Effect of Oxytetracycline on Anaerobic Digestion of Cattle Manure

By

Moniba Mushtaq

(00000277251)

Institute of Environmental Sciences and Engineering (IESE)
School of Civil & Environmental Engineering (SCEE)
National University of Sciences and Technology (NUST)
Islamabad, Pakistan

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Certified that the contents and form of the thesis entitled **“Effect of Oxytetracycline on Anaerobic Digestion of Cattle Manure”** submitted by **Ms. Moniba Mushtaq** has been found satisfactory for partial fulfilment of the requirements of the degree of Masters of Science in Environmental Science

Supervisor: ______________________

Dr. Zeshan

Associate Professor

IESE, SCEE, NUST

GEC Member: ______________________

Dr. Muhammad Arshad

Professor

IESE, SCEE, NUST

GEC Member: ______________________

Dr. Muhammad Ali Inam

Assistant Professor

IESE, SCEE, NUST

THESIS ACCEPTANCE CERTIFICATE

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Dedicated to

My exceptional mother and beloved siblings whose endless prayers, tremendous support and encouragement led me to this wonderful accomplishment

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List of Abbreviations

Abbreviations	**Description**
ΔAIC	Delta Akaike Information Criteria
ΔBIC	Delta Bayesian Information Criteria
$\mu g.kg^{-1}$	Microgram Per Kilogram
a-Apo-OTC	a-apo-oxytetracycline
AD	Anaerobic Digstion
AEDB	Alternative Energy Development Board
AIC	Akaike Information Criteria
APHA	American Public Health Association
ARGs	Antibiotic Resistance Genes
b-Apo-OTC	b-apo-oxytetracycline
BIC	Bayesian Information Criteria
C/N	Carbon to Nitrogen Ratio
CaO	Lime
CH_3COOH	Acetic Acid
CH_4	Methane
CO_2	Carbon dioxide
CPEC	China-Pakistan Economic Corridor
CTC	Chlortetracycline
Dmax	Maximum Daily Biogas Yield
EDCs	Endocrine disrupting compounds
EOTC	epi-Oxytetracycline
$g.kg^{-1}$	Gram Per Kilogram
GDP	Gross Domestic Product
GHGs	Greenhouse Gases

GWh	Gigawatt hour
gVS/L	Gram of Volatile Solids Per Liter
GWP	Global Warming Potential
H_2	Hydrogen
H_2S	Hydrogen Sulfide
HCl	Hydrochloric Acid
HRT	Hydraulic Retention Time
lb	Pound
LCFAs	Long Chain Fatty Acids
M	Molar Solution
m^3	Cubic Meter
MW	Megawatt
$mg.kg^{-1}$	Milligrams Per Kilogram
$mg.L^{-1}$	Milligrams Per Liter
N_2O	Nitrous Oxide
Na_2CO_3	Sodium Carbonate
$NaHCO_3$	Sodium Bicarbonate
NaOH	Sodium Hydroxide
NH_3	Ammonia
NmL CH_4/gVS	Normal Milliliter of Methane Per Gram of Volatile Solids
NmL/gVS	Normal Milliliter Per Gram of Volatile Solids
OLR	Organic Loading Rate
OTC	Oxytetracycline
PCRET	Pakistan Council of Renewable Energy Technology
R^2	Coefficient of Determination
RE	Renewable Energy

Rm	Maximum Biogas Production Rate
RMSE	Root Mean Square Error
rRNA	Ribosomal RNA
TKN	Total Kjeldahl Nitrogen
TOC	Total Organic Carbon
TS	Total Solids
TWh	Terawatt hour
VAs	Veterinary Antibiotics
VFAs	Volatile Fatty Acids
VS	Volatile Solids
λ	Lag Phase

Abstract

Cattle manure is rich in organic matter and nutrients, but it may also contain harmful substances such as residual antibiotics and other toxic compounds. Oxytetracycline (OTC) is a widely used veterinary antibiotic and its presence in manure can affect the subsequent anaerobic digestion process. This study evaluated the effect of OTC concentrations viz. 0.12, 0.3, 0.6, 1.2, 3, 6 and 12 mg L^{-1} on batch mesophilic anaerobic digestion of cattle manure. The results showed that cumulative biogas yield decreased by 25, 29 and 55% at 3, 6 and 12 mg OTC L^{-1} as compared to control. Volatile solids removal was 39% for control which reduced to 13% in 12 mg L^{-1} OTC spiked reactor. Effect on stability parameters were significant at OTC concentrations from 1.2 to 12 mg L^{-1}. The modified Gompertz model best fitted to the experimental data.

Keywords

Oxytetracycline, Cattle manure, Anaerobic digestion, Model analysis

Chapter 1

Introduction

1.1 Background

Energy is the most important commodity for individual's quality of life and considered as the backbone of an economy (Abbas et al., 2017) as it provides employment opportunities necessary for economic growth and development (Amir et al., 2020). Increase in population, gap in demand and supply (El-Nahhal et al., 2020) and change in world economy due to technological and industrial revolution demands more use of energy thereby leading to consumption of fossil fuels (Kohli et al., 2019). In order to protect the natural biodiversity from depletion and environmental impacts of greenhouse gases (GHGs), the only available solution is shift towards renewable energy (Rizvi et al., 2020).

