The occurrence of cyanobacteria in pulp and paper waste-treatment systems

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Abstract: Pulp and paper secondary waste-treatment systems in Brazil, Canada, New Zealand, and the U.S.A. contained dynamic cyanobacterial communities, some of which exceeded heterotrophic bacterial biomass. No other viable photoautotrophic populations were detected in the ponds. Regardless of geographical location, Oscillatoriales including Phormidium, Geitlerinema, and Pseudanabaena were the dominant taxa. As well, Chroococcus (Chroococcales) was an important genus in Brazil and New Zealand. The possible impact of cyanobacteria on waste-treatment efficiency deserves further study given their large biomass and diverse metabolic characteristics.

Key words: cyanobacteria, blue-green algae, heterotrophic bacteria, community structure, pulp and paper secondary waste treatment.

Résumé: On a observé au Brésil, au Canada, en Nouvelle-Zélande et aux États-Unis que les systèmes de traitement secondaire des rejets d’usines de pâtes et papiers contenaient des populations dynamiques de cyanobactéries dont certaines dépassent même les valeurs de la biomasse bactérienne hétérotrophe. Aucune autre population de photoautotrophes n’a été détectée dans les bassins. Indépendamment de la localisation géographique, les taxons dominants appartaient aux Oscillatoriales incluant Phormidium, Geitlerinema et Pseudanabaena. Au Brésil et en Nouvelle-Zélande Chroococcus (Chroococcales) était aussi un genre important. L’impact potentiel des cyanobactéries sur l’efficacité du traitement des rejets mérite d’être étudié plus à fond, compte tenu de la biomasse élevée et des caractéristiques métaboliques variées de ces bactéries.

Mots clés : cyanobactéries, algues bleues-vertes, bactéries hétérotrophes, organisation de la population, traitement secondaire des rejets d’usines de pâtes et papiers.

The pulp and paper industry discharges millions of tons of effluent into aquatic environments each year with varying ecological effects. Negative impacts on water quality include increases in turbidity, colour, nutrient loads, and the addition of toxic and persistent compounds. Bleached-kraft mills were once responsible for high discharges of numerous chlorinated compounds, including highly toxic dioxins and furans. During the last decade, the majority of bleached-kraft mills have replaced elemental chlorine with chlorine dioxide as a bleaching agent, resulting in a relatively large decrease of chlorinated organics in effluents (LaFleur 1996; Wiegand et al. 1999). Although the decline of elemental chlorine bleaching has resulted in a reduction of organochlorine-associated toxicity, whole-effluent chronic toxicity is still a concern (Ahtiainen et al. 1996). There remain nonorganochlorine components of bleached-kraft effluents, specifically natural wood extractives (resin and fatty acids, terpenes, sterols, and other unidentified compounds), that may be inducing chronic toxicity effects, such as endocrine disruption in fish (Axegard et al. 1993).

Bleached-kraft mills utilize secondary treatment systems to reduce the organic load in wastewater prior to discharge into receiving waters. Either aerated stabilization basins (ASBs) or activated sludge (AS) systems are employed to house a large community of heterotrophic bacteria to degrade the organic constituents in wastewater. To promote high densities of bacteria, influent to the waste-treatment system is maintained at a relatively constant temperature (28°C–33°C) and pH (7.3–7.7). Nitrogen and phosphorus levels can differ among mills, depending on the wood source and fertilizer addition during secondary treatment. Using available nutrient data from mills in our study and from the literature, total dissolved nitrogen can vary from 0.6 to 3.6 mg/L, and total phosphorus can vary from 0.4 to 4 mg/L.

Although the removal of organic compounds from pulp and paper waste is highly dependent on biological treatment, relatively little is known about the populations of bacteria involved in biodegradation. Some work has been done to char-
acterize the heterotrophic communities in pulp and paper secondary treatment systems (Fulthorpe et al. 1993; Fortin et al. 1998). To our knowledge, there are no published reports on the presence of cyanobacteria (previously known as blue-green algae) in pulp and paper secondary treatment systems or their possible impact on waste-treatment efficiency. As a group, the cyanobacteria are widely distributed and have a wide range of habitats (Hoffman 1996). However, the presence of a large and diverse cyanobacterial community in an environment with very low light penetration (Secchi depth < 0.10 m) and numerous toxic constituents deserves further study.

