# REMOVAL OF MICROPOLLUTANTS WITH LIMITED AERATION ASSISTED ANAEROBIC DIGESTION

By

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# Abstract

To cope up with the rapid industrialization, urbanization and population it is very important to make use of sustainable water treatment technologies to reuse the processed water. Now a days, with technological advancements in anaerobic digestion (AD) process it is possible to recover some of the resources. AD can be coupled with the membrane to provide the complete biomass retention ensuring the growth of slow growing bacteria. Considering these aspects an innovative configuration of Anaerobic Membrane Bioreactor (AnMBR) with limited aeration is developed aiming to improve the reactor performance. This AnMBR is tested, comparing its performance before and after aeration to assess the impact of subjected aeration especially on the activity of methanogens monitoring the biogas production and its composition. Results show that limited aeration helped in achieving significantly lower overall COD removal without adversely affecting the biogas production as well as composition. It also improved the biodegradability of less biodegradable ovalbumin (protein).

Furthermore, limited aeration might improve the biotransformation of some of the recalcitrant micropollutants (MPs) possibly by activating mono-oxygenase enzymes of micro-organisms. They might convert the aromatic hydrocarbons into less recalcitrant phenolic intermediates, whose degradation process could occur anaerobically. From the group of available MPs, diclofenac (DCF), metoprolol (MPT), sulphamethoxazole (SMX), trimethorprim (TMP) are selected based on different characteristics. All the selected MPs are analysed for their removal through biodegradation and adsorption under anaerobic conditions. MPs are tested from liquid phase in dissolved form through liquid chromatography coupled with mass spectroscopy (LC-MS) technique while MPs sorbed onto the sludge surface are not analysed because of complex extraction process.

Results shows that SMX and TMP are removed more than 99% through AD while DCF is removed with removal efficiency of more than 90%. Adsorption is found to be a major contributor for the removal of DCF, MPT and TMP while SMX is mainly removed through biodegradation. DCF and TMP are adsorbed well (>90%) compared to SMX and MPT (around 30%) onto the sludge contributing to overall removal. Octanol water coefficient ($K_{ow}$) which defines the hydrophobicity of compound, is the key parameter deciding the extent of adsorption. Additionally, sludge retention time (SRT) and compound structure play major role in deciding the fate of MPs through biodegradation. Different aerations had not shown any significant impact on the removal of DCF, SMX and TMP. While in case of metoprolol effect of aeration was not completely understood. Considering the overall removal, DCF was found to be bio-degraded more with increased concentration. Granular activated carbon (GAC) used for adsorption removed all the four MPs with more than 90% efficiency. Further studies can be conducted on possibilities of adding GAC to the reactor so that they get adsorbed and later on degraded.

**Keywords**: Adsorption, AnMBR, biodegradation, limited aeration, micropollutants, octanol water coefficient

# List of Abbreviations

| | | |
|---|---|---|
| AD | : | Anaerobic Digestor |
| AMO | : | Ammonium mono-oxygenase |
| ANOVA | : | Analysis of variance |
| AnMBR | : | Anaerobic membrane bioreactor |
| CAS | : | Conventional activated sludge |
| COD | : | Chemical oxygen demand |
| DCF | : | Diclofenac |
| GAC | : | Granular activated carbon |
| HRT | : | Hydraulic retention time |
| LC-MS | : | Liquid chromatography-mass spectroscopy |
| MP | : | Micro-pollutant |
| MPT | : | Metoprolol |
| ORP | : | Oxidation reduction potential |
| SCFA | : | Short chain fatty acids |
| SMX | : | Sulphamethoxazole |
| SRT | : | Solids retention time |
| SS | : | Suspended solids |
| TOC | : | Total organic carbon |
| TMP | : | Trimethoprim |
| UASB | : | Up-flow anaerobic sludge blanket |
| VFA | : | Volatile fatty acids |
| VS | : | Volatile solids |
| WAS | : | Waste activated sludge |
| WWTP | : | Wastewater treatment plant |

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# Introduction

## 1.1 Project Background

The rapid industrialization, urbanization, and population growth resulted in increasing volumes of untreated domestic and industrial wastewater. Adequate treatment of wastewater is important because all water eventually becomes a part of the water cycle, including used and disposed water. Very often, the treated water from wastewater treatment plants ends up in surface water deteriorating the quality of surface and ground water. If not treated properly, the wastewater can contain harmful or toxic substances that will affect the organisms and plants that live in water and also human beings and animals that come in contact with water. Treated wastewater can also contribute to groundwater replenishment and mitigate water inadequacy.

Water covers 70% of our Earth's surface (Eurekalert.org, 2015). Despite this, water shortage due to lack of access and improper infrastructure or due to physical shortage of water in certain regions is causing water scarcity. According to the United Nations report 2018, over 2 million people live in countries experiencing water stress and two-thirds of the world's population experience severe water scarcity during at least one month of a year. Integrated water resource management is one strategy developed to ensure sustainable water use (Global Water Partnership) of which the reuse and recycling of water is an important aspect. Globally, more than 330 $km^3$ per year of municipal wastewater alone is produced of which only 24 $km^3$ per year undergoes tertiary treatment globally and is fit for reuse. (Global Wastewater Database, CGIAR). In India, according to the Central Pollution Control Board 2015 reports, 61,754 Million Liters per Day of sewage is produced of which only 37% is treated.

Local Treatment of Urban Sewage streams for Healthy Reuse ($LOTUS^{HR}$) is a Dutch-Indian collaborative initiative aiming to develop a novel holistic wastewater approach for recovery of water, energy and nutrients from urban wastewater(lotushr.org). The Barapullah Drain in the central part of New Delhi, India is the location of study. The project is categorized into three research lines among which sewage pre-treatment and energy recovery is one of them wherein anaerobic treatment is selected due to its ability to produce biogas and leave behind biological nutrients which can further be recovered. Integrated technology combining anaerobic reactors with membranes called Anaerobic MBR technology (AnMBR) and with Dissolved Air floatation called AD-DAF is tested and compared for improving water quality. This study will contribute to the $LOTUS^{HR}$ project by studying removal efficiency of selected micropollutants present in wastewater by employing AnMBR treatment technology.

## 1.2 Anaerobic Digestion (AD) & AnMBR

Biological Wastewater treatment is a technology that relies on a complex ecosystem of microorganisms that decompose organic pollutants and remove nutrients and pathogens. It can be

broadly classified into aerobic treatment and anaerobic treatment. Anaerobic treatment of wastewater is an attractive process for the biological treatment of wastewater.

The energy crisis in 1970s lead to the focus on sustainable treatment of wastewater and thus on the anaerobic biological wastewater treatment technology (Jules B. van Lier. et al., 2015)(Lucas, 1998). The fermentation process in which organic material is degraded and biogas (composed of mainly methane and carbon dioxide) is produced, is referred to as anaerobic digestion (Jules B. van Lier, 2012). Anaerobic wastewater treatment technology was initially developed for high strength industrial wastewater with chemical oxygen demand (COD) concentration greater than 1500 mg/l (Kayranli and Ugurlu, 2011) at an ideal temperature of 25-35$^0$C (Kalogo & Verstraete, 2001)(Foresti, Zaiat and Vallero, 2006).

Anaerobic treatment is characterized by four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Hydrolysis is the conversion of slowly biodegradable complex COD in particulate, colloidal or dissolved form to readily biodegradable COD. Hydrolysis is most often considered the rate-limiting step and hence the reactor design is often determined based on the rate of hydrolysis. Acidogenesis is the conversion of hydrolysis products through fermentation or anaerobic oxidation. Further, the products formed during acidogenesis, except acetate, is converted to $CO_2$, acetate and hydrogen gas by acetogenic bacteria. During the final methanogenesis stage, the organic compounds are converted to biogas (methane & carbon dioxide) by methanogens (Jules B. van Lier. et al., 2012).

Anaerobic wastewater treatment is a very attractive technology due to the following reasons:

Anaerobic wastewater treatment reactors can handle high volumetric load, almost 5-10 times higher than aerobic systems in the range of 20-35 kg COD per $m^3$ of reactor per day (Jules B. van Lier 2012), hence reducing the reactor volume and in turn reducing carbon foot print.

Due to slow growth of microorganisms such as the methanogens, the production of excess sludge (sludge yield) is less which also reduces the issues concerning sludge handling (treatment and disposal) (Jules B. van Lier 2012).

Anaerobic wastewater treatment is a sustainable technology not only because of the very low consumption of fossil fuel but also because of the potential energy recovery in the form of biogas. Biogas has high calorific values and can be used for energy generation (Chernicharo et al., 2015) (Jules B. van Lier 2012). Thus, the anaerobic digestors could result in net energy production making it a sustainable technology when compared to aerobic treatments which require high energy input.

Lately, anaerobic wastewater treatment technology is being developed for low strength domestic wastewater (Foresti, Zaiat, and Vallero 2006) and for efficient operation at low temperatures (Bandara et al. 2012). However, there are certain drawbacks of using anaerobic technology for the treatment of dilute wastewaters such as municipal wastewater. As it is subject to changes in operational and environmental conditions due to diurnal variations (which affects the load) and seasonal variations (which affects the temperature). The depolymerization of complex substrates- hydrolysis, is a process that is very sensitive to temperature and the fluctuations in temperature (Jules B. van Lier 2012). At low temperatures (15$^0$C), the rate of hydrolysis is significantly reduced, leading to accumulation of suspended solids which in turn reduces the reactor volume available for active biomass (Lettinga, Rebac, and Zeeman 2001). Methanogenic microorganisms have a very slow growth rate, making it difficult to retain them in the anaerobic digestors treating low strength domestic wastewaters at short

hydraulic retention times (Guo et al. 2016). Deterioration of methanogenic activity of the sludge due to accumulation of slowly biodegradable COD was also reported by (Giraldo-Gomez, 1991). Slow growing methanogens along with poor settleability leads to low net biomass yield and loss of biomass to the effluent (Lin et al. 2013) thereby affecting both methanogenic activity and effluent quality.

To summarize, hydrolysis or methanogenesis can be the rate limiting processes in anaerobic digestion depending upon the influent and hence requires longer SRT. For the conventional anaerobic digestors which do not decouple the retention times, longer SRT for optimal hydrolysis would entail large reactor volume and lower organic loading rate, thus compromising its competitiveness against aerobic reactors. Poor settleability leading to loss of biomass to effluent would mean employing a post-treatment step to meet the effluent standards. The Anaerobic Membrane Bioreactors (AnMBRs) offsets these limitations. An AnMBR can be simply defined as a biological treatment process operated without oxygen that uses a membrane to provide solid–liquid separation (Lin et al. 2013). The main advantages of AnMBR include higher biomass retention to attain a full growth of slow-growing methanogenic consortia, improving both effluent quality and the probability of biogas recovery (Lin et al. 2013). AnMBR can operate at reduced foot print compared to conventional anaerobic reactors while ensuring better effluent quality and reduced sludge handling (Guo et al. 2016). Thus, AnMBR offers independent control of HRT and SRT (Martinez-Sosa, Helmreich and Horn 2012).

AnMBR systems are implemented based on two configurations, external/side-stream configuration and submerged/ immersed configuration (Lin et al. 2013). The external configuration allows higher fluxes and provides ease in membrane cleaning, replacement while leading to higher energy consumption (of the order 10 kWh/m3 product) (Le-Clech, Chen, and Fane 2006). Membrane clogging and cross flow affecting the biomass activity are two drawbacks of AnMBR. Moreover, high cross-flow velocity has been reported to have a negative impact on biomass activities in AnMBR systems (Choo and Lee 1996).

### 1.2.1 Limited Aeration in AD

From the above discussion on conventional anaerobic reactors and AnMBRs, it is clear that the anaerobic digestion process can be bettered if the rate-limiting hydrolysis process can be made more efficient. It is commonly known that hydrolysis rates are significantly higher under aerobic and anoxic conditions compared to anaerobic conditions (Henze and Mladenovski 1991). However, oxygen is considered to be toxic to anaerobic digestion consisting of strict anaerobes whose growth might be inhibited (Chu et al. 2005). In fact, the inoculums used in anaerobic digesters are de-aerated or dosed with oxygen scavenging chemicals before commencing reactor operation (Jarrell 1985).

However, micro aeration or limited aeration can be employed to enhance hydrolysis which will increase the rate of conversion of slowly biodegradable COD to readily biodegradable COD. This might be helpful in preventing the accumulation of suspended solids in the reactor thereby affecting the treatment efficiency and biogas production (Jagadabhi, Kaparaju, and Rintala 2010)(Botheju 2011) demonstrated the possibility of the existence of an optimum oxygenation level which would yield a maximum methane generation in AD. The studies on limited aeration in AnMBR is not much researched due to the misconception of the toxicity of oxygen to anaerobic reactors. Amount of oxygen that can be safely added should be carefully selected because too much or too little aeration might have adverse effects on the treatment process.

## 1.3 Emerging contaminants in wastewater: a cause of concern

The presence of micropollutants in treated wastewater is an issue of emerging concern. In the European Market alone, 1,43,000 micropollutants were registered in 2012 (Das et al. 2017). The study of toxicity of micropollutants to the aquatic environment, the animals and humans who come in contact with water and consume the water (non-potable purposes) is multiplied by the fact that the properties of all micropollutants is not fully known. Only those substances that are commonly found in known concentrations and have well known and proven impacts on human and the ecosystem are included in the legal regulations (Water Framework Directive 2000). Many substances fall outside this category and are thus unregulated. This poses a larger threat since the ones that do not fall under the legal framework may also not be targeted during treatment, before disposal.

Ecotoxicity and genotoxicity such as endocrine disruption, antibiotic resistance, inability to reproduce, loss of sensitive species etc., are a few of the numerous problems that micropollutants may cause. Micropollutants can be present both in domestic wastewater and in industrial wastewater. The origin of micropollutants in wastewater is aplenty, such as in pharmaceuticals, personal care products, flame retardants, industrial chemicals, pesticides and so on and so forth. These micropollutants are said to enter the aquatic system mainly through the treated wastewater that is disposed onto the surface water (Corominas et al., 2013)(Rogowska et al., 2020). The micropollutants in wastewater are degraded through adsorption or through biodegradation.

### 1.3.1 Effect of limited aeration on MP removal

Most of the micropollutants presents in the water bodies are recalcitrant offering resistance to the degradation. Hence research is being carried out to assess the removal of these recalcitrant MPs through different technologies. These available technologies are broadly divided into biological and non-biological processes. It was observed that removal efficiencies of anaerobic treatments are much lower than those of aerobic treatment systems (Alvarino et al., 2016)(De Graff et al., 2011)( Joss et al., 2004). Hence it is important to know the mechanism with which it is possible to remove the MPs. Limited aeration offers advantages in this case as aerobic degradation of most of the MPs is likely to happen.

Given the complex structure containing aromatic rings, most of the MPs are not biodegradable under anaerobic conditions and required oxygen for their degradation. In this case the provided limited aeration can help to degrade these compounds. Moreover, oxygen from the limited aeration might favour the co-metabolic reactions involved in micropollutants removal. Therefore, It will be interesting to investigate the effect of limited aeration on MPs removal.

## 1.4 Research Scope

The anaerobic digestion of wastewater is considered a sustainable technology not only because of its ability to treat wastewater with less or no use of fossil fuels but also because of the ability to recover energy in the form of biogas. Other advantages include high volumetric loading leading to reduced carbon foot print, ability to recover nutrients, reduced sludge yield etc., The few drawbacks related to treating low-strength wastewater with high percentages of slowly biodegradable substrates include: inability to adapt to fluctuating temperature because of the sensitivity of the rate limiting hydrolysis step, accumulation of suspended solids which hampers treatment efficiency and reduces the SRT

among others limitations. These drawbacks are mitigated by employing integrated anaerobic bioreactor with membranes (AnMBR) which will allow for the decoupling hydraulic retention time and solids retention time. As a result, slow growing biomass (methanogens) will be retained in the system, thereby also improving biogas production. Limited aeration might enhance the rate of hydrolysis which would further reduce the accumulation of slowly biodegradable suspended COD, thus improving the overall treatment efficiency.

Micropollutants in wastewater are a cause for concern because of its ecotoxicity and genotoxicity when left untreated in wastewater. Limited aeration aids in removal of micropollutants by improving their co-metabolism in presence of $O_2$ and further degrading them under anaerobic conditions.

To summarize, this study focusses on investigating the removal efficiency of selected micropollutants in an AnMBR system assisted with limited aeration and comparison of reactor performance before and after subjected aeration.

The following research areas are dealt with in this report by answering the research questions below,

1. The effects of limited aeration on reactor performance in terms of effluent quality with overall COD removal, fate of nutrients, biogas production and composition as well as stability of reactor in terms of reactor pH monitoring the VFA accumulation
2. The efficiency of removal of selected micropollutants in anaerobic digestors with limited aeration. This will be further investigated as
    - Removal of MPs through biodegradation
    - Removal of MPs through adsorption
3. The effect of increase in aeration on the removal efficiency of selected micropollutants.

## 1.5 Thesis Outline

The thesis starts with introduction giving a short project background and then general introduction of AD and importance of limited aeration and how it can be helpful in treatment of recalcitrant MPs. Along with this, the research goals of this study are explained in **chapter 1**. In **chapter 2** available scientific knowledge on AD and limited aeration are reviewed. Special emphasis is given to the micropollutants analysing their fate in anaerobic treatment. Depending on the characteristics, four MPs are selected to investigate their removal efficiency in this AnMBR with limited aeration. **Chapter 3** describes the research methodology along with the analysis techniques applied in this study. **Chapter 4** provides the results obtained from the experiments performed and **Chapter 5** has separate discussion on each of the results obtained with the limitations if any. **Chapter 6** presents the limitation of this study and recommendations for further research while **Chapter 7** provides the conclusion of this research.

# 2

# Literature review

## 2.1 Anaerobic Digestion (AD) with limited aeration

AD is a multistage process in which degradable organic matter is microbiologically converted in absence of oxygen to produce methane ($CH_4$) and carbon dioxide ($CO_2$). The four sequential stages of AD - hydrolysis, acidogenesis, acetogenesis, and methanogenesis are illustrated in Figure 1. During hydrolysis, complex substrates such as carbohydrates, lipids and proteins are broken down to produce soluble organics which are then fermented in the acidogenesis stage to produce volatile fatty acids (VFAs), alcohols, $H_2$ and $CO_2$ (Appels et al., 2008). In the acetogenesis stage of AD process, the VFAs and alcohols are further degraded into acetate, $H_2$, and $CO_2$ by acetogenic bacteria (van Lier et al., 2008). The last stage of AD, methanogenesis is defined by the conversion of acetate, $H_2$, and $CO_2$ by the acetoclastic and hydrogenotrophic methanogens to produce gaseous $CH_4$ and $CO_2$ (van Lier et al., 2008).

AD of complex organic matter is a four-step complex process that converts degradable organic compounds to methane ($CH_4$) and carbon dioxide ($CO_2$) in the absence of elemental oxygen. These four stages of AD are hydrolysis, acidogenesis, acetogenesis, and methanogenesis as shown in Figure 1. Hydrolysis - the first step, is the breakdown of complex high molecular weight substrates such as carbohydrates, lipids, proteins to soluble organic substances such as sugars, amino acids, long-chain fatty acids (LCFA), glycerol by exo-enzymes secreted by hydrolytic and fermentative bacteria (Appels et al., 2008).

The second step - acidogenesis is where dissolved compounds produced during hydrolysis enter the cells of fermentative bacteria and are converted to volatile fatty acids (VFAs), alcohols, $NH_3$, $H_2$, $CO_2$, and $H_2S$ and then excreted from the cells (van Lier et al., 2008). The third step - acetogenesis is the anaerobic conversion of VFAs and alcohols produced in the acidogenesis step into acetate, $H_2$, and $CO_2$ by acetogenic bacteria. The most common acetogenic substrates are propionate and butyrate - key intermediates in the AD process (van Lier et al., 2008).

Methanogenesis is the final step in AD where acetate, $H_2$, and $CO_2$ are converted to gaseous $CH_4$ and $CO_2$. This step is accomplished by two groups of bacteria – acetotrophic methanogens and hydrogenotrophic methanogens. Acetotrophic methanogens decarboxylate acetate to form $CH_4$ while hydrogenotrophic methanogens reduce $CO_2$ using $H_2$ as an electron donor (van Lier et al., 2008).

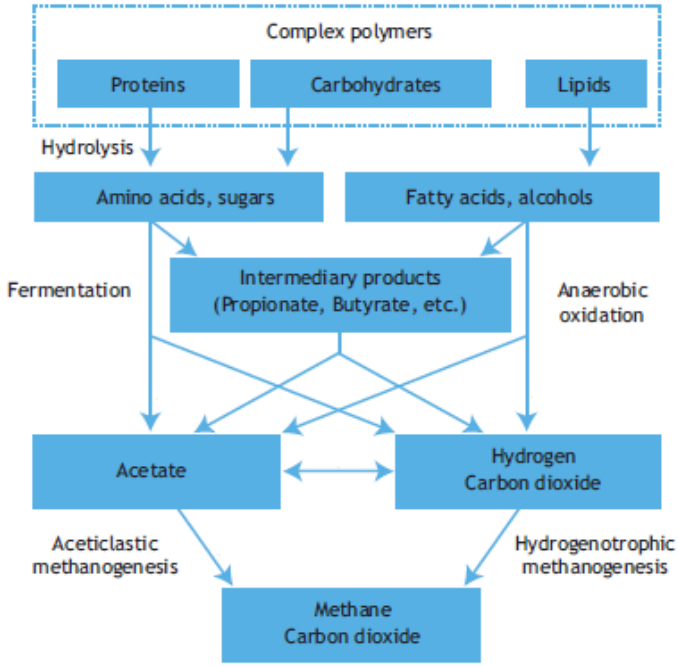


*Figure 1: Anaerobic Digestion process (Gujer & Zehnder, 1983; van Lier et al., 2008)*

Performance of this anaerobic digestion can be improved by adding micro aeration or limited aeration by improving the rate of conversion of slowly biodegradable COD to readily biodegradable COD. This way reactor provides stable performance without the accumulation of VFAs. Botheju et al.,(2011) demonstrated the possibility of the existence of an optimum oxygenation level which would yield a maximum methane generation in AD. Though limited aeration had not been widely studied because of the inhibitory characteristics of $O_2$ which might affect the activity of methanogens.

