Aerostalagmus cinnabarinus. This interrelationship produces rubbery, elongated bodies (52).

Antagonistic relationships between microorganisms is an aspect of paper mill microbiology that has received little attention. Such relationships are referred to by Wimpenny (67) as ammensalism. The production of antibiotics, fermentation products and metabolic heat are cited as factors which could adversely affect the growth of other organisms. In a situation where organisms are living in close proximity the effect of the production by one

organism of an inhibitory metabolite would be greater than if the organisms were in a dispersed state. Thus, less of the metabolite would be required to effect a change. However, in a situation where the organisms are deriving more benefit from the closer association thereby being able to utilize more energy for replication, to spend time producing inhibitory substances might be self defeating Secondly, although the concentration of such a secondary metabolite required to inhibit growth of others is low, if an organism did embark on such an excercise, it would have to overcome the ever increasing numbers of other organisms it proposes to inhibit.

Production of antibiotics by fungi is not a problem which would be encountered in a submerged area. The fungi in such a situation are either dead or dormant.

Fermentation products such as acids and gas, if in sufficient quantity, could serve to alter the entire microbial environment. The greatest effect of fermentation would be the lowering of the pH which, in due course, would halt the growth of the fermentor itself. A self-limiting action yes, but particularly advantageous if carried out by a spore-former which could then sporulate and lie dormant until conditions become favourable.

1.5 Physical Conditions in the Mill and Slime Control

In its simplest form slime control can be partly achieved by alteration of controlling physical parameters. Redox

potential, pH, temperature and nutrient concentration are major factors in determination of ecological flora in the mill (7). Fluctuations in any of the above factors occur from time to time and microorganisms which are capable of adjustment to periodic changes are at a selective advantage over those which cannot. Many of the paper mill slime formers are commonly detected in a wide range of environmental habitats which indicates that they are capable of growth under a variety of environmental conditions.

Temperature has been shown to directly influence 80% of bacterial density fluctuations in paper mill process water (64). This effect was seen particularly where temperatures were altered to just above or just below the microbial growth maxima or minima respectively.

Elevating paper machine temperature has been previously proposed as a method by which bacterial numbers could be controlled (14, 52). Continuous and not sporadic heating would achieve the desired effect although temperatures in excess of 60°C would be desirable since many organisms were found to be capable of efficient growth at the comparitively high mill functioning temperatures - i.e., 40°C and over.

The pH of the machine would be expected to influence the overall flora in the system. Under acid sizing conditions fungi and yeasts are expected to dominate and indeed

in South African paper mills, dithiocarbamate slimicides, which are primarily effective against these organisms are used. An alkaline sized system would be expected to support growth of a wider range of bacteria.

Despite lower pHs being less favourable to bacterial growth, the occurrence of bacterial slime in an acid sized machine is not uncommon. The reason could be attributed to one of two factors: the pH is sufficiently high to permit growth of bacteria which do not have absolute growth limits with regard to pH; or the protection afforded to the cell by the slime mass might be sufficient to buffer the effects of lower pH. In a more dispersed state a low pH could have an adverse effect on the individual organism.

The pH was neverthless found to be a significant regulator of bacterial numbers in process water (64). Increases in pH caused increases in numbers of pseudomonads and Klebsiella pneumoniae.

Redox potential fluctuations are expected to affect only obligate aerobes and anaerobes. Facultative anaerobes could be excluded from this category since their physiological character allows them to survive under both aerobic and anaerobic conditions. Uneven aeration of process water resulting in anaerobic pockets is a problem common to much machinery involved in a water requiring process, particularly older machinery. As such, redox

potential in one machine could vary quite significantly from area to area and indeed the routine occurrence of all three types of microorganism in any one mill is proof that this situation exists. If it were possible to maintain a machine at either a very low or a very high redox potential the growth of aerobes or anaerobes respectively could be controlled. However, unless a specific problem is encountered eg. fermentation (56), little would be achieved by such an excercise since much of the slime problem is caused by facultative anaerobes (12, 17, 54, 56). Thus, redox potential plays a role inasmuch as it determines the location of certain organisms, but should not be a prime consideration to combat a slime problem.

Nutrients are generally freely available in a paper mill (58), although the type of nutrient being dependant upon the process, may be a controlling factor. Nitrogen is however limited, resulting in a promotion of polysaccharide production (10)..

1.5.1 Slimicides

An effective slime control programme does not merely involve the addition of a slimicide. The following are important considerations for slimicide usage (41).

a) If practical, alteration of machine conditions to make them less favourable to microbial growth.

b) An effective cleanliness and washup programme.

c) Coordinated use of additives.

d) Correct choice of biocide and correct application.

Slimicides fall into two categories viz the bacteriostatic agents and the bacteriocidal agents. The former slow down growth of microorganisms but do not kill them and the latter kill microorganisms. In low concentrations the effect of a bacteriocide might approximate the action of a bacteriostat. For most situations a bacteriostat is sufficient, but should a specific problem arise a bacteriocide is often preferable.

The requirements of a good slimicide are as follows (12,41):

a)Proven track record - with the advent of new slimicides and improved experimental testing this point becomes a little irrelevant, although for the most part it still applies.

b)Compatibility with other machine additives and other biocides.

c)Low mammalian toxicity as paper products might be used in food packaging.

d)If possible, biocides should display complementary action.

e)Acceptable odour as bad odours might find their way into the final product.

f)Stable and readily dispersable.

g)No undesirable side effects.

h)Non-hazardous to handle.

i)Competitive price.

Until the mid sixties the most widely used slimicides were the toxicants based on the salts of heavy metals, chlorinated phenols and chlorine (61). Although chlorine is still

in use today, it has a number of disadvantages. Firstly, chlorine cannot be used except in conjunction with chlorine fast dyes when coloured paper is being produced. Secondly, chloramine tends to pit brass chute wires (57), and thirdly and most recently some organisms have been found to be resistant to chlorine (23,46). However, a well chlorinated fresh water supply is still recognized as being an important prerequisite for smooth paper machine functioning (53).

FDA legislation has reduced the use of heavy metal toxicants (61). Although effective, mammalian toxicity levels are high posing problems both in handling and in the manufacture of paper used for food packaging.

Organo-sulphurs and quaternary ammonium compounds were introduced in the mid sixties, followed shortly afterwards by organohalogens. Chlorinated phenols were, however, still used as a result of their success as fungicides (61).

By 1974 blends of slimicides, solvents and dispersants were being marketed. It had been found that to effectively reduce or control slime, the constituents should be in *a dispersed state. The dispersants serve a dual purpose by* not only aiding in dispersal of the slime masses, but by maintaining potential slime formers in a dispersed state (58). The major slimicides in use were polychlorinated phenates and phenols, methylene bisthiocyanate, sodium dithiocarbamates and bis trichloromethyl sulphone (58, 61).

Slimicides in use today are, with a few refinements, much the same as those used in the seventies. Blends of dithiocarbamates and thiocyanates are popularly used.

A breakthrough in slimicide technology has taken the form of an enzyme said to break down the polysaccharide capsule thereby dispersing the organisms in the slime (20, 28 , 32).

Based on the finding that most bacterial slimes in the paper mill are composed largely of levan (20), an enzyme capable of breakdown of this homopolymer was sought. Although Economics Laboratory Inc., the firm which has developed this product, have not divulged the methods employed, it is clear that they have developed a method of rapidly producing this enzyme.

The enzyme preparation is being marketed under the name EDC-1 by Soilax Ltd. and is currently being successfully used in some Scandinavian countries and in some North American paper mills.

The use of an enzyme as opposed to a chemical substance holds a number of advantages (20):

a)Enzymes are specific in their action.

b)Enzymes have no effect on the action of dispersants, fillers, defoamers, drainage and retention aids or any other paper machine additive.

c)They are environmentally safe.

d)They are non-corrosive.

e)They do not emit dangerous fumes.

f)They are not persistent (32).

1.6 Aims

The major goal of this project is to make a contribution to the elucidation of the ecology of paper mill slime forming organisms, their origins and buildup by studying the operating conditions and environment of a specific paper machine. Further, the project has been targeted at increasing the understanding and knowledge of the effects of slimicide usage with the objective of formulating some recommendations which may lead to a more efficient slimicide dosage and formulation.

2. METHODS AND MATERIALS

2.1 Machinery and Operating Conditions

The paper machine selected for the purposes of this work was used exclusively for the production of fine paper, typically copy paper and bond. It runs under acid conditions producing over 100 000 kg per day. The machine is shut down for monthly maintenance checks and repair.

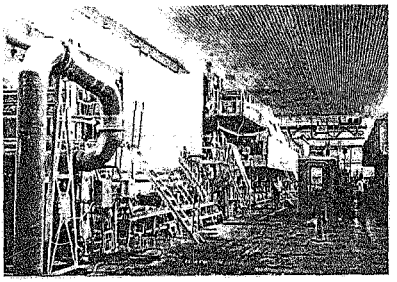


Figure 3 : Paper machine 3. Sheet-forming wire and felt displayed in foreground.



