Bull. Environ. Contam. Toxicol. (2005) 75:135–142

DOI: 10.1007/s00128-005-0729-5

Bulletin of Environmental Contamination and Toxicology

Speciation and Stability of Particle-Bound Pulp and Paper Mill Sourced Resin Acids in Sodium Azide Preserved Recipient Water

S. Ali-Kanber, A. G. Langdon, A. L. Wilkins

Chemistry Department, The University of Waikato, Private Bag 3105,
Hamilton, New Zealand

Received: 29 January 2005/Accepted: 27 April 2005

We have previously reported an investigation of the levels of free and bound resin acids present in Tarawera River, New Zealand, water samples collected at the State Highway 30 (SH30) bridge down stream of the discharge point of the aerated lagoons of two pulp and paper mills (Ali Kanber et al. 2000). Our analyses showed that typically >50% of recoverable resin acids were bound to particles in the range 0.025-15 µm. More recently, others (e.g. Kostamo et al. 2004) have also reported that a large proportion of discharged wood extractives are adsorbed to particulates. A difficulty encountered in our investigations was the continuing degradation of resin acids during reproducibility, settling and sequential filtration experiments, which typically were performed several days, or weeks, after collection. This constraint prompted us to investigate the possibility that post-collection biodegradation of resin acids could be inhibited by the addition of 0.1% sodium azide to the water samples at the time of collection. In this paper we report the outcomes of experiments which showed (a) sodium azide addition inhibited the biodegradation of resin acids in Tarawera River water samples for periods of up to 90 days at 8°C, (b) sequential filtration of azide preserved river water afforded results which were comparable to those previously reported for non-preserved water samples, (c) some settling during prolonged storage occurred but (d) there was no evidence of non reversible aggregation on standing. We also report SEM evidence for the presence of Fe, Al, C and O rich surface deposits on particulate matter recovered by filtration of river water samples.

MATERIALS AND METHODS

Water samples (SH30) in screw capped 2.5 L glass Winchesters or 20 L plastic containers were collected from the Tarawera River at the State Highway 30 Bridge. After the addition of 0.1% of sodium azide, water samples were stored at 4°C (or 8°C; preliminary experiments) until required for analyses. Turbidity was determined using a Hach 2100 turbidimeter. Absorbances at 270, 340 and 440 nm were determined using a 1 cm quartz cell and a Hitachi 15-20 spectrometer. The pH of water samples was determined using an EPM-120 pH meter, calibrated against pH 6.86 and 4.0 buffer solutions. Total suspended solid (TSS) levels were determined gravimetrically by weighing material collected on pre-weighed filter

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Correspondence to: A. G. Langdon

papers (Whatman 0.45 μm), after oven drying at 105°C. Combustion at 550°C overnight afforded the level of inorganic suspended solids (ISS). Organic suspended solids (OSS) were calculated as TSS-ISS. Free and bound resin acid levels (μg/L) of filtered river water, were determined for well mixed sub-samples of sodium azide stabilised Tarawera River water using previously reported liquid/liquid or Soxhlet extraction and selected ion GC-MS methodologies (Ali Kanber et al. 2000). The recovery of *O*-methylpodocarpic acid was typically 75-105%. Sodium azide stabilized river water, stored in a 20 L plastic container for 5 and 90 days, was thoroughly shaken and allowed to stand for 30 min before analytical samples (2 x 1 L and 1 x 6 L) were withdrawn from a tap located 50 mm from the bottom of the container. The 6 L sample was sequentially filtered through Whatman glass fiber (6 L) and Millipore membrane filters (3 μm, 4.9 L; 0.8 μm, 3.8 L; 0.45 μm, 2.7 L; 0.2 μm, 1.6 L and 0.05 μm, 0.6 L). After each filtration step, the filters and 1 L (or 0.6 L for the 0.05 μm filtered sample) portions of the filtered water were Soxhlet or liquid/liquid extracted respectively. Filtered and unfiltered water samples were prepared and liquid/liquid or Soxhlet extracted (filters) on the same day.

