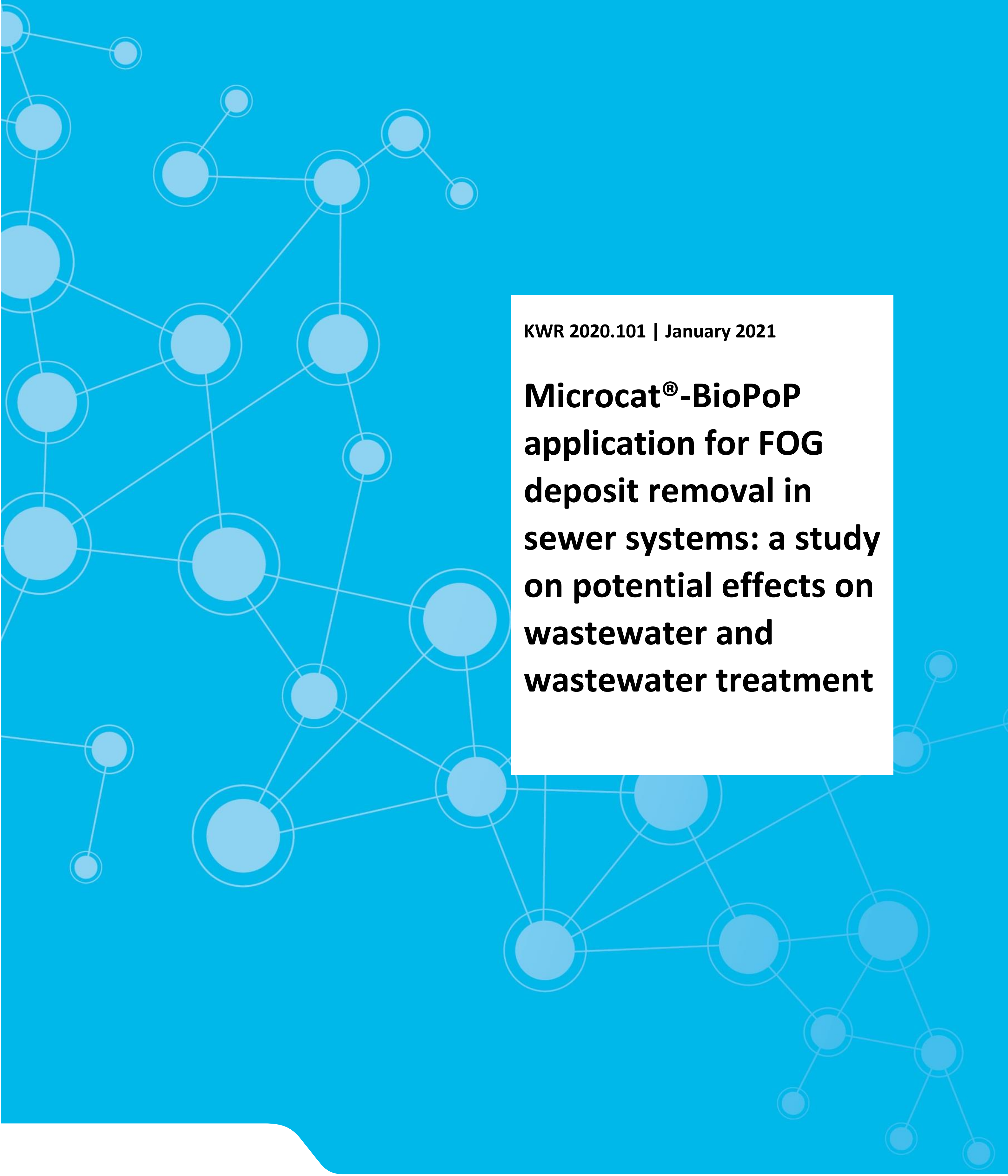

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# Microcat®-BioPoP application for FOG deposit removal in sewer systems: a study on potential effects on wastewater and wastewater treatment

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# Rapport

## Microcat®-BioPoP application for FOG deposit removal in sewer systems: a study on potential effects on wastewater and wastewater treatment

### KWR 2020.101 | January 2021

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# Samenvatting

Het lozen van vet, olie en smeer (fat, oil and grease (FOG)), bijvoorbeeld via etensresten, gebeurt onder meer via de riolering. In de riolering kan dit FOG samenklonteren en zich afzetten tegen de voorzieningen. Dit kan leiden tot verstoppingen waardoor mogelijk systemen, zoals pompgemalen, uit kunnen vallen. In de praktijk komt het steeds vaker voor dat FOG veroorzaker is van correctief onderhoud. Dit brengt hoge (maatschappelijke) kosten met zich mee. De veroorzakers van FOG in de riolering kunnen niet eenvoudig getraceerd worden. FOG lozers kunnen grofweg in drie groepen onderverdeeld worden: horeca, bedrijven en huishoudens. Door bezuinigingen zijn controles van vetafscheiders bij horeca en/of bedrijven vaak geen prioriteit meer. Omdat de lozingsnorm van FOG is vervallen, is juist controle van de vetafscheiders een must. Er wordt niet enkel benedenstrooms van horeca/bedrijven vaak FOG-blokkades aangetroffen maar in de gehele riolering. Het aandeel FOG van individuele huishoudens en vergunningsvrije kleine horeca (zgn 'hobbybakkers') dat geloosd wordt op de gemeentelijke riolering is aanzienlijk en is zeer moeilijk terug te herleiden.

Microcat®-BioPoP is een product dat op verscheidene locaties in Nederland wordt ingezet om vetblokkades te voorkomen in (riool)netwerksystemen van industrie en gemeenten. Dit leidt volgens de gebruikers tot lagere onderhoudskosten en minder overlast van FOG in het rioolstelsel. Ondanks dat dit product al veelvuldig wordt toegepast, zijn de eventuele gevolgen van deze toepassing op de bedrijfsvoering op een rioolwaterzuiveringsinstallatie (RWZI) echter nog niet onderzocht.

Onderzoeksvraag

Als projectgroep hebben we samen mogelijke effecten van het toepassen van de Microcat®-BioPoP in het riool op de bedrijfsvoering van de RWZI ingeschat, aangevuld met mogelijke effecten geïnventariseerd op basis van literatuurstudie en interviews van vakmensen. Ondanks dat experts minimale effecten verwachten, is er wel een duidelijke wens uitgesproken vanuit de waterschappen om dit te toetsen. De onderzoeksvraag is als volgt geformuleerd: "kunnen er negatieve of schadelijke effecten voor de bedrijfsvoering van de RWZI verwacht worden als gevolg van de toepassing van Microcat®-BioPoP in het rioolstelsel in strijd tegen vetophoping? Na een eerste inventarisatie zijn de volgende mogelijke, ook al werd de kans klein geacht, effecten nader onderzocht. Heeft het toepassen van Microcat®-BioPoP in het rioolstelsel effect op de samenstelling van afvalwater (hoofdstuk 4,5 en 6), 2) nutriëntenverwijdering op de RWZI (hoofdstuk 7.1), 3) anaerobe vergisting (hoofdstuk 7.4), en 4) vrij komen van organische microverontreinigingen (hoofdstuk 7.3)? Door middel van laboratoriumonderzoek is gekeken of het toepassen van Microcat®-BioPoP in de strijd tegen FOG-ophoping in het riool (of gemaal) effect heeft op de procesvoering van de RWZI (op basis van chemisch zuurstof verbruik (CZV) en stikstof (N)) en/of de efficiëntie van de anaerobe vergisting. Daarnaast is gekeken naar het vrijkomen van ongewenste organische verontreinigingen.
KWR heeft geen enkele relatie met de producent en aanbieders van Microcat®-BioPoP, en onderschrijft ook niet het (bio)product of het gebruik ervan. De resultaten en conclusies in dit rapport staan volledig los van enig commercieel doel.

Materiaal en methoden

Om deze onderzoeksvragen te beantwoorden zijn verschillende methoden toegepast. Het effect van Microcat®-BioPoP op de RWZI is in de eerste plaats onderzocht met bekerglasexperimenten, waarbij met synthetisch afvalwater het effect van Microcat®-BioPoP en FOG in verschillende combinaties is getoetst. Bovendien is er een onderscheid gemaakt tussen synthetisch FOG, en FOG uit de praktijk van een industrieel of municipaal rioleringsstelsel (of een combi).

Daarnaast is met behulp van bioreactoren over een duur van 100 dagen het effect van Microcat®-BioPoP op het actiefslibproces onderzocht. Er zijn twee bioreactoren operatief gesteld, elk gevoed met synthetisch afvalwater en FOG afkomstig van een rioleringsstelsel met zowel industrieel als municipaal afvalwater. Voor een van de reactoren is in de voedingstank Microcat®-BioPoP toegevoegd. Verder is het mogelijke effect van Microcat®-BioPoP op de vergisting onderzocht met het AMPTS II systeem (accumulated methane potential test system) Met behulp van dit systeem kan de snelheid en mate van biogasproductie worden gemeten. Ten slotte zijn monsters van het bekerglasexperiment en monsters van het effluent van de bioreactor, genomen op verschillende momenten, geanalyseerd op basis van non-target screening en suspect target screening. Hiermee is onderzocht of mogelijk ongewenste organische microverontreinigingen zijn vrijgekomen. Het eventueel vrijkomen van microverontreinigingen zou een risico kunnen vormen voor de bedrijfsvoering van de RWZI. De concentratie Microcat®-BioPoP was voor deze experimenten relatief hoog. In een praktijksituatie zal deze hoeveelheid vele malen lager liggen, en zullen eventuele effecten in de praktijk kleiner verwacht mogen worden.

Effect op samenstelling afvalwater (hoofdstuk 4, 5 en 6)

Bekerglasexperimenten speelden een belangrijke rol bij de eerste labverkenningen. In deze testen werden verschillende condities uitgeprobeerd in absentie van actief slib. De aanwezigheid van Microcat®-BioPoP in synthetisch afvalwater laat nauwelijks variatie zien in P, nitraat en nitriet. Ondanks de afwezigheid van actiefslib is er wel ammoniumomzetting waargenomen. Het toevoegen van synthetisch FOG verslechterende ammoniumomzetting, maar deze verbeterde door aanwezigheid van Microcat®-BioPoP . Voor CZV was dit effect anders: door het toevoegen van FOG werd de totale en opgeloste CZV-concentratie direct bij aanvang van de test verhoogd. Dit effect was overigens sterker voor FOG met een industriële oorsprong dan voor municipale FOG. Alleen wanneer Microcat®-BioPoP aanwezig was, nam de totale CZV-concentratie verder toe gedurende de eerste dagen van deze batchtest. Er is geen significant effect op fosfaat waargenomen door toevoeging van FOG of Microcat®-BioPoP.

Effect op actief slib (en bedrijfsvoering) (hoofdstuk 7.1)

Het vervolgonderzoek bestond uit een langeduurtest van 100 dagen in twee bioreactoren. De bioreactoren werden gevoed met synthetisch afvalwater met een toevoeging van FOG uit een gecombineerde industrieel en municipaal rioolstelsel. Bij één van de reactoren is Microcat®-BioPoP toegevoegd, bij de ander niet. Op basis van visuele bevindingen bleek dat in aanwezigheid van Microcat®-BioPoP er meer FOG-desintegratie plaatsvindt in de voedingstank. Omdat de (mate van) FOG-desintegratie verder niet het doel van dit onderzoek was, is dit niet verder onderzocht. Er is in beide reactoren geen sulfide- of schuimproductie waargenomen. In de reactor waarbij Microcat®-BioPoP is toegevoegd aan de influenttank, was sprake van een significante verwijdering van opgelost CZV (>75%) en N (>90%). Wel was de CZV-verwijdering een fractie lager voor de reactor waarbij Microcat®-BioPoP was toegevoegd. Er was geen sprake van fosfaatverwijdering, dit was echter ook niet het geval in de reactor zonder Microcat®-BioPoP . In het influent van de reactor gevoed met Microcat®-BioPoP lag de FOG-concentratie hoger dan voor de reactor zonder Microcat®-BioPoP . Voor beide reactoren geldt dat de FOG-concentratie in het effluent onder de detectielimiet was. Wel was er in de reactor met Microcat®-BioPoP sprake van FOG-films aan de wand van de reactor, dit is echter niet nader onderzocht.

Effect op vergisting (hoofdstuk 7.2)

Het slib dat na 100 dagen in deze bioreactoren (wel of niet Microcat®-BioPoP ) is gegroeid, is vervolgens getest met de AMPTSII om de efficiëntie van vergisting te onderzoeken. Er is geen significant verschil gevonden in biogasproductiesnelheid en yield voor slib gevoed met influent dat wel behandeld met Microcat®-BioPoP in vergelijking met slib dat deze behandeling niet had.

Effect op microverontreinigingen (hoofdstuk 7.3)

Het influent en effluent van de bioreactoren, evenals samples van het bekerglasexperiment zijn getoetst met non-target screening en suspect-target screening. Voor zowel de positieve als negatieve ionisatie methode zijn er geen significante verschillen te koppelen aan de aanwezigheid van Microcat®-BioPoP . Deze labresultaten laten zien dat er geen sprake is van significante toevoeging van ongewenste organische microverontreinigingen door toepassing van Microcat®-BioPoP .

Conclusie
Samengevat is in dit onderzoek onderzocht of er mogelijke negatieve of schadelijke gevolgen kunnen optreden bij de toepassing van Microcat®-BioPoP  als preventief middel tegen vetophoping in de riolering. Er is met succes een bioreactor actief geweest dat gevoed werd met influent bestaande uit synthetisch afvalwater, FOG uit municipaal/industrieel riolering en Microcat®-BioPoP. De verwijdering van opgelost CZV (>75%) en stikstof (>90%) in de reactor waarbij de voeding behandeld was met Microcat®-BioPoP  werd niet negatief beïnvloed. Vergistingsproeven lieten zien dat er geen significante verschillen zijn in biogasproductie en yield voor slib uit deze reactoren. Er zijn verschillen waargenomen in samenstelling tussen samples al dan wel of niet behandeld met Microcat®-BioPoP  voor wat betreft organische microverontreinigingen, maar op basis van de uitgevoerde suspect-target screening bleek dit niet significant. Deze labresultaten geven aan dat er geen negatieve effecten op de bedrijfsvoering van een RWZI verwacht worden als gevolg van de preventieve inzet van Microcat®-BioPoP  vetophoping in het riool. Kortom, bij toepassing van Microcat®-BioPoP in hoge concentraties, resulteerde in het behandelingsproces niet tot substantiële verschillen. In de praktijk is er echter sprake van verdunde omstandigheden, omdat Microcat®-BioPoP wordt toegepast op een deelstroom en in lagere doseringen, er worden daarom ook geen (substantiële) verschillen verwact in de praktijk.

# Contents

# 1 Introduction

## 1.1 Background

### 1.1.1 Fat, oil and grease (FOG) deposits in the sewer system

Discharges of fat oil and grease (FOG) can occur for instance by food waste disposal through the sewer system. In the sewage this FOG can agglomerate and deposit against the facilities. Excessive amounts of FOG deposits can cause blockages and system failure, but also cause unwated overflows (Post et al. 2016, U.S.EPA 2004). To solve this, corrective maintained is required, which is accompanied by high (social) costs. The source of FOG deposits in the sewage cannot be traced easily. Within the Netherlands, FOG dischargers can roughly be divided into three groups: the catering industry, companies and households.  Due to cutbacks, inspection of grease traps from companies are often no longer a priority. As FOG discharge standards are expired, the urgency of grease traps inspection increased. Not only downstream catering establishment/companies FOG deposits are found, but also in the entire sewer system. The fraction of FOG from individual household or unlicensed catering establishment (so called- hobby bakers’) that is discharged in toe the sewer system is considerable and hard to trace.

There are differences in the origin of FOG among countries. The FOG deposits in sewers in Canada, the United States, and Great Britain are mainly caused by wastewater from (fast food) restaurants and other companies that prepare food (Bowen 2006, Keener et al. 2008, Williams et al. 2012). According to sewerage staff, the FOG blockages occur between 50 and 200 m downstream of the restaurants and approximately every 30 days to 2 years (Keener et al. 2008). However, in the Netherlands, companies that process food (including restaurants, slaughterhouses) are required to install a FOG separator. Separators should be emptied monthly and FOG should be disposed to special FOG processing companies. This is a legal requirement which is enforced by the inspectorate. For places where mineral oils are used, an oil separator is required (Rijkswaterstaat). Huge fatbergs as reported in the UK or the US will not occur in NL. Nevertheless, FOG is present in sewers, mainly from domestic use.

Several mechanisms can lead to FOG deposits in the sewerage. Because FOG cools down in the sewer system, it can harden and deposit against the walls or other obstacles in the sewer (un-reacted free fatty acids) (He et al. 2011, Keener et al. 2008). Besides, the unreacted free fatty acids (FFAs) in the boiled or heated oil can react with calcium to form a calcium soap through saponification, which also deposits in the sewer system (see Figure 1)  (He et al. 2011, Williams et al. 2012).

The deposits investigated have a grainy, sandy texture with a high yield strength (Keener et al., 2008). A high yield strength ensures that the deposit cannot easily be deformed. Because the deposits have a sticky character, debris can easily settle and accumulate in the deposit (Iasmin et al. 2014, Keener et al. 2008). When a FOG deposit has formed, it often contains FFAs that have not reacted yet. Due to the charge of these FFAs, a surplus of calcium can be attracted by van der Waals attraction and electrostatic repulsion (He et al. 2013, He et al. 2011). These mechanisms can explain the high calcium levels found in deposits (He et al. 2011, Keener et al. 2008). In addition, water hardness can also influence the calcium content. Williams et al. (2012) found that higher water hardness resulted in higher calcium levels in the deposit which was also harder and had a higher melting point than deposits with lower calcium contents.

A possible source of calcium can be concrete corrosion. However, wastewater also contains calcium, which means that a change of material from the sewer pipes does not necessarily lead to fewer FOG deposits (He et al. 2013). The different sources of calcium can lead to different deposits with regard to texture and adhesion (Iasmin et al. 2014).

Most deposits are found above the waterline and obstructions in the sewer system (Williams et al. 2012). These obstructions can be roots of trees or other deformations in the pipe (Dominic et al. 2013). Also, so-called 'fat bergs' can be found in pumping stations where the water level is constantly changing (Williams et al. 2012). This is an indication that potential deposits are floating on the water and the actual adhesion is formed only upon drying, which explains the deposits above the waterline and obstructions.

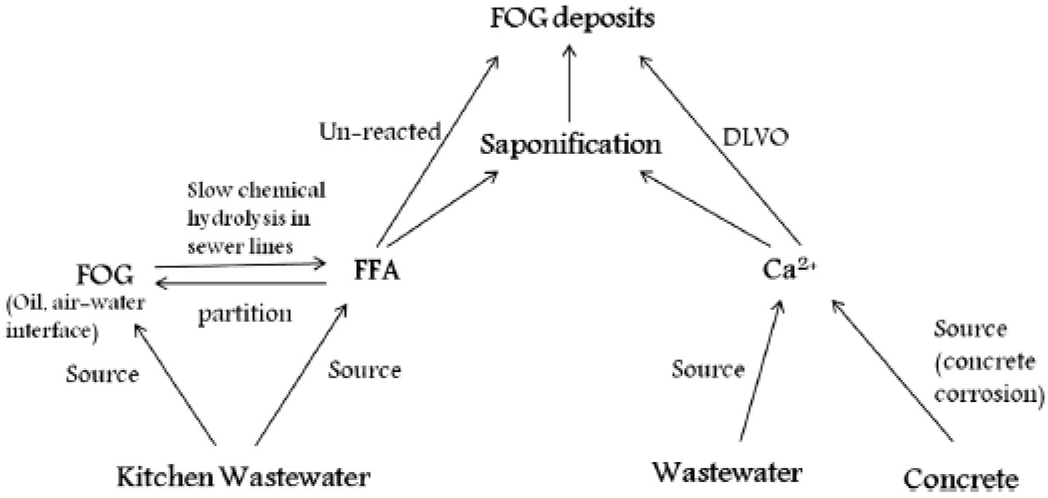


*Figure 1 Several proposed mechanisms of FOG deposits (He et al., 2013).*

### 1.1.2 Fat, Oil and Grease (FOG)

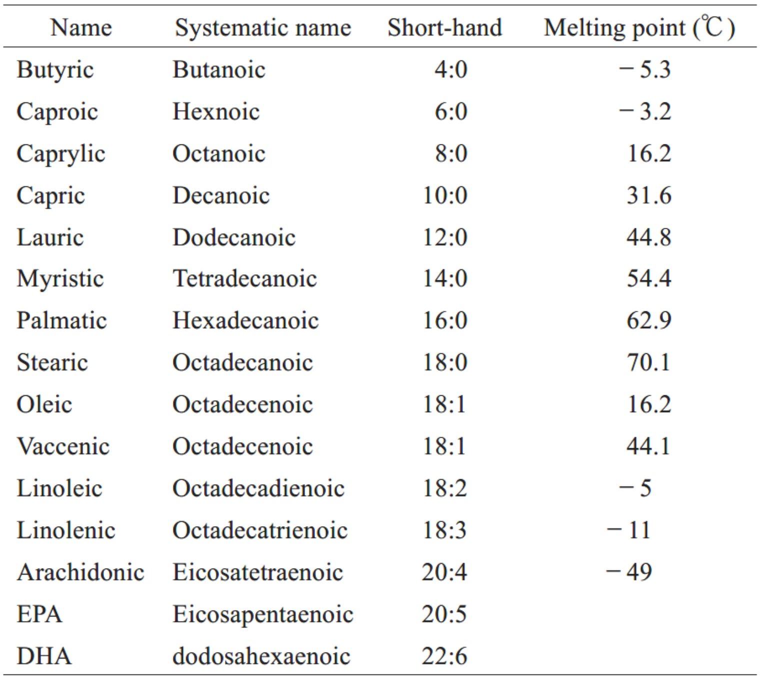
Fat, oils and grease (FOG) are organic molecules mainly byproducts of cooking. FOG includes matter such as food scraps, meat fats, lard, tallow, cooking oil, butter, margarine, sauces, gravy, dressings, deep-fried food, bake goods, cheese, and butter. FOG can be solid or viscous liquid depending on the saturation of carbon chain. Oils and fats are subsection of lipids that are composed of fatty acids (mainly hydrophobic) , tryacylglycerols, and lipid-soluble hydrocarbons that are minor bur important components of FOG (Husain et al., 2014). In high pH conditions these fats and oils will hydrolyse (saponification), and with calcium they will form insoluble complexes.

### 1.1.3 FOG removal in wastewater treatment plants (WWTP)

Many WWTPs have a FOG trap installed in the intake and will remove a significant amount at the influent side, before the primary clarifier. The most abundant FFA in food are shown in Table 1 (Husain et al. 2014). Free fatty acids are expected to be biodegradable and therefore converted by biological processes in the wastewater treatment plant (Chipasa and Mędrzycka 2006). However, fats have a negative influence on the transport of oxygen in biofilms, as a result of which the micro-organisms get less oxygen and achieve lower growth rates. The fats can thus be removed biologically, but in general, the biological oxygen demand (BOD) in the effluent is higher when the influent BOD contains fats.

Moreover, the fats adhere to the biomass, and influence the sedimentation of the biomass. The latter causes problems with sludge removal, because foaming can take place and the sludge can continue to float (Chipasa and Mędrzycka 2006). Due to the adhesion of fats to biomass, the anaerobic digestion can be influenced by the fact that the biomass tends to float and foam formation can take place (Lienen et al. 2013). Long-chain fatty acids can also have an inhibiting effect on the anaerobic micro-organisms (Chipasa and Mędrzycka 2006) and the microbial community can shift (Lienen et al. 2013). Nevertheless, FOG conversion in anaerobic reactors leads to higher biogas production (Long et al. 2012).

*Table 1 Most common free fatty acids (Husain et al., 2014)*



| Name | Systematic name | Short-hand | Melting point (℃) |
|---|---|---|---|
| Butyric | Butanoic | 4:0 | − 5.3 |
| Caproic | Hexnoic | 6:0 | − 3.2 |
| Caprylic | Octanoic | 8:0 | 16.2 |
| Capric | Decanoic | 10:0 | 31.6 |
| Lauric | Dodecanoic | 12:0 | 44.8 |
| Myristic | Tetradecanoic | 14:0 | 54.4 |
| Palmatic | Hexadecanoic | 16:0 | 62.9 |
| Stearic | Octadecanoic | 18:0 | 70.1 |
| Oleic | Octadecenoic | 18:1 | 16.2 |
| Vaccenic | Octadecenoic | 18:1 | 44.1 |
| Linoleic | Octadecadienoic | 18:2 | − 5 |
| Linolenic | Octadecatrienoic | 18:3 | − 11 |
| Arachidonic | Eicosatetraenoic | 20:4 | − 49 |
| EPA | Eicosapentaenoic | 20:5 | |
| DHA | dodosahexaenoic | 22:6 | |

### 1.1.4 FOG removal by commercial microbiological products

In this project we investigate the effect of Microcat®-BioPoP as a bioremediator for FOG in sewers. However, Microcat®-BioPoP is not the only commercial product in the field of bioremediation of FOG. Several other commercial products are mentioned by Cammarota and Freire (2006), however, their effects are not mentioned. The action of micro-organisms on the removal of FOG depends on the type of oil and fat and the micro-organisms that are used for bioremediation. This was also supported by the research of Wakelin and Forster (1997) in which different pure cultures, but also mixed cultures, were tested on different FOG. Active sludge was found to be the most effective to remove different FOG, especially when the activated sludge was acclimatized to FOG.

Bio-Amp is a biological addition of Eco Bionics (Irving, TX, USA), consisting of high levels of 5 different active microorganisms (*Pseudomonas fluorescens, Pseudomonas putida, Bacillus subtilis, Bacillus licheniformis, and Bacillus thuringiensis*) that are known to FOG removal (Tang et al. 2012). A study in which Bio-Amp was added in a grease separator showed that it was able to convert FOG, resulting in up to 40% fewer FOG deposits in the sewer. Additionally, fewer fatty acids were measured in the months that Bio-Amp was used (59% lower concentration). Furthermore, other compounds were converted, resulting in lower levels of chemical oxygen demand (COD), total nitrogen, and total phosphate being supplied to the WWTP. It was also found that the fraction of easily biodegradable COD was increased after the application of Bio-Amp, which could lead to a possible improvement of biological phosphate removal at the WWTP. Overall no negative effects of the application of Bio-Amp were found.

In addition to commercial products, enzymes and micro-organisms have also been tested for the degradation of FOG in WWTPs. The anaerobic treatment of dairy effluent by the addition of an enzyme pretreatment has been investigated by (Gomes et al. 2011). This showed that pretreatment with the enzyme pancreatin led to lower efficiency of the fermentation and also to a less stable process. This was due to an inhibitory effect of the enzyme on the biomass in the digester, which reduced COD removal.

The pretreatment with microorganism *Candida rugosa* for anaerobic treatment of dairy effluent showed that there was a positive effect on methane production (Domingues et al. 2015). This was also the case for the enzymes produced by *Penicillium sp*. As demonstrated by (Rosa et al. 2009). Here, a marked reduction in COD removal was measured when the pretreatment was stopped. Pre-treatment with microorganism *Geotrichum candidum*, however, led to an inhibitory effect of the biomass in the anaerobic digester (Domingues et al. 2015). In conclusion, the impact on the biological treatment process depends on the microorganisms and the type of FOG.

### 1.1.5 FOG bioremediation by Microcat®-BioPoP

Microcat®-BioPoP is bioremediator product for FOG in sewers systems. According to the manufacturer and suppliers, the Microcat®-BioPoP are rugged, semi-submersible solid forms that slowly dissolve and release safe, naturally-occurring microbes that can reduce odor and improve fat, oil, and grease (FOG) degradation. The Microcat®-BioPoP is composed of slowly dissolving, biodegradable matrix material packed inside a durable, porous mesh netting. They are designed to be dropped into or suspended in used water containing systems (drains, grease traps, lift stations, sewer lines, etc) by a line or rope. Microcat®-BioPoP contains only environmentally–safe, naturally-occurring preselected microbes. The microbes are specially selected for their ability to break down (not just dissolve) the organic contaminants common in food preparation and manufacturing wastewaters and domestic water discharges. For optimal applications, the water pH should be 6-9 (optimum 7), and the temperature range 10-40°C (optimum 35°C).

### 1.1.6 User's experience with Microcat®-BioPoP

In consultation with the partners, interviews were carried out at two locations: Zoetermeer and Alkmaar municipalities. In both cases, a large proportion of the wastewater is treated with Microcat®-BioPoP. The persons who were approached, and the questionnaire that served as a guideline for the discussion are included in Appendix I. Additionally, two experts in the field of wastewater treatment were consulted their opinion on what possible effects on the treatment could be if all the wastewater in the sewer has been treated with Microcat®-BioPoP.

Based on interviews outcomes (see Appendix II), the overall picture from the municipalities about the use of Microcat®-BioPoP to reduce fat, oil and grease accumulation in the sewer is positive. The impression of the municipalities is that there are fewer calamities with regard to fat accumulation and that odor nuisance has been reduced. Unfortunately this cannot be confirmed with figures/data/facts.

On the other hand, wastewater technologists experts suggested that hardly a negative effect is expected on the WWTP as a result of the Microcat®-BioPoP application in the sewer system. If an effect is noticeable, it is unanimously expected to appear first in the fermentation, or in the activity of anaerobic bacteria.

## 1.2 Aim and objectives

Fats, oils, and greases (FOGs) in wastewater create issues including the blockage of sewer lines. Removal of FOGs from wastewater is thus important to assure smooth disposal of the wastewater to the treatment plants. There are several commercial products in the market mentioned by Cammarota and Freire (2006) that have been used for such purpose. Microcat®-BioPoP is one of the products in the field of removal of FOG in the sewer. However the effects on the wastewater and treatment system are not mentioned.

This study aims to identify the main research question:

**Should one expect negative or damaging effects on the operation of the WWTP as a result of the application of Microcat®-BioPoP in the sewage system to avoid fat accumulation?**

In order to answer this research question the following subresearch-questions were formulated:

- What is the effect on the wastewater matrix? (chapter 4, 5 and 6)
- What is the consequence of these effects on the performance (COD, N, P removal) of the activated sludge process at the sewage treatment plant? (chapter 7.1)
- What is the consequence for the digestion process? (chapter 7.2)
- What is the effect on the release of organic micropollutants (chapter 7.3)

For each subresearch-question it is indicated in which chapter the results are presented.

## 1.3 Wastewater matrix effect (Batch tests)

In order to gain insight into the effect of Microcat®-BioPoP on the wastewater matrix, tests are carried out in the presence and absence of Microcat®-BioPoP , and also in the presence/absence of boiled fat, oil, and grease (FOG). For the first experiments, artificial wastewater and FOG are chosen instead of a sample (wastewater / FOG) from practice. Experiments based on an artificial medium in controlled conditions are more feasible to perform. This has the advantage that the composition is known, making the data more manageable to be analyzed.
After the first experiments with synthetic wastewater and artificial FOG, experiments with practical samples of solid FOG and synthetic wastewater follow.
Therefore, the following starting points were taken into account for the experiments:

- Synthetic wastewater is used; as described in van den Brand et al., 2014.
- Synthetic FOG: as described in Aiyuk and Verstraete (2004), Synthesis (ratio 1:4 soy oil to milk powder FOG).
- The oil was boiled (about 15 minutes), in practice this is also the case for the majority (frying fat and similar); and influences the effect as shown in the literature study.
- No addition of activated sludge during the experiments to avoid that the results are disturbed by the introduction of a not well defined matrix and/or by biological conversion.
- There is no need to work sterile because in practice there are also microorganisms present. Moreover, there is a continuous supply of mixed bacteria consortium.
- The system is mixed gently to simulate the flow and turbulence in the sewer.

## 1.4 Activated sludge process effect: Bioreactors performance

In order to determine the effect of Microcat®-BioPoP on the activated sludge process, two bioreactors are operated with the presence and absence of Microcat®-BioPoP in the influent wastewater, both with the presence of boiled fat, oil, and grease (FOG). For the experiments, artificial wastewater and solid FOG from a combined municipal/industrial sewage were used. Experiments based on artificial wastewater in controlled conditions are more feasible to perform and make the analysis of the effects of Microcat®-BioPoP on the treatment process and effluent quality more appropriate.
Therefore, the following starting points were taken into account for the experiments:

- Synthetic wastewater is used; as described in van den Brand et al. (2014).
- There is no need to work sterile with the influent because in practice there are also micro-organisms present. Moreover, there is a continuous supply of mixed bacteria consortium from the Microcat®-BioPoP.
- The system is completely mixed to maintain the sludge in suspension and avoid dead zones.
- The reactors are operated in a sequencing batch mode to avoid activated sludge wash-out.
- The same amount of FOG is added to both reactor influent tanks.

## 1.5 Non-target screening

One of the concerns with the addition of products to the sewer is the formation or release of toxic and inhibiting compounds which may end up causing issues in the wastewater treatment system. In principle, during the saponification of FOGs, glycerol and free fatty acids (FFAs) are formed. Fatty acids are linear molecules or branched linear hydrocarbons with carbonic acid groups. If they are biodegraded, smaller molecules, most likely, shorter chain fatty acids and volatile fatty acids are formed. However, micropollutants or other subtances trap withing the FOG, could be also released.

Early identification of these unknown micropollutants in water is not only essential for carrying out a risk assessment, but it is also necessary to predict the behavior of a substance in the environment and in the water treatment process. Through a non-target screening method, data can be screened from databases for candidate substances in the water samples, including exact mass, isotope pattern, MS2 fragmentation pattern, and metadata (McEachran et al. 2017, Schymanski and Williams 2017). If a substance is missing in available databases - such as transformation products - then it must be identified manually. Depending on how unambiguous the identification is, the identified structure is provided with a certain level of confidence (Schymanski et al. 2014a). Depending on the pattern of the peaks of the non-target screening, it will be decided whether further identification will be performed.

### 1.5.1 Data analysis of non-target screening

With statistical and visualization tools the data can be analyzed, and it can be examined whether there are significant differences in peaks between samples from different treatment steps. These tools can make trend profiles and patterns visible in the data of unknown substances, for example before and after treatment steps. They show whether and, if so, which transformation products are formed, and unknown substances can be characterized and grouped.

### 1.5.2 Identification of unknowns

The bottleneck in non-target screening is the unambiguous identification (structure elucidation) of unknown compounds from high-resolution MS (/ MS) data. Knowing the identity of a compound is not only essential to be able to perform a good (humane) risk assessment, it is also necessary to predict the behavior of a substance in the environment and in water purification. Certainly, in the event of a calamity, rapid identification of an unknown connection is essential. It is often possible to determine the correct gross formula (CiHxNyOz ...) from high-resolution data from the unknown connection. From one gross formula, the number of possible structures can vary from several to thousands of existing connections. The number of possible compounds is even greater in practice because many substances can be converted by processes in the body, the environment, and water purification (transformation products) and these are often not yet known let alone form part of the various chemical databases. Besides, knowledge of chromatography (retention time) and ionization mode (positive/negative), hydrophobicity (log P), and the presence/absence of functional groups (e.g., acidic or basic groups) of a compound play an important role in the identification process.