Figure 4 : Paper machine 3. Completed "jumbo rolls" and another in the process of being completed displayed in the foreground

2.2 Raw Materials

Principal raw materials are pine, wattle and broke (paper repulped as a result of a paper break) pulps and water. The water is mainly treated sewage water, together with small amounts of potable water. Spent (process) water is recycled after treatment flocculation and removal of fibre.

Other raw materials include bleaches, sizes, slimicides, starch, fillers and defoamers.

The quantities of these products used are very small relative to volumes of pulp and water used. Starch is however, a good substrate for microbial growth. The nature and mix of the minor raw materials used depends on the exact type of paper to be produced.

2.3 Sampling

Although paper machine 3 (PM 3), the machine selected for this work, presents the mill with no exceptional problems in terms of slime accumulation, slime buildups do occur. Depending on location and/or size of the slime deposit, they may or may not ultimately contribute to a slime break.

Investigation of previous records of total bacterial counts together with physical investigation of the machine showed the machine thickener walls to be an area where slime regularly accumulated (see fig. 5). Thus a small extension of the thickener tank was chosen as the sampling site because of its suitability, ease of access and safety (see fig. 6).

By virtue of the large volumes of pulp and water used in paper production, it would be expected that they contribute significantly to microbial inoculation of the system. In addition, because of the nature of these raw materials both in terms of availability and in range of organic matter, they would be expected to be capable of supporting the growth of a wide range of microorganisms.

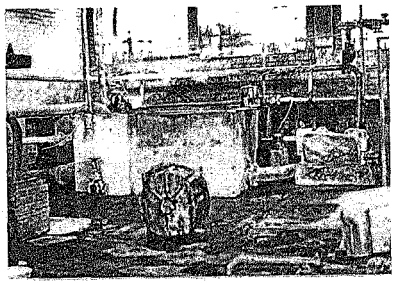


Figure 5 : PM 3 thickener tank.

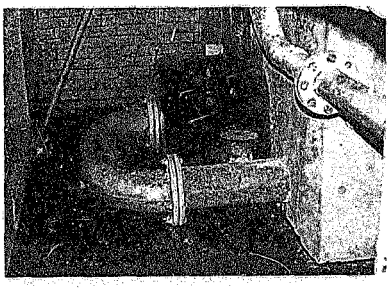


Figure 6 : Small extension of PM 3 thickener tank where samples were taken.

All major raw materials were sampled twice, at the same time of day, in duplicate.

Though it is unlikely that raw materials such as dyes, bleaches and defoamers are sterile, most contain only small amounts of organic matter. Organisms with exacting nutritional requirements growing on these raw materials would therefore be expected to die or persist in low numbers once inoculated into the system.

Mixed sour smelling starch, healthy starch, and both raw potato and corn starches were sampled as above for major raw materials.

All samples were taken in sterile refrigerated glass McCartney bottles. They were stored and transported on ice, time between sampling and treatment being approximately 60 minutes.

2.4 Culture

Based upon results obtained from a study to identify major slime forming genera in a variety of South African paper mills (Harris, I. A., unpublished results) the following agar media were chosen for culture of PM 3 slime formers :

a)Tryptone Glucose Yeast Extract Agar (PCA)

b)Nutrient Agar (NA)

c)Sabouraud Dextrose Agar (SDA)

d)Dextrose Tryptone Agar (DTA)

e)Pseudomonas Aeromonas Agar (PAA)

One gram or one millilitre quantities, whichever applicable, of samples were measured and diluted in sterile distilled water, or in sterile Ringer's solution in the case of the successional analysis. Aliquots (0,1ml) of these dilutions were applied and spread onto appropriate media using a flamed glass spreader. Each slime sample was inoculated onto all of the above media. Samples of starch mixture were plated onto PCA and onto Brewers Anaerobic Agar (see appendix) and samples of the raw corn and potato starches were applied to corn starch agar plates and potato starch agar plates respectively (see appendix), in addition to being inoculated onto Brewers Anaerobic Agar plates.

Slime samples, including those in the successional analysis, were incubated at 30°C for 48 hours. Although this temperature might not have been conducive to growth of some organisms present in the slime, it represented the average mill temperature and this should encourage prolific growth of the major slime formers in the machine. For this reason this temperature was also used for culture of organisms in the pulps.

Plates inoculated with samples of the starch mixture were incubated for 48 hours at 37°C, 39°C and 55°C in aerobic and anaerobic environments. Since the temperature of the starch mixture in the mixing tank is consistently higher than that of the actual paper machine, higher temperatures were used.

2.5 Isolation and Identification

The inoculated agar plates were assessed from visual inspection and counting of colonies for the numerically significant organisms.

2.5.1 Pure Cultures

Samples of colonies were streaked onto fresh agar media. In addition smears of colonies were prepared and gram stained to provide verification of pure cultures.

2.5.2 Gram Stain

The method was used as described by Salle (50).

2.5.3 Oxygen Relations

Samples of pure cultures were plated in triplicate onto the media from which they were originally isolated and incubated in a Forma Scientific Anaerobic Cabinet model 1024. Slime samples were incubated for 48 hours. In addition, duplicate plates were incubated in a candle extinction jar for 48 hours. Temperatures of incubation were the same as was used for aerobic culture.

2.5.4 Motility

Unstained wet mounts of each culture were viewed under oil immersion at a magnification of 1000 x.

2.5.5 Catalase Test

Hydrogen peroxide was applied dropwise, using a pasteur pipette, to growth on the agar plate and observed for effervescence.

2.5.6 Oxidase Test

A 1% solution of tetramethyl-p-phenylene diamine dihydrochlorine was prepared in glass distilled water. Loopfuls of culture were smeared onto a drop of the above solution on filter paper and observed for the formation of a purple colour.

2.5.7 Metabolism

Carbohydrate fermentation media were prepared using commercially prepared purple broth base. Organisms were inoculated into test tubes of these media and incubated at 30°C for 48 hours after which tubes were observed for acid and gas production.

2.5.8 Fluorescent Pigments

Plate cultures of organisms were placed under ultra violet light and growth was observed for fluorescent pigment.

2.5.9 Citrate Utilization

Commercially prepared Koser's citrate medium was reconstituted and dispensed into test tubes for sterilization. After inoculation they were incubated for 3 days at 30°C, after which tubes were inspected to assess whether any change in indicator colour had occurred.

2.5.10 Methyl Red Test

Method as described by Salle (50).

2.5.11 Voges-Proskauer Test

Method as described by Salle (50).

2.5.12 Indole Production

Method as described by Salle (50).

2.5.13 pH of the Starch Mixture

pH was measured using a Metrohm Herisau pH meter.

2.5.14 Gas Chromatography

Where appropriate, 20ml samples of raw material were centrifuged at 3000 g for 15 minutes to remove particulate matter. Samples (5µl) were analysed for the presence of volatile fatty acids on a PYE Unicam gas chromatograph using a Chromosorb 101 column, 1,8m long and with a 3mm internal diameter. Injector temperature was 220°C.

2.6 Succession of Microorganisms

2.6.1 Sampling

Slime deposits tend to attach themselves to and increase in size on solid surfaces. The walls of the machine thickener tank are naked rough plaster and a simulated medium was achieved by the use of unglazed ceramic tiles.

Ceramic tiles 53mm x 53mm x 5mm with a small hole to facilitate wire attachment were used. The tiles together with affixed wire were flame sterilized and submerged in the thickener tank suspended on the wires which were anchored to the edge of the tank. Over the month (from one washup to the next) tile were

removed at the following times - 48 hours, 5, 9, 16 and 29 days. In each case a tile was placed in 50 ml quarter strength Ringer's solution at 4°C. Beakers containing the tiles were transported in a cool polystyrene box.

The low temperature of the Ringer's solution facilitated detachment of the biofilm and shaking of the vessel served to disperse the organisms to form a suspension.

2.7 Microbial Interrelationships

Three different methods were used to assess potential microbial relationships. Two of these methods were found to be reliable indicators but the third method failed to produce accurate results.

2.7.1 Method 1 - Parallel Streaking

Prior experimentation with Bacillus, Staphylococcus and Micrococcus showed this method to give a good qualitative indication of a synergistic relationship between two organisms.

The method involves making single parallel streaks of two different organisms on the nutrient medium. The streaks were less than 6 mm apart. Single streaks of the organisms at the sides of the plate served as controls.

PCA plates were used as the nutrient medium. After inoculation these plates were inverted and incubated at 30°C for 8 days. After growth had occurred the plates were

examined for any possible dependance or synergism.

2.7.2 Method 2 - Filled Wells

This method was undertaken as verification for method 1.

Using overnight broth cultures of the major slime formers, seeded agar plates (using PCA and NA plates) were made. A sterile cork borer was used to cut holes in the seeded agar and the wells were filled with aliquots of broth culture of the different organisms. Sterile distilled water was placed in some holes to serve as the controls.

