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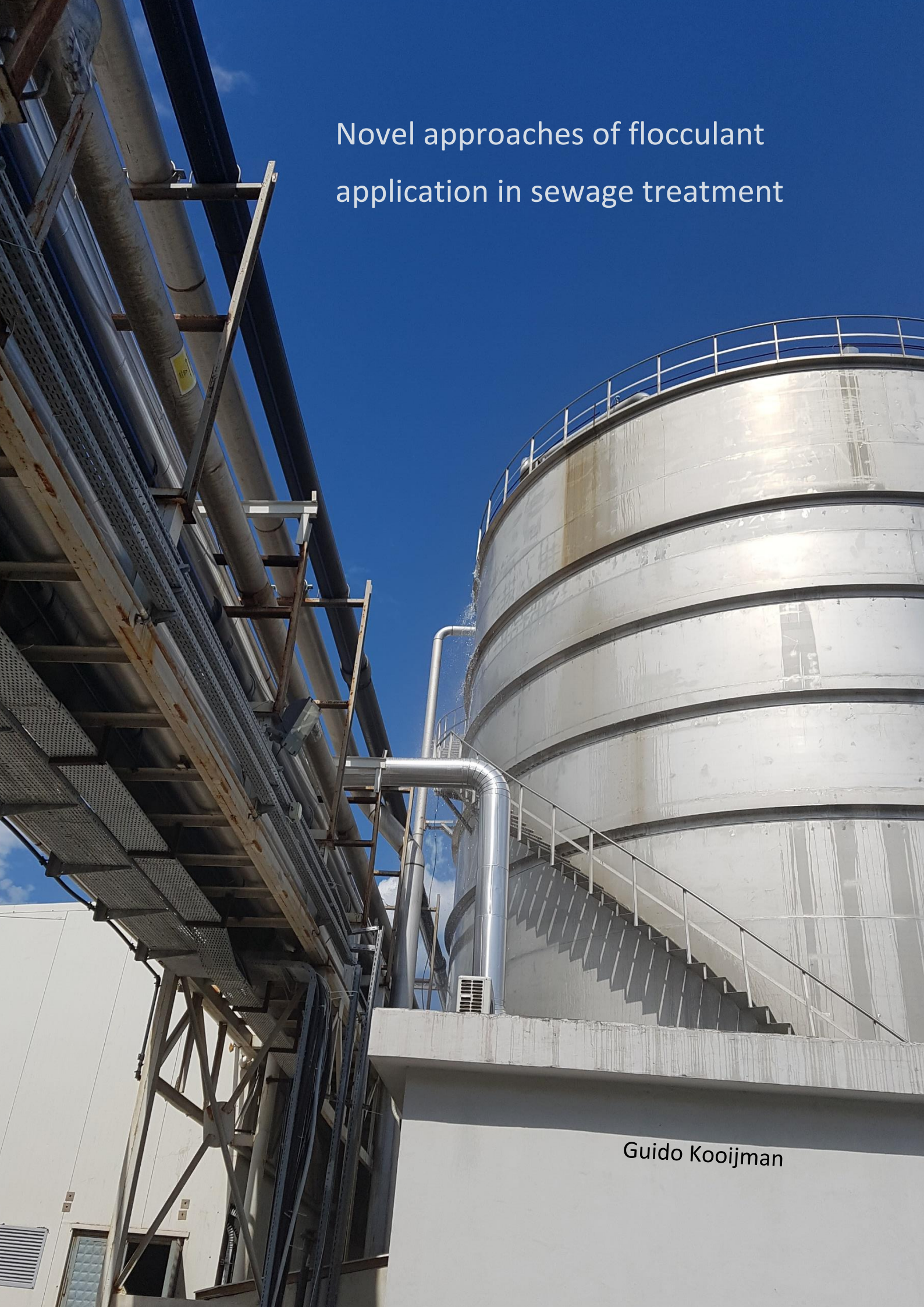

Guido Kooijman

Novel approaches of flocculant application in sewage treatment

Dissertation

for the purpose of obtaining the degree of doctor

at Delft University of Technology

by the authority of the Rector Magnificus, Prof.dr.ir. T.H.J.J. van der Hagen

chair of the Board for Doctorates

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by

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Contents

Abstract

Organic flocculants are typically only applied in the sludge line and sometimes in quaternary treatment of conventional sewage treatment plants (STPs) that aim for enhanced nutrient removal. However, with the ongoing societal changes directing towards a higher degree of circularity of resources and a higher degree of wastewater treatment demands, there is a need to re-asses the potentials that flocculants may offer in new wastewater treatment concepts. Our work investigated new possible applications of flocculants in an STP.

Applying flocculants for chemically enhanced primary treatment (CEPT) increases the primary sludge production for biogas production, which may lead to a more positive energy balance of an STP. Results showed that 66% more influent COD could be used for biogas production via anaerobic digestion (AD), meanwhile the aerobic oxidation of this COD in the aeration tanks was prevented. However, removing the COD in CEPT with cationic flocculants led to a COD/N ratio of 3.75 g COD/g N in the water line, which is lower than the minimum ratio that is required for a conventional biological nutrient removal STP (BNR-STP). However, recently, novel N removal technologies have been introduced that function well at low COD/N ratios, such as N removal over nitrite, or that do not need any COD at all, such as the Anammox process in the waterline of an STP. With the application of these novel N removal techniques, CEPT with flocculants could be advantageous for the overall energy balance and space requirements of the future STP. Besides the STP energy balance, the application of cationic flocculants for CEPT also impacted the AD: the additional COD that was removed by CEPT was more readily biodegradable, leading to a 9% higher biomethane potential of the primary sludge. Also, in separate batch tests, it was found that flocculants decreased the viscosity of the sludge and, concomitantly, an increase in the hydrolysis rate up to 27% was observed. However, in contrast to the rate of digestion, the results showed that refractory polyacryl amide (PAM) flocculants, irreversibly bound the particles, and thus partially reduced the biomethane potential.

Besides the energy aspects of an STP, also there are increasing challenges in the treatment of micro pollutants. STP effluents are one of the main sources of pharmaceuticals in the environment. Literature reports that a large part of the pharmaceuticals in an aquatic matrix, such as present in an STP, are sorbed to colloids. Since flocculation can remove colloids, flocculants in principle could be used to concentrate pharmaceuticals into a smaller sludge flow that subsequently could be treated more efficiently. The possibility of concentrating pharmaceuticals by flocculation in the primary settler was investigated. A jar test showed that pharmaceuticals were hardly removed from sewage with coagulation/flocculation. To investigate the discrepancy between reported colloidal sorption and the lack of removal when removing colloids, we tested a commonly applied experimental setup for determining the colloidal sorption of pharmaceuticals. Colloids were removed from a solution containing pharmaceuticals in two ways: by ultra-filtration (UF) and by flocculation. Both methods showed similar removal of colloids. However, during UF the observed retention of pharmaceutical was 93±4%. In contrast, when removing the colloids with flocculation, no pharmaceuticals removal was observed. These results strongly indicate that an analysis bias is introduced when using UF membranes in the determination

of colloidal sorption of pharmaceuticals. Very likely, a direct retention of pharmaceuticals on the UF membrane occurred. Overall results of current work showed that pharmaceuticals hardly sorb to colloids and herewith the absence of removal of pharmaceuticals during coagulation/flocculation is explained. Therefore, flocculation does not seem to be a viable option for concentrating pharmaceuticals from sewage streams.

As the cities grow, and the land becomes scarcer, there is an increasing requirement for compact STPs. To achieve this reduction in footprint, the digester volume may be decreased by uncoupling the solids retention time (SRT) from the hydraulic retention time (HRT). Separation of liquid and solids retention is a typical feature of an anaerobic membrane bioreactor (AnMBR) where a membrane keeps the solids inside the reactor, while the liquid can permeate. Membrane filtration of sludge will immediately result in the formation of a cake layer on top of the membrane's surface. This cake layer or fouling layer forms an excellent barrier for solids and acts as a secondary membrane during the filtration process. Therefore, a simple woven cloth can also act as a support for this cake layer, avoiding the need for purchasing actual membranes, which would decrease the investment costs significantly. An AnMBR equipped with a woven cloth as filter medium is referred to as an anaerobic dynamic membrane bioreactor (AnDMBR).

Challenges in operating an An(D)MBR are the filterability and viscosity of the sludge, which limits the maximum SRT that can be achieved. Our results showed that flocculants reduced the viscosity and increased the filterability of the emulsion. Therefore, flocculants may play a positive role in the optimization of an AnDMBR. Our results showed that increased filterability was only obtained after adding a high concentration of flocculants. However, these high concentrations caused a significant decrease in biomethane potential of the sludge, as the VS destruction was lowered from 32% to 24% after adding the flocculants. In addition, a decrease in the mean particle size (d50) was observed from 58 µm to 32 µm. This was likely to be caused by refractory flocculants that shielded small particles which were turned refractory as well, a phenomenon that is described in literature as well. Likely, the accumulation of these small refractory particles affected the filterability of the sludge, which led to a doubling of the trans membrane pressure (TMP) from about 150 mbar to 300 mbar. Therefore, adding flocculants to an AnDBMR did not yield the benefits that were initially expected. A potential solution to prevent irreversible binding, and thus a decreased filterability, is to use biodegradable flocculants. Further research is needed to evaluate this possibility.

As the disposal of sludge forms a large part of the operational costs of an STP, waste sludge reduction in an STP may have a big impact on the operational costs. A proven concept for waste sludge reduction in an STP, although not yet applied in practice, is predation of sludge by aquatic worms. In literature, several reactor configurations were studied in which secondary sludge is successfully predated by aquatic worms in lab-scale bioreactors. However, in contrast to AD, secondary sludge reduction by aquatic worms will cost energy for aeration, meanwhile the converted organic matter is not available anymore for energy recovery. Therefore, the ideal configuration would be to have AD followed by worm predation (WP) of the digested sludge, allowing for both energy recovery and a larger extent

of sludge reduction. So far, this had not been considered a viable option, due to the low ammonia tolerance of aquatic worms and the high ammonium concentrations present in the anaerobic digester. However, by flocculating anaerobically digested sludge, the sludge solids can be easily separated from the ammonia-rich liquid creating the possibility of WP of digested sludge, reducing the amounts of solids that need to be disposed. Our results revealed an additional removal of 40% VS of the digested sludge in 12 days when applying worm predation. The solids remained well separated from the liquid, which facilitates further treatment. However, the cationic flocculants caused mortality of the used worms, due to toxicity. The assessed 4-day LD_{50} value was between 50 and 100 mg/L. For the possible full-scale application of WP for AD sludge degradation, a process could be designed with continuous worm addition. However, a non-toxic flocculant could also be the solution prevent mortality of the worms. Further research is needed to elucidate the best option and to validate the full-scale possibilities.

The main barrier identified in this thesis for the application of flocculants in the current and future BNR-STP are i) the economic viability of flocculant applications other than the conventional applications ii) the refractory characteristics of flocculants and iii) the toxicity to aquatic worms. A solution for the above-mentioned challenges may lie in the production of polysaccharide bio-based flocculants such as alginate, chitosan, cellulose and starch. The performance of bio-based flocculants has been investigated for specific cases in numerous successful laboratory and full-scale studies. However, full-scale application is still marginal due to the high costs of production. A possible solution to make bio-based flocculants cost-effective is to find an organic waste source from which these polymers could be synthesized or extracted. However, thus far, there is only limited research in waste-based flocculants in STPs. Therefore, more research is needed in this field that could lead to new bio- and waste-based flocculants to be applied in sewage treatment.

Samenvatting

Organische flocculanten worden vooral gebruikt in de sliblijn en soms in de quaternaire zuivering van een conventionele rioolwaterzuivering (RWZI), ontworpen voor verregaande nutriënt verwijdering. Maar door maatschappelijke veranderingen op het gebied van duurzaamheid is er een grotere vraag naar circulariteit van grondstoffen en een hogere graad van zuivering. Daarom is het tijd voor een her-evaluatie van het potentieel dat flocculanten hebben in nieuwe afvalwaterzuiveringsconcepten. Dit werk laat het onderzoek zien naar nieuwe mogelijke toepassingen van flocculanten in de RWZI.

Het toepassen van flocculanten in fysisch-chemische voorzuivering zorgt voor meer primair slib dat kan worden omgezet naar biogas. Dit leidt tot een positievere energiebalans van een RWZI. Resultaten laten zien dat 66% meer chemisch zuurstof verbruik (CZV) uit het ruwe afvalwater gebruikt kan worden voor biogas productie via anaerobe vergisting (AV). Tegelijkertijd, hoeft de naar biogas omgezette CZV ook niet meer te worden afgebroken met zuurstof in de aeratietank, wat beluchtingsenergie bespaard. Maar het fysisch-chemisch verwijderen van CZV in de voorbehandeling met cationische flocculanten leidde tot een CZV/N-verhouding van 3.75 g CZV/g N in de waterlijn, wat minder is dan de minimaal benodigde ratio voor conventionele biologische N-verwijdering. Maar recent zijn er nieuwe N-verwijderingstechnieken geïntroduceerd, welke goed functioneren met lage CZV/N-ratio's zoals N-verwijdering via nitriet. Ook zijn er N-verwijderingstechnieken die helemaal geen CZV nodig hebben zoals het Anammox proces in de waterlijn van een RWZI. Met het toepassen van deze nieuwe technieken, kunnen flocculanten in de chemisch-fysische zuivering voordelig zijn voor de energiebalans op een RWZI. Naast de energiebalans werd ook de AV beïnvloed door flocculantgebruik in de voorzuivering: het extra CZV dat was verwijderd in de voorzuivering was makkelijker afbreekbaar, en leidde tot een 9% hogere biomethaan potentiaal van het primaire slib. Ook werd in afzonderlijke batch-testen gevonden dat flocculanten de viscositeit van de suspensie verlaagden, terwijl ook een 27% hogere hydrolyse snelheid werd waargenomen. In tegenstelling tot de hogere hydrolysesnelheid, zorgen de moeilijk afbreekbare poly acrylamide flocculanten, irreversibel gebonden aan de slibdeeltjes, voor een lagere biomethaan potentiaal.

Naast de energieaspecten van een RWZI, zijn er ook toenemende uitdagingen in de verwijdering van microvervuilingen. RWZI's zijn één van de belangrijkste bronnen van medicijnresten in het milieu. Volgens de literatuur zijn een groot gedeelte van de medicijnresten in een aquatisch milieu, gesorbeerd aan colloïden. Omdat flocculanten colloïden kunnen verwijderen zouden flocculanten gebruikt kunnen worden om medicijnresten te verwijderen uit afvalwater. De medicijnresten zouden dan in een meer geconcentreerde vorm in de slibstroom efficiënter behandeld kunnen worden. De mogelijkheid van medicijnverwijdering uit afvalwater door middel van flocculanten is onderzocht. Batch testen hebben laten zien dat medicijnen nauwelijks te verwijderen zijn met coagulatie/flocculatie. Om het verschil tussen gerapporteerde colloïdale sorptie en geringe verwijdering van medicijnen tijdens coagulatie/flocculatie te verklaren, hebben we een experiment uitgevoerd waarin colloïden in water met medicijnen werden verwijderd op twee manieren: door ultrafiltratie en door flocculatie. Beide manieren lieten een

vergelijkbare graad van verwijdering van colloïden zien. In de ultrafiltratie werden ook medicijnen verwijderd tot wel 93±4%. Maar in het experiment waarin de colloïden met flocculanten werden verwijderd werd er geen verwijdering van medicijnen waargenomen. Deze resultaten wijzen in de richting van een mogelijkheid tot verkeerde interpretatie van analyseresultaten wanneer UF wordt gebruikt om colloïdale sorptie van medicijnen te bepalen. Zeer waarschijnlijk leidt de kleine poriegrootte van UF tot directe retentie van niet sorbeerde medicijn moleculen. De resultaten van dit onderzoek laten zien dat medicijnen niet aan colloïden sorberen, en daarmee is de afwezigheid van verwijdering tijdens het coaguleren/flocculeren van colloïden verklaard. Daarom zijn flocculanten geen oplossing voor het concentreren van medicijnen in de slibstroom.

Omdat steden groeien en land schaarser wordt, is er een toenemende behoefte aan compacte RWZI's. Om de RWZI te verkleinen, kan het volume van de vergister verkleind worden. Dit kan door de vaste stoffen verblijftijd (VVT) te ontkoppelen van de hydraulische verblijftijd (HVT). Het scheiden van VVT en HVT is een typische eigenschap van een anaerobe membraan bioreactor (AnMBR) waar het membraan de vaste stoffen in de reactor houdt, terwijl de vloeistof de reactor kan verlaten door het membraan. Bij membraanfiltratie van slib vormt er cake laag op het membraanoppervlak. Deze cake laag, of vuil laag, vormt een uitstekende barrière voor vaste stoffen, en fungeert dan ook als een tweede membraan tijdens het filtratieproces. Daarom kan een simpel geweven canvas ook dienstdoen als de support voor deze cake laag van slib wat de noodzaak van een kostbaar membraan overbodig maakt. De AnMBR voorzien van een geweven canvas als filter medium wordt een anaerobe dynamische membraan bioreactor genoemd (AnDMBR).

De uitdagingen in het bedrijven van een AnDMBR zijn de filtreerbaarheid en de viscositeit van het slib, welke de maximum haalbare VVT bepalen. Onze resultaten laten zien dat flocculanten de viscositeit kunnen verlagen en de filtreerbaarheid kunnen verhogen. Daarom zouden flocculanten een positieve rol kunnen spelen in de optimalisering van een AnDMBR. De filtreerbaarheid werd alleen verhoogd werd na toevoeging van veel flocculanten. Maar deze grote hoeveelheden flocculanten zorgden voor een significante afname van het biomethaan potentiaal: de volatile deeltjes (VD) afbraak werd verminderd van 32% naar 24% na toevoeging van flocculanten. Daar bovenop werd de gemiddelde deeltjesgrootte (d50) verlaagd van 58 µm naar 32 µm. Dit is waarschijnlijk veroorzaakt door moeilijk afbreekbare flocculanten die kleine slibdeeltjes afschermen, die daardoor ook niet meer afgebroken kunnen worden. Dit fenomeen is ook elders in de literatuur beschreven. Waarschijnlijk heeft de accumulatie van kleine moeilijk afbreekbare deeltjes de transmembraandruk verhoogd van ongeveer 150 naar 300 mbar. Daarom levert de toevoeging van flocculanten aan een AnDMBR niet de gewenste verwachtte voordelen op. Een mogelijke oplossing om het moeilijk afbreekbaar maken van kleine deeltjes tegen te gaan, is het gebruik van biologisch afbreekbare flocculanten. Meer onderzoek is nodig om deze mogelijkheid te evalueren.

Het afvalslib vorm een groot deel van de operationele kosten van een RWZI. Daarom kan afvalslibreductie een groot effect hebben. Een bewezen concept op lab- en pilotschaal voor afvalslibreductie in een RWZI, is het afbreken van slib door aquatisch wormen. In de

literatuur zijn er verschillende reactor concepten bestudeerd waarin secundair slib succesvol wordt afgebroken door aquatisch wormen op lab schaal. Maar in tegenstelling tot AV, kost de afbraak van secundair slib door wormen energie in de vorm van beluchting. En ook is een deel van de omgezette energie uit het slib niet meer beschikbaar voor energie terugwinning naar biogas. Daarom zou de ideale configuratie zijn om AV te hebben, gevolgd door worm afbraak van het vergiste slib. Dit zorgt voor energie terugwinning en een extra reductie van afvalslib. Dit werd tot nu toe niet als mogelijke optie gezien omdat aquatisch wormen een lage tolerantie hebben voor ammonium, dat in grote mate aanwezig is in vergist slib. Maar door het vergiste slib te flocculeren, kunnen de vaste stoffen gemakkelijk gescheiden worden van de ammonium-rijke vloeistof wat de mogelijkheid opent voor afbraak door wormen. Onze resultaten lieten zien dat de geflocculeerde vaste stoffen met 40% VV konden worden afgebroken in 12 dagen, wanneer blootgesteld aan wormen. De vaste stoffen bleven goed gescheiden van de vloeistof, wat verdere behandeling vergemakkelijkt. Maar de wormen gingen dood door de cationische flocculanten omdat deze giftig bleken. De 4-daagse LD_{50} waarde was tussen de 50 en 100 mg/L. Voor de full-scale toepassing zou een proces kunnen worden ontworpen met continue worm toevoeging. Maar ook werken met niet-toxische flocculanten zou een oplossing kunnen zijn. Meer onderzoek is nodig om de beste opties te vinden voor een full-scale oplossing.

De grootste barrières in deze thesis voor het gebruik van flocculanten in de huidige en toekomstige RWZI's met biologische nutriënt verwijdering zijn i) de economische haalbaarheid van het toepassen van flocculanten voor anders dan de conventionele toepassingen ii) de moeilijke afbreekbaarheid van flocculanten en iii) de giftigheid van flocculanten voor aquatisch wormen. Een mogelijke oplossing voor de bovengenoemde uitdagingen zou kunnen liggen in de productie van polysaccharide flocculanten van biologische aard, zoals alginaat, chitosan, cellulose en zetmeel. De werking biologische flocculanten is onderzocht voor specifieke gevallen en vele succesvolle laboratorium testen. Maar full-scale toepassingen zijn nog marginaal vanwege de hoge kosten van productie. Een mogelijke oplossing om biologische flocculanten kosteneffectiever te maken, is om deze te synthetiseren of extraheren uit een organische afvalstroom. Maar tot nu toe is er weinig onderzoek naar afval-gebaseerde flocculanten in de RWZI. Daarom is er meer onderzoek nodig in dit veld nodig dat kan leiden tot nieuwe biologische en op afval gebaseerde flocculanten die in de RWZI kunnen worden toegepast.

1. Introduction

1.1. Introduction

Today, coagulation and flocculation processes are essential in a variety of diverse disciplines, such as cheese and rubber manufacturing, biochemistry and in wastewater and drinking water treatment. The most ancient reference to the application of coagulation is found in drinking water. The Sus'ruta Samhita, Sanskrit writings about medical issues, which dates back to 2000 B.C, mentions the use of crushed nuts of the Nirmali tree as a mean to clarify water (Cohen, 1958). This is in fact a reference to flocculation as the extracts of the Nirmali seeds are anionic poly electrolytes, thus a chemical to promote flocculation (Tripathi et al., 1976). The first description of metal coagulants was found in the writings of Pliny the Elder, a roman philosopher (ca. 77 A.D.), where he mentions the use of alum as a coagulant in potable water production (Faust and Aly, 1998). As in the past the production of potable water was vital for life, today the challenge for maintaining good health (see text in blue below) also extent to the treatment of used water: sewage treatment. And also, in this challenge today, flocculant and coagulant application are standard practice in the sludge line and sometimes in quaternary treatment.

Brief wastewater treatment history

In contrast to potable water, the need for treating wastewater is relatively new. Centuries ago, transporting wastewater out of the cities was considered sufficient. As early as 800 B.C., the Romans created *Cloaca Maxima*, a system which transported sewage from public latrines and bath houses in Rome into the Tiber (Henze et al., 2008). But after the fall of the Roman Empire, a period of utterly poor sanitation, also known as the *Sanitary Dark Ages*, started (Lofrano and Brown, 2010). In these *Sanitary Dark Ages*, the standard practice of sewage management became mere emptying buckets in the streets. Due to health concerns of this practice, it was no surprise that the readers in 2007 of British Medical Journal (BMJ) chose the *Sanitary revolution* during the nineteenth century as the most important medical milestone. Since early nineteenth century, there was a tremendous improvement in sanitary conditions: between 1800 and 1900, many cities installed sewage collection systems and from the early 20th century sewage treatment was developed, starting with trickling filters after which activated sludge processes became standard practice, which it still is up to date. During the 20th century the focus of wastewater treatment shifted from biological oxygen demand removal to nutrient removal to prevent eutrophication of water bodies. And in the last decades, micro pollutant removal and a higher energy-efficiency in wastewater treatment are gaining increasing attention.

1.2. Coagulants and flocculants

Coagulants are chemicals that cause destabilization of a suspension. Polymeric coagulants or flocculants are added to both destabilize a suspension and to accelerate the flocculation process. Both coagulants and flocculants are described below.

1.2.1. Metal coagulants

Metal coagulants are generally inexpensive and are therefore widely used in sewage treatment. The commonly used metal coagulants fall into two categories: those based on aluminium and those based on iron. Metal coagulants are typically used for two reasons in wastewater treatment, i.e. lowering the sludge volume index (SVI) in case of sludge bulking and to chemically bind and remove phosphate (figure 1.1).

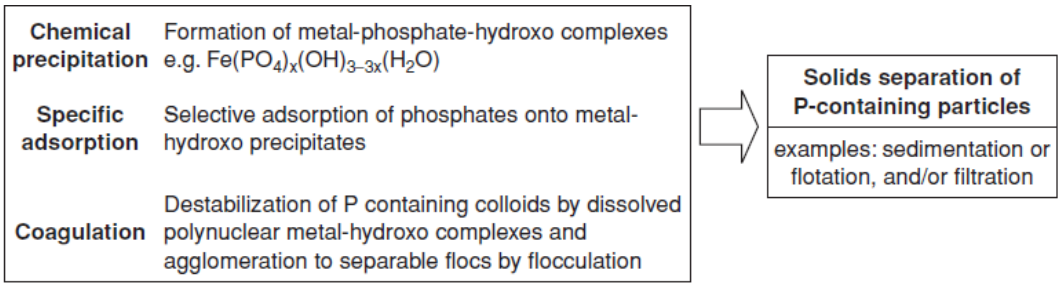


Figure 1.1. Summary of phosphorus removal from wastewater by using metal salt addition. Scheme adapted from Bratby (2006).

Ortho-phosphate is removed from wastewater in three ways (Figure 1.1): firstly, by direct precipitation with metal ions. Secondly, to lesser extent, by absorption of ortho-phosphates to the solid metal hydroxide complexes (Thistleton et al., 2002). And thirdly, by enmeshing colloidal matter containing phosphate into the solid metal hydroxides structures during settling. This form of induced settling is referred to as sweep coagulation. The metal hydroxide formed in a solution is mainly depending on the pH which is shown in Figure 1.2.

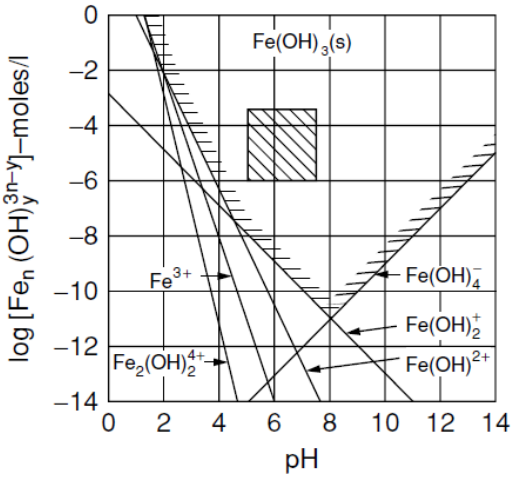


Figure 1.2. Equilibrium solubility products of iron hydroxides in water as function of the pH. The dashed area is the working area in wastewater treatment. Adapted from Bratby (2006).

1.2.2. Polymeric coagulants, or flocculants

Polymeric coagulants, or flocculants, are water soluble polymers consisting of long chains of monomers. The most common synthetic polymers are those based on polyacrylamide (PAM). Generally, three types of flocculants exist: cationic, anionic and non-ionic. Ionic polymers can also be called polyelectrolytes. The most important characteristics of flocculants are molar weight (MW) and charge density (CD) for poly electrolytes. Conventional flocculants have MWs in the range of $<10^5$, 10^5-10^6 and $>10^6$ kDa, which is also termed as low, medium and high MW, respectively (Bolto and Gregory, 2007). Furthermore, the CD of a flocculant can be experimentally determined with the colloid titration technique (Kam and Gregory, 1999). CD is expressed in mili-equivalents per gram of polymer (meq/g) or as percentage of charged monomers. It is broadly accepted to classify polymer CD as low, medium or high, where it describes a CD of 10%, 25%, or 50-100% respectively (Bolto and Gregory, 2007). The cationic polymer family has a greater variety of molecular structure than any other polymer type. But generally, it contains quaternary ammonium groups (Bolto, 1995) as shown in Figure 1.3 which bears a positive charge. Anionic polymers contain mostly weakly acidic carboxylic acid groups. Therefore, their CD will depend on the pH. Sometimes stronger acidic groups are used, for example sulphonic acid which are fully ionized at neutral pH.

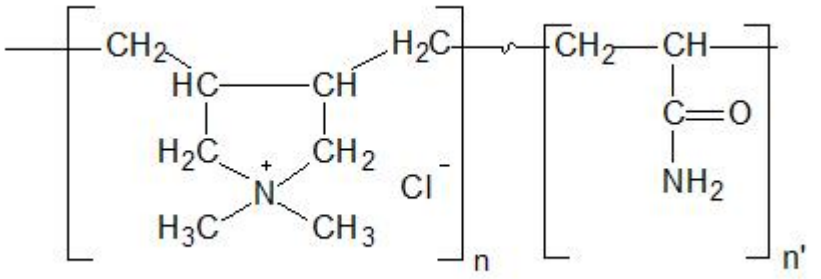


Figure 1.3. Example of a part of the cationic acrylamide based polymer with quaternary ammonium group with a positive charge (left) and acryl amide group (right). Source: http://www.luyuechemical.com.

1.3. Destabilization: mechanism of action

There are two ways in which colloids and fine particulates can form large flocs with the help of coagulants or flocculants (Kitchener, 1972). First, colloids can be destabilized by coagulants, which creates small flocs. Then flocculants help to aggregate the small flocs to larger flocs (shown below in scheme a). Flocculants can also directly create large flocs by destabilizing and aggregating to large floc in one single step (shown in scheme b).

(a) Colloids $\xrightarrow{+\text{ coagulants}}$ Unstable colloids $\xrightarrow{+\text{ time (conditioning)}}$ Small flocs $\xrightarrow{+\text{ flocculants}}$ Large flocs

(b) Colloids $\xrightarrow[+\text{ time (conditioning)}]{+\text{ flocculants}}$ Large flocs

There can be several types of binding of flocculants to colloids or particles:

1. Electrostatic bonding where flocculants bind to a surface with opposite valence: e.g. cationic polymer that binds a negatively charged surface.
2. hydrogen bonding: Non-ionic poly acrylamide (PAM) can absorb to surfaces with dipole-dipole interactions (Griot and Kitchener, 1965).
3. Ion bonding: Ion bonding is where there is electrostatic attraction between oppositely charged ions.

Despite electrostatic repulsion, in many cases anionic polyelectrolytes can bind to negatively charged particles. However, this only occurs with a sufficiently high concentration of divalent cations (O'Gorman and Kitchener, 1974) which can form a bridge between the polymer and the particle. Flocculants attach to particles on many different places. Therefore, each separate binding might be based on a weak force. Since it is very unlikely that all binding sites are released simultaneously, the polymer binding to a particulate is considered permanent (Hogg, 1999).

The attachment to the particle initiates the flocculation process. The mechanism of flocculation of particles can be described by three phenomena (Bolto and Gregory, 2007):

- Polymer bridging
- Charge neutralization
- Depletion of flocculant

Depletion of flocculation is most likely not of any influence in water treatment (Bolto and Gregory, 2007) and is therefore is not discussed in this work.

1.3.1. Polymer bridging

Long chained polymers that are attached to a particle may have their chains extended into the solution, prone for attaching to another particle as shown in Figure 1.4. The phenomenon of a polymer connecting to particles is referred to as 'bridging'. There are several factors important for bridging. It is favoured with high MW linear polymers. The dosage must be such that there is only partial coverage of the particles by polymers. Overdosing will cause the with polymer covered particulate surfaces to have the same charge which compromises coagulation. To further extent the chains of the polymers, the ionic strength needs to be low, and there is an optimum CD in which bridging takes place (Bratby, 2006).

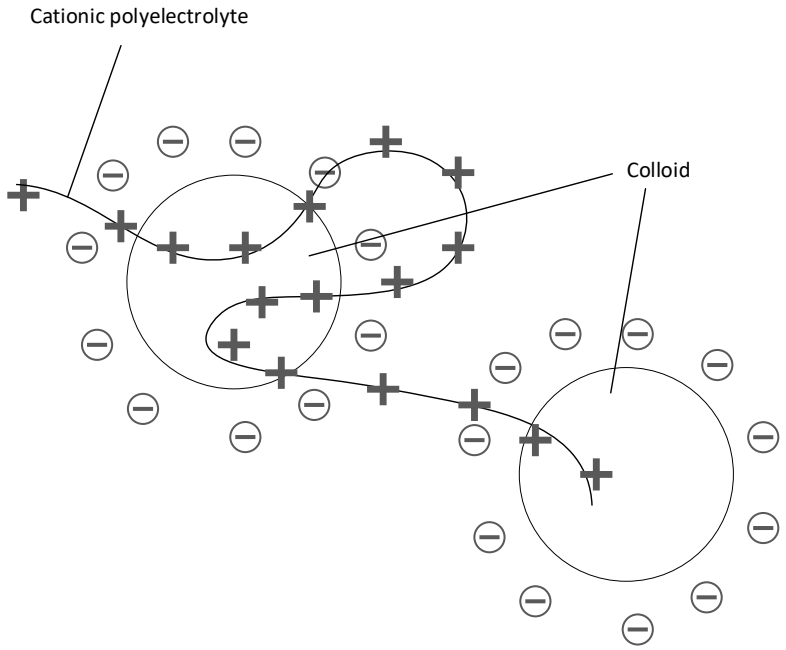


Figure 1.4. Polymer bridging of cationic polyelectrolyte between two negatively charged colloids.

1.3.2. Charge neutralization

Another way of removing particles with oppositely charged flocculants is by charge neutralization (Figure 1.5). Many studies found that the optimal flocculant dosage is a dosage that neutralizes the particle charge (Kleimann et al., 2005). To effectively neutralize the charge, high CD polymers are more effective, simply because they bear more charge per gram of polymer. In addition, high CD polymers tent to absorb in a flat manner rather than with extended chains (Bolto and Gregory, 2007). Although the nett charge of particles may be zero, there can be patches of polymers on the particle surface as shown in Figure 1.5. In this way the particles contain positively and negatively charged areas. Two patches of opposing valence on two particles attract each other which causes agglomeration.