To minimize the use of non-renewables, biomass energy is regarded as the sustainable and cleaner environmental energy source (Kataki et al., 2017). Biomass such as biogas is the most viable renewable energy resource which is produced from waste (animals, agriculture, municipal and industries) to generate electrical energy and also to use for cooking purposes which is easily and widely accessible (Tareen et al., 2018).

1.2 Energy situation in Pakistan

Being a developing country and an increased population growth rate, Pakistan requires energy security and balance between energy supply and demand (Mufti et al., 2016). Due to continuous energy crises in Pakistan, different renewable energy (RE) options are in consideration (Rajput et al., 2018). In Pakistan, fossil fuels contribute about 37% of total energy mix which is quite expensive due to continuous change in oil prices. The oil, gas, coal, nuclear and hydropower are all primary energy sources. Pakistan had launched its RE policy in 2006 (Khan et al., 2020). By 2030, government has decided to increase the use of renewable energy sources from 1.1 to 5% in total energy mix (Irfan et al., 2020). Alternative Energy Development Board (AEDB) was established in May 2003 to promote, encourage and facilitate renewable energy production in Pakistan (Pandey & Bajgain, 2007).

In the start of this era, Pakistan faced widespread gas and energy shortages especially in rural areas during the peak winter and summer months, respectively (Malik et al., 2019). More precisely, the period of power outrage has reached 6 to 8 hours and 12 to 16 hours in urban and rural areas respectively (Irfan et al., 2020). In 2008, the energy shortage was 4000 MW which was expected to increase to 8000 MW by 2010. This energy crisis will grow at the pace of 5.67% per year (Kamran, 2018).

The energy plays a very important role for economy of any country in the world, likewise in Pakistan. China-Pakistan Economic Corridor (CPEC) comes with several benefits for both countries that is China and Pakistan which helps to overcome many challenges mainly energy shortage. According to the Board of Investment (BOI), hydro, wind, coal and solar energy projects constitutes 74% of total CPEC projects. For that purpose, China had financed $33 billion from 2018 to 2020 in 22 energy projects which greatly helped to lessen energy crisis in Pakistan (Zahid et al., 2018).

In order to achieve the set target of renewable energy Pakistan, biogas is the best viable option. Waste from cattle and poultry dung, metropolitan and agricultural residues can be used for production of biogas (Ahmad et al., 2019). The production of bio energy from cattle dung has the potential to reduce fossil fuel usage (Mittal et al., 2018). In Pakistan, the power production from livestock dung is 4800 to 5600 MW (Irfan et al., 2020).

1.2.1 Biogas potential of livestock in Pakistan

Waste to energy technology can play a vital role to avoid and lessen the power shortage especially in developing countries like Pakistan (Rasheed et al., 2019). The livestock sector plays a pivotal role in uplifting the socioeconomic conditions, managing long lasting power production and alleviating poverty especially in rural areas (Jabbar et al., 2015). Livestock sector is growing 4% annually in Pakistan (Chaudhry et al., 2009). Annually, the consequential animal manure is 368,434,650 kg used in biogas production with the energy potential of 23,654 GWh (Saghir et al., 2019). Pakistan is blessed with self-sufficient biomass resources (Kanwal et al., 2020) therefore, Pakistan holds a strong potential for biogas production (Batool et al., 2020).

According to 2019-2020 survey, livestock sector contributes 11% of GDP in Pakistan by contributing 60% to agriculture in Pakistan (Wang et al., 2020). Another survey showed that 652 million kg dung per day is produced by 172.2 million livestock which is sufficient enough for biogas (Amir et al., 2020; Kanwal et al., 2020; Saleem et al., 2019; Saleh, 2012) generating 21 million tons of organic fertilizers daily (about 20 and 60% nitrogen and phosphorous) and 16.3 m^3 per day biogas (Batool et al., 2020; Jan & Akram, 2018). The estimated potential of biogas production in Pakistan is about 14.25 106 m^3 per day (Javed et al., 2016). 1 m^3 biogas and 2.5 kWh electricity is generated by 20 kg manure. In Pakistan, almost 112 million people are living in rural-sub urban areas and their monthly and annual money worth 7672 and 92,062 PKR can be saved from biogas digester of 10 m^3 (Kamran, 2018; Yasar et al., 2017). Research results showed that 1 kg solid animal dung can produce 0.19 m^3 of biogas at 15°C and can double the biogas by increasing the temperature to 27°C (Amir et al., 2020).

Livestock is the chief producer of biogas in Pakistan. The country's potential to produce biogas from livestock manure and other residues is 8.8-17.2 billion m^3 gas per year (Amjid et al., 2011; Jabbar et al., 2015; Saghir et al., 2019; Wang et al., 2020) which is equal to 55 to 106 TWh of energy per year (Harijan et al., 2009). The Pakistan Council of Renewable Energy Technology (PCRET) website stated that Pakistan has livestock to produce about 16 million m^3 biogas per day which is an efficient source of fuel (Pandey and Bajgain, 2007). There are numerous uses of this biogas such as to be used for cooking and lighting in industries and homes, transportation and lesson the pressure on imports of fossil fuels by generating electricity (NRSP, 2011).