To assess the diversity and abundance of cyanobacteria in pulp and paper secondary effluent, we collected near-surface grab samples from the centre of six bleached-kraft pulp and paper secondary treatment basins in Brazil, Canada, New Zealand, and the U.S.A (Table 1). An aliquot of each sample was preserved with Lugol’s acid-iodine for subsequent enumeration and identification. Effluent samples collected from two mills were preserved with 2% formalin for total bacterial counts using the 4,6-diamidino-2-phenylindole (DAPI) staining protocol (Porter and Feig 1980). Aliquots of the original fresh effluent were transferred to a 1-L Nalgene™ bottle with head space and shipped immediately to the laboratory. To verify cyanobacterial viability, fresh effluent was both inoculated into sterile BG-11 culture medium (Allen 1968) and left as whole-effluent samples when placed into an illuminated incubator at 28°–32°C. All effluent samples had visible cyanobacterial growth within 2–7 days.

Cyanobacteria from the Lugol’s preserved effluent samples were settled for 24 h in settling chambers. A Zeiss D-7082 microscope with oil immersion at 1000X magnification was used for cyanobacterial identification and enumeration. Cyanobacteria were identified according to Komarek and Anagnostidis (1986) and Anagnostidis and Komarek (1988). Cyanobacterial cells and filaments were counted along one radius (40 fields) per sample. Biovolume was calculated by approximation to geometric figures and converted to mass units by assuming a specific gravity of 1.

All waste-treatment systems surveyed in our study had measurable cyanobacterial populations (Table 2). It is interesting to note that photosynthetic eukaryotes were conspicuously absent in most mill samples. Cyanobacterial biomass varied from about 1 mg/L in Alberta, Brazil, and New Zealand to 150 mg/L at the Quebec 2 site. The relatively large standard error value for the Brazil site suggests substantial biomass variability from month to month in contrast with Idaho and New Zealand.

To assess the relative abundance of cyanobacteria to the rest of the bacterial community, we performed subsequent total counts using epifluorescence microscopy on effluent samples from two mills (Table 3). These counts represent a conservative estimate of cyanobacterial numbers because only cyanobacterial filaments, which were readily distinguishable from the other bacteria, were enumerated. Although cyanobacterial numbers are generally an order of magnitude lower than the rest of the bacterial community, relative biomass values show cyanobacterial biomass to be the same (Maine) or higher (Quebec 2) than bacterial biomass. Both of these mills are dominated by cyanobacterial filaments, specifically Phormidium, which greatly influences total community biomass. This indicates that at least in these two mills, cyanobacteria are a significant component of the microbial community.

In all waste-treatment sites, filaments of Oscillatoriales dominated the cyanobacterial communities (Fig. 1). Phormidium accounted for 90% of cyanobacterial biomass in the Quebec sites and about 50% in the Alberta and Idaho sites. Geitlerinema, a subgenus of Phormidium, was found at all sites in proportions varying from 5% in New Zealand to 45% in Alberta. Pseudanabaena was present in Idaho (40%), New Zealand (25%), and Quebec 1 (10%). The Chroococcales had a greater representation in Brazil and New Zealand (Fig. 1). Chroococcus accounted for about 45% of cyanobacterial biomass in Brazil and about 30% in New Zealand. The presence of the same genera (Phormidium, Geitlerinema, Pseudanabaena, and Chroococcus) in all the communities, regardless of the geographical location, indicates that conditions at all sites are rather similar, and hence, are selecting for the same cyanobacterial genera. For instance, temperature, an important regulator of cyanobacterial abundance and composition (Paerl and Tucker 1995), was fairly high and uniform (28°C–33°C) in these waste-treatment systems and may partially explain the similarity in cyanobacterial taxa at all sites.

The data for Alberta, Brazil, Quebec 1, and Quebec 2 reflect one sample date, and thus, are only a "snapshot" of resident cyanobacterial genera in each waste-treatment system. Waste-treatment systems in Idaho and New Zealand sampled on several dates show substantial changes of cyanobacterial community composition over time (Fig. 2). The Idaho site consistently contained higher cyanobacterial biomass (by an order of magnitude) than the New Zealand site. Samples from April and May showed a community dominated by Phormidium, but by June Phormidium was replaced by Pseudanabaena. Cyanobacterial biomass doubled between May and June, suggesting that environmental conditions in the waste-treatment system became ideal for Pseudanabaena growth and the emergence of Pseudocapsa. By July, biomass decreased to pre-June levels and included the reemergence of Phormidium as the dominant genus. Pseudanabaena became the dominant taxon again in September but at much lower biomass levels than in June. Geitlerinema never dominated the community but was consistently present in all samples.

