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Studies on slime-forming organisms of a paper mill—slime production and its control

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Bacteria, yeast and filamentous fungi were isolated from various sites within a paper mill. *Bacillus alvei* and *Aerobacter aerogenes* were the most prevalent contaminating bacteria. Maximum slime was produced by *A. aerogenes* (4.2 mg ml^{-1}) at pH 6.5 and by *B. alvei* (7.2 mg ml^{-1}) at pH 7.5 in white water. The optimum temperature was 40°C for maximum slime production by both organisms. In the presence of levanase, a 25% reduction in dosages of a biocide (Bioplus®) was observed. Killing of *A. aerogenes*, which was achieved in 8 h with 20 ppm Bioplus®, could be obtained in 6 h with the combined use of levanase and a lower concentration of Bioplus® (15 ppm). With *B. alvei* almost the same inhibitory effect (4.22-log decrease) was obtained at 20 ppm Bioplus®, and in combination with a lower concentration of Bioplus® (15 ppm) and enzyme. The paper properties did not show any adverse effect after treatment with levanase and Bioplus®.

Keywords: slime production; *Bacillus alvei*; *Aerobacter aerogenes*; coated broke; white water; biocide (Bioplus®); levanase

Introduction

Slime formation in a paper-making system is the result of two primary mechanisms: microbial activity and deposition of organic and inorganic compounds, but the former is more important than the latter in causing web breaks, corrosion, foul odours, shrinkage and sheet defects such as holes and scabs resulting in overall increased production costs [4,17]. Unfortunately, with the recent trend towards more closure of the white water system because of environment constraints, and the use of higher secondary fiber along with temperature and pH ranges optimal for microbial growth, the scale has tilted towards microbial deposits. These may be defined as the accumulation of microorganisms which may be bacteria, filamentous fungi or yeast, along with pulp fiber, filler dirt and other materials. Many of these organisms develop a capsular material, a viscous substance around the cell, which enables the cells to attach to each other as well as to inert surfaces and helps to entrap other debris within the system, eventually gluing all the parts together to form a final deposit called slime [19]. Due to complex and varied paper-making systems, the nature of slime varies and hence its control. Therefore, at the onset of a slime problem in a paper mill, the options for its control are either to shut down the machine for boilout or to alter the biocide and increase biocide usage. It is known that the slime restricts the contact of biocide with the target. In today's market, with the paper industry's emphasis on economics, production efficiency and sheet quality, the prevailing measures do not provide a satisfactory solution. An alternative approach could involve use of nontoxic slime-hydrolysing enzymes [8], the use of which could eliminate slime from the system, thereby exposing cells directly to biocides at lower dosages and indirectly creating lesser environmental problems. Hence, the present study was planned to elucidate the nature of the slime problem and to recommend various means of control in one of the biggest paper mills in North India, Shree Gopal Paper Mill, Yamunanagar, India.

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Materials and methods

Materials

Biocide (Bioplus®) was purchased from The Bombay Oil Ind Ltd (Bhandup, Bombay, India). Levanase was obtained by cultivating *Rhodotorula* sp, an isolate from the coated broke chest of Shree Gopal Paper Mill, Yamunanagar, India [5]. All other chemicals used were of analytical grade.

Isolation and identification of microbiota

Samples were collected every month for a year from different sampling sites. They were streaked on nutrient agar (Hi-Media, Bombay, India) for bacteria, potato dextrose agar (Hi-Media) for filamentous fungi and malt agar media (malt extract, 20 g; glucose, 20 g, dihydrogen ammonium phosphate, 1 g; peptone, 1 g; yeast extract, 1 g; and agar 20 g per liter, pH 4.5) for yeast. Nutrient agar plates were incubated at 37°C while potato dextrose agar and malt agar plates were incubated at 25°C for 48–72 h. Representative colonies that were quantitatively numerous or more frequent in occurrence were picked and purified. Bacteria and yeast were quantified by the standard plate count method and identified according to biochemical characteristics as per 'Bergey's Manual of Determinative Bacteriology' [13]. Fungi were identified according to their morphology (microscopic studies). *A. aerogenes* and *B. alvei* isolates were mucoid and most commonly encountered throughout the year, hence these were selected for further studies.

