European J. Appl. Microbiol.
Biotechnol. 5, 87–93 (1978)

European Journal of Applied Microbiology and Biotechnology

Sulphate-Reducing Bacteria in Paper Machine Waters and in Suction Roll Perforations

J. Soimajärvi[1], M. Pursiainen[1], and J. Korhonen[2]

[1]Department of Biology, University of Jyväskylä, SF-40100 Jyväskylä
[2]Material Research Department, Valmet Oy, Rautpohja Works, Jyväskylä, Finland

Summary. To define some aspects of the biological corrosion sulphate-reducing bacteria were studied in paper machine waters and in plugged perforations of a suction roll. The desulphuricants were most active on passive fiber recipients. Most bacteria found in fiber plugs taken from the perforations of suction rolls belonged to the genus *Desulfovibrio*. Desulphuricants were found mainly at the outer ends of plugged perforations, where corrosion of the roll metal is most evident.

Sulphate is dissimilatory reduced by bacteria of the genera *Desulfovibrio* and *Desulfotomaculum*. Desulphuricants may be involved in corrosion either indirectly, by producing hydrogen sulphide that is oxidized to sulphuric acid by Thiobacilli, or directly by depolarizing the cathodic regions of iron (cf., Piluso, 1972). In the latter case, hydrogen sulphide produced may be released as a gas or may precipitate ferrous ions as ferrous sulphide. The corrosion products usually consist of ferrous sulphide and iron hydroxide (Sharpley, 1973).

In paper machine water systems the precipitation of FeS can lead to blackening of paper pulp. Furthermore, the precipitation of metal sulphides or the corrosion of steel may harm the equipment. Desulphuricants may be found under slime and precipitates at various points of the circulation system (Wolfson and Michalski, 1964). Bacteria of the genus *Desulfovibrio*, discovered under slime accumulated in a headbox, have been shown to relate to corrosion in the same area (Mueller and Muhonen, 1972).

This work investigates the occurrence of desulphuricants in two paper machines. A special emphasis has been put on suction rolls, where the perforations are frequently corroded.

Significance of steel quality and other technical aspects in suction roll corrosion are described in another paper of this project (Vestola and Korhonen, 1976).

Materials and Methods

Paper Machines and Water Analyses. Two paper machines were studied having different water circulation systems. A simplified block diagram of the water circulation

0340–2118/78/0005/0087/$ 01.40

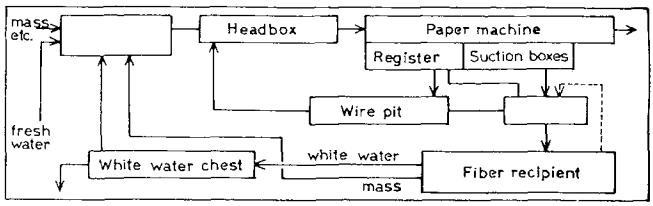

Fig. 1. Partial block diagram of water circulation system of paper machine

is shown in Figure 1. The circuit of machine A included as fiber recipient a rectangular save-all provided with a scraper chain at the bottom of the settling tank (approx. 1000 m^3 capacity) and a white water chest subdivided into several compartments of varying capacities and retention times. The circuit of the newer machine B had a simple white water system, and fiber recovery was carried out with a vertical clarified (approx. 3000 m^3) located outside.

In two dates (interval 6 weeks) duplicate water samples were taken with a Ruttner sampler from different areas of the water circulation systems for duplicate analyses and culturing. In recipients that contained sediments because of a low velocity of flow, the samples were collected close to the top of the sediment layer.

Plug Material of Suction Roll Perforations. To remove water from paper mass to the suction boxes (Fig.1) the paper machines were provided with cylindrical suction rolls (diameter ca. 150 cm). Figure 2 shows a partial cross section of the suction roll with numerous perforations (4.3 x 70 mm) through its metal part and rubber cover. Some of the perforations had become plugged by fiber material, filler clay, etc., so that the plugs restricted water flowing in the perforations. The sample plugs were removed with a sterile spiral drill and were left unaltered for chemical analyses and microbe cultures. To study the longitudinal distribution of desulphuricants, a few plugs were cut up in 10 mm lengths that were suspended in a lactate sulphate medium for cultivation.

Analyses. The water samples were subjected to simultaneous measurements of pH, temperature, and redox potential (E_X, using an Ag/AgCl reference electrode). The Hydrobiological Research Institute of Jyväskylä measured the oxygen saturation, the $KMnO_4$ demand from a 0.8 μm filtrate, the concentrations of NO_3^-, NH_4^+ and CO_2, the total phosphorus, total sulphur, and SO_4^{-2} contents according to the APHA Standard Methods of 1971. Hydrogen sulphide was measured as sulphide in 1 N sodium hydroxide with an Ag^+/S^{2-} electrode.

