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REPORT

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Abstract

The ability of an IWTM Elysator to inactivate microbes has been tested. Tap water enriched with *Klebsiella pneumoniae* and *Pseudomonas fluorescens* was circulated through the Elysator in a closed loop with a water reservoir. The organic content, pH and oxygen levels were manipulated to try out the system. All tests were performed at 27-29 °C.

A high pH alone or anaerobic conditions alone did not seem to be sufficient to get significant inactivation of bacteria by the Elysator. But when operated properly and, hence, both anaerobic conditions and a pH above 10 were obtained, the Elysator appeared to inactivate *Pseudomonas fluorescens* and *Klebsiella pneumoniae*. Low bacterial counts were found both in the water and in the sludge deposited on the bottom of the Elysator. Due to the generally unfavourable growth conditions enforced by the Elysator, as well as the nature of the bacterial strains tested, this bactericidal effect is likely to be rather general. However, complete wipe-out of bacteria or "sterile" conditions in the long run is highly unlikely. Nevertheless, the Elysator will contribute to low bacterial numbers in the water.

To verify operational stability of the Elysator, dissolved oxygen and pH levels should be checked and the anode sticks should be cleaned regularly.

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4. Magnesium hydroksid-utfelling	4. Magnesium hydroxide precipitation

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The ability of an Elysator to inactivate bacteria

Preface

International Water treatment Maritime AS (IWTM) is a distributor of Elysators, an equipment designed to control corrosion in cold and hot water systems. IWTM's chief engineer, Jan Ebbestad contacted NIVA to test the Elysator system for potential bactericidal effects, which could be of interest in controlling bacterial growth in the same water systems.

A pre-test was performed in January 2002 at NIVA's laboratories in Oslo, but it was decided that a more thorough test would be necessary to draw any conclusions on the matter. These were done in February and March 2002, also at NIVA's laboratories in Oslo. All tests were done with the same Elysator, provided by IWTM.

NIVA's research scientists Harry Efraimsen and Christian Vogelsang performed the tests.

Oslo, 14.05.2002

Christian Vogelsang

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Definitions of bacteria-related terms

Anti-microbial agent: a substance that prevents microbes from growing, but not necessarily having any direct bactericidal effect

Bactericidal effect: causing death of bacteria

Bacterial inactivation: is dependant on what type of activity is measured; here: growth when supplied a rich medium (see Cultivatable bacteria). The "inactive" bacteria may still be alive, surviving in a dormant phase.

Bacterial proliferation: increase in bacterial cell numbers

Cultivable bacteria: bacteria that are able to grow on a given specified medium

Colony forming units, CFU: in a sufficiently diluted sample each cultivatable bacterium will form an isolated colony when grown on a solid rich agar medium, hence the CFU number relates directly to the density of cultivatable bacteria in the sample.

Opportunistic bacteria: specialised to exploit newly opened habitats

Pathogenic bacteria: causing disease

Summary

The ability of an IWTM Elysator to inactivate microbes has been tested. Tap water enriched with *Klebsiella pneumoniae* and *Pseudomonas fluorescens* was circulated through the Elysator in a closed loop with a water reservoir. The organic content, pH and oxygen levels were manipulated in consecutive tests to try out the system. All tests were performed at 27-29 °C.

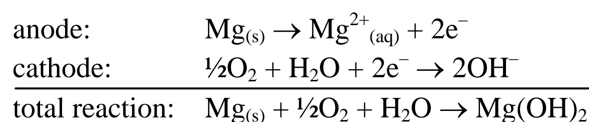
A high pH alone or anaerobic conditions alone did not seem to be sufficient to obtain significant bactericidal effect by the Elysator. But when operated properly and, hence, both anaerobic conditions and a pH above 10 were obtained, the Elysator appeared to show bactericidal effect on *Pseudomonas fluorescens* and *Klebsiella pneumoniae*. Low bacterial counts were found both in the water and in the sludge deposited on the bottom of the Elysator. Due to the generally unfavourable growth conditions enforced by the Elysator, as well as the nature of the bacterial strains tested, this bactericidal effect is likely to be rather general. However, complete wipe-out of bacteria or "sterile" conditions in the long run is highly unlikely. Nevertheless, the Elysator will contribute to low bacterial numbers in the water.

Dissolved oxygen and pH levels should be checked regularly. An anaerobic environment seems to coincide with a reduced anode current output, but should be verified with standard oxygen electrode measurements from time to time. For stable operation the anode sticks in the Elysator should be rinsed free from the developed magnesium hydroxide film regularly. However, finding the necessary cleaning-frequency has not been a part of this project.

The Elysator is typically applied in many different systems, and the experiments carried out have not been designed to test all possible environmental conditions. The most important factors that have not been taken into (adequate) consideration are the capacity of the Elysator (how the efficiency is correlated with the size of the system, e.g. total volume and flow of water), continuous (heavy) nutrient loading, and temperature.

1. Introduction

The Elysator system is a non-chemical water treatment system to prevent all kinds of corrosion, and it has been applied in different heating, cooling and steam generating systems. The function of the Elysator as a corrosion inhibitor is based on the anodic/cathodic principle, letting a less noble metal (here: magnesium) to be corroded instead of more vital metal parts of the system being protected. In the process magnesium (at the anode) is oxidised to $Mg(OH)_2$ in a redox process given by the two half-cell reactions (Ref 1):



anode:	$Mg_{(s)} \rightarrow Mg^{2+}_{(aq)} + 2e^-$
cathode:	$½O_2 + H_2O + 2e^- \rightarrow 2OH^-$
total reaction:	$Mg_{(s)} + ½O_2 + H_2O \rightarrow Mg(OH)_2$

Oxygen is consumed in the process, eventually giving anaerobic conditions if the influx rate of oxygen to the system is less than the consumption rate. $Mg(OH)_2$ is relatively poorly soluble in water, but nevertheless contributing to an increased pH in the water due to the release of OH^-. Its solubility product $K_S = 7.1 \cdot 10^{-12}$ indicates a theoretical maximum pH of 10.28. $Mg(OH)_2$ is also an excellent coagulant at high pH (optimum at approx. pH 9). Hence, the Elysator may also remove organic matter from the water by precipitation.