1.3 Need for cattle manure treatment through anaerobic digestion

Being an important worldwide economic activity, the livestock production and its waste management varies from country to country (Ghirardini et al., 2020). Although livestock sector provides job opportunities to millions of people but the environmental problems associated with this sector cannot be foreseen. These environmental challenges include eutrophication, acidification, GHG emissions and biodiversity loss (Magrí et al., 2013; Mottet et al., 2017).

The manure of livestock is traditionally handled by storing manure heap in an open site and later on to spread on agricultural field. Secondly, dung cakes are being made in rural areas to use them for cooking purpose. These manure-handling methods results in emission of ammonia and GHGs. The cattle manure management is becoming difficult in many South Asian countries due to rapid increase in animal production. The manure management in Pakistan produced 10.58 Tg CO_2 equivalent of annual GHG emissions (Ali et al., 2019). The on-farm sources of GHGs emissions included 25% as enteric methane (CH_4) from the cow, 24% as CH_4 and nitrous oxide (N_2O) from manure, the field releases 19% as N_2O and carbon dioxide (CO_2), and farm energy (4% as CO_2) (Wattiaux et al., 2019).

Antibiotics are intensively used in livestock industry (Habib et al., 2015). The main reason for application of antibiotics in animal husbandry is to use as growth promoters, treatment and prevention of infections in livestock (Tylová et al., 2010). The indiscriminate use of antibiotics results in manure contaminated environment. The “post-antibiotic era” term is being proposed and gives alarming future insights of excessive use of antibiotics (Bloem et al., 2017). Based on livestock data, use of veterinary antibiotics applied in livestock farming is expected to increase up to 67% during next years (Spielmeyer, 2018). The major medium for animal and human antibiotic’s transmission in the environment is excretion (Jjemba, 2002). Veterinary antibiotics (VAs) are added in feed or drinking water at lower doses. Vary in structure, antibiotics are transmitted into animal feces and liquid manure due to poorly digested or partially absorb in animal gut (Ur Rahman & Mohsin, 2019).

In world’s animal producing countries, Pakistan being among top 10 countries for intense farming practices (Page & Gautier, 2012) relies heavenly on antibiotics commonly used as growth promoters and disease prevention in animals and spray on plants that lead to antibiotic resistance (Ali et al., 2018). In Pakistan the “misuse and overuse” of antibiotics is widespread with up to 70% being used inappropriately (Khan et al., 2018). The presence of VAs and their excretion varies depending on kind of animal and antibiotic type, method of antibiotic use and duration, and collection time of VAs containing manure after treatment (Gaballah et al., 2021; Jayalakshmi et al., 2017). Approximately, 600,000 quacks and 50,000 unnecessary registered VAs are further worsening the situation. During an interview, different VAs suppliers and famers had mentioned the frequent use of veterinary medicines such as lactam and macrolides classes of antibiotics mainly penicillins and erythromycin, tetracyclines, oligosaccharide and many others used as growth promoters. Several studies by different authors such as Ahmed & Gareib 2016, Singh et al., 2016 and Habib et al., 2015 had also reported the presence of VAs in dairy milk and meat, and also in poultry meat. The antibiotic residues mentioned in those studies are oxytetracycline, gentamicin, chloramphenicol, amoxicillin, benzylpeni-cillin, streptomycin, and tylosin (Ur Rahman & Mohsin, 2019). Nisar et al., 2017 found that multidrug resistant Campylobacter spp was present in about 90% of meat samples which were resistant to 79.2% enrofloxacin, 77.6% tylosin, 25.6% gentamicin, 32.8% neomycin, ciprofloxacin and amoxicillin were 71.2% each. Studies conducted in different areas in Pakistan also reported different concentrations of antibiotics in meat, milk, and eggs suggesting high usage of antibiotics in food producing animals (FPAs) (Khan et al., 2018). Public health is of great concern due to presence of different concentrations of antibiotic residues in milch animals. A study was conducted to analyze the presence of β-lactam residues in retail dairy milk and reported that 36% of 1367 milk samples were found positive for ß-lactam antibiotics residues. Out of these, 56% were positive for amoxicillin and 48% for ampicillin (Malik et al., 2008). Another study conducted in Sindh showed that 38% of 300 raw beef samples were positive for antibiotic residues (Mangsi et al., 2014).

Anaerobic digestion (AD) is an effective process for biogas production, waste reduction and management, pollution extenuation, renewable energy use and reduced GHG emission. Anaerobic digestion is a biochemical process with many advantages (Yao et al., 2020). The small scale anaerobic digesters produce biogas which is used as fuel for cooking, to heat water or buildings and to generate electricity. Being used for treatment and management of organic waste and its problems associated in confined and large animal feeding operations, anaerobic digestion of livestock waste is an efficient and alternate waste management method (Ileleji et al., 2015; Kinyua et al., 2016). AD is a sustainable alternative pathway to avoid the direct discharge of cattle manure in the environment (soil and aquatic ecosystems) as this method results in biogas and bio-fertilizer production, whilst reducing the microbial load of the surrounding environments (Resende et al., 2014).

