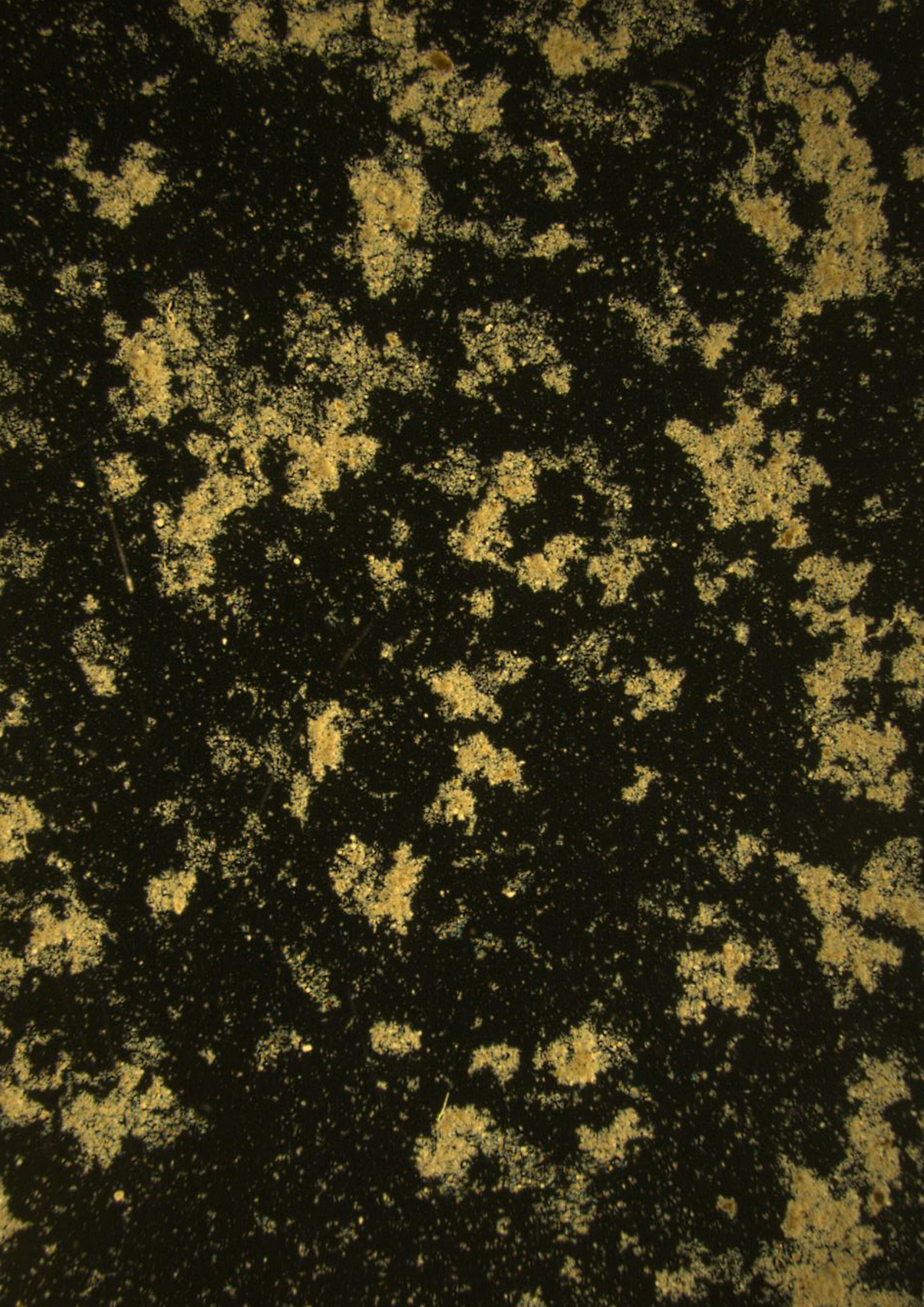

Reactor characterization and the application of biological selective pressure for granulation in a CAGS pilot

BSc Thesis Life Sciences and Technology

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Abstract

Treatment of wastewater with aerobic granular sludge (AGS) has major advantages compared to activated sludge. The only commercial system that uses AGS is the Nereda® batch process. The disadvantage of this system is the lack of existing infrastructure. AGS in continuous systems could be a solution to this disadvantage. That's why the HARKOS pilot is built. This pilot is based on the enhanced biological phosphorus removal (EBPR) process. The aim of this study is to make sure this pilot runs according to the EBPR process (reactor characterization) and to apply biological selective pressure for granulation. This biological selective pressure consists out of four pillars. Prevention of COD overshoot into the aerobic zone, as much COD in the anaerobic zone to storage polymers, enrichment for bio-p organisms and compartmentalization of the reactor.

The results from the characterization showed that the pilot behaves according the EBPR process. The HPLC results showed that no acetate entered the aerobic zone. The maximum COD uptake of the reactor rose from 0,17 to 1,19 kgCOD/kgTss/d, this means that more COD is taken up as a storage polymer. This is similar to the Willem Anna polder. The phosphate uptake increased from 11,3 to 12,7mgPO4-P/hour/gTSS. A normal EBPR process has a phosphate uptake of around 2,2mgPO4-P/hour/gTSS. This shows that the pilot is highly enriched for bio-p organisms. The rates of the control batch test and the pilot were similar, this means that the compartmentalization is good enough to prevent COD overshoot. If it's enough for diffusion into granules isn't clear yet because no granules were present in the pilot.

It's concluded that the pilot is characterized, that no COD overshoot is present, that more COD is stored as storage polymers, that the sludge is enriched for bio-p organisms and that the reactor is well enough compartmentalized. The biological selective pressure is applied. If enough physical selective pressure is applied granulation will start in the HARKOS pilot.

Table of contents

§1 Introduction

The treatment of wastewater has an important role in society. Without the proper sanitation of sewage, nutrient levels of phosphate and ammonium would rise drastically in surface waters, which can lead to eutrophication and in extreme cases to diseases like cholera (Chislock et al., 2013). Since 1923 a mixed culture of bacteria are used to lower the levels of phosphate, ammonium and carbohydrates (COD) in sewage treatment processes (Beychok et al., 1967). These bacteria consume COD, ammonium and phosphate to grow and these compounds are thus converted to biomass. Since biomass is a solid phase, it can be separated from the water by means of decanting. This is how you clean the water. This mixed culture of bacteria is also known as activated sludge.

Though activated sludge is still the most common method, it has some unwanted features. An example of such a feature is the low settleability, compared to other solid particles such as sand, this means you need more space to separate the activated sludge from the effluent (more clarifiers). This also means the plant has to put more energy in moving the sludge around. To make a particle settle faster, there are a few things you can do, make it denser and reduce the hydrodynamic drag.

The most common waste water treatment process technique in the Netherlands is Enhanced biological phosphorous removal (EBPR), more than 95% of the household wastewater is treated according to this process (Van Dijk, 2018). The EBPR process is a continuous system that uses activated sludge and has three different stages. The anaerobic stage, the aerated stage and the clarifier stage. The goal of the EBPR system is to select for phosphate accumulating organisms (PAO's). These organisms can store phosphate into poly-phosphate polymers in their cells under aerobic conditions, which can later be use as energy source to anaerobically store readily biodegradable COD as PHB. This feature of PAOs makes it easy to select for them with a feast and famine regime. In the anaerobic stage (famine) phosphate polymer is broken down to yield energy for storing readily biodegradable COD as PHB. In the aerobic stage phosphate is taken up and stored as a polymer (feast). In the clarifiers the sludge is separated from the effluent and part of the sludge is discarded. This is how phosphorous is removed from the wastewater. Other pollutants like ammonium are also removed, by nitrification followed by denitrification.

After 70 years of little change in the treatment of wastewater, in the nineties, aerobic granular sludge was discovered. This is a type of activated sludge that has a granular. The size of the granules differ between 0,2 and 2 mm (Liu et al.). This improves the settleability significantly. Where activated sludge settles at a speed of 8-10 m/h, aerobic granular sludge settles with a speed of 30 to 70m/h (Liu et al.). Another advantage of the granular morphology is that larger concentrations of biomass can be sustained in the reactor, which makes it possible to treat higher volumetric loads of wastewater. Image 1.1 shows a schematic intersection of a granule. Because a granule is relatively large in size, oxygen does not fully penetrate into the granule due to mass transfer limitation, mass transport limitation and due to the simultaneous consumption by micro-organisms (Philip Stewart, 2003). Therefore the granule can be divided into three zones. The aerobic zone (green), the anoxic zone (yellow) and the anaerobic zone (red). The different zones make it possible to have different kinds of metabolism at

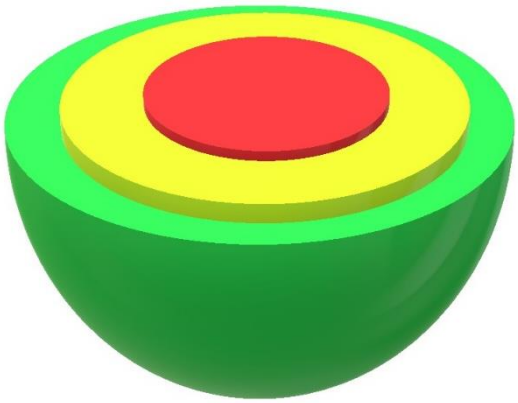

Image 1.1 *"intersection of a granule, with the green aerobic zone, yellow anoxic zone and red anaerobic zone"*

the same time. For example phosphate uptake in the green zone and denitrification in the red zone. It is estimated that replacing the activated sludge with aerobic granular sludge can save 20% in operational costs and 75% in surface area (de Kreuk et al., 2004).

In 2003 the TU Delft in partnership with RoyalHaskoningDHV started a pilot for a new technique in waste water treatment, which eventually would be called Nereda®. This process uses aerobic granular sludge in a sequential batch configuration. To handle a continuous flow of waste water, multiple batch reactors are built in parallel to each other or an influent buffer is used. Just like the EBPR the Nereda® has an anaerobic stage followed by an aerated stage and in the end a settling stage. The EBPR and the Nereda® sound similar and in fact they are. That's how this study came about.

The Harnaschpolder korrelslib pilot (HARKOS) (image 1.2) is a new cooperation between the TU Delft, Hoogheemraadschap (HH) Delfland, HH Rijnland, Evides, Delffluent services B.V. and RoyalHaskoningDHV. This pilot is a 7 to 9m^3 EBPR system. Compared to the Nereda, granulation inside an EBPR system has the advantage that most WWTP in the Netherlands are EBPR systems, no additional infrastructure is needed which means it effectively reduces the cost to get the advantages of aerobic granular sludge.

Image 1.2 *"a picture of the HARKOS pilot installation, located on the Harnaschpolder WWTP."*

The aim of this study

The goal of this pilot is to create, as fast as possible granules from activated sludge. To achieve this goal you need to types of selective pressure, physical and biological. This study focusses on the latter of the two. The aim of this study is to show that the anaerobic zone of an EBPR process is suitable to obtain and maintain granulation. There are four important biological parameter/pillars of granulation, if the requirements of these pillars are met the pilot is in an ideal state conversion wise. However to really start granulation still selective pressure on the morphology side has to be applied.

The data found in this study is then compared to two other systems. The first system is the Pilot Nereda Utrecht (PNU) is a pilot for the Nereda system located in Utrecht. This pilot has a batch volume of 1500m^3 and was built to find design parameters for big scale Nereda WWTP's. The second system is the Willem Anna polder this is a WWTP with an EBPR system. In the summer of 2017 the plant spontaneously formed granules. Comparing the data from the HARKOS pilot to these two systems could reveal some interesting insights.

The first pillar is the prevention of readily degradable COD into the aerobic zone. If easy degradable COD enters the aerated zone. Filamentous organisms will have an advantage over the slow growing bio-p organisms. This will create a bloom of these filamentous organisms. The drag caused by these filamentous organisms will have a negative impact on the Sludge Volume Index (SVI) and so the settleability (Pronk, 2015). Used measurements are the concentrations of VFA's and filtered COD.

The second pillar is the conversion of COD into storage polymers during the anaerobic time. These values are especially interesting because they cannot be measured inside the PNU The higher this percentage the better the sludge is enriched for slow growing organisms, like bio-p and glycogen accumulating organisms (GAOs). These organisms contribute to granulation. The used measurement is the anaerobic COD uptake, which is compared to data from the Willem Anna polder WWTP.

The third pillar is enrichment for bio-p organisms, these organisms are vital for granulation and have two important roles in the granulation process. They create the strong biofilm that holds the granule together. They also store phosphate, by eventually discarding the granules phosphate is removed from the water. The used measurement is the phosphate uptake rates, these are compared to the Pilot Nereda® Utrecht and EBPR data from literature (Brdanovic et al., 1998).

The fourth pillar is the compartmentalization of the reactor. This pillar has two goals the first is to prevent overshoot (the same objective as pillar 1). If there would be only one well mixed tank in anaerobic zone than COD overshoot would be certain. The second goal of the plug flow is to maximize the readily biodegradable COD concentration in the anaerobic stage. The diffusion penetration depth is dependent on the difference in concentration between the bulk liquid and the biofilm. The higher the difference in concentration the deeper the penetration of COD into the biofilm /granules (Philip Stewart, 2003). The deeper the penetration depth the bigger the granules can get. The used measurements are the acetate uptake and phosphate release of the reactor and the control batch test. Where the control batch test figures as a model for the perfect plug flow. If the sludge contains granules. This experiment could be used to compare the effect of plug flow on the diffusion inside the granules.

