O.2.1. Methodology of spruce extracts as biocides replacement in a wastewater system

A.2.1. Testing and evaluation of spruce bark extracts as antimicrobial agent in pilot scale investigations

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The overall objectives and achievements

Greener Solutions from Forests: Tannins from Spruce Bark as a Sustainable Biocide Alternative

In today's drive toward sustainable industry, the paper and wastewater sectors face a shared challenge: how to manage microbial growth and harmful gas emissions without relying on synthetic biocides. These chemicals, though effective, often come with environmental risks and high costs. This project, developed within the CEforestry initiative, explores an innovative solution hidden in plain sight—Norwegian spruce bark, a by-product of forestry operations.

A Natural Ally: Tannins in Spruce Bark Extract

Rich in phenolic compounds known as tannins, the hot water extract from spruce bark has shown remarkable potential as a bio-based antimicrobial agent. In the processing of recycled pulp—especially cardboard containing starch—it reduced both acidification and gas formation, two key indicators of microbial activity. Notably, it slowed the buildup of hydrogen gas (H_2) to non-hazardous levels, a critical safety improvement in pulp storage.

Comparable to Industry Biocides—But Greener

Laboratory tests revealed that the spruce extract matches or even surpasses Fennosan, a widely used synthetic biocide, in inhibiting a wide range of bacterial strains. These include harmful spore-forming bacteria like Clostridium and Bacillus, as well as filament-forming microbes such as Thiothrix. Importantly, it also showed inhibitory effects on hydrogen sulfide (H_2S) producing bacteria, which are notorious for creating toxic, corrosive, and foul-smelling gases in wastewater systems.

From Lab Bench to Pilot Scale: Promising, But Complex

Field studies confirmed that H_2S emissions occur mainly within biofilms in stagnant, nutrient-rich zones, such as near food-processing discharges. While lab results are promising, these real-world complexities underline the need for targeted application strategies and further testing.

Towards Circular, Clean, and Chemical-Free Processes

By transforming what was once waste into a functional bioproduct, this study aligns with circular economy principles. It offers a safer, sustainable alternative to synthetic biocides, contributing to both cleaner paper production and improved wastewater management. The implications are wide-reaching: industries can reduce their chemical footprint while maintaining microbial control—paving the way for more environmentally responsible industrial practices.

Summary

This study is an output of the CEforestry project and explores the use of a spruce bark extract, derived from forestry by-products rich in phenolic compounds, as sustainable alternatives to conventional synthetic biocides in both processing of recycled pulp and in wastewater treatment.

In systems containing recycled materials, here card bord, a tannin rich hot water bark extract from Norwegian spruce, was shown to significantly reduce acidification and gas formation in the pulp. The addition mitigated the typical pH drop during incubation and strongly inhibited microbial gas production, especially hydrogen (H_2) and carbon dioxide (CO_2). In stored pulp, hydrogen concentrations reached levels considered hazardous in hours, but the inclusion of spruce bark extract reduced these levels drastically. These results were comparable to previous studies involving cationic tannins (acacia-based) and non-ionic tannins (quebracho-based), suggesting that tannins effectively inhibit fermentative bacterial activity, particularly under conditions of high starch content and process stagnation.

The antimicrobial potential of the spruce bark extract was evaluated. Laboratory tests demonstrated its ability to inhibit the growth of different types of bacterial species isolated from pulp and wastewater facilities. As compared to the synthetic biocide Fennosan, a product commonly used in the paper industry, the extract offered wider efficacy among various bacterial types, including some hydrogen sulphide (H_2S) producing species and spore forming bacteria like *Bacillus* sp. and *Clostridium* sp. The growth of filament-forming species such as *Thiothrix* sp. was slowed down.

In wastewater systems, the extract's capacity to inhibit H_2S formation was of particular interest. H_2S is a bad smelling, highly toxic, and corrosive gas associated with biofilm-forming sulphur-reducing bacteria. Although standard wastewater samples did not produce measurable H_2S in laboratory conditions, however microbial sampling confirmed the extract's activity against H_2S-producing strains at low concentrations.

Importantly, field/pilot observations revealed that H_2S production occurred within biofilms lining the pipe walls—particularly at locations with stagnant flow and high nutrient input, such as near a local dairy outlet. These conditions complicate treatment strategies and indicate that any application of spruce bark extract must be carefully targeted.

In conclusion, spruce bark extract, contains tannin as active compound, demonstrated strong potential as environmentally friendly biocide replacement. In pulp processing, it helps maintain chemical stability and reduce microbial growth and gas emissions. In wastewater systems, it offers a bio-based approach to controlling microbial activity and mitigate harmful gas formation. While the laboratory results are promising, the complexity of real-world systems calls for further field trials to assess optimal dosing strategies and long-term effectiveness. These natural compounds could play a key role in the development of greener industrial practices.

Objectives of the project

CEforestry – Innovation in Forestry Biomass Residue Processing: Advancing Circular Forestry with High-Value Products

The Baltic Sea region (BSR) generates vast amounts of underutilized forestry biomass residues such as bark, needles, and cones. While these side streams often are used for low-value applications like bioenergy, they hold significant potential as a source of high-value compounds for a wide range of uses.

CEforestry aims to transform the way forestry residues are utilized by developing innovative practices and circular economy concepts tailored to the forestry sector. The project focuses on creating novel solutions for the effective use of forestry side streams across the BSR.

This goal will be achieved through cross-sector collaboration involving researchers, SMEs, large companies, and other key stakeholders. The solutions will be tested and demonstrated in pilot facilities to ensure practical applicability.

A Circular Economy business model was developed based on project outcomes, along with recommendations for how BSR countries better can utilize forestry side streams. These efforts will support the objectives of the EU Green Deal, the EU Circular Economy Action Plan, and the Baltic Sea Region Bioeconomy Strategy.

The project will directly benefit the circular forest economy and businesses interested in developing sustainable, innovative products. CEforestry involves a strong network of stakeholders, including 12 project partners and 17 associated partners from Poland, Latvia, Lithuania, Finland, and Sweden.

Objective of WP2 and activity 2.1 (A.2.1): Testing and evaluation of spruce bark extracts as antimicrobial product in pilot scale investigations

Figure 1 illustrates the application of **spruce bark extract**, which is obtained through **pilot-scale hot water extraction**, as a **biocide replacement and antioxidant** in two key processes aimed at reducing or eliminating the formation of harmful or unwanted gases during biomass and wastewater treatment.

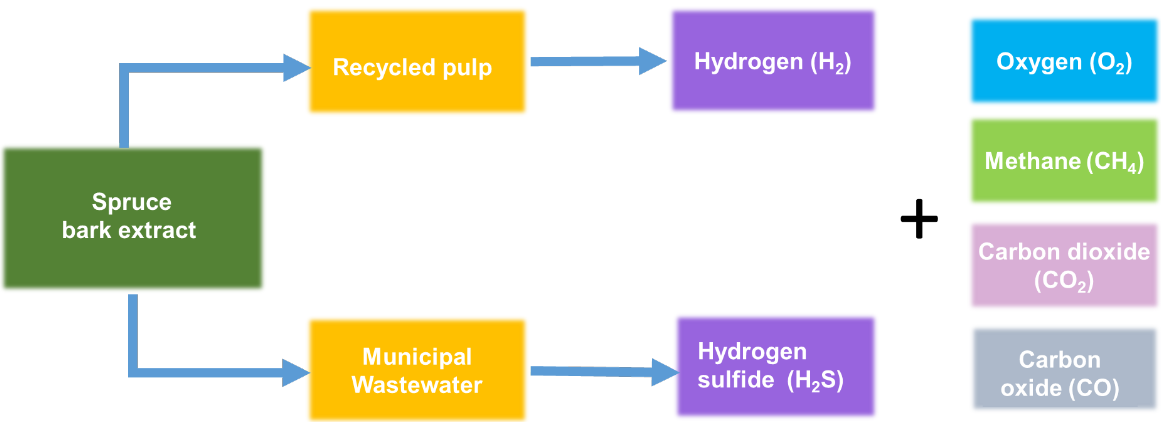


Figure 1. SLU and Umeå University objectives. Spruce bark extract for treatment of recycled pulp and municipal wastewater to reduce/eliminate undesired gas formation.

Spruce bark extract is applied in the following two pathways:

1. **Recycled Pulp Treatment**:
   In this process, spruce bark extract is added to **recycled pulp systems** to inhibit both **microbial activity** and **oxidation reactions**, which are primary contributors to the formation of gases such as:
   - **Hydrogen (H_2)**
   - **Hydrogen sulphide (H_2S)**
   - **Oxygen (O_2)**
   - **Methane (CH_4)**
   - **Carbon dioxide (CO_2)**
   - **Carbon monoxide (CO)**

By suppressing these biological and chemical pathways, the extract helps **reduce or prevent gas generation**, leading to a more stable and environmentally friendly production process.

2. **Municipal Wastewater Treatment**:
   Similarly, in **municipal wastewater treatment**, the extract functions as both a biocide and antioxidant to limit microbial activity and oxidative reactions responsible for the formation of **hydrogen sulphide (H_2S)**. This results in a **significant reduction of H_2S emissions**, improving safety and reducing environmental impact.

This dual-function approach showcases the potential of spruce bark extract, produced from a forestry side stream, as a valuable component in **circular and sustainable bio-based solutions**. By using bark residues through green extraction methods, the process contributes to cleaner industrial systems and supports the principles of a **circular bioeconomy**.

A. Treatment of recycled pulp to mitigate hydrogen gas formation and microbial growth during storage of recycled pulp

Background

Microbial contamination presents both environmental and economic challenges in paper production, contributing to issues such as **biofilm formation, corrosion, and unwanted gas emissions**. The generation of **hydrogen (H_2)** poses a serious safety risk, as it can accumulate to explosive levels in pulp storage towers at paper mills. Meanwhile, the formation of **hydrogen sulphide (H_2S)** not only results in foul odours and equipment corrosion but also degrades product quality. At high concentrations, H_2S is also toxic to humans (Flemming et al., 2013).

