FATE AND REMOVAL OF PESTICIDES IN WASTEWATER TREATMENT PLANTS – CASE OF YEŞİLIRMAK BASIN

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submitted by **KUMRU KOCAMAN** in partial fulfillment of the requirements for the degree of **Master of Science in Environmental Engineering Department, Middle East Technical University** by,

Prof. Dr. Halil Kalıpçılar
Dean, Graduate School of **Natural and Applied Sciences** _____

Prof. Dr. Bülent İçgen
Head of Department, **Environmental Eng.** _____

Prof. Dr. Filiz Bengü Dilek
Supervisor, **Environmental Eng., METU** _____

Prof. Dr. Ülkü Yetiş
Co-Supervisor, **Environmental Eng., METU** _____

Examining Committee Members:

Assoc. Prof. Dr. Tuba Hande Bayramoğlu
Environmental Eng., METU _____

Prof. Dr. Filiz Bengü Dilek
Environmental Eng., METU _____

Prof. Dr. Ülkü Yetiş
Environmental Eng., METU _____

Prof. Dr. Erkan Şahinkaya
Bioengineering Dept., İstanbul Medeniyet University _____

Assist. Prof. Dr. Yasemin Dilşad Yılmazel
Environmental Eng., METU _____

Date: 28.01.2019

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Surname: Kumru Kocaman

Signature:

ABSTRACT

FATE AND REMOVAL OF PESTICIDES IN WASTEWATER TREATMENT PLANTS – CASE OF YEŞİLIRMAK BASIN

Kocaman, Kumru
Master of Science, Environmental Engineering
Supervisor: Prof. Dr. Filiz Bengü Dilek
Co-Supervisor: Prof. Dr. Ülkü Yetiş

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The effluent of wastewater treatment plants (WWTP) had been shown to be a significant source of micropollutants in surface waters. In this study, the aim was to investigate the biological treatment of commonly found micropollutants in Yeşilırmak river basin, with special emphasis on the effects of operational conditions on their removal in conventional biological WWTPs. Based on the monitoring results of TÜBİTAK project (115Y013) "Management of Point and Diffuse Pollutant Sources in Yeşilırmak River Basin" carbendazim, imidacloprid and aclonifen pesticides were observed to exceed the relevant Environmental Quality Standards (EQSs) in one or more sampling campaign therefore these pesticides were selected to be studied. To study the influence of SRT and pesticide concentration on the overall treatment performance laboratory scale instantaneously fed Sequencing Batch Reactors(SBRs) with 5 different SRTs (3, 8, 10, 20 and 30 days) were operated and the effects of having the pesticides in the influent on the COD removal performance of the reactors were sought. Also, the removals of these pesticides, either individual or in mixture, were studied under different SRTs and influent pesticide concentrations (0-400μg/L). COD utilization capacity of the reactors operated with a single pesticide were not disrupted remarkably until introduction of 50 μg/L pesticide. However, COD

utilization capacity of the reactors operated with mixture of pesticides were disrupted beyond the addition of 25 μg/L of each pesticide. There exists no clear correlation between the elimination of pesticides and SRT.Removal efficiencies of aclonifen and carbendazim pesticides were better when the pesticides were spiked as mixture.

Keywords: Carbendazim,Imidacloprid, Aclonifen, Activated Sludge, COD Removal

ÖZ

ATIKSU ARITMA TESİSLERİNDEKİ PESTİSİTLERİN AKIBETİ VE GİDERİMİ – YEŞİLIRMAK HAVZASI

Kocaman, Kumru
Yüksek Lisans, Çevre Mühendisliği
Tez Danışmanı: Prof. Dr. Filiz Bengü Dilek
Ortak Tez Danışmanı: Prof. Dr. Ülkü Yetiş

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Atıksu arıtma tesislerinin (AAT) çıkış sularının önemli mikrokirletici kaynakları olduğu gözlemlenmiştir. Bu çalışmada Yeşilırmak nehir havzasında yaygın olarak tespit edilen mikrokirleticilerin biyolojik gideriminin, özellikle operasyonel koşulların konvansiyonel biyolojik giderim üzerindeki etkilerinin dikkate alınarak araştırılması amaçlanmıştır. 115Y013 numaralı "Yeşilırmak Havzası Noktasal ve Yayılı Kirlilik Kaynakları Yönetimi Projesi" TÜBİTAK projesinin izleme sonuçlarına göre karbendazim, imidakloprid ve aklonifen pestisitleri bir veya daha fazla örnekleme kampanyasında ilgili Çevresel Kalite Standartlarını (ÇKS) aştığı gözlenmiştir. Bu nedenle karbendazim, imidakloprid ve aklonifen pestisitleri çalışılmak için seçilmiştir. Çamur yaşı ve pestisit konsantrasyonunun giderim performansı üzerindeki etkilerini incelemek için 5 farklı çamur yaşında (3, 8, 10, 20 ve 30 gün) çalıştırılan laboratuvar ölçekli ardaşık kesikli reaktörler kurulmuştur ve pestisit içeren atıksuların KOİ giderim performansı üzerindeki etkileri araştırılmıştır. Ayrıca, bu pestisitlerin, tek tek veya karışım halinde, farklı çamur yaşlarında ve pestisit konsantrasyonlarında (0-400μg/L) arıtılabilirliği çalışılmıştır. KOİ giderim verimi tek pestisit ile işletilen reaktörlerde 50 μg/L pestisit eklenineye kadar belirgin bir şekilde bozulmazken, pestisit karışımı ile çalışan reaktörlerin KOİ giderim verimi her pestisitten 25 μg/L

eklenmesinden sonra bozulmuştur. Çamur yaşı ile pestisitlerin giderimi arasında net bir ilişkiye rastlanılamamıştır. Karbendazim ve aklonifen reaktörlere karışım halinde verildiğinde daha yüksek giderim verimleri elde edilmiştir.

Anahtar Kelimeler: Karbendazim, İmidakloprid, Aklonifen, KOİ, Aktif Çamur

To my mother

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CHAPTER 1

INTRODUCTION

1.1. General

Micropollutants, also known as contaminants of emerging concern are present at trace concentrations in the environment, ranging from a few ng/L to several μg/L. Although, micropollutants are not commonly monitored in the environment, they have the potential to enter the environment and cause adverse ecological and human health effects (US EPA, 2010). Origin of micropollutants in the environment are diverse yet a significant amount of these pollutants originates from mass produced materials. According to their intended use micropollutants can be categorized under six main categories: pesticides, personal care products, pharmaceuticals, industrial chemicals, steroid hormones and surfactants.

The occurrence of micropollutants in the aquatic environment has become a global environmental concern. Even though these pollutants are present in trace amounts in the environment their presence is a growing concern since trace amounts of these pollutants in the aquatic environment can induce interference with the endocrine system, create antibiotic resistance and accumulate in animals, soil and plants (Ahmed et al., 2017) .

Emerging contaminants enter water bodies via different pathways. For example, personal care products and pharmaceuticals are not completely metabolized and are discharged to sewers from households and hospitals. Steroid hormones used in animal farming enter water bodies through the effluents and manure. Pesticides from

agricultural land and runoff from urban areas including biocides and other chemicals from automobile emissions, dry and wet atmospheric deposition contribute to diffuse pollution (Eggen et al., 2014).

Micropollutants are expected to be found at very low concentrations in receiving waters and wastewater treatment plants. However, due to their extensive use the amount of pesticides found in wastewater treatment plants (WWTPs) and in receiving waters are increasing day by day. Increasing concentrations of micropollutants have led to adverse effects on the environment and human health.

In this respect, EU Water Framework Directive 2000/60/EC (WFD) aims to restore degraded ground and surface water to "good status". By definition, good status implies the status attained by a water body when both its chemical status and its ecological status are at least good, which are classified in accordance with Annex V of WFD. A water body which is in good status requires to meet Environmental Quality Standards (EQS) for priority substances.

Priority substances are substances which are classified in accordance with Article 16(2) of the WFD (2000/60/EC). Priority substances, which are regulated and monitored at EU level, pose serious risks for the water environment and are listed in Annex X of WFD (2000/60/EC), which has been reviewed by Directive 2013/39/EU. In addition to the priority substances list, EU Member States and EU candidate countries have listed river basin specific pollutants. Specific pollutants are pollutants of regional or local importance, which pose risks either on river basin level or national level. EU Member States and EU candidate countries are responsible for identification of specific pollutants, providing EQS values, monitoring and defining regulatory measures and actions. Turkey, as an EU candidate country, has conducted a

comprehensive study on determination of specific pollutants. As a result of this study, 250 specific pollutants (133 non-point sourced and 117 point sourced) and their national EQSs were determined (Orhon et al., 2017). Priority substances and specific pollutants are listed in Surface Water Quality Regulation (SWQR)[1].

Indeed, WWTPs acts as barriers against the spread of these pollutants. However, conventional WWTPs are not designed to eliminate micropollutants. Therefore, when emerging compounds are not completely removed, conventional WWTPs effluents become the major source of micropollutants (Joss et al., 2004). Upgrading WWTPs with advanced treatment processes, such as advanced oxidation processes, activated carbon adsorption, ozonation, membrane bioreactors, nanofiltration and reverse osmosis is a way to overcome this issue (Andersen et al., 2003; Eggen et al., 2014; Joss et al., 2008; Margot, 2015a; Margot et al., 2013). However, the economic cost of upgrading and operating WWTPs are big challenges (Jones et al., 2007). Alternatively, measuring the existing biological process performances of the WWTPs and optimizing the operation conditions is a vital issue.

This thesis study was performed as a part of TÜBİTAK project (115Y013) on "Management of Point and Diffuse Pollutant Sources in Yeşilırmak River Basin". The principal goal of 115Y013 numbered TÜBİTAK project is to provide technical support to The General Directorate of Water Management (Ministry of Agriculture and Forestry) about developing an approach on the management of point and diffuse pollution sources in Yeşilırmak river basin in accordance with the WFD. In addition, below mentioned actions were planned to be done within the scope of the TÜBİTAK project:

[1] Official Journal dated August 10,2016 No: 29797

- Preparation of a comprehensive pollutant inventory, identification of the principal point and non-point pollution sources and pollutants discharged from these sources
- Prioritization of the identified river basin specific pollutants (RBSPs)
- Determination of EQS values for the pollutants whose EQS values were not previously allocated by the Ministry
- Determination of EQS based discharge standards by using Tiered approach (Directive 2008/105/EC) and tolls such as Discharge Test
- Evaluation of the processes and performances of existing treatment plants and development of improvement recommendations
- Assessment of advanced treatment processes for the pollutants exceeding the EQS values

Yeşilırmak river is 519 kilometers long and is the second largest river of Turkey. The river basin is 38,732 kilometer square and passes through 5 big cities enclosing 14 wastewater treatment plants (TÜBİTAK MAM, 2010). Over the course of this project, more than 50 stations in Yeşilırmak river basin were monitored seasonally. According to the outcomes of these monitoring studies the striking micropollutant exceeding the EQS were pesticides. This was an expected outcome since agriculture is the main economic activity conducted in Yeşilırmak river basin.

1.2. Aim and Scope of the Study

In this thesis, the aim was to study the biological treatment of commonly found micropollutants in Yeşilırmak river basin, with special emphasis on the effects of operational conditions on their removal in conventional biological wastewater treatment plants.

Within the scope of the study, pesticides, namely, carbendazim, imidacloprid and aclonifen pesticides were selected to study, based on the monitoring results of the aforementioned project. These pesticides were observed to exceed the relevant EQSs in one or more sampling period. One other reason for selecting aclonifen was that there exists no treatability study in the literature to the best of our knowledge.

The effects of operational conditions (sludge retention time (SRT) and pesticide concentration) on the overall treatment performance were investigated. In this respect, laboratory scale instantaneously fed SBRs were operated and the effects of having the pesticides in the influent on the COD removal performance of the reactors were sought. Also, the removals of these pesticides, either individual or in mixture, were studied under different SRTs and influent pesticide concentrations.

CHAPTER 2

LITERATURE REVIEW

2.1. Micropollutants

2.1.1. General Information

The occurrence of micropollutants in the aquatic environment has become a global environmental concern. Micropollutants are synthetic or naturally occurring chemicals which are not commonly monitored in the environment, however these chemicals have the potential to enter the environment and cause adverse ecological and human health effects (US EPA, 2010). As individual compounds or as part of complex mixtures micropollutants are relevant for water quality.

In the environment micropollutants are present at trace concentrations, ranging from a few ng/L to several μg/L. Micropollutants are also referred as contaminants of emerging concern (CECs) due to the new technologic achievements in creating analytic methods sensitive enough to detect at environmentally relevant trace concentrations. Micropollutants are also known as trace contaminants considering that micropollutants are present in trace amounts in environmental samples. CECs are also referred as persistent organic compounds since most of the micropollutants are non-biodegradable and they are also known as xenobiotic compounds because most of the micropollutants are of anthropogenic origin (US EPA, 2010).

Origin of micropollutants in the environment are diverse and an important amount of these pollutants originate from mass produced materials. Generally, micropollutants can be grouped under six categories, namely personal care products, pharmaceuticals,

pesticides, steroid hormones, surfactants and industrial chemicals. Table 1 summarizes the major categories of some important micropollutants in the aquatic environment.

Table 1 Sources of Micropollutants in the Aquatic Environment (Ahmed et al., 2017; Schwarzenbach et al., 2006)

Category	**Important Micropollutants**	**Major Sources**
Personal Care Products	Sun screens, fragrance, disinfectants, insect repellents, cosmetics	Domestic Wastewater Industrial Wastewater (from Product Manufacturing) Landfill Leachate
Pharmaceuticals	Antibiotics, β-blockers, NSAIDs, lipid regulators, antiseptics, analgesics, food supplements	Hospital Wastewater Domestic Wastewater Industrial Wastewater (from Product Manufacturing) Landfill Leachate
Pesticides	Insecticides, herbicides and fungicides	Agricultural Runoff Domestic Wastewater Industrial Wastewater (from Product Manufacturing) Landfill Leachate
Steroid Hormones	Estrogens	Domestic Wastewater Runoff from Animal Farms Industrial Wastewater (from Product Manufacturing)
Surfactants	Non-ionic surfactants	Domestic Wastewater Industrial Wastewater
Industrial Chemicals	Fire retardants, plasticizers	Domestic Wastewater Industrial Wastewater (from Product Manufacturing) Landfill Leachate

2.1.2. Threat for the Environment

Micropollutants enter water bodies via different pathways. Figure 1 demonstrates the related cycle. Pesticides from agricultural land and runoff from urban areas including biocides and other chemicals from automobile emissions, dry and wet atmospheric deposition contribute to diffuse pollution (Eggen et al., 2014). Pharmaceuticals and personal care products, industrial chemicals and numerous other chemicals are not completely metabolized and are discharged into sewers from households. Consumer products, used in households, are a major source of trace contaminants as well as the chemicals used in industry.

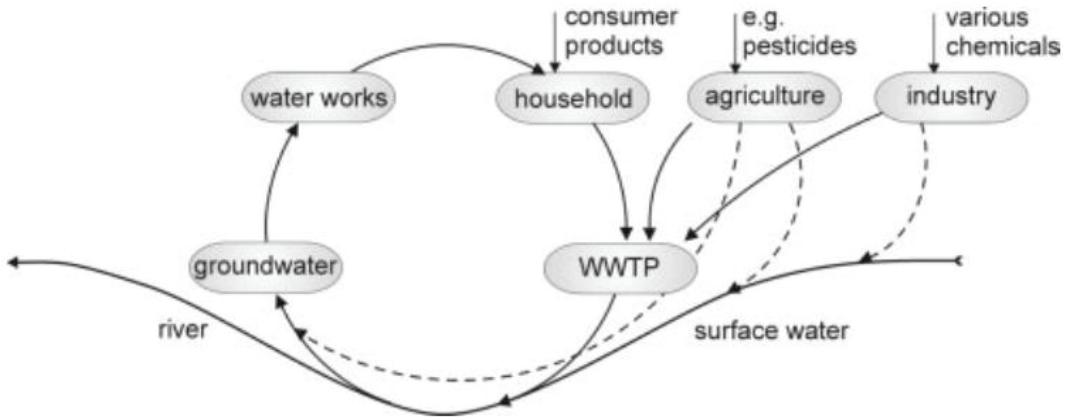

Figure 1 Micropollutants Transport in Water Cycle (Reemtsma et al., 2006)

As mentioned before, emerging contaminants are a large and relatively new group of chemicals and can potentially cause damage in human and aquatic life with their trace existence in environment (Table 2). These pollutants are constitutes of wastewater, municipal sewage, hospital wastewater and landfill leachate. Micropollutants' environmentally relevant concentrations are a growing concern since such concentrations in the aquatic environment can induce interference with endocrine

system, antibiotic resistance and accumulation in animals, soil and plants (Ahmed et al., 2017) .

The micropollutants which received the greatest attention are endocrine disruptors. These compounds disturb the highly functional endocrine system by mimicking, blocking and disturbing the function of hormone (Bolong et al., 2009). Endocrine disruptors can cause problems in reproductive system, breakage of the eggs of fishes, birds and turtles, reduction of sperm in the male human reproductive organ, increase the risk of prostate and breast cancer (Esplugas et al., 2007).

Pharmaceuticals are one of the largest groups of micropollutants including antibiotics, non-steroid anti-inflammatory drugs (NSAIDs). It is discovered that the bioaccumulation of these compounds infuriates the abnormal hormonal control leading to reproductive impairments, increases incidence of testosterone and breast cancer, and can encourage the development of antibiotic resistant genes (Ahmed et al., 2015).

Surfactants present in aquatic environment are also responsible of the endocrine activity by affecting the physical stability of human growth hormone (Katakam et al., 1995).

Furthermore, pesticides, being another important group of micropollutants, may appear in the receiving water bodies via either surface runoffs from agricultural areas or direct discharges from industries producing pesticides. They alter the functions of endocrine system in mammals and fishes, and can cause changes in tissue structure (Dunier et al., 1993). Further examples are listed in Table 2.

Table 2 Examples of Ubiquitous Water Pollutants and Health Effects

Micropollutants	**Usage**	**Health Effects**	**Reference**
Bisphenol A (BPA)	Epoxy resin and polycarbonate plastics	Increases breast cancer risk in humans, estrogenic effects in rats	(Krishnan et al., 1993) (Dodds et al., 1938)
Triclosan	Toothpaste, hand soap	Development of bacteria resistance towards triclosan, persistent degradation product	(McMurry et al., 1998) (Lindström et al., 2002)
Polychlorinated biphenyls (PCBs)	Lubricant used in transformers and capacitors	Effects brain development and causes IQ decrease in children.	(Routledge, Sheahan, et al., 1998)
Sulfonamides, tetracycline, penicillin	Antibiotics	Creates bacterial resistance	(Kolpin et al., 2002)
Parabens	Antimicrobial preservative used in food, cosmetics etc.	Weakens estrogenic activity	(Routledge, Parker, et al., 1998)
Nonylphenol	Detergent	Endocrine active transformation product	(Ahel et al., 1994)
Butylated Hydroxyanisole(BHA)	Food antioxidant	Simulates human and rainbow trout estrogen receptors	(Jobling et al., 1995)
Dichloro diphenyl tricloroethane (DDT)	Insecticide	Hormonal effect, causes behavioral changes thinning of eggshells, damages male productivity	(Colborn, 1995)
Atrazine	Herbicide	Effects primary producers	(Pape-Lindstrom et al., 1997)
Penconazole	Fungicide	Effects thyroid	(McKinney et al., 1994)
Prochloraz	Fungicide	Can affect pituitary gland weight	(McKinney et al., 1994)

Micropollutants	Usage	Health Effects	Reference
Propiconazole	Fungicide	Effects steroid metabolism	(McKinney et al., 1994)
Tridemorph	Fungicide	Can cause cystic ovaries	(McKinney et al., 1994)
Epoxyconazole	Fungicide	Can cause ovarian tumors	(McKinney et al., 1994)

2.2. Treatability of Micropollutants

Micropollutants cleansing from waters is an extremely challenging task. Given the numbers of these compounds and the diversity of their use, removal from the water cycle necessitates a complementary approach. Source control, best management practices and end of pipe solutions must be implemented. Regulations can be set for specific compounds to eliminate the entry of critical compounds to water bodies. Nonylphenol is a good example for this, since the EU Directive 2003/53/EC nonylphenol in water bodies decreased successfully (Eggen et al., 2014; European Commission, 2003). However, keeping in mind the variety of micropollutants, it is impossible and impractical to implement compound specific regulations for each and every pollutant. In this aspect, wastewater treatment plants play a vital role in micropollutants fate. WWTPs acts as barriers against the spread of these pollutants.

Emerging compounds originate from different point and diffuse sources and enter the water cycle via different pathways and WWTPs work as barriers by collecting pollutants before entering water bodies. As a result, when micropollutants are not completely removed, conventional WWTPs effluents become the major source of micropollutants (Joss et al., 2004).

Conventional WWTPs are designed to achieve a common set of objectives:

- To enhance hygienic conditions of the receiving waters through functioning as a barrier for pathogens and fecal bacteria

- To enhance the water quality of receiving waters
- To remove nitrogen and phosphorus which are causing eutrophication of water ecosystems

The increase of micropollutants pose new challenges to conventional WWTPs, since approximately only half of the micropollutant load is eliminated in these plants (Luo et al., 2014). Micropollutants in WWTPs are mostly eliminated by either sorption to sludge or by degradation. Nonetheless, countless compounds with hydrophilic characteristics do not sorb to sludge and are either persistent over the retention time in the WWTP or are transformed into unknown byproducts, which are constantly discharged to receiving waters (Eggen et al., 2014; Schymanski et al., 2014). Consequently, conventional WWTPs are not capable of removing most of the micropollutants and therefore micropollutants enter the water bodies.

To overcome this issue, many scientists investigated the benefits of upgrading conventional WWTPs (Andersen et al., 2003; Eggen et al., 2014; Joss et al., 2008; Margot, 2015a; Margot et al., 2013). Advanced treatment processes, such as advanced oxidation processes, activated carbon adsorption, ozonation, membrane bioreactors, nanofiltration and reverse osmosis can accomplish high micropollutant removal (Luo et al., 2014). Advantages and challenges of different treatment technologies for removal of micropollutants are outlined in Table 3. Nevertheless, the economic cost of implementing and operating are big challenges (Jones et al., 2007). Instead, measuring the existing biological process performances and optimizing operation conditions to improve micropollutants removal is a vital issue.

Table 3 Advantages and Challenges of Treatment Processes in the Removal of Micropollutants

Treatment Process	Advantages	Challenges	Reference
Activated Sludge	Lower operational and capital costs than AOPs	Large amount of sludge including micropollutants Inefficient for wastewaters with high COD levels	(Bolong et al., 2009; Clara et al., 2003; Luo et al., 2014)
Coagulation-Flocculation	Elimination of micropollutants with high K_{ow} values (e.g. diclofenac and nonylphenol)	Ineffective elimination of most micropollutants Large amount of sludge Coagulant salts are introduced to the aqueous environment	(Luo et al., 2014; Suarez et al., 2009)
Activated Carbon Adsorption-PAC	Effective for treating persistent organic compounds. Problematic by-product formation is eliminated.	Disposal difficulty of sludge Maintaining suitable PAC dose is challenging	(Luo et al., 2014; Margot, 2015b; Margot et al., 2013)
Activated Carbon Adsorption-GAC	Considerable removals of steroids and pharmaceuticals	GAC regeneration poses risk	(Grover et al., 2011; Luo et al., 2014)
Membrane Bioreactors	Effective removal of recalcitrant micropollutants High SRTs Small footprint	Pharmaceuticals are not removed Fouling problem	(Clara, Strenn, et al., 2005; Luo et al., 2014; Radjenović et al., 2009; Spring et al., 2007)
Ozonation	Very effective, non-selective micropollutant removal	Formation of unknown, reactive byproducts Operation difficulties Cost	(Esplugas et al., 2007; Margot et al., 2013; Snyder et al., 2006; Thomas A. Ternes et al., 2003)

Treatment Process	Advantages	Challenges	Reference
Advanced Oxidation Processes (AOPs)	Effective micropollutant removal (e.g. pesticides, pharmaceuticals)	Formation of toxic disinfection byproducts Operational and maintenance cost	(Esplugas et al., 2007; Homem et al., 2011; Ribeiro et al., 2015)
Constructed Wetlands	Low operation and maintenance cost High removal of estrogens and pesticides	Large area requirement Biofilm growth and seasonal dependency	(Töre et al., 2012)

2.2.1. Activated Sludge Process

The principals behind the operation of all aerobic biological systems are the same. Treatment systems shows an alteration depending on the system constrains. In activated sludge systems, the main system constrains are the mixing regime and sludge return. As illustrated in Figure 2 two type of mixing regime exists: completely mixed and plug flow. Theoretically in completely mixed systems, the influent and reactor content are promptly and exhaustively mixed. Therefore, the reactor content and the effluent have the same compound concentrations. Completely mixed activated sludge systems are usually circular or square. Mixing in these systems are generally carried out by diffused air bubble aeration or mechanical aerators. Aerated lagoons, extended aeration plants, single reactor completely mixed activated sludge plants and Pasveer ditches are examples of completely mixed systems.

Plug flow systems are generally long channel type reactors. The influent is introduced from one end and theoretically the reactor is divided into volume elements which are assumed to remain unmixed. Sludge from the settling tank is recycled to the aerobic reactor to inoculate the influent with microorganisms. The sludge return generates an intermediate flow. Depending on the magnitude of the aforementioned flow the

reactor may deviate from plug flow conditions. For instance, in conventional activated sludge systems the sludge return ratios are 0.25 to 3 times the influent flow. At high recycle ratios, the mixing regime of the reactor are completely mixed.

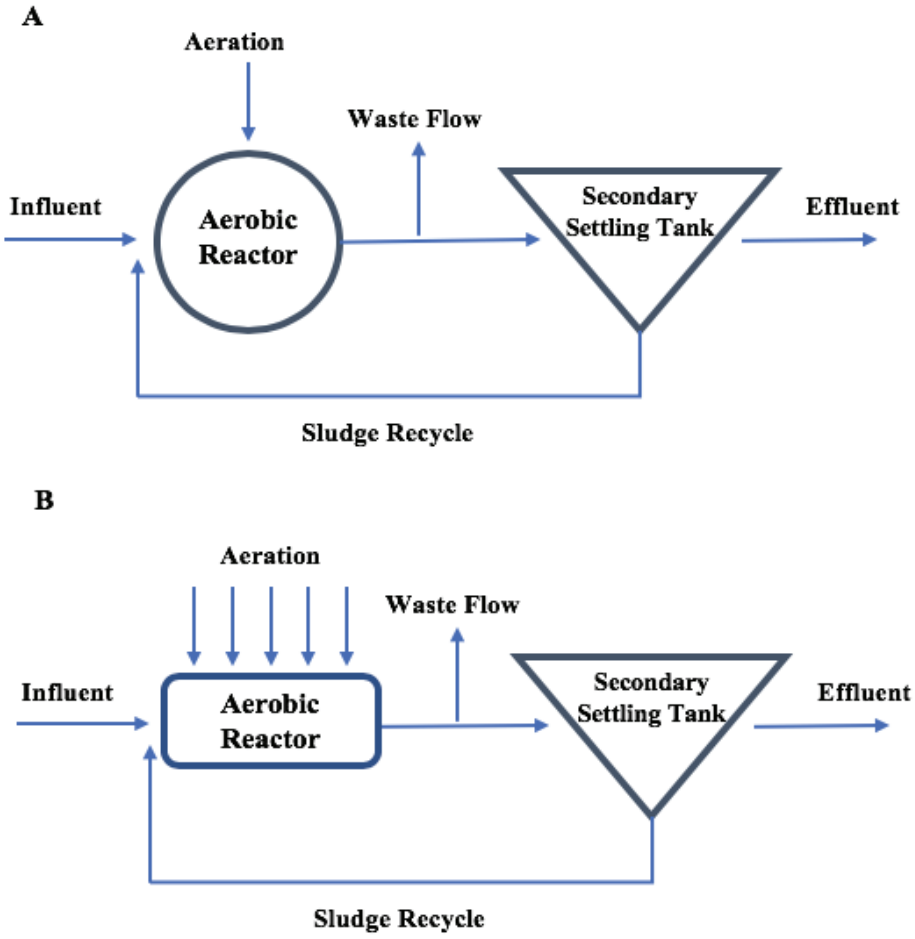

Figure 2 Activated Sludge System with Completely Mixed Reactor (A), a Plug Flow Reactor (B)

2.2.1.1. Some Operational Aspects of Activated Sludge Process

2.2.1.1.1. Sludge Retention Time (SRT)

Sludge retention time (solids retention time or sludge age: SRT) is a commonly used operational parameter in activated sludge systems to control the plant efficiency. By definition, SRT is the amount of time, in days, that bacteria or solids are under aeration (Gerardi, 2002). Thus, SRT is an operational parameter used to maintain the appropriate amount of activated sludge in the aeration basin.

SRT is defined by:

$$SRT = \frac{Mass\ of\ sludge\ in\ reactor}{Mass\ of\ sludge\ wasted\ per\ day} \quad (1)$$

The common practice is to withdraw waste sludge from the secondary settling tank underflow. However hydraulic control of SRT can be maintained by withdrawing sludge directly from the aerobic tank. The sludge concentrations of the biological reactor and the waste flow are the same in the case where sludge is abstracted directly from the biological reactor. For example, to set the SRT to 10 days, 10 % of the reactor must be wasted on a daily basis. Thereby, SRT can be defined as:

$$SRT = \frac{X_t * V_p}{X_t * Q_W} = \frac{V_p}{Q_W} \quad (2)$$

where:

X_t: Total active biomass concentration (mg/L)

V_p: volume of the aeration tank (L)

Q_w: flow rate of the sludge wasted form the reactor (L/day)

2.2.1.1.2. Hydraulic Retention Time (HRT)

Another important parameter in activated sludge systems is hydraulic retention (HRT).The amount of time, in hours, for wastewater to pass through the aeration tank is defined as HRT (Gerardi, 2002).

$$HRT = \frac{Volume\ of\ aeration\ tank}{Daily\ average\ influent\ flow\ rate} \quad (3)$$

2.2.1.1.3. Effect of Inhibition

As the name implies biological processes deal with living organisms, therefore these systems are exposed to inhibitory and toxic agents. Inhibition in activated sludge processes can be related to various factors such as oxidation-reduction potential of the medium, temperature, pH, etc. Furthermore, various substances present in wastewaters cause inhibitory and toxic effects on the biological activity of the microorganisms. Inhibition in biological wastewater treatment is defined as the deterioration of enzymatic system of the microorganism or destruction of the cell structure, eventually resulting in the slowing down of microbial activity. In the case which inhibited biochemical reactions are critical to the cell the agent is classified as toxic. The effect of toxicity is demonstrated by microbial cultures increased difficulty in nutrient removal and decline in growth rate. In other words, in WWTPs degradation rate and biomass activity decreases.

Kinetic models for inhibition

Presence of chemicals in the feed of a WWTP or a metabolism product can reduce the rate of degradation. The enzymatic kinetics of inhibition can be explained with the Michaelis-Menten expression. In other words, Michaelis-Menten expression can be used to understand the enzymatic mechanisms in such cases where inhibitory compounds intrusion occurs.

Equation 4 describes the mechanism of enzyme kinetics, where substrates and enzyme attach to yield a product. S and E represent the substrate and the enzyme, respectively Whereas, S* and P represent the active complex and the product, respectively.

$$S + E \overset{k_1}{\leftrightarrow} S^* \overset{k_2}{\rightarrow} P + E \tag{4}$$

Because the controlling step of the equation is the last step (k_2), enzyme substrate attachment step can be regarded in the equilibrium. Due to this, the Michaelis-Menten kinetic expression is defined as (Henze et al., 2008):

$$r_s = \frac{r_{max} * S}{k_s + S} \tag{5}$$

Where r_s represents enzymatic reaction rate and r_{max} represents maximum enzymatic reaction rate. Kinetic constant k_s is a combination of both steps, $k_s=(k_1+k_2)/ k_1$. Activated complex (S*) decomposition is a slow process and k_s defines the equilibrium coefficient.