Slime production and its estimation

A. aerogenes and *B. alvei* were inoculated separately into 20 ml of modified Park medium (1% peptone, 1% yeast extract and 20% sucrose, pH 6.0) in 100-ml Erlenmeyer flasks [19] and incubated on a rotary shaker (100 rpm) at 37°C for 18 h. Five millilitres of this inoculum were added to 200 ml sterilized white water (used as growth medium) in a 1-L flask. The flasks were incubated on a rotary shaker (100 rpm) at 37°C for 48 h and the contents were then centrifuged ($12\,000 \times g$) for 20 min at 4°C. The cellmass was washed three times with sterile distilled water and suspended aseptically in sterile sucrose solution. The pH was adjusted to 6.5 with 1 N NaOH. The cells were incubated at 37°C and samples were withdrawn at regular intervals and processed for the estimation of slime [7]. Fructose in the hydrolyzate was estimated by Seliwanoff's method [2]. For the effect of pH and temperature on slime production, experiments were carried out with white water at different pHs (5.5–8.5) and at different temperatures (30–50°C).

Inhibition by Bioplus® in combination with levanase

A 0.5-ml sample of precultivated culture of each organism prepared as above was used to inoculate 50 ml of white water containing 0.5% sucrose. Three types of treatments were given to each organism: (i) Bioplus® alone; (ii) levanase alone; and (iii) levanase + Bioplus® with one positive control. Bioplus® at 10, 15 and 20 ppm was added to the biocide-labelled flasks. The flasks were incubated at 37°C on a rotary shaker (100 rpm) for 24–26 h. One millilitre of concentrated enzyme solution (1.35 units mg^{-1} protein) from *Rhodotorula* sp [5]) was added to the enzyme-designated flasks and incubated at 37°C on a rotary shaker (100 rpm) for 8 h. The samples withdrawn at different time intervals were processed by standard plate count.

Treatment with Bioplus® and Bioplus® + levanase on paper sheets

A 100-g sample of furnish bleached pulp was suspended in 2.5 L distilled water in a bucket. Two treatments were given to the pulp with: (i) 20 ppm Bioplus®; and (ii) 20 ppm Bioplus® + 50 ml levanase (1.35 units mg^{-1} protein) along with one control. One set of each type was beaten for refining in a PFI mill No. 297 after incubation at 28 ± 2°C for 6 h; the other set was not beaten. Handsheets were prepared from the pulps and strength properties were studied by standard Tappi procedures.

Results and discussion

Isolation and identification of microbiota

A wide variety of viable and culturable microbiota were isolated (Table 1). The most prevalent microorganisms were *Aerobacter* spp, *Bacillus* spp, *Citrobacter* spp, *Pseudomonas* spp, *Arthrobacter* spp, *Enterobacter* spp, *Klebsiella* sp, *Aspergillus flavus* and *Rhodotorula* sp. The presence of such a wide variety of microbiota could be accounted for due to the varied and complex paper-making system where many factors such as a wide temperature range (30–37°C) and pH range (5.0–8.0) [22], use of different additives [3], long dwelling time in coated broke chest, and recycling of white water [10] are able to establish the necessary conditions for their growth. The consistent presence of the yeast *Rhodotorula* sp in all the samples from coated broke could be due to the conditions prevailing there for its growth.

Table 1 Microorganisms isolated from six sites in the paper mill

Sampling site	Organisms	Probability of occurrence
Head box	*Bacillus alvei*	0.75
	Enterobacter sp	0.58
	Aerobacter aerogenes	0.25
		0.16
Hydrofoil	*Enterobacter* sp	0.08
	Aerobacter cloacae	0.33
	Asperillus sp	0.58
	Citrobacter sp	0.16
A Street final washer pulp	Not characterized	
Flat box	*Bacillus licheniformis*	0.58
	Arthrobacter sp	0.08
	Citrobacter sp	0.33
	Penicillium sp	0.41
	Citrobacter sp	0.41
Back water	*A. aerogenes*	0.75
	Klebsiella pneumoniae	0.58
	Aspergillus niger	0.25
Coated broke	*B. alvei*	0.83
	B. circulans	0.75
	Enterobacter sp	0.41
	Pseudomonas sp	0.58
	Citrobacter sp	0.58
	Rhizopus sp	0.33
	Alternaria sp	0.58
	Aspergillus flavus	0.33
	Rhodotorula sp	0.91

