SEQUENTIAL ANAEROBIC-AEROBIC TREATMENT OF HERBICIDES IN WATER

Thesis

Submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

MAHESH G.B.

(165113CV16F11)

DEPARTMENT OF CIVIL ENGINEERING
NATIONAL INSTITUTE OF TECHNOLOGY KARNATAKA
SURATHKAL, MANGALURU – 575 025

JANUARY, 2020

SEQUENTIAL ANAEROBIC-AEROBIC TREATMENT OF HERBICIDES IN WATER

Thesis

Submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

MAHESH G.B.
(165113CV16F11)

Under the Guidance of

Dr. BASAVARAJU MANU

DEPARTMENT OF CIVIL ENGINEERING
NATIONAL INSTITUTE OF TECHNOLOGY KARNATAKA
SURATHKAL, MANGALURU – 575 025

JANUARY, 2020

DECLARATION

I hereby *declare* that the Research Thesis entitled **"Sequential anaerobic-aerobic treatment of herbicides in water"** which is being submitted to the **National Institute of Technology Karnataka, Surathkal** in partial fulfilment of the requirements for the award of the Degree of **Doctor of Philosophy** in **Civil Engineering**, is a *bonafide* report of the research work carried out by me. The material contained in this Research Thesis has not been submitted to any University or Institution for the award of any degree.

(MAHESH G.B.)
Register No.165113CV16F11
Department of Civil Engineering

Place: NITK, Surathkal

Date: 13-01-2020

CERTIFICATE

This is to *certify* that the Research Thesis entitled **"Sequential anaerobic-aerobic treatment of herbicides in water"** submitted by **Mr. MAHESH G.B.** (Register Number: **165113CV16F11**) as the record of the research work carried out by him, is accepted as the Research Thesis submission in partial fulfilment of the requirements for the award of degree of Doctor of Philosophy.

Dr. BASAVARAJU MANU
Associate Professor
Research Guide
(Signature with date and seal)

Prof. K. Swaminathan
Chairman - DRPC
(Signature with date and seal)

ACKNOWLEDGEMENT

First of all, I sincerely thank to my parents, my brother and my sister for their encouragement, moral support, love and care.

I take this opportunity to express my deep sense of thankfulness and gratitude to my honourable Guide **Dr. Basavaraju Manu**, for his continuous support of my Ph.D. research work, and for his encouragement, patience, enthusiasm and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I am highly indebted to him.

Besides my supervisor, I would like to thank **Prof. S. Shrihari**, for his insightful comments and encouragement during the progress of the work. I acknowledge my thanks to **Prof. K Swaminathan**, Head Civil Engineering Department, for his support and timely help. My heartfelt thanks to all the **Professors and previous HODs** of Civil Engineering Department, for their good will, and support.

My sincere thanks to **Prof. K Vidya Shetty**, Department of Chemical Engineering, for the comments and suggestions during the research progress.

I would like to extend my sincere thanks to **Mr. Manohar K. Shanbhogue**, Senior Technical Assistant, Civil Engineering Department, for his kind help, suggestions, and cooperation during the course of my experimental work in the Environmental Engineering laboratory.

I would like to thank my friend **Mr. Marsh M Bandi** for his contribution in this research and all the fellow Research Scholars of Civil Engineering Department and other Departments of NITK, for their support and productive interaction.

I also extend my thanks to the staff members of Civil Engineering Department and the Institute for their support in execution of this research.

Mahesh G.B.

NITK, Surathkal

ABSTRACT

Herbicides are toxic compounds which cause deterioration of the surface and ground water resources, cause harm to all living organisms. Various treatment methods like physicochemical and biological processes and in combination of aforementioned treatment techniques have been suggested for removal of pesticides from water. Under anaerobic reducing conditions, herbicides undergo dehalogenation, dechlorination and demethylation reactions and form substituent which can be further mineralized under aerobic conditions. Therefore, this study was conducted to evaluate the sequential anaerobic-aerobic treatment of three herbicides namely (2-ethylamino)-4-(isopropylamino)-6-(methylthio)-s-trazine) (ametryn), 3,6-dichloro-2-methoxybenzoic acid (dicamba) and 2,4-dichlorophenoxyacetic acid (2,4-d), and their mixtures in different formulations. The performance was evaluated at hydraulic retention time (HRT) of 48 h, neutral pH between 6.5 – 7.5 and at ambient reactor liquid temperature (27 – 32.2^{o}C). A preliminary study was conducted in four set of sequential anaerobic-aerobic system influent herbicides concentrations of 25 mg/L of 2,4-d, ametryn and dicamba separately and keeping one set as control. The preliminary study was conducted to evaluate the treatment potential of the reactors; significant removal efficiency was achieved for both the herbicides. The long term study was conducted using 4 anaerobic and aerobic reactors namely R1 (anaerobic control with no herbicide), R2 (anaerobic reactor fed with ametryn), R3 (anaerobic reactor fed with dicamba), R4 (anaerobic reactor fed with 2,4-d and ametryn mixture), and R5 (anaerobic reactor fed with 2,4-d ametryn and dicamba mixtures). Effects of increased herbicides concentration when they are treated separately (ametryn and dicamba), and in mixtures (2,4-d with ametryn and 2,4-d, ametryn with dicamba) during 400 – 430 days of treatment period. Five aerobic reactors were operated simultaneously to give post treatment to the anaerobic effluent. The reactors performance was evaluated by monitoring herbicide removal efficiency of ametryn, dicamba, chemical oxygen demand (COD) and biogas production. The reactors stability parameters pH, alkalinity, volatile fatty acids (VFA) and oxidation reduction potential (ORP) were monitored on daily basis. All the anaerobic reactors were

stabilized using 2 g/L of starch with total organic loading rate (OLR) of 0.21 – 0.215 kg-COD/m^3/d during 48 days, and aerobic reactors were stabilized in 14 days using anaerobic effluent as feed having OLR of 0.02 to 0.038 kg-COD/m^3/d. After achieving the quasi-state condition the influent was fed with known herbicide concentrations to the respective anaerobic reactors. The maximum removal efficiency obtained for different influent herbicide concentrations under anaerobic treatment from R2 reactor was 88 – 100% for ametryn and 85 – 92% for COD, similarly from R3 about 68 – 80% for dicamba and 77 – 85% for COD respectively. Sequential anaerobic-aerobic removal efficiency was found to be greater than the efficiency of anaerobic reactor, complete removal of ametryn with COD >95% in A2, and >88% for dicamba and COD in A3 was achieved. The mixed herbicides removal efficiency was evaluated based on COD removal efficiency only, the overall COD removal efficiency achieved for different influent concentrations of herbicides mixture was >85%, and >88% respectively from A4 and A5 respectively. Addition of anthraquinone-2,6-disulphonate (AQS) as a redox mediator enhanced the herbicides removal efficiency in the anaerobic reactors R2 and R3 by 12 – 20%, and a slight improvement in the COD removal in the R3 and R4 reactors by 5 – 10%. The GC-HRMS and LC-MS analysis was conducted to identify the transformation products (TPs) formed during the treatment process. Commonly identified TPs of anaerobic treatment include long chain fatty acids, esters, and alcohols from all the reactors, which were oxidised in the aerobic reactors and TPs of herbicides were different for the specific herbicides, ametryn TPs were biodegradable under anaerobic condition itself (in R2), while some TPs of dicamba were mineralised in aerobic post treatment step. The effluent from R4 – A4 and R5 – A5 contained different TPs which were not mineralised completely, but removed to a maximum level. Therefore, sequential anaerobic-aerobic treatment is found to be effective and efficient for the removal of selected herbicides from wastewater.

Keywords: Ametryn, Dicamba, 2,4-d, Biodegradation, SBR, ASBR, Sequential anaerobic-aerobic treatment

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS

λ_{max}	Maximum wavelength
ASBR	anaerobic sequential batch reactor(s)
COD	Chemical oxygen demand
d	Day(s)
DO	Dissolved oxygen
g	Gram
HRT	Hydraulic retention time
mL	milli litres
h	hour(s)
mV	milli volts
µg	micro gram
KOH	Potassium hydroxide
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
min	minute(s)
$NaHCO_3$	Sodium bicarbonate
nm	nanometer(s)
OLR	Organic loading rate
ORP/redox	Oxidation reduction potential
SBR	sequential batch reactor(s)
SRT	Solids retention time
STP	Sewage treatment plant
TP	transformation product(s)
UASB	Upflow anaerobic sludge blanket
VFA	Volatile fatty acid(s)

CHAPTER 1

INTRODUCTION

Water is an essential component for the existence and survival of life on earth. The various uses of freshwater include domestic, industrial, agricultural, institutional, recreational, and environmental activities. India is having only 4% of freshwater source, 90% of which is consumed in the agricultural activities (Dhawan 2017), and the remaining water is used for drinking, household, and industrial purpose. The agriculture activities receive water from various sources like surface and groundwater bodies. The intensive agriculture activities use a different type of agrochemicals like fertilizers, pesticides, herbicides, fungicides, and bio-pesticides to enhance crop productivity by eradicating pests, weeds, and fungus in the cropland.

Agriculture runoff joins the downstream water bodies, which fall under non-point source type of pollution, and its treatment becomes tedious. The used agrochemicals particularly the pesticides/herbicides are toxic, which gets transported to surface and groundwater bodies and cause pollution. On the other hand, an increase in population leading to excessive consumption of freshwater, reduction in the catchment area, and production of a large quantity of wastewater. The future water demands rely on the freshwater resources, thus it is essential to sustain the freshwater through various water reclamation methods.

To fulfil the future water demand, integrated strategies like water conservation and wastewater recycle methods have to be practiced. With these techniques, loss of water at the source can be prevented, and wastewater treatment and recycling reduce the dependency on freshwater bodies. Recycling of treated wastewater has several advantages like reduced or no pollution on receiving bodies, cost of treatment, and reduced water scarcity.

1.1 Background and motivation

India is an agriculture-based country, having its 60 – 70% of the total population depends on agriculture contributing around 16 – 17% of total gross

development product (GDP). Around 15 – 25% of crop production is lost due to pests, weeds, and diseases. India produces only 3 tons/hectare of yield, which is very less when compared to China, Brazil and the USA (Deshpande 2017). The total population of India is currently around 17.84% of the world population, has 2.4% of land area and 4% of water resources. The dependency on food increasing day by day as the population of the country increasing, which intern leading to adopt some advanced techniques to increase the crop yield and to protect the crops.

The advanced technology in the agricultural field focused mainly on increasing the crop yield by way of controlling weeds using chemicals, seed treatment agronomy, biotechnology, etc. India is the fourth-largest global producer of agrochemicals after the US, Japan, and China. Around 50% of the agrochemicals are consumed in the country, and the remaining is being exported, and this consumption rate may increase in the future (Tata strategic report 2016). India consumes around 0.5 kg/hectare of herbicide (De at al. 2014), which is very less in comparison with the quantity consumed by Japan (15 kg/hectare), United States (2 kg/hectare) and European Union (4 kg/hectare) during 2001 - 2003 (OECD 2008). The herbicide consumption was found to be 62183 Metric Tonnes for the year 2018 as observed from the data (Statistical database 2019).

The agrochemicals include pesticides, herbicides, fungicides, and bio-pesticides to eradicate pests, weeds, and fungus in the agricultural and non-agricultural fields. Herbicides are of prime importance as their usage is increasing due to a lack of available labor to remove weeds in various crop fields. The used herbicides and their derivatives have become a major concern in the field of environmental engineering as they join freshwater bodies through agricultural runoff and then join the downstream water bodies and increase its toxicity. The application of herbicides before rainfall has laid to the increase in herbicide load on the downstream water bodies (Conte et al. 2016). The sources of herbicides include pesticide manufacturing units, soil leaching, surface runoffs, accidental spills, improper disposal, etc. Some of the primary herbicide compounds used are 2,4 – dichlorophenoxy acetic acid (2,4-d), 2-ethylamino-4-isopropylamino-6-methyl-thio-s triazine (ametryn) and 3,6-dichloro-2- methoxybenzoic acid (dicamba) in different

combinations (Sangami and Manu 2017a). Agricultural runoff contained up to 500 mg/L of pesticide (Chiron et al. 2000), runoff from sugarcane fields, for instance, contained 24.5 mg/L of 2,4-d, 3.4 mg/L of ametryn and 93.7 mg/L dicamba (Sangami and Manu 2017a). The herbicide application in the crop fields is expected to rise in future due to the lack of availability of labors, increased food crisis, usage awareness, and cropland expansion.

Ametryn is a phytotoxic aromatic organic herbicide mainly used in large scale to kill unwanted plants like a different type of broadleaf weeds in various crop fields like corn, sugarcane, pineapple (Peters et al. 2014). Ametryn is considered to be more toxic to dicots than that of monocots, and the toxic risk has been detected in terrestrial species that have depended on the grasses and broadleaf plants for their food (USEPA 2010). Ametryn is ubiquitous in surface and in groundwater due to its low soil sorption capacity, around 3.4 mg/L of ametryn was detected in the agricultural runoff water (Sangami and Manu 2017a), wastewater treatment plants (Navaratna et al. 2016), and nearby the agricultural fields (Allan et al. 2017). Ametryn belongs to s-triazine group of herbicides, has less water solubility (209 mg/L at 25°C), melting point of 80°C, and has pKa value of 4 (Frías et al., 2004). It is known to be as an endocrine disruptor (Sanderson et al. 2000), aquatic ecosystem disruptor (Velisek et al. 2017), and can cause various health effects to human and animals (USEPA 2010). It belongs to the class III herbicide category (moderately toxic to fish, large mammals, and humans), and is highly toxic to crustaceans and molluscs (Hurley 1998). Usage of such type of herbicides has been banned in the European Union (EU) since 2002 due to their environmental consequences (Liu et al. 2016).

Dicamba is mainly used to control the post-emergence of broadleaf type of weeds in the crop field (González et al. 2006). Due to its high water solubility (4500 mg/L), half-life period (28.3 day), high mobility in soil (Comfort et al. 1992) and exists in water as anions, which makes it weakly adsorbed (Ghoshdastidar and Tong 2013). Application of dicamba is not limited to the agricultural field but also used to eradicate weeds in railway embankments, drainages, and gardens, and it is often detected more in surface than ground-water. Dicamba can cause various health effects on aquatic life, animals, and also on human (Shin et al. 2011). Due to its potential

risk of toxicity, dicamba has been banned for some years in the United States, and a temporary consent has been issued to use it for two years (USEPA 2018). Application of a mixture of herbicides is being considered as an intensive agriculture practice to remove a different type of weeds effectively and efficiently (Sangami and Manu 2017a). A mixture of 2,4-d and ametryn are being used to remove weeds that are resistant to triazine herbicides in maize and sugarcane crops (Sandoval-Carrasco et al. 2013). Use of different herbicide mixtures in the crop field to remove weeds that are resistant to individual herbicides may also affect on the non-target plants. Application of complex herbicide mixtures may accumulate over the soil due to poor solubility and volatilization.

Herbicides are highly mobile and form stable compound during chemical hydrolysis and are well-known endocrine-disrupting chemicals and exposure to these chemicals affects to eyes, thyroid, liver, kidney and nervous system of the human beings (USEPA 2005). The used herbicides and their derivatives have become a major concern in the field of environmental engineering as they join freshwater bodies through agricultural runoff and increase the toxicity of the water. Persistence of these herbicides in the soil leads to contamination of both surface and groundwater. Various concentrations of 2,4-d in surface and groundwater were detected even after the chemical was not used for a long time (Laganàet al. 2002). The effects of these three herbicides would be detrimental to human and also to other living organisms. Hence, herbicides have to be removed before discharging into water bodies. Therefore various authorities have prescribed standard limit for these herbicides in surface water, 2,4-d = 29 (surface water) – 10 µg/L (groundwater) (WHO 2003), ametryn = 14 (surface water) – 1.4 µg/L (groundwater) (USEPA 2010) and dicamba = 200 µg/L (surface water) – 14 µg/L (groundwater) (Hamilton et al. 2003).

The different Physico-chemical treatment methods have been adopted to remove herbicides including chemical oxidation processes, granular activated carbon adsorption, radiolytic degradation, advanced oxidation processes like Fenton's, electro-Fenton's, photo-Fenton's, photoelectro-Fenton's, electro-oxidation, photocatalysis, UV irradiation, electrolysis with UV irradiation photoelectrolysis and ozone treatment methods. Though the treatment mentioned above have shown

considerable treatment efficiency, there is always a production of the complex intermediate product and which has to be treated further. Since most of the intermediate compounds are unstable and may not survive, they can be removed in the post treatment itself.

Thus the physicochemical processes become tedious and uneconomical to operate. Some of the aforementioned Physico-chemical methods have also been associated with biological methods to increase treatment efficiency and to reduce the cost. However, aromatic herbicides are removed partially due to their strong link between benzene ring and halogens, in general, Physico-chemical treatment methods produce toxic intermediates which may pose toxicity than the parent compound. Biological treatment processes are cheaper than the Physico-chemical methods in terms of investment and operation costs. The cost for biological treatment methods is range from 5 to 20 times less than chemical ones such as ozone and hydrogen peroxide in the case of the advanced oxidation process. The treatment cost can be reduced by 3 to 10 times in the case of biological methods (Marco et al. 1997).

To overcome some of the limitations of Physico-chemical methods and to reduce toxic byproduct formation, biological treatment processes are extensively being adopted in recent years. Apart from the shock loading effects and slow biomass stabilization, the biological methods are environmental friendly, easy to operate and utilize locally available resources for the treatment. Several biological treatment methods have been developed by many researchers to treat herbicide present in water and wastewater. It was found that microbial biomass can detoxify the herbicides by consuming herbicides as their carbon source (Mangat and Elefsiniotis 1999).

Biological treatment methods to remove 2,4-d, ametryn and dicamba include the following methods: anaerobic biodegradation (Milligan and Häggblom 1999), biodegradation (Szewczyk et al. 2018); packed bed biofilm reactors (Sandoval-Carrasco et al. 2013; González-Cuna et al. 2016), membrane bioreactors (Navaratna et al. 2012; Ghoshdastidar and Tong 2013), UASB reactors (Sponza and Ulukoy 2006), submerged biological anaerobic/aerobic filter (Nasseri et al. 2014), sequential batch reactors (Mangat and Elefsiniotis 1999; He and Wareham 2011). Herbicide removal

using pure cultures isolated from algae, fungi, and bacteria have also been used to remove herbicides efficiently (Szewczyk et al. 2018; Bhaskar et al. 2019). But their suitability is limited to a particular type of herbicides, and practically it is difficult to maintain in pure form on a large scale at field conditions.

The conventional sequential batch reactors (SBR) are considered as an effective treatment option in the biological wastewater treatment methods because they are simple, flexible, and economically viable (Irvine et al. 1989). SBR in aerobic, anaerobic, and anoxic conditions could yield better removal efficiencies of herbicides (Chin et al. 2005). The mixed microbial consortia present in the reactor biomass can degrade different type of herbicides at various influent concentration levels even at different environmental conditions. Aerobic SBR has a drawback of formation of the recalcitrance of the herbicide which becomes difficult to degrade. Despite issues like formation of recalcitrant substances for some halogenated herbicides like ametryn, dicamba, etc. the aerobic SBR has been widely used to treat phenoxy herbicides including 2,4-d (Chin et al. 2005; Celis et al. 2008), 2,4,6-trichlorophenol by modifying the existing SBR (Khorsandi et al. 2018).

The herbicides contain halogens in the aromatic ring make them structurally stable and aerobically persistent can be treated efficiently under reductive conditions in anaerobic reactors, which can support the biotransformation of halogenated compounds (Field et al. 1995). However, under anaerobic reducing reactions, the halogens can be separated through dehalogenation, dechlorination reactions (Suflita et al. 1982). Thus anaerobic sequential batch reactor (ASBR) can be used effectively in support of the reductive reactions. ASBR was used in the treatment of different pollutants including herbicides like dicamba (Taraban et al. 1993), 2,4-d (Sponza and Ulukoy 2006; Celis et al. 2008), and refractory organic chemicals (Weinberg and Teodosiu 2012). There were no significant researches have been reported to remove ametryn and dicamba by SBR method, but available studies are limited for the treatment of 2,4-d (Chin et al. 2005).

It was found that some of the recalcitrant compounds that are aerobically persistent can be removed under anaerobic conditions. Some studies reported that the

biodegradation of herbicides primarily due to breaking up of bond between the benzene ring and the substituent group by methanogens in the presence of electron donor microbes (Ghattas et al. 2017). Under reducing reactions, there is a greater potential of mineralization of herbicides along with the formation of transformation products (TPs) of herbicides. Some studies have revealed the complete mineralization of organic compounds over a long operation period, while some have reported the formation of TP (He and Wareham 2011; Derakhshan et al. 2018). The anaerobic reducing reactions can be enhanced by the addition of redox mediators like anthraquinone-2,6-disulphonate (AQS). They increase the rate of reaction by shuttling electrons from primary electron donors or from bulk electron donors to the electron-accepting organic compounds.

The TPs remained in the anaerobic reactor can be aerobically degraded at a longer acclimation period (Tan et al. 1999; Donlon et al. 1996). Therefore, it becomes effective if the refractory halogenated aromatic compounds which cause recalcitrance are treated firstly by ASBR followed by aerobic SBR. The investigation reported on the sequential anaerobic-aerobic treatment of various environmental pollutants including azo dyes removal (Manu and Chaudhari 2002), textile wastewater treatment (Abiri et al. 2017) but none have been found for the treatment of ametryn and dicamba.

In the present research, a lab-scale sequential anaerobic-aerobic reactor was established and evaluated for possible biotransformation and mineralization of herbicides present in the simulated water. The simulated water contained ametryn, dicamba, a mixture of 2,4-d with ametryn and mixture of 2,4-d, ametryn, and dicamba. The reactors were stabilized using starch, and then after achieving the reactor stabilization, the simulated water with different herbicide concentration was fed. Impact of different concentration of redox mediator on the treatment processes was evaluated.

1.2 OBJECTIVES

The main objective of this study is to evaluate the efficiency of a sequential anaerobic-aerobic technique for possible mineralization of herbicides selected for the study.

1.2.1 The specific objectives

Evaluating the effect of

- Varying influent herbicide concentration on the performance of sequential anaerobic-aerobic treatment
- Mixture of 2,4-d + ametryn, and mixture of 2,4-d + ametryn + dicamba on the performance of sequential anaerobic-aerobic treatment
- AQS redox mediator on the performance of sequential anaerobic-aerobic treatment

1.3 ORGANIZATION OF THESIS

The dissertation has been divided into 5 chapters. **Chapter 1** explains about the introduction to the herbicides, their effects on the environment and the different treatment techniques, emphasising anaerobic-aerobic treatment, need significance, and objective of research and the listing of objectives of the present study. **Chapter 2** provides a comprehensive literature survey about the presence of ametryn, dicamba, and 2,4-d in wastewater, their sources, health effects, and the available treatment methods for their removal from water. This chapter also presents a summary of the literature and gaps in the literature review. **Chapter 3** presents the details of different materials used, experimental methodology, and analytical techniques adopted for achieving the objectives of this study. **Chapter 4** contain the results obtained, and detailed discussion about the results in comparison with the studies reported in the literature. **Chapter 5** summarises the conclusions drawn out of the present research work towards achieving the objectives. The **Appendix** includes the HPLC chromatographs, UV-VIS spectrum, calibration curves, GC-HRMS, and LC-MS of the herbicides and their derivatives obtained during the treatment processes.

CHAPTER 2

LITERATURE REVIEW

Water is an essential component required for all living organisms on earth. India accounts for about 17% of the world's population and about 4% of freshwater resources. As a world's 2nd largest producer of agriculture output, India generates about 13.7% of GDP by agriculture in 2013 and employed 50% of the population (Dhawan 2017). Irrigation consumes 90% of the freshwater than industrial and domestic activities in India. The total cultivable land in India is about 140 million ha, in which 42% of land lies in drought-prone areas (Dhawan 2017). The underground and surface water resources are depleting due to pollution, overexploitation, poor water management, and irrigation systems. On the other hand, about 15 – 25% of potential crop productions lost due to pests, weeds, and diseases. The effective water management in agriculture can avail water even during non-rainy seasons, which can help in obtaining more agriculture products. To maintain the supply of food with a growing population, the best ways to protect the crop from losses and increase the crop yield are challenging. The latest technologies practiced around the globe to enhance the crop yield and to protect crop are crop protection chemicals, agronomy, fertigation, seed treatment, biotechnology development, etc. In India, the insecticide consumption rate is about 0.5 kg/hectare in which 80% of insecticide, 15% of herbicide, 2% fungicide and 3% another type of pesticide (De et al. 2014). The growth of weed is considered to be high in the warm season than in cold season, and a shortage of labors lead to an increase in consumption of herbicides. (Tata strategic report 2016).

In the processes of controlling weeds and pests at the crop fields by chemical usage in the form of pesticides and herbicides would lead to contaminate the water resources. The sources of herbicides contributing to water pollution are manufacturing units, mainly the process wastewater from product purification, process area cleanup wastes, aqueous wastes from the centrifuges, filters or decanters, scrubber water from dryer units, production area wash waters, wash water from manufacturing equipment

clean, and laboratory drains (Weinberg and Teodosiu, 2012). Another major source of herbicide release is the agricultural activities and accidental spillages. Use of agrochemicals to support more agricultural production by removing the weeds is considered to cause significant water pollution due to the accumulation of herbicides in soil. Agricultural activities accumulate herbicides and their substituent compounds in the water, soil, and air (WHO 2003). The large scale production of pesticide/herbicides to maintain their demands increases the load on the environment. It has been reported that the herbicides could enter the food web of living beings and cause detrimental health impacts.

Herbicides can disrupt human body cells, damage deoxyribonucleic acid (DNA), genotoxicity, endocrine disruption, cancers, and affects the human immune system (Balagué 2002; WHO 2003; Briggs 1992; Cox 1994). The applied pesticides/herbicides react with available components in the field and form different transformation products (Roberts and Hutson 1999). These products tend to move vertically downward and contaminate groundwater (Broholm et al. 2001); also these byproducts can move laterally and join the surface water bodies and pollute water ecosystem (Aga and Thurman 2001).

Release of herbicides from production units and the agriculture sector led to water pollution. It is often difficult to collect and treat agriculture runoff water. Moreover, some of the manufacturing industries fail to install herbicide removal mechanisms in their plants due to lack of space, technical human resources, and often finances. With an increase in production and consumption of pesticide/herbicide, there is an increase in wastewater release. The untreated effluent released from agriculture and industrial activities is known to cause significant damage to the environment and living organisms. Suitable treatment methods are being researched to remove these harmful compounds effectively from water/wastewater. The treatment methods, mainly the combination of physical, chemical, and biological methods to remove biodegradable and non-biodegradable compounds from water, are being used.

The physical and chemical methods are not economically feasible, though they are efficient. Herbicides are considered to be organic compounds that can be biodegraded in the biological treatment systems. Among biological treatment methods, the sequential anaerobic-aerobic treatment seems to be an effective and promising technique to remove the herbicides. Under anaerobic conditions, dehalogenation, dechlorination, and dealkylation reactions take place (Suflita et al. 1982) and produce simpler by-products which become susceptible to aerobic mineralization.

2.1 The herbicides and intermediates

The different herbicides used in the agricultural activities include round-up (glyphosate), 2,4-d, 2,4-dichlorophenol, ametryn, dicamba, bensulfuron-methyl, Isoproturon, pendimethalin, acetochlor, diuron, pentachlorophenol, metolachlor, and triclopyr, etc. Among all the primary herbicides like 2,4-d, ametryn and dicamba are being used in large quantities in the agricultural sector to eradicate the weeds in croplands like rice, sugar cane, maize, wheat, etc. 2,4-d and dicamba are inexpensive types of herbicides used to control plantain Plantago and white clover broadleaf type of weeds.

Around 1500, pesticides contain 2,4-d as the main ingredients (Chu et al., 2004). Ametryn is used to control the moneywort type of broadleaf weeds in the sugarcane, corn, citrus, pineapple, tea, and other crops (Asongalem and Akintonwa 1998). A mixture of 2,4-d and ametryn are being used to remove weeds in maize and sugarcane crops, as it helps destroy the weeds that are resistant to triazine herbicides (Sandoval-Carrasco et al. 2013). Herbicides 2,4-d, ametryn, and dicamba are used in the mixture to remove different types of broad leave weeds (Sangami and Manu 2017a). The selected herbicides are halogenated compounds containing stable structures associated with chlorine, methyl, and hydroxyl groups.

2,4-dichlorophenoxyaceticacid (2,4-d) is considered as one of the main chlorinated organic herbicide, used to remove a different type of broadleaf weeds in the cereal crops and also on water bodies. 2,4-d is used in the form of flakes, powder, crystalline powder, and in salt material. It is stable at a melting pint of 135 – 142°C

(EPA 1988). 2,4-d forms water-soluble salts with alkali and amines, and it is soluble in most of the solvents. 2-ethylamino)-4-(isopropylamino)-6-(methylthio)-s-triazine (ametryn) is a methylthio-triazine herbicide widely used in the form of powder and grains. Ametryn tends to move vertically and laterally in soil due to its high water solubility of 209 mg/L at 25°C (Wang et al. 1995). This herbicide has been detected in surface and groundwater (Laabs et al. 2002). If this water joins the drinking water source, it is important to identify the ultimate fate of this chemical. 3,6-dichloro-2-methoxybenzoic acid (dicamba) is a phenoxy herbicide, a most used agrochemical for plant protection worldwide (Aspelin and Grube 1999).

Dicamba is being used in different forms as dimethylamine salt and sodium salt. It has a pKa value of 1.95 (Koskinen et al. 1998); it is highly mobile in water and has polluted ground and surface water; it has a half-life of 31 days (Krueger et al. 1991). It is volatile and known to drift for long distances after applications at high temperatures (Drzewicz et al. 2005). The total worldwide pesticide consumption is in the ratio of herbicides: 47.5%, insecticide: 29.5% and fungicides: 17.5% and others: 5.5% (De et al. 2014). The three major herbicides, consumption rate, and their health impacts are tabulated in Table 2.1.

Table 2.1: The herbicides, quantity consumed and their health effects

Herbicide	Consumed quantity (Kg)	Country	Effects
2,4-Dichlodiphenoxyacetic acid (2,4-d)	1.5 million Kg/year (Canada and Environment Canada 2005)	Canada, New Zealand,	Soft-tissue sarcoma and non-Hodgkin lymphoma (Balagué 2002), affects algae, small invertebrates, amphibians, and fishes, during their juvenile stages (Tomlin 2006)
2-ethylamino-4-isopropylamino-6-methyl-thio-s-triazine (ametryn)	Applied on more than 7 million hectares worldwide (Smith et al. 2008)	Australia (Briggs,1992), USA (Hurley, 1998)	extremely phytotoxic PSII herbicide (Sandoval-Carrasco et al. 2013) moderately toxic to fish, large mammals and humans (WHO 2003; Briggs 1992), organic form is highly toxic to crustaceans and molluscs (Hurley 1998)
3,6-dichloro-2-methoxybenzoic acid (dicamba)	250-500 tonnes/year (Environment Canada 2005), 11 million lbs in 1990 (Milligan and Häggblom 1999)	Canada, US	Mutagenicity and carcinogenicity (Cox 1994)

In India, the herbicide consumption is in the increasing tread, at present India consumes around 0.5 kg/hectare of herbicide (De at al. 2014). There is a likely chance of an increase in the consumption of pesticides/herbicides in order to increase the food production by removing the pest/weeds in the crop field to fulfil the demands of a growing population. Some of the herbicides are detected in the agriculture runoff water, which is much above the discharge standards.

2.2. Overview of treatment methods adopted to remove herbicides in water

The different type of Physico-chemical, biological and their combinations are generally adopted to remove herbicides from water. According to literature, biological methods have proven to be efficient and economical. An overview of the available treatment options for herbicides removal is given in Table 2.2

Table 2.2: Overview of treatment options adopted for herbicides removal in water

Method	Physico-chemical processes/Advanced oxidation processes (AOP)	Biological processes	Combined/Integrated processes/AOP
	Chemical oxidation processes, GAC adsorption, Radiolytic degradation, Fenton's reagent, Electro-Fenton's, Photo-Fenton's, Photoelectro-Fenton's, Electro-oxidation, Photocatalysis, UV irradiation/H_2O_2, Electrolysis, UV irradiation, Photoelectrolysis, Ozone	Pure cultures (bacterial, fungal and algal), mixed cultures, aerobic and anaerobic process	Physico-chemical/AOP followed by biological, biological followed by Physico-chemical/AOP, integrated anaerobic-aerobic, sequential anaerobic-aerobic
Remarks	Herbicide removal is possible	Herbicide removal is efficient	Effective removal and mineralization of herbicides is possible

2.2.1 Physico-chemical treatment methods of herbicides

Physico-chemical treatment methods include chemical oxidation processes, including coagulation/flocculation using lime, alum, iron salts, polyelectrolytes are effective in removing the herbicides and COD from water. But the treatment process generates a large quantity of hazardous sludge which poses collection, handling, and disposal problems. Removal of herbicides using granular activated carbon (GAC), powdered activated carbon (PAC), and other low adsorbents are also used to remove herbicides. But they do not completely remove herbicides and are inefficient. Advanced oxidation processes like Fenton's reagent, Photo-Fenton, ozonation, UV irradiation, etc. are also considered as effective in removing the herbicides. They are reliable but are costly and commercially unattractive, which makes it difficult to adopt in treating herbicides as either pre or post-biological treatment.

2.2.2 Biological methods for herbicides removal

The biological treatment methods are cost-effective and are suitable to remove herbicides in single or in a combination of two processes. Biological methods are cheaper than other methods in investments (5 – 10 times) and operation costs (3 – 10 times) (Marco et al. 1997). Some of the studies on the biological treatment of herbicide containing wastewater are discussed in details.

2.2.2.1 Removal of herbicide using pure and mixed bacterial cultures

The recent advancement with respect herbicide removal is by using the isolated cultures of fungi, mixed bacterial cultures, and algal cultures, having capabilities of growing over different type of herbicides under both aerobic and anaerobic conditions. Several researchers have reported the degradation of triazine herbicide like atrazine using different bacterial strains, some of them are Rhodococcus rhodochrous (Jones et al. 1998), Pseudomonas sp. (Katz et al. 2001), Acinetobacter spp. (Singh et al. 2004).

Removal of dicamba using pure cultures of bacteria were studied under aerobic conditions with the formation of 3,6-dichlorosalicylic acid (3,6-DCSA) as its metabolite (Krueger et al. 1989). Szewczyk et al. (2018) have reported up to 12% reduction in 100 mg/L of initial ametryn concentration after 17 days of the incubation period, using an isolated fungus Metarhizium brunneum. Herbicide ametryn acted as a potential inhibitor to the fungal strain and required an easily degradable substrate like glucose, and the removal was mainly due to the conversion of ametryn to its by-products rather than degradation. However, the removal of herbicide using pure cultures depends on the type of herbicides, and the application of pure cultures for treating on a large scale is impractical.

Degradation of xenobiotic herbicides like 2,4-d, ametryn, and dicamba is also carried out by mixed cultures. Many researchers have reported a high rate of biodegradation and mineralization of herbicides under co-metabolic conditions. Taraban et al. (1993) have used anaerobic consortium to degrade dicamba and reported that the biotransformation of dicamba occurred under demethylation

reactions over 60 days of inoculation. Sandoval-Carrasco et al. (2013) have reported removal of 97% of xenobiotic over 50 days of culturing in a biofilm reactor using a 6 strain bacterial mixed culture isolated from sugar cane cultivated the soil. Another example was the use of activated sludge containing mixed bacterial consortia for ametryn removal during 214 days of culture in the hybrid membrane bioreactor with an influent concentration of 1 mg/L and found about 46% reduction in ametryn (Navaratna et al. 2012). Removal of 2,4-d was carried out using C. necator JMP134(pJP4) plasmid augmented in an SBR and reported complete removal of 2,4-d by developing 2,4-d degrading trans-conjugants resulting from indigenous bacteria in the SBR (Tsutsui et al. 2013). Atrazine removal studies using mixed bacterial consortia were reported previously, Aerobacterium sp., Microbacterium sp., Bacillus sp., Micrococcus sp., Deinococcus sp., and Delftia acidovorans (Vargha et al. 2005). These bacteria were isolated from the soil contaminated with atrazine, and bacteria could able to utilize atrazine completely as their carbon source.

