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ANAEROBIC TOXICITY OF RESIN ACIDS IN CHEMITHERMOMECHANICAL PULP WASTEWATER

by
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A thesis
submitted under the supervision of
Dr. Ronald L. Droste

presented to the University of Ottawa
in partial fulfillment of the requirements
for the degree of Master of Applied Science
in Civil Engineering

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GLOSSARY

FUNDAMENTAL QUANTITIES

T = time (d,h,m,s)

L = length (m, cm, or mm)

M = mass (kg, g, mg, μg, or mole)

EQUATION CONSTANTS AND VARIABLES

A = empirical constant

b = bacterial die-off constant $[T^{-1}]$

B = empirical constant

G = rate of methane production $[L^3T^{-1}]$

I = toxicant concentration $[ML^{-3}]$

k = specific substrate utilization rate $[T^{-1}]$

k* = specific substrate utilization rate in the presence of an inhibitor $[T^{-1}]$

Ki = toxicant coefficient $[ML^{-3}]$

k_1 = rate constant $[T^{-1}]$

k_2 = rate constant $[T^{-1}]$

Ks = Monod half-velocity constant [M]

K*s = half velocity constant in the presence of inhibitor [M]

Q = flow rate $[L^3T^{-1}]$

r_G = rate of bacterial growth $[MT^{-1}]$

r_{su} = rate of substrate utilization $[ML^{-3}T^{-1}]$

S = substrate concentration $[ML^{-3}]$

t = time [T]

t_d = hydraulic retention time [T]

V = volume of reactor $[L^3]$

Y = substrate specific yield factor of bacterial growth

X = concentration of reactor biomass $[ML^{-3}]$

Xa = active mass concentration $[ML^{-3}]$

ABBREVIATIONS

ATA = anaerobic toxicity assay

ATIR = activity test inoculum reactor

BCTMP = bleached chemithermomechanical pulp

BCTMP-N = bleached chemithermomechanical pulp, normal fines

BCTMP-NC = bleached chemithermomechanical pulp, normal fines centrifuged

COD = chemical oxygen demand

CODr = chemical oxygen demand (removed)

CODs = chemical oxygen demand (soluble)

CODt = chemical oxygen demand (total)

CTMP = chemithermomechanical pulp

DOM = Domtar

GDW = glass distilled water

HRT = hydraulic retention time [T]

KEC = Kraft evaporator condensate

MATA = modified anaerobic toxicity assay

MIF = methanol insoluble (pulp) fiber

min = minute

MSF = methanol soluble (pulp) fiber

NRC = National Research Council of Canada

OLR = organic loading rate $[ML^{-3}T^{-1}]$

RA = resin acid(s)

RRI = reactor resistance to inhibition

SLR = specific loading rate

SRT = solids retention time [T]

SS = suspended solids

TSS = total suspended solids $[ML^{-3}]$

UASB = upflow anaerobic sludge blanket reactor

VSS = volatile suspended solids $[ML^{-3}]$

ABSTRACT

Chemithermomechanical pulp wastewaters are being treated anaerobically in spite of the presence of inhibitory compounds. These compounds are neither well identified nor are they placed in an order of priority in terms of inhibition to anaerobic bacteria. In this study anaerobic toxicity assays were used to quantify the relative toxicity of resin acids to the total toxicity in bleached chemithermomechanical wastewater. Resin acids are partitioned between the soluble wastewater and the fiber phases. Therefore fiber toxicity was also compared to raw bleached chemithermomechanical wastewater toxicity. Resin acids were found to be toxic to anaerobic bacteria but were not found to be responsible for all the toxicity in the wastewater. Toxicity associated with the fiber is soluble in methanol. The bulk of the fiber which is not methanol soluble exhibits no toxicity.

Preliminary batch studies on resin acid anaerobic degradation were inconclusive but do indicate the possibility of isomerization of other resin acids to the energetically stable dehydroabietic form.

Transfer of information obtained from batch modified anaerobic toxicity assays to continuous anaerobic treatment systems was also evaluated. Modified anaerobic toxicity assay data was compared to continuous toxicity assays using an upflow anaerobic sludge bed reactor. The continuous reactor was relatively unaffected by acute or chronic exposure to resin acids at concentrations that invoked inhibition in batch tests. Inferences from these tests indicated that batch modified anaerobic toxicity assay results may be unreliable when applied to continuous systems.

CHAPTER 1

1.0 INTRODUCTION

1.1 Wastewater Treatment

1.1.1 Traditional Pulp and Paper Wastewater Treatment

Wastewaters from the pulp and paper industries have been traditionally treated by sedimentation or oxidation. Oxidative processes include combustion and various aerobic biological processes (Webbs, 1984). Until recently, the mainstay of the biological processes used in treating pulp and paper wastewater was the aerated lagoon (Norrman, 1982). Aerated lagoons require a large area of land, input of power for aeration, addition of nutrients, produce copious quantities of sludge and are known for high suspended solids in the effluent and consequent lower effluent quality. Anaerobic processes were ignored because of high loading requirements and perceived difficulties in operation, in spite of the advantages of lower power requirement, production of methane and decreased nutrient requirements. A resurgence in research in anaerobic processes coupled with the following alterations in pulp and paper production has led to full scale anaerobic treatment of pulp and paper effluents.

1. Recycling of mill effluents with the consequent increase in concentration of chemical oxygen demand (COD).

2. Subsequent increase in effluent temperature due to recycling (Cocci et al., 1982).

1.1.2 High Rate Anaerobic Treatment

With the development of high rate anaerobic reactor systems, anaerobic treatment of pulp and paper waste has moved from the laboratory and pilot project stages to fully operational plants (Webb, 1984). Among these full scale operating systems, one finds the upflow anaerobic sludge blanket (UASB) reactor, which was developed by Lettinga (1982). Presently there are three full scale operational UASB systems in Canadian pulp mills.

1.1.3 Treatment Efficiency

Treatment efficiency is usually measured as the reduction of chemical oxygen demand (COD) or biochemical oxygen demand (BOD). Another measure of efficiency is the amount of suspended solids (SS) that is exiting the treatment system. To meet regulated effluent standards it is becoming recognized that for many wastewaters a combination of anaerobic and aerobic processes may be needed to meet effluent requirements (Vochten et al., 1988). Typically high strength wastewaters can be first treated anaerobically and then be polished with an aerobic treatment.

1.2 Toxicity in Pulp and Paper Industry Wastewaters

Treatment of any kind for wastewater from the pulp and paper industry must deal with the presence of an undetermined number of toxic compounds (to both aerobes and anaerobes), which may vary from production day to production day, and which may affect treatment efficiency. The treatment plant operation requires that any toxicant detrimental to the biological processes within the plant be removed or masked so that efficient treatment be obtained. Ministry of Environment guidelines require that effluent quality be met and criteria for effluent quality are being toughened yearly.

1.2.1 CTMP Wastewater

One wastewater of interest is from the chemithermomechanical pulp (CTMP) production system. In the past decade CTMP mills have become popular due to high yield of pulp per tonne of tree harvested. However the CTMP process also produces a wastewater so high in COD as to make the cost of aerobic treatment prohibitive. Unlike wastewater from the Kraft process, that from the CTMP mills has no chemicals present to make evaporation and recovery of the chemicals and burning of the condensate cost effective. Therefore anaerobic treatment of the effluent may be the only viable alternative.

1.2.2 Anaerobic Treatment of CTMP Wastewater

Anaerobic treatment of CTMP effluent has been shown to be possible in spite of the presence of compounds that inhibit anaerobic bacteria (Schnieder *et al*., 1987; Welander, 1988; Richardson, 1989; Hall and Cornacchio, 1988). To date there has been little work done to characterize the compounds present in CTMP wastewater which are toxic to anaerobes. Resin acids are one such group of compounds that are suspected of causing inhibition but there is a paucity of literature on the proportion of anaerobic toxicity present in CTMP wastewater caused by resin acids.

1.3 Anaerobic Toxicity Assay

A standard serum bottle test is available to help identify whether or not a substance is toxic to anaerobic bacteria. The anaerobic toxicity assay (ATA) developed by Owen et al., (1979) and later modified (MATA) by Cornacchio et al., (1988) is the standard method. This assay is based on having many serum bottles with the same biomass but with different concentrations of the toxicant(s). The change in rate of methane production and/or cumulative methane production are monitored and compared to that of a control. The technique primarily tests inhibition of methanogenic bacteria but will also indicate inhibition of other types of bacteria if substrates are wisely chosen (Wu et al., 1989).

1.4 Purpose of Study

The objectives of the present study are: to investigate the role of resin acids in the anaerobic toxicity of CTMP wastewater in 1) batch assays and 2) a continuous system (UASB); to investigate the nature of fiber toxicity with respect to total CTMP toxicity; and to do preliminary work to study the fate of resin acids in anaerobic treatment.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Basic Anaerobic Digestion

Anaerobic processes by definition occur in the absence of oxygen. Natural habitats of anaerobic bacteria include marshes, hot springs, piles of organic matter and deep oceans. Many species of bacteria are involved in the breakdown of complex organics to methane and carbon dioxide. The steps involved are summarized in Fig. 2.1.

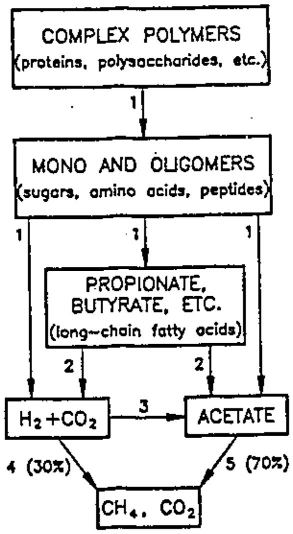


Figure 2.1-- Microbial groups involved in the flow of carbon from polymeric materials to methane in an anaerobic bioreactor. Group 1, fermentative bacteria; group 2, hydrogen-producing acetogenic bacteria; group 3, hydrogen-consuming bacteria; group 4, CO2-reducing methanogens; group 5, acetoclastic methanogens (from Zinder, 1984).

The end product of anaerobic digestion is methane. Methane, being insoluble in aqueous environments, is lost to the atmosphere. Thus unlike aerobes, anaerobes have an alternate source to chemically bind energy, in lieu of producing new cells. Methane production is the main reason why anaerobic bacteria produce less biomass.

2.2 Biological Treatment of CTMP Wastewater

Biological treatment of CTMP wastewater has been characterized extensively by Richardson (1989). The following is a brief summary. The CTMP process produces a combined waste stream high in COD (COD levels in CTMP vary from 6 000 - 9 000 mg COD L^{-1}) and BOD with an elevated COD:BOD ratio. Relative to other pulping processes, high concentrations of extractives (which include terpenes, resin acids and fatty acids) are released in the production of CTMP. Cursory examination of the bacterial toxicity of the extractives, which will be reported later, concluded that extractives are toxic to bacteria. Extractives are therefore suspected to cause major problems in biological treatment.

2.2.1 Anaerobic Treatment

In spite of the presence of toxic compounds, CTMP effluent has been successfully treated in anaerobic bench scale reactors (Wilson et al., 1988; Richardson, 1989), anaerobic pilot treatment plants, (Schneider, 1988; Andersson et al., 1985; Welander and Andersson, 1985) and finally in full scale operations (Welander, 1988). Two CTMP plants in Canada have full scale UASB reactors in operation, one in Quesnel, B.C., and another in Bathurst, N.B..

2.2.2 Aerobic Treatment

CTMP wastewater has been treated aerobically, with varying degrees of success, using a carrousel process (bench scale) by Reeser and Johnson (1988) and by fully operational aerated lagoons (McAllen, 1988).

2.2.3 Physical Chemical Treatment

Both Welander (1985) and Bennett et al., (1988) used detoxification methods to remove toxicity of CTMP wastewater prior to biological treatment. Welander employed ferrous and aluminum chlorides to precipitate the toxic compounds while Bennett et al., used lime for the same purpose. It is interesting to note that other researchers have treated up to 100% CTMP without detoxifying the wastewater (Richardson, 1989; Kudo et al., 1989).

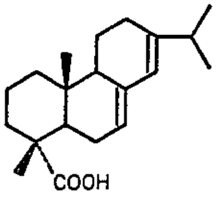
2.2.4 Anaerobic Process Problems

Problems associated with shutdown and startup of bench scale UASB reactors being fed CTMP wastewater have occurred (Richardson 1989; Kudo et al., 1989). There has also been one reported case of a full scale process failure after shutdown (Gorur, 1989). There is speculation that accumulation of toxins (McFarlane and

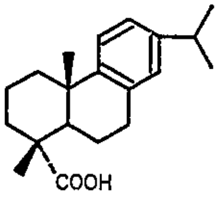
Clark, 1988) and the accumulation of fiber (Richardson, 1989) in the sludge bed, in conjunction with the absence of an easily degradable soluble carbon source may lead to increased inhibition of the biomass activity. Fiber and/or toxin accumulation are suspected to cause loss of granulation as well as loss of activity. Experiments are underway to investigate this hypothesis (Kennedy, 1989).

2.3 Toxins in Pulp and Paper Wastewaters

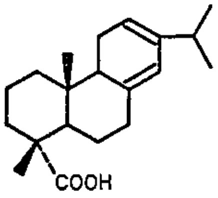
There are many chemicals naturally found in wood that may have toxic effects on anaerobic bacterial life (Kringstad and Lindstrom, 1984). Among the compounds highly suspect are those compounds traditionally labeled "extractives" which include tall oil, and terpenes (Rogers et al., 1975). The total content of extractives varies from species to species of tree, and can even vary within the same stem (Kringstad and Lindstrom, 1984). The resin acids shown in Fig. 2.2 are present in tall oil. It is thought that the purpose of resin acids in the tree is that of protecting the tree from biological attack. The biologically active component of the tall oil has been identified to be in the resin acid fraction and has long been used in traditional folk medicine (Svikle et al., 1978). It would not be surprising, therefore, to find that resin acids are toxic to tree pathogens and conceivably to anaerobic bacteria.



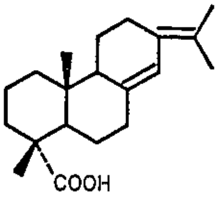
abietic acid



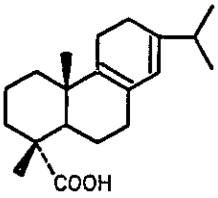
dehydroabietic acid



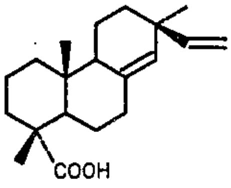
levopimaric acid



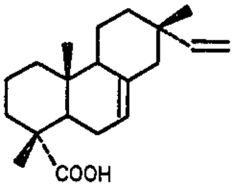
neoabietic acid



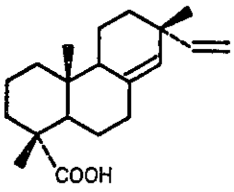
palustric acid



pimaric acid



isopimaric acid



sandaracopimaric acid

Figure 2.2--- Resin acids commonly found in Pulp and Paper wastewaters (from Swan, 1973).

2.3.1 Resin Acids

It is well documented that resin acids are highly toxic to fish at concentrations of less that 1 mgL^{-1} (Leach and Thakore, 1976; Walden and Howard, 1981). Therefore the presence of concentrated resin acids in waste effluents which flow into streams and lakes does have serious consequences to aquatic life.

Resin acids are found in many forest industry wastewaters (Table 2.1) and are found in particularly high concentrations in CTMP waste. The high concentrations are a result of the process used to render the pulp soluble as well as a function of the species of tree (furnish) used for the pulp. Oven dried percentage weights of resin acids with respect to the total wood vary in softwoods from 0.02 - 7% of the wood. Canadian hardwoods do not contain resin acids. Concentrations in pine species range from 2.4-7%, whereas concentrations in fir and spruce range from 0.2-0.48% and 0.12-0.27%, respectively (Swan, 1973).

Resin acid concentration in pulp and paper wastewater is dependant on the dilution of the wastewater within the plant and is site specific. General ranges of concentrations of resin acids are outlined in Table 2.1.

Table 2.1 Recorded Resin Acid Concentrations in Pulp and Paper Industry Wastewaters. All concentrations as mgL^{-1}.

No. of SAMPLES	RANGE	TMP	CTMP	KEC	SCREW PRESS	SOURCE
2	25-28	26				Bennett et al.
20	14-62	28±9.4				Leach & Thakore
4	12-23	16±4.1				Leach & Thakore
4	101-177	143±31				McCarthy
1		52				Richardson & Bloom (1982)
1		2				Richardson & Bloom (1983)
1			121			Bennett et al.
22	36-701		223±169			McCarthy
1				0.6		Qiu et al.
1					1360	McFarlane,Clark

2.4 Resin Acid Removal by Biological or Chemical/Physical Treatment

2.4.1 Aerobic Removal

Several studies have shown that resin acids can be removed from pulp and paper wastewater by biological and/or physical treatment. Rogers et al., (1988) demonstrated that resin acids could be 99% removed from Kraft evaporator condensate (KEC) by aeration for 99 hours. Richardson and Bloom (1982) demonstrated 99% removal of resin acids after 5 days in an activated sludge pilot plant treating thermomechanical pulp (TMP) effluent. The same authors published similar results in 1983.

2.4.2 Physical Chemical Removal

Andersson and Welander (1985) detoxified abietic acid with a mixture of ferrous and calcium chloride.

Welander (1988) detoxified CTMP using 1 mM of each of $AlCl_3$, $CaCl_2$, and $FeCl_2$. Studies by de Vegt (1988) on CTMP wastewater showed that there was little effect on anaerobic microorganism toxicity by addition of the same metal ions. Bennett et al., (1988) used lime to pre-treat CTMP wastewater to reduce resin acid concentration and toxicity. Resin acids were reduced on average from 26 mgL^{-1} to 10 mgL^{-1} in TMP and from 121 mgL^{-1} to 12 mgL^{-1} in CTMP by lime addition and sedimentation. The main drawback to pretreatment of a wastewater is the cost involved, not only in the materials, but also in these specific cases for the requirement of another clarifier.

2.4.3 Combination of Anaerobic and Aerobic

Combined anaerobic and aerobic treatments have also demonstrated a substantial decrease in effluent fish toxicity (Jurgensen et al., 1985; Wilson et al., 1985). Qiu et al., (1988) reported that anaerobic treatment of KEC did not reduce resin acid concentration. Influent to effluent concentrations for a UASB anaerobic reactor changed from 595 μgL^{-1} to 1 111 μgL^{-1}, suggesting leaching of resin acids from the biomass, while aerobic polishing in an activated sludge basin reduced the resin acid concentration to detection limits.

2.5 Degradation of Resin Acids in Anaerobic Treatment

McFarlane and Clark (1988) reported that resin acids are degraded in an UASB reactor treating CTMP wastewater. All resin acids were reported to be quantifiably degraded with the exception of dehydroabietic acid. It was also concluded by the same authors that anaerobic bacteria could acclimatize to high levels of dehydroabietic acid. Qiu et al., (1988) reported similar findings to those of McFarlane and Clark with respect to degradation of resin acids in an UASB reactor treating KEC. Resin acid concentrations in KEC are normally much lower than those in CTMP. The concentration of four of the seven nonchlorinated resin acids decreased significantly when influent and effluent resin acids were monitored. On the other hand, Qui et al., (1988) reported an eight-fold increase in dehydroabietic acid concentration from influent to effluent, which suggested possible isomerization of resin acids to the more energetically stable dehydroabietic acid.

2.6 Adsorption of Resin Acids unto the Biomass and Possible Degradation

Adsorption of resin acids onto the biomass was demonstrated by McFarlane and Clark (1988) where 1 750 mgL^{-1} of combined resin acids were found in the sludge of an UASB reactor treating a screw press wastewater which had a resin acid concentration of 1 360 mgL^{-1}. [Adsorption and accumulation of resin acids on biomass was also reported by Kennedy (1989)]. Gas chromatographs of the influent and effluent displayed a disappearance or diminishment of most of the resin acid peaks. It should be noted that the effluent sample monitored was a soluble sample and that the influent was a total sample.

By demonstrating that a significant concentration of resin acids could be adsorbed on anaerobic biomass, McFarlane and Clark have shown that consideration of only influent and effluent concentrations of resin acids for signs of degradation is an oversimplification. However, McFarlane and Clark did not consider the resin acid concentration in the non-soluble portion of the effluent, and thus the conclusion of degradation is premature. The high concentration of dehydroabietic acid in the biomass might not be only a result of accumulation and lack of degradation, but could possibly be a result of isomerization. Until the total effluent is monitored and a mass balance is done on the resin acids, a conclusion of degradation is weak. Possible breakdown products of resin acids cited in the McFarlane and Clark paper are also evidence of degradation, although as yet such evidence is not convincing.

The publication by McFarlane and Clark, though not complete, is the most comprehensive report on the fate of resin acids in biological treatment. The papers cited above which claim removal of resin acids from wastewater did not consider accumulation in the biomass or possible isomerization. The full story is yet to be disclosed.

2.7 Toxicity of Resin Acids to Anaerobic Bacteria

There is little known about the toxicity of resin acids to anaerobic treatment plant micro-organisms. Andersson and Welander (1985) reported an abietic acid/oleic acid mixture to be inhibitory to anaerobic bacteria at 1 178 mgL^{-1}. However Koster and Cramer (1987) reported inhibition to fifty percent of control activity by oleic acid at 1 235 mgL^{-1} which left some doubt as to the effect of abietic acid. Field et al., (1988) found 50% of control activity for abietic acid at 114 mgL^{-1}. The assay done by Field et al., (1988) was based on rendering abietic acid soluble in a basic solution and concentration was reported on a mg COD L^{-1} and not as true concentration of abietic acid. Depending on the purity of the abietic acid used, which was not reported in the paper, the basic solution may not have contained only abietic acid. Welander and Andersson (1985) and Welander (1988) reported abietic acid and all resin acids respectively to be inhibitory, but no experimental evidence was provided with the conclusions.

2.8 Effects of Toxicants and Modeling

Toxicity to methanogenic bacteria, even to the extreme, where a period of zero gas production occurs, does not necessarily mean dieoff of the methanogens

(Speece, 1985; Parkin et al., 1983). The effects of most toxicants are reversible at low to medium doses (McCarty, 1964). Acclimatization to toxicants and/or biodegradation of toxicants may play a role in reversibility in batch tests. Acclimatization, according to Speece, is observed but not understood. Speece also remarked that toxicity of compounds is related to their molecular structures and is somewhat predictable. For example, the longer the aliphatic chain of carbons, the greater the toxicity. The more chloro-substitutions on an aromatic ring the more toxicity (also see Wu et al., 1989).

Modeling inhibition of bacterial activity is usually generalized from the enzyme inhibition model. However, a bacterium has many enzymes and it is conceivable that a toxin could affect more than one enzyme. For example, enzyme A could be effected competitively whereas enzyme B, noncompetitively. The problem is exacerbated in biological treatment. Since bacterial populations in biological wastewater treatments are heterogeneous, any one toxic substance could conceivably effect the activity of separate enzymes of different bacteria in varying ways.

2.9 Inhibition Mechanisms

Three main enzyme inhibition mechanisms are outlined by Lehninger (1975): competitive, uncompetitive and noncompetitive. A fourth inhibition mechanism would be a combination of competitive and either noncompetitive or uncompetitive inhibition and is called mixed inhibition (Volskay and Grady, 1988). Competitive inhibition occurs when the inhibitor or toxin forms a reversible complex with the active site of the enzyme. Uncompetitive signifies the formation of an enzyme-substrate-inhibitor complex. Noncompetitive inhibition follows when the inhibitor combines with either the free enzyme (though not on the active site) or the enzyme-substrate complex.

Competitive inhibition can be reversed by increasing the substrate concentration since the substrate and the inhibitor are competing for the same active site. Neither uncompetitive nor noncompetitive inhibition are affected by substrate concentration. Mixed inhibition could conceivably be partly reversed by substrate addition.

2.10 Inhibition Models

2.10.1 Introduction

Conceptually understanding what is happening within the biological system upon introduction of a toxicant is aided by models. Modeling toxicity in anaerobic digestion has been focused on the methanogenic group of bacteria for the following reasons. In anaerobic treatment, acetoclastic methanogenic toxicity is considered the most important since methanogens are thought to be very susceptible to toxic shock. It is generally accepted that 70% of methane production eventually comes from acetate conversion by methanogens (Jeris and McCarty, 1965; Kasper and Whurman, 1978; Smith and Mah, 1966; and McCarty, 1964).

Parkin and Speece (1982) suggested three models for mathematically describing toxicity to methanogens. The first is empirical and is based on an equation similar to the Streeter-Phelps equation used in the dissolved oxygen sag model. The other two models are based on Monod kinetics.

2.10.2 Model 1--Recovery Pattern Model

The "Recovery Pattern Model" is based on the equation

$$G = Ae^{-k1t} + Be^{k2t} \tag{2.1}$$

where,

G is the methane production mLd^{1}

A,B are empirical constants

t is the time after addition of toxicant

k_1,k_2 are rate constants (d^{-1})

The general form of the equation is

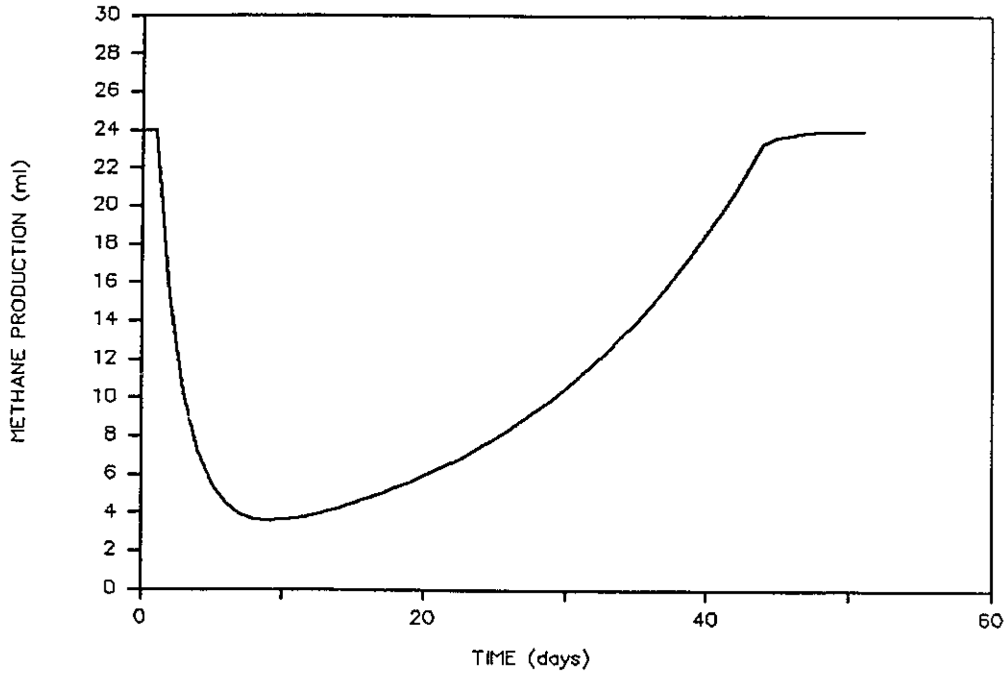


Figure 2.3 Response curve of methane production after toxicant addition (Parkin and Speece, 1982)

The rate constant k_1 is related to the slope of the downward curve in Fig. 2.1.. The constant k_2 is the recovery constant and is related to the slope of the upswing curve. A, B, k_1 and k_2 are dependant on toxicant type and toxicant concentration. The model as described does not consider specific loading of the toxicant. However, the model does allow for prediction of both zero gas production and a threshold toxicant concentration both of which are experimentally observed phenomena.