Besides providing the improved reactor performance, limited aeration offers advantages in degradation of some of the recalcitrant MPs. According to Aquino, Brandt, and Chernicharo (2013), some of the MP are not biodegradable under anaerobic conditions because they contain phenolic aromatic rings which can be degraded only in presence of $O_2$. In this case the provided limited aeration can help to degrade these compounds. Additionally, limited aeration can enhance biodegradation of non-readily biodegradable organic by activating monooxygenase enzymes of micro-organisms (Batt et al., 2006; Vader et al., 2000). They might convert the aromatic hydrocarbons into less recalcitrant phenolic intermediates, whose degradation process could occur anaerobically. Moreover, oxygen might favour the co-metabolic reactions involved in micropollutants removal.

## 2.2 Introduction to Micropollutants (MPs)

Micropollutants (MP) will be treated as emerging contaminants posing the potential danger to the environment, significantly to the aquatic life present around. These compounds termed as Micropollutants as they are present in very less concentration about micro grams to nano grams per litre(A. Stasinakis and G. Gatidou, 2010).

### 2.2.1 Occurrence of MPs

Large number of variety of compounds are included in this category of “micropollutants” such as pesticides, personal care products (PCPs), flame retardants, per-fluorinated compounds,

pharmaceuticals, surfactants, pharmaceuticals, steroid hormones, drugs of abuse and others (Ribeiro et al., 2015). More than 10,000 of such different MPs are ubiquitously present everywhere and applied on regular basis, finally ending up in the domestic water after their use.

### 2.2.2 Fate of MPs

Although in very small concentrations, literature have identified the presence of these compounds in the water bodies with the advanced techniques available for their measurement (Kolpin et al., 2002; Loos et al., 2009). Based on their application and use, these compounds finally end up in the environment. As seen, the major contribution is through agricultural and urban runoff as shown in the Figure 2 below. Besides, municipal and industrial as well as hospital wastewater discharge, sludge disposal and accidental spills or contamination through landfill leachates are responsible for this contamination (Ashton et al., 2004)(Becker et al., 2008)(Mompelat et al., 2009).

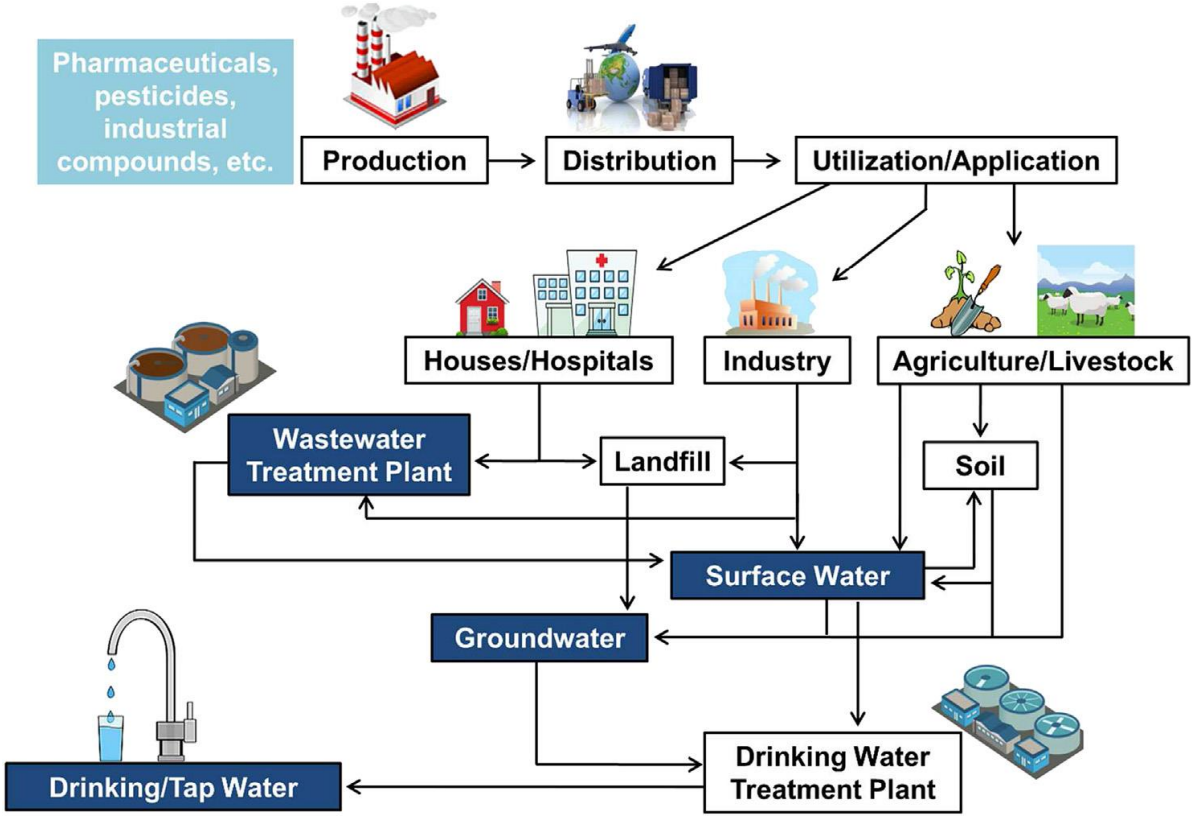


*Figure 2:Occurrence and fate of MPs(Mompelat et al., 2009)*

Once released, these MPs undergo different processes such as sorption onto the surfaces available, distribution between different phases, decompositions, biological and abiotic degradation in the environment (Hebberer, 2002a)(Birkett and Lester, 2003)(Farre et al., 2008). In this way MPs gets removed in the environment through the different processes taking place as mentioned. So It is important to understand these processes in details as removal efficiency of MPs can vary depending on physico-chemical properties of these compounds (polarity, partitioning coefficient, water solubility, vapor pressure) and the type of the environment where the micropollutants are present (groundwater, surface water, sediment, wastewater treatment systems, drinking water facilities)(A. S. Stasinakis, G. Gatidou, 2010). Because of which, different transformation reactions take place, degrading and generating their intermediates depending on the environment and their parent compound.

### 2.2.3 Toxic effects of MPs

Advanced techniques available today made it possible to detect the MPs present at very low concentrating enabling to identify their effects on environment and humans. Though it was possible to identify occurrence and fate of MPs, their actual ecological effects in the environment are still poorly understood (A. Stasinakis and G. Gatidou, 2010).

Due to daily usage of umpteen variety of MPs they present in mixtures rather than being present separately. Brian et al. observed toxic effects of oestrogens as a mixture on fishes.

So far, several effects of these MPs have been reported in abundance, such as acute and chronic toxicity, endocrine disruption, bioaccumulation and biomagnifications (Oaks et al., 2004)(Fent et al., 2006)(Darbre and Harvey, 2008).Additionally, they are responsible for aquatic toxicity, increase in pathogenic bacteria resistance, genotoxicity, increase in breast and prostate cancer incidence, endometriosis, and other endocrine disorders (Aquino, Brandt, Chernicharo, 2013)(Kummerer, 2010).

## 2.3 Removal mechanism of MPs

Once ended up in the environment these MPs may get removed by different processes as discussed earlier. Some of the MPs are recalcitrant and only possibly degraded with particular mechanism. Therefore, quite a lot of studies have been done to investigate the removal mechanism of the MPs.

These processes can be divided in 2 major types first being biological and other is non-biological processes (physical, chemical, physicochemical). Non-biological processes consist of advanced filtration technologies based on physical separation such as nano filtration, reverse osmosis, adsorption techniques with zeolite, activated carbon and other developed adsorbent as well as advanced oxidation, ozonation for MPs removal (De La Cruz et al.,2012)(Vidal et al.,2015). However, these non-biological treatments are expensive with high capital investment with their installation and operating cost. Additionally, physical processes need further treatment to dispose of the MPs already removed/adsorbed/extracted during the process (Aquino, Brandt, Chernicharo, 2013)(Pessoa et al.,2014)

Considering this point, some investigations into micropollutants removal by biological treatment have been carried out. These researchers have analysed biological treatment with respect to both aerobic and anaerobic reactors (Patricia et al., 2019). It was observed that removal efficiencies of anaerobic treatments are much lower than those of aerobic treatment systems (T. Alvarino et. al., 2016)(De Graff et al., 2011)(Joss et al., 2004).

Removal of micropollutants within biological process can be seen through the below mentioned 3 mechanisms (Figure 3):

- Volatilisation (by stripping or by surface volatilisation),
- Sorption to the sludge (biosorption),
- Biological conversion (biodegradation),

As shown in figure below each of the process mentioned above indicates the MPs concentrations in gas, dissolved and solid compartments. The process of volatilisation and sorption is driven by the equilibrium mechanism between dissolved-gas and dissolved-solid compartments. Therefore, MPs will be removed by transferring to gas in volatilisation and by getting adsorbed onto the solid sludge in sorption. While in case of biotransformation, MPs from solid compartment (previously adsorbed onto

sludge) or dissolved phase (in liquid- dissolved compartment) are degraded by the micro-organisms present (M. pomies et. al.,2013).

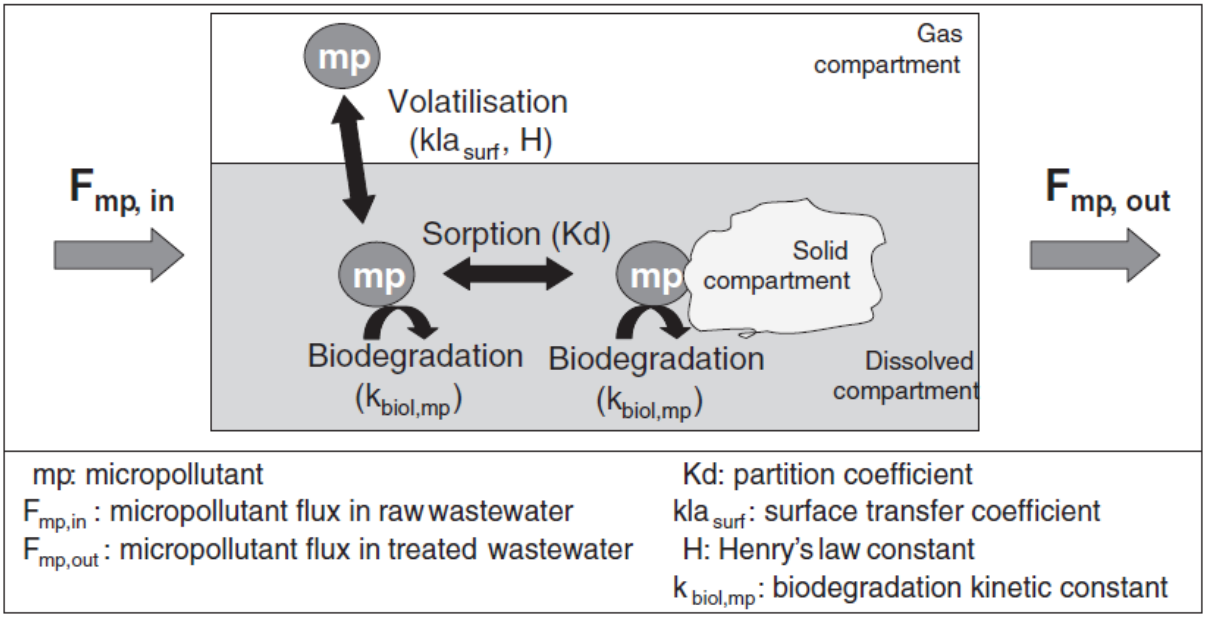


*Figure 3: Removal mechanism of MPs (M. Pomies et. al.,2014).*

### 2.3.1 Sorption

Adsorption is physical phenomenon where the adsorbate gets adsorbed onto the adsorbent. Here in this process MPs will be getting adsorbed in sludge surface (adsorbent). This process is also called as biosorption as the adsorbent used in this case of biological sludge. The idea of biosorption is originated in 1986 by the 'Solvent Engineering Extraction and Ion Exchange Group of the Society of Chemical Industry' in the UK and biosorption was regarded as an emergent technology (Apel and Torma, 1993).

As shown in Figure 3 above, two compartments, namely the aqueous compartment which is the dissolved phase, and the solid phase of the sludge (bio-sorbent) are involved in the sorption process. (M. Pomies et al.,2013).

The extent of sorption depends on characteristics of the MPs getting sorbed. Hydrophobicity, solubility, octanol-water coefficient ($K_{ow}$) are some of the very important characteristics of the MPs to be considered for sorption(Garcia et. al., 2002; Ilani et. al., 2005; Yu and Huang, 2005). Additionally, sorption behaviour can be evaluated with the sorption coefficient ($K_d$, solid-water distribution coefficient) and it is dependent on the characteristics of the compound as well as on the sludge type (Caliman and Gavrilescu,2009). Temperature, pH, mixing are the external parameters that influence the adsorption significantly.

Besides this knowledge, Sorption mechanism is difficult to analyse separately as happens continuously with biodegradations. Also, very few researchers have worked on isolating and assessing this sorption process for the removal of MPs. Moreover, the values used for $K_d$ and $K_{ow}$ for organic micropollutants are not sufficiently accurate to be used for predictions as they vary with the type of sludge and environment present (Barret et al., 2010b).

### 2.3.2 Volatilisation or abiotic degradation

Abiotic degradation can be defined as degradation of organic compounds without the presence of micro-organism but with chemical or physical reactions (Doll and Frimmel, 2003)(Iesce et al., 2006). This can be both natural as well as triggered. As far as biodegradation of MPs is concerned, abiotic processes are not significantly important (Stangroom et al., 2000)(Lalah et al., 2003)(Soares et al., 2006)(Katsoyiannis and Samara, 2007).

Volatilisation is basically a process where MPs from dissolved phase gets volatilised to gaseous phase. The extent of this process essentially depends on the physicochemical properties of the micropollutant (H: Henry's law constant) and on the operating conditions of the process (i.e., aeration, agitation, temperature and atmospheric pressure).However pharmaceutical compounds, hormones did not include volatilisation because it is not considered a significant removal mechanism for these families (Wang et al., 2003)(Urase and Kikuta, 2005)(Plosz et al., 2010).

Byrns (2001) investigated the effect of Henry's constant on volatilisation for range of MPs and posited that threshold value for volatilisation to be significant is 100 $Pa.m^3mol^{-1}$. MPs with H less than this threshold did not contribute significantly to volatilisation. Universally, volatilisation is applicable only for volatile MPs, but the limit of volatility is not clearly mentioned (M. Pomies et al.,2013).

### 2.3.3 Bio-degradation

Biodegradation of MPs will be defined as biotransformation or consumption of MPs by micro-organism present in the sludge. MPs being recalcitrant it takes large time (>10 number of days) in general to degrade these MPs, while some of them cannot be degraded at all. Biodegradation of MPs depends from which compartment (Figure 2) the MPs are available such as:

- Biodegradation of MPs from the dissolved phase
- Biodegradation of MPs that are sorbed onto sludge surface
- Simultaneous Biodegradation of Both dissolved and sorbed MPs.

Other than that, MP removal through biodegradation also depends on multiple other factors such environment (aerobic, anaerobic, anoxic), sludge characteristics (microbial community, type of sludge,VSS,TSS). Biodegradation of MPs varies depending on properties of MPs.

## 2.4 Characteristics of MPs

As mentioned in the section 2.3 for the removal of MPs through sorption, biodegradation and volatilisation, characteristics of MPs plays major role in deciding the extent of removal through these processes.

MPs possesses wide range of chemically complex structures with distinct physico-chemical properties such as octanol water partitioning coefficient, solubility, Henry's constant, dissociation constant, molecular structure, nature (anionic/cationic). Some MPs are polar with high solubility making them hydrophilic hence difficult to be removed from water (dissolved phase) while some MPs are highly insoluble in water. Many of them contain aromatic groups with substituted compounds making them very specific in their identity. Before making the choice of treatment, MPs to be closely evaluated with respect to these intrinsic properties to achieve maximum removal with the specific treatment.

In addition to the properties of MPs, operational parameters such as SRT, HRT, temperature, mixing are the factors which affect the overall removal (Suarez et al., 2008)(Caliman and Gavrilescu, 2009)(Liu et al., 2009)(Wick et al., 2009).

### 2.4.1 HRT/SRT

Longer the HRT/SRT, longer the duration for which MPs will stay in the system so that they can be removed through the process of adsorption or biodegradation.

Kirk et. al., observed that with higher HRTs oestrogens were removed more. The same phenomenon was observed by Gros et. al, (2007), where it was found that removal efficiencies were highest for most of selected MPs at high HRTs (25-33 hrs), while same were poorly removed with lower HRTs (8 hrs). Although, due to the different characteristics of the MPs, a general removal trend was hard to establish.

Same observations were made by many authors in case of SRT concluding, higher the SRT higher was the removal. (Choubert et al., 2011)(Strenn et al., 2004)(Clara et al., 2005)(Carucci et al., 2006). Longer SRT ensures growth of all the micro-organisms including slow growing species making the diverse bacterial culture, as result increasing the potential for removal of MPs through bio-transformation (Kreuzinger et al., 2004)(Suarez et al., 2010). Moreover, a high SRT combined with a reduced food/microorganism ratio seemed to favour the biodegradation of antibiotics (Gobel et al., 2007).

Therefore sufficiently high SRT is essential for the removal and degradation of MPs in wastewater, Although increasing the SRT more than that will not contribute positively in their removal (Joss et al., 2005)(Vieno et al., 2007).

For shorter SRTs about 2 days, Clara et al. (2005b) found out that DCF was not eliminated, the same was removed up to 44% to 85% with MBR containing SRTs of 190–212 days (Gonzalez et al., 2006).

### 2.4.2 Co-metabolism

Literature mentioned that observed low concentrations of personal care products (PCPs) in the sewage will be due to co-metabolism. Meaning biodegradation of micropollutants (MPs) can occur during the conversion of macro-pollutants. Therefore, presence of other substrate like cellulose, acetate(which provides readily biodegradable COD) is must as co-substrate, as MPs cannot act as the source of carbon or energy for the biomass to satisfy their growth (M. Pomies et. al., 2007).

In fact, co-metabolism has found to be the main mechanism which posited removal of hormones under nitrifying conditions, through the enzyme ammonium monooxygenase (AMO). Consequently, several researchers seconded this by even establishing the link between the removal of PCPs to the nitrification process (T. Alvarino et. al., 2014).

### 2.4.3 Octanol water partitioning coefficient ($K_{OW}$)

Octanol water partitioning coefficient is vital parameters which defines the hydrophobicity of the compound. Extent of adsorption of the selected compound is significantly depends on its nature (Hydrophobic: repel water, hydrophilic: attract water). More the hydrophobic compound more it will likely to get removed from water (Yu and Huang, 2005).

Compounds with log $K_{OW}$ value less than 2.5 are characterized by high bioavailability and their sorption to activated sludge is not expected to contribute significantly to adsorption. For compounds with log

$K_{ow}$ between 2.5 and 4, moderate sorption is expected while for values greater than 4 are significantly removed through adsorption. (Rogers, 1996)(Ter Laak et al., 2005).

### 2.4.4 Dissolved oxygen (DO)/pH

For those MPs which can be biodegraded easily in aerobic conditions while offering resistant to degrade under anaerobic conditions, dissolved oxygen (DO) might act as most important parameter (Furuichi et al., 2006)

Cirja (2007) reported that the pH can influence the removal of MPs affecting the activity of micro-organisms. Depending on their dissociation constant ($pK_a$) values, MPs can exist in anionic or cationic from depending on pH. For instance, at pH 6 – 7 tetracyclines are neutral without any charge hence effectively removed through adsorption mechanism (Kim et al., 2005). In addition to biodegradation, adsorption also get affected with the pH. Therefore, Cirja (2007) suggested that the control of the pH value might be a solution for the removal of micropollutants in WWTPs.

### 2.4.5 Temperature

Removal of MPs through biodegradation and sorption is greatly affected by temperature. The very fact that biodegradation affected by the temperature is because biomass activity is closely linked to the temperature (Price and Sowers, 2004).

Solubility is function of temperature and it increases with temperature except for the compounds with reverse solubility. Adsorption is affected by the solubility (hydrophobic/hydrophilic nature). Therefore, it increases with decrease in solubility.

So, for most compounds, sorption equilibrium decreases with increase of temperature, whereas the biodegradation is less efficient at lower temperatures.

Effect of temperature is evident with the seasonal change. Vieno et.al.,(2005) observed that during the summer(17°C), removal of DCF was increased compared to that of winter (7°C). Carballa et. al.,(2005) also noticed the similar phenomenon.

### 2.4.6 Molecular structure

The structure of a MP can significantly decide its removal in that particular environment. Simple compounds tend to undergo degradation readily during the biological treatment rather than the compounds with complex structure. This is very reason which indicates recalcitrant behaviour of large number of MPs. For examples, complex compounds are identified by their specific structure which shows the ionic nature of that particular compound. Hence method of removal to be chosen accordingly as ketoprofen and naproxen were removed with membrane bioreactors while but not with conventional treatments (Kimura et al., 2007). It was observed that MPs with complex structure are mostly recalcitrant because of having two aromatic rings making the compound more resistant to degradation processes. Also, MPs with halogenated compound structure (DCF) shows recalcitrance.

## 2.5 Selection of MPs

Wide range of micropollutants are analysed so far. In general, diclofenac is the most analysed MP with all the types of processes (M. Carbala et al., 2007). Choice of the MPs was rather depend on the availability of the analytical data (M. Pomies et. al., 2013). Because of the fact the MPs present in very

low concentrations (about nano grams to micro grams per litre) their precise detection with analytical techniques is costly.