2.2.2.2 Aerobic methods of herbicide removal

The wastewaters with fluctuating quantity, quality, and temperature make it difficult to remove pollutants in continuous biological treatment methods. In a sequential batch reactor (SBR), the long sludge retention time (SRT) support adaptability of bacteria to the toxic environmental condition of the reactor. It has been found that the long sludge retention in the bioreactor would positively contribute to the efficiency of the system (Navaratna et al. 2012); meanwhile, sequential batch reactors (SBR) also provide the flexibility of handling long solids retention time as the sludge will be retained the reactor. SBR works on the simple principle of fill, react, settle, and draw, has advantages like low sludge production, easy operation, economically driven which make them self-sustainable (Chin et al. 2005). Thus the bacteria present in the reactor sludge gain the tendency of neutralizing the toxic substances by getting adapted to the toxic nature of the compound and utilize it as their carbon source. The sequencing batch reactors are considered to be most suitable, flexible, easy to operate and economical alternatives (Irvine et al. 1989).

Treatment of different type of herbicides has been conducted using aerobic treatment by many researchers. The reported studies have shown mixed outcomes for the different type of herbicides/pesticides, some of the chlorinated herbicides have been removed efficiently than another type of halogenated herbicides. Aerobic SBR has a drawback of forming recalcitrant substances which become difficult to degrade and sometimes it may become difficult to treat in aerobic reactors. Despite some issues like formation of recalcitrant substances for complex chemicals, the aerobic SBR has been widely used to treat various type of organic chlorinated chemicals including 2,4-d by Orhon et al. (1989), and claiming that the acclimation period of 35 to 45 days was required to degrade 100-400 mg/L. It has been reported that the addition of substrates like glucose was required for the reactor acclimation along with the herbicide, after reactor acclimation the biomass was able to degrade the herbicide 2,4-d without any substrate (Mangat and Elefsiniotis 1999).

The mechanism of 2,4-d removal was mainly due to biodegradation, and negligible adsorption and volatilization were reported (McTernan and Pereira 1991). SBR used as a pre-treatment process to reduce the shock load (Yeruva et al. 2015). Studies in the literature reported the treatment of herbicide wastewater using either aerobic or anaerobic SBR system (Chin et al. 2005). It was proved that the 2,4-d concentration of 500 mg/L was non-toxic to aerobic bacteria (Celis et al. 2008). The herbicides like ametryn and dicamba are not uniformly susceptible to degradation by aerobic treatment process like activated sludge processes, because they have highly complicated and strong chemical structures associated with different halogen groups. Removal of such compounds has required a long operation period than the treatment time required for simpler organic compounds under aerobic treatment conditions.

Mangat and Elefsiniotis (1999) have studied a bench-scale aerobic sequential batch reactor system for the removal of chlorinated herbicides like 2,4-d during 300 days at an HRT of 16 to 48 h. Two SBRs were operated to remove 2,4-d in the presence and absence of supplemental materials like phenol and dextrose. The reactor stabilization was achieved in 2 months; reactors were operated to acclimate 2,4-d (40 mg/L) in the presence of supplemental materials and reported higher 2,4-d removal efficiency in the reactor containing phenol than in the reactor containing dextrose.

Maximum removal in the reactor was attributed to the structural similarity of the compounds. Both the reactors exhibited complete removal within 20 days of operation, and then the initial concentration was raised to 100 mg/L, which had not influenced the removal of supplemental material. No considerable 2,4-d removal was detected till 110 days of operation, and hence the HRT was increased from 16 to 48 h, a two-fold increase in the supplementary material and the reactor MLVSS concentration using fresh activated sludge. It was found to be successful for complete removal of 2,4-d in the phenol containing reactor within 3 days, whereas the other reactor took 5 weeks for complete removal.

A reactor without supplemental material was also used to study the 2,4-d removal at an HRT of 48 h, there was no inhibition reported, and the biomass was able to utilize 2,4-d as carbon source. Long operation period required for 2,4-d degradation was mainly due to low reactor MLVSS, HRT (16 h) and low SRT (1.5 days). The reactor with no supplemental carbon source could able to remove >99% of 2,4-d with influent concentrations of 200 mg/L at an HRT of 12 h, and the treatability limit was reported during the treatment of 300 mg/L of 2,4-d.

Celis et al. (2008) reported the extensive research on aerobic and anaerobic SBR for treating water containing 2,4-d and isoproturon during long operation period at an HRT of 48 h. Complete removal of 2,4-d was achieved in the aerobic reactor with an influent concentration of 300 to 500 mg/L in the presence of glucose as a supplementary carbon source. Increase in influent 2,4-d concentration to 700 mg/L did not show the degradation and reduction in glucose removal of 70% were reported. No significant removal in the isoproturon was reported, the major reason was inferred to the chemical nature of the compound, limiting its bioavailability for the bacterial degradation. A mixture of biomass previously exposed to herbicides and the fresh biomass was able to reduce the reactor acclimation period for the herbicide removal.

Baghapour et al. (2013) have reported that atrazine removal efficiencies of 98% for initial concentrations of 10 mg/L. Acclimation of the aerobic reactor was achieved in 25 days, then after the aerobic reactor containing mixed bacterial consortium was tested for atrazine removal with varying influent concentrations at

different HRTs. They claim that the submerged aerobic reactor was able to biodegrade the high concentrations of atrazine mainly due to the concentration gradient, high influent concentration has higher chance to be exposed, penetrate the cell and thus biodegraded. Co-metabolism of atrazine with primary carbon sources was found to be effective, as the microbes utilize primary carbon/nitrogen sources and produce enzyme/cofactor, which was then be used in the degradation of secondary substrates like atrazine.

Khorsandi et al. (2018) have studied the treatment of 2,4,6-trichlorophenol (TCP) up to 430 mg/L at 8 h HRT using a modified SBR. The reactor was able to remove 99% of TCP along with >92% removal COD was reported. The reactors were acclimated to 3.5 mg/L of TCP over 28 days, and further, the influent TCP was raised from 5 to 430 mg/L over 150 days of reactor operation. The reactor showed a decline in the TCP removal efficiency at higher concentrations (430 mg/L) indicated biomass inhibition, which required long reactor acclimation period of 20 days — the transformation of TCP intermediates during the treatment process impacted on the COD removal efficiency of the reactor.

Moreover, the presence of multiple halogens and nitrogen groups in some of the herbicides like ametryn and dicamba become difficult to a breakdown in aerobic condition. Due to the production of large quantities of industrial effluents, it demands large space for the accommodation of effluent wastewater to be treated. Therefore, this conventional method has to be developed further to improve their treatment performance and to reduce the treatment period.

Advantages of aerobic sequential batch reactors than Physico-chemical and other biological treatment methods

- Provide long solids retention time (SRT), which allow the development of specific bacteria suitable for the degradation of targeted herbicide
- The time required for acclimation is less
- Easy to operate and cost-effective
- Less sludge production

2.2.2.3 Anaerobic methods of herbicide removal

The anaerobic degradation processes generally produce VFA during the conversion of entrapped solids and organic compounds and then into biogas as the end product (He and Wareham 2011). Anaerobic sequential batch reactor (ASBR) was used in the treatment of different pollutants, as it allows for the reducing reactions leading to dehalogenation, dechlorination, and demethylation of herbicides (Suflita et al. 1982). It has been reported that dicamba was degraded due to demethylation and followed by dechlorination, this was indicated by the presence of 3-chlorobenzoate which was not degraded by dicamba degrading bacteria and thus the dehalogenation facilitated with the presence of hydroxyl group at ortho position (Taraban et al. 1993). Halogens present at meta position are more susceptible to microbial attack than ortho and para isomers; this mainly occurs in the case of multiple halogenated substrates.

ASBR can offer both the suspended and attached growth type of treatment within a single reactor. There will be no moveable components as in the case of anaerobic moving bed bioreactor (AMBBR), no supporting material for biofilm development, can be operated in closed batch mode (i.e., Fill and draw-type of operation), does not require much supervision. Anaerobic treatment process converts the organic matter to a very less quantity of sludge and can produce a high quantity of biogas (Ghosh and Philip 2004). ASBR can generate less sludge due to endogenous decay (Li and Wu 2014), and can provide long sludge retention time (Chin et al. 2005). At long SRTs, the bacterial adaptation and development of the required metabolic pathway to degrade targeted pollutant is high (Koh et al. 2008).

Anaerobic co-treatment of toxic compounds can be diluted with biodegradable organic compounds, which can enhance the biogas production and buffering capacity of the reactor (Xu et al. 2018). Starch was used as co-substrate as it is a simple organic compound which can be digested anaerobically through carbohydrate degradation cycle and it may also contribute to the degradation of toxic compounds (Wang et al. 2018). This type of co-treatment may be conducted for the effluents containing toxic compounds in anaerobic batch reactors (ASBR). In ASBR the

anaerobic/facultative bacteria attack on functional groups like methyl thio, isopropyl amino, and ethyl amino attached to ring in reductive steps and can use them as a carbon source, and the N-alkyl groups in the ametryn structure may serve as electron acceptors during the anaerobic processes and support rapid growth of the bacteria (Gibson and Harwood 2002). Few studies have been reported to treat s-triazine type of herbicides like atrazine with 55-60% removal using anaerobic moving bed bioreactor (Ghosh and Philip 2004; Derakhshan et al. 2018).

It was observed that the 2,4-d concentration of up to 120 mg/L was non-toxic and was treated by anaerobic bacteria (Celis et al. 2008). Anaerobic sequential batch reactor (ASBR) with acidogenic bacteria were able to degrade 130 mg/L of 2,3-d over long acclimatization period of 100 days (Chin et al. 2005). It has was found that some of the recalcitrant compounds that are aerobically persistent were transformed under anaerobic conditions. Ghattas et al. (2017) have reported that many of the organic compounds including herbicides have been partially biodegraded due to breaking up of the bonds between the benzene ring and the substituent group by methanogens in the presence of electron donor microbes.

Chin et al. (2005) have reported the treatment of 2,4-d from 20-200 mg/L using anaerobic SBR under acidogenic condition. 2,4-d was treated along with the starch as supplemental material. 2,4-d removal was found to be negligible during the first 100 days of operation while the glucose consumption occurred within the first 3 h of operation. And from 101 days after the 2,4-d removal efficiency started slowly and attained complete removal of 20 mg/L, which indicated the establishment of required biochemical mechanisms for biodegradation within the reactor. The influent 2,4-d concentration was raised to 100 mg/L and was found that the reactor adapted gradually over 10 days and was able to remove >90% of influent 2,4-d. Further increased to 95% on the continued operation. Raise in reactor temperature to 33°C did not contribute to reactor performance.

Further influent 2,4-d concentration was raised to 200 mg/L and found similar removal efficiencies within two days without causing toxicity. The reactor was able to

remove 130 mg/L of 2,4-d completely. The major transformation product of acidogenic degradation of 2,4-d was VFA, which imparted the 65% of effluent COD.

He and Wareham (2011) have used a sequential batch reactor the treatment of 2,4-d using 22 L capacity steel reactor. Anaerobic acid digestion process was carried out for the influent 2,4-d concentration of 30 to 100 mg/L. The reactor acclimated within 24 days, and then after 30 mg/L of 2,4-d was introduced and about 90% removal was reported during the day 80. The influent concentration was raised to 50 mg/L and reported the reduced removal efficiency due to toxicity, but the quick biomass adaptation contributed to reaching the removal efficiency >90% on day 82. Further, increase in the concentration from 50 to 100 mg/L, led to the drop in MLSS concentration from 4 g/L to 2.6 g/L along with drop-in 2,4-d removal to 63% and then stabilized again on day 127 with an increase in 2,4-d removal up to 93%. It was found that utilization of 2,4-d started only after the consumption of available nutrient in the reactor and 2,4-d acted as an electron donor under anaerobic condition.

Derakhshan et al. (2018) have reported the treatment of atrazine using a pilot-scale anaerobic membrane bioreactor of 15 L capacity by inoculating anaerobic sludge MLSS concentration of 30 g/L, MLVSS/MLSS ratio of 0.8 and filled with effluent with COD concentration of 10 g/L using sucrose as supplemental material. OLR was maintained lower (0.5 g COD/L d) during the start-up, then raised to 2 g COD/L, and the reactor acclimation was achieved with COD removal of 95%. The acclimation to atrazine was carried out with fresh feed containing 0.1 mg/L of atrazine. The study was conducted to evaluate the atrazine removal efficiencies from 0.1 to 10 mg/L with variation in HRT of 6 – 24 h. It was found that the higher HRT was found to efficient in removing the atrazine.

After reactor stabilization, different concentration of COD and atrazine was introduced. The removal efficiency of the reactor dropped initially with the introduction of herbicides, and it increased removal efficiency was reported over the continued operation, which indicated a temporary shock load on the biomass. The rise in atrazine after 27 days of operation does not affect on the COD removal efficiency, but increase in supplemental carbon source from 500 to 1000 mg/L reduced the

atrazine removal efficiency. There was no adsorption of the compound on to reactor sludge reported during the study.

Advantages of anaerobic treatment of herbicides

- Dehalogenation, dechlorination, and demethylation produces simpler end products
- Biotransformation of complex organic herbicides into biogas through reductive reactions
- Reduced sludge production and biogas recovery
- Highly efficient and minimum operation

2.2.2.4 The sequential anaerobic-aerobic treatment of herbicides

As discussed earlier that the herbicides are refractory aromatic compounds which can cause recalcitrance in the aerobic treatment, and lead to the formation of complex compounds which can be complicated that the parent compound. Under the anaerobic reducing condition, the dehalogenation, dechlorination and demethylation reactions take place, which can break down the herbicides to simpler end products. The end products of anaerobic degradation are organic compounds that include volatile fatty acids, esters, alcohols, etc. as reported in the literature (Chin et al. 2005). Thus post-treatment of such anaerobic effluent in the aerobic reactor would significantly oxidize the organic compounds (Gaunt and Hester 1989; Ratledge 1992; Murphy et al. 2009). A combined anaerobic-aerobic SBR treatment has several advantages over aerobic or anaerobic treatment system, under the ASBR process though removal is slower due to long acclimation period, it can withstand high toxicity, and allows resource recovery.

The aerobic SBR found to be faster in the initial acclimation, the treatment of complex organic compounds like herbicides is difficult due to recalcitrance and thus suitable as a post-treatment step. Several researchers have reported the application of sequential anaerobic-aerobic treatment of various environmental pollutants including azo dyes removal (Manu and Chaudhari 2002; Penha et al. 2005; Frijters et al. 2006),

textile wastewater treatment (Abiri et al. 2017), but there have been no such studies reported for the treatment of herbicides like ametryn, dicamba, and their mixtures.

Simulated textile wastewater was treated using the sequential anaerobic-aerobic system by O'Neill et al. (2000). They have used a lab-scale reactor set up having UASB reactor capacity of 30 L with an HRT of 24 h followed by aerobic reactor with 20 L capacity and HRT of 16 h. The treatment of azo dye C.I. Reactive Red 141 was used in varying concentration from 150 to 759 mg/L along with the starch concentration of 1.9 to 3.8 g/L. The UASB reactor was able to remove 66% of COD for 1.9 and 0.15 g/L of starch and dye concentrations, and the post-treatment was able to remove 14% COD, and thus yielding a total of 80%. After anaerobic treatment, 59% of colour removal was achieved for the influent concentrations of 3.8 and 0.15 g/L of starch and dye concentrations. This removal efficiency was further increased to 18% in the following aerobic reactor, with a total of 77%. Effluent COD values were above the discharge standards, and therefore, a Physico-chemical treatment was required to reduce the COD to the discharge limits.

Kapdan and Oztekin (2006) have used a sequential anaerobic-aerobic batch reactor to treat textile dyestuff containing Remazol Rot RR at varying residence time of 2 – 19 hand with influent COD concentrations of 400 to 1800 mg/L. It was found that anaerobic reactor alone was able to remove 90% of 60 mg/L of dyestuff within 4 – 6 h residence time along with COD removal of 85%. It was reported that the anaerobic reactor COD removal efficiency was limited to 50%, while the aerobic reactor performance increased up to 80% after aerobic step; thus aerobic step acted as post-treatment after colour removal. Abiri et al. (2017) have investigated the treatment of textile wastewater using the sequential anaerobic-aerobic batch reactor for 90 days. The activated sludge was previously fed with dyeing wastewater over 90 days to achieve the bacterial adaptability. Overall colour removal efficiency achieved in anaerobic was about 72% at an optimum duration of 34 h in anaerobic reactor and a further 50% reduction in the aerobic reactor at 22 h.

The combined system consumes less energy, produces less sludge, easy to operate, and are efficient (Von Sperling and Chernicharo 2005). To overcome the

demerits of physicochemical, and other biological treatment methods, the sequential anaerobic-aerobic method was developed to investigate the treatment performance. By combining both anaerobic and aerobic SBR in series, the better performance of the system can be achieved.

Advantages of sequential anaerobic-aerobic batch reactors over anaerobic or aerobic treatment methods

- The higher removal efficiency of organic compounds in the effluent.
- Production of effluent which falls under the effluent discharge limits.
- Formation of intermediates, which can be reduced without further recalcitrance.
- Reduction in the overall treatment time required.
- Lower treatment costs for the treatment of complex organic compounds.
- Easy operation and maintenance of the system enable for filed applications.

2.2.2.5 Factor influence on the anaerobic-aerobic treatment processes

Several parameters and operational conditions can affect the biological treatment processes, mainly pH, temperature, alkalinity, biogas, etc. influence anaerobic reactions (Björnsson et al. 2000). These parameters have to be properly monitored during the conduction of experimentation; this will allow in evaluating the optimum reactor conditions at which maximum degradation can be achieved.

Effect of influent herbicide concentration

Influent herbicide concentration to any of the biological reactor is an important parameter. The bacteria in the bioreactor are sensitive to influent toxic compounds before adaptation. A high concentration of influent herbicide may lead to biomass inhibition, which requires long operation days for reactor recovery and also the addition of fresh activated sludge may be required (Derakhshan et al. 2018). Dicamba acted as a toxic inhibitor on microbial community even at low concentrations and appeared as persistent over 112 days even at low concentrations of 3.5 mg/L (Ghoshdastidar and Tong 2013). Dicamba concentration of 19.7 mg/L was treated up to 77% using aerobic packed bed reactor over 150 days operation. Another

study reported complete mineralization of dicamba to CO_2 and water in anaerobic reducing condition (Milligan and Häggblom 1999). Navaratna et al. (2016) have reported reactor inhibition for raise in influent ametryn concentration from 1 to 2 mg/L. Therefore, low initial herbicides concentration was chosen in this study.

Effect of pH, alkalinity, and temperature

The biological processes mainly depend on the pH of the reactor. Anaerobic reactor pH may vary significantly, 6.8 – 7.7 was found to be the optimum for anaerobic reactions (Pirsaheb et al. 2017). The anaerobic transformation product of organic compounds mainly produces VFA in the absence of molecular oxygen. Further accumulation of VFA in the absence of methanogenic bacteria lead to reduced pH in the anaerobic reactor (Bonakdarpour et al. 2011). The degradation of VFA formed in the anaerobic reactor raises the pH during the sequential aerobic treatment step (Sponza and Isik 2002). Alkalinity indicates the reactor stability, and high alkalinity indicates the toxicity in the reactor while the low alkalinity refers to stable reactor condition. Alkalinity is depended on the anaerobic intermediate compounds formed during the treatment process (Manu and Chaudhari 2002) and further accumulation of high concentration of VFA may also contribute to high alkalinity concentrations (Chin et al. 2005). Addition of sodium bicarbonate to maintain the optimum pH may also contribute to reactor alkalinity. Long chain fatty acids contributed to high VFA concentration, became toxic to the sensitive methanogens and leading to unstable digestion process (Shin et al. 2003). VFA accumulation resulted in unbalanced microbial consortia, which was detrimental in the anaerobic process operation and led to the total system failure (Mohan 2005). Alkalinity indicates the buffering capacity within the reactor and it should not be higher than the required range. Here the alkalinity up to 2000 mg-CaCO3/L may be considered as favourable for the anaerobic condition. Addition of $NaHCO_3$ has contributed to the high VFA and alkalinity of the reactor, which has been reported by Hasan et al (2015).

It was stated that when an organic compound degraded a cation is released which contributes to alkalinity and it also comes from the addition of ammonium and sodium hydroxide on daily feed (Sambusiti et al. 2013). Therefore, it is important to monitor the pH and alkalinity of the reactors to find out the optimum conditions for herbicides removal. Temperature plays a vital role during the biological treatment processes. At mesophilic temperature ranges, the methanogenic activity is found to be higher. Temperature affects significantly on biogas production, high-temperature ranges can produce high biogas.

Effect of oxidation-reduction potential (ORP)

The efficiency of anaerobic biotransformation of herbicides in water can be improved by using redox mediators, which increases the rate of reaction by shuttling electrons from primary electron donors in biological oxidation processes or from bulk electron donors to the electron-accepting organic compounds. An efficient biological reactor should have optimal ORP of -320 mV (Van der Zee and Cervantes 2009). The different type of mediators that are being used in the pollutant removal processes are 1-29 hydrozybenzotriazole (HBT), N-hydroxyphthalimide (HPI), 2,2,6,6-Tetramethyl-1-30 piperidinyloxy (TEMPO), violuric acid (VA), syringaldehyde (SA), vanillin (VA), and 2,2′-31 azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), anthraquinone-2,6-disulphonate (AQS), and other naturally available mediators.

The redox mediator like anthraquinone-2,6-disulphonate (AQS) can accelerate the reaction by lowering the activation energy of a reaction and which more efficient than any other type of redox mediators (Rau et al. 2001). These redox mediators (electron shuttles) are the organic molecules which can be either reduced or oxidized reversibly (Van der Zee and Cervantes 2009). The redox mediators are capable of transferring electron over a wide variety of organic and inorganic compounds. Reduction of redox mediators can be promoted through chemical reactions of anaerobic environments in the presence of reductants like sulfides, cysteine (Curtis and Reinhard 1994).

The reduction of redox mediator can be linked to anaerobic oxidation of organic matter by microorganisms. It has been reported that some of the electron-withdrawing compounds accept the electron from reduced redox mediators, such re-oxidation was observed with azo dyes (Rau et al. 2001) and some polyhalogenated compounds (Kappler and Haderlein 2003). In the presence of redox mediator, the polychlorinated pollutant removal reported 6 fold reduction rates (Cervantes et al. 2004), the enhanced removal efficiency of nitroaromatic pollutants like aniline was observed for AQS amended reactions (Tratnyek et al. 2001). Several redox-mediated treatment processes have received ample of attention for the treatment of a different type of pollutants and impact of the addition of different concentrations of AQS (5 – 20 mg/L) on anaerobic dicamba removal was studied by monitoring the ORP in the reactor.

2.2.3 Application of treatment processes for the removal of herbicides

Milligan and Häggblom (1999) have reported conversion of dicamba to 3,6-dichlorosalicylate under anaerobic O-methylation condition over 80 days of operation. The biotransformation of dicamba was mainly influenced by the presence of electron acceptors. Complete biotransformation of dicamba was achieved in the reactor containing sediment soil previously exposed to dicamba, whereas under methanogenic condition up to 40% reduction was achieved over 80 days of treatment. On continued treatment 3,6-dichlorosalicylate was converted to 6-chlorosalicylate within 110 days, no dehalogenation of 6-chlorosalicylatewas reported in the presence of sulfate-reducing condition. It has been reported that the recalcitrance of 6-chlorosalicylate was dehalogenation to salicylate under the methanogenic condition and it was depended on inoculum and the incubation length. In the presence of high inoculum density has contributed to the increased electron donors, which was indicated by the elevated methanogenesis.

Shawaqfeh (2010) have reported the sequential anaerobic-aerobic batch treatment of pesticide Vydine. The lab-scale rector set up was designed to provide both anaerobic and also combined anaerobic-aerobic sequential treatment facility. The system was operated for 230 days with influent Vydine concentration of 25 - 30 mg/L

under varying HRT. Glucose (1 - 2 g/L) was used as a co-substrate and found that the optimum ratio for Vydine to glucose was 1:75. The Vydine and COD removal efficiency of the system was greater than 95 to 98%. High HRT was found to be effective for Vydine removal. The reactor inhibition was reported during the 30^{th} day of operation due to the formation of short-term toxic intermediates, but after 160 days of operation, the raise in Vydine concentration from 25 – 30 mg/L did not cause the reactor inhibition.

Navaratna et al. (2012) have reported from their study that 1 mg/L of ametryn was removed from a membrane bioreactor (MBR) over 145 days of operation. After addition of ametryn has caused significant changes in the reactor biomass, variation in COD removal efficiency of the reactor until 2 weeks, and further high COD removal efficiency was achieved (>95%). Ametryn acted as a nutrient source, which has consumed by the bacteria. Maximum removal was achieved over long hydraulic retention time (HRT), and the removal was accounted for the amount of ametryn adsorbed on to the reactor sludge, filtered on to the fouled membrane surface and biodegradation. MBR was then expanded with UV disinfection and a granular GAC unit to enhance the ametryn removal efficiency. This combined biochemical process seems efficient, but the cost of treatment may be high.

Sandoval-Carrasco et al. (2013) have developed a packed bio-barrier reactor using soil collected from different crop fields as a bacterial source to treat mixer of 2,4-d and ametryn herbicides with an initial concentration of 31.5 mg/L. About 35 - 90% of 2,4-d and 80-90% of ametryn was removed over 50 days. They have identified the bacterial strains which can biodegrade the formulation of the herbicide, including Gesapax-H, 2,4-d, ametryn, and cyanuric acid. The bacterial isolates Chryseobacterium, Variovorax, Aeromonas and Xanthobacter, were able to grow the herbicides mixtures. Use of such bacterial consortium is effective in the treatment of wastewater from a non-point source containing a mixture of herbicides. This study showed that the treatment of a mixture of different herbicide formulations is efficient if the isolated bacterial strain is previously exposed to the herbicide toxicity. The applicability of this method may be limited to lab-scale study.

Navaratna et al. (2016) have reported the 65% removal of 1 – 2 mg/L of ametryn using a hybrid MBR associated with Ultra-Violet (UV) disinfection system and a GAC unit during 214 days of the treatment period. The maximum removal of ametryn at a lower initial concentration of 1.31 mg/L and higher removal (61%) at a higher initial concentration of 5.34 mg/L was reported due to the different nature of biodegradation of ametryn under the distinct microbial composition of MBR sludge. Adsorption on to reactor sludge was not considered as a removal mechanism. Up to 46% of ametryn can be biodegraded in the anoxic reactor at 15.6 h HRT while the MBR required 24 h HRT for the same. The long term operation of MBR revealed that the ametryn adsorption on to biomass is negligible.

2.3 SUMMARY THE OF LITERATURE REVIEW

Agriculture runoff water contains a different type of organic and other toxic pesticides. The release could cause different physiological, chemical, and biological problems in the receiving ecosystem. Therefore they have to be removed to the discharge standards prescribed by the regulating authorities. Various Physico-chemical and biological treatment process are used to remove the pesticides/herbicides and organic compounds from water. However, the combination of the aforementioned method is suggested, and combined sequential anaerobic-aerobic type of biological treatment methods is considered as effective to meet the effluent discharge standards.

- Physico-chemical treatment methods are found to be inefficient in the herbicide removal, and their removal using advanced oxidation processes are efficient but are costlier.
- Biological treatment processes are economical and have several advantages over Physico-chemical methods to remove herbicides from water.
- Herbicide removal using pure cultures of algae, bacteria, and fungi are limited to a particular type of herbicides and maintenance of pure form of strains in large scale is difficult, and hence it is limited in the actual field applications. However, mixed bacterial cultures are considered to be efficient for herbicide removal.

- Herbicide biodegradation under anaerobic conditions is a simpler and non-specific process which involves the reduction of halogen bonds between the benzene ring and further reduction of the benzene ring. Some of the herbicides are completely biodegraded under anaerobic conditions through biotransformation, but it is uncertain for some herbicides. Transformation products sometimes accumulate during higher influent load of herbicides, which may be biodegraded over long operation period after proper reactor acclimation. Some transformation products are resistant to anaerobic reduction, which may be further oxidized under aerobic conditions. Major transformation products obtained under anaerobic biotransformation of herbicides are long-chain fatty acids, 3,6-dichlorosalicylate, and 6-chlorosalicylate for dicamba, and VFA, cyanuric acid for ametryn. These compounds may be further biodegraded through the β-oxidation pathway and further oxidized to CO_2 via the tricarboxylic acid cycle.
- Combined anaerobic-aerobic treatment of herbicides, wherein the anaerobic pre-treatment (dehalogenation and dechlorination) followed by the aerobic treatment may mineralize the anaerobic metabolites seems to be a promising technique for the treatment of agricultures and industrial effluents.
- The herbicides removal efficiency using combined biological treatment systems can be enhanced by the addition of external redox mediators like anthraquinone-2,6-disulphonate, under anaerobic conditions the redox mediator accelerates the electron transfer between the substrates and herbicides by shuttling the electrons, which can enhance the anaerobic biotransformation in the reactor.

2.4. LITERATURE GAP

- Only a few studies have reported the biological treatment of ametryn and dicamba.
- Sequential anaerobic-aerobic treatment of ametryn and dicamba have not been reported in the literature
- The existing biological methods are limited to treat low concentrations of ametryn (2 mg/L) and dicamba (31.5 mg/L), but these compounds are detected

at high concentrations (ametryn = 3.4 mg/L and dicamba = 93.7 mg/L) for which the sequential anaerobic-aerobic treatment can be conducted.

- Use of redox mediators like AQS is not reported during the herbicide treatment in biological treatment methods, which likely enhances the treatment process.
- Chemical treatment processes produce excess sludge and sometimes uneconomical for treating a large quantity of wastewater, and therefore, sequential anaerobic-aerobic treatment can be used as an effective alternative.
- Treatment of herbicides mixture has not been studied using sequential anaerobic-aerobic treatment.
- Biodegradation of mixture of herbicides is of potentially important and is of a real-time challenge to Environmental Engineers; hence, this novel and cost-effective treatment method will have to be developed.

CHAPTER 3

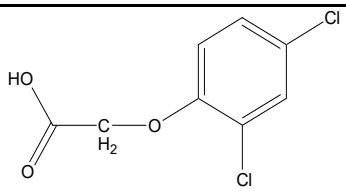
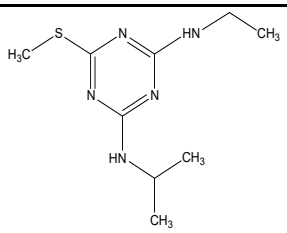
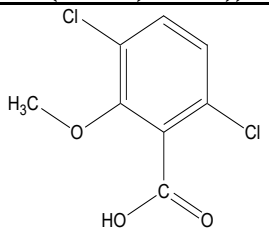
MATERIALS AND METHODS

In this chapter, the various materials used and the detailed experimental methodology adopted are discussed.

3.1 MATERIALS

The herbicides used in this study are 2,4-d, ametryn, and dicamba. The Physico-chemical properties of these selected herbicides are listed in Table 3.1. The instruments and chemicals used in this study are tabulated in Table 3.2.

Table 3.1: Physical and chemical properties of herbicides

Properties	2,4 –d ($C_8H_6Cl_2O_3$)	Ametryn ($C_9H_{17}N_5S$)	Dicamba ($C_6H_2Cl_2(OCH_3)CO_2H$))
Structure			
Synonym	2,4-Dichlorophenyloxy acetic acid	(2-ethylamino)-4-(isopropylamino)-6-(methylthio)-s-triazine	3,6-dichloro-2-methoxybenzoic acid
Molecular weight	221 g/mol	227 g/mol	221 g/mol
Solubility in water (mg/L)	890 at 20^oC	209 at 25^oC	4500 at 25^oC
Melting point	140.5^oC	85^oC	115^oC
Boiling point	160^oC	337^oC	200^oC

Source: Sangami and Manu 2017a.

The anaerobic seed sludge was collected from the outlet of UASB reactor of sewage treatment plant (STP) of Mangaluru Municipal Corporation, located at Kavoor, Mangaluru, India and seed sludge for the aerobic reactor was collected from the primary settling tank of STP located in NITK campus, Surathkal, India. The seed biomass was processed by passing through 250μm sieve to get uniform solids and characterized for MLVSS and MLSS.

Table 3.2: The instruments and chemicals used in this study

	Particulars	Manufacturer
Instruments	Gas Chromatography – High-Resolution Mass spectrometry (GC-HRMS)	GC – Agilent, MS – Jeol
	Liquid Chromatography-Mass Spectrometry (LC-MS)	Shimadzu
	High Performance Liquid Chromatography (HPLC)	Agilent
	UV-VIS double beam Spectrophotometer	Systronics
	pH and ORP meter	Hanna
	High-Speed Centrifuge	Remi
Chemicals	2,4-d, ametryn, dicamba, methyl-tert-butyl ether	Sigma-Aldrich
	Starch and sodium hydrogen carbonate	Himedia
	Potassium hydroxide (99% purity), potassium dichromate, silver sulfate, mercuric sulfate, potassium iodide, sodium thiosulphate, ferrous ammonium sulfate, HPLC grade methanol, and ultra-pure water	Merck

3.2 METHODOLOGY

3.2.1 Characterization of standard herbicides solutions

Spectral characterization of the standard herbicide solution was carried using UV-VIS spectrophotometer. The maximum wavelength (λ_{max}) of the respective standard herbicide solution was found to be 224, 230, and 274nm for ametryn, 2,4-d, and dicamba, respectively. The standard calibration curve was developed for different concentration of herbicide. These calibration curves are used to determine herbicide concentration in the influent and effluent during the treatment process.

3.2.2 Reactor set up and operation

The treatment process was carried out using a sequential batch reactor in anaerobic followed by an aerobic reactor, as showed in the flow diagram in Figure 3.1. The reactors were operated manually with operating cycle include processes like feeding (10 min), reaction (23 h), settling (30 min), and decanting (20 min) (Chin et al. 2005). Initially, the anaerobic reactors were operated with feed water containing 2 g/L of starch as carbon source and 4 g/L of $NaHCO_3$ as a buffer; aerobic reactors were fed with effluent from the anaerobic reactors as feed.

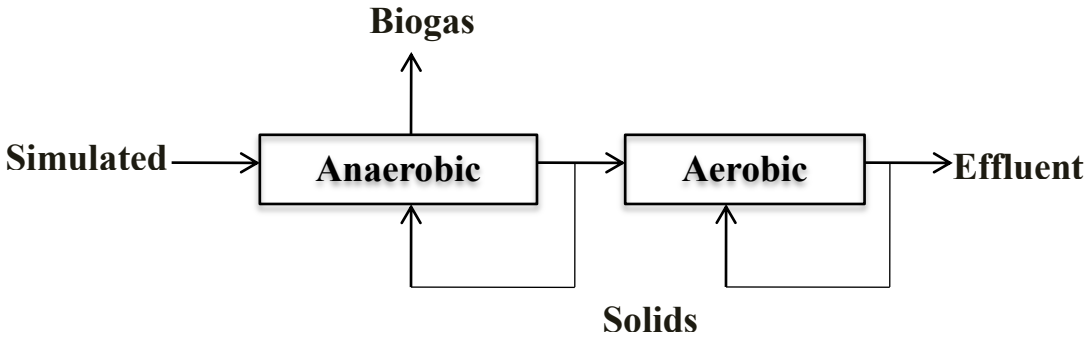
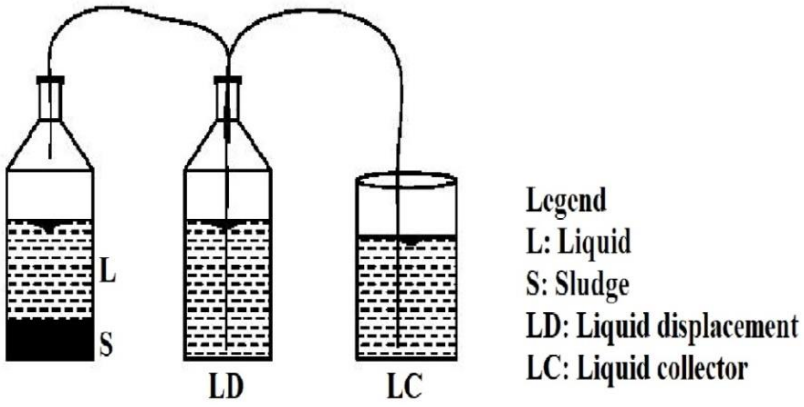
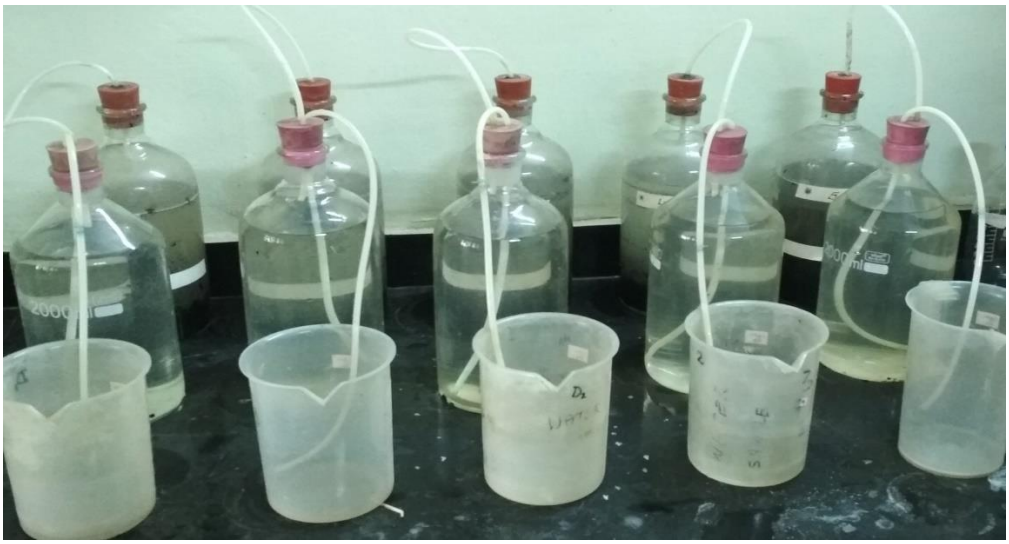


Figure 3.1: Flow diagram of sequential anaerobic-aerobic treatment process

The anaerobic laboratory-scale reactors were made using 2 L capacity glass containers (Figure 3.2). One litre of seed sludge was inoculated with 9 g/L of MLVSS (sludge characteristics: MLSS = 76 g/L, MLVSS = 36 g/L) to each anaerobic reactor, (i.e., 250 mL of anaerobic sludge was diluted with 750 mL of water) and 1 L of simulated feed water containing 2g/L starch was added to maintain the total volume of 2 L.



