Biodegradation Studies of Recycled Vegetable Oils, Surface-Active Agents, and Condensing Wastewaters

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BIODEGRADATION STUDIES OF RECYCLED VEGETABLE OILS, SURFACE-ACTIVE AGENTS, AND CONDENSING WASTEWATERS

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Biodegradation is an aerobic or anaerobic degradation reaction where bacteria use organic materials as an energy source. In the aerobic biodegradation reaction, bacteria need oxygen as an electron acceptor, whereas an anaerobic reaction takes place in the absence of oxygen. Compounds degrade totally or partially, and produce simple inorganic species, such as CO$_2$, CH$_4$, NH$_3$, NO$_3^-$, and H$_2$O, as well as by-products that may be non-biodegradable and/or toxic.

In this thesis, the biodegradability of recycled vegetable oils, surface-active agents, and condensing waters from the process of wood drying were studied using the manometric respirometric BOD OxiTop method. The biodegradation of organic compounds was measured under the standard conditions (OECD 301F), and also in other matrices, such as different waters and soils. These are very different environments with respect to the biodegradation reaction in nature. The main differences in waters and soils are their organic and inorganic nutrient contents, bacteria strains, and temperatures.

The BOD OxiTop method is based on automatic pressure detection in a closed reactor vessel. Oxygen is consumed and carbon dioxide is formed in the aerobic reaction. The pressure decrease is detected after the carbon dioxide is adsorbed into a NaOH pellet or solution. The pressure change is dependent on oxygen consumption. The degree of biodegradation is calculated from the BOD value of the sample.

The studied recycled vegetable oils were found to be 60–83% biodegradable, and the added surface-active agent did not affect their biodegradation. Biodegradation of tall oil soaps was also examined in sand, topsoil, groundwater, and surface water, as well as under OECD 301F standard conditions. Tall oil soaps were proven to be 50–85% biodegradable. Concrete solvent agent (CSA) was also proven to be 78–83% biodegradable under standard conditions. Another detergent, cetyltrimethylammonium bromide (CTAB), was found to be toxic, whereas Triton X-100 biodegraded by only 6% in solution. Biodegradation of the soil matrix was found to be enhanced with added surface-active agents. This can be explained by better wetting of small pores with surface-active agents, as compared to the behavior of pure water. The biodegradation of the matrix occurred even with toxic surface-active agents. Organic pollutants of wastewaters from the process of wood drying were 25–61% biodegradable during a 28-day period, and were proven to be quite pure when considering the carbon content of the samples. Based on these results, the disposal into drainage of condensing waters from wood drying may be regarded as safe, which from an economical viewpoint is a very important conclusion.

**Keywords:** biodegradation, BOD, concrete washing agent, condense, manometric respirometric method, recycled vegetable oil, surface-active agent

Tiivistelmä

Biohajoavuus on luonnollinen aerobinen tai anaerobinen hajoamisprosessi, jossa bakteerit käyttävät orgaanista materiaalia energian lähteenä. Aerobisessa reaktiossa bakteerit tarvitsevat happea elektrien vastaanottajaksi, kun taas anaerobinen reaktio tapahtuu hapetettomissa olosuhteissa. Yhdisteet hajoavat joko täysin tai osittain sekä tuottavat yksinkertaisia epäorgaanisia yhdisteitä, kuten CO₂, CH₄, NH₃, NO₃ tai H₂O. Reaktiossa voi myös muodostua sivutootteita, jotka voivat olla biohajoamattomia ja/tai toksisia.


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Abbreviations and symbols

\( \alpha \)  Bunsen absorption coefficient
ATU  allylthiourea
BOD  biochemical oxygen demand
BTEX  benzene, toluene, ethylbenzene, xylenes [volatile, monocyclic aromatic compounds]
COD  chemical oxygen demand
CSA  concrete solvent agent
CTAB  cetyltrimethylammonium bromide
\( \Delta m \)  change in mass [g]
DOC  dissolved organic carbon
\( \Delta p \)  change in pressure [J L\(^{-1}\)]
LCA  life cycle assessment
M  molecular weight [g mol\(^{-1}\)]
OECD  Organisation for Economic Co-operation and Development
PAH  polycyclic aromatic hydrocarbons
R  gas constant [8.314 J mol\(^{-1}\)K\(^{-1}\)]
RBT  ready biodegradability test
SRT  sludge retention time
ThOD  theoretical oxygen demand
TOC  total organic carbon
Triton X-100  octylphenol ethylene oxide
\( V_{fr} \)  free gas volume of the vessel [L]
wt\%  weight percent [%]
WWTP  wastewater treatment plant
List of original articles

This thesis consists of the following articles, which are referred to by their Roman numerals:


The writer has been the main author of all articles I–IV. The design of analyses, the bulk of the experimental work, as well as the analysis of the results related to Articles I, II and IV were the work of the first author. In article III, the present writer has done a part of the designing of the analyses, the laboratory work, and the analysis of the results.
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1 Introduction

Biodegradation is one of the most important factors when considering the environmental friendliness of different compounds. It is nature’s own way to dispose of materials. Almost all natural compounds and a portion of the synthetic ones are biodegradable. It is nowadays a general goal to promote the manufacturing of biodegradable products. In biodegradation reactions, the compounds are broken down, either aerobically or anaerobically, by microorganisms.

The measurement of biodegradation is performed with the manometric respirometric method used in this study. Biodegradation measurements in different environments are important, considering biodegradation in nature. Measurements under standard conditions are tailored to the most affordable growth environments for bacteria and other micro-organisms that are present in wastewater, and which are used as inocula. Because of the different behavior of the organic materials in nature, measuring conditions outside of the laboratory are of considerable importance. The biodegradation reaction in nature is usually slower than it is under standard conditions, and therefore the rates of biodegradation in different circumstances need to be measured or evaluated. Evaluation of biodegradation can be done by determining the kinetics of the reaction. With BOD (biochemical oxygen demand) measurements, the degree of purification in different environments can be estimated.

In the European Union, the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulations came into force on 1st June 2007. These regulations were established to improve the protection of human health and the environment from the risks that can be posed by chemicals, while at the same time enhancing the competitiveness of the EU chemical industry. REACH also promotes alternative methods for the hazard assessment of compounds in order to reduce the number of tests on animals (http://echa.europa.eu/fi). Biodegradability is one of the testing methods for toxicity, but it is only a part of the procedure. New testing methods are investigated regularly with the goal of removing and replacing animals in toxicity testing. (Combes et al. 2003, Shukla et al. 2010).

Pollution and the exploitation of natural resources are two main effects caused by our consuming of materials in everyday life. This has caused a new way of thinking, called material efficiency, to emerge. Motivations for material efficiency include reducing energy demand, reducing emissions and other environmental impacts of industry, and increasing national resource security. The
argument of Allwood et al. (2011) is that, with a growing population and increasing wealth, the processing of chemicals, consumer goods, and material intake from nature are likely to double by 2050. Avoiding disposable materials and consumables is the top goal of the waste hierarchy that falls under the idea of material efficiency. The best way to deal with waste is, firstly, to prevent waste formation and, secondly, to minimize unavoidable waste formation (see Paper I).

By recycling, the virgin materials that were gathered previously from nature can be left untouched. The price of recycling is the most restrictive factor, and everyone should take responsibility for it. Recycling waste vegetable oils fits perfectly into material-efficiency thinking. Another important matter is to replace harmful products with more environmentally-friendly ones. Harmful compounds can be replaced by using or refining the by-products from industry, such as tall oil soap, which is an environmentally safe product.

In this thesis, the ecological choice and replacement of harmful compounds and manufacturing with biodegradable products are studied. In addition, the environmentally-friendly method for drying wood chips is also examined.
2 Biodegradation of organic compounds

2.1 Aerobic biodegradation

The micro-organisms that biodegrade organic compounds are either prokaryotes, including bacteria and archaeabacteria, or eukaryotes, such as various fungi, molds, algae, and protozoans. (Madigan et al. 2012). Micro-organisms need energy for macromolecular synthesis and for cell growth. This energy is taken from three different sources: light, inorganic compounds, and organic compounds. Micro-organisms produce those enzymes that are responsible for biodegrading compounds. Micro-organisms consume oxygen in a biodegradation reaction when they use organic compounds as the energy source. A BOD determination gives a measure of the amount of organic material in the water that can be oxidized by the micro-organisms present in the water. The consumed amount of oxygen is the BOD value of the sample. In aerobic biodegradation reactions, energy is released and carbon dioxide, water, and new biomass are produced. The nonbiodegradable by-products appear when the reaction does not reach the final stage. Gaseous nitrogen compounds (NOX), as well as nitrite and nitrate in water, are formed by nitrification, if the sample contains nitrogen in ammonium form. Aerobic biodegradation is represented in the simplified reaction equation (1). (Madigan et al. 2012).

\[ C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O (+ \text{energy} + \text{new biomass}) \] (1)

In the Organization for Economic Cooperation and Development (OECD) guidelines (1992), the oxygen-consuming elements are listed: carbon, hydrogen, chlorine, nitrogen, sodium, phosphorus, and sulfur. Any of these are taken into account if the sample contains them in significant amounts.

A compound is considered to be readily biodegradable if a biodegradation degree of 60% is achieved during a ten-day period, starting from 10% biodegradation. If the biodegradation degree rises to 60% after ten days, the compound is considered to be moderately biodegradable. (OECD guidance 1992). The rate of biodegradation and the percentage values of biodegradation degree are both used; the degree of the biodegradation may rise well over 80%, yet the compound may still be only moderately biodegradable.

For the manometric respirometric test instructions given in OECD 301F standard conditions are given for sample concentration, dilution water, time, temperature, and inoculation. These standard conditions are tailored to create an
environment that is optimal for the micro-organisms. Aerobic biodegradation measurements under standard conditions give information on the maximum degree and rate of biodegradation for the specific compounds, and the results are comparable with results from other laboratories. Furthermore, the standard conditions for the biodegradation of organic compounds can be measured also in other waters.

2.2 Anaerobic biodegradation

With the depletion of oxygen, anaerobic bacteria start to grow. These are archaeabacteria. Anaerobic biodegradation is a valuable source of methane, but when it is not controlled methane can drift into the atmosphere. Methane (CH₄) is an important greenhouse gas that, by mass, has 25 times the global warming potential of carbon dioxide (CO₂) (Yvon-Durocher et al. 2014). On the other hand, it is a “pure” fuel when collected. Anaerobic biodegradation is an important and globally growing area of biological waste treatment. In anaerobic digestion, bacteria take energy from the organic material and produce methane and carbon dioxide. Anaerobic biodegradation is used widely to produce biogas from animal manure or other biomasses, such as municipal solid waste (sludge), various types of fruit and vegetable solid wastes, leaves, grasses, woods, weeds, and marine and freshwater biomass. (Gunaseelan, 1997). Biogas contains about 50–70% methane (Aftab et al. 2014), biogas formed from anaerobic biodegradation consists of about 46 liters per 1 kg of substrate (Ilyin, 2004). The final composition depends upon the substrate.

The anaerobic biodegradation process consists of at least six independent, parallel, and sequential reactions, conveyed by different micro-organisms. These reactions include i) anaerobic hydrolysis, where hydrolysable, complex, particulate organics, such as insoluble cellulose and hemicellulose, are converted into monomers, such as amino acids, sugars, and long-chain fatty acids; ii) fermentation, where amino acids and sugars are converted to volatile fatty acids; iii) acetogenesis, where long-chained fatty acids are converted to acetate and hydrogen; iv) anaerobic oxidation, where intermediate products, such as volatile fatty acids, are converted to acetate and hydrogen; v) aceticlastic methanogenesis, where acetate is converted to methane by acid-utilizing methanogens; and vi) hydrogenotrophic methanogenesis, where hydrogen is used to form methane by hydrogen-utilizing methanogens. (Gujer & Zehnder 1983, Myint et al. 2007).
In an aerobic respiration reaction, oxygen is the electron acceptor. However, micro-organisms have the ability to use a variety of alternative electron acceptors for anaerobic respiration. Nitrate and sulfate are well-known acceptors in the absence of oxygen. Other inorganic electron acceptors are oxidized metal ions, such as iron and manganese, and oxy-anions, such as selenate, arsenate, and uranate. In methanogenic environments, bicarbonate and protons act as terminal electron acceptors. (Alfons 2006). Sulfate reduction is a part of the anaerobic reactions. In biological sulfate reduction, bacteria convert sulfate to sulfide, which in turn precipitates as sparingly-soluble metal sulfides. (Battersby 2000).