Next two achieving the goals of granulation, it's also important to know if the pilot is in fact behaving like an EBPR system. Therefore another aim of this study is to do a reactor characterization.

§2 Material and methods

The Pilot Set-up

Reactor configurations

The pilot set-up consists out of 14 vessels configured in series(image 2.1). All tanks except for the clarifiers are mechanically mixed. The first 6 tanks (1 to 6) are cilindrical anaerobic reactors with a volume that can vary between 75 and 300 liters. Tank 7 to 9 are hybrid reactors, they can be used anaerobic and aerobic, the volume is 1000 liters and have a cubical shape. Vessel 10 to 12 are cubical aerated tanks. They can be aerated with two types of bubbles. Fine bubbles, and coarse bubble aeration. Aeration is controlled by actuated needle valves. In tank 12 there is a suction tube installed that can apply selective pressure by wasting bad settling sludge. At the end two parralel clarifiers are used to separate the mixed liquor into effluent and return sludge.

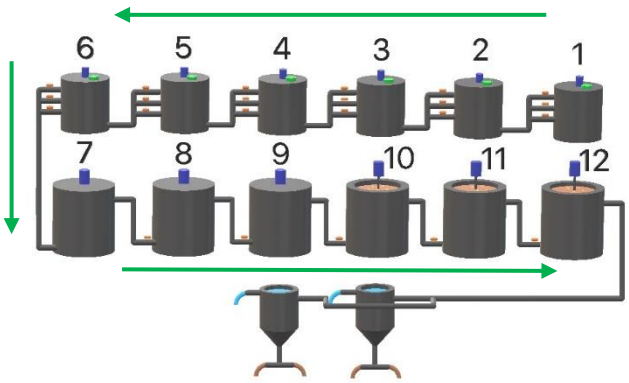

Image 2.1 *"animation of the HARKOS pilot set-up"*

Influent and inoculum

The influent was retrieved from the VBT at the Harnaschpolder WWTP. The influent flow was set on 500 liter per hour. Every three to four days the influent was measured for total COD, .45 um filtered COD, Ortho phosphate, total phosphate, ammonium and kjeldahl nitrogen. The Figures for the influent characteristics over time can be found in the results. The reactor was inocculated with activated sludge that was retrieved from the return sludge line at distribution station E30 at Harnaschpolder WWTP.

Residence time distribution tracer experiment

Objectives

The aim of this experiment is to determine the residence time distribution of the reactor, which can be used to identify whether the reactor has shortcuts, flows or dead zones compared to the theoretical residence time distribution of a tanks-in-series reactor configuration with a recycle.

Set-up

491*Volume(m3) of NaCl is prepared. The amount of salt rises the conductivity measurements with 1000µS/cm. This makes the signal significant. The conductivity probe is installed in the middle of tank 12, 15 cm under the surface.

Starting the experiment

First tank 1 was closed and the influent pump was stopped. Next the NaCl was added. After 3 minutes of mixing the valve was opened and the influent pump started. This is an important step, to prevent shortcuts.

Data processing

The numerical procedure described in Battaglia et al. (1993) was used to determine the single-pass residence time from the conductivity measurement recorded at the outlet, since the systems contains a recycle from the clarifiers to the first anaerobic tank.

Compartment mixing time experiments

Objectives

The aim of this test is to make sure the vessels are well mixed. If this is not the case, the agitator's rotational speed should be increased. If the mixing time is under 2 minutes you can safely say that there are no shortcuts.

Set-up

In every tank an amount of salt was added that would raise the conductivity in the tank 1000 us/cm. this means that for tank 5, 147,3 gr of NaCl is prepared and for tanks 7, 8, 9 and 12 491gr of NaCl is prepared. The conductivity probe was installed in the middle of the tank at a depth of 15 cm. The agitators were set on position 3.

Starting the experiment

To start the experiment al the valves around the tank were closed. Next the salt was added to the reactor, preferably to the side of the reactor. Then the conductivity was consulted to see whether the experiment was coming to an end (stable conductivity).

Data processing

The data was retrieved from the pilot system and the mixing time of the tanks was calculated, from the moment the tracer was added until a stable signal was retrieved.

Anaerobic phosphate release batch test

Summary

To make aerobic granular sludge the enrichment of bio-p bacteria is very important. An indicator for the amount of bio-p per amount of biomass is the phosphate release per biomass. Hence the fact that bio-p bacteria are the only bacteria that store phosphate. The phosphate release is measured in a batch test, where return sludge is added to effluent based acetate solution. This duration of this experiment is around 8 hours.

Objectives

The objective of this test is to find out the maximum phosphate release of the pilot in mgPO4-P/gTSS, this information is then used to calculate the percentage of phosphate release in the pilot and on what level the sludge is enriched for bio-p bacteria (PAO's). When granules are formed this will be a good indication on how much you have to enrich for bio-p to get granules to form.

Set-up

A 500ml infusion flask is used as a batch reactor. The reactor is agitated by a magnetic stirrer on a moderate speed, there is no vortex. The flask is closed with a membrane cap. Three objects penetrate this membrane. Object one is an aeration tube where nitrogen gas is suspended. Object two is a syringe for taking samples. Object three is a hollow needle, this needle makes it possible for the surplus of nitrogen gas to escape.

Method

Starting the experiment

First effluent is retrieved from the Harnaschpolder WWTP. 250 ml of this effluent is put in the flask. Next return sludge is taken from the pilot 250 ml is put in the flask and the remaining part is used for a dry weight measurement. The goal of this dry weight measurement is to obtain the concentration of sludge inside the return sludge. Then a HEPES buffer solution is added until the concentration of HEPES is 50mM, this is to make the pH stable. Thereafter acetate is added until the desired concentration of acetate is reached. This concentration is based on the expected amount of phosphate inside the biomass. For example if you expect a biomass concentration (B) of 1g/l and a phosphate release(P) of 30mg/l. You use this formula. A=(P*B*2)*1.2. For the example this would be 72mg/l acetate in the batch test.

After the acetate is added the bottle cap is put in place and the solution is flushed with nitrogen gas during the whole time to make it anaerobic.

Sampling

After the acetate is added the experiment starts. Samples are taken according to table 9.1 in the appendix. You can change the time of sampling and the amount of samples by your expectations. This can save time and tests.

Before a sample is taken through the syringe it is flushed with the batch fluid three times. Then the sample is taken and filtered through a 0.45µm filter. Because this sampling is not done under sterile conditions it's important to cool the samples. If testing of the samples take longer than 24 hours it's recommend to freeze them prior to analysis.

At the end of the experiment the pH is measured to confirm that the solution was sufficiently buffered.

Sample analysis

From all the samples the Phosphate concentration is measured. This is done with Hach Lange cuvettes, the LCK series. The samples are also ran through a HPLC machine to measure the amount of acetate that is left.

Plug-flow reactor control batch test

Summary

To make sure granules are formed it's important to have a sustainable diffusion gradient. The driving force for this diffusion rate is the concentration difference of readily biodegradable COD inside and outside the granule. This means that a good plug flow is very important to maintain granules. A batch

test is used to mimic the pilot's anaerobic stage with perfect plug flow. A batch test has a perfect plug flow because it starts with the highest concentration and ends with the lowest and changes graduate over time, where a continuous system has as many different concentrations as compartments. Data from this experiment is used to determine to which extent the plug flow differs from the batch tests. The duration of this experiment is the same as the anaerobic time of the pilot.

Objectives

The aim of this test is to see whether the pilot mimics a good enough plug flow. A batch test is the perfect plug flow. Information retrieved from this experiment can be used to compare with the pilot. If the measures of plug flow differ significantly with the pilot, modifications should be made to enhance the plug flow. Without a plug flow the diffusion gradient will be too low to get nutrients inside the granule.

Set-up

The set-up is exactly the same as the anaerobic phosphate release batch test except there is also a conductivity sensor which is hooked up to the logging system of the pilot, the conductivity has a relationship with the phosphate concentration that's why the conductivity is measured.

Starting the experiment

First Influent is taken from the pilot. Depending on the return sludge/influent ratio a certain volume is added to the flask. Thereafter the flask is topped up to a total volume of 500ml with return sludge. For example, if the ratio is 0,7 then 295ml of influent and 205ml of return sludge are added to the flask. HEPES solution is added so that the concentration of HEPES is 50mM. This is to maintain a neutral pH level of 7,35.

After the buffer solution is added the bottle cap is put in place and the solution is flushed with nitrogen gas during the whole time to make it anaerobic.

Sampling

Samples are taken according to table 9.2 in the appendix. It is important to take samples in the early stage more frequent, since the initial release rate is much higher. Because one can expect the concentration profile to resemble Figure 3.11.1. The experiment should last the same time as the anaerobic time in the reactor.

Samples are taken in the exact same way as the anaerobic phosphate release batch test. At the end of the experiment the pH is measured this is to ensure that the buffer solution worked.

Sample analysis

From all the samples the Phosphate and COD concentration is measured. This is done with Hach lange cuvettes LCK series. The samples are also ran through a HPLC machine to measure the amount and kind of volatile fatty acids that are left. The VFA's that are measured are formate, acetate, propionate, butyrate and (iso)-valerate.

Concentration profiles in the pilot-scale reactor

Summary

To monitor the reactor the whole reactor is measured twice a week. Phosphate, COD, VFA, Redox, conductivity and ammonium concentration are measured. The total sampling time of the reactor was approximately one hour.

Objectives

To see whether the pilot is heading in the right direction, the phosphate, COD, VFA, Redox, conductivity and ammonium concentration is measured. With this data the following rates are calculated. Acetic acid uptake rate, the phosphate release rate, the anaerobic COD uptake rate, the phosphate uptake rate and the ammonium sludge adsorption. These rates are key indicators for our pilot.

Set-up

All disposables and materials needed for the sampling of the pilot are prepared and washed. If the 0.45μm filters are not flushed they are flushed with demi water. The conductivity probe is hooked up to the electronic logging system.

Starting the experiment

Sampling

Prior to taking the first sample the reactor the syringe and connecting tube are rinsed by pumping water from the reactor back and forth three times. Then, the sample is taken and filtered through a fine coffee filter and stored in a plastic cylinder. This filtered most of the insoluble compounds. Subsequently the sample is filtered through a syringe filter of .45um, this sample is used for COD and HPLC measurements. The tanks are measured in series starting at tank 1 and ending at tank 12. Before a sample is taken the conductivity probe is installed in the tank of interest.

Anaerobic hydrolysis batch test

Objectives

The aim of this test is to find out, how much easy degradable COD is hydrolyzed during the anaerobic stage. If the hydrolyze rate is substantial, more COD than measured is taken up by the biomass. When all the phosphate is released no significant COD is taken up by the biomass. But hydrolysis will create more VFA's. This production term is very important in black box calculations.

The Set-up

The set-up is exactly the same as the set-up from the anaerobic batch test

Starting the experiment

First influent is taken from the pilot. The amount that is added depends on the phosphate release and the concentration of acetate in the influent. The goal was to add an amount of acetate to obtain the maximum phosphate release as determined in another experiment. Thereafter return sludge is

added to the reactor. To maintain a neutral pH level a HEPES buffer is added, so the concentration inside the experiment is 50mM.

After the buffer solution is added the bottle cap is put in place and the solution is flushed with nitrogen gas during the whole time to make it anaerobic.

The Sampling

Samples are taken according to table 9.3 in the appendix. It's important to take samples in the early stage more frequent, because of the small amount of sludge the maximal phosphate release is quickly reached. After this the hydrolysis rate can be measured. The hydrolysis experiment should take approximately 125% the amount of time as the anaerobic time of the pilot. The samples are taken according to table 9.3 in the appendix.

§3 Results

The results are divided in 3 main sections. The first part is the characterization of the reactor. The main goal of this section is to make sure that the data retrieved from the reactor is trust worthy and can be worked with. These results include the influent, sampling, mixing time, retention time, ammonium absorption and nitrogen hydrolysis.

The second part of the results are the results retrieved during the first period. This period lasted from 3oth of April till the 29th of May 2018. The results contain phosphate and COD measurements.

The third and last part of the results is about the second period that lasted from the 1st till the 28th of June. This part is divided in two sub categories. The anaerobic and the aerobic results. The anaerobic results show the COD uptake, Phosphate release, hydrolysis, maintenance rate, batch test, control test and the enrichment for bio-p organisms (PAO's). The aerobic result shows the phosphate uptake and the enrichment for bio-p.

Reactor characterization

3.1 The influent

The influent is characterized by measuring the PO4-P, total phosphate, NH_4^+, Kjeldahl Nitrogen, total COD, .45μm filtered COD and VFA's. The main goal of these measurement is to see how these concentration change over the course of this spring when the sewer temperature rises and to use this data in models for recomputing influent concentrations.

Phosphate concentration

In Figure 3.1.1 the concentration of phosphate in the influent is set out over time. The orange line shows the measured total phosphate, which differs between 7,75 and 9,75 mg PO4-P/l. The blue line shows the concentration of phosphate that is dissolved in the influent. These concentrations differ between 4 and 7 mgPO4-P/l. The results show that the phosphate levels in the influent are quite stable.