In New Zealand, the first sample date in February exhibited both the highest cyanobacterial biomass (3.9 mg/L) and the most diversity. On the remaining sample dates, community biomass was less than 1 mg/L. Pseudanabaena was dominant on all dates except February. In February, the cyanobacterial community was dominated by Chroococcus, which then disappeared until October. Samples taken in February and October reflect the end and beginning of summer in New Zealand, respectively. The appearance of Chroococcus in this waste-treatment system may depend on the higher light intensities and warmer temperatures prevalent during summer.

Generally, both the Idaho and New Zealand sites had consistent dominant genera over time, supporting the notion that a relatively constant environment is maintained in these waste-treatment systems. Although system perturbation events may explain community composition shifts, the relatively short retention time of these systems (Idaho, 7 days;
New Zealand, 10 days) probably expedites the return of pre-
dominant cyanobacterial taxa witnessed under stable condi-
tions. All of the dominant cyanobacterial taxa identified in
preserved effluent samples were isolated and cultured in the
laboratory indicating that they are viable and metabolically
functional. The dynamic changes in community total bio-
mass and composition over time confirms this conclusion.

The cyanobacteria are a cosmopolitan group of organisms
characterized by broad environmental tolerances (Adhikary
1996; Hoffman 1996). In aquatic systems, cyanobacterial
contribution to total phytoplankton biomass increases with
lake trophic state and accounts for nearly the entire biomass
in hypertrophic lakes (Watson et al. 1997). The environmen-
tal characteristics of ASBs are not unlike the conditions of
shallow, hypertrophic lakes in that both systems tend to be
well mixed, turbid, and have high nutrient concentrations.

According to Wetzel (1983), the cyanobacterial biomass val-
ues measured in this study classify the Idaho and Quebec
waste-treatment systems as hypertrophic (>10 mg/L) and the
Alberta, Brazil, and New Zealand mills as mesotrophic (1–
3 mg/L).

Available nutrients, specifically phosphorus and nitrogen,
can be the key controlling factors of cyanobacterial abun-
dance and composition (Schindler 1977; McQueen and Lean
1987; Lung and Paerl 1988). The addition of nitrogen and
phosphorus fertilizers can be a conventional practice in pulp
and paper waste treatment, but many mills, including those
in our study, do not add nutrients during secondary treat-
ment. Some mills achieve sufficiently high nitrogen and
phosphorus levels from the process wastewater without fer-
tilizer additions (Jarvinen 1997; Rantala and Wirola 1997).

Table 1. Pulp and paper mills included in this survey.

<table>
<thead>
<tr>
<th>Mill location</th>
<th>Feed-stock source</th>
<th>Bleaching type</th>
<th>Secondary treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alberta</td>
<td>Mixed hardwood and softwood</td>
<td>ECF</td>
<td>AS</td>
</tr>
<tr>
<td>Brazil</td>
<td>Eucalypt</td>
<td>ECF, TCF</td>
<td>ASB</td>
</tr>
<tr>
<td>Idaho</td>
<td>Mixed softwood</td>
<td>ECF</td>
<td>ASB</td>
</tr>
<tr>
<td>Maine</td>
<td>Hardwood</td>
<td>ECF</td>
<td>ASB</td>
</tr>
<tr>
<td>New Zealand</td>
<td>Mixed softwood and eucalypt</td>
<td>ECF</td>
<td>ASB</td>
</tr>
<tr>
<td>Quebec 1</td>
<td>Mixed hardwood</td>
<td>ECF</td>
<td>ASB</td>
</tr>
<tr>
<td>Quebec 2</td>
<td>Mixed hardwood</td>
<td>ECF, TCF</td>
<td>ASB</td>
</tr>
</tbody>
</table>

Note: ECF, elemental chlorine free; TCF, total chlorine free; AS, activated sludge; ASB, aerated stabilization basin.

Table 2. Cyanobacterial biomass estimates from six pulp and paper waste-treatment sites.

<table>
<thead>
<tr>
<th>Mill location</th>
<th>Sample size (n)</th>
<th>Mean biomass (mg/L)</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alberta</td>
<td>1</td>
<td>0.6</td>
<td>NA</td>
</tr>
<tr>
<td>Brazil</td>
<td>2</td>
<td>0.5</td>
<td>0.38</td>
</tr>
<tr>
<td>Idaho</td>
<td>5</td>
<td>20</td>
<td>4.2</td>
</tr>
<tr>
<td>New Zealand</td>
<td>6</td>
<td>1.2</td>
<td>0.59</td>
</tr>
<tr>
<td>Quebec 1</td>
<td>1</td>
<td>14</td>
<td>NA</td>
</tr>
<tr>
<td>Quebec 2</td>
<td>1</td>
<td>150</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Sample size refers to the number of monthly samples taken at each site over a 1-year period.

