Soil Microbial Responses to Potassium-Based Black Liquor from Straw Pulping

C. Xiao, M. Fauci, D. F. Bezdicek, W. T. McKeen, and W. L. Pan*

ABSTRACT

Sodium-based black liquor from fiber pulping for papermaking creates challenging waste disposal issues. By substituting NaOH with KOH in the pulping process, the resulting black liquors may be land applied as an environmentally beneficial disposal alternative. Incubation studies examined the effect of KOH-based black liquor on soil pH, electrical conductivity (EC), microbial biomass, CO2 evolution, and soil enzyme activities in a silt loam soil. Amended soils with black liquor at rates up to 67.2 mL kg⁻¹ soil, corresponding to 1200 kg K ha⁻¹ were incubated at 24°C for 60 d. Increasing application rates increased soil pH, indicating that black liquor has potential as a fluid liming material. Soil EC increased with black liquor application rates, but only up to 1.04 dS m⁻¹, suggesting that black liquor application at these rates would not cause a salinity problem. Carbon dioxide evolution rate peaked at 2 d of incubation, and then gradually declined until the end of incubation. Metabolic quotient significantly increased with increasing application rates. Soil microbial biomass, CO2 evolution, dehydrogenase, β-glucosidase, and arylsulfatase activities generally increased with increasing application rates throughout the incubation period. In contrast, increasing soil pH with KOH alone generally decreased CO2 evolution and soil dehydrogenase, β-glucosidase, and arylsulfatase activities, indicating that this liquor effects in increasing soil microbial activity were possibly attributable to organic constituents contained in this liquor rather than its high pH.

PULPING BLACK LIQUOR is an environmental concern for the pulp and paper industry due to its high levels of biological oxygen demand, chemical oxygen demand, suspended solids, and lignin and their derivatives (Juwarkar and Subrahmanyam, 1987; Hamner, 1988). The discharge of black liquor into surface waters is not only a serious aesthetic problem, but decreased penetration of solar radiation reduces algal and aquatic plant productivity. Presently, strict environmental protection regulations and higher public awareness have limited the discharge of untreated black liquor (Ali and SrEEKIrshnan, 2001). As the pulp and paper industry begins to use crop straw in its feedstock stream to supplement wood-based fiber, the development of straw pulping processes should account for these environmental concerns.

A current common disposal approach is through a combustion recovery process where black liquor is concentrated, burned for producing energy, and recycled for recovering inorganic chemicals (Grover et al., 1999). However, this disposal approach is neither cost effective nor environmentally friendly (Girovich, 1996). Traditionally, NaOH is used in straw pulping, producing Na-based black liquor. Land application of this liquor raises concern for deterioration of soil structure (Balba, 1995). Increased soil erodibility (Singer et al., 1982), diminished plant growth and reduced soil permeability have been attributed to high concentrations of Na (Jorgensen, 1970; Guarri, 1973). Reducing Na content of black liquor by substituting KOH for NaOH for pulping should render black liquor that can be used as a K fertilizer and soil amendment while offering an environmentally acceptable disposal alternative. Cox et al. (1997) observed that application of black liquor increased plant nutrients, soil organic matter, cation exchange capacity (CEC), water holding capacity, and tilth. Other effects on soil quality parameters are less well defined, but the alkalinity and soluble organic matter in the black liquor could be beneficial.

The objectives of this study were to: (i) determine the temporal impacts of KOH-based black liquor on soil biological and chemical indicators of soil quality, and (ii) distinguish the causal factors of these impacts by the black liquor between pH modifications vs. other factors.

MATERIALS AND METHODS

Three soil incubation experiments were conducted to address the objectives stated above. A Shano silt loam soil (coarse-silty, mixed, Xeric Haplocambids) was collected from the Washington State University experiment station in Othello, WA. Selected characteristics were as follows: pH 7.5; EC 0.26 dS m⁻¹; organic C 6.4 g kg⁻¹ soil; total N 0.70 g kg⁻¹ soil; Olsen K 125 mg kg⁻¹ soil; CEC 15 cmol kg⁻¹ soil; and water holding capacity 147 mL kg⁻¹ soil.