2.10.3 Monod Kinetic Models

The two remaining models proposed by Parkin and Speece are based on Monod kinetics which is described by the equation

$$\frac{dS}{dt} = - \frac{k\ S\ Xa}{Ks + S} = r_{su} \qquad (2.2)$$

where,

S	is the concentration of the substrate (mgL^{-1})
t	is time (d^{-1})
k	is the specific substrate utilization rate expressed as mg substrate utilized per day per mg of methane bacteria.
Xa	is the concentration of active methane bacteria (mgL^{-1}) which is usually measured as VSS.
Ks	is the Monod half-velocity constant (mgL^{-1})
r_{su}	is the rate of substrate utilization ($mgL^{-1}d^{-1}$)

Operational Parameters of Biological Reactors

To better understand the kinetic models it is best to consider the operation parameters of a biological reactor. The rate of substrate utilization is related to the rate of growth of biomass by

$$r_G = -Yr_{su} - bXa \tag{2.3}$$

where,

r_G is the rate of growth of biomass (mgd^{-1})
Y is the yield factor specific to substrate
b is the die-off constant (d^{-1})

Considering a steady state complete mix reactor with no biomass entering the system we can write the following mass balance

$$-QX - (-Yr_{su} - bX)V = 0 \tag{2.4}$$

where,

Q is the flow into the reactor (m^3d^{-1})
X is the concentration of biomass within the reactor (mgL^{-1})
V is the volume of the reactor (m^3)

Equation 2.4 can be simplified recognizing that V/Q is equivalent to the hydraulic retention time (t_d).

$$\frac{1}{t_d} = - Yr_{su} - b \tag{2.5}$$

The solids retention time (SRT) of the system is equal to the mass in the system divided by the mass exiting the system or VX/QX. Thus,

$$\frac{1}{SRT} = - Yr_{su} - b = - Y \left[\frac{k\ S\ Xa}{Ks + S}\right] - b \qquad (2.6)$$

Examining Eq. 2.6 it can be concluded that if a decrease in rate of substrate utilization occurs due to a decrease in k, the specific utilization rate, or an increase in Ks, the substrate concentration at half the maximum velocity, then there will be a consequent increase in the required SRT. Or conversely, to safeguard against changes in the rate of substrate utilization a safety factor in the design SRT should be applied to make it sufficiently large. A sludge age with a substantial safety factor is required for anaerobic reactors receiving varying toxicant loadings.

2.10.3.1 Monod Kinetics -- Model 2

The second model proposed by Parkin and Speece uses the Monod equation as proposed. The Monod equation, although empirical, is similar to the theoretical enzyme kinetic equation proposed by Michaelis-Menton (Aida, 1973).

Toxicant loading affects k and Ks in many ways, and depends upon the type of inhibition. The effects are perhaps best described in a table.

Table 2.2--Effects of different types of inhibitors on the kinetic parameters for biodegradation of non-inhibitory organic matter (Volskay and Grady, 1988)

Inhibitor type	Effect on k	Effect on Ks
Competitive	None	Increase
Noncompetitive	Decrease	None
Uncompetitive	Decrease	Decrease
Mixed	Decrease	Increase

Competitive inhibition is the special case in that it is the only type where the specific substrate utilization rate is not decreased. This follows from the fact that competitive inhibition is overcome by increasing the concentration of substrate and when S>>Ks then equation 2.2 becomes first order at the maximum specific utilization rate. In competitive inhibition the value of Ks is increased, thus increasing the concentration of substrate needed to obtain the maximum substrate utilization rate.

2.10.3.2 Monod Kinetics -- Model 3

The second model does not incorporate actual toxicant concentration or toxicant type, although calibrated values of k and Ks would be specific to concentration and type of inhibitor as well as being dependant on acclimatization. The third model attempts to relate changes to k and Ks that may appear in the presence of a toxicant to the concentration of the toxicant, I, and to a toxicant coefficient, Ki. The effects on k and Ks are summarized in Table 2.3. k* is the specific utilization rate in the presence of inhibitor. K*s is the half velocity constant in the presence of inhibitor.

Table 2.3--Quantification of inhibitory effects using simple reversible linear inhibition models (Volskay and Grady, 1988).

Inhibitor type	Effect on k	Effect on Ks
Competitive	k* = k	K*s = Ks(1 + (I/Ki))
Noncompetitive	k* = k/(1 + I/Ki)	K*s = Ks
Uncompetitive	k* = k/(1 + I/Ki)	K*s = Ks/(1 +(I/Ki))
Mixed	k* = k/(1 + I/Ki)	K*s = Ks(1 + (I/Ki))

Actual values of Ki are not available, and until the inhibitory process is better understood, the value of this third model is purely conceptual.

2.10.4 Lewandowski Model -- Reactor Resistance to Inhibition

Lewandowski (1985) demonstrated that a continuously fed complete mix reactor displayed a reactor resistance to inhibition (RRI) when exposed to toxicant loading. He concluded that the RRI is that maximum concentration of toxicant compound that causes no change in reactor treatment efficiency. In a theoretical paper, Lewandowski (1987) supported the observation of RRI using Monod kinetics and reactor operational parameters.

Lewandowski (1987) states that interaction of a toxic substance and a mixed bacterial culture is too complex to be analyzed in the same way as pure enzyme inhibitor reactions have come to be understood. Rather than try to relate toxic effects to enzyme substrate interactions, Lewandowski maintained that relating toxicity to reactor operating parameters would be more fruitful.

Reactor operational parameters investigated by Lewandowski were the biomass needed for complete conversion and the retention time needed for the same purpose. He stated that most reactors have a buffer zone of biomass and/or retention time so that if a toxicant is present that does affect activity, then there will be enough active mass and/or time allowed by the system to maintain treatment efficiency.

2.11 Summary of Inhibition and Modeling

Modelling methanogenic toxicity is at a state of infancy. The interaction of toxicant and biomass is complex and may not be transferable from basic enzyme kinetics. Process efficiency stability does not in itself indicate the absence of toxicity, but rather that the reactor has a certain tolerance. All the studies stressed the importance of solids retention time to overcome the effects of toxicity.

Some factors not considered by either Parkin and Speece or by Lewandowski in the development of a model are:

1. Excessive biomass washout in continuous systems which is not related to a de-

> crease in yield of biomass, but rather to a detrimental change in settling characteristics of the biomass. The washout could perhaps be modelled by a change in "b" or dieoff coefficient and may be related to toxicant type.
>
> 2. Accumulation of toxicant within the biomass due to adsorption with concomitant increase in toxicity and possibly acclimatization.

It is interesting to note that Lewandowski concurred with several authors that specific loading of an inhibitor is more important in metal ion toxicity than the actual concentration of the ions (Sujarittanonta and Sherrard, 1981; Randall and Buth, 1984; Directo and Multon, 1962; McDermott et al., 1963; Ayres et al., 1965; Lamb and Tollefson, 1973). By basing his model on operational parameters, Lewandowski incorporated these effects.

2.12 Mechanism of Toxicity of Compounds Related to Resin Acids

Resin acids are hydrophobic and are termed insoluble in water at neutral pH (Merck Index, 1983). At pH 11 in the presence of sodium ion, they become much more soluble as an undissociated salt in the aqueous solution. However, pH 11 does not exist in functioning biological treatment. The fact that resin acids appear in the soluble phase of pulp and paper wastewaters must be due to the surfactant nature of the waste. It is more likely that the resin acids in wastewater are in a colloidal state rather than being truly soluble. It is also possible that micelle formation occurs (for literature review of micelle formation see Valsaraj and Thibodeaux, 1988). Micelles are formed by ionic surfactants and have a hydrophobic inner layer and a somewhat hydrophylic outer layer.

It is widely agreed that for a toxin to have an effect in an aqueous environment it must be soluble. This cannot be the case with many hydrophobic compounds which cause toxicity. What must be considered in a biological treatment reactor is that there is more than just the aqueous phase. One must also consider the biomass phase which has in part a nature of hydrophobicity. Therefore colloidal-biomass or micelle-biomass phase transfer of toxins should also be considered in the solubility/toxicity problem.

Long chain fatty acids, which are water insoluble and form micelles, have been shown to cause inhibition to methanogencic bacteria (Koster and Cramer, 1987; Hanaki et al., 1981). Long chain fatty acids are similar to resin acids in that they are mainly hydrophobic with a single carboxylic acid functional group. A significant difference is that resin acids are ringed structures as opposed to long aliphatic chains.

Some general points from the paper by Koster and Cramer are as follows; Inhibition increased as carbon chain length increased from 10 to 18; Synergism was evident in that mixtures of fatty acids were much more toxic than equivalent concentrations of either; toxicity was related to concentration rather than to specific loading.

As mentioned earlier, it is widely accepted that metal ion toxicity is related to specific loading of the ions to the biomass (Section 2.11). It is interesting to postulate why metal ions would be toxic with respect to specific loading whereas fatty acids are toxic according to concentration. One possible factor may be that metal ion concentration within the cells is regulated by protein pumps and that excess ions or exotic ions interfere with the pump mechanism. The ion-pump interaction may be stoichiometric and therefore inhibition is related to specific loading. For fatty acids the mechanism may be purely obstructive, in that the fatty acids adsorb onto and absorb into the cell membrane, and subsequently inhibit general cell function. Adsorption onto the membrane would be diffusion related and therefore related to concentration.

Not enough is known to make a judgement on this unexplained phemonenum. Benjamin et al., (1983) tested the toxicity to methanogens of compounds present in sulfite evaporator condensate (SEC). Some of the compounds tested were hydrophobic in nature. He did not consider specific loading in his analysis.

Field and Lettinga (1987) tested the toxicity of hydrolyzable tannin. Again specific loading was not considered but the mechanism of the toxicity of the gallotannic acid was suspected to be that of "tanning" available proteins. One would expect such toxicity to be related to specific loading.

2.13 Conclusion

CTMP wastewater is being treated anaerobically in spite of the presence of inhibitory compounds. Some anaerobic processes for treatment of CTMP seem to require detoxification and others do not. Certain compounds are suspected to be causing the inhibition and among those are the resin acids. Resin acids are common to most pulp and paper industry wastewaters but are found in relatively high concentrations in CTMP. Defining the role of resin acids in anaerobic toxicity should be a priority in order to better design treatment works for CTMP wastewater.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Experimental Plan

The experimental plan had five (5) stages and each subsequent phase was dependant on the results from the previous phase. The phases were as follows

1) To investigate if the bleached chemi-thermomechanical pulp (BCTMP) wastewater was toxic to anaerobic bacteria.

2) To investigate the portion of the toxicity (if any) was caused by fiber present in the wastewater.

3) To ascertain whether resin acids, as a mixture or individually, were toxic to anaerobic bacteria in batch assays.

4) To do a preliminary investigation as to the fate of resin acids in batch reactors.

5) To investigate the possible toxicity of resin acids in a continuous system.

3.2 Modified Anaerobic Toxicity Assay

Most of the work of this thesis was done using the MATA. In summary, biomass, varying concentrations of suspected toxins and/or wastewater, a nutrient supplement and dilution water are mixed in a serum bottle which is then closed. A spike of acetate/propionate is then introduced to all bottles and gas production is measured by acidified water displacement and gas composition is done by gas chromatograph. Effects of the toxicants and/or wastewater on the activity of the biomass are shown by rate of production of methane with respect to a control. A more detailed description of this test is given in Appendix A.

The method was slightly modified from that of Cornacchio et al., (1986). The modifications were:

1) Methanol was used to dissolve the resin acid mixture and therefore the first spike for these samples also includes methanol degradation. The volume of methanol used was 200 μL which provided an additional food source of 6 000 mg COD L^{-1}.

2) After gas production ceased in all the serum bottles a second spike of acetate/propionate was given which provided a final serum bottle soluble COD of 3 300 mg L^{-1}.

3) Occasionally additional VOA spikes were given.

Each study was run in duplicate with controls. The same volume of methanol was added to the controls. Serum bottles (150 mL) were agitated throughout the experiment in a room controlled at 35°C. The final concentration of volatile suspended solids (VSS) in the serum bottles was 5 to 7 gL^{-1}.

Three sludge sources were used for the MATA. In Experiment #1 the activity test inoculum reactor (ATIR) sludge was compared to the Domtar sludge with respect to BCTMP toxicity. The ATIR actively received a sucrose/acetate feed and the sludge had a high acetoclastic activity. The Domtar sludge was stored at 4°C and was inactive. The third sludge used was in the final MATA of the study. Originally the sludge was obtained from Domtar, but had been actively receiving acetate feed in an UASB reactor for 77 days prior to the test.

3.3 Resin Acids

Pure resin acids were purchased from Helix Biotech. Scientific Ltd., Richmond, B.C.. Abietic acid (85%) was purchased from Sigma Biochemical. Resin acid analysis was carried out by Econotech Laboratory, New Westminster, British Columbia.

3.4 Bleached Chemithermomechanical Pulp Wastewater

Bleached chemithermomechanical pulp (BCTMP-N) wastewater, with a resin acid concentration of 36 mgL^{-1}, was provided by Quesnel River Pulp Company, Quesnel, British Columbia (The "N" in BCTMP-N indicates that the wastewater has normal fines/fiber content. Quesnel River Pulp Company also produces a higher fines/fiber wastewater when producing certain special grades of pulp). The mill has an alternating thermomechanical/BCTMP process with peroxide bleaching. Wastewater was stored upon receipt at -20°C until it was used.

3.5 BCTMP Preparation

One-half of the BCTMP-N waste was thawed and was then centrifuged at a relative centrifugal force (RCF) of 12 100 g for 30 minutes. Supernatant was stored and labelled BCTMP-NC (normal fines centrifuged) and was assumed to be fiber-free. (Not all of the material in the fines is fiber but fines and fiber will be used interchangeably in this thesis). The centrifugate, raw fiber, was collected and labelled fiber. Fiber in BCTMP-N was 0.6% (w/v) respectively to the raw fiber and wastewater. The second half of the wastewater was stored as BCTMP-N. All wastewater was stored at -20°C until it was used.

3.6 Fiber Preparation

A known weight of wet fiber was washed four times in HPLC grade methanol pouring off the supernatant each time. The pellet was saved and labelled methanol insoluble fiber (MIF). The combined supernatant, collected from washing the fiber, was again centrifuged at a RCF of 12 100 g for 30 minutes. The pellet was combined with the MIF. The supernatant was labelled methanol soluble fiber (MSF). Methanol was aspirated off and the dried MIF and MSF were weighed. Solutions were made up on a proportionality basis with respect to the weight of the original wet fiber.

For example, if the separation started with 2.4 g of wet fiber, which is the equivalent of 400 mL (0.4 L X 6 gL^{-1} = 2.4 g) BCTMP-N, then to make 100 mL of MIF or MSF solutions that have a fiber content equivalent to BCTMP-N one fourth of the MIF or MSF is required.

3.7 Toxicity of MSF VS MIF

Single and double strength solutions of MIF and MSF were tested. Single strength represents that amount of

MIF or MSF that would be present if there was 100% wastewater in the serum bottle. To test the toxic effect of raw fiber, a parallel study was run using raw fiber at single strength.

3.8 Toxicity of Resin Acids

A resin acid mixture was prepared that matched as closely as possible the mean resin acid composition reported in BCTMP-N wastewater from Quesnel River Pulp Company on five separate occasions. Seven resin acids were used and their percent composition in the final mixture is indicated in Table 3.1. A series of resin acid concentrations ranging from 20 to 700 mgL^{-1} were assayed using the ATA.

Table 3.1 Resin acid concentration in BCTMP-N Wastewater

Resin acid	Percentage
Pimaric	7
Sandarocopimaric	6
Isopimaric	12
Levopimaric	21
Dehydroabietic	22
Abietic	26
Neoabietic	6

	100

3.9 Upflow Anaerobic Sludge Blanket Reactor

A schematic of the UASB which was located in a temperature controlled room maintained at 35 ± 1°C, is in Fig. 3.1. One liter of effluent from a continuous reactor receiving an artificial sucrose-acetate soluble carbon source was placed in the reactor and both reactor and effluent were purged with an 80%/20% nitrogen/carbon dioxide gas mixture. The reactor was then inoculated with 1 200 grams of sludge previously acclimatized to recycled paper wastewater in Roermond, Holland. The sludge was stored in a tightly lidded container at 4°C until use. Finally, 80 biorings were added as well as enough deoxygenated tap water to bring the liquid volume to 3.45 L and the reactor was capped, purged with an 80%/20% nitrogen/carbon dioxide gas mixture and allowed to sit overnight with recycle. Synthetic acetate wastewater, as described by Kennedy (1985), was used as the carbon source at a concentration of 3 000 mg acetate per liter. The hydraulic retention time of the reactor was slowly decreased until an HRT of 1 day was obtained. Acids within the recycle line of the reactor were monitored daily and were maintained below 500 mgL^{-1}.

A variable speed Masterflex pump (Cole-Parmer model 7555-10, 1-600 rpm) provided recycle at a rate of 0.15 L $(min)^{-1}$. Feed was maintained at 4°C in a methanol cooled feed tank and was pumped to the reactor by a Harvard peristaltic pump (model 5006-033). The floating biorings were made of polyethylene plastic and were manufactured by Flexiring Koch Inc.. Each bioring measured 1.5 cm in diameter and length with a surface area to volume ratio of 235 m^2 m^{-3}. The biorings were at the gas-liquid interface and had a primary function of preventing solids from exiting from the overflow port. Gas exiting the reactor was volume measured by a wet tip gas meter and was scrubbed by a steel wool filter prior emission to a hooded vent. Effluent exited via the overflow port and was collected in a food grade plastic bucket.

Sampling ports were located in both the feed line and the recycle line. The ports were glass tees fitted with a screw cap and butyl rubber septum.

3.9.1 Inoculum

Anaerobic granular sludge (Domtar) from a corrugated paper mill using recycle paper was used as an inoculum. The sludge originated in Roermond, Holland and was the initial inoculum for the pilot plant at Quesnel River Pulp Company.

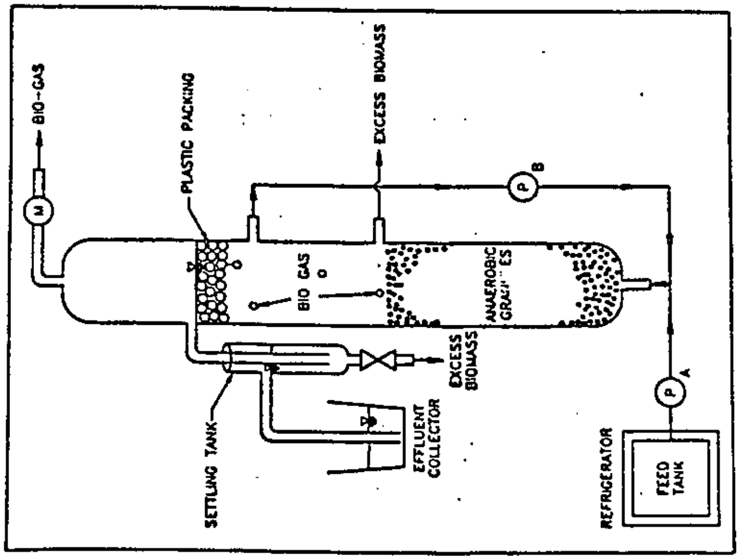


Figure 3.1 Schematic ot the UASB reactor P = pump; A = Feed pump, B = Recycle pump

3.9.2 Acetate Synthetic Wastewater

A buffered acetate synthetic wastewater (20 000 mg acetate per liter) was made according to the method of Kennedy (1985). Prior feeding to the UASB reactor the waste was diluted to 3 000 mg acetate per liter. Wastewater was stored at 4°C both prior and after dilution.

3.9.3 Abietic Acid Supplement to Acetate Synthetic Wastewater

Abietic acid was made soluble in the acetate feed according to the method of Field _et al_., (1988). Briefly, dry NaOH was added to the diluted acetate feed to adjust the pH to 11. Abietic acid (85%) was then added and the pH was maintained above pH 11 as the abietic acid dissolved. The pH was then readjusted to 5.4 with concentrated HCl and prior feeding it to the reactor. At pH 5.4 the feed became cloudy indicating that abietic acid had come out of solution. The cloudy solution of abietic acid did not clear overnight which is indicative of good dispersion of the abietic acid.

3.10 Analytical Methods

3.10.1 pH

A Radiometer/Copenhagen pH meter, model 26 with a glass conduction electrode, was used for all pH measurements. The meter was standardized prior to pH measurements and had a sensitivity of ± 0.05 pH units.

3.10.2 Alkalinity

Alkalinity, measured as $CaCO_3$, was done using a Radiometer/Copenhagen pH meter equipped with an automatic titrator. The endpoint of titration was at pH 3.75 and 0.1 N HCl was used as titrant.

3.10.3 Biogas Measurement

For the reactor study gas production was monitored with a wet tip gas meter (Wet Tip Gas Company) which was calibrated prior use. For the MATA gas was measured by displacement of acidified water in a graduated manometer.

3.10.4 Biogas Composition

The composition of the biogas was measured by gas chromatography using a Hewlett Packard model 5710 in conjunction with a Hewlett Packard integrator model 3386A. The column was a Porapack T 50/80 mesh and was maintained at 70^{o}C. Helium at a flow rate of 40 mL $(min)^{-1}$ was the carrier gas. The injection port was set at 100^{o}C. Percent biogas fractions were corrected to standard temperature and pressure.

3.10.5 Volatile Fatty Acids

Acetate, butyrate and propionate were measured by the method of Ackman (1972) using a Hewlett Packard GC model 5730A equipped with an automatic sampler model 7671A and integrator model 3380A. A Chromosorb 101 glass column and injection port were maintained at 180^{o}C and 200^{o}C, respectively. The carrier gas was helium at a flow rate of 15 mL $(\mathrm{min})^{-1}$. The helium was passed over formic acid prior entering the GC. All samples were prepared for analysis by centrifuging in a Fisher Micro-centrifuge model 235A, and then diluting the sample with an equal portion of internal standard. The internal standard was a solution of isobutyric acid at a concentration of 1 000 mgL^{-1}.

3.10.6 Methanol

Methanol exiting the UASB reactor was measured using the same method as for the VOA with the exceptions that the oven temperature was set at 150°C and isopropynol, at a concentration of 1000 mgL^{-1}, was used as an internal standard.

3.10.7 Chemical Oxygen Demand

COD was measured according to the method of Knechtel (1978) with the minor modification that the samples were heated at 150°C for 3 hours instead of 2 hours.

3.10.8 Solids

Total suspended solids (TSS), VSS and fixed suspended solids (FSS) were measured according to Standard Methods (APHA, 1985).

3.10.9 Specific Activity Test

Acetoclastic specific activity was measured as follows. Three 10 mL samples of sludge were diluted with a reduced phosphate buffer and was transferred anaerobically to serum bottles. A 0.2 mL volume a 100 gL^{-1}

substrate (acetate) solution was added to each of two bottles. An equal volume of reduced phosphate buffer was added to the third which would serve as the control. Bottles were then placed on a New Brunswick Scientific shaker model 4556 rotating at 40 rpm in a temperature controlled room kept at 35°C. Samples were withdrawn periodically and the acetate concentration was measured by GC. The period of withdrawal depended on the activity of the biomass. The rate of substrate removal was related to the VSS present and the specific substrate activity was calculated.

3.10.10 Sludge Settleability Test

Sludge settleability was done according to the method of Andras <u>et al</u>., (1989).

A 10 mL sample of sludge is placed in a 20 cm long and 2.50 cm I.D. glass column equipped with an outflow port near the top and is subjected to ever increasing upflow velocities for set time intervals. At each of 10 velocities the mass-fraction of biomass exiting at the overflow port is collected on prepared and preweighed filter papers, photographed, and the dry weight of each fraction is determined. A profile of biomass exiting the column with respect to upflow velocity can be constructed which gives a characteristic exit curve for the state of the sludge.

CHAPTER 4

4.0 RESULTS

4.1 Introduction

BCTMP wastewater was used in many of the experiments that will be presented in this chapter. Data on the wastewater and dilutions used in the experiments are summarized in Tables 4.1 and 4.2.

TABLE 4.1 -- Average BCTMP wastewater characteristics

	SS (gL^{-1})	VSS (gL^{-1})	CODt (mgL^{-1})	CODs (mgL^{-1})
BCTMP-N	1.63	1.36	7300	5400

Fiber present in BCTMP-N was 6.0 gL^{-1} wet weight and was 1.63 gL^{-1} measured as SS. BCTMP-NC had a COD of 5 400 mgL^{-1} by definition. Dilution of BCTMP-N and BCTMP-NC produced the concentrations summarized in Table 4.2.

Table 4.2 -- COD of diluted BCTMP wastewater

Wastewater Concentration (%)	CODt (mgL^{-1})	CODs (mgL^{-1})
20	1460	1080
40	2920	2160
60	4380	3240
80	5840	4320

4.1.1 Sludges Used in Experiments

Experiments #1 and #2 use both the ATIR sludge and the Domtar sludge. In all subsequent experiments only the Domtar sludge was used as the inoculum.