1.3.1 Inhibitors of anaerobic digestion

Bio-methanation can be inhibited by the presence of detergents, heavy metals and antibiotics used in livestock rearing (Kossmann & Pönitz, 2011). The major source of organic pollutants are manure and sewage sludge in the environment. Some of the major threats that arise from the use of organic wastes as fertilizers include the emission of pathogens, the generation of microbial resistance to released antibiotics and the emission of endocrine disrupting compounds (EDCs), such as steroid hormones that may induce strong adverse endocrine responses in wildlife and humans, even in the ng L^{-1} to mg L^{-1} range (Rodríguez-Navas et al., 2013). The anaerobic digestion factors like pH and temperature and compounds like volatile fatty acids (VFAs), ammonia (NH_3), sulfide and hydrogen (H_2) can inhibit the biogas yield (Zhang et al., 2016). The other contaminants to be considered for better animal manure characterization are pathogenic and indicator microorganisms (Ghirardini et al., 2020).

The effect of antibiotics on AD depends on the antibiotic type, concentration, and digestion conditions mainly reactor type, temperature, and organic loading rate (OLR) (Cai et al., 2021). The main reason for studying anaerobic digestion along with presence of antibiotics is to see its effect on biogas/methane yield. Three conclusions have been drawn so far (1) Inhibitory effect, which shows that antibiotic residues inhibit the AD process. The Oxytetracycline (OTC) concentrations of 10, 50 and 100 mg L^{-1} has inhibited the biogas production by 56, 60 and 62%, respectively (Arikan et al., 2006). Huang et al., (2014) reported that within 7 days, the biogas production was reduced by 12-15% due to presence of Chlortetracycline (CTC). It was previously determined that 0, 10, 25, 50 and 100 mg CTC per L inhibited the cumulative methane yield by 93 ± 5, 94 ± 2, 85 ± 1, 74 ± 0, and 75 ± 8 mL CH_4/gVS (Lee et al., 2020). Mitchell et al., (2013) found that biogas yield reduced by 10-38% with the addition of Tylosin concentration between 130 and 913 mg L^{-1}. The different concentrations of Florfenicol i.e. 6.4, 36 and 210 mg L^{-1} decreased biogas by 5, 40 and 75%, respectively. Another study reported that anaerobic digesters containing

10 mg L^{-1} monensin produced 75% less methane as compared to control (Arikan et al., 2018). (2) No observed effect. Results of some studies showed no evident effect of antibiotic residues on AD process. Lallai et al., (2002) reported that methane production was not effected with the addition of CTC concertation of 120-125 mg L^{-1} during anaerobic digestion of animal manure. (3) Stimulating effect. Few studies have reported increased in biogas/methane production with the antibiotic's presence. The addition of different antibiotics such as OTC, sulfadimethoxine (SDM), sulfamethoxazole (SMX), enrofloxacin (ENR), ciprofloxacin (CPFX), ofloxacin (OFX) and norfloxacin (NRFX) with 100 mg L^{-1} concentration exhibited a strong stimulating effect on CH_4 yield (Zhi & Zhang, 2019).

1.4 Novelty of study

Antibiotics are being used in livestock husbandry for both therapeutic and prophylactic use but indiscriminate use led the manure contaminated environment. A lot of studies have been conducted for antibiotic's presence in milk and meat of livestock in Pakistan but no study has been done for effect of antibiotics particularly oxytetracycline on anaerobic digestion of cattle manure. This study will give an insight on effect of different concentrations of oxytetracycline antibiotic on anaerobic digestion of cattle manure.

1.5 Objectives

- To determine the effect of different concentrations of oxytetracycline on biogas production from anaerobic digestion of cattle manure
- To identify the effect of oxytetracycline concentrations on stability parameters of anaerobic digestion of cattle manure
- To compare the kinetic model results on biogas yield from cattle manure containing oxytetracycline

Chapter 2

Literature Review

This chapter will provide an insight of past work that has been done on anaerobic digestion of cattle manure along with the effect of antibiotics especially oxytetracycline on biogas and methane yields.

2.1 Nutrients and recalcitrant compounds in cattle manure

Cow dung is an excrete of bovine animals in form of undigested residue of consumed food. It is a mixture of urine and feaces in the ratio of 1:3 and mainly contains lignin, cellulose and hemicelluloses (Gupta et al., 2016). It also contains trace amount of sulphur, magnesium, copper, cobalt and manganese and 24 micronutrients (iron, vitamins, zinc) and macronutrients (proteins) (Leip et al., 2019; Randhawa & Kullar, 2011). Manure being a fibrous material contains around 2.6% nitrogen content which is significantly higher than any fibrous material such as wheat straw (Chen et al., 2003). Cow manure is a rich complex substrate comprised of fibrous, soluble or particulate matters. It also contains lipids, fats, proteins and carbohydrates in the form of hemicellulosic and cellulosic fibers and VFAs. Dairy manure contains around 40-50% VFAs which are lignocellulosic biodegradable biomass to produce CH_4 (Massé & Cata Saady, 2015). Roughly 10 lb nitrogen, 5 lb phosphate and 10 lb potash per ton are present in cow feces (Neshat et al., 2017). Recalcitrant compounds are also present in cow manure even in highest concentration. Carbohydrates present in animal feed especially leaves are recalcitrant in manure (Kinyua et al., 2016).

