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ORIGINAL PAPER

Pseudoxanthomonas bacteria that drive deposit formation of wood extractives can be flocculated by cationic polyelectrolytes

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Abstract Runnability problems caused by suspended bacteria in water using industries, have, in contrast to biofilms, received little attention. We describe here that *Pseudoxanthomonas taiwanensis*, a wide-spread and abundant bacterium in paper machine water circuits, aggregated dispersions of wood extractives ("pitch") and resin acid, under conditions prevailing in machine water circuits (10^9 cfu ml^{-1}, pH 8, 45°C). The aggregates were large enough (up to 50 μm) so that they could be expected to clog wires and felts and to reduce dewatering of the fiber web. The *Pseudoxanthomonas* bacteria were negatively charged over a pH range of 3.2–10. Cationic polyelectrolytes of the types used as retention aids or fixatives to flocculate "anionic trash" in paper machines were effective in flocculating the *Pseudoxanthomonas* bacteria. The polyelectrolyte most effective for this purpose was of high molecular weight (7–8 × 10^6 g mol^{-1}) and low charge density (1 meq g^{-1}), whereas polyelectrolytes that effectively zeroed the electrophoretic mobility (i.e., neutralized the negative charge) of the bacterium were less effective in flocculating the bacteria. Based on the results, we concluded that the polyelectrolytes functioning by bridging mechanism, rather than by neutralization of the negative charge, may be useful as tools for reducing harmful deposits resulting from interaction of bacteria with wood extractives in warm water industry.

Keywords Flocculation · *Pseudoxanthomonas taiwanensis* · Pitch · Papermaking · Cationic polyelectrolyte

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Introduction

Deposits generated by microorganisms, colloidal, and finely divided substances from wood ("extractives", "pitch"), and papermaking raw materials are a common fouling problem in paper machines. These deposits can impede paper machine runnability by clogging wires and felts of the machines, causing web breaks and down time, and lower paper quality (e.g., spots and holes in the paper) [2, 7, 9, 10]. Deposits may also cause problems in the dry end of paper machines by fouling on-line calanders.

Various dispersants are used to alleviate the fouling, but they also tend to deactivate cationic retention and fixation aids [10], i.e., the additives used to bind fine material to the paper web. Moreover, deposits may accumulate in spite of the use of dispersants. Biofilms in the paper industry have been investigated in many studies [17–19, 21, 31, 33], but the role of suspended microorganisms in fouling of the process equipment is not well understood.

We investigated the role of suspended bacteria in interactions between colloidal wood extractives and the ability of cationic polyelectrolytes commonly used in papermaking [3, 27] to flocculate the model bacterium, *Pseudoxanthomonas taiwanensis*. This bacterium has been shown prevalent in the water circuits of many pulp and paper mills [9, 22, 25, 26]. We showed that the white water bacteria *Psx. taiwanensis* aggregated with wood

extractives and resin acid to droplets of visible size and that cationic polyelectrolytes could be used to flocculate this bacterium.

Materials and methods

Bacteria

Pseudoxanthomonas taiwanensis strain JN11306 (formerly cited as *Thermomonas* sp., [17] was cultured in R2 broth (Lab M Ltd., Bury, UK) made in spring water (Tuuslähde, Kerava, Finland), 2 days, 45°C, 160 rpm. The biomass was harvested by centrifugation at 2,800 × *g*, 10 min. The supernatant was discarded and the pellet suspended to 10 mg ml^{-1} in sterile 10 mM NaCl or buffered with 1 mM $NaHCO_3$ or 10 mM Tris–Cl (pH 8). If not otherwise stated, the bacterial suspensions were thermostated to 45 ± 1°C in a water bath for ≥1 h before each measurement.

Polyelectrolytes

Poly(diallyldimethyl)ammoniumchloride (polyDADMAC, Sigma-Aldrich Chemie GmbH, Steinheim, Germany), cationized polyacrylamide, (C-PAM, Kemira Oyj, Vaasa, Finland) and cationized starch (CS, Ciba Specialty Chemicals, Raisio, Finland) were used (Table 1). PolyDADMAC and C-PAMs were used as delivered. The starches are described in detail by Tammelin et al. [30]. The stock solutions of the polyDADMAC and the C-PAMs were prepared in water by stirring the polyelectrolyte at 22–24°C overnight. Solutions of starches were prepared by autoclaving a mixture of CS and water for 10 min at 120°C. Stock solutions of all polyelectrolytes (2.3–7.2 g l^{-1}) were prepared on the day before measurements. For the electrophoretic mobility and turbidity measurements, the stock solutions were diluted to 0.42 or 0.042 g l^{-1} with 1 mM $NaHCO_3$ in 10 mM NaCl on the day when used and stirred ≥1 h before use.

Wood extractives

Unbleached thermomechanical pulp (TMP, Norway spruce) was freeze-dried and extracted with hexane in a Soxhlet apparatus. The solvent was evaporated; the residue was dissolved in acetone and stored at −20°C. This solution was concentrated to the final stock solution concentration of 100 mg ml^{-1}. Dispersions of extractives were prepared as described by Sundberg et al. [28] by injecting the stock solution into aqueous solution resulting in precipitation of colloidal particles of extractives. Stock solution of abietic acid (technical grade 75%, Fluka, Buchs, Switzerland) was prepared in acetone (60 mg ml^{-1}) and stored at −20°C. Dispersions were prepared as described for the extractives.