4.2 Experiment #1--Granular Sludge Selection

MATA were done using two types of granular sludge and BCTMP wastewater. Both BCTMP-N and BCTMP-NC wastewaters were tested. After gas production ceased in all bottles a second acetate/propionate spike was given. Both spikes 1 and 2 results are shown graphically (to be specified later) as cumulative methane production versus time.

4.2.1 Results for ATIR Sludge

For the ATIR sludge, which was continuously fed sucrose/acetate, four wastewater concentrations were assayed: 20%; 40%; 60%; and 80%. Spike 1 results for BCTMP-N and BCTMP-NC are shown in Figs. 4.1 and 4.2, respectively.

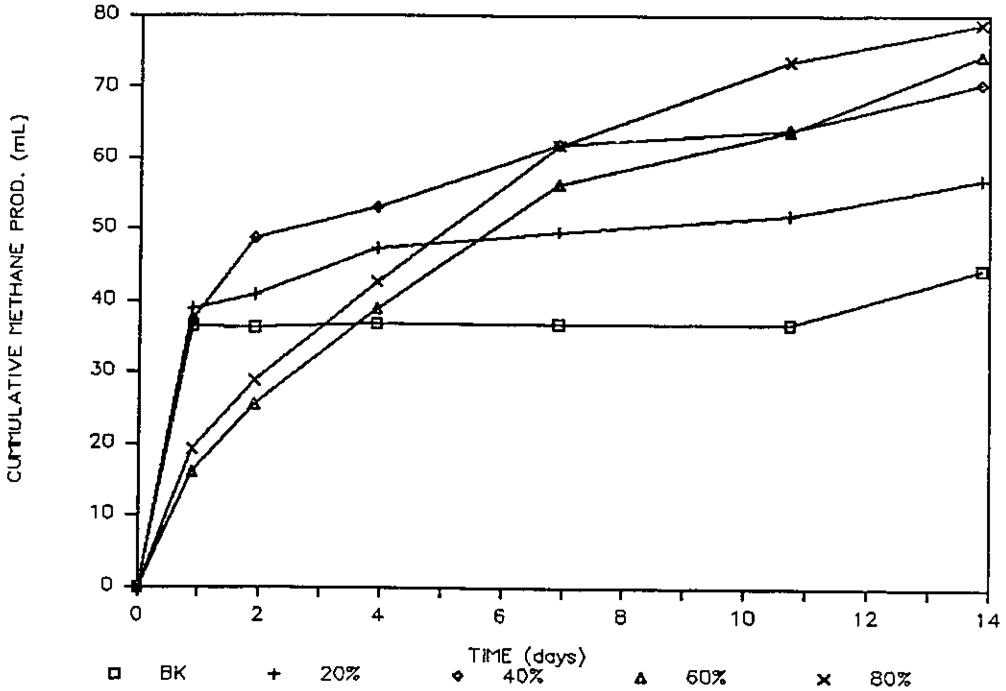


Figure 4.1 BCTMP-N wastewater; ATIR sludge (spike 1)

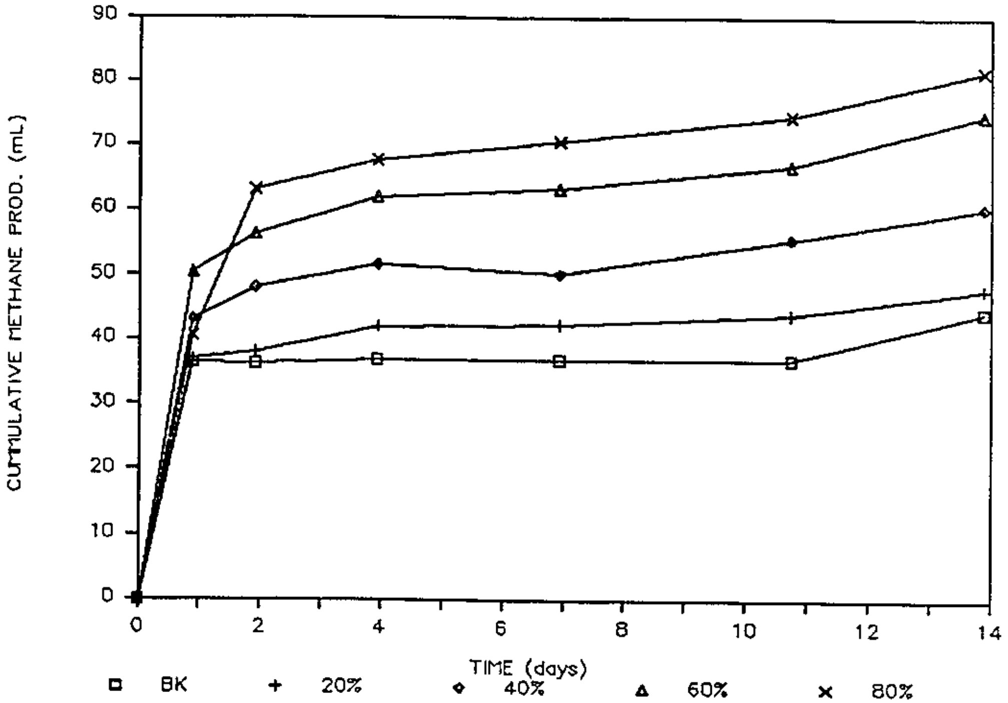


Figure 4.2 BCTMP-NC wastewater; ATIR sludge (spike 1)

Spike 2 initial gas production results for Experiment #1 are shown in Figs. 4.3 and 4.4.

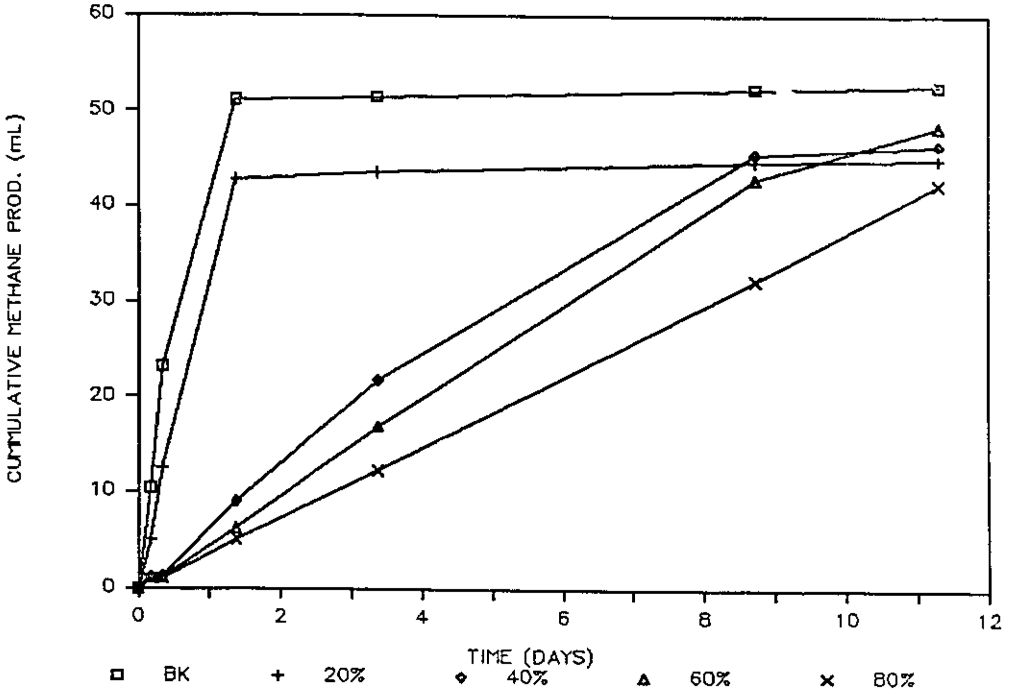


Figure 4.3 BCTMP-N wastewater; ATIR sludge (spike 2)

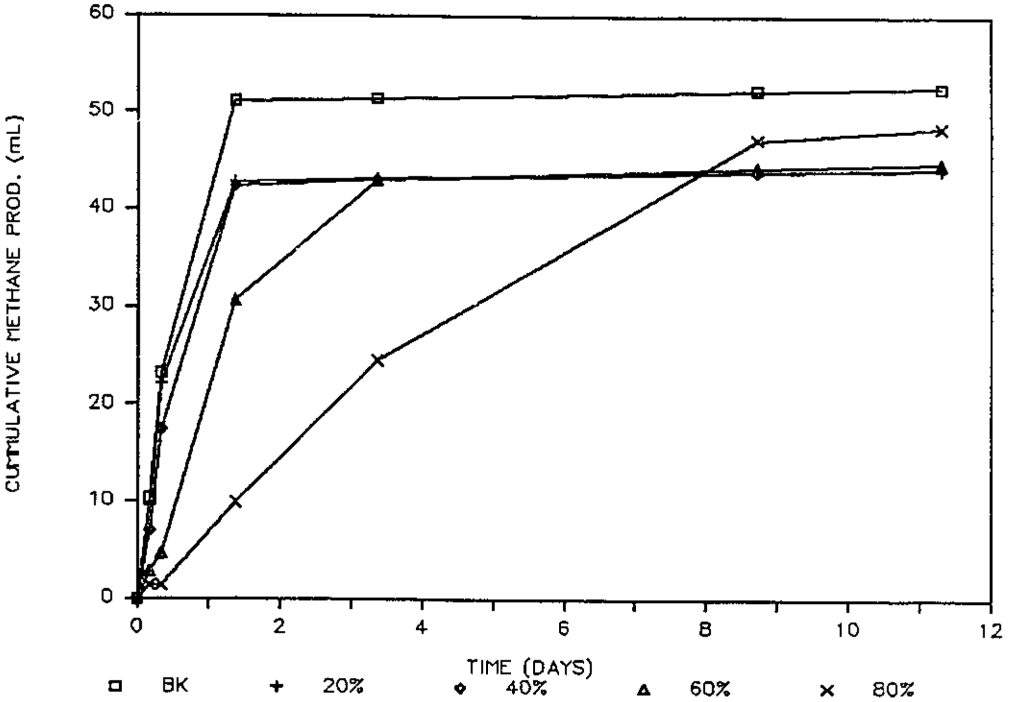


Figure 4.4 BCTMP-NC wastewater; ATIR sludge (spike 2)

4.2.2 Results for the Domtar Sludge

Wastewater concentrations tested on the Domtar sludge were 20, 40 and 60 percent. Spike 1 results are shown in Figs. 4.5 and 4.6 and results for spike 2 are shown in Figs. 4.7 and 4.8.

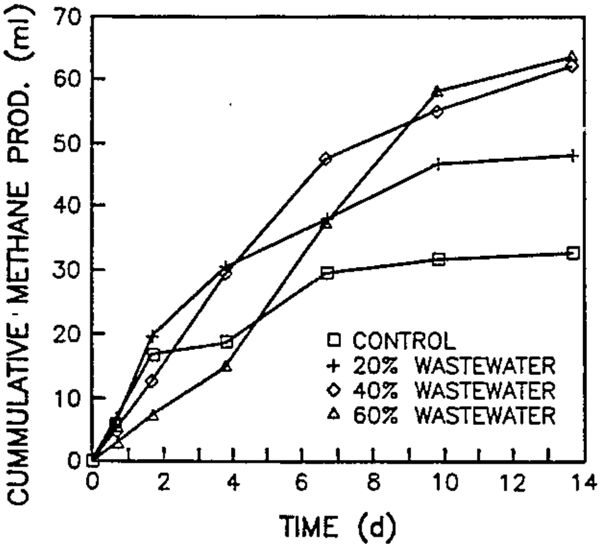


Figure 4.5 BCTMP-N wastewater; Domtar sludge (spike 1)

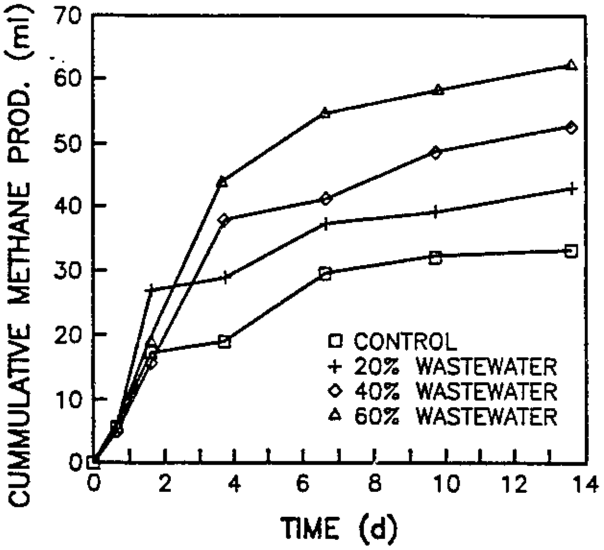


Figure 4.6 BCTMP-NC wastewater; Domtar sludge (spike 1)

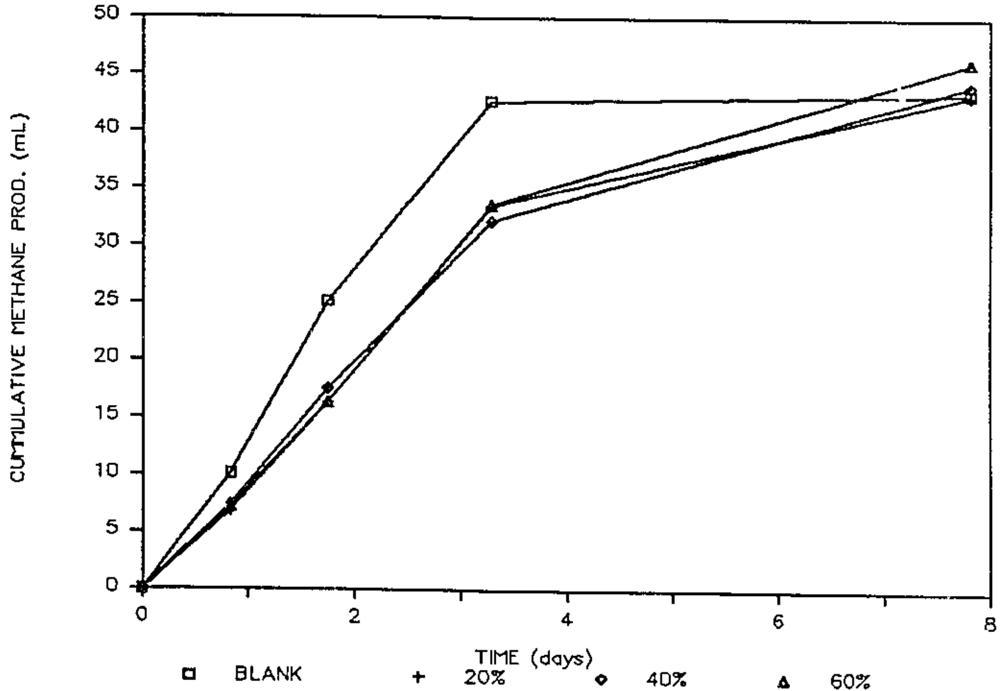


Figure 4.7 BCTMP-N wastewater; Domtar sludge (spike 2)

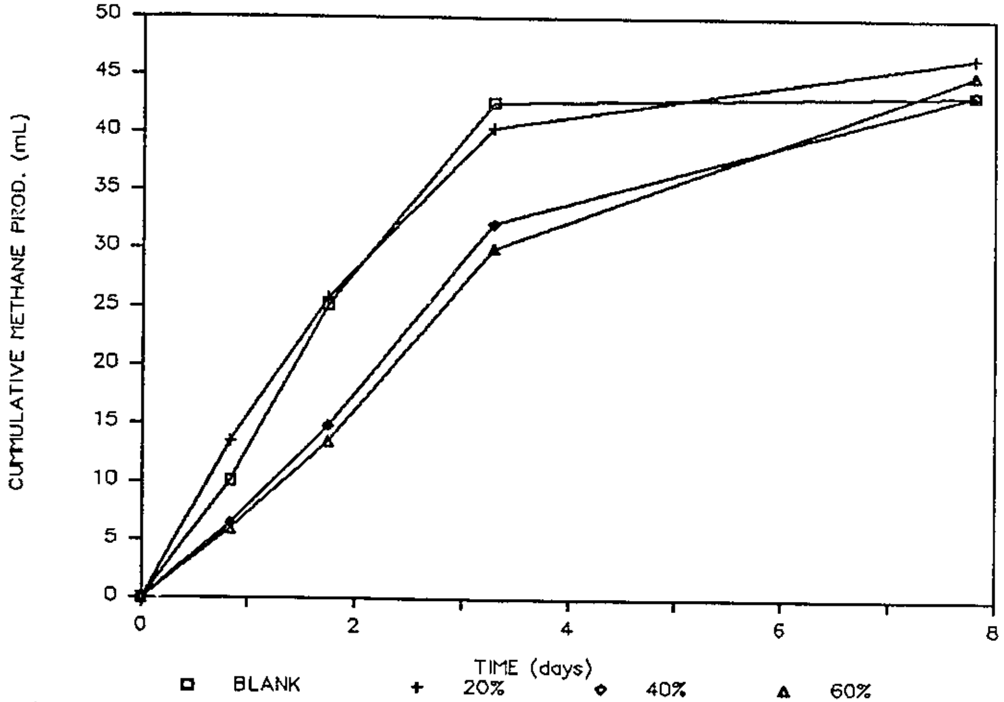


Figure 4.8 BCTMP-NC wastewater; Domtar sludge (spike 2)

4.3 Experiment #2--Preliminary Studies on Resin Acid Toxicity

4.3.1 Abietic Acid Toxicity

This experiment was conducted in two parts. The first study involved inoculating selected pairs of bottles from Experiment #1 with a third spike of acetate/propionate along with 500 mgL^{-1} abietic acid. The purpose was to give the serum bottles a moderately high dose of resin acid to test toxicity and provide information on the concentrations of abietic acid that should be investigated later.

In the second part of the experiment a series of abietic acid concentrations were tested for inhibitory effects on the Domtar sludge.

4.3.1.1 ATIR Sludge and Abietic Acid

For the ATIR sludge, the 20% wastewater serum bottles were selected from Experiment #1 and assayed along with the controls from Experiment #1. Duplicate pairs were split with one bottle receiving 500 mgL^{-1} of 85% pure abietic acid dissolved in 250 μL methanol and the other bottle was a control receiving only methanol. Both bottles received acetate/propionate along with the methanol. The result of adding 500 mgL^{-1} of abietic acid (85% purity) can be seen in Fig. 4.9.

4.3.1.2 Domtar Sludge and Abietic Acid

Figure 4.10 shows the effect of adding the abietic acid to the 60% wastewater serum bottles of the Domtar sludge.

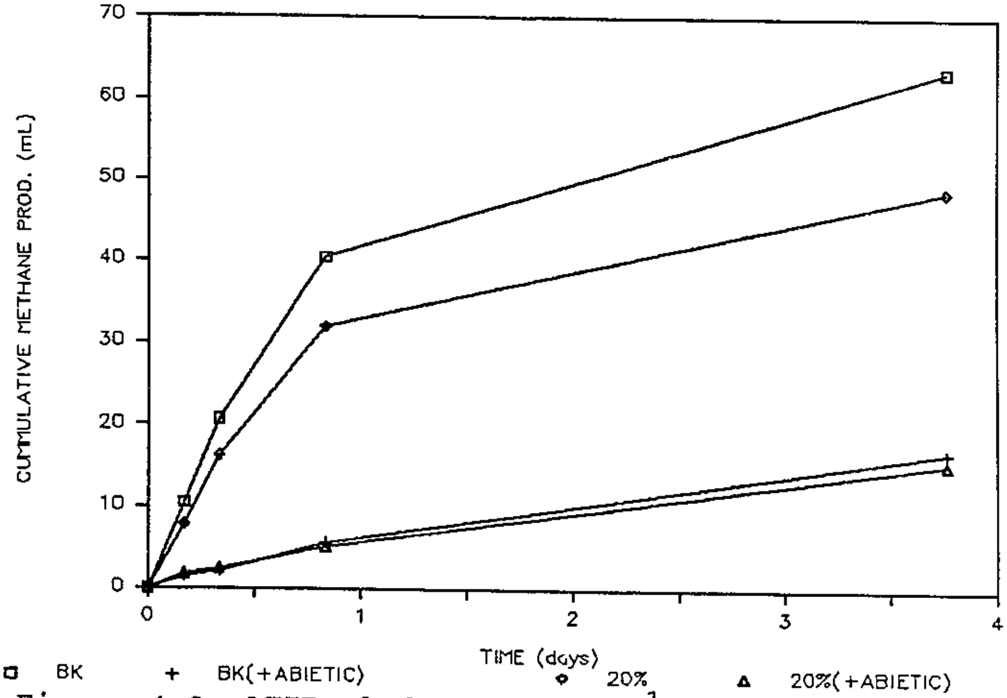


Figure 4.9 ATIR sludge; 500 mgL^{-1} abietic acid

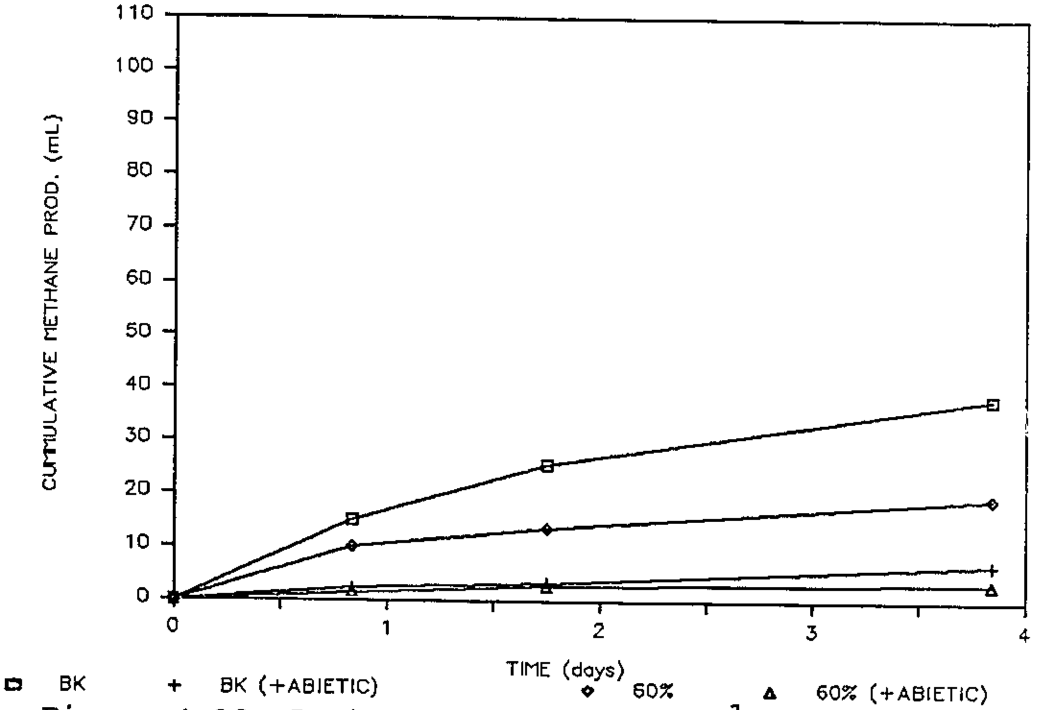


Figure 4.10 Domtar sludge; 500 mgL^{-1} abietic acid

4.3.2 Subsequent Investigation on Abietic Acid Toxicity

The second part of the investigation was performed on Domtar sludge using a series of abietic acid concentrations ranging from 50 to 350 mgL^{-1}. The abietic acid was injected into the serum bottles in 175 μL of methanol in spike 1 which was the only carbon source. Acetate/propionate was injected in spike two after all gas production ceased.

Results are demonstrated for spike 1 and 2 in Figs. 4.11 and 4.12, respectively.

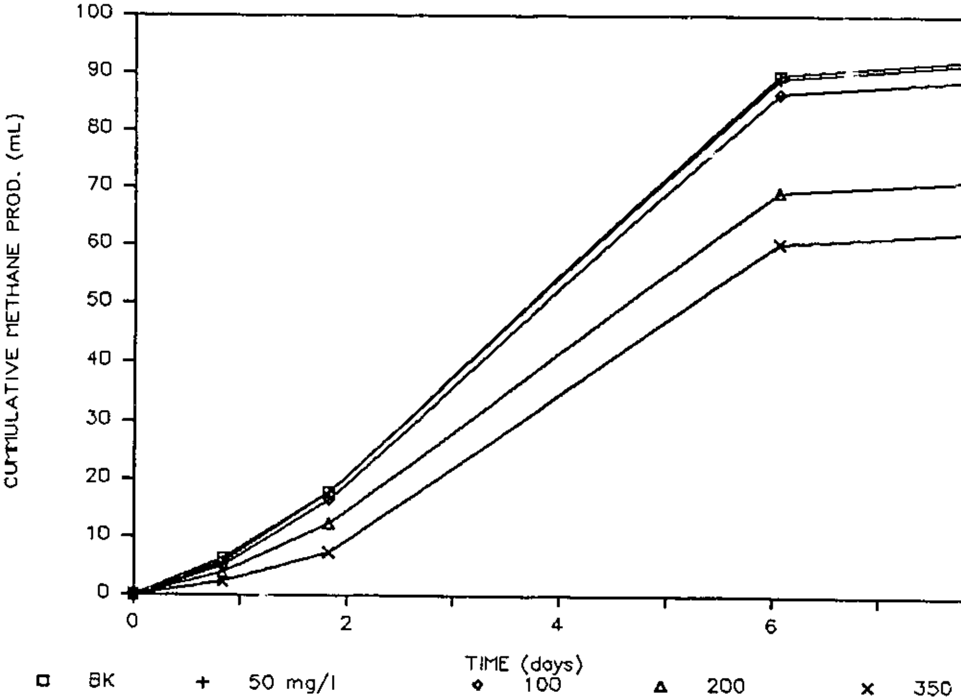


Figure 4.11 Domtar sludge; abietic acid toxicity (spike 1)

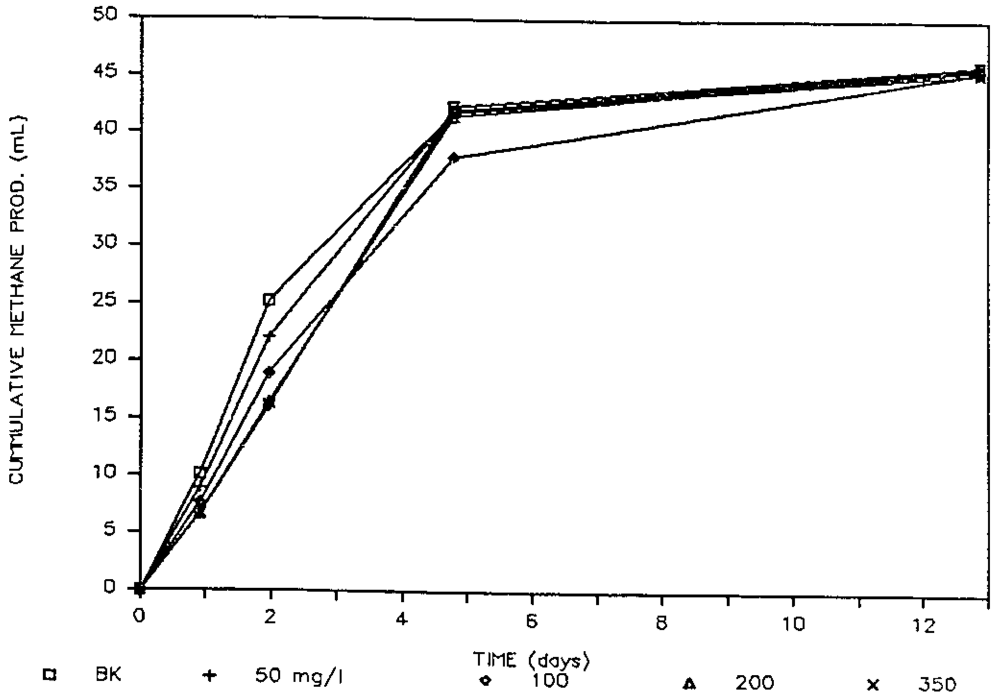


Figure 4.12 Domtar sludge; abietic acid toxicity (spike 2)

4.4 Experiment #3--BCTMP Fiber Toxicity

Fiber collected in the preparation of BCTMP-NC was washed in methanol and therefore separated into two fractions: the methanol soluble fraction (MSF); and the methanol insoluble fraction (MIF). Proportional amounts of the two fractions were tested by MATA. The total fiber was also tested for toxicity using the MATA for comparison purposes.

