THE ANAEROBIC
BIODEGRADABILITY AND
METHANOGENIC TOXICITY OF
PULPING WASTEWATERS

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ABSTRACT

The objective of this study was to evaluate the effect of various pulping conditions and different lignocellulosic
feedstocks on the anaerobic treatability of pulping wastewaters. Wastewaters were prepared from lignocellulosic
feedstocks commonly used in the forest industry, namely, pine, spruce and birch wood, and wheat straw. The
pulping conditions used were representative of those applied in TMP and soda pulping processes.

The anaerobic biodegradability and the methanogenic toxicity of the various wastewaters were evaluated in
standardized batch bioassays using anaerobic granular sludge. The acidification of the TMP wastewaters (conversion
to CH₄ and VFA) ranged from 68 to 87% of the total COD, indicating their high anaerobic biodegradability. TMP
wastewaters were non-toxic to methane bacteria at concentrations expected in paper mill wastewaters. No inhibition
was observed at 10 g COD/l. In contrast, wastewaters prepared in alkaline conditions were poorly biodegradable
(approx. 50% acidification) and they caused severe inhibition of the methanogenic activity. The 50% inhibitory
concentrations ranged from 2.1 to 5.4 g COD/l.

Additional experiments showed that wood resin components, poorly solubilized at acidic to neutral pH, but easily
extractable in alkali, are responsible for most of the methanogenic toxicity observed in alkaline pulping
wastewaters. These results indicated that contact of wood with alkali contributes significantly to increase the
methanogenic toxicity of the pulping wastewater.

KEY WORDS

Anaerobic digestion; methanogenic toxicity; biodegradability; wood; straw; wood resin; pulping wastewater; TMP
wastewater; soda pulping wastewater.

INTRODUCTION

The forest industry utilizes wood and other lignocellulosic feedstocks as raw materials for the production of paper.
The major constituents of wood are cellulose, hemicellulose, lignin and resin. Softwoods, hardwoods and straw have
different proportions of chemical components as shown in Table 1.

The processing of wood in paper mills involves various operations including debarking, pulping and bleaching that
result in the discharge of highly polluted wastewaters. The quantity and types of pollutants in these effluents vary
with the type of lignocellulosic feedstock used as raw material, the process conditions applied (pH, temperature,
pressure, chemical and mechanical treatments) and the specific water consumption (Gottsching et al., 1977;
Stemberg and Norberg, 1977). Chemical additions, and to a lesser extent high pressures and temperatures, result in
an increased release of organic matter into the process water and extensive lignin solubilization. Therefore, the
pollution loads and the color due to dissolved lignic compounds (Corson and Lloyd, 1978; Virkola and Honkanen,
1985) is very high for chemical as compared to mechanical pulping effluents. The COD loads associated with
mechanical pulping processes range from 20–50 kg COD per ton of pulp (Stemberg and Norberg, 1977) whereas
TABLE 1 Literature Averaged Composition of Hardwoods, Softwoods and Straw (Misra, 1980; Fengel and Wegener, 1984; Tewari and Nemerow, 1982).

<table>
<thead>
<tr>
<th>CONSTITUENT</th>
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</tr>
<tr>
<td></td>
<td>25-32</td>
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</tr>
<tr>
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</tr>
<tr>
<td></td>
<td>55-61</td>
</tr>
<tr>
<td></td>
<td>33-38</td>
</tr>
<tr>
<td>Extractives</td>
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<td>6-8</td>
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</table>

* alcohol-benzene extract followed by hot water extraction.
** ether extract.

those corresponding to soda pulping processes may be as high as 500-900 kg COD per ton of pulp (Anonymous, 1986). Nevertheless, the black liquors originating from kraft and soda processes are usually burnt to recover the pulping chemicals and the calorific power from the organic components, diminishing to a great extent the environmental impact associated with these pulping processes. Conventional recovery processes are not economically viable in small paper mills and in those using non-woody raw materials with a high silica content (Velasco et al., 1985; Anonymous, 1986). Black liquors represent a very important pollution source in several countries where small scale mills are common (Velasco et al., 1985; Anonymous, 1986; Gonen et al., 1990).