Other chemicals

The other chemicals were of analytical grade. The deionized water was purified with a Millipore Synergy UV unit.

Electrophoretic mobility of *Pseudoxanthomonas* bacteria

Electrophoretic mobilities were determined with a Coulter Delsa 440 Laser-Doppler electrophoresis instrument (Coulter Electronics Ltd., UK) at 0.5 mA using a run time of 120 s. pH dependence of *Psx. taiwanensis* (1,000 mg l^{-1} in 10 mM NaCl) was measured from pH 2 to 10 (adjusted with HCl or NaOH). For determination of the effect of polyelectrolytes on the electrophoretic mobility of *Psx. taiwanensis* in 10 mM NaCl, pH was first adjusted to 8 using 1 mM $NaHCO_3$. Polyelectrolyte solutions were added to the bacterial suspensions under stirring (350 rpm). The total volume was adjusted to 55 ml with 1 mM $NaHCO_3$ in 10 mM NaCl (1,000 mg bacterial biomass wet wt l^{-1}). Each sample was stirred for 15 min prior to measuring the mobility. Each measurement was repeated 2–3 times.

Table 1 Properties of cationic polyelectrolytes

Type of cationic polyelectrolyte	Notation	$M_w \times 10^6$ g mol^{-1}	Charge density meq g^{-1}
Poly(diallyldimethyl)-ammonium chloride, 20% w/w aq. soln	polyDADMAC	0.4–0.5	6.1
Cationized polyacryl-amide, granular	$C\text{-}PAM_{HL}$	7–8	1
Cationized polyacryl-amide, 27% w/w aq. soln	$C\text{-}PAM_{LL}$	0.2	1
Cationized starch, DS[a] 0.2	CS_{HL}	0.88	0.5
Cationized starch, DS[a] 0.75	CS_{HH}	0.87	1.5
Cationized starch, DS[a] 0.75	CS_{LH}	0.45	1.5

[a] Degree of substitution

Flocculation by polyelectrolytes

Pseudoxanthomonas taiwanensis was suspended in 1 mM $NaHCO_3$ with 10 mM NaCl. Polyelectrolyte solutions were added to the bacterial suspension under stirring (350 rpm) at 22–24°C. The volume was adjusted to 210 ml with 1 mM $NaHCO_3$ in 10 mM NaCl (200-mg bacterial biomass wet wt l^{-1}). The sample was continuously stirred (350 rpm) and pumped through the cylindrical glass measurement cell of a Turbiscan On-line instrument (Formulaction, France) with a flow rate of 600 ml min^{-1} controlled by a peristaltic pump. This instrument measures transmission of light through the sample using a near-infrared LED (light emitting diode $\lambda = 850$ nm) as a light source.

For particles that absorb no light, the transmission (T) of light through the Turbiscan cell decreases exponentially with increasing diameter of the particles at a given volume fraction and T can thus be used to monitor particle growth when the bacteria flocculate [4, 5]. With the samples studied here, the changes in transmission due to flocculation were very low due to the low particle concentrations ($\approx 10^{11}$ cells l^{-1}). Therefore, rather than attempting to calculate changes in particle size using light scattering theory, we used the change in transmission during the first 60 s after the addition of polyelectrolyte as a simple measure for comparing the efficiency of the different polyelectrolytes as flocculants.

Flow cytometry

Particle-size distributions were measured by flow cytometer (BD LSR II with FACSDiva software, BD Franklin Lakes, NJ, USA). Bacterial suspensions were made in 1 mM $NaHCO_3$ or in 10 mM Tris–Cl with and without 10 mM NaCl (pH 8). Measurements were executed after mixing the bacteria with the dispersion and incubating for 1 h at 45°C. The measurements were calibrated by measuring the forward scatter values for standard microspheres (Flow Cytometer Size Calibration Kit, Invitrogen, Eugene, OR, USA) in 1 mM $NaHCO_3$ with 10 mM NaCl. After the flow cytometric measurement, the same mixtures were examined with a phase-contrast microscope (Carl Zeiss Axioscope 40).

Results

Electrophoretic mobility of *Pseudoxanthomonas taiwanensis* depends on pH and the presence of cationic polyelectrolytes

Figure 1 shows the electrophoretic mobility of *Psx. taiwanensis* in a non-buffered, low-salt medium at pH values from 2 to 10 and the influence of cationic polyelectrolytes on the mobility. In the absence of any polyelectrolyte (Fig. 1a), the point of zero charge (*pzc*), i.e., the pH at which the mobility of the bacteria without adsorbed polymer was zero, also denoted the isoelectric point of the bacteria, occurred at pH 3.2. Above pH 3.2, *Psx. taiwanensis* was negatively charged and the charge increased in parallel with the pH value.