Similar types of bacteria were isolated from a waste paper reprocessing mill in Germany [15], in process water of a paper mill in Southern Finland [23] and in coated broke from a paper mill in South Africa [11]. Various species of *Pseudomonas*, *Enterobacter*, and *Acinetobacter*, as well as *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *Escherichia coli*, *Proteus vulgaris* and *Citrobacter freundii* were isolated from three paper mills in the USA [17]. However, in deposits from a Canadian paper mill, filamentous organisms ie iron bacteria (*Sphaerotilus*) and sulfur bacteria (*Beggiatoa*) were isolated [10]. Fuwa *et al* [9] reported that closed water cycling, more neutral conditions and use of coated broke in large quantities resulted in an increase in moulds. Though the sampling sites in the paper mills of New Zealand were similar to ours, different selective media were used to support the growth of various types of microorganisms [16]. The slime-causing organisms identified in these mills were mostly similar to those encountered in our study, with the following notable exceptions: *Alcaligenes* sp, *Micrococcus* sp, *Staphylococcus* sp and *Coryneform* sp among the bacterial species, the filamentous fungi *Trichoderma* sp, *Aureobasidium* sp, *Paecilomyces* sp, *Phoma* sp, and the yeasts *Trichosporum* sp and *Geotrichum* sp.

Effect of pH and temperature on slime production

Figure 1 illustrates slime production at different pH values by *B. alvei* and *A. aerogenes*. The range of pH and tempera-

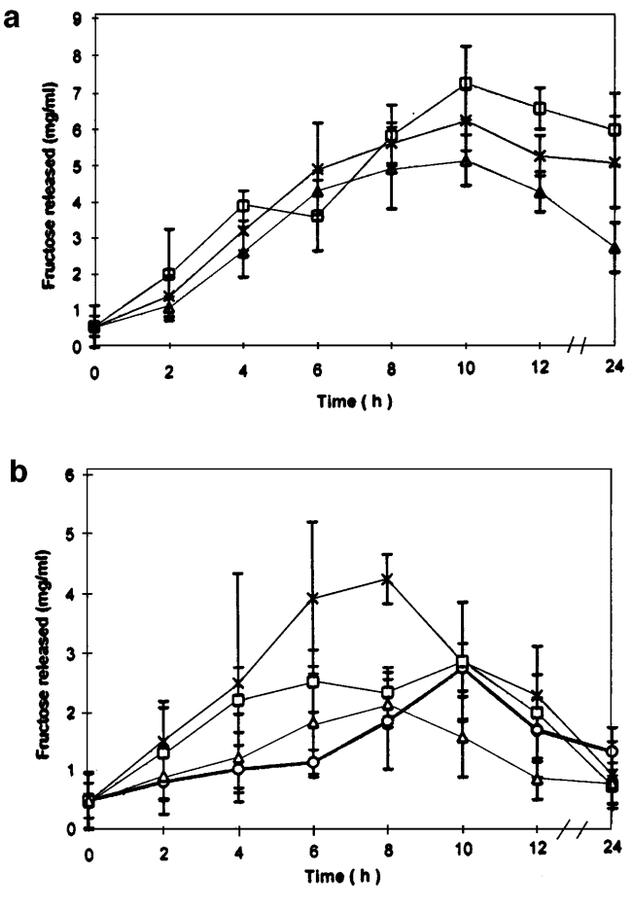

Figure 1 Effect of pH on slime production (calculated as fructose released) by *Bacillus alvei* (a) and *Aerobacter aerogenes* (b). The error bars indicate standard deviation. –○–, pH 5.5; –X–, pH 6.5; –□–, pH 7.5, –△–, pH 8.5.