Product formation can be disrupted by various ways namely:

- an interfering substance may interact with the same specific site of the enzyme (competitive inhibition)
- an interfering substance may interact with a different site of the enzyme (non-competitive inhibition)
- an inhibitor may interact with the activated complex (un-competitive inhibition)

Competitive inhibition

In competitive inhibition an inhibitor binds to the enzyme in the same place as the substrate, since the inhibitor occupies substrate's site the reaction does not lead to the product (Henze et al., 2008).

$$I + E \leftrightarrow I^{*} \tag{6}$$

Where I represents inhibitor and E represent the enzyme.

In competitive inhibition the kinetic expression transforms to:

$$r_s = \frac{r_{max} * S}{k_s(1 + \frac{I}{K_I}) + S} \tag{7}$$

Here K_I, which is inversely related to the inhibition power, represents the affinity of the inhibitor.

Non-competitive inhibition

$$S + E \leftrightarrow S^* + I \leftrightarrow SI^* \quad (8)$$

$$I + E \leftrightarrow I^* + S \leftrightarrow IS^* \quad (9)$$

When the inhibitor (I) gets attached to the enzyme since it changes the composition, the product formation gets precluded. Therefore, the complexes SI* and IS* will not form a product. In this kind of inhibition, where there is no competition between substrate and inhibitor, kinetic constant k_s remains constant since the affinity of the substrate is not altered. Although k_s remains constant, with the presence of inhibitor r_{max} decreases. In non-competitive inhibition the kinetic expression transforms to (Henze et al., 2008):

$$r_s = \frac{r_{max} * S}{(k_s + S) * (1 + \frac{I}{K_I})} \quad (10)$$

Some examples of non-competitive inhibition are effect of metals on denitrification process (Gumaelius et al., 1996), effect of non-ionic surfactants in activated sludge processes (Carvalho et al., 2001).

Un-competitive inhibition

$$S + E \leftrightarrow S^* + I \leftrightarrow SI^* \tag{11}$$

In un-competitive inhibition after the active complex is formed, the inhibitor attaches to it and ends up blocking the product formation. Both parameters k_s and r_{max} decreases at the presence of inhibitor. In un-competitive inhibition the kinetic expression transforms to:

$$r_s = \frac{r_{max} * S}{k_s + S * (1 + \frac{I}{K_I})} \tag{12}$$

The aforementioned inhibition methods are known as classic forms of inhibition. Figure 3 illustrates the Lineweaver-Burk representations of competitive, non-competitive and un-competitive inhibition.

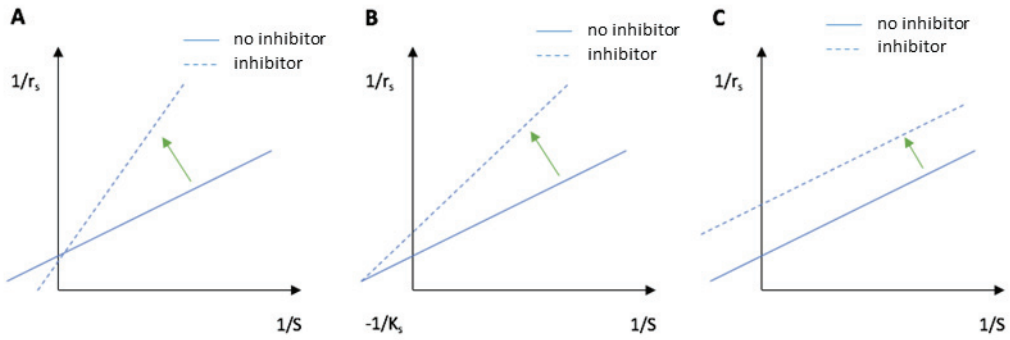

Figure 3 Lineweaver-Burk Representations Competitive (A), Non-Competitive (B) and Un-Competitive (C) Inhibition (Henze et al., 2008)

In competitive inhibition r_{max} is not affected but k_s increases. In non-competitive inhibition r_{max} decreases while k_s remains constant. In uncompetitive inhibition both r_{max} and k_s decreases.

2.2.1.2. Micropollutant Removal by Activated Sludge Process

Activated sludge systems are widely used secondary treatment processes. In Table 4 several micropollutant removal efficiencies in full-scale activated sludge processes are outlined. As mentioned before only a limited number of micropollutants are removed in this process whereas many studies have proven that activated sludge has a proven ability to remove a great variety of micropollutants when modifications to advance treatment processes, such as advanced oxidation processes, activated carbon adsorption, ozonation, membrane bioreactors are made (Clara, Kreuzinger, et al., 2005; Falås et al., 2016; Joss et al., 2005a).

Table 4 Removal Efficiencies of Micropollutants in Activated Sludge Processes

Category	Micropollutant	Source	Influent (μg/L)	Effluent (μg/L)	Removal (%)	Reference
Pesticide	Diuron	WWTP	0.10	0.04	60	(Rosal et al., 2010)
Pesticide	Atrazine	WWTP	0.98	0.73	25	(Luo et al., 2014)
β-blocker	Atenolol	WWTP	0.15	0.03	80	(Miège et al., 2009)
Analgesic	Ibuprofen	WWTP	14.6	1.96	87	(Miège et al., 2009)
Analgesic	Paracetamol	WWTP	80	0	100	(Miège et al., 2009)
Personal Care Product	Tonalide musk	WWTP	2.1	0.32	85	(Rosal et al., 2010)
Endocrine Disruptor	Nonylphenol	WWTP	0.020	0.0016	92	(Liu et al., 2009)

To investigate the effect of the important WWTP design parameter SRT on the elimination of micropollutants, Clara et al. (2003) conducted a study with lab scale experiments in 3 different SRTs (1, 16 and 35 days). Removal of common pharmaceuticals were studied, only one antibiotic (Sulfamethoxazole) was degraded up to 70% at the reactors working at SRT 1 day. As the SRT increased to 16 days, significant removals were observed. Although there were significant increases in the removal rates for most of the pollutants, the compound diclofenac was an outlier. As the SRT increased, the removal rate decreased for diclofenac. Clara et al. (2003) stated that this behavior was due to the fact that as SRT increases, excess sludge production decreases and in an equilibrated system, daily excess sludge production stands for the adsorbent, thus the adsorption capacity decreases. Further increase in SRT did not demonstrate any significant increase in the elimination of micropollutants.

Additional studies were carried out in this topic by the same scientist. Clara et al. (2005) determined specific SRTs for different micropollutants. Furthermore, the removal efficiencies of conventional activated sludge systems and a membrane bioreactor working at the same SRTs (1, 5, 13 and 26 days) were compared. No notable difference was observed in the mentioned treatment techniques, although it must be kept in mind that it is possible to achieve higher SRTs in membrane bioreactors. With increasing SRT, enhanced removals were detected for most of the micropollutants. Almost complete removal of most of the compounds were detected at SRT 10 days. Similar enhanced removal performances after increasing SRT were reported by Joss (2004). With setting the critical SRT as 10 days it can be concluded that, WWTPs with nitrogen removal are also successful in eliminating degradable micropollutants. However, contradicting results were also obtained since some of the pharmaceuticals did not degrade in any studied SRT.

Kreuzinger et al. (2004) conducted a study to compare removal efficiency of activated sludge plants working under different SRTs. The aim of the study was to describe an approach to understand comparable removal rates for activated sludge systems based on SRT and mass balance. In this manner, Kreuzinger et al. (2004) investigated a group of 15 micropollutants including endocrine disruptors and pharmaceutical compounds. This group of investigated compounds included commonly studied micropollutants such as Bisphenol-A, Carbamazepine, Diclofenac, Galaxolide and Ibuprofen. Investigation on the behavior of selected compounds in the course of wastewater treatment were performed at different scales. Laboratory scale experiments in which the reactors were fed with synthetic wastewater were run under four different SRTs, which were 1, 5, 15 and 35 days. For the scope of this study, a membrane pilot plant with an ultrafiltration membrane was operated under 3 different SRTs of 11, 20 and 41 days. Finally, sampling campaigns were conducted at 4 full scale WWTPs with activated sludge process. No removal was detected for the plants

working at SRT 1 day. Kreuzinger et al. (2004) observed that with increasing SRT the biodegradation of micropollutants increased.

Strenn et al. (2004) conducted a study to understand the influence of SRT on the elimination of Benzafibrate, Carbamazepine, Diclofenac and Ibuprofen. Laboratory scale plants were operated while the removal of the aforementioned micropollutants were monitored at 12 full scale WWTPs. According to this study, in the lab scale high loaded sequenced batch reactor with SRT of 1 day no significant removal was achieved for all four micropollutants. Reactors were operated at SRT 1, 4, 17 and 29 days. Micropollutants Carbamazepine and Diclofenac did not demonstrate a notable removal in all four SRTs. No dependency on the SRT was correlated for these compounds. Zwiener et al. (2001) also noted that Diclofenac removal in pilot WWTPs were 1-6%. While no elimination of Ibuprofen and Benzafibrate was observed in SRT 1 day, it was shown that removal efficiencies over 90% could be achieved with increasing SRT up to 4 days and more. An apparent dependency of the removal efficiencies on SRT was ascertained for these compounds. In the full-scale plants, an apparent dependency of SRT was detectable for benzafibrate. As in lab scale experiments, no dependency of SRT was detected for Carbamazepine and Diclofenac since no significant removal was detected.

As mentioned before micropollutant removal is influenced by activated sludge process' variable SRT, many researchers have focused on this subject to optimize maximum removals of a diverse range of micropollutants (Clara, Kreuzinger, et al., 2005; Hamid et al., 2012; Maeng et al., 2013; Strenn et al., 2004). SRT ≥ 10 days is considered necessary to enhance removal of biodegradable micropollutants (Clara, Kreuzinger, et al., 2005; McAdam et al., 2010). On the other hand, it is reported that metals are solubilized by chelators produced by biomass at SRT ≥ 10 days which ended up in increased metal concentration in the effluent (Santos et al., 2010).

A study was carried out with a wide range of micropollutants including steroid estrogens, metals and nonylphenolics in which SRT and HRT was focused separately and their individual effect on microbial removal was evaluated. The pilot scale activated sludge process was operated under 3 SRTs (3, 10 and 27 days) while HRT was set to 8 hours and 3 HRTs (8, 16 and 24 hours) while SRT was kept constant at 27 days (Petrie et al., 2014). At SRT 27 days, maximum achievable removal was achieved for all compounds. Moreover, when HRT was increased to 24 hours organic biodegradation was enhanced, especially for recalcitrant estrogens (Petrie et al., 2014).

Falås et al. (2016) conducted a 10 years long study to investigate the limits of organic micropollutant removal in biological wastewater treatment with 15 diverse biological reactors. Short and long-term experiments were run. Three parallel sequencing batch reactors were operated at SRTs of 25, 40 and 80 days. These reactors were fed with synthetic wastewater for a year without any exposure to micropollutants, after a year micropollutants were spiked. Following this exposure, degradation of micropollutants started immediately. On this basis, Falås et al. (2016) concluded that long-term exposure to micropollutants is not an essential trigger for micropollutant degradation in biological WWTPs. In the activated sludge reactors fed with synthetic wastewater, no correlation between removal rate constants and SRT was observed. However, it must be kept in mind that even the lowest SRT tested by Falås et al. (2016) was 25 days, which is above the critical SRT of 10 days reported by Clara et al.(2005). Another outcome of this study is that the micropollutant removal rate constants depend on the compound rather than the biomass, in other words degradation rates k_{bio} (L/gSS.d) were not affected by SRT increase.

Moreover, inhibition of main substrate removal in the presence of micropollutants has been reported for some antibiotics (Plósz et al., 2010) and estrogens (Li et al., 2008). The same behavior, was seen when trimethoprim was spiked into synthetic wastewater (Falås et al., 2016). In the content of this study, Falås et al. (2016) also examined the

removal of carbendazim pesticide which is studied in the scope of this thesis. For carbendazim, a strong inhibitory response was not detected.

Furthermore, Falås et al. (2016) conducted long term experiments with municipal wastewater. A significant increase in removal of several micropollutants were observed when compared to the activated sludge reactors fed with synthetic wastewater. Oxic biofilm treatment and anaerobic treatment also improved the removal of specific recalcitrant pollutants.

2.3. Pesticides Studied

Pesticides are organic compounds which are used in the management of pests. These chemicals act against pests by several ways such as mitigation, destruction, prevention and repulse. Pesticides are categorized based on their use. The most important categories are herbicides, insecticides and fungicides. The mass production of synthetic organic compounds for pest control initiated with the discovery of DDT in 1938 and the exponential increase in production and use continued since (Matthews, 2006). Although pesticides are used for a good purpose, extensive use of these synthetic compounds resulted in environmental contamination problems worldwide along with deleterious effects on humans and ecosystem (Virkutyte et al., 2010).

The following sub-sections present the general information on the pesticides studied in this thesis.

2.3.1. Carbendazim

Carbendazim is a broad spectrum benzimidazole fungicide. It is colorless crystalline odorless solid. Carbendazim is used to control fungal diseases while growing pears, grapes, wheat, rice, strawberries, apples, lemons, lentil and several other crops. Carbendazim is not allowed any longer in European Union since 2014 due to its toxic effects. After the expiration of approval, EU announced the grace period of usage as 31/05/2016 and now no member state is using carbendazim as an antifungal (European Commission, 2013).

It is proven that carbendazim has deleterious effects on reproduction (Carter et al., 1987). Other than its sole usage, carbendazim ends up in the environment after application of benomyl fungicide as a daughter product. Many studies have been carried out for years to investigate benomyl and its metabolites' deleterious effects on reproduction (Gray et al., 1990; Jeffay et al., 1996; Lazzari et al., 2008; Moffit et al., 2007; Yu et al., 2009). Carbendazim is also referred as a genotoxic substance (PAN Europe, 2014). Studies showed that the compound causes liver tumor in mice (McCarroll et al., 2002) and abnormalities in sperm (Amer et al., 2003). Moreover, carbendazim is a potent endocrine disruptor (Kim et al., 2009; Morinaga et al., 2004). Carbendazim is listed as a specific pollutant for Turkey.

Annual average environmental quality standard (AA-EQS) is derived for controlling the effects of long-term pollution and maximum allowable concentration environmental quality standard (MAC-EQS) is derived for controlling the effects of short-term pollution. For inland waters, annual average environmental quality standard (AA-EQS) is set as 2.7 μg/L, while maximum allowable concentration (MAC-EQS) is set as 77 μg/L (Ministry of Forestry and Water Affairs, 2016). Additional information can be found in Table 5.

2.3.2. Imidacloprid

Neonicotinoids are synthetic compounds mimicking nicotine. Imidacloprid is a neonicotinoid insecticide and it is used to control termites, sucking insects, soil insects and fleas (European Commission, 2015).

Imidacloprid is used while growing a broad range of products including, grapes, tobacco, cotton, beans, pears, okra, apples, potatoes, tomatoes, pistachio and many other (EFSA, 2016).

Imidacloprid is not carcinogenic to human (Harada et al., 2016) whereas, it is highly toxic to honeybees and birds (European Commission, 2005). Imidacloprid is listed as a specific pollutant for Turkey. For inland waters, AA-EQS is set as 0.14 μg/L, while MAC-EQS is set as 1.4 μg/L (Ministry of Forestry and Water Affairs, 2016). Additional information can be found in Table 5.

2.3.3. Aclonifen

Aclonifen is an herbicide used in monocotyledonous and dicotyledonous plant protection products. The compound is classified as a nitrophenyl ether herbicide and inhibits carotenoid biosynthesis. Aclonifen is a systemic, selective herbicide (EFSA, 2008).

Aclonifen is used to control grass and broad-leaved weeds. It is applied while growing various products including sunflowers, soybeans, beans, peanut, rice, peas and carrots. The usage of aclonifen is allowed by European Union.

Toxicity tests has been carried out on rats for aclonifen. The results showed that aclonifen is of very low acute toxicity. Neither skin nor eye irritancy were detected in tests with guinea pigs. Aclonifen is not classified as genotoxic (EFSA, 2008). Aclonifen is classified as a priority substance. For inland waters, AA-EQS is set as 0.12 μg/L, while MAC-EQS is set as 0.012 μg/L (European Commission, 2013 ; Ministry of Forestry and Water Affairs, 2016). Additional information can be found in Table 5.

Table 5 EQS for Carbendazim, Imidacloprid and Aclonifen

Pesticide	Priority/ Specific Pollutant	AA-EQS (Inland Surface Waters)	AA-EQS (Other Surface Waters)	MAC-EQS (Inland Surface Waters)	MAC-EQS (Other Surface Waters)
Carbendazim	Specific Pollutant	2.7 μg/L	77 μg/L	2.7 μg/L	77 μg/L
Imidacloprid	Specific Pollutant	0.14 μg/L	1.4 μg/L	0.14 μg/L	1.4 μg/L
Aclonifen	Priority Pollutant	0.12 μg/L	0.012 μg/L	0.12 μg/L	0.012 μg/L

2.4. Fate of Micropollutants in WWTPs

The factors affecting the fate of micropollutants in WWTPs can be grouped as internal factors and external factors. Internal factors are micropollutant related factors, in other words, the physical and chemical characteristics of compounds. Whereas, external factors are WWTP related factors, such as the treatment conditions. Various treatment technologies can be employed for the removal of micropollutants however no matter what technology is used, the removal depends on the physical and chemical properties of micropollutants and treatment conditions (Luo et al., 2014).

In biological WWTPs, micropollutants can be removed through transformation, sorption and volatilization (Falås et al., 2016). Whereas, biodegradation and sorption are the two major removal mechanisms. Most of the micropollutants are non-volatile as the selected pesticides for this thesis; carbendazim, imidacloprid and aclonifen.

The octanol-water partitioning coefficient (K_{ow}) can be used to understand the sorption behavior of chemicals. Sorption of compounds to solids mostly depends on the hydrophobicity, and octanol-water partitioning coefficient can be used in this manner. A rule of thumb for estimating the sorption potential can be provided: $\log K_{ow} < 2.5$ indicates low sorption potential, $2.5 < \log K_{ow} < 4$ indicates medium sorption potential, and $\log K_{ow} > 4$ indicates high sorption potential (Rogers, 1996).

The solid-water distribution coefficient (K_d) is defined as the partition of a compound between the water phase and sludge in activated sludge processes (Luo et al., 2014). Scientists have proposed K_d as an accurate indicator of sorption behavior when taken into consideration both with $\log K_{ow}$ and pK_a (Joss et al., 2005b; T. A. Ternes et al., 2004). It was reported that for compounds with K_d values below 300 L/kg sorption

onto secondary sludge was considered insignificant (Luo et al., 2014). In a recent study it was reported that, for the micropollutants with K_d values between 10-500 L/kg sorption was insignificant, the micropollutant fraction removed by excess sludge was less than 10% (Falås et al., 2016). Furthermore, micropollutants estrone and nonylphenol (log K_d > 3.2) were easily removed (> 85%) by sorption to secondary sludge (Tadkaew et al., 2011).

With the knowledge of the solid-water distribution coefficient, it is possible to assess the sorption behavior of micropollutants unless WWTPs (or experimental reactors) are exposed to iron supplements. High iron dosage may change the sorption characteristics of the sludge (Carballa et al., 2004). Therefore, sorption assessment through K_d values becomes challenging. Nevertheless, Falås et al. (2016) reported that an iron dosage up to 0.5 g/L does not significantly increase the micropollutant removal by sorption. It should be noted that the synthetic wastewater fed to the reactors throughout this study contained 0.45 g/L of iron.

Biodegradability of micropollutants depends on the bioavailability of compounds. Compound structure plays a major role since the first step of biodegradation is the uptake of by cell (Siegrist et al., 2005). As the complexity of the compound increases the degradation gets harder. Similarly, the presence of functional groups obstructs the biodegradation process. For instance, the persistent micropollutants usually possess halogens, sulfates or electron withdrawing functional groups (Joss et al., 2005a; Tadkaew et al., 2011).

2.4.1. Carbendazim

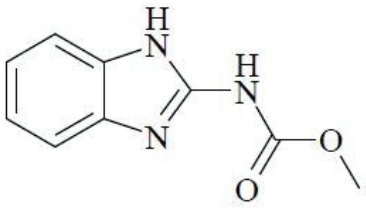

Figure 4 Structural Formula of Carbendazim (European Commission, 2007)

Table 6 Physical and Chemical Properties of Carbendazim (EFSA, 2014; European Commission, 2007; PAN Europe, 2014; Wick et al., 2011)

Name of Property	**Explanation/Value**
Common Name (ISO)	Carbendazim
Chemical Name (IUPAC)	methyl benzimidazol-2-ylcarbamate
Synonyms	BCM, Methyl 2-benzimidazolecarbamate, Methyl benzimidazol-2-ylcarbamate
CAS Number	10605-21-7
EC Number	234-232-0
Molecular Formula	$C_9H_9N_3O_2$
Molecular Mass	191.21 g/mol
Form	Almost clear crystalline odorless solid
Melting Point	Above 302 – 307 °C
Vapor Pressure	1.5 x 10^{-4} Pa (25 °C)
Solubility in Water	30 mg/L at pH 4, 8 mg/L at pH 7 and 1.49 mg/L at pH 8 (20 °C)
Acid Dissociation Constant (pK_a)	4.2

Name of Property	Explanation/Value
Octanol-Water Partition Coefficient ($logK_{ow}$)	1.48 at pH 7 (20 °C)
Solid-Water Distribution Coefficient (K_d)	20 L/kg

Carbendazim, is not expected to adsorb to solids due its low solid-water distribution coefficient of 20 L/kg and octanol-water partition coefficient of 1.48. Luo et al (2014) reported that adsorption potential of compounds with K_d values below 300 L/kg are insignificant (Table 6). Furthermore, Rogers (1996) stated that if a chemical's log K_{ow} value is lower than 2.5 the sorption potential is low. As a result, carbendazim is not expected to adsorb to solids due its low solid-water distribution coefficient of 20 L/kg and octanol-water partition coefficient of 1.48.

2.4.2. Imidacloprid

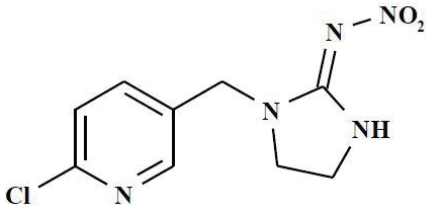

Figure 5 Structural Formula of Imidacloprid (European Commission, 2008)

Table 7 Physical and Chemical Properties of Imidacloprid(EFSA, 2016; European Commission, 2008; Flores-Céspedes et al., 2002)

Name of Property	**Explanation/Value**
Common Name (ISO)	Imidacloprid
Chemical Name (IUPAC)	(E)-1-(6-Chloro-3-pyridinylmethyl)-N-nitroimidazolidin-2- ylideneamine
CAS Number	138261-41-3
EC Number	428-040-8
Molecular Formula	$C_9H_{10}C_lN_5O_2$
Molecular Mass	255.7 g/mol
Form	White odorless powder
Melting Point	144 °C
Vapor Pressure	4.0 X 10^{-07} mPa (25 °C)
Solubility in Water	610 mg/L
Acid Dissociation Constant (pK_a)	-
Octanol-Water Partition Coefficient ($logK_{ow}$)	0.57 at pH 7 (20 °C)
Solid-Water Distribution Coefficient (K_d)	2.56 L/kg

The K_{ow} value can be used to understand the sorption behavior of pollutants. The rule of thumb suggested by Rogers (1996) is that if a chemical's log K_{ow} value is lower than 2.5 the sorption potential is low. Imidacloprid has an octanol-water partition coefficient of 0.57 which is way lower than 2.5 thus the sorption potential of imidacloprid is very low. Additionally, as it can be also seen from Table 7 imidacloprid is not expected to adsorb to solids given its low solid-water distribution coefficient 2.56 L/kg and high water solubility 610 mg/L.

2.4.3. Aclonifen

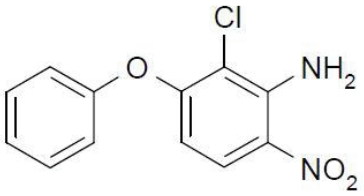

Figure 6 Structural Formula of Aclonifen (European Commission, 2012)

Table 8 Physical and Chemical Properties of Aclonifen (EFSA, 2008, 2015; European Commission, 2012)

Name of Property	**Explanation/Value**
Common Name (ISO)	Aclonifen
Chemical Name (IUPAC)	2-chloro-6-nitro-3-phenoxyaniline
CAS Number	74070-46-5
EC Number	277-704-1
Molecular Formula	$C_{12}H_9ClN_2O_3$
Molecular Mass	264.7 g/mol
Form	Yellow odorless powder
Melting Point	81.2 °C
Vapor Pressure	0.016 mPa (25 °C)
Solubility in Water	1.4 mg/L (20 °C) at pH 5 to pH 9
Acid Dissociation Constant (pK_a)	Not measurable, by calculation -3.15
Octanol-Water Partition Coefficient ($logK_{ow}$)	4.37
Organic Carbon-Water Partition coefficient (K_{oc})	7126

Name of Property	Explanation/Value
Solid-Water Distribution Coefficient (K_d)	892 L/kg

Aclonifen, is a hydrophobic pollutant with an octanol-water partition coefficient of 4.37 (Table 8). According to Rogers (1996), pollutants with log K_{ow} values higher than 4 have high sorption potential. The sorption potential is also supported by the organic carbon-water partition coefficient of 7126. Additionally, the solid-water distribution coefficient is higher than both the ranges determined by Falås et al. (2016) and Luo et al. (2014).

CHAPTER 3

MATERIAL AND METHODS

3.1. Source of Microbial Culture

In this study, the microbial culture used as seed in the reactors was obtained from the return activated sludge line following the secondary sedimentation tank of Ankara Central Wastewater Treatment Plant, which is the largest WWTP of Turkey with a treatment capacity of 765,000 m^3/day. The WWTP is also known as Tatlar WWTP taking its name from the village it is located, Tatlar village, which is 45 km away from Ankara's city center.

Ankara Central WWTP is designed as a conventional activated sludge system, the excess sludge is anaerobically digested and from the biogas obtained, electricity is produced. The produced electricity is used within the WWTP. The treated effluent is discharged to Ankara Creek.

Sludge samples were taken from the return activated sludge line following the secondary sedimentation tanks. The collected sludge samples were immediately brought to the laboratory. Prior to starting the experiments, the sludge samples were sieved to eliminate any large particle. After sieving, the samples were left for 2 hours to settle and to increase the solid concentration. As soon as settling had occurred, the supernatant was drained. Finally, the remaining sludge samples were aerated for 1 day before preparing the experimental setups.

3.2. Synthetic Wastewater

In the scope of this study, the reactors were operated with synthetic wastewater. The composition of synthetic wastewater is given in Table 9**.** The carbon and energy source was provided by Oxoid branded proteose peptone. The concentration of peptone was fixed to attain 500 mg/L COD content in synthetic wastewater. Although, the concentration of peptone was fixed, COD analysis of synthetic wastewater was done constantly to ensure exactly 500 mg/L COD was attained. The proteose peptone also served as nitrogen source to the microbial culture, which corresponds to around 250 mg/L protein (Dilek et al., 1998).

Stock solutions of carbendazim, imidacloprid and aclonifen pesticides were prepared by using ultra-pure water. The stock solutions were stored in 100 mL borosilicate glass volumetric flasks. The flasks were covered to eliminate the entrance of light. Stock solutions of carbendazim and aclonifen were stored in refrigerator at +4°C whereas imidacloprid stock solution was stored in room temperature. In order to adjust the required concentrations, pesticides were spiked into the prepared synthetic wastewater.

Table 9 Composition of Synthetic Wastewater (adapted from Orhon, 2014)

Ingredient	**Concentration (mg/L)**
Proteose-Peptone	470 (500 mg/L as COD)
NaCl	156.70
Na_2SO_4	17.20
K_2HPO_4	44.60
KH_2PO_4	20.00
$MgCl_2.6H_2O$	3.700
$FeCl_2.4H_2O$	4.520
$CaCl_2$	2.794
$MnSO_4.H_2O$	0.0638
$ZnSO_4.7H_2O$	0.0819
$CoCl_2.6H_2O$	0.0753
$CuSO_4$	0.0760
$(NH_4)_6Mo_7O_{24}.4H_20$	0.0338

Synthetic wastewater was prepared by using tap water, immediately after preparation pH was measured. In all experimental setups, the initial pH was in between pH 7-7.4.

3.3. Pesticides Studied

In this study, three different pesticides, namely, carbendazim, imidacloprid and aclonifen were used. The pesticide concentrations studied were: 10 μg/L, 25 μg/L, 50 μg/L, 100 μg/L, 200 μg/L, 300 μg/L and 400 μg/L. This concentration range was determined with the consideration of several factors to begin with the toxicity thresholds' of carbendazim, imidacloprid and aclonifen pesticide were considered. Secondly, the monitoring results of Yeşilırmak River Basin were taken into account

as well as the results of literature survey on pesticide concentrations encountered in wastewaters. According to the monitoring results of the TÜBİTAK project Carbendazim, Imidacloprid and Aclonifen exceeded their relevant EQSs more than once. Carbendazim was found to be 0.800 μg/L, Imidacloprid was found to be 599.022 μg/L and Aclonifen was found to be 0.972 μg/L. The biggest limitation was the analysis capability of HPLC device. In all 6 methods developed, the limit of detection was 10 μg/L. Owing to this limitation, the lowest concentration to work was determined as 10 μg/L. The highest concentration was set as 400 μg/L considering both the solubility of studied pesticides and monitoring results.

3.4. Experiments

To understand the effects of concentration and sludge age 20 instantaneously fed SBRs were operated throughout this study. Besides the effect of concentration and sludge age, the effect of individual pollutant treatment and mixed pollutant treatment was studied by operating different reactors including pesticides individually or as mixtures. As mentioned previously, the reactors were operated with the sludge samples taken from Ankara Central WWTP. In this study, the reactors were operated under 5 different solids retention time (SRT) conditions which are 3, 8, 10, 20 and 30 days. These reactors were fed daily with synthetic wastewater which was spiked with different concentrations (10-400 μg/L) of a specific pesticide or mixture of pesticides. The reactors operated during the scope of this study are listed in Table 10.

Table 10 Instantaneously fed SBRs Operated

Reactor Name	Pesticide Spiked	SRT (day)
Reactor 1	Carbendazim	3
Reactor 2	Carbendazim	8
Reactor 3	Carbendazim	10
Reactor 4	Carbendazim	20
Reactor 5	Carbendazim	30
Reactor 6	Imidacloprid	3
Reactor 7	Imidacloprid	8
Reactor 8	Imidacloprid	10
Reactor 9	Imidacloprid	20
Reactor 10	Imidacloprid	30
Reactor 11	Aclonifen	3
Reactor 12	Aclonifen	8
Reactor 13	Aclonifen	10
Reactor 14	Aclonifen	20
Reactor 15	Aclonifen	30
Reactor 16	Carbendazim + Imidacloprid +Aclonifen	3
Reactor 17	Carbendazim + Imidacloprid +Aclonifen	8
Reactor 18	Carbendazim + Imidacloprid +Aclonifen	10
Reactor 19	Carbendazim + Imidacloprid +Aclonifen	20
Reactor 20	Carbendazim + Imidacloprid +Aclonifen	30

Amber glass bottles of 2.5 L were used as reactors to eliminate the risk of photodegradation (Figure 7). Also glass reactors were preferred instead of plastic reactors to avoid the possibility of adsorption of pollutants to inner surfaces of the reactor. Although, the volume of a bottle was 2.5 L the net volume used in the experiments were 2 L. Initially, the reactors' liquid composition was arranged by

adding 0.2 L of sludge and 1.8 L of synthetic wastewater. Once the reactors were set, they were placed in water baths to provide the temperature control. Water baths were set to work at 25 °C and aeration was provided by air pumps to obtain dissolved oxygen concentration of at least 3 mg/L.