## 1.6 Suspect-target screening

Target screening methods cover a relatively small proportion of organic contaminants. This may result in bias due to the pre-selection of chemicals in advance (Gago-Ferrero et al. 2015). The presence of an unlimited number of compounds can be investigated at the required sensitivity, without pre-selection of analytes or even without having reference standards available. The ultimate suspect and non-target approaches reported for environmental matrices (water and sediments) are based on semi-quantitative analysis (using peak intensities) if standard substances are not available (Park et al. 2018) or identification as the last step (Schymanski et al. 2014b). However, they are still challenges providing quantitative information about identified compounds is yet not well-resolved. Suspect screening usually implies processing against a “target” library, for example, a compound class such as pesticides. Even though they are technically “unknowns” because you used a generic acquisition strategy (not defined any target analytes for acquisition), you are only concerned with the pesticides, or whatever is in the library that is giving you the hits.

# 2 Materials and methods

The aim of this chapter is to describe the materials, setups, methods of analysis and procedures carried out to enable answering the research questions.

## 2.1 Experimental design Batch tests

An experimental design was proposed to assess the research question: "what is the effect of the Microcat®-BioPoP on the wastewater matrix?" by using beaker experiments as shown in Table 2. Two preliminary experiments were carried out to determine changes in Microcat®-BioPoP weight, COD, and pH, and get familiar with working FOG and Microcat®-BioPoP. The preliminary experiment 2b was carried out with only demineralized water and Microcat®-BioPoP to identify its release of COD over time.
Experiments from 3 and 4 were carried out with artificial (liquid) FOG to explore the effects on Microcat®-BioPoP on the wastewater matrix and investigate potential organic micropollutants coming from the transformation of liquid FOG. Experiments 6 to 10 were performed with FOG from the field, from different sewer systems containing municipal, industrial, and combined wastewaters. The effects of adding Microcat®-BioPoP with these different solid FOGs were assessed by measuring COD, N-$NH_4$, P-$PO_4$, and pH.

*Table 2 Beaker experiments*

| Experiment | Water type | Presence of Microcat®-BioPoP | Presence of FOG |
|---|---|---|---|
| Preliminary 1a | Synthetic | Yes | Artificial |
| Preliminary 1b | Synthetic | No | No |
| Preliminary 2a | Synthetic | Yes | Artificial |
| Preliminary 2b | Demiwater | Yes | No |
| 1 | Synthetic | No | No |
| 2 | Synthetic | Yes | No |
| 3 | Synthetic | Yes | Artificial |
| 4 | Synthetic | No | Artificial |
| 5 | Synthetic | Yes | Municipal |
| 6 | Synthetic | No | Municipal |
| 7 | Synthetic | Yes | Industrial |
| 8 | Synthetic | No | Industrial |
| 9 | Synthetic | Yes | Industrial+Municipal |
| 10 | Synthetic | No | Industrial+Municipal |

### 2.1.1 Experimental setup and operation

Experiments were carried out using a continuos jar-test set-up as shown in Figure 2. Typical features of this set-up are the ability to control each of the impellers rotation speed and to take samples from the bottom and top fraction of each jar.

*Figure 2 Set-up used for the experiments*

During the experiments a gentle mixing of 50 rpm was used, simulating the mixing that takes place in the sewer. All beakers have four baffles installed to improve mixing. The experiments were done in triplicate, having three beakers containing the synthetic wastewater with Microcat®-BioPoP and FOG, and three with FOG and no Microcat®-BioPoP.

### 2.1.2 Measurement schedule

According to information from the supplier, the expected initiation of bacterial activity of Microcat®-BioPoP is about 30 minutes after dosing and the doubling time of the bacteria population is about 6-8 hours (Robert Wagenveld, personal communication). Therefore, it was expected that it was not necessary to take many samples immediately after the start-up. Samples were taken every 24 h, up to a maximum of 264 hours, and analysed as indicated.

### 2.1.3 Microcat®-BioPoP dosage

The distribution of bacteria is not completely homogeneously distributed over the Microcat®-BioPoP . Normally the advice is to place a Microcat®-BioPoP in a system with a flow of 200 - 400 m3 / day. To scale this back to beaker level would mean that very little product should be used and that would not yield a representative sample. A core sample with a diameter of 6 mm x 6 cm through an entire Microcat®-BioPoP was placed in the beakers, with an average weight of about 32 gr Microcat®-BioPoP . This provides enough bacteria to the system.

## 2.2 Experimental setup and operation: Bioreactors

Experiments were carried out using two fully controlled bioreactors (Applikon) as shown in Figure 3.
The experiments were performed using two laboratory-scale completely stirred tank reactors (CSTR) operated as Sequencing Batch Reactors (SBR), both with an effective volume of 2.5 L. A schematic diagram of the configuration of the reactors is depicted in Figure 1. The bioreactors were equipped with pH, temperature, and dissolved oxygen sensors and feed and effluent pumps (Watson-Marlow), acid (HCl 0.1 M), and base (NaOH 0.1 M) pumps, and air supply tube were installed. The setups were controlled by an ez-Control and computer running ezControl software (version 1.40, Applikon) (Figure 4).

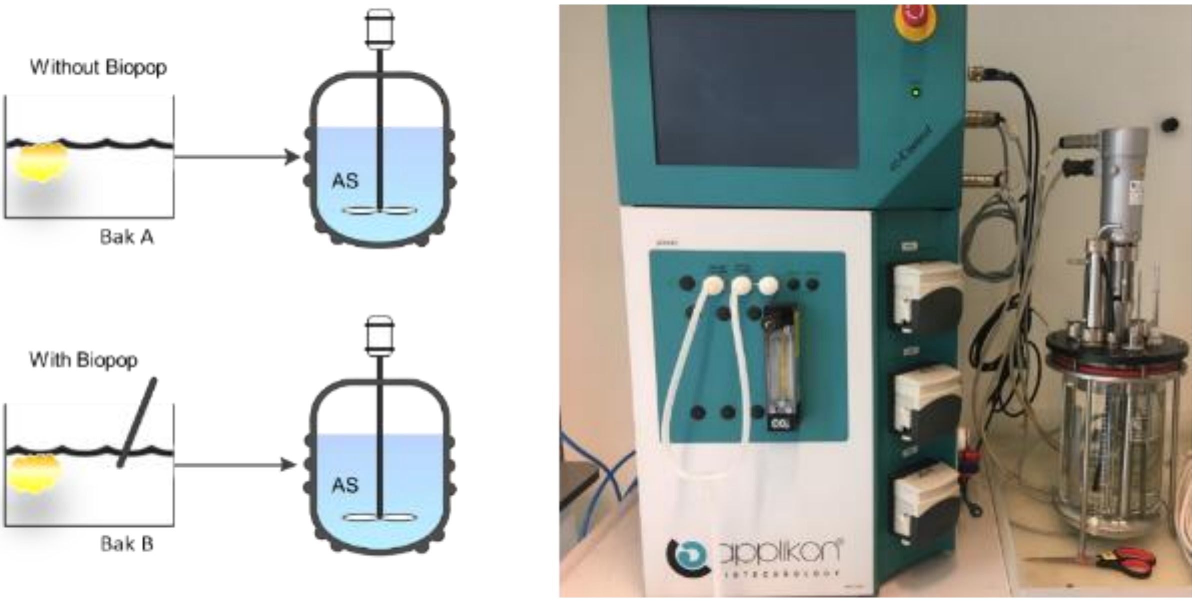


*Figure 3 Scheme of the experimental set-up.*

A feeding tank simulating the pump stations were FOG accumulates in the sewer was installed for each of the bioreactors. Both reactors were fed from these feeding tanks containg solid FOG from a combined municipal and industrial wastewater sewer. In one of the tanks Microcat®-BioPoP was added (Figure 5). The reactors were operated during 100 days (after a 30 days operation time for stabilisation) in a sequencing batch mode. The operation cycled through four phases as indicated in Table 3 (Ferrer-Polonio et al. 2018). Figure 6 shows the reactor at the end of the sedimentation phase. The parameters that were controlled during the operation of the bioreactors were pH (7.5), dissolved oxygen (DO), and hydraulic retention time (HRT) as shown in Table 4. Typical HRTs in WWTPs (16-24 h) (Ferrer-Polonio et al. 2018). HRT was increased towards improving ammonium oxidation.
The solid retention time was kept between 20-30 days. Any operational changes were always applied to both reactors. The reactors were operated at a room temperature of 18°C.

*Figure 4 Experimental set-up of the reactors.*

*Figure 5 FOG in the feeding tank.*

*Figure 6 Settled activated sludge.*

*Table 3 SBR reactors operation mode*

| Phase | Time (min) |
|---|---|
| Filling (anoxic) + aerobic | 360 |
| Sedimentation | 90 |
| Draw + Idle | 30 |
| Cycles/day | 3 |

*Table 4 HRT and DO variations during the experiment.*

| Days | HRT [h] | Dissolved Oxygen [%] |
|---|---|---|
| 0-23 | 16 | 20 |
| 24-61 | 22 | 20 |
| 62-100 | 29 | 30 |

### 2.2.1 Anaerobic digestion test with activated sludge from Bioreactors

To better understand any possible effect of the Microcat®-BioPoP on the anaerobic digestion process, the activated sludge from the bioreactors was digested under anaerobic conditions in a batch test.
Experiments were carried out with activated sludge from the bioreactors with and without Microcat®-BioPoP . The activated sludge was taken after the activated sludge experiments were finished. COD of the sludges were determined in triplicate before the start of the experiment. Reactors (0.6 L glass bottles) were seeded with anaerobic sludge obtained from a full-scale reactor treating municipal wastewater/sludge (Nieuwegein, The Netherlands).
An inoculum to substrate ratio of 1:2 was used in the anaerobic reactors. Macronutrients (6 mL.L$^{-1}$), trace elements (0.6 mL.L$^{-1}$) and phosphate buffer solutions (50 mL.L$^{-1}$) as described by table 5 were added to the reactors. A liquid working volume of 0.4 L was used in all reactors.

*Table 5 Anaerobic tests medium solutions*

| Stock Solutions | Content | Concentration |
|---|---|---|
| Phosphate | $K_2HPO_4.3H_2O$<br>$NaH_2P0_4.2H_20$ | 45.65 $gL^{-1}$<br>31.20 $gL^{-1}$ |
| Macronutrients | $NH_4Cl$<br>$CaCl_2.2H_2O$<br>$MgSO_4.7H_2O$ | 170 $gL^{-1}$<br>8 $gL^{-1}$<br>9 $gL^{-1}$ |
| Micronutrients | $FeCl_3.4H_2O$<br>$Na_2SeO_3.5H_2O$<br>$CoCl_2.6H_2O$<br>$NiCl_2.6H_2O$<br>$MnCl_2.4H_2O$<br>EDTA<br>$CuCl_2.2H_2O$<br>HCl 36%<br>$ZnCl_2$<br>$HBO_3$<br>Yeast extract<br>$(NH_4)6Mo7O_2.4H_2O$ | 2 $gL^{-1}$<br>100 $mgL^{-1}$<br>2 $gL^{-1}$<br>50 $mgL^{-1}$<br>0.5 $gL^{-1}$<br>1 $gL^{-1}$<br>30 $mgL^{-1}$<br>1 $mLL^{-1}$<br>50 $mgL^{-1}$<br>50 $mgL^{-1}$<br>2 $gL^{-1}$<br>90 $mgL^{-1}$ |

The anaerobic digestion tests were performed using an Automated Methane Potential Test System AMPTS II (Bioprocess Control, Sweden) (Figure 7) following the protocols suggested by Holliger et al. (2016), Loosdrecht et al. (2016). The AMPTS calculates and records the volume of gas under normal conditions (NmL, 0°C, 100 kPa). The experiments were carried out at 35 °C. $CO_2$ and $H_2S$ gas were stripped from the biogas by a 3 M NaOH solution before entering the methane- flow cell meter. The tests were conducted in triplicate.
The cumulative methane production, or methane yield, is the net methane production per gram substrate COD or VS added during the entire incubation period of 30 days at standard temperature and pressure (T = 0 °C and P = 1 atm) which is expressed as NmL $CH_4.gVS^{-1}$.

*Figure 7. Automated Methane Potential Test System AMPTS II used for anaerobic tests.*

## 2.3 Synthetic wastewater, and Fat, Oil and Grease (FOG) dosage

The artificial wastewater was based on the medium described in Van den Brand et al. (2014) except that in this case, no aquarium salt was used (Table 6). The micronutrient solution was prepared based on Lau et al. (2006)(Table 7). All components were dissolved in demineralized water.

In the case of bioreactor experiments, the artificial wastewater was kept inside a refrigerator (MMM Medcenter$^{TM}$ Friocell$^{TM}$ , 55 L) at 10°C to avoid degradation. In each cycle of the sequence batch mode, the artificial wastewater was pumped automatically to the feeding tank to maintain always the same level and amount of wastewater in contact with the FOG taken from the field and/or Microcat®-BioPoP .

*Table 6 Media for synthetic wastewater (Van den Brand et al. (2014))*

| Stock solution | Compounds and concentration (mg.L$^{-1}$) | |
|---|---|---|
| C | 364. 63 | $NaAc\cdot 3H_2O$ |
| | 110.31 | NaPr |
| | 37.97 | $K_2HPO_4$ |
| | 14.16 | $KH_2PO_4$ |
| N | 382 | $NH_4Cl$ |
| | 70.06 | $MgCl_2\cdot 6H_2O$ |
| | 43.79 | $CaCl_2$ |
| Trace elements | As described in Lau et al. (2006). See Table 6. | |

*Table 7 Trace metals solution (from Lau et al., 2006)*

| Compound | Concentration synthetic wastewater (mg.L$^{-1}$) |
|---|---|
| $FeCl_3.6H_2O$ | 0,316 |
| $H_3BO_3$ | 0,032 |
| $CuSO_4$ | 0,004 |
| KI | 0,006 |
| $MnSO_4.H_2O$ | 0,021 |
| $(NH_4)6Mo_7O_{24}.4H_2O$ | 0,008 |
| $ZnSO_4.7H_2O$ | 0,025 |
| $CoCl_2.6H_2O$ | 0,032 |
| $C_6H_5Na_3O_7.2H_2O$ | 0,211 |

### 2.3.1 Synthethic FOG in batch experiments

In the batch tests, soy oil, and milk powder were used to mimic FOG in the synthetic wastewater following the approach of Aiyuk and Verstraete (2004). The ratio of soy oil and milk powder (Milk Powder, 35 g Fat / 100 g) was 1: 4 (based on weight). The soy oil was cooked (about 15 minutes) before it was used in the experiments.

### 2.3.2 FOG dosage to bioreactors

The fat, oil, and grease added to the bioreactors was taken from a combined municipal/industrial sewer system (Wargaren, Municipality Oss, Netherlands) (Figure 8). FOG was in the solid form, with visible food remnants. In each of the feeding tanks 65 gr of the FOG sample was added in a mesh. During operation a futher 5.0±0.8 g were added on days 22, 41, 62 and 83.

*Figure 8 Combined municipal/industrial sewer from where the FOG was sampled.*

### 2.3.3 Microcat®-BioPoP dosage to bioreactors

The bacteria are not completely homogeneously distributed over the Microcat®-BioPoP. A core sample with a diameter of 6 mm x 6 cm through an entire Microcat®-BioPoP was placed in one of the feeding tanks, with an average weight of about 56.6±2.2 gr Microcat®-BioPoP . The addition of new Microcat®-BioPoP was repeated during the operation of the reactors on days 0, 22, 41, 62, and 83.

## 2.4 Analytical methods

### 2.4.1 Chemical oxygen consumption and nutrient determinations

All measurements are made with the corresponding Hach-Lange test method, as shown in Table 8. The chemical oxygen demand (COD), subdivided in total COD (CODt) and the dissolved fraction COD (CODs), were determined using the same type of Hach-Lange measurement. For soluble COD, the sample was filtered over a 0.45 µm filter. For ammonium, nitrate, nitrite, and soluble phosphate, the samples were also filtered.

*Table 8 Influent and effluent characterization analysis.*

| Analysis | Hach Lange methods |
|---|---|
| COD | LCK 514 |
| Ammonium | LCK 302, 303, 304 |
| Nitrate | LCK 348 |
| Nitrite | LCK 341 |
| Soluble Phosphate | LCK 350 |
| Total Phosphate | LCK 350 |

### 2.4.2 FOG content

The FOG content was measured using the Soxhlet extraction method described in the NEN 6672:2013 by AL-West B.V. (Agrolab Group, Deventer). In the method, FOG soluble in petroleum ether is extracted from the sample and determined gravimetrically. The minimum level of detection was 20 mg.L$^{-1}$.

### 2.4.3 Solids determination (sludges)

As support measurements for the operation of the reactors (activated sludge and anaerobic), the total solids (TS), volatile solids (VS), total suspended solids (TSS), and volatile suspended solids (VS) were measured on weight base (gL$^{-1}$) according to the standard methods for the examination of water and wastewater (APHA 1998).

### 2.4.4 Sulfide measurement

The sulfide measurement is based on an adapted methylene blue method (APHA 1998). For this adapted method, it has been shown that fluctuations in salt, sulfate, COD, N, P, and the like have no effect on the measurement.
Required solutions:

- 10% Zinc Acetate (500 mL): 50 gr ZnAcetate. Make up to 500 ml with demineralised water.
- Reagent A (1L): 2 g dimethyl paraphenyldiamine, 200 ml demineralized water, 200 ml $H_2SO_4$ (concentrated). Make up to 1 liter with demi water and wrap in aluminum foil.
- Reagent B (100mL): 10 gr Fe (III) (NH4) (SO4) ¬2.12H2O, 50 ml demineralised water, 2 mL H2SO4 (concentrated). Make up to 1 liter with demi water and wrap in aluminum foil.

The sulfide concentration was determined by adding the following into a cuvette: 10 µl sample, 490 µl zinc acetate solution, 1280 µl demi water, 200 µl Reagent A, 20 µl Reagent B. This cuvette was mixed well. After 30 minutes the absorbance was measured at 675 nm. The sulfide concentration was determined based on a previously made calibration curve. The calibration curve for the sulfide test was made with a standard solution of 0.2 gram $Na_2S$ in 200 ml demineralized water. Then the following dilution series were tested: diluted 1, 2, 4, 5, 10, and 20 times.

### 2.4.5 Foaming

When foaming occurs, antifoam will be added until the foaming has disappeared. The antifoam dosage (time and amount) can then serve as a measure for the foam formation.

### 2.4.6 Organic micropollutants (suspect and non-target screening)

#### Samples and sample preparation

Samples from experiments 1, 2, 3, and 4 of the batch tests with the presence or not of artificial FOG and Microcat®-BioPoP were used for the initial non-target screening (see Table 2).
Artificial wastewater, inlet wastewater (from feeding tank), and effluent samples from the bioreactors were used for the suspect and non-target screening. Also, samples from the batch tests with FOG from the field and their corresponding field wastewaters were included. All samples collected were stored in HDPE containers in the freezer (-25 $^{o}$C) until sample pre-treatment.
Fifty mL of thawed sample was transferred to a 50 mL flask to which the internal standards atrazin-d5 (CDN isotopes, Pointe-Claire, Canada) and bentazone-d6 (LGC, Almere, the Netherlands) were added (1 µg.L$^{-1}$) for quality control purposes. The samples were filtered using a Phenex™-RC 15 mm Syringe Filters 0.20 µm (Phenomenex, Torrance, USA) and transferred to an autosampler vial for LC-HRMS analysis.

#### LC-HRMS analysis

Liquid chromatography – high-resolution mass spectrometry (LC-HRMS) analysis was performed using a Vanquish UHPLC system coupled to an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The system was controlled by Xcalibur 4.2 and Chromeleon 7.2 software (Thermo Fisher Scientific). The chromatographic analysis was performed on an Xbridge C18 XP column (2.1 × 150 mm, 2.5 µm, Waters, Etten-Leur, the Netherlands) preceded by a SecurityGuard Ultra C18 column (2.0 mm × 2.1 mm, Phenomenex, Torrance, USA) at a temperature of 25 °C. The mobile phase solvents were ultrapure water produced through purification of demineralized water in an Elga Purelab Chorus ultrapure water system (High Wycombe, UK), and acetonitrile (HPLC grade, Avantor Performance Materials B.V., Deventer, the Netherlands) both with 0.05% formic acid (v/v) (Fluka Analytical, Sigma-Aldrich). The mobile phase consisted of solutions A and B. A: Ultrapure water + A: Ultrapure water + 0.05% formic acid, B: Acetonitrile + 0.05% formic acid. A gradient mobile phase was carried out as described in Table 9.

*Table 9 Applied mobile phase gradient in Non-target screening Liquid chromatography.*

| Time (min) | B (%) |
|---|---|
| 0 | 5 |
| 1 | 5 |
| 25 | 100 |
| 29 | 100 |
| 29.5 | 5 |
| 34 | 5 |

The mass spectrometer was operated in the positive and negative electrospray ionization mode. The source voltage was set to 3.0 an -2.5 kV for positive and negative modes, respectively. The RF lens was set to 50 %. The vaporizer and capillary temperature were both maintained at 300 °C. Sheath, auxiliary, and sweep gas were set to arbitrary units of 40, 10, and 5. Full scan accurate MS and MS/MS mass spectra were recorded from 80 to 1300 m/z with a resolving power of 120,000 and 15,000 FWHM (at m/z 200), respectively. The data-dependent acquisition was performed using stepped High Collision Dissociation (HCD) energy at 20, 35, and 50%. The Orbitrap Fusion mass spectrometer was externally calibrated using the Pierce FlexMix calibration solution to obtain a mass accuracy < 2 ppm. All samples including the blank were analyzed in triplicate (If possible).

For the batch tests, a data analysis filtering (based on the chromatograms) for the transformation products of the synthetic wastewater (SWW) samples with FOG, Microcat®-BioPoP , and both were applied as follows:
Background

- Ratio sample / SWW degraded (blank) > 5
- Ratio (area)
- Ratio SWW + FOG + Microcat®-BioPoP / SWW + Microcat®-BioPoP > 25
- Ratio SWW + FOG + Microcat®-BioPoP / SWW + FOG > 25

P-value

- P-value SWW + FOG + Microcat®-BioPoP / SWW + Microcat®-BioPoP < 0.05
- P-value SWW + FOG + Microcat®-BioPoP / SWW + FOG < 0.05

**Data analysis - suspect screening**
The non-target data were processed with Compound Discoverer 3.1 (Thermo Fisher Scientific) for peak picking, componentization, and suspect screening. A threshold of 100.000 counts was used for peak picking in the positive and negative mode. Features detected in the samples with a ratio < 10 compared to blank samples (0.20 µm filtered ultrapure water), were marked as background. The output of Compound Discoverer is a feature list, i.e. a table with accurate mass/retention time pairs (features) and their intensity. The feature intensity is reported as the peak area. The response of each technical triplicate is reported individually.
The suspect screening was performed using two spectral libraries, the online mzCloud library and the offline MassBank of North America library. Compounds with an MS/MS database score of 90 or higher received a level 2 identification (Schymanski et al. 2014a). Compounds which obtained a level 1 identification were unambiguously identified using a

reference standard. Only compounds that have an area greater than 2e5 in the positive mode and 1e5 in the negative mode were reported in the suspect screening results.

**Processing, analysis, and interpretation of NTS data**
The Compound Discoverer output was imported into R Studio for further data analysis and visualization (R_CoreTeam 2017). Data preprocessing in R included the application of a retention time cut-off of 2.4 min, a coefficient of variation (CV) cut-off between triplicate responses of 15% (i.e. in at least one sample group the CV had to be smaller than 15%), and both a blank and SWW control cut-off of 10 (i.e. in at least one sample group the response had to exceed 10 times the blank and/or SWW control response).

Principal Component Analysis (PCA) provided an overview of the differences between the samples and treatment groups (Masiá et al. 2014). The R package *factoextra* was used for PCA visualization. After normalization of the data through the division of feature areas across samples by the maximum area of the respective feature, both samples and features were clustered together based on Euclidean distances using the complete method (Everitt 1974., Schollee et al. 2016). Hierarchical clustering results were visualized in a heat map using the R package *pheatmap*. The data-based.Lists used for finding matches were:

- TKI_vetten_TP_match: features that were detected in the batch experiments with artificial FOG as potential transformation products.
- KWR_suspect_match is a database of environmentally relevant compounds that can be expected to reach the water cycle in the Netherlands (Sjerps et al. 2016).
- LOA600_suspect are the compounds that were also used in the suspect target screening

Significance testing and fold change filtering was applied to identify differences between the batch samples with and without the addition of Microcat®-BioPoP . Results were illustrated using Volcano plots displaying log2FC and the negative log10-transformed p-values of features (Cui and Churchill 2003). Differences in retention time (RT and molecular weight (MW) density distribution of features were visualized in violin plots.

# 3 Preliminary batch tests results

The goal of the preliminary tests was two fold. Firstly, to determine changes in Microcat®-BioPoP weight, COD, and pH, and get familiar with working with FOG and Microcat®-BioPoP. Secondly, to identify how the Microcat®-BioPoP product release its organics compounds (COD) over time. This chapter describes the findings and observations of the experiments carried out.

The synthetic wastewater on preliminary experiment 1a (see Table 2) contained 31.25 mg.L$^{-1}$ (Soy Oil) and 125 mg.L$^{-1}$ (Milk Powder, 35 g Fat / 100 g) in addition to the wastewater media described in Table 6.

Results showed a significant increase in total and soluble COD in time from an initial value of 612 mg.L$^{-1}$ before adding the Microcat®-BioPoP (Figure 9) to a maximum total COD of about 2351 mg.L$^{-1}$ at 248 hours. Soluble COD was about 58% of the total COD after the initial 136 hours.
Microcat®-BioPoP weight decrease trend inferred that most of the COD in the first 48 hours is released from the Microcat®-BioPoP. About 13% of weight loss was observed after 48 hours, with a weight of about 68% from the initial at the end of the experiment. Overall pH changes were minimum, with a slightly decrease at the end of the experiment.

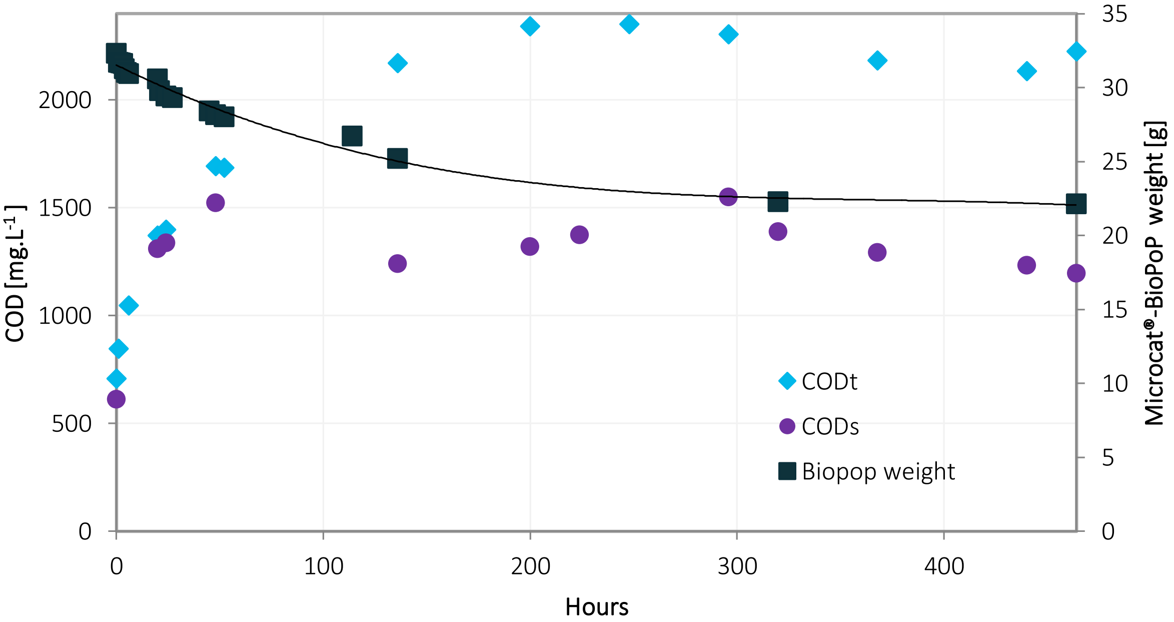


*Figure 9 Preliminary experiment 1. FOG: 31.25 mg.L$^{-1}$ soy oil, 125 mg.L$^{-1}$ milk powder. pH was stable during the experiment around 7.12±0.06.*

Overall, COD measurements did not provide insight into the degradation of fat, oil, and grease (FOG). Sampling was not easy since the FOG attached easily to any surface, for example, the pipet, impeller, and wall of the beaker (Figure 10).

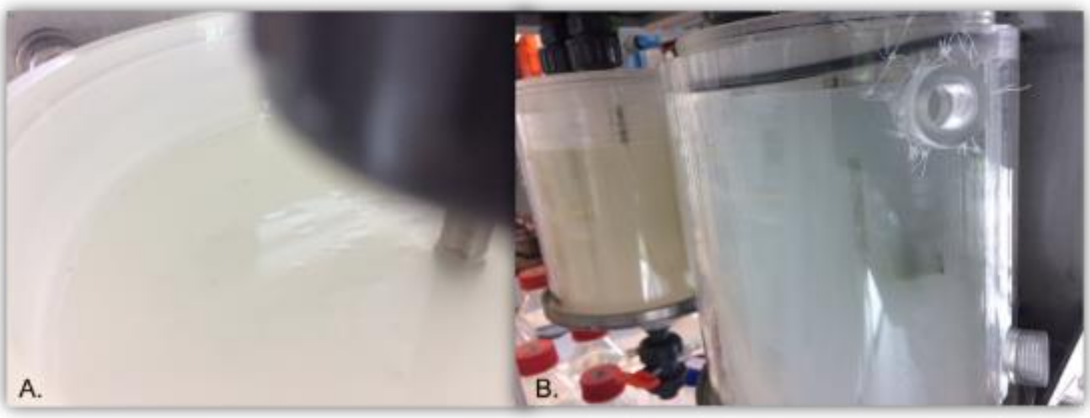


*Figure 10 A. top view of the beaker. B. Beaker with and without FOG.*

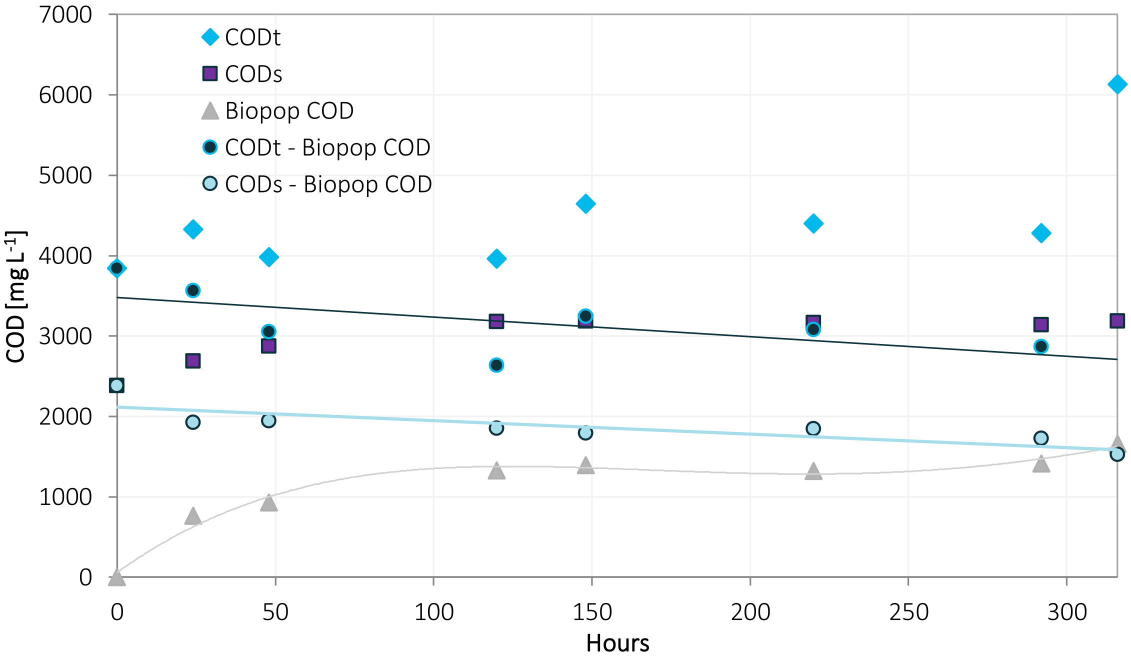


*Figure 11 Preliminary experiment 2. FOG: 460 mg.L$^{-1}$ Soy Oil, 1840 mg.L$^{-1}$ Milk Powder. Lines represent the trend from the subtraction between COD total and soluble and the COD released by the Microcat®-BioPoP .*

To elucidate better how much COD was released by the Microcat®-BioPoP , a second preliminary experiment was carried out. For this purpose, a higher FOG (14 times higher) concentration of 460 mg.L$^{-1}$ Soy Oil and 1840 mg.L$^{-1}$ Milk Powder (35 g Fat / 100 g) was added to the synthetic wastewater medium, and Microcat®-BioPoP pieces with approximately the same weight (32 g) were used.

The total and soluble COD from the FOG added were 3208 mg.L$^{-1}$ and 1748 mg.L$^{-1}$ (Figure 11), respectively, while the synthetic medium without FOG was about 635 mg.L$^{-1}$. Total COD increased by about 10% in the first 24 hours. Soluble COD increased by 13% and 20% at 24 h and 48 h, respectively. An average ratio CODs: CODt of 0.7 was found along with the experiment. An increasing trend of Microcat®-BioPoP total COD in water was observed. A COD of 931 mg.L$^{-1}$ was observed at 48 h, representing 70% of the steady COD concentration found after 220 hours at the end of the experiment. By subtracting the Microcat®-BioPoP COD from both total and soluble COD in the synthetic wastewater, an indication of COD degradation in the beaker was determined by a 36% removal of the soluble COD.

Analysis of the supernatant using colorimetric MQuant® Free fatty acids (Merck) test at the end of the experiment did not show free fatty acids (<0.2 mg KOH.g$^{-1}$ ) as initially expected, but a visual difference on the top layer of the synthetic wastewater was significant in time (Figure 12).
The Microcat®-BioPoP COD test (Preliminary test 2b, Table 2) showed that 32 mg.L$^{-1}$ h of COD is released within the first 24 h (Figure 13), and indicated that most of the COD content from the Microcat®-BioPoP matrix is released after 72 h reaching at steady-state around 248 h, in which a rate of about 5 mg.L$^{-1}$ h$^{-1}$ was observed (Figure 14). The latter was set as the evaluation time for the rest of the batch experiments.

