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The Microbiological Ecology of Slime Formation in a Paper Mill

Alexander E. Maccubbin

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THE MICROBIOLOGICAL ECOLOGY OF SLIME FORMATION
IN A PAPER MILL

by
Alexander E. Maccubbin

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment
of the
Degree of Master of Arts

Western Michigan University
Kalamazoo, Michigan
April 1976
ACKNOWLEDGEMENTS

I wish to thank the National Council of the Paper Industry for Air and Stream Improvement for the financial support of a fellowship which allowed this project to be undertaken. I further thank Dr. Stephen Friedman and Dr. Allan Springer for their criticism and help. I must also extend thanks and appreciation to Dr. Melvin Carter and Mr. Marshall Brunden for their extensive help and guidance in the statistical analysis of the data. Finally, I give my greatest thanks to my advisor, Dr. Darwin Buthala, whose constant support and encouragement were greatly appreciated.
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LITERATURE REVIEW

The Roles of Microorganisms in the Paper Industry

The roles of microorganisms in the paper industry have been discussed by several authors. Microorganisms affect the industry's profits from the time a tree is planted to be used by the industry through the time the finished product is delivered to the customer (Buckman and Kirchen, 1958). Buckman and Kirchen (1958) stated that some of the effects of microorganisms included decreased pulp quality, lost production time, decreased equipment performance, loss of heat and chemicals and destruction of finished product. More specifically, microorganisms decay pulp and wood (Buckman, 1950), cause odors (Russell, 1959), and form masses of slime (Sanborn, 1933). These slime masses can break loose and cause spots, holes, and breaks in the paper sheet. The slime masses may also plug felts, screens, and pipes causing reduced efficiency of the mill operation (Dietzel and Kirchen, 1959). These microorganisms may be bacteria, fungi and other microscopic organisms (Sanborn, 1965). Zabel (1959) detailed the losses due to fungi. They included tree diseases, heart-rots, slime formation, pulp deterioration, and decay of buildings. The most important and major of all losses have been due to slime formation (Coster, 1968).
Slime and Slime Control

The losses attributed directly to slime deposits have been detailed (Buckman, 1950). They included lost time due to wash-ups and squirt-ups not required by changes in orders, cleaning of wires, washing felts and felt repairs, cleaning up breaks and other cleaning chores not ordinarily required. Decreased equipment performance as manifested by decreased speed of operation and increased stream consumption for drying also was found. A third type of loss described was the loss of heat, chemicals, filler, fiber, and water. The loss due to decreased life of felts, wires and corrosion of machine parts was mentioned. Finally the loss of finished product due to spots, holes, and other poor qualities was considerable. These economic losses have forced the paper industry to examine slime formation and its control.

There have been several types of slime formations described. Sanborn (1965) has characterized them as gelatinous and adherent, stringy or ropey, thick and viscous, pasty and rubbery and leathery, horny, hard or matted. The type of slime developed depends on the conditions of the particular mill system. Several varieties of microorganisms have been described as being involved slime build-ups. They are summarized in Table 1. It can be seen from Table 1 the nature of microorganisms found varies widely. Of the genera included in Table 1, Enterobacter, Pseudomonas, Flavobacterium, and Alcaligenes are the most prolific slime producing organisms. The word slime in this sense describes the material produced by the individual organism.
Table 1
Microorganisms Isolated from Slime Accumulation

<table>
<thead>
<tr>
<th>Type</th>
<th>Genus</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Algae</td>
<td>Oscillatoria</td>
<td>Martin and Dobson, 1945</td>
</tr>
<tr>
<td></td>
<td>Spirogyra</td>
<td>Martin and Dobson, 1945</td>
</tr>
<tr>
<td></td>
<td>Ulothrix</td>
<td>Martin and Dobson, 1945</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Achromobacter</td>
<td>Martin and Dobson, 1945, Lundgren, 1959, Sanborn, 1965a, Coster, 1968</td>
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<tr>
<td></td>
<td>Arthrobacter</td>
<td>Eveleigh and Brewer, 1965</td>
</tr>
<tr>
<td></td>
<td>Chromobacterium</td>
<td>Eveleigh and Brewer, 1965</td>
</tr>
<tr>
<td></td>
<td>Clostridium</td>
<td>Wolfson and Michalski, 1964, Coster, 1968, Starnes, 1973</td>
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<tr>
<td></td>
<td>Crenothrix</td>
<td>Martin and Dobson, 1945</td>
</tr>
<tr>
<td></td>
<td>Desulphovibrio</td>
<td>Coster, 1968, Starnes, 1973</td>
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<tr>
<th>Type</th>
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<tr>
<td></td>
<td>Paracolobacterium</td>
<td>Eveleigh and Brewer, 1965</td>
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<tr>
<td></td>
<td>Micrococcus</td>
<td>Lundgren, 1959, Coster, 1968</td>
</tr>
<tr>
<td></td>
<td>Mycobacterium</td>
<td>Tuleuova, 1969</td>
</tr>
<tr>
<td></td>
<td>Sphaerotilus</td>
<td>Tuleuova, 1969, Starnes, 1973</td>
</tr>
<tr>
<td>Diatoms</td>
<td>Acrostalagmus</td>
<td>Martin and Dobson, 1945</td>
</tr>
<tr>
<td>Fungi</td>
<td>Alternaria</td>
<td>Lundgren, 1959, Zabel, 1959</td>
</tr>
<tr>
<td></td>
<td>Aureobasidium</td>
<td>Eveleigh and Brewer, 1965</td>
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<tr>
<td></td>
<td>Botrytis</td>
<td>Zabel, 1959, Sanborn, 1965a</td>
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<tr>
<td></td>
<td>Candida</td>
<td>Eveleigh and Brewer, 1964</td>
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<tr>
<td></td>
<td>Cephalosporium</td>
<td>Zabel, 1959, Eveleigh and Brewer, 1964</td>
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<tr>
<td></td>
<td>Chaetomium</td>
<td>Zabel, 1959</td>
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<tr>
<td></td>
<td>Citromyces</td>
<td>Zabel, 1959, Lundgren, 1959</td>
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<td></td>
<td>Cladosporium</td>
<td>Coster, 1968</td>
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<tr>
<td></td>
<td>Clonostachys</td>
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</tr>
<tr>
<td></td>
<td>Actinomycetes</td>
<td>Zabel, 1959, Yang, 1961</td>
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<tr>
<td>Fusarium</td>
<td>Lundgren, 1959, Zabel, 1959</td>
<td></td>
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<tr>
<td>Geothichum</td>
<td>Eveleigh and Brewer, 1965, Sanborn, 1965a</td>
<td></td>
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<td>Gliocladium</td>
<td>Zabel, 1959</td>
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<tr>
<td>Hormiscium</td>
<td>Eveleigh and Brewer, 1965</td>
<td></td>
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<tr>
<td>Margarinomyces</td>
<td>Eveleigh and Brewer, 1965</td>
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<tr>
<td>Monilia</td>
<td>Zabel, 1959, Wolfson and Michalski, 1964, Sanborn, 1965a</td>
<td></td>
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<tr>
<td>Mucor</td>
<td>Zabel, 1959</td>
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<tr>
<td>Mycelia</td>
<td>Eveleigh and Brewer, 1965</td>
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<tr>
<td>Nectria</td>
<td>Eveleigh and Brewer, 1965</td>
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<tr>
<td>Oidium</td>
<td>Zabel, 1959</td>
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<tr>
<td>Oospora</td>
<td>Sanborn, 1965a</td>
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<tr>
<td>Phialocephala</td>
<td>Eveleigh and Brewer, 1965</td>
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<td>Phialophora</td>
<td>Brewer, 1959, Eveleigh and Brewer, 1965</td>
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</tr>
<tr>
<td>Pseudoplectania</td>
<td>Eveleigh and Brewer, 1965</td>
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<td>Pullalaria</td>
<td>Coster, 1968</td>
<td></td>
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<tr>
<td>Rhizopus</td>
<td>Zabel, 1959</td>
<td></td>
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<tr>
<td>Spicaria</td>
<td>Zabel, 1959</td>
<td></td>
</tr>
<tr>
<td>Sporotrichum</td>
<td>Zabel, 1959, Eveleigh and Brewer, 1965</td>
<td></td>
</tr>
<tr>
<td>Type</td>
<td>Genus</td>
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<td>------------------------------------------------</td>
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<tr>
<td></td>
<td>Stachybotrys</td>
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</tr>
<tr>
<td></td>
<td>Thamnidium</td>
<td>Zabel, 1959</td>
</tr>
<tr>
<td></td>
<td>Trichodermia</td>
<td>Zabel, 1959, Eveleigh and Brewer, 1965</td>
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<tr>
<td></td>
<td></td>
<td>Coster, 1968</td>
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This material is extracellular in nature and is accumulated in a sticky mass around the cell. It may be organized into a capsule or microcapsule or it may be a loosely formed gelatinous mass in which the cell is embedded. There is no apparent metabolic function for the slime layer and organisms have been shown to survive when it has been removed. The amount and type of slime depends on the organism being studied. The slime layer has been shown to be chemically diverse. Some of the components include carbohydrates, protein, deoxyribonucleic acid and glutamyl peptide. The type and relative amount of the components varies from species to species (Salton, 1961 and Lamanna et al., 1973). It is this slime layer that has been postulated to be important in trapping debris which can accumulate into a slime deposit in the paper mill. The variation in types of organisms and their varied slime components, and the physical and chemical environment found in paper mills have made the control of slime deposits a complicated task.