Table 2.1 shows the presence of nutrients and recalcitrant compounds present in animal manure. Microorganisms beneficial to enhance soil structure and biological activity are present in cow manure. Nonetheless, the practice of manure application on farms has decreased for the past 50 years mainly due to separate breeding and cultivation farms for livestock and crop prodcution, cost constraints in terms of manure transportation along with increased production, cheaper rates and availability of synthetic fertilizers. The management of daily bulk manure production is challenging and requires a wise solution for manure usage on farms. The manure rich in nutrients is more inbevitable threat to the environment and public health than to be used as valuable commodity. The imporper manure handling may result in ground water pollution, destrcutive run-off and loss of nutrients during manure collection, storage, distribution and application (Neshat et al., 2017).

Table 2.1 Nutrient contents and refractory compounds in livestock manure

Parameters	**Unit**	**Swine**	**Cow**	**Poultry**
Lignin	%VS	2.2-16	7.9-10	3.4-5.2
Cellulose	%VS	15-20	17-25	11-15
Hemicellulose	%VS	20	22	11-17
Nitrogen	Kg day^{-1} animal	0.037-0.95	0.22-0.33	0.002-0.01
Phosphorus	Kg day^{-1} animal	0.024-0.25	0.08-0.14	0.001-0.37
Potassium	Kg day^{-1} animal	0.028-0.26	0.12-0.19	0.001-0.46

The major hindrance in sustainable development is mishandling of manure treatment and recycling results in GHG emissions. According to the global warming potential (GWP), the manure storage releases 8-10 times more CH_4 into the environment than CO_2 and contributes 4% of all anthropogenic CH_4. In order to avoid emissions, an efficient manure management is required. For manure treatment, several technical options are available and classified as thermochemical, aerobic and anaerobic methods (Awasthi et al., 2019; Kafle & Chen, 2016). The best and efficient technology to manage, treat and convert livestock manure into biogas and digestate is anaerobic digestion. It results in environmental pollution control, energy production and removal of toxic pollutants and organic matter (Duan et al., 2018; Li et al., 2018).

2.2 Emergence and use of veterinary antibiotics

Among two emerging pollutants which have adverse effects on human and environment are antibiotics and nanoparticles (Zhao et al., 2019). Antibiotics are bioactive metabolites (Inyinbor et al., 2018) used to kill microorganisms or to inhibit and treat infections (Min et al., 2020). Antimicrobial drugs are natural or synthetic chemical substances extensively used in human and animal medicine mainly to prevent infection, treat disease and to promote growth (Reyes-Contreras & Vidal, 2015; Tylová et al., 2010). Some antibiotics are recalcitrant after excretion (Du & Liu, 2012). Public health protection is of great concern due to indiscriminate use and harmful effects of antibiotic residues (Mangsi et al., 2014) due to overuse of antibiotics for, human, veterinary and agricultural purposes (Çelik et al., 2018). From 100,000 to 20,000 tons per year antibiotics are estimated to be used worldwide and almost 70% of them are being sold for veterinary use (Albero et al., 2018; Tasho & Cho, 2016).

In late 1940's, antibiotics were introduced in animal farming. For more than 60 years, antibiotics are used in cattle feed and its addition in animal feed for enhanced animal growth and increase in milk yield was discovered by an American Cyanamid publication (Khan et al., 2018). The gradual shift towards livestock production results in increased production of veterinary antibiotics (VAs) (Brooks et al., 2015). In 2010, with the development of livestock sector about 63,151 tons of VAs were consumed across the globe which will increase to 105,596 ± 3605 tons (over 100 thousand tons, 67% increase) by year, 2030. It has been estimated that the global average annual consumption of antimicrobials per kilogram of animal produced was 45 mg kg^{-1}, 48 mg kg^{-1} and 172 mg kg^{-1} for cattle, chicken, and pigs (Ahmad et al., 2019; Gurmessa et al., 2020).

2.2.1 Environmental risks of veterinary antibiotics in manure

The development of livestock has attracted worldwide attention due to excessive use of antibiotics in feedstock and their discharged into the environment via animal manure (Cetecioglu et al., 2013; Zhang et al., 2019a). Antibiotics are used extensively in animal husbandry to heal, prevent and control the spread of infectious diseases. They are also utilized to stimulate growth and food rate (Granados-Chinchilla & Rodriguez, 2017). When antibiotic is administered, some is partially absorbed (Ghirardini et al., 2020) and remaining dose is excreted via animal urine and faeces (Berendsen et al., 2015). As the VAs are poorly absorbed in animal gut and about 30 to 90% is pass out of the body as active and non-active metabolites that end up in animal manure (Wu et al., 2011).

Livestock manure is the major source and reservoir of VAs (Lu & Lu, 2019) and have previously been detected in animal feces from low µg kg^{-1} range up to the g kg^{-1} range high levels (Berendsen et al., 2018)**.** Animal manure and slurries contain nutrients essential for crops growth. Besides providing nutrients, manure also contain VAs used in animal husbandry (Gros et al., 2019). As raw manures are not treated nor regulated and applied on land as fertilizer so they are an important route of entry into the environment (Brooks et al., 2015).