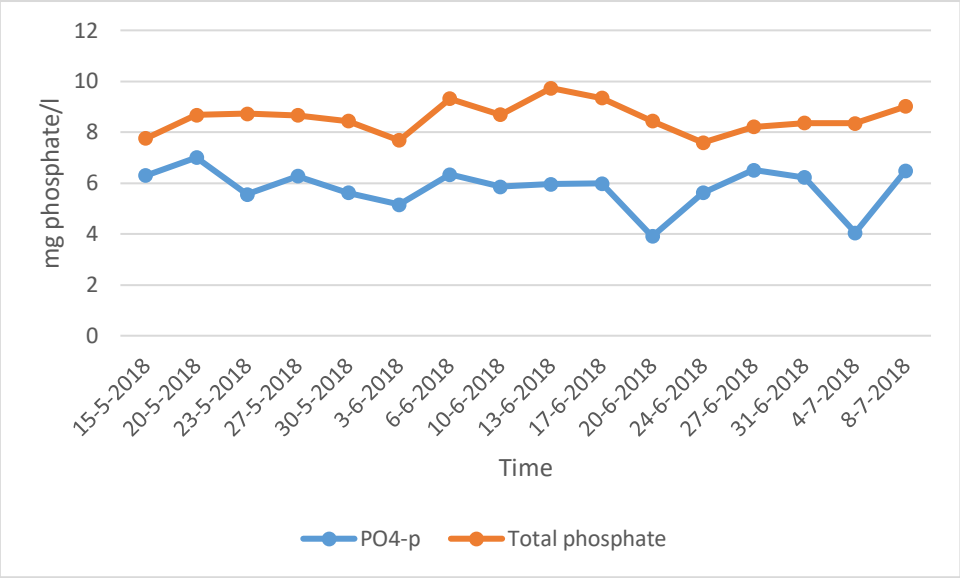

Figure 3.1.1 *"the dissolved and total phosphate concentrations in the influent over 2 months"*

Figure 3.1.2 shows the average of the phosphate measurements accompanied by error bars based on the standard deviation. The standard deviation shows that the influent has quite stable concentrations of phosphate. The dissolved phosphate adds up to 67% of the total phosphate.

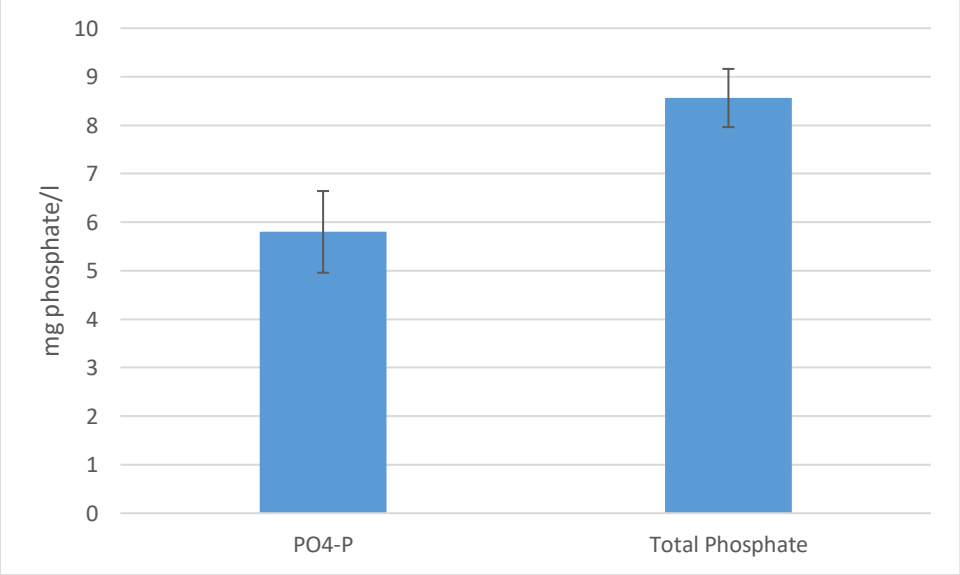

Figure 3.1.2 "the average concentration of dissolved and total phosphate of the influent"

Nitrogen containing compounds

In Figure 3.1.3 the concentration of ammonium and Kjeldahl nitrogen in the influent is set out over time. The nitrogen is measured in two groups, NH_4^+ (ammonium) and Kjeldahl. Kjeldahl is ammonium plus the bound ammonium groups (for example in amino acids). The blue line represents the ammonium which differs between 46 and the 62 mgNH4/l. The orange line represents the kjeldahl nitrogen that differs between 52 and 75 mgNH4-N/l. The closer this is to one the more hydrolysis has taken place in the sewers. As you can see the ratio starts to drop upward of the 6th of June.

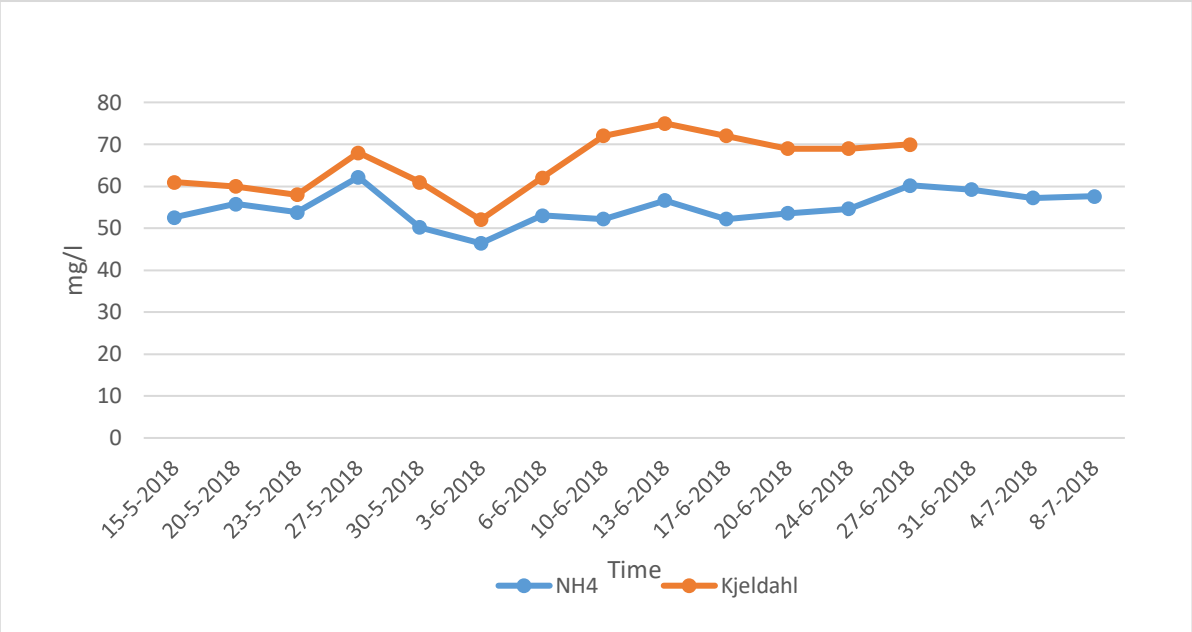

Figure 3.1.3 *"the ammonium and Kjeldahl concentrations in the influent over two months"*

Figure 3.1.4 shows the average nitrogen data from the influent. The error bars are based on the standard deviation. The standard deviation shows that the influent has quite stable concentrations of ammonium and Kjeldahl nitrogen. The ammonium adds up to 84% of the total Kjeldahl Nitrogen.

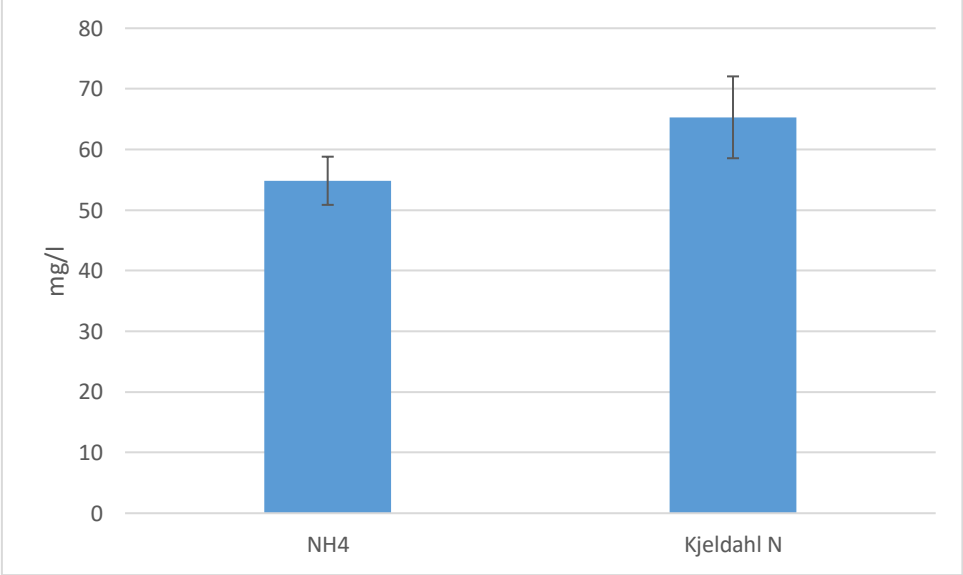

Figure 3.1.4 "the average ammonium and Kjeldahl concentration of the influent, from 15th of May till the 8th of June"

COD concentration

To keep track on the organic compounds of the influent, the COD was measured. The COD was measured on three levels. The 'total COD' this contains all Types of COD, for example, VFA's, other dissolved COD (like sugars), colloidal COD and COD particles. The .45µm COD only contains dissolved and colloidal COD that is smaller than .45µm. The Acetate COD contains only acetate. The trend

shows that the amount of acetate rises in the influent when time passes, this could be due to rising temperatures in the sewage which promote hydrolysis.

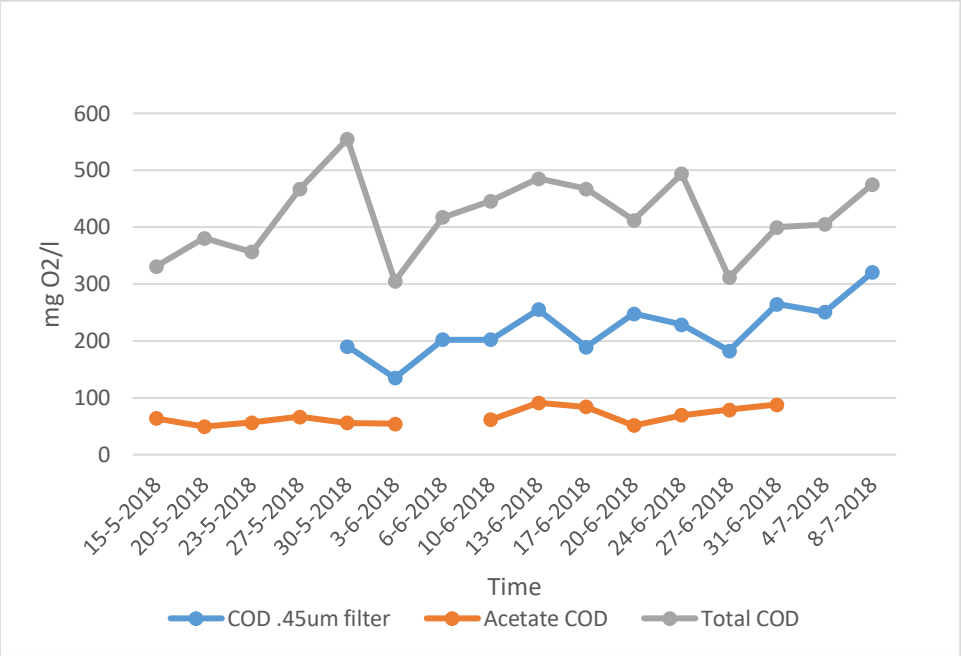

Figure 3.1.5 *"the concentrations of total, filtered and acetate COD in the influent over two months"*

Figure 3.1.6 shows the average COD data from the influent. The error bars are based on the standard deviation. The data deviates more than the other concentrations.

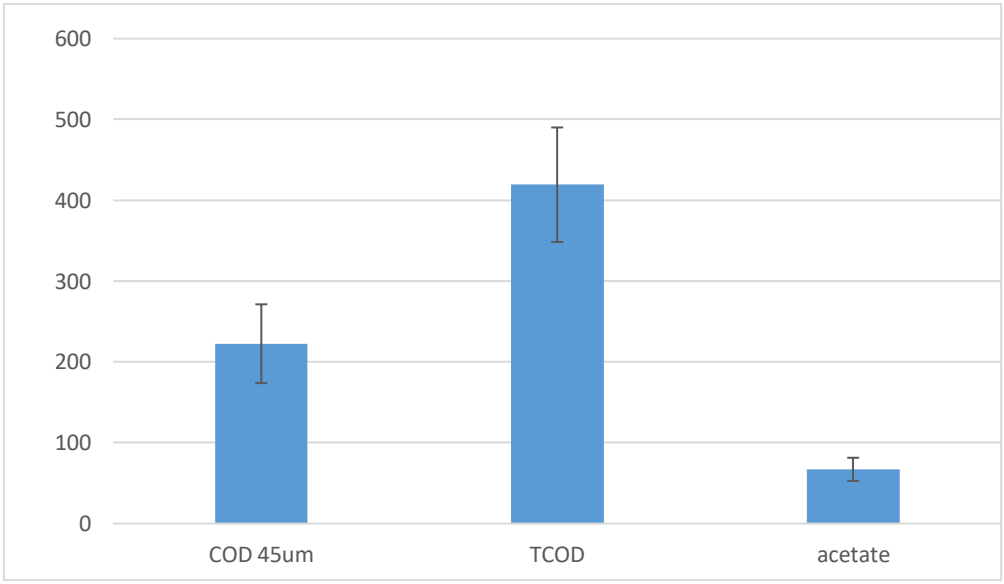

Figure 3.1.6 "The average concentration of filtered, total and acetate COD in the influent"

3.2 Sampling

Sampling is a big part of this research and it's important to make sure that the sampling doesn't affect the results. There are two main risks in sampling. The first risk is the sampling of the average influent. This sampling is done by a vacuum pump, this can evaporate the VFA's out of the liquid. To see if this happened influent samples are taken with and without the vacuum pump and are

compared to each other. The results showed that 5% of the acetate was lost due to the vacuum sampling.