Formation of Hydrogen Sulphide in water samples during incubation at 42°C (the system temperature in most areas was over 40°C) was studied by means of the following system: 50 ml vol sample water was transferred to a gas washing bottle of 250 ml capacity. Pure industrial nitrogen was washed with water and passed continuously through the test bottle at a rate of about 10 ml per min. The hydrogen sulphide formed in the test bottle was transferred by the nitrogen to a third bottle, where it was trapped in 1 N NaOH. Potentiometric measurements of sulphide were made at intervals by inserting electrodes into the trap bottle.

Culture Conditions. Colony counts were performed of bacteria capable of growth on a nutrient agar (Difco) at 30°C. Anaerobic bacteria were counted by plating on TSHGA agar (Nurmikko and Kärhä, 1961) in a nitrogen chamber at 28°C. The presence of Thiobacilli was indicated by growth and a drop in pH during incubation for 1–2 weeks in the dark at 30°C in a thiosulphate mineral medium (London, 1963). Sulphate-reducing bacteria were indicated by FeS blackening after incubation in screw-capped culture tubes filled to the brim with Postgate's liquid nutrient medium C (Postgate, 1966). The sulphate-reducing bacteria were enriched in the same Postgate medium by serial tube cultivation or by means of the Söhngen double bottle technique in a hydrogen atmosphere.

Microscopy. For routine tests, the suspended samples were monitored with a phase-contrast microscope. Alternatively, the cells were stained with aqueous 1% phosphotungistic acid and examined by electron microscopy.

Results

Process Waters and Microbial Counts. When water samples were taken on two dates from the paper machines and subjected to chemical analysis, results listed in Table 1 were obtained. In all samples there was relatively high $KMnO_4$ demand and high concentrations of phosphorus, sulphur, and CO_2. Sulphate accounted for over half the total sulphur in all waters. The total sulphur, SO_4^{2-}, and CO_2 content of the samples from machine A were higher than from machine B, clearly in consequence of process differences. The E_x was negative in fiber recipients, except for one sample (200 mV in the settling tank, 2nd date).

Table 1. Range limits from the analysis and colony counts of the water circulation systems of two paper machines

	Paper machine A		Paper machine B	
	Fiber recipient	Wire pit or White water	Fiber recipient	Wire pit or White water
Temperature, °C	44; 45	29; 48	33; 34	47; 50
pH	5.1; 5.2	5.3; 5.5	4.6; 5.2	5.1; 5.2
$KMnO_4$ demand, mg/*l*	1300; 1400	1230; 1440	1340	ND
Redox, mV	-150; 200	115; 280	-420; -90	200
O_2 saturation, %	3; 28	7; 37	0; 18	3; 5
Nitrate, mg N/*l*	0.03	0.01; 0.18	0.04; 0.15	0.03; 0.14
Ammonium, mg N/*l*	0.07; 0.09	0.07; 0.88	0.19; 0.23	0.24; 0.34
Phosphate, mg P/*l*	0.24; 0.29	0.12; 0.90	0.18; 0.31	0.31; 0.37
Total phosphorus, mg/*l*	0.58	0.76; 2.23	0.76	ND
Sulphate, mg S/*l*	25; 34	27; 34	18; 20	19; 20
Total sulphur, mg/*l*	42; 56	39; 56	23; 43	25; 30
Nutrient agar count/ml, x 10^{-6}	1.3; 3.6	2.9; 7.8	0.9; 5.1	0.2; 0.3
Anaerobe count/ml, x 10^{-5}	0.7; 2.0	0.5; 2.3	0.3	0.2; 1.7

ND, not determined

Thiobacilli were shown to exist in the white water of machine A and in several samples coming from the settling tank and wire pit A, but respective indication was not obtained in the samples of machine B.

Table 1 includes two microbial counts of the different areas. No significant difference was revealed between the two machines in either nutrient count or anaerobe count. The significance of correlation coefficients between the logarithm of either microbe count and the other variables in Table 1 was tested; the available number of samples was generally 10–12, consisting of the separate water pools of both machines on the two dates. The anaerobe count had a negative but not significant ($P < 0.2$) correlation to the E_x and there was no close connection of either count with pH. Both counts correlated negatively but not significantly ($P < 0.2$) to the temperature. The nutrient count correlated ($P < 0.05$) to the O_2 saturation percentage and to the SO_4^{2-} content ($P < 0.05$), but the correlation of the anaerobe count to the SO_4^{2-} was not significant ($P < 0.2$). A negative trend ($P < 0.1$) was observed between each count and the NH_4^+ content. The contents of NO_3^-, PO_4^{3-}, total P, total S, and CO_2 had no close connection with the counts.