The control programs used by mills have been described as a combination of chemical treatment and good housekeeping (Sanborn, 1965). Chemical treatment methods have been shown to be effective in both mill and laboratory studies (Shema, et al., 1948, Buckman, 1949, Shema, et al., 1949, Appling, 1949, DeGroff, 1953, Stern, 1959, Dietzel, 1960, Michalski, et al., 1963, Sanborn, 1965b, Coster, 1968, Bendt, 1970, and Andre, 1973). The main forms of chemical treatment have been biocides and biostats which were active against bacteria and fungi. The most widely used chemicals have been methylene-bis thiocyanate, organosulphur, and bromine or chlorophenate compounds (McLeish and Shockett,
1973). The use of dispersent agents has also become popular and studies have shown their effectiveness (Nelson, 1962 and Michalski, et al, 1963). Housekeeping procedures vary but have generally consisted of wash-ups, squirt-ups, and hypenate boil-outs preferably with some biocide compound (Sanborn, 1965c). While these methods have emphasized the killing of microorganisms as an adequate way of stopping slime build-up, the results in the mill have not always been satisfactory. Several reasons have been discussed trying to explain this phenomenon. The first and most obvious one was that not all slime deposits were caused by microorganisms. Sanborn (1965c) and Buckman and May (1970) have reviewed the topic of mill deposits and have documented several other forms of nonmicrobiological deposits. Other deposits included pitch and scale. Additional nonbiological deposits may be of a chemical or fibrous nature caused by some physical attraction of particles to parts of the paper machine. Dietzel and Kirchen (1959) have stated that an effective control program may be hampered by poor application in the mill due to lax control of the biocide treatment. A final reason for unsuitable control of slime may be the incorrect control program being applied to the system because of changes in the nature of the slime problem. Several authors have stressed the importance of constant monitoring of the mill conditions in order to control slime (Dietzel and Kirchen, 1959, Eddy, 1958, 1960, Buckman and Kirchen, 1958, DeGroff, 1953, and Sanborn, 1965c).

More recently the overkill method of removing all the organisms from the system has been questioned. Bucht (1969) discussed the
possibility of biological control instead of chemical control. This approach would entail a more detailed understanding of the overall ecology of the mill system. The idea of understanding the mill as an ecological system has not received widespread attention. However, Appling (1955) and Eveleigh and Brewer (1964 and 1965) have suggested the need for such work. Bendt (1971) suggested that the increase usage of slimicide may not be the only answer to slime control. A long term program of control that was well planned would benefit the mill in the long run.

Slime Control and Closure of Mill Water Systems

As stated previously, the question of slime control has not been adequately resolved. The problems encountered in slime control have been compounded with still another factor, the closing of mill water systems. Mills have closed their systems to reclaim fiber and water and to abate pollution (Bendt, 1971). The effects of closure have been explored by some investigators. The process of closing has been described by Kirchen (1949) as the replacing of fresh water with white water when the operation of the paper machine is not hampered. The process as described would save fresh water, heat and chemicals (Kirchen, 1949). An increase in slime accumulation associated with mill closure has been noted by several authors (Kirchen, 1948, Neal and Jennings, 1948, Buckman and Garcia, 1973 and Shema, 1973). Neal and Jennings (1948) stated the losses incurred through slime accumulations countered any gains from closing. Manogue (1954) and Martin-Lof (1973) have suggested increasing the temperature to 70°C and above would cause a decrease in the
slime accumulation. Janes and Aldrich (1971) and Aldrich (1970) surveyed mills which had closed white water systems and the most often reported problem caused by closure was an increase in slime accumulation and the accompanying problems it brings. It would seem, therefore, the study of slime accumulation in the paper industry is not fully completed. With this idea in mind, the following research was undertaken.
MATERIALS AND METHODS

Mill Survey

The mill

The mill, monitored in the study, manufactured paperboard for food boxes and containers. There were two cylinder type machines employed in the board production. Machine #1 had seven cylinders and produced about 200 tons of board per day. Machine #2 had eight cylinders and produced about 170 tons of board per day. Early in the study, the mill used about 8,000 to 10,000 gallons of fresh water per ton of board produced. Later in the study, the mill was partially closed and 4,000 to 5,000 gallons of fresh water were used per ton of board. The fresh water was treated with chlorine to a residual of .2 to .3 parts per million (ppm). The temperature, if controlled, was by the addition of steam to the water supply. The pH when controlled was adjusted in the stock by the addition of alum. Other environmental conditions affecting the growth of microorganisms were not controlled with the exception of the addition of slimicide. The pulp was mainly from waste paper.

The slime control program consisted of the addition of a methylene-bis thiocynate slimicide to the white water and periodic wash-ups. The slimicide was applied as slug doses at the machine chests just before the machine liner and filler vats. The amount of slimicide was .5 pounds per ton of fiber. The liner vats received slimicide for thirty minutes for each hour while the filler vats received
received treatment for forty-five minutes of each hour. Wash-ups occurred only when the slime problem was deemed to be out of control, or when the machine was down for repair or lack of production. The wash-ups consisted of hosing off vats and other surfaces which had accumulations. The slime growth was mainly in the vats and manifested itself by clogging pipes and showers. The slime build-up was also responsible for spots on the paper sheet, breaks in the paper sheet, and foul odors. The main problem areas were the filler and topliner vats.

It was hoped that by monitoring certain parameters of the mill system the factors of importance, for this mill, in the development of slime deposits could be defined.

The parameters studied

It was necessary to make an educated guess as to what factors might be affecting the build-up of slime in the system. After examining the literature and discussing the problem with mill officials, the slime build-up was concluded to be essentially micro-biological in nature. Factors that affected the growth of micro-organisms were then chosen for sampling. The sampling points were the topliner and filler vats of both machines.

Temperature of the white water was taken in situ with a mercury filled thermometer. The pH of the water was measured by a pH meter immersing the electrode into the vat. Water samples were taken and fixed at the mill, for later titration at the laboratory, for determination of dissolved oxygen by the azide modification of the Winkler method. Another water sample was taken for determination, in the lab, of total
Kjeldahl nitrogen and total phosphorus. The vanadyl molybdate method for phosphorus was used. The methods for dissolved oxygen, nitrogen and phosphorus were taken from the 13th Edition of Standard Methods (1971). Water samples were taken in sterile test tubes for total soluble carbohydrates by the indole method (Aswell, 1957) and total viable count of microorganisms as described in Standard Methods (1971). Slime accumulation was measured in situ by a qualitative scale based on several observations of slime accumulation conditions found at different times in the mill. The qualitative scale used is presented in Table 2. Samples of slime were taken in sterile containers for later microscopic examination. The fresh samples were examined using phase contrast microscopy for an assessment of the components included in the deposit. A portion of the sample was fixed in glutaraldehyde (3% in .1 M phosphate buffer) for subsequent processing for scanning electron microscopy (SEM). This portion of the sample was post-fixed with osmium tetraoxide (1% in .1 M phosphate buffer) for 2 hours and then washed several times with distilled water. The fixation was followed by dehydration through graded alcohols to absolute ethanol. For further processing the sample was run through graded ethanol-amyl acetate washes to 100% amyl acetate. The sample was then critical point dried (Anderson, 1951), coated with carbon and gold by a vacuum evaporator, and mounted in silver paint for SEM examination.
Table 2  
The Qualitative Slime Scale

<table>
<thead>
<tr>
<th>Qualitative Scale</th>
<th>Description of Deposit</th>
<th>Problem Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Clean, no accumulation</td>
<td>These levels of slime presented essentially</td>
</tr>
<tr>
<td></td>
<td></td>
<td>no problem.</td>
</tr>
<tr>
<td>1</td>
<td>Very thin layer, feels slippery</td>
<td>These levels were a potential problem with</td>
</tr>
<tr>
<td></td>
<td></td>
<td>some sloughing of deposit and spotting the</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sheet.</td>
</tr>
<tr>
<td>2</td>
<td>Thin layer, less than 1/8 inch gray color</td>
<td>These levels represented the worst</td>
</tr>
<tr>
<td></td>
<td></td>
<td>accumulations with much sloughing.</td>
</tr>
<tr>
<td>3</td>
<td>Moderate loose gray-mass or jelly-like mass about 1/8 inch</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1/8-1/4 inch gray slippery mass or layer on sides of vat</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1/4 inch gray slimy layer with jelly-like masses</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1/2 inch thick, much like 5 only thicker layer</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1/2-1 inch thick whitish gray mass, very loose and runny</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Very thick, greater than 1 inch, whitish gray mass often</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>with black gritty interior</td>
</tr>
<tr>
<td>9</td>
<td>Same as 8, with long strings or whiskers</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Extremely thick mat with many long strings, often with</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>odor and black center</td>
</tr>
</tbody>
</table>
Analysis of data

The data gathered on the various physical, chemical, and biological parameters were subjected to statistical analysis. A multiple linear regression run on the observations of slime accumulation (dependent variable) versus the observations for total count, nitrogen, phosphorus, carbohydrate, dissolved oxygen, pH, temperature, and the days elapsed since the last wash-up. The analysis was done on the computer using the MAD program developed at Brigham Young University (Bryce and Carter, 1974). Several other statistical analyses were performed by hand using a calculator.