### 2.3 Inoculation

Inoculation is needed to ensure the optimal conditions for biodegradation reactions during incubation. Usually, the wastewater used to inoculate samples is from a wastewater treatment plant (WWTP).

Sometimes the bacteria strain that is responsible for a certain biodegradation reaction cannot be isolated from wastewater. Many isolated bacterial strains in a laboratory may be good for biodegrading, but do not have an important role under natural conditions (Wackett 2004), and it is not surprising that many yet unknown species take part in a biodegradation reaction. (Curtis et al. 2002). It has been estimated that less than 1% of the natural bacterial population can be cultured in laboratory conditions. (Torsvik et al. 2002, Torsvik & Ovreas 2002).

When the inoculum is used after being stored even two months at 4 °C, there are no changes to the biodegradability values of different samples, according to the author’s empirical studies during the last 12 years. This reveals that the most important bacteria in the biodegradation reaction survive during long periods of storage.

Bacteria can be used to control the living conditions of other bacteria in many ways, for example, through competition for food, excretion of toxic compounds, parasitism, and the struggle for living space (Schuler et al. 1992). This is one of the reasons why the biological reaction differs from the chemical reaction. It is difficult to know which bacteria are best in biodegrading if the used inoculum is broad-spectrum. The interaction between different bacteria is very diverse. Major interactions between two bacteria species are competition, neutralism, mutualism, commensalism, amensalism, and prey-predator interaction. The symbiosis is a situation where two organisms live together. This interaction may be mutualistic,
neutralistic, parasitic, or commensalistic, to mention. (Shuler & Kargi 1992, Madigan et al. 2012).

In biodegradation studies, the bacteria in a vessel with constant stirring are found to move to the vessel’s edges. Solid surfaces indirectly affect the bacteria function in both negative and positive directions. (Van Loosdrecht et al. 1990). Many investigations have been made concerning the net charge of bacteria influencing their adhesion onto solid interfaces (Dickson & Koochmar 1989, Castellanos et al. 2006). The initial step in colonization—the adhesion of bacteria to the artificial solid surface—is governed mainly by long-range van der Waals and electrostatic interactions between the solid surface and the bacterial cell. While van der Waals forces are generally attractive, the usually negative charges of bacteria and solid surfaces lead to electrostatic repulsion. (Jucker et al. 1996). The majority of the bacteria are hydrophilic, indicating the importance of cell-surface hydrophobicity for bacterial adhesion in sludge, and for the overall success of the wastewater treatment process (Zita & Hermansson 1997). Usually the bacteria interface is charged, and this may have an effect on the biodegradation of surface-active agents, which can be either cationic or anionic.

After exposing the bacteria to new compounds at the beginning of the biodegradation measurement, an adaptation time is required for the bacteria to grow in the absence of a compound that they are able to biodegrade, and thereafter the ability to biodegrade is restored. This can be seen in the biodegradation graph as a lag phase at the start of the measurement. According to the author’s empirical observations, the time needed for adaptation ranges from a few hours to 6 days. Adaptive enzymes have been defined: they are produced only when required, and their formation depends upon the adaptation of the organism to a specific compound. There are also constitutive enzymes that are formed by the cell and are independent of the composition of the medium in which the cell is grown. (Gale 1943, Madigan et al. 2012). Rolfe et al. (2012) stated that, although the cells are not dividing during the lag phase, they are increasing in size.

2.4 Effect of temperature

Bacteria and archaea can be divided into three categories, depending on the optimum growth-range temperature: psychrophiles, growing mainly in the temperature range of 0–15 °C mesophiles, at 30–35 °C, and thermophiles, at temperatures over 55 °C (Madigan et al. 2012). The mesophilic temperature range is used mostly for example in composting, but the thermophilic range enables
faster biodegradation and at the same time provides a higher level of hygienization (Droste 1997). Hyperthermofiles can thrive up to 122 °C (Madigan et al. 2012).

Manzano et al. (1999) studied the effect of temperature on the degree of biodegradation of a surface-active agent (nonylphenol polyethoxylate), and found biodegradation degree to be highly dependent on temperature. In the study of Manzano et al. (1999), the lowest and highest BOD values (30% and 70%) were observed at 7 °C and 25 °C, respectively. It is known that when bacteria are frozen and then thawed, their survival depends on many parameters, such as cooling and warming rates, holding temperature, and duration after freezing. The freeze-thaw cycle can be used for disinfection of wastewater, but it is not as effective a technique as pasteurization. (Gao et al. 2006).

Nadarajah et al. (2007) observed that a change in temperature from 30–45 °C caused a shift in both the bacterial community structure and function. However, even cooling to 35 °C can be a challenge for some of the industries whose effluents are produced at significantly higher temperatures. In the pulp and paper industry, cooling towers are used to decrease the temperature of fresh effluent from above 60 °C to 30–35°C, before entry into mesophilic secondary WWTPs. However, due to recycling of process water and a high surrounding temperature in summer months, the temperature in the treatment systems may frequently rise above 50 °C, leading to bulking, solids separation difficulties, and increased turbidity. (Carpenter et al. 1968, Lee et al. 1978, Morgan-Sagastume & Allen 2003, 2005).

If it is the intention to examine biodegradation in natural waters, measuring the temperature should be considered. The temperature in surface waters in Finland is at 20 °C or above for not more than a few months per year. Groundwater temperature throughout the year is about 6–8 °C. (Järvinen et al. 1994). The mean temperature of the ground is 2–3 °C in northern Finland, and 6–8 °C in southern Finland.

2.5 Theoretical oxygen consumption

The equation used to calculate the theoretical oxygen consumption is presented in Paper I, in which is presented one model from the OECD 301F standard for calculating the theoretical value of oxygen consumption. In the OECD standard are two models: one method suggests nitrogen in the sample to be an ammonium ion, while the second method assumes that nitrogen in the sample results in a
nitrate. Both nitrogen compounds can be present in the sample solution. It is impossible to determine, based on the biodegradation measurements only, whether the nitrogen is in ammonium form or in nitrate form at the end of the incubation time, so the consumed amount of oxygen for nitrification is impossible to evaluate. Nitrification is a slow process and it is prevented in BOD measurements by adding allylthiourea into solution. Many times the carbon, hydrogen, and oxygen compositions are difficult to measure, especially for liquids, and so the calculation has to be performed, for example, based on the carbon content only. This usually gives too optimistic an evaluation for the degree of biodegradation, and therefore with readily and totally biodegradable products the calculated degree of biodegradation may rise up to 120%.

2.6 Biodegradation of organic compounds in solid phase

In a solid phase there are two types of measurements in use; either the solid sample alone or a sample in different solid matrices. Possible crucial factors to consider, besides the water content, may be physical conditions, nutrition, the ratios of various structural hydrocarbons that are present, the bioavailability of the substrate and the diversity of the bacterial communities involved, pH, water-holding capacity of the soil, and micro-organisms (Sihag *et al.* 2014).

Composting is a widely used application where aerobic biodegradation serves to degrade organic wastes. The biodegradation of a compound is defined by the biological, physical, and chemical characteristics of the soil environment (Davis & Madsen, 1996). The characteristics of soil used as a matrix are very different. Sand (carbon content in this study was under 1%) may be practically out of carbon compared to topsoil which may contain over 30% of organic carbon. Pores in the ground are of different sizes and the nutrient content varies a lot between different soils. Furthermore, water-retention capacity is dependent on the soil structure. The study of Margesin *et al.* (2006) states that low-moisture content is a more limiting factor for composting than low temperature. Pommier *et al.* (2007) observed that the degraded substrate content increases with increasing initial water content. Obviously, the water environment is a better place than solid phase for bacteria to function.
2.7 Biodegradation of organic compounds in groundwater

Biodegradation measurements in groundwater can be performed in both aerobic and anaerobic conditions. The result from anoxic conditions is closer to the real conditions in nature, since the groundwater is practically oxygen-free. If groundwater is contaminated, the purification may take a very long time because the biodegradation in these conditions is slow. Ramos et al. (2014) have studied that removal of BTEX (“benzene, toluene, ethylbenzene, xylenes;” which are monocyclic aromatic compounds) and PAH (polycyclic aromatic hydrocarbons) compounds begins only 0.7 year after the release. The slow biodegradation in groundwater leads to a situation where the natural purification is unusable, and tiny amounts of contaminants are more adverse than in, for example, river water.

2.8 Biodegradation of organic compounds in wastewater treatment

Biodegradation in WWTPs is utilized in biological purification of wastewaters. Purification of wastewaters is nowadays increasingly required. It is calculated that 2.2 million people die every year from dirty water, according to the World Health Organization (WHO 2015). Furthermore, 25% of the world population is affected by the lack of pure water (González et al. 2007). The actual value may be even greater because of domestic wastewater contaminations. For a long time, BOD measurements have been the basic method in monitoring the purification level in wastewater treatment plants. The efficiency of purification is dependent on many factors: biological and physical features such as temperature and pH; qualitative factors as well as different levels of substance, such as nutrients, inhibitors, and toxic compounds; and the retention time in a wastewater treatment plant (Clara et al. 2005). The purifying result is also dependent on the hydraulic load and the amount of organic matter. These parameters can vary greatly, making the controlling more difficult compared to watching a steady load. (Jinkeun 2012).

In wastewater purification, the activated sludge system is a widely used method that is based on aerobic biodegradation. Basically, there are three steps in purification: i) the removal of solids, ii) the biological treatment, and iii) the removal of suspended solids and the rest of the soluble organic material that is left after the second step. Wastewaters should be conducted via sewers whenever possible because water containing high levels of biodegradable compounds flowing directly into, for example, a lake, might cause depletion of oxygen, and
those life forms needing oxygen suffer. In rivers, the situation is better because running water dissolves and releases gases more efficiently than stagnant water.

2.9 Nitrification and denitrification

The purification of nitrogen compounds in WWTPs can be done either by the removal of nitrogen from aerobic or anaerobic systems, or from a combination of the two. In an aerobic system, nitrogen is oxidized, while in an anaerobic system, nitrogen is reduced. The removal of nitrogen in a nitrification/denitrification process means that the ammonium ion (NH$_4^+$) oxidizes to an intermediate product NH$_2$OH, which then oxidizes to nitrite NO$_2^-$, and at the last stage to nitrate NO$_3^-$. The anaerobic process, which changes nitrate to molecular nitrogen, takes place as progressive degradation from nitrate to nitrite, and then to nitrogen oxide or directly to nitrogen gas via intermediate N$_2$O to N$_2$. (Hwang et al. 2006). In biological purification the nitrification consumes oxygen. Because this is a complicated process, the nitrification should be prevented during the BOD measurements. The time needed for beginning nitrification is long; longer than other reactions in wastewater purification. The level of nitrification cannot be directly known, and it is impossible to find this out without measuring the nitrogen levels.

2.10 Kinetics of aerobic biodegradation

The kinetics of the biodegradation reactions in this work were studied by comparison to zeroth-, first-, and second-order reactions, of which the pseudo first-order kinetics was observed to be the most suitable model. The zeroth-order was found to be unsuitable for describing the kinetics of the reaction. The second-order reaction rate was reduced to pseudo first-order reaction kinetics, because the followed parameter was oxygen consumption, and not the reacted carbon and hydrogen. The surrounding amount of oxygen could be set constant. The followed parameter was the pressure change, and the equipment calculates the consumed oxygen based on this information. The measuring system is discussed in Chapter 4.