The second risk is the filtering of the samples with the .45µm. Often glycerol is used to smoothen the filters. But glycerol has a significant COD value, some test where performed to find out how much COD is left in the filters. The results show that 10mgCOD/l is due to this problem. The data are corrected for this.

3.3 Compartment mixing time

For the design of the reactor it's very important to know if the tanks are well mixed. The mixing time needs to be an order of magnitude faster than the transfer of oxygen into the liquid. This means the mixing time has to be around 60 seconds. Figure 3.3.1 shows that after adding NaCl the conductivity went up and stayed stable after only 90 seconds. If this time is compared to the total time of this reactor (+-3600 seconds) this is well enough. Al the tanks were measured and all the mixing times were <120seconds.

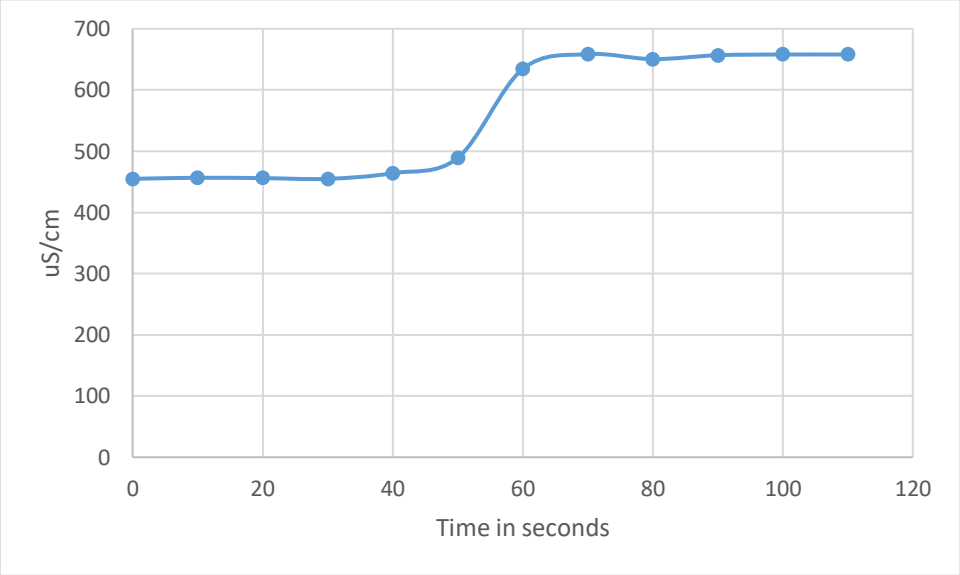

Figure 3.3.1 "one of the results of the mixing time experiments."

3.4 Residence time distribution

The residence time distribution is the time it takes for a particle to run through the pilot, the peak of the curve gives you the average retention time, the broader the curve the more backwash. It is important to check whether the pilot has more or less the same curve as an ideal CSTR in series. The data is shown in Figure 3.4.1 and it shows that the RTD is as expected, no significant short cuts or

delays and well distributed.

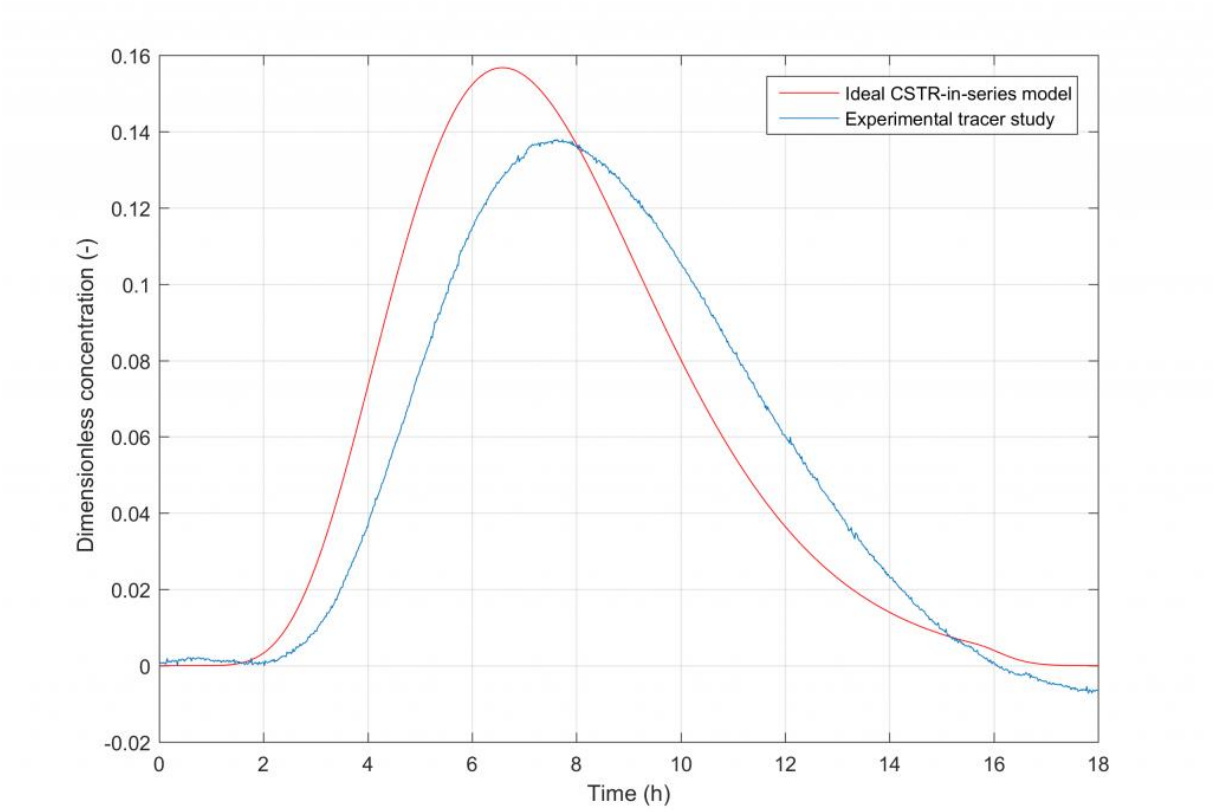

Figure 3.4.1 *"the results of the retention time experiment and the model"*

3.5 Ammonium absorption

It's possible that ammonium absorbs to the sludge. It then 'hides' from the measurements, to check whether this is the case. It is important to check this because the ammonium concentration at the end of the anaerobic zone can be used to calculate influent concentrations. It was calculated that 0,75-1 out of the 50 mgNH4-N/l was absorped. This is within the error margins of the tests performed.

3.6 Hydrolysis of N-containing organics

When organic material such as aminoacids break down, ammonia levels will rise. To see whether this happens, the ammonium levels were measured every week. Figure 3.6.1 shows the ammonium concentration of the anaerobic zone on the 15th of June. This data is also representable for other weeks. It shows that the concentration of ammonium stays the same. This means no significant breakdown of N-containing organics occurs in the anaerobic time.

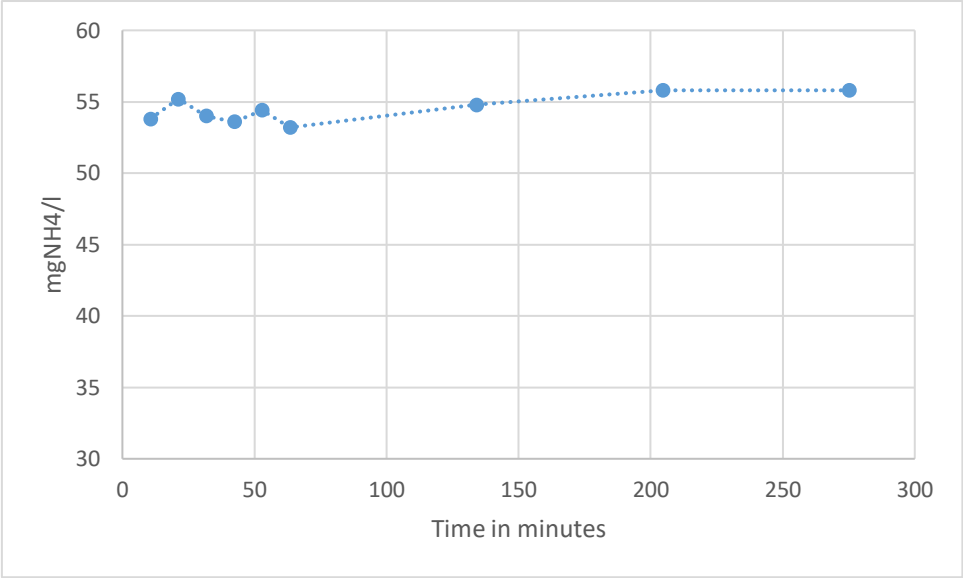

Figure 3.6.1 “the ammonium concentration in the anaerobic zone of the pilot on the 15th of June 2018”

The first period (30th of April → 29th of May)

The first period of the reactor started with the inoculation of the return sludge of the Harnaschpolder WWTP into the pilot. The following section shows the results of the measurements that resulted in the restart of the pilot.

Process parameters:
- Total sludge load 0,34 kg COD/kg TSS/d total time
- Anaerobic sludge load 2,5 kg COD/kg TSS/d anaerobic time average
- Aerobic sludge load 0,4 kg COD/kg TSS/d aerobic average
- DO-setpoint aerobic: 0.5 mg O_2/l
- Total anaerobic volume 0,9-1m^3
- Average SRT 5 days

3.7 Increasing SVI and COD-overshoot

After the inoculation of the reactor the SVI went up every week, as is shown in Figure 3.7.1. Of course the goal is to lower the SVI. This meant that there was a problem in the reactor that has to be solved. After the first HPLC results came back, the problem was found

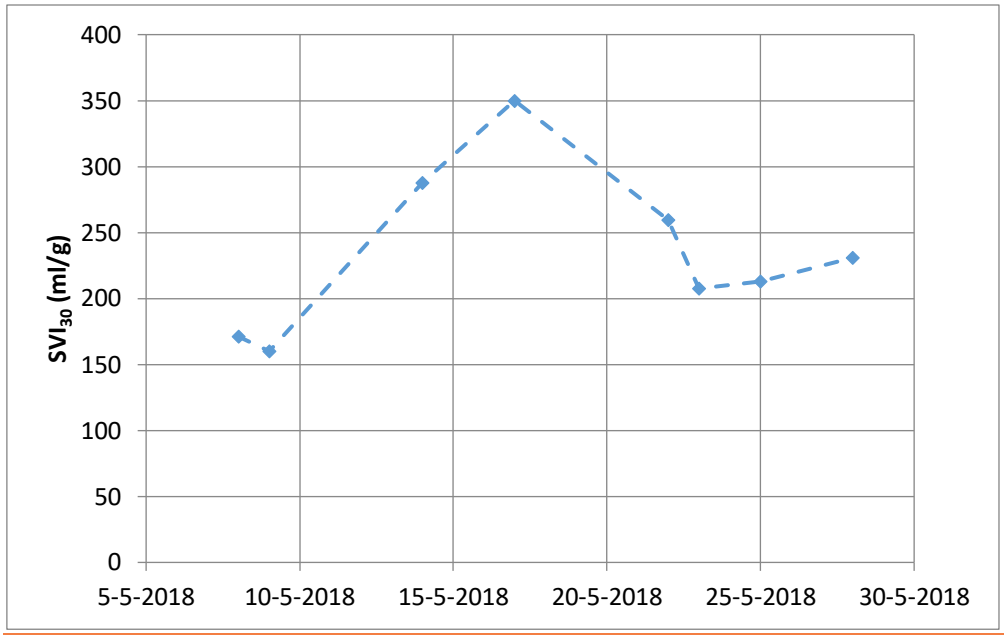

Figure 3.7.1 *"SVI measurements over the first period"*

Figure 3.7.2 shows the concentration of acetate and phosphate over time in the anaerobic zone. Almost half of the acetate goes through the anaerobic zone and into the aerobic zone. As a consequence filamentous organisms bloom because they outcompete slow growing organisms like bio-p.