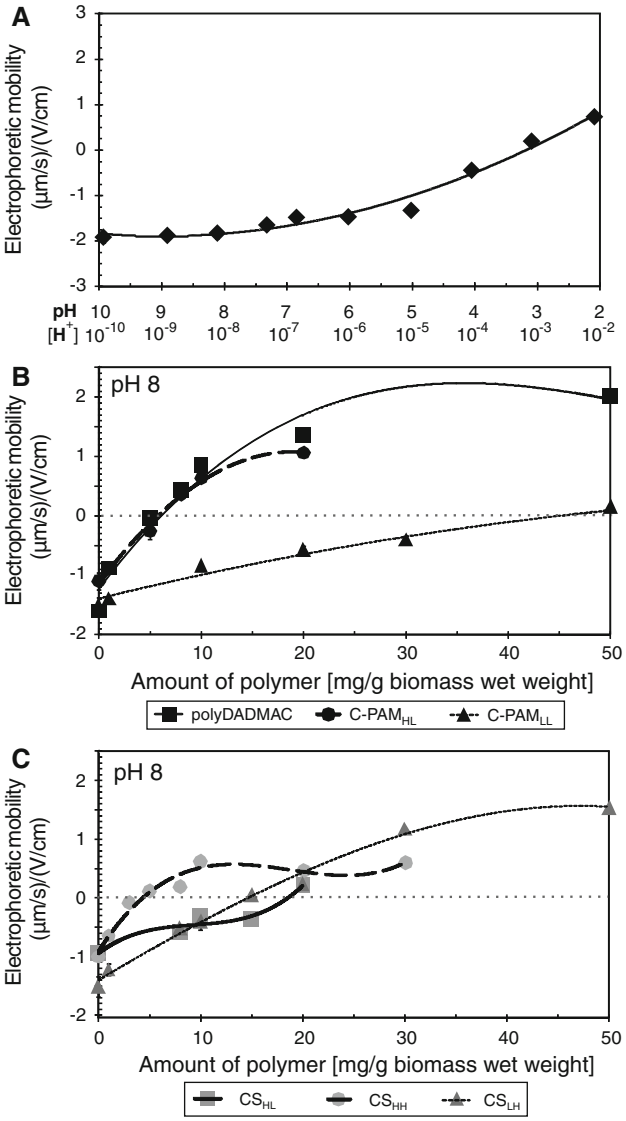

Fig. 1 Electrophoretic mobility of live *Pseudoxanthomonas taiwanensis* in the absence and presence of cationic polyelectrolytes. Electrophoretic mobility of live cells of *Psx. taiwanensis* JN11306 (1 g wet wt l^{-1}) was measured in different media. **a** Aqueous 10 mM NaCl with pH adjusted with NaOH or HCl. **b**, **c** Aqueous 1 mM $NaHCO_3$ in 10 mM NaCl (pH 8) with cationic polyelectrolytes (in Table 1) added to concentrations indicated at the *x*-axis. **b** PolyDADMAC, C-PAM_{HL}, C-PAM_{LL}; **c** CS_{LH}, CS_{HH} and CS_{HL}. The *dotted lines* indicate zero electrophoretic mobility. SD $\leq$ 0.2. The initial electrophoretic mobility of bacteria at pH 8 varied due to characteristics of the living material

Table 2 The dosages of cationic polyelectrolytes required for reducing the electrophoretic mobility of live *Pseudoxanthomonas taiwanensis* to zero

Polyelectrolyte	Dosage of polyelectrolyte required for reducing the mobility to zero mg g^{-1}	Efficiency in decreasing the mobility $-[\mu m\ s^{-1}/V\ cm^{-1}/$ mg $g^{-1}]$
polyDADMAC	5	0.17
C-PAM$_{HL}$	6	0.18
C-PAM$_{LL}$	45	0.03
CS$_{HL}$	18	0.05
CS$_{HH}$	4	0.23
CS$_{LH}$	14	0.10

Psx. taiwanensis JN11306 was suspended in 1 mM $NaHCO_3$ (pH 8) with 10 mM NaCl and the electrophoretic mobility measured. The dosage of polyelectrolyte required to zero the electrophoretic mobility of the bacteria was estimated from Fig. 1 (shown by *dotted lines*). The efficiency of decreasing the electrophoretic mobility of the bacteria to zero was extrapolated from the curves shown in Fig. 1b, c. SD $\leq$ 30%. The properties of the polyelectrolytes are shown in Table 1

When cationic polyelectrolytes were added at pH 8 (Fig. 1b, c), the net-negative charge was first neutralized, observed as zero electrophoretic mobility, and then, at higher dosages of polyelectrolyte, the net charge of the bacteria became positive. These events show that the cationic polyelectrolytes adsorbed onto the surface of the bacteria. The decrease of mobility of the bacteria per added mass of polyelectrolyte is shown in Table 2. It shows that three of the polyelectrolytes (polyDADMAC, C-PAM$_{HL}$, and CS$_{HH}$) were more effective than the other three (C-PAM$_{LL}$, CS$_{HL}$, and CS$_{LH}$) in reducing the mobility towards zero. PolyDADMAC has the highest charge density of the used polyelectrolytes and required the lowest dosage (5 mg g^{-1}) for neutralizing the charge of the bacteria. The two C-PAMs have the same charge densities but different molecular weights. Much higher dosages of low-molecular-weight C-PAM was required for neutralization than of high-molecular-weight C-PAM. CS$_{HL}$ has a lower charge density than CS$_{HH}$ and, hence, more of this polymer had to be added to achieve neutralization. The polymers with high molecular weight and low charge density (C-PAM$_{HL}$, CS$_{HH}$) neutralized the charge of bacteria effectively.

