The Role of Natural Wood Constituents on the Anaerobic Treatability of Forest Industry Wastewaters

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
dr. H. C. van der Plas,
in het openbaar te verdedigen
op vrijdag 12 oktober 1990
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen.

STELLINGEN

1. The higher sensitivity of methanogenic bacteria to toxic compounds as compared to aerobic bacteria is often cited as an important disadvantage of anaerobic wastewater treatment systems. The bulk of the toxicity data available in the literature indicates that this concept is incorrect.

2. Björklund-Jansson (1980) overestimated the lignin content of fiber board wastewater since the analytical method utilized lumped tannins together in the lignin measurement.

   Björklund-Jansson (1980). TAPPI 63(7):78

3. The high toxicity of bleaching wastewaters is usually attributed to the presence of chlorinated phenols, while in fact other compounds like wood resin constituents are responsible for a greater portion of the toxicity.

4. The fact that phosphates are no longer present in detergents does not mean that detergents can be advertised as being environmentally sound products.

5. The major spanish political parties have abused the environmental protection theories of "sustainable economic development". According to their interpretation, a new destructive phase must be tolerated in order to achieve an economic level in which the excess wealth can be employed to pay for pollution control measures.

6. Genetic manipulation of crops in order to improve their resistance to certain pesticides instead of improving their resistance against pests, is an unethical application of biotechnology.

7. The exaggerated faith in numbers implies that all which cannot be quantified is too often left completely out of the calculation.

8. The Dutch regulations concerning shopping hours discriminate against single people who live alone.

9. If men were primarily interested in human well-being and not in production, conceived as a good in itself, they should abandon economic development as the priority objective of politics in favour of a policy which searches for applying more selective criteria of well-being.

   E. J. Mishan. 1983. Los costes del desarrollo económico. ("Growth the price we pay"). Ed. Orbis, S. A.

10. War does not result from an instinct, but it is an invention. It is unknown to animals and it is a purely human institution, like science or administration.

J. Ortega y Gasset, La rebelión de las masas. Ed. Espasa-Calpe

11. Aphids (Aphis fabae) can be considered as the 'milking cows' of the ant world.

F. Dominguez García-Tejero, Plagas y enfermedades de las plantas cultivadas. Edit. Dossat

Stellingen behorende bij het proefschrift "The Role of Natural Wood Constituents on Anaerobic Treatability of Forest Industry Wastewaters" van R. Sierra-Alvarez.

Wageningen, 12 oktober 1990

a mis padres

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ABSTRACT

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Anaerobic treatment has been shown to be an efficient and energy conserving method for treating various types of readily biodegradable non-inhibitory forest industry wastewaters. However, the high toxicity of paper mill effluents derived from chemical wood processing operations has hampered the wide spread application of anaerobic treatment in the forest industry.

This dissertation describes research on the anaerobic treatment of inhibitory wastewaters from the forest industry. The main objective was to determine the role of natural wood-derived organic constituents on the methanogenic toxicity of these wastewaters.

Lignocellulosic feedstocks were pulped by processes commonly applied in the forest industry, namely thermomechanical (TMP) and alkaline pulping processes, to determine which factors are responsible for the extraction of toxic substances. Batch anaerobic biodegradability and methanogenic toxicity assay results indicated that the pulping conditions applied had a significant effect on the anaerobic treatability of the resulting wastewaters. TMP effluents were highly biodegradable and non-inhibitory. Soda pulping liquors contain important fractions of recalcitrant organic matter and exerted severe toxicity. Wood resin constituents were shown to be the major inhibitors present in pulping wastewaters. Wood resin is composed of fatty constituents which are poorly soluble in water at neutral to acid pH values. The increased solubility of resin at high pH values, indicates that the contact of alkali with wood contributes strongly to producing toxic wastewaters by extracting resinous compounds. The alkali promotes lignin solubilization and thereby also contributes to a lowered biodegradability of the wastewater.

Compounds representative of the major wood resin constituents were assayed for methanogenic toxicity. The high toxicity of a variety of resin compounds including volatile terpenes, resin acids and apolar phenols was demonstrated. Concentrations causing 50% inhibition ranged from 20 to 330 mg/l.

Aside from the resinous wood constituents, lignin derived compounds are also potential sources of toxicity in pulping wastewaters. The methanogenic toxicity of lignin mixtures isolated from paper mill effluents was determined. Experiments with ultrafiltered lignins revealed that the toxicity of various wastewater lignins originated from the low molecular weight (MW) fraction. Studies with selected low MW lignin model compounds showed that their inhibitory activity was related to the functional groups on the aromatic ring. Compounds with aldehyde groups or apolar substituents were highly toxic; whereas, those with carboxylic groups were distinctly less toxic.

The effect of chemical structure on the methanogenic toxicity of aromatic compounds was investigated. Some basic structure-toxicity relationships were evident. In general, the toxicity increased with increasing the length of aliphatic side-chains and increasing the number of alkyl or chlorine groups. On the other hand, the toxicity decreased as polar functional groups were introduced on the alkylic side chains. The partition coefficient n-octanol/water, an indicator of hydrophobicity, was observed to be positively correlated with the methanogenic inhibition. The results indicate that hydrophobicity is an important factor contributing to the high toxicity of numerous aromatic compounds. Therefore, highly hydrophobic compounds such as resin constituents, apolar lignin derivatives and chlorinated aromatics and are the primary suspect toxicants in forest industry effluents.

The susceptibility of important organic toxins in forest industry effluents to anaerobic biodegradation was assessed. The results indicated that anaerobic treatment technologies have a limited capacity to mineralize natural wood toxins. Although the degradation of a lignin derivative, guaiacol, and long chain fatty acids was demonstrated, other important wood toxins such as volatile terpenes and resin acids were persistent.

Finally, the treatability of TMP and soda pulping effluents was evaluated in lab-scale upward-flow anaerobic sludge blanket (UASB) reactors. TMP wastewaters were found highly suitable for anaerobic treatment. Despite the inhibitory character of soda pulping liquors, anaerobic systems were feasible for removing the biodegradable COD if, prior to biological treatment, the wastewaters were diluted to subtoxic levels or detoxified by pretreatment with the adsorbent Amberlite-XAD-2.

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CHAPTER 1

TREATMENT OF PULP AND PAPER MILL WASTEWATERS: GENERAL INTRODUCTION

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1. FOREST INDUSTRY WASTEWATERS

1.1. INTRODUCTION

Pulp and paper manufacturing is a water-intensive industry responsible for the discharge of highly polluted wastewaters. In average, wastewater discharge volumes range between 80 and 150 m^3 per ton of finished product (59,168,181). Considering the magnitude of the world production of paper and paperboard, estimated at 227 million tons in 1987 (189), the forest industry can be considered as a particularly important source of wastewater. Pulp and paper mill effluents are highly heterogeneous. Their composition and concentration depend on the lignocellulosic materials used, and more importantly on the process conditions applied (i.e., chemical and mechanical treatments). Fig. 1 illustrates the main sources of wastewater in the pulp and paper industry.

Pulp is the cellulose rich fibrous mass used as intermediate product during the manufacturing of paper and paperboard. Pulp is a generally prepared from woody plants. Non-woody plants, such as bagasse, cotton linters, hemp and wheat, barley, rice and flax straw are also occasionally used to prepare pulps for papermaking.

The major constituents of wood are cellulose, hemicellulose and lignin. Extractives and mineral substances are minor components that tend to vary in amount and composition depending on the wood species (42,43). The average overall composition of wood is illustrated in Fig. 2. The organic compounds commonly found in the wood extractives (also known as wood resin) are long chain fatty acids (LCFA), terpenes, resin acids, lignans and apolar phenols. The averaged composition of the resin fraction of softwoods and hardwoods is summarized in Table 1.

The production of wood pulp involves various operations including debarking, pulping and bleaching. The first stage in the papermaking process is the removal of bark from the woody feedstocks. Frequently, wet processes are used for debarking which generate wastewaters containing substantial amounts of organic matter dissolved from the bark (49).

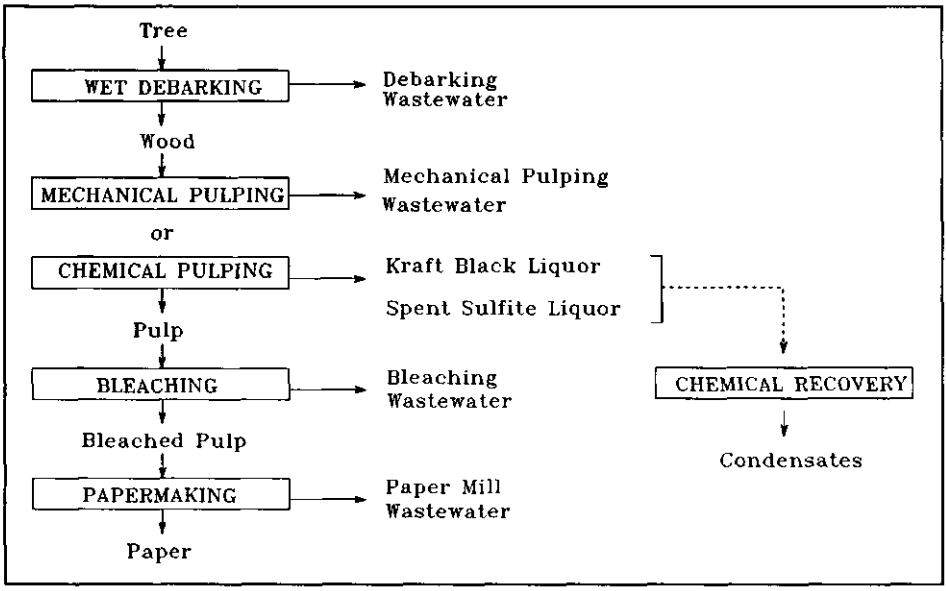

FIG. 1. The main sources of wastewater in the pulp and paper industry.

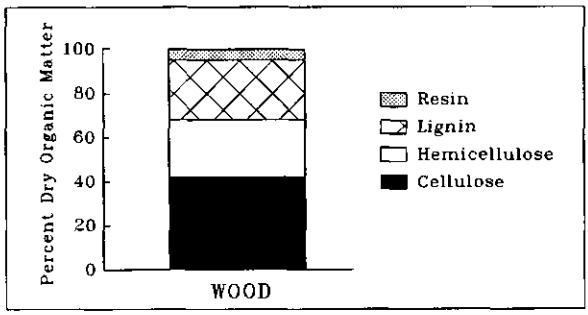

FIG. 2. The average overall composition of wood.

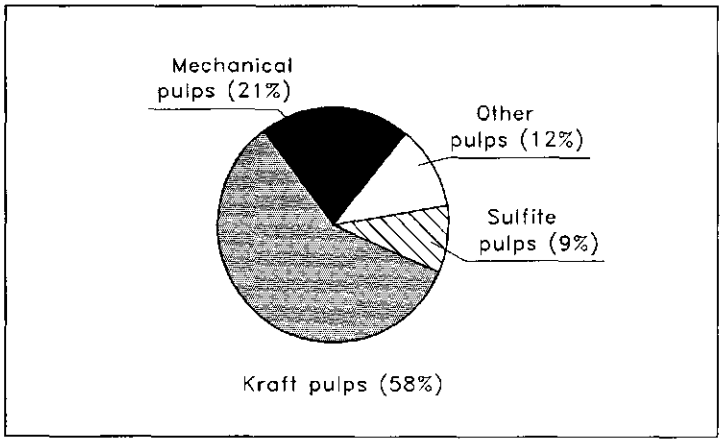

FIG. 3. Contribution of different pulping technologies to the total world pulp production 1979 (46).

The debarked wood is chopped into chips which are then available for pulping. Pulping is accomplished by mechanical or chemical means. In mechanical pulping (MP) the wood is disintegrated into a fibrous mass without the use of chemicals. The removal of wood components during MP is very low and as much as 93 to 98% of the original wood weight is recovered as pulp (126). The chemical processing of wood utilizes strong alkaline or acidic reagents in order to separate the cellulosic fibers from the lignin. The yields of chemical pulping (CP) processes are low (45-50%), because 90 to 98% of the lignin and 50 to 80% of the hemicelluloses are removed from the wood and extracted into the process water (122). Several types of pulps are produced by a combination of chemical and mechanical procedures. The yields of these semichemical or chemi-mechanical processes are intermediate between those of MP and CP, ranging from 65% to 85% (122,126). The specific water consumption and pollution loads in typical mechanical and chemical pulp mills are shown in Table 2.

The kraft pulping process has a dominant role in the industry representing more than half of the total pulp production and nearly three quarters of the chemical pulps as shown in Fig. 3. Sulfite pulping accounts for only about 9% of the total world pulp production, and its application is likely to decrease in the future. Although mechanical pulps represent only 21% of the total world pulp production, thermomechanical and chemo-thermomechanical processes are becoming increasingly popular.

TABLE 1. Literature averaged composition of wood and bark resin (42,43,164).

COMPOUNDS	Hardwoods	Softwoods	
	Wood	Wood	Bark
	<--- % total resin		--->
resin acids	T*	30	7
volatile terpenes	NM+	2	NM
triterpenols	36	T	3
LCFA#	48	33	13
apolar phenols	NM	25	NM
lignans	NM	10	NM

T* = traces, NM+ = not measured, # = long chain fatty acids

TABLE 2. The specific water consumption and pollution loads in typical mechanical and chemical pulp mills (65,84,180,189,152).

PROCESS	Water Consumption (m^3/ton pulp*)	BOD_7 Kg/ ton pulp*	COD
Wet debarking	5 - 25	3 - 14	5 - 20
GWP	10 - 15	8 - 15	15 - 32
TMP			
unbleached	10 - 30	15 - 25	40 - 60
bleached	10 - 30	20 - 30	50 -120
CTMP			
unbleached	10 - 15	30 - 45	70 -120
bleached	10 - 15	40 - 60	100 -180
NSSC	20 - 80	15 - 60	30 -120
Kraft			
unbleached	40 - 60	8 - 20	40 - 60
bleached	60 - 90	20 - 40	100 -140
Ca-sulfite			
unbleached	80 -100	30 - 70	NA
bleached	150 -180	45 - 85	150 -180
Mg-sulfite			
unbleached	40 - 60	25 - 50	60 -120
Papermaking	10 - 50	1 - 5	NA

Pulp* = oven dried pulp.

Unbleached chemical pulps are usually dark in color due to the residual lignin remaining in the fibers. For the manufacture of high-grade white papers, the pulp must be purified in bleaching processes. The bleaching of chemical pulps involves various chemical treatments aimed at the removal of the residual lignin. Together with lignin, other components (i.e., extractives and hemicelluloses) may also be partly extracted during the bleaching stages.

1.2. MECHANICAL and THERMOMECHANICAL PULPING PROCESSES

Groundwood pulping (GWP) and refiner mechanical pulping (RMP) processes are the most important of the mechanical processes. GWP involves wet grinding of wood by means of a large revolving grindstone. GWP effluents are high in carbohydrates (80-90%) and organic acids (10-20%), and they also contain minor amounts of extractives (85,180). The original RMP is a process derived from GWP in which chips are fiberized in rotatory grooved disk refiners at atmospheric pressure. RMP has been largely replaced by using pressurized presteaming and refining (e.g., thermomechanical pulping) and applying chemicals (e.g., chemi-thermomechanical pulping).

Thermomechanical pulping (TMP) is the most important refining process applied in modern paper mills (46,104). The effluents generated in the manufacture of TMP pulps contain mainly carbohydrates and organic acids (40-60%), and lignin (15-30%) (90). The wood resin constituents are poorly soluble in acidic conditions (13), and therefore only low concentrations are expected in TMP effluents. GWP and TMP effluents are relatively diluted, with concentrations generally ranging between 1,000 and 4,500 mg COD/l (90,159).

Chemi-thermomechanical pulping (CTMP) is a modification of the TMP process in which the chips are usually impregnated with an alkaline solution of sodium sulfite prior to thermomechanical refining. CTMP effluents have intermediate COD strengths, generally ranging between 6,000 and 13,000 mg COD/l (157,163,195). The principal constituents in these effluents are polysaccharides (10-15%), organic acids (35-40%) and lignin (30-40%) (147,195). Resin and fatty acids are also present in considerable amounts in CTMP effluents (195).

Pollution from MP mills is considerably less than from CP mills due mainly to their higher pulping yields. TMP and CTMP mills will, however, produce higher pollution loads compared to GWP (119,189). Presteaming of the wood chips at high pressures and elevated temperatures increases somewhat the dissolution of wood components in TMP effluents (181,199). Additionally, alkaline pretreatment of wood chips solubilizes lignin and extractives to a greater extent in CTMP as compared to other MP processes. The introduction of CTMP processes will radically increase the specific BOD and COD loads as shown in Fig. 4.

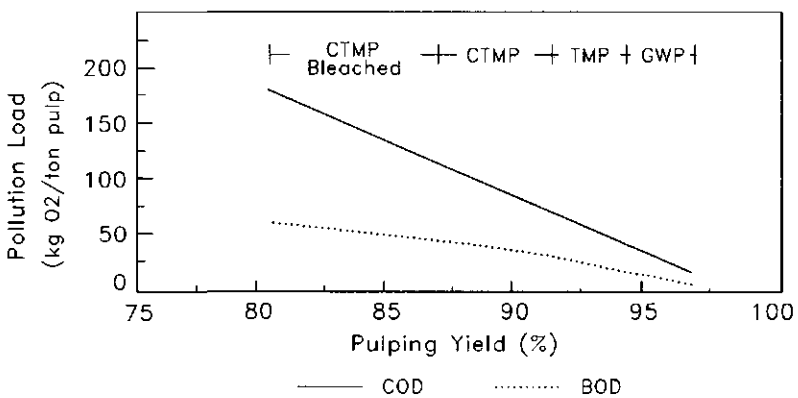

FIG. 4. The specific BOD and COD loads as a function of the pulping yield for different mechanical pulping processes. Groundwood (GWP); Thermomechanical (TMP); Chemi-thermomechanical (CTMP). (References in Table 2).

1.3. CHEMICAL PULPING PROCESSES

Chemical pulping processes separate wood into its constituent fibers by dissolving the lignin from the wood. The pollution loads and the color due to dissolved lignic compounds are consequently higher for chemical as compared to mechanical pulping effluents (Table 2).

1.3.1. Alkaline chemical pulping processes. The soda process and the sulfate process (also known as "kraft") are the principal alkaline pulping methods used. Sodium hydroxide is the principal cooking chemical in both processes; however, during sulfate pulping sodium sulfide is utilized as an additional reagent. Both processes are denominated for the compounds added during chemical recovery to compensate for the loss of NaOH, namely sodium carbonate and sodium sulfate, respectively. The soda process has been largely replaced by the kraft process for wood feedstocks, but it is still an important process for the production of non-wood fiber pulps (130).

Pulping in alkaline conditions solubilizes to a large extent both the hemicellulosic and lignic fractions of the feedstocks. Alkaline delignification systems operate on the basis of hydrolytic depolymerization of phenylalkyl-ethers (45,62). The cleavage of ether bonds contributes essentially to reduce the size of the lignin molecule and simultaneously generate phenoxide ions (Fig. 5), rendering the lignin more soluble in alkali. The presence of sulfide ions serves to degrade lignin more rapidly during kraft as compared to soda pulping (19,62). However, the carbohydrate dissolution rate is similar in both pulping processes.

The black liquors generated by alkaline pulping processes are highly polluted. The solids content normally ranges from 14 to 18% (6,19). These liquors contain some residual alkali from the cooking and generally have a pH value of 10 to 13. Degradation products of the carbohydrates and lignin account for about 40% and 50%, respectively, of the total solids in alkaline pulping liquors (55,94,123). The resin and fatty acids (tall oil) of the wood are soluble in the alkaline medium and are largely extracted into the black liquor. With resinous wood species, the concentration of these tall oils is high enough that they can be separated by skimming and sold as an important by-product of the process (19). The tall oil soaps consist mainly of resin and fatty acids, with minor amounts of unsaponifiable materials, including hydrocarbons, alcohols and sterols (19).

Chemical recovery is an integral part of alkaline pulping because the process is more economical if costs are reduced by chemical recovery and reuse. Conventional chemical recovery involves the evaporation of the black liquor followed by incineration of the organic solids. The incineration of black liquors fulfills various functions (19). Firstly, it destroys most of the dissolved organic matter generated during the pulping stage, eliminating a major potential source of water pollution. Furthermore, it allows the regeneration of pulping reagents and the incineration itself produces usable energy. Nonetheless, it is noteworthy to mention that chemical recovery is not possible in small paper mills were the capital cost of incineration units is not permissible (6,130). Therefore, black liquors represent a major environmental problem at such paper mills (6,130,187).

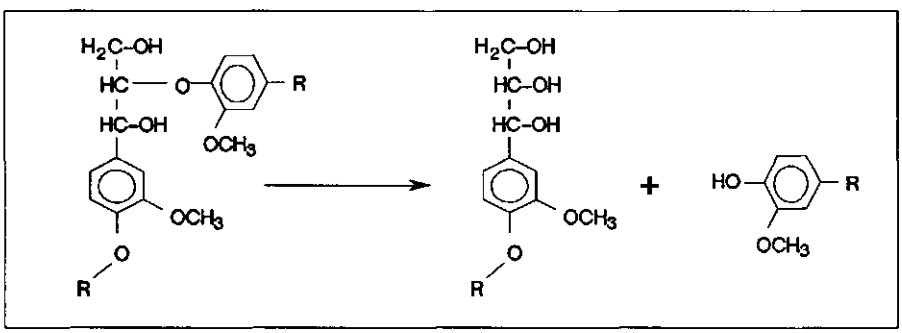

FIG. 5. Cleavage of ß-aryl ether bonds in lignin units during alkaline pulping.

The evaporation of kraft black liquors produces condensates which primarily contain methanol along with substantial amounts of reduced sulfur compounds, such as hydrogen sulfide (H_2S) and methyl mercaptan (CH_3SH) (14,19). Resin acids, fatty acids, volatile terpenes and ethanol are also present in condensates at lower concentrations (14,189).

Environmental problems due to the odor and toxicity of H2S and other reduced sulfur compounds have brought about some renewed interest for sulfur-free alkaline pulping processes. Sulfur-free processes based on the application of additives such as anthraquinone (soda-AQ-process) and soda oxygen delignification procedures (soda-oxygen process) will very likely increase in importance in the future (19,46,62).

1.3.2. Sulfite Chemical Pulping. Since the first sulfite mill was constructed in 1874, this pulping method rapidly became the principal technology for producing chemical pulps. However, the importance of this process rapidly declined with the development of efficient kraft recovery systems in the early 1930's. In 1937, kraft pulp production already exceeded that of sulfite pulps (189).

Delignification of wood with various sulfite and bisulfite containing aqueous solutions has resulted in the development of a substantial number of industrially accepted sulfite pulping processes. At present, a number of different sulfite pulping processes are known which differ in the composition of the cooking liquor, the choice of the base and pH (20,46).

Acid chemical sulfite delignification systems are based on the nucleophilic action of sulfur dioxide in water. The increase in lignin water solubility is due to the formation of lignin sulfonates (Fig. 6) and may also be attributed in part to a certain reduction in molecular size (62).

Spent sulfite liquors (SSL) are generally highly concentrated with COD and BOD values ranging from 120,000 to 220,000 mg/l and 25,000 to 45,000 mg/l, respectively (22,63,91). The major organic constituents in SSL are lignosulfonates (50-60%) and carbohydrates (15-25%) (55,61,91). Although the pollution load in sulfite pulping significantly decreased as recovery processes became available, incineration of SSL at many paper mills is not economically feasible. Also, it is not technically feasible to recover the cooking chemicals from calcium-based sulfite liquors. The high BOD content of the spent pulping liquor creates a serious water pollution problem where chemical recovery is not practiced.

In contrast to alkaline pulping, incineration of sulfite spent liquor has been of marginal interest and it has only been extensively adopted when effluent quality regulations made it necessary. The evaporation of spent sulfite liquors produces an acidic condensate that has a relatively high concentration of dissolved organic matter, 10,000 to 50,000 mg COD/l (8,18). Acetic acid is the principal organic constituent in sulfite evaporator condensates (SEC), with substantial quantities of methanol and furfural also present (8,18). In addition, these waste streams may contain considerable concentrations of sulfur as sulfurous acid and as organic sulfonates (18,40,168). Sulfur dioxide and acetic acid are responsible for the high acidity and low pH (1.5-2.0) in SEC.

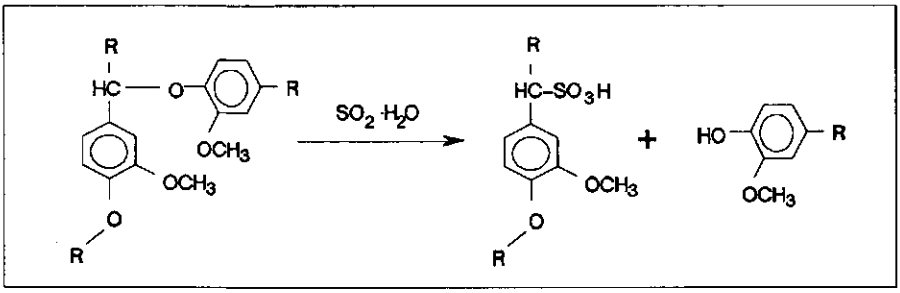

FIG. 6. Sulfonation of lignin units during acidic sulfite pulping.

1.4. SEMICHEMICAL PULPING PROCESSES: The NSSC Process

The neutral sulfite semichemical (NSSC) process is the most important semichemical pulping method. This is a two-stage process which involves a mild chemical pretreatment of the wood chips in an neutral or slightly alkaline sodium sulfite liquor at high temperatures (160-190°C) followed by a mechanical refining step. Yields of NSSC pulps are generally about 10 to 40% higher than those of conventional chemical pulping processes because only 25 to 50% of the lignin and 30 to 40% of the hemicelluloses in the wood are removed (122).

Delignification is the predominate reaction in the NSSC process. Similar to sulfite chemical pulping, the mechanism of NSSC attack involves primarily sulfonation of a portion of the lignin in the lignin-carbohydrate complex, followed by hydrolysis to soluble lignosulfonic acids and soluble carbohydrates.

NSSC spent liquors represent a major environmental problem because their relatively low organic matter content render them generally unsuitable for energy purposes. However, when NSSC pulping is integrated in a kraft mill, the spent liquor is recovered in a so-called "cross-recovery" system to reduce the environmental pollution load (46).

1.5. BLEACHING

The principal aim of bleaching is to increase pulp brightness. The chromophoric components in unbleached pulps are predominantly functional groups of degraded and altered residual lignin. Bleaching can be performed by converting and stabilizing cromophoric groups without loss of substances ("lignin-preserving bleaching") or by removing the lignin ("lignin-removing bleaching").

1.5.1. Bleaching of Mechanical Pulps. Bleaching of mechanical pulps usually applies lignin-preserving procedures in order to maintain the advantage of their high yields. Peroxide and dithionite are the dominating chemicals used for bleaching. Peroxide in alkaline medium (pH 10-11) is an oxidizing agent that will react with chromophoric lignin structures (i.e., quinones, etc.) and decolorize them (45,118). The brightness obtained in dithionite bleaching can be attributed to the reduction of chromophoric groups (118). Both processes use chelating agents (e.g., DPTA, EDTA) to minimize decomposition of the bleaching agents by metallic ion impurities.

The dissolution of organic substances in dithionite bleaching is low (2 to 8 kg BOD/ton pulp) in comparison with peroxide bleaching processes (5 to 20 kg BOD/ton pulp) (118,152). Peroxide bleaching effluents contain mainly carbohydrates accounting for 60% of the solids, whereas methanol and organic acids account for 40% (189).

1.5.2. Bleaching of Chemical Pulps. Delignification in commercial chemical pulping is completed when the lignin content in the fibers has decreased to a low residual percentage (5-10%) and the cellulose has become more vulnerable to chemical attack than the remaining lignin. For the manufacture of high-grade white papers, the remaining, darkly colored lignin, can be removed by bleaching processes that are more selective in their attack on lignin as compared to the chemical pulping processes.

Lignin-removing bleaching is usually performed in multi-stage procedures with acid oxidative stages combined with at least one alkaline extraction step. Exceptions from this rule are hypochlorite, oxygen, and peroxide bleaching, where the oxidation is carried out in alkaline medium.

Chlorine and chlorine compounds are the best established and most widely used bleaching agents for chemical pulps. In the chlorination stage, delignification is achieved by oxidative depolymerization resulting in lignin fragmentation along with chlorination and hydroxylation that contribute to increased water solubility (62,118). Chlorination converts residual lignin in alkali soluble degradation products, and therefore alkaline extraction is usually the following step to remove these components.

The effluents from bleach plants contribute very significantly to increase the pollution loads caused by pulp and paper mills (Table 2). In modern conventional kraft mills, bleaching wastewaters account for 50-60% of the total BOD load (189). Chlorination and the alkali extraction stages are the major sources of organic pollution in bleaching plants (118,189). Bleachery effluents mainly contain degradation products of lignin, accounting for 50 to 80% of the total solids (35,167), with smaller amounts of degradation products originating from carbohydrates and wood extractives (35,87,167). Due to their high lignin content, these effluents are darkly colored. They are responsible for 85 to 90% of the color pollution at modern kraft pulp mills (118,189).

Chlorine dioxide (ClO_2) is gradually displacing chlorination in the first stage of pulping. This development is the result of several advantages of ClO_2, e.g., substantial decrease of the BOD in the effluents, significant reduction in the formation of chlorinated lignin fragments, etc. (118). In contrast to chlorination, ClO_2 promotes exclusively oxidative reactions of phenolic lignin units (44). Chlorinated phenols however are still derived to some extent from the action of Cl_2 generated by partial decomposition of ClO_2.

The development of new chlorine-free bleaching processes also offers interesting perspectives to reduce the environmental impact of bleachery effluents. Bleaching sequences including an oxygen bleaching stage appear to be the most promising processes for the future. The main advantage of oxygen bleaching is that the resulting effluents do not contain chlorinated lignin fragments and they can be processed within the normal kraft liquor incineration systems. With oxygen delignification, the BOD load can be reduced by 25-50% and the color in the effluent by 65%-80% compared to conventional bleaching (60,189).

2. ENVIRONMENTAL EFFECTS RELATED TO THE DISCHARGE OF PULP AND PAPER MILL WASTEWATERS

2.1. INTRODUCTION

Although environmental control regulations have been mostly concerned with conventional parameters such as BOD, suspended solids (SS) and pH, more restrictive regulations will be promulgated in the near future focusing on the reduction of toxic pollutants. Concerning forest industry waste streams, extensive toxicity testing programs are being conducted in numerous countries in order to establish toxicity discharge standards. Sweden has already issued regulations limiting total organic chlorine (TOCL) discharge (138). In Canada, aquatic toxicity discharge standards have been established and effluent fish toxicity testing is obligatory (109,189).

Despite the forceful measures which have been taken in the past decade to reduce the BOD load caused by pulp and paper mills (119,189), the forest industry must introduce new processes or modify existing processes to fulfill future environmental requirements and reduce toxic discharges. For example, new bleaching sequences should be considered that reduce the formation of chlorophenolics. Specific external treatment facilities will be built to reduce effluent toxicity and decrease color discharge. These measures will comprise both biological and physical-chemical treatment systems (81).

2.2. ENVIRONMENTAL EFFECTS

Pulp and paper mill effluents display acute toxicity to fish and other aquatic organisms (Table 3). The toxicity of forest industry effluents can be attributed to a complex mixture of wood extractives, including resin and fatty acids (87,112,162); tannins (184); some lignin degradation products (191), and low molecular weight (MW) chlorinated lignin derivatives (5,110). Table 4 lists the inhibitory concentrations of relevant compounds to fish.

The aquatic toxicity of mechanical pulp mill effluents is mainly associated with the wood extractives, particularly resin acids and unsaturated fatty acids (111,127,199,200). Therefore,

pulping of softwood species with high resin and fatty acid contents (e.g., pines) is expected to increase the aquatic toxicity of mechanical pulping effluents (111).

When the logs are debarked on the mill site, up to 50% of the total toxicity originates from debarking effluents (189). Tannins (184) and resin acids (87,131) are the most important inhibitory compounds in debarking effluents. Particularly the high content of tannins has been shown to be responsible for the severe aquatic toxicity of these effluents (184).

The toxicity emissions of chemical pulp mills originate mainly from condensates and from bleaching effluents (189). The concentration of highly inhibitory reduced sulfur compounds such as H_2S, methyl mercaptan (CH_3SH) and dimethyl sulfide ($(CH_3)_2S$), is the dominant factor causing toxicity to aquatic organisms in kraft condensates (14). Resin and fatty acids were also identified as important contributors to the toxicity.

When considering the total toxicity discharge (ie., water consumption and inhibitory concentration) of the different effluents generated at a typical kraft paper and pulp mill (Table 3), bleaching effluents are in fact not the most important source of aquatic toxicity. In any case, bleaching effluents have received the most attention due to the growing concern about the release of toxic chlorinated aromatic compounds in the environment. Many of these compounds have been shown to be considerable resistant to biological degradation and are persistent in the environment (79,117).

Bleaching plant effluents exert strong toxic effects on fish (5,101), invertebrates (74,177), and algae (149,177). The chlorinated phenols formed in the chlorination and subsequent alkaline extraction stages are generally considered responsible for a major portion of the toxicity in bleaching effluents. Particularly, the chlorinated aromatic compounds formed in the chlorination and subsequent alkaline extraction stages are important inhibitory compounds in bleaching effluents. Although 65 to 95% of the organically bound chlorine is in the form of high MW chlorolignins (100,167) which is non-toxic to aquatic organisms (74,167), the small fraction of low MW chlorinated phenolics displays high aquatic toxicity (5,110,170). Resin acids and chlorinated resin compounds also contribute to the toxicity of bleaching effluents (110,131,149).

Bleaching spent liquors have significant ecological effects on fish and crustacea communities. Sublethal effects such as morphological deformations, hampered reproduction and reduced inmuno defense have been related to exposure to bleaching effluents (10,107,172). Furthermore, bleaching effluents contain many hydrophobic components susceptible to bioaccumulation in food-chains. Numerous reports indicate the accumulation of chlorinated aromatics and lipophilic resin acids and terpenes in the flesh of aquatic fauna (1,183). Pulp and paper industries also contribute to the presence of xenobiotics (e.g., chlorinated dioxines, PCBs) in natural sediments (1,71).

TABLE 3. The toxicity of untreated pulp and paper mill wastewaters to fish.

Effluent	Toxicity range LC_{50} (96 h)* (%)	TEF+ (m3/ton pulp)	Reference
Debarking	0.2 - 5	1000 -1600	109,133,189
GWP	4 - 10	200 - 300	80,199
TMP	1 - 10	150 - 400	80,127,199,200
Sulfite mill	30 - 50	130 - 150	109
Kraft evaporator condensate	0.1 - 17	1700	14,133
Bleached kraft whole mill	15 - 50	150 - 600	60,101,109,189
Bleaching	1 - 20	200 - 500	60,59,87,118,133,170,189

* LC_{50} (96 h) refers to the dilution at which the effluent is lethal to 50% of the test organisms after a 96 h exposure period.