Black liquor was obtained from wheat straw pulping with KOH instead of NaOH according to modified Universal Pulping (M. Jackson, consultant, Tolovana Park, OR, personal communication, 2000). Briefly, wheat straw was mixed with KOH, concentrated HNO3, and alum [Al2(SO4)3.14H2O] at a ratio of 10:1, 100:1, and 1000:1 on a weight basis, respectively, and water was added to keep the ratio of water to straw of 10:1. The mixture was cooked under the ambient pressure and a temperature of 90°C for 1 h. After cooling, black liquor was separated from the pulped straw by draining, and tested for the residual alkalinity based on acid titration neutralization (M. Jackson, consultant, Tolovana Park, OR, personal communication, 2000). The separated liquor was reused as part of the cooking liquor for the next cook after fortification with KOH to the target level (mass ratio of straw/KOH = 10:1). Black liquor was recycled eight times. General characteristics of this eight times, recycled liquor are shown in Table 1.

Application rates were based on the K concentration of black liquor. The rates ranged from the field corn recommended K rate (200 kg K ha⁻¹) in Washington, to a very high rate (1200 kg K ha⁻¹).

Abbreviations: CEC, cation exchange capacity; EC, electrical conductivity.


© Soil Science Society of America
677 S. Segoe Rd., Madison, WI 53711 USA
Table 1. General characteristics of eight times, recycled KOH-based black liquor (n = 3).

<table>
<thead>
<tr>
<th>Contents</th>
<th>W/V</th>
<th>W/V dry solid</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>9.95</td>
<td></td>
</tr>
<tr>
<td>Electrical Conductivity (EC)</td>
<td>27.4 dS m⁻¹</td>
<td></td>
</tr>
<tr>
<td>Solid content</td>
<td>57.9 g L⁻¹</td>
<td></td>
</tr>
<tr>
<td>Total organic C</td>
<td>21.2 g L⁻¹</td>
<td>366 g kg⁻¹</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>12.3 g L⁻¹</td>
<td>212 g kg⁻¹</td>
</tr>
<tr>
<td>Lignin</td>
<td>6.5 g L⁻¹</td>
<td>112 g kg⁻¹</td>
</tr>
<tr>
<td>K</td>
<td>14.9 g L⁻¹</td>
<td>257 g kg⁻¹</td>
</tr>
<tr>
<td>Total N</td>
<td>6.6 g L⁻¹</td>
<td>114 g kg⁻¹</td>
</tr>
<tr>
<td>P</td>
<td>62 mg L⁻¹</td>
<td>1071 mg kg⁻¹</td>
</tr>
<tr>
<td>Ca</td>
<td>64 mg L⁻¹</td>
<td>1105 mg kg⁻¹</td>
</tr>
<tr>
<td>Mg</td>
<td>22 mg L⁻¹</td>
<td>380 mg kg⁻¹</td>
</tr>
<tr>
<td>Si</td>
<td>116 mg L⁻¹</td>
<td>2003 mg kg⁻¹</td>
</tr>
<tr>
<td>Mn</td>
<td>0.28 mg L⁻¹</td>
<td>4.8 mg kg⁻¹</td>
</tr>
<tr>
<td>Cu</td>
<td>0.23 mg L⁻¹</td>
<td>4.0 mg kg⁻¹</td>
</tr>
</tbody>
</table>

**Experiment 1**

Air-dried soil was passed through a 2-mm sieve and amended with black liquor at rates of 0 (nonamended control), 200 (recommended K rate), 400, 800, and 1200 kg K ha⁻¹. Black liquor application rate for each treatment was calculated based on its K concentration (1.49 g K 100⁻¹ mL⁻¹). The corresponding volumes of black liquor for treatments were 0, 11.2, 22.4, 44.8, and 67.2 mL kg⁻¹ soil, respectively, assuming a soil bulk density of 1.19 g cm⁻³ and a soil depth of 10 cm (1.19 × 10⁶ kg soil ha⁻¹). The black liquor was thoroughly mixed with a 25-g air-dried soil for each treatment. There were four replications for each treatment. Additional water was added to keep the soil moisture content at 60% of the water holding capacity.

