The use of ultrasound to prevent bacteriological biofouling in pipelines

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Abstract

The main issue to disinfecting water is the inactivation or removal of bacteria. Bacteria can form biofilms that clog pipelines and other material to transport water. Chemicals are often used to remove the biofouling. A new technology to prevent biofouling is the use of ultrasound. Especially the use of non-cavitational ultrasound is of great interest. Most research done until now involved ultrasound with cavitation, but this is due to the high energy inputs neither feasible nor sustainable, therefore it would be very interesting to find out whether ultrasound without cavitation could also prevent biofouling in pipelines. An experiment was done three times, using four straight pipelines (with and without ultrasound). Biofouling was measured by means of calculating the light extinction coefficient out of the light intensity that went through the pipelines. Results show that during the starting phase, the biofouling with ultrasound is slowed down, but only in case of a very clean (disinfected) pipeline. The ultrasound is extending the first phase of biofilm growth, resulting in a possibility of extending the periods of cleaning for companies. However, when the ultrasound is applied to a pipeline with biofouling already present, the biofilm growth is stimulated by the ultrasound. This is probably due to the better distribution of nutrients towards, and wastes from, the biofilm, caused by the ultrasonic waves. So non-cavitational ultrasound does not kill, or remove bacteria, nor does it stop the biofilm growth, it is only able to extend the starting phase of the biofouling. It takes longer for bacteria to settle on the surface of the pipelines when ultrasound is applied on a clean pipeline.

Keywords: Ultrasound, biofouling, bacteriological, disinfection, water

1. Introduction

1.1 Water treatment

The use of chemicals to disinfect and clean water is increasingly considered to be harmful for public health and the environment. Therefore newly developed technologies for water treatment focus on durability and minimal, or no use of chemicals. The main issue to disinfecting water is the inactivation or removal of bacteria. Especially pathogenic bacteria are a threat to public health. The accumulation of microorganisms like bacteria is called biofouling (Figure 2). Bacteria can form biofilms (Figure 1) that clog pipelines and other material to transport water. Furthermore, biofilms support settlement of pathogenic bacteria and removal of biofouling is costly. Chemicals (like chlorine) are often used for removal.

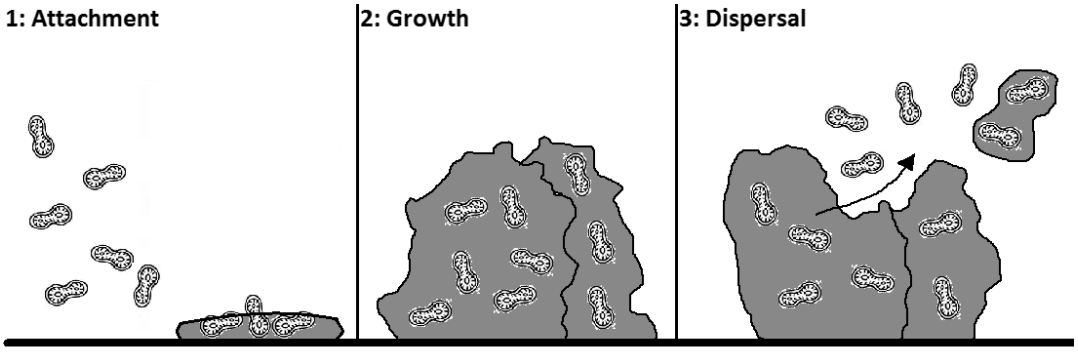

Figure 1 Biofouling: The formation of a biofilm. Bacteria settle on a surface by the excretion of a slimy substance. When settled, the colony can grow and disperse.

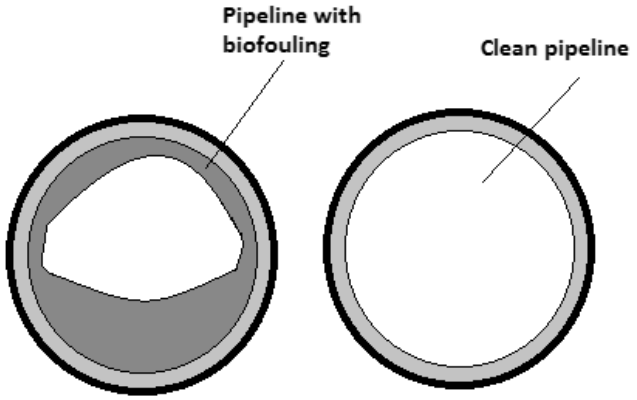

Figure 2 Biofouling in pipelines.

1.2 New technologies

A relatively new technology to prevent biofouling is the use of ultrasound. When ultrasound is applied in water at high energy levels, cavitation will take place. Cavitation is the formation of resonating cavities or bubbles. In water cavitation occurs with ultrasound excited with a power more than 0,33W/cm^2 (Adewuyi, 2001). There are two types of cavitation: Non-inertial cavitation and inertial cavitation (Leighton, 2007). When non-inertial cavitation occurs, the cavitation bubble remains stable during each sound cycle. During inertial cavitation the bubble grows and becomes instable in the end, collapses and generates high pressure and temperatures, causing the formation of reactive free radicals, like the hydroxyl radical (Adewuyi, 2001). Due to the mechanical, physical and chemical forces created by cavitation, destructive effects on living cells can be expected (Antoniadis, 2007).

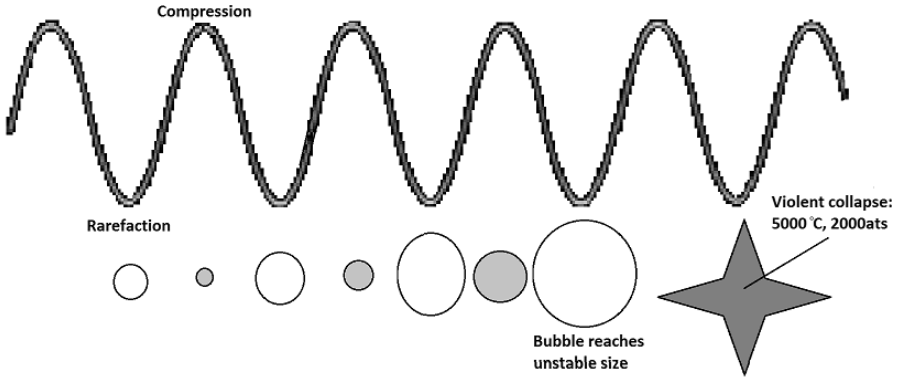

Figure 3 Sound. The transport of sound circles forming a wave (compression and rarefaction). Below the formation of a cavitation bubble, in this case being inertial.