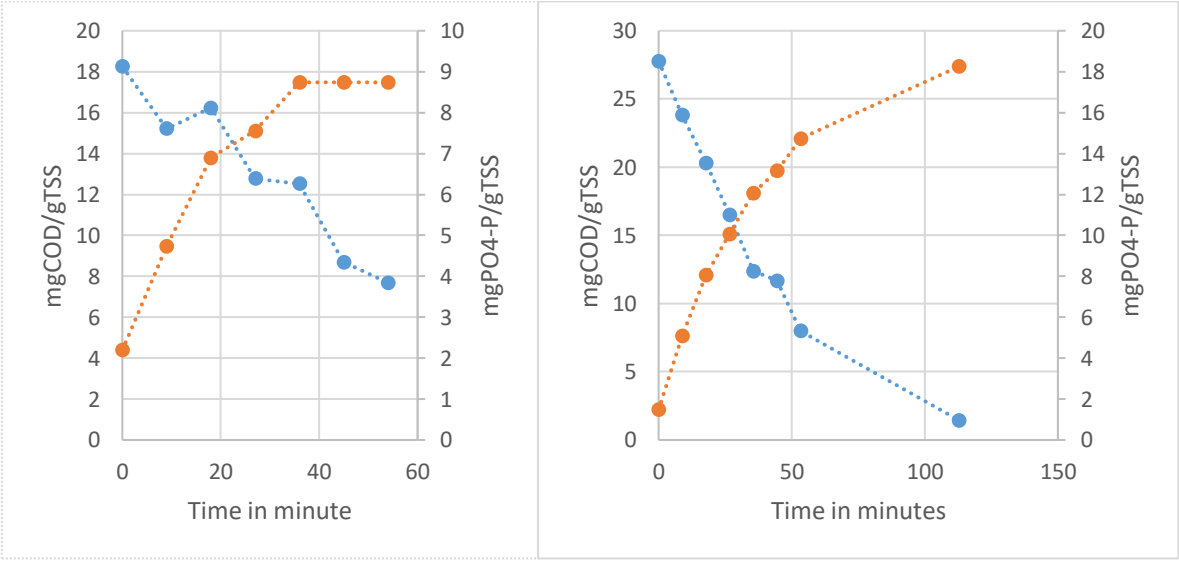

Figure 3.7.2 "on the left the concentration of COD and phosphate corrected for the dry weight in the pilot on the 16th of May, on the right the same but then for 29th of May."

When the anaerobic time was elongated with 60 minutes on the 29th of May the acetate was almost depleted, however the phosphate concentration still increased. The anaerobic sludgeload is way to high, it was concluded that the best option to solve this problem is to re-inoculate the reactor and add another 120 minutes of anaerobic time. Image 3.7.1 shows a microscope picture of the sludge on the 14th of May, a little over two weeks after starting the pilot. It's clearly visible that there are a lot of filamentous organisms present.

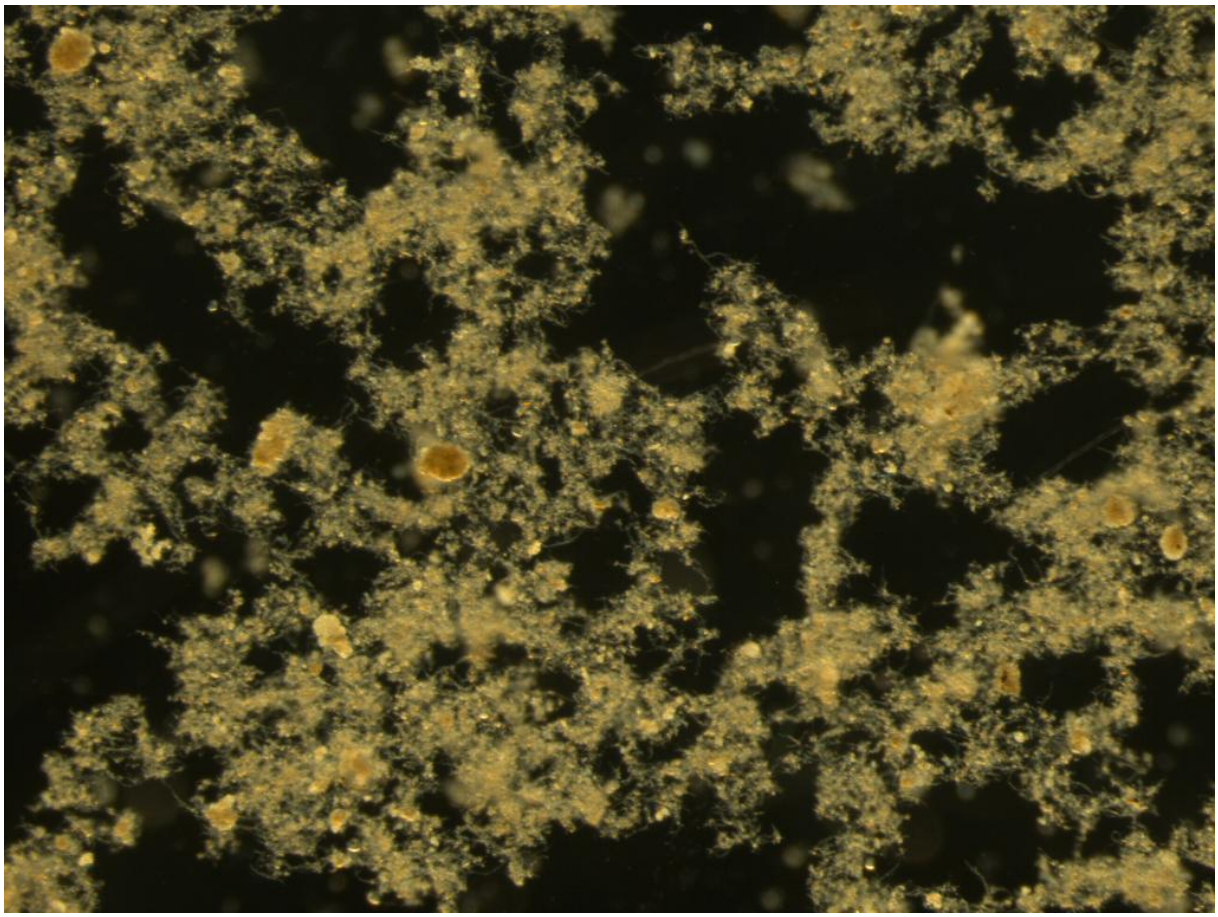

Image 3.7.1 “a picture of the sludge on the 14th of May, filamentous organisms are clearly present”

Second period (1st of June → 28th of June)

The second period of the reactor started with the re-inoculation of the return sludge of the Harnaschpolder WWTP into the pilot. And it ended with the last measurement on the 28th of June.

Process parameters:
- Total sludge load 0,22 kg COD/kg TSS/d total time
- Anaerobic sludge load 0,4 kg COD/kg TSS/d anaerobic time average
- Aerobic sludge load 0,52 kg COD/kg TSS/d aerobic average
- DO-setpoint aerobic: 0.5 mg O_2/l
- Total anaerobic volume 3,95 m^3
- Average SRT 8 days

Anaerobic

3.8 COD uptake

The COD uptake during the stable period can be seen in Figure 3.8.1. The initial concentration (t=0) is based on the rate of the first 4 data points. The slope of these data points determent the t=0 concentration of COD.

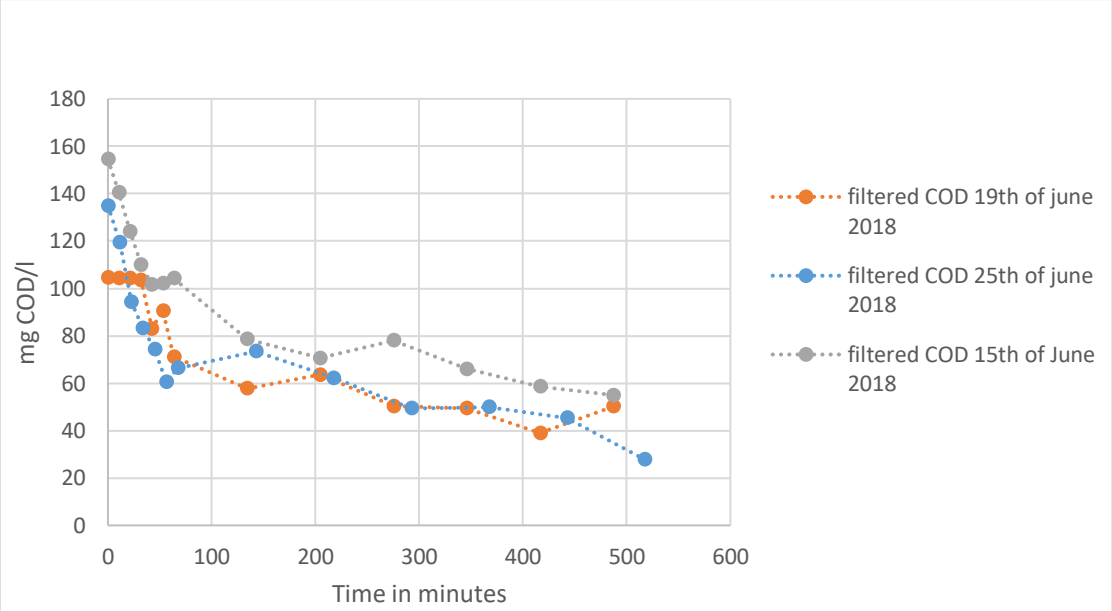

Figure 3.8.1 "the filtered COD concentrations in the pilot after the 300 minutes the aerobic zone starts"

Figure 3.8.2 shows that the max COD uptake quadruples during the second period. This means that the sludge can take up more COD during the anaerobic period. This means that the sludge is more enriched for organisms that use storage polymers, examples of these kind of organisms are bio-p and glycogen accumulating organisms (GAO's). Figure 3.8.3 shows that between 40 and 65% of the filtered COD is used as a storage polymer. The amount of inert COD is calculated by deviding the fraction left after the aerated zone (t=500+) by the COD concentration entering the reactor. The results show that 15 to 25% of the filtered COD is inert.

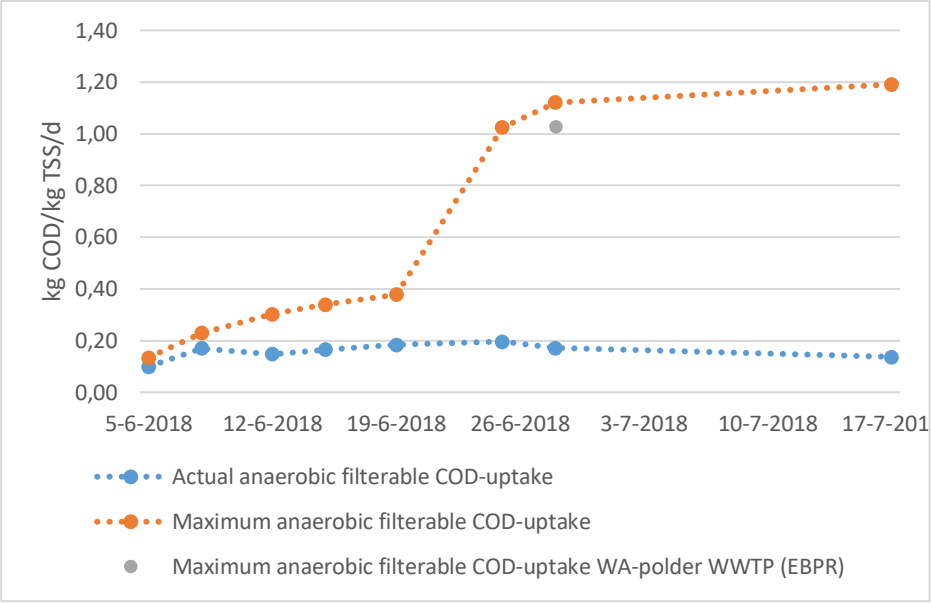

Figure 3.8.2 "the COD uptake, maximal uptake and Willem Anna polder uptake over time"

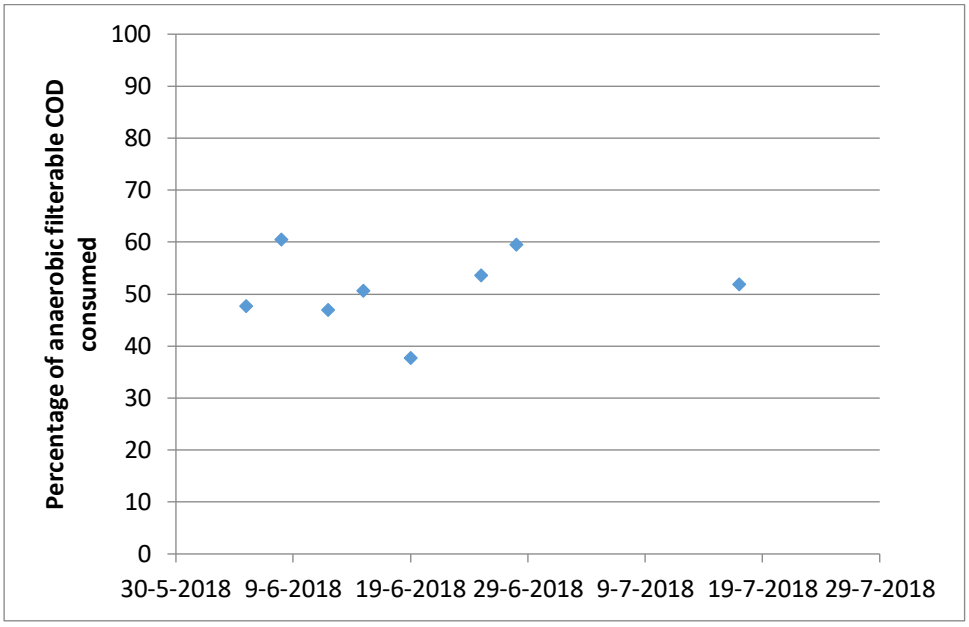

Figure 3.8.3 – Percentage of filterable COD stored anaerobically (including non-biodegradable fraction) over time

3.10 Volatile fatty acids (VFA's)

VFA's are the easiest carbohydrates to burn for microorganisms, they are often at the beginning of metabolic pathways. To see which kind of VFA's are important in the reactor, HPLC was performed on the reactor. Figure 3.10.1 shows the uptake of 5 kinds of VFA's and its total in COD/TSS.