Pulp and paper mill effluents can cause considerable damage to receiving waters if discharged untreated. The environmental impact associated with these wastewaters is not only restricted to the oxygen demand; but also numerous effluents from the forest industry display acute toxicity to fish and other aquatic organisms (Rogers 1973; Leach and Thakore, 1976; Juhani et al., 1982). Furthermore, these wastewater streams often exert inhibitory effects to microorganisms that can disturb biological treatment systems (Benjamin et al., 1984; Ferguson and Benjamin, 1985; Minami et al., 1986; Welander, 1988).

Aerobic treatment systems have traditionally been applied for reducing the pollution caused by the pulp and paper industry effluents. However, in the last years, the rising energy prices and the relatively high operation costs of conventional aerobic systems have resulted in an increasing application of the anaerobic wastewater treatment technologies. Non-inhibitory forest industry wastewaters rich in readily biodegradable organic matter such as paper recycling wastewaters, mechanical pulping effluents and sulphite evaporator condensates (Frostell et al., 1985; Habets and Knelissen, 1985; Maat and Habets, 1987) are successfully treated in full-scale anaerobic reactors. However, the number of full-scale applications to toxic and more complex forest industry wastewaters is still very limited. An evaluation of the factors determining the composition of paper mill wastewaters, such as the lignocellulosic feedstock used and the conditions applied during pulping or bleaching, as well as a good understanding of the fate of the wastewater components in anaerobic systems is necessary to determine the potentials and limitations of the anaerobic technologies for the treatment of these toxic wastewater streams.

The purpose of this study was to evaluate the effect of mechanical and soda pulping processes on the anaerobic treatability of pulping wastewaters originating from various lignocellulosic feedstocks.

MATERIALS AND METHODS

Preparation of wood and straw pulping wastewaters

Extracts were prepared from lignocellulosic feedstocks commonly used in the forest industry, namely, pine (Pinus sylvestris), spruce (Picea abies) and birch wood (Betula verrucosa), and wheat straw (Triticum aestivum). Birch wood samples were collected in a local forest from birch logs that were recently cut. Spruce and pine chips were obtained from a paper factory (Parenco, Renkum, The Netherlands). Wheat straw was purchased in a local shop. The pulping conditions used were representative of those applied in thermo mechanical pulping (TMP) and soda pulping processes. Prior to pulping, debarked wood chip samples and straw were air dried (48 h at 70°C) and ground in a cross mill. A slurry containing 100 g of ground wood or straw per litre of water was cooked at 120°C during 2 h. When soda pulp waters were prepared 8 g NaOH were also added. The remaining pulp was separated from the pulp water by centrifugation and subsequent filtration. Finally, the pulp waters were neutralized by addition of NaOH or HCl, as required, and stored in refrigerated containers.
Treatments of the pulping wastewaters

**Ether extraction.** Resin was extracted from the pulping wastewater by liquid-liquid extraction with diethyl ether under strongly acidic (pH 2-3) conditions (Bjorklund-Jansson, 1980; Voss and Rapsomatiotis, 1985). The sample was extracted three successive times with an equal volume of ether (50:25:25 % of the total ether volume in the 1st:2nd:3rd extraction). The aqueous fraction was partially evaporated in a rotatory evaporator to remove traces of ether. The required volume of water was added to reach the initial sample volume, and the solution was neutralized with NaOH.

**XAD treatment.** 1 l of the pulping wastewater (5 to 10 g COD/l) at pH 9 was shaken with 71.5 g of a polymeric resin (XAD-2) for 5 hours. Subsequently, the sample was paper filtered and neutralized with HCl.

**Ca²⁺ precipitation.** 1 l of the pulping wastewater (5 to 10 g COD/l) was treated at pH 11 with 1000 mg/l of Ca²⁺, supplied as CaCl₂·2H₂O. Precipitation was allowed to occur overnight after which the wastewater was paper filtered and neutralized with HCl.

**PVP treatment.** Pulping wastewaters were treated with an insoluble polyamide, polyvinylpyrrolidone (PVP), to specifically remove the fraction with tannic qualities. The treatment was in accordance with the method described by Field *et al.*, (1988); (14.3 g PVP/l for 1 hour shaking followed by filtering the wastewater).

**Acid precipitation.** 1 l of the wastewater (5 to 10 g COD/l) was adjusted to pH 2 with HCl. The precipitation was allowed to occur overnight. The pulp water was centrifuged and the precipitate and filtrate quantitatively recovered. The precipitate was repeatedly washed with a diluted HCl solution (pH 2) to removed traces of the mother liquid, centrifuged and suspended in 1 l of H₂O. Finally, the filtrate (soluble fraction) and suspended precipitate (insoluble fraction) were neutralized.