4.4.1 Methanol Soluble Fiber

Initial gas production for 100% and 200% MSF is shown in Figs. 4.13. and 4.14 for spikes 1 and 2, respectively. Spike 1 was injected with both acetate/propionate and 500 μL of methanol. Spike 2 received only the acetate/propionate.

4.4.2 Methanol Insoluble Fiber

Similarly, in Figs. 4.15 and 4.16, cumulative methane production is reproduced for 100% and 200% MIF. Neither spike 1 nor 2 received methanol.

4.4.3 Total Fiber

Total fiber at 6.0 gL^{-1} or 100% of the normal wastewater concentration was tested. Spike 1 results are shown in Fig. 4.17 and spike 2 results are displayed in Fig. 4.18. As with MIF, there was no methanol injected in either spike 1 or 2.

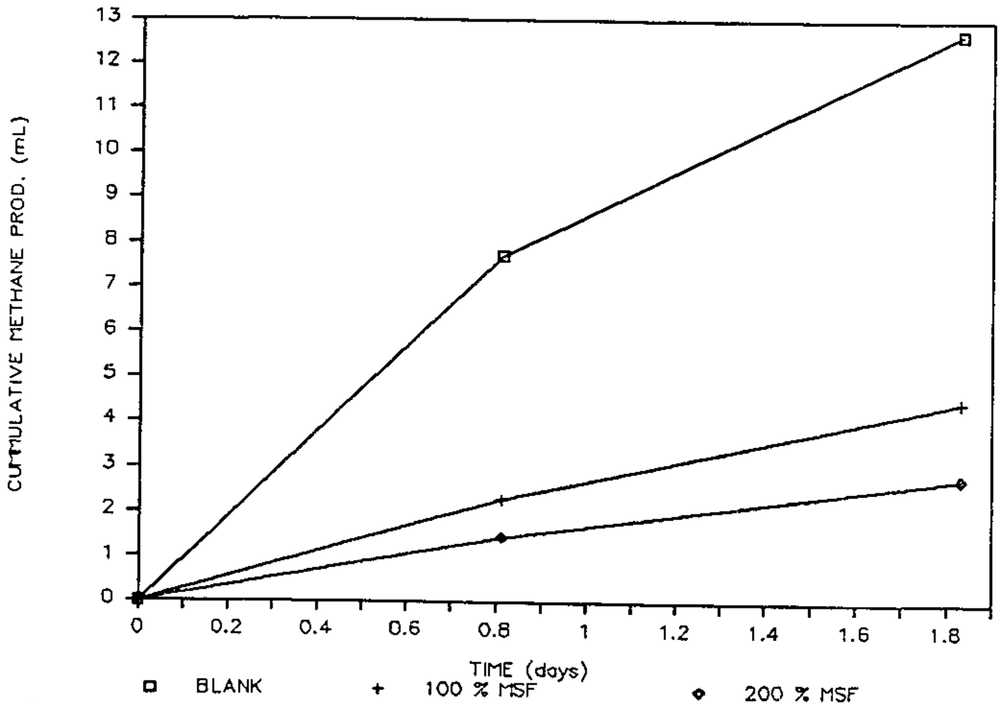


Figure 4.13 MSF--Spike 1

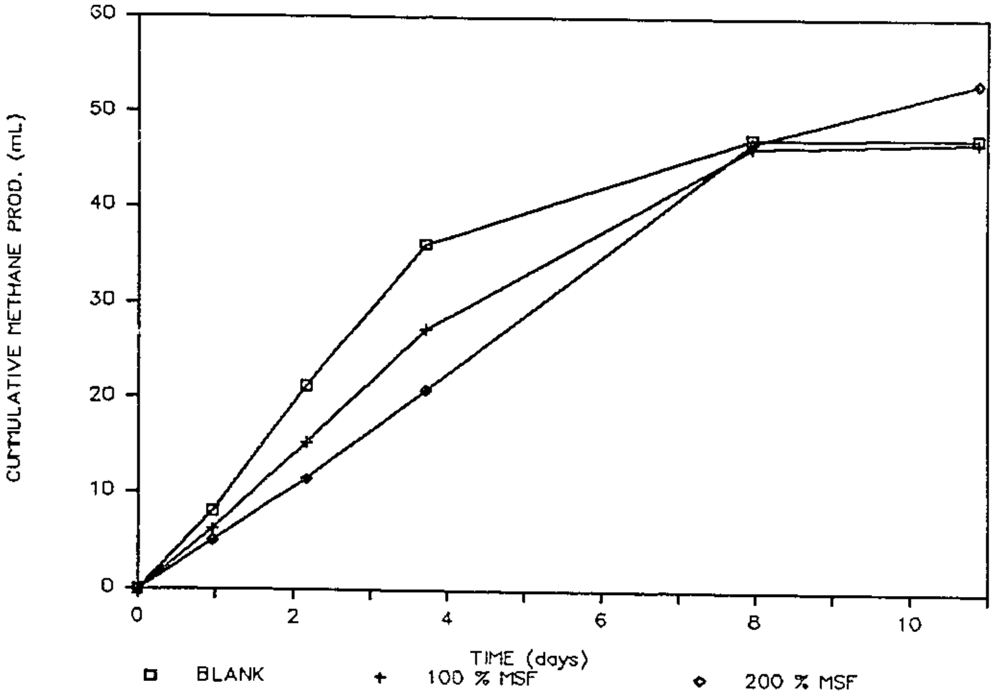


Figure 4.14 MSF--Spike 2

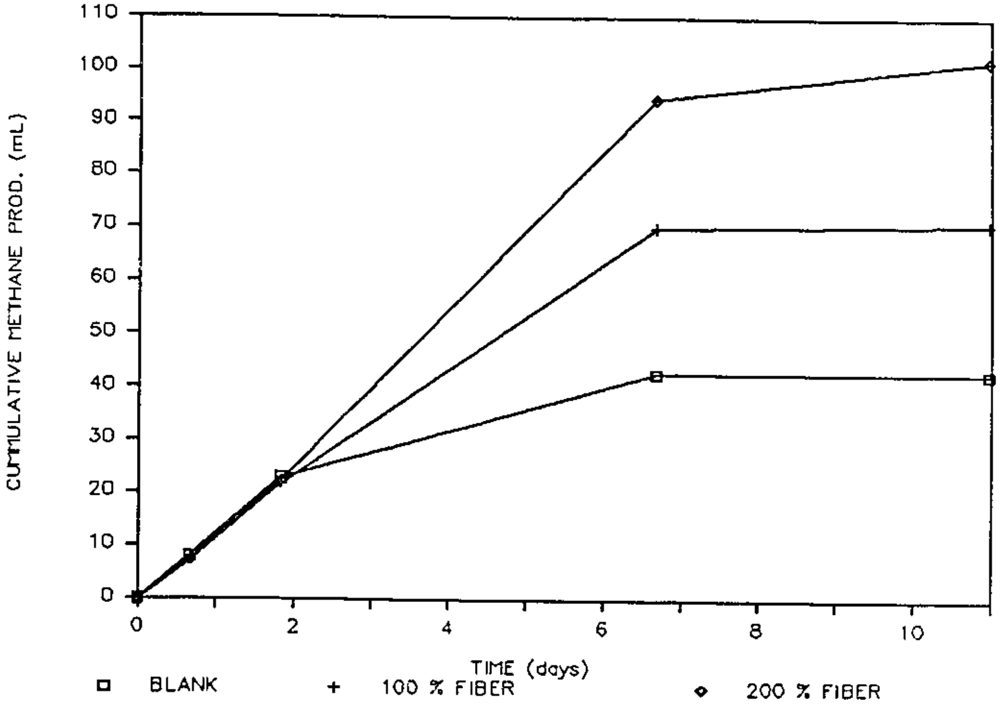


Figure 4.15 MIF--Spike 1

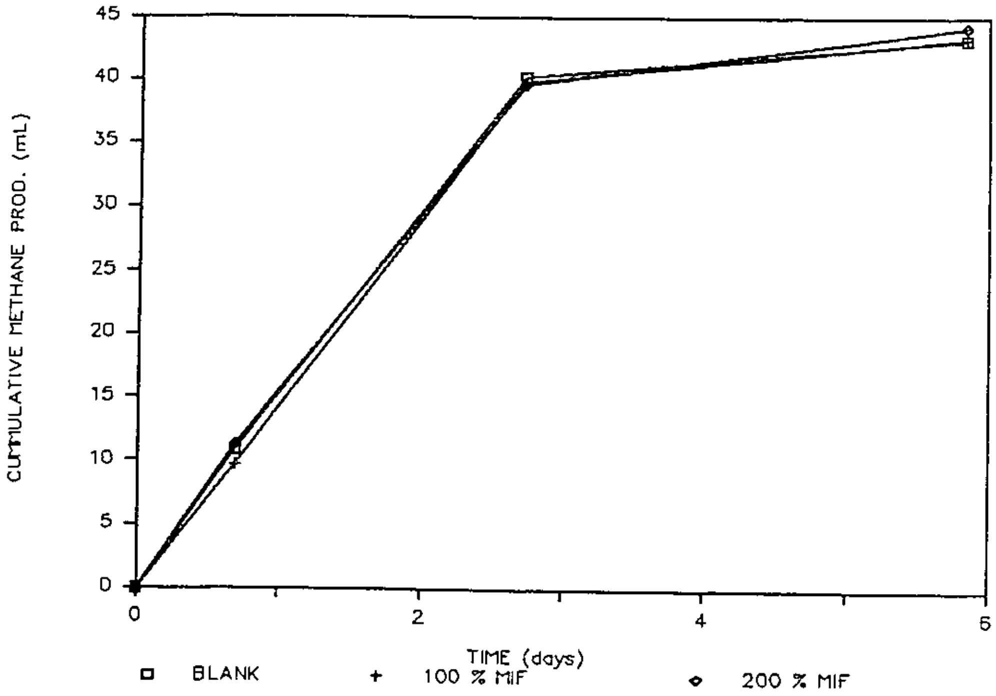


Figure 4.16 MIF--Spike 2

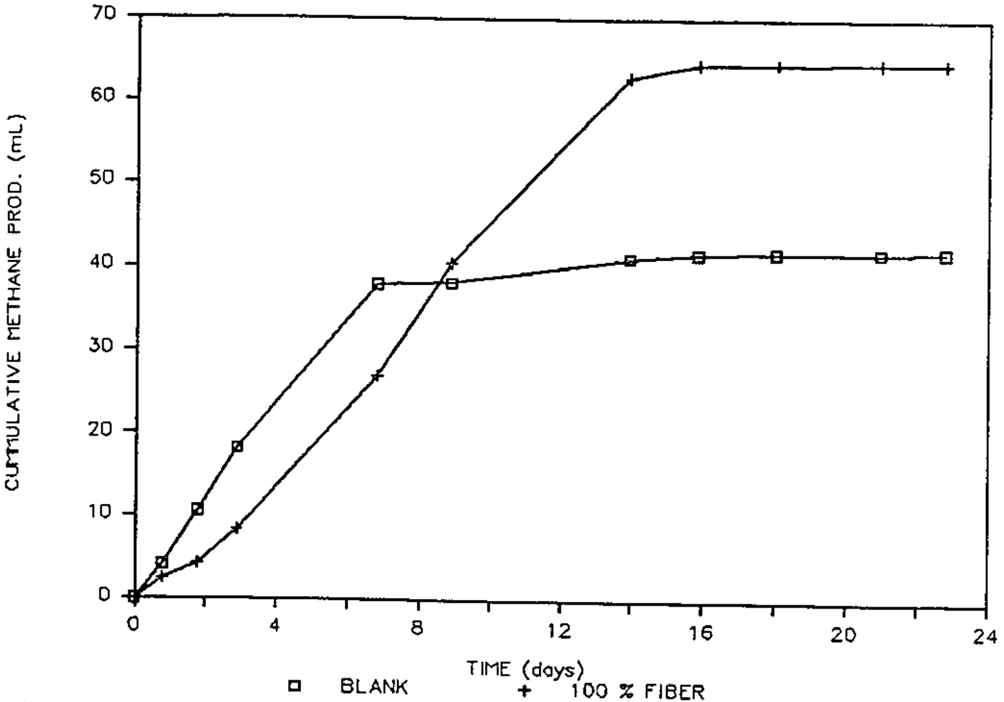


Figure 4.17 Total Fiber--Spike 1

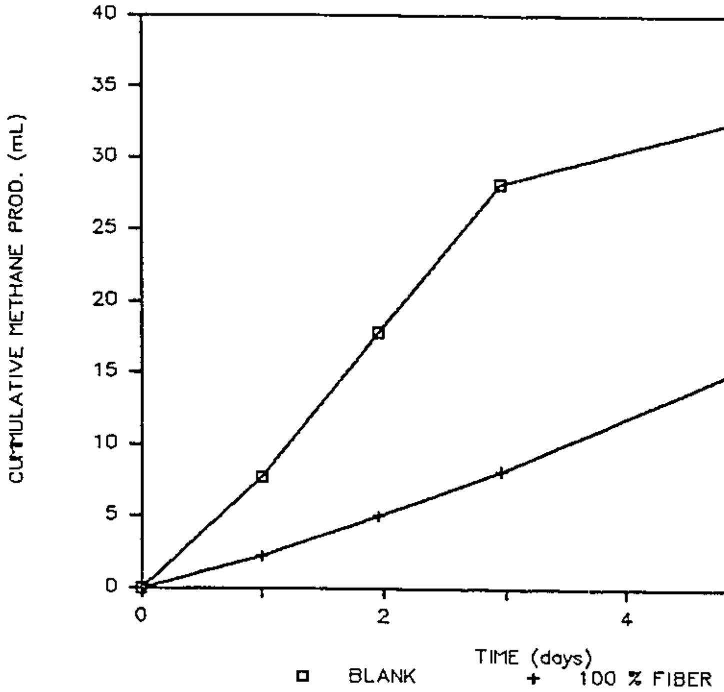


Figure 4.18 Total fiber--Spike 2

4.5 Experiment #4--Resin Acid Cocktail Toxicity

A resin acid cocktail was prepared (as described in Sec. 3.8) and a MATA was used to investigate the toxicity displayed by the mixture. Included in the first spike was 200 μL of methanol used to dissolve the resin acids.

Resin acid concentrations of 20 to 700 mgL^{-1} were assayed for toxicity. Representative graphs for spike 1 and spike 2 are displayed in Figs. 4.19 and 4.20, respectively. Spike 2 (acetate/propionate) was administered on day 18 of spike 1.

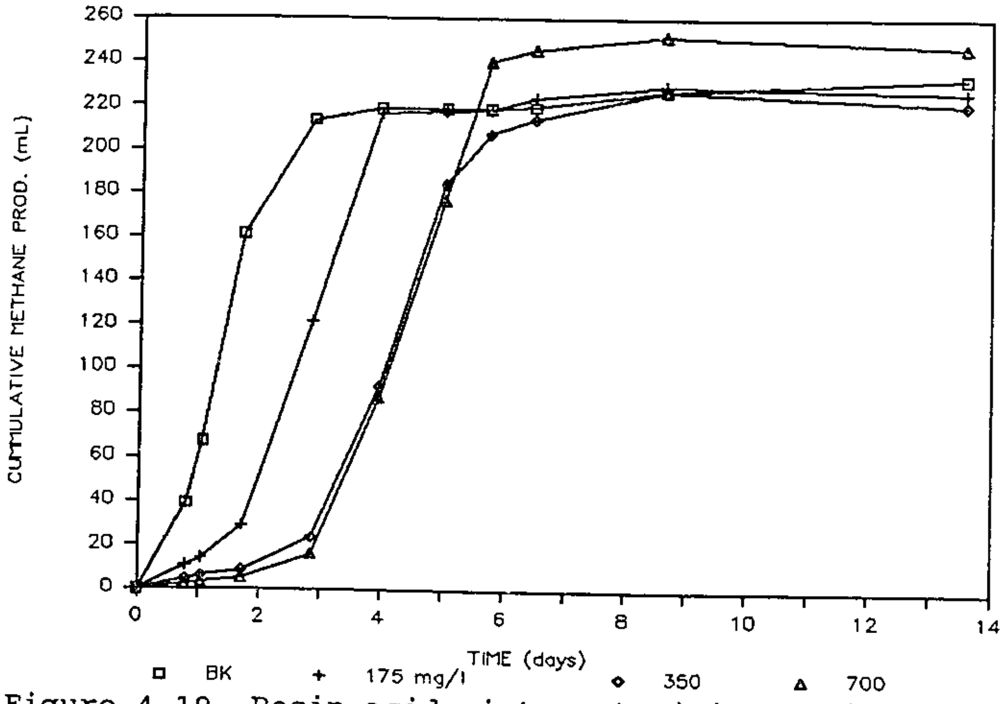


Figure 4.19 Resin acid mixture toxicity--Spike 1

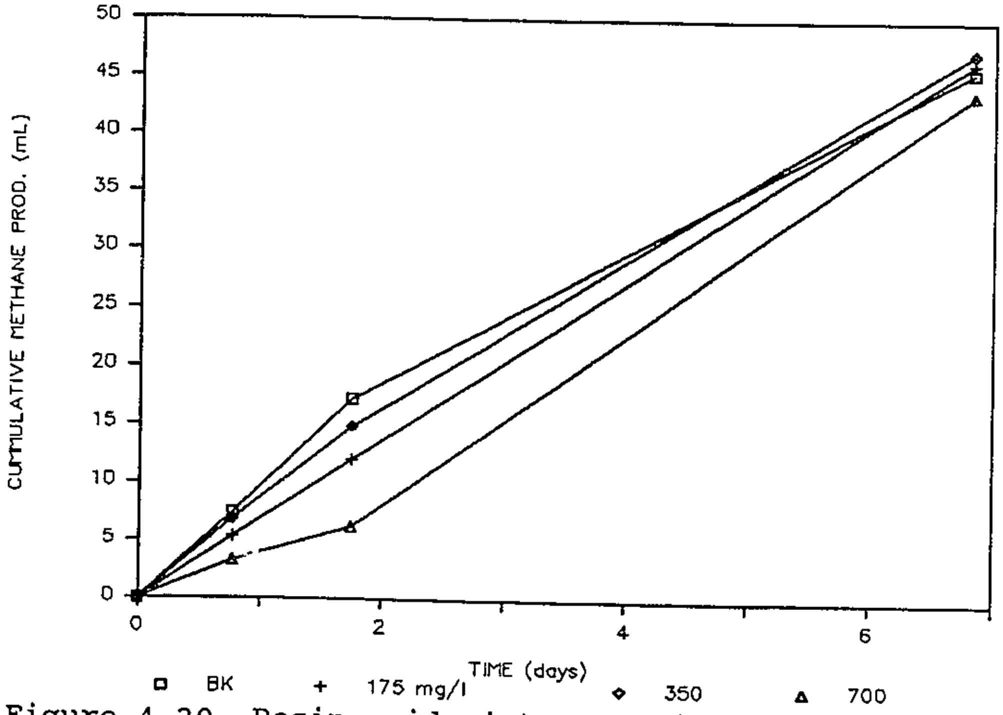


Figure 4.20 Resin acid mixture toxicity--Spike 2

4.6 Summary of Experiments 1 Through 4

Initial methane production rates can be estimated from the initial slopes of the cumulative methane production curves. For each experiment, the rate of methane production is compared to that of the control in that experiment. Percent of control rates are summarized in Table 4.3.

Table 4.3 Summary of Percent Control Activity for Experiments 1 - 4 for Domtar sludge

(All numbers under spike 1 and 2 are based on percentage of control activity.)

Toxicant	Spike 1	Spike 2
BCTMP-N 20%	117	65
40%	78	70
60%	48	65
BCTMP-NC 20%	150	109
40%	91	70
60%	118	54
MSF (100%)	33	73
MSF (200%)	13	56
MIF (100%)	95	96
MIF (200%)	94	100
Total fiber (100%)	39	41
Resin acids		
20 mgL^{-1}	102	100
40 mgL^{-1}	119	84
80 mgL^{-1}	97	84
160 mgL^{-1}	63	65
320 mgL^{-1}	41	59
700 mgL^{-1}	NA*	38

* -- acetate/propionate was not given to these bottles in spike 1

Resin acid data are presented in graphic form in Fig. 4.21.

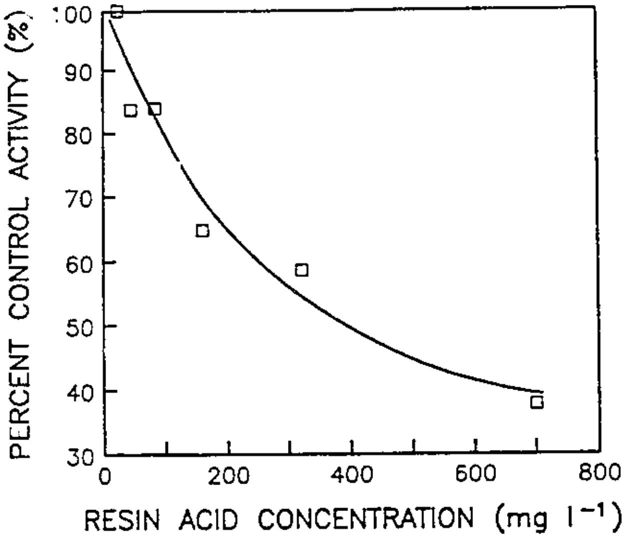


Figure 4.21 Percent control activity vs. resin acid concentration

4.7 Experiment #5--Individual Resin Acid Toxicity

Seven resin acids were individually tested for toxicity using the MATA at a single concentration of 140 mgL^{-1}. Second third and fourth spikes of acetate/propionate were given to the serum bottles. Each serum bottle received 200 μL of methanol in which was dissolved the specific resin acid. Resin acid purities and chromatographs are given in Appendix D.

Cumulative methane production graphs for the acetate/propionate spike are given in Figs. 4.22 and 4.23.

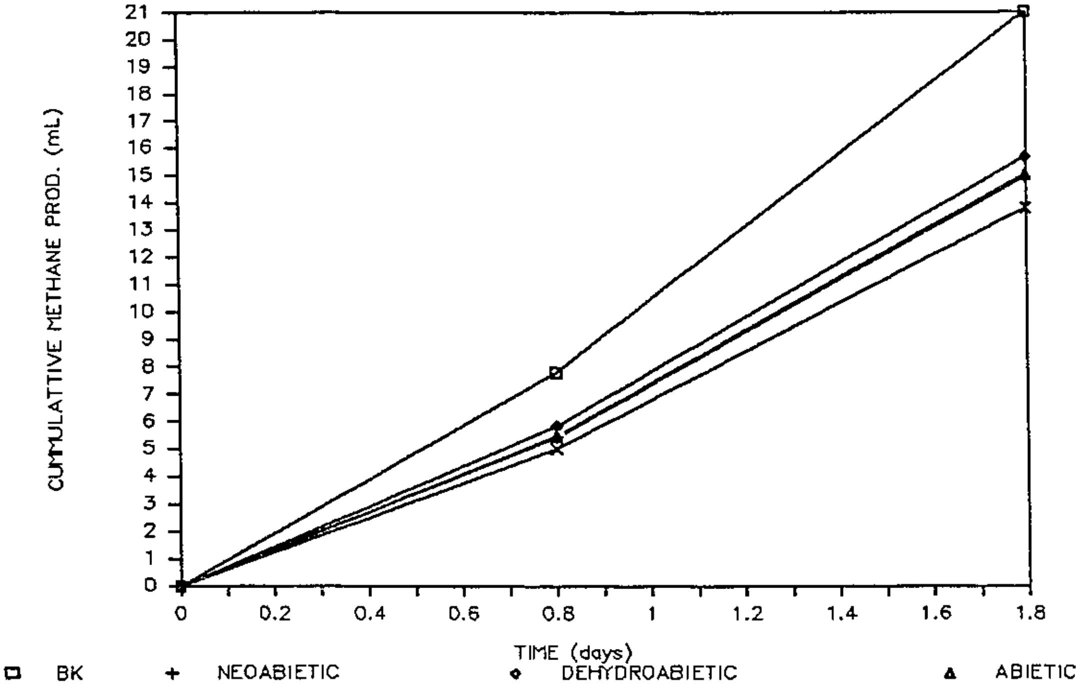


Figure 4.22 Abietic series of resin acids--acetate/propionate spike.