The increasing reliance on recycled raw materials, combined with progressively stricter environmental regulations, underscores the urgent need for **sustainable alternatives to synthetic biocides**, which are currently used to manage these problems.

The aim of this project was to explore the potential of **forestry side streams** as sources for natural biocide alternatives. **Spruce bark**, a low-value forestry by-product, is rich in **bioactive phenolic compounds**, particularly tannins (Nisca et al., 2021; Jyske et al., 2023). A tannin-rich spruce bark extract was produced using green extraction methods and was subsequently chemically characterized.

To assess its functionality, the extract was tested for **antimicrobial activity** against bacterial strains isolated from industrial paper production processes. In addition, its impact on **gas formation in recycled pulp systems** was evaluated.

SCA Obbola Liner Board, production and microbial challenges

SCA's Obbola mill produces approximately **800,000 tons of linerboard annually**, consisting of various grades. Roughly, **60 % of the production is based on virgin forest materials**, while the remaining **40 % comes from recycled cardboard** (Fig 2). Many operational challenges stem from the use of recycled material, which contains **high amounts of starch.** Based on the use of recycled cardboard and the starch content (4%), about 20 tons of starch is added to the circulation every day. This **native starch, the glue in the cardboard, enters** the system during the **initial milling stage** and becomes part of the process water circulation. In addition to the native starch, an **unknown but notable amount of cationic starch** is also introduced in the paper machine as a **retention aid**. Altogether, it is estimated that **approx. 20 tons of starch per day** are circulated within the system.

Figure 2. Storage of bales containing recycled cardboard.

The process environment — characterized by **abundant nutrients (starch)**, **elevated temperatures (about 45 °C)**, and **long retention times**, due to a complex circulation system — is highly favourable for **microbial growth** (Johansson et al., 2001). A key problem is the proliferation of **slime-forming bacteria**, which produce **biofilms** that accumulate in pipes and equipment, ultimately leading to **defects in the final product**, such as visible "stickies" and spots.

A particularly problematic microbial process is **dark fermentation**, where bacteria metabolize starch into gases and organic acids. These metabolic pathways result in:

1. **Gas formation**, primarily H_2 and CO_2
    - High concentrations of H_2 are hazardous—**levels above 6% are explosive**.
    - At the SCA Obbola mill, continuous gas monitoring has shown that **production interruptions** can lead to **rapid hydrogen accumulation** in the pulp storage towers within just a few hours (Fig. 3).

2. **Production of short-chain fatty acids**, mainly **acetic acid** and **butyric acid**, during fermentation.
    - These acids are typically produced by bacteria such as ***Clostridium*** sp., which has been identified in the pulp.
    - The resulting **pH drop** in the stored pulp increases the **solubility of calcium compounds**, which negatively impacts the **paper quality**.
    - A **moderate acidification** is preferred to avoid this issue, as excessive pH drops can destabilize the system.

Representative fermentation reactions include (Kamran M, 2021):

$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2$

$C_6H_{12}O_6 + 6H_2O \rightarrow 2CO_2 + 2CH_3COOH + 4H_2$

$C_6H_{12}O_6 + 6H_2O \rightarrow 2CO_2 + CH_3CH_2CH_2COOH + 2H_2$

These reactions illustrate how glucose, a breakdown product of starch is converted into gases and organic acids, posing both **safety and quality risks** in paper production. ***Bacillus*** sp. and some types of **clostridia** are known to utilize starch and produce H_2 (Miłek J and Lamkiewicz J, 2022; Masset J, et al., 2012)

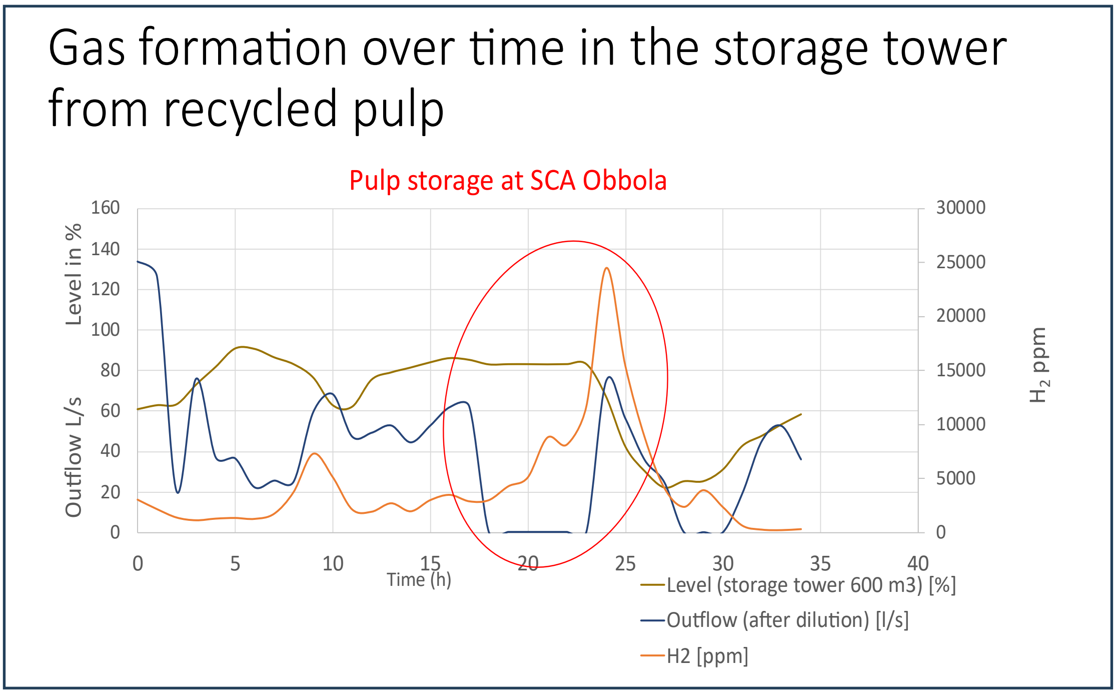


Figure 3. H_2 concentration in the pulp storage tower related to production data from SCA Obbola. Hydrogen concentration in the storage tower is plotted against production data from the regular process control system at the factory.

Monitoring gas concentrations in the pulp storage tower at the Obbola paper mill - previous studies

Before the start of this project (CE-forestry) we conducted studies on the factory related to this project, the SCA paper mill in Obbola. (Fig. 3). We therefor find it relevant to account for this, i.e. the two following points concerning tannins and bacterial processes. Hydrogen gas formation poses safety risks in pulp processing. Past incidents have involved explosions in storage towers due to H_2 accumulation. Methane (CH_4) levels, however, were found to be negligible.

In the Obbola mill, pulp is stored in a 600 m³ tower, continuously fed from the top and discharged from the bottom. Under normal conditions, retention time is controlled. However, during process interruptions or mechanical failures, stagnation can lead to undesirable microbial activity and gas formation.

H_2 levels were monitored using an ECOM probe inserted from the top, suspended approximately 2 meters above the pulp surface. The H_2 data was compared to registrations from the production line. Level means the level in the tower; filling grade and outflow means the flow through the storage tower. Interruptions in flow (e.g., standstills) were clearly associated with rapid increases in H_2 concentration above the stored pulp. This trend suggests that gas buildup begins within 4 hours of halted processing.

The generation of H_2S by microbes is a shared problem in paper mills and wastewater systems (Torén et al., 1996; Zhang et al., 2023; Bergeron and Pelletier, 2004; Henriet et al., 2017). *Thiothrix* is a genus of filamentous bacteria that plays a role in sulphur cycling, particularly in sulphur-reducing environments. They can oxidize reduced sulphur compounds, like H_2S and store the oxidized sulphur (sulphur globules) within their cells. When oxygen is limited or absent, *Thiothrix* can reduce these stored sulphur compounds, potentially impacting nutrient cycling and wastewater treatment processes. In closed systems where anaerobic conditions are present, corrosion and odour emission will arise. In addition to odour and corrosion, increased concentration of H_2S is a risk factor for human health.

Material and methods

Spruce bark extract preparation

The bark from Norwegian spruce, collected at a local sawmill (SCA Wood, Rundvik) used in this study was supplied by the Swedish University of Agricultural Sciences (SLU), Biomass Technology Centre (BTC). The bark was first shredded and sieved using a 30 mm mesh to ensure appropriately sized particles for extraction. The prepared bark was packaged, frozen, and shipped to LUKE (Natural Resources Institute Finland) in Espoo, Finland, for extraction.

Pilot-scale hot water extraction

A total of 92.8 kg of thawed fresh spruce bark was loaded into a 300-liter pilot-scale digester. The extraction was carried out in batch mode, using 281 L of pure water at 92 °C as the solvent. The system was heated with steam to a temperature of 100 °C, and the extraction process was maintained for 40 minutes. The procedure yielded 226.5 kg of aqueous extract with a pH of 4.9.

The total dissolved solids were measured, resulting in an extraction efficiency of 10.3%, calculated based on the dry weight of the bark.

Ultrafiltration concentration

The crude extract was concentrated by ultrafiltration using a tubular PCI unit equipped with a modified polyether sulfone (PES) membrane (EM006, molecular weight cut-off: 6000 Da).
From the initial 226.5 kg of extract, a 30.4 kg concentrate was obtained, while 275 kg of permeate was separated during the process. The concentrate was frozen and sent to SLU for further testing and analysis.

Extract composition

The chemical composition of the final spruce bark extract concentrate is summarized in Table 1.

Table 1. Proportion and bioactive properties of compound analysed in the produced spruce bark extract.