Analyses

Samples for COD and volatile fatty acid (VFA) determination were filtered (Schleicher & Schull paper filter no. 589-1). COD (colorimetric method with dichromate), TSS and VSS were determined according to Standard Methods (American Public Health Organization, 1985). The pH was determined with a Knick 511 meter and a Scot Gerade N61 double electrode immediately after sampling in order to avoid a pH rise due to the loss of carbon dioxide from the liquid. VFA were analyzed by gas chromatography using a Packard Becker model 417 equipped with a 2 m x 4 mm glass column packed with Supelcoport (100-120 mesh) coated with 10% Fluorad FC 431. The temperature of the column, the injection port and the flame ionization detector were 130, 220 and 240°C, respectively. Nitrogen saturated with formic acid was used as carrier gas at a flow rate of 50 ml/min. The analysis of methanol and ethanol were based on a similar chromatographic procedure modifying the temperature of the column, the injection port and the flame ionization detector to 80, 180 and 200°C, respectively.

The carbohydrate content in the wood extracts was determined colorimetrically at 480 nm by the sulfuric-phenol method (Dubois *et al.*, 1956), using analytical grade glucose as a standard.

The ultra violet absorbance at 280 nm (UV₂₈₀) was determined in a 1 cm quartz cuvette by diluting the samples to less than 0.8 absorbance units in a 0.02 M borate buffer, providing a pH of 9.1. The lignin content in the analyzed samples was estimated from the UV₂₈₀ using an absorptivity coefficient of 22.3 l per g per cm (Hill, 1985; Kim *et al.*, 1987). This spectrophotometric method is based on the distinct absorption of the aromatic ring at 280 nm (Fengel and Wegener, 1984a). It should be noted that other liquor components, especially aromatic extractives, also absorb light at this wavelength.

Resin content in wood was determined by extraction of a known amount of the wood sawdust with a hexane:methanol (2:1 in v/v) solution in a Shoxlet apparatus for 8 hours, and subsequent gravimetric determination of the amount of wood resin after drying the residue at 105°C.

Chemicals

The chemicals used were purchased from Merck (Darmstadt, West Germany). The yeast extract was supplied by Gist-Brocades (Delft, The Netherlands). XAD-2 and PVP were purchased from Janssen Chimica (Beers, Belgium).

Biomass

The granular sludge used in these experiments was obtained from a full-scale UASB reactor treating distillery wastewater (Nedalco, Bergem op Zoom, The Netherlands) or potato processing wastewater (Aviko, Steenderen, The
Netherlands). The sludges were elutriated to remove the fines and stored at 4 °C under nitrogen gas. The sludge used was not acclimated to the pulping wastewaters prior to the toxicity assays.

**Biological Assays**

All assays contained macronutrients (N, P and S) and trace elements required for bacterial growth as outlined previously (Sierra-Alvarez and Lettinga, 1990). Batch fed assays were conducted in 0.6 or 1.2 l glass serum flasks sealed with a rubber septum and a screw cap. The assay medium was flushed with nitrogen gas prior to incubation of the serum vials in a temperature controlled room at 30 ± 2°C. The serum flasks were not shaken during the assay period.

Methane production was monitored periodically during the assays with modified Mariotte flasks. These flasks were filled with a 3% NaOH solution which served to remove the CO₂ contained in the biogas.

**Anaerobic toxicity assay.** In this study, two types of toxicity experiments were conducted as outlined follows:

**Type 1.** This method was used to determine the methanogenic toxicity of soda pulp liquors. The assays were carried out in two consecutive feedings. In the first feeding, tap water, granular sludge (1.5 g VSS/l) and known amounts of wastewater COD were transferred to the serum flasks containing nutrient solution. No wastewater was added to the substrate controls. Subsequently, distilled water was added to complete a medium volume of 0.5 l and afterwards, the substrate controls and treatments (assays containing wastewater) were supplied with 4 g COD/l of a neutralized volatile fatty acid (VFA) solution. The composition of the VFA solution that served as substrate was 100:100:100 g acetate:propionate:butyrate per kg. Finally, 1 g NaHCO₃ per gram of biodegradable wastewater COD was added to the treatment containing the highest assay concentration to buffer eventual accumulations of VFA. The same quantity of NaHCO₃ was also supplied to the other treatments and to the substrate controls.

