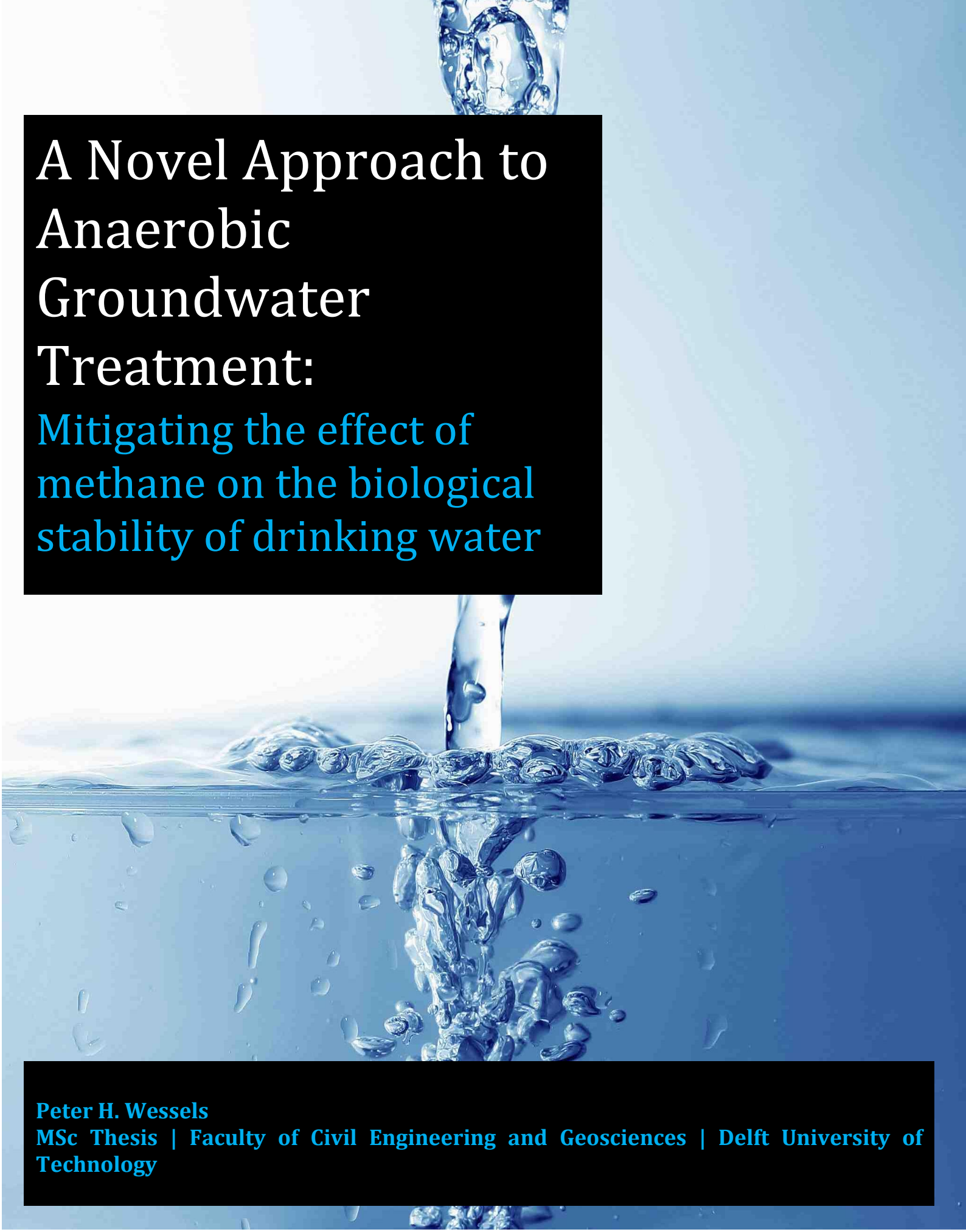

A Novel Approach to Anaerobic Groundwater Treatment:

Mitigating the effect of methane on the biological stability of drinking water

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oasen

Challenge the future

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Source cover picture: http://mureepakistan.blogspot.nl/2011/05/drinking-water.html

PREFACE

Over the course of the last eight months I have had the pleasure of working together with Oasen N.V. in Gouda on this exciting topic. It was a great experience; working together with colleagues on a new treatment approach, which I hope I have been able to contribute to.

I want to express my appreciation for Oasen as a company for giving me this opportunity and all my colleagues for their helpful comments and support. Also, thank-you for making me feel at home. I have learned a lot in this period, both about water treatment and about the way in which drinking water companies work tirelessly to provide their customers with clean and safe drinking water. This has really inspired me!

I would also like to use this opportunity to thank all my friends and family for their moral support and for providing me with the much needed distractions during the weekends. I would especially like to thank Bernardo and Nataša for taking me into their home over the past two months. I hope I did not burden you too much! I had a great time with you guys and it was a pleasure to get to know you even better.

Finally, I would of course, like to thank the members of my graduation committee for their input. I very much appreciate the fact that you were always available if I needed you. The discussions we had, but especially your enthusiasm about my research, gave me a lot of energy and motivated me to keep on thinking about solutions to the encountered challenges.

This document marks the end of an era: my career as a student in Delft on which I look back with great pleasure. I hope it also marks the beginning of a new career in the drinking water sector, which I am very much looking forward to!

Peter H. Wessels

EXECUTIVE SUMMARY

Introduction

This thesis is a consequence of Oasen vision "*to produce pristine water of impeccable quality and to deliver this water flawlessly to its customers*". In this vision, Oasen expresses concern for so-called "unknown" pollutants which are increasingly being found in its water sources. In light of these unknown pollutants and to improve the overall water quality produced by the company, a novel treatment approach is to be implemented by 2018. The new treatment will be built at the site of an existing treatment plant called ZS de Hooge Boom in Kamerik which needs replacement.

The novel treatment approach centers on reverse osmosis (RO) as a primary barrier against virtually all pollutants (microbial, organic and inorganic). RO will treat the complete stream of water directly abstraction, whilst it is still anaerobic. By doing so, frequently occurring issues such as fouling and scaling are minimized.

This approach introduces a couple of challenges to be dealt with, one of them being the removal of methane which is no longer (partly) removed biologically; in a conventional treatment facility this usually happens in two stages, first aeration and then (sand) filtration in which the remaining methane is broken down biologically. Methane therefore needs to be removed sufficiently by a single post-treatment system, which is challenging especially when very low methane concentrations are to be achieved. In order to overcome this challenge, the main goal of this research was to:

Determining the most optimal post-treatment technology for the removal of sufficient amounts of methane in order to prevent biological regrowth in the distribution system thereby complying with Oasen's goal to "produce pristine water of impeccable quality and a flawless distribution"

Since there is currently no legislation pertaining to the maximum concentration of methane in drinking water, a novel design parameter had to determined; the acceptable level of methane in drinking water to prevent biological regrowth in the distribution system. With the determined parameter, three treatment techniques were then tested to determine which would be the most optimal for application at Kamerik.

The research was structured under two main research questions and a large variety of sub questions pertaining to these:

1. What is the acceptable level of methane considering the growth potential of bacteria on methane?
2. Which technique shows the potential to achieve the target level of methane and does so most efficiently?

A bulk parameter for growth on methane and AOC

A new method was developed to measure the growth potential of bacteria on different concentrations of methane. The method is based on the "new" assimilable organic carbon (AOC) assay which measures the growth potential of a water. A natural inoculum (originating from backwash water at Kamerik) was used to inoculate sample batches containing different concentrations of methane. Different concentrations of methane in these batches were achieved by diluting a prepared methane concentrate solution. Finally, a control batch, containing no methane, was prepared in a similar fashion. After preparation, batches are incubated at 30°C and flow-cytometric measurements are done over a 10 day period. The flow cytometer is used to determine the increase in total cell counts (TCC) over time and also to give an indication of the cell size.

Two experimental runs were carried out during this research using the newly developed growth potential method. With the resulting data, it was found that the yield of methane-oxidizing most probably falls within the range of 8.6×10^5-1.7×10^6 cells/µg CH_4. It was also found that in samples containing methane, the median cell volume was a factor 2.16 larger than in samples with no methane

(control). It was assumed that the latter sufficiently represents the cell distribution of non-methane-oxidizing bacteria, in other words AOC bacteria.

With the factor relating the cell volume of MOB to AOC bacteria, the calculated yield of MOB and the known yield of AOC bacteria, a new bulk parameter for the biological stability of drinking water was defined:

$$Bulk\ GP\ =\ C_{AOC}\ +\frac{C_{CH_4}}{2.7}\quad [\mu g\ AOC\ eq./L]$$

With:

$$C_{AOC} = AOC\ content\ of\ the\ water\ sample\ [\mu g\ AOC/L]$$
$$C_{CH_4} = Methane\ concentration\ of\ the\ sample\ [\mu g\ CH_4/L]$$

This parameter takes into account the difference in Total Cell Yields but also the difference in produced biomass due to the difference in cell volume between MOB and AOC bacteria. With this new bulk parameter for biological growth, drinking water companies can measure the AOC content and CH_4 separately by standard procedures and then combine the measurements to determine the total effect on the biological stability of their drinking water.

Implications of the bulk parameter for Oasen

Oasen wants to produce water that complies with, but preferably exceeds the quality standards which are dictated by Dutch drinking water law. With the requirements for methane (<10 µg/L) and AOC (<1 µg/L) as stated in its project plan, the calculated Bulk GP becomes 4.7 µg AOC eq. /L. This is far lower than the value which is generally accepted (10 µg AOC/L). It also needs to be considered that methane removal below 10 µg/L, though preferable, becomes increasingly difficult. By this reasoning 10 µg/L CH_4 was determined as being the maximum acceptable methane level for the new treatment concept. It is assumed herein that the AOC content will indeed be <1 µg/L.

Methane Removal Techniques

With the requirement of a maximum effluent methane concentration being set to 10 µg CH_4 /L, the minimum removal efficiency that is needed from a viable post-treatment system is 99.6 %. Three methane removal techniques were compared to each other by pilot testing and conceptual dimensioning to determine their energy consumption and costs. Where necessary, additional information was gathered from literature. It was found that all three tested techniques have the potential to facilitate the minimum removal capacity requirement.

Tower Aerators

Tower aerators have been around for a long time and have been used in a wide variety of applications. They therefore have a well-established reputation and a lot of research has been done into their optimization. They are easy to design, very reliable and can accommodate more than sufficient removal capacities to comply with the requirements (>99.8%). They are also very flexible in operation and can easily cope with varying flow rates.

Plate Aerators

Plate aerators have also been used a lot for the removal of methane in drinking water in the Netherlands. Removal efficiencies of 99.7% have been found in literature. The design seems to be based mainly on experience as it is very difficult to model this type of system. The removal efficiency is dependent on a lot more variables and as such is deemed more vulnerable than tower aerators. It is also less capable of dealing with fluctuations in flows. It was however found that plate aerators use a lot (5-16x) less energy and that the total cost of ownership (TCO) is a lot (1.25-2 x) lower the other two techniques.

Membrane Contactors

Membrane contactors have never been applied in large scale drinking water treatment or for the removal of methane. There are therefore at present still a lot of questions about their functionality; can they provide sufficient removal efficiency? How would they cope in a drinking water installation? Pilot results in this research have not yet yielded sufficient data to answer these questions. Due to their modularity however, they are very interesting: They flow capacity is easily expanded by adding more trains and most importantly, by placing more units in series, very high removal efficiencies (>99.9%) are deemed possible. Additionally, of the three techniques, membrane contactors are the only ones that provide the possibility of gas exchange without mixing the air and the water phases. This means contaminations in the air will not be distributed in the water. Unfortunately, it was found that these systems require large amounts of energy and the costs are a lot higher (1.6-2 x) higher than tower and plate aerators respectively. The higher costs are mainly caused by the periodical replacement of membranes currently estimated to be about 7 years.

Taking these considerations into mind, a multi-criteria analysis was executed, testing the alternatives on: Removal efficiency, safety, reliability, feasibility within the time frame of the project, costs, energy consumption and expandability. The result clearly demonstrates that tower aerators are the most optimal technique.

Conclusions

Tower aerators have been shown to be the most optimal post-treatment technique for the new the new facility at Kamerik. Using this technique Oasen will be able to remove methane to concentrations < 10 µg/L (estimated 5.5 µg/L), thereby improving the bulk growth potential to < 4.7 µg AOC eq./L (estimated 3 µg AOC eq./L) which should comply with Oasen's vision "*to produce pristine water of impeccable quality and to deliver this water flawlessly to its customers*".

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1 INTRODUCTION

1.1 General

Oasen NV is a drinking water company located in the eastern part of South Holland which provides drinking water to 750 000 people and 7 200 businesses (Oasen NV, 2014). The company is planning to build a new treatment plant at location Kamerik to replace the existing conventional treatment plant, called Zuiveringsstation (ZS) De Hooge Boom, which has reached its End-of-life (EOL). The location of the company's service area in the Netherlands as well as the location of ZS de Hooge Boom with its service area are shown in figure 1. An aerial view of the treatment facility is also provided.

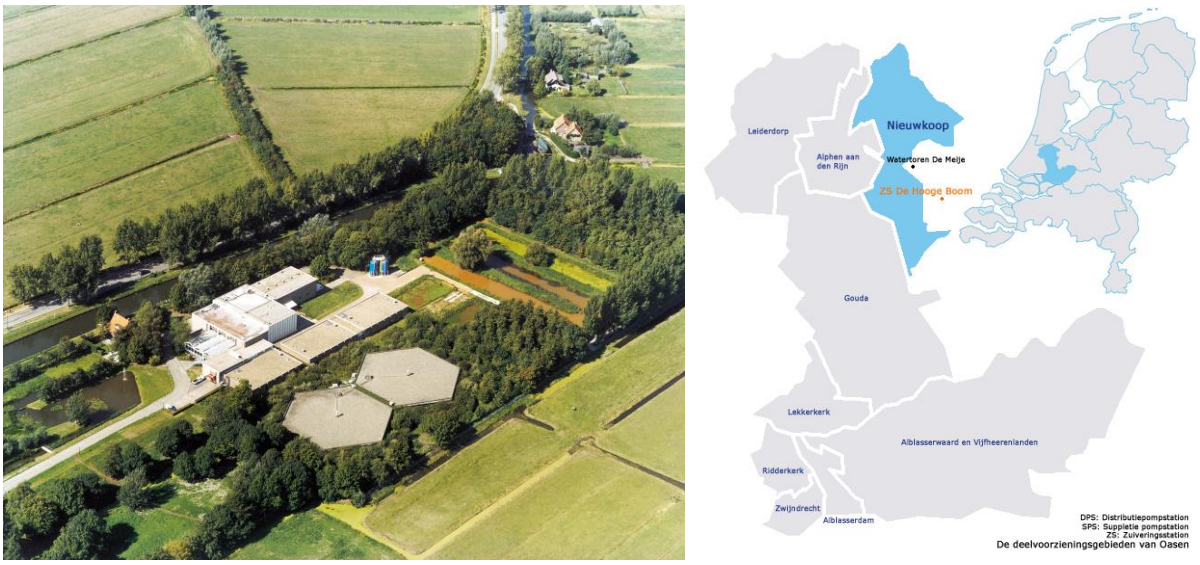


Figure 1 Aerial photograph of the existing treatment facility at ZS de Hooge Boom (left) and the service area of Oasen and ZS de Hooge Boom (right) (Oasen NV, 2014)

The first parts of the current treatment plant were built as early as the 1930's. Later, in the 1980's, the plant was upgraded with additional treatment steps. The treatment scheme as it is currently, is shown in Figure 2. The treatment plant is relatively small with an average production capacity of 300m³/h and a yearly production of 3Mm³. Due to its age, the plant is outdated and needs to be completely revised or replaced.

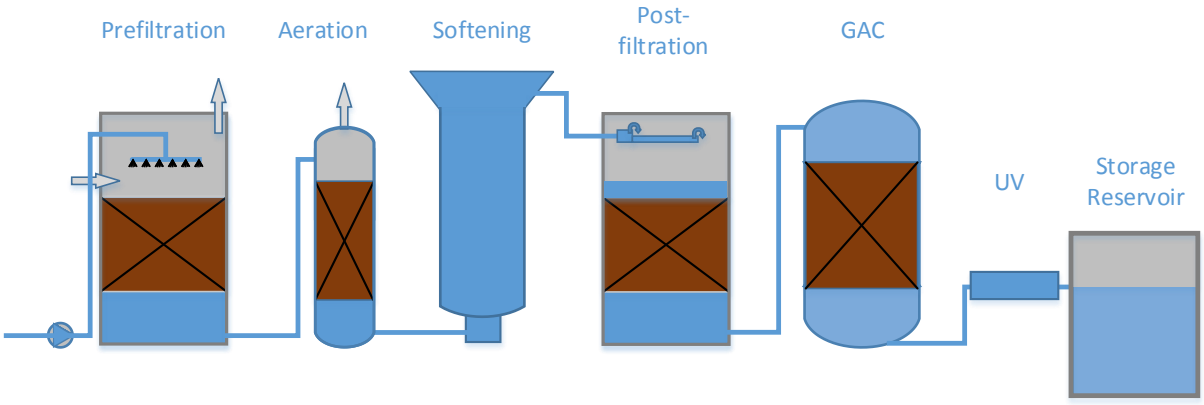


Figure 2 Current treatment Scheme at ZS De Hooge Boom

1.2 Oasen's Vision

The company wants to use this opportunity as a stepping stone towards implementing its ambition to introduce a new standard for water supply which it has summarized as the "*production of pristine drinking water of impeccable quality and flawless delivery to our customers*". This vision is described in "Programma Kamerik" in which Oasen strives towards the best water supply in the 21st century called "Better than Possible" (Van der Laan, 2013) . With this vision the company expresses its concern for so-called "unknown" pollutants which are increasingly being found in source waters in the Netherlands. Though for most of these pollutants, it is not yet known whether they are harmful or not, they are preferably removed especially since the concentrations are expected to increase in the future.

At present, research is being done into the optimal configuration of a new treatment concept, which is capable of removing unknown pollutants and improve the quality of the produced water on all other aspects. This new concept will then be implemented at Kamerik by 2018.

1.3 A novel treatment Approach

In order to achieve its vision, Oasen has opted for a novel approach, choosing reverse osmosis (RO) membranes as a primary barrier treating the entire stream of groundwater directly after abstraction. The novelty of this approach lies in the fact that entire flow of water is treated anaerobically; there is no pre-treatment and no water by-pass.

RO membranes remove virtually all known and "unknown" pollutants as well as nutrients from the water, thereby producing (almost) pure water. Under aerobic conditions RO membranes treating water containing high levels of organic substances are very susceptible to biofouling (Ridgway, et al., 1991). The raw water at ZS de Hooge Boom, however, is anaerobic; by treating the water anaerobically, it is thought there will be less risk of fouling and clogging of the membranes so that no pre-treatment is necessary. Though RO membranes are highly efficient in the removal of most pollutants, some additional post-treatment steps are needed to insure the production of impeccable water:

- Initial tests have shown that ammonium will not be sufficiently removed. Therefore, an ion-exchange system is added as a first post-treatment step to remove ammonium to concentrations <0.02 mg/L.
- A consequence of the fact that the entire stream (i.e. no by-pass) is treated by RO membranes is that the water will no longer contain essential minerals such as calcium and magnesium. These mineral therefore need to be added in a post-treatment step (remineralisation) in order to comply with Dutch drinking water standards.
- Finally, the raw water contains volatile gasses which will not be removed by RO. Some of these gasses, such as MBTE, vinyl chloride and cis-1.2 dichloroethylene are harmful to human health, whilst methane (CH_4) is a carbon source which could potentially disrupt the biological stability of the water. All the aforementioned gasses are therefore unwanted and are to be removed in a third post-treatment step. This step should also add oxygen to the water in order to comply with Dutch drinking water standards.

The new treatment approach, with full-stream RO and post-treatment steps, are depicted in the order in which they are currently thought most optimal. The order of the last two steps is currently being debated, with the depicted order having the preference of Oasen.

Figure 3 Treatment steps of the future treatment facility at ZS de Hooge Boom as currently planned by Oasen

Currently, Oasen is carrying out extensive research into the feasibility of this new approach and the capability to achieve the envisioned results. A pilot plant has been set-up, which contains all of the aforementioned processes. In this plant the capabilities of individual processes, the interaction between processes and the quality of the end product are being tested and optimised so as to guarantee a new standard in drinking water treatment.

1.4 Purpose of this research

This document is part of the ongoing research into the feasibility and optimisation of the novel treatment approach. Its focus is on third post-treatment process which should:

1. Provide sufficient removal of volatile gasses (MBTE, vinyl chloride & cis-1.2 dichloroethylene)
2. Provide sufficient removal of methane (CH_4)
3. Provide sufficient addition of oxygen (O_2).

The purpose of the document is to determine the most optimal post-treatment system in order to comply with these conditions. To determine the most optimal post-treatment system, however, this report focusses on determining the most optimal treatment system for the removal of sufficient amounts of methane because it is assumed that:

1. The required removal efficiency for methane is much higher than that required for the removal of volatile gasses and the addition of oxygen.
2. Volatile gasses are removed more easily than methane.

Therefore, it is assumed that the system providing sufficient methane removal will also provide more than sufficient removal of volatile gasses and addition of oxygen.

2 PROBLEM DEFINITION & GOALS

For over a century it has been known that methane can be present in groundwater. As water infiltrates through various layers of the sub-surface, bacteria will utilise oxygen, forming CO_2. Depending on the amount of organic matter available this can happen quite rapidly, so that the oxygen is depleted after only a few meters of soil passage. Once depleted, nitrate is used as an electron donor (denitrification) to oxidise organic material in the soil after which the water is subjected to a variety of anaerobic processes. Anaerobic bacteria will continue to oxidise organic material, if present (e.g. in peat), producing methane (CH_4) while utilizing carbon dioxide and bicarbonate as oxidators. Biological production of methane (Methanogenesis) is the last process in a sequence of redox processes and will only occur after manganese, iron and sulphates have been reduced (De Vet, 2011).

Due to the high pressure of the water column pressing down, the water is able to dissolve much higher concentrations of methane than is possible under "normal" surface pressure conditions causing oversaturation. When the pressure of the water is reduced, for example by pumping it up to the surface, methane will escape back to the atmosphere (Helm, van der, 1998).

In European countries which do not use chlorination as a residual disinfectant such as the Netherlands, Switzerland, Germany and Austria, the presence of methane poses a threat to the biological stability; these countries rely on the production of drinking water in which microbial regrowth is limited through limitation of nutrients essential for growth, usually focusing on organic carbon (Hammes, et al., 2010).

Methane is a carbon source which could potentially be utilised by bacteria in drinking water under aerobic conditions. Research in the Netherlands has shown that the presence of methane in the influent of treatment plants has an impact on the number of *Aeromonas* found in the distribution system (Reijnen, 1994). The given reason for this is not that there was methane in the effluent, but rather that the biological breakdown of methane in the filtration steps led to high levels of biomass production which in turn was not sufficiently retained by the sand filters. The higher concentrations of biomass led to regrowth of *Aeromonas* in the distribution system; not the presence of methane itself. Finally, literature gives a wide range of biomass yields for methane oxidizing bacteria (methanogens); 0.5-0.8 g DW/g CH_4 (Leak, et al., 1986). Another source states that the yield of bacteria on methane is 6 times higher than the yield on ammonium (Helm, van der, 1998). The aforementioned research, leads experts to believe that even very small amounts of methane could lead to significant growth in the distribution system.

To the author's knowledge, however, no attempt has been made to quantify the direct effect of the presence of methane on the biological stability of the produced drinking water. In addition, there is no current legislation dictating the maximum level of methane in drinking water. Conventional treatment of anaerobic groundwater usually involves some form of aeration as a pretreatment step to add oxygen to the water, which is needed in the subsequent treatment of the water in filters. During aeration, methane and volatile gasses are largely removed, leaving low concentration of methane in the water. Sand filters will break down remaining methane biologically by methane oxidizing bacteria (MOB) so that the effluent contains no more methane.

By switching to RO membranes Oasen is confronted with a new challenge; it can no longer assume that all methane will be removed in the treatment as RO membranes do not remove dissolved gasses. A single post-treatment step is therefore introduced to provide sufficient removal of methane. This will have to be a chemical or physical removal technique, as biological removal would lead to recontamination of the already very pure RO filtrate. To remove all methane without biological treatment of the water is however virtually impossible as this would have a large impact on the cost of water production and also demand large amounts of energy. This begs the question how much methane should be removed from the water for it to be biologically stable without having to over-dimension the removal step. Various removal techniques exist which from literature show the

potential to achieve high removal efficiencies for methane. The question is which of these techniques is capable of achieving the required removal to guarantee biologically stable water most optimally.

2.1 Goal

The concept of methane removal needs to be rethought in order to fit the novel approach envisioned by Oasen. The goal of this thesis therefore, can be summarized as:

Determining the most optimal post-treatment technology for the removal of sufficient amounts of methane in order to prevent biological regrowth in the distribution system thereby complying with Oasen's goal to "produce pristine water of impeccable quality and a flawless distribution"

2.2 Requirements

In order to provide sufficient removal, the post-treatment step must comply with Oasen's goal to produce pristine water of impeccable quality and a flawless distribution to its consumers. As such the requirements which relate to this step have been extracted from the original research plan (Van der Laan, 2013).

The quality of the produced water must be as high as possible and should at minimum comply with the following requirements:

- The AOC content of the water should be <1 µg/L.
- The methane concentration must be <10 µg/L.

Though the treatment step does not have a direct effect on the assimilable organic carbon (AOC) value, it should be tested whether the combination of the growth on these concentrations of AOC and methane leads to biologically stable water in terms of compliance with the generally accepted AOC concentration of 10 µg/L. This is seen as the absolute maximum value allowable in the produced water. As Oasen wants to produce better than possible water, much lower values would be preferable.

2.3 Research questions & structure

In order to determine the most efficient post-treatment technique complying with the aforementioned requirements, the following research questions were formulated:

1. Generally speaking, at which concentration of methane does the biological stability reach acceptable levels?
    a. What is the yield of MOB?
    b. How does this relate to the yield of bacteria on AOC in terms of Total Cell Counts (TCC) and produced biomass (TCC x cell volume)?
2. Which techniques could potentially be used to sufficiently remove methane from the water?
    a. How do these techniques perform in a pilot plant?
    b. What is the maximum removal efficiency for methane?
    c. How would the treatment technique look in a full-scale set-up?
    d. How much energy is needed for each technique?
    e. How much would each technique costs?
3. What are the implications for the needed removal efficiency at ZS de Hooge Boom taking into account Oasen's vision to produce impeccable drinking water and the limitations of achieving this removal efficiency with one single post-treatment step?

4. Which technique is most optimal for Kamerik with respect to:
    a. Removal efficiency
    b. Safety
    c. Reliability
    d. Feasibility (within the time frame of the project)
    e. Costs
    f. Energy Consumption
    g. Expandability

From the answers obtained in researching these questions, the treatment technique best suited for application at Kamerik will be defined. Figure 4 illustrates the research structure and how the questions mentioned above relate to each other.

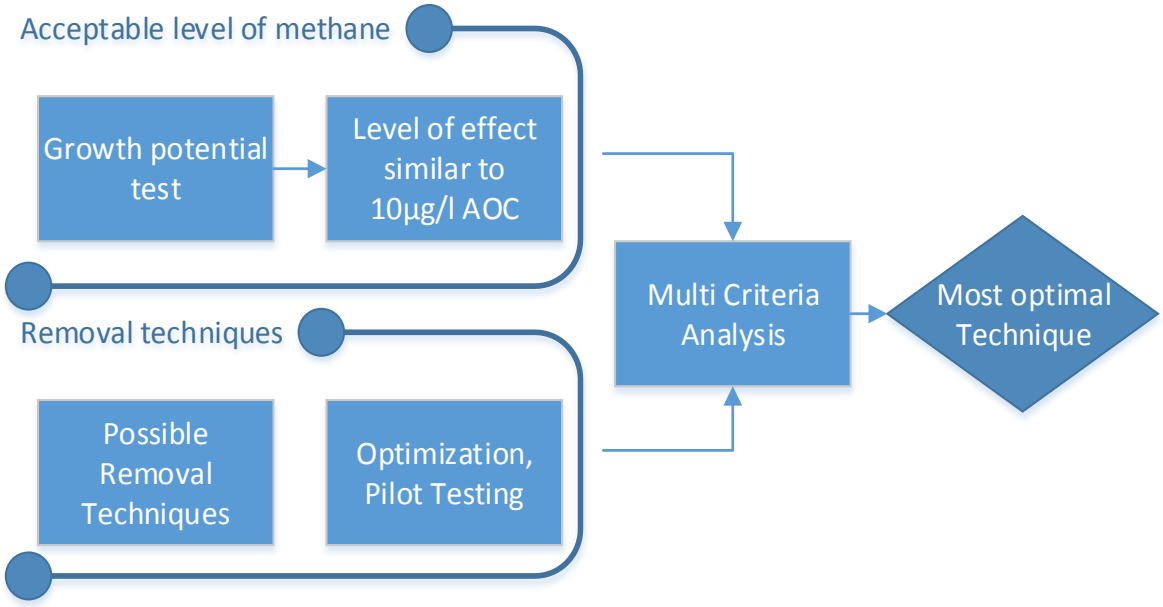


Figure 4 Research structure: Steps for the selection of the most optimal Post-treatment technique for the production of biologically stable drinking water at ZS de Hooge Boom.

The report has been structured similarly:

- **Chapter 3** reports on the development of a novel method to quantify the impact of methane in water on the growth of microorganisms. It then relates this growth to the AOC concentration of drinking water taking into account the difference in dry weight biomass production, concluding with a recommendation as to the maximum acceptable level of methane in drinking water without chlorination.
- **Chapter 4** elaborates on physical processes which influence the removal of methane, justifies the choice for three techniques for pilot testing and the results of each technique in the pilot plant. It then continues into the superficial dimensioning of each system for a full-scale treatment facility after which energy use and costs are calculated. After all these factors have been considered a multi-criteria analysis is performed to determine the most optimal treatment technique.
- **Chapter 5** Draws conclusions from the research done in chapter 3 and 4. It ends with recommendations for future research needed to further improve and complement the results found over the course of this research.

3 DETERMINING THE ACCEPTABLE LEVEL OF METHANE

This chapter defines a novel method to determine the biological growth potential of a water for methane oxidizing bacteria. The method is based on existing methods for determining the biological stability of drinking water. The section explains the set-up and methods used and presents the preliminary results from the executed experiments. Finally, conclusions are drawn with respect to the acceptable level of methane in order to produce biologically stable drinking water.

3.1 Approach

In the Netherlands, the most commonly used approach for the assessment of biological stability of water is the assimilable organic carbon (AOC) assay. The conventional AOC analysis method was developed in the Netherlands in the early 1980's (Kooij, et al., 1982). It quantifies bacterial growth as the increase in number of colony forming units (cfu) in a water sample from inoculation to the stationary phase usually performed as an endpoint measurement only. Pure cultures of either *Pseudomonas fluorescens (P. fluorescens)* strain P-17 or *Spirillum* strain NOX are used as test organisms with which a water sample is inoculated. The water sample is then incubated at 15°C. The microbial growth is quantified on agar plates at t=7, 8 and 9 days. The net measured growth is related to the growth of the test organism on acetate, the yield of which needs to be predetermined (Hammes, et al., 2005). AOC therefore is expressed in µg acetate-C eq. /L. Later on, a couple of improvements on this method were suggested including; increasing the inoculum density from 500 cfu/mL to 2-4 x 10^4 cfu/mL (Kaplan, et al., 1993) and a higher incubation temperature (22 °C) (LeChevallier, et al., 1993).

Generally speaking the consensus in the Netherlands is that water with an AOC content of <10 µg acetate-C eq. /L is sufficient to guarantee biologically stable water. The problem with the AOC measurement, however, is that the pure cultures of bacteria which have been used previously do not incorporate methane and ammonium oxidizing bacteria. Therefore, even if a considerable amount of methane and/or ammonium were present, this would not be detected in the measurements, as the test organism could not utilize it.

More recently a new approach has been suggested to improve the traditional AOC method. The method replaces plate counting, which is time consuming and not always accurate, with flow cytometry. This laser based technique is able to count individual bacterial cells in a sample thereby giving a very accurate cell count and providing the possibility of detecting inactive and unculturable bacteria. The new approach also suggests using a natural consortium of bacteria as inoculum as it would be able to utilize a wider range of substrates, thus providing a more representative view of the actual growth potential of the tested water. The calculated yield of a natural consortium of bacteria is assumed to be approximately 1x10^7 cells/µg AOC (Hammes, et al., 2005).

The approach used in the present work to determine the acceptable level of methane in drinking water is based on the aforementioned AOC techniques. Both AOC methods have made use of a known amount of AOC (typically acetate) to determine the yield of bacteria. The methods then relate a certain amount of growth in any sample to a given concentration of AOC.

A similar approach can also be applied for methane, in order to determine the yield on methane separately from the yield on AOC. The two can then be combined into a single parameter for the biological stability of the measured sample.