Cationic polyelectrolytes flocculated *Pseudoxanthomonas taiwanensis*

To answer the question of whether flocculation of suspended *Psx. taiwanensis* was due to neutralization of the negative surface charge of the bacterium or to some other mechanism, the rates of flocculation and flock sizes were measured. Cationic polyelectrolytes were added to suspensions of *Psx. taiwanensis* and the time-dependent

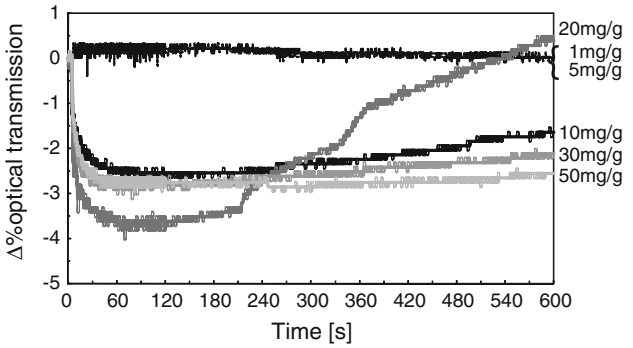

Fig. 2 Changes in optical transmission of a suspension of live *Pseudoxanthomonas taiwanensis* in response to different dosages of C-PAM$_{HL}$. *Psx. taiwanensis* JN11306, 0.2 g wet wt l^{-1} (equivalent to $\approx 10^{11}$ bacterial cells l^{-1}) was suspended in aqueous 1 mM $NaHCO_3$ (pH 8) with 10 mM NaCl. C-PAM$_{HL}$ was dosed under constant stirring and the resulting changes in the optical transmission were constantly recorded. The properties of C-PAM$_{HL}$ are shown in Table 1

decrease of optical transmission was measured as an indicator of flocculation of the bacteria (Fig. 2). It showed that when the cationic polyacrylamide (C-PAM$_{HL}$) was mixed into the suspension of *Psx. taiwanensis*, a rapid initial decrease of the optical transmission was detected. This indicates that the bacteria aggregated into larger particles. The rate of transmission change, i.e., the rate of particle growth, increased with increasing dosage of the C-PAM$_{HL}$ until the dosage of 20 mg per g of bacteria (wet wt) within 60 s. During prolonged stirring (180 s and beyond) the optical transmission eventually reverted to its initial value, indicating that the formed aggregates broke apart. At higher dosages of C-PAM$_{HL}$ (>20 mg g^{-1}), aggregates remained smaller than with 20 mg g^{-1} and did not break down as easily.

Experiments similar to that shown in Fig. 2 were conducted with polyDADMAC, C-PAM$_{LL}$ and the three cationized starches listed in Table 1. The changes of the optical transmission after 60 s in response to the dosed cationic polyelectrolyte are shown in Fig. 3. The arrows in Fig. 3 indicate the dosages of the polyelectrolytes where electrophoretic mobility of the *Pseudoxanthomonas* bacteria was zero (Fig. 1b, c, dotted lines). For five of the cationic polyelectrolytes (C-PAM$_{HL}$, polyDADMAC, CS$_{HL}$, CS$_{HH}$, CS$_{LH}$), a higher dosage of the polyelectrolytes was needed for the flocculation than for the neutralization of the bacteria. Figure 3 shows that C-PAM$_{HL}$ induced the largest decrease in the optical transmission, indicating that it was more effective in flocculating the bacteria than the other five cationic polyelectrolytes (C-PAM$_{LL}$, polyDADMAC, CS$_{HL}$, CS$_{HH}$ and CS$_{LH}$). This difference between the efficiencies of C-PAM$_{HL}$ and CS$_{HH}$ in flocculating *Pseudoxanthomonas* bacteria is interesting. It shows that the power to neutralize the electrophoretic

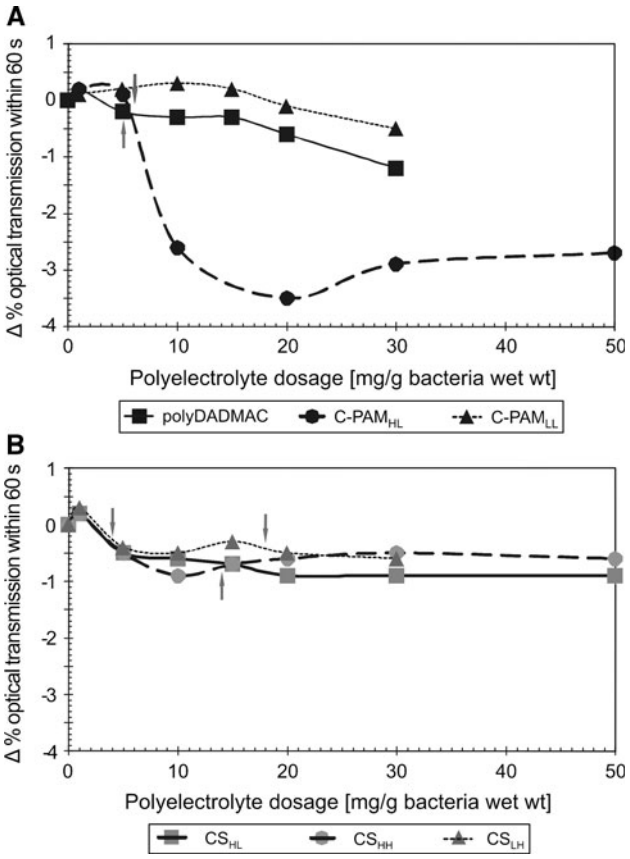

Fig. 3 Decrease in optical transmission of suspended *Pseudoxanthomonas taiwanensis* JN11306 in response to additions of polyelectrolytes. The change was extrapolated from the data of similar measurements to that shown in Fig. 2. PolyDADMAC, C-PAM$_{HL}$, C-PAM$_{LL}$ (**a**) and CS$_{LH}$, CS$_{HH}$ and CS$_{HL}$ (**b**). SD $\leq$ 30%. The properties of the polyelectrolytes are shown in Table 1. *Arrows* mark the dosage of the polyelectrolyte where the electrophoretic mobility of the bacteria was zero as detected based on the data shown in Fig. 1b, c