For the pseudo first-order reaction (oxygen concentration [O$_2$] constant), the rate law can be written as follows:

$$ v = -k_1[s] = k_2[C][O_2], $$

(2)
where \([s]\) represents the concentration of a sample. Because there is an excess of oxygen in the reaction vessel, it can be approximated that

\[
[O_2] \approx [O_2]_{t=0}
\]  

(3)

Therefore, the reaction-rate law simplifies to equation (4), where \(k_{obs}\) is the observed first-order reaction-rate constant (Atkins 1995),

\[
v = k_{obs}[C],
\]

(4)

where

\[
k_{obs} = k_2[O_2]_{t=0}
\]

(5)

Thus, the reaction follows pseudo first-order reaction kinetics. Since the OxiTop measuring system can observe very small changes in pressure - in this case, in oxygen concentration - the changes in carbon and hydrogen concentrations are equal to changes in oxygen concentration (See equation (6)) (Atkins 1995).

\[
C + O_2 \rightarrow CO_2, \quad 2H + \frac{1}{2}O_2 \rightarrow H_2O
\]

(6)

Oxygen is consumed in the reaction along with carbon and hydrogen, and it has been taken into account in the calculations using the initial concentrations of carbon and hydrogen. The term \([O_2]\) represents the oxygen consumed during the biodegradation reaction. The progress of the biodegradation reaction can be followed by determining the changes in pressure. (Atkins 1995). The general, empirical, integrated form of the first-order rate law is

\[
\ln([O_2]_t/[O_2]_0) = -k_{obs}t
\]

(7)

and its rearranged form is presented in equation (8), where \(\ln[O_2]_0\) is constant.

\[
\ln[O_2]_t = -k_{obs}t + \ln[O_2]_0
\]

(8)

Thus, the observed reaction rate constant can be calculated, according to equation (9), by plotting \(\ln[O_2(\text{initial}) - O_2(\text{measured})] vs. \(t\),

\[
\ln[O_2(\text{initial}) - O_2(\text{measured})]_t = -k_{obs}t + \ln[O_2]_0,
\]

(9)

where \(O_2(\text{initial}) = \) the maximum value of oxygen consumption [mol L\(^{-1}\)], \(O_2(\text{measured}) = \) the oxygen consumed during the reaction [mol L\(^{-1}\)], \(t = \) the time of reaction [d].

The half-life \((t_{1/2})\) of the first-order and pseudo-first-order reactions can be calculated as follows:

\[
t_{1/2} = \ln2/k_{obs} = 0.693/k_{obs}.
\]

(10)
It should be noted that, in first-order reaction kinetics, the half-life is not dependent on the initial concentration of the parent compound (Bobrovnik 2000). In this work, equations (9) and (10) were used to calculate numerical values for the parameters $k$ and $t_{1/2}$ (Atkins 1995).
3 Aims of this work

The main goal of this work was to measure degrees of biodegradation for recycled vegetable oil, tall oil soaps, concrete washing agent, and wastewaters from wood-chip drying. The biodegradations were measured according to the OECD 301F method. In addition, this work addressed many other questions concerning biodegradation of different samples.

Vegetable oils are usually biodegradable, but the samples in this study were recycled products manufactured from waste vegetable oils, and the biodegradation of these products had not been studied previously (Karhu et al. 2012). The change in the degree of biodegradation with the addition of Triton X-100 (a slightly biodegradable, non-toxic, surface-active agent) was also studied, in order to find out if the surface-active agent enhances the biodegradation of the vegetable oil-based product, as compared to the untreated sample. Also, the inhibition effect on the biodegradation of recycled oils was also considered from measurements under OECD 301F conditions. (Paper I)

The examined tall oil soaps are by-products of kraft pulping and these are surface-active compounds. The purpose of this examination, beside the biodegradation degree under OECD 301F conditions, was to clarify the rates of the biodegradation reactions under different circumstances, and to determine whether kinetic calculations are a practical tool for predicting the half-lives of the biodegradation reactions, especially in the long-term. For this purpose, biodegradation degrees were studied under the following circumstances: OECD 301F, two groundwaters at 10 °C and 20 °C, anaerobic conditions, two tap waters, and two river waters. The kinetics were calculated based on these results. (Paper II)

Concrete washing agent contains only 1% of the surface-active agent. Other surface-active agents that were studied as reference compounds were cetyltrimethylammonium bromide (CTAB) and Triton X-100. They were all measured under OECD 301F standard conditions, in river water, in groundwater, and in tap water. The biodegradation in different environments was studied in order to find out the possible harmful effects on nature, given that slow biodegradation may affect living organisms and accumulation in sediments. The biodegradation of surface-active agents were also measured in nutrient-rich gardening soil. A main question of this research was whether surface-active agents enhanced the biodegradation of the soil itself. A further question was if the
moisture content that affects biodegradation in the ground was either anaerobic or aerobic. (Paper III)

The biodegradation of organic pollutants in condensing water from wood drying was investigated. This investigation also contained a part where the effects of pH and inoculation on the biodegradation rate and final level of biodegradation were examined. Condensates from wood drying were studied in order to find out the effectiveness of the drying system from an ecological viewpoint, and if the condensates can be fed to the sewer. (Paper IV)

The effect of freezing and temperature on the biodegradation were also investigated, given that the temperatures in both ground and natural waters are often much lower than 20 °C, which is used in standard conditions. The effects of the amount of inocula and nutrients were also studied, because natural waters contain fewer of these than are used in standard conditions.

Sometimes the biodegradation reaction starts almost immediately, but there are measurements indicating that it may take a couple of days to start (Spain & Van Veld 1983). This is called an adaptation time. The lag phase (delayed biodegradation reaction) at the beginning of the measurement was investigated with descending series of inocula to find out if too low a concentration of bacteria extends this time.
4 Materials and methods

4.1 Biodegradation measurements

Biodegradation measurements in this thesis were performed with a manometric respirometric BOD OxiTop method. The BOD OxiTop control equipment measures the pressure change while keeping the temperature (here 20.0 ± 0.2 °C) constant during the incubation. The equipment provides 360 data points during the measurement, and the time between the points is proportional to the measuring time. The experimental setup has different concentration areas, starting from 0–40 mg L\(^{-1}\) and extending to 0–4000 mg L\(^{-1}\), while the filling volume ranges from 432 mL to 22.7 mL. The filling volume was adjusted so that the amount of oxygen in the airspace was sufficient for the total biochemical oxidation of the sample.

Dilution water was prepared according to the OECD 301F standard. The final content of the dilution water is presented in Table 1. Inocula were taken from Oulu’s WWTP. Wastewater was filtered with coarse filter paper so that the used solution contained no solid material. Solid material causes inhomogeneity to the samples, and the blank sample cannot be deduced properly.

Table 1. Nutrient content in dilution water under OECD 301F standard conditions.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH(_2)PO(_4)</td>
<td>0.625 mmol L(^{-1})</td>
</tr>
<tr>
<td>K(_2)HPO(_4)</td>
<td>1.249 mmol L(^{-1})</td>
</tr>
<tr>
<td>Na(_2)HPO(_4) (\cdot) 7 H(_2)O</td>
<td>1.246 mmol L(^{-1})</td>
</tr>
<tr>
<td>NH(_4)Cl</td>
<td>0.318 mmol L(^{-1})</td>
</tr>
<tr>
<td>MgSO(_4) (\cdot) 7 H(_2)O</td>
<td>0.092 mmol L(^{-1})</td>
</tr>
<tr>
<td>CaCl(_2)</td>
<td>0.248 mmol L(^{-1})</td>
</tr>
<tr>
<td>FeCl(_3) (\cdot) 6 H(_2)O</td>
<td>0.0009 mmol L(^{-1})</td>
</tr>
<tr>
<td>Inoculum</td>
<td>2 mL L(^{-1})</td>
</tr>
</tbody>
</table>

In the BOD OxiTop method for liquid measurements (Fig. 1), oxygen is consumed from the airspace of the bottle and the released carbon dioxide is absorbed into NaOH pellets. The formed water stays in the liquid phase or condenses on the walls of the bottle, because the moisture content in a sealed vessel is balanced during the incubation. From the consumed amount of oxygen, the pressure decrease was calculated by the equipment, using the modified ideal
gas equation. In solution measurements, the instrument calculates the BOD value in the desired unit [mg L⁻¹] using equation (11),

\[
BOD = \frac{M(O_2)}{RT_m} \cdot \left\{ \frac{(V_{tot} - V_l)}{V_l} + \alpha \frac{T_m}{T_0} \right\} \Delta p(O_2), \tag{11}
\]

where \( M(O_2) \) is the molecular weight of oxygen [32.00 g mol⁻¹],
\( R \) is the gas constant [83.144 L hPa mol⁻¹K⁻¹],
\( T_m \) is the measurement temperature [293.15 K],
\( T_0 \) is 273.15 K,
\( V_{tot} \) is the bottle volume [mL],
\( V_l \) is the liquid phase volume [mL],
\( \alpha \) is a Bunsen absorption coefficient [0.03103, given by the manufacturer],
\( \Delta p(O_2) \) is the difference in partial oxygen pressure [hPa].

The Bunsen absorption coefficient, \( \alpha \), is defined as the volume of the gas at STP (273 K and 1 atm) dissolved by a unit volume of the solvent at a given temperature under a partial pressure of 1 atm of the gas. At 293 K, the values of \( \alpha \) for oxygen and carbon dioxide are 0.0028 and 0.088, respectively. (Negi 2004). It is calculated using equation (12),

\[
\alpha = \frac{V_0}{Vp}, \tag{12}
\]

where \( V_0 \) is the volume of the dissolved gas [L],
\( V \) = volume of the solvent [L],
\( p \) = partial pressure of the gas in atmosphere [atm].
Fig. 1. The BOD OxiTop measuring system for liquid and solid samples.

The calculations of biodegradation degree from solid-phase measurements are as follows:

\[ \Delta m = \Delta p \cdot V_{fr} \cdot \text{MO}_2 / (R \cdot T), \]

where the \( \Delta m \) is the amount of consumed oxygen [g],
\( \Delta p \) is change in the pressure [J L\(^{-1}\)],
\( V_{fr} \) is the free gas volume of the vessel [L],
\( \text{MO}_2 \) is the molecular weight of oxygen [32.00 g mol\(^{-1}\)],
\( R \) is the gas constant [8.3144 J mol\(^{-1}\)K\(^{-1}\)],
\( T \) is the measuring temperature [293.15 K].

\( \Delta m \) is calculated for both vessels containing the sample and the blank. The blank value is then deduced from the result of the sample. For calculation of the free gas
volume, the density of the sample has to be known. The biochemical oxygen demand (BOD [g g\(^{-1}\)]) of the sample is calculated using equation (14):

\[
\text{BOD (g g}^{-1}) = \frac{\Delta m}{m_{\text{sample}}}, \quad (14)
\]

\[
\text{ThOD (g g}^{-1}) = \frac{m_C \cdot (M_{O_2}/M_C)}{m_{\text{sample}}}, \quad (15)
\]

where \(m_C\) is the mass of carbon,
\(M_C\) is the molar mass of carbon,
\(M_{O_2}\) is the molar mass of the oxygen.

The degree of biodegradation is calculated in the same manner for both liquid- and solid-phase measurements:

\[
\text{Degree of biodegradation [%]} = \left(\frac{\text{BOD}}{\text{ThOD}}\right) \cdot 100\%, \quad (16)
\]

where the BOD is from equation (14) and the ThOD is from equation (15) for solid-phase measurements. (Vähäoja 2006). The sample amount should be adjusted before measurement so that the pressure stays under the detection limit of \(-200\) hPa.