Treated soils were placed individually in sealed 1-L Mason jars, each containing two vials, one with 5 mL of 1 M KOH to trap evolved CO₂, and one with 10 mL of CO₂-free water to replace the CO₂ trapping vials at 2, 5, 10, 20, 40, and 60 d. The treated soils were destructively sampled at Days 5, 20, 40, and 60 for the determination of pH, EC, microbial biomass C, and soil enzyme activities.

**Experiment 2**

The same rates of black liquor used in Exp. 1 were applied to 500 g of air-dried soils with four replications per treatment. The treated soils were incubated at 24°C for 60 d using a randomized complete block design. Incubation jars were opened to refresh the incubation air and to remove and replace the CO₂ trapping vials at 2, 5, 10, 20, 40, and 60 d.

**Experiment 3**

Experiment 2 demonstrated that soil microbial activity and soil pH increased with increasing black liquor application rates, raising a question of whether pH was a controlling factor of microbial activity. To separate the chemical and pH modifying effects of black liquor, KOH was added alone to soil. Addition of black liquor at a rate of 11.2 to 67.2 mL kg⁻¹ soil in the Exp. 1 increased soil pH by 0.1 to 0.5 units over the 60 d of incubation. A preliminary soil incubation determined that the addition of 0.40 M KOH to the same soil at a rate of 10, 30, and 50 mL kg⁻¹ soil increased soil pH to 7.7, 7.9, and 8.0, respectively, mimicking the soil pH modifications found in Exp. 2 (data not shown).

Twenty five grams of air-dried soil samples were amended with 0.40 M KOH at a rate of 0, 10, 30, and 50 mL kg⁻¹ soil with four replications for the determination of CO₂ evolution over 60 d of incubation under the conditions as described in the Exp. 1. The same rates of 0.40 M KOH were applied to 200 g air-dried soils with four replications under conditions as described in the Exp. 2. The treated soils were destructively sampled at Days 5, 20, 40, and 60 for the determination of CO₂ evolution and soil enzyme activities.

**Black Liquor and Soil Sample Analysis**

The pH and EC of black liquor were measured with a pH meter (211/digital pH meter, Orion Research Inc., Boston, Mass) and an EC meter (YSI Model 35, Yellow Springs Instrument, Co., Inc., Yellow Springs, OH), respectively. Black liquor subsamples (5 mL) were oven-dried at 85°C for 24 h for determination of solid content. Polysaccharides and lignin in black liquor were determined as described by Sun and Tomkinson (2001). Black liquor was digested using HNO₃–H₂O₂ (Jones and Case, 1990). The digestion solution was analyzed for the determination of K, Ca, Mg, Si, Mn, Cu, and P concentrations. A 0.5-mL subsample of black liquor was used for the determination of total C and N by a Leco analyzer (CNS2000, LECO, St. Joseph, MI).

A water-saturated soil paste was equilibrated for 1 h for the determination of pH by a pH meter (211/digital pH meter, Orion Research Inc., Boston, MA), and EC by an EC meter (YSI Model 35, Yellow Springs Instrument, Co., Inc., Yellow Springs, OH).

Carbon dioxide absorbed in NaOH was measured by titration (Anderson, 1982). The cumulative CO₂ amounts were expressed as μg CO₂–C g⁻¹ dry soil, and CO₂ evolution rates as μg CO₂–C g⁻¹ dry soil d⁻¹. The apparent percentage of black liquor-derived C evolution was calculated based on the equation:

\[
\text{Apparent percentage of black liquor-derived C evolution} = \left( \frac{\text{cumulative CO}_2 \text{ evolution in the black liquor amended treatment} - \text{cumulative CO}_2 \text{ evolution in the non-amended control}}{\text{total black liquor derived C}} \right) \times 100
\]

Soil microbial biomass C was determined by the fumigation-incubation method (Horwath and Paul, 1994). Following the removal of chloroform, 25 g of fumigated dry weight equivalent, and non-fumigated soils at soil moisture with 60% of water-holding capacity were then incubated in a 0.5-L Mason jars at 24°C in the dark for 10 d. The CO₂ flush from fumigated and unfumigated soils were trapped according to the method as described in the Exp. 1. The soil microbial biomass C was calculated based on the equation:

\[
\text{Soil microbial biomass C (μg g⁻¹ dried soil)} = (F - UF)/K, \text{ where, } F = \text{CO}_2 \text{ flush from the fumigated soil, } UF = \text{CO}_2 \text{ produced by the unfumigated soil, and } K, \text{ coefficient} = 0.41 \text{ (Anderson and Domsch, 1978).}
\]

Soil dehydrogenase, β-glucosidase, and ariysulfatase activities were measured by the method of Tabatabai (1994).