mobility of the bacteria (Table 2) is not the feature that determines the power to flocculate the same bacterium (Table 2). The data in Table 2 together with that shown in Fig. 3 indicate that the properties determining high capacity of a polycations to flocculate *Pseudoxanthomonas* bacteria are high molecular weight combined with low charge density (C-PAM$_{HL}$).

The white water bacterium *Pseudoxanthomonas taiwanensis* forms aggregates with wood extractives

When dispersion of wood extractives (TMP from spruce wood) were incubated with *Psx. taiwanensis* it was seen that aggregates visible to the naked eye resulted from mixing the dispersed wood extractives with *Psx. taiwanensis* (Fig. 4a, top panel). The wood extractives, *Psx. taiwanensis*, and mixtures of these two were analyzed for particle size distribution by flow cytometry and examples of the histograms are shown in Fig. 4a (lower panels). The forward scatter values in Fig. 4a were translated into particle sizes based on calibration done with size-certified polystyrene beads (sizes from 0 to 15 μm). Figure 4b summarizes the flow cytometer results. The results show that the particle sizes of dispersed wood extractives alone ranged from <1 to 4 μm and the bacteria were <1 μm. However, when the bacteria were mixed with the wood extractives, particles larger than the single components emerged, indicating aggregation (Fig. 4a, b). Light microscopic images (Fig. 5) of the same suspensions show that polydisperse agglomerates up to >50 μm in size were generated (Fig. 5c). The agglomerates were built of hundreds of bacterial cells glued together by the dispersed wood extractives. No such agglomerates were observed in either of the components separately (Fig. 5 a, b). The largest agglomerates (Fig. 5c) are not visible in the histograms (Fig. 4a), since the cytometer settings cut-off value was set at 15 μm.

Resin acids are the main lipophilic components of spruce wood extractives. The interactions of *Psx. taiwanensis* with resin acid were investigated using abietic acid as a model substance. Dispersions of abietic acid were incubated with and without *Psx. taiwanensis* and analyzed by flow cytometry. Figure 6 shows no aggregates in the dispersion of abietic acid or in the suspension of *Psx. taiwanensis*, but massive aggregation, visible to the naked eye, was observed when these two were mixed. Figure 6a shows the forward scatter histograms. The dispersion of abietic acid and the suspension of *Psx. taiwanensis* had particles size distributions corresponding to <1–4 μm when measured separately, but 4–15 μm when the bacteria were mixed with the abietic acid. Thus, Fig. 6a shows that polydisperse aggregates emerged when *Psx. taiwanensis* was mixed with abietic acid.

Figure 6b compiles the average particle size distributions of three parallel experiments. Aggregates larger (>2 μm) than the cells of *Psx. taiwanensis*, and larger than the droplets of dispersed abietic acid, emerged similarly in three of the buffers when the dispersion of abietic acid and the suspension of *Psx. taiwanensis* were mixed, the exception being Tris–Cl buffer with no NaCl. Figure 7 displays phase-contrast micrographs of a large agglomerate >50 μm (Fig. 7c), typically formed when *Psx. taiwanensis* bacteria were mixed with abietic acid. It shows droplets of abietic acid trapped inside the biofilm-like rafts consisting of the *Psx. taiwanensis* cells. No such agglomerates were seen in abietic acid in absence of the bacteria.

Discussion

In this paper, we showed that bacteria from paper-machine white water aggregated wood extractives and resin acid to