Figure 7 Reactors used in the study

The reactors were operated in fill and draw mode at five different SRTs. For each reactor depending on its SRT every day at the same time the following procedure was applied in the following order; the reactors were mixed completely, a specific portion of reactor content was wasted, the reactor was allowed to settle for 30 minutes, a portion of the supernatant was drained and finally 1 L of synthetic wastewater was added. The synthetic wastewater influent to the reactors were set to be 1 L/day and the active volume of the reactors were kept constant at 2 L which gives 2 d of HRT in the

reactors. The portions of wastewater wasted and supernatant drained from the reactor to ensure SRTs are given in Table 11.

Table 11 Portion of Reactor Content Wasted and Supernatant Drained at Different SRTs

SRT (day)	Wastewater Wasted (mL/day)	Supernatant Drained (mL/day)
3	670	330
8	250	750
10	200	800
20	100	900
30	67	933

Initially the reactors were operated without pesticides. The reactors were operated for at least 2 SRTs to reach steady state condition. Steady state conditions were followed by daily mixed liquor suspended solids (MLSS) and COD measurements. Once steady state was reached, samples were taken from the reactors to conduct COD, MLSS, pH measurements. After completing aforementioned measurements, the same reactors were operated with pesticides. As mentioned before, pesticides were spiked into 1 L synthetic wastewater, then the synthetic wastewater was fed to the reactors on a daily basis. This is the case for individual pollutant experiments. For the reactors working with mixed pollutants, the equal concentrations of 3 pesticides, namely carbendazim, imidacloprid and aclonifen were spiked to the synthetic wastewater. The initial concentration studied both in individual reactors and reactors fed with mixture of pesticides was 10 μg/L. Initially the reactors were fed with synthetic wastewater bearing 10 μg/L pesticide a daily basis. Steady state conditions were followed by daily MLSS and COD measurements. Once steady state was reached, samples were taken from the reactors to conduct COD, MLSS, pH and pesticide measurements. Prior to

HPLC analysis, the supernatants taken from the reactors were passed through 0.22 μm pore sized filters. After completing aforementioned measurements, the pesticide concentrations fed to the reactors were increased. From this point on the same procedure was followed for each concentration studied.

3.5. Analytical Methods

3.5.1. MLSS

Daily MLSS measurements were done during the throughout this study. These measurements were performed in accordance with the Standard Methods 2540B procedure (APHA/AWWA/WEF, 2012). To begin with, evaporating dishes were prepared by cleaning, heating the dishes in 103 to 105°C for 1 hour and cooling in a desiccator. Then the dishes were weighed. Well mixed wastewater samples were taken from each reactor and 5 mL samples were passed through a 0.45 μm pore sized filter by using a Whatman vacuum filtration apparatus. The filters were dried in a drying oven at 103 to 105°C for 1 hour. Following this, the dishes were cooled in a desiccator by doing so the effect of humidity is overruled. Finally, the dishes were weighed through the use of an analytical balance, which is capable of weighing to 0.1 mg. The described procedure was repeated 3 times for each wastewater sample. MLSS concentration was calculated as follows:

$$MLSS = \frac{(M_2 - M_1) * 1000}{mL\ of\ sample} \quad (14)$$

where:

M_2= weight of dried residue + filter + evaporating dish, mg

M_1=weight of filter + evaporating dish, mg

3.5.2. COD

COD values of synthetic wastewater and reactor supernatants were measured in accordance with Hach 8000 method (HACH, 2014). Hach branded COD digestion vials; both high range (100 mg/L - 2000 mg/L) and low range kits (15 mg/L - 150 mg/L) were used. Due to the high number of reactors in this study and the need for working in triplicates, in some experiments self-prepared COD kits were also used. Laboratory prepared COD kits were made according to Standard Methods 5220 (APHA/AWWA/WEF, 1999). The COD measurements were done as follows, 2 mL wastewater sample was put into COD kits, mixed completely and digested for 2 hours at 150°C. After cooling in a dark place, COD concentrations were determined by using Hach DR/2500 spectrophotometer.

3.5.3. pH

Hach HQ40D portable multi meter was used to measure pH values of synthetic wastewater and reactor supernatants. The portable multi meter was calibrated once a month by using Hach pH 4 and pH 9 buffer solutions.

3.5.4. Pesticides

Analysis of micropollutants in wastewater samples is difficult due to their low concentrations. To analyze carbendazim, imidacloprid and aclonifen liquid chromatography was used. During this study, 2 different HPLC devices were used. The initial experiments were carried out using Shimadzu LC10AT equipped with Nucleosil C18 column (inner diameter 4.6mm, length 250mm, particle size 5μm) and

SPD-10Avp UV/VIS detector. The injection volume of the device is fixed as 20µL (Figure 8). Analysis methods for carbendazim, imidacloprid and aclonifen were developed for this HPLC.

Figure 8 Shimadzu LC10AT HPLC Device

Even though the Shimadzu works perfectly, the biggest drawbacks of this HPLC is that the device does not have an auto sampler and a degasser. Therefore, injecting every analysis manually takes a lot of time, as well as degassing the mobile phase. As a result of these drawbacks, in this study another HPLC device which has recently been brought to the laboratory was also used (Figure 9) after the initial phase of the study. The Agilent 1200 branded HPLC is equipped with an auto sampler and a degasser which improved the experiment conditions, by reducing idle time. Agilent

1200 HPLC is also equipped with a Zorbox Eclipse Plus C18 column (inner diameter 3.5 mm, length 100 mm, particle size 3.5 μm) and 1260 Infinity II Variable Wavelength Detector. The injection volume of the devices can vary between 5 μL – 60 μL. Analysis methods for carbendazim, imidacloprid and aclonifen were developed for this Agilent branded HPLC as it was for Shimadzu HPLC. Although these 2 HPLC devices are both equipped with C18 columns, the properties of these columns differ from each other. Due to these differences, the analysis methods developed differ from each other.

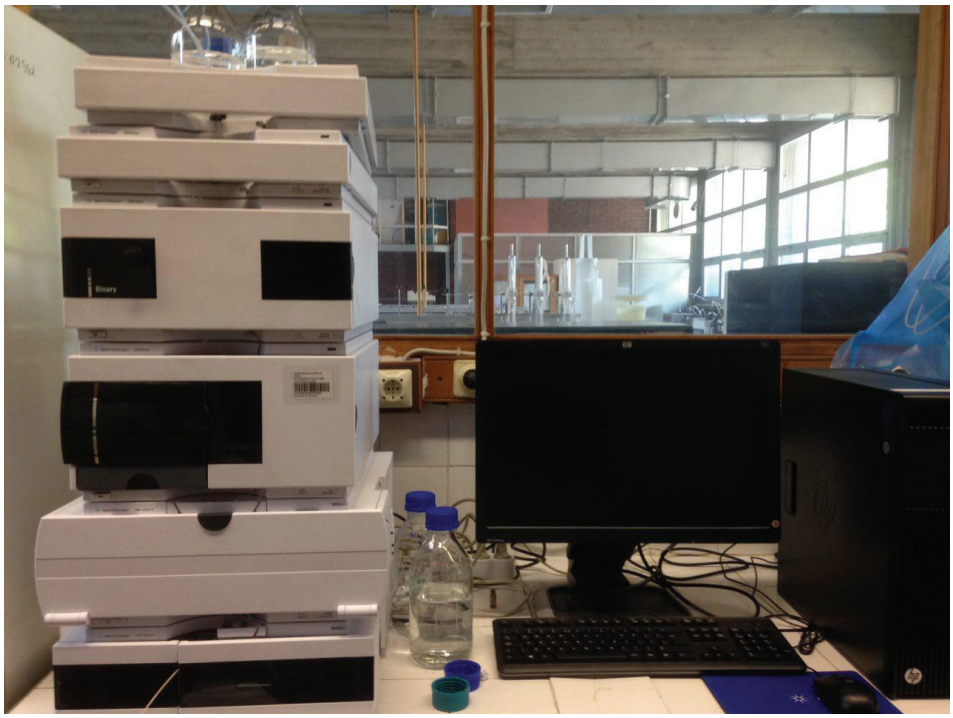

Figure 9 Agilent 1200 HPLC Device

The developed methods for the two HPLC devices are given in Table 12, Table 13 and Table 14, for carbendazim, imidacloprid and aclonifen, respectively. In carbendazim analysis method methanol and water mixture was used as mobile phase.

Whereas in imidacloprid and aclonifen analysis acetonitrile-water mixture was used as mobile phase. Calibration curves of the methods are presented in Appendix A.

Table 12 Detail of Carbendazim Determination Methods

Analyzed Pollutant	Carbendazim	Carbendazim
HPLC Brand	Shimadzu	Agilent
Mobile Phase	50% Methanol 50% Ultra-pure Water	50% Methanol 50% Ultra-pure Water
Flow Rate	0.9 mL/min	1 mL/min
Oven Temperature	40 °C	40 °C
Retention Time	8 min	2.3 min
Wavelength	254 nm	254 nm
Injection Volume	20 μL	20 μL

Table 13 Detail of Imidacloprid Determination Methods

Analyzed Pollutant	Imidacloprid	Imidacloprid
HPLC Brand	Shimadzu	Agilent
Mobile Phase	40% Acetonitrile 60% Ultra-pure Water	40% Acetonitrile 60% Ultra-pure Water
Flow Rate	1.5 mL/min	0.75 mL/min
Oven Temperature	30 °C	30 °C
Retention Time	3.2 min	2.26-2.4 min
Wavelength	270 nm	270 nm
Injection Volume	20 μL	20 μL

Table 14 Detail of Aclonifen Determination Methods

Analyzed Pollutant	Aclonifen	Aclonifen
HPLC Brand	Shimadzu	Agilent
Mobile Phase	60% Acetonitrile 40% Ultra-pure Water	60% Acetonitrile 40% Ultra-pure Water
Flow Rate	1.5 mL/min	1.5 mL/min
Oven Temperature	40 °C	40 °C
Retention Time	8.3-8.9 min	2.8 min
Wavelength	220 nm	220 nm
Injection Volume	20 μL	20 μL

3.6. Chemicals

Analytical standards of carbendazim, imidacloprid, and aclonifen, HPLC grade acetonitrile (gradient grade, ≥99.9) and HPLC grade methanol (gradient grade, ≥99.9), Proteose-peptone and synthetic wastewater minerals were purchased from Merck KGaA,Germany. COD kits were purchased from Hach Co.,USA.

3.7. Laboratory Devices and Equipment

Before usage, all apparatus (glass pipettes, beakers, volumetric flasks, amber glass bottles, micro-spoons etc.) were washed and dried to ensure that any kind of pollutants attached to these materials were eliminated in order to remove the risk of pollution. The following steps were done to eliminate possible interferences. To begin with, all apparatus were placed in a mixture of hot water and Alconox detergent for a night,

scrubbed and rinsed with hot tap water followed by ultra-pure water and the final rinsing was done either by HPLC grade methanol or acetonitrile depending on the intended usage. The cleaning procedure was finalized by drying the apparatus at 105ºC for at least 1 hour after cleaning.

Laboratory devices and equipment used among the period of this thesis are listed in Table 15.

Table 15 Laboratory Devices and Equipment

Name	**Model**	**Intended Use of Device**
HPLC	Shimadzu LC-10AT	Pesticide analysis
HPLC	Agilent 1200	Pesticide analysis
Ultrapure water purification system	MilliporeMilli-Q Simplicity 188	Water purification
Magnetic stirrer	Isolab 613.03.001	Preparation of solvents
Furnace	Nüve FN/032/055/120 Dry Heat Sterilizer	MLSS analysis and drying apparatus
Analytical balance	Sartorius GC8035-OCE	Measuring the weights of chemicals and filters
Refrigerator		Safe storage of solutions and samples
Water Baths	Thermo Fisher Scientific	Optimized temperature control
Hach COD Reactor	P/N 45600-02	COD analysis digester
Hach Spectrophotometer	Odyssey DR/2500	COD analysis
Multi meter	HQ40d multi	pH measurement

CHAPTER 4

RESULTS AND DISCUSSION

In this section, results obtained through the experimentations performed toward the treatment of selected pesticides, namely, carbendazim, imidacloprid and aclonifen, via the activated sludge process are presented and discussed. In doing so, the effect of SRT, as a most important operational parameter, is primarily concerned. Also, results belonging to the effects of these pesticides on the operational behavior of activated sludge process are presented and discussed on the basis of MLSS concentrations and COD removals.

4.1. Carbendazim Removal

To explore the carbendazim removal in activated sludge systems, as well as its effect on the general treatment performance, 5 laboratory scale instantaneously fed SBRs with different SRTs were utilized. In this section carbendazim removal in reactors working at SRTs 3, 8, 10, 20 and 30 days is discussed. Effect of SRT on MLSS concentration, COD removal and carbendazim removal are discussed separately. The corresponding complied data of the operated reactors are presented in Appendix B.

4.1.1. Effect of SRT on MLSS and COD Removal in the Presence of Carbendazim

Figure 10 demonstrates the steady-state MLSS concentrations in reactors working at SRT 3, 8, 10, 20 and 30 days, as a function of the influent carbendazim concentration. As expected, in reactors devoid of carbendazim, MLSS concentrations are the lowest at the reactor working at SRT 3 and the highest MLSS concentrations are observed at

reactors working at SRT 20 and 30 days. This trend is also kept in reactors receiving carbendazim.

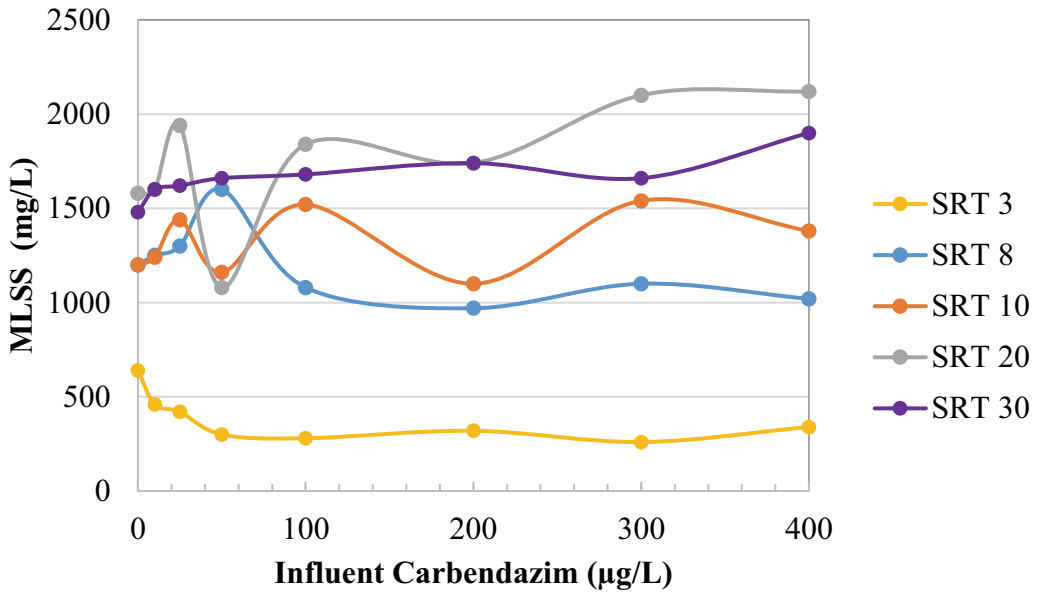

Figure 10 MLSS Variations at Reactors Receiving Carbendazim

However, when the variation in MLSS with the influent carbendazim concentration is examined in individual reactors, it is seen that the changes in MLSS concentrations are not consistent for all reactors. To illustrate, after the introduction of carbendazim to the reactors, an apparent decrease was observed in MLSS concentrations of SRT 3 days. However, this behavior was not noticed in reactors working at SRT 8, 10, 20 and 30 days. As a matter of fact, decline of MLSS concentrations are expected at lower SRTs after introduction of a pollutant, since the microbial cultures are not diverse as the cultures of high SRTs and thus the reactors working at lower SRTs are more sensitive to external inputs.

As seen from Figure 10, MLSS concentrations reach to almost constant level in the reactor at SRT 3 days after spiking 50 µg/L carbendazim. After this point, no drastic decline in MLSS concentration was observed even though the influent carbendazim concentration increased constantly. The same happens in the reactor operated at SRT 8 days, but after spiking 100 µg/L carbendazim instead of 50 µg/L. A peak observed in MLSS in this reactor when received 50 µg/L carbendazim remained unexplained. On the other hand, interestingly, unlike for SRT 8 days, sudden drops in MLSS were observed at SRT 20 and 30 days after spiking 100 µg/L carbendazim, which also remained unexplained. However, it is evident that some fluctuation in MLSS did occur until carbendazim concentration of 100 µg/L in reactors of SRT 8, 10 and 20 days. It could be attributed to the possibility that microorganisms are trying to get acclimatized to the elevated carbendazim concentrations. The reason for not observing such fluctuation at SRT 30 days could be the longer contact time which allowed the microorganisms to get acclimatized better to the carbendazim. Moreover, no fluctuation, but a steady decrease in MLSS observed at SRT 3 days could support this aforementioned attribution. Because, 3 days of SRT probably was not sufficient to reach to the acclimatization and hence, direct response of decrease in MLSS was observed. It can then be inferred that in reactors having SRT 8, 10 and 20 days, microorganisms were able to acclimate to the carbendazim till after supplying 100 µg/L carbendazim to the reactors. In other words, it became possible to reach the acclimatization within the time spent to reach this concentration in these reactors, thus the MLSS concentrations are either stabilized or a slight increase is observed even though the concentrations of the spiked carbendazim increased constantly.

When the effect on COD removal is concerned, effluent COD concentrations increased gradually as the influent carbendazim concentration increased in all reactors studied, as illustrated in Figure 11. This observation suggests that the ability of the microorganisms to degrade the easily degradable substrate (i.e. peptone) was adversely affected.

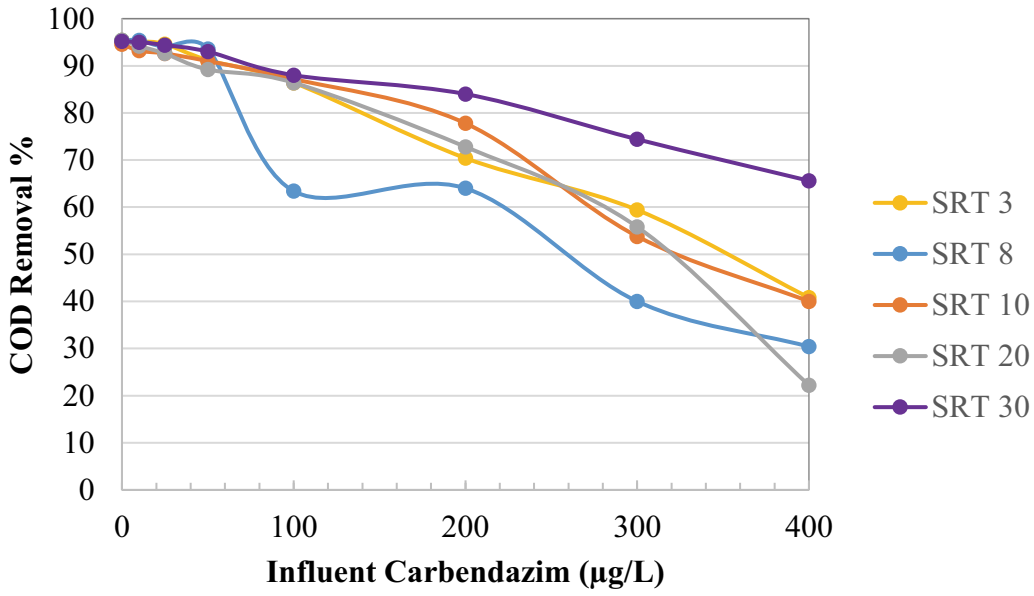

Figure 11 COD Removal Efficiencies in Carbendazim Receiving Reactors

However, the COD utilization ability of the reactors were not disrupted remarkably until introduction of 100 µg/L carbendazim except for the reactor working at SRT 8 days. In this reactor, the COD utilization was unfavorably affected immediately after injection of 100 µg/L carbendazim the COD removal efficiency decreased to 63 % from its base-line value of 95 %. This observation is in accordance with the sudden drop in MLSS as indicated in Figure 10. COD removal remained constant until 200 µg/L carbendazim exposure; thereafter effluent COD values increased constantly. In other words, from this point on, the removal efficiencies of COD decreased drastically as carbendazim concentration increased. It can be interpreted that the greater concentrations of carbendazim was toxic to the microbial cultures, which resulted in deficiency of substrate utilization, hence remarkable increase in COD effluent.

As can be depicted from Figure 11, as the carbendazim concentration increased, COD removal performances for SRT 3, 10 and 20 days were quite similar. On the other hand, the removal efficiencies attained for the reactor of SRT 30 days was quite distinct than the others. Despite the fact that the COD removal efficiencies decreased as the influent carbendazim concentration increased in all reactors, the reactor of SRT 30 days outperformed the others. This can be attributed to the longer contact between the microorganisms and the carbendazim, hence better acclimatization to the carbendazim at SRT 30 days. So, undoubtedly the reactor working at SRT 30 days was the best in COD removal, among the others. At the highest carbendazim concentration, which happens to be 400 μg/L for this study, 66% of the initial COD was removed in SRT 30 days. On the contrary, only 41, 34, 40, and 22% of the initial COD was removed at SRT 3, 8, 10 and 20 days, respectively.

Even in the reactor working at SRT 30 days in which the COD removal efficiency is highest, the adverse effect of carbendazim is evidently present, especially pronounced beyond 100 μg/L carbendazim. In other words, inhibition of the microorganisms by the carbendazim is of concern. Considering that the carbendazim is of organic structure, it can be inferred that competitive inhibition is more probable to be in effect. With this assumption, effluent COD data as a function of SRT (i.e. 1/μ) were analyzed to calculate the inhibition coefficient, K_I, for each reactor, according to Eq. 7. Calculations are presented in Appendix C. Figure 12 depicts the calculated K_I values at different SRTs. As can be seen from this figure, K_I value for the reactor of SRT 30 days, is remarkably different than for the others. As known, the greater the K_I value, the lower the inhibition coefficient (α) and hence the lower inhibition is. Therefore, highest value of K_I obtained for SRT 30 days supports the best COD removal at SRT 30 days, as compared to the others. These findings make it clear that, even in the best scenario there is a drastic effect of carbendazim exposure to wastewaters. From this point of view, discharge of wastewaters containing carbendazim to WWTPs will cause major decrease in actual performance of these plants in terms of COD removal and

eventually result in undesired effluent characteristics which are not complied by the existing discharge standard for COD stated in Water Pollution Control Regulation[1] Table 21.

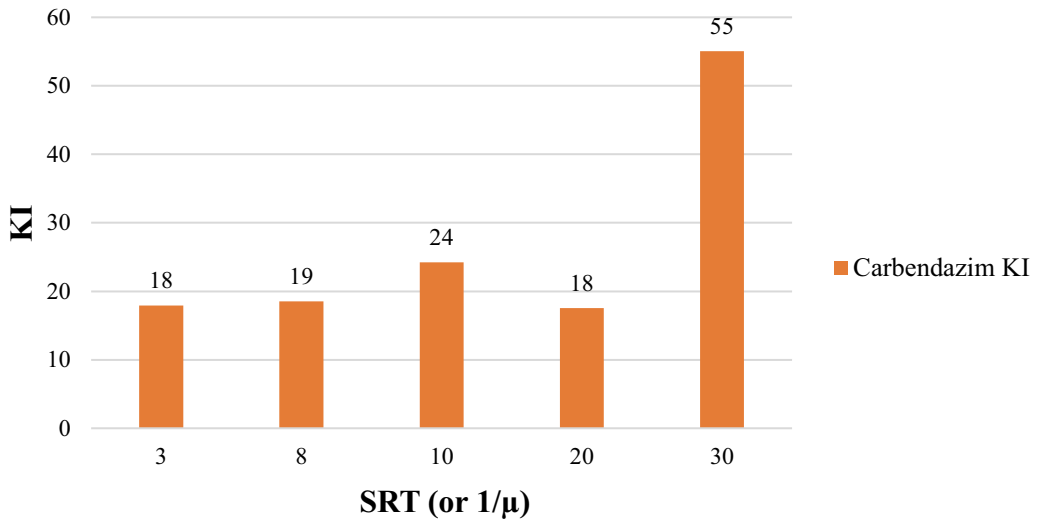

Figure 12 KI values of Carbendazim at Different SRTs

Figure 13 depicts the effect of carbendazim on MLSS and COD together over the entire range of SRT. As seen, washout of microorganisms occurred at around SRT 1 day. It is also clearly seen that the COD removal efficiency deteriorates more as the carbendazim concentration gets increased in the influent for a given SRT. Also, the trend lines for the MLSS concentrations as a function of SRT indicates the existence of the maintenance energy requirement at SRT 30 days. This maintenance energy

[1] Official Journal dated December 31,2004 No: 25687

requirement seems, though not so clearly observable, decreased at higher carbendazim concentrations. Indeed, this is in accordance with the attributions done above, i.e. better acclimatization to carbendazim at longer SRTs. Also, this can be attributed to the smaller carbendazim/biomass ratio observed at longer SRTs. As seen from Figure 10, especially for higher carbendazim concentrations, MLSS concentration is higher at longer SRT. So, carbendazim per unit biomass is smaller.

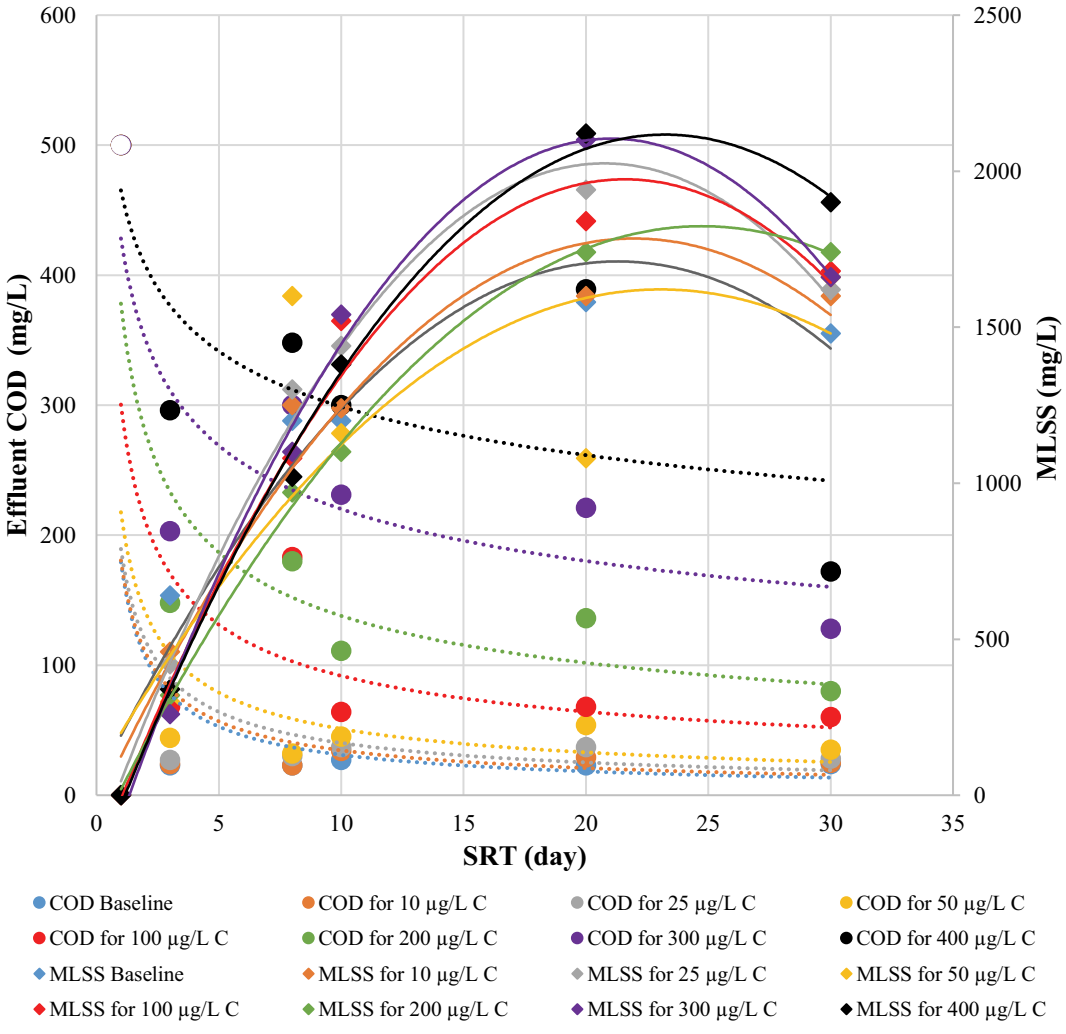

Figure 13 Variation of MLSS and Effluent COD with SRT in Reactors Receiving Carbendazim (C: Carbendazim)

4.1.2. Effect of SRT on Carbendazim Removal

Removal efficiencies of carbendazim pesticide as a function of the influent carbendazim concentration at reactors operated at different SRTs are demonstrated in Figure 14. This figure also shows the concurrent COD removal efficiencies attained in the reactors. The bar graphs demonstrate COD removal efficiencies, and the lines demonstrates carbendazim removal.

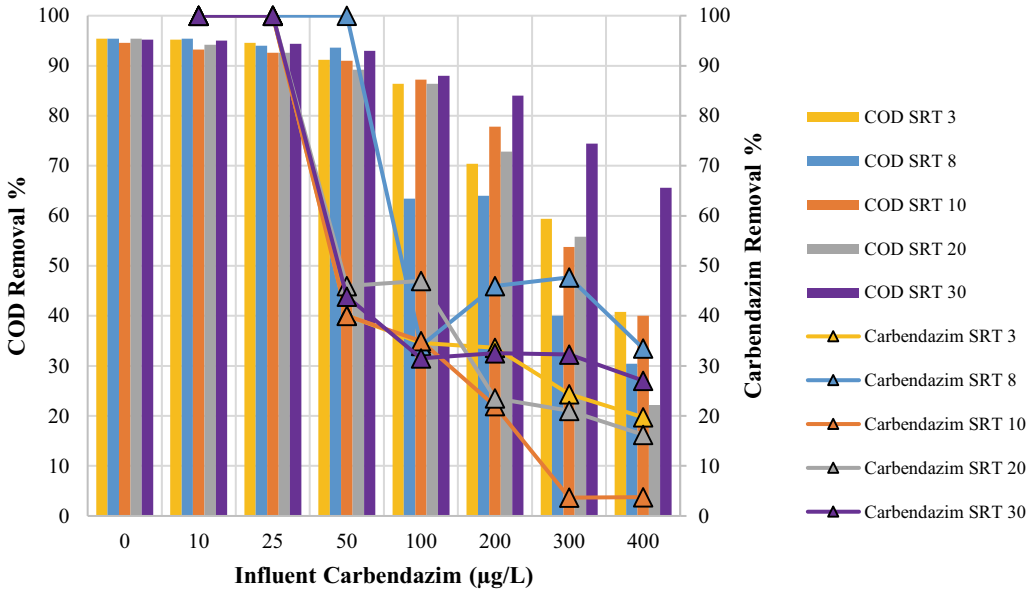

Figure 14 Removal Efficiencies of Carbendazim and COD at Different SRTs

As seen from this figure, all of the reactors were capable of removing carbendazim by almost 100% (as the effluent carbendazim measurements were below LOD of 10 µg/L), when 10 µg/L and 25 µg/L carbendazim was supplied to the reactors. Thus, no correlation was observed between carbendazim removal and SRT for these two concentrations (10 µg/L and 25 µg/L). However, as the influent carbendazim concentration increased removal performances of the reactors deteriorated remarkably. Immediately after supplying 50 µg/L of carbendazim to the reactors,

carbendazim elimination decreased significantly down to 40%, except in the reactor with SRT 8 days. However, this exceptional case disappeared at 100 µg/L and all the reactors performed similarly. From this point on, although a fluctuation in the carbendazim removal efficiency among the reactors depending on SRTs was observed, a decreasing trend in the carbendazim removal performance with the increase in carbendazim concentration was almost common to all reactors. When 400 µg/L carbendazim is reached, the carbendazim removal efficiency attained was less than 35% in all reactors. However, there exists no clear correlation between the carbendazim removal and SRT. Fluctuation observed is more appreciable beyond 100 µg/L and the effect of SRT on the carbendazim removal becomes unpredictable as a function of influent carbendazim concentration. When the carbendazim removal efficiencies are examined for the influent carbendazim concentrations beyond 100 µg/L at a closer look, it can be seen that removals were quite similar at SRT 3, 20 and 30 days, unlike for SRT 8 and 10 days. This can be attributed to the possible mechanism of carbendazim removal by the microbial culture. As known, biodegradation/biotransformation could be expected at longer SRTs owing to energy metabolism while biosorption is more likely to occur at shorter SRTs owing to cellular synthesis being dominant. So, at SRT 3 days, carbendazim is removed probably by biosorption while at SRTs 20 and 30 days removal is mainly by biodegradation/biotransformation. However, very different behavior between SRT 8 and 10 days could not be explained with such attributions. Therefore, it is safer to state that beyond 50 µg/L carbendazim, the treatment system is really upset. Supportively, COD removal decrease becomes also noticeable beyond 50 µg/L especially in reactors operated at SRT<30 days. At SRT 30 days, decrease in COD removal efficiency was less as compared to the other SRTs, probably due to the better acclimatization of the culture to the carbendazim at longer contacts provided, as also stated above.