In Table 2, the measurements performed with different samples are presented.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Measurement environment</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recycled vegetable oils</td>
<td>OECD 301F, addition of surfactant, inhibition, and emulsification</td>
<td>I</td>
</tr>
<tr>
<td>Tall oil soaps</td>
<td>OECD 301F, inhibition, two groundwaters at 10 °C and 20 °C, anaerobic conditions, two tap waters, and two river waters. Topsoil, forest soil, and sand</td>
<td>II</td>
</tr>
<tr>
<td>CSA</td>
<td>Two reference samples, OECD 301F, groundwater, two tap water, and two river water. Topsoil, forest soil, and sand</td>
<td>III</td>
</tr>
<tr>
<td>Wastewater</td>
<td>Raw, pH adjusted, and inoculated</td>
<td>IV</td>
</tr>
<tr>
<td>Adaptation time</td>
<td>Changing amount of inocula</td>
<td></td>
</tr>
<tr>
<td>Temperature effect</td>
<td>Three measuring temperatures, two freezing times</td>
<td></td>
</tr>
<tr>
<td>Amount of nutrients</td>
<td>Dilution series of nutrients</td>
<td></td>
</tr>
</tbody>
</table>
4.2 Recycled vegetable oils (paper I)

4.2.1 Materials

The samples investigated were recycled waste vegetable oils from deep-fat frying. These oils, in turn, have been manufactured from waste vegetable oils collected from deep-fat frying. These oils are an excellent raw material for recycling, because the necessary polymerization that takes place during heating has already occurred. Recycling is also recommended, because it diminishes the carbon footprint compared to products manufactured from mineral oil, and produces no waste. Combusting is not the best option for dealing with these oil wastes, as it releases three times the pollutants from biodiesel. Most recycled vegetable oils that were studied here are made from recycled palm oil and mixtures of different oils. The total amount of waste cooking oil collected for recycling in the EU exceeded about 0.5 million tons in one year (1999) (Rice 1999). Supple et al. (2002) states that based on estimates from seven countries, a total of about 0.4 Mt is collected within the EU, mainly from the catering industry, while the amount that could be collected is estimated to be considerably higher, possibly from 0.7 to 1 Mt.

Hydraulic systems operate under high pressures, and leaks or failures from hoses, seals or cylinders can result in large amounts of hydraulic oil being released into the environment (Battersby 2000). Chain oils are used in chain saws for forestry; mold oil is used in the casting of concrete; universal oil is used in many situations. This is why it is very important that polluting spills need to be from biodegradable oils.

The increasing demand for vegetable oils can be seen from an increase in the volume and number of shipments. As a consequence of this, accidental spills of vegetable oils have increased. They do not contain the acutely toxic compounds that are present in crude oil and refined petroleum products, but they can have a harmful effect on sensitive aquatic organisms and ecosystems. (McKelvey et al. 1980, Mudge 1995). Biodegradation is nature’s own way to deal ecologically with biodegradable wastes, and it is true that minor oil spills can be left to nature’s care. However, with larger oil spills, such as from crashes of oil tankers, this is impossible. Vegetable oils are no better than crude oil in these circumstances, as their effect on nature is similar at such high levels. (Crump-Wiesner & Jennings 1975).
Fats and oils are heated at high temperatures during baking, grilling, and pan frying. However, deep-fat frying (about 190 °C) is the most common method of high-temperature treatment, and it is a popular food-preparation method, as it produces a desirable fried-food flavor, golden brown color, and crisp texture, while oxidized products of fatty acids give off flavors and odors to the frying medium and fried foods. (Warner 1999, Lin et al. 2001). The reactions in deep-fat frying depend on factors such as replacement with fresh oil, frying conditions, the original quality of the frying oil, food materials, the type of fryer, and the type and concentrations of antioxidants and oxygen (Sanchez-Muniz et al. 1992). Other factors, such as frying temperature, quantity of frying, and initial content of the oil also affect the oxidative stability and overall quality of the oil during the frying process (Melton et al. 1994). Physicochemical changes that occur during the frying process and the compounds formed in deteriorated frying oil have been extensively studied by previous researchers (Andrikopoulos et al. 2002). In general, deep-fat frying increases foaming, color, viscosity, density, the amount of polymeric and polar compounds, and the free fatty-acid content of frying oils (Man 2010). During frying, different compounds are released from the fried food, such as animal fats, and other compounds. These compounds have to be removed before refining the oils.

4.2.2 Experimental

Oil samples were weighed and placed directly in the reaction vessel and dilution water was added. Dilution water content was presented in Table 1. The sample concentration should be 100 mg L\(^{-1}\) according to OECD 301F conditions, but due to the difficulties in weighing sample concentrations varied from 99.0 to 724.2 mg L\(^{-1}\). Measurement range was selected as 0–400 mg L\(^{-1}\). For inhibition tests, Triton X-100 was diluted with distilled water and both the sample and surface-active agent were added to the reaction vessel. The biodegradation examination for recycled vegetable oils was performed also with the surface-active agent, so that the effect of dissolution on the biodegradation could be examined. Inoculation was needed in measurements for OECD 301F conditions; the added amount was 2 mL L\(^{-1}\). Allylthiourea was added at 20 drops to the liter. Measuring time was 28 days and incubation temperature was 20 °C. Biodegradability of oil-in-water emulsion was measured too.
4.3 Surface-active agents (papers II and III)

4.3.1 Materials

The global production of surface-active agents in 2000 was $15 \times 10^6$ t, from which the amount of soaps were $8 \times 10^6$ t. Of this, alkylbenzenesulfonates were quantitatively the most important class ($2 \times 10^6$ t a$^{-1}$) (Kosswig 2000). Detergents are formulations designed to have cleaning/solubilization properties. These formulations consist of surface-active agents together with subsidiary components, including builders, boosters, fillers, and auxiliary compounds. (Scott & Jones 2000).

Surface-active agents can be divided into two main classes: non-ionic and ionic. Within the class of ionic surface-active agents, there are three different types: anionic, cationic, and amphoteric. Anionics includes sulphonates, sulphates, and fluorinated molecules. Soaps are also included among these compounds. Cationic surface-active agents consist usually of molecules with nitrogen-containing functional groups, such as quaternary ammonium or derivatives of imidazole or pyridine. Amphoteric compounds contain a group of hydrophilic compounds that possess cationic and anionic natures. The agents in the non-ionic class, as the name suggests, have non-ionic hydrophilic elements, such as nonylphenols, alkylphenolethoxylates, and alcohol ethoxylates. (Olkowska et al. 2010).

Soap is one of the oldest detergents known to man, and it is still widely used. Soaps biodegrade in both aerobic and anaerobic environments, and are a fairly safe alternative to the other commercial detergents. Soaps are precipitated in hard-water environments with metal ions, and this influences the biodegradation rates. Sodium soap salts have mineralization rates of 80–90%, whereas calcium soap salts have significantly less, with only 67% mineralization. (Scott & Jones 2000). The tall oil soaps included in this thesis (paper II) are a complex mixture of resin acids, fatty acids, and unsaponifiables. The composition of crude tall oils, manufactured from tall oil soap, is presented in Table 3. Tall oil soap is a by-product of the kraft-pulping industry, where the majority of production is used to manufacture tall oil, while only a part is sold in soap form. Tall oil soap is gathered from the top of a black liquor. The alkaline pulping process converts the resin acids and fatty acids to their sodium salts. (Norlin 2012, Foran 2006). According to the information provided by manufacturers, soap contents of the samples were 15–30%, 20%, and 5–15% for samples 1, 2, and 3, respectively.
Concrete solvent agents (CSAs) are used to wash concrete molds, which can be manufactured from different materials. The agent has to be acidic for it to be used to dissolve concrete. In this study, CSA contains 23% glycolic acid (pH ≈ 2); 1% surface-active, non-ionic tenside; and a pH elevating, readily biodegradable compound. The pH of the product is about 3. Even this low amount of surface-active agent affects the properties of the solution by dehomogenizing it. This also distorts the results of the total organic carbon (TOC) measurement.

CTAB (N,N,N-Trimethyl-1-hexadecanaminium bromide) is a cationic detergent that is 1 M soluble in water at 20 °C. It is only slightly soluble in acetone and readily soluble in alcohol. It is commonly used in the preparation and purification of genomic DNA from bacteria, including DNA mini preps for sequencing. CTAB forms complexes with both polysaccharide and residual protein and its critical micelle concentration is 1 mM. (Chem spider http://www.chemspider.com/Chemical-Structure.5754.html).

Triton X-100 is a non-ionic detergent, and it has no antimicrobial properties that could affect the biodegradation reaction. The pH of a 5% aqueous solution ranges from 6.0 to 8.0. Critical micelle concentration (CMC) is from 0.22 to 0.24 mM. Triton X-100 is soluble in all proportions at 25°C in water, benzene, toluene, xylene, trichloroethylene, ethylene glycol, ethyl ether, ethanol, isopropanol, and ethylene dichloride. (Sigma Product Information Sheet, 10.9.2015).

Most surface-active agents are at least partially biodegradable. However, as a rule they have high molecular weights and complex structures, and thus biodegradation involves many reactions. (Jimenez et al. 1991, van Ginkel 1996). The formed products may be less biodegradable than the parent compounds (Griffiths et al. 1986, van Ginkel 1996, Bokern & Harms 1997, Bennie 1999, Itrich & Federle 2004). Primary biodegradation can be defined after the molecule has lost its surface-active properties. Ultimate biodegradation occurs when a surface-active agent molecule has been mineralized to CO₂, CH₄, water, mineral salts, and biomass. (Scott & Jones 2000).

### Table 3. Composition of crude tall oil.

<table>
<thead>
<tr>
<th></th>
<th>South eastern USA</th>
<th>Northern USA and Canada</th>
<th>Scandinavia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid number ¹</td>
<td>160–175</td>
<td>125–135</td>
<td>120–140</td>
</tr>
<tr>
<td>Resin acids %</td>
<td>35–45</td>
<td>25–35</td>
<td>20–30</td>
</tr>
<tr>
<td>Fatty acids %</td>
<td>45–55</td>
<td>50–60</td>
<td>50–60</td>
</tr>
<tr>
<td>Unsaps. %</td>
<td>7–10</td>
<td>12–18</td>
<td>18–24</td>
</tr>
</tbody>
</table>

¹Acid number is an equivalent of NaOH required to neutralize the tall oil. (Foran, 2006).
4.3.2 Experimental

Tall oil soaps were weighed in the reaction vessel, because the solution made from the sample, due to the surface-active nature, was inhomogeneous. All measurements were performed at measuring area 0–400 mg L⁻¹. The OECD 301F test was performed as earlier (Chapter 4.2.2.) presented. Test in natural waters were performed by weighing the sample in water. No inoculation or nutrients were needed. CSA, CTAB, and Triton X-100 were measured using the same procedure.

The BOD OxiTop measurement in the solid phase is based on the same principle as used in liquid measurements. Samples were placed in the reaction vessel amongst the soil matrix. NaOH was as a solution in a separate flask. The equipment gave pressure values, from which the BOD was calculated. For some of the measurements, an extra amount of water was added in order to examine whether the larger moisture content affects the biodegradation reaction.

4.4 Condensing waters from wood drying (paper IV)

4.4.1 Materials

Before industrialization, wood was the main energy source. It was later replaced globally, mainly by fossil fuels. Wood is considered carbon neutral, although CO₂ emitting during combustion is gained back during photosynthesis. If we will replace fossil fuels with wood to produce energy, we would reduce the net amount of CO₂ emissions to the atmosphere by over 90%. (Jong et al. 2003, Hagedorn et al. 2003). In 2008, the EU commission put forward a proposal for a new directive on renewable forms of energy. Each of the member states should increase its share of renewable energies from the total current value of 8.5% to a value of 20% by 2020. In Finland, the national target is to increase from the current level of 28.5% to 38%. Wood drying is a necessary step before combustion, because it increases the calorific heat value and prevents self-ignition in the wet pile. (Ince 2013, Kuokkanen 2013). If wood can be more efficiently dried, more is saved in energy costs. Drying is the most energy demanding part of the mechanical forest industry, representing 70–85% of the energy used in the entire industry (Lydersen 1985).

The main factors affecting wood-chip drying are the wood species, size, and temperature, as well as the air and mass flows. Depending on the surrounding
conditions, the moisture content of the wood is also affected by the felling season, location, and storage duration. (Thuvander et al. 2002). The boiler design is also an important factor. In Finland, due to its northern location, the most common tree species include one type of pine (*Pinus sylvestris*), one type of spruce (*Picea abies*), one type of aspen (*Populus tremula*), and two types of birch (*Betula pendula* and *Betula pubescens*). There are numerous other tree species, but they are quite rare or cultivated, and they are not used commonly by industry for combustion purposes, so they are not included here. The moisture content range, in Finland, of freshly-felled small-sized Scots pine and Norwegian spruce is 50–60%, and 40–50% for birch (Laurila et al. 2014). The wood has to be dried to about 10–15 wt% (wet weight), depending on the purpose of use (Fagernäs et al. 2010, Kuokkanen 2013, McKendry 2002). An important feature of drying is that it should be slow, because the dry surface in wood controls the drying rate until the mean moisture content in the bulk decreases to the fiber saturation level. The water from wood evaporates from the cell cavity first and then from the cell walls. This point, when cavity has been emptied, is called the fiber saturation point (FSP), of which the FSP is about 28 to 30 wt% of dry matter and 23% of wet matter. (Bousquet 2000, Laurila et al. 2014).