1.3 The effects of ultrasound on bacteria and biofouling

The effect of ultrasound on living bacteria has already been investigated in several studies. Whether ultrasound could kill bacteria, depended on the type of ultrasound that was applied and on the bacterial species. For instance, *E.coli* was eliminated according to several researchers (Antoniadis, 2007), (Blume, 2004) while *Bacillus globigii* was extremely resistant (Holm, 2008). The research mentioned above used high intensities, which means cavitation occurred. Whether ultrasound without cavitation can kill bacteria, has not been investigated yet.

The effect of ultrasound on biofouling was already confirmed by several researches. Research already showed a negative effect of ultrasound on the formation of a biofilm. With this research it was assumed that bacteria experienced resonance when they were exposed to ultrasound. Because of this, the bacteria no longer excreted the extracellular

polymeric substances (EPS) to settle on the surface of the pipelines, probably because the bacteria experienced the sound waves as turbulent water (G.Hutchinson, 2008). There is also research that shows a positive effect of ultrasound on the formation of a biofilm. Suggested was that the sound waves support the transport of oxygen and nutrients towards the biofilm, enhancing the biofilm growth (Pitt, 2003). Above mentioned research applied ultrasound without cavitation. Ultrasound with cavitation also showed removal of biofilm (Pitt, 2003).

1.4 New research to stop biofouling

Most research that has been done until now involved cavitation, requiring a high energy input. Therefore the technique is neither feasible nor sustainable. The main aim is to find out if ultrasound without cavitation could also stop biofouling. If so, this application would be a durable solution to prevent biofouling in pipelines. This would reduce cleaning costs and the use of chemicals. Secondly it is interesting to find out whether or not bacteria are killed by low intensity ultrasound (Rajasekhar, 2012).

2. Methods

2.1 The system

A pilot system was made to test if ultrasound without cavitation could be a suitable solution to prevent biofouling in pipelines. The pilot consisted of two similar systems with two transparent pipes of 49mm diameter each. In one system the water inside the pipes was exposed to ultrasound (20W electrical power) by transducers, type VAM Sonic (Van Antwerpen Milieutechniek, NL), which were inserted at the bottom of each pipe. Each system had its own water storage. The water that was used was salt groundwater (28g/l). The water was pumped from the bottom to the top of the pipelines, and then recirculated to the storage. The volume used per system was approximately 50- 100L. Each pipeline had a flow-meter installed and a valve, so that the flow could be adjusted. The flow was approximately 850l/h for each pipeline. The flow defines partly the growth of a biofilm: the faster the flow, the longer it takes for bacteria to settle. During the experiments, the pipelines where covered with isolating foam, resulting in formation of bacterial biofilm instead of algae which need light to grow. The experiment was done three times.

To stimulate the bacterial growth, several nutrients were added to the water with the use of feeding pumps (appendix):

1. Glucose
2. Yeast extract
3. Peptone
4. Sodium phosphate ($Na_2HPO_4.2H_2O$)

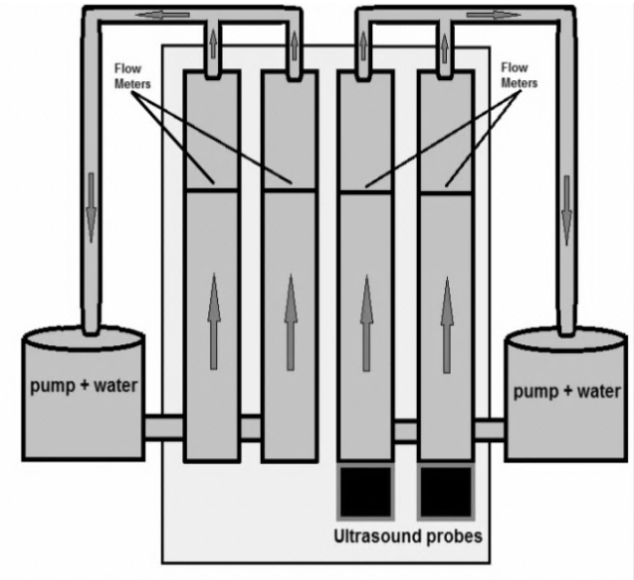

Figure 4 The pilot system. Two systems with two pipes each. One system had ultrasonic probes installed. The arrows display the flow direction of the water. The nutrients were added in the barrels.

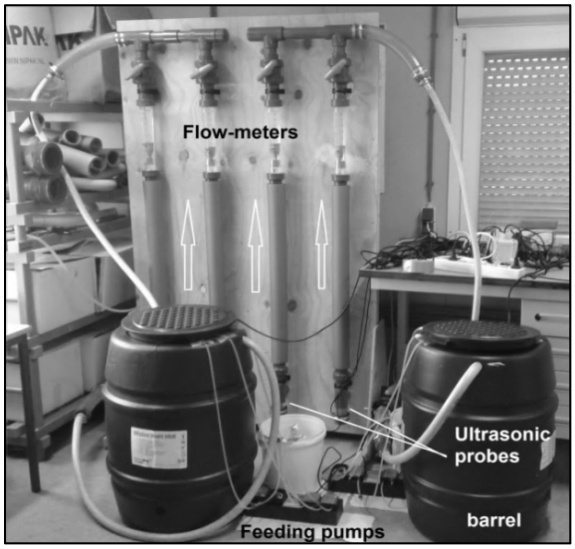

Figure 5 Photograph of the pilot system. The white arrows indicate the flow-direction. During the experiment every pipeline (also the flow-meters) where covered with a grey cover as can be seen in this picture, in order to prevent algae growth in the system.

2.2 Monitoring

The biofouling was monitored by means of light transmission through the pipes. As soon as biofouling took place, the light transmission of the pipelines decreased. A light intensity meter was used to measure the light transmittance.