The physicochemical properties of particular MP had significant impact on its removal. Moreover, removal efficiencies vary depending on operating conditions, such as sludge retention time (SRT), hydraulic retention time (HRT) and temperature, pH, mixing, making the selection of MPs very important.

MPs selected for this study are chosen from different groups as mentioned in the Table 1 Different characteristics of specific MPs are then analysed to draw the conclusion from the experiments performed.

Considering the different classes and characteristics 4 numbers of MP were selected to assess their removal as shown in the Table 1 below:

*Table 1: Expected extent removal of selected MPs under different biological environment*

| Micro-pollutants | Class | Aerobic | Anaerobic | Adsorption |
|---|---|---|---|---|
| **DCF** | **Anti-inflammatory** | ++ | - | ++ |
| **MPT** | **Beta-blocker** | + | - | + |
| **TMP** | **Antibiotic** | - | +++ | - |
| **SMX** | **Antibiotic** | - | +++ | + |

Considering the removal under anaerobic conditions in AnMBR subjected to limited aeration, MPs selected such that each MP is expected to be degraded in different environment.

As shown in table above SMX and TMP are known to be biodegraded under the lower redox potential (T. Alvarino, 2014)(T. Alvarino,2016)(M. Carbala,2007). Therefore, removal efficiency of these two is expected to be high with the operated system.

While removal efficiency of DCF and MPT is very low under the anaerobic conditions (A. Ternes, 2010) though low removal of these 2 respectively was observed during aerobic treatment step though bio-degradation via monooxygenation (Zhang et al., 2008). Reason behind choosing these 2 MPs was that limited aeration might help their degradation even under anaerobic conditions.

(Kimura et al., 2005) explained the recalcitrant behaviour of DCF. He proposed that compounds with complex structure containing halogens and nitro group exhibit resistance to biodegradation and tend to have very low removal. As shown in Annexure 1 (Molecular structure for selected MPs). DCF has complex structure with $Cl_2$ as halogenated compound.

Furthermore, Removal of TMP was explained which has relation to its structure. Substituted heterocyclic compounds are more prone to biotransformation under anaerobic conditions (N.R. Adrian and M. Suflita,1994). As shown in Annexure 1 (Molecular structure for selected MPs) TMP pyrimidine has substituted with 2 amide($-NH_2$) groups. SMX was degraded under anaerobic conditions

through reduction due to the presence of electron-withdrawing sulphonyl groups as shown in Annexure 1 (Molecular structure for selected MPs) (J. Field et. al.,2002).

*Table 2: Specific properties of selected MPs*

| Micro-pollutants | Molecular Formula | Log $K_{ow}$ | Charge | $pK_a$ | Solubility @ $25^0$C (mg/l) | Henry's constant (atm.$m^3$/mol) |
|---|---|---|---|---|---|---|
| **DCF** | $C_{14}H_{11}Cl_2NO_2$ | 4.6 | - ve | 4.15 | 2.37 | 4.73E-12 |
| **MPT** | $C_{15}H_{25}NO_3$ | 1.8 | + ve | 9.6 | >1000 | 2.11E-11 |
| **TMP** | $C_{14}H_{18}N_4O_3$ | 1.2 | + ve | 7.12 | 400 | 2.14E-14 |
| **SMX** | $C_{10}H_{11}N_3O_3S$ | 0.7 | - ve | 5.12 | 610 | 6.40E-13 |

*Data for the selected MPs retrived from: National Center for Biotechnology Information (2020). PubChem Compound Summary for CID 5329. https://pubchem.ncbi.nlm.nih.gov/compound/.*

Octanol-water partitioning coefficient ($K_{ow}$) is important parameter defining the hydrophobicity of compound. More the $K_{ow}$ value compound is less soluble in reducing its polarity. This can be seen from the Table 2 above. Therefore, these MPs selected have a range of $K_{ow}$ from 4.6 highest for DCF to 0.5 lowest for SMX.

Biomass has a high potential as an adsorbent due to its physico-chemical characteristics (M. Rafatullaha et.al.,2010). This mechanism of bio-adsorption is linked to the interaction between cell surface and ionic components. The cell surface of biomass has negative charge so it attracts positively charged compounds. It is now recognized that ion-exchange is responsible for the efficiency and the selectivity of MP-adsorption to microbial biomass (O. Sahu and N. Singh, 2009).

$pK_a$ values of the selected MPs decides its cationic/anionic nature. Anionic nature of DCF can be explained by its dissociation constant ($pK_a$) of 4.15 while an estimated $pK_a$ of metoprolol is 9.6, indicates MPT exist in the cationic form and gets adsorb to organic carbon and clay. Similarly, $pK_a$ values of TMP and SMX indicates their nature which is tabulated above. With the different charge it will be interesting to see the effect on their removal through adsorption.

Moreover, as indicated in the Table 2: Specific properties of selected MPs Henry's constant H for all selected MPs is lower than the threshold value making their removal through volatilisation insignificant (M. Pomies et. al., 2013).

## 2.6 Research Gaps

As of now, wide range of MPs investigated for their removal through different processes. Diclofenac (DCF) was observed to the most studied MP, on the contrary metoprolol (MPT) was rarely selected. Researchers also dealt with effect of different conditions such as biological environment (aerobic, anaerobic, anoxic), temperature, pH, mixing, on the removal of different MP. Additionally, MPs with specific physico-chemical properties are also dealt with the literature to decide their fate in the environment as well as in the removal processes. Though almost all properties are analysed, nature of the MPs (cationic/anionic) was something which was not taken into account by any researchers.

Research done till now is mostly focussed in removal of MPs under aerobic or anaerobic conditions separately and very few authors combined these processes to investigate their effect on MPs removal. T. Alvarino et. al. (2014,2016), combined up-flow anaerobic sludge blanket (UASB) reactor with conventional activated sludge (CAS) process and published the results.

Adsorption of MPs through sludge is investigated by very few researchers as analysing the very low concentration with analytical techniques available is complex considering the laborious process of extraction from solid matrix. Moreover, assessing the adsorption separately was challenging which required the Inhibition of biomass.

Ternes et al. (2002) reported the removal of DCF through anaerobic bank filtration. However, he did not explain whether the removal was through sorption or it was through biodegradation. Likewise, authors commented on overall removal but most of them failed to distinguish the mechanism of removal.

Some findings showed that removal efficiencies MPs under aerobic treatment were more than that of anaerobic reactors (T. Alvarino et.al., 2014)(De Graaf et al.,2011)(Joss et. al.,2004). Also, recent investigations indicated adding low oxygen concentrations to anaerobic systems could improve the initial degradation of recalcitrant compounds, such as monoaromatic hydrocarbons (BTEX) (Firmino et. al.,2018)(Siqueira et. al.,2018). However, to the best of the author's knowledge, no researcher had investigated MPs removal with limited aeration except Patricia et. al., (2019), who investigated MPs removal with micro-aerated anaerobic reactor.

# 3

# Materials and Methods

## 3.1 AnMBR assisted with limited aeration

A lab scale AnMBR was installed as part of LOTUS$^{HR}$ project as mentioned in the Introduction. Seven litres of glass reactor was coupled with an anaerobic membrane to increase the sludge retention and to provide the better-quality effluent. Feed for this reactor was synthetic prepared blackwater with a recipe adapted from Ozgun et. al.,2013. The Sludge used for this reactor was obtained from NIOO, KNAW, Wageningen, from a 1 $m^3$ anaerobic reactor treating blackwater. Reactor had 7 nozzles of different diameter connected to different port as per the requirement. Synthetic blackwater feed was prepared in a bucket of 15 litres which was pumped to the reactor from the top. Effluent was then removed from the bottom of the reactor and was fed to anaerobic membrane. This was reinforced, PVDF, hollow tubular, helix membrane with a pore size of 0.3 µm. Membrane had two outlets and single inlet. Out of 2, one outlet was used to withdraw permeate from the outside of the membrane while other which was from the inside, was recirculated to the reactor bottom. Permeate was collected through permeate pump in a permeate tank (bucket of 15 L). Biogas was collected from the top and measured with gas ritter. Limited aeration (14.7 mlair/Lrec/d) was provided from the bottom of the reactor through calibrated pump.

Synthetic black water feed was prepared by altering the recipe from Ozgun et al.,2013 to make the average chemical oxygen demand (COD) of 5g/L. Feed was prepared by adding the macro and micro nutrients as mentioned in Table 3.

*Table 3: Recipe for preparation of feed and micronutrients (adapted from Ozgun et al.,2013)*

| Micronutrient Solution Compound | Unit | Value |
|---|---|---|
| $FeCl_3.6H_2O$ | mg/L | 1000 |
| $CoCl_2.6H_2O$ | mg/L | 1000 |
| $MnCl_2.4H_2O$ | mg/L | 250 |
| $CuCl_2.2H_2O$ | mg/L | 15 |
| $ZnCl_2$ | mg/L | 25 |
| $H_3BO_3$ | mg/L | 25 |
| $(NH_4)_6Mo_7O_{24}.4H_2O$ | mg/L | 45 |
| $Na_2SeO_3.H_2O$ | mg/L | 50 |
| $NiCl_2.6H_2O$ | mg/L | 25 |
| EDTA | mg/L | 500 |
| HCl 36% | mL/L | 0.5 |

| Micronutrient Solution Compound | Unit | Value |
|---|---|---|
| Urea | mg/L | 1200 |
| $NH_4Cl$ | mg/L | 2000 |
| $CH_3COONa.3H_2O$ | mg/L | 7400 |
| Ovalbumin | mg/L | 450 |
| $MgSO_4.7H_2O$ | mg/L | 180 |
| $KH_2PO_4.3H_2O$ | mg/L | 1400 |
| $CaCl_2$ | mg/L | 264.9 |
| Starch | mg/L | 6400 |
| Milk powder | mg/L | 1500 |
| Yeast extract | mg/L | 600 |
| Sunflower oil | mg/L | 1000 |

| | | | | | |
|---|---|---|---|---|---|
| Resazurin sodium salt | mg/L | 250 | Micronutrients | mL/L | 26.6 |
| Yeast extract | mg/L | 1000 | Humic and Fulvic acid | mL/L | 0.2 |

10 L of feed was prepared twice a week. Feed bucket had mechanical agitator to ensure complete mixing through the feeding process to avoid settling of particle. Additionally, while preparing the feed after the addition of all the nutrients they were mixed properly with the hand blender particularly to mix oil droplets homogeneously. COD of the feed was checked daily to make sure reactor is fed with constant COD load. Feed was then fed to the bioreactor through pump with a flow of 2.5 L/day. Flow from the inlet pipe was measured on weight basis to made sure that flow remained constant during the entire operation.

Permeate was removed through creating suction from the membrane and collected in a permeate tank. Permeate flow was maintained at 2.3 L/day. Feed and permeate flow were adjusted in such a way that it allowed the 200 ml of sludge wastage on daily basis. Reactor level was maintained at 5.5 L with a HRT of 2.2 days and SRT of 27.5 days. Reactor temperature was maintained at $37^0$C to create mesophilic conditions by jacketing the reactor with circulated warm water. Other than parameters mentioned above, reactor was operated under following design parameters

*Table 4: AnMBR set-up operating parameters*

| Parameters | Value | Units |
|---|---|---|
| Reactor volume | 5.5 | L |
| Temperature | 37 | $^0$c |
| Solids retention time | 28 | days |
| Inflow | 2.5 | L/d |
| Hydraulic retention time | 2.3 | days |
| Permeate flow | 2.3 | L/d |
| Waste sludge flow | 0.2 | L/d |
| Flux | 10 | LMH |
| Recycle flow | 1190 | L/d |
| Organic loading rate | 5.42 | gCOD/L/d |

Membrane used in this set up was ultra-filtration membrane with a characteristic mentioned in the Table 5.

*Table 5: Membrane characteristics*

| Membrane characteristics | | |
|---|---|---|
| **Parameter** | **Value** | **Unit** |
| Pore size | 30 | nm |
| Type | Tubular, inside out | |
| Brand | Pentair | |
| Diameter | 5.2 | mm |
| Length | 64 | cm |
| Cross sectional area | 2.10E-05 | $m^2$ |
| Membrane area | 0.01 | $m^2$ |
| Cross flow velocity | 0.6 | $m.s^{-1}$ |

The membrane used in this study was a Reinforced PVDF membrane from Pentair. This membrane was connected through 3 joints out of which the top and bottom openings were connected to the bio-reactor and 3rd was connected to permeate pump. Pressure at all the 3 points were monitored continuously and transmembrane pressure (TMP) was calculated using

$$TMP = \frac{P1+P2}{P2} - P3 \qquad \textit{Equation 1}$$

$P_1$ pressure at the inlet (PIA-152) ..... see the Figure 4 for reference.
$P_2$ pressure at the inlet (PIA-151)
$P_3$ pressure at the inlet (PIA-150)

Biogas from the top of reactor was removed and vented to the atmosphere through the gas ritter. Gas ritter was used to measure the daily biogas production. Biogas sample was analysed for its composition.

Limited aeration of 14.7 mlair/Lrec/d was applied directly to the reactor through the pump which was equivalent to 3.1 $mlO_2$/Lrec/d. Girotto et al., concluded that 3 $mlO_2$/L/d was optimum oxygen amount that can be provided to have a positive effect on methane production, when the aeration was applied during the AD. Initially this aeration was applied gradually to avoid sudden change in the anaerobic environment. The aeration was divided in 3 cycles (8 hrs each) per day with each cycle consisting 4 hours aeration followed by 4 hours resting time. Automated timer was used as a signal to start and stop the pump for the set intervals.

This AnMBR set up was operated for more than a year without aeration and then 6 months with limited aeration. Automation for the said AnMBR was provided by by "CARYA Automatisering, The Netherlands" as represented in Figure 4.

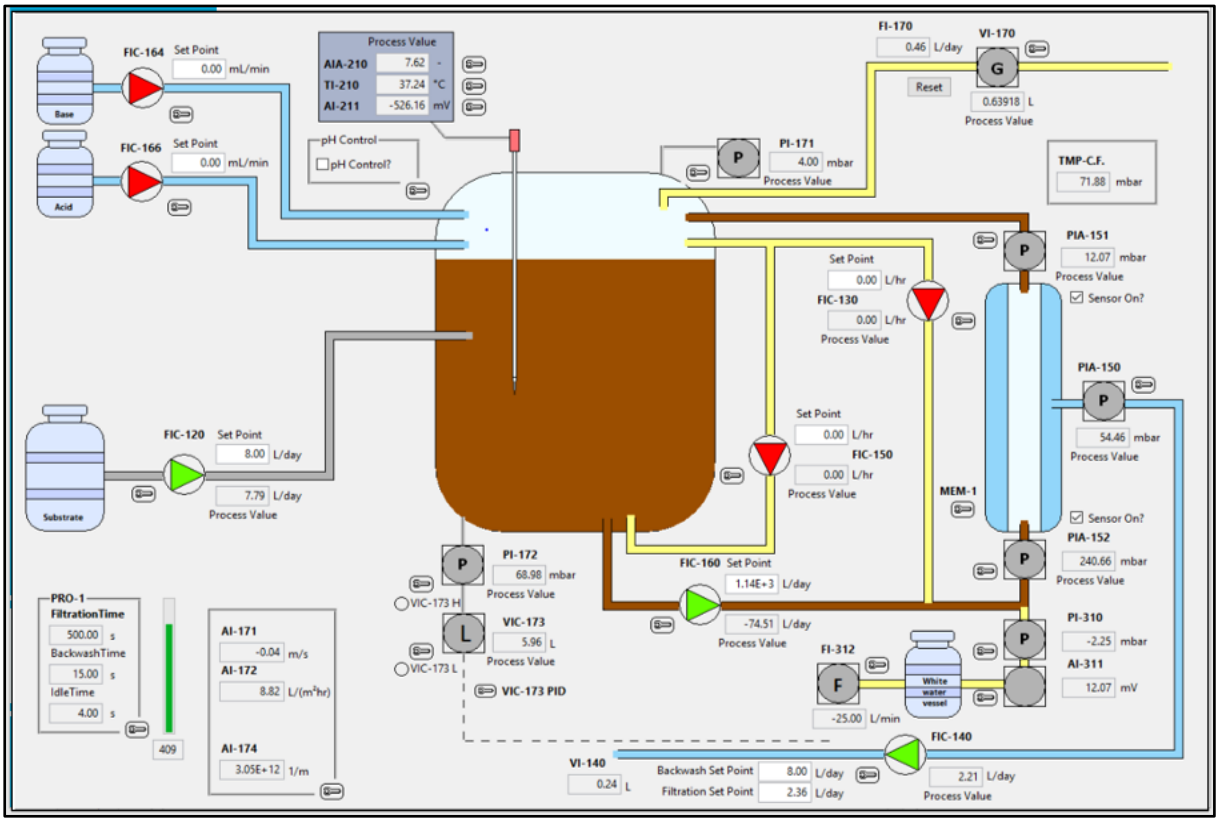


*Figure 4: AnMBR set -up by CARYA automation (Process flow diagram along with the instruments)*

Input to the system was enabled by the software. Pump operation as well as change in flow was done using this software. Besides this automation, manual operation was also possible. As shown in piping and Instrumentation diagram(Figure 4), software recorded data for the reactor through the sensors such as pH(AIA-210), ORP(AI-211), temperature(TI-210), pressure(PI-171), level(VIC-173) and stored in the database. System was provided with interlocks to avoid possible damage to the reactor. It shuts down the system if the set parameter goes beyond stipulated range to ensure safety.

The data obtained from the software was logged in the system for every 2 hours. The pH, temperature and ORP were measured by the sensor probe and was recorded daily. The biogas flow through the gas ritter was also recorded manually every day. COD was measured on a daily basis during the anaerobic run of the reactor and then measured on every alternate day. Other than COD, nutrients such as ammonium($NH_4^+$-N), ortho phosphate($PO_4$-P), nitrate($NO_3$-N) and sulphate($SO4^{-2}$) were measured once every 2 weeks. Sludge and permeate samples were analysed for volatile fatty acids (VFA) once in 2 weeks. Also, VSS and TSS of the sludge and influent was measure once in a week.

## 3.2 Analytical tests

### 3.2.1 Chemical Oxygen Demand (COD)

Chemical Oxygen Demand(COD) tests were carried out on every alternate day for influent and effluent. The COD of sludge and soluble COD of the influent were also measured to check the COD balance in the system. The COD tests were performed for the influent and sludge with the help of Hach Lange's LCK014 COD cuvettes for the range 1000- 10000mgCOD/L. For the effluent, Hach Lange's LCK314 COD cuvettes for the range 15-150mgCOD/l were used. The methods as described in the COD kit were used and the samples were digested in Hach Lange's LT200 oven for 2 hours at $148^0$C.

### 3.2.2 Nutrients and TSS/VSS

TSS and VSS of the sludge/feed were tested using Standard Methods described in APHA, 1992. The nutrients analysed for the study were $NH_4$-N, $NO_3$-N, $PO_4$-P and $SO_4^{-2}$. The analysis was done for the influent and effluent samples once every week. The samples were tested using Hach Lange's kits and the methods were followed as instructed in the kits. $NH_4$-N, $NO_3$-N, $PO_4$-P and $SO_4^{-2}$ were tested using LCK303, LCK350, LCK339 and LCK153 kits respectively. After the test procedure, the samples were analysed in spectrometer from Hach Lange.

### 3.2.3 Gas Chromatography

#### *3.2.3.1 Volatile Fatty Acids (VFAs)*

The composition of VFA in samples extracted from effluent and sludge was analysed by Gas Chromatography (GC) with a flame ion detector (FID). The samples were collected in 2 mL eppendorfs every day. The collected samples were prepared by centrifugation for ten minutes at 10,000.g in a high-speed mini-centrifuge (Microspin 12, Biosan). The supernatant from the centrifuged sample was extracted with a syringe and subsequently filtered through a 0.45 $\mu$m glass filter membrane. The filtered samples were then diluted with an internal standard, pentanol in a 1:1 ratio to obtain a 1.5 mL sample. About 10 $\mu$l of formic acid was added to each sample to further reduce microbiological activity and decrease the pH of the prepared samples for analysis. To prevent carry overs from adjacent samples queued for GC analysis, blanks of 1.5 mL were prepared using demineralized water and 10 $\mu$l of formic acid. The prepared samples were refrigerated at 4˚C until the time of analysis.

The composition of VFAs in the prepared samples was analysed by gas chromatograph (Agilent tech 7890A, U.S) with a capillary HP-FFAP column size of 25 m x 0.32 mm x 0.50 µm (Agilent 19091F-112, U.S.) using helium as a carrier gas (pressure = 11 psi, flow rate = 2.45 mL · $min^{-1}$). The temperatures of detector and injector were maintained at 225˚C and 240˚C respectively.

#### *3.2.3.2 Biogas*

For the analysis of biogas, the gas samples were collected using 1.5 mL syringe(s) after which they were immediately injected into a GC with thermal conductivity detector (TCD). To analyse the composition of the gas samples, two separate columns, HP-PLOT U and a Molesieve GC column (Agilent 19095P-MS6, U.S.) of 60 m x 0.53 mm x 200 µm were used along with helium as the carrier gas. The operational temperatures for the injector and detector were maintained at 200˚C each.

### 3.2.4 LCMS: MPs concentration

Liquid chromatography coupled mass spectrometry (LC–MS) was technique used to measure the very low concentrations of MPs. This technique used physical separation in tandem with the mass analysis capabilities providing the accurate results even at low concentrations.

#### *3.2.4.1 Preparation of standard MPs solution:*

Diclofenac (DCF), Metoprolol (MPT), Sulphamethoxazole (SMX) and Trimethoprim (TMP) were the 4 MPs ordered from Sigma Aldrich used for this study. Standard solution of these four selected MPs were prepared by mixing 1 mg of each of the MP in 1 l of ultrapure water and stored at -$20^0$C. After adding all the selected MPs, solution was stirred for 1 hr using magnetic stirrer to ensure homogeneity.