**Statistical Analyses**

Analyses of variance (ANOVA) were conducted on each parameter. There was interaction between treatments and sampling date for all measured parameters except for soil pH and EC. Therefore, separate analyses were performed for microbial parameters at each sampling date. Protected LSD test was performed when treatments were significant at p ≤ 0.05 (SAS Institute, 2002) to compare the means of all different treatments within the same sampling date and the same parameter.

**RESULTS AND DISCUSSION**

**Soil pH and EC**

The addition of black liquor (pH 10) resulted in rapid increases in soil pH by 0.1 to 0.5 units within 2 d and then
Carbon Dioxide Evolution

Cumulative CO₂ evolution differed among the five treatments over the incubation period (Fig. 1a), ranging from 316 μg CO₂-C g⁻¹ soil in the unamended control to 831 μg CO₂-C g⁻¹ soil in the amended soil receiving the highest black liquor rate (67.2 mL kg⁻¹ soil). There was also a linearly increase with increasing rates of black liquor (r² = 0.99; p < 0.01).

The CO₂ evolution rates were the most rapid during the first 2 d of incubation, and treatment differences were most apparent during this initial time period (Table 2). The evolution rates for all treatments drastically decreased to a low level by 10 d after application where treatment differences diminished. For example, at 2 d of incubation, CO₂ evolution rate was 180 μg CO₂-C g⁻¹ soil d⁻¹ in the highest rate of black liquor (67.2 mL kg⁻¹ soil), 4.5 times higher than that in the unamended control (40 mL kg⁻¹ soil) in the amended soil receiving the highest black liquor rate (67.2 mL kg⁻¹ soil). In contrast, at 60 d of incubation CO₂ rates were 3.9 and 2.6 μg CO₂-C g⁻¹ soil d⁻¹ in the highest application rate and unamended control, respectively. Bardgett et al. (1995) reported similar findings with addition of silage effluent to soils.

The CO₂ evolution rate is also an index for organic matter turnover (Debosz et al., 2002) and an indicator of the effect of organic waste amendments on soil microbial activity (Anderson, 1982). This increase in CO₂ evolution rate in our study may have been related to the supply of the easily decomposable polysaccharides (1.23 g 100 g⁻¹ dry soil) (Table 1) contained in black liquor. In addition to the 138 to 827 μg polysaccharides g⁻¹ soil, application of black liquor also provided other nutrients such as N, K, P, Ca, Mg, and Zn for utilization by soil microorganisms. The black liquor itself may contain microorganisms. The black liquor itself may contain microorganisms that survive the pulping process or proliferate between processing and application. The decline in CO₂ evolution in later stages of the incubation was probably caused by depletion of polysaccharides or essential nutrients, or the accumulation of toxic metabolites during the incubation (Saviozzi et al., 1993; Wong and Wong, 1986). Later in the incubation, additional nutrients from the higher application rates may have stimulated greater microbial utilization of recalcitrant substrates such as lignin (0.65 g 100 mL⁻¹) (Table 1) contained in black liquor.