In order to investigate particle settling, freshly sampled sodium azide stabilized river water in a 20 L plastic container was thoroughly shaken (t = 0), and allowed to stand without being disturbed for 30 days. Sub-samples (2 or 3 L) were gently drawn off from a tap located 50 mm above the bottom of the container after 15 and 30 min, 1 and 8 h, and 14, 26 and 30 days. The residue (1.6 L) remaining in the container (below the tap) at the end of the sampling period was thoroughly mixed and divided into 2 x 800 mL sub-samples which were filtered through Whatman No 1 and 0.45 μm filters (residue 1), or a 0.45 μm filter (residue 2), prior to liquid/liquid extraction or Soxhlet extraction.

Surface examination of the filtered particulate matter was undertaken using a Hitachi S4000 scanning electron microscope (SEM). Energy dispersive X-ray analysis was performed using a Kevex microanalyser. River water (0.5 L) was filtered using a 0.45 μm filter and the filtrate was freeze dried (DYNAVAC FD12). Small portions of the freeze residue, or a small piece of filter covered with suspended solid material were smeared on double sided conductive carbon sellotape (Shrinton, Japan), and mounted on an SEM stub and coated with 200 to 500 Å of platinum-palladium using a diode sputtering system (Hitachi E1030). Distribution maps identified the location of surface concentrations of elements.

RESULTS AND DISCUSSION

Our earlier finding that post collection biodegradation of resin acids present in natural (unstabilized) Tarawera River water samples stored at 4°C proceeded with a half life of c 19 days (Ali Kanber et al. 2000) prompted us to investigate whether preventing biological activity by azide addition would affect resin acid particle binding and aggregation. The effectiveness of azide in preventing degradation of resin acids is demonstrated by the data of Figure 1 (levels of natural and azide stabilized SH30 water samples stored for 1-14 days at 8°C), Table 1 (levels of

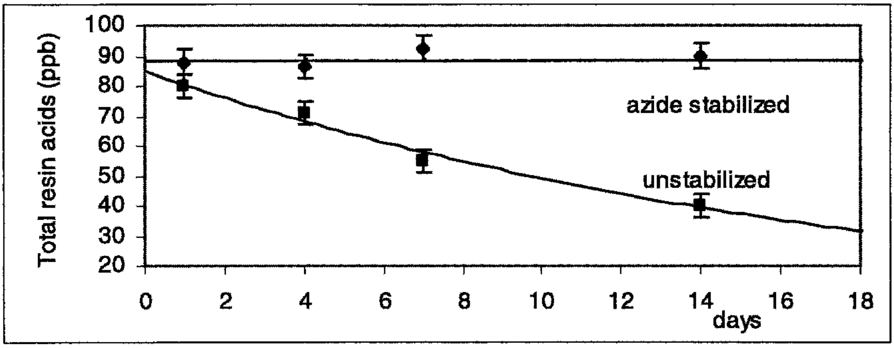

Figure 1. Resin levels determined for azide stabilized and unstabilized SH30 water collected 14/1/99 and stored at 8°C.

Table 1. Resin acid levels (μg/L) determined for unfiltered azide stabilized SH30 water samples collected 25/2/99.

storage time	secol/2	pim	18-Ab	DHAA	13-ene	Cls	TRA
5 days, + azide (n = 2)[a]	21.9	9.4	25.0	17.5	24.9	3.6	102
60 days, + azide (n = 2)[a]	16.4	7.0	24.7	18.7	26.4	2.9	96
90 days, + azide (n = 2)[a]	17.0	6.1	25.6	20.3	28.1	4.3	101

[a]Mean of n = 2 analyses. Abbreviations: secol/2 = secodehydroabietic acids-1 and 2; Pim = pimaric acid, 18-Ab = abietan-18-oic acid; DHAA = dehydroabietic acid; 13-ene = abiet-13-en-18-oic acid; Cls = 12-chloro, 14-chloro and 12,14-dichlorodehydroabietic acids; TRA = total resin acids.

resin acids recovered from duplicate unfiltered azide stabilized SH30 water samples, collected on 25/2/99 and stored for 5, 60 and 90 days at 4°C) and Table 2 (levels fresh and aged river water samples collected on 4/3/2000 and 3/4/2000). Within the expected uncertainty, there was no degradation of resin acids in the azide stabilized systems but c 50% of the resin acids were degraded in the unstabilized system. Azide addition did not affect the recovery of resin acids.