- Assuming an AOC concentration of 10µg/l or less guarantees biological stability and the yield on AOC = 1 x 10^7 cells/µg, the maximum amount of bacteria acceptable in water is equal to 1x10^8 cells/L.

- Therefore, the total growth caused by AOC + CH_4 must not exceed $1x10^8$ cells/L, in other words:

$$c_{CH_4} x\, Y_{CH_4} + c_{AOC} x\, Y_{AOC} \leq 1x10^8 cells/L \quad (1)$$

With:

$$c_{CH_4} = methane\ concentration\ (\mu g/L)$$
$$c_{AOC} = AOC\ content\ (\mu g/L)$$
$$Y_{CH^4} = yield\ CH_4\ (cells/\mu g\ CH_4)$$
$$Y_{AOC} = yield\ AOC = 1x10^7\ (cells/\mu g\ CH_4)$$

The yield of methane-oxidizing bacteria is determined by growth potential tests. In short, the method builds forth on the "new" AOC method suggested by Hammes et al. (2005). In a single test run, batches with three different concentrations of methane are made as well as one batch which contains no methane. The batch without methane is used as a control sample to account for growth of bacteria on substances other than methane (i.e. AOC and possibly ammonium). The net growth potential is calculated by subtracting control growth from the total growth on each methane concentration. The yield is then equal to:

$$Y_{CH4} = \frac{total\ growth - control\ growth}{CH_4\ concentration} \quad (2)$$

Some minor adaptations to the "new" AOC method were implemented to overcome difficulties presented by the fact that methane will diffuse from water to air if the concentration is higher than the equilibrium concentration[1] and to ensure the presence of methanotrophs in the experiment:

1. In the method as described by Hammes et al. (2005), sample vials are not fully filled to limit contact between the water and the bottle cap which could cause extra growth by leaching of organic substances from the material of the cap. Methane however, will diffuse into the headspace of a bottle, if a small concentration gradient exists, thereby lowering the concentration in the water. As the growth potential on methane is to be determined, all methane should be consumed by the bacteria and no methane should be allowed to escape from the sample. The bottles are therefore not allowed to have any headspace and must be fully filled.
2. A sample will lose part of its methane content as soon as it comes into contact with open air. Every sample therefore, can only be tested once and is thrown away after it has been tested. This means that for each test setting the number of samples collected is equal to the number of flow cytometer analysis which is to be done (in triplicate). The number and exact times at which each flow cytometer analysis is to be carried out is predetermined.
3. In a general growth potential test, the total potential with respect to biological growth is to be determined, whether limited by carbon, phosphate or nitrogen. In the conducted tests the effect of methane alone is to be measured. To prevent other nutrient from being limiting to the growth, a solution containing phosphate and nitrate is added.
4. Samples are inoculated with an inoculum cultivated from backwash water from the existing sand filters at ZS de Hooge Boom instead of using tap water as inoculum. Though there is a high probability of methanotrophs being present in tap water, it was thought even more probable for them to occur in the backwash water of the sand filters. The cultivation of the inoculum will be described in subsequent paragraphs.

[1] See paragraph 4.2 for an elaborate explanation of this phenomenon

3.2 Materials & Methods

As described in previous paragraph, the method used to determine the yield of methanogens is based on the old (Kooij, et al., 1982) and new (Hammes, et al., 2005) AOC methods. Many of the procedures and protocols described in the following paragraph are therefore identical or very much similar. On the other it is to be noted that some adaptations had to be made so that the method would be suitable for evaluation of the methane growth potential. A significant amount of tweaking was needed before achieving that which is presented here.

Each experimental run consisted of four batches of samples:

- 3 batches containing methane, each batch with a different concentration
- 1 control batch containing no methane

Each batch consisted of 15 sample vials (5 time steps measured in triplicate). For the control sample, 18 vials were made initially, three of which were used to confirm the initial cell count. It was assumed that this is sufficiently accurate and represents the initial concentration in the other samples.

Each individual sample is prepared in a 24 ml vial (volume estimated when fully filled up to the cap, including the neck). Special caps with a septum were used to seal the vials. The septum consisted of a PTFE layer, which is in contact with the sample and a butyl rubber layer on the outer side of the sample. Butyl rubber septa allow very little gas to diffuse out of the sample (Lange, et al., 2008) so that little methane is lost over the course of the experiment. The PTFE layer serves as a protection layer between the butyl rubber septum and the water sample to prevent leaching of organic substance into the sample.

The fully filled vials were initially tested to confirm that only a limited amount of methane will be lost from the sample during the experiment. This was done by filling 9 vials with a mixture of methane and water and measuring the methane concentrations at t=0, 2 and 4 days. The results are shown in Table 1. The maximum loss of methane during the growth phase of the experiments (4 days) is 5 % which is acceptably low. In reality the loss will be much lower, as a large part of the methane will be utilized by bacteria before it can diffuse from the vial. Therefore, this loss is considered negligible.

Table 1 Results of methane loss experiments from vials: methane concentrations in time from triplicate vials

Time (h)	CH_4 concentration (μg/L)	% lost
0	867	0.0%
44	850	1.9%
91	825	4.9%

Three additional samples were taken during the preparation of each batch to analyze the methane content of the batch. These samples were taken in vials provided by a specialized drinking water lab and were delivered there for analysis. The actual concentration of methane in a batch is therefore only known after the analysis has been completed and the results have been received from the lab (approximately 3 working days).

The samples were incubated at 30 °C only to be removed once they were to be analyzed using flow cytometry. These analyses took place at (approximately) t= 0, 2, 3, 4, 7 and 10 days. Figure 5 gives an overview of the various aspects included in the new method. It cannot be emphasized enough that the vials used in growth potential tests must be completely filled, preferably with no bubbles at all. The table at the bottom right of the figure shows when the FC measurements take place and demonstrates the total number of vials needed per batch and overall.

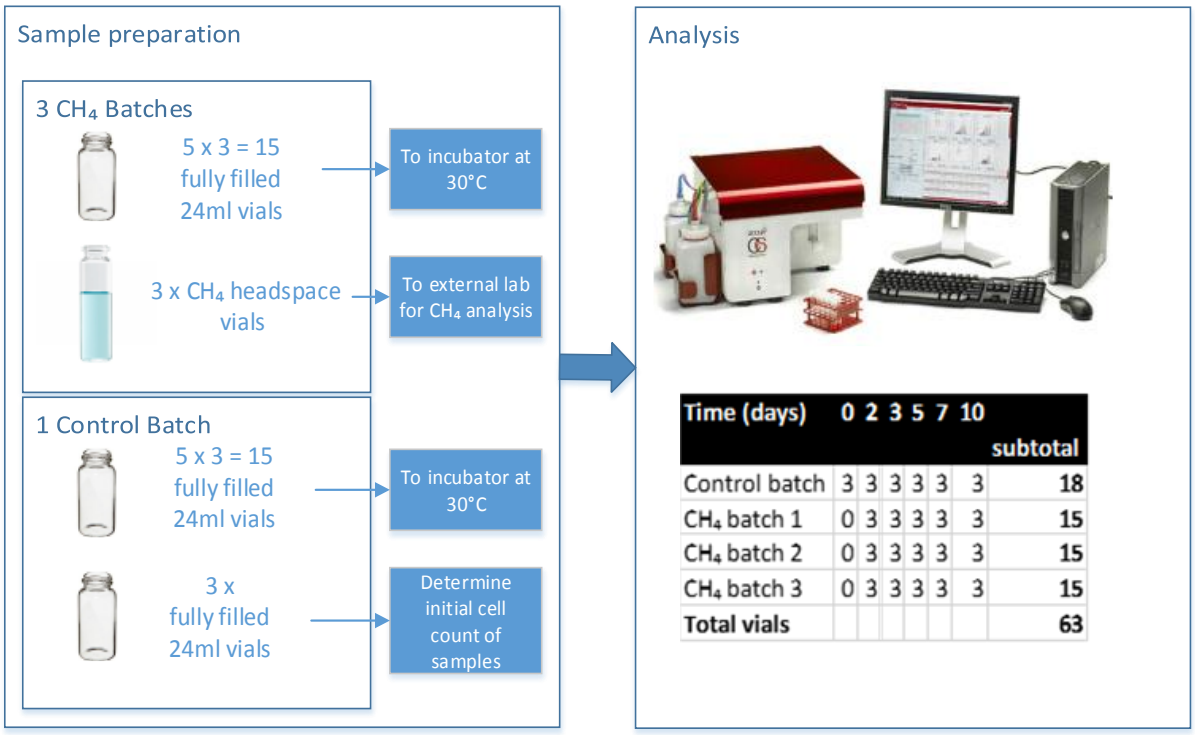


Figure 5 Illustration of the various aspects of the new method for growth potential tests on methane (sources illustrations: http://www.upci.upmc.edu/cytometry/analytical.cfm & www.amazonsupply.com)

The following paragraphs provide a detailed overview of each aspect of the method individually, from the cleaning protocols, preparation of samples and finally the analysis using flow cytometry.

3.2.1 Cleaning protocols

Assimilable organic carbon (AOC) can be utilised by bacteria causing growth in a water sample. In order to obtain reliable and reproducible results, both in a normal AOC determining procedure or as in the experiments conducted in this thesis, it is of utmost importance that all materials which are in direct contact with the sample are AOC-free. Because the equipment used during these experiments was made of different materials, it was not possible to clean everything in the same way. Two cleaning procedures were used depending on the resistance to heat: one for heat resistant glassware and one for remaining equipment (mostly plastics).

Cleaning procedure for heat resistant glassware

The most effective way to rid a material of all carbon on it is by oxidation. For all glassware (except syringes) that is therefore able to resist extreme heat, the following procedure is recommended as being the most optimal and effective.

1. If the glassware has been used before: wash with MQ or demineralised water containing 6 g/l Alconox® detergent, then rinse three times with MQ and air-dry overnight. If not used before continue to step 2.
2. Soak overnight in 0.2 mol HCl solution, rinse with MQ and air-dry overnight
3. Cover the opening of each separate container or vial with aluminium foil
4. Place the glassware in a muffle oven at 550°C for 6 hours
5. The bottles are now AOC free. They should be handled as little as possible before being used. Leave the aluminium foil on the bottle until it is to be used, or even close the vial with a (cleaned) screw cap.

Cleaning procedure for vial caps, glass syringes and other plastic materials

1. If the glassware has been used before: wash with MQ or demineralised water containing 6 g/l Alconox detergent, then rinse three times with MQ and air-dry overnight. If not used before continue to step 2.
2. Soak in 0.2 mol HCl solution overnight
3. Rinse with mQ and air-dry overnight
4. After drying, submerge in $Na_2S_2O_8$ and heat to 60° C for 1 hour, rinse with mQ or demineralised water and air-dry before use.
5. Once again it is important to handle the AOC free caps and other items as little as possible to prevent AOC-recontamination. Also it is best to prevent those surfaces which are in direct contact with the sample from coming into contact with air. For example, vials caps should be placed upright during storage. Syringes and other equipment can be covered with aluminium foil.

Note that recontamination can take place by a variety of sources, from touching with the bare hand (body fats) to volatile substances in the lab environment (e.g. perfumes of lab participants or ethanol used for disinfection). Storage of the materials after having been cleaned is therefore also vital. Preferably one would find a room in which few people walk in and out, no volatile substances are being used and also no wastewater experiments are being conducted.

3.2.2 Total cell count enumeration with flow cytometer

The total cell counts (TCC) were analysed using a flow cytometer. The methods and procedures which will be described briefly in the following paragraphs, have only been developed and standardised recently for the drinking water sector.

Flow cytometry, on the other hand, has existed for many years and is being used in a wide range of applications; cell counting, cell sorting, biomarker detection and other protein engineering applications.

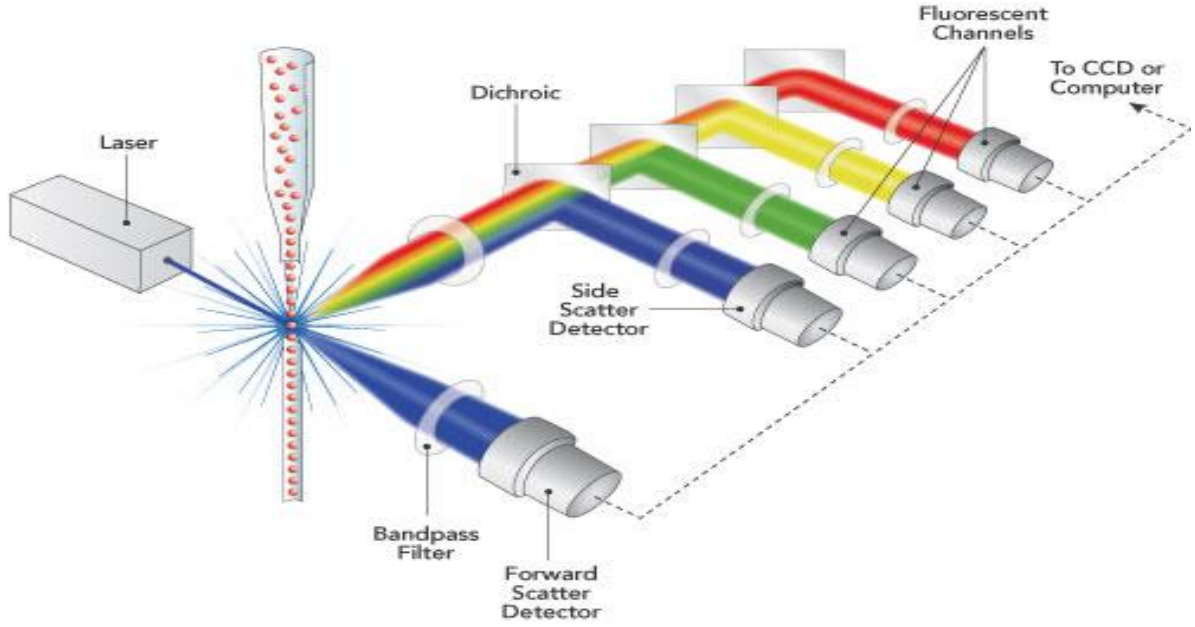


Figure 6 Principle of flow cytometric measurements (Semrock, Inc., 2014)

During flow cytometric measurements, particles are suspended in a sheath fluid and flow through a narrow flow chamber in such a way that only one particle passes at a time. The rate at which this happens can be as much as 10 000 events/s. As the particles move through a laser beam, the light bounces off the passing objects, scattering into different directions. The scattered light is detected by

sensors, yielding information about the number of events (particles passing through the laser beam) and characteristics of the cells such as size and shape.

Different kinds of particles will always be present in natural waters. In order to distinguish between bacterial cells and other particles (e.g. inorganic), a fluorescent stain is used that binds preferably to nucleic acids. The stain, SYBR® Green I, gives each bacterium a signature fluorescence in the green (approx. 520 nm) and red (>615 nm) wavelengths. Depending on the nucleic acid content of the cells, smaller or larger amounts of SYBR Green I bind to the cell, resulting in different fluorescence intensities when excited by the laser beam. Using filters selective to a specific range of wavelengths, the intensity can then be analysed to identify certain particles of interest (in this case bacterial cells) and to distinguish between these particles and others (background noise, i.e. crystalized minerals, suspended solids). This can be done by specialized software packages in which electronic gates are defined.

The advantage of flow cytometry over other enumeration methods is that it yields fast (2 minute measurements) and reproducible results. Using standardized staining and measurement protocols will lead to very small errors (<3%). For other enumeration methods, such as heterotrophic plate counting (HPC), the analysis is much more time consuming (results cultivation of bacteria on a growth medium obtained after 2-10 days) and prone to error (human and/or mechanical counting). Most importantly, these techniques do not detect all bacteria (most natural drinking water bacteria are not cultivable on growth media) (Hammes, et al., 2008; Prest, et al., 2013).

Flow Cytometer procedure

Measurements were performed using a BD Accuri C6® flow cytometer equipped with a 488 nm wavelength laser. BD Accuri provides an accompanying software platform called CFlow® which easily facilitates changes in settings and analysis of data during or after experiments. Fluorescence intensity was collected by sensor at two channels FL1= 533 nm and FL3 > 630 nm.

All measurements were done using the same procedures, which were derived from a standardized method used in Switzerland (SLMB, 2012). Prest et al. (2013) showed that the staining time and temperature are the most influential parameters in the reproducibility of results. The stain used (SYBR Green I) is diluted 100 times before use (stock solution). The following staining protocol is then to be followed precisely:

1. Warm sample in heating block (35 °C) for 5 min
2. Stain sample in a ratio of 1:100 with the SYBR® Green I stock solution (in this research: 5:500 µL)
3. Mix sample on vortex
4. Warm stained sample at 35 °C for exactly 10 minutes in the dark (closed heating block)

The analyzed volume of each sample is 500 µL. The flow cytometric measurements were performed at medium flow rate (35 µL/min). The flow cytometer was calibrated to measure the number of particles in 50 µL of a 500 µL sample. During a measurement the upper limit of detection is approximately 2 x 10^5 cells/ml. If this value is exceeded the sample must be diluted. This is done with 0.22 µm filtered Evian water. All samples, except the initial (T=0h) measurements, were diluted 50 times during the executed experiments.

3.2.3 Sample preparation

Water used in experiments

At first samples were taken directly from the pilot plant, using a small tower aerator to produce batches containing different concentrations of methane. The reason that this would have been handy, was that it would simultaneously have given an indication of the growth due to AOC and Ammonium in the water. It was however, soon discovered that it would be difficult to continue using this method because:

1. The pH after aeration (without reconditioning) was very low (<6), therefore it was unclear whether bacteria would grow and whether this would be representative for situations in water with a normal pH.
2. The concentration of methane in the raw water was unknown during sampling (analysis is done later in the lab) making it very difficult to estimate appropriate tower aerator settings.
3. Samples would have to be taken at ZS de Hooge Boom and subsequently transported to Delft. There they would have to be opened to dose inoculum and nutrient solution, which would lead to a part of the methane escaping from the samples. If not done consistently for every sample, this would mean a single batch may have large variations in methane concentrations.
4. Finally, it was very difficult to make sure the control sample contained no methane without contaminating the sample with additional AOC from tools used and from the surroundings.

Due to these factors it was decided to manufacture water with different concentrations of methane in the lab. Ideally, the water used would contain no AOC and would be of extremely high quality so that growth could be directly attributed to the concentration of methane in the water. Milli-Q water was thought of, but, it was determined that it would lack trace elements to support growth and might be prone to pH fluctuations due to the lack of buffering capacity. Therefore, finally, bottled Evian water was determined as being the most optimal choice; It has a stable quality (low growth potential) and provides (part of) the trace elements necessary for bacteria to grow.

The Evian water was filtered with a 0.22µm vacuum filter in order to remove already present bacteria, so that the initial concentration of bacteria in the samples was very low and comes only from the inoculum which is added.

Inoculum: synthesis and dosing

For a normal AOC test pure cultures of either *Pseudomonas fluorescens* strain P-17 or *Spirillum* strain NOX are most often used as test organisms, because they are easily culturable and can grow on a complex pool of bioavailable carbon compounds (Hammes, et al., 2005). These bacteria, however, cannot oxidise methane so that a new inoculum must be found which includes MOB.

Hammes et al. (2005) have suggested the use of a natural microbial consortium as inoculum and have proven that in doing so one could simulate more accurately the regrowth potential of natural waters. This is due to the fact that the natural consortium of a water system has adapted substrates available in that specific system, optimising their ability to utilise the available energy sources.

This same principle applies to waters containing methane, so that the natural consortium will contain a community of bacteria which probably includes methanotrophs (or other methane oxidising bacteria) which have been found to occur naturally in most environments where methane is present (Ward, et al., 2004). The existing sand filters were identified as most probable treatment stage for large amounts of methanotrophs to be. Backwash water from the first filtering stage was therefore used to synthesize an inoculum.

Backwash water was dosed into vials with filtered Evian containing a high dose of methane concentrate and phosphate and nitrate solution. These were then incubated at 30° C until the stationary stage was reached after approximately 3-4 days. The process was repeated two times to insure a strong competitive advantage of the MOB (enrichment). After the initial synthesis of the inoculum, a new batch of inoculum was made every time a new experimental run was executed.

Though it has not practically been confirmed that the growing bacteria were actually methanotrophs, the explosive growth during the inoculum incubation with high concentrations of methane leaves no doubt that these microorganisms grew mainly on methane as a carbon source.

The desired inoculum dose for the conducted growth potential experiments was 5 000 cells/ml as used by Hammes et al (2005). On the day a new run was started, the total cell count (TCC) was determined using flow cytometry. The needed dose of inoculum was then calculated by the following formula:

$$inoculum\ dose = \frac{desired\ cell\ concentration\ x\ volume\ sample}{measured\ TCC\ in\ inoculum} \quad [ml] \qquad (3)$$

Nutrients added

As the conducted experiments are aimed at determining the growth potential of MOB on methane, the growth should not be limited by the absence of other nutrients (N and P) in the used water. Therefore a nutrient solution containing phosphate and nitrate was added to the samples. The composition of this supplement solution and calculation of the necessary dose are elaborated on in Appendix A A dose of 5 µL was used in all experiments.

No further investigation into the limitation of growth by trace elements has been carried out in this research. It was assumed that these are sufficiently available which, from the results, seems to be accurate.

Production of methane concentrate and dilution in different batches

It is quite difficult to make a concentrated methane solution and subsequently dose it in such a way that all samples in a single batch will contain the same concentration. Additionally, it is difficult to dilute this concentrate in a similar fashion for both growth potential vials (which are fully filled) and methane analysis vials (which are only half full) as pipettes cannot be used. The following procedure has been designed to make a concentrated methane solution and to subsequently dilute relatively accurately into various vials.

1. Making a concentrated mixture of methane with water (Figure 7):
    a. A glass syringe (30ml) was filled with 25ml of filtered Evian water.
    b. The remaining 5ml was filled with highly pure methane gas and the syringe shut with a 3-way valve which is fitted to the luer of the syringe. The solution was then shaken for 1 minute to insure gas exchange between gas and liquid phases.
    c. The gas was then released from the syringe by slowly pushing the plunger back until some water escapes. The valve was then closed. The syringe now contained a concentrated methane solution.

    Even if done very accurately, the concentrate will vary in concentration every time a new mix is made in the syringe, especially with the rudimental equipment described above. For this reason a new methane concentrate solution was made for every new batch of samples. There in total this process demonstrated in Figure 7 is repeated 3 times (control samples do not contain methane).

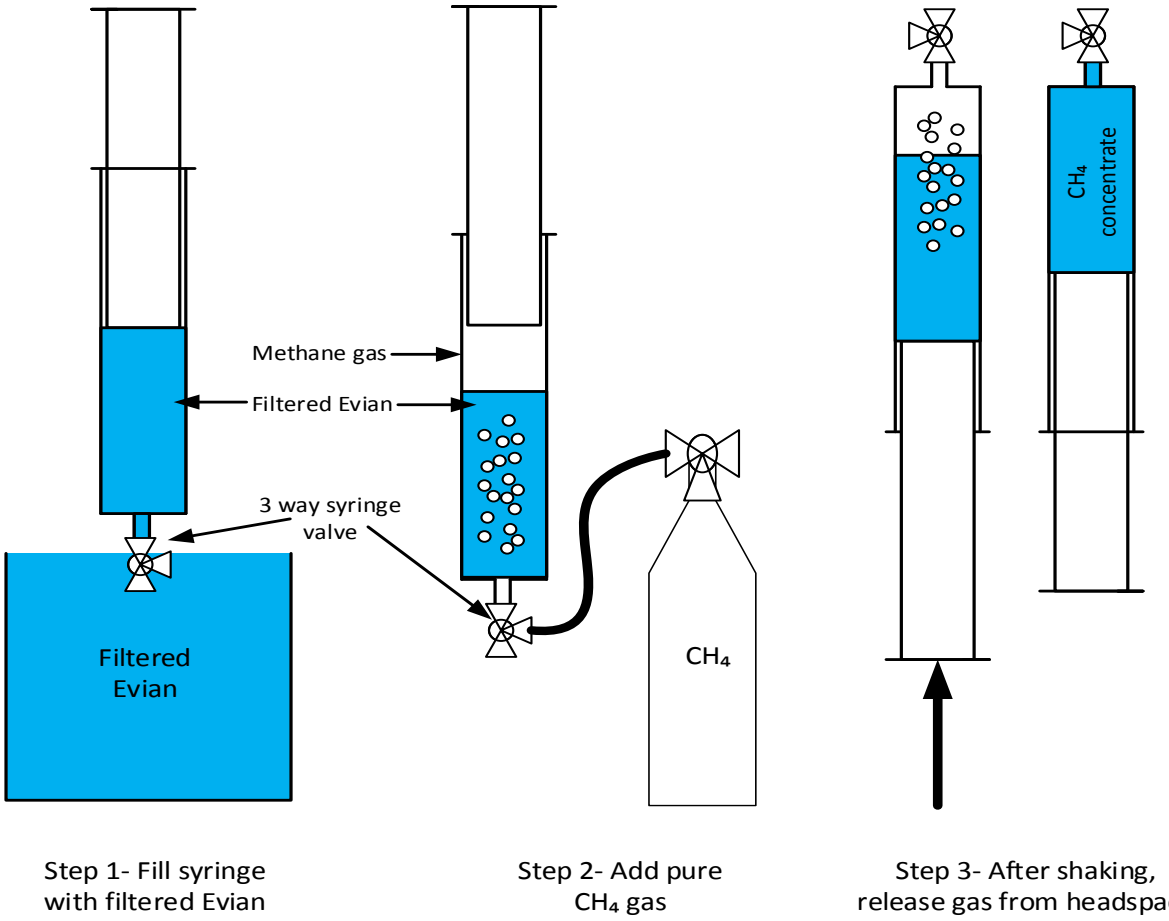


Figure 7 Illustration of the process used for making a concentrated methane solution: 1. Fill syringe with filtered Evian, 2. Add methane gas and shake for 1 minute, 3. Release gas from headspace.

2. In each experiment, three batches were prepared. Each batch consisted of 15 vials containing the same methane concentration. To achieve this, the concentrate is diluted (Figure 8) to a predetermined ratio. For example, if one wants a batch with a concentration 5 mg/l the concentrate is to be diluted approximately 3-4 times. Before the experiments, the objective concentration is determined and the corresponding dilution calculated (Table 2). This dilution must then be strictly applied to all samples of the same batch. Dilution takes place in two stages (Figure 8):
    a. A preliminary dilution was performed by injecting the methane concentrate into a larger (100ml) glass syringe containing a certain amount of filtered Evian. The difference in concentration between batches was achieved by varying the dilution (CH_4 concentrate and filtered Evian) in the larger syringe.
    b. Vials already containing inoculum and nutrient solution were filled with 20 ml of filtered Evian using a glass pipette. The total volume of the vials (if fully filled) is 24ml. The 15 vials thus prepared were filled one by one until completely full (4ml of diluted methane concentrate from large syringe) and were immediately closed with a vial cap. It is of utmost importance that the vials are closed immediately after being filled and that the time between filling and closing the samples is always the same. Table 2 gives an example of the dilution factors of methane concentrate after the first dilution procedure stage and the final dilution after the second stage. Control samples were also added to the vials from the 100ml syringes for the sake of homogeneity.

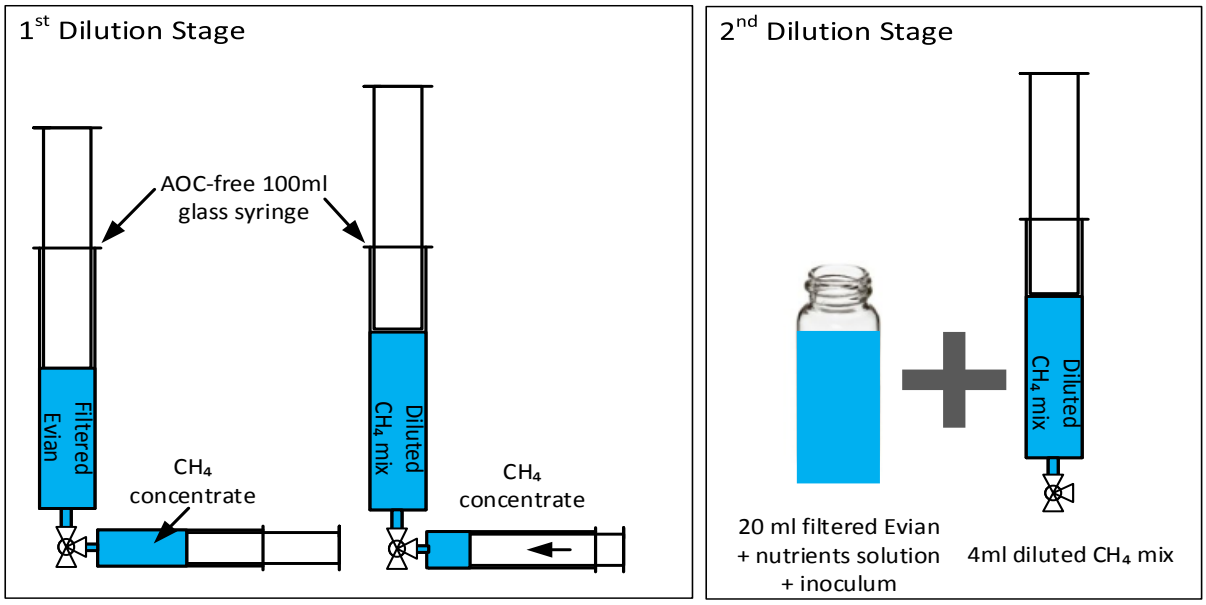


Figure 8 Two stage dilution of methane concentrate in samples

For the analysis of the methane concentration, samples were taken according to the sampling protocol from the Vitens Laboratory. The samples are only filled halfway so that the headspace methane concentration can be analysed by gas chromatography. After sampling, the vials were immediately sealed with crimp caps and further analysed for methane concentration by specialised personnel. The results were communicated after a few days.

Table 2 Dilution factors for different batches in the 100 ml glass syringe and final dilution factor in the prepared sample vials

Setting #	Filtered Evian in large syringe (ml)	Added CH_4 concentrate (ml)	Dilution factor in 100ml syringe	Final dilution factor in sample vial
Control	75	0	-	6
Batch 1	50	25	3	18
Batch 2	72	18	5	30
Batch 3	80	10	9	54

3.2.4 Incubation

Hammes et al. (2005) used two incubation settings (15 and 30 °C) and demonstrated that at the higher temperature, the growth rate and therefore the stationary phase was reached after approximately 40 h while at the lower temperature the stationary phase was only reached after 70-130 h. They also discovered that at the higher temperature, the yield (Total cell count/µg acetate-C) was higher.

In this research, it was decided to start with an incubation temperature of 30°C as this yields faster results, thereby, making optimal use of the time that was available. This may however lead to an overestimation of the yield since the temperature in the distribution system will be much lower. Further research may be required at low temperatures in order to get a more accurate prediction of the yield of methane oxidising bacteria in the distribution system.

3.2.5 Data Analysis

Yield calculations

The objective of the growth potential tests conducted in this research was to determine the yield of methane oxidising bacteria on methane. As explained, the growth of bacteria in three batches containing different concentrations of methane was measured at regular intervals. The total cell concentrations (TCC), measured by flow cytometry in time for each batch, were then plotted to show the development of the bacterial growth over time. The same was done for a control batch which did not contain methane. This sample is used to correct for bacterial growth due to other nutrients which will inherently be present in the sample.

The yield has been calculated with two different methods:

1. Hammes et al. (2005) have shown that growth potential experiments conducted at an incubation temperature of 30 °C result in a stationary phase after 3-4 days. The AOC content is therefore determined by the net growth after 3-4 days. Using this same principle, the yield of each batch was calculated individually from:

$$Y_{xCH4} = \frac{TCC_{x,t=3-5d} - control_{t=3-5d}}{C_x} \quad (4)$$

With:

$$Y_{xCH4} = yield\ of\ batch\ x$$
$$TCC_{x,t=3-5d} = average\ of\ all\ (6)\ TCC\ of\ batch\ x, between\ t = 3\ and\ 5\ days$$
$$control_{t=3-5d} = average\ of\ all\ (6)\ control\ TCC, between\ t = 3\ and\ 5\ days$$
$$C_x = CH_4\ concentration\ of\ batch\ x$$

Using this method, however, introduces a large error because there will be inevitably be large variation between measured values of different vials in a batch. Averaging may therefore give an incomplete figure for the yield. On the other hand, this method has one big advantage; it produces a single value for the yield which can then easily be compared to the yields of other batches.