*Figure 12 Preliminary experiment 2 at A. 48h B. 148h. C. 292h*

*Figure 13 Preliminary experiment 2. Microcat®-BioPoP COD release test.*

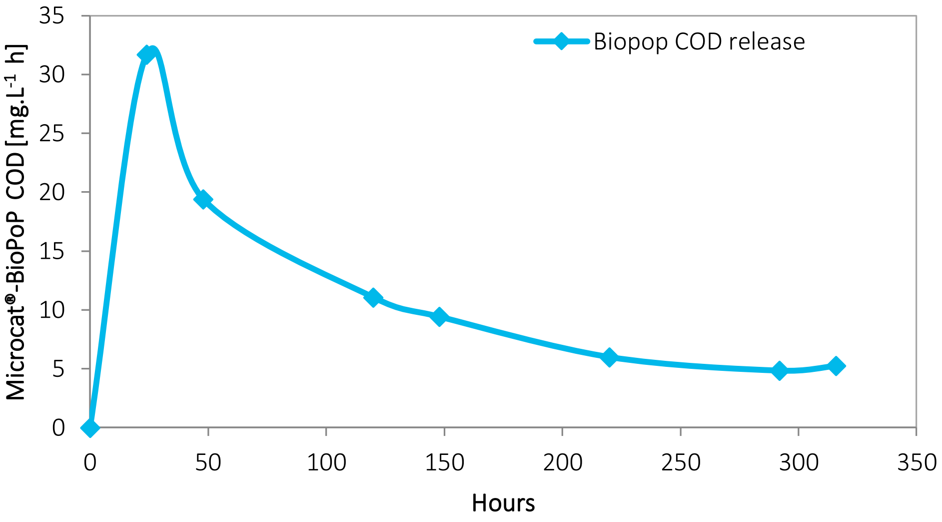


*Figure 14 Preliminary experiment 2b. Microcat®-BioPoP  COD release test in beaker with only demiwater and Microcat®-BioPoP .*

Based on the observations, we concluded that Microcat®-BioPoP weight loss of 13% was observed after 48 hours, and 22%  after 248 h. Total COD increase as the weight of Microcat®-BioPoP decreased, without any significant pH changes. Moreover, the main hydrophilic/soluble part of the Microcat®-BioPoP matrix which was measured as COD is released within the first 72 h.

# 4 Beaker (Jar) tests results with artificial FOG

After the preliminary tests for exploration presented in Chapter 3, batch tests with artificial fat, oil, and grease (FOG) were carried out to assess the effect of adding Microcat®-BioPoP in the synthetic wastewater matrix under control conditions. Focus will be on COD, change in nitrogen compounds ($NH_4$, $NO_2$, $NO_3$) and phosphate. Control experiments with synthetic wastewater without FOG and with FOG were performed to determine the impact of applying Microcat®-BioPoP to the medium.
In addition the formation of byproducts has been investigated by application of a non-targed screening analysis

## 4.1 Synthetic wastewater without Microcat®-BioPoP and without FOG (Experiment 1)

In the experiments without FOG, soluble and total COD were consumed asymptotically by 85% and 60% respectively (Figure 15.A), confirming the nature of easily degrading COD from the synthetic wastewater along with the experiment. Interestingly, at 144 h, about 84% and 53% of the soluble and total COD were already consumed. A final total concentration of about 233±95 mg.L$^{-1}$ of total COD and 90±73 mg.L$^{-1}$ of soluble was observed.
Ammonium concentration decreased from an initial concentration of about 19 mgL$^{-1}$ by 37% at 240 h (Figure 15. B). The latter indicates that less biomass uptake occurred compared to when Microcat®-BioPoP is applied. Therefore, less ammonium oxidation took place with 1% of the N-$NH_4$ ending up as N-$NO_2$ and 0.4% as N-$NO_3$ (Figure 15. C). interestingly, nitrite concentration was higher than nitrate along with the experiment.
Orthophosphate decreased by 14% within 72h whereas the total phosphate increased by 22%. However, at the end of the experiment, total phosphate concentration was found to be about the same as the initial concentration (19 mg.L$^{-1}$) (Figure 15. D).

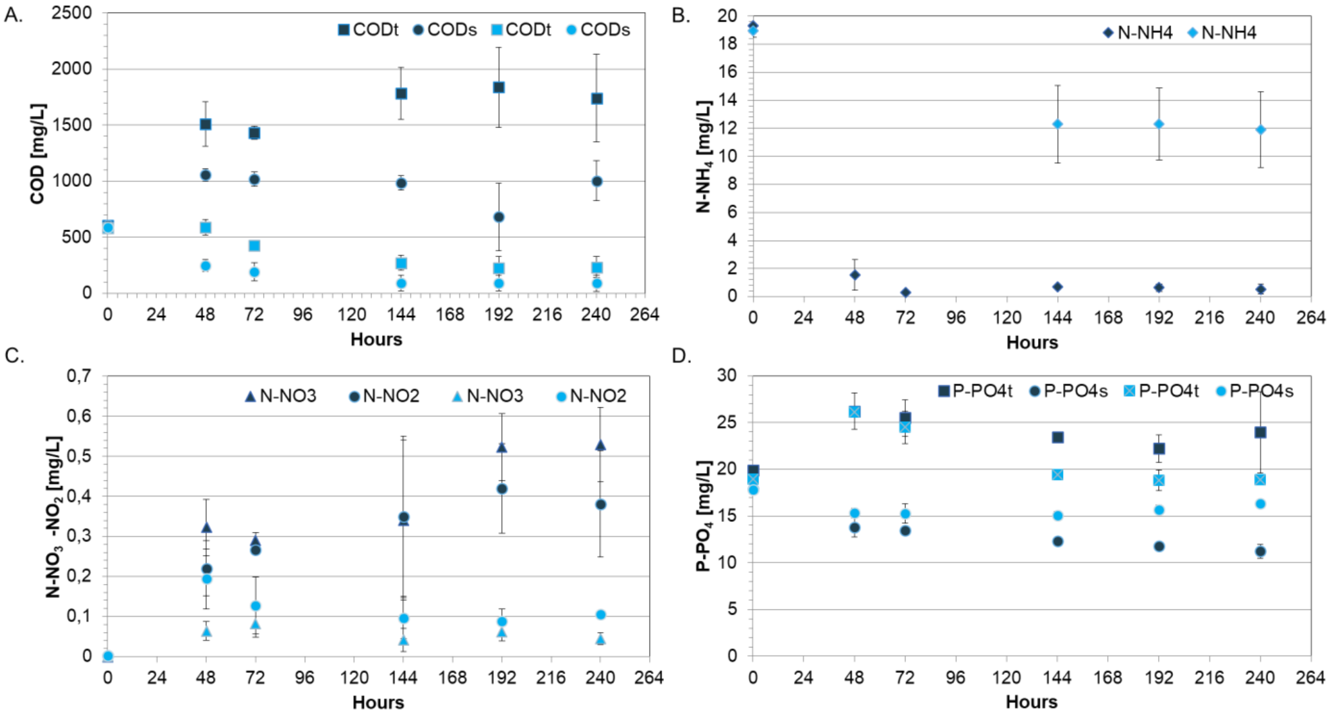


*Figure 15 Synthetic wastewater without Microcat®-BioPoP and without FOG (**Experiment 1:** light blue) , Effect of Microcat®-BioPoP in synthetic wastewater without FOG (**Experiment 2:** dark blue).*

## 4.2 Synthetic wastewater with Microcat®-BioPoP and without FOG (Experiment 2)

The effects on COD and nutrients concentration due to the presence of the Microcat®-BioPoP on the synthetic wastewater without FOG (See Table 2) on time are summarized in Figure 15.
As expected from preliminary experiments, the total and soluble COD increased particularly during the first 48 h to about 1500 mg.L$^{-1}$ (Figure 15. A) due to the release of COD by Microcat®-BioPoP itself. It is important to point out that this Microcat®-BioPoP COD released was not substracted from the COD measurements due to the difficulty to predict the COD release on time for each piece of Microcat®-BioPoP.
In the case of nutrients, remarkably, 98% of the N-$NH_4$ concentration was depleted in 72 hours inferring a high microbial activity/uptake. However, no completed depletion was observed at 240 h with a concentration of 0.5 mg.L$^{-1}$ N-$NH_4$ (Figure 15. B). N-compounds from the oxidation of N-$NH_4$ such as N-$NO_3$ and N-$NO_2$ were determined. A slightly increasing trend was observed for both nitrate and nitrite along with the experiments (Figure 15. C). No aeration was supplied to any of the reaction vessels. This would indicate that the oxygen would be depleted as well and no nitrification would take place as oxygen is essential for nitrification.
P-$PO_4$ in its soluble (orthophosphate) referred to as P-$PO_4$s and total (P-$PO_4$t) form was also determined. Soluble P-$PO_4$ decreased about 37% at the end of the experiment, which might be due to biomass uptake (Figure 15. D).
High variations in concentration were observed in COD attributed to the differences in the composition of the Microcat®-BioPoP pieces used in each of the triplicates. N-$NO_3$ and N-$NO_2$ analysis showed significant high variations in nitrification considered that N-$NH_4$ was stable in all reaction vessels.

## 4.3 Synthetic wastewater with Microcat®-BioPoP and with FOG (Experiment 3)

The observed effects of the Microcat®-BioPoP on synthetic wastewater with FOG (see Table 2), are summarized in Figure 16. Contrary to what was observed without FOG, the total COD increased by about 67% to a maximum of 4512 mg.L$^{-1}$, mainly as a result of Microcat®-BioPoP COD release and corresponding biomass growth. Interestingly, soluble COD was maintained relatively constant during the whole experiment (Figure 16. A) which confirms that the insoluble or colloidal fraction is the one that increased. It may be also the possibility that the rate of solubilization of COD from FOG and CODs conversion (as observed in experiment 1) was similar, and therefore the CODs concentration did not change substantially.
The highest ammonium uptake of about 83% occurred within 72 hours most likely due to biomass growth. About 93% of ammonium was depleted in 240 hours (Figure 16. B). Higher nitrification was observed, compared to the experiments without FOG. About 17 times higher N-$NO_3$ and double N-$NO_2$ maximum concentrations were found respectively at 144 h (Figure 16. C). Furthermore, P-$PO_4$ total varied with an increasing and decreasing trend within the experiment (Figure 16.D). Apparently, about 20% of the P-$PO_4$ total was released from Microcat®-BioPoP . In contrast, observed soluble P-$PO_4$ at 48h and 240 h were about 17±0.3 mg.L$^{-1}$, indicating that about 16% of the orthophosphate was taken up.
When comparing with experiment 2, it can be seen that the presence of FOG reduces the N-$NH_4$ conversion rate, and that P-$PO_4$ is not substantially affected. It should be pointed out that compared to experiment 1 without Microcat®-BioPoP and without FOG, the soluble COD at the end of the experiment was about 10 times higher when having Microcat®-BioPoP and FOG.

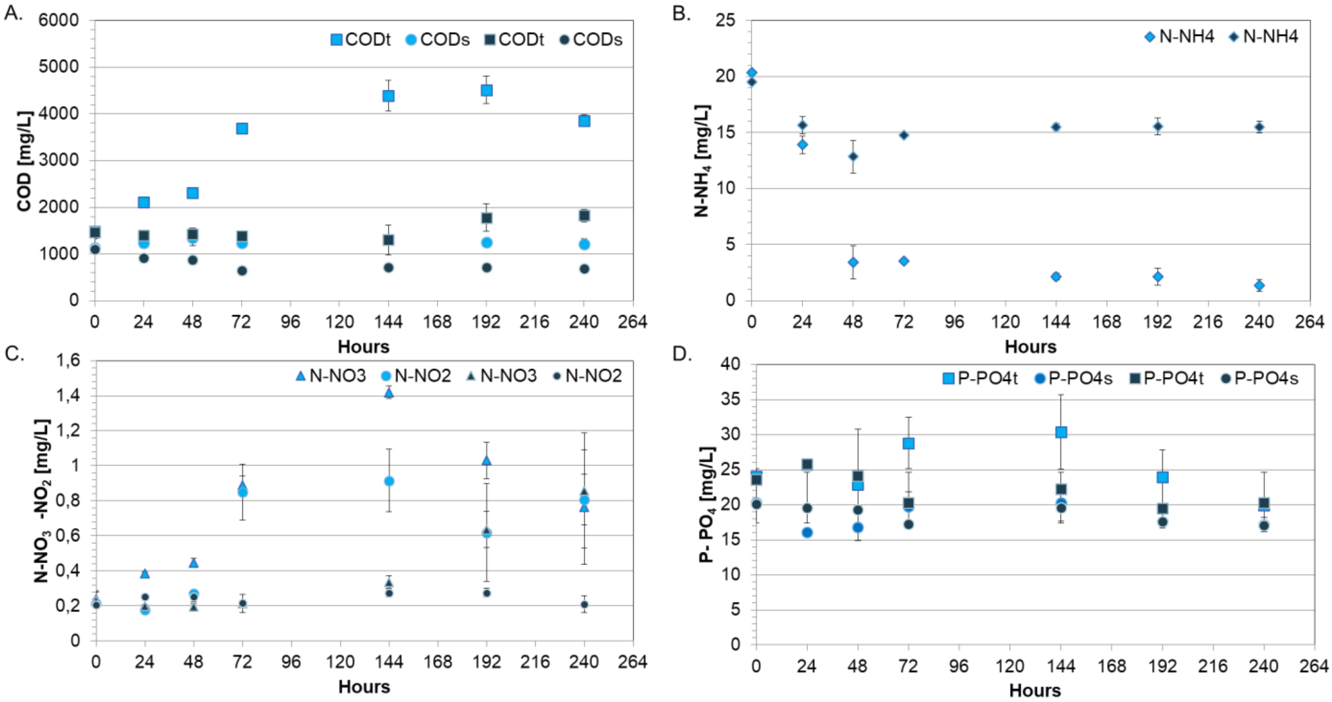


*Figure 16 Effect of Microcat®-BioPoP in synthetic wastewater with FOG (125 mg.L$^{-1}$ soy oil, 500 mg.L$^{-1}$ milk powder) (**Experiment 3:** light blue); Synthetic wastewater without Microcat®-BioPoP and with FOG (125 mg.L$^{-1}$ boiled soy oil, 500 mg.L$^{-1}$ milk powder) (**Experiment 4:** dark blue).*

FOG analysis revealed an average removal of about 94%. The average final FOG concentration was about 12 ±13.8 mg.L$^{-1}$. Two of the triplicates were found to achieve 98% removal while the other one was found to be about 88% (Figure 17). The initial FOG concentration was determined as 195 mg.L$^{-1}$. Continuous reactor experiments are expected to avoid any nutrient limitation due to fast N-NH$_4$ uptake/removal by feeding influent containing N-NH$_4$ continuously.

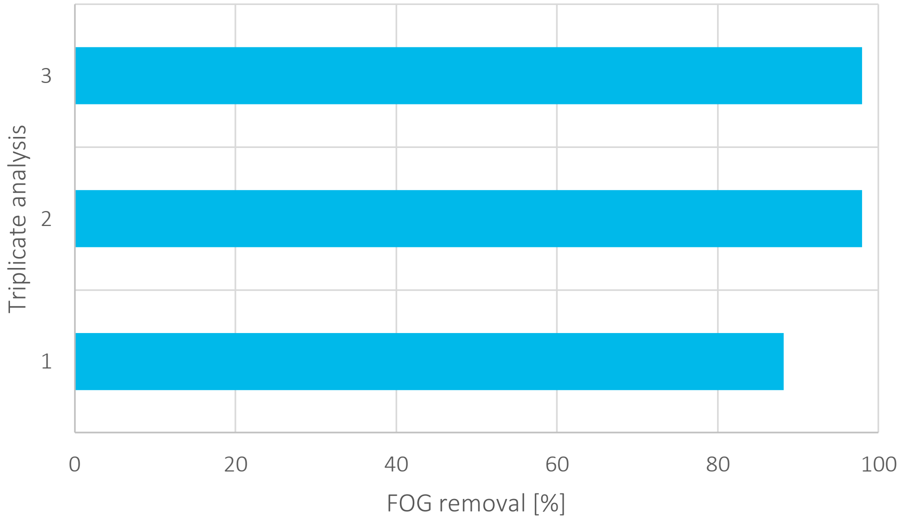


*Figure 17 FOG removal % based on fog balance from experiment 3 (triplicate).*

## 4.4 Synthetic wastewater without Microcat®-BioPoP and with FOG (Experiment 4)

In experiment 4 synthethic wastewater was tested with only FOG added. The soluble COD conversion was about 41% within 72 h, but further conversion was not found (Figure 16. A). The final concentration was about 693 mg.L$^{-1}$. On the other hand, total COD increased by 24% at the end of the experiment, with a notable trend after 144 h. The latter indicated that easily degraded COD was rapidly consumed and the other 59%, most likely coming from FOG or degradation compounds was not converted.
The ammonium concentration decreased by 24% within 72 h (Figure 16. B). N-$NO_3$ and N-$NO_2$ concentrations remained constant at about 0.2 mg.L$^{-1}$ during 72 h. However, a significant increase to about 0.8 mg.L$^{-1}$ of nitrate concentration was observed at the end of the experiment (Figure 16. C).
Total phosphate decreased by 11.7% while orthophosphate uptake of about 15.4% was found at the end of the experiment (Figure 16. D).
By comparing with experiment 1 with Microcat®-BioPoP and FOG, it can be seen that the soluble COD decreased to about 693 mg.L$^{-1}$ , but still, it does not decrease as observed in experiment 1, when no FOG. The later may also indicate that the soluble COD from the FOG is not easily biodegradable. Overall, the concentrations from N-$NH_4$ and P-$PO_4$ did not change substantially with the presence of FOG in the wastewater matrix, and final concentrations were similar to experiment 1, without Microcat®-BioPoP and FOG.

Overall, we could conclude that the addition of Microcat®-BioPoP led ro an increase in total and soluble COD, crearly observed during the initial 48 h. It can be inferred that the presence of FOG in synthetic wastewater reduces the COD conversion rates but did not have a significant effect on the nutrients uptake. On the contrary, when compared to the experiments in which Microcat®-BioPoP was applied, soluble COD conversion was minimum and total COD increased mainly due to biomass growth, whereas a rapid ammonium uptake of average 90% (99% without FOG and 82% with FOG) occurred within the first 72 h. In the case of orthophosphate, the concentration was comparable in all experiments but a higher decrease was observed when Microcat®-BioPoP was applied. Both nitrite and nitrate concentrations varied significantly along the experiments having a high standard deviation. Values higher than 0.4 mg.L$^{-1}$ were mainly observed with Microcat®-BioPoP experiments.

## 4.5 Non-target screening (NTS) analysis results in batch tests

The objective of the non-target screening (high-resolution mass spectrometry) was the detection of the transformation products in the synthetic wastewater samples of experiment 3, which could be identified as organic micropollutants, with both artificial FOG and Microcat®-BioPoP after a degradation time of 240 hours. The detected number of features in the positive and negative mode of the analysis for the synthetic wastewater with only Microcat®-BioPoP or only FOG and with both Microcat®-BioPoP and FOG are presented in Figure 18. The peak extraction and alignment of all peaks found in the samples resulted in a maximum value of about 4071 in the positive mode and a minimum of 852 in the negative mode for the samples with Microcat®-BioPoP and with FOG respectively. Chromatograms of the triplicates from the samples of the experiments 1 to 4 are shown in Appendix I.

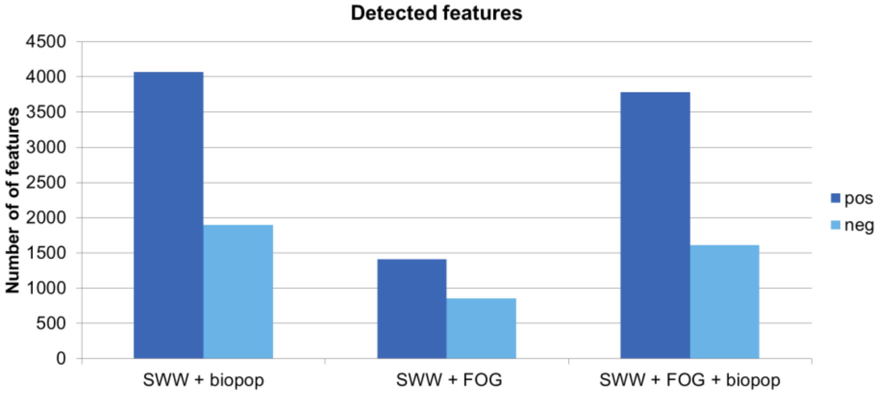


*Figure 18 Detected features by non-target screening in the positive and negative modes of ionization.*

Features that were present in the samples from synthetic wastewater (SWW) and Microcat®-BioPoP , or SSW and FOG, and in the samples from SWW with both Microcat®-BioPoP and FOG were not included in the analysis to only focus on features resulting from the application of Microcat®-BioPoP for FOG removal. After detection frequency and blank filtration, manual inspection to exclude features with a poor spectral library match, and exclusion of compounds because of the poor statistical reproducibility among the triplicates, a total of 12 (Table 10) and 17 (Table 11) transformation features were identified from the synthetic wastewater sample with Microcat®-BioPoP and FOG in the positive and negative modes of ionization, respectively. An internal standard concentration equivalent is proposed, to have a relative comparison of the concentrations of the compounds. However, this concentration will not necessarily relate to the actual concentration of the compounds in the samples.

*Table 10 Detected transformation products in positive mode (p-value < 0.05)*

| Feature nr | Molecular weight | Formula | Retention time (min) | Area | Concentration ISTD equiv. ($\mu g.L^{-1}$) |
|---|---|---|---|---|---|
| 1 | 221,1051 | $C_{12} H_{15} N O_3$ | 8,00 | 59776 | 0,25 |
| 2 | 205,1103 | $C_{12} H_{15} N O_2$ | 9,93 | 50068 | 0,21 |
| 3 | 316,1994 | $C_{15} H_{28} N_2 O_5$ | 11,66 | 27686 | 0,12 |
| 4 | 172,0735 | $C_8 H_{12} O_4$ | 6,48 | 25759 | 0,11 |
| 5 | 218,1055 | $C_{12} H_{14} N_2 O_2$ | 7,64 | 22015 | 0,09 |
| 6 | 272,1733 | $C_{13} H_{24} N_2 O_4$ | 11,90 | 19987 | 0,08 |
| 7 | 330,2151 | $C_{16} H_{30} N_2 O_5$ | 11,87 | 16622 | 0,07 |
| 8 | 448,1747 | $C_{24} H_{24} O_5 N_4$* | 10,21 | 11437 | 0,05 |
| 9 | 260,1193 | $C_{11} H_{20} N_2 O_3 S$ | 6,36 | 10466 | 0,04 |
| 10 | 384,1395 | $C_{14} H_{20} N_6 O_7$ | 11,99 | 9871 | 0,04 |
| 11 | 300,1118 | - | 6,36 | 9351 | 0,04 |
| 12 | 296,0285 | $C_{12} H_{12} N_2 O_3 S_2$* | 10,75 | 6199 | 0,03 |

* Uncertain chemical formula because of too low signal/no good isotopic pattern

From the composition of the above-identified transformation product features, up to 14931 isomers hits were found from databases for the feature with the highest internal standard equivalent concentration of 0.82 $\mu g.L^{-1}$ , which makes the process of partial identification highly analytical and time-demanding. None of the detected transformation products are present in mass spectrometry databases.

Furthermore, it is quite uncertain that these transformation products are listed in compound databases such as ChemSpider, PubChem, and EPA chemistry dashboard. The normal approach for identifying these compounds would be comparing the detected mass spectrometry $MS^2$ spectrum with the theoretical $MS^2$ spectrum (in silico) of the compounds in the ChemSpider or PubChem database. However, if these compounds are not present in these databases, identification using this approach is not possible. Matching compounds with similar structures can provide information about the type of compound and functional groups that are present. Therefore, the highest confidence level that is likely to be obtained for these transformation products is level 3. (1-5 scale, 1 = identified).

*Table 11 Detected transformation products in negative mode (p-value < 0.05)*

| Feature nr | Molecular Weight | Formula | Retention time (min) | Area | Concentration ISTD equiv. ($\mu g.L^{-1}$) |
|---|---|---|---|---|---|
| 1 | 291,1833 | $C_{17} H_{25} N O_3$ | 17,64 | 77368 | 0,82 |
| 2 | 275,1553 | $C_{13} H_{25} N O_3 S$ | 16,12 | 41219 | 0,44 |
| 3 | 273,1575 | $C_{13} H_{23} N O_5$ | 13,06 | 25588 | 0,27 |
| 4 | 307,1780 | $C_{17} H_{25} N O_4$ | 14,75 | 23359 | 0,25 |
| 5 | 100,0154 | $C_4 H_4 O_3$* | 6,54 | 6440 | 0,07 |
| 6 | 229,1674 | $C_{12} H_{23} N O_3$ | 15,25 | 4257 | 0,05 |
| 7 | 271,9536 | unknown Cl compound | 5,67 | 4193 | 0,04 |
| 8 | 233,1081 | $C_{10} H_{19} N O_3 S$ | 10,53 | 4177 | 0,04 |
| 9 | 221,1047 | $C_{12} H_{15} N O_3$ | 8,07 | 3868 | 0,04 |
| 10 | 343,0944 | $C_{12} H_{17} N_5 O_5 S$ | 9,52 | 3776 | 0,04 |
| 11 | 218,1050 | $C_{12} H_{14} N_2 O_2$ | 7,70 | 3433 | 0,04 |
| 12 | 258,1075 | $C_8 H_{14} O_4 N_6$* | 3,75 | 3348 | 0,04 |
| 13 | 433,1876 | - | 12,61 | 2797 | 0,03 |
| 14 | 247,1239 | $C_{11} H_{21} N O_3 S$ | 15,32 | 2633 | 0,03 |
| 15 | 241,0581 | $C_{10} H_{11} N O_6$* | 6,45 | 2628 | 0,03 |
| 16 | 330,2153 | $C_{16} H_{30} N_2 O_5$ | 11,96 | 1695 | 0,02 |
| 17 | 247,1239 | $C_{11} H_{21} N O_3 S$ | 13,04 | 1648 | 0,02 |

* Uncertain chemical formula because of too low signal/no good isotopic pattern

The lowest internal standard concentration equivalent found was about 0.02 µg.L$^{-1}$. These relative concentrations (range 0.02-0.82 µg.L$^{-1}$) are rather low taking into account that the beakers contained a higher FOG (liquid) concentration and Microcat®-BioPoP content compared to what it is expected in a real sewage system. Moreover, by comparing with the concentration range (3-40 µg.L$^{-1}$) of the top 20 highest detected compounds in the influent of a sewage treatment plant (see Table 12), it may be inferred that no effects will be noticeable in the wastewater treatment system. Nevertheless, this still needs to be examined as will be done with bioreactors in the following study.

*Table 12 Top 20 highest detected compounds in a Sewage Treatment Plant influent reference sample (positive mode).*

| Name | Molecular Weight | Formula | RT (min) | Area | Concern. ISTD equiv. (µg.L$^{-1}$) |
|---|---|---|---|---|---|
| Caffeine | 194,0802 | $C_8 H_{10} N_4 O_2$ | 6,83 | 13276339 | 40 |
| 2-Aminohexadecanoic acid | 271,25092 | $C_{16} H_{33} N O_2$ | 16,99 | 3182183 | 10 |
| Cetylamine | 241,27681 | $C_{16} H_{35} N$ | 20,74 | 2626552 | 8 |
| 1-Tetradecylamine | 213,24548 | $C_{14} H_{31} N$ | 17,88 | 2479331 | 8 |
|  | 227,2611 | $C_{15} H_{33} N$ | 20,24 | 2149197 | 7 |
| DEET | 191,13084 | $C_{12} H_{17} N O$ | 14,84 | 1946236 | 6 |
|  | 356,27126 | $C_{24} H_{36} O_2$ | 18,89 | 1866200 | 6 |
| PEG n7 | 326,19371 | $C_{14} H_{30} O_8$ | 6,86 | 1740058 | 5 |
| PEG n8 | 370,21995 | $C_{16} H_{34} O_9$ | 7,35 | 1712108 | 5 |
| Nonaethylene Glycol | 414,24618 | $C_{18} H_{38} O_{10}$ | 7,75 | 1640528 | 5 |
|  | 594,34168 | $C_{33} H_{46} N_4 O_6$ | 11,95 | 1635365 | 5 |
| PEG n6 | 282,16753 | $C_{12} H_{26} O_7$ | 6,19 | 1627148 | 5 |
| D-(+)-Pipecolinic acid | 129,07879 | $C_6 H_{11} N O_2$ | 2,26 | 1612521 | 5 |
|  | 194,1152 | $C_8 H_{18} O_5$ | 2,66 | 1482051 | 5 |
| PEG n5 | 238,14143 | $C_{10} H_{22} O_6$ | 4,92 | 1462772 | 4 |
| Bis(2-Ethylhexyl) Phthalate | 390,27666 | $C_{24} H_{38} O_4$ | 17,51 | 1226562 | 4 |
| 2-Acetamidophenol | 151,06307 | $C_8 H_9 N O_2$ | 4,54 | 1218416 | 4 |
|  | 327,2252 |  | 6,19 | 1021384 | 3 |
|  | 199,2298 | $C_{13} H_{29} N$ | 17,45 | 1020517 | 3 |
| Paraxanthine | 180,06451 | $C_7 H_8 N_4 O_2$ | 5,23 | 1001790 | 3 |

We concluded from this initial screening that a limited amount of compounds were formed during the artificial FOG degradation by Microcat®-BioPoP with apparent low concentrations. It is foreseen that effects of these transformation products will not be noticeable in the wastewater treatment process. Nevertheless, this is examined further in Chapter 6 with the bioreactors. Matching compounds with similar structures can provide information about the type of compound and functional groups. The highest confidence level that is likely to be obtained for these transformation products is level 3 (1-5 scale, with 1 = identified).

# 5 Beaker (jar) tests results with FOG from the field

FOG samples were taken in different sewer systems, transporting municipal, industial, and combined (industrial and municipal) wastewaters (see Appendix IV). The FOG samples were used to carry out beaker experiments in similar experiments as describe in the previous chapter. All the experiments were carried out in triplicate having about 5 g solid FOG in a net in 2 L synthetic wastewater during 264 hours as shown in Figure 19. The goal of these experiments was to elucidate any effects of adding Microcat®-BioPoP in the synthethic wastewater when using solid FOG from the sewer, based on COD, N and P .

*Figure 19 Solid municipal FOG in beaker (jar) experiments*

## 5.1 Synthetic wastewater with Microcat®-BioPoP and municipal FOG (Experiment 5)

The effects of Microcat®-BioPoP with municipal FOG on COD and nutrients conversion in the reaction vessels are shown in Figure 20. As expected from the previous experiment with artificial FOG, total and soluble COD increased with the presence of Microcat®-BioPoP . During the initial 72 hours, the total COD reached values of 1577 ± 618 mg.L$^{-1}$, indicating a high variability within the replicates. The final total COD was close to the soluble COD, however visually the FOG did not solubilize much (see Figure 21). The soluble COD was 63% higher concentration at the end of the experiment compared to the initial.

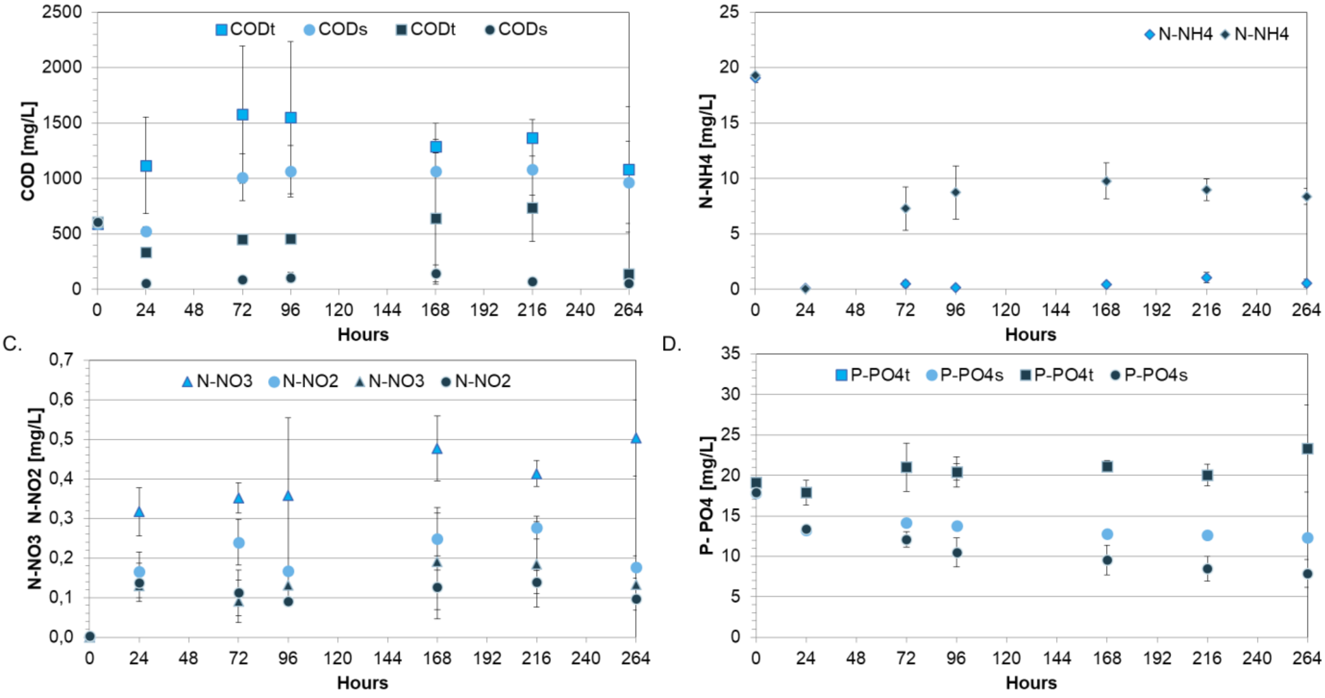


*Figure 20 Synthetic wastewater with Microcat®-BioPoP and municipal fog (**Experiment 5:** light blue), Synthetic wastewater without Microcat®-BioPoP with municipal FOG (**Experiment 6:** dark blue).*

N-NH$_4$ was depleted in the first 24 hours, decreasing from 19.1 mg.L$^{-1}$to 0.12 mg.L$^{-1}$ (Figure 20. B). This is in accordance with what was observed before with the Microcat®-BioPoP experiments, however, such a low concentration was not found. This could be attributed to the fact that the FOG also contains bacteria that could uptake the nitrogen from synthetic wastewater, or simply that nitrifiers bacteria from Microcat®-BioPoP are not hindered by solubilised FOG as in the artificial FOG experiments . Nitrification was observed and maximum concentrations of N-NO$_3$ and N-NO$_2$ were found of about 0.5 and 0.28 mg.L$^{-1}$ respectively (Figure 20. C). Orthophosphate decreased by 31% at the end of the experiment, but no significant difference was found in the total P-PO$_4$.

*Figure 21 Solid FOG after 168 (left) and 264 hours (right).*

## 5.2 Synthetic wastewater without Microcat®-BioPoP and with municipal FOG (Experiment 6)

The COD conversion of municipal FOG was different from what was observed with artificial FOG. In this case, total COD did not show a constant decreasing trend, which could be attributed to the presence of FOG particles released. On the other hand, soluble COD decreased by 92% which is significantly higher than the 41% converted with artificial FOG (Figure 20. A).
$N\text{-}NH_4$ was rapidly depleted during the first 24 h, but at the end of the experiment, a concentration of about half of the initial $N\text{-}NH_4$ concentration was observed (Figure 20. B). This phenomenon may be explained by the nitrogen release due to protein degradation from the FOG. The nitrogen coming from nitrate and nitrate concentration was in the range of 0.05 to 0.3 $mg.L^{-1}$ (Figure 20. C)
Phosphorus increased by 22% in the insoluble fraction (total) while the soluble decreased asymptotically by 56%. The latter was remarkably different from experiment 5 with the presence of Microcat®-BioPoP , inferring that orthophosphate is less taken up when Microcat®-BioPoP is present (Figure 20. D).
Clearly, the bacteria present in the municipal FOG accelerated the process of conversion of COD, $N\text{-}NH_4$, and orthophosphate. It seems that municipal FOG introduces less total and soluble COD to the system than artificial FOG from chapter 4.