4.2. Imidacloprid Removal

To explore the imidacloprid removal in activated sludge systems, as well as its effect on the general treatment performance 5 laboratory scale instantaneously fed SBRs with different SRTs were utilized. In this section imidacloprid removal in reactors working at SRTs 3, 8, 10, 20 and 30 days is discussed. Effect of SRT on MLSS concentration, COD removal and imidacloprid removal are discussed separately. The corresponding complied data of the operated reactors are presented in Appendix B.

4.2.1. Effect of SRT on MLSS and COD Removal in the Presence of Imidacloprid

Figure 15 demonstrates the steady-state MLSS concentrations in reactors working at SRT 3, 8, 10, 20 and 30 days, as a function of the influent imidacloprid concentration. As expected, in reactors devoid of imidacloprid MLSS concentrations are the lowest at the reactor working at SRT 3 and the highest MLSS concentrations are observed at the longest SRT, which happens to be 30 days for this study. This trend is also kept in reactors receiving imidacloprid.

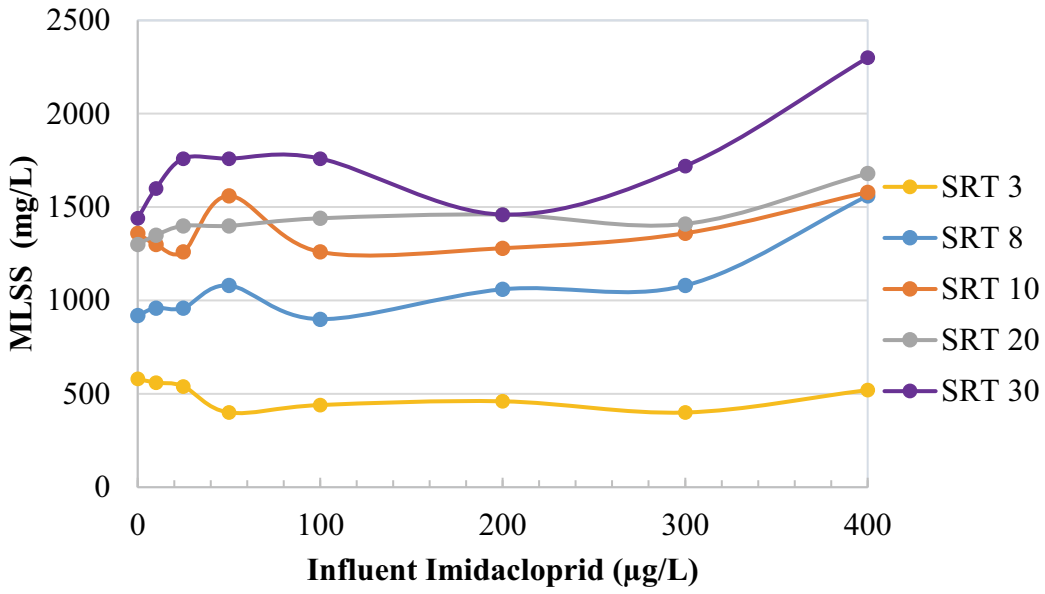

Figure 15 MLSS Variations at Reactors Receiving Imidacloprid

Nevertheless, when the variation in MLSS with the influent imidacloprid concentration is examined in individual reactors, it is seen that the changes in MLSS concentrations are not consistent for all reactors. For the reactor with the shortest sludge age (SRT 3 days), the MLSS concentration decreased constantly until 50 µg/L imidacloprid dosed. From this point on, the changes in the MLSS concentration was not considerable. For this particular reactor, it can be interpreted that the microbial culture was immediately affected from the introduction of imidacloprid pollutant. The same behavior was not observed in the other reactors, this can be related to the diversity of the microbial cultures. In order words, decline of MLSS concentrations are possible at lower SRTs after introduction of a pollutant, since the microbial cultures are not diverse as the cultures of high SRTs and thus the reactors working at lower SRTs are more sensitive to external inputs.

It is evident that some fluctuation in MLSS did occur until imidacloprid concentration of 100 µg/L in reactors of SRT 8 and 10 days (Figure 15). It could be attributed to the possibility that microorganisms are trying to get acclimatized to the elevated imidacloprid concentrations. Such fluctuations were not observed at SRT 20 and 30 days, except the low spot appearing at SRT 30 days after injecting 200 µg/L of imidacloprid. Indeed, beyond the imidacloprid concentration of 200 µg/L, there observed a steady increase in MLSS in all reactors (except SRT 3 days), indicating the possible acclimatization of the culture to the imidacloprid. Even further, an increase in MLSS was noticeable at longer SRTs (especially at 30 days) at elevated imidacloprid concentrations, maybe supporting its possible stimulatory effect on biomass synthesis.

When the effect on COD removal is concerned, in general, COD removal efficiencies decreased gradually as the influent imidacloprid concentration increased (Figure 16). This observation suggests that the ability of the microorganisms to degrade the easily degradable substrate (i.e peptone) was adversely affected.

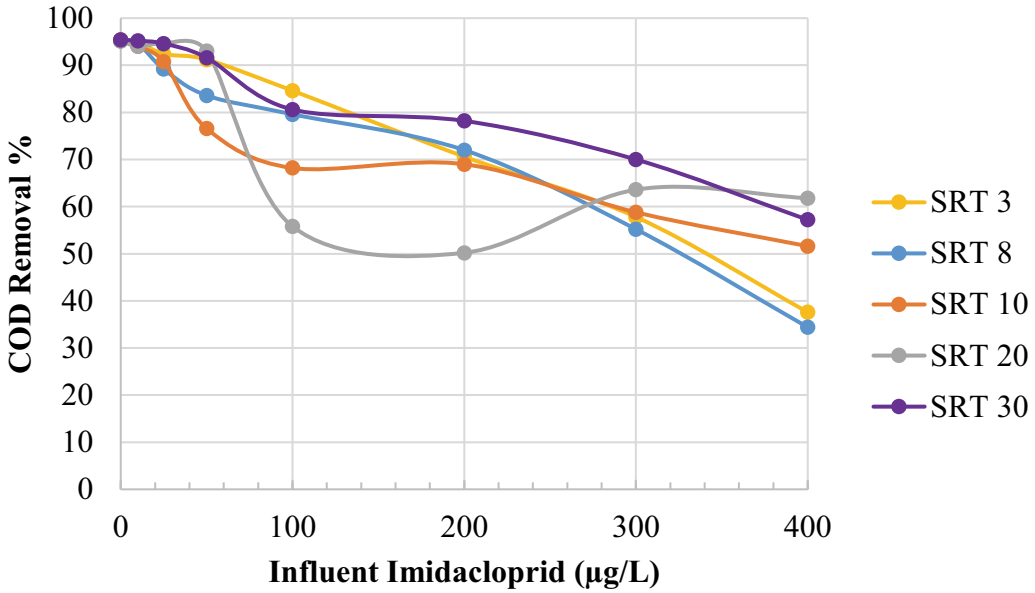

Figure 16 COD Removal Efficiencies at Imidacloprid Reactors

As seen from Figure 16, COD removal efficiencies were not affected adversely until the imidacloprid concentration of 25 µg/L, in all reactors. Then, it starts to decrease with the increase in the imidacloprid concentration. But, this decrease was not same in all reactors. The COD elimination efficiency of the reactors operated at SRT 3 and 8 days decreased to 71% and 72%, respectively, right after exposure to 200 µg/L of imidacloprid. Whereas, the COD removal performance of the reactor operated at SRT 10 days, decreased to 76% after injecting 50 µg/L imidacloprid. Besides these differences, especially the reactor operated at SRT 20 days behaved differently than the others (Figure 16), which happens to be the hardest one to understand. The removal percentage of COD decreased from 93% to 56% when 100 µg/L imidacloprid was supplied to the system. Following this, a slight decrease (50%) in COD removal was observed after 200 µg/L imidacloprid injection. The interesting fact about this reactors' performance is that, unlike the other reactors from this point on the COD removal efficiency increased. This behavior cannot be related to the effects of prolonged sludge age since no such behavior was detected for the reactor operated at

SRT 30 days. Also, this reactors COD utilization was affected deleteriously right after supplying 100 µg/L imidacloprid. None of the other reactors were affected as much as this reactor. Therefore, it should be concluded that these behaviors must be related to another unknown parameter rather than the effect of SRT. Among all reactors, the reactor working at SRT 30 days was the best in COD removal, when all of the concentration levels were considered. For 400 µg/L imidacloprid concentration, the COD removal efficiency of the reactor operated at SRT 20 days (62%) was slightly better than the one operated at SRT 30 days (57%). However, considering all concentration levels it could be concluded that the reactor operated at SRT 30 days performed better.

At the highest imidacloprid concentration, which happens to be 400 µg/L for this study, 62% of the initial COD was removed in SRT 20 days and 57% of the initial COD was removed in SRT 30 days. On the contrary, only 34% of the initial COD was removed at SRT 8. So, it can be stated that COD removal efficiencies became higher at longer SRTs, due to better acclimation to the imidacloprid at longer SRTs, as expected. On the other hand, at lower concentrations, it is not possible to relate the removal efficiencies of COD and SRT. However, for the higher concentrations there is a definite correlation between the COD removal efficiencies and the SRTs as can be seen in Figure 16. As a result of this correlation, there is a significant COD removal performance difference between the reactors working at longer SRTs (SRT 10, 20 and 30 days) and shorter SRTs (SRT 3 and 8 days). To recapitulate, at higher pollutant concentrations COD removal increases with the prolonged sludge ages.

The deleterious effect of imidacloprid in the COD removal efficiency is evidently present even in the reactor working at SRT 30 days. That is to say, considering the organic structure of imidacloprid, inhibition (especially competitive inhibition) of the microorganisms by the imidacloprid is of concern. Effluent COD data as a function of SRT (i.e. 1/μ) were analyzed to calculate the inhibition coefficient, K_I, for each imidacloprid reactor. Figure 17 illustrates the calculated K_I values at different SRTs. As seen from this figure, K_I values for the reactors of SRT 10, 20 and 30 days, are quite close to each other and are remarkably higher than the remaining reactors (SRT 3 and 8 days). As known, the greater the K_I value, the lower the inhibition coefficient (α) and hence the lower inhibition is (Eq. 7). Therefore, high values of K_I obtained for SRT 10, 20 and 30 days supports the better COD removal efficiencies at these reactors, as compared to the others (SRT 3 and 8 days).

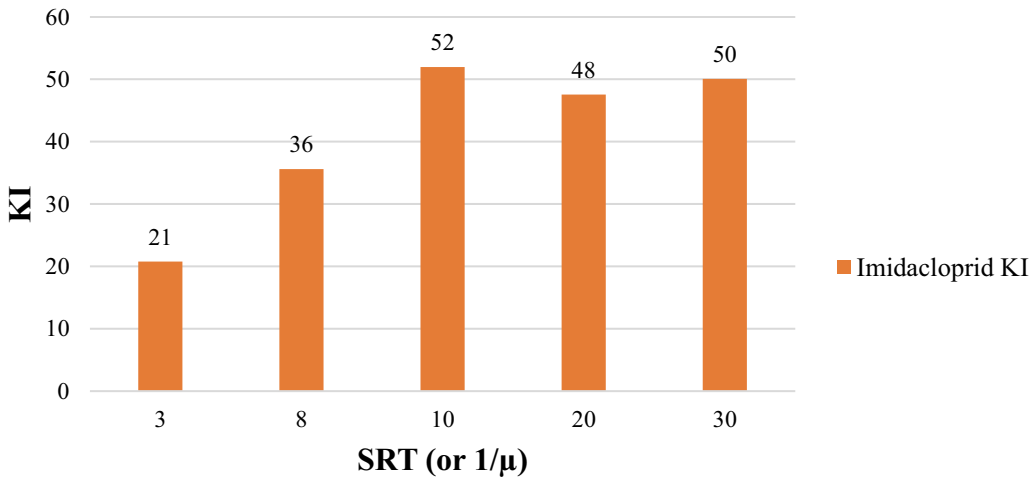

Figure 17 KI values of Imidacloprid at Different SRTs

Figure 18 illustrates the effect of imidacloprid on MLSS and COD together over the whole range of SRT. As seen, washout of microorganisms occurred at around SRT 1 day. It is also clearly seen that the COD removal efficiency deteriorates more as the imidacloprid concentration gets increased in the influent for a given SRT. There is a clear correlation between COD removal and SRT. The COD removal performances of the reactors increased as SRT increased. Moreover, the trend lines for the MLSS concentrations as a function of SRT indicates the existence of the maintenance energy requirement at SRT 30 days. This maintenance energy requirement seems, though not so clearly observable, decreased at higher imidacloprid concentrations. Indeed, this is in accordance with the attributions done above, i.e. better acclimatization to imidacloprid at longer SRTs. Also, this can further be attributed to the smaller imidacloprid/biomass ratio observed at longer SRTs. As seen from Figure 15, especially for higher imidacloprid concentrations, MLSS concentration is higher at longer SRT. So, imidacloprid per unit biomass is smaller.

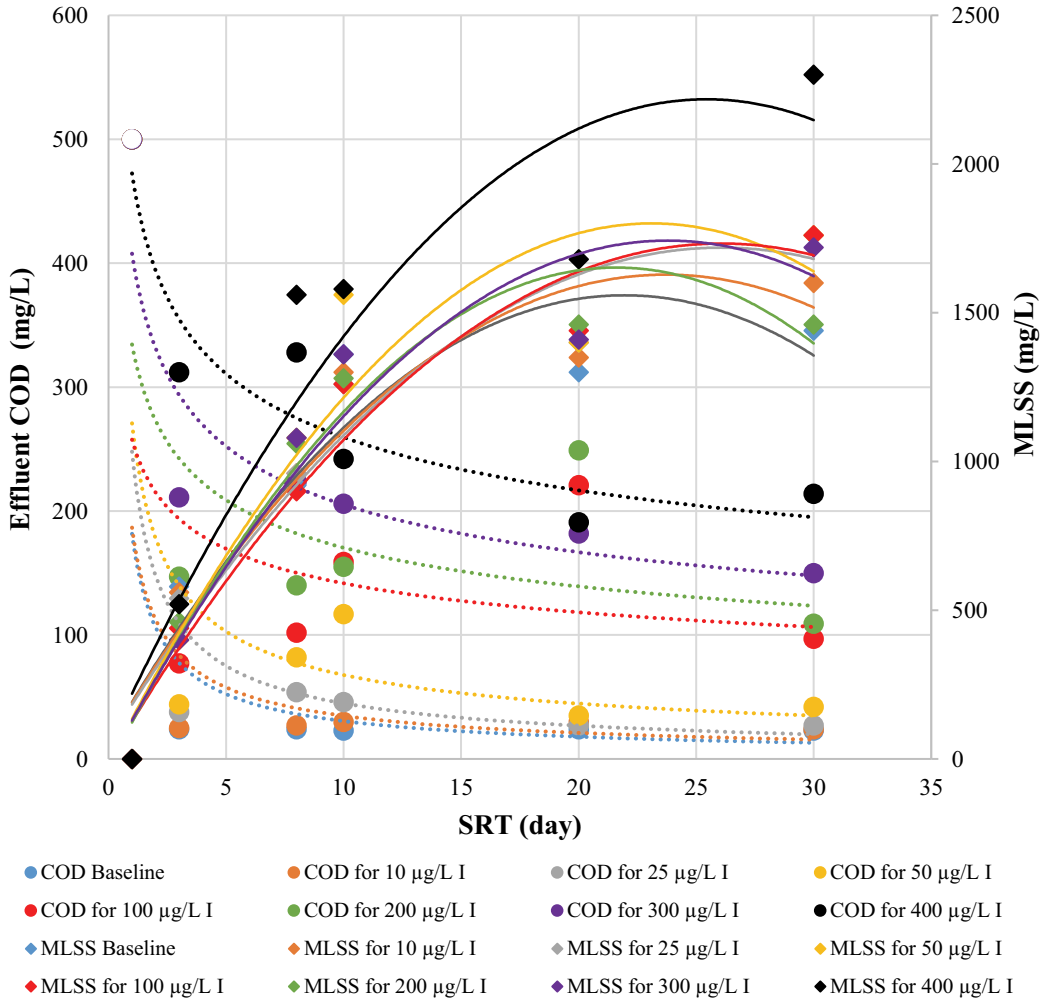

Figure 18 Variation of MLSS and Effluent COD with SRT in Reactors Receiving Imidacloprid (I: Imidacloprid)

4.2.2. Effect of SRT on Imidacloprid Removal

Removal efficiencies of imidacloprid pesticide as a function of the influent imidacloprid concentration at reactors operated at different SRTs are demonstrated in Figure 19. This figure also shows the concurrent COD removal efficiencies attained in the reactors. The bar graphs demonstrate COD removal efficiencies, and the lines demonstrates imidacloprid removal.

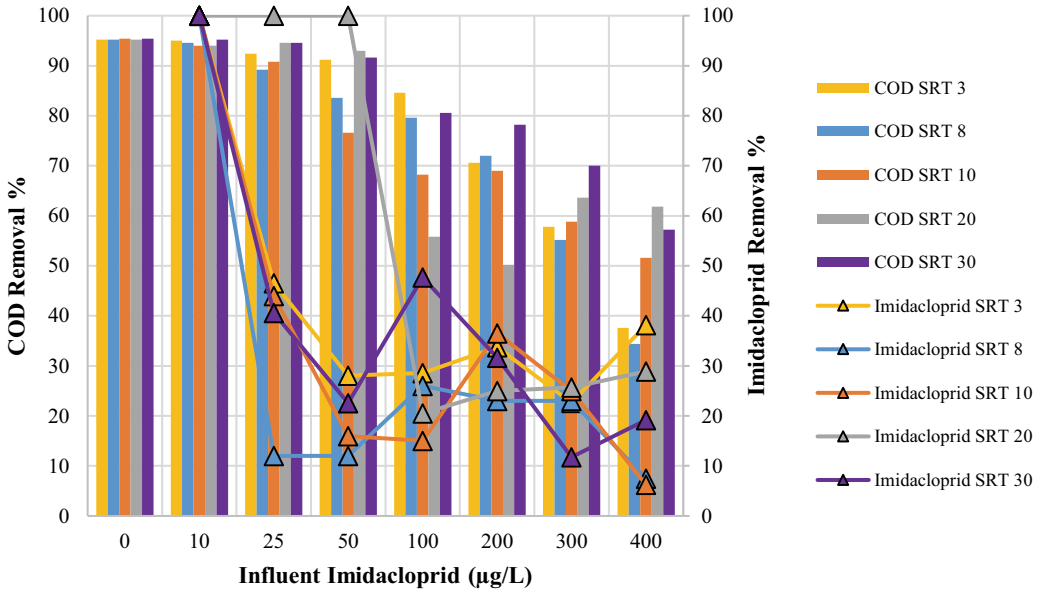

Figure 19 Removal Efficiencies of Imidacloprid and COD at Different Reactors

As Figure 19 indicates, all of the reactors removed imidacloprid by almost 100%, when 10 µg/L imidacloprid was supplied to the reactors. Therefore, for the lowest concentration studied, no correlation was observed between imidacloprid removal and SRT. However, as the influent imidacloprid concentration increased removal performances of the reactors deteriorated remarkably. Immediately after increasing the influent imidacloprid concentration to 25 µg/L, imidacloprid removal efficiency decreased down to 40% in the reactors operated at SRT 3, 10 and 30 days. Two

exceptional cases were recorded at this concentration level. Firstly, the reactor operated at SRT 20 days was able to remove imidacloprid by almost 100%, secondly the one operated at SRT 8 days was able to remove only 12% of influent imidacloprid.

However, the exceptional case of SRT 8 days disappeared at 50 µg/L and the reactors performed similarly (except for SRT 20 days). Moreover, the reactor operated at SRT 20 days performed similarly after 100 µg/L imidacloprid was supplied to the reactor.

After 100 µg/L imidacloprid, although there were observed a fluctuation in the imidacloprid removal efficiency among the reactors depending on SRTs, a decreasing trend in the imidacloprid removal performance with the increase in imidacloprid concentration was almost common to all reactors. For 400 µg/L imidacloprid, the imidacloprid removal efficiency achieved was less than 38% in all reactors. Nevertheless, there exists no clear correlation between SRT and imidacloprid removal.

Again for 400 µg/L imidacloprid, it can be seen that imidacloprid removals of SRT 3, 20 and 30 days were distinctly higher than the ones operated at SRT 8 and 10 days. This can be related to the possible mechanisms of imidacloprid removal by the microbial culture. At longer SRTs, as a result of the energy metabolism biodegradation/biotransformation is expected whereas at shorter SRTs biosorption is more likely owing to cellular synthesis being dominant. Therefore, at SRT 20 and 30 days imidacloprid is removed possibly by biodegradation/biotransformation while at SRT 3 days removal is primarily by biosorption. However, the removal behaviors of SRT 8 and 10 days is not possible to be explained by these attributions.

4.3. Aclonifen Removal

To explore the aclonifen removal in activated sludge systems, as well as its effect on the general treatment performance, 5 laboratory scale instantaneously fed SBRs with different SRTs were utilized. In this section aclonifen removal in reactors working at SRTs 3, 8, 10, 20 and 30 days is discussed. Effect of SRT on MLSS concentration, COD removal and aclonifen removal are discussed separately. The corresponding complied data of the operated reactors are presented in Appendix B.

4.3.1. Effect of SRT on MLSS and COD Removal in the Presence of Aclonifen

Figure 20 demonstrates the steady-state MLSS concentrations in reactors working at SRT 3, 8, 10, 20 and 30 days, as a function of the influent aclonifen concentration. As expected, in reactors devoid of aclonifen, MLSS concentrations are the lowest at the reactor working at SRT 3 days and the highest MLSS concentrations are observed at the longer SRT, which happens to be 20 and 30 days. This trend is also kept in reactors receiving aclonifen.

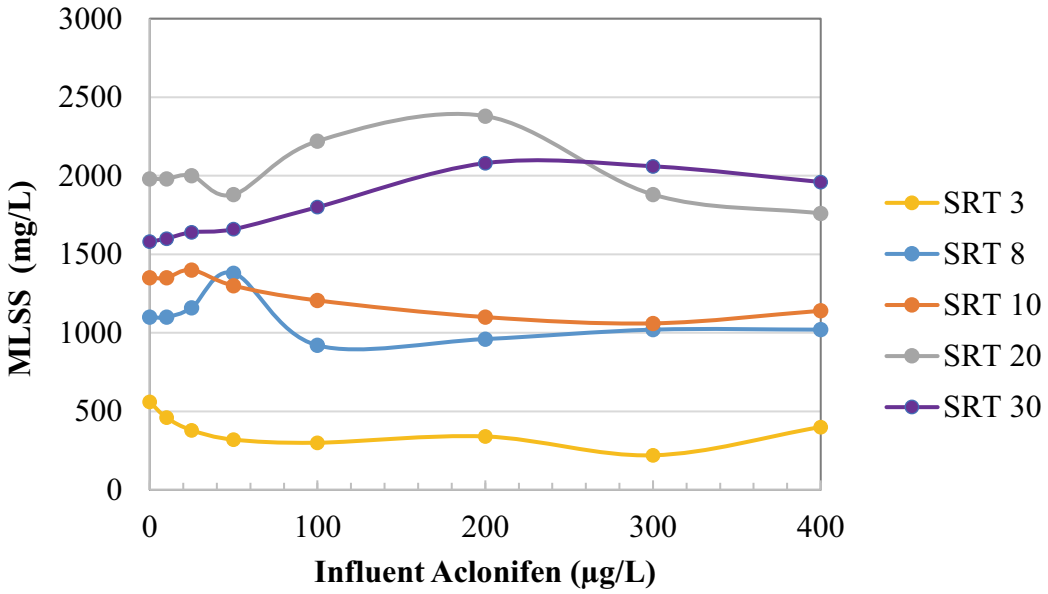

Figure 20 MLSS Concentration Variations at Aclonifen Reactors

Nevertheless, when the variation in MLSS with the influent aclonifen concentration is examined in individual reactors, it is seen that the changes in MLSS concentrations are not consistent for all reactors. For the reactor with the shortest sludge age (SRT 3 days), the MLSS concentration decreased constantly until 50 µg/L aclonifen dosed. From this point on, the changes in the MLSS concentration was not considerable, except the low spot appearing at SRT 3 days after injecting 300 µg/L of aclonifen. For this particular reactor, it can be interpreted that the microbial culture was immediately affected from the introduction of aclonifen pollutant. The same behavior was not observed in the other reactors, this can be related to the diversity of the microbial cultures. In order words, decline of MLSS concentrations are possible at lower SRTs after introduction of a pollutant, since the microbial cultures are not diverse as the cultures of high SRTs and thus the reactors working at lower SRTs are more sensitive to external inputs.

As seen from Figure 20, MLSS concentrations reach to almost constant level in the reactors at SRT 8 and 10 days after spiking 100 µg/L aclonifen. For these reactors, after 100 µg/L, no drastic decline in MLSS concentration was observed even though the influent aclonifen concentration increased constantly. A peak observed in MLSS in the reactor operated at SRT 8 days when received 50 µg/L aclonifen remained unexplained. However, it is apparent that some fluctuation in MLSS did occur as aclonifen concentration of 100 µg/L in reactors of SRT 8, 10 and 20 days. This could be related to the possibility that microorganisms are struggling to get acclimatized to the elevated aclonifen concentrations. Aforementioned fluctuations were not observed at SRT 30 days, the reason for this could be due to the longer contact time provided, which allowed the microorganisms to get acclimatized better to aclonifen. Additionally, the steady decrease noted in MLSS at SRT 3 days could support this aforementioned attribution. Since, 3 days of SRT probably was not sufficient to reach to the acclimatization and hence, direct response of decrease in MLSS was observed. It can then be inferred that in reactors having SRT 8 and 10 days microorganisms were able to acclimate to the aclonifen till after supplying 100 µg/L aclonifen to the reactors. By way of explanation, it became possible to reach the acclimatization within the time spent to reach this concentration in these reactors, hence the MLSS concentrations are either stabilized or a slight increase is observed even though the concentrations of the spiked aclonifen increased constantly.

When the effect on COD removal is concerned, effluent COD concentrations increased gradually as the influent aclonifen concentration increased in all reactors studied, as demonstrated in Figure 21. This finding suggests that the ability of the microorganisms to degrade the easily degradable substrate (i.e. peptone) was adversely affected.

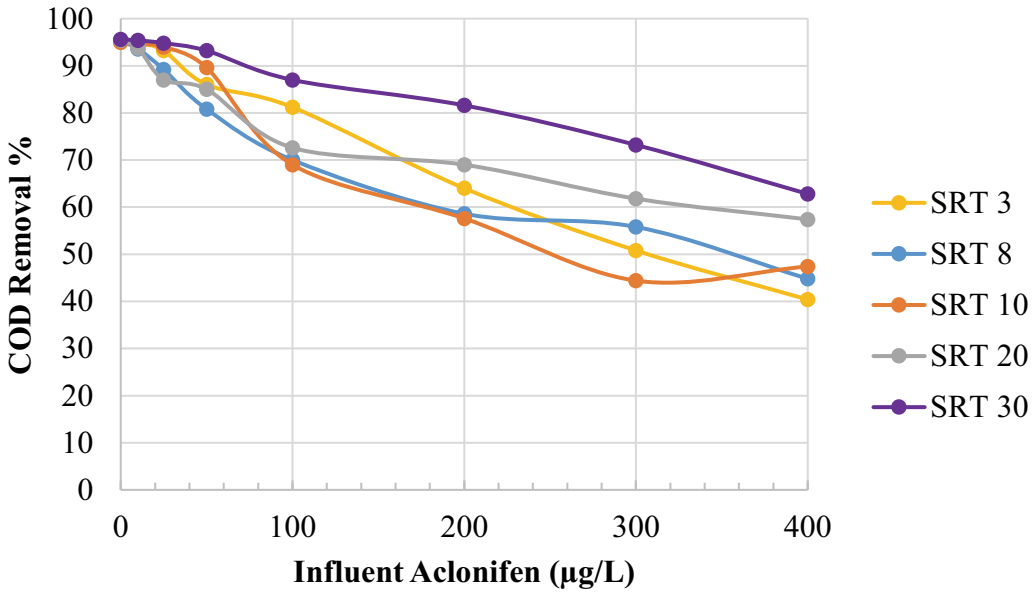

Figure 21 COD Removal Efficiencies at Aclonifen Reactors

As seen from Figure 21, COD removal efficiencies were not affected adversely until the aclonifen concentration of 50 µg/L, in all reactors. From this point on, COD removal efficiencies started to decrease with the increase in the aclonifen concentration. However, this decrease was not same in all reactors. For instance, immediately after injection of 50 µg/L, COD elimination efficiency of the reactor operated at SRT 8 days decreased to 81%. Whereas, the efficiency of the reactor operated at SRT 30 days was 93%. COD elimination performances of the reactors differentiated more as the pollutant concentrations increased.

At the highest aclonifen concentration, which happens to be 400 µg/L for this study, 63% of the initial COD was removed in SRT 30 days. On the contrary, only 41% of the initial COD was removed at SRT 3 days. Hence, it can be stated that COD removal

efficiencies became higher at longer SRTs, due to better acclimation to the aclonifen at longer SRTs, as expected. Nevertheless, at lower concentrations, it is not possible to define a correlation between removal efficiencies of COD and SRT. Although, for the higher concentrations there is a clear correlation between the COD removal efficiencies and the SRTs as can be seen in Figure 21. On account of this correlation, there is a significant COD removal performance difference between the reactors working at longer SRTs and shorter SRTs.

Among all reactors, the reactor working at SRT 30 days was the best in COD removal, for the entire concentration range. Even in the reactor working at SRT 30 days in which the COD removal efficiency is highest, the efficiency decreased to 63%. These findings make it clear that, even in the best scenario there are deleterious effects of aclonifen exposure to wastewaters. From this point of view, discharge of wastewaters containing aclonifen to WWTPs will cause major decrease in actual performance of these plants in terms of COD removal and eventually result in undesired effluent characteristics which are not complied by the existing discharge standard for COD stated in Water Pollution Control Regulation[1] Table 21.

The adverse effect of aclonifen is evidently present, especially pronounced beyond 50 µg/L aclonifen. Inhibition of microorganisms' activity by aclonifen is of concern. Considering that aclonifen is of organic structure, it can be inferred that competitive inhibition is more probable to be in effect. With this assumption, effluent COD data as a function of SRT (i.e. $1/\mu$) were analyzed to calculate the inhibition coefficient, K_I, for each reactor, according to Eq. 7. Figure 22 depicts the calculated K_I values at different SRTs. As can be seen from this figure, K_I values of the reactors are relatively

[1] Official Journal dated December 31,2004 No: 25687

close to each other. As known, the greater the K_I value, the lower the inhibition coefficient (α) and hence the lower inhibition is.