Experimental Work

Shaker flask apparatus for laboratory evaluation of slime accumulation

A method for multiple determination of slime accumulation was needed and the following apparatus evolved. The required number of 125 ml Erlenmeyer flasks, which had been preweighed and sterilized, were incubated for 48 hours in a New Brunswick controlled environment shaker incubator. The flasks were filled with 50 ml of the water sample to be studied. During the course of the incubation period, an accumulation of slime would build up along the air water interface. After 48 hours, the water was removed from the flask by suction and the flasks, with the accumulation, were dried at 105°C overnight. The dried flasks were placed in a dessicator and cooled for at least one hour. The cooled flasks were weighed and the difference between the initial dry weight and the final dry weight was taken as a dry weight accumulation of slime. This method was used for several experiments described herein.
The basic shaker flask method was modified twice for certain experiments. The first modification consisted of placing, into the flasks, applicator sticks, to which clean glass coverslips had been attached. The flasks were stoppered with cotton and dry heat sterilized. The flasks were then filled with a water sample and incubated as before. Slime accumulated on the coverslip and was sampled at different times. The second modification was actually the adoption of the technique of Stern (1959). Tongue depressors were inserted into the flasks instead of applicator sticks and a slime deposit accumulated on them. This slime was sampled at different times and processed for SEM as described previously.

**Slime board technique for laboratory evaluation slime accumulation**

The slime board technique has been used for measuring slime accumulation by the paper industry. It has the drawback of being able to analyze one sample at a time. However, it was felt necessary to evaluate the qualitative scale and shaker flask method in comparison with this technique in order to be able to generalize the results of the other two methods. The slime board apparatus used followed the procedures developed at the National Council of the Paper Industry for Air and Stream Improvement, Inc. (Springer, 1973).

**Experimental examination of the factors effecting slime accumulation**

A factorial design experiment was run to look for the most obvious factors contributing to slime accumulation. The experimental design was a $2 \times 2^5$ design with split-plot confounding (Kempthorne, 1962). The split-plot was necessary to examine the temperature effect because
only one incubator was available for use. The shaker flask apparatus described above was used for this experiment. The six factors of temperature, pH, dissolved oxygen, nitrogen, phosphorus, and carbohydrate were varied while the number of microorganisms was held constant. A white water sample was analyzed for the above variables and then adjusted to a high and low level of each of the factors except temperature. The low levels were obtained by dilution with deionized distilled water of the original sample. The high levels were obtained as follows. Nitrogen was adjusted by the addition of anhydrous ammonium chloride, phosphorus by the addition of monobasic potassium phosphate and the carbohydrate by addition of glucose. The dissolved oxygen was at a high level of saturation at 150 rpm's on the shaker while the low level was obtained by the addition of nitrogen gas above the water sample and stoppering of the flask with a rubber stopper. The pH was adjusted with the addition of acid or base. The temperature was adjusted for the entire incubator by a thermostat. Table 3 contains the combinations of treatments for this experiment.

The data generated from this experiment design were analyzed using the MAD program (Bryce and Carter, 1974). The analysis of the data was performed on all the combinations shown in Table 3 and for the temperature by ABCDE interaction, replica effects, and temperature effects alone.

A second series of experiments was run to examine in detail the factors which were shown to be most influential in slime accumulation. Each factor was varied separately with all other variables being held constant. It was hoped that from the two experiments the
Table 3

Treatment Combination for the $2 \times 2^5$ Design

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dissolved $O_2$ (A)</th>
<th>pH (B)</th>
<th>Carbohydrate (C)</th>
<th>P (D)</th>
<th>N (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td>e</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td>e</td>
</tr>
<tr>
<td>3</td>
<td>a</td>
<td>B</td>
<td>c</td>
<td>d</td>
<td>e</td>
</tr>
<tr>
<td>4</td>
<td>a</td>
<td>b</td>
<td>C</td>
<td>d</td>
<td>e</td>
</tr>
<tr>
<td>5</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td>E</td>
</tr>
<tr>
<td>6</td>
<td>A</td>
<td>B</td>
<td>c</td>
<td>d</td>
<td>e</td>
</tr>
<tr>
<td>7</td>
<td>A</td>
<td>b</td>
<td>C</td>
<td>d</td>
<td>e</td>
</tr>
<tr>
<td>8</td>
<td>A</td>
<td>b</td>
<td>c</td>
<td>D</td>
<td>e</td>
</tr>
<tr>
<td>9</td>
<td>A</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td>E</td>
</tr>
<tr>
<td>10</td>
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<td>B</td>
<td>c</td>
<td>d</td>
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<td>11</td>
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<td>c</td>
<td>D</td>
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<td>B</td>
<td>c</td>
<td>d</td>
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<td>C</td>
<td>D</td>
<td>e</td>
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<td>C</td>
<td>d</td>
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<td>D</td>
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</tr>
<tr>
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<td>B</td>
<td>c</td>
<td>D</td>
<td>e</td>
</tr>
<tr>
<td>19</td>
<td>A</td>
<td>B</td>
<td>c</td>
<td>d</td>
<td>E</td>
</tr>
</tbody>
</table>
Table 3 (continued)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dissolved O$_2$ (A)</th>
<th>pH (B)</th>
<th>Carbohydrate (C)</th>
<th>P (D)</th>
<th>N (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>A</td>
<td>b</td>
<td>C</td>
<td>D</td>
<td>e</td>
</tr>
<tr>
<td>21</td>
<td>A</td>
<td>b</td>
<td>C</td>
<td>d</td>
<td>E</td>
</tr>
<tr>
<td>22</td>
<td>A</td>
<td>b</td>
<td>c</td>
<td>D</td>
<td>E</td>
</tr>
<tr>
<td>23</td>
<td>a</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>e</td>
</tr>
<tr>
<td>24</td>
<td>a</td>
<td>B</td>
<td>C</td>
<td>d</td>
<td>E</td>
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<td>c</td>
<td>D</td>
<td>E</td>
</tr>
<tr>
<td>26</td>
<td>a</td>
<td>b</td>
<td>C</td>
<td>D</td>
<td>E</td>
</tr>
<tr>
<td>27</td>
<td>A</td>
<td>B</td>
<td>c</td>
<td>D</td>
<td>E</td>
</tr>
<tr>
<td>28</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>e</td>
</tr>
<tr>
<td>29</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>d</td>
<td>E</td>
</tr>
<tr>
<td>30</td>
<td>A</td>
<td>b</td>
<td>C</td>
<td>D</td>
<td>E</td>
</tr>
<tr>
<td>31</td>
<td>a</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
</tr>
<tr>
<td>32</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
</tr>
</tbody>
</table>

$^a$A small letter indicated the low level

$^b$A capital letter indicated the high level
major contributors to slime accumulation could be found and then examined in depth.

After some initial work the question of the effect of slimicide on the shaker flask response was raised and an experiment was set up to test the effect of various dilutions of slimicide on the slime accumulation in the shaker flasks. The technique was as before and the dry weight accumulation and total viable count were recorded for each dilution of slimicide. Two sets of experiments were run. The total count for the first set was measured by plate counts and by Biostix test strips (Ames Company, Elkhart, Indiana). The second set had total count determined by the Biostix only.

Sequential experiments

It was felt that the sequence of events in slime accumulation could be monitored using the modified shaker flask technique as described previously. The first experiment was run for 48 hours at 30°C and total counts, slime accumulation, and microorganisms present were monitored. The microorganisms were isolated and pure cultures were made. Identification of the organisms was from the pure culture. The slime accumulations were processed for SEM as described. Counts were by plate count and Biostix. Samples were taken at 0, 2, 8, 12, 24, and 48 hours.

The second experiment used the Stern technique and a 1 mg sample of slime, in addition to the other samples, was taken for microorganism identification.
Identification of microorganisms

Media:

1. enriched nutrient broth (ENB)
   - 5 gm Bacto peptone
   - 5 gm Bacto yeast extract
   - 5 gm glucose
   - 3 gm Bacto beef extract
   - 1 L distilled water

2. enriched nutrient agar
   - ENB plus 15 gm Bacto agar

3. Saboroud agar

4. Vaspar
   - 70 gm petrolatum
   - 30 gm paraffin

5. Carbohydrate utilization medium (Hugh and Leifson, 1953)

6. MR-VP medium

7. Koser citrate broth

Methods and stains:

1. Gram stain
2. spore stain
3. Houwink and Van Iterson (1950) method for flagella
4. oxidase test (Kovacs, 1956)
5. catalase test

After performing the various tests the results were interpreted using the following: Buchanan and Gibbons (1974), Sanborn (1965a), Skerman (1967), Funder (1953), Barnett and Hunter (1972), Hendrie and Shewan (1966), Carpenter, et al (1966), Baird-Parker (1966) and Shewan (1963).
Chemicals

The chemicals used for SEM preparations were obtained from E. J. Fullam Co. (Schenectady, New York). Unless otherwise stated all other chemicals were of reagent grade and obtained from a chemical supply house.
RESULTS

Mill Survey

Survey of the mill began in March, 1974 and continued through April, 1975. During that period, samples as described in Materials and Methods were taken at various times during the week from both machines' topliner and filler vats. The temperature, pH, nitrogen, phosphorus, dissolved oxygen, carbohydrate, qualitative slime accumulation, total viable count, and day elapsed from the last wash-up were recorded. Not all of these parameters were taken from each machine on each visit to the mill due to losses of samples, breakdown of equipment, changes in parameters as the study progressed, and other miscellaneous errors in sampling. A complete set of all nine parameters was collected eighty-three times and this set of complete data were analyzed on the computer by multiple regression analysis using the MAD program (Bryce and Carter, 1974) developed at Brigham Young University.