2. A second method which overcomes the problem of averaging was also used to analyze the yield results. Here instead of using the average of the measured values between t=3-5 days for each batch, the yield of each individual measurement is calculated for all vials measured between 3 days (when the stationary phase starts) and 7 days (measurements after 7 days are very irregular):

$$Y_{xCH4} = \frac{TCC_{x,t} - control_t}{C_x} \quad (5)$$

With:

$$Y_{xtCH4} = yield\ of\ sample\ batch\ x\ at\ time\ t$$
$$TCC_{x,t} = indivdual\ result\ of\ sample\ of\ batch\ x, measured\ at\ time\ t$$
$$control_t = average\ measured\ control\ TCC\ at\ time\ t$$
$$C_x = CH_4\ concentration\ of\ batch\ x$$

For the control batch the average value is still used, as the deviation between values isn't very large. This method gives a much larger data set which can be plotted as a boxplot to give the range of yields and a clear overview of the measured range of yields, outliers, and the effect outliers have on the mean yield. Clear outliers, defined as falling outside the 1.5 x interquartile range (IQR) value, can be identified and deleted. This gives a more accurate and complete view of the range of probable yields to be expected from growth of MOB on methane.

The first method was used to present the results of each experiment and to analyze the yield based on total cell counts or on the concentration of low- or high- nucleic acid cell groups as explained hereafter.

It is a simple tool to quickly analyze and present the measured data in a compact fashion. The second was applied to calculate the individual yields of each measurement, which are then used to draw conclusions about the range of yields one can expect to see from MOB and to derive from this yield the acceptable level of methane in drinking water.

Fluorescence distribution plots

Analysis of the fluorescence distribution of a FCM measurement in a drinking water sample usually yields two basic clusters of cells. These clusters are commonly referred to as high nucleic acid (HNA) and low nucleic acid (LNA) bacteria (Ramseier, et al., 2011). HNA and LNA clusters on the flow cytometric dot plots (raw data) usually represent an entire bacterial community in a drinking water sample and discussions are ongoing as to the meaning of this division. Some researchers (Lebaron, et al., 2001) have argued that the LNA region represents the inactive fraction and the HNA region the active fraction cells of the same community, whilst others ((Wang, et al., 2009) later showed that the LNA cells are actually viable and active at low nutrient concentrations. Another research added that LNA cells might be classified as viable-but-non-cultivable (VNBC) cells. From the above it is clear that there is currently no general consensus as to the meaning of the LNA/HNA division. Since it has been shown however, that LNA cells are viable and active, the assumption can be made that LNA cells detected by flow cytometry are in fact active bacteria, and that these are different bacterial groups than those measured in the HNA region.

The measured fluorescence distributions were used to get an indication of the region in which it is most likely that MOB can be observed, so that results from different experiments could be compared to each other and validated.

Forward scatter analysis

Forward scatter (FSC) data was used to obtain insights into the size distributions of cells and more importantly the difference in bio-volume (or dry weight biomass production) due to bacteria growing on methane and those growing on other substances.

Though the data should be seen as an estimative measurement of the size of a cell passing by the laser beam, various researchers have used the FSC light as an indicator of the particle size, also for bacteria (Hammes, et al., 2010).

The median of the measured FSC value of a sample is assumed hereafter as giving an indication of the cross-sectional area of the bacteria in the sample. It is assumed that the difference in median values between samples is proportional to the area of the cross-section (of a spherical bacterium). With round cells, the ratio between the cross-sectional areas is then equal to the ratio of the median FSC values. As is clear from Figure 9, the spread in FSC values is quite large and as such, should not at all be seen as being an exact measure for the difference in cell size or biomass between different samples, but rather as an estimate.

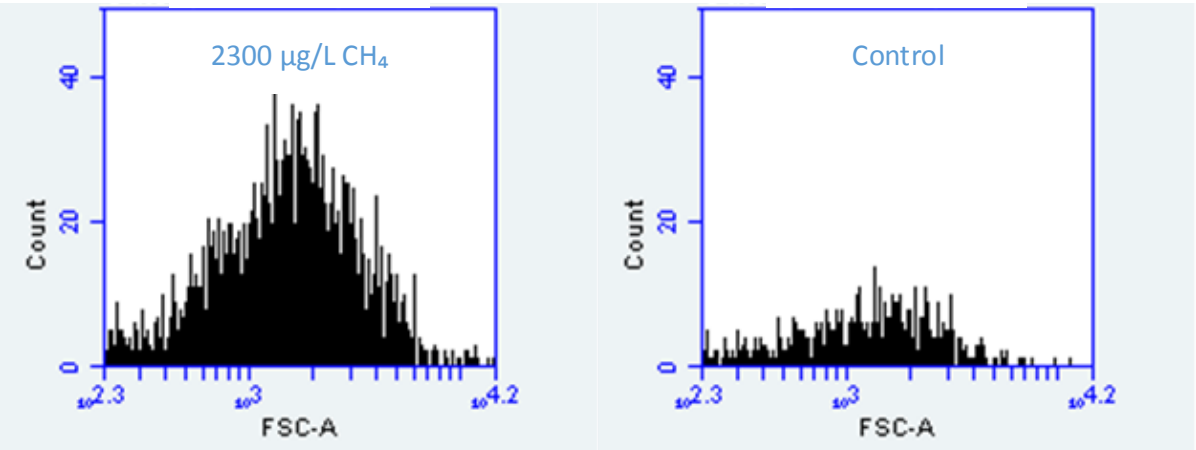


Figure 9 Forward scatter data from two samples of the first experiment: One with MOB (left) and one without MOB (right)

It has been assumed that the cells are round so that the volume is equal to:

$$V = \frac{1}{6} x\ \pi\ x\ D^3\ with\ D = diameter\ of\ the\ cell \tag{6}$$

The previously stated assumption that the ratio of the measured FSC value is equal to the ratio of the cross-sectional areas can be defined as:

$$\frac{A_{MOB}}{A_{AOC}} = \frac{median\ FSC\ MOB}{median\ FSC\ AOC} \tag{7}$$

The factor (Df) between MOB cell diameter and non-mob cell diameter is determined by:

$$Df = \frac{D_{MOB}}{D_{AOC}} = \sqrt{\frac{A_{MOB}}{A_{AOC}}} = \sqrt{\frac{median\ FSC\ MOB}{median\ FSC\ AOC}} \tag{8}$$

So that the difference in cell volume is equal to:

$$Vf = \frac{V_{MOB}}{V_{AOC}} = Df^3 = \left(\frac{median\ FSC\ MOB}{median\ FSC\ AOC}\right)^{1.5} \tag{9}$$

This volume factor (Vf) can then be used as a weighing factor between the yield of MOB and non-MOB's.

3.3 Results and discussions Growth potential experiments

This section shows the results acquired from two growth potential experiments executed over the course of the research. The results of individual experiments are presented and discussed separately, after which the calculated yields are demonstrated and conclusions are drawn concerning the relation between growth on methane and AOC.

3.3.1 Results first run

The experiment protocol of this run deviates slightly from that mentioned in paragraph 3.2 as the method was still in development; some of the lessons learnt from the results of this experiment therefore helped further improve the method to its current state. The deviations are:

- Evian was filtered using plastic syringes
- Different methane concentrations were achieved with a single dilution by dosing concentrated methane solution directly into the sample vials (from the 100ml syringe) which contained different amounts of filtered Evian for each batch.

Finally, the last measurement was done at t=264h (or 11 days) instead of 240 h. It is not probable that this last factor has much influence on the results as the growth is in the stationary phase at this point.

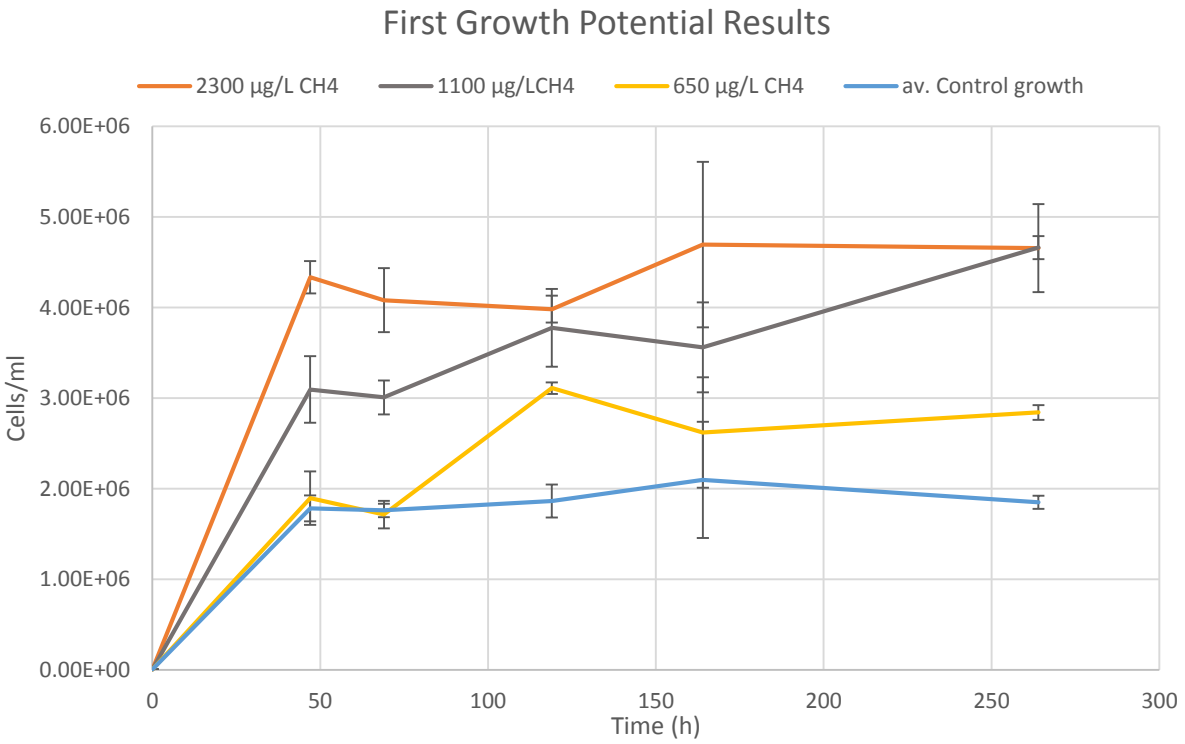


Figure 10 Results of the first growth potential run, showing the increase in bacterial cell concentrations over time in three batches containing various methane concentrations and in the control sample. Each point on the curve represents the average of a triplicate measurement from three separate vials. The error bars represent the standard deviation between triplicates. The initial cell count is 6000 cells/ml for all curves.

The growth curves from the first experiment are shown in Figure 10. The figure clearly shows that a higher methane concentration leads to a higher bacterial growth.

An important observation is that the control growth is much higher than expected; Hammes et al. (2005) found an approximate growth of 1 x 10^5 cells/ml in Evian water. The higher growth therefore indicates contamination of the samples, due to improper cleaning of the materials and equipment used. A large part of this can probably be attributed to the use of plastic syringes to filter Evian water. In later runs, water was filtered using a glass vacuum cylinder instead and more stringent cleaning

procedures were implemented. The fact that vials are completely filled may be another explanation for the higher control growths observed. The caps and septa are made of carbon based substances (i.e. plastics and rubber), which may leach into the sample.

There is a significant standard deviation in measured TCC's of triplicate samples, sometimes reaching values of 1 000 000 cells/ml. This is partly inherent to growth potential tests, but can also indicate contamination of the samples or errors in the flow cytometer analysis. There may also have been some inaccuracy in the methane dosing (as the dosing method was improved after this experiment). Finally, some methane may have escaped from the sample due to improper sealing of the caps or due to air bubbles in the vial.

One very interesting and valuable observation was made in the measured data. The distribution of the green fluorescence (FL1) indicates much higher growth in the HNA part than in the LNA part. This is demonstrated in Figure 11.

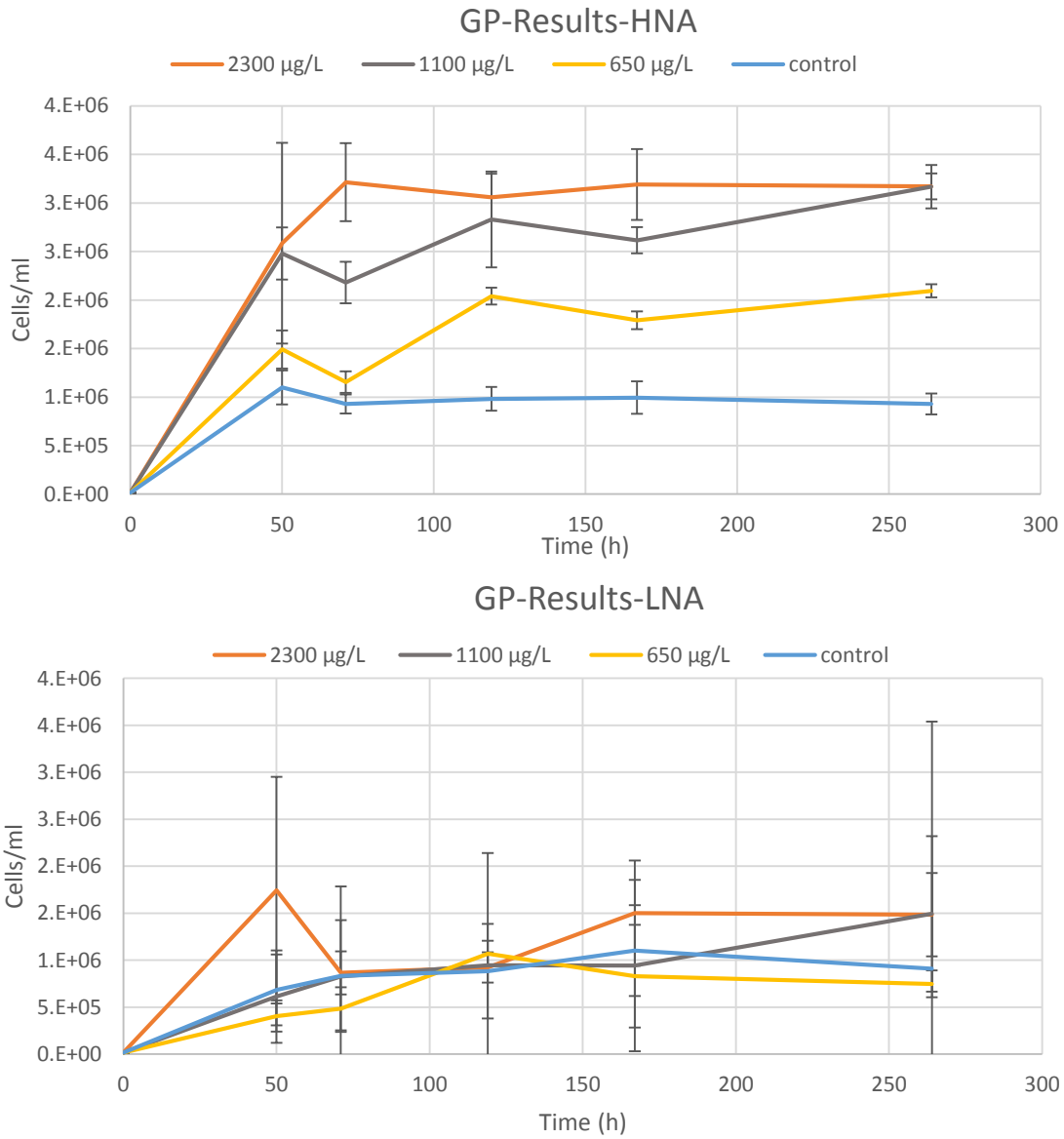


Figure 11 Average growth curves of the High Nucleic Acid content bacteria (HNA, top) and Low Nucleic Acid Bacteria (LNA, bottom) for each batch: The growth of HNA cells is clearly related to the methane concentration, while no difference in growth is observed for cells in the different conditions.

Quite clearly there is a relationship between the overall growth (Figure 10) and the HNA growth. The LNA curves on the other hand show no significant difference in growth between different batches

(different methane concentrations and control) and no relation to the overall growth. Interestingly, it seems that the LNA growth varies a lot between triplicates introducing a larger standard deviations to the overall result while the triplicate measurements of HNA region have relatively small errors.

In fact, this relationship is demonstrated even more clearly by the average yields calculated from the overall growth (TCC) and HNA growth (Table 3). For this calculation equation (4) was used. These yields are almost identical for all three batches. The LNA growth only contributes to a very minute part of the overall yield.

Table 3 Overall and HNA yields shown direct relation between HNA fluorescence measurements and the growth of bacteria on CH_4

		batch 1	batch 2	batch 3
CH_4 conc. µg/L		**2300**	**1100**	**650**
Overall yield	cells/µg CH_4	9.65E+05	1.44E+06	9.24E+05
HNA yield	cells/µg CH_4	9.48E+05	1.41E+06	9.87E+05
LNA	cells/µg CH_4	1.59E+04	2.62E+04	-6.45E+04

The FSC data was also analysed for this experiment. Due to the difference in behaviour between the LNA and HNA cells during the first experiment, the test was deemed to give a good insight into the cell sizes of bacterial communities growing on methane (batches containing methane, hereafter denoted by MOB) and those of growing only on other substances present in the water sample (control samples, hereafter denoted by AOC). The median FSC value of the cells grown in the HNA region of the fluorescence distribution was gathered for all samples measured between t=3-7 days. This data was then sorted into two groups; median FSC value of the AOC bacteria (i.e. control samples) and mean FSC values of the MOB. The average and median of the two groups was then found calculated. The resulting values are shown in Table 4 together with the calculated factors for the diameter (eq. (8)) and volume (eq. (9)) which were calculated as discussed in 3.2.5.

Table 4 shows the average (mean) and median forward scatter values of the samples containing methane (MOB) and of the control samples (AOC) measured from only HNA bacteria. The diameter factor (Df) and the factor for the volume difference (Vf) between MOB and AOC cells are also given.

	Median FSC MOB	Median FSC AOC	Df	Vf
mean	1205	720	1.29	2.16
median	1227	735	1.29	2.16

From the measured FSC values, there seems to be a factor 2.16 difference in cell volume between MOB and AOC bacteria. This means that a correction is needed in the TCC yield calculation to account for the difference in cell size leading, for a same number of cells produced, to a different biomass production. The mean and median values of the gathered data both produce the same value so that it does not matter which of the two is used.

In conclusion, the results of this experiment can be used to get a first indication of the range of expected yields on methane. Furthermore, it is highly probable that the growth of methane oxidizing bacteria is mostly occurring in the HNA region of the fluorescence distribution curve while the LNA growth is similar for all methane concentrations. It has also been shown that a large part of the measured standard deviations on triplicates samples is caused by large variations in the growth of LNA bacteria while HNA bacteria give relatively reproducible results. From this it was concluded that analyzing the HNA region FSC data yields the most accurate insight into the cell sizes in the executed experiments. Analysis of this data showed that there is a factor 2.16 difference in cell volume between samples containing methane and those without methane.

3.3.2 Results final run

All protocols described in the methods section of this chapter were followed accurately for the final run. The growth curves for each CH_4 concentration over time is shown in Figure 12.

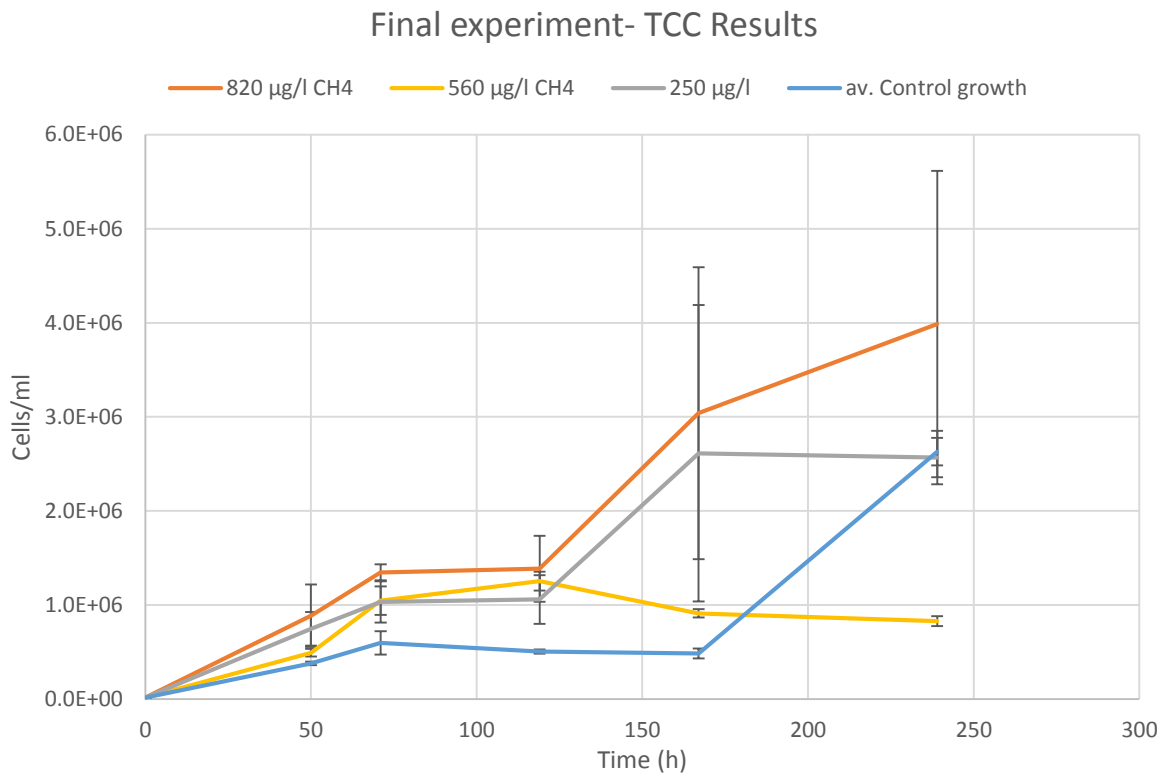


Figure 12 Results of the final growth potential run, showing the increase in bacterial cell counts over time in three batches containing various methane concentrations and in the control sample. Each point on the curve represents the average of a triplicate measurement from three separate vials. The error bars represent the standard deviation between triplicates. The initial cell count for all four curves is 14 960 cells/ml.

Once again the curves show that higher growth occurred at higher methane concentrations. Additionally, the control growth has declined and has stabilized at approximately $5x10^5$ cells/ml, so that one can assume little contamination of the samples in this experiment.

The growth in all 4 settings was relatively uniform until t=119h. After t=119h there was a sudden increase in measured TCC's in some samples (of a triplicate measurement). At t=167h this increase was measured only in samples from the 820 µg/L and 250 µg/L batches, while in the final measurement at t=239h, all batches showed at least one of the triplicate samples with this peculiar high growth.

Though these results are somewhat puzzling, analysis of the measured fluorescence data gives some insights which might help to explain these deviations:

1. Samples which show extensive growth have a different fluorescence distribution than those observed in the first GP experiment. Figure 13 compares the fluorescence distribution of a "normal" sample and of a sample with extensive growth. Both are from the same batch (250 µg/L) and are measured at the same time (t=167h). The measured TCC's are shown at the bottom of each graph. The left distribution (normal sample) is similar to that observed in previous experiments while the right graph (sample with excessive growth) shows a large peak in the LNA part of the fluorescence distribution. All samples which show extensive growth have this peak in the LNA part of the fluorescence distribution. The extensive growth observed in some of the samples can therefore be attributed to this peak.

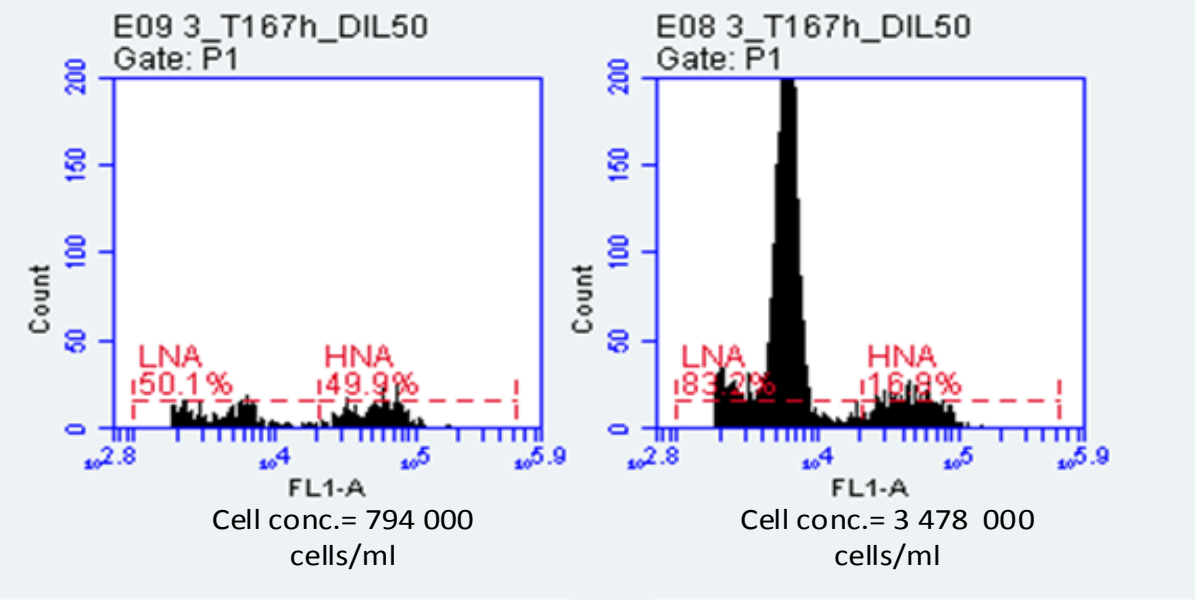


Figure 13 Fluorescence distributions of two 250 µg/L CH_4 samples at t=167h: The left graph shows a normal distribution similar to that observed in the first run, while the right graph shows a large peak in the LNA part indicating the growth of organisms which did not occur in the first run.

2. The yields calculated from the TCC, the HNA and the LNA regions do not correspond to each other. Even in the initial stationary phase (t=3-5 days), the HNA and LNA yields are both lower than the TCC yield. Based on the results from the first experiment, the HNA yield should be similar to the TCC yield and the LNA yield should be much lower. Table 5 shows that at least half of the growth occurring in all samples was caused by LNA bacteria. On the other hand it is fascinating to observe that the overall yields found in this experiment are very much similar to those in the previous experiment. This makes sense since the total amount of biomass produced in the sample must be proportional to the amount of carbon available. The overall yields should therefore remain useable to determine the acceptable methane level. Further conclusions pertaining to the bacterial community should however not be based on the results of this run.

Table 5 Overall, HNA and LNA yields of batch 1, 2 and 3 calculated from the average TCC concentrations between days 3 and 5

		Setting 1	Setting 2	Setting 3
CH_4 concentration		**820 µg/L**	**560 µg/L**	**250 µg/L**
TCC yield	Cells/µg CH_4	9.95E+05	9.41E+05	1.98E+06
HNA yield	Cells/µg CH_4	3.48E+05	5.27E+05	7.31E+05
LNA yield	Cells/µg CH_4	6.46E+05	5.41E+05	1.25E+06

3. At t=239h, the control samples show a similar spike in growth in the LNA region of the fluorescence distribution graph. Though this is delayed with respect to other batches, it is an indication that the observed peak is not caused by bacteria growing on methane.

All of the aforementioned points lead to believe that except for MOB, another bacterium was growing in the samples. These bacteria have a LNA content and there is no evidence to show they grow on methane. These bacteria either inhibited the growth of MOB or possibly scavenged on them, thereby causing lower growth of HNA bacteria and decreasing the HNA yield.

It is not clear where the bacteria originated from and why they were not found in large numbers in previous experiments. One hypothesis is that they were present in the inoculum and developed a competitive advantage over the period during which experiments were carried out; thereby making it

possible for them to compete with methanogens even in environments where other nutrients were scarce. The development of these LNA bacteria over time is shown clearly by the fluorescence distribution graphs of the inoculum used for the different runs.

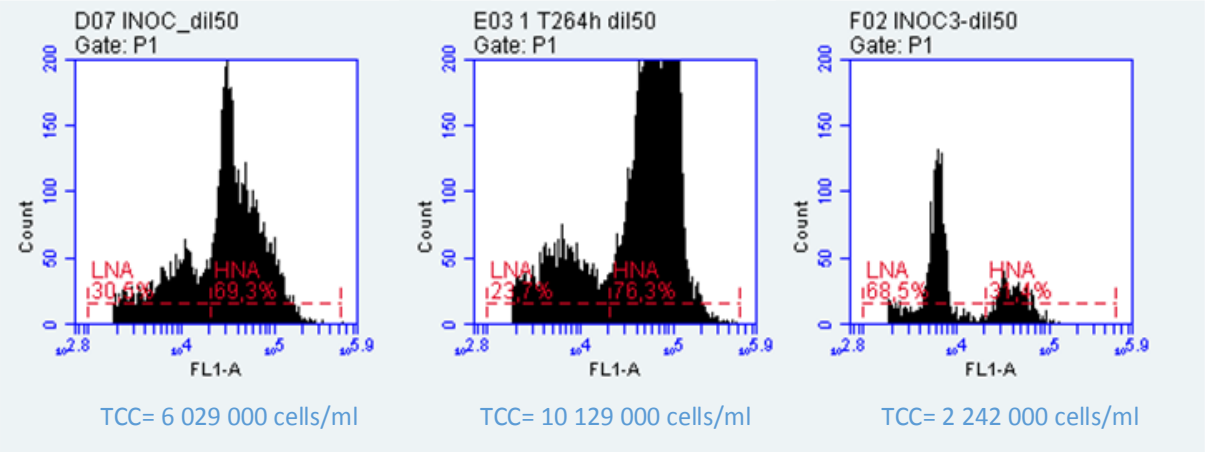


Figure 14 TCC & fluorescence distribution graphs of the inoculum used for the three experimental runs. Note that the cell count in the last inoculum is lower due to lower initial dose of methane during making of this batch. There is a clear peak in the LNA part of this inoculum which explains the rapid growth in the final growth potential experiment.

3.3.3 Concluding remarks on the growth potential method and results

The results of both experiments demonstrate that higher methane concentrations can be related to higher growth of bacteria and therefore confirming the initial concern of Oasen that methane in the produced water may cause biological instability.

Results of the first experiments indicate that there is a strong relation between the overall growth of methane-oxidizing bacteria and the growth observed in the HNA region of the flow cytometric fluorescence distribution graphs. The growth in the HNA region in this experiment was almost similar to the overall growth, while the growth in the LNA region was the same for all four batches independent on the methane concentration. Analysis of the HNA region therefore should give more accurate insight into the cell size difference between MOB and bacteria growing on AOC. The forward scatter data of the first experiment revealed a difference in volume between MOB and AOC bacteria. The factor between the differences in volume was calculated from the results and was found to be 2.16.

The results of the second experiment do not show any correlation between HNA, LNA and overall growth. It has been demonstrated that this can most probably be attributed to the growth of a different type of bacterium which originated from the used inoculum. This bacterium can be easily distinguished because of the large peaks in the LNA region of the fluorescence distribution. Due to the high productivity of this organism, the contribution of the LNA region was significant and the HNA growth was lower than observed in the other experiments. The overall yield however, was quite similar to that observed in previous runs, so that it is concluded that these can be used for further analysis of the acceptable methane level. Due to uncertainty about the population in the samples, the data from this experiment pertaining to the distribution of the population (i.e. cell sizes) have not been shown and will not be used hereafter.

Generally speaking, the author is confident that the developed method can be applied for the determination of growth potential on methane and can be very useful in determining the effect of methane oxidizing bacteria on the biological stability of drinking water.

There is, however, still a lot of uncertainty in the results obtained from these experiments. This is largely inherent to the unpredictable nature of bacterial behavior in such tests. The outcome of an experiment can be largely influenced by minute contamination occurring during cleaning and storage

of equipment and during the preparation of samples. After analyzing the data from the experiments it is clear that these can at most be used to get a preliminary indication of the yield of methane oxidizing bacteria. Many more runs must be executed and the method further improved and complimented with other measuring techniques, in order to obtain a reliable and large set of data which can improve the accuracy of the yield calculations. In particular, the difference in cell size of MOB and AOC bacteria needs to be investigated in more detail as the FCM does not give an absolute measure of the produced biomass.

Tips for future Researchers

- Experiments (especially the cleaning of vials and preparation of samples) should be carried out in extremely clean environments. Preferably an isolated room where air contamination can be minimized (i.e. no volatile carbon sources such as perfumes and ethanol).
- Start with a freshly prepared batch of inoculum for each new experiment, making sure the LNA peak does not develop. Reusing the same inoculum every time increases the risk of strengthening the competitive advantage of LNA bacteria. It may also be possible to find a way to store the inoculum for longer time spans (by refrigeration) whilst keeping it stable and unchanged.
- Alternatively, use pure cultures of methane oxidizing bacteria (such as methylococcus capsulatis) instead of natural inoculum. This will help identify a more specific region in the fluorescence distribution plot in which one should count the cells and will yield results with less noise thereby improving the yield calculation.
- This research did not include an investigation into whether or not other elements than phosphate and nitrate were growth limiting. In future experiments, the addition of trace elements should be considered or it should at least be proven that these are sufficiently available.
- Flow cytometry is a very nice and simple technique for quantifying the TCC in a sample. It is however not sufficiently equipped for an accurate determination of the cell volume. Other techniques should therefore be added to the presented method in order to complement and improve the estimation of the biomass yield.