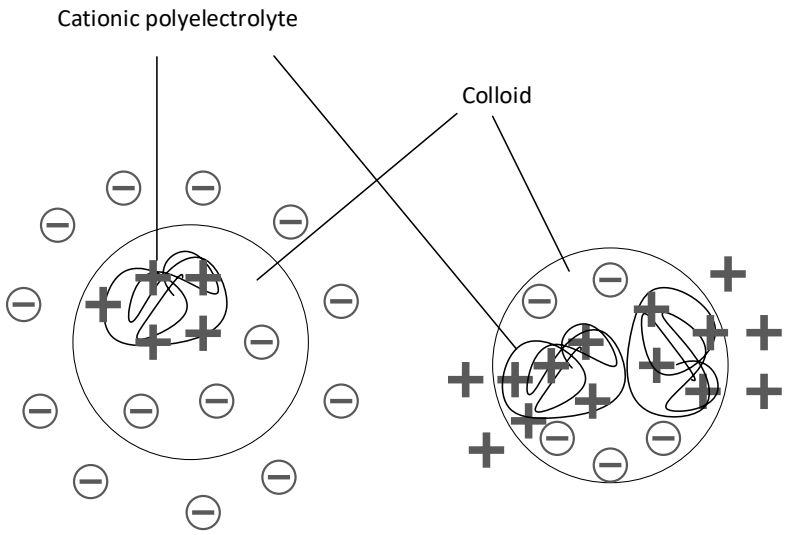


Figure 1.5. Charge neutralization. Cationic flocculant patches the negatively charged colloids after which it become oppositely charged or even positively charged. Now the repulsion between colloids is compromised and coagulation can start.

1.4. Kinetics

When flocculants are added to a suspension, several processes simultaneously and subsequently take place and proceed at different rates (Figure 1.6). After addition of a concentrated solution of flocculant, vigorous mixing is required to prevent local differences in concentration causing either saturation or too low concentrations of the flocculant. Non-uniform absorption may result in reestablishment of the stability of colloids. During stirring the absorption to particles takes place. The rate of absorption depends primarily on the concentration of particles: the higher the concentration of particles, the higher the absorption rate (Gregory, 1988). In diluted wastewaters, the absorption can be achieved in the order of minutes. In highly concentrated sludges, the absorption can take place in 1 s (Bolto and Gregory, 2007). For low MW polymers, the absorption process is mainly guided by diffusion. For medium and high MW polymers, the absorption is shear induced and depends on collision of particle and polymer. This means that the rate of polymer absorption depends on the mixing conditions. After mixing and absorption there is a period of rearrangement of absorbed chains which may take several seconds for high MW polymers (Pelssers et al., 1990). The rearrangement may result in tails of chains free to bind other particles. This is referred to as direct flocculation. It may also result in altering the surface valence by for example electrostatic patching. In this way, the flocculant acts as a coagulant and coagulation subsequently takes place. The final step is denominated as flocculation: when particles are covered with sufficient polymer, they become destabilised. Collisions then will result in aggregation of particles to form larger particles.

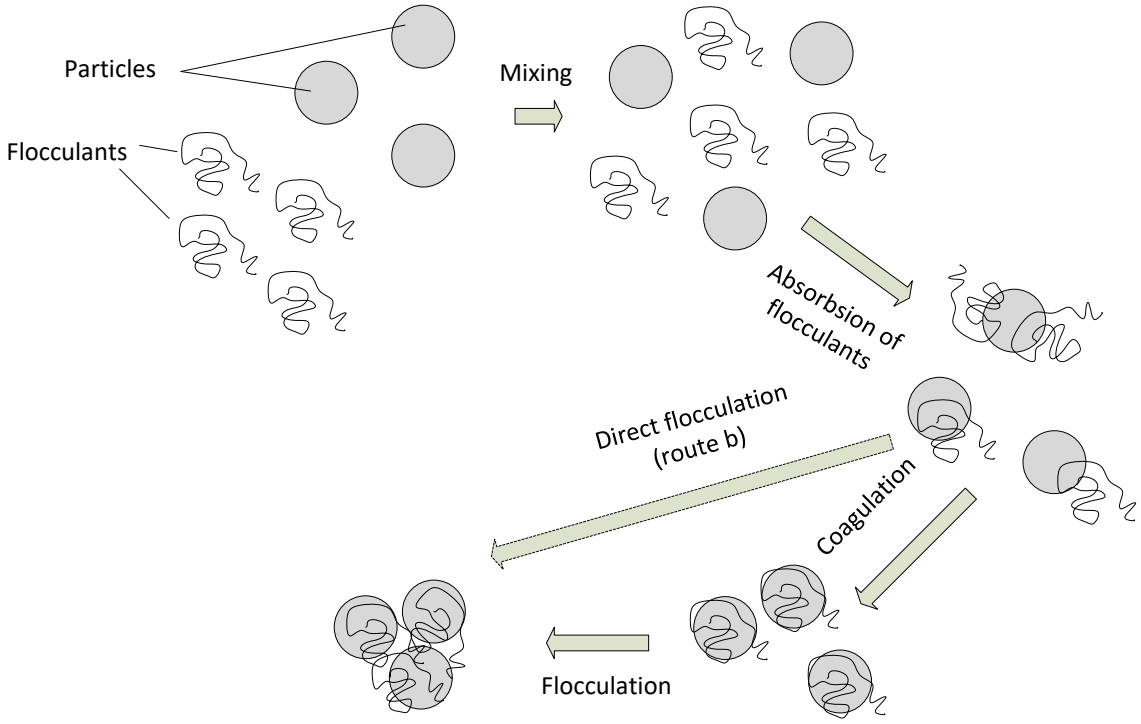


Figure 1.6. Processes involved in flocculation of particles. Adapted from Gregory (1988).

1.5. Dosage

The terminology 'effective polymer addition' is used when the dosage is less than required for complete saturation of the particles (Kitchener, 1972). Most optimally, the dosed amount of flocculants suffices to cover half or less of the colloid or particle surface with flocculants (Bratby, 2006). In practice this means about 0.05 to 0.5 mg polymer per gram of solids (Hogg, 1999) or even as high as 1 mg polymer per g of suspended solids (Bolto and Gregory, 2007). But several factors play a role in optimised dosing: for example, the particle size distribution (PSD). Flocculants generally do not easily absorb to small particles, unless high concentrations of flocculants are used (Hogg, 1999). Since the flocculants need to cover the surface, the dosage depends on the particle surface area of the suspension (Taylor et al., 2002). Small particulates have a large surface to mass ratio (Table 1.1). Therefore, more flocculant is required to destabilize smaller particles. However, since small particles have only a small total surface area, they are easily saturated. This leads to hindered flocculation, resulting in repulsion of the with polymer saturated small particles to other saturated small particles. This causes a suspension with a low average particle size, forming a bimodal floc size distribution (Figure 1.7). As a result, more flocculant is required to come to a large uniformly distributed average particle size (Rattanakawin and Hogg, 2001).

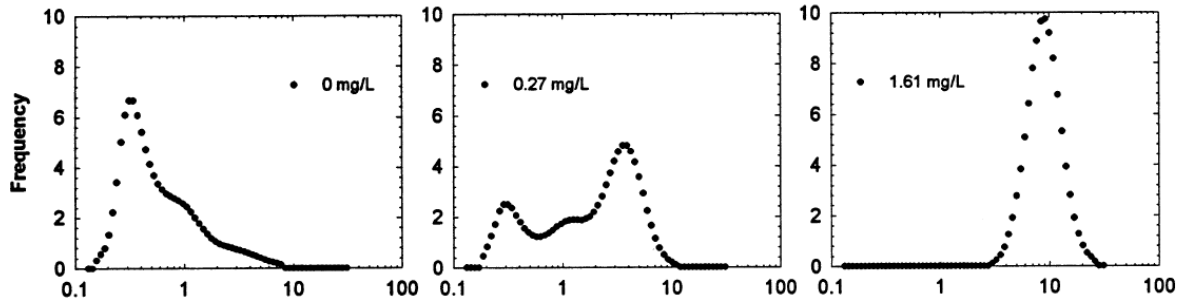


Figure 1.7. PSD graphs of aluminium oxide suspension. The initial stable solution without flocculant addition (0 mg/L) shows a mono-modal PSD with a small particles size. After the addition of a limited (0.27 mg/L) amount of cationic polymer, bigger particles were formed, but some of the small particles remained stable due to flocculant saturation, which causes a bimodal PSD of the solution. After the addition of additional cationic flocculant to a concentration of 1.61 mg/L, a mono-modal PSD is obtained at higher particle size. Taken from Rattanakawin and Hogg (2001).

1.6. Current application of coagulants/flocculants in sewage treatment

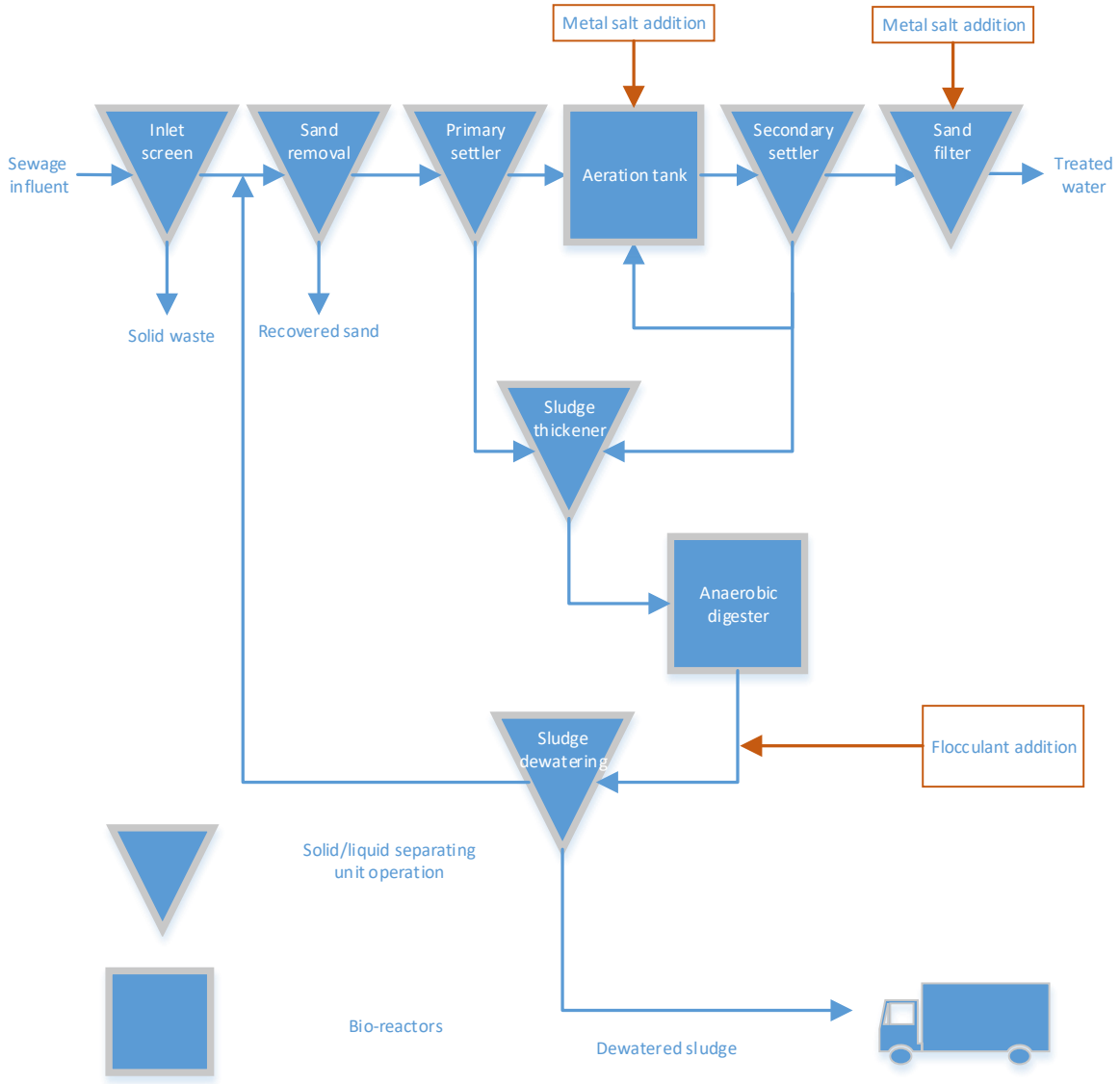


Figure 1.8. Schematic overview of a typical sewage treatment plant with biological and chemical nutrient removal.

In conventional STPs in the Netherlands, schematically depicted in Figure 1.8, there are several steps where solids-liquid separation takes place to remove the wide range of solid sewage constituents. Sewage comprises dissolved compounds, colloids, and suspended solids, among which there is a variety of biological forms such as viruses, bacteria, protozoa and even higher life forms such as aquatic worms or in rare cases even crocodiles (Snyder, 2010). Besides the soluble contaminants, also the suspended solids and colloids need to be removed in sewage treatment.

A crocodile is easy to remove from wastewater as it is strained by a strainer. Wastewater particulates of $\geq 10^{-2}$ mm will pass the strainer but will be settled in the primary settler to form primary sludge (Van Nieuwenhuizen *et al.*, 2000). The smallest organic constituent of sewage, organic dissolved matter, is consumed by microorganisms present in the STP and is converted to mainly gaseous compounds, water and biomass. The produced aerobic

biomass forms flocs inside the sewage treatment plant and is settled in the secondary settler to form secondary sludge.

A more challenging sewage constituent is colloids. Colloids have a very low mass and a very large specific surface area (Table 1.1). Because of this very large specific surface area, the electrostatic force interactions on the surfaces of these colloids are dominant compared to the gravitational force. Therefore, it takes years for colloids to settle. However, they are largely removed from wastewater treatment by incorporation into the sludge flocs (Li *et al.*, 2006), where they are hydrolysed or removed as solids with the secondary sludge.

The primary and secondary sludges are typically thickened before they are pumped to the anaerobic digester. Secondary sludge is often thickened with polymer to increase the solids concentration. In the anaerobic digester, the solids are hydrolysed and converted to methane and CO_2. Small refractory parts are not digested and accumulate as colloidal material in the digestate (Elmitwalli *et al*., 2001). Due to the presence of colloids, settling is not an option for the separation of solids from the liquid. Therefore, the digestate is dewatered by centrifuges and belt presses after adding polymers. The dewatered sludge leaves the sewage treatment plant by truck. In addition to solids removal in a sewage treatment plant, coagulants are also sometimes used for precipitating phosphate to reach the restrictive limits.

Table 1.1. Specific surface area and settling times of different constituents of sewage. Adapter from Bratby, (2006).

Particle size	**Classification**	**Examples**	**Specific surface area**	**Time required to settle 100 mm***
[mm]	[-]	[-]	[m^2/cm^3]	[-]
10^3	Unusual objects in sewage	Crocodiles	$6*10^{-6}$	0.02 s**
10	Coarse dispersion (visible with naked eye)	Gravel, coarse sand, mineral substances, precipitated and flocculated particles, silt macroplankton and other organisms	$6*10^{-4}$	0.1 s
1			$6*10^{-3}$	1 s
10^{-1}			$6*10^{-2}$	13 s
10^{-2}	Fine particulate dispersion (visible under microscope)	Mineral substances, precipitated and flocculated particles, silt, micro organisms	0.6	11 min
10^{-3}			6	20 hours
10^{-4}			60	80 days
10^{-5}	Colloidal dispersion	Mineral substances, hydrolysis and precipitation products, macromolecules, biopolymers and viruses	600	2 years
10^{-6}			6000	20 years
$<10^{-6}$	Solution	Inorganic simple and complex ions, (polymeric) molecules, organic molecules	-	-

*When specific gravity is 2.65

** In case of a dead crocodile as a live one could swim

1.6.1. Inlet works and settlers

Solids separation starts in the inlet works of an STP where coarse material is removed by a coarse screen (left figure in Figure 1.9). Large particles (typically larger >2-6 mm, depending on the screen) are sieved from the water and are disposed as solid waste. After the coarse screen, cyclones or a discrete settling step can be used to induce settling of sand particles (small but heavy). This is done to reduce wearing of the pumps and to prevent deposition of sand in other treatment units.

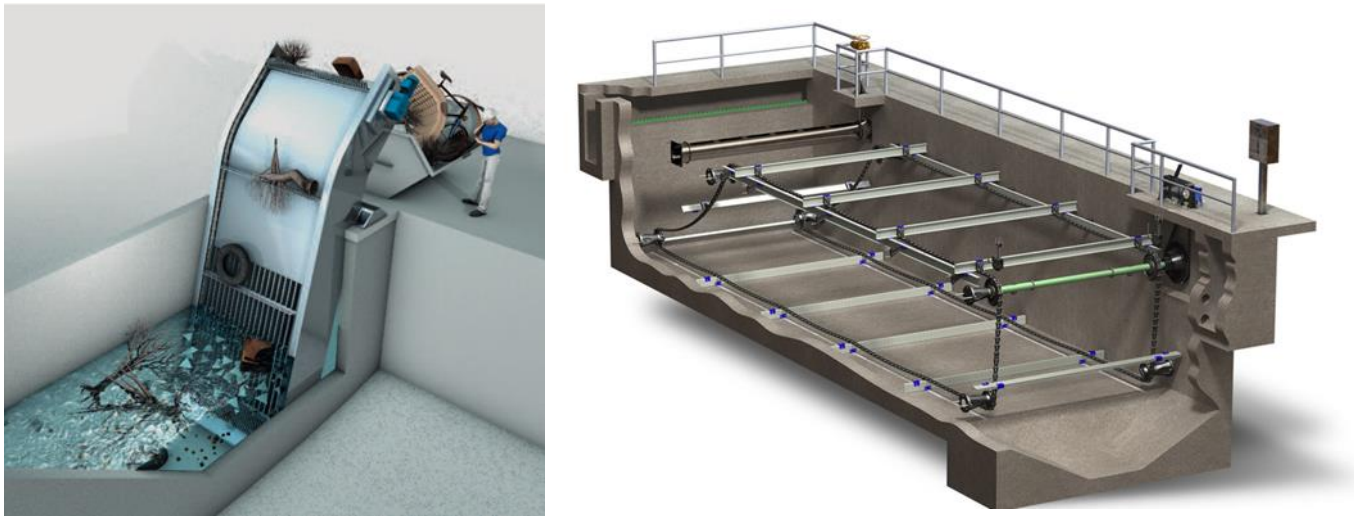


Figure 1.9. Solid-liquid separation in a sewage treatment plant. Coarse screens to remove large incoming particles (left, source: www.huber.de) and a cross section of a primary settler (right, source: www.brentwoodindustries.com).

After the sand removal unit, the water in many STPs is led over a primary clarifier (right figure in Figure 1.9) where the water is retained up to several hours allowing the particles of > 0.45 µm to settle (Van Nieuwenhuijzen *et al.*, 2004). The settled sludge particles form primary sludge and are pumped into the sludge line. The primary sludge is first thickened in a sludge thickener where compaction of the sludge bed by gravity force yields a higher solids concentration.

Another sludge stream that is compacted is generated in the secondary settler where activated sludge is removed from the water line. Part of the secondary sludge is pumped back to the activated sludge basin and part is compacted in a sludge thickener, often aided by the addition of flocculants.

After compaction, the secondary sludge, together with the compacted primary sludge, is send to the anaerobic digester where it is kept at about 35 °C for 15-30 days. Anaerobic digestion (AD) serves mainly to destruct solids, but also generates biogas that can be used for electricity generation and heating. Alternatively, the biogas is upgraded to biomethane which is growing in popularity with an annual growth of number of installations between 20% and 30% (Angelidaki et al., 2018). Due to heavy metals content of the sludge, agricultural application is abandoned in the Netherlands. Therefore, apart from some excess sludge transported to neighbouring countries, the vast majority of the excess sewage sludge in the Netherlands is incinerated (Kacprzak et al., 2017).

Prior to incineration, the water content of the sludge needs to be drastically reduced. This is because evaporating water requires a large amount of energy. To illustrate: the energy required to evaporate 1L of water is over six times higher than the energy required to heat 1L of water from 15 to 100 °C. For instance, dewatered sludge with 24% dry solids (DS) contains 240 kg of solids and 760 kg of water. The solid part, which comprises of digested sludge, typically contains about 70% VS, which means 168 kg of VS per ton of dewatered sludge. Per kg of VS, there is about 1.42 kg of COD. The lower heating value (LHV) of COD is

12.56 MJ/kgCOD (Schaum, *et al.*, 2016). Therefore, the energy produced by combusting the 240 kg of dewatered sludge is given by formula 1.1.

$$240[kgTS] * 0.7\left[\frac{kgVS}{kgTS}\right] * 1.42\left[\frac{kgCOD}{kgVS}\right] * 12.56\left[\frac{MJ_{LHV}}{kgCOD}\right] = 2996[MJ] \qquad (1.1)$$

For the evaporation of the 760 kg of water, 1707 MJ is required and for the heating of the sludge from 15 to 100 C, 355/ton MJ is required. Therefore, the nett thermal energy yield from combusting dewatered sludge (with 24% DS) is 934 MJ/ton. Increasing the DS from 24% to 25%, increases the nett energy yield with about 16%. Also, an increased DS will have a positive impact on the transportation and storage costs. Therefore, it is beneficial from an energy perspective to dewater the sludge to a large extent.

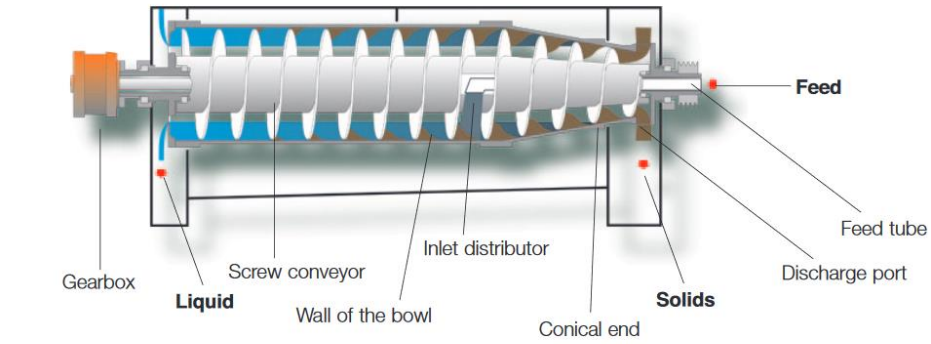
AD reduces the average particle size of sludge (Novak et al., 2003, 1988). Because of the decreased average particle size of digested sludge, plain settling and compaction is not an option for dewatering. Similarly, direct filtration is not feasible either. As the formula of Carmen-Kozeny equation suggests (see formula 1.2), a lower particle size, increase the pressure drop with the square root.

$$\frac{\Delta P}{L} = \frac{180\mu}{\phi_S^2 * D_P^2} * \frac{(1-\epsilon)^2}{\epsilon^3} * \upsilon_S \qquad (1.2)$$

In this formula the ΔP is the pressure drop; L is the bed length; μ is the viscosity of the fluid; Φ_S is the sphericity; ϵ is the bed porosity; υ_s is the superficial velocity. Novak et al. (1988) reported that a mean particle size lower than 40 μm has a detrimental effect on the overall sludge filterability. In addition to the small particle size, the presence of both small and large particles in digested sludge compromises to an extra extent (Karr and Keinath, 1976; Novak et al., 1988). This is caused by the phenomenon of blinding: during filtration small particles migrate into filter cake pores, blocking the water flow (Sorensen et al., 1994). Therefore, the average particle size needs to be increased before water can be removed. This is done by adding flocculants.

1.6.2. Dewatering equipment

Because of the low energy yield of incineration and the high inorganic fraction of dewatered sludge, the costs of disposing sludge in the Netherlands is high: about 60 €/ton dewatered sludge (Duin *et al.*, 2016) and can amount up to 50% of the operational costs of the treatment plant (Wei, et al., 2018). In order to transport less water and increase the energy yield for incineration, the challenge is to reach a DS concentration that is the highest that the incinerators can handle. There are three sludge dewatering techniques that are most commonly used in the Netherlands: centrifuges, belt presses and chamber filter presses. Centrifuges reach a dry solids content of 23-24% with a flocculant dosage of 13-15 g per kg DS. Belt presses yield a DS content of 19-21% with 8-9 g of flocculant per kg DS. Filter presses yield a similar DS concentration but there is no data on the flocculant dosage (Korving, 2011). In Figure 1.10, a centrifuge (top) and belt press (bottom) are displayed.



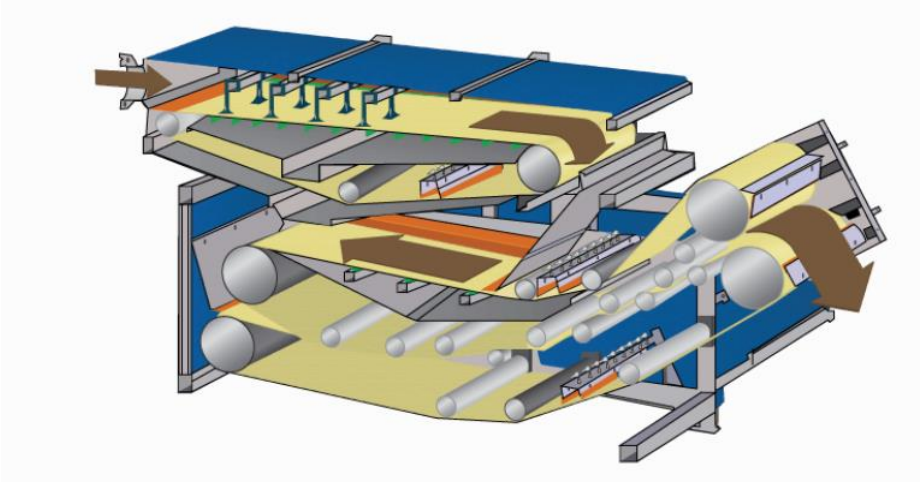


Figure 1.10. Dewatering centrifuge (top, source www.alfalaval.com). Sludge is fed on the right, dewater sludge leaves the centrifuge on the right, liquid leaves the centrifuge on the left. Belt press (bottom, source: frcsystems.com) presses sludge between two belts that are tightened over course of the path through the machine.

1.7. Novel application of flocculation aids in wastewater treatment

Despite the fact that there are several solids/liquid separation processes in a conventional STP, the use of flocculants in municipal sewage treatment is typically limited to sludge dewatering and sludge thickening (Figure 1.11): the use of flocculants in other sewage treatment separation processes is often not economically feasible or not possible because of the critical COD/N ratio for denitrification (see Chapter 2). Technically, however, there are several possibilities to benefit from flocculants in sewage treatment. And as society changes and technical innovation changes the future STP, these novel applications may become an option for the future STPs. Where in the past sewage management consisted of merely transporting it out of the cities to prevent pathogenic outbreaks, nowadays sewage management should be energy efficient; compact; cost effective, oriented to resource recycling, and thorough. To keep sewage treatment in line with societies latest demands such as the need for more renewable energy; micro pollutant removal, and footprint reduction in the ever-growing cities, a renewed perspective on flocculant application in sewage treatment is required. Figure 1.11 shows novel applications of flocculants with the possible benefits. Below, the chapters of this thesis are briefly introduced.

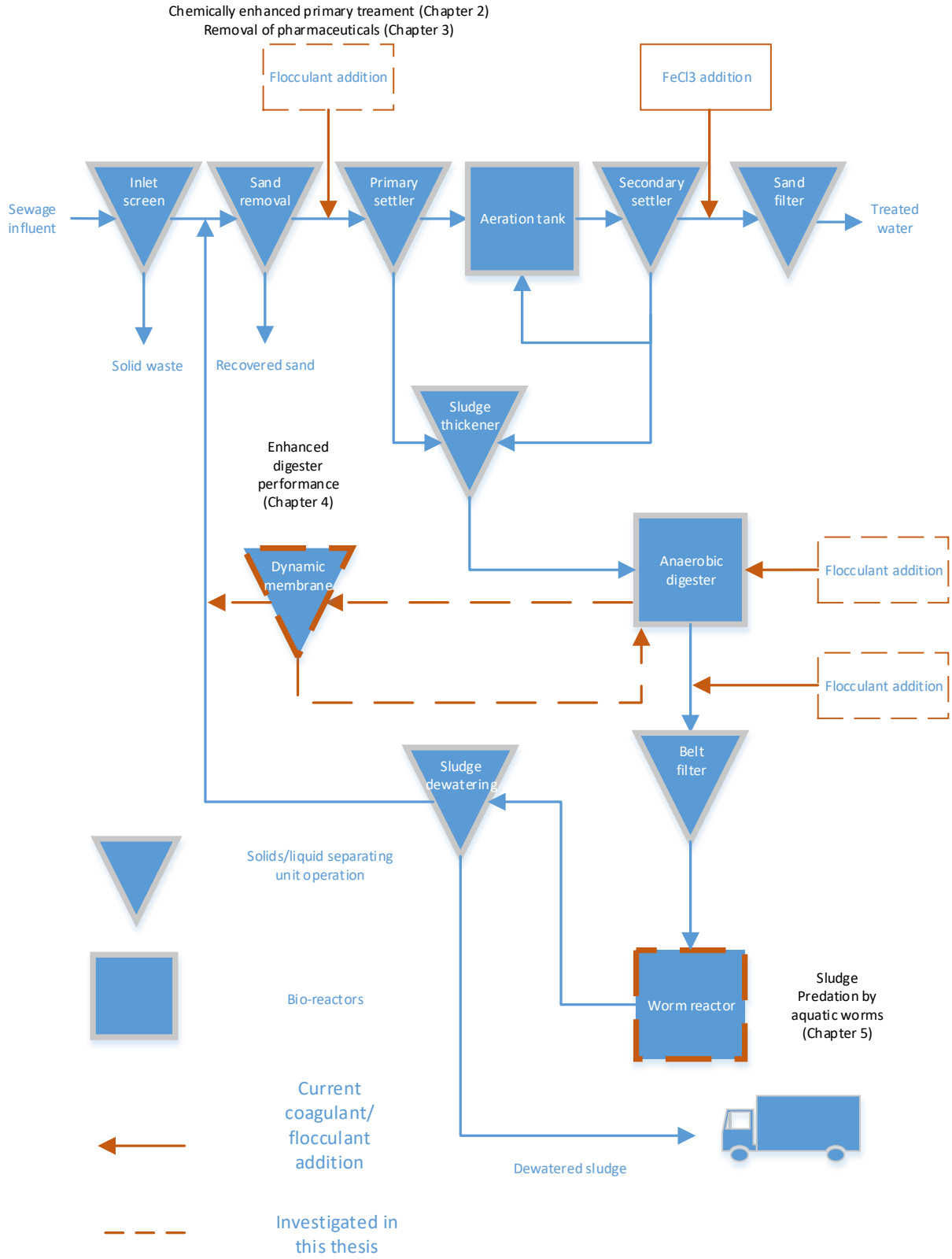


Figure 1.11. Overview of novel flocculant applications investigated in this thesis.

1.7.1. Chemically enhanced primary treatment – Chapter 2

Flocculants can be used for chemically enhanced primary treatment (CEPT). In this way, more influent COD could be used for biogas production via AD, instead of the current

aerobic oxidation of COD in the aeration tanks as schematically shown in Figure 1.12. Therefore, CEPT could lead to a more positive energy balance of the STP. However, thus far, CEPT is not applied in the Netherlands. The critical COD/N for denitrification can be negatively influenced by extensive primary treatment, compromising efficient nitrogen removal due to a lack of COD for denitrification (Van Nieuwenhuijzen et al., 2000). However, with the perspective of a low COD/N tolerant STP, CEPT could be advantageous for the energy balance of an STP. For example, N removal over nitrite or completely autotrophic N removal by using e.g. the mainstream anammox technology in the waterline of sewage treatment (Hoekstra, 2017; Lotti, 2016). The effects and possible benefits of application of CEPT in the current wastewater treatment plant are explained in Chapter 2.

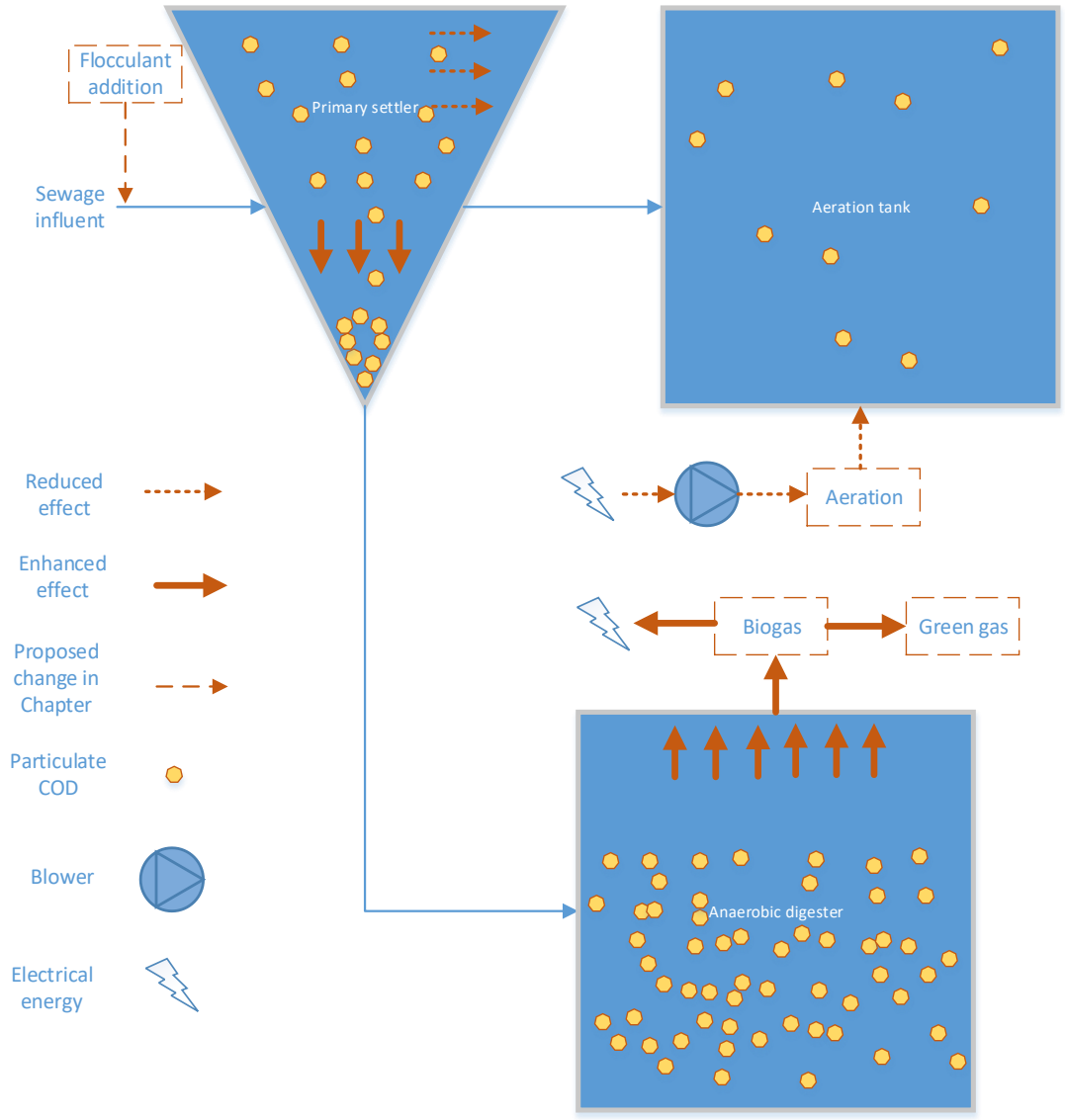


Figure 1.12. Schematic overview of possible benefits of applying chemically enhanced primary treatment: more particulate COD is directed to the anaerobic digester instead of the aeration tank, yielding more biogas and a reduced electrical energy consumption for aeration.