The MAD program is "capable of analyzing unbalanced (and balanced) univeriate and multivariate analysis of varience problems as well as univariate and multivariate regression problems." It allows a model to be made and variations of the model to be described. It will then perform analysis of the model and the variations given the data for various variables. Any number of variables can be analyzed as the dependent variable with all or part of the other variables being the independent variables.
The initial experimental design model analyzed included all the parameters, the second order interaction terms and the square terms of the parameters. The dependent variable was slime accumulation which was expressed as the qualitative slime scale described in Material and Methods plus the constant one (1). This was done to remove the value of zero which would not be properly analyzed by the program. The independent variables included pH, temperature, nitrogen, phosphorus, carbohydrate, dissolved oxygen, log of total viable count, day from wash-up, machine, and vat. The machine and vat terms of the model were used to pick out differences between machine responses and vat differences. The machine and vat terms were in the model as main effects and the two-way interaction. All the other variables were in the model as two factor interactions and square terms in order to describe any curvature in the response. Subsequent analysis reduced the model by removing the interactions only, the square terms only, and both the interactions and the squares and analyzing the data based on these factors only. Analysis of the different variations of the full model allowed reduction to the simplest experimental design model of slime accumulation. The method of testing the validity of reducing the model, in the various ways, employed the F-test. The "F" statistic was determined for reduction of the model by the equation:

\[ F = \frac{[\text{SSE}(\text{reduced}) - \text{SSE}(\text{full})][\text{df}(\text{reduced}) - \text{df}(\text{full})]}{\text{MSE}(\text{full})} \]

where \( \text{df} \) = degrees of freedom in the error term
reduced = reduced experimental model
full = full experimental model
SSE = sum of squares of error term
MSE = mean square of error

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The degrees of freedom for the F statistic are calculated by subtracting the degrees of freedom of the full model from the df of the reduced model (the numerator). The denominator is the df of the full model. Table 4 gives an example of this calculation. A summary of all the calculations of all the models tested is found in Table 5. From the results shown in Table 5, it was concluded that the interaction terms could be dropped from the experimental design model and the main effects plus their squares would be the simplest experimental design model for the data. The analysis of variance of the data using this model is found in Table 6.

The analysis showed that a significant amount of the regression of the model describing slime accumulation was due to the day from wash-up, the total count and their square terms. The slime accumulation increased with day from wash-up but as the day increased the accumulation was decreased due to the square of the day. The same relationship was shown with total count. These results are confounded by the fact there was a significant difference between the topliner and filler vats on the different machines. Figure 1 shows this difference. Figure 2 shows the shape of the theoretical curves of slime accumulation with respect to the factors shown significant and all of the others being held constant.

As stated before, not all the parameters were sampled every time or at all sample stations. A summary of all the data collected is found in Table 7.

The monthly variation of the various parameters is shown in Figures 3-5. There was a good deal of fluctuation from month to month.
Table 4

Testing a Possible Reduced Model of the Slime Accumulation

<table>
<thead>
<tr>
<th></th>
<th>Full</th>
<th>Reduced</th>
</tr>
</thead>
<tbody>
<tr>
<td>df</td>
<td>35</td>
<td>43</td>
</tr>
<tr>
<td>SSE</td>
<td>65.132</td>
<td>129.77</td>
</tr>
<tr>
<td>MSE</td>
<td>1.8609</td>
<td></td>
</tr>
</tbody>
</table>

\[ F(8,35) = \frac{(129.77-65.123)/8}{1.809} = 4.342^{b} \]

\(^a\) The full model was run with all terms as described above and then reduced by eliminating the square terms.

\(^b\) The test F from the table for df (8,35) is 2.49 at the .05 level. Therefore the F value calculated is significant and the square terms alone cannot be excluded from the experimental design model.
Table 5
A Summary of the F Tests for All Reduced Models of Slime Accumulation

<table>
<thead>
<tr>
<th>Model</th>
<th>df</th>
<th>SSE</th>
<th>MSE</th>
<th>&quot;F&quot;</th>
<th>Sign.</th>
</tr>
</thead>
<tbody>
<tr>
<td>All main effects, interactions</td>
<td>35</td>
<td>65.1</td>
<td>1.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(two way) and squares of main effects.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effects plus two way interactions.</td>
<td>43</td>
<td>129.9</td>
<td>3.02</td>
<td>4.34</td>
<td>*a</td>
</tr>
<tr>
<td>Main effects plus squares</td>
<td>63</td>
<td>146.8</td>
<td>2.33</td>
<td>1.57</td>
<td>N.S.</td>
</tr>
<tr>
<td>Main effects alone</td>
<td>71</td>
<td>187.9</td>
<td>2.64</td>
<td>1.83</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

*a significant at .05 level

b N.S. = not significant at the .05 level
Table 6
Multiple Regression Analysis of Mill Data

<table>
<thead>
<tr>
<th>Source</th>
<th>Prediction Coefficient</th>
<th>&quot;F&quot;</th>
<th>Significance at .05 Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day from last wash-up</td>
<td>.18</td>
<td>13.60</td>
<td>*&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Temperature</td>
<td>.20</td>
<td>.37</td>
<td>n.s.</td>
</tr>
<tr>
<td>Dissolved oxygen (D.O.)</td>
<td>.04</td>
<td>.00</td>
<td>n.s.</td>
</tr>
<tr>
<td>pH</td>
<td>1.32</td>
<td>.23</td>
<td>n.s.</td>
</tr>
<tr>
<td>Total Kjeldahl nitrogen (N)</td>
<td>.06</td>
<td>2.34</td>
<td>n.s.</td>
</tr>
<tr>
<td>Total phosphate (P)</td>
<td>.09</td>
<td>.12</td>
<td>n.s.</td>
</tr>
<tr>
<td>Total soluble carbohydrate (C)</td>
<td>.00</td>
<td>3.31</td>
<td>n.s.</td>
</tr>
<tr>
<td>Log count</td>
<td>1.33</td>
<td>7.09</td>
<td>*</td>
</tr>
<tr>
<td>Day square term</td>
<td>-.01</td>
<td>7.07</td>
<td>*</td>
</tr>
<tr>
<td>Temperature square term</td>
<td>-.01</td>
<td>1.04</td>
<td>n.s.</td>
</tr>
<tr>
<td>D.O. square term</td>
<td>-.01</td>
<td>.25</td>
<td>n.s.</td>
</tr>
<tr>
<td>pH square term</td>
<td>.12</td>
<td>.26</td>
<td>n.s.</td>
</tr>
<tr>
<td>N square term</td>
<td>.00</td>
<td>.79</td>
<td>n.s.</td>
</tr>
<tr>
<td>P square term</td>
<td>.05</td>
<td>.27</td>
<td>n.s.</td>
</tr>
<tr>
<td>C square term</td>
<td>.00</td>
<td>1.53</td>
<td>n.s.</td>
</tr>
<tr>
<td>Count square term</td>
<td>-.13</td>
<td>6.71</td>
<td>*</td>
</tr>
</tbody>
</table>
Table 6 (continued)

<table>
<thead>
<tr>
<th>Source</th>
<th>Prediction Coefficient</th>
<th>&quot;F&quot;</th>
<th>Significance at .05 Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Machine</td>
<td>.46</td>
<td>1.42</td>
<td>n.s.</td>
</tr>
<tr>
<td>Vat</td>
<td>.59</td>
<td>4.1</td>
<td>*</td>
</tr>
<tr>
<td>Machine/vat interaction</td>
<td>-.73</td>
<td>7.86</td>
<td>*</td>
</tr>
</tbody>
</table>

*a* significant  

b n.s. not significant
Figure 1

Estimated mean slime accumulation as given by the MAD experimental design model.
Theoretical slime accumulation based on the experimental design model generated by the MAD program. These curves are for Machine #2 filler vat (vat #2) and three different log counts. The symbols for the three counts are as follows:

- log count = 2  ○
- log count = 4  ▲
- log count = 6  □

The equation for these curves is:

\[ Y = 2.32 + .18 \text{ day} + 1.33 \text{ count} - .01 \text{ day}^2 - .13 \text{ count}^2 + .59 \text{ vat} - .73 (\text{machine})(\text{vat}) \]

Where \( Y \) is slime accumulation:

- Day is \# of days elapsed since wash-up
- Count is log total viable count/ml
- Vat is a constant equal to 1 for topliner and 2 for filler
- Machine is a constant equal to 1 for machine #1, and 2 for machine #2
Table 7
Summary of Mill Data\(^a\)