Condensing water often includes varying amounts of volatile organic compounds (VOCs) that are released during drying (Samuelsson et al. 2006). A part of the burnable material may be lost if the wood is dried too much or at too high a temperature. Since the surface area/volume ratio of wood chips and wood dust changes with particle size, it follows that surface dry-out should increase with decreasing particle size. (Banerjee 1998a). Terpenes are released from wood, both in kiln drying and forced drying (Banerjee 2001). The proportion of the most common extractives (terpenes) in wood ranges from 2 to 5% (Rathke & Stratev 2013); pine contains more extractives than spruce (Bengtsson 2004).

Softwood drying releases VOC’s in two stages. There is an initial surge from surficial VOCs, followed by a second burst when the surface water dries out, and the furnish temperature rises. (Banerjee et al. 1995). VOCs from softwood are mostly α- and β-pinenes. The reactions may occur in the gas phase and not all of the compounds are necessarily from wood. (Milota 2000). It follows, however, that the amount of VOCs released per unit of water lost is greater at higher temperatures, especially beyond about 30% weight loss. This corresponds to the onset of the falling rate period, where the transport of water to the surface becomes rate-limiting in drying (Banerjee et al. 1998b). Hazardous air pollutants (HAPs) are also released in wood drying, and these are included among VOCs.
4.4.2 Experimental

The samples for BOD measurements were placed in the vessels without dilution. Inocula were added to some samples, and their pH levels were adjusted. The effect of added inocula was studied to determine whether the wastewater already contains sufficient amounts of bacteria and other micro-organisms. These waters were slightly acidic, and the effect of pH on biodegradation was analyzed.

4.5 Other related topics

4.5.1 Adaptation time

Adaptation time was studied, using a series of different concentrations of inocula. The amounts of inocula were 20, 10, 5, 2, 1.5, 1, 0.5, 0.1, and 0.05 mL L\(^{-1}\). The mineral solution was the dilution water made according to the OECD 301F standard. The sample was the same glucose solution added in the same amount to each bottle, and the final concentration of the glucose was 205.4 mg L\(^{-1}\).

4.5.2 Temperature effect

The effect of temperature on biodegradation was studied at 10 °C, 20 °C, and 35 °C. BOD value was measured before and after freezing the samples for 2 weeks and after 1 month. The sample was fresh wastewater taken from the municipal WWTP.

4.5.3 Amount of nutrients

Glucose samples were weighed directly in the vessel and different amounts of nutrients were added in a descending series. The 100% addition was the same amount used in the OECD 301F standard conditions. The descending dilution series was 100%, 50%, 25%, 10%, 5%, 2.5%, 1%, and 0.5%.

4.6 Other measurements beside the biodegradation

Other measurements are presented in original papers: i) (paper I) elemental analysis, density, surface tension, viscosity, water content, heat value, acid number, and metals: Cd, Co, Cr, Cu, Ni, Pb, V, Zn, Fe, As, Ba, Mg, Sn, and Sb; ii)
(Paper II) chemical oxygen demand (COD), elemental analysis, solid content, viscosity, surface tension, density, pH, and dissolved organic carbon (DOC); iii) (paper III) total organic carbon (TOC); iv) (paper IV) pH, electrical conductivity, COD, DOC, thermogravimetric (TG) analysis, moisture content, size distribution, heat values, elemental analysis, ash content, and metals: Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, and Zn.
5 Results and discussion

All examined products, recycled vegetable oils, tall oil soaps, and concrete solvent agents, proved to be either moderately or readily biodegradable and organic pollutants in wastewater were moderately biodegradable.

5.1 Recycled vegetable oils

The recycled vegetable oil samples were mold oil, which comprised three runs taken from different batches; chain oil with three runs; one run with hydraulic oil; and one run with universal oil. The samples’ carbon and hydrogen contents are presented in Table 4 in which the degrees of biodegradation of the samples under OECD 301F conditions are also presented. The mold oils biodegraded best, with a value of 83%.

![Biodegradation graphs of mold (upper) and chain oils (lower) samples.](image)

Mold oils 1 and 2 were readily biodegradable. Mold oil 3, hydraulic oil, universal oil, and chain oils 1, 2, and 3 were moderately biodegradable (see Fig. 2 and Table 4). Some of the oils continued the biodegradation reaction after the 28-day measuring time, which indicates that the actual biodegradation degree is much higher than the values reached during the 28 days of incubation time in this trial.
The biodegradation of an oil emulsion was measured too, and when the emulsion sample was prepared with emulsifier, there was no change in the oil’s biodegradability, which was about 73% for both pure oil (universal oil) and the oil emulsion manufactured from universal oil. Small differences in the biodegradation degrees depended on the batch from which a sample was taken. This is due to the differences between starting materials. As a conclusion, recycling of waste vegetable oils is an excellent choice, from an ecological viewpoint, when compared to combustion.

Table 4. The carbon and hydrogen contents of oil samples and degrees of biodegradation calculated with carbon and hydrogen contents (28 days).

<table>
<thead>
<tr>
<th>Sample</th>
<th>C [%]</th>
<th>H [%]</th>
<th>Degree of biodegradation [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mold oil 1</td>
<td>77.5</td>
<td>12.6</td>
<td>82</td>
</tr>
<tr>
<td>Mold oil 2</td>
<td>76.2</td>
<td>10.3</td>
<td>83</td>
</tr>
<tr>
<td>Mold oil 3</td>
<td>77.0</td>
<td>11.5</td>
<td>75</td>
</tr>
<tr>
<td>Chain oil 1</td>
<td>77.1</td>
<td>10.9</td>
<td>69</td>
</tr>
<tr>
<td>Chain oil 2</td>
<td>76.9</td>
<td>10.1</td>
<td>60</td>
</tr>
<tr>
<td>Chain oil 3</td>
<td>75.8</td>
<td>13.2</td>
<td>62</td>
</tr>
<tr>
<td>Universal oil</td>
<td>76.7</td>
<td>10.9</td>
<td>73</td>
</tr>
<tr>
<td>Hydraulic oil*</td>
<td>76.5</td>
<td>11.0</td>
<td>69</td>
</tr>
<tr>
<td>Oil emulsion</td>
<td>23.0</td>
<td>3.3</td>
<td>73</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>62.2</td>
<td>10.1</td>
<td>6</td>
</tr>
</tbody>
</table>

*Carbon and hydrogen contents have been estimated from earlier measurements.

The shapes of the biodegradation-degree graphs were similar in all measurements under OECD 301F conditions, including an adaptation time at the beginning; only the final biodegradation degree varied.

In addition, the densities and viscosities of mold oils are lower than the corresponding values of chain oils. For example, the densities [g cm⁻³] for two samples were as follows: mold oil 1, 0.9045; chain oil 1, 0.9192; and the viscosities [mPa·s] were as follows: mold oil 1, 56.3; chain oil 1, 91.0. Other measured physico-chemical properties, such as surface tension, water content, heat values, and acid numbers are quite same in the tested samples of mold oil and chain oil. (Paper I)

The biodegradation measurement was successful, even without the added surface-active agent, Triton X-100. The inhibition of a universal oil sample was examined by investigating its behavior with different sample amounts. The results for the inhibition of the universal oil sample are presented in Table 5 and in Fig 3.
The sample concentration in the first measurement concerning the inhibition effect of recycled oil was 290 mg L$^{-1}$, although the requirement in OECD 301F is 100 mg L$^{-1}$. This increase in concentration does not lower the resulting degree of biodegradation. As can be seen in Table 5, the added surface-active agent does not raise the result of the biodegradation degree for an oil sample. There are variations in the results at the same concentration level, but these values are still quite similar, as can be seen in Fig. 4. There is a clear inhibition effect when the concentration rises over 600 mg L$^{-1}$, and the effect on the results is large when the concentration reaches 4000 mg L$^{-1}$. In concentrations over 10,000 mg L$^{-1}$, the biodegradation reaction almost stops completely due to inhibition. Therefore, it may be concluded that the decreased biodegradation degree when the sample concentration becomes larger is due to inhibition (see Fig. 5). From the results, it also may be concluded that these oils do not biodegrade during storage when there is no water present. In the literature, the enhanced biodegradation degrees of crude oil after the addition of a surface-active agent have been reported (Zahed et al. 2010, Kaczorek 2012).

![Fig. 3. The inhibition of a recycled vegetable oil sample.](image-url)
Table 5. Degree of biodegradation of recycled vegetable oil sample, with and without added surface-active agent.

<table>
<thead>
<tr>
<th>Sample concentration [mg L⁻¹]</th>
<th>Triton X-100 concentration [mg L⁻¹]</th>
<th>Degree of biodegradation [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>290</td>
<td>0</td>
<td>82</td>
</tr>
<tr>
<td>290</td>
<td>230</td>
<td>91</td>
</tr>
<tr>
<td>620</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>660</td>
<td>230</td>
<td>40</td>
</tr>
<tr>
<td>3700</td>
<td>0</td>
<td>9.2</td>
</tr>
<tr>
<td>3800</td>
<td>5000</td>
<td>7.1</td>
</tr>
<tr>
<td>12,000</td>
<td>0</td>
<td>2.8</td>
</tr>
<tr>
<td>13,000</td>
<td>5000</td>
<td>1.5</td>
</tr>
</tbody>
</table>

5.2 Surface-active agents

Biodegradation of tall oil soaps were studied in seven different environments. First, there were four water phases, and then three soils. Used matrix were river water and water at OECD 301F standard conditions. In addition, the biodegradations in groundwater, under both aerobic and anaerobic conditions, were examined. Finally, the temperature effect on the biodegradation in groundwater was examined.

The biodegradation degrees for concrete washing solvent agent (CSA), Triton X-100, and CTAB were studied in four different waters: OECD 301F, groundwater, surface water, and tap water, and also in three different soils: two topsoils and sand.

5.2.1 Biodegradation results of tall oil soaps in different water environments

Biodegradation degree in OECD 301F standard conditions was the largest during 28 days, and these values varied between 50–84%. The results are presented in Table 6. Results were not always repeatable, and sample 3 biodegraded best, with a value of about 84%. Sample 1 biodegraded by 50–54%, and sample 2 by 57–63%. A large effect on biodegradation degree by surface-active agents originates from the charges of both the surface-active agent and the bacteria. Some manufacturers provided information on the biodegradability of tall oil soaps, and their values differed in ways similar to those observed in this study. The inhibition effect on biodegradation was clear, as the sample concentration was almost 15 times greater than that used in standard conditions (2.3 g L⁻¹ and 0.16 g L⁻¹). The
biodegradation of samples 1 and 2 decreased by 24–36%, and sample 3 has the best biodegradation. When comparing the additives in the samples, sample 1 contained the most additives. These compounds were preservatives, which probably caused the diminishing in the degree of biodegradation. No information on additive content was found for sample 2, while sample 3 contained nothing more than soap (manufacturer’s information). Biodegradation degree of the sample 3 in OECD 301F conditions was the largest.

Measurements in groundwater were performed in two different groundwaters. Groundwater 1: first at concentrations around 0.5, and 0.3, g L⁻¹, and second at levels a tenth as great (0.046–0.033 g L⁻¹). Two different temperatures, 10 °C and 20 °C, were used for the groundwater 1. In the 99-day measurement for groundwater 1 with low concentration, the degree of biodegradation was 60% for all of the samples. The biodegradation was lower in cold water (10°C) than at 20 °C. During the 28-day measurement period, biodegradation values rose from 1%, 4%, and 3% to 26%, 34%, and 8%, when temperatures increased from 10 °C to 20 °C, respectively. This indicates that temperature is a large factor when considering the biodegradation in groundwater. Groundwater 1 contained more iron and manganese than groundwater 2, and this had a clear effect on the biodegradation degree. Values rose from 2%, 0.5%, and 3% (groundwater 2) to 26%, 34%, and 8% (groundwater 1), respectively. In anaerobic conditions, no biodegradation reaction was detected. This indicates that tall oil soaps do not biodegrade in cold and anaerobic conditions at all, or that the rate of biodegradation is very slow and large degrees of biodegradation may take several years, as may be seen from the calculated half-lives presented in Table 6 in aerobic conditions. Based on these results, the infiltration of these compounds into groundwater should be prevented.