On day 14, all serum flasks were provided with a second substrate feeding in order to evaluate the residual activity of the sludge after exposure to the pulp water. The supernatants were carefully decanted to avoid losses of methanogenic sludge and replaced, while maintaining N₂ flushing in the head space, with a nutrient supplemented medium containing 4 g VFA-COD/l. No wastewater was included in the replacement medium. Finally, the serum flasks were incubated for 1 to 2 weeks.

**Type 2.** This method was applied to assay the methanogenic toxicity of TMP wastewaters at concentrations ranging 5 to 20 g COD/l. The method described in assay type 1 is not reliable for determining the methanogenic toxicity when the wastewater provides a high concentration of substrate to the medium. The large differences in the VFA concentration of the various treatments complicate the comparison of the treatment activities with that of a single substrate control.

During the first feeding each treatment was paired with a corresponding substrate control which contained an equal concentration of biodegradable COD supplied as VFA. The composition in COD basis of the neutralized VFA stock solution utilized in the substrate controls was 75:20:5 acetate:propionate:butyrate, similar to that of the completely acidified wastewater. The treatments were not supplied with VFA as the wastewater itself provided the substrate for the toxicity assay. To buffer eventual accumulations of VFA, 1 g NaHCO₃ per gram of biodegradable COD was added to the treatments. The second feeding was the same as previously described in the toxicity assay type 1.

The specific methanogenic activity, expressed as the amount of CH₄ produced by 1 g of sludge VSS per day (g CH₄-COD per g VSS per day), was calculated in all toxicity assays from the slope of the methane production versus time curve and the quantity of VSS. As an example, the cumulative methane production versus time curves obtained in a toxicity experiment with birch alkaline liquor are shown in Figure 1. The methanogenic activities in the first and second feeding for each concentration point were calculated in the time interval corresponding to the maximum control activity. The inhibited activity was expressed as percentage of the control activity, and it is abbreviated as (% ACT). The percentage inhibition (% I) is defined as: % I = 100 - % ACT. The wastewater concentration that caused 50% and 80% inhibition of the methanogenic activity is referred to as "50% IC" and "80% IC", respectively.

It should be noted, that the first feeding is usually less reliable than the second assay feeding. This is due to the different rates and levels of acidification in the treatments and their respective controls and to the fact that the toxins often do not fully express their inhibiting activity prior to the time period used to calculate the methanogenic inhibition in the first feeding.

**Detoxification experiments.** The detoxification obtained by selectively removing specific wastewater components was evaluated in anaerobic toxicity assays fed the original and treated pulping wastewater, following the procedure previously described in the toxicity assay type 1. A concentration close to the 80% IC was chosen as a standard concentration for the "detoxification" experiments.
Biodegradability and toxicity of pulping wastewaters

Fig. 1. The cumulative methane production of VFA fed assays containing birch soda pulping liquor in the first (A) and second assay feeding (B). The wastewater assay concentrations (in g COD/l) were: 0 (■), 2 (○), 4 (▲), 6 (▲), 8 (▲) and 10 (▲). x = the time period used to determine the methanogenic activity.

Anaerobic biodegradability assay. The biodegradability experiments were conducted in 0.6 l flasks. Granular sludge (5 g VSS/l), distilled water and a known amount of wastewater COD were transferred to the flasks containing 0.1 l of the nutrient solution. The assay COD concentration after dilution to the final volume (0.5 l) ranged from 4 to 5 g COD/l. The biodegradability assays of the soda pulp liquors were supplied with a lower substrate concentration (approx. 2 g COD/l) to minimize methanogenic inhibition during the assays. The latter experiments were conducted in 1.2 l serum flasks to allow a more accurate determination of the methane production. All experiments included a sludge blank lacking substrate. The treatments and blanks were supplied with 1 g NaHCO₃ per gram of biodegradable COD. The percentage acidification of the wastewater COD was calculated by the sum of the cumulative CH₄-COD and the media VFA-COD for a given assay period. The acidification results reported are corrected for the acidification of the sludge controls.

RESULTS

The average composition of the wood and straw pulping wastewaters used in this study and the yield of soluble organic matter from the various lignocellulosic feedstocks are listed in Table 2.