1.7.2. Micro pollutant removal – Chapter 3

Colloids are reported in literature to sorb pharmaceuticals to a large extent (Cheng *et al.*, 2017; Duan *et al.*, 2013; Holbrook *et al.*, 2004; Maskaoui and Zhou (2010); Zhou *et al.*, 2007; Yang *et al.*, 2011). Flocculants are known to remove colloids from the water (Bratby, 2006). Therefore, pharmaceuticals could be removed from the water line in an STP with flocculants and concentrated into the sludge (see Figure 1.13 for schematic overview). This is investigated in Chapter 3.

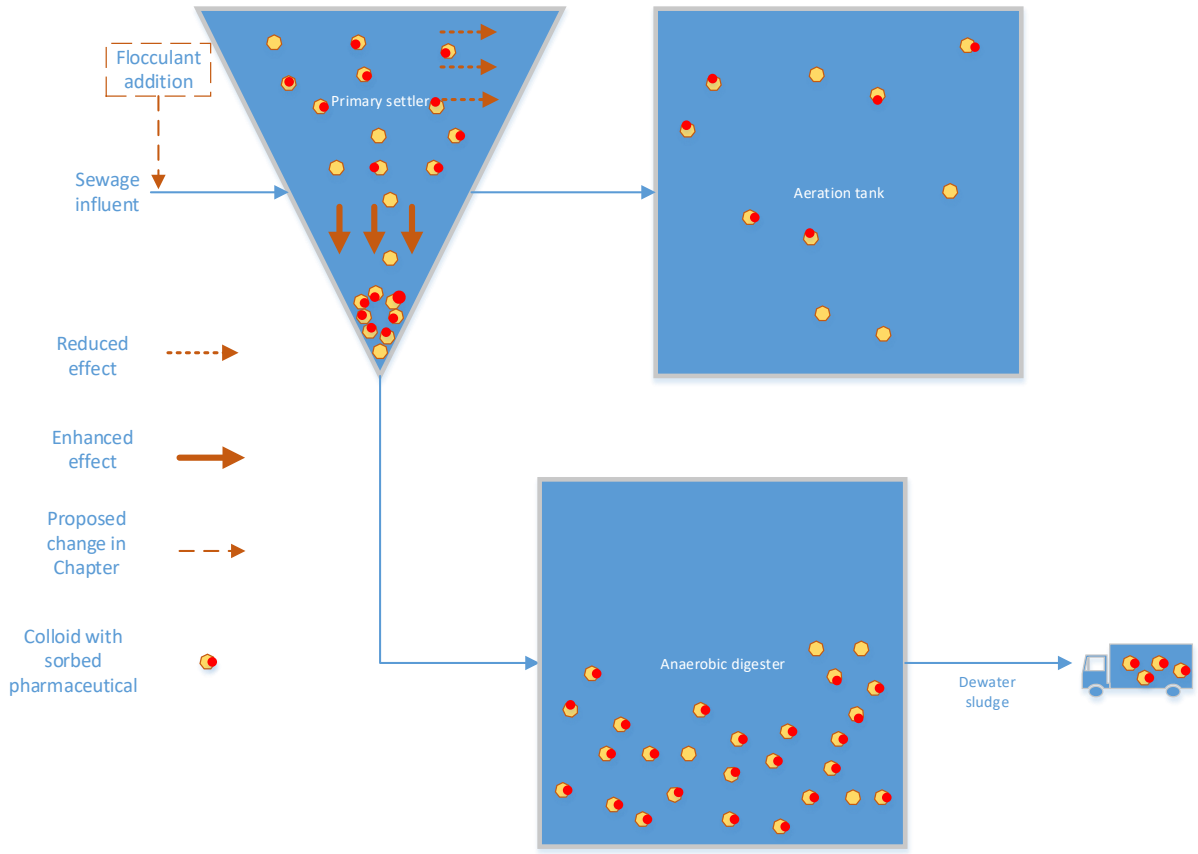


Figure 1.13. Schematic overview of possible benefits of applying chemically enhanced primary treatment: more colloidal bound pharmaceuticals are directed to the smaller sludge stream to facilitate treatment and/or disposal after sludge dewatering.

1.7.3. Improved anaerobic digestion – Chapter 4

A development in AD is the application of anaerobic dynamic membrane bioreactor (AnDMBR) where a simple cloth is used to uncouple the SRT from the HRT in an anaerobic digester (Ersahin et al., 2012) as schematically shown in Figure 1.14. In AnDMBRs treating sludge, increased viscosity due to increasing solids accumulation forms the limit of the highest attainable SRT (Meabe et al., 2013). The challenge in the separation of solids and liquid in anaerobic digestion is to overcome the low filterability of digested sludge. Flocculants are known to reduce the viscosity of digested sludge and increase the filterability. Therefore, the addition of flocculants in an AnDMBR treating sludge could increase the sludge filterability and decrease the liquid viscosity. This would enhance the economic feasibility of replacing the conventional continuous stirred tank reactor (CSTR) digester of STPs by an AnDMBR with a 25% smaller reactor. In Chapter 4 the application of flocculants in an AnDMBR treating waste activated sludge is investigated.

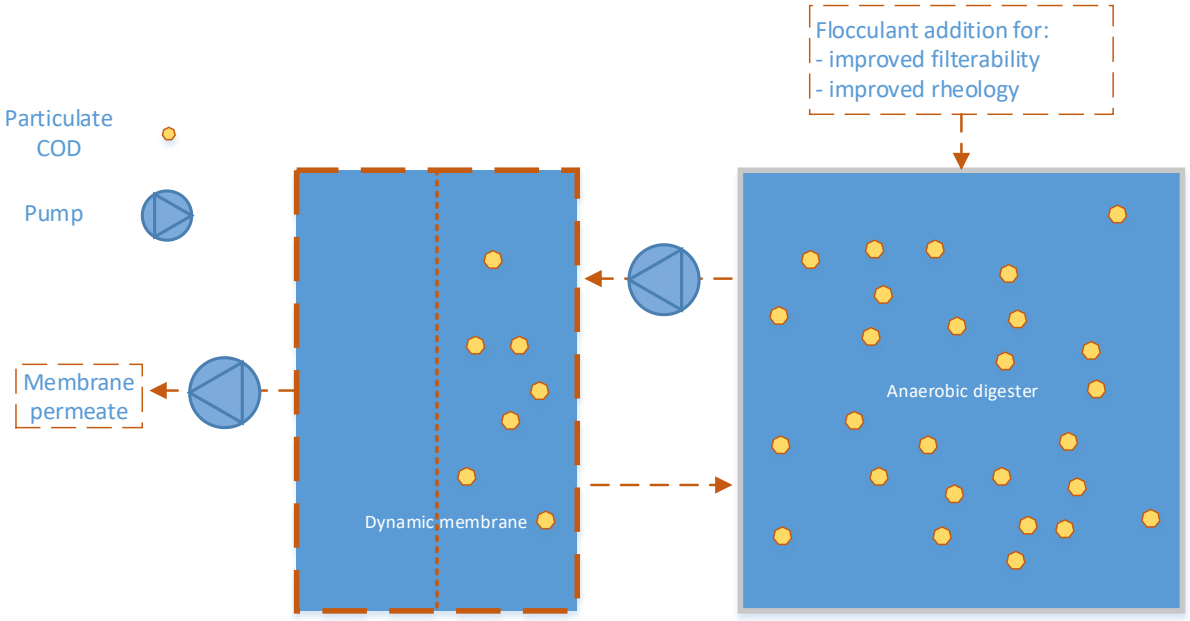


Figure 1.14. Schematic overview of setup where a dynamic membrane is applied to uncouple the hydraulic retention time and solids retention time. Flocculants can be added to improve the filterability and the rheology in the anaerobic digester.

1.7.4. Reduced sludge production – Chapter 5

Sludge disposal is one of the largest cost factors of sewage treatment (see Section 1.6). Therefore, relatively costly treatment techniques reducing the amount of waste sludge can become economically feasible. Waste activated sludge predation by aquatic worms is a proven concept and allows for substantial sludge reduction (Tamis, et al., 2011). Sludge predation of anaerobically digested sludge would allow for both energy recovery and additional sludge reduction. So far, this was considered not viable due to the low ammonia tolerance of aquatic worms (Hendrickx et al., 2010). By flocculating anaerobic sludge, the sludge solids can be separated from the ammonia containing reject water, creating the possibility of sludge predation by aquatic worms. In Chapter 5, the possibility of aquatic worm predation with *Limnodrilus* and *T. tubifex* was investigated of flocculated digested sludge solids.

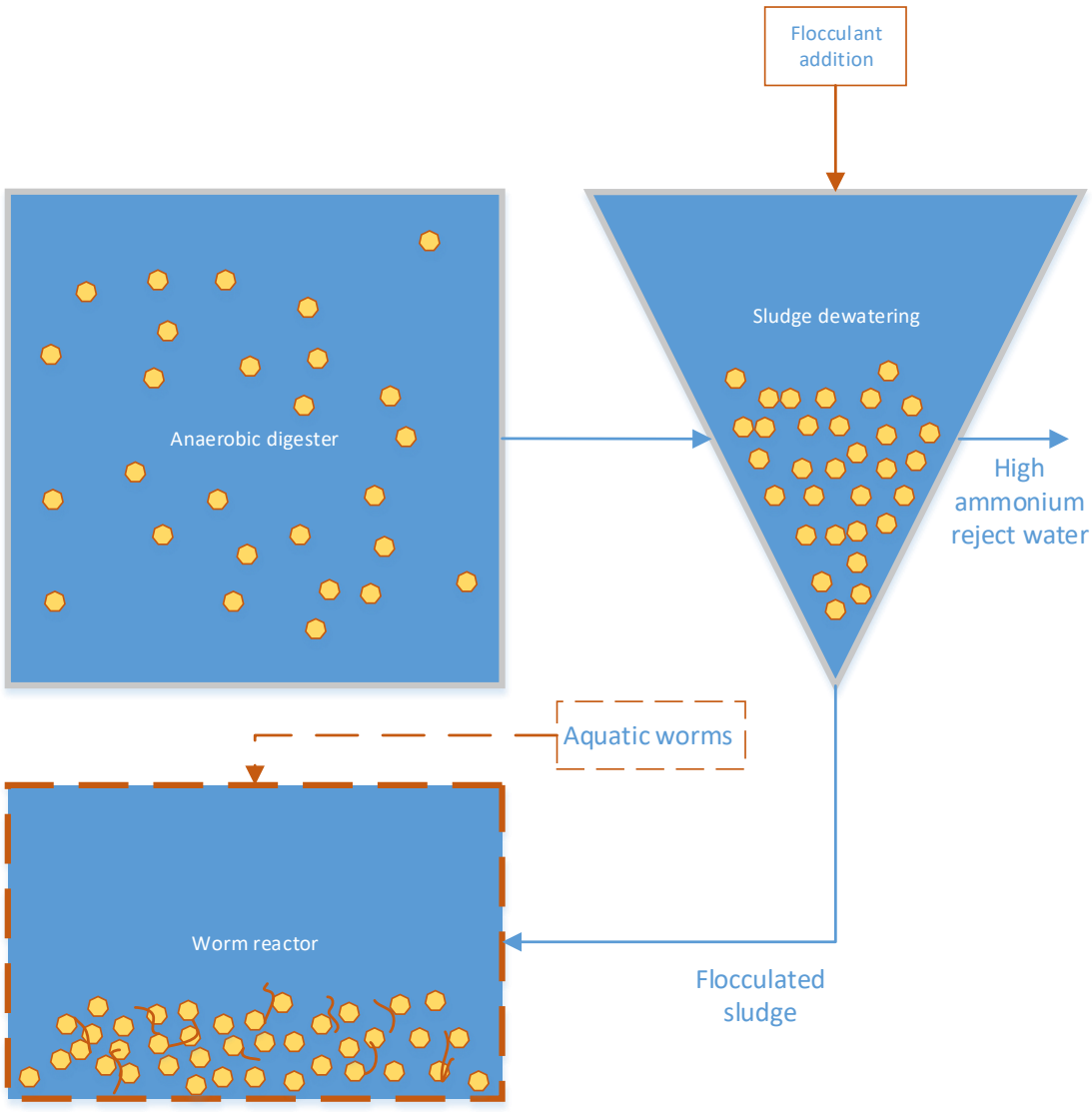


Figure 1.15. Schematic overview of setup where a dynamic membrane is applied to uncouple the hydraulic retention time and solids retention time. Flocculants can be added to improve the filterability and the rheology in the anaerobic digester.

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2. Influence of chemically enhanced primary treatment on anaerobic digestion and dewaterability of waste sludge

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2.1. Abstract

To lower energy consumption at a sewage treatment plant (STP), primary settling could be enhanced to direct more COD to anaerobic digestion (AD) for increased biogas production and decreased aeration. Primary settling can be chemically enhanced by applying flocculants. Flocculants are refractory compounds that may affect all sludge treatment facilities. In this study the consequences are investigated of the application of flocculants in chemically enhanced primary treatment (CEPT) on AD and subsequent dewatering of digested sludge in a conventional STP. It was found that flocculants maintain their effect throughout all sludge processing facilities. With CEPT, more readily degradable solids were removed, resulting in a higher bio-methane potential of the primary sludge. In AD, flocculants lowered the viscosity, meanwhile an increased hydrolysis rate was observed. But flocculants also partially irreversibly bound substrate in such way that it is not available for biological degradation anymore. In subsequent dewatering of digested sludge, a higher dry solids concentration was observed with CEPT. A computer simulation showed that in a conventional biological nutrient removal (BNR)-STP, CEPT would not be economically feasible. However, several benefits were discussed that can make CEPT an interesting option for future low COD/N tolerant STPs with, for example, main stream Anammox processes for N-removal.

2.2. Introduction

In industrial wastewater treatment, there are many different applications for flocculants. For example, the solids of poultry or pig manure can be concentrated using cationic flocculants to allow for smaller anaerobic digester volumes (Campos et al., 2008; Liu et al., 2016; Møller et al., 2007). In an upflow anaerobic sludge blanket (UASB), pre-treatment of the flocculent biomass with cationic flocculant can improve the COD removal performance at low hydraulic retention times (HRTs) (Garcia et al., 2008). Uncoupling HRT and solids retention time (SRT) can be applied in a continuously stirred tank reactor by returning flocculated solids from the effluent using flocculants (Cobbledick et al., 2016). In conventional sewage treatment plants (STPs), the application of flocculants is commonly limited to sludge dewatering. Using flocculants in the primary settler, however, could lead to a more positive energy balance of STPs: more influent COD could be used for biogas production via anaerobic digestion (AD), instead of the current aerobic oxidation of COD in the aeration tanks.

Chemically enhanced primary treatment (CEPT) with flocculants is thus far not combined with conventional biological nutrient removal (BNR)-STPs because of the critical COD/N ratio

for denitrification. Since COD is required for denitrification, extensive COD removal by CEPT can compromise efficient nitrogen removal due to a lack of COD for denitrification (Van Nieuwenhuijzen et al., 2000). According to the electron balance, the minimum amount of COD required for nitrification and subsequent denitrification is 2.86 gCOD/gN. However, the organisms in sewage treatment require COD for growth as well. Therefore, in practice, a COD/N ratio of 6 to 30 gCOD/gN is required (Golterman, 1985; Roy et al., 2010; Sobieszuk and Szewczyk, 2006; Yadu et al., 2018). Recently, novel low COD/N tolerant N removal technologies were introduced such as N removal over nitrite or Anammox processes in the waterline of the STP (Hoestra, 2017; Lotti, 2016). With Anammox, for example, a COD/N ratio as low 1.2 to 2.5 gCOD/gN is shown to be sufficient for N removal in sewage (Hoekstra, 2017; Ji et al., 2018; Lotti, 2016; Miao et al., 2018; Wang et al., 2018;). With the perspective of low COD/N tolerant BNR-STPs, CEPT with flocculants could be advantageous for the energy balance and space requirements of a BNR-STP in the future.

However, there is not much known about the effects of flocculants on anaerobic digestion and subsequent sludge treatment facilities. It is known that flocculants are not readily biodegradable anaerobically (Chang et al., 2001; Chu et al., 2003). Therefore, flocculants applied in CEPT may retain their flocculating capacity throughout all subsequent sludge processing facilities and affect the entire STP. For example, flocculants present in the anaerobic digester are hypothesized to reduce the polymer consumption for subsequent sludge dewatering (Cobbledick et al., 2016). But also, the solids content of dewatered sludge may be improved. Since sludge disposal is one of the largest cost factors of sewage treatment and can amount up to 50% of the total operational costs (Spinosa and Vesilind, 2001), small differences in dry solids percentage of dewatered sludge can have large effects on the operational costs of STPs.

This study presents a renewed perspective on the application of CEPT, using solely organic flocculants and coagulants (referred to as flocculation aids, or FAs in this section) to increase primary sludge production and enhance dewaterability of the digested waste sludge. Also, the effects of the presence of FAs on the AD process are investigated. Finally, a mathematical simulation of a reference STP using the Biowin software package was performed in which the experimental results were used, to determine the economic effects of CEPT.

2.3. Materials and methods

2.3.1. Chemically enhanced primary treatment experiment (CEPT Experiment)

The *CEPT Experiment* is a settling experiment. Results will show the effects of FAs in the primary settler, meanwhile flocculated and settled sludges are being produced to be used in the AD and dewatering experiments. The *CEPT Experiment* was performed at STP Leiden Noord, The Netherlands (150.000 P.E.), during dry weather flow conditions. Four grab samples of 80 L were collected at the same time, after coarse screening. The samples are representative for the influent at this STP, since measured concentrations were in the range of the yearly averages for COD (557±120 mg/L), TN (53±10 mg/L) and TP (9.3±1.8 mg/L). Three different settling situations using flocculants were compared to the reference settling

(situation RS), where there was settling without dosing flocculants (Table 2.1). The used FAs were cationic flocculant (situation C), anionic flocculant (situation A) and a mix between coagulant and cationic flocculant (situation M). The applied concentrations were based on optimal turbidity removal (data not shown). Flocculants were added during 3 minutes of vigorous stirring. Then 60 s of slow stirring was followed by a 30 min settling period. The supernatant water was syphoned off.

Table 2.1. Overview of settling, BMP and SMA experiments performed in this study. FA = flocculation aid; C_{FA} = cationic flocculant; A_{FA} = anionic flocculant; M_{FA} = organic coagulant.

Reference in paper	Type of experiment	Flocculant dosage or substrate	Description
CEPT Experiment	Primary sedimentation	Substrate: influent at STP Leiden Noord Situation RS*:* no FAs Situation C: 10 ppm C_{FA} Situation A: 10 ppm A_{FA} Situation M: 10 ppm M_{FA} and 2 ppm C_{FA}	Screened influent of STP Leiden Noord was settled, with and without CEPT
BMP_1	BMP test	Substrate: mixture of secondary sludge and sludge samples of *CEPT experiment*	Sludge samples of *CEPT Experiment* were used in BMP test
BMP_2_C_{FA}	BMP test	Substrate: Secondary and primary sludge Flocculant dosage: 0, 5, 7.5 and 10 g/kg C_{FA}	BMP test with primary and secondary sludge as substrate, with C_{FA} in different concentrations added to the BMP test bottles at t=0
BMP_3_Unstirred	BMP test	Substrate: Secondary and primary sludge FA dosage: 0 and 5 g/kg C_{FA}	Unstirred BMP test with and without C_{FA} addition at t=0
SMA_FA	SMA test	Substrate: acetate FA dosage: 5 g/kg C_{FA}	SMA test with flocculated and non-flocculated inoculum

The FAs (Table 2.2) were selected from a set of commercially available FAs. The applied concentrations (Table 2.1) were based on turbidity removal performance in screened dry weather wastewater of STP Leiden Noord. Both cationic and anionic poly-electrolytes were used, as well as an organic coagulant.

Table 2.2. Flocculants and coagulant used for the experiments on the flocculation of sludge.

Name	Reference in paper	Type	Active compounds	MW	Charge	COD [mg/g]
Core Shell	C_{FA}	Cationic organic flocculant	Acrylamide based polymer	-	Low	86±3
Ultimer	A_{FA}	Anionic organic flocculant	Acrylamide based polymer	Medium MW	Medium	23±1
Nalco	M_{FA}	Organic coagulant	Poly-ampholytic	High MW	-	42±2

2.3.2. Biomethane potential and specific methanogenic activity tests

Biomethane potential (BMP) tests assessed the extent of methane production (Angelidaki et al., 2009; Holliger et al., 2016). The BMP tests performed are summarized in Table 2.1. In all BMP tests the substrate consisted of primary sludge, either from the *CEPT Experiment* or directly taken from the STP Leiden Noord, and secondary sludge. The substrate, i.e. primary and secondary sludge was mixed in a 3:1 volatile solids (VS) ratio, respectively, to resemble the composition fed to the anaerobic digester at STP Leiden Noord. As an inoculum, digested sludge of STP Leiden Noord was used with total suspended solids (TSS) concentration of 33.6±0.4 g/L and volatile suspended solids (VSS) concentration of 23±0.5 g/L. A VSS inoculum:VS substrate ratio (VSS_I/VS_S) of 2 was used. A control with only inoculum was included to correct for the biogas produced by the inoculum in the *BMP_1* experiment. All BMP tests were done in triplicates in bottles of 400 mL and were incubated in a New Brunswick Scientific Innova 44 at 35 °C and stirred at 150 rpm, except for the experiment under non-stirred conditions. The produced biogas was scrubbed with a 3M NaOH solution to remove the CO_2. The methane production was measured over time with an automated methane potential test system (AMPTS), Bioprocess Control (Lund, Sweden). The BMPs were terminated when three consecutive days of <1% biogas increase was measured (Holliger et al., 2016) except for the *BMP_3_Unstirred* experiment, where we were only interested in the initial rate of digestion. The specific methanogenic activity (SMA) test was performed with the same equipment and settings. The $VSS_{inoculum}$:$COD_{substrate}$ ratio was 2 with sodium acetate in a buffer (10 mM phosphate, pH 7) together with micro and macro nutrients according to Zhang et al. (2014).

2.3.3. Analytical techniques

Particles counting was performed with a Met One particle counter in the size range of 2-100 µm and equipped with a LB1020 sensor (Beckman Coulter, Bra, United States). An Anton-Paar USD200 rheometer with Z2 DIN and TEZ 180 bob (Graz, Austria) measured the viscosity of the sludge mixtures. Merck kits (Germany) determined the ammonium-N (10 – 150 mg-N/L), COD (25-15000 mg L^{-1}) and P concentrations (0.015 – 5 mg-P L^{-1}). Total solids (TS), VS, TSS, and VSS were measured according APHA (1999). The protein measurements were done with protein reagent 0.01% (w/v) Coommassie Briljant Blue, 4.7% (w/v) ethanol and 8.5% (w/v) phosphoric acid with a BSA calibration line and measured at 595 nm (Bradford, 1976).

Turbidity was determined with a Hach 2100N turbidity measurement device. Fluorescence spectra were recorded using a Perkin-Elmer LS-50B luminescence spectrophotometer, which uses a 450W xenon lamp. All samples were diluted with carbon-free electrolyte solution at pH about 7.5. The fluorescence excitation emission matrix (F-EEM) tests were carried out at a concentration of 1 mg C L^{-1} to minimize the inner-filter effect. The acquisition interval and integration time were maintained at 0.5 nm and 0.1 s, respectively. Right-angle geometry was used for liquid samples in a 10 mm fused-quartz cuvette. Three dimensional spectra were obtained by repeatedly measuring the emission spectra within the range of 280-600 nm, with excitation wavelengths from 200 to 400 nm, spaced at 10 nm intervals in the excitation domain. Spectra were then concentrated into an excitation-emission matrix (EEM).

2.3.4. Dewatering parameters

Capillary suction time (CST) measurements were done with a Triton Electronics Type 304m (Essex, England). CST was used to indicate the optimal PE dosing values for the dewatering experiments. For the dewatering test, a mini filter press was used, which was custom made by Mareco (Kortehemmen, the Netherlands). The pressure was set at 3.5 bar with 200s pressing time. The piston speed was set to ~1 cm min^{-1}. The specific resistance to filtration (SRF) tests were done with Grade 1 Whatman filters with 1 bar of pressure and a sludge volume of 100 mL. The values for SRF and blinding index β were calculated according to Novak et al. (1988).

2.3.5. Biowin simulation

To evaluate the impact of CEPT on the activated sludge process, the Biowin 3.1 simulator was used. The STP of Leiden Noord was simulated using a flow of 20.000 m^3 d^{-1} and the standard parameters of Biowin. To simulate flocculation in the primary settler (PS), the removal percentages of the PS in the Biowin package was varied to match the removal observed in the batch experiment and thus varied per FA used: 60% for the reference situation (RS), 80% for M and 95% for C. Also the ortho-phosphate fraction (fPO4) was adjusted to obtain a similar phosphate removal as in the flocculation tests. The operational temperature was set to 15 °C.

2.4. Results and discussion

In order to determine the effects of FAs on anaerobic digestion and sludge dewatering, primary sludge was produced in the *CEPT Experiment*. As expected, settling situation C and settling situation M showed a significant improvement in terms of COD, TSS and VSS removal, when compared to situation RS (Table 2.3). Dosage of 10 ppm C_{FA} (settling situation C) showed a 66% increased COD removal, 120% increased TP removal and 45% increase in TSS removal. During settling situation M, these removal efficiencies increased with 47%, 88% and 28%, respectively. Application of 10 ppm A_{FA} (situation A) did not improve the removal at all which can be explained by the fact that particles in domestic sewage are negatively charged (Elmitwalli et al., 2001; Rytwo et al., 2014) and thus are generally not bound by the negatively charged anionic FAs.

Table 2.3. Supernatant and influent characteristics of the CEPT Experiment. Samples were taken at STP Leiden Noord (150.000 P.E.).

	COD [mg/L]	**TP** [mg/L]	**TN** [mg/L]	**TSS** [mg/L]	**VSS** [mg/L]	**Turbidity** [NTU]
Supernatant situation RS	324±11	7.9±0.1	60±1	106±0	89±2	71
Supernatant situation C	206±14	6.4±0.0	55±1	45±14	35±2	25
Supernatant situation A	326±17	8.0±0.1	60±2	101±20	76±13	67
Supernatant situation M	240±11	6.8±0.0	54±4	68±2	58±1	38
Influent CEPT Experiment	503±7	9.2±0.1	63±5	240±10	207±4	99

2.4.1. Anaerobic digestion

During a BMP test (*BMP_1*), the methane production per gram VS of primary sludges from the *CEPT Experiment* was followed over time (Figure 2.1). The produced methane of the sludges during the first two days were comparable for all samples. The produced methane of sample A was similar to the methane produced by sample RS over the whole period. This was expected because situation A did not show any improved removal during settling and therefore is not likely to have affected the settled sludge in composition. However, between two and five days, samples C and M displayed a significantly ($p<0.1$) higher methane production rate. Also, the BMPs of samples of situation C and M were significantly higher than the BMP of sample RS.

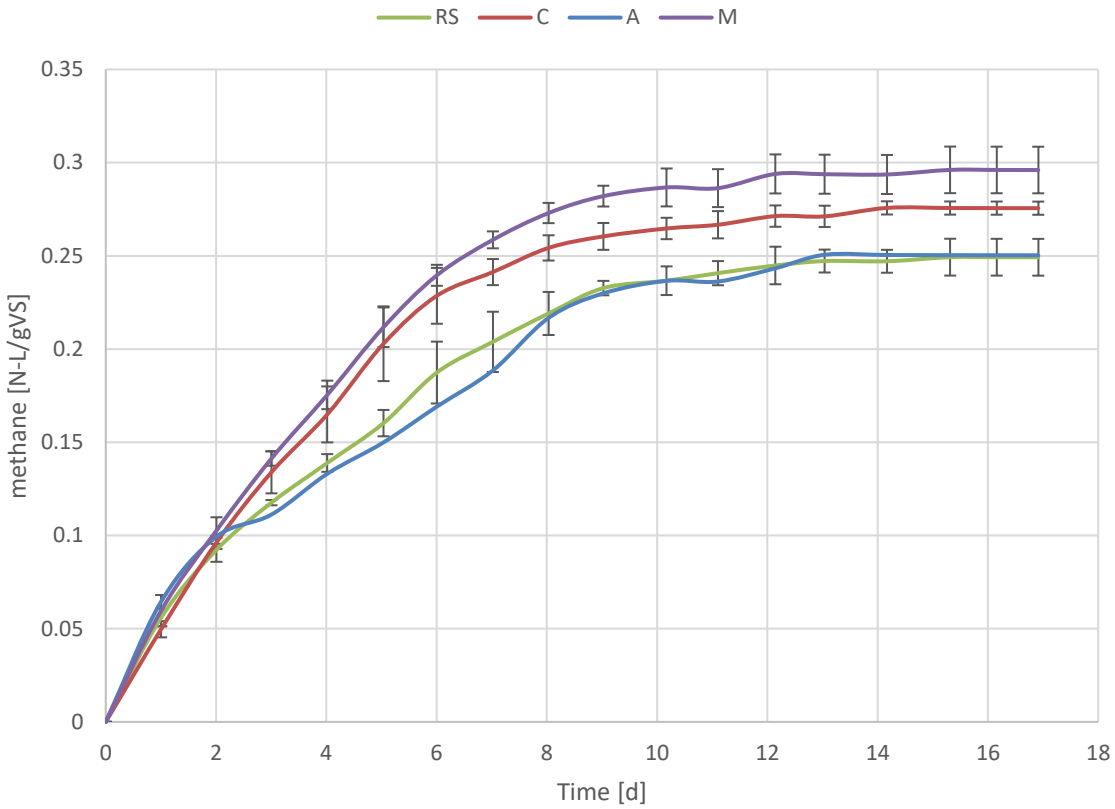

Figure 2.1. Methane production during the BMP (BMP_1) test in N-mL methane per g VS substrate of primary sludges of the CEPT Experiment. C refers to sludge produced from sewage influent with 10 ppm cationic flocculation aid (FA); A refers sludge produced by with 10 ppm anionic FA; and M refers to sludge produced with 10 ppm organic coagulant and 2 ppm cationic FA. RS is the reference situation where sludge was produced by plain settling without FA additions.

Since FAs could be partially anaerobically digested (Campos et al., 2008; Chang et al., 2001), the added FAs may have contributed to the biogas production for samples C and M. However, the COD added by the addition of FAs was too low (equivalent to <1 mL CH_4 gVS^{-1}) to explain the differences in BMP and methane production rate. With CEPT, additional small particles can be removed that would not have settled without FA dosing. These particles may have had a different biodegradability and therefore may have caused the increased digestion rates and higher BMPs for C and M. To distinguish between the physiological effects on the sludge that FAs establish, such as change in viscosity, and the difference in flocculated material (substrate), a new experiment was performed: *BMP_2_C_{FA}*. In *BMP_2_C_{FA}*, cationic FA (C_{FA}) was added in different concentration at t=0 to the incubation bottles of the BMP experiment with the same quantity of primary and secondary sludge as substrate. The methane production is shown in Figure 2.

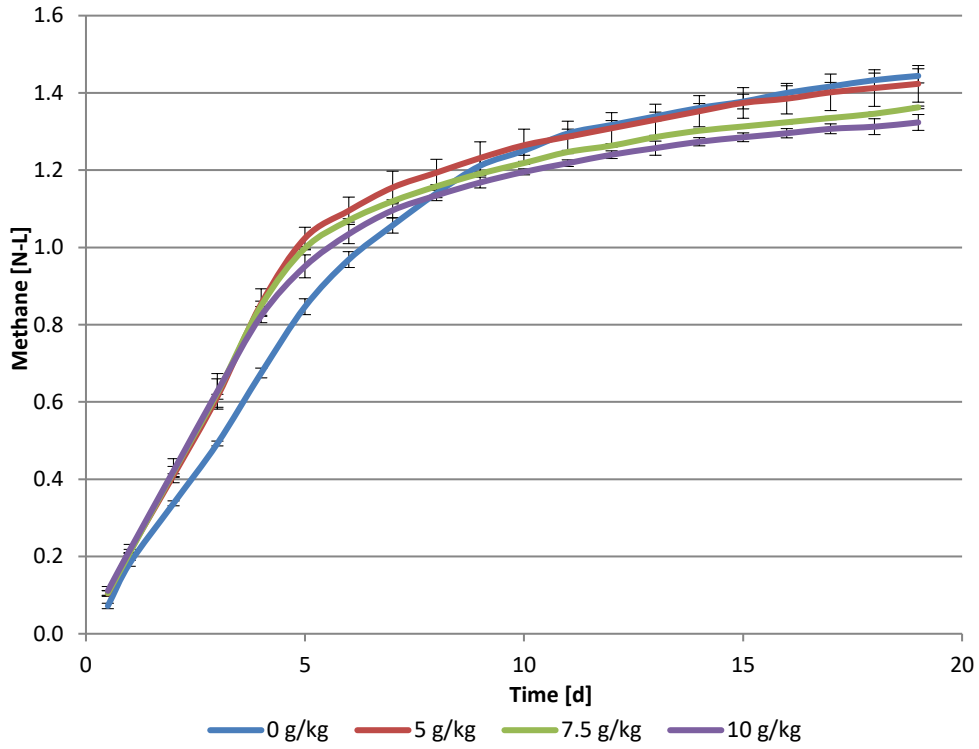

Figure 2.2. Methane production during a BMP test with primary, secondary sludge as substrate. C_{FA} was added directly to the BMP test bottles at t=0 in different concentrations (BMP_2_C_{FA}). All samples contained the same amount and type of primary and secondary sludges.