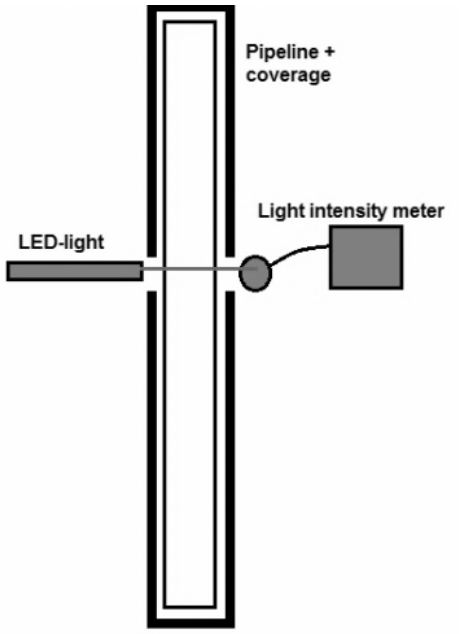

Figure 6 The way of monitoring of the biofouling in the pipelines.

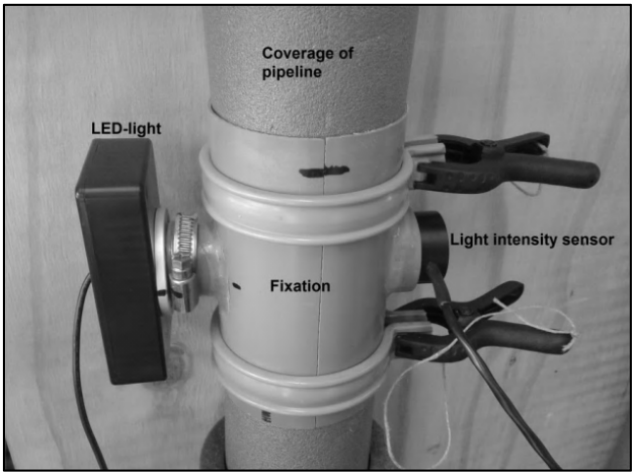

Figure 7 Picture of the way of monitoring the biofouling in the pipelines.

Transmission was determined using a LED light source on one side of the pipe and measuring intensity at the opposite side. The LED-light that was used had a beam angle of 3°. Because of this low angle, the light aimed directly at the sensor, without the light being scattered through the entire pipeline. The device that was used to measure the light transmittance was the Skye Instruments LTD with Quantum Sensor (range 0-20.000 µmol/sec/m^2). For each pipeline three fixed measuring points were taken. A special cover, holding the LED on one side and the sensor on the other, was placed around the pipeline during the measurements to ensure consistency in positioning. Each point was measured twice per measurement.

Blank pipeline 1	Blank pipeline 2	Ultrasound pipeline 1	Ultrasound pipeline 2
B1,1	B2,1	US1,1	US2,1
B1,2	B2,2	US1,2	US2,2
B1,3	B2,3	US1,3	US2,3

Tabel 1 Definition of the measurement points for each series.

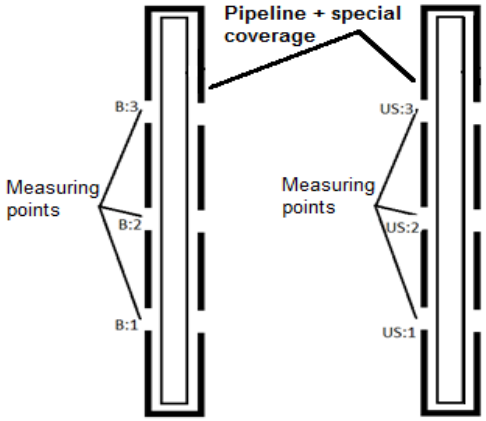

Figure 8 The fixed measurement points, with the use of The special cover.

Several steps needed to be taken to determine the bacterial growth:

1. First the maximum light transmission through the pipelines had to be measured. This was with clear water in a separate, clean test tube. The values measured at the pipelines could never exceed this value due to the biofouling and other bacteria present in the system.
2. With the same test tube samples of water were taken from the barrels and the light transmittance was measured again. This value defined the possible presence of bacteria in the water, thus causing increased turbidity and declining the light transmission through the pipelines.
3. At last the measurements through the four pipelines took place. The light that was transmitted is the light that could get through the biofouling present on the wall of the pipelines, and the bacteria in the water, measured in the second step.

To process the data of the above mentioned steps, the law of Lambert-Beer was applied:

$I_{out} = I_{in} * e^{-Kx}$

- I_{in} was the maximum value that could be measured (the test tube with clear water). This was a fixed value: 0,42(µmol/sec/m^2).
- I_{out}-was the value that was measured through the pipelines and the test tube with water from the barrels. This value was a variable and was dependent on the presence of biofouling and/or bacteria in the water.
- K was the light extinction coefficient. This was proportional to the biofouling at the wall of the pipelines. K needed to be calculated.
- X was the light path, the diameter of the pipelines. This was a fixed value: 0.049m

The light absorption that was caused by the biofouling and the bacteria in the water could be calculated by the transformation of the above mentioned formula into:

$K= -(\ln(I_{out}/I_{in})/x)$

For the determination of the difference between the light absorption caused by the biofouling and the bacteria in the water, the following was done:

- Calculation of the K that represents both the biofouling and the bacteria in the water together (measurement of the four pipelines): K_T
- Calculation of the K that represents the bacteria in the water (measurement through the test tube): K_W
- The K value representing the biofouling is then: $K_B=K_T-K_W$

3. Results

3.1 Experiment I

Halfway the first experiment, some adjustments were made to the test system, splitting the experiment into two phases: phase I before the change and phase II after. The reason for this is that during the first weeks the biofouling did not really seem to start. Therefore it was necessary to make some adjustments in favor of the biofouling. The details will be discussed below. The results will be discussed per phase.

The starting phase of the experiment, phase I (5-4-2013 until 19-4-2013):

- The addition of nutrients was done with feed pumps. During the test it appeared that the dosing of the pumps was irregular and often was not similar to the calculations of the dosing.
- Each barrel contained a volume of 100L water. This was done to obtain an uninterrupted water flow. The water of the outflow was directly dosed into the water of the barrels, without there being a free outlet through air.
- The water of the barrels was never refreshed, making sure that the bacterial culture in the barrels remained stable. In this way a constant climate was created.
- The barrels were not aerated to prevent an explosive growth of bacteria in the water.