Parameter	**Value**
Dry matter content	104.6 mg/mL
Tannin content	30.2 mg/mL
Total phenolic compounds	411.4 mg/g
Ferric reducing capacity	2397 µM/g
Antioxidant activity (ORAC)	18,841 µM/g
Polysaccharides	45.0 mg/mL

(Korpinen, R., 2025)

Laboratory-scale trials: Proof of concept

To evaluate the potential of spruce bark extract in reducing gas formation, **recycled thick pulp** (10% dry solids) was collected from the SCA Obbola factory and transported to the laboratory at **SLU** for testing.

Experimental procedure

- **Preparation of pulp samples**
  Recycled thick pulp (1 kg) was diluted with 1 L of tap water, as the untreated control sample.

- **Bark Extract Treatment**
  Spruce bark extract (10 %) was diluted to **1%** to improve mixing with the pulp, and 1 L of this bark extract solution was mixed with 1 kg of thick pulp for one minute.

- **Incubation**
  The control sample and the treated sample were transferred to two sealed cylinders and placed in a **37 °C heating cabinet**. Gas formation was monitored at **24 and 48 h**, with data recorded over **90 seconds** using the ECOM gas analyser.

Initial experiments were conducted using the **gas washing bottle method** to measure the total volume of gas produced (Fig. 4). Subsequently, a more precise method, referred to as the **cylinder method**, was adopted. This setup allowed for direct gas analysis using an **ECOM gas analyser** (Fig. 4).

Figure 4. Gas Washing Bottle Setup. Total gas production with gas washing bottle (left) and with different concentrations of added bark extract over time (right). N=3 for each concentration. Amounts of depressed water was recorded/10 min with a camera.

Measurement Techniques

- **Cylinder Setup with ECOM Analyzer** was used to quantify specific gases. The ECOM instrument measures **O_2, CO, CO_2, CH_4, H_2S, and H_2**. The setup includes sealed cylinders containing the treated and untreated pulp, respectively and a **gas probe**, with real-time output (Fig. 5).

Figure 5. Experimental setup. Cylinders with pulp and the measuring probe (left), and data output (right).

The gases produced were primarily CO_2 and H_2, consistent with expected microbial fermentation pathways. These trials served as a **proof of concept** for the effectiveness of **spruce bark extract** in mitigating microbial gas formation in recycled pulp, particularly $\mathbf{H_2}$ and $\mathbf{H_2S}$, which are known to cause both safety hazards and product quality issues.

Microbial content in pulp and process water

Samples of pulp and process water obtained from different environments at the local paper mill (SCA, Obbola) were collected on several occasions and examined for microbial communities by culture techniques (Fig. 6). The samples were serially diluted in sterile distilled water (MQ water) and 100 µL from each dilution were spread on selective and non-selective agar media. Plates containing Fennosan (0.5 mL/L) and spruce bark extract (0.5 %) respectively, were included to spot resistant strains. The total number of cultured bacteria was determined, and representative isolates of dominating species were sub cultured and examined further by microscopy and biochemical methods.

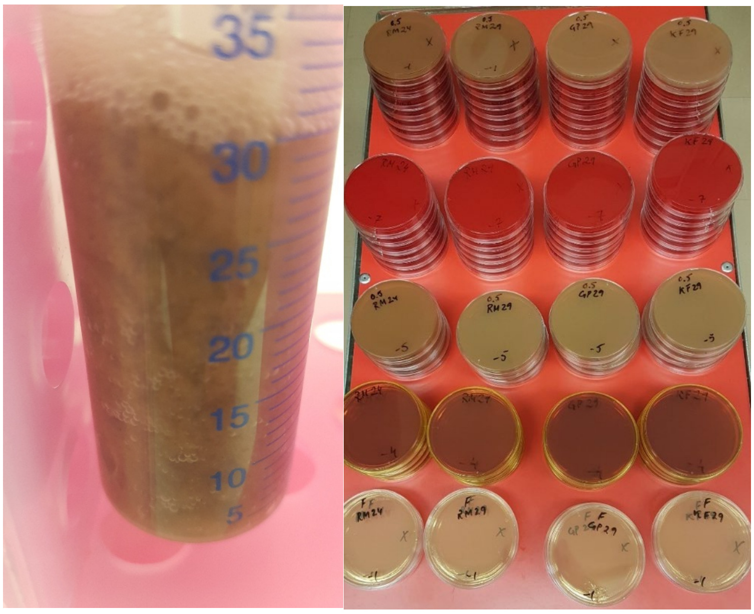
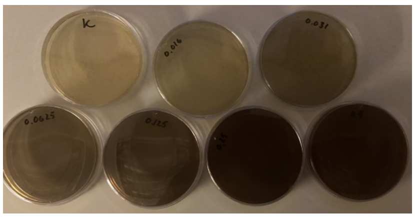


Figure 6. Test tube with diluted pulp exposing visible gas bubbles (left) and the set-up of agar media to analyse four samples of pulp and process water collected at different areas along the production line at the SCA Obbola papermill (right).

Determination of minimum inhibitory concentrations of spruce bark extract

Minimum inhibitory concentrations (MIC) (Ref. EUCAST 2000) of spruce bark extract were determined for different bacteria isolated from recycled pulp and process water by agar dilution technique (Fig. 7). Aerobic and anaerobic, Gram-positive and Gram-negative bacteria, spore-formers and non-spore-formers were investigated.

- A serial 2-fold dilution of spruce bark extract was prepared in MQ-water in test tubes
- 1.0 mL of each dilution was added to 19 mL of melted agar
- Plates were casted



- Standardised inoculates of the bacteria to investigate were prepared
- Steer's replicator was used to inoculate the plates
- One µL of each bacterial strain tested was picked up and transferred to the spruce bark plates

Figure 7. Illustration of the agar dilution method used for antimicrobial testing of spruce bark extract. The lowest concentration of spruce bark extract in the agar plates are determined for each tested bacterial strain, noted as MIC values.

Pilot trials

Effect of spruce bark extract on gas emissions

Recycled pulp was transported from the factory to our experimental facilities for pilot-scale testing. A total of 250 kg of wet pulp was diluted with 250 L of water (42 °C) in two separate tanks of 1 m³ (Fig. 8). To this mixture, 15 L water (control) or 750 g of spruce bark extract (total solids, TS) in 15 L water were added, resulting in a concentration of 1.5 g spruce bark extract /L of the total 500 L, or equivalently, 3 g/kg pulp.

During the experiment, the pulp-spruce bark extract-water mixture was continuously stirred to ensure homogeneity and maintained at a constant temperature of 35-37 °C to simulate optimal microbial activity conditions. The temperature in the container with the untreated control increased to 45-46 °C.

Pulp samples obtained from the two containers in the pilot trail were treated as described above. During the 48-h incubation period pulp samples were collected at different time points and immediately frozen at -20 °C for later analysis. At the same timepoints, the concentrations of the gases formed were analysed and recorded by the ECOM system.

Figure 8. Recycled pulp in 1 m³ containers with heaters to control the temperature during transport from the paper mill to the SLU pilot facilities.

Results and discussion

Recycled pulp is heavily contaminated with various microbes. The total bacterial number is approximately 10^8 colony forming units (CFU)/g pulp (Fig. 9).

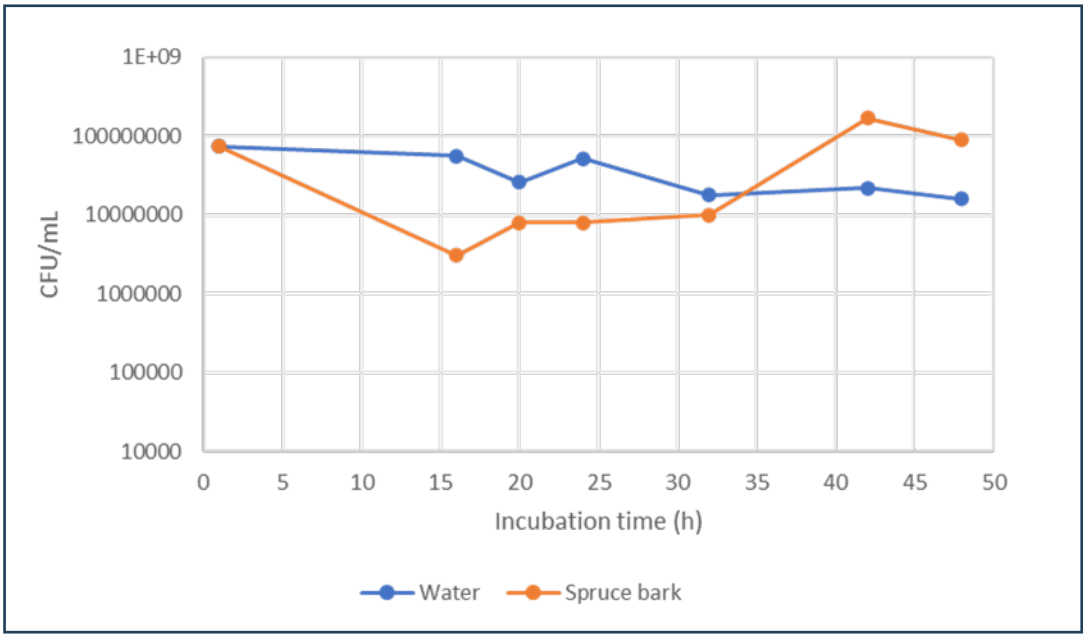


Figure 9. Total number of cultured bacteria over time in the pilot trail containers with addition of spruce bark extract (red) and untreated pulp (blue). At 16 h the bacterial population was reduced by 96% in presence of spruce bark extract.

Microscopic analysis revealed a dominant presence of *Thiothrix* sp., a filament-forming microbe (Figure 10 a and b). The true number of *Thiothrix* was difficult to determine though, as handling of the samples broke the long filaments into smaller pieces giving rise to new small “colonies”, called rosettes, in the culture media. The size of the filaments of *Thiothrix* most certainly make them dominate the pulp sample volume, even if their actual number is lower than the regular sized bacteria. Filament forming bacteria are common in the environments in nature and are known to cause problems, like biofilm formation, in facilities involving water.