Tests for sulphate-reducing bacteria gave positive results in most samples of the various waters. During incubation in lactate sulphate medium, a ferrous sulphide precipitate formed first in the samples coming from settling tank A and from clarifier B (4–7 days). The white water and wire pit samples also gave positive results, but the induction period generally took longer. In the Spearman's rank correlation test, the order of desulphuricant indication correlated with the anaerobe count ($P < 0.05$) in respective samples, but not significantly with the other variables in Table 1; a possible, negative trend was observed with temperature ($P < 0.1$) and E_x ($P < 0.2$). The sulphate-reducing bacteria detected in various water samples, after an enrichment cultivation in sulphate lactate medium under nitrogen or hydrogen atmosphere, were morphologically identical gram-negative curved rods with a single polar flagellum and without endospores (Fig. 4). These characteristics denote them *Desulfovibrio* species.

Formation of Hydrogen Sulphide in Different Waters. When samples from different waters were placed in the incubation apparatus at 42°C, the formation of H_2S was detected, after an induction period of 50–100 h, initially in the sample originating from the settling tank. After 200 h, when maximum content in the trap bottle was achieved, the recovery of H_2S corresponded to 55% of the original sulphate sulphur concentration. The formation of H_2S was slower and stopped earlier in the other water samples, taken from clarifier B or from the wire pit and various sections of the white water chest.

To study the use that sulphate-reducing bacteria make of the nutrient elements, 500 mg/l of reagent sulphur as several sulphur compounds was added to the settling tank samples in the incubation apparatus at 42°C. The accumulation of H_2S in the trap bottle started after ca. 100 h and the H_2S content reached its maximum after 200 h. Among the chemicals studied, sodium sulphate and sodium thiosulphate most clearly accelerated the formation of H_2S, so that it corresponded to ca. 80 mg/l of sulphur. At the same time, the formation of H_2S from control samples without added sulphur compounds corresponded to ca. 50 mg/l of sulphur. Sodium sulphite and sodium bisulphite in the applied concentrations inhibited the formation of H_2S, so that it was less than 20% when compared with the control.

Desulphuricants in Suction Roll Perforations. The plug material obtained from self-plugged perforations of suction roll had the same 1 cm^3 maximum volume as the roll perforations. The plugs had an average weight of 0.26 g and a dry content of 14%. The total sulphur content of the plug material accounted for 6% of the plug dry weight.

The main part of bacteria originating from plugs suspended in a liquid medium was morphologically identical with the sulphate reducers of process waters. The similarity in morphology and biochemical requirements also were retained during enrichment.

The longitudinal distribution of sulphate-reducing bacteria in plugged perforations was studied in 9 plugs. In 6 plugs, desulphuricants were indicated in cultivation. Figure 2 summarizes the observed distribution of sulphate-reducers along the plugs. In all desulphuricant-positive plugs, bacterial growth was initially detected in the cultivation of sections originating from the outer (roll face) end of the roll perforation. Desulphuricants also grew in the central sections in one plug and even in all sections in another plug.

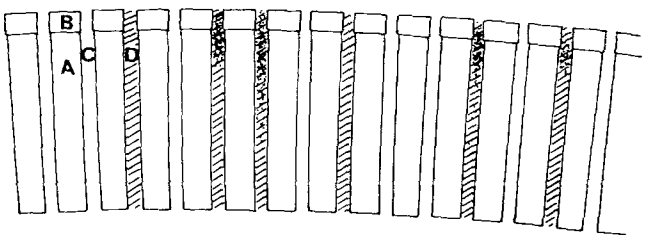

Fig. 2. Distribution of desulphuricants in suction roll perforations. This is a schematic cross section of a suction roll shell (*A*), its rubber cover (*B*), and drilled perforations (*C*). Plugged perforations (*D*, shadowed) frequently had their highest activity of desulphuricants close to the rubber cover (dotting)

Also, perforated samples of roll shell material that had been submerged in the wire pits contained desulphuricants after a period of three months, in perforations that had been plugged with headbox stock at the beginning of the test as well as in those holes that had become plugged during the test. The sulphate reducers grew along the entire length of the plugs or close to the outer ends, where sulphide blackening and corrosion pits could be observed. Figure 3 represents a specimen cut after the submersion period along the rows of perforations. A visual assessment indicated that the plugged holes contained the greatest amount of corrosion traces and that the holes left open had corroded least.