A feature of the results reported above is the very good reproducibility that was achieved. Recoveries were generally in the range of from 90 to 100% and cvs were always less that 10 % and often less than 5%. These results compare very favorably with the results of workers using other extraction, derivatization and analytical protocols (Morales et al. 1992; Lee et al. 1990; Richardson and Bloom 1982; Voss and Rapsomatiotis 1985). The results of sequential filtration experiments performed using azide stabilized river water which had been stored undisturbed for 5 and 90 days prior to analysis are presented in Table 3. These results show that for azide stabilized river water samples stored for 5 days and 90 days, particle associated resin acids levels and speciation effects were comparable to those which we have previously reported for freshly collected, unstabilized, river water samples (Ali Kanber et al. 2000).

Table 2. Mean resin acid levels (μg/L) identified in replicate analyses of unfiltered (UF) and sintered glass filtered (SGF) SH30 water.

Storage time, t (day)	seco1/2	pim	18-Ab	DHAA	13-ene	Cls	TRA	recv
t = 0, no azide, UF, 4/3/00	8.0	5.9	15.1	9.5	26.6	1.6	66.8	95%
stdev (n = 6)	0.39	0.28	0.66	0.46	1.3	0.12	2.8	
cv	4.9%	4.7%	4.4%	4.9%	4.9%	7.5%	4.2%	
t = 30, azide, UF, 4/3/00	8.1	5.9	14.8	9.4	24.9	1.7	64.6	96%
stdev (n = 6)	0.62	0.40	1.3	0.60	2.1	0.17	4.9	
cv	7.7%	6.8%	8.8%	6.4%	8.4%	10%	7.6%	
t = 0, no azide, SGF, 3/4/00	14.2	15.8	16.7	19.7	101	2.3	170	93%
stdev (n = 6)	0.69	0.61	1.22	1.95	3.65	0.08	7.7	
cv	4.9%	3.9%	7.3%	9.9%	3.6%	3.5%	4.5%	
t = 30, azide, SGF, 3/4/00	13.9	16.1	16.6	19.2	103	2.6	172	93%
stdev (n = 5)	0.37	0.72	0.98	0.60	3.9	0.16	6.5	
cv	2.7%	4.5%	5.9%	3.1%	3.8%	6.2%	3.8%	

Abbreviations: seco1/2 = secodehydroabietic acids-1 and 2; Pim = pimaric acid, 18-Ab = abietan-18-oic acid; DHAA = dehydroabietic acid; 13-ene = abiet-13-en-18-oic acid; Cls = 12-chloro, 14-chloro and 12,14-dichlordehydroabietic acids; TRA = total resin acids; recv = % recovery of *O*-methylpodocarpic acid.

There is no evidence for aggregation that is not reversed by shaking 30 minutes before sampling. Our results are also consistent with the finding of Hall and Liver (1996) that, in the biomass system which they investigated, azide addition inhibited resin acid degradation during partitioning experiments, and with the findings of Hoel and Aarsand (1995) that in a TMP effluent, resin acids were associated with particulate and colloidal fractions. Since our results showed that, in both stabilized and unstabilized Tarawera River water samples, part of the recoverable resin acids is associated with particulate material, we reasoned that allowing a bulk sample to stand for an extended period may result in particle settling and an accumulation of resin acid in water drawn from the bottom of the sample container. Thus settling was studied in 2.5 L Winchesters and a bulk 20 L container. In the latter case, sub-samples (2 or 3 L) were withdrawn from a tap located 50 mm from the bottom of the 20 L container after t = 0 and 15 min, 1 and 8 h, and 14, 26 and 30 days. Portions of each of the water sub-samples were liquid/liquid extracted to determine the total resin acid (TRA) content or filtered through 0.45 μm and liquid/liquid extracted (filtrate) and Soxhlet extracted (residue) to determine particulate and 0.45 μm filterable resin acid (Table 5).