#### *3.2.4.2 Preparation of samples for LCMS:*

Samples (0.7ml) were taken out from each bottle at the required time interval and then immediately centrifuged at a speed of 10000 rpm for 10 mins. Solid and liquid phases were separated to analyse them further. These centrifuged samples are then filtered through 0.45 um filter. In this way filtered samples were collected and then stored at -$20^0$C, discarding the solid phase(sludge). Before running the LC-MS these filtered samples were again filtered through 0.2 um filter to ensure to particles will enter the LC-MS. Limit of quantification (LOQ) for this LC-MS for selected MPs was 0.001 to 10 ug/l therefore samples were diluted accordingly.

#### *3.2.4.3 Preparation of internal standards for LC-MS:*

An internal standard for each of the MP was used to track the analyte in the LC-MS quantitation based on the certain ratio of peak area of the analyte to that of the internal standard. This was used to adjust for the losses involved in the matrix or measurement. The internal standard for each of the MPs was prepared using their isotopes to ensure that it accurately replicates the behaviour of that particular MP. The LC-MS machine was capable of separating these two compounds.

The internal standard solution was prepared in ultrapure water (ELGA). Internal standard (0b as mentioned in the Table 6: Preparation of standards for LC-MS. was prepared by dissolving targeted MPs to get concentrations of 10 mg/l. Then 50 µL internal standard solutions (0b) should be added to 4950 µL of ELGA, to the final concentration of 100 µg/l. This was termed as “iSTDMix” and stored at -$20^0$C.

#### 3.2.4.4 *Preparation calibration curve for LC-MS:*

The calibration line standard was considered as an external standard in this condition which was prepared manually. Calibration curve was used to establish a standard and which was then compared to the actual concentrations of MPs detected by LC-MS. This curve was established by making standard solutions with respect to targeted MPs at concentrations ranging from 0 to 10 µg/l. Stock solutions "stock 1" and "stock 2" were prepared using internal standards (iSTDMix) as shown in the Table 7 below and then further diluted progressively to make calibration curve. All the calibration samples were prepared by 1st adding iSTDMix followed by (ELGA) ultra-pure water 2nd and lastly sample as indicated in below Table 7. By measuring standard solutions at a certain concentration, a linear calibration line was obtained in LC-MS and applied for the sample measurement.

The detailed instruction of the specific procedure to make the solutions were provided in appendix

*Table 6: Preparation of standards for LC-MS.*

| General preparations | name | concentration [µg/L] | Volume [µL] |
|---|---|---|---|
| **Step 0 a** | "single substance stock 0" | *10000* | *1000* |
| **Step 0 b** | "single iStd. stock" | *10000* | *1000* |
| **Step 1** | "iStd. Mix" | *100* | *1500* |
| **Step 2** | "stock 1" | *50* | *1000* |
| **Step 3** | "stock 2" | *0.5* | *500* |

*Table 7: Preparation of standards for calibration*

| Calibration standards how-to guide | | | | |
|---|---|---|---|---|
| **stock name =>** | **iStd. Mix** | **stock 1** | **stock 2** | **ELGA water** |
| **Target conc.** | **V to add** | **V to add** | **V to add** | **V to add** |
| **[µg/L]** | [µL] | [µL] | [µL] | [µL] |
| ***0*** | 10 | | 0 | 990 |
| ***0.0025*** | 10 | | 5 | 985 |
| ***0.005*** | 10 | | 10 | 980 |
| ***0.01*** | 10 | | 20 | 970 |
| ***0.05*** | 10 | | 100 | 890 |
| ***0.1*** | 10 | | 200 | 790 |
| ***0.5*** | 10 | 10 | | 980 |
| ***1*** | 10 | 20 | | 970 |
| ***2.5*** | 10 | 50 | | 940 |
| ***5*** | 10 | 100 | | 890 |
| ***10*** | 10 | 200 | | 790 |
| Sequence | iStd. Mix: 1st | stock: 3rd | stock: 3rd | ELGA water: 2nd |

## 3.3 Biodegradability tests

Biodegradability tests were conducted to assess specifically the biodegradability of selected 4 MPs. This was done in 2 batch tests: firstly, with changing the concentrations of MPs and later on changing the amount of air. This was done in order to assess the effect of MP's concentration and aeration on their removal through degradation respectively.

The BMP tests were carried out in 180 ml serum bottles as shown in Figure 5. The serum bottles used for this purpose were fitted with rubber stopper and further sealed with aluminium crimp using clamper. In this way bottles were sealed completely preventing any passage to the gas generated during the operation. The batch tests were performed following the protocol mentioned by Holliger et al.,2016, only instead of cellulose, sodium acetate trihydrate was used as a substrate.

*Figure 5: Serum bottles used for batch experiments*

Sludge withdrawn from the aerated anaerobic reactor was used as an inoculum for this batch tests. This reactor was operated with synthetic black water feed for more than 90 days. As mentioned above in the set-up, daily 200ml of sludge was wasted from the reactor and collected over a period of 20 days such that there was enough sludge to run the each of the batch tests. This collected sludge is stored in the fridge at $4^0$C to avoid any biomass activities. This inoculum was tested for COD, VS, TS, TSS and VFA before performing the batch tests and these values were tabulated for the sludge characterisation. This matrix is provided in the Appendix. Both the batch tests were performed at $37^0$C temperature to replicate similar environment as that of the reactor. Temperature was maintained by placing these bottles in the incubator. To create the mixing all these 180 ml bottles were rotated @ 120 revolutions per minute (RPM).

As per the literature there will endogenous generation of gas, particularly methane from the biomass itself even in the absence of substrate (Holliger et al.,2016). Therefore, accumulated sludge was subjected to incubation at $37^0$C to prevent this unwanted gas production. Additionally, blank samples were added which only contained sludge to analyse this biogas production through biomass without feeding anu substrate. Sludge was analysed for VSS,VS,TSS,TS before and after the incubation as some of biomass got volatilised decreasing its VS value.

Sodium acetate trihydrate (98% purity from Sigma-Aldrich) was used as a substrate for both the batch tests. For each of test, triplicates (3 bottles) were used. Weight of the substrate was calculated based on setting up the ration of VS of the inoculum to the VS of substrate as 2(Holliger et al.,2016). In addition to the acetate micronutrients were added and further diluted with demineralised (DEMI) water to make the total volume of 100 ml. bottles were then purged with $N_2$ to create the anaerobic environment stripping the air present inside the headspace.

Exactly similar experiments were performed with non-aerated anaerobic sludge stored previously.

#### *3.3.1.1 Batch test with different MPs and same aeration*

In addition to the above substrate (acetate and micronutrients) 4 numbers of selected MPs were added to the bottles with different concentrations. For the first set of batch test, 3 different concentrations of selected MPs were used. Concentrations used were in the increasing order as 100/200/300 ug/l to assess their effect on MPs degradation. In all the cases aeration was kept constant at 3.9 mlair/batch/d which was 2.03 % of the sludge VSS used in the reactor. Standard solution prepared for selected MPs as mentioned in the section 3.2.4, were used in this test.

#### *3.3.1.2 Batch test with different aeration and same MPs concentration:*

In this batch test all the selected MPs were added in same concentration which was 100 ug/l. Similar standard solution prepared for selected MPs as mentioned in the section 3.2.4, were used in this test too. But in this case, 3 different aerations were used which were 3.9/7/11 mlair/batch/d which were 2.03/3.1/4.85 % of the sludge VSS used in the reactor respectively.

For both batch tests, the acetate was used as a simple substrate for co-metabolism of MPs. Different MPs concentrations (with same aeration) were used in 1$^{st}$ batch while different aerations (with same MPs concentrations) were used in 2$^{nd}$ batch test. The aeration was provided for the first 5 days after substrate addition. Accordingly, the required amount of air was injected into the liquid phase, through a syringe.

The degradation of acetate along with MPs under these conditions were compared to standards to assess the biogas production. Standards were prepared by adding acetate only as a substrate without MPs and without aeration. 1ml of gas samples were collected and analysed for the first 4 days after incubation and then once every week, for biogas composition. The pressure was recorded twice a day.

The bottles were depressurised to atmospheric pressure using the glass syringe to prevent the over pressurisation. Calibration of gas syringes gave direct measurement for the gas production. These glass syringes were specially made for this purpose and were very sensitive enabling the accurate measurements. Utmost care was taken to maintain the incubator temperature at 37$^0$C as gas volume might change with the temperature.

## 3.4 Adsorption test

Adsorption was the important to analyse as some of the selected MPs were hydrophobic with high octanol-water coefficients. This adsorption test enabled the distinction of overall removal of MPs between the biodegradation and adsorption.

Exactly the similar serum bottles of 180 ml as mentioned for biodegradability test (Figure 5) were used for the adsorption test. This batch experiment was particularly focussed on adsorption of MPs onto the sludge therefore it was important to deactivate the biomass completely. To inhibit the activity of biomass, experiments were performed at very low temperature about 11$^0$C (Sergio et al., 2001). To create the mixing, all these 180 ml bottles were kept on shaker with speed of 120 RPM(refer Figure 6) Adsorption being a physical phenomenon was very fast process hence this experiment was planned for 8hrs. Additionally, bottles were kept open to atmosphere instead of sealing them which in way benefited to deactivate biomass further inhibiting the MPs undergoing biodegradation.

*Figure 6: Set-up of batch experiment (left: shaker with sample bottles, right: centrifuge)*

For this batch test both types of anaerobic sludge namely aerated and non-aerated were used. Additionally, granular activated carbon (GAC) was used to compare the adsorption kinetics of MPs onto sludge. Also blank was prepared to observe if there was any decomposition/hydrolysis of MPs without biomass and without GAC. Exactly similar substrate (sodium acetate trihydrate along with micronutrients diluted with DEMI water) was used so that results will be compared to that of the batch tests of biodegradation. Also, MPs used were having 3 different concentration as 100/200/300 ug/l as used before. Figure 7 shows each type of inoculum mentioned above with different concentrations of selected MPs.


*Figure 7: Sample bottles used for adsorption experiment (from left A1: Anaerobic aerated sludge with MPs 100 ug/l, B1: Anaerobic aerated sludge with MPs 200 ug/l,C1: Anaerobic aerated sludge with MPs 300 ug/l,D1: Anaerobic non-aerated sludge with MPs 100 ug/l,G1: GAC without sludge)*

Initially samples were taken after every 45 mins for 3 hrs and then after that samples were taken hourly. After taking the samples they were immediately centrifuged with a centrifuge shown in Figure 6 and filtered through 0.2 um filter and stored at -20$^0$C. These stored sampled were later on diluted while preparing for LC-MS analysis.

# 4

# Results

## 4.1 Reactor performance

Reactor was operated for more than 1.5 years. Initially it was fed with the synthetically prepared blackwater for about a year and later subjected to stepwise aeration. From May 2020, reactor was subjected to continuous aeration of 14.7 $ml_{air}/l_{rec}/d$. After 3 SRTs reactor had reached the stable conditions. Reactor performance was assessed comparing the dataset available during the adaptation period of aeration and after complete adaptation to the aeration.

### 4.1.1 COD and Nutrients removal

Limited aeration adapted reactor had shown better removal in all the aspects comparing the COD as well as the nutrients ($NH_4^+$,$NO_3^-$,$PO_4$,$SO_4^{-2}$) refer the Table 8 below

*Table 8: ANOVA results for different parameters indicating reactor performance*

| Aeration | Parameters | Influent | Effluent | Removal | ANOVA |
|---|---|---|---|---|---|
| | | average mg/l | average mg/l | average % | P-value for removal |
| B | COD | 5171 ± 212 | 88 ± 5.55 | 98.2 ± 0.1 | 4.19E-09 |
| A | | 5712 ± 778 | 75 ± 13 | 98.9 ± 0.3 | |
| B | $NH_4^+$-N | 244 ± 3.5 | 616 ± 95 | -151 ± 36 | 0.557* |
| A | | 347 ± 23 | 896 + 53 | -158 ± 19 | |
| B | $NO_3^-$-N | 1.24 ± 0.2 | 0.64 ± 0.01 | 47.8 ± 1.9 | 4.49E-07 |
| A | | 1.45 ± 0.2 | 0.57 ± 0.02 | 59.8 ± 5.6 | |
| B | $SO_4^{-2}$ | 273 ± 2.6 | 30.1 ± 1.6 | 89 ± 0.5 | 0.611 |
| A | | 182 ± 27 | 19.5 ± 2.4 | 89.2 ± 1.2 | |
| B | $PO_4$-P | 68.1 ± 2.5 | 53.4 ± 1.5 | 21.4 ± 5 | 0.375 |
| A | | 58.2 ± 3.2 | 46.6 ± 3.6 | 19.8 ± 4.3 | |

*Where, B indicates the phase before and during the aeration and A represents phase of a reactor after complete adaptation to subjected aeration. (-ve removal efficiency indicates Ammonia has increased in effluent).*

P-value greater than 0.05 in Table 8, indicated the null hypothesis can be accepted and there is no significant difference between the two groups. Meaning, the observed change in the average values upon adaptation of the reactor to the subjected aeration of 14.7mlair/Lrec/d were insignificant. Therefore, the added aeration had no significant effect on that particular parameter.

From the table above there was no significant change in increase of $NH_4^+$-N as well as removal of $SO_4^{-2}$ and $PO_4$(P>0.05). However, the COD removal and $NO_3$ removal was significantly different as indicated by the P value which was less than 0.05 in both cases.

Additionally, It was observed that there was significant difference (P value < 0.05) in average values of influent as well as effluent for COD and all the four nutrients ($NH_4^+$,$NO_3$,$PO_4$,$SO_4^{-2}$) between the groups during the adaptation period and complete adaptation to aeration.

### 4.1.2 Other parameters

Reactor performance was monitored for other critical parameters like ORP, pH, Temperature, Biogas production and composition, presence of VFAs. Regarding pH, before the complete adaptation average reactor pH was 7.5 which was increased to 7.7. Variations in the feed pH were minimised by replacing magnetic stirrer to mechanical agitator. Feed pH was stable at 6.3 with a standard deviation of 0.05, earlier which was fluctuating in a range of 6 to 8. During the adaptation period ORP was initially reduced to -500 mV upon subjected aeration and then improved to -520 mV during the adaptation. After the complete adaptation ORP was stable to -530 mV.

During the initial phases of aeration VFA accumulation was observed with detection of I C6 (iso-caproic acid) and C6 (Caproic Acid) in the sludge. After the complete adaptation to the provided aeration this VFA concentration was found to be nil.

The biogas flow from the reactor was measured through the reading of gas ritter. Before aeration the average biogas production was observed to be in the range of 2.3-2.5 l/d. Even after the complete adaptation the value remained the same and no significant difference was observed. However, biogas composition had changed increasing the methane content from 84% to 89% while rest was $CO_2$. This biogas composition did not change significantly even after the complete adaptation.

## 4.2 Total removal of MPs

Selected four numbers of MPs were tested to assess their removal in a batch test. Two sets of batch tests were performed, one with different MPs concentrations and other with different aerations.

### 4.2.1 Different concentration

Aerated anaerobic sludge withdrawn from the reactor was used to evaluate the removal efficiencies of selected MPs through biodegradation experiments. Additionally, previously stored strictly anaerobic sludge was also used for the same for comparison. Selected MPs were used in 3 different concentrations as 100 ug/l, 200 ug/l, 300 ug/l to assess the effect of different concentration on their removal efficiencies. Figure 8 below, describes the graphical results for the batch experiments.

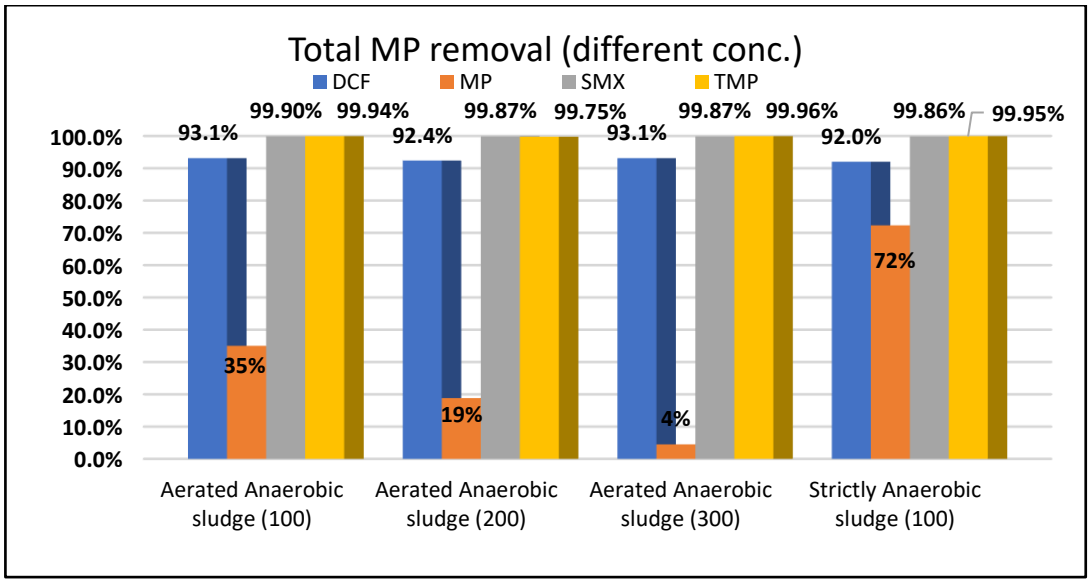


*Figure 8: Removal of MPs with different concentrations (*100/200/300 indicated the MPs concentration in ug/l respectively)*

As shown in the Figure 8, SMX and TMP were removed more than 99% and there was no effect of increase in concentrations on their removal as it was almost same in each case. This removal was observed at the end of 15 days when acetate was completely consumed giving the calculated biogas production.

For Diclofenac removal efficiency (92%) was lesser than SMX,TMP(>99%) and also it was observed that the removal efficiency did not change with concentrations.

For all the above mentioned MPs(DCF,SMX,TMP) there was no significant difference observed between aerated anaerobic sludge and strictly anaerobic sludge.

For metoprolol, removal efficiency decreased with increase in concentration. Also, with strict anaerobic sludge removal efficiency was significantly higher (72%) compared to that of aerated anaerobic sludge (35% maximum).

### 4.2.2 Different aerations

Aerated anaerobic sludge withdrawn from the reactor was used to evaluate the removal efficiencies of selected MPs through biodegradation experiments. For this batch test bottles were prepared with the same concentration of all the 4 selected MPs which was 100 ug/l. These bottles were subjected to 3 different aerations as, 3.9/7/11 mlair/batch/d to assess the effect of different aerations on their removal efficiencies.

Figure 9 below, describes the results for the batch experiments.

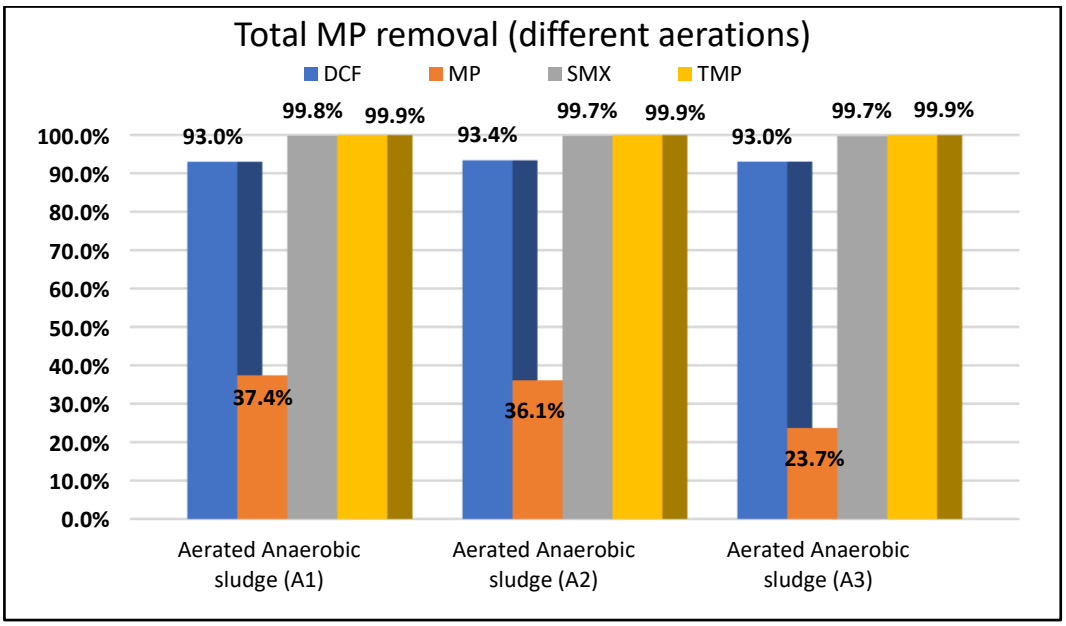


*Figure 9: Removal of MPs with different aerations (*A1/A2/A3 indicated different aerations 3.9/7/11 mlair/batch/d respectively)*

As shown in the Figure 9, SMX and TMP were removed more than 99% and there was no effect of aeration on their removal as it was exactly same in each case. This removal was observed at the end of 15 days when acetate was completely consumed giving the calculated biogas production.

For Diclofenac, removal efficiency was a less (93%) than that of above 2 MPs (SMX,TMP more than 99%) but then the removal efficiency did not change with different aerations.

For metoprolol, removal efficiency decreased with increase in aeration from 37.4% maximum for 3.9 mlair/batch/d to 23.7% minimum for 11 mlair/batch/d.

## 4.3 Adsorption of MPs

Aerated anaerobic sludge withdrawn from the reactor as well as previously stored strictly anaerobic sludge was used to evaluate the removal efficiencies of selected MPs through the adsorption experiments. Additionally, granular activated carbon (GAC) was also used to compare the adsorption kinetics. Selected MPs were used in 3 different concentrations as 100 ug/l, 200 ug/l, 300 ug/l to assess the effect of different concentration on their removal efficiencies through adsorption.

Figure 10, below describes the results for the batch experiments.