It turns out that acetate is the biggest contributor to the COD concentration. Another interesting fact is that it's the only VFA that has a significant uptake rate in the anaerobic zone. This suggest that only acetate is used as a substrate for the release of phosphate. The second most

significant VFA is iso-valerate. There is no big significant decrease in valerate until the acetate concentration drops. Eventually iso-valerate will be completely oxidated in the aerobic zone. The VFA's propionate, butyrate and formate aren't significant in this data.

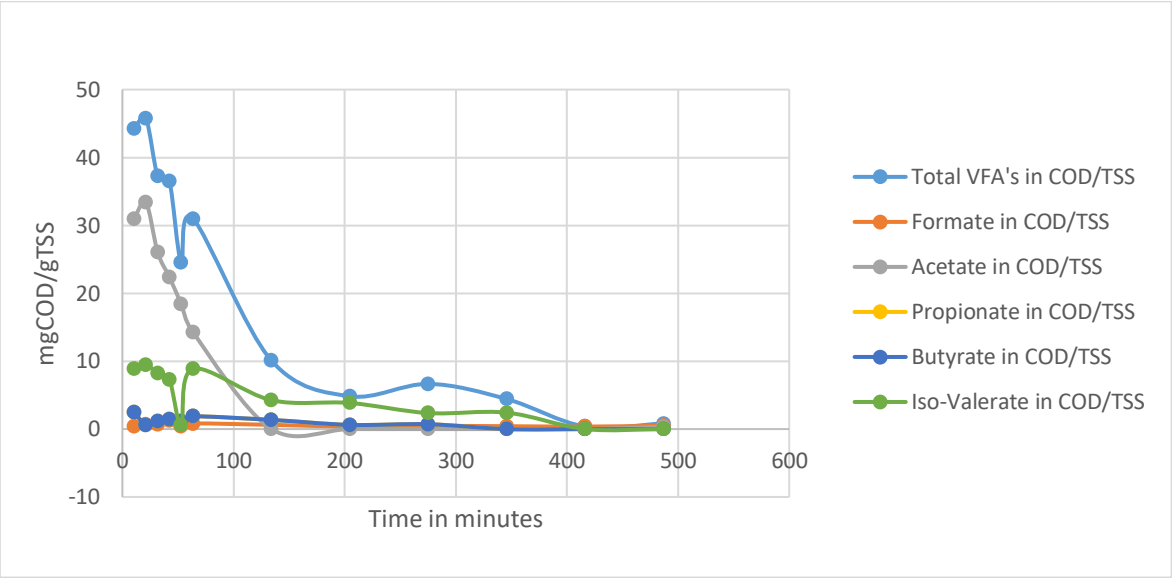

Figure 3.10.1 "the VFA's in the HARKOS pilot corrected for the dry weight on the 19th of June"

3.9 Acetate uptake

Acetate is used by the PAO's as a carbon and energy source.

The acetate uptake during the stable period can be found in Figure 3.9.1. The acetate is shown in COD value. The initial concentration (t=0) is based on the rate of the first 4 data points. The slope of these data points determent the t=0 concentration of COD. The data shows that there is no overshoot of acetate into the aerated tanks.

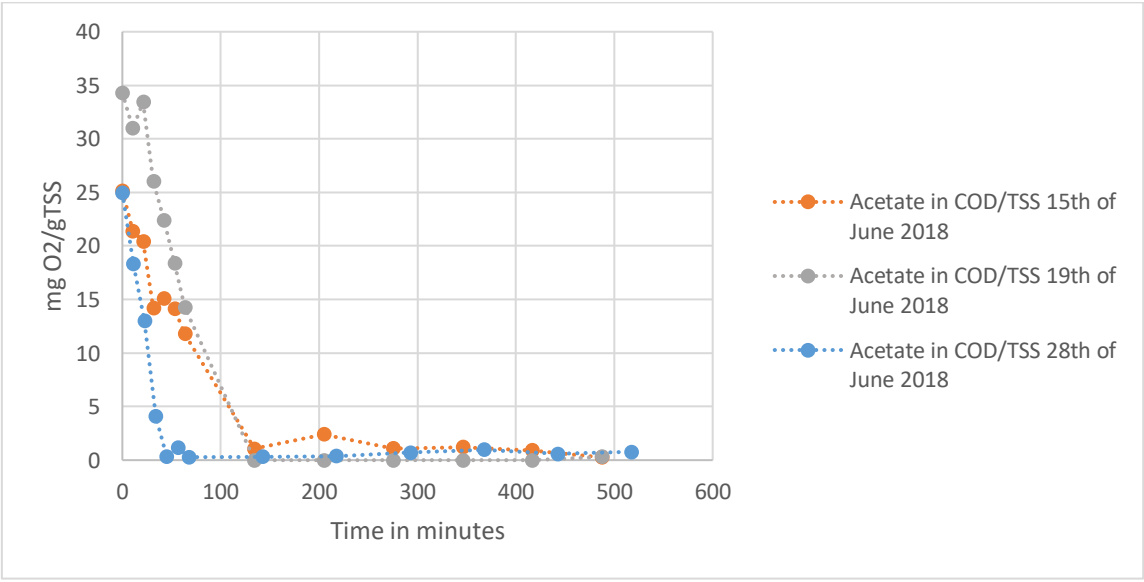

Figure 3.9.1 "Acetate concentrations in COD corrected with the dry weight in the pilot of several weeks, the anaerobic tanks stops at data point 9"

3.11 Phosphate release

The phosphate release is an important parameter for the performance of the reactor. It gives insides in how much the reactor is enriched for the bio-p metabolism. These are eventually the micro-organisms that start the granulation process.

Figure 3.11.1 shows the phosphate release over the reactor on four moments in the second period. It shows that the amount of phosphate release and its rates are more or less similar. The 19th of June has a higher maximum and the phosphate release rate of the 15th is slightly higher.

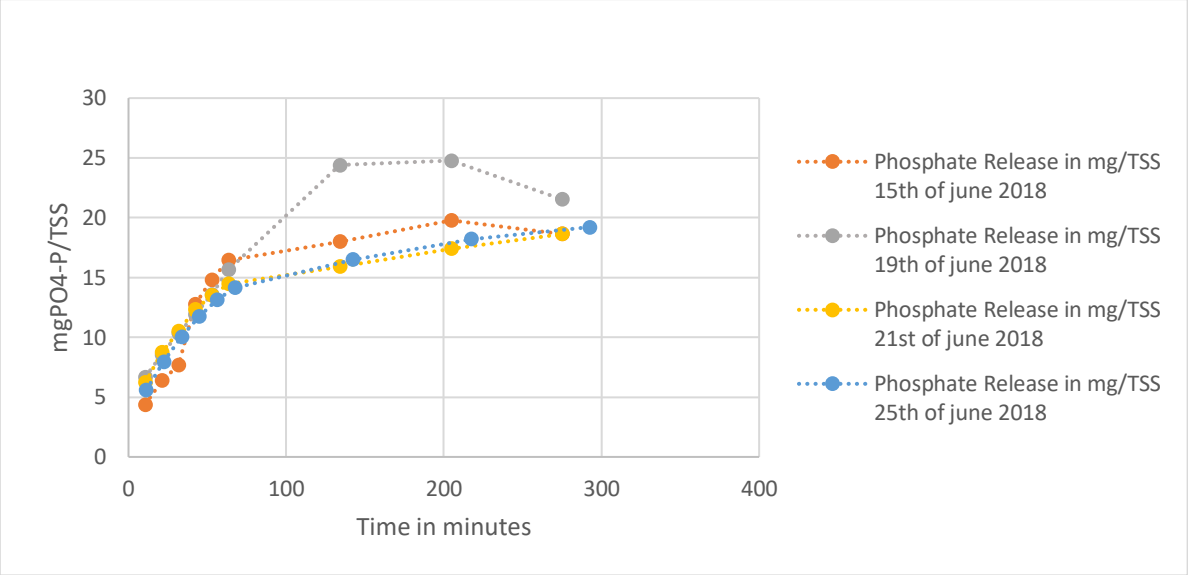

Figure 3.11.1 *"The phosphate release over two weeks in the second period corrected for the dry weight concentration"*

Figure 3.11.2 shows that the phosphate release rates initially significantly lower than the phosphate release rate of the Willem Anna polder. However the upward trend shows that phosphate release rate of the pilot approaches the rate of the Willem-Anna polder.

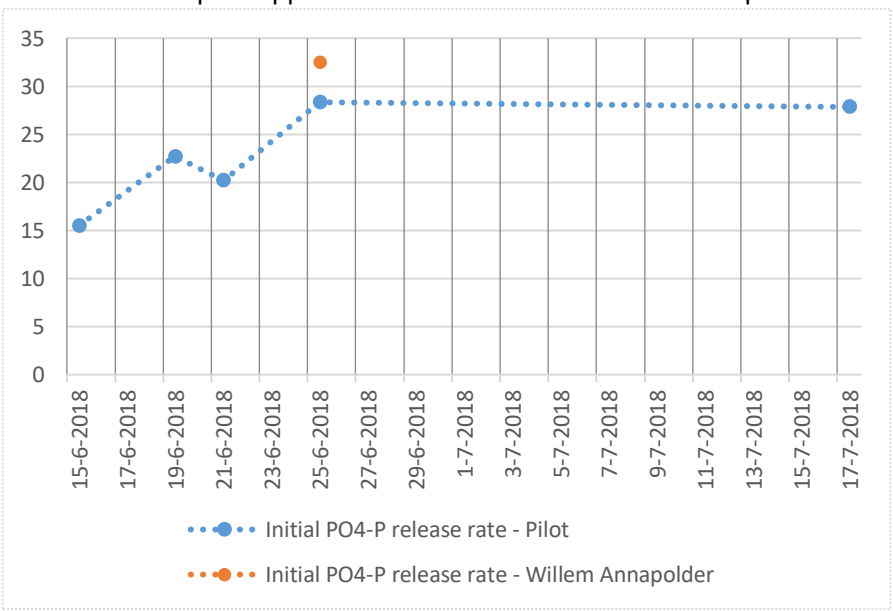

Figure 3.11.2 *"the phosphate release rate over a ten day period"*

3.12 Phosphate release over COD uptake ratio

The phosphate/COD ratio is an important parameter in a published paper of Cañizares et al., it is found that bio-p organisms have a phosphate/COD ratio of 0,55. The data in Figure 3.12.1 shows that this is exactly the case. This is proof that most of the COD is stored as a storage polymer.

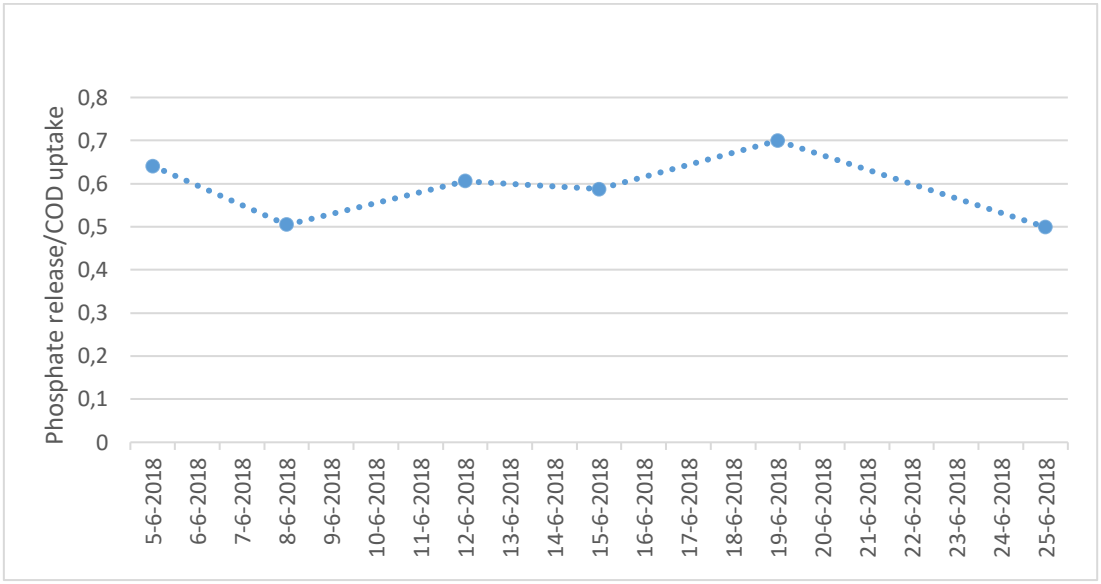

Figure 3.12.1 *"phosphate uptake over filtered COD ratio over time"*

3.13 Total phosphorous release batch test

A batch test was performed to find the maximum phosphate release and the maintenance rate. The batch test shows that the maximum phosphate release on the 20th of June is 6mg/gTSS. Another remarkable result is that the acetate is still being consumed while the phosphate remains stable.