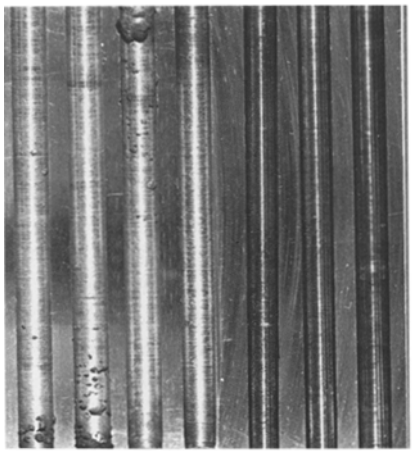

Fig. 3. Cross section of a specimen of suction roll shell material after 3 months submersion period in the wire pit, cut along the rows of perforations. The plugged holes (left) give a visual assessment of the degree of corrosion. Compare with perforations left open (right)

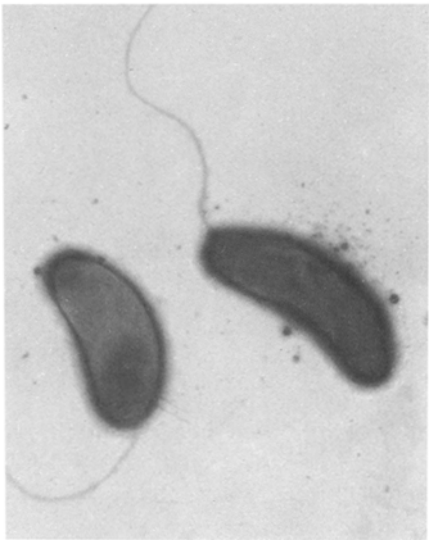

Fig. 4. Electron micrograph of negatively stained *Desulfovibrio* cells of general strain from paper machine water, cultivated in lactate sulphate medium. 30,000 X

Discussion

The number of bacteria in the water circulation system of paper machine was not definitively controlled by the usual physical and chemical process conditions that were closely similar to those reported elsewhere (e.g., Eklund and Ahlers, 1969; Korhonen et al., 1973). The correlation between the nutrient count and the O_2 saturation, together with the trends between either nutrient or anaerobic count and the E_X and temperature, nevertheless refer to an influence of these variables on the spectrum of bacteria. The connection of the bacterial counts to the sulphate and ammonium content in process water seems possible, although the evidence is insufficient.

Sulphate-reducing bacteria were indicated in nearly all areas of the water circuits. The ranking of samples by means of the order of indication revealed a correlation of the sulphate-reducing bacteria to the anaerobic count. Respective ranking also gives a sign that the sulphate reducers have a similar connection with the process temperature and E_x as the anaerobic count has. These findings might be helpful in evaluating the progressive microbiologic corrosion, as it has been reported that there is no correlation to the number of sulphate-reducing bacteria that will create a corrosion problem in a given paper mill system (Piluso, 1972). The thiosulphate and sulphate activation of H_2S formation during incubation was not uncommon, but the sulphite inhibition cannot be interpreted without additional data on concentration effects and apparent nonspecific agents. Kobayashi et al. (1974) have reported a K_m value 3.6×10^{-3} M of purified sulphite reductase for sulphite in *Desulfovibrio vulgaris*.

Evidence of microbiologic corrosion of suction roll is shown by observations that desulphuricants concentrate in the same areas of plugged perforations where the metal is generally and most severely corroded. Except for the question of roll steel quality, inhibiting desulphuricants might be discussed in prevention of corrosion. Elimination by changing process conditions or by adding antimicrobial agents is possible to a certain extent (e.g., Wolfson and Michalski, 1964), but seems not quite practicable. We propose to control geometric and other causes that can lead to depositions and plugging in the perforations. Such a control can prevent local conditions favorable to the sulphate reducers.

Acknowledgements. We wish to thank Professor M. Raatikainen (University of Jyväskylä) and J. Vestola, Research Engineer of Valmet Oy in Jyväskylä, for their interest in this work. Our thanks also to Dr. I. Nuuja, who performed the EM and to P. Niutanen for the photography.

References

American Public Health Association. (1971). Standard methods for the examination of water and wastewater, 13th ed., New York: American Public Health Association, Inc.

Eklund, D., Ahlers, P.-E. (1969). SCAN forsk. rapport nr 7. Oy Keskulaboratorio, Helsinki

Kobayashi, K., Seki, Y., Ishimoto, M. (1974). J. Biochem. **75**, 519–529

Korhonen, J., Lumme, P., Kinnunen, L.J. (1973). Paperi ja puu-Paperi och Trä **55**, 559–570; 646–656

London, J. (1963). Arch. Microbiol. **46**, 329–337

Mueller, W.A., Muhonen, J.M. (1972). TAPPI **55**, 35–41

Nurmikko, V., Kärhä, E. (1961). Ann. Acad. Sci. Fenn. Chem. 104

Piluso, A. (1972). Paper Trade J. **148**, 46–48

Postgate, J.R. (1966). Lab. Prac. **15**, 1239–1244

Sharpley, J.M. (1973). Microbiological corrosion and its control. In: Corrosion inhibitors, C.C. Nathan, ed., pp. 228–235. Houston: NACE

Vestola, J., Korhonen, J. (1976). TAPPI **59**, 130–133

Wolfson, L.L., Michalski, R.J. (1964). TAPPI **47**, 197–199

Received August 15, 1977