\+ Toxicity emission factor (TEF) = (Q * 100 / LC_{50}), where Q is the specific water consumption (m^3/ton pulp); and LC_{50} is expressed as % dilution of wastewater.

TABLE 4. The lethal concentrations of various forest industry wastewater constituents to fish and the sources of aquatic toxicity.

CHEMICAL COMPOUNDS	LC_{50} (96 h)* (mg/l)	TOXICITY# SOURCES	References
Bark tannins	16.2	D	184
Resin acids		D, KP, MP, SP	
Dehydroabietic acid	1.1		112
Abietic acid	0.7		112
Pimaric acid	0.8		112
LCFA		D, KP, MP, SP	
Oleic	5.0		111
Linoleic	5.0		111
Diterpene alcohols		D, MP	
Pimarol	0.3		112
Dehydroabietol	0.8		112
Abietol	1.8		112
Chlorinated phenolics		KB	
3,4-dichlorocatechol	0.3		87
2,4,6-trichlorophenol	2.2		87
3,4,5-trichloroguaicol	0.8		110
3,4,5,6-tetrachloroguaicol	0.3		110
pentachlorophenol	0.3+		97
Lignin derivatives		SP	
phenol	60.0+		97
p-cresol	7.1		92
p-methoxyphenol	31.6		92
Chlorinated resin		KB	
monochlorodehydroabietic acid	0.6		110
dichlorodehydroabietic acid	0.6		110

* LC_{50} (96h)= Concentration that results in 50% death of the test fish during a 96 hours exposure period.

\# D= Debarking, MP = Mechanical pulping, SP = Sulfite pulping, KP = Kraft pulping, KB = Bleaching of kraft pulp.

\+ LC_{50} (24h)

Screening for the mutagenic properties in forest industry effluents indicates the presence of mutagenic materials in most of the discharges within the industry (106,191). Chlorination-stage bleachery effluents have been identified as the principal source of mutagenicity in paper mills (106,191). Concerning the responsible molecular species, recent investigations have indicated the mutagenic effects of a variety of wood derived chlorinated hydrocarbons (101). Dehydroabietic and neoabietic have also been shown to be mutagenic to bacteria and yeast (132).

3. ABATEMENT OF POLLUTION FROM PULP AND PAPER MILLS

Although pulp and paper production has continuously increased during the last decades, the pollution loads of pulp and paper mills have experienced a significant decrease since the 1970's due to the enforcement of environmental regulations limiting BOD and SS discharges (119,189). The reduction in the discharge of organic pollution in the Finnish pulp and paper industry is illustrated in Fig. 7. To comply with regulations controlling water pollution, the forest industry has adopted internal and external measures. Water consumption has been reduced by closing water circuits and by modernizing technologies which utilize water more efficiently. Additionally, new processes and modification of existing ones have been introduced in order to reduce the dissolution of organic material. External treatment facilities i.e., mechanical sedimentation facilities and biological treatment systems, also have been built to further reduce final pollution loads.

3.1. MECHANICAL TREATMENT OF FOREST INDUSTRY WASTEWATERS

Mechanical treatment (clarification) is an established method for the removal of SS from paper mill effluents which has been practiced for more than 20 years. Settling basins were initially built to recover fibrous materials. However at present, most paper mills have installed clarifiers for environmental reasons. Mechanical treatment can be combined with chemical precipitation in order to achieve a relatively high removal of COD. These treatments result in considerable SS reductions (60-95%) (144); however, they do not adequately eliminate BOD because an important fraction of the biodegradable organic matter is present as dissolved components.

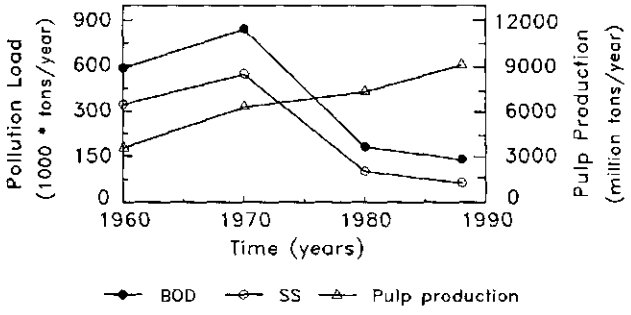

FIG. 7. Development of the pulp production and the pollution loads in the Finnish forest industry. The data plotted in this figure were obtained from Puhakka (152).

3.2. AEROBIC TREATMENT OF PULP AND PAPER WASTEWATERS

In order to combat the BOD pollution loads, biological treatment methods were introduced. Aerated lagoons were the first biological purification units constructed for treating paper mill effluents. The BOD reductions achieved in aerated lagoons range from 40-70% (119,142,144). Activated sludge treatment systems were introduced in the early 1980's. At present, there is an important number of activated sludge plants in operation treating effluents from mechanical and chemical paper mills. The loading rates achieved in activated sludge plants range from 0.6 to 3.9 g BOD/l·day (144). The BOD removal efficiencies are high, in most cases between 65 and 85% (34,88,144). The COD reductions, however, vary between 40-70% at paper and board mills and range between 25-55% at chemical pulp mills (88). Generally, little or no color removal is obtained by activated sludge treatment (191). The poor COD and color reduction observed during aerobic treatment of chemical pulping wastewaters is due to high content of poorly biodegradable lignin in these effluents.

Aerobic treatment has been shown to be effective in reducing the aquatic toxicity from various types of forest industry wastewaters (59,87,131,197). A variety of mechanisms may contribute to the detoxification observed. Non-biological elimination mechanisms may include the stripping of volatile compounds and the physical removal of toxins by precipitation or adsorption on biomass (117,124,193). Biological degradation of toxic organic constituents also contributes to the detoxification mechanisms in aerobic treatment systems. Toxic wood extractives, such as LCFA and resin acids, are known to be biodegradable in aerobic systems (34,87,109). In contrast, microbial degradation of chlorinated aromatics is slow, and these compounds often survive aerobic treatment at pulp mills (87,117). Several authors have reported reductions of chlorinated phenolic compounds in aerated lagoons and activated sludge processes ranging from 30 to 50% (87,101,169). Leuenberger *et al.* (117) have demonstrated that non-biological elimination mechanisms (sorption on sludge, volatilization) significantly contributed to the removal of various chlorinated organics in an activated sludge plant treating bleaching effluents.

An important drawback of aerobic biological treatment systems is that they produce large amounts of excess sludge, approximately 0.5 g of sludge volatile suspended solids (VSS) per g $BOD_{removed}$ (38). Treatment and particularly dewatering of the excess biological sludges is both troublesome and expensive. It has been estimated that up to 50% of the total costs of wastewater treatment are due to sludge management (105). Usually, dewatered sludges are landfilled or incinerated. These practices may have secondary environmental impacts since chlorinated organics known to be adsorbed on waste activated sludge from bleached kraft effluent treatment (174) are potentially transformed to dioxins during burning or might be released to the ground water from landfills.

3.3. ANAEROBIC TREATMENT OF PULP AND PAPER WASTEWATERS

3.3.1. Anaerobic Versus Aerobic Wastewater Treatment Methods. Anaerobic wastewater treatment is a relatively recent development in the field of environmental technology. The application of anaerobic wastewater treatment technologies at paper mills is enjoying a rapidly increasing popularity. Compared to conventional aerobic methods, the anaerobic wastewater treatment concept offers a number of important benefits (115,125,179). These include lower energy requirements and operating costs as well as the production of a useful energy by-product in the form of methane gas. Additionally, anaerobic treatment systems reduce considerably the volume of excess sludge produced due to the low cell yields of anaerobic bacteria. The low excess sludge production (0.03-0.15 g sludge-VSS per g $BOD_{removed}$ (78)) makes anaerobic treatment methods particularly attractive since waste sludge disposal is becoming a major problem for aerobic treatment systems (154,174). The low nutrient requirements of anaerobic bacteria is also an advantage in the treatment of nutrient deficient wastewaters such as those from pulp and paper mills (121). Furthermore, the improved biomass retention in high rate anaerobic treatment systems allows the application of high organic loading rates, reducing considerably the required reactor volume.

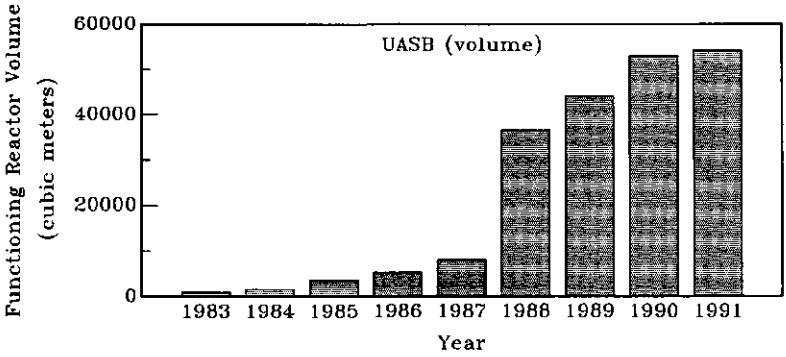

FIG. 8. The growing application of UASB reactors for the treatment of forest industry wastewaters (Lettinga et al. (113)).

The most significant drawbacks which have hampered the widespread application of anaerobic systems for wastewater treatment in the past are related to the low growth rates and yields of anaerobic bacteria. Thus, maintenance of high biomass concentrations within the reactor is required in order to achieve high reaction rates inspite of the slow growth rates of methanogenic bacteria. Consequently, relatively long start-up periods are needed for anaerobic reactors in order to obtain high concentrations of active biomass. Likewise, recovery of the methanogenic population after a toxic shock may be quite time consuming.

3.3.2. The Development of Anaerobic Technologies for Forest Industry Wastewaters. Anaerobic treatment systems were not considered until recently as viable technologies for water pollution control in the forest industry. Pulp and paper mill wastewaters were mostly considered too diluted for feasible anaerobic treatment. However, in the past few years, paper and pulp wastewaters are becoming increasingly more concentrated due to the trend towards increasing closure of water circuits in modern mills (189). The temperature of the process water is also increasing, sometimes reaching 70°C (135). On the other hand, the development of high-rate anaerobic treatment systems has enabled anaerobic treatment of relatively diluted wastewaters, since the biomass retention is not longer coupled to the hydraulic rate (116). Anaerobic processes were also previously regarded as being too sensitive to inhibitory compounds. The recent advances in the identification of inhibitory substances in paper mill effluents as well as the increasing insight over the biodegradative capacity and the toxicity tolerance of anaerobic microorganisms, has helped to demonstrate that anaerobic treatment of various inhibitory wastewaters is feasible (78,114,145). In cases where the effluents are highly toxic, a variety of detoxification technologies are being developed as pretreatments to anaerobic digestion systems as will be discussed later.

Anaerobic treatment technologies are already in use for many types of forest industry effluents. At present, at least 33 full scale anaerobic treatment plants are in operation at pulp and paper industries in 14 countries (146). The upward-flow anaerobic sludge blanket (UASB) and the contact process are the most widely applied systems. The increasing application of UASB reactors to forest industry effluents since 1983 (Fig. 8) reflects the increased popularity of anaerobic treatment systems.

A great majority of the existing anaerobic full-scale plants are treating non-inhibitory forest industry wastewaters rich in readily biodegradable organic matter (i.e., carbohydrates and organic acids) such as paper recycling wastewaters, mechanical pulping and thermomechanical pulping effluents (58,68,120,146,157). Likewise, evaporator condensates which are composed mostly of alcohols and volatile fatty acids are increasingly treated in anaerobic systems (8,32,58,136,146).

The organic loading rates achieved for these types of forest industry effluents in full-scale UASB plants range from 5 to 27 g COD/l·day. The BOD efficiencies are high, in most

cases between 75 and 99%, indicating that anaerobic treatment is particularly useful for the elimination of readily biodegradable organic matter. Anaerobic treatment of mechanical pulping wastewaters provides COD efficiencies of about 80% of the total COD (146). Likewise, the anaerobic biodegradability of kraft evaporator condensates (137,155) and sulfite evaporator condensates (47,102) usually exceeds 80%. High concentrations of SO_2 and organic sulfur compounds may occasionally result in methanogenic inhibition during anaerobic treatment of sulfite evaporator condensates (11,47,102).

In contrast to the readily biodegradable effluents, the number of anaerobic full-scale applications to chemical, semichemical, and chemo-thermomechanical pulping liquors, as well as bleaching and debarking effluents, is limited (146). These effluents often exert inhibitory effects on microorganisms that can disturb biological treatment systems. Additionally, they generally contain important fractions of poorly biodegradable organic matter.

3.3.3. The Methanogenic Toxicity of Forest Industry Wastewaters. Common toxic organic substances in forest industry wastewaters include: resin compounds (11,194); chlorinated phenolics (134,171) and tannins (50). These compounds are highly toxic to methane bacteria at very low concentrations (Table 5). A number of low MW lignin derivatives have also been identified as microbial inhibitors (11,27,190,205). Furthermore, inorganic wastewater components, such as sulfate, sulfite, hydrogen peroxide and other chemicals added during pulping and bleaching processes also exert inhibitory effects on methanogenic bacteria (40,153,196,194). Some toxicity data available in the literature are summarized in Table 6.

A number of forest industry effluents are notorious for their high methanogenic toxicity. These include debarking effluents, CTMP wastewaters, spent liquors of chemical pulping and bleaching effluents. Various research groups have initiated studies to define the toxicity of these waste streams to anaerobic bacteria, evaluate their anaerobic treatability and, in some cases, develop detoxification pretreatments. The results of these investigations will be discussed case by case in the following sections.

3.3.3.1. Debarking Wastewaters. Debarking wastewaters are particularly important for biological treatment since they significantly contribute to the total BOD discharge at numerous paper mills. Initial lab- and pilot-scale studies concerning the anaerobic treatment of debarking effluents have generally led to poor results due to the deterioration of the methanogenic activity (108,157,158). Tannins were identified as the principal methanogenic inhibitors in these wastewaters in the investigations of Field *et al.* (50). The concentration of these inhibitors in debarking effluents is rather high, accounting for 13 to 30% of the soluble COD (50). The 50% inhibitory concentration of bark tannins to methanogenic activity averages 350 mg/l. Pulping processes using wood and bark (masonite, fiber board, card board) may also result in wastewaters containing substantial amounts of tannins solubilized from the bark, and therefore can potentially exert high methanogenic toxicity. Due to the high toxicity of bark solubles, successful anaerobic treatment is only feasible by diluting these wastewaters considerably in other waste streams. In cases were sufficient dilution cannot be achieved, than detoxification measures must be considered. One potential method of detoxification recently developed involves the polymerization of the toxic tannins to high MW humus polymers which are non-toxic to methanogenic bacteria (54). The polymerization can be achieved by short term aeration pretreatments at elevated pH values. Anaerobic treatment of autoxidized aqueous bark extracts in lab-scale UASB reactors was demonstrated at high loading rates (40 g COD/l·day) with 98% removal of the biodegradable COD (49).

3.3.3.2. Chemi-thermomechanical Pulping Wastewaters. Growing interest for the anaerobic treatment of CTMP wastewaters has resulted from the rapidly increasing application of this type of pulping technology in modern paper mills. The effluents originating from CTMP processes exert severe methanogenic inhibition (147,194). The toxicity of CTMP wastewaters is mostly due to the presence of toxic wood resin constituents (195). The microbial toxicity

TABLE 5. The methanogenic toxicity of various paper mill wastewater constituents.

COMPOUNDS	Concentration (mg/l)	Substrate*	Inhibition (%)	References
LIGNIN and TANNIN DERIVATIVES				
Aromatic compunds				
p-coumaric acid	3300	CE	50	141
ferulic acid	4850	CE	0	141
vanillic acid	5040	CE	0	141
benzoic acid	4880	C2	35	26
gallic acid	3200	C_2, C_3, C_4	50	52
L-dopa	1000	C_2, C3, C_4	50	53
pyrogallol	3000	C_2, C_3, C_4	50	52
hydroquinone	4400	C_2	35	26
resorcinol	3190	C_2	50	26
catechol	2640, 3000	C_2	50	16,26
catechol	1500	C_3	50	16
guaiacol	2200#	C_2	50	11
phenol	2200, 2444	C_2	50	11,16
phenol	875	C_3	50	16
eugenol	250#	C_2	50	11
Chlorinated aromatics				
2,5-dichlorophenol	600	C_2	100	192
pentachlorophenol	0.2	C_2, C_3	50	67
WOOD RESIN CONSTITUENTS				
LCFA				
lauric acid	525, 869	C_2	50	26,98
capric acid	1027	C_2	50	98
myristic acid	1104	C_2	50	98
oleic acid	1235	C_2	50	98
linoleic	897	H_2/CO_2	50	150
linolenic	501	H_2/CO_2	50	150
linolenic	278	pyruvate	50	31
mixture LCFA	250#	C_4	50	75
mixture LCFA	250#	H_2	50	75
Alcohols				
octyl alcohol	500	glucose	99	178
Resin acids				
abietic acid:oleic acid+	1178	C_2	99	2
abietic acid	114	C_2, C_3, C_4	50	50
Volatile terpenes				
p-cymene	500#	C_2	50	11
limonene	250#	C_2	50	11
α-pinene	122	C_2	0	29

* the assay substrate: CE = cellulose, C2 = acetate, C3 = propionate, C_4 = butyrate, PH = phenol.

\# the inhibitory concentrations were estimated from available reported data.

\+ abietate : oleate ratio on a dry weight basis was 52 : 48.

TABLE 6. The methanogenic toxicity of inorganic compounds common in forest industry wastewaters.

COMPOUND		Concentration (mg/l)	Inhibition (%)	References
Inorganic Sulfur Compounds				
Sulfate	(SO_4^{2-})	10,000	50	160
Sulfite	(SO_3^{2-})	125	50	202
Hydrogen sulfide	(H_2S^*)	530	50	99
Dithionite	($S_2O_4^{2-}$)	1,500	99	153
Chelating Agents+				
DTPA		5	99	4
EDTA		83	0	198
Miscellaneous				
Hydrogen peroxide	(H_2O_2)	<< 200	NR#	195
Chlorine	(Cl_2)	12	99	198

* H_2S = Total concentration of hydrogen sulfide (H_2S + HS^-) at pH 6.8; only the unionized H_2S form is toxic.

+ DPTA = Diethylenetriaminepenta-acetic acid; EDTA = Ethylenediaminetetra-acetic acid

\# NR = Data not reported.

of wood resin constituents should be suspected because these components increase the decay resistance in trees (7,165,89). The high methanogenic toxicity of resinous wood components such as resin acids and volatile terpenes has indeed been reported in the literature (Table 5). Crude pine resin and the resin acid, abietic acid, were shown to cause a 50% inhibition of the methanogenic activity at very low concentrations, 57 and 115 mg/l, respectively (50). Wood extractives are dissolved in considerable amounts during alkaline wood pretreatment and may account for up to 10% of the wastewater COD (1000 mg/l) (195). CTMP effluents may also contain pulping chemicals such as sulfur compounds, hydrogen peroxide and chelating agents which interfere with anaerobic treatment systems (194,195).

Pichon et al (147) could achieve a 70% BOD removal at an organic loading rate of 2.6 g COD/l·day during anaerobic treatment of CTMP wastewaters in a fixed-bed reactor. However, the application of organic loads exceeding 3 g COD/l·day resulted in a significant decrease of the process efficiency due to methanogenic inhibition. The toxicity was attributed to the presence of sulfur compounds in the wastewater, however the authors did not provide evidence to support this conclusion. An other research group (4,194), developed a detoxification process for CTMP wastewaters based on the biocatalytic removal of hydrogen peroxide in an aerobic stirred tank reactor (140) followed by precipitation of long chain fatty acids and resin acids with aluminum, calcium and iron salts (2). This pretreatment method was found to be effective in removing the high methanogenic toxicity of CTMP effluents. With detoxified CTMP wastewaters, BOD removal efficiencies of 90% were achieved at an organic loading rate of 4 g COD/l·day in upflow anaerobic fixed-film reactors (194).

3.3.3.3. Chemical and Semichemical Pulping Wastewaters. Chemical alkaline and sulfite pulping processes generates highly polluted liquors. Fortunately, the black liquors originating from kraft and soda processes and the spent liquors from the acidic bisulfite process are

usually incinerated for pulping chemical recovery, reducing to a great extent the environmental impact. Nonetheless, chemical recovery is not often practiced at NSSC mills nor at small scale kraft and soda paper mills. Also, calcium-base sulfite liquors are not incinerated due to the lack of suitable processes for the recovery of cooking chemicals. Thus, an important sector of the forest industry does in fact discharge these highly polluted liquors and there is a great need to study their biological treatment.

The methanogenic toxicity of alkaline pulping liquors has not been studied. The chemical processes during alkaline pulping generally lead to the solubilization of toxic wood resin compounds. Therefore, one should expect high methanogenic inhibition from these wastewaters. In any case, a full-scale contact process in Spain has been reported which treats diluted black liquor derived from the soda pulping of straw (187). The BOD treatment efficiencies obtained in the anaerobic plant are over 90%; but the organic loading rates applied are not reported. In China, continuous anaerobic lab-scale studies have been conducted with black liquors mixed with acidic wastewaters to enhance acid precipitation of the lignin (206). Under these conditions, 85% COD reductions were observed at organic loading rates of 5 g COD/l·day. The acidic precipitation is also potentially effective in removing the toxic resin constituents.

The methanogenic toxicity of spent sulfite liquors has been observed during anaerobic treatability studies conducted by Chave *et al.* (22). The toxicity was attributed to phenolic compounds in the spent liquor since stripping of SO_2 did not result in alleviating the inhibition. Additionally, NSSC spent liquors have been found to be inhibitory at high concentrations (exceeding 20,000 mg COD/l) in batch toxicity bioassays (70). In pilot-scale studies, the methanogenic toxicity could be effectively reduced by dilution with other mill waste streams. Organic loading rates of 10-15 g COD/l·day could be accommodated with BOD removal efficiencies exceeding 80%. Based on the promising results obtained in pilot-scale studies, various full-scale UASB reactors have been constructed for the treatment of NSSC wastewaters (48,70,146).

3.3.3.4. Bleaching Wastewaters. Bleaching effluents are major sources of toxicity at paper mills. The effluents from chlorination and the first alkaline extraction have been shown to be highly toxic to methanogenic bacteria (134,155,169,203). The inhibitory effects of bleaching wastewaters have been attributed to the chlorinated organic compounds formed during the chlorination stage (134,156,194). The concentration of low MW chlorinated phenols reported in bleaching effluents ranges from 0.1 to 2.6 mg/l (72,83,87,134). Resin constituents dissolved during the alkaline extraction stage are also likely to be important methanogenic inhibitors in bleaching effluents. Concentrations of resin derived components ranging from 45 to 410 mg/l have been determined in kraft bleaching effluents (35,87).

Relatively little is known about the toxicity of chlorinated phenols. In any case, pentachlorophenol (PCP) was found to inhibit methanogenesis in unacclimated cultures at concentrations exceeding 0.2 mg/l (67). Exposure of the anaerobic sludge to gradually increasing concentrations of PCP was shown to result in significant acclimation of the microorganisms to the inhibitor. Upon acclimation, PCP removal exceeded 99.9% during anaerobic treatment of an influent containing 5 mg PCP/l. Dichlorophenol was also found to be highly toxic to methanogenic activity (192). Concentrations of 600 mg/l caused complete methanogenic inhibition.

Anaerobic treatment of the highly inhibitory alkaline extraction bleaching effluents has been shown to be feasible after considerable dilution of the wastewater. For example, dilution of bleaching wastewaters from the alkali extraction stage with kraft condensate (39,155) and sulfite evaporator condensate (134,173) prior to anaerobic treatment has been shown to be an efficient measure for reducing the methanogenic toxicity. Furthermore, alkali extraction bleaching effluents are suitable for neutralizing acidic sulfite evaporator condensate, reducing neutralization costs. Anaerobic treatment of the combined wastewaters was shown to be feasible at high organic loading rates (8 to 12 g COD/l·day) with BOD reductions ranging from 70 to 80% (134,155).

A two-step process consisting of an anaerobic fluidized bed reactor followed by an aerobic trickling filter has also been applied for treating bleaching effluents from the chlorination and alkali extraction stage (169). This process results in removal efficiencies of

about 95% BOD and 50% of the COD. The anaerobic treatment step was not effective in eliminating BOD, however, it served as a pretreatment for the aerobic system as it contributed to detoxify and to increase the aerobic biodegradability of the wastewater.

3.3.4. Anaerobic Biodegradability of Pulp and Paper Mill Wastewater Constituents.

3.3.4.1. Chlorinated Phenols. Among the potential benefits of anaerobic systems, is the ability of anaerobic microorganisms to participate in the biodegradation of halogenated compounds. Anaerobic microbial communities have recently been shown to degrade a variety of halogenated aromatic hydrocarbons, including several chlorinated phenols (17,67,71,128,185). The initial step in the degradation of these compounds is the removal of chlorine atoms from the aromatic ring by a reductive dechlorination reaction (185). Dechlorination is strongly dependent on the number and relative position of the chlorine atoms on the aromatic ring and the acclimation of the microbial consortia. Highly chlorinated substances are more readily dechlorinated, while the last chlorine atoms are more persistent (185). Unacclimated bacteria have been shown to preferentially remove chlorine substituents in the ortho position to the phenolic hydroxy groups (17,82,128). Acclimation of the sludge to the chlorophenols was shown to favor dechlorination of previously persistent compounds and to decrease the lag phases preceding dechlorination (17,67).

A number of anaerobic treatment technologies have been shown to be effective in eliminating the chlorinated organic matter of bleached pulp kraft effluents (41,73,74,156) and in synthetic wastes containing related chlorinated aromatics (67,103,201).

The degradative capacity of anaerobic treatment systems towards chlorinated aromatic compounds appears to be superior as compared to that of conventional aerobic methods. The results of a comparative study on the removal of chlorinated organic compounds from bleached kraft pulp effluents indicated that anaerobic treatment in fluidized bed reactors resulted in higher removal efficiencies (68-80%) as compared to aerobic treatment in activated sludge units or aerated lagoons (30%-50%) (171). Furthermore, anaerobic treatment was found more effective in removing di-, tri-, tetra- and penta-chlorophenols and their derivatives from bleaching effluents as compared to the aerobic treatment methods.

The ability of anaerobic consortia to dechlorinate and detoxify chlorinated phenolics in bleaching effluents makes anaerobic processes attractive as a treatment alternative to conventional aerobic systems. Particularly, anaerobic reductive dechlorination may be an important step to obtain complete mineralization of highly chlorinated compounds which are often resistant to aerobic bacterial attack (185).

3.3.4.2. Compounds Causing Aquatic Toxicity. The anaerobic treatment systems have a limited capacity to decrease the aquatic toxicity of forest industry wastewaters (197). Resin compounds such as terpenes and resin acids are poorly biodegraded by anaerobic microorganisms (155,175,186). Low MW tannins of bark are only partially degraded during anaerobic treatment (49). Monomeric chlorinated phenols, on the other hand are highly metabolized during anaerobic treatment, as was previously discussed, which can result in significant aquatic toxicity removal (73,74). Particularly, the decrease in chlorine atom number by anaerobic bacteria contributes to decreasing the aquatic toxicity.

3.3.4.3. Lignin and Lignin Derived Aromatics. Lignin derivatives are major components of the wastewater streams generated in the chemical processing of wood. The delignification treatments applied during chemical pulping and bleaching processes modify and partially depolymerize the lignin, increasing its solubility in the process water. Kraft lignins and lignosulfonates are the most important lignins produced by commercial pulping operations.

The presence of lignin in forest industry wastewaters represents a major source of organic matter resistant to biological wastewater treatment. Anaerobic bacteria have a very limited capacity to degrade lignin (12,69,204). Although the lignin polymer is inert anaerobically, low MW aromatics have been shown to be metabolized in anaerobic

environments. Numerous reports have indicated that lignin monomeric model compounds representative of those derived from alkaline, heat treated lignin are degradable under strict anaerobic conditions (9,66,76,77). Lignin related dimers are also decomposed by anaerobic microbial processes (24-26,204). In the latter studies with lignin dimers, the various types of intermonomeric bonds characteristic of native lignin were shown to undergo anaerobic metabolism. This indicates that the presence of intermonomeric lignin bonds does not *per se* limit the anaerobic biodegradability of lignin. Furthermore, kraft lignins were partially decomposed in studies with mixed methanogenic cultures under mesophilic (204) and thermophilic conditions (12). Zeikus *et al.* (204) observed that degradation was limited to the low MW material (MW <600 dalton), whereas lignin moieties with MW greater than 850 dalton were not mineralized. Similarly, Colberg & Young (28) demonstrated substantial anaerobic biodegradation of natural lignin chemically solubilized to fragments of MW ranging from 200 to 1400 dalton, with more extensive metabolism occurring in the lower MW fractions. Native lignin and synthetic high MW lignins are highly refractory to anaerobic biodegradation (12,69,139). As in the case of the anaerobes, it appears that degradation of lignin by aerobic bacteria is also limited to the low MW material (93). Lignin related dimeric compounds (64,188) and even tetrameric compounds (86) are readily metabolized by aerobic bacteria. Based on the cited literature, a positive correlation of the bacterial recalcitrance with the molecular size of the lignin fragments is clearly indicated.

3.3.4.4. Anaerobic Biodegradability of Pulp and Paper Mill Wastewaters. Paper mill effluents generated by chemical treatments which effectively attack lignin, such as those from chemical sulfite alkaline pulping (22); NSSC pulping processes (70,137) and bleaching processes (134,168,173) are poorly biodegradable. The COD reductions obtained during anaerobic treatment of these types of wastewaters are generally only 50% or less. Similarly, chemi-thermomechanical pulping liquors contain significant fractions of lignin and are thus not fully biodegradable. The COD reductions observed in various anaerobic treatability studies with CTMP effluents varied from 45 to 60% (90,147,194).

3.4. REMOVAL of COLOR from PULP AND PAPER MILL WASTE STREAMS

Color is an important characteristic associated with lignin and other high MW polyphenolics (51,182). The limited capacity of bacteria to degrade the lignin chromophoric structures indicates that combined technologies, including physical-chemical and enzymatic or fungal treatments should be applied to remove the color bearing lignic COD which is resistant to anaerobic as well as conventional aerobic wastewater treatment.

A variety of methods for removing the highly colored dissolved lignin from forest industry wastewaters have been proposed in the literature. Alkali lignin is easily separated by acidification of the spent liquors and subsequent filtration (94). Precipitation processes based on the addition of lime (33), polyamines (96) and other coagulants (161) have been investigated for controlling paper mill color discharge. Enzymatic pretreatments have been applied to enhance the precipitability of lignin and polyphenols in pulp mill effluents, either directly (30,56,143) or in combination with precipitation processes (21,56,129,176). Significant decolorization can also be accomplished by adsorption with activated charcoal (15), ultrafiltration (57) and chemical oxidations with H2O2 (148). Finally, numerous studies utilizing white-rot fungi demonstrate that fungal treatment of bleaching wastewaters resulted in high decolorization efficiencies (35,37,151,182). The high degradative capacity of white rot fungi towards lignin (95) indicates the potentials of utilizing fungi for the treatment of forest industry wastewaters. A combination of fungal treatments with conventional aerobic or anaerobic treatment should be considered in future research. Such a treatment configuration could possibly allow for complete biological removal of the organic matter in forest industry wastewaters.

4. SCOPE OF THIS THESIS

This thesis describes research on the anaerobic treatment of inhibitory wastewaters from the forest industry. The main objective was to determine the role of natural organic wood constituents on the inhibitory effects of these wastewaters.

In the first part of the thesis (Chapter 2), lignocellulosic feedstocks were pulped by different processes to determine which factors are responsible for the extraction of toxic substances. Thereafter (Chapters 3 to 5), compounds representative of inhibitory wood constituents were assayed for methanogenic toxicity. This included evaluating the role of chemical structure on methanogenic toxicity. In Chapter 6, the anaerobic biodegradability of selected inhibitory wood derived compounds was determined. Finally (Chapter 7), the continuous anaerobic treatment of toxic pulping effluents was investigated.

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CHAPTER 2

The Anaerobic Biodegradability and Methanogenic Toxicity of Pulping Wastewaters

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THE ANAEROBIC BIODEGRADABILITY AND METHANOGENIC TOXICITY OF PULPING WASTEWATERS

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ABSTRACT

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

The anaerobic biodegradability and the methanogenic toxicity of the various wastewaters were evaluated in standardized batch bioassays using anaerobic granular sludge. The acidification of the TMP wastewaters (conversion to CH_4 and VFA) ranged from 68 to 87% of the total COD, indicating their high anaerobic biodegradability. TMP wastewaters were non-toxic to methane bacteria at concentrations expected in paper mill wastewaters. No inhibition was observed at 10 g COD/l. In contrast, wastewaters prepared in alkaline conditions were poorly biodegradable (approx. 50% acidification) and they caused severe inhibition of the methanogenic activity. The 50% inhibitory concentrations ranged from 2.1 to 5.4 g COD/l.

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

1. INTRODUCTION

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

The processing of wood in paper mills involves various operations including debarking, pulping and bleaching that result in the discharge of highly polluted wastewaters. The quantity and types of pollutants in these effluents vary with the type of lignocellulosic feedstock used as raw material, the process conditions applied (pH, temperature, pressure, chemical and mechanical treatments) and the specific water consumption (Gottsching *et al.* 1977; Stemberg & Norberg, 1977). High pressures and temperatures, and particularly chemical addition result in an increased release of organic matter into the process water and extensive lignin solubilization. Therefore, the pollution loads and the color due to dissolved lignic compounds (Corson & Lloyd, 1978; Virkola & Honkanen, 1985) is very high for chemical as compared to mechanical pulping effluents. The COD loads associated with mechanical pulping processes range from 20-50 kg COD per ton of pulp (Stemberg & Norberg, 1977) whereas those corresponding to soda pulping processes may be as high as

500-900 kg COD per ton of pulp (Anonymous, 1986). Nevertheless, the black liquors originating from kraft and soda processes are usually burnt to recover the pulping chemicals and the calorific power from the organic components, diminishing to a great extent the environmental impact associated with these pulping processes. Conventional recovery processes are not economically viable in small paper mills and in those using non-woody raw materials with a high silica content (Velasco *et al.* 1985; Anonymous, 1986). Black liquors represent a very important pollution source in several countries where small scale mills are common (Velasco *et al.* 1985; Anonymous, 1986; Gönenç *et al.* 1989).

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

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

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

TABLE 1. Literature Averaged Composition of Hardwoods, Softwoods and Straw (Misra, 1980; Fengel and Wegener, 1984; Tewari and Nemerow, 1982).

	Composition (% total dry weight)		
CONSTITUENT	Hardwood	Softwood	Straw
Lignin	17-26	25-32	17-19
Hemicellulose	22-34	15-18	27-32
Cellulose	58-64	55-61	33-38
Extractives	3-8[+]		1-2[++]
Mineral matter	1		6-8

[+] alcohol-benzene extract followed by hot water extraction
[++] ether extract.