These plates were incubated in an upright position at 30°C for 8 days. Plates were visually assessed for growth.

2.7.3 Method 3 - Agar Discs

This method was proposed by Kovrov et. al. (37).

Using glass rings approximately 1 cm high and with a diameter not exceeding the size of the petri dish used. NA or PCA is poured to a maximum thickness of 2 mm. The discs are allowed to solidify and the rings removed. The discs are picked up (plastic gas sterilized knives were used for this purpose) and placed in the centre of a NA or PCA plate.

Fifty µl of a 24 hour broth culture of each organism to be tested (the test culture in this context) was inoculated onto the agar disc and spread using a flamed glass spreader.

These plates were incubated for 10 days at $30^{o}C$ in an upright position.

After incubation the agar discs with microbial growth were removed (a sterile plastic knife again used for this purpose). Point inoculation using broth cultures of each microorganism were carried out in the area previously occupied by the disc. Plates not previously treated with microorganism were also point inoculated to serve as controls. These plates were incubated in an inverted position at $30^{o}C$ for 4 days.

2.8 Effect of Slimicide on Bacterial Growth

One of the slimicides commonly used in the mill is a dithiocarbamate, which is primarily effective against fungi, but possess bacteriostatic qualities. The concentrations of slimicide used in the mill are recommended on the basis of the minimum concentration which gives favourable results with respect to slime accumulation under normal running conditions. As such, the effects on microbial growth of the maximum administered dose and some lower doses were assessed.

2.8.1 Construction of Growth Curves

Samples of nutrient broth (100 ml) in Erlenmeyer flasks (250 ml) were inoculated with 1 ml of overnight broth cultures containing 10^{6} organisms per ml of the four major slime formers.

The following slimicide concentrations were tested: 200 ppm, 100 ppm, 50 ppm and 20 ppm. The recommended dosage for the paper machine was 200 ppm.

Uninoculated flasks with the appropriate concentrations of slimicide served as controls. This was necessary since the slimicide tended to make the media cloudy.

All flasks were incubated at 30°C at 100 rpm. Hourly absorbance readings were taken for up to 8 hours for the experimental flasks with 200 ppm slimicide concentrations. Readings were taken for up to 16 hours for the three lower concentrations. All readings were taken on a Corning colorimeter using a green filter.

2.9 *Photography*

Photographs were taken with a Fujica St 605 camera using an Agfa 100 ASA film. Processing was done by a commercial photographic laboratory.

3. RESULTS

3.1 Major Organisms in the Slime

3.1.1 Total and Selective Counts

Preliminary experiments revealed that a 10^{-4} dilution gave countable colonies. Subsequently total viable bacterial counts on both NA and selective media were carried out. While solid agar media such as NA or any enrichment media have been reported to give lower total counts than using direct counting methods, notwithstanding a number of inactive cells, it was decided that provided the same method of counting was applied throughout, this would be satisfactory when considered on a comparative basis. Secondly, raw process water on microscopic examination reveals a tangled network of fibre which makes counting difficult. Thirdly, the standard plate count is the method employed by the industrial organisations concerned with microbiological quality control in paper mills, and finally, it also allows for rapid and simple isolation of microorganisms for identification purposes.

Aqueous samples were taken at approximately the same time in the morning from the machine standpipe, a well aerated, open vessel. Samples were all taken from a depth of 10 cm from the middle of the tank. This represented an area which could be reached relatively easily and where samples could be taken with the minimum delay and without having to employ the use of old unwashed sampling buckets. Contamination could therefore be kept to a minimum.

Medium	Bacterial Count day 1.	Bacterial Count day 2.	Bacterial Count day 3	Mean	Standard Deviation	Organism Code	Mean Individual Count	% Total
NA	72 x 10^4 214 x 10^4 33 x 10^4	49 x 10^4 102 x 10^4	113 x 10^4 110 x 10^4	99 x 10^4	59 x 10^4	1	46 x 10^4	46,5
PAA	143 x 10^5 98 x 10^5 66 x 10^5	203 x 10^5 32 x 10^5	241 x 10^5 169 x 10^5	136 x 10^5	27,7 x 10^4	2	32 x 10^5	25,5
SDA	[illegible] 16[illegible] x [illegible] 243 x [illegible]	211 x 10^3 256 x 10^3	59 x 10^4 155 x 10^3	296 x 10^3	283 x 10^4	3	57 x 10^3	19,3
NA	72 x 10^4 214 x 10^4 33 x 10^4	49 x 10^4 102 x 10^4	113 x 10^4 110 x 10^4	99 x 10^4	59 x 10^4	4	97 x 10^3	9,8

Table 1 : Total and selective counts of slime formers in PM3 process waters. Process water was sampled on three consecutive days in triplicate in the first instance and in cuplicate on the second and third days. Selective counts are the mean counted of samples obtained on day 1.

Organism number / Characteristic	1	2	3	4
Shape and arrangement	fat rods in pairs	rods in pairs	small rods	small, fat rods
Gram reaction	+	-	-	-
Motility	+	+	+	+
Respiration	F	A	F	F
Catalase	+	+	+	+
Oxidase	+	+	-	+
Fluorescent pigment	-	+	-	-
Spore	+	-	-	-
Glucose fermentation	ND	ND	ND	acid
Arabinose	ND	ND	+	+ +
Sucrose	ND	ND	+	ND
Indole	ND	ND	+	ND
Methyl Red	ND	ND	+	ND
Voges-Proskauer	ND	ND	-	ND
Citrate	ND	ND	+	+
Average size	1,1 by 4	0,8 by 2,6	0,9 by 2,3	1,1 by 3,4

Table 2 : Characteristics of isolates from PM 3.
(F - facultatively anaerobic; A - obligate aerobe)

For the purposes of total counts both on NA and on selective media, sampling was carried out on three consecutive days, three times in the first instance and twice each on the second and third days. Samples from day one were plated out in triplicate and those from days two and three in duplicate. Table 1 gives the mean numbers of organisms obtained from the counting procedure.

The fact that similar results were obtained in this study to the preliminary experiments six months earlier may suggest that the bacteria themselves do not fluctuate significantly.

On the first day of sampling numerically significant colony types were ascertained and subsequently cultured and identified.

The characteristics of the four numerically significant organisms suggest that the isolates were Bacillus, Pseudomonas, Citrobacter and Aeromonas respectively (see Table 2 and figs. 7 - 10).

3.2 Raw Materials

3.2.1 Sewage and Potable Waters

The sewage could not be sampled independantly, therefore samples of the sewage and potable water mixture were taken. The water mixing tank served as the sampling site with samples being taken at a depth of 10 - 15 cm. This source was sampled twice in October giving a two week

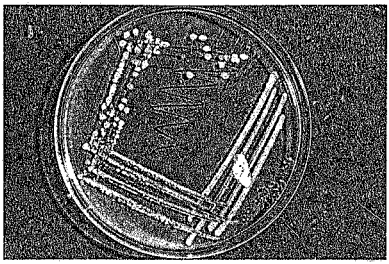


Figure 7 : Colonies of Bacillus on Nutrient Agar.

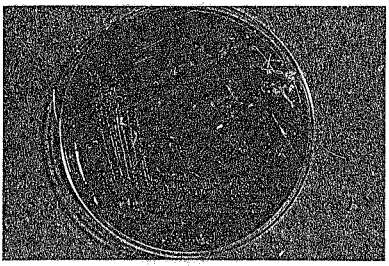


Figure 8 : Colonies of Pseudomonas on Pseudomonas Aeromonas Agar.

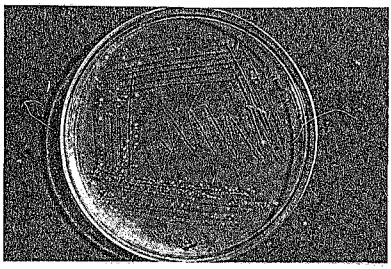


Figure 9 : Colonies of Citrobacter on Sabouraud Dextrose Agar.

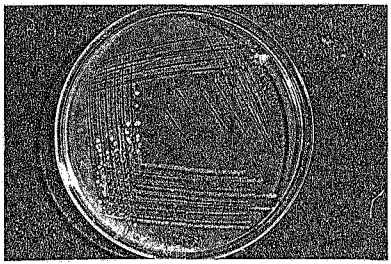


Figure 10 : Colobies of Aeromonas on Nutrient Agar.

interval between sampling. In each case, two samples were taken and each diluted and plated in duplicate. All commonly occurring colony types were subcultured and characteristics assessed using the methods described in section 2.5. The details of those organisms are given in table 3.

An organism occuring in both replicates of each sample was considered to be persistently present at a fairly high level at that time of the year. Attention was also given to identifying those organisms whose colony morphology appeared similar to organisms isolated from the machine process water itself.

Considering the characteristics (see table 3) the organisms in the water sample can be classified as follows: Streptococcus, Alcaligenes, Listeria, Pseudomonas, Aeromonas, Bacillus and Flavobacterium.