<table>
<thead>
<tr>
<th>Machine</th>
<th>Vat</th>
<th>Temperature (^b)</th>
<th>pH</th>
<th>Dissolved Oxygen (^c)</th>
<th>Total Kjeldahl nitrogen (^c)</th>
<th>Total phosphate (^c)</th>
<th>Total Soluble Carbohydrate (^c)</th>
<th>Log(^d) Count</th>
<th>Slime(^e) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Topliner</td>
<td>18</td>
<td>3.8</td>
<td>1.0</td>
<td>2.0</td>
<td>1.1</td>
<td>100</td>
<td>2.00</td>
<td>0 Minimum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>42</td>
<td>7.7</td>
<td>8.6</td>
<td>50.8</td>
<td>13</td>
<td>4408</td>
<td>6.34</td>
<td>7 Maximum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32.9</td>
<td>5.2</td>
<td>4.2</td>
<td>22.8</td>
<td>3.3</td>
<td>1517</td>
<td>3.32</td>
<td>0 Average</td>
</tr>
<tr>
<td>Filler</td>
<td>30</td>
<td>3.9</td>
<td>1.1</td>
<td>5.5</td>
<td>0.5</td>
<td>100</td>
<td>2.00</td>
<td>0</td>
<td>Minimum</td>
</tr>
<tr>
<td></td>
<td>53</td>
<td>7.7</td>
<td>6.6</td>
<td>67.3</td>
<td>9.4</td>
<td>5980</td>
<td>7.58</td>
<td>10</td>
<td>Maximum</td>
</tr>
<tr>
<td></td>
<td>40.8</td>
<td>6.3</td>
<td>3.8</td>
<td>20.5</td>
<td>3.3</td>
<td>1644</td>
<td>4.93</td>
<td>2</td>
<td>Average</td>
</tr>
<tr>
<td>2</td>
<td>Topliner</td>
<td>28</td>
<td>3.9</td>
<td>1.9</td>
<td>6.8</td>
<td>0.7</td>
<td>100</td>
<td>3.07</td>
<td>0 Minimum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>46</td>
<td>7.9</td>
<td>9.9</td>
<td>57.0</td>
<td>6.9</td>
<td>4000</td>
<td>7.61</td>
<td>9 Maximum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36.5</td>
<td>7.1</td>
<td>5.2</td>
<td>18.9</td>
<td>3.1</td>
<td>1780</td>
<td>5.99</td>
<td>3 Average</td>
</tr>
<tr>
<td>Filler</td>
<td>27</td>
<td>3.8</td>
<td>1.9</td>
<td>6.7</td>
<td>0.1</td>
<td>60</td>
<td>3.70</td>
<td>0</td>
<td>Minimum</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>8.1</td>
<td>6.9</td>
<td>93.1</td>
<td>6.6</td>
<td>2900</td>
<td>7.68</td>
<td>9</td>
<td>Maximum</td>
</tr>
<tr>
<td></td>
<td>37.2</td>
<td>7.4</td>
<td>4.5</td>
<td>19.9</td>
<td>2.5</td>
<td>1189</td>
<td>6.46</td>
<td>3</td>
<td>Average</td>
</tr>
</tbody>
</table>
Table 7 (continued)

A summary of the data collected during the mill survey. The table includes the minimum, maximum, and average values recorded for the various parameters on each machine and vat.

\(^a\) Degrees Centigrade

\(^b\) Milligrams per liter

\(^c\) Organisms per milliliter (viable)

\(^d\) Qualitative scale for slime

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Figure 3

Average monthly variation of the parameters

All values plotted are the averages of the observations made during the particular month. Any spaces in the line connecting the points indicates a lack of sufficient data for that parameter during that month.

Symbols:  
- Machine #1 topliner  ○
- Machine #1 filler  ●
- Machine #2 topliner  ▲
- Machine #2 filler  □
Figure 4

Average monthly variation of the parameters

All values plotted are the averages of the observations made during the particular month. Any spaces in the line connecting the points indicates a lack of sufficient data for that parameter during that month.

Symbols:  
- Machine #1 topliner  ○
- Machine #1 filler  ●
- Machine #2 topliner  ▲
- Machine #2 filler  □
Figure 5

Average monthly variation of the parameters

All values plotted are the averages of the observations made during the particular month. Any spaces in the line connecting the points indicates a lack of sufficient data for that parameter during that month.

Symbols:
- Machine #1 topliner  ○
- Machine #1 filler     ●
- Machine #2 topliner  ▲
- Machine #2 filler     □
for most parameters. It was noted that pH routinely was lower on
Machine #1 topliner vat. The filler vat on Machine #1 had, for the
most part, a lower pH than all others except the topliner vat of
Machine #1. The pH was stabilized at about 7.5 for topliner and 7.3
for filler on Machine #2 during the last two thirds of the study.
Slime accumulation was nearly always 0 on Machine #1, topliner vat.
The filler vat of Machine #1, while higher than the topliner,
generally had a lower accumulation than either vat on Machine #2.
The slime accumulation in the topliner and filler vat of Machine #2
was alternately higher than all others.

The other parameters seemed to vary randomly with one
exception. During the last four months of the study, the nitrogen
levels of all sample points steadily decreased.

The mill data collected during the survey were subjected to
an additional analysis. This was done to pick up any one variable
as being a significant factor in slime accumulation. The parameters
of log count, total soluble carbohydrate, nitrogen, phosphorus, pH,
temperature, and dissolved oxygen were compared to slime accumulation
using Chi-square contingency tables. The slime accumulation was
grouped according to the relative problem it would cause as described
in Materials and Methods. The other variables were grouped on an
arbitrary scale of low, medium, and high as observed during the study.
The Chi-square analyses are summarized in Table 8.
Table 8
Chi-square Analysis of Data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chi-square</th>
<th>df</th>
<th>Significance</th>
<th>Contingency Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day from last wash-up</td>
<td>8.89733</td>
<td>4</td>
<td>.01</td>
<td>.1443005</td>
</tr>
<tr>
<td>Temperature</td>
<td>3.34841</td>
<td>4</td>
<td>.50</td>
<td>.0706228</td>
</tr>
<tr>
<td>pH</td>
<td>13.89966</td>
<td>4</td>
<td>.01</td>
<td>.1427716</td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>4.79874</td>
<td>4</td>
<td>.31</td>
<td>.0849607</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>9.32188</td>
<td>4</td>
<td>.05</td>
<td>.1209406</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>3.69643</td>
<td>4</td>
<td>.16</td>
<td>.1216704</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>4.51399</td>
<td>4</td>
<td>.34</td>
<td>.0957350</td>
</tr>
<tr>
<td>Log count</td>
<td>9.14099</td>
<td>4</td>
<td>.06</td>
<td>.1920193</td>
</tr>
</tbody>
</table>

Slime Accumulation Observations

During the course of the mill survey several samples of slime deposits were collected and examined grossly and microscopically. Fresh samples were examined by phase contrast microscope under oil immersion. There were several different types of materials found in the deposits. Bacteria consisting of motile rods and nonmotile rods and cocci were often found in varying concentrations. Flat flake-like materials determined to be clay particles were found, round irregular particles
possibly either sand or glass particles were seen and often had a green or orange color. The orange particles might also have been oxidized iron particles. Fibers of various lengths were found both singularly and matted together. These constituents were often held together in a matrix of amorphous jelly-like material. Not every deposit had all the constituents described above and all the relative amounts varied from sample to sample. Several samples were fixed and examined by scanning electron microscopy. Figures 6 and 7 show some typical observations of this material.

The gross appearance of the various slime samples varied. Some samples were a loose gray and white material of runny consistency. Many samples were gray to gray-white intermixed with a neutral, clear, jelly-like mass. A few samples were found to have a black and odoriferous center indicating an anaerobic condition at the center of the deposit. Some dark gray colored samples were noted and a number of samples contained a reddish orange pigment.

Laboratory Examination of Some Factors Affecting Slime Accumulation

After the mill survey was completed, the shaker flask apparatus was used to test, under controlled conditions, the importance of the various parameters as they affected slime accumulation. The $2^5$ factorial design with split-plot confounding was used (Kempthorne, 1962). The purpose of this design was to screen the parameters of a high level and a low level, based on the mill observations, and to pick out major contributors in slime accumulation. The shaker flask technique was
Figure 6

SEM photomicrograph of a slime accumulation

The photo shows the accumulation of clay particles, sand-like particles and fibers. 3000x.
Figure 7

SEM photograph of a slime deposit

The closely intertwined nature of the fibers and associated materials is apparent. This deposit is at the stage where bacteria become entrapped and begin to lay down a gelatinous slimy mass. 150x.
described in Materials and Methods. Table 9 contains the parameters and the levels at which they were run.

The results of the experiment were analyzed using the MAD program (Bryce and Carter, 1974) and the analysis of variance table is presented in Table 10. The main effects of pH, carbohydrate, and phosphorus were the most dominant factors in slime accumulation. The slime accumulation increased with increased pH while it decreased with increased carbohydrate and phosphorus. The estimated mean effects are shown in Figure 8.

Further investigation of the factors involved with slime accumulation was limited to examination of the three main effects discovered in the $2 \times 2^5$ design experiment. Water samples were treated by adjusting the pH or carbohydrate or phosphorous level while holding all the other factors constant. Table 11 details the levels explored in this set of experiments. The results of these experiments are shown in Figure 9. The effects of pH and carbohydrate while varying over the range had the same relative effect at the extremes. Phosphorus did not show the same magnitude of responses as was seen previously.