In *BMP_2_C_{FA}*, a significant decrease in BMP was found with an increased C_{FA} addition (7.5 and 10 g kg^{-1}), when results were compared to the BMP without additions (0 g kg^{-1}). This indicates that increased BMP values in *BMP_1* of samples C and M flocculated sludge is attributable to an increased fraction of readily biodegradable material in these sludges, which were removed from the sewage during the *CEPT Experiment*.

Decreased biodegradability in *BMP_2_C_{FA}* due to FA addition was observed before in literature. Chu et al. (2003) showed that the BMP of waste activated sludge was lowered with a high cationic flocculant dosage (>5 g kg^{-1}). Chu et al. (2005) hypothesized that substrate mass transfer resistance causes decreased biodegradability. However, mass transfer resistance may lower the rates of AD, but will not determine the BMP, since it is a measure for the ultimate biodegradability of the substrate. Chang et al. (2001) reported that parts of the flocculant are not available for anaerobic digestion. The structure of these refractory fractions might shield substrate from hydrolysis when irreversibly bound, which could be the reason for the observed reduced BMP values observed in literature and in *BMP_2_C_{FA}*. This reduced BMP was observed earlier with the digestion of flocculated sludge (Campos et al., 2008). It was postulated that there was an accumulated of refractory colloids. However, no proof was presented for this hypothesis.

The rates of anaerobic digestion in BMP_2_C_{FA} were also affected by the addition of C_{FA}. The initial digestion rates were enhanced significantly ($p<0.1$) in all the flocculated samples compared to the non-flocculated sample (0 g kg^{-1}): the production rate in the first four days was 169±6 N-mL d^{-1} for the non-flocculated sludge batch and 214±3, 212±0 and 206±0 N-mL

d^{-1} for the 5, 7.5 and 10 g kg^{-1} FA-batches, respectively. On day 1, the volatile fatty acids (VFA) concentration of the non-flocculated sludge batches were below the detection limit, indicating a direct conversion of VFA intermediates to methane. Apparently, methanogenesis was not the rate limiting step during the BMP assays. However, on day four, 84, 125, and 169 mg L^{-1} of total VFA was found in the 5, 7.5 and 10 g kg^{-1} FA-batches, respectively. An SMA test with flocculated and non-flocculated inoculum (*SMA_FA*) revealed that the methanogenic activity was slightly lowered by the addition of C_{FA} yielding an SMA of 0.19±0.00 gCOD $gVSS^{-1}$ d^{-1} for the non-flocculated and 0.18±0.00 gCOD $gVSS^{-1}$ d^{-1} for the flocculated sludge. Based on these SMA results, the expected maximum rate of methane production in this setup was 206 N-mL d^{-1} for the non-flocculated and 195 N-mL d^{-1} for the flocculated sludge batches. The observed increased methane production rates at the start of the experiment indicated that the initial hydrolysis rates were enhanced by the addition of C_{FA}. On the other hand, the observed VFA accumulation indicated that the methanogenic activity became rate limiting.

In literature, a similar phenomenon was observed by Chu et al. (2003), who reported that the addition of cationic FA to waste activated sludge digestion yields increased initial methane production rates as well, however this was not statistically supported. In addition, Li et al. (2013) reported about the enhanced digestion rates in the presence of cationic FA. It was postulated that increased digestion rates were the result of enhanced microbial interactions due to the reduced distance between acidogens, acetogens, methanogens, with the addition of cationic FA. However, no evidence was presented for this hypothesis.

We hypothesize that FA addition indeed increased the hydrolysis rates by the increased interactions between inoculum bacteria and the substrate surface. Increased enzymatic hydrolysis effects can be provoked by higher particulate substrate concentrations in the vicinity of the organisms excreting the exo-enzymes. Obtained results supports this hypothesis, since in *BMP_2*, where FA was added to a mixture of substrate and inoculum, all solids (inoculum and substrate) were effectively bound, thus provoking reduced distances between the exo-enzyme producers and the substrate.

2.4.2. Fluorescence excitation emission matrix (F-EEM) analyses

To investigate if FA addition enhances the interaction between substrate and exo-enzymes, the protein concentrations and the protein nature of the supernatant on day 7 of BMP_2_C_{FA} were examined. If these interactions were indeed enhanced by binding exo-enzyme producing organisms and substrate together, lower exo-enzyme concentrations in the bulk would be expected. Tryptophan is a microbial synthesized amino acid and its concentration is an indication to what extend the proteins present in the supernatant are of microbial nature (Vivian and Callis, 2001). With a F-EEM measurement of the bulk liquid (Figure 2.3) it was observed that with increasing FA concentration, less microbiologically derived proteins, characterised by tryptophan, could be observed in the supernatant on day 7, whilst the total amount of proteins in the sample was similar in all bottles (RS: 59.3±0.6 mg/L, C: 52.4±0.6 mg L^{-1}, A: 52.2±2.2 mg L^{-1}, M: 51.1±1.0 mg L^{-1}). The lower fraction of microbial proteins in the supernatant of flocculated batches is an indication that substrate and protein – among which exo-enzymes – synthesizing organisms were bound together

leading to less exo-enzymes being free in the supernatant. These observations support our above described hypothesis.

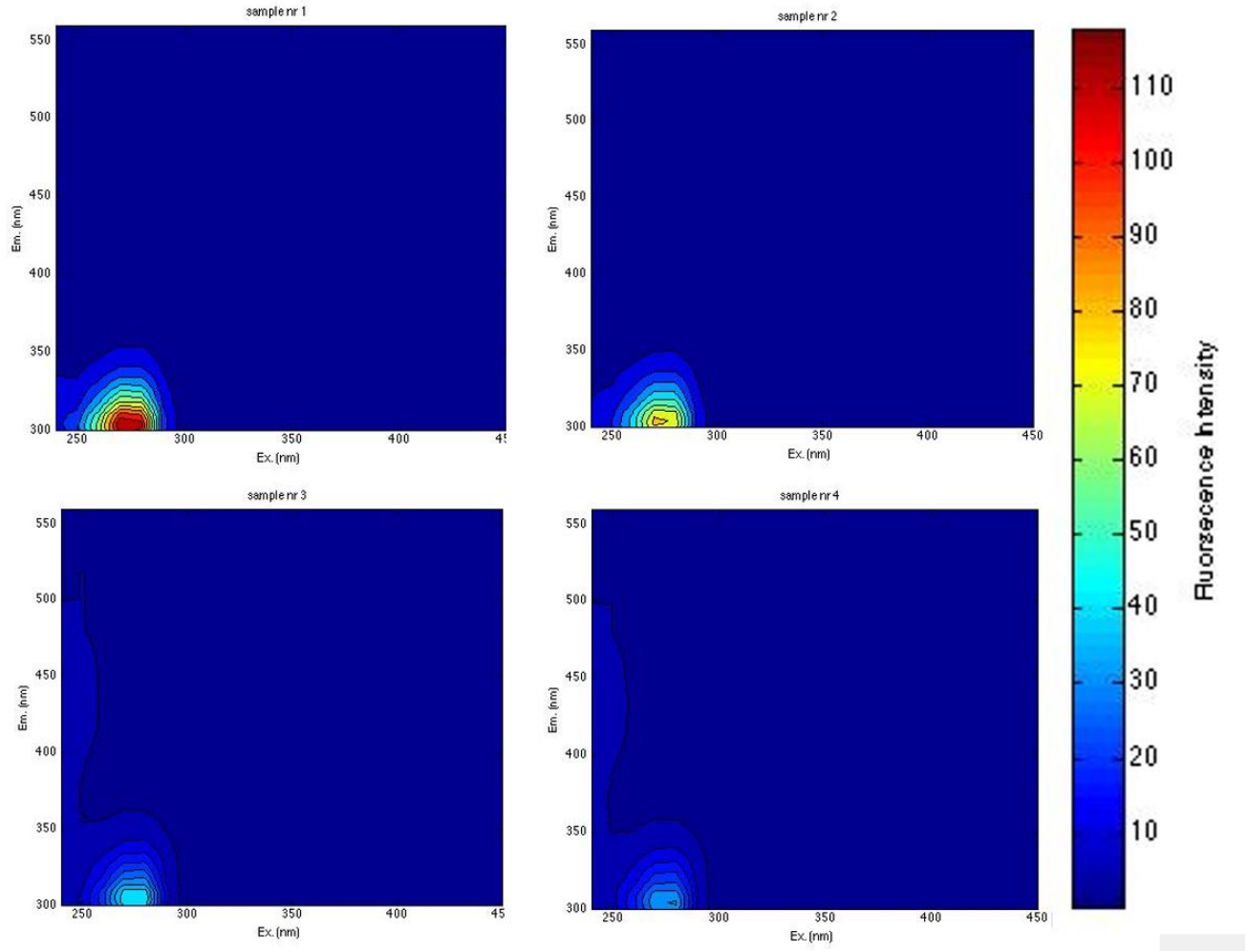

Figure 2.3. F-EEM images of the supernatant of BMP_2_C_{FA} on day 7 with 0 g kg^{-1} (sample nr. 1), 5 g kg^{-1} (sample nr. 2), 7.5 g kg^{-1} (sample nr. 3) and 10 g kg^{-1} (sample nr. 4). On 275 nm (Ex.) and 310 nm (Em.) is the area where fluorescence of tryptophan, characterising microbial derived proteins, is located. The higher the density in this area, the more microbially derived protein is present.

If interactions between exo-enzyme producing organisms and the substrate were enhanced by dosing FA, this enhancing effect on the digestion rate should become more profoundly visible when dosing FAs to a BMP test where no stirring is applied. This is because in case of an absence of stirring, the substrate and exo-enzyme interaction is only prone to diffusion and not by convection. Under these non-stirred conditions, the hydrolysis is likely to benefit most from the enhanced substrate – exo-enzyme interactions. This was tested, but no significant difference could be observed in digestion rates between the flocculated and non-flocculated samples in non-stirred conditions (*BMP_3_U* in Figure 2.4). Apparently, the chosen test set-up was insufficient to make this effect visible. The viscosity of the non-flocculated batch was 0.268 Pa.s while the flocculated batch was 0.226 Pa.s, thus 19% lower.

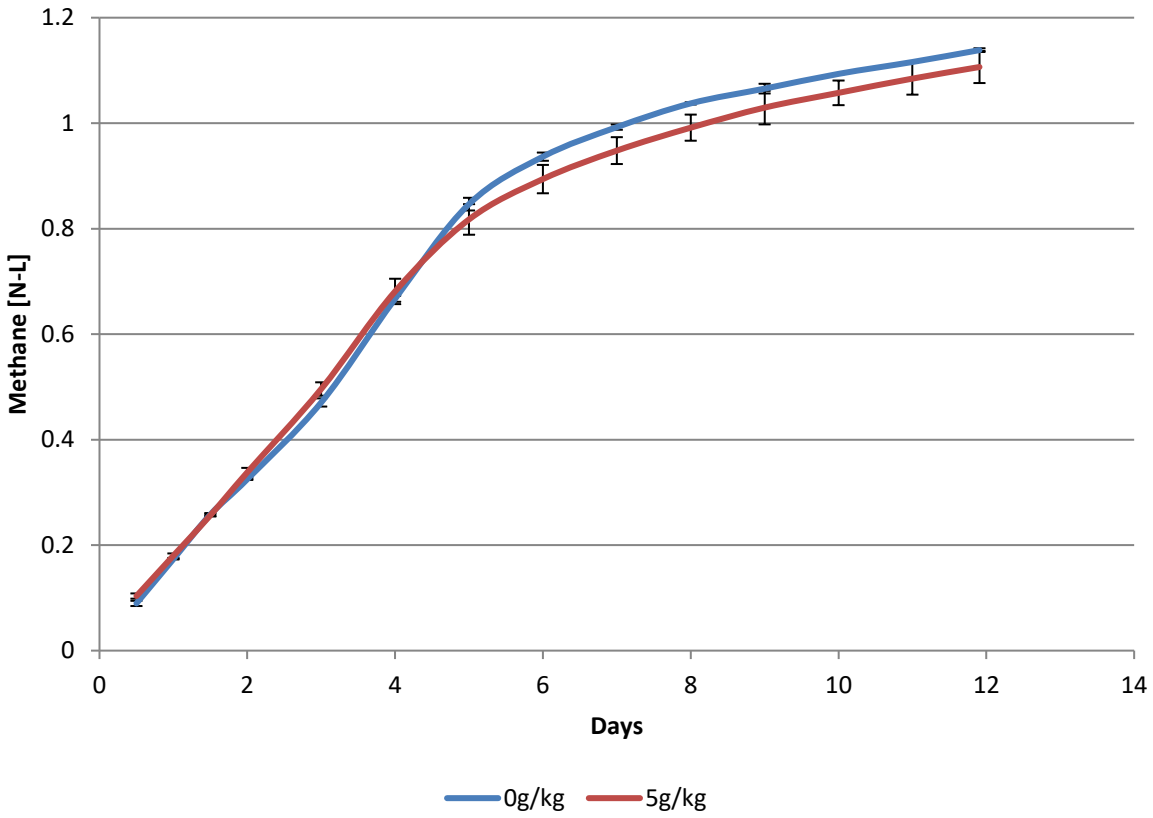

Figure 2.4. BMP test with non-stirred conditions (BMP_3_Unstirred) with flocculated sludge (5 g kg-^{1}) and non-flocculated sludge (0 g kg^{-1}).

To the authors' knowledge, the effect of viscosity on the hydrolysis in AD has not been studied to date. The effect of viscosity on hydrolysis in human intestines, however, has been studied in detail. Increasing the viscosity in human intestines is shown to lower the hydrolysis rate of starch (Dartois et al., 2010; Ellis et al., 1988). The relation between hydrolysis rate and viscosity could be explained by the diffusion rates of enzymes and hydrolysis products, which are enhanced by decreasing the viscosity (Einstein, 1905). Low concentrations of hydrolysis-products favour the enzymatic hydrolysis rates because of minimised product inhibition. Lower diffusion rates of hydrolysis products in high viscous solutions results in higher local product concentrations, subsequently leading to lower conversion rates. Therefore, alternative to enhanced microbial binding to the solid substrate, the increased hydrolysis rates observed in this study, also may be explained by the lowered viscosity of the medium due to the presence of C_{FA} or M_{FA}. However, further research is needed in which a kinetic model can help to understand the factors involved in the increased hydrolysis rates (Zhen et al., 2015).

2.4.3. Sludge dewatering

As expected, due to their refractory characteristics, the FAs were capable of altering the sludge characteristics throughout the digestion process. CST results of the different digested sludges (of equal TS concentration) of *BMP_1*, shown in Table 2.4, indicate a positive effect of dosed FA (C_{FA} and M_{FA}) after digestion. The lower CST values in the flocculated sludges (C

and M) compared to non-flocculated sludges (RS) are probably due to the lower fraction of small particles in the flocculated sludges (Figure 2.5).

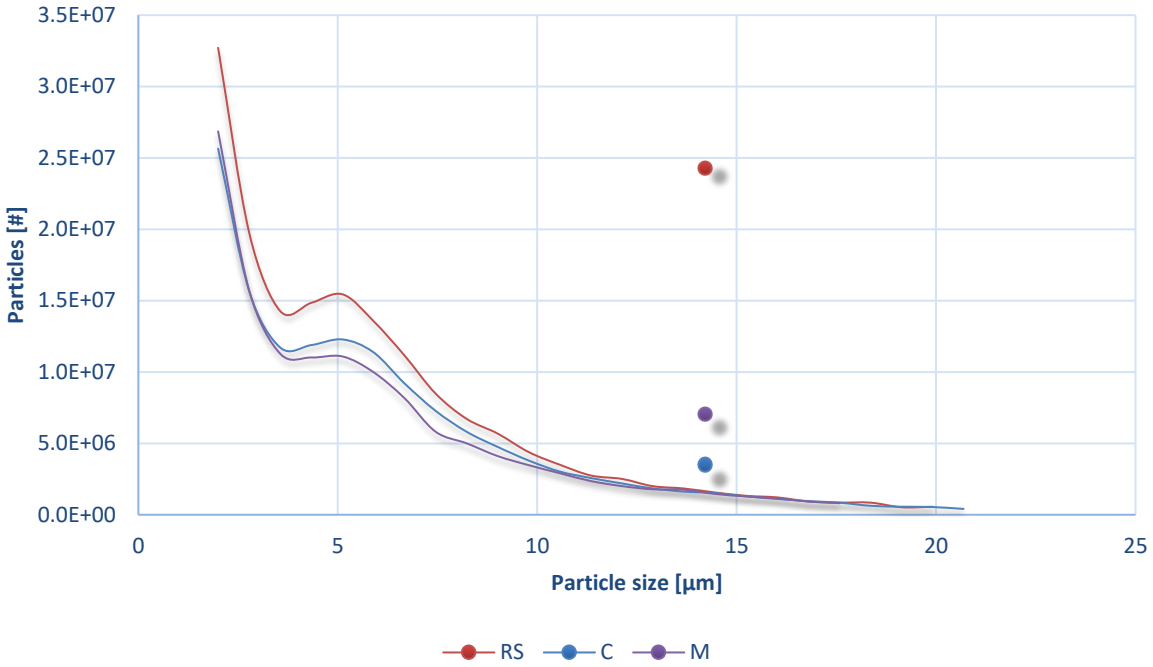

Figure 2.5. Particle count of the digested flocculated sludges (C and M) and the digested reference sludge (RS).

The application of CEPT (C and M) increased the percentage cake dry solids content when the sludge mixture was dewatered with the Mareco bench scale filter press compared to RS (Table 2.4). The best dewatering results were obtained with C_{FA} flocculated sludge but also the sludge treated with M_{FA} shows improved cake DS content compared to the digested raw settled sludge.

Table 2.4. Dewatering characteristics: capillary suction time (CST), specific resistance to filtration (SRF) and blinding index (β, positive value indicates blinding).

Sample	Cake	Sludge dewatering variables			Effluent filter press		
	Cake DS	**CST**	**SRF**	**B**	**Proteins**	**TP**	**COD**
[-]	[%]	[s]	[cm/g]	[-]	[mg/L]	[mg/L]	[mg/L]
RS	18.8±0,4	159±11	3.99E+10	0.037	46.4±0.0	175±11	549±10
C	20.0±0,7	130±13	3.80E+10	0.089	39.3±0.0	167±1	472±6
M	19.3±0,1	128±12	3.43E+10	-0.221	38.2±0.1	165±1	510±8

2.4.4. Evaluating Economic consequences using Biowin simulations

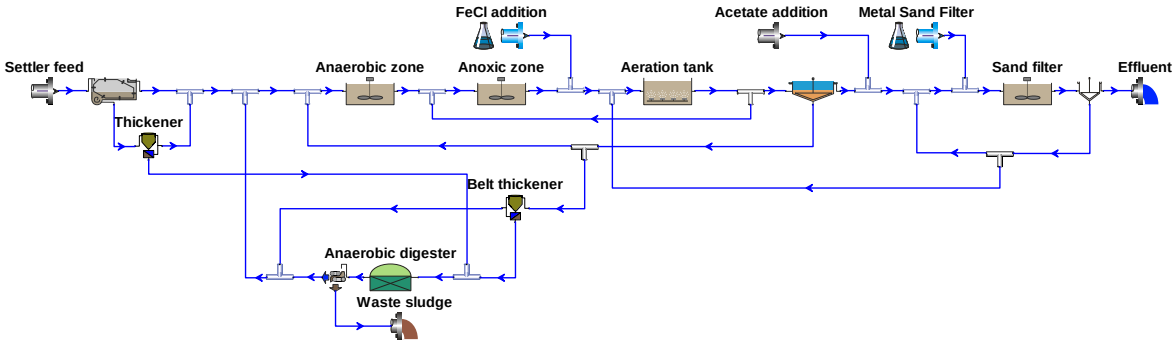

Figure 2.6. Biowin print-out representing a schematic overview of the STP Leiden Noord including CEPT combined with anaerobic digestion of excess sludge (150.000 P.E.).

The evaluation of the possible economic consequences of CEPT application in combination with anaerobic digestion was based on a steady-state Biowin simulation (Figure 2.6) of the STP Leiden Noord (the Netherlands) during dry weather flow conditions. The addition of flocculants resulted in a lower COD load fed to the water line; higher biogas production rates and an increased dewaterability and thus lower sludge disposal costs (Table 2.5). Since the COD/N ratio for STP Leiden Noord is already too low for complete biological denitrification, additional denitrification capacity is reached by sand filtration with acetic acid dosage. Therefore, flocculating/coagulating COD in the primary settler increases the acetate demand, impacting the operational costs considerably. The simulation shows that a COD/N of 8.88 would be sufficient for biological denitrification without acetate dosing.

Table 2.5. Operational costs of STP Leiden Noord in the reference situation (RS+A) and with the application of 10 g kg^{-1} cationic flocculant (C) and 10 g kg^{-1} coagulant (M) in the primary settler.

	RS	M	C
	[EUR/d]	[EUR/d]	[EUR/d]
Solids disposal	1639	1584	1535
Energy cost belt filter	7.5	7.5	7.5
Costs PE	297	295	297
Costs Acetic acid	273	373	518
Costs Floc./Coag.	0	696	480
Costs FeCl	475.9	486.0	486.0
Costs aeration	255	242	240
Biogas revenue	177	221	257
Balance	**3124**	**3905**	**3821**

Following the Biowin simulations, the final digested sludge disposal for C and M flocculated sludge is decreased compared to RS, but it could not compensate for the increased costs of acetic acid and of the FA itself. In a BNR-STP with a higher COD/N ratio or with N removal over nitrite or autotrophic N removal, the outcome can be different. In these cases, the dosage of a cationic coagulant in the primary settler can be considered due to the avoided sludge disposal costs and increased biogas production. Moreover, the footprint of the BNR-STP can be decreased, since the dosage of FA will allow the use of a belt filter instead of a primary settler plus a sludge thickener. This will also lead to a higher dry solids content that can be fed to the digester, and thus to a lower digester volume. The effect of this last aspect on the anaerobic digestion process itself should be further studied.

2.5. Conclusion

FAs added to the primary settler maintain their effect throughout all sludge processing facilities. In the primary settler, more readily degradable biomass is removed by flocculation, resulting in a higher BMP of the primary sludge. In the anaerobic digester, FAs lower the viscosity which correlates to an increased hydrolysis rate. The latter is either caused by increased diffusion rates of the metabolic intermediates due to the lowered viscosity, or by the enhanced binding of hydrolytic biomass to the solid substrate. However, FAs addition also result to irreversible substrate binding, such that it is no longer fully available for anaerobic digestion and thus lowering the biomethane potential. The subsequent dewatering of digested sludge was also affected: an increased dry solids concentration was observed after CEPT. The filterability of the digested CEPT sludge improved and larger sludge flocs facilitated dewatering. A known drawback of CEPT is that

the COD/N ratio can be lowered to such extent that it may compromise the biological denitrification capacity. Therefore, even though increased biogas production and decreased sludge disposal costs can be achieved, the application of CEPT in a conventional STP targeting nutrient removal will not be economically feasible. However, with the prospective of a low COD/N tolerant STP, such as incorporating mainstream Annamox in the treatment, CEPT may become economically feasible. In such case, CEPT will increase the biogas production, decrease sludge production and reduce the footprint of the primary treatment, aeration tank and anaerobic digester.

2.6. Acknowledgements

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3. Perspectives of coagulation/flocculation for the removal of pharmaceuticals from domestic wastewater; a critical view at experimental procedures

This chapter is based on: Kooijman G, Kreuk MK De, Houtman C, Lier JB Van. Perspectives of coagulation / flocculation for the removal of pharmaceuticals from domestic wastewater : A critical view at experimental procedures. Published in Journal of Water Process Engineering 2020;34:101161. doi:10.1016/j.jwpe.2020.101161.

3.1. Abstract

Literature frequently reports that colloids in aqueous matrices sorb a large fraction of pharmaceuticals. Since coagulation/flocculation removes colloids, it is expected that coagulation/flocculation in principle should be useful in concentrating pharmaceuticals in wastewater treatment, which would facilitate the treatment of these refractory compounds. In our present work, we researched the potential of coagulation/flocculation for removing pharmaceuticals from raw sewage. Results from jar tests showed that pharmaceuticals are hardly removed from sewage with coagulation/flocculation. To investigate the discrepancy between reported colloidal sorption and the lack of removal when removing colloids, we tested a commonly applied experimental setup, which makes use of ultra-filtration (UF), for determining the colloidal sorption of pharmaceuticals. The UF method under research was compared with an assessment making use of flocculation. Both methods, UF and flocculation, showed similar removal of colloids. However, during UF, the retention of pharmaceuticals reached values up to 93±4%. In contrast, when removing the colloids with flocculation, no pharmaceutical removal was observed. These results confirm that it is very likely to introduce an analysis bias in using UF membranes in the determination of colloidal sorption of pharmaceuticals. In fact, results predict an over-estimation caused by a direct retention of pharmaceuticals without any binding to colloidal matter. Overall results of the current work show that pharmaceuticals hardly sorb to colloids and herewith the absence of removal of pharmaceuticals during coagulation/flocculation is explained.

3.2. Introduction

In general, pharmaceuticals consumed by humans are subsequently transferred into the sewer through human excreta. Since pharmaceuticals in many cases are recalcitrant towards biological degradation, sewage treatment plants (STPs) often do not completely remove these pharmaceutical compounds (Suárez et al., 2008). Therefore, the main source of pharmaceuticals in surface waters is STP effluent discharge (Gaw et al., 2014). Although the pharmaceutical concentrations in these discharges are low (ng/L to µg/L) (Carballa et al., 2005; Dionisi et al., 2006; Loos et al., 2013; Ternes, 1998), enhanced removal is necessary in order to prevent adverse effects on ecology and accumulation in the aquatic environment, especially when considering the increased pharmaceutical consumption in Europe over time (Fekadu et al., 2018).

3.2.1. Mechanisms of removal of organic micro pollutants with coagulation/flocculation

Treatment of organic micro pollutants (OMPs) in low concentrations, such as pharmaceuticals, is challenging. Despite recent efforts to equip STPs with quaternary micro pollutant treatment (Grelot, 2019), typically, current practice of treatment in STPs is not sufficient yet (Cheng et al., 2017a; Loos et al., 2013; Luo et al., 2014). A feasible strategy to enhance the treatment effectiveness might be to concentrate OMPs in the sludge stream prior to sludge disposal techniques such as incineration and super critical sludge gasification. The research platform of the Dutch Water Authorities showed that supercritical gasification of sewage sludge is potentially a feasible option for future STPs (Korving, 2016) and this treatment would likely destroy all pharmaceutical molecules. In relatively clean water such as drinking water or ultra-pure water, the removal of pharmaceuticals by coagulation/flocculation is very poor (Huerta-Fontela et al., 2011; Stackelberg et al., 2007; Ternes et al., 2002; Vieno et al., 2006). However, adding organic matter before coagulation/flocculation can increase the pharmaceutical removal significantly (Vieno et al., 2006). In wastewaters rich in organic compounds, such as sewage, OMP removal up to 80% is observed using coagulation/flocculation (Carballa et al., 2005; Suarez et al., 2009). Choi et al. (2008) showed that antibiotics are removed to approximately 50% from river water applying poly-aluminium chloride. These results indicate that the presence of organic matter may substantially enhance the removal of pharmaceuticals during coagulation/flocculation.

Coagulation/flocculation comprises of two different processes (Bratby, 2006): 1. the tendency for suspended matter to form larger aggregates promoted by altered surface properties and 2. the removal of dissolved matter by precipitation. To our knowledge, precipitation of pharmaceuticals in wastewater as a mean of removal has not been reported in literature. Therefore, if removal of pharmaceuticals due to coagulants/flocculants is observed, the responsible removal mechanism is likely linked to the removal of suspended matter that acts as a vehicle for pharmaceuticals. This would imply that there is a relation between sorption of pharmaceuticals to suspended matter (expressed by the adsorption-desorption distribution coefficient K_d [L/kg]) and their removal efficiencies by coagulation/flocculation. This hypothesis is supported by results of Carballa et al. (2005) and Suarez et al. (2009), that show that coagulation in raw sewage yields a more or less linear relationship between removal efficiencies and log K_d values of OMPs (Figure 1); the higher the sorption, the higher the removal efficiency. In addition, the observation is made that pharmaceuticals in MilliQ water, so without solids, are hardly removed by coagulation (Vieno et al., 2006).

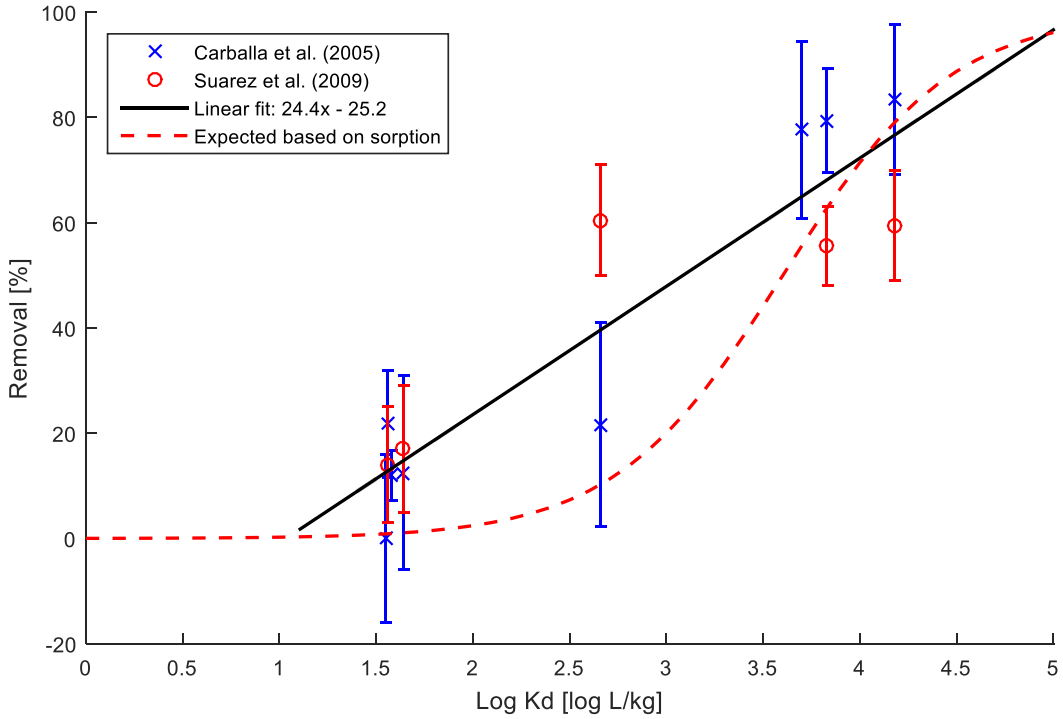

*Figure 3.1. Removal efficiency of pharmaceuticals and fragrances by coagulation of raw sewage measured by Carballa et al. (2005) and Suarez et al. (2009), plotted against their log K_d values (in log L/kg). A linear fit through all data points yielded the following equation: Removal % = 24.4*Log Kd – 25.2, with r^2 = 0.83. The dashed red line represents the percentage of pharmaceuticals sorbed to solids in wastewater with a typical total suspended solids concentration of 250 mg/L, which is in fact the percentage that can be expected to be removed by flocculation/coagulation. The K_d values are taken from Ternes et al. (2004) except for celestolide (taken from Fernandez-Fontaina et al. (2013) and naproxen taken from Barron et al. (2009)).*

3.2.2. Plain sorption mechanism

The pharmaceuticals sorbed to solids, described by the sorption coefficient K_d, may be removed from wastewater by removing the solids. In that case, pharmaceutical removal during coagulation/flocculation can be predicted based on the K_d values. In this study, this mechanism is referred to as *plain sorption*. In Figure 3.1 the percentage of sorbed pharmaceuticals in wastewater with a typical total suspended solids (TSS) concentration of 250 g/L is displayed (red dashed line), plotted against the log K_d. When a suspended solids removal efficiency of 100% due to coagulation/flocculation is assumed, the red dashed line describes the removal of pharmaceuticals by the *plain sorption mechanism*. The formula of this line is given in Eq. 3.1, with K_d as sorption coefficient (in L/kg) and TSS as total suspended solids (in kg/L).

$$\text{Removal percentage} = 100 \cdot \frac{k_d}{\left(\frac{1}{TSS} + k_d\right)} \qquad (3.1)$$

In the higher log K_d range (>3.5), the observed removal percentages correspond well with the percentages of predicted removal. However, in the lower log K_d ranges (<3.5), lower removal is predicted by the *plain sorption* mechanism then what was measured. In order to optimize the removal efficiency of pharmaceuticals, the mechanism of removal by

flocculation and coagulation should be understood, starting with the explanation of the difference in predicted and empirically observed removal in raw sewage.

3.2.3. The colloid mechanism

In Figure 3.1 there seems to be a discrepancy between predicted removal based on the K_d value (red dashed line) and observed removal in the low K_d value range. This discrepancy could be explained by the role of colloids. Colloids are often said to play an important role in the fate of pharmaceuticals (Duan et al., 2013; Thiele-Bruhn, 2003). Table 3.1 shows the sorption to colloids reported in literature which indicates that even pharmaceuticals with low reported K_d values, such as carbamazepine, can have strong affinity with colloids. Since coagulation/flocculation can be applied to remove particulates of colloidal size (Bratby, 2006) and colloids are reported to bind a disproportionally large fraction of pharmaceuticals, colloids could explain the difference between expected removal of pharmaceuticals during coagulation/flocculation and observed removal. The possibility of removal of colloidally sorbed pharmaceuticals with coagulation/flocculation, is referred to as the *colloid mechanism* in this study. With the *colloid mechanism*, a larger fraction of pharmaceuticals can be removed from water with coagulation/flocculation than what is expected based on the K_d value (*plain sorption mechanism*) because there is a disproportionally large fraction of pharmaceuticals sorbed to colloids.

Table 3.1. Overview of reported colloidal sorption of pharmaceuticals and endocrine disruptors.