<table>
<thead>
<tr>
<th>BL rates (mL kg⁻¹ soil)</th>
<th>Incubation days</th>
<th>CO₂–C evolution rate, μg CO₂–C g⁻¹ dry soil d⁻¹</th>
<th>Microbial biomass C, μg C g⁻¹ dry soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L.S.D₀.₀₅</td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td>44 c†</td>
</tr>
<tr>
<td>11.2</td>
<td></td>
<td></td>
<td>65 d</td>
</tr>
<tr>
<td>22.4</td>
<td></td>
<td></td>
<td>90 c</td>
</tr>
<tr>
<td>44.8</td>
<td></td>
<td></td>
<td>135 b</td>
</tr>
<tr>
<td>67.2</td>
<td></td>
<td></td>
<td>180 a</td>
</tr>
<tr>
<td>LSD₀.₀₅</td>
<td></td>
<td></td>
<td>7.7</td>
</tr>
</tbody>
</table>

† Different letters mean significant differences among treatments at the same day of incubation at p ≤ 0.05, and NS: no significant differences among treatments.
A similar percentage (18.3% at Day 2 and 39.1% at Day 60) of black liquor derived C was evolved as CO2–C across all treatments during incubation period (Fig. 1b). This suggests that black liquor at 11.2 to 67.2 mL kg⁻¹ soil had no detrimental effect on soil microbial activity in terms of CO2 evolution, with a similar proportion of total added C evolved as CO2 regardless of application rates within this range.

**Microbial Biomass Carbon**

Soil microbial biomass C increased with increasing application rates except at Days 20 and 40 (Table 2). Studies reported that additions of silage effluent and urban wastewater (Bardgett et al., 1995; Meli et al., 2002) increased soil microbial C, partly due to the addition of readily available nutrients into soil, and partly due to the input of microorganisms into the soil from the waste effluents. In our study, this increase in microbial biomass was probably attributed to the addition of easily decomposable substrate such as polysaccharides (Table 1).

**Metabolic Quotient**

The metabolic quotient (specific respiration, qCO₂) is the ratio of microbial respiration CO₂–C rate to microbial biomass C. In our study, the qCO₂ increased with increasing application rates throughout the incubation period except at Day 60 (Table 3).

An increase in qCO₂ can be explained as a response by soil microorganisms to disturbance or environmental stress (Anderson and Domsch, 1990). Some researchers reported the increased qCO₂ as response to input of easily decomposable substrates (Bäath and Arnebrant, 1994). In contrast, others observed decreased qCO₂ due to addition of easily decomposable substrates (Kandeler and Eder, 1993).

In our study, increased qCO₂ due to the increasing application rates (Table 3) may not mean that soil microorganisms were stressed. Soil microbial biomass C and CO₂ evolution rates (Table 2) both increased with increasing application rates. This indicated no stress to the microorganisms (Badalucco et al., 1992). In addition, there were no differences in the proportion of black liquor derived C evolved as CO₂ (Fig. 1b).

The increased soil pH induced by the black liquor rates could result in a number of changes that affected the composition of the microbial community (Dick et al., 2000). High salinity and heavy metal contents of sludge can inhibit microbial activity (Tester and Parr, 1983; Wong and Lai, 1996). Black liquor obtained by wheat straw pulping with KOH had undetectable levels of heavy metals (data not shown). Application of black liquor at rates up to 67.2 mL kg⁻¹ soil resulted in low levels of soil EC. Therefore, increased qCO₂ were not likely a result of the heavy metals or soil salinity.

**Enzyme Activities**

Black liquor increased soil dehydrogenase activity over the incubation period (Fig. 2a). Soil dehydrogenase activity was the highest at the start of our incubation and decreased over time. Soil dehydrogenase activity was considered an index of total viable soil microorganisms (Taylor et al., 2002). Soil β-glucosidase activity was relatively stable throughout the incubation, and generally in-

---

**Table 3. Metabolic quotient (qCO₂) as influenced by black liquor (BL) application rates.**

| BL rates (mL kg⁻¹ soil) | Incubation days | Metabolic quotient  
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>0</td>
<td>0.21 d†</td>
<td>0.055 c</td>
</tr>
<tr>
<td>11.2</td>
<td>0.22 d</td>
<td>0.063 c</td>
</tr>
<tr>
<td>22.4</td>
<td>0.27 c</td>
<td>0.064 c</td>
</tr>
<tr>
<td>44.8</td>
<td>0.44 b</td>
<td>0.089 b</td>
</tr>
<tr>
<td>67.2</td>
<td>0.57 a</td>
<td>0.11 a</td>
</tr>
<tr>
<td>LSD0.05</td>
<td>0.038</td>
<td>0.015</td>
</tr>
</tbody>
</table>

† Different letters mean significant differences among treatments at the same day of incubation at p ≤ 0.05; NS represents no significant differences.