Most microorganisms grow well at normal pH (~6-8) and oxygen conditions (~1-10 mg/l) and when offered a steady supply of nutrients. Deviations from this are likely to cause a general reduced growth of microorganisms. However, the more than three billion years history of these little creatures, have made them, as a group, extremely adaptive to the most extreme conditions. Hence, even in a highly unpleasant environment there is likely to be at least some types of microorganisms surviving, and maybe even growing well. This also applies to standard disinfection routines with chlorine; it is not possible to obtain sterile conditions in an open system. A general suppression of microbial growth is a good way to maintain low microbial numbers. However, this should be done in a manner so that also the potentially pathogenic (and often opportunistic) microbes are taken care of. Hence, the "allowed" number of a particular microbe should be related to its potential harmfulness.

The opportunistic pathogenic bacteria *Klebsiella pneumoniae* and the proposed slightly pathogenic bacteria *Pseudomonas fluorescens* were used as test organisms in this study (Ref 2). They are both heterotrophs (need organic carbon) that grow readily under both aerobic and anaerobic conditions. However, *P. fluorescens* is frequently used as an anti-microbial agent because of its ability to out-compete more pathogenic microbes on substrate. Any good data on pH-dependency of growth of the two bacteria has not been found, however, most enzyme systems get increasingly unstable at pH above 9.5. *Klebsiella* sp. and *Pseudomonas* sp. typically have their pH optimums for growth between 7 and 8 (Ref 2), hence it is not likely that significant growth will be observed at pH above 9.5.

The purpose of this project was to test whether the Elysator has any anti-microbial effect and, if so, what this effect was caused by and its limiting factors.

2. Test description

2.1 Experimental overview

The Elysator's ability to inactivate microbes was tested by circulating tap water enriched with *Klebsiella pneumoniae* and *Pseudomonas fluorescens* through the Elysator in a closed loop with a water reservoir. The organic content, pH and oxygen levels were manipulated in consecutive tests to try out the system. Oxygen transfer from the atmosphere was minimised by flushing the headspace of the reservoir with CO_2 or N_2, hence lowering the oxygen concentration in the gas phase above the water.

1. **Test I** (50 hours)
   Reservoir: A 5-L closed glass flask. Total volume: 30 L
   Bacteria: *K. pneumoniae* and *Ps. fluorescens* were added at the start, giving estimated initial concentrations of 10^4 CFU per ml of each strain.
   Organic substrates: The water contained 0.5‰ nutrient growth medium.
   pH: Semi-constant pH conditions (pH 6.5-8).
   Oxygen: The headspace of the reservoir was flushed with CO_2.

2. **Test II** (326 hours)
   Reservoir: A 100-L plastic container with a large oxygen transfer surface. Total volume: 130 L.
   Bacteria: 500-ml cultures of *K. pneumoniae* and *Ps. fluorescens* were added at the start, giving estimated initial concentrations of $1.2 \cdot 10^4$ CFU/ml and $3.9 \cdot 10^4$ CFU/ml, respectively.
   Organic substrates: The water contained 1.0‰ nutrient growth medium.
   pH: Free floating.
   A) Initialisation (0-50 h):
   The Elysator was disconnected, letting the bacteria grow freely in the system.
   Oxygen: Open reservoir.
   B) Open reservoir (50-92 h):
   The Elysator was connected.
   Oxygen: Open reservoir.
   C) CO_2-flushing of reservoir (92-208 h):
   The Elysator was connected. In the period 104-155 h the circulation between the Elysator and the reservoir was accidentally turn off. Around 110 h the CO_2 gas also stopped.
   Oxygen: The headspace of the reservoir was flushed with CO_2 gas.
   D) N_2-flushing of reservoir (208-326 h):
   The Elysator was connected.
   Oxygen: The headspace of the reservoir was flushed with N_2 gas.

3. **Test III** (167 hours)
   Reservoir: A 30-L plastic container with a minimised oxygen transfer surface. Total volume: 60 L.
   Bacteria: The Elysator and reservoir was filled up with 6.5 L (10%) of the water after part 1 and fresh tap water (preheated to 60ºC and cooled down). The water was kept in circulation with the Elysator disconnected for 24 hours, giving initial concentrations of *K. pneumoniae* and *Ps. fluorescens* of 110 CFU/ml and $3.5 \cdot 10^4$ CFU/ml, respectively.
   Organic substrates: The water contained 1.0‰ nutrient growth medium.
   pH: Free floating.
   A) N_2-flushing of reservoir (0-71 h):
   The Elysator was connected.
   Oxygen: The headspace of the reservoir was flushed with N_2 gas.
   B) Increased organic and microbial load (71-167 h)
   The Elysator was connected.
   Organic substrates: An extra 65 ml 100% nutrient growth medium was added to the reservoir, increasing the concentration with 1.0‰.