3.4 Yield calculations

This section summarizes the yields calculated from the results of the two growth potential experiments. In paragraph 3.2.5 two methods were discussed to calculate the yield. Equation (4) is based on the average TCC values of all samples (triplicates) in batch measured at t=3 and 5 days. This equation was used to calculate the yields shown in the section 3.3.1 and 3.3.2 because it provides a single value, enabling a quick comparison with other results. The issue with this method is that it pulls a larger data set (6 values) into one average value. Due to this averaging, outliers are less visible and may lead to incomplete or even inaccurate conclusions.

To get better insight of the range of expected yields the second method (equation (5)) was therefore used to analyze results of both experiments in this section. The yields of each flow cytometric measurement executed over the course of the experiment (between t=3-7 days) were calculated separately.

The average value of the (triplicate) control samples was subtracted from individual TCC of three different CH_4 batches. This is valid as the control batches did not show significant standard deviations on triplicates; the error introduced by averaging them is acceptably low. The individual yields were calculated for the measurements done at t=3, 5 and 7 days during which both experiments were in the stationary phase. This gives a total data set of 27 values for each experiment (3 batches, measured in triplicate, at three different time intervals).

With the individual yields calculated, the data was analyzed and sorted to find the 1st, 2nd and 3rd quartile (25th percentile, median and 75th percentile respectively) values for the individual experimental datasets (first and final experiments) and the combined datasets.

Table 6 Distribution of yields for experimental runs and overall results

	First Experiment	Final Experiment	Overall dataset
Number of values	**27**	**27**	**54**
min	9.54E+04	6.11E+05	9.54E+04
Q1	8.84E+05	8.31E+05	8.63E+05
Median	1.13E+06	1.11E+06	1.12E+06
Q3	1.75E+06	1.54E+06	1.70E+06
Max	2.12E+06	4.26E+06	4.26E+06

Next, the interquartile range (IQR) was calculated from:

$$IQR = 3^{rd}\ quartile\ value - 1^{st}\ quartile\ value \tag{10}$$

Outliers are defined as those values <1st – 1.5 x IQR or >3rd quartile + 1.5 x IQR. The remaining data was then plotted using boxplots as shown in Figure 15. The error bars indicate the maximum and minimum values which fall within the 1.5 x IQR.

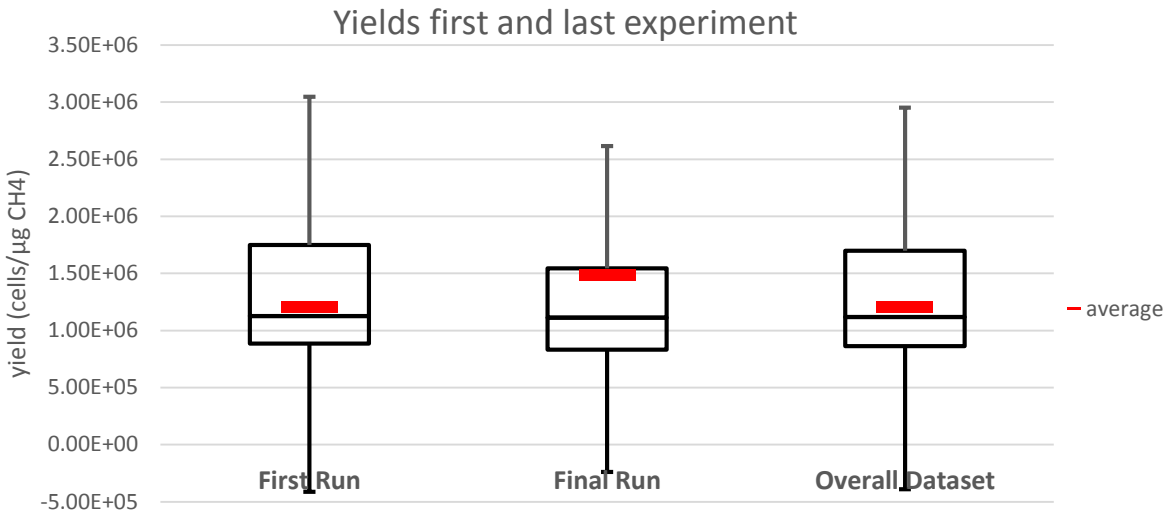


Figure 15 Boxplots of yields calculated from the two experimental runs, after deleting outliers, and of the overall range of yields including the results of both experiments. The error bars indicate the 1.5 x IQR lower and upper limits.

Since the overall dataset seems to show no significant deviation from the results of the individual experiments, it can be used to give a range of values in which the yield of MOB is expected to fall. From this the following conclusions can be drawn:

1. The range within which the yield of methanotrophs falls is most likely 8.6 x 10^5-1.7 x 10^6 cells/µg CH_4. This covers all values between the 25th and 75th percentile values.
2. It is likely that the yield of methanotrophs on methane is close to 1.1 x 10^6 cells/µg CH_4. This is the mean value of all results and corresponds very well with the individual mean values of the two experiments.

3.5 The acceptable methane level

The most used parameters for biological stability in drinking water in the Netherlands is AOC. At present, water containing an AOC content of 10 µg/L is generally accepted as being biologically stable. Hammes et al. (2006) estimated the yield of a natural consortium of bacteria on AOC to be 1 x 10^7 cells/µg AOC. Looking only at the TCC and assuming these can be compared on a 1-to-1 basis (assuming no difference in cell size), the maximum acceptable methane level would be equal to:

$$Acc.methane\ level\ = \frac{Y_{AOC} x\ acc.AOC\ concentration}{Y_{CH_4}} = \frac{1x10^8}{Y_{CH_4}}\ \{\mu g\ CH_4\ /\ L] \qquad (11)$$

With the range of MOB yields mentioned in the previous paragraph, the range of acceptable methane levels were calculated, yielding a range between 58-117 µg/L CH_4. Note that this is the maximum acceptable level of methane (equivalent to 10 µg/L AOC in terms of number of cells produced) assuming that there is no AOC in the water. For the purpose of conservatism the lower value (58 µg/L CH_4) of this range is used for further estimation of the methane level.

In terms of yields the methane and AOC can also be related to each other as:

$$\frac{Y_{AOC}}{Y_{MOB}} = 5.8 \qquad (12)$$

In other words, the number of bacterial cells produced on 5.8 µg of CH_4 is equal to the number of cells produced on 1µg of AOC. However, this comparison is not complete as it has been shown from the results of the first experiment, that there is a difference in cell size between samples with methane and control samples. Thus, for a same amount of cells growing on methane and AOC, the resulting production of biomass (measured in weight) will be higher in a sample containing methane.

The calculated difference in volume is expressed as a volume factor (Vf). This factor is derived from the difference in the mean FSC data which were demonstrated in the results of the first experiment. Assuming that the weight of a cell is directly related to its size, this factor can be incorporated into equation (12) so that the yield is corrected for this difference in volume (and therefore in biomass production):

$$Y_f = \frac{Y_{AOC} x V_{AOC}}{Y_{CH_4} x V_{MOB}} = \frac{Y_{AOC}}{Y_{CH_4} x Vf} = 2.7\ \mu g\ CH_4/\mu g\ AOC \quad (13)$$

The bulk growth potential (the growth due to AOC + CH_4) can then be written in the following manner:

$$Bulk\ GP = C_{AOC} + \frac{C_{CH_4}}{Y_f} = C_{AOC} + \frac{C_{CH_4}}{2.7} \quad [\mu g\ AOC\ eq./L] \quad (14)$$

By this method, both parameters (AOC concentration and methane concentration) can be measured individually in a sample after which the bulk growth potential can be determined.

3.6 Recommendations about the acceptable level of methane for Oasen

As discussed in the goals and requirements (sections 2.1 and 2.2) of this report, the goal of this research is to determine the most optimal post-treatment technology for the sufficient removal of methane in order to produce water with a sufficient biological stability. In the Netherlands, water containing <10 µg/L AOC is found to be acceptably stable. Oasen, however, wants to produce water with a better quality. Requirements stated in Oasen's original project description (Van der Laan, 2013) has two parameters pertaining to the biological stability:

1. The AOC content must be <1 µg/L
2. The CH_4 concentration must be <10 µg/L

Filling these values into the equation for the bulk growth potential (eq. (14)) yields the following value for biological stability:

$$Bulk\ GP = 4.7\ \mu g\ AOC\ eq./L.$$

This is much lower than the generally accepted value of 10 µg AOC/L and as such is found to be acceptably low. It must also be considered that it may be possible to decrease the methane concentration to even lower values than 10 µg/L, but that this becomes increasingly difficult.

Thus, it is recommended to Oasen to take 10 µg/L as a maximum acceptable concentration to which all potential removal techniques must comply. Techniques capable of higher removals without requiring unreasonable amounts of energy or investment costs are then preferable over those capable of providing the bare minimum removal requirement.

4 METHANE REMOVAL TECHNIQUES

4.1 Introduction

Conventional treatment plants treating anaerobic groundwater usually consist of a multi-barrier system. In the Netherlands these plants usually include some form of aeration and at least one sand filtration step, both of which provide removal of methane to some extent; it is advised to reduce the methane concentration to <0.1 mg/l by aeration before filtration (Reijnen, 1994). It is then assumed that all remaining methane will be removed in the sand filters and the effluent concentration will be negligible with respect to bacterial regrowth. This assumption has proven to be valid as an inquiry from REWAB[2] showed no measured CH_4 concentration (detection limit 5 µg/L) in any drinking water produced in the Netherlands between 2009 and 2013.

There is currently no legislative norm on the allowable concentration of methane in water. As evidence of biological growth due to the presence of methane has been shown before (see introduction chapter 3), however, the assumption at the start of this research was always that very low concentrations of methane would have to be achieved in order to produce biologically stable water. The goal was therefore to look only at those techniques which from past experience have been shown to accommodate high removal rates or to find possible new techniques which in theory could do so.

During the process of the research presented in the preceding chapter combined with Oasen's goal to produce water of a pristine quality it became clear that the maximum allowable concentration would have to be 10 µg/l CH_4. The minimum, average and maximum CH_4 concentrations in the raw water at Kamerik are shown in Table 7. For an elaborate overview of all water quality parameters, the reader is referred to Appendix B.

Table 7 Minimum, Average and Maximum raw water methane concentrations at ZS de Hooge Boom

	Raw water CH_4 concentration	Unit
Minimum	1600	µg/L
Average	2000	µg/L
Maximum	2700	µg/L

Since the treatment must produce biologically stable water at all times, it needs to be able to cope with the maximum concentration of the raw water, which, as Table 7 shows, is equal to 2700 µg/L. With a maximum effluent concentration of 10 µg/L, any viable removal system will have to provide minimum removal efficiency of 99.6%.

In Oasen's new treatment concept, the removal of methane has to be achieved by one post-treatment step. Three possible mechanisms could be utilised to do so: biological, chemical or physical removal.

As one can conclude from previous experience in the Netherlands, sand filtration has been an effective medium to accommodate methane removal. RO filtrate, however, is of a very high quality and a biological post-treatment step could partly diminish this. Therefore, biological removal has not been considered as a potential treatment mechanism.

Methane is a 28 times (over a 100 year period) more potent greenhouse gas than CO_2 (Myhre, et al., 2013) and, as such, is of big concern with respect to climate change. With traditional aeration techniques, the gas is stripped from the water to the gas phase and released straight into the atmosphere. In the initial phase of this thesis it was investigated whether it would be possible to oxidize methane either using catalysts or chemically. Several discussions with experts, however, have revealed that this does not seem to be a viable solution because the gas is extremely stable; Methane

[2] REWAB is a benchmarking tool used to gather water quality parameters from all Dutch drinking water companies. The data was obtained via KWR Water-cycle Research Institute and showed no presence of methane.

is very difficult to oxidize, especially at low concentrations and temperatures. To use chemical oxidation as a removal process would therefore require large contact areas with catalysts, high doses of chemicals and long contact times.

As a means of comparison, the rate constant of methane with OH radicals (which are also formed by ozonation or UV/peroxide) in air is $6.3\ x\ 10^{-15}\ M^{-1}s^{-1}$ (Sander, et al., 2011) compared to other chemicals for which advanced oxidation processes (AOP's) are beeing used which have rate constants in the order of $10^{8} - 10^{10} M^{-1}s^{-1}$ (Glaze, et al., 1989). This means that the reaction is a magnitude of 23 times slower than for other substances removed by advanced oxidation processes. It was therefore deemed highly unlikely that the reaction rate will become high enough to make advanced oxidation a viable solution. Therefore, unfortunately, chemical methane oxidation does not seem to be an attractive solution both from an environmental as well as an economical perspective.

From the above it becomes clear that only physical removal (gas exchange) of methane is a viable option. Physical removal techniques have been used in a variety of forms and applications and there is therefore a lot of literature on these type of systems. Three techniques which, from literature, showed the potential to facilitate extremely high methane removal efficiencies were selected. This chapter provides a brief introduction into the physical removal mechanism, followed by descriptions of the three removal techniques, pilot results, cost and energy estimations and finally a multicriteria analysis (MCA) which was used to determine the most optimal post-treatment step.

4.2 Physical Removal processes

Gas exchange systems (GES) make use of the principles of mass transfer to remove gasses from or add gasses to water. GES's utilize different methods to facilitate contact between water and air, thereby accommodating and stimulating mass transfer. This chapter explains the principles which drive this mass transfer, starting with the equilibrium concentration, the air/water ratio and finally, Fick's law.

4.2.1 Equilibrium concentration

When water containing dissolved gasses comes into contact with, air gas exchange will take place until the equilibrium concentration of that gas is reached. The direction of the exchange depends on the concentration difference; if the water is oversaturated the gas will diffuse to the air and vice-versa (Crittenden, et al., 2012).

The equilibrium concentration for a certain gas is determined by the distribution coefficient as described in Henry's Law.

$$k_D = \frac{c_s}{c_g} \tag{15}$$

With

$$c_s = conc.volatile\ matter\ in\ water,\ c_g = conc.volatile\ matter\ in\ gas$$

$$k_D = distribution\ coefficient$$

And

$$c_g = \frac{p_a * MW}{RT} \tag{16}$$

With

$$p_a = partial\ pressure = atm\ pressure * molar\ fraction\ gas\ in\ air \qquad [Pa]$$

$$atm.pressure = 101325\ Pa$$

$$MW = molar\ weight\ [\frac{g}{mol}]$$

$$R = universal\ gas\ constant = 8.3143\ [J/mol/K]$$

$$T = Temperature\ [°K]$$

The distribution coefficient of a certain gas in water is dependent on the temperature. These coefficients have been determined for a wide variety of gasses and can be found in literature. Table 8 gives an overview of the distribution coefficients and some other properties of gasses which are important for drinking water treatment.

Table 8 properties of various gasses in found in drinking water: Molar Mass, Molar fraction in Air and Distribution Coefficients at three different temperature commonly occurring in drinking water.

Gas	Molar mass [g/mol]	Molar fraction in air {mol/mol}[3]	Distribution coefficient[4]		
			0 °C	10 °C	20 °C
CH_4	16.0143	0.000002	0.0556	0.0433	0.0335
NH_3	17.0306	trace	1.3	0.943	0.763
N_2	28.0134	0.78084	0.023	0.0192	0.0166
O_2	31.9988	0.20948	0.0493	0.0398	0.0337
H_2S	34.0799	trace	4.69	3.65	2.87
CO_2	44.01	0.0004	1.71	1.23	0.942
Air	-	-	0.0288	0.0234	0.02

The distribution coefficient can be extrapolated linearly between the values shown in the above table. Linear extrapolation of values at temperatures occurring in drinking water treatment only leads to a very small error (approximately 1.5%) compared to the actual values. This is due to the fact that the standard enthalpy change is negligible at these low temperatures (Helm, van der, 1998).

In order to demonstrate the approximate equilibrium concentration for CO_2 and CH_4 in the treated water at Kamerik, the values shown in Table 9 have been calculated assuming a water temperature of 10°C.

Table 9 Equilibrium concentration in water at treatment plant

	Molar Fraction (mol/mol)	Partial pressure (Pa)	Molar Weight (g/mol)	Conc. In air (mg/l)	Distribution Coefficient (283K)	Equilibrium Conc. In water (mg/l)
CH_4	$2 * 10^{-6}$	0.20265	16.0143	0.00138	0.043	$6*10^{-5}$
CO_2	0.0004	40.53	44	0.758	1.2	0.9

4.2.2 Maximum theoretical removal efficiency

The equilibrium concentration will eventually be reached provided that water is in contact with air for a very long time, sufficient exchange area is available and the air is continuously refreshed. Refreshing the air keeps the concentration of methane low and close to that shown in Table 8.

In process of aeration the molar fraction of a certain gas in air will change because part of the gas will escape from the water to the gas phase or vice versa. The concentration of gas (c_g) will therefore also change increasing the saturation concentration (CS). The removal efficiency is therefore dependent on the amount of fresh air available compared to the flow of water. This is quantified by the air to water ratio called RQ.

[3] (Lide, 1997), values for air at 15°C and 101325 Pa

[4] (Helm, van der, 1998)

The RQ is one of the main factors influencing exchange of gas between air and water. The maximum removal efficiency for a certain gas (with a certain k_D) at a certain RQ can be calculated using the following formula which is derived from a simple mass balance[5]:

$$k = \frac{RQ}{RQ + k_D} = \frac{c_0 - c_e}{c_0 - c_s} \tag{17}$$

With:

$$k = removal\ efficiency$$

$$RQ = \frac{Q_{air}}{Q_w}$$

$$k_D = distribution\ coefficient\ of\ gas$$

$$c_s = equilibrium\ concentration\ in\ water$$

This form does not take into account the inefficiency of the aeration system and assumes that sufficient air/water surface is created and sufficient contact times are achieved (i.e. $k_2T \rightarrow \infty$). It therefore gives the theoretical maximum removal for a certain RQ and a certain k_D. In reality the removal will be lower due to limitations of each system. To demonstrate the effect of the RQ, eq. (17) has been plotted (Figure 16) for a range of RQ's and two different gasses.

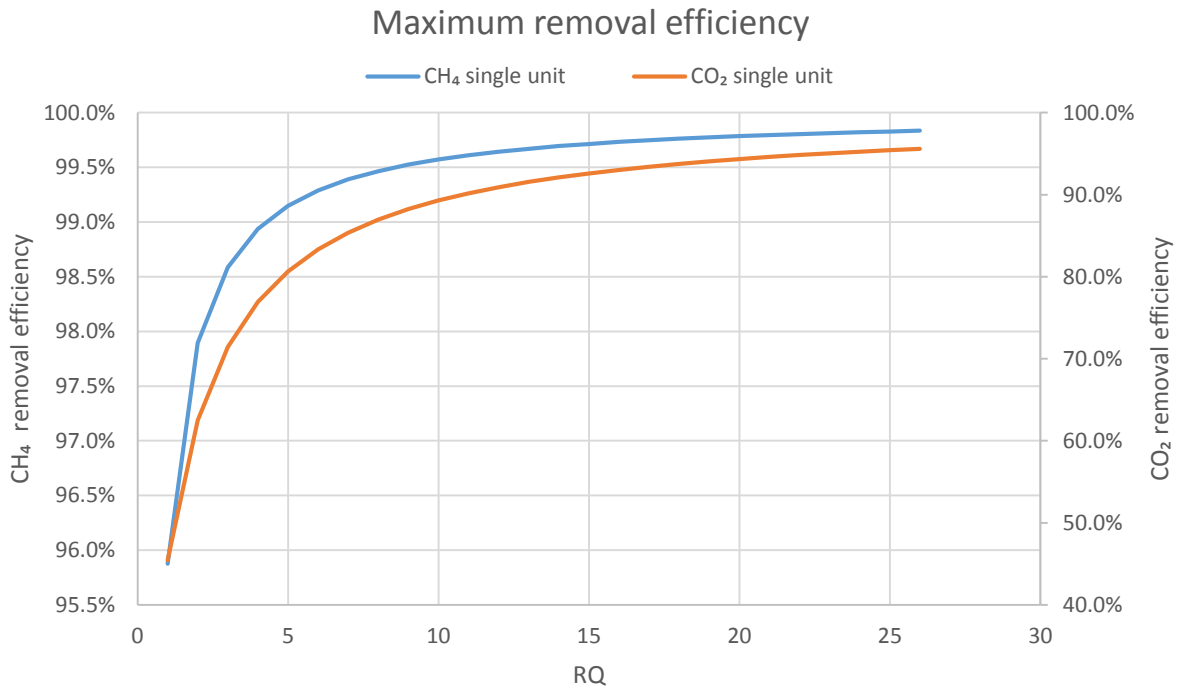


Figure 16 Theoretical Removal efficiencies for CO_2 and CH_4 for a single step aeration

As observed in the graph, the removal efficiency increases with a larger RQ. At the higher RQ's however, the change in removal is minimal, increasing the RQ above a value of 10 does not have a very large impact on the removal efficiency. On the other hand, increasing the RQ does increase the energy costs of a treatment step as more air needs to be pumped through the aerator. There is therefore a trade-off between high removal capacities and energy use of an aeration system.

[5]Appendix C : This formula only holds for completely mixed and co-current plug-flow systems; If $k_2T \rightarrow \infty$ in eq.(19) and (20).

The maximum theoretical removal of counter-current systems is higher; if $k_2T \rightarrow \infty$ in eq. (21), $k \rightarrow 1$

Another option to achieve very high removal rates with low RQ's is to place two aeration systems in series. Since Henry's Law holds for very low concentrations of gasses, the removal (in percentages) of each separate systems will be the same, so that the removal of multiple units can be calculated by:

$$K = 1 - (1 - k)^n \tag{18}$$

With:

$$K = overall\ removal$$
$$k = removal\ in\ each\ step$$
$$n = number\ of\ units\ in\ series$$

With eq. (17), the removal for the same two gasses demonstrated in Figure 16 will be a lot higher at lower RQ's. Figure 17 demonstrates this phenomenon for two units in series, each with the same RQ.

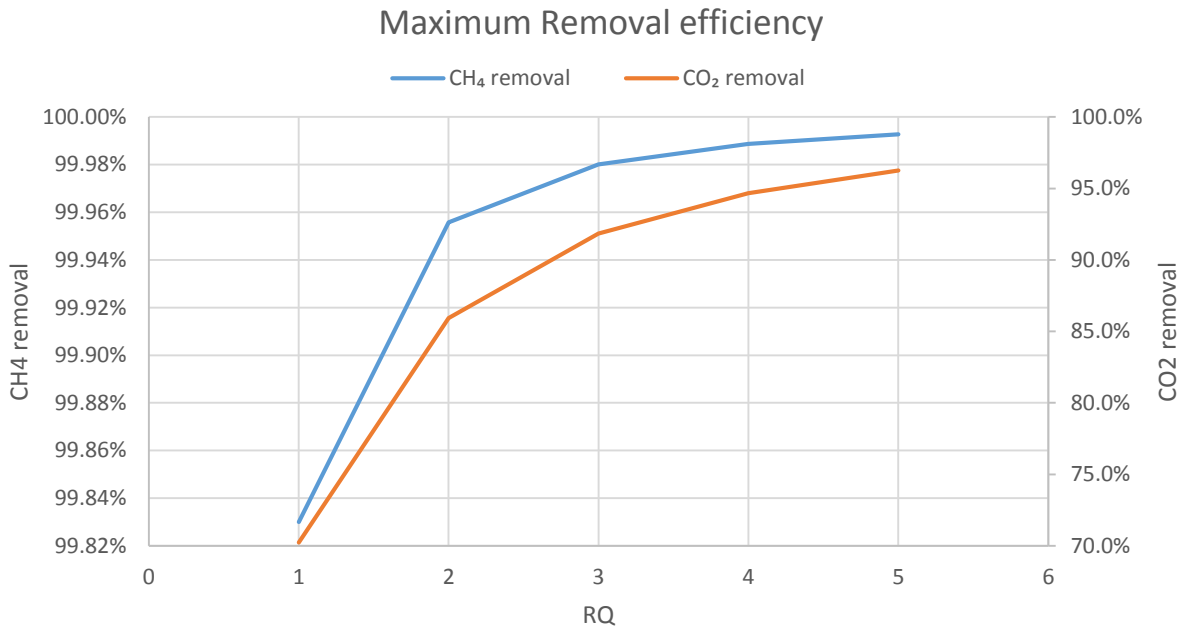


Figure 17 Maximum removal efficiencies for two units in series

The removal efficiencies demonstrated previously do not take into account the properties of aeration systems. The actual capacity of a system to approach the theoretical maximum removal efficiency is determined by its capacity to facilitate diffusion from the gas to the liquid and vice versa. To understand factors that are limiting and prevent the actual removal of a system from being the same as the theoretical removal, a couple of mechanisms need to be understood.

Fick's Law

The rate and direction of diffusion is determined by the difference in concentration in the gas and liquid phase, the magnitude of this diffusion per unit of diffusive area being proportional to the concentration gradient (Helm, van der, 1998). This is known as Fick's Law which states:

$$\phi_d'' = -D \times \frac{dc}{dy}$$

With:

$$\phi_d'' = the\ rate\ of\ diffusion\ per\ unit\ of\ area = \left[\frac{g}{m^2.s}\right]$$
$$D = the\ diffusion\ coefficient\ of\ the\ gas\ \left[\frac{m^2}{s}\right]$$

$$\frac{dc}{dy} = the\ concentration\ gradient\ of\ the\ gas\ \left[\frac{g}{m^4}\right]$$

The negative right hand side of the equation indicates that diffusion will take place from a high concentration to a low concentration.

The diffusion constant D as defined in Fick's Law is dependent on:

- Type of gas: Different gasses have different molecular masses, therefore the Brownian movement will be different.
- Type of medium: Air or Water, the Brownian movement in air is interrupted less by collisions than in water because the respective distance between molecules is bigger.
- The temperature; Influences the speed at which molecules are moving

Mass-balance

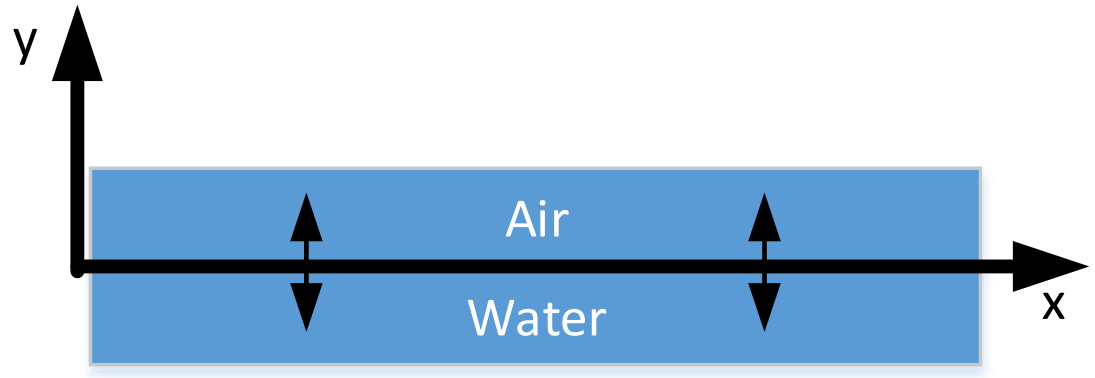


Figure 18 Axis definitions

By making the mass balance over the length of the gas-exchange module, the change in concentration can be determined. Taking the coordinate system as demonstrated in Figure 18, the change in liquid-phase concentration is derived (Helm, van der, 1998):

$$\frac{dc_l}{dt} = v_{lx}\frac{dc_l}{dx} + D_1\frac{d^2c_l}{dy^2} - r_l$$

$$\frac{dc_l}{dt} = v_{lx}\frac{dc_l}{dx} + k_2 \times \left(k_D \times c_g - c_l\right) - r_l$$

With:

$$c_l = concentration\ in\ liquid\ \left[\frac{mg}{l}\right]$$

$$v_{lx} = flow\ velocity\ \left[\frac{m}{s}\right]$$

$$r_l = production\ per\ unit\ of\ time = 0\ for\ methane$$

$$k_2 = k_L \times a$$

The mass balance for the air side of the system yields a formula similar to the one shown above, namely:

$$\frac{dc_g}{dt} = v_{lx}\frac{dc_g}{dx} - \frac{k_2}{RQ} \times \left(k_D \times c_g - c_l\right) - r_g$$

With:

$$c_g = concentration\ in\ gas\ phase\ \left[\frac{mg}{l}\right]$$

$$r_g = production\ per\ unit\ of\ time = 0\ for\ methane$$

The so called k_2 *or* $k_l a$ value are device specific parameters, which need to be determined during the pilot plant research; they are dependent on a device's capacity to facilitate surface area for exchange to take place and the residence time. An aeration techniques' main purpose is to provide the most optimal combination of both, however they will never be able to reach the theoretical removal efficiency; there will always be limitations to the provided surface area and the residence time.

It is therefore essential to determine the device specific parameters of each technique and the settings necessary to ensure sufficient removal. This is done by demonstration, optimisation and comparison of techniques in pilot studies.

The following sections describe the characteristics and operational parameters influencing the actual removal capacity.

4.2.3 Modelling the removal efficiency

Various attempts have been made to find an analytical equation which describes the capacity of a certain technique to remove a certain gas. These analytical equations are very handy because they can then be used to predict the removal of a certain technique from a few measurements in a pilot plant. Once the device specific parameters, k_L *and* a $(or\ k_{2)}$ have been determined in a pilot plant, the equation can be used to predict the removal at different residence times, different RQ's and for different gasses, thereby making it possible to scale up to a full-scale treatment step.

Spread in residence time: Plug-flow vs. completely mixed

The spread in residence time is a key factor influencing the structure of the analytical equation. In a pure plug-flow system, there will be no spread in residence time, so that all molecules will have the same residence time, equal to the average residence time. The gas exchange in such reactors takes place continuously over the length of the flow. The consequence is that a concentration gradient will develop along the length of flow.

In a completely mixed system, particles entering the reactor are continuously mixed so that the spread of the residence time can be anything from zero to infinity. Gas exchange takes place as soon as the water and air come into contact with each other and the concentration of the gas in the air and water is constant everywhere and equal to the effluent concentration (Helm, van der, 1998).

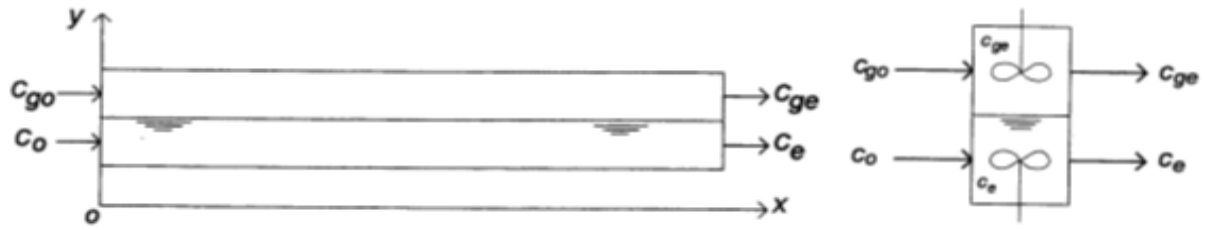


Figure 19 demonstration of the principle of Plug-flow (left) and completely mixed reactors (right) (Helm, van der, 1998)

In practice aeration systems will never be pure plug-flow or mixed reactors. However, the spread in residence time can be estimated to determine which model is most accurate.

Direction of water and air flow

Another important factor in the derivation of an analytical solution is the direction of the water and air flows. Three types of configuration are imaginable as shown in Figure 20.

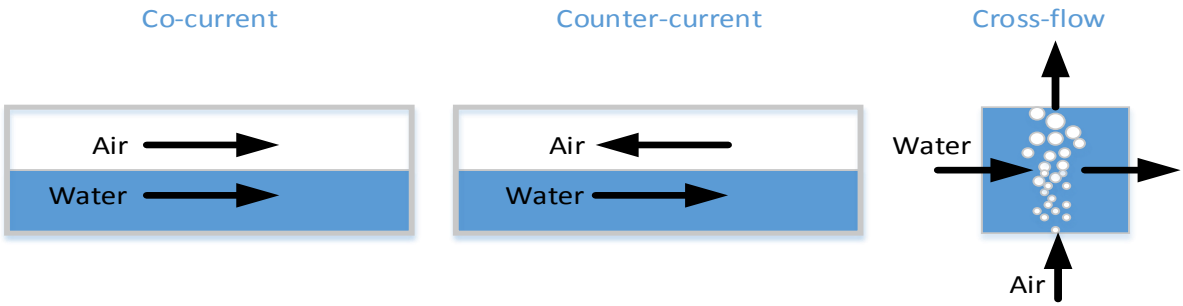


Figure 20 flow configuration in aeration systems

If flows are parallel to each other, as in the co- or counter- current situations, the concentration gradient will be in the direction of the flow which makes it relatively easy to solve these equations analytically. For cross-flow systems, it is not possible to do so because the direction of the flows are perpendicular to each other making it impossible to relate them to each other. Numerically, one can attempt to model these type of systems as a series of completely mixed systems (Helm, van der, 1998).