The Effect of Slimicide on Slime Accumulation as Measured by Shaker Flasks

During the course of the study, it became increasingly apparent that some factor was not being monitored and controlled thus causing some inconsistent results. It was thought that the level of slimicide was causing responses to vary. The effects that slimicide exerted
Table 9
Levels for Variables in $2 \times 2^5$ Design

<table>
<thead>
<tr>
<th>Parameter</th>
<th>High Level</th>
<th>Low Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>50°C</td>
<td>30°C</td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>5.9 mg/L. @30°C</td>
<td>.7 mg/L. @30°C</td>
</tr>
<tr>
<td></td>
<td>2.9 mg/L. @50°C</td>
<td>.3 mg/L. @50°C</td>
</tr>
<tr>
<td>pH</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1.5 mg/L.</td>
<td>10 mg/L.</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>.7 mg/L.</td>
<td>100 mg/L.</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>420 mg/L.</td>
<td>4000 mg/L.</td>
</tr>
<tr>
<td>Total viable count</td>
<td>held constant at $10^5-10^6$ cells/ml</td>
<td></td>
</tr>
</tbody>
</table>

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### Table 10

#### Analysis of Variance, $2^5$ Design

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>S.S./M.S.</th>
<th>Significance .05</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>127</td>
<td>169,788,300</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Whole plot</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replicas</td>
<td>1</td>
<td>8,373,260</td>
<td>8,373,260</td>
<td>7.35</td>
<td>n.s.</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>3,447,130</td>
<td>3,447,130</td>
<td>3.29</td>
<td>n.s.</td>
</tr>
<tr>
<td>ABCDE</td>
<td>1</td>
<td>1,144,370</td>
<td>1,144,370</td>
<td>1.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>TxABCD</td>
<td>1</td>
<td>2,090,109</td>
<td>2,090,109</td>
<td>1.84</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Whole plot error</strong></td>
<td>3</td>
<td>3,415,140</td>
<td>1,138,380</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Split plots</strong></td>
<td>60</td>
<td>67,301,000</td>
<td>1,121,680</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td>50,721</td>
<td>50,721</td>
<td>.05</td>
<td>n.s.</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>21,849,400</td>
<td>21,849,400</td>
<td>19.48</td>
<td>*c</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>5,428,510</td>
<td>5,428,510</td>
<td>4.84</td>
<td>*</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>6,993,800</td>
<td>6,993,800</td>
<td>6.24</td>
<td>*</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td>84,769</td>
<td>84,769</td>
<td>.08</td>
<td>n.s.</td>
</tr>
<tr>
<td>AB</td>
<td>1</td>
<td>71,537</td>
<td>71,537</td>
<td>.06</td>
<td>n.s.</td>
</tr>
<tr>
<td>AC</td>
<td>1</td>
<td>254,720</td>
<td>254,720</td>
<td>.23</td>
<td>n.s.</td>
</tr>
<tr>
<td>AD</td>
<td>1</td>
<td>4,261,010</td>
<td>4,261,010</td>
<td>3.79</td>
<td>n.s.</td>
</tr>
<tr>
<td>AF</td>
<td>1</td>
<td>32,896</td>
<td>32,896</td>
<td>.03</td>
<td>n.s.</td>
</tr>
<tr>
<td>BC</td>
<td>1</td>
<td>327,848</td>
<td>327,848</td>
<td>.29</td>
<td>n.s.</td>
</tr>
<tr>
<td>BD</td>
<td>1</td>
<td>1,701,550</td>
<td>1,701,550</td>
<td>1.52</td>
<td>n.s.</td>
</tr>
<tr>
<td>BF</td>
<td>1</td>
<td>78,606</td>
<td>78,606</td>
<td>.07</td>
<td>n.s.</td>
</tr>
<tr>
<td>CD</td>
<td>1</td>
<td>625,801</td>
<td>625,801</td>
<td>.56</td>
<td>n.s.</td>
</tr>
<tr>
<td>CE</td>
<td>1</td>
<td>90,100</td>
<td>90,100</td>
<td>.08</td>
<td>n.s.</td>
</tr>
<tr>
<td>DE</td>
<td>1</td>
<td>13,203</td>
<td>13,203</td>
<td>.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>BCDE</td>
<td>1</td>
<td>108,345</td>
<td>108,345</td>
<td>.10</td>
<td>n.s.</td>
</tr>
<tr>
<td>AT</td>
<td>1</td>
<td>1,093</td>
<td>1,093</td>
<td>.00</td>
<td>n.s.</td>
</tr>
<tr>
<td>TBCDE</td>
<td>1</td>
<td>156,101</td>
<td>156,101</td>
<td>.14</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

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Table 10 (continued)

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>S.S./M.S.</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACDE</td>
<td>1</td>
<td>297,606</td>
<td>297,606</td>
<td>.27</td>
<td>n.s.</td>
</tr>
<tr>
<td>BT</td>
<td>1</td>
<td>1,077,150</td>
<td>1,077,150</td>
<td>.96</td>
<td>n.s.</td>
</tr>
<tr>
<td>TACDE</td>
<td>1</td>
<td>1,181,570</td>
<td>1,181,570</td>
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<td>n.s.</td>
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<td>1</td>
<td>289,180</td>
<td>289,180</td>
<td>.26</td>
<td>n.s.</td>
</tr>
<tr>
<td>CT</td>
<td>1</td>
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<td>4,118</td>
<td>.00</td>
<td>n.s.</td>
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<tr>
<td>TABDE</td>
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<td>1,319,910</td>
<td>1,319,910</td>
<td>1.18</td>
<td>n.s.</td>
</tr>
<tr>
<td>ABCE</td>
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<td>2,271,650</td>
<td>2,271,650</td>
<td>2.03</td>
<td>n.s.</td>
</tr>
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<td>2.18</td>
<td>n.s.</td>
</tr>
<tr>
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<td>.20</td>
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</tr>
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<td>ABCD</td>
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<td>1,245,040</td>
<td>1,245,040</td>
<td>1.11</td>
<td>n.s.</td>
</tr>
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<td>ET</td>
<td>1</td>
<td>10,013</td>
<td>12,013</td>
<td>.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>CDE</td>
<td>1</td>
<td>1,030,690</td>
<td>1,030,690</td>
<td>.92</td>
<td>n.s.</td>
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<td>ABT</td>
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<td>1,301</td>
<td>1,301</td>
<td>.00</td>
<td>n.s.</td>
</tr>
<tr>
<td>TCDE</td>
<td>1</td>
<td>60,552</td>
<td>60,552</td>
<td>.05</td>
<td>n.s.</td>
</tr>
<tr>
<td>BDE</td>
<td>1</td>
<td>102,491</td>
<td>102,491</td>
<td>.09</td>
<td>n.s.</td>
</tr>
<tr>
<td>TAC</td>
<td>1</td>
<td>2,414,500</td>
<td>2,414,500</td>
<td>2.15</td>
<td>n.s.</td>
</tr>
<tr>
<td>TBDE</td>
<td>1</td>
<td>903,168</td>
<td>903,168</td>
<td>.80</td>
<td>n.s.</td>
</tr>
<tr>
<td>BCE</td>
<td>1</td>
<td>293,187</td>
<td>293,187</td>
<td>.26</td>
<td>n.s.</td>
</tr>
<tr>
<td>ADT</td>
<td>1</td>
<td>58,996</td>
<td>58,996</td>
<td>.05</td>
<td>n.s.</td>
</tr>
<tr>
<td>TBCE</td>
<td>1</td>
<td>412,232</td>
<td>412,232</td>
<td>.36</td>
<td>n.s.</td>
</tr>
<tr>
<td>BCD</td>
<td>1</td>
<td>51,681</td>
<td>51,681</td>
<td>.05</td>
<td>n.s.</td>
</tr>
<tr>
<td>AET</td>
<td>1</td>
<td>157,220</td>
<td>157,220</td>
<td>.14</td>
<td>n.s.</td>
</tr>
<tr>
<td>TBCD</td>
<td>1</td>
<td>88,937</td>
<td>88,937</td>
<td>.08</td>
<td>n.s.</td>
</tr>
<tr>
<td>ADE</td>
<td>1</td>
<td>699,449</td>
<td>699,449</td>
<td>.62</td>
<td>n.s.</td>
</tr>
<tr>
<td>BCT</td>
<td>1</td>
<td>525,825</td>
<td>525,825</td>
<td>.47</td>
<td>n.s.</td>
</tr>
<tr>
<td>TADE</td>
<td>1</td>
<td>371,522</td>
<td>371,522</td>
<td>.33</td>
<td>n.s.</td>
</tr>
<tr>
<td>ACE</td>
<td>1</td>
<td>530,192</td>
<td>530,192</td>
<td>.47</td>
<td>n.s.</td>
</tr>
<tr>
<td>BDT</td>
<td>1</td>
<td>3,977,610</td>
<td>3,977,610</td>
<td>3.54</td>
<td>n.s.</td>
</tr>
<tr>
<td>Source</td>
<td>Degrees of Freedom</td>
<td>Sum of Squares</td>
<td>Mean Square</td>
<td>S.S./M.S.</td>
<td>Significance .05</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------</td>
<td>----------------</td>
<td>-------------</td>
<td>-----------</td>
<td>-----------------</td>
</tr>
<tr>
<td>TACE</td>
<td>1</td>
<td>467,545</td>
<td>467,545</td>
<td>.42</td>
<td>n.s.</td>
</tr>
<tr>
<td>ACD</td>
<td>1</td>
<td>1,710,330</td>
<td>1,710,330</td>
<td>1.52</td>
<td>n.s.</td>
</tr>
<tr>
<td>BET</td>
<td>1</td>
<td>113,168</td>
<td>113,168</td>
<td>.10</td>
<td>n.s.</td>
</tr>
<tr>
<td>TACD</td>
<td>1</td>
<td>66,887</td>
<td>66,887</td>
<td>.06</td>
<td>n.s.</td>
</tr>
<tr>
<td>AEB</td>
<td>1</td>
<td>431,753</td>
<td>431,753</td>
<td>.38</td>
<td>n.s.</td>
</tr>
<tr>
<td>CDT</td>
<td>1</td>
<td>43,808</td>
<td>43,808</td>
<td>.04</td>
<td>n.s.</td>
</tr>
<tr>
<td>TABE</td>
<td>1</td>
<td>568,178</td>
<td>568,178</td>
<td>.51</td>
<td>n.s.</td>
</tr>
<tr>
<td>ABD</td>
<td>1</td>
<td>3,137,510</td>
<td>3,137,510</td>
<td>2.80</td>
<td>n.s.</td>
</tr>
<tr>
<td>CET</td>
<td>1</td>
<td>32,832</td>
<td>32,832</td>
<td>.03</td>
<td>n.s.</td>
</tr>
<tr>
<td>TABD</td>
<td>1</td>
<td>1,180,190</td>
<td>1,180,190</td>
<td>.99</td>
<td>n.s.</td>
</tr>
<tr>
<td>ABC</td>
<td>1</td>
<td>1,696,480</td>
<td>1,696,480</td>
<td>1.51</td>
<td>n.s.</td>
</tr>
<tr>
<td>DET</td>
<td>1</td>
<td>342,999</td>
<td>342,999</td>
<td>.31</td>
<td>n.s.</td>
</tr>
<tr>
<td>TABC</td>
<td>1</td>
<td>180,540</td>
<td>180,540</td>
<td>.16</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

a n.s. not significant

b A--dissolved oxygen
B--pH
C--carbohydrate
D--phosphorus
E--nitrogen
c*--significant
Figure 8

Figure 8a: The mean effects of pH as estimated through $2 \times 2^5$ model

Slime accumulation is in mg/50 ml white water.