	Colloid fraction	**Propranolol**	**Sulfonamides**	**Carbamazepine**	**Indomethacine**	**Diclonfenac**	**Estrogens**	**Endocrine disruptors**	**Tamoxifen**	**Meclofenamic Acid**	**Ketoprofen**	**Naproxen**	**Clofibric Acid**	**Ibuprofen**	**Reference**
STP effluent, river water and ground water	1 kDa-7µm	45%	40%	22%	39%	37%									Maskaoui and Zhou (2010)
STP effluent	1 kDa-1.5 µm						1-60%								Holbrook et al. (2004)
STP effluent, river and sea water	1 kDa-0.7 µm							10-29%							Zhou et al. (2007)
STP effluent	1 kDa-0.7 µm	10-40%	4-12%						31-43%	6.5-26%					Yang et al. (2011)
River water	5 kDa-0.7 µm					22-33%					10-14%	17-36%	22-33%	9-28%	(Duan et al., 2013)
Lake water	1 kDa – 0.7 µm		7%-35%												(Cheng et al., 2017b)

3.3. Aims of this study

The aim of this study is to verify if pharmaceuticals can be removed from wastewater by coagulation/flocculation. This was tested by studying the removal of 16 measured pharmaceuticals in raw wastewater when applying coagulation/flocculation. After it appeared that pharmaceuticals could not be removed in the mentioned test, we investigated the discrepancy between reported colloidal sorption of pharmaceuticals and the lack of removal when removing colloids. To this end we tested a commonly applied experimental setup for determining the colloidal sorption of pharmaceuticals. Colloids were removed from a solution containing pharmaceuticals in two ways: by commonly applied ultra-filtration (UF) and by flocculation. The removal efficiencies of pharmaceuticals were compared.

3.4. Materials and methods

3.4.1. Flocculation experiment

The flocculation of wastewater was conducted using municipal sewage of the sewage treatment plant (STP) Leiden Noord, The Netherlands (140.000 P.E.) with the following characteristics: pH = 7.3; T = 18.0°C; TSS = 250.5±6.3 mg/L and volatile suspended solids (VSS) = 185±6.3 mg/L. Raw sewage was collected as a grab sample during dry weather conditions. On this batch of sewage, three types of settling conditions were applied. As a reference condition (RS), sewage was settled without the addition of coagulants/flocculants. In a second batch, cationic acrylamide based low charged flocculant (Core Shell 71305) was dosed to a final concentration of 10 ppm (sample C). The third sample was treated with a mixture of organic coagulant (Nalco 8190; poly ampholitic; high MW) and the cationic flocculant Core Shell 71305 with final concentrations of 10 and 2 PPM respectively (sample M). The flocculant and/or coagulant were added during 3 minutes of vigorous stirring, followed by 60 s of slow stirring and a 30 min settling period. Pharmaceutical concentrations as well as general wastewater parameters were analysed.

3.4.2. Humic substance removal experiments

A test was performed to verify the possibility of an analysis bias in ultra-filtration (UF) for the determination of colloidal sorption of pharmaceuticals. To this end, humic substances (HS) removal in combination with pharmaceutical removal by UF was compared to removal by coagulation/flocculation. A 1.0 g/L stock solution of HS was prepared by adding humic salts (Sigma 53680) into a 100 mM phosphate buffer adjusted to pH 13 and stirred for 1h. The pH was adjusted to 7 with hydrochloric acid and the stock solution was filtered over AP40 glass fibre filters under vacuum. From the stock solution, 100 mg/L HS solutions were prepared for the UF and coagulation/flocculation experiments. The solution contained 43 commonly used pharmaceuticals (Table 3.3) in concentrations of 600, 100 and 20 ng/L (depending on the compound). In half of the samples, HS were removed by coagulation/flocculation using Caldic (Rotterdam, the Netherlands) P1502 cationic flocculant. Pharmaceutical and HS concentrations before and after flocculation were measured in triplicate to determine the removal efficiencies. For the other half of the samples, UF was performed with a ceramic 1 kDa tubular membrane as described in Shang

et al. (2014), operated at 5 bar trans membrane pressure and 1 m/s cross flow velocity. A 20L stock solution was used. After 30 minutes of operating the UF setup, a sample of 1L was taken from the permeate and feed solution and analysed for pharmaceutical and HS concentrations. The pharmaceutical removal efficiencies were determined with clean water removal (10 mM phosphate buffer) as a blank.

3.4.3. Analytical techniques

Test kits (Hach Lange, Germany) were used to measure the concentrations of total phosphorus (LCK 350), total nitrogen (LCK338) and COD (LCK 514). TSS and VSS were measured according to standard methods (APHA, 1999). Turbidity was measured with a Hach 2100N (Hach Lange, Germany). The pharmaceutical concentrations were measured, using an ultra-performance liquid chromatograph coupled to a mass spectrometer (UPLC-triple quad MS; Waters Micromass, United States, MA). Before analysis, 100 mL of the wastewater samples were 10x diluted and pre-treated with solid phase extraction (SPE) using 6CC HLB Waters Oasis cartridges and eluted with HPLC grade methanol. In the analysis, 43 pharmaceutical compounds were measured as named in Table 3.3. The pharmaceuticals were separated by injection of 50 µL extract on an UPLC (Waters Acquity; Waters, Etten-Leur, the Netherlands) equipped with a binary pump, a Waters Acquity UPLC BEH C18 column. The eluate was ionised using electrospray ionisation and the pharmaceuticals were analyzed on a Quattro Xevo triple quadrupole Mass selective Detector (Waters Micromass). Quantification was performed using an external calibration series of 8 concentrations of a standard mixture of the selected pharmaceuticals. Details of the analysis method can be found in Houtman *et al.*, 2013. The recovery of pharmaceuticals from wastewater during the SPE-extraction and analysis on UPLC-tQ-MS was investigated by spiking a parallel sample. Data with the following criteria were included in the results if the concentrations were > 10 ng/L; the recoveries between 50% and 140% and the variation coefficient of removal as < 10% points. Flocculated wastewater was filtered through a Whatman Grade 1 filter (11 µm) and diluted (10x) before analysing the particles size distribution (PSD). The HS concentrations were determined spectrophotometrically by absorption at 465 nm in combination with a calibration line. PSD was determined using a Hiac (Indianapolis, United States) particle counter within the range 0.4 µm - 5 µm. The volume percentages of the particles in the wastewater fractions were determined using a Malvern Mastersizer 2000 and was performed by Delft Solids Solutions in Delft, the Netherlands.

3.4.4. K_d value determination

The K_d values of 43 pharmaceuticals were determined by using an adjusted method of Carballa et al. (2008). 1L of primary sludge from STP Leiden Noord (TS = 17 g/L) was spiked 50 to 1500 ng/L with 43 pharmaceuticals and incubated overnight at 4°C. An unspiked sample was incubated under the same conditions. From both samples, both the solid and the liquid phases were analysed for pharmaceutical concentrations. An extra internal standard spiked before injection in the UPLC showed that there was a strong suppression of the signal by the solid matrix. Therefore, K_d values were determined based on the aqueous

phases of the experiments only. The assumption was made that there is no bioconversion during the over-night incubation.

3.5. Results and discussion

3.5.1. Flocculation of raw sewage

To test the possibility of removing pharmaceuticals from sewage, a jar test was performed. The addition of coagulant (sample M) and flocculant (sample C) showed to have a positive effect on the removal of suspended solids and COD compared to settling without chemical addition (RS). COD removal was enhanced with 54% and 52% in sample C and sample M, respectively. The TSS removal was doubled in sample C and M compared to RS. With flocculant (C) and coagulant (M) addition, the removal of small particles of 0.4 to 5.0 µm (Figure 3.2) was increased with 65% and 50%, respectively.

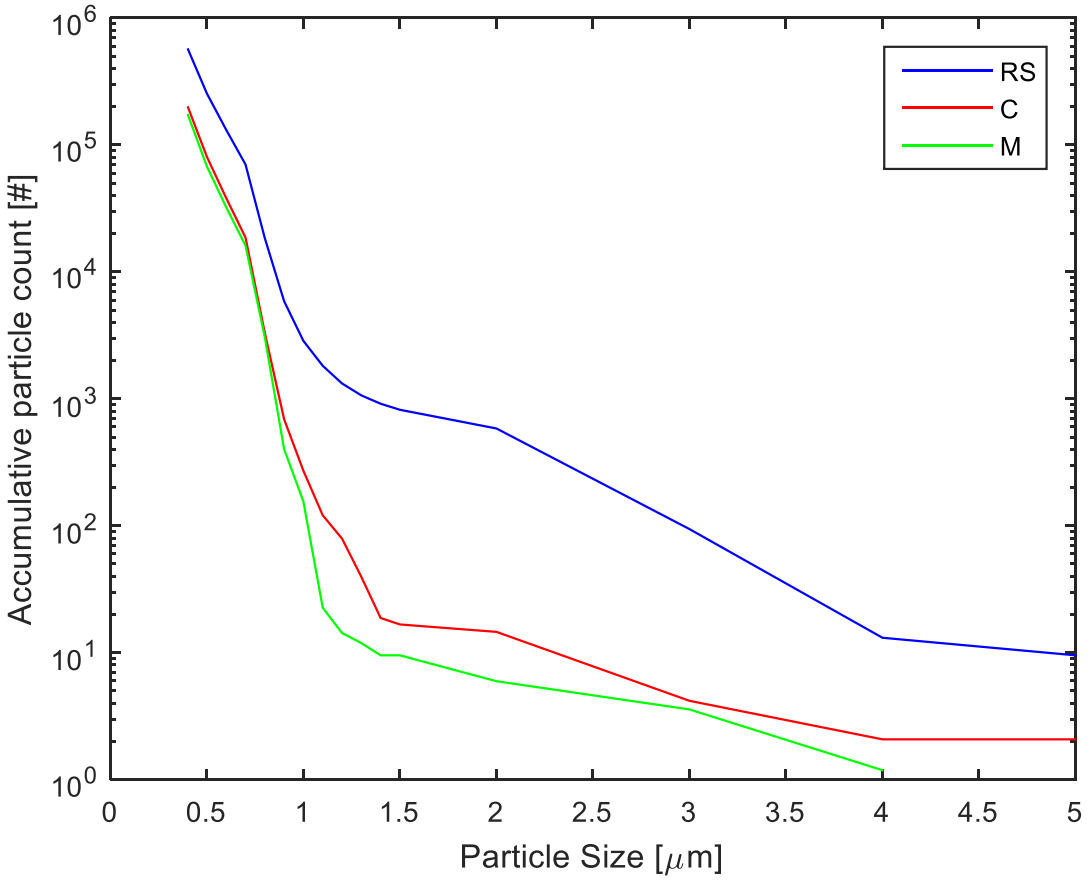

Figure 3.2. Particles counts between 0.4 and 5.0 µm of raw sewage after settling (RS) and after chemically enhanced settling with flocculant (C) and coagulants (M).

The concentrations of 43 pharmaceuticals were determined before and after settling in the jar tests. The removal efficiencies by settling, with or without coagulant/flocculant dosing were calculated (Table 3.2). Because the sewage was not spiked with pharmaceuticals, only pharmaceuticals already present in the sampled sewage were detected. In the column 'Sewage concentration' of Table 3.3, the influent concentrations are given.

The data show that there is almost no removal of the measured pharmaceuticals in any settling method (Table 3.2). This is in concordance with the *sorption only mechanism*: the log K_d values of the pharmaceuticals range from 0.68 (hydrochlorthiazide) to 2.49 (trimethoprim) and thus a removal between 0% to 7% was predicted following this theory. The negative removals were caused by the variation of the measurement.

Table 3.2. Removal efficiencies (%) of pharmaceuticals during settling without chemical additions (RS); flocculation with cationic (C) flocculant and flocculation with organic coagulant (M).

	RS	C	M
Atenolol	14±4	-5±6	2±8
Atorvastatin	-17±1	-2±1	7±1
Bezafibraat	-23±5	21±3	-5±3
Carbamazepine	14±6	-11±5	13±6
Enalapril	3±3	-10±3	1±8
Gemfibrozil	12±4	-4±6	-5±4
Hydrochlorthiazide	-6±6	-7±7	4±6
Ibuprofen	-5±5	-13±6	-3±5
Lidocaine	-9±8	-9±7	-4±7
Losartan	-10±9	-13±7	-18±9
Metoprolol	28±4	-10±4	3±7
Oxazepam	-2±3	16±2	19±2
Sotalol	16±3	-7±3	4±7
Temazepam	-6±4	15±2	19±4
Theophylline	16±5	-7±4	-32±6
Trimetoprim	-17±4	-7±3	-2±4

3.5.2. Discrepancy between colloidal sorption and removal observed in this work

Although colloids were removed with coagulation/flocculation (difference between RS and C or M in Figure 3.2), no clear pharmaceutical removal was observed (Table 3.2). A removal was expected regarding the reported sorption of pharmaceuticals to colloids in literature (Table 3.1). This difference may be explained by an analysis bias in the quantification of colloidally bound pharmaceuticals: in many studies colloidal sorption is determined by UF with a nominal size exclusion cut-off level for colloids as low as 1 KDa (Holbrook et al., 2004; Maskaoui and Zhou, 2010; Yang et al., 2011; Zhou et al., 2007). This is very close to the weight of pharmaceutical molecules themselves (0.2-0.3 kDa). Using these small pore sizes in the filtration of colloids raises the question if retaining pharmaceuticals is a matter of sorption to retained colloids, as is often stated, or mere retention of non-sorbed pharmaceutical molecules in the filter during filtration. In matrices with relatively little amounts of colloids such a ground and drinking water, a filtration over a filter with nominal pore sizes between 0.09 kDa – 0.3 kDa retains over 90% of the pharmaceuticals (Nghiem et al., 2005; Radjenović et al., 2008; Verliefde et al., 2009). In the lower range of the cut-off (0.09 kDa), the retention is dominated by steric size exclusion. In the higher range (0.270 kDa) both steric size exclusion and electrostatic repulsion causes the removal of these large

molecules (Nghiem et al., 2005; Urase and Sato, 2007; Yangali-Quintanilla et al., 2010). However, also larger pore sizes have been shown to retain pharmaceuticals; Burba et al. (2005) showed that over 70% of diclofenac in colloid free water is retained with a 1 kDa cut-off polyethersulfon (PES) membrane. Therefore, at a cut-off of 1 kDa, the filtration itself may retain pharmaceuticals.

3.5.3. Ultra-filtration of pharmaceuticals

To test the possibility of direct removal (retention) of unbound pharmaceuticals by UF, an experiment was performed in which colloids in a solution with pharmaceuticals, were removed in two ways: by UF and by flocculation. The pharmaceutical removal during the removal of colloids (in the form of HS) by UF was compared to pharmaceutical removal with colloids removal by flocculation. In Figure 3.3 the pharmaceutical removal efficiencies of the UF and flocculation experiment are shown. In both cases, the removal of HS was near complete: 91% with UF and 85% with flocculation. However, the pharmaceuticals were not removed in case of flocculation. When UF was applied, concomitant with the removal of colloids, the pharmaceuticals avorstatin, bezafibrate, enalapril, iopromide, ketoprofen, lidocaine, losartan, metoprolol and pravastatine were removed with efficiencies exceeding 40%. No correlation was found between removal percentage of pharmaceuticals obtained by UF and the log K_d value or the octanol partition coefficient log K_{ow} (Table 3.3). Because the removal of HS with UF and flocculation were comparable, the difference in pharmaceutical removal efficiency cannot be explained by sorption to HS. These results show that the use of UF for determining colloidal sorption, may lead to overestimation of pharmaceuticals sorbed to colloids. What factors play a role in the removal with UF should be investigated further.

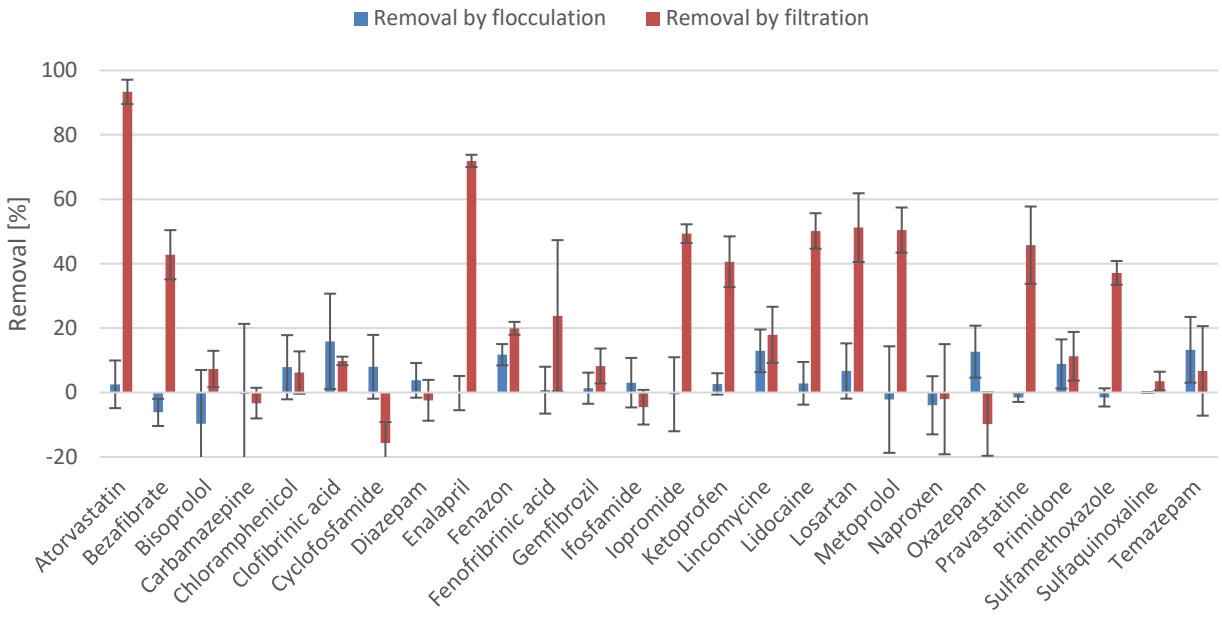

Figure 3.3. Removal efficiencies of pharmaceuticals when removing humic substances by flocculation and ultra-filtration. When using ultra-filtration, significant removal can be observed of pharmaceuticals, often leading to the erroneous conclusion that pharmaceuticals are attached to colloids.

3.6. Conclusion

Pharmaceuticals were not removed from sewage by settling, even when coagulation/flocculation was applied. Despite the reports of colloidal sorption of pharmaceuticals in literature, and the fact that colloids are removed during coagulation/flocculation, the measured 16 pharmaceuticals were apparently not attached to these colloids. For the determination of colloidal sorption of pharmaceuticals, many authors use a cut-off as low as 1 kDa for colloids during UF filtration. In a comparison of pharmaceutical removal in an experiment where colloids were removed by coagulation/flocculation and an experiment where colloids were removed by UF, it was observed that the 1 kDa cut-off can cause direct retention of pharmaceuticals. Direct retention may lead to an overestimation of colloidal sorption of pharmaceuticals. This possible overestimation using UF for estimating colloidal sorption of pharmaceuticals may explain why there is no observed removal of pharmaceuticals when applying coagulation/flocculation on sewage. It can be concluded that coagulation/flocculation is not a good method to concentrate pharmaceuticals during the treatment of municipal sewage.

Table 3.3. The log K_d, log K_{ow} and native concentrations in wastewater of the investigated pharmaceuticals. Log K_{ow} values obtained from Chemspider.com. Pharmaceuticals, which shows a native concentration in wastewater are not bound to colloids, except for oxazepam, metoprolol, temazepam and paroxetine.

Compound	Log K_{ow}	Charge at neutral pH	Measured Log K_d primary sludge	Log K_d of primary sludge from literature	Sewage concentration
	[]	[]	[log L/Kg]	[log L/Kg]	[ng/L]
Atenolol	0.43	1	1.66±0.00	1.04[4] 1.98±0.63[5]	2844±26
Atorvastatin	5.08	-1	2.04±0.05	2.00±0.17[7]	54±2
Bezafibraat	3.99	-1	1.913±0.00	-	258±5
Bisoprolol	2.20	1	2.39±0.00	-	50±1
Carbamazepine	2.77	0	1.66±0.14	1.55[2] 1.40[4] 2.50±0.65[5] 1.95±0.37[9]	917±11
Chloramphenicol	0.88	0 to –1	3.09±0.61	-	N.D.

Clofibrinic acid	3.84	0	1.573±0.01[1]	0.7[4]	N.D.
Coffeine	-0.55	0	N.A.	1.15[4]	30923±1205[1]
Cyclofosfamide	0.10	0	1.889±0.01	1.74±0.36[3]	N.D.
Diazepam	3.08	0	2.345±0.03	1.64±0.59[3] 2.14±0.19[9]	N.D.
Diclofenac	4.26	-1	2.310±0.02[1]	1.82±0.35[2] 2.66±0.07[3] 2.02[4] 2.29±0.69[5] 2.13±0.25[6] 2.18±0.22[9]	N.D.
Enalapril	0.59	0 to -1	1.87±0.03	-	277±11
Fenazon	1.22	0	1.945±0.02	-	N.D.
Fenofibrate	5.28	0	N.A.	-	30±39
Fenofibric acid	4.36	0	2.023±0.00	-	N.D.
Furosemide	1.75	-1	1.449±0.05	2.10±0.32[6] 220[10]	1377±68
Gemfibrozil	4.39	1 to 2	2.40±0.07	1.36±1.00[5] 2.11±0.27[9]	299±26
Hydrochlorthiazide	-0.58	0	0.68±0.38	1.91±0.23[6]	2752±233
Ibuprofen	3.84	1 to 2	2.26±0.00[1]	1.58±0.38[3] 0.98±0.33[5] 2.32±0.23[2]	4103±360
Ifosfamide	0.10	0	1.90±0.03	1.34±0.64[3]	N.D.
Iopromide	-0.44	0 to 1	1.86±0.00	0.84[2]	40517±478
Ketoprofen	3.61	0	1.76±0.04	2.35±0.80	N.D.

				[5]	
Lidocaine	2.84	1	2.33±0.00	-	234±12
Lincomycin	-0.32	1	1.94±0.03	-	N.D.
Losartan	5.08	0 to -1	1.26±0.81[1]	-	3877±50
Metformin	-1.36	1	2.34±0.00	-	89298±916[1]
Metoprolol	1.76	1	1.30±0.00	1.26[4]	1127±16
Naproxen	2.88	0	1.85±0.00	1.00[2] 1.56[4] 2.16±0.23[9]	2797±56
Oxacillin	1.70	1	2.18±0.03	-	4±14
Oxazepam	2.92	0	1.89±0.32	2.90 with (R^2=0.90)[8]	602±3
Paracetemol	0.91	0	N.A.	1.51[4]	29305±268[1]
Paroxetine	3.15	0	N.A.	4.15 with (R^2=0.96)[8]	112±10
Pravastatin	1.65	-1	1.93±0.00	-	1694±98
Primidone	1.12	0	1.99±0.00	-	9±2
Propranolol	2.58	1	2.29±0.00	2.52[4] 2.81±0.75[5]	24±2
Salicylic acid	1.98	-1	2.30±0.54	1.36[3]	34535±1165
Sotalol	-0.40	1	2.34±0.00		3012±14
Sulfametoxazol	0.79	0 to -1	2.30±0.10	1.36[2] 1.18[4] 0.51±1.41[5] 2.21 with (R^2=0.77)[8] 2.43±0.38[9]	288±7
Sulfaquinoxaline	3.08	1	2.25±0.03	-	N.D.
Temazepam	2.79	0	2.27±0.31	-	355±4
Theophylline	-0.77	0 to 1	1.40±0.40	-	3811±98
Tiamulin	4.50	1	2.88±0.00	-	N.D.
Trimetoprim	1.28	1	2.49±0.00	1.83[4] 2.63±0.56[5] 2.59 with (R^2=0.98)[8] 2.30±0.16[9]	122±3

1. Values are an indication.
2. (Carballa et al., 2008) values for mesophilic digested sludge
3. (Ternes et al., 2004)
4. (Barron et al., 2009), values for digested sludge

5. (Radjenovic et al., 2009)
6. (Jelic et al., 2012)
7. (Jelic et al., 2012), values for wastewater
8. (Hörsing et al., 2011)
9. (Hyland et al., 2012), values for secondary sludge
10. (Stuer-Lauridsen et al., 2000), unknown what type of sludge is used

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4. Impact of Coagulant and Flocculant Addition to an Anaerobic Dynamic Membrane Bioreactor (AnDMBR) Treating Waste Activated Sludge

This chapter is based on: Kooijman, G., Lopes, W., Zhou, Z., Guo, H., de Kreuk, M., Spanjers, H., & van Lier, J. B. (2017). Impact of coagulant and flocculant addition to an anaerobic dynamic membrane bioreactor (AnDMBR) treating waste-activated sludge. Published in: *Membranes*, *7*(2). https://doi.org/10.3390/membranes7020018

4.1. Abstract

In this work we investigated the effects of coagulants and flocculants, referred to as flocculation aids (FAs), addition to an anaerobic dynamic membrane bioreactor (AnDMBR) (7L, 35^{o}C) treating waste activated sludge (WAS). The experiment consisted of three distinct periods. In period 1 (day 1 – 86) the reactor was operated as a conventional anaerobic digester with a solids retention time (SRT) and hydraulic retention time (HRT) of 24 days. In period 2 (day 86 – 303) the HRT was lowered to 18 days with the application of a dynamic membrane while the SRT was kept the same. In period 3 (day 303 – 386) a cationic flocculant in combination with $FeCl_3$ was added. The additions led to a lower viscosity, which was expected to lead to an increased digestion performance. However, the FAs caused irreversible binding of the substrate, lowering the VS destruction from 32% in period 2 to 24% in period 3. An accumulation of small particulates was observed in the sludge, lowering the average particle size by 50%. These particulates likely caused pore blocking in the cake layer, doubling the trans-membrane pressure. The methanogenic consortia were unaffected. Dosing coagulants and flocculants into an AnDMBR treating sludge leads to a decreased cake layer permeability and decreased sludge degradation.

4.2. Introduction

High rate anaerobic treatment is a consolidated concept in industry due to the high COD removal capacity, energy recovery and low waste sludge production (van Lier, 2008, van Lier et al., 2015). The success of high rate anaerobic reactors depends on the extent to which hydraulic retention time (HRT) and solids retention time (SRT) can be uncoupled in a system, to keep the slow growing methanogens in the system. The HRT and SRT uncoupling in an anaerobic membrane bioreactor (AnMBR) cannot be disturbed by, for example, high influent concentrations of total suspended solids or high fats that can compromise the biomass retention in expanded granular sludge bed reactors and upflow anaerobic sludge blanket systems. AnMBRs have become a well-studied concept in wastewater treatment over the last decades with an increasing number of full-scale references (Pacheco-Ruiz *et al.*, 2019). The main drawbacks of (An)MBR systems in general are the energy consumption, membrane fouling and relatively high investment costs (Krzeminski et al., 2016). Membrane fouling limits the operational flux that can be achieved. Cationic flocculants in an AnMBR treating wastewater were shown to temporarily increase the permeate flux, which was ascribed to an increased average particle size, creating a higher permeability of the cake layer (Díaz et al., 2014; Zhang et al., 2017). Simultaneously, a higher effluent quality was obtained because of lowered soluble microbial product (SMP) concentrations (Díaz et al.,

2014; Zhang et al., 2017). In an AnMBR treating sludge instead of wastewater, flocculants can have additional benefits. Flocculants may lower the viscosity in anaerobic sludge digestion (see Chapter 2). Earlier studies indicate that a lower viscosity may increase the hydrolysis rate (Chapter 2; Dartois et al., 2010) which is considered the rate limiting step in anaerobic sewage sludge digestion (Eastman and Ferguson, 1981). Therefore, the application of flocculants and coagulants (referred to as flocculation aids, or FA) in anaerobic sludge digestion can lead to increased digestion rates by lowering the viscosity (Chapter 2; Chu et al., 2003), although there are also reports of lower biogas production rates with the addition of FA (Yu et al., 2015). A second advantage of FA addition to an AnMBR treating sludge is the increase in applicable SRT that can be achieved. However, at higher SRTs, the solids concentration and viscosity in an AnMBR will be higher as well. Since increased viscosity limits the highest attainable SRT because of increasing solids accumulation (Meabe et al., 2013), lowering the viscosity with FAs could increase the maximally attainable SRT in an AnMBR. Besides membrane fouling, another disadvantage mentioned before is the high investment and operational costs. These high costs are for a large part caused by the capital costs related to the membranes. However, the cake layer that would normally form on the membrane during filtration is dense and compact and will form an excellent barrier for solids (Jeison et al., 2008). Therefore, instead of using a membrane, a simple cloth can act as a support for the cake layer as well, lowering the investment and operational costs (Ersahin et al., 2014). A membrane bioreactor equipped with such a cloth instead of a membrane is referred to as anaerobic dynamic membrane bioreactor (AnDMBR). To the authors' knowledge, the effects of FAs in an AnDMBR treating sludge have not been investigated yet. However, Yu et al. (2015) investigated the application FAs in an AnMBR treating sewage sludge. Unfortunately, their experiments in which the effects of FAs on sludge digestibility were investigated were limited to batch tests and the results were for a part inconclusive. Therefore, in this study we investigated the effects of cationic FA addition in a continuous flow AnDMBR treating waste activated sludge (WAS), supported by batch-wise fed experiments. We investigated the methanogenic activity, the extent of sludge degradation, changes in sludge characteristics and trans-membrane pressure (TMP). Conventional anaerobic digestion (AD) at an SRT and HRT of 24 days was compared to an AnDMBR with an HRT of 18 days and an SRT of 24 days, including a period without and a period with FA addition.

4.3. Material and methods

4.3.1. AnDMBR setup and operation

Table 3.1 gives an overview of the experimental set-up. In period 1, the digester was operated as a daily fed sewage sludge digester without solids retention. The reactor had a volume of 7 L and was operated at 35 °C. In period 2 and 3, the digester was coupled to a dynamic membrane module with a total filtration area of 0.025m^2.

Table 3.1. Description of the three periods where different operational parameters where applied.

	Period	Reactor operation	Substrate	HRT	SRT	FA addition
[-]	[d]	[-]	[-]	[d]	[d]	[-]
Period 1	0-86	Conventional anaerobic digester	WAS	24	24	-
Period 2	86-303	AnDMBR	WAS	18	24	-
Period 3	303-386	AnDMBR	WAS	18	24	Calfloc 1502 + $FeCl_3$

The cross-flow velocity over the external dynamic membrane was 0.044 $m.s^{-1}$, which corresponded to a recirculation flow of 240 $L.h^{-1}$. The membrane surface was relatively large for the required liquid extraction from the digester and thus the applied fluxes were very low reaching only 0.10 $L/m^2.h$. No backwash was required. Since in this work we focussed on the biological processes no flux optimisation studies were performed. A mono-filament woven fabric made of polypropylene material (Lampe B.V., Sneek, the Netherlands) was used as support material for the cake layer of the dynamic membrane. Filtration was carried out by using a constant flux strategy set by a peristaltic pump (3 in Figure 4.1) at the permeate side. The biogas was measured with a biogas flow meter described in Appendix I. The feed and sludge withdrawal were carried out manually, once a day, 6 times per week (not on Sundays). The volume of sludge withdrawal varied and was determined by the sludge concentration and the total sludge mass in the reactor in relation to the desired SRT, i.e. 24 days.

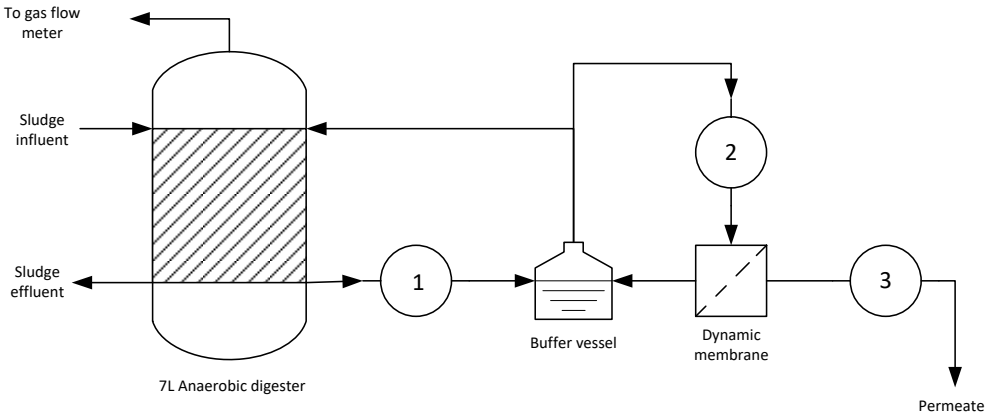

Figure 4.1. Schematic overview of the AnDMBR setup used. The first pump (1) transports the sludge to the buffer vessel from which it is circulated over the membrane by pump 2. The permeate pump (3) creates the pressure difference over the membrane by removing permeate.

The WAS substrate was taken from the municipal wastewater treatment plant Harnaschpolder (Den Hoorn, The Netherlands). The WAS had a total solids (TS) concentration between 55 and 65 $g.L^{-1}$. The influent total solids concentration was set to a constant value of 48 $g.L^{-1}$ by diluting the WAS using tap water. The characteristics of the final feed to the digester are shown in Table 3.2.

Table 3.2. Characteristics of the waste activated sludge used as feed.

Parameter	Unit	Average value
Total Solids	g L^{-1}	48.2 ± 1.7
Total Volatile Solids	g L^{-1}	34.9 ± 1.0
Total Suspended Solids	g L^{-1}	45.6 ± 1.6
Volatile Suspended Solid	g L^{-1}	33.9 ± 1.0
Total COD	g L^{-1}	50.1 ± 3.2
Total Nitrogen	g L^{-1}	2.49 ± 0.52
Total Phosphorus	mg L^{-1}	2.444 ± 0.15

2.2. FA selection and addition

FAs were selected by comparing the capillary suction time (CST) and specific resistance to filtration (SRF) of sludge from the AnDMBR, which was treated with 24 cationic flocculants and coagulants. Sludge samples were taken at day 120. An initial screening was carried out by using a CST tests. The six best performing FAs (Table 4.3), with the shortest CST, were subjected to an SRF test.

Table 4.3. Six best performing flocculants.