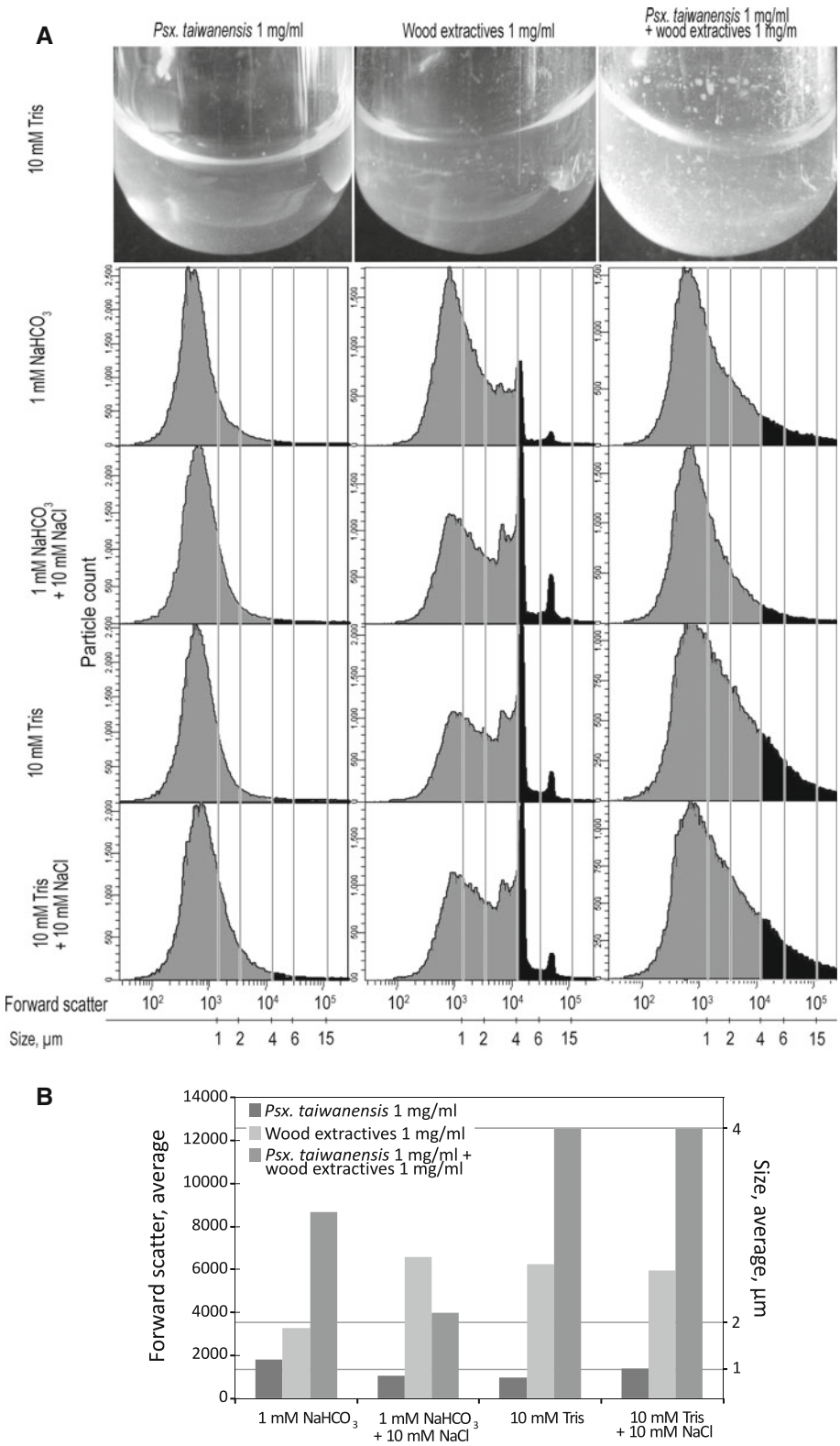

Fig. 4 **a** Aggregation of dispersed wood extractives by *Psx. taiwanensis* JN11306. Suspensions of *Psx. taiwanensis* JN11306 were incubated with and without added wood extractives at 45°C for 1 h at pH 8. *Top panel*, photographs of the test tubes. Histograms show the particle size distributions analyzed by flow cytometry. *Y*-axis shows particles count, *x*-axis the forward scatter values and the calibrations obtained with standard microspheres (1, 2, 4, 6, and 15 µm in diameter). The *grey areas* indicate the numbers of particles <4 µm and the *black areas* from 4 to 15 µm in diameter. **b** Averaged forward scatter values and particle sizes of *Psx. taiwanensis* JN11306 suspensions and dispersed wood extractives, separately and in combination, in buffers with different ionic compositions, pH 8. The *columns* indicate the fraction of particles in the window from 0 to 4 µm in diameter from the measurements in Fig. 4a. The total number of particles measured per injection was 10^5

droplets of visible size. *Pseudoxanthomonas taiwanensis* is a frequent and abundant contaminant of paper machines [9, 22, 31]. Abietic acid was used as the model resin acid because it is a major component in spruce wood pitch [15, 32]. The ability of *Psx. taiwanensis* to aggregate wood extractives may not be unique to this species, as similar phenomenon has been observed with *Burkholderia* spp. isolated from paper machines [20].

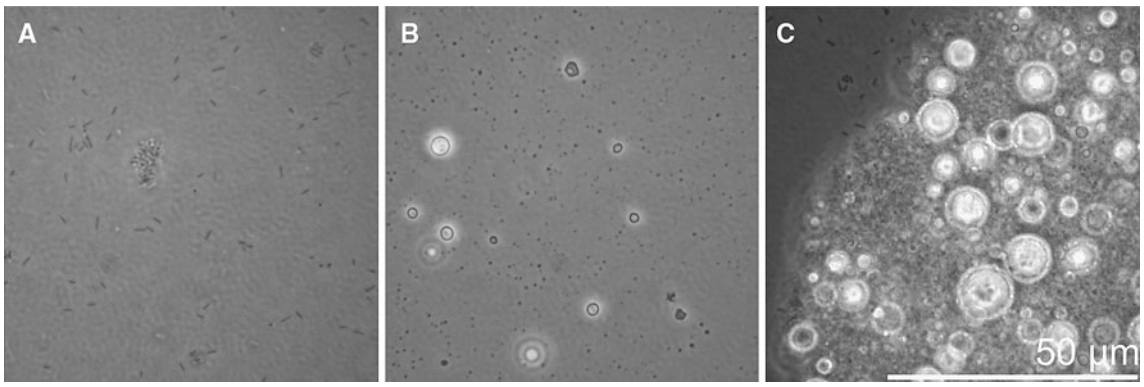

Fig. 5 Light microscopic views of the suspensions analyzed by flow cytometry in Fig. 4. *Psx. taiwanensis* JN11306 (10^9 cells ml^{-1}, **a**); 1 mg ml^{-1} of dispersed wood extractives (**b**); aggregates formed when the bacteria were mixed with the dispersed wood extractives (**c**). Buffer, 10 mM Tris (pH 8). Aggregates were larger than the bacteria or the wood extractives alone