The adjustments made, resulting in the second phase of the experiment (22-4-2013 until 3-5-2013):

- Aeration was added to the barrels to stimulate bacterial growth.
- Therefore the water was refreshed every two days (removal of bacteria in the water) to favor the biofouling in the pipelines. Only the water inside the barrels was refreshed and not the water of the pipelines, because the removal of water out of the pipelines could have disturbed the biofouling.
- Instead of 100L of water now 50L of water was used for each barrel, this to shorten the residence time of the water in the barrels. In this way the biofouling was favored because bacteria in the water had not as much time to develop.
- The feed pumps where disconnected and the nutrients were added manually.

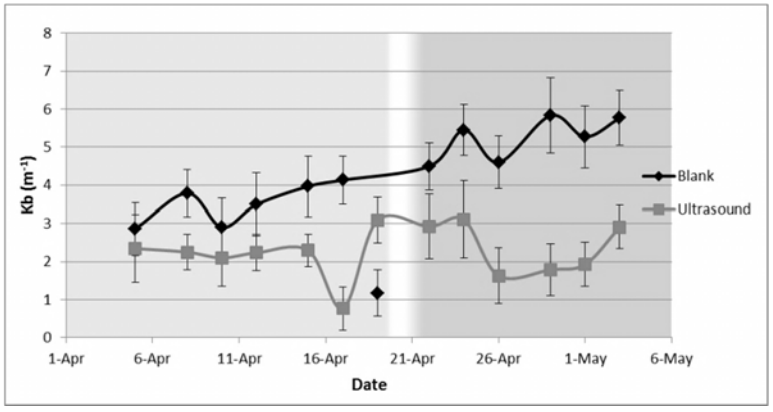

Figure 9 The development of the light extinction coefficient representative for the biofouling (Kb). The color change in the background on 21 April shows the two phases of the experiment. The unexpected outlier at 19-4 of the Blank is shown separately.

Figure 9 shows a clear difference between the biofouling in the blank pipelines and the biofouling in the pipelines with ultrasound. Especially during the second phase the growth in the blank pipelines was clearly visible, while the biofouling in the pipelines with ultrasound remained negligible. The values were significantly different after the first measurement. For both treatments, there was no significant difference between the positions 1, 2 and 3 of the pipelines. Figure A3 in appendix A shows the development of the bacteria in the water for both cultures and phases.

In figure 10 the pictures show a clear difference between the blank pipelines and the pipelines with ultrasound. The biofouling showed best in the flow-meters which are visible in the pictures.

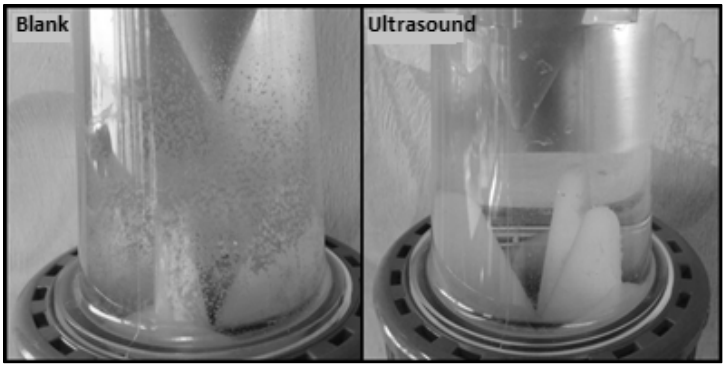

Figure 10 Pictures of the flow meters at the end of the experiment. In the blank, biofouling is clearly visible.

3.2 Experiment II

After the first experiment the system was cleaned and prepared for the second experiment (14-8-2013 until 9-9-2013). At the start of this experiment the ultrasound probes were switched on at the second day, which means the water was not influenced by ultrasound the first day. Therefore the biofilm (for both treatments) was able to grow during the first day, which could explain the difference between the two (Figure 12). The first measurement was done at the second day. It is clearly visible that the biofilm, exposed to ultrasound, had a shock as soon as the ultrasound was switched on. However, after the second day, the biofilm exposed to ultrasound started to grow and eventually exceeded the blank. At the end of this experiment all results were significantly different.

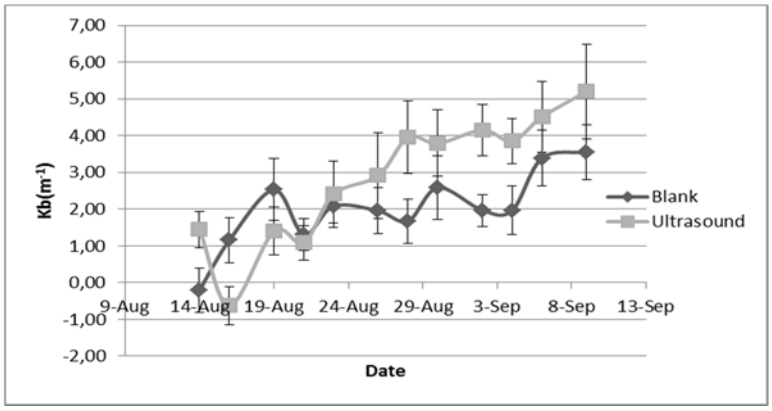

Figure 11 The development of the light extinction coefficient representative for the biofouling (Kb) during the second experiment.