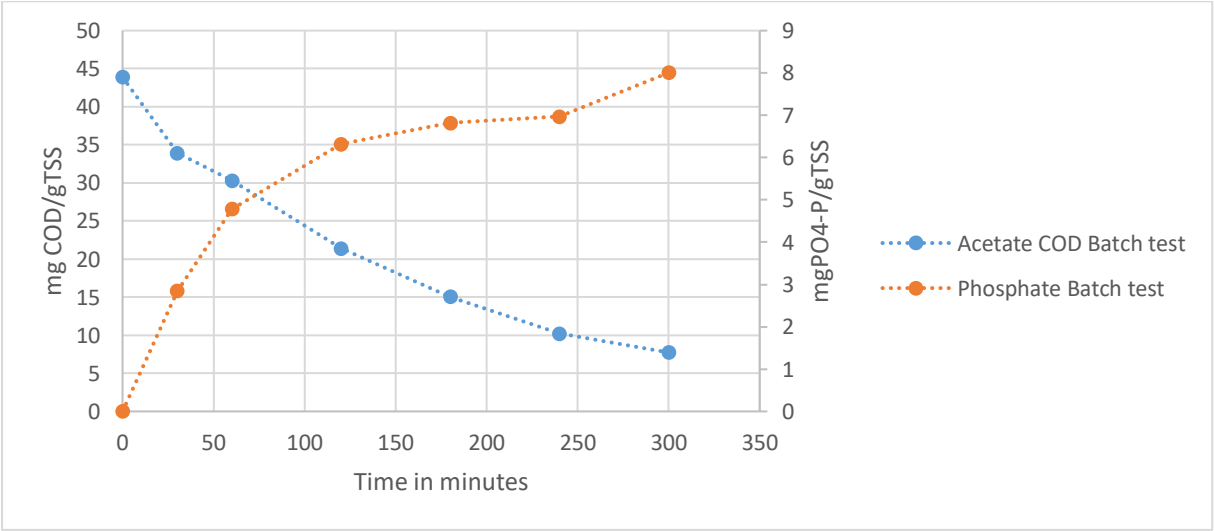

Figure 3.13.1 "*batch test performed on the 20th of June. The PO4-P and acetate concentrations are shown"*

Figure 3.13.1 shows data from a failed batchtest attempt on the 27th of june. But because the acetate stays stable the maintenance rate could be calculated. The maintenance rate is 0,738 mgPO4-P/gTSS/hour.

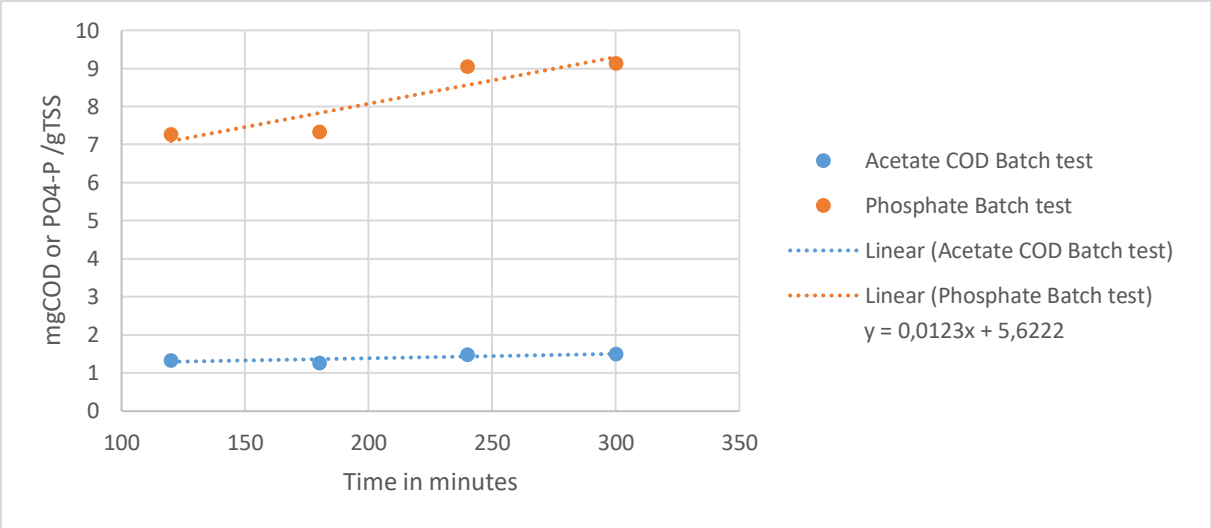

Figure 3.13.1 "Part of the failed batch test performed on the 27th of June"

3.14 Control test

The control test is designed to see whether the reactor behaves the same as an ideal 'batch' version of the reactor. If they have big differences, than something is wrong. Figure 3.14.1 show the COD and the phosphate concentration over time, the control (orange) is compared to the pilot (blue). The results show that the rates are similar.

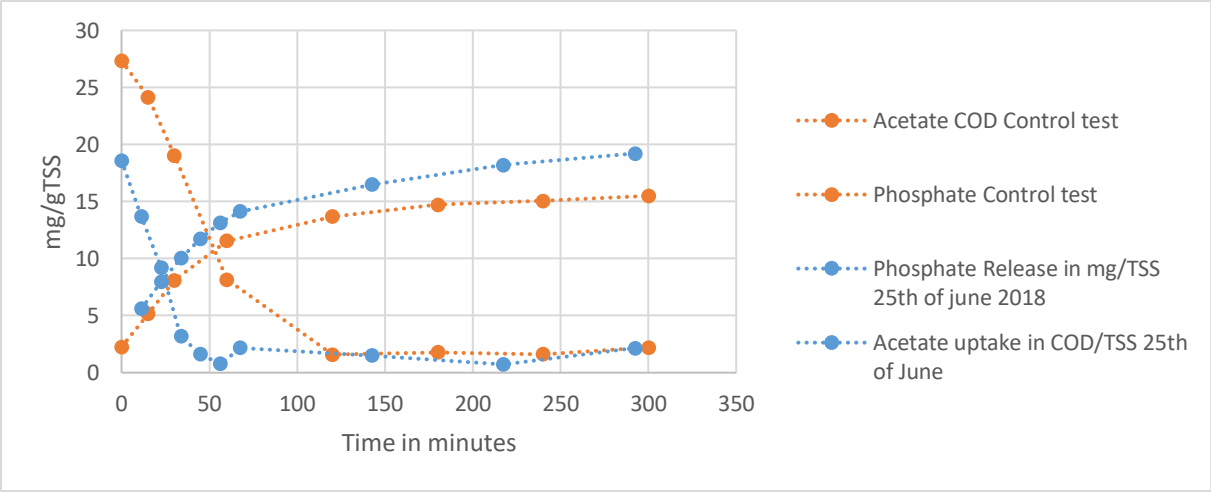

Figure 3.14.1 *"Control batch test performed on the 27th of June"*

Aerobic

3.15 Phosphate Uptake

When the anaerobic time is followed by the aerated time, bio-p organisms start to store phosphate as a polymer. This is called the feast regime. The rate in which phosphate is taken up by these organisms is determined by the amount of bio-p organisms. The phosphate uptake rate is therefore a parameter for the enrichment of the sludge for bio-p organisms.

Figure 3.15.1 shows the results of the phosphate measurements. The rates increase gently from 11,3 to 13,2 mgPO4-P/gTss.

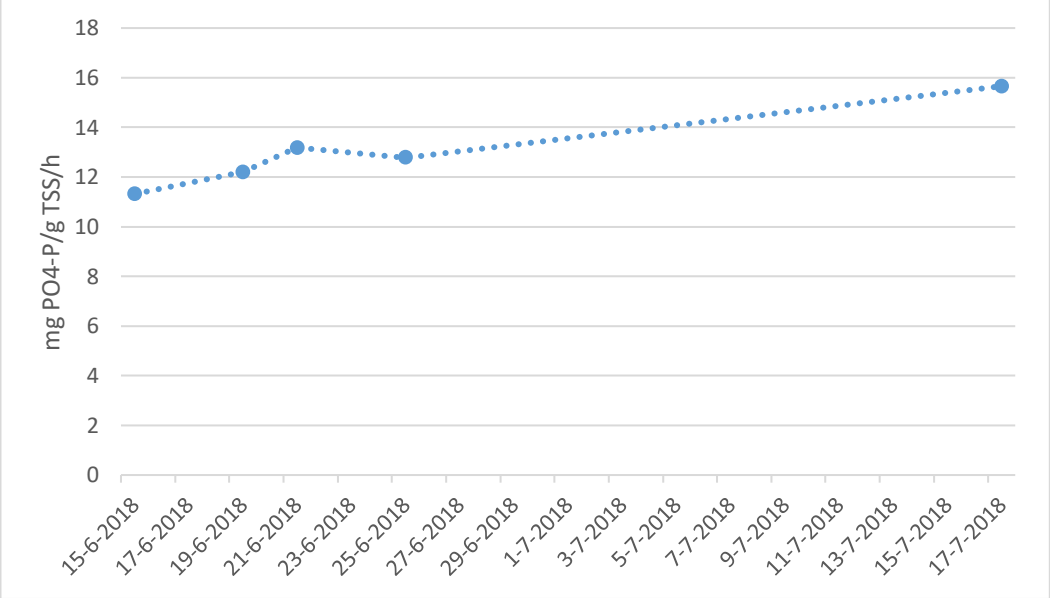

Figure 3.15.1 “Phosphate uptake rates in the second period”

3.16 SVI measurements

Figure 3.16.1 shows the SVI measurments of the second period, it shows a downward trend. This is proof that no filamentous organism growth takes place inside the reactor.

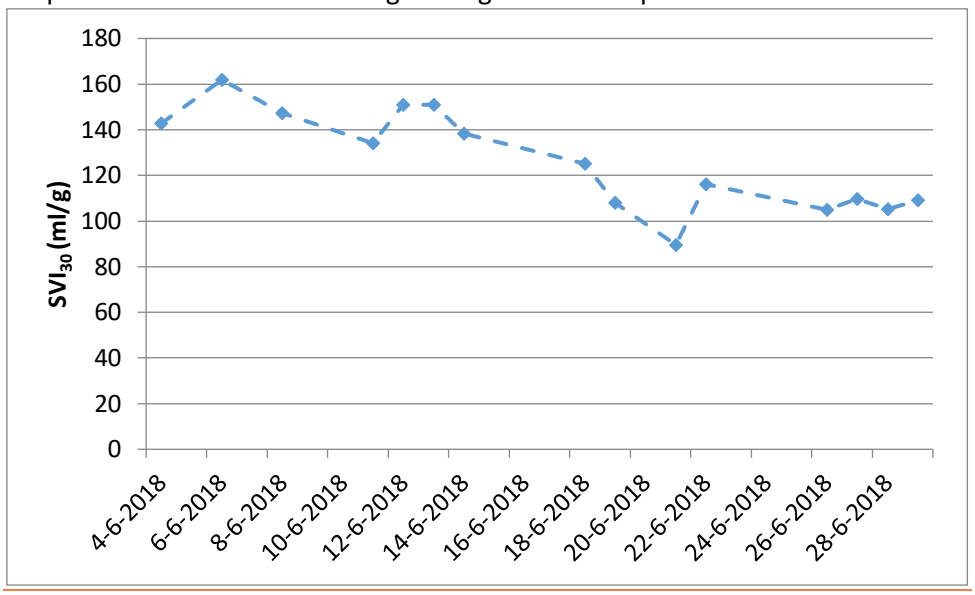

Figure 3.16.1 ”SVI measurement over the second period”

§4 Discussion

4.1 Reactor characterization

The total COD values have a rather high standard deviation this is due to the fact that there are a lot of big solid particles floating around in the influent. These solid particles have a big impact on the COD concentration.

The ratio between ammonium and Kjeldahl nitrogen drops while it's suspected to rise because of the rising temperatures in the sewage. The concentration of Kjeldahl nitrogen can be affected by the amount digestate that is returned to the waterline via the digested sludge centrifuges. All the acetate data was corrected for the loss of acetate due to vacuum pump sampling method. The COD values were corrected for the amount of COD inside the .45µm filters if necessary.

The mixing time was short enough for the tanks to be called well mixed. The retention time of the whole pilot showed great resemblance with the theoretical model though the there was some degree of dispersion. The results showed that no significant hydrolysis of N containing organic compounds is present in the pilot, the ammonium concentration during the anaerobic time didn't change. And that no significant amount of ammonium is absorbed by the sludge.

After the reactor characterization the different pillars of granulation are discussed.

4.2 Prevention of COD overshoot

During the first period the SVI went from 150 to 250ml/g. This almost a doubling of the SVI of the Harnaschpolder WWTP. Results from the HPLC showed that there was 8 mg/l of acetate left at the end of the anaerobic zone. This failure led to a bloom of filamentous organisms. The morphology of these organisms caused an increase in the drag of sludge flocs which caused the SVI to rise.