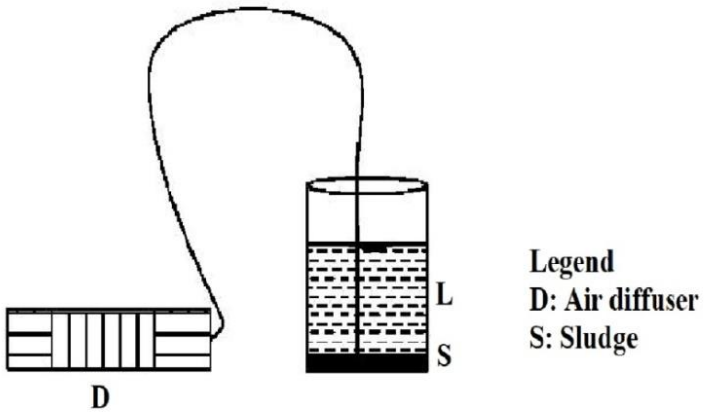
(a)



(b)

Figure 3.2(a-b): Schematic diagram and Laboratory scale set up of the anaerobic reactors

The aerobic laboratory-scale reactor models were prepared by using 2 L capacity plastic beakers (Figure 3.3). One litre of seed sludge was inoculated to each aerobic reactor with 2500 mg/L of MLVSS (Sludge characteristics: MLSS = 6.3 g/L, MLVSS = 4.5 g/L), (i.e., 560 mL of aerobic sludge was diluted with 440 mL of water). And 1 L of tap water was added to maintain the total volume of 2 L on the first day and, the aerobic reactors were aerated using air diffusers.



(a)

(b)

Figure 3.3(a-b): Schematic diagram and Laboratory scale set up of aerobic sequential batch reactors

Starch was prepared by dissolving 10 g of starch powder in 250 ml of hot water. The composition of trace metal solution was prepared as per the protocols (Prakash and Gupta 2000; Manu and Chaudhari 2002). The trace metal solution include in g/L; $COCl_2.6H_2O$:1.613, $FeSO_4$:8.39, $MgSO_4.7H_2O$:5, H_3BO_3:0.1, $ZnCl_2$:0.0473, $CuSO_4.5H_2O$:0.0782, $NiSO_4.6_2O$:1.698, $(NH_4)_6MO_7O_{24}.4H_2O$:0.54, $CaCl_2$:7.776, $MnCl_2.4H_2O$:7.863.

The feed to anaerobic reactors was prepared using stock solution by diluting to the required concentration, with the addition of 4 g/L of $NaHCO_3$, 2 mL/L of trace metal solution and stock herbicide solution to give required herbicide concentration in 1 L volumetric flask containing water. The anaerobic reactors were operated at ambient temperature; 1 L of supernatant was decanted and fed with 1 L of fresh feed. The reactors were decanted manually by transferring 1 L of supernatant liquid to a beaker and capped instantly after feeding the fresh solution to avoid oxygen entry in to the reactor. The aerobic reactors were operated simultaneously by feeding 0.5 L of anaerobic effluent after 24 h by decanting 0.5 L supernatant. Influent and effluent characteristics of feed water and effluent water were analyzed for pH, temperature, ORP, alkalinity, COD, and herbicide removal efficiency.

The reactors were operated for several days till the quasi-steady condition was achieved. After achieving the quasi-steady-state condition, simulated water containing 25 mg/L of ametryn, dicamba and 2,4-d was fed to the respective reactors during phase – I (Table 3.3), and different concentrations of ametryn, dicamba, mixtures of 2,4-d with ametryn, and mixtures of 2,4-d, ametryn, dicamba was fed to the respective anaerobic reactors during phase – II and III (Table 3.4).

Table 3.3: Reactors treating the different herbicides during the phase - I

Reactor No.	Herbicide treated
An1	Anaerobic control (reactor with no herbicide)
An2	Ametryn treatment reactor
An3	Dicamba treatment reactor
An4	2,4-d treatment reactor
A1	Aerobic control (reactor treating An1 effluent)
A2	Reactor treating An2 effluent
A3	Reactor treating An3 effluent
A4	Reactor treating An4 effluent

Table 3.4: Reactors treating the different herbicides during the phase – II & III

Reactor No.	Herbicide treated
R1	Anaerobic control (reactor with no herbicide)
R2	Ametryn treatment reactor
R3	Dicamba treatment reactor
R4	2,4-d and ametryn treatment reactor
R5	2,4-d, ametryn and dicamba treatment reactor
A1	Aerobic control (reactor treating R1 effluent)
A2	Reactor treating R2 effluent
A3	Reactor treating R3 effluent
A4	Reactor treating R4 effluent
A5	Reactor treating R5 effluent

All the anaerobic reactors were capped after each feeding and connected to a liquid displacement system to record the biogas production. The methane gas production was recorded at every 2 h interval; the liquid contains 5% KOH. The methane gas production was recorded for a period of 12 h, and the supernatant from the anaerobic reactor was decanted after 24 h. This procedure was continued for 3 – 5 consecutive days. The performance of R2 and R3 reactors were evaluated by

determining herbicide removal, COD removal efficiency and biogas production rate while the reactors R1, R4 and R5 were evaluated by determining COD removal and biogas production rate.

The removal efficiency of herbicide and COD was calculated using the following equation,

$$(\eta) = (C_{in} - C_f) / C_{in} * 100; \quad (1)$$

where,

(η) : Removal efficiency (%).

C_{in} : concentration of herbicide or COD in the influent feed water (mg/L).

C_f : concentration of herbicide or COD in the effluent of reactors (mg/L).

The solids retention time in the reactors was calculated using the equation below,

$$SRT = \frac{VtXTc}{VwXw24} \quad (2)$$

where, SRT is the solids retention time (days); Vt is the total reactor volume (L); X is the MLSS in the reactor (mg/L); Tc is the total operating cycle (h); Vw is the volume of MLSS wasted (L); and Xw is the MLSS wasted (mg/L).

3.3. Analytical methods

3.3.1. Determination of herbicide and transformation products

The liquid samples were prepared for HPLC analysis as per the protocol developed by Sangami and Manu (2017a). Sample preparation for herbicides was conducted separately for specific herbicide of concern. Samples were centrifuged using at 4000 rpm for 8 minutes and filtered in 0.2 µm filter paper. Thus prepared samples were analyzed in HPLC for determining herbicides using the method developed as tabulated in Table 3.5. And the determination of a mixture of herbicides in HPLC was performed but due to the limitations like low detection limits <0.05 mg/L, no specific λ_{max} value and hence the formation of several intensity peaks, which yielded improper concentrations.

Table.3.5: HPLC method developed for the different herbicides

Herbicide	Flow rate (mL/min)	Mobile phase ratio (Methanol: water)	Wavelength (nm)	Retention time (min)
Ametryn	1.1	50:50	224	12.9
Dicamba	1	50:50	274	1.2
2,4-d	0.6	70:30	230	9.5
Temperature: 26^{o}C; sample volume:10 µL;				

The sample extraction to detect transformation product using GC-HRMS was carried as per method 1699 (USEPA 2007). Samples were analysed in GC-HRMS to detect the biotransformation products using the standard GC – HRMS method: column type: capillary; column class: standard non-polar; active phase: RTX-1; column length: 60m; carrier gas: He; column diameter: 0.22 mm; phase thickness: 0.25µm; data type: linear RI; program type: Ramp; start temp: 60^{o}C; end temp: 230^{o}C; heat rate: 10 K/min; end time: 35 min.

3.3.1.1 Sludge characterization for determining the herbicide adsorption

The sludge from herbicide treating reactors was characterized to determine the herbicide adsorption o the sludge as per the method adopted by Weaver et al. (2004). The sludge was resuspended using 20 mL 100% methanol, and the sample (covered with an aluminium foil) was allowed to mix in a mechanical shaker (at 150 rpm) for 20 h. Then centrifuged (6000 g) and filtered using a 0.2 µm filter and then herbicide concentration was measured using HPLC. Mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS) concentrations were measured as per the standard methods (APHA 2005).

3.3.2. Water quality parameters

The various parameters, like alkalinity and chemical oxygen demand (COD) by closed reflux titrimetric method), were measured as per the protocols of standard methods (APHA 2005). pH and ORP values were measured in the reactors using the portable digital meter (edge®, Hanna Instruments, India). Dissolved oxygen (DO) in the aerobic reactors was measured using DO meter (HI 9741). Volatile fatty acid

(VFA) concentration was measured using the standard operating methods (Baxter 2014) and the components of VFA were measured in gas chromatography (GC, Thermo Scientific) fitted with flame ionization detector (FID) using the method developed (Mkhize et al. 2014). The protocol includes sample preparation as follows: All the samples were diluted 10 times using deionized water. 10 mL sample was vortexed along with 10 mL of methyl-tert-butyl ether (MTBE) for approximately 10 min. The supernatant organic phase was transferred quantitatively to a dry beaker and dried using anhydrous magnesium sulphate. The extract was filtered, and 1 µL was injected into the GC.

GC method includes: Carrier gas – Helium; Flow rate – 1 mL/min; initial oven temperature – 35^{o}C and rose to 240^{o}C at 10^{o}C/min, Auxiliary temperature – 230^{o}C, run time – 28 min.

3.3. Herbicide toxicity studies of anaerobic reactors

The methanogenic activity study was conducted following the protocol (Isa et al. 1993). The reactors were tested for maximum initial concentrations of 25 mg/L of ametryn and dicamba separately, with starch as the carbon source and sodium bicarbonate ($NaHCO_3$) as a buffering agent. The constant herbicide dose of 25 mg/L was selected in the preliminary study (Phase – I) to check the shock loading impact on the biomass. The reactors were capped after each feed and connected to a gas-liquid displacement system to record the gas production rate. The anaerobic reactors performance was monitored mainly based on biogas production, and it is one of the most important indicators, which represent the capability of anaerobic condition (Chen et al. 2015).

The displacement of 5% potassium hydroxide (KOH) solution from the liquid bottle was collected and measured as methane gas. The methane yield was measured every 2 h for 12 hours. The feeding, decanting, and gas recording procedure was followed for three consecutive days. The maximum slope obtained on the graph of methane yield (quantity) versus time indicates the methanogenic activity of the sludge (kg-CH_4 – COD/kg.VSS/d).

CHAPTER 4

RESULTS AND DISCUSSION

The agriculture runoff water contains herbicides associated with different halogens, which cause water pollution of surface and groundwater bodies. Several methodologies have been suggested for the removal of herbicides from water, but each one has advantages and limitations. The biological methods are considered to be cost-effective and environmentally sustainable alternatives for the treatment of wastewater (He 2006), but the conventional methods are inefficient for removing the toxic chemical like pesticides (Meric et al. 2003). Biological treatment methods, including aerobic SBR methods, are not efficient enough to remove herbicides, due to the formation of recalcitrant by-products. Herbicides contain electron-deficient bond with substituent groups, and hence under reducing condition, these bonds easily broke and forms demethylated and dechlorinated compounds (Suflita et al. 1982; Taraban et al. 1993; Weinberg and Teodosiu 2012). However, anaerobic degradation produces intermediate compounds and may require aerobic conditions for their complete degradation.

The herbicides and their biotransformation products (TPs) are toxic to anaerobic biomass at a certain level; therefore due consideration of herbicides and their TPs toxicity is to be accounted for the proper functioning of anaerobic reactors. Various biological systems, pure cultures (fungi and bacteria) and mixed culture treatment techniques have been adopted to treat different type of herbicides. However, the use of pure cultures proved better treatment efficiency of herbicides, but they have limited to the confined environment. Mixed culture treatment methods are suitable for treating the herbicides with synergetic effects of bacteria. Sequential batch reactor (SBR), particularly of aerobic type treatment, was started long back in the year 1914 for treatment of domestic and industrial wastewaters. SBR technology is widely used in recently to remove different xenobiotic compounds and herbicides from wastewaters (Mohan et al. 2005; Chin et al. 2005). SBR technology is developed on the basic scientific assumption that periodic exposure of the microorganisms to defined process conditions and is effectively achieved in a fed batch system wherein

exposure time, frequency of exposure and amplitude of the respective concentration can be set independently of any inflow condition (Wilderer et al. 2001). The xenobiotic compounds are stable, have low water solubility and degradability due to lack of toxic pressure generation. For improving the biodegradation of such herbicides sufficient toxic pressure must be exerted on the biomass to induce the enzymatic modifications, this can be achieved in SBR (Singh 2004). The development of anaerobic sequential batch reactors (ASBR) seems to be well suited for herbicides treatment than aerobic sequential batch reactors (aerobic SBR) treatment alone. In ASBR and aerobic SBR, it is possible to maintain high solids retention time (SRT) which is required for the degradation of recalcitrance compounds like herbicides. Use of an anaerobic reactor is important for VFA production, due to higher substrate–cell surface interaction and higher microorganism activity (Sentürk et al. 2010). Therefore, the combination of anaerobic-aerobic system is thought to produce effluent with minimum organic matter with high system performance.

4.1 Sequential anaerobic-aerobic treatment of herbicides

The anaerobic sequential batch reactor (ASBR) has been used in the treatment of complex organic compounds, including dyes, pesticides, and herbicides. Compared to other types of anaerobic biological systems like anaerobic membrane bioreactor, anaerobic biofilm reactor and anaerobic fluidized bed/baffled bioreactor the anaerobic SBR can retain high biomass concentrations and can handle the high organic load (Chin et al. 2005; Khan et al. 2011). ASBR can be operated at lower costs than other anaerobic processes, liquid and solid separation takes place within a reactor, no additional supporting media required for the biomass attachment, no operating issues like clogging, and continuous power supply. Therefore, the use of ASBR in the treatment of wastewater is becoming an attractive method (Chin et al. 2005). SBR is a traditional method used to treat different type of domestic and industrial effluents. The aerobic sequential batch reactor has been used to treat different type of phenoxy herbicides (Mangat and Elefsiniotis 1999). Herbicides were treated first in ASBR followed by the aerobic reactor to improve the treatment efficiency. The mechanism of treatment involves dehalogenation, dechlorination, and demethylation under reducing conditions and then followed by oxidation of anaerobic intermediates.

The results of this study are sub-divided into 3 phases. Phase – I deals with a short term study of 60 days, including the reactors stabilization and for herbicide shock loading of 25 mg/L. Phase – II deals with the stabilization of anaerobic and aerobic reactors for the influent containing 2 g/L of starch and herbicide concentration of 0.1 mg/L. In phase – III studies, herbicide removal with increased influent concentrations was undertaken in sequential anaerobic-aerobic reactor system in the presence and absence of redox mediator.

4.2 Phase – I: Short term study

A short term study was conducted to evaluate the impact of herbicide shock load on the anaerobic-aerobic batch reactor system. Before the introduction of herbicides, the reactors were stabilized; three anaerobic and three aerobic reactors were operated for more than 28 days with similar feed and draw-off processes, while the bacterial biomass acclimatized to the controlled environment. The aerobic reactors attained quasi-steady-state condition after 14 days, showing constant COD removal efficiency of 82% for 3 consecutive days. The anaerobic reactors took 26 days to attain steady-state conditions. From the day 28 onwards the anaerobic and aerobic reactors were fed with the simulated water containing herbicides, with a constant OLR of 0.2025 kg-COD/m^3/d (2 g-starch/L and 25 mg-herbicide/L). The reactor HRT was maintained constant for 48 h throughout the treatment in the anaerobic-aerobic system. Reactors liquid temperature was between 27 and 32.1^oC during the treatment. Each run was carried out daily by decanting 1 L of supernatant and feeding with fresh influent.

The aerobic reactors A1, A2, A3, and A4, were operated using the effluents from An1, An2, An3, and An4 reactors respectively, as feed. The OLR was observed between 0.02 and 0.038 kg-COD/m^3/d. 0.5 L of supernatant was decanted from the aerobic reactors before each fresh feed. Details of influent quality are given in Table 4.1.

Table 4.1: Influent characteristics to the reactors

Parameter / Reactor	pH	Alkalinity (mg-$CaCO_3$/L)	COD (mg/L)	ORP (mV)	Temperature (^oC)
Anaerobic	7.5 – 8.2	1900 – 2100	1700 – 2100	10 – 20	25 – 27.5
Aerobic	5.6 – 7.3	1800 – 2400	160 – 1200	-105 to -275	28 – 31.5

4.2.1 Experiments in the control reactors (An1 and A1)

The control reactor performance was monitored by measuring COD removal efficiency and biogas production, as shown in Figure 4.1. Effluent parameters, including ORP, alkalinity, pH, and temperature, were monitored throughout the study period. Reactor pH was always between 6.6 and 7.7, which is within the acceptable range for a methanogenic reactor (Ross 1992). The alkalinity ranged from 1,900 to 2,600 mg-$CaCO_3$/L, the higher concentrations may be attributed to the addition of $NaHCO_3$, accumulated organic matter and VFA.

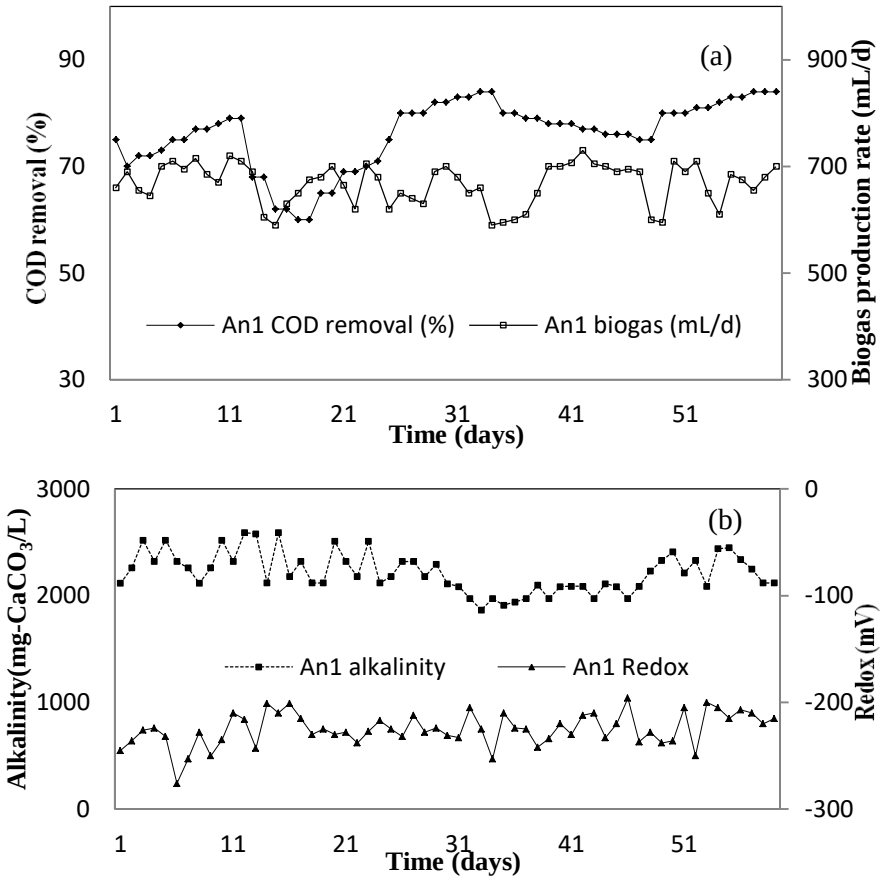


Figure 4.1(a-b) Performance of An1 reactor

It was stated that when an organic compound degraded a cation is released which contributes to alkalinity and it also comes from the addition of ammonium and sodium hydroxide on daily feed (Sambusiti et al. 2013). COD removal exceeded 90% after 5 days of operation but fell to some extent (to 60%), which has caused by the presence of sodium salts and sulphides in the feed, which could inhibit methanogen activity. The issue was overcome by adding a 2 ml/L solution of trace metals to create favourable conditions for and strengthen the methanogens (Manu and Chaudhari 2002). After this, the COD removal efficiency consistently exceeded 80%.

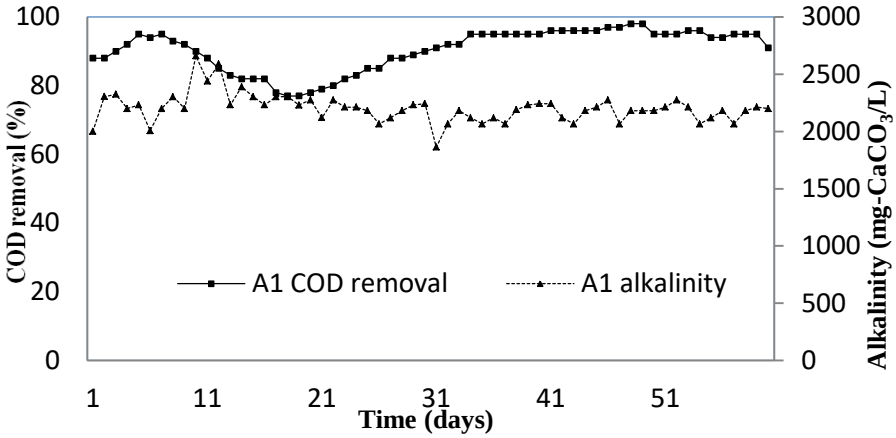


Figure 4.2: Performance of A1 reactor

The performance of A1 and its effluent alkalinity are shown in Figure 4.2. The reactor's ORP was between approximately -250 and -300 mV, the level required for anaerobic reactions occurring under reducing conditions (Van der Zee and Cervantes 2009). The 24 h average methane gas production in the control reactor (An1) was in the range 300 to 350 mL/d, and the total gas yield (including methane and other biogas components) averaged 550 to 710 mL/d. Gas production variation is directly proportional to reactor temperature, higher temperatures (32.1^{o}C) favouring anaerobic degradation processes. Treatment of the anaerobic effluent in the aerobic reactor (A1) improved the total COD removal efficiencies to more than 95%, perhaps by oxidation of volatile fatty acids to water and CO_2 through the β-oxidation pathway (Gaunt and Hester 1989).

4.2.2 Experiments in the An2 reactor

The reduction in ametryn and COD concentrations in An2 and the gas production rate are shown in Figure 4.3(a-b). The maximum ametryn removal efficiency achieved was 22% on day 54. Acute initial toxicity was indicated by the reduced reactor performance when the data are compared with the control. The toxicity was overcome after day 45, with increased COD and ametryn removal, and higher biogas production. The maximum ametryn removal obtained due to biotransformation, indicated by the HPLC report. The spectrophotometer wavelength scan reported lower absorbance intensity at 223 nm (Sandoval-Carrasco et al. 2013). The GC-HRMS analysis confirmed the formation of transformation products (TPs) such as esters and fatty acids. The An2 pH was between 6.9 and 7.5, which is favourable for methanogenesis. Reactor temperature in An2 was higher than in control (An1) at between 30.3 to 31.3^{o}C. At the higher temperatures, higher ORP between -250 and -280 mV was recorded, indicating that the reactor was performing better, which was confirmed by its higher gas yield and better COD removal efficiency.

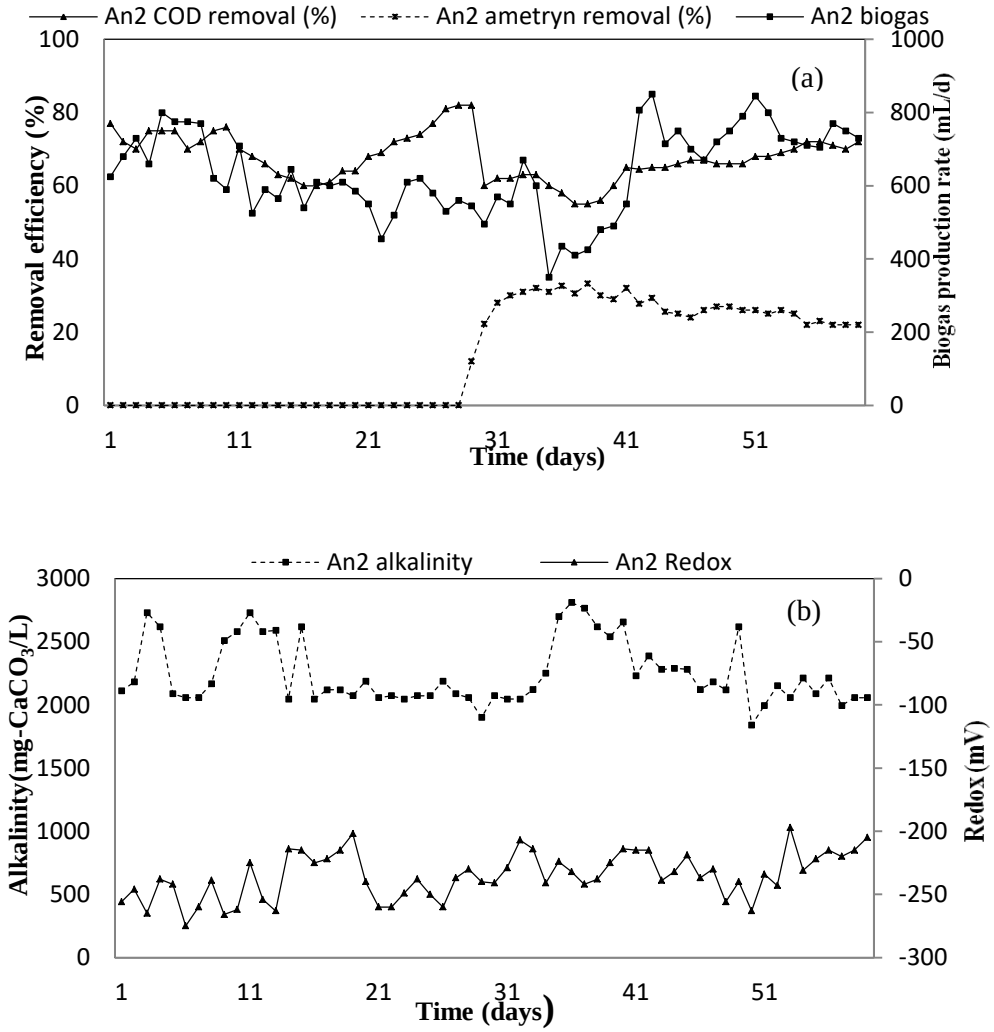


Figure 4.3(a-b): Performance of anaerobic reactor treating ametryn (An2)

The high effluent alkalinity obtained for low COD removal and methane yield between days 37 and 41 indicates slightly toxic condition arising from the formation of VFA, but no other toxic inhibitions were reported. The low proportional removal of COD may also indicate undegraded organic compounds in the effluent (González-Cuna et al. 2016).

Ametryn adsorption onto the reactor sludge was investigated because the solids were retained throughout the study. The sludge had adsorbed around 35 mg/mg-MLVSS of herbicide on the day 40. No further adsorption was found as the process continued, however, possibly due to the high pKa value of ametryn (Navaratna et al. 2016). The 80% COD removal efficiency and high methane yields

(14%) than the control suggest that the methanogens adapted suitably and were important in the treatment process.

4.2.3 Sequential anaerobic-aerobic treatment of ametryn (A2)

In A2, 72% ametryn removal efficiency was achieved between days 55 and 58, with COD removal efficiency of 86% (Figure 4.4). Comparison with the 92% COD removal in the control reactor indicates that a portion of intermediate organic compounds was not digested by the aerobic bacteria initially. After an initial lag, the aerobic reactor performance improved and became stable, with constant COD removal efficiency. The reactor sludge contained no trace of ametryn. The HPLC and UV spectra reports for the effluent indicate extensive degradation of ametryn metabolites. The GC-HRMS analysis showed that the metabolites formed during anaerobic treatment were oxidised to their end products in the aerobic phase (Mahesh and Manu 2019a).

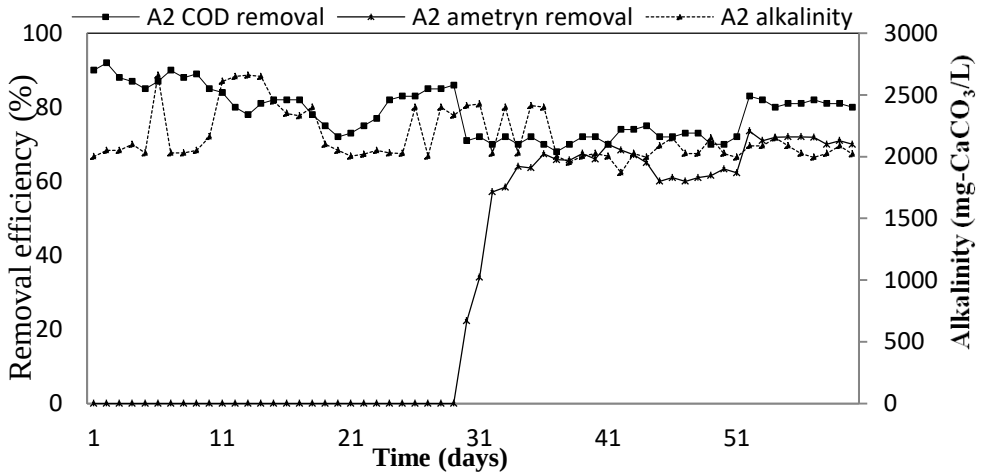


Figure 4.4: Performance of aerobic reactor treating ametryn (A2)

The anaerobic TPs (different fatty acids) contain more carbon atoms than functional groups (halogens and alkyls), which can be oxidized to CO_2 and water through the tricarboxylic acid cycle (Ratledge 1992). Oxidative reduction of fatty acids through β-oxidation produces acetyl-Coenzyme A by successive loss of C_2 units. Though the treatment in the anaerobic reactor limited due to biomass inhibitions, the sequential aerobic treatment was found to be effective in reducing the ametryn as well

as COD. Thus sequential anaerobic-aerobic treatment of ametryn is more efficient than the individual treatment.

4.2.4 Experiments in An3 reactor

The maximum dicamba and COD removal efficiencies observed, 58 and 72%, were achieved on day 56, along with high biogas production showed in Figure 4.5(a-b). The pH in An3 was between 6.8 and 7.8, and the temperature between 30.3 and 32.1^{o}C. In practice, the stability parameters remained within the required ranges, like those in the control reactor.

Dicamba degradation in the reactor was monitored using HPLC; the changed peak sets in the chromatogram indicating dicamba TP formation. The high COD removal efficiency and methane yield exceeding that in the control reactor by 12 to 14% suggest that dicamba was processed predominantly by the methanogenic bacteria. The maximum dicamba removal efficiency may arise from the formation of more oleic acid groups as TPs, and possibly the degradation and adsorption of oleic acid onto the sludge leading to high CH_4 yields (Pereira et al. 2002). No dicamba was adsorbed onto the reactor sludge, so there were no related peaks in the chromatogram, probably because of the compound's high water solubility (4,500 mg/L) and its low soil sorption capacity (Magga et al. 2008).

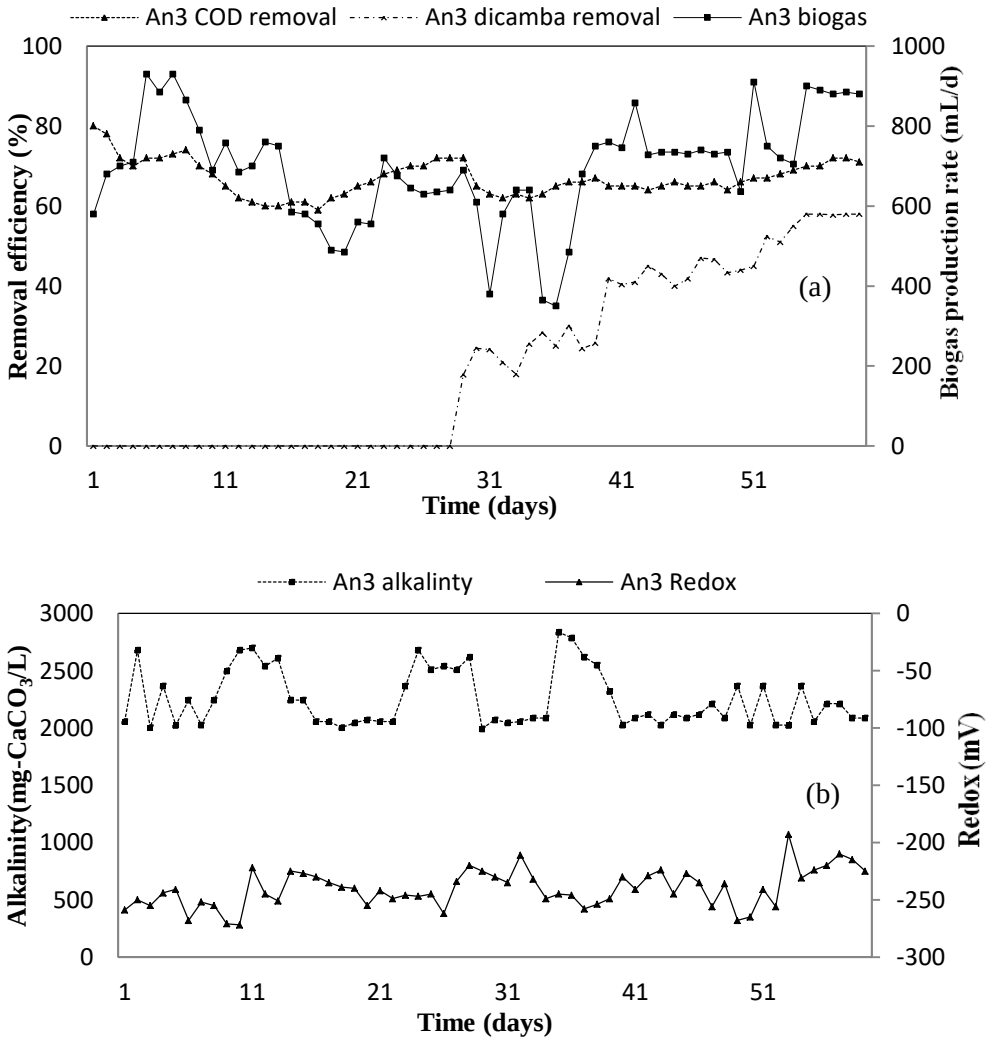


Figure 4.5(a-b): Performance of anaerobic reactor treating dicamba (An3)

4.2.5 Sequential anaerobic-aerobic treatment of dicamba in A3

The effluent from An3 was treated further in A3 reactor to remove dicamba TPs. A3's low COD removal efficiency compared to A1 indicates that these TPs are recalcitrant to aerobic treatment initially. Up to 78% of the dicamba TP were removed, with 85% COD removal showed in Figure 4.6. The increased COD removal efficiency in the later stages may be attributed to the 48 h HRT, as HRT is important in the biological treatment and high HRT provide greater treatment efficiencies (Wang et al. 2014). The anaerobic effluent contained high concentrations of oleic acid, whose mineralization to water and CO_2, would have lowered the COD

concentration in A3 effluent. Continued operation with similar treatment conditions might have also contributed to higher removal efficiencies due to gradual adaptation and development of anaerobic biomass over operating periods.