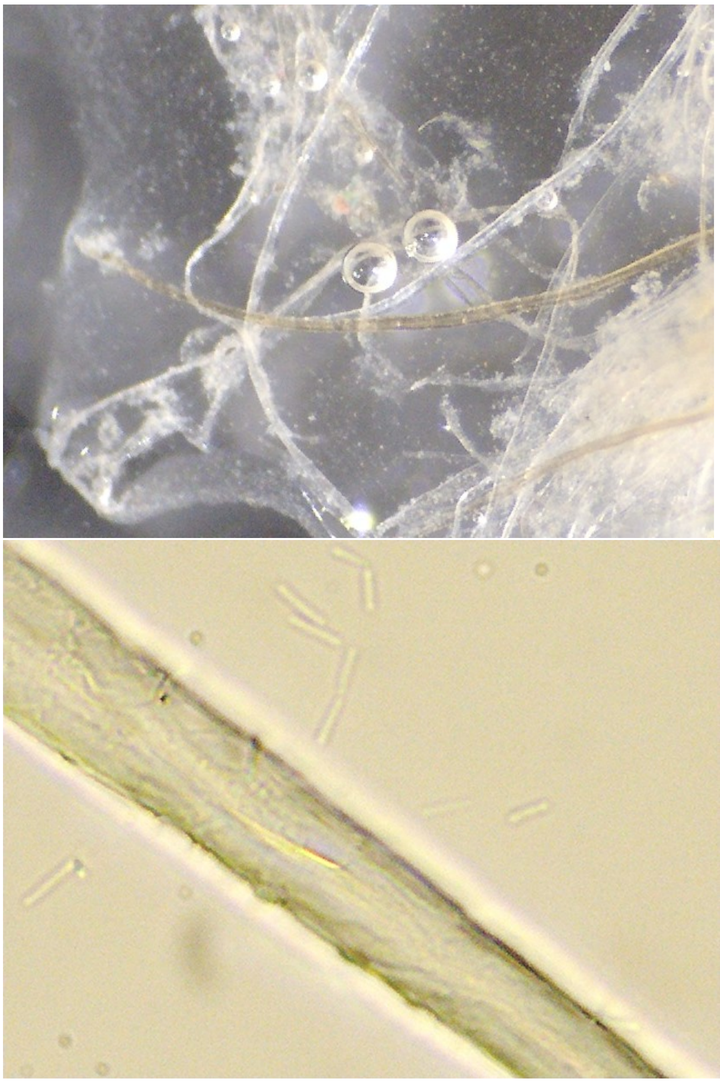


Figure 10. a) Direct microscopy of pulp mixed with a drop of water. The filaments of Thiothrix sp. can be seen by the naked eye. Note the gas bubbles). b) Cells and spores of Bacillus surrounding an enormous Thiothrix filament.

Effect of spruce bark extract on H_2 production – pilot study

This pilot study demonstrated rapid increases in H_2 concentration in untreated samples, reaching 60,000 ppm—the detection limit of the ECOM analyser probe. In contrast, the tank treated with spruce bark extract showed significantly reduced and delayed hydrogen production (Fig. 11). While delayed gas formation may still occur over days, these findings indicate that tannins effectively suppress H_2 generation. Concentrations above 60,000 ppm are considered hazardous due to explosion risk.

Microbiological sampling conducted during gas production in pilot experiments revealed a clear correlation between microbial activity and H_2S formation (Fig. 12).

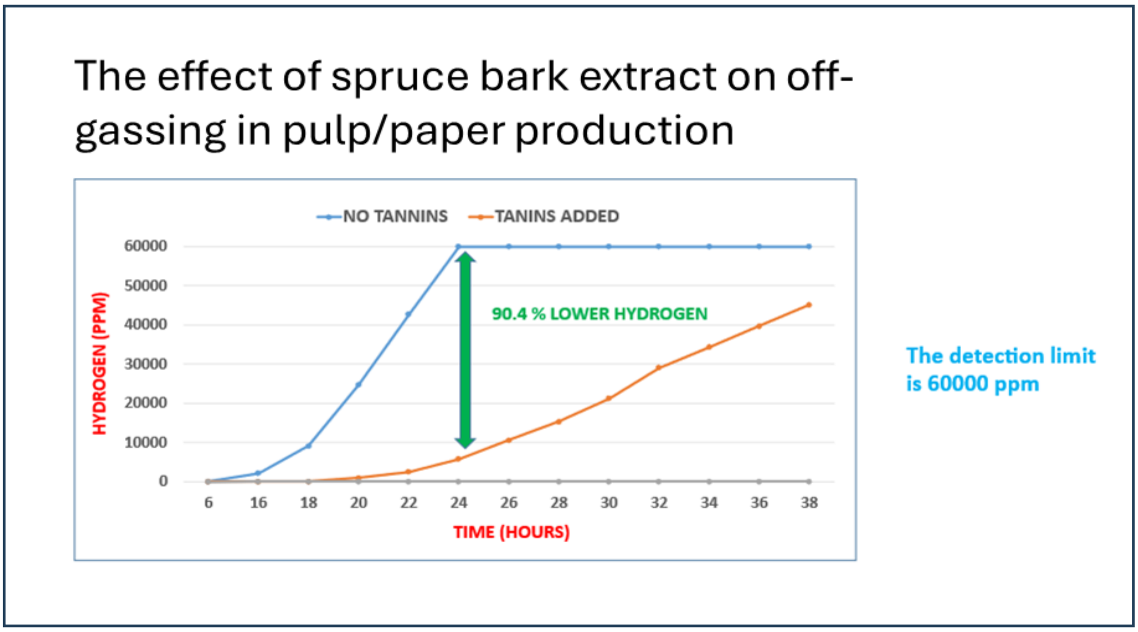


Figure 11. H_2 concentration over time in the experimental containers with spruce bark extract (tannins) added and (red) and untreated pulp sample (blue).

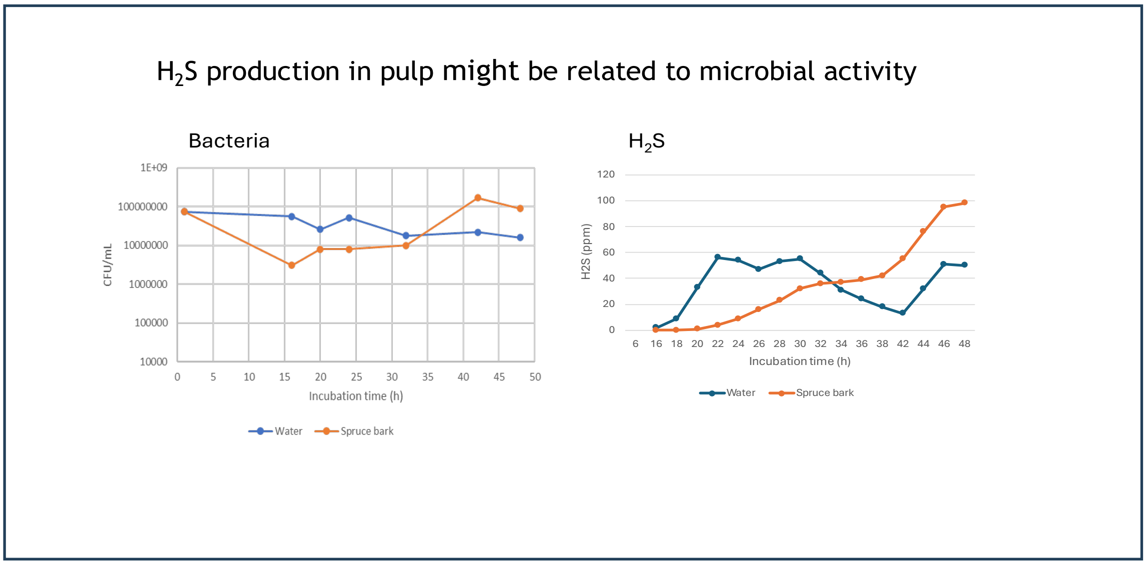


Figure 12. H_2S concentration over time in the experimental containers with spruce bark extract (tannins) added and (red) and untreated pulp sample (blue).

Antimicrobial testing of spruce bark extract

Microbial samples were collected from pulp and process water. To isolate resistant strains, some samples were cultured in the presence of Fennosan R20V (0.5 mL/L), a commonly used synthetic biocide in the paper industry (Fig.13). Most isolates investigated were susceptible at levels below 0.5% spruce bark extract (Table 2; Fig. 14). Interestingly, many colonies of *Bacillus* sp. isolates from Fennosan-containing plates were observed to carry fragments of *Thiothrix* filaments after subculture. These same *Bacillus* strains, when cultured on blood agar and on medium containing 0.5% spruce bark extract, showed no growth on spruce bark plates of either *Bacillus* or *Thiothrix*, indicating a strong antimicrobial effect. On blood agar, both microbes produced good growth when subcultured.

As compared to Fennosan, the bark extract demonstrated a broader antimicrobial activity. The inhibitory effects of spruce bark extracts on the different bacterial species associated with pulp and process-water indicate that the extract has the potential to be used in the production line. The extract could either replace the synthetic biocide (Fennosan) presently used or be added as a supplement in specific critical locations in the process.