## 5.3 Synthetic wastewater with Microcat®-BioPoP and industrial FOG (Experiment 7)

Both total and soluble COD increased by 51 and 63% respectively during the first 72 hours of the experiment (Figure 22. A). Contrary to what was observed when using artificial FOG, the soluble COD was not partially degraded, inferring that FOG contained soluble compounds that were not easily degradable.

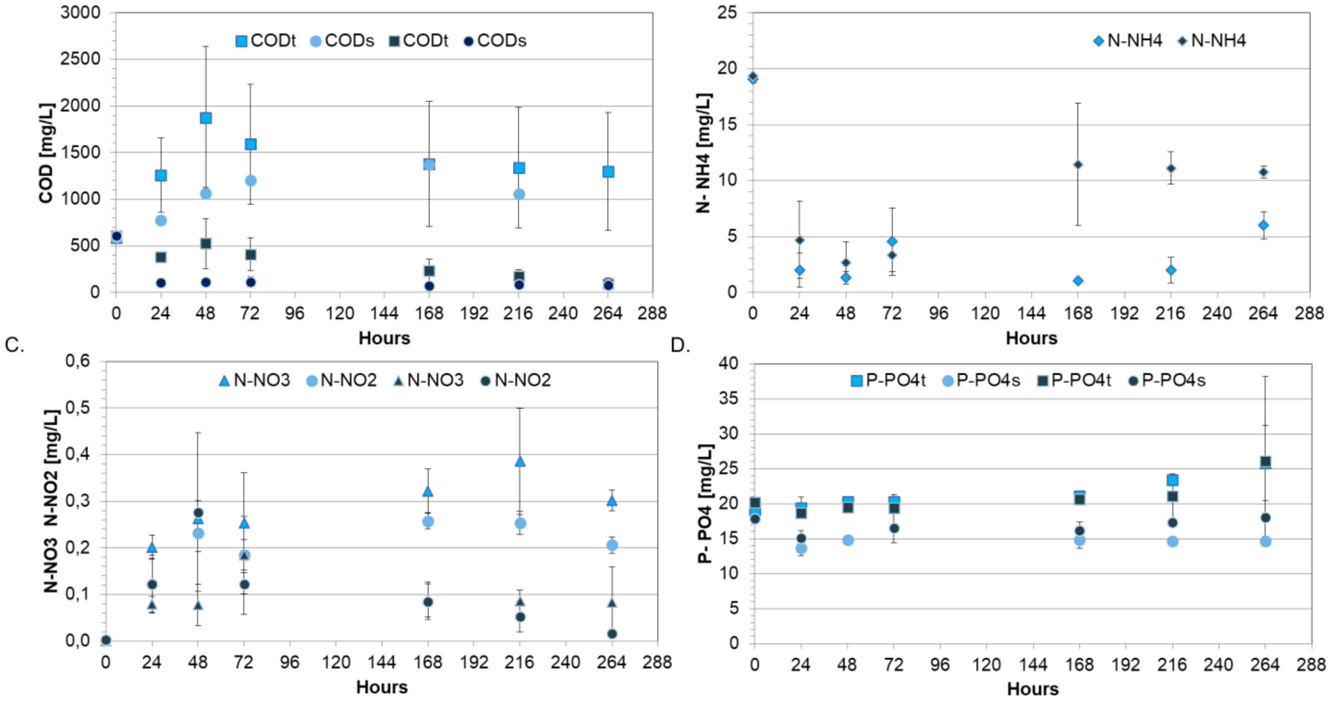


*Figure 22 Synthetic wastewater with Microcat®-BioPoP and industrial FOG (**Experiment 7:** light blue) ; Synthetic wastewater without Microcat®-BioPoP and industrial FOG (**Experiment 8:** dark blue)*

Similarly to before, $N\text{-}NH_4$ was consumed about 90% during the first 24 hours. However, an increase of 1 $mg.L^{-1}$ to about 6 $mg.L^{-1}$ was observed after 168 h (Figure 22. B). The latter was not observed with municipal FOG. This may be due to the solubilization of proteins contained in the FOG. However, the changes in the visual structure of the FOG after the experiment were minimum (Figure 23).

*Figure 23 Industrial FOG at 168 (left) and 264 hours (middle, right)*

Nitrite and nitrate concentration increased to 0.25 and 0.4 $mg.L^{-1}$ within 216 hours, decreasing by 23 and 16% respectively in the last phase of the experiment. Orthophosphate was taken up by 23% in the first 24 hours, and remained rather constant until the end of the experiment. Similarly to municipal FOG, total phosphate increased after 168 hours, most likely due to insoluble fraction release from the FOG (Figure 22. D).

## 5.4 Synthetic wastewater without Microcat®-BioPoP and with industrial FOG (Experiment 8)

Industrial FOG did not contribute as much to the total COD of the synthetic wastewater when compared to municipal FOG. Both total and soluble COD were degraded by about 87% at the end of the experiment (Figure 22. A.). In the case of nutrients, a decrease of about 83% of $N\text{-}NH_4$ was observed within 72 hours, but an increase in concentration to about 10.75 $mg.L^{-1}$ was observed at the end of the experiment comparable to what was observed with municipal FOG (Figure 22. B.). Nitrate and nitrite were in the range of 0.02-0.28 $mg.L^{-1}$. High variability was found within the triplicates during the first 72 hours (Figure 22. C.). $P\text{-}PO_4$ remained rather constant during the entire experiment, with an increase of 30% in the insoluble fraction at the end of the experiment (Figure 22. D.)

## 5.5 Synthetic wastewater with Microcat®-BioPoP and combined industrial/municipal FOG (Experiment 9)

The effects of the combined FOG and Microcat®-BioPoP in synthetic wastewater degradation are shown in Figure 24. The behavior of all parameters analyzed was more similar to the municipal than to the industrial FOG. However, total and soluble COD concentration showed similar concentrations with the combined FOG. Both increased by 60% at the end of the experiment. N-NH4 was consumed mainly for 24 hours as expected, reaching a concentration of about 1.9 $mg.L^{-1}$. An increase to 4.25 $mg.L^{-1}$ was also observed at the end of the experiment.
Likewise, experiment 5, $N\text{-}NO_3$ and $N\text{-}NO_2$ concentrations showed high variability within the triplicates and along with the experiment. The maximum concentrations of about 0.42 $mg.L^{-1}$ $N\text{-}NO_3$ and 0.2 $mg.L^{-1}$ $N\text{-}NO_2$ were obtained at 264 hours. Orthophosphate decreased by 13% for 24 hours and stay rather constant for the rest of the experiment, while total phosphate increased by 11% (Figure 24).

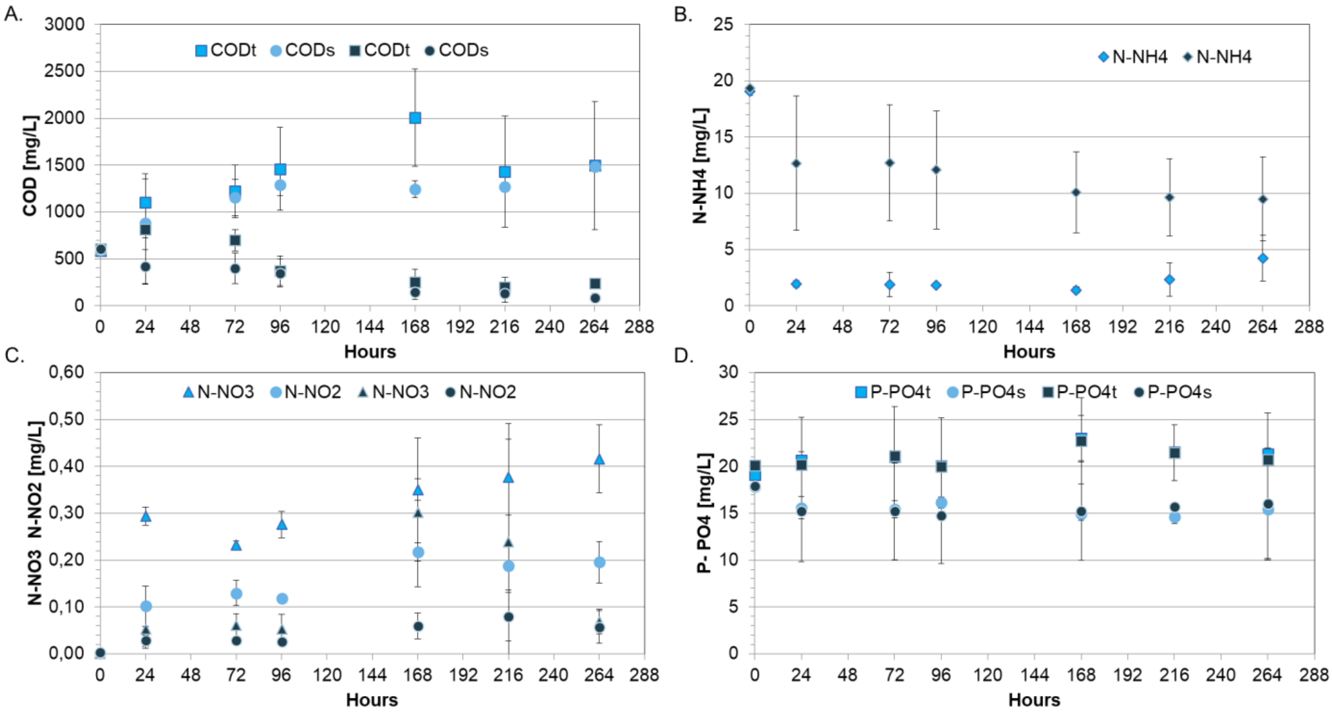


*Figure 24 Synthetic wastewater with Microcat®-BioPoP and combined industrial/municipal FOG (**Experiment 9:** light blue), Synthetic wastewater without Microcat®-BioPoP and with combined industrial/municipal FOG (**Experiment 10:** dark blue).*

## 5.6 Synthetic wastewater without Microcat®-BioPoP and with combined industrial/municipal FOG (Experiment 10)

The synthetic wastewater with the combined FOG obtained a high COD degradation of about 60% for the total and 85% for the soluble COD (Figure 24. A). N-NH$_4$ was consumed slowly similarly to what was observed with artificial FOG to a concentration of about 9.5 mg.L$^{-1}$. However, higher variability was found within the triplicates compared to all other experiments (Figure 24. B). N-NO$_3$ and N-NO$_2$ were under 0.1 mg.L$^{-1}$ in most of the experiments. Between 168 hours and 216 hours, N-NO$_3$ concentration increased up to 0.3 mg.L$^{-1}$ (Figure 24. C). The soluble form of phosphate decreased by 15% during 24 hours and remained constant until the end. P-PO$_4$ total slightly increased, having a significant variability within the triplicates compared to other experiments (Figure 25).

*Figure 25 Synthetic wastewater with combined industrial/municipal FOG at 96 and 264 hours.*

What must be highlighted is that by comparing the experiments with and without Microcat®-BioPoP , and with the presence of FOG, is that the introduction of fat, oil, and grease does not influence the ammonium removal/uptake, while the addition of Bipop remarkably has an impact on high ammonium conversion.

# 6 Evaluation of the effects of Microcat®-BioPoP in the wastewater matrix

An evaluation of the effects of Microcat®-BioPoP was carried out based on a results matrix displayed as Table 13, targeting COD, ammonium, and phosphate removal and nitrate and nitrite concentration increase (nitrification). Negative values in the removal percentage indicated that the substance was released, produced, or increased concentration when compared with the initial value. Negative values in nitrification indicated consumption/conversion of N-NO$_3$, N-NO$_2$ while positive values indicated the production of these compounds.

*Table 13 Effects of experiments on the wastewater matrix: COD, ammonium, phosphate removal, and occurrence of any nitrification (N-NO$_3$, N-NO$_2$ produced).*

| | | | Removal [%] | | | Nitrification [mg.L$^{-1}$] | | Removal [%] | | Time [h] |
|---|---|---|---|---|---|---|---|---|---|---|
| Exp. Nr. | | Description | CODt | CODs | N-NH$_4$ | N-NO$_3$ | N-NO$_2$ | P-PO4$_t$ | P-PO4$_s$ | |
| 1 | | Synthetic wastewater without artificial FOG | -1 | 58 | 100 | 0.06 | 0.19 | -38 | 14 | 48 |
| SWW | ☒ | | 27 | 67 | 100 | 0.08 | 0.13 | -29 | 14 | 72 |
| Microcat®-BioPoP | ☐ | | 53 | 84 | 35 | 0.04 | 0.09 | -2 | 15 | 144 |
| FOG | ☐ | | 61 | 84 | 35 | 0.06 | 0.09 | 1 | 12 | 192 |
| | | | 60 | 85 | 37 | 0.04 | 0.10 | 0 | 8 | 240 |
| | | **Final parameter Concentration [mg.L$^{-1}$]** | **233** | **90** | **12** | **0.04** | **0.1** | **18.9** | **16.4** | |
| 2 | | Microcat®-BioPoP in synthetic wastewater without artificial FOG | -148 | -74 | 92 | 0.32 | 0.22 | -32 | 23 | 48 |
| SWW | ☒ | | -136 | -68 | 99 | 0.29 | 0.26 | -28 | 25 | 72 |
| Microcat®-BioPoP | ☒ | | -194 | -62 | 96 | 0.34 | 0.35 | -18 | 31 | 144 |
| FOG | ☐ | | -203 | -12 | 97 | 0.52 | 0.42 | -12 | 34 | 192 |
| | | | -186 | -65 | 97 | 0.53 | 0.38 | -20 | 37 | 240 |
| | | **Final parameter Concentration [mg.L$^{-1}$]** | **1740** | **1000** | **0.5** | **0.53** | **0.38** | **24** | **11.2** | |
| 3 | | Microcat®-BioPoP in synthetic wastewater with artificial FOG | -43 | -9 | 32 | 0.15 | -0.04 | -7 | 21 | 24 |
| SWW | ☒ | | -57 | -19 | 83 | 0.21 | 0.06 | 5 | 17 | 48 |
| Microcat®-BioPoP | ☒ | | -151 | -9 | 83 | 0.65 | 0.64 | -20 | 3 | 72 |
| FOG | ☒ | | -197 | -17 | 89 | 1.18 | 0.70 | -26 | 0 | 144 |
| | | | -206 | -10 | 89 | 0.79 | 0.40 | 0 | 13 | 192 |
| | | | -161 | -7 | 93 | 0.52 | 0.59 | 17 | 15 | 240 |
| | | **Final parameter Concentration [mg.L$^{-1}$]** | **3851** | **1215** | **1.4** | **0.8** | **0.8** | **19.9** | **17.2** | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 4 | | Synthetic wastewater with artificial FOG | 4 | 17 | 20 | -0.03 | 0.05 | -10 | 3 | 24 |
| SWW | ☒ | | 3 | 20 | 34 | -0.04 | 0.04 | -3 | 4 | 48 |
| Microcat®-BioPoP | ☐ | | 6 | 41 | 24 | -0.02 | 0.01 | 14 | 14 | 72 |
| FOG | ☒ | | 11 | 36 | 21 | 0.10 | 0.07 | 6 | 3 | 144 |
| | | | -21 | 35 | 20 | 0.40 | 0.07 | 17 | 13 | 192 |
| | | | -24 | 37 | 21 | 0.63 | 0.00 | 14 | 15 | 240 |
| | | **Final parameter Concentration [mg.L$^{-1}$ ]** | **1827** | **693** | **16** | **0.86** | **0.2** | **20.3** | **17** | |
| 5 | | Synthetic wastewater with Microcat®-BioPoP and municipal FOG | -90 | 12 | 99 | 0.32 | 0.16 | 7 | 26 | 24 |
| SWW | ☒ | | -168 | -71 | 97 | 0.35 | 0.24 | -10 | 21 | 72 |
| Microcat®-BioPoP | ☒ | | -163 | -80 | 99 | 0.36 | 0.16 | -7 | 23 | 96 |
| FOG | ☒ | | -119 | -80 | 98 | 0.48 | 0.25 | -10 | 29 | 168 |
| | | | -132 | -82 | 95 | 0.41 | 0.27 | -5 | 29 | 216 |
| | | | -83 | -63 | 97 | 0.50 | 0.18 | -22 | 31 | 264 |
| | | **Final parameter Concentration [mg.L$^{-1}$ ]** | **1080** | **964** | **0.56** | **0.5** | **0.18** | **23.3** | **12.4** | |
| 6 | | Synthetic wastewater with municipal FOG | 45 | 91 | 100 | 0.13 | 0.14 | 11 | 25 | 24 |
| SWW | ☒ | | 26 | 85 | 62 | 0.09 | 0.11 | -12 | 32 | 72 |
| Microcat®-BioPoP | ☐ | | 25 | 83 | 55 | 0.13 | 0.09 | -1 | 41 | 96 |
| FOG | ☒ | | -5 | 76 | 49 | 0.19 | 0.12 | -1 | 47 | 168 |
| | | | -21 | 88 | 54 | 0.18 | 0.14 | 1 | 53 | 216 |
| | | | 77 | 91 | 57 | 0.13 | 0.09 | 9 | 56 | 264 |
| | | **Final parameter Concentration [mg.L$^{-1}$ ]** | **141** | **54** | **8.4** | **0.13** | **0.1** | **23.3** | **7.9** | |
| 7 | | Synthetic wastewater with Microcat®-BioPoP and industrial | -114 | -30 | 89 | 0.20 | 0.12 | -1 | 24 | 24 |
| SWW | ☒ | | -218 | -80 | 93 | 0.26 | 0.23 | -6 | 17 | 72 |
| Microcat®-BioPoP | ☒ | | -170 | -103 | 76 | 0.25 | 0.18 | -6 | 100 | 96 |
| FOG | ☒ | | -134 | -131 | 94 | 0.32 | 0.26 | -10 | 17 | 168 |
| | | | -127 | -79 | 90 | 0.39 | 0.25 | -22 | 18 | 216 |
| | | | -120 | -79 | 69 | 0.30 | 0.20 | -35 | 18 | 264 |
| | | **Final parameter Concentration [mg.L$^{-1}$ ]** | **1298** | **1059** | **6** | **0.3** | **0.2** | **25.8** | **15** | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 8 | | Synthetic wastewater with industrial FOG | 38 | 83 | 76 | 0.08 | 0.12 | 8 | 16 | 24 |
| SWW | ☒ | | 14 | 81 | 86 | 0.08 | 0.27 | 3 | 100 | 72 |
| Microcat®-BioPoP | ☐ | | 33 | 81 | 83 | 0.18 | 0.12 | 4 | 8 | 96 |
| FOG | ☒ | | 62 | 89 | 41 | 0.09 | 0.08 | -2 | 10 | 168 |
| | | | 71 | 86 | 42 | 0.09 | 0.05 | -5 | 3 | 216 |
| | | | 84 | 87 | 44 | 0.08 | 0.01 | -29 | -1 | 264 |
| | | **Final parameter Concentration [mg.L$^{-1}$]** | **95** | **77** | **10.8** | **0.08** | **0.02** | **26** | **18** | |
| 9 | | Synthetic wastewater with Microcat®-BioPoP and combined industrial/municipal FOG | -88 | -49 | 90 | 0.29 | 0.10 | -8 | 13 | 24 |
| SWW | ☒ | | -108 | -95 | 90 | 0.23 | 0.13 | -10 | 14 | 72 |
| Microcat®-BioPoP | ☒ | | -148 | -118 | 90 | 0.28 | 0.12 | -5 | 10 | 96 |
| FOG | ☒ | | -241 | -110 | 93 | 0.35 | 0.22 | -20 | 16 | 168 |
| | | | -143 | -114 | 88 | 0.38 | 0.19 | -12 | 18 | 216 |
| | | | -154 | -150 | 78 | 0.42 | 0.19 | -11 | 13 | 264 |
| | | **Final parameter Concentration [mg.L$^{-1}$]** | **1496** | **1481** | **4.25** | **0.42** | **0.2** | **21.3** | **15.5** | |
| 10 | | Synthetic wastewater with combined industrial/municipal FOG | -34 | 31 | 34 | 0.05 | 0.03 | 0 | 15 | 24 |
| SWW | ☒ | | -15 | 34 | 34 | 0.06 | 0.03 | -5 | 15 | 72 |
| Microcat®-BioPoP | ☐ | | 39 | 42 | 38 | 0.05 | 0.02 | 1 | 17 | 96 |
| FOG | ☒ | | 58 | 76 | 48 | 0.30 | 0.06 | -13 | 15 | 168 |
| | | | 67 | 79 | 50 | 0.24 | 0.08 | -7 | 12 | 216 |
| | | | 61 | 86 | 51 | 0.07 | 0.05 | -3 | 10 | 264 |
| | | **Final parameter Concentration [mg.L$^{-1}$]** | **237** | **87** | **9.5** | **0.07** | **0.06** | **20.8** | **16.1** | |

Remarkably, the final concentrations found in the beaker experiments indicated that the presence of Microcat®-BioPoP induced higher COD and lower N-NH$_4$ concentrations independently of the presence of FOG. Municipal and combined FOG showed higher content of COD compared to the industrial FOG, but the final total and soluble COD concentrations when Microcat®-BioPoP was used were 1496, 1298, 1080 mg.L$^{-1}$ and 1481, 1059, and 964 mg.L$^{-1}$ for Combi, Industrial, and municipal FOG, respectively, meaning most of the COD was dissolved.

In these batch experiments both production and consumption occur. In order give more insight in both processes Figure 26, Figure 27 and Figure 28 were derived From Table 13, the effects of Microcat®-BioPoP on the wastewater were quantified by correcting the values for the corresponding beaker (jar) experiment carried out without addition of Microcat®-BioPoP. For every time slot the relative removal or consumption for each batch test was calculted. Then the average removal/consumption value from the whole experiment are presented in Figure 26, Figure 27 and Figure 28.

The effect of Microcat®-BioPoP on the COD of the wastewater showed that in the case of total COD, the Microcat®-BioPoP added more COD to the wastewater and therefore the total COD removal was negatively impacted (Figure 26). The lowest removal found was in experiment 2 with Microcat®-BioPoP without artificial FOG, whereas the Microcat®-

BioPoP with industrial FOG showed the most positive effect. Soluble COD was removed by 25% and 31% in the experiments with municipal and industrial FOG respectively. The latter could be attributed also to the bacteria contained in the FOG that contributed to the degradation of FOG. However, the effect of Microcat®-BioPoP in the soluble COD with the combined FOG was not positive since without Microcat®-BioPoP both total and soluble COD were significantly removed.

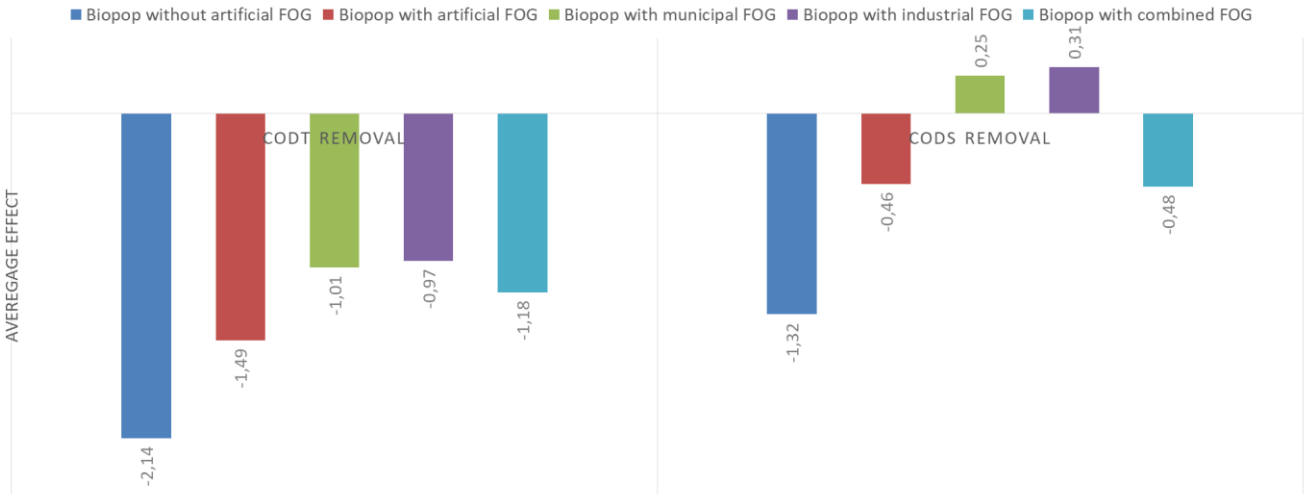


*Figure 26 Effect of Microcat®-BioPoP on the total and soluble COD removal [ratio] in all experiments. The average effect was calculated by subtracting the values from experiments with Microcat®-BioPoP from the corresponding experiment without Microcat®-BioPoP . The average was calculated with the last three values on time.*

The effect of Microcat®-BioPoP on N-NH$_4$ removal was positive, with a removal higher than 20% in all experiments. The Microcat®-BioPoP contributed 64% to the removal of N-NH$_4$ in the experiment with artificial FOG, and as a result concentrations of produced N-NO$_3$ and N-NO$_2$ of about 0.87 and 0.92 mg.L$^{-1}$ were observed. With the FOGs from the field, about 46%, 35%, and 23% of the N-NH$_4$ was removed due to the Microcat®-BioPoP for the combined, municipal, and industrial FOGs, correspondingly (Figure 27). Microcat®-BioPoP did not contribute to reaching concentrations higher than 0.26 mg.L$^{-1}$ of N-NO$_3$ neither from N-NO$_2$ in the experiments with FOGs from the field.

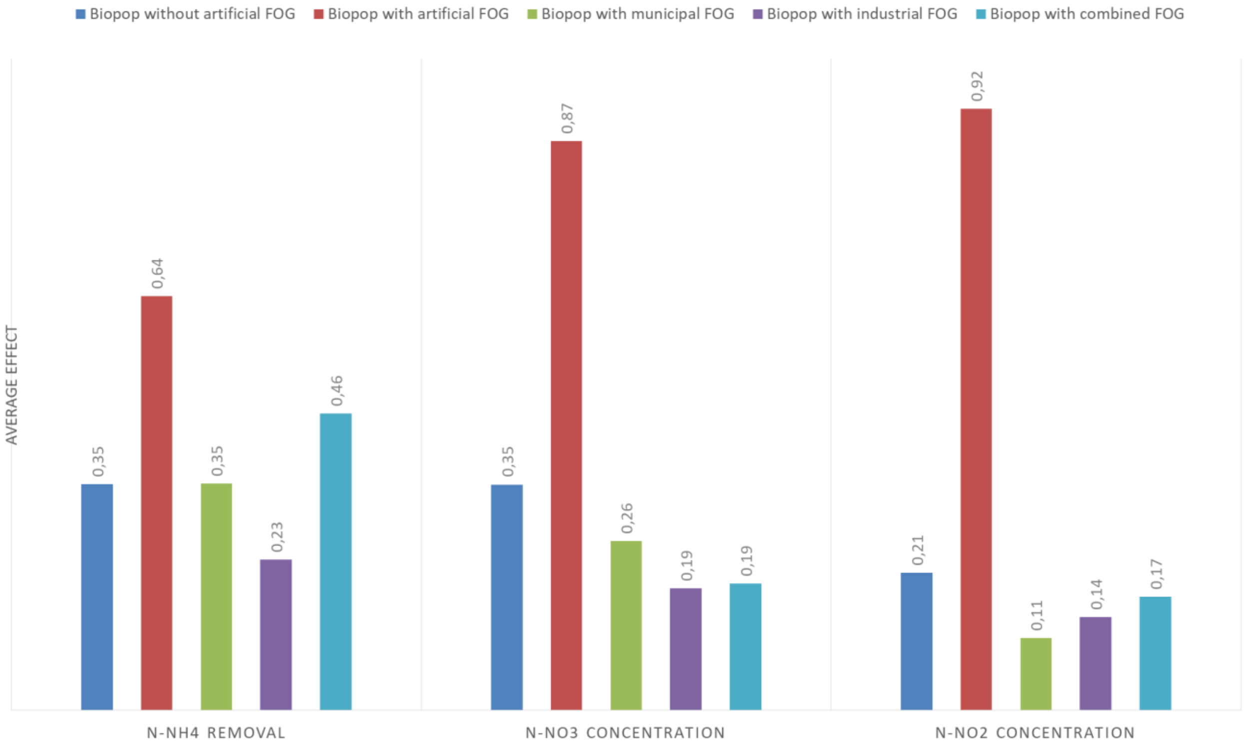


*Figure 27 Effect of Microcat®-BioPoP on the N-$NH_4$ removal [ratio] and N-$NO_3$, N-$NO_2$ production (nitrification), or concentrations [mg/L] increase in the wastewater. The average effect was calculated by subtracting the values from experiments with Microcat®-BioPoP from the corresponding experiment without Microcat®-BioPoP . The average was calculated with the last three values on time.*

Finally, the effect of Microcat®-BioPoP on the total phosphate removal was negative but comparable in all the experiments, inferring that apparently, the Microcat®-BioPoP matrix could release an average of about 10% of total P-$PO_4$. The effect of the Microcat®-BioPoP on the removal of orthophosphate (P-$PO_4$s) was positive for the experiments without artificial FOG and industrial FOG with about 17% and 10% removal, respectively (Figure 28). On the other hand, the Microcat®-BioPoP did not show a significant effect on the soluble phosphate removal for the artificial and combined FOG. About 16% higher soluble P-$PO_4$s was observed with municipal FOG.

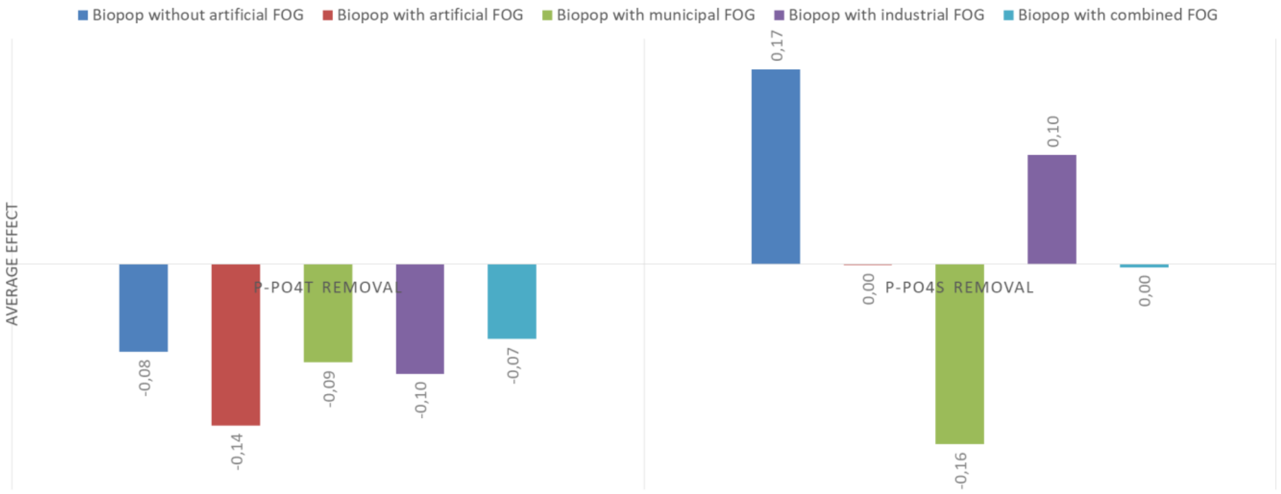


*Figure 28 Effect of Microcat®-BioPoP on the total and soluble phosphate P-$PO_4$ removal [ratio]. The average effect was calculated by subtracting the values from experiments with Microcat®-BioPoP from the corresponding experiment without Microcat®-BioPoP . The average was calculated with the last three values on time.*

It must be pointed out that the amount of Microcat®-BioPoP used in the experiments per volume of wastewater is extremely high when compared to the real application in the sewer. Therefore, it cannot be concluded from this analysis that Microcat®-BioPoP increased the COD in the sewer or the phosphate concentration, or that Microcat®-BioPoP will increase the N-$NH_4$ conversion in the sewage system. Further analysis with the bioreactors will assess any negative/positive impact, but still looking at a high amount of Microcat®-BioPoP per volume wastewater.

# 7 Results and Discussion Bioreactors

## 7.1 Bioreactors performance comparison: the effect of Microcat®-BioPoP on the activated sludge system process

In this chapter, the effect of using Microcat®-BioPoP in a sewer system on a conventional activated sludge treatment process wast tested. For this, two bioreactors simulating a wastewater treatment plant were operated and were fed with wastewater containing a combined industrial/municipal (Combi) FOG. One reactor was operated with Microcat®-BioPoP in the feeding tank, while the second was operated without. The following sections described the overall results observed based on the main parameters of water quality, i.e., chemical oxygen demand (COD), nitrogen compounds ($NH4^+$, $NO_3^{-2}$, $NO^{2-}$), phosphate ($PO_4^{3-}$), and particularly in this case the FOG. The results with and without Microcat®-BioPoP are described and compared.

### 7.1.1 COD removal

The removal of the organic content in the activated sludge system with Microcat®-BioPoP is presented in Figure 29. Along with the experiment, the activated sludge process with Microcat®-BioPoP in the feeding tank removed an average of 84.6 ±11.7 % of the total COD. On the other hand, the soluble COD removed was about 78.8±21.1%, which indicated a higher variability on the biodegradability of the soluble fraction of the COD. It should be pointed out that both the total (606±307 $mg.L^{-1}$) and soluble (419±271 $mg.L^{-1}$) COD varied substantially in the feeding tank mainly due to the addition of new Microcat®-BioPoP , FOG, and partial degradation throughout the cycles, whereas in the effluent a more stable trend was observed especially in the soluble COD after day 41 under HRT ≥ 22 days (Figure 30). During the first 23 days (HRT 16 h) the CODs/CODt ratio in the influent presented a decreasing trend suggesting that the solid FOG in the feeding tank started to fall apart (Figure 31). Thereby, particulate COD increased in the influent of the reactor with Microcat®-BioPoP . Based on this, an increase from 16 to 22 hours of the HRT in the reactor was applied to both reactors to allow longer reaction time to the particulate COD coming into the reactor. However, as expected, the change of HRT affected the stability of the reactor leading to variations (increase/decrease) of the absolute COD removed from this day onwards with an average of 532±307 total COD and 368±267 soluble COD removed (see Figure 49, Appendix I ).