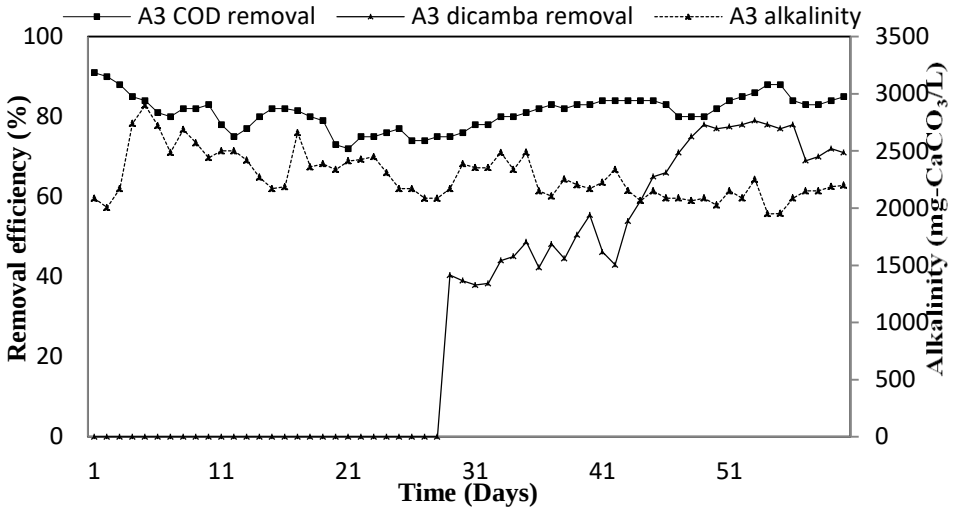


Figure 4.6: Performance of aerobic reactor treating dicamba (A3)

A previous study reported that over 100 days of long operation period was required to achieve maximum removal of phenoxy acetic acid herbicide (Chin et al. 2005). In this present study, the sequential anaerobic-aerobic system could able to remove dicamba within a short time of 30 days without inhibitions (Mahesh and Manu 2019a). The UV spectra obtained for the influent, and An3 and A3 effluents, in the trial reported here showed up to 78% removal of dicamba.

4.2.6 Experiments in An4 reactor

The comparative analysis of COD removal efficiency in control and experimental along with 2,4-d degradation efficiency are depicted in the Figure 4.7(a-b). Methane gas production in the An4 was observed to be greater than 14 – 18% of the An1. It can be observed that there was a drop in COD removal efficiency and total gas yield corresponding to reduced MLVSS (<8500 mg/L) concentration after the herbicide introduction. It may be due to toxic inhibition on the reactor biomass due to accumulation of VFA >1000 mg/L in the effluent (Liu et al. 2018).

After 8 days of lag phase, MLVSS concentration recovered due to increased biological activity. Increased CH_4 gas yield and COD reduction may be due to

increased biomass concentration in the reactor over the time. It was observed that 100% herbicide biotransformation was achieved on 18th day of treatment with COD removal of 75%. Chin et al. (2005) have reported 65% COD removal for complete removal of 130 mg/L of 2,4-d at 48 h HRT using glucose as carbon source. VFA in the An1 was observed to be <800 mg/L, whereas the An2 contained >1000 mg/L, high COD levels in the An2 may be due to VFA (Aramrueang et al. 2016). HPLC obtained for the influent and effluent samples indicate the formation of intermediates of 2,4-d in the effluent sample with the appearance of different peaks.

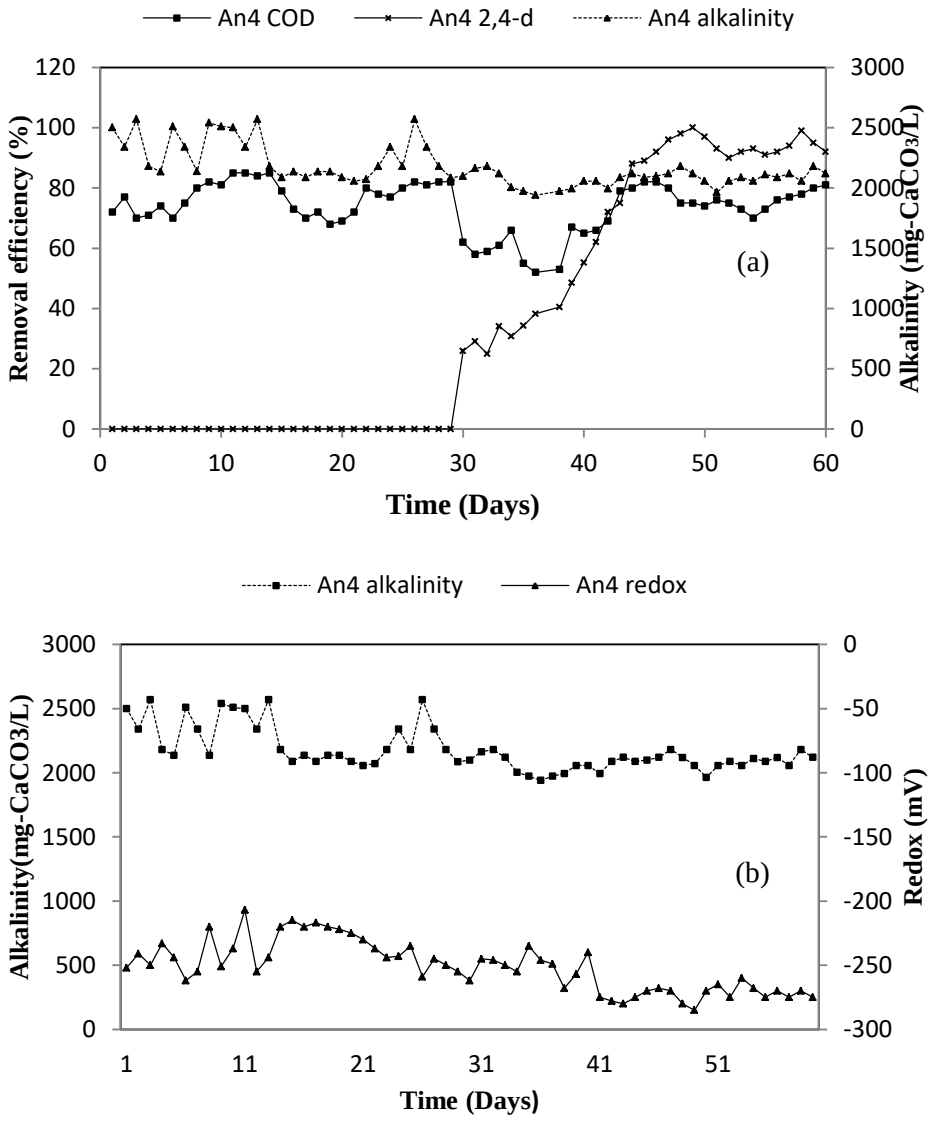


Figure 4.7(a-b): Performance of anaerobic reactor treating 2,4-d (An4)

Then the samples were analysed in the LC-MS to track the metabolites and some of the major transformation products are identified as esters and different fatty acid groups. HPLC results obtained for liquid extracted from sludge samples did not show intensity peak, indicate 2,4-d was not adsorbed on to the reactor sludge. pH and alkalinity varied fairly stable during the initial period of operation (before introducing 2,4-d) and are at required level for an anaerobic digestion process. After introducing 2,4-d to An2, the pH was reduced to 6.2 and the alkalinity reported >2900 mg-$CaCO_3$/L. pH was then increased and was in between 6.5 to 7.3, which was considered as suitable for anaerobic methanogenic digestion process (Pirsaheb et al. 2018). Low pH <6.5 – 6.2, observed during the initial days of operation may be due to the acedogenic condition of the reactor.

The anaerobic reactors mainly buffered to maintain the required pH level. Ambient temperature was recorded regularly and was reported between 28 ± 0.5 to 31 ± 0.5^{o}C and ORP was observed in the range of -250 to -300 mV. The digestion process is considered to be highly efficient at high temperature and low ORP ranges. ORP indicates type of reaction mechanism with in a reactor. Negative ORP value indicates the reducing reactions taking place in the reactor. It was reported that the ORP of an efficient anaerobic biological reactor should have ORP of -320 mV (Van der Zee and Cervantes 2009). As the negative ORP indicates reductive biochemical activity in anaerobic reactor supported by the added substrates, ORP of An2 was observed to be much lower than the An1. Lower ORP than the control may suggest 2,4-d being acted as electron acceptor, starch as electron donor and thus transformed to fatty acids under reducing reactions (Van der Zee and Cervantes 2009).

4.2.7 Sequential anaerobic-aerobic treatment of 2,4-d in A4

Post treatment of anaerobic effluents in the subsequent aerobic reactor was conducted to enhance the system efficiency; the performance A1 and A2 is shown in the Figure 4.8. Complete degradation 2,4-d metabolites within the 12 days of operation was observed, which is supported by the maximum COD removal efficiency (>99 %). The degradation pattern followed similar trend as observed during treatment before 2,4-d introduction.

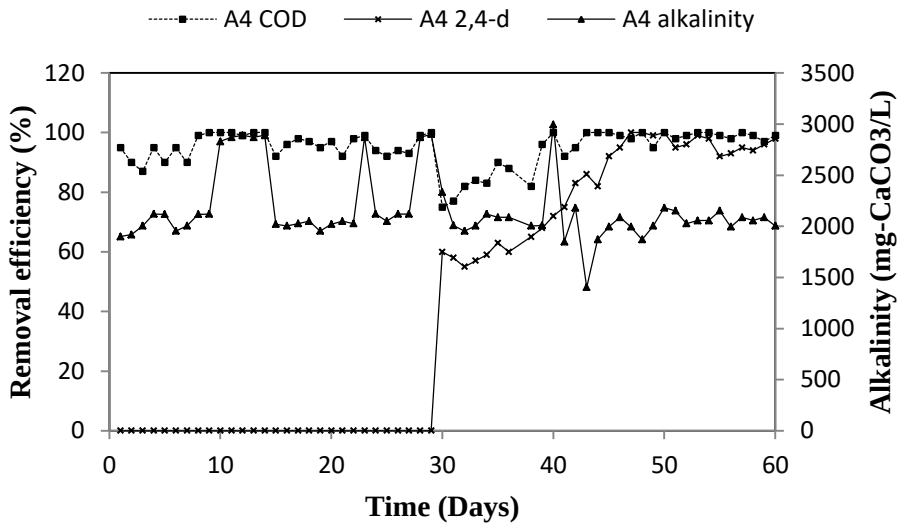


Figure 4.8: Performance of aerobic reactor treating 2,4-d (A4)

Celis et al. (2008), have reported that aerobic reactor removed 500 mg/L of 2,4-d at 48 h HRT during 185 days of operation. In this study aerobic reactor acted as a polishing step, which completely mineralised the 2,4-d transformation products within 10 days at 24 h HRT. No VFA compounds have been detected in the aerobic effluent was observed in GC analysis. HPLC report obtained for the aerobic effluent shows no formation of intensity peaks at retention time of 9.5 min, which supports the disappearance of compound. The DO in the reactor was observed to be 2 – 3.5 mg/L and at this level the maximum COD removal was observed. Maximum COD removal may indicate the degradation of organic compounds in the reactor (Liu et al. 2018). Alkalinity was found to be <1900 mg-$CaCO_3$/L indicates a favourable condition for the aerobic reaction. pH in the reactor was observed in the range of 7.5 – 8.3, which was similar to the influent water.

4.3 Phase – II: Long term treatment of herbicides

The preliminary study conducted during 60 days of short term period showed the scope for long term operation, as biological treatment processes require long time for the acclimation to xenobiotic compounds so as to develop specific metabolic pathway (Baghapour et al. 2013; Derakhshan et al. 2018). Hence, it was assumed that long term operation of reactors would effectively contribute to the herbicide removal due to the development of specific metabolic pathway for herbicide degradation.

4.3.1 Start-up and reactor stabilization

Navaratna et al. (2016) have reported that longer SRT is required to remove herbicides in water. Since the older cells with reduced nutrient supply have proved better removal efficiency and hence the reactors like SBR may help in the degradation of herbicides (Khan et al. 2011). Therefore, sequential batch reactors are specifically suitable for the degradation of herbicides which are recalcitrance, as in these reactors higher HRT and SRT can be maintained simultaneously, and thereby efficient COD and herbicides can be achieved. Some of the studies have been conducted in batch reactors at the controlled temperature (28 – 31^{o}C) and very few reports are available on the treatment of actual/simulated agriculture wastewater containing different type of herbicides. Also the complex agriculture wastewater containing herbicides and various other agrochemicals and salts is not taken into account.

It is known that the degraded under reducing environments, but very few studies have monitored the oxidation-reduction potential (ORP) in the reactors. Mostly, the anaerobic reactors in the field are operated under sub-mesophilic temperature (25 – 31^{o}C). Therefore, this study aimed at studying the herbicides removal phenomena by monitoring various parameters such as ORP, pH, alkalinity, herbicides removal and COD removal at the sub-mesophilic temperatures (25 – 31^{o}C) with simulated agriculture wastewater containing herbicides. In view of the above, the experiments were conducted at an HRT of 24 h. Five anaerobic sequential batch reactors were started with similar influent (contain no herbicide) and the performance

was evaluated by monitoring COD removal. The influent feed contained 2 g/L of starch and 4 g/L sodium bicarbonate, and pH in the range of 7.5 – 8.3.

During the start-up period phase, for about 14 days high COD removal efficiency >80% was observed, due to the adsorption of starch on to biomass leading to high COD removal in the effluent. Adsorption of starch on to the reactor biomass is in agreement with several literatures reported and hydrolysis of starch followed surface limited adsorption reaction kinetics and suggested that starch adsorption occurred on the biological floc before it was hydrolyzed (Mino et al. 1995). The anaerobic biomass property to adsorb substrate is utilized in the contact stabilization activated sludge process (Tchobanoglous et al. 2003). This observation was also supported by low biogas production due to lower conversion of starch to methane and CO_2. From then onwards for period of up to 39 days, COD removal decrease gradually till 73%. From 40^{th} day onwards, the COD removal efficiencies started to increase may be due to starch adsorption was completed, and biomass was acclimated to the influent substrate (starch), growth of biomass and hence starch degradation yielding methane and CO_2.

COD removal was observed to be similar and almost constant in all the reactors from 45 to 48 days, and hence it was assumed that constant anaerobic biological activity was taking place in all the reactors (Mahesh and Manu 2019b; 2019c). COD removal efficiency varied from 70 – 80% from day 25 to 48. Quasi steady-state condition indicated by variation of COD removal efficiency up to 10% for 5 consecutive feedings (Polprasert and Haas 1995; Yeruva et al. 2015) have reported that aerobic SBR and anoxic SBR have shown 95 and 92% COD reduction, respectively (out of 3000 mg/L) while the reactor acclimation is achieved. Anaerobic SBR acclimation can take more time than that of aerobic reactor (Speece 1996). The anaerobic reactor acclimatization may take 25 days and even more depending on the feed characteristics and other environmental parameters (Khorsandi et al. 2018). Therefore it seemed that quasi steady-state condition has been achieved in all the bioreactors and hence from the day 48 onwards the herbicide simulated water was fed. One reactor was fed with simulated water (no herbicide) and other four reactors were fed with simulated water containing different herbicides. The anaerobic batch reactors

were then operated for about 200 days. pH in the reactors was found to be constant between 6.6 – 7.7 and it was in the acceptable range for a methanogenic reactor (Ross 1992). Effluent alkalinity ranged from 1900 – 2600 mg-$CaCO_3$/L, and the increased alkalinity may be due to conversion of sulphite to sulphide in anaerobic reactor (McCartney and Oleszkiewicz 1991). The effluent pH was in the range of 6.6 – 7.7, volatile fatty acid (VFA) level in the reactors effluent was in the range 400 – 600 mg/L suggest that better reactor performance. For better reactor performance the VFA should be <1000 mg/L (Stronach et al. 1986). High alkalinity in the effluent up to 2400 compared influent alkalinity of 1900 mg-$CaCO_3$/L was observed.

ORP in the reactor was reported in the range of -250 to -280 mV, indicate reducing condition of the reactors. An ORP value below -250 mV is required for a better anaerobic reactor performance as reported previously (Manu and Chaudhari 2003; Van der Zee and Cervantes 2009). The observed ORP in the control reactor (R1) was found to be optimum for the degradation of influent starch. The 24 h average methane gas production in the control reactor was found to be in the range of 100 – 250 mL/d and the total gas averaged at 450 – 550 mL/d and methane gas production observed in this study is equivalent to 350 mL/day (48 h) per gram of COD removed reported (Isa et al. 1993) The methane gas production depends on the composition and biodegradability of organic carbon source and also the rate of methane gas production depends on population of microorganisms and their growth conditions. The average COD removal during last four consecutive feedings was ~ 1500 mg/L. Hence, theoretically the methane production should be 450 – 600 mL/day for 48 h, and the methane gas obtained practically was in agreement with the expected yield. The variation in gas production is in directly proportionate to the reactor temperature. Higher temperature (30^oC) in the reactor was observed to be favourable for anaerobic degradation process, with greater adaptability of anaerobes at high temperature ranges 25 – 35^oC (Zaiat et al. 2001).

Aerobic sequential batch reactors (SBR) operation was started simultaneously from day 2 as a post treatment step to the ASBR effluent. Five aerobic batch reactors were started with anaerobic effluent as feed and the performance was evaluated by monitoring COD removal. The influent feed contained COD in the range of 410 to

730 mg/L, and pH in the range of 6.4 – 7.7. The effluent showed high pH in the range of 7.5 – 8.5, high alkalinity of 200 – 350 mg-$CaCO_3$/L than the influent, VFA in the range of 250 – 300 mg/L. The dissolved oxygen in the reactor was maintained in the range of 3 – 4 mg/L, as sufficient DO is required for the oxidation of anaerobic metabolites. The COD removal in the reactor was observed to be >85%, this would indicate the oxidation of VFA to CO_2, and end products (Mahesh and Manu 2019b). This condition was observed till the day 10 during the start-up, and thereafter the COD removal efficiency increased up to 98% between 12 to 18 days and the quasi steady-state condition was thus confirmed on day 14. The steady state conditions of the reactors vary with respect to different operating and influent conditions, it may be achieved in some hours and up to 25 days (Khorsandi et al. 2018). Reduction in the VFA to below 250 mg/L with high COD removal efficiency of >98% was achieved over long operation period.

4.4 Phase – III: Treatment of herbicides

After achieving the reactors acclimation, the actual treatment process was carried out with different influent herbicide concentrations. The herbicides treated separately are ametryn and dicamba from 48 to 430 days and mixture of ametryn with 2,4-d, and mixture of ametryn, 2,4-d and dicamba are treated up to 400 days. The treatment process was carried out in separate reactors and the results are discussed in separately by considering the particular type of herbicide and details about the number of operation days with respect to experimental conditions are tabulated in the respective section.

4.4.1 Treatment of ametryn in ASBR

Ametryn was treated in the anaerobic and aerobic batch reactors R2 and A2 respectively, and the control being R1. The influent concentration of ametryn was increased from 4 to 10 mg/L over 430 days of continued operation. The experimental conditions studied during the course of treatment are tabulated in the Table 4.2.

Table 4.2: Operational conditions maintained during the sequential anaerobic-aerobic treatment of ametryn

Sl. No	Reactor operation (Days)	Experimental condition studied
1	0 – 48	Reactor start-up and acclimation using 2 g/L starch and 0.1 mg/L ametryn, (OLR = 0.21 – 0.215 kg-COD/m^3/d)
2	2 onwards	Anaerobic effluent fed to corresponding aerobic reactor
2	49 – 97	Influent ametryn concentration = 4 mg/L
3	98 – 150	Influent ametryn concentration = 6 mg/L
4	151 – 284	Influent ametryn concentration = 8 mg/L
5	249 – 341	Addition of AQS = 5 mg/L
6	285 – 430	Influent ametryn concentration = 10 mg/L
7	342 – 430	Addition of AQS = 10 mg/L

After successful acclimation of anaerobic reactors, the actual treatment process was carried out in the R2 reactor at 24 h HRT, 0.21 - 0.215 kg-COD/m^3/d of OLR and at ambient reactor liquid temperature of 28.5 – 31.4^oC. Performance of R1 and R2 reactors during the treatment period of 430 days with influent ametryn concentration of 4 to 10 mg/L is depicted in the Figure 4.9(a-d). Influent ametryn concentration was increased after observing the maximum removal (100%) of previous influent dose, considering the COD and other reactor components are at constant. Ametryn removal efficiency on the day 49 was observed to be 20.5% with a drop of COD removal efficiency from 80 to 45%, while the biogas production equal to that of R1 reactor. Sudden decrease in the COD removal efficiency may be attributed to ametryn addition being appeared as a temporary shock to anaerobic biomass. Reactor did not recovered even after 30 days for high atrazine treatment study (Derakhshan et al. 2018). The higher VFA in the range of 700 – 950 mg/L, and alkalinity of 1950 – 2400 mg-$CaCO_3$/L, may indicate the toxicity condition in the reactor. The ametryn removal efficiency was increased gradually from 38 – 99% on

day 79. Initial biotransformation was indicated by the formation of different intensity peaks in the HPLC.

Complete reduction of ametryn was observed within 50 days of operation, whereas membrane bioreactor (MBR) operated for about 214 days was able remove up to 65% of 1 – 2 mg/L of ametryn (Navaratna et al. 2016). Dehalogenation, dechlorination and demethylation reactions under reducing conditions supported the dissociation of ametryn to its primary metabolites, which further reduced to simple end products. The ametryn adsorption on reactor sludge was monitored with high priority throughout the study period, and it is discussed further. COD removal was also observed to be greater than 60 and maximum of 81% was achieved on day 95, total gas production was greater than the control by around 270 mL/d. Higher total gas production was an indication of ametryn being converted to its TPs, and ultimately to nitrogen, hydrogen and carbon dioxide gases (Sene et al. 2010). High effluent COD (400 – 480 mg/L) in the R2 indicate incomplete degradation of transformation products of ametryn and starch, this was reported similarly during 2,4-d removal (Celis et al. 2008). Anaerobic sludge granulation after 83 days of ametryn introduction may indicate active bacterial development due to the conversion of ametryn to nitrogen source (Cook and Huetter 1981).

The influent ametryn concentration was raised to 6 mg/L, the degradation pattern and reactor performance can be observed from 98 – 150th day onwards. Reduced COD removal may be attributed to formation of high concentrations of VFA up to 1300 mg/L due to increased ametryn loading, which might have become non-degradable in the ASBR, however the VFA in the R1 reactor remained between 300 – 550 mg/L. Formation of long chain fatty acids contributed to high VFA concentration, it has appeared toxic to the sensitive methanogens and leading to unstable digestion process (Shin et al. 2003). Another reason could be the appearance of high concentrations of ammonia nitrogen (60 – 75 mg/L) compared to <20 mg/L of R1 reactor. These observations are reported in the case of atrazine and s-triazine treatment, but none of the studies have reported such observations for ametryn.

As described in the cyanuric acid degradation pathway, production of nitrogen with increased ametryn concentration and excessive nitrogen formation might be

another reason for toxicity of methanogens in the sludge. Alkalinity observed was in the range of 1700 – 2400 mg-$CaCO_3$/L, and low biogas production up to 645 mL/day till 130^{th} day. From 132^{nd} day onwards the reactor recovered, indicated by reduced ammonia nitrogen (20 – 35 mg/L), alkalinity (<1200 mg-$CaCO_3$/L, high biogas production (>730 mL/day). The reactor biomass recovered due to the biotransformation N-alkyl groups of ametryn at low initial concentrations, and over long operation periods, these observations are in line with their study involving atrazine removal (Derakhshan et al. 2018). Further, complete mineralization was achieved from day 147 with high biogas production up to 800 mL/d, and COD removal efficiency >85% was observed in the R2 reactor, whereas R1 was able to produce biogas of 590 mL/d and COD removal efficiency of 77% (Mahesh and Manu 2019c). Variation in biogas production and COD removal efficiency of both R1 and R2 may evidence the enhanced biotransformation and followed by mineralization of ametryn up to 6 mg/L.

Ametryn biodegradation studies using fungal and bacterial isolates have reported incomplete removal efficiencies <15% and mainly due to the biotransformation to its metabolites (Szewczyk et al. 2018). However the complete removal in the present study is mainly due to the anaerobic reducing reactions, stable bacterial adaptability, co-metabolism and utilization of ametryn as carbon/nutrient source. The complete removal of ametryn indicated by the disappearance of intensity peak in HPLC chromatogram, and similar observations was reported by Sánchez-Sánchez et al. (2013). The characterization of influent, anaerobic and aerobic effluent was analysed in spectrophotometer, which indicated the complete transformation of the ametryn and the UV spectra is shown in the Figure S1.

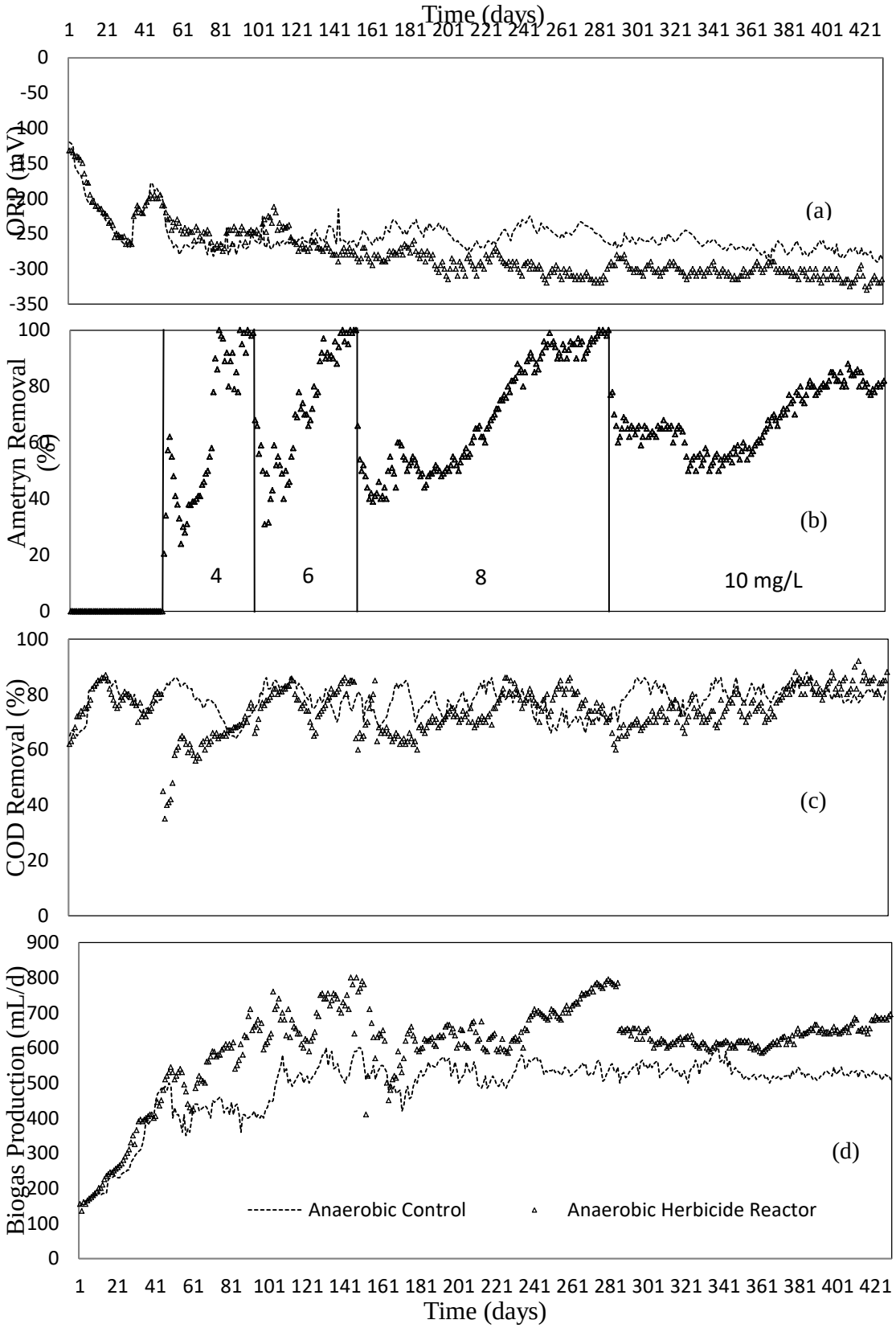


Figure 4.9(a-d): Variation of performance parameters during the anaerobic treatment of ametryn (R2) compared with anaerobic control (R1)

The maximum ametryn removal is supported by high total gas production up to 710 mL/d than 410 mL/d of control reactor and sludge granulation. Granules formation may be due to the presence of N-alkyl groups of ametryn might have served as electron acceptors during the anaerobic processes and helps for the rapid growth of the bacteria (Gibson and Harwood 2002). Anaerobic sludge granulation during this period may be the significant indicator of active biomass growth (Mahesh and Manu 2019c). The sludge of anaerobic control and experimental reactor are depicted in the Figure 4.10.

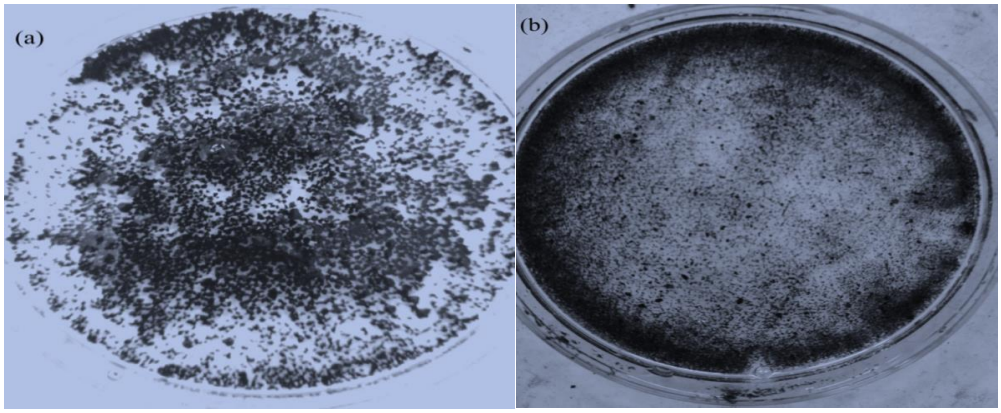


Figure 4.10: Anaerobic sludge of (a) R2 and (b) R1 reactors

Influent ametryn concentration of 8 mg/L was fed from 151st day, the removal efficiency dropped to 40 from 99% (6 mg/L). About 90% of ametryn was removed till the day 245, and the COD removal efficiency observed was in the range of 77 – 80%, total gas production in the range of 690 – 720 mL/d and the ORP observed was in the range of -290 to -300 mV. After the introduction of 8 mg/L ametryn the R2 reactor performance reduced significantly. Previously, complete biodegradation of 4 – 6 mg/L of ametryn was achieved in 48 to 50 days, whereas during treatment of 8 mg/L more than 90 days were required to achieve 90% removal efficiency. The reasons attributed to slower rector recovery are the high concentration of ametryn leading to reactor instability due to biomass inhibition. Biomass can withstand the toxic load up to certain limit (saturation limit), and it can cause toxicity after that limit as there will be high concentrations of intermediate compound formation. Formation of large quantity of intermediates accumulates and often leads to cause biomass inhibition till the biomass get acquainted to existing reactor toxicity level.

Thus, it can be said that the high influent concentration developed high concentration of intermediates which lead to instability and further recovered over the continued operation. The ametryn removal during this stage was limited up to 90% and then to improve the reactor performance, about 5 mg/L of AQS was added with the influent from 249th day onwards. The addition of AQS could able to enhance the reactor performance indicated by reduced ORP in the range of -300 to -320 mV. High reactor performance was observed for lower ORP values, which is indicated by enhanced redox reactions after AQS addition. Ametryn removal was observed >99%, biogas production was >780 mL/d, COD removal >80%, this observation indicated the effective biodegradation of 8 mg/L of ametryn.

The HPLC reports obtained for influent and effluent of R2 are showed in the Figure S2, the influent having absorbance peak at 12.9 min, effluent obtained on the day 200 and 280 have the reduced and no peak corresponding to the same retention time. The gradual reduction and further complete disappearance of intensity peak imply that the compound is being degraded over continued operation. These observations are in line with the previous studies (Sandoval-Carrasco et al. 2013). The UV-spectra for the influent and anaerobic effluent obtained during different treatment stages (<70%) and (>98%) showed reduction in the ametryn compound (refer Figure S1). Similar wavelength scan for samples was performed in spectrophotometer resulted in the reduction of absorbance intensity for intensity at the 223 nm (Sandoval-Carrasco et al. 2013).

Influent ametryn concentration was raised to 10 mg/L from 284th day onwards after achieving the complete biotransformation of 8 mg/L. AQS concentration of 5 mg/L was added since the beginning to maintain the existing ORP level. At this stage, the ametryn removal efficiency reduced to 77% and remains stable at around 65%. Later reduced to 50% gradually over 342 days of operation, with COD removal efficiency of 75 – 78%, biogas production of 600 – 630 mL/d. Influent AQS was raised to 10 mg/L from 342nd day, and observed a lower ORP of -300 to -320 mV, and the maximum removal observed during over long operation period of 126 days was 85% with COD removal of 85 – 90% and biogas production of 670 mL/d. It has been reported that high influent herbicide concentration to the bioreactors creates a greater

chance of herbicide exposure for bacterial metabolism (Baghapour et al. 2013). But it was not observed in the anoxic MBR reactor, it was able to remove only about 46% of ametryn. The assumption was seems to be failed during MBR study, indicated by poor bacterial adoptability and possible recalcitrance (Navaratna et al. 2016).

Reduced granules size after the introduction of 8 mg/L of ametryn with reduced MLVSS in the reactor and inhibition of sludge indicated by poor sludge quality. This unstable condition is in par agreement with the initial days of ametryn introduction. Though the compound is being treated from the past 150 days with a stable bacterial adoptability, the reactor took more than 70 days to recover and to function effectively. It may indicate that the biomass has reached its maximum tolerable limit for the ametryn. Addition of AQS supported the reactor recovery alongside the long operation period, which has contributed to the bacterial adoptability, growth of inactive bacteria, and such observations have been reported previously by many researchers for treating different type of herbicides.

The increased ametryn concentration (10 mg/L) caused reduction in anaerobic biomass initially, but recovered faster than the previous stage. It may be observed that even after long operation period of more than 125 days, the removal of ametryn remained at 85% in the R2 reactor. The biotransformation followed by biodegradation was observed similarly over long operation period as obtained previously. The wavelength scan for samples was performed in spectrophotometer resulted in the reduction of absorbance intensity for intensity at the 223 nm (Sandoval-Carrasco et al. 2013). The effluent sample was analysed in LC-MS and major TPs identified are indicated in the MS report (Figure S3). Some of them are 2-Nitro-1-propanol (105), 2-Chloro-N-ethylacetamide (121), Ethyl 3-isothiocyanatopropionate (159), 4-Nitrobenzoic acid, 3-chloroprop-2-enyl ester (141), methyl ester (269), Dichloroacetamide (323), Trichlamide (339), Benzoylprop-ethyl (365), and pentadecyl ester (433). The TPs identified are having more number of carbon atoms, which were oxidised in aerobic reactor. Effluent obtained during the maximum removal efficiency has indicated presence of oleic acid, cyanuric acid and biuret, which were further transformed nitrogen and CO_2 (Sene et al. 2010).

4.4.1.1 Biodegradation of ametryn and pathway proposal

The major intermediate compounds produced were identified using GC-HRMS and the biodegradation pathway is proposed as shown in the Figure 4.11. The degradation pathway derived revealed the formation of intermediate compounds like n-ethyl-6-(methylsulfanyl)-1,3,5-triazin-2,4-diamin, deisopropylhydroxyatrazine, 2,4-dihydroxy-6-(N'-ethyl)amino-1,3,5-triazine, hydroxyatrazine, and n-isopropylammelide.