2. MATERIALS AND METHODS

2.1. Preparation of wood and straw pulping wastewaters

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

2.2. Treatments of the pulping wastewaters

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

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

2.2.3. Ca^{+2} precipitation. 1 l of the pulping wastewater (5 to 10 g COD/l) was treated at pH 11 with 1000 mg/l of Ca^{+2}, supplied as $CaCl_2.2H_2O$. Precipitation was allowed to occur overnight after which the wastewater was paper filtered and neutralized with HCl.

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

2.2.5. Acid precipitation. 1 l of the wastewater (5 to 10 g COD/l) was adjusted to pH 2 with HCl. The precipitation was allowed to occur overnight. The pulp water was centrifuged and the precipitate and filtrate quantitatively recovered. The precipitate was repeatedly washed with a diluted HCl solution (pH 2) to removed traces of the mother liquid, centrifuged and suspended in 1 l of H_2O. Finally, the filtrate (soluble fraction) and suspended precipitate (insoluble fraction) were neutralized.

2.3. Analyses

Samples for COD and volatile fatty acids (VFA) determination were filtered (Schleicher & Schull paper filter no. 589-1). COD (colorimetric method with dichromate), TSS and VSS were determined according to Standard Methods (American Public Health Association, 1985). The pH was determined with a Knick 511 pH-meter and a Scot Gerade N61 double electrode immediately after sampling in order to avoid a pH rise due to the loss of carbon dioxide

from the liquid. VFA were analyzed by gas chromatography using a Packard Becker model 417 equipped with a 2 m x 4 mm glass column packed with Supelcoport (100-120 mesh) coated with 10% Fluorad FC 431. The temperature of the column, the injection port and the flame ionization detector were 130, 220 and 240°C, respectively. Nitrogen saturated with formic acid was used as carrier gas at a flow rate of 50 ml/min. The analysis of methanol and ethanol were based on a similar chromatographic procedure modifying the temperature of the column, the injection port and the flame ionization detector to 80, 180 and 200°C, respectively.

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

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

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

2.4. Chemicals

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

2.5. Biomass

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

2.6. Biological Assays

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

Methane production was monitored periodically during the assays with modified Mariotte flasks. These flasks were filled with a 3% NaOH solution which served to remove the CO_2 contained in the biogas.

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

Type 1. This method was used to determine the methanogenic toxicity of soda pulp liquors. The assays were carried out in two consecutive feedings. In the first feeding, tap water, granular sludge (1.5 g VSS/l) and known amounts of wastewater COD were transferred to

the serum flasks containing nutrient solution. No wastewater was added to the substrate controls. Subsequently, distilled water was added to complete a medium volume of 0.5 l and, afterwards, the substrate controls and treatments (assays containing wastewater) were supplied with 4 g COD/l of a neutralized volatile fatty acid (VFA) solution. The composition of the VFA solution that served as substrate was 100:100:100 g acetate:propionate:butyrate per kg. Finally, 1 g $NaHCO_3$ per gram of biodegradable wastewater COD was added to the treatment containing the highest assay concentration to buffer eventual accumulations of VFA. The same quantity of $NaHCO_3$ was also supplied to the other treatments and to the substrate controls.

On day 14, all serum flasks were provided with a second substrate feeding in order to evaluate the residual activity of the sludge after exposure to the pulp water. The supernatants were carefully decanted to avoid losses of methanogenic sludge and replaced, while maintaining N_2 flushing in the head space, with a nutrient supplemented medium containing 4 g VFA-COD/l. No wastewater was included in the replacement medium. Finally, the serum flasks were incubated for 1 to 2 weeks.

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

During the first feeding each treatment was paired with a corresponding substrate control which contained an equal concentration of biodegradable COD supplied as VFA. The composition in COD basis of the neutralized VFA stock solution utilized in the substrate controls was 75:20:5 acetate:propionate:butyrate, similar to that of the completely acidified wastewater. The treatments were not supplied with VFA as the wastewater itself provided the substrate for the toxicity assay. To buffer eventual accumulations of VFA, 1 g $NaHCO_3$ per gram of biodegradable COD was added to the treatments. The second feeding was the same as previously described in the toxicity assay type 1.

The specific methanogenic activity, expressed as the amount of CH_4 produced by 1 g of sludge VSS per day (g CH_4-COD per g VSS per day), was calculated in all toxicity assays from the slope of the methane production versus time curve and the quantity of VSS. As an example, the cumulative methane production versus time curves obtained in a toxicity experiment with birch alkaline liquor are shown in Figure 1. The methanogenic activities in the first and second feeding for each concentration point were calculated in the time interval corresponding to the maximum control activity. The inhibited activity was expressed as percentage of the control activity, and it is abbreviated as (% ACT). The percentage inhibition (% I) is defined as: % I = 100 - % ACT. The wastewater concentration that caused 50% and 80% inhibition of the methanogenic activity is referred to as "50% IC" and "80% IC", respectively.

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

2.6.2. Detoxification experiments. The detoxification obtained by selectively removing specific wastewater components was evaluated in anaerobic toxicity assays fed the original and treated pulping wastewater, following the procedure previously described in the toxicity assay type 1. A concentration close to the 50% IC was chosen as a standard concentration for the "detoxification" experiments.

2.6.3. Anaerobic biodegradability assay. The biodegradability experiments were conducted in 0.6 l flasks. Granular sludge (5 g VSS/l), distilled water and a known amount of wastewater COD were transferred to the flasks containing 0.1 l of the nutrient solution. The assay COD concentration after dilution to the final volume (0.5 l) ranged from 4 to 5 g COD/l. The biodegradability assays of the soda pulp liquors were supplied with a lower substrate

concentration (approx. 2 g COD/l) to minimize methanogenic inhibition during the assays. The latter experiments were conducted in 1.2 l serum flasks to allow a more accurate determination of the methane production. All experiments included a sludge blank lacking substrate. The treatments and blanks were supplied with 1 g $NaHCO_3$ per gram of biodegradable COD. The percentage acidification of the wastewater COD was calculated by the sum of the cumulative CH_4-COD and the media VFA-COD for a given assay period. The acidification results reported are corrected for the acidification of the sludge controls.

3. RESULTS

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

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

3.1. The anaerobic biodegradability of wood and straw pulping wastewaters

The average acidification of the wastewater COD (conversion to CH_4 and VFA) observed during the batch anaerobic digestion of the TMP and the soda pulping wastewaters prepared from wood (pine, spruce or birch) and wheat straw are reported in Table 3.

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

TABLE 2. The Average Composition of the Wood and Straw Pulping Wastewaters Used in this Study and the Yield of Soluble Organic Matter from the Various Lignocellulosic Feedstocks.

	Pulping method and lignocellulosic feedstock*							
	TMP				SODA			
COMPONENT (% COD)	P	S	B	St	P	S	B	St
VFA	1	2	6	5	8	12	28	10
Alcohols++	ND	ND	ND	ND	0	0	0	0
Sugar	ND+	ND	ND	ND	11	12	ND	35
Lignin	20	16	ND	32	40	46	ND	ND
pH	5	4	5	7	11	11	11	11
Yield#	42	30	38	ND	250	187	259	384

* P = pine, S = spruce, B = birch, St = straw. + ND = not determined; ++ Methanol and ethanol; # Yield = organic matter dissolved in g COD per kg of dried wood or straw.

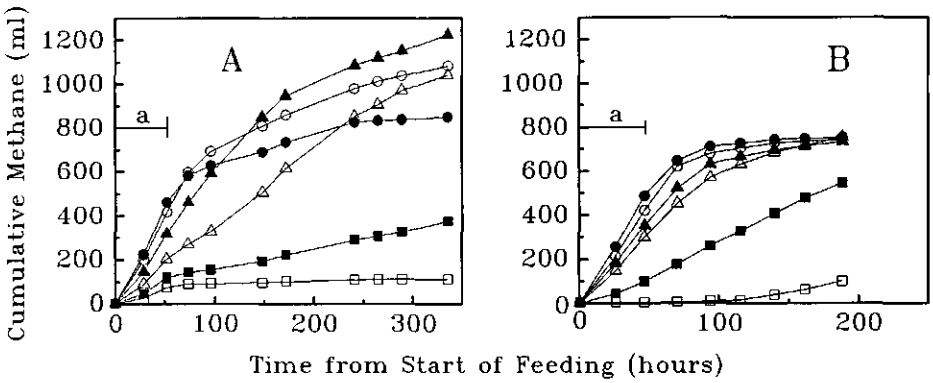

FIG. 1. The cumulative methane production of VFA fed assays containing birch soda pulping liquor in the first (A) and second assay feeding (B). The wastewater assay concentrations (in g COD/L) were: 0 (●), 2 (○), 4 (▲), 6 (△), 8 (■) and 10 (□); a = the time period used to determine the methanogenic activity.

3.2. The methanogenic toxicity of wood and straw pulping wastewaters

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

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

Batch digestion experiments with repeated feedings of straw and spruce soda pulp water were also performed to evaluate the short-term adaption capacity of the sludge to these wastewaters. The procedure for the additional feedings was similar to that applied to assay the methanogenic toxicity of the soda pulp liquors in the first feeding. The 50% and 80% IC resulting from exposure of granular sludge to five consecutive feedings of straw alkaline liquor are given in Figure 3. According to these results, a significant increase in the inhibiting concentration with feeding number was observed, indicating that granular sludge can adapt to a great extent to the inhibitory effects of straw soda pulp liquor. In contrast, repeated feedings with spruce soda pulping wastewater did not result in any adaption of the granular sludge, and the 50% and 80% IC values determined in the first and third assay feeding were very similar.

3.3. Sources of methanogenic inhibition in soda pulping wastewater

Additional experiments were conducted in an attempt to identify the sources of the inhibition in soda pulping liquors. The detoxification obtained by selectively removing specific wastewater components was evaluated in anaerobic toxicity assays fed the original and treated wastewaters. The treatments applied included liquid-liquid extraction with ether, XAD and PVP adsorption, calcium and acid precipitation. The extraction of wood derived wastewaters with ether removes hydrophobic resin components, such as fatty acids, resin acids, esters, waxes and sterols (Bjorklund-Jansson, 1980; Voss & Rapsomatiotis, 1985). Adsorption onto XAD-2 under alkaline (pH 9) conditions has previously been used to isolate wood resin compounds from pulp and paper effluent samples (Rogers, 1973; Leach & Thakore, 1976). PVP is a polyamide that specifically removes the fraction of the wastewater with tannic qualities (Field *et al.* 1988). The calcium can precipitate an important fraction of the dissolved lignin, but its effectiveness is restricted to the intermediate to high molecular lignin derivatives (Dugal *et al.* 1975; Schmidt & Joyce, 1980). Fatty and resin acids are also removed by calcium precipitation (Easty *et al.* 1978). Acidification precipitates lignin and resin compounds similarly as calcium (Bjorklund Jansson & Back, 1975; Kim *et al.* 1987).

The elimination of COD in the treatments with PVP, XAD, calcium and ether was low, ranging between 13 and 20% of the total wastewater COD. In contrast, precipitation in acidic medium resulted in removal of 67% of the total wastewater COD.

The influence of various treatments of pine alkaline pulp liquor on the methanogenic activity of granular sludge exposed to the resulting wastewaters is illustrated in Figure 4.

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

Pulping method	Lignocellulosic material	Acidification (%)
TMP		
	Pine	78.4
	Spruce	79.8
	Birch	86.5
	Straw	67.5
SODA		
	Pine	50.6
	Spruce	46.7
	Birch	67.3
	Straw	45.4

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

PULP.	LIGNOCELLULOSIC MATERIAL	1st. feeding		2nd. feeding	
		50% IC	80% IC	50% IC	80% IC
TMP					
	Pine	11.5	14.0	12.5	14.4
	Spruce	13.0	22.0	22.0	>>22.0
	Birch	13.7	17.0	17.4	22.0
	Straw	NT*	NT	NT	NT
SODA					
	Pine	2.1	2.7	2.0	2.8
	Spruce	5.4	8.3	4.8	7.3
	Birch	5.3	7.3	6.8	8.6
	Straw	4.4	7.3	7.5	9.7

*NT= non-toxic at 3 g COD/l, the highest concentration tested

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

PINE WOOD	1st feeding		2nd. feeding	
	50% IC	80% IC	50% IC	80% IC
UNTREATED	2.1	2.7	2.0	2.8
RESIN-FREE	9.0	20.0	>>12.0	>>12.0

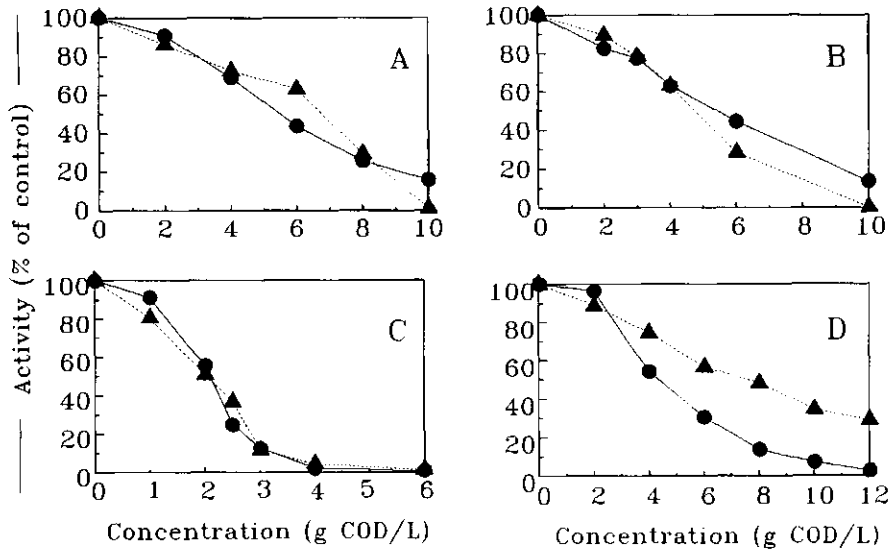

FIG. 2. The methanogenic activity (as percentage of the control activity) of granular sludge exposed to soda liquors prepared from wood and straw: birch (A), spruce (B), pine (C) and wheat straw (D), respectively, versus the wastewater assay concentration. The activity of the sludge in the first feeding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

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

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

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

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

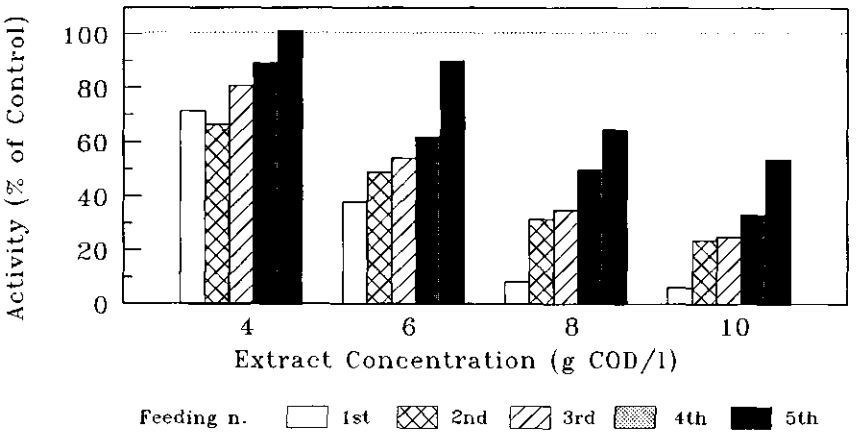

FIG. 3. The effect of consecutive feedings of straw soda pulping liquor on the activity of methanogenic granular sludge.

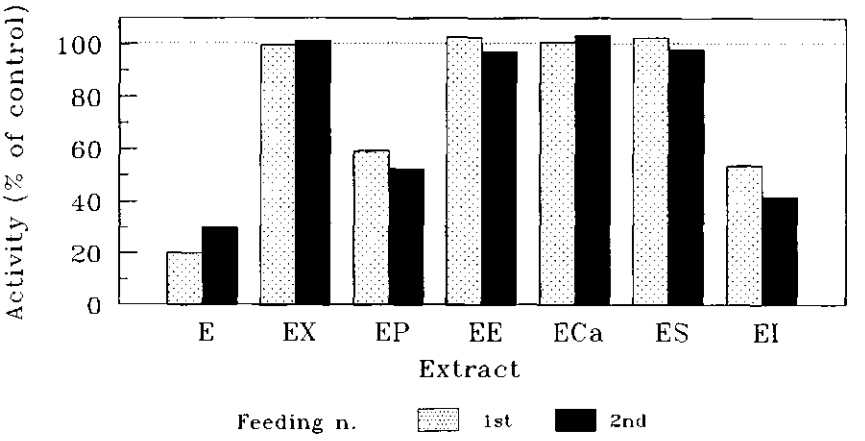

FIG. 4. The influence of various treatments of pine soda pulping liquor on the methanogenic activity of granular sludge exposed to the different extracts in the first and in the second assay feeding. Untreated wastewater (E), wastewater treated with XAD-2 (EX), with PVP (EP), with ether (EE), with calcium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

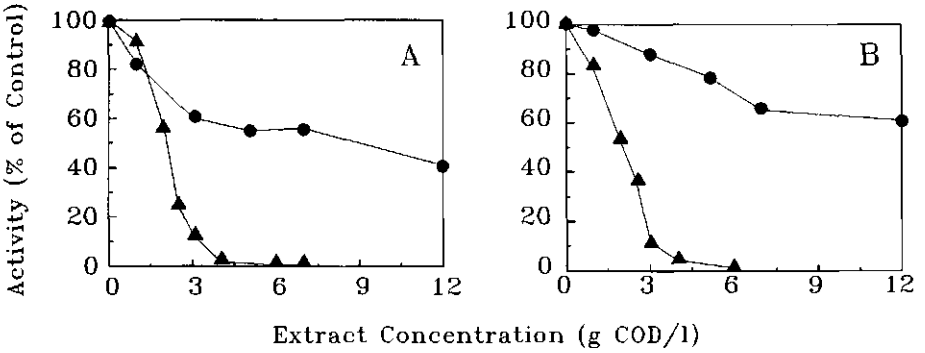

FIG. 5. The effect of soda pulping wastewaters from untreated pine wood (▲) and treated resin-free pine wood (●) on the methanogenic activity of granular sludge. (A) first assay feeding; (B) second assay feeding.

4. DISCUSSION

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

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

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

The anaerobic biodegradability of TMP effluents was high due to the large fraction of readily biodegradable carbohydrates in these wastewaters. The distinctly lower biodegradability of alkaline pulping liquors can be explained by their higher lignin content. Past research indicates that anaerobic bacteria have a very limited capacity to degrade lignin (Hackett *et al.* 1977; Zeikus *et al.* 1982; Benner *et al.* 1984). The recalcitrance of lignin in anaerobic environments is related to its characteristic chemical heterogeneity and high molecular weight. In any case, the anaerobic metabolism of various lignin monomers

and oligomers (MW< 800 dalton) has been reported (Colberg & Young, 1985; Chen *et al.* 1985a, 1985b, 1987). Higher MW lignin is not degraded by anaerobic bacteria (Zeikus *et al.* 1982; Colberg & Young, 1985).

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

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

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

Although wood resin was responsible for most of the methanogenic toxicity, the soda pulp liquor derived from resin-free pine wood exerted a small inhibitory effect, indicating that non-resinous compounds may also contribute to a certain extent to the toxicity of soda pulp wastewaters. These non-resinous components most likely correspond to lignin derived phenolics. Low molecular weight lignin derivatives have previously been identified as microbial inhibitors in aqueous lignocellulosic extracts (Clark & Mackie, 1984; Jung, 1988) and in studies with model compounds (Zemek *et al.* 1979; Vohra *et al.* 1980; Benjamin *et al.* 1984).

The high methanogenic toxicity of soda pulp liquors as compared to those of TMP demonstrate that the contact of wood with alkali has an important effect in increasing the methanogenic toxicity of wood derived wastewaters. The forest industry applies various bleaching and chemical pulping processes where wood is subjected to alkaline treatment. This will generate effluents with high concentrations of resin compounds that are responsible for severe methanogenic toxicity.

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CHAPTER 3

The Methanogenic Toxicity of Wood Resin Constituents

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THE METHANOGENIC TOXICITY OF WOOD RESIN CONSTITUENTS

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ABSTRACT

The purpose of this study was to evaluate the inhibitory effect of representative wood resin compounds on the activity of methanogenic bacteria. Resin is by definition the mixture of wood components that are extractable with apolar solvents. Major resin constituents are long chain fatty acids, terpenes, resin acids, lignans and apolar phenols.

The methanogenic inhibition was determined at a temperature of 30 °C in standardized toxicity assays utilizing anaerobic granular sludge.

An apolar phenol, 4-hydroxystilbene, was the most toxic of the compounds studied, with a 50% inhibiting concentration of 20 mg/l. Resin acids and volatile terpenes were also highly toxic to methanogenic activity. Concentrations causing 50% inhibition ranged from 43 to 330 mg/l. In contrast, triterpenes were nontoxic at relatively high concentrations, 1000 to 1300 mg/l.

These results suggest that wood resin constituents play an important role in the anaerobic inhibition exerted by several forest industry wastewaters.

1. INTRODUCTION

Resin is by definition the mixture of wood components extractable with apolar solvents. Major resin constituents are long chain fatty acids (LCFA), terpenes, resin acids, lignans and apolar phenols. The averaged composition of the wood resin from softwoods and hardwoods is shown in Table 1. The resin content in wood usually ranges from 3 to 5% of the wood dry weight, but it can be much higher in certain tropical and subtropical woods (Fengel & Wegener, 1984a). Generally, softwoods are richer in resin than hardwoods. Softwoods extractives also differ in composition with respect to those of hardwoods. The resin of coniferous trees contains all classes of terpenes from monoterpenes to tri- and tetraterpenes, whereas in deciduous wood mainly higher terpenes are present (Fengel & Wegener 1984b).

The pulp and paper industry is a major source contributing to the discharge of wood extractives in the environment. Examples of other waste streams containing wood extractives or related compounds include citrus processing effluents and chemical manufacture wastewaters.

In paper mills wood is subjected to pulping and bleaching operations in order to purify the cellulose. As a result of these treatments, hemicellulose, lignin and extractives are separated to different extents from the cellulose and extracted into the process waters. The resin content of various forest industry wastewaters reported in the literature is shown in Table 2.

Pulp and paper mill wastewaters often exert inhibitory effects on microorganisms that can disturb biological treatment systems. They are derived either from extracted wood components, from chemicals added or products formed in the industrial processes.

TABLE 1. Literature averaged composition of wood and bark resin (Fengel & Wegener, 1984a; Rudloff & Sato, 1963).

COMPOUNDS	Hardwoods	Softwoods	
	Wood	Wood	Bark
	<---	% total resin	--->
resin acids	T*	30	7
volatile terpenes	NM+	2	NM
triterpenols	36	T	3
LCFA#	48	33	13
apolar phenols	NM	25	NM
lignans	NM	10	NM

* = traces, + = not measured, # = long chain fatty acids.

TABLE 2. Resin content in paper mill wastewaters.

Wastewater[#]	Extractives (mg/l)	LCFA[*] (mg/l)	Resin Acids (mg/l)	References
Debarking	51	2	7	20
GWP	105	NR[+]	NR	48
BB	69-154	NR	NR	4
CM	228	NR	NR	31
TMP	90	NR	NR	48
TMP	394	NR	NR	17
TMP	NR	43	380	5
TMP	NR	46	245	5
TMP	NR	37-53	63-143	8
TMP	NR	NR	48-370	53
TMP	NR	NR	15- 62	19
CTMP	1000	NR	NR	51
Acidic bleaching of KP	46	8	0.2	20
Alkaline bleaching of KP	185	9	0.4	20
H_2O_2-bleaching of GWP	100	NR	NR	48

[*] LCFA = Long Chain Fatty Acids

[#] GWP = Groundwood Pulping, BB = Building Board, CM = Corrugated medium, TMP = Thermomechanical Pulp CTMP = Chemi-thermomechanical Pulping, KP = Kraft Pulping.

[+] NR = Not reported

The methanogenic toxicity of inorganic wastewater components, such as sulfate, sulfite, hydrogen peroxide and other chemicals added during pulping and bleaching processes has been extensively investigated (Eis *et al.* 1983; Puhakka *et al.* 1985; Ruffer & Boeck, 1988; Rinzema & Lettinga, 1988; Welander 1988). In contrast, very limited information describing the fate of natural organic wood components in anaerobic systems is available.

The microbial toxicity of wood resin constituents should be suspected because these components increase the decay resistance in trees. Timber durability has been related to the antifungal activity of resin components (Anderson & Scheffer, 1962; Jurd & Manners, 1980; Rudman, 1962). Additionally, the toxicity of resin constituents to aquatic organisms has been extensively investigated (Leach & Thakore, 1976; Rogers 1973; Kutney *et al.* 1981a; 1981b; 1982) and a substantial amount of data is available to indicate that resin containing paper mill wastewaters display acute toxicity to fish and other aquatic organisms. Resin acids and other resin constituents have been identified as important fish toxins. Leach & Thakore (1976) reported a LC_{50}(96h) for various resin acids and LCFA ranging from 0.3 to 5.0 mg/l with Rainbow trout as a test organism.

The methanogenic toxicity of resin constituents has been studied to a lesser extent. Some toxicity data available in the literature are summarized in Table 3. In this table one can observe that mostly the effects of LCFA on methanogenic mixed cultures have been investigated whereas very little data are available concerning the effects of other resin components in anaerobic environments.

The purpose of this study was to evaluate the inhibitory effect of various representative wood resin compounds on the activity of methanogenic bacteria.

2. MATERIALS AND METHODS

2.1. Biomass

The granular sludge used in these experiments was obtained from a full scale upward-flow anaerobic sludge blanket (UASB) reactor treating distillery wastewater. The sludge was elutriated and stored at 4 °C under nitrogen gas. The sludge used was not acclimated to the toxic compounds prior to the toxicity assays.

2.2. Basal medium

The inorganic macro- and micro-nutrients were supplied to the assay media as a five-fold concentrated solution. After dilution the basal medium used in the anaerobic toxicity assay contained (mg/l): $NaHCO_3$ (400), NH_4Cl (280), $CaCl_2 \cdot 2H_2O$ (10), K_2HPO_4 (250), $MgSO_4 \cdot 7H_2O$ (100), yeast extract (100), H_3BO_3 (0.05), $FeCl_2 \cdot 4H_2O$ (2), $ZnCl_2$ (0.05), $MnCl_2 \cdot 4H_2O$ (0.05), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.05), $AlCl_3 \cdot 6H_2O$ (0.09), $CoCl_2 \cdot 6H_2O$ (2), $NiCl_2 \cdot 6H_2O$ (0.05), $CuCl_2 \cdot 2H_2O$ (0.03), $Na_2SeO_3 \cdot 5H_2O$ (0.1), EDTA (1), resazurin (0.2) and 36% HCl (0.001 ml/l). All chemicals were of analytical grade (Merck, Darmstadt, FRG). The yeast extract was supplied by Gist-Brocades (Delft, The Netherlands).

2.3. Analyses

Volatile fatty acids (VFA) were determined with a gas chromatograph equipped with a 2 m x 4 mm glass column packed with Supelcoport (100-120 mesh) coated with 10% Fluorad FC 431. The temperature of the column, the injection port and the flame ionization detector were 130, 220 and 240 °C, respectively. Nitrogen saturated with formic acid was used as carrier gas at a flow rate of 50 ml/min. The pH was determined immediately after sampling with a Knick 511 pH-meter and a Scot Gerade N61 double electrode. COD (colorimetric micro-method) and volatile suspended solids (VSS) were determined according to Standard Methods (American Public Health Assoc., 1985).

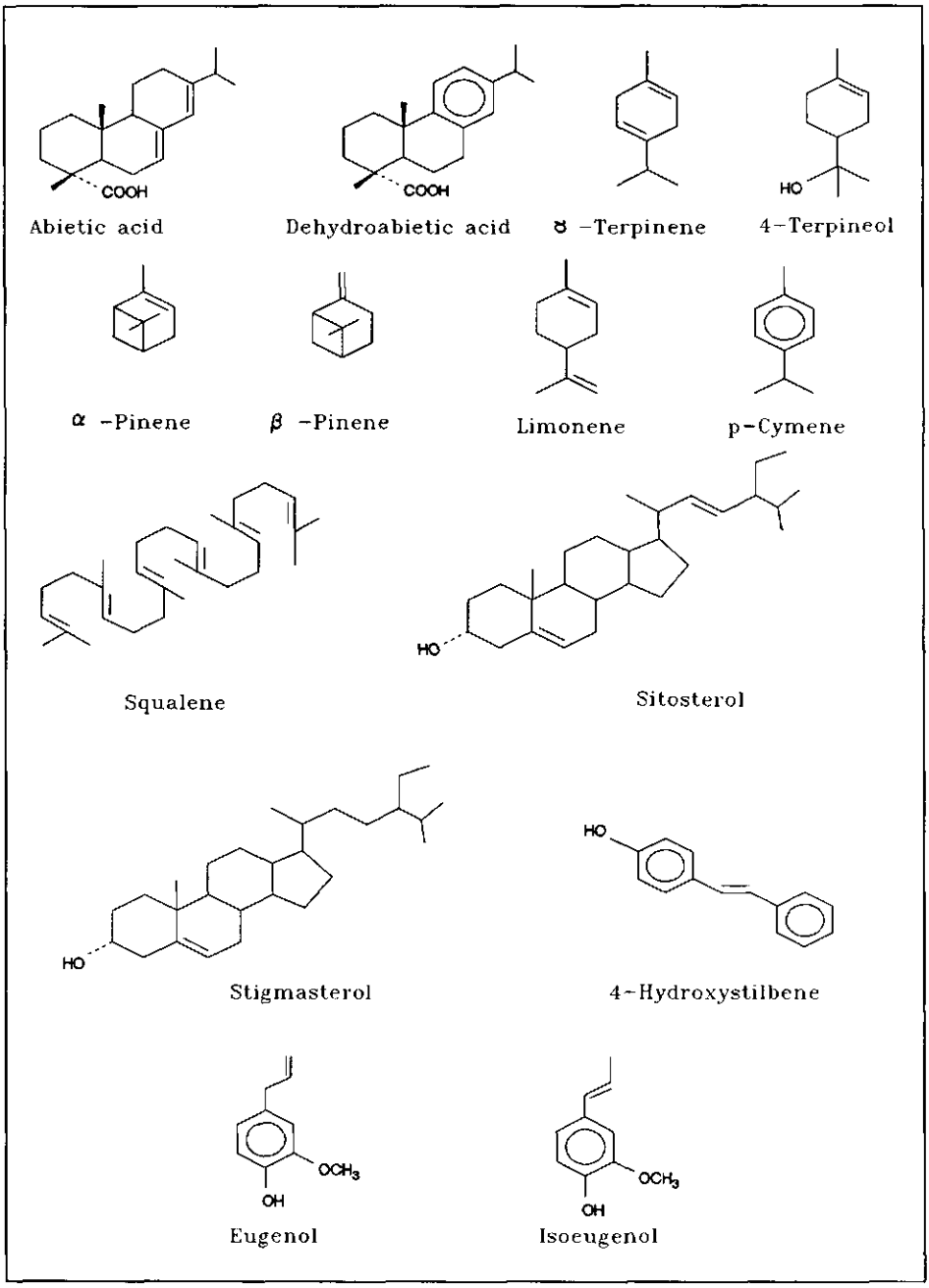

FIG. 1. Chemical structure of the wood resin components investigated in this study.

TABLE 3. Literature data on toxicity of wood resin components and related compounds.

COMPOUND	Substrate*	50% IC (mg/l)	References
Long Chain Fatty Acids			
lauric acid	C_2	525	Chou et al. 1978
lauric acid	C_2	869	Koster & Kramer, 1987
capric acid	C_2	1027	Koster & Kramer, 1987
myristic acid	C_2	1104	Koster & Kramer, 1987
oleic acid	C_2	1235	Koster & Kramer, 1987
linoleic	H_2/CO_2	897	Prins et al. 1972
linolenic	H_2/CO_2	501	Prins et al. 1972
linolenic	pyruvate	278	Demeyer & Henderickx, 1967
mixture LCFA	C_4	250#	Hanaki et al. 1981
mixture LCFA	H_2	250#	Hanaki et al. 1981
Alcohols			
octyl alcohol	glucose	500 (99%)+	Sonoda & Seiko, 1968
Resin acids			
abietic acid:oleic acid**	C_2	1178 (99%)+	Andersson & Welander, 1985
abietic acid	C_2, C_3, C_4	114	Field et al. 1988
Volatile terpenes			
p-cymene	C_2	500#	Benjamin et al. 1984
limonene	C_2	250#	Benjamin et al. 1984
limonene	C_2	122 (79%)	Crane et al. 1957
α-pinene	C_2	122 (0%)	Crane et al. 1957
Resin related aromatics			
eugenol	C_2	250#	Benjamin et al. 1984

* C_2 = acetic acid, C_3 = propionic acid, C_4= butyric acid.

\+ The number in the parenthesis indicates the inhibition observed at the reported concentration.

** Abietate : oleate ratio on a dry weight basis was 52 : 48.

\# Approx. 50% inhibitory concentration estimated from reported data.

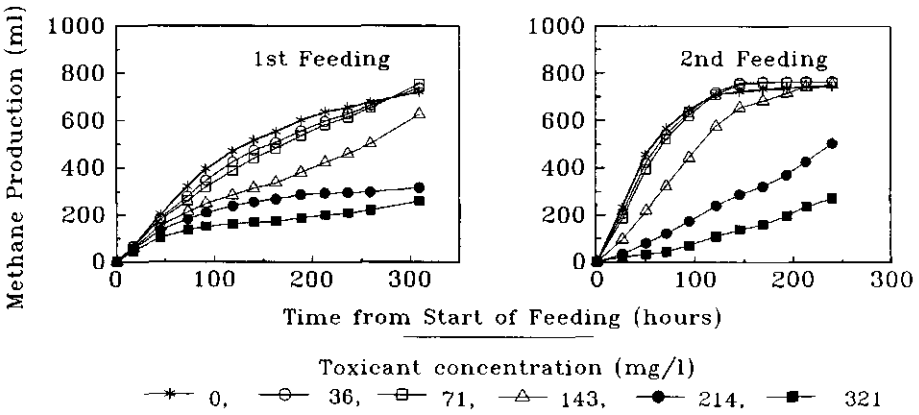

FIG. 2. The cumulative methane production during the methanogenic toxicity assay of abietic acid.

2.4. Anaerobic toxicity assay

Methanogenic activity measurements were performed in 0.6 l glass serum flasks. Distilled water, granular sludge (1.5 g VSS/l) and known amounts of toxicant were transferred to the flasks containing 100 ml of the concentrated nutrient solution. The toxicant concentrations supplied were chosen to provide an inhibition of the methanogenic activity ranging from 0 to 100%. Substrate controls were based on assays where no toxicant was added. Subsequently, all serum flasks were supplied with 4 g COD/l of a neutralized VFA solution, containing 100:100:100 g acetate:propionate:butyrate per kg, that served as substrate. Finally, distilled water was added to complete a medium volume of 500 ml. The liquid was flushed with nitrogen gas and the flasks were sealed with a rubber septum and a screw cap and incubated in a temperature controlled room at 30±2 °C. All experiments were mechanically shaken unless otherwise indicated. Methane production was monitored periodically during the 14 days that followed with modified Mariotte flasks. These flasks were filled with a 3% NaOH solution which served to remove the CO_2 contained in the biogas.