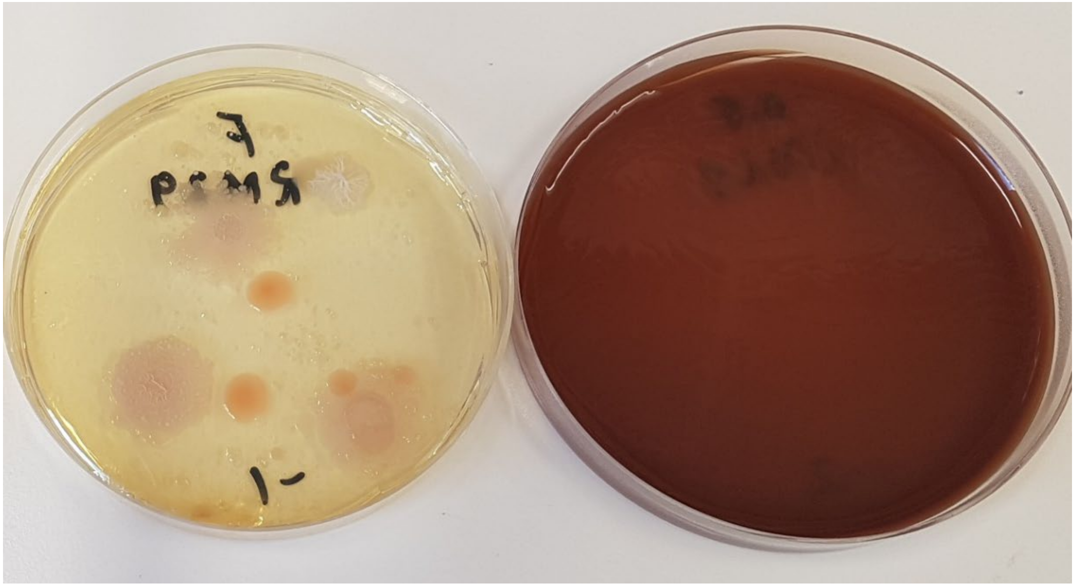


Figure 13. Sample of pulp cultured for 48 h at 37 °C in air. Some aerobic bacteria in recycled pulp are less susceptible to 0.5 mL/L Fennosan R20V (left) than to 0.5 % spruce bark extract (right), where no microbial colonies are visible.

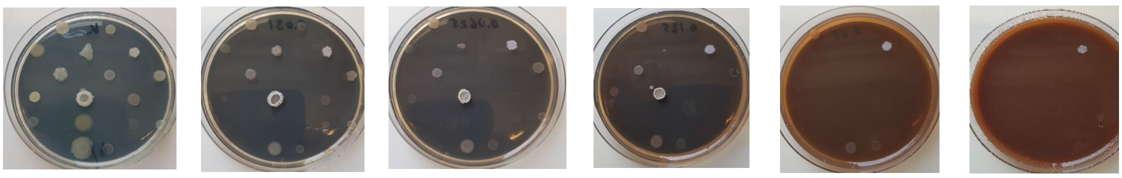


Figure 14. The MIC was defined as the lowest concentration of spruce bar extract clearly inhibiting growth. It was demonstrated that a majority of the studied bacteria were susceptible to the spruce bark extract and had MICs ≤0.5 % (w/v).

Table 2. Minimum Inhibitory Concentrations (MIC) of spruce bark extract for selected bacterial species from Obbola paper mill and wastewater systems.

Aerobic bacteria	**MIC (% w/v)**	**Notes**
Pulp, process water		
H2 Gram- rod	0.5	
H6 Gram- rod	0.125	
H11 *Beggiatoa* sp.	>0.5	Filament forming
T44 *Cloacibacterium* sp.	0.25	
BV1 *Bacillus* sp.	0.25	Carries *Thiothrix* (*Ttx*)
RM24:19 *Bacillus* sp.	0.25	Fennosan R
RM24:21 *Bacillus* sp.	0.0625	Fennosan R/ Carries *Ttx*
RM24:22 *Bacillus* sp.	0.25	Fennosan R
RM29:7 Gram+ cocci	0.25	10^8 CFU/ mL
RM29:13 *Bacillus*	0.25	Fennosan R/Carries *Ttx*
RM29:14 *Bacillus*	0.0625	Fennosan R
Wastewater		
SW1 *Shewanella xiamenensis*	0.125	H_2S producing
F1 Gram- rod	0.031	
F3 Gram- rod	0.125	
F5 Gram- rod	0.125	
F6 Gram- rod	0.25	
Anaerobic bacteria		
Pulp		
RM24:24 *Clostridium* sp.	0.25	
RM24:27 *Clostridium* sp.	0.25	Aero tolerant
RM24:28 *Clostridium* sp.	>0.5	
RM29:20 *Clostridium* sp.	>0.5	
RM29:22 *Clostridium* sp.	0.5	Aero tolerant
GP29:13 *Clostridium* sp.	0.5	Aero tolerant
GP29:24 *Clostridium* sp.	0.25	Aero tolerant
Wastewater		
SW6 *Clostridum sulfidigenes*	0.125	H_2S producing
Reference strains		
E. coli CCUG 10 979	0.5	
L. plantarum DSMZ 13 890	>0.5	

Previous studies - effect of cationic acacia tannins and raw tannins from spruce and quebracho on total gas production

In previous unpublished studies, the aim was to evaluate tannins extracted from spruce bark and compare their effects with commercially available cationic acacia tannins (TANAC A/S, Brazil).

A general pattern emerged when tannins—whether acacia or other types—were added to the pulp (Fig. 15): gas production was delayed initially (on average 17h), as the bacterial community adapted to the new environment. This adaptation phase typically lasted between 17 and 20 hours, based on repeated experiments.

Gas production was found to be dose-dependent; higher concentrations of tannins led to reduced gas formation. In the untreated control (zero sample), approximately 800 mL of gas was produced from 1 kg of thick pulp (dry solids content = 10%).

The acacia tannins used in this study were cationized through a Mannich reaction, which involves a small amount of formaldehyde, a compound known for its antimicrobial properties. According to the safety data sheet (MSDS), the formaldehyde content was <0.1%.

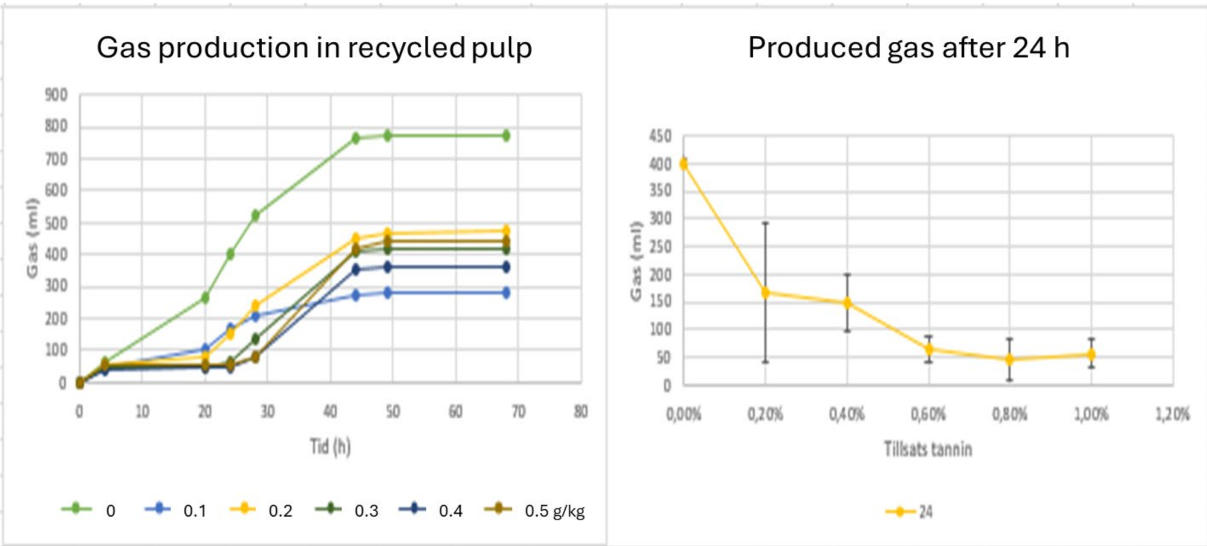


Figure 15. Left) Total gas production over time in control sample and added cationic acacia tannins (0-0,5 g tannin /kg thick pulp). Each data point represents the average of N=3. Right) Total gas production after 24h with different doses of tannins (0 – 1 %).

Effect of non-ionic (raw) bark extracts from spruce and Quebracho

To evaluate the effects of non-ionic tannins, 0.125 g of extract (per kg recycled pulp) from either spruce bark extract or Quebracho was added. The same method described in Fig. 4 was used. Gas volume was recorded every 10 minutes using a camera and results are presented as cumulative gas production with ±2 standard deviations.