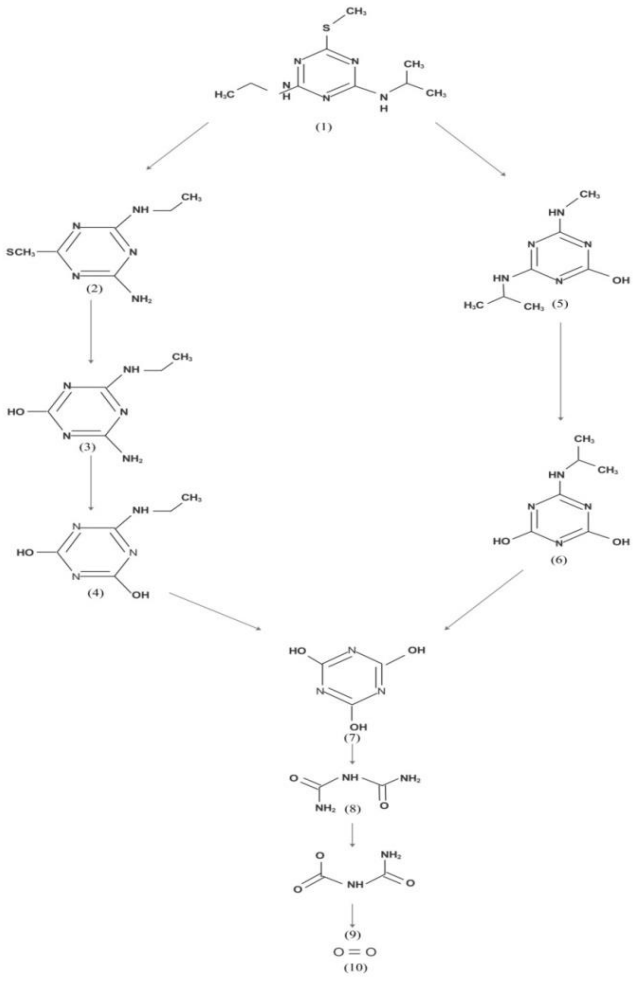


Figure 4.11: Proposal of ametryn biodegradation pathway

The compounds identified from the biodegradation pathway shown in Figure 4.11 are: (1) – ametryn, (2) - n-ethyl-6-(methylsulfanyl)-1,3,5-triazin-2,4-diamin, (3) – deisopropylhydroxyatrazine, (4) – 2,4-dihydroxy-6-(N'-ethyl)amino-1,3,5-triazine, (5) – hydroxyatrazine, (6) – n- isopropylammelide, (7) – cyanuric acid, (8) – biuret,

(9) – allophanate, (10) – CO_2. These compounds further undergo enzymatic reactions and can produce CO_2 through cyanurc acid pathway. The appearance of cyanuric acid and biuret in the MS analysis support these observations (Figure S4).

Intermediate compounds proposed are in agreement with the compounds produced during the biodegradation of s-triazine (Cook and Huetter 1981) and atrazine (Derakhshan et al. 2018). Szewczyk et al. (2018), claims the formation of 2-hydroxy atrazine, ethyl hydroxylated ametryn, s-demethylated ametryn, and deethylametryn, and Bhaskar et al. (2019), have reported 2 ‐ acetamido ‐ 4 (isopropylamino) ‐ 6 ‐ (methylthio) ‐ s triazine) and 2 ‐ amino ‐ 4 (ethylamino) ‐ 6 ‐ (methylthio) ‐ s triazine as the intermediate compounds during biodegradation of ametryn. These intermediate compounds are of great concern to environment due to their toxic risks, which need to be removed completely (Velisek et al. 2017). However, in the present study the major TPs identified were cyanuric acid, biuret, and long chain fatty acids including 9-Octadecenal, and oleic acid (Mahesh and Manu 2019c). Formation of long chain fatty acids were mainly the anaerobic fermentation products of starch, whereas cyanuric acid and biuret can be considered as TPs of ametryn. The degradation of cyanuric acid degraded to biuret, ammonia nitrogen, CO_2, and further contributed to the methanogenesis process (Cook et al. 1985; Sene et al. 2010). Therefore, successive reduction of ametryn to different type of intermediate compounds and finally to carbon/nitrogen source, which has contributed to high methane gas production.

The biogas production was measured during the treatment period, before and after the herbicide introduction in both the reactors. Methane gas production was measured using the 5% KOH solution displaced from the gas liquid displacement system (on regular intervals of 10 days). Methane gas production in the R2 reactor was higher than the R1 by 280 – 350 mL/d (i.e., 35 – 41% v/v). This may indicate rich nutrient condition prevailed over continued operation due to conversion of ametryn to nitrogen or carbon source leading to enhanced methanogenesis in R2 (Cook and Huetter 1981). Biogas production varied with respect to ametryn and COD removal efficiencies, this is also in correlation with variation in MLVSS.

4.4.1.2 Anaerobic sludge characterization: MLVSS and ametryn adsorption studies

Ametryn variation in MLVSS concentration between the R1 and R2 reactor over the course of treatment is tabulated in Table 4.3. Ametryn adsorption on to the reactor sludge was considered with high priority because the solids have been retained throughout the study period. Around 2.3 mg/g.MLVSS of ametryn was adsorbed on to reactor sludge till the day 70 and later that, there was no adsorption detected. This was mainly due to ametryn desorption from the sludge, biotransformation and also due to the high dissociation constant (pKa) value of ametryn (Frías et al. 2004). Ametryn was adsorbed initially during the present study and no further adsorption was detected over the long operation due to desorption, similar findings are reported by Navaratna et al. (2016). MLVSS was varying significantly from day 49 to 80 for about 6.4 to 9.2 g/L, it clearly indicates that there was a slight toxicity inhibited by the herbicide. Increase in the influent ametryn concentration caused toxicity on the biomass as expected, which has led to the deterioration of granules and loss of biomass due to poor sludge quality. Biomass regenerated over continued operation and MLVSS was found to be > 9.6 g/L, which was greater than the MLVSS concentration of R1 reactor (by 0.3 – 0.4 g/L).

The sludge stabilization ratio (MLVSS/MLSS) was observed in the range of 0.67 to 0.82 in the R2, and it was in the range of 0.67 – 0.72 in the R1 reactor. Increase in MLVSS/MLSS ratio up to 0.82 in the R2 reactor may indicate significant reduction in SRT, and further, reduced MLVSS/MLSS ratio of 0.67 – 0.77 contributed to high SRT (Derakhshan et al. 2018). The SRT and MLVSS/MLSS ratios are inversely proportional to each other, and impact of SRT has been reported previously by Metcalf and Eddy (1991). In our previous study, the impact of SRT on dicamba removal was studied, wherein long operation period contributed to high SRT in the reactor (Mahesh and Manu 2019b). There was a low SRT (35 – 50 days), during the first 10 – 20 days of ametryn introduction indicates poor sludge quality due to slight toxicity. High SRT was observed during the long treatment period, long SRT of 100 – 150 days during the acclimatization period and 150 – 180 days on day 98th and 170 – 210 days on 150th day was observed. Long operation period promoted the

active biomass growth in the presence of ametryn, which has improved the sludge quality and contributed to long SRT.

Table 4.3: Ametryn adsorption, and characterization of MLSS, MLVSS, and sludge stabilization ratio (MLVSS/MLSS) of anaerobic reactor (R2)

Run (Days)	**Ametryn adsorbed (mg/g.MLVSS)**	**MLVSS concentration (g/L)**		**MLSS concentration (g/L)**		**MLVSS/MLSS**	
Reactor	R2	R1	R2	R1	R2	R1	R2
0 – 48	-	9.2	9.3	13	13.2	0.71	0.71
49 – 60	2 – 2.3	9.3	6.4 – 6.7	13.1	8 – 8.7	0.7	0.77 – 0.87
61 – 71	1.4 – 2	9 – 9.3	7.2 – 8.1	12.7 – 13.1	9.4 – 10	0.7 – 0.71	0.76 – 0.81
83 – 97	0	9.1 – 9.3	9.2 – 9.3	12.7 – 13.8	12. 8 – 13.5	0.67 – 0.71	0.68 – 0.71
98 – 108	1.3 – 2	9.2 – 9.3	8 – 8.2	12.1 – 12.8	10.5	0.68 – 0.72	0.8 – 0.82
109 – 119	1 – 1.2	9.1 – 9.4	9 – 9.2	12.5 – 13.1	11.6 – 11.9	0.71 – 0.72	0.77
142 – 150	0	9.2 – 9.4	9.3 – 9.6	12.7 – 13.1	12.9	0.72	0.72 – 0.74
151 – 210	2 – 4.5	9.1 – 9.3	7.6 – 8.1	12.7 – 13.8	9.7 – 10.2	0.67 – 0.71	0.78 – 8.1
211 – 280	0	9.1 – 9.4	9.6 – 10.8	12.5 – 12.8	13.2 – 14.2	0.72 – 0.73	0.72 – 0.76
281 – 365	2 – 4	9.2 – 9.3	7.2 – 9.4	12.7 – 13.1	9.0 – 12.9	0.7– 0.73	0.72 – 0.8
366 – 430	0	9.1 – 9.4	9.2 – 10.5	12.2 – 13	12.8 – 13.8	0.72 – 0.74	0.71 – 0.76

Long SRT of 180 days was reported in MBR treatment, and excessive sludge toxicity of influent ametryn dose (2.76 mg/L) demanded the sludge wasting to maintain the required SRT (Navaratna et al. 2016). ASBR is found to be very

effective for yielding high SRT and fast recovery for herbicide toxicity than the previous studies (Koh et al. 2008; Wang et al. 2018).

Anaerobic sludge granulation with increased MLVSS concentration >9.8 g/L indicate the adoptability of anaerobic bacteria, and ametryn being acted as nutrient source. These observations including granulation and the size of granules greatly influence the reactor performances (Gao et al. 2011). Anaerobic sludge granulation during this period may be the significant indication of active biomass growth; sludge obtained from R1 and R2 reactor (Figure 4.10). The seed sludge to both the anaerobic reactors contained grains size < 250 micron at the time of start-up, and the granules size varied from 0.2 – 0.5 mm in size in R2 reactor from the day 70 to 430 days, but there was no granulation observed in the control reactor throughout the study period. The granulation was observed from 70^{th} day, and sized up to 0.5 mm till the day 80. Further, granule size was reduced to 0.3 mm on day 81 of ametryn rise to 6 mg/L, and again reached to 0.5 mm from 130 day onwards. Further raise in the influent ametryn concentration (8 – 10 mg/L) led to reduced granule size along with variation in MLVSS/MLSS ratio as explained and regenerated over continued operation.

4.4.1.3 Factors influencing on the performance of anaerobic reactor

pH and alkalinity

pH in the reactor was observed between 6.5 – 7.5 and it is considered as favourable range for better methanogens activity, reactor temperature was observed to be higher than the control, in the range of 30.3 – 31.3^{o}C. The pH in the reactor was maintained in the neutral range of 6.6 to 7.7 as required for methanogenic treatment (Pirsaheb et al. 2018), by using 4 g/L of sodium bicarbonate. High alkalinity, low COD removal and low methane gas production between days 10 to 17 indicate a slight toxicity and it was recovered gradually. There was no much deviation from the required pH level was observed during the study period. Alkalinity in the influent was in the range of 950 – 1300 mg-$CaCO_3$/L, whereas the effluent contained 1800 – 2400 mg-$CaCO_3$/L for R1 and 1650 – 2500 mg-$CaCO_3$/L R2. High alkalinity was reported at low COD removal rates may be due to accumulation of inorganic substrates like sulphates, nitrates causing toxicity on biomass (Manu and Chaudhari 2002). Addition

of sodium bicarbonate to maintain the pH would also contribute to high alkalinity of reactor effluent.

ORP and temperature

During high temperature ranges higher ORP value of -250 to -280 mV was recorded, at this condition the reactor had performed better, which is indicated by high total gas production and COD removal efficiency. High reduction reactions in the experimental reactor indicated by low ORP values implies that ametryn acted as electron acceptor, the functional groups had attacked by the methanogens in the reducing environment (Gibson and Harwood 2002). The anaerobic conditions exhibits negative ORP values and reducing reactions in the experimental reactor indicated by low ORP values implies that ametryn acted as electron acceptor, the functional groups had attacked by the methanogens in the reducing environment (Gibson and Harwood 2002).

ORP in the R2 and R1 reactors varied between -230 to -310 mV and -200 to -280 mV respectively. Low ORP values in the R2 compared to R1 reactor indicate highly active biomass in R2, which is in agreement with studies reported by Manu and Chaudhari (2002). The complete removal of 4 – 6 mg/L of influent occurred within 48 to 50 days with all the existing conditions with ORP in the range of -230 to -310 mV. However, addition of 5 – 10 mg/L of redox mediator during the treatment of 8 to 10 mg/L of influent ametryn was able to enhance the treatment efficiency by 12 – 15%. Temperature plays a crucial role in anaerobic degradation processes and at high ambient temperatures ranges 30 – 31.4^{o}C maximum reactor performances was observed. During the treatment period anaerobic experimental reactor temperature varied between 28.5 to 31.4^{o}C, while the control reactor temperature was observed to be lower than R2 reactor by 0.4 ± 0.1^{o}C.

4.4.1.4 Sequential anaerobic-aerobic treatment of ametryn

Anaerobic biotransformation products mainly constituted with some long and short chain fatty acid, which can be easily oxidised as the VFA consumed by aerobic/facultative bacteria (Gaunt and Hester 1989). The aerobic SBR was operated as post treatment to anaerobic effluent and the performance of both control (A1) and ametryn treating reactor (A2) is depicted in the Figure 4.12(a-b). Ametryn entering in to the aerobic reactor mainly in the form of its anaerobic TPs except start up step and it was at low concentrations as the maximum was removed in the anaerobic step. Initially there was a maximum ametryn removal of >80% observed in the effluent and then reduced to 65% on day 12, with a COD removal efficiency of 81%. Ametryn intermediates formed during anaerobic treatment might have become toxic to the aerobic bacteria on initial days.

After initial lag phase, the aerobic reactor performance improved and became stable with a constant COD removal efficiency of >90%. HPLC report and UV spectra obtained for initial, anaerobic and aerobic effluents indicates degradation of ametryn. The HPLC obtained for the aerobic effluent on maximum herbicide removal can be seen in the Figure S5. TPs might have been oxidised to end products like water and CO_2, the maximum COD removal efficiency support the reduced levels of the compounds in the effluent. It can be observed that the aerobic reactor was able to degrade the anaerobic TPs of ametryn and starch, but the presence of ametryn in the aerobic effluent convey aerobic persistence of the compound. But it is commendable to mention that sequential anaerobic-aerobic treatment has better treatment efficiency for removal of COD and other nondegraded organic compounds.

A combined MBR/UV/GAC study for removal of 5 mg/L of ametryn was able to remove about 61% (Navaratna et al. 2016), whereas in the present study 100% removal was achieved in the ASBR reactor alone. Anaerobic biotransformation products mainly constituted by long and short chain fatty acid due to the fermentation of starch, which have can be oxidised by aerobic/facultative bacteria (Gaunt and Hester 1989), and also long chain fatty acids loses carbon atoms by β-oxidation pathway produces acetyl-CoA, and further oxidised to CO_2 via tricarboxylic acid cycle (Ratledge 1992). Initially there was a low COD removal efficiency in the R4

reactor, may be due to toxicity of fatty acids and the toxicity was found to be reduced after the VFA biodegradation commenced (Gaunt and Hester, 1989). Inhibitions in the aerobic reactor lasted within 10 – 15 days of operation due to low influent ametryn and TPs concentrations, but the reactor recovery took more than 90 days while treating high concentrations of dicamba (60 mg/L previously). Anaerobic metabolites of ametryn and starch were removed, which was indicated by the COD removal efficiencies >95% and low VFA in the range of 55 – 80 mg/L.

Most of the previous studies on aerobic treatment of refractory organic compounds have resulted in limited treatment efficiency, formation of either recalcitrance or loss of biomass (Manu and Chaudhari 2002; Sandoval-Carrasco et al. 2013). But in the present study the TPs have not caused such negative effect on the aerobic biomass as most of the compounds are mineralised in the previous anaerobic step. HPLC and GC-HRMS results also supported the complete mineralization ametryn and its anaerobic TPs. Thus a sequential anaerobic-aerobic system can be a novel addition to remove ametryn and its metabolic by-products from water. Removal of ametryn and TPs was observed >70% with COD removal efficiency >80% after increasing the influent ametryn concentration to 8 mg/L.

The maximum removal of ametryn and COD >90% was observed after 230 days of operation. There was no ametryn detected in the aerobic sludge. The VFA compounds from the influent might have caused the slight toxicity in the reactor during the ametryn raise, but it recovered over continued treatment observed previously. After initial lag phase, the aerobic reactor performance improved and became stable with a constant COD removal efficiency of >90%. HPLC report and UV spectra obtained for initial, anaerobic and aerobic effluents indicates degradation of ametryn.

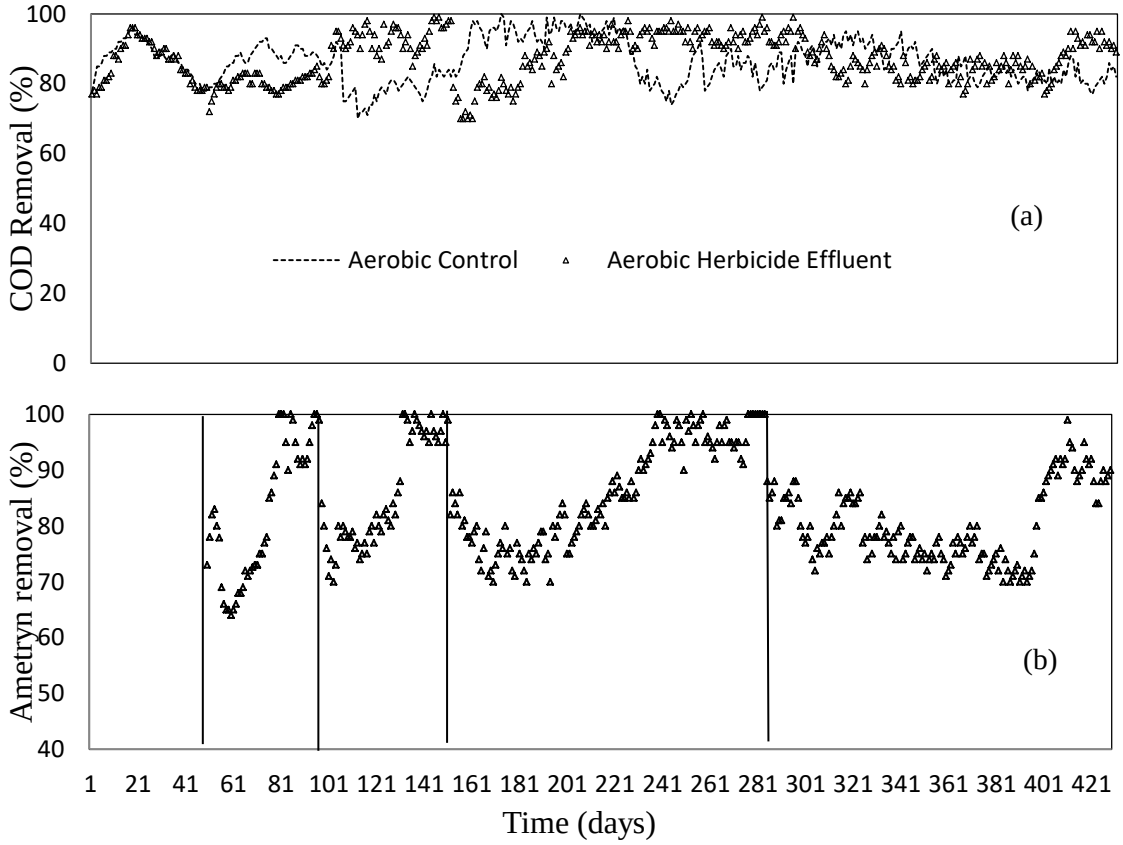


Figure 4.12(a-b): Performance of aerobic reactor treating ametryn (A2) compared with aerobic control (A1)

Effluent from A2 reactor contained ametryn concentrations of about 1.4 – 2 mg/L during incomplete anaerobic biotransformation. Complete removal of anaerobic metabolites was achieved after 238th day for influent ametryn concentration of 8 mg/L, whereas the removal efficiency was limited to 90 – 95% for influent concentration of 10 mg/L. High VFA in the aerobic reactor effluent was also detected during the initial days of every raise in influent ametryn concentrations. The effluent from A2 reactor contained VFA in the range of 200 – 400 mg/L and <100 mg/L in the A1 reactor as observed during the long operation period. Increased COD removal and reduced alkalinity indicate VFA reduction. The pH and alkalinity of A1 and A2 reactors varied in the range of 7.4 – 8.3 and 2300 – 2850 mg-$CaCO_3$/L respectively. A2 reactor effluent had high alkalinity (200 – 450 mg-$CaCO_3$/L) and VFA than the A1 reactor; this was observed mainly during incomplete ametryn biodegradation period.

4.4.2 Treatment of dicamba in ASBR

Herbicide dicamba was treated in the sequential anaerobic-aerobic batch reactor R3 and A3, the influent dicamba concentration tested was 10 to 100 mg/L over the treatment period of 430 days. The details of experimental condition studied are tabulated in the Table 4.4.

Table 4.4: Operational conditions maintained during the sequential anaerobic-aerobic treatment of dicamba

Sl. No	Reactor operation (Days)	Experimental condition studied
1	1 – 48	Reactor start-up and acclimation to 2 g/L of starch (OLR = 0.21 – 0.215 kg-COD/m^3/d)
2	2 onwards	Anaerobic effluent fed to corresponding aerobic reactor
3	49 – 113	Influent concentration of dicamba = 10 mg/L
4	89 – 142	Addition of AQS = 5 mg/L
5	114 – 164	Influent concentration of dicamba = 20 mg/L
6	165 – 242	Influent concentration of dicamba = 40 mg/L
7	165 – 211	Addition of AQS = 10 mg/L
8	212 – 300	Addition of AQS = 15 mg/L
9	243 – 339	Influent concentration of dicamba = 60 mg/L
10	301 – 339	Addition of AQS = 20 mg/L
11	340 – 390	Influent concentration of dicamba = 80 mg/L
12	340 – 389	Addition of AQS = 10 mg/L
13	391 – 430	Influent concentration of dicamba = 100 mg/L
14	390 – 430	Addition of AQS = 15 mg/L

The performance of anaerobic reactor treating different concentrations of dicamba is shown in the Figure 4.13(a-d). Biomass inhibition was observed during the first 27 days of introduction and it was indicated by reduced COD removal and biogas production than the control. The COD in the effluent of acclimated reactor was 900 - 1300 mg/L, whereas the control reactor COD was at 300 – 460 mg/L. High effluent

COD may indicate the incomplete degradation of dicamba (González-Cuna et al. 2016). Initial dicamba removal between days 52 – 75 was due to the accumulation of compound through adsorption on to reactor sludge, it was confirmed after characterising the sludge. Adsorption was in the range of 5 – 8 mg/g.MLVSS, indicating that significant amount of dicamba was adsorbed on to sludge. Adsorption of dicamba was reduced over continuous operation, may be due to high water solubility of the compound (4500 mg/L). Effluent from the dicamba acclimated anaerobic reactor contained biotransformation products and it was due to inability of anaerobic bacteria to completely biodegrade the compound under anaerobic conditions (González-Cuna et al. 2016).

Further, decline in COD concentration and stable biogas production after 65 days indicate the anaerobic sludge was restored slowly with a consistent biological activity in the reactor, which may be an indication of acclimatization to 10 mg/L of dicamba. Acclimation to 20 mg/L of 2,4-d took more than 80 days to aerobic reactor and inhibitory effects of herbicide was avoided in the presence of glucose (Chin et al. 2005). Therefore treatment was continued with the same influent dicamba concentration and the anaerobic reactor reported 65% of dicamba removal. It was found that at low ORP in the anaerobic reactor the reductive reactions like demethylation and dechlorination reactions occur, which lead to the breakup of methyl, chlorine and halogen group. It was observed that the ORP in the reactor was limited to around -250 mV and believed that varying the ORP value would support increase in biodegradation.

It has been reported that the addition of quinones like AQS activated the ability of unacclimated biomass to degraded azo dyes (Rau et al. 2002). Therefore, addition of AQS to influent of the reactor was started from 5 – 20 mg/L. Then addition of 5 mg/L AQS has reduced ORP from -250 to -300 ± 10 mV which was able increase the treatment efficiency by 5 – 12% (Mahesh and Manu 2019b). Increase in AQS to 10 mg/L has showed increased dicamba removal efficiency but there was a raise in TPs concentration indicated by high effluent COD when compared to control.

The initial dicamba concentration was increased to 20 mg/L after the consistent removal of 10 mg/L (from 108th day). Toxic effect of dicamba was appeared to be negligible when compared to the previous stage as the bacteria in the reactor have been acquainted to the compound over the 113 days of operation. After increase in the influent dicamba concentration, there was raise in effluent dicamba concentration and COD in the effluent which may be attributed to increased toxicity load which inhibited on the anaerobic sludge. High COD in the effluent is also an indication of incomplete dicamba degradation (González-Cuna et al. 2016). During this period from 140 – 160 days, it may also be noted that the acclimatised bacteria were able to degrade the compound partially, indicate presence of insufficient dominant bacteria to degrade dicamba. Increase in AQS supported the dicamba removal along with reduced effluent COD, may indicate the development of dicamba degrading bacteria favoured by redox mediator. ORP during this period was observed to be around -300 mV with existing influent AQS concentration of 10 mg/L. It has been reported that the addition of quinones like AQS activated the ability of unacclimated biomass to degraded azo dyes (Rau et al. 2002). At ORP of -270 to -320 mV anaerobic reactor performance was reported to be stable for dicamba removal, incomplete degradation in anaerobic reactor followed the previous degradation pattern.

Performance of the anaerobic reactor for the treatment of 40 mg/L dicamba was conducted during 166 – 243 days of operation in acclimated biomass. The reduced of anaerobic biological activity was indicated by high effluent dicamba concentration, high COD, and reduced biogas production. The reduced sludge activity in anaerobic reactor than the previous stage and control reactor may be due to sudden toxicity, but it was not observed in the aerobic reactor. There was no herbicide adsorption detected in the sludge extract. The operation was preceded using 10 mg/L of AQS till 45 days (between 166 – 211 days), ORP remained at -270 to -320 mV and the treatment efficiency for dicamba and COD was 74 and 77% respectively. AQS was increased to 15 mg/L (from day 243) and ORP was reduced slightly (-5 mV), it was almost negligible sometimes. It was assumed that long operation period of 40 days would have supported the degradation indicated by consistent reactor

performance introducing 40 mg/L of dicamba. It may be due to development of specific degradation pathway by the microorganisms leading to increased dicamba removal after long operation period (Koh et al. 2008).

It may be observed from the graph that at average ORP values around -310 mV the anaerobic reactor has showed >70% reduction in dicamba with COD removal efficiency of 81%. The dicamba removal efficiency of Fenton's treatment process reported 85% of 86.1 mg/L of dicamba with COD removal of 83% (Sangami and Manu 2017b). Maximum dicamba removal may be due to the formation of more oleic acid groups as TP, possible degradation and adsorption of oleic acid on to the sludge thus produced high CH_4 gas (Pereira et al. 2002). There was no dicamba adsorption on to the reactor sludge, as there are no peaks observed in the chromatogram. High water solubility of 4,500 mg/L and low soil sorption capacity of dicamba may be the reason for no adsorption (Magga et al. 2008). The reactor was slightly limited, may be due to inhibition of long chain fatty acids and VFA on anaerobic bacteria (Dasa et al. 2016).

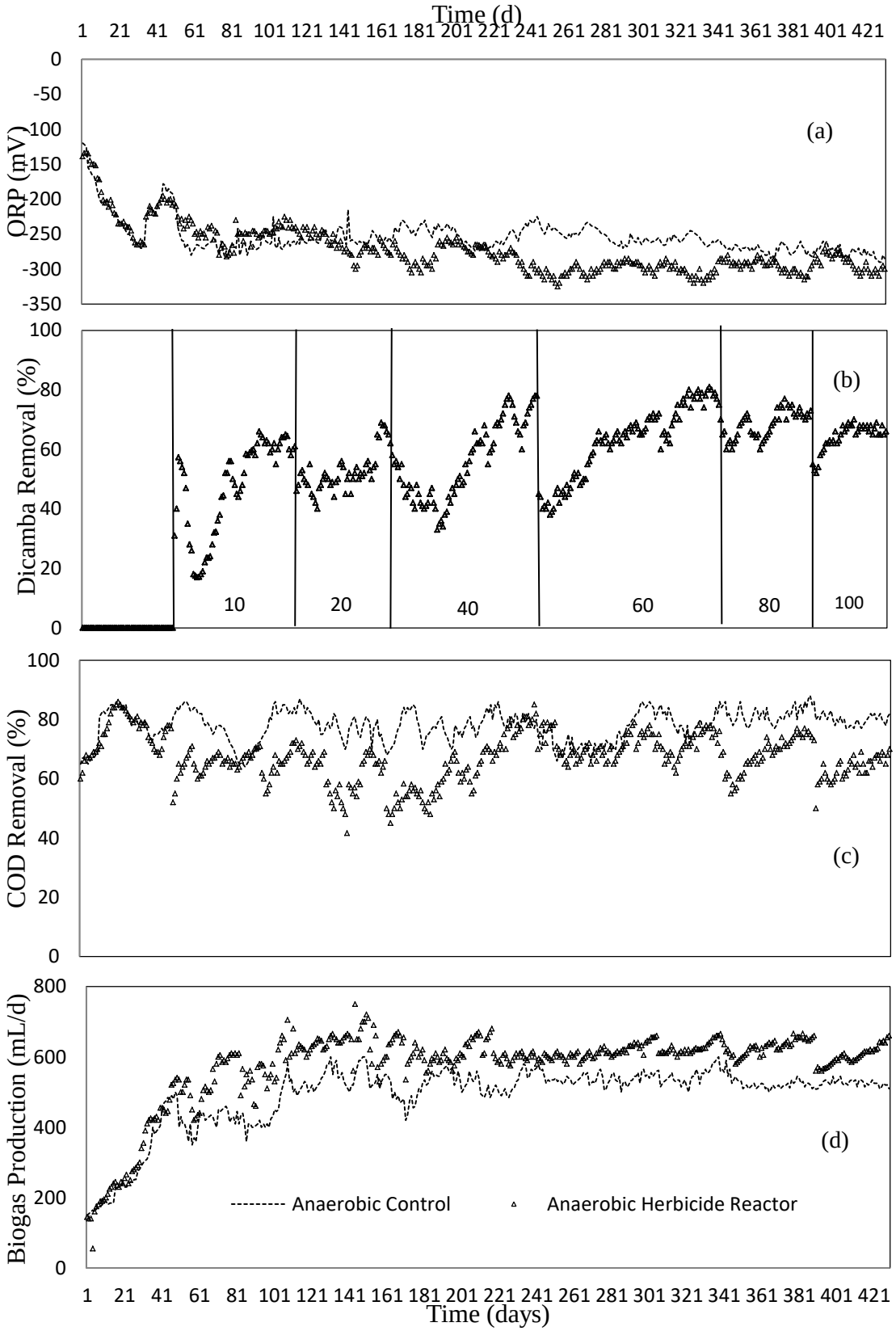


Figure 4.13(a-d): Variation of performance parameters during the anaerobic treatment of dicamba (R3) compared with anaerobic control (R1)

The treatment process was carried out with influent dicamba concentrations from 60 – 100 mg/L during operation period of 180 days from 243 – 430 days. The dicamba inhibitions on anaerobic sludge was observed up to 31 days of introducing the 60 mg/L dicamba, low COD removal (65%) and biogas production than the control (630 mL/d). High effluent COD may indicate the incomplete degradation of dicamba, as described previously also (González-Cuna et al. 2016). Accumulation of compound through adsorption on to reactor sludge was negligible; it was confirmed on characterising the sludge continuously.

Further, dicamba was removed up to 80% with consistent COD removal efficiency of 75 – 80% and total gas production >620 mL/d on 328 – 335 days of operation. The improved reactor performance during this stage may indicate the reactor acclimatization to 60 mg/L of dicamba faster than the previous stage. Acclimation to 20 mg/L of 2,4-d required more than 80 days to aerobic reactor and inhibitory effects of herbicide was avoided in the presence of glucose (Chin et al. 2005). It was found that at low ORP in the anaerobic reactor supported the demethylation and dechlorination reductive reactions to occur, which lead to the breakup of methyl, chlorine and halogen group. It was observed that the ORP in the reactor was limited to around -310 mV and to enhance the biotransformation, AQS concentration was raised up to 20 mg/L from the day 301. Dicamba removal efficiency of up to 80% was achieved on day 335, and the effluent COD removal was around 77%.

The influent dicamba concentration was increased to 80 mg/L from 340th day onwards after the consistent removal of 60 mg/L. There was a slight reduction in the dicamba removal efficiency after the raise, but the effects were recovered quickly than the previous stage. Inhibitions during this period was negligible, may be due to the bacterial adoptability for high concentrations (>60 mg/L). Up to 77% reduction in dicamba and COD was observed on 375th day. Due to the presence of dicamba degrading bacteria in the acclimated biomass, it might have become easy for their metabolism. Increase in AQS supported the dicamba removal along with reduced effluent COD, may indicate the development of dicamba degrading bacteria favoured by redox mediator. ORP during this period was observed in the range of -270 to -310

mV with influent AQS concentration of 10 mg/L. At ORP of -270 to -310 mV anaerobic reactor performance was reported to be stable for dicamba removal and there was an improved dicamba removal observed during this stage.

Performance of the anaerobic reactor for the treatment of 100 mg/L dicamba was conducted from 390 – 430 days of operation in the acclimated biomass. There was no herbicide adsorption detected in the sludge extract. The operation was preceded using 15 mg/L of AQS, ORP was at -270 to -310 mV and the treatment efficiency for dicamba and COD was about 67%. The HPLC chromatogram obtained for influent and anaerobic effluent of R3 reactor is depicted (Figure S6). It was assumed that AQS dosage of 10 – 15 mg/L was significant in the removal of dicamba with long operation period, and hence the influent AQS was limited up to 15 mg/L to avoid the toxicity of AQS. Long operation period enriched the development of specific degradation pathway by the microorganisms might have led to the increased dicamba removal over long operation period (Koh et al. 2008).

It was observed from the graph that at average ORP values around -310 mV the anaerobic reactor has showed >70% reduction in dicamba and COD removal efficiency of 81%. Maximum dicamba removal was due to the formation of more oleic acid groups as TP, possible degradation and adsorption of oleic acid on to the sludge thus produced high CH_4 gas (Pereira et al. 2002). There was no dicamba adsorption on to the reactor sludge, as there are no peaks observed in the HPLC chromatogram. The reactor performance observed to be limited; this may be due to inhibition of long chain fatty acids on anaerobic bacteria (Dasa et al. 2016).

MLSS and MLVSS detected in the effluent was considerably less (i.e., MLSS: 800 – 6000 mg/L and MLVSS: 600 – 2800 mg/L). The maximum and minimum SRT calculated was 200 days and 26 days during the stabilization period and dicamba treatment period respectively. After addition of 10 mg/L of dicamba the MLVSS in the reactor was reduced to 7200 mg/L (quantification of MLVSS was done after and before the raise in influent dicamba to avoid biomass loss). The loss of MLSS and MLVSS found in the effluent during daily decanting was considered to be very small, of the order of 2200 – 2600 mg/L and 1800 – 2000 mg/L, respectively, and the

corresponding SRT was 60 – 70 days. Khan et al. (2011) have reported SRT of 20 days for 5 % loss of biomass. With increase in operation period, the sludge quality was improved indicated by the lower appearance of sludge in the effluent (<1200 mg/L). The SRT calculated during the long operation period (89 – 113 days) was >150 days. The long operation period supported the growth of slow and inactive biomass to get adapted to the toxic condition, and hence, the increased reactor performance was observed.

During the first 20 days of each stage with increased dicamba concentration there was always lower SRT (<55 days) compared to the second phase of that stage. Increase in influent dicamba concentration to 20 mg/L followed the same pattern as observed in the previous stage, whereas further raise to 40 mg/L has appeared as shock loading with reduced sludge activity. The shock load impacts are gradually overcome due to adaptation of bacteria over long operation period (Chin et al. 2005) and the SRT found was 28 – 35 days during the first 10 days. MLVSS concentration dropped to <6500 mg/L during stage III and recovered over 76 days. Further raise in dicamba concentration to 60 mg/L had low impact on the biomass compared to previous stage. Long SRT in a biological reactor enable growth of slowly growing micro-organisms which have further enhanced the removal of endocrine disrupting chemicals (Koh et al. 2008). Further increase in influent dicamba concentration to 80 – 100 mg/L was observed to be similar to the previous stages but reactor recovered comparatively faster.

4.4.2.1 Biodegradation of dicamba

The major intermediate compounds produced in the anaerobic effluent were identified using GC-HRMS. The effluent contained biotransformation compounds like 3,6-dichlorosalicylate, salicylate and dicamba as identified in the GC-HRMS (Figure S7). Dehalogenated and dechlorinated compounds like 3,6-dichlorosalicylate, 6-chlorosalicylate have been detected as intermediate compounds (Milligan and Häggblom 1999). Another study reported major intermediates of dicamba as halogens and benzoates, end products are CH4 and CO2 (Suflita at al. 1982). The formation 3,6-dichlorosalicylate, Salicylate, along with long chain fatty acids like oleic acid ($C_{18}H_{34}O_2$), 2-hydroxy-1-(hydroxymethyl) ethyl ester ($C_{21}H_{40}O_4$), trans-13-

octadecenoic acid ($C_{18}H_{34}O_2$) were observed. These intermediate compounds are of great concern to environment due to their toxic risks, which need to be removed completely (Velisek et al. 2017).