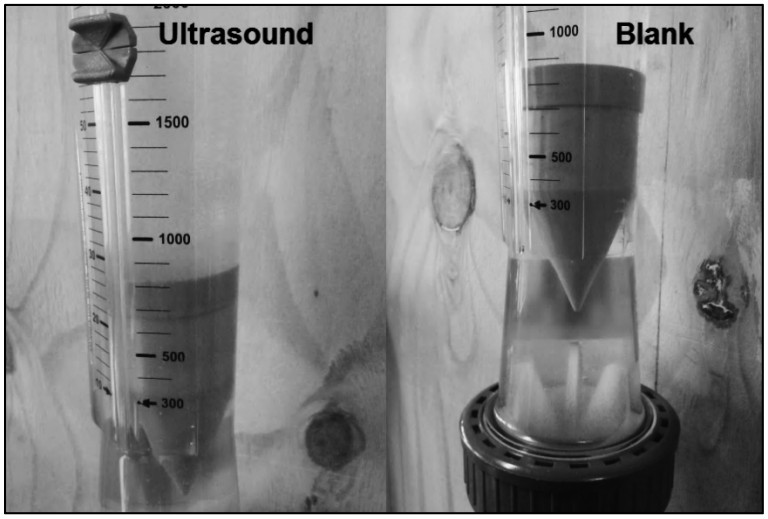

Figure 12 Filmy biofouling visible in the flow meters with ultrasound. In the blank system no biofouling is visible in the flow meters

3.3 Experiment III

In preparation of the third experiment (13-9-2013 until 11-10-2013) the system was cleaned again. This time, the ultrasound was switched on as soon as there was water in the system, this to avoid the immediate fouling found in experiment II. Figure 13 shows that in the starting phase of the experiment the blank was growing immediately while the biofilm exposed to ultrasound started to grow half-way the experiment. At the end of this experiment the results were not significantly different, this was partly due to the high standard deviation caused by the blank. This standard deviation was high due to a higher biofilm growth at one point compared to the other points.

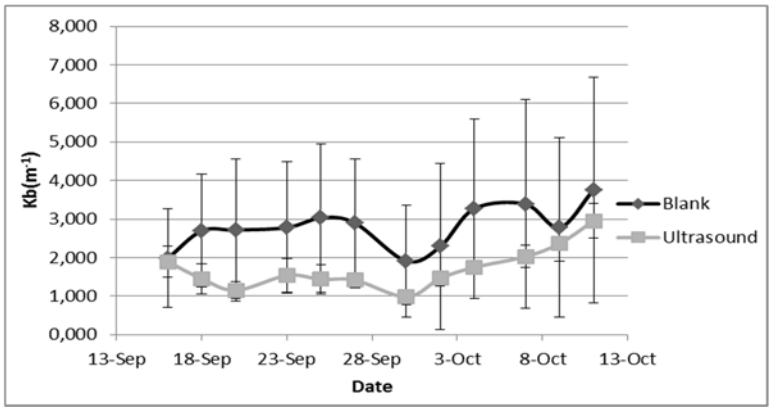

Figure 13 The development of the light extinction coefficient representative for the biofouling (Kb) during the third experiment.

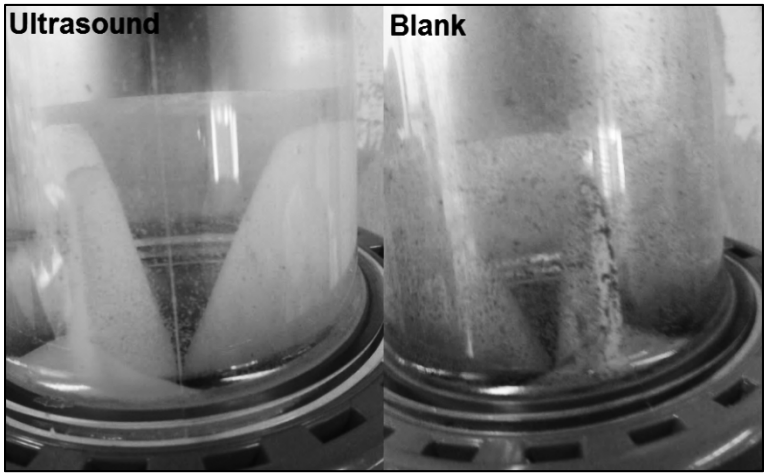

Figure 14 Picture of the biofouling in the flow meters at the end of the experiment. In both systems biofouling is visible, in the blank more than in the ultrasound system.

4. Discussion

4.1 Experiment I

During the first phase of the first experiment the biofouling is already lower in the pipelines with ultrasound compared to the pipelines without. During the first phase however, the amount of bacteria in the water itself is increasing with ultrasound while the water of the blank becomes brighter. The reason for the decline of bacteria in the water of the blank pipelines is not clear, maybe the biofouling is already dominating here (Appendix A, figure A 3).

The effects of the adjustments made during the experiment are quite clear in the second phase. As soon as the regular refreshment of the water in the barrels has started, the biofouling in the blank pipelines (K_b) starts to increase. The light extinction coefficient is increasing from that point. This means that the nutrients, now added manually, are consumed by the biofilm bacteria and no longer by the bacteria in the water, which is also visible in the clarity of the water (K_w) in both barrels. The K_b of the pipelines with ultrasound remains low, indicating the absence of biofouling. This result is similar to the result of (G.Hutchinson, 2008). Secondly, there are also indications that ultrasound can stimulate biofouling (Pitt, 2003). During this research it was assumed that ultrasound increases the nutrient supply towards the biofouling and the waste disposal from the biofouling, which results in a stimulation of biofilm growth. Apart from the study mentioned, no scientific literature can be found that proves the usefulness of low energy ultrasound.

4.2 Experiment II

During the second experiment the results are completely different compared to the first experiment. At the end of this experiment the K_b of the pipes with ultrasound is significantly higher compared to the K_b of the blank pipes. The ultrasound is switched on at the second day of the experiment instead of immediately at the beginning of the experiment, like during the first experiment. Because of this, the bacteria were able to settle on the surface of the pipes the first day. This process of settlement goes very fast (IR. Jan Rijstenbil). The first measurement was at the second day of the experiment. The K_b of the pipes with ultrasound is already higher compared to the blank during this first measurement. As soon as the ultrasound is switched on, the biofilm that already had developed during this first day, seems to have a shock as during the second measurement the K_b is clearly lower compared to the first measurement. The biofilm seemed to have disappeared at this point or at least, was not measureable. At the end of this experiment the K_b of the pipes with ultrasound is significantly higher than the K_b of the blank pipes. The question is whether some biofilm remained at the surface of the pipes which is not visible in the measurements. The biofilm that remains is then

stimulated by the ultrasound (better transport of nutrients and wastes), which results in a faster growth (Pitt, 2003). After the first and the second experiment it could be said that ultrasound is only able to prevent biofilm growth when switched on immediately after cleaning the system. As soon as the biofilm has a chance to grow, it is only stimulated by the ultrasound. In this case ultrasound has no use.