The potable water sampled independantly gave only one major isolate which was the Bacillus.

The Pseudomonas, Aeromonas and Bacillus appeared to be similar to those isolated from the machine.

3.2.2 Broke, Wattle and Pine Pulps

The three paper pulps, namely broke, wattle and pine were each sampled twice in duplicate. Samples were taken from temporary holding tanks.

Organism number / Characteristic	1	2	3	4	5	6	7	8
Shape and arrangement	cocci in pairs	rods	rods in v-formation	thin rods	cocci in clumps	rods	large rods	thin rods
Gram reaction	+	-	+	-	+	-	+	-
Motility	ND	+	+		ND	+	+	-
Respiration	F	A	F		A	F	F	F
Catalase	-	-	+	+	+	+	+	ND
Oxidase	-	+	+	+	+	+	ND	ND
Pigment	cream	cream	clear - cream	fluor-escent	pale yellow	lemon yellow	off white	dark yellow
Spore	ND	-	-	-	ND	-	+	-
Indole	ND	-	-	ND	ND	-	-	-
Arabinose	ND	ND	ND	ND	ND	ND	acid	ND

Table 3 : Characteristics of major isolates from a mixture of sewage and potable waters. (F - facultatively anaerobic; A - obligate aerobe)

Organism number / Characteristic	1	2	3	4	5	6	7	8
Shape and arrangement	cocci in pairs	rods	rods in v-formation	thin rods	cocci in clumps	rods	large rods	thin rods
Gram reaction	+	-	+	-	+	-	+	-
Motility	ND	+	+	+	ND	+	+	-
Respiration	F	A	F	A	A	F	F	F
Catalase	-	-	+	+	+	+	+	ND
Oxidase	-	+	+	+	+	+	ND	ND
Pigment	cream	cream	clear - cream	fluorescent	pale yellow	lemon yellow	off white	dark yellow
Spore	ND	-	-	-	ND	-	+	-
Indole	ND	-	-	ND	ND	-	-	-
Arabinose	ND	ND	ND	ND	ND	ND	acid	ND

Table 3 : Characteristics of major isolates from a mixture of sewage and potable waters. (F - facultatively anaerobic; A - obligate aerobe)



Organism number / Characteristic	1 (broke)	2 (broke)	3 (broke)	4 (wattle)	5 (wattle)	6 (pine)
Shape and arrangement	cocci in pairs	rods	fat rods	rods	rods	rods
Gram reaction	+	-	+	+	-	+
Motility	ND	+	+	+	+	+
Respiration	F	F	F	F	F	+
Catalase	+++	+	+	+	-	+
Oxidase	-	-	+	+	-	+
Pigment	orange	cream	cream	cream	cream	cream
Spore	ND	-	+	+	-	+
Arabinose	ND	+	ND	ND	+	ND
Sucrose	ND	+	ND	ND	ND	ND
Fructose	ND	ND	ND	ND	+	ND
Indole	ND	+	ND	ND	-	ND
Methyl Red	ND	+	ND	ND	+	ND
Voges-Proskauer	ND	-	ND	ND	ND	ND
Citrate	ND	+	ND	ND	+	ND

Table 4 : Characteristics of the major isolates from broke, wattle and pine pulps. (F - facultatively anaerobic; A - obligate aerobe)

Having already ascertained how the bulk of the slime formers were being inoculated into the system i.e. via the sewage water, the scale of pulp investigation was narrowed and in most cases only colonies resembling those produced by major slime formers were subcultured and subsequently identified. Exact counts were not made.

Results in table 4 show that of the three organisms isolated from the broke pulp, two have already been implicated as potential slime formers. Wattle pulp yielded two different genera and pine pulp one.

Characteristics of these organisms, presented in table 4 show that the organisms isolated from the pulps were (in order) Staphylococcus, Citrobacter, Bacillus, Bacillus, Klebsiella and Bacillus.

3.2.3 Starch Mixture

Raw starch is mixed with purified sewage water to form a 4% solution. Before 1983 only imported potato starch was used for this purpose. Exclusive use of this potato starch was dispensed with early in 1983 when a locally produced corn starch was introduced. These two starches mixed in a 1 : 1 ratio were used to form the required solution. Shortly after this change was brought about, the mill started using greater volumes of sewage in an attempt to conserve water due to pressure caused by drought conditions.

Starch Mixture	pH
Healthy	7,21
Sour smelling	4,09

Table 5 : pH of the different starch mixtures

Concomitant with these changes was the onset of a spasmodic sour smell of the starch mixture. This situation deteriorated and the smell was found to persist in the paper, an unacceptable situation.

The colour of the starch remained unchanged when the smell occurred. The smell was indicative of a mixture of volatile fatty acids. It was thus suspected that the smell had resulted from a fermentation. This idea was supported by recorded pH values (table 5).

When the starch was inoculated onto various media and maintained at $37^{0}C$, $39^{0}C$ and $55^{0}C$ under aerobic and anaerobic conditions, positive growth occurred only at $37^{0}C$ on Brewers Anaerobic Agar incubated anaerobically.

When a 10 fold (v/v) dilution was made of the two respective starch mixtures, inoculated onto Brewers Anaerobic Agar, and incubated anaerobically at $37^{0}C$, the sour smelling starch was found to have a count of 128×10^{5} cells/ml compared to a count of less than 10 cells ml^{-1} for healthy starch. In addition, the smell of the growth from the sour smelling starch had the same sour smell evident in the sour mixture.

Staining of growth on Brewers Anaerobic Agar showed a pure culture of gram positive rods (see fig.11).

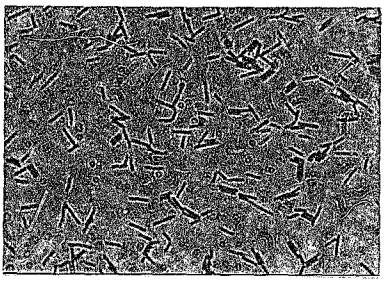


Figure 11 : Gram positive rods cultured from sour smelling starch on Brewer's Anaerobic Agar at 37°C

Gas Chromatographic Analysis

In preparation for gas chromatographic analysis of any volatile fatty acids this organism was expected to be producing, samples of this growth were inoculated into 10ml volumes of commercially prepared Robertson's Cooked Meat Medium with 1% glucose. Since the organism had been isolated at 37°C, media were incubated at that temperature for 48 hours under anaerobic conditions.

Gas chromatographic analysis yielded a total of three peaks in the sour smelling starch (see fig. 12). The retention times suggest the presence of large amount of acetic and butyric acid and smaller amounts of lactic acid. However, the retention times did not exactly

parallel those found to be typical fermentation products used in the standards. Therefore a 50 : 50 mixture of sample and standard was made and injected onto the column. No extra peaks or shoulders resulted and the peaks obtained from the sample were thus according to the standard (see figs 13 and 14).

This procedure confirmed the presence of an anaerobic fermentor in the sour smelling starch.

Identification of the Organism

The volatile fatty acids produced were indicative of either Clostridium or Eubacterium sp (6). The genus Eubacterium is very similar to that of the genus Clostridium, although the latter are spore-formers and the former not. No spores were seen, but because of the similarity of the two genera the organism could not be classified with confidence as belonging to one or the other genus.

3.2.4 Raw Potato and Corn Starch

In order to determine if the organisms were present in the raw powdered starch, or arose from the sewage water, samples were cultured on corn and potato starch agar and incubated at 37°C aerobically and anaerobically.

The organisms isolated from both starch samples had the same colonial morphology and were microscopically alike, being large gram positive rods which tended towards gram negativity as the cultures aged. They were non-motile, facultatively

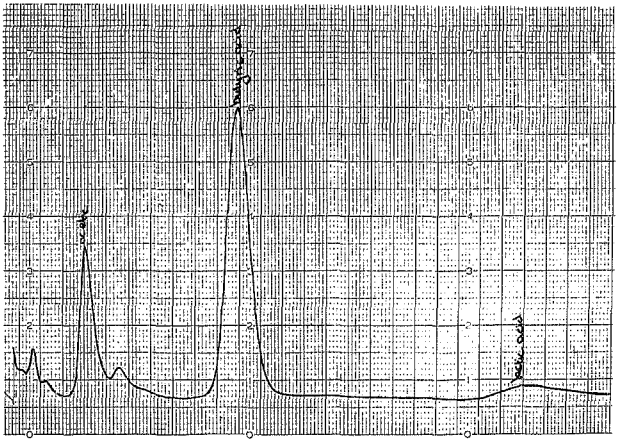


Figure 12 : Gas chromatographic analysis of volatile fatty acids produced by an organism isolated from sour smelling starch. The organism was inoculated into 10ml Robertson's Cooked Meat Medium with 1% glucose, incubated anaerobically for 48 hours at 37°C, the medium centrifuged to remove any particulate matter and 50µl aliquots analysed on a Chromosorb 101 column, 1.8m long with an internal diameter of 3mm and injector temperature of 220°C.