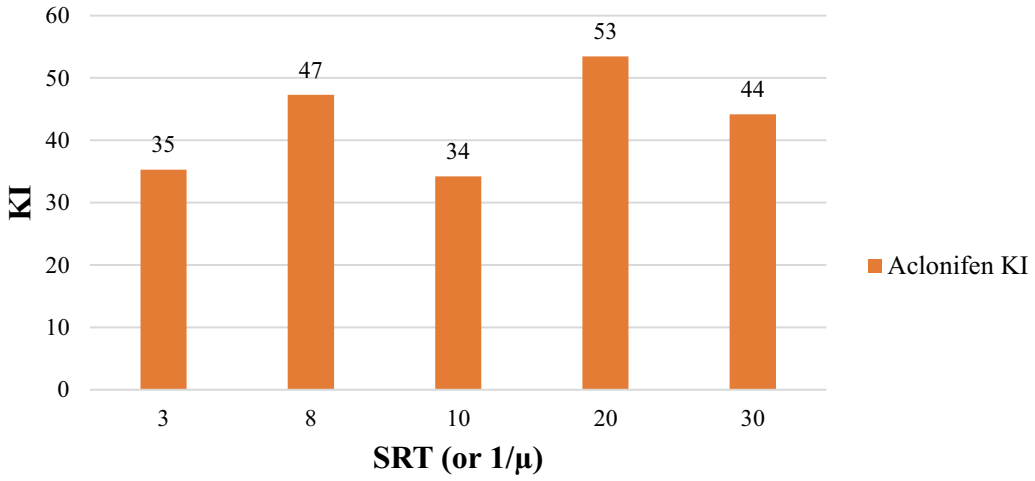

Figure 22 KI values of Aclonifen at Different SRTs

Figure 23 depicts the effect of aclonifen on MLSS and COD together over the entire range of SRT. As seen, washout of microorganisms occurred at around SRT 1 day. It is also clearly seen that the COD removal efficiency deteriorates more as the aclonifen concentration gets increased in the influent for a given SRT. Also, the trend lines for the MLSS concentrations as a function of SRT indicates the existence of the maintenance energy requirement at SRT 30 days. This maintenance energy requirement decreased at aclonifen concentration of 100 μg/L and above. This maintenance energy requirement seems, though not so clearly observable, decreased at higher aclonifen concentrations. Indeed, this is in accordance with the attributions done above, i.e. better acclimatization to aclonifen at longer SRTs. Also, this can further be attributed to the smaller aclonifen/biomass ratio observed at longer SRTs.

As seen from Figure 20, especially for higher aclonifen concentrations, MLSS concentration is higher at longer SRT. So, aclonifen per unit biomass is smaller.

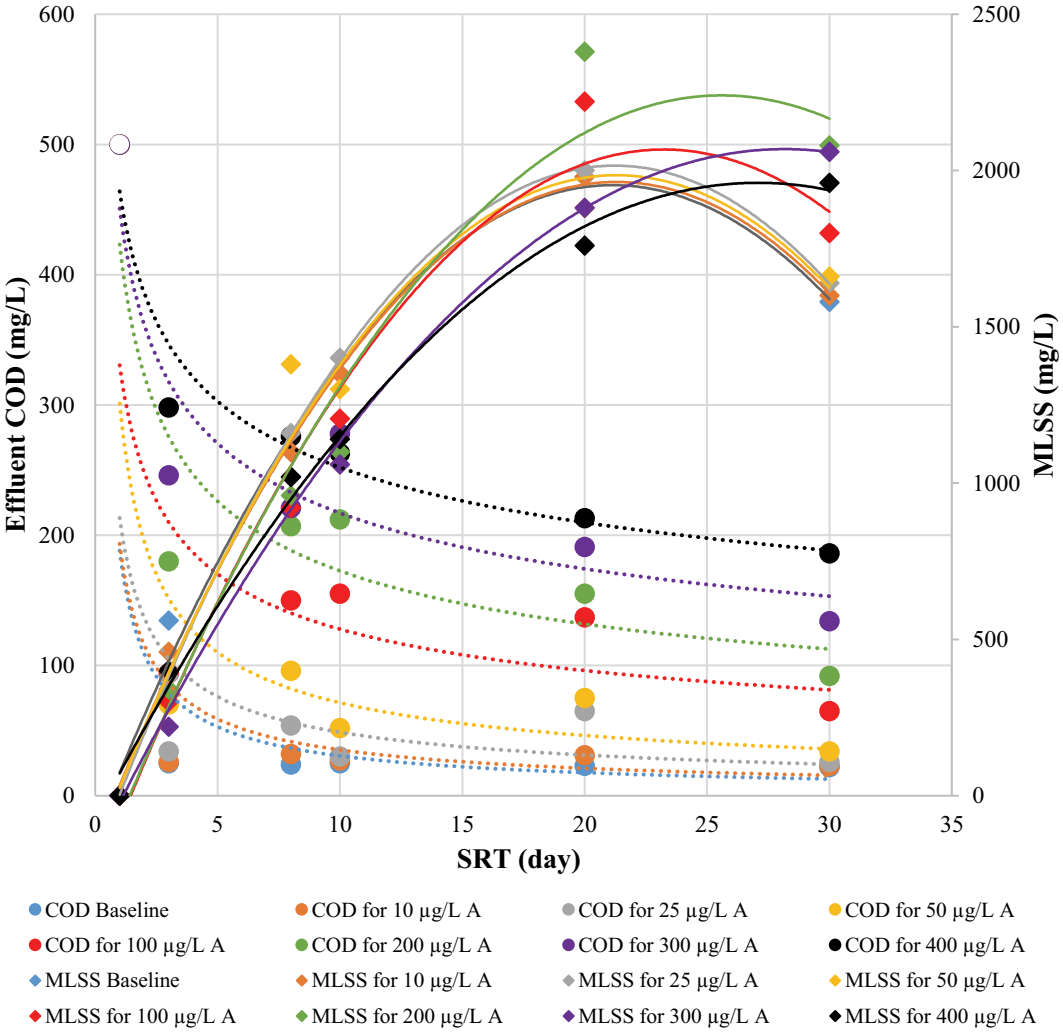

Figure 23 Variation of MLSS and Effluent COD with SRT in Reactors Receiving Aclonifen (A: Aclonifen)

4.3.2. Effect of SRT on Aclonifen Removal

Removal efficiencies of aclonifen pesticide as a function of the influent aclonifen concentration at reactors operated at different SRTs are demonstrated on Figure 24. This figure also shows the concurrent COD removal efficiencies attained in the reactors. The bar graphs demonstrate COD removal efficiencies, and the lines demonstrates aclonifen removal.

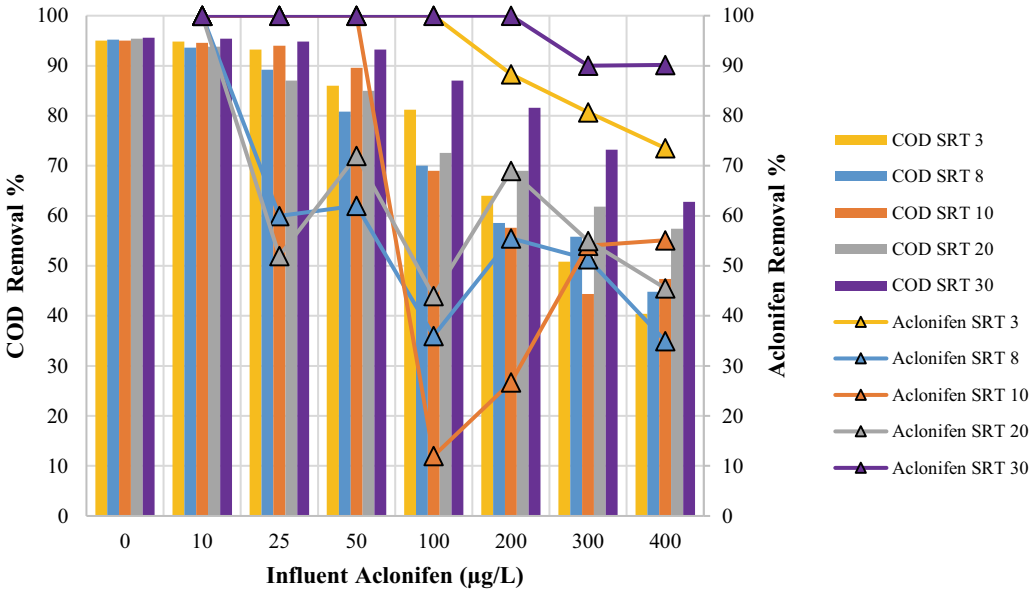

Figure 24 Removal Efficiencies of Aclonifen and COD at Different Reactors

As demonstrated on Figure 24, all of the reactors were capable of removing aclonifen by almost 100% (as the effluent aclonifen measurements were below LOD of 10 µg/L), when 10 µg/L aclonifen was supplied to the reactors. Therefore, no correlation was observed between aclonifen removal and SRT for the concentrations of 10 µg/L. However, as the influent aclonifen concentration increased removal performances of the reactors deteriorated remarkably. After supplying 25 µg/L of aclonifen to the reactors, aclonifen elimination decreased significantly down to 60% and 52% at the

reactors SRT 8 and 20 days, respectively. Until addition of 100 µg/L aclonifen reactors SRT 3, 10 and 30 days were capable of removing aclonifen by almost 100%. Immediately after supplying 100 µg/L of aclonifen to the reactors, aclonifen removal performance of the reactor SRT 10 days decreased drastically from almost 100% down to 12%. The reactor SRT 3 was capable of removing aclonifen by almost 100% until the addition of 200 µg/L aclonifen while SRT 30 days removed almost 100% of the influent aclonifen until supplying 300µg/L aclonifen. When 300 µg/L aclonifen was supplied to the reactors, aclonifen elimination of reactor SRT 30 decreased to 90%. Furthermore, the performance of the reactor operated at SRT 3 days decreased from 88% to 81% resulting in an effluent concentration of 58 µg/L. At this point, performances of the reactors operated at SRT 8, 10 and 20 days were very similar. The effluent concentrations of these reactors varied between 135-146 µg/L. When 400 µg/L aclonifen is reached, the aclonifen removal efficiencies attained in the reactors were spectacularly different. The best removal was attained at SRT 30 days, in which 90% aclonifen was removed. The second best aclonifen removal was attained at SRT 3 days (74%). However, aclonifen removal efficiencies attained at reactors SRT 8, 10 and 20 days were less than 55%. As a matter of fact, only 35% of the influent aclonifen was removed at SRT 8 days.

When the aclonifen removal efficiencies are examined for the influent aclonifen concentrations at a closer look, it can be seen that removals were quite similar at SRT 3 and 30 days, unlike for SRT 8, 10 and 20 days. This can be related to the possible mechanism of aclonifen removal by the microbial culture. Aclonifen, is a hydrophobic pollutant with an octanol-water partition coefficient of 4.37. Given its high octanol-water partition coefficient and low solubility, aclonifen is expected to have high sorption potential. As known, biosorption could be expected at shorter SRTs owing to cellular synthesis being dominant whereas biodegradation/biotransformation is more likely to occur at longer SRTs owing to energy metabolism. Therefore, at SRT 3 days, aclonifen is removed probably by biosorption while at SRT 30 days removal is mainly

by biodegradation/biotransformation. However, the behavior of the remaining reactors could not be explained with such attributions.

4.4. Removal in Reactors Fed with Pesticide Mixtures

To explore the pesticide removal in activated sludge systems, as well as its effect on the general treatment performance, 5 laboratory scale instantaneously fed SBRs with different SRTs were utilized. In this section removal of carbendazim, imidacloprid and aclonifen pesticides in reactors fed with mixtures working at SRTs 3, 8, 10, 20 and 30 days are discussed. Effect of SRT on MLSS concentration, COD removal and pesticide removal are discussed separately. The corresponding complied data of the operated reactors are presented in Appendix B.

4.4.1. Effect of SRT on MLSS and COD Removal in the Presence of Carbendazim, Imidacloprid and Aclonifen Mixture

Figure 25 demonstrates the steady-state MLSS concentrations in reactors working at SRT 3, 8, 10, 20 and 30 days, as a function of the influent carbendazim, imidacloprid and aclonifen concentration. As expected, in reactors devoid of pesticides, MLSS concentrations are the lowest at the reactor working at SRT 3 and the highest MLSS concentrations are observed at reactors working at SRT 20 and 30 days. This trend is also kept in reactors receiving carbendazim, imidacloprid and aclonifen mixtures.

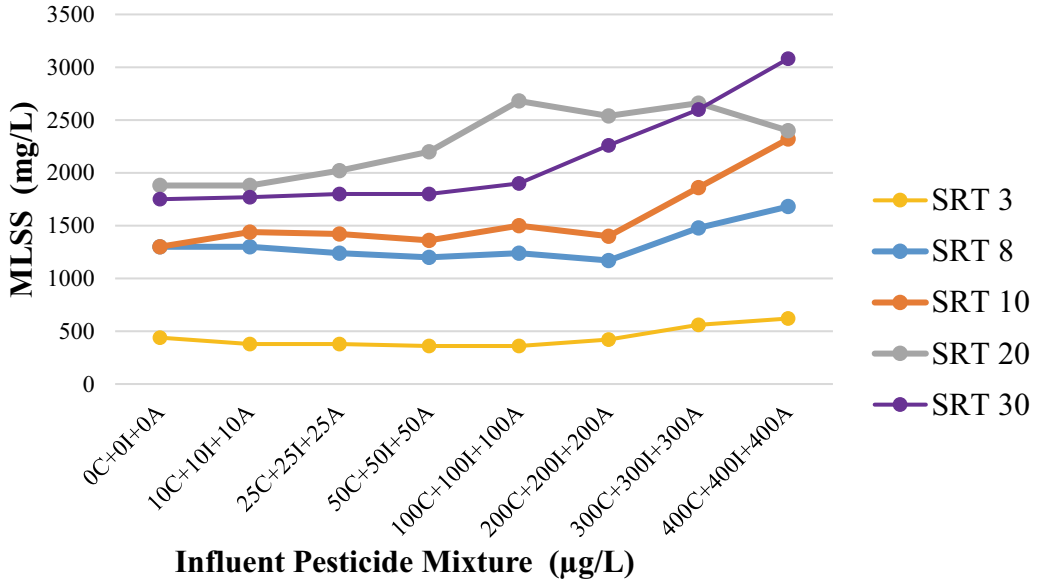

Figure 25 MLSS Concentration Variations in Reactors Fed with Pesticides Mixture

To recall, after the introduction of pesticides to the reactors in individual studies, an apparent decrease was observed in MLSS concentrations at shorter SRTs, SRT 3 and 8 days. Unlike the individual pollutant studies discussed in the previous sections, no major changes were observed in the MLSS concentrations of the reactors operated at SRT 3 and 8 days. As a matter of fact, decline of MLSS concentrations are expected at lower SRTs after introduction of a pollutant, since the microbial cultures are not diverse as the cultures of high SRTs and thus the reactors working at lower SRTs are more sensitive to external inputs. Though, when the pesticides are fed to the reactors as mixtures aforementioned effect was not observed.

As seen from Figure 25, MLSS concentrations of the reactor with the shortest SRT (SRT 3 days), are almost constant until the addition of 200 µg/L of each pesticide. From this point on, a slight but constant increase is observed in the MLSS concentrations of SRT 3 days. The same happens in the reactor operated at SRT 8 and

10 days, however the increase in MLSS concentrations of these reactors (SRT 8 and 10 days) are far more apparent than SRT 3 days. No drastic decline in MLSS concentration was observed even though the influent pesticide mixture concentration increased constantly. The same situation was also valid for the longer SRTs, SRT 20 and 30 days. The MLSS concentrations for SRT 30 days were almost constant until the addition of 100 μg/L of each pesticide, after this concentration an apparent increase in MLSS concentration was observed at SRT 30 days. The variation of MLSS concentrations of SRT 20 days was different when compared to SRT 30 days, because MLSS concentrations increased immediately after the addition of 10 μg/L of each pesticide. Although an apparent increase was observed in the overall MLSS concentration in the studied SRT range, fluctuations in MLSS concentrations were also observed. In other words, the increase pattern of MLSS concentrations was not as smooth as SRT 30 days.

When the effect on COD removal is concerned, effluent COD concentrations increased gradually as the influent pesticides' concentration increased in all reactors studied, as illustrated in Figure 26. This observation suggests that the ability of the microorganisms to degrade the easily degradable substrate (i.e. peptone) was adversely affected.

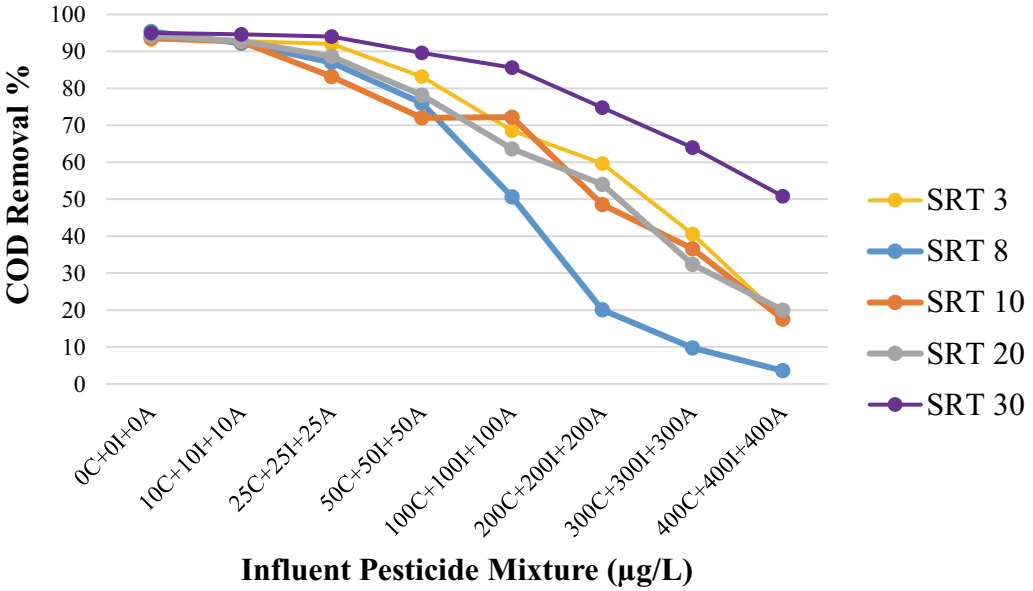

Figure 26 COD Removal Efficiencies of Reactors Fed with Mixture Pesticides

In the experiments, where individual pollutants were supplied to the reactors, the COD utilization ability of most of the reactors were not disrupted remarkably until introduction of 100 µg/L pollutant. However, for the reactors, which were fed with mixture of pesticides carbendazim, imidacloprid and aclonifen, the COD utilization ability were disrupted immediately after supplying 25 µg/L of each pesticide. For instance, the COD removal efficiency of the reactor operated at SRT 10 days decreased to 83%. COD removal remained relatively constant until 50 µg/L pesticide mixture supplied; thereafter effluent COD values increased constantly. Particularly, from this point on, the removal efficiencies of COD decreased drastically as pesticide mixture concentration increased. It can be interpreted that the greater concentrations of carbendazim, imidacloprid and aclonifen was toxic to the microbial cultures, which resulted in deficiency of substrate utilization, consequently remarkable increase in COD effluent.

As can be depicted from Figure 26, as the pesticides concentration increased, COD removal performances for SRT 3, 10 and 20 days were quite similar. The reactor operated at SRT 8 days performed the worst. On the other hand, the removal efficiencies attained for the reactor of SRT 30 days was quite distinct than the others. Regardless of the fact that the COD removal efficiencies decreased as the influent carbendazim, imidacloprid and aclonifen concentration increased in all reactors, the reactor of SRT 30 days outperformed the others. The closest COD removal performance to the reactor working at SRT 30 days belongs to the second longest sludge age, SRT 20 days, in which only 20% of COD was removed when a mixture of carbendazim, imidacloprid and aclonifen was supplied (400 µg/L each). The reason behind this outstanding performance can be related to the longer contact between the microorganisms and pesticides. In other words, with longer SRTs better acclimatization to pesticides are achieved. At the highest pesticide mixture concentration, which happens to be 400 µg/L for this study, 51% of the initial COD was removed in SRT 30 days. However, only 18, 4, 18, and 20 % of the initial COD was removed at SRT 3, 8, 10 and 20 days, respectively.

Even in the reactor working at SRT 30 days in which the COD removal efficiency is highest (51%), deleterious effect of pesticides is undoubtedly present. These findings make it clear that, even in the best scenario there are severe effects of pesticide exposure to wastewaters. From this point of view, discharge of wastewaters containing carbendazim, imidacloprid and aclonifen pesticides to WWTPs will cause major decrease in actual performance of existing plants and eventually result in undesired effluent characteristics which are not complied by the existing discharge standard for COD stated in Water Pollution Control Regulation[1] Table 21.

[1] Official Journal dated December 31,2004 No: 25687

Figure 27 illustrates the effect of the mixture of pesticide (carbendazim, imidacloprid and aclonifen) on MLSS and COD together over the entire range of SRT. As seen, washout of microorganisms occurred at around SRT 1 day. It is also clearly seen that the COD removal efficiency deteriorates more as the pesticide concentration gets increased in the influent for a given SRT. Moreover, the trend lines for the MLSS concentrations as a function of SRT indicates the existence of the maintenance energy requirement at SRT 30 days. This maintenance energy requirement seems decreased at pesticide concentrations beyond 200 µg/L. Normally, especially in the absence of inhibition, one should expect that maintenance energy requirement will be more pronounced at longer SRTs, due to endogenous respiration to occur. However, here, this decreased maintenance energy requirement at higher pesticide concentration could be attributed to the longer contact time between biomass and pesticides, hence better acclimatization of the culture to the pesticides. As in the cases of individual pesticides, this can further be attributed to the smaller pesticide/biomass ratio observed at longer SRTs. As seen from Figure 25, especially for higher pesticide concentrations, MLSS concentration is higher at longer SRT. So, pesticide per unit biomass is smaller.

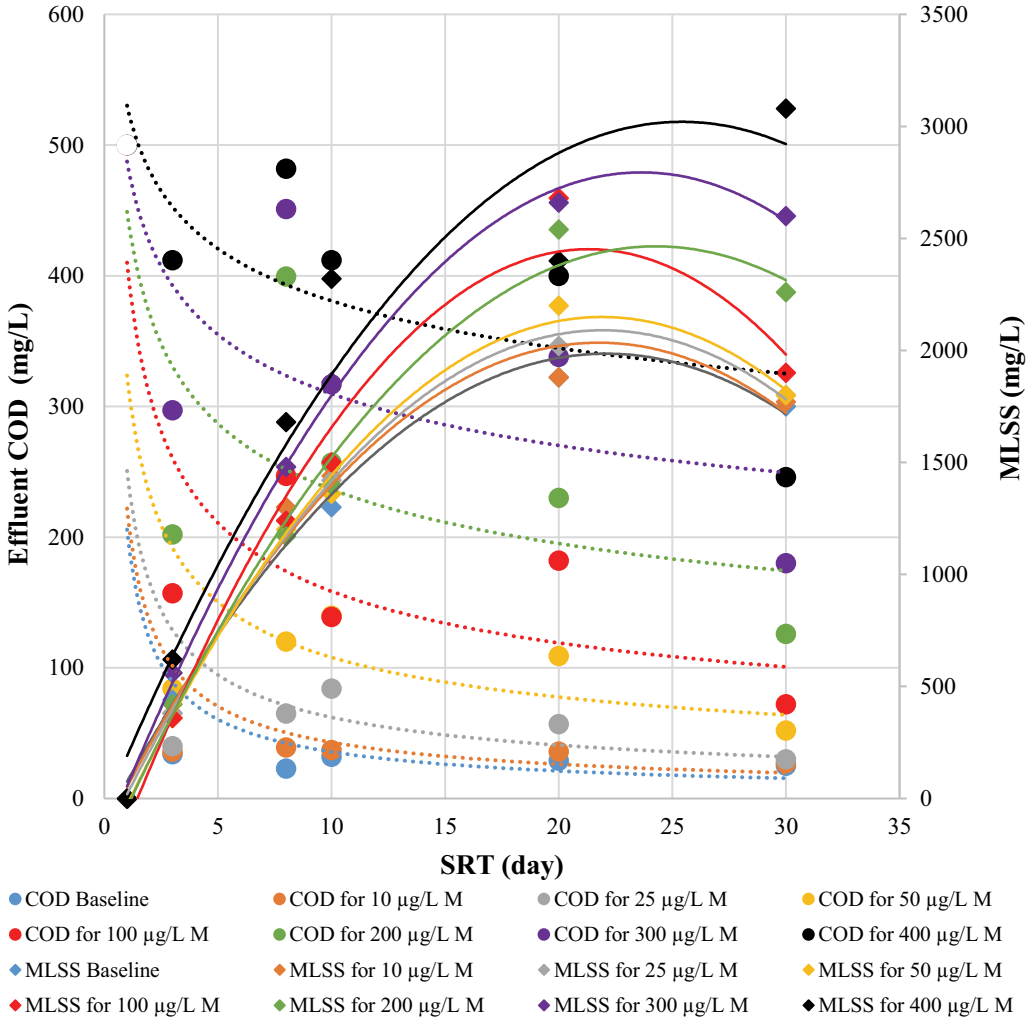

Figure 27 Variation of MLSS and Effluent COD with SRT in Reactors Receiving Mixture of Pesticides (M: Carbendazim + Imidacloprid + Aclonifen)

4.4.2. Effect of SRT on Carbendazim Removal at Reactors Fed with Pesticide Mixtures

Removal efficiencies of carbendazim pesticide as a function of the influent pesticide concentration at reactors operated at different SRTs are demonstrated on Figure 28. The reactors were fed with mixture of pesticides containing the same amount of carbendazim, imidacloprid and aclonifen. For instance, the first concentration studied was 10 µg/L. For this experiment, a synthetic wastewater bearing 10 µg/L of carbendazim, 10 µg/L of imidacloprid and 10 µg/L of aclonifen was fed to the reactors.

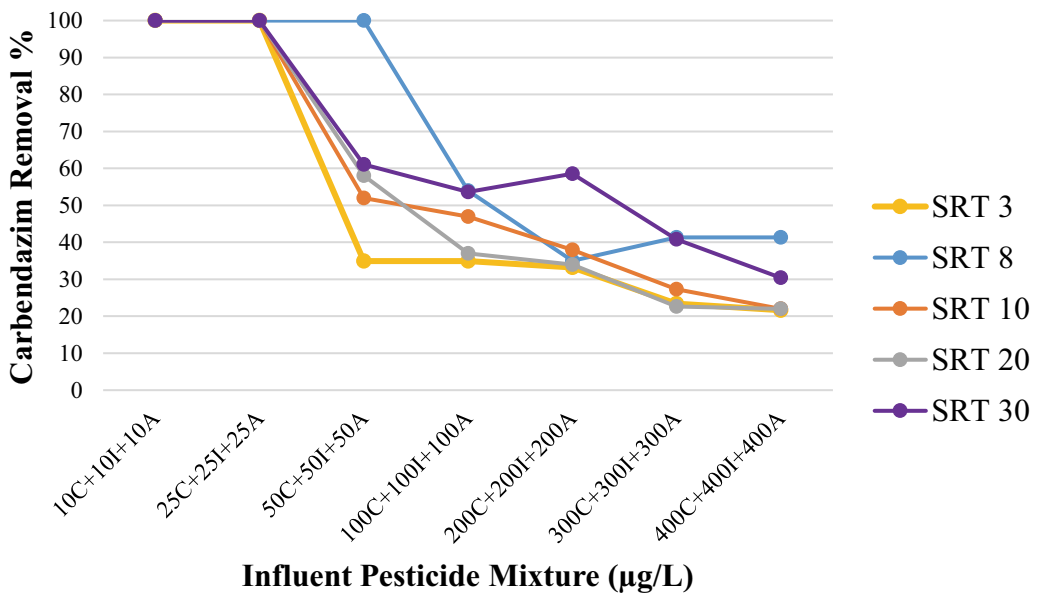

Figure 28 Removal Efficiencies of Carbendazim at Reactors Fed with Mixture Pesticides

As presented in Figure 28, all of the reactors were capable of removing carbendazim by almost 100% , when pesticide mixtures bearing 10 µg/L and 25 µg/L carbendazim was supplied to the reactors. Figure 29 presents the carbendazim removal efficiencies

attained when 10 µg/L carbendazim was supplied to the reactors both individually and as mixture while Figure 30 presents carbendazim removal efficiencies attained when 25 µg/L carbendazim was supplied. As seen from the figures, all of the reactors were capable of removing carbendazim by almost 100% regardless of whether carbendazim was supplied individually or as a mixture. Consequentially, no correlation was observed between carbendazim removal and SRT for the lowest concentrations (10 µg/L and 25 µg/L). Yet, as the influent carbendazim concentration increased carbendazim elimination decreased remarkably.

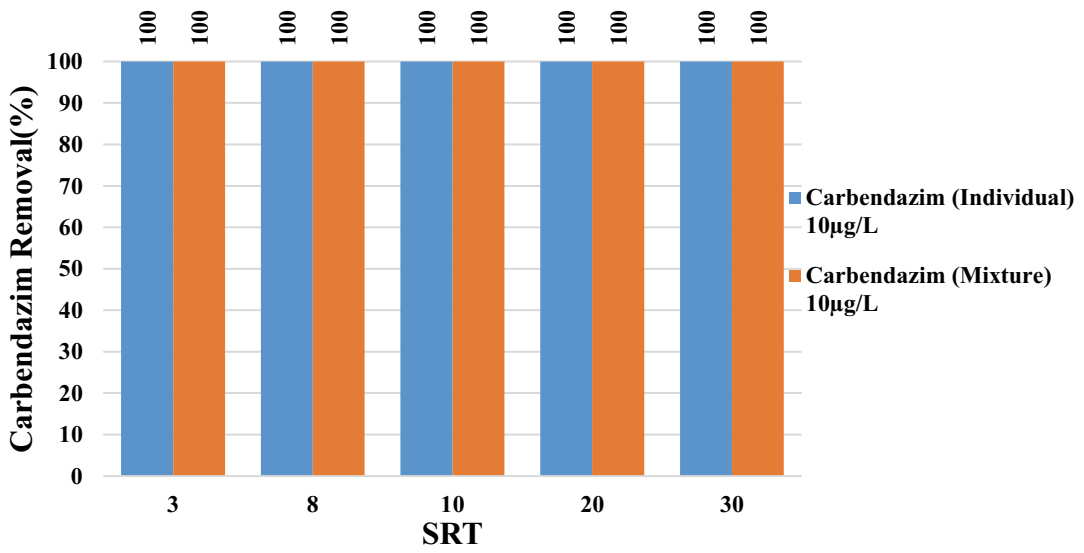

Figure 29 Removal Efficiencies of 10 µg/L Carbendazim Pesticide in Relation to the Sludge Retention at 25°C

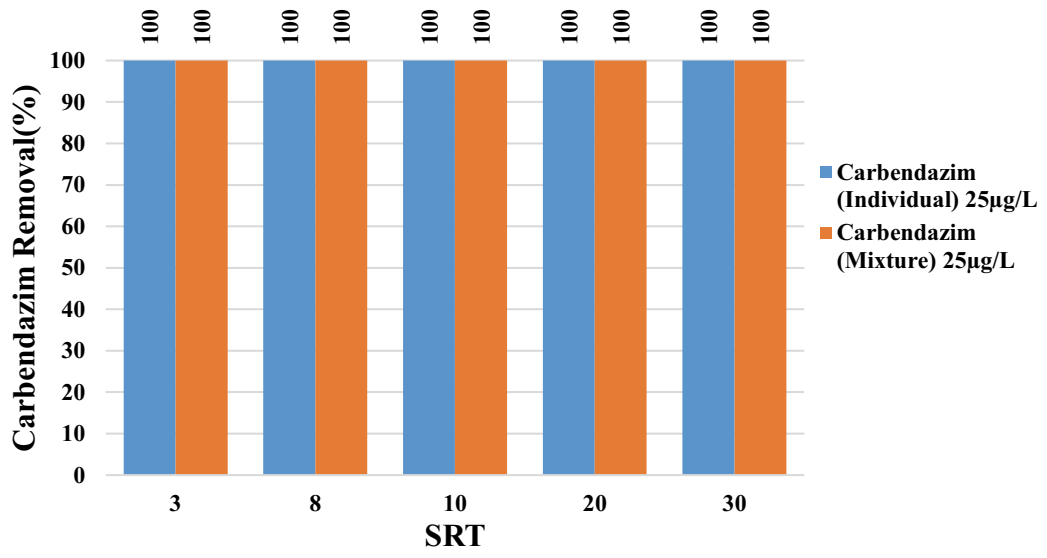

Figure 30 Removal Efficiencies of 25 µg/L Carbendazim Pesticide in Relation to the Sludge Retention at 25°C

After supplying 50 µg/L of carbendazim, imidacloprid and aclonifen to the reactors, carbendazim elimination decreased significantly, except for the reactor operated at SRT 8 days. Anyhow, as can be seen in Figure 28, this exceptional case of reactor SRT 8 days, in which nearly 100 % of the supplied pesticides are removed, disappeared at 100 µg/L. After this point, the reactors performances were relatively similar in removing the supplied carbendazim pesticide and a decreasing trend in the carbendazim removal performance with the increase in carbendazim concentration was almost common to all reactors.