Autoclaving the aqueous wood slurry (120°C, 2h) at the natural wood pH resulted in yellow colored, weakly acidic (pH 4.7) pulping wastewaters of intermediate COD strength (3 to 5 g COD/l). The amount of the lignocellulosic material dissolved after pulping ranged from 33 to 42 g COD per kg of dried wood. Soda pulp liquors, on the other hand, were dark brown colored and contained high concentrations of dissolved organic matter (20 to 40 g COD/l). The organic matter dissolved in the alkaline liquors ranged from 187 to 384 g COD per kg of dried wood or straw, which are values that are significantly higher than those observed for the TMP wastewaters.

<table>
<thead>
<tr>
<th>Pulping method and lignocellulosic feedstock*</th>
<th></th>
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<tbody>
<tr>
<td>COMPONENT (%) COD</td>
<td>TMP</td>
<td></td>
<td></td>
<td></td>
<td>SODA</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>S</td>
<td>B</td>
<td>St</td>
<td>P</td>
</tr>
<tr>
<td>VFA</td>
<td>1.0</td>
<td>1.5</td>
<td>5.8</td>
<td>5.3</td>
<td>8.1</td>
</tr>
<tr>
<td>Alcohol**</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.0</td>
</tr>
<tr>
<td>Sugar</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>10.8</td>
</tr>
<tr>
<td>Lignin</td>
<td>20.3</td>
<td>16.3</td>
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<td>4.4</td>
<td>4.6</td>
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<td>10.5</td>
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<tr>
<td>Yield</td>
<td>42</td>
<td>30</td>
<td>38</td>
<td>ND</td>
<td>250</td>
</tr>
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</table>

* P = pine, S = spruce, B = birch, St = straw. ** ND = not determined; "" Methanol and ethanol; ♯ Yield = organic matter dissolved in g COD per kg of dried wood or straw.
The anaerobic biodegradability of wood and straw pulping wastewaters

The average acidification of the wastewater COD (conversion to CH₄ and VFA) observed during the batch anaerobic digestion of the TMP and the soda pulping wastewaters prepared from wood (pine, spruce or birch) and wheat straw are reported in Table 3.

The acidification of the TMP wastewaters ranged from 68% to 87% of total COD, indicating their high anaerobic biodegradability. In contrast, wastewaters prepared in alkaline conditions were poorly biodegradable (approx. 50% acidification), indicating the presence of recalcitrant organic matter in the soda pulp liquors.

The methanogenic toxicity of wood and straw pulping wastewaters

The concentration of the various TMP and soda pulping wastewaters resulting in a 50% and 80% inhibition of the methanogenic activity are listed in Table 4. The effect of soda pulping liquors on the methanogenic activity is also shown in Figure 2.

As shown in Table 4, TMP wastewaters exerted low toxicity on methane bacteria and did not cause any significant inhibition at concentrations expected in the effluents of paper mills utilizing TMP processes. The 50% IC values obtained in the first feeding ranged 11.5 to 13.7 g COD/I, whereas those observed in the second assay feeding were significantly higher, indicating partial recovery of the methanogenic activity. In contrast, soda pulp liquors were highly inhibitory to the activity of methanogenic bacteria, indicating that the contact of wood with alkali contributes significantly to increase the methanogenic toxicity of the pulping wastewaters. The COD concentrations resulting in 50% inhibition in the first assay feeding ranged from 2.1 to 5.4 g COD/I, respectively. Alkaline pine wood liquors, with 50% IC corresponding to 2.1 g COD/I, were distinctly more inhibitory than soda pulp liquors prepared from other lignocellulosic feedstocks. The residual methanogenic activities determined in the second feeding that followed the 2 week exposure to the pine and spruce soda pulp liquors were slightly lower as compared to those obtained in the first feeding. The persistence of the inhibition beyond exposure indicates a damaging effect of the toxicants on the sludge. Partial recoveries of the methanogenic activity were evident in the toxicity assays with alkaline liquors prepared from birch wood and straw, since somewhat higher inhibitory concentrations were observed in the second compared to the first feeding (Table 4).

Batch digestion experiments with repeated feedings of straw and spruce soda pulp water were also performed to evaluate the short-term adaption capacity of the sludge to these wastewaters. The procedure for the additional feedings was similar to that applied to assay the methanogenic toxicity of the soda pulp liquors in the first feeding. The 50% and 80% IC resulting from exposure of granular sludge to five consecutive feedings of straw alkaline liquor are given in Figure 3. According to these results, a significant increase in the inhibiting

### Table 3: The Average Acidification of the Wastewater COD during Batch Anaerobic Digestion of Pulping Wastewaters Prepared from Wood (Pine, Spruce or Birch) and Wheat Straw. The Acidification Data Reported were Determined on the 15th Day of the Assay.