In order to evaluate the residual activity of the sludge after exposure to the toxic compound, on day 14 all the serum flasks were provided with a second substrate feeding lacking the toxicant. The supernatants were carefully decanted to avoid losses of methanogenic sludge and replaced, while maintaining N_2 flushing in the head space, with a nutrient supplemented medium containing 4 g VFA-COD/l. The bottles were reincubated again for 1 to 2 weeks.

The specific methanogenic activity, expressed as the amount of CH_4 produced by 1 g of sludge VSS per day (g CH_4-COD/g VSS·day), was calculated from the slope of the methane production versus time curve and the quantity of VSS. The methanogenic activities for each toxicant concentration were calculated in the time interval corresponding to the maximum control activity. The inhibited activity was expressed as percentage of the control activity, and it is abbreviated as (% ACT). The percentage inhibition (% I) was defined as: % I = 100 - % ACT. The compound concentrations that caused 50% and 80% inhibition of the methanogenic activity are referred to as "50% IC" and "80% IC", respectively.

2.5. Chemicals

The chemicals used were purchased from Janssen Chimica, (Tilburg, The Netherlands), Hicol (Oud Beijerland, The Netherlands), Merck (Darmstadt, FRG). The chemical structures of the compounds investigated in this study are illustrated in Fig. 1. Spruce crude resin was collected in a local forest from spruce *(Picea abies)* logs that had been recently cut.

3. RESULTS

The effects of several individual resin constituents on the activity of methane bacteria were evaluated in this study. Fig. 2 shows an example of the cumulative methane productions as a function of time obtained from a typical toxicity assay. The 50% and 80% IC, as determined in the first and second feedings of the assays, are listed in Table 4. These toxicity data were obtained by interpolation of the %ACT versus concentration curves determined for each toxicant. Fig. 3 illustrates the curves obtained for abietic acid, p-cymene, α-pinene and isoeugenol, respectively.

An apolar phenol, 4-hydroxystilbene, was the most toxic compound studied. The 50% IC and 80% IC of this compound in the first feeding were only 20 and 25 mg/l, respectively. Resin acids and monoterpenes had inhibitory concentrations in the same order of magnitude. The 50% IC's for abietic acid and dehydroabietic acid were 43 and 89 mg/l, respectively. The 50% IC's found for various volatile terpenes ranged 45 to 110 mg/l. The only monoterpenol tested, 4-terpineol, with a 50% IC of 330 mg/l, was distinctly less toxic than the homologous terpene lacking the hydroxyl functional group. Two apolar lignin monomers, eugenol and isoeugenol, were also tested; these compounds had 50% IC's of 274 and 96 mg/l, respectively. In contrast, the triterpenic compounds tested (squalene, ß-sitosterol and stigmasterol) did not cause any decrease of the methanogenic activity at the highest concentration tested. These corresponded to 1300 mg/l and 1000 mg/l for the sterols and squalene, respectively.

The residual methanogenic activities determined in the second feeding that followed the 2 week exposure to the toxicants were much lower than that of the control residual activity. The persistance of the inhibition beyond exposure indicates a damaging effect of the resin compounds on the sludge. However, partial recoveries of the methanogenic activity were evident since higher 50% IC and 80% IC were observed in the second compared to the first feeding (Table 4).

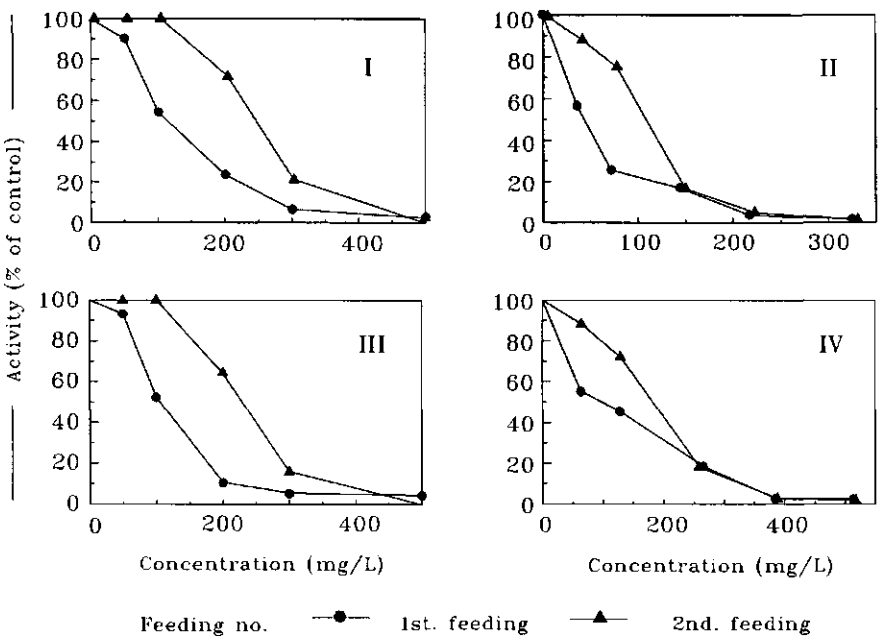

FIG. 3. The methanogenic toxicity of (I) abietic acid, (II) p-cymene, (III) α-pinene and (IV) isoeugenol in the first and second VFA fed assay.

TABLE 4. The effect of the wood resin components investigated on the methanogenic activity.

COMPOUND	1st. Feeding		2nd. Feeding	
	50% IC	80% IC	50% IC	80% IC
	<-------- mg/l -------->		<-------- mg/l -------->	
Resin acids				
abietic acid[U]	89	139	235	206
dehydroabietic acid[U]	43	105	123	141
Volatile terpenes				
α-pinene	105	230	180	290
β-pinene	110	210	165	390
δ-terpinene	42	67	112	94
4-terpineol	330	505	267	470
p-cymene	110	245	220	310
limonene	90	175	150	250
Triterpenols				
β-sitosterol	NT*	NT	NT	NT
stigmasterol	NT*	NT	NT	NT
Triterpenes				
squalene	NT#	NT	NT	NT
Apolar phenols				
4-hydroxy-stilbene	20	25	42	56
isoeugenol[U]	96	182	235	252
eugenol[U]	274	479	428	569

[U] Toxicity data obtained in unshaken experiments.

NT* = the compounds were nontoxic at the highest concentration tested, 1300 mg/l.

NT# = nontoxic at the highest concentration tested, 1000 mg/l.

Spruce crude resin was used as a raw material for evaluating the methanogenic inhibition caused by a natural resin mixture. Crude spruce resin extracts were obtained upon prolonged extraction of 2.5 g resin in 1 l of water or diluted NaOH solutions. The solubility of resin components depended very much on the pH during extraction. This was indicated by the increasing COD concentrations in the filtered extracts of increasing pH values. Concentrations of 42, 121, 503, 3153 mg COD/l (1 g resin = 2.8 g COD) corresponded to solutions extracted at pH values of 7, 9, 11, 12, respectively. The toxicity of the filtered extracts was determined after neutralization with HCl. The results of the toxicity experiments show that alkaline extracted natural wood resin was highly inhibitory to the activity of methanogenic bacteria (Fig. 4).

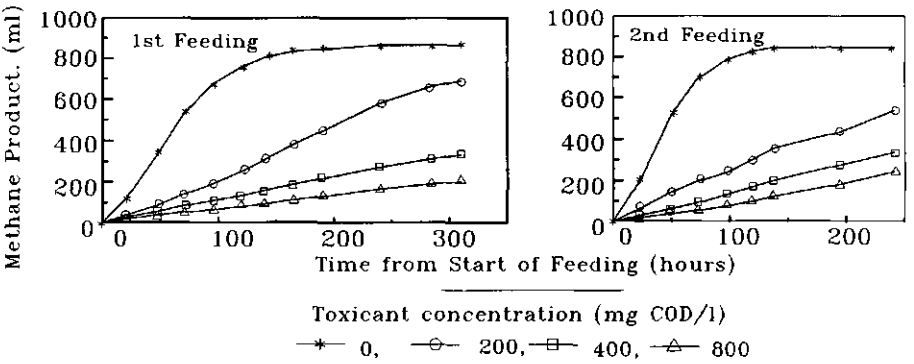

FIG. 4. The cumulative methane production during the methanogenic toxicity assay of a crude spruce resin solution extracted at pH=12.

4. DISCUSSION

In this study we have demonstrated that various wood resin compounds are highly toxic to methane bacteria. The results show that apolar phenols, volatile terpenes and resin acids caused a 50% inhibition of the maximum specific methanogenic activity at concentrations ranging from 20 to 110 mg/l. Eugenol and 4-terpineol with 50% inhibitory concentrations of 274 mg/l and 330 mg/l, respectively, were somewhat less toxic. The nontoxicity of several triterpenes was also established in this study. Compounds corresponding to this group were nontoxic at the highest concentration tested, 1000 to 1300 mg/l.

These observations are supported by previous literature reports where the toxicity of a few individual resin constituents was studied. Our results are in good agreement with those of Benjamin *et al.* (1984) who reported the acetoclastic toxicity of eugenol and two monoterpenes, p-cymene and limonene. The high toxicity of volatile terpenes was reported earlier by McNary *et al.* (1951) in studies concerning the anaerobic digestion of citrus processing wastewaters. They observed that the presence of orange essential oil, composed mostly of limonene, at concentrations higher than 50 mg/l in the wastewater resulted in methanogenic inhibition. Terpenic essential oils extracted from numerous plants as well as various compounds isolated from the oils were also found to be inhibitory to the methanogenic activity of rumen bacteria (Oh *et al.* 1967; 1968). Furthermore, Minami *et al.* (1986) have identified several monoterpenes and sesqui-terpenes as the major constituents of the woody oil that inhibited the anaerobic digestion of kraft evaporator condensate. Finally, at least two reports are available (Field *et al.* 1988; Andersson & Welander, 1985) indicating that abietic acid, a resin acid, is highly inhibitory to methane bacteria.

LCFA are distinctly less toxic to methane bacteria than other resin constituents. The concentrations of various individual LCFA causing a 50% acetoclastic inhibition reported by Koster & Kramer (1987) as well as Chou *et al.* (1978) ranged 525 to 1235 mg/l.

The toxicity of wood resin compounds to microorganisms other than methane bacteria is also indicated in the literature. Crane *et al.* (1957) observed that cellulose digestion by ruminal microorganisms was inhibited by the monoterpene hydrocarbons limonene and α-pinene. The inhibitory effect of the terpenic Juniper berry oils on ethanol fermentation has been noted by Veljkovic *et al.* (1988). The fungicidal activity of heartwood extractives including stilbene related compounds and other wood resin aromatic derivatives has been demonstrated by several research groups (Jurd & Manners, 1980; Lyr 1962; Rudman 1962; Rudman, 1963). In an other study, various terpenic compounds extracted from commercial spices caused complete fungal growth inhibition (Hitokoto *et al.* 1980). Zemek *et al.* (1979) have also described the inhibitory effect of several resinous aromatic compounds on the growth of selected bacteria, yeast and fungi. Isoeugenol was found to be the most potent

inhibitor of the compounds tested in their experiments; concentrations that completely inhibited the growth of the organisms studied ranged from 50 to 250 mg/l.

The factors determining the methanogenic toxicity of wood extractives are not yet fully described but the hydrophobic character of these compounds probably plays an important role. Lipid solubility and surface activity have been previously correlated with the antimicrobial actions of lipophilic organic compounds (Cavill *et al.* 1949; Oka, 1964). The inhibitory effect of LCFA has been attributed to their adsorption on the bacterial cell walls. Destruction of cell membranes, disturbance of cell division by changes in surface tension and non-defined chemical interactions are postulated as possible toxicity mechanisms (Florence *et al.* 1984; Kodicek & Woorden, 1945).

Structural features of volatile terpenes, resin acids and apolar phenols associated with increasing hydrophobicity include aliphatic side chains on aromatic or alicyclic hydrocarbons, the presence of benzene functional groups and a general lack of polar substituents. Likewise, an increase in alkyl substitutions and the presence of benzene groups has been reported to be associated with an increasing toxicity of organic compounds (Jurd & Manners, 1980; Zemek et al. 1979; Chou *et al.* 1978; Wayne-Schultz *et al.* 1978). The alkyl side chain structure plays an important role in antimicrobial activity. Zemek *et al.* (1979) have observed that the introduction of polar functional groups on aliphatic side chain results in a decrease of the microbial toxicity of aromatics. Kutney *et al.* (1982) also found a decrease in resin acid toxicity when these compounds were hydroxylated by fungi. In our study, it was also evident that the presence of hydroxyl groups on terpenes can be associated somewhat to a decrease in toxicity. In contrast with these observations, Oh *et al.* (1967) found that oxygenated monoterpenes strongly inhibited gas production by rumen microorganisms; whereas most of the monoterpene hydrocarbons tested were non-toxic.

Triterpenic compounds, though highly hydrophobic, did not cause methanogenic inhibition at relatively high concentrations. A distinct feature of triterpenes compounds as compared to the very toxic volatile terpenes is their higher molecular weight. Molecular weight has been found to be an important factor in determining the toxicity of resinous compounds. Anderson and Scheffer (1962) as well as Lyr (1962) observed that polymerization of aromatic terpenes was associated with a decrease in toxicity towards fungi. Monomeric aromatic compounds have also been found to be more effective inhibitors than their dimeric homologs (Zemek *et al.* 1979).

Anaerobic biotransformations of organic toxins, that may result in less inhibitory products or cause complete mineralization, also have an important role in determining the methanogenic inhibition of resinous compounds. The toxicity of LCFA is known to be largely affected by the anaerobic biodegradability of these compounds. Low concentrations of LCFA, which are readily biodegraded in anaerobic environments, have a stimulatory effect on the methanogenic activity (Rinzema et al. 1988; Koster & Kramer, 1987; Hanaki *et al.* 1981). Furthermore, an immediate recovery of the methanogenic activity has been observed in experiments inhibited by LCFA when the anaerobic degradation of LCFA occurred (Hanaki *et al.* 1981).

As opposed to LCFA, typical resin components like terpenic hydrocarbons and resin acids are believed to be recalcitrant or slowly biodegradable in anaerobic environments. In agreement with this idea, a poor removal of resin acids has been observed during anaerobic treatment of bleaching wastewaters (Qiu *et al.* 1987). Furthermore, Schink (1985) suggested in his study on the anaerobic metabolism of unsaturated hydrocarbons, that factors such as branched carbon chains, hydrocarbon rings and lack of polar functional groups, like carboxyl and hydroxyl groups, prevent anaerobic microbial mineralization. In any case, it should be noted that partial degradation of squalene, a triterpenic compound, has been reported (Schink,1985).

Natural wood resins are composed of a variety of fatty constituents (Table 1). These components are hydrophobic and poorly soluble in water at neutral to acid pH values. In alkaline conditions, resin solubility is increased due to the solubilization of acidic compounds, like resin acids and LCFA. A higher solubility of crude spruce resin extracted in alkaline solutions as compared to neutral aqueous solutions was observed in our experiments. The toxicity results showed that alkaline aqueous extracts of natural spruce resin inhibited the methanogenic activity of the granular sludge used in this study. Extracts concentrations causing a 50% inhibition corresponded to 130 mg COD/l (1 mg resin = 2.8 mg COD).

The increased solubility of resin at high pH, indicates that resin toxicity will be more important in forest industry wastewaters generated from pulping or bleaching processes that use alkaline conditions. Additionally, resin toxicity is more likely greater in wastewaters derived from soft- as compared to hardwoods. Coniferous wood resin contains high proportions of resin acids and monoterpenes whereas deciduous wood mainly contains triterpenes, but does not include resin acids nor monoterpenes.

5. CONCLUSIONS

This study indicates that wood resin is highly toxic to methane bacteria. Extracts of crude spruce resin resulted in high methanogenic toxicity. The 50% inhibiting concentration was 50 mg/l. The most toxic constituents of wood resin are resin acids, volatile terpenes and apolar phenols, which caused 50% inhibition of the maximum specific methanogenic activity at concentrations ranging 20 to 274 mg/l. In contrast, triterpenes were nontoxic at the highest concentrations tested, 1000 to 1300 mg/l.

These results suggest that the potential toxicity of wastewaters containing wood resin constituents or related compounds should not be neglected when evaluating the feasibility of anaerobic wastewater treatment process.

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CHAPTER 4

The Methanogenic Toxicity of Wastewater Lignins and Lignin Related Compounds

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THE METHANOGENIC TOXICITY OF WASTEWATER LIGNINS AND LIGNIN RELATED COMPOUNDS

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ABSTRACT

Lignin derivatives are major components of wastewater streams generated in the chemical processing of wood. The objective of this study was to evaluate the inhibitory effect of various lignins isolated from forest industry wastewaters and selected lignin model compounds to methanogenic bacteria. The methanogenic inhibition was determined at a temperature of 30°C in standard toxicity assays utilizing anaerobic granular sludge as inoculum. The wastewater lignins differed considerably in their inhibitory activity. Some lignin samples were non-toxic, whereas others caused 50% inhibition at concentrations ranging from 3320 to 5950 mg COD/l. Experiments with ultrafiltered lignins revealed that the toxicity of the inhibitory lignin samples originated from the low molecular weight fraction. In additional studies with low molecular weight lignin model compounds, it was observed that the inhibitory activity of these compounds was related to the functional groups on the aromatic ring. Compounds with aldehyde groups or apolar substituents were highly toxic; whereas, those with carboxylic groups only caused significant inhibition at high concentrations. These results indicate that low molecular weight lignin derivatives in forest industry wastewaters are potential inhibitors of anaerobic treatment systems.

1. INTRODUCTION

Lignin is a major waste product in pulp and paper manufacturing. The chemical processing of wood aims at separating the cellulose fiber component from the lignin. The delignification treatments applied during chemical pulping and bleaching processes modify and depolymerize the lignin in order to increase its solubility in the process water. The complex mixtures of dissolved lignin fragments resulting from these treatments significantly differ from native lignin in chemical composition, molecular weight (MW) distribution and physical characteristics (Glasser & Kelley, 1987). Kraft lignins and lignosulfonates are the most important lignins produced by commercial pulping operations. The properties of wastewater lignins are not uniform due to the different lignocellulosic feedstocks used and the various processes applied in the pulp mills (Fengel & Wegener, 1984; Glasser 1980).

The presence of lignin in wastewater presents a major source of organic matter resistant to biological wastewater treatment systems. The microbial recalcitrance of lignin is due to its high molecular weight, chemical heterogeneity and the absence of regular hydrolyzable intermonomeric linkages (Kirk & Farrell, 1987; Zeikus *et al.* 1982). The degradation of lignin by anaerobic environments is limited to the low molecular weight derivatives. Only monomers and oligomers of lignin (<600 daltons) have been shown to be mineralized (Chen *et al.* 1985a, 1985b, 1987; Colberg & Young, 1985), whereas higher molecular weight lignin is not anaerobically degradable (Benner *et al.* 1984; Hackett *et al.* 1977; Zeikus *et al.* 1982). It appears that aerobic bacteria are also not able to significantly metabolized high molecular weight lignin (Jokela *et al.* 1985; Kern & Kirk, 1987; Pellinen & Salkinoja, 1985; Vikuña *et*

al. 1987). Research to date indicates that the capacity for the complete decomposition of lignin by single microorganisms is limited to white rot fungi and related basidiomycetes (Ander *et al.* 1980; Kirk & Farrell, 1987; Kirk & Shimada, 1985). These fungi have the unique enzymatic capacity to promote non-specific extracellular oxidative reactions that depolymeraze the lignin macromolecule.

In spite of the fact that lignin is an important component of forest industry wastewaters very little research has been focused on its toxicity to microorganisms involved in biological wastewater treatment. However, some research is available indicating that low molecular weight fragments of lignin can cause toxicity to yeast, fungi and bacteria. (Ando *et al.* 1986; Benjamin *et al.* 1984; Clark & Mackie, 1984; Vohra *et al.* 1980; Zemek *et al.* 1979).

The objective of this study was to evaluate the inhibitory effect of various lignins isolated from forest industry wastewaters and selected lignin model compounds to methanogenic bacteria.

2. MATERIALS AND METHODS

2.1. Analyses

The chromatographic determination of the methane content in the gas samples has been described elsewhere (Sierra-Alvarez & Lettinga, 1990). Samples for volatile fatty acids (VFA) and COD determination were filtered (Schleicher & Schull paper filter no. 589-1). VFA were determined by gas chromatography (Sierra-Alvarez & Lettinga, 1990). COD (colorimetric micro-method) and volatile suspended solids (VSS) were determined according to Standard Methods (American Public Health Assoc., 1985).

The pH was determined with a Knick 511 pH-meter and a Scot Gerade N61 double electrode immediately after sampling in order to avoid a pH rise due to the loss of carbon dioxide from the liquid.

Lignin molecular weight distribution was determined by gel filtration chromatography in a 1.05 x 100 cm column packed with Sephadex G-50. A 1 ml aliquot of the lignin solution (pH 9.0) was applied to the column and eluted with a 0.02 M borate buffer providing a pH 9.1 (Amy *et al.* 1987). The ultraviolet absorbance at 280 nm of the fractions collected was used as an indicator of their lignin content (Fengel & Wegener, 1984). The absorbance was determined in a 1 cm quartz cuvette by diluting the samples to less than 0.8 absorbance units in the borate buffer. The biochemicals used for column calibration included aprotinin (MW = 6500), cytochrome C (MW = 12400), carbonic anhydrase (MW = 29000) and albumin (MW = 66000). Blue dextran (MW = 2000000) was used to determine the void volume of the packed column.

The lignin aqueous solutions (pH 8.0) were fractionated by ultrafiltration in an Amicon 2000A stirred cell provided with a Spectra/Por type C ultrafiltration membrane (Labinco BV, Breda, The Netherlands) with a nominal cut-off value of 10000 daltons. A nitrogen atmosphere provided a pressure ranging 320 kPa. The sample size was 2 l and the concentration factor was 10-20. Subsequently, the concentrated retentate was diluted in two occasions with 2 x 0.3 l distilled water and each time the ultrafiltration was allowed to proceed until the retentate volume had decreased to 0.1 l. The retentate and the filtrate were stored in refrigerated containers at 4°C.

2.2. Biological Assays

All assays contained macronutrients (N, P and S) and trace elements required for bacterial growth as outlined previously (Sierra-Alvarez & Lettinga, 1990). Batch fed assays were conducted in 0.3 or 0.6 l glass serum flasks sealed with a rubber septum and a screw cap. The assay medium was flushed with nitrogen gas prior to incubation of the serum vials in a temperature controlled room at 30 ± 2°C.

2.2.1. Biomass. The methanogenic granular sludge used in these experiments was obtained from a full scale anaerobic reactor treating distillery wastewater (Nedalco, Bergem op Zoom, The Netherlands). The sludge was elutriated to remove the fines and stored at 4°C under nitrogen gas.

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

Type 1. This method was applied to assay the methanogenic toxicity of the lignin model compounds. It is based on the use of acetate as the sole substrate. The activity is evaluated after short exposure periods enabling the rapid assesment of the toxicity of numerous compounds.

Specific methanogenic activity measurements were performed in 0.3 l glass serum flasks. Granular sludge (1 g VSS/l) was transferred to the serum vials containing 0.1 l of the basal medium and acetate was added from a neutralized stock solution to obtain a final concentration of 2 g COD/l. Subsequently, the flasks were sealed and placed in a reciprocating shaker at 30 ± 2°C.

After 1 day of incubation the acetate concentration in the assay media was measured and replenished to obtain a concentration of 2 g COD/l. The required amount of inhibitory compound was added to each flask to provide the toxic concentration to be investigated. Compounds were neutralized, if needed, prior to their addition to the assay medium. Substrate controls were based on assays where no toxicant was added. After two days of exposure to the toxicants, the acetate concentration was replenished to 2 g COD/l in order to assay the specific methanogenic activity. The bottles were reincubated for 1 hour prior to the determination of the methane production rate. The methane composition in the head space content of each serum flask was determined periodically during the 4 to 5 hour period that followed.

Type 2. This method was used to determine the methanogenic toxicity of the wastewater lignin samples. Specific methanogenic activity measurements were performed in 0.6 l glass serum flasks. The assays were carried out in two consecutive feedings. In the first feeding, distilled water, granular sludge (1.5 g VSS/l) and known amounts of toxicant were transferred to the serum vials containing 0.1 l of the concentrated nutrient solution. Substrate controls were based on assays where no toxicant was added. Distilled water was added to complete a medium volume of 0.5 l. Afterwards, the substrate controls and treatments (assays containing lignin) were supplied with 4 g COD/l of a neutralized VFA solution. The composition of the VFA solution that served as substrate was 100:100:100 g acetate:propionate:butyrate per kg. Finally, the flasks were sealed and incubated in a temperature controlled room at 30 ± 2°C.

On day 14, all serum flasks were provided with a second substrate feeding in order to evaluate the residual activity of the sludge after exposure to the toxicants. The supernatants were carefully decanted to avoid losses of methanogenic sludge and replaced, while maintaining N_2 flushing in the head space, with a nutrient supplemented medium containing 4 g VFA-COD/l. No toxicants were included in the replacement medium. Finally, the serum flasks were incubated again for 1 to 2 weeks.

Methane production was monitored periodically during the assays with modified Mariotte flasks. These flasks were filled with a 3% NaOH solution which served to remove the carbon dioxide from the biogas. The serum flasks were not shaken during the assay period.

The specific methanogenic activity, expressed as the amount of CH_4 produced by 1 g of sludge VSS per day (g CH_4-COD/(g VSS·day)), was calculated in all assays from the slope of the methane production *vs.* time curve and the quantity of VSS. The methanogenic activities for each toxicant concentration were calculated in the time interval corresponding to the maximum control activity (Figure 1). The inhibited activity was expressed as percentage of the control activity, and it is abbreviated as (% ACT). The percentage inhibition (% I) was defined as: % I = 100 - % ACT. The compound concentrations that caused 50 and 80% methanogenic inhibition are referred to as "50% IC" and "80% IC", respectively.

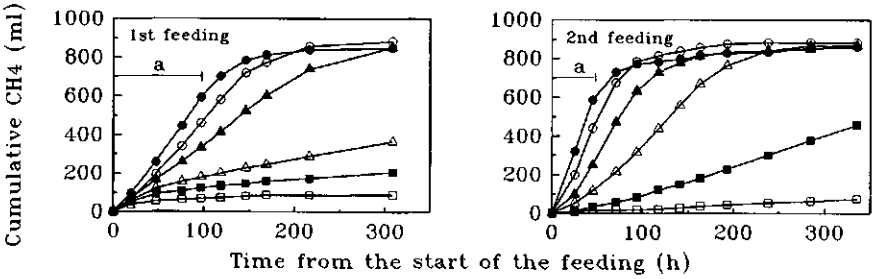

FIG. 1. The cumulative methane production during the methanogenic toxicity assay of the lignin sample L1. The lignin concentrations (in g COD/l) supplied to the medium where: 0 (●), 2 (○), 4 (▲), 6 (△), 8 (■) and 10 (□), respectively. "a" = the time period chosen to determine the methanogenic activity of the various treatments and the substrate control. Assay conditions: see 2.2.2. Anaerobic toxicity assay, type 2.

2.3. Chemicals

The chemicals used were purchased from Janssen Chimica, (Tilburg, The Netherlands), BDH (Brunschwig Chemie, Amsterdam, The Netherlands) and Merck (Darmstadt, West Germany). Sephadex G-50 coarse (dry bead diameter 100-300μ) and the molecular weight markers for gel filtration were supplied by Sigma (St. Louis, MO, USA).

Steam explosion lignin from poplar was a gift from Bio-regional Energy Associates, Ltd. (Floyd, Va, USA). Indulin C, a kraft pine lignin, and Polyfon F and Polyfon H, sulfonated kraft lignins, were kindly provided by Westvaco (Charleston Heights, SC, USA). Serla-sol Pan, a sodium lignosulfonate, and a kraft lignin sample (mostly pine based) were a gift from Metsa-Serla (Tampere, Finland). In this study the name of these lignins will be abbreviated as follows: Indulin C (L1); Polyfon F (L2); Polyfon H (L3); kraft lignin from Metsa-Serla (L4); Serla-sol Pan (L5) and steam explosion lignin (L6).

The chemical structures of the lignin model compounds investigated in this study are illustrated in Figure 2.

3. RESULTS

The inhibitory effects of various wastewater lignins on the activity of granular anaerobic sludge were evaluated in this study. The 50% IC and 80% IC values determined in the first and second assay feeding of the toxicity experiments are summarized in Table 1. The methanogenic inhibition versus lignin COD concentration curves obtained for the lignin samples L1, L2, L3 and L6 are illustrated in Figure 3. The sulfonated kraft lignin L2, with a 50% IC in the first assay feeding of only 3,320 mg/l, was the most toxic lignin studied. The kraft lignin L1, the sulfonated kraft lignin L3 and the steam explosion lignin L6 were somewhat less inhibitory to methane bacteria. The 50% IC values corresponding to these lignins ranged from 4,050 to 5,950 mg/l. In contrast, 8,000 mg/l of the kraft lignin L4 and 11,300 mg/l of the lignin sulfonate L5 did not cause any decrease in the methanogenic activity.

The residual methanogenic activities determined in the second assay feeding that followed the 2 week exposure to the lignin samples L1 to L4 were lower than that of the control activity. The persistance of the inhibition beyond exposure indicates a damaging effect of the toxic lignins on the sludge. However, partial recoveries of the methanogenic activity were evident since somewhat higher inhibiting concentrations were observed in the second compared to the first feeding (Table 1).

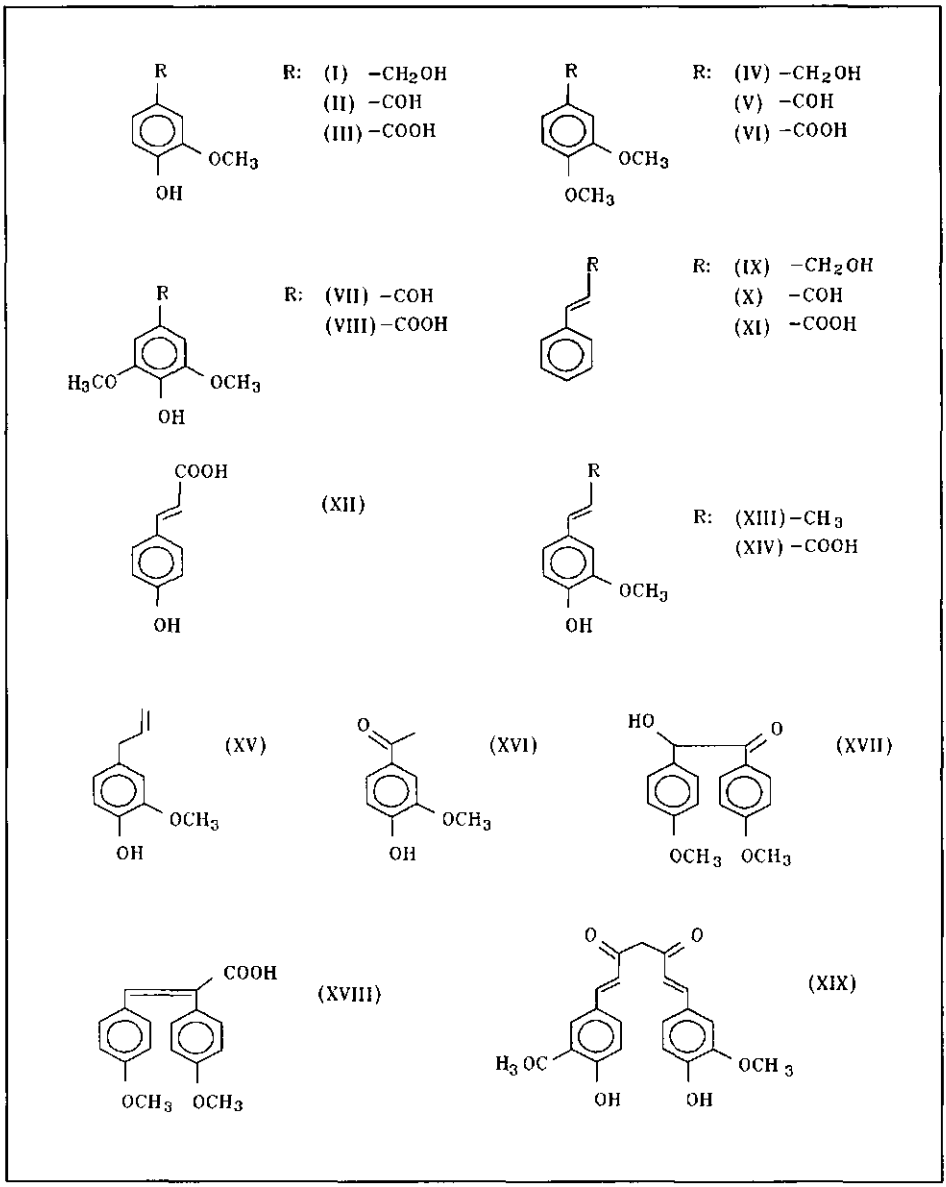

FIG. 2. The chemical structures of the compounds investigated in this study. (1) vanillyl alcolhol, (2) vanillin, (3) vanillic acid, (4) veratryl alcohol, (5) veratraldehyde, (6) veratric acid, (7) syringaldehyde, (8) syringic acid, (9) cinamyl alcohol, (10) cinnamyladehyde, (11) cinnamic acid, (12) coumaric acid, (13) isoeugenol, (14) ferulic acid, (15) eugenol, (16) acetovanillone, (17) anisoin, (18) 2,3 bis (4-methoxyphenyl) acrylic acid, (19) curcumin.

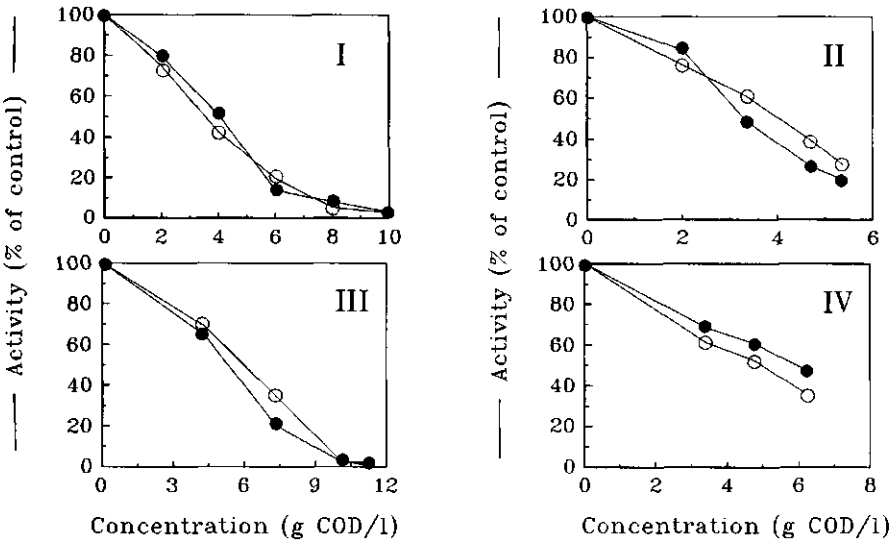

FIG. 3. The methanogenic activity (expressed as percentage of the control methanogenic activity) of VFA-fed granular sludge exposed to the lignin samples (I) L1, (II) L2, (III) L3 and (IV) L4 in the first (●) and second (○) VFA-fed assay. The second VFA feeding was started after removing the lignin-containing medium to determine the sludge activity after a two-week exposure period to the toxicants. Assay conditions: see 2.2.2. Anaerobic toxicity assays, type 2.