To avoid aggregation of wood extractives, tools are needed for eliminating *Psx. taiwanensis* and other similarly responding bacteria in paper-machine water circulation. The net charge of *Psx. taiwanensis* at common paper-making pH values was negative and the *pzc* of the bacteria was 3.2, similar to many other bacteria [13, 16, 23], and reflects the pK_a value of carboxylic groups of polysaccharides (uronic acids, 3.6–3.8). Bacterial cells are charged and their surface structure is complex, rendering the location of the slipping plane between the bacteria and the surrounding solution during the measurements of electrophoretic mobilities uncertain. Therefore, we reported only primary electrophoretic mobility data rather than converting them in the conventional way to zeta potentials using, e.g., the Smoluchowski equation [12], which applies to spherical, non-deformable particles. If polyvalent simple cations are added to the bacterial suspension, they effect on the stability of the bacterial cells. Polyvalent cations reduce the thickness of the electrical double layer and may also bind specifically to anionic polysaccharides. Both effects will reduce the repulsion between the bacterial cells, leading to flocculation. The addition of the polyvalent cations will decrease the electrophoretic mobility of the cells. These kinds of phenomena have been observed earlier in studies of colloidal wood resin [24, 29].

Of the six polymers tested in this study, polyDADMAC had by far the highest charge density and thus the amount required to neutralize the charge of the bacteria was low. The two cationic PAMs have the same charge density but a much higher amount of the polymer with low molecular weight was required for neutralization. A simple explanation would be that each molecule of the high-molecular-weight C-PAM_{HL} attached to the bacterium surface spans over a larger number of cationic groups than a polymer with low molecular weight, explaining why less of the former was required for neutralization. A similar argument would explain the differences between CS_{HH} and CS_{LH}, which have the same charge density. CS_{HL} has a lower charge density than CS_{HH} and, hence, more of this polymer was required for achieving neutralization. In summary, either a polymer with a very high charge density (polyDADMAC) or polymers with low charge density and high molecular weight (C-PAM_{HL}, CS_{HH}) neutralize the charge of bacteria effectively. The interaction of the polycations with the polymer layer surrounding the bacteria will be complex, the driving forces for adsorption being a combination of electrostatic attraction, entropic factors (associative phase separation) [14], and perhaps also interactions specific to each type of polymer.

The present results showed that C-PAM_{HL}, with a molecular weight of an order of magnitude larger than any of the other polymers (M_w 7–8 $\times$ 10^6 mol g^{-1} and charge density 1 meq g^{-1}), was more effective than any of the other cationic polyelectrolytes (C-PAM_{LL}, polyDADMAC, CS_{HL}, CS_{HH} and CS_{LH}) studied in flocculating the bacteria. Polymers with high M_w and low charge density may flocculate particles by forming bridges, whereas polymers with low M_w and high charge density tend to flocculate particles by neutralization of their charge [1, 8]. Based on the results presented in this paper, we suggest that the polyelectrolyte that most effectively will flocculate *Pseudoxanthomonas* bacteria functions by the bridging mechanism (C-PAM_{HL}), whereas charge neutralization is less effective. This is in line with Hughes et al. [11], who showed that bacteria (*Escherichia coli* and *Bacillus subtilis*) flocculated in the presence of cationic polyacrylamides with a high M_w (1–1.8 $\times$ 10^7), and charge densities similar to those of C-PAM_{HL}, and with study by Eriksson and Härdin [6].

Conclusions

The aggregates of white water bacteria with wood extractives ("pitch") and resin acid observed in this investigation