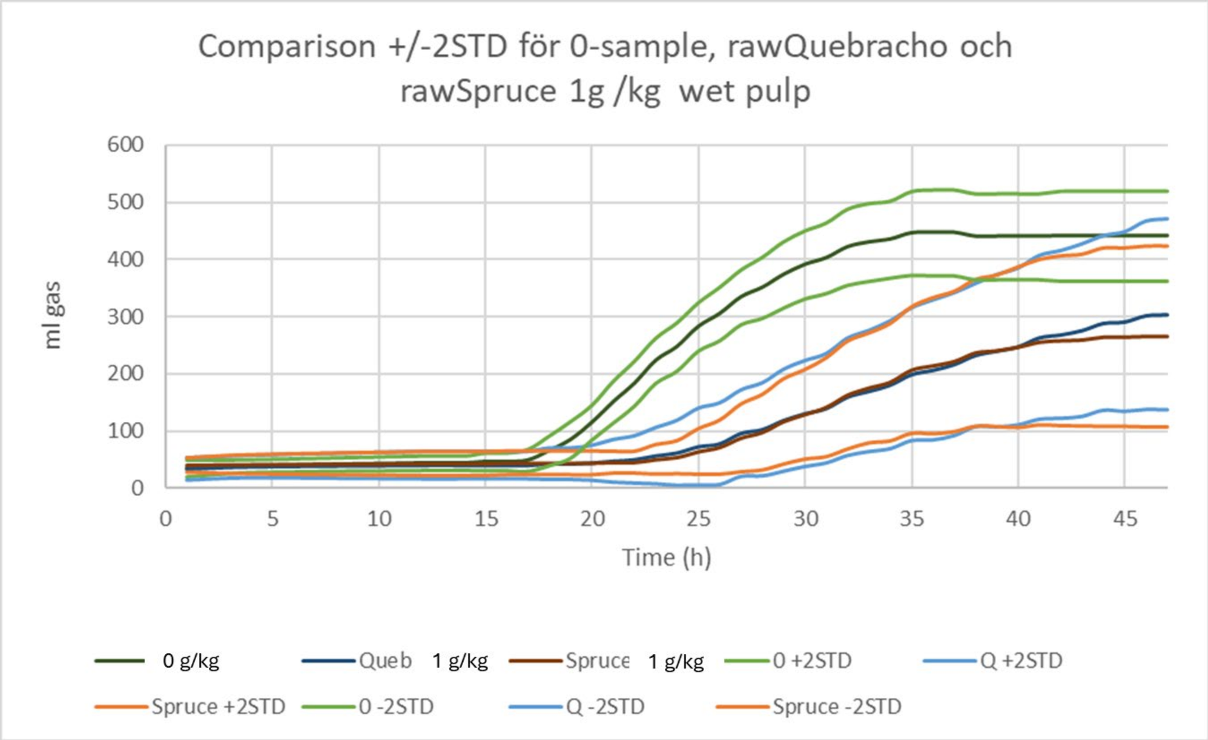


Figure 16. Cumulative gas production from recycled pulp. Zero sample and added Quebracho and Spruce tannins (1g/kg pulp. The blue arrow shows values for Spruce and Quebracho tannins (+ - 2STD).

The results show that non-ionic tannins also reduced gas production compared to the control. Total gas production in the zero sample was approximately 500 mL. Notably, the applied dose (1 g/kg) for non-ionic tannins was higher than that used for cationic Acacia tannins.

In both cases as described in Fig. 15 and Fig. 16 the reduction of gas production was reduced to approx. 50 % compared to the zero sample. However, it is problematic to compare between samples taken on different days depending on several unpredictable factors.

Effect of spruce bark extract on gas production in recycled pulp

Both cationic and non-ionic tannins significantly affected microbial gas production. Overall, cationic tannins (Acacia) were found to be more effective, requiring roughly one-third the dosage of non-ionic tannins to achieve a similar effect. Whether this is due to formaldehyde presence or another mechanism remains unclear.

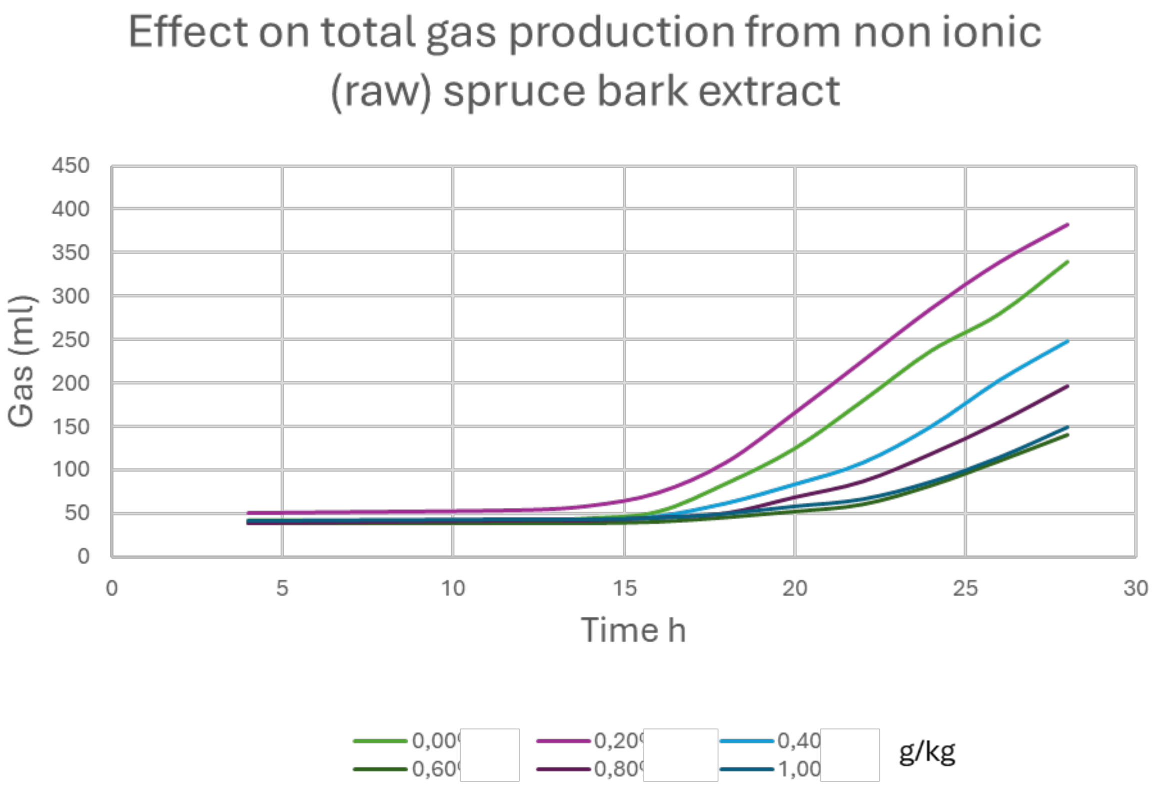


Figure 17. Effect on total gas production with added doses of spruce bark extract

Dose-dependent effect of spruce bark extract on total gas production (Fig 17). At 1 g/kg wet pulp, gas production decreased from about 400 mL to 100–200 mL. Similar trends were observed in other experiments.

Gas production from different pulp treatments

The thick pulp used in the tests contained 10 % fibres and 90 % water. It was initially diluted with white water—recirculated process water known to contain starch and other soluble organic materials. We hypothesized that white water itself might contribute significantly to gas formation by carrying either bacteria or soluble carbohydrates (Fig 18).

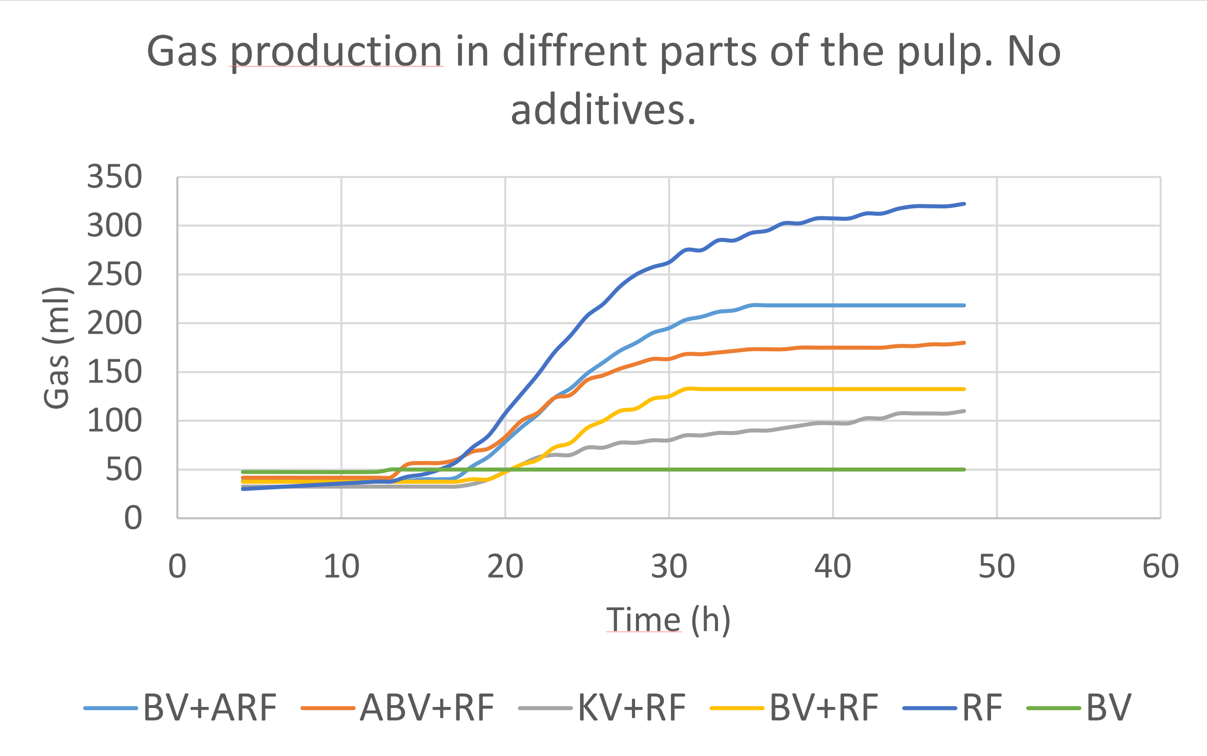


Figure 18. Total gas production from pulp with different treatment and white water. BV=white water. ABV= Autoclaved white water. ABV= Autoclaved white-water RF= Recycled cardboard, the pulp. KV= Tap water

The key observation was that white water alone did **not** generate significant amounts of gas, contrary to the expectations. The effects of sterilization (autoclaving) and dilution with tap water showed no clear trends, and experimental variability may have affected results.

Effect of tannins on acidification of recycled pulp

Based on prior experience, we applied non-ionic (raw) tannins at three times the dose of cationic tannins (Acacia, AC) to evaluate their impact on pulp acidification (Fig. 19). The acidification in stored pulp probably mainly depends on the bacterial processes shown above, i.e. the dark fermentation process resulting in H_2 and short organic acids.