In order to investigate the role of the lignin molecular weight on the inhibition exerted by the wastewater lignins, aqueous solutions prepared from the lignin samples L1 to L3 were fractionated by ultrafiltration in a retentate (MW > 10000 dalton) and a filtrate (MW < 10000 dalton) and their methanogenic toxicities were subsequently evaluated. The specific conditions of the toxicity experiments conducted are listed in Table 2. As shown in Figure 4, the low MW lignin fractions (filtrates) were highly toxic at low COD concentrations. In contrast, the high MW fractions (retentates) were not inhibitory at 7000 g COD/l, a concentration that caused high inhibition with the unfractionated lignins. These results indicate that the toxicity of the lignin samples L1, L2 and L3 originated from the low MW fraction.

The low MW fraction (MW < 10000 daltons) in the lignins L1, L2, L3, L5 and L6, as calculated from the corresponding MW distributions obtained by gel filtration, averaged 26.4, 18.4, 28.0, 19.8 and 6.2 % of the total lignin, respectively. Figure 5 illustrates the gel filtration chromatograms of a toxic and a non-toxic lignin samples. It should be noted that the lignin samples (L4 and L5) did not cause any methanogenic inhibition (Table 1), even though at the high concentrations tested the concentration of total low MW aromatics (MW < 10000) in both samples was comparable to those corresponding to the 50% IC of the inhibitory lignin samples. This indicates that not all the low MW compounds present in the wastewater lignins are necessarily responsible for toxicity.

In order to investigate the nature of the inhibitory low MW lignin derivatives, the methanogenic toxicity of various lignin model compounds was also evaluated in this study. The compound concentrations resulting in 50% and 80% inhibition of the acetoclastic activity are listed in Table 3. The aromatic aldehydes tested were highly toxic to methane bacteria. Phenolic compounds with apolar alkylic substitutions, like eugenol and isoeugenol, also inhibited methane production at very low concentrations. The 50% IC corresponding to these compounds were 225 and 140 mg/l, respectively. In contrast, the carboxylic acids were only mildly inhibitory at concentrations ranging from 6000 to 10500 mg/l. The inhibitory effect of

TABLE 1 The concentration (in mg COD/l) of the various wastewater lignins evaluated in this study resulting in a 50% and 80% inhibition of the methanogenic activity.

LIGNIN SAMPLE	1st. feeding		2nd. feeding	
	50% IC	80% IC	50% IC	80% IC
L1	4050	5600	3550	5950
L2	3320	5360	3950	5900
L3	5300	7350	6030	8480
L4	NT*	NT	NT	NT
L5	NT	NT	NT	NT
L6	5950	8050	5050	7500

* NT= non-toxic at the highest concentration tested: 8000 and 11300 mg COD/l for the lignins L5 and L6, respectively.

TABLE 2. The bioassay parameters of the toxicity experiments conducted with the retentates and the filtrates obtained by ultrafiltration of the lignin samples L1, L2 and L3.

LIGNIN SAMPLE	Assay concentration (mg COD/l)	f_{280}*
Lignin L1		
Filtrate	1370	5.4
Retentate	7000	9.7
Lignin L2		
Filtrate	2130	5.7
Retentate	7000	15.7
Lignin L3		
Filtrate	2330	6.6
Retentate	7000	12.6

* f_{280} = lignin absortivity factor (in l/(g COD · cm)) at 280 nm.

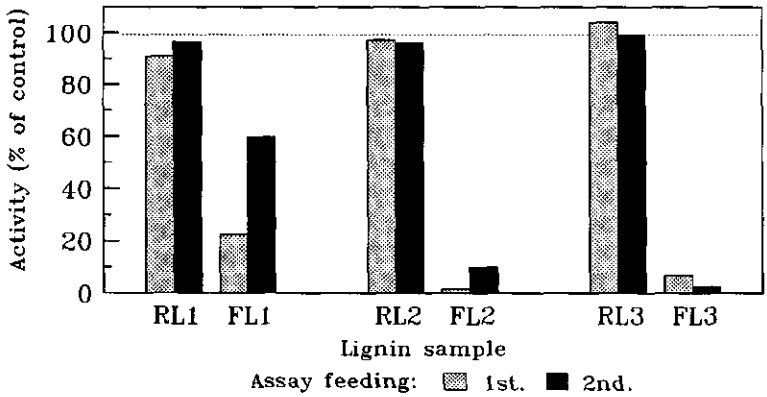

FIG. 4. The methanogenic activity (expressed as a percentage of the control methanogenic activity) of VFA-fed granular sludge exposed to the retentates (RL1, RL2, RL3) and filtrates (FL1, FL2, FL3) obtained by ultrafiltration of the lignin samples L1, L2 and L3 in the first and second VFA fed assay. The second VFA feeding was started after removing the lignin-containing medium to determine the sludge activity after a two-week exposure period to the toxicants. Assay conditions: see 2.2.2. Anaerobic toxicity assays, type 2.

the aromatic alcohols was generally intermediate between that of the homologous aldehyde and carboxylic acid. As an example, the 50% IC obtained for cinnamaldehyde, and for the homologous alcohol and carboxylic acid were 80, 510 and 7590 mg/l, respectively.

4. DISCUSSION

Industrial lignins, regardless of the method of manufacture and purification, are very complex mixtures of molecules which differ in size and often in chemical structure (Glasser 1980). As anticipated from the heterogenic nature which is characteristic of lignin, in this study we have observed a high variability in the methanogenic toxicity of various wastewater lignin samples. The 50% inhibitory concentrations observed for the kraft lignin L1, the lignosulfonate samples L2 and L3, as well as the steam explosion lignin L6, ranged from 3320 to 5950 mg/l. In contrast, high concentrations of the lignosulfonate L4 and the kraft lignin L5 did not cause any decrease of the methanogenic activity. To our knowledge we are only aware of one other study in which the methanogenic toxicity of purified wastewater lignin was directly tested. In that study a lignosulfonate sample at a concentration of 15000 mg/l did not cause any methanogenic inhibition (Puhakka *et al.* 1985).

Molecular weight is an important factor determining the inhibitory effect of lignin derivatives towards methanogenic bacteria. In our study we demonstrated that the removal of the low MW fraction (MW < 10000) from the inhibitory lignin samples resulted in an almost complete detoxification, indicating that the methanogenic toxicity originated from compounds present in the low MW fraction. In a similar fashion, Clark *et al.* (1984) showed that the removal of the low MW phenolics from pine wood hydrolysate by dialysis resulted in its detoxification towards yeast. With respect to other phenolic compounds, Field *et al.* (1989 & 1990) demonstrated that the methanogenic inhibition of tannins was limited to the low MW polymers. The excessive size of the high MW tannin polymers (MW > 3000 daltons) was postulated to hinder their penetration into the sensitive sites of bacteria, rendering the compounds atoxic.

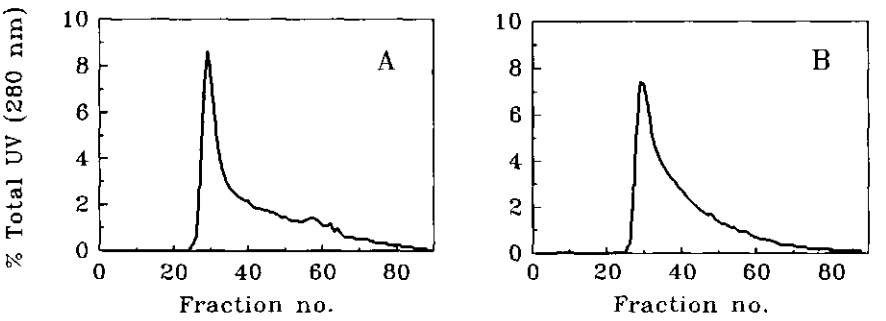

FIG. 5. The gel filtration chromatograms of the lignin sample (A) L1 and (B) L5. Column (1.05 x 100 cm) packed with Sephadex G-50; the mobile phase was borate buffer (pH 9.1).

Although, the methanogenic toxicity of the inhibitory lignins was shown to be associated to their low MW fraction, the concentration of low MW lignin derivatives does not appear to be a proper index for estimating the methanogenic toxicity of different lignin samples. In this respect, it should be noted that two of the lignin samples tested in this study did not cause any methanogenic inhibition (Table 1), even though they also contained appreciable amounts of low MW components.

These observations suggest that the various low MW compounds present in wastewater lignin mixtures differ considerably in their inhibitory potentials. The toxicity data obtained in studies with low MW lignin derivatives confirmed this hypothesis. The wide range of the 50% IC data listed in Table 3 indicates that highly variable toxic activities corresponded to the different aromatic compounds tested. Lignin derivatives with aldehyde groups or apolar substituents were highly toxic to methane bacteria. The aromatic carboxylic acids, on the other hand, were only mildly toxic. Similar results are also evident in various studies on the inhibitory effects of lignin related compounds towards a variety of test organisms. Ando *et al.* (1986) reported that inhibition of yeast fermentation by monomeric lignin derivatives was strongly related to the functional groups in the aromatic compounds. In agreement with our results, compounds with apolar alkyl substitutions and aldehyde groups were found to be the most effective inhibitors. Zemek *et al.* (1979) observed that lignin model compounds bearing carboxylic functional groups exhibited a low toxicity towards fungi, yeast and bacteria, while compounds with apolar functional groups were highly antibiotic. Clark & Mackie (1984) observed that vanillic acid caused lower inhibition to yeast fermentation as compared to its corresponding aldehyde, vanillin. Op de Camp *et al.* (1988) tested the toxicity of several lignin model phenolic acids to the anaerobic degradation of cellulose and observed that the acids only caused inhibition of the methane production at very high concentrations. Benjamin *et al.* (1984) evaluated the methanogenic toxicity of various lignin derived monomers present in kraft condensates and found that eugenol, with an apolar side chain, was more toxic as compared to guaiacol, lacking the side chain.

Fragmentation of the lignin macromolecule by alkalis or acids is the basic step of all chemical procedures involved in wood processing, therefore the presence of low MW lignin-derived compounds, similar to those described in this paper, is to be expected in chemical pulping and bleaching effluents from the forest industry. Liquors produced from biomass via hydrolysis routes and related processes may also contain low MW lignin derivatives. Microbial inhibition resulting from the low MW lignin products formed in these processes is indicated in the literature. The acid hydrolysis of wood releases low MW phenolics derived from lignin which have been identified as important inhibitors disturbing yeast fermentation of the wood hydrolysate (Clark & Mackie, 1984; Lee & McCaskey, 1983). The inhibitory effect exerted by effluents from steam explosion of wood on yeast fermentation as well as on anaerobic cellulose degradation has been suggested to result from aromatic compounds derived from lignin (Ando *et al.* 1986; Khan, 1988). Alkali oxidation of klason (acid

TABLE 3. The 50% IC and 80% IC determined in this study for various lignin model compounds.

	COMPOUND	50% IC (mg/l)	80% IC (mg/l)
(1)[#]	vanillyl alcolhol	1525	1865
(2)	vanilline	1800	1880
(3)	vanillic acid	9230 (16%)[+]	NM[*]
(4)	veratryl alcohol	6000 (34%)	NM
(5)	veratraldehyde	1650	2630
(6)	veratric acid	10500 (37%)	NM
(7)	syringaldehyde	4400	7374
(8)	syringic acid	6500 (30%)	NM
(9)	cinamyl alcohol	510	940
(10)	cinnamaldehyde	80	140
(11)	cinnamic acid	7590	9810
(12)	coumaric acid	7100	NM
(13)	isoeugenol	140	200
(14)	ferulic acid	6050	14100
(15)	eugenol	225	330
(16)	acetovanillone	1925	2100
(17)	anisoin	5000 (0%)	NM
(18)	2,3 bis (4-methoxyphenyl)-acrylic acid	1000 (0%)	NM
(19)	curcumin	1000 (0%)	NM

[#] Compound number in Figure 2.

[+] The value in the parenthesis indicates the inhibition observed at the highest concentration tested.

[*] NM = Not measured.

precipitated) lignins leads to the formation of degradation products that display fungistatic activity (Telysheva *et al.* 1968). Alkaline heat treatment of peat, which in its natural form is non-toxic to methane bacteria, resulted in the formation of highly methanogenic inhibitory products (Owen *et al.* 1979). A substantial increase in the methanogenic toxicity of the peat degradation products was observed with increasing treatment temperatures.

A good understanding of the role of the various industrial processes involving lignin depolymerization on the presence of inhibitory low MW compounds in the resulting wastewaters would be of considerable value when evaluating the feasibility of anaerobic treatment. Our results indicate that one should expect greater toxicity from processes that result in the formation of highly apolar low MW lignin derivatives as well as phenolic aldehydes. Further research relating the structure with the methanogenic toxicity of lignin derivatives could significantly contribute to facilitate the selection of the process conditions minimizing the formation of inhibitors and also to the development of detoxification processes leading to the transformation of the inhibitors to non-toxic compounds.

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CHAPTER 5

The Effect of Aromatic Structure on the Inhibition of Acetoclastic Methanogenesis in Granular Sludge

(Submitted for publication)

THE EFFECT OF AROMATIC STRUCTURE ON THE INHIBITION OF ACETOCLASTIC METHANOGENESIS IN GRANULAR SLUDGE

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ABSTRACT

Benzene derivatives are important constituents of various industrial wastewaters, e.g. certain effluents of the pulp and paper industry, petrochemical and chemical industries. The anaerobic treatment of these wastewaters can be limited due to the methanogenic inhibition exerted by the aromatic compounds.

The objective of this study was to evaluate the effect of aromatic structure on the acetoclastic methanogenic inhibition. The toxicity to acetoclastic methanogens was assayed in serum flasks utilizing granular sludge as inoculum. Among the monosubstituted benzenes, chlorobenzene, methoxybenzene and benzaldehyde were the most toxic with 50% inhibition occurring at concentrations of 3.4, 4.2 and 5.2 mM; respectively. In contrast, benzoate was the least inhibitory, concentrations up to 57.3 mM were nontoxic. In general, the toxicity of aromatic compounds increased with increasing length of aliphatic side-chains, increasing the number of alkyl or chlorine groups. The logaritm of the partition coefficient n-octanol/water (log P), an indicator of hydrophobicity, was observed to be positively correlated with the methanogenic inhibition. The results indicate that hydrophobicity is an important factor contributing to the high toxicity of the most inhibitory aromatic compounds.

1. INTRODUCTION

Aromatic compounds are present in natural environments as derivatives of lignin, tannins, phenolic amino acids, and other aromatic plant components. Human activities also contribute to the presence of aromatics in the environment. Waste incineration, mining and the discharge of wastewater streams generated by petrochemical factories, paper manufacturing and chemical industries, among others are important sources of aromatic pollution.

The presence of aromatic xenobiotics in the environment may create serious public health and environmental problems. Some aromatics are mutagenic or carcinogenic and some may bioaccumulate, additionally, man-made aromatics are often resistent to biodegradation and toxic to microorganisms.

Traditionally, biological treatment of aromatic containing wastewaters was based on aerobic processes. Recently, anaerobic processes are being used more often for the treatment of these complex wastewaters (Borghans & Van Driel, 1988; Kim *et al.* 1986; Salkinoja-Salonen *et al.* 1983; Suidan *et al.* 1983); however, their application is restricted by the limited data available on the behavior of aromatic compounds in anaerobic treatment systems.

Although the anaerobic biodegradation of aromatic compounds has been extensively investigated as reviewed by Colberg (1988), Hollinger *et al.* (1988), Schink (1988) and Young (1984), less attention has been given to the toxic effects of these compounds on anaerobic microbial communities.

The purpose of this study was to evaluate the methanogenic toxicity of several aromatic compounds to acetoclastic methanogens in granular sludge. The inhibitory effects of homologous series of aromatic compounds were assessed to study the relationships between chemical structure and methanogenic toxicity.

2. MATERIALS and METHODS

2.1. Biomass.

Elutriated methanogenic granular sludge from a full scale upward-flow anaerobic sludge blanket (UASB) reactor treating distillery wastewater was used as inoculum. The sludge was stored at 4 °C, and reactivated by incubation at 30 °C in the presence of acetate. The sludge used was not acclimated to aromatic compounds prior to the experiments.

2.2. Basal medium.

The basal medium used in the anaerobic toxicity assay contained (mg/l): $NaHCO_3$ (400), NH_4Cl (280), $CaCl_2 \cdot 2H_2O$ (10), K_2HPO_4 (250), $MgSO_4 \cdot 7H_2O$ (100), yeast extract (100), H_3BO_3 (0.05), $FeCl_2 \cdot 4H_2O$ (2), $ZnCl_2$ (0.05), $MnCl_2 \cdot 4H_2O$ (0.05), $CuCl_2 \cdot 2H_2O$ (0.03), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.05), $AlCl_3 \cdot 6H_2O$ (0.09), $CoCl_2 \cdot 6H_2O$ (2), $NiCl_2 \cdot 6H_2O$ (0.05), $Na_2SeO_3 \cdot 5H_2O$ (0.1), EDTA (1), resazurin (0.2) and 36% HCl (0.001 ml/l). All chemicals were of analytical grade (Merck, Darmstadt, West Germany). The yeast extract was supplied by Gist-Brocades (Delft, The Netherlands).

2.3. Analyses.

Acetic acid was determined with a gas chromatograph equipped with a 2 m x 4 mm glass column packed with Supelcoport (100-120 mesh) coated with 10% Fluorad FC 431. The temperature of the column, the injection port and the flame ionization detector were 130, 220 and 240 °C, respectively. Nitrogen saturated with formic acid was used as carrier gas at a flow rate of 50 ml/min.

The methane content in the gas samples was determined by gas chromatography. The column was packed with a molecular sieve 5A (mesh 60-80). The temperature of the column, the injection port and the flame ionization detector were 60, 200, 220 °C, respectively. The carrier gas was nitrogen at a flow rate of 14.3 ml/min.

The pH was determined immediately after sampling with a Knick 511 pH-meter and a Scot Gerade N61 double electrode.

All the other analytical determinations were performed according to Standard Methods (American Public Health Assoc., 1985).

2.4. Anaerobic toxicity assay.

Specific acetoclastic methanogenic activity measurements were performed in 315 ml glass serum flasks. Granular sludge (1 g volatile suspended solids (VSS) per liter) was transferred to the serum vials containing 100 ml of the basal medium and acetate was added from a neutralized stock solution to obtain a final concentration of 30 mM. Subsequently, the liquid and head space were flushed with nitrogen gas. The flasks were sealed with a rubber septum and a screw cap and placed in a reciprocating shaker at 30 ± 2°C.

After 1 day of incubation the acetate concentration in the assay media was measured and replenished to obtain a concentration of 30 mM. The required amount of inhibitory compound was added to each flask to provide the toxic concentration to be investigated. Acidic test compounds were neutralized prior to their addition to the assay medium. Substrate controls were based on assays where no toxicant was added. Finally, after flushing the head space with nitrogen gas, the flasks were again incubated.

After two days of exposure to the toxicants, the acetate concentration was replenished to 30 mM in order to assay the specific methanogenic activity. The head space was flushed with nitrogen gas and the bottles were reincubated for 1 hour prior to the determination of the methane production rate. The methane composition in the head space content of each serum flasks was determined periodically during the 4 to 5 hour period that followed. The

maximum specific acetoclastic methanogenic activity was calculated from the slope of the methane production versus time curve. The inhibited activity was expressed as a percentage of the control activity, and it is abbreviated as (% ACT). The percentage inhibition (% I) was defined as: % I = 100 - % ACT. The compound concentrations that caused 50% and 80% inhibition of the methanogenic activity are referred to as "50% IC" and "80% IC", respectively.

2.5. Chemicals.

All chemicals were purchased from Janssen Chimica (Tilburg, The Netherlands) and Merck (Darmstadt, West Germany).

3. RESULTS and DISCUSSION

3.1. The Effect of Aromatic Structure on Methanogenic Inhibition

The inhibitory effects of 34 aromatic compounds on the activity of acetoclastic methanogenic bacteria were evaluated in this study. The compound concentrations resulting in a 50 and 80% IC of the acetoclastic activity are summarized in Table 1. Those compounds that were not inhibitory at a concentration lower than 40 mmol/l were considered to be non-toxic as shown in the table.

The various aromatic compounds caused different levels of inhibition as indicated by the wide range of the 50% IC values observed (Table 1). Pentachlorophenol was the most toxic compound studied, causing a 50% inhibition at 0.03 mM, whereas compounds such as benzoate were nontoxic at concentrations as high as 57.3 mM.

The experimental results suggest that some general relationships between the molecular structure of aromatic compounds and their inhibitory effects on methanogenic bacteria can be established. Bioassay results indicated that ring substitution is an important factor determining the inhibition caused by aromatic compounds. The toxicity of the monosubstituted benzenes (Fig. 1) was found to increase in the following substituent order:

$$COOH < SO_3H < H < OH < CH_3 < CHO < OCH_3 < Cl$$

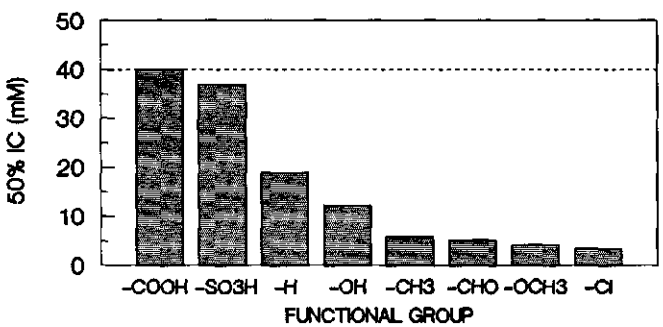

FIG. 1. The effect of the functional group on the methanogenic toxicity exhibited by mono-substituted benzenes.

TABLE 1. The 50% and 80% IC observed in this study for various aromatic compounds.

COMPOUNDS	Molecular weight	50% IC.	80% IC.	log P[#]
		<------ (mmol/l) ------>		
Miscellaneous				
(1[+]) benzene	78.1	18.91	23.04	1.95
(2) 4-methylbenzaldehyde	120.1	4.25	6.18	1.98
(3) benzaldehyde	106.1	5.03	7.92	1.48
(4) methoxybenzene	108.1	4.61	7.40	2.11
(5) 2-methylanisole	122.2	2.74	3.69	2.61
(6) 1,3,5-trimethoxybenzene	168.2	1.58	2.08	NA[*]
(7) 4-hydroxystilbene	196.3	0.33	0.43	4.31
Alkyl-benzenes				
(8) methylbenzene	92.1	6.76	8.14	2.69
(9) ethylbenzene	106.2	4.95	5.65	3.15
(10) 1,2-dimethylbenzene	106.2	4.23	5.03	3.15
(11) 1,3,5-trimethylbenzene	120.2	2.59	3.34	3.42
(12) allylbenzene	118.2	2.13	3.40	3.23
(13) n-propylbenzene	120.2	1.66	3.54	3.68
(14) styrene	105.2	0.09	0.38	3.00
Chloro-benzenes				
(15) chlorobenzene	112.6	3.38	4.18	2.84
(16) 1,2-dichlorobenzene	147.0	1.22	1.77	3.53
(17) 1,2,4-trichlorobenzene	181.5	0.52	0.66	4.26
Chloro-phenols				
(18) 2-chlorophenol	128.6	3.19	4.01	2.17
(19) 2,4-dichlorophenol	163.0	0.49	0.64	3.15
(20) 2,4,6-trichlorophenol	197.6	0.59	0.91	3.38
(21) 3-chloro-5-methoxyphenol	158.6	0.41	0.79	NA
(22) pentachlorophenol	266.3	0.03	0.05	5.01
Apolar phenols				
(23) 1,2-dihydroxybenzene	110.1	16.47	24.66	1.01
(24) 2-methoxyphenol	124.1	9.39	14.95	1.48
(25) phenol	94.1	11.69	18.38	1.46
(26) 4-methylphenol	108.1	5.26	7.23	1.94
(27) 4-ethylphenol	122.2	2.13	4.09	2.66
Alcohols				
(28) 2-phenylethanol	122.2	46.53	66.46	NA
(29) phenylmethanol	108.1	31.74	42.17	1.10
Carboxilic and sulfonic acids				
(30) benzoic acid	122.1	NT[**]	NT	1.87
(31) 3-phenylpropionic acid	150.2	NT	NT	NA
(32) phenylacetic acid	136.2	5.27	9.18	1.41
(33) 4-phenolsulfonic acid	174.2	NT	NT	NA
(34) sulfonic acid	158.2	36.86	NT	-2.25

[#] log P values were obtained from the literature (Keuning & Janssen 1987; Laane et al. 1986; Leo et al. 1971; Verscheuren 1983; Vighi & Calamari 1987). [+] compound identification number.

[*] NA= non-available; log P value was not available.

[**] NT= nontoxic; compounds were considered to be nontoxic if the 50% IC was higher than 40 mM.

Structure-toxicity relationships were also evident for aromatic compounds with more complex substitution patterns. Structural features associated with an increasing inhibition included increasing the number and the length of alkyl substitutions, as well as an increasing number of chlorine-atoms on the aromatic compound. Fig. 2 illustrates the higher toxicity of the methyl substituted aromatics as compared to their homologous counterparts with less or no methyl groups. The inhibitory effect of alkylbenzenes with increasing carbon chain length is compared in Fig. 3. Benzene caused the least inhibition, whereas propylbenzene, with the longest alkyl side chain, was the most inhibitory of this homologous series. Fig. 4 illustrates the distinct effect of chlorine atoms on increasing the toxicity of aromatic compounds. These structure-toxicity relationships are not unique to acetoclastic methanogens, as similar results are evident in literature reports concerning the toxicity of aromatic compounds towards a wide range of organisms (Bringmann & Kuhn, 1980; Chou *et al.* 1978; Jurd & Manners, 1980; Ruckdeschel *et al.* 1987).

Our toxicity results show that the addition of a functional group containing an oxygen or sulfur hetero-atom, such as carboxylic and sulfonic substitutions, often decreased the toxicity of aromatics. However, the aldehyde group was an exception since it caused increased inhibition. The effect of the hydroxyl group on the methanogenic inhibition of aromatic compounds tested in our study was diverse. The addition of the first hydroxyl group to the aromatic ring increased somewhat the toxicity of the aromatic compound. However, a dihydroxy-benzene (catechol) was less inhibitory than phenol, suggesting that further introduction of hydroxyl groups may lead to less inhibitory compounds. Decreasing methanogenic inhibition has been found to correspond with increasing hydroxyl substitutions in studies with phenolic monomeric compounds including phenol, catechol, resorcinol, pyrogallol and phloroglucinol (Chou *et al.* 1978; Field *et al.* 1987; Field & Lettinga 1989). Furthermore, we observed that adding a polar functional group to alkylic side chain generally contributed to a significant decrease in the aromatic toxicity (Fig. 5). The latter observation suggests that the alkyl side chain structure plays an important role in the antimicrobial activity of aromatic compounds. Similarly, literature reports show that the presence of polar functional groups on aliphatic side chain often results in a decrease of the microbial toxicity of aromatics (Zemek *et al.* 1979).

3.2. Correlations of toxicity with Octanol/Water Partition Coefficient

The n-octanol/water partition coefficient (P) is defined as the ratio of the equilibrium concentration of a given compound in a two-phase sytem consiting of two largely inmiscible solvents, in this case n-octanol and water. The logarithm of the n-octanol/water partition coefficient is widely used as an indicator of the hydrophobic character of organic compounds (Leo *et al.* 1971; Verscheuren 1983). Increasing hydrophobicity leads to easier passage of the organic compound trough membranes and greater distribution to lipophilic areas of the organism (Florence *et al.* 1984; Galbraith & Miller 1973; Parsons & Opperhuizen, 1987). Consequently, log P has been successfully used to predict partitioning and bioconcentration phenomena of hydrophobic organic pollutants in aqueous systems (Chiou et al. 1977; McKay 1984; Neely et al. 1974). It is also interesting to note that in numerous studies on the relationships between toxic activity and molecular structure (Quantitative Structure-Activity Relationship (QSAR) studies), the toxicological properties of a wide array of organic chemicals correspond inversely with water solubility and directly with log P (Hansch & Dunn 1972; Kamlet et al. 1986; Wayne Schultz *et al.* 1978). The toxic effects observed in these studies were attributed to adsorption or bioaccumulation of the aromatic compounds on the organisms tested.

The results of this study indicated that hydrophobicity is also an important factor contributing to the methanogenic toxicity of aromatic compounds. As shown in Fig. 6, a moderate correlation was found between the methanogenic toxicity and hydrophobicity when log (50% IC) was plotted against log P for all the data points of this study. In contrast, very good correlations between toxicity and hydrophobicity were observed within series of homologous compounds such as alkylbenzenes, alkylphenols, chlorobenzenes and chlorophenols, respectively, as shown in Fig. 7. However, the high correlation disappeared when the compounds from each of these series were combined (Table 2). These results imply

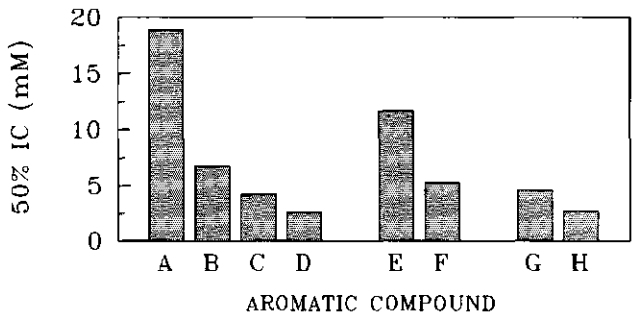

FIG. 2. The effect of methyl groups on the toxicity of aromatic compounds. A= benzene, B= methylbenzene, C= 1,2-dimethylbenzene, D= 1,3,5-trimethylbenzene, E= phenol, F= 4-methylphenol, G= methoxybenzene, H= 2-methylanisole.

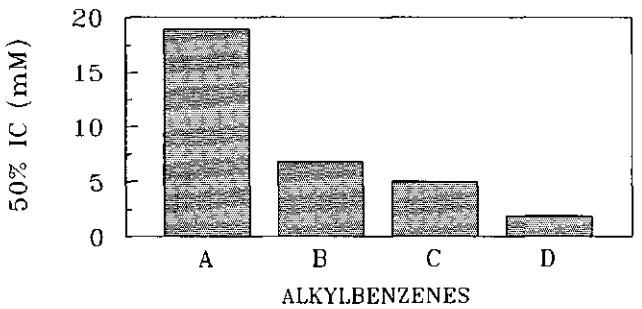

FIG. 3. The methanogenic toxicity of several alkylbenzenes with increasing carbon chain length. A= benzene, B= methylbenzene, C= ethylbenzene, D= n-propylbenzene.

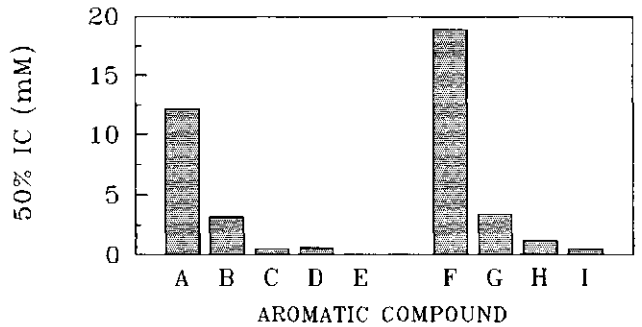

FIG. 4. The methanogenic toxicity of several chlorinated aromatics with increasing chlorine atom number. A= phenol, B= 2-chlorophenol, C= 2,4-dichlorophenol, D= 2,4,6-trichlorophenol, E= pentachlorophenol, F= benzene, G= chlorobenzene, H= 1,2-dichlorobenzene, I= 1,2,4-trichlorobenzene.

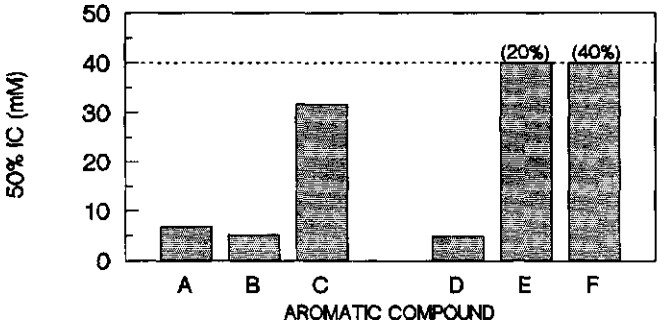

FIG. 5. The effect of adding a hydroxyl or carboxylic group to the alkyl side chain on the methanogenic toxicity of aromatics. A= methylbenzene, B= phenylacetic acid, C= phenylmethanol, D= ethylbenzene, E= 3-phenylpropionic acid, F= 2-phenylethanol. Numbers in parenthesis indicate the inhibition (%) at the highest concentration tested.

that log P is only strongly related to toxicity within homologous series of toxicants that have a similar mode of inhibitory action. Strong positive correlations between toxicity and partition coefficient within series of structurally related organic contaminants have also been reported earlier by several research groups using aerobic bacteria (Liu *et al.* 1982), yeast (Kwasniewska & Kaiser, 1983), ciliates (Wayne Schultz *et al.* 1978), crustacean (Kopperman *et al.* 1974) and fish (Kaiser *et al.* 1984) as test organisms.

The slope and the intercept values of the linear regression between log (50% IC) and log P provide numerical indexes for comparing structure-toxicity relationships. The slope is a measure of the response of the biological system to increasing hydrophobicity within a series of homologous toxicant compounds, whereas the intercept indicates the intrinsic toxicity of the series. The intercept is also related to the sensitivity of the test organism or biochemical system to the toxicants (Hansch & Dunn, 1972).

In our study, the chloro-aromatics have similar slopes, regardless if they are derivatives of benzenes or phenols. In a similar fashion the alkylbenzenes and alkylphenols had nearly equal slopes, however these were distinctly lower than those observed for chloro-aromatics. This results demonstrate that the increment of the toxic activity resulting from increasing hydrophobicity clearly depends on the type of functional group added (i.e. chlorine or methyl). Therefore, log P is not the sole factor determining toxicity but rather it is a parameter indicating the response of bacteria to the hydrophobic effects of a determined toxicant series.

Furthermore, when considering the intercept values obtained, the toxicity of alkyl- or chlorophenols at a given level of hydrophobicity was superior to that of the corresponding benzene derivatives. This indicates that phenol derivatives have an additional mode of action beyond that which would be predicted from the hydrophobicity of the benzene derivatives. This additional toxicity mechanism is perhaps related to the hydrogen bonding phenomena of the phenol group (Kamlet *et al.* 1986).