However, in the present study the 3,6-dichlorosalicylate and salicylate can be considered as TPs of dicamba, while long chain fatty acids can be considered as TPs of starch. 3,6-dichlorosalicylate and salicylate may become stable under anaerobic conditions as reported previously and hence anaerobic treatment of dicamba was not efficient. The biogas production was measured during the treatment period, before and after the herbicide introduction in both the reactors and methane gas production was measured using the 5% KOH solution displaced from the gas liquid displacement system (on regular intervals of 10 days). Methane gas production in the R3 reactor was higher than the R1 by 100 – 200 mL/d. This may indicate partial degradation of dicamba to intermediate compounds, and the conversion of nondegraded organics leading to high biogas production than the control.

4.4.2.2 Factors influencing on anaerobic treatment of dicamba

Influent dicamba concentration

Anaerobic reactor was found be under the toxic risk on introduction of dicamba which can be evaluated based on the reduction in MLVSS concentration and biogas production. The averaged MLVSS concentration and biogas production was compared with respect to the influent dicamba concentration. It can be observed that the inoculated 9000 mg/L sludge concentration was raised up to 11000 mg/L initially in both the reactors. The variation in MLSS, MLVSS, sludge stabilization ratio (MLVSS/MLSS), and concentration of dicamba sorption on to the reactor sludge is tabulated as shown in Table 4.5.

After 48 days of total stabilization period, 10 mg/L of dicamba was introduced to one of the anaerobic reactor. In the beginning it was appeared that there was slight toxicity which reduced MLVSS concentration (7000 mg/L), whereas in the control it was 9300 mg/L. The reduced sludge activity was an indication of toxicity induced by the transformation of products (TPs) of dicamba on bacteria than the dicamba itself (Ghoshdastidar and Tong 2013; Kuppusamy et al. 2017). MLVSS concentration was

restored in the dicamba treating reactor with continued operation on 85th day. On the 113th day MLVSS in the control was 9800 mg/L and in the dicamba containing reactor it was 9400 mg/L and then influent dicamba concentration was raised to 20 mg/L the toxicity inhibition was also followed similar pattern as before and restored on continued treatment due to the adoptability of bacteria to the TPs of dicamba.

In spite of the toxicity the biogas production was found to be consistently higher than that of control biogas production, this would be another evidence for well adopted dominant anaerobic bacteria. Further the concentration was doubled to 2 times (40 mg/L) which appeared as toxic load (shock load) on the sludge (Weinberg and Teodosiu 2012) and reduced the MLVSS concentration (< 6000 mg/L). Then the sludge activity was improved over 40 days with regeneration of MLVSS concentration and improved biogas production. Further increase in dicamba concentration of 60 mg/L has lead in the reduction of MLVSS to 7000 mg/L initially and recovered over 40 days of operation which indicate the stable bacterial performance over long SRT. It can be observed that different influent dicamba concentration was removed gradually over time and dicamba (in the form of TPs) remaining in the effluent after anaerobic and anaerobic-aerobic treatment.

The raise in influent dicamba concentration from 80 – 100 mg/L was found to follow the stable treatment efficiency. A slight reduction in MLVSS concentration after the raise was observed, but it recovered during the continued operation. The MLVSS/MLSS ratio in the reactor varied between 0.66 – 0.85, the lower value in the reactor indicate an effective reactor performance and active biomass growth. The higher value indicates the unstable reactor performance due to dicamba loading, it may be considered as a toxic condition of the reactor.

Table 4.5: Dicamba adsorption, variation in MVSS, MLVSS, and sludge stabilization ratio (MLVSS/MLSS) in anaerobic reactor (R3)

Run (Days)	Dicamba adsorbed (mg/g.MLVSS)	MLVSS (g/L)		MLSS (g/L)		MLVSS/MLSS	
		R1	R3	R1	R3	R1	R3
0 – 49	0	9.2	9.2	13	13	0.71	0.71
50 – 88	3 – 5	9.3	7.1 - 8.2	13.1	9.2 – 10	0.7	0.77 – 0.8
89 – 113	2	9 – 9.3	9.3 – 9.4	12.7 – 13.1	12.8 – 13	0.7 – 0.71	0.72
114 – 142	4 – 6	9.2 – 9.3	8.0 - 8.3	12.8 – 13.3	10 – 10.1	0.69 – 0.71	0.8 – 0.82
143 – 165	0	9.1 – 9.3	8.5 - 9.3	12.7 – 13.8	12 – 12.9	0.67 – 0.71	0.7 – 0.72
166 – 211	6 – 8	9.2 – 9.3	6.4 - 7.7	12.1 – 12.8	8.1 - 9	0.68 – 0.72	0.79 – 0.85
212 – 243	0	9.1 – 9.4	8.0 - 9.0	12.5 – 13.1	9.8 - 11	0.71 – 0.72	0.80
244 – 300	6 – 10	9.2 – 9.3	7.0 - 8.5	12.8 – 13.1	9.1 – 10.5	0.7 – 0.72	0.76 – 0.8
301 – 340	0	9.1 – 9.3	8.0 – 9.2	12.6 – 13	11.1 - 13	0.7 – 0.71	0.7 – 0.72
341 – 389	2 – 4	9.2 – 9.4	8.2 – 9.0	12.7 – 13.1	10.8 – 12.8	0.71 – 0.72	0.7 – 0.76
390 – 430	2 – 4	9.1 – 9.4	8.1 – 9.1	12.2 – 13	12.2 – 13	0.71 – 0.72	0.66 – 0.77

ORP and temperature

ORP is an important parameter which has greatly influenced on the dicamba treatment in the anaerobic reactor. AQS produces the free radicals which enhances the redox reaction in reductive environments by oxidising various types of organic and inorganic compounds (Van der Zee and Cervantes 2009), by transferring the electrons from electron donors (starch) to electron acceptor (dicamba) (Da Silva et al. 2012). ORP in the anaerobic reactors during the stabilization period was in the range of -220 to -270 mV at the ambient temperature ranges of 28 to 30^{o}C and ORP was depended on temperature as observed from the experiment. The variation of ORP was linked to redox reactions between the various substrates and hence degradation of dicamba at different ORP and biogas production has been compared with control.

Introduction of 10 mg/L dicamba after the stabilization has activated redox reaction under reducing condition indicated by reduced ORP (-260 to -285 mV). Decrease in the ORP was observed for addition of 5 mg/L AQS solution at the similar ambient temperature ranges and which increased the degradation efficiency of dicamba. In the second stage with 20 mg/L of influent dicamba concentration ORP remain the same around -270 to -290 mV it was sufficient enough enhance the anaerobic degradation, where the control ORP ranged from -220 to -270 mV. The raise in AQS by 10 mg/L has improved the redox reactions with further reduction of ORP (-10 ± (-4) mV) at the ambient temperature. Da Silva et al. (2012) have reported increased dye removal for addition of AQS which enhanced the colour removal by mediating the redox reactions in the acidogenic and anaerobic reactors. Then the dicamba concentration was doubled to 40 mg/L keeping all the other dosages constant. At this stage the ORP was found to be -310 ± (-12) mV indicates that there were active substrates (dicamba) available for the redox reaction for the anaerobes; this was a clear indication of compound being transformed to its metabolites.

The effluent water contained high COD up to 750 to 1200 mg/L, it was believed that there may be excess substrates which can be degraded in anaerobic reactor and hence the AQS was increased to 15 mg/L. Though there was a reduction in ORP (around -12 mV) the reduction in the residual compounds took place only after certain days of operation from herbicide introduction and also after the

introduction of AQS. The acclimatization of bacteria over 20 – 45 days after raise in dicamba had indicated the reduced risk of dicamba for shock loads and contributed to biodegradation. Dicamba concentration was raised to 60 mg/L and the ORP remained same may be due the inability of bacteria to undergo redox reactions at existing AQS of 15 mg/L. Even after raise in AQS to 20 mg/L there was no significant change in ORP was observed but the removal was taking place around 70%. It was observed that attainment of saturation kinetics during the decolouration studies (Field and Brady 2003), may be due to reduced ambient temperature in the reactor (28 – 29.2^{o}C) influenced on the redox reactions. ORP remained around -270 to -300 mV but the effluent contained significant amounts of residual concentration which contributed to high COD values.

pH and alkalinity

pH in the reactor was observed between 6.4 – 7.7 and it is considered as favourable range for better methanogens activity, reactor temperature was observed to be higher than the control by 0.5 – 1.2^{o}C. The pH in the reactor was maintained in the neutral range of 6.6 to 7.7 as required for methanogenic treatment (Pirsaheb et al. 2018), by using 4 g/L of sodium bicarbonate. High alkalinity (2300 – 2800 mg-$CaCO_3$/L), low COD removal and low methane gas production indicates anaerobic toxicity. Alkalinity in the influent was in the range of 950 – 1300 mg-$CaCO_3$/L, whereas the effluent contained 1800 – 2400 mg-$CaCO_3$/L for R1 and 2250 – 2800 mg-$CaCO_3$/L for R2. High alkalinity was reported at low COD removal rates may indicate accumulation of inorganic substrates like sulphates, nitrates causing toxicity on biomass (Manu and Chaudhari 2002). Alkalinity variation within the reactor also depends on the concentration of COD loading and the addition of $NaHCO_3$ (Hasan et al 2015). Thus high effluent alkalinity may be due to accumulated non-biodegraded organic matter contributing to high COD in the reactor.

4.4.2.3 Sequential anaerobic-aerobic treatment of dicamba

The performance parameters of aerobic reactor treating dicamba are depicted in Figure 4.14(a-b). The effluent from the anaerobic reactor (R3) was treated further in the aerobic reactor (A3) to remove TPs of dicamba. The low COD level than that of

control reactor indicate that TPs are recalcitrant to aerobic treatment in the beginning. With the introduction of dicamba including TPs of dicamba and starch to aerobic reactor through the anaerobic effluent, the dicamba and COD removal observed was 65 and 70% respectively. Dicamba removal raised to 81% with COD removal constant, indicates a adsorption of TPs and dicamba initially 58^{th} day, then dropped to 55%. Then reactor performance increased gradually attaining >98% dicamba and 85% COD removal efficiencies on the 90^{th} day. The aerobic effluent showed negligible TPs with effluent COD of 45 – 110 mg/L. In contrast the aerobic treatment was able to remove >99% (3.5 mg/L) dicamba over 112 days of treatment (Ghoshdastidar and Tong 2013). Appearance of sludge granulation on day the 98 was an indication of active growth of aerobic bacteria by utilizing the anaerobic TPs as their nutrient sources (Mahesh and Manu 2019b); aerobic sludge granulation is depicted in the Figure 4.15.

After raise in the influent dicamba (20 mg/L) at the anaerobic influent, has impacted on the aerobic biomass indicating low dicamba and COD removal on the day 115. The dicamba removal efficiency in the aerobic reactor was fluctuated initially between 55 – 99% till the 113^{th} day during the treatment of R3 effluent with influent dicamba of 10 – 20 mg/L. Consistent removal of dicamba >92% and COD >95% suggests that the TPs of dicamba have been degraded in the aerobic reactor. COD removal efficiency of A3 was observed to be greater than the control reactor (A1). High herbicide removal in the aerobic reactor was due to low influent load (COD <400 mg/L) and presence of readily oxidising long chain fatty acids.

Removal efficiency of dicamba and COD reduced below to 70% on the 166^{th} day with increased influent dicamba to 60 mg/L. The high COD removal in A1 reactor than the A3 reactor suspects the accumulation of nondegraded organic compounds. This condition was observed was supported by the high alkalinity (>2600 mg- $CaCO_3$/L) and VFA (800 – 1400 mg/L) concentration from the aerobic effluent. After the continued operation, the A3 reactor COD removal efficiency increased above 90% leaving low effluent COD.

Increase in the herbicide concentration to 60 mg/L from 244th day onwards, the removal efficiencies of dicamba reduced to 69% on 252nd day, and increased to 88% on 270th day. The maximum dicamba removal corresponding to COD removal efficiency (>95%) indicates degradation of accumulated organic compounds. Further, the dicamba removal efficiency reduced to 66%, indicating the toxicity of added compounds due to the accumulation. After continuation, stable removal efficiency was observed, dicamba and COD by 88 and 95% respectively. The A3 reactor performance was observed to be increased even after raising the influent dicamba concentration to 80 mg/L.

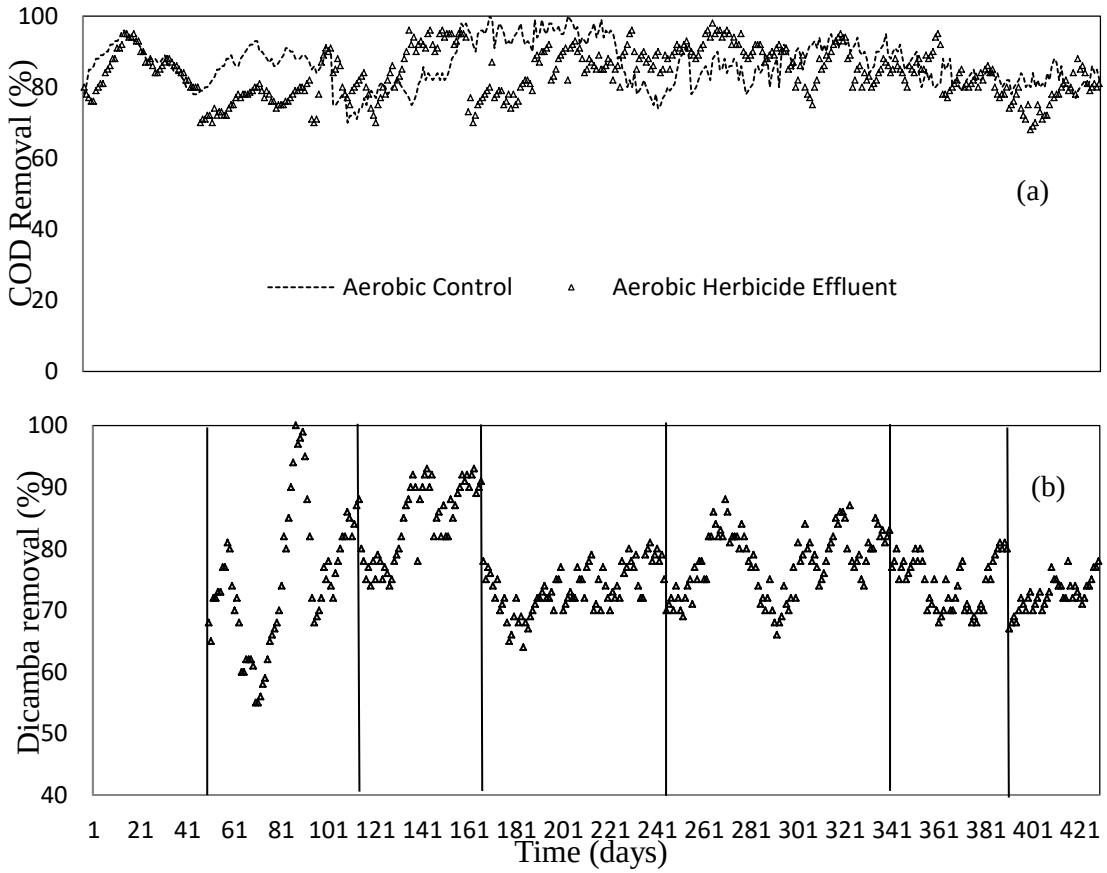


Figure 4.14(a-b): Performance parameters of aerobic reactor treating dicamba (A3) compared with aerobic control (A1)

The lowest dicamba removal efficiency observed was 68% with the COD removal of 80%. Similar removal efficiency for dicamba and COD was observed for the influent concentration of 100 mg/L, with reduction initially and increase over the continuation. The maximum removal observed on 427th day was 71% and 81% for

dicamba and COD respectively. Addition of AQS to anaerobic reactor was found to be significant during the aerobic post treatment reactor throughout the study period. The maximum removal efficiencies obtained during the study suggest that the compound dicamba and its anaerobic TPs acted as carbon source. The active bacterial adoptability to the condition supported the growth of dense microbial consortia with the development of sludge granules from the 98th day onwards.

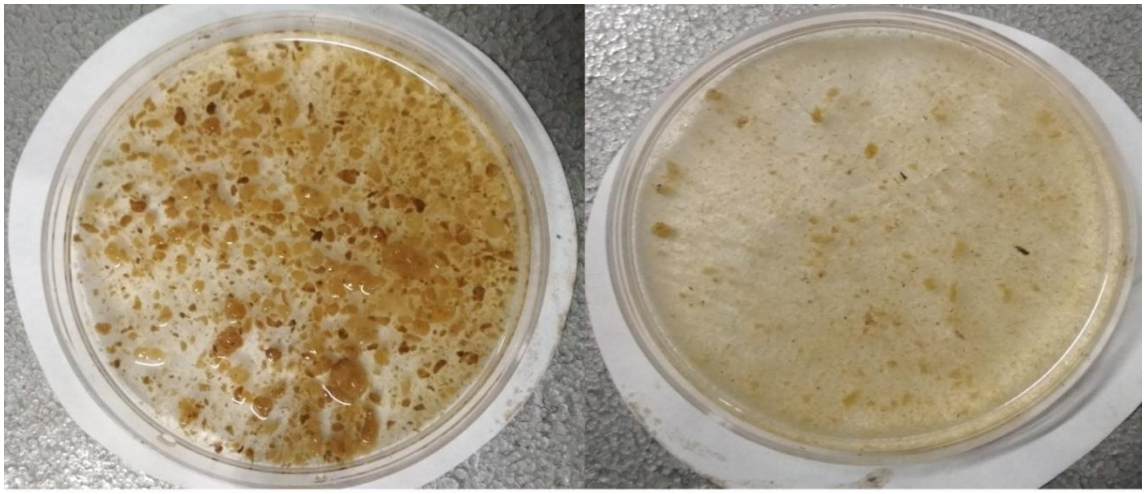


Figure 4.15: Aerobic sludge granulation of (a) dicamba treatment reactor (A3), and (b) control reactor (A1)

Formation of sludge granules may be due to the reactor operating conditions promoted by the dense microbial consortia of different bacterial species, supporting the degradation of dicamba and similar observations are reported (Dutta and Sarkar 2015). The TPs formed during the anaerobic treatment of dicamba have tendency to get oxidised when they are treated in aerobic reactor (Mahesh and Manu 2019b). Aerobic reactor was able to remove dicamba TPs up to 85% with COD removal of 92%. The dicamba TPs have been degraded by the aerobic bacteria in the reactor indicated by disappearance of intensity peak at retention time of 1.2 min in HPLC (Figure S8). The HPLC report obtained for the aerobic effluent indicates that the transformation products of dicamba are mineralised.

The different type of fatty acids and other TPs formed during the anaerobic treatment of dicamba can be used by aerobic bacteria as nutrient and supported sludge granulation. Degradation of fluroaromatics compounds (type of herbicides) by aerobic bacteria in the presence of oxygenase enzyme has supported our findings (Murphy et al. 2009). Granules are cultivated to treat xenobiotic compounds in aerobic SBR as the bacteria uses the compound as their sole carbon source (Khan et al. 2011). The growth of active biomass with the formation of granules indicates that anaerobic TPs have served the nutritional requirements of the aerobes and supported their growth in this study.

DO in the reactor was maintained consciously in the required range and it was found to be similar to the DO of control reactor. The increased removal of TPs in the later stage may be attributed to less influent concentration and oxidation of long chain fatty acids to simple end products and hence the COD removal efficiency has increased. Since the anaerobic effluent has high concentration of oleic acid, the aerobic treatment supported the mineralization of such compounds to water and CO_2. Oleic acid was the only fatty acid group remained after aerobic treatment of anaerobic effluent.

4.4.3 Treatment of 2,4-d and ametryn mixtures in ASBR

The herbicide mixtures of ametryn and 2,4-d were treated in sequential anaerobic-aerobic batch reactors R3 and A3 with the influent concentrations of ametryn:2,4-d = 2:5 to 4:10. The operational condition followed during the treatment of herbicide mixture is tabulated in the Table 4.6.

Table 4.6: Operational conditions maintained during the sequential anaerobic-aerobic treatment of ametryn and 2,4-d mixtures

Sl. No	Reactor operation (Days)	Experimental condition studied
1	1 – 48	Reactor start-up and acclimation to 2 g/L of starch (OLR = 0.21 – 0.215 kg-COD/m^3/d)
2	2 onwards	Anaerobic effluent fed to corresponding aerobic reactor
3	49 – 190	Influent herbicide concentration = (ametryn:2,4-d) = (2:5) mg/L
4	116 – 142	Addition of AQS = 5 mg/L
5	143 – 190	Addition of AQS = 10 mg/L
6	191 – 400	Influent herbicide concentration = (ametryn:2,4-d) = (4:10) mg/L
7	305 – 359	Addition of AQS = 5 mg/L
8	360 – 400	Addition of AQS = 10 mg/L

R4 was fed with herbicide mixtures (ametryn: 2 and 2,4-d: 5 mg/L) from day 49 onwards. The study was carried out up to 190 days with this concentration and the influent concentration was raised to two fold from day 191 onwards and the same concentration was maintained for 400 days. The performance of reactor treating 2,4-d and ametryn mixture is shown in Figure 4.16(a-c). COD removal of R4 dropped down to 44% after herbicide introduction along with drop in biogas production (330 mL/day) than the control reactor (430 mL/day) on day 50. This condition is a clear indication of sludge toxicity and the poor sludge settling behaviours have also highlighted the reactor condition.

On continued operation COD removal efficiency improved and reached to a maximum of 69% with biogas yield of 500 mL/day on day 141, whereas the R1 COD removal was at 70% and gas yield was 510 mL/day. The equal reactor performances of both the R1 and R4 at this stage may indicate that the despite the toxicity, bacteria were able to digest the starch almost completely. This would suggest that the bacteria were able to survive at the toxicity level and they may degrade over long operation

period. Decreased COD removal may be attributed to the production of different intermediate compounds from the incomplete mineralization of herbicides mixture. Similar observations were reported during the treatment of 2,4-d (Chin at al. 2005), and 2,4-d with multiple chlorophenols (Ma et al. 2012).

It was observed that the herbicide mixtures toxicity inhibited the treatment efficiency for longer time up to 115 days, than it was reported for individual compound treatment. The studies reported 2,4-d up to 100 mg/L was removed completely during 100 days operation (Chin et al. 2005), 3.5 mg/L dicamba was removed (99%) in 112 days (Ghoshdastidar and Tong 2013), about 1 mg/L of ametryn was removed (46%) during 216 days of operation (Navaratna et al. 2016). Toxicity induced on the anaerobic biomass was due to the synergic effect of mixed herbicides, and similar observations were reported for different influent concentration with treatment performance (Celis et al. 2008; Ghoshdastidar and Tong 2013; Ma et al. 2012). Reducing condition in anaerobic reactor favour dechlorination, demethylation, dealkylation and dechlorination reactions produce different intermediate compound, which often induce toxicity for bacterial degradation (Ghoshdastidar and Tong 2013; Kuppusamy et al. 2017). GC-HRMS analysis of the treated effluent showed that intermediate compounds were mainly the long chain fatty acids and alcohols in the R1 and presence of cyanuric acid along with fatty acids observed in the R4 reactor.

The influent concentration of herbicides was raised to two fold (i.e., ametryn: 4 and 2,4-d: 10 mg/L) from day 191 onwards, it can be observed that the treatment efficiency was reduced slightly. Though there was an inhibition up to 65 days after raise, the reactor recovered comparatively faster than the previous step. The early recovery may be due to the bacterial adaptation over the past several days of exposure to similar herbicides condition, similar observations have been reported during the treatment phenoxy herbicides (Chin et al. 2005; Ma et al. 2012). pH was in the range of 6.5 – 7.3 and temperature remained 29.6^{o}C. ORP was reduced to -275 mV in the absence of AQS, and hence 10 mg/L of AQS was added to enhance the redox reaction further and it also promotes the exchange of electrons between the substrate and herbicide (Da Silva et al. 2012).

COD removal efficiency improved significantly at lower ORP (-300 mV), may be the significant contribution of AQS addition. After observing the stable reactor performance even after 100 days of treatment period, it was believed that the further continuation may not produce significant outcome due to toxicity of mixed herbicides. The ORP and biogas production of the reactor were observed to be similar to that of control during first 8 days. The herbicide removal increased gradually from 35 – 45% over a period of 26 days (from 90 – 116 days), maximum COD removal was around 50%. ORP in the reactor was equal to control and the biogas production was slightly lower than the R1.

It was thought that the anaerobic biomass was inhibited by the toxicity of mixed herbicides. As the ORP in the reactor observed to around -255 mV, therefore redox mediator AQS was introduced to R4 from day 116. The reactor performance increased slowly over 20 days of operation. The sudden drop in reactor performance after AQS raise was an indication of increased sludge toxicity initially may be due to toxicity induced by excess sodium accumulation. This was considered as a limiting stage for removal of herbicide mixtures due to lack of methanogenic bacterial development for existing toxicity even during long operation period of 140 days. Increase in biogas production than the control on some days suggests that there may be bacteria which are resistant to toxicity of herbicide mixtures.

Maximum removal of individual compounds ametryn or dicamba was achieved within 50 days of operation without using redox mediator (Mahesh and Manu 2019c). The R4 reactor required long operation period of 140 days to remove 70% of COD. This indicates the inability of anaerobic bacteria to get adapted to the synergic toxicity of herbicide mixtures. After the raise in influent herbicide concentration, the reactor performance was continued without lag, may be due to the adaptation of bacteria to existing toxic conditions as observed earlier.

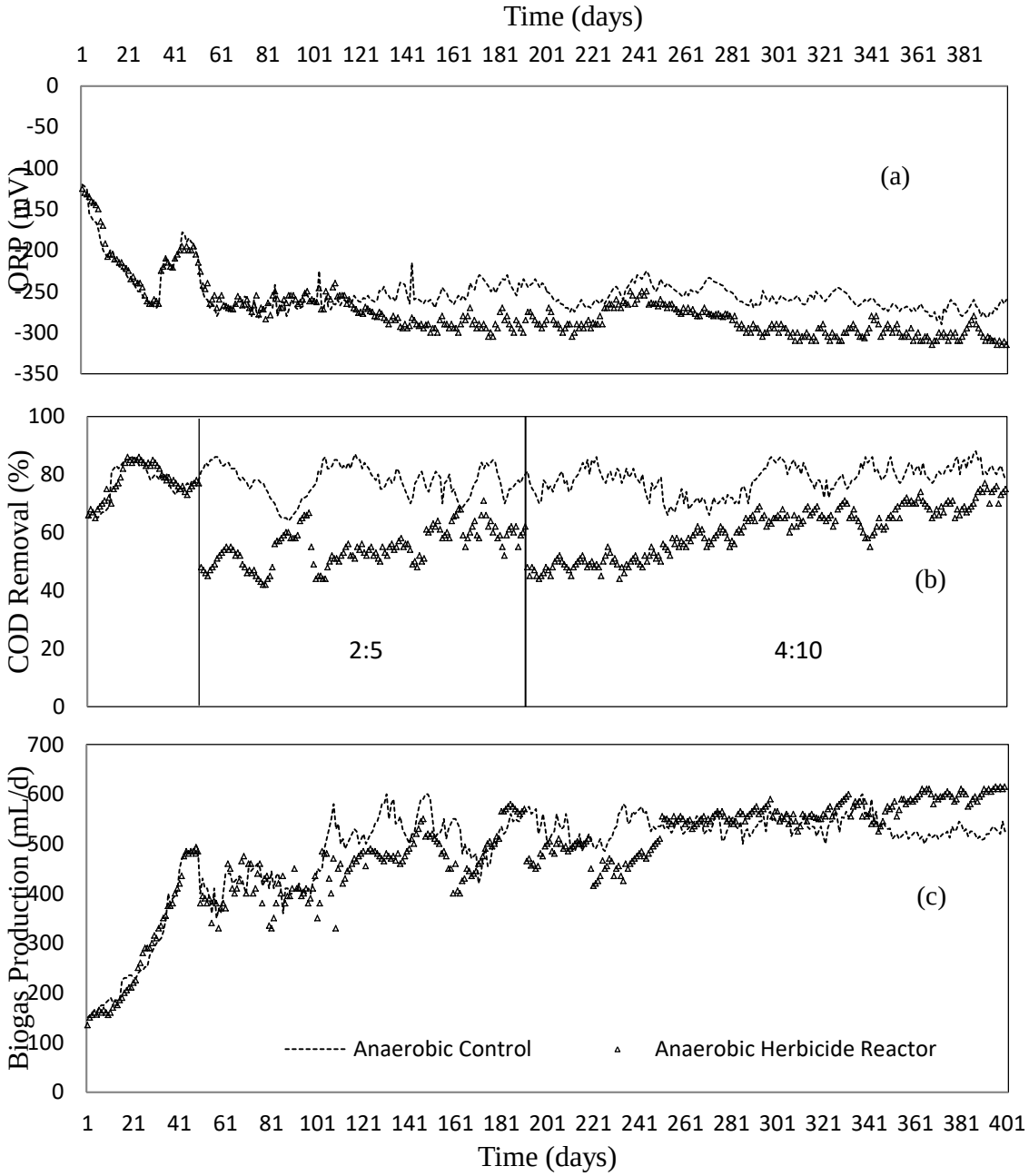


Figure 4.16(a-c): Variation of performance parameters during the anaerobic treatment of 2,4-d and ametryn mixture (R4) compared with the anaerobic control (R1)

During this time the removal of herbicides up to 65% was achieved within 40 days of raise, which may indicate that the anaerobic bacteria have developed to degrade the 2,4-d and ametryn mixtures. High COD values than the control in the effluent suggest presence of biodegradable organic matter. Though the reactor recovery and biomass adaptation was slower, it has exhibited the treatment pattern as observed during the treatment of ametryn alone.

AQS produces the free radicals which enhances the redox reaction in reductive environments by oxidising various types of organic and inorganic compounds as explained previously and starch acts as electron donor herbicides as electron acceptor (Da Silva et al. 2012). ORP in the anaerobic reactors during the stabilization period was varied in the range of -220 to -270 mV at the ambient reactor temperature ranges of 28 to 30^{o}C and ORP was depended on temperature as discussed earlier. Introduction of herbicide mixture after the stabilization did not impacted on the redox reactions within the anaerobic reducing condition and ORP remained almost equal ORP of R1 reactor. The addition of 5 mg/L of AQS reduced the ORP by -10 to -15 mV and the ORP of control being -250 mV. Addition of AQS was significantly contributed to the maximum COD removal of 66% and raise in total gas production of 20 – 40 mL/d than that of R1 reactor.

Further, the influent AQS solution was raised to 10 mg/L from the day 143, the COD removal efficiency increased slightly. But it was found to be limited as there high effluent COD and high VFA concentration (>850 mg/L). During the treatment of high influent herbicide load from 191 day onwards the ORP of R4 remained lower than the R1 by -20 – 30 mV, about 5 mg/L of AQS was added from 305th day and it was raised to 10 mg/L from 360th day. Addition of AQS contributed to enhanced reactor performance with high COD removal >70% towards the end of treatment period.

pH in the reactor was observed between 6.4 – 7.7 and it is considered as favourable range for better methanogens activity, reactor temperature was observed to be higher than the control by 0.5 – 1.2^{o}C. The pH in the reactor was maintained in the neutral range of 6.5 to 7.7 as required for methanogenic treatment (Pirsaheb et al. 2018), by using 4 g/L of sodium bicarbonate. High alkalinity (2300 – 2800 mg-$CaCO_3$/L), low COD removal and low methane gas production indicates anaerobic toxicity. Low pH (6.4 – 6.6) in the R4 reactor was mainly conferred to the accumulation of high VFA (2000 – 2400 mg/L) and acedogenic bacterial activity during initial days of herbicide introduction and similar observations have been reported in Hasan et al. (2015). On continued operation pH increased up to 7.7

indicating the development of methanogens inside the anaerobic reactor and VFA observed was below 1400 mg/L along with a slight increase in biogas production.

The effluent alkalinity was between 1800 – 2400 mg-$CaCO_3$/L for R1 and 2250 – 2800 mg-$CaCO_3$/L for R4 while influent alkalinity being 1900 – 2100 mg-$CaCO_3$/L. The high alkalinity obtained during low COD removal period may indicates the possibility presence of inorganic substrates like sulphates, nitrates accumulation leading to toxicity on the biomass (Manu and Chaudhari 2002). Alternatively high alkalinity may also be due to addition of sodium bicarbonate, and high alkalinity has caused a buffer effect and similar phenomenon was reported by Sentürk et al. (2010). In addition, bicarbonate alkalinity was formed in the reactor by the reaction of ammonia with carbon dioxide and water to form ammonium bicarbonate (Hasan et al. 2015). Alkalinity concentrations within the range of 1800 – 2200 mg-CaCO3/L has shown better reactor performance and hence can be regarded as suitable alkalinity range for herbicides removal.

4.4.3.1 Sequential anaerobic-aerobic treatment of herbicide mixtures (ametryn and 2,4-d)

Figure 4.17 shows the performance of A4 reactor treating anaerobic effluent of two herbicide mixtures. The aerobic reactor showed low performance for about 60 days with low COD removal efficiency of <70%. High COD concentration in the aerobic effluent indicated the incomplete biodegradation of organic compounds. On further continuation with the same influent herbicides concentration, the COD removal efficiency raised up to 80% on 140^{th} day. As discussed previously during the treatment of individual herbicides, it is clear to presume that the anaerobic metabolites of herbicides were degraded comparatively at higher efficiencies in the A4 reactor. A4 reactor was able to remove 80% of COD, alkalinity was between 2500 – 2850 mg-$CaCO_3$/L and VFA accounted for about 600 – 750 mg/L till the 190^{th} day.

After increasing the influent herbicide concentration the aerobic reactor performance reduced, COD removal efficiency reduced by 20% than the control. But interestingly the COD removal efficiency increased up to 92% within 40 days after raise. From then onwards the removal efficiencies dropped to lower level (<70%), but

this was lasted on continued operation and maximum COD removal efficiency of (>80%) was achieved finally.

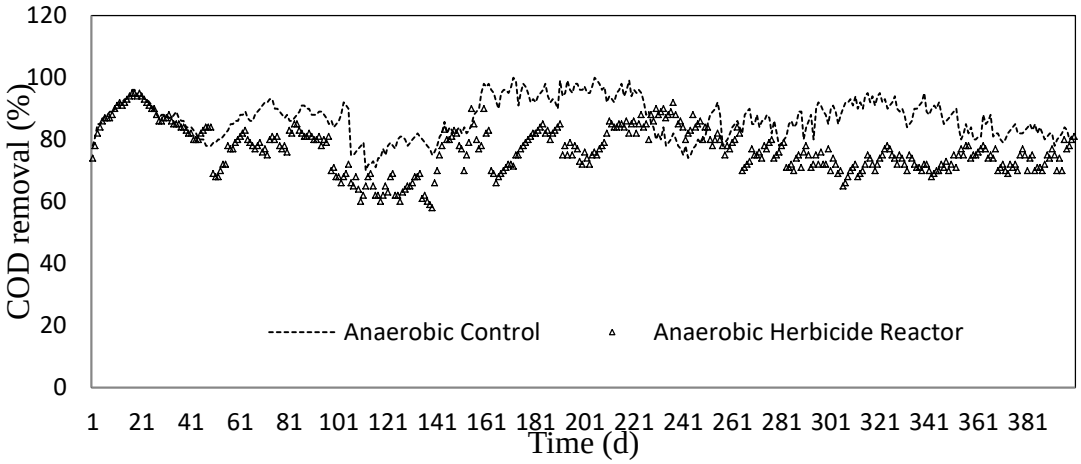


Figure 4.17: Performance parameters of aerobic reactor treating 2,4-d and ametryn mixture (A4) compared with aerobic control (A1)

About 700 – 1100 mg/L of VFA concentration was detected, which indicates the presence of biodegradable organic compounds. High COD removal efficiencies in the effluent may indicate the selected herbicide mixture did not cause inhibitions and also suggests that there may be high chances of herbicides being removed.

4.4.4 Treatment of 2,4-d, ametryn and dicamba mixtures in ASBR

The sequential anaerobic-aerobic treatment of mixture of ametryn, 24-d and dicamba was carried out in R5 and A5 reactor and the reactor operation condition is tabulated in the Table 4.7. The reactor performance was monitored through COD removal efficiency and biogas production due to difficulty of detecting herbicide mixture concentration using HPLC. Figure 4.18(a-c) shows the profiles of COD removal, biogas production and ORP as a function of time during anaerobic treatment three mixed herbicides in R5 reactor. After the introduction of herbicides mixture on day 49, the COD removal reduced to 44% and biogas production <460 mL/d, which is less than the control and all other reactors (R1, R2, R3 and R4).