Figure 31 presents the carbendazim removal efficiencies attained when 50 µg/L carbendazim was supplied to the reactors both individually and as mixture. As seen, except for the reactor operated at SRT 3 days, carbendazim was removed better in reactors fed with mixture of pesticides rather than the reactors fed only with

carbendazim. Additionally, carbendazim removal performances of the reactors operated at SRT 8 days were same, nearly 100 % of the supplied carbendazim pesticide was removed in both reactors.

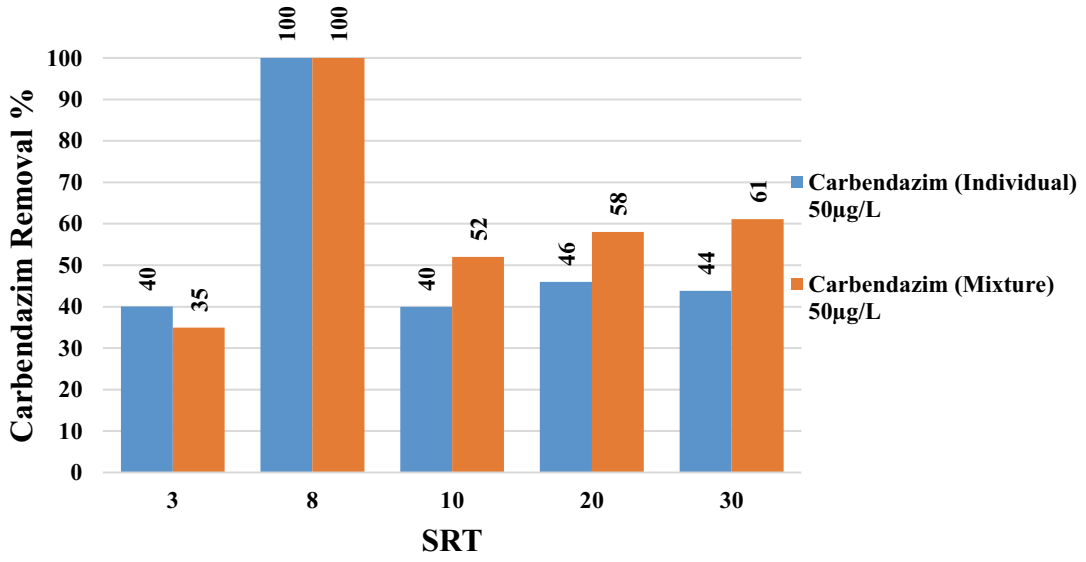

Figure 31 Removal Efficiencies of 50 µg/L Carbendazim Pesticide in Relation to the Sludge Retention at 25°C

As presented in Figure 28, after 100 µg/L of pesticide mixture was supplied, a decreasing trend in the carbendazim removal performance was recorded with the increase in pesticide concentration for almost all of the reactors. To recall, the same situation was recorded during the experiments in which carbendazim was studied individually.

Figure 32 presents the carbendazim removal efficiencies attained when 100 µg/L carbendazim was supplied to the reactors both individually and as mixture. As seen, except for the reactors operated at SRT 3 and 20 days, carbendazim was removed

better in reactors fed with mixture of pesticides rather than the reactors fed only with carbendazim. In both reactors operated at SRT 3 days, regardless of whether carbendazim was supplied individually or as a mixture, removal of carbendazim was 35 %. Carbendazim removal performance of the reactor operated at SRT 20 days which was only fed with carbendazim outperformed the reactor operated at SRT 20 days which was fed with mixture of pesticides.

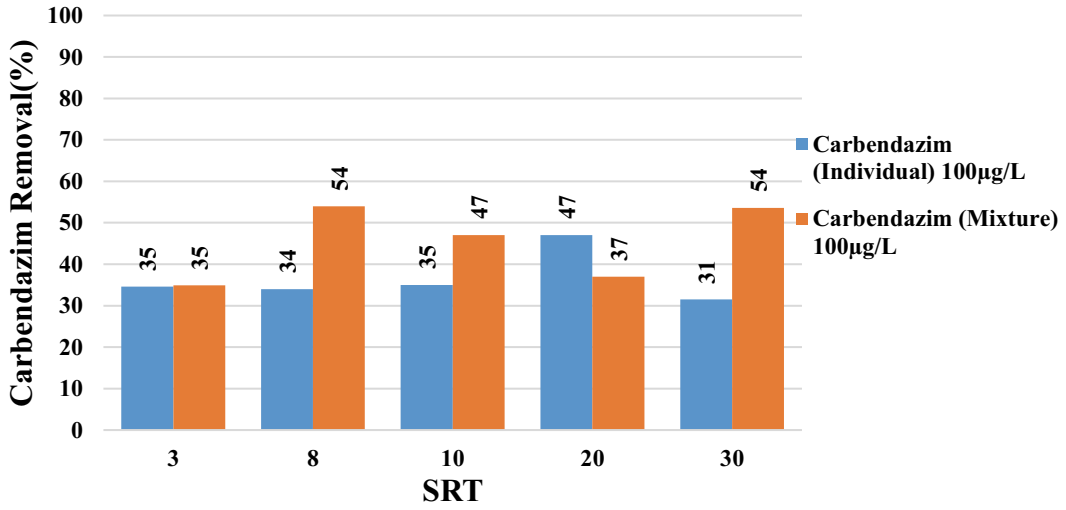

Figure 32 Removal Efficiencies of 100 µg/L Carbendazim Pesticide in Relation to the Sludge Retention at 25°C

Figure 33 presents the carbendazim removal efficiencies attained when 200 µg/L carbendazim was supplied to the reactors both individually and as mixture. When the carbendazim removal efficiencies are examined for the influent carbendazim concentrations of 200 µg/L at a closer look, it can be seen that removals were quite similar at SRT 3, 8, 10 and 20 days in reactors fed with mixture of pesticides when compared to the ones which were bearing only carbendazim pesticide. These

mentioned reactors, working with mixtures of pesticides, performed similarly by removing less than 38 % of carbendazim. However, at SRT 30 days 59% of carbendazim was removed. Supportively, COD removal decrease becomes also noticeable especially in reactors operated at SRT<30 days. To recall at SRT 30 days, decrease in COD removal efficiency was less as compared to the other SRTs (Figure 26), probably due to the better acclimatization of the culture to the carbendazim at longer contacts provided.

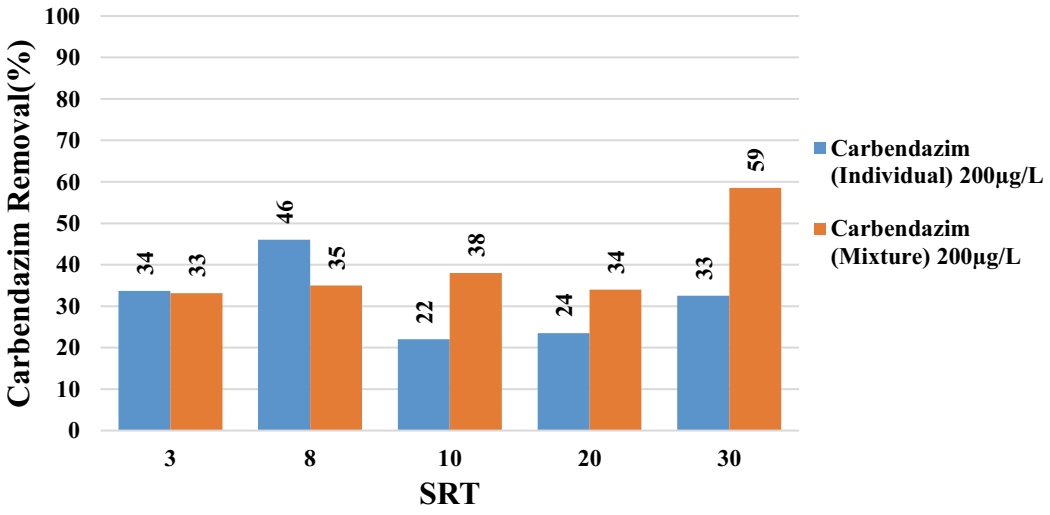

Figure 33 Removal Efficiencies of 200 µg/L Carbendazim Pesticide in Relation to the Sludge Retention at 25°C

As presented in Figure 34, once 300 µg/L carbendazim, imidacloprid and aclonifen was supplied, the carbendazim removal efficiency attained was less than 41% in all reactors fed with mixture of pesticides. However, there exists no clear correlation between the carbendazim removal and SRT since for the mentioned concentration SRT 8 days performed the best by removing 41% of carbendazim, while SRT 3, 10

and 20 days performed similarly by removing less than 30% of carbendazim. Additionally, for the specific concentration level of 300 µg/L it is not possible to conclude whether carbendazim is removed better in reactors bearing only carbendazim or in reactors bearing a mixture of pesticides.

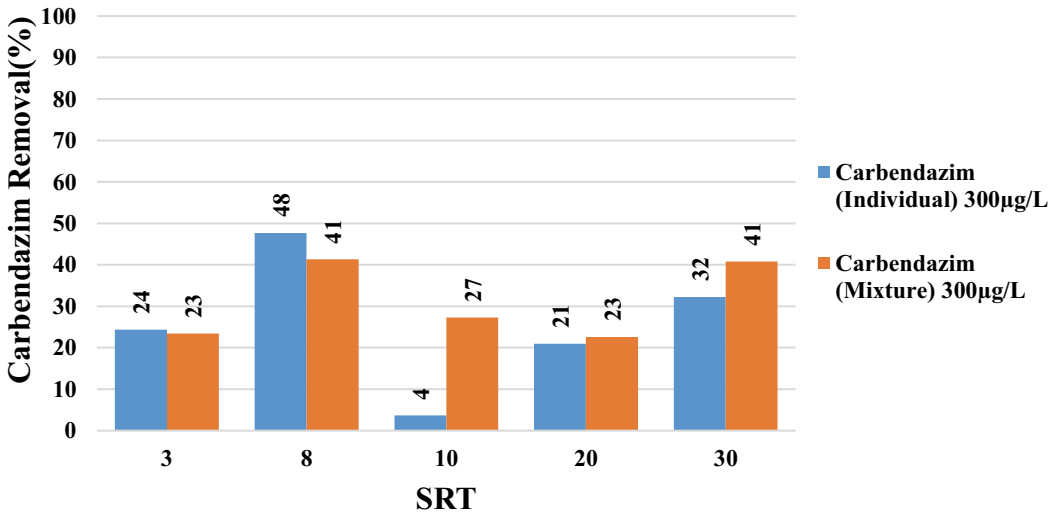

Figure 34 Removal Efficiencies of 300 µg/L Carbendazim Pesticide in Relation to the Sludge Retention at 25°C

Once 400 µg/L carbendazim, imidacloprid and aclonifen was supplied, the carbendazim removal efficiency attained was less than 41% in all reactors (Figure 35). There exists no clear correlation between the carbendazim removal and SRT since for the mentioned concentration SRT 8 days performed the best by removing 41% of carbendazim, while SRT 3, 10 and 20 days removed 22% of carbendazim. As the influent concentrations increased, the performance difference between the reactors fed with pesticides mixtures and reactors fed only with carbendazim increased. Figure 35 presents the removal efficiency of 400 µg/L carbendazim in individual reactors and

reactors fed with mixture of pesticides. The carbendazim removal efficiency of the reactors fed with mixture of pesticides was better in all SRTs. As seen, most remarkable change was observed for the reactor operated at SRT 10 days, only 3.75% of the initial carbendazim was removed at the reactor where only carbendazim was supplied. However, the removal efficiency increased to 22% when the pesticides carbendazim, imidacloprid and aclonifen were supplied together.

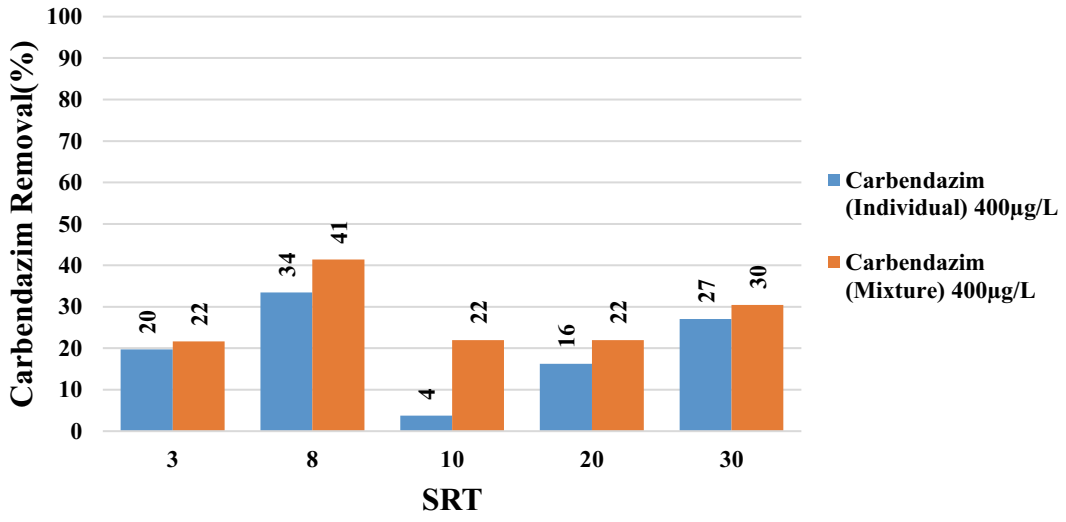

Figure 35 Removal Efficiencies of 400 µg/L Carbendazim Pesticide in Relation to the Sludge Retention at 25°C

4.4.3. Effect of SRT on Imidacloprid Removal at Reactors Fed with Pesticide Mixtures

Removal efficiencies of imidacloprid pesticide as a function of the influent pesticide concentration at reactors operated at different SRTs are demonstrated on Figure 36. The reactors were fed with mixture of pesticides containing the same amount of imidacloprid, carbendazim and aclonifen. For instance, the first concentration studied was 10 µg/L. For this, a synthetic wastewater bearing 10 µg/L of imidacloprid, 10 µg/L of aclonifen and 10 µg/L of carbendazim was fed to the reactors.

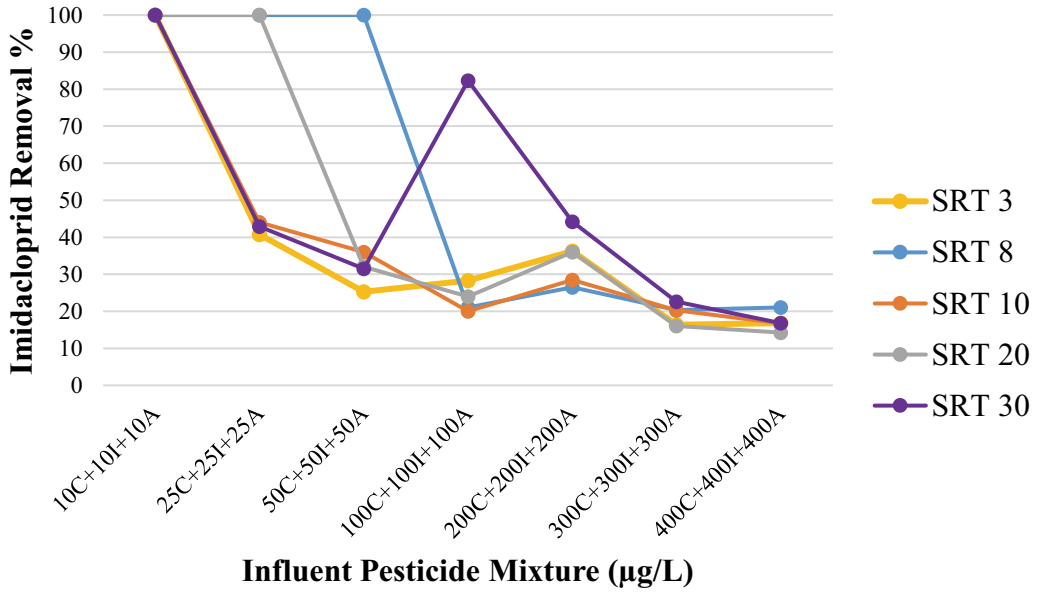

Figure 36 Removal Efficiencies of Imidacloprid at Reactors Fed with Mixture Pesticides

As presented on Figure 36, all of the reactors were capable of removing imidacloprid by almost 100% when pesticide mixtures bearing 10 µg/L imidacloprid was supplied to the reactors. To recall, same situation was observed during the experiments in which imidacloprid was studied individual as can be seen on Figure 37. Accordingly, no

correlation was observed between carbendazim removal and SRT for the lowest concentration, 10 µg/L. However, as the influent imidacloprid concentration increased imidacloprid elimination decreased remarkably.

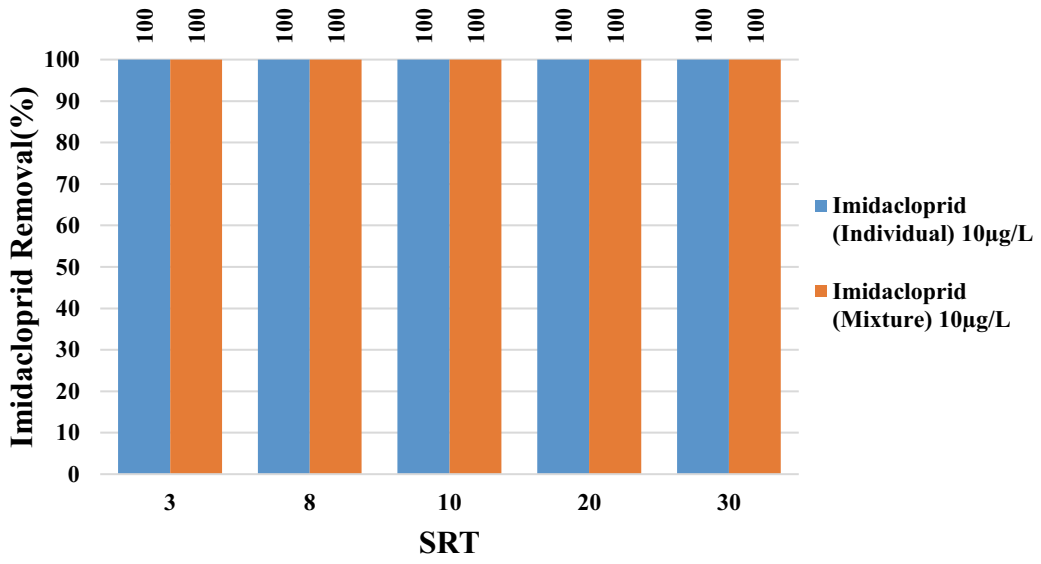

Figure 37 Removal Efficiencies of 10 µg/L Imidacloprid Pesticide in Relation to the Sludge Retention at 25°C

Immediately after increasing the influent pesticide mixture concentration to 25 µg/L, imidacloprid removal efficiency decreased down to 41%, 44% and 43% in the reactors operated at SRT 3, 10 and 30 days, respectively (Figure 36). For 25 µg/L, two reactors, SRT 8 and 20 days, were able to remove imidacloprid by almost 100%. However, these high removal performances decreased as the influent pesticide concentration increased.

Figure 38 presents the imidacloprid removal efficiencies attained when 25 µg/L imidacloprid was supplied to the reactors both individually and as mixture. As seen,

except for SRT 8 days, the imidacloprid removal performances of reactors bearing only imidacloprid and reactors bearing carbendazim, imidacloprid and aclonifen were very similar.

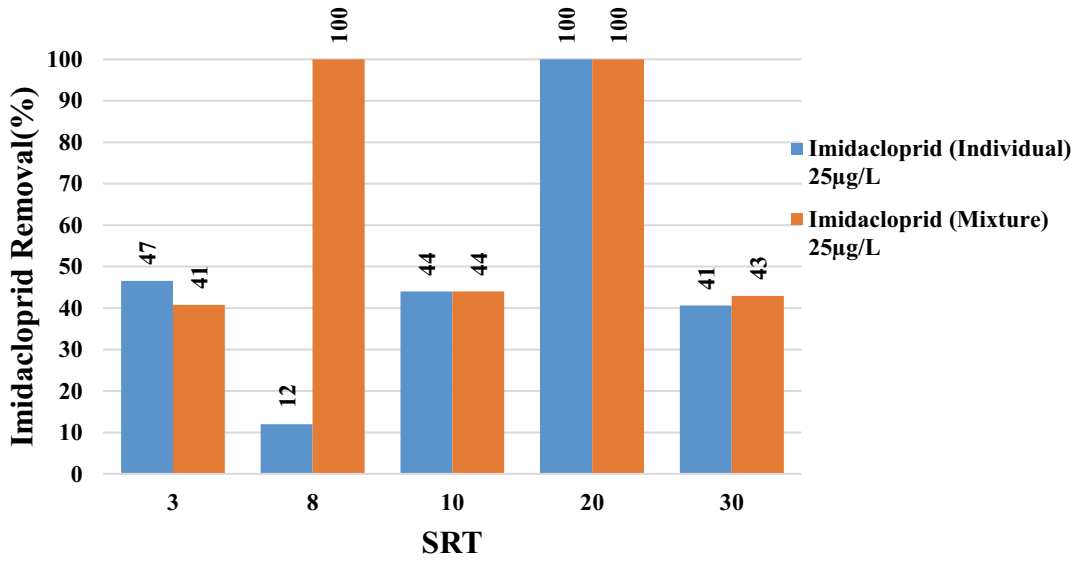

Figure 38 Removal Efficiencies of 25 µg/L Imidacloprid Pesticide in Relation to the Sludge Retention at 25°C

After supplying 50 µg/L of imidacloprid, carbendazim and aclonifen to the reactors, imidacloprid elimination performance achieved after supplying 25 µg/L pesticide mixture decreased in all of the reactors operated except for SRT 8 days (Figure 36). Anyhow, as can be seen in Figure 39, this exceptional case of reactor SRT 8 days, in which nearly 100 % of the supplied imidacloprid is removed, disappeared at 100 µg/L.

Figure 39 presents the imidacloprid removal efficiencies attained when 50 µg/L imidacloprid was supplied to the reactors both individually and as mixture. As seen, except for SRT 20 days, the imidacloprid removal performances of reactors bearing

mixture of pesticides were either better or similar to imidacloprid removal of the reactors bearing only imidacloprid.

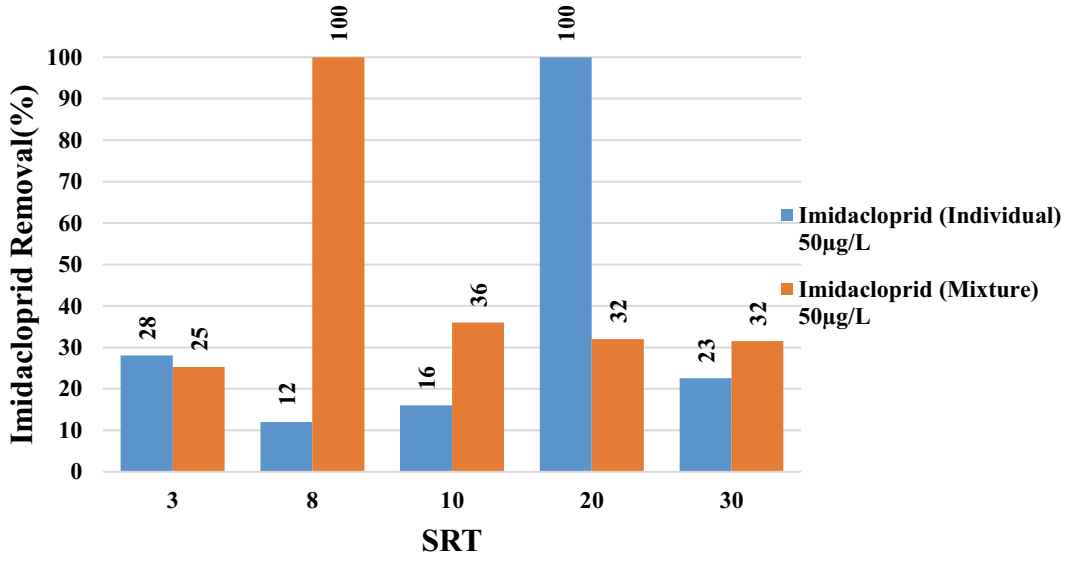

Figure 39 Removal Efficiencies of 50 µg/L Imidacloprid Pesticide in Relation to the Sludge Retention at 25°C

As expected, removal performances decreased as the influent pesticide concentration increased. The removal performances of the reactors (except SRT 30 days), followed the same removal trend after supplying 100 µg/L. A peak observed in imidacloprid removal in the reactor operated at SRT 30 days when received 100 µg/L pesticide mixture remained unexplained, since the peak disappeared after receiving 200 µg/L (Figure 36). From this point on, the removal efficiencies of the reactors were consistent with others as the concentration increased. Moreover, as can be seen form Figure 36, imidacloprid removal performance of the reactor operated at SRT 30 days was better than the rest of the reactors at most of the concentration levels.

Figure 40 presents the imidacloprid removal efficiencies attained when 100 µg/L imidacloprid was supplied to the reactors both individually and as mixture. The removal efficiencies of imidacloprid at reactors fed with mixture of pesticides were very similar to the individual reactors. The only exception was for 100 µg/L imidacloprid. For this specific influent concentration, the best removal efficiency (48%) at individual reactors was observed at SRT 30 days. However, when the pesticides were fed to the reactors as a mixture the removal efficiency increased to 82%. Thus, it is possible to say that imidacloprid removal increases when the pesticides are fed to reactors as mixtures.

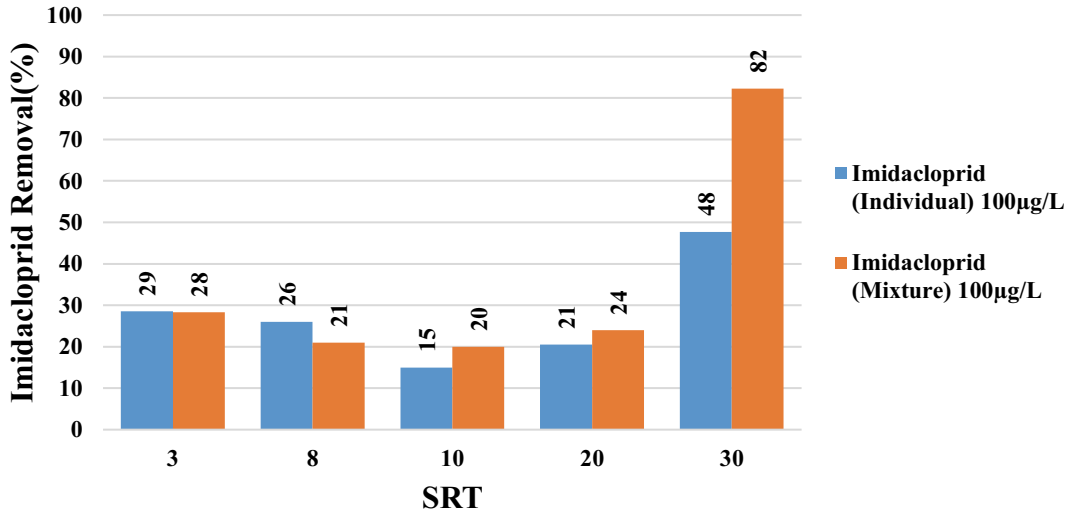

Figure 40 Removal Efficiencies of 100µg/L Imidacloprid Pesticide in Relation to the Sludge Retention at 25°C

Figure 41 presents the imidacloprid removal efficiencies attained when 200 µg/L imidacloprid was supplied to the reactors both individually and as mixture while Figure 42 presents the imidacloprid removal efficiencies attained when 300 µg/L is

supplied. For the higher concentrations, 200 µg/L and 300 µg/L, imidacloprid elimination performances of the reactors were quite similar, regardless of SRT. Thus, it is not possible to define a correlation between SRT and imidacloprid removal both for individual and mixture experiments.

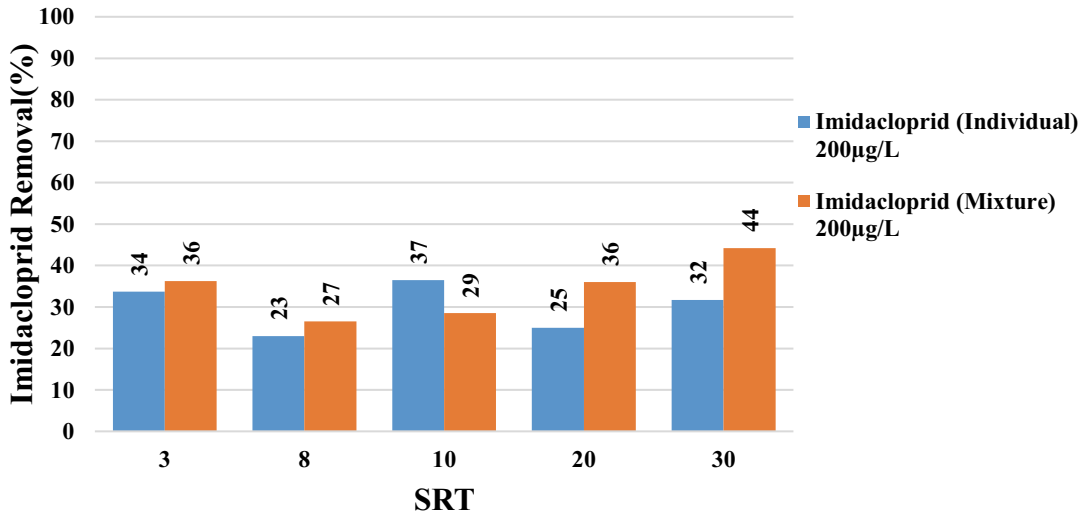

Figure 41 Removal Efficiencies of 200µg/L Imidacloprid Pesticide in Relation to the Sludge Retention at 25°C

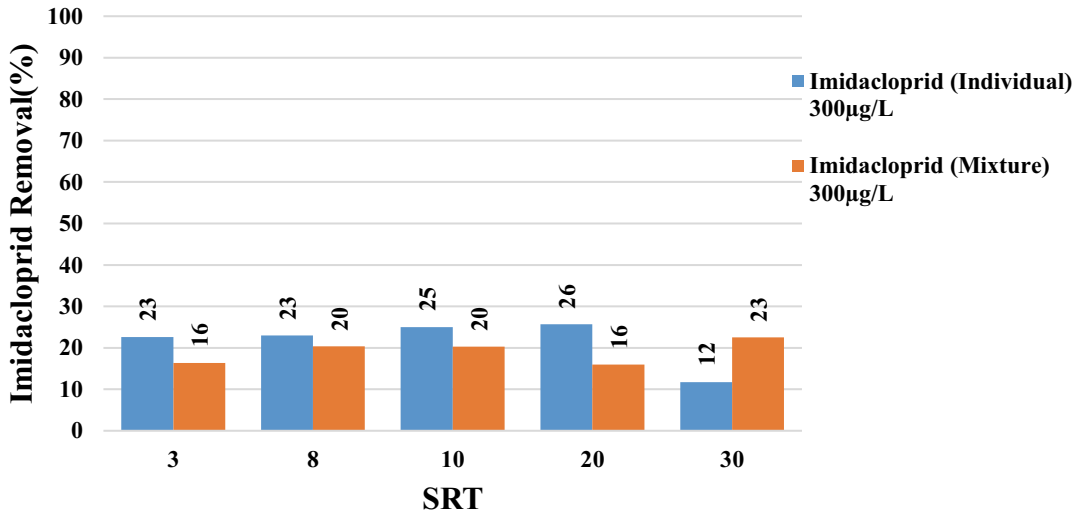

Figure 42 Removal Efficiencies of 300µg/L Imidacloprid Pesticide in Relation to the Sludge Retention at 25°C

As presented in Figure 43, for 400 µg/L imidacloprid, reactors operated at SRT 3 and 20 days performed better at the individual imidacloprid removal experiments when compared to the other individual reactors. On the other hand, when carbendazim, imidacloprid and aclonifen was supplied as a mixture, these reactors' elimination performances were the same with the rest of the reactors. However, it must be noted that, sharp drops on removal efficiencies of SRT 8 and 10 days were eliminated when the pesticides were supplied as mixture (Figure 43).