Figure 8b: The mean effects of carbohydrate as estimated through the $2 \times 2^5$ model

Slime accumulation is in mg/50 ml white water and carbohydrate is in mg/L.

Figure 8c: The mean effects of phosphorus as estimated through the $2 \times 2^5$ model

Slime accumulation is in mg/50 ml white water and phosphorus is in mg/L.
<table>
<thead>
<tr>
<th>pH</th>
<th>4.0</th>
<th>4.7</th>
<th>5.5</th>
<th>6.3</th>
<th>7.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>phosphorus</td>
<td>1.5mg/L</td>
<td>3.5mg/L</td>
<td>6.5mg/L</td>
<td>8.5mg/L</td>
<td>11.5mg/L</td>
</tr>
<tr>
<td>carbohydrate</td>
<td>420 mg/L</td>
<td>1.3 g/L</td>
<td>2.3 g/L</td>
<td>3.7 g/L</td>
<td>5.0 g/L</td>
</tr>
</tbody>
</table>
Figure 9

The main effects of pH, carbohydrate, and phosphorus. Each variable was changed while all the others were held constant. The level of each was plotted versus the mean accumulation of a set of flasks which received a certain combination of variables.
SLIME ACCUMULATION (mg)

\[ PpH \]

CARBOHYDRATE (mg/liter)

PHOSPHORUS (mg/liter)

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on slime accumulation in the shaker flasks was examined in two different experiments. Various dilutions of the slimicide used in the mill were added to the shaker flasks. The results of the slime accumulation observations and the total viable count are presented in Figures 10 and 11. While the total count was decreased in the sample of water with increased slimicide, the accumulation fluctuated randomly. Another result from these experiments was the discovery of the reliability of Biostix for determining total viable counts in white water samples. The observations and the correlation of Biostix with plate counts are found in Table 12 and Figure 12. The high positive correlation of the Biostix with plate counts enabled them to be used for determination of counts thus cutting the time and cost of monitoring microbial count.

Sequential Experiments

These experiments were aimed at determining if a sequence of events led to the formation of slime deposits and the part, if any, that microorganisms played in the sequence. The shaker flask method was modified as described in Materials and Methods. Scanning electron photomicrographs of stages of slime accumulation are included in Figures 13-16. The types of organisms found and identified, and the relative importance of these isolates are included in Tables 13-17. The SEM photographs show the gradual build-up that was characteristic of slime accumulation in the shaker flasks. Initially, Figure 13, there was an attraction of particles by the glass surface. This was probably due to an electrostatic physical attraction as the cover glass

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Figure 10

First dilution experiment

Mean slime accumulation as measured by the shaker flask method and mean log count as measured by plate counts versus the slimicide dilution.

□ = log total count

○ = slime accumulation

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Figure 11

Second dilution experiment

The slime accumulation as measured by the shaker flask method and the log total count as measured by the Biostix method are plotted versus the dilution of slimicide.

□ = log total count cells/ml

• = slime accumulation mg
Mean Slime Accumulation (mg)

SLIMICIDE (mg/l)

Mean Log Viable Count

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Table 12
Total Viable Counts by Biostix and Plate Count

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Log count by plate</th>
<th>Log count by Biostix</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.63</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>3.04</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>7.26</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>7.21</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>6.78</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>7.15</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>7.05</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>6.52</td>
<td>6</td>
</tr>
<tr>
<td>9</td>
<td>6.93</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>3.70</td>
<td>5</td>
</tr>
<tr>
<td>11</td>
<td>4.34</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td>5.20</td>
<td>6</td>
</tr>
<tr>
<td>13</td>
<td>5.51</td>
<td>6</td>
</tr>
<tr>
<td>14</td>
<td>6.38</td>
<td>8</td>
</tr>
<tr>
<td>15</td>
<td>6.03</td>
<td>7</td>
</tr>
<tr>
<td>16</td>
<td>7.21</td>
<td>8</td>
</tr>
<tr>
<td>17</td>
<td>7.15</td>
<td>8</td>
</tr>
</tbody>
</table>
Figure 12

Comparison of log counts by dilution plates and test strips

\[ y = 0.867X + 1.37 \]

\[ r = 0.9116 \]
Figure 13

SEM photograph of a slime accumulation on a glass cover slip

The photo was taken of a cover slip which had been dipped into the white water sample and then fixed. 2900x.
Figure 14

SEM photomicrograph of slime accumulation on a glass cover-slip.

The coverslip had been immersed in the white water for 2 hours and then fixed. 2900x.
Figure 15

SEM photomicrograph of slime accumulation on a glass coverslip

The coverslip had been in the white water sample for 8 hours and then fixed. 2870 x.
Figure 16

SEM photomicrograph of slime accumulation on a glass coverslip

The coverslip had been immersed in the white water sample for 12 hours. 1700x.
Table 13
First Sequential Experiment

<table>
<thead>
<tr>
<th>Organism</th>
<th>0 Hour</th>
<th>2 Hour</th>
<th>8 Hour</th>
<th>12 Hour</th>
<th>24 Hour</th>
<th>36 Hour</th>
<th>48 Hour</th>
<th>Dominant Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alicaligenes</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>XX</td>
<td>X</td>
<td>XX</td>
<td>XX</td>
<td>X</td>
<td>X</td>
<td>0,2 hours</td>
</tr>
<tr>
<td>Bacillus</td>
<td>XX</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>all times except 0,2 hours</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavobacterium</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micrococcus</td>
<td>X</td>
<td>XX</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>An X indicates that genus was found at that time period more than one X indicates a different species based on colony morphology and some varying biochemical test responses.
Table 14
Second Sequential Experiment 26°
Topliner White Water

<table>
<thead>
<tr>
<th>Organism</th>
<th>0 Hour</th>
<th>2 Hour</th>
<th>8 Hour</th>
<th>12 Hour</th>
<th>24 Hour</th>
<th>48 Hour</th>
<th>72 Hour</th>
<th>100 Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tw</td>
<td>Ts</td>
<td>Tw</td>
<td>Ts</td>
<td>Tw</td>
<td>Ts</td>
<td>Tw</td>
<td>Ts</td>
</tr>
<tr>
<td>Alvaligenes</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Bacillus⁸</td>
<td>X</td>
<td>X</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>X</td>
<td>X</td>
<td>XX</td>
</tr>
<tr>
<td>Flavobacterium</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Micrococcus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>X</td>
<td>XX</td>
<td></td>
<td>X</td>
<td>XX</td>
<td>XXX</td>
<td>XX</td>
<td>X</td>
</tr>
</tbody>
</table>

⁸Bacillus dominated at all times except 72 hours at which time Pseudomonas was the most abundant genus.
Table 15
Second Sequential Experiment 26°
Filler White Water

<table>
<thead>
<tr>
<th>Organism</th>
<th>0 Hour</th>
<th>2 Hour</th>
<th>8 Hour</th>
<th>12 Hour</th>
<th>24 Hour</th>
<th>48 Hour</th>
<th>72 Hour</th>
<th>100 Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fw</td>
<td>Fs</td>
<td>Fw</td>
<td>Fs</td>
<td>Fw</td>
<td>Fs</td>
<td>Fw</td>
<td>Fs</td>
</tr>
<tr>
<td>Bacillus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Geothichum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X X X X X</td>
</tr>
<tr>
<td>Monilia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Micrococcus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Penicillum</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>
Table 16
Second Sequential Experiment 45°Topliner White Water

<table>
<thead>
<tr>
<th>Organism</th>
<th>0 Hour</th>
<th>2 Hour</th>
<th>8 Hour</th>
<th>12 Hour</th>
<th>24 Hour</th>
<th>48 Hour</th>
<th>72 Hour</th>
<th>100 Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tw</td>
<td>Ts</td>
<td>Tw</td>
<td>Ts</td>
<td>Tw</td>
<td>Ts</td>
<td>Tw</td>
<td>Ts</td>
</tr>
</tbody>
</table>

- **Alicigenes**  
  - X  
  - X  
  - X  
  - X

- **Bacillus**  
  - X  
  - XX 
  - XX 
  - XX  
  - XX 
  - XX 
  - XX 
  - XXX 
  - XX

- **Flavobacterium**  
  - X  
  - X

- **Pseudomonas**  
  - X  
  - X 
  - XX  
  - X  
  - X  
  - X  
  - XXX  
  - XXX

*Bacillus* dominated at all times except 100 hours when *Pseudomonas* was in abundance as well as *Bacillus*. X indicates the presence of that genus at that time, more than one X indicates more than one species as determined by colony morphology and various biochemical tests.