---

**Fig. 2. (a) Dehydrogenase, (b) β-glucosidase, and (c) arylsulfatase activities as influenced by black liquor application rates. I: LSD₀.₀₅ bars: significant differences among treatments at each sampling date at p ≤ 0.05; and NS: no significant differences among treatments at each sampling date.**

Reproduced from Soil Science Society of America Journal. Published by Soil Science Society of America. All copyrights reserved.
creased from 10 through 40 d (Fig. 2b). Soil β-glucosidase, an important extracellular enzyme, one of the three enzymes responsible for decomposition of cellulose, has potential as a biological indicator of soil quality (Turner et al., 2002). Similar to soil dehydrogenase and β-glucosidase activities, soil arylsulfatase activity increased with increasing application rates except at Days 2 and 5 (Fig. 2c). Soil arylsulfatase is an enzyme that catalyzes the hydrolysis of an arylsulfate anion by cleavage of the O-S bound and is considered partly responsible for soil S cycling and also as an indicator of fungi activity in soil (Oshrain and Wiebe, 1979). The addition of organic substances due to application of organic wastes, stimulated microbial activities and subsequent enzyme synthesis (Sastre et al., 1996). Kannan and Oblisami (1990b) found that 15 yr of irrigation of paper mill waste effluent increased soil dehydrogenase, amylase, and phosphatase activities. They attributed the increases in enzyme activities to the addition of organic matter and nutrients. Increased soil enzyme activities in treated soils in our study may be related to the addition of polysaccharides.

**Effects of Increased Soil pH on Soil Microbial Activity**

Increased soil pH by KOH alone generally decreased soil respiration, dehydrogenase, β-glucosidase, and arylsulfatase activities over the 60 d of incubation (data not shown). Increased soil pH may have decreased the proliferation of microbial species already present in soil that were relatively active, thus reducing soil microbial respiration, or altered the diversity of the soil microbial community; thus decreasing soil enzyme activities (Dick et al., 2000). This result differed with other studies that found soil respiration increased with increasing soil pH in strongly acid soils that were adjusted to near neutral pH (Neale et al., 1997; Zimmermann and Frey, 2002).

**CONCLUSIONS**

Addition of KOH-based black liquor caused rapid increases in soil pH, suggesting that it may have potential as a fluid liming material. Black liquor slightly increased soil EC, indicating that KOH-based black liquor at rates up to 67.2 mL kg⁻¹ soil would not cause a salinity problem. Addition of KOH-based black liquor also increased soil microbial biomass and soil respiration, and soil enzyme activities. Increased soil pH by KOH alone generally decreased soil microbial activity in terms of CO₂ evolution, and soil enzyme activities, suggesting that black liquor effects in increasing soil microbial activity may have been attributable to organic constituents contained in black liquor rather than the pH effects. Application of KOH-based black liquor up to 67 mL kg⁻¹ soil in this study, corresponds to nearly 80 000 L ha⁻¹ in the field. Therefore, 1 ha of soil can receive KOH-based black liquor generated from production of approximately 10 tons of paper generated from 20 tons of straw. Field application rates may be higher, but potential problems due to high EC, high pH or K imbalances should be monitored. Costs of transporting this dilute material limit its range of applicability to local fields unless steps are taken to increase nutrient concentrations. Further experiments are needed to clarify upper limits of KOH-based black liquor application rates, and whether some combination of Na, K, and Ca hydroxide might provide reasonable pulping conditions that might also allow for higher rates of black liquor application.

**ACKNOWLEDGMENTS**

This project was supported by the Washington State University Agricultural Research Center, USDA Grass Seed Cropping Systems for Sustainable Agriculture and USDA NRI project no 2003-35504-12861. The authors thank Eric Harwood, Ron Bolton, Mark Lewis, Margaret Davies and Deb Bikfasy for their technical assistance.

**REFERENCES**

ing and plant analysis. 3rd ed. SSSA Book Series. 3. SSSA, Madison, WI.