<table>
<thead>
<tr>
<th>Pulping Method</th>
<th>Lignocellulosic Material</th>
<th>Acidification (%)</th>
</tr>
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<tr>
<td>TMP</td>
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<td></td>
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<tr>
<td>Pine</td>
<td>78.4</td>
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<tr>
<td>Spruce</td>
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<tr>
<td>Birch</td>
<td>86.5</td>
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<tr>
<td>Straw</td>
<td>67.5</td>
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</tr>
<tr>
<td>Soda</td>
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<tr>
<td>Pine</td>
<td>50.6</td>
<td></td>
</tr>
<tr>
<td>Spruce</td>
<td>46.7</td>
<td></td>
</tr>
<tr>
<td>Birch</td>
<td>67.3</td>
<td></td>
</tr>
<tr>
<td>Straw</td>
<td>45.4</td>
<td></td>
</tr>
</tbody>
</table>
Biodegradability and toxicity of pulping wastewaters

Fig. 2. The methanogenic activity (as percentage of the control activity) of granular sludge exposed to soda liquors prepared from wood and straw: birch (A), spruce (B), pine (C) and wheat straw (D), respectively, versus the wastewater assay concentration. The activity of the sludge in the first feeding (---) and the residual sludge activity in the second feeding (- -) of the batch anaerobic toxicity assays.

TABLE 4 The Concentrations (in g COD/l) of the Various TMP and Soda Pulping Wastewaters Evaluated in this Study Resulting in a 50% and 80% Inhibition of the Methanogenic Activity.

<table>
<thead>
<tr>
<th>PULP. LIGNOCELLULOSIC MATERIAL</th>
<th>1st. feeding</th>
<th>2nd. feeding</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>50% IC</td>
<td>80% IC</td>
</tr>
<tr>
<td>TMP</td>
<td></td>
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</tr>
<tr>
<td>Pine</td>
<td>11.5</td>
<td>14.0</td>
</tr>
<tr>
<td>Spruce</td>
<td>13.0</td>
<td>22.0</td>
</tr>
<tr>
<td>Birch</td>
<td>13.7</td>
<td>17.0</td>
</tr>
<tr>
<td>Straw</td>
<td>NT*</td>
<td>NT</td>
</tr>
<tr>
<td>SODA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pine</td>
<td>2.1</td>
<td>2.7</td>
</tr>
<tr>
<td>Spruce</td>
<td>5.4</td>
<td>8.3</td>
</tr>
<tr>
<td>Birch</td>
<td>5.3</td>
<td>7.3</td>
</tr>
<tr>
<td>Straw</td>
<td>4.4</td>
<td>7.3</td>
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</table>

* NT= non-toxic at 3 g COD/l, the highest concentration tested.
concentration with feeding number was observed, indicating that granular sludge can adapt to a great extent to the inhibitory effects of straw soda pulp liquor. In contrast, repeated feedings with spruce soda pulping wastewater did not result in any adaption of the granular sludge, and the 50% and 80% IC values determined in the first and third assay feeding were very similar.

**Sources of methanogenic inhibition in soda pulping wastewater**

Additional experiments were conducted in an attempt to identify the sources of the inhibition in soda pulping liquors. The detoxification obtained by selectively removing specific wastewater components was evaluated in anaerobic toxicity assays fed the original and treated wastewaters. The treatments applied included liquid-liquid extraction with ether, XAD and PVP adsorption, calcium and acid precipitation. The extraction of wood-derived wastewaters with ether removes hydrophobic resin components, such as fatty acids, resin acids, esters, waxes and sterols (Bjorklund-Jansson, 1980; Voss and Rapsomatitits, 1985). Adsorption onto XAD-2 under alkaline (pH 9) conditions has previously been used to isolate wood resin compounds from pulp and paper effluent samples (Rogers, 1973; Leach and Thakore, 1976). PVP is a polyamide that specifically removes the fraction of the wastewater with tannic qualities (Field et al., 1988). The calcium can precipitate an important fraction of the dissolved lignin, but its effectiveness is restricted to the intermediate to high molecular lignin derivatives (Dugal et al., 1975; Schmidt and Joyce, 1980). Fatty and resin acids are also removed by calcium precipitation (Easty et al., 1979). Acidification precipitates lignin and resin compounds similarly as calcium (Bjornlund Jansson and Back, 1975; Kim et al., 1987).