Analytical removal equations

In the case that a certain technique approaches either plug-flow or completely mixed conditions, the differential equations for the gas and liquid side concentrations can be solved analytically. This requires two assumptions:

1. The influent concentrations do not fluctuate over time. Though this is not completely true, this can be used to model the removal efficiency of a system during a time period with a constant concentration.
2. There is no chemical or biological degradation of the gas during the aeration step ($r_g and\ r_l = 0$). For methane this is valid, while for some other gasses there may be a fraction of degradation, even during the very small residence times which occur in aeration systems.

The direction of the flows (counter- or co- current) and the type of reactor (plug-flow or completely mixed) influences the outcome of the solutions for the gas and liquid side effluent concentrations. Once these solutions have been determined they can then be used to derive a formula for the removal efficiencies of different set-ups (Helm, van der, 1998).

Table 10 Analytical removal equations adapted from: (Helm, van der, 1998)

Flow Regime	Removal Equation	
Completely mixed	$k = \frac{1}{1 + \frac{1}{k_2 T} + \frac{k_D}{RQ}}$	(19)
Plug-flow co-current	$k = \frac{1 - e^{-k_2 T(1+\frac{k_D}{RQ})}}{1 + \frac{k_D}{RQ}}$	(20)
Plug-flow counter-current	$k = \frac{1 - e^{-k_2 T(1-\frac{k_D}{RQ})}}{1 + \frac{k_D}{RQ} * e^{-k_2 T(1-\frac{k_D}{RQ})}}$	(21)

4.3 Physical removal techniques

Physical removal techniques or gas exchange systems (GES's) are widely used in the drinking and wastewater sectors. A wide variety of GES's exists ranging from very simple spray or bubble aeration systems and cascades to tower aerators and more recently, so-called membrane contactors. For the purpose of this study, the goal of Oasen, from the beginning has been to maximise the methane removal capacity. Therefore three techniques were identified which have previously been shown to facilitate high removal efficiencies; these are the packed tower aerator (PTA), the INKA intensive aeration system (also called plate aerators in the Netherlands), and membrane contactors (MC). This section gives a general introduction into each system, their advantages and limitations, then presents the results of the performed pilot research and the energy and cost estimations.

4.3.1 Packed Tower Aerator

Packed tower Aerators are mostly circular towers that are filled with an irregularly shaped inert packing material which is meant to disperse the water into droplets in order to create and renew the air-water contact interface. This is very important as the air-water interface determines the possibility and speed of mass transfer between the gas and the liquid phase. Packing materials of different shapes, sizes and physical properties are available commercially (Crittenden, et al., 2012).

Tower aerators are operated under counter-current or co-current conditions, which means the flow of air and water is in opposite direction or in the same direction respectively. Because the flow of air and water are independent of each other, the Air/Water ratio (RQ) can be very high (1-100) leading to high removal efficiencies for methane while the surface loading of the device can also be relatively high to limit the footprint of these devices($100 m^3/m^2/h$). In practice, tower aerators have been shown to have a very large removal efficiency (Reijnen, 1994; Helm, van der, 1998; Crittenden, et al., 2012) under similar conditions to those at Kamerik which is a promising prospect for the pilot research.

Generally speaking, counter-current tower aerators can achieve the highest removal efficiencies of all the techniques which are currently being used in drinking water treatment. This is mainly holds for those towers operated under countercurrent flows. With the flows in opposite directions, the concentration gradients in the air and water point in opposite directions and the concentration gradient between both media is always maximized. Due to the need for high removal efficiencies, only counter-current towers have been investigated in this research.

One disadvantage of tower aerators is that they are usually relatively high (+/- 6m) which makes them difficult to fit into already existing structures or leads to unnecessarily high treatment buildings. The latter can be solved by letting the towers stick out through the roof of the building. Flooding can also occur if tower aerators are operated at extremely high loading rates and RQ's. At ZS de Hooge Boom this should not be an issue, because the loading rate will fall well below the upper limit of rates found in literature.

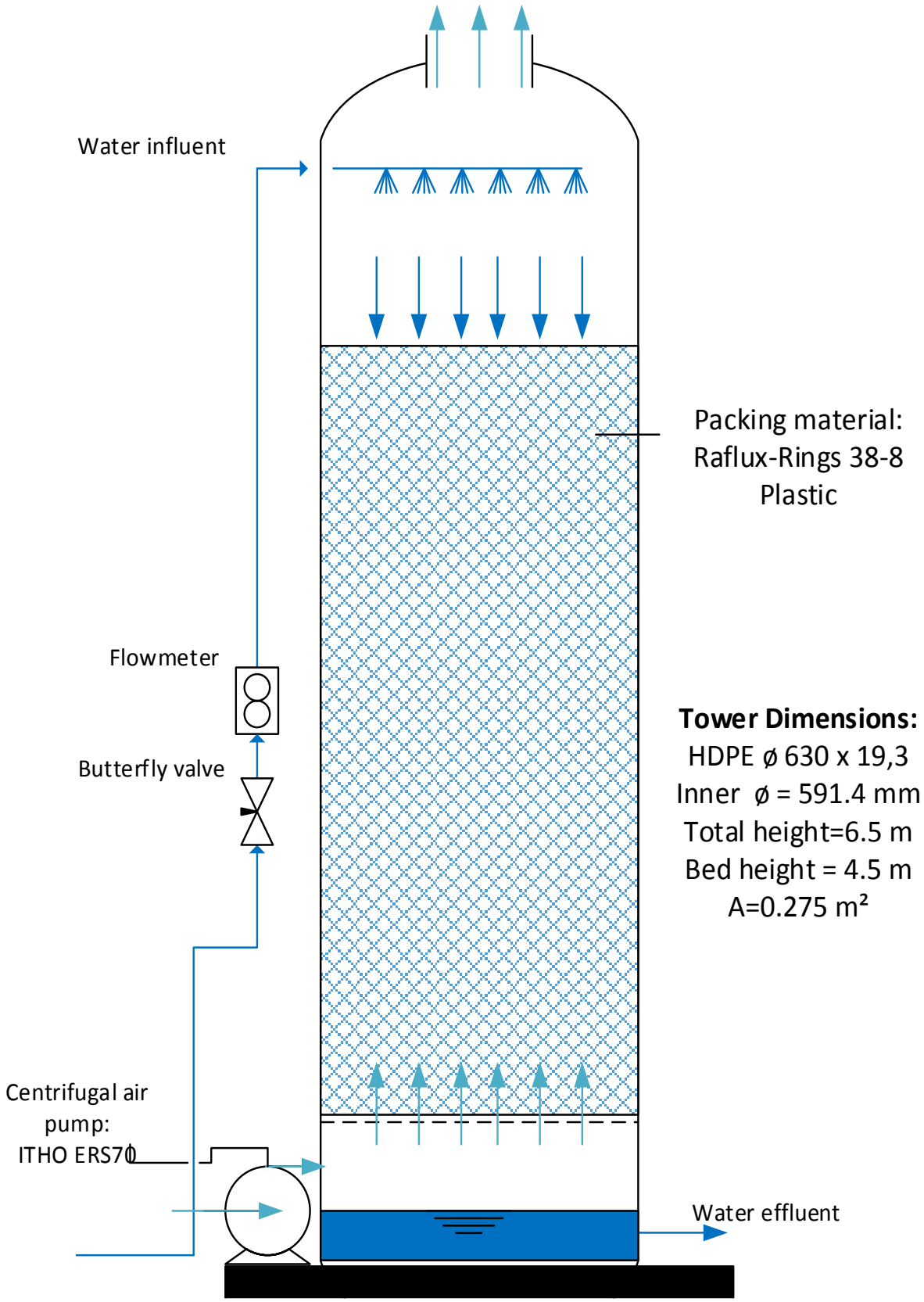


Figure 21 Illustration of packed tower aerator used in pilot research demonstrating its dimensions and working processes

Factors influencing the removal efficiency

A number of factors may affect the removal efficiency of a packed tower aerator. Van der Helm (1998) lists the following factors as having an influence:

- Packing material:

  Packing materials have been made in a variety of different shapes, sizes and materials. Their main goal is to distribute the flow of water over the bed height, creating and refreshing the air-water interface. This facilitates the creation of large surface areas in order to maximise the diffusion of gasses between the gas and the liquid phase. In drinking water applications, packing materials are usually suspended loosely into the tower aerator. Various studies have shown that the packing material must be < $1/8^{th}$ x the tower diameter in order to insure sufficient distribution of the flow.
- The height of the packed bed

  As mentioned previously, the tower is not completely filled with water. The fall height is therefore the main factor determining the residence time. In practice, the most common bed heights used in installations with a focus on methane removal are in the range of 3-4m.
- Water distribution over the packed bed:

  It is essential that the water is spread evenly over the whole surface area of the packed bed. It is also important to make sure water does not run down the wall of the tower as this will cause shortcutting and insufficient air-water surface renewal.
- Loading rate

  The loading rate (S) of a tower aerator is given by:

$$S = \frac{Q_W}{A_s} \tag{22}$$

  With:

$$Q_W = water\ flow\ rate\ [m^3/h], \qquad A_s = surface\ area\ of\ the\ tower\ [m^2]$$

  As the tower is not completely filled, the loading rate does not have large effect on the residence time as long as the tower does not fill up. A minor increase will be observed with an increase in flow due to the increase in air resistance. For methane removal loading rates in the order of 40-100 m/h have been applied. For very high removal rates as envisioned at Kamerik, the lower values of this range are probably the most realistic. On the other hand, higher loading rates mean fewer tower aerators which results in lower investment costs.

- Residence time

  The actual residence time is difficult to determine but is one of the main factors contributing to the removal. A way to determine the residence time is determine the "holdup," which is a measure for the amount of water in the tower aerator. Another approach to estimate the residence time has been to divide the bed height by the loading rate. This does not yield accurate results as the tower is not completely filled and the fall height is more important than the loading rate.
- RQ

  Tower aerators have the capacity to accommodate a large range in RQ's (up to 120), meaning they are quite flexible to variations in water quality. A high air to water ratio keeps the concentration increase in the gas phase at a minimum, thereby maximising the concentration gradient and the removal efficiency. If the RQ goes to infinity, the effluent concentration will approach the saturation concentration. Though high RQ's can theoretically be applied, it should be kept in mind that this requires more of energy.

Formula for removal efficiency

Packed tower aerators can be modelled using the previously presented equation (21) for counter-current plug-flow systems:

$$k = \frac{c_e - c_0}{c_s - c_0} = \frac{1 - e^{-k_2 \times T \times (1 - \frac{k_D}{RQ})}}{1 - \frac{k_D}{RQ} e^{-k_2 \times T \times (1 - \frac{k_D}{RQ})}}$$

With:

$$k = removal\ efficiency$$

$$k_2 = the\ device\ specific\ gas\ transfer\ coefficient (also\ known\ as\ k_L a)[s^{-1}]$$

$$T = average\ residence\ time$$

A pilot study was used to determine the $k_2\ and\ T$ values. Once known, eq. (21) is used to extrapolate for data under different operating conditions (e.g. different RQ's). Normally, $k_2\ and\ T$ are determined separately, however van der Helm (1998) advises to determine the parameters as one for packed tower aerators because it is difficult to determine the actual average residence time T. Some studies have used the "fictitious" residence time dividing the loading rate by the fall height, however this does not give a good view of the residence time as the tower is not completely filled with water, and an increase in flow does not translate directly into an increase in residence time.

Note that $k_2 T$ is influenced by the residence time and the contact area, both of which are dependent on tower height and loading rate. If the height and loading rate are changed in the full-scale design, the value of $k_2 T$ will change.

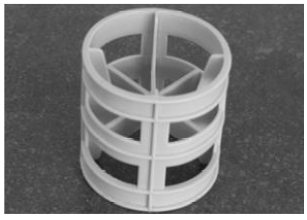
Pilot testing: Packed Tower Aerator

The tower aerator which has been used for the purpose of this research was hired from a company which specialises in the construction of drinking and wastewater treatment equipment. The specifications of the tower are shown in Figure 21. The tower is built from a HDPE Ø630 pipe with a total height of 6.5 m. The tower sticks through the roof of the pilot building so that the pumped air can escape directly to the surroundings and does not change the consistency of the air in the building. There is no possibility for recirculation of air in this specific set-up. The water is divided over the width of the tower by one overflow-channel and is not spread out evenly over the whole area of the tower.

The packed bed height is 4.5m filled with plastic 38-8 Raflux-Rings which are shown in Table 11. The packing volume is 1.24 m³.

Table 11 Specifications of packing material used in pilot research (source: rvtpe.de)

Description	Dimension
Nominal size	38mm
Weight	68 kg/m³
Surface area	175 m²/m³
Void fraction	92%



The centrifugal air pump (ITHO type ERS70) is a single speed pump which is connected directly to the packed tower aerator. The maximum flow capacity of this pump is approximately 550 m³/h. The air flow is regulated by a ball valve which partly shuts off the air intake. The air flow is measured with an air flow sensor (PVM-620) which measures the velocity. This is done five times and the average of the measurements is used to calculate the flow by multiplying the velocity with the area of the pipe.

Similarly the water flow can be regulated using a ball valve. A flow meter was installed to measure the flow of water entering the top of the tower. During this research this flow meter had a maximum capacity of 6500 l/h which yields (from eq. (22)) a very low loading rate:

$$S = \frac{Q_w}{A} = \frac{6.5\ m^3/h}{0.275 m^2} = 23.6\ m/h$$

Note that this falls below the lower range of loading rates found in literature. Though van der Helm (1998) has shown the effect of the loading rate to be minimal, it is highly doubtfull whether this holds true if the loading rate is doubled. This issue will be discussed more in the results section.

4.3.2 Plate Aerators

Plate Aerators, as they are commonly called in the Netherlands, are also called INKA intensive aeration or low profile aeration systems. Hereafter, they will be referred to by the Dutch name as this report has been composed for a Dutch drinking water company. This system comes in two basic forms: a single level tray (Figure 22) or multiple stacked trays (Figure 23); the basic principle is the same for both set-ups. Water is pumped to the top of the stripper where it is distributed evenly over the width of the device by an inlet weir. The weir controls the height of the bubble bed. The water then flows over a steel plate with small holes. Air is blown up through these holes, so that bubbles are formed which rise up through the water. Due to the high air flow highly turbulent conditions are created which maximize the contact of water and air. A minimum RQ of 20 is required to prevent water from seeping through the aeration holes (Stocking, et al., 1999; Helm, van der, 1998; van Dijk, 2007).

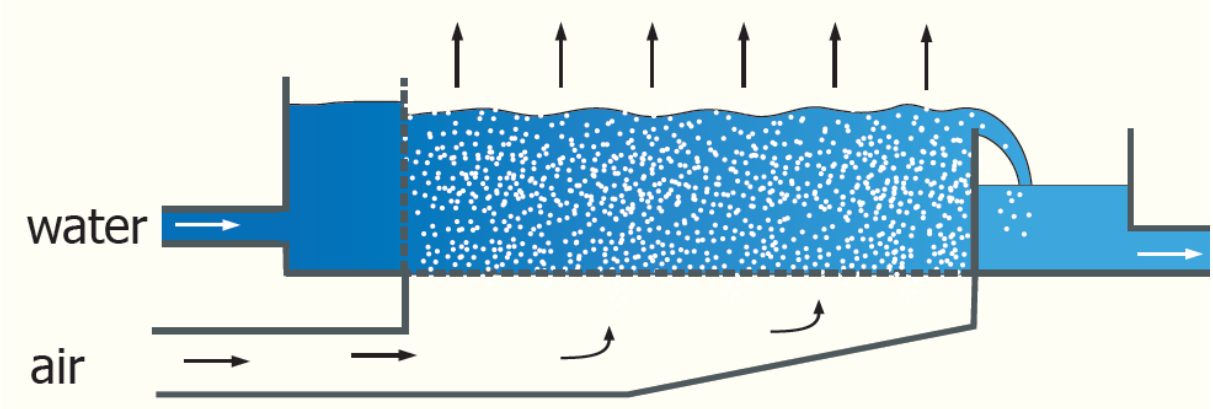


Figure 22 Schematic representation of a single plate aerator (van Dijk, 2007)

In case stacked trays are applied, the water flows from the upper tray to lower trays via overflow weirs. The air rises through each tray from bottom to top subsequently, thereby forming a series of linked cross-flow reactors. The concentrations of methane in the air increases from bottom to top while the concentration in the water decreases in each subsequent tray.

Typical operating RQ's found in literature are in the range of 20-60 for which removal efficiencies up to 99.7% are achievable (Helm, van der, 1998). In the Netherlands it seems that most of the used plate aerators consist of only one tray and never multiple stacked trays. The reason for this is that the systems have traditionally been installed in conventional treatment plants where they were placed on top of the sand filter to save building space. Note that in the new treatment plant at Kamerik, sand filters are no longer present so that this traditional benefit no longer holds.

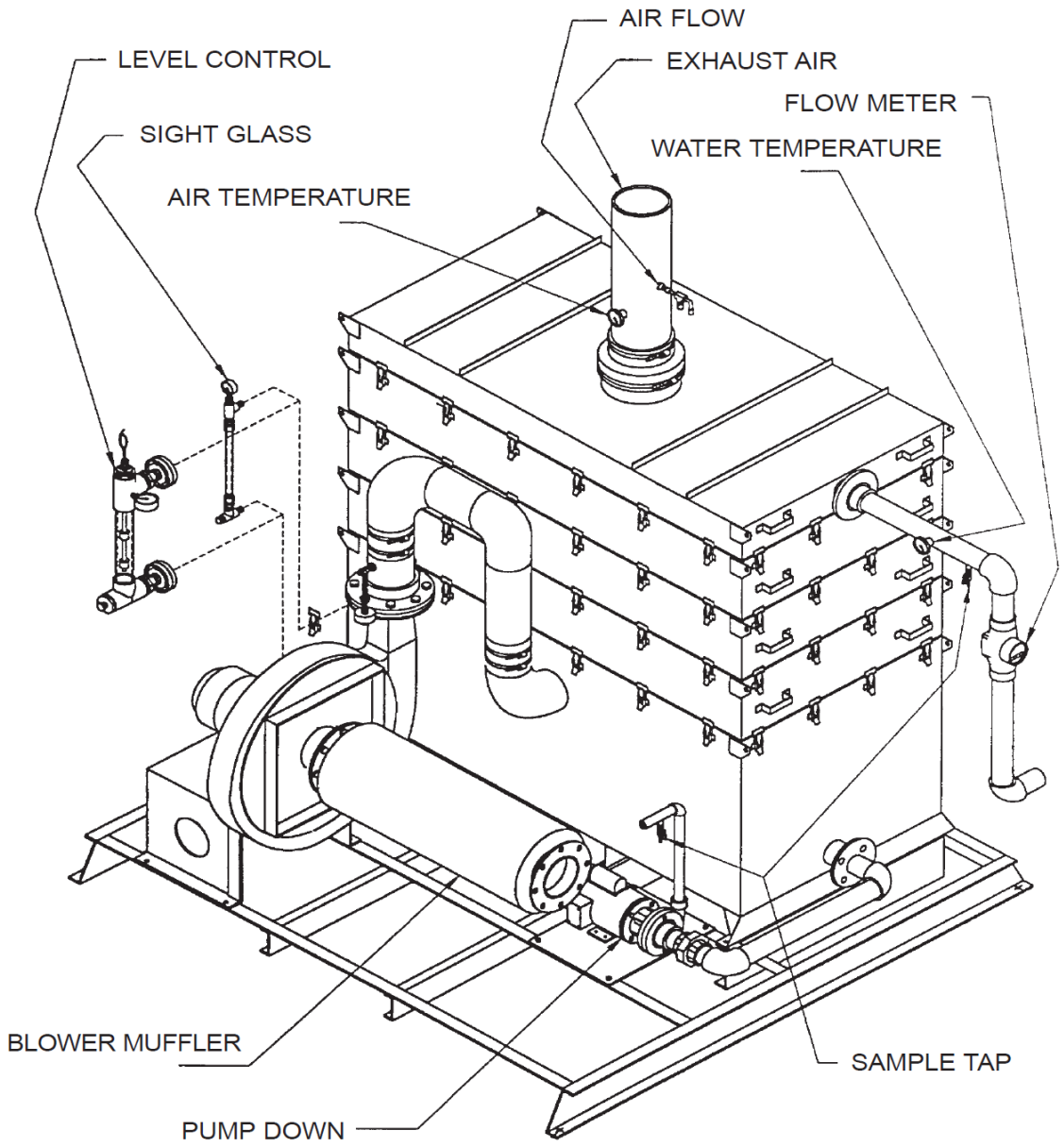


Figure 23 Schematic drawing of a multiple tray plate aerator similar to the one used in pilot research at Kamerik ((Stocking, et al., 1999)

Factors influencing the removal efficiency of plate aerators

Various factors, most of them similar to those of tower aerators influence the removal efficiency of plate aerators. The most important ones from literature (Helm, van der, 1998; Stocking, et al., 1999; Reijnen, 1994) are mentioned:

- Stability of the bubble bed
  For optimal working of the plate aerator a stable bubble bed needs to be created. This is accomplished by proper division of the air over the plate and by the correct ratio between the number and diameter of the air holes in the plate; Values found indicate that between 2500-25000 holes/m2 and 1-3mm respectively.
- Length to width ratio
  The length to width ratio is very important due to two factors. An increasing width causes increased spread of residence time and therefore decreases the removal efficiency. An

increasing length increases the contact time of the water in the plate aerator. A long narrow plate is therefore more effective than a short broad one. The spread in residence time could also be reduced by placing baffles along the length of the plate, thereby creating long and narrow flow channels.

- Surface loading
  The surface loading rate of plate aerators is defined as the flow of water (m^3/h)/perforated plate area (m^2). Typical values for surface loading of plate aerators are 30-35 m/h. The surface loading has direct effect on the residence time and therefore on the removal capacity. A higher loading rate yields a lower removal efficiency.
- Number of stacked trays
  The removal efficiency for stacked systems increases with the number of trays (Stocking, et al., 1999), however it is questionable that this will be comparable to the increase achieved by increasing the length of a single tray system (no literature compares the removal of single and multiple stacked plate aerators).

Analytical modelling

As mentioned in paragraph 4.2.3 there is no analytical solution for plate aerators because the flow of the water and air are perpendicular to each other (cross-flow). To scale up from pilot scale to full scale, the only way the removal can exactly be replicated is by installing multiple systems with identical dimensions as those tested in the pilot plant. If however, the width of the flow channels and loading rate are not increased and the length and number of trays is not reduced, upscaling the system will yield similar or possibly better results. Designs of plate aerators seem to be based mainly on experience and extensive pilot research.

Pilot set-up

The plate aeration system used at Kamerik consists of three stacked trays. The trays have a dimension of 540 x 540 mm which gives an area of 0.29 m^2 and a total area of 0.87 m^2. The total flow length is 1.62 m and the bubble bed height is controlled by overflow weirs with a height of 0.15 m.

The prescribed water flow rate was 3-5 m^3/h. The flow was adjusted with a valve and a digital flow meter was used to ensure the correct flow setting. A single speed centrifugal air pump (ITHO ERS60) with a maximum capacity of 240 m^3/h was used to supply air to the bottom tray. A valve was used to regulate the air flow to the appropriate value, using a handheld velocity measuring device (PVM-620) to check the flow.

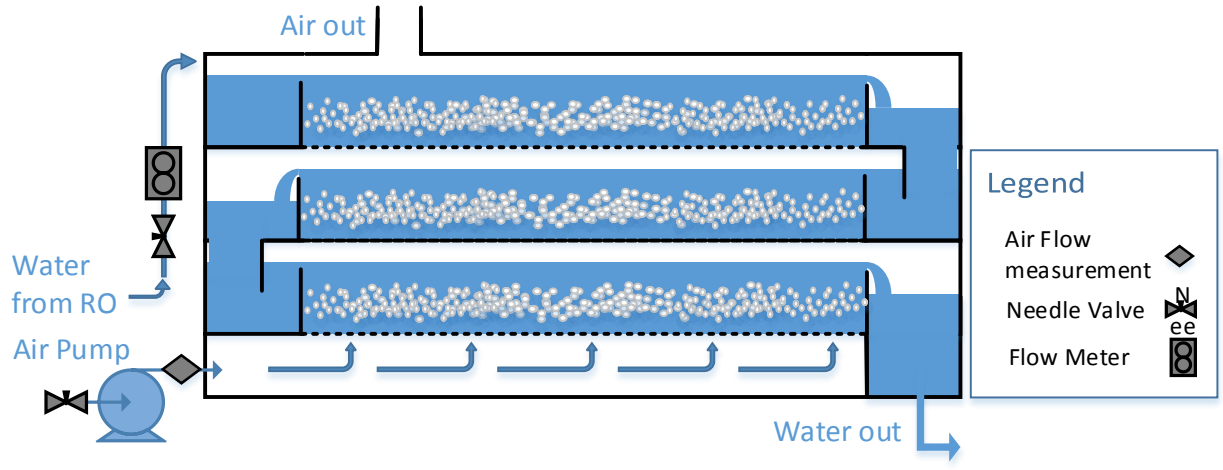


Figure 24 Schematic representation of the pilot scale system set-up used at ZS de Hooge Boom

The system was delivered as a ready to use installation which had been designed for use in another pilot installation. Therefore, the applicable settings were given by the manufacturer and are not necessarily the most economical; the applicable loading rates were 3.5 – 5.7 m/h which is low compared to the normal loading rates found in literature.

4.3.3 Membrane Contactors

Membrane contactors are a relatively new technique for degasification processes. Since their introduction more and more applications are being found, most of them industrial. They are being used to add or remove O_2, CO_2 and N_2 to or from liquids in the semiconductor, power, pharmaceutical photographic and food and beverage industries (Buonomenna, 2013). However, to the authors knowledge, there are currently no large scale application of this technology in the production of drinking water, at least not for the specific goal of removing methane from water.

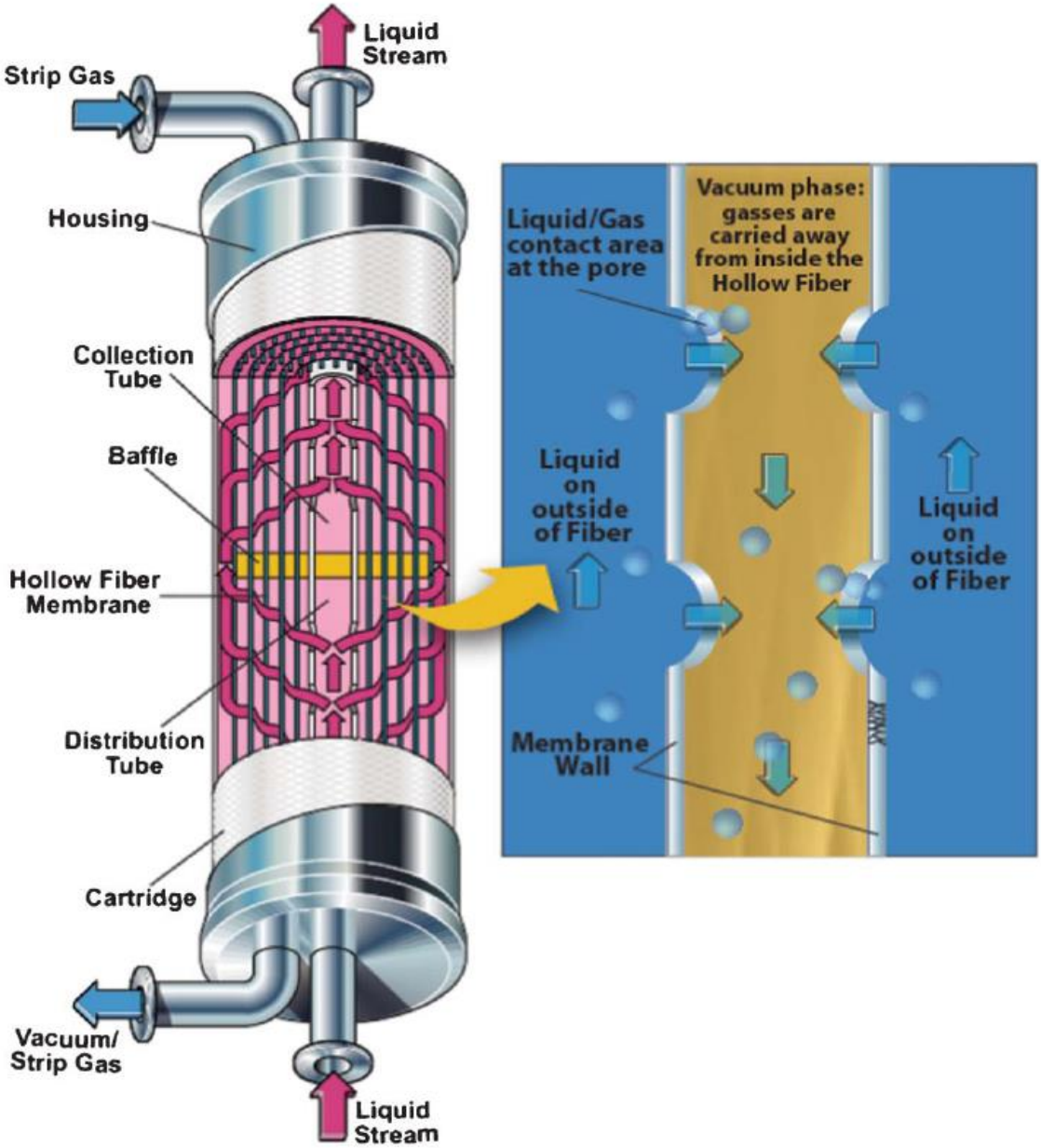


Figure 25 Processes and components of a liqui-cel ® membrane contactor (Buonomenna, 2013)

Membrane contactors are modular systems consisting of a cartridge which contains superphobic hollow fibres. The sweep gas (air) passes through the hollow fibres while the water remains on the

outside, so that the flows are completely separated from each other. The air and water flows are in opposite direction. Due to the very small diameters (3 µm) up to 10 000 hollow fibres can be fit into one single unit, providing a large exchange interface in a very compact system with small footprints and lighter weights (Sengupta, et al., 1998).

The baffle in the liquid side forces the water, which enters the unit through the distribution tube, to flow outwards, insuring turbulent mixing and increasing the contact between the air (in the fibres) and the water. In essence, therefore, this technique can be classified as a cross-flow system, which makes it very difficult to solve for the removal efficiency analytically.

Due to the separation of the liquid and gas phase (unlike other gas exchange systems), the two phases are completely independent of each other so that problems such as flooding at high surface loading and unloading at low surface loads do not occur. The maximum liquid and air flow rates are limited only by the pressure drop over the membrane unit, which should not become too high. Additionally, contamination of the air in the gas side will not enter the liquid which is very advantageous for ZS de Hooge Boom; as the aeration is a post-treatment step to RO, there is no barrier for contamination after this step.

Other advantages named in literature are (Sengupta, et al., 1998; Li, et al., 2005):

- Differences in pressures between the two phases does not affect the mass transfer
- The available surface area remains undisturbed at high and low flow rates.
- The modularity of the contactors, making them easy to integrate into systems and to scale up as the increase in capacity is linear to the amount of units applied. To increase the removal capacity, it is also possible to place multiple units in series.
- The membrane contactors are retrofitable and expandable

Membrane contactors also have some disadvantages (Gabelman, et al., 1999), the most important ones are named here:

- Membranes are subject to fouling and clogging, which may be a problem in case they are to applied after the remineralisation step.
- Membranes have a finite life[6]; the cost of periodical replacement should be considered.
- The unit introduces an additional resistance to the mass-transfer which is not found in the traditional mass-transfer systems; the resistance of the membrane itself. This is however over-compensated by the increase in surface area.

Factors influencing removal capacity

The following factors are most influential on the removal capacity of the membranes:

- The installed exchange interface of a system:
  This is largely dependent on the unit size of the membrane that is applied. Membrane contactors come in many different, diameters, lengths and number of fibers. Manufacturers provide this kind of information in the datasheet of a product.
- Residence time:
  The residence time determines how much gas exchange will take place. Increasing the length and the diameter of a membrane contactor will increase the residence time. The water flow rate is also an important factor; the residence time increases with a decrease in flow rate.
- Diameter:
  As discussed in the introduction, membrane contactors contain a baffle which forces the water to flow in the radial direction across the hollow fibres. Except for an increase in residence time, therefore, an increase in diameter also means that the water will pass more fibers filled with air, so that the removal capacity is larger with a larger diameter.

[6] Average life of a membrane for high quality water such as RO filtrate is 7 years according to the manufacturer (Membrana GMBH, 2014)

Set-up of membrane contactors in pilot installation

For the pilot installation two 2.5 x 8" liqui-cel ® extra-flow membranes were placed in series. Each unit provides a 1.4 m² exchange interface. The maximum flow capacity of these units is 0.7 m³/h and 1.7 m³/h for water and air respectively. The maximum applicable water pressure is 7 bar. The setup is shown schematically in Figure 26.