Bacteria: More *K. pneumoniae* was added to the reservoir, giving an estimated density of $5.8 \cdot 10^3$ CFU/ml.
Oxygen: The reservoir was flushed with N_2 gas.

At the start of Test I and just before starting the Elysator in Tests II and III, 250-ml samples were taken out from the respective reservoirs and used as control cultures for turbidity and microbial growth analysis.

2.2 Test system

A schematic description of the test system is shown in figure 1. A 25-L Elysator and convenient flexible stainless steel transmission lines were provided by IWTM (International Water Treatment Maritime AS). The water was pumped through the system at a flow of 3-5 L/min, giving a residence time in the Elysator of approximately 5-8 min. In the 100-L reservoir slow stirring (50 rpm) was applied to ensure good mixing. The anode current signal of the Elysator was monitored. Dissolved oxygen, pH, and turbidity (only pH and dissolved oxygen in Test I) were measured in the upper part of the water reservoirs. Water samples for bacterial analysis and for analysis of total organic carbon (TOC) and dissolved organic carbon (DOC) were taken out from the upper part of the reservoirs after gentle stirring at indicated times (see figures 2, 3 and 4). Bacterial analysis was also done of the biofilm developed on the inner wall of the Elysator and of deposited sludge at the bottom of the Elysator at the end of Tests II and III. Control cultures for turbidity and microbial growth were taken out from the reservoir right before starting the Elysator.

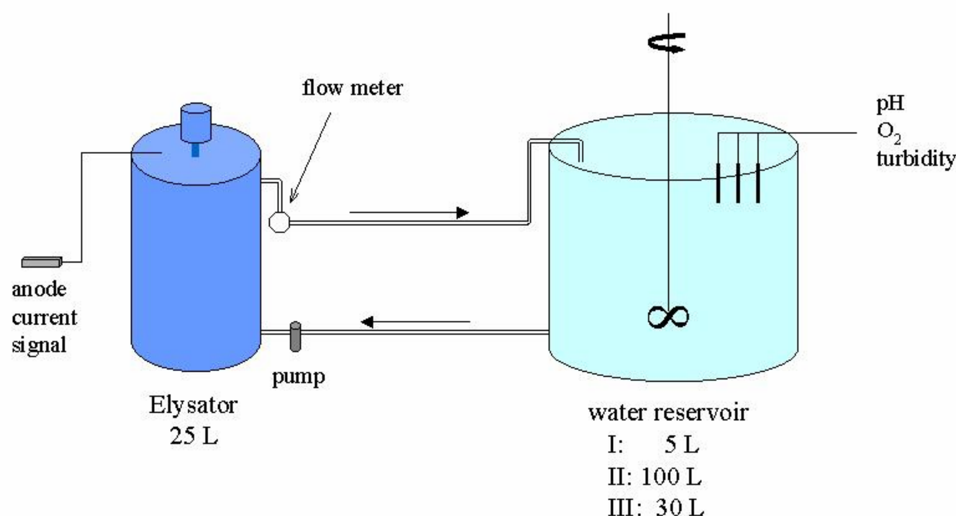


Figure 1. The Elysator test system. Reservoir I was used in the pre-test, and the reservoirs II and III were used in the main test part 1 and 2, respectively. Turbidity was not monitored in the pre-test.

The water used in the tests was tap water supplied from the water facility Oset serving major parts of Oslo City. No chlorine residue was detected in the water. Except from in Test I, the tap water used was always preheated to 60°C to inactive indigenous bacteria and then cooled down to 30°C before inoculation of test organisms.

The water was slightly enriched in readily degradable organic matter by adding a limited amount of nutrient broth (NS-ISO 6222); 0.5 ‰ in Test I and 1.0 ‰ in the other tests. This increased the total organic carbon (TOC) concentration from the natural background of 3.5-4 mg C/l to 5.7 mg C/l and 7.7 mg C/l, respectively.

The external oxygen transfer was controlled by continuously flushing the headspace of the reservoir with either CO_2 gas or N_2 gas. When flushing with CO_2, the pH decreased significantly (from pH ~10 to pH ~5.5) due to the increased carbonate concentration when CO_2 was dissolved in the water.

During Test I semi-constant pH conditions (pH 6.5-8) were obtained by slightly increasing the buffer capacity of the water (by adding K_2HPO_4 up to 0.2 mM), and adjusting the pH below pH 8 with (25 ml) 1M HCl during the test period. Otherwise the pH was left floating.

All tests were performed at 27-29 °C.

2.3 Cultivation and enumeration of test organisms

Bacterial strains
The following bacterial strains were used in the test:
- *Klebsiella pneumoniae* (SLV-186, Statens livsmedelsverk. Sweden)
- *Pseudomonas fluorescens* (ATCC 49642)

Pre cultivation
The micro-organisms were cultivated on nutrient broth (NS-ISO 6222; 3 g yeast extract and 6 g tryptone per litre) in separate tubes over night at 30°C. The cultures were stored at 4°C no more than four days before use. 10 ml of each of the prepared suspensions of bacteria was added to the reservoirs at the times indicated.

Reference cultures
Reference cultures were taken from the reservoir right before each time the Elysator was started and kept in heat-treated (at 60°C for a few hours) 250-ml glass flasks. The flasks were kept in the same temperated room as the test system. Samples for measuring turbidity and microbial analysis were taken out aseptically.