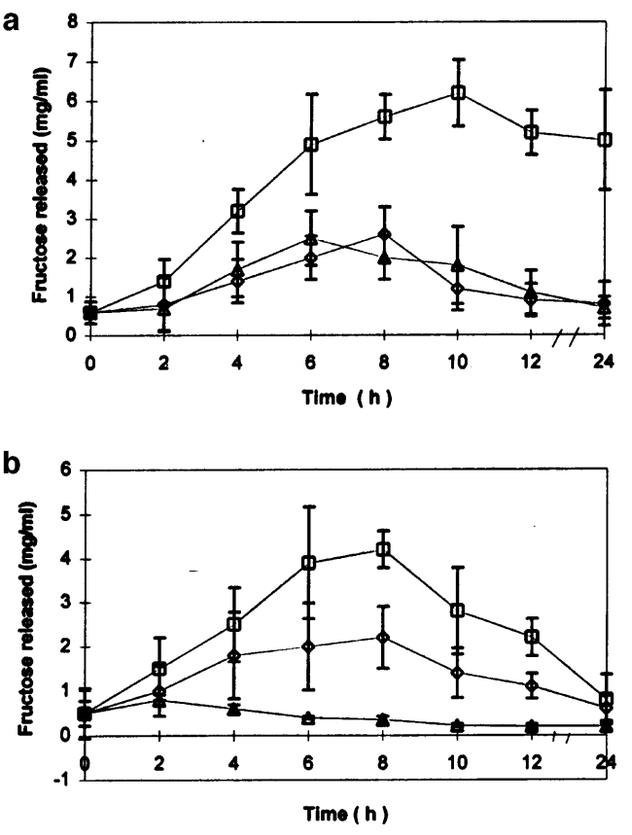

Figure 2 Effect of temperature on slime production (calculated as fructose released) by *Bacillus alvei* (a) and *Aerobacter aerogenes* (b). The error bars indicate standard deviation. –◇–, 30°C, –□–, 40°C, –△–, 50°C.

ture was chosen to represent the conditions prevailing in different paper mill systems. For *B. alvei*, pH 7.5 was optimum for slime production (7.2 mg ml^{-1} in 10 h) as compared to pH 6.5 and 8.5. Slime production at pH 8.5 was less than at pH 6.5. However, the fructose released was insignificant at all pH levels ($P<0.05$). *B. alvei* did not grow at pH 5.5. On the other hand, with *A. aerogenes*, maximum slime production (4.2 mg ml^{-1} in 8 h) occurred at pH 6.5 followed by pH 5.5, 7.5, and 8.5. The amount of fructose released was significantly different (*t*-test, $P<0.05$) at pH 6.5 and 8.5. Neutral to alkaline conditions favour the slime production by *A. aerogenes*. With *B. alvei* and *A. aerogenes* maximum slime production (6.2 mg ml^{-1} in 10 h and 4.2 mg ml^{-1} in 8 h, respectively) was observed at 40°C (Figure 2). At 50°C growth of both organisms was inhibited. The effect was more pronounced with *A. aerogenes* than with *B. alvei*. However, fructose released by *B. alvei* was significantly different than that by *A. aerogenes* at different temperatures.

Both pH and temperature affect bacterial growth patterns. Most slime bacteria prefer to grow at neutral pH and at 37°C [12]. This was confirmed by our results. However, Jaquess and Sorrelle [14] reported that there was no significant difference in slime production by slime-forming bacteria which grew between pH 5.0–8.0. They also showed that though the microorganisms were metabolically more active at pH 9.0 than at pH 5.0 or 6.0, they produced less extracellular carbohydrate at pH 9.0. It is apparent from our results that slime production by both organisms was minimal at 50°C. Therefore, to restrict slime formation, the temperature of various slurries of the paper mill should be raised to 50°C. Similar suggestions for raising the temperature of process water [23] and starch slurry systems [21] have been made for controlling slime problems in other systems.

Biocontrol of slime-forming organisms

Among eight biocides tested for inhibition against both organisms, Bioplus® was the most promising (data not shown). The slime produced by both organisms was levan as determined by ^{13}C NMR studies (data not shown). Therefore, levanase was used in order to remove the slime around the cells and facilitate the entry of biocide at lower dosages. The use of concentrated levanase in conjunction with Bioplus® showed that complete killing of *A. aerogenes* (Figure 3), which was earlier attained in 8 h by the use of 20 ppm Bioplus®, could be achieved in 6 h by the combined use of levanase and Bioplus® (15 ppm). In *B. alvei* the inhibitory effect (4.22-log decrease) which was obtained by Bioplus® at 20 ppm could also be achieved at a lower concentration of Bioplus® (15 ppm) with the addition of levanase (4.34-log decrease). With the enzyme it was poss-