Table 4.7: Operational conditions maintained during the sequential anaerobic-aerobic treatment of ametryn, 24-d and dicamba mixtures

Sl. No	Reactor operation (Days)	Experimental condition studied
1	1 – 48	Reactor start-up and acclimation to 2 g/L of starch (OLR = 0.21 – 0.215 kg-COD/m^3/d)
2	2 onwards	Anaerobic effluent fed to corresponding aerobic reactor
3	49 – 190	Influent herbicide concentration = (ametryn:2,4-d:dicamba) = (2:5:10) mg/L
4	116 – 142	Addition of AQS = 5 mg/L
5	143 – 190	Addition of AQS = 10 mg/L
6	191 – 400	Influent herbicide concentration = (ametryn:2,4-d:dicamba) = (4:10:20) mg/L
7	305 – 359	Addition of AQS = 5 mg/L
8	360 – 400	Addition of AQS = 10 mg/L

The COD removal efficiency remained lower than the R1, which indicated the presence of significant biodegradable organic compounds. ORP of the reactor (R5) was almost equal to the R1 reactor, and hence 5 mg/L of AQS was added from 116 day, and further reduce the ORP by -10 to -20 mV than the control was observed. AQS was raised to 10 mg/L from day 143 to improve the reactor efficiency then the ORP by -5 mV, but the maximum COD removal observed was about 70% and the biogas production was 550 mL/d. The R1 reactor was able to yield high COD removal efficiency (>80%) and biogas production (>600 mL/d), and suggests the accumulation of toxic intermediate compounds within the R5 reactor. Hence, the mixture of three herbicides is considered to be more toxic and difficult to remove under anaerobic conditions. High effluent COD, VFA and reduced MLVSS concentration indicates the anaerobic sludge toxicity by the accumulated compounds (Dasa et al. 2016).

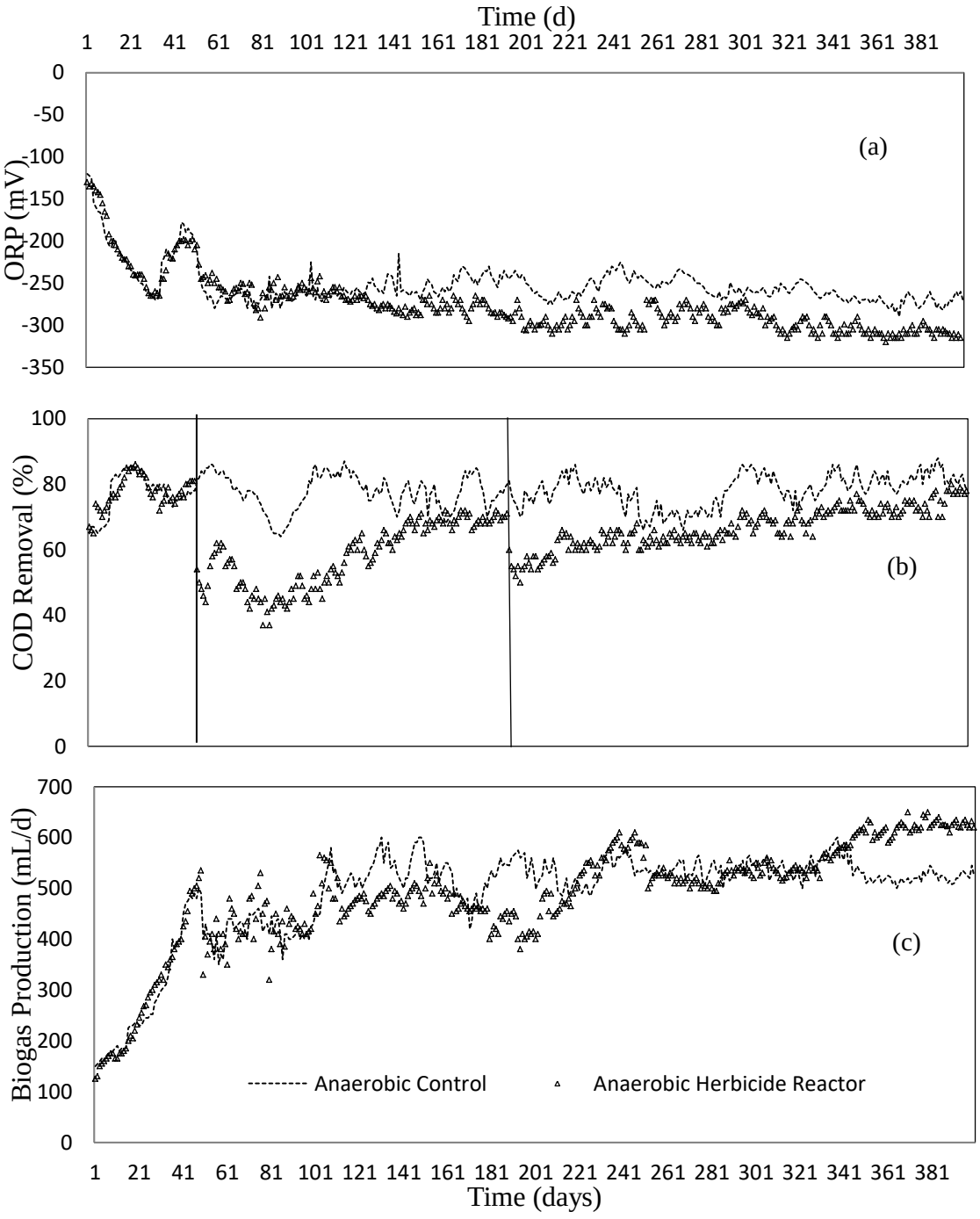


Figure 4.18(a-c): Variation of performance parameters during the anaerobic treatment of 2,4-d, ametryn and dicamba mixtures (R5) compared with anaerobic control (R1)

It is obvious that the mixture of three selected herbicides would cause more toxicity than the herbicides treated individually and also in mixture of two types. As expected the introduction of herbicides reactor performance reduced and observed that which followed similar pattern as obtained previously. While it has been reported

that the maximum removal of individual herbicides, 2,4-d (Chin et al. 2005); Celis et al. 2008), dicamba (Ghoshdastidar and Tong 2013) and ametryn (Navaratna et al. 2016) was achieved spontaneously. The treatment period required during the removal of individual herbicides was comparatively lesser than the number of days required during the treatment of mixture of three herbicides; this is observed even in the present study during ametryn and dicamba treatment.

The presence of high concentrations of chlorine groups would become toxic and reduces the biodegradability of chlorophenols (Annachhatre and Gheewala 1996). After observing a stable COD removal efficiency, the influent herbicides concentration was raised to two fold from 191st day. The COD removal reduced to 45%, further increased gradually (65%) on continued operation. The VFA and alkalinity observed was higher as expected during a toxic condition and the possible reasons are attributed to various parameters as discussed. High COD removal and biogas production observed than the control towards the end of treatment period. This behaviour may be an indication of bacterial adoptability and hence it may be considered that the mixture of three selected herbicides have tendency to get biodegraded anaerobically.

The biogas production in the reactor (R5) was much lower than control (R1) as observed from the Figure 4.18. But on a continued operation with the similar herbicide and reactor conditions and in the presence of AQS (5 – 10 mg/L), the biogas production started increasing above the control. The continued operation supports the biomass to get acquainted with the feed condition and thus can develop metabolic pathway required for the herbicide degradation. The increased biogas production of R5 than the R1 reactor indicates the herbicides have been converted to intermediates, VFA and further in to end products as CO_2/methane gas as observed during the treatment of individual herbicides. The toxicity of herbicides can be minimised in the presence of a co-substrate like starch and thus converted to TPs and then to biogas.

4.4.4.1 Sequential anaerobic-aerobic treatment of three herbicide mixtures

Figure 4.19 shows the profile of COD removal as a function of time during aerobic treatment of R5 effluent. COD removal efficiency is observed to be >80% till day 130 and it was comparatively lower than the control. The liquid content of the reactor turned to dark grey colour even at continued aeration (DO: 3 – 4 mg/L), indicates accumulation TPs. The poor sludge settling, low COD removal efficiencies observed till 80th day due to sludge toxicity. The grey colour was disappeared and high COD removal efficiency was achieved after 100 days. The continued operation supported the aerobic bacteria to slowly acquainted with system and allowed the activation of inactive biomass to metabolize the organic matter leading increased COD removal efficiency.

After raise in influent herbicide concentration, COD removal was around 82%, which suggest that the bacteria adapted to the existing influent condition to degrade particular compounds. Hence, further rise in herbicides concentration promotes enhanced removal efficiency than the previous stage. Such observations have been discussed previously by Ma et al. (2012), where 2,4-d was removed >99% in the presence of high influent chlorophenol concentrations. The significant amount of TPs of herbicides mixture leaving the system was indicated by high effluent COD and VFA (>900 mg/L).

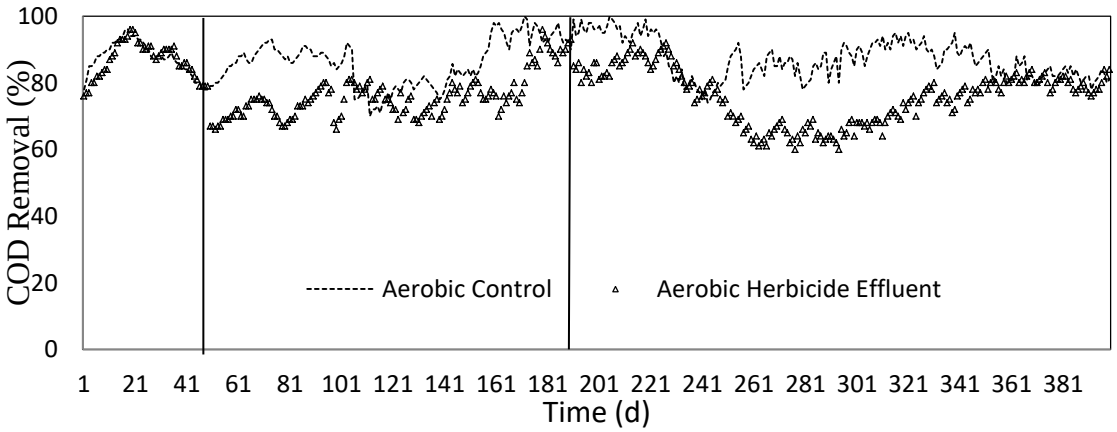


Figure 4.19: Performance parameters of aerobic reactor treating 2,4-d, ametryn and dicamba mixtures (A5) compared with the aerobic control (A1)

The COD removal efficiency of both A1 and A5 remained same for few days up to 243 days and A5 COD removal reduced to 60%, further gradual increase in the COD removal was observed over 75 days. The low COD removal efficiency in the A5 reactor than the A1 reactor by 15 – 22% would indicate the inability of aerobic biomass to degrade the TPs, which had contributed to high VFA concentration up to 1600 mg/L. This may be a toxic condition as discussed previously, and the alkalinity observed during this period was 2400 – 2800 mg-$CaCO_3$/L. The continued operation supported the degradation of VFA thereby reducing the effluent COD level. A stable COD removal efficiency of 80 – 85% was achieved from A1 and A5 reactors after 360 days of operation.

The similar COD removal efficiencies obtained for both control and herbicide treatment reactor and high VFA concentration indicates that anaerobic transformation products of three mixed herbicides have not completely degraded. High alkalinity observed on the initial days of herbicide introduction and also rises in the influent concentration for the second time. Eventually, it was observed that the reactor responded positively with increased COD removal, with reduction in alkalinity below 2400 mg-$CaCO_3$/L. This reactor is actually able to remove high COD removal than the A4 reactor treating only two herbicide mixtures; this may be an indication of transformation of herbicide compounds at different dosages. Thus the sequential anaerobic-aerobic system may be considered as efficient method to detoxify and also to remove mixture of herbicides which are potentially significant.

CHAPTER 5

SUMMARY AND CONCLUSIONS

In the present study, laboratory scale sequential anaerobic-aerobic reactors have been set up and operated to treat priority herbicides like ametryn, dicamba, mixtures of 2,4-d and ametryn, and 2,4-d, ametryn and dicamba. The research was conducted using starch as a primary carbon source and sodium bicarbonate as a buffer to maintain the pH favourable for methanogenic bacteria. The HRT was maintained constant as 48 h, pH between 6.4 – 7.7 and constant reactor liquid temperature. The reactors stability parameters pH, alkalinity, volatile fatty acids (VFA) and oxidation reduction potential (ORP) were monitored on daily basis

The experiments were carried out in 3 phases, in phase – I comprise of preliminary study during 60 days, which include reactor stabilization and treatment of constant herbicides dosage of 25 mg/L. Anaerobic reactor stabilization was achieved in 28 days and aerobic reactors were stabilized in 14 days.

In phase – II & III long term study was conducted for 400 – 430 days, which includes the reactor stabilization and varying herbicides loading along with redox mediator (AQS). The anaerobic reactors were stabilized using 2 g/L of starch within 48 days of operation and aerobic reactors were stabilized within 15 days of operation using anaerobic effluent as feed. This study has been found to be an efficient method for removal of selected herbicides and the significant outcomes of this study are listed as follows:

- The preliminary study conducted for 60 days with an influent OLR of 0.2025 kg-COD/m^3/d including 25 mg/L of 2,4-d, ametryn and dicamba in three separate reactors, the removal efficiencies obtained in the respective anaerobic reactor was >99% for 2,4-d, 22% for ametryn, and 58% for dicamba with a maximum COD removal of >80%.
- The overall removal efficiency of the system during the 60 days treatment was 2,4-d by 100%, ametryn by 72%, dicamba by 78%, and maximum COD removal observed was >85%.

- Complete mineralization of 4 – 6 mg/L of ametryn and >90% of COD removal efficiency was achieved in 50 days in the anaerobic reactor. The anaerobic biomass was able to convert ametryn in to carbon and nitrogen sources and it was deduced in the ametryn degradation pathway.
- The anaerobic sludge developed the granules after 30 days of ametryn introduction, which contained ANNAMOX bacteria responsible for anaerobic degradation of the compound and its transformation products.
- Treatment of ametryn concentration by 8 – 10 mg/L required more than 90 days for removal of ametryn and COD by >90%, and the addition of AQS (5 – 10 mg/L) enhanced the reactor performance with increased COD and ametryn removal by 10 to 12%.
- Ametryn acted as a nutrient source to the biomass instead of causing toxicity, the introduction of low ametryn and further increased concentration over long operation period contributed to stable biomass growth with enhanced removal efficiency up to 10 mg/L along with high biogas production in the R2 than the control (R1) by 300 – 450 mL/d.
- The aerobic reactor was able to remove ametryn completely up to 8 mg/L with COD of >90%. Further, higher ametryn removal efficiency than the anaerobic reactor up to 99% for initial 10 mg/L with 95% COD removal was achieved.
- The R2 reactor sludge stabilization (MLVSS/MLSS) ratio in the range of 0.68 – 0.82 was an indication of stable reactor condition. About 2.3 mg/g.MLVSS of ametryn adsorption was observed on to the reactor sludge initially, and it was desorbed further and no adsorption was observed on continued operation.
- The anaerobic dicamba removal efficiency was 68% with COD removal 80 – 95% for 10 – 40 mg/L of influent concentration was achieved. The maximum removal was achieved in the aerobic reactor by >92% for both dicamba and COD.
- Aerobic reactor developed sludge granulation after 98 days of dicamba treatment and the granules continued to develop thought the treatment period.

- High influent dicamba concentration between 60 – 100 mg/L was carried out with 88% removal in the presence of 5 – 20 mg/L of AQS in the anaerobic reactor. AQS was able to increase the dicamba removal efficiency by 15 – 20% with a maximum COD removal of >95%.
- The sludge stabilization (MLVSS/MLSS) ratio in the anaerobic dicamba treatment reactor was observed within 0.82 as required for the anaerobic treatment. About 5 – 8 mg/g.MLVSS of dicamba was adsorbed during the initial days of operation and later no adsorption was detected.
- The biogas production in the R3 reactor was slightly greater than the control by 150 – 200 mL/d due to conversion of dicamba to CO_2/CH_4.
- COD removal efficiency of R4 reactor was 71 – 75% in the presence of AQS (5 – 10 mg/L) over 400 days of operation for increased herbicides concentration. The addition of AQS has contributed to enhanced COD removal by 5 – 10% in the anaerobic reactor.
- The overall COD removal during the anaerobic-aerobic treatment of two herbicides mixture was 78 – 85%.
- The anaerobic COD removal efficiency greater than 78% was achieved for R5 reactor, and the addition of AQS (5 – 10 mg/L) contributed to increased COD removal efficiency by 5 – 10%. The anaerobic-aerobic system was able to remove the COD by 84 – 95%.
- The gradual adaptation of biomass to the toxic condition in both R4 and R5 reactors treating mixture of two and three herbicide compounds were able to produce increased biogas than the control by 50 – 150 mL/d after sufficient days of operation.

5.1 Recommendations for Future Research

- Treatment of high concentrations of ametryn and dicamba using sequential anaerobic-aerobic method may be developed, which can be suitable to treating pesticide industry effluents.
- Impact of change in operating conditions on herbicide removal efficiencies can be evaluated for improving the treatment efficiency at a faster rate.
- Development of methodology for quantifying the mixture of herbicides and their transformation products can be conducted.
- Further studies may be conducted to improve the treatment efficiency of herbicide mixtures in water.
- The integrated research involving the treatment of real time agriculture and industrial effluents containing herbicides along with domestic effluents for efficient removal of pollutants and recovery of biogas.
- Studies may be conducted to quantify the concentrations of intermediate compounds formed due to herbicides biotransformation.
- Research may be extended to find out the type of bacteria present in the reactor sludge by polymer chain reaction (PCR) amplification and 16 S rRNA sequencing.

REFERENCES

Abiri, F., Fallah, N., and Bonakdarpour, B. (2017). "Sequential anaerobic-aerobic biological treatment of colored wastewaters: Case study of a textile dyeing factory wastewater." *Water Sc. Technol.,* 75(6), 1261–1269.

Aga, D. S., and Thurman, E. M. (2001). "Formation and transport of the sulfonic acid metabolites of alachlor and metolachlor in soil." *Environ. Sc. Technol.,* 35(12), 2455-2460.

Allan, H. L., van de Merwe, J. P., Finlayson, K. A., O'Brien, J. W., Mueller, J. F., and Leusch, F. D. (2017). "Analysis of sugarcane herbicides in marine turtle nesting areas and assessment of risk using in vitro toxicity assays." *Chemosphere,* 185, 656-664.

Annachhatre, A. P., and Gheewala, S. H. (1996). "Biodegradation of chlorinated phenolic compounds." *Biotechnol. Advances,* 14(1), 35-56.

APHA, 2005 21st Edition Standard Methods for the Examination of Water and Wastewater. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC.

Aramrueang, N., Rapport, J., and Zhang, R. (2016). "Effects of hydraulic retention time and organic loading rate on performance and stability of anaerobic digestion of Spirulina platensis." *Biosys. Eng.,* 147, 174-182.

Asongalem, E. A., and Akintonwa, A. (1998). "Evaluation of effects of oral exposure to Ametryn on development of mice." *Pest. Sc.,* 53(1), 1-8.

Aspelin, A.L., and Grube, A.H. (1999). "Pesticides industry sales and usage in 1996 and 1997 market estimated." U.S. Environmental Protection Agency report no. 733-R-99-001.

Baghapour, M. A., Nasseri, S., and Derakhshan, Z. (2013). "Atrazine removal from aqueous solutions using submerged biological aerated filter." *J. Environ. Heal. Sci. Eng.,* 11(1), 1–9.

Balagué, C. E., Ruiz, C. S. De, Rey, R., Duffard, A. M. E. De, and Nader-Macías, M. E. (2002). "Effect of the herbicide 2,4-dichlorophenoxyacetic acid on uropathogenic Escherichia coli virulence factors." *Toxicology,* 177(2–3), 143–155.

Bhaskar, S., Manu, B., and Sreenivasa, M. Y. (2019). "Bacteriological synthesis of iron hydroxysulfate using an isolated Acidithiobacillus ferrooxidans strain and its

application in ametryn degradation by Fenton's oxidation process." *J. Environ. Manag.*, 232, 236-242.

Björnsson, L., Murto, M., and Mattiasson, B. (2000). "Evaluation of parameters for monitoring an anaerobic co-digestion process." *Appl.Micro.Biotechno.*, 54(6), 844-849.

Bonakdarpour, B., Vyrides, I., and Stuckey, D. C. (2011). "Comparison of the performance of one stage and two stage sequential anaerobic–aerobic biological processes for the treatment of reactive-azo-dye-containing synthetic wastewaters." Int. Biodet. Biodeg., 65(4), 591-599.

Briggs, S. A. (1992). "Basic guide to pesticides: their characteristics and hazards." Taylor and Francis.

Broholm, M. M., Rügge, K., Tuxen, N., Højberg, A. L., Mosbæk, H., and Bjerg, P. L. (2001). "Fate of herbicides in a shallow aerobic aquifer: a continuous field injection experiment (Vejen, Denmark)." *Water Res. Res.*, 37(12), 3163-3176.

Canada. Environment Canada and Environment Canada Pesticide Program Coordinating Committee. (2005). Pesticide utilization in Canada: A compilation of current sales and use data. Environment Canada.

Celis, E., Elefsiniotis, P., and Singhal, N. (2008). "Biodegradation of agricultural herbicides in sequencing batch reactors under aerobic or anaerobic conditions." *Water Res.*, 42(12), 3218–3224.

Cervantes, F. J., Vu-Thi-Thu, L., Lettinga, G., and Field, J. A. (2004). "Quinone-respiration improves dechlorination of carbon tetrachloride by anaerobic sludge." *Appl. Microbiol. Biotechnol.*, 64:702–11.

Chen, S., Zhang, J., and Wang, X. (2015). "Effects of alkalinity sources on the stability of anaerobic digestion from food waste." *Waste Manag. Res.*, 33(11), 1033-1040.

Chin, H., Elefsiniotis, P., and Singhal, N. (2005). "Biodegradation of 2, 4-dicholophenoxyacetic acid using an acidogenic anaerobic sequencing batch reactor." *J. Environ. Eng. Sc.*, 4(1), 57-63.

Chiron S., Fernandez-Alba A., Rodriguez A. & Garcia-Calvo E. 2000 Pesticide chemical oxidation: state-of-the-art. *Water Res.*, 34(2), 366-377.

Chu, L., Zhang, X., Yang, F., and Li, X. (2006). “Treatment of domestic wastewater by using a microaerobic membrane bioreactor.” *Desalination*, 189(1-3), 181-192.

Comfort, S. D., Inskeep, W. P., and Macur, R. E. (1992). “Degradation and Transport of Dicamba in a Clay Soil.” *J. Environ. Qual.*, 21(4), 653 – 658.

Conte, L. O., Schenone, A. V., and Alfano, O. M. (2016). “Photo-Fenton degradation of the herbicide 2, 4-D in aqueous medium at pH conditions close to neutrality.” *J. Environ. Manag.*, 170, 60-69.

Cook, A. M., and Huetter, R. (1981). “S-triazines as nitrogen sources for bacteria.” *J. Agricul. Food Chem.*, 29, 1135-1143.

Cook, A. M., Beilstein, P., Grossenbacher, H., and Hütter, R. (1985). “Ring cleavage and degradative pathway of cyanuric acid in bacteria.” *Biochem. J.* 231, 25-30.

Cox, C. (1994). “Dicamba.” *J. Pestic. Reform*, 14, 30–35.

Curtis, G. P., and Reinhard, M. (1994). “Reductive dehalogenation of hexachloroethane, carbon tetrachloride, and bromoform by anthraquinone disulfonate and humic acid.” *Environ. Sc. Technol.*, 28, 2393–401.

Da Silva, M. E. R., Firmino, P. I. M., and Dos Santos, A. B. (2012). “Impact of the redox mediator sodium anthraquinone-2, 6-disulphonate (AQDS) on the reductive decolourisation of the azo dye Reactive Red 2 (RR2) in one-and two-stage anaerobic systems.” *Biores. Technol.*, 121: 1-7.

Dasa, K. T., Westman, S. Y., Millati, R., Cahyanto, M. N., Taherzadeh, M. J., and Niklasson, C. (2016). “Inhibitory effect of long-chain fatty acids on biogas production and the protective effect of membrane bioreactor.” BioMed. Res. Intern.

Statistical database, “Directorate of plant protection, quarantine & storage,” Govt. of India. (2019). http://ppqs.gov.in/statistical-database?page=1.

De, A., Bose, R., Kumar, A., and Mozumdar, S. (2014). “Targeted delivery of pesticides using biodegradable polymeric nanoparticles.” New Delhi: Springer India.

Dhawan, V. (2017). “Water and Agriculture in India.” *Backgr. Pap. South Asia Expert panel Dur. Glob. Forum Food Agric.*, 28.

Drzewicz, P., Gehringer, P., Bojanowska-Czajka, A., Zona, R., Solar, S., Nałęcz-Jawecki, G., Sawicki, J., and Trojanowicz, M. (2005). “Radiolytic degradation of the herbicide dicamba for environmental protection.” *Arch. Environ. Contam. Toxicol.*, 48(3), 311–322.

Dutta, A., and Sarkar, S. (2015). "Sequencing Batch Reactor for Wastewater Treatment: Recent Advances." *Curr. Pollut. Reports*, 1(3), 177–190.

Deshpande, T. 2017. *State of Agriculture in India.* PRS Legislative Research, 6-7. http://prsindia.org/sites/default/files/parliament_or_policy_pdfs/State%20of%20Agriculture%20in%20India.pdf (Accessed on: 20.04.2018).

Field, J. A., and Brady, J. (2003). "Riboflavin as a redox mediator accelerating the reduction of the azo dye Mordant Yellow 10 by anaerobic granular sludge." *Water Sc. Technol.*, 48(6), 187-193.

Field, J. A., Stams, A. J. M., Kato, M., and Schraa, G. (1995). "Enhanced biodegradation of aromatic pollutants in cultures of anaerobic and aerobic bacterial consortia." *Antonie van Leeuwenhoek*, 67(1), 47-77.

Frías, S., Sánchez, M.J., and Rodríguez, M.A. (2004). "Determination of triazine compounds in ground water samples by micellar electrokinetic capillary chromatography." *Anal. Chim. Acta* 503, 271–278.

Frijters, C. T. M. J., Vos, R. H., Scheffer, G., and Mulder, R. (2006). "Decolorizing and detoxifying textile wastewater, containing both soluble and insoluble dyes, in a full scale combined anaerobic/aerobic system." *Water Res.*, 40(6), 1249–1257.

Gao, N. Y., Deng, Y. and Zhao, D. (2009). "Ametryn degradation in the ultraviolet (UV) irradiation/hydrogen peroxide (H_2O_2) treatment." *J. Haz. Mater.*, 164(2), 640-645.

Gaunt, P., and Hester, K. W. (1989). "A kinetic model for volatile fatty acid biodegradation during aerobic treatment of piggery wastes." *Biotechnol. Bioeng.*, 34(1), 126-130.

Ghattas, A. K., Fischer, F., Wick, A., and Ternes, T. A. (2017). "Anaerobic biodegradation of (emerging) organic contaminants in the aquatic environment." *Water Res.*, 116, 268-295.

Ghosh, P.K., and Philip, L. (2004). "Atrazine degradation in anaerobic environment by a mixed microbial consortium." *Water Res.*, 38, 2277 – 2284.

Ghoshdastidar, A. J., and Tong, A. Z. (2013). "Treatment of 2,4-D, mecoprop, and dicamba using membrane bioreactor technology." *Environ. Sc. Pollut. Res.*, 20(8), 5188–5197.

Gibson, J., and S. Harwood, C. (2002). "Metabolic Diversity in Aromatic Compound

Utilization by Anaerobic Microbes." *Annu. Rev. Microbiol.*, 56(1), 345–369.

González-Cuna, S., Galíndez-Mayer, J., Ruiz-Ordaz, N., Murugesan, S., Piña-Escobedo, A., García-Mena, J., and Santoyo-Tepole, F. (2016). "Aerobic biofilm reactor for treating a commercial formulation of the herbicides 2, 4-D and Dicamba: Biodegradation kinetics and biofilm bacterial diversity." *Int. Biodeter. Biodeg.*,107, 123-131.

Hamilton, D. J., Ambrus, A., Dieterle, R. M., Felsot, A. S., Harris, C. A., Holland, P. T., and Wong, S. S. (2003). "Regulatory limits for pesticide residues in water (IUPAC Technical Report)." *Pure Appl. Chem.*, 75(8), 1123-1155.

He, X. (2006). "The use of naturally generated volatile fatty acids for pesticide removal during the denitrification process." Ph.D. Thesis, The University of Canterbury, Christchurch, New Zealand.

He, X., and Wareham, D. G. (2011). "2,4-D removal via denitrification using volatile fatty acids." *Water Sc. Technol.*, 63(1), 178–183.

Hurley P. M. (1998) "Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents." *Environ. Health Perspec.*, 106(8), 437.

Irvine, R. L., Ketchum Jr, L. H., and Asano, T. (1989). "Sequencing batch reactors for biological wastewater treatment." *Critical Rev. Environ. Sc. Technol.*, 18(4), 255-294.

Isa, M. H., Farooqi, I. H., and Siddiqi, R. H. (1993). "Methanogenic activity test for study of anaerobic processes." *Indian J. Environ. Health*, 35, 1-8.

Jones, L.R., Owen, S.A., Horrell, P., and Burns, R.G. (1998). "Bacterial inoculation of granular activated carbon filters for the removal of atrazine from surface water." *Water Res.*, 32, 2542 - 2549.

Kapdan, I. K., and Oztekin, R. (2006). "The effect of hydraulic residence time and initial COD concentration on color and COD removal performance of the anaerobic–aerobic SBR system." *J. Haz. Mater.*, 136(3), 896-901.

Kappler, A., and Haderlein, S.B. (2003). "Natural organic matter as reductant for chlorinated aliphatic pollutants." *Environ. Sc. Technol.*, 37:2714–9.

Katz, I., Dosoretz, C.G., Mandelbaum, R.T., and Green, M. (2001). "Atrazine degradation under denitrifying conditions in continuous culture of *Pseudomonas* ADP." *Water Res.*, 35, 3272 –3275.

Khan, M. Z., Mondal, P. K., Sabir, S., and Tare, V. (2011). "Degradation pathway, toxicity and kinetics of 2, 4, 6-trichlorophenol with different co-substrate by aerobic granules in SBR." *Biores. Technol.,* 102(13), 7016-7021.

Khorsandi, H., Ghochlavi, N., and Aghapour, A. A. (2018). "Biological Degradation of 2, 4, 6-Trichlorophenol by a Sequencing Batch Reactor." *Environ. Proc.*, 5, 907-917.

Koh, Y. K. K., Chiu, T. Y., Boobis, A., Cartmell, E., Scrimshaw, M. D., and Lester, J. N. (2008). "Treatment and removal strategies for estrogens from wastewater." *Environ. Technol.,* 29(3), 245-267.

Krueger, J.P., Butz, R.G, and Cork, D.J. (1991). "Aerobic and anaerobic soil metabolism of dicamba." *J. Agric. Food Chem.,* 39, 995–999.

Kuppusamy, S., Jayaraman, N., Jagannathan, M., Kadarkarai, M., and Aruliah, R. (2017). "Electrochemical decolorization and biodegradation of tannery effluent for reduction of chemical oxygen demand and hexavalent chromium*." J. Water Process Eng.*, 20, 22-28.

Laabs, V., Amelung, W., Pinto, A. A., Wantzen, M., da Silva, C. J., and Zech, W. (2002). "Pesticides in surface water, sediment, and rainfall of the northeastern Pantanal basin, Brazil." *J. Environ. Qual.,* 31(5), 1636-1648.

Laganà, A., Bacaloni, A., De Leva, I., Faberi, A., Fago, G. And Marino, A. (2002). "Occurrence and determination of herbicides and their major transformation products in environmental waters." *Anal. Chimi. Acta,* 462(2), 187-198.

Li, B., and Wu, G. (2014). "Effects of sludge retention times on nutrient removal and nitrous oxide emission in biological nutrient removal processes." *Int. J. Environ. Res. Public Health,* 11(4), 3553–3569.

Liu, H., Han, P., Liu, H., Zhou, G., Fu, B., and Zheng, Z. (2018). "Full-scale production of VFAs from sewage sludge by anaerobic alkaline fermentation to improve biological nutrients removal in domestic wastewater." *Biores. Technol., 260,* 105-114.

Liu, Z., Wang, Y., Zhu, Z., Yang, E., Feng, X., Fu, Z., and Jin, Y. (2016). "Atrazine and its main metabolites alter the locomotor activity of larval zebrafish (Danio rerio)." *Chemosphere,* 148, 163-170.

Ma, J. Y., Quan, X. C., Yang, Z. F., and Li, A. J. (2012). "Biodegradation of a mixture of 2, 4-dichlorophenoxyacetic acid and multiple chlorophenols by aerobic granules cultivated through plasmid pJP4 mediated bioaugmentation." *Chem. Eng. J.*, 181, 144-151.

Magga, Z., Tzovolou, D. N., Theodoropoulou, M. A., Dalkarani, T., Pikios, K. and Tsakiroglou, C. D. (2008). "Soil column experiments used as a means to assess transport, sorption, and biodegradation of pesticides in groundwater." *J. Environ. Sc. Health Part B,* 43(8), 732-741.

Mahesh, G. B., and Manu, B. (2019a). "Biodegradation of ametryn and dicamba in a sequential anaerobic-aerobic batch reactor: A case study." *Water Pract. Technol.,* 14(2), 423 – 434.

Mahesh, G. B., and Manu, B. (2019b). "Biological Treatment of 3,6-Dichloro-2-Methoxybenzoic Acid Using Anaerobic-Aerobic Sequential Batch Reactor." *Environ. Process.,* 6(2), 493-509.

Mahesh, G. B., and Manu, B. (2019c). "Removal of ametryn and organic matter from wastewater using sequential anaerobic-aerobic batch reactor: A performance evaluation study." *J. Environ. Manag.,* 249.

Mangat, S. S., and Elefsiniotis, P. (1999). "Biodegradation of the herbicide 2, 4-dichlorophenoxyacetic acid (2, 4-D) in sequencing batch reactors." *Water Res.,* 33(3), 861-867.

Manu, B., and Chaudhari, S. (2002). "Anaerobic decolorisation of simulated textile wastewater containing azo dyes." *Bioresour. Technol.*, 82(3), 225–231.

Manu, B., and Chaudhari, S. (2003). "Decolorization of indigo and azo dyes in semicontinuous reactors with long hydraulic retention time." *Process Biochem.,* 38(8), 1213–1221.

Marco, A., Esplugas, S., and Saum, G. (1997). "How and why combine chemical and biological processes for wastewater treatment." *Water Sc. Technol., 35*(4), 321-327.

McCartney, D. M., and Oleszkiewicz, J. A. (1991). "Sulfide inhibition of anaerobic degradation of lactate and acetate." *Water Res.,* 25(2), 203–209.

McTernan W. F., and Pereira J. A. (1991). "Biotransformation of lindane and 2,4-D in batch enrich-ment cultures." *Water Res.,* 22, 1417-1423.

Meric, S., Eremektar, G., Ciner, F., and Tunay, O. (2003). “An OUR-based approach to determine the toxic effects of 2,4-dichlorophenoxyacetic acid in activated sludge.” *J. Haza. Mater.,* 101 (2), 147–155.

Metcalf, E., Eddy, H., 1991.Wastewater Engineering: Treatment. Disposal, and Reuse, vol. 3.

Milligan, P. W., and Häggblom, M. M. (1999). “Biodegradation and biotransformation of Dicamba under different reducing conditions.” *Environ. Sc. Technol.,* 33(8), 1224-1229.

Mino, T., Pedro, D. C. S., and Matssuo, T. (1995). “Estimation of rate slowly biodegradable COD (SBCOD) hydrolysis under anaerobic, anoxic and aerobic conditions by experiments using starch as model substrate.” *Wat. Sc. Technol.,* 31, 95-103.

Mkhize, N. T., Msagati, T. A., Mamba, B. B., and Momba, M. (2014). “Determination of volatile fatty acids in wastewater by solvent extraction and gas chromatography.” *Physics Chem. Earth, Parts A/B/C,* 67, 86-92.