The influence of various treatments of pine alkaline pulp liquor on the methanogenic activity of granular sludge exposed to the resulting wastewaters is illustrated in Figure 4. Extraction with ether, method specific for wood resin components, almost completely removed wastewater toxicity. Other methods which remove resin compounds such as XAD-2 adsorption, acid and calcium precipitation were also able to completely detoxify the wastewater. PVP adsorption, on the other hand, had only a small effect on removing the toxicity, indicating that organics with tannic qualities did not significantly contribute to the high toxicity of the soda pulp waters. Finally, the inhibitory effect of the insoluble fraction upon acid precipitation was close to that exerted by the untreated wastewater. Although not illustrated, these treatments gave similar results with spruce soda pulp liquors. These results indicate that the inhibitory components in coniferous wood extracts were insoluble at acidic pH and could be precipitated by calcium. Furthermore, the non-toxicity of the ether-extracted liquors strongly suggests that the major inhibitory compounds are the wood resin constituents.

Finally, it should be noted that the composition of the apolar extractives of straw significantly differs from that of wood. The methanogenic toxicity of straw apolar extractives was not assessed and therefore it is not certain whether the inhibitory potential of straw soda pulp liquor is caused by resinous or other lignocellulosic derivatives released during alkaline pulping.

**The methanogenic toxicity of soda pulp liquors from resin-free coniferous wood.**

To further investigate the inhibitory role of resin compounds, pine wood was pre-extracted with organic solvents to remove resin prior to alkaline pulping. The effect of alkaline liquors prepared from both untreated pine wood and treated resin-free pine wood on the methanogenic activity was tested. As shown in Figure 5, removing the wood resin resulted in an almost complete detoxification of the soda pulp water. It should be noted that non-resinous components may also contribute somewhat, though to a much lesser extent, to the inhibitory activity of alkaline liquors as indicated by the low inhibition caused by the resin-free pulp liquor (Table 5).

**TABLE 5 The Concentrations (in g COD/l) of Soda Pulping Liquors from Pine Wood and Resin-free Pine Wood Resulting in a 50% and 80% Methanogenic Inhibition**

<table>
<thead>
<tr>
<th>PINE WOOD</th>
<th>1st feeding</th>
<th>2nd feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50% IC</td>
<td>80% IC</td>
</tr>
<tr>
<td>UNTREATED</td>
<td>2.1 2.7</td>
<td>2.0 2.8</td>
</tr>
<tr>
<td>RESIN-FREE</td>
<td>9.0 20.0</td>
<td>&gt;&gt;12.0 &gt;&gt;12.0</td>
</tr>
</tbody>
</table>
Biodegradability and toxicity of pulping wastewaters

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**Fig. 3.** The effect of consecutive feedings of straw soda pulping liquor on the activity of methanogenic granular sludge.

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**Fig. 4.** The influence of various treatments of pine soda pulping liquor on the methanogenic activity of granular sludge exposed to the different extracts in the first and in the second assay feeding. Untreated wastewater (E), wastewater treated with XAD-2 (EX), with PVP (EP), with ether (EE), with calcium (ECa); fraction soluble at pH 2 (ES) and fraction insoluble at pH 2 (EI).
DISCUSSION

In this study we have demonstrated that the anaerobic treatability of pulping wastewaters is largely dependent on the pulping method applied and, to a smaller extent, on the type of lignocellulosic feedstocks used as raw material. Wastewaters derived from soda pulping were less biodegradable and they caused significantly higher methanogenic inhibition as compared to those derived from TMP. The significant differences observed in the anaerobic biodegradability and methanogenic toxicity of TMP compared to soda pulping effluents can be explained by the distinct effect of each pulping method in controlling the type and quantity of lignocellulosic components solubilized into the wastewater.

During mechanical pulping processes, carbohydrates are the principal components extracted. The wood is subjected to high pressures and temperatures under slightly acidic conditions in which hemicellulose is largely dissolved and lignin is attacked only to a minor extent. In the resulting effluents carbohydrates may account for 50 to 70% and lignin for 15% to 30% of the total wastewater dry solids (Stemberg and Norberg, 1977; Jarvinen et al., 1980). The wood resin constituents are poorly soluble in acidic conditions and therefore only low concentrations are expected in mechanical pulping effluents (Bjorklund-Jansson and Back, 1975).