Product	Characteristics	Charge
Calfloc L1408	Branched, cationic, emulsion	Medium
Calfloc L111	Branched, cationic, emulsion	Medium
Calfloc L1401 LMW	N.A.	Medium/high
Calfloc P1502	Linear, cationic, powder	High
Calfloc LS1423	Polyamine	Medium
Nalco 71305	Acryl-amide based	High

The CST and SRF tests were done using a 5 g/kg dosage, which means 5 grams of active FA component per kg of TS. From day 267, the best performing FA was dosed to the AnDMBR applying a dosage of 7.5 g/kg. Because no effect was observed at this point in time, from day 303, the Nalco 71305 was replaced with the Caldic (Rotterdam, the Netherlands) cationic FA Calfloc 1502 (10 g/kg) in combination with 40% FeCl3 (0.13 mL FeCl3 gTS^{-1}).

4.3.2. Analytical methods

Merck Spectroquant kits (Germany) were used to assess ammonium-N (10-2000 mg-N/L), COD (25-15000 mg/L) and P concentrations (0.015 – 5 mg-P/L). CST was measured by a Triton Electronics Model 304M CST device (Essex, England). The specific resistance to filtration (SRF) was measured by applying a pressure of 1 bar to a Whatman Grade 1 filter with 100 mL of sludge sample. The permeate volume was measured over time during 2

hours. The SRF calculations were done following the procedure of Novak et al. (1988). An Anton-Paar USD200 rheometer with Z2 DIN and TEZ 180 bob (Graz, Austria) was used to measure viscosity. The particle size distribution (PSD) was analysed by a Donner Technologies DIPA-2000 laser scanner with B100 lens, and with 10-2000 µm measuring range. The soluble microbial products of poly saccharide nature (SMP-PS) were measured following the procedure of Ersahin et al. (2014). The soluble microbial products of protein nature (SMP-PN) were measured according to Bradford (1976) The median particle size (D_{50}) was calculated from the volume-based PSD. The specific methanogenic activity (SMA) and biomethane potential (BMP) tests were done as previously (Chapter 2; Kooijman et al., 2017). The BMP test was done in duplicate, and to each bottle 10 mL antifoam (100x dilution with water) was added. SMA tests were carried out in triplicate. VFA concentrations were analysed using a GC with an FID detector (Agilent 7890A, USA). Helium was used as carrier gas with a flow rate of 1.8 mL/min. Column used was an Agilent 19091F-112, with injector temperature of 240 °C, 25 m x 320 µm x 0.5 µm, Oven temperature: 80 °C. The remaining parameters were assessed following Standard Methods (APHA, 1999).

4.4. Results

4.4.1. Performance of the conventional sludge digester and the AnDMBR (period 1 and 2)

In order to study the effect of uncoupling HRT and SRT in sludge digestion, the laboratory scale sludge digester was firstly operated as a conventional digester with an SRT equal to the HRT of 24 days, being fed once per day (period 1). Secondly, in the subsequent period (period 2), the HRT was lowered to 18 days by operating the reactor as an AnDMBR. During the first period the VS destruction was about 37% (Figure 4.2). After lowering the HRT to 18 days during period 2, the VS destruction lowered and stabilised at about 32% after 4 months of operation. A slight decrease in SMA could be observed after installing the membrane unit lowering the SMA from 0.19±0.01gCOD gVS^{-1} d^{-1} to 0.14±0.02 gCOD gVS^{-1} d^{-1} at the end of period 2.

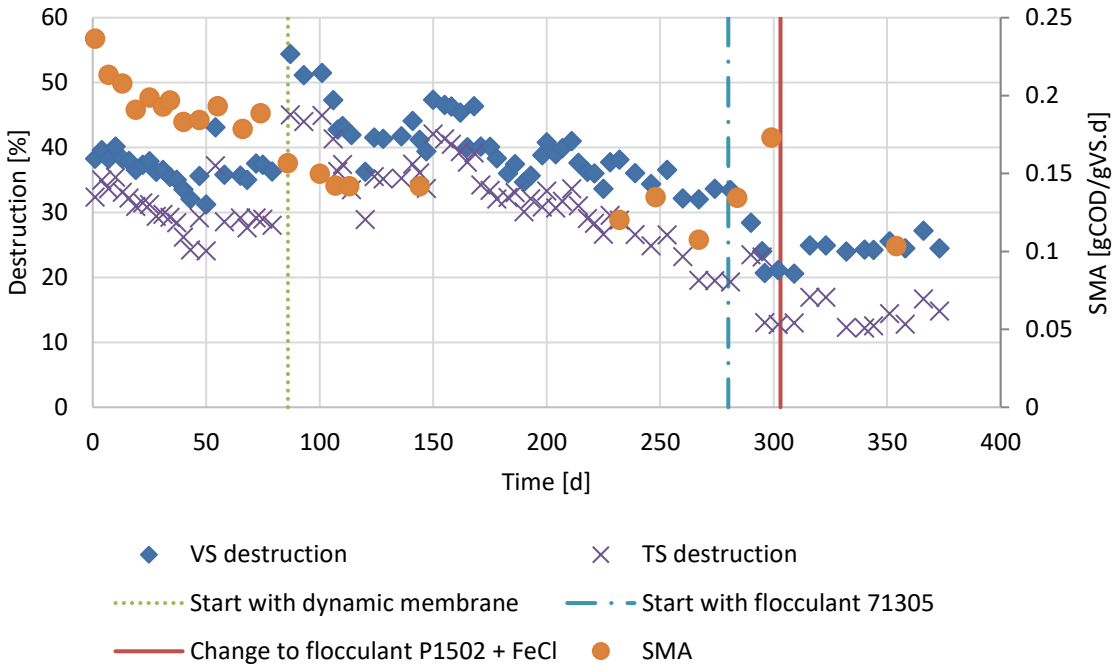

Figure 4.2. TS and VS destruction and SMA of the reactor operated as conventional anaerobic digester in period 1 (day 1 - 86), operated as an AnDMBR in period 2 (day 86 - 303) and operated as an AnDMBR with flocculant addition in period 3 (day 303 - 386).

The concentrations of propionate and butyrate remained close to 0 mg/L (Figure 4.3) during all three periods. Apparently, acetogenic conversions were not rate limiting in the digester.

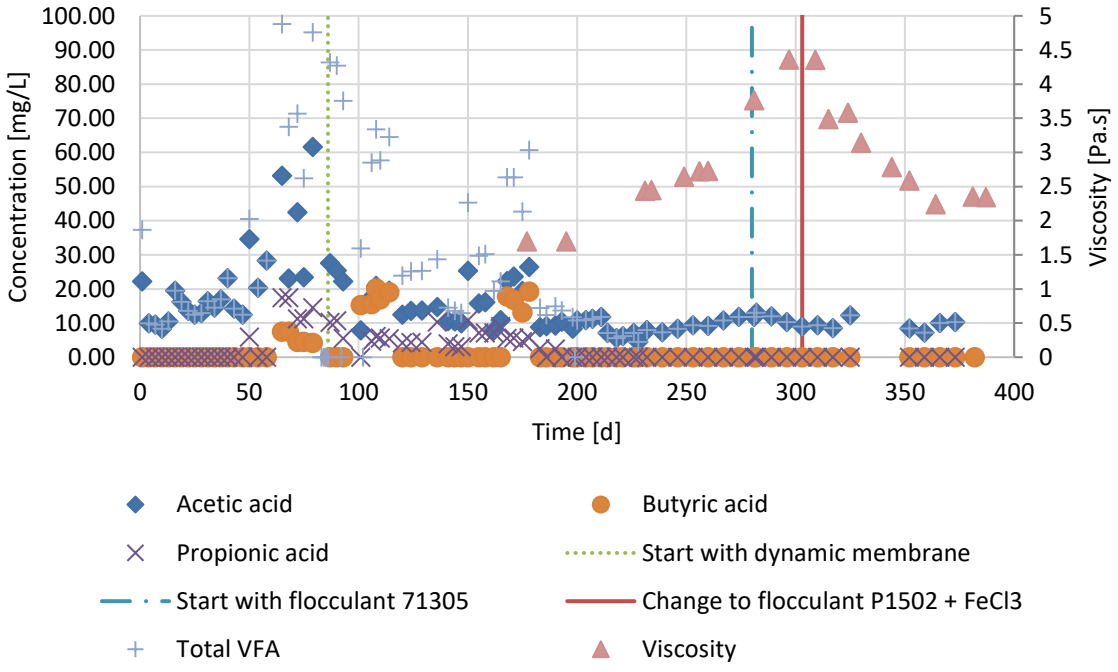

Figure 4.3. VFA concentrations of the reactor and viscosity of the reactor operated as conventional anaerobic digester in period 1 (day 1 - 86), operated as an AnDMBR in period 2 (day 86 - 303) and operated as an AnDMBR with flocculation aid addition in period 3 (day 303 - 386).

4.4.2. FA selection

24 cationic FAs were tested on the digested sludge from the AnDMBR prior to selection around day 130. The best performing FA in terms of CST and SRF was Nalco 71305. From day 267 onwards, 7.5 g/kg of this FA was dosed. However, FA addition did not result in a visible flocculation in the reactor, the SRF was only shorty affected and CST even increased (Figure 4.4). Also, repeated CST and SRF tests with an increased dosage of 15 g/kg of Nalco 71305 did not show a clear improvement. Therefore, after one week, Nalco 71305 dosing was stopped. After a new testing phase, the applied FA was changed to a combination of cationic FA Calfloc 1502 (10 g/kg) with $FeCl_3$ (0.13 mL $FeCl_3$ g TS^{-1}). Hereafter, the CST was lowered from ~2000s to ~1000s. From day 303 a dosage of 10 g kg^{-1} 1502 and 0.13 mL $FeCl_3$ g TS^{-1} was applied. Because of the FAs built-up in the reactor, the FAs concentrations were lowered to 6.6 g kg^{-1} and 0.09 mL $FeCl_3$ g TS^{-1} from day 330 and to 3.3 g/kg and 0.04 mL $FeCl_3$ g TS^{-1} from day 354. The SRF and CST were successfully lowered in the reactor (Figure 4.4). When dosing the new combination of FAs, foaming problems occurred that were mitigated by adding an antifoam emulsion.

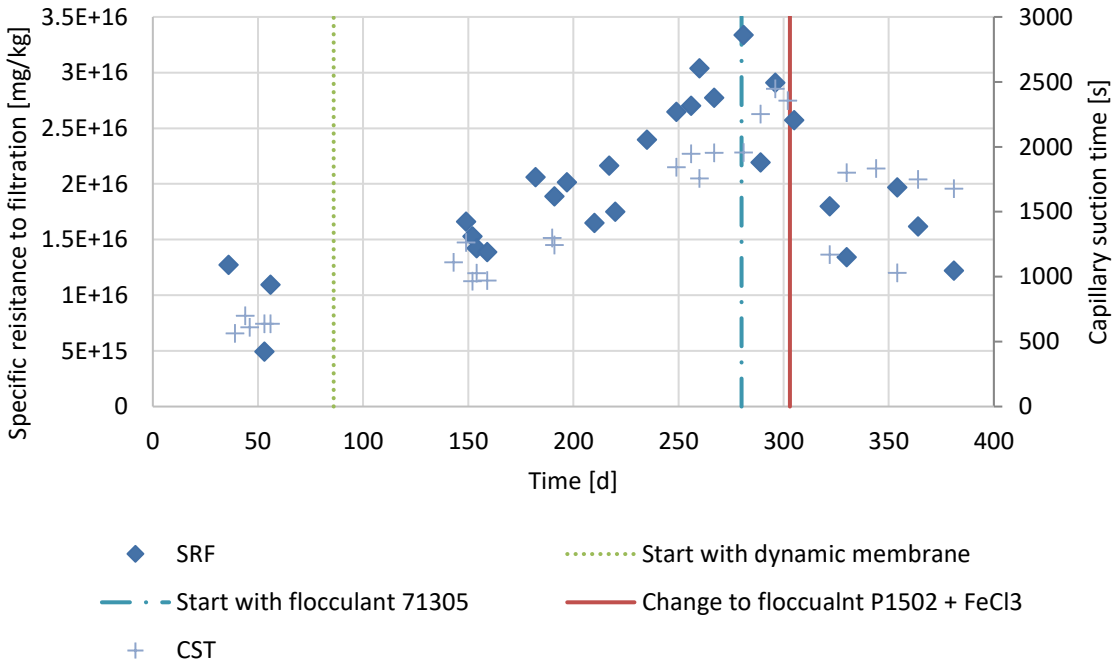

Figure 4.4. The specific resistance to filtration (SRF) and the capillary suction time (CST) of the effluent sludge of the reactor operated as conventional anaerobic digester in period 1 (day 1 - 86), operated as an AnDMBR in period 2 (day 86 - 303) and operated as an AnDMBR with flocculation aid addition in period 3 (day 303 - 386).

4.4.3. Performance of the AnDMBR with FAs dosing (period 3)

During period 3 the sludge viscosity was significantly lowered due to FA addition (Figure 4.3). However, in the same period, the VS destruction decreased to about 24% (Figure 4.2). A BMP test was carried out to examine the possibility of irreversible substrate binding. Results showed that there was irreversible binding of substrate by FA already with dosages as low as 5 g/kg Calfloc 1502 and 0.07 mL $FeCl_3$ kg TS^{-1} (Figure 4.5).

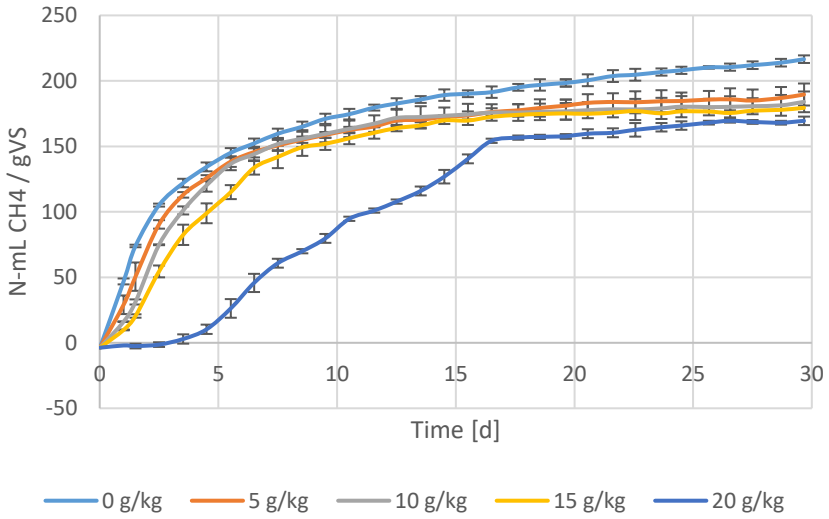

Figure 4.5. BMP tests of WAS with different flocculant concentrations. Increased flocculant concentration decreases the BMP values.

The addition of FAs in period 3 in the reactor lowered the SRF with about 40% despite the higher TS concentration that reached to 57 g L^{-1} in period 3. Also, the CST decreased in period 3. The average particle size (D_{50}) in period 2 was 58 µm (determined on day 256). Surprisingly, the D50 was reduced after the addition of FAs in period 3 to 32 µm (determined on day 353). The TMP in period 2 was about 150 mbar but it doubled to about 300 mbar in period 3, when FA was added to the digester. The effluent quality increased, as the SMP-PS concentrations in the permeate were lowered in period 3 (Figure 4.6). At the same time, the SMP-PS concentration in the supernatant of the reactor increased. The permeate SMP-PN concentration remained equal in period 3 compared to period 1. However, the SMP-PN concentrations in the reactor supernatant increased in period 3.

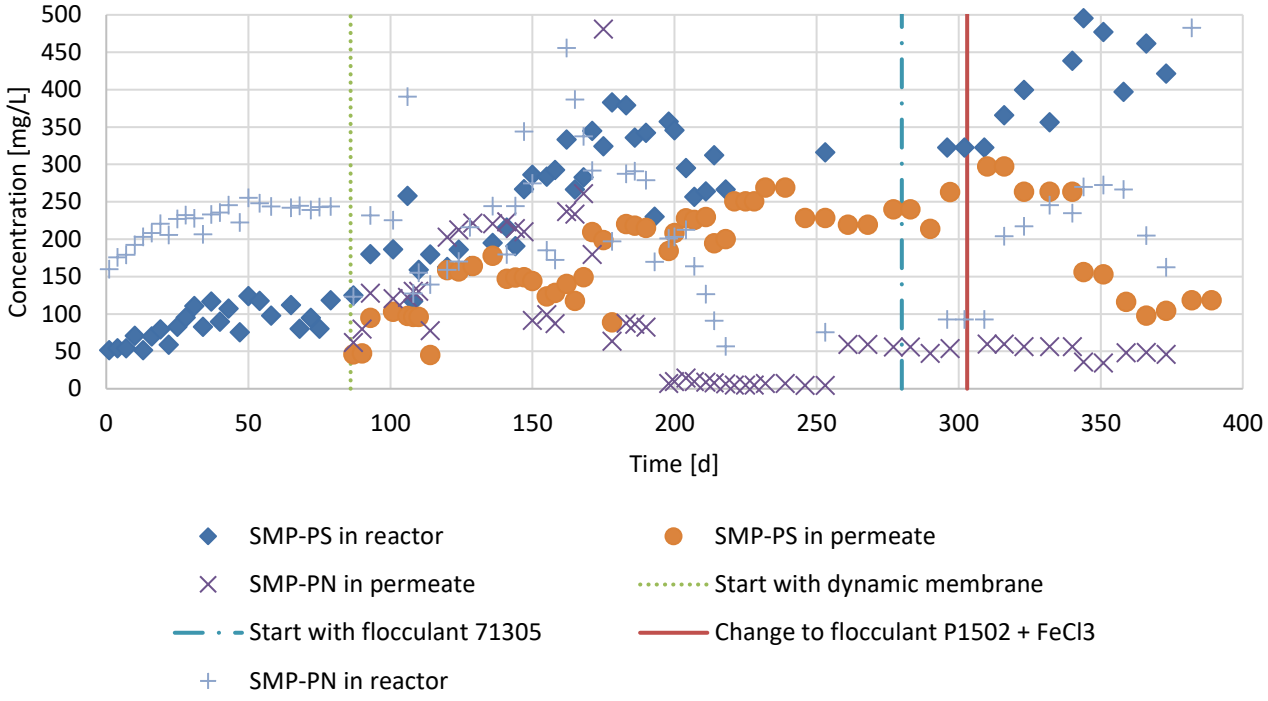

Figure 4.6. Concentrations of SMP-PS (polysaccharides) and SMP-PN (polypeptides) in the supernatant and the permeate of the reactor operated as conventional anaerobic digester in period 1 (day 1 - 86), operated as an AnDMBR in period 2 (day 86 - 303) and operated as an AnDMBR with flocculant addition in period 3 (day 303 - 386).

The concentrations of ortho-phosphate (PO4-P) decreased in period 3 (Figure 4.7). The concentrations were similar for the reactor and permeate. For ammonium (NH4-N), there was an increase in reactor concentration in period 3, while the permeate concentrations remained the same.

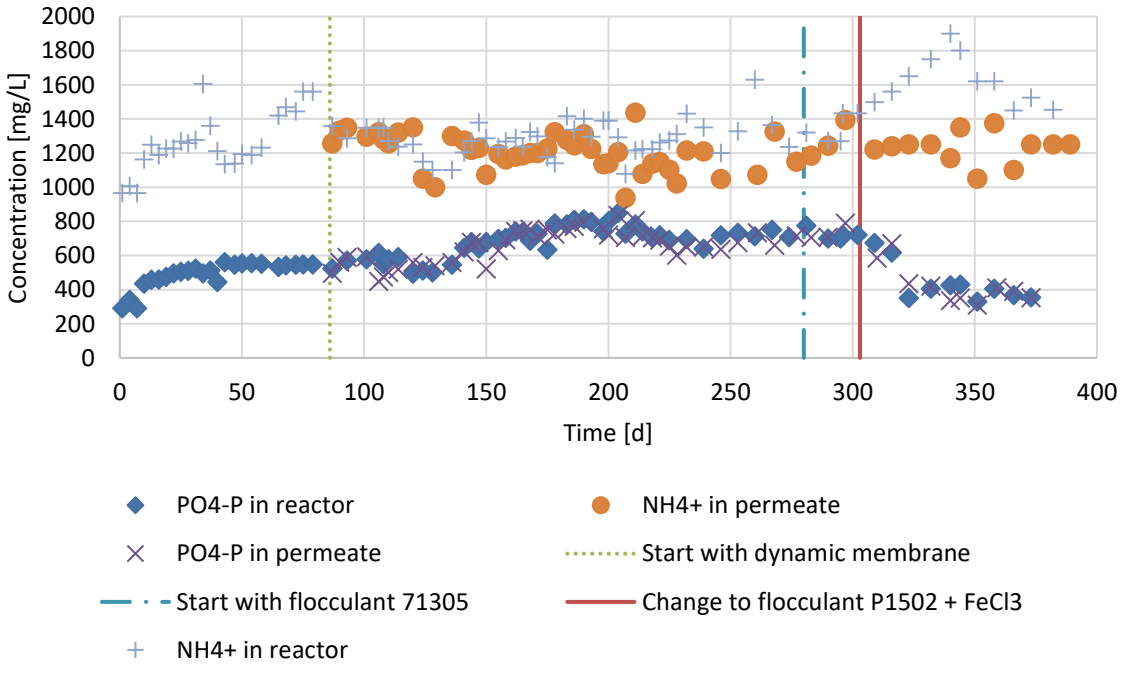

Figure 4.7. Ortho-phosphate (PO4-P) and ammonium (NH4+) concentrations of the reactor operated as conventional anaerobic digester in period 1 (day 1 - 86), operated as an AnDMBR in period 2 (day 86 - 303) and operated as an AnDMBR with flocculant addition in period 3 (day 303 - 386).

4.5. Discussion

4.5.1. Digestion performance in period 2

After installing a dynamic membrane at the beginning of period 2, while keeping the SRT on 24 days, the VS destruction decreased compared to period 1 from 37% to about 32% after 4 months of continuous operation. A lower digester performance was reported earlier, when using an external membrane (Brockmann and Seyfried, 1996; Choo and Lee, 1996; Ghyoot and Verstraete, 1997). It was postulated that the shear forces caused by pumping the reactor content through the side stream membrane unit caused disruption of the microbial consortia (Ghyoot and Verstreate, 1997). However, no accumulation of propionic acid or butyric acid was however observed (Figure 4.3), indicating that syntrophic acetogenic consortia were not notably affected. The treatment performance was also not likely to be affected by free ammonium inhibition as the free ammonium in period 2 was about 30±4 mg/L which is well below the concentration that is found to be inhibiting (Rajagopal et al., 2013). Since the VFA concentrations remained low in period 2, the decrease in VS destruction was likely caused by a decreased hydrolysis rate. The higher solids concentration in the reactor compared to period 1 caused a higher viscosity, which possibly negatively impacted the hydrolysis rate (Dartois et al., 2010).

4.5.2. Digester performance in period 3

In period 3, despite the lower viscosity, the VS destruction further lowered to 24%. FAs are considered to be non-toxic to anaerobic consortia (Campos et al., 2008; Chu et al., 2003; Kooijman et al., 2017). The acetotrophic methanogens were indeed not notably affected by the FAs, indicated by the similar SMA values in period 2 and 3. In addition, acetogenic conversions and methanogenesis were not the rate limiting step during reactor operation as evidenced by the low VFA concentrations. The treatment performance was also not likely to be affected by free ammonium inhibition as the free ammonium in period 3 was more or less similar as before, about 32±2 mg/L. In our previous work we showed that FAs can irreversibly bind to solids, such that they are not available for bioconversion anymore (Chapter 2). Results of the BMP test showed that apparently, there was irreversible binding of substrate by FA already with dosages as low as 5 g/kg Calfloc 1502 and 0.07 mL $FeCl_3$ kg TS^{-1} (Figure 4.5). Therefore, with the applied concentrations in the AnMBR, it can be concluded that part of the solids indeed was irreversibly bound, explaining the lower observed VS destruction.

4.5.3. Filtration performance and nutrient concentrations in period 2 and 3

The SRF dropped about 40% in period 3 compared to period 2 and also the CST dropped slightly. The low drop in CST in period 3 despite the FA addition compared to period 2 may have been caused by the higher TS concentration, which results in a higher CST. The observed lower SRF and CST after FA addition agrees with earlier studies in an aerobic MBR (Huyskens et al., 2012) and in an AnMBR (Yu et al., 2015). However, the TMP doubled from

150 mbar in period 2 to 300 mbar in period 3. Other studies with AnMBRs, show an increase in filterability due to FA addition (Díaz et al., 2014; Yu et al., 2015). The reason for the higher TMP may be increase in small particles in the reactor in period 3 compared to period 2. Small particles are known to clog the pores in the cake in AnMBRs (Lin et al., 2011). During AD, colloids are usually rapidly degraded (Elmitwalli et al., 2001). However, cationic FAs irreversibly bind solids (Hogg, 1999) and since cationic FA are known to be partially non-biodegradable (Chang et al., 2001), the irreversibly bound organic particles can become refractory to biological degradation. The hypothesis of colloidal accumulation when digesting flocculated sludge has been postulated before (Campos et al., 2008), however no proof wat presented. These supposed small, accumulated and refractory colloids could be causing a higher TMP in period 3 compared to period 2.

The lower average particle size in period 3 compared to period 2 may be caused by accumulation of colloidal matter, irreversibly bound to FA. As mentioned before, it should be noted that no backwash was applied. From these results it can be concluded that the filtration in terms of TMP did not benefit from the FAs addition due to the accumulation of small refractory particles that accumulated in the reactor. Typically, the SMP concentrations are lowed by to the addition of FAs (Hwang et al., 2007; Yu et al., 2015). However, in period 3, the SMP-PS and SMP-PN concentration increased in the reactor (Figure 6). The increase in SMP in the reactor in period 3 can be explained by the SMP present in the refractory small particles as mentioned above. At the same time SMP concentration in the effluent decreased in period 3 which can be explained by the decreased permeability of the clogged cake in period 3. In Figure 4.7, it can be observed that despite a lower VS destruction the NH4+ concentration in the reactor increases in period 3 with respect to period 2. This increase is most likely due to the degradation of cationic FA, a phenomenon that was observed before when anaerobically degrading cationic FAs (Chang et al., 2001). This hypothesis is supported by the fact that the measured NH4+ reactor concentration lowered shortly after lowering the FA dosage on day 330. The PO4-P concentration in the reactor and effluent decreased. This was most likely a consequence of the $FeCl_3$ dosing.

4.6. Conclusions

An increased viscosity in the reactor, after lowering the HRT to 18 days with a filter cloth, eventually caused a lower VS destruction, possibly due to a lower hydrolysis rate caused by an increased viscosity. Subsequently lowering the viscosity with FAs, did not improve the VS destruction. This was explained by an irreversible binding of the substrate, hampering bioconversion. Irreversible binding of colloidal organic matter by partially non-biodegradable cationic flocculation aid, led to an accumulation of small non-degradable particulates in the reactor. These particulates may have caused a higher TMP caused by pore blocking. The FA concentrations did not notably affect the methanogenic activity of the system. It can be concluded that FA dosage is not beneficial for WAS treating AnDMBRs.

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5. Aquatic worm predation of flocculated digested sludge

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5.1. Abstract

Sludge disposal is one of the largest cost factors of sewage treatment. Therefore, waste sludge reduction will have a large economic impact on the operation of a sewage treatment plant. The most commonly applied method for on-site sludge reduction is anaerobic digestion (AD), which concomitantly allows for energy recovery. Another well-researched approach for waste activated sludge reduction is predation by aquatic worms (WP). Despite the large extent of sludge reduction that might be attained by WP, the disadvantage of WP is the need for aeration, and the oxidation of organic matter to CO_2, reducing the energy recovery potential. Therefore, an energy-wise optimised configuration of interest for sludge reduction would be an AD step followed by WP of the digested sludge, allowing for both energy recovery and a high degree of sludge reduction. So far, the AD-WP concept has not been considered a viable option due to the low ammonia tolerance of aquatic worms. However, by effectively separating the anaerobically digested sludge from the reactor broth using flocculants, WP can then be performed in a separate unit at low ammonia concentrations. In this study, we investigated the possibility of WP of anaerobically digested sludge by aquatic worms *Limnodrilus* and *T. tubifex*. We found an additional maximum removal of 40% volatile solids. However, the used cationic flocculants had a negative impact on the worm viability and caused mortality of the used worms, due to toxicity. The assessed 4 day LD_{50} value was between 50 and 100 mg/L. Due to the observed toxicity of the flocculants, WP of anaerobic solids is only of interest when non-toxic flocculants can be used, which is a prerequisite for making the AD-WP concept a viable option.

5.2. Introduction

Sludge disposal is one of the largest cost factors in sewage treatment, that can amount up to 50% of the total operational costs (Spinosa and Vesilind, 2001). In 2010, in the Netherlands alone 300.000 ton of dry solids (DS) was dewatered by centrifuges, filter presses and belt presses in sewage treatment plants (STPs) (Korving, 2012). Since application in agriculture is prohibited and landfills are abandoned in the Netherlands, most of the sludge is incinerated, with disposal costs of 60 €/ton dewatered sludge (Duin et al., 2016). Due to the high sludge disposal costs, relatively costly sludge treatment techniques can become economically feasible. An often mentioned and investigated biological approach to lower the sludge production in an STP is by sludge predation with higher organism such as protozoa or aquatic worms.

During the transfer of chemically bound energy from bacteria to higher organisms such as aquatic worms, energy is lost due to inefficient energy conversion. Energy that is lost cannot be used for growth and therefore biomass production is minimized (Ratsak et al., 1996). Minimized biomass production results in reduced sludge production at an STP. Aquatic worms are the main predators that are found in STPs, and apparently are particularly suitable for grazing on sludge (Ratsak et al., 1993). In particular *Tubifex tubifex* is associated with nutrient rich environments and can feed on sewage sludge (de Valk et al., 2016;

Finogenova and Lobasheva, 1987). Blooms of aquatic worms are occasionally observed in STPs (Elissen, 2007) and lead to an increase in the sludge settleability (Ratsak et al., 1993). For these reasons, several authors have used aquatic worms in pursue of an efficiency increase in the STP by having worms grazing on waste activated sludge (Elissen et al., 2006; Hendrickx et al., 2009a; Tamis et al., 2011; Wang et al., 2011). Despite the report of a successful and stable worm population in an STP in the Netherlands over four years (Tamis, 2011), there are still questions about the performance stability (de Valk et al., 2016).

In the Netherlands, the most commonly applied on-site sludge reduction technique is anaerobic digestion (AD). The main goal of AD in STPs in the Netherlands is to reduce waste sludge but also to recover energy in the form of methane. Since aquatic worms are able to grow on digested sludge (Hendrickx et al., 2010), the most energy efficient position of worm predation in an STP would be after the AD because then energy is recovered in the form of methane and a higher degree of sludge reduction is achieved by a combination of AD and WP. However, aquatic worms are sensitive for high concentrations of ammonia and, therefore, worm predation of anaerobic sludge is considered not possible (Hendrickx et al., 2010). Nonetheless, since flocculants can contribute efficiently to separate solids from the ammonium containing liquid, the flocculated digested solids then can be subjected to predation by aquatic worms. In this study, we investigate the possibility of sludge reduction of flocculated AD sludge by sessile *Tubificidae* and *Lumbriculidae*.

5.3. Material and methods

In this study, digested sludge was used from STP Harnaschpolder (1.3 million P.E.), Den Hoorn, the Netherlands. The sludge was flocculated with Nalco 71305 cationic polymer with a concentration of 19 g/kg dry solids (DS) which was the optimal flocculant dosage yielding the lowest capillary suction time.

5.3.1. Bottle experiment

In the *bottle experiment*, six 2L Schott flasks were filled with 1.6 L tap water and 3.0 g DS of flocculated sludge. Bottle 1 – 5 contained 22 g of wet worms (12.3 g wet worms/g VS). Aquatic worms were bought at a pet shop and consisted of *Limnodrilus* and *T. tubifex* (Elissen, 2007). The flasks were incubated in the dark at room temperature (19-23 °C) and aerated such that dissolved oxygen concentrations of 6 - 9 mg/L were reached, which was considered sufficient for optimal sludge predation (Cai et al., 2016). Aeration was performed at approximately 15 cm above the sludge to prevent shear stress. Flask 6 was the reference with only endogenous respiration of the flocculated sludge and no worms. At different time intervals (0, 3, 6, 9 and 12 days), the flasks were sacrificed in ascending order and analysed. Flask 6, containing only flocculated sludge, was sacrificed at day 12 together with flask 5. To determine the removal of volatile solids (VS) by worm predation, both sludge VS and volatile suspended solids (VSS) of the water phase were measured and summed up to come to a total of VS present in a flask. To calculate VS degradation, the VS degradation in the WP bottles was lowered with the VS degradation values of endogenous degradation of flask 6. The wet weight of the worms was determined by removing all excess water with a paper napkin before weighing. The wet weight was used to determine the worm density because it

correlates well with the dry weight (Reynoldson et al., 1996). The solids were settled on the bottom of the flasks and the aquatic worms were crawling through the solids (Figure 5.1).

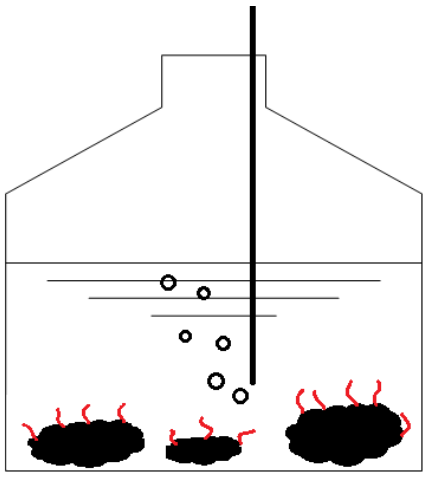

Figure 5.1. Schematic representation of bottle experiment. Flocculated sludge (black mass) accumulated on the bottom of the flask. The worms waved their tail through the water for oxygen uptake. The flocculated sludge solids remained separated from the liquid over the course of the 12 d experiment. Aeration is performed at approximately 15 cm above the sludge bed to prevent shear stress.

5.3.2. Comparison experiment

To verify if the applied worm population was able to survive on anaerobic sludge and to assess the potential toxicity of flocculated sludge, a comparison was made between worm survival during 25 days by grazing on 3.0 g of digested solids either separated by centrifugation or digested solids separated by flocculation (19 g/kg DS of Nalco 71305). For each type of separation method, 2L bottles were prepared with 1.6 L tap water and 100 aquatic worms in each bottle. To mitigate the potential negative influence of NH_4^+, the water phase was regularly replaced/refreshed with tap water. After 25 days of incubation in the dark and at room temperature, the living worms were counted, and the survival rate was determined. The experiment was performed in triplicate.