Mohan, S.V., Rao, N.C., Prasad, K.K., and Sarma, P.N. (2005). “Bioaugmentation of an anaerobic sequencing batch biofilm reactor with immobilized sulphate reducing bacteria for the treatment of sulphate bearing chemical wastewater.” *Proc. Biochem.,* 40(8), 2849–2857.

Murphy, C. D., Clark, B. R., and Amadio, J. (2009). “Metabolism of fluoroorganic compounds in microorganisms: Impacts for the environment and the production of fine chemicals.” *Appl. Microbiol. Biotechnol.,* 84(4), 617–629.

Navaratna, D., Elliman, J., Cooper, A., Shu, L., Baskaran, K., and Jegatheesan, V. (2012). “Impact of herbicide Ametryn on microbial communities in mixed liquor of a membrane bioreactor (MBR).” *Biores. Technol.,* 113, 181–190.

Navaratna, D., Shu, L., and Jegatheesan, V. (2016). “Evaluation of herbicide (persistent pollutant) removal mechanisms through hybrid membrane bioreactors.” *Biores. Technol*., 200, 795-803.

O’neill, C., Hawkes, F. R., Hawkes, D. L., Esteves, S., and Wilcox, S. J. (2000). “Anaerobic–aerobic biotreatment of simulated textile effluent containing varied ratios of starch and azo dye.” *Water Res.,* 34(8), 2355-2361.

OECD. 2008: *Environmental performance of agriculture at a glance*. Organisation for Economic Co-operation and Development. http://www.oecd.org/greengrowth/sustainable-agriculture/40953155.pdf/ (accessed on 20.11.2017).

Orhon D., Talinli I., and TuÈ nay O. (1989). "The fate of 2,4-D in microbial cultures." *Water Res.,* 23, 1423-1430.

Penha, S., Matos, M., and Franco, F., (2005). "Evaluation of an integrated anaerobic/aerobic SBR system for the treatment of wool dyeing effluents." *Biodegradation,* 16, 81-89.

Pereira, M. A., Pires, O. C., Mota M., and Alves, M. M. (2002). "Anaerobic degradation of oleic acid by suspended and granular sludge: identification of palmitic acid as a key intermediate." *Water Sc. Technol.*, 45(10), 139-144.

Peters, L. P., Carvalho, G., Martins, P. F., Dourado, M. N., Vilhena, M. B., Pileggi, M., and Azevedo, R. A. (2014). "Differential responses of the antioxidant system of ametryn and clomazone tolerant bacteria." *PloS one*, 9(11), e112271.

Pirsaheb, M., Mohamadi, S., Rahmatabadi, S., Hossini, H., and Motteran, F. (2018). "Simultaneous wastewater treatment and biogas production using integrated anaerobic baffled reactor granular activated carbon from baker's yeast wastewater." *Environ. Technol.*, 39(21), 2724–2735.

Polprasert, C., and Haas, C. (1995). "Effect of sulphate on anaerobic processes fed with dualsubstrates." *Wat. Sc. Technol.,* 31(9), 101-107.

Prakash, S. M., and Gupta, S. K. (2000). "Biodegradation of tetrachloroethylene in upflow anaerobic sludge blanket reactor." *Biores. Technol.*, 72(1), 47–54.

Ratledge, C. (1992). "Microbial oxidations of fatty alcohols and fatty acids." *J. Chem. Technol. Biotechnol.,* 55: 399-400.

Rau, J., Knackmuss, H. J., and Stolz, A. (2002). "Effects of different quinoid redox mediators on the anaerobic reduction of azo dyes by bacteria." *Environ. Sc. Tech.,* 36(7), 1497-1504.

Roberts, T., and Hutson, D. (1999). "Metabolic pathways of agrochemicals-part one herbicides and plant growth regulation." *The Royal Soc. Chem.,* London, 188-215.

Ross, W. R. (1992). “Anaerobic Digestion of Waste-Water Sludge: Operating Guide. Water Research Commission.” Water Institute of Southern Africa, Pretoria, South Africa.

Sambusiti, C., Ficara, E., Malpei, F., Steyer, J. P., and Carrère, H. (2013). “Benefit of sodium hydroxide pretreatment of ensiled sorghum forage on the anaerobic reactor stability and methane production.” *Biores. Technol*., 144, 149-155.

Sánchez-Sánchez, R., Ahuatzi-Chacón, D., Galíndez-Mayer, J., Ruiz-Ordaz, N., and Salmerón-Alcocer, A. (2013). “Removal of triazine herbicides from aqueous systems by a biofilm reactor continuously or intermittently operated.” *J. Environ. Manag.*, 128, 421–426.

Sanderson, J. T., Seinen, W., Giesy, J. P., and Van den Berg, M. (2000). “2-Chloro-s-triazine herbicides induce aromatase (CYP19) activity in H295R human adrenocortical carcinoma cells: a novel mechanism for estrogenicity?.” *Toxicol. Sc.*, 54, 121-127.

Sandoval-Carrasco, C. A., Ahuatzi-Chacón, D., Galíndez-Mayer, J., Ruiz-Ordaz, N., Juárez-Ramírez, C., and Martínez-Jerónimo, F. (2013). “Biodegradation of a mixture of the herbicides ametryn, and 2,4-dichlorophenoxyacetic acid (2,4-D) in a compartmentalized biofilm reactor.” *Biores. Technol.*, 145, 33–36.

Sangami, S., and Manu, B. (2017a). “Fenton’s treatment of actual agriculture runoff water containing herbicides.” *Water Sc. Technol.*, 75(2), 451–461.

Sangami, S., and Manu, B. (2017b). “Optimization of Fenton’s oxidation of herbicide dicamba in water using response surface methodology.” *Appl. Water Sc.*, 7(8), 4269–4280.

Sangami, S., and Manu, B. (2018). “Catalytic efficiency of laterite-based FeNPs for the mineralization of mixture of herbicides in water.” *Environ. Technol.,* 1–13.

Sene, L., Converti, A., Secchi, G. A. R., and Simão, R. d C. G. (2010). “New aspects on atrazine biodegradation.” *Brazilian Arch. Biol. Technol.*, 53(2), 487–496.

Sentürk, E., Ince, M., and Engin, G. O. (2010). “Treatment efficiency and VFA composition of a thermophilic anaerobic contact reactor treating food industry wastewater.” *J. Haz. Mater.,* 176(1-3), 843-848.

Shawaqfeh, A. T. (2010). “Removal of pesticides from water using anaerobic-aerobic biological treatment.” *Chinese J. Chem. Eng.,* 18(4), 672-680.

Shin, E.H., Choi, J.H., Abd, El-Aty, A.M., Khay, S., Kim, S.J., Im, M.H., and Shim, J.H. (2011). "Simultaneous determination of three acidic herbicide residues in food crops using HPLC and confirmation via LC-MS/MS." *Biomed. Chromatogr.,* 25(1-2): 124-135.

Shin, H. S., Kim, S. H., Lee, C. Y., and Nam, S. Y. (2003). "Inhibitory effects of long-chain fatty acids on VFA degradation and β-oxidation." *Water Sc. Technol.,* 47(10), 139–146.

Singh, A., Ward, O.P. (Eds.), 2004. Biodegradation and Bioremediation. Springer, Berlin, Germany.

Singh, P., Suri, C.R., and Cameotra, S.S. (2004). "Isolation of a member of *Acinetobacter* species involved in atrazine degradation." *Bioch. Bioph. Res. Comm.,* 317, 697 - 702.

Smith, D. T., Richard Jr, E. P., Santo, L. T., LeBaron, H., McFarland, J., and Burnside, O. (2008). "Weed control in sugarcane and the role of triazine herbicides." *The Triazine Herbicides*, 50, 185-197.

Speece, R.E. (1996). "Anaerobic biotechnology for industrial wastewaters." Archae Press, Nashville, Tenn.

Sponza, D. T., and Işik, M. (2002). "Decolorization and azo dye degradation by anaerobic/aerobic sequential process." *Enzyme Micro. Technol.,* 31(1-2), 102-110.

Sponza, D. T., and Uluköy, A. (2005). "Treatment of 2, 4-dichlorophenol (DCP) in a sequential anaerobic (upflow anaerobic sludge blanket) aerobic (completely stirred tank) reactor system." *Proc. Biochem.,* 40(11), 3419-3428.

Sponza, D. T., and Uluköy, A. (2006). "Treatment of 2,4 dichlorophenol (DCP) in a sequential anaerobic (upflow anaerobic sludge blanket) aerobic (completely stirred tank) reactor system at increasing organic loading rates." *Desalination*, 195(1–3), 235–250.

Stronach, S. M., Rudd, T., and Lester, J. N. (1986). "Anaerobic digestion processes in industrial wastewater treatment." Springer-Verlag, Berlin Heidelberg, Germany.

Suflita, J. M., Horowitz, A., Shelton, D. R., and Tiedje, J. M. (1982). "Dehalogenation: a novel pathway for the anaerobic biodegradation of haloaromatic compounds." *Science*, 218(4577), 1115-1117.

Szewczyk, R., Kuśmierska, A., and Bernat, P. (2018). "Ametryn removal by Metarhizium brunneum: Biodegradation pathway proposal and metabolic background revealed." *Chemosphere*, 190,174-183.

Taraban, R. H., Berry, D. F., Berry, D. A., and Walker, H. L. (1993). "Degradation of dicamba by an anaerobic consortium enriched from wetland soil." *Appl. Environ. Microbiol.,* 59(7), 2332-2334.

Tata strategic report, (2016). "Next Generation Indian Agriculture - Role of Crop Protection Solutions, a report on Indian Agrochemical Industry FICCI." http://ficci.in/spdocument/20744/Agrochemicals-Knowledge-report-2016.pdf (accessed on 15.07.2017).

Tchobanoglous, T., Burton, F. L., and Stensel, H. D. (2003). "Wastewater Engineering – Treatment and Reuse." New Delhi: Tata McGraw-Hill Publishers.

Terry, E. Baxter. (2014). "Standard Operating Procedure: Approximate Volatile Acids by Titration". https://www.cefns.nau.edu/~teb/ambl/ambl_SOPs.html (accessed on 10.01.2017).

Tomlin, C. D. S. (2006). "The pesticides manual: a world compendium." *British Crop Protec. Council,* 14, 351.

Tratnyek, P. G., Scherer, M. M., Deng, B. L., and Hu, S. D. (2001). "Effects of natural organic matter, anthropogenic surfactants, and model quinones on the reduction of contaminants by zero-valent iron." *Water Res.,* 35, 4435–43.

Tsutsui, H., Anami, Y., Matsuda, M., Hashimoto, K., Inoue, D., Sei, K., Soda, S., and Ike, M. (2013). "Plasmid-mediated bioaugmentation of sequencing batch reactors for enhancement of 2,4-dichlorophenoxyacetic acid removal in wastewater using plasmid pJP4." *Biodegradation*, 24(3), 343–352.

United States Environment Protection Agency. (2005). "Prevention, Pesticides and Toxic Substances (7508C). Registration Eligibility Decision for 2,4-D." http://archive.epa.gov/pesticides/reregistration/web/pdf/24d_red.pdf (accessed on 31.01.2017).

USEPA (2010). "Reregistration eligibility decision (red) for ametryn." https://archive.epa.gov/pesticides/reregistration/web/pdf/Ametryn_red.pdf (accessed on 21.06.20.18).

USEPA, (2018) “Registration Decision for the Continuation of Uses of Dicamba on Dicamba Tolerant Cotton and Soybean”
https://www.regulations.gov/document?D=EPA-HQ-OPP-2016-0187-0968 (accessed on 08.12.2018).
USEPA. (2007). Method 1699: Pesticides in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS.
Van der Zee, F. P., and Cervante, F. J. (2009). “Impact and application of electron shuttles on the redox (bio) transformation of contaminants: a review.” *Biotechnol. Advances,* 27(3), 256-277.
Vargha, M., Takats, Z., and Márialigeti K. (2005). “Degradation of atrazine in a laboratory scale model system with Danube river sediment.” *Water Res.,* 39, 1560 - 1568.
Velisek, J., Stara, A., Zuskova, E., and Kouba, A. (2017). “Effects of three triazine metabolites and their mixture at environmentally relevant concentrations on early life stages of marbled crayfish (Procambarus fallax f. virginalis).” *Chemosphere,* 175, 440-445.
Von Sperling, M., and De Lemos Chernicharo, C. A. (2017). “Biological wastewater treatment in warm climate regions (p. 857).” IWA publishing.
Wang, D., Ji, M., and Wang, C. (2014). “Degradation of organic pollutants and characteristics of activated sludge in an anaerobic/anoxic/oxic reactor treating chemical industrial wastewater.” *Brazilian J. Chem. Eng.*, 31(3), 703–713.
Wang, H., Li, X., Gong, Z., Wang, X., Liang, H., and Gao, D. (2018). “Co-metabolic substrates enhanced biological nitrogen removal from cellulosic ethanol biorefinery wastewater using aerobic granular sludges.” *Environ. Technol.,* 1–22.
Wang, Y. S., Duh, J. R., Liang, Y. F., and Chen, Y. L. (1995). “Dissipation of three s-triazine herbicides, atrazine, simazine, and Ametryn, in subtropical soils.” *Bull. Environ. Contam. Toxicol.,* 55(3), 351-358.
Weaver, M. A., Zablotowicz, R. M., and Locke, M. A. (2004). “Laboratory assessment of atrazine and fluometuron degradation in soils from a constructed wetland.” *Chemosphere,* 57(8), 853-862.
Weinberg, B., and Teodosiu, C. (2012). “Monitoring and assessment of herbicides removal by industrial wastewater treatment.” *Environ. Eng. Manag. J.,* 11(1), 215–

224.

Wilderer, P. A., Irvine, R. L., and Goronszy, M. C. (Eds.). (2001). Sequencing batch reactor technology. IWA publishing.

World Health Organization. (2003). "2,4-D in Drinking-water." *2,4-D Drink.*, 1–16.

Xu, B., Gao, N. Y., Cheng, H., Hu, C. Y., Xia, S. J., Sun, X. F., and Yang, S. (2009). "Ametryn degradation by aqueous chlorine: kinetics and reaction influences." *J. Haz. Mater.*, 169(1), 586-592.

Yeruva, D. K., Jukuri, S., Velvizhi, G., Kumar, A. N., Swamy, Y.V., and Mohan, S.V. (2015). "Integrating sequencing batch reactor with bio-electrochemical treatment for augmenting remediation efficiency of complex petrochemical wastewater." *Biores. Technol.*, 188, 33-42.

Zaiat, M., Rodrigues, J. A. D., Ratusznei, S. M., Camargo, E. F. M. De, and Borzani, W. (2001). "Anaerobic sequencing batch reactors for wastewater treatment: A developing technology." *Appl. Microbiol. Biotechnol.*, 55(1), 29–35.

APPENDIX

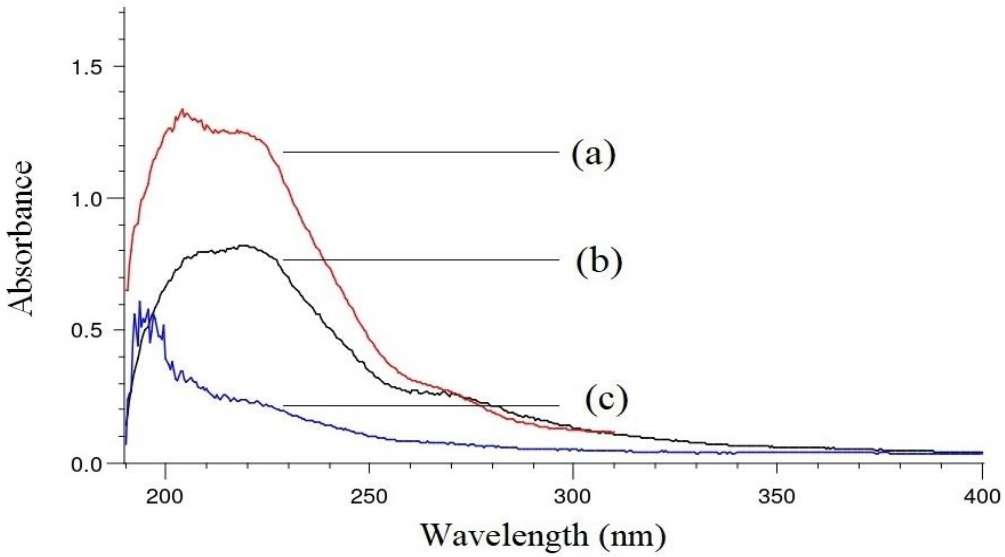
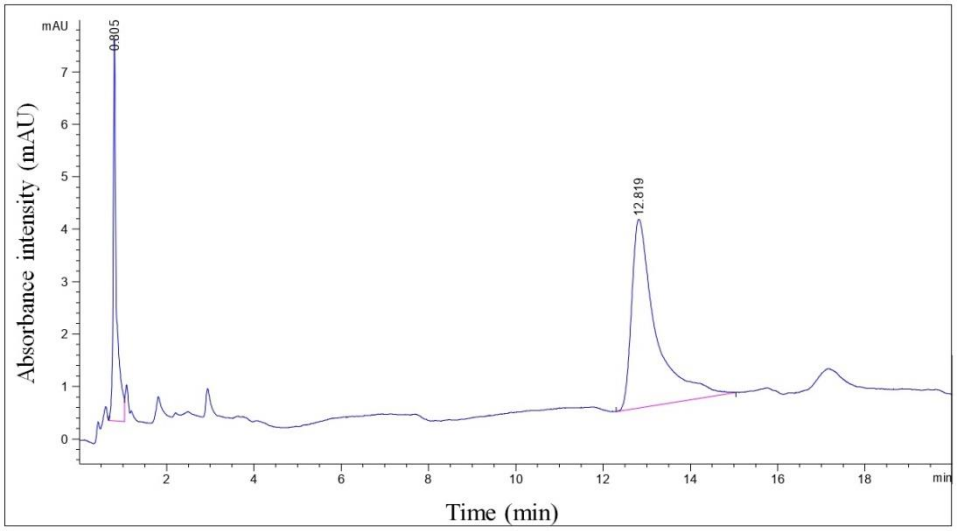
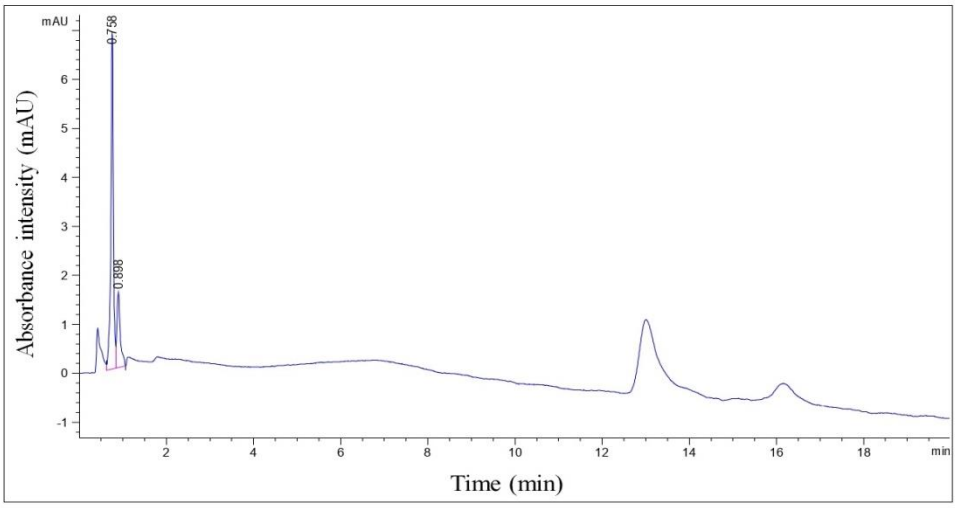


Figure S1: UV-Spectra obtained for: (a) influent, (b) anaerobic effluent on day 80, and (c) on day 97



(a)



(b)

Figure S2: The HPLC reports obtained for (a) influent and (b) effluent R2 reactor

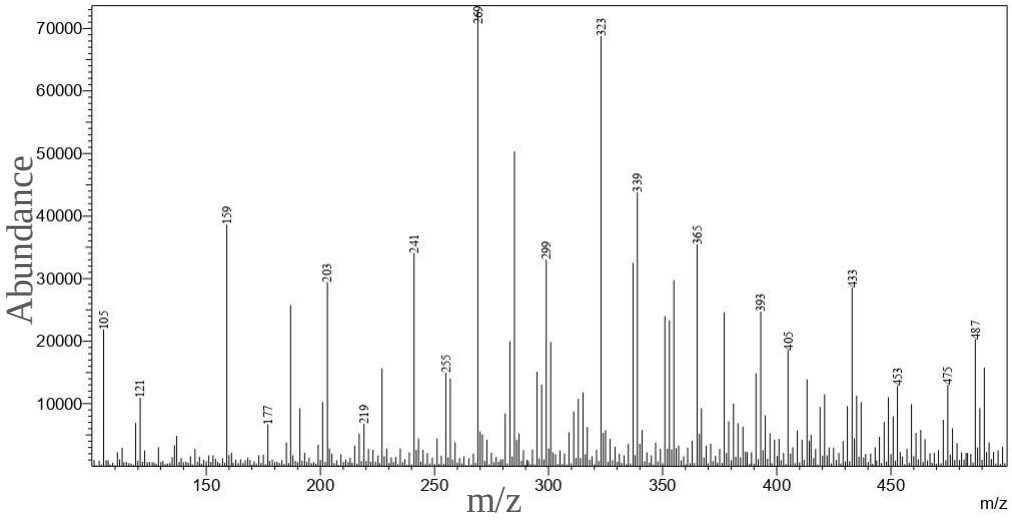


Figure S3: The LC-MS report showing the major TPs of ametryn

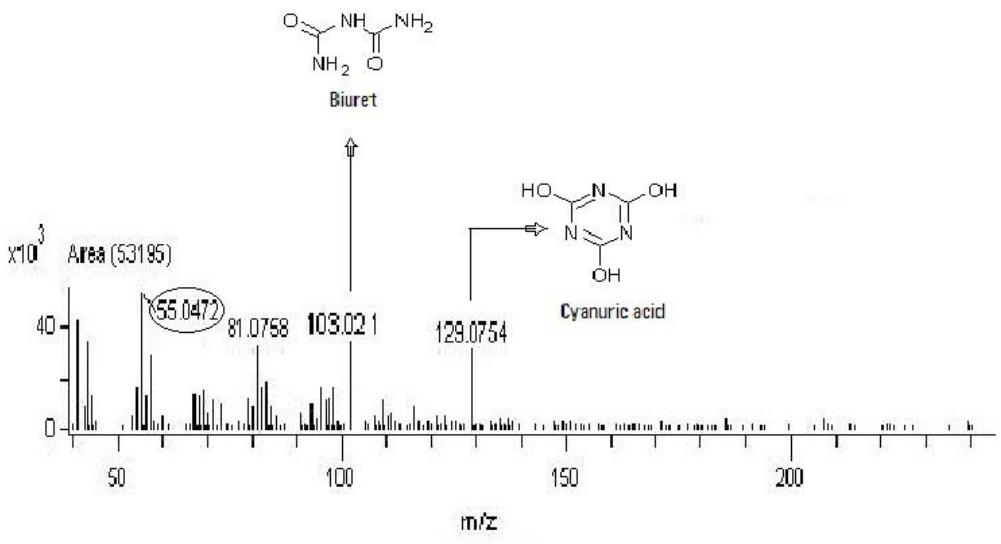


Figure S4. GC-HRMS result obtained for R2 effluent showing the cyanuric acid, and biuret

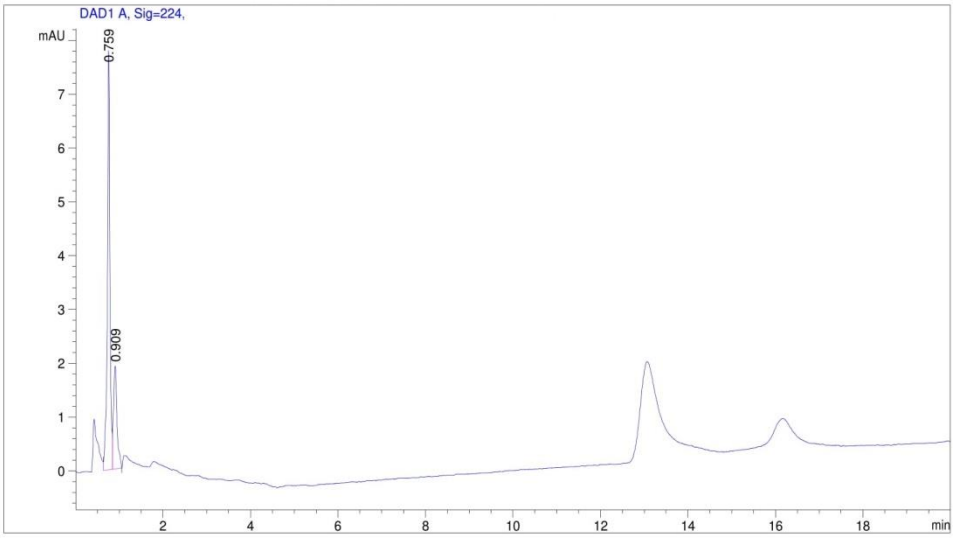
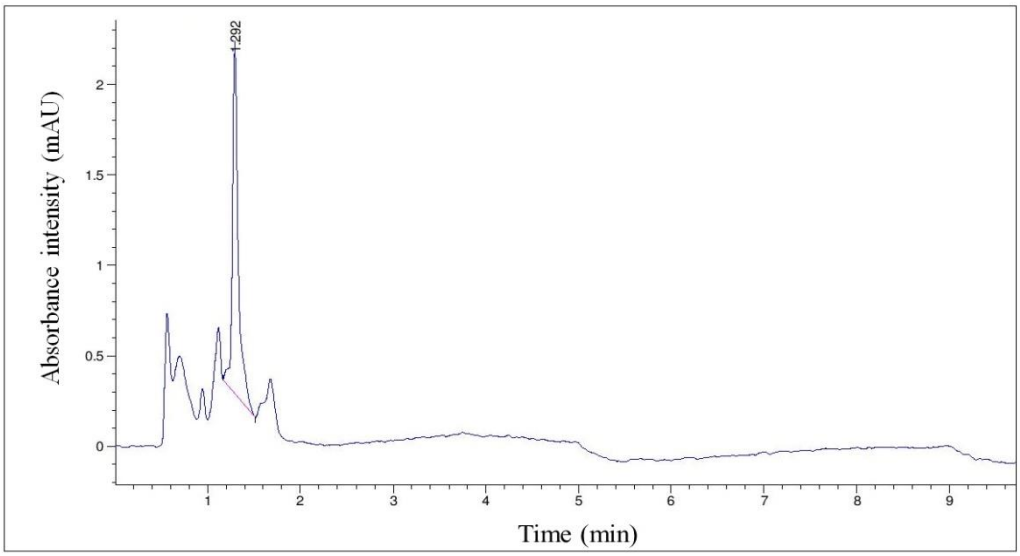
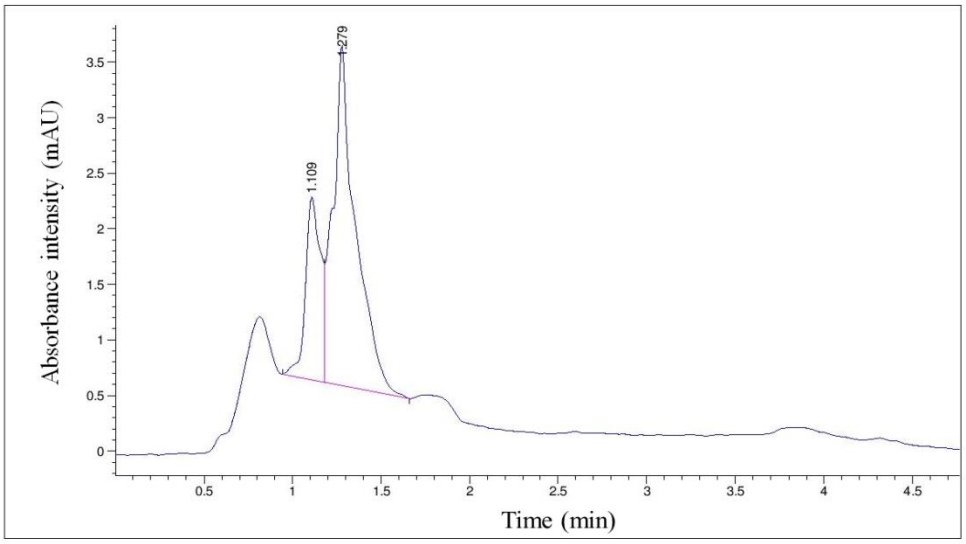


Figure S5: HPLC obtained for the effluent from A2 reactor



(a)



(b)

Figure S6: HPLC chromatogram obtained for (a) influent containing (40 mg/L) dicamba, and (b) effluent of R3

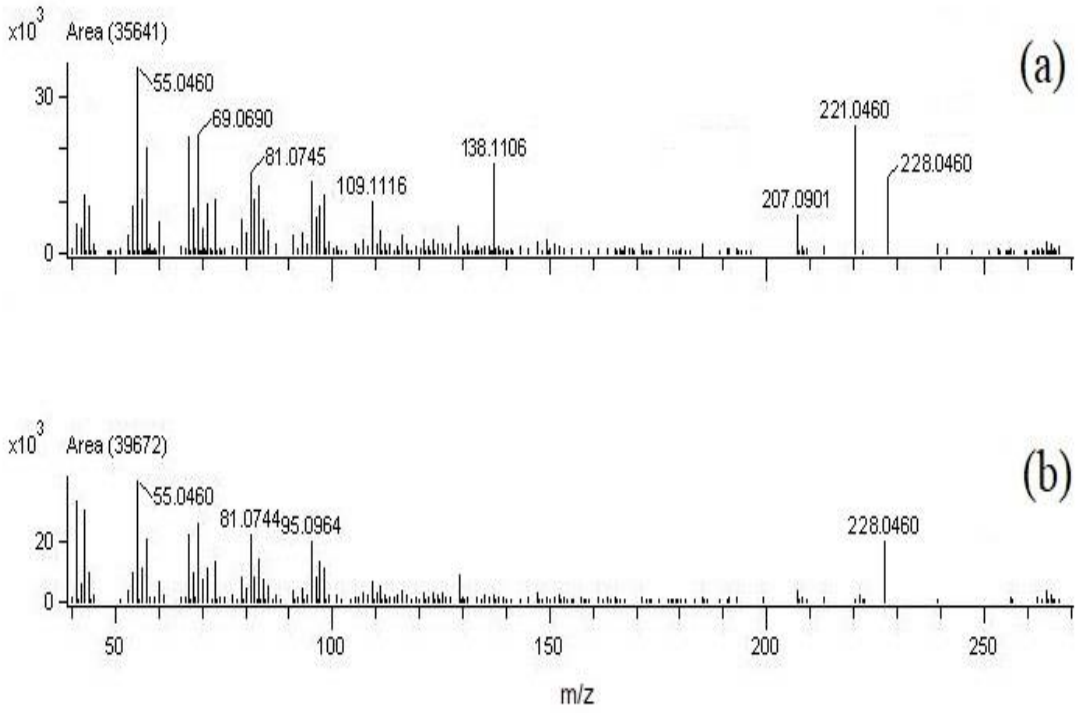


Figure S7: (a) GC-HRMS result obtained for R3 effluent showing dicamba (221.046), 3,6-dichlorosalicylate (207.09), salicylate (138.11) and other long chain fatty acids, (b) GC-HRMS obtained for the control effluent

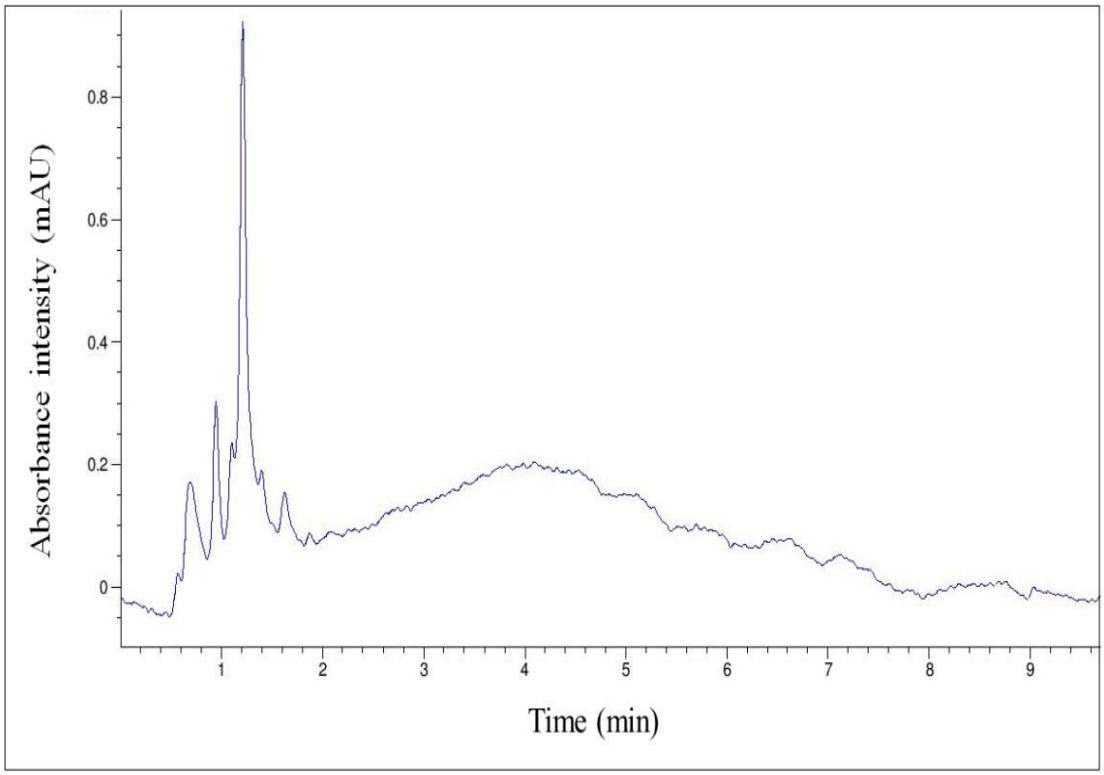


Figure S8: HPLC chromatogram obtained for the A3 effluent showing complete disappearance of dicamba

LIST OF PUBLICATIONS

(a) List of papers published in Journals

1. Mahesh, G. B., and Manu, B. (2019a), "Biodegradation of ametryn and dicamba in a sequential anaerobic-aerobic batch reactor: A case study." *Water Practice and Technology*, vol. 14(2), pp 423 – 434. DOI: 10.2166/wpt.2019.027**.**
2. Mahesh, G. B., and Manu, B. (2019b), "Biological treatment of 3, 6-dichloro-2-methoxybenzoic acid using anaerobic-aerobic sequential batch reactor". *Environmental Processes*, vol. 6(2), pp 493 – 509. DOI: 10.1007/s40710-019-00375-w.
3. Mahesh, G. B., and Manu, B. (2019c), "Removal of ametryn and organic matter from wastewater using sequential anaerobic-aerobic batch reactor: A performance evaluation study." *Journal of Environmental Management*, vol. 249. DOI: 10.1016/j.jenvman.2019.109390.
4. Mahesh, G. B., and Manu, B. "Anaerobic co-digestion of herbicide 2,4-dichlorophenoxyacetic acid with starch and post treatment in aerobic reactor." (Lecture Notes in Civil Engineering, Springer - Under review).

(b) List of conference presentations

5. Mahesh, G. B., and Manu, B. "Biodegradation potential of sequential anaerobic-aerobic reactor for the mixture of herbicides in water", at International conference on Affordable Strategies for Health and Environment, 23rd May, 2019, held at NMAMIT, Nitte.
6. Mahesh, G. B., and Manu, B. "Anaerobic co-digestion of herbicide 2,4-dichlorophenoxyacetic acid with starch and post treatment in aerobic reactor", at International conference on Civil Engineering Trends and Challenges for Sustainability, 24th May, 2019, held at NMAMIT, Nitte.

BIODATA

Name	Mahesh G.B.
Fathers Name	Sri Basappa N.
Mothers Name	Smt. Chowdamma
Date of Birth	20.12.1988
Siblings	Rajesh G B (Brother) Pavithra G B (Sister)
Permanent Address	Gajanuru (Village & Post), Malavalli (T.), Mandya (Dist.) – 571430
Email id:	gbcmahesh@gmail.com
Mobile No.	8861868447

Educational Qualification

Degree	College	University	Year
B.E. (Environmental Engineering)	Sri Jayachamarajendra College of Engineering , Mysore	VTU, Belgaum	2010
M.Tech. (Industrial Pollution Control)	NITK, Surathkal	NITK, Surathkal	2013