On the other hand, pulping in alkaline conditions solubilizes to a large extent both the hemicellulosic and lignin fractions. The lignin content of the alkaline pulping liquors can account for up to 50% of the total solids (Forss, 1982; Kim et al., 1987). The high lignin content is responsible for the characteristic strong brown color of these wastewaters (Sundman et al., 1981). Alkaline pulping processes also effectively extract wood resin constituents, resulting in the presence of important quantities of resin-derived components in the process relief gases and in the black liquor (Bryce, 1980).

The anaerobic biodegradability of TMP effluents was high due to the large fraction of readily biodegradable carbohydrates in these wastewaters. The distinctly lower biodegradability of alkaline pulping liquors can be explained by their lignin content. Past research indicates that anaerobic bacteria have a very limited capacity to degrade lignin (Hackett et al., 1977; Zeikus et al., 1982; Benner et al., 1984). The recalcitrance of lignin in anaerobic environments is related to its characteristic chemical heterogeneity and high molecular weight. In any case, the anaerobic metabolism of various lignin monomers and oligomers (MW < 800 dalton) has been reported (Colberg and Young, 1985; Chen et al., 1985a, 1985b, 1987). Higher MW lignin is not degraded by anaerobic bacteria (Zeikus et al., 1982; Colberg and Young, 1985).

Numerous types of forest industry wastewaters contain important amounts of lignin. Chemical processes such as bleaching, kraft, soda and sulfite pulping effectively extract lignin into the wastewater. The limited capacity of anaerobic microorganisms to degrade lignin indicates that other technologies, including physical-chemical and enzymatic or fungal treatments (Schmidt and Joyce, 1980; Campbell and Joyce, 1983; Eaton, 1985; Milstein et al., 1988) should be applied to remove the color bearing lignic COD which is resistant to anaerobic as well as conventional aerobic wastewater treatment (Pellinen and Salkinoja-Salonen, 1985; Larrea et al., 1989).

The toxicity of pulping wastewaters was found to depend strongly on the pulping conditions used. Wastewaters derived from the TMP process were only mildly toxic to methane bacteria. In contrast, the wastewaters of soda
pulping caused severe methanogenic inhibition at low concentrations (Table 4). The toxicity of soda pulp wastewaters also depended on the type of lignocellulosic feedstock used. Pine wood was responsible for the soda pulp liquor with the highest toxicity. Birch wood and straw alkaline pulp liquors were the least inhibitory.

Selective removal of various fractions from the soda extract with different physical-chemical treatments indicated that wood resin constituents were responsible for most of the toxicity associated with the alkaline liquors of wood. The methanogenic toxicity of wood resin was confirmed by comparing the inhibitory effect of soda pulp waters prepared from pine wood and resin-free pine wood. These observations are supported by previous literature reports indicating the high methanogenic toxicity of individual resin constituents such as volatile terpenes (McNary et al., 1951; Benjamin et al., 1984; Sierra-Alvarez and Lettinga, in press), resin acids (Field et al., 1988; Sierra-Alvarez and Lettinga, in press) and long-chain fatty acids (Demeyer and Henderickx, 1967; Hanaki et al., 1981; Koster and Kramer, 1987). Moreover, resin compounds are also implicated in the aquatic toxicity of forest industry effluents (Rogers, 1973; Leach and Thakore, 1976; Walden, 1980).

Although wood resin was responsible for most of the methanogenic toxicity, the soda pulp liquor derived from resin-free pine wood exerted a small inhibitory effect, indicating that non-resinous compounds may also contribute to a certain extent to the toxicity of soda pulp wastewaters. These non-resinous components most likely correspond to lignin-derived phenolics. Low-molecular-weight lignin derivatives have previously been identified as microbial inhibitors in aqueous lignocellulosic extracts (Clark and Mackie, 1984; Jung, 1988) and in studies with model compounds (Zemek et al., 1979; Vohra et al., 1980; Benjamin et al., 1984). The high methanogenic toxicity of soda pulp liquors as compared to those of TMP demonstrate that the contact of wood with alkali has an important effect in increasing the methanogenic toxicity of wood-derived wastewaters. The forest industry applies various bleaching and chemical pulping processes where wood is subjected to alkaline treatment. This will generate effluents with high concentrations of resin compounds that are responsible for severe methanogenic toxicity.

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LITERATURE CITED