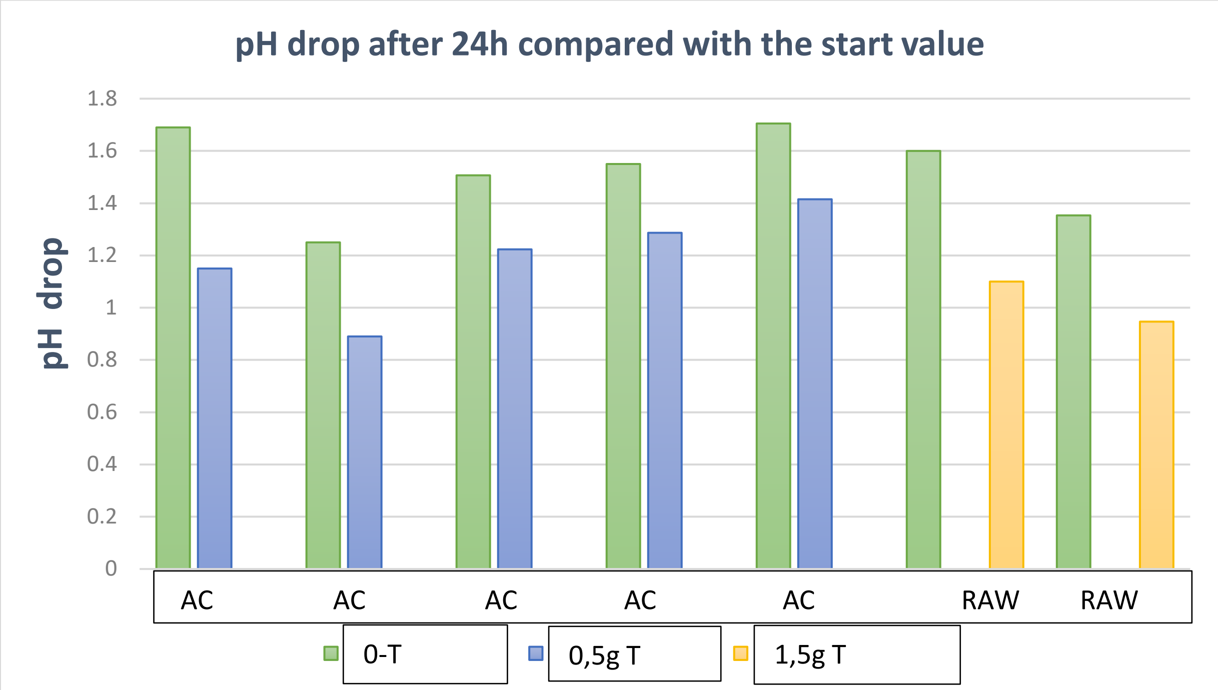


Figure 19. pH -drop after 24h in recycled pulp with (purple) and without (green) addition of tannins.AC refers to cationic acacia tannins 0.5gT/kg wet pulp and blue is addition of 1.5g non-ionic Quebracho (raw) tannins/kg wet pulp.

Conclusions

A key challenge in these studies is the variability of pulp samples collected from the production line. Pulp quality fluctuates due to raw material differences and process interruptions, including unplanned standstills. Additionally, synthetic biocides are dosed three times daily to control microbial growth, and the timing of sample collection relative to biocide application was unknown. The biocide used has a reported half-life of seven hours, further contributing to sample variation.

Despite this variability, consistent trends emerged across experiments. Tannin addition, both cationic and non-ionic, significantly reduced gas production in recycled pulp. Total gas volume—primarily CO_2 and H_2—ranged from 250 mL to 800 mL per sample depending on conditions. The formation of other gases, such as H_2S and CH_4, was minimal (Fig. 20).

Figure 20. Gas concentration readout from ECOM analyser showing levels of CO_2, H_2, and other gases.

The gas production process is primarily driven by *dark fermentation*, in which starch is broken down into CO_2, H_2, and short-chain organic acids. Methane (CH_4) production appears negligible, likely due to the absence of methanogenic Archaea in the microbial community.

Production of short-chain fatty acids is not only relevant for pH changes but may also have economic implications. The process is time-dependent, with gas and acid production increasing over hours.

A major contributor to H_2 production is the high starch content entering the process. This starch fuels microbial growth, particularly of hydrogen-producing *Bacillus* and *Clostridium* species, identified in pulp samples. Another critical factor is time—specifically, stagnation in the process flow. Our data show that after a production standstill, H_2 levels increase rapidly within a few hours. Notably, the ECOM probe measures gas above the pulp surface, not within the pulp mass itself.

Further insight into H_2 production capacity comes from the pilot study (Figure 8), in which 250 kg of recycled pulp was mixed with 250 L of water in a closed system. Within 24 hours, H_2 concentrations reached 60,000 ppm—the maximum detectable limit of the analyser and a concentration considered hazardous due to explosion risk. However, the addition of tannins significantly reduced H_2 levels.

These studies consistently show that tannins reduce total gas production in a dose-dependent manner and offer a promising alternative or supplement to synthetic biocides in pulp processing.

B. Treatment of Sewage Water to Mitigate H_2S Formation

Background

Microbial contamination in wastewater systems leads to significant environmental and operational issues, including biofilm formation, infrastructure corrosion, and the generation of gases such as hydrogen sulphide (H_2S). H_2S is not only malodorous but also highly corrosive and toxic at elevated concentrations.

The overarching goal of this project is to evaluate the use of forestry by-products—specifically, spruce bark—as a source of bioactive compounds that could serve as sustainable biocide alternatives. Spruce bark, typically considered a low-value waste product, contains high levels of phenolic compounds, including tannins, known for their antimicrobial properties.

A tannin-rich extract was produced from spruce bark and chemically characterized. Its antimicrobial efficacy was tested against bacterial strains isolated from wastewater systems. Additionally, the extract's effect on gas formation—especially H_2S—was assessed in laboratory-scale experiments.

Umeå Wastewater System Overview

The municipal wastewater treatment plant (WWTP) in Umeå is designed to handle the load from approximately 200,000 population equivalents (PE). It is fed by a network of pipelines and multiple pump stations (Fig. 21). Specific locations within the system are monitored for H_2S production, with one site—receiving effluent from a local dairy—exhibiting persistently high levels of H_2S due to elevated nutrient content.

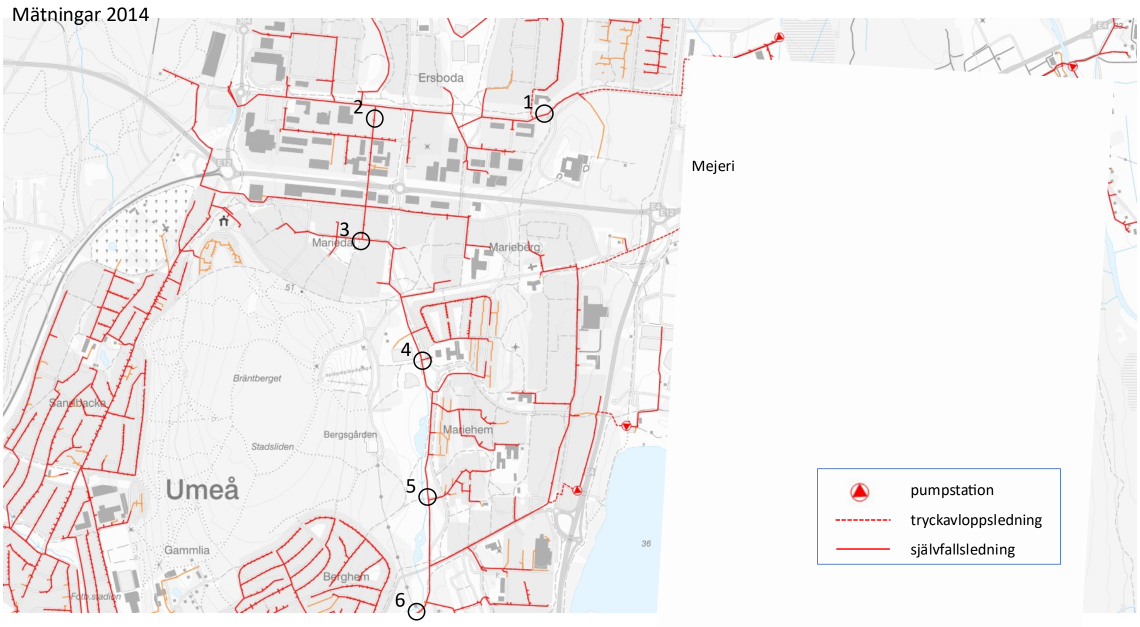


Figure 21. Map of wastewater pipeline network in northeastern Umeå, showing pump stations 1–6. Each pump station presents conditions favorable for H_2S generation due to long retention times and intermittent pumping.

High levels of H_2S is recorded at this site and it depends on the specific situation. At first, the ww contains high amounts of nutrition (from the diary) and that the tube has a pulsed flow before it enters a downflow to the wastewater treatment plant. The pumping regime here is not continuous but in pulses which gives us that the ww have a longer resident time in the tube leading to fluctuating levels of H_2S (Fig. 22).