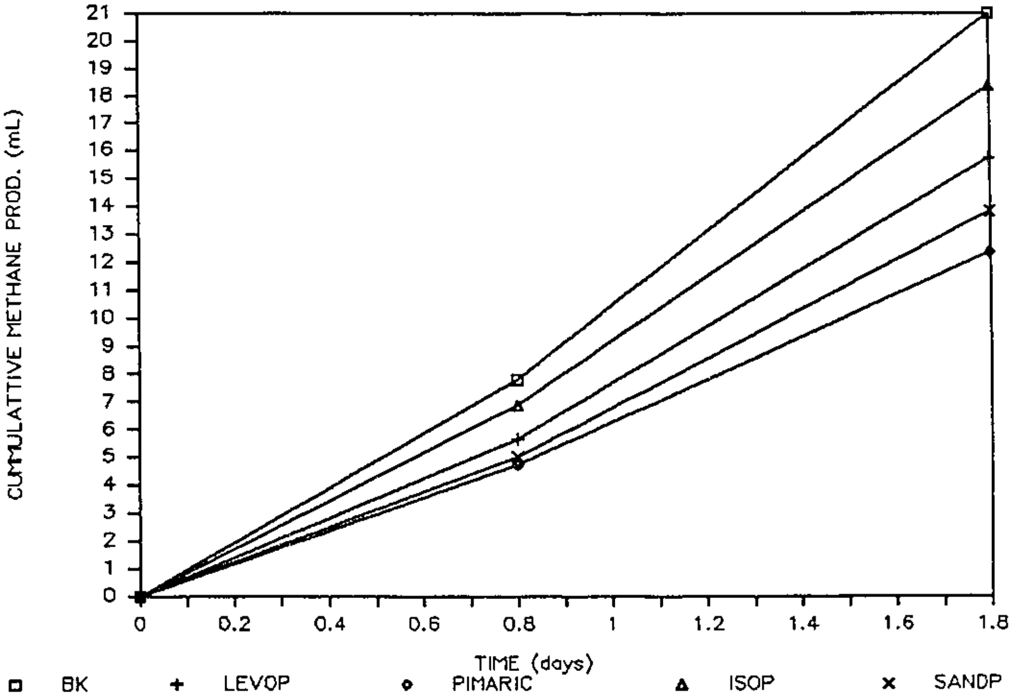


Figure 4.23 Pimaric series of resin acids--acetate/propionate spike.

4.8 Experiment #6--Toxicity of BCTMP Wastewater Spiked with Resin Acid

BCTMP-NC was spiked with the resin acid cocktail at concentrations of 40 and 80 mgL^{-1}. Toxicity was compared to a control which had no additional resin acid added to the wastewater.

In spike 1, the addition of the resin acids had a marked effect on BCTMP-NC wastewater. With the addition of 40 mgL^{-1}, methane production was 59% of the control. With the addition of 80 mgL^{-1} of the resin acid mixture, methane production decreased to 29% of the control. However subsequent spikes showed no inhibition and perhaps slight improvement of activity over the control with 40 mgL^{-1} performing at 138% of control activity and 80 mgL^{-1} at 125% of the control. BCTMP-N spiked with the resin acid mixture showed similar results.

4.9 Experiment #7--Anaerobic Degradation of Resin Acids

Pure dehydroabietic acid and levopimaric acid were studied for possible degradation and/or adsorption onto the biomass. Resin acid concentrations are summarized in Table 4.4. For each resin acid, three sets of conditions were used and resin acid analysis was done on both the supernatant and the biological (sludge) phases. The first condition had no biomass present and served as a control. The second and third conditions allowed for exposure to Domtar sludge for periods of 1 hour and 1 month respectively. In the one month exposure acetate/propionate was injected twice into the serum bottles at concentrations equivalent to the MATA. Both supernatant and sludge samples were sent to the Econotech Laboratory to be analyzed for resin acid content and composition.

Experimental conditions and results are summarized in Table 4.4. Recovery of the resin acids was poor and is summarized in graph format in Fig. 4.24.

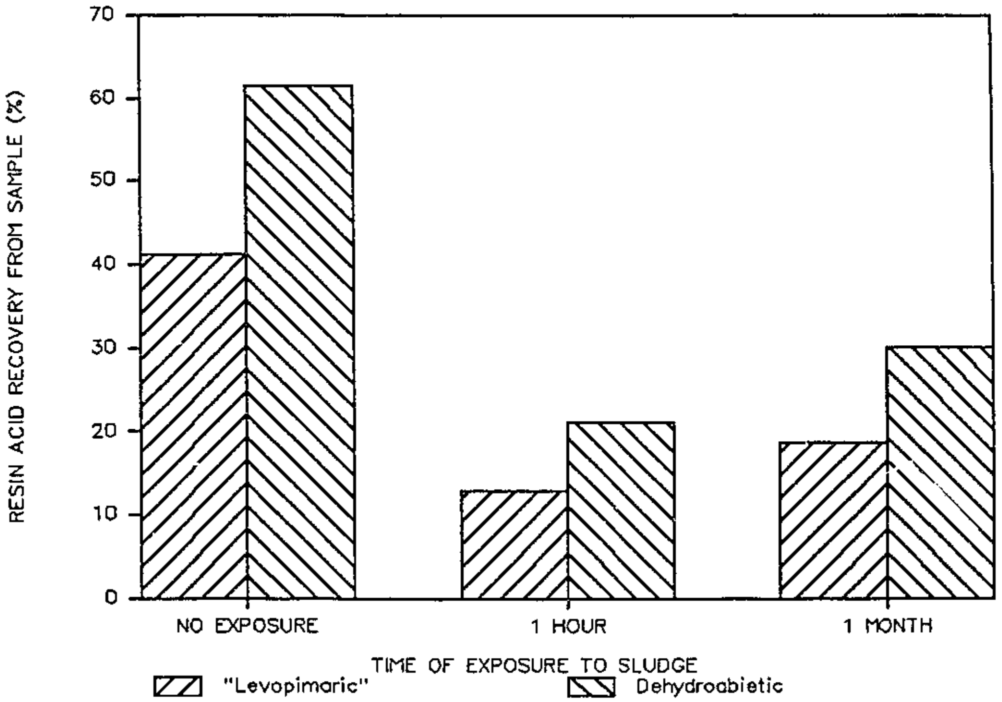


Figure 4.24 Recovery of Resin Acids after Sludge Exposure

Serum Bottle	Defined Medium Vol. (mL)	Water Vol. (mL)	Volatile Organic Acid (mL)	Sulfide Vol. (mL)	Methanol Vol. (mL)	Resin A. in Bottle (mg)	Sludge Vol. (mL)	Sludge Sample Vol. (mL)	Total Vol. (mL)
L-no exposure	100.0	360.0	0.0	0.0	1.0	60.8	0.0	NA	521.8
L-1 hour	100.0	360.0	0.0	6.0	1.0	61.6	100.0	110.0	628.6
L-1 month	100.0	360.0	48.0	6.0	1.0	63.0	100.0	110.0	678.0
D-no exposure	100.0	360.0	0.0	0.0	1.0	62.1	0.0	NA	523.1
D-1 hour	100.0	360.0	0.0	6.0	1.0	60.6	100.0	105.0	627.6
D-1 month	100.0	360.0	48.0	6.0	1.0	61.5	100.0	95.0	676.5

LABORATORY ANALYSIS

CALCULATIONS

Serum Bottle	Total Supern. Vol. (mL)	Supern. Resin a. Conc. (mg/L)	Sludge Resin a. (mg/L)	Sludge Fraction Recovery (mg)	Supern. Fraction Recovery (mg)	Total Recovery of Original (mg)	Total Recovery of Original (%)	Supern. Recovery (%)	Sludge Recovery (%)
L-no exposure	521.8	48.0	0.0	0.0	25.0	25.0	41.2	100.0	0.0
L-1 hour	518.6	9.6	27.1	3.0	5.0	8.0	12.9	62.6	37.4
L-1 month	568.0	18.6	10.9	1.2	10.6	11.8	18.7	89.8	10.2
D-no exposure	523.1	72.9	0.0	0.0	38.1	38.1	61.4	100.0	0.0
D-1 hour	522.6	16.7	38.7	4.1	8.7	12.8	21.1	68.2	31.8
D-1 month	581.5	26.5	32.2	3.1	15.4	18.5	30.0	83.4	16.6

Table 4.4 Levopimaric acid and dehydroabietic acid degradation in the presence of DOM sludge. (L-Levopimaric Acid, D-Dehydroabietic acid)

The levopimaric acid that was placed in the three serum bottles was 99% pure (see chromatograph in Appendix D). However, the resin acids recovered from the levopimaric acid experiments were a mixture of three resin acids: levopimaric, abietic (predominantly); and dehydroabietic. The proportion of each changed with respect to length of exposure to the biomass. Whereas, abietic acid was predominant with no exposure, after 1 month, only substantial amounts of dehydroabietic remained. The shift in predominance of resin acids is shown in Fig. 4.25.

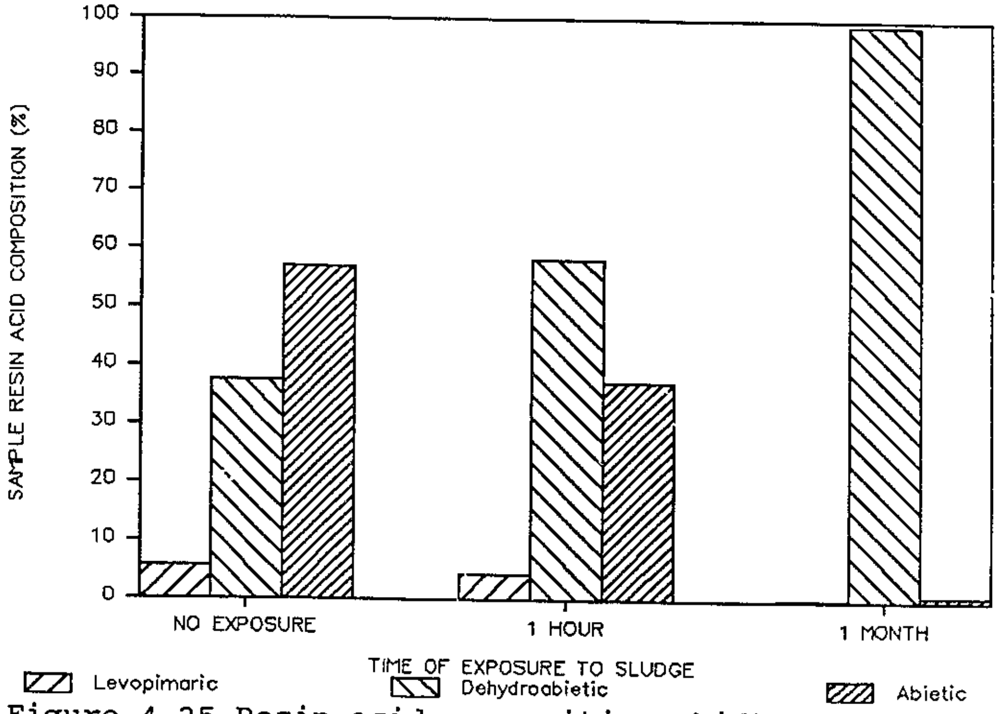


Figure 4.25 Resin acid composition shift after sludge exposure

4.10 Experiment #8--Resin Acid Toxicity in a UASB Reactor

After a steady baseline was obtained in a UASB reactor being fed acetic acid as the soluble carbon source, a series of acute and chronic resin acid toxicity studies were carried out. First, both the resin acid cocktail and 85% abietic acid were used to spike the reactor. After the spike experiments, 85% pure abietic acid was fed continuously to the reactor at several concentrations. Reactor performance was monitored by measuring biogas volume and percentage methane, acetic acid concentrations in influent and effluent, pH, and alkalinity.

4.10.1 General Reactor Performance

A baseline was obtained after 21 days. The preferred reactor HRT of 1 day was reached after 20 days with an organic loading of 3 g COD L^{-1} d^{-1}. Reactor performance is shown in Fig. 4.26 by daily gas production, HRT, and influent/effluent acetic acid concentrations.

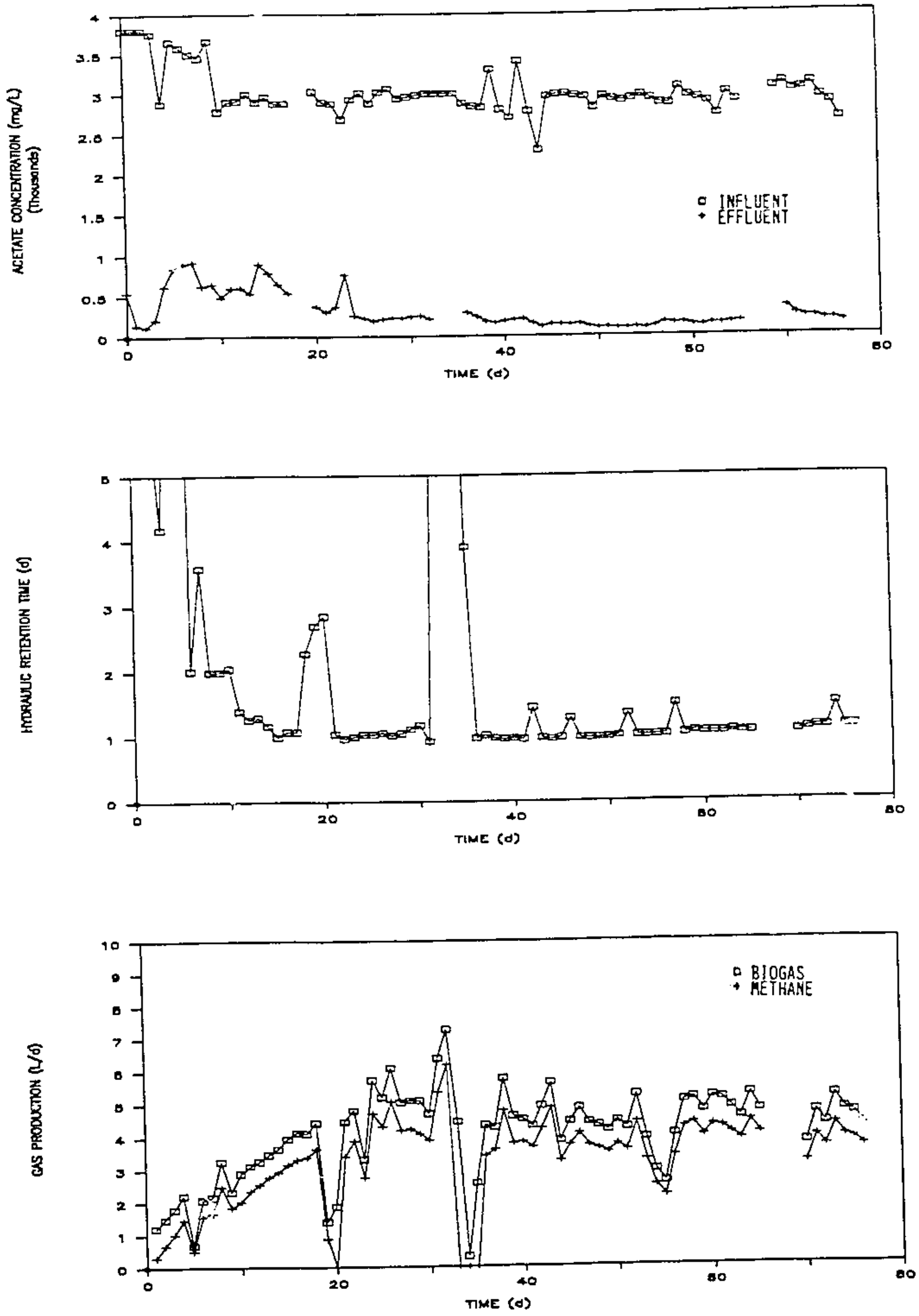


Figure 4.26 General UASB reactor performance.

4.10.2 Specific Activity

Specific acetoclastic activity was measured twice. On day 30 just prior to toxicant addition and again on Day 65, just before shutdown of the reactor for a period of four days. On Day 65, the reactor had been continuously fed acetic acid containing 300 mgL^{-1} abietic acid for a period of 4 days. The results are listed in Table 4.4.

Table 4.4 UASB biomass specific activity

Time	Specific Activity [mg CODr $(g\ VSS)^{-1}$ d^{-1}]
Day 30	192
Day 65	155

4.10.3 Methanol Spike Exit Curve

Other than the first resin acid mixture spike, spikes of the resin acid mixture or abietic acid were given to the UASB reactor dissolved in methanol. Prior to resin acid addition, a spike of methanol without resin acid, was administered to the reactor to monitor the possible effect that methanol would have on reactor performance. Reactor performance was monitored hourly after methanol addition. The concentration of methanol in the reactor over time was monitored by GC. When a different volume of methanol was given to the UASB, the concentration exiting over time was again monitored, as well as reactor performance. Results are shown in Appendix B.

4.10.4 Resin Acid Toxicity

The reactor was spiked with the resin acid mixture (without methanol) on day 31 to make a final reactor concentration of 100 mgL^{-1}. Reactor pH, gas production and composition, and influent/effluent acetic acid concentrations were monitored hourly. The resin acid addition had no effect on reactor performance (See Appendix B for all spike profiles). On day 45 the same concentration was again injected into the reactor in methanol. Again there was no effect. On day 56, abietic acid was spiked into the reactor to make a final reactor concentration of 400 mgL^{-1}, and again had no discernable effects.

Continuous feeding of 150 mgL^{-1} abietic acid was commenced on day 57 and was stepped up to 300 mgL^{-1} on day 64 until day 67. There was no noticeable effect on reactor performance with the possible exception of increased biomass washout. After a shut down of 3 days, the reactor was re-started at the hydraulic retention time (HRT) and organic loading rate (OLR) which existed just prior to shutdown. The reactor performance stabilized at the previous level after two days. On day 73, abietic acid at a concentration of 600 mgL^{-1} was added to the feed of the reactor. Reactor efficiency and toxic loading are summarized in Fig. 4.27.

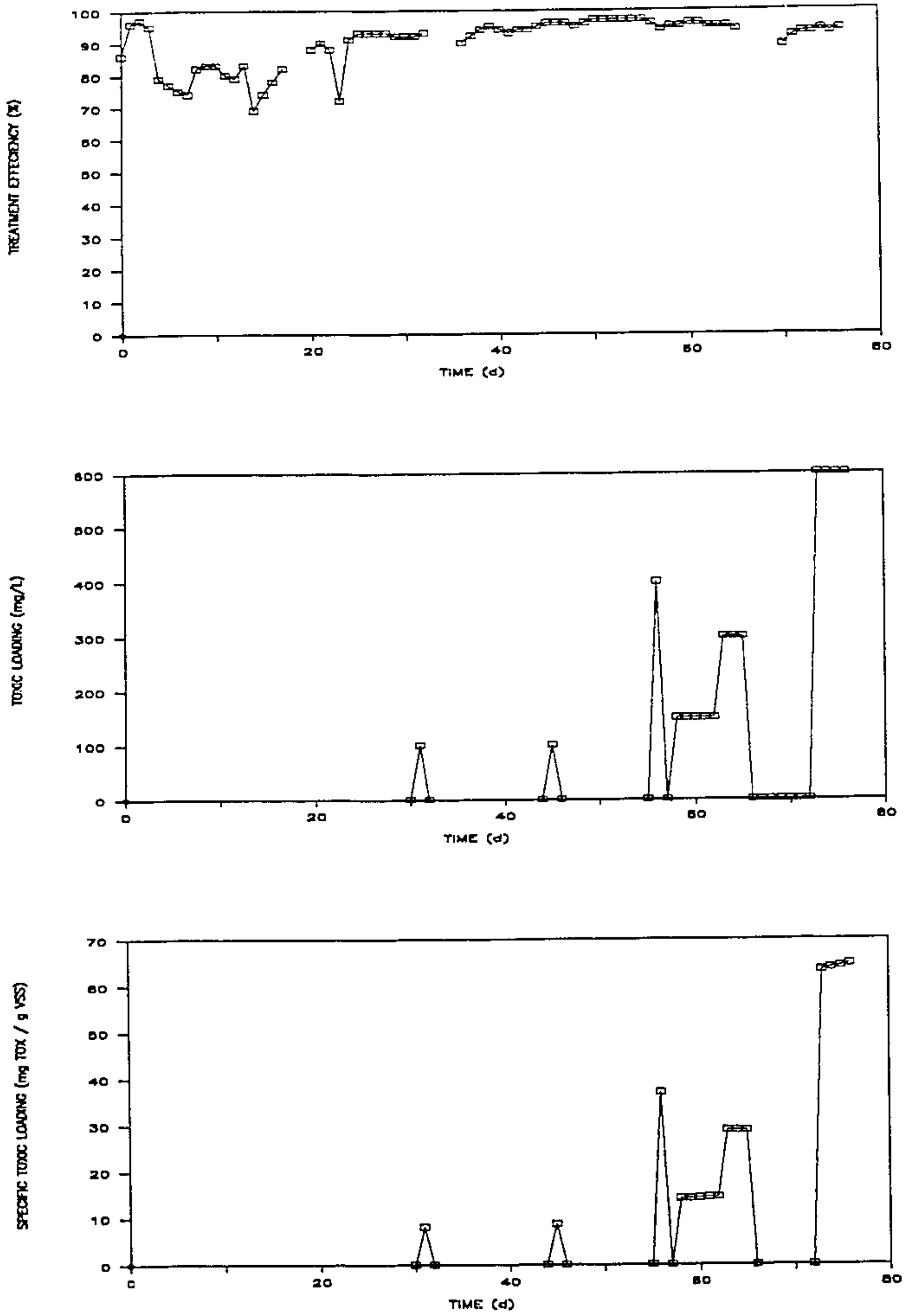


Figure 4.27 Efficiency of treatment of the UASB reactor under toxic loading

4.10.5 Sludge Settleabillity

Sludge settleabillity of the UASB reactor biomass was monitored on day 30, prior toxic loading and after baseline establishment, and again on day 65, after continuous feeding of 300 mgL^{-1} abietic acid. The results of the test are summarized in Fig. 4.28.

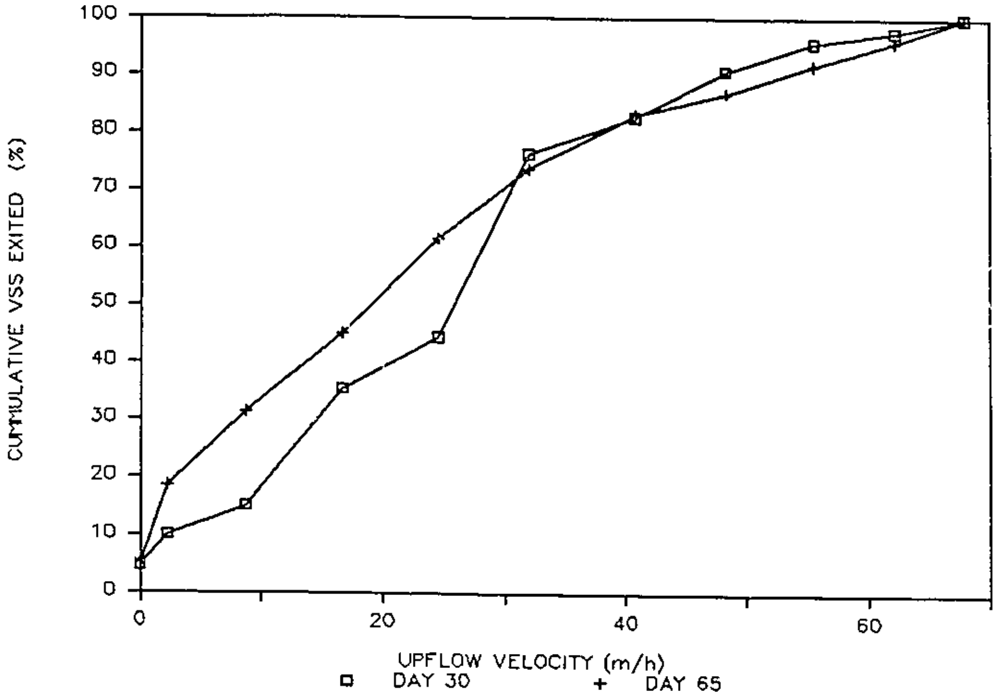


Figure 4.28 Sludge settleability on Day 30 and Day 65

4.10.6 Solids Loss, pH, and Alkalinity

Alkalinity and pH were measured frequently throughout the experiment. Alkalinity remained at approximately 2 400 mgL^{-1} throughout the experiment and pH varied from 7.4 to 7.8. These data and those from VSS/TSS analyses are summarized in Fig. 4.29.