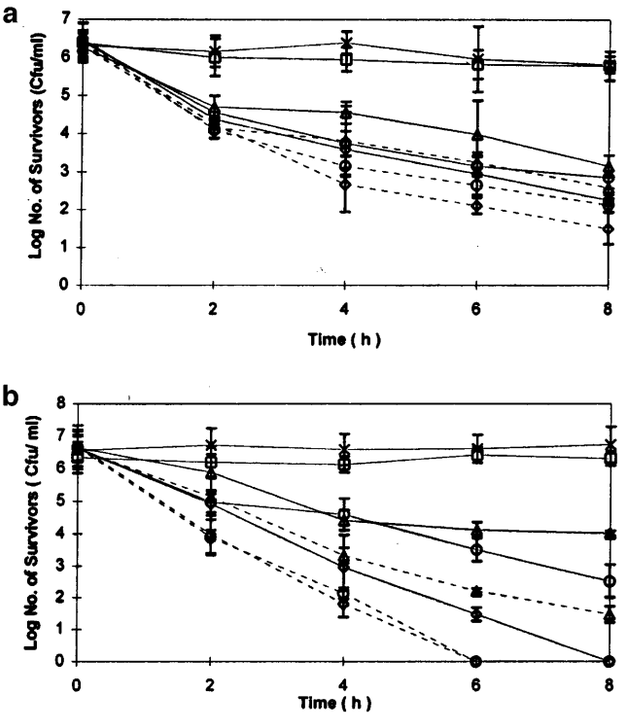

Figure 3 Effect of Bioplus® and Bioplus® + levanase on the growth of *Bacillus alvei* (a) and *Aerobacter aerogenes* (b). The error bars indicate standard deviation. –X–, Control; –△–, Bioplus® 10 ppm; –○–, Bioplus® 15 ppm; –◇–, Bioplus® 20 ppm; –□–, levanase; ....△...., levanase + Bioplus® 10 ppm; ....○...., levanase + Bioplus® 15 ppm, ...◇...., levanase + Bioplus® 20 ppm.

ible to reduce the biocide concentration by 25%. On the contrary, other authors have reported much greater reduction in biocide usage with the enzyme. This may be due to the source of enzyme, its efficiency as well as the type of targeted organisms. Fries [8] showed that combined treatment with 20 ppm methylene-bisthiocyanate (MBT) and 40 ppm levan hydrolase lowered the colony count of *Aerobacter levanicum* and *Bacillus subtilis* from 10^9 and 10^4 CFU ml^{-1} to 10^6 and 10^3 CFU ml^{-1}, respectively. Field studies in a fine paper mill also exhibited population reduction of bacteria in the head box from 10^7 to 10^5 with combined treatment using 10 ppm MBT and enzyme. Further it was shown that on application of enzyme (0.10 kg metric tonne^{-1}) on machines producing printing grade papers, it was possible to reduce the biocide concentration from 0.13 to 0.02 kg metric tonne^{-1}, while maintaining a constant level of microorganisms. Similar reduction in biocide concentration was achieved in a backwater system of a paper mill by the use of an enzyme preparation called EDC-1 [6,18]. Levan hydrolase has also been used in white water of a board mill [20]; breaks were reduced from three per day to three per month. It was also possible to reduce the biocide concentration by 50%. Another enzyme product, NOPCO EDC-1, a nontoxic enzyme, has also been used in the USA, Japan, UK and Scandinavia for better slime control with a lower biocide level [1].

Table 2 Effect of Bioplus® and Bioplus® + levanase on hand sheet properties of unrefined (U) and refined (R) bleached mixed wood pulp

Property	Control		Bioplus®		Bioplus® + levanase	
	U	R	U	R	U	R
Freeness (ml)	539	390	549	370	579	400
Fold (double folds)	14	73	10	97	13	89
Opacity (%)	83	74	84	77	86	78
Basis weight (g m^{-2})	63.6	62.7	62.2	63.2	62.5	63
Bulk (cm^3 g^{-1})	1.75	1.38	1.8	1.33	1.82	1.36
Burst factor	21	43.2	17.5	45.6	16.9	42.4
Breaking length (km)	3.70	6.174	3.59	6.22	3.48	6.24
Tear factor	80.2	81.3	85.2	71	87.9	71.2
Brightness (% pv)	76	71.7	75.7	70.6	76.5	72.1
Thickness (mm)	0.113	0.0868	0.112	0.0844	0.113	0.0859

Effect of biocontrol on sheet properties

Before recommending the implementation of an enzyme usage programme, it is essential that information on its effect on the paper properties be known. Table 2 shows the effect of various treatments on paper properties. Upon the addition of levanase along with Bioplus®, the values of the burst factor decreased by 19.5% in unrefined and 1.85% in refined compared to the corresponding control. In refined pulp, the treatment using levanase and Bioplus® showed a 21.9% increase in double fold (paper property) as compared to the control, while no change was observed in unrefined pulp. The tear factor showed a 9.6% increase and a 12.4% decrease on combined treatment of enzyme and Bioplus® in unrefined and refined pulps respectively, compared to the control. The addition of enzyme did not result in much change in such properties as basis weight, freeness, thickness, bulk, breaking length, brightness and opacity. Thus, the small changes in certain properties after the addition of enzyme along with biocide did not adversely affect the overall handsheet properties. Colasurdo and Wilton [6] also observed that EDC-1 enzyme preparation did not affect the paper-making process; rather it improves the paper quality.