Sampling of bacteria
Bacterial sampling of 10-100 ml water samples was taken aseptically from the reservoir using sterile pipettes. Biofilm on the inner wall of the Elysator was sampled by sequential scrubbing with heat-treated (at 60°C for a few hours) cotton pins dipped in sterile buffer and rinsing with sterile buffer. The rinse water was collected using a sterile pipette. Sludge deposited on the bottom of the Elysator was sampled through the bottom outlet after emptying the Elysator for water. The sludge was gathered together using a heat-treated (at 60°C for a few hours) plastic scraper, and washed out through the drain with sterile buffer.

Detection and enumeration of bacteria
A selective medium, m-Endo broth MF (DIFCO) was used for detection of *Klebsiella pneumoniae*. The membrane filtration technique, with membrane filters (Sartorius filters) of a pore size of 0.45 µm, was used. Samples were diluted in sterile buffer at different 10 log dilutions in the first 4 hours of samplings before the filtration steps, and for the 8 hours, 24 hours and 50 hours samplings up to 100 ml was filtrated.

Standard plate count media (NS-ISO 6222) was used for the enumeration of *Pseudomonas fluorescens*. The pour plate technique was used. These results include “all” cultivable heterophic bacteria present in the water, including *K. pneumoniae*.

The number of colonies was counted after 24 hours of incubation at 35°C for *K. pneumoniae*, and after 3 days of incubation at 22°C for “*P. fluorescens*”.

2.4 Measurements and chemical analysis

The pH was measured using a Radiometer combined electrode (GK2401C) connected to an ORION 720 instrument.

The dissolved oxygen concentration was measured using a WTW oxygen meter, OXI 340, with a CellOx 325 standard electrode.

The total organic carbon (TOC) content of the water was analysed according to NIVA’s internal method G4-2. The dissolved organic carbon content was measured using the same method on water samples filtered through a GC/C-filter.

The turbidity was measured as the absorbance at 550 nm using a 2500 Odyssey spectrophotometer from HACH.

3. Results and discussion

3.1 Test I

In Test I the pH was kept relatively constant (between pH 6.8 and 7.7). A small reservoir volume (5 L) compared to the 25-L Elysator and a minimised oxygen transfer from the outside, were expected to give a rapid depletion in the dissolved oxygen concentration. Within approximately 24 hours anaerobic conditions were reached, as seen in Figure 2.

As figure 2 also shows, at this point the total amount of bacteria (*K. pneumoniae* and *Ps. fluorescens*) had increased almost 3-log. However, almost no *Klebsiella pneumoniae* was found (more than 4-log decrease). In the control culture, which was water removed from the Elysator before it was started, the amount of *Pseudomonas fluorescens* had inclined considerable less than in the Elysator – a little bit more than 1-log – but Klebsiella had "only" declined from 5500 to 1260 CFU/ml (0.6-log). A minor acidification was observed in the control water, from pH 7.2 at start to pH 6.5 at the end of the test. The dissolved oxygen concentration was then 7.1 mg/l.

A very rapid removal of *K. pneumoniae* happened between two and four hour's duration time. Within this period the oxygen level was reduced from around 90% to a little bit less than 60%, and the pH fell from 7.7 to 6.8 after addition of hydrochloric acid, hence both oxygen and pH conditions were favourable for growth. At this point there should also be plenty of substrate for growth. The solitary removal of *K. pneumoniae* excludes precipitation as a plausible removal mechanism.