Table 17
Second Sequential Experiment 45°Filler White Water

<table>
<thead>
<tr>
<th>Organism</th>
<th>0 Hour</th>
<th>2 Hour</th>
<th>8 Hour</th>
<th>12 Hour</th>
<th>24 Hour</th>
<th>48 Hour</th>
<th>72 Hour</th>
<th>100 Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fw</td>
<td>Fs</td>
<td>Fw</td>
<td>Fs</td>
<td>Fw</td>
<td>Fs</td>
<td>Fw</td>
<td>Fs</td>
</tr>
</tbody>
</table>

- **Bacillus**  
  - X  
  - X  
  - X  
  - X  
  - X  
  - X  
  - X

- **Monilia**  
  - X  
  - X
was merely dipped into the white water sample and removed for processing. The other figures show how the cover glass became littered with flat particles and fibers. After 48 hours, the cover glass had a build-up of 5-6mm consisting of fibers, clay, and bacteria.

Examination of the microbial flora gave some interesting results. In the first sequential, *Alcaligenes* dominated the initial 2 hours after which time *Bacillus* dominated in terms of numbers. During all times fairly large numbers of slime-producing organisms were found. These included *Alcaligenes*, *Pseudomonas*, and *Flavobacterium*.

The second experiment tested two different types of white water at two different temperatures. The types of microorganisms differed between the samples but the amount of slime was the same. Likewise, the numbers and different types of organisms were different at the different temperatures but the amount of slime accumulated was essentially the same. This indicated that something other than microorganisms was important in the initial, at least, accumulation of slime.

Comparison of Slime Evaluation Techniques

Two methods of slime evaluation were used in this study. The qualitative slime scale and the shaker flask accumulation methods are described elsewhere in this paper. A third method of slime accumulation is used by laboratories examining the potential for slime accumulation of various water samples. This method is the slime board accumulation technique of the National Council of the Paper Industry for Air and Stream Improvement, Inc. (Springer, A.M. 1973). Samples of water

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from the various sample points were taken and analyzed using all three methods. The samples taken and the accumulations observed are in Table 18 and Figures 17-19. The Figures show that a high correlation existed between the techniques. While not completely interchangeable, the techniques all picked up the same trends.
Table 18
Comparison of Slime Evaluation Techniques

<table>
<thead>
<tr>
<th>Source of Sample</th>
<th>Qualitative</th>
<th>Slime Board (mg)</th>
<th>Shaker Flask (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2 7/15</td>
<td>2</td>
<td>109.1</td>
<td>7.3</td>
</tr>
<tr>
<td>T2 7/15</td>
<td>8</td>
<td>279.0</td>
<td>32.0</td>
</tr>
<tr>
<td>F1 7/15</td>
<td>0</td>
<td>85.0</td>
<td>11.1</td>
</tr>
<tr>
<td>T1 7/15</td>
<td>4</td>
<td>190.2</td>
<td>16.9</td>
</tr>
<tr>
<td>T2 7/14</td>
<td>8</td>
<td>212.5</td>
<td>11.2</td>
</tr>
<tr>
<td>F1 7/14</td>
<td>0</td>
<td>130.0</td>
<td>9.8</td>
</tr>
<tr>
<td>T1 7/14</td>
<td>6</td>
<td>200.9</td>
<td>11.8</td>
</tr>
<tr>
<td>F2 7/3</td>
<td>4</td>
<td>107.5</td>
<td>1.5</td>
</tr>
<tr>
<td>T2 7/3</td>
<td>8</td>
<td>323.0</td>
<td>42.8</td>
</tr>
<tr>
<td>F1 7/3</td>
<td>2</td>
<td>97.5</td>
<td>11.8</td>
</tr>
<tr>
<td>T1 7/3</td>
<td>0</td>
<td>190.9</td>
<td>10.6</td>
</tr>
<tr>
<td>F1 6/28</td>
<td>0</td>
<td>70.0</td>
<td>0.7</td>
</tr>
<tr>
<td>T1 6/28</td>
<td>4</td>
<td>247.7</td>
<td>13.2</td>
</tr>
<tr>
<td>F1 6/25</td>
<td>2</td>
<td>115.4</td>
<td>2.3</td>
</tr>
<tr>
<td>T1 6/25</td>
<td>4</td>
<td>134.2</td>
<td>5.8</td>
</tr>
<tr>
<td>T1 6/4</td>
<td>6</td>
<td>288.7</td>
<td>39.7</td>
</tr>
</tbody>
</table>
Figure 17

Comparison of slime board versus the qualitative scale
SLIME BOARD ACCUMULATION (mg/1000 cm²)

QUALITATIVE SLIME SCALE + 1

\[ y = 21.06x + 76.44 \]

\[ r = 0.7799 \]
Figure 18

Comparison of shaker flask accumulation versus qualitative scale

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y = 2.78X + 1.40
r = .6393
Figure 19

Comparison of slime board accumulation versus shaker flask accumulation

\[ y = 5.35X + 97.39 \]
\[ r = 0.8624 \]
DISCUSSION

It was hoped at the start of the study that the factors affecting slime accumulation could be discovered and studied in detail. It must be said at the beginning of this discussion that this goal was not fully realized and that the factors involved in the accumulation of slime are much more complex and numerous than the ones explored in this study. The work presented above was based on the hypothesis: slime accumulation was directly related to the microorganisms found in the system, the factors affecting the growth and increase in microorganisms were important in the slime accumulation, and that the mill system could be accurately and simply reproduced in the laboratory.

The simulation of mill conditions by the shaker flask method while not completely accurate did give a valid simulation and potentially useful results. The unexpected but satisfyingly high correlation with the qualitative scale and the slime board technique showed this to be the case. The shaker flask technique evolved out of a need to have a fairly simple and rapid way of analyzing a large number of samples. It was the chosen method after examining several original and previously reported methods.

The mill survey data analyzed and interpreted gave results that were fairly consistent with the laboratory work and thus the mill system was reasonably well simulated in the laboratory for the factors which were explored. However, intrinsic differences between vat and machine as seen in the mill data analysis indicated that some factor
other than those examined was involved in slime accumulation. Among the factors that could have been involved were variation in slimicide treatment and dosage, machine speed, product being made and any other of a number of factors contributed to the system by addition of materials needed for certain grades of paper.

The results of the work described were hard to explain in some cases. That pH caused a decrease in slime accumulation has been shown in other work (Rudolf and Nemerow, 1950, 1951) and seemed to be a valid and explainable phenomenon. Oppermann and Wolfson (1961) have stated that the attachment of fimbriated and nonfimbriated forms of bacteria was effected by the pH and that at lower pH levels they did not attach so readily. Duguid (1950) has also presented evidence that slime formation around cells did not take place at lower pH levels. If these two factors are the mechanisms of slime formation, when micro-organisms are involved, then lowering the pH would necessarily be important in decreasing the slime accumulation. Likewise, if chemical additives such as clay and titanium dioxide are the cause of the formations being built up, neutralizing their anionic charges by the increased concentration of $H^+$ ions at lower pH would help to decrease slime accumulations. The experimental results found concerning the carbohydrate and phosphorous responses were not so easily explained. The accumulation of slime around a cell has been shown often to depend on the amount of nutrients available to the organisms (Duguid, 1950, Salton, 1960, and Brock, 1966). Normally a high concentration of carbohydrate and limiting amounts of phosphorus, nitrogen or sulfur are needed to allow the accumulation of large amounts of slimy layer or capsular
material to be formed. Lamanna et al (1973) have stated that an upper
limit where no more slime is made has been shown and, possibly, this
upper limit has been reached in the experimental work described above.
The decrease with increased phosphorus might have been due to the
monobasic potassium phosphate used to adjust the phosphorous level.
The increased amount of H\textsuperscript{+} ion might have effectively neutralized the
charges existing thus causing decreased accumulation. This effect is
speculative and not conclusively proved as the follow-up experiments
showed the response of accumulation to phosphorus varied somewhat
from the 2 X 2\textsuperscript{5} experiment. This might be due to the complexity of
accumulation and the experiments not taking in to account all the
necessary variables.

The results of this study have led to some conclusions about
this particular system. The slime accumulation in this mill was not
exclusively of microorganism origin. The SEM photomicrographs taken
of accumulations during the sequential experiments showed this to be
true. The time zero slides had an immediate accumulation of particles
and at no time during the first 48 hours did microorganisms appear to
be dominant in the accumulation on the slide although they were present
as determined by total counts. This led to the conclusion that the
first steps of slime accumulation were of a physical-chemical nature.
The microorganisms probably become involved in the accumulation at some
later time. The dilution experiments showed this in a slightly different
manner. Despite reduced total count of viable organisms with increased
slimicide, the accumulation did not decrease accordingly. This would
indicate that either the slime producing organisms are not affected
by the slimicide or that the slime accumulation is not dependent on the viability of microorganisms. The former does not seem to be likely as a great deal of this product has been sold as a slime control agent and its ability to kill all forms of troublesome slime producing organisms has been reported (Starnes, 1969). The conclusion is that the accumulation is due to factors other than microorganisms. The nonmicrobiological nature of slime accumulations has been reported and discussed elsewhere in this paper.

It would also seem that nature of the microorganisms was not crucial in the formation of slime deposits in this particular system. This was seen in the sequential experiments where two different patterns of microorganism dominance was shown. One white water system had bacteria, mainly *Bacillus*, dominating, while the other had a predominant fungal population. These two waters did not have any appreciable difference in accumulation in the shaker flask system.

The ultimate conclusion which can be reached about this mill system and about slime accumulation in general is that the mechanisms involved and the study of these mechanisms is extremely complex and difficult. Reduction of the system to some simple model is virtually impossible and as Sanborn (1965) has stated the only judgment of adequate control of slime in a paper mill is performance.
LITERATURE CITED


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