Resin acid levels identified in the filtered liquid/liquid and Soxhlet extracts (eg a total resin acid level of 107 μg/L for the 14 day sub-sample) were in good agreement with those determined for the corresponding unfiltered sub-sample (110 μg/L). Marginally higher levels of TRAs in the unfiltered samples were detected in the 0-8 h sub-samples than was the case for the 14-30 day sub-samples. A similar downward trend in TRA levels for the filtered samples (sum of unfiltered and filtered levels) of the 0 min to 30 day settling times was just discernable at the precision (approximately ±10%) of the experiments. After 30 days the residual water (1.6 L) remaining in the 20 L container was shaken and

Table 3. Resin acid levels (μg/L) identified in sequentially filtered well mixed azide stabilized SH30 water, collected 25/2/99 and stored 5 and 90 days.

	seco1/2	pim	18-Ab	DHAA	13-ene	Cls	TRA	recv
5 days, (n = 2)	21.9	9.4	25.0	17.5	24.9	3.6	102.2	100%
glass fibre (liq/liq)	17.1	7.1	15.5	12.2	15.9	2.7	70.5	69%
3 μm (liq/liq)	15.6	5.3	11.7	10.9	12.5	2.3	58.3	57%
0.8 μm (liq/liq)	13.6	4.6	10.0	8.7	10.8	2.1	49.8	49%
0.45 μm (liq/liq)	14.8	3.5	8.9	9.8	5.7	1.9	44.7	44%
0.2 μm (liq/liq)	12.6	2.4	4.8	8.9	4.5	1.2	34.3	34%
0.05 μm (liq/liq)	7.0	1.0	1.5	12.2	1.5	0.6	24.4	22%
glass fibre (Soxhlet)	3.7	6.2	6.8	6.1	9.4	1.7	34.0	33%
3 μm (Soxhlet)	0.5	0.9	2.2	1.7	2.4	0.3	7.9	8%
0.8 μm (Soxhlet)	0.7	1.1	2.7	2.0	2.7	0.3	9.4	9%
0.45 μm (Soxhlet)	0.4	0.7	1.8	1.4	1.5	0.2	6.0	6%
0.2 μm (Soxhlet)	0.9	1.2	3.2	2.6	3.0	0.6	11.4	11%
0.05 μm (Soxhlet)	0.8	1.3	2.7	2.3	2.0	1.3	10.2	9%
90 days, (n = 2)	17.0	6.1	25.6	20.3	28.1	4.3	101.4	100%
glass fibre (liq/liq)	13.4	4.6	16.8	14.7	21.2	2.0	72.8	72%
3 μm (liq/liq)	14.0	3.8	10.7	10.4	12.9	1.2	53.1	52%
0.8 μm (liq/liq)	13.9	3.1	10.2	11.2	9.7	1.0	49.1	48%
0.45 μm (liq/liq)	13.0	2.3	7.2	9.5	8.3	1.2	41.6	41%
0.2 μm (liq/liq)	13.7	2.7	4.9	8.8	5.6	0.7	36.4	36%
0.05 μm (liq/liq)	10.0	-	1.7	13.8	2.3	0.5	28.3	28%
glass fibre (Soxhlet)	2.1	2.4	13.2	6.0	13.3	1.1	38.0	37%
3 μm (Soxhlet)	0.7	0.8	3.7	1.9	3.8	0.4	11.3	11%
0.8 μm (Soxhlet)	0.1	0.2	0.8	0.5	0.7	0.1	2.4	2%
0.45 μm (Soxhlet)	0.3	0.4	1.2	0.7	1.2	0.1	3.9	4%
0.2 μm (Soxhlet)	-	-	2.6	1.6	2.8	0.4	7.4	7%
0.05 μm (Soxhlet)	0.2	0.6	1.2	1.0	0.9	0.1	4.0	4%