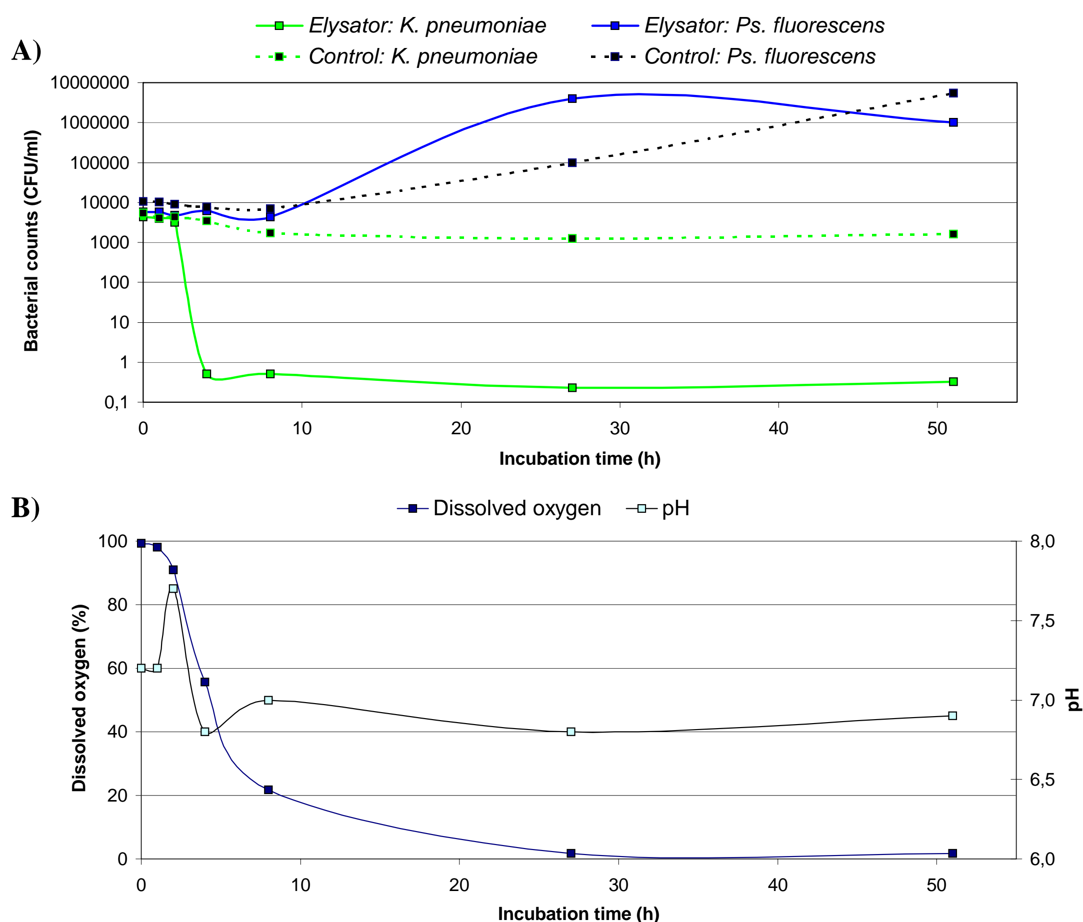


Figure 2. Results from Test I, using pH control. **A)** Bacterial density of *Klebsiella pneumoniae* and *Pseudomonas fluorescens* in the Elysator system and in the control culture taken out before starting the Elysator. **B)** Dissolved oxygen concentration and pH in the reservoir.

3.2 Test II: effects of pH and dissolved oxygen

During this more than 300-h test period several (intentional and non-intentional) changes in the environment were carried out to evaluate possible effects caused by different pH and dissolved oxygen levels. The results are given in figure 3, the heading showing the main changes made. A brief description of the results in the six indicated periods follows:

1. Initialisation (0-50 hours)

The Elysator had not been started, and the intention was to let the bacteria grow freely in the system. The dissolved oxygen concentration dropped from around 85% to 70%, and the pH was stable at about pH 7. A more than 2-log increase in *Ps. fluorescens* and an approximately 0.5-log increase in *K. pneumoniae* were found after 50 hours. The turbidity data indicates exponential growth the first 24 hours, after which it levelled off. This is in accordance with growth patterns observed during pre cultivation. This tells us three important things:

1) Bacteria grew well in the system, though the growth of *K. pneumoniae* was significantly restricted compared to that of *Ps. fluorescens*. The latter probably out-compete *K. pneumoniae* on substrate, an effect often observed in mixed cultures with *Ps. fluorescens* when the substrate concentration is low (Ref 2).
2) The bacterial growth (increase in numbers) had stopped, most probably because of lack of available substrates.
3) Turbidity data seems to describe the growth of bacteria well.

2. Elysator connected to an open reservoir (50-92 hours)

Starting the Elysator, an immediate response in the water's pH was seen. It increasing from pH 7 to more than pH 9.5 in 16 hours and above pH 10 after 8 hours more (at 84 h). Due to high external oxygen transfer into the water reservoir, the dissolved oxygen level in the system was still around 60% at 92 hours. This resulted in a slight decrease in turbidity and an apparent decrease in both bacterial strains of approximately 1-log. However, the bacterial counts of *Ps. fluorescens* may have been underestimated by up to 2-3X because of to low dilutions during enumeration (to many colonies on the agar plates to be counted accurately). Furthermore, a similar reduction in the bacterial counts were found in the control cultures.

This showed that a high pH alone would not significantly remove or inactivate the bacteria.

3.-5. Flushing the reservoir headspace with CO_2 (3: 92-104 h; 4: 104-155 h; 5: 105-208 h)

3: By flushing the headspace of the reservoir with CO_2 one influences three important factors: 1) the external oxygen transfer is minimised because the oxygen concentration in the air above the water surface is minimised, 2) the pH in the water is decreased due increased carbonate content caused by raised dissolution of CO_2, and 3) increased buffer capacity of the water due to the higher carbonate content. As can be seen in figure 3, the pH dropped almost immediately from above pH 10 to about pH 5.6 and the dissolved oxygen concentration started dropping.

4: An accidental stop in the water circulation between 104 and 155 h followed, and the CO_2-flushing also stopped around 110 h. This led to a slow increase in the pH and a marked increase in the dissolved oxygen concentration in the reservoir.

5: When starting the water circulation and CO2 flushing again at 155 h, the oxygen concentration once again dropped and the pH increased more rapidly. However, the high buffer capacity of the water made it difficult to reach a pH above 10, and it also seemed difficult to obtain anaerobic conditions.

During this almost five-day period a slight decrease in turbidity was found, indicating continued high bacterial counts. One could expect a significant reduced proliferation of bacteria due to the stress caused by the rapid change in pH.