4.3 Experiment III

As mentioned, the ultrasound was switched on immediately at the start of this experiment. This was done because the biofilm growth seemed to be stimulated a lot during experiment II. At the starting phase of this experiment the biofilm growth of the blank pipelines is higher and the growth of the pipelines with ultrasound remains very low and here no growth is visible. However, halfway the experiment a biofilm starts to grow in the pipelines with ultrasound. From that point onward the biofilm is only growing. Again this experiment indicated a delayed growth when ultrasound is applied, but it does not stop the growth. Over time bacteria seem to adjust themselves to the ultrasound and are able to settle on the surface. Probably ultrasound only affects the bacteria in their settlement phase, and stimulates the biofilm growth after settlement.

5. General discussion and conclusion

After three experiments several discussions and conclusions can be made. The biofilm growth behaves different during each experiment compared to the other experiments. Biofilm never grows the same way. Bacteria are of course organisms that have a certain behavior and that respond to environmental conditions. Also not much research has been done yet on low-energy ultrasound, making it hard to refer this research to other research. The first experiment shows a very positive result on the inhibiting effect of ultrasound on biofilm growth, but it is possible that the experiment stopped at the right time when no biofilm growth was present yet. When the experiment would have been performed over a longer period, the result might have been similar to the results of the following experiments. However the results of the three experiments are different mutually, still some conclusions can be made concerning the use of ultrasound.

It is quite clear that when the ultrasound is applied on a dirty pipeline, with biofilm already present, it has no effect. This second experiment indicates that ultrasound even has a stimulating effect (Pitt, 2003). However, when ultrasound is applied to a very clean pipeline, the biofilm growth is delayed by a few weeks with ultrasound, as experiment I and III show. In this way the cleaning period for companies can be extended. This indicates that bacteria somehow need to adjust to the ultrasound before they can settle on the surface of the pipelines. As soon the bacteria are settled, the biofilm growth is stimulated again by the ultrasound (experiment II and III). Experiments on the growth of biofilm in tanks exposed to ultrasound gave similar results (not presented here) as the results presented in this article.

6. Recommendations

Several recommendations towards the use of low-energetic ultrasound to stop biofilm growth can be made:

- Low energetic ultrasound should only be applied to a very clean pipeline, otherwise ultrasound has no use and is only stimulating the biofilm growth.
- As soon the bacteria are settled and stimulated by the ultrasound the system should be cleaned again. The system should therefore often be visually checked on biofilm growth.
- Low energetic ultrasound does not stop the biofilm growth, it only extends the period of cleaning.
- To what extent the use of low-energetic ultrasound is cost effective could not be determined. Cleaning costs however will decrease.

Recommendations for further research:

- It might be useful to see if the biofilm growth with ultrasound eventually will exceed the growth of the blank. Therefore a long term test is necessary, starting with a clean pipeline.

- Also it might be interesting to investigate what ultrasound really does to the bacteria by a microscopic test.
- The use of light to determine the biofilm growth is very applicable. However it should be noticed that the intensity meter probably does not ‘see’ all the biofilm growth. This because it was noticed during all experiments that at some points no, or little growth was measured, while some biofilm was visible. Therefore the experiments should always be visually checked, or a more sensitive intensity meter should be applied.

7. Acknowledgements

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Appendix A: Data of experiments

Experiment 1

Table A 1: Data figure 9: The exact K_B for the blank and ultrasound.

Date	K_B (m^{-1}) Blank	K_B (m^{-1}) Ultrasound
05-04-2013	2,86	2,35
08-04-2013	3,79	2,25
10-04-2013	2,90	2,10
12-04-2013	3,49	2,23
15-04-2013	3,97	2,29
17-04-2013	4,13	0,77
19-04-2013	1,18	3,09
22-04-2013	4,50	2,92
24-04-2013	5,45	3,10
26-04-2013	4,60	1,63
29-04-2013	5,83	1,79
01-05-2013	5,27	1,93
03-05-2013	5,76	2,91

Table A 2: Data figure 10: The exact K_W for the blank and ultrasound.

Date	K_W (m^{-1}) Blank	K_W (m^{-1}) Ultrasound
05-04-2013	5,55	4,31
08-04-2013	4,31	5,55
10-04-2013	4,62	6,87
12-04-2013	4,92	7,56
15-04-2013	3,72	8,64
17-04-2013	3,15	10,59
19-04-2013	4,92	4,01
22-04-2013	2,04	5,55
24-04-2013	1,78	4,01
26-04-2013	2,86	3,72
29-04-2013	1,51	3,72
01-05-2013	1,00	1,51
03-05-2013	1,00	1,51

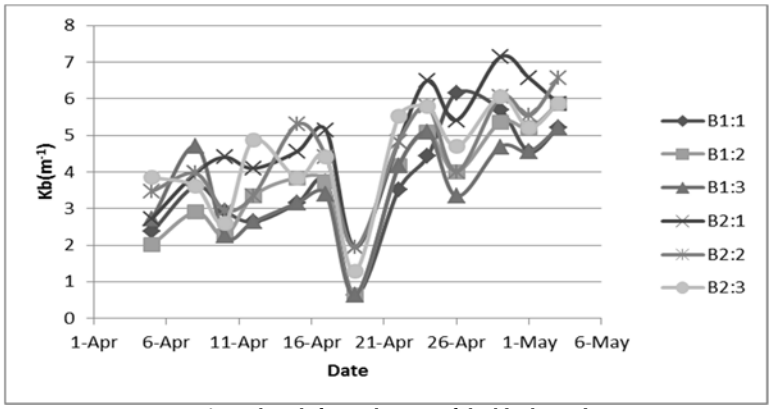

Figure A 1: The Kb for each point of the blank pipelines.