Abbreviations: seco 1/2 = secodehydroabietic acids-1 and 2; Pim = pimaric acid, 18-Ab = abietan-18-oic acid; DHAA = dehydroabietic acid; 13-ene = abiet-13-en-18-oic acid; Cls = 12-chloro, 14-chloro and 12,14-dehydroabietic acids; TRA = total resin acids; recv = % recovery relative to mean of n = 2 unfiltered SH30 water samples.

divided into 2 x 800 mL sub-samples (residues 1 and 2). Greater levels of resin acids (363 and 335 μg/L respectively) were identified in the two residual samples. These can be attributed to the gradual settling of resin acid carrying particulate matter. The mass balance determined for the bulk container experiment is summarized in Table 4. A more detailed analysis of the levels of free and particle bond resin acids identified in the two residual water samples is given in Table 5. The accumulation of particle associated resin acids in the two residual samples was readily apparent. The average level of particle associated resin acids found in the residual water samples (c 350 μg/L) corresponds to the settling of c 13 μg/L of particle associated resin acids from the 18 L of water removed during the experiment with 19.6 L of SH30 water. However, it was not possible to recognize a downward trend in the > 0.45 μm fraction, even though the majority of the settled resin acid was associated with particles removed by 0.45 μm filtration.

Table 4. Total resin acid (TRA) levels and mass balance determined for filtered and unfiltered water sub-samples from a settling experiment performed using 19.6 L of azide stabilized SH30 water.

time (sample)	unfiltered (liquid/liquid)			0.45 µm filtered		
	TRA (µg/L)	volume (mL)	mass (µg)	liq/liq (µg/L)	Soxhlet (µg/L)	TRA (µg/L)
0 min	123	3000	369	52	72	124
15 min	127	3000	381	58		
1 hour	117	3000	351	46	75	121
8 hour	118	2000	236	50		
14 days	110	3000	330	54	53	107
26 days	94	2000	188	60		
30 days	105	2000	210	50	70	120
30 days, residue 1	363[a]	800	290[a]	79	284	363[a]
30 days, residue 2	335[a]	800	268[a]	67	268	335[a]
TRA (recovered)		19600	2623 (109%)			
TRA (t = 0 min)	123	19600	2411[b]			

[a]See Table 7. [b]Mass of resin acid present at commencement of the experiment (t = 0).

Table 5. Resin acid levels (µg/L) identified in liquid/liquid and Soxhlet extracts of two filtered residual water samples from the 30 day settling experiment.

sample	seco1/2	pim	18-Ab	DHAA	13-ene	Cls	TRA
Residue 1 sub-sample							
Soxhlet (Whatman No 1)	16.7	22.9	113	31.1	71.6	7.0	262
Soxhlet (0.45 µm)	1.6	1.8	8.8	3.2	6.1	0.4	22
Liq/liq (What + 0.45 µm)	29.2	5.9	12.3	15.4	14.2	1.6	79
Total	47.5	30.6	134	49.7	91.9	9.0	363
Residue 2 sub-sample							
Soxhlet (0.45 µm)	18.2	26.6	113	27.9	75.5	6.4	268
Liq/liq (0.45 µm)	27.0	5.2	8.3	13.2	12.0	1.5	67
Total	45.2	31.8	121	41.1	87.5	7.9	335

Abbreviations: seco1/2 = secodehydroabietic acids-1 and 2; Pim = pimaric acid, 18-Ab = abietan-18-oic acid; DHAA = dehydroabietic acid; 13-ene = abiet-13-en-18-oic acid; Cls = 12-chloro, 14-chloro and 12,14-dichlorodehydroabietic acids; TRA = total resin acids.