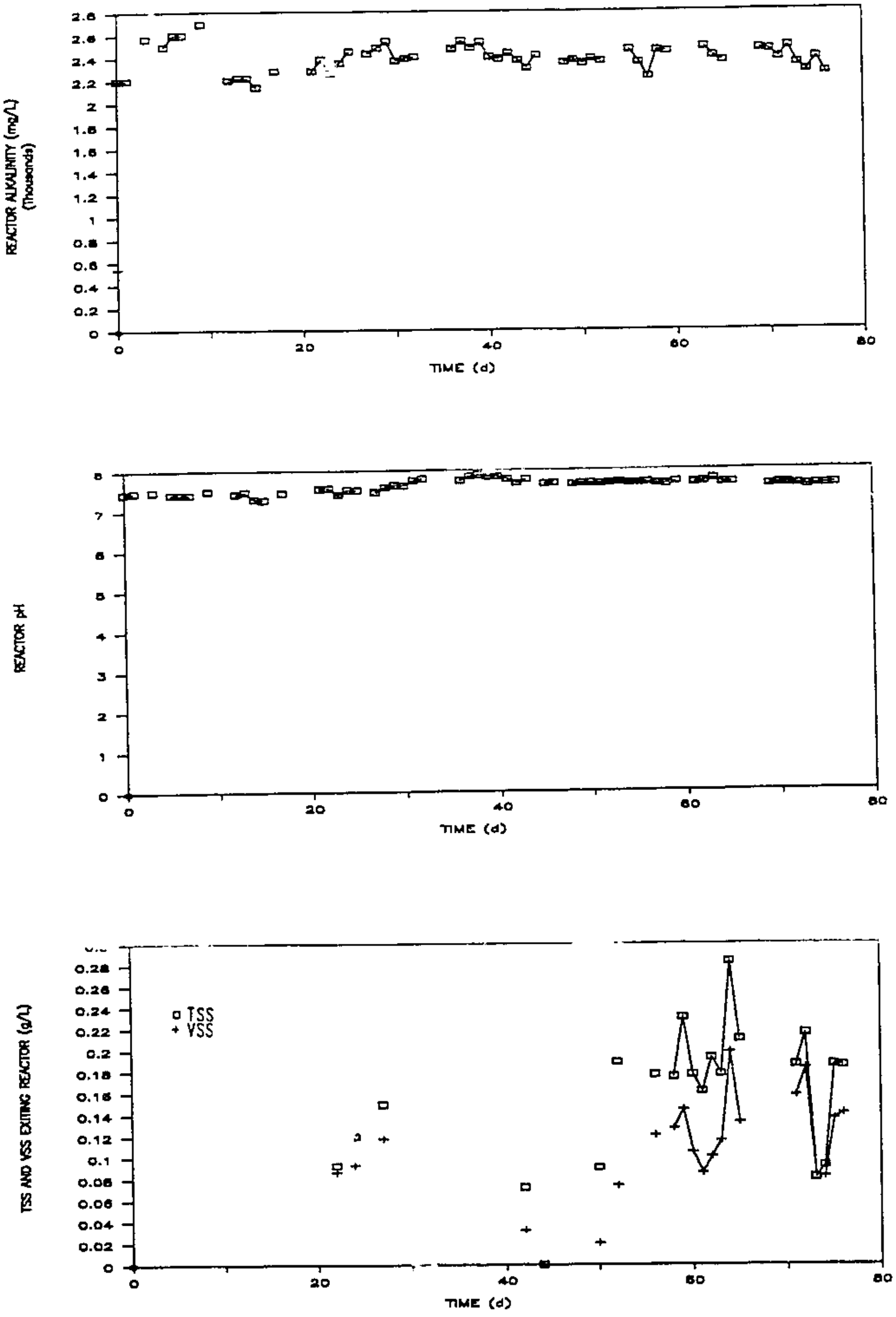


Figure 4.29 Alkalinity, pH, and Solids exiting for UASB reactor

4.10.7 Possible Resin Acid Accumulation

It was noticed during shutdown on Day 65 that a white mass was floating in the interstitial spaces of the biorings in the UASB reactor. The mass could have been a substantial amount of resin acid but analysis was not conducted.

4.10.8 MATA on UASB Reactor Biomass

On Day 77 a MATA was done on the active sludge from the UASB reactor. The resin acid mixture and the 85% abietic acid were both dissolved in defined medium adjusted to pH 11 with concentrated NaOH. This was the same method that was used to disperse the abietic acid in the resin acid feed, and is discussed in Sec. 3.9.3. After the resin acids dissolved (the resin acid mixture never completely dissolved), the pH was readjusted to 7.0. Duplicate serum bottles with concentrations of a resin acid mixture and abietic acid of 300 mgL^{-1} were prepared. After the serum bottles were reduced, they were inoculated with sludge directly and anaerobically transferred from the continuous reactor. Controls were treated in the same fashion except that no resin acid was included. Methanol was not used since it was not needed to render the resin acids soluble. Results of acetate/propionate spikes 1 and 2 are shown in Figs. 4.30 and 4.31.

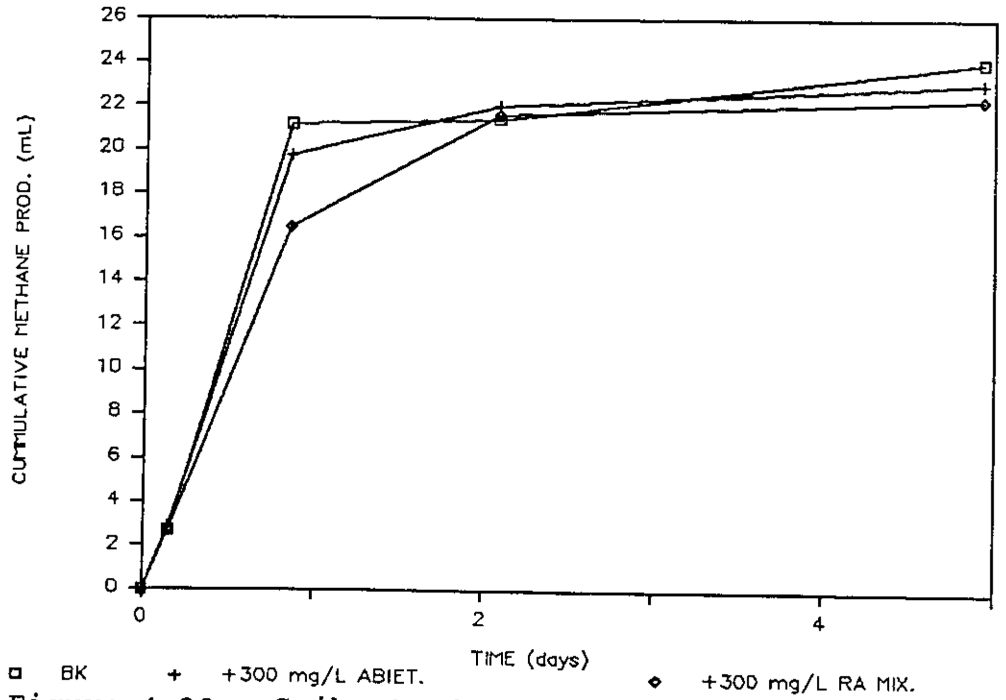


Figure 4.30 Spike 1 of MATA on UASB biomass--acetate propionate spike

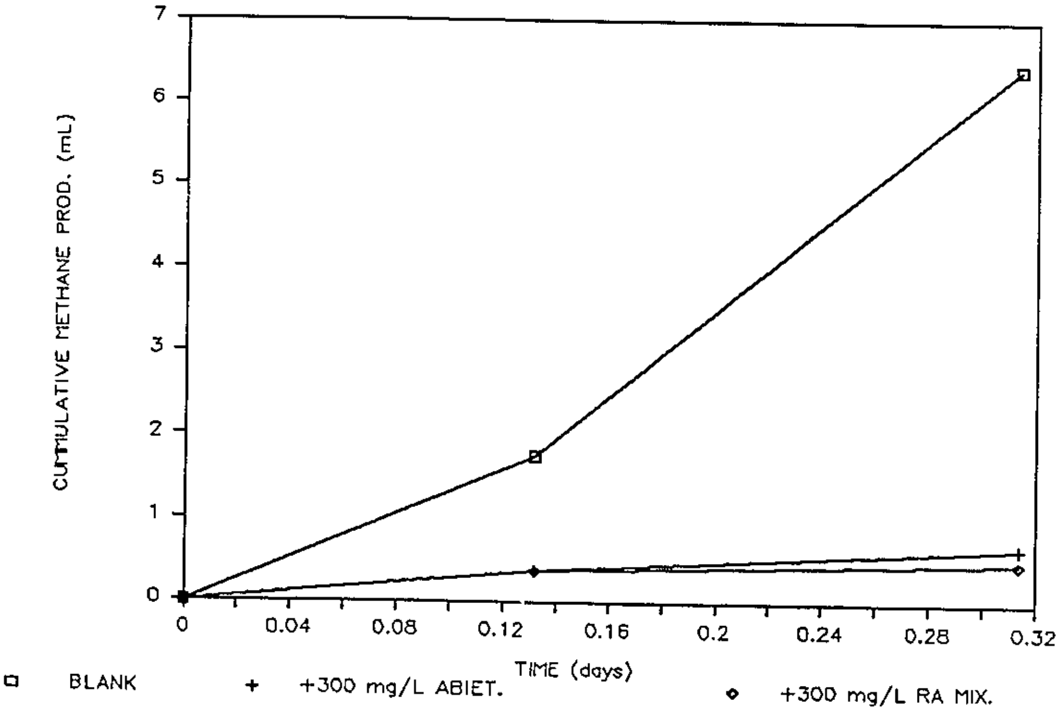


Figure 4.31 Spike 2 of MATA on UASB biomass--acetate/propionate spike

4.11 Specific Activity and Specific Loading for Serum Bottle Experiments

4.11.1 Sludge selection experiments--VSS Concentration, Specific Activity of Biomass

The VSS concentration and specific activity for the sludge selection experiments are presented in Table 4.5. All numbers are averages for the duplicate serum bottles used for the experiments.

4.11.2 Resin Acid Experiments--VSS Concentration, Specific Loading of Resin Acid, Specific Activity of Biomass

The VSS concentration, specific loading of resin acid mixture or individual resin acid and the specific activity of the biomass for the serum bottle experiments are presented in Table 4.6. All numbers are averages of the duplicate serum bottles for each experiment.

EXPERIMENT #	WASTEWATER CONC. (%)	VSS (g/L)	SPIKE ONE		SPIKE TWO		SPIKE ONE SPECIFIC ACTIVITY (gCOD/gVSS/d)	SPIKE TWO SPECIFIC ACTIVITY (gCOD/gVSS/d)
			CH4 PROD. (mL/d)	COD REM. (mgCOD/d)	CH4 PROD. (mL/d)	COD REM. (mgCOD/d)		
DOM SLUDGE WITH BCTMP WASTEWATER	CONTROL	5.05	9.35	23.97	13.80	35.38	0.095	0.140
BCTMP-NC	20.00	5.40	14.00	35.90	15.10	38.72	0.133	0.143
"	40.00	5.84	8.55	21.92	8.25	21.15	0.075	0.072
"	60.00	5.40	11.05	28.33	7.50	19.23	0.105	0.071
BCTMP-N	20.00	7.15	10.95	28.08	9.05	23.21	0.079	0.065
"	40.00	6.75	7.30	18.72	9.70	24.87	0.055	0.074
"	60.00	6.95	4.50	11.54	9.10	23.33	0.033	0.067
ATIR SLUDGE WITH BCTMP WASTEWATER	CONTROL	4.86	40.95	105.00	39.50	101.28	0.432	0.417
BCTMP-NC	20	5.20	41.30	106.67	33.70	86.41	0.411	0.333
"	40	4.07	48.60	124.62	32.30	82.82	0.613	0.407
"	60	4.23	56.75	145.51	21.70	55.64	0.688	0.263
"	80	4.50	40.75	104.49	7.05	18.08	0.464	0.080
BCTMP-N	20	5.36	44.00	112.82	31.40	80.51	0.421	0.300
"	40	4.59	42.00	107.69	6.35	16.28	0.469	0.071
"	60	4.85	18.06	46.29	4.50	11.54	0.191	0.048
"	80	4.34	21.65	55.51	3.65	9.36	0.256	0.043

TABLE 4.6 VSS concentrations and Specific activity of DOM and ATIR sludges: Spike 1 for BCTMP wastewater; Spike 2 for acetate/propionate spike

EXPERIMENT #	RESIN ACID CONC. (mg/L)	VSS (g/L)	RESIN A (mg/L)	SPECIFIC LOADING (mg RES. A PER g VSS)	SPIKE ONE COD REM. (mgCOD/d)	SPIKE TWO COD REM. (mgCOD/d)	SPIKE ONE SPECIFIC ACTIVITY (gCOD/gVSS/d)	SPIKE TWO SPECIFIC ACTIVITY (gCOD/gVSS/d)
MIXED RESIN A.	CONTROL	8.30	0.00	NA	21.13	16.86	0.051	0.041
	20.00	7.36	20.00	2.72	21.56	16.94	0.059	0.046
	40.00	7.35	40.00	5.44	25.10	14.10	0.068	0.038
	80.00	7.45	80.00	10.74	20.60	14.14	0.055	0.038
	160.00	7.15	160.00	22.38	13.32	10.96	0.037	0.031
	320.00	7.34	320.00	43.60	8.59	8.59	0.023	0.023
IND. RESIN ACID	CONTROL	7.55	0.00	NA	20.51	20.26	0.054	0.054
	NEOABIETI	7.53	140.00	18.59	12.95	9.10	0.034	0.024
	DEHYDROAB	7.39	140.00	18.94	11.67	13.21	0.032	0.036
	ABIETIC	7.52	140.00	18.62	10.51	13.46	0.028	0.036
	PIMARIC	7.54	140.00	18.57	22.18	18.21	0.059	0.048
	SANDAROCO	7.61	140.00	18.40	8.21	12.31	0.022	0.032
	LEVOPIMAR	8.14	140.00	17.20	10.51	11.15	0.026	0.027
	ISOPIMARI	7.71	140.00	18.16	7.31	11.79	0.019	0.031
UASB MATA	CONTROL	6.12	0.00	NA	63.33	53.08	0.207	0.173
	ABIETIC	6.10	300.00	49.18	58.72	5.51	0.193	0.018
	RES. A. M	6.75	300.00	44.44	47.18	3.97	0.140	0.012

Table 4.7 VSS concentration and Specific Activity of DOM sludge under varying specific loadings of Mixed Resin Acids and Individual Resin Acids.

CHAPTER 5

5. DISCUSSION

5.1 Sludge Selection

The first issue addressed in this study was that of sludge selection for the batch assays. Experiment #1, while proving that BCTMP wastewater had inhibitory effects on methane production rates in batch assays (and which will be discussed further in Section 5.3.2) also served as the experiment to select the sludge that would be used in the subsequent studies.

5.1.1 Domtar sludge

Two granular sludges were available for the study. Both had advantages and disadvantages. The Domtar sludge originated in Roermond, Holland, where it had been used to treat recycle paper wastewater in an UASB reactor. Upon receipt at NRC, this sludge was stored at 4°C and therefore was inactive. Domtar sludge had been successfully used at NRC as seed to many UASB reactors, as was the case in the concurrent study on BCTMP wastewater treatability by Richardson (1989).

5.1.1.1 Advantages of Domtar Sludge

The advantages of using the Domtar sludge were twofold. 1) The sludge had some exposure to pulp and paper industry wastewater. The advantage of using a sludge somewhat acclimatized to the wastewater would be that the study results would have greater bearing on industrial application. 2) The sludge had a constant but low initial activity upon revival. Studies by Marquis (1988) have shown that sludge, when stored at 4°C maintained activity. Starting each experiment from the same baseline was considered beneficial to final results interpretation.

5.1.2 ATIR sludge

The ATIR sludge was actively receiving a 50/50 sucrose/acetate feed at an organic loading rate of 5 g COD L^{-1} d^{-1}. Levels of VFA were checked daily and were usually at or near zero, indicating a starved reactor condition.

5.1.2.1 Advantages of ATIR Sludge

The perceived advantage of using the ATIR sludge was that its activity was high. In Experiment #1, spike 1 activity of the sludge on 20% BCTMP-N wastewater was 0.41 g COD removed per g VSS per day (g CODr $gVSS^{-1}$ d^{-1}) and 0.33 CODr $gVSS^{-1}$ d^{-1} for spike 2 (see Table 4.5). Higher specific activity meant that each set of experiments would take less time to complete.

The disadvantage of using the ATIR sludge was the possibility of reactor failure leading to irregularity in starting biomass conditions. The main disadvantage of the Domtar sludge was the low activity. For example, in Experiment #1, the activity of the sludge on 20% BCTMP-N wastewater was 0.08 g CODr $gVSS^{-1}$ d^{-1} for spike 1 and 0.06 g CODr $gVSS^{-1}$ d^{-1} for spike 2 (see Table 4.5).

5.1.3 Sludge Selection Analysis

The effect of BCTMP wastewater on ATIR sludge is shown in Figs. 4.3 and 4.4. In general the higher the wastewater percentage in the serum bottle, the lower the initial methane production rate. Methane production rate of 60% BCTMP-N wastewater (Fig. 4.3) was only 7% of the initial control rate. 60% BCTMP-NC wastewater (Fig. 4.4) had 22% of the control activity. 80% wastewater exhibited still lower methane production rates.

The effect of BCTMP wastewater on Domtar sludge activity is shown in Figs. 4.5 and 4.6 for spike 1 and Figs. 4.7 and 4.8 for spike 2. In general, the effect on methane production based on percentage of control activity was less for the Domtar sludge than for ATIR sludge. BCTMP-N wastewater at 60% reduced activity to 65% of the control in spike 2. BCTMP-NC reduced activity to 54% for the same spike. This was much less than the effect caused by the wastewater on ATIR sludge activity.

Total methane production by both sludges for the wastewater was approximately the same in spike 1 (Figs. 4.1, 4.2, 4.5, and 4.6). When examining the total gas production it should be noted that in Figs. 4.5 and

4.6, the control had 10 mL of methane (total) less than the controls in Figs. 4.1 and 4.2.

5.1.4 Choice of Sludge--Domtar over ATIR

On the basis that 1) both sludges were affected by the wastewater, 2) both sludges produced similar quantities of methane, albeit at different rates and 3) Domtar sludge was less effected by the presence of the wastewater on a percent control activity basis and 4) the advantages that Domtar sludge hadbecause it was acclimated to pulp and paper industry wastewater, it was decided to continue work with the Domtar sludge.

5.2 Preliminary Study on Abietic Acid Toxicity

When 500 mgL^{-1} of abietic acid was introduced into one of a pair of serum bottles containing the ATIR sludge (Fig. 4.9), and into the bottle containing Domtar sludge (Fig. 4.10), methane production was reduced considerably compared to the other of the pairs that did not receive the toxic load. Based on this reduction in initial methane activity, a series of abietic acid concentrations from 50 to 350 mgL^{-1} were assayed by MATA using the Domtar sludge. First and second spike results are shown in Figs 4.11 and 4.12, respectively. Generally the rate of methane production decreased as abietic acid concentration increased for both spikes. Total methane production in Fig. 4.11 should have been a constant value for all concentrations. The fact that it was not was probably caused by a leaky syringe that was used to inject the pure methanol and which was not noticed until after the injections had started. A small change in volume of methanol leads to a large change in methane production.

Field et al., (1988) reported inhibition to 50% control activity with 114 mgL^{-1} abietic acid. The purity of the abietic acid used by Field was not given and his concentration was based on a COD value of the solution. In this experiment 85% pure abietic acid was used and concentrations reported are corrected to actual abietic acid concentrations. For spike 1 (Fig. 4.11), 50% control activity was caused by an abietic acid concentration in the order of 300 mgL^{-1}. In spike 2 (Fig. 4.12), the assay did not go high enough to predict the value, although it would be above 350 mgL^{-1} of original abietic acid placed in the serum bottles.

5.3 Experiments Using Domtar Sludge

5.3.1 Toxicity of BCTMP-N vs BCTMP-NC Wastewaters

The results of the anaerobic toxicity assays for the analyses which used Domtar sludge are summarized in Table 4.2. For the centrifuged wastewater, BCTMP-NC, only the 40% dilution displayed slight inhibition in spike 1. Inhibition increased with wastewater concentration for spike 2 for the same wastewater. Soluble toxic substances which inhibit anaerobic bacteria were therefore masked in spike 1 degradation and their effects were evident in the spike 2 results. The 20%

BCTMP-NC wastewater never displayed inhibition which suggested that dilution to 20% and/or removal of the fiber rendered the wastewater free of toxic effects. In fact there may be a net beneficial effect at this dilution since 20% BCTMP-NC waste performed better than the control for both spikes 1 and 2.

In contrast to BCTMP-NC, the raw wastewater (BCTMP-N) toxicity increased with concentration for spike 1. The difference can be attributed to the presence of the fiber in BCTMP-N. All concentrations of BCTMP-N inhibited methane production in spike 2. Since 20% BCTMP-N wastewater was toxic in the second spike, it can be concluded that to remove the toxic effects, not only is dilution to 20% wastewater required, but also the fiber must be removed.

It must be noted that for spike 1 of both BCTMP-NC and BCTMP-N, wastewater was being degraded as well as the acetate/propionate spike. Higher wastewater concentration increases the organic loading which may have a beneficial or detrimental effect on the anaerobic microbial activity depending on the organic loading and the consequent increase in toxicant concentration. This may explain the higher than control activity of BCTMP-NC at two of the three wastewater concentrations in spike 1. It is not clear why BCTMP-NC at 40% in spike 1 exhibited slightly lower activity than that of the 60% wastewater but it does suggest that the interaction of organic loading and toxicant concentration is complex.

BCTMP-N wastewater in the second spike neither exhibited greater inhibition with concentration increase nor a greater inhibition at the 60% level than 60% BCTMP-NC wastewater. This was unexpected since the presence of the fiber added toxicity in 20% wastewater. It can be seen in Figs. 4.5 and 4.6 that 60% BCTMP-N wastewater degradation took much longer than either the blank or BCTMP-NC 60% wastewater degradation. As a result the BCTMP-NC bottles were inactive longer without an easily degradable carbon source before the introduction of the second spike. It is thought that the length of time the serum bottles remain in the presence of toxic substances without an easily degradable carbon source may have an effect on the apparent toxicity. Results on the shutdown and re-startup of laboratory scale continuous reactors fed with BCTMP-N waste also suggested that the length of time the sludge is without an easily degradable carbon source in the presence of BCTMP-N wastewater has an adverse effect on anaerobic activity (Kennedy (1989)). Experiments are underway to investigate this hypothesis.

5.3.2 Fiber Toxicity

5.3.2.1 Fiber Preparation

It should be noted that any volatile compounds would be aspirated off in the process so that compounds such as terpenes would not exist in significant concentrations in either MSF or MIF. The toxic compounds remaining in MSF and MIF are therefore non-volatile.

5.3.2.2 Toxicity of MSF VS MIF

Figs. 4.14 and 4.16 demonstrate that most if not all of the inhibitory substances contained in the fibers are soluble in methanol. Results listed in Table 4.2 indicate that MIF has no inhibition in either spike 1 or 2 whereas MSF inhibition is 27% at 100% concentration in spike 2 and 67% in spike 1. 200% MSF is even more inhibitory. It is possible that the methanol wash dissolved material that would normally not be dissolved in BCTMP wastewater or in the defined medium plus water. This was tested with raw fiber at 0.6 g/100 mL or the same proportionate strength as 100% MSF.

In Table 4.2 the activity for spike 1 single strength raw fiber content is similar to that caused by 100% MSF (see Figs. 4.17 and 4.18). In spike 2 the raw fiber inhibition was even greater than both 100% and 200% MSF. Two factors may explain this response. It may indeed take longer for the toxicants to dissolve from the raw fiber in the serum bottle than in methanol which would both help to decrease the toxicity in spike 1 and increase the toxicity in spike 2. Secondly, it was stated earlier that MSF was void of volatile substances. These substances may play a role in increasing the toxicity in spike 1 and to a lesser extent, as gasses escape, in spike 2.

5.3.3 Resin Acid Toxicity

Resin acid toxicity is also summarized in Table 4.2 and spike 2 toxicity is displayed graphically in Fig. 4.21. Generally in both spike 1 and 2 inhibition increases as the concentration of resin acids increases. At a resin acid concentration of 40 mgL^{-1} there is an improvement in activity with respect to the control in spike 1 and only 16% inhibition over the control in spike 2. Spike 1 of the resin acids exhibits a classical inhibition curve as discussed by McCarty (1964). Inhibition decreases as concentration increases until a certain level of toxicant is attained at which point an actual beneficial effect is seen. After this point, as the concentration of the toxicant increases, the inhibition increases.

5.3.4 Toxicity of Resin Acids VS BCTMP Wastewater

For this comparison only spike 2 will be considered since in the second spike all serum bottles received identical organic loadings. From Table 4.2, 40% BCTMP-NC exhibits 70 percent control activity. If Fig. 4.21 is entered on the abscissa at 70 percent control activity, then 40% BCTMP-NC wastewater corresponds to 140 mgL^{-1} resin acids. The actual resin acid concentration is 36 mgL^{-1} in the BCTMP-N wastewater. There is at most 14 mgL^{-1} resin acid in 40% raw wastewater and even less in centrifuged wastewater. Similarly 60% BCTMP-NC corresponds to 320 mgL^{-1} resin acids. Barring a synergistic effect of resin acids with a non-toxic compound in the wastewater it can be concluded that not all of the toxicity of BCTMP-N wastewater is caused by resin acids.

5.3.5 Toxicity of Methanol Soluble Fiber VS Resin Acids

Analyses have been done on the partition of resin acids into the soluble and non-soluble (fiber) phases of the wastewater (de Vegt 1988). At the very least 35% of the resin acids remain in the wastewater. More generally however, filtered wastewater contains 60% or more of the resin acid concentration present in the raw wastewater. Since the MSF was prepared from wastewater that had a resin acid concentration of 36 mgL^{-1}, 65% or 23 mgL^{-1} is the maximum possible concentration of resin acids in the reconstituted fiber. In order to achieve the same inhibition attained by MSF (100%) a resin acid concentration of 140 mg/L would have had to be present (see Fig. 4.21). It can be concluded that resin acids were not responsible for all the inhibition caused by MSF.

5.3.6 Individual Resin Acid Toxicity

The question remained to this point of the study whether all resin acids were inhibitory, or if only selected resin acids were. Figures 4.22 and 4.23 demonstrate that all resin acids tested slowed down the initial methane generation rate. This is not a surprising result given the structural similarity of the isomers in question (see Fig. 2.2). Pimaric acid seemed to have the least effect, but it was noted that on subsequent spikes, the percent control activity inhibition increased in the serum bottles containing pimaric acid (data are not included).

5.4 Specific Activity and Specific Loading

5.4.1 Specific Loading

The specific loading of the resin acid mixture (and for abietic acid) in the serum bottle experiments is given in Table 4.6. These loadings can be compared to the specific loading of resin acids to the reactor biomass which is given in Fig. 4.29. The 80 mgL^{-1} concentration of the resin acid mixture in the serum bottle was roughly equivalent to the 100 mgL^{-1} resin acid mixture spike in the continuous experiment. These comparable values indicate that the lack of inhibition found in the continuous system (which will be discussed later) could not be accounted for by lower specific loading of toxicant, or by the actual concentration of the toxicant.