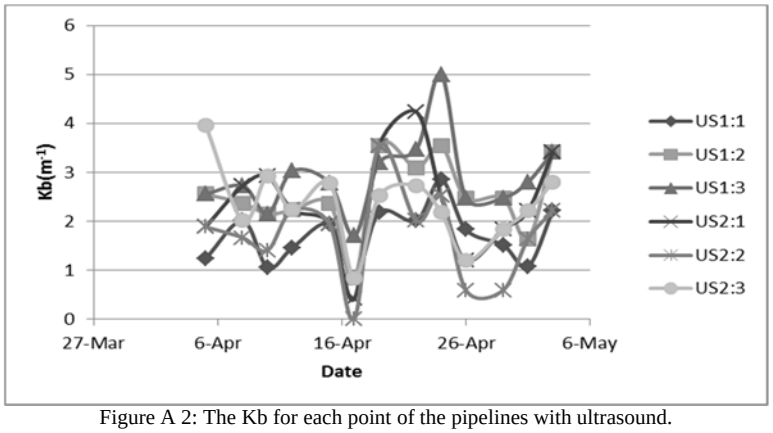

Figure A 2: The Kb for each point of the pipelines with ultrasound.

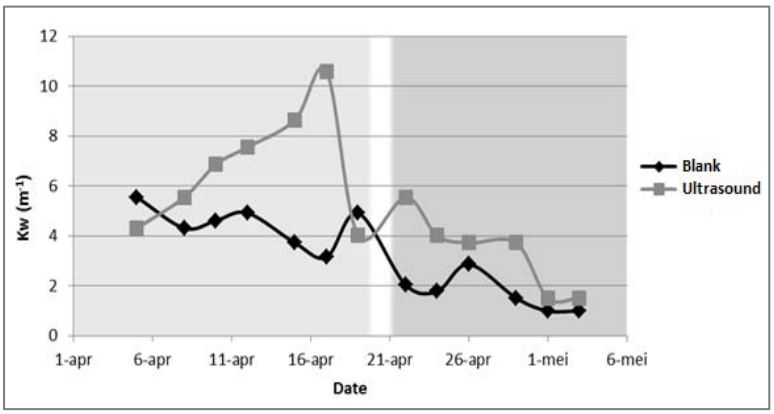

Figure A 3: The development of the light extinction coefficient of the bacteria in the free water column (Kw). The color difference in the background displays the two phases of the experiment.

Experiment II

Table A 3: Data figure 12: The exact K_B for the blank and ultrasound.

Date	K_B Blank	K_B Ultrasound
14-08-2013	-0,20	1,44
16-08-2013	1,15	-0,62
19-08-2013	2,53	1,41
21-08-2013	1,31	1,08
23-08-2013	2,09	2,41
26-08-2013	1,96	2,91
28-08-2013	1,67	3,96
30-08-2013	2,59	3,79
02-09-2013	1,97	4,15
04-09-2013	1,97	3,85
06-09-2013	3,39	4,51
09-09-2013	3,56	5,21

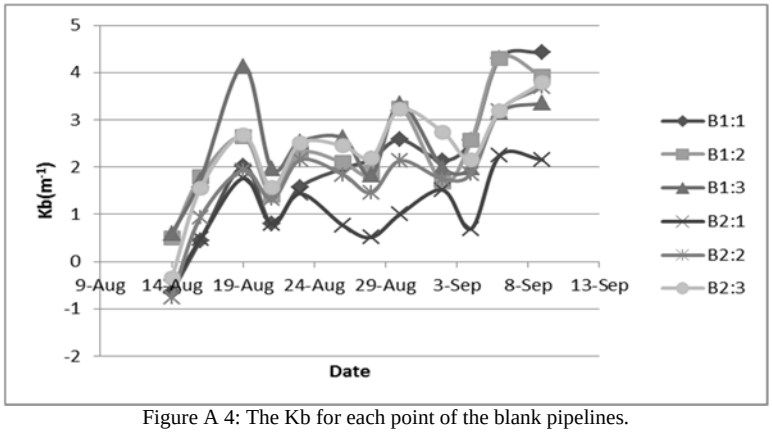

Figure A 4: The Kb for each point of the blank pipelines.

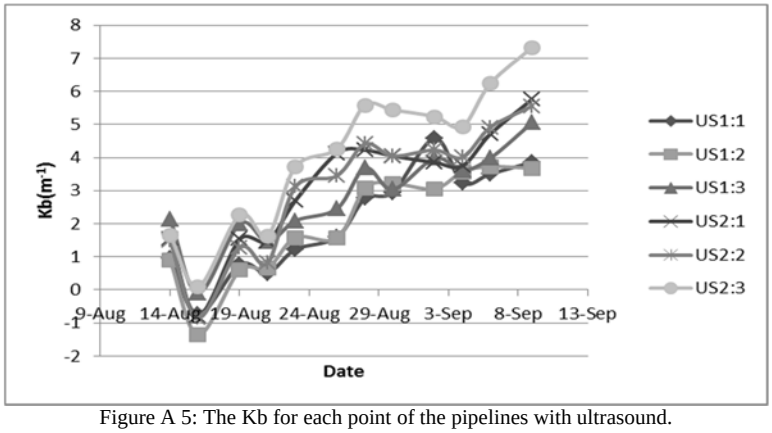

Figure A 5: The Kb for each point of the pipelines with ultrasound.

Experiment III

Table A 4: Data figure 14: The exact K_B for the blank and ultrasound.

Date	K_B Blank	K_B Ultrasound
16-09-2013	1,98	1,89
18-09-2013	2,70	1,45
20-09-2013	2,72	1,15
23-09-2013	2,78	1,54
25-09-2013	3,03	1,44
27-09-2013	2,90	1,42
30-09-2013	1,91	0,98
02-10-2013	2,29	1,46
04-10-2013	3,27	1,75
07-10-2013	3,39	2,03
09-10-2013	2,78	2,37
11-10-2013	3,76	2,96

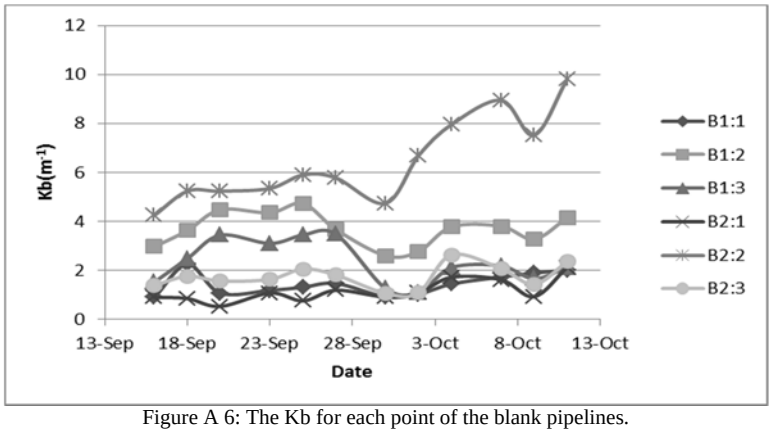

Figure A 6: The Kb for each point of the blank pipelines.