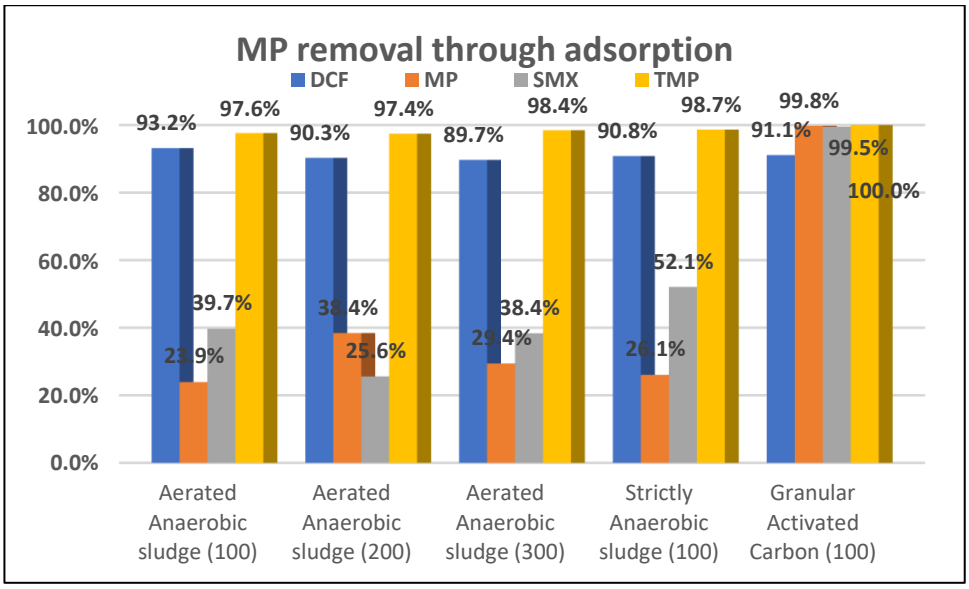


*Figure 10: Removal of MPs with different concentrations (*100/200/300 indicated the MPs concentration in ug/l respectively)*

From the above Figure 10, DCF was well adsorbed on both the types of sludge as well as on GAC with more than 90% removal. It was observed that with increase in concentration of DCF it adsorbed less. For lower concentration (100ug/l DCF) removal efficiency was highest (93.2%).

Adsorption of Metoprolol and SMX were low (23 to 39%) on the aerated anaerobic sludge. There was no trend increasing/decreasing observed which proves the effect of different concentrations on their removal. Though it was observed that metoprolol adsorbed more on the strict anaerobic sludge (52.1%).

TMP showed the highest removal efficiency through adsorption with more than 97% removal in each case. Its removal was slightly increased with increase in concentration.

GAC being the popular adsorbent showed the highest removal efficiency for all the 4 selected MPs. Adsorption of DCF on GAC was lesser (91.1%) compared to that of other three (MPT,SMX,TMP) which is above 99%.

## 4.4 Biodegradation of MPs

Results from the section 4.2 and 4.3 were used to determine the removal of MPs through biodegradation. Total removal of each of the MPs through biodegradation and adsorption was shown in the Table 9.

*Table 9: Summary of removal of all the four selected MPs with respect to biodegradation and adsorption (*100/200/300 indicated the MPs concentration in ug/l respectively).*

| Type of sludge | Removal efficiency of Micropollutants | | | | | |
|---|---|---|---|---|---|---|
| | Removal of Diclofenac (DCF) | | | Removal of Metaprolol (MP) | | |
| | Total | Adsorption | Biodegradation | Total | Adsorption | Biodegradation |
| Aerated Anaerobic sludge (100) | 93.1% | 93.2% | 0.0% | 35.0% | 23.9% | 11.1% |
| Aerated Anaerobic sludge (200) | 92.4% | 90.3% | 2.1% | 18.8% | 38.4% | -19.6% |
| Aerated Anaerobic sludge (300) | 93.1% | 89.7% | 3.5% | 4.5% | 29.4% | -24.9% |
| Strictly Anaerobic sludge (100) | 92.0% | 90.8% | 1.2% | 72.3% | 26.1% | 46.3% |
| Granular Activated Carbon (100) | | 91.1% | | | 99.8% | |

| Type of sludge | Removal efficiency of Micropollutants | | | | | |
|---|---|---|---|---|---|---|
| | Removal of Sulphamethaxazole (SMX) | | | Removal of Trimethorpim (TMP) | | |
| | Total | Adsorption | Biodegradation | Total | Adsorption | Biodegradation |
| Aerated Anaerobic sludge (100) | 99.9% | 39.7% | 60.2% | 99.9% | 97.6% | 2.3% |
| Aerated Anaerobic sludge (200) | 99.9% | 25.6% | 74.3% | 99.7% | 97.4% | 2.3% |
| Aerated Anaerobic sludge (300) | 99.9% | 38.4% | 61.5% | 100.0% | 98.4% | 1.5% |
| Strictly Anaerobic sludge (100) | 99.9% | 52.1% | 47.8% | 100.0% | 98.7% | 1.3% |
| Granular Activated Carbon (100) | | 99.5% | | | 100.0% | |

For all the selected MPs overall removal efficiency was higher than 92% except metoprolol.

In case of metoprolol, it was around 35%, which was maximum for aerated anaerobic sludge. However, strict anaerobic sludge shown better overall removal up to 72.3% predominantly though biodegradation (46.2%) followed by adsorption (26%).

Increase in concentration of MPs did not show clear trend on how it affected the actual removal of selected MPs. Though, metoprolol showed clear decline in their total removal efficiency at higher concentration.

The combined results clearly indicated that adsorption was dominant compared to biodegradation in the total removal of all selected MPs except SMX. While on the contrary, in case of SMX biodegradation dominated the overall removal efficiency.

# 5

# Discussion

This section contains the detailed discussion and analysis of the results from the previous section 4. This section provides insights of some useful results obtained from the experiments performed, considering their limitations.

## 5.1 Reactor performance: COD and nutrients

As mentioned in the results section reactor performance was assessed in terms of nutrients ($NH_4^+$,$NO_3$,$PO_4$,$SO_4^{-2}$) and overall COD removal.

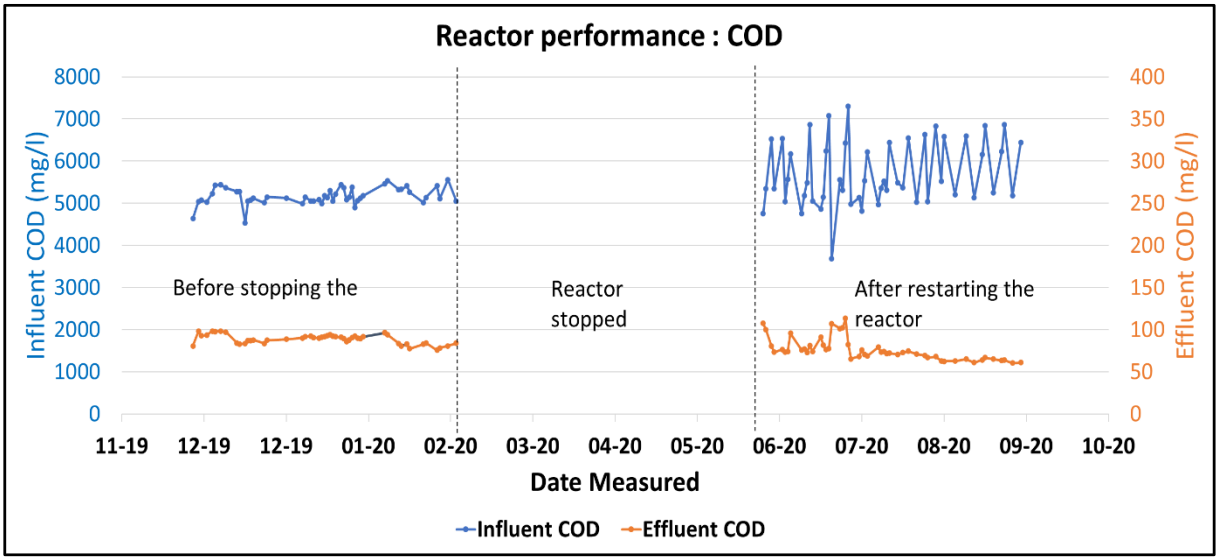


*Figure 11: COD in mg/l for influent and effluent before and after the aeration.*

As seen the from the Figure 11, large variation in the feed COD (avg. COD 5732 mg/l with std. deviation of 778 mg/l) was observed in the later period of reactor operation. Reason behind that may be the change in mixing of the feed due to replacement of magnetic stirrer by mechanical rotor as shown in Figure 12 below. Mechanical rotor created intense mixing avoiding the settling of particles in the feed bucket. Therefore, these undissolved particles always stayed in suspension and carried along in the tubes feeding reactor. To analyse it further, samples from feed bucket were compared to the samples taken from the inlet tube of the reactor.

It was observed that after the preparation of feed there were no accumulation of particles inside the tubes but over the time, these particles got settled inside of the tubes, increasing the COD. Figure 12, shows the picture how the particles were accumulated inside of the tubes.

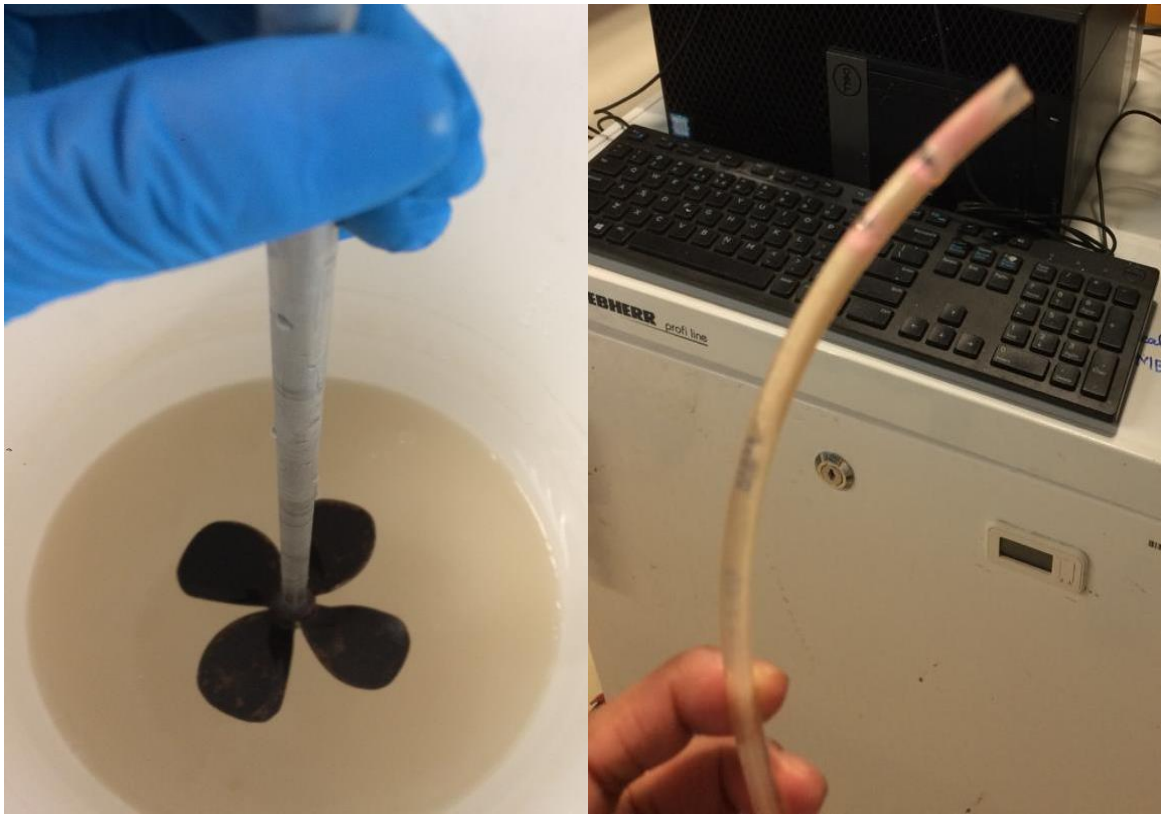

*Figure 12: Replaced new mechanical rotor (left), clogged tube feeding the reactor(right)*

Though, reactor was fed with the higher average COD compared to the previous period, It was observed that COD removal efficiency was significantly improved reducing the permeate COD value to less than 70 mg/l compared to previously reported 85 mg/l. W. Zhou et. al., 2007 also reported 40% increase in COD removal after the aeration. This improved quality might be due to the better biodegradation of not readily degradable compounds such as humic acids, ovalbumin, sunflower oil due to introduction of limited aeration.

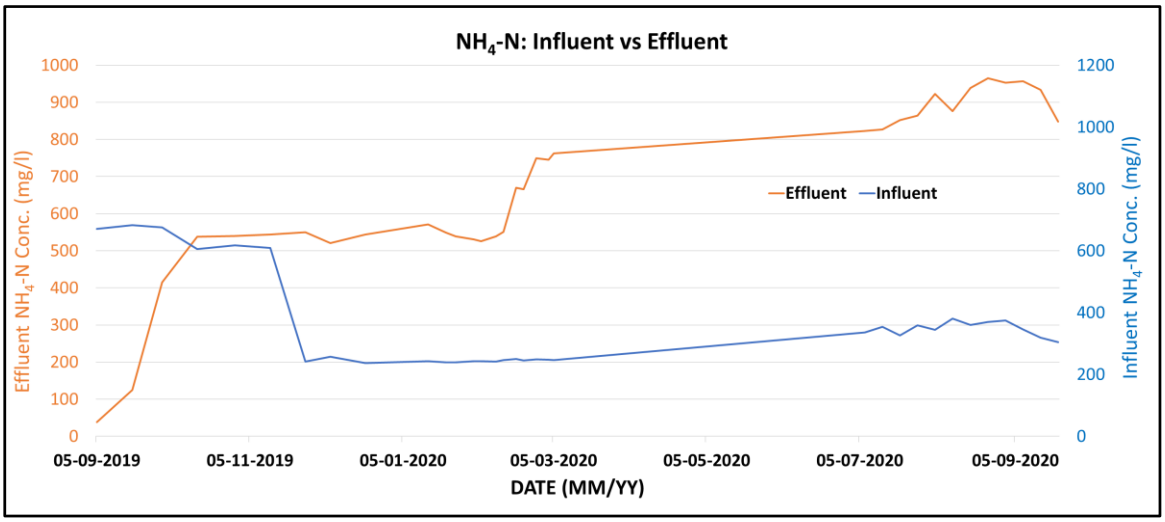


*Figure 13: Ammonia-N mg $NH_4^+$/l in influent and effluent before and after the aeration*

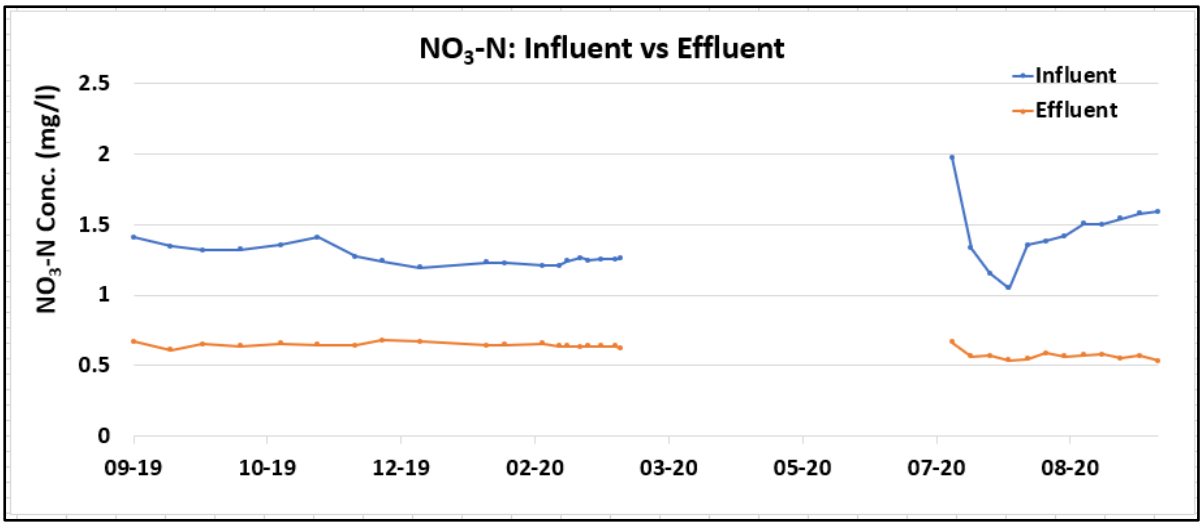


*Figure 14: Nitrate-N mg $NO_3$/l in influent and effluent before and after the aeration*

Ammonification of organic nitrogen increases the ammonia during the anaerobic treatment process (A. Smith et al.,2013)(C. Chernicharo et al., 2006). Inline with this research considering the $NH_4^+$-N as shown in the Figure 13, concentration had increased in the permeate, though, overall increase, compared to the data before and after the aeration, was not significantly different. Initial changes observed in the Figure 13, was due to change in feed composition.

According to Stickland reaction during acidogenesis of amino acids ammonia is produced (J. Lim, J. Chiam and J.Wang, 2014). Additionally, Diak et al., reported increase in the effluent ammonium concentration by 7% with aeration. Hence, the increase in $NH_4$-N concentration could be due to degradation of proteinaceous compounds like ovalbumin also it could be related to a better degradation of nitrogen containing substrates such as proteins and urea. This increase in $NH_4$-N supports the fact that overall COD reduction was improved by limited aeration (Joss et al., 2013).

For Nitrates, as shown in Figure 14, removal efficiency was significantly improved. This is because $NO_3$ in the feed had increased as can be seen from the Figure 14, while in the effluent it remained constant increasing total removal. This might be due homogeneous mixing of feed with respect to installation of mechanical mixer.

To make the cycle complete and balance the Nitrogen, Total $N_2$ along with the $NO_2$ were measured with the kits. Table 10 showed the overall N balance:

*Table 10: Overall N- balance for the reactor (all concentrations are in mg/l).*

| Nutrients | Influent avg. | Effluent avg. |
|---|---|---|
| **$NO_3^-$-N** | 1.09±0.03 | 0.64±0.06 |
| | 1.33±0.07 | 0.56±0.01 |
| **$NO_2^-$-N** | 0.28±0.04 | 0 |
| | 0.29±0.04 | 0 |
| **$NH_4^+$-N** | 324±64 | 845±45 |
| | 315±34 | 860±13 |
| **Total N** | 877±26 | 885±45 |
| | 904±37 | 895±10 |

Ammonium present in the feed was due the ammonium chloride, while ammonium in the effluent contains the ammonia through the release of amide group of urea as well as degradation of amino group present in proteinaceous ovalbumin (Henderson et al., 2007). Nitrite were tested in both influent and effluent but was very low to contribute to the $N_2$ balance. Ideally total $N_2$ in the effluent should be less than that of influent but here it showed some increase this might be due to manual error or sampling gap. Though, from the Table 10, it was clear that $NH_4$-N dominated the total $N_2$ balance and contribution from nitrates and nitrite was negligible.

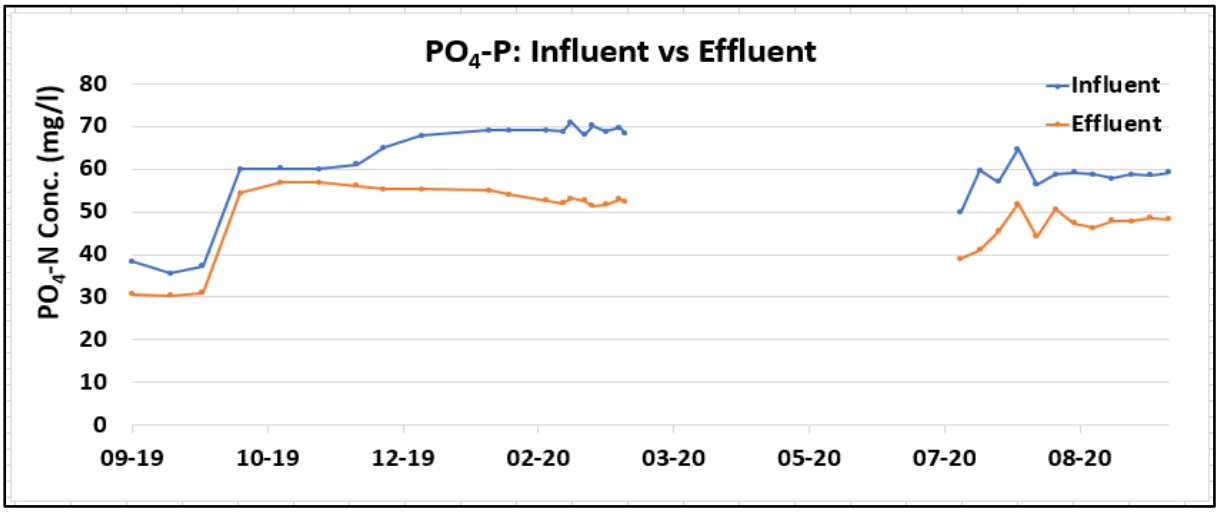


*Figure 15: Ortho phosphate-P mg $PO_4$/l in influent and effluent before and after the aeration*

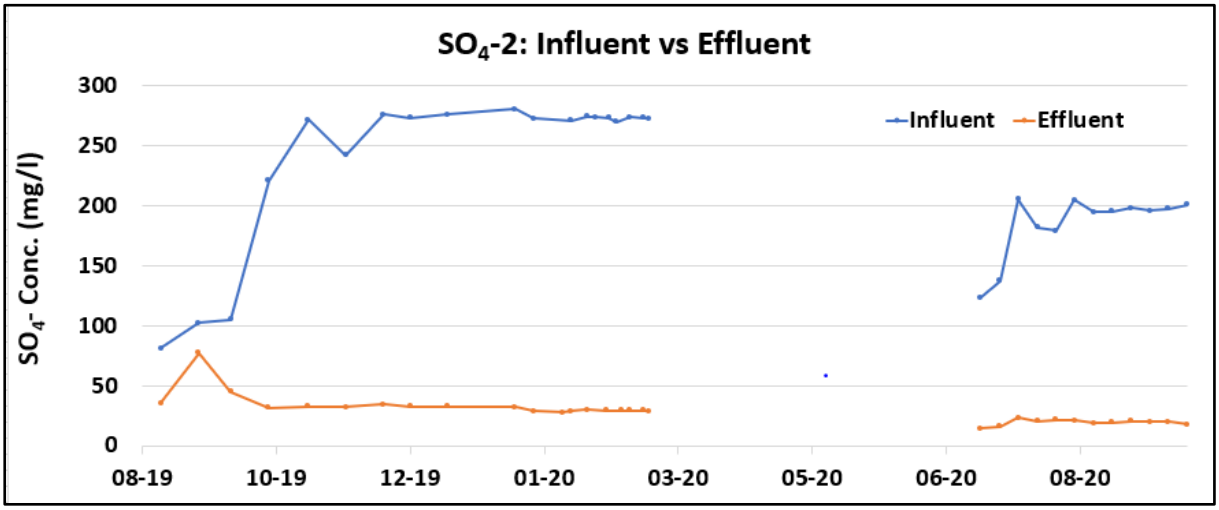


*Figure 16: Sulphate mg $SO_4^{-2}$/l in influent and effluent before and after the aeration*

$SO_4^{-2}$ and $PO_4$ both were removed but removal efficiency was not significantly different.