A solution to this problem is to elongate the amount of anaerobic time by converting the 7th tank of the reactor from aerobic to anaerobic. This will lower the anaerobic sludge load that was 2,5 kgCOD/kgTSS/d. While the anaerobic sludge load of the PNU was around 0,8 (Haaksman personal communication). The results showed that even extra time was essential. That's why 2 additional tanks with a total time of around 120 minutes were converted from aerobic to anaerobic. This is the only way to drastically lower the sludge load because the influent pump is set on 500l an hour. Adding additional anaerobic time would eventually take care of the filamentous organisms, the sludge retention time (SRT) was 7-8 days. After 3 cycles most of the old sludge is gone. This means that it would take 21-24 days to get back to the original Harnaschpolder SVI. That's why the whole reactor was drained on the 30th of May and re-inoculated with Harnashpolder return sludge.

It was confirmed by the acetate measurements that no COD overshoot is present anymore. The VFA measurements showed that the most important VFA is acetate, around 80% of the COD value comes from acetate. The second biggest VFA in terms of COD is iso-valerate, however it's debatable if the iso-valerate is that significant. The COD factor of iso-valerate is 6,5 that's much higher than the acetate factor which is 2. This makes the measurement sensitive to proper integration of the peak in the HPLC spectrum The unstable baseline in the HPLC spectrum therefore hampers correct integration. Primary effluent contains a variety of other compounds, which decreases the signal to noise ratio. It can be concluded that acetate is the only VFA that is important to follow.

4.3 Anaerobe conversion of COD to storage polymers

One of the objectives then is to transform as much COD as possible to storage polymers. A great parameter for this is the COD uptake. The results show that this parameter frist grew from 0,13to 0,2 kgCOD/kgTSS/d and subsequently dropped back to 0,13 kgCOD/kgTSS/d. But if the concentration of COD rises than the uptake could rise with it. This could happen if the temperature rises. That's why the maximum COD uptake was calculated. The result shows that the maximum COD uptake grew 0,17 to 1,19 kgCOD/kgTSS/d. This means that the sludge is enriched for organisms that store COD in storage polymers. If the data is compared to the data from the Willem Anna polder. It shows that the maximum COD uptake is higher than the actual COD uptake from the Willem Anna polder. This shows that the sludge has great conversion capacity of COD into storage polymers. It was calculated that around 40 to 60 percent of the filterable COD was converted into storage polymers, no trend was visible.

The batch test showed that while the release of ortho-phosphate was finished, acetate continued to be taken up by microorganisms. This suggests that, next to bio-p activity, other metabolisms are also present. An example of such type of organisms are GAO's. Apparently there are also other readily biodegradable COD compounds in the influent that can be stored in polymers by releasing poly-phosphate. Therefore it's impossible to calculate the maximal phosphate release, with a batch test with only acetate as substrate.

4.4 Enrichment for bio-p organisms

The phosphate uptake rate mirrors the selection for bio-p organisms. This is a parameter to see how enriched the sludge is for these organisms The results show that the phosphate uptake rises from 11,3 to 12, 7mg PO4-P/gTSS/h in a time frame of ten days. The most recent measurement performed on the 18th of july showed that the phosphate uptake has risen even further to 16,9 mgPO4-P/hour/gTSS. If you compare this with EBPR systems you'll find that the phosphate uptake is only 2,8 mg PO4-P/gTSS/h (Brdanovich, 1998). These test were conducted in April 1998, this means the temperature was lower than it is now in the pilot, this can explain the big difference. Even the PNU falls short on the Pilot rate. The most recent PNU rate is 15,6mgPO4-P/hour/gTSS (Edward van Dijk, personal communication). The pilot out performes these two systems mainly because it's now focused on phosphate removal and not nitrogen removal, this means that in the pilot all the focus can be put on the enrichment of bio-p organisms. This data shows that the pilot succeeded in this step.

The paper (Cañizares et al) shows that for highly enriched EBPR systems the ratio between the phosphate release and the filtered COD has a value of 0,55. The results in section 3.12 show ratios that surround this number.

4.5 Compartmentalization and degree of plug-flow

The plug-flow control batch test showed that the current compartmentalization is good enough to overcome overshoot problems. Since it was configured according to the most used EBPR-process configurations (modified UCT and 3-stage Phoredox). Compartmentalization has another function in the formation and stabilization of granules, since it determines the diffusion penetration depth of compounds into the granule and therefore determines how big the granules can get. (Phillip Stewart, 2003) The similarity between the uptake rates of acetate in the control as in the pilot shows that this

rate isn’t effected by the concentration of acetate, this was also found by student Wladimir Rios, 2018.

§5 Conclusion

In this study, two separate subjects were examined. The Reactor characterization of the Harkos pilot and the pillars of granulation (conversion wise).

The influent was characterized during a time frame of two months. No major deviating values were found. Two risks were identified in the sampling and data was corrected for this. The tanks turned out to be well mixed and the retention time had some dispersion compared to the model but did not deviate drastically. No ammonium adsorption was present and no hydrolysis of nitrogen containing organic compounds took place in the anaerobic time, this means it's possible to use the data in the last anaerobic tank to recompute the influent concentrations.

In the second period no more COD overshoot was present inside the HARKOS pilot thanks to decreased anaerobic sludge load. This was concluded by the acetate measurements and the control batch test. The maximal COD uptake rises during the second period and eventually outperformed the COD uptake from the Willem Anna polder. This showed that the HARKOS pilot is selecting for organisms that use storage polymers and that it has enrichment compared to other systems that already have granulation. The phosphate uptake outperformed a regular EBPR system and was similar to the PNU system. This concludes that the system is enriched for bio-p organisms. The compartmentalization of the anaerobic zone is good enough to prevent COD overshoot and it also shows great resemblance with the control batch test. This means that the difference in the maximum driving force for diffusioninto a biofilm is marginal compared to a perfect-plug flow.

In the end, it was concluded that the reactor is well characterized and that the goals of the four pillars of granulation are met:

- Prevention of COD overshoot
- Anaerobe conversion of COD to storage polymers
- Enrichment for bio-p organisms
- Compartmentalization and degree of plug-flow

The HARKOSpilot biological selective pressure is well performed and a success.

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§6 Outlook

Now that the conversions in the HARKOS pilot are granulation ready one should focus on the morphology side. This means putting selective pressure on the morphology side. Waste the bad settling sludge and elongate the sludge retention time for well settling particles. A study of different types of selective pressures could be interesting.

When granulation is achieved one could look at other aspects of waste water treatment. Like the removal of nitrogen. The failed batch tests makes it interesting to install a better controlled lab scale reactor at the pilot to see how real waste water influent influences certain experiments, while maintaining the direct control lab scale offers.

Another interesting topic that this study didn't go into is the hydrolysis of COD inside the anaerobic zone. Because of the simultaneous uptake and consumption this is hard to measure. A batch test experiment were all VFA's are removed from the influent could give answers to the COD hydrolysis in the anaerobic zone. It might be possible to use the phosphate uptake data from this study to find the hydrolysis rate.

§7 Acknowledgements

I would like to thank Viktor Haaksman for his support, enthusiasm and above all his incredible knowledge about wastewater treatment. Furthermore I would like to thank Stephy Lin for helping me out with measurements and maintaining the HARKOS pilot.

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§9 Appendix

Table 9.1 “the order of sampling for the maximal phosphate release batchtest”

Sample	Time (min)
1	0
2	30
3	60
4	120
5	180
6	240
7	300

Table 9.2 “The order of sampling for the control batch test”

Sample	Time (min)
1	0
2	15
3	30
4	60
5	120
6	180
7	240
8	300

Table 9.3 “the order of sampling for the hydrolysis batch test”

Sample	Time (min)
1	0

2	15
3	30
4	60
5	120
6	180
7	240
8	300

Calculation ammonium absorprtion

Maximum COD uptake calculation

The maximum COD uptake was calculating according the following formula

$$MaxCODUptake = \frac{CODUptake}{F}$$

Where F is the following factor. The anaerobic time till the filtered COD uptake stagnates divided by the total anaerobic time. This is illustrated in Figure 9.1

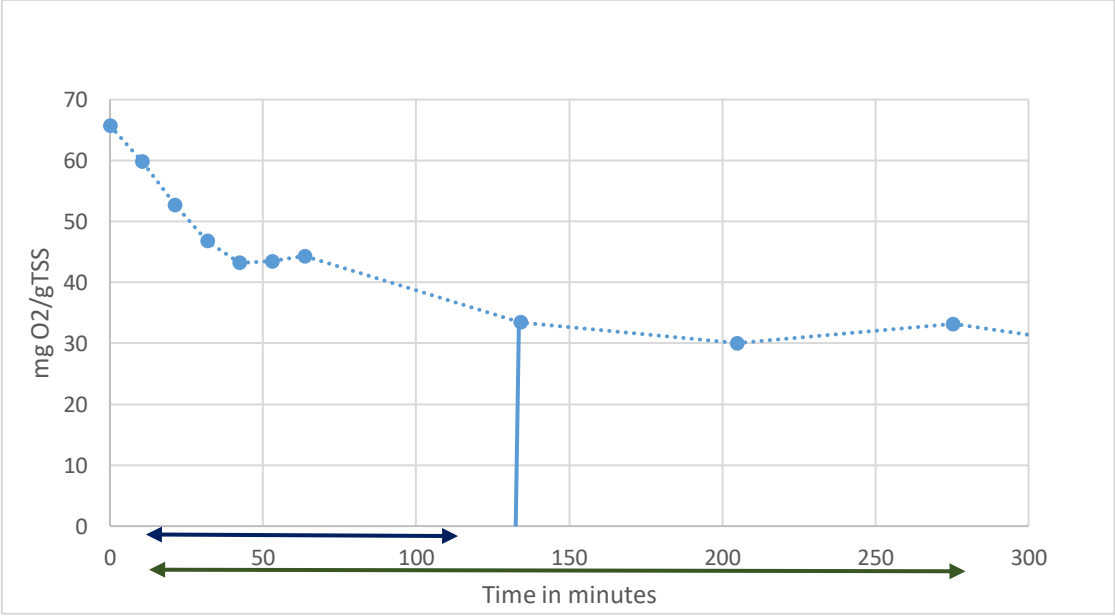

Figure 9.1 *"illustration how factor F is calculated"*

COD factor of VFA's calculation

The COD calculation are based on the following formula

$$COD\ factor = C + \frac{H}{4} - \frac{O}{2} - \frac{3}{4N}$$

	Formate	Acetate	Proprionate	Butyrate	Iso-Valerate	Valerate
C	1	2	3	4	5	5
H	2	4	6	8	10	10
O	2	2	2	2	2	2
COD factor	0,5	2	3,5	5	6,5	6,5
MW	46	60	74	88	102	102

Events

This section is used to describe the main events and incidents that had effect on the continuity of the reactor and/or had some other effects on the outcome of experiments. These events were logged in either the pilot Grafana® logging system or the writers lab journal.

Monday 30th of April 2018. “Start of the reactor”

The reactor was inoculated with 1500 liters of secondary return sludge from the Harnaschpolder WWTP. The SVI of this sludge was 146ml/gr. The Hybrid tanks were aerated and all aerated tanks were aerated with microbubbles.

Wednesday 2nd of May 2018 → Wednesday 30th of May. “Adding of antifoam”

During the first days a foam layer had appeared on tanks 7 to 9. This foam layer blocked the flow which caused flooding of tank 7. To tackle this problem antifoam was added to the system. The amount that was added is the same as what the operators of the Harnaschpolder WWTP uses in the sludge digester, which is 50 ppm. The effect was immediate and worked well. However the antifoam flushes out eventually this is why an anti-foam pump was used to add a continuous flow of anti-foam. This antifoam pump put in 4,3 ml of antifoam an hour. On the 30th of May we decided to stop the antifoam because it was building up. A significant amount would stay in the return sludge and comeback into the reactor.

Wednesday 30th of May. “rebooting/re-inoculating the reactor”

Due to a COD overshoot into the aerated tanks the growth of filamentous organisms was stimulated. This resulted in a terrible SVI of over 200. To fix this problem it was faster to re-inoculate the reactor with new sludge. By switching tank 7 to 9 to anaerobic tanks, the anaerobic time was enlarged and the COD overshoot was tackled.

Wednesday 6th of June. “Harnaschpolder blackout”

Due to maintenance on the Harnaschpolder WWTP there was no power, this meant all the flows and recording stopped. During this time the clarifiers were modified, now both clarifiers could be used.

Thursday 7th of June. “Sludge wasted and influent supply stopped”

Due to an exploding return sludge pipe a considerable amount of sludge was lost. The influent pump was stopped for 14 hours due to an HH alarm.

Sunday 17th of June. “Sludge wasted”

A significant amount of sludge was lost due to a spill in tank 10.

Monday 1st of July. “Adding of antifoam”

Excessive Foam in tank 10 resulted in a spill, to tackle the foam 5,5ml of antifoam was added to tank 10.

Monday 2nd of July. “Sludge wasted”

Clarifier couldn’t handle sludge load a considerable amount of sludge was wasted through the effluent.

Friday 6th of July. “Sludge wasted”

Sludge was wasted due to human mistakes.