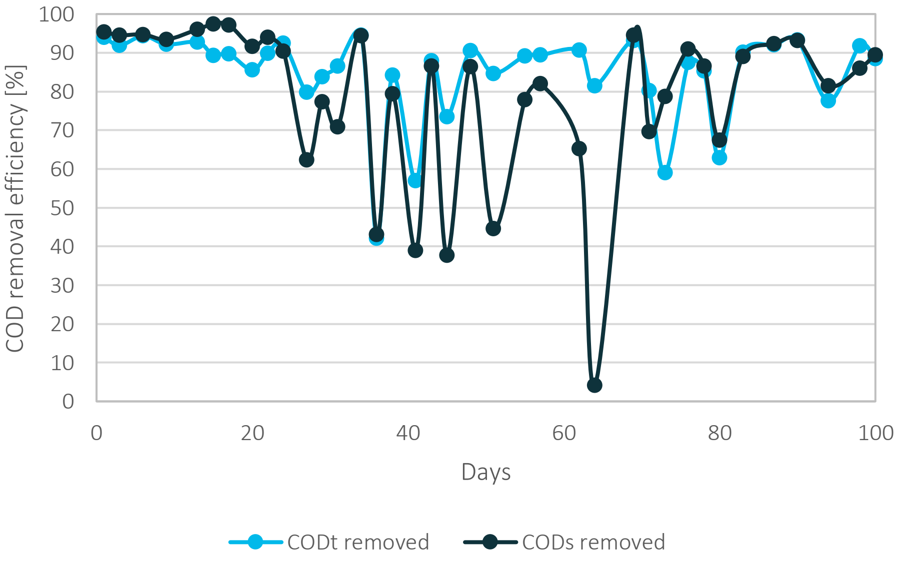
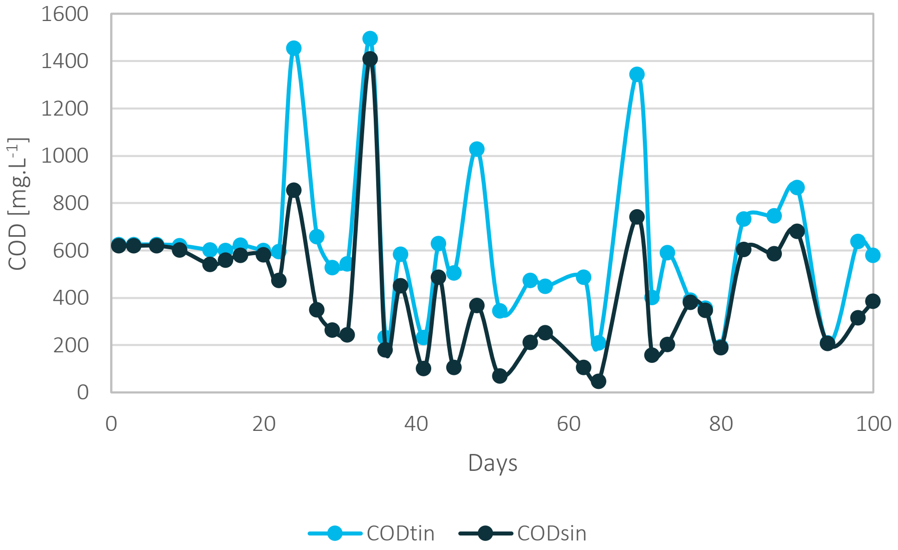


*Figure 29 Total (t) and soluble (s) COD removal efficiency of activated sludge system with Microcat®-BioPoP and FOG in the feeding tank.*

A.



B.

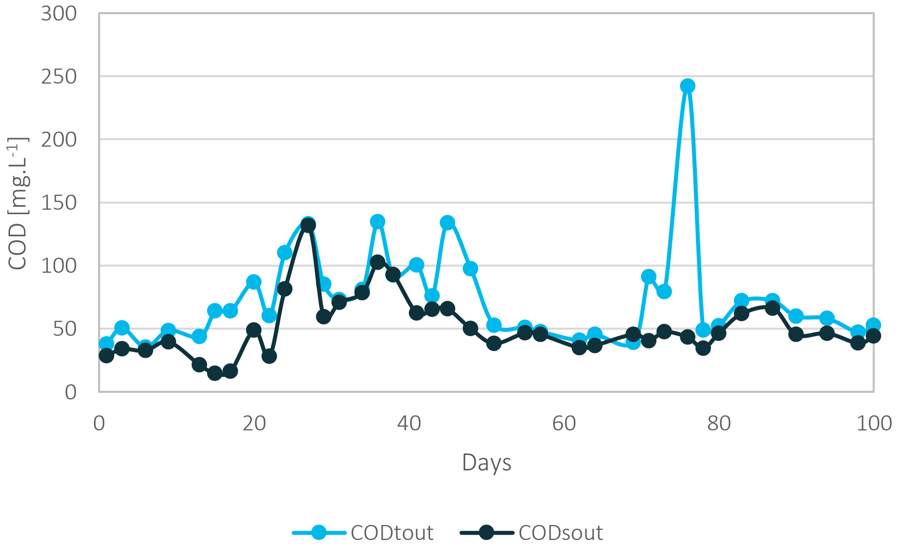


*Figure 30 Total (t) and soluble (s) COD at the influent (A) and effluent (B) of the activated sludge process in the reactor with Microcat®-BioPoP and FOG in the feeding tank. CODt is total COD, CODs is soluble COD.*

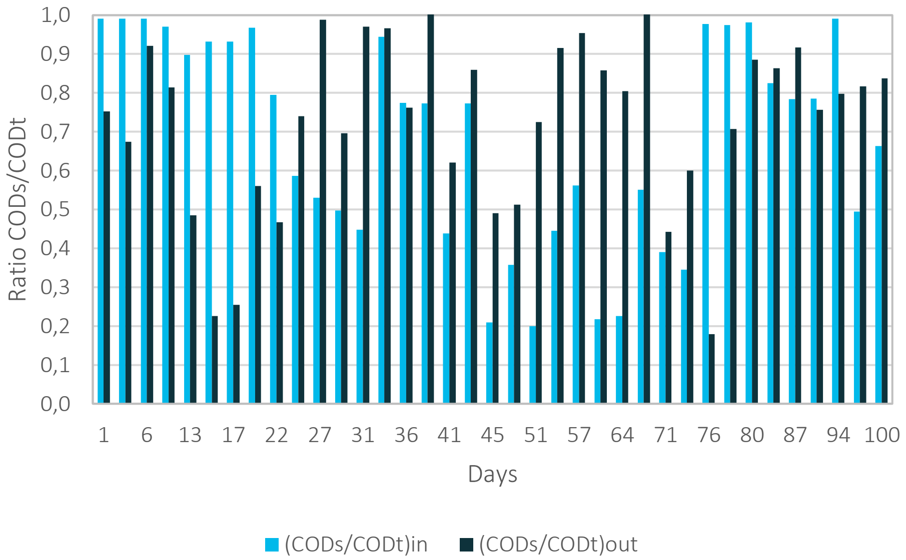


*Figure 31 Soluble (s) to total (t) COD ratio in the influent and effluent of the reactor with Microcat®-BioPoP and FOG in the feeding tank*

The COD removal efficiency in the activated sludge system without Microcat®-BioPoP in the feeding tank showed an average of 87.1 ±10.1 % of the total COD and 90.3±7.4%, of the soluble COD (Figure 32). The influent variability was less than the reactor with Microcat®-BioPoP in the feeding tank, with a total and soluble COD of 576±172 mg.L$^{-1}$ and 460±137 mg.L$^{-1}$ respectively (Figure 33). The latter confirms the observation made with the batch tests about the soluble COD that is added by the Microcat®-BioPoP material (see section. The soluble COD in the effluent was 36±17 mg.L$^{-1}$ which is about 30% lower compared to the effluent of the reactor with Microcat®-BioPoP in the feeding tank. Also, the ratio CODs/CODt showed that during most of the experimental period, the influent of the reactor was mostly soluble COD. However, the effluent was not the same which suggested that biomass washed-out especially during the first 30 days (Figure 34). Furthermore, compared to the other reactor, it can be confirmed that Microcat®-BioPoP in the feeding tank induced detachment of FOG particles and more particulate COD entering the reactor in the lab-setup (no pretreatment if compared to a conventional activated sludge system). Moreover, by looking at the actual COD removed (see Figure 50 Appendix I), without Microcat®-BioPoP, an average of 505±179 total and 425±139 soluble COD were removed.

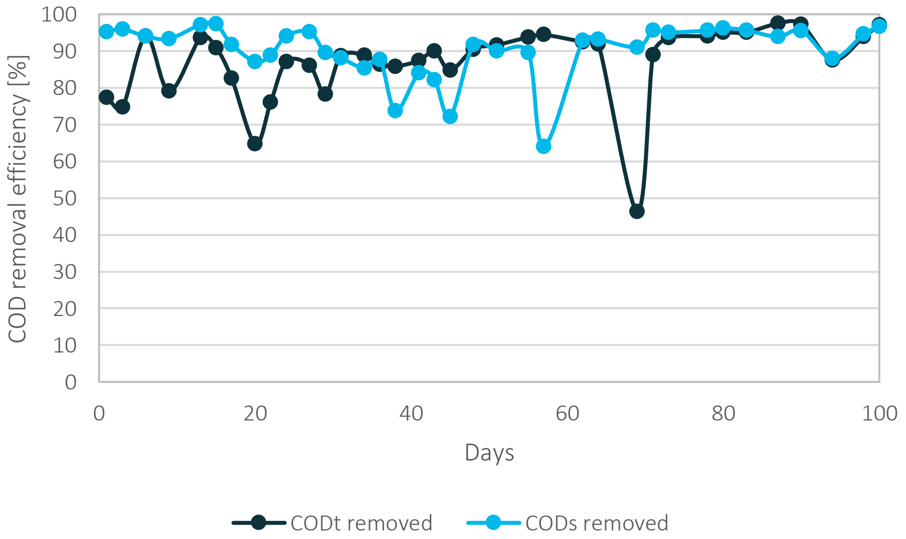
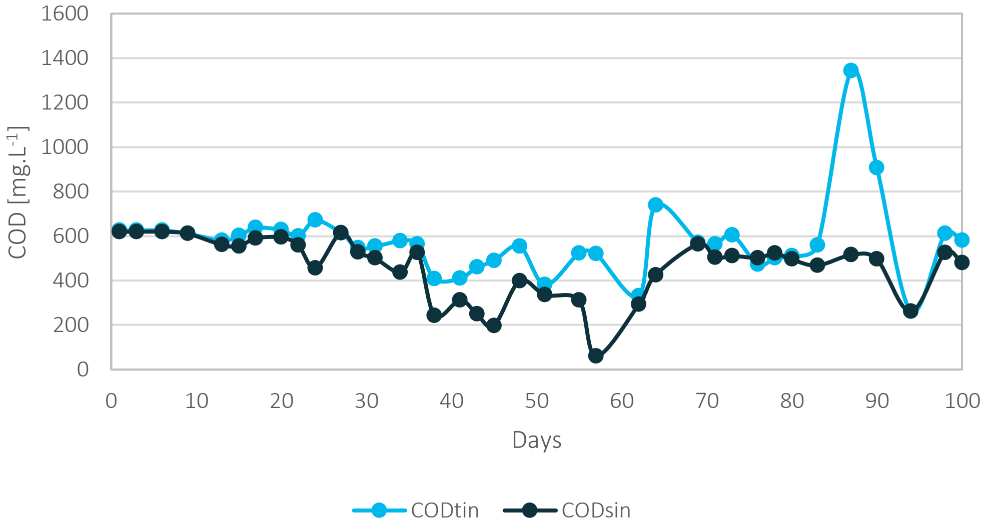


*Figure 32 Total (t) and soluble(s) COD removal efficiency of the activated sludge system without Microcat®-BioPoP and with FOG in the feeding tank*

A.



B.

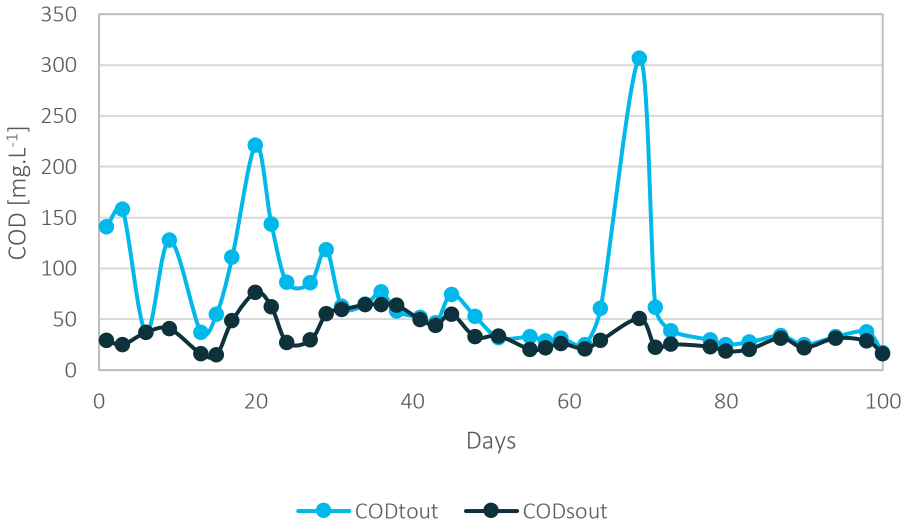


*Figure 33 Total (t) and soluble (s) COD at the influent (A) and effluent (B) of the activated sludge process in the reactor without Microcat®-BioPoP and with FOG in the feeding tank.*

Overall, higher COD removal was observed in the reactor without Microcat®-BioPoP in the feeding tank, with a difference of about 2% in the total COD and 11% in the soluble COD. The increase of the HRT from 16 to 29 did not appear to have a significant impact on the COD removal, neither the increase of DO from 20% to 30% as described in the methods section.

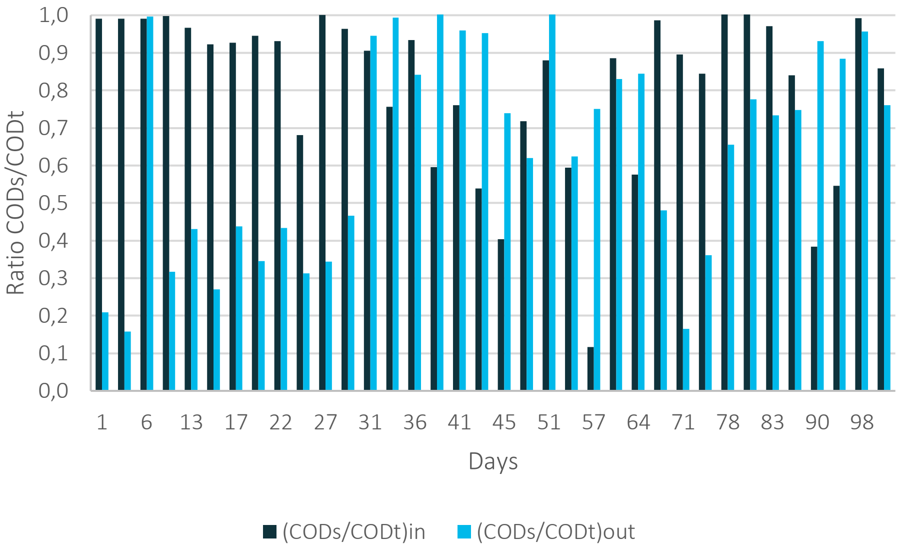


*Figure 34 Soluble to total COD ratio in the influent and effluent of the reactor without Microcat®-BioPoP and with FOG in the feeding tank.*

### 7.1.2 N-compounds conversion

The ammonium (N-$NH_4^+$) removal efficiencies from the reactors with and without Microcat®-BioPoP in the feeding tank are shown in Figure 35. Initially, N-$NH_4^+$ removal did not seem to have a difference between the two reactors. Thus, synthetic wastewater with a higher N-$NH_4^+$ concentration of about 160 mg.$L^{-1}$ was supplied to the reactors. Immediately, the removal of N-$NH_4^+$ decreased to about 20%. Owing to the high N-$NH_4^+$ uptake induced by the Microcat®-BioPoP and observed during batch tests, a higher N-$NH_4^+$ conversion was expected to occur on the reactor with the presence of the bioproduct in the feeding tank. However, only after increasing the HRT from 16 h to 22h on day 22, a clear difference between the two reactors was observed. Moreover, the N-$NH_4^+$ concentration was decreased on day 43 to about 65 mg.$L^{-1}$ and further to 25 mg.$L^{-1}$. Even by increasing the DO from 20% to 30% in both reactors on day 62, only a slightly improved on the N-$NH_4^+$ removal was observed by the reactor without Microcat®-BioPoP . Significantly higher removal efficiency of 70±37 % was obtained in the reactor with Microcat®-BioPoP compared to a low 18±23% of the reactor without.

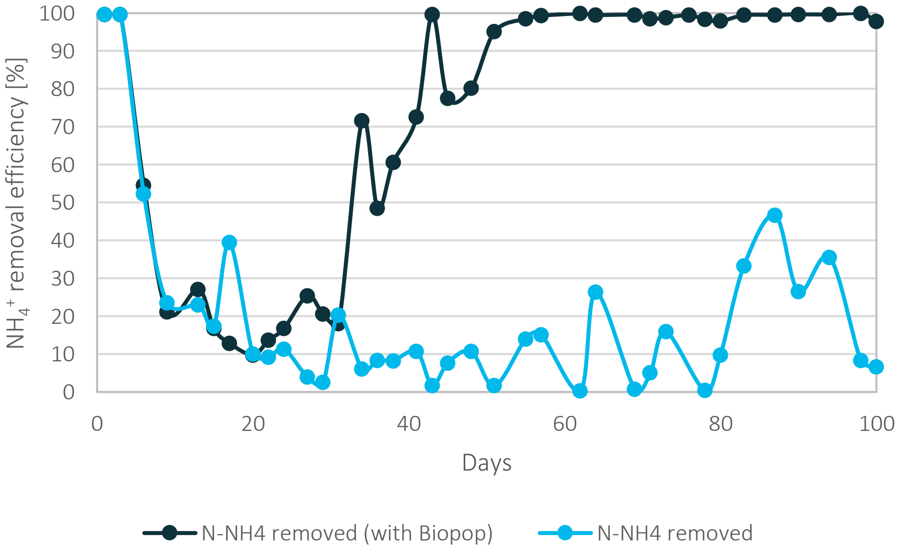


*Figure 35 N-$NH_4^+$ removal efficiencies from the reactors with and without Microcat®-BioPoP and with FOG in the feeding tank*

On the other hand, the N-removal efficiency by both denitrification and assimilation (biomass growth) mechanisms was determined by making a nitrogen (N-) balance ($\Delta N= (N\text{-}NH_4^+ + N\text{-}NO_3 + N\text{-}NO_2)_{in} - (N\text{-}NH_4^+ + N\text{-}NO_3 + N\text{-}NO_2)_{out}$) (Morales 2014). The N-removed at the beginning of the experiment was about 9% higher in the reactor without Microcat®-BioPoP in the feeding tank, and after the increase of the influent ammonium concentration. However, after the change of the HRT to 22 days, a trend improving the N-removal efficiency was observed in the reactor with Microcat®-BioPoP (Figure 36). An average of 50±27% of N- removal efficiency was observed compared to 18±20% in the reactor without Microcat®-BioPoP in the feeding tank. On the other hand, minimum nitritation and nitrification were occurring in the feeding tank based on the influent concentrations. The N-$NO_3$ and N-$NO_2$ concentrations were 0.06±0.05 mg.$L^{-1}$ and 0.03±0.04 mg.$L^{-1}$ respectively for the reactor without Microcat®-BioPoP . In the case of the reactor with Microcat®-BioPoP , similar concentrations of 0.09±0.06 mg.$L^{-1}$ and 0.05±0.04 mg.$L^{-1}$ for N-$NO_3$ and N-$NO_2$ were found (Figure). On the contrary, the effluent concentrations of the reactor with Microcat®-BioPoP were substantially higher (Figure B.). Nitrite concentrations up to 45 mg.$L^{-1}$ were observed on day 38 at an HRT of 22 h and a DO of 20%. Apparently partial nitrification to N-$NO_2$ took place during days 34-62. With the increased to DO 30% and HRT to 29 h on day 62, nitrite concentrations decreased substantially to concentrations below 1 mg.$L^{-1}$. On the contrary, the reactor without Microcat®-BioPoP exhibited $NO_3$ and $NO_2$ concentrations in the effluent of 1.3±2.5 mg.$L^{-1}$ and 0.12±0.1 mg.$L^{-1}$, which ideally would be desired, however, N-$NH_4^+$ concentration was still high indicating the lack of nitrification/denitrification within the activated sludge process (Figure D). The reason for not achieving nitrification in the system without Microcat®-BioPoP after increasing the N-$NH_4$ at the beginning of the experiment may be attributed to the biomass washout observed. Sludge transfer from the reactor with Microcat®-BioPoP to the one without to maintain the sludge retention time similar in both reactors did not improve either the nitrification activity.

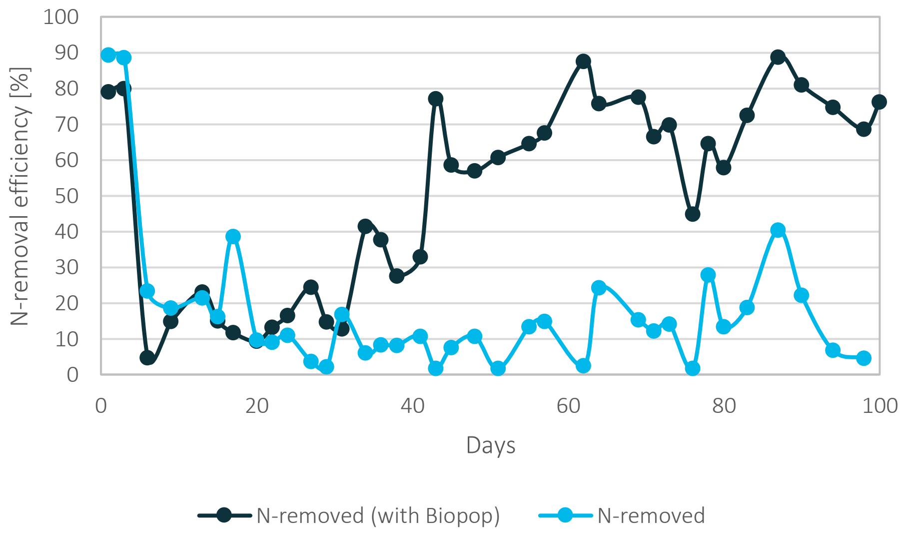
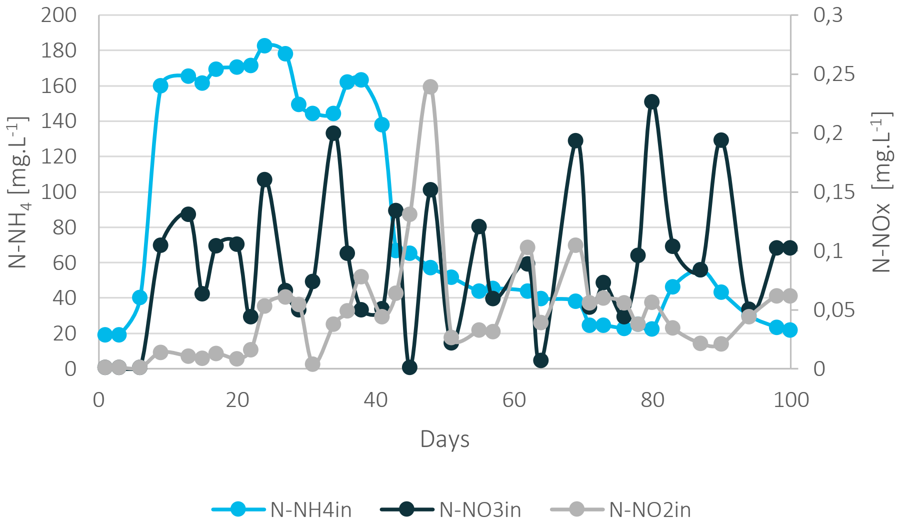
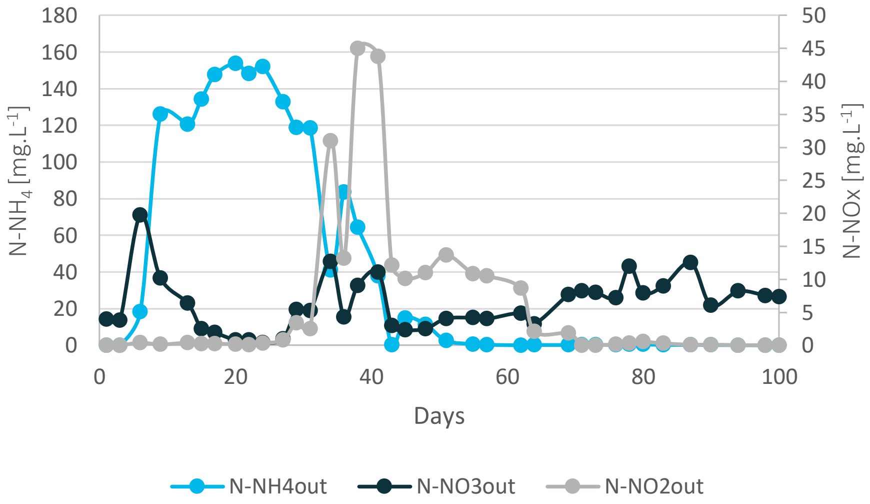


*Figure 36 N-removal efficiency by both denitrification and assimilation in both reactors.*

A.



B.



C.

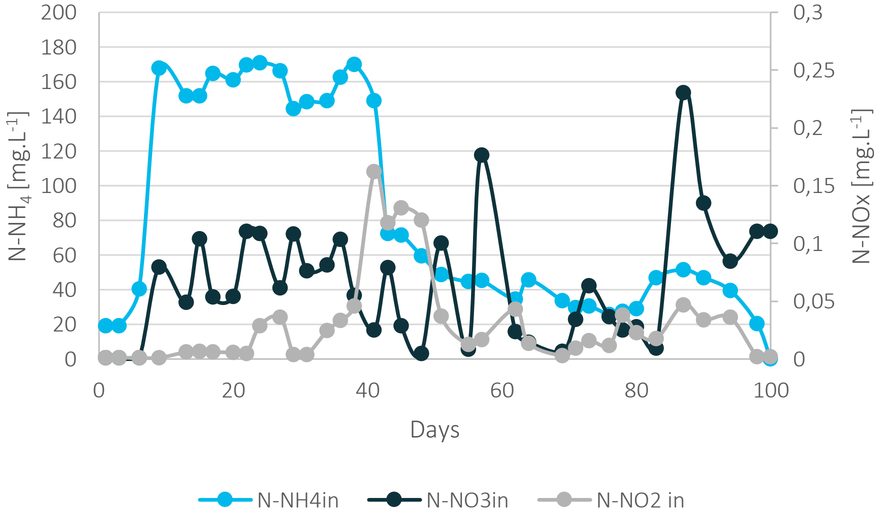


*Figure 37. N-compounds concentration in the influent (in) and effluent (out) of the reactors with FOG, with (A, B) and without (C, D) Microcat®-BioPoP in the feeding tank.*

D.

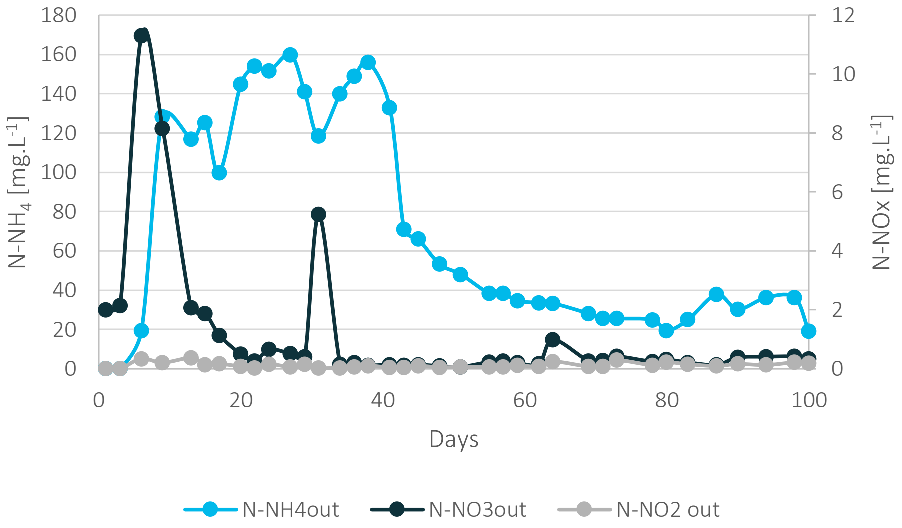
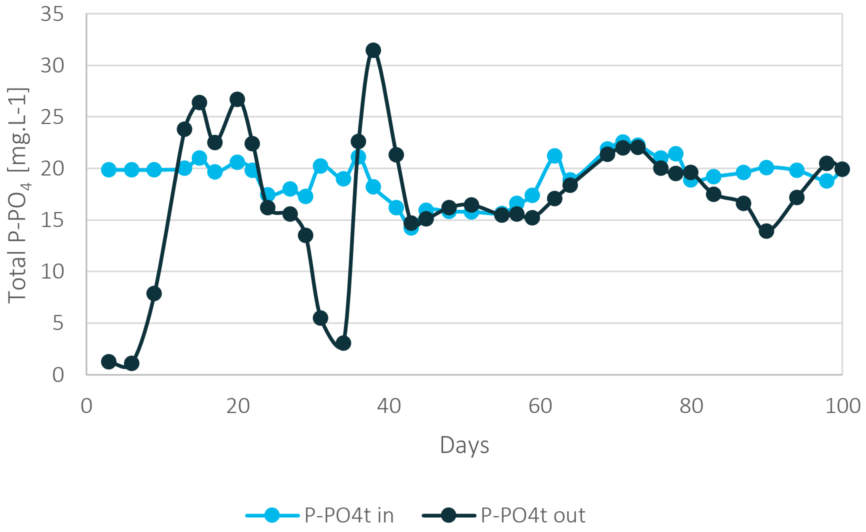


*Figure 37. N-compounds concentration in the influent (in) and effluent (out) of the reactors with FOG, with (A, B) and without (C, D) Microcat®-BioPoP in the feeding tank.*

### 7.1.3 Phosphate

The phosphate conversion through the experiment did not show significant differences. The total phosphate average influent and effluent concentrations were 19±2 mg.L$^{-1}$ and 17±7 mg.L$^{-1}$ for the reactor with Microcat®-BioPoP in the feeding tank (Figure 38 A). On the other hand, the soluble phosphate was about 16.2±2 mg.L$^{-1}$ and 15.2±6 mg.L$^{-1}$ in the influent and effluent, respectively (Figure 38 B). The fact that in several days the concentration of phosphate was higher in the effluent that in the influent can be attributed to the fact that the solid FOG and Microcat®-BioPoP matrix could contain phosphate that was released during the degradation process. The days when there was a significant phosphate uptake in the activated sludge process may be attributed to anoxic phosphate uptake with simultaneous denitrification.

A.



B.

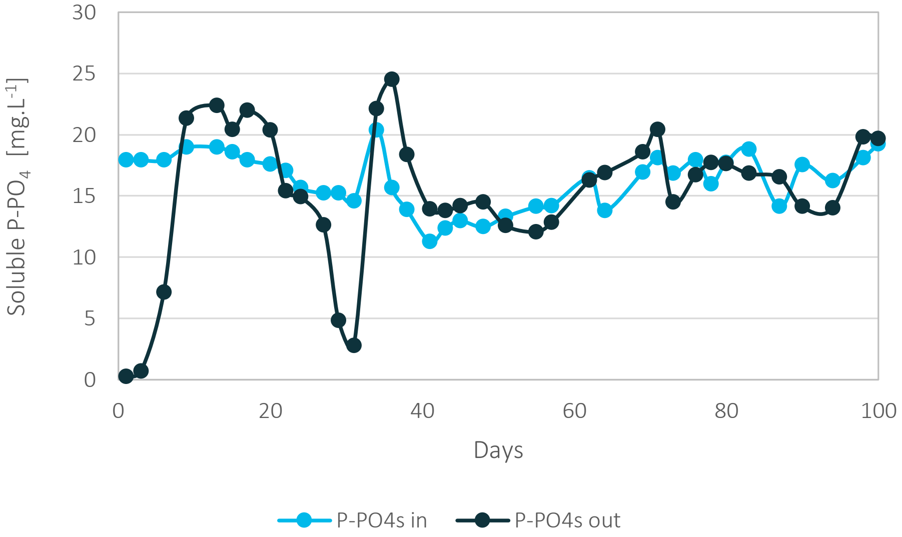
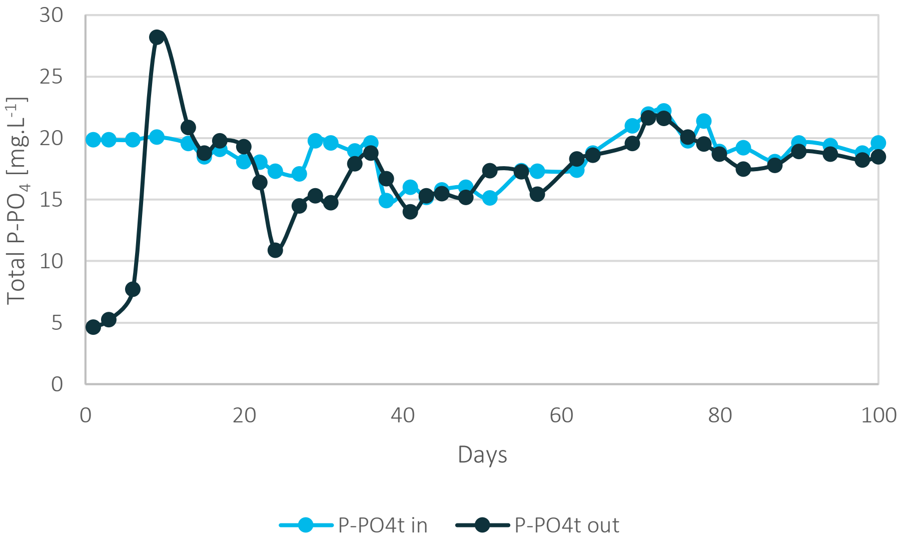


*Figure 38 Total (A) and soluble (B) phosphate in the influent (in) and effluent (out) of the reactor with Microcat®-BioPoP and FOG in the feeding tank.*

In the case of the reactor without Microcat®-BioPoP , similar behavior was observed. The total phosphate at the influent and effluent were 19±2 mg.L$^{-1}$ and 17±4 mg.L$^{-1}$, whereas for the orthophosphate a 17±3 mg.L$^{-1}$ and 16±5 mg.L$^{-1}$ were observed in the influent and effluent correspondingly.

A.



B.

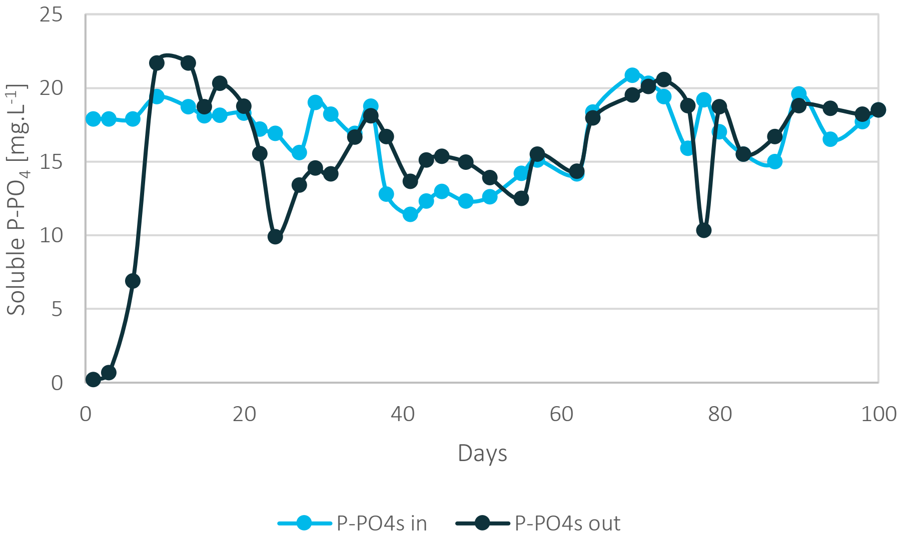


*Figure 39 Total (A) and soluble (B) phosphate in the influent (in) and effluent (out) of the reactor without Microcat®-BioPoP and with FOG.*

### 7.1.4 Fat, Oil, and Grease (FOG) analysis

The concentration of FOG release entering the activated sludge process from the feeding tanks with and without Microcat®-BioPoP was assessed along with the experiment. There was a clear indication of a higher concentration of FOG in the samples from the influent on days 22, 38, 62, and 84 of the reactor with Microcat®-BioPoP compared to the reactor without, suggesting that the Microcat®-BioPoP may contribute to the disintegration of the solid FOG. Compared to the reactor without Microcat®-BioPoP in the feeding tank about 51%, 93%, and 43% higher FOG concentration was observed on days 22, 38, and 84 respectively. On days 41 and 100, both FOG concentration in the influent showed to be under the limit of detection (<20 mg.L$^{-1}$). This variability in the influent can be also attributed to the fact that on days 22, 41, 62, and 83 additional 5 gr of solid FOG was added to the feeding tanks. The release of FOG was not constant, and it was observed that in some days the feeding wastewater contained more small pieces of FOG than in others.