In contrast to the literature (Ziang et al., 2013) where phosphate was released under the anaerobic conditions here ortho phosphate was removed. This might be because of lower COD:P ratio which had to be maintained in anaerobic reactor as phosphorus is one of the vital nutrient for microorganisms (van Lier et al., 2008). Additionally, limited aeration had increased the pH of the reactor which might had helped precipitating the phosphate (Ramos and Gwaka, 2004). This phosphate precipitation could be in the form of calcium and magnesium salts (Möller and Müller, 2002). Bouropoulos and

Koutsoukos (2010), posited that at pH higher than 8.5, phosphate precipitates as struvite for pH higher than 8.5

Sulphate might had been reduced by the sulphur reducing bacteria. Though reduction of sulphate to sulphite or elemental sulphur was not known as GC could not measure the $H_2S$ from biogas. As shown in the Figure 16, variation was observed in the influent where the value was lesser than previous feed. However, it did not alter overall removal significantly as effluent value had also been reduced accordingly.

## 5.2 Reactor performance: Other parameters

Reactor alkalinity is determined by the feed composition and concentration of its components. Generally, large amount of proteinaceous wastes is related to higher alkalinity of reactor. The alkalinity is the result of the release of amino groups ($-NH_2$) and production of ammonia ($NH_3$) as the proteinaceous wastes are degraded.

Alkalinity is present primarily in the form of bicarbonates that are in equilibrium with carbon dioxide in the biogas at a given pH. When organic compounds are degraded, carbon dioxide is released. When amino acids and proteins are degraded, carbon dioxide and ammonia are released. Therefore, equilibrium between carbonic acid, bicarbonate alkalinity, and carbonate alkalinity as well as ammonia and ammonium ions is a function of digester pH.

Reactor has stable performance upon induced aeration as amino acids and proteins are degraded, and alkalinity is produced increasing the reactor pH.

Biogas quality in terms of methane content had improved with limited aeration. Similar results were observed by Fu et al.,(2016) Nguyen et al. 2007. Additionally, Ahn et. al.,(2014) observed 25% increase in methane yield while aerating the system treating sewage sludge with 0.05 Lair/Lrec/min for 24 hours.

Reactor performance was stable with no VFA accumulation after adapting to the subjected aeration. In previous studies it was already observed by Ramos and Fdz-Polanco (2010), Botheju et al.,(2010) that limited aeration reduces the VFA accumulation. J. Lim, J. Chiam and J.Wang, (2014) reported that the short-chain fatty acids (SCFAs) are readily get converted to simple acetic acid with the limited aeration while mentioning that effect of aeration might be different in case of nature of inoculum.

## 5.3 Removal of MPs (different concentration of MPs and different aerations)

Removal of MPs through adsorption had to be considered although the experiments were performed to assess the biodegradation. Experiments were performed with acetate as simple substrate for 12 days till the acetate was completely consumed. From the graphs attached in Annexure 3 it was observed that concentration was decreased sharply on 1$^{st}$ day itself for all the selected MPs except for metoprolol. This removal must be due to adsorption as removal through biodegradation will take more time for activation of biomass and to start degradation. Additionally, MPs being recalcitrant were not expected to be removed on the 1$^{st}$ day itself through biodegradation.

As mentioned in the results, it was observed that concentration for SMX and TMP were gradually decreased over the time period indicating their bio-transformations, as samples were taken after every 3 days. In the literature also, it was reported that SMX and TMP were removed through

biotransformation under the anaerobic conditions (T. Alvarino et al.,2016)(Marta Carballa et al.,2007) (T. Alvarino et al.,2014). Past studies posited that, substituted heterocyclic compounds are more incline to undergo anaerobic degradation. Therefore, presence of substituted pyrimidine group in the structure of TMP might be responsible for its bio degradation. While SMX has sulphonyl group present in its structure. Due to electron withdrawing nature of sulphonyl group it gets degraded under anaerobic conditions (T. Alvarino et al.,2016).

In case of DCF, its concentration remained constant over the entire period indicating no biodegradation over the entire time period. In the literature it was observed that limited aeration triggered the removal of DCF through ammonium monooxygenase (AMO) enzyme and helped to degrade it even under the anaerobic conditions (T. Alvarino et al.,2014) Unfortunately, this phenomenon was not observed in this experiment. Reason could be the amount of air supplied during the experiment was less as Patricia et al.,(2019) used 0.1 ml/min of air for their experiments.

Metoprolol showed interesting behaviour decreasing the concentration up to 6 days and then further increasing it at the end of the experiment. This could be related to the subjected aeration, as the aeration was provided for initial 5 days and then after it was discontinued. This phenomenon was not clearly understood, also no literature was found to support this.

However, metoprolol degraded well under strict anaerobic (without aeration) conditions giving the higher removal about 72% compared to maximum 35% in case of aerated sludge.

Effect of increase in concentration had negligible effect on the removal of all the selected MPs except metoprolol. Metoprolol degraded better with lower concentration. Additionally, amount of air supplied might have affected its removal.

Similar experiments were performed with different aerations with constant MPs concentrations but no significant change in their removal was observed. This might be because the air was introduced in pulse manner at once instead of stepwise. Providing the sequential aeration (say after every 2 hrs) was not feasible because of the time constraint and manual work involved in it. This might have resulted in inefficient use of provided aeration. Air might have escaped in the head space available in the bottes which was observed with increase in pressure of the bottles after the pulse aeration.

Also, it might be possible that sludge behaviour has changed upon the induced aeration.

## 5.4 Removal of MPs through adsorption

To assess the MPs removal only through adsorption it was necessary to inhibit the microorganism making sure that there was no biodegradation happening. From the methods available for biomass inhibition, low temperature technique was used to deactivate this mesophilic biomass obtained from the reactor. Temperature was maintained low at $11^0$C throughout the experiment. Adsorption being the physical process, very fast in nature. Therefore, experiment was performed for 8 hrs. This also helped prohibiting the removal of MPs through biodegradation given the very less time designed for the experiment. Furthermore, measured initial and final COD of blank (avg. 4830 mg/l and avg. 5120 mg/l respectively) ensured there was no biodegradation.

Purpose of this experiment was to find the kinetics of the adsorption process as in the previous biodegradation experiments it could not be assessed being fast nature of this process. Therefore, experiment was planned for 8 hrs focussing on initial 4 hours where the adsorption was expected to

be very fast with sampling frequency of 30 mins. To assess the kinetics, granular activated carbon (GAC) was used for comparison. Additionally, previously stored strict anaerobic sludge was also used to assess the adsorption.

Besides the estimation on how fast the adsorption could be, the results obtained for the adsorption experiment were found to be similar to that of previously performed degradation experiments. First samples were taken at t=0 but considering the number of samples it took 10 mins to collect all the samples. Unfortunately, adsorption was so fast that all selected MPs got adsorbed so quickly that the concentration almost remained constant thought the experiment for all the samples taken thereafter. This can be observed from the graphs presented in Annexure 5. Though, in case of TMP, concentration was decreased gradually contradicting the results observed for other MPs. Also, with GAC it was observed that concentration was somewhat decreased gradually after initial sharp fall for TMP and SMX.

Though, kinetics of the adsorption was not analysed, some important findings were obtained about the adsorption of MPs.

Octanol water coefficient ($K_{ow}$) played vital role in deciding the overall adsorption removal efficiency. In case of DCF with high $K_{ow}$(4.2), It got readily adsorbed due to highly hydrophobic nature compared to others with comparatively lower $K_{ow}$(<2) values. It was evident that higher the $K_{ow}$, higher the adsorption. Rogers (1996) and Ter Laak et al., (2005) also observed similar results with their experiments.

Metoprolol was selected being the cationic in the nature. Sludge being negatively charged it was expected that the metoprolol would adsorb more given the interaction between opposite charges. This hypothesis did not hold true. On the other hand, besides having anionic nature, DCF adsorbed best amongst all the 4 selected MPs. It clearly proved the importance of $K_{ow}$ over the electrical charge of selected MPs.

As discussed DCF having the highest $K_{ow}$ value, highly hydrophobic in nature. As a result, solubility of DCF in water was very low about 2.37 mg/l @ $25^0$C (Finn A et al.,1986). During the adsorption experiments temperature was maintained around $11^0$C reducing the DCF's solubility further. This might be the reason behind the high adsorption of DCF which might be difficult at higher temperature. L. Zilnika et. al., (2007) investigated the effect temperature on DCF solubility and posited that it decreased with decrease in temperature.

## 5.5 Removal of MPs through volatilisation

Volatilisation process can be defined as when MP from the dissolved phase enter to the gaseous state and therefore getting removed from the system. This transfer from liquid to gas phase is driven by physicochemical properties of the micropollutant (Henry's constant, H) and on the operating conditions of the process (i.e., aeration, agitation, temperature and atmospheric pressure).

Generally pharmaceutical compounds, hormones and metals did not include volatilisation because it is not considered a significant removal mechanism for these families (Wang et al., 2003)(Urase and Kikuta,2005)(Plosz et al., 2010).

Additionally, Byrns (2001) analysed large range MPs for their volatilisation and concluded that 9.86e-4 atm.m3mol−1 is the threshold value of Henry's constant (H) for the volatilisation to occur. For MPs

less than this threshold value volatilisation is not significant. As mentioned in the Table 2 in section 2.5, for all the selected MPs H value is less than the threshold mentioned hence they were not tested for volatilisation.

## 5.6 Applicability of the batch results to reactor operation

Although, total removal was observed to be more than 90% for all the selected MPs except metoprolol the time required to achieve this removal was around 12 to 15 days which is not feasible with the reactor as it has the HRT of 2 days. Therefore, while spiking the reactor with the MPs, it should be noted that total removal can be significantly reduced compared to that of batch experiments. For the close estimation, removal of MPs after 2 days in case of batch experiments will be considered as final removal while operating the reactor.

Strictly anaerobic sludge showed better removal in case of metoprolol but then time required was significantly more. Hence value obtained as total removal should always be used in context of the time required to achieve this removal.

Furthermore, reactor is coupled with AnMBR which was not possible in case of batch tests. Once adsorbed, MPs might stay there as a result of retention by the membrane at least up to SRT based on the size exclusion principle. These adsorbed MPs might be degraded later on in the reactor.

All the batch experiments were performed with 120 rpm rotation, to replicate the mixing happening in the reactor. Though the actual mixing taking place in the reactor might produce different results.

Regarding the aeration, reactor might perform better as aeration can be done in sequential manner in contrast with the pulse aeration that was applied during the batch experiments.

Adsorption experiments were done at low temperature ($11^0$C) to prevent removal of MPs through biodegradation. Temperature plays an important role in determining the extent of adsorption. Therefore, adsorption might change in case of reactor which is operated at $37^0$C. Because of the very low solubility at lower temperature($11^0$C) DCF might have adsorbed more which might not be possible with the reactor due to higher temperature($37^0$C).

# 6

# Recommendations

The study uses various experimental and statistical methods to investigate the removal of MPs through anaerobic digestion provided with limited aeration. This chapter provides some recommendations to improve the experimental methods used in this study and further research that could provide a better insight into the research.

Reactor performance was evaluated after the complete adaptation to the subjected aeration with respect to COD and other nutrients ($NH_4^+$-N, $NO_3$-N, $PO_4$-P, $SO_4^{-2}$), VFA, biogas production and composition. Total $N_2$ to be measured additionally to provide the $N_2$ balance. Sulphate was getting removed through SRBs. To get better insights besides influent and effluent, sulphate to be measured in the sludge as well. Additionally, $H_2S$ to be measured in the biogas as limited aeration has improved the removal efficiency of sulphate. In that way it will be well understood that how the sulphate is getting removed.

To avoid the COD fluctuations in the feed to the reactor, higher flow with higher velocity to be used with intermittent feeding to ensure the accumulation of particle as less as possible inside the tube. Biogas to be recirculated to the reactor to ensure the proper mixing. Sludge characteristics to be measured to assess the impact of limited aeration.

In this study, MPs used were selected based on their distinctive properties and degradation followed in different environment. More number of MPs can be assessed to see the reliable trends among the different groups. MPs selected were used in higher concentration than that of actually present in normal blackwater, to assess their removal and impact of different aerations as well as different concentrations. It would be interesting to see how it affects their removal when used in their actual concentrations present in blackwater.

Reactor was operated with synthetically prepared blackwater. However, batch tests were performed with simple sodium acetate trihydrate along with the concentration of selected MPs. It will be interesting to observe the behaviour of these MPs in presence of other compounds present in blackwater.

LC-MS was used for the analysis of MPs. This technique can measure multiple MPs at once so there is possibility to test more MPs in addition to the selected four. LC-MS used was calibrated to measure the samples in a range of 1 to 10 ug/l. There is a possibility to alter this calibration. Hence, If MPs used are in high concentration then new calibration can be used to minimise the error through the dilutions.

Batch tests were performed to assess the biodegradation of selected MPs. Additionally, to analyse the impact of different aerations on their removal, calculated amount of air was injected to the bottles at once. This was done for initial 5 days. Instead air can be provided in sequential manner for a long run to achieve estimated benefits.

Batch test was performed to assess kinetics of adsorption for selected MPs. But unfortunately, adsorption was too fast to measure the rate/kinetics. Adsorption was measured for aerated anaerobic

sludge, strict anaerobic sludge as well as for GAC at once. This increased the total number of samples making it difficult to sample for close interval. This can be avoided and only single sample set to be tested with a sampling duration as less as possible to have a clear trend which can be used to represent rate of removal of particular MP.

GAC had shown better performance, adsorbing all the four selected MPs more than 90% hence further studies to be done on possibilities of adding GAC to the reactor as the adsorption of MPs facilitates their removal in the biological processes. Xueqing Li, Faisal I. Hai, Long D. Nghiem observed that addition of powdered activated carbon (PAC) directly into the MBR can lead to their retention through adsorption which can be later degraded.

MPs were analysed only in liquid phase because of the complexity of the method used for the extraction of MPs from solids (sludge). In future this can be done which will be useful in getting the insights of the fate of the MPs closing the complete cycle.

# 7

# Conclusions

AD coupled with AnMBR was an innovative technique due to use of limited aeration. Limited aeration had improved the performance of the digestor in several aspects. The value of selected aeration had not shown any adverse effects on the performance of AD. On the contrary, improved reactor performance in several aspects. Moreover, micro-pollutants being the most recalcitrant compounds under the anaerobic conditions, their removal was of very much interest. Hence removal of selected MPs was investigated in this study. Therefore, objectives of this study were

1. To understand the performance of the reactor with limited aeration

2. To assess the removal of MPs

3. To investigate the effect of aeration of MPs removal.

Observations were made by operating the continuous AnMBR with limited aeration for 6 months. Results were obtained by performing the batch experiments with the aerated sludge. Conclusions drawn from this study were summarised as follows:

- increasing the Overall COD removal was significantly improved indicating the biodegradation of not readily biodegradable oil and ovalbumin present in black water. Reactor was stabilised increasing reactor pH with almost no production of VFA.
- Quality of biogas had improved with increase in methane content.
- SMX and TMP were removed almost completely (>99%) under anaerobic conditions. DCF was also well removed (90%). No effect of increase in MPs concentrations were found on their removal. Removal efficiency for metoprolol was decreased with increase in concentration. Additionally, it was found that metoprolol removed much better under strictly anaerobic conditions without aeration, provided HRT more than 10 days, while DCF, SMX and TMP showed similar results under strict anaerobic sludge.
- Different aerations had not shown any significant impact on the removal of DCF, SMX and TMP. While in case of metoprolol effect of aeration was not completely understood.
- DCF and TMP were adsorbed well (>90%) compared to SMX and Metoprolol (around 30%) onto the sludge contributing to overall removal. It was observed that higher the octanol water coefficient higher was the adsorption due to increased hydrophobicity.
- Adsorption was dominant removal mechanism for all the selected MPs (DCF, MPT and TMP) except SMX where biodegradation was found the major contributor in its overall removal.
- GAC used for comparison showed better removal through adsorption giving more than 90% for all the four selected MPs.
- Considering the overall removal, DCF was found to be bio-degraded more with increased concentration.

## Annexure 1 (Molecular structure for selected MPs)

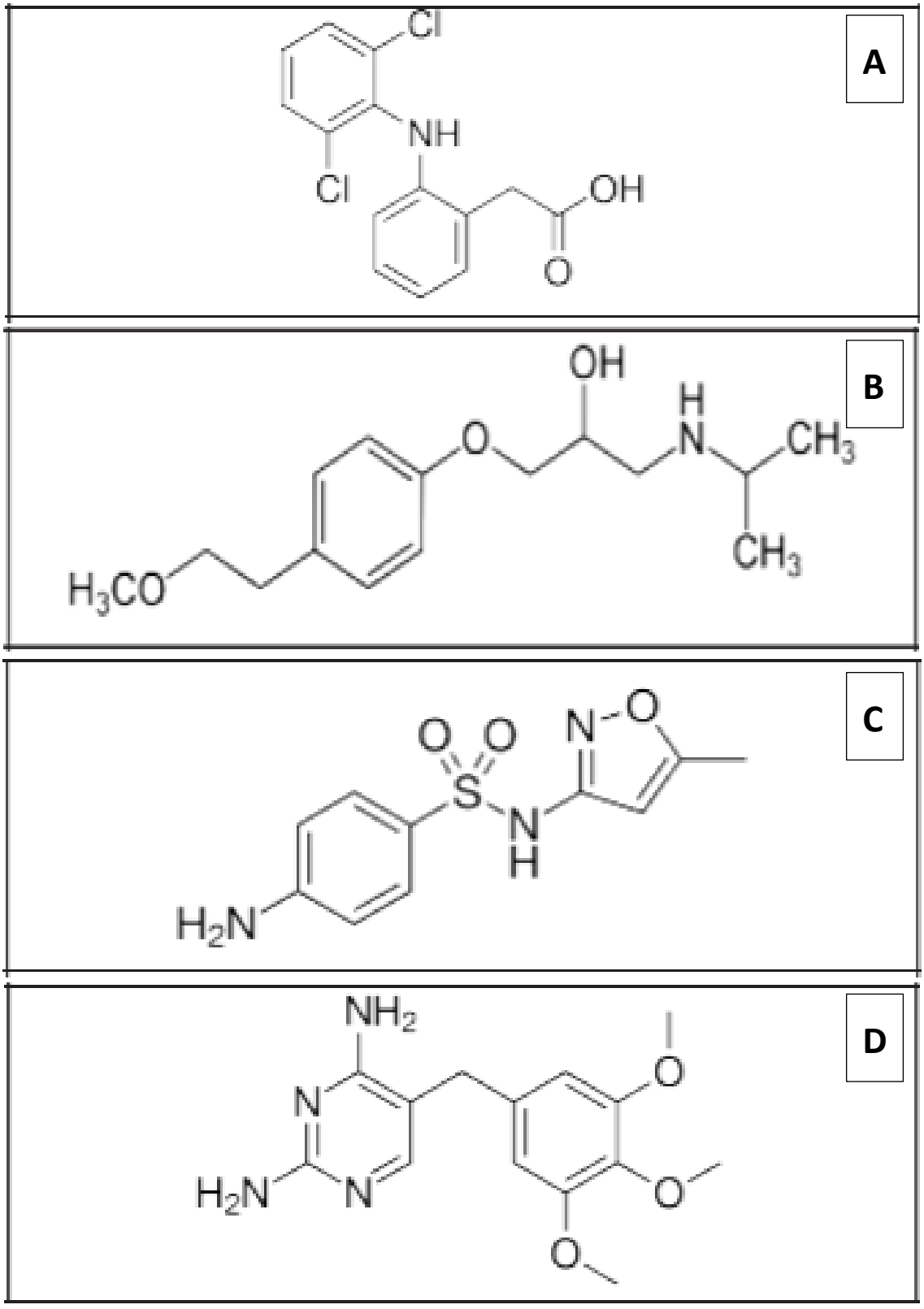


*Figure 17: Molecular structure of A Diclofenac (DCF), B: Metoprolol (MPT), C: Sulphamethoxazole (SMX), D: Trimethoprim (TMP).*

## Annexure 2a (ANOVA: results COD)

ANOVA results for the data of influent, effluent and removal efficiency of COD before and during the adaptation period and after adapted to the subjected aeration.