5.3.3. Toxicity assessment

To assess the direct toxicity of the Nalco 71305 cationic polymer on the worms, individual worms were subjected to a concentration range. Stock solutions containing 0, 1, 3.2, 10, 32, 50 and 100 mg/L polymer were prepared in tap water. To properly count the worms, 35 worms were distributed individually in Eppendorf tubes containing 0.5 mL of the respective polymer concentration. Every 2 days the polymer solutions in the Eppendorf tubes were replaced. Mortalities were recorded at 24 to 48h intervals. Worms were considered dead when no response to external stimuli was observed. A bright light source was found to be sufficient to induce a physical response.

5.3.4. *Analytical methods*

For analysis, the bulk liquid, the worms and the solids were separated. The determination of COD, ammonium, nitrate and phosphate was done with Merck kits (Massachusetts, United States). TS, VS, TSS and VSS were determined according to APHA (Clesceri, 1999).

5.4. *Results and discussion*

5.4.1. *Bottle experiment*

To assess the feasibility of WP on flocculated sludge, an indicative experiment was performed where worms were incubated during 12 d with flocculated sludge. During the 12 d experiment, the flocculated sludge remained separated from the water. The degradation of volatile solids continued over the course of the experiment and reached almost 40% on day 12 (Figure 5.2). The percentage of VS in the sludge was lowered from 58% on day 0 to 34% on day 12, which was similar to the resulting VS concentration of 41% after worm predation reported by Hendrickx et al. (2010). The latter publication also describes that *Lumbriculus variegatus* is able to grow on anaerobic digested sludge. Our results, however, show that the weight of the worm population declines over time.

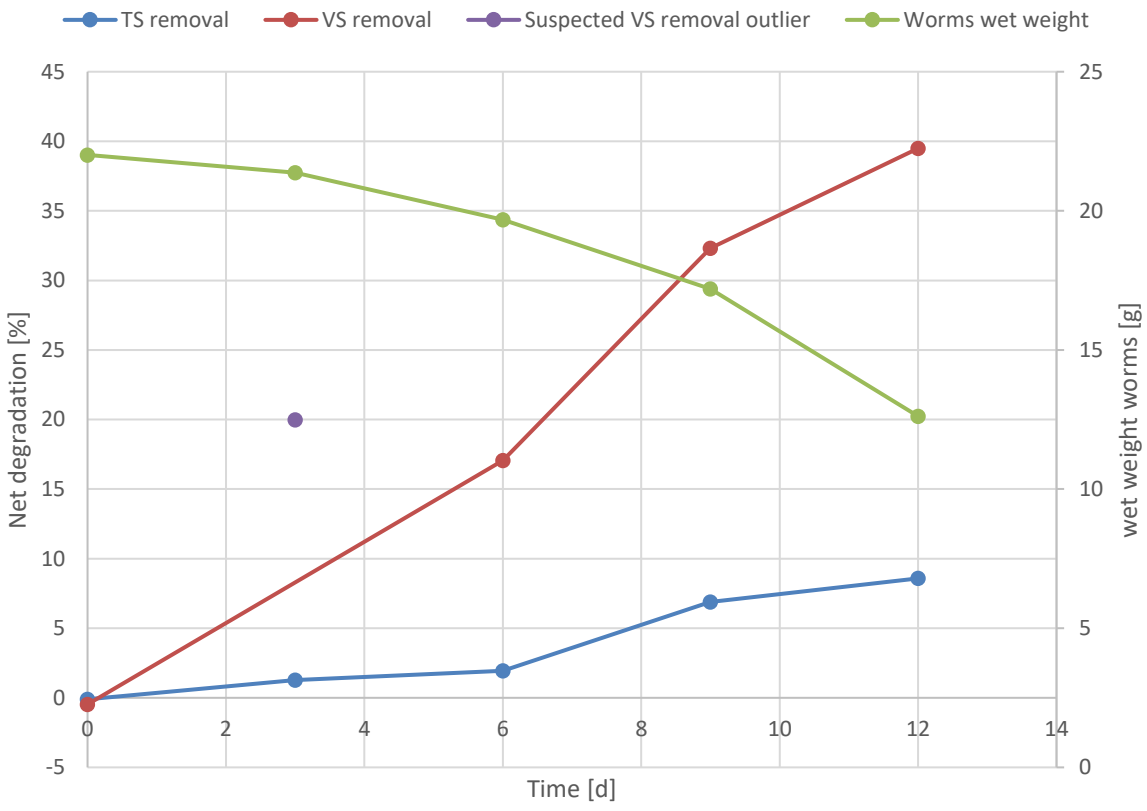

Figure 5.2. Degradation of TS, VS and the quantity of worms in the 12 day experiment

There can be several reasons for this decline in worm biomass. The decline may be caused by the relatively high concentration of total ammonium in water, varying between 23 and 62 mg-N/L over the course of the experiment (Figure 5.3). The LD50 of non-ionised NH_3 for aquatic worms are reported to range between 0.29 and 3.22 mg-N/L (Besser et al., 1998;

Hickey and Vickers, 1994; Schubaur-Berigan et al., 1995; Whiteman et al., 1996). Hendrickx et al. (2009b), however, reports that *Lumbriculus variegatus* can survive non-ionised NH_3 concentration of 8.00 mg-N/L. In our experiment, the concentration non-ionised NH_3 varied between between 0.25 and 3.72 mg-N/L, which is sometimes above and sometimes below the lowest reported LD50 value. As the worm biomass showed a decline, also in periods when the NH_3 concentration was low, it is concluded that the possible NH_3 toxicity is not the main driver for the worm biomass loss.

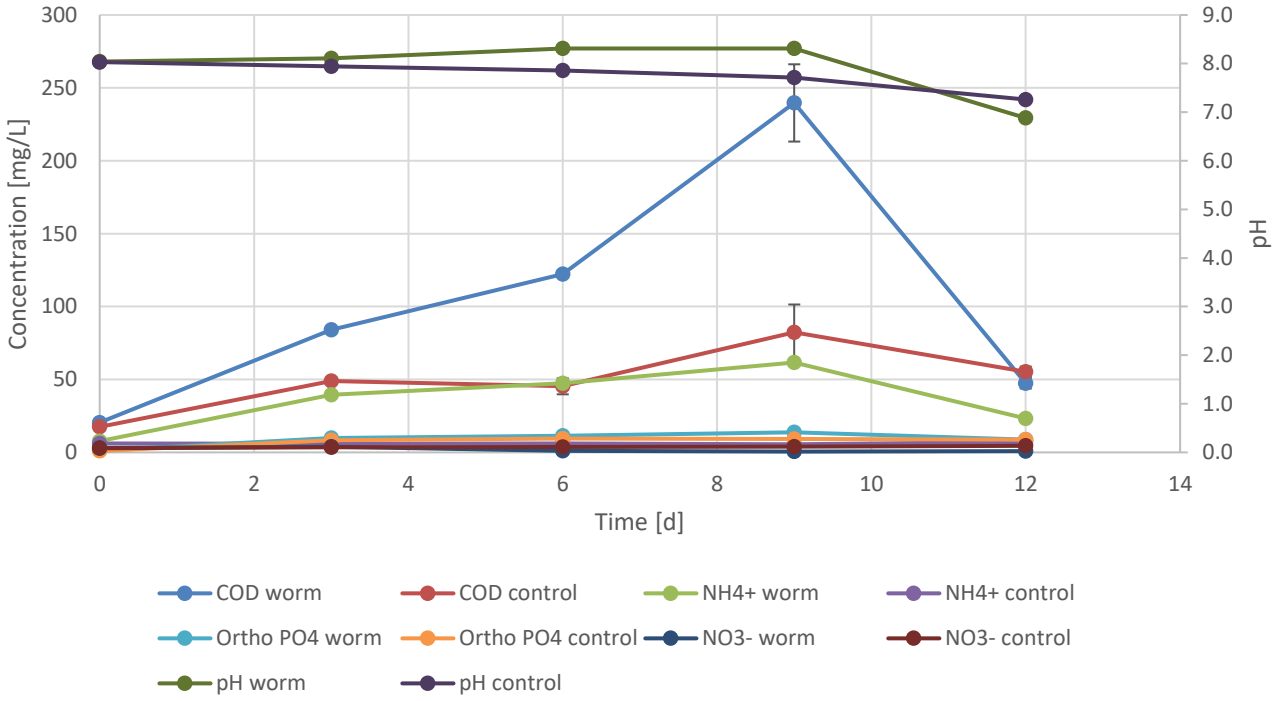

Figure 5.3. Development of concentrations and pH of the experiment with worms (worm) and the endogenous respiration (control).

During the experiment, the temperature varied between 19 and 23 °C which can be considered high since the highest biomass conversion rates with *Lumbriculus variegatus* were obtained at 15°C (Hendrickx et al., 2009b). However, the latter study reports that a decline in worm biomass only occurred at >25°C. Therefore, the applied temperatures in our study are considered suitable for worm survival. This was supported by the high viability of worms (98 ± 5 %) in the *comparison experiment* (see below), which was conducted at the same temperature (between 19 and 23 °C). Also, previous experiments with the same worm population and secondary sludge as substrate showed that the worms were able to grow in the applied temperature (data not shown). Therefore, the temperature was concluded not to be of a negative influence on the worm population.

It is known that flocculants irreversibly bind solids such that they are not available anymore for bioconversion in AD (Chapter 2 and 4). Therefore, a possible explanation for the decline in worm biomass might be a reduced bioavailability of the substrate (sludge mass) to the worms. However, *toxicity experiment* (see below) showed that worms can survive for many weeks without substrate. Moreover, the *bottle experiment* showed a net decrease in sludge VS when the sludge was incubated with living worms, indicating that the worms were

indeed responsible for VS degradation. Therefore, it is not very likely that a reduced bioavailability of the substrate caused the worm biomass to decline.

5.4.2. Comparison experiment

To explain the decline in worm biomass while feeding the flocculated sludge, a test was performed where a fixed number of worms were fed flocculated anaerobic sludge and compared to a fixed number of worms that was fed centrifuged anaerobic sludge. In both cases, the supernatant was replaced with tap water. After an incubation period of 25 days, the number of worms fed with centrifuged anaerobic sludge remained stable with 98 ± 5 % survival of the incubated worms. In contrast, only 68 ± 23 % of the worms survived that were fed with flocculated sludge. The difference in worm counts between flocculated and non-flocculated sludge shows that flocculation has a negative impact on the viability of the worms. Apparently, the applied cationic flocculants exerted a toxic effect on the worm population.

5.4.3. Toxicity experiment

Cationic flocculants are markedly more toxic than anionic and non-ionic polymers to aquatic organisms and therefore are rated at moderately to highly toxic (Hamilton et al., 1994). Although cationic flocculants are non-toxic to microorganisms (Campos et al., 2008; Chu et al., 2003), higher aquatic organism such as fish are known to suffer from flocculants toxicity of cationic polymers (Timofeeva et al., 1994). Therefore, also on aquatic worms, cationic flocculants likely may have a toxic effect. Natural cationic flocculants, i.e. chitosan, showed to have 4 day LD_{50} values of 1.72±0.08 g/L on *T. tubifex* (Mosleh et al., 2007). Also, the growth rate of the worms was negatively influenced by the presence of cationic flocculants: at all concentrations tested (25, 62 and 125 mg/L chitosan) a mortality was observed.

To assess the potential toxicity of the cationic flocculant applied in our experiment (Nalco 71305), a *toxicity experiment* was conducted. In this experiment, individual worms were subjected to a concentration range of polymer. The results are summarized in Figure 5.4. The Nalco 71305 was shown to exert a high toxicity to aquatic worms. The 4 day LD_{50} value of Nalco 71305 was shown to be between 50 and 100 mg/L which is more toxic than the chitosan polymer, reported by Mosleh et. Al (2007). Although, with flocculated sludge, the flocculants concentration is expected to be negligible in the water phase as it is well bound to the sludge. We hypothesize that the worms only will be exposed to higher concentrations of flocculants whilst grazing on the anaerobic sludge.

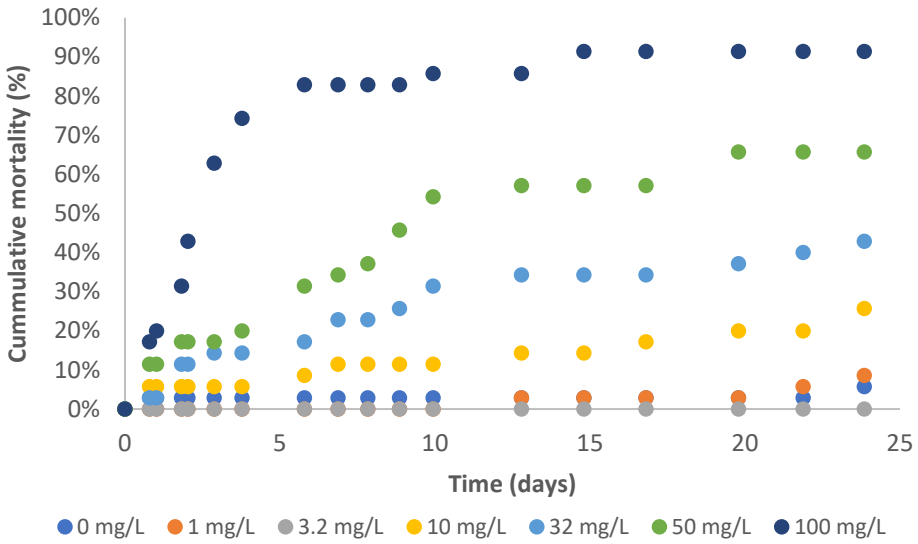

Figure 5.4. Cumulative mortality of aquatic worms exposed to different concentrations of cationic flocculant during 24 days.

5.5. Conclusions

Pet shop mixtures of *Limnodrilus* and *T. tubifex* efficiently converted the VS fraction of digested sludge, while grazing on anaerobic sludge separated by centrifugation. Also when the anaerobic solids were separated by flocculation, degradation of the VS of 40% after 12 days was observed. After 12 days the predated solids remain separated from the liquid and therefore, further treatment of the solids is facilitated. However, the worm population declined showing a higher degree of mortality when exposed to flocculated sludge. It was found that the cationic flocculant exerted a toxic effect to the aquatic worms with a 4 day LD_{50} of between 50 and 100 mg/L. Results show that flocculating anaerobic solids is an efficient way of separating digested solids from high ammonia concentration that hinders worm predation. However, subsequent worm predation appeared not to be feasible due to the toxicity of the applied cationic flocculants.

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6. Conclusions and future perspectives

Even though there are several solids/liquid separation processes in a conventional STP, the use of flocculants in sewage treatment is typically limited to sludge dewatering and to a lesser extent to sludge thickening. However, with the ongoing societal changes directing to a higher degree of circularity of resources and a higher degree of wastewater treatment demands, there is a need to re-asses the potentials that flocculants may offer in new wastewater treatment concepts. In countries like The Netherlands, STPs are increasingly redesigned as resource recovery factories. At present, the Dutch water authorities aim to maximise the recovery of energy and other resources, such as phosphorus, from the wastewater that they treat. In the meantime, they are asked to minimise fossil fuel consumption and are also facing the challenge to address the societal concerns on micropollutants emissions with the effluent.

This thesis investigated new perspectives on flocculant application in the sewage treatment plant of tomorrow. Figure 6.1 shows the set-up of a possible future STP based on mainstream Anammox for N removal and chemical P removal in which the potential application of flocculants is indicated. In the sections below, the applications of flocculants are further discussed.

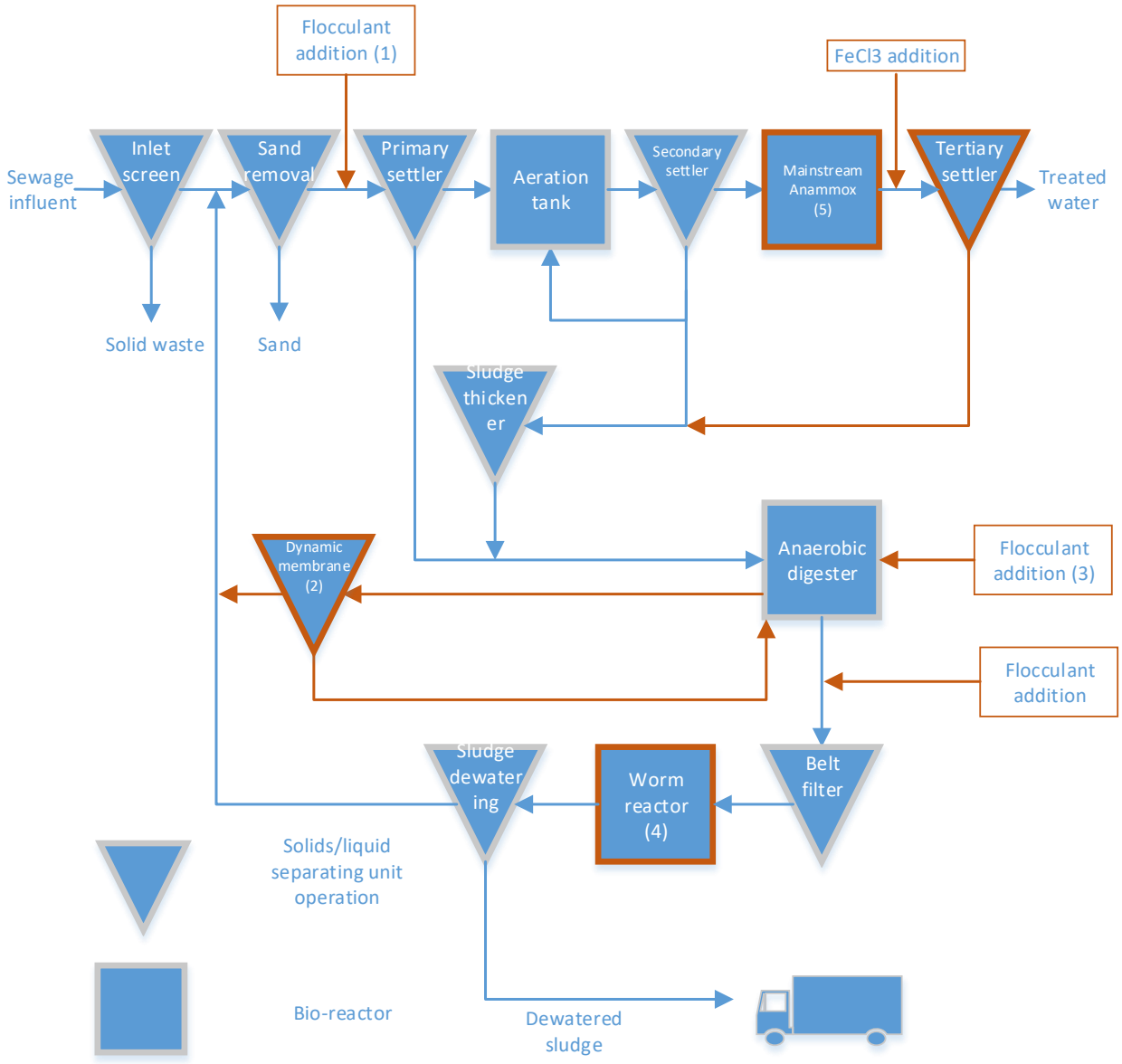

Figure 6.1. Possible future sewage treatment plant where primary sludge production is maximized, and sludge production and footprint are minimized. Possible applications of flocculant additions are indicated. The numbers 1 – 5 are further discussed in the text below.

6.1. Flocculant application for chemically enhanced primary treatment.

Applying flocculants for chemically enhanced primary treatment (CEPT) (Figure 6.1, Nr. 1), will increase the primary sludge production for biogas production, which leads to a more positive energy balance of an STP. Results in Chapter 2 indicate that 66% more influent COD could be used for biogas production via anaerobic digestion (AD), meanwhile the aerobic oxidation of this COD in the aeration tanks is prevented. Removing the COD in CEPT with cationic flocculants leads to a COD/N ratio of 3.75 g COD/g N in the water line, which is lower than the minimum ratio that is required for a conventional biological nutrient removal STP (BNR-STP): 6 to 30 gCOD/gN (Golterman, 1985; Roy et al., 2010; Sobieszuk and Szewczyk, 2006; Yadu et al., 2018). However, recently, novel N removal technologies were introduced that function well at low COD/N ratios such as N removal over nitrite, or that do not need any COD at all, such as the Anammox process in the waterline of an STP (Hoekstra,

2017; Lotti, 2016). The so-called “mainstream Anammox” process is also indicated in Figure 6.1 (Nr. 5). With the application of these novel N removal techniques, CEPT with flocculants could be advantageous for the overall energy balance and space requirements of the future STP. Besides the STP energy balance, as was described in Chapter 2, the application of cationic flocculants for CEPT also impacted the AD: the additional COD that was removed by CEPT was more readily biodegradable, leading to a 9% higher biomethane potential of the primary sludge. Also, in separate batch tests, it was found that flocculants decreased the viscosity of the sludge and, concomitantly, an increase in the methane production rate up to 27% was observed. In contrast to the rate of digestion, batch tests also confirmed literature reports that the refractory polyacryl amide (PAM) flocculants, irreversibly bound the solids, and thus reduced the biomethane potential.

6.2. Flocculation for pharmaceutical removal

Besides the increasing demand for energy efficiency and the ongoing pursue for lower operational costs by lowering waste sludge production, future BNR-STPs will also face challenges related to more stringent water quality criteria, particularly related to emerging pollutants. The increased consumption of pharmaceuticals is causing increased concentrations of these compounds in the sewage (Fekadu et al., 2018). With the current applied wastewater treatment techniques these refractory compounds are only partly removed from the wastewater effluent. Daughton and Ternes (1999) already stated that STP effluents are the main sources of pharmaceuticals in the environment. Literature reports that a large part of the pharmaceuticals in an aquatic matrix, such as present in an STP, are sorbed to colloids (Cheng *et al.*, 2017; Duan *et al.*, 2013; Holbrook *et al.*, 2004; Maskaoui and Zhou (2010); Zhou *et al.*, 2007; Yang *et al.*, 2011). Since flocculation can remove colloids, flocculants in principle should be useful to concentrate pharmaceuticals into a smaller sludge flow that subsequently could be treated more efficiently (Figure 6.1, Nr. 1). In Chapter 3, the possibility of concentrating pharmaceuticals by flocculation in the primary settler is investigated. A jar test showed that pharmaceuticals are hardly removed from sewage with coagulation/flocculation. To investigate the discrepancy between reported colloidal sorption and the lack of removal when removing colloids, we tested a commonly applied experimental setup for determining the colloidal sorption of pharmaceuticals. Colloids were removed from a solution containing pharmaceuticals in two ways: by ultra-filtration (UF) and by flocculation. Both methods showed similar removal of colloids. However, during UF up to 93±4% retention of pharmaceuticals was observed. But when removing the colloids with flocculation, no pharmaceutical removal was observed. These results strongly indicate that an analysis bias is introduced when using UF membranes in the determination of colloidal sorption of pharmaceuticals. Very likely, a direct retention of pharmaceuticals on the UF membrane occurs. Overall results of current work show that pharmaceuticals hardly sorb to colloids and herewith the absence of removal of pharmaceuticals during coagulation/flocculation is explained. Therefore, flocculation does not seem to be a viable option for concentrating pharmaceuticals from sewage streams.

6.3. Flocculants in an AnDMBR for sewage sludge treatment

A more concentrated sludge stream and increased digestion rates by applying CEPT would allow for smaller anaerobic digesters. However, a smaller digester volume can also be achieved by uncoupling the solids retention time (SRT) from the hydraulic retention time (HRT) (Figure 6.1, Nr. 2). Separation of liquid and solids retention is a typical feature of an anaerobic membrane bioreactor (AnMBR) where a membrane keeps the solids inside the reactor, while the liquid can permeate. In industrial wastewater treatment, especially in dairy, AnMBR application is already a proven concept with various full-scale references (Pacheco-Ruiz *et al.*, 2019). Thus far, application of membranes to shorten the HRT in sludge digesters only has been limitedly researched. In the late eighties and early nineties of the past century, the research group of Cape Town University applied cross-flow microfiltration units for enhanced sludge retention (Ross and Strohwald, 1994). But research was not followed-up because of the high investment and operational costs. However, membrane costs significantly dropped in the past decades and the interest in membrane application for enhanced sludge retention increased (van Lier *et al*., 2019). Membrane filtration of sludge will immediately result in the formation of a cake layer on top of the membrane's surface. This cake layer or fouling layer forms an excellent barrier for solids and acts as a secondary membrane during the filtration process (Jeison et al., 2008). Therefore, a simple woven cloth can also act as a support for this cake layer, avoiding the need for purchasing actual membranes (Ersahin et al., 2016, 2014), which would decrease the investment costs significantly. An AnMBR equipped with a woven cloth as filter medium is referred to as an anaerobic dynamic membrane bioreactor (AnDMBR) (Ersahin et al., 2014).

Challenges in operating an An(D)MBR are the filterability of the sludge and the viscosity which limits the maximum SRT that can be achieved. In Chapter 2 it was shown that flocculants can reduce the viscosity and increase the filterability of the sludge. Therefore, flocculants may play a positive role in the optimization of an AnDMBR. This concept was investigated further in Chapter 4, where coagulants and flocculants, or flocculation aids (FAs), were added to a waste activated sludge digesting AnDMBR. It appeared that the sludge required a high concentration of FAs to affect the filterability. However, these high concentrations caused a significant decrease in biomethane potential of the sludge as the VS destruction was lowered from 32% to 24% after adding the FAs. In addition, a decrease in the d50 (mean particle size) was observed from 58 µm to 32 µ, probably caused by small particles shielded by the refractory flocculants. Apparently, the accumulation of these small particles affected the filterability of the sludge, which led to a doubling of the trans membrane pressure (TMP) from about 150 mbar to 300 mbar. Therefore, adding flocculants to an AnDBMR does not yield the benefits that were initially expected. A potential solution to prevent irreversible binding, and thus a decreased filterability, is to use biodegradable flocculants. Further research is needed to evaluate this possibility.

6.4. Sludge reduction by worm predation

As the disposal of sludge forms a large part of the operational costs of an STP, waste sludge reduction in an STP may have a big impact on the operational costs (Wei *et al.*, 2018). A proven concept for waste sludge reduction in an STP is predation of sludge by aquatic

worms (Elissen, 2007; Hendrickx et al., 2010) (Figure 6.1, Nr. 4). In literature, several reactor configurations were studied in which secondary sludge is successfully predated by aquatic worms in lab-scale bioreactors (Elissen et al., 2006; Hendrickx et al., 2010, 2009; Serrano et al., 2016; Wang et al., 2011). However, in contrast to AD, secondary sludge reduction by aquatic worms will cost energy for aeration, meanwhile the converted organic matter is not available anymore for energy recovery. Therefore, the ideal configuration would be to have AD followed by worm predation (WP) of the digested sludge, allowing for both energy recovery and a larger extent of sludge reduction. So far, this had not been considered a viable option due to the low ammonia tolerance of aquatic worms and the high ammonium concentrations present in AD reject water. However, by flocculating anaerobically digested sludge, the sludge solids can be easily separated from the ammonia-rich liquid creating the possibility of WP of digested sludge, reducing the amounts of solids that need to be disposed. In Chapter 5, the AD-WP concept is further investigated. Results revealed an additional removal of 40% VS of the digested sludge in 12 days. Even though the solids remained well separated from the liquid, which facilitates further treatment, the cationic flocculants caused mortality of the used worms, due to toxicity. The assessed 4 day LD_{50} value was between 50 and 100 mg/L. For the possible full-scale application of WP for AD sludge degradation, a process could be designed with continuous worm addition. However, a non-toxic flocculant could also be the solution for the current mortality of the worms. Further research is needed to elucidate the best option and to validate the full-scale possibility.

6.5. Future perspectives

Flocculants today are only limitedly applied in STPs. However, they have a huge potential to play a role in the future STPs contributing to i) a smaller STP footprint, ii) improved energy balance and iii) lower environmental burden of wastewater treatment. But there are a few challenges to overcome.

Firstly, there is the environmental impact of flocculants. Flocculants can be considered a bulk chemical due to the vast worldwide use. Currently, the commonly applied oil-based PAM flocculants are increasing the carbon footprint of the STP. Switching to bio-based polymers could reduce this carbon footprint.

Secondly, there is the health impact of the flocculant. The monomers of poly acryl amide flocculants are known to be carcinogenic and neurotoxic (Okaiyeto et al., 2016; Yang et al., 2016). Changing to a non-PAM flocculant avoids these negative health consequences.

Thirdly, PAM flocculants are non-biodegradable (Chapter 3 and 4). And as flocculants are irreversibly bound to solids, the organic solids treated with flocculants become refractory and biological degradation will become partly impossible. This can lead to less sludge destruction and a lower energy recovery in AD for example. But also, the accumulation of small refractory compounds in AnDMBRs are detrimental for the filterability of the sludge. The use of biologically degradable flocculants would solve these issues and added flocculants may even be converted to biogas in the anaerobic digester.

Fourthly, the PAM flocculants are toxic for aquatic worms (Chapter 5), compromising the possibility of worm predation of digested flocculated sludge. Possibly, a bio-based flocculant may exert no toxicity to the worms and could create the possibility of sustainably degrading anaerobically digested solids while producing protein rich aquatic worms.

Finally, the economic feasibility of the use of flocculants needs to be carefully assessed. Currently, applying flocculants for purposes other than sludge dewatering and thickening is not economically feasible in the main water line of an STP, because of the vast volumes of water and the costs of flocculants. Commonly applied flocculants are fossil fuel based, which is a valuable commodity. A way to lower the price, may be to find an organic bulk waste stream that can be used for the production of flocculants.

A solution for the above-mentioned challenges may lie in the production of polysaccharide bio-based flocculants such as alginate, chitosan, cellulose and starch. These bio-based flocculants are generally derived from seaweeds, arthropods and plants (Salehizadeh *et al*., 2018). Bio-based flocculants are composed of carbon from renewable sources and have gained interest over the years due to the fact that they do not contribute, with respect to the raw materials, to greenhouse gas emissions. In addition, these bio-based flocculants are biodegradable and non-toxic (Kaith *et al*., 2010; Lanthong *et al*., 2006; Yang *et al*., 2016).

The performance of bio-based flocculants has been investigated for specific cases. For example, a starch based flocculant (STC-G-PDMC) showed to perform very well in the dewatering of digested sludge (Wang *et al*., 2013): with similar dosage the specific resistance to filtration (SRF) was $0.04x10^{13}$ m/kg which was much lower compared to the SRF of $1.51x10^{13}$ m/kg achieved with a PAM flocculant. In paper mill wastewater, alginate combined with a PAC coagulant, removed 93% of the colour of a synthetic dye wastewater (Wu *et al*., 2012). In the wastewater treatment of a paper mill factory, chitosan-based flocculant removed 80% of turbidity and COD (Renault *et al*., 2009). Despite numerous successful laboratory studies on bio-based flocculants, full-scale application is still marginal due to the high costs of production (Yang *et al*., 2016) and due to the – in general – inferior performance compared to oil-based flocculants (Salehizadeh *et al*., 2018).

A possible solution to make bio-based flocculants cost-effective is to find an organic waste source from which these polymers could be synthesized or extracted. An example of such waste source is alginate like exopolymers (ALE) from aerobic granular sludge (Lin *et al*., 2010). It already has been shown that ALE from this sludge is applicable for paper coating (Lin *et al*., 2015) or can act as soil conditioner or water repellent concrete coating (www.kaumera.com). However, thus far, to the authors knowledge, there is no research yet on the application of this ALE for flocculation. In contrast to ALE, EPS extracted from biodiesel wastewater treatment is shown to have comparable performance as a polyacrylamide flocculant (Ajao *et al.*, 2018). However, there are no full-scale examples of this concept to the authors knowledge. Therefore, more research is needed to prove the feasibility at full scale. Another topic of research should be directed to the improvement of flocculation performance of these polymers. For example, by adding special active groups to the bio-based polymers, these flocculants would become more broadly applicable for different processes, meanwhile flocculation performance could be enhanced. However, the

research for this is still in its infancy state and further development is required (Salehizadeh *et al*., 2018).

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7. List of abbreviations

abs	Absolute Value
AD	Anaerobic Digestion
AnDMBR	Anaerobic Dynamic Membrane Bio Reactor
BNR	Biological Nutrient Removal
BMP	Biomethane Potential
BOD	Biological Oxygen Demand
CD	Charge Density
CEPT	Chemically Enhanced Primary Treatment
COD	Chemical Oxygen Demand
CST	Capillary Suction Time
CSTR	Continuously Stirred Tank Reactor
DS	Dry Solids
EEM	Excitation-Emission Matrix
EGSB	Expanded Granular Sludge Bed
FA	Flocculation Aid
hPa	100 pascal
HRT	Hydraulic Retention Time
MFC	Mass Flow Controller
MW	Molar Weight
OLR	Organic Loading Rate
PAC	Poly-aluminium chloride
PAM	Polyacryl Amide
PC	Personal Computer
PSD	Particle Size Distribution
RPM	Rotation Per Minute
SMA	Specific Methanogenic Activity
SMP	Soluble Microbial Product
SRF	Specific Resistance to Filtration
SRT	Solids Retention Time
std	Standard Deviation
STP	Sewage Treatment Plant
TMP	Trans Membrane Pressure
TS	Total solids
TSS	Total suspended solids
USB	Universal serial bus
VS	Volatile solids
VSS	Volatile Suspended Solids
WAS	Waste Activated Sludge
WP	Worm Predation

8. Acknowledgements

Most former PhD researchers told me at forehand that a PhD track impacts your life for its personal development rather than for its technical development. I didn't think my PhD was going to be like that. But along the way, there had been the classic downs (also some ups), the frustration, the despair and even the moment where I intended to quit. Just as the people predicted. So, I tried to fully specialize into a subject, only to find myself being a typical graduated PhD, telling everybody who considers a PhD the same thing others had warned for before.