Interestingly, the FOG concentration was <20 mg.L$^{-1}$ in the effluent in all the samples taken indicating that there was not a significant difference in the activated sludge process even though according to the results from Table 14 the reactor with Microcat®-BioPoP in the feeding tank was summited to higher influent concentrations. However, it must be pointed out that at the end of the experiment, when the reactors were emptied, a layer was observed stick around the walls and baffles of the reactors (Figure 40), which inferred that not soluble and slowly biodegradable FOG compounds also accumulated in the reactors. Nevertheless, when compared to a real wastewater treatment with an activated sludge process in which substantially lower Microcat®-BioPoP will be found in the influent, and a higher volume of reactor and contact area in the sewer, the probability of this phenomenon occurring at the wastewater treatment plant is negligible.

*Table 14 FOG concentrations determined at the influent and effluent of the reactors with and without Microcat®-BioPoP in the feeding tank.*

| | Reactor with FOG | Reactor with FOG+ Microcat®-BioPoP | Reactor with FOG | Reactor with FOG + Microcat®-BioPoP |
|---|---|---|---|---|
| Day | Influent [mg.L$^{-1}$] | | Effluent [ mg.L$^{-1}$] | |
| 22 | 35 | 71 | < 20 | < 20 |
| 38 | 33 | 440 | < 20 | < 20 |
| 41 | < 20 | < 20 | < 20 | < 20 |
| 62 | < 20 | 39 | < 20 | < 20 |
| 84 | 86 | 150 | < 20 | < 20 |
| 100 | < 20 | < 20 | < 20 | < 20 |

*Figure 40 Sticky layer in the wall of the reactor with Microcat®-BioPoP at the end of the experiment.*

### 7.1.5 Sulfide and foam formation

The presence of $H_2S$ formation was also tested in the reactors by checking its concentration in the liquid phase. $H_2S$ concentration was not found in the liquid phase, and no typical $H_2S$ odor (rotten egg) was observed in the feeding tank. Furthermore, any presence of foaming in the activated sludge process was not observed at any time of the experiment.

## 7.2 Anaerobic activated sludge digestion

Anaerobic tests of the activated sludge digestion from the two reactors at the end of the continuous experiment were carried out by using an AMPTS device. The results of the production of methane per gram of volatile solids (VS) of the activated sludge showed that the methane yield of the two activated sludge process that contained Microcat®-BioPoP and no Microcat®-BioPoP in the feeding tank was of the same magnitude. A final methane yield of about 497.6±36.5 Nml $CH_4.gVS^{-1}$ was determined with the activated without Microcat®-BioPoP compared to 466.2±35.6 Nml $CH_4.gVS^{-1}$ with Microcat®-BioPoP, indicating that the methane yield of the sludges was similar with only about 6% difference between them (Figure 41). Interestingly, these methane yield values were comparable to primary sludge rather than secondary sludge, suggesting the presence of proteins/FOG or other slowly biodegradable compounds in the sludge.

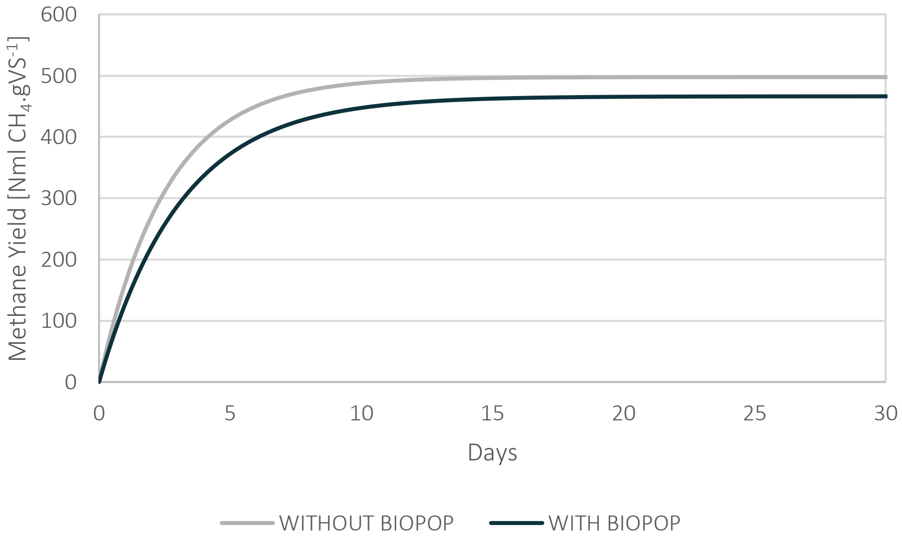


*Figure 41 Average methane yield observed with the activated sludge from the reactors with and without Microcat®-BioPoP .*

## 7.3 Chemicals/compounds identification

### 7.3.1 Non-target screening (NTS)

Overview of NTS features

As described in the methods section, the non-target screening procedure included that the mass spectrometer was operated in the positive and negative electrospray ionization mode (for more details see Appendix 0). In the following sections, the results obtained in each of the modes are described.

A total of 31880 features (possible chemical compounds) were found in all the samples analyzed. Each of the samples was identified with a number and their description can be found in Table 15 a total of 9 blanks samples were used.

*Table 15 Analysed Samples Description. SWW: Synthetic wastewater, N.A: not applicable, Combi: FOG form combined industrial/municipal site.*

| Sample Number | Origin FOG | Condition | Type |
|---|---|---|---|
| 10 | N.A | SWW | Control |
| 11 | N.A | SWW | Control |
| 12 | N.A | SWW | Control |
| 13 | Combi | SWW + FOG + Microcat®-BioPoP | Influent |
| 14 | Combi | SWW + FOG + Microcat®-BioPoP | Influent |
| 15 | Combi | SWW + FOG + Microcat®-BioPoP | Influent |
| 16 | Combi | SWW + FOG + Microcat®-BioPoP | Effluent |
| 17 | Combi | SWW + FOG + Microcat®-BioPoP | Effluent |
| 18 | Combi | SWW + FOG + Microcat®-BioPoP | Effluent |
| 20 | Combi | SWW + FOG | Influent |
| 21 | Combi | SWW + FOG | Influent |
| 22 | Combi | SWW + FOG | Influent |
| 23 | Combi | SWW + FOG | Effluent |
| 24 | Combi | SWW + FOG | Effluent |
| 25 | Combi | SWW + FOG | Effluent |
| 26 | Combi | SWW + FOG + Microcat®-BioPoP | Effluent |
| 28 | Combi | SWW + FOG + Microcat®-BioPoP | Batch |
| 29 | Combi | SWW + FOG + Microcat®-BioPoP | Batch |
| 30 | Combi | SWW + FOG + Microcat®-BioPoP | Batch |
| 31 | Combi | SWW + FOG | Batch |
| 32 | Combi | SWW + FOG | Batch |
| 33 | Combi | SWW + FOG | Batch |
| 34 | Municipal | Municipal WW | Control |
| 35 | Industrial | Industrial WW | Control |
| 36 | Combi | Combi WW | Control |
| 38 | Municipal | SWW + FOG + Microcat®-BioPoP | Batch |
| 39 | Municipal | SWW + FOG | Batch |
| 40 | Industrial | SWW + FOG + Microcat®-BioPoP | Batch |
| 41 | Industrial | SWW + FOG | Batch |

### Principal component Analysis

Large datasets are increasingly widespread in many disciplines. In order to interpret such datasets, methods are required to drastically reduce their dimensionality in an interpretable way, such that most of the information in the data is preserved. For this purpose, Principal component analysis (PCA) is one of the most widely used technique. Its idea is simple, to reduce the dimensionality of a dataset while preserving as much variability (i.e. statistical information) as possible (Jolliffe and Cadima 2016). The aim with the PCA analysis is to get an insight on how different the samples are in terms of their composition (features).

*Positive ionization mode*

The principal component analysis (PCA) of all samples from the positive mode is shown in Figure 42. From the PCA analysis can be concluded that the wastewater (municipal, industrial, combi) control samples (34-36) are clearly separated from the other samples meaning their features differed among them. Similarly, the samples that are coming from synthetic wastewater with FOG and Microcat®-BioPoP (SWW+FOG+ Microcat®-BioPoP ) from municipal and industrial FOG origin (38 and 40) are separated from the samples with the same FOG origin but without Microcat®-BioPoP (SWW+FOG) (39 and 41). The batch SWW+FOG+ Microcat®-BioPoP samples from combined origin (28-30) cluster to each other. The rest of the samples that are from the bioreactors experiments (influent and effluent) all with the same Combi FOG origin (13-26) are very close to each other regardless of the addition of Microcat®-BioPoP (see zoom-in- Figure 43 for more detail) indicating the presence of similar features in all of them. Interestingly, the batch without Microcat®-BioPoP samples (31-33) cluster with the influent (13-15, 20-22) and effluent samples (16-18, 23-26).

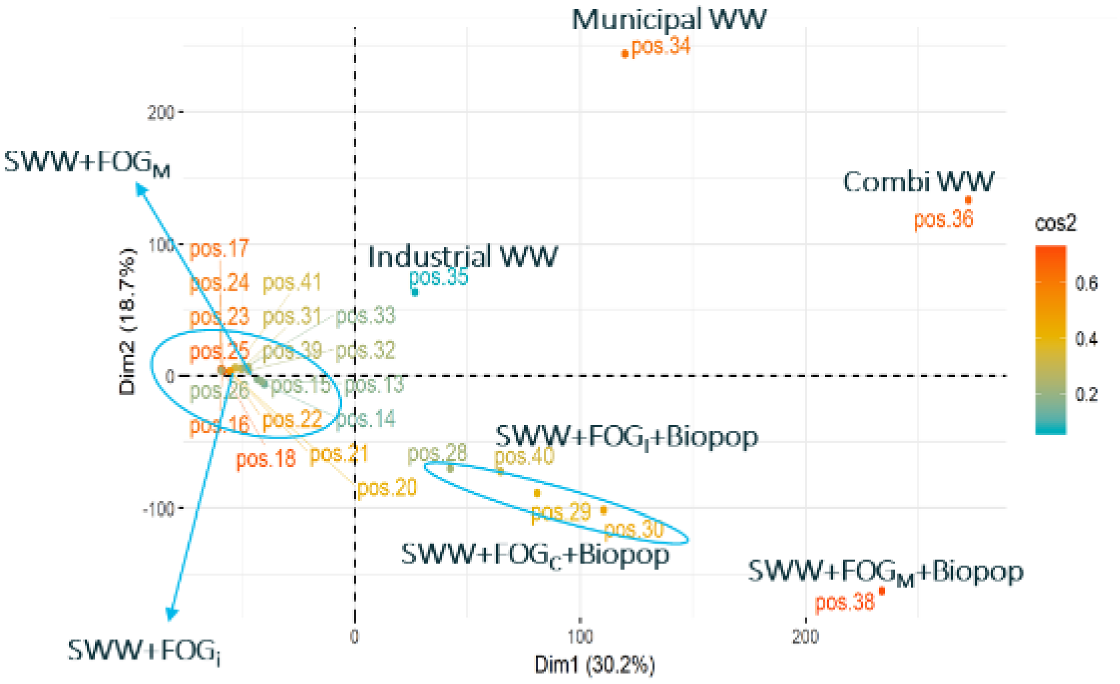


*Figure 42 PCA analysis of all individual samples in the positive ionization mode. Each component (Dim1 and Dim2) explain the variances of the whole set of data by 18.7% and 30.2% respectively. Cos2 scale, is the quality of representation of the variables of the principal components, meaning how much of a variable(sample) is represented in a given component.*

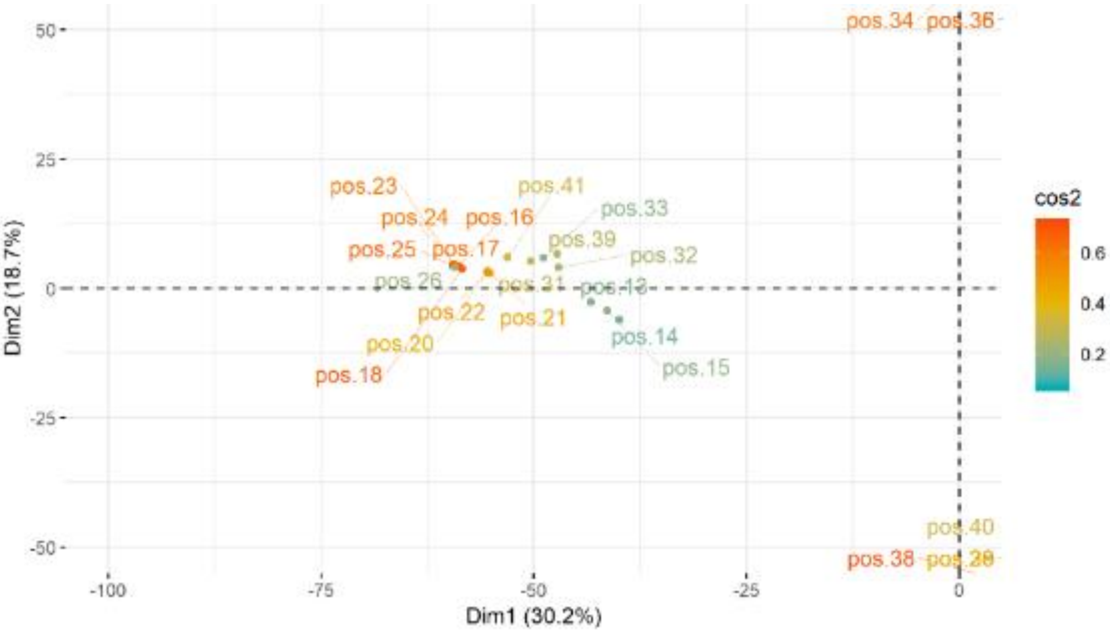


*Figure 43 PCA analysis of all individual samples in the positive ionization mode (zoom-in). Each component (Dim1 and Dim2) explain the variances of the whole set of data by 18.7% and 30.2% respectively. Cos2 scale, is the quality of representation of the variables of the principal components, meaning how much of a variable(sample) is represented in a given component.*

### *Negative ionization mode*

In the case of the negative ionization mode, the sample that was separated from the others was the 34, which is the municipal wastewater, while the industrial (35) and Combi (36) wastewaters clustered together. The latter indicated that in the negative ionization mode the features found in the municipal wastewater are very different from the ones found in the other two. The samples of batch tests with Combi FOG and Microcat®-BioPoP (28-30) clustered together with the batch tests of municipal (38) and industrial (40) FOG. The rest of the samples with Combi FOG with and without Microcat®-BioPoP clustered together, inferring similarities in the chemical compounds found in their chemical matrix.

*Figure 44 PCA analysis of all individual samples in the negative ionization mode. Each component (Dim1 and Dim2) explain the variances of the whole set of data by 18.8% and 22% respectively. Cos2 scale, is the quality of representation of the variables of the principal components, meaning how much of a variable(sample) is represented in a given component.*

### Hierarchical clustering

The hierarchical clustering based on the areas found from the LC-MS analysis showed how different samples cluster depending on the origin of the FOG (industial, municipal, combi), the condition with or without Microcat®-BioPoP or wastewater(industial, municipal, combi), and the experimental type as from bioreactor (influent and effluent), batch or control experiments. The normalized heat map scale (0-1) indicates that dark red (1) the highest intensity of the feature across samples, and dark blue (0) means low intensity. The hierarchical clustering orders the rows (features) and/or the columns (samples) based on similarity. This facilitates to observed correlation/similarities between samples. Four screening suspect lists were scanned (TKIvetten, KWR suspect, LOA600, MzCloud score), molecular weight, the retention time of the features and background are also presented, and the columns in the left side are colored with their corresponding intensities (scales).

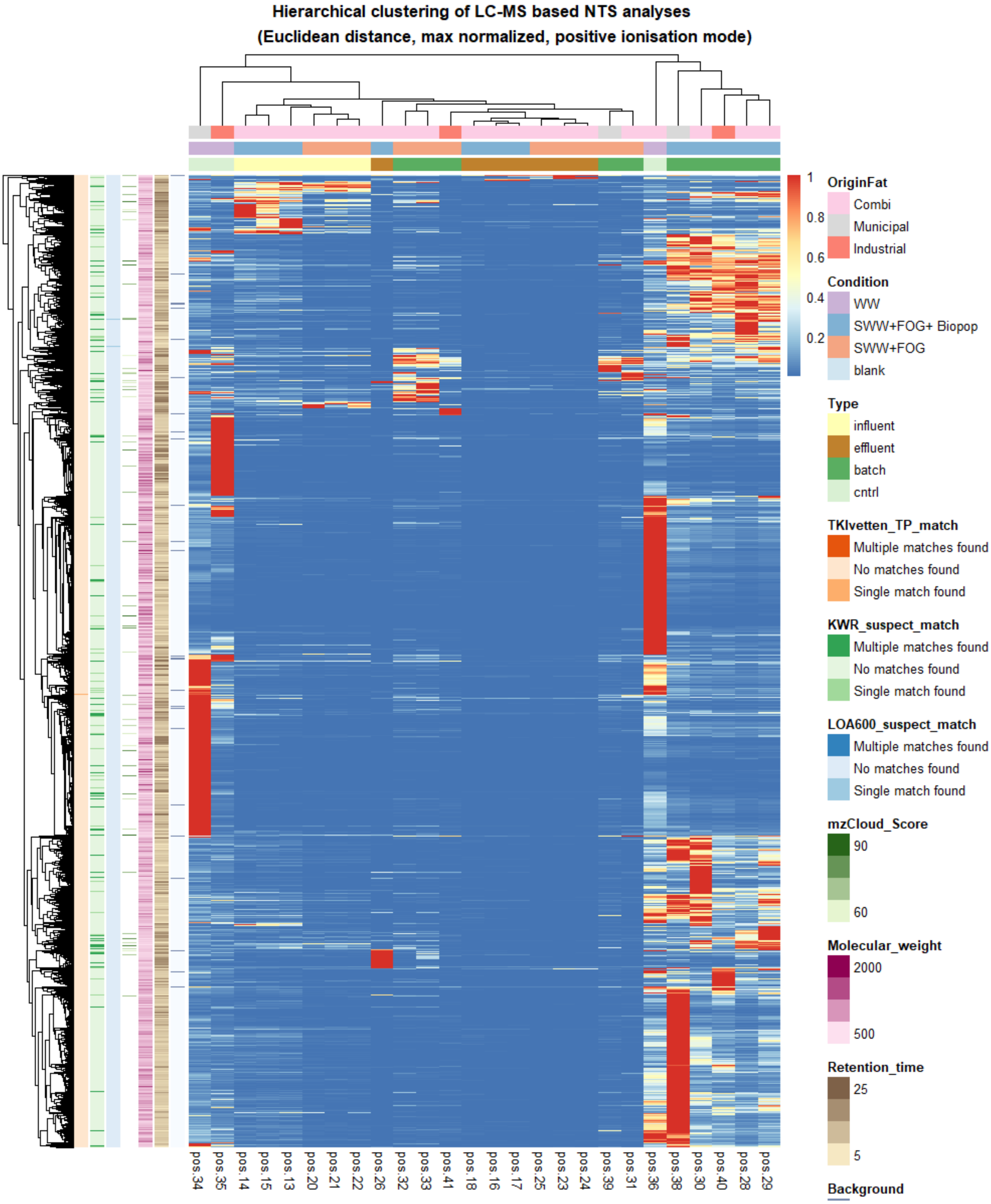

**Hierarchical clustering of LC-MS based NTS analyses**
**(Euclidean distance, max normalized, positive ionisation mode)**

*Figure 45 Hierarchical clustering of analyzed samples in the positive ionization mode.*

*Positive ionization mode*

Effluent and influent are separated, within influent the respective triplicates cluster together, with and without Microcat®-BioPoP samples separated. The intensity of features observed in the effluent is low (mainly dark blue) compared to the influent (some in red, orange, and yellow) (Figure 45), inferring the degradation of these features within the activated sludge process. All the batch samples with Microcat®-BioPoP (SWW+FOG+Microcat®-BioPoP ) clustered together, and are the samples with the largest amount of features with high intensities after the control samples. The wastewater control samples from the three origins (industrial, municipal, and combi) have different highly present features (dark red). Overall, clustering seems to be driven by experimental condition first (influent/effluent or batch), then with Microcat®-BioPoP (SWW+FOG+Microcat®-BioPoP ) or without (SWW+FOG), and fat origin doesn't seem to influence much the clustering. Feature matches were found mainly with the KWR_suspect_match database, and only a single match found with the TKI_vetten_match (list of compounds found with batch tests and artificial FOG).

*Negative ionization mode*

In the negative ionization mode, influent with and without Microcat®-BioPoP were clearly separated, while most of the effluent samples clustered together independently of the condition of with or without Microcat®-BioPoP . The wastewater controls Combi and Industrial clustered together, while the municipal showed a larger number of features with high intensity compared to the other two. The batch samples with Microcat®-BioPoP showed a high number of features with high intensity compared to the samples without and from the bioreactors (influent and effluent). Effluent samples showed only a few features with high intensity and the majority with low intensity when compared to influent samples, suggesting the degradation of most of them and the appearance of a few in both cases with and without Microcat®-BioPoP (see Figure 46).

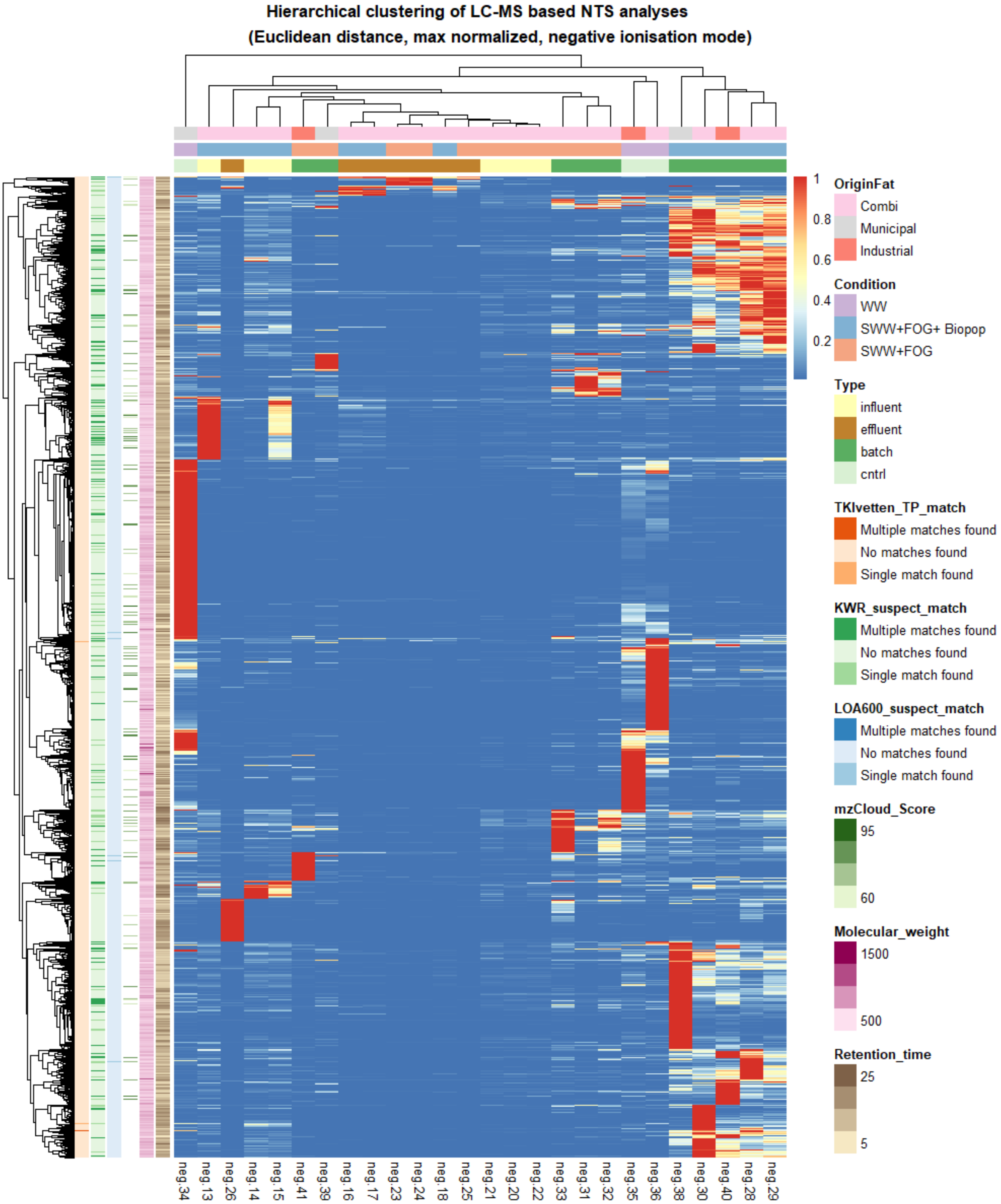


*Figure 46 Hierarchical clustering of analyzed samples in the negative ionization mode.*

## Log2 fold change analysis

Fold change is a measure describing how much a quantity changes going from an initial to a final value, in our case, changes in features composition observed by comparing samples with and without Microcat®-BioPoP . A volcano log2 fold change analysis was made (see Appendix  from comparing the batch samples with (28-30, 38, and 40) and without

Microcat®-BioPoP (31-33, 39 and 41) (see Table 15). The top five features with the highest and lowest log2 fold chance between batch samples with and without Microcat®-BioPoP are shown in Table 16.

*Table 16 Top five features with the highes and lowest log2 fold chance between batch samples with and without Microcat®-BioPoP .*

| FeatureID | Formula | Ionization | Annotation Source: Predicted Compositions | Annotation Source: ChemSpider Search | Number of ChemSpider Results |
|---|---|---|---|---|---|
| 599,4 / 12,8 | C28 H55 F N9 O2 P | Positive | Full match | No results | 0 |
| 427,2 / 12,8 | C27 H29 N3 O2 | Positive | No match | Full match | 5 |
| 427,2 / 12,7 | C27 H29 N3 O2 | Positive | No match | Full match | 5 |
| 599,4 / 12,7 | C26 H55 F2 N7 O6 | Positive | Full match | No results | 0 |
| 515,3 / 8,7 | C22 H51 N3 O6 P2 | Positive | Full match | No results | 0 |
| 385,3 / 24,2 | C24 H51 N O2 | Positive | Full match | Full match | 1 |
| 371,3 / 23,6 | C23 H49 N O2 | Positive | Full match | No results | 0 |
| 371,3 / 23,8 | C23 H49 N O2 | Positive | Full match | No results | 0 |
| 141,0 / 3,2 | C10 H7 N | Positive | Full match | Full match | 20 |
| 218,2 / 14,9 | C16 H26 | Positive | Full match | Partial match | 27 |
| 277,1 / 9,4 | C13 H27 N O5 | Negative | Full match | Full match | 5 |
| 97,9 / 2,1 | H2 O4 S | Negative | No results | Full match | 1 |
| 405,3 / 16,5 | C21 H43 N O6 | Negative | Full match | Full match | 1 |
| 310,1 / 10,2 | C16 H24 N O5 | Negative | No match | Full match | 6 |
| 97,9 / 2,6 |  | Negative | No results | No results | 0 |
| 97,9 / 2,2 | H3 O4 P | Negative | No results | Full match | 1 |
| 439,2 / 13,4 | C20 H41 N O7 S | Negative | Full match | Full match | 1 |
| 394,1 / 8,2 | C17 H22 N4 O7 | Negative | Not the top hit | Full match | 7 |
| 251,8 / 2,0 |  | Negative | No results | No results | 0 |
| 195,9 / 2,1 | C5 H6 Te | Negative | No results | Full match | 5 |

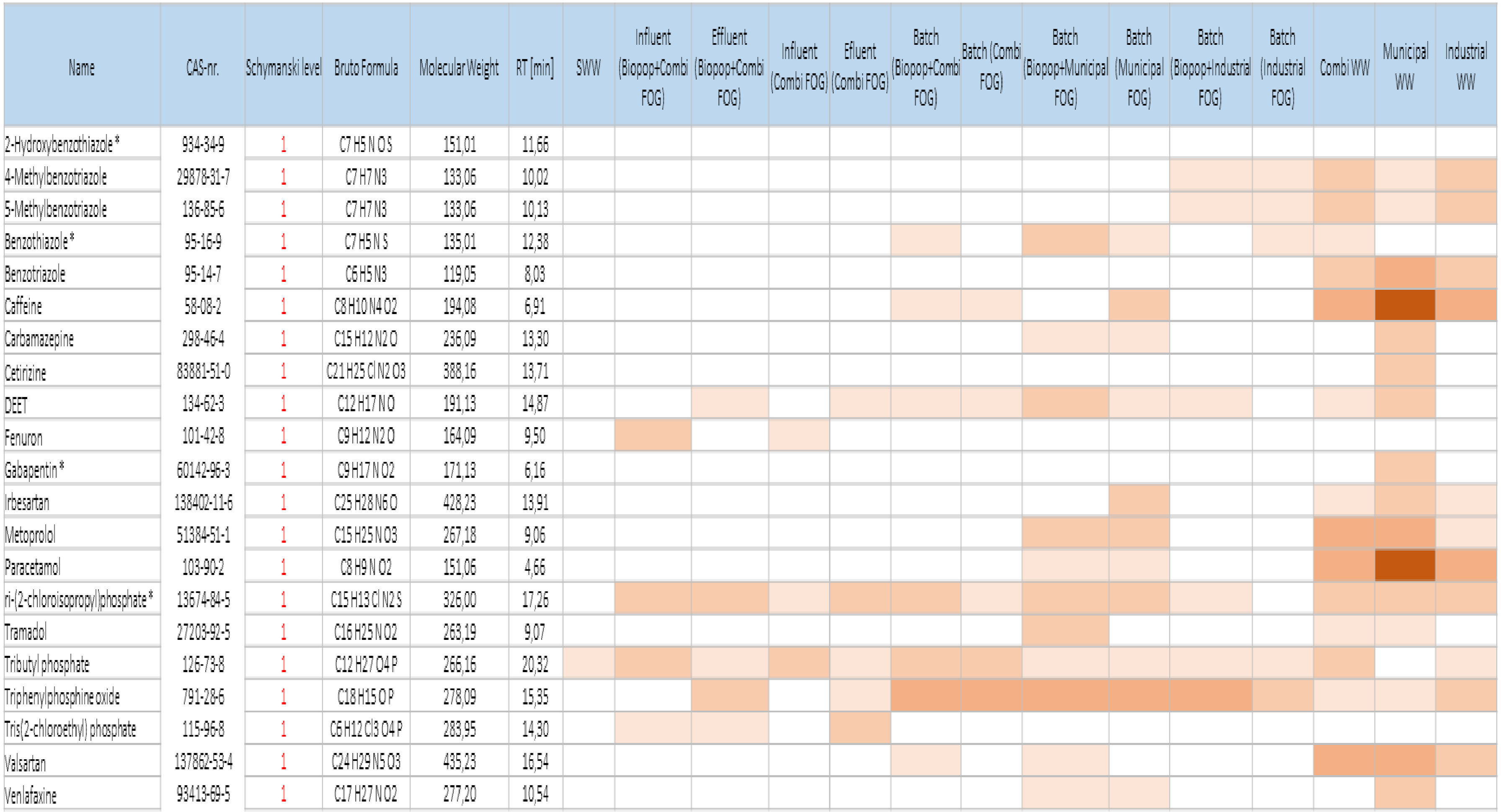
From these features, none matches with a spectral library (Annotation sources: mzCloud and mzVault) were found. A spectral library match is based on both MS$^1$ and MS$^2$ data and the most confident match. 19/20 features do not match any suspect lists. Only the feature 218.2 / 14.9 matches a suspect list, namely the SusDat list. Suspect list matches are based on accurate mass only. As multiple chemical structures can have the same mass, an accurate mass match is ambiguous and needs to be confirmed based on MS$^2$ information or reference standards. As SusDat list consists of European environmental relevant compounds of about ~40k suspects. Furthermore, 7/20 features do not match with ChemSpider. ChemSpider is a database of chemical structures and basically a very large suspect list. If a feature cannot be matched to ChemSpider, its identification is something challenging and time-consuming and often does not lead to identification. In conclusion, the features found in the batch samples with Microcat®-BioPoP corresponded with existent chemical compounds formulas, but since the fact they did not correspond with any suspect lists, makes its further identification to a higher level difficult. Additionally, as it was shown in the hierarchical clustering from the point of view of the effect on a wastewater treatment system, the features appearing in the effluent mostly have a lower intensity than in the influent, independently of samples with or without Microcat®-BioPoP . Therefore, it was decided to further proceed with suspect target screening to identify whether there are significant differences in samples in the influent or effluent of the activated sludge system.

### 7.3.2 Suspect-target screening (STS)

The objective of applying NTS and STS techniques for chemical compounds identification was the detection of transformation products and any organic micropollutant released which could be identified in both bioreactor experiments with FOG from the combined sewer (industrial/municipal), and batch experiments with FOG from combined, industrial and municipal sewer. The results showed 21 compounds identified with level 1 of the Schymanski matrix (see Appendix VIII), meaning full identified compounds. These compounds are shown in a heat map intensity based on the peak area in the mass spectrometric detection (Figure 47). The compound with the highest intensity on most of the batch test was Triphentlphosphine oxide. The highest intensity (dark red) compounds found in the municipal wastewater sample was Caffeine and Paracetamol. Other pharmaceutical compounds with the high intensity found in the wastewater samples were Valsartan and Metoprolol. Chemicals such as corrosion inhibitors Benzotriazole, 4- and 5-Methylbenzotriazole showed also high intensity. Overall, there were no differences determined due to the presence of Microcat®-BioPoP in samples.