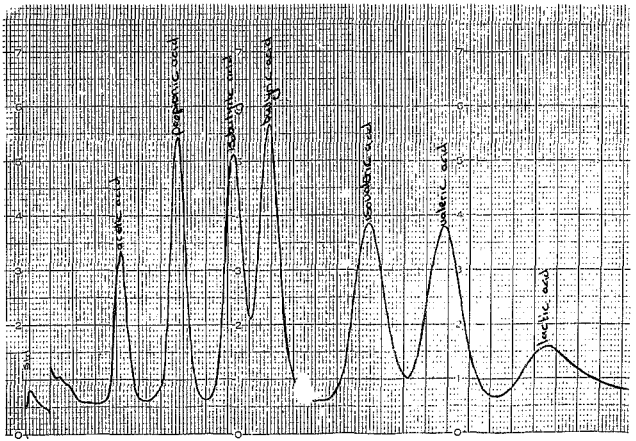


Figure 13 : Gas Chromatographic analysis of a standard mixture of volatile fatty acids on a Chromosorb 101 column, 1,8m long, 3mm internal diameter and injector temperature of 220°C.

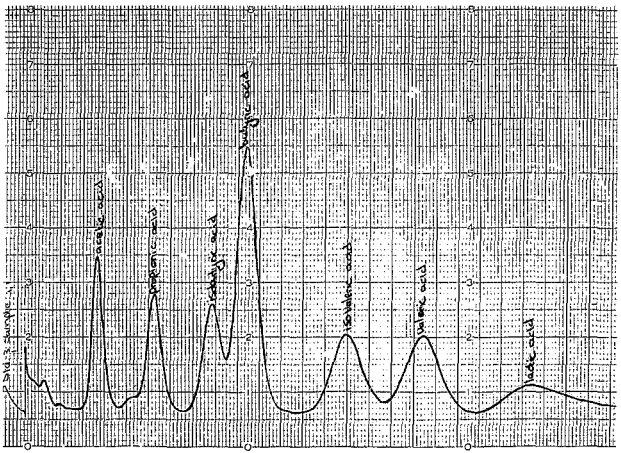


Figure 14 : Gas chromatographic analysis of a 50 : 50 mixture of a standard volatile fatty acid mixture and supernatant containing volatile fatty acids produced by an *organism isolated from sour smelling starch*. The organism was inoculated into 10ml of Robertson's Cooked Meat Medium with 1% glucose, incubated anaerobically for 48 hours at 37°C, the medium centrifuged to remove particulate matter and then added to the standard mixture. Analysis was done on a Chromosorb 101 column, 1.8m long, internal diameter 3mm and with an injector temperature of 220°C.

anaerobic and displaying a negative catalase reaction.

These results suggest the presence of lactobacilli in the raw starches themselves but the absence of the obligate anaerobe.

3.3 Succession of Microorganisms

Tiles were placed in the machine thickener tank suspended from wires. At increasing time intervals (see table 6) two tiles at a time were removed so that the microbial flora present could be assessed. The numbers of predominant organisms were assessed using methods described in sections 2.5 and 2.6.

Visual inspection of the tiles after removal from the tank showed no noticeable microbial mass adherent to the tiles until the sampling on the sixteenth day. Tiles removed at day 16 and particularly day 29 were covered by a distinct slime layer. The thickness of the layer was estimated to be 4 mm on the tiles removed on day 29. Spots of "pink slime" were clearly visible on each of the fifth set of tiles, but did not appear on culture plates, even in the undiluted state indicating that these organisms were dramatically outnumbered by the other slime forming microorganisms in the tile. "Pink slime" is a common problem in paper mills and is caused by the yeast Rhodotorula which has previously been shown to grow well on both NA and SDA.

Plots of total bacterial number per tile versus time (see

Sampling time / Genus	48 hours	5 days	9 days	16 days	29 days
Bacillus	186×10^3	235×10^4	102×10^6	32×10^7	240×10^9
Pseudomonas	80	230	67×10^2	250×10^4	48×10^5
Citrobacter	30	190	280	64×10^3	210×10^3
Aeromonas	-	-	184	276×10^3	88×10^5
Rhodotorula	-	-	-	51×10^3	-

Table 6 : Total bacterial counts per tile for each slime forming genus as recorded over one month. Counts represent the mean counts for two tiles. Tiles which were suspended in the machine thickener tank were removed at appropriate intervals, put into 50 ml cold sterile Ringer's solution and shaken well to dislodge and disperse the adherent growth into the medium. Aliquots were pipetted off and used for serial dilutions from undiluted to 10^{-10}.

fig. 15) show a steadily increasing number of bacteria, the growth slowing at day 9 for Bacillus which predominates, and day 16 for Citrobacter, Aeromonas and Pseudomonas.

3.4 Interrelationships

In assessing the possibility of interrelationships, all possible permutations of any two organisms were tested. Method 1 (parallel streaking), having been shown previously to be a good indicator of synergism, showed no synergistic or antagonistic effects between any two organisms. Results from Method 2 (filled wells) were in agreement with the former.

Results using Method 3 (agar discs) were erratic and unreliable. In incubation of experimental plates with discs inoculated with bacteria any metabolite which might in any way influence the growth of another organism would be expected to diffuse into the agar below. Following removal of the disc, point inoculation by one or numerous other organisms should then result in growth of colonies, the diameters of which can be measured. Comparison of these diameters with those obtained from a control (an untreated agar plate point inoculated in the same way as the experimental plates) should then give a qualitative and quantitative assessment of any possible synergistic or antagonistic relationship.

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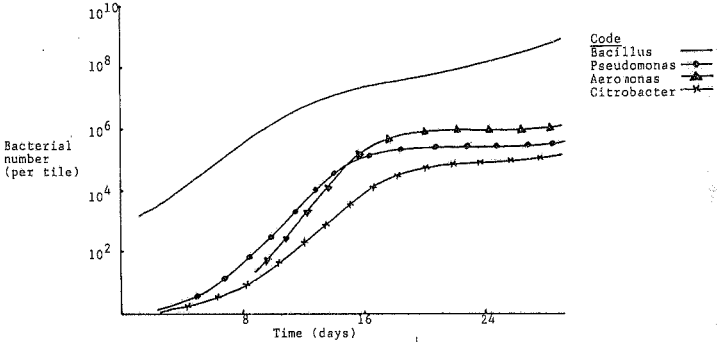


Figure 15: Graph of bacterial number cultured per tile vs time.

growth around the point of inoculation on control plates. In cases where growth occurred on experimental plates, it was delayed and sporadic.

Since no evidence of interrelationships was obtained from Methods 1 and 2, variations of Kovrov's method were tried in an attempt to produce consistent results with this technique. The medium was altered in the form of a basal medium (see appendix), the discs poured thinner and the incubation time lengthened, but results were still unsatisfactory with this procedure.

From experiments using an uninoculated agar disc on the .rol (storage for 10 to 14 days and removal prior to point inoculation) it would appear that the actual presence of the disc altered the agar medium below thus rendering it unsuitable for microbial growth. Inoculation of the above plates produced the same erratic and sporadic growth as seen on experimental plates.

3.5 Effect of Slimicide on Bacterial Growth

Slimicide at the appropriate concentration was added to flasks inoculated with Bacillus, Pseudomonas, Citrobacter and Aeromonas and incubated for 16 hours as described in section 2.8.1. Two samples from each flask were taken every hour except for between 3 and 5 hours where a 2 hour time interval was used. Optical densities were recorded using NB and slimicide blanks (see figs. 16 - 19).

A sixteen hour growth experiment was estimated to allow the organisms to reach the log phase. The results show that only Citrobacter was capable of delayed growth at the two lower concentrations of slimicide. Bacillus, Pseudomonas and Aeromonas displayed no growth although all control cultures inoculated with the same organisms showed growth which in all cases gave the classical growth curve.

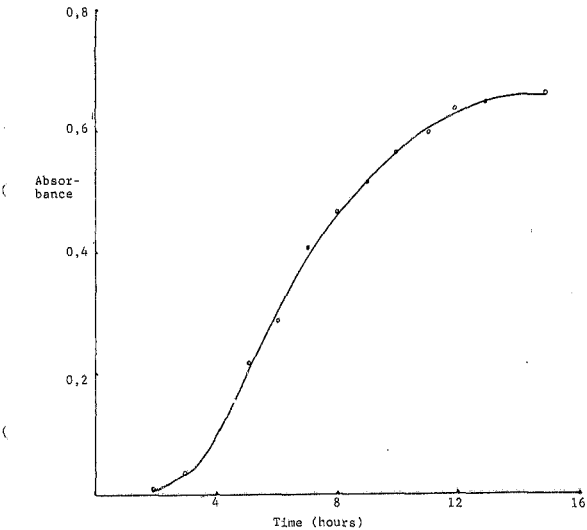


Figure 16 : *Graph of Absorbance vs Time for* Bacillus. Organism inoculated into 100 ml nutrient broth and incubated aerobically at 30°C and 100 rpm.