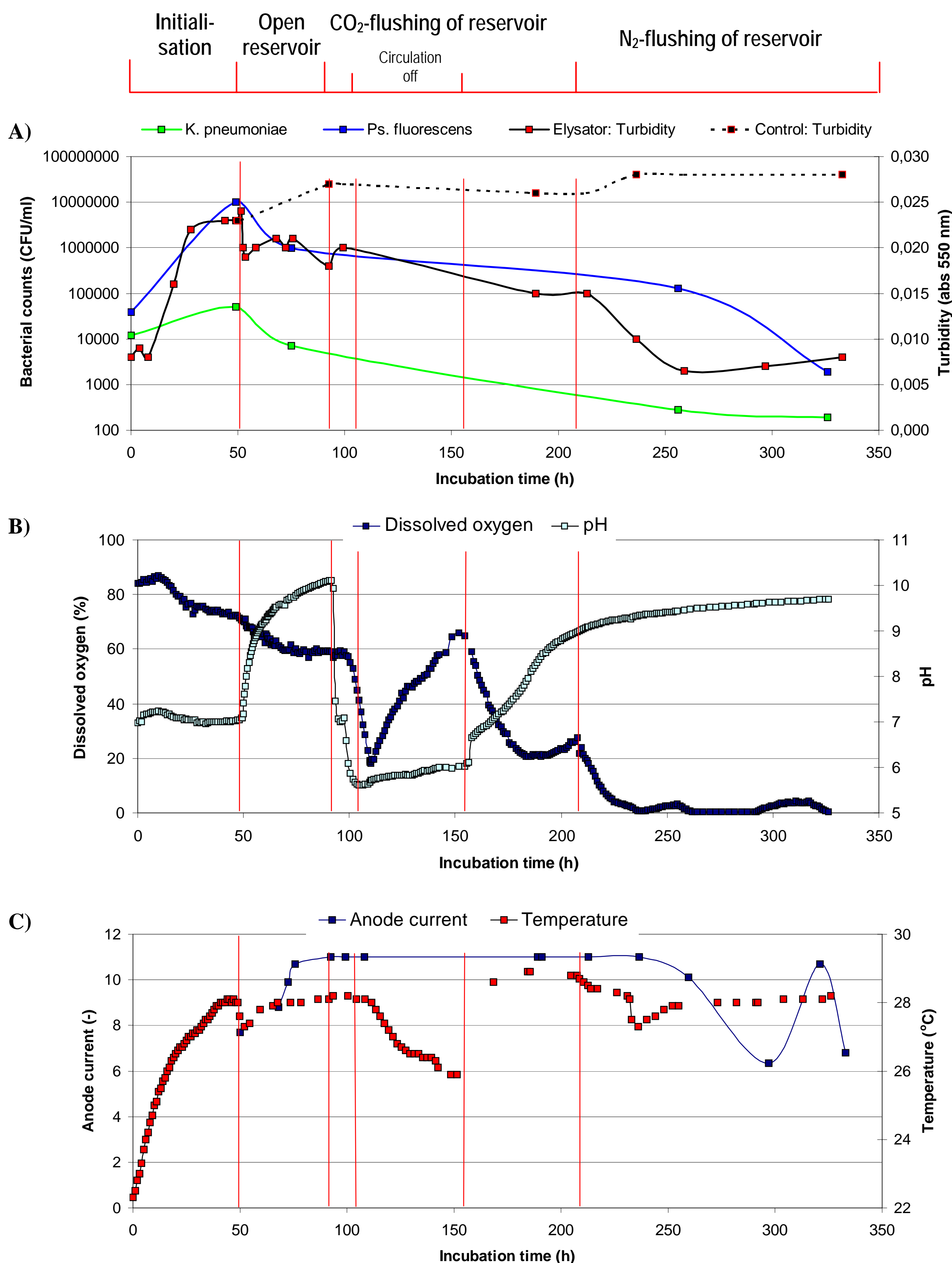


Figure 3. Results from Test II. The main changes made during the 326-h test period are indicated in the heading of the figure and the times of changes is indicated with red lines. **A)** Turbidity and bacterial density of *Klebsiella pneumoniae* and *Pseudomonas fluorescens* in the Elysator system. **B)** Dissolved oxygen concentration and pH in the reservoir. **C)** Anode current of the Elysator and temperature in the reservoir.

6. **Flushing the reservoir headspace with N2 (208-326 hours)**
   The CO2-flushing was replaced by N2-flushing, which eventually led to anaerobic conditions around 236 h. However, it was difficult to keep a steady N2 flow, so the dissolved oxygen concentration fluctuated between 4% and 0.2% the next 100 hours.

   When anaerobic conditions were reached, the turbidity had halved, equalling the turbidity of tap water. However, at this point the bacterial counts of *Ps. fluorescens* were still high (130.000 CFU/ml), though about 2-log lower than when the Elysator was started around 200 hours earlier. During the following 70 hours, with a constant high pH and low oxygen level, the bacterial counts of *Ps. fluorescens* were reduced by almost 2 logs (to 1900 CFU/ml), and a concomitant slight increase in turbidity. *K. pneumoniae* were found in low numbers (280 CFU/ml and 190 CFU/ml) in the same period.

3.3 Test III: effects of increased organic and microbial loads

In Test III the purpose was to test the Elysator under “realistic” conditions. 90% of the highly buffered water was replaced by fresh water and more nutrients were added (1.0 ‰ nutrient broth) to promote additional bacterial growth. To ease the “anaerobification” of the system, the 100-L reservoir was replaced by a 30-L reservoir with a narrow bottleneck. Before the Elysator was turned on, the water was circulated for 24 hours, giving bacterial counts of *Ps. fluorescens* and *K. pneumoniae* of 35.000 CFU/ml and 110 CFU/ml, respectively.

As figure 4 shows, anaerobic condition was reached around 27 hours after the Elysator was started and remained anaerobic to the end of the experiment. The pH was then 10.2 and stabilised later at pH 10.3-10.4, which was held throughout the remaining part of the experiment (the apparent low pH response in the beginning was due to instrumental problems and not real). In the same period the turbidity had increased slightly, probably as a result of continued growth. After this the turbidity levelled out and decreased significantly, though, still somewhat above the turbidity of tap water. An almost two-log decrease in bacterial counts of *Ps. fluorescens* (down to 500 CFU/ml) were found after 70 h, and the counts of *K. pneumoniae* had decreased to 5 CFU/ml. At the same time, bacterial counts in the control culture of the respective bacteria were 130.000 CFU/ml and 40 CFU/ml. Hence, compared to the control culture, the *Ps. fluorescens* numbers had been decreased by more than 99% and the numbers of *K. pneumoniae* by almost 90%.