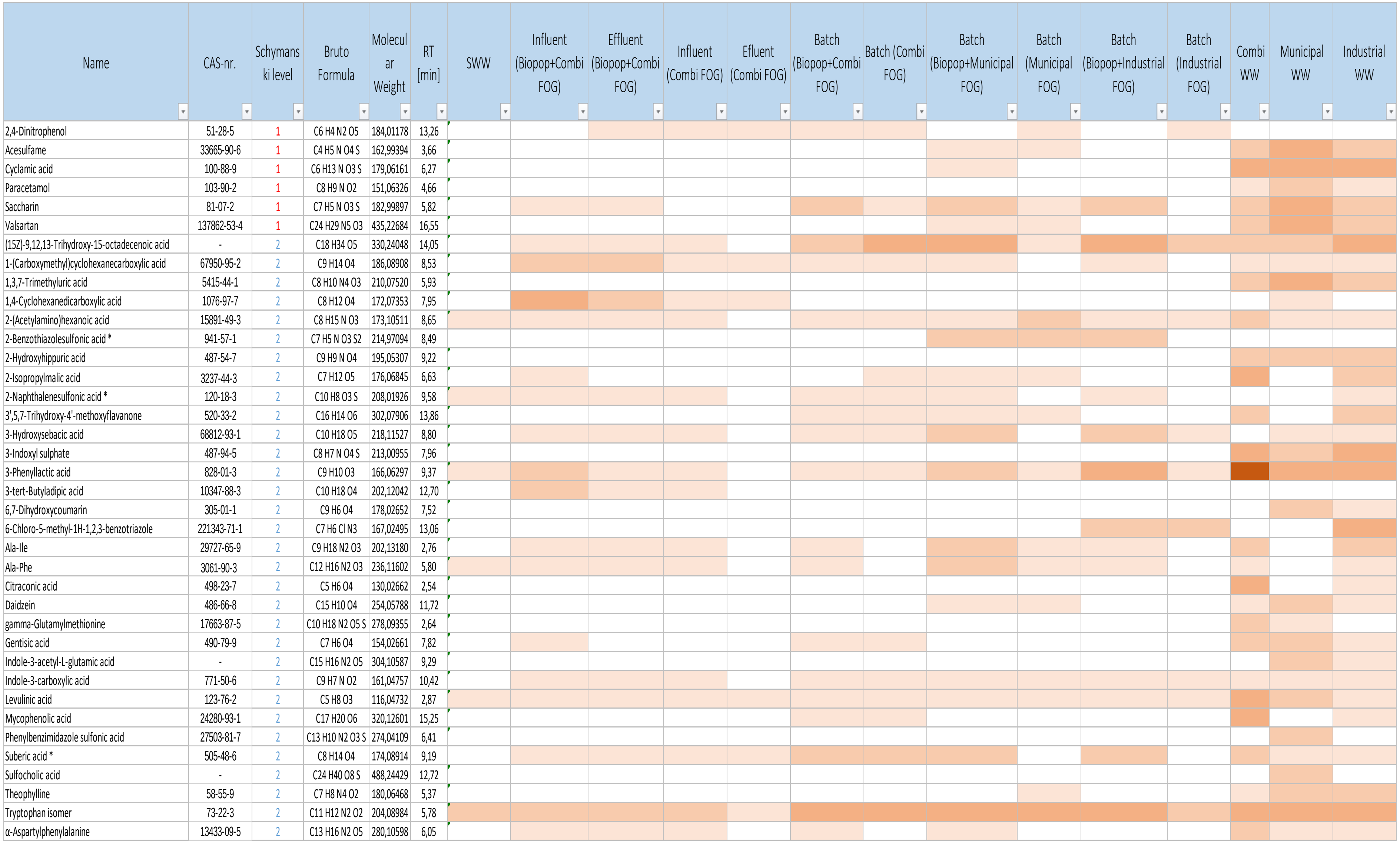
A total of 85 compounds with level 2 of identification (probable structure) were also found in the positive ionization mode (see details in Figure 55 , Appendix VIII ). As observed with confirmed compounds, there were no differences identified due to the presence of Microcat®-BioPoP among samples. The differences in intensity between the influent and effluent samples with and without Microcat®-BioPoP may infer the degradation of several compounds.

In the negative ionization mode, a total of 6 and 33 compounds were determined with a level 1 and 2 of identification confidence, respectively (Figure 48). 2,4-dinitrophenol, acesulfame, cyclamic acid, paracetamol, saccharin, and valsartan showed a higher peak intensity in the wastewater samples. No representative differences attributed to the presence of Microcat®-BioPoP among samples were found in the negative ionization mode. Furthermore, some of the differences observed in intensity from influent and effluent samples may suggest the degradation of certain compounds during the activated sludge process.



| Name | CAS-nr. | Schymanski level | Bruto Formula | Molecular Weight | RT [min] | SWW | Influent (Biopop+Combi FOG) | Effluent (Biopop+Combi FOG) | Influent (Combi FOG) | Efluent (Combi FOG) | Batch (Biopop+Combi FOG) | Batch (Combi FOG) | Batch (Biopop+Municipal FOG) | Batch (Municipal FOG) | Batch (Biopop+Industrial FOG) | Batch (Industrial FOG) | Combi WW | Municipal WW | Industrial WW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2-Hydroxybenzothiazole* | 934-34-9 | 1 | $C_7H_5NOS$ | 151,01 | 11,66 | | | | | | | | | | | | | | |
| 4-Methylbenzotriazole | 29878-31-7 | 1 | $C_7H_7N_3$ | 133,06 | 10,02 | | | | | | | | | | ░ | ░ | ▒ | ░ | ▒ |
| 5-Methylbenzotriazole | 136-85-6 | 1 | $C_7H_7N_3$ | 133,06 | 10,13 | | | | | | | | | | ░ | ░ | ▒ | ░ | ▒ |
| Benzothiazole* | 95-16-9 | 1 | $C_7H_5NS$ | 135,01 | 12,38 | | | | | | ░ | | ▒ | ░ | | ░ | ░ | | |
| Benzotriazole | 95-14-7 | 1 | $C_6H_5N_3$ | 119,05 | 8,03 | | | | | | | | | | | | ▒ | ▓ | ▒ |
| Caffeine | 58-08-2 | 1 | $C_8H_{10}N_4O_2$ | 194,08 | 6,91 | | | | | | ░ | ░ | | ▒ | | | ▓ | █ | ▓ |
| Carbamazepine | 298-46-4 | 1 | $C_{15}H_{12}N_2O$ | 236,09 | 13,30 | | | | | | | | ░ | ░ | | | | ▒ | |
| Cetirizine | 83881-51-0 | 1 | $C_{21}H_{25}ClN_2O_3$ | 388,16 | 13,71 | | | | | | | | | | | | | ▒ | |
| DEET | 134-62-3 | 1 | $C_{12}H_{17}NO$ | 191,13 | 14,87 | | | ░ | | ░ | ░ | ░ | ▒ | ░ | ░ | | ░ | ▒ | |
| Fenuron | 101-42-8 | 1 | $C_9H_{12}N_2O$ | 164,09 | 9,50 | | ▒ | | ░ | | | | | | | | | | |
| Gabapentin* | 60142-96-3 | 1 | $C_9H_{17}NO_2$ | 171,13 | 6,16 | | | | | | | | | | | | | ▒ | |
| Irbesartan | 138402-11-6 | 1 | $C_{25}H_{28}N_6O$ | 428,23 | 13,91 | | | | | | | | | ▒ | | | ░ | ▒ | ░ |
| Metoprolol | 51384-51-1 | 1 | $C_{15}H_{25}NO_3$ | 267,18 | 9,06 | | | | | | | | ▒ | ▒ | | | ▓ | ▓ | ░ |
| Paracetamol | 103-90-2 | 1 | $C_8H_9NO_2$ | 151,06 | 4,66 | | | | | | | | ░ | ░ | | | ▓ | █ | ▓ |
| ri-(2-chloroisopropyl)phosphate* | 13674-84-5 | 1 | $C_{15}H_{13}ClN_2S$ | 326,00 | 17,26 | | ▒ | ▒ | ░ | ▒ | ▒ | ░ | ▒ | ▒ | ░ | | ▒ | ▒ | ▒ |
| Tramadol | 27203-92-5 | 1 | $C_{16}H_{25}NO_2$ | 263,19 | 9,07 | | | | | | | | ▒ | | | | ░ | ░ | |
| Tributyl phosphate | 126-73-8 | 1 | $C_{12}H_{27}O_4P$ | 266,16 | 20,32 | ░ | ▒ | ░ | ▒ | ░ | ▒ | ▒ | ░ | ░ | ░ | ░ | ▒ | | ░ |
| Triphenylphosphine oxide | 791-28-6 | 1 | $C_{18}H_{15}OP$ | 278,09 | 15,35 | | | ▒ | | ░ | ▓ | ▓ | ▓ | ▓ | ▓ | ▒ | ░ | ░ | ▒ |
| Tris(2-chloroethyl) phosphate | 115-96-8 | 1 | $C_6H_{12}Cl_3O_4P$ | 283,95 | 14,30 | | ░ | ░ | | ▒ | | | | | | | | | |
| Valsartan | 137862-53-4 | 1 | $C_{24}H_{29}N_5O_3$ | 435,23 | 16,54 | | | | | | ░ | | ░ | | | | ▓ | ▓ | ▒ |
| Venlafaxine | 93413-69-5 | 1 | $C_{17}H_{27}NO_2$ | 277,20 | 10,54 | | | | | | | | ░ | ░ | | | | ▒ | |

*Figure 47 Heat map intensity of compounds (21) with level 1 of identification confidence (confirmed structure) identified from samples in the positive ionization mode.*



| Name | CAS-nr. | Schymanski level | Bruto Formula | Molecular Weight | RT [min] |
|---|---|---|---|---|---|
| 2,4-Dinitrophenol | 51-28-5 | 1 | C6 H4 N2 O5 | 184,01178 | 13,26 |
| Acesulfame | 33665-90-6 | 1 | C4 H5 N O4 S | 162,99394 | 3,66 |
| Cyclamic acid | 100-88-9 | 1 | C6 H13 N O3 S | 179,06161 | 6,27 |
| Paracetamol | 103-90-2 | 1 | C8 H9 N O2 | 151,06326 | 4,66 |
| Saccharin | 81-07-2 | 1 | C7 H5 N O3 S | 182,99897 | 5,82 |
| Valsartan | 137862-53-4 | 1 | C24 H29 N5 O3 | 435,22684 | 16,55 |
| (15Z)-9,12,13-Trihydroxy-15-octadecenoic acid | - | 2 | C18 H34 O5 | 330,24048 | 14,05 |
| 1-(Carboxymethyl)cyclohexanecarboxylic acid | 67950-95-2 | 2 | C9 H14 O4 | 186,08908 | 8,53 |
| 1,3,7-Trimethyluric acid | 5415-44-1 | 2 | C8 H10 N4 O3 | 210,07520 | 5,93 |
| 1,4-Cyclohexanedicarboxylic acid | 1076-97-7 | 2 | C8 H12 O4 | 172,07353 | 7,95 |
| 2-(Acetylamino)hexanoic acid | 15891-49-3 | 2 | C8 H15 N O3 | 173,10511 | 8,65 |
| 2-Benzothiazolesulfonic acid * | 941-57-1 | 2 | C7 H5 N O3 S2 | 214,97094 | 8,49 |
| 2-Hydroxyhippuric acid | 487-54-7 | 2 | C9 H9 N O4 | 195,05307 | 9,22 |
| 2-Isopropylmalic acid | 3237-44-3 | 2 | C7 H12 O5 | 176,06845 | 6,63 |
| 2-Naphthalenesulfonic acid * | 120-18-3 | 2 | C10 H8 O3 S | 208,01926 | 9,58 |
| 3',5,7-Trihydroxy-4'-methoxyflavanone | 520-33-2 | 2 | C16 H14 O6 | 302,07906 | 13,86 |
| 3-Hydroxysebacic acid | 68812-93-1 | 2 | C10 H18 O5 | 218,11527 | 8,80 |
| 3-Indoxyl sulphate | 487-94-5 | 2 | C8 H7 N O4 S | 213,00955 | 7,96 |
| 3-Phenyllactic acid | 828-01-3 | 2 | C9 H10 O3 | 166,06297 | 9,37 |
| 3-tert-Butyladipic acid | 10347-88-3 | 2 | C10 H18 O4 | 202,12042 | 12,70 |
| 6,7-Dihydroxycoumarin | 305-01-1 | 2 | C9 H6 O4 | 178,02652 | 7,52 |
| 6-Chloro-5-methyl-1H-1,2,3-benzotriazole | 221343-71-1 | 2 | C7 H6 Cl N3 | 167,02495 | 13,06 |
| Ala-Ile | 29727-65-9 | 2 | C9 H18 N2 O3 | 202,13180 | 2,76 |
| Ala-Phe | 3061-90-3 | 2 | C12 H16 N2 O3 | 236,11602 | 5,80 |
| Citraconic acid | 498-23-7 | 2 | C5 H6 O4 | 130,02662 | 2,54 |
| Daidzein | 486-66-8 | 2 | C15 H10 O4 | 254,05788 | 11,72 |
| gamma-Glutamylmethionine | 17663-87-5 | 2 | C10 H18 N2 O5 S | 278,09355 | 2,64 |
| Gentisic acid | 490-79-9 | 2 | C7 H6 O4 | 154,02661 | 7,82 |
| Indole-3-acetyl-L-glutamic acid | - | 2 | C15 H16 N2 O5 | 304,10587 | 9,29 |
| Indole-3-carboxylic acid | 771-50-6 | 2 | C9 H7 N O2 | 161,04757 | 10,42 |
| Levulinic acid | 123-76-2 | 2 | C5 H8 O3 | 116,04732 | 2,87 |
| Mycophenolic acid | 24280-93-1 | 2 | C17 H20 O6 | 320,12601 | 15,25 |
| Phenylbenzimidazole sulfonic acid | 27503-81-7 | 2 | C13 H10 N2 O3 S | 274,04109 | 6,41 |
| Suberic acid * | 505-48-6 | 2 | C8 H14 O4 | 174,08914 | 9,19 |
| Sulfocholic acid | - | 2 | C24 H40 O8 S | 488,24429 | 12,72 |
| Theophylline | 58-55-9 | 2 | C7 H8 N4 O2 | 180,06468 | 5,37 |
| Tryptophan isomer | 73-22-3 | 2 | C11 H12 N2 O2 | 204,08984 | 5,78 |
| α-Aspartylphenylalanine | 13433-09-5 | 2 | C13 H16 N2 O5 | 280,10598 | 6,05 |

*Figure 48 Heat map intensity of compounds with level 1 (6) and 2 (33) of identification confidence from samples in the negative ionization mode.*

# 8 Conclusions

Presence of fat, oil and grease (FOG) in wastewater can cause accumulation in sewers. Often collars of hardened fats will accumulate in tanks and manholes. In severe circumstances this can lead to plugging and damage of the sewer conduits.
Some companies have developed products that can release microorganisms of which they claim that they can break down FOG in the sewer, and in that way reducing the problems caused by accumulated FOG. Consequently, the sewer maintenance costs can be reduced. However it is unclear, how effective these products are for reducing FOG accumulation in sewers, what the effect on the wastewater quality is in terms of COD, N and P levels, how the change in water quality and the presence of the released microorganisms can impact the operation of the activated sludge system at the sewage plant, and whether breakdown chemical products can be formed or organic micropollutants can be released.

The scope of this project was to determine wheter possible negative side effects of the use of a FOG bioremediation product, Microcat®-BioPoP, in the quality of the wastewater and the performance of the biological wastewater treatment process might be expected. The actual functioning of the Microcat®-BioPoP on the breakdown of accumulated FOG in the sewer was not part of this project.

The subresearch-questions formulated on the project were:

- What is the effect on the wastewater matrix? (chapter 4, 5 and 6)
- What is the consequence of these effects on the performance (COD, N, P removal) of the activated sludge process at the sewage treatment plant? (chapter 7.1)
- What is the consequence for the digestion process? (chapter 7.2)
- What is the effect on the release of organic micropollutants (chapter 7.3)

To find answers, two types of experiments were conducted: beaker (jar) experiments (batch) on the wastewater matrix and bioreactors experiments (continuous) to assess the effect on the activated sludge process. Experiments with and without Microcat®-BioPoP were conducted in both setups. Finally, samples were taken in both systems for analysis with non-target screening, and suspect-target screening techniques to find any organic micropollutants that could be released by the FOG decomposition.

The main conclusions drawn from this study are presented as follows:

- The effects of the Microcat®-BioPoP on the wastewater matrix were mainly an increase of the COD and total phosphate by the release of its components, and increased the N-$NH_4$ depletion/removal most likely due to microbial uptake. The addition of Microcat®-BioPoP to the wastewater led to an increase in total and soluble COD. When artificial FOG was added, the soluble COD concentration remained rather constant around 1200 mg.$L^{-1}$, while the total COD increased to a maximum of 4500 mg.$L^{-1}$. Moreover, after the addition of Microcat®-BioPoP, the N-$NH_4$ was almost depleted after 72 hours which was attributed to microbial growth/N-uptake, leading to higher $NO_3$ and $NO_2$ concentrations when compared to the case in absence of Microcat®-BioPoP . The end concentration of soluble P-$PO_4$ (orthophosphate) was lower due to Microcat®-BioPoP addition, while the final concentration of total P-$PO_4$ was higher. An average 10% of total phosphate seemed to be released by the Microcat®-BioPoP product matrix.

- The addition of Microcat®-BioPoP to the wastewater was reflected as a negative effect on the total COD and P-$PO_4$ removal/degradation on time. On the contrary, a substantially positive effect in the N-$NH_4$ removal was observed with FOG collected from the field. A positive effect of about 25% and 31% on the removal of soluble COD was observed in the experiments with municipal and industrial FOG. However, these side effects will considerably decrease in real application since a high dilution of the Microcat®-BioPoP content through the operation of the sewer system is expected. The use of municipal, industrial and combined (industrial/municipal) solid FOG showed clear differences in the effects of the Microcat®-BioPoP, mainly due to the solubilization of COD and possibly nitrogen like compounds (proteins) contained in the different solid FOG matrix, as well as the microorganisms already present in the solid FOG. Municipal and combined FOG showed higher content of COD compared to the industrial FOG, but the final total and soluble COD were similar for combined and municipal FOG when Microcat®-BioPoP was added.

- The effects of using Microcat®-BioPoP on the performance of the activated sludge process at a typical municipal wastewater treatment plant by emulating the process with a bioreactor were overall minimal. A marginally lower soluble COD removal was observed in the activated sludge process with Microcat®-BioPoP in the feeding tank, with a difference of about 11% when compared to the one without Microcat®-BioPoP. Moreover, N-$NH_4$ and N- removal efficiency by assimilation and denitrification in the reactor with Microcat®-BioPoP was about 52% and 32% higher than the reactor without, confirming the observations made previously with the beaker (jar) experiments of high N-$NH_4$ uptake.

- No side effects such as $H_2S$ production or foam formation were observed neither in batch tests nor in the experiments in bioreactors with the activated sludge process.

- The anaerobic tests showed no substantial (<10%) differences on the methane yield of activated sludge digestion between the reactors with and without Microcat®-BioPoP in the feeding tank. Even though the FOG conversion in the feeding tank was not evaluated, the concentrations of FOG determined in the influent on the reactors, and visual observation indicated that the addition of Microcat®-BioPoP promoted the FOG disintegration from its solid form.

- Non-target screening results showed that a limited amount of compounds were formed with the artificial FOG and Microcat®-BioPoP, with relatively low concentrations, when compared with the internal standard concentrations of the 20 highest detected compounds in sewage treatment plants influent.

- Chemical identification methods suggested dissimilarities between the non-target features in the wastewater samples with and without Microcat®-BioPoP as indication of biodegradation of FOG from the field, but no main differences with the targeted compounds identified on levels 1 and 2 (the highest levels of identification). Although suspect and non-target analysis has grown rapidly, they are still uncommon for most environmental monitoring agencies and environmental scientists. The techniques still represent a challenge because their inability to provide quantitative information about identified compounds.

Therefore, the consequences of the overall observed effects at the municipal wastewater treatment plant are not expected to hamper the performance of the activated sludge process. Based on the laboratory experiments, it can be concluded that the addition of Microcat®-BioPoP may not produce any adverse side effects on the conventional municipal treatment process or in its effluent quality. Under high concentrations of Microcat®-BioPoP the treatment process outcome did not result on substantial differences, and therefore under diluted conditions such as in practice, they are not expected. However, it must be pointed out that this study did not specifically look at the actual Microcat®-BioPoP capability to degrade fat, oil, and grease, or its microbial conversion capacity for lipids, and therefore no conclusions on this matter were provided. KWR does not have any liase with the Microcat®-BioPoP producer and providers, neither is endorsing the bioproduct or its use. The results and conclusions presented in this report are completely independent from any commercial purpose.

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# I Appendix: Interviews

## Contactpersonen

Tabel 1 Contactpersonen

| Locatie | Gemeente | Waterschap |
|---|---|---|
| Zoetermeer | Hans van Denderen | Paul Weij<br>Klaas Appeldoorn |
| Almaar | Arie Swets | Maaike Hoekstra |

## Vragenlijst

Algemeen

- Bent u bekend met biopop? En via welke route kent u het?
- Wat weet u van biopop?
- Wat is voor u de reden dat u voor of tegen de toepassing van biopop bent?

Gemeenten:

- Wat is voor u de hoofdreden om de biopop toe te passen?
- Wat zijn uw ervaringen m.b.t. het toepassen van biopop?
    - Zijn er opvallende positieve trends zichtbaar?
    - Zijn er opvallende negatieve trends zichtbaar?
- Heeft u één van de volgende waarnemingen gedaan na toepassing van de biopop?
    - Verandering in geur/stankoverlast?
    - Verandering in corrosie problematiek?
    - Verandering in continuering van bedrijfsprocessen?
    - Verandering in aantal verstoppingen/benodigde reiniging?

  - Verandering in klachten bewoners (aantal en aard van klacht)?
- Welke mogelijke gevolgen van de biopop toepassing voorziet u voor het riool?
  - En van welke acht u de kans het grootst? En waarom?
- Heeft u zich ook verdiept in de juridische kant van de toepassing? Wat kwam daar naar voren?
- Zijn er nog andere issues in rioolsystemen die niet aangepakt worden met biopop?

Waterzuiveringstechnoloog

- Wat zijn uw ervaringen m.b.t. vet voor de bedrijfsvoering van de RWZI?
- Wat zijn uw ervaringen m.b.t. het toepassen van biopop?
  - Zijn er opvallende positieve trends zichtbaar?
  - Zijn er opvallende negatieve trends zichtbaar?
- Heeft u één van de volgende waarnemingen gedaan na toepassing van de biopop?
  - Verandering in geur/stankoverlast?
  - Verandering in corrosie problematiek?
  - Verandering in continuering van bedrijfsprocessen?
- Welke mogelijke gevolgen van biopop toepassing voorziet u voor de afvalwaterzuivering?
  - En van welke acht u de kans het grootst? En waarom?
- Heeft u zich ook verdiept in de juridische kant van de toepassing? Wat kwam daaruit naar voren?

# II Appendix: Samenvatting interviews

### a. Arie Swets van Stadswerk

Contact: 5-1-2018 en aanvulling per email op 15-1-2018

In 2016 heeft Macero contact opgenomen met de gemeente Alkmaar over de mogelijkheid om biopop in te zetten om vetophoping in riolen te voorkomen. De gemeente is hier op ingegaan, omdat ze 1) de overlast voor burgers als gevolg van het aantal verstoppingen wilden verminderen en 2) innovatieve ontwikkelingen graag een kans willen geven. Er is besloten dit toe te gaan passen op locaties die gevoelig zijn voor verstoppingen (zoals zinkers en verzakte riolen). Momenteel gaat het om een tiental locaties; dit jaar wordt dit uitgebreid met drie extra locaties. Het algehele gevoel/gedachte is dat er minder verstoringen zijn als gevolg van ophopingen, maar dit kan helaas niet bevestigd worden door concrete getallen. Stadswerk072 geeft aan dat de gemeente vooral op korte termijn oplossingen moet bieden bij verstoppingen, en dat verstoppingen en registraties daarvan niet plaatsvinden. Eén collega van stadswerk072 gaf aan dat op een drietal locaties het maandelijks nodig was om het riool te ontstoppen, en dat er sinds de plaatsing van de biopop geen overlast meer was (en er dus geen maatregelen genomen hoefden te worden) in het afgelopen jaar (persoonlijke ervaring). Het kon niet geverifieerd worden of een dergelijk overlast vrij jaar al eerder op deze locatie heeft plaatsgevonden (om weersomstandigheden, of lozingsgedrag gebruiker uit te sluiten), evenals of op vergelijkbare locaties zonder toepassing van biopop ditzelfde verschijnsel plaatsvond (aanvulling per email 15-1-2018).

De volgende waarnemingen zijn gedaan op basis van gevoel, niet te onderbouwen met getallen: minder stankhinder (inspecteur op locatie), minder ontstoppingen en minder klachten. Effect op corrosie of continuering van bedrijfsprocessen is niet inzichtelijk. Stadswerk072 verwacht geen negatieve effecten in het riool als gevolg van de toepassing van biopop.

De kosten voor de toepassing van biopop blijft een aandachtspunt. Het blijft een afweging tussen het toepassen van biopop en de kosten voor het reinigen van het riool en de overlast van de burger bij een calamiteit. Ook in de toekomst is dit moeilijk in kaart te brengen, omdat de overlast van de burger lastig in geld is uit te drukken. Recentelijk is stadswerk072 begonnen met registratie van verstoppingen.

### b. Hans van Denderen

Contact: 19-3-2018 en aanvulling per email op 5-4-2018

Het is belangrijk om te realiseren dat in Zoetermeer het vuilwater en regenwater apart wordt afgevoerd. Het vuilwater wordt daardoor afgevoerd door middel van een rioolstelsel met een kleine diameter. Het is daardoor gevoeliger voor verstoppingen met vet. Om deze reden wordt er preventief elke vier jaar alle vuilwaterleidingen doorgespoeld.

Verder vind er additionele schoonmaak plaats wanneer er klachten gemeld worden. Er wordt frequenter schoon gemaakt als er meer klachten zijn en vice versa. Voor de toepassing van Microcat®-BioPoP stonden 28 locaties in bestek. Sinds de toepassing van Microcat®-BioPoP zijn er nauwelijks klachten binnengekomen, waardoor er minder ingegrepen hoeft te worden. Jaarkosten voor schoonmaak en inspectie laten zien dat er een kostenbesparing van 20-25% is gerealiseerd.

Door de beheerder wordt aangevuld dat er sinds de toepassing van Microcat®-BioPoP minder stankoverlast is rondom de putdeksel en er nu veel minder schollen met vet uit het riool gehaald worden. Bovendien zijn er geen negatieve effecten gezien van rioolputten die nu relatief schoon zijn. Er is geen verandering met betrekking tot corrosie in het riool waargenomen.

### c. Paul Weij

Contact: 2 januari 2018

Het principe achter biopop wordt kort toegelicht aan Paul, werkzaam bij Delfluent Services BV(uitvoerder op RWZI Harnaschpolder waar het afvalwater van Zoetermeer gezuiverd wordt). Zij zijn bekend met vergelijkbare systemen waarbij nevel/sproeiers worden ingezet om de vetlaag in de voorbezinktank te reduceren. Men past dergelijke systemen niet toe, omdat de kosten niet opwogen tegen de baten.

Het afvalwater van Zoetermeer komt via het Oostergemaal binnen bij RWZI Harnaschpolder, en is verantwoordelijk voor 20% van het influent dat op deze RWZI behandeld wordt. Er zijn geen verschillen in bedrijfsvoering van de RWZI sinds de toepassing van biopop in Zoetermeer.

Paul verwacht dat wanneer vetophopingen niet meer in het riool worden verwijderd, er dus meer vet aankomt op de RWZI. Dit zal via de skimmer worden afgevangen en naar de vergisting worden gestuurd. Er is dus een mogelijk effect van de toepassing van biopop op de vergisting. Wel hangt de kans of dit schadelijk is af van de manier waarop het vet vrij komt; als pulse (bij inzet op vetophoping), of geleidelijk (bij onderhoud van vetophopingsgevoelige locatie). Praktijktesten moeten dit uitwijzen, maar als de C:N:P ratio klopt worden er geen problemen verwacht.

### d. Maaike Hoekstra

Contact: 25-1-2018

Er zijn nog vervolgvragen gestuurd (een reminder op 26-2-2018). Daarna zal ik hier een samenvatting van de resultaten opnemen. In het zeer kort: bevestigd beeld dat Mark van Loosdrecht en Willy Verstraete beschrijven.

### e. Prof. Mark van Loosdrecht TU Delft / KWR

Contact: 9-12-2017 (email)

Het afvalwater bevat van nature al bacteriën die lipases produceren; vandaar dat vooral verwacht wordt dat er een fysisch limiet is aan de vetverwijdering in riolen in plaats van een biologisch limiet. Toch kan niet uitgesloten worden dat er een positief effect in het riool is. Door de hoge activiteit van de bacteriën in een RWZI zal daar geen positief effect zijn.

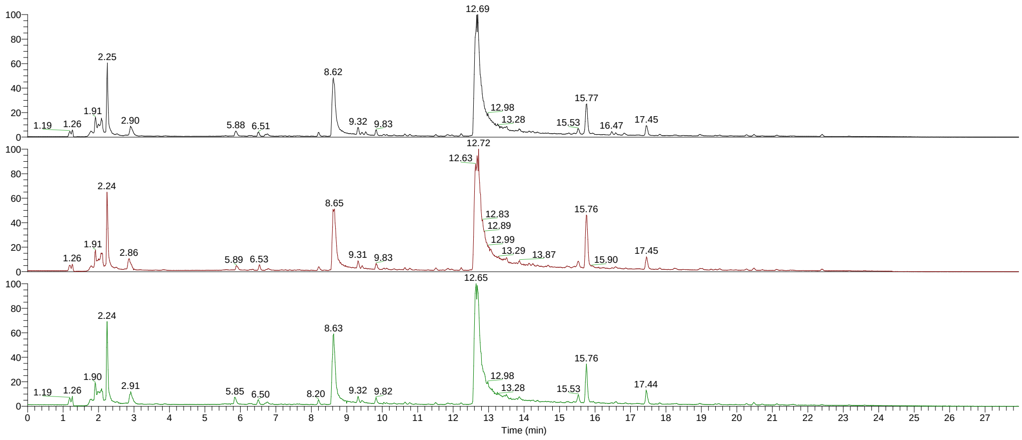
### f. Prof. Willy Verstraete Universiteit Ghent / KWR

Contact: 8-12-2017

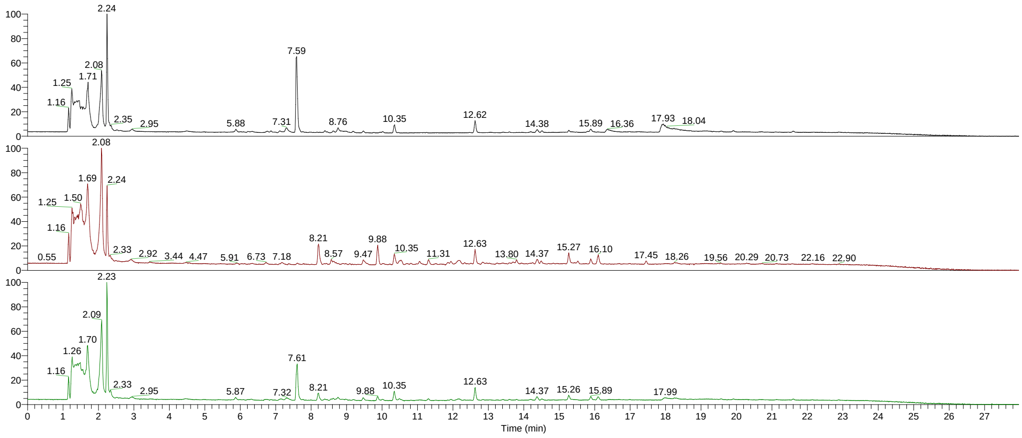
Willy Verstraete verwacht geen negatieve invloed van een biologische activiteit van biopop op het afvalwater. Sterker nog, als het vet in oplossing gebracht wordt, kan het zelfs de bedrijfsvoering continueren. Bij een fysisch effect (met een solvent) kan het vet in een pulse vrijkomen, dit zou problemen kunnen geven op de RWZI. Echter, gezien aangegeven wordt dat biopop een biologische werking heeft, wordt een langzame(re) omzetting verwacht. In dit geval zal er geen sprake zijn van piekbelasting op de RWZI: en dus niet resulteren in calamiteiten op de RWZI.

Als er sprake zou kunnen zijn van een negatief effect op de RWZI door toepassing van biopop wordt deze als eerste verwacht op het anaerobe slib (wel een kleine kans). Zowel, omdat een toenemende hoeveelheid vet deze kant zal opgaan, als de mogelijkheid dat vet chlorerende verbindingen ophopen.

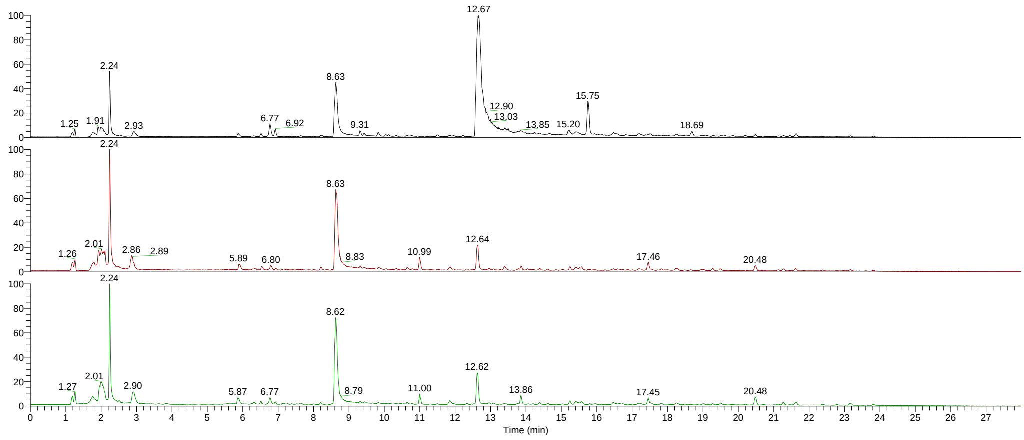
# III Appendix: Non-target screening (NTS) chromatograms(triplicates) in the positive mode



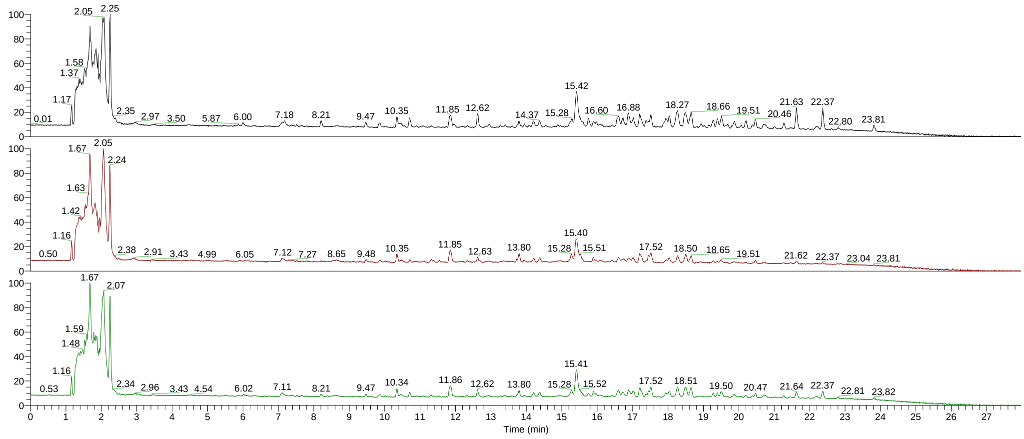
EXPERIMENT 1 (DEGRADED SYNTHETIC WASTEWATER WITH MICROCAT®-BIOPOP ).



EXPERIMENT 2 (DEGRADED SYNTHETIC WASTEWATER).