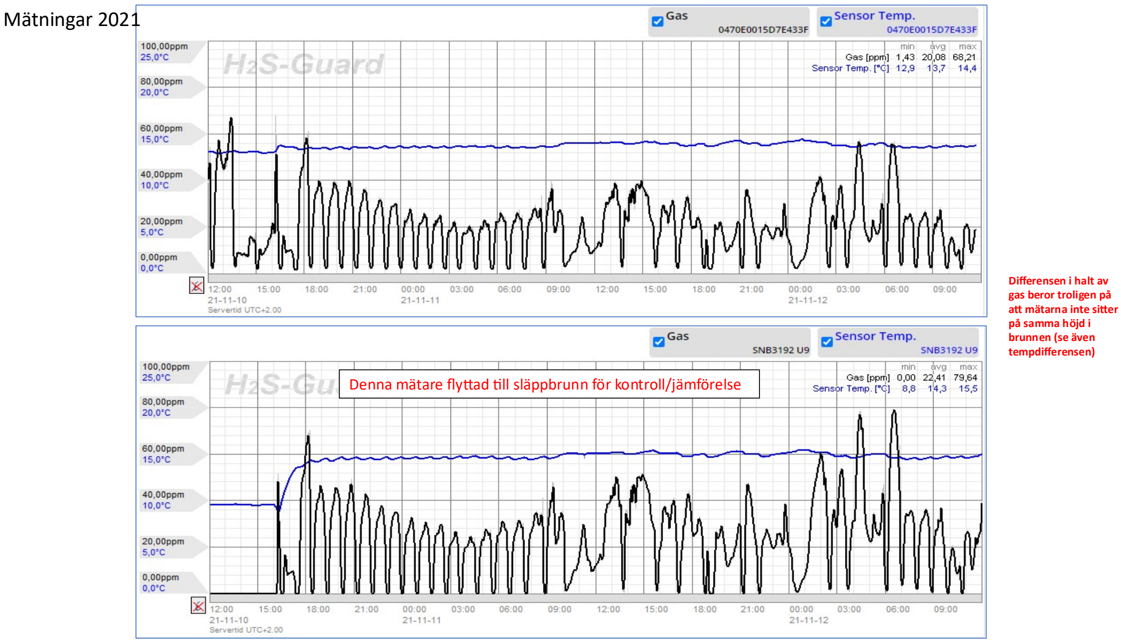


Figure 22. H_2S levels fluctuate due to pulse pumping and the geometry of the pipeline, resulting in conditions ideal for anaerobic bacterial activity.

Continuous high concentrations of H_2S leads to several issues:

- Odor complaints from nearby residents
- Chemical conversion of H_2S to sulphuric acid (H_2SO_4), which corrodes concrete pipes (Fig. 23)
- Development of biofilms that most probably harbour sulphur-reducing bacteria

High and continuous levels of H_2S entails several problems. First that it causes inconveniences for residents and further that H_2S forms H_2SO_4 which erodes the concrete tubes (Fig. 19). Another observation is that these practical circumstances lead to a colonisation of bacteria (possible S-reducing bacteria), in the slime stuck to the pipe walls.

Figure 23. Degradation of concrete pipe due to H_2SO_4 formation from H_2S.

The conventional mitigation strategy involves the addition of ferric chloride to precipitate sulphides as FeS. Alternative solutions, such as commercial bacterial amendments, exist but are costly. This study evaluates whether spruce bark extract could serve as a cost-effective and sustainable alternative.

Materials and Methods

Laboratory-scale gas formation tests

Wastewater samples were collected from various pump stations within the Umeå municipal network. Laboratory trials were conducted using 3L of wastewater placed in transparent Plexiglas cylinders. Spruce bark extract was added at concentrations ranging from 0.2 to 1.2 g/L. A control (zero-sample) was included in each trial.

Gas concentrations (H_2, H_2S, CO_2) were monitored daily for up to 144 hours using ECOM gas analysis equipment.

Antimicrobial effect of spruce bark extract in wastewater microbes

Samples obtained from different environments at the local waste-water treatment plant (VAKIN) were collected on several occasions and examined for microbial communities by culture techniques. Minimum inhibitory concentrations (MIC) of spruce bark extract were determined for different bacteria isolated from wastewater (Figure 5). Aerobic and anaerobic, Gram-positive and Gram-negative bacteria, spore-formers and non-spore-formers were investigated.

Results and Discussion

Gas formation and H_2S suppression

Initial trials with samples from the dairy outlet (Area 1, Fig. 22) showed no detectable H_2S under laboratory conditions, with or without the addition of spruce bark extract. Similar results were observed in samples from the wastewater treatment plant inlet and a remote pump station (Ersforsen), suggesting that conditions in the test system did not favour H_2S formation.

To eliminate confounding effects from possible ferric chloride residues, "clean" wastewater samples from Ersforsen were selected.

Since no significant H_2S was formed, further experiments were designed to artificially simulate H_2S-generating conditions. Wastewater samples were amended with a culture of sulphur reducing bacteria (*Shewanella xiamenensis*) and a sulphur source (L-cyctein) in tryptic soy broth. Spruce bark extract was again added, and the objective was to determine whether the extract could inhibit H_2S production under favourable conditions. Unfortunately, also under these experiment conditions H_2S levels remained negligible. However, an increase in H_2 was observed over time in samples treated with spruce bark extract, possibly due to sugars present in the extract promoting hydrogen genic bacterial activity.

Antimicrobial test in wastewater and associated bacterial isolates

The bacterial isolates collected from wastewater were susceptibility to spruce bark extract at concentrations between 0.031% and 0.25% (Table 2). Some of these isolates are known to contribute to biofilm formation and H_2S production in wastewater systems.

These findings support the hypothesis that spruce bark extract can suppress H_2S formation by inhibiting the growth of sulphur-reducing bacteria. Biofilm analyses from the wastewater pipeline revealed that H_2S was not generated in the liquid phase but inside the pipe walls, where bacterial biofilm had formed (Fig. 24).

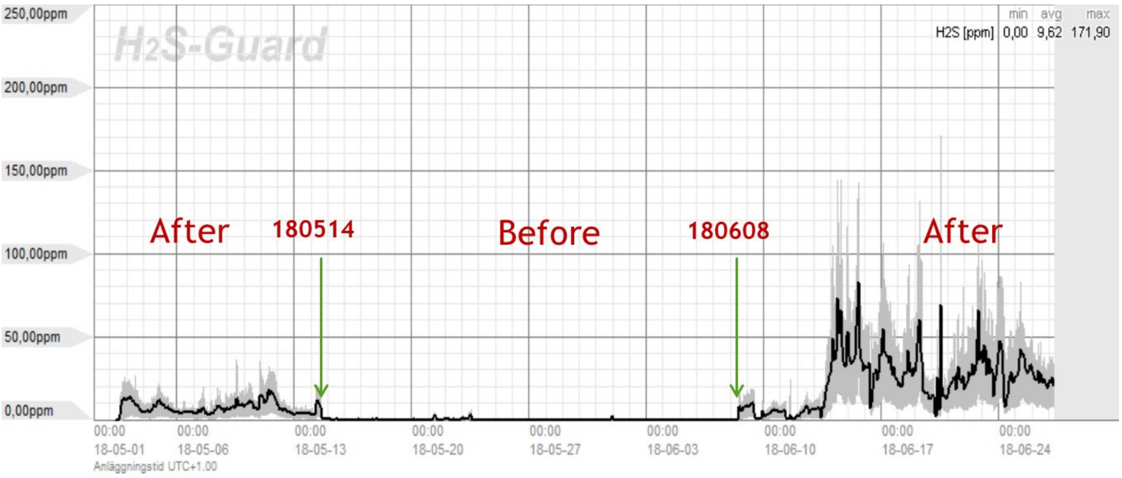


Figure 24. Measurement of H_2S concentration in wastewater from the local dairy industry. Location of the measurement probe showed that H_2S is produced inside the tube without any production in the wastewater itself. Measurements ***before*** *the counter stream tube showed no detectable generation of H_2S, while measurements* ***after*** *the tube showed that a substantial generation of H_2S took place inside the tube.*

Conclusions

The antimicrobial properties of spruce bark extract indicate a potential to be used as antimicrobials in paper mills and wastewater systems. The effect of the extract has been verified against different bacterial species isolated from these environments. In addition, the extract was shown to inhibit H_2 production in a pilot test with pulp containing recycled cardboard. The reduction of gas formation followed the inhibition of bacterial growth in this test. The need for new environmentally friendly alternatives for controlling microbial growth in paper mills indicates a potential for use of spruce bark extract in these systems.

Concerning the microbial-related generation of H_2S in the wastewater system, the situation is more complex. It is no doubt that spruce bark extracts inhibit growth of H_2S producing bacteria. However, the cross talk between nutrients in the water streams and the biofilm microbes on the pipe surface indicates the need of a complex test system for evaluating the effect of the extract. These aspects showed that the extract cannot be randomly applied to the system. There is a need to test the effect of the extract on selected areas with known microbial H_2S generation in future studies.

Laboratory trials demonstrate that tannin-rich spruce bark extract has strong antimicrobial properties and potential to suppress the formation of H_2S in wastewater systems. While H_2S was not generated under standard experimental conditions, the extract showed clear inhibition of sulphur-reducing bacteria in enriched systems.

Given the corrosive nature of H_2S and the high costs associated with chemical treatments like $FeCl_x$, spruce bark extract represents a promising bio-based alternative. Field trials in sections of the pipeline network—particularly those downstream of high-nutrient industrial sources—are recommended to evaluate real-world efficacy.

Overall Conclusions

- The spruce bark extract showed strong promises as bio-based alternative to treatment with synthetic products in pulp industries and wastewater facilities.
- Their antimicrobial effects are evident, but field validation is essential due to system complexity and variability.
- Integration into current treatment routines will require dose optimization, targeted application, and cost-benefit analysis.

Recommendations for Future Work

1. Pulp Treatment Studies

- Conduct controlled dose-response trials for different tannin types.
- Investigate synergistic effects between spruce bark extract/tannins and current biocides.
- Explore tannin interactions with different starch levels and pulp retention times.

2. Spruce Bark Extract Biocide Development

- Chemical fingerprinting (e.g., phenolic profile) of extract batches to standardize active ingredients.
- Long-term pipe segment trials downstream from high-H_2S sources (e.g., dairy outlet).
- Monitor biofilm thickness, microbial composition, and corrosion rates pre/post-treatment.
- Assess feasibility of dosing routines.

3. Environmental & Economic Assessment

- Compare life-cycle costs and environmental impact of spruce bark extract vs ferric chloride/Fennosan.
- Evaluate waste valorisation potential: turning forestry by-product into value-added treatment.

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