Table 6. Color, turbidity and suspended solid levels determined for a bulk (19.6 L) azide stabilized SH30 water sample, collected 25/2/99.

settling time	absorbance			turbidity (NTU)	TSS mg/L	ISS mg/L	OSS mg/L
	270 nm	340 nm	440 nm				
0 min	0.281	0.095	0.032	7.30	8.5	4.2	4.3
15 min	0.281	0.092	0.029	7.14	6.8	2.1	4.7
1 hour	0.277	0.089	0.028	6.59	5.4	2.2	3.2
8 hour	0.275	0.089	0.028	4.91	5.6	1.8	3.8
14 days	0.278	0.087	0.024	3.67	5.2	1.5	3.7
26 days	0.261	0.079	0.020	3.34	4.0	0.5	3.5

TSS = total suspended solids; ISS = inorganic suspended solids; OSS = organic suspended solids.

Table 7. Resin acid levels (µg/L) determined in settling experiments performed using 2.5 L Winchesters (A, B, C) and azide stabilized SH30 water.

sample	seco1/2	pim	18-Ab	DHAA	13-ene	Cls	TRA
A well shaken, t = 0	19.0	12.0	30.7	19.9	34.1	3.7	119
A well shaken, t = 30 days	19.6	12.0	29.8	20.2	34.7	3.6	120
B well shaken, t = 0	20.1	10.2	32.1	24.7	39.1	4.4	131
B well shaken, t = 30 days	20.1	12.3	33.0	23.1	36.4	4.1	129
C not shaken, upper layer	17.1	8.0	19.5	14.7	22.4	2.3	84
C not shaken, lower layer	20.1	11.7	28.7	20.3	33.8	3.7	118

Abbreviations: seco1/2 = secodehydroabietic acids-1 and 2; Pim = pimaric acid, 18-Ab = abietan-18-oic acid; DHAA = dehydroabietic acid; 13-ene = abiet-13-en-18-oic acid; Cls = 12-chloro, 14-chloro and 12,14-dichlorodehydroabietic acids; TRA = total resin acids.

The downward trends in turbidity and suspended solid levels (Table 6) are consistent with the gradual settling of fine particulate matter. A moderate decrease in color levels may be attributed to either the adsorption of some colored species (chromophoric lignin molecules) onto particulate matter and/or the slow aggregation (flocculation) and settling of chromophoric molecules. The results of experiments performed using water stored in 2.5 L Winchesters were consistent with these observations. Similar levels (to within 6-10%) were identified in duplicate, well shaken, t = 0 and 30 day 1 L sub-samples taken from the same Winchester. However a significantly lower level of resin acids was identified in a 1 L sub-sample taken from the top of a Winchester that had been allowed to stand undisturbed for 30 days (84 µg/L), compared to the level of resin acids identified in a second 1 L sub-sample withdrawn from the bottom of the same Winchester (119 µg/L). It is apparent that if a bulk water sample is allowed to stand, slow settling of particulate matter will lead to elevated levels of resin acids in analytical samples taken from the bottom of the container.

SEM analyses of particulate matter recovered after 0.45 µm filtration and freeze drying of the filtrate revealed high surface concentrations of silica rich deposits, consistent with a well document geothermal input of silica to the river (McIntosh 1995) together with a lower level of deposits which exhibited a combination of Fe, Al, O and C responses, possibility attributable to Fe and/or Al induced flocculation and surface adsorption of organic species such as resin acids, microbial biomass and/or phenolic lignin molecules.

Our results show that the majority of resin acid in both freshly sampled and aged Tarawera River water is associated with small particles. While some settling of this material occurs, the process is slow with no more than 10% of the total particulate resin acid settling in 30 days. However, after prolonged periods and where quiescent conditions prevail, significant amounts of resin acids can be expected to settle. This could lead to the accumulation of resin acids in sediments and onto benthic surfaces far removed from the point of discharge or in media through which recipient water permeates (Wilkins et al. 1996a; 1996b).

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