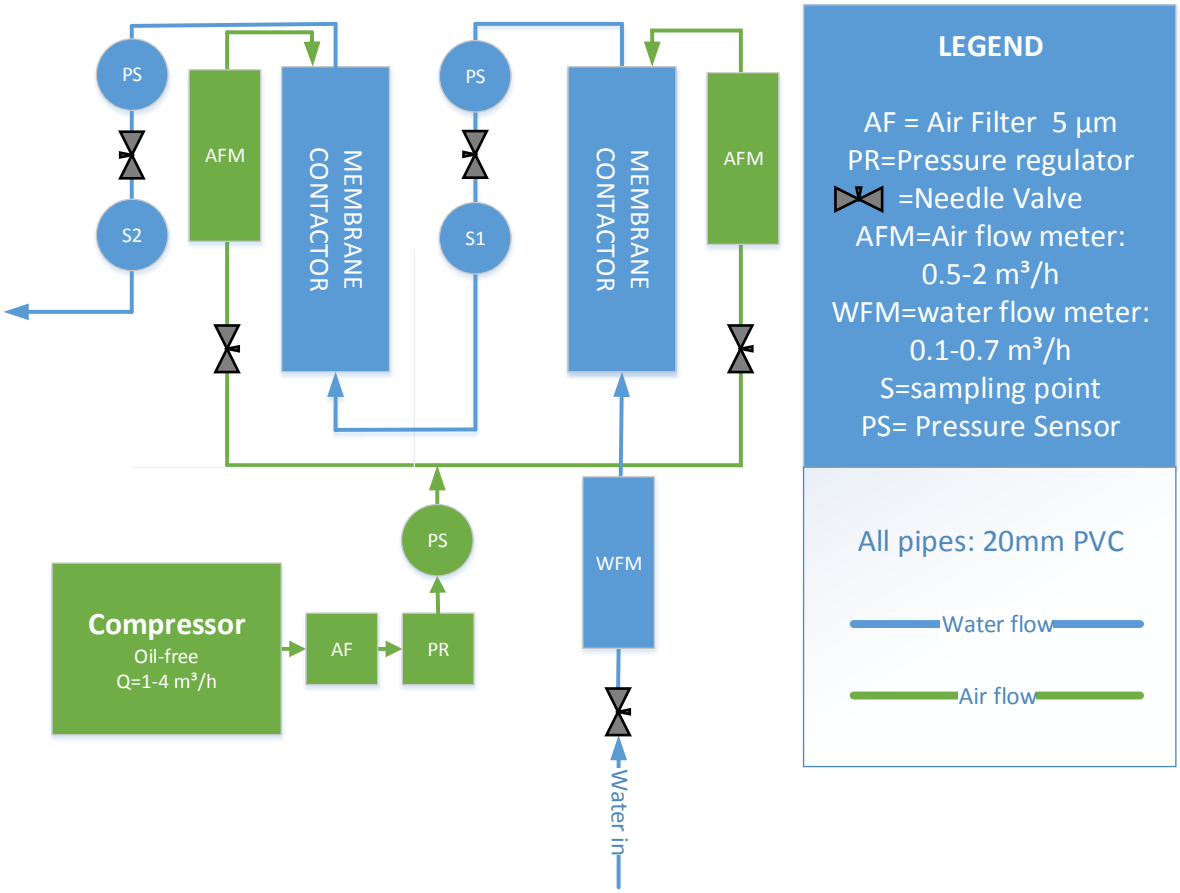


Figure 26 Schematic set-up of pilot membrane contactor system; two 2.5 x 8" Liqui-cel ® membranes in series.

Pressure sensors were installed before and after every unit in order to monitor the pressure drop occurring at different water flow settings. It was found that the pressure drops correspond with those given in the datasheet of the 2.5 x 8"membranes which can be found on the liqui-cel ® webpage[7]. For further in depth information on the modules, the reader is referred to the data sheet.

[7] http://www.liquicel.com/product-information/data-sheets.cfm

4.4 Pilot research results

In order to test different envisioned treatment processes, their individual performance and influence on other treatment steps, as well as the functionality of the complete treatment cycle, Oasen has set-up a pilot plant. The set-up is similar to that of the planned future plant with an RO unit, IEX reactors, a remineralisation step and various aeration techniques.

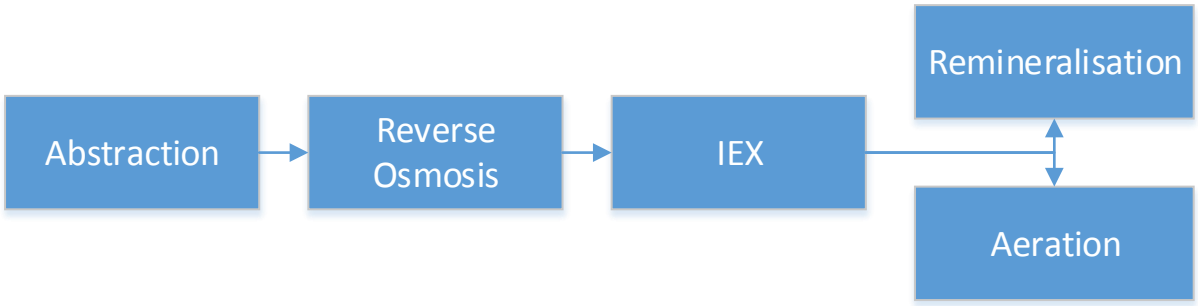


Figure 27 Schematic set-up of the treatment facility

The pilot plant has a maximum capacity of 12 m3/h. The RO filtrate is treated in the IEX columns to remove residual ammonium. The aeration and remineralisation steps are set-up in such a way that they can be connected separately or in series (first Aeration, then remineralisation or vice versa). This has been done because the order of these steps in the future treatment plant has yet to be determined, and the pilot plant will be used to study the effects of these change in the order. For the purpose of this study, however, the aeration set-ups were connected directly after the IEX columns so that the efficiency could be determined without effects from the remineralisation step.

As mentioned previously, three aeration systems were tested. The descriptions of each pilot set-up and the range of settings have been given in the previous sections. This section presents the results attained from the pilot plant and discusses them in terms of complying with the objective removal capacity and factors to keep in mind for upscaling.

The removal efficiency (k) for a certain technique was determined by:

$$k = \frac{c_0 - c_e}{c_0 - c_s} \tag{23}$$

With:

$$k = removal\ efficiency$$

$$c_s = equilibrium\ concentration\ methane\ in\ water = 0.0575\ \mu g/L\ \ at\ 10°C$$

$$c_0 = influent\ methane\ concentration\ [\mu g/L]$$

$$c_e = effluent\ methane\ concentration\ [\mu g/L]$$

The influent and effluent for a certain setting are sampled and sent to a specialized drinking water lab (Vitens Laboratory) for further analysis. The lab uses the headspace analysis method to determine the concentration of methane in the water. With this method, the detection limit for methane is 5 µg/L[8]. Note that the saturation concentration for methane in water at 10°C is used in all subsequent calculations unless stated otherwise; the concentration is 0.0575 µg/l.

[8] Personal correspondence with Vitens Drinking Water Lab

4.4.1 Tower Aerator Results

The goal for this technique was to determine the methane removal efficiency of the tower aerator at different RQ's and loading rate. The removal efficiencies were then used to determine the device specific parameter k_2T which can be used to predict the removal efficiency of tower aerators at different RQ's (provided the loading rate and packed tower height remain the same) and to design a full-scale treatment facility at a later stage.

Table 12 lists the air and water flow settings which were implemented, the measured CH_4 concentrations in the influent and effluent and the calculated removal efficiency. These values were then used to calculate the k_2T value for the given loading rate by rewriting eq. (21) which yields (Appendix D):

$$k_2T = \frac{\ln\left(\frac{1 - R * \left(\frac{k_D}{RQ}\right)}{1 - R}\right)}{1 - \frac{k_D}{RQ}} \tag{24}$$

The settings implemented and the results corresponding to those settings are shown in Table 12. Note that the air capacity is always the same and equal to the maximum capacity of the centrifugal pump connected to the tower.

Table 12 Air and water flow settings and results from pilot testing of the tower aerator

Flow data					CH_4 concentrations			Removal	k_2T
Velocity	Qa	Qw	S	RQ	Cs	C_o	C_e	k	-
m/s	m3/h	m3/h	m/h	-	µg/l	µg/l	µg/l	%	-
18.17	551.5	3.6	13.2	152	0.0572	1500	6	99.60%	5.53
18.17	551.5	4.0	14.6	137	0.0572	710	6	99.16%	4.78
18.17	551.5	4.3	15.5	129	0.0572	1500	8	99.47%	5.24
18.17	551.5	5.9	21.6	93	0.0572	1700	19	98.89%	4.50
18.17	551.5	6.0	21.8	92	0.0572	990	13	98.69%	4.34
18.17	551.5	7.6	27.5	73	0.0572	530	9	98.31%	4.08

It is clear that even though extreme high RQ's were used in the tower aerator, it was not possible to achieve a removal of more than 99.6 %. The highest removal being achieved only with an extremely low loading rate (3.6 m/h) and a very high RQ (152). This has come as a surprise since values found in literature show much more promising results, i.e. higher removal efficiencies at higher loading rates and lower RQ's.

Though van der Helm (1998) states that the loading rate does not have a large effect on the removal capacity, it is clear from Table 12 that the k_2T value decreases significantly with increasing loading rates and therefore this does not seem to be valid for the tested tower aerator. Since it was not possible to operate the tower at higher loading rates, it is not clear what the value of k_2T will become at higher loading rates.

A possible explanation for the lower than expected removal efficiencies and the fast decline in the calculated k_2T would be that the division of water in the top of the tower was not properly designed. If water is not spread properly over the area of the tower or some of the water is sprayed directly onto the wall of the tower aerator, this can cause shortcutting which leads to a reduction of k_2T and therefore a reduction in removal efficiency.

The loading rate is a key factor in the economic feasibility of the tower aerator. The investment cost decreases with a higher loading rate as this decreases the tower surface needed to treat the required water flow. As the loading rate does however seem to have some impact on the removal efficiency it

is advisable to keep the loading rates in the lower range of applicable values found in literature; approximately 50 m/h. With such loading rates and a proper water division in the tower, the author is confident that the objective removal efficiency is achievable at reasonable RQ's (50-100).

4.4.2 Plate Aerator Results

The pilot installation was situated inside a building so that the CH_4 concentration in the room increased during the aeration process. This effects the removal capacity of the system so that additional background concentration measurements had to be done. These measurements therefore add an additional error to the results. Note that due to this issue, the saturation of methane in water (c_s) used in the calculation of the removal efficiency was derived from the measured air concentration and is therefore different from the c_s value presented in the other results.

Table 13 Plate Aerator flow settings applied during pilot testing and results

flow data				**CH_4 concentration**				**removal efficiency**
Air flow	Water flow	loading rate	RQ	Cg	Cs	C_o	C_e	k
m³/h	m³/h	m/h	-	µg/L	µg/L	µg/L	µg/L	%
61	3	3.45	20	17	0.731	1540	157	89.85%
239	3	3.45	80	13	0.559	1540	17	98.93%
60.1	3	3.45	20	17	0.731	1345	122	90.98%
181.9	3	3.45	61	16	0.688	1360	31	97.77%
78.9	4	4.60	20	5	0.215	1078	110	89.81%
238.2	4	4.60	60	8	0.344	1041	18	98.30%

Six measurements of the removal efficiency were done over a three week period. The water flow rates applied were 3 and 4 m³/h. The maximum water capacity of 5 m³/h could not be tested due to operational circumstances in the pilot at the time of testing. The air flow rate varied between 60 and 240 m³/h, the latter being the maximum capacity of the installed centrifugal pump.

The loading rates are very low (<5 m/h) compared to those found in literature. Nonetheless, the found removal efficiencies are not very high. The highest removal efficiency (98.93%) was found for a loading rate of (only) 3.45 m/h and an RQ of 80. This while removal efficiencies found in literature indicate efficiencies of >99.6% can be achieved with much higher loading rates (30-35 m/h).

Two explanations for this "low" efficiency are:

- The tested system was a multiple tray system while the previously stated values found in literature are for single tray aerators. As the air rises up to each next tray, the concentration of methane is higher than it would be if a single tray was used, thereby, decreasing the driving force between the gas and liquid phase.
- The combined length of the three stacked plates (1.62 m) is much smaller than the length of the plates found in literature (>3 m). This reduces the contact time of the air and water significantly.

Nonetheless, the author is positive that plate aerators, given the appropriate design, could achieve the removal efficiencies required. However, to achieve such removals, a single tray plate must be used and the length must be increased to >3.5 m. Literature sources (Reijnen, 1994; Helm, van der, 1998) show that single tray plate aerators with a length of >4 m and a width of <2m with an RQ of 50-100 should be able to provide efficiencies of more than 99.6 %. The applied surface load can then be in the range of 30-35 m/h.

4.4.3 Membrane Contactor Results

Each membrane unit comes with a datasheet which shows the removal efficiency for oxygen (O_2) against the water flow rate. The removal curve for 2.5x8" liqui-cel ® membrane units is shown in Figure 28. The gas flow rate is kept constant and at the maximum allowable gas flow rate of the unit. As the curve shows the removal achieved at the maximum possible RQ for each water flow setting, it represents the maximum removal capacity of the unit for oxygen, using N_2 as a sweep gas at 20°C.

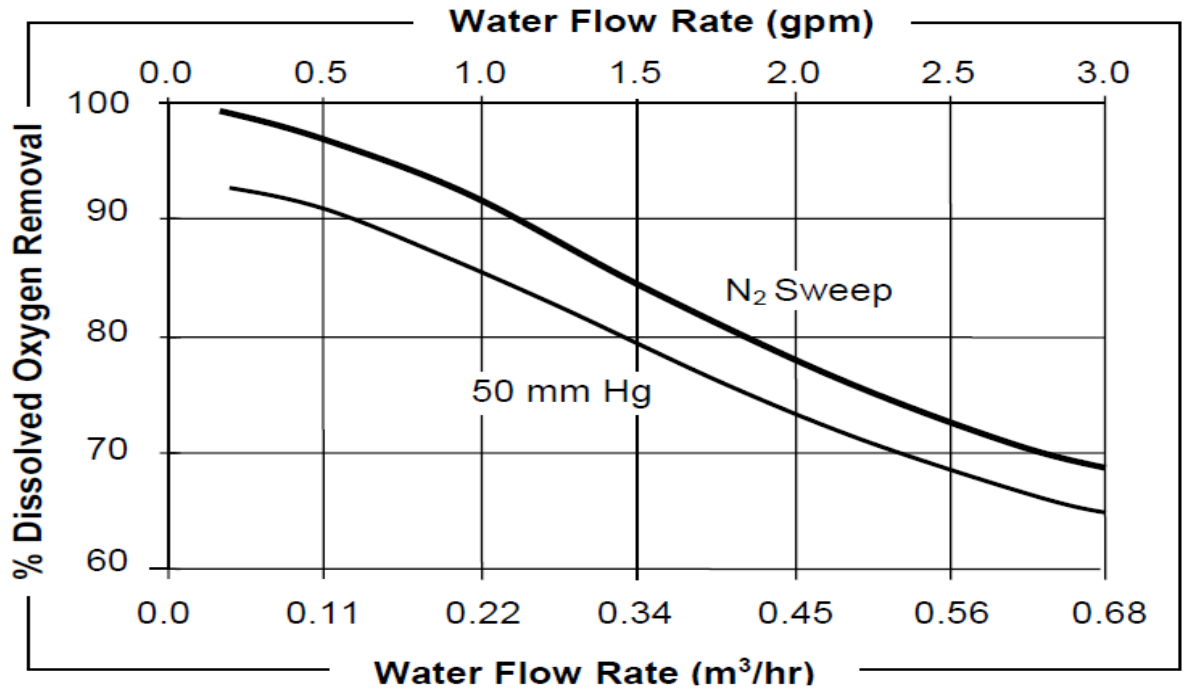


Figure 28 Maximum removal curve of 2.5x8 inch liqui-cel unit for different water flow rates at 20°C (Membrana, 2014)

In order to compare the removal of methane with the oxygen removal curves given in the datasheets, this same method was employed in the pilot plant. The air flow was therefore set to the maximum rate given in the datasheet; 1.7 m³/h. During the testing the water temperature varied between 10 and 11.5 °C, which is lower than the normal testing temperature (20° C). Four measurements were done with water flow settings varying between 0.3-0.6 m³/h.

Table 14 Settings, measured methane concentrations and calculated removal efficiencies for pilot membrane contactor research

flow data			CH_4 concentration		removal efficiency
Air flow	Water flow	RQ	C_o	C_e	k
m3/h	m3/h	-	µg/l	µg/l	%
1.7	0.6	2.83	1350	599	56%
1.7	0.5	3.40	1350	543	60%
1.7	0.4	4.25	1350	489	64%
1.7	0.3	5.67	1350	388	71%

Note that in the above table, the water flow decreases from top to bottom, increasing the RQ (as the air flow remains constant) and therefore the removal efficiency. Additionally, with a decreasing water flow, the contact time increases linearly so that the residence time at the bottom is twice as high as at the top.

As is clear, the removal efficiency of a single module of this size is not sufficient to achieve anything near the objective capacity of 99.6%. This however, does not necessarily mean this technique is not applicable.

The removal efficiency of a single membrane unit is highly dependent on the length and diameter of the unit. The contact time increases proportionally to the length; the diameter effects the contact time, but also the amount of hollow fibres which are crossed while water passes through the unit. A larger membrane unit will therefore be more efficient than a smaller one.

This effect is clearly demonstrated in the datasheets; As a comparison, the maximum removal efficiencies of the tested 2.5 x 8 " membranes are shown together with the removal efficiencies of 14 x 40 " membranes, which are the most optimal type (in terms of flow- and removal- capacity) for full-scale application at ZS de Hooge Boom.

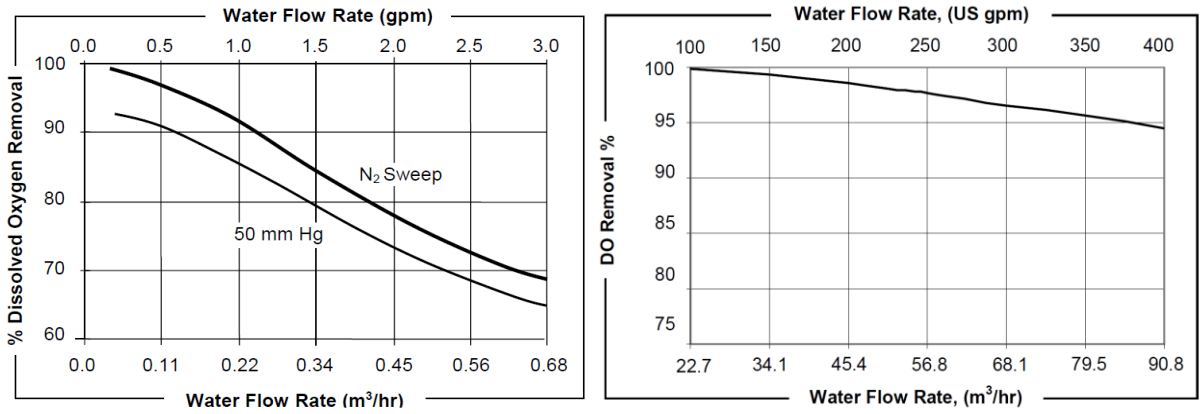


Figure 29 The maximum removal efficiencies for 2.5 x 8 inch (left) and 14 x 40 inch (right) liqui-cel® membrane contactors as shown in datasheets of both products.

It is clear that the larger membrane units have a much higher maximum removal capacity than their smaller counterpart. Since a large-scale plant would require the use of these larger membranes due to their larger capacity, it can be concluded that the methane removal efficiency achievable with membrane contactors will be much higher than demonstrated by the results of the pilot installation.

Comparing the O_2 removal of the 2.5 x 8" unit and the measured CH_4 removal in the pilot installation (Figure 30), it becomes clear that the measured CH_4 removal is much lower than the O_2 removal found in the datasheets.

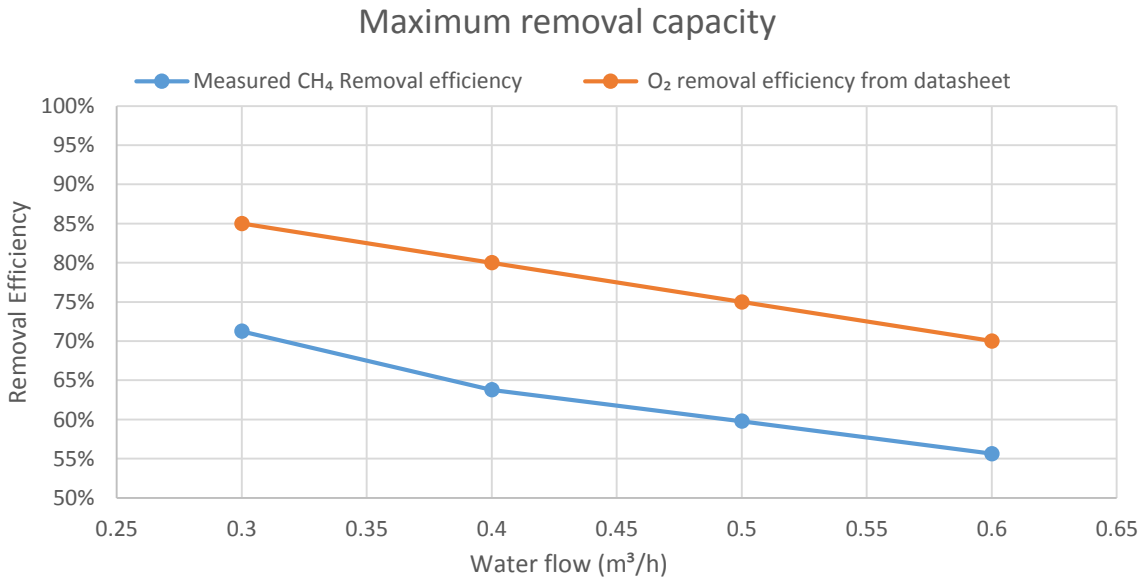


Figure 30 Comparison of the maximum O_2 removal efficiency at 20°C (estimated from data sheet graph) and measured CH_4 removal efficiencies at approximately 10°C

This can be explained by the fact that there is a difference in k_D values of the two gasses at the different temperatures for which the removal were measured; 0.0433 and 0.0337 (Table 8) respectively. The removal of O_2 will be higher at 20°C than the removal of CH_4 at 10°C. At 20°C however, the removal of both gasses would probably be very much similar since the difference in k_D is only 0.0002 at this temperature.

Therefore, it is not unreasonable to conclude that the O_2 removal curves from the datasheets gives an indication of the removal capacities achievable for methane at lower temperatures. It should however be taken into account that the CH_4 removal at 10°C will always be a few percent lower than that shown in the curves. The exact difference is difficult to estimate and should be found by testing the larger units in a pilot set-up.

With these considerations in mind, the methane removal efficiency of a 14x40" membrane with a water flow rate of 60 and 80 m³/h at 10°C, the removal capacity was estimated (from Figure 29). These are merely estimates based on the percentual difference (approximately 10-13 %) observed in the measured removal efficiencies and the values estimated from the O_2 removal curves found in the datasheet of the 2.5 x 8" units (Figure 30). The pressure drops over a single membrane unit as found in the datasheets of the 14 x 40" units at the given flow rates are also shown.

Table 15 Estimated removal efficiencies and pressure drops of 14x40" membrane contactors for 60 and 80 m3/h flow rates

Flow m³/h	Lower Limit	Upper Limit	Pressure drop m
60	84 %	87 %	0.3
80	82 %	85%	0.48

The membrane contactors are modular and extremely compact so that it would be very easy to place multiple units in series in order to achieve higher overall removal efficiencies. The overall efficiency after each step can then be calculated from:

$$K = 1 - (1 - k)^n \tag{25}$$

$$with\ k = removal\ in\ each\ step\ and\ n = number\ of\ steps$$

With the range of removal efficiencies for 60 and 80 m³/h flows estimated previously (Table 15), the removal efficiencies of multiple units in series are demonstrated in Table 16.

Table 16 lower and upper limit removal of multiple units based on an estimation of the removal capacity from datasheets

Flow rate	One unit		Two units		Three Units		Four Units	
m³/h	lower limit	upper limit	lower limit	upper limit	lower limit	upper limit	lower limit	upper limit
60	84.0%	87.0%	97.4%	98.3%	99.6%	99.8%	99.9%	100.0%
80	82.0%	85.0%	96.8%	97.8%	99.4%	99.7%	99.9%	99.9%

The needed removal efficiency (99.6%) is only achievable with at least 3 units in series. Additionally, a minimum removal efficiency of 84% per unit is needed in order to achieve the required removal capacity with three membranes in series. If the removal proves to be less than 84% four membranes are needed to provide sufficient removal. Table 16 demonstrates that with four membranes contactors in series, extremely high removal efficiencies can be achieved even if the efficiency of a

single element is much lower than expected. With a single element having a removal capacity of >75%, the objective removal of 99.6% is still reached.

In order to determine whether the demonstrated estimations are accurate (i.e. whether the removal efficiencies can be achieved), it is recommended to test the 14 x 40" membranes in a pilot plant.

4.4.4 Conclusions Removal efficiency of Aerations Systems

Given the maximum raw water concentration of 2.7 mg/L which, with a required maximum concentration of 10 µg/L, corresponds to a minimum removal efficiency requirement of 99.6 %, the following conclusions can be drawn with respect to the removal capacity of each system:

- All three techniques have the potential to achieve a removal capacity of 99.6 %. The estimated maximum removal efficiency of every alternative is shown in Table 17. These are estimates based on data found in literature or datasheets.
- Tower Aerators, from literature, are found to achieve the highest removal capacities and are very flexible in operation (large range of RQ's and loading rates applicable). To ensure sufficient removal, it is recommended to apply relatively low loading rates (50 m/h) and high RQ's (50-100) with a tower height of at least 6 m.
- Plate Aerators, similarly, have been shown to be able to achieve removal efficiencies above 99.6%. To achieve these, however, it is recommended to use a single tray with a long (>4 m) narrow (<2m) perforated plate instead of multiple tray system which seems to be much less efficient. With these conditions, a loading rate of 30 m/h can be applied.
- There is still a lot of uncertainty about the removal efficiency of membrane contactors. It is recommended to use the largest (14 x 40") liqui-cel® membrane contactors. Initial estimations show that with a low flow rate (≤60 m^3/h), a removal of >84 % can be achieved which would provide sufficient removal capacity if three membranes are placed in series. If the removal efficiency falls between 75% and 84% (at higher flow rates) per unit, it will be necessary to install 4 membranes in series. With four membranes in series, the removal efficiency becomes very high; even if the individual capacity of a membrane is as low as 75%, the objective removal of 99.6% can be achieved. To determine the exact removal efficiency, the 14 x 40" membranes should be tested in the pilot plant.

Table 17 gives a summary of the estimated removals as found from literature. It also demonstrates the estimated methane concentration corresponding to that removal if applied at Kamerik.

Table 17 Estimated maximum removal efficiencies and effluent concentrations of the considered alternatives

Alternative	Estimated maximum removal	Effluent concentration at Kamerik (µg/L)
Tower Aerator	99.8 %	5.5
Plate Aerator	99.7 %	8.1
MC alternative 1	99.6 %	10.0
MC alternative 2	99.9 %	2.7

4.5 Conceptual dimensioning treatment steps

Oasen has expressed its wish to implement the new treatment facility with four treatment trains; each with a capacity of 120 m³/h, thus giving a total capacity of 480 m³/h. This section briefly describes the most important dimensions for each technique based on the estimated ranges of flow rates (water and air) as well as other factors mentioned in the previous sections. These are then translated into an initial indication of the footprint of each installation. Note that this conceptual design is made for the sole purpose of estimating the energy use and costs of the three techniques.

As an overall assumption for all the subsequent techniques, it has been assumed that a working space of 1m is needed between units and on all sides of the unit.

4.5.1 Dimensions of the Tower Aerator

As mentioned in paragraph 4.3.1 and 0 the dimensioning of a tower aerator is based mainly on the surface loading rate. The standard values found in literature (Helm, van der, 1998; van Dijk, 2007) gives a range of surface loading rates between 40-100 m/h as being normal for tower aerators. As shown in the pilot installation, the removal efficiency decreases with increased loading rate. It has therefore been recommended apply a loading rate of 50 m/h to insure sufficient removal of methane.

Tower Aerators are found in a wide variety of sizes, including diameters and heights. In drinking water treatment it is often found that 6 m height provides sufficient removal. This was also the height of the tower which was tested in the pilot installation. It has been assumed that with proper design, a tower with a height of 6 m is sufficient.

The diameters of tower aerators in literature vary between 1.5-2.5m; high, slender towers are preferred to short, broad ones because this will reduce the spread in residence time (Helm, van der, 1998). With the given loading rate of 50 m/h and a capacity of 120 m³/h the needed tower area per train is 2.4 m². Since the towers are round, the needed diameter is equal to 1.75 m which falls within the range of diameters found in literature.

The total area needed for the whole treatment step with four towers and a workspace of 1m is equal to 45 m² and the volume of the treatment step (given a height of 6 m) is 270 m³. Note that the needed building volume is very much dependent on whether the towers are to be inside the building or whether they can stick through the roof so that a normal building height of approximately 3 m determines the volume needed. For now it has been assumed the building is dimensioned to contain the complete tower.

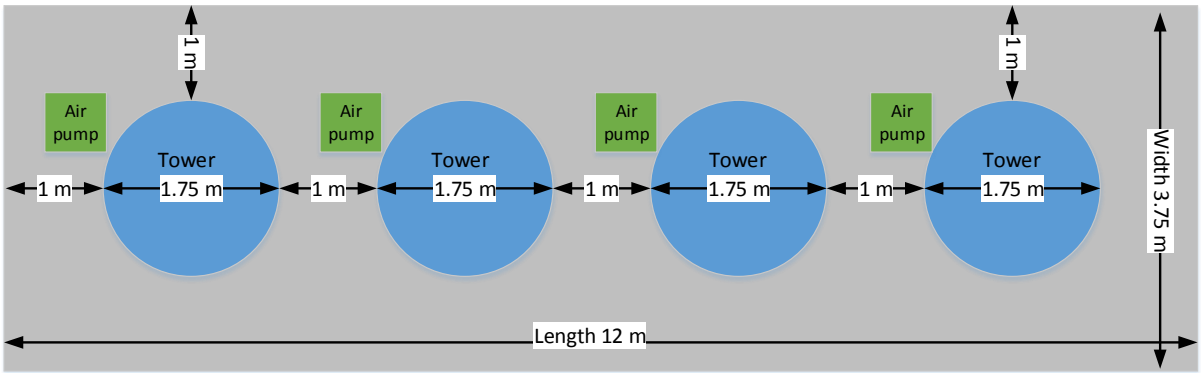


Figure 31 Dimensions of the Tower aerators and total area needed for four treatment trains estimated from findings in this research

The capacity of the centrifugal pump is dependent on the maximum water flow rate and RQ to be applied in the future set-up. For now, an RQ of 100 is being assumed, so that the centrifugal pump needs a capacity of 12 000 m³/h. Each train has a separate air pump. An overview of all the settings including head-losses is given in table xx

Table 18 Overview of all dimensions, settings and other aspects of the tower aerator

Aspects		Unit	Per train	Total
Flows	Air	m^3/h	12000	48000
	Water	m^3/h	120	480
	RQ	-	100	100
	Loading Rate	m/h	50	50
Tower Dimensions	Diameter	m	1.75	
	Area	m^2	2.41	9.62
	Height	m	6	
Building space required	total area	m^2		45
	total volume	m^2		270
Head-loss	Air	m	1	1
	Water	m	6	6
Pump capacity	Air	m^3/h	12000	48000

4.5.2 Dimensions of the Plate Aerator

Normal loading rate for plate aerators fall within 30-40 $m^3/m^2.h$ (van Dijk, 2007). Since the pilot results are currently not sufficient to draw any conclusions about the most optimal loading rate, a rate of 30 m/h has been chosen to insure sufficient removal.

The plate aerator consists of a single tray and with the preference for a long and narrow plate. A plate aerator set-up with a very high removal efficiency (99.7 %) was found which had a length of 4 m and a width of 2 m (Helm, van der, 1998). It has been assumed that a plate aerator with a length of at least 4m and a width of less than 2m will provide similar or better results. The length is set to 4m and the width is determined by the width needed in order to obtain the correct surface load (30 m/h). The needed plate area is 4 m^2, so that the width becomes 1 m.