5.4.2 VSS Concentration in the Serum Bottle Experiments

Serum bottle VSS concentrations are recorded in Tables 4.5 and 4.6. Concentrations ranged from 4.07 to 8.30 gL^{-1}, but were generally constant within each set of experiments.

5.4.3 Specific Activity in Serum Bottle Experiments

The specific activity of the Domtar sludge varied from 0.14 gCODr (g VSS)$^{-1}$ d^{-1} for the BCTMP wastewater experiment to 0.012 gCODr (g VSS)$^{-1}$ d^{-1} for the MATA done on the UASB reactor biomass. Variation in control specific activity for spike 1 is to be expected as the substrates varied from BCTMP wastewater to acetate/propionate to methanol. The variation that occurred in control activity for spike 2 was not expected.

Spike 2 variations in control specific activity varied from 0.14 to 0.041 gCODr (g VSS)$^{-1}$ d^{-1}. In spike 2 all bottles received the same injection of acetate/propionate, and none of the controls received a toxic dose or wastewater. However, the controls did receive varying amounts of methanol in spike 1, which can have toxic effects, and which may help to explain the discrepancy. Change in experimental conditions may have also played a role in the spike 2 control activity variation. The pH of the serum bottles was monitored and did not vary from neutrality so is not considered to be a possibility (data not included). Activity data from the MATA was not included as the biomass was active prior to the start of the experiment.

5.5 Degradation or Adsorption of Resin Acids

The first part of this work leads to the conclusion that resin acids are inhibitory to anaerobic bacteria in anaerobic batch assays. The fate of the resin acids in an anaerobic environment is the subject of this section. As mentioned earlier, resin acids have been reported to be degraded in a UASB reactor by McFarlane and Clark (1988). The object of this study was to do a mass balance on resin acids in a batch assay and to determine whether or not degradation and/or adsorption occurs.

Dehydroabietic acid was not degraded according to McFarlane and Clark. Therefore, it was chosen as a control. Levopimaric acid was said to be degraded by the same authors. Since the purity of levopimaric acid as supplied was greater than 99%, it was chosen as a representative of the "degradable" resin acids.

Two durations of time were used for exposure of the biomass to resin acids. Studies by Liver (1989) have indicated that substantial adsorption of resin acids onto biomass in batch studies occurs within the first few hours of exposure. For this reason a 1 hour length of exposure was chosen. A longer exposure was chosen to give some opportunity for the biomass to degrade the resin acids, and 1 month was chosen arbitrarily.

The purpose of the experiment was foiled by two factors. 1)The total recovery of resin acids from the system was low ranging from 12 to 60% of the initial concentration of resin acid (Fig. 4.24). If the recovery over time had gone consistently down then degradation may have been concluded. However, total recovery after 1 month of exposure to the biomass was greater than the recovery after 1 hour of exposure. More surprisingly, recovery of resin acid from the aqueous solution with no sludge exposure was only 42% for the levopimaric bottle and 62% for the dehydroabietic bottle. 2) Levopimaric acid (99% pure) as recovered from all three bottles was a mixture of three resin acids (Fig. 4.25). Therefore, the combination of poor recovery, and unanticipated impurity of the recovered resin acids, made it impossible to conclude adsorption or degradation.

There are two possible reasons for low recovery. 1) the purity of the resin acids from the supplier was lower than indicated. 2) The method of resin acid extraction and quantification is not satisfactory. Chromatographs of the purity of the resin acids which were included with the shipment preclude the former. Inconsistency in recovery (i.e. recovery was greater after 1 month than after 1 week) and problems associated with resin acid extraction and quantification support the latter (Hall, 1989).

Regardless of the problems associated with resin acid extraction, the result cited in Fig. 4.25 remains to be explained. As mentioned earlier, the pure levopimaric acid recovered in this experiment was a mixture of three resin acids when analyzed. The proportion of each resin acid changed over time, with dehydroabietic acid becoming predominant after 1 week exposure, and almost exclusive after 1 month. In the serum bottles that were spiked with pure dehydroabietic acid, only dehydroabietic acid was found (data not shown). Assuming that the errors in extraction were consistent in each of the assays, it can be concluded that the shift in isomer predominance of resin acids is a real phenomenon. And future conclusions of degradation of resin aids must also consider possible isomerization.

5.6 Resin Acids in a UASB Reactor

The batch assays gave evidence of resin acid inhibition of anaerobic bacteria. However, batch assays results are not necessarily transferable to the continuous reactor treatment systems used in industry. Therefore, a UASB laboratory scale reactor was set up to evaluate resin acid toxicity. Acetic acid was used as the sole soluble carbon source to simplify the system for two reasons. By using acetic acid, any decrease in reactor performance could be directly attributable to interference with acetoclastic methanogen activity. Also, treatment efficiency could be easily measured by measuring influent and effluent acetic acid concentrations.

5.6.1 General Performance of UASB

General reactor performance, as presented in Sec. 4.10.2.1 and in Fig. 4.26., was stable upon baseline generation (after 20 days of operation). Poor performance occurred during days 18-21 because of problems with the recirculation pump and broken feed lines. During days 31-35 the reactor drain tube plugged continually and the recycle pump control blew several fuses before being replaced. Other than these two periods, the reactor run of 77 days went well. The break in the graph data from Days 67-70 came as a result of a planned shutdown of the reactor.

5.6.2 UASB Performance Under Toxic Load

Fig. 4.27 compares reactor efficiency (based on influent/effluent acetic acid concentrations) to toxic loadings. Toxic load of resin acids is given as a pure concentration and also as a specific loading. The specific loading is the ratio of actual mass of resin acid in the reactor to the reactor biomass as VSS. Efficiency was not affected by the presence of the resin acid mixture, neither at a spike load of 100 mgL^{1} nor by abietic acid at a spike load up to 400 mgL^{-1}. In addition, treatment efficiency was not affected by continuous feed of abietic acid up to a concentration of 600 mgL^{-1}.

5.6.3 Increased Effluent VSS

Reactor pH, alkalinity and effluent TSS/VSS are given in Fig. 4.29. Both pH and alkalinity were stable throughout the run. Average values of effluent VSS changed as a result of resin acid loading. With no resin acid load, average VSS was 70 mgL^{-1}. Under toxic load, the average VSS jumped to 120 mgL^{-1}. Some of the effluent VSS could have been insoluble resin acid. However, as indicated in the results, there was a visible white precipitate floating in the interstitial space between the biorings which was presumably resin acid. Assuming that some of the resin acid accumulated in the biomass, and considering the possible amount of resin acid lodged in the floating precipitate, then the resin acid exiting the reactor would not account for the increase in effluent VSS. Total reactor biomass volume decreased from 1.2 L to 850 mL over the course of the run which corresponded to a reactor biomass concentration decrease from 13.9 to 9.9 gL^{-1}. Evidence indicates that a good deal of the biomass loss was caused by the toxic loading.

5.6.4 Reactor Biomass Settleability

Settleability data, which are summarized in Fig. 4.28, supports the idea that toxic loading led to increased biomass washout. One measure of biomass washout is the upflow velocity at which 50% of the biomass has exited. Prior to toxic loading, the upflow velocity causing 50% of biomass to exit was 27 mh^{-1} (V_{50}). When settleability was measured again on Day 65, after the reactor had been fed continuously abietic acid at a concentration of 300 mgL^{-1}, the V_{50} dropped to 19 mh^{-1}. Such a drop indicates a substantial change in settleability characteristics of the biomass and is predictive of a greater effluent VSS.

5.6.5 UASB Reactor Biomass Specific Activity

Specific activity of the UASB reactor biomass was measured on the same days as the settleability and is recorded in Table 4.4. The measured activity dropped by 19% over the time period between Day 30 and Day 65. No loss of overall reactor performance resulted.

5.6.6 UASB Reactor Shutdown

On day 65, the UASB reactor was shutdown for a period of three and one-half days. The purpose of the shutdown was to mimic a maintenance shutdown at a full scale UASB reactor that resulted in reactor failure. The full scale plant was shutdown for approximately three days. Resin acid accumulation was suspected to be a cause of the failure. To this point in the run, 6 850 mg of resin acid had been fed to the reactor. If all of the resin acid had accumulated the specific loading of resin acid would be 200 mg of resin acid per g VSS [mg RA (g VSS)$^{-1}$] . Sludge recovered from the plant that failed had a resin acid concentration of 900 mgL^{-1} which would represent a specific loading (assuming 40 gVSS*L^{-1}) of 22 mgRA* (gVSS)$^{-1}$ (Gorur, 1989). Therefore, if only 10% of the resin acid fed to the reactor had accumulated, there would have been a similar specific load to that of the failed full scale reactor.

The laboratory reactor performed well during and after startup. The HRT and OLR in use just prior to shutdown were used for the startup conditions. The only difference was that resin acids were not mixed with the acetate feed (see Appendix C for startup profile).

5.6.7 UASB Reactor Biomass MATA

After the reactor had been continuously fed abietic acid at a concentration of 600 mgL^{-1} and before the reactor was finally shut down, a MATA was done to test the toxicity of resin acids on active biomass. Methanol was not used in this MATA to dissolve the resin acids in order to mimic the feed to the UASB. Spike 1 results are illustrated in Fig. 4.30. Little effect is shown by the addition of 300 mgL^{-1} of abietic acid. The addition of 300 mgL^{-1} of the resin acid mixture displayed slightly more inhibition.

A very interesting result occurred when the serum bottles were spiked with acetate/propionate for the second time (Fig. 4.31). The second spike took place just after the final reading of spike 1 on Day 5. In both pairs of serum bottles which had the resin acid mix or abietic acid spike, there was a substantial decrease in the initial rate of methane production compared to that of the control. The resin acids which had shown little toxicity to the biomass which was fresh out of the reactor and which had been continuously exposed to a degradable carbon source, proved highly toxic to the biomass that had been sitting a few days without a easily degradable soluble carbon source.

Actual concentrations of resin acid mixture or abietic acid in the serum bottles cannot be assumed to be 300 mgL^{-1}. Since the biomass had been continuously receiving acetic acid feed containing abietic acid at a concentration of 600 mgL^{-1}, the concentration of resin acid provided to the serum bottles by the biomass was not assessed. It is possible that this additional amount of resin acid, in conjunction to the spike, lead to the drastic reduction in specific activity (see Table 4.6).

5.6.8 Summary

Treatment efficiency in the UASB reactor was maintained in spite of 1) the presence of resin acids which have proved inhibitory in batch assays 2) VSS washout, 3) a slight decrease in apparent biomass specific activity. Biomass freshly drawn from the UASB was not significantly affected by the presence of 300 mgL^{-1} of the resin acid mixture or by the same concentration of abietic acid, but a dramatic decrease in methane production was evident on the addition of a second spike of acetate/propionate to the same biomass (see Table 4.6). A similar increase in toxicity was seen in the experiment using BCTMP-NC wastewater and Domtar sludge where BCTMP-NC wastewater at higher concentrations was more toxic than the same wastewater with fiber (Sec. 5.3.1). It was suspected to be caused by the presence of a toxin(s) in conjunction with a prolonged period of time without a readily available soluble carbon source.

CHAPTER 6

CONCLUSIONS

The following three conclusions are based on a sludge that was not acclimatized to CTMP wastewater and are a result of the MATA work.

1) Inhibition can be masked in the first spike of the MATA. Differential organic loading caused by variation in wastewater concentrations may be a cause.

2) Anaerobic toxicity in BCTMP-N wastewater is partly soluble and partly fiber associated. The anaerobic toxicity that is removed with the fiber is methanol soluble. The methanol washed fiber has no toxicity.

3) Resin acids inhibit anaerobic activity but BCTMP wastewater toxicity to anaerobic bacteria can only be partially explained by the presence of resin acids. Experiments suggest that less than 10% of the inhibition can be directly attribuble to resin acids. The possible synergistic effects that other compounds may have in conjunction with the resin acids in BCTMP-N wastewater toxicity have not been excluded.

The remaining conclusions stem from the work done on resin acid degradation and resin acid toxicity in a continuous system

4) Acute shocks of resin acid of concentrations up to 400 mgL^{-1} and of specific loadings up to 38 mg RA (g VSS)$^{-1}$ d^{-1}, to a UASB reactor have little effect on reactor efficiency. The concentrations and specific loadings of the resin acid shocks given to the batch system, and which proved inhibitory, were comparable to those administered to the continuous system.

5) Chronic dosing of concentrations of abietic acid up to 600 mgL^{-1} to a UASB reactor did not effect reactor efficiency.

6) The transfer of inhibition information from batch assays to continuous systems should be done with caution.

CHAPTER 7

FUTURE RESEARCH

1) Develop a dependable method of resin acid extraction and quantification.

2) Investigate adsorption kinetics of resin acids onto biomass.

3) Try to reproduce reactor failure of the full scale plant by continuous feeding of resin acid to a continuous system with periodic shut-downs, but with an understanding of (2) above.

4) Depending on (1), investigate degradation of resin acids in an anaerobic system using a mass balance on the whole system.

5) Depending on (4), investigate breakdown products of resin acids for inhibition of anaerobic metabolism.

6) Investigate whether resin acid concentration or specific loading is the governing factor in batch test inhibition.

7) Investigate synergistic effects causing inhibition between resin acids and another compound(s) present in BCTMP wastewater. Long chain fatty acids should be a priority (see Wu *et al.*, 1989). The investigation should include both batch assays and a continuous system.

8) Possible isomerization between resin acids should be investigated prior making any conclusions on resin acid degradation.

9) Why do resin acids inhibit activity of batch assay biomass but do not affect continuously fed biomass or biomass fresh from a continuously fed reactor?

10) Are there other compounds, perhaps more toxic than resin acids, present in BCTMP wastewater?

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APPENDIX A

APPENDIX A

INHIBITION TEST: MODIFIED ANAEROBIC TOXICITY ASSAY

In the Modified Anaerobic Toxicity Assay (MATA), varying concentrations of wastewater, a nutrient supplement, and an acetate/propionate substrate are combined and inoculated with an active anaerobic culture in a closed serum bottle. Toxic effects of the wastewater on the methanogenic and/or acetogenic microorganisms may be indicated by differences in methane production patterns (eg. rates of methane production or by the presence of lag periods).

Test Procedure

1. Prepare the defined medium, volatile organic stock, sulfide solution, and the dilution water (NOTE: make all solutions with glass distilled water, GDW).

2. Maintain all assay constituents at 35^{o} C in a water bath (or 35^{o} C room) and keep oxygen-free during dispensing by continuous gassing with 20/80 CO_2/N_2. This includes solutions, sludges, wastewater and equipment (eg. bottles, syringes, pipettes, etc.).

3. Gravity settle the wastewater for approximately 30 minutes to reduce the suspended solids concentration if necessary.

4. Deoxygenate the wastewater (supernatant) by flushing with CO_2/N_2 gas (approximately 10 min). Adjust the pH to approximately 7.0 with 10 N NaOH or H_2SO_4.

5. Anaerobically transfer 10 to 30 mL of wastewater into calibrated 160 mL serum bottles and weigh capped. Depending on the concentration of the wastewater, select final assay volume fractions of 5% to 60% (volume wastewater/total liquid volume). For high strength wastewater, select the highest volume fractions to produce maximum final assay soluble COD concentration of 12 000 mg/L.

6. Add dilution water to bring the interim assay volumes to 40 mL and weigh capped.

7. Add 0.5 mL sulfide solution to each serum bottle and weigh. Buffle flush the serum bottle contents until the resazurin redox indicator becomes colourless.

8. Sludge treatment: anaerobically transfer a quantity of sludge to a pre-weighed, pre-flushed erlenmyer flask and weigh. Add defined medium to the sludge in the ratio 5:4 by weight.

9. While magnetically stirring the sludge/defined medium mixture, anaerobically dispense 18 mL of the mixture into the serum bottles, cap, seal, and weigh. Incubate at 35^{o} C on a shaker.

NOTE: When dispensing the mixture into the serum bottles is half finished, dispense 18 mL into two pre-weighed centrifuge tubes. Use these for SS/VSS measurement.

10. Each bottle should be duplicated. Tests should be performed in triplicate (eg. 5%, 35%, and 60% v/v) with 2 spiked controls. The controls are treated identically as the other 6 bottles except dilution water is substituted for the wastewater.

11. After 30 minutes, inject 2 mL of the volatile organic acid solution with a glass syringe (t=0) and weigh. Total volume 50.5 mL.

12. Measure the initial soluble COD of each bottle. After spike injection, mix the bottle contents, sample 1 mL of mixed liquor (16G needle), micro-centrifuge, and perform a COD on the supernatant. Total volume 49.5 mL. Weigh.

13. Adjust the headspace to 1 atm by first putting an overpressure of CO_2/N_2 gas in the serum bottle. Then insert a needle to release the excess gas. The pressure may be checked with a gauge if desired. Place on a shaker.

14. Monitor gas quality initially (t=0) and at least once for every 10 mL biogas produced. Recommend sampling directly from manometer when gas quantity is measured. Experiment ends when methane production is low and constant (endogenous activity).

NOTE: Base the sampling schedule on the known methane production rate of the sludge or perform a trial run to establish the appropriate schedule.

15. Estimate the initial SS/VSS concentration. Determine the SS/VSS concentration of the sludge/defined medium mixture (see 9.) and the wastewater in duplicate. Calculate the SS/VSS concentration in each serum bottle.

16. If the serum bottles are to be reinjected go to step 17. Measure the final COD (total and soluble), pH, and SS/VSS of each serum bottle.

17. Weigh the bottles. Mix the bottle contents, sample 1 mL mixed liquor (16 gauge needle), micro-centrifuge, and perform a COD on the supernatant. Weigh the bottles. Flush for 5 minutes to ensure a 20/80 CO_2/N_2 gas mix (no methane). Inject 2 mL of the volatile organic acid solution with a glass syringe (t=0) and weigh. Repeat steps 12. to 16..

NOTE: Inhibitory effects of the wastewater on the anaerobic micro-organisms is identifiable by comparing the rate and extent of the methane production in the MATAs to that observed in the spiked controls. One might express percent inhibition as follows:

$$\%\ \text{inhibition} = \left[1 - \frac{\text{activity of exposed sludge}}{\text{activity of control}}\right] *100$$

$$\text{where activity} = (x\ \text{L}\ CH_4/\text{d})*(2.53\ \text{g COD/L}\ CH_4\ @\ 35\ \text{C}) / (\text{g VSS in bottle})$$

$$= \text{g CODr/g VSS.d}$$

Inoculum

Most reliable results obtainable when an inoculum that is fresh, active, consistent, and acclimated to the wastewater is used. Immerse the sludge in a 35^{o} C water bath and gas with CO_2/N_2 during dispensing.

Manometer

During a gas volume reading, slightly agitate the serum bottle.

Adjust the pressure in the manometer to 1 atm by raising or lowering the reservoir to equilibrate the liquid level to that in the measuring cylinder.

DEFINED MEDIUM

Combine the stock solutions in the proportions listed.

Boil for 5 minutes.

Add 3.4 g of $NaHCO_3$.

Cool to 35 C.

Top to 1 L with GDW.

Flush with CO_2/N_2.

Store in fridge.

Volatile Organic Acid (VOA) Stock

Dissolve 37.5 g sodium acetate + 13.25 g sodium propionate in 1 L GDW.

Flush with CO_2/N_2 gas.

Store in fridge.

Sulfide Solution

25 g/L $Na_2S.9H20$

Prepare in small aliquots in freshly boiled GDW just prior to use.

Dilution Water

Deoxygenate GDW by boiling 5 min while flushing with CO_2/N_2 gas. While cooling, flush the headspace. Transfer anaerobically to serum bottles, cap and seal. Cool to incubation temperature (35° C) in a freezer in tightly capped bottles.

NOTE: for all anaerobically treated solutions stored in serum bottles, ensure an overpressure of CO_2/N_2 gas. Flush syringes before removing liquid and repressurize bottles as required.

APPENDIX B

APPENDIX B

UASB REACTOR PROFILES

Methanol spike profiles

First Methanol Profile--Day 47

In this profile, the methanol concentration was not monitored. Profile data are listed in Table B.1.

Table B.1 Injection of one (1) mL of methanol with no resin acids--Day 47

	Time (h)	pH	Feed Acids (mgL^{-1})	Recirc. Acids (mgL^{-1})	Gas Prod. (*100mL)	CH4 (%)
	-1.0	7.78	2700	195	0	85
	-0.5	7.78			1	85
INJ.	0.0	7.78		190	2	85
	0.5	7.78	2700	183	3	86
	1.0	7.78		178	4	86
	2.0	7.81		182	5	86
	3.0	7.77	2660	180	7	86
	4.0	7.78		190	9	86
	5.0	7.81		168	11	86
	6.0	7.86		168	13	86
	7.0	7.85	2540	171	15	86
	8.0		3120*			

*	new acetate feed was added
"-" time	indicates prior injection
INJ.	time when methanol was injected

Second Methanol Profile--Day 57

In this profile, the methanol concentration was monitored. Profile data are listed in Table B.2.

Table B.2 Injection of five (5) mL of methanol with no resin acids--Day 57

Time (h)	pH	Feed Acids (mgL^{-1})	Recirc. Acids (mgL^{-1})	Recirc. Methanol (mgL^{-1})	Gas Prod. (*100mL)	CH4 (%)
-1.0	7.65	2962	96	0	0	84
INJ. 0.0	7.65	2920	110	0	2	84
0.25			105	1254	2	84
0.5	7.65		104	1093	3	84
1.0	7.66		101	1030	4	84
2.0	7.67	2972	94	911	6	84
3.0	7.66		102	873	8	84
4.0	7.65		93	789	10	84
5.0	7.67		100	751	12	84
6.0	7.68	2925	98	723	14	84
9.8	7.68		91	570	22	84
11.8	7.67		88	500	27	84
23.0	7.67	2926	91	96	51	84
28.0	7.67		85	0	63	83

"-" time indicates prior injection

Resin Acid Injection Profiles

Resin Acid Mixture Profile Without Methanol--Day 36

This was the first resin acid spike and was done without dissolving the resin acids in methanol. Profile data are listed in Table B.3.

Table B.3 Injection of 336 mg of the resin acid mixture making a final reactor concentration of 100 mgL^{-1}---Day 36

	Time (h)	pH	Feed Acids (mgL^{-1})	Recirc. Acids (mgL^{-1})	Gas Prod. (*100mL)	CH4 (%)
	-.75	7.75	2930	250	0	84
INJ.	0.0	7.73	2930	250	1	84
	0.5	7.72		254	2	84
	1.0	7.73	3030	246	4	85
	2.0	7.72		237	6	84
	3.0	7.72		248	8	85
	4.0	7.71		243	11	85
	5.0	7.72	3090	240	13	84
	6.0	7.70		233	16	84
	7.0	7.70		233	18	85
	30	7.79	3000	210	74	85

Resin Acid Profiles with Methanol

Resin Acid Mixture to 100 mgL^{-1} Reactor Concentration - Day 52

The resin acid mixture was dissolved in 1.0 mL of HPLC methanol and injected into the UASB reactor. The reactor reaction to the resin acids is tabulated in Table B.4.

Table B.4 Injection of 349 mg of the resin acid mixture dissolved in 1.0 mL of methanol making a final reactor concentration of 100 mgL^{-1}---Day 52

	Time (h)	pH	Feed Acids (mgL^{-1})	Recirc. Acids (mgL^{-1})	Gas Prod. (*100mL)	CH4 (%)
	-1.0	7.65	3040	140	0	84
	-0.5	7.64	2965	135	1	84
INJ.	0.0	7.77		149	2	84
	0.5	7.67		150	4	84
	1.5	7.64	2924	154	5	84
	2.5	7.62		174	7	84
	3.5	7.67	2995	171	9	84
	4.5	7.62		171	12	84
	5.5	7.67		164	14	84
	8.5	7.68	3022	171	21	84
	12.5	7.63	3055	136	32	84
	24.5	7.67	2990	134	67	84

Abietic Acid Spike to 400 mgL^{-1} Reactor Concentration - Day 62

A mass of 2.013 g of abietic acid was dissolved in 5.0 mL of HPLC methanol and injected into the UASB reactor (see Table B.5).

Table B.5 Injection of 2.013 g of 85% abietic acid dissolved in five (5) mL of methanol to bring the reactor concentration to 400 mgL^{-1}--Day 62

	Time (h)	pH	Feed Acids (mgL^{-1})	Recirc. Acids (mgL^{-1})	Recirc. Methanol (mgL^{-1})	Gas Prod. (*100mL)	CH4 (%)
	-1.0		2904	107	0	0	
	-0.5	7.68			0	1	84
INJ.	0.0	7.68	2935	108	0	2	84
	0.25			111	869	3	84
	0.5			123	867	4	
	1.5	7.69		144	745	6	84
	2.5	7.70	2972	122	716	8	83
	3.5	7.67		134	688	10	84
	4.5	7.66	2854	140	661	12	84
	5.5	7.65		154	627	14	84
	6.5	7.62	2845	156	604	16	84
	7.5	7.63		158	578	18	84
	10.3	7.70	2840	131	475	27	84
	11.5	7.70		146	441	29	84
	23.0	7.64	2870	161	206	51	84
	24.0			171	206	54	84
	25.0			151	177	56	
	27.0			140	137	61	
	28.0	7.70	2743	136	111	63	84
	31.0	7.73		127	<50	70	84

APPENDIX C

APPENDIX C

START-UP PROFILE OF UASB REACTOR ON DAY 71

UASB Start-up Profile on Day 71

The UASB reactor was started with a 1.0 day HRT and a feed concentration of 3000 mgL^{-1} acetic acid, which were the conditions at the time of shutdown. The gas meter was not functioning well at the beginning of the start-up which explains the poor gas production.

Table C.1 Start-up of UASB on day 71

Time (h)	pH	Feed Acids (mgL^{-1})	Recirc. Acids (mgL^{-1})	Gas Meter (*100mL)	CH4 (%)
0.0	7.60	3057	14	0	
1.0			134	0	80
2.0			214	0	
3.0			269	0	
4.0			325	0	
5.0	7.60	3047	371	0	
6.0			406	2	
7.0	7.57		426	3	81
8.0			436	5	
24.0	7.62	3114	336	37	
53.3	7.62	3027	250	95	84
76.3	7.60	3040	222	141	84

APPENDIX D

APPENDIX D

Resin Acid Information and Chromatographs

Analyses of Resin Acids

Column - Chromatographic Specialties OV 101, Capillary Fused Silica, 15 M length.
Products are derivatized to Methyl Esters, with N-Nitrosylmethyl urea.