**Influent COD**

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Column 1 | 51 | 263761.3 | 5171.791 | 45097.48 |
| Column 2 | 51 | 291346.8 | 5712.683 | 605286.9 |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 7460386 | 1 | 7460386 | 22.94147 | 5.8E-06 | 3.936143 |
| Within Groups | 32519219 | 100 | 325192.2 | | | |
| Total | 39979605 | 101 | | | | |

**Effluent COD**

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Column 1 | 51 | 4515.467 | 88.53856 | 30.86091 |
| Column 2 | 51 | 3861.543 | 75.71654 | 171.2993 |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 4192.311 | 1 | 4192.311 | 41.47513 | 4.2E-09 | 3.936143 |
| Within Groups | 10108.01 | 100 | 101.0801 | | | |
| Total | 14300.32 | 101 | | | | |

**COD Removal Efficiency**

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Column 1 | 50 | 4914.329 | 98.28658 | 0.014298 |
| Column 2 | 50 | 4931.655 | 98.6331 | 0.12986 |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 3.001744 | 1 | 3.001744 | 41.64506 | 4.19E-09 | 3.938111 |
| Within Groups | 7.063764 | 98 | 0.072079 | | | |
| Total | 10.06551 | 99 | | | | |

## Annexure 2b (ANOVA: results Nitrate)

ANOVA results for the data of influent, effluent and removal efficiency of nitrate before and during the adaptation period and after adapted to the subjected aeration.

**Influent Nitrate**

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Column 1 | 12 | 14.82667 | 1.235556 | 0.000463 |
| Column 2 | 12 | 17.38333 | 1.448611 | 0.054963 |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 0.272356 | 1 | 0.272356 | 9.827763 | 0.004815 | 4.30095 |
| Within Groups | 0.609684 | 22 | 0.027713 | | | |
| Total | 0.88204 | 23 | | | | |

**Effluent Nitrate**

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Column 1 | 12 | 7.734333 | 0.644528 | 0.000234 |
| Column 2 | 12 | 6.844333 | 0.570361 | 0.001151 |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 0.033004 | 1 | 0.033004 | 47.65638 | 6.23E-07 | 4.30095 |
| Within Groups | 0.015236 | 22 | 0.000693 | | | |
| Total | 0.04824 | 23 | | | | |

**Nitrate Removal**

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Column 1 | 12 | 573.7099 | 47.80916 | 3.664401 |
| Column 2 | 12 | 718.5004 | 59.87503 | 31.4698 |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 873.5119 | 1 | 873.5119 | 49.7243 | 4.49E-07 | 4.30095 |
| Within Groups | 386.4763 | 22 | 17.5671 | | | |
| Total | 1259.988 | 23 | | | | |

## Annexure 2c (ANOVA: results Ammonia)

ANOVA results for the data of influent, effluent and removal efficiency of ammonia before and during the adaptation period and after adapted to the subjected aeration.

**Influent Ammonia**

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Column 1 | 12 | 2935.333 | 244.6111 | 12.48148 |
| Column 2 | 12 | 4173.333 | 347.7778 | 539.5825 |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 63860.17 | 1 | 63860.17 | 231.3506 | 3.71E-13 | 4.30095 |
| Within Groups | 6072.704 | 22 | 276.032 | | | |
| Total | 69932.87 | 23 | | | | |

**Effluent Ammonia**

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Column 1 | 12 | 7395.333 | 616.2778 | 9030.684 |
| Column 2 | 12 | 10761.33 | 896.7778 | 2853.32 |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 472081.5 | 1 | 472081.5 | 79.44823 | 9.37E-09 | 4.30095 |
| Within Groups | 130724 | 22 | 5942.002 | | | |
| Total | 602805.5 | 23 | | | | |

**Ammonia Removal**

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Column 1 | 12 | 1819.245 | 151.6038 | 1298.666 |
| Column 2 | 12 | 1903.316 | 158.6096 | 357.9673 |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 294.4929 | 1 | 294.4929 | 0.355532 | 0.557084 | 4.30095 |
| Within Groups | 18222.97 | 22 | 828.3166 | | | |
| Total | 18517.46 | 23 | | | | |

## Annexure 2d (ANOVA: results Sulphate)

ANOVA results for the data of influent, effluent and removal efficiency of sulphate before and during the adaptation period and after adapted to the subjected aeration.

**Influent Sulphate**

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Column 1 | 12 | 3284.2 | 273.6833 | 7.172424 |
| Column 2 | 12 | 2194.6 | 182.8833 | 759.1104 |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 49467.84 | 1 | 49467.84 | 129.1112 | 1.13E-10 | 4.30095 |
| Within Groups | 8429.111 | 22 | 383.1414 | | | |
| Total | 57896.95 | 23 | | | | |

**Effluent Sulphate**

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Column 1 | 12 | 361.2667 | 30.10556 | 2.776936 |
| Column 2 | 12 | 234.4333 | 19.53611 | 5.678678 |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 670.2789 | 1 | 670.2789 | 158.5406 | 1.57E-11 | 4.30095 |
| Within Groups | 93.01176 | 22 | 4.227807 | | | |
| Total | 763.2907 | 23 | | | | |

**Sulphate Removal**

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Column 1 | 12 | 1068.026 | 89.00217 | 0.30155 |
| Column 2 | 12 | 1070.451 | 89.20428 | 1.535656 |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 0.245099 | 1 | 0.245099 | 0.266817 | 0.610629 | 4.30095 |
| Within Groups | 20.20927 | 22 | 0.918603 | | | |
| Total | 20.45437 | 23 | | | | |

## Annexure 2e (ANOVA: results Phosphate)

ANOVA results for the data of influent, effluent and removal efficiency of phosphate before and during the adaptation period and after adapted to the subjected aeration.

**Influent Phosphate**

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Column 1 | 13 | 885.4 | 68.10769 | 6.445028 |
| Column 2 | 13 | 756.36 | 58.18154 | 10.12199 |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 640.4354 | 1 | 640.4354 | 77.3145 | 5.69E-09 | 4.259677 |
| Within Groups | 198.8042 | 24 | 8.28351 | | | |
| Total | 839.2397 | 25 | | | | |

**Effluent Phosphate**

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Column 1 | 13 | 694.2 | 53.4 | 2.369815 |
| Column 2 | 13 | 606.83 | 46.67923 | 12.97322 |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 293.5968 | 1 | 293.5968 | 38.27101 | 2.16E-06 | 4.259677 |
| Within Groups | 184.1165 | 24 | 7.67152 | | | |
| Total | 477.7133 | 25 | | | | |

**Phosphate Removal**

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Column 1 | 13 | 278.6142 | 21.43186 | 25.285 |
| Column 2 | 13 | 257.0888 | 19.77606 | 18.26357 |

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 17.82088 | 1 | 17.82088 | 0.818437 | 0.37463 | 4.259677 |
| Within Groups | 522.5829 | 24 | 21.77429 | | | |
| Total | 540.4038 | 25 | | | | |

## Annexure 3 (Removal of MPs with different concentrations)

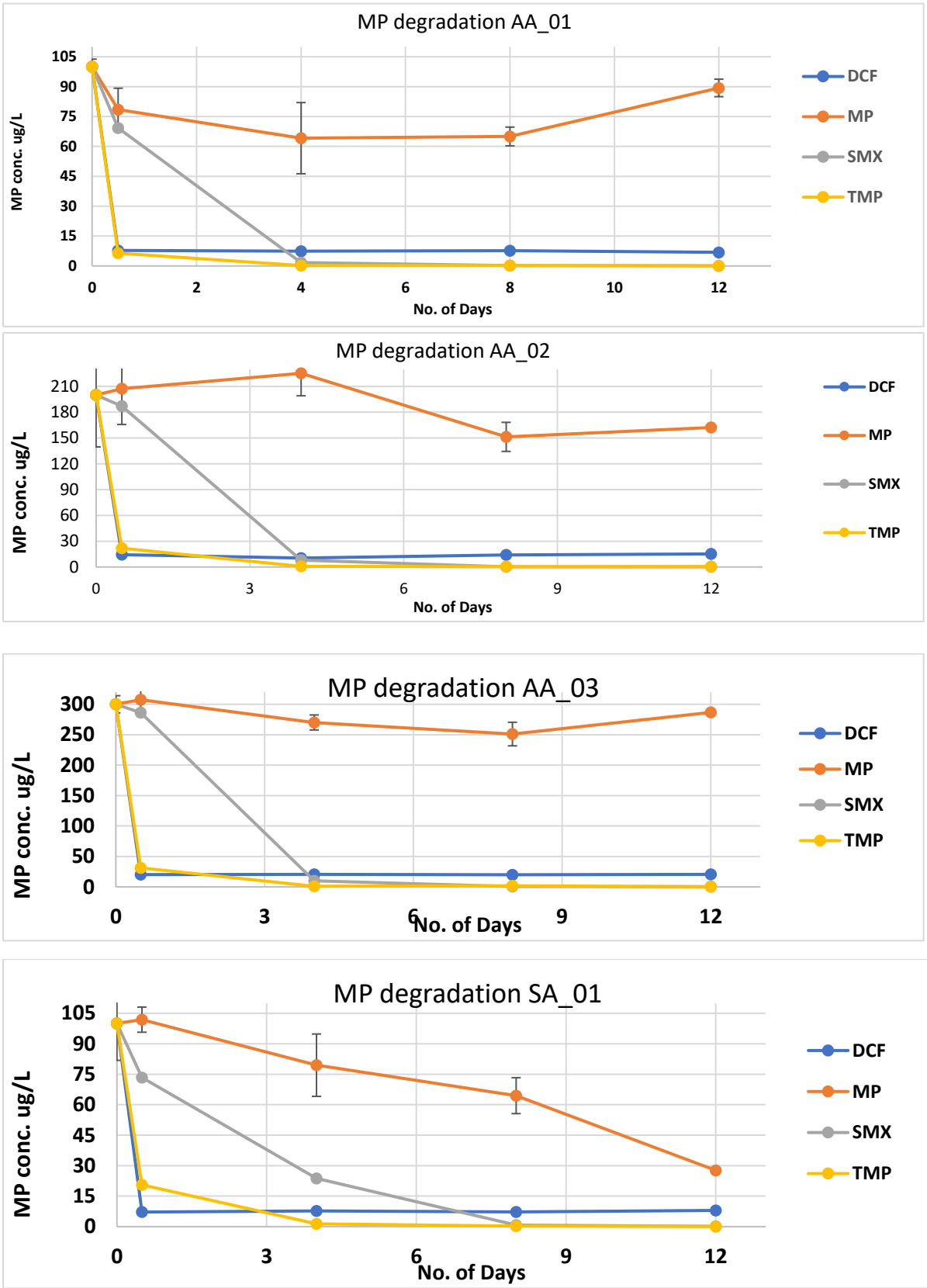


*Figure 18: Total removal of selected MPs (AA: Aerated Anaerobic sludge SA: Strict Anaerobic sludge with *01/02/03 indicating concentrations of MPs 100/200/300 in ug/l respectively)*

## Annexure 4 (Removal of MPs with different aerations)

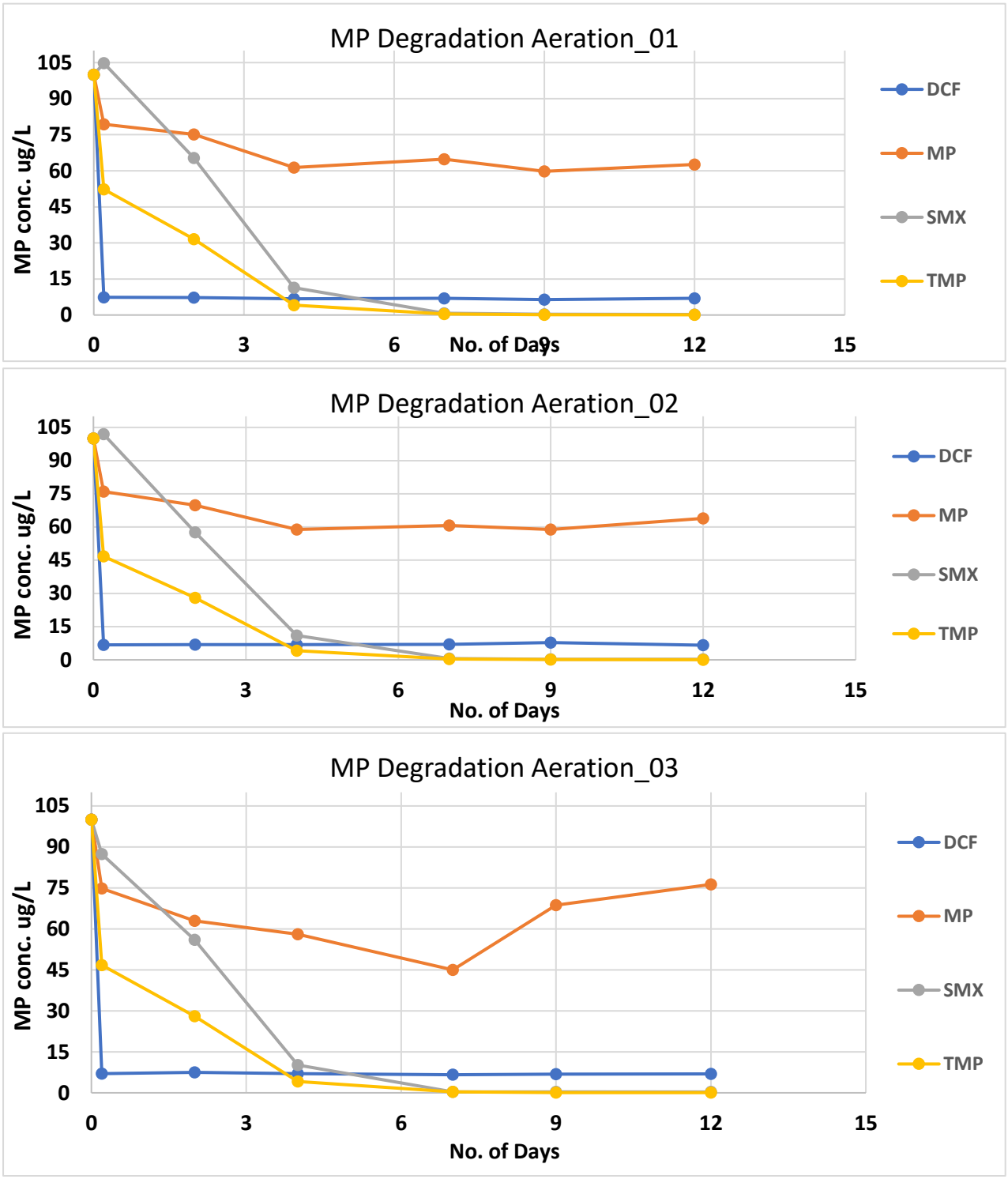


*Figure 19: Total removal of selected MPs (with Aeration*01/02/03 indicating different aerations aerations 3.9/7/11 mlair/batch/d respectively)*

## Annexure 5 (Removal of MPs through Adsorption)

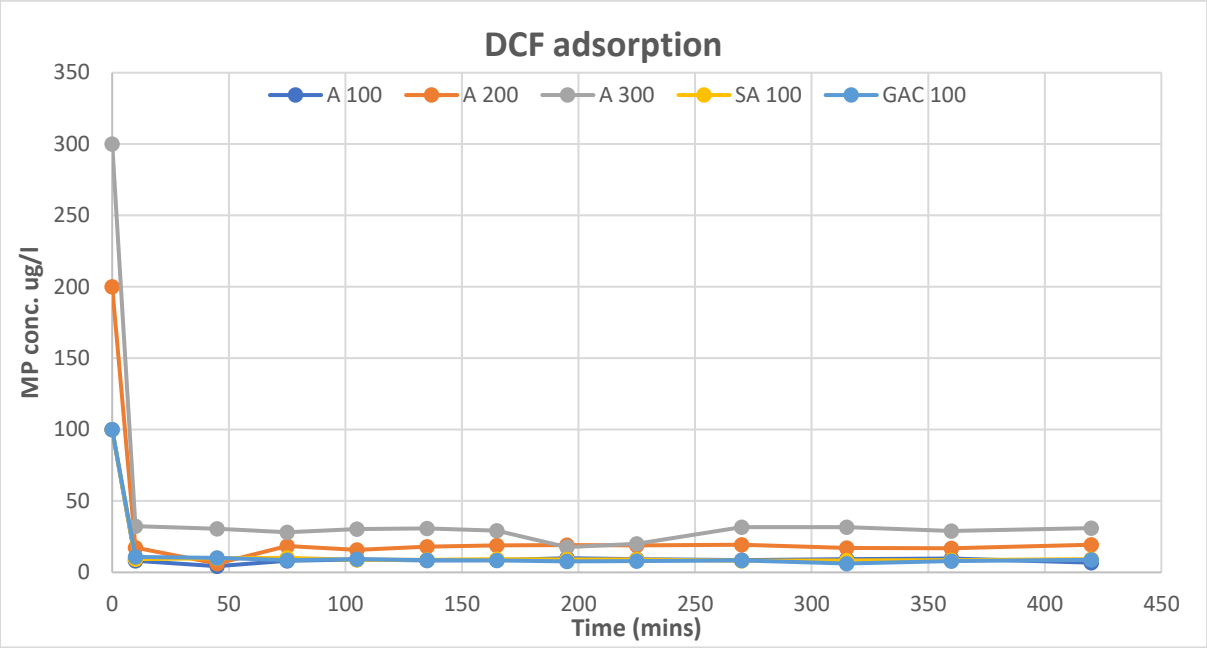


*Figure 20: Adsorption of DCF over the time (A: Aerated sludge, SA: Strict anaerobic GAC: Granular activated carbon while *100/200/300 indicating concentrations of MPs 100/200/300 in ug/l respectively)*

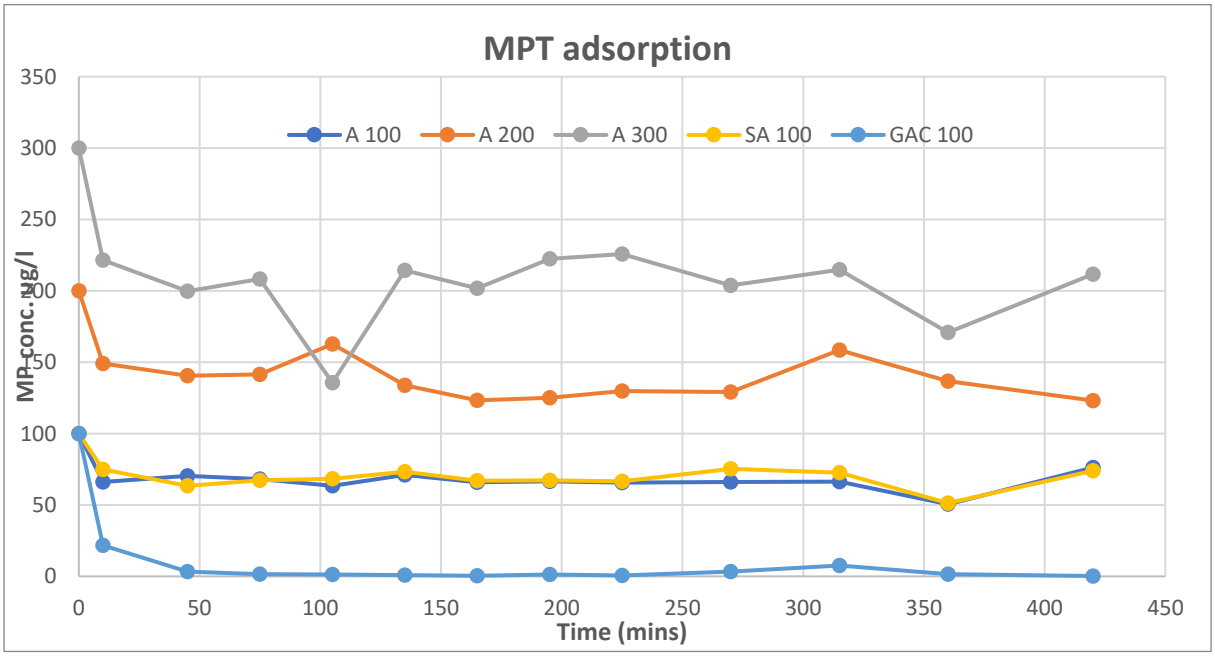


*Figure 21: Adsorption of MPT over the time (A: Aerated sludge, SA: Strict anaerobic, GAC: Granular activated carbon while *100/200/300 indicating concentrations of MPs 100/200/300 in ug/l respectively)*

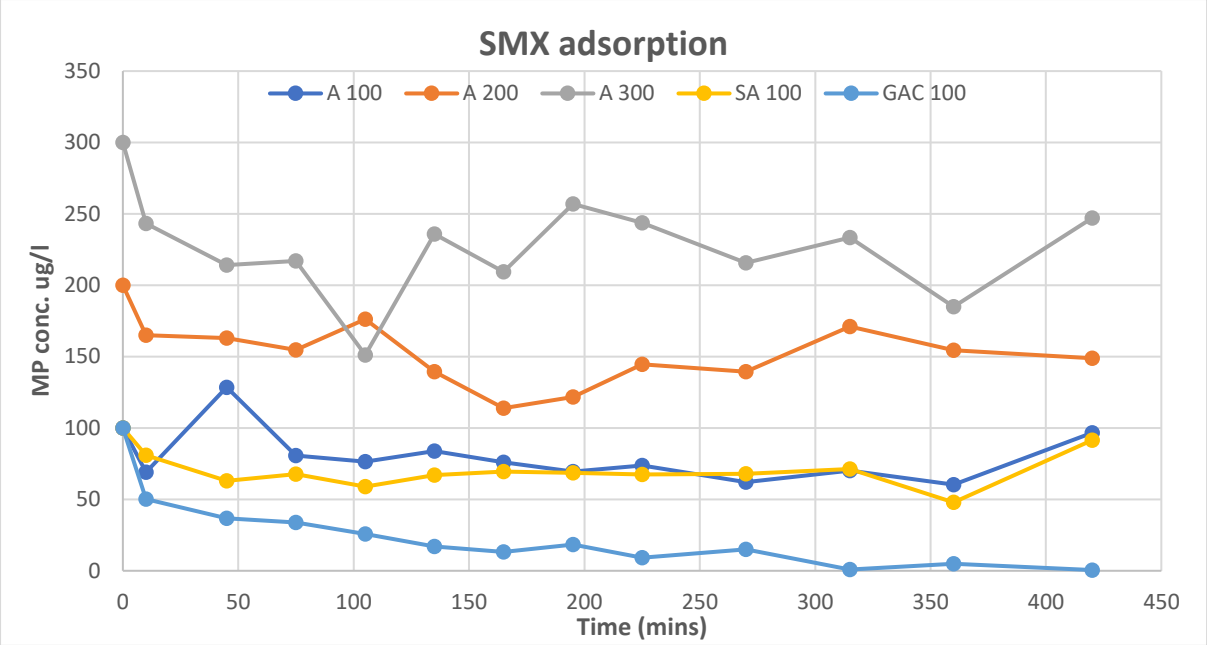


*Figure 22: Adsorption of SMX over the time (A: Aerated sludge, SA: Strict anaerobic GAC: Granular activated carbon while *100/200/300 indicating concentrations of MPs 100/200/300 in ug/l respectively)*