There are several sources for the entry of antimicrobial residues in the environment (Halling-Sørensen et al., 2003) as shown in Figure 2.1. The connection between antibiotic use and the emergence of antibiotic resistant bacteria (ARB) is well reported (Zhang et al., 2013). Manure contained antibiotics can pollute the groundwater, soil, surface and even drinking water (Tylová et al., 2010). A major risk of antibiotic resistant when applied on land as fertilizer, it results in over spread of VAs in water and soil (Heuer et al., 2011; Massé et al., 2014). The soil ecosystem mainly contains heavy metals, VAs and antibiotic resistance genes (ARGs) which enter into the environment via organic fertilizers such as manure (Li et al., 2018). Soil and leachate samples showed presence of antibiotics indicating soil exposure to antibiotic contamination (Nurk et al., 2019). The aquatic environment is also at risk of antibiotic’s release as its being fed to aquaculture animals (Yuan et al., 2010).

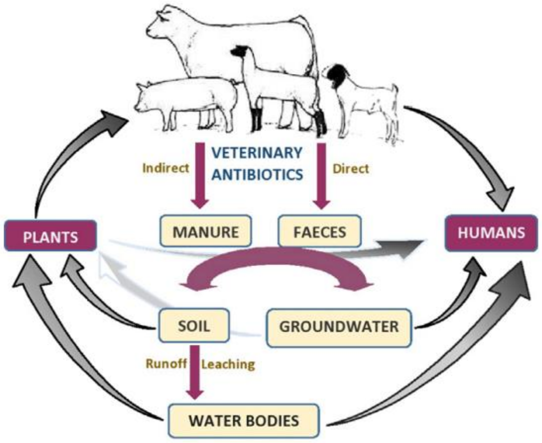


Figure 2.1 Fate of veterinary antibiotics used in livestock industry

The weather conditions, soil properties like pH, ion and cation exchange strength and organic matter presence as well as physicochemical properties (volatility, water solubility and sorption capacity) determine the fate and effect of released antibiotic on environment. Therefore, the presence of antibiotic can be for a day to weeks or several months depending upon the chemical structure of antibiotic and environmental factors like pH and temperature as well as hydrological effects. The major factor involved for antibiotic's availability and transport is the sorption of antibiotics to soil. This sorption characteristic results in transport of antibiotics to aqueous or solid phase of soil thereby available for plant uptake (Albero et al., 2018; Massé et al., 2014).

2.2.2 Types of antibiotics

Due to extensive livestock farming, (Yin et al., 2019) several types of veterinary antibiotics are present in animal manure (Insam et al., 2015) to increase growth and reduce diseases (Yannarell and Mackie, 2012) are shown in Table 2.2. VAs have different classes which mainly differ from each other due to difference in mode of action, metabolism, environmental effect and fate and chemical structure of antibiotic. The antibiotic's contamination level on manure varies depending on animal types (Bloem et al., 2017). The main types of antibiotics include tetracylines, sulphonamides, macrolides, ß-lactams, and quinolones. These classes differ from each other due to difference in pH conditions and can have neutral, charged (cationic or anionic) or zwitterionic effect based on different functionalities (Kümmerer, 2009; Pan & Chu, 2016).

Table 2.2 Group of antibiotics in swine, cattle and poultry

Antibiotic	**Swine**	**Cattle**	**Poultry**
Tetracycline	✓	✓	✓
Sulfonamides	✓	✓	✓
Macrolides	✓	✓	✓
β-Lactams	✓	✓	✓
Streptogramins	✓	✓	✓
Aminoglycosides	✓	✓	✓
Lincosamides	✓		✓
Polepeptides	✓		✓
Ionophores		✓	✓
Fluoroquiniolones		✓	✓
Chloramphenicol		✓	

Antibiotic classes have different action of mechanism based on their functional groups as shown in Figure 2.2. So based on functional groups, antibiotics are categorized as the inhibition of cell wall, protein or nucleic acid synthesis, change in cell membranes, and metabolic or non-competitive antagonism (Grenni et al., 2018; Sengupta et al., 2013).

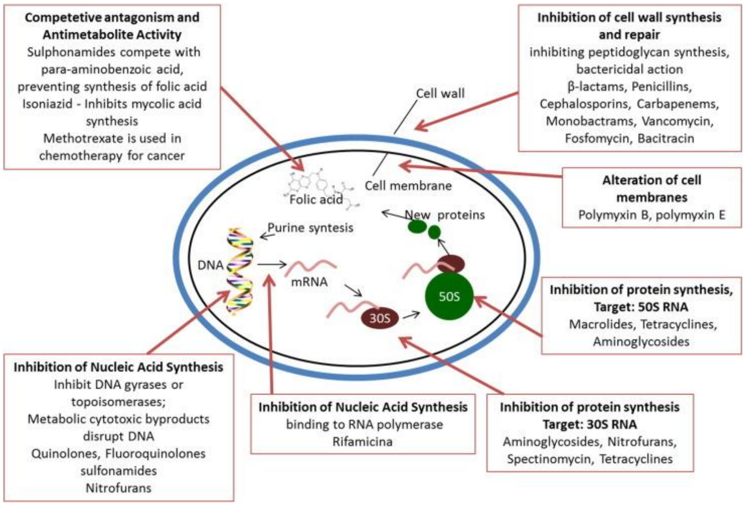


Figure 2.2 Mechanism of action of antibiotics

Source: Grenni et al., 2018

2.2.3 Half-life of antibiotics

Different VAs have different half-lives reported in studies which suggests that significant amount of parent antibiotic might have degraded before land application (Chee-Sanford et al., 2009). Based on manure handling and storage, half-life of antibiotic range from 100 days (for very persistent antibiotic, tetracyclines) to less than 2 days (for least persistent, macrolides) (Ghirardini et al., 2020; Insam et al., 2015). The antibiotic's concentration in animal manure can be less as 1 mg kg^{-1} to 10 mg kg^{-1} or L^{-1} to high level such as 200 mg kg^{-1} or per Liter (Massé et al., 2014). Table 2.3 shows the persistence of antibiotics in animal manure.