The river waters tested were taken in autumn and in summer in order to find out whether the season has an effect on the biodegradation degree. As a result, the biodegradation was more effective in autumn than in summer, being 15%, 15%, and 14% in summer, and 32%, 34% and 32% in autumn, respectively. This can be explained, at least from these measurements, by the river water containing more nutrients in autumn than in summer. There are mainly two reasons: i) seasonal fluctuations in the lakes, or ii) nutrients washing out from, for example, swamps or fields during autumn rains. In nature, lower temperatures exist than in OECD measurements, which inhibit the biodegradation reaction during most of the year. This temperature effect on biodegradation should be considered when estimations of biodegradation rates in different environments are made. According to
Kolehmainen et al. (2010), the consumption of oxygen in lake water is higher in summer than in autumn.

With lower concentrations in the 99-day experiment, the biodegradation degree was as high as under OECD 301F standard conditions. In both river water and in groundwater, the enhanced biodegradation indicated that the tall oil soaps had an inhibitive effect on biodegradation. However, an explanation of this mechanism is not known. The graphs of biodegradation degrees for the tall oil soap samples in water at OECD 301F conditions, two river waters, and groundwater is presented in Fig. 4. Measurements in solid phase were inconclusive and are not presented here.

The rates of the biodegradation reactions were studied in liquid measurements. The values of the half-lives, measured concentrations, and biodegradation degrees are presented in Table 6. The relationship between \( k \) values and half-lives differs for different reaction orders. Therefore, the half-lives were calculated from the first-order reaction kinetics using equation (10): \( t_{1/2} = \frac{\ln 2}{k} = 0.693/k \), as presented previously in section 2.10.

### Table 6. Biodegradation degrees, measured concentrations, and half-lives of tall oil soaps in different water environments.

<table>
<thead>
<tr>
<th></th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>OECD standard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>degree of biodegradation [%]</td>
<td>24</td>
<td>31</td>
<td>36</td>
</tr>
<tr>
<td>concentration [g L⁻¹]</td>
<td>2.3</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>half-life up to [d]</td>
<td>103</td>
<td>67</td>
<td>50</td>
</tr>
<tr>
<td>OECD standard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>degree of biodegradation [%]</td>
<td>54</td>
<td>57</td>
<td>85</td>
</tr>
<tr>
<td>concentration [g L⁻¹]</td>
<td>0.54</td>
<td>0.68</td>
<td>0.35</td>
</tr>
<tr>
<td>half-life [d]</td>
<td>5 - 16</td>
<td>4 - 9</td>
<td>3 - 8</td>
</tr>
<tr>
<td>OECD standard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>degree of biodegradation [%]</td>
<td>50</td>
<td>63</td>
<td>83</td>
</tr>
<tr>
<td>concentration [g L⁻¹]</td>
<td>0.16</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>half-life [d]</td>
<td>5 - 20</td>
<td>4 - 9</td>
<td>3 - 4</td>
</tr>
<tr>
<td>Groundwater (1) 10°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>degree of biodegradation [%]</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>concentration [g L⁻¹]</td>
<td>0.49</td>
<td>0.46</td>
<td>0.49</td>
</tr>
<tr>
<td>half-life up to [d]</td>
<td>693</td>
<td>301</td>
<td>433</td>
</tr>
<tr>
<td>Groundwater (1) 20°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>degree of biodegradation [%]</td>
<td>26</td>
<td>34</td>
<td>8 (3 days)</td>
</tr>
<tr>
<td>concentration [g L⁻¹]</td>
<td>0.30</td>
<td>0.27</td>
<td>0.27</td>
</tr>
<tr>
<td>Sample</td>
<td>Sample 2</td>
<td>Sample 3</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td>half-life up to [d]</td>
<td>78</td>
<td>54</td>
<td>50</td>
</tr>
<tr>
<td><strong>Groundwater (1) 99 days</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>degree of biodegradation [%]</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>concentration [g L(^{-1})]</td>
<td>0.046</td>
<td>0.033</td>
<td>0.034</td>
</tr>
<tr>
<td>half-life up to [d]</td>
<td>42</td>
<td>60</td>
<td>32</td>
</tr>
<tr>
<td><strong>Groundwater (2) 20 °C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>degree of biodegradation [%]</td>
<td>2</td>
<td>0.5</td>
<td>3</td>
</tr>
<tr>
<td>concentration [g L(^{-1})]</td>
<td>0.67</td>
<td>0.67</td>
<td>0.63</td>
</tr>
<tr>
<td>half-life up to [d]</td>
<td>693</td>
<td>990</td>
<td>533</td>
</tr>
<tr>
<td><strong>Surface water (autumn)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>degree of biodegradation [%]</td>
<td>32</td>
<td>34</td>
<td>32</td>
</tr>
<tr>
<td>concentration [g L(^{-1})]</td>
<td>0.24</td>
<td>0.16</td>
<td>0.15</td>
</tr>
<tr>
<td>half-life up to [d]</td>
<td>55</td>
<td>54</td>
<td>50</td>
</tr>
<tr>
<td><strong>Surface water (summer)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>degree of biodegradation [%]</td>
<td>15</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>concentration [g L(^{-1})]</td>
<td>0.41</td>
<td>0.42</td>
<td>0.40</td>
</tr>
<tr>
<td>half-life up to [d]</td>
<td>121</td>
<td>126</td>
<td>122</td>
</tr>
<tr>
<td><strong>Surface water (autumn) 99 days</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>degree of biodegradation [%]</td>
<td>80</td>
<td>63</td>
<td>60</td>
</tr>
<tr>
<td>concentration [g L(^{-1})]</td>
<td>0.030</td>
<td>0.027</td>
<td>0.029</td>
</tr>
<tr>
<td>half-life up to [d]</td>
<td>14</td>
<td>18</td>
<td>14</td>
</tr>
</tbody>
</table>
5.2.2 Biodegradation results of CSA, Triton X-100, and CTAB in different water environments

Biodegradation degree of CSA under OECD 301F standard conditions is about 80%, and the sample was shown to be readily biodegradable according to the OECD protocol. In river water gathered in autumn, the biodegradation degree was larger than in summer due to the larger content of nutrients and humic acids in autumn. Furthermore, the biodegradation degree was higher in Kiiminkijoki river water than in Oulujoki river water for the same reason. The biodegradation degree in groundwater varied between 24–100%, depending on the concentration of the sample. The fluctuation originates from the surface-active nature of the sample. In tap water from Oulu, the biodegradation degree of CSA was better when compared to tap water from Haukipudas, because it was purified river water and in Haukipudas the corresponding water was groundwater. This is probably because the groundwater contains fewer nutrients than the river water. In Fig. 5, there are presented biodegradation graphs of CSA for water at OECD 301F.
conditions, surface water, and groundwater. The studied toxic effect of CTAB is due to the molecule itself and not the bromide ion, as was tested by comparison with NaBr. CTAB is not biodegradable under any circumstances, and it inhibits the biodegradation of glucose. Triton X-100 is not toxic, and the biodegradation degree is 6% under OECD 301F conditions. In Fig. 6, the biodegradation graphs of CTAB and Triton X-100 in water at OECD 301F conditions are presented. Values of biodegradation degrees for CSA, Triton X-100, and CTAB in different water environments are presented in Table 7. The TOC values, and the amounts of inorganic carbon of different dilution waters, are given in Table 8.

![Graph of biodegradation degrees](image)

**Fig. 5.** Biodegradation degrees of CSA in water at OECD 301F conditions, surface water, and groundwater. The identification of the curves is presented in the same order as in the figure.
Fig. 6. Biodegradation degrees of CTAB and Triton X-100 under the OECD 301F standard. The identification of the curves is presented in the same order as in the figure.

Table 7. Values of biodegradation degrees for CSA, Triton X-100, and CTAB in different water environments.

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample</th>
<th>C [g L(^{-1})]</th>
<th>Water phase</th>
<th>Time [d]</th>
<th>Calculated biodegradation degree [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>CSA</td>
<td>0.37</td>
<td>OECD 301F</td>
<td>28</td>
<td>83</td>
</tr>
<tr>
<td>conditions</td>
<td>CSA</td>
<td>1.12</td>
<td>OECD 301F</td>
<td>28</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>CSA</td>
<td>1.28</td>
<td>OECD 301F</td>
<td>28</td>
<td>78</td>
</tr>
<tr>
<td>Standard</td>
<td>CTAB</td>
<td>0.12</td>
<td>OECD 301F</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>Conditions</td>
<td>CTAB</td>
<td>0.12</td>
<td>OECD 301F</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>CTAB</td>
<td>0.12</td>
<td>OECD 301F</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>CTAB</td>
<td>0.12</td>
<td>OECD 301F</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>CTAB</td>
<td>0.12</td>
<td>Glucose + OECD 301F</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>CTAB</td>
<td>0.12</td>
<td>Glucose + OECD 301F</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>Inhibition</td>
<td>NaBr</td>
<td>0.02</td>
<td>Glucose + OECD 301F</td>
<td>28</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>NaBr</td>
<td>0.02</td>
<td>Glucose + OECD 301F</td>
<td>28</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>CTAB</td>
<td>0.10</td>
<td>OECD 301F</td>
<td>28</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>CTAB</td>
<td>0.10</td>
<td>OECD 301F</td>
<td>28</td>
<td>7</td>
</tr>
<tr>
<td>Inoculated with soil</td>
<td>CTAB</td>
<td>0.10</td>
<td>OECD 301F</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>CTAB</td>
<td>0.10</td>
<td>OECD 301F</td>
<td>28</td>
<td>2</td>
</tr>
</tbody>
</table>
Inocula addition Triton 0.11 OECD 301F 28 -3
Triton 0.11 OECD 301F 28 4
Inoculated with soil Triton 0.11 OECD 301F 28 23
Triton 0.11 OECD 301F 28 0
Inocula addition CSA 0.07 Surface water 60 37
CSA 0.81 Surface water 60 9
Oulujoki river CSA 0.67 Surface water 60 76
Kiiminkijoki river CSA 0.89 Surface water 60 57
CSA 1.04 Surface water 60 67
Groundwater CSA 0.06 Groundwater 60 84
CSA 0.06 Groundwater 60 100
CSA 0.31 Groundwater 60 72
CSA 0.97 Groundwater 60 24
CSA 1.03 Groundwater 60 42
Tap water from Oulu CSA 0.06 Tap water 60 68
Tap water from Haukipudas CSA 0.07 Tap water 60 24
Haukipudas CSA 0.07 Tap water 60 46

As can be seen from Table 8, river waters contain more organic carbon than groundwaters and tap waters. Most of the carbon is humic acid.

**Table 8. The TOC values, and the amount of inorganic carbon of different dilution waters.**

<table>
<thead>
<tr>
<th>Dilution water</th>
<th>TOC [ppm]</th>
<th>IC [ppm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haukipudas tap water</td>
<td>6.2</td>
<td>8.85</td>
</tr>
<tr>
<td>Oulu tap water</td>
<td>4.3</td>
<td>9.36</td>
</tr>
<tr>
<td>River Kiiminkijoki</td>
<td>36.1</td>
<td>1.55</td>
</tr>
<tr>
<td>River Oulujoki</td>
<td>11.3</td>
<td>2.24</td>
</tr>
<tr>
<td>Haukipudas groundwater</td>
<td>6.3</td>
<td>15.6</td>
</tr>
</tbody>
</table>

**5.2.3 Biodegradation results of CSA, Triton X-100, and CTAB in solid phase (paper III)**

Three soil matrices used in solid-phase measurements consisted of two nutrient-rich topsoils that could be bought commercially. For topsoil 1, the mean value of the carbon content was 33.6%, hydrogen 3%, nitrogen 0.4%, sulfur <2%, and oxygen 23%. Topsoil 2 contained less carbon than topsoil 1, with a mean value of 24.3%. The carbon content of topsoil 1 fluctuated between 28.9%–43.8%,
indicating that the soil material is quite heterogeneous. In sand, the carbon content was less than 1%.