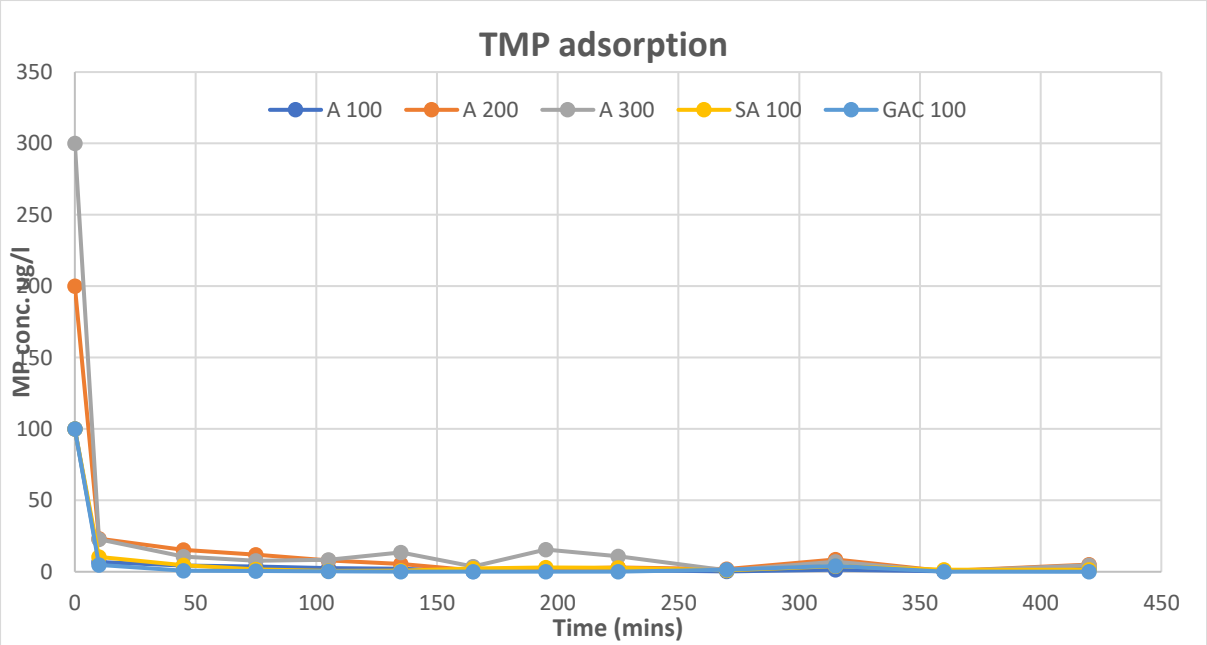


*Figure 23: Adsorption of TMP over the time (A: Aerated sludge, SA: Strict anaerobic GAC: Granular activated carbon while *100/200/300 indicating concentrations of MPs 100/200/300 in ug/l respectively)*

## Annexure 6 (Total removals for MPs through biodegradation and adsorption)

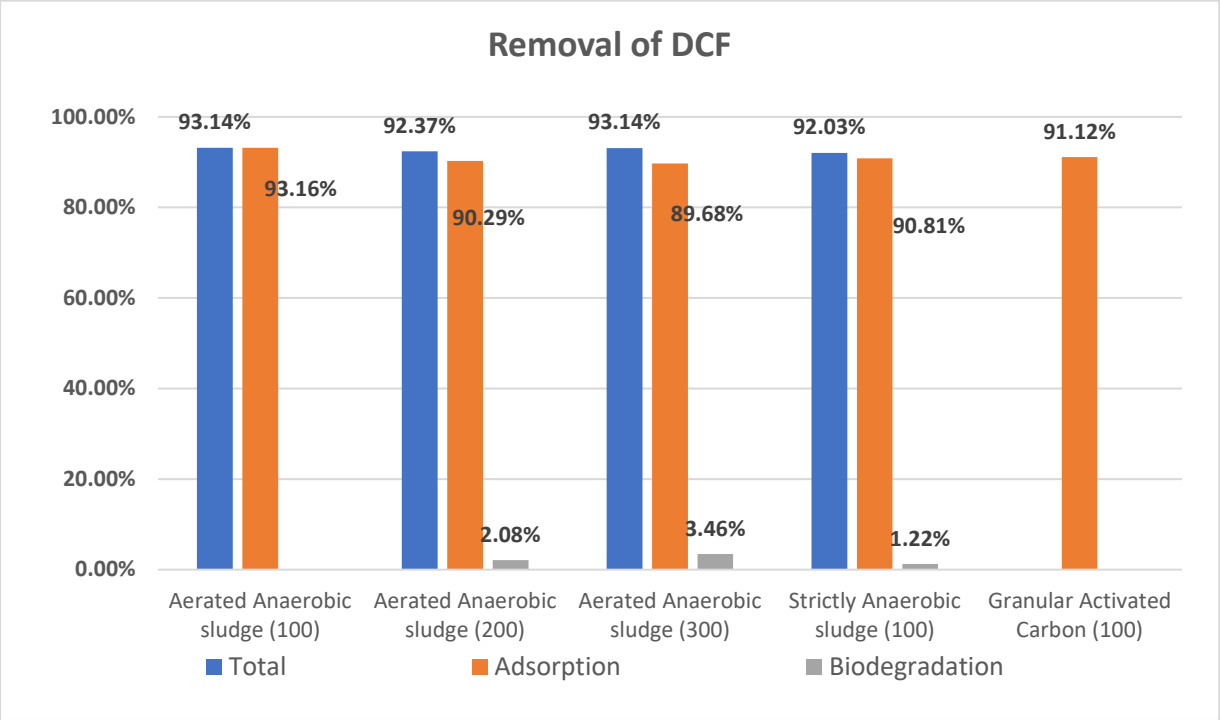


*Figure 24: Overall removal of DCF through biodegradation and adsorption (where*100/200/300 indicating concentrations of MPs 100/200/300 in ug/l respectively)*

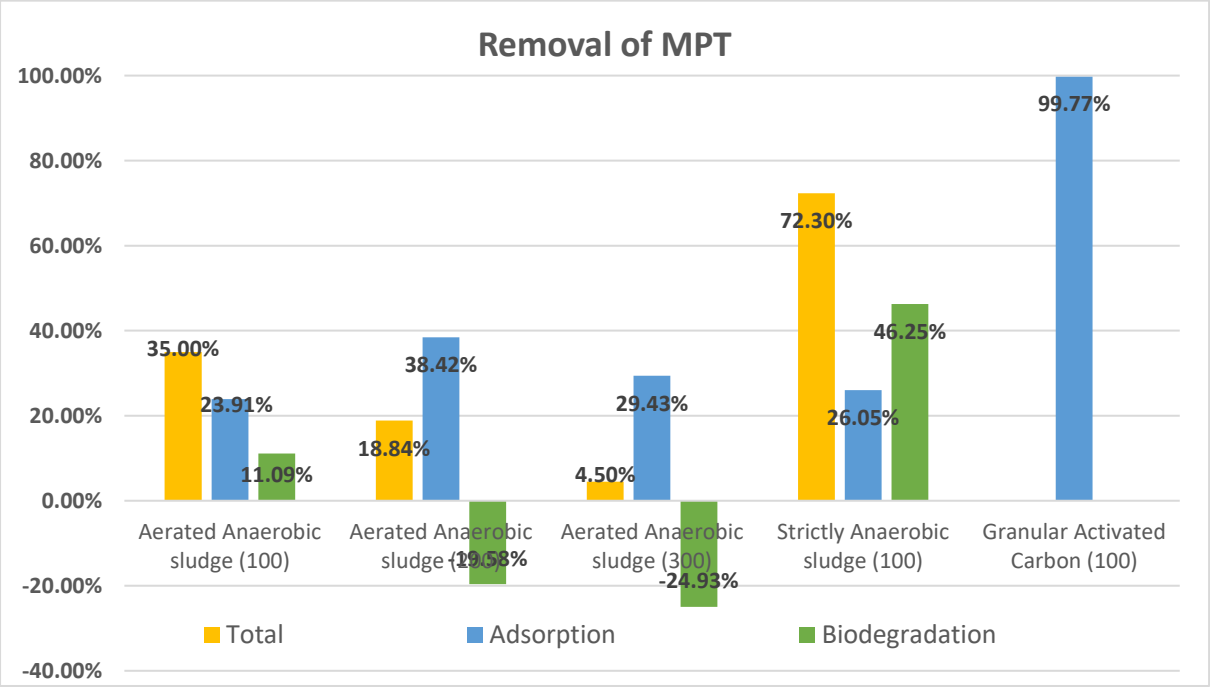


*Figure 25: Overall removal of DCF through biodegradation and adsorption (where*100/200/300 indicating concentrations of MPs 100/200/300 in ug/l respectively)*

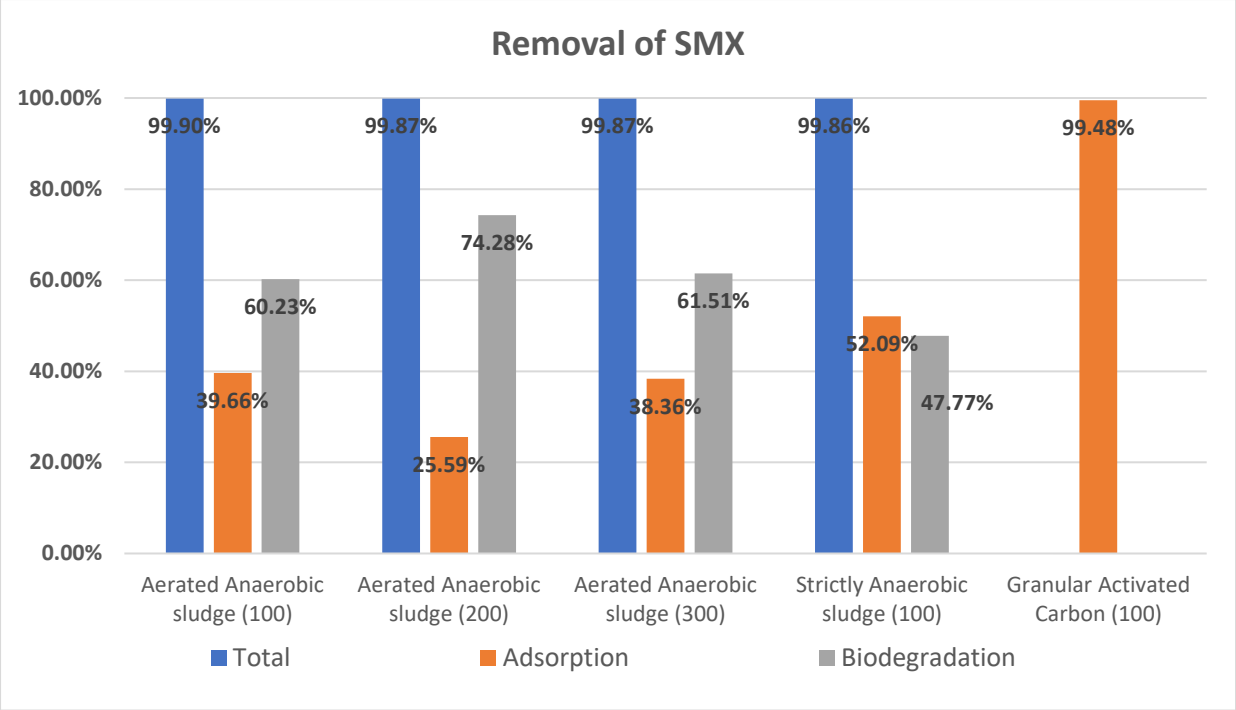


*Figure 26: Overall removal of DCF through biodegradation and adsorption (where*100/200/300 indicating concentrations of MPs 100/200/300 in ug/l respectively)*

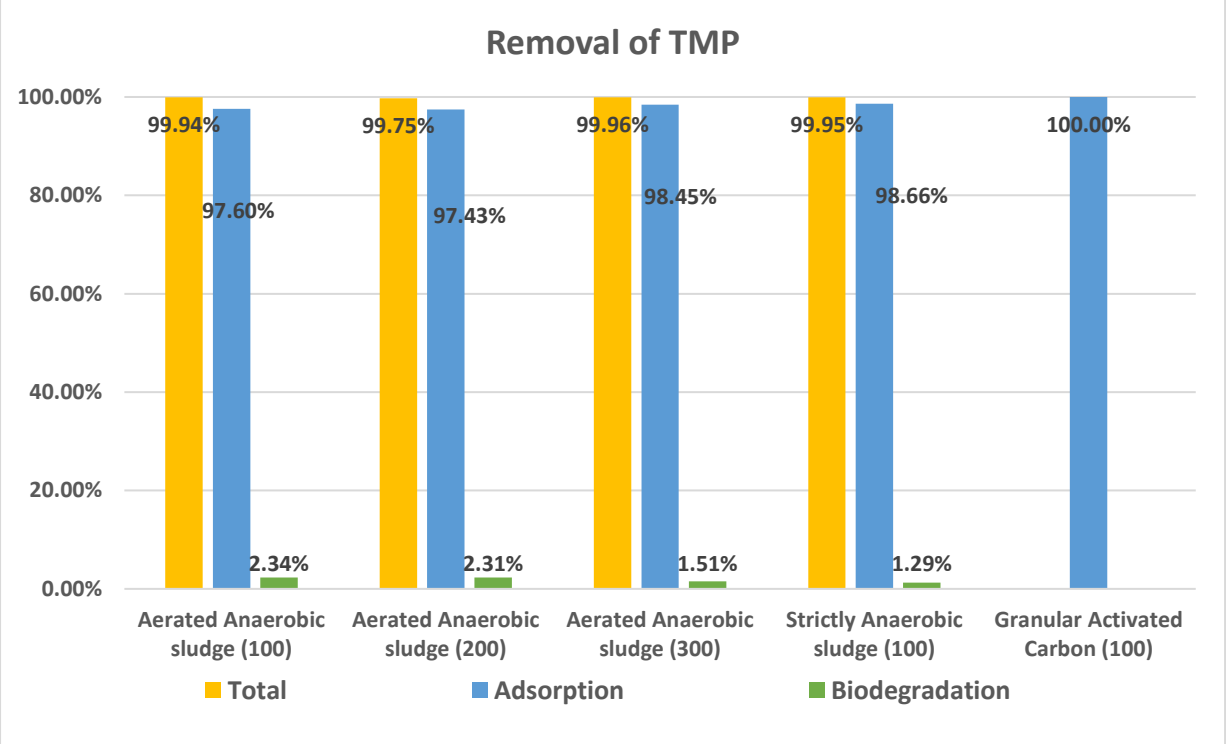


*Figure 27: Overall removal of DCF through biodegradation and adsorption (where*100/200/300 indicating concentrations of MPs 100/200/300 in ug/l respectively)*

## Annexure 7a (Matrix for biodegradation test with different aerations)

| ottle N | Sample Type | Aeration (ml/bottle/day) | Total Volume (ml) | Inoculum Volume (mL) | Inoculum VS (g) | Substrate C VS (g/g) | Substrate C mass (g) | MPs conc.(ug/l) | Micronutrients Volume (ml) | COD of substrate (g) | Methane production (ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | negative control | 0 | 99 | 66.00 | 0.2200 | 0.00 | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| 2 | negative control | 0 | 99 | 66.00 | 0.2200 | 0.00 | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| 3 | negative control | 0 | 99 | 66.00 | 0.2200 | 0.00 | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| 4 | positive control | 0 | 99 | 66.00 | 0.2200 | 0.21 | 0.5192 | 0.00 | 0.12 | 0.26 | 89.94 |
| 5 | positive control | 0 | 99 | 66.00 | 0.2200 | 0.21 | 0.5192 | 0.00 | 0.12 | 0.26 | 89.94 |
| 6 | positive control | 0 | 99 | 66.00 | 0.2200 | 0.21 | 0.5192 | 0.00 | 0.12 | 0.26 | 89.94 |
| 7 | Sodium acetate | 4.8 | 99 | 66.00 | 0.2200 | 0.21 | 0.5192 | 100.00 | 0.12 | 0.26 | 89.94 |
| 8 | Sodium acetate | 4.8 | 99 | 66.00 | 0.2200 | 0.21 | 0.5192 | 100.00 | 0.12 | 0.26 | 89.94 |
| 9 | Sodium acetate | 4.8 | 99 | 66.00 | 0.2200 | 0.21 | 0.5192 | 100.00 | 0.12 | 0.26 | 89.94 |
| 10 | Sodium acetate | 7 | 99 | 66.00 | 0.2200 | 0.21 | 0.5192 | 100.00 | 0.12 | 0.26 | 89.94 |
| 11 | Sodium acetate | 7 | 99 | 66.00 | 0.2200 | 0.21 | 0.5192 | 100.00 | 0.12 | 0.26 | 89.94 |
| 12 | Sodium acetate | 7 | 99 | 66.00 | 0.2200 | 0.21 | 0.5192 | 100.00 | 0.12 | 0.26 | 89.94 |
| 13 | Sodium acetate | 11 | 99 | 66.00 | 0.2200 | 0.21 | 0.5192 | 100.00 | 0.12 | 0.26 | 89.94 |
| 14 | Sodium acetate | 11 | 99 | 66.00 | 0.2200 | 0.21 | 0.5192 | 100.00 | 0.12 | 0.26 | 89.94 |
| 15 | Sodium acetate | 11 | 99 | 66.00 | 0.2200 | 0.21 | 0.5192 | 100.00 | 0.12 | 0.26 | 89.94 |

## Annexure 7b (Matrix for biodegradation test with different MPs concentrations)

| Bottle No. | Sample Type | Aeration (ml/bottle/d) | Total Volume (ml) | Inoculum Volume (mL) | Inoculum VS (g) | Substrate C VS (g/g) | Substrate C mass (g) | MPs conc.(ug/l) | MPs Stock Solution Volume (ml) | Micronutrients Volume (ml) | COD of substrate (g) | Biogas production (ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | negative control | 0 | 99 | 66.00 | 0.2468 | 0.00 | 0.00 | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| 2 | negative control | 0 | 99 | 66.00 | 0.2468 | 0.00 | 0.00 | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| 3 | negative control | 0 | 99 | 66.00 | 0.2468 | 0.00 | 0.00 | 0.00 | 0.00 | 0.12 | 0.00 | 0.00 |
| 4 | positive control | 0 | 99 | 66.00 | 0.2468 | 0.21 | 0.5825 | 0.00 | 0.00 | 0.12 | 0.29 | 100.92 |
| 5 | positive control | 0 | 99 | 66.00 | 0.2468 | 0.21 | 0.5825 | 0.00 | 0.00 | 0.12 | 0.29 | 100.92 |
| 6 | positive control | 0 | 99 | 66.00 | 0.2468 | 0.21 | 0.5825 | 0.00 | 0.00 | 0.12 | 0.29 | 100.92 |
| 7 | Sodium acetate | 5.1 | 99 | 66.00 | 0.2468 | 0.21 | 0.5825 | 100.00 | 10.00 | 0.12 | 0.29 | 100.92 |
| 8 | Sodium acetate | 5.1 | 99 | 66.00 | 0.2468 | 0.21 | 0.5825 | 100.00 | 10.00 | 0.12 | 0.29 | 100.92 |
| 9 | Sodium acetate | 5.1 | 99 | 66.00 | 0.2468 | 0.21 | 0.5825 | 100.00 | 10.00 | 0.12 | 0.29 | 100.92 |
| 10 | Sodium acetate | 5.1 | 99 | 66.00 | 0.2468 | 0.21 | 0.5825 | 200.00 | 20.00 | 0.12 | 0.29 | 100.92 |
| 11 | Sodium acetate | 5.1 | 99 | 66.00 | 0.2468 | 0.21 | 0.5825 | 200.00 | 20.00 | 0.12 | 0.29 | 100.92 |
| 12 | Sodium acetate | 5.1 | 99 | 66.00 | 0.2468 | 0.21 | 0.5825 | 200.00 | 20.00 | 0.12 | 0.29 | 100.92 |
| 13 | Sodium acetate | 5.1 | 99 | 66.00 | 0.2468 | 0.21 | 0.5825 | 300.00 | 30.00 | 0.12 | 0.29 | 100.92 |
| 14 | Sodium acetate | 5.1 | 99 | 66.00 | 0.2468 | 0.21 | 0.5825 | 300.00 | 30.00 | 0.12 | 0.29 | 100.92 |
| 15 | Sodium acetate | 5.1 | 99 | 66.00 | 0.2468 | 0.21 | 0.5825 | 300.00 | 30.00 | 0.12 | 0.29 | 100.92 |
| 16 | Sodium acetate | 0 | 99 | 66.00 | 0.2211 | 0.21 | 0.5217 | 100.00 | 10.00 | 0.12 | 0.26 | 90.39 |
| 17 | Sodium acetate | 0 | 99 | 66.00 | 0.2211 | 0.21 | 0.5217 | 100.00 | 10.00 | 0.12 | 0.26 | 90.39 |
| 18 | Sodium acetate | 0 | 99 | 66.00 | 0.2211 | 0.21 | 0.5217 | 100.00 | 10.00 | 0.12 | 0.26 | 90.39 |