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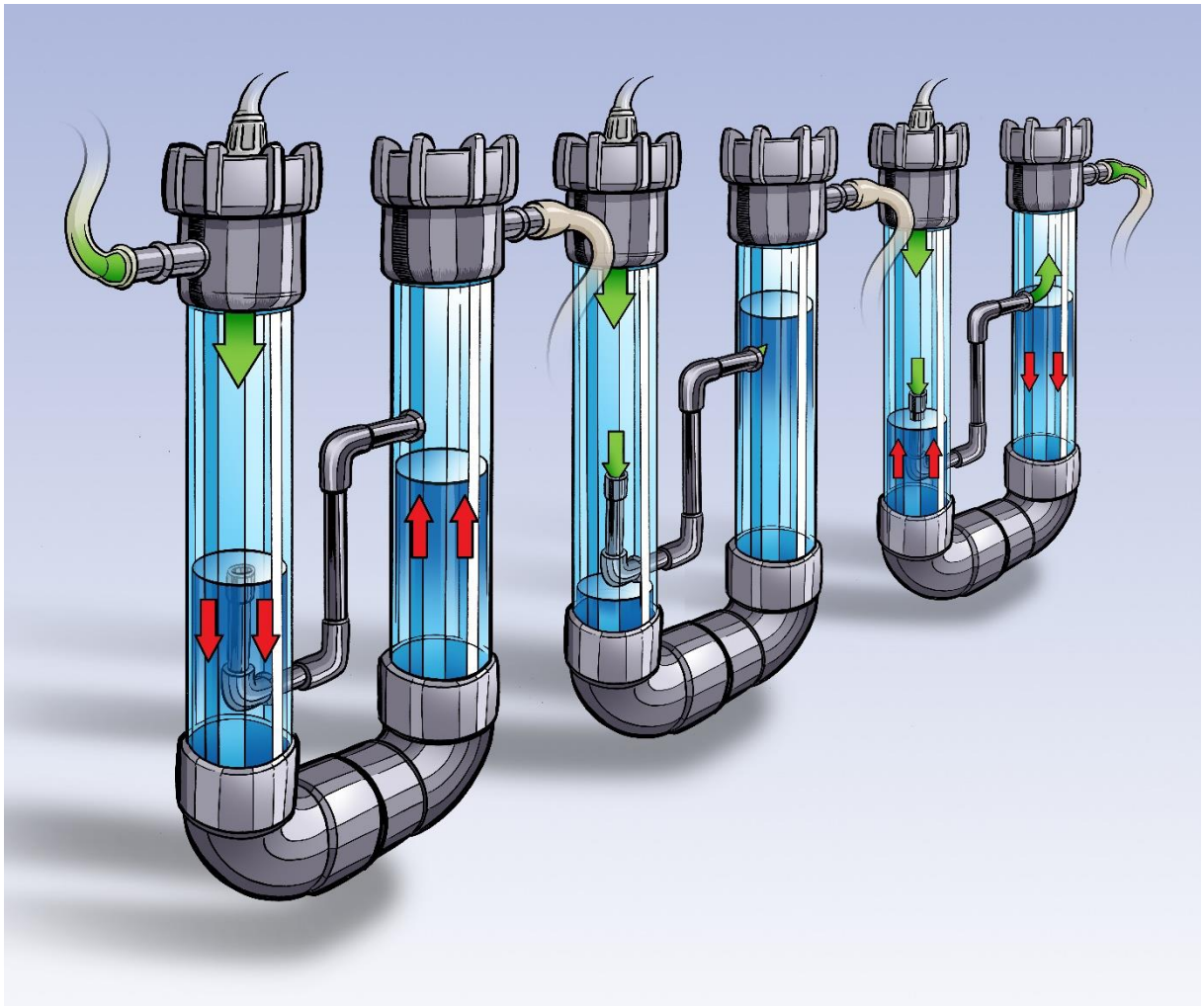
9. Appendix I: A novel biogas flow metering principle for laboratory-scale anaerobic digesters

Submitted to: Measurement Science and Technology.

By Guido Kooijman, Merle K. de Kreuk, Henri Spanjers and Jules B. van Lier.

9.1. Graphic abstract



Artwork: Stephan Timmers

9.2. Abstract

In this work, a novel type of biogas flow meter is presented. This gas flow meter combines the robustness of a hydraulic valve with temperature and pressure measurements. The gas flow meter with integrated micro-controller does not contain any moving parts and automatically corrects for the temperature and pressure of the biogas, computing gas volumes in standard temperature and pressure values. The micro controller that calculates the gas flow value can be connected to a PC by USB connection. The gas flow meter was constructed, calibrated and tested with a mass flow controller and with a CO_2 production experiment. The gas flow meter has an accuracy of >99% and precision of <1% coefficient of variation over the full range of gas flows between close to 0 and 15 N-L/h. It can handle any type of gas and the resolution of 0.4 N-mL is considered high for laboratory and bench-scale use. With this gas flow meter we solved the most common problems in bench scale biogas flow measurement such as increasing inaccuracies of moving parts, corrosion of metal parts, inaccurate measurements due to pressure and temperature variations and a too high cut-off value for accurate measuring of low-level biogas flow rates.

9.3. Introduction

In anaerobic reactor technology, the chemical oxygen demand (COD) balance is generally used to monitor the treatment performance of the reactor. For closing the COD balance of an anaerobic digester, accurate measurements of the biogas production are considered essential. The volumes of laboratory scale digesters generally range from 1 – 50 L (Hochfeld and William, 1994). The organic loading rate (OLR) of a continuously stirred tank reactor (CSTR) is typically about 5 kg COD m^{-3} d^{-1}. In case of a 90% conversion to methane and 60% methane content, this yields a maximum biogas production of 6 L/h in a 50 L reactor. Expanded granular sludge bed (EGSB) reactors can convert up to 45 kg COD m^{-3} d^{-1} (Van Lier, 2008). A maximally loaded 10 L EGSB reactor could therefore produce a biogas flow rate of about 10 L/h. Hence, it is reasonable to assume a maximum biogas flow rate of 15 N-L/h in a lab setup.

In literature there is a wide variety of laboratory gas flow measurement solutions, all with their advantages and disadvantages (Table 9.1). The earliest report of a biogas flow meter dates back from more than a century ago where a soap bubble flow meter was mentioned in literature (Exner, 1875). More recent, these soap bubble flow meters were combined with automatic optical detection (Lashkari and Kruczek, 2008; Tauber et al., 2011). However, currently, most applied gas flow measurements consist of either volumetric or manometric methods (Bonn, 2008). In manometric measurement devices, the pressure of the head-space gradually increases until the moment when a pressure threshold is reached and a solenoid valve releases the gas. The pressure can also be released manually (I. Angelidaki et al., 2009). A disadvantage of manometric methods is that high headspace pressure can result in increased CO_2 solubility, which significantly can disturb the microbial pathway and/or activity (Theodorou et al., 1994). The latter is the main reason why the in-situ headspace pressure should remain low during the biogas production rate determinations in anaerobic biodegradability tests (Rozzi and Remigi, 2004). The range and accuracy of

manometric methods is limited (Parajuli, 2011). Therefore, the majority of the gas flow assessments are based on liquid displacement, avoiding the drawbacks mentioned above. Biogas flow meters based on volumetric displacement are simple, economic and require little maintenance. The simplest and most well-known implementation of the liquid displacement principle is Mariotte's bottle (McCarthy, 1934). An advantage of this system is that it can be easily automated for continuous measurement with a balance connected to a computer, collecting the displaced liquid. The drawbacks are i) the displaced liquid periodically needs to be replenished, ii) the biogas flow should be low, iii) liquid displacement is affected by atmospheric pressure changes, and iv) leakages in connection tubing may occur. Another popular volumetric method is the use of a U-shaped tube (U-tube), where gas is introduced into one leg thereby pushing the water up in the other leg until a solenoid valve releases the gas and resets the system. Such automated U-tube system are either optically controlled (Angelidaki, 1992; Dissing et al., 1984; Liu et al., 2004; van den Berg et al., 1974), by inductive detection (Glauser et al., 1984) or controlled by liquid contact with electrodes (Moletta and Albagnac, 1982; Nilsson et al., 1988).

Currently, the most popular commercially available gas flow meters biogas flows of < 2 L/h are buoyancy driven tilting mechanisms where the number of tilts are counted of a gas hood submerged in a liquid under which gas is alternatingly collected and released. Examples of these gas flow meters are the Ritter Milligas Counter and the AMPTS of Bioprocess Control AB (Liu, 2012). For biogas flows higher than 2 L/h, a drum type gas flow meter are often used. In drum-type gas flow meters, biogas is introduced in a drum with packing liquid. The biogas flow causes the internals in the drum to rotate. The rotations are counted to determine the gas flow. Disadvantage of this the drum-type is that the lower flow limit is relatively high: 0.5 – 1 L/h but an advantage is that the upper flow limit can be up to several thousands of L/h.

Finally, there is a hydraulic valve system as depicted in Figure 9.2. In such a system, liquid displacement is automatically reset without the need for an electronic or mechanical control of the system. Due to the absence of system controls or moving parts this type of gas flow meters is intrinsically robust. In literature, examples of this system are described with discrete optical detection (Mata-Alvarez et al., 1986) or electrodes to count the number of systems resets (Veiga et al., 1990).

Table 9.1. Overview of commonly used lab scale gas flow metering methods.

Method	Advantage	Disadvantage	References
Bubble gas flow meters	Accurate measurement in low flow range	Low flows and narrow flow range	(Exner, 1875; Lashkari and Kruczek, 2008; Tauber et al., 2011)
Manometric gas flow meters	Accurate measurement in low flow range	Low flows and narrow flow range; dissolved CO_2 can disturb microbial activity	Oxytop (Weilheim, Germany), (I Angelidaki et al., 2009)
Volumetric devices: liquid displacement (e.g. Mariotte's bottle)	Inexpensive; no moving parts	Low flow range; labour intensive; increased risk for leakages; influenced by atmospheric pressure changes	(McCarthy, 1934)
Volumetric devices: mechanical detection	No labour required	Either low or high flow range; expensive	Ritter Milligascounter; Ritter Series TG; Biorpocess Control AMPTS
Volumetric devices: U-shaped tube with discrete displacement detection and active controlling	No labour required	Low resolution; Complex due to actively controlled valves;	(Angelidaki, 1992; Dissing et al., 1984; Glauser et al., 1984; Liu et al., 2004; Moletta and Albagnac, 1982; Nilsson et al., 1988; van den Berg et al., 1974)
Volumetric devices: hydraulic valve with discrete detection	Robust, no moving parts	Low resolution; gas amount per click depends on dimensions anaerobic reactor; relatively unreliable due to bubbles compromising good detection	(Mata-Alvarez et al., 1986; Veiga et al., 1990)

Every gas flow meter has its advantages and disadvantages (Table 9.1). But the most important factors affecting the precision and accuracy of biogas flow measurements, are errors due to varying temperatures and pressure (Walker et al., 2009). These errors are in most cases not addressed in the above-mentioned volumetric gas flow meters. In addition, the on-going contribution to literature with new principles of gas flow metering indicates that there is still a need for a low cost, more reliable, more precise and more accurate system.

In our present study, a continuous, low cost gas flow meter is presented covering the complete range of expected flows in lab scale digesters. The device also corrects for pressure and temperature changes of the gas. The presented gas flow meter is a hydraulic

valve where, instead of measuring the discrete resets of the system, the liquid level is gradually followed with a manometer in the gas phase of the first leg of the U-tube (P in Figure 9.2). The gas flow meter was patented (Kooijman, 2014), constructed and tested. The results of the tests are presented in this paper.

9.4. Material and methods

9.4.1. Gas flow meter description

The gas flow meter was constructed of conventional pluming 40 mm PVC and consists of two 90° bends that form the bottom the U-shaped tube and two transparent PVC pipes of 40 cm that for the legs (Figure 9.1).

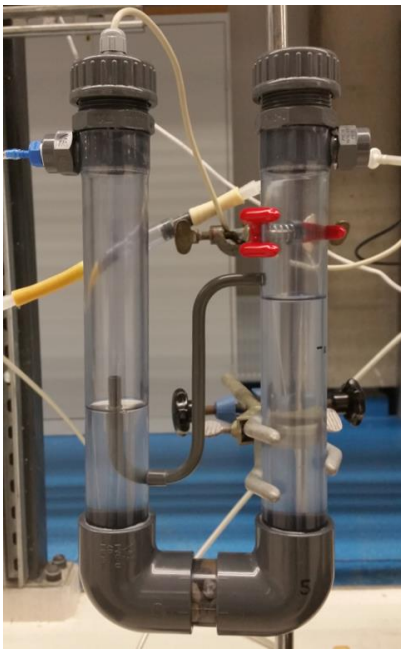


Figure 9.1. Photo of the gas flow meter used in this study. The gas flow meter is made of 40 mm PVC piping.

The gas flow meter is schematically depicted in Figure 9.2. The gas flow meter is filled with liquid, 1 cm above point 8 in Figure 9.2. The pressure and temperature were continuously measured in the headspace of the first tube (nr. 2) with an MS5803 pressure sensor (P) of Measurement Specialities (Hampton, Virginia, United states). The data was recorded and processed with an Arduino Uno micro controller. The gas flow data was transferred by a USB cable to a PC. Upstream of the inlet of the gas flow meter (nr. 1), an orifice with a round hole with a diameter of 0.8 mm was installed.

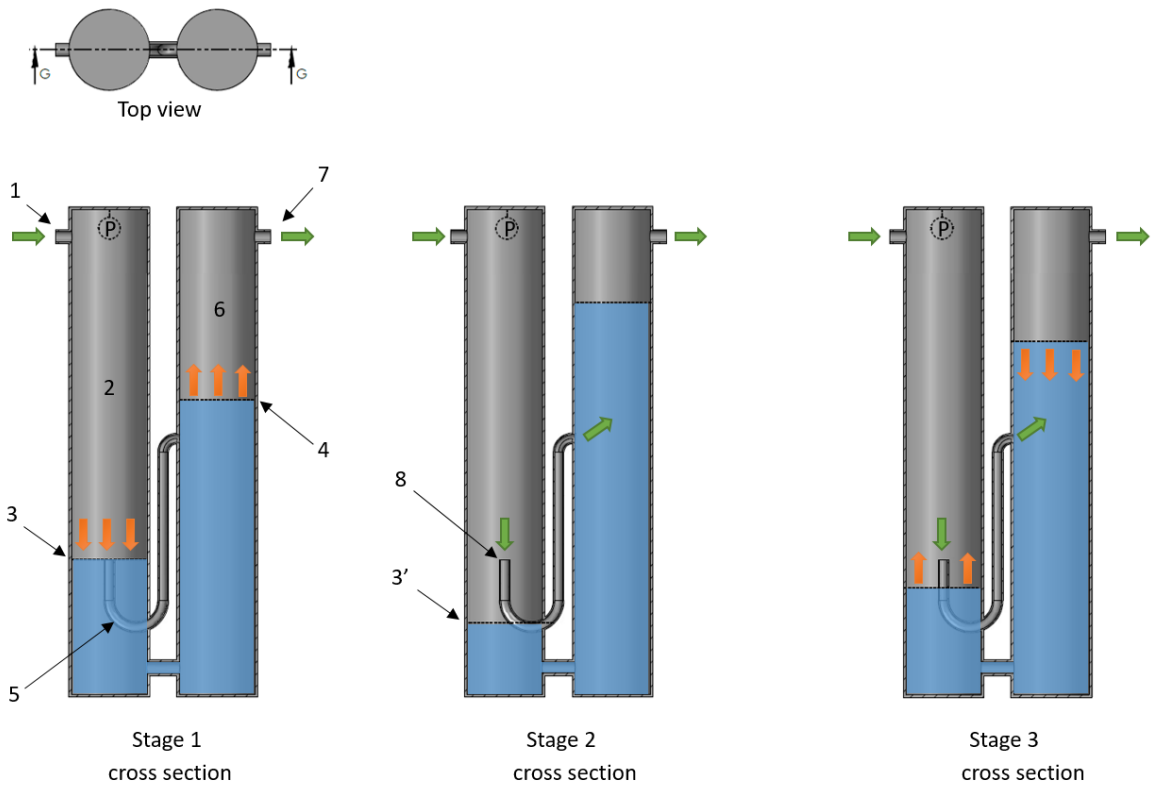


Figure 9.2. Schematic overview of physical functioning of the gas flow meter equipped with a pressure sensor (P) coupled to an Arduino Uno micro controller. All stages are cross sections of the device at line 'G' of the top view. The green arrows represent the gas flow and the orange arrows represent the water movement.

The physical working mechanism of the gas flow meter (Figure 9.2) is as follows: the starting position in represented as stage 1. Gas enters into the gas flow meter at nr. 1 and accumulates in the gas phase of the inlet leg (nr. 2) pushing the liquid level (nr. 3) down. Correspondingly, the liquid level (nr. 4) of the right leg is pushed up. This continues until the liquid level (nr. 3) reaches position nr. 3'as shown in stage 2. When 3' is reached, the gas can escape through the 'J' shaped tube (nr. 5) from the left head space (nr. 2) to the right head space (nr. 6) and subsequently to the atmosphere via the exit of the device (nr. 7), which is open to the air. Due to escaping gas from nr. 2 to nr. 6, the pressure drops in nr. 2 and the liquid level goes back up again, a process which is represented in stage 3. During stage 3, the gas continuous to escape from nr. 2 to nr. 6 until the liquid level nr. 3 reaches the lower opening of the J-shaped tube (nr. 8), which is then filled with liquid, blocking the free flow of gas from nr, 2 to nr. 6 via the J-shaped tube. Now the liquid levels in both tubes reach their starting positions again (stage 1). The process of alternating water levels is repeated, while the gas continues to flow. An event of zeroing the liquid levels (stage 3) is referred to as a 'click' and occurs in a matter of seconds. In the bottom of the gas flow meter, gravel was used as a kind of packing material delivering resistance to prevent too heavy back flow of liquid from the right to the left tube during a click.

9.4.2. Embedded software description

In the micro controller, the temperature and pressure signals are converted into a gas volume over time that is periodically sent to the PC over the USB. The main calculation is the conversion of the pressure increase (ΔP) to a volume. This conversion is done as follows:

$$\Delta Volume = \Delta P * converion\ factor$$

The conversion factor is determined for each gas flow meter individually. The embedded software consists of one main software loop, which is schematically shown in Figure 9.3. This software loop is executed by the micro controller each 0.5 seconds (when not in a 'click'). In the first step (step 1), the pressure and temperature are measured. Then follows a step (step 2) where it is determined if there is a 'click' event. A 'click' is recognized on when the measured pressure is lower (with a threshold) than the previously measured pressure (negative ΔP). In case of a 'click 'event, the pressure is repeatedly measured with a 250 millisecond interval until there are six successive stable pressure increases measured (step 2a). These stable pressure measurements are shown in Figure 9.4 as red circles. In step 2b, these six pressure measurements are used to extrapolate back over the 'click' period (red line in Figure 9.4). With this extrapolation, the gas flow is estimated that has escaped through the gas flow meter during the 'click'. In case of no 'click' event, the increase in measured gas volume is determined based the difference between the current and previous pressure measurement. The calculated volume is converted to standard conditions (1013.25 hPa (absolute) and 273.15 K) with the measure pressure and temperature before sending the value to the PC.

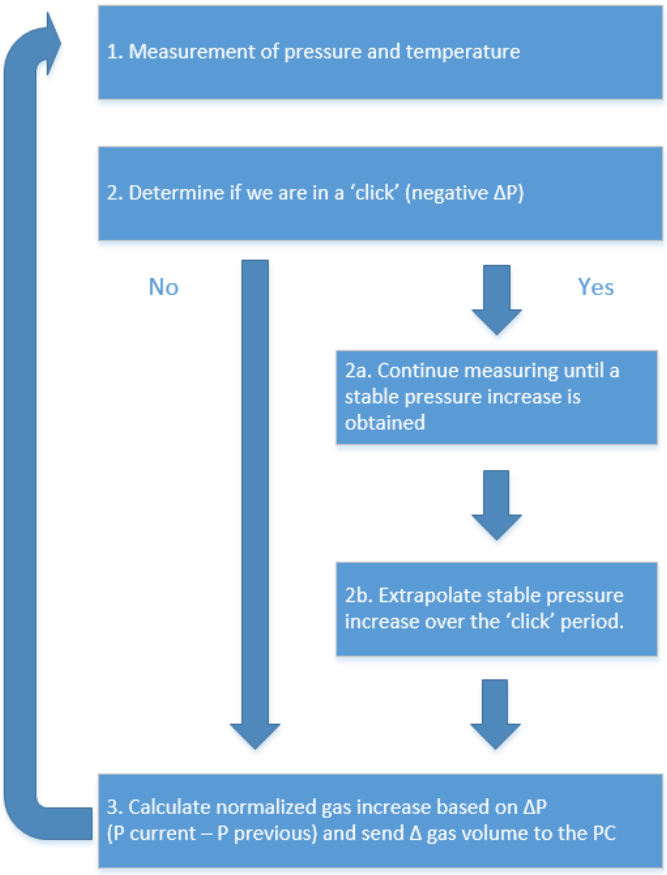


Figure 9.3. Schematic representation of the software loop that is executed in the Arduino Uno to measure the gas flow.

9.4.3. Calibration

In the micro controller, a calibration factor was used to convert the pressure increase to volume. The calibration factor was determined using a calibrated Bronkhorst (Ruurlo, the Netherlands) F-201CV mass flow controller (MFC) by using compressed air with a flow of 5 N-L/h for 15 minutes. The calibration was performing using the calibration settings. The calibration settings were: the gas flow meter filled with tap water 1 cm above point nr. 8; gravel was used as packing material for creating resistance in the bottom part of the gas flow meter, 8-12 mm gravel was used. The gas flow meter was positioned horizontally using a spirit level. Gas flow and volume values were normalized to 1013.25 hPa (absolute) and 273.15 K.

9.4.4. Performance evaluation

The precision was assessed by repeating ten times a gas flow measurement of 5 N-L/h for 30 minutes. The precision was expressed as coefficient of variation and calculated according the following formula (Brown, 1998):

$$Precision\ [\%] = 100 * \frac{std(measured\ gas\ flow)}{mean(measured\ gas\ flow)}$$

The accuracy of the gas flow meter was determined between 0.677 and 14.010 N-L/h, by supplying a known amount of air. Between 5 N-L/h and 14 N-L/h, the airflow was dosed by using the MFC setup. Air flows < 5 N-L/h were supplied by a Whatson Marlow 120u peristaltic pump. To relate the RPMs and the normalized gas flows of the peristaltic pump, a 2 L cylinder was positioned up-side-down in the water, completely filled with water. The time required to reach a certain volume of air underneath the cylinder in combination with temperature and atmospheric pressure was used to calculate the RPM-normalized gas flow relation. The accuracy was expressed as:

$$Accuracy\ [\%] = 100 - 100 * \frac{abs(expected\ gas\ flow - \ mean(measured\ gas\ flow))}{expected\ gas\ flow}$$

9.4.5. Correction for temperature

An experiment to assess the accuracy of the temperature sensor for volume flow correction was performed by placing the gas flow meter in an incubator at 25 °C, 30 °C and 40 °C (New Brunswick Scientific Innova 44). The MFC airflow was set to 5 N-L/h.

1.1. Critical factors affecting the gas measurement

To identify the critical factors affecting accurate biogas flow measurements several tests were performed. Each time, one of the following factors was changed compared to the calibration settings. The effect of liquid type was tested by using silicon oil (Sigma-Aldrich, 482412) instead of tap water. The effect of gravel type as a resistance in the gas flow meter was evaluated by using 4-8 mm gravel instead of 8-12 mm gravel. And the effect of orientation of the gas flow meter was tested by tilting the gas flow meter 4.3 ° to the left and to the right compared to an upright position.

9.4.6. Effect of different gas composition

The gas flow meter was calibrated with air using a calibrated MFC. Although, the nature of the gas is not expected to have any influence on the performance of the gas flow meter, a test with a different gas composition, in this case pure CO_2, was performed to confirm this. This CO_2 was produced in a precise and controlled way by acidifying a 90.24 g calcium carbonate (Sigma-Aldrich C6763) suspension in demineralized water. A 30% (w/w) hydrochloric acid (Boom, the Netherlands) was gradually added with a constant flow using a peristaltic pump (Watson Marlow 120u).

9.5. Results

9.5.1. Resistance in the gas flow

The gas flow rate meter was calibrated, by using air with a flow of 5 N-L/h. In a test the gas flow meter was connected to an EGSB reactor containing water, with air supplied on the bottom by the MFC. In this setup, the gas flow meter measured about 30% lower gas flow than was expected based on the MFC output. To prevent free, uncontrollable flow from the head space of the EGSB through the gas flow meter during a click, an orifice (diameter of 0.8 mm) was installed between the EGSB and the gas flow meter. The pressure profile in the gas phase of the first leg (nr. 2) is displayed in Figure 9.4, including the extrapolation that the

micro controller makes to estimate the undetected gas flow. The remaining experiments were done after installing an orifice and recalibration of the device.

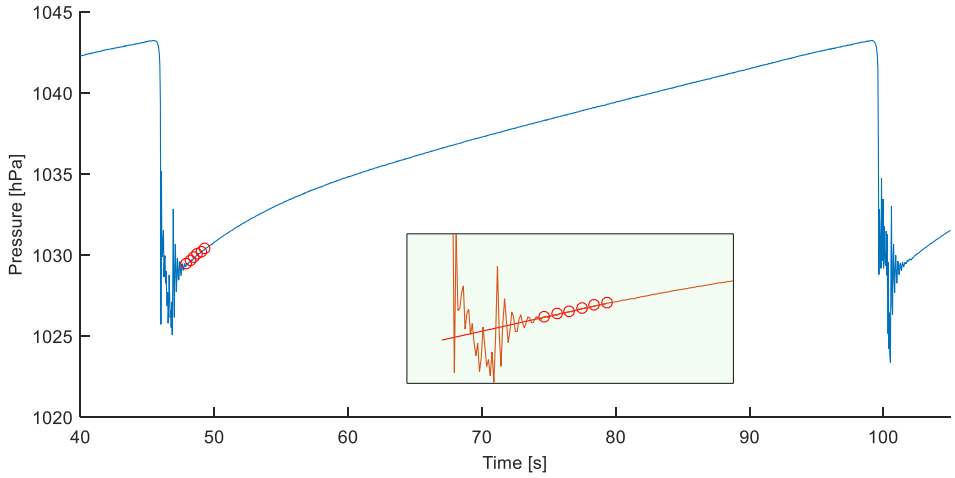


Figure 9.4. Example of pressure built-up during pressure equalization between the gas collector of the reactor and the gas phase of first tube of the gas flow meter at a flow of 5 N-L/h. The extrapolation (in red) is used to estimate the pressure built-up during the click from which the gas flow during the click can be estimated.

9.5.2. Precision and accuracy

The precision was determined by repeatedly measuring a flow of 5 N-L/h during 30 minutes. The coefficient of variation of the measured gas flows was 0.20%. The accuracy was determined by measuring over the whole range at different flows. The accuracy, determined with the MFC in the range 5 to 15 N-L/h, showed to be >99%. By using a peristaltic pump in the range 0.677 and 14.010 N-L/h, the accuracy was also >99%.

9.5.3. Factors affecting gas flow measurement

Various factors that affect accurate and precise gas flow measurements were investigated to assess the boundary conditions of the gas flow meter. In Table 9.2 the results are summarised. The error indicates the error with the respect to the reference conditions: calibration settings.

Table 9.2. Evaluation of effects on gas flow measurements. All deviations from calibration settings affected the measured gas flow, except for liquid amount in the gas flow meter. The column with error describes the deviated flow from the calibration settings in percentage.

Parameter	**Value**	**Error**	**Value**	**Error**	**Value**	**Error**
[-]	[N-L/h]	[%]	[N-L/h]	[%]	[N-L/h]	[%]
Calibration settings	1.49±0.01	-	18.71±0.08	-	39.69±0.06	-
Oil as liquid	1.38±0.01	7.97	16.21±0.02	15.42	34.33±0.07	15.61
Tilted right	1.56±0.01	4.49	18.83±0.04	0.64	39.28±0.34	1.04
Tilted left	1.54±0.01	3.25	18.96±0.12	1.32	40.03±0.30	0.85
Different resistance	1.57±0.03	5.10	19.22±0.08	2.65	40.30±0.17	1.51
3 cm more water	1.49±0.01	0.00	18.80±0.05	0.48	39.78±0.17	0.23

9.5.4. Temperature correction

To test the capability of the software connected to the gas flow meter to correct for temperature, a test was performed with a 5 N-L/h flow at 25 °C, 30 °C and 40 °C. The accuracy of the measured flows where 0.23%, 0.54% and 0.50% respectively. It can be concluded that the gas flow meter successfully corrected for the temperature as the variation in non-normalized gas flow was up to 5% in case of 40 °C compared to 25 °C.

9.5.5. Alternative gas composition

The gas flow meter was calibrated using air. To assess if an alternative gas composition affects the measurements due to solubility, an experiment was performed in which CO_2 was produced by adding hydrochloric acid to an emulsion of calcium carbonate. The produced CO_2 was measured with the gas flow meter. The expected CO_2 production of the acidification of calcium carbonate was 20208.83 N-mL. During the experiment 217 mL of 30% HCl was pumped in. Over the course of the experiment the gas meter measured 20158.03 N-mL which shows an accuracy of 0.25%.

9.6. Discussion

9.6.1. Resistance in the gas flow discussion

The deviation of the gas flow meter measurement from the actual gas flow in the EGSB set-up was caused by a pressure build-up of ~15 hPa in the gas phase of first leg (nr. 2 in Figure 9.2). The pressure build-up was caused by the difference in liquid level nr. 3 and nr. 4. Gas phase nr. 2 was directly connected via a tube to the gas collector of the EGSB. Therefore, there was also a pressure built-up in the gas collector of the EGSB, maximally reaching up to ~15 hPa at the moment a click occurred. During a click, the open gas connection between nrs. 2 and 6 also effectively connected the pressurised gas collector of the reactor with the atmosphere for about 2 seconds. The duration of a click was long enough to release the ~15 hPa overpressure from the EGSB gas collector. The overpressure gas escaped without being

detected which caused the measured gas flow to be too low. For example, a reactor with a gas collector volume of 1 L at 1000 hPa absolute pressure, will release ~15 mL each click event because of the 15 hPa pressure drop in the 1 L gas collector. Because of the geometry of the gas flow meter, click events occur every ~60 mL of produced gas. Therefore, every 60 mL, 15 mL of gas will escape from the head space undetected which will result in a 20% too low gas flow measurement. With a gas collector volume of 2 L this would be 40% too low measurement. The overpressure release is an issue that is inherently present when using hydraulic valves (and also U-shaped tubes). However, the issue of measuring a gas flow that is too low has not been mentioned or addressed before in literature.

To prevent a sudden escape of gas at each click event a restrictive plate – or orifice – was installed at the gas inlet of the device (nr. 1 in Figure 9.2). The orifice was a plastic insert with a round hole of 0.8 mm diameter. The restrictive plate prevented a sudden escape of the pressurised gas from the gas collector to the atmosphere due to the head loss during a click event. With a restrictive plate installed, at the end of a click event when the J-shaped tube was filled with water again, the pressure inside the gas collector was not in equilibrium with left leg of the device nor with the atmosphere. Therefore, the equilibration between the gas collector pressure and the pressure in the left leg (nr. 2 in Figure 9.2) continued after the click event until equilibrium was reached. In the early stage of this pressure equilibration, there is a steady pressure built-up in nr. 2 as shown in Figure 9.4. This steady build-up was then used to extrapolate the non-measured gas flow over the period when there were no viable data. By quantifying this extrapolation, the escaped gas can be estimated. After installing the restrictive plate and adapting the embedded software, the gas flow out of the EGSB was accurately (>99 accuracy) and precisely (<1% coefficient of variation) measured. However, the consequence of the restrictive plate was that the maximum flow rate that could be measured with the gas flow meter was 15 N-L/h. Flows of >15 N-L/h would result in too high (>20 hPa) gas pressure beneath the gas collector and possible gas loss in the EGSB. In addition to this, the accurate gas flow is only measured correctly after a ‘click’ had occurred. Therefore, the measured gas values before a ‘click’ will not be accurate as the head space effect is not yet considered into the measurement.

9.6.2. Factor affecting flow measurements

Liquid type

Because gasses can dissolve or react with the liquid in the gas flow meter, it may be desirable to use a different type of liquid such as silicon oil instead of water. When silicon oil is used, there is an under-determination of 7.3% for the lowest flow and 13.5% for the highest flow. Therefore, with a different type of liquid, the gas meter needs to be recalibrated.

Tilting the gas flow meter

The effect of orientation was investigated by tilting the gas meter to 4.3° to the right and 4.3° to the left (randomly chosen angles). In both cases applying a low flow there was an overestimation of the gas flow of 4.6% and 3.4%, respectively. These results indicate that it

is essential to mount the gas meter in the same orientation as the orientation during calibration.

Increasing the liquid volume

Due to cooling of the gas when flowing from the reactor to the gas flow meter, water may condensate in the first tube of the gas flow meter. Therefore, the liquid levels may change. The effect of the amount of liquid in the gas flow meter was studied by topping up the water level to 3 cm higher than the calibration level. The measurement error was < 0.5% (Table 9.2). Therefore, it can be concluded that the amount of liquid in the gas flow meter does not affect the performance of the gas flow meter.

9.6.3. Costs of the gas flow meter

The gas flow meter is constructed from a pressure sensor, and Arduino Uno micro controller, PVC pipes and junctions, wiring and glue, which adds up to less than 80 euros. The saw tooth pressure profile, induced by a gas flow, is converted to a gas flow with a calibration factor. Because the calibration factor is determined for each copy of the gas flow meter, the manufacturing can be done with relatively high tolerances and thus will not require expensive precision tools.

9.6.4. Future improvements

An advantage of the gas flow meter presented in this paper is that there are no moving parts that can corrode or get stuck. The part that is expected to be most vulnerable is the pressure sensor. In theory the pressure sensor could corrode due to corrosive gasses such as H_2S, often present in biogas. The sensitivity of the pressure sensor for H_2S gas was not investigated and will most likely depend on the nature of the pressure sensor sealing and the quality of the biogas. Therefore, a further improvement may be to physically separate the pressure sensor from the gas phase by a flexible membrane that transduces the pressure but forms a barrier for corrosive gases.

9.7. Conclusion

The continuous gas flow meter presented in this study has an accuracy of >99% and precision of <1% coefficient of variation with a high resolution (0.4 N-mL). It can handle any type of gas and has a flow range between close to 0 N-mL/h to 15 N-L/h, which makes it capable for measuring all laboratory scale biogas flows. Due to temperature and pressure monitoring, the micro controller can provide a normalized gas flow. The signal can be sent via a USB connection to a computer. With this new concept, we created a gas flow meter with a user-friendly interface; an acceptable accuracy and precision and which is expected to be reliable on the long term due to the lack of moving parts.

9.8. Acknowledgements

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