### EXPERIMENT 3 (DEGRADED SYNTHETIC WASTEWATER WITH MICROCAT®-BIOPOP AND FOG)



### EXPERIMENT 4 (DEGRADED SYNTHETIC WASTEWATER WITH FOG).

# IV Appendix: Memo gemeente Oss

| | |
|---|---|
| Aan | **TKI projectteam oplossen van vet in riolering** |
| Betreft | **Vetten in riolering/monstername vet en afvalwater 3 locaties in Oss** |
| Van | Ronnie Hurkens |
| Datum | 22 november 2018 |

GEMEENTE OSS

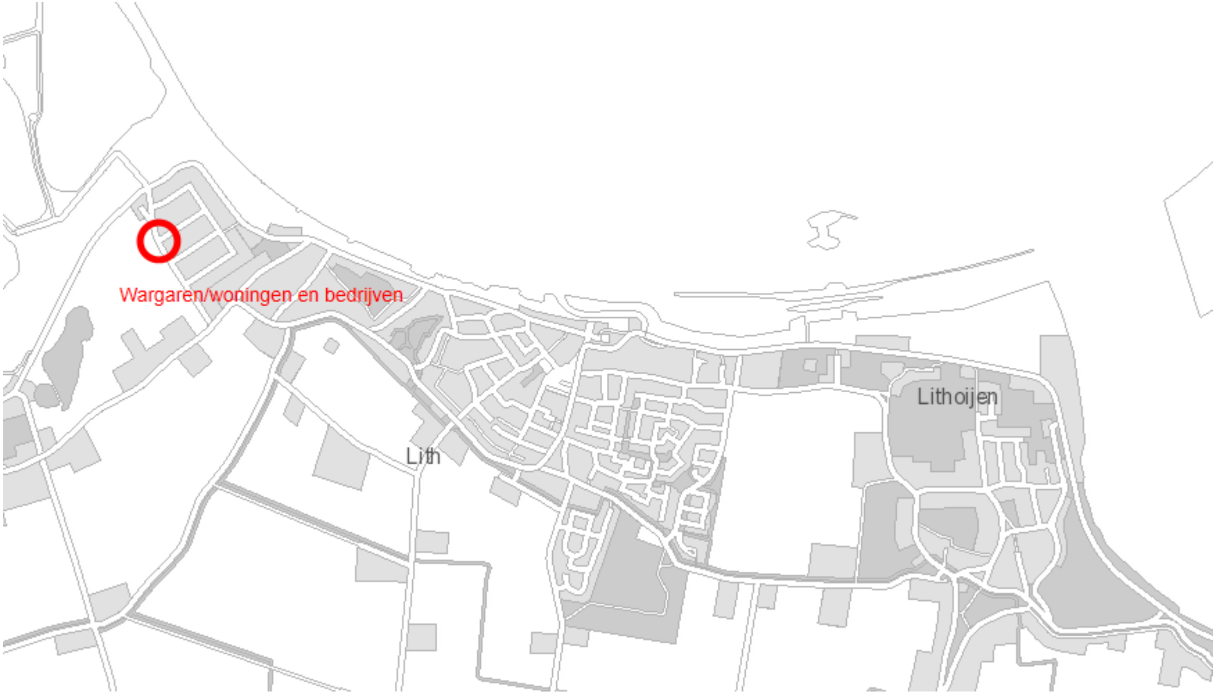
## Algemeen

Tijdens het overleg van 12 november jl. is afgesproken om het gebruik van de Microcat®-BioPoP met echt rioolvet en afvalwater te testen. Het vet en afvalwater zal uit 3 gemalen met verschillende aansluitingen worden gehaald.
1 locatie (gemaal) waar alleen woningen op aangesloten zijn;
2 locatie (gemaal) waar alleen bedrijven (industrie) op aangesloten zijn;
3 locatie (gemaal) waar woningen en bedrijven op aangesloten zijn.
Het type stelsel (gemengd of gescheiden) is niet van belang omdat er geen verschil is in hoeveelheid vet dat wordt aangetroffen.

Locatie Harnas en De Geer Oss

Gemaal Harnas betreft een gemaal waar alleen woningen op lozen.
Gemaal De Geer betreft een gemaal waar alleen bedrijven op lozen.

Locatie Wargaren Lith



Gemaal Wargaren betreft een gemaal waar bedrijven en woningen op lozen.

Op 22 november jl. is op drie locaties een monster van vet en afvalwater uit de gemalen genomen. Dit is gedaan in samenwerking met een medewerker van Macero.
De zes monsters zijn die middag naar het KWR in Nieuwegein gebracht om verdere testen mee uit te voeren.

Hieronder ter verduidelijking enkele situatiefoto’s van de gemalen tijdens de monstername.
Harnas (woningen):

De Geer (bedrijven):

Wargaren (woningen en bedrijven):

# V Appendix: Absolute COD removed in the activated sludge process

## V.I Absolute COD removed with Microcat®-BioPoP

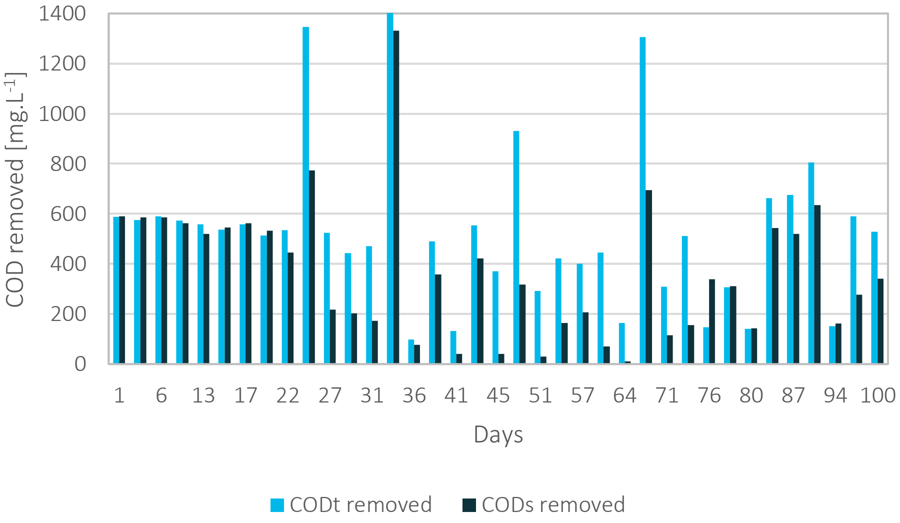


*Figure 49 Total and soluble COD removed of activated sludge system with Microcat®-BioPoP in the feeding tank.*

## V.II Absolute COD removed without Microcat®-BioPoP

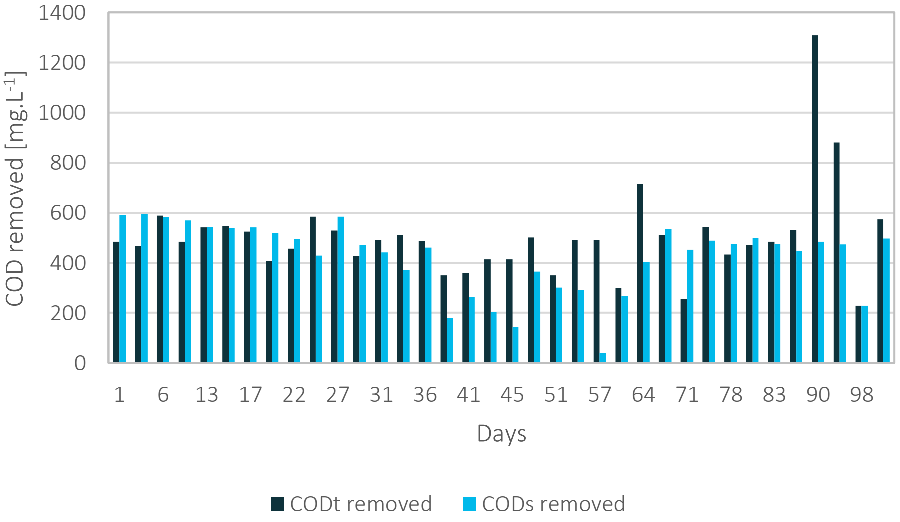


*Figure 50 Total and soluble COD removed of activated sludge system without Microcat®-BioPoP in the feeding tank.*

# VI Appendix: Non target screening features

*Positive ionization mode*

The area of detectable chemicals or molecular features is related to the concentration of a compound in the samples. However, mass spectrometry is not a quantitative technique as ionization efficiencies of compounds vary strongly. All samples analysis data were processed together, after the data curation procedure (see below). The features in the positive ionization mode presented a molecular weight [Da] ranging from 80 Da to 2000 Da, and the highest density distribution was found below the retention time of 15 min (Figure 51 A).

The retention time (RT) of a compound in reverse phase (RP) chromatography using a C18 column corresponds to the polarity/hydrophobicity of the compound. More polar compounds elute earlier, and hydrophobic compounds later. Compounds that elute before 2.4 minutes are considered to not interact with the C18 column, and therefore they flow through the system (Figure 51 B). Therefore, all the features with a retention time less than 2.4 minutes were discarded (Figure 51 C). A total of 34145 features from the positive ionization were identified without data cut-off. After removing the features with a retention time of less than 2.4 min, 31171 features remained. By further removing features that did not exceed 10 times the blank area, the total of features could be reduced to 27377. Similarly, after removing features that did not exceed 10 times the synthetic wastewater (control), a total of 27174 features could be removed leaving 203 features with positive ionization found in the synthetic wastewater.

*Negative ionization mode*

The molecular weight of the features in the negative ionization mode was in the range of 80 to 1750 Da, but most of them were below 500 Da (Figure 52), and a density distributed mainly between 5- 20 min RT.

The total features of the negative ionization data was 9862. Compared to the positive ionization this is about 3.5 times less. After applying a cut-off of 2.4 min RT the number of features reduced to 9002. A total of 6641 features were left after removing features that did not exceed 10 times the blank samples. Finally, a total of 4706 features could be removed that did not exceed 10 times the synthetic wastewater (control), which indicated only 27 features with negative ionization were present in the synthetic wastewater.

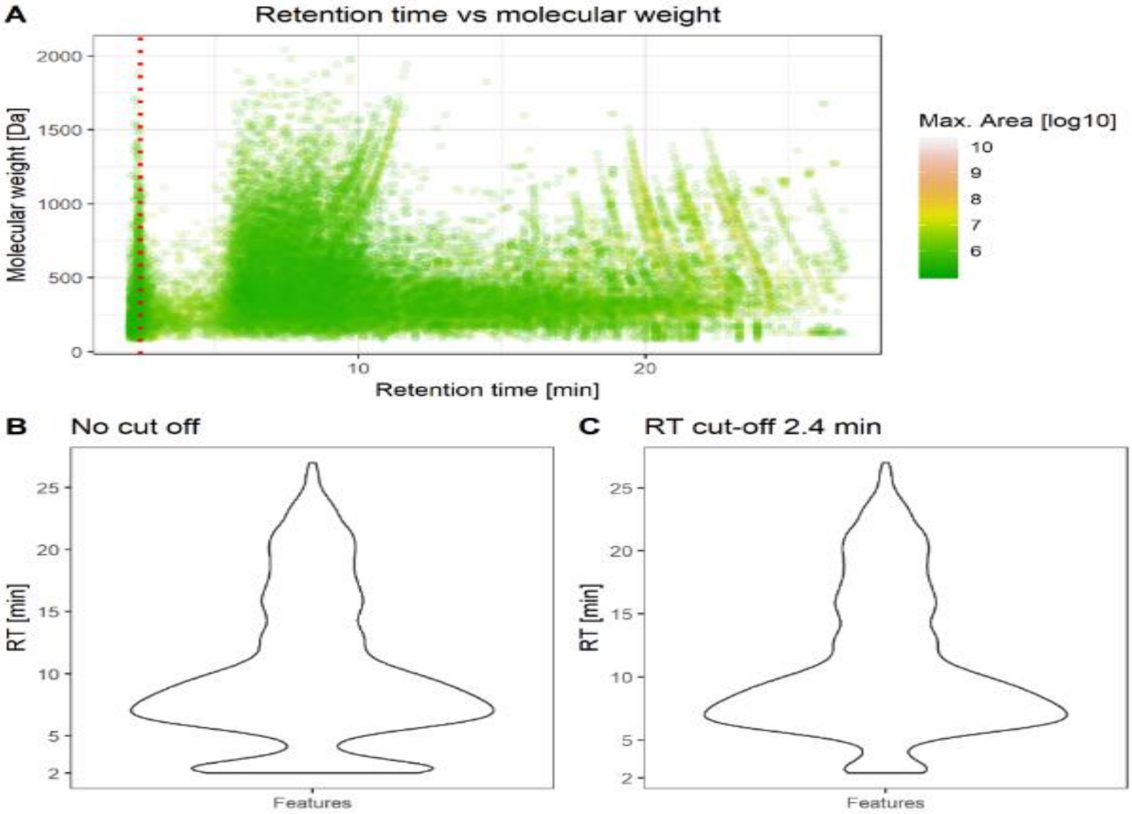


*Figure 51 Retention time vs Molecular weight and maximum area (A) in the positive ionization mode. Features distribution without a cut-off (B) and with 2.5 min as cut-off (C).*

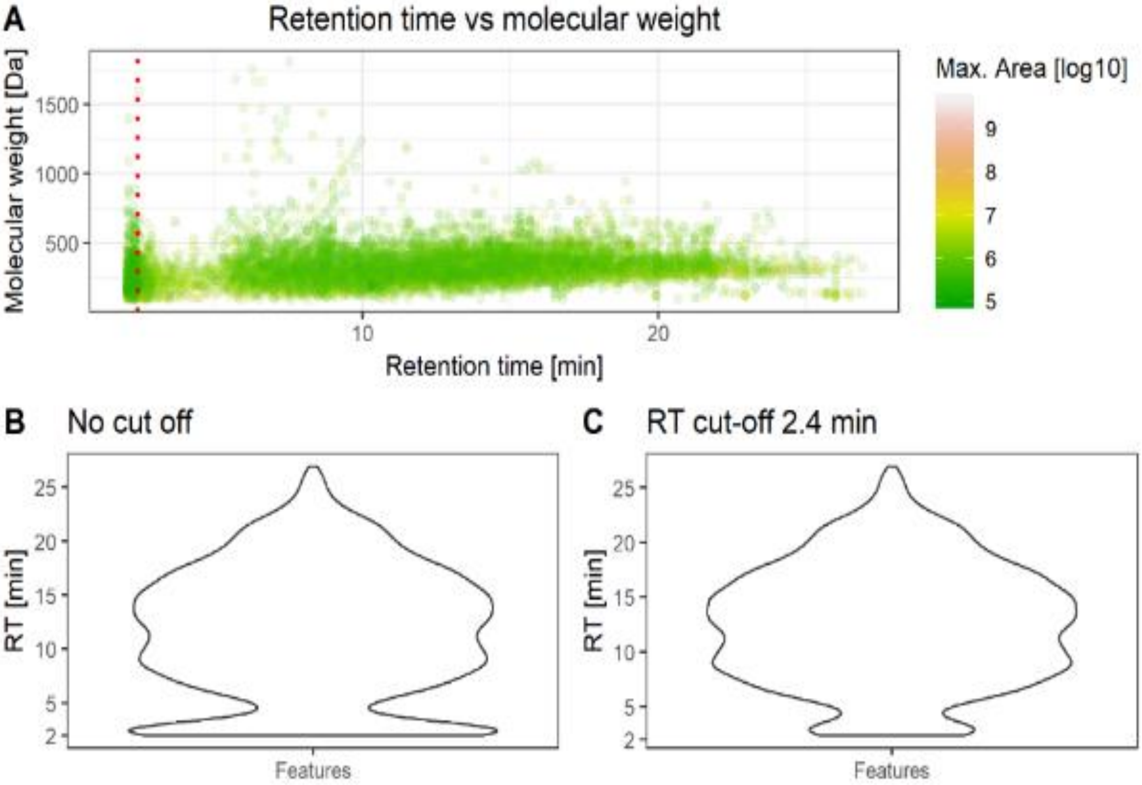


*Figure 52 Retention time vs Molecular weight and maximum area (A) in the negative ionization mode. Features distribution without a cut-off (B) and with 2.5 min as cut-off (C).*

# VII Appendix: Log2 fold change analysis

From the analysis in both positive (Figure 53 A) and negative (Figure 53 B) ionization modes, the five features that increase the most (highest log2 fold change) in the batch samples with Microcat®-BioPoP compared to without are highlighted in red. Similarly, the five features that decrease the most (lowest log2 fold change) in the samples with Microcat®-BioPoP were highlighted in blue, corresponding to the scale of intensity. These features that increase/decrease the most might be a good starting point for further identification.

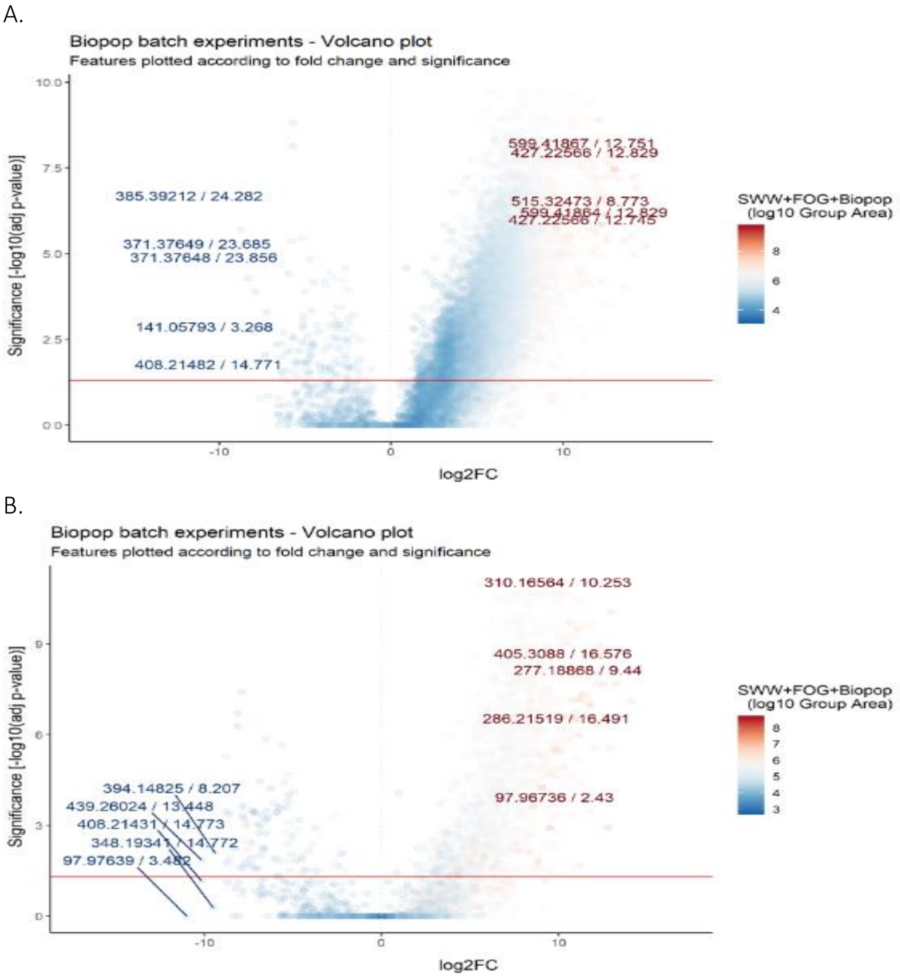


*Figure 53 Volcano plots of log2 fold change analysis with samples with and without Microcat®-BioPoP . A. Positive ionization mode. B. Negative ionization mode.*

# VIII Appendix: Suspect target screening identification

The suspect-target screening was carried out with all the samples and the different suspects' list presented in the methods section. The results in the positive ionization mode after applying a cut-off of peak area intensity (threshold > 2x$10^5$) showed 21 compounds with level 1 of the Schymanski matrix (see Figure 54), i.e., confirmed structure.

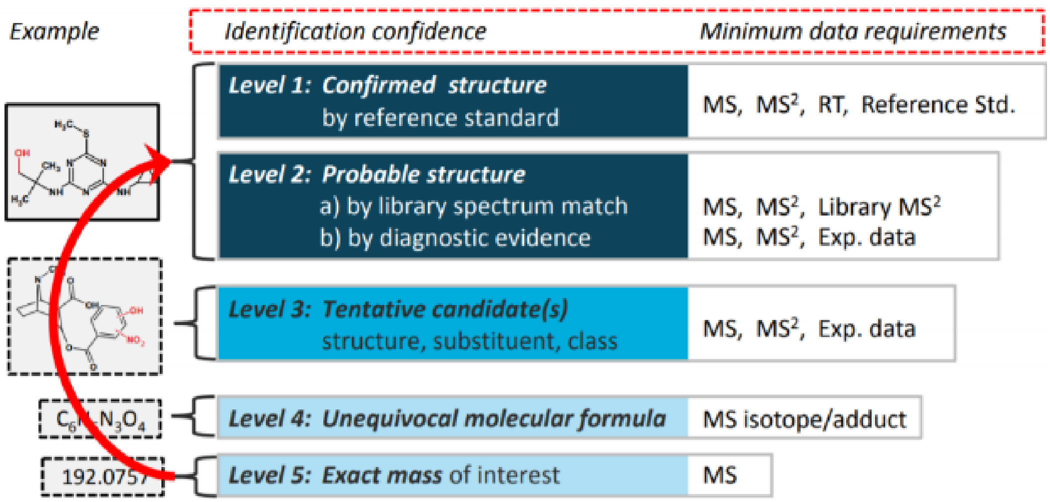
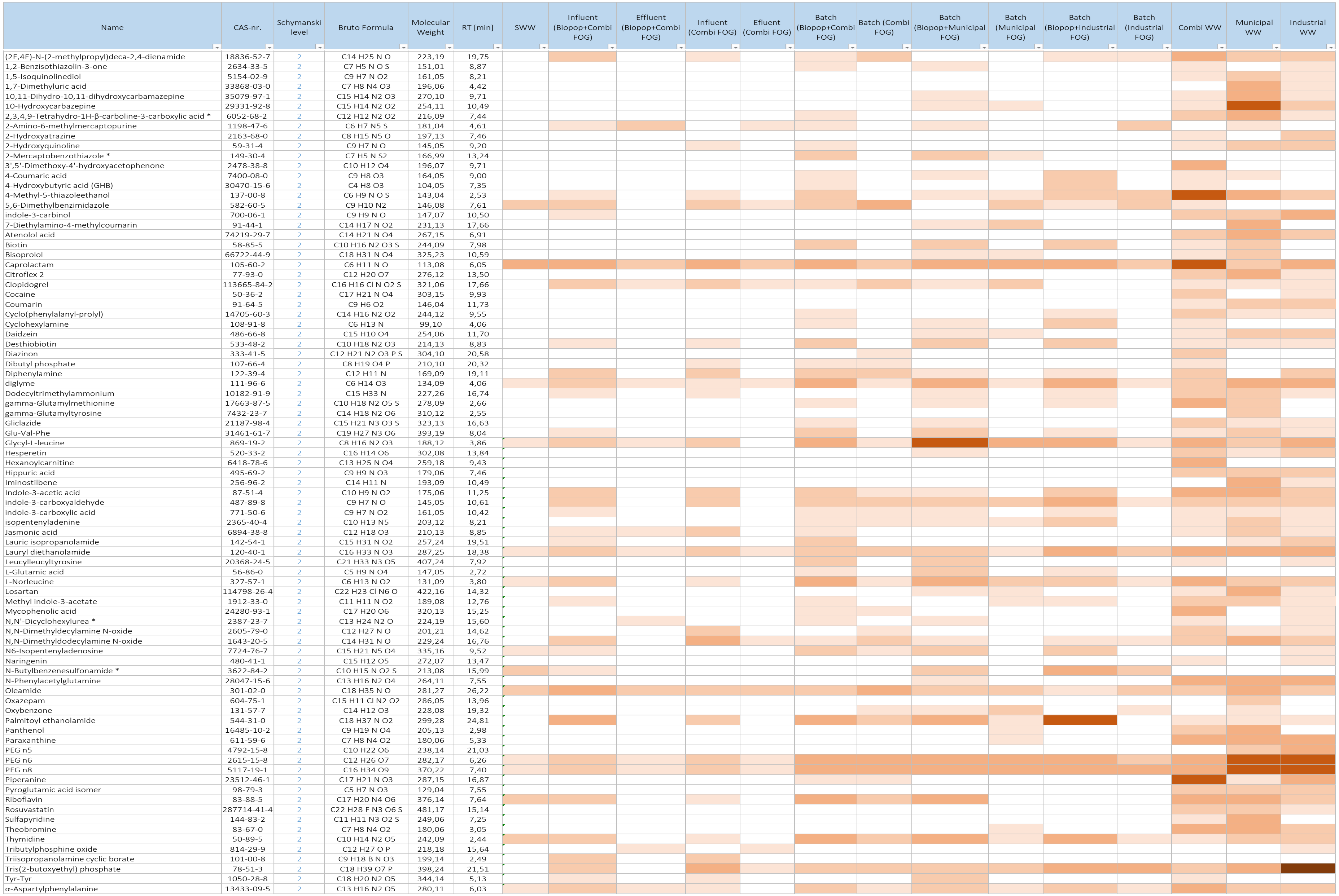


*Figure 54 Levels of identification confidence based on the Schymanski matrix (Schymanski et al. 2014a).*



| Name | CAS-nr. | Schymanski level | Bruto Formula | Molecular Weight | RT [min] |
|---|---|---|---|---|---|
| (2E,4E)-N-(2-methylpropyl)deca-2,4-dienamide | 18836-52-7 | 2 | C14 H25 N O | 223,19 | 19,75 |
| 1,2-Benzisothiazolin-3-one | 2634-33-5 | 2 | C7 H5 N O S | 151,01 | 8,87 |
| 1,5-Isoquinolinediol | 5154-02-9 | 2 | C9 H7 N O2 | 161,05 | 8,21 |
| 1,7-Dimethyluric acid | 33868-03-0 | 2 | C7 H8 N4 O3 | 196,06 | 4,42 |
| 10,11-Dihydro-10,11-dihydroxycarbamazepine | 35079-97-1 | 2 | C15 H14 N2 O3 | 270,10 | 9,71 |
| 10-Hydroxycarbazepine | 29331-92-8 | 2 | C15 H14 N2 O2 | 254,11 | 10,49 |
| 2,3,4,9-Tetrahydro-1H-β-carboline-3-carboxylic acid * | 6052-68-2 | 2 | C12 H12 N2 O2 | 216,09 | 7,44 |
| 2-Amino-6-methylmercaptopurine | 1198-47-6 | 2 | C6 H7 N5 S | 181,04 | 4,61 |
| 2-Hydroxyatrazine | 2163-68-0 | 2 | C8 H15 N5 O | 197,13 | 7,46 |
| 2-Hydroxyquinoline | 59-31-4 | 2 | C9 H7 N O | 145,05 | 9,20 |
| 2-Mercaptobenzothiazole * | 149-30-4 | 2 | C7 H5 N S2 | 166,99 | 13,24 |
| 3',5'-Dimethoxy-4'-hydroxyacetophenone | 2478-38-8 | 2 | C10 H12 O4 | 196,07 | 9,71 |
| 4-Coumaric acid | 7400-08-0 | 2 | C9 H8 O3 | 164,05 | 9,00 |
| 4-Hydroxybutyric acid (GHB) | 30470-15-6 | 2 | C4 H8 O3 | 104,05 | 7,35 |
| 4-Methyl-5-thiazoleethanol | 137-00-8 | 2 | C6 H9 N O S | 143,04 | 2,53 |
| 5,6-Dimethylbenzimidazole | 582-60-5 | 2 | C9 H10 N2 | 146,08 | 7,61 |
| indole-3-carbinol | 700-06-1 | 2 | C9 H9 N O | 147,07 | 10,50 |
| 7-Diethylamino-4-methylcoumarin | 91-44-1 | 2 | C14 H17 N O2 | 231,13 | 17,66 |
| Atenolol acid | 74219-29-7 | 2 | C14 H21 N O4 | 267,15 | 6,91 |
| Biotin | 58-85-5 | 2 | C10 H16 N2 O3 S | 244,09 | 7,98 |
| Bisoprolol | 66722-44-9 | 2 | C18 H31 N O4 | 325,23 | 10,59 |
| Caprolactam | 105-60-2 | 2 | C6 H11 N O | 113,08 | 6,05 |
| Citroflex 2 | 77-93-0 | 2 | C12 H20 O7 | 276,12 | 13,50 |
| Clopidogrel | 113665-84-2 | 2 | C16 H16 Cl N O2 S | 321,06 | 17,66 |
| Cocaine | 50-36-2 | 2 | C17 H21 N O4 | 303,15 | 9,93 |
| Coumarin | 91-64-5 | 2 | C9 H6 O2 | 146,04 | 11,73 |
| Cyclo(phenylalanyl-prolyl) | 14705-60-3 | 2 | C14 H16 N2 O2 | 244,12 | 9,55 |
| Cyclohexylamine | 108-91-8 | 2 | C6 H13 N | 99,10 | 4,06 |
| Daidzein | 486-66-8 | 2 | C15 H10 O4 | 254,06 | 11,70 |
| Desthiobiotin | 533-48-2 | 2 | C10 H18 N2 O3 | 214,13 | 8,83 |
| Diazinon | 333-41-5 | 2 | C12 H21 N2 O3 P S | 304,10 | 20,58 |
| Dibutyl phosphate | 107-66-4 | 2 | C8 H19 O4 P | 210,10 | 20,32 |
| Diphenylamine | 122-39-4 | 2 | C12 H11 N | 169,09 | 19,11 |
| diglyme | 111-96-6 | 2 | C6 H14 O3 | 134,09 | 4,06 |
| Dodecyltrimethylammonium | 10182-91-9 | 2 | C15 H33 N | 227,26 | 16,74 |
| gamma-Glutamylmethionine | 17663-87-5 | 2 | C10 H18 N2 O5 S | 278,09 | 2,66 |
| gamma-Glutamyltyrosine | 7432-23-7 | 2 | C14 H18 N2 O6 | 310,12 | 2,55 |
| Gliclazide | 21187-98-4 | 2 | C15 H21 N3 O3 S | 323,13 | 16,63 |
| Glu-Val-Phe | 31461-61-7 | 2 | C19 H27 N3 O6 | 393,19 | 8,04 |
| Glycyl-L-leucine | 869-19-2 | 2 | C8 H16 N2 O3 | 188,12 | 3,86 |
| Hesperetin | 520-33-2 | 2 | C16 H14 O6 | 302,08 | 13,84 |
| Hexanoylcarnitine | 6418-78-6 | 2 | C13 H25 N O4 | 259,18 | 9,43 |
| Hippuric acid | 495-69-2 | 2 | C9 H9 N O3 | 179,06 | 7,46 |
| Iminostilbene | 256-96-2 | 2 | C14 H11 N | 193,09 | 10,49 |
| Indole-3-acetic acid | 87-51-4 | 2 | C10 H9 N O2 | 175,06 | 11,25 |
| indole-3-carboxyaldehyde | 487-89-8 | 2 | C9 H7 N O | 145,05 | 10,61 |
| indole-3-carboxylic acid | 771-50-6 | 2 | C9 H7 N O2 | 161,05 | 10,42 |
| isopentenyladenine | 2365-40-4 | 2 | C10 H13 N5 | 203,12 | 8,21 |
| Jasmonic acid | 6894-38-8 | 2 | C12 H18 O3 | 210,13 | 8,85 |
| Lauric isopropanolamide | 142-54-1 | 2 | C15 H31 N O2 | 257,24 | 19,51 |
| Lauryl diethanolamide | 120-40-1 | 2 | C16 H33 N O3 | 287,25 | 18,38 |
| Leucylleucyltyrosine | 20368-24-5 | 2 | C21 H33 N3 O5 | 407,24 | 7,92 |
| L-Glutamic acid | 56-86-0 | 2 | C5 H9 N O4 | 147,05 | 2,72 |
| L-Norleucine | 327-57-1 | 2 | C6 H13 N O2 | 131,09 | 3,80 |
| Losartan | 114798-26-4 | 2 | C22 H23 Cl N6 O | 422,16 | 14,32 |
| Methyl indole-3-acetate | 1912-33-0 | 2 | C11 H11 N O2 | 189,08 | 12,76 |
| Mycophenolic acid | 24280-93-1 | 2 | C17 H20 O6 | 320,13 | 15,25 |
| N,N'-Dicyclohexylurea * | 2387-23-7 | 2 | C13 H24 N2 O | 224,19 | 15,60 |
| N,N-Dimethyldecylamine N-oxide | 2605-79-0 | 2 | C12 H27 N O | 201,21 | 14,62 |
| N,N-Dimethyldodecylamine N-oxide | 1643-20-5 | 2 | C14 H31 N O | 229,24 | 16,76 |
| N6-Isopentenyladenosine | 7724-76-7 | 2 | C15 H21 N5 O4 | 335,16 | 9,52 |
| Naringenin | 480-41-1 | 2 | C15 H12 O5 | 272,07 | 13,47 |
| N-Butylbenzenesulfonamide * | 3622-84-2 | 2 | C10 H15 N O2 S | 213,08 | 15,99 |
| N-Phenylacetylglutamine | 28047-15-6 | 2 | C13 H16 N2 O4 | 264,11 | 7,55 |
| Oleamide | 301-02-0 | 2 | C18 H35 N O | 281,27 | 26,22 |
| Oxazepam | 604-75-1 | 2 | C15 H11 Cl N2 O2 | 286,05 | 13,96 |
| Oxybenzone | 131-57-7 | 2 | C14 H12 O3 | 228,08 | 19,32 |
| Palmitoyl ethanolamide | 544-31-0 | 2 | C18 H37 N O2 | 299,28 | 24,81 |
| Panthenol | 16485-10-2 | 2 | C9 H19 N O4 | 205,13 | 2,98 |
| Paraxanthine | 611-59-6 | 2 | C7 H8 N4 O2 | 180,06 | 5,33 |
| PEG n5 | 4792-15-8 | 2 | C10 H22 O6 | 238,14 | 21,03 |
| PEG n6 | 2615-15-8 | 2 | C12 H26 O7 | 282,17 | 6,26 |
| PEG n8 | 5117-19-1 | 2 | C16 H34 O9 | 370,22 | 7,40 |
| Piperanine | 23512-46-1 | 2 | C17 H21 N O3 | 287,15 | 16,87 |
| Pyroglutamic acid isomer | 98-79-3 | 2 | C5 H7 N O3 | 129,04 | 7,55 |
| Riboflavin | 83-88-5 | 2 | C17 H20 N4 O6 | 376,14 | 7,64 |
| Rosuvastatin | 287714-41-4 | 2 | C22 H28 F N3 O6 S | 481,17 | 15,14 |
| Sulfapyridine | 144-83-2 | 2 | C11 H11 N3 O2 S | 249,06 | 7,25 |
| Theobromine | 83-67-0 | 2 | C7 H8 N4 O2 | 180,06 | 3,05 |
| Thymidine | 50-89-5 | 2 | C10 H14 N2 O5 | 242,09 | 2,44 |
| Tributylphosphine oxide | 814-29-9 | 2 | C12 H27 O P | 218,18 | 15,64 |
| Triisopropanolamine cyclic borate | 101-00-8 | 2 | C9 H18 B N O3 | 199,14 | 2,49 |
| Tris(2-butoxyethyl) phosphate | 78-51-3 | 2 | C18 H39 O7 P | 398,24 | 21,51 |
| Tyr-Tyr | 1050-28-8 | 2 | C18 H20 N2 O5 | 344,14 | 5,13 |
| α-Aspartylphenylalanine | 13433-09-5 | 2 | C13 H16 N2 O5 | 280,11 | 6,03 |