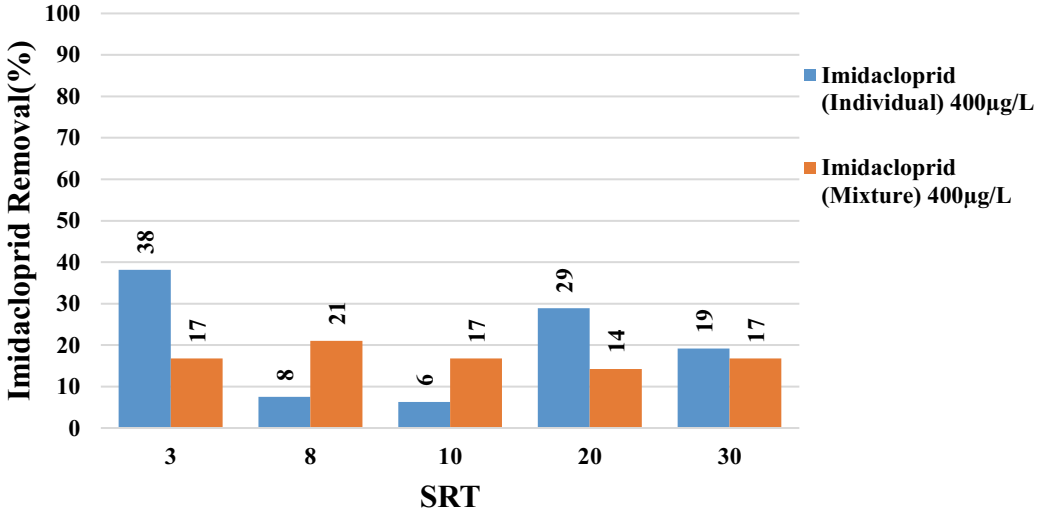

Figure 43 Removal Efficiencies of 400µg/L Imidacloprid Pesticide in Relation to the Sludge Retention at 25°C

4.4.4. Effect of SRT on Aclonifen Removal at Reactors Fed with Pesticide Mixtures

Removal efficiencies of aclonifen pesticide as a function of the influent pesticide concentration at reactors operated at different SRTs are demonstrated on Figure 44. The reactors were fed with mixture of pesticides containing the same amount of aclonifen, carbendazim and imidacloprid. For instance, the first concentration studied was 10 µg/L. For this, a synthetic wastewater bearing 10 µg/L of aclonifen, 10 µg/L of imidacloprid and 10 µg/L of carbendazim was fed to the reactors.

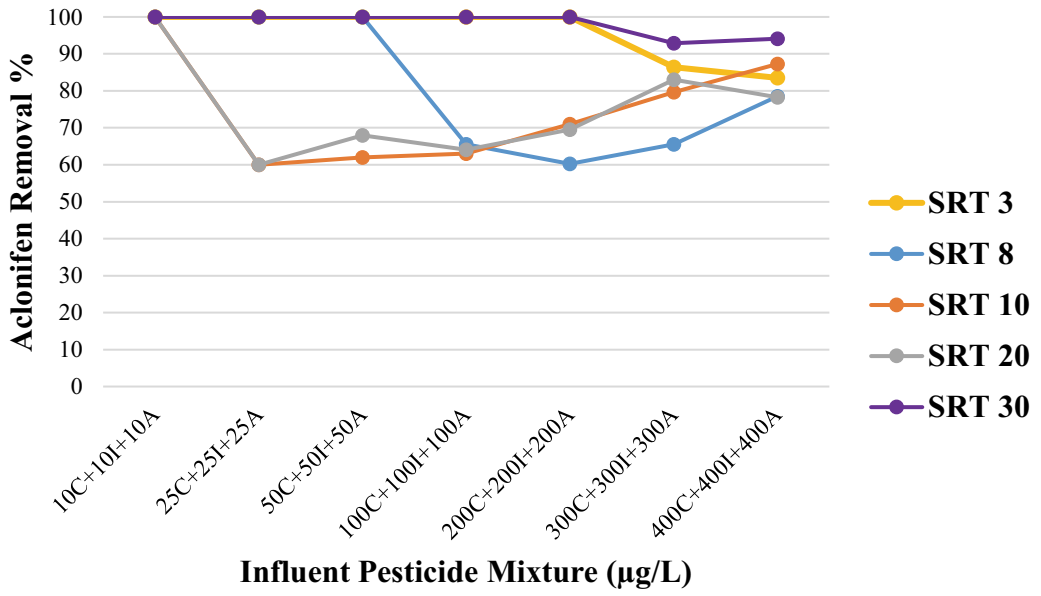

Figure 44 Removal Efficiencies of Aclonifen a at Reactors Fed with Mixture Pesticides

As illustrated on Figure 44, at the lowest concentration of this study, 10 µg/L, aclonifen was removed by almost 100% at all of the reactors, regardless of the SRT. To recall, when 10 µg/L aclonifen was supplied to the reactors in individual studies all of the reactors removed aclonifen by almost 100% (Figure 45). The lowest concentration of this study, 10 µg/L, was the only concentration in which all the reactors behaved the same as they all removed aclonifen by almost 100%. From this point on, as the influent aclonifen, imidacloprid and carbendazim concentration increased removal performances of the reactors deteriorated remarkably.

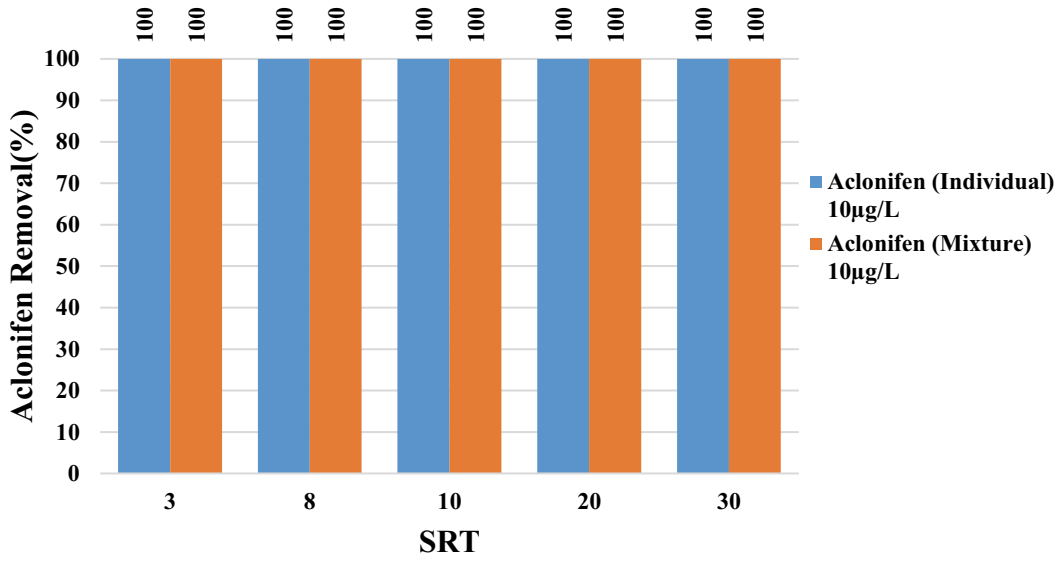

Figure 45 Removal Efficiencies of 10 µg/L Aclonifen Pesticide in Relation to the Sludge Retention at 25°C

Figure 46 presents the aclonifen removal efficiencies attained when 25 µg/L aclonifen was supplied to the reactors both individually and as mixture After supplying 25 µg/L of each pesticide (mixture of pesticides carbendazim, aclonifen and imidacloprid) to the reactors, aclonifen elimination decreased significantly down to 60% at the reactors SRT 10 and 20 days.

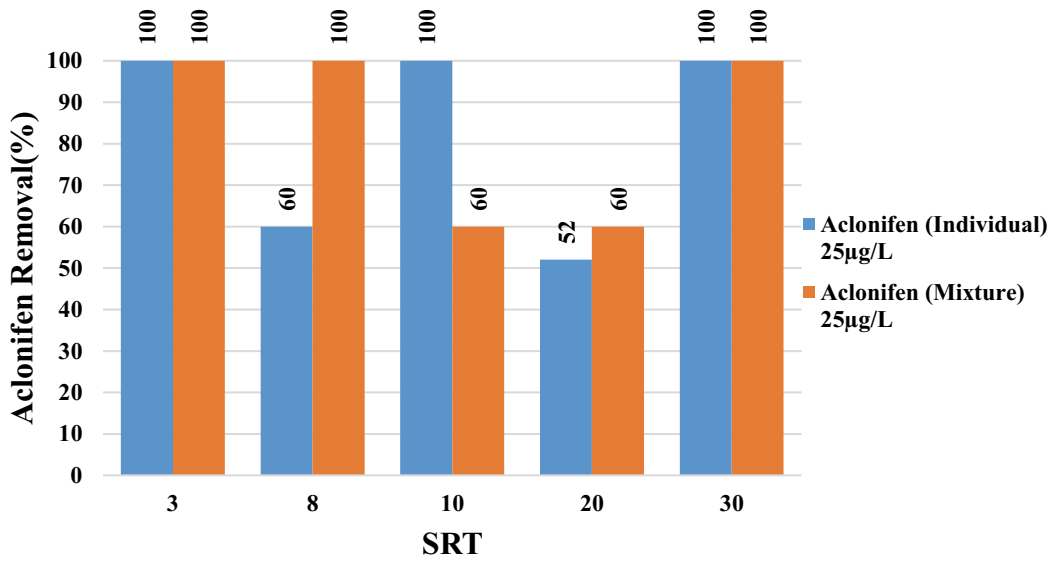

Figure 46 Removal Efficiencies of 25 µg/L Aclonifen Pesticide in Relation to the Sludge Retention at 25°C

Figure 47 presents the aclonifen removal efficiencies attained when 50 µg/L aclonifen was supplied to the reactors both individually and as mixture. As seen, although the influent pesticide concentration was doubled to 50 µg/L, aclonifen removal did not deteriorate, actually even a slight improvement was recorded after supplying 50 µg/L of aclonifen, imidacloprid and carbendazim.

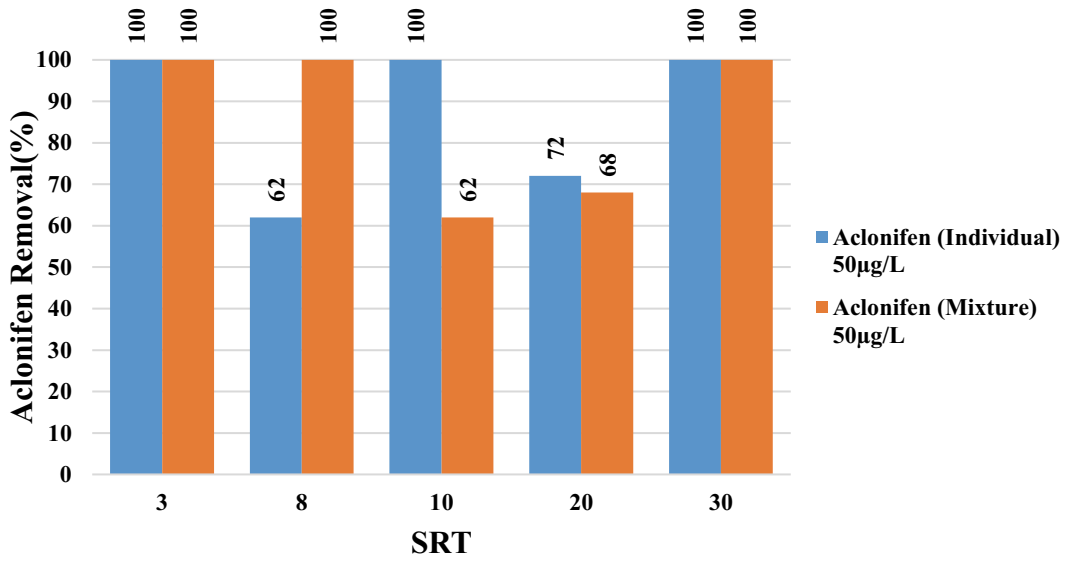

Figure 47 Removal Efficiencies of 50 µg/L Aclonifen Pesticide in Relation to the Sludge Retention at 25°C

Until 100 µg/L, reactors operated at SRT 3, 8 and 30 days were capable of removing aclonifen by almost 100%. Following the addition of 100 µg/L of pesticide mixture (100 µg/L aclonifen, 100 µg/L carbendazim and 100 µg/L imidacloprid) to the reactors, aclonifen elimination of reactor SRT 8 days decreased to 64%. However, it must be noted that, as it can be seen clearly on Figure 44 after 100 µg/L, despite the increase of influent pesticide concentration aclonifen removal efficiencies of SRT 10 and 20 days were not disrupted on the contrary the removal efficiencies increased. In fact, after supplying 200 µg/L to the reactors, the aclonifen elimination efficiencies of the reactors SRT 10 and 20 days increased to 71% and 70%, respectively (Figure 49). Moreover, the same situation was recorded for the reactor operated at SRT 8 days after supplying 300 µg/L. The behavior of the reactors SRT 8, 10 and 20 days, could be explained by the acclimatization of microbial cultures. As it is clearly seen on Figure 44, especially after 100 µg/L, aclonifen removal performance of the aforementioned reactors increased even though the influent pesticide concentration increased.

Supportively, as seen on Figure 25 the MLSS concentrations also increased after supplying 100 µg/L although the concentrations of the spiked carbendazim, imidacloprid and aclonifen increased constantly.

Figure 48 presents the aclonifen removal efficiencies attained when 100 µg/L aclonifen was supplied to the reactors both individually and as mixture. As seen, aclonifen was removed better in reactors fed with mixture of pesticides rather than the reactors fed only with aclonifen. It should also be noted that, the sharp performance drop observed at SRT 10 days, in which aclonifen was supplied individually was not observed during the experiments conducted with mixed pesticides.

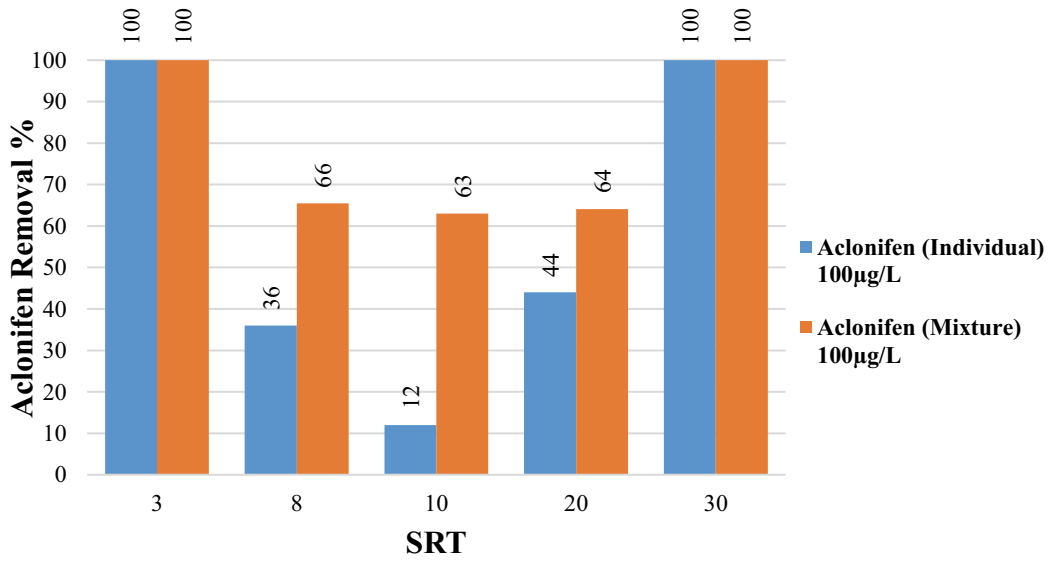

Figure 48 Removal Efficiencies of 100 µg/L Aclonifen Pesticide in Relation to the Sludge Retention at 25°C

Figure 49 presents the aclonifen removal efficiencies accomplished when 200 µg/L aclonifen was supplied to the reactors both individually and as mixture. As mentioned

before, after supplying 200 µg/L of aclonifen, imidacloprid and carbendazim to the reactors, the aclonifen elimination efficiencies of the reactors SRT 10 and 20 days increased from 63% and 64% to 71% and 70%, respectively .When aclonifen removal is compared in individual and mixed experiments for 200 µg/L, although the removal efficiencies of the reactors operated ta SRT 20 days were quite similar, in the rest of the reactors aclonifen was removed better when the reactors were fed with mixture of pesticides rather than the only with aclonifen.

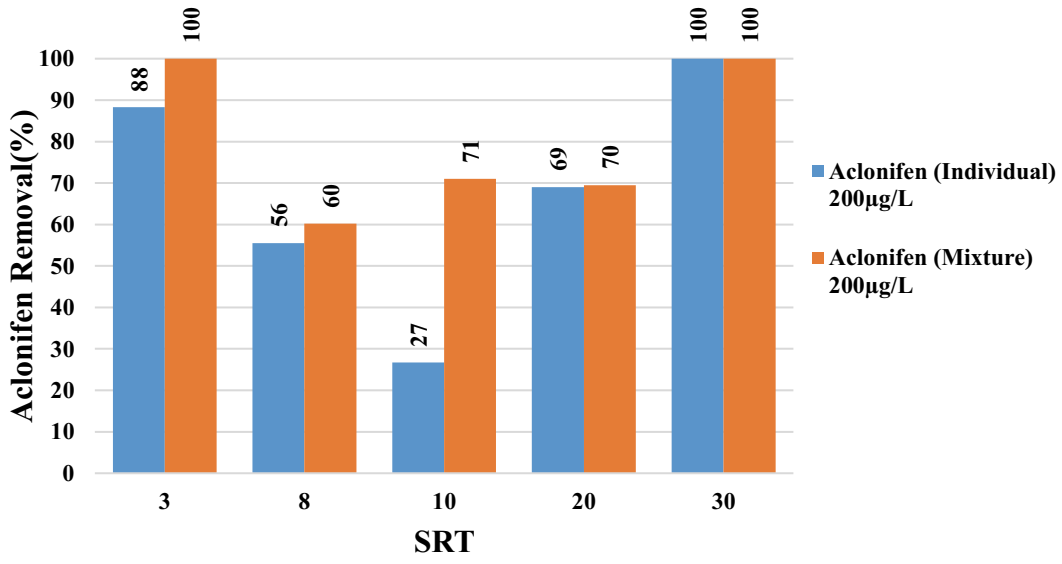

Figure 49 Removal Efficiencies of 200 µg/L Aclonifen Pesticide in Relation to the Sludge Retention at 25°C

Until the addition of 300 µg/L, reactors SRT 3 and 30 days were capable of removing aclonifen by almost 100%. As it can be seen from Figure 24, the reactors SRT 3 and 30 days were also very successful when only aclonifen was fed to the reactors until the addition of 300 µg/L. After supplying 300 µg/L of aclonifen, imidacloprid and carbendazim to the reactors, the aclonifen elimination efficiencies of the reactors SRT 3 and 30 days decreased to 86% and 93%, respectively. Although a noticeable

decrease was observed in the aforementioned reactors performances', attained aclonifen removal was better in these reactors where pesticides were supplied as mixture when compared with the reactors ran with only aclonifen (Figure 50). Additionally, the aclonifen elimination efficiencies of the reactors SRT 8, 10 and 20 days increased from 60%, 71 % and 70% to 66%, 80% and 83%, respectively.

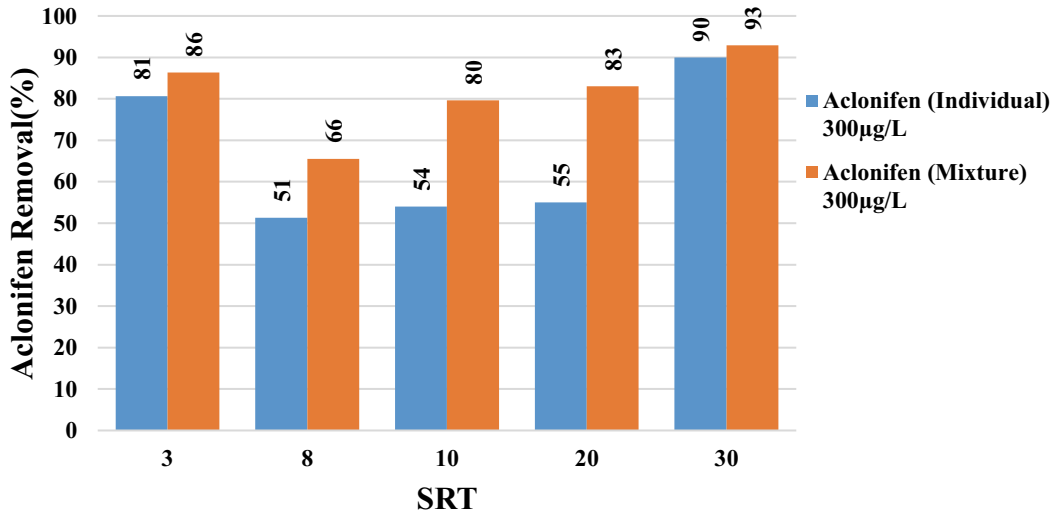

Figure 50 Removal Efficiencies of 300 µg/L Aclonifen Pesticide in Relation to the Sludge Retention at 25°C

Figure 51 presents the removal efficiency of 400 µg/L aclonifen in individual reactors and reactors fed with mixture of pesticides. As the influent concentrations increased, the performance difference between the reactors fed with pesticides mixtures and reactors fed only with aclonifen increased. Once 400 µg/L aclonifen, imidacloprid and carbendazim was supplied, the aclonifen removal efficiency attained was more than 78% in all reactors (Figure 51). It must be noted that, aclonifen removal efficiency of the reactors fed with mixture of pesticides was better in all SRTs when compared to

the reactors operated with only aclonifen. As seen, most remarkable changes were observed for the reactors operated at SRT 8, 10 and 20 days, for instance for SRT 8 days only 35% of 400 µg/L supplied aclonifen was removed when only aclonifen was supplied to the reactors. However, when 400 µg/L aclonifen was supplied to the reactor with 400 µg/L carbendazim and 400 µg/L imidacloprid the removal ratio of aclonifen increased up to 79%. For the highest concentration of this study, 400 µg/L, aclonifen elimination efficiency of reactor SRT 3 days decreased down to 84% whereas SRT 30 days achieved to remove 94% of the influent aclonifen supplied to the system. Overall, as it can be clearly seen from Figure 44, it is possible to conclude that for aclonifen, SRT 30 days is the best condition to remove aclonifen since this reactor performed the best in all concentration levels studied.

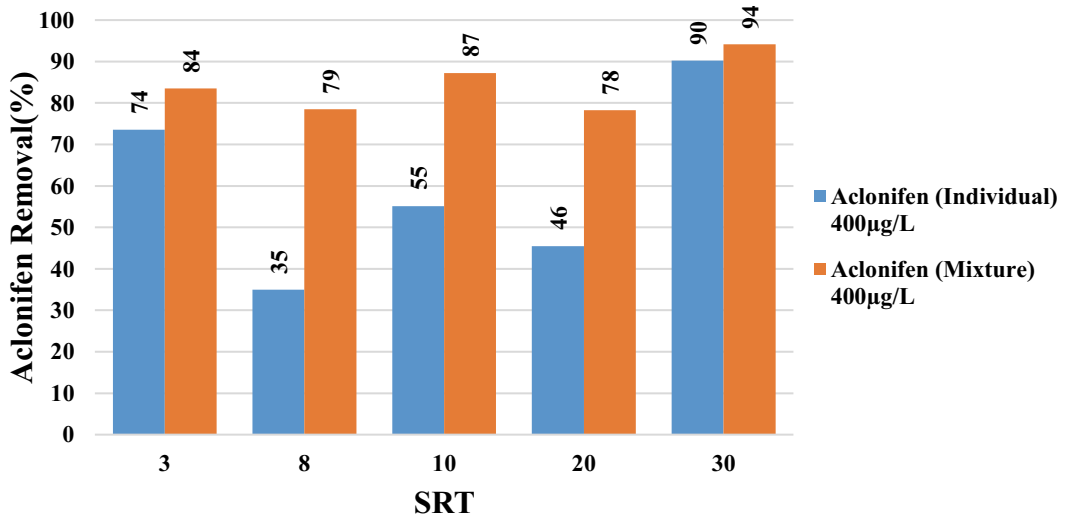

Figure 51 Removal Efficiencies of 400 µg/L Aclonifen Pesticide in Relation to the Sludge Retention at 25°C

CHAPTER 5

CONCLUSION

Within the scope of this thesis, the biological treatability of carbendazim, imidacloprid and aclonifen bearing wastewaters were investigated in detail. The main conclusions that can be drawn from this study is as follows:

5.1. Carbendazim Removal

1. In all reactors regardless of the SRT, microorganisms were able to acclimate to carbendazim after supplying 100 µg/L carbendazim to the reactors. Although the concentrations of the spiked carbendazim increased constantly the MLSS concentrations were either stabilized or a slight increase was observed after this concentration level.

2. COD utilization ability of the reactors were not disrupted remarkably until the introduction of 50 µg/L carbendazim. After spiking 50 µg/L, COD utilization ability of the reactors were adversely affected. When 50 µg/L carbendazim was spiked, all the reactors were capable of removing >89% of the initial COD. As the spiked carbendazim concentration increased from 50 µg/L to 400 µg/L, the COD utilization performance of the reactors decreased from >89% to a range of 22% to 66%.

3. Inhibitory effect of carbendazim on COD removal was recorded the least in SRT 30 days (K_I=55).

4. Minimum SRT required to treat carbendazim bearing wastewater was 1 day.

5. There exists no clear correlation between the carbendazim removal and SRT.

6. For the higher concentrations, 300 µg/L and 400 µg/L carbendazim removal efficiencies are more or less similar.

7. Considering both carbendazim removal and COD utilization, it is safer to state that beyond 50 µg/L carbendazim, the treatment system is really upset. When 50 µg/L carbendazim was spiked to the reactors, carbendazim removal varied between 40% to 100%.As the spiked carbendazim concentration increased from 50 µg/L to 400 µg/L, carbendazim removal efficiencies decreased significantly. At 400 µg/L carbendazim removal varied between 4% -34%.

5.2. Imidacloprid Removal

1. When the variation in MLSS with the influent imidacloprid concentration is examined in individual reactors, it is seen that the changes in MLSS concentrations are not consistent for all reactors. In other words, it is not possible to explain the fluctuations in MLSS concentration by SRT or by concentration.

2. Among all reactors, the reactor working at SRT 30 days was the best in COD removal, when all of the concentration levels were considered.

3. For the higher concentrations studied there is a definite correlation between the COD removal efficiencies and the SRTs. There is a significant COD removal performance difference between the reactors working at longer SRTs (SRT 10, 20 and 30 days) and shorter SRTs (SRT 3 and 8 days) at 300 µg/L and 400 µg/L. When 400 µg/L carbendazim was spiked, the reactors working at longer SRTs were capable of removing >52% of the initial COD while reactors working at SRT 3 and 8 days were capable of removing 38% and 34%, respectively. Better COD removal in longer SRTs is also supported by the inhibition coefficients (K_I)

calculated. High values of K_I obtained for SRT 10 (K_I=52), SRT 20 (K_I=48) and SRT 30 (K_I=50) days supports the better COD removal efficiencies at these reactors, when compared to the reactors operated at shorter SRTs (SRT 3 and 8 days).

4. Minimum SRT required to treat imidacloprid bearing wastewater was 1 day.

5. Above 50 µg/L imidacloprid a decreasing trend in the imidacloprid removal performance with the increase in imidacloprid concentration was almost common to all reactors.

6. For 400 µg/L imidacloprid, the imidacloprid removal efficiency achieved was less than 38% in all reactors.

7. There exists no clear correlation between SRT and imidacloprid removal.

5.3. Aclonifen Removal

1. In all reactors, COD removal efficiencies were not affected adversely until the aclonifen concentration of 50 µg/L.

2. At lower concentrations, it is not possible to define a correlation between removal efficiencies of COD and SRT. However, for the higher concentrations (300 µg/L and 400 µg/L) there is a clear correlation between the COD removal efficiencies and SRT. When 400 µg/L was spiked to the reactors, COD removal efficiencies differed between 40% to 63%, depending on the SRT.

3. Minimum SRT required to treat aclonifen bearing wastewater was 1 day.

4. When the aclonifen removal efficiencies are examined it can be seen that removals were quite similar at SRT 3 and 30 days, unlike for SRT 8, 10 and 20 days. This can be related to the possible mechanisms of the microbial culture; adsorption at low SRT (3 days) and metabolism at high SRT (30 days).

5. Considering both aclonifen removal and COD utilization, it is safer to state that beyond 50 µg/L aclonifen, the treatment system is upset. When 50 µg/L aclonifen was spiked to the reactors, aclonifen removal varied between 62% to 100%.As the spiked aclonifen concentration increased from 50 µg/L to 400 µg/L, aclonifen removal efficiencies decreased noticeably. At 400 µg/L aclonifen removal varied between 35% -90% in the reactors operated at SRT 3, 8, 10, 20 and 30 days.

5.4. Removal in Reactors Fed with Pesticide Mixtures

1. In the experiments with individual pollutants the COD utilization ability of most of the reactors were not disrupted remarkably until introduction of 50 µg/L pollutant. However, for the reactors, which were fed with mixture of pesticides the COD utilization ability were disrupted immediately after supplying 25 µg/L of each pesticide. COD utilization efficiency varied between 83% to 94% after spiking 25 µg/L of carbendazim, 25 µg/L of imidacloprid and 25 µg/L of aclonifen. As the pesticide mixture increased to 400 µg/L, COD removal efficiencies decreased significantly to a range of 4% -50%.

2. COD removal efficiency attained at SRT 30 days was quite distinct than the other reactors.

3. Minimum SRT required to treat mixtures of carbendazim, imidacloprid and aclonifen bearing wastewater was find to be 1 day.

4. Aclonifen and carbendazim removal was better when pesticides were spiked as mixture. These improvements in aclonifen and carbendazim removal were especially recorded at 300 and 400 µg/L.