In the solid environment, the matrix used consisted of nutrient-rich topsoil and sand (Roppola 2009a, Vähäoja 2006). The biodegradation of tall oil soaps in a solid environment were inconclusive. An odd behavior was observed, and the idea was that the surface-active agent enhances the biodegradation of organic carbon-rich topsoil. When the surface tension is lowered, the wetting of the soil is higher than without the surface-active agent, and the organic material is available to bacteria for the biodegradation reaction. This explains the situations when the calculated biodegradation degree rose to values of many thousands percent. In addition to this, there are three other possible reasons for the measurement results in soil when the pressure values fluctuated between negative and positive values. Possibilities for this kind of behavior may be the releasing of trapped gases after mixing the soil; anaerobic biodegradation that occurs with oxygen depletion, and which is mainly due to higher water contents; or nitrogen-containing reactions. Furthermore, different fungi may distort the results, as they are numerous in the soil. In all measurements the zero-sample is not possible to deduce, because in the zero-sample not all phenomena appear (See Fig. 7). In sand, the biodegradation of tall oil soaps was not observed.

Even though CTAB is toxic to micro-organisms and it does not inhibit soil biodegradation, the values of the biodegradation degree were still lower than with CSA. The solubility of CTAB did not affect the results.
As can be seen in Fig. 7, the values of the biodegradation degree were for some measurements reasonable, and yet very high after calculation. The biodegradation degree rose dramatically when the calculation was based on the theoretical value of the sample only. When based on the theoretical value of the soil also, degrees of biodegradation decreased to almost zero. It is impossible to say which biodegradation values apply to the matrices, and which ones are the samples’ results. At first, the lower values in Fig. 7 were considered to be the values of the sample, but after closer examination, it became clear that the situation was not so easy to interpret. Topsoil 1 was stored at room temperature for many years before these tests were made, and all fast-composting reactions had happened during this time, but slow biodegradation reactions may have continued to occur.
Table 9. Biodegradation degrees for CSA, Triton X-100, and CTAB in different solid environments.

<table>
<thead>
<tr>
<th>Sample</th>
<th>C [g kg⁻¹]</th>
<th>Moisture content [%]</th>
<th>Solid phase</th>
<th>Time [d]</th>
<th>Calculated biodegradation degree [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSA</td>
<td>3.36</td>
<td>70</td>
<td>Topsoil 1</td>
<td>60</td>
<td>590</td>
</tr>
<tr>
<td>CSA</td>
<td>3.36</td>
<td>70</td>
<td>Topsoil 1</td>
<td>30</td>
<td>-30</td>
</tr>
<tr>
<td>CSA</td>
<td>1.68</td>
<td>70</td>
<td>Topsoil 1</td>
<td>30</td>
<td>-80</td>
</tr>
<tr>
<td>CSA</td>
<td>1.68</td>
<td>70</td>
<td>Topsoil 1</td>
<td>60</td>
<td>1100</td>
</tr>
<tr>
<td>CSA¹</td>
<td>1.05</td>
<td>55</td>
<td>Topsoil 1</td>
<td>30</td>
<td>500</td>
</tr>
<tr>
<td>CSA¹</td>
<td>1.30</td>
<td>55</td>
<td>Topsoil 1</td>
<td>30</td>
<td>-110</td>
</tr>
<tr>
<td>CSA¹</td>
<td>1.10</td>
<td>55</td>
<td>Topsoil 1</td>
<td>30</td>
<td>530</td>
</tr>
<tr>
<td>CSA</td>
<td>0.12</td>
<td>64</td>
<td>Topsoil 1</td>
<td>60</td>
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</tr>
<tr>
<td>CSA</td>
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<td>64</td>
<td>Topsoil 1</td>
<td>60</td>
<td>-220</td>
</tr>
<tr>
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<td>64</td>
<td>Topsoil 1</td>
<td>60</td>
<td>-1800</td>
</tr>
<tr>
<td>CSA</td>
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<td>54</td>
<td>Topsoil 2</td>
<td>55</td>
<td>-4700</td>
</tr>
<tr>
<td>CSA</td>
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<td>54</td>
<td>Topsoil 2</td>
<td>55</td>
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</tr>
<tr>
<td>CSA</td>
<td>1.68</td>
<td>70</td>
<td>Topsoil 1</td>
<td>60</td>
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</tr>
<tr>
<td>CSA</td>
<td>3.34</td>
<td>70</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>Sand</td>
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<td>-84</td>
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<td>CSA</td>
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<td>20</td>
<td>Sand</td>
<td>30</td>
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</tr>
<tr>
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<td>20</td>
<td>Sand</td>
<td>30</td>
<td>-132</td>
</tr>
<tr>
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<td>0.03</td>
<td>20</td>
<td>Sand</td>
<td>60</td>
<td>430</td>
</tr>
<tr>
<td>CSA</td>
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<td>20</td>
<td>Sand</td>
<td>60</td>
<td>-430</td>
</tr>
<tr>
<td>CSA</td>
<td>0.79</td>
<td>21</td>
<td>Sand + topsoil 1</td>
<td>60</td>
<td>-89</td>
</tr>
<tr>
<td>CSA</td>
<td>0.79</td>
<td>21</td>
<td>Sand + topsoil 1</td>
<td>60</td>
<td>-39</td>
</tr>
<tr>
<td>CSA</td>
<td>0.03</td>
<td>21</td>
<td>Sand + topsoil 1</td>
<td>60</td>
<td>-143</td>
</tr>
<tr>
<td>CSA</td>
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<td>Sand + topsoil 1</td>
<td>60</td>
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</tr>
<tr>
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<td>64</td>
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<td>60</td>
<td>230</td>
</tr>
<tr>
<td>CTAB</td>
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<td>64</td>
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<td>190</td>
</tr>
<tr>
<td>CTAB</td>
<td>0.11</td>
<td>64</td>
<td>Topsoil 1</td>
<td>60</td>
<td>-250</td>
</tr>
<tr>
<td>Triton</td>
<td>0.11</td>
<td>64</td>
<td>Topsoil 1</td>
<td>60</td>
<td>-50</td>
</tr>
<tr>
<td>Triton</td>
<td>0.11</td>
<td>64</td>
<td>Topsoil 1</td>
<td>60</td>
<td>-100</td>
</tr>
<tr>
<td>Triton</td>
<td>0.11</td>
<td>64</td>
<td>Topsoil 1</td>
<td>60</td>
<td>220</td>
</tr>
<tr>
<td>Triton</td>
<td>0.19</td>
<td>60</td>
<td>Topsoil 1</td>
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<td>-370</td>
</tr>
<tr>
<td>Triton</td>
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<td>60</td>
<td>Topsoil 1</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td>CTAB</td>
<td>0.11</td>
<td>54</td>
<td>Topsoil 2</td>
<td>55</td>
<td>-277</td>
</tr>
<tr>
<td>CTAB</td>
<td>0.11</td>
<td>54</td>
<td>Topsoil 2</td>
<td>55</td>
<td>10</td>
</tr>
<tr>
<td>Triton</td>
<td>0.11</td>
<td>54</td>
<td>Topsoil 2</td>
<td>55</td>
<td>-515</td>
</tr>
<tr>
<td>Triton</td>
<td>0.11</td>
<td>54</td>
<td>Topsoil 2</td>
<td>55</td>
<td>-15</td>
</tr>
</tbody>
</table>
5.3 Condensing waters

In this research, condensed water samples were gathered from the drying of four different species of wood. Two samples were condensates from birch dried at 80 °C, two condensate samples came from a mixture of 80% aspen and 20% pine dried at 115 °C and 130 °C, three samples were condensates from spruce samples dried at 60–66 °C, and finally condensates from pine samples dried at 70 °C. VOCs from the dryer to the atmosphere were not measured. Wood dust samples consisted of spruce, pine and birch; wood chips were made up of spruce or pine. The drying rate for the wood chips was studied with oven drying at 105 °C. Thermogravimetric (TG) analysis was performed on the wood dust samples, which had a small amount of water remaining. Recovery of condensed water was about 30% in wintertime. Biodegradation graphs are presented in Figures 8 and 9.

Fig. 8. Biodegradation degrees of condensates from hard wood samples. The identification of the curves is presented in the same order as in the figure.
Fig. 9. Biodegradation degrees of condensates from soft wood samples. The identification of the curves is presented in the same order as in the figure.

TG analysis of the wood dusts was used in this study to determine the optimal combustion temperature, the temperature of evaporation of water, and the products from combustion. TG analysis can be used for many different situations. In a previous study by Yorulmaz & Atimtay (2009), thermogravimetric analysis was used to study the kinetics of combustion mechanisms, thermal kinetics, and phases of combustion for waste wood samples and one fresh wood. Bakirtzis et al. (2014) used TGA to examine the flammability of nanocomposites, Strzemiecka et al. (2014) studied the surface chemistry of carbon black, and Gheno et al. (2015) used it to determine the degradation kinetics of two polyester thermosetting powder coatings.

Organic pollutants in condensates have been measured to be moderately biodegradable (25–60%). Wood contains bacteria that have adapted to biodegrade the extractives in wood, and these bacteria seem to be responsible for the biodegradation of organic compounds in wastewater. This can be noted in results where the biodegradation degree is similar or lower than in the results without added inocula, indicating that the inoculation was not needed. The pH was not an issue, and settling most likely lowered the degree of biodegradation values. This, too, is due to an untreated environment where the circumstances are already favorable. Waters proved to be very pure, thus the drying method was very
efficient and ecological. The drying temperature does not affect the biodegradation degree of wastewaters from wood drying.

In a sieve analysis, the largest sieve was 1.41 mm, and only pine sawdust contained a portion (9%) that was greater than this limit. All three samples (birch, spruce, and pine dust) had identical TG curves, indicating that at these moisture levels the species of tree does not affect the drying rate and behavior. The drying rate of the wood chips measured by oven drying increased in the beginning and then slowed down.

5.4 Biodegradation results of other related topics

Some clarifying measurements concerning the effects of the adaptation time, nutrients, and temperature on the biodegradation reactions were performed. The results of the biodegradation studies showed that the initial amount of inoculum affects the so-called adaptation time by lengthening the time needed to start the biodegradation reaction. This may be due to the population of the bacteria needing to reach a suitable level, and not only from adaptation. A BOD value of 5.6 mg L⁻¹ was achieved during periods of 0.23, 0.23, 0.23, 0.47, 1.4, 1.4, 1.56, 1.63, and 1.94 days (see Table 9). Roppola et al. (2009b) studied the effect of larger concentrations of inocula, and concluded that these inocula concentrations had no effect on the final BOD value.

As can be seen from Table 10, the initial amount of inocula affected not only the rate of biodegradation, but also the biodegradation degree when the measurement time was 28 days. The lowest added amounts achieved the stationary phase during this time, while the highest amounts added continued the biodegradation reaction after the 28 days. This result shows that the length of the adaptation time can be determined with a series of varying amounts of inocula.
Table 10. Time taken for 5.6 mg L\(^{-1}\) to be achieved, and the final biodegradation degree of a glucose sample (concentration 205.4 mg L\(^{-1}\)) with different inocula amounts added.