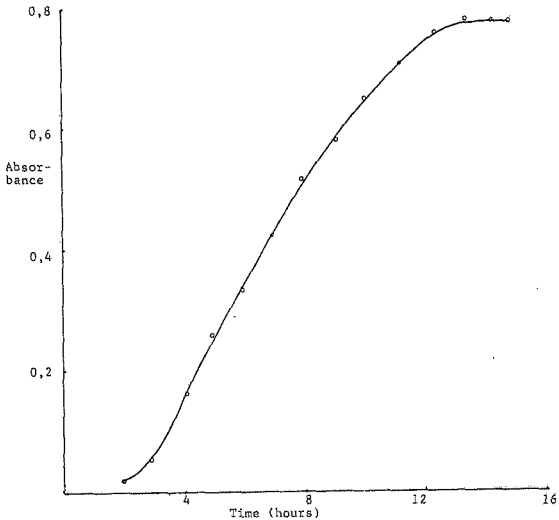


Figure 17 : Graph of Absorbance vs Time for *Pseudomonas*. Organism inoculated into 100 ml nutrient broth and incubated aerobically at 30°C and 100 rpm.

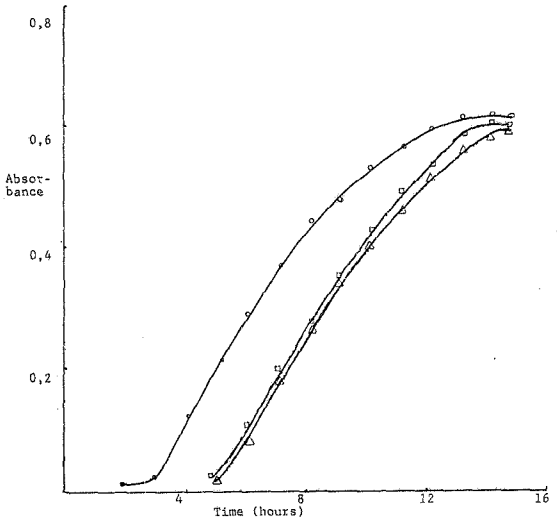


Figure 18: Graph of Absobance vs Time for growth of Citrobacter in 100 ml nutrient broth without slimicide and with 20 ppm and 50 ppm slimicide added. Inoculated flasks were incubated aerobically at 30°C and 100 rpm.

Code
No slimicide —○—
20 ppm slimicide —□—
50 ppm slimicide —△—

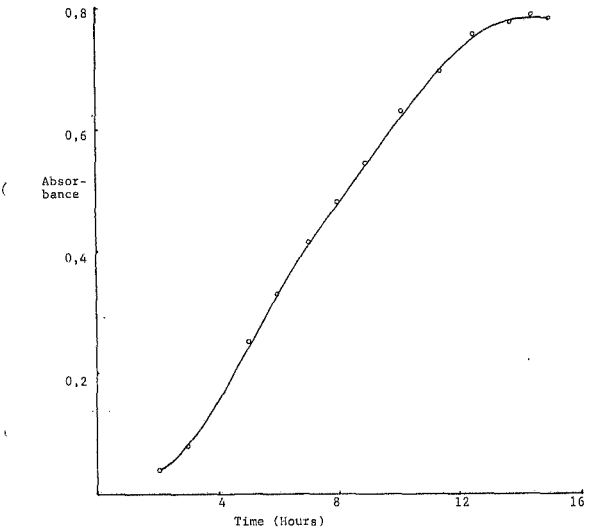


Figure 19 : Graph of Absorbance vs Time for Aeromonas. Organism inoculated into 100 ml nutrient broth and incubated aerobically at 30°C and 100 rpm.

4. DISCUSSION

The slime in PM 3 was predominantly bacterial in nature, the four major genera present being Bacillus, Pseudomonas, Aeromonas, and Citrobacter (see tables 1 and 2). This is consistent with the findings of Nason et. al. (42). who reported the slimes of an Ontario sulphite mill to be predominantly bacterial in nature, citing Bacillus as an important slime former. A list of important slime formers compiled by Sanborn (56) includes Bacillus and Pseudomonas, and later work by Eveleigh and Brewer (17) again showed Bacillus and Pseudomonas to be important organisms in slime formation.

The dominance of bacteria in PM 3 slime is likely to be reflective of the type of slimicide used in the machine - a dithiocarbamate derivative. Dithiocarbamates are predominantly fungicidal in nature, but do possess some bacteriostatic qualities (60). Thus, an effective dithiocarbamate based slimicide would be expected to keep fungal numbers down. However, by so doing, the chances for rapid proliferation by bacteria, resulting from the reduced competition, if there was any competition previously, would be enhanced. The environmental conditions in the mill are an important factor in determination of the range of microorganisms present. The rich nutrient supply and temperature of approximately 30°C encountered in PM 3 tend to provide a favourable environment to a wide range of microorganisms.

The genus Pseudomonas was shown to make up 25,5% of the organisms present on PAA (see tables 1 and 2). Although its presence in paper mill slimes and in other slime accumulations has previously been reported by a number of workers (17, 56), numbers have previously not been quoted.

Pseudomonas is a common inhabitant of soils, fresh and salt waters, decomposing matter and sewage (29). Since it is a strict aerobe is would be expected to be prominent in the outer, more aerobic layers of the biofilm. The slime produced by this organism probably plays an important role in maintaining the integrity and cohesion of the slime mass. Other organisms which themselves do not produce much slime may embed themselves in this slime, further increasing the size of the deposit.

Pseudomonas is a relatively unfastidious organism, capable of breaking down a number of polysaccharides P. stutzeri being the organism responsible for the breakdown of the latter. Levan formation is not a distinguishing feature of the genus, but occurs in some biotypes of P. fluorescens, P. chlorophis, P. syringae and P. sereofaciens (6).

Economics Laboratory's work on some slime formers from North American paper mills showed the polysaccharide capsule of the slime forming bacteria to be composed largely of levan (29). Owing to the ubiquitous nature of the

slime formers, including Pseudomonas, it could be possible that the polysaccharide slimes elaborated by microorganisms at different geographic locations might be the same. Thus, although the microorganisms were not identified to species level, it is possible that the Pseudomonas species isolated from PM 3 is a levan producer.

The predominance of Citrobacter (see table 1 and 2) could be due to the temperature at which the machine runs (generally averaging 30°C). Citrobacter itself has not previously been implicated as a major cause of slime problems although other members of the Enterobacteriaceae are commonly isolated from slimes. A common inhabitant of water, food, faeces and urine (29), its appearance in PM 3 could be due to the use of large volumes of treated sewage water in the machine.

The genus Aeromonas has not been previously cited as a major slime former. However, since it is commonly water borne and relatively unfastidious it is not an unlikely candidate for habitation of paper mills. It is, in addition, a slime producer (59), and has previously been found in other South African paper mills (Harris, I. A. unpublished results). A. hydrolytica and A. punctata are both inhabitants of water and sewage (6) and as Aeromonas was found in the sewage water used, it is likely that the Aeromonas organisms found in the paper mill are one or a mixture of the above-mentioned species.

Aeromonas, Citrobacter and Pseudomonas are all gram negative rod shaped bacteria, a characteristic of many organisms with a preference for water as an environment (26). In addition, they are all motile.

Although motility in water might appear to be a luxury, it is advantageous in that it allows the microorganism to direct its own movement towards anchorage or a nutrient pocket. The necessity for motility in a flowing water system such as a paper mill becomes correspondingly less significant, although ability to direct movement of the cell itself, by itself, lessens the initial chances of being washed out. Non-motile organisms have to rely on random collisions with solid objects for possible adhesion.

The presence of the spore-forming Bacillus was expected. It is not only a common paper mill inhabitant, but is found on a variety of substances including wood, paper leather and even drugs (21). Its ability to produce spores ensures that even if conditions become unfavourable it can persist. This obviously poses a problem with regard to control, particularly since it is capable of fermentation, the products of which are generally undesirable in large quantities in a paper mill.

This work has shown the treated sewage to make the most significant contribution in terms of introduction of slime formers to the system (see tables 3 and 4).

Sewage water was found to harbour 75% of the species of major slime formers in addition to numerous bacteria previously cited as troublesome in paper mills (12, 17, 56). These include Listeria, Staphylococcus, Alcaligenes, Micrococcus and Flavobacterium. Although not numerically significant in the slime sampled from PM 3 in October, these organisms might assume greater numbers at different times of the year. It has been suggested that, depending on the season, the nature of the dominant species might change but that the overall flora would remain constant. This phenomenon is known as the seasonal fluctuation.

Paper mills do not require drinking quality waters for production. Even in the case of production of food wrapping and packaging, most harmful organisms would be killed by the high temperature of the drier rolls (34, 52). Thus, even after treatment of the incoming sewage, the level of microbial contamination is expected to be high.