Temperature Gradient -
190° + $1C^{\circ}$/min. for 10 min.
+ $2C^{\circ}$/min. for 10 min.
+ $3C^{\circ}$/min. for 10 min.
To $250C^{\circ}$
Internal Standard with Unknowns is C-17.

Gas - Helium

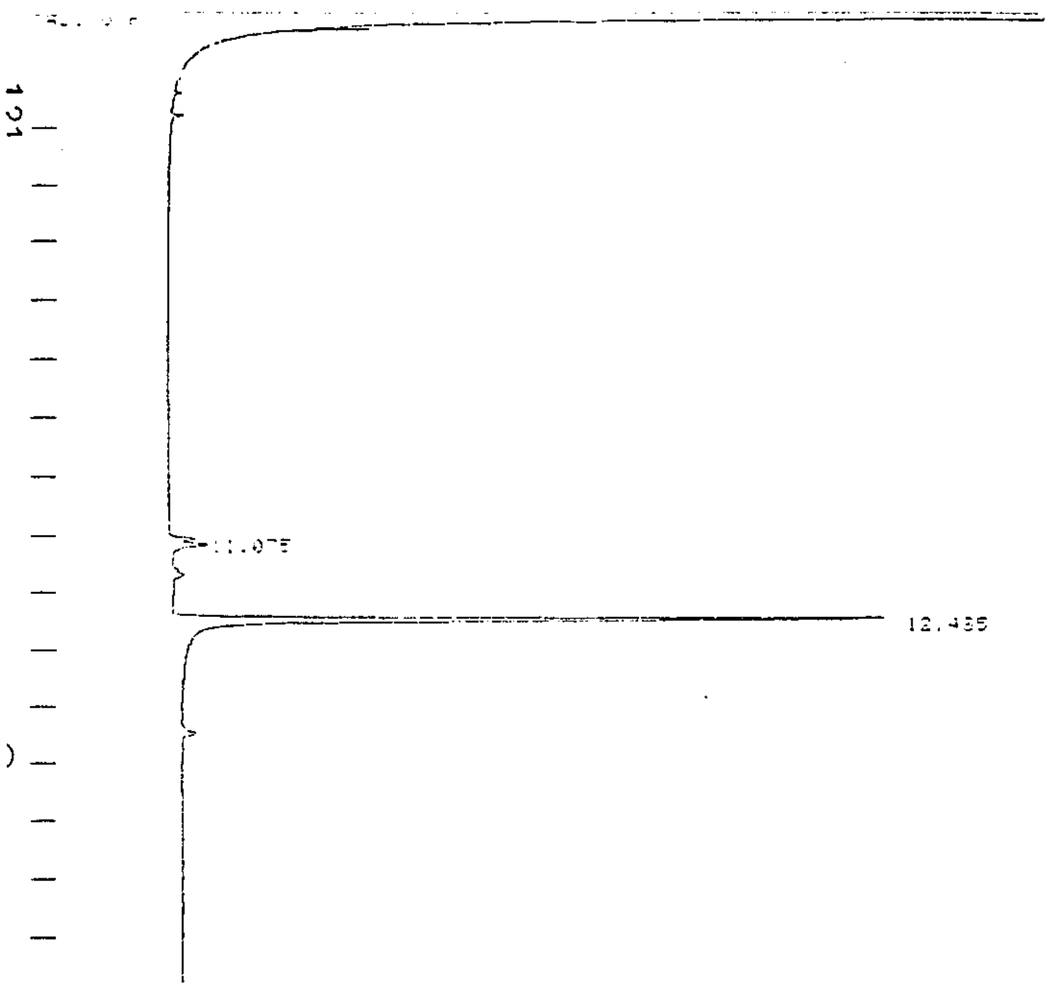
Flow Rate -
Capillary column 0.24 mm.
10 - 12 psi head on column
30 mL/min. of madeup gas through detector.

Resin Acid Purity

The level of purity of the resin acids along with the contaminants are listed in Table D.1. Chromatographs are given in Figs D.1 to D.7.

Table D.1 Purity of the Individual Resin Acids

Resin Acid	%	Contaminant(s)	%
Abietic	92	Other resin acids	8
Dehydroabietic	99+	Other resin acids	<1
Neoabietic	99+	Other resin acids	<1
Isopimaric	99+	Other resin acids	<1
Levopimaric	99+	Other resin acids	<1
Isopimaric	99+	Isopimaric acid	<1
Pimaric	88	Other resin acids	12
Sandaracopimaric	89	Isopimaric acid	11



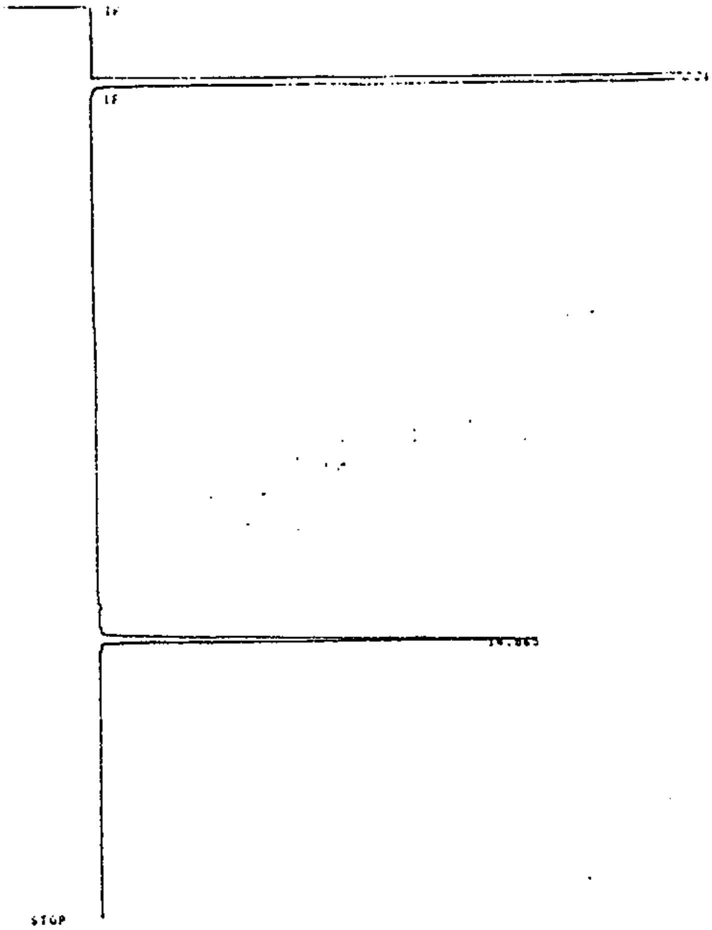
ABIETIC ACID 97+%

```
TITLE:                                              21:46    1 JAN 83

CHANNEL NO: 1       SAMPLE:                        METHOD: 1

PEAK   PEAK          RESULT       TIME         AREA     SEP
 NO    NAME                       (MIN)        COUNTS   CODE
   1                 99.4444      1.990       2740730   BV
   2                  0.0115      2.242           319   T
   3                  0.0037      3.782           107   BB
   4                  0.0148     11.075           407   BV
   5                  0.0287     11.169           791   VB
   6                  0.4967     12.489         13690   BB

TOTALS:             100.0000                  2756040
```

Figure D.1 Chromatograph of Abietic Acid

```
* RUN # 404     MAY 19, 1988  10:47:55
START-Source
```

R-001 LOT: SW2



```
Closing signal file M:SIGNAL  .BNC

RUN#   404            MAY 19, 1988  10:47:55

SIGNAL FILE: M:SIGNAL.BNC

DEHYDROABIETIC ACID

AREA%
       RT      AREA TYPE  WIDTH       AREA%
   14.065    100127   PB    .083  100.00000

TOTAL AREA= 100127
MUL FACTOR=1.0000E+00
```

Figure D.2 Chromatograph of Dehydroabietic Acid

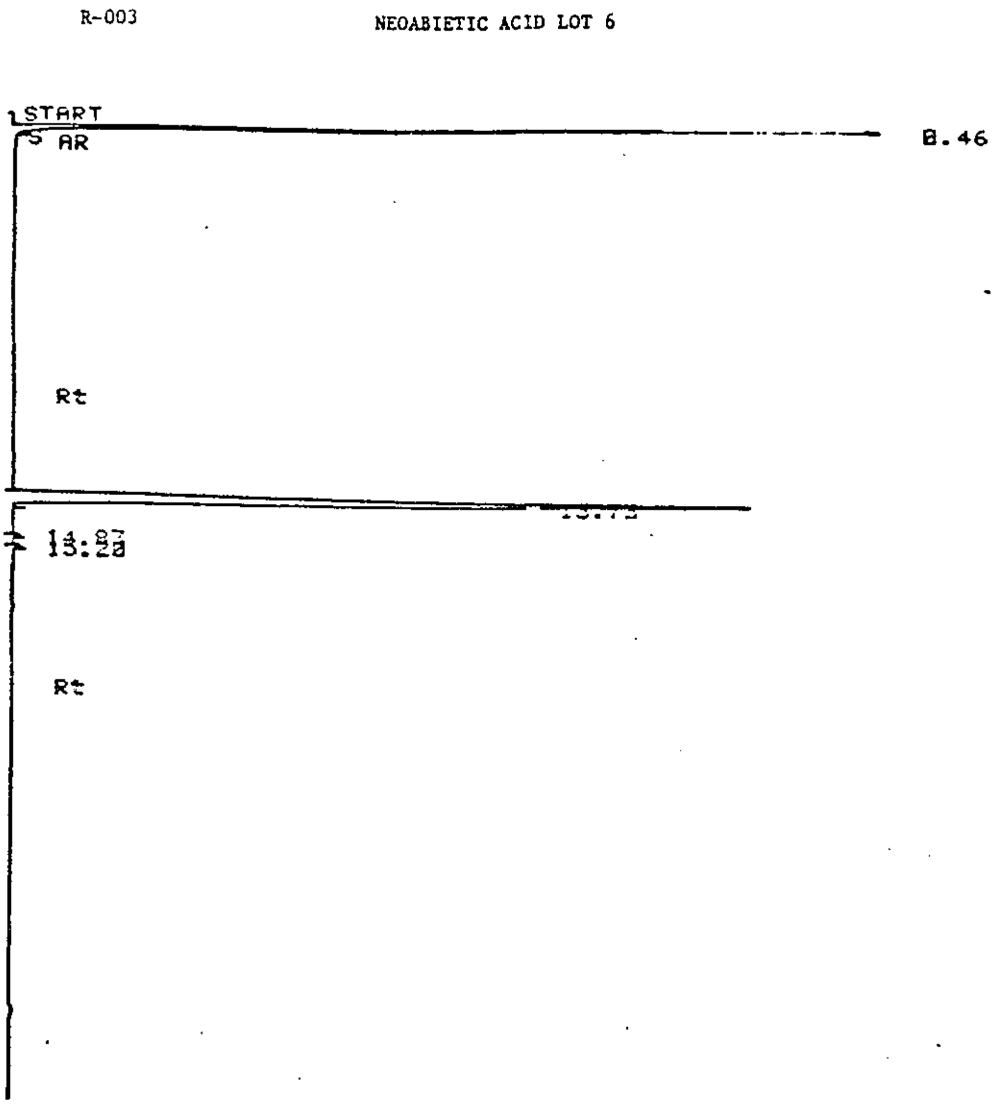
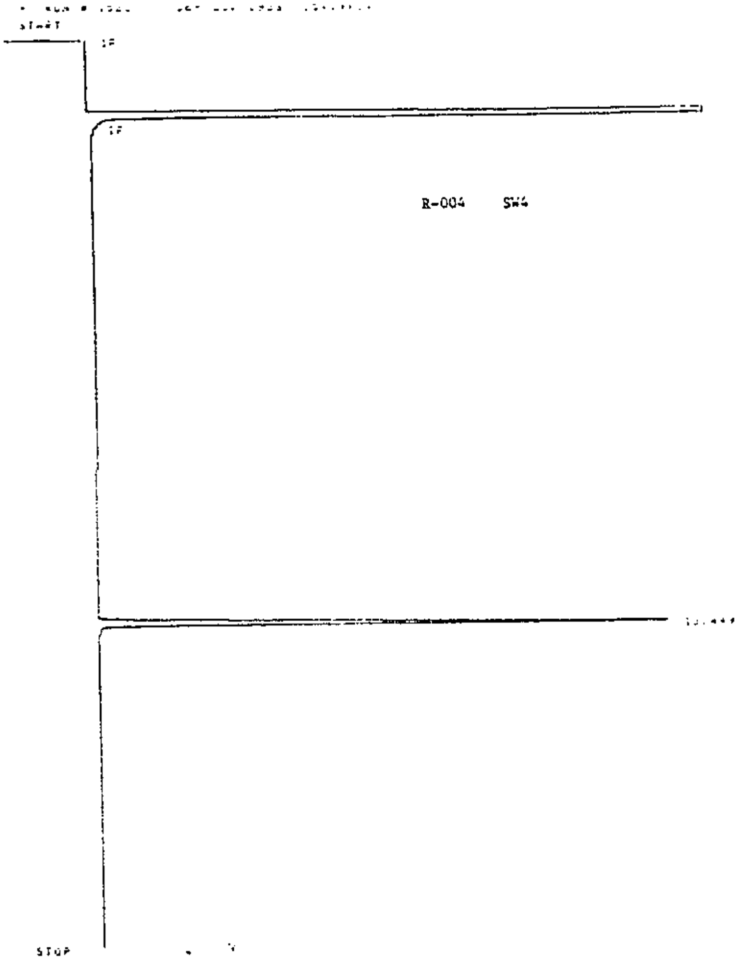
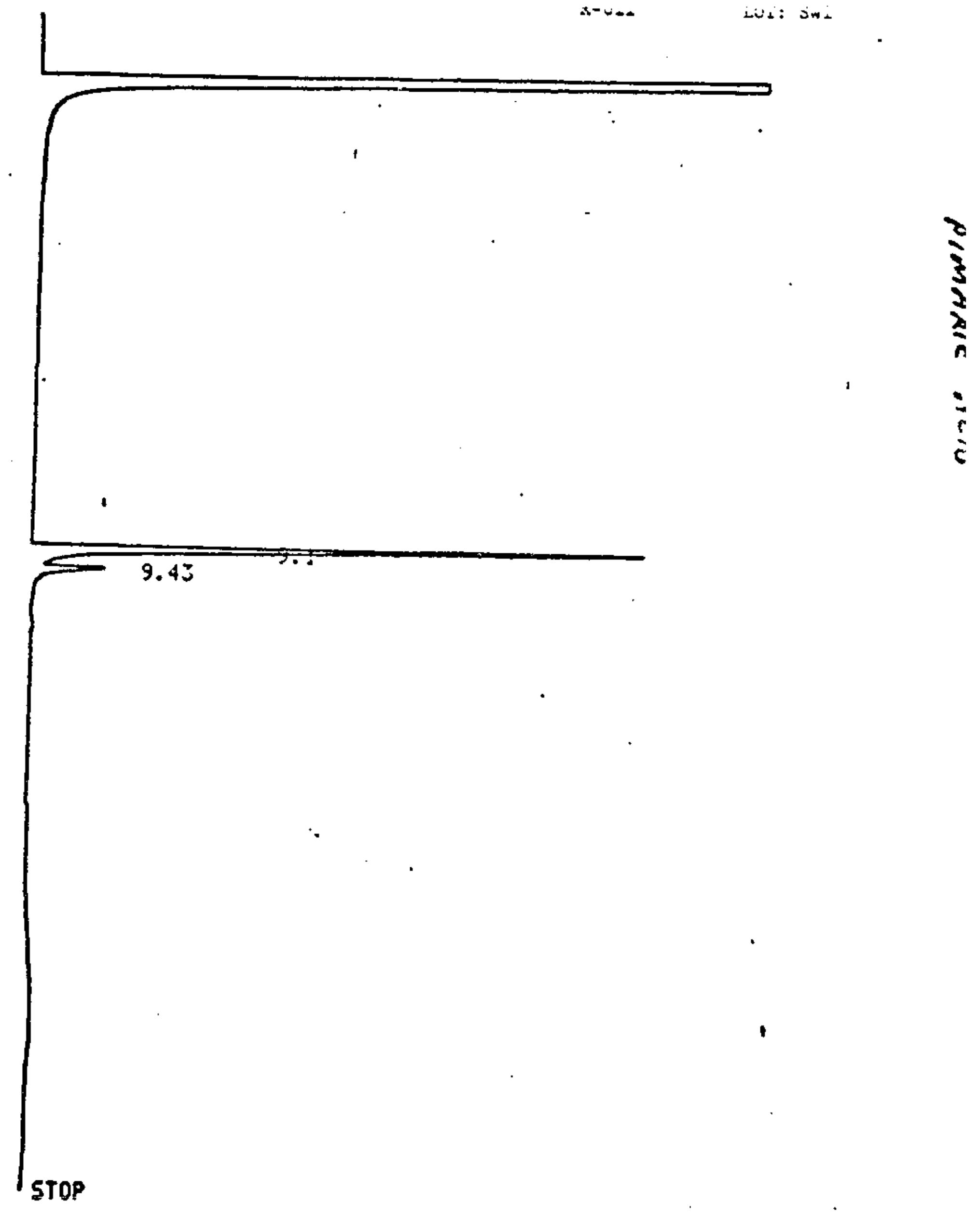


Figure D.3 Chromatograph of Neoabietic Acid



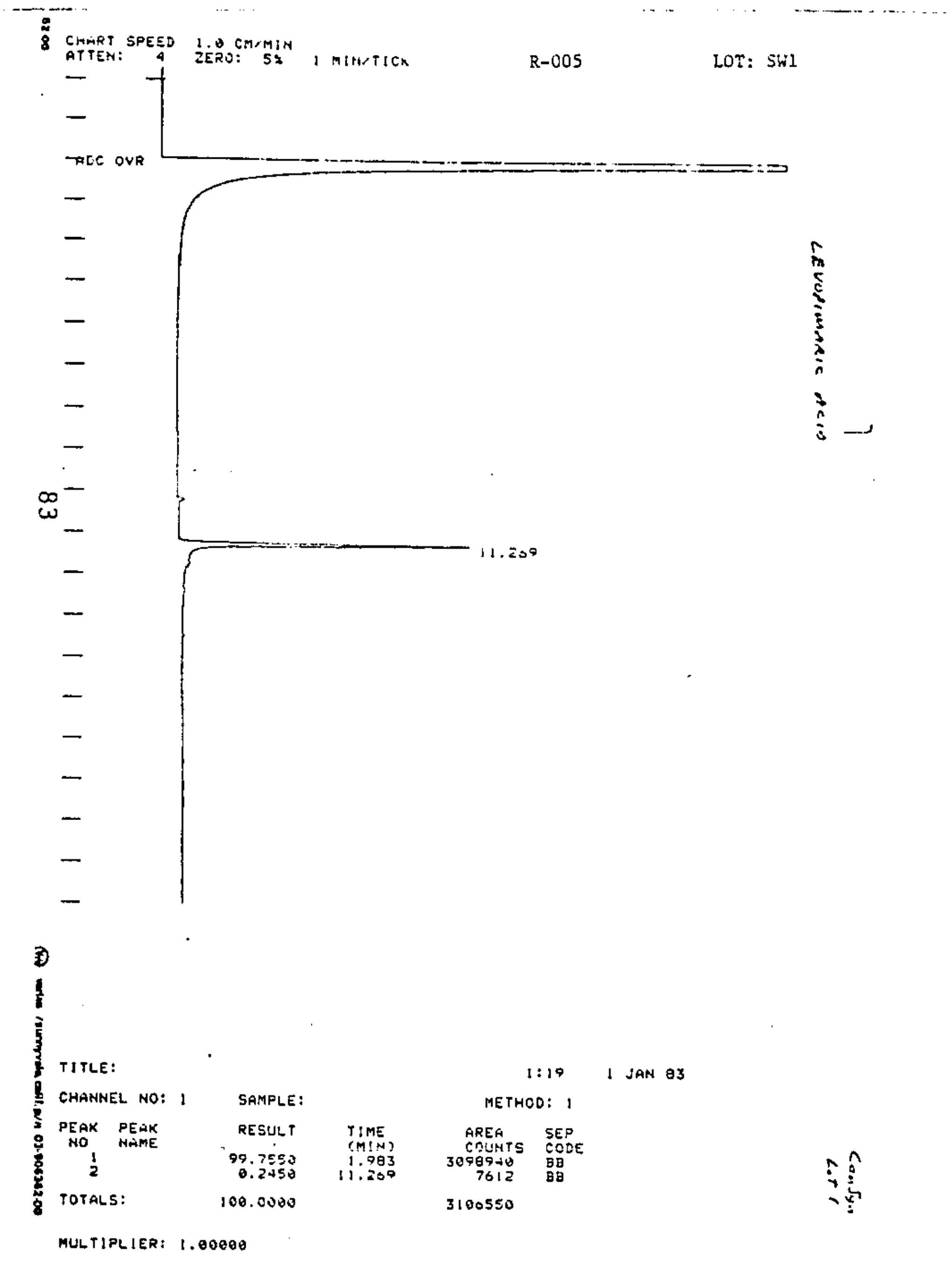
```
Closing signal file R:SIGNAL  .BNC

RUN# 1523            SEP 23, 1988  15:19:54

SIGNAL FILE: R:SIGNAL.BNC

ISOPIMARIC ACID

AREA%
      RT       AREA TYPE  WIDTH      AREA%
   13.449    140627   PB    .081  100.00000

TOTAL AREA= 140627
MUL FACTOR=1.0000E+00
```

Figure D.4 Chromatograph of Isopimaric Acid



```
C-R1A
SMPL #      00
FILE #       2
REPT #    4210
METHOD      41

    #   NAME   TIME        CONC      MK       AREA
    0          9.1         87.6592            78113
    0          9.43        12.3407    V       10994
```

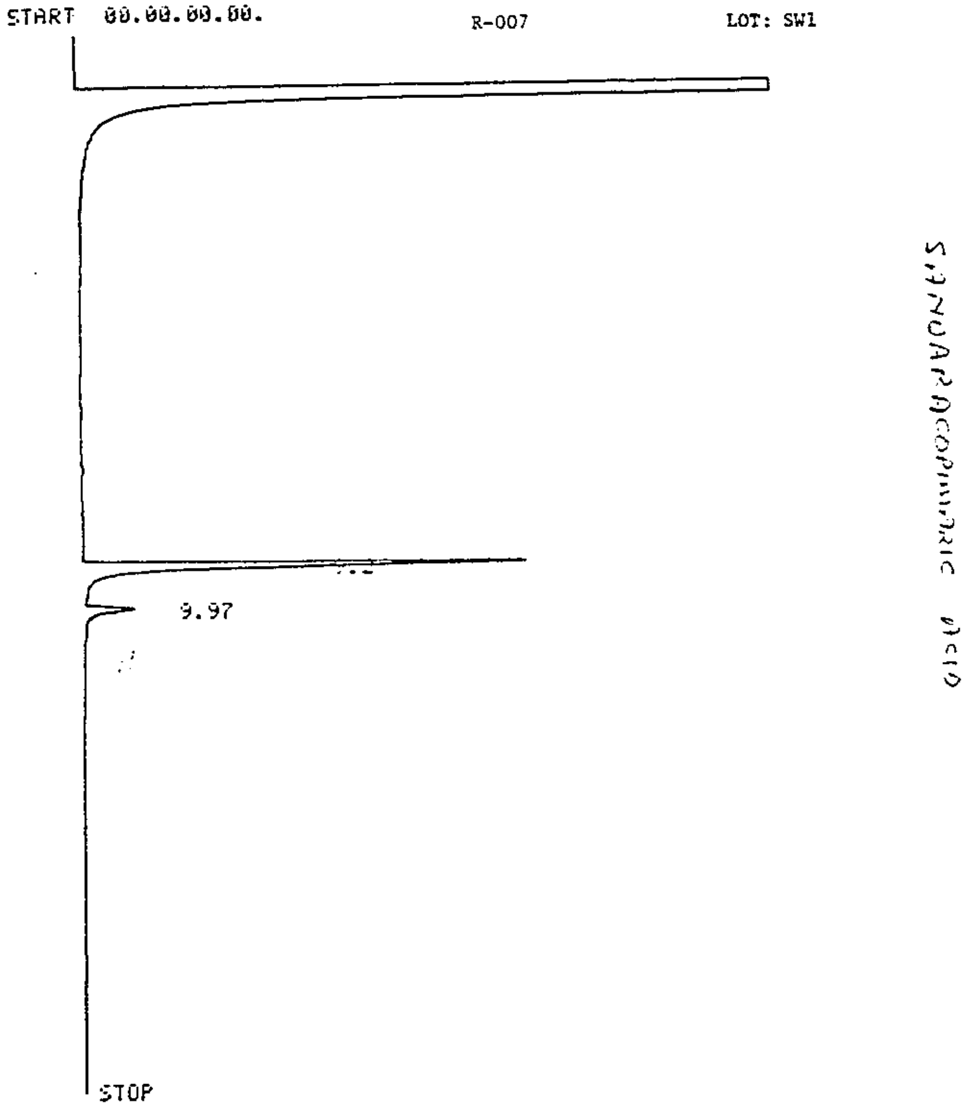
Figure D.5 Chromatograph of Pimaric Acid



```
CHART SPEED  1.0 CM/MIN
ATTEN:    4   ZERO:  5%   1 MIN/TICK           R-005            LOT: SW1
```

```
TITLE:                                                   1:19    1 JAN 83

CHANNEL NO: 1        SAMPLE:                         METHOD: 1

PEAK  PEAK           RESULT      TIME          AREA    SEP
 NO   NAME                       (MIN)        COUNTS   CODE
   1                 99.7550      1.983      3098940   BB
   2                  0.2450     11.269         7612   BB

TOTALS:             100.0000                 3106550

MULTIPLIER: 1.00000
```

Figure D.6 Chromatograph of Levopimaric Acid



```
C-R1A
SMPL #      00
FILE #       2
REPT #    4530
METHOD      41

   #   NAME   TIME          CONC      MK        AREA
   0           9.2          89.4859             64197
   0           9.97         10.514               7542
         TOTAL              99.9999             71740
```

Figure D.7 Chromatograph of Sandaracopimaric Acid