In the following 70-h period, after additional *K. pneumoniae* (to 5800 CFU/ml) and nutrients (1.0 ‰ nutrient broth) had been added to the reservoir (at 71 h), no sampling were done. But at the end of this period the number of *K. pneumoniae* had decreased to 14 CFU/ml and the number of *Ps. fluorescens* had been halved (to 260 CFU/ml). The turbidity had decreased further and levelled out close to the turbidity of tap water.

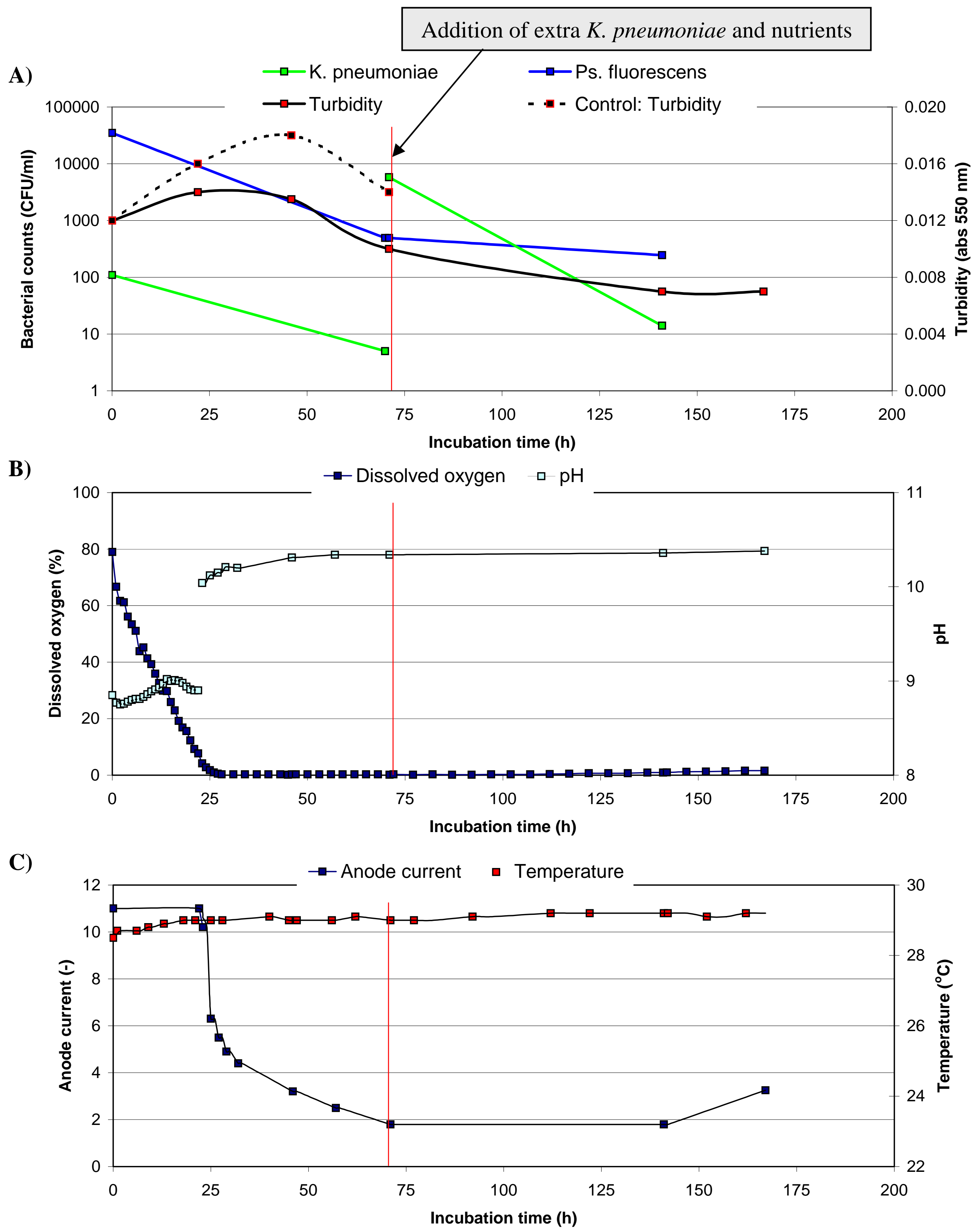


Figure 4. Results from Test III. The time (70 h) of addition of extra *Klebsiella pneumoniae* and nutrients is indicated with a red line. **A)** Turbidity and bacterial density of *Klebsiella pneumoniae* and *Pseudomonas fluorescens* in the Elysator system. **B)** Dissolved oxygen concentration and pH in the reservoir. **C)** Anode current of the Elysator and temperature in the reservoir.

3.4 Where are the bacteria?

The results give strong indications that the Elysator, when operated properly and hence giving anaerobic conditions and a pH above 10, eliminates "the bacterial activity" from the water. Bacterial analysis of the sludge deposited on the Elysator bottom (at the end of Tests II and III) revealed very low numbers of cultivatable bacteria. Though the organic content of the sludge was as high as 42-43% (dry weight), active bacteria constituted not more than 0.00001% of this[1]. The numbers are too uncertain to estimate the share of the total bacteria deposited on the bottom of the Elysator (or in the reservoir), but the turbidity data indicates that a main part has been removed from the water.

The remaining part of the sludge (57-58%) is inorganic, and most probably its content is mainly precipitates of magnesium hydroxide. It is likely that these precipitates have influenced the deposition of bacteria, though, bacteria when stressed (e.g. by highly unfavourable conditions) tend to flocculate into larger aggregates that may sediment without the help of a coagulant such as magnesium hydroxide.