In general, the sewage water used in the paper mill is simply flocculated and chlorinated and in the light of this it can be understood why such high numbers and such a diverse range of microorganisms are still present after purification. In addition, due to the nature of the nutrients in the sewage water, many microorganisms are able to thrive.

Since purification of incoming sewage remains the same

irrespective of degree of fouling, periodic gluts of microorganisms are expected. A good example of this is the water that arrives following a Monday washday when large amounts of foam floating in the flocculation tank can be seen. Although a more rigorous purification programme for this type of situation could be embarked upon, implementation is not simple. Flocculation is the first and most important purification step, but due to the flocculation equipment design, it is only after this process or very near the end that the level of initial fouling can be assessed. Equipment to facilitate reflocculation would therefore be necessary.

Investigation of pulp samples showed the broke pulp to harbour one of the main slime formers in the form of Citrobacter. Because the broke used in PM 3 is composed largely of repulped paper it is a more homogeneous mixture of the microbial nutrient. Pure pulps such as pine and wattle, although also richly endowed with certain microbial nutrient substrates, tend to be able to support only those microorganisms capable of breakdown of the nutrient offered by that particular pulp. Conversely, broke pulp, being a mixture of paper, board, other pulps and even small quantities of bagasse in some cases offers a wider range of microbial nutrient and would thus be expected to support growth of a wider range of microorganisms. It is therefore likely that problem-causing microorganisms would be found in broke pulp. Sanborn (56) has previously reported broke pulp

to play a significant role in inoculation of slime formers into the system.

It is interesting to note the presence of Klebsiella, another member of the Enterobacteriaceae in the wattle pulp (see table 4). This could be a function of the favourable storage temperature of the pulp. Klebsiella is a bacterial genus which has frequently been cited as a major contributor in the formation of slimes (9,44).

The presence of the spore-forming Bacillus in the sewage, potable water and all the pulps is an indication of the versatility of this organism. Its ability to produce spores in the face of adverse conditions ensures that it survives many of the harsh preparatory processes in paper making, such as high temperature, acid treatment and chlorination. (56).

Although Bacillus itself does not produce excessively large amounts of extracellular polysaccharide (6), it could contribute to slime formation by functioning as an initial colonizer. However, as very large numbers were consistently isolated from the mill and because of its routine isolation from paper mill slimes, it must be considered a major slime former. Being a facultative anaerobe it could also bring about unwanted fermentations if anaerobic conditions arose. Finally, once fermentation products rendered the environment unfavourable for growth, spores would be formed and the organism would go into a state

of dormancy (6, 21).

One of the starch mixtures used in the paper process was observed to become sour smelling. This was found to be the result of a saccharolytic action by either Clostridium or Eubacterium species. The production of large amounts of acetic and butyric acids with lesser amounts of lactic acid caused a drop in pH accompanied by the sour smell. Results show total anaerobic counts to be five fold higher for the sour smelling starch when compared with results from the fresh starch (see section 3.2.3).

Investigation of the raw potato and corn starches used to make the starch mixture, showed both to be free of the organism responsible for the fermentation. The water, which is exclusively treated sewage was therefore the most likely source of contamination.

Clostridium and Eubacterium are both frequently isolated from soil, human and animal faeces and the human intestinal tract (6, 15, 21). They are therefore likely inhabitants of the sewage water.

Some doubt exists as to exactly when the problem of the sour starch became serious. It appeared that the change from exclusive use of potato starch to a 50 :50 mixture of potato and corn starch had an effect on the problem. It was also at more-or-less the same time that the mill began its use of treated sewage. Although analysis showed

the Clostridium or Eubacterium not to be introduced via either of the two starches (see section 3.2.3) the newly used corn starch could offer a more favourable substrate to the bacteria. The "cooking" of the starch serves to disrupt the granular structure although not altering the molecular structure. This, in itself, would aid in making the starch a more accessible substrate to bacteria. The "cooking" could affect the granular structures of the two starches in different ways.

The fresh starch mixture did not smell sour after three weeks storage. It is, thus, more likely that the problem arose due to overloading of the treatment plant with a consequent failure to treat the sewage satisfactorily.

Microorganisms are always found where there is a concentration of nutrient. Lactobacilli in the two raw starches are thus not unexpected since they are known starch utilizers (29).

Results show that a distinct succession of microorganisms exists in PM 3 (see table 6). Colonization began with Bacillus, followed by Pseudomonas and Citrobacter which colonized simultaneously, and finally Aeromonas.

Initial colonization numbers of Bacillus were much higher than for any of the other three genera, and Bacillus numbers remained consistently higher than those of the other organisms during the 29 day sampling period.

In a washed out system the water used to restart was a mixture of treated sewage water and potable water (the standard mixture), thus, all the major slime formers were likely to be present at the time of restart, although in a dispersed state. Colonization of solid surfaces is expected to follow.

It is likely that the Bacillus was initially present in the restart water in higher numbers than the other organisms. This is suggested by counts carried out on water samples (see table 1). If initial colonization by Bacillus is not entirely by chance, then being present in higher numbers may represent an advantage for colonization and then facilitate quick establishment of a slime mass.

Paper mill slime formers are not generally noted to be fastidious organisms and it is therefore possible that many are capable of initial colonization. However, prior colonization by an organism present in higher numbers and producing even a small amount of extracellular polysaccharide would function to present a more favourable surface for other bacteria, possibly masking charge effects of the wall or by presenting a more sticky surface. However, the reliance of other slime formers on prior colonization by Bacillus is probably not absolute.

On a numerical basis, Pseudomonas, an obligate aerobe was the second colonizer (see table 6). Since it is an

aerobe one would expect this organism to be a primary colonizer since it is at the early stages of biofilm development that conditions would be expected to be more aerobic. Thus, this secondary colonization by Pseudomonas tends to support the theory that Bacillus (a facultative anaerobe) colonization is not due to chance and that its presence actually serves a purpose with respect to providing a more favourable surface for colonization by other organisms.

The colonization by Pseudomonas represents an important point in the succession. Being an organism noted for its copious slime production, its presence could accelerate the growth and diversity of other genera involved in production of the slime mass as follows: the outward radiating polysaccharide fibres would serve as a network to which other passing organisms, not necessarily slime formers, could adhere. In addition, owing to the likely occurrence of aerobic conditions, it is possible that fungi would become associated with the mass at this stage of development. The hyphae produced by the fungi would tend to increase the size of the network and in turn trap debri, again increasing the size of the deposit.

The growth of Pseudomonas is ultimately self-limiting in that the more microorganisms that become entangled in their polysaccharide fibres, the less the chances of maintaining an oxygen supply sufficient to ensure aerobic conditions. Thus, as oxygen becomes depleted the pseudo-

monads would die, but the network created would remain in the mass thereby contributing to the maintenance of the integrity of the slime mass. Once the mass gets sufficiently large and the shear force of the water exceeds the adhesive properties of the slime, parts will begin to slough off. This would also occur if one entire layer died and lysed simultaneously creating a weak spot which was unable to hold the outer layers.

Despite the fact that those pseudomonads which were first to colonize eventually die, continual colonization of the outer surface by this genus will occur. The aerobes in turn will die once oxygen becomes depleted and in such a way a cycle is set up. The nutrients liberated by death and lysis of the cells tend to diffuse to the outer aerobic surface of the slime mass for reuse (33, 47, 67).

Colonization by Citrobacter and Pseudomonas occurred simultaneously (see table 6) although Pseudomonas always outnumbered Citrobacter despite the ability of this organism to survive in anaerobic conditions.

Citrobacter could be a slower growing organism and therefore only ever present in lower numbers. It is also likely that by maintaining themselves in a stable state in low numbers, competition between organisms of this genus for a particular nutrient is reduced. Alternative nutrient sources are more likely to be utilized by organisms with less exacting nutritional requirements.

After nine days Aeromonas made its first appearance in relatively high numbers (see table 6). The indication is therefore that colonization must have commenced shortly after sampling on the fifth day.

It is likely that this organism requires a fairly high degree of colonization by other organisms prior to becoming associated with the deposit. This could either be because it lacks the ability to colonize a naked surface, due possibly to the nature of the polysaccharide capsule, or to the nature of the surface available for colonization. It may require an existing polysaccharide network for binding purposes. If the latter argument is correct this requirement would be well met by the production of copious polysaccharide by Pseudomonas and some other organisms already established in the slime.

Plotting the buildup of each organism tested against time shows a curve which resembles the lag and log phases of the classical growth curve (see fig.15). Bearing in mind that the stationary and death phases rely on the depletion of nutrients, as would be experienced in a closed container with a finite nutrient concentration, the lack thereof in this instance is likely to be an indication that the nutrient supply is continual and that dead cells are replaced by living cells. This nutrient supply could be gained not only from the process water, but also from nutrient recycling in the slime mass.

Although these phases are obviously not an indication of the generation time of each individual organism, they could represent the phases in growth of the slime mass as a whole.