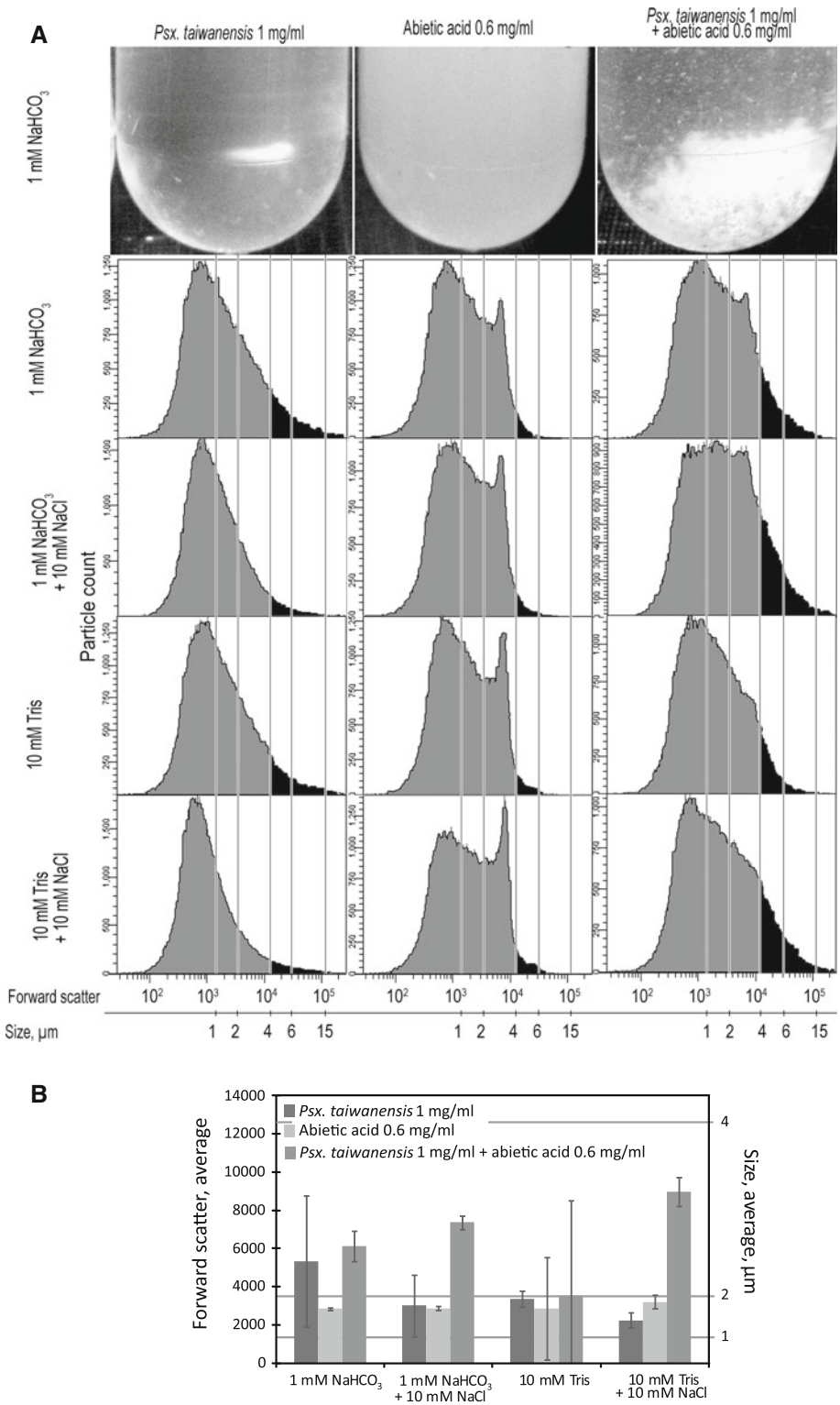

Fig. 6 **a** Aggregation of dispersed abietic acid by *Psx. taiwanensis* JN11306. *Top panel*, photographs of the test tubes. The measurements were made like those in Fig. 4a. **b** Averaged forward scatter values and particle sizes of *Psx. taiwanensis* JN11306 suspensions and dispersed abietic acid, separately and in combination, in buffers with different ionic compositions, pH 8. The *columns* indicate the fraction of particles in the window from 0 to 4 µm in diameter from the measurements in Fig. 6a. The total number of particles measured per injection was 10^5. The *bars* show standard deviations, calculated from three separate measurements

are large enough so that they could clog wires and felts and reduce dewatering of the fiber web in the paper machine by aggregating wood extractives and pitch to droplets of visible size. The aggregates could deposit on the surface of the paper web and parts of paper machines and subsequently induce fouling of hot surfaces of the on-line calanders at the dry end of the paper machine. The present work gives indications for tools to prevent these problems by using cationic polyelectrolytes of the types also used as retention aids or fixatives to flocculate "anionic trash" in paper

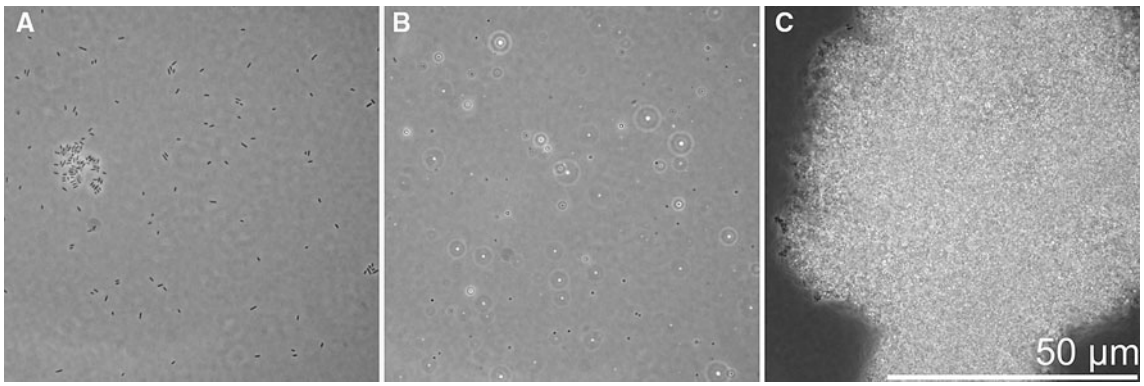

Fig. 7 Light microscopic views of the suspensions analyzed by flow cytometry in Fig. 6a. *Psx. taiwanensis* JN11306 (10^9 cells ml^{-1}, **a**); 0.6 mg ml^{-1} of dispersed abietic acid (**b**); a large aggregate typically formed when the bacteria were mixed with the dispersed abietic acid (**c**). Buffer, 1 mM $NaHCO_3$ (pH 8). Aggregates were larger than the bacteria or the abietic acid alone

machines. The cationic polyelectrolyte most effective for this purpose was the one with a high molecular weight and low charge density.

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