Table 3. Numerical abundance of total bacteria (i.e., the whole bacterial community) and ratios that compare the relative abundances of cyanobacteria with the remaining bacterial community in two pulp and paper secondary waste-treatment systems.

<table>
<thead>
<tr>
<th>Mill location</th>
<th>Total bacteria (cells/mL)</th>
<th>Cyanobacteria–bacteria (no. of cells)</th>
<th>Cyanobacteria–bacteria (biomass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maine</td>
<td>$3.9 \times 10^7$</td>
<td>0.02:1</td>
<td>1:1</td>
</tr>
<tr>
<td>Quebec 2</td>
<td>$5.4 \times 10^7$</td>
<td>0.07:1</td>
<td>7:1</td>
</tr>
</tbody>
</table>

Fig. 1. Cyanobacterial community composition based on sample means for the six pulp and paper waste-treatment systems.
Studies performed downstream of effluent discharge indicate a marked increase in both bacteria and periphyton (Bothwell 1992; Mohamed et al. 1998). The hypertrophic state of some sites in our study is consistent with these observations, as is the presence of large populations of *Phormidium*, a genus known to proliferate in high-nutrient environments (Ohkubo et al. 1993; Canizares Villanueva et al. 1994).

The large cyanobacterial communities in pulp and paper waste-treatment systems have the potential to affect waste-treatment efficiency in various ways. In the light, photosyn-

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**Fig. 2.** Time course of cyanobacterial biomass and composition in the Idaho and New Zealand pulp and paper waste-treatment systems.

**Fig. 3.** Cyanobacterial biomass and composition across the Brazil pulp and paper waste-treatment system on two sample dates. Site 1, initial point of secondary treatment; site 4, half-way point of secondary treatment; and site 6, final point of secondary treatment.
thesizing cyanobacteria would produce oxygen to the benefit of the aerobic heterotrophic bacteria. However, they could also adversely influence the size and nature of the heterotrophic bacterial community by competing for organic substrates in the dark (Droop 1974; Feuillade and Feuillade 1989) and by increasing the biological oxygen demand (BOD) of the system. The treatment systems in our study have influent BOD of about 250 mg/L. Using the conversion formula of Strathmann (1967), the amount of carbon contributed by the cyanobacterial biomass in this study ranged from 0.2 mg/L carbon (Brazil) to 26 mg/L (Quebec 2). In high biomass systems, such as Quebec 2, cyanobacteria could increase the wastewater BOD by 28%–40%, depending on their total contribution of carbohydrates, lipids, and proteins.

Cyanobacteria could also affect bacterial growth through competition for available nitrogen and phosphorus. Nitrogen-fixing cyanobacteria may be a component of pulp and paper waste-treatment systems considering that influent inorganic nitrogen is quickly removed during biological waste treatment (Jarvinen 1997). Although none of the cyanobacterial taxa identified in this survey were heterocystous (i.e., morphologically predisposed to fixing nitrogen), all have species capable of nitrogen fixation in low-oxygen environments (Bergman et al. 1997). Pulp and paper waste-treatment systems, which have dissolved oxygen levels varying between 1 and 2 mg/L, would provide a suitable environment for nitrogen fixation to occur.

High abundances of cyanobacteria can be detrimental to the functioning of engineered systems, such as water-purification plants, fish ponds, and aquaculture systems (Sevrin-Reyssac and Pletikosic 1990; Paerl and Tucker 1995; Hu and Chiang 1996). Many cyanobacteria are known producers of secondary metabolites that can pose either esthetic (e.g., musty odour) or toxicity (e.g., neurotoxins) problems. One strain of musty odour producing Phormidium was isolated and cultured from two sites in our study. It is particularly interesting to note that cyanobacteria can produce a range of sterols and fatty acids (Carr and Whitton 1973). Sterols have received close scrutiny in recent years as compounds of toxicological concern in pulp and paper effluents, particularly as potential endocrine disrupters. Cook et al. (1997) found an increase in sterols over the course of pulp and paper biological treatment, which corresponds with our finding of an increase in cyanobacterial biomass across a treatment system (Fig. 3).

Such varied metabolic characteristics of cyanobacteria give rise to a number of concerns relating to their impact on waste-treatment efficiency. How the presence of cyanobacteria in pulp and paper waste-treatment systems affect the end points of waste treatment, particularly toxicity removal, warrants further study and is currently under investigation.

References


Mohamed, M.N., Lawrence, J.R., and Robarts, R.D. 1998. Phosphorus limitation of heterotrophic biofilms from the Fraser...