It should be noted that the toxicity of some compounds distinctly differ from that expected on the basis of their hydrophobicity, suggesting that their toxic activity is not controlled by partitioning and that it may involve specific mechanisms. In this respect, carboxylic and sulfonic acids, with very low toxicities and intermediate log P values, deviate significantly from the correlation. Log P may not be an appropriate hydrophobicity indicator for organic acids and bases since their aqueous solubility may vary drastically with changes in pH. Furthermore, compounds bearing aldehyde groups or containing side chains with double bonds caused significantly higher methanogenic inhibition than anticipated when only considering their hydrophobic character. Likewise, greater toxicities to various organisms than predicted by QSAR have been reported for various aldehydes (Kamlet *et al.* 1986) and allylic organics (Hansch & Dunn, 1972). Certain polar phenols also form an important exception to the trend relating increasing hydrophobicity with toxicity. Tannins, which are

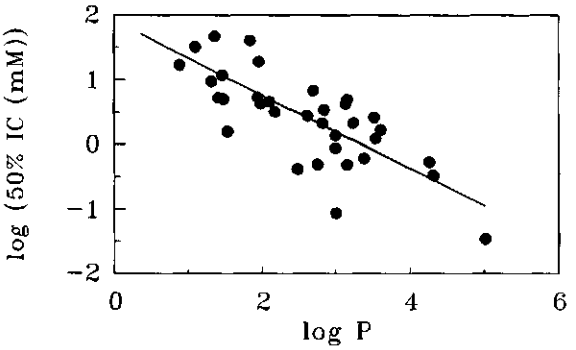

FIG. 6. The effect of hydrophobicity (log P) on the methanogenic toxicity (log (50% IC)) exerted by the aromatic compounds evaluated in this study.

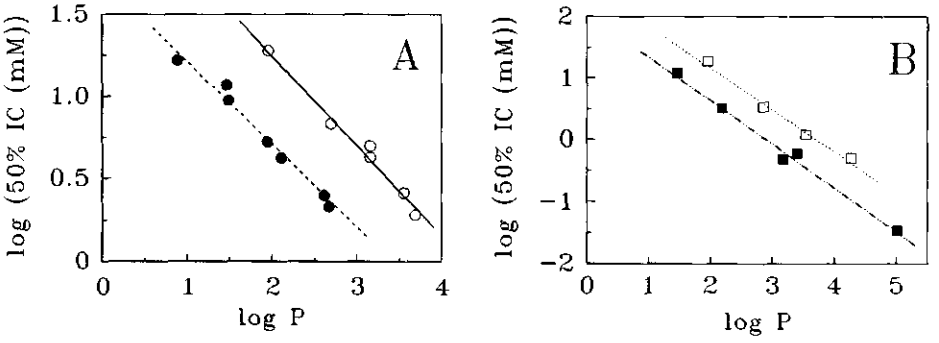

FIG. 7. The effect of hydrophobicity on the methanogenic toxicity of homologous series of aromatic compounds. A: (○) alkylbenzenes, (●) alkylphenols; B: (□) chlorobenzenes, (■) chlorophenols. Compounds included in the series were (1, 8-11, 13), (4, 5, 23-27), (1, 15-17) and (18-20, 22, 25), respectively. Compound numbering system corresponds to the list in Table 1.

TABLE 2. Linear relationships hydrophobicity-methanogenic toxicity (log (50% IC) = b * log P + a).

HOMOLOGOUS SERIES	b	a	n	r^2
(A) alkylbenzenes	-0.549	2.350	6	0.983
(B) alkylphenols	-0.544	1.792	7	0.989
(C) chlorobenzenes	-0.684	2.548	4	0.988
(D) chlorophenols	-0.712	2.076	5	0.990
series A, B, C & D combined	-0.551	1.951	20	0.735

highly hydrophilic phenols, are highly toxic to methanogenic bacteria (Field & Lettinga 1987; Field *et al.* 1988). The inhibition results from strong hydrogen bonding interactions between bacterial proteins and tannins (Field *et al.* 1989).

In conclusion, strong hydrophobicity-toxicity correlations should only be expected for organic toxicants wherein factors like partitioning and transport are controlling their toxic effects and within homologous series of compounds having a similar mode of action. Deviations from this trend should be anticipated for toxicants with biological effects that are mainly determined by specific toxicity mechanisms.

4. ACKNOWLEDGEMENTS

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CHAPTER 6

The Anaerobic Biodegradability of Paper Mill Wastewater Constituents

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THE ANAEROBIC BIODEGRADABILITY OF PAPER MILL WASTEWATER CONSTITUENTS

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ABSTRACT

The biodegradability of ten paper mill wastewater constituents under methanogenic conditions was evaluated in this study. The compounds studied included wood resin constituents and low molecular weight lignin derivatives. Biodegradation was assessed in batch bioassays inoculated with anaerobic granular sludge at 30 ± 2°C. The assays were supplied with low concentrations of the test chemicals (35-200 mg/l) in order to prevent methanogenic inhibition. The long chain fatty acids, oleic and linoleic acid, were readily biodegradable under anaerobic conditions. Guaiacol was also mineralized after a 40 days lag period. However, no indication of methanogenic degradation was obtained with eugenol, benzene, the resin acids (abietic and dehydroabietic acid), the volatile terpenes (pinene and limonene) and the unsaturated hydrocarbon squalene.

1. INTRODUCTION

Pulp and paper manufacture is a water-intensive industry responsible for the discharge of large quantities of highly polluted wastewaters. The environmental impact associated with these waste streams is not restricted to the oxygen demand resulting from degradation of organic compounds, since numerous effluents from the forest industry are highly colored and they often display acute toxicity to fish and other aquatic organisms. Furthermore, certain chlorinated compounds formed during wood-processing operations exhibit strong mutagenic effects (Walden 1980).

Biological wastewater treatment methods have been shown to be effective in reducing the high BOD loads in paper mill effluents. Anaerobic treatment methods are gaining importance (Latola 1985; Maat & Habets, 1987; Pearson 1989; Salkinoja-Salonen *et al.* 1985) due to the the recent development of high rate anaerobic reactors which combine technical and economical advantages compared to conventional aerobic biological methods (Hulshoff Pol & Lettinga, 1986). Nevertheless, the application of anaerobic methods is still largely limited to the treatment of non-inhibitory paper mill effluents which mainly contain readily degradable organic compounds (i.e. carbohydrates, volatile fatty acids, alcohols). The toxic compounds present in inhibitory pulp and paper mill effluents can interfere with biological treatment. Most noteworthy are wood resin compounds (Benjamin *et al.* 1984; Crane *et al.* 1957; Sierra-Alvarez & Lettinga, 1990a), low molecular weight (MW) lignin derivatives (Op den Camp *et al.* 1988; Sierra-Alvarez & Lettinga, 1990b) and chlorinated organic compounds (Guthrie *et al.* 1984; Sierra-Alvarez & Lettinga, 1990c; Wang *et al.* 1989), which have been identified as important toxins to methanogenic bacteria. These types of components also contribute to the aquatic toxicity of paper mill effluents (Junna *et al.* 1982; Leach & Thakore, 1976; Leach *et al.* 1978; Rogers, 1973). The susceptibility of the organic toxins to biotransformation or complete mineralization under anaerobic conditions will determine the effectiveness of

anaerobic wastewater treatment systems to reduce the aquatic toxicity of forest industry wastewaters. Likewise, the biodegradability of these compounds will decrease their toxicity to methanogenic bacteria, facilitating the removal of BOD.

The purpose of this study was to evaluate the anaerobic biodegradability of various organic compounds representative for the resinous wood constituents and low MW lignin derivatives in paper mill effluents.

2. MATERIALS AND METHODS

2.1. Analyses

Volatile fatty acids (VFA) were determined by gas chromatography (Sierra-Alvarez & Lettinga, 1990a). COD (colorimetric micro-method), total suspended solids (TSS) and volatile suspended solids (VSS) were determined according to Standard Methods (American Public Health Assoc., 1985). The pH was determined with a Knick 511 pH-meter and a Scot Gerade N61 double electrode. The methane content in the headspace of the serum flasks was determined by gas chromatography. A 100μl gas sample was injected in a gas chromatograph equipped with a molecular sieve 5A (mesh 60-80) column. The temperature of the column, the injection port and the flame ionization detector were 60, 200 and 220 °C, respectively. The carrier gas was nitrogen at a flow rate of 14.3 ml/min.

2.2. Biomass

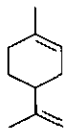
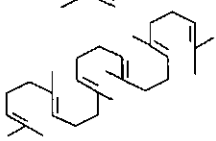
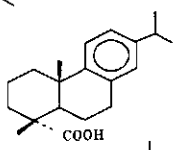
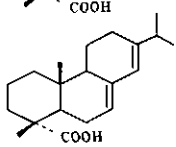
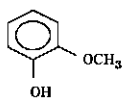
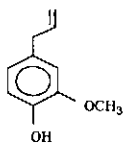
The methanogenic granular sludge used in these experiments was obtained from a full-scale anaerobic reactor treating distillery wastewater (Nedalco, Bergem op Zoom, The Netherlands). The sludge was elutriated to remove the fines and stored at 4°C under nitrogen gas. The sludge contained 9.6% TSS and 8.4% VSS. The maximum specific methanogenic activity of the sludge, as determined in standard batch activity tests (Sierra-Alvarez & Lettinga, 1990a), was 569 mg CH_4-COD/(g VSS·day).

2.3. Anaerobic Biodegradability Assay

The batch anaerobic biodegradability assays were conducted in 119 ml glass serum flasks. The basal medium used in the bioassays contained (mg/l): $NaHCO_3$ (400), NH_4Cl (280), $CaCl_2{\cdot}2H_2O$ (10), K_2HPO_4 (250), $MgSO_4{\cdot}7H_2O$ (100), yeast extract (100), H_3BO_3 (0.05), $FeCl_2{\cdot}4H_2O$ (2), $ZnCl_2$ (0.05), $MnCl_2{\cdot}4H_2O$ (0.05), $CuCl_2{\cdot}2H_2O$ (0.03), $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.05), $AlCl_3{\cdot}6H_2O$ (0.09), $CoCl_2{\cdot}6H_2O$ (2), $NiCl_2{\cdot}6H_2O$ (0.05), $Na_2SeO_3{\cdot}5H_2O$ (0.1), EDTA (1), resazurin (0.2) and 36% HCl (0.001 ml/l). Anaerobic granular sludge (0.5 g VSS per liter of medium), mineral solution and known amounts of the test compounds (Table 1) were transferred to the serum flasks in an anaerobic chamber. Oxygen-free distilled water was added to complete a final medium volume of 52 ml. Liquid hydrocarbons were supplied with chromatography microsyringes while solid substrates were dissolved in concentrated neutralized stock solutions and then added to the medium. The flasks were sealed with butyl rubber stoppers and subsequently incubated without shaking in a temperature controlled room at 30 ± 2°C. Sludge blanks, to correct for gas production from the sludge, were based on assays where no test compounds were provided. All biodegradability data are average of quadruplicate run experiments, except the sludge blanks which utilized ten serum flasks to assess the methane production.

The methane composition in the head space of each serum flask was monitored periodically during the assays. The flasks were shaken vigorously before gas measurements were taken. Methane production was calculated from the volume of the head space and the methane composition in the gas. In order to allow for an accurate determination of the CH_4 production, the head space was flushed with N_2 gas when the methane content exceeded 3%.

TABLE 1. The anaerobic biodegradability of paper mill wastewater constituents.

Compound	Structure	Conc.[a] (mg/l)	M (% TMP)	SE[b] (% TMP) M	SE[b] (% TMP) BM	Lag Phase (days)	Degradation potential*
LCFA							
oleic acid		170	87.6	3.6	1.6	< 3	+
linoleic acid		200	90.8	3.3	1.4	5	+
Volatile terpenes							
α-pinene		75	-17.7	8.4	10.2	> 123	-
limonene		75	-20.9	9.2	10.2	> 123	-
Triterpenes							
squalene		200	0.1	2.2	3.6	> 123	-
Resin acids							
dehydroabietic acid		35	58.1	6.1	26.0	> 123	±
abietic acid		75	2.0	6.4	11.7	> 123	±
Aromatics							
guaiacol		200	89.6	4.3	6.1	> 123	+
eugenol		150	-0.9	3.6	6.9	> 123	-
benzene		200	-2.6	0.6	4.3	> 123	-

[a] concentration of the test compound in the bioassay. [b] SE = standard deviation (as % TMP) of the methane production from the test compound (M) and background methane production (BM)

* Biodegradation potential: (+) completely degradable; (-) non degradable; (±) partial biodegradation.

Net methane production was calculated by subtracting background methane production in the controls from that in the test bottles. The corrected methane production (M) was expressed as a percentage of the theoretical methane production (TMP) expected from the test chemical mineralization based on the Buswell equation (Tarvin & Buswell, 1934).

2.4. Chemicals

Nitrogen and methane gas were purchased from Hoekloos (Schiedam, The Netherlands). The yeast extract was supplied by Gist-Brocades (Delft, The Netherlands). Abietic acid was purchased from Hicol (Oud Beijerland, The Netherlands). All other organic chemicals were obtained from Janssen Chimica (Tilburg, The Netherlands) and Merck (Darmstadt, FRG).

3. RESULTS

3.1. Evaluation of the anaerobic biodegradability assay

Glucose, a readily biodegradable non-inhibitory substrate and formaldehyde, a biodegradable highly inhibitory substrate, were used as reference compounds to assess the accuracy of the bioassay method used. The concentrations of glucose and formaldehyde in the assay medium were 100 and 54 mg COD/l, respectively. The total COD concentration in the bioassay with formaldehyde was 74 mg/l because the formaldehyde reagent used contained 10% (w/w) methanol. Both compounds were completely degraded after 5 days of incubation (Fig. 1). The ultimate conversion of the substrate COD to methane equal 91.0 and 88.1% for glucose and formaldehyde, respectively. The background methane production corresponded to 38.4 and 52.0% of the TMP expected from glucose and formaldehyde, respectively. Reproducibility of the methane production among replicate serum flasks was found to be satisfactory, with standard deviations generally lower than 5.4%.

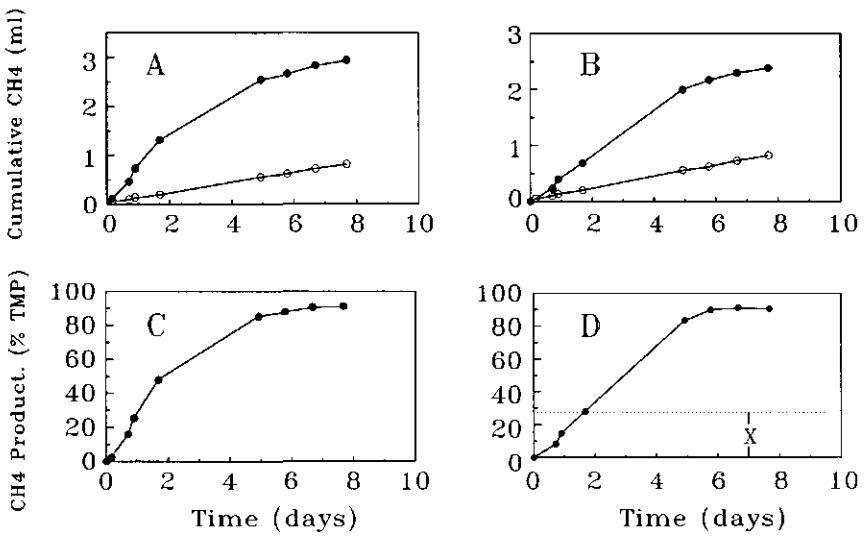


FIG. 1. The cumulative CH_4 production of the test compound (●) and the sludge blank (○) in the bioassay with glucose (A) and formaldehyde (B). The percentage of the theoretical methane production from glucose (C) and formaldehyde (D). X = percentage of the TMP equivalent to the COD of the methanol supplied with the formaldehyde.

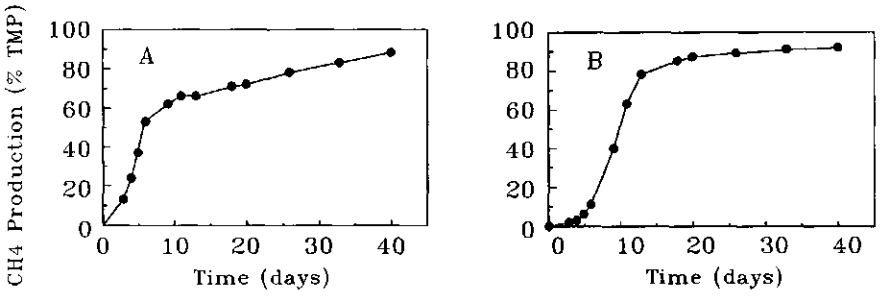


FIG. 2. The methane production (as percentage of the theoretical methane production) in the batch biodegradability assays with oleic (A) and linoleic (B) acid.

3.2. The anaerobic biodegradability of paper mill wastewater constituents

The biodegradability of selected paper mill wastewater constituents under methanogenic conditions was evaluated in this study. The organic compounds tested included wood resin constituents and low molecular weight lignin derivatives (Table 1). Test chemicals in the former group were selected as to be representative for the principal classes of compounds in wood resin (i.e. long chain fatty acids, terpenes and resin acids).

The various compounds assessed, with the exception of squalene, have been shown to be inhibitory towards methanogenic bacteria (Benjamin *et al.* 1984; Koster & Kramer, 1988; Sierra-Alvarez & Lettinga, 1990a; 1990b; 1990c). The concentration of the test compounds in the bioassay medium (Table 1) were carefully chosen to prevent significant methanogenic inhibition during the bioassays. In view of the relatively high background methane production obtained in the assays with formaldehyde and glucose, the anaerobic sludge used in these experiments was preincubated for 1 month in unfed conditions. Sludge preincubation minimized the readily degradable organic matter in the sludge and thus minimized the background methane production. In the various bioassays, the reproducibility of the methane production in replicates was satisfactory as indicated by the average standard deviations reported in Table 1. The ultimate methane production from the sludge inoculum was 5.0 ml, corresponding to 237 mg COD/l.

The long chain fatty acids (LCFA) were readily biodegradable under anaerobic conditions. Oleic acid degradation was very rapid and started within the first day of incubation (Fig. 2). Conversion of linoleic acid to methane began after a 5 day lag phase (Fig. 2). Both compounds were completely mineralized after 2 weeks of incubation as indicated by the high conversion of the substrate COD to methane which was equal to 87.6 and 90.8% for oleic and linoleic acid, respectively.

In contrast with the rapid and extensive mineralization observed for the LCFA, significant methanogenic degradation was not evident with the other classes of resinous components examined after 123 days of incubation. The serum flasks amended with the volatile terpenes, pinene and limonene, provided an amount of methane comparable to that of the sludge blanks (Fig. 3) during the first 90 days of the experiment. Afterwards, the methane production rate in the test bottles was somewhat lower than that of the sludge controls, suggesting methanogenic inhibition. Likewise, no evidence of methanogenic degradation was obtained with the unsaturated hydrocarbon squalene (Fig. 3).

The biodegradability assays with the resin acids compounds, dehydroabietic and abietic acid, resulted in positive net methane productions corresponding to 58.1 and 32.0% of the maximum theoretical methane production, respectively (Fig. 4). However, it is questionable if these results corresponded to partial degradation. After the prolonged incubation period (123 days), the background CH_4 production was high as compared to the TMP expected from the low concentrations used in the bioassay with dehydroabietic acid (35 mg/l), and thus does not permit any definite conclusions (Fig. 5). The standard deviation of the CH_4 production in the blanks corresponded to 26% of the TMP expected from dehydroabietic degradation.

Furthermore, the abietic acid reagent used contained 15% (w/w) unidentified impurities, which could have been responsible for some of the excess methane production beyond the controls.

The anaerobic biodegradability of the low molecular weight lignin derivative, guaiacol, was also tested. Guaiacol was completely mineralized after 45 days of incubation. During the initial period (day 0-40) of the experiment, the conversion of this substrate to methane proceeded slowly compared to that of readily biodegradable substrates such as glucose or oleic acid. The methane production rate increased considerably after day 40 (Fig. 6), and the methane conversion reached a maximum of 89.6% on day 45. In contrast, no evidence of anaerobic degradation was obtained in the bioassays conducted with eugenol and benzene after 123 days of incubation (Fig. 6).

4. DISCUSSION

4.1. Evaluation of the anaerobic biodegradability assay

Batch bioassays are relatively simple and inexpensive methods for assessing the anaerobic biodegradability of organic compounds. A number of approaches to determine the degradation potential of organic compounds under methanogenic conditions have been proposed (Fedorak & Hrudey, 1984; Field *et al.* 1988; Healy & Young, 1979; Owen *et al.* 1979; Shelton & Tiedje, 1984). These serum flask techniques are based on the measurement of the excess gas volume (CH_4 + CO_2) produced during the incubation of a test chemical with digested sewage sludge. The CH_4 composition in the biogas is generally estimated from the compound degradation stoichiometry or determined by gas chromatography. However, some of these screening tests suffer from important limitations since methods where mineralization is monitored by measuring the head space pressure or total gas production are subject to errors due to abiotic production of CO_2 gas. Furthermore, the presence of CH_4, the terminal product of methanogenesis, is established indirectly. Other methods recommend the use of a fixed concentration of the test chemical, without considering the toxicity of these compounds (Shelton & Tiedje, 1984). The assessment of the degradation potential of numerous inhibitory compounds is not feasible at toxic concentrations (Battersby & Wilson, 1989). Furthermore, the use of relatively unstable digested sewage sludge results in a relatively high background methane production as compared to the TMP of the test compounds (Shelton & Tiedje, 1984).

In this study, mineralization was monitored by direct measurement of the CH_4 composition in the serum flask head space. This method was found to be reliable for assessing the anaerobic biodegradation potential of organic compounds at relatively low concentrations (54 mg COD/l). The test compounds were provided to the assay medium at subtoxic concentrations in order to prevent inhibition of the anaerobic biodegradability by disturbances of the methanogenesis. Background methane production was reduced by using relatively low concentrations of preincubated sludge. Furthermore, anaerobic granular sludge was used instead of digested sewage sludge. The higher methanogenic activity and stability of granular sludge allows for the use of low sludge concentrations, which reduces methane generation from the blanks. In any case, the background methane production was found to interfere with the determination of the biodegradation potential in bioassays amended with low concentrations of highly inhibitory compounds which are persistent or slowly degradable, as was the case with dehydroabietic acid (Fig. 5). Therefore, alternative methods should be considered for highly toxic environmental pollutants that cannot be supplied at high concentrations. More sophisticated bioassay methods may be required for testing the anaerobic biodegradability of these types of compounds (e.g. carbon labelling; slow release).

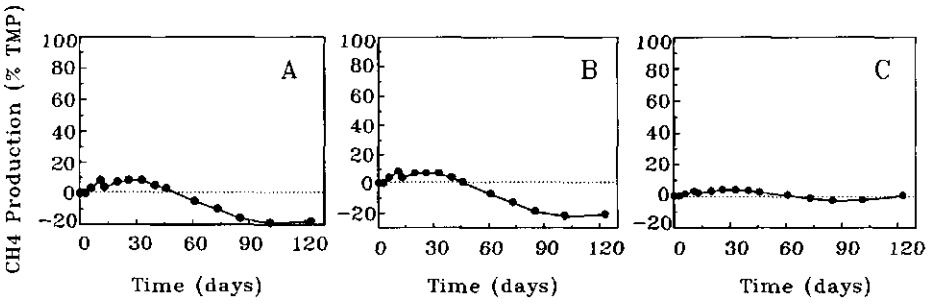


FIG. 3. The methane production (as percentage of the theoretical methane production) in the batch biodegradability assays with pinene (A), limonene (B) and squalene (C).

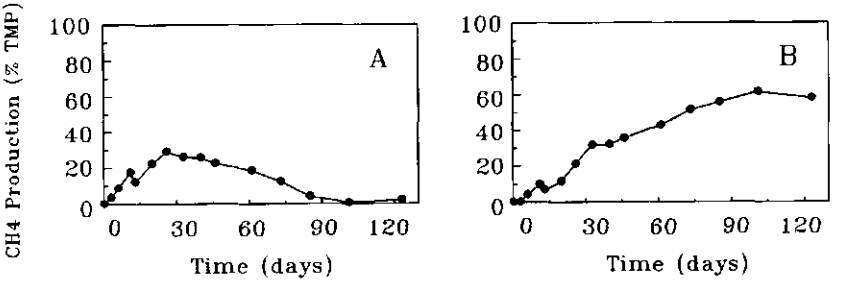


FIG. 4. The methane production (as percentage of the theoretical methane production) in the batch biodegradability assays with abietic (A) and dehydroabietic (B).

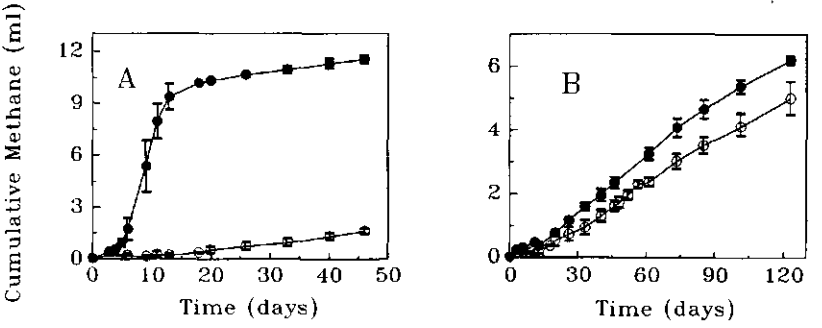


FIG. 5. The cumulative methane production in the bioassays with (A) oleic acid an example of a readily biodegradable compound and (B) dehydroabietic acid an example of a highly toxic and poorly biodegradable compound: (●) test compound, (○) sludge blank.

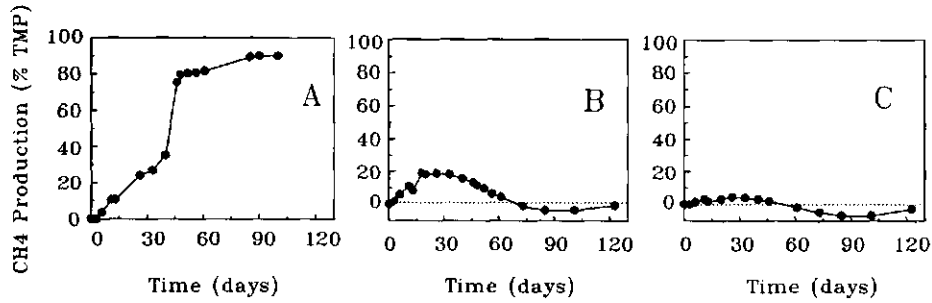


FIG. 6. The methane production (as percentage of the theoretical methane production) in the batch biodegradability assays with guaiacol (A), eugenol (B) and benzene (C).

4.2. The anaerobic biodegradability of paper mill wastewater constituents

The anaerobic biodegradability of various organic compounds representative for the resinous wood constituents and low MW lignin fragments in paper mill effluents was evaluated in this study. Batch bioassays indicated that oleic and linoleic acid were readily biodegraded in anaerobic environments. The rapid mineralization of LCFA at low concentrations was in agreement with previous reports (Hanaki *et al.* 1981; Novak & Carlson, 1970). Tracer studies have shown that ß-oxidation is the primary mechanism in anaerobic biodegradation of LCFA (Jeris & McCarty, 1965).

As opposed to LCFA, typical resin components like resin acids and terpenic hydrocarbons were not significantly degraded after prolonged incubation periods (123 days). Whereas mineralization of resin acids and terpenic compounds by aerobic bacteria has been demonstrated in numerous reports (Britton 1984; Junna *et al.* 1982; Leach *et al.* 1978; Trudgill 1984), relatively little is known about the anaerobic degradability of these compounds. We are not aware of any previous investigation on the anaerobic degradability of resin acids supplied as a sole substrate. In any case, a poor removal of resin acids has been observed during anaerobic treatment of bleaching wastewaters (Qiu *et al.* 1987). In accordance with our results, the recalcitrance of volatile terpenes has also been observed in batch bioassays with digested sewage sludge (Benjamin *et al.* 1984). Although unsubstituted hydrocarbons are considered highly resistant to microbial biodegradation in the absence of oxygen, evidence for the anaerobic metabolism of a limited number of unsaturated hydrocarbons, including the terpenoid hydrocarbon squalene, has recently been provided (Schink 1985). The initial hydroxylation of the unsubstituted hydrocarbons is the major hindrance to anaerobic degradation (Britton 1984; Schink 1985). Schink (1985) postulated that anaerobic degradation of hydrocarbons is possible if unsaturated bonds exist which allow hydrations and carboxylations that act as primary reactions of substrate activation.

Guaiacol, a lignin monomeric derivative, was highly biodegraded in the present study. Likewise, numerous studies have demonstrated the complete anaerobic degradation of simple lignin-related compounds such as coumaric (Tarvin & Buswell, 1934), ferulic (Grbic-Galic 1981; Healy *et al.* 1980), syringic (Healy & Young, 1979; Kaiser & Hanselmann, 1982) and vanillic (Healy & Young, 1979; Horowitz *et al.* 1981) acids. In contrast to guaiacol, eugenol (4-allyl-guaiacol) was not significantly degraded in this study, indicating that the presence of alkyl side chains increases the resistance of the aromatic compound to bacterial attack. Benzene was also persistent under anaerobic conditions which is consistent with the fact that in numerous previous studies (Battersby & Wilson, 1989; Horowitz *et al.* 1981; Schink 1985) aromatic hydrocarbons lacking polar functional groups (e.g. benzene and alkylbenzenes) are generally not found to be degradable. However, Grbic-Galic (1986) and Vogel & Grbic-Galic (1986) have recently reported evidence indicating that these compounds can be transformed anaerobically.

The results of this study indicate that anaerobic treatment technologies have a limited capacity to mineralize natural wood toxins. Although the degradation of long chain fatty acids and lignin monomers was demonstrated, other important wood toxins such as volatile terpenes and resin acids were found to be persistent under methanogenic conditions. These compounds are implicated in the aquatic toxicity of forest industry wastewaters. Therefore, anaerobic systems should be combined with other treatment steps in order to eliminate residual toxicity in anaerobically treated paper mill effluents.

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CHAPTER 7

The Continuous Anaerobic Treatment of Pulping Wastewaters

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THE CONTINUOUS ANAEROBIC TREATMENT OF PULPING WASTEWATERS

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ABSTRACT

The anaerobic treatability of thermomechanical pulping (TMP) effluents and soda pulping liquors was evaluated in this study. Continuous experiments were conducted in lab-scale UASB reactors inoculated with granular sludge at 30 ± 2°C.

TMP wastewaters were found highly suitable for anaerobic treatment. The application of high organic loadings (31 g COD/l·day) was feasible by the end of the continuous experiment with TMP wastewaters, with 68 and 98%, total COD and biodegradable COD elimination efficiencies, respectively. Unlike TMP effluents, soda pulping wastewaters were highly inhibitory to methanogenic bacteria and they contained important fractions of recalcitrant organic matter. Wood resin constituents were shown to be responsible for most of the methanogenic inhibition in these wastewaters. Nonetheless, anaerobic wastewater treatment was feasible for removing the biodegradable substrate in soda pulping wastewaters if, prior to biological treatment, the wastewaters were diluted to subtoxic levels or detoxified by pretreatment with the adsorbent Amberlite XAD-2.

Low COD removal efficiencies were observed during the continuous experiment (45 to 50%) with soda pulping liquors, due to the high amounts of recalcitrant lignin in these wastewaters. The elimination of UV_{280} absorbance (15 to 20%) indicated partial removal of some lignin components by anaerobic treatment. The lignic fraction removed or biotransformed anaerobically corresponded to low molecular weight lignin derivatives as confirmed by gel chromatography results.

1. INTRODUCTION

Numerous pilot- and full-scale experiences have shown that anaerobic treatment is an efficient and energy conserving method for treating various types of forest industry wastewaters including condensates, recycling-paper wastewaters as well as mechanical and thermomechanical pulping effluents (Pearson 1989). However, considerably less full scale plants have been built for treating paper mill waste streams derived from chemical wood processing operations. Chemical pulping and bleaching wastewaters contain recalcitrant organic compounds and toxic substances such as lignin derived aromatics, chlorinated phenols and resinous constituents, that have hindered the application of anaerobic treatment technologies. However, with the increasing insight over the biodegradative capacity and toxicity tolerance, anaerobic systems may in fact be well suitable for the complex waste streams of the forest industry.

Some aromatic compounds, previously considered recalcitrant, have been shown to be mineralized in anaerobic environments (Hollinger *et al.* 1988; Schink 1988; Woods *et al.* 1989). Anaerobic technologies appear to have a superior degradative capacity towards certain chlorinated aromatics as compared to conventional aerobic systems (Salkinoja-Salonen *et al.*

1983; Bosma *et al.* 1988). In addition, anaerobic bacteria are known to have a remarkable ability to adapt to numerous inhibitory compounds (Chou *et al.* 1979; Fedorak & Hrudey, 1986). Bacterial sludge immobilization and high biomass retention potential, which are features characteristic of high-rate anaerobic systems, improve the tolerance of methanogenic bacteria to toxic substances (Dwyer *et al.* 1986; Blum *et al.* 1986).

In this study, we have evaluated the continuous anaerobic treatment of black liquors. To date, the anaerobic treatment of alkaline pulping liquors has not received much attention, probably because a majority of these waste streams are usually burnt for recovery of pulping chemicals (Bryce 1980). However, conventional recovery processes are not economically viable in small paper mills and in those using non-woody materials with a high silica content (Anonymous 1986; Velasco *et al.* 1985). As a result, soda pulping liquors represent a very important pollution source in several countries. The study of black liquors is also important for understanding the anaerobic treatment of semi-chemical pulping and bleaching processes which also involve alkaline treatments of wood and, therefore, produce wastewaters containing similar toxic and recalcitrant by-products.

2. MATERIALS AND METHODS

2.1. Analyses

Samples for volatile fatty acid (VFA) and COD determination were filtered (Schleicher & Schull paper filter no. 589-1). VFA were determined by gas chromatography (Sierra-Alvarez & Lettinga, 1990a). COD (colorimetric micro-method) and volatile suspended solids (VSS) were determined according to Standard Methods (American Public Health Assoc., 1985).

The pH was determined with a Knick 511 pH-meter and a Scot Gerade N61 double electrode.

The lignin content in the wastewater samples was estimated from the ultraviolet absorbance of the samples at 280 nm (UV_{280}) using an absortivity coefficient of 22.3 l/ g·cm (Kim *et al.* 1987). The UV_{280} was determined in a 1 cm quartz cuvette by diluting the samples to less than 0.8 absorbance units in a 0.02 M borate buffer providing a pH 9.1.