Every paper mill is unique as far as the presence of different microorganisms is concerned. Thus it becomes imperative to understand the problem of a particular mill and recommend control strategies accordingly. The control strategies may be a combination of two or more measures. Though biocides have been in use for a long time for the control of slime, their increased use over the years has become a growing concern as far as the environment is concerned. Hence, an approach towards ecofriendly measures which can reduce the level of biocide was sought. To implicate these strategies in Indian mills, further research is needed for pilot scale investigations regarding enzyme efficacy, stability and economic production.

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References

1 Anonymous. 1990. Slime control using an enzyme product-NOPCO EDC-1. Papeterie 140: 32.
2 Asaoka H. 1985. Isomerization of D-glucose by disodium pentasilicate in methanol-water solutions. Carbohyd Res 137: 99–109.
3 Baker ED. 1981. Using chlorine dioxide for slime control in alkaline paper machine systems. Tappi J 64: 91–93.
4 Bandala LY and MJ Boho. 1987. An innovative method for monitoring microbiological deposits in pulp and paper mills. Tappi J 70: 68–71.
5 Chaudhary A, LK Gupta, JK Gupta and UC Banerjee. 1996. Purification and properties of levanase from *Rhodotorula* sp. J Biotechnol 46: 55–61.
6 Colasurdo AR and VS Brozel. 1991. SONOCO utilizes enzymes to control problems with slime and deposits. Pulp and Paper 62: 89–93.
7 Dawes EA, DW Ribbon and DA Rees. 1968. Sucrose utilization by *Zymomonas mobilis*: formation of a levan. Biochem J 98: 804–811.
8 Freis RE. 1984. The effect of specific enzyme on biocide use. Tappi J 67: 100–102.
9 Fuwa M, F Ueda and T Numakura. 1990. Slime problems and slime control. Jap J Paper Technol 7: 19–25.
10 Goldstein SD. 1987. Some overlooked fundamentals of slime control. Appita 40: 213–216.
11 Harris IA and H Garnett. 1989. Using unglazed ceramic tiles to sample attached microbial flora in paper machines. Tappi J 72: 179–180.
12 Hollis CG. 1985. Alkaline paper making systems and microbial activity. pp 48–52, Tappi Alkaline Paper Makers Conference, Atlanta GA, USA.
13 Holt JG (ed). 1984–1989. Bergey's Manual of Systematic Bacteriology. Vol 4. Williams & Wilkins, Baltimore, MD.
14 Jaquess PA and PH Sorrelle. 1988. The role of pH on microbial activity. pp 312–317, Papermakers Conference, Tappi proceedings, Atlanta, GA, USA.
15 Jung WK and HJ Kutzner. 1978. Microbiological problems associated with closed process water systems in the paper industry. Eur J Appl Microbiol Biotechnol 5: 215–224.
16 Kregten MC. 1988. Slime flora of New Zealand paper mills. Appita 41: 470–474.
17 Martin HC. 1988. Identification and implications of troublesome slime forming bacteria found in paper mill systems. Paper Maker Conference, Chicago, IL, USA, 11–13 April. Tappi Press, Atlanta, GA, USA.
18 Moore AH and MJ Hatch. 1984. Enzyme to control microbiological deposits. pp 15–19, Pira Conference 'Slime and its Control SPB/3', UK.
19 Park YK, MPL Mortatti and HH Sato. 1983. Study on levan formation during fermentation of *Zymomonas mobilis* on sucrose. Biotechnol Lett 5: 515–518.
20 Patterson JV. 1986. Enzyme for improved deposit control. Tappi Seminar notes, pp 23–27, Paper making Chemical Processing Aids.
21 Poock SJ. 1985. Microbial contamination in the use of starch. Tappi J 67: 78–80.
22 Tomlin JL. 1986. Microbiological growth and control in the paper making process. Tappi Seminar notes, pp 15–22. Paper making Chemical Processing Aids.
23 Vaatanen P and SI Niemela. 1983. Factors regulating the density of bacteria in process waters of a paper mill. J Appl Bacteriol 54: 367–371.