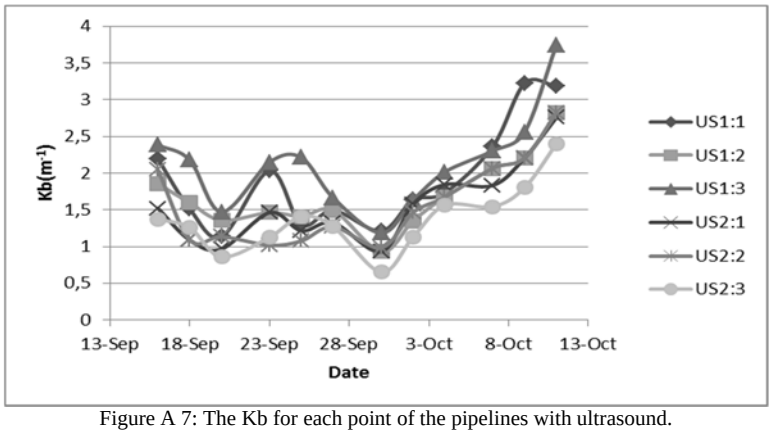

Figure A 7: The Kb for each point of the pipelines with ultrasound.

Appendix B: statistic tests

Results compared by independent samples t-tests. Both the EVA (Equality of Variances Assumed) and EVNA (Equality of Variances Not Assumed) values are shown.

Experiment I

Table B 1: Output t-tests experiment I

Date	EVA or EVNA	Output independent samples t-test	Significantly different?
5-4-2013	EVA	0,130	No
8-4-2013	EVA	0,000	Yes
10-4-2013	EVA	0,017	Yes
12-4-2013	EVNA	0,000	Yes
15-4-2013	EVA	0,000	Yes
17-4-2013	EVA	0,000	Yes
19-4-2013	EVA	0,000	Yes
22-4-2013	EVA	0,000	Yes
24-4-2013	EVA	0,000	Yes
26-4-2013	EVA	0,000	Yes
29-4-2013	EVA	0,000	Yes
1-5-2013	EVA	0,000	Yes
3-5-2013	EVA	0,000	Yes

Experiment II

Table B 2: Output t-tests experiment II

Date	EVA or EVNA	Output independent samples t-test	Significantly different?
14-8-2013	EVA	0,000	Yes
16-8-2013	EVA	0,000	Yes
19-8-2013	EVA	0,001	Yes
21-8-2013	EVA	0,230	No
23-8-2013	EVNA	0,289	No
26-8-2013	EVNA	0,022	Yes
28-8-2013	EVA	0,000	Yes
30-8-2013	EVA	0,003	Yes
2-9-2013	EVA	0,000	Yes
4-9-2013	EVA	0,000	Yes
6-9-2013	EVA	0,005	Yes
9-9-2013	EVA	0,001	Yes

Experiment III

Table B 3: Output t-tests experiment III

Date	EVA or EVNA	Output independent samples t-test	Significantly different?
16-9-2013	EVNA	0,825	No
18-9-2013	EVNA	0,014	Yes
20-9-2013	EVNA	0,014	Yes
23-9-2013	EVNA	0,030	Yes
25-9-2013	EVNA	0,016	Yes
27-9-2013	EVNA	0,011	Yes
30-9-2013	EVNA	0,049	Yes
2-10-2013	EVNA	0,212	No
4-10-2013	EVNA	0,045	Yes
7-10-2013	EVNA	0,110	No
9-10-2013	EVNA	0,556	No
11-10-2013	EVNA	0,356	No

Appendix C: Specifications ultrasonic probes

The ultrasonic probes are delivered by Van Antwerpen Milieu Techniek B.V. The control box of the probes has four different jumper settings which regulate the electrical input power which is measured on the power plug:

- J1; 17 Watt
- J2; 30 Watt
- J3; 31 Watt
- J4; 30 Watt

For the experiment the probes are set on the second jumper setting :Voltage 220 V, Frequency range: 35-52 kHz, Continuous signal.

Appendix D: General Groundwater quality

Locatie	SEA-Lab Vlissingen
Date	jul-09
Laboratorium	Aqualab Zuid
Depth (m)	28,8
Salinity (g/l)	29,7
pH	7,03
mg/l	
Bicarbonaat(HCO3)	442
Chloride(Cl)	16500
Ammonium(NH4)	11
Nitraat(NO3)	<10,0
Nitriet(NO2)	<0,010
N-totaal	
Ortho-fosfaat(PO4)	
P-totaal	0,65
Sulfaat(SO4)	2060
Kalium(K)	310
Magnesium(Mg)	1000
Calcium(Ca)	460
Natrium(Na)	8900
Aluminium(Al)	0,38
Borium(B)	
Ijzer(Fe)	17
Koper(Cu)	0,003
Mangaan(Mn)	0,28
Molybdeen(Mo)	
Silicium(Si)	
Zink(Zn)	0,02
Verhoudingen	
Na:K	28,7
Mg:Ca	2,2
N:P	16,7
Growth experiences	
Algen	++
Schelpdieren	++
Wormen	++
Vis	
Kreeftachtigen	++

Appendix E: feed for the biofouling

During the second phase of experiment I, experiment II and experiment III nutrients were added directly to the water as follows:

1,009922	gram **glucose** in the groundwater barrels after each refreshment
2,019844	gram **peptone** in the groundwater barrels after each refreshment
0,010099	gram **yeast extract** in the groundwater barrels after each refreshment
0,087917	gram **Sodium phosphate** in the groundwater barrels after each refreshment

The amounts of nutrients are calculated with a model designed by I.R. J.Rijstenbil. The amounts are calculated according to the biomass increase per day per m^2. This was estimated on 2,5g/m^2 from the growth on whatman filters that were placed in the tanks.