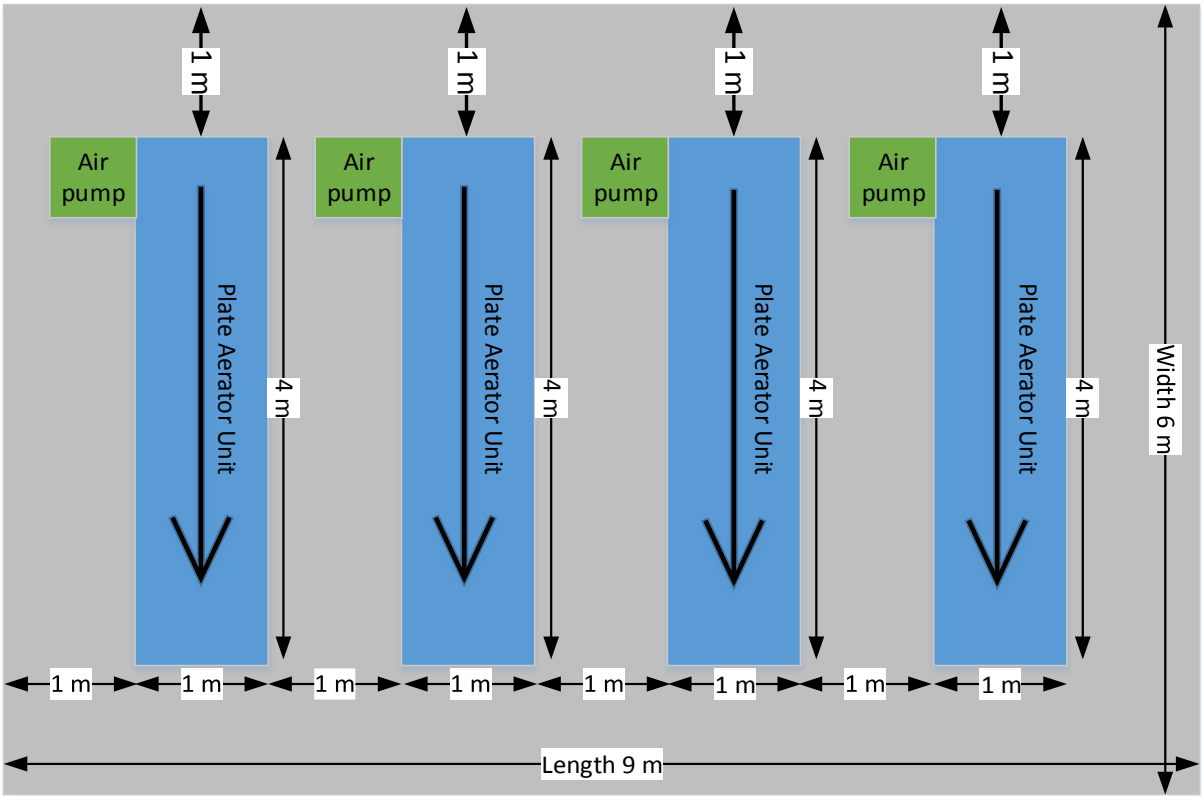


Figure 32 Layout and dimensions of plate aerators

The total area taken up by the four trains including workspace is equal to 54 m^2, which is more than the tower aerators. Plate aerator are very low (approximately 1m); since the area below or above the plates cannot be utilized however, the needed volume is estimated from the height of a normal building, which is 3m. The total volume requirement is then 162 m^3.

As with the tower aerators, the RQ is being used to predict the needed centrifugal pump capacity. For plate aerators, the maximum needed RQ is also assumed to be 100. Therefore the pump capacity per train is equal to 12 000 m^3/h.

Table 19 Dimensions, settings and other aspects of plate Aerators

Aspects		Unit	Per train	Total
Flows	Air	m^3/h	12000	48000
	Water	m^3/h	120	480
	RQ	-	100	100
	Loading Rate	m/h	30	30
Plate Aerator	Length	m	4	
	width	m	1	
	Area	m^2	4	16
	Height	m		3
Building space requirement	total area	m		54
	total volume	m		162
Head-loss	Air	m		2
	Water	m		1
Required pump capacity	Air	m^3/h	12000	48000

4.5.3 Dimensions of the Membrane contactors

As shown in the previous section, a minimum of three 14x40" liqui-cel® membrane contactors are to be place in series in order to provide sufficient removal capacity. These units have maximum flow capacity of 120 m^3/h for both air and water. If however, the water flow rate of 120 m^3/h is applied, the resulting pressure drop is very high (6m), as demonstrated by Figure 33. Additionally, with a higher flow rate, the removal efficiency decreases. Therefore, it would be best to apply flow rates in the range of 60-80 m^3/h as recommended earlier.

Two alternatives are presented hereafter, the first with four treatment trains, containing two membrane streets, each treating 60 m^3/h. The second only has three treatment trains with two membrane streets, each with a capacity of 80 m^3/h. This done to investigate whether four treatment trains are the most optimal set-up if membrane contactors were to be applied.

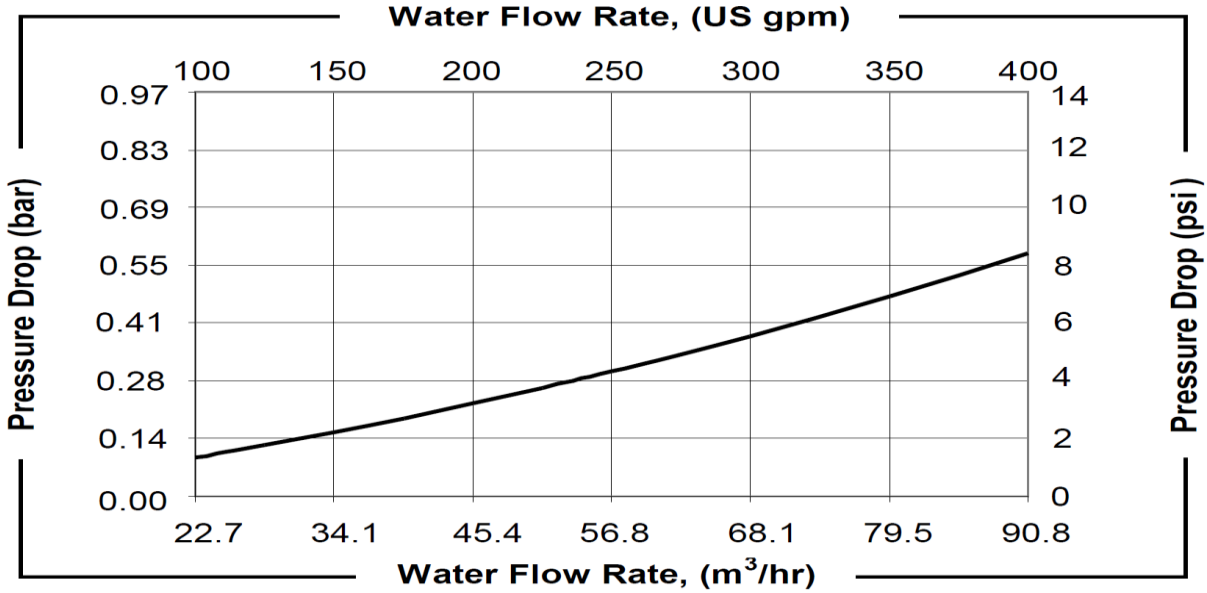


Figure 33 liquid side pressure drop from different water flow rate in 14 x 40" liqui-cel membranes

Alternative with four treatment trains.

In order to have four treatment trains, two membrane streets are needed each with a capacity of 60 m³/h. Each street contains three membranes in series to provide sufficient removal capacity. With four trains, containing 2 streets of 3 membranes in series, the total number of membranes needed is equal to 24. The membranes are placed vertically and have a diameter of approximately 0.5 m. The total needed area is equal to 44 m².

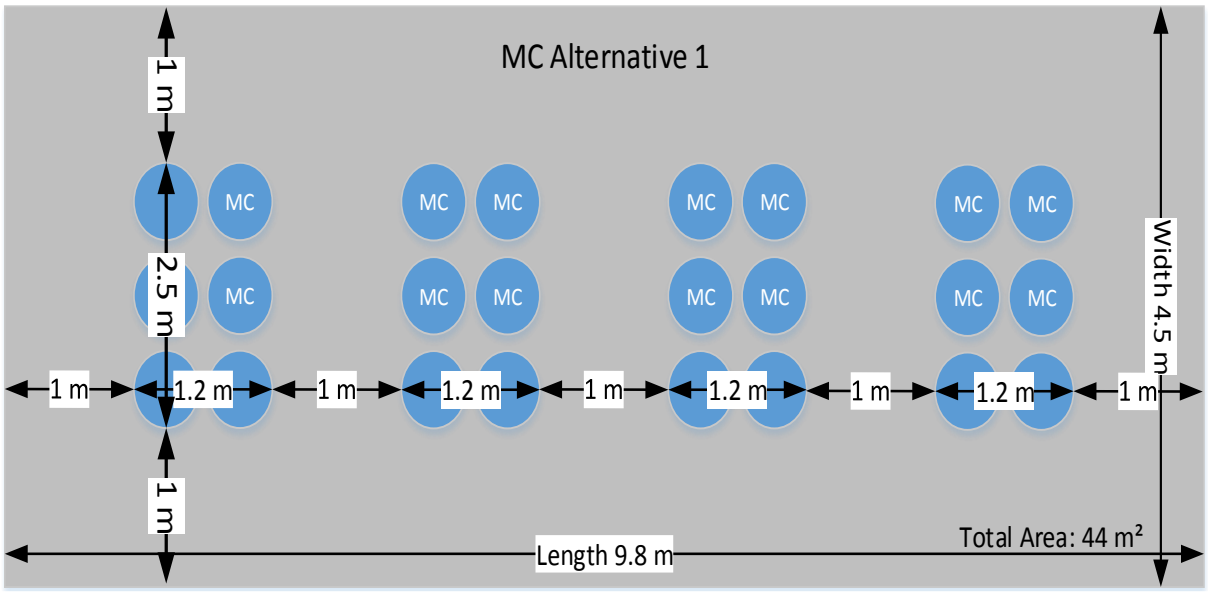


Figure 34 Layout and dimensions of membrane contactors in four treatment trains, first alternative

The maximum RQ per unit is dictated by the maximum allowable air flow through a membrane (120 m³/h) which, with a water flow of 60 m³/h corresponds to an RQ of 2. The capacity of the centrifugal pump is equal to the maximum flow of air multiplied by the number of membranes in a train, so that the total capacity per train is 720 m³/h.

Alternative with three treatment trains

Due to the possibility of increasing the capacity to 80 m³/h, an alternative is presented which consists of three treatment trains instead of four. The capacity per treatment train is then equal to 160 m³/h (two streets with 80 m³/h).

As it is unlikely that at this flow rate the membranes will provide sufficient removal capacity with only three membranes in series (i.e. the removal efficiency per membrane <84%), an additional membrane needs to be added. There are 3 treatment trains with 2 streets containing 4 membranes in series so that the total number of membranes used is exactly equal to that of alternative 1 (24 units). This alternative has the added benefit that it will facilitate high removal capacities even if the individual removal in each membrane unit is very low (75%) so that it is almost certain that this alternative will provide sufficient removal.

The maximum RQ per unit is dictated by the maximum allowable air flow through a membrane (120 m³/h) which, with a water flow of 80 m³/h corresponds to an RQ of 1.5. The capacity of the centrifugal pump is equal to the maximum flow of air multiplied by the number of membranes in a train, so that the total capacity per train is 960 m³/h.

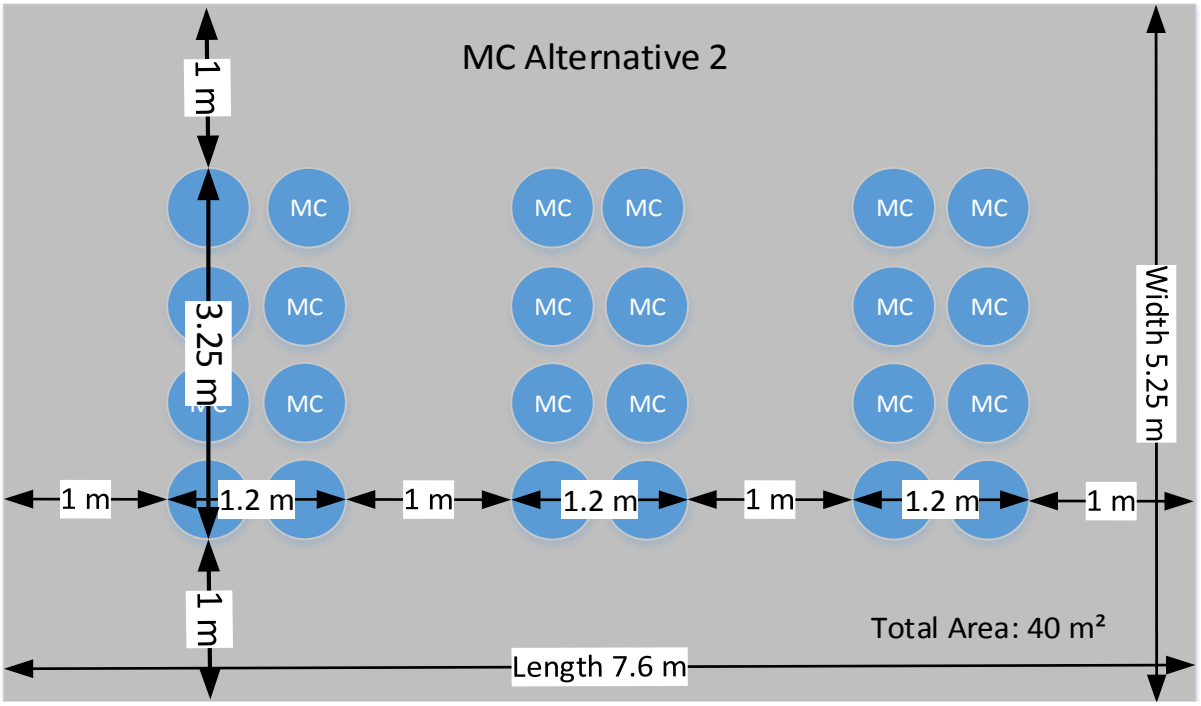


Figure 35 Layout and dimensions of membrane contactors in three treatment trains, second alternative.

Both alternatives will be discussed in terms of costs, energy use and both will be subjected to a multi-criteria analysis, to determine the more optimal of the two and compare them to the tower and plate aerators. The set-ups as currently envisioned by the author are shown in Figure 34 and Figure 35. Table 20 summarizes all aspects of both alternatives, including the head-losses.

Table 20 Dimensions, flow settings and other aspects of membrane contactor set-up alternatives 1 & 2

			Alternative 1		Alternative 2	
Aspects		Unit	per train	Total	per train	Total
Flows	Air	m³/h	720	2880	960	2880
	Water	m³/h	120	480	160	480
	RQ	-	2	6	1.5	100
	water flow/MC	m³/h	60	60	80	30
Membranes	# streets	-	2	8	2	8
	# MC's in series	-	3		4	
	Total	-	6	24	8	32
Building space needed	Height	m		3		3
	workspace	m		1		1
	total area	m²		44		45
	total volume	m		132		135
Head-loss	Air	m	1	1	1	1
	Water	m	3	9	5	20
Pump capacity	Air	m³/h	720	2880	960	2880

4.6 Estimation of energy use

The energy use of the three aeration steps has been estimated from head loss measurements gathered in the pilot plant, literature and interpretation of datasheets (for membrane contactors). The energy calculation is based on the pumping energy needed to pump air and water which is dependent on the flow rate to be pumped, the liquid density and the head loss. Finally the efficiency of the pump determines the electrical power needed. Losses due to friction in pipes, corners and valves have been neglected in this calculation.

The following formula was used to calculate the hydraulic power and electric power needed:

$$P_H = \frac{Q * \rho * g * h}{3.6\ x\ 10^6} \quad [kW] \tag{26}$$

$$P_E = \frac{P_H}{\eta} \quad [kW] \tag{27}$$

$$with: P_H = hydraulic\ power\ and\ P_E = electrical\ power$$

$$Q = flow\ water\ or\ air\ [m^3/h]$$

$$\rho = density = 1000\ and\ 1.2\ for\ water\ and\ air\ respectively\ [kg/m^3]$$

$$g = gravity = 9.81\ [m/s^2]$$

$$h = headloss\ [m]$$

$$\eta = pump\ efficiency = 0.7\ [-]$$

The liquid side pressure drop in the tower is equal to the fall height of the water which is equal to the tower height (6m). The liquid pressure drop of the plate aerator is estimated based on the used pilot system (1m). For membrane contactors the pressure drop was estimated from the available product data sheets. The pressure drop are 3 m and 5 m for flows of 60 and 80 m³/h respectively. The total pressure drop is equal to the pressure drop per unit times the number of units in series, so that the total liquid pressure drops for alternatives 1 and 2 are 9m and 20m respectively.

The air pressure for tower aerators and membrane contactors is equal to 1 m (0.1 bar). For plate aerators the pressure is a higher because the air needs to be squeezed through the tiny holes in the plates and needs to overcome the hydrostatic pressure of the water on top of the plate. Keeping these factors in mind, the air pressure drop for plate aerators was estimated at 2 m (0.2 bar).

With all other factors needed for the energy calculations having been mentioned previously, the energy requirements were calculated using eq. (26) and (27). The results as well as some important aspects are shown in Table 21.

Table 21 Energy use: estimated air and water flow rates, pressure losses and calculated consumption for aeration systems

ENERGY CALCULATIONS									
		Tower Aerator		Plate Aerator		MC Alternative 1		MC Alternative 2	
		Water	**Air**	**Water**	**Air**	**Water**	**Air**	**Water**	**Air**
Flow	m³/h	480	48000	480	48000	480	2880	480	2880
Head loss	m	6	1	1	2	9	1	20	1
Power	kW	7.85	0.16	1.31	0.31	11.77	0.01	26.16	0.01
Electricity	kW	11.21	0.22	1.87	0.45	16.82	0.01	37.37	0.01
Total	**kW**	**11.44**		**2.32**		**16.83**		**37.38**	
kWh/m³		**0.024**		**0.005**		**0.035**		**0.078**	
Yearly	**kWh/y**	**1.00E+05**		**2.03E+04**		**1.47E+05**		**3.27E+05**	

Table 21 demonstrates that the energy consumption varies greatly between different systems. Clearly, the energy consumption is determined almost completely by the head loss in the liquid side so that even with very high RQ's, the air flow rate has very little impact on the total energy consumption. This is caused by the difference in density between air and water (factor 1000) and the small head losses in the gas side compared to the liquid side (with the exception of plate aerators).

The second MC alternative consumes much (more than 2 x) more energy than the first alternative. This is due to the fact that four membranes are placed in series and the higher flow rate applied in this alternative. In terms of energy therefore, the first alternative is better than the second.

It also becomes very clear from the calculations, that the plate aerators require by far the least amount of energy, using 5 times less than the tower aerator and 6-13 times less energy than the membrane contactors. The tower aerator uses 1.5-3.3 times less energy than membrane contactors.

4.7 Total Cost of Ownership (TCO) estimation of treatment alternatives

The costs estimation was executed on the basis of Oasen own cost model which estimates the costs of treatment step from the investment costs and operational costs over a time frame of 30 years also called the total cost of ownership (TCO). The different aspects for investment costs are listed in Table 22 which also demonstrates the recovery period for each of these aspects as prescribed by Oasen investment policy.

Table 22 Breakdown of investment costs and corresponding recovery periods

Aspect	Recovery period (years)
Civil	40
Mechanical	20
Electrical	10
Process Automation	10

The operational costs consist of energy costs, maintenance costs (estimated 1% of total investment cost) and for membrane contactors, replacement of the membrane units. For the costs shown in the subsequent overview, a replacement period of 7 years has been used which is an estimate based on information from the manufacturer. Note that this investment period may decrease significantly if aerated water precipitates calcium so that scaling occurs. This vulnerability should be investigated in future pilot research.

Further descriptions and breakdown of costs are given in Appendix E , which entails a complete overview and includes the calculations for energy, maintenance and investment costs as well as a description of the method used to calculate the TCO for each alternative.

Table 23 Estimated TCO's for three aeration systems; membrane contactors contain 2 alternatives: one with four treatment trains and two membranes in series (left) and with three trains and three units in series (right)

	Tower Aerator	Plate Aerator	Membrane Contactors	
			Alternative 1	Alternative 2
Capex	€ 644 500.00	€ 551 300.00	€ 642 700.00	€ 551 000.00
Opex (€/y)	€ 15 000.00	€ 7 000.00	€ 54 000.00	€ 69 000.00
TCO	€ 2 496 000.00	€ 2 041 000.00	€ 3 904 000.00	€ 4 025 000.00

Table 23 demonstrates clearly that there is a large difference between the TCO of the 4 alternatives. The capital expenditures (CAPEX) or investment costs do not vary much between alternatives. The lower investment costs for MC alternative 2 in comparison to MC alternative 1 is due to the reduction in the number of treatment trains; This leads to a reduction because less additional equipment (air pumps, sensors and electrical) is needed.

The main difference in TCO is caused by the difference in operational costs which are caused by:

- The difference in energy consumption in the four alternatives. As demonstrated previously, there is a factor 5-16 difference between plate aerator and other alternatives in terms of energy consumption which translates linearly to costs. The difference in operational costs between the two membrane contactor alternatives is caused only by differences in energy consumption.
- The large difference in operational costs between membrane contactors and the other two alternatives is caused by the replacement of membranes every 7 years. This adds an additional 34 000 €/y to the operational costs of both MC alternatives.

4.8 Multi-Criteria Analysis

In order to decide which of the four previously described alternatives most optimally fits the future treatment facility, a multi-criteria analysis (MCA) was carried out. This section briefly describes the criteria on which the alternative were tested and justifies the weights accredited to each criterion. The individual scores of each alternative are extensively discussed in Appendix F. After showing and discussing the resulting scores for each alternative, the section concludes with the most optimal treatment technique for the future treatment facility at ZS de Hooge Boom.

Each alternative was accredited a score for each criterion, based on the previously presented results, literature and the authors insights gained from testing the systems in a pilot plant. The scores range between a value of 1-5; 5 being the most optimal and 1 representing a less than average aspect. This MCA was made specifically with the situation at ZS de Hooge Boom in mind and does not necessarily have to be the same at another location.

In addition to a score for each alternative, each criterion was given a weight factor, indicating its relative importance, both in overall terms and in relation to other criteria. The criteria are (in order of importance, from top to bottom):

- **Removal efficiency**: The main goal of the treatment step discussed in this report is the sufficient removal of methane. This is therefore the most important criterion. If a certain technique or alternative would not provide sufficient removal (≥99.6 %), it would not be a viable option and would therefore not be considered in the MCA at all. Since this has not been proven, all alternatives are considered. This means that the accredited scores show the

capability of a certain technique to exceed the minimum required removal capacity, thereby, improving the biological stability of the water even more.

- **Safety**: Drinking water companies want to produce safe drinking water for their customers at all times. Safety of a treatment technique is therefore very important, especially, since there will be no disinfection or barrier for other contaminations after the aeration step.
- **Reliability**: The reliability of a treatment step is dependent on a wide variety of aspects. It is important that a technique be robust, so that it is capable of dealing with fluctuations in flows and methane concentrations. Also it is important that the drinking water company can depend on the continuous functioning of the system, with frequent repairs and replacements.
- **Feasibility**: This criterion pertains to the time frame of the project. Oasen has the goal for the new treatment to be operational by 2018, which means building should start in 2016 or 2017. Since some techniques are easier to design than others and some require more research to prove their capabilities and expose their vulnerabilities, this criterion expresses the author's certainty as to whether or not a technique could be completed by 2018.
- **Costs**: The economic viability of a system is of course also a very important aspect, especially since there is a large difference in costs between the alternatives. When compared to the TCO and cost/m³ of the whole treatment facility, it becomes clear that the costs are relatively low, even for the more expensive techniques. This criterion gets a relatively low weight compared to those named previously.
- **Energy consumption**: This criterion is often related to the environmental sustainability of a technique. It is therefore always important to take energy consumption into account when comparing techniques to each other. As with the costs, however, the energy consumption is relatively small compared to the overall energy consumption of a complete treatment plant, especially one that uses RO as a primary treatment step. Therefore, this aspect also gets a relatively low weight.
- **Expandability**: This criterion expresses the ease with which a technique can expanded in the future, for example to accommodate capacity expansions or the need for higher removal rates.

The aforementioned criteria were described in order of relative importance to each other and to the overall treatment facility. Weights between 1 and 10 were accredited to each of the criteria. These were justified by the reasons presented before. The accredited weights are shown in Table 24. The table also shows the scores given to each alternative and the outcome of the multi-criteria analysis.

Table 24 Weights and individual accredited scores per criterion and total score per alternative as determined in MCA.

Criterion	Weight	Tower Aerator	Plate Aerator	MC Alternative 1	MC Alternative 2
Removal Efficiency	10	4	2	1	5
Safety	9	1	1	5	5
Reliability	8	5	3	2	2
Feasibility	8	5	4	2	2
Costs	6	4	5	1	1
Energy	6	3	5	2	1
Expandability	4	3	2	4	4
Total		**183**	**153**	**121**	**155**

It is clear from the outcome of the multi-criteria analysis that the tower aerator is the most optimal treatment technique. This is because it scores well on all criteria, making it a very dependable technique with an extremely high removal efficiency.

Plate aerators provide sufficient removal efficiency but are more vulnerable to fluctuations and issues with fouling may cause a decreasing removal efficiency which is highly unwanted, especially since this could occur without the problem being visible. Even though plate aerators are cheaper and less energy consuming, tower aerators are found to be the more safe option, especially since the removal of methane is dependent on only one system.

Membrane contactors have a two large disadvantages; their energy consumption and their costs. This makes them less optimal for application at ZS de Hooge Boom. Additionally, because this technique has never been used for the currently envisioned application, it is unclear which other factors need to be considered and dealt with. Therefore it would require more time to intensively test various aspects of their functionality (e.g. removal capacity and vulnerability to scaling and fouling). It is unlikely that this will be completed within the time frame of this project.

Alternative 1 is cheaper and uses less energy, however, it is unlikely to provide a significantly higher removal efficiency than 99.6 % and even that may prove difficult. Alternative 2, is estimated to provide extremely high removal efficiencies. This gives it one mayor advantage over the other techniques; if higher removal efficiencies are needed in the future, membrane contactors with 4 (or more) membranes in series, become the only viable alternative. It is therefore recommended that further research be carried out into the capabilities and vulnerabilities of membrane contactors for future treatment plants.

For the new treatment plant at Kamerik it is found that tower aerators offer the most optimal alternative.

5 CONCLUSIONS & RECOMMENDATIONS

This thesis is a consequence of Oasen vision "*to produce pristine water of impeccable quality and to deliver this water flawlessly to its customers*". In light of this goal a novel treatment approach is to be implemented by 2018, utilizing RO membranes as a primary barrier against upcoming pollutants. RO membranes will be used to treat all the abstracted groundwater whilst it is still anaerobic, thereby preventing fouling and scaling issues which would occur if the water was to be aerated first.

This approach introduces a couple of new issues to be dealt with, one of them being the removal of methane which is no longer (partly) removed biologically; Methane therefore needs to be removed sufficiently by a single post-treatment system, which is challenging especially when very low methane concentrations are to be achieved. In order to overcome this challenge, the main goal of this research was to:

Determining the most optimal post-treatment technology for the removal of sufficient amounts of methane in order to prevent biological regrowth in the distribution system thereby complying with Oasen's goal to "produce pristine water of impeccable quality and a flawless distribution"

Since there is currently no legislation pertaining to the maximum concentration of methane in drinking water, a novel design parameter had to determined; the acceptable level of methane in drinking water to prevent biological regrowth in the distribution system. With the determined parameter, three treatment techniques were then tested to determine which would be the most optimal for application at Kamerik.

The research was therefore structured under two main research questions and a large variety of sub questions pertaining to these:

3. What is the acceptable level of methane considering the growth potential of bacteria on methane?
4. Which technique shows the potential to achieve the target level of methane and does so most efficiently?

These topics have been discussed extensively in preceding chapters, results have been presented and discussed and preliminary conclusions drawn. This section compiles all this information into a short summary which concludes with the most optimal technique to be used in Oasen's new treatment facility and some recommendations for future research.

5.1 Conclusions

The following conclusions can be drawn from the various questions researched:

- A novel method was developed to determine the effect of methane oxidizing bacteria (MOB) on the biological stability of drinking water.
    - The method is capable of demonstrating the growth potential of bacteria on methane and the results are easily related to the AOC assay. It is therefore a good tool for continued research into the effect of methane on the biological stability of water.
    - Preliminary results show that the yield of MOB most likely falls in the range of 8.6 x 10^5-1.7 x 10^6 cells/µg CH_4. Additionally, indications are present that the cell volume of MOB is approximately 2 times larger than that of bacteria growing on AOC. The total cell yield therefore needs to be compensated by the cell volume to get an accurate estimate of the biomass produced by MOB compared to AOC bacteria.
- Having quantified the effect of the presence of methane on biological stability in water and the relation between this effect and the effect of AOC a new parameter for biological stability of drinking water (or growth potential) was formulated (eq. (14)):

$$Bulk\ GP = C_{AOC} + \frac{C_{CH_4}}{Y_f} = C_{AOC} + \frac{C_{CH_4}}{2.7} \quad [\mu g\ AOC\ eq./L]$$

    - With this bulk parameters, the AOC and CH_4 concentrations can be measured separately by standard procedures after which the bulk growth potential is calculated with the aforementioned equation. This bulk parameter can then be compared to the objective biological stability expressed in AOC content.
- Oasen has set itself the goal to produce water of a pristine quality and to limit issues occurring in the distribution of that water. Biological stability is an essential part of this. In its plan, Oasen states it wants to achieve an AOC content of 1 µg/L and a CH_4 concentration of <10 µg/L. This yields a bulk growth potential of 4.7 µg AOC eq. /L. Since this is far less than the generally accepted stability parameter (<10 µg/L AOC), and taking into account the increasing complexity of achieving lower values, the author believes this to be an acceptable target value. 10 µg CH_4/L is therefore recommended as the maximum acceptable level of methane for Oasen.
- With a maximum methane concentration of 10 µg/L, three techniques and four alternatives were discussed in order to determine the most optimal post-treatment step to be implemented in the future treatment facility. The minimum removal efficiency corresponding to the maximum acceptable concentration of methane is 99.6 %. All techniques have been shown to potentially provide sufficient removal capacity, each with their own advantages and limitations:
    - Tower aerators provide more than sufficient removal efficiencies and are very flexible in operation, making them extremely reliable and easy to use. Additionally, it is relatively easy to design a tower aerator so that it should be a problem to complete the project by 2018.
    - Plate aerators are much cheaper and less energy consuming than the tower aerators and membrane contactors respectively. They are however reliant on a range of variables in order to work properly and this makes them less reliable and prone to operational issues. This could potentially lead to insufficient removal.
    - Membrane contactors show the potential to provide extremely high removal efficiencies and have the added benefit of keeping the water and air flow separated, so that potential contamination from the air to the water is limited. Membrane contactors are however extremely expensive and energy consuming. Finally, additional research is needed before membrane contactors can be applied in a new treatment facility. It is therefore unlikely this can be rounded up before 2018.

- Taking these considerations into mind, a multi-criteria analysis was executed, testing four alternatives on: Removal efficiency, safety, reliability, feasibility within the time frame of the project, costs, energy consumption and expandability. The result clearly demonstrates that tower aerators are the most optimal technique to be used in Oasen new treatment facility.
- Using tower aerators, it might be possible to achieve methane concentrations slightly lower than 10 µg/L CH_4 (estimated: 5.5 µg/L) so that the bulk growth potential becomes even less than the previously stated value (estimated: 3 µg/L), thereby complying with Oasen's goal "*to produce pristine water of impeccable quality and to deliver this water flawlessly to its customers*".

5.2 Recommendations

The conclusions pertaining to the growth potential of methane-oxidizing bacteria are drawn on the basis of two conducted experimental runs which in some aspects show very contradictory results. It is therefore essential that Oasen continue conducting research on the effect of methane before implementing its future plans. This can be done with the method presented in chapter 3. Recommendations relating to possible adaptations and improvements to this method are presented in that chapter.

Future research should include:

- A deeper investigation into the relation between total cell yields of methane-oxidizing bacteria. Complementing the flow-cytometric measurements with other quantification methods would help.
- A better quantification cell volume of methane-oxidizing bacteria and natural consortium of bacteria growing on AOC so as to obtain better insight into the biomass produced on each of these parameters.
- Identification of the bacteria responsible methane oxidation in drinking water. Pure cultures of this bacteria can then be synthesized in order to obtain more straight-forward results.
- A comparison of the effect of methane with other biological stability parameters such as the BDOC content.

With more measurements and more accurate results, the author is confident that the bulk parameter can become a very useful parameter in the design and control of future drinking water plants treating anaerobic groundwater.

As for the removal techniques presented in chapter 4, the author is relatively certain that Tower Aerators are the most optimal post-treatment technique for application at Kamerik. The results from the pilot research are however not sufficient to design a full-scale treatment facility. Therefore alternative methods should be found to insure optimal dimensions and settings of the future treatment.

- Over the course of this research, it has become clear that pilot research, can only yield a limited amount of information about the removal efficiency and can only be useful if the dimensions in the pilot plant are very much similar to those implemented in the later treatment facility.
- As it is very expensive to build a large scale system for pilot research, the author recommends Oasen to base its design of the future tower aerator on experience in other treatment plants with high removal efficiencies, rather than on pilot research. Various models exist to further optimize the removal once appropriate dimensions have been determined.