3.5 General validity and limitations

As described in the introduction, *Pseudomonas fluorescens* is a bacterium that thrives under many different conditions because of its low nutritional demands. As an opportunistic bacterium *Klebsiella pneumoniae* also can stand rather rough conditions. Hence, it is likely that the apparent bactericidal effect shown on these bacteria by the conditions enforced upon them by the Elysator will show a similar effect on most bacteria. The high pH will most likely also repress the growth of yeasts. However, it must be stressed that complete "sterile" conditions are highly unlikely to be obtained in the long run, and that it is not possible to rule out that an opportunistic bacterium could have a large out-brake in the system. That goes for standard disinfection procedures as well.

The Elysator is applied in many different systems, and the experiments that have been carried out have not been designed to test all possible environmental conditions. The most important factors that have not been taken into (adequate) consideration are the capacity of the Elysator (how the efficiency is correlated with the size of the system, e.g. total volume and flow of water), continuous (heavy) nutrient loading, and temperature. It is to be expected that the larger the system is (e.g. increased reservoir volume compared to the internal Elysator volume), the longer it takes to obtain "bactericidal conditions". Leakage of nutrients and oxygen into the system will also constitute a potential heavy load on the system.

Yet another important factor is exhaustion with time. After each experiment a thin film (probably) of magnesium hydroxide had developed on the anode sticks. It is likely that there exist a certain limit when this film gets so thick that it restricts the diffusion of oxygen into non-oxidised magnesium. Hence, the anode should be checked and cleaned regularly. How often this should be done is not possible to say from the results herein.

3.6 Possible effects on *Legionella* sp. and algae in cooling towers

Hot water systems such as cooling towers, evaporative condensers and hot water services offer an environment that may promote the proliferation of pathogenic *Legionella* sp. This rather common bacteria are usually found in low numbers, but since it grows well at temperatures up to 50ºC it may be found in so high numbers (> 10.000 per litre) in these hot water systems that they may represent a

[1] 50 mg organic material removed from the Elysator after part 2 contained 35.000 CFU. Given the normal dimensions of these bacteria (0.8-1 µm x 1.5-2 µm), and an expected dry weight of 15-30%, these bacteria would in total contain ~10 ng (10^{-9} g) organic material, or ~0.00002% of the total. The corresponding numbers of part 1 were 21.300 CFU in 45 mg organic material, giving ~0.000004% live organic material compared to the total.

great health risk to humans. They usually grow up inside biofilms attached to surfaces in the system. However, they only constitute a real threat when transported in aerosols (small water droplets) of a size small enough to reach the lungs. The growth of *Legionella* sp. is usually controlled by regular disinfection and cleaning of the hot water system. Addition of a biocide to the water is also often recommended.

All *Legionella* sp. are aerobic, hence they can not grow under anaerobic conditions. And their optimum pH for growth is reported to be 6.8-7.0, which further indicates that the conditions obtained by the Elysator under optimum operation will suppress the proliferation of *Legionella* sp. However, cooling towers are constructed to maximise the contact between air and water for heat transfer, giving a steady rich supply of oxygen to the water. It is not likely that the Elysator will be able to provide anaerobic conditions in this type of system. The high pH may still prevent rapid growth of *Legionella* sp., and magnesium hydroxide precipitates may still cause the removal of bacterial cells from the water. Regular disinfection and cleaning should still be recommended. See also 3.7 Operation control, below.

Algae are a large and diverse group of so-called eucaryotic organisms (in contrast to bacteria that are procaryotes) with a mutual and literally sole nutritional necessity: light. This makes it difficult to prevent the growth of algae in systems exposed to direct sun light. As before; regular disinfection and cleaning is recommended.

3.7 Operation control

A high pH alone or anaerobic conditions alone do not seem to be sufficient to obtain the desired bactericidal effect by the Elysator. The combination of pH above 10 and en anaerobic environment seems to be necessary.

From the anode current data given in figures 3 and 4 there is a striking correlation between a measurable reduction in the anode current (from the maximum ~11 units) and anaerobic conditions. This is not unexpected, as the flow of electrons from the anode to the cathode is dependent on oxygen as an “electron sink”. This indicates that the amplitude of the anode current may be used as an indicative parameter for whether oxygen is present or not in the Elysator. By comparing the dissolved oxygen concentration and anode current read-out during the last operating hours in part 1 of the main experiment (figures 3b and c), it appears to be a relatively sensitive parameter.

To verify high pH values (above pH 10) water samples should be taken regularly, the frequency depending on the instability of the system. This instability can be measured as fluctuations in pH and/or anode current output.

4. Conclusions

When operated properly and hence giving anaerobic conditions and a pH above 10, the Elysator appears to show bactericidal effect on *Pseudomonas fluorescens* and *Klebsiella pneumoniae*. Low bacterial counts were found both in the water and in the sludge deposited on the bottom of the Elysator. Due to the, for most microbes, unfavourable conditions enforced by the Elysator and the nature of the tested bacterial strains, this bactericidal effect is likely to be rather general. However, complete wipe-out of bacteria or “sterile” conditions in the long run is highly unlikely. Nevertheless, the Elysator will strongly contribute to low bacterial numbers in the water.

Dissolved oxygen and pH levels should be checked regularly. An anaerobic environment seems to coincide with a lowered anode current output, but should be verified with standard oxygen electrode measurements from time to time. For stable operation the anode sticks in the Elysator should be cleaned regularly, finding the necessary cleaning-frequency has not been a part of this project.

5. References

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