Lignin molecular weight distribution of pine and spruce samples was determined by gel filtration chromatography in a 1.05 x 100 cm column packed with Sephadex G-50. A 1 to 2 ml aliquot of the wastewater (pH 9.0) was applied to the column and eluted with a $NaHCO_3$ (0.025 M)-NaOH buffer solution (pH 10.5) containing 0.5 g/l of polyethylene glycol (Pellinen & Salkinoja-Salonen, 1985). The molecular weight (MW) distribution of the lignic fraction in straw pulping liquors was determined in a 1.05 x 110 cm column packed with Sephadex G-75. A 0.02 M borate buffer providing a pH 9.1 was used as eluent (Amy *et al.* 1987). Elution was monitored by measuring the UV_{280} of the fractions collected. The biochemicals used for column calibration included aprotinin (MW = 6500), cytochrome C (MW = 12400), carbonic anhydrase (MW = 29000) and albumin (MW = 66000). Blue dextran (MW = 2000000) was used to determine the void volume of the packed column.

The color of the wastewaters was indicated by measuring the visible light absorbance at 440 nm as outlined previously for the UV_{280}.

2.2. Preparation of wood and straw pulping wastewaters

Extracts were prepared from lignocellulosic feedstocks commonly used in the forest industry, namely, pine (*Pinus sylvestris*) wood, spruce (*Picea abies*) wood and wheat (*Triticum aestivum*) straw. Spruce and pine chips were obtained from a paper factory (Parenco, Renkum, The Netherlands). Wheat straw was purchased in a local shop.

The pulping conditions used were representative of those applied in thermomechanical pulping (TMP) and soda pulping processes. Prior to pulping, debarked wood chip samples and straw were air dried (48 h at 70°C) and ground in a cross mill. A slurry containing 100 g of ground wood or straw per liter of water was cooked at 120°C during 2 hours.

When soda pulp waters were prepared, 8 g of NaOH were also added. The remaining pulp was separated from the pulp water by centrifugation and subsequent filtration. Finally, the pulp waters were neutralized by addition of NaOH or HCl, as required, and stored in refrigerated containers.

The treatment of the soda pulping liquors with Amberlite XAD-2 (XAD) was performed as following: 1 l of the wastewater (3 to 7 g COD/l) at pH 9.0 was shaken with 71.5 g of XAD for 5 hours, after which the samples were paper-filtered and neutralized with HCl. Adsorption onto XAD under alkaline conditions has previously been used to isolate wood resin compounds, such as fatty acids, resin acids, waxes and sterols, from paper mill effluent samples (Leach & Thakore, 1976; Rogers 1973).

2.3. Biomass

The anaerobic granular sludge used in these experiments was obtained from a full-scale anaerobic reactor treating distillery wastewater (Nedalco, Bergem op Zoom, The Netherlands). The sludge was elutriated to remove the fines and stored at 4°C under nitrogen gas.

2.4. Methanogenic Activity Assay.

All assays contained macronutrients (N, P and S) and trace elements required for bacterial growth as outlined previously (Sierra-Alvarez & Lettinga, 1990b). The serum flasks were not shaken during the assay period.

Maximum specific methanogenic activity measurements were conducted in 0.6 l glass serum flasks sealed with a rubber septum and a screw cap. The anaerobic sludge (1.5 g VSS/l) was transferred to a serum flask containing 0.5 l of a nutrient supplemented medium. Subsequently, the assays were supplied with 4 g COD/l of a neutralized VFA solution. The composition of the VFA substrate solution was 73:22:5, acetate:propionate:butyrate on a COD basis. Finally, after flushing the head space with nitrogen gas, the flasks were sealed and incubated in a temperature controlled room at 30 ± 2°C. In order to determine the methanogenic activity of the sludge after adaption to the VFA substrate, the assays were provided with a second VFA feeding (4 g VFA-COD/l). The substrate was replenished when 80% of the substrate supplied in the first feeding was converted to methane.

Methane production was monitored periodically during the assays with modified Mariotte flasks. These flasks were filled with a 3% (w/v) NaOH solution which served to remove the carbon dioxide from the biogas.

2.5. UASB Experiments

The continuous experiments were performed in glass UASB reactors with a liquid volume of 0.15 l (Fig. 1), placed in a temperature controlled room at 30 ± 2°C. In order to prevent wash-out of floating sludge, the UASB reactors were equipped either with a glass ball or a screen supported on a ring placed above the sludge bed. All reactors were inoculated with 20 g VSS/l anaerobic granular sludge. The reactors were started-up with neutralized VFA solutions (C_2:C_3:C_4, 23:34:41 in COD basis) at concentrations ranging from 2.5 to 5.0 g COD/l. The influent flow rate was adjusted to provide a loading ranging from 5 to 10 g COD/l·day. After this 15 days adaption period to the VFA substrate, the column experiment was started by replacing the influent VFA solution for a influent solution consisting of a pulping wastewater (day 0 of the experiment). All influent solutions contained macronutrients (N, P and S) and trace elements required for bacterial growth as outlined previously for the activity test. The pulping liquors were supplied with 1 g $NaHCO_3$ per gram of biodegradable wastewater COD to buffer eventual accumulations of VFA. The influent solutions were stored under N_2 gas in a refrigerator.

The methane production was measured with 10 l Mariotte flasks filled with a 3% (w/v) NaOH solution to scrub out the carbon dioxide from the biogas.

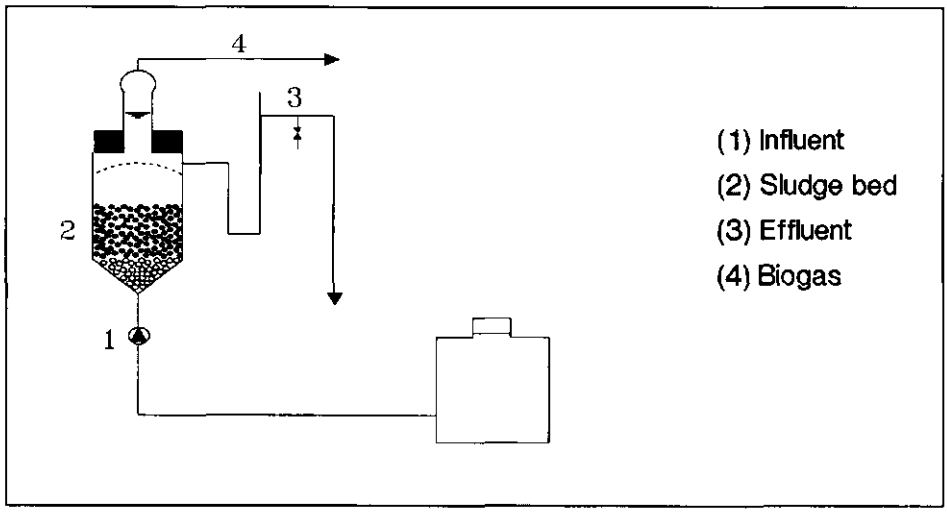

FIG. 1. Squematic representation of the UASB reactors used in this study.

In this study three separate experiments were conducted. The continuous anaerobic treatment of TMP and soda pulping liquors prepared from spruce wood was evaluated in experiment A. The anaerobic treatability of soda liquors from straw was studied in experiment B. Finally, the anaerobic biodegradability of the lignic fraction in diluted soda pulping liquors derived from pine and spruce wood was evaluated in experiment C. The experiments are outlined in Table 1.

The following parameters were used to indicate the performance of the UASB reactors: COD_{in} = Influent COD (mg COD/l); M = % conversion COD_{in} to methane; VFA = effluent VFA concentration as % of COD_{in}; A = % conversion COD_{in} to VFA (A = M + VFA); E = % COD_{in} removed based on the filtered effluent COD concentration; BD = % biodegradable COD_{in} (BD = E + VFA); E_{BD} = % biodegradable COD_{in} removed (E_{BD} = E/BD); E_{UV} = % UV_{280} elimination based on the filtered effluent UV_{280}.

2.6. Chemicals

The chemicals used were purchased from Janssen Chimica, (Tilburg, The Netherlands) and Merck (Darmstadt, West Germany). Sephadex G-50 coarse, Sephadex G-75 fine and the molecular weight markers for gel filtration were supplied by Sigma (St. Louis, MO, USA).

TABLE 1. Overview of the continuous experiments conducted in this study.

EXPERIMENT	REACTOR	INFLUENT
A	R1	Spruce TMP wastewater
	R2	Spruce soda pulping liquor
	R3	R2 influent pretreated with XAD
B	R4	Straw soda pulping liquor
C	R5	Pine soda pulping liquor
	R6	Spruce soda pulping liquor

3. RESULTS

3.1. Experiment A : The anaerobic treatment of TMP and soda pulping liquors prepared from spruce wood.

Three laboratory scale UASB reactors (R1, R2 and R2) were run in parallel in order to investigate the continuous anaerobic treatment of TMP and soda pulping liquors prepared from spruce wood. The reactors R1 and R2 were fed with the TMP and soda pulping wastewater, respectively. The latter soda pulping influent was pretreated with XAD and served as influent feed stock for reactor R3. This reactor was operated in order to determine the role of apolar wastewater constituents (ie., resin constituents) on the methanogenic inhibition exerted by the spruce soda liquors.

The average influent concentration, hydraulic retention time (HRT), organic loading rate (OLR) and treatment efficiency in the various periods of the experiment are listed in Table 2.

During the first part of the experiment (day 0-66), the reactors R1 and R2 were fed at influent COD levels ranging from 2.0 to 3.0 g COD/l. The concentration of the influent provided to the reactor R3 was on the average 80% of the COD compared to the influent R2, as pretreatment with XAD removed 20% of the wastewater COD. The treatment performance of all reactors during this initial period was satisfactory, as the percentage biodegradable substrate eliminated exceeded 95%. The high efficiencies were maintained even when the reactors R1, R2 and R3 were operated up to loading rates of 31.2, 27.1 and 15.7 g COD/l·day, respectively.

The pulping conditions applied had a significant effect on the anaerobic treatability of the resulting wastewaters (Figs. 2, 3 and 4). Alkaline pulping resulted in wastewaters with a lower anaerobic biodegradability as compared to thermomechanical pulping. This was indicated by the lower COD eliminations obtained with the column fed the soda pulping liquor compared to the column operated with the TMP wastewater. The average COD elimination corresponding to these reactors was 48.9 and 67.5%, respectively. On the other hand, anaerobic treatment of the XAD pretreated influent provided higher COD removal efficiencies as compared to the untreated soda liquor (Table 2). However, the COD elimination obtained with the latter reactors was comparable if this parameter is calculated on the basis of the COD concentration in the untreated soda pulping wastewater.

From day 66 to 75, all reactors were fed an influent containing solely VFA in order to determine the maximum methanogenic capacity of the reactors. The influent was supplied at 5 g COD/l. The composition of the influent on a COD basis was 73:22:5 of C_2:C_3:C_4, similar to that of the acidified wastewaters. During this period the effluent VFA concentrations were maintained above 600 mg COD/l to prevent substrate limitation (Koster *et al.* 1986). The maximum methanogenic capacities measured in this period for reactors R1, R2 and R3 averaged 61.5, 38.2 and 57.2 g CH_4-COD/l·day, respectively.

In a final period (day 76-89), reactors R2 and R3 were again fed with the soda pulping liquors in order to evaluate the treatability of these wastewaters at increased concentrations. Increasing the concentration of the untreated black liquor to 7.6 g COD/l caused a rapid deterioration of the treatment performance. The average loading applied was 20.1 g biodegradable COD/l·day, which was considerably lower than the maximum uninhibited methanogenic capacity of the reactor R2 during the preceding period (day 66-75) with the VFA containing influent (38.2 g COD/l·day). The average conversion of the influent COD to methane decreased from 38.6 to 26.8% as a result of an increasing VFA concentration in the effluent. This indicated inhibition of the methanogenic bacteria by the spruce soda pulping liquor. On the other hand, the XAD pretreatment of soda pulping liquor effectively removed the methanogenic toxicity from the wastewater. This was evident because the performance of the reactor fed concentrated XAD pretreated influent (R3) was comparable to that observed in the initial period of the experiment (Table 2). Furthermore, no accumulation of VFA was observed in the anaerobic effluent.

The specific methanogenic activity of the sludge in each reactor was determined at the end of the experiment (Table 3). The methanogenic activity of the sludges obtained from the

TABLE 2. The average influent concentration, hydraulic retention time (HRT), organic loading rate (OLR) and treatment efficiency corresponding to the UASB reactors fed spruce TMP wastewater (R1), spruce soda pulping liquor (R2) and XAD-treated spruce soda pulping liquor (R3) in the various periods of the continuous experiment.

	Reactors and experimental period[a]				
	R1	R2		R3	
PARAMETER[b]	I	I	II	I	II
Operation Parameters:					
CODin (g COD/l)	2.5	2.6	7.6	2.1	5.7
OLR (g COD/l·day)	12.1	12.0	40.1	8.2	25.0
HRT (days)	0.2	0.3	0.2	0.3	0.2
Efficiency:					
% E	67.5	48.9	39.3	61.1	60.3
% E_{BD}	98.8	97.3	72.8	98.9	98.5
% M	54.9	38.6	26.8	50.1	57.7
% A	55.7	39.9	36.1	50.8	58.6
% VFA	0.8	1.3	10.7	0.7	0.9
% E_{UV}	ND[c]	22.0	ND	19.1	ND

[a] Experimental period: (I) day 0-66, (II) day (76-89).
[b] Abbreviations are defined in Materials and Methods.
[c] ND = Not determined

TABLE 3. The specific methanogenic activity of the sludge in the reactors fed spruce TMP wastewater (R1), spruce soda pulping liquor (R2) and XAD treated spruce soda pulping liquor (R3).

	SLUDGE ACTIVITY[a] (g CH_4-COD/g VSS·day)	
SLUDGE	1st Feeding	2nd Feeding
R1	1.4	1.4
R2	1.2	1.2
R3	0.6	0.8

[a] The max. spec. methanogenic activity of the seed sludge after VFA-acclimation was 0.9 g CH_4-COD/g VSS·day

reactors operated with the TMP and the XAD pretreated soda pulping influent (R1 and R3) increased during the course of the experiment. In contrast, the activity of the sludge from the column fed soda pulping liquor (R2) decreased during the experiment and was 50% lower than that of the sludge exposed to the XAD pretreated wastewater.

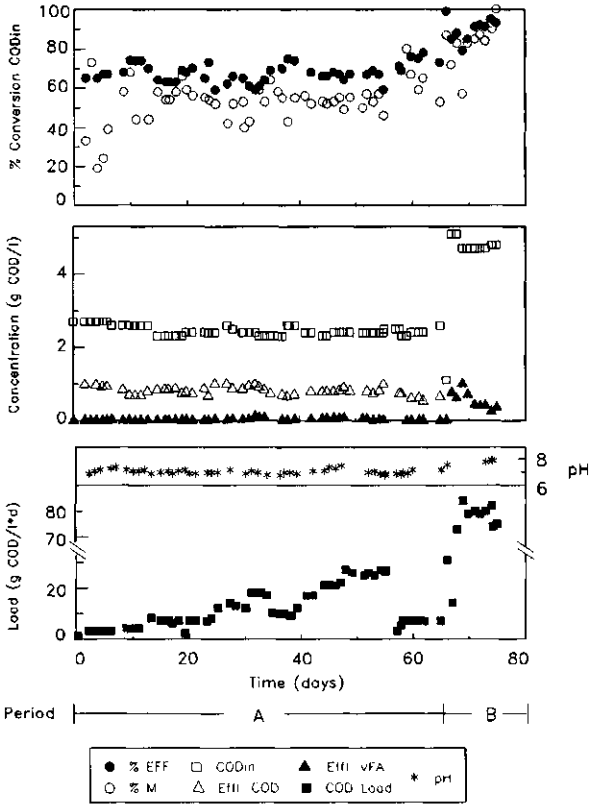

FIG. 2. Operation and efficiency during the anaerobic treatment of spruce TMP wastewater in the UASB reactor (R1). Experimental periods: (A) day 0-66, (B) day 67-75.

3.2. Experiment B : The anaerobic treatment of soda pulping liquors prepared from wheat straw.

Experiment B investigated the continuous anaerobic treatment of soda liquors from wheat straw in a laboratory scale UASB reactor (R4). The influent was supplied to the reactor at concentrations that ranged from 4.8 to 14.7 g COD/l.

The operation parameters and efficiency during the continuous column experiment is illustrated in Fig. 5. The average COD elimination efficiency, acidification and methanogenesis of the influent COD are listed in Table 4.

The treatment performance of the reactor was excellent, and high influent concentrations (14.7 g COD/l) were treated at loadings up to 50.0 g COD/l·day with over 95% removal of the biodegradable COD. However, the average elimination of the total COD was only 46.7% indicating that the straw soda pulping influent, similarly to that prepared from spruce, contained large amounts of organic matter recalcitrant to anaerobic treatment. During the course of the experiment, the biodegradability of the wastewater COD increased from 41.2 to 54.3% (Table 4) indicating some adaption of the sludge to the biodegradability of certain wastewater components.

In this experiment, 21.4% UV elimination was observed, indicating a partial removal of the lignic fraction in the straw soda pulping liquor. Gel chromatography results show that the lignic matter eliminated was restricted to low MW lignin derivatives (Fig. 6).

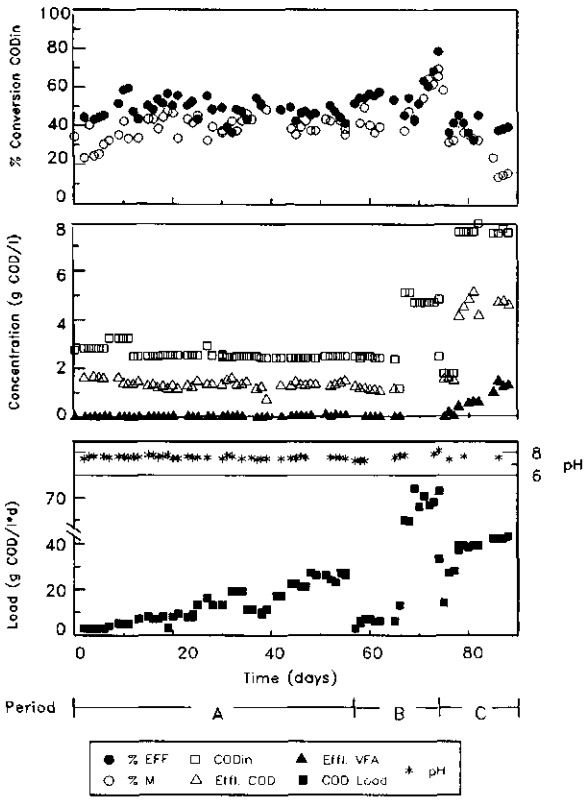

FIG. 3. Operation and efficiency during the anaerobic treatment of spruce soda pulping liquors in the UASB reactor (R2). Experimental periods: (A) day 0-66, (B) day 67-75, (C) day 76-89.

3.3. Experiment C: the anaerobic biodegradability of the lignic fraction in soda pulping liquors

Laboratory columns (R5 and R6) were utilized to investigate the anaerobic biodegradability of the lignin fraction present in soda pulping wastewaters. The influent of the reactors R5 and R6 consisted of soda liquors prepared from pine and spruce wood, respectively.

The reactors were fed with diluted pulping liquors to prevent methanogenic inhibition at a HRT of 0.3 days (Table 5). The loadings applied in both reactors were kept constant at values that did not exceed the maximum methanogenic capacity of the reactors. These measures were taken to guarantee low VFA levels in the anaerobic effluent in order to favor anaerobic degradation of phenolic compounds. High VFA concentrations have been reported to hinder the anaerobic degradation of phenolic intermidates (Field 1989).

The operation and efficiency of the reactors during the course of the continuous experiment is illustrated in Figs. 7 and 8 and summarized in Table 5.

The average COD elimination efficiencies observed for the reactors fed pine and spruce soda pulping liquors were very similar, corresponding to 45.6 and 46.7% of the influent COD, respectively. However, the average methanogenesis and acidification of the influent COD was somewhat higher for the spruce as compared to the pine soda pulping liquor. The lignin content in the effluent of the reactors R5 and R6 was equal to 65 and 78% of the effluent

TABLE 4. The average influent concentration, HRT, organic loading rate and treatment efficiency during the continuous anaerobic treatment of straw soda pulping liquor in the various periods of the reactor operation.

PARAMETER[a]	EXPERIMENTAL PERIOD (day no. start/end)			
	AVG	I	II	III
Operation Parameters:				
CODin (g COD/l)	7.4	5.3	6.3	10.5
OLR (g COD/l·day)	30.6	10.4	31.4	40.4
HRT (days)	0.3	0.5	0.2	0.2
Efficiency:				
% E	46.7	40.8	46.3	53.7
% E_{BD}	97.6	99.0	96.1	98.9
% M	37.5	34.0	38.1	39.4
% A	38.6	34.4	39.9	40.0
% VFA	1.1	0.4	1.8	0.6

[a] Abbreviations are defined in Materials and Methods.
[b] Experimental period: (AVG) average entire experiment, (I) day 0-20, (II) day 20-80, (III) day 80-110.

TABLE 5. The average influent concentration, HRT, organic load rate and treatment efficiency during the continuous operation of the UASB reactors fed diluted pine (R5) and spruce (R6) soda pulping liquors.

PARAMETER[a]	R5	R6
Operation Parameters:		
COD_{in} (g COD/l)	2.0	3.9
OLR (g COD/l·day)	6.6	13.5
HRT (days)	0.3	0.3
Efficiency:		
% E	46.7	45.6
% E_{BD}	99.6	95.2
% M	35.7	40.8
% A	35.9	43.0
% VFA	0.2	2.2
% E_{UV}	20.1	15.1
% Color	0.0	0.0

[a] Abbreviations are defined in Materials and Methods.

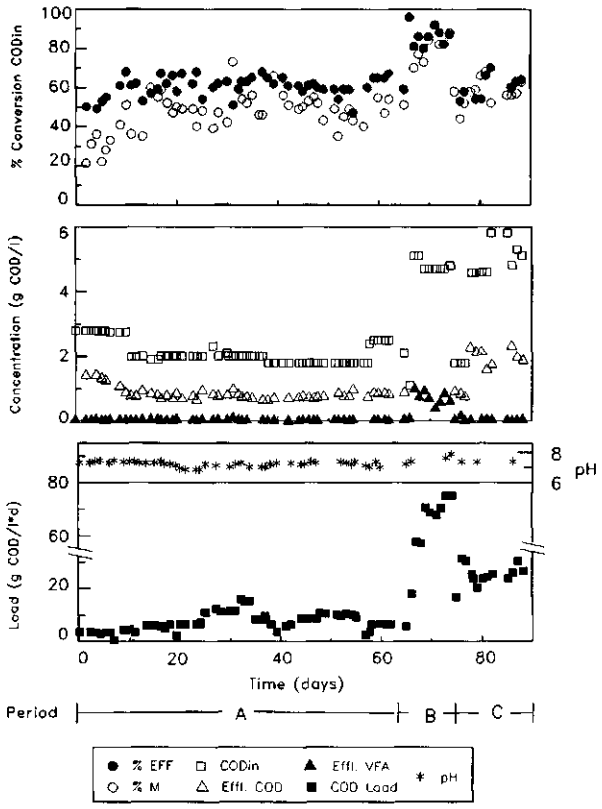

FIG. 4. Operation and efficiency during the anaerobic treatment of XAD treated spruce soda liquor in the UASB reactor (R3). Experimental periods: (A) day 0-66, (B) day 67-75, (C) day 76-89.

COD, respectively. This implies that the COD recalcitrant to anaerobic treatment in the soda liquor can be accounted mostly for by lignin derivatives.

The elimination of UV_{280} absorbance by anaerobic treatment was monitored regularly during the experiment. The UV_{280} elimination in the reactors fed the pine liquor and spruce liquor averaged 20 and 15% of the influent UV_{280}, respectively, indicating a partial removal of some lignin components. The lignic fraction removed or biotransformed anaerobically corresponded to low MW components, as illustrated in Fig. 9. Adaption of the sludge to the biodegradability of the recalcitrant wastewater components was not evident, as the COD and UV_{280} elimination did not increase during the course of the experiments.

Finally, it should be noted that continuous anaerobic treatment of the soda liquors was not effective in removing the high color levels associated to these wastewaters. No elimination of the color was observed in effluent samples analyzed for absorbance at 440nm immediately after collection. However, it was noticed that exposure of the anaerobic effluent to the air increased the color of the effluent to levels exceeding that of the influent.

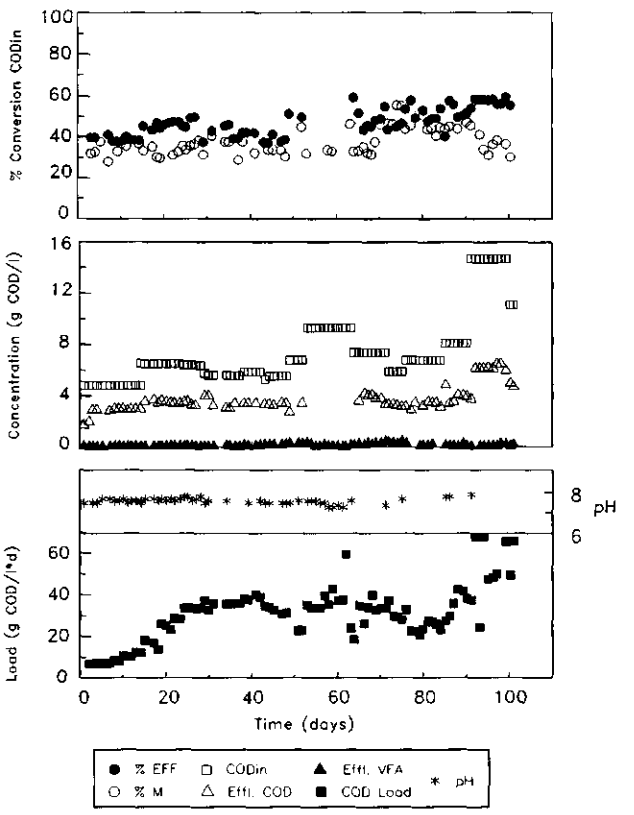

FIG. 5. Operation and efficiency during the anaerobic treatment of straw soda pulping liquor in the UASB reactor (R4).

4. DISCUSSION

The forest industry is a major source of pollution responsible for the discharge of high BOD loads in surface waters. Regulations restricting the discharge of untreated wastewaters and the relatively high costs of conventional aerobic systems have triggered research on the potentials of anaerobic wastewater treatment technologies for reducing the environmental impact associated with pulp and paper industry effluents. In this study, the anaerobic treatment of TMP and soda pulping wastewaters was evaluated in lab-scale UASB reactors using anaerobic granular sludge.

4.1. The anaerobic treatability of pulping wastewaters

The continuous anaerobic treatment of TMP wastewaters was feasible at high organic loading rates (31.2 g COD/l·day) with total COD and biodegradable COD removal efficiencies of 67.5 and 98%, respectively. The influent COD concentration of 2.5 g COD/l which was maintained throughout the experiment is comparable with those expected in industrial TMP effluents. High treatment efficiencies have also been reported in earlier laboratory studies on the anaerobic treatability of wastewaters derived from mechanical pulping and thermomechanical pulping processes (Jurgensen *et al.* 1985; Rintala & Vuoriranta, 1988).

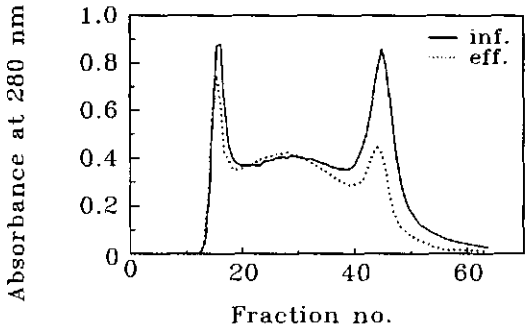

FIG. 6. The molecular weight distribution of the influent and the effluent of the UASB reactor fed straw soda pulping liquor.

Furthermore, numerous full-scale and pilot scale experiences (Pearson 1989) have demonstrated that anaerobic technologies are highly suitable for the removal of the organic load in these types of forest industry effluents.

On the other hand, soda pulping liquors should be regarded as relatively more difficult for anaerobic treatment. Unlike TMP effluents, soda pulping wastewaters are highly inhibitory to methanogenic bacteria and they contain important fractions of poorly biodegradable organic matter. Furthermore, these wastewater streams are generally highly concentrated with values ranging from 75 to 170 g COD/l (Velasco *et al.* 1985; Anonymous 1986).

4.1.1. The methanogenic toxicity of pulping liquors. Results of continuous experiments indicated that spruce soda pulping wastewater was highly inhibitory to methanogenic bacteria (Fig. 3) at concentrations of 7.5 g COD/l. Straw soda pulping liquors were less toxic, and influent concentrations exceeding 10 g COD/l could be accommodated with high treatment efficiencies. Although the continuous anaerobic treatment of pine soda liquor was also investigated, conclusions concerning its methanogenic toxicity cannot be drawn as the influent concentration was maintained at subtoxic levels during the experiment.

The results of batch anaerobic toxicity assays in previous works (Sierra-Alvarez & Lettinga, 1990a), indicated that soda pulping liquors derived from pine, spruce and straw resulted in 50% methanogenic inhibition at concentrations of 2.1, 5.4 and 4.4 g COD/l, respectively. A comparison of the toxicity assay results with those obtained in the long term treatability experiments of this study suggests that significant adaption of the methanogenic bacteria to the inhibitory compounds in the straw soda liquor occurred during the continuous experiment. A similar level of adaption was not evident in the reactor fed spruce soda wastewater.

The inhibitory characteristics of soda liquors are most likely due to the presence of wood resin compounds and low molecular weight lignin derivatives in these waste streams. These compounds are highly solubilized in the alkaline conditions prevailing during soda pulping (Bryce 1980). Resinous wood components such as resin acids and volatile terpenes, are highly inhibitory to methanogenic bacteria (Benjamin *et al.* 1984; Sierra-Alvarez & Lettinga, 1990b). Various resin constituents were shown to cause 50% methanogenic inhibition at concentrations ranging from 20 to 330 mg/l (Sierra-Alvarez & Lettinga, 1990b). Microbial inhibition resulting from the low MW lignin products formed during chemical pulping operations is also indicated in the literature (Sierra-Alvarez & Lettinga, 1990c; Zemek *et al.* 1979).

In order to determine the importance of resin compounds on the methanogenic toxicity observed during the continuous anaerobic treatment of spruce soda pulping liquors, a parallel experiment was conducted with the wastewater pretreated with XAD, an adsorbent known to

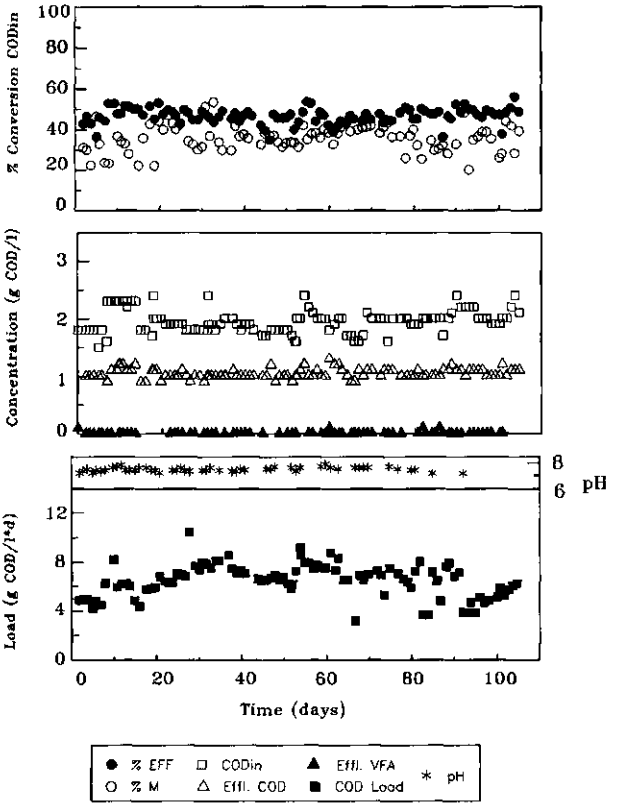

FIG. 7. Operation and efficiency during the anaerobic treatment of diluted pine soda pulping liquor in the UASB reactor (R5).

be effective in removing resinous compounds from forest industry wastewaters (Leach & Thakore, 1976; Rogers 1973). The improved reactor performance observed during the experiment with the XAD-treated wastewater (Table 2), confirmed that the wood resin constituents were responsible for methanogenic inhibition.

Despite the inhibitory character of soda pulping liquors, high biodegradable COD removal efficiencies were obtained in the various reactors (R2 to R6) operated with these types of wastewaters in so far as the influent COD concentrations were maintained at subtoxic levels. Moreover, under these conditions of operation, high organic loading rates could be applied in the various experiments without significant deterioration of the treatment performance. Therefore, anaerobic treatment technologies can be successfully applied for reducing the organic load in soda pulping liquors if these wastewaters are adequately diluted. Dilution of the wastewater will prevent methanogenic toxicity and favor possible microbial adaption to the inhibitory compounds. In practice, considerable dilution might be feasible with other waste streams generated in the paper mill.

In cases where dilution is not technically or economically viable, the feasibility of applying pretreatment methods leading to detoxification of the wastewater should also be considered. In this study, the XAD treatment was shown to be highly effective in removing the toxicity in soda pulping liquors. However, the high costs of XAD and the need for periodic regeneration of this resin raise doubts if the application of this pretreatment method at industrial scale would be economically acceptable. In any case, the use of a similar adsorbent material (Amberlite XAD-8) for color removal from kraft mill effluents has been claimed to have economic advantages over existing technologies (Rogers 1973).

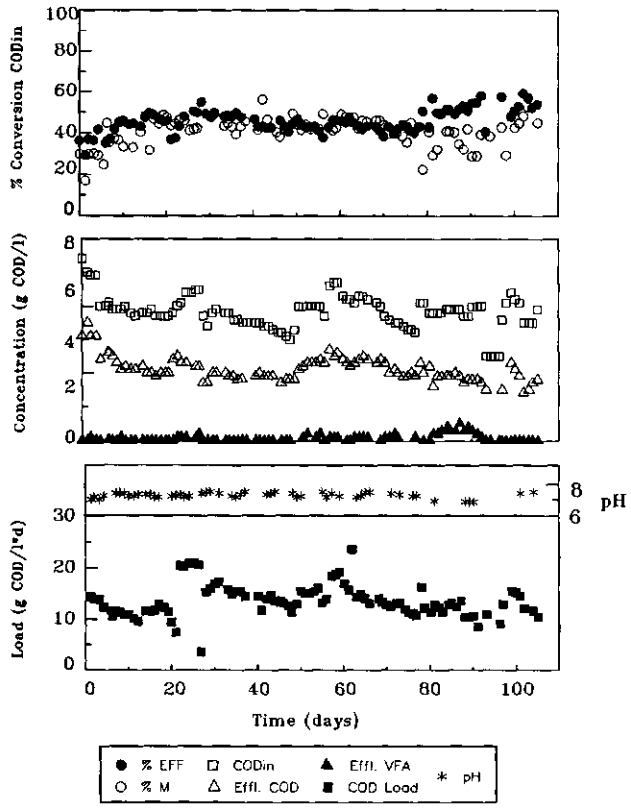

FIG. 8. Operation and efficiency during the anaerobic treatment of diluted spruce soda pulping liquor in the UASB reactor (R6).