<table>
<thead>
<tr>
<th>Amount of inocula [mL L(^{-1})]</th>
<th>BOD = 5.6 [mg L(^{-1})] achieved in days</th>
<th>Degree of biodegradation [%] 28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.23</td>
<td>24</td>
</tr>
<tr>
<td>10</td>
<td>0.23</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>0.23</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>0.47</td>
<td>14</td>
</tr>
<tr>
<td>1.5</td>
<td>1.4</td>
<td>13</td>
</tr>
<tr>
<td>1</td>
<td>1.4</td>
<td>16</td>
</tr>
<tr>
<td>0.5</td>
<td>1.56</td>
<td>16</td>
</tr>
<tr>
<td>0.1</td>
<td>1.63</td>
<td>8</td>
</tr>
<tr>
<td>0.05</td>
<td>1.94</td>
<td>10</td>
</tr>
</tbody>
</table>

Temperature is a significant factor when considering the biodegradation of a sample. Reducing it by 10 degrees, a much lower result than at 20 °C was obtained (see Table 11). Furthermore, a freezing and thawing cycle slows down the biodegradation reaction. When the temperature effect was studied, the biodegradation of wastewater decreased as the temperature decreased, and rose when the temperature increased. Temperatures were between 10–35 °C. Fresh wastewater taken from the WWTP biodegraded better than other sources, because the bacteria strains in the WWTP are selected so that they function at a temperature range around 20 °C. Freezing was found to slow the biodegradation reaction in the melted samples. In the SFS standard, it is stated that samples should be seeded after freezing, as it affects the biodegradation value.

Table 11. Effect of freezing and temperature on BOD value of wastewater.

<table>
<thead>
<tr>
<th>Temperature [°C]</th>
<th>Fresh wastewater</th>
<th>Frozen two weeks</th>
<th>Frozen one month</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>275</td>
<td>140</td>
<td>150</td>
</tr>
<tr>
<td>20</td>
<td>350</td>
<td>115</td>
<td>220</td>
</tr>
<tr>
<td>10</td>
<td>150</td>
<td>100</td>
<td>75</td>
</tr>
</tbody>
</table>

The amount of nutrients plays a large role in the biodegradation reaction. When the amount is low enough, the reaction stops completely. Then the variation in nutrient content has a strong effect on the biodegradation degree. The lack of nutrients is one reason why biodegradation in natural waters is lower than under standard conditions. In this research, at the end of the 28 days, measurement of
the degree of biodegradation was between 45–60% for dilutions of 100%, 50%, 25%, and 10%. In the 25% solution, the biodegradation rate was slower than in the 10% solution. The biodegradation degree decreased in more diluted solutions (2.5% and 1%) to 31%, and to 20% for the 5% concentration (see Table 12). With a 0.5% concentration, no biodegradation reaction was detected. The 100% nutrient-content dilution water was made according to the OECD 301F standard. All results are presented in Table 12, and the sample used in this research was glucose. The effect of nutrients on the biodegradation degree is remarkable, at least according to this study. However, Roppola et al. (2009a) studied the effect of ten-fold concentrated nutrient solution (from 100% solution) on biodegradation, and found that it did not affect the results.

Table 12. Degrees of biodegradation of glucose sample with different nutrient concentrations.

<table>
<thead>
<tr>
<th>Sample concentration [g L⁻¹]</th>
<th>Nutrient content [%]</th>
<th>Degree of biodegradation [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.144</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td>0.143</td>
<td>50</td>
<td>55</td>
</tr>
<tr>
<td>0.340</td>
<td>25</td>
<td>45</td>
</tr>
<tr>
<td>0.173</td>
<td>10</td>
<td>52</td>
</tr>
<tr>
<td>0.172</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>0.177</td>
<td>2.5</td>
<td>31</td>
</tr>
<tr>
<td>0.150</td>
<td>1</td>
<td>31</td>
</tr>
<tr>
<td>0.135</td>
<td>0.5</td>
<td>-2</td>
</tr>
<tr>
<td>0.155</td>
<td>100</td>
<td>54</td>
</tr>
</tbody>
</table>
6 Critical inspection of biodegradation results

The law concerning waste disposal, for example, in wastewater purification is unambiguous that the waste must not be diluted. This is why wastewater treatment plants have both numerical and percentage demands for reducing the BOD value to an accepted purification level. It is a different situation to monitor the purification level or to measure the right BOD value. Usually, for monitoring the purification level, the old closed-bottle method is used. In this method, the wastewater has to be diluted, and the dilution factor may rise to several tens of thousands. The result after dilution is much higher than without dilution if the studied water is inhibitive. However, because wastewaters from industry are usually highly inhibitive, the realistic BOD value for these waters has to be measured for a non-diluted sample. The inhibiting effect on the biodegradation may be caused by many different factors, and it is not always a toxic effect.

Partial biodegradation is one question in biodegradation measurements that is not usually regarded. The exact biodegradation degree should always be mentioned when giving the results for a single compound, not only that the compound is biodegradable or readily biodegradable. Some industrial and common consumer products may be only partially biodegradable, and the non-biodegradable decomposition products are as dangerous as they would be as an original substrate. So, if the (BOD/ThOD)*100% is 50%, the product may still be environmentally unsafe due to the possible toxicity of the resulting degradation compounds. Biodegradation of intermediates may be enhanced if the toxic or inhibitive compound is pre-degraded (for example, photochemically) before biodegradation (González et al. 2007). Many synthetic compounds are not biodegradable or are only partially biodegradable. The bacteria may be very selective in which materials they are able to biodegrade, and the biodegradation reaction is based on the degrading ability of enzymes. Enzymes are very specific catalysts for the biodegradation reaction of the initial compounds. (Stryer 1988)

The degree of biodegradation is calculated by dividing the BOD value with the theoretical oxygen consumption (ThOD). If the theoretical value was the weight of the whole sample, it would give a proper knowledge of the biodegradation degree of the sample in question. Usually, this situation occurs when the sample contains large amounts of inorganic material. For example, an acceptable adjustment is the moisture content, which is a variable that should be deduced when calculating the degree of biodegradation. If a sample contains 1% of organic material, and half of it biodegrades, the biodegradation degree is 50%
for the sample, although the right value calculated with the sample weight would be considerably lower. This can be seen in such samples like ashes and soils that contain a large proportion of inorganic material. This inorganic material is left out in calculations.

The so called adaptation time in the beginning of the measurement is not always due to adaptation. Sometimes the number of bacteria has to increase to a suitable level. This can be seen in measurements when the initial bacteria content is very low, and so the final degree of biodegradation is lower, than in a situation when the bacteria content is higher at the beginning. This can be examined with dilution series.
7 Conclusions

The biodegradation degrees of recycled vegetable oil products were between 46–83%, depending on the concentrations of the samples. The mold-oil products were readily biodegradable at about 100 mg L\(^{-1}\) concentrations. The recycled vegetable oils did not contain any harmful metals at measurable low levels. Sometimes surface-active agent addition is used to dissolve the sample in the dilution water, but according to the results, oils can be measured under these standard conditions without adding the surface-active agent. An added surface-active agent does not affect to the biodegradation degree of the oil sample, and the dissolution does not affect the result. The inhibition is dependent on the sample’s concentration and not how it dissolves. A clear inhibition effect on the biodegradation degree was observed when the concentration of the sample increased to 600 mg L\(^{-1}\). Biodegradation stopped completely when the concentration was greater than 10,000 mg L\(^{-1}\). Based on these results, the addition of a surface-active agent to oil spillages in nature is not necessary, at least in these concentrations. The results indicate that recycled vegetable oils are biologically stable during storage and transport, as long as there is no water present. The degree of biodegradation for the oil emulsion was the same as that for the universal oil from which it was made. By recycling vegetable oils, the carbon footprint can be diminished, as compared to manufacturing the same products from mineral oil.

For surface-active agents, the following conclusions may be drawn. Some of the tall oil soaps are readily or moderately biodegradable under OECD 301F conditions (around 39–83%). The lowest values of biodegradation degrees were found in cold groundwater, where the biodegradation was only slight. Tall oil soaps biodegraded moderately or very little in natural waters. In river water, the biodegradation degrees were about 14–50%, and in groundwater the biodegradation degrees were between 1% and 34%. Under these conditions, the biodegradation is dependent on the temperature, nutrients, and oxygen and bacteria contents, as well as the concentration of the sample. These products are inhibitive in large concentrations. In water environments, the biodegradation results were not repeatable, but a clear biodegradation reaction was detected. The values of biodegradation degree ranged from 9–76% in surface water, 24–100% in groundwater, and 24–68% in tap water. Tall oil soaps did not biodegrade in anaerobic conditions. In sand, no biodegradation reaction was observed. In topsoil, all tall oil soaps enhanced the biodegradation of the soil itself. From these
results, it can be concluded that the infiltration of tall oil soaps into the ground should be prevented, because the biodegradation is slow in sand and in groundwater.

The calculated half-lives of the biodegradation rate for these three tall oil samples were very much in line with the biodegradation results, while the calculation of half-lives gives quite good estimation of the reaction rate. Pseudo first-order kinetics can be applied to estimate the rate of biodegradation accurately, and to calculate the \( t_{1/2} \) values. This knowledge is very important and practical for predicting the rate of slow biodegradation reactions.

Concrete solvent agent (CSA) is readily biodegradable under OECD 301F conditions (~80%), and moderately biodegradable in surface and tap water. These results were repeatable. In groundwater and aerobic conditions that material biodegrades only slightly. CTAB did not biodegrade at all, and Triton X-100 biodegraded 6% under OECD 301F conditions, as shown earlier. In surface water and groundwater, the biodegradation of CSA was moderate, but when the sample amount was diminished to about 0.06 g L\(^{-1}\), the biodegradation in groundwater increased to 84–100%. The concentration of the sample, nutrient content of the matrix, and temperature affected the final biodegradation degree. This indicates that, in highly diluted form, biodegradation in nature can be as large as in measurements at standard conditions, and that CSA is not harmful to nature.

A correct biodegradation degree for the surface-active agents is very difficult to obtain, because they probably interact with the cell walls, which are micellar, double-layered, and charged.

In solid phase, the biodegradation of the matrix was found to increase due to a more effective wetting of the smallest pores of the soil, where the nutrients, biodegradable material, and bacteria are located. The studies of CSA in different solid environments revealed two main behaviors. The first was the aerobic biodegradation of a sample and matrix, and the second was the anaerobic biodegradation that occurred with the depletion of oxygen. The calculated biodegradation degrees varied from \(-4800\) to \(3350\)%, with the negative values originating from anaerobic biodegradation. This was also found when the surface-active agent was toxic CTAB and non-biodegradable Triton X-100, although the mean values were much lower than with CSA. Some of the graphs fluctuated within a range that was roughly from 100% to \(-100\)%. As a result, it can be stated that, in these measurements, the theoretical oxygen consumption of the soil is so high that the biodegradation of the sample is very difficult to separate from the gathered data. The calculated biodegradation degree values are closer to zero.
when the carbon content of the soil is taken into account. The amount of water is a large factor, considering the biodegradation in the ground. The biodegradation increases with increased wetting, indicating that the water environment is more suitable than dry soil for the growing of bacteria.

The following conclusions for wood drying are that the condensing waters contain their own bacteria strains, which are able to biodegrade the organic pollutants in the water without any added inocula. The biodegradation degrees of pollutants in condensing waters varied between 25% and 61%. Adjusting the pH did not affect the biodegradation reaction of organic compounds in condensates.

The biodegradation of pollutants in these condensing waters reveals that they are moderately biodegradable and, because the biodegradation reaction continues after 28 days measurement time, it can be concluded that they contain very little if any harmful or toxic compounds. This is supported by the information that the water does not contain any inhibitive compounds, although the measurement was performed using non-diluted samples. The TOC values were also quite low, and therefore, they can be disposed directly to drainage, which is a very important conclusion from a practical and economical viewpoint.

From the thermogravimetric analyses (TG), the maximum drying temperature of the wet wood chips was about 200 °C, and the optimum drying temperature of wood for combustion purposes is when looking only at the TG graph, between 100 and 150 °C.

When the amounts of nutrients and inocula were lowered, the biodegradation reaction slowed down, and for this reason the biodegradation in different natural situations is slower than under standard conditions.

The adaptation time can be examined by a dilution series, where the amount of inocula varies. The correct value for adaptation time is a value, which does not increase when the amount of inocula increases. With smaller amounts, the time required for the onset of the biodegradation reaction is longer than with larger amounts. This is because of the increasing number of the bacteria, and not only because they are growing in size. This also can be detected indirectly from the lowered degree of biodegradation value when the initial amount of bacteria is fewer.
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BIODEGRADATION STUDIES OF RECYCLED VEGETABLE OILS, SURFACE-ACTIVE AGENTS, AND CONDENSING WASTEWATERS

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