Results of research studies have confirmed the presence of VAs in animal manure whether its fresh or in storage tanks, ground, underground and on soil surface. A study had been done by De Liguoro et al., (2003) whose results found insignificant concentration of Tylosin and OTC in water and soil while 0.11 mg kg^{-1} and 10 mg kg^{-1} of these antibiotics were found in fresh manure, respectively. Another study conducted by Storteboom et al., (2007), authored that OTC is more persistent in dairy manure with half-life of 17.7 days as compared to horse manure having half-life of 8.4 days.

Table 2.3 Persistence of antibiotics in livestock manure

Antibiotic Class	Half-life (d)
Aminoglycosides	30
B-lactams	5
Macrolides	<2-21
Quinolones	100
Sulfonamides	<8-30
Tetracyclines	100

2.2.4 Tetracyclines and oxytetracycline

An extensively used antibiotic for disease treatment in humans and animal farming (Zhang et al., 2013) is tetracycline which is a broad antibiotic class (Keßler et al., 2019). Tetracycline being an important antibiotic (Granados-Chinchilla & Rodriguez, 2017) can be detected worldwide in animal manure (Nurk et al., 2019) from level ranges mg kg^{-1} to more than 100 mg kg^{-1} due to its excessive use (Yannarell and Mackie, 2012). Being a broad spectrum antibiotic type, authors reported this class concentration from 0.001 to 150 mg kg^{-1} in animal manure (Bousek et al., 2018). Oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC) are widely used antibiotics of tetracycline class. They are used to promote growth, to control and treat diseases both at therapeutic and sub-therapeutic levels in animal farming worldwide (Kasumba et al., 2020). Mechanism of action of tetracycline class and its use according to WHO are given in Table 2.4.

Oxytetracycline is widely used in dairy cattle production primarily to prohibit disease and enhance growth and increase milk production (Beneragama et al., 2013) favored by the fact that it is cost-effective (Turker et al., 2018) and has wide range of antimicrobial activity against gram-positive and gram-negative bacteria (Prasad & Rao, 2010). OTC has three degradation products named as epi-Oxytetracycline (EOTC), a-apo-oxytetracycline (a-Apo-OTC) and b-apo-oxytetracycline (b-Apo-OTC) (Arikan et al., 2006). About 60% of OTC indigested dose is absorbed and widely spread in the body) (Sarker et al., 2018). The fate and effect of OTC is of great concern as its toxicity not only effect the human health via food chain but also effects the presence and behavior of microbes in an ecosystem (Li et al., 2019). OTC is detected in surface waters and soil due to its extensive use and persistence in nature. The treatments systems such as nitrifying and anaerobic digesters can be negatively affect due the presence of antibiotic residues or metabolites in animal manure (Arikan et al., 2006).

Table 2.4 Class, mechanism of action, uses and World Health Organization classification of tetracyclines based on importance to human health

Class	**Mechanism of action**	**Uses/WHO classification**
Tetracycline sTetracycline, chlortetracycline, oxytetracycline, doxycycline	Inhibit bacterial protein synthesis	Human Animals Growth promoter Highly important

2.2.4.1 History of oxytetracycline

The tetracycline antibiotic era had started in 1948 by Benjamin Duggar who discovered chlortetracycline from Streptomyces aureofaciens. Due to its yellow color, it was named as aureomycin. Finaly and his colleagues discovered OTC in soil samples having actinomycete known as Streptomyces rimosu. The extensive use of natural tetracycline results in antibiotic resistance. To overcome prevalent resistance issue, tetracycline was being made semi-synthetically. After the approval from Food and Drug Administration in 2005, the third generation of tetracycline with tigecycline was developed (Priya & Radha, 2014).

2.2.4.2 Structure of oxytetracycline

OTC belongs chemically to the polyketides. OTC is chemically known as [4S-(4α, 4aα, 5α, 5aα, 6β, 12aα)] - 4 – (Ddimethylamino) - 1,4,4a, 5,5a,6,11,12a-octahydro-3,5,6,10,12,12a, -hexahydroxy-6-methyl-1,11-dioxo-2-naphthacene- carboxyamide or 5-Hydroxytetracycline. The chemical structure of OTC is shown in Figure 2.3.

OTC is odorless, bitter and yellow in color hygroscopic compound which decomposes at 180ºC. It is soluble, insoluble and slightly soluble in water, ether and chloroform and ether, respectively. OTC is available as OTC freebase ($C_{22}H_{24}N_2O_9$; MW 460.4 g/mol). Oxytetracycline hydrochloride (C_{22} $H_{24}N_2O_9$. HCl; MW 496.9 g/mol), oxytetracycline dihydrate (C_{22} $H_{24}N_2O_9$.$2H_2O$; MW 496.4 g/mol) and oxytetracycline calcium ([$C_{22}H_{24}$ N_2O_9] 2. Ca), MW 958.9 g/mol). OTC is a strong chelating agent, and both its antibacterial and pharmacokinetic properties are influenced by the chelation of metal ions present in food and the biological environment (Sversut et al., 2017).