Precipitation treatments have also been proposed to reduce the methanogenic toxicity resulting from wood resin in forest industry effluents. Welander (1988) developed a detoxification process based on precipitation of long chain fatty acids (LCFA) and resin acids with aluminum, calcium and iron salts. This method was found to be effective in removing the high methanogenic toxicity of chemi-thermomechanical pulping wastewaters. Likewise, in a previous study (Sierra-Alvarez & Lettinga, 1990a), we observed that both calcium and acid precipitation resulted in a complete removal of the methanogenic toxicity in soda pulping liquors. The implementation of such precipitation processes on a mill scale might be limited because precipitated sludges are often poorly settleable and difficult to dewater. Moreover, continuous chemical additions generally result in high operation costs. Therefore, further research is still needed for the development of more economically viable detoxification processes.

4.1.2. The anaerobic biodegradability of pulping liquors. In this study, TMP effluents could be treated with high COD efficiencies. The high biodegradable is due to the large fraction of readily biodegradable carbohydrates present in these wastewaters (Jurgensen *et al.* 1985). In contrast, the COD removal efficiencies obtained during the continuous anaerobic treatment of soda pulping liquors ranged from 45 to 50%, regardless of the lignocellulosic feedstock used. The distinctly lower biodegradability of alkaline pulping liquors results from their high lignin content. Unlike TMP processes, the alkaline treatment of wood results in

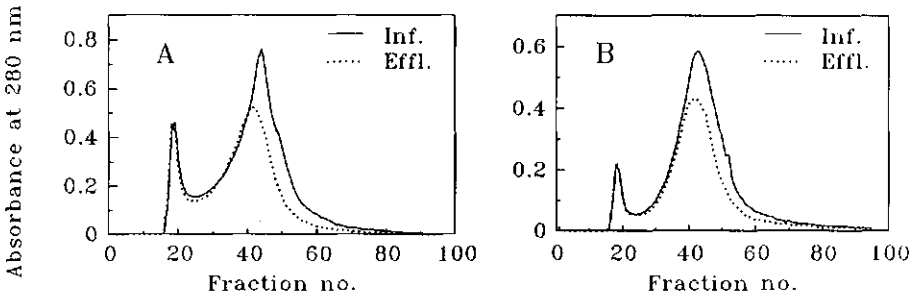

FIG. 9. The molecular weight distribution of the influent and the effluent of the UASB reactors fed diluted spruce (A) and pine soda liquor (B).

extensive lignin solubilization. The lignin content of the alkaline pulping liquors can account for up to 50% of the total solids (Forss 1982; Kim *et al.* 1987). In a similar fashion, poor anaerobic biodegradabilities have been reported for paper mill effluents generated by chemical treatments which effectively attack lignin, such as sulfite pulping (Norrman & Narbuvold, 1984; Hall *et al.* 1986) and bleaching wastewaters (Nitchals *et al.* 1985; Salkinoja-Salonen *et al.* 1985; Särner 1988).

In this work, anaerobic treatment of the soda pulping liquors resulted in a 15 to 20% elimination of the UV absorbance, indicating partial removal of some lignin components. The lignic fraction removed or biotransformed anaerobically corresponded to the low MW lignin derivatives as confirmed by gel chromatography results. Both the COD and UV_{280} elimination efficiencies remained fairly constant during the continuous treatment of pine and spruce soda liquors, indicating that adaption to the biodegradability of the recalcitrant lignic matter did not occur after 110 days of reactor operation.

The fact that high MW lignin is highly resistant to biodegradation by both anaerobic and aerobic bacteria is well established in the literature (Hackett *et al.* 1977; Kern & Kirk, 1987; Odier & Monties, 1983; Zeikus *et al.* 1982). The occurrence of lignin degradation in anaerobic environments is generally restricted to low MW derivatives (Colberg & Young, 1985; Healy & Young, 1979; Zeikus *et al.* 1982). Considering that the majority of the lignin present in chemical pulping wastewaters belongs to the high MW fraction (Glasser & Kelley, 1987; Pellinen & Salkinoja-Salonen, 1985), very little removal of lignin can be expected by biological wastewater treatment.

The presence of lignin is responsible for the high color levels in many industrial effluents from the paper and pulp industry. In accordance with the limited ability of anaerobic bacteria to degrade the lignin chromophoric structures, anaerobic treatment was not effective in removing the high color levels of soda pulping liquors. On the contrary, it was noticed that upon exposure of the anaerobic effluent to air, the color of the effluent increased to levels exceeding that of the influent. Increasing color levels have been previously observed upon aeration of anaerobically treated paper mill wastewaters (Rintala & Vuoriranta, 1988). This phenomenon is perhaps related to an increase in the phenolic groups due to biological removal of methoxy groups from aromatic ring structures which is known to occur under anaerobic conditions (Kaiser & Hanselmann, 1982). The resulting phenolic compounds bearing neighboring hydroxyl groups are susceptible to autoxidative modifications that lead to the formation of colored compounds (Field & Lettinga, 1989).

Biological wastewater treatment systems should be combined with other technologies in order to remove the recalcitrant color bearing lignin COD. A variety of methods have been investigated for controlling paper mill color discharge. These include precipitation by lime (Dugal *et al.* 1975) and polyamines (Kisla & McKelvey, 1978), adsorption by activated carbon (Bloodgood & El-nagaar, 1961), ultrafiltration (Fremont & Kleper, 1980), chemical oxidation (Prat *et al.* 1988), enzymatic and fungal treatments (Eaton *et al.* 1982; Forss *et al.* 1989).

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CHAPTER 8

SUMMARY

CHAPTER 8

Summary

Pulp and paper manufacturing is a water intensive industry responsible for the discharge of highly polluted effluents. The environmental impact of forest industry wastewaters is largely due to the release of biodegradable organic matter (ie. BOD) and inhibitory constituents which are toxic to aquatic organisms. Additionally, the characteristic dark color of these effluents is due to the presence of large amounts of solubilized lignic materials that are resistant to biodegradation.

Both, environmental regulations restricting the discharge of untreated wastewaters as well as the relatively high costs of conventional aerobic wastewater treatment systems have triggered research on the potentials of anaerobic technologies for reducing the environmental impact associated with pulp and paper industry effluents.

Compared to conventional aerobic processes, the anaerobic wastewater treatment method offers a number of important benefits such as lower energy requirements and operating costs, the production of a useful energy by-product in the form of methane gas and a considerable reduction in the volume of excess sludge produced. The low excess sludge production makes anaerobic treatment methods particularly attractive since sludge disposal is becoming a major problem for aerobic treatment systems. Furthermore, improved biomass retention in anaerobic reactors allows the application of high organic loading rates, reducing to a large extent the required reactor volume.

Anaerobic wastewater treatment processes are generally considered to be more sensitive to toxic substances compared to the aerobic systems. Consequently, the high toxicity of a variety of pulp and paper mill effluents is one of the major factors hampering the wide spread application of anaerobic treatment technologies in the forest industry. The recent advances in the identification of inhibitory substances as well as the increasing insight over the biodegradative capacity and toxicity tolerance of anaerobic microorganisms has helped to demonstrate the feasibility of anaerobic treatment systems for many types of inhibitory wastewaters.

In the case of the forest industry effluents, the bulk of the previous research attention has been directed towards the methanogenic toxicity of inorganic substances, such as sulfur containing compounds. Although the anaerobic biodegradability of chlorinated aromatic compounds has been investigated with great enthusiasm, almost no research results are available which describe the toxicity of these compounds to anaerobic microorganisms. Moreover, there is a distinct deficiency of information regarding the toxicity of natural wood derived organic compounds to anaerobic microorganisms.

This thesis describes research on the anaerobic treatment of inhibitory wastewaters from the forest industry. The main objective was to determine the role of natural organic wood constituents on the methanogenic toxicity of these wastewaters. In the first part of the thesis (Chapter 2), lignocellulosic feedstocks were pulped by different processes to determine which factors are responsible for the extraction of toxic substances. Thereafter (Chapters 3 to 5), compounds representative of inhibitory wood constituents were assayed for methanogenic toxicity. This included evaluating the role of chemical structure on methanogenic toxicity. In Chapter 6, the anaerobic biodegradability of selected inhibitory wood derived compounds was determined. Finally (Chapter 7), the continuous anaerobic treatment of toxic pulping effluents was investigated.

General Introduction

Chapter 1 presents a general introduction reviewing the available literature data concerning the characteristics and composition of pulp and paper mill effluents. The environmental impact related to the discharge of these effluents is also discussed. Finally, different wastewater treatment technologies are reviewed which are presently being applied to reduce pollution loads at pulp and paper mills.

Pulping Conditions Leading to the Extraction of Toxic Substances in Wastewaters

In Chapter 2, wastewaters were prepared from different lignocellulosic feedstocks, commonly used in the forest industry, under conditions that were representative of thermomechanical pulping (TMP) or soda pulping processes. The pulping conditions applied had a significant effect on the anaerobic treatability of the resulting wastewaters. The TMP wastewaters were not toxic to methanogenic bacteria at concentrations of 10 g COD/l, which is in excess of the concentration expected in TMP mill effluents. TMP wastewaters were also found to be highly biodegradable with 68 to 87% of the COD being acidified (converted to methane and volatile fatty acids) in biodegradability assays. In contrast, wastewaters prepared under the alkaline conditions of soda pulping caused severe inhibition to the methanogenic activity. The 50% inhibitory concentrations of soda pulping liquors ranged from 2.1 to 5.4 g COD/l. The soda pulping liquors were also poorly biodegradable (approximately 50% acidification).

Selective removal of various organic fractions from the soda pulping liquors with different physical-chemical treatments indicated that wood resin compounds were responsible for the majority of the toxicity. This was confirmed by comparing the methanogenic toxicity of pulping liquors prepared from normal and resin free (organic solvent extracted) wood.

Resin is not extracted from wood under the acidic conditions that prevail during mechanical pulping. On the other hand, the contact of wood with alkali during chemical pulping and bleaching processes results in extensive solubilization of the resinous compounds and consequently contributes to the formation of toxic wastewaters. Additionally, the alkali solubilizes to a great extent lignin and thereby also contributes to a lowered biodegradability of the wastewater.

Toxicity of Forest Industry Wastewater Constituents

Based on the results of Chapter 2, resinous compounds constitute one of the most important sources of toxicity in pulping wastewaters. The major resinous constituents of wood are long chain fatty acids, terpenes, resin acids, lignans and apolar phenols. In Chapter 3, the high methanogenic toxicity of a variety of resin compounds was demonstrated. Of all the compounds tested, an apolar phenol (4-hydroxystilbene) was the most toxic, causing 50% inhibition of the methanogenic activity at only 20 mg/l. Resin acids and volatile terpenes were also found to be highly toxic. Concentrations responsible for 50% inhibition ranged from 43 to 330 mg/l. In contrast, triterpenes were relatively non-toxic at concentrations of 1000 mg/l or greater. An alkaline extract of natural spruce wood resin was also assayed and was found to cause 50% inhibition at only 50 mg solids/l.

Aside from the resinous compounds in lignocellulosic feedstocks, lignin derived compounds are also potential sources of toxicity in pulping wastewaters. The objective of Chapter 4 was to evaluate the methanogenic toxicity of various lignins isolated from forest industry wastewater and selected lignin model compounds. The wastewater lignins differed considerably in their inhibitory activity. Some lignin samples were non-toxic, whereas others caused 50% inhibition at concentrations ranging from 3.3 to 6.0 g COD/l. Experiments with ultrafiltered lignins revealed that the toxicity of the inhibitory lignin samples originated from the low molecular weight (MW) fraction. This would be expected from the poor penetration of high MW lignin polymers to the sensitive sites of bacteria. In additional studies with low MW lignin model compounds, it was observed that the inhibitory activity of these compounds was related to the functional groups on the aromatic ring. Compounds with aldehyde groups or apolar substituents were highly toxic; whereas those with carboxylic groups only caused significant inhibition at high concentrations. Fragmentation of the lignin macromolecule by alkalis or acids is the basic step of all chemical procedures involved in wood processing leading to variable amounts of low molecular weight lignin-derived compounds in chemical pulping and bleaching effluents. However, the content of low MW lignin is in itself not an adequate index for estimating the contribution of lignin to the wastewater toxicity since in fact only certain types of lignin fragments are inhibitory.

The effect of aromatic structure on methanogenic inhibition was evaluated in detail in Chapter 5 in order to better understand the role of these aromatic derivatives on the toxic characteristics of forest industry wastewaters. The toxicity of different monosubstituted benzenes to acetoclastic methanogens was compared. Chlorobenzene, methoxybenzene and benzaldehyde were the most toxic with 50% inhibition occurring at concentrations of 381, 498 and 534 mg/l; respectively. Toluene (methylbenzene), phenol (hydroxybenzene) and benzene itself displayed intermediate toxicity with 50% inhibiting concentrations of 623, 1100 and 1477 mg/l, respectively. In contrast, benzoic acid was the least inhibitory, concentrations up to 7000 mg/l were non-toxic. When numerous benzene derivatives were evaluated, some basic structure-toxicity relationships were evident. In general, the toxicity of aromatic compounds increased with increasing the length of aliphatic side-chains and increasing the number of alkyl or chlorine groups. On the other hand, the toxicity decreased as polar functional groups were introduced on the alkylic side chains. The logarithm of the partition coefficient n-octanol/water, an indicator of hydrophobicity, was observed to be positively correlated with the methanogenic toxicity of aromatic compounds. The results indicate that hydrophobicity is an important factor contributing to the high toxicity of the most inhibitory aromatic compounds. Therefore, strongly hydrophobic compounds such as apolar lignin derivatives, chlorinated aromatics and wood resin constituents are the primary suspect organic toxicants in forest industry effluents.

Biodegradability of Toxic Forest Industry Wastewater Constituents

In Chapter 6, the susceptibility of important organic toxins in forest industry effluents to biodegradation under anaerobic conditions was assessed. Biotransformation or mineralization of inhibitory compounds can potentially reduce the aquatic toxicity of forest industry wastewaters. Likewise, the biodegradability of these compounds would decrease their toxicity to methanogenic bacteria, facilitating the removal of BOD during anaerobic treatment processes.

The compounds studied included wood resin constituents and low molecular weight lignin derivatives. Biodegradation was assayed by monitoring the methane gas production from the test compounds which were diluted to sub-toxic concentrations in batch anaerobic bioassays. The long chain fatty acids, oleic and linoleic acid, were rapidly biodegraded. Guaiacol (methoxyphenol), a lignin derivative, was also mineralized after a 40 day lag period. In contrast, no indication of degradation was obtained from an apolar phenol, eugenol, unsubstituted benzene, resin acids, volatile terpenes, nor the unsaturated hydrocarbon, squalene. These results indicate that important compounds implicated in the aquatic of forest industry wastewaters are persistent under methanogenic conditions. Therefore, anaerobic treatment systems should be combined with other treatment steps to adequately remove the aquatic toxicity.

Continuous Anaerobic Treatment of Pulping Wastewaters

Chapter 7 describes investigations on the treatability of thermo-mechanical pulping (TMP) effluents and soda pulping liquors in laboratory scale upward-flow anaerobic sludge blanket (UASB) reactors using granular sludge.

TMP wastewaters were found highly suitable for anaerobic treatment. The continuous anaerobic treatment of TMP wastewaters was feasible at high organic loading rates (31 g COD/l·day) with total COD and biodegradable COD removal efficiencies of 68 and 98%, respectively. The influent COD concentration of 2.5 g COD/l which was maintained throughout the experiment is comparable with those expected in industrial TMP effluents.

On the other hand, soda pulping liquors should be regarded as relatively more difficult for anaerobic treatment. Soda pulping wastewaters were highly inhibitory to methanogenic bacteria and they contain important fractions of recalcitrant organic matter. Furthermore, these wastewater streams are generally highly concentrated with values ranging from 75 to 170 g COD/l.

Despite the inhibitory character of soda pulping liquors, anaerobic wastewater treatment was feasible for removing the biodegradable substrate if, prior to biological treatment, the wastewaters were diluted to subtoxic levels or detoxified by pretreatment with the adsorbent, Amberlite XAD-2. Moreover, under these conditions of operation, high organic loading rates could be applied in the various experiments without significant deterioration of the treatment performance. Biodegradable COD removal efficiencies exceeded 95% when organic loading rates were applied at 7 to 14 g COD/L·day with wood derived black liquors diluted to 2-4 g COD/l, and up to 40 g COD/l·day with straw derived black liquors diluted to 10 g COD/l.

Low COD removal efficiencies (45 to 50%) were observed during the continuous experiment with soda pulping liquors due to the high amounts of recalcitrant lignin in these wastewaters. The elimination of UV_{280} absorbance (15 to 20%) indicated partial removal of some lignin components by anaerobic treatment. The lignic fraction removed or biotransformed anaerobically corresponded to low molecular weight lignin derivatives as confirmed by gel chromatography results.

The presence of lignin is responsible for the high color levels in forest industry effluents. In accordance with the limited ability of anaerobic as well as aerobic bacteria to degrade the lignin macromolecule, biological wastewater treatment systems are not expected to be effective in removing color. Biological treatment systems should be combined with other technologies, e.g. physical-chemical, enzymatic and fungal treatments, in order to effectively remove the recalcitrant color bearing lignin.

HOOFDSTUK 8

SAMENVATTING

Samenvatting

Bij de fabricage van pulp en papier wordt veel water gebruikt, hetgeen tot de lozing van ernstig vervuild afvalwater leidt. De milieubelasting door dit afvalwater is grotendeels een gevolg van de lozing van biologisch afbreekbare organische stof (d.w.z. BZV) en remmende bestanddelen, die giftig zijn voor aquatische organismen. Kenmerkend is ook de donkere kleur van deze afvalwaters, een gevolg van de aanwezigheid van een grote hoeveelheid opgeloste lignine-derivaten, die biologisch slecht kunnen worden afgebroken.

Beperkende maatregelen voor de lozing van ongezuiverd afvalwater, alsmede de relatief hoge kosten van conventionele aërobe zuiveringssystemen, hebben onderzoek op gang gebracht naar de mogelijkheden om met anaërobe zuiveringstechnologieën de milieuvervuiling door de lozing van afvalwater van de pulp- en papierindustrie te verminderen. In vergelijking tot conventionele aërobe zuiveringsprocessen biedt anaërobe afvalwaterzuivering een aantal belangrijke voordelen, zoals de lagere energiebehoefte en bedrijfskosten, het vrijkomen van een bruikbaar eindprodukt in de vorm van methaangas en de forse vermindering van de hoeveelheid surplusslib. Met name de lage surplusslibproduktie maakt anaërobe zuivering aantrekkelijk, omdat de slibverwerking een van de grootste problemen is bij aërobe zuiveringssystemen. Door de goede slibretentie in anaërobe reaktoren is het mogelijk hoge belastingen toe te passen, waardoor het benodigde reaktorvolume aanzienlijk kan worden verkleind.

In het algemeen acht men anaërobe zuiveringssystemen gevoeliger voor toxische stoffen dan aërobe systemen. Daarom is de toxiciteit van verschillende afvalwaters van pulp- en papierfabrieken een van de belangrijkste faktoren, die een brede toepassing van anaërobe behandeling van dit type afvalwater in de weg heeft gestaan. De recente vooruitgang die is geboekt met de identificatie van remmende stoffen, alsmede het toegenomen inzicht in de eigenschappen van anaërobe microorganismen (zoals hun vermogen om bepaalde stoffen biologisch af te breken en hun weerstand tegen toxische verbindingen), hebben de toepasbaarheid van anaërobe zuiveringssystemen voor veel soorten remmende afvalwaters helpen aantonen. In het geval van het afvalwater van de houtverwerkings- en papierindustrie is het merendeel van het tot dusverre uitgevoerde onderzoek gericht geweest op de methanogene toxiciteit van anorganische verbindingen, zoals zwavelhoudende stoffen. Alhoewel de anaërobe afbraak van gechloreerde aromatische verbindingen met veel geestdrift is onderzocht, zijn er bijna geen onderzoeksresultaten die een beschrijving geven van de giftigheid van deze verbindingen voor anaërobe organismen. Bovendien is er een duidelijk gebrek aan informatie over de giftigheid voor anaërobe microorganismen van de organische verbindingen, die uit hout vrijkomen.

Dit proefschrift beschrijft het onderzoek naar de anaërobe behandeling van verschillende afvalwaters van de houtverwerkingsindustrie, waarvan bekend is dat ze de biologische aktiviteit van anaërobe microorganismen remmen. De belangrijkste doelstelling was het vaststellen van de rol, die natuurlijke organische houtbestanddelen spelen, bij de toxiciteitsverschijnselen veroorzakt door dit type afvalwater. In het eerste deel van het proefschrift (Hoofdstuk 2) werden lignocellulose bevattende grondstoffen op verschillende wijze verpulpt om vast te stellen welke faktoren verantwoordelijk zijn voor de extraktie van giftige stoffen. Daarna (Hoofdstuk 3 t/m 5) werd de methanogene toxiciteit bepaald van stoffen die representatief zijn voor remmende verbindingen uit hout, waarbij ook het effekt van de chemische struktuur van deze verbindingen op de mate van remming van de methanogene aktiviteit werd geëvalueerd. In Hoofstuk 6 werd de anaërobe afbreekbaarheid van een aantal geselekteerde uit hout verkregen verbindingen bepaald. Ten slotte (Hoofdstuk 7) werd de continue anaërobe zuivering van giftige pulp-afvalwaters onderzocht.

Algemene Inleiding

In Hoofdstuk 1 wordt een algemene inleiding gegeven met een overzicht van de beschikbare literatuur over de eigenschappen en samenstelling van afvalwaters van de pulp- en papierindustrie. De milieuvervuiling door deze afvalwaters wordt ook besproken. Ten

slotte, worden verschillende afvalwaterzuiveringstechnologieën behandeld, die thans worden toegepast bij het bestrijden van de milieuvervuiling ontstaan als gevolg van pulp- en papierfabricage.

De Invloed van de Pulpmethode op de Aanwezigheid van Giftige Stoffen in het Afvalwater

In Hoofdstuk 2 werden afvalwaters bereid van verschillende, algemeen gebruikte grondstoffen onder omstandigheden die representatief waren voor thermo-mechanische pulping (TMP) of loog pulping. De verschillende toegepaste verpulpingstechnieken hadden een sterk effekt op de anaërobe afbreekbaarheid van het verkregen afvalwater. De TMP-afvalwaters waren niet giftig voor methanogene bacteriën bij concentraties van 10 g CZV/l, hetgeen meer is dan de concentraties, die in het afvalwater van een TMP-pulpfabriek kunnen worden verwacht. TMP-afvalwaters bleken bovendien uitstekend biologisch afbreekbaar te zijn. Uit afbreekbaarheidstests bleek 68 tot 87% van de CZV te worden verzuurd (omgezet in methaan en vluchtige vetzuren). De afvalwaters, die daarentegen onder de alkalische omstandigheden van de loog pulping werden gevormd, bleken een ernstige remming van de methanogene aktiviteit te veroorzaken. De concentraties van het afvalwater van loog pulping, die 50% remming veroorzaakten, lagen tussen 2.1 en 5.4 g CZV/l. Deze afvalwaters waren ook slecht afbreekbaar (ongeveer 50% verzuring).

Selektieve verwijdering van verschillende frakties van de loog pulping afvalwaters door middel van verschillende fysisch-chemische methoden, gaf aan dat harsen uit het hout verantwoordelijk zijn voor het grootste deel van de toxiciteit. Dit werd bevestigd door het vergelijken van de methanogene toxiciteit van pulpafvalwater bereid uit normaal en harsvrij (met organische oplosmiddelen geëxtraheerd) hout.

Hars wordt niet uit hout geëxtraheerd onder de zure omstandigheden, die bij de mechanische pulping voorkomen. Aan de andere kant zal het kontakt van loog met de lignocellulosehoudende grondstoffen, tijdens de chemische pulping en het bleekproces, de extraktie van harsachtige verbindingen bevorderen en daardoor een sterke bijdrage leveren aan de vorming van toxische afvalwaters. Bovendien zal het loog voor een groot deel de aanwezige lignine oplossen en daardoor aanleiding geven tot een verlaging van de afbreekbaarheid van het afvalwater.

Toxiciteit van Stoffen uit het Afvalwater van Houtverwerkingsbedrijven

Op grond van de resultaten van Hoofdstuk 2 is het duidelijk, dat harsachtige verbindingen de belangrijkste bronnen voor de toxiciteit van pulpafvalwaters vormen. De belangrijkste harsachtige verbindingen in hout zijn hogere vetzuren, terpenen, harszuren, lignanen en apolaire fenolen. In Hoofdstuk 3 werd de hoge methanogene toxiciteit van verschillende harsen aangetoond. Van alle verbindingen die werden onderzocht, was een apolaire fenolachtige stof (4-hydroxy-stilbeen) het meest giftig. Deze stof veroorzaakte een remming van 50% van de methanogene aktiviteit bij slechts 20 mg/l. Harszuren en vluchtige terpenen bleken ook zeer giftig te zijn. Concentraties van 43 tot 330 mg/l leidden tot een remming van 50%. Tri-terpenen waren daarentegen relatief weinig giftig bij concentraties van 1000 mg/l of hoger. Er is ook een alkalische extract van sparrenhars onderzocht en het bleek, dat er een 50% remming werd verkregen bij slechts 50 mg droge stof/l.

Van de lignocellulose houdende grondstoffen zijn behalve de harsachtige verbindingen ook verbindingen die uit lignine worden verkregen potentiële bronnen van toxiciteit. De doelstelling van Hoofdstuk 4 was het vaststellen van de methanogene toxiciteit van verschillende lignines, die geïsoleerd werden uit het afvalwater van houtverwerkingsbedrijven, alsmede van enkele geselecteerde model lignines. De remming door de verschillende lignines uit het afvalwater bleek aanzienlijk te variëren. Sommige lignine monsters waren niet giftig, terwijl anderen een 50% remming veroorzaakten bij concentraties van 3.3 tot 6.0 g CZV/l. Ultrafiltratie van de lignines toonde aan, dat de toxiciteit van de remmende lignine monsters zijn oorsprong vindt in de fraktie aan verbindingen met een laag molecuulgewicht. Dit valt te verklaren uit de hypothese, dat hoogmoleculaire lignines de bacterie-cel niet goed kunnen binnendringen en daardoor de kwetsbare plaatsen in de

bacterie nauwelijks bereiken. Aanvullende proeven met lignineachtige stoffen met een laag molecuulgewicht lieten zien dat de remmende werking van deze stoffen verband hield met de aard van de functionele groepen aan de aromatische ring. Verbindingen met aldehyde-groepen of apolaire substituenten waren zeer giftig, terwijl verbindingen met carboxyl-groepen alleen bij hoge concentraties een duidelijke remming veroorzaakten. Splitsing van het lignine macromolecuul door inwerking van loog of zuur is de basisstap van alle chemische behandelingen, die worden gebruikt bij de verwerking van hout tot pulp, waarbij een afvalwater onstaat met verschillende hoeveelheden uit lignine verkregen laag moleculaire stoffen. Het gehalte aan laag moleculaire lignine is op zich echter geen geschikte maat voor het schatten van de bijdrage van de lignines aan de giftigheid van het afvalwater, omdat alleen bepaalde lignine-fragmenten een remmende werking uitoefenen.

Het effekt van de aromatische struktuur op de remming van de methanogene aktiviteit werd in detail bestudeerd in Hoofdstuk 5 met het doel een beter begrip te verkrijgen van de rol van deze struktuur op de giftige eigenschappen van het afvalwater van de houtverwerkingsindustrie. De giftigheid van verschillende mono-gesubstitueerde benzenen werd met elkaar vergeleken. Monochloorbenzeen, methoxy-benzeen en benzaldehyde waren het meest giftig met een 50% remming van de methanogene aktiviteit bij concentraties van resp. 383, 498 en 534 mg/l. Tolueen (methyl-benzeen), fenol (hydroxy-benzeen) en benzeen zelf vertoonden een gemiddelde toxiciteit met een remming van 50% bij concentraties van resp. 623, 1100 en 1477 mg/l. Benzoëzuur was daarentegen het minst giftig; concentraties tot 7000 mg/l waren niet toxisch. Bij vergelijking van een groot aantal benzeen-derivaten bleken er duidelijke verbanden te bestaan tussen de struktuur en de toxiciteit. De giftigheid van aromatische verbindingen nam in het algemeen toe met een toenemende lengte van de alifatische zijketen en een toenemend aantal alkyl- of chloor-groepen. Aan de andere kant nam de giftigheid af bij aanwezigheid van meer polaire groepen in de alifatische zijketens. Het bleek, dat de logaritme van de partitie coëfficiënt n-octanol/water, een maat voor de hydrofobiciteit, positief gecorreleerd is met de remming van de methanogene aktiviteit. Deze resultaten geven aan, dat de hydrofobiciteit een belangrijke rol speelt bij de sterke toxiciteit van de meest giftige aromatische verbindingen. Daarom zijn sterk hydrofobe verbindingen, zoals harsachtige houtbestanddelen, apolaire lignine-derivaten en gechloreerde aromaten de meest verdachte gifstoffen in het afvalwater van de houtverwerkingsindustrie.

De Afbreekbaarheid van Gifstoffen uit het Afvalwater van de Houtverwerkingsindustrie

In Hoofdstuk 6 werd de afbreekbaarheid van belangrijke organische gifstoffen in het afvalwater van de houtverwerkingsindustrie onderzocht. Biologische omzetting of mineralisatie van giftige stoffen kan leiden tot een vermindering van het toxische effekt van dit afvalwater op het aquatisch milieu. De biologische omzetting van deze verbindingen zal ook een verminderde remming van de aktiviteit van de methanogene bacteriën tot gevolg hebben, waardoor de BZV-verwijdering tijdens de anaërobe zuivering zal worden vergemakkelijkt.

Onderzocht werden harsen uit hout en laag moleculaire lignine-derivaten. De biologische afbreekbaarheid werd getest door de produktie van methaangas te volgen, gebruik makend van test-verbindingen, die werden verdund tot sub-toxische niveau's. Hogere vetzuren, oleïnezuur en linolzuur, werden snel afgebroken. Guaiacol (methoxy-fenol), een lignine-derivaat, werd na een lag-periode van 40 dagen ook gemineraliseerd. Er waren echter geen aanwijzingen voor afbraak van eugenol (een apolaire fenol), ongesubstitueerde benzeen, harszuren, vluchtige terpenen noch de onverzadigde koolwaterstof squaleen. Deze resultaten geven aan, dat belangrijke stoffen in het afvalwater van de houtverwerkingsindustrie, anaëroob moeilijk afbreekbaar zijn. Het is daarom aan te bevelen anaërobe zuiveringssystemen te koppelen aan andere zuiveringsstappen om de aquatische toxiciteit van het afvalwater afdoende te verwijderen.

Continue Anaërobe Zuivering van Pulpafvalwater

Hoofdstuk 7 beschrijft het onderzoek naar de mogelijkheid het afvalwater van thermomechanische pulping (TMP) en loog pulping op laboratoriumschaal in een Upflow Anaerobic Sludge Blanket (UASB) reactor geënt met korrelslib te zuiveren.

Het bleek, dat TMP-afvalwaters uitstekend anaëroob gezuiverd konden worden. Continue anaërobe zuivering van TMP-afvalwater was mogelijk bij hoge volumebelastingen (31 g CZV/l·d), waarbij een totale CZV- en een biodegradeerbare CZV-verwijdering van resp. 68% en 98% werd verkregen. De influent-concentratie van 2.5 g CZV/l, die gedurende het gehele experiment werd gehandhaafd, is vergelijkbaar met de concentraties die in industriële TMP-effluenten kunnen worden verwacht.

Het afvalwater, dat bij pulpen met loog wordt verkregen is daarentegen relatief lastig anaëroob te zuiveren. Deze afvalwaters werken sterk remmend op de aktiviteit van methanogene bacteriën en ze bevatten een grote fraktie recalcitrant organisch materiaal. Deze afvalwaters zijn bovendien sterk geconcentreerd, met CZV-gehaltes die lopen van 75 tot 170 g/l.

Ondanks het giftige karakter van het afvalwater van loog pulping kon het biologisch afbreekbare substraat anaëroob worden gezuiverd, wanneer voorafgaand aan de biologische zuivering het afvalwater werd verdund tot sub-toxische concentraties of werd ontgift met het adsorbens Amberlite XAD-2. Onder deze omstandigheden konden zelfs hoge volumebelastingen worden toegepast, zonder betekenisvolle verslechtering van het zuiveringsresultaat. Het verwijderings-rendement van biodegradeerbare CZV was meer dan 95% bij volumebelastingen van 7 tot 14 g CZV/l.d met tot 2-4 g CZV/l verdund afvalwater, dat werd verkregen met de chemische verpulping met loog van hout. Hetzelfde resultaat werd verkregen bij een volumebelasting tot 40 g CZV/l met afvalwater van met behulp van loog verpulpte stro, dat werd verdund tot 10 g CZV/l.

Er werden lage CZV-verwijderingsrendementen (45 tot 50%) waargenomen bij de continue experimenten met afvalwater van loog pulping, als gevolg van het hoge gehalte aan lignines in deze afvalwaters. De vermindering van de absorptie van UV-licht (bij 280 nm) van 15 tot 20% wees op een gedeeltelijke verwijdering van enkele componenten van het lignine tijdens de anaërobe zuivering. Met gel-chromatografie werd bevestigd, dat de ligninefraktie die werd verwijderd, overeenkomt met de in het afvalwater aanwezige laagmoleculaire lignine-derivaten.

De aanwezigheid van lignine in het afvalwater van de houtverwerkingsindustrie is verantwoordelijk voor de sterke verkleuring van dit water. Als gevolg van het beperkte vermogen van zowel anaërobe als aërobe bacteriën het lignine macromolecuul af te breken, zullen deze biologische waterzuiveringssystemen weinig effektief zijn in het verwijderen van de waterkleuring. Om de door recalcitrante lignines veroorzaakte kleur te verwijderen, zullen biologische zuiveringssystemen gekoppeld moeten worden aan andere technologieën, zoals bijvoorbeeld een fysisch-chemische of enzymatische behandeling of een zuivering met behulp van schimmels.

CURRICULUM VITAE

The author of this dissertation, Reyes Sierra Alvarez, was born on December 18th, 1961 in León (Spain). She received her high school diploma in 1979 from the Universidad Laboral of Cáceres, Cáceres (Spain). In 1984, she obtained her bachelor of science degree from the Faculty of Sciences (Department of Chemistry) at the University of Valladolid, Valladolid (Spain). From the same University, she was granted her master of science degree from the Department of Chemical Engineering (Section of Environmental Technology) in 1985. The topic of the master thesis was the investigation on the stability of anaerobic reactors. Between December 1985 until 1989, the author has been a colleague of the Department of Environmental Technology at the Agricultural University in Wageningen (The Netherlands).