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APPENDICES

A. Calibration Curves

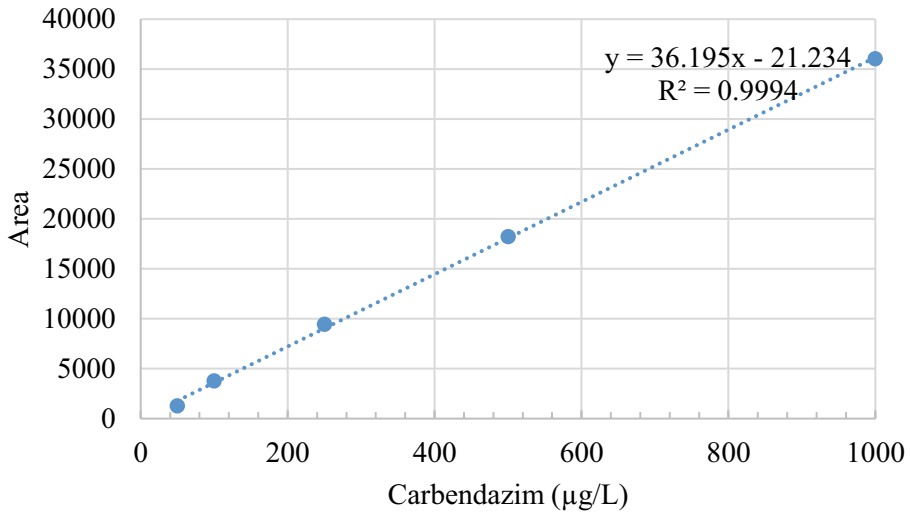

Figure 52 Calibration Curve for Carbendazim (Shimadzu)

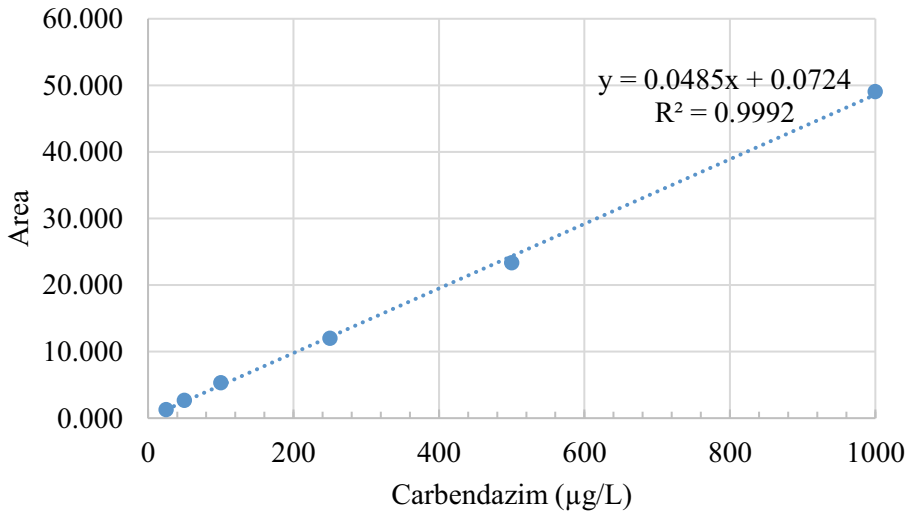

Figure 53 Calibration Curve for Carbendazim (Agilent)

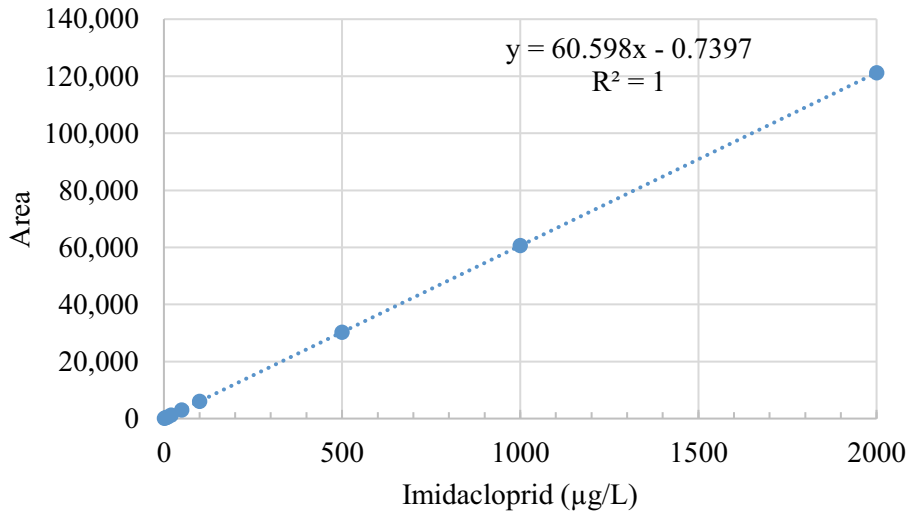

Figure 54 Calibration Curve for Imidacloprid (Shimadzu)

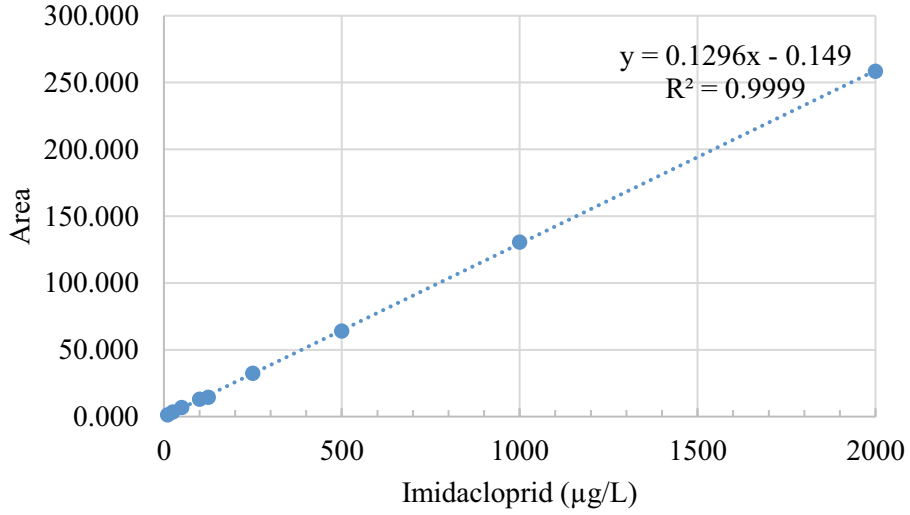

Figure 55 Calibration Curve for Imidacloprid (Agilent)

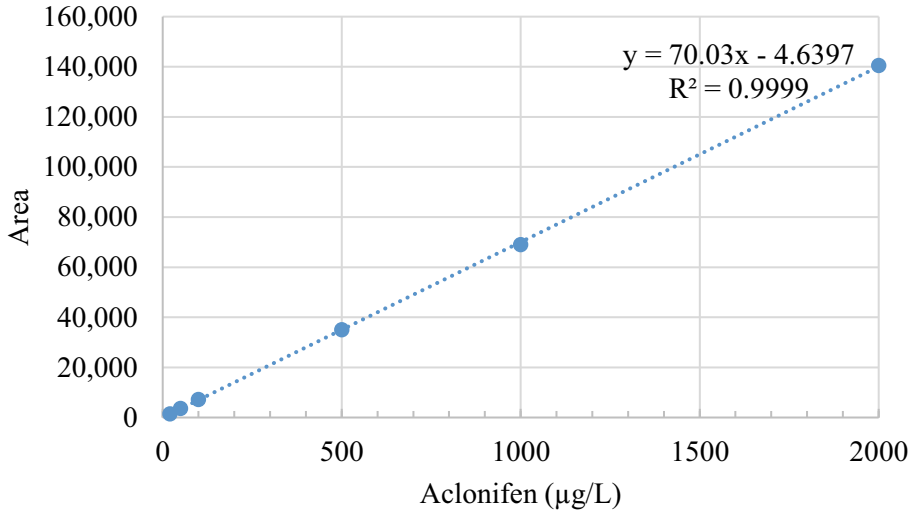

Figure 56 Calibration Curve for Aclonifen (Shimadzu)

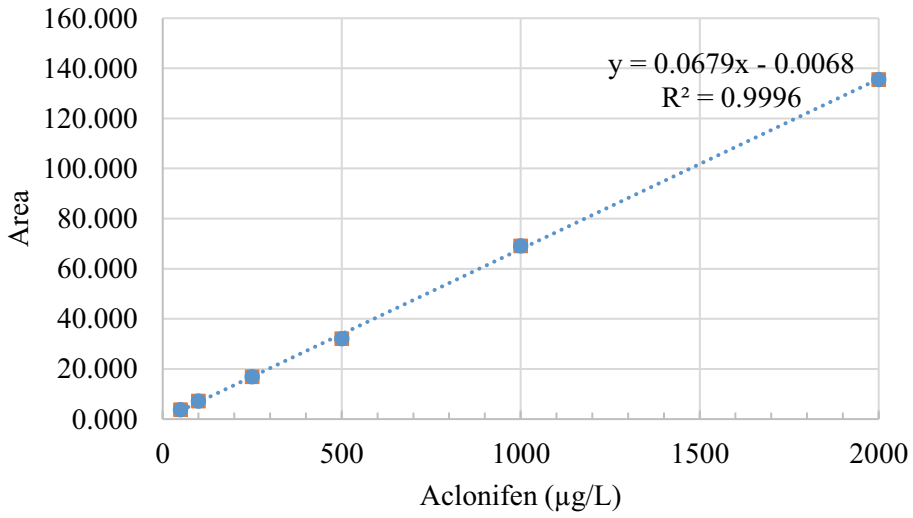

Figure 57 Calibration Curve for Aclonifen (Agilent)

B. Reactor Data

Table 16 Steady State Characteristics of Reactor 1

SRT 3				
Influent Carbendazim Concentration (µg/L)	**Effluent Carbendazim Concentration (µg/L)**	**COD (mg/L)**	**MLSS (mg/L)**	**pH**
0	0	23	640	7,4
10	0	24	460	7,42
25	0	27	420	7,4
50	30	44	300	7,4
100	65	68	280	7,47
200	133	148	320	7,5
300	227	203	260	7,47
400	321	296	340	7,43

Table 17 COD and Carbendazim Removal Efficiencies in Reactor 1

SRT 3		
Influent Carbendazim Concentration (µg/L)	**Carbendazim Removal (%)**	**COD Removal (%)**
0	0	95
10	100	95
25	100	95
50	40	91
100	35	86
200	34	70
300	24	59
400	20	41

Table 18 Steady State Characteristics of Reactor 2

SRT 8				
Influent Carbendazim Concentration (µg/L)	**Effluent Carbendazim Concentration (µg/L)**	**COD (mg/L)**	**MLSS (mg/L)**	**pH**
0	0	23	1200	7,4
10	0	23	1250	7,39
25	0	30	1300	7,42
50	0	32	1600	7,4
100	66	183	1080	7,47
200	108	180	970	8,01
300	157	300	1100	7,47
400	266	348	1020	7,57

Table 19 COD and Carbendazim Removal Efficiencies in Reactor 2

SRT 8		
Influent Carbendazim Concentration (µg/L)	**Carbendazim Removal (%)**	**COD Removal (%)**
0	0	95
10	100	95
25	100	94
50	100	94
100	34	63
200	46	64
300	48	40
400	34	30

Table 20 Steady State Characteristics of Reactor 3

SRT 10				
Influent Carbendazim Concentration (µg/L)	**Effluent Carbendazim Concentration (µg/L)**	**COD (mg/L)**	**MLSS (mg/L)**	**pH**
0	0	27	1200	7,41
10	0	34	1240	7,7
25	0	37	1440	7,85
50	30	45	1160	7,47
100	65	64	1520	8,01
200	156	111	1100	8,19
300	289	231	1540	8,2
400	385	300	1380	8,24

Table 21 COD and Carbendazim Removal Efficiencies in Reactor 3

SRT 10		
Influent Carbendazim Concentration (µg/L)	**Carbendazim Removal (%)**	**COD Removal (%)**
0	0	95
10	100	93
25	100	93
50	40	91
100	35	87
200	22	78
300	4	54
400	4	40

Table 22 Steady State Characteristics of Reactor 4

SRT 20				
Influent Carbendazim Concentration (µg/L)	**Effluent Carbendazim Concentration (µg/L)**	**COD (mg/L)**	**MLSS (mg/L)**	**pH**
0	0	23	1580	7,4
10	0	29	1600	7,42
25	0	37	1940	7,5
50	27	54	1080	7,31
100	53	68	1840	7,7
200	153	136	1740	8,01
300	237	221	2100	8,07
400	335	389	2120	8,44

Table 23 COD and Carbendazim Removal Efficiencies in Reactor 4

SRT 20		
Influent Carbendazim Concentration (µg/L)	**Carbendazim Removal (%)**	**COD Removal (%)**
0	0	95
10	100	94
25	100	93
50	46	89
100	47	86
200	24	73
300	21	56
400	16	22

Table 24 Steady State Characteristics of Reactor 5

SRT 30				
Influent Carbendazim Concentration (µg/L)	**Effluent Carbendazim Concentration (µg/L)**	**COD (mg/L)**	**MLSS (mg/L)**	**pH**
0	0	24	1480	7,42
10	0	25	1600	7,42
25	0	28	1620	7,45
50	28	35	1660	7,39
100	69	60	1680	7,42
200	135	80	1740	7,7
300	203	128	1660	7,72
400	292	172	1900	7,6

Table 25 COD and Carbendazim Removal Efficiencies in Reactor 5

SRT 30		
Influent Carbendazim Concentration (µg/L)	**Carbendazim Removal (%)**	**COD Removal (%)**
0	0	95
10	100	95
25	100	94
50	44	93
100	31	88
200	33	84
300	32	47
400	27	66

Table 26 Steady State Characteristics of Reactor 6

SRT 3				
Influent Imidacloprid Concentration (µg/L)	**Effluent Imidacloprid Concentration (µg/L)**	**COD (mg/L)**	**MLSS (mg/L)**	**pH**
0	0	24	580	7,41
10	0	25	560	7,4
25	13	38	540	7,3
50	36	44	400	7,42
100	71	77	440	7,48
200	133	147	460	7,42
300	232	211	400	7,43
400	247	312	520	7,53

Table 27 COD and Imidacloprid Removal Efficiencies in Reactor 6

SRT 3		
Influent Imidacloprid Concentration (µg/L)	**Imidacloprid Removal (%)**	**COD Removal (%)**
0	0	95
10	100	95
25	47	92
50	28	91
100	29	85
200	34	71
300	23	58
400	38	38

Table 28 Steady State Characteristics of Reactor 7

SRT 8				
Influent Imidacloprid Concentration (µg/L)	**Effluent Imidacloprid Concentration (µg/L)**	**COD (mg/L)**	**MLSS (mg/L)**	**pH**
0	0	24	920	7,4
10	0	27	960	7,35
25	22	54	960	7,2
50	44	82	1080	7,02
100	74	102	900	6,59
200	154	140	1060	5,55
300	231	224	1080	6,09
400	370	328	1560	6,82

Table 29 COD and Imidacloprid Removal Efficiencies in Reactor 7

SRT 8		
Influent Imidacloprid Concentration (µg/L)	**Imidacloprid Removal (%)**	**COD Removal (%)**
0	0	95
10	100	95
25	12	89
50	12	84
100	26	80
200	23	72
300	23	55
400	8	34

Table 30 Steady State Characteristics of Reactor 8

SRT 10				
Influent Imidacloprid Concentration (µg/L)	**Effluent Imidacloprid Concentration (µg/L)**	**COD (mg/L)**	**MLSS (mg/L)**	**pH**
0	0	23	1360	7,41
10	0	30	1300	7,5
25	14	46	1260	7,84
50	42	117	1560	7,5
100	85	159	1260	7,4
200	127	155	1280	7,46
300	225	206	1360	7,56
400	375	242	1580	6,88

Table 31 COD and Imidacloprid Removal Efficiencies in Reactor 8

SRT 10		
Influent Imidacloprid Concentration (µg/L)	**Imidacloprid Removal (%)**	**COD Removal (%)**
0	0	95
10	100	94
25	44	91
50	16	77
100	15	68
200	37	69
300	25	59
400	6,3	52

Table 32 Steady State Characteristics of Reactor 9

SRT 20				
Influent Imidacloprid Concentration (µg/L)	**Effluent Imidacloprid Concentration (µg/L)**	**COD (mg/L)**	**MLSS (mg/L)**	**pH**
0	0	24	1300	7,41
10	0	30	1350	7,4
25	0	27	1400	7,39
50	0	35	1400	7,4
100	79,5	221	1440	8,51
200	150	249	1460	5,47
300	223	182	1410	8,35
400	284,5	191	1680	8,39

Table 33 COD and Imidacloprid Removal Efficiencies in Reactor 9

SRT 20		
Influent Imidacloprid Concentration (µg/L)	**Imidacloprid Removal (%)**	**COD Removal (%)**
0	0	95
10	100	94
25	100	95
50	100	93
100	21	56
200	25	50
300	26	64
400	29	62

Table 34 Steady State Characteristics of Reactor 10

SRT 30				
Influent Imidacloprid Concentration (µg/L)	**Effluent Imidacloprid Concentration (µg/L)**	**COD (mg/L)**	**MLSS (mg/L)**	**pH**
0	0	23	1440	7,41
10	0	24	1600	7,45
25	15	27	1760	7,46
50	39	42	1760	7,38
100	52	97	1760	7,4
200	137	109	1460	7,46
300	265	150	1720	7,48
400	323	214	2300	7,47

Table 35 COD and Imidacloprid Removal Efficiencies in Reactor 10

SRT 30		
Influent Imidacloprid Concentration (µg/L)	**Imidacloprid Removal (%)**	**COD Removal (%)**
0	0	95
10	100	95
25	41	95
50	23	92
100	48	81
200	32	78
300	12	70
400	19	57

Table 36 Steady State Characteristics of Reactor 11

SRT 3				
Influent Aclonifen Concentration (µg/L)	**Effluent Aclonifen Concentration (µg/L)**	**COD (mg/L)**	**MLSS (mg/L)**	**pH**
0	0	25	560	7,44
10	0	26	460	7,42
25	0	34	380	7,38
50	0	70	320	7,26
100	0	94	300	7,36
200	23	180	340	7,5
300	58	246	220	7,47
400	106	298	400	7,43

Table 37 COD and Aclonifen Removal Efficiencies in Reactor 11

SRT 3		
Influent Aclonifen Concentration (µg/L)	**Aclonifen Removal (%)**	**COD Removal (%)**
0	0	95
10	100	95
25	100	93
50	100	86
100	100	81
200	88	64
300	81	51
400	74	40

Table 38 Steady State Characteristics of Reactor 12

SRT 8				
Influent Aclonifen Concentration (µg/L)	**Effluent Aclonifen Concentration (µg/L)**	**COD (mg/L)**	**MLSS (mg/L)**	**pH**
0	0	24	1100	7,41
10	0	32	1100	7,35
25	10	54	1160	7,3
50	19	96	1380	7,11
100	64	150	920	7,01
200	89	207	960	6,71
300	146	221	1020	6,01
400	260	276	1020	6,5

Table 39 COD and Aclonifen Removal Efficiencies in Reactor 12

SRT 8		
Influent Aclonifen Concentration (µg/L)	**Aclonifen Removal (%)**	**COD Removal (%)**
0	0	95
10	100	94
25	60	89
50	62	81
100	36	70
200	56	59
300	51	56
400	35	45

Table 40 Steady State Characteristics of Reactor 13

SRT 10				
Influent Aclonifen Concentration (µg/L)	**Effluent Aclonifen Concentration (µg/L)**	**COD (mg/L)**	**MLSS (mg/L)**	**pH**
0	0	25	1350	7,4
10	0	27	1350	7,35
25	0	30	1400	7,3
50	0	52	1300	7,12
100	88	155	1206	6,32
200	147	212	1100	6,52
300	138	278	1060	7,17
400	180	263	1140	7,03

Table 41 COD and Aclonifen Removal Efficiencies in Reactor 13

SRT 10		
Influent Aclonifen Concentration (µg/L)	**Aclonifen Removal (%)**	**COD Removal (%)**
0	0	95
10	100	95
25	100	94
50	100	90
100	12	69
200	27	58
300	54	44
400	55	47

Table 42 Steady State Characteristics of Reactor 14

SRT 20				
Influent Aclonifen Concentration (µg/L)	**Effluent Aclonifen Concentration (µg/L)**	**COD (mg/L)**	**MLSS (mg/L)**	**pH**
0	0	23	1980	7,41
10	0	31	1980	7,45
25	12	65	2000	7,54
50	14	75	1880	7,36
100	56	137	2220	7,6
200	62	155	2380	7,97
300	135	191	1880	8,06
400	218	213	1760	7,41

Table 43 COD and Aclonifen Removal Efficiencies in Reactor 14

SRT 20		
Influent Aclonifen Concentration (µg/L)	**Aclonifen Removal (%)**	**COD Removal (%)**
0	0	95
10	100	94
25	52	87
50	72	85
100	44	73
200	69	69
300	55	62
400	46	57

Table 44 Steady State Characteristics of Reactor 15

SRT 30				
Influent Aclonifen Concentration (µg/L)	**Effluent Aclonifen Concentration (µg/L)**	**COD (mg/L)**	**MLSS (mg/L)**	**pH**
0	0	22	1580	7,4
10	0	23	1600	7,43
25	0	26	1640	7,42
50	0	34	1660	7,36
100	0	65	1800	7,42
200	0	92	2080	7,45
300	30	134	2060	7,54
400	39	186	1960	7,43

Table 45 COD and Aclonifen Removal Efficiencies in Reactor 15

SRT 30		
Influent Aclonifen Concentration (µg/L)	**Aclonifen Removal (%)**	**COD Removal (%)**
0	96	96
10	95	95
25	95	95
50	93	93
100	87	87
200	82	82
300	73	73
400	63	63

Table 46 Steady State Characteristics of Reactor 16

SRT 3								
Influent Concentration (µg/L)			**Effluent Concentration (µg/L)**			**COD (mg/L)**	**MLSS (mg/L)**	**pH**
C*	**I****	**A*****	**C***	**I****	**A*****			
0	0	0	0	0	0	34	440	7,41
10	10	10	0	0	0	36	380	7,44
25	25	25	0	15	0	40	380	7,52
50	50	50	33	37	0	84	360	7,71
100	100	100	65	72	0	157	360	8,01
200	200	200	134	127	0	202	420	8,11
300	300	300	230	251	41	297	560	7,89
400	400	400	313	333	66	412	620	8,3

*Carbendazim, **Imidacloprid, ***Aclonifen

Table 47 COD and Pesticide Removal Efficiencies in Reactor 16

SRT 3						
Influent Concentration (µg/L)			**Removal (%)**			**COD Removal (%)**
C*	**I****	**A*****	**C***	**I****	**A*****	
0	0	0	0	0	0	93
10	10	10	100	100	100	93
25	25	25	100	41	100	92
50	50	50	35	25	100	83
100	100	100	35	28	100	69
200	200	200	33	36	100	60
300	300	300	23	16	86	41
400	400	400	22	17	84	18

*Carbendazim, **Imidacloprid, ***Aclonifen

Table 48 Steady State Characteristics of Reactor 17

SRT 8								
Influent Concentration (µg/L)			**Effluent Concentration (µg/L)**			**COD (mg/L)**	**MLSS (mg/L)**	**pH**
C*	**I****	**A*****	**C***	**I****	**A*****			
0	0	0	0	0	0	23	1300	7,4
10	10	10	0	0	0	39	1300	7,5
25	25	25	0	0	0	65	1240	7,47
50	50	50	0	0	0	120	1200	7,7
100	100	100	46	79	35	247	1240	8,1
200	200	200	130	147	80	400	1170	8,1
300	300	300	176	239	104	451	1480	8,8
400	400	400	235	316	86	482	1680	8,74

*Carbendazim, **Imidacloprid, ***Aclonifen

Table 49 COD and Pesticide Removal Efficiencies in Reactor 17

SRT 8						
Influent Concentration (µg/L)			**Removal (%)**			**COD Removal (%)**
C*	**I****	**A*****	**C***	**I****	**A*****	
0	0	0	100	100	100	95
10	10	10	100	100	100	92
25	25	25	100	100	100	87
50	50	50	100	100	100	76
100	100	100	54	21	66	51
200	200	200	35	27	60	20
300	300	300	41	20	66	10
400	400	400	41	21	79	4

*Carbendazim, **Imidacloprid, ***Aclonifen

Table 50 Steady State Characteristics of Reactor 18

SRT 10								
Influent Concentration (µg/L)			Effluent Concentration (µg/L)			COD (mg/L)	MLSS (mg/L)	pH
C*	I**	A***	C*	I**	A***			
0	0	0	0	0	0	32	1300	7,42
10	10	10	0	0	0	37	1440	7,55
25	25	25	0	14	10	84	1420	7,85
50	50	50	24	32	19	140	1360	7,5
100	100	100	53	80	37	139	1500	7,71
200	200	200	124	143	58	257	1400	8,88
300	300	300	218	239	61	317	1860	8,77
400	400	400	312	333	51	412	2320	8,97

*Carbendazim, **Imidacloprid, ***Aclonifen

Table 51 COD and Pesticide Removal Efficiencies in Reactor 18

SRT 10						
Influent Concentration (µg/L)			Removal (%)			COD Removal (%)
C*	I**	A***	C*	I**	A***	
0	0	0	100	100	100	94
10	10	10	100	100	100	93
25	25	25	100	44	60	83
50	50	50	52	36	62	72
100	100	100	47	20	63	72
200	200	200	38	29	71	49
300	300	300	27	20	80	37
400	400	400	22	17	87	18

*Carbendazim, **Imidacloprid, ***Aclonifen

Table 52 Steady State Characteristics of Reactor 19

SRT 20								
Influent Concentration (µg/L)			**Effluent Concentration (µg/L)**			**COD (mg/L)**	**MLSS (mg/L)**	**pH**
C*	**I****	**A*****	**C***	**I****	**A*****			
0	0	0	0	0	0	29	1880	7,39
10	10	10	0	0	0	36	1880	7,61
25	25	25	0	0	10	57	2020	7,88
50	50	50	21	34	16	109	2200	7,5
100	100	100	63	76	36	182	2680	8,01
200	200	200	132	128	61	230	2540	8,51
300	300	300	232	252	51	338	2660	8,42
400	400	400	312	343	87	400	2400	8,47

*Carbendazim, **Imidacloprid, ***Aclonifen

Table 53 COD and Pesticide Removal Efficiencies in Reactor 19

SRT 20						
Influent Concentration (µg/L)			**Removal (%)**			**COD Removal (%)**
C*	**I****	**A*****	**C***	**I****	**A*****	
0	0	0	100	100	100	94
10	10	10	100	100	100	93
25	25	25	100	100	60	89
50	50	50	58	32	68	78
100	100	100	37	24	64	64
200	200	200	34	36	70	54
300	300	300	23	16	83	32
400	400	400	22	14	78	20

*Carbendazim, **Imidacloprid, ***Aclonifen

Table 54 Steady State Characteristics of Reactor 20

SRT 30								
Influent Concentration (µg/L)			**Effluent Concentration (µg/L)**			**COD (mg/L)**	**MLSS (mg/L)**	**pH**
C*	**I****	**A*****	**C***	**I****	**A*****			
0	0	0	0	0	0	25	1750	7,40
10	10	10	0	0	0	27	1770	7,62
25	25	25	0	14	0	30	1800	7,77
50	50	50	19	34	0	52	1800	7,5
100	100	100	46	18	0	72	1900	8,12
200	200	200	83	112	0	126	2260	8,11
300	300	300	178	232	21	180	2600	8,04
400	400	400	278	333	23	246	3080	8,20

*Carbendazim, **Imidacloprid, ***Aclonifen

Table 55 COD and Pesticide Removal Efficiencies in Reactor 20

SRT 30						
Influent Concentration (µg/L)			**Removal (%)**			**COD Removal (%)**
C*	**I****	**A*****	**C***	**I****	**A*****	
0	0	0	0	0	0	95
10	10	10	100	100	100	95
25	25	25	100	43	100	94
50	50	50	61	32	100	90
100	100	100	54	82	100	86
200	200	200	59	44	100	75
300	300	300	41	23	93	64
400	400	400	30	17	94	51

*Carbendazim, **Imidacloprid, ***Aclonifen

C. Inhibition Calculation Example

$$\mu = \frac{\mu^{max} * S}{\alpha * k_s + S}$$

where μ: specific growth rate (1/time)
μ^{max}: maximum specific growth rate (1/time)
S: substrate concentration (mass/volume)
k_s: half-saturation constant (mass/volume)
α: inhibition coefficient

$$In\ chemostat\ culture\ at\ Steady\ State\ \mu = D$$
$$where\ D: Dilution\ rate$$

$$D = \frac{\mu^{max} * \bar{S}}{\alpha * k_s + \bar{S}}$$

$$\bar{S} = \frac{\alpha * k_s * D}{\mu^{max} - D}$$

$$if\ \beta = \frac{k_s * D}{\mu^{max} - D}$$

$$\bar{S} = \alpha * \beta$$

$$\bar{S} = \beta + \frac{I * \beta}{K_I}$$

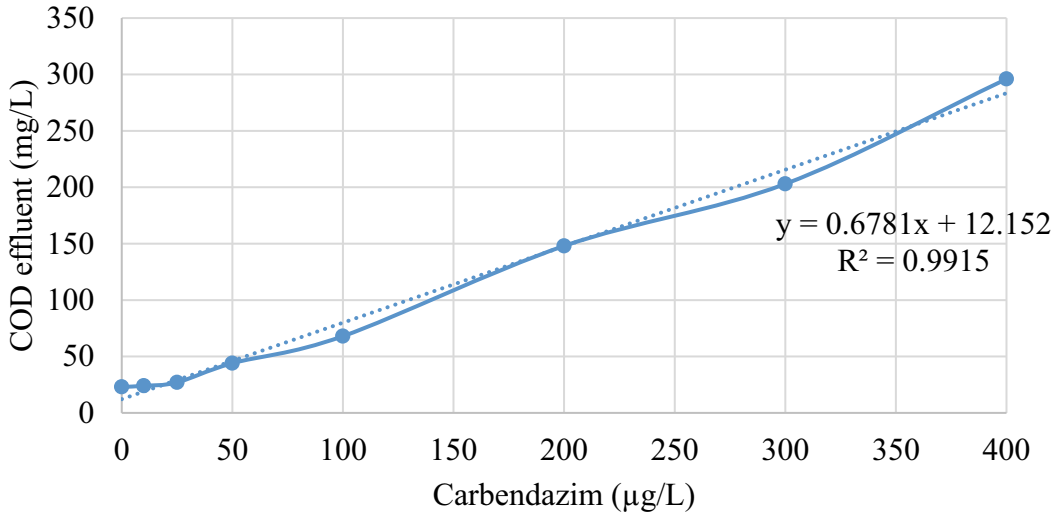

Figure 58 Carbendazim vs COD Effluent at the Reactor SRT 3 days

$$Slope = \frac{\beta}{K_I}$$
$$Intercept = \beta$$
$$\beta = 12$$
$$K_I = 18$$

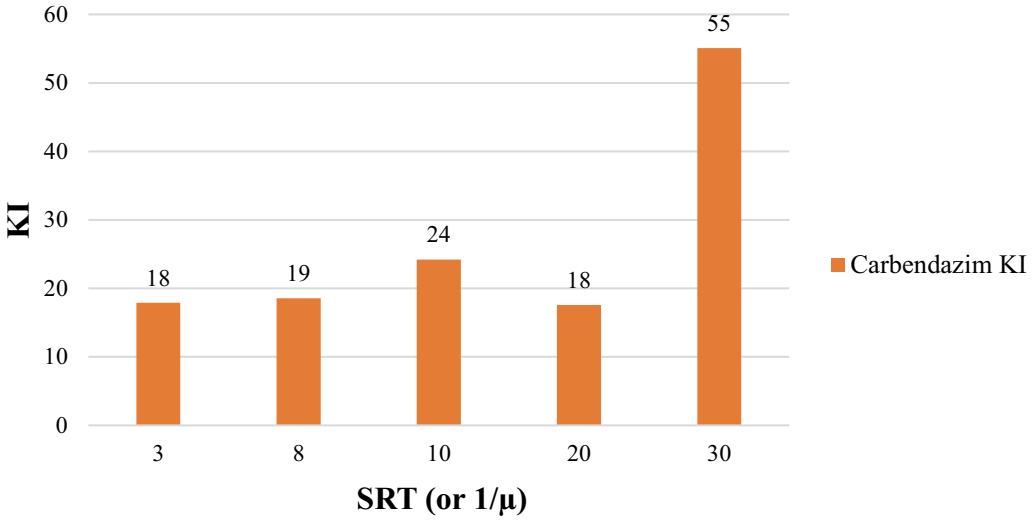

Figure 59 KI values of Carbendazim at Different SRTs