Membrane Contactors are less optimal compared to Tower Aerators for the currently investigated case. This is justified by the much higher energy consumptions and costs of this technique compared to tower aerators. Due to their modularity, it is however possible to place multiple units in series so that very high removal efficiencies are possible; in the case extremely higher removal efficiencies (>99.8%) are needed (exceeding the removal capabilities of tower aerators) they may therefore become the only viable solution. This could occur if:

- Further investigations into the growth potential of methane-oxidizing bacteria shows higher yields than that which has currently been found.
- If a new treatment facility with the same set-up is to be built at another location with a higher methane concentration in the raw water.

In this respect, it is therefore recommended that Oasen continue its research into the removal efficiency of this new and exciting treatment technology.

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APPENDIX A – SYNTHESIS OF A NUTRIENT SOLUTION AND CALCULATION OF THE REQUIRED DOSE

The nutrient solution used in this thesis was a supplement solution which contained both phosphate and nitrate.

Making the solution

The production of this solution was taken directly from the BPP-test protocol made by KWR (KWR, 2011):

1. Dissolve 1.5 g of KH_2PO_4 and 6 g of KNO_3 in 1 L of MQ water in an AOC free glass flask.
2. Autoclave for 15 min at 125°C

The solution can be stored for up to a year if store in a refrigerator at 5°C.

Dosing calculation

According to Hammes et al (2005), the ratio of C:N:P needed for growth of micro-organisms in a medium is 100:10:1. The following calculation is based on the phosphate concentration needed for unrestrained growth, the ratio of C:P needed is 100:1. The amount of phosphate present in water is unknown and is neglected in the calculation.

An initial estimation of the maximum methane concentration that may occur during the dosing experiments (assuming the maximum dose=4 ml of methane mix) was **4 mg/l of CH_4** which equates to **0.25 mmol/L**.

As stated previously, the ratio of C:P needed is 100:1 (in moles). Therefore, the maximum concentration needed is **0.0025 mmol/l.** This is equal to 75 µg P /L or 238 µg PO_4/L.

The molar mass of KH_2PO_4 is 136 mg/mmol. The concentration of KH_2PO_4 in the sample therefore must be: 136mg/mmol x 0.0025 mmol/L = 0.340 mg/l.

With a sample size of 24ml or 0.024L the amount of KH_2PO_4 to be dosed is:

0.340 mg/l x 0.024= 0.008 mg

Finally, the nutrient solution KH_2PO_4 concentration is 1500 mg/l so that the dose needed is calculated from: 0.008 mg / 1500 mg/l = 5 x 10^{-6} l = 5 µl.

All experiments carried out in this research, unless otherwise stated, were done using the same dosage of 5 µl per sample for the sake of reproducibility. For samples containing lower concentrations of carbon, it is assumed that the additional phosphate does have any effect on the growth of micro-organisms.

APPENDIX B – RAW WATER QUALITY AT ZS DE HOOGE BOOM

25 Minimum, Average and Maximum values of quality parameters measured in individual wells

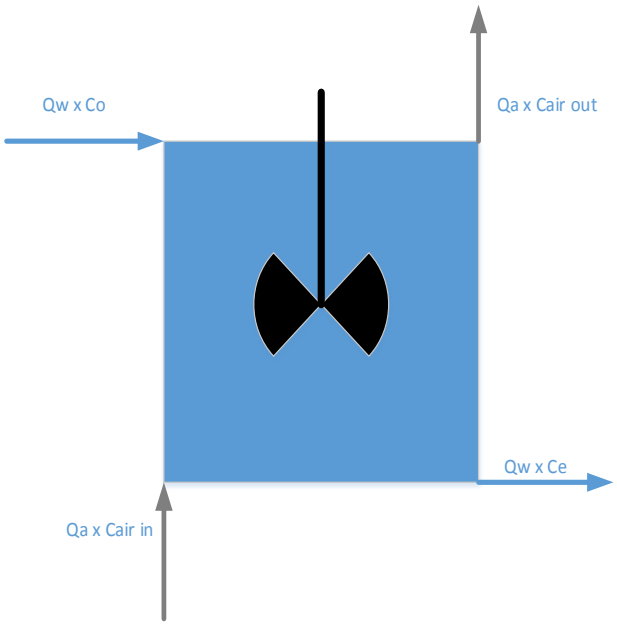
Parameter	Symbol	Units	Minimum	Average	Max
Temperature	-	°C	11.1	11.6	12
Acidity	-	pH	7	7.1	7.5
Dissolved oxygen	DO	mg/l	-	0.4	-
Methane	CH_4	µg/l	39.8	1962	4193.3
Conductivity 20 °C	EC	mS/m	55.7	81.7	102.3
Total hardness		mmol/l	2.3	3.6	4.8
Calcium	Ca	mg/l	73.8	113.6	152.4
Magnesium	Mg	mg/l	10.3	17.1	21.2
Sodium	Na	mg/l	20	52.3	69.7
Potassium	K	mg/l	3.4	5.7	6.9
Iron	Fe	mg/l	1.8	8.3	11.3
Manganese	Mg	mg/l	0.1	0.5	1
Ammonium (N)		mg/l	0.6	2.9	3.8
Ammonium (NH_4)	NH_4	mg/l	0.7	3.7	5
Aluminium	Al	µg/l	0.2	2.6	1.7
Barium	Ba	µg/l	-	107.6	-
Cadmium	Cd	µg/l	-	0	-
Copper	Cu	µg/l	-	0.1	-
Lead	Pb	µg/l	0	0	0.5
Zinc	Zn	µg/l	0	1.2	0.6
Bicarbonate	HCO_3	mg/l	259.4	385.9	477.3
Chloride	Cl	mg/l	32.1	77.8	133.4
Nitrate	NO_3	mg/l	0	0.2	0
Sulphate	SO_4	mg/l	0.7	42.3	109.2
Fluoride	F	mg/l	0.1	0.1	0.2
Orthophosphate (P)		mg/l	0.2	0.5	1.1
Orthophosphate (PO_4)	PO_4	mg/l	-	1.6	-
Total phosphate (PO_4)	PO_4	mg/l	-	2	-
Total phosphate (P)		mg/l	0.1	0.5	1.1
Nitrite	NO_2	mg/l	0	0	0
Total organic carbon	TOC	mg/l	2.9	7.7	9.2
Silicate	Si	mg/l	-	7.6	-
Boron	B	µg/l	-	76.4	-

APPENDIX C – MAXIMUM REMOVAL EFFICIENCIES OF DIFFERENT AERATION SYSTEMS

Derivation of the maximum removal efficiency of a completely mixed system

The following derivation is made assuming that all systems, given sufficient time for diffusion will behave as a completely mixed reactor as demonstrated in the figure.

Given:



(1) $k = \frac{c_0 - c_e}{c_0 - c_s}$

$k = removal\ efficiency\ [-]$

$c_0 = initial\ water\ conc.\ [\mathrm{mg/l}]$

$c_e = effluent\ water\ conc.\ [\mathrm{mg/l}]$

$c_s = saturation\ conc.\ [\mathrm{mg/l}]$

(2) $RQ = \frac{Q_A}{Q_w}\ [-]$

$Q_A = air\ flow\ \left(^{l}/_{s}\right)$

$Q_w = water\ flow\ \left(^{l}/_{s}\right)$

(3) $k_D = \frac{c_w}{c_A}$

Which gives:

$$c_{air\ in} = \frac{c_s}{k_D}\ and\ c_{air\ out} = \frac{c_e}{k_D}$$

The following mass balance can be set up for this system:

$$Q_W x C_0 + Q_A x C_{air\ in} = Q_W x C_e + Q_A x C_{air\ out}$$

Dividing both sides by Q_W and using relation **(2)** gives**:**

$$C_0 + RQ x C_{air\ in} = C_e + RQ x C_{air\ out}$$

Then using **(3)** and reorganising the equation yields:

$$C_0 = C_e\left(1 + \frac{RQ}{k_D}\right) - \frac{RQ}{k_D} C_s$$

Filling this into **(1)** gives:

$$k = \frac{\frac{RQ}{k_D} c_e - \frac{RQ}{k_D} c_s}{(c_e - c_s)(1 + \frac{RQ}{k_D})} = \frac{RQ}{RQ + k_D}$$

This can also be derived from the analytical formula for completely mixed systems, which is:

Completely Mixed

$$k = \frac{1}{1 + \frac{1}{k_2 T} + \frac{k_D}{RQ}} \qquad (28)$$

If $k_2 T \longrightarrow \infty$ (which means that the contact time and exchange area are infinitely large), then eq. (18) simplifies to:

$$k = \frac{1}{1 + \frac{k_D}{RQ}} = \frac{RQ}{RQ + k_D}$$

Derivation of the maximum removal efficiency of a co-current plug-flow systems

Similarly, if $k_2 T \longrightarrow \infty$ in the formula of a co-current plug-flow system, the maximum removal efficiency is found to be:

Plug-flow co-current

$$k = \frac{1 - e^{-k_2 T(1+\frac{k_D}{RQ})}}{1 + \frac{k_D}{RQ}} \quad (29)$$

if $k_2 T \longrightarrow \infty$

$$k = \frac{1}{1 + \frac{k_D}{RQ}}$$

Thus, the maximum removal efficiency is equal for co-current plug-flow and completely mixed systems.

Derivation of the maximum removal efficiency of a counter-current plug-flow systems

For counter-current system the analytical formula reads:

Plug-flow counter-current

$$k = \frac{1 - e^{-k_2 T(1-\frac{k_D}{RQ})}}{1 + \frac{k_D}{RQ} * e^{-k_2 T(1-\frac{k_D}{RQ})}} \quad (30)$$

if $\boldsymbol{k_2 T \longrightarrow \infty}$ and $\boldsymbol{RQ > k_D}$

$$k = 1$$

The maximum removal efficiency of counter-current systems is 100% if if $k_2 T \longrightarrow \infty$ and provided $RQ > k_D$. Since the k_D value for CH_4 is 0.043, the can be very low to achieve high removal efficiencies.

Table C-1 Analytical equations for the removal in three different types of systems

Flow Regime	Removal Equation	
Completely mixed	$k = \frac{1}{1 + \frac{1}{k_2 T} + \frac{k_D}{RQ}}$	(31)
Plug-flow co-current	$k = \frac{1 - e^{-k_2 T(1+\frac{k_D}{RQ})}}{1 + \frac{k_D}{RQ}}$	(32)
Plug-flow counter-current	$k = \frac{1 - e^{-k_2 T(1-\frac{k_D}{RQ})}}{1 + \frac{k_D}{RQ} * e^{-k_2 T(1-\frac{k_D}{RQ})}}$	(33)

APPENDIX D - DERIVATION OF FORMULA FOR K_2T

The calibration formula for packed tower aerators is given by eq. (21):

$$k = \frac{1 - e^{-k_2T(1-\frac{k_D}{RQ})}}{1 + \frac{k_D}{RQ} * e^{-k_2T(1-\frac{k_D}{RQ})}}$$

With:

$$k = removal\ efficiency\ [\%]$$

$$k_2T = device\ specific\ calibration\ constant\ dependent\ on\ the\ available\ exchange\ area\ and\ the\ residence\ time\ [-]$$

$$k_D = Henry's constant\ for\ CH_4\ [-]$$

$$RQ = \frac{Air}{Water}\ [-]$$

During the pilot plant experiments performed in this study, various air and water flow setting (i.e. various RQ's) were tested. At the start of the experiment, the tower aerator is set to the aimed at RQ and the air and water flows are recorded. During the experiments the temperature of the water was constant and close to 10°C.

Samples were then taken according to protocol and sent to the lab for analysis of the methane concentration. Once the analysis is completed, the removal efficiency (k) can be calculated. All parameters in the above formula are then known and the device specific parameter k2T can be calculated.

Eq. (21) is rewritten to yield this parameter as follows:

$$k = \frac{c_e - c_0}{c_s - c_0} = \frac{1 - e^{-k_2 \times T \times (1-\frac{k_D}{RQ})}}{1 - \frac{k_D}{RQ} e^{-k_2 \times T \times (1-\frac{k_D}{RQ})}}$$

1. Supplementing $k_2 \times T$ by x and $\frac{k_D}{RQ}$ by a and rewriting the formula yields:

$$R = \frac{1 - e^{-x+xa}}{1 - ae^{-x+xa}} = \frac{e^{ax} - e^{x}}{ae^{ax} - e^{x}}$$

2. Reorganizing the equation yields:

$$Rae^{ax} - Re^{ax} = -Re^{x} + e^{x}$$
$$(1 - Ra)e^{ax} = (1 - R)e^{x}$$
$$\ln(1 - Ra) - \ln(1 - \mathrm{R}) = \mathrm{x}(1 - a)$$
$$x = \ln(\frac{1 - Ra}{1 - R})/(1 - a)$$

Which gives:

$$k_2T = \frac{\ln\left(\frac{1 - R * \left(\frac{k_D}{RQ}\right)}{1 - R}\right)}{1 - \frac{k_D}{RQ}}$$

It is to be noted that the k_2T value will change if the loading rate or the height of the tower aerator is changed. For each loading rate, therefore, a separate k_2T must be determined. In this study, the height of the tower was the same for all experiments.

APPENDIX E – ELABORATION ON COST CALCULATION MODEL

The costs model as explained in chapter 4, is based on a previously made model for Oasen's own use. This appendix describes the structure of the model and justifies the costs that have been used as input for the model.

The cost model consists of three tabs in a spread-sheet:

1. A tab used to calculate the investment costs
2. A tab used to calculate the operational costs
3. A tab which calculates the total cost of ownership (TCO) over a period of 30 years.

Each of these aspects is discussed separately, including assumptions that have been made, issues encountered and a justification of costs of separate units that have been implemented. The calculated costs are only shown for the alternatives with four treatment trains. The membrane contactor alternative with three trains was calculated by changing relevant parameters in the model and is therefore difficult to demonstrate simultaneously with others.

Calculation of the investment costs

The investment costs, or capital expenditures (Capex), are split into 4 categories. Each category has its own investment costs as prescribed by Oasen's investment policy. The categories and prescribed recovery periods are shown in table E-1.

Table E–1 Capex aspects and recovery periods as prescribed by Oasen's investment policy

Aspect	Recovery period (years)
Civil	40
Mechanical	20
Electrical	10
Process Automation	10

The recovery period indicates after how long the investment needs to be paid back and is an estimate of the period after which the aspect is to be replaced. Each of the aspects is discussed separately hereafter.

In the investment costs tab, there is a table with highlighted green fields. These fields represent interchangeable variables influencing mostly the investment costs, but sometimes also the operational costs. If these change the TCO obviously also changes. The model variables are shown in table E-2.

Table E-2 Investment cost model variables

Name	value	Unit	Description
n_train	4	-	Number of treatment trains
trn_cap	120	m3/h	water capacity per treatment train
tot_cap	480	m3/h	total water capacity of treatment facility
h_norm	3	m	normal building height
n_mmc	2	-	Number of streets per train (only for membrane contactors)
r_install	25%		additional costs of installation membrane contactors
n_ser	3		# of membrane contactors in series
r_airmmc	0.5	-	cost reduction due to smaller capacity air pump MC
CC_civ	350	**€/m³**	building price/m3
UC_mmc	10000	**€**	price/ membrane unit

Capex-Civil

The costs per building unit as used by Oasen is 350 €/m3[9]. The volume for an air pump comes from the Oasen model and includes the area needed for electrical equipment, pipes and filtration equipment. With the areas and heights of each of the three techniques, the needed building area and volume per treatment train was calculated. These are simply adjusted in the cost model to find the civil capital expenditures for each alternative. The model for capital expenditures is split up into treatment trains so that the number of trains can be varied by changing only on parameters. A breakdown of each of the alternatives, the needed area (m²) and volume (m³) per train are shown as well as the costs of the building needed to incorporate these volumes is given in Table E-3.

Table E- 3 Breakdown of the civil costs based on initial dimensioning of alternatives

	A (m²)	H (m)	V (m³)	Price per Train	Total (4 Trains)
Tower Aerator	11.3	6	67.5	€ 23 625.00	**€ 94 500.00**
Air pump			25	€ 8 750.00	**€ 35 000.00**
Total					**€ 129 500.00**
Plate Aerator	13.5	3	40.5	€ 14 175.00	**€ 56 700.00**
air pump			25	€ 8 750.00	**€ 35 000.00**
Total					**€ 91 700.00**
Membrane contactors	11	3	33	€ 11 550.00	**€ 46 200.00**
air pump			10	€ 3 500.00	**€ 14 000.00**
Total					**€ 60 200.00**

Capex-Mechanical

Mechanical cost were estimated for each of the alternatives as shown in Table E-4. The unit prices are based on prices found in Oasen's cost model and include installation costs such as piping and placement:

- For the tower aerator:
  - The unit price per tower is assumed to be similar to GAC filter with the same capacity. The unit price given for a GAC filter is 60 000 €/unit. (2012 price estimation)
  - The air pump for the tower aerator has approximately the same capacity as the air pump for which the price was estimated in 2012. The same price is therefore used in this model.
- For the plate Aerator:
  - The unit price of the plate aerator and air pump are taken directly from the Oasen model
- Membrane Contactors:
  - The unit price a 14 x 40"liqui-cel® membrane is 10 000 €/unit. An additional 25% is added to this costs to account for other installation necessities (i.e. pipes and valves). The price per train is calculated by the number of units in series x the number of streets in a train x 1.25.
  - Because the air pump needs a much lower capacity than the other two alternatives (720 m³/h instead of 12 000 m³/h). The price is therefore estimated as being a factor

[9] Most unit prices shown in the model are taken directly from the Oasen cost calculation model. These values originate from estimations made in 2012 and have not been corrected for inflation. The author has made the assumption that these prices sufficiently represent the current costs.

0.5 lower than for the other two alternatives. This is probably still an overestimation of the price.

Table E-4 Breakdown of the mechanical costs based on initial dimensioning of alternatives

	Price per Train		total	
Tower Aerator	€	60 000.00	€	240 000.00
Air pump	€	31 250.00	€	125 000.00
Total			**€**	**365 000.00**
Plate Aerator	€	46 150.00	€	184 600.00
air pump	€	31 250.00	€	125 000.00
Total			**€**	**309 600.00**
Membrane contactors	€	75 000.00	€	300 000.00
air pump	€	15 625.00	€	62 500.00
Total			**€**	**362 500.00**

Capex-Electrical

The electrical capital expenditures are taken directly from the costs estimation for plate aerators as found in Oasen's cost model. Since there is no reason to expect difference in electrical costs these are similar for all three alternatives. An exception is made for membrane contactors; these require a pressure sensor between each of the modules placed in series, so that the required amount is estimated to be twice as high as that of the other two alternatives.

Table E-5 Breakdown of electrical costs based on Oasen's cost model

	Price per Train		total	
Tower Aerator	€	15 000.00	€	60 000.00
Air pump	€	10 000.00	€	40 000.00
Total			**€**	**100 000.00**
Plate Aerator	€	15 000.00	€	60 000.00
air pump	€	10 000.00	€	40 000.00
Total			**€**	**100 000.00**
Membrane contactors	€	30 000.00	€	120 000.00
air pump	€	10 000.00	€	40 000.00
Total			**€**	**160 000.00**

Capex-Process Automation

All the alternatives due not need complex steering or automation equipment. It was, however, found wise to incorporate an online CH_4 measuring device into the process, so that processes can be adapted to compensate for fluctuations in the raw water. Two sensors are needed; one controlling the inflow concentration and one controlling the outflow concentration. A sensor which is well suited for this job is the Contros HISEM® sensor which costs approximately 25 000 €/unit. This gives a total of 50 000 €/installation (independent of the amount of trains applied). An additional 10 000 € was added to the process automation costs for membrane contactors to take into account monitoring software for pressure drops over the membranes.

Table E-6 Estimated process automation costs

Alternative	total	
Tower Aerator	**€**	**50 000.00**
Air pump		
Total	**€**	**50 000.00**
Plate Aerator	€	50 000.00
air pump		
Total	**€**	**50 000.00**
Membrane contactors	€	60 000.00
air pump		
Total	**€**	**60 000.00**

With all the individual costs for civil, mechanical, electrical and process automation, the total investment costs for the three alternatives with four treatment trains is added up to yield the total investment costs required. The total investment costs as calculated in the demonstrated model are shown in table E-7.

Table E-7 Calculated investment costs of the three alternatives with four treatment trains

Alternative	**Total investment costs**	
Tower Aerator	**€**	**644 500.00**
Plate Aerator	**€**	**551 300.00**
Membrane contactors (alternative 1)	**€**	**642 700.00**

Calculation of the operational costs

The operational costs (Opex) consist have been calculated in three categories the results of which are demonstrated in table E-8:

- Energy costs
- Membrane replacement
- Maintenance costs

Table E-8 Operational costs in individual aspects and total

	Energy Cost	**Membrane replacement**	**Maintenance**	**Total Opex**
	€/y	€/y	€/y	€/y
Packed Tower Aerator	€ 9 015.87	€ -	€ 6 445.00	**€ 15 460.87**
Plate Aerators	€ 1 826.75	€ -	€ 5 513.00	**€ 7 339.75**
MC-alternative 1	€ 13 269.24	€ 34 285.71	€ 6 427.00	**€ 53 981.96**

The membrane replacement is an additional operational cost which is only valid for membrane contactor alternatives. As in the previously demonstrated investment costs, only the costs of the first alternative will be shown in this paragraph. Factors influencing only the operational cost aspects and

not the investment costs are once again marked with green fields in the Opex tab. The variables and their description are shown in Table E-9.

Table E-9 Values influencing operational cost calculation in the model

Variable	value	unit	Description
RQ_PT	100	-	Tower Aerator RQ
RQ_PA	100	-	Plate Aerator RQ
RQ_MC	2	-	Membrane contactor RQ (single element)
dp_PT	6	m	Water pressure drop Tower Aerator
dp_PA	1	m	Water pressure drop Plate Aerator
dp_MC	3	m	Water pressure drop MC (per element!)
ro_air	1.2	kg/m³	Density air
ro_water	1000	kg/m³	Density water
g	9.81	m/s²	
mu_pump	0.7	-	
p_energy	0.09	€/KWh	Energy price
t_mem_rep	**7**	years	Time after which membranes are replaced

Energy Costs

The energy cost calculation is based on the energy consumption calculation as shown in section 4.6 of this report. In fact, the energy calculation model is integrated into the cost calculation, so that both costs and energy are calculated simultaneously by changing the related variables (RQ, pressure drop, density air and water, number of membranes, number of trains). The energy price that has been used to estimate the energy use is 0.09 €/kWh as found in the original Oasen model.

Maintenance Costs

The maintenance costs were estimated to be 1% of the investment cost of each alternative.

Membrane Replacement Costs

Membranes in the membrane contactor installation have to be replaced after a certain period. The manufacturer estimates (given that the water applied is RO permeate) that the membranes will last for approximately 7 years. The cost is then equal to the total number of membranes in the installation (number of trains x number of streets x number of membranes in series) x the cost of a membrane unit/ the replacement time of the membranes. It is clear that the operational costs of membrane contactors will therefore increase significantly if the replacement time decreases. As there is currently no data about the replacement time the value given by the manufacturer has been used. Since the aeration step in the future plant may be occurring after the demineralization, it should however, be tested whether this will not cause a significant decrease in the replacement time (due to scaling of the fibers).

Total cost of ownership (TCO) calculations

The total cost of ownership is an estimation of the bulk costs of each alternative over a 30 year period. The interest rate and inflation rate used in this calculation are 5% and 2% respectively. The calculation consists of two parts:

- The investment costs, taking into account the recovery period of each of the individual aspects.
- The operational costs: these are multiplied by the number of years in period over which the TCO is to be calculated.

As has been shown previously in table E-1, the Capex are split into four categories (civil, mechanical, electrical and process automation), each with an assigned recovery period (40, 20, 10 and 10 years respectively). After the recovery period has passed, the aspects falling under a specific category are to be replaced, requiring a reinvestment. The number of investments needed within the time frame of the TCO calculation can therefore be calculated from:

$$n_{r,x} = \frac{t_{TCO}}{R_x} - 1$$

With:

$n_{r,x} = number\ of\ reinvestments\ (round\ up\ to\ nearest\ 1)\ needed\ for\ aspect\ x$ [-]

$$TCO\ time\ frame = 30\ years$$

$$R_x = recovery\ period\ for\ aspect\ x\ [years]$$

The total investment needed for each aspect is the equal to:

$$inv_{tot,x} = \left(1 + n_{r,x}\right) * \ inv_x$$

With:

$$inv_{tot,x} = total\ investment\ for\ aspect\ x\ over\ TCO\ time\ frame\ [€]$$

Table E-10 Recovery periods, number of reinvestments, initial investment cost and total investment costs for each aspect

Alternative	Aspect	R_x	$n_{r,x}$	inv_x [€]	$inv_{tot,x}$ [€]
Tower Aerator	Civil	40	0	129 500.00	129 500.00
	Mechanical	20	1	365 000.00	730 000.00
	Electrical	10	2	100 000.00	300 000.00
	P.A.	10	2	50 000.00	150 000.00
Plate Aerator	Civil	40	0	91 700.00	917 000.00
	Mechanical	20	1	309 600.00	619 200.00
	Electrical	10	2	100 000.00	300 000.00
	P.A.	10	2	50 000.00	150 000.00
MC alternative 1	Civil	40	0	60 200.00	60 200.00
	Mechanical	20	1	362 500.00	725 000.00
	Electrical	10	2	160 000.00	480 000.00
	P.A.	10	2	60 000.00	180 000.00

The resulting total investment needed for every aspect is shown in table E-10. This is however, the amount of money that would have to paid without interest. The cost model also incorporates the additional sum from interest paid during the TCO time frame.

This once again is calculated separately for each aspect from:

$$interest_x = inv_x * i_r$$

With:

$$interest_x = interest\ paid\ per\ year\ for\ aspect\ x\ [€/y]$$

$$i_r = interest\ rate\ [\%]$$

The interest to be paid during the recovery period of an aspect is:

$$interest_{R,x} = \sum_{0}^{n} (interest_x)/(1 + inflation)^n$$

With:

$$interest_{R,x} = sum\ of\ the\ interest\ paid\ for\ aspect\ x\ in\ years\ 0\ to\ R_x$$

$$n = years\ from\ 0\ to\ R_x$$

For some aspects (civil and mechanical) the (last) investment period lasts longer than the time frame of the TCO calculation. The remaining years of these aspects is calculated from:

$$t_r = R_x - (n_{inv.} * R_x - t_{TCO})$$

With:

$$t_r = time\ of\ last\ investment\ cycle\ within\ TCO\ timeframe$$

$$n_{inv.} = total\ number\ of\ investment\ cycles = 1 + n_{r,x}$$

$$t_{TCO} = TCO\ time\ frame = 30\ years$$

The interest paid during the last investment cycle if the last investment cycle is not over after the end of the TCO time frame is calculated by:

$$interest_{t_r,x} = \sum_{0}^{n} (interest_x)/(1 + inflation)^n$$

With:

$$n = years\ from\ 0\ to\ t_r$$

The total amount of interest to be paid over the TCO time frame per aspect, is then dependent on whether the last investment cycle last longer than the TCO time frame:

If $t_r = 0$;

$$Interest_{t,x_{TCO}} = n_{inv.} * interest_{R,x}$$

Otherwise;

$$Interest_{t,x_{TCO}} = n_{r,x} * interest_{R,x} + interest_{t_r,x}$$

The total cost of ownership is then equal to:

$$TCO = t_{TCO} * Opex + \sum inv_{tot,x} + Interest_{t,x_{TCO}}$$

With:

$$Opex = operational\ expenditures\ of\ each\ alternative\ [€/y]$$

The total investment costs, interest and operational costs as well as the TCO are shown in Table E-11.

Table E-11 Investment costs, interest rate and operational costs paid over a period of 30 years and the TCO calculated for 30 years for the three alternatives with four treatment trains.

	Tower Aerator	Plate Aerator	MC-alternative 1
Total Capex	€ 1 309 500.00	€ 1 160 900.00	€ 1 445 200.00
Total interest	€ 825 660.47	€ 710 905.85	€ 839 478.07
Total Opex	€ 463 826.16	€ 220 192.36	€ 1 619 458.70
TCO	€ 2 598 986.63	€ 2 091 998.21	€ 3 904 136.77

It is the author's opinion that the method used to calculate the total interest to be paid over a 30 year period is flawed. The reason being that the method does not take into account the yearly reduction of the debt over the recovery period (investment/recovery period). The definition of a recovery period is that after the period is over (for a certain aspect), the debt has been paid back completely; this would mean that the yearly paid interest rate decreases over time until it is 0 at the end of the recovery period. The process then repeats itself in the next investment (or recovery) cycle.

It is therefore thought that the interest calculation as currently presented is a gross overestimation of the actual amount to be paid in terms of interest, leading to an overestimation of the TCO. To keep the results of this model comparable to those from Oasen's own model, however, the interest calculation has not been changed.

APPENDIX F – ELABORATION ON MULTI-CRITERIA ANALYSIS

The multi-criteria analysis was based on eight criteria, which in order of importance are: Removal efficiency, safety, reliability, feasibility, costs, energy consumption and expandability. The justification of the weight accredited to each criterion is given in the main report. This appendix gives a justification for the scores given to the alternatives in terms of each criterion.

Each alternative was accredited a score for each criterion, based on the previously presented results, literature and the authors insights gained from testing the systems in a pilot plant. The scores range between a value of 1-5; 5 being the most optimal and 1 representing a less than average aspect. This MCA was made specifically with the situation at ZS de Hooge Boom in mind and does not necessarily have to be the same at another location. The given scores are shown in table F-1 followed by a justification in the order in which the criteria are given.

Table F-1 Weights and individual accredited scores per criterion and total score per alternative as determined in MCA.

Criterion	Weight	Tower Aerator	Plate Aerator	MC Alternative 1	MC Alternative 2
Removal Efficiency	10	4	2	1	5
Safety	9	1	1	5	5
Reliability	8	5	3	2	2
Feasibility	8	5	4	2	2
Costs	6	4	5	1	1
Energy	6	3	5	2	1
Expandability	4	3	2	4	4
Total		**183**	**153**	**121**	**155**

Removal efficiency:

Note that all the alternatives comply with the minimum removal efficiency of 99.6 %. The scores are therefore based on the additional removal (therefore providing additional biological stability).

- MC alternative 2 most probably provides the highest removal capacity.
- Tower aerators also provide high removals, but slightly less than MC alternative 2.
- Plate aerators provide a little bit more removal than the minimum requirement.
- MC alternative 1 most probably provides the lowest removal capacity and it is unclear whether it will even be able to achieve this value.

Safety:

- Membrane contactors have the added benefit of separation (or barrier) between air and water so that potential contaminations in the air cannot enter the water.
- The other two techniques do not have this benefit. Additional filters are needed to insure the pumped air is sufficiently clean.

Reliability:

- Tower aerators are very simple in operation. The only variables are the water and air flow rates. This makes them very reliable and the author sees no reason to assume sudden problem due to fouling and scaling occurring in this type of system.
- Plate aerators are dependent on a variety of variables (bed height, air division, average residence time) all of which are influenced by the air and water flow rates. One very important variable is the division of air over the whole plate. If clogging and fouling occur on the plate, this may distort the air division leading to incomplete removal, which is very disadvantageous. Additionally, these changes can occur without changes in the flows, so that it is difficult to monitor.
- It is currently not known how reliable membrane contactors are. In the author's opinion, these membranes may be very prone to scaling, especially if applied after the remineralisation step in the new treatment facility. If this happens it will not directly influence the removal efficiency, but will lead to a shorter replacement time, so that the costs of the alternatives increase even further.

Feasibility:

- Tower aerators can be designed from previous experience and models exist which can assist in further optimisation. This makes it relatively easy to design& construct a tower aerator within the time frame of the current project.
- Plate aerators similarly, are designed mostly on previous experience. Often some additional testing is however required by means of pilot research, because it is difficult to model these systems.
- There are currently too many unknowns about membrane contactors. Their removal efficiency, but also their sensitivity to fouling in the currently envisioned treatment scheme need to be tested extensively before they can be used in a future treatment plant. It is unlikely that this can be executed within the timeframe of the current project.

Costs

- As demonstrated clearly in section 4.7 the TCO varies greatly between alternatives. Plate aerators are the cheapest, but the difference with tower aerators is not very big (18%). Both membrane contactor alternatives are a twice as expensive as the plate aerator.

Energy

- As demonstrated in section 4.6 the differences in energy consumptions between different techniques are huge. Plate aerators by far use the least amount of energy followed by tower aerators (5 x). Membrane contactor alternatives both use consume very high amounts of energy: alternative 1 (7.3 x) and alternative 2 (16 x).

Expandability

- Membrane contactors are modular making it is to expand the capacity in terms of flows as well as removal capacity. Due to their size, they can be fit into op spaces in an existing treatment plant.
- Tower aerators require more space than membrane contactors, but less than plate aerators.
- Plate aerators require the largest amount of additional space, thereby making them the least expandable.