The phenomenon of a long lag period following microbial attachment has been reported by Marshall (39). This is particularly prevalent in nutrient poor environments where, prior to attachment, the organisms might be in a dormant state as a result of severe nutrient limitation. Although a paper machine cannot be considered to be a nutrient poor environment, the relative nutrient concentrations of the process water and the interface of water and wall should be sufficiently different to constitute a nutrient poor and nutrient rich environment respectively.

Lag phase represents initial colonization where the organisms would channel much energy into establishing a niche, rather than replication. A longish lag phase as seen with Aeromonas suggests longer time to establish and induce enzymes capable of using the nutrient present.

The events occurring during the apparent log phase would be twofold. Active proliferation would occur culminating in gradual death of some organisms as a result of conditions becoming unfavourable. The death of these organisms coincides with recolonization of the surface layers of the mass by more organisms of the same genus and

this would prevent any significant drop in numbers of that particular genus. Furthermore, the death of the initial colonizers may occur simultaneously with the period of adjustment and establishment by the secondary colonizers resulting in a net lag phase as indicated in figure 15.

The occurrence of alternate periods of microbial growth and prolonged dormancy is a phenomenon that has been previously reported by Jannasch (31).

The studies to ascertain dependance on a metabolite produced by one organism to support another did not demonstrate any interdependance. However, the techniques used all involved growth on a solid medium. Conditions in a fluid environment are more conducive to production of capsule and extracellular material and this may explain the discrepancy. Further, any capsular material produced is unlikely to readily diffuse through the agar utilized in the tests. It is also possible that the sequential colonization observed in situ is due to the physical properties of the slime as well as the nutrient composition, a factor which these procedures would not test.

Under laboratory conditions, growth of Bacillus, Pseudomonas and Aeromonas was found to be inhibited by slimicide concentrations of 20 ppm to 200 ppm. However, Citrobacter grew in the presence of slimicide at conc-

entrations of 20 ppm and 50 ppm, although a short delay preceeding growth was noted (see fig.18).

The abovementioned organisms had been isolated from a slime accumulation in the machine thickener tank and from the process water earlier in the study during a time when the dithiocarbamate slimicide tested here was in routine use. Failure of Bacillus, Pseudomonas and Aeromonas to grow at all slimicide concentrations tested and at 100 ppm and 200 ppm concentrations for Citrobacter could be attributed to one of the following factors: The theoretical dosage of slimicide is not necessarily the same as that applied in practice. The dosage equipment used for PM 3 is crude and has previously been seen to malfunction resulting in erratic dosage or stoppage of slimicide administration. Secondly, fluctuations in slimicide concentrations could occur as a result of uneven water flow rates giving rise to pockets of high or low slimicide concentrations in much the same way as anaerobic pockets would occur. The problem is compounded by the fact that influent and effluent water volumes vary. Thus, where large volumes of water are used such as in a paper mill where more than 11 tonnes of water are required to produce one tonne of paper, variations might not be as critical to paper production as they would be to slimicide concentration.

Finally, the occurrence of tolerant or resistant organisms,

particularly at low levels of antimicrobial substance, is a problem which might be experienced in a system where the antimicrobial is used on a routine basis and has also been used as such for a long time. An example of mass resistance to a routinely used antimicrobial is the decrease in effectivity of penicillin which, although still in use today, is far less effective than it was in the 1930s when discovered by Alexander Fleming.

The occurrence of resistance is an important consideration particularly when dealing with relatively unfastidious organisms capable of growth in a variety of environments.

Although it is likely that the slimicide killed the Pseudomonas and Aeromonas, it probably induced the Bacillus culture to sporulate. All cultures in the laboratory were maintained on NA slants and the mixture of nutrient broth and slimicide would therefore represent an unfavourable environment. This would either stop or slow down growth, kill the organisms, or in the case of a spore-former such as Bacillus, induce sporulation. If the effectiveness of the slimicide were to decrease over time, germination of spores would ultimately be expected. This did not occur over the 16 hours of incubation, although no conclusions can be drawn from this observation since data regarding effective life of slimicides was not available.

Results showed Citrobacter (figure 18) to be resistant

to slimicide concentrations of 20 ppm and 50 ppm. Growth was delayed by 5 hours during which time it is likely that the organism was making minor physiological adjustments to the presence of the slimicide.

This work serves as an example of the major disadvantage of using a broad spectrum biocide. In this excercise, one gram positive and three gram negative organisms were used to test slimicide effectivity. Considering the diversity of genera already found in this study and in others (17, 56), it is unrealistic to assume that one biocide is capable of killing such a broad group of microorganisms as all the gram positive or all the gram negative bacteria. In addition, more adaptable organisms would be likely to be the first to develop resistance and it is precisely these which have been shown as important slime formers.

The situation is however, not as depressing as it may sound. Only 25% of the slime formers isolated in this study were resistant, but not to the higher slimicide dosages. Bearing in mind that one of the characteristics of slime is the diversity of genera present, to retard growth or kill 75% of genera present does make the biocide an agent well worth consideration. Dosage equipment should, however, be properly monitored and wherever possible influent and effluent water volumes kept constant.

To reduce the risk of resistance, rotation of slimicides is practical, particularly if the mode of action of the

slimicides differ. In addition, pulse feeding of slimicide also merits consideration. Here the systems would be allowed to function for sufficient time to allow growth of organisms, but not large accumulations (this time would have to be experimentally determined for each individual machine), followed by a dose of slimicide sufficiently large to kill most of the organisms present. Thus, provided no accumulation had formed, these organisms would be washed out of the system without causing a problem. Such a dosage programme is best suited to closed systems, those which recycle water and those which experience long water retention times.

While this system holds obvious disadvantages, it does, at least in the short term, offer a divergence from the routine administration of slimicide, which if not tightly controlled (and a number of practical problems regarding this point have already been discussed in this section) could be a major contributing factor to microbial resistance.

Long term strategies are discussed in section 4.1.

4.1 General Conclusion and Future Work

It would appear that in dealing with the problem of slime accumulations in paper mills, the problem is multi-pronged. By virtue of the rich nutrient supply offered by the raw materials, temperatures, pH and redox potential, a paper machine is a favourable microbial habitat. In addition, older machinery often enhances the presence of

isolated and protected pockets as a result of uneven aeration and water flow. The ecology of the slime accumulation is a subject which is poorly documented and it is only by gaining knowledge of the problem to be dealt with that slimicide formulation can be tailored more specifically to meet the requirements. Secondly, use of slimicides appears to be too heavily dependant on academic criteria rather then the problem experienced by each individual mill. This reduces slimicide dosage to being a hit and miss process.

Ecological studies are very often fraught by contradictions, are very time consuming and can involve tedious work, which is possibly why it is not always a subject attractive for study. Currently there is little ongoing research into paper mill microbiology. The majority of scientific papers in the major paper journals are concerned with machine technology. Those papers published on microbial ecology are generally not directly addressed to the paper mill slime problem.

The nature of the slime and the range and types of genera present has been previously studied in other environments and this study supports the fact that certain microorganisms are common. Insight into the nature of the succession of the microorganisms coupled with gathering of knowledge pertaining to the possible relationships between these organisms could prove valuable in elucidating the problem with which the slimicide has to deal.

A microbial succession does exist in PM 3 although the growth of each individual organism may not directly depend on any other. Further studies should be performed to ascertain the reason for the succession observed.

If organisms are dependant upon one another it is of interest how the relationship is affected by the slimicide. For example, by killing of one partner of an antagonistic relationship, growth of another might thereby be stimulated. Thus, the effect of slimicide on such a relationship in the context of the slime mass as a whole is an aspect of paper mill microbiology which warrants further investigation.

Finally, more research interest should be directed towards improvement of existing slimicides and development of new types of slimicide.

5. APPENDIX

Basal Medium

Constituent	Amount g/l
$MgSO_4.7H_2O$	0,2
K_2HPO_4	1,0
$NaNH_4HPO_4.7H_2O$	1,0
$FeSO_4.7H_2O$	0,1
NaCl	0,1
$CaCl_2$	0.1
Agar	10 - 15
Distilled water	1 l

Mix salts and agar in distilled water and set to pH 7,2.
Sterilize by autoclaving and use medium as required.

Brewers Anaerobic Agar

Constituent	Amount g/l
Proteose peptone	10,0
Peptone from meat	5,0
L-cystine	0,4
Yeast extraxt	5,0
D(+) glucose	10,0
NaCl	5,0
Sodium thioglycollate	2,0
Methylene blue	0,002
agar	12,6

Dissolve 51 g of the above in 1 l distilled water.
Boil for 10 minutes and set pH to 7,2.
Sterilize by autoclaving and pour plates anaerobically.

Pure Starch Agar

Constituent	Amount g/l
Starch	20
Agar	12
Distilled water	1 l

Dissolve the above in distilled water and set pH to 7,2.
Sterilize by autoclaving and pour plates thickly.

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