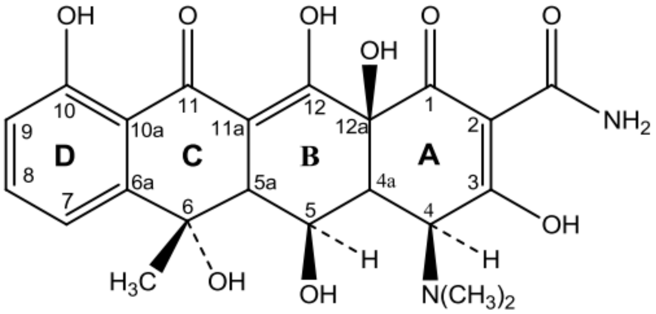


Figure 2.3 Chemical structure for oxytetracycline

Source: Sversut et al., 2017

OTC is a complex, zwitterionic macromolecular organic compound and consists of a complex four ring system with a number of ionizable functional groups. The B, C and D rings mainly absorb light at wavelengths of 250–300 and 340–380 nm (Li et al., 2019).

2.2.4.3 Mechanism of action

OTC is a bacteriostatic antibiotic which inhibits bacterial protein synthesis by binding to the ribosomal complex. It inhibits bacterial protein synthesis by binding the 30S ribosomal subunit to prevent the association of the aminoacyl-tRNA to the ribosomal acceptor site. It also causes structural change in 16S rRNA (Demerdash et al., 2018).

2.3 Detection of antibiotics in cattle manure

A number of research had been published in last decade regarding occurrence rate, fate and effect of incipient environmental pollution from livestock manure. The major pressure and threat of using manure as fertilizer includes the discharge of pollutants and pathogens and microbial resistance generation even in ng level in released antibiotics (Rodríguez-Navas et al., 2013). Table 2.5 shows the reported concentrations of tetracycline class in animal manure. Studying veterinary antibiotics in manure is very effective as it will give insight to relation between antibiotic residues and bacterial resistance in animal's gut, the spread of VAs in environment and their eco-toxicological effects, and non-invasive sampling in farms to visualize trend of antibiotic use in feedlots. Lastly, policies on antibiotic's usage accompanied with the prevention of off-label and illegal use of antibiotics. Hence, it is concluded that analysis of antibiotic contaminated manure will be informative leading to need for developing multi-methods for detection of VAs in faeces (Berendsen et al., 2015).

Table 2.5 Reported concentration of tetracycline, oxytetracycline and chlortetracycline in cattle manure

Substrate	**Antibiotic**	**Concentration (mg kg^{-1})**	**Reference**
Cow manure	Tetracycline	0.052-5.36	Alavi et al., (2015)
	Oxytetracycline	0.07-0.62	
Dairy manure	Tetracycline	0.43-2.69	Li et al., (2013)
	Oxytetracycline	0.21-10.37	
	Chlortetracycline	0.61-1.94	
Cow manure	Chlortetracycline	Up to 267.59	Zhao et al., (2010)
	Oxytetracycline	Up to 59.59	
Fresh cattle manure	Oxytetracycline	0.06	Karcı et al., (2009)
Fresh cattle manure	Oxytetracycline	871.7	De Liguoro et al., (2003)
Beef cattle manure	Chlortetracycline	5.3	Patten et al., (1980)
	Oxytetracycline	11.3	

2.3.1 Treatment technologies for removal of antibiotics

Research for the removal of emerging pollutant that is antibiotics, are under consideration (Bernet & Béline, 2009). The well-established treatment technologies for reduction of antibiotic residues in livestock manure are composting, aerobic and anaerobic digestion.

As the antibiotic's presence may impact the efficiency of digestion and their degradation during treatment for their antimicrobial properties (Du & Liu, 2012).

Anaerobic digestion is considered as one of the best choice for treatment of effluent as it reduces and even remove antibiotics and ARGs. In order to see the antibiotic's removal efficacy using activated carbon (AC), a study was conducted by Zhang et al., 2019a. They reported that Amoxicillin and Ofloxacin antibiotic's removal rate was 33–60% without AC while it was close to 100% with AC. Another treatment technology which has been published for treatment of wastes is two phase anaerobic reactors. In this way the toxic materials will be removed in first phase thereby not affecting the methanogens. Therefore, a study was conducted to see the removal rate and effect of Oxytetracycline antibiotic in two-phase cattle manure anaerobic digester. The results showed that higher methane yields of OTC with two-phase and single-phase digesters were achieved i.e, 99 ± 8 and 72 ± 9 mL CH_4/g VS. OTC removal rate in first and two-phase digester was 48 and 38% respectively (Akyol et al., 2016b). Another study was conducted by Yin et al., 2018, which investigated the degradation of OTC (40 mg kg^{-1} TS) and CTC (60 mg kg^{-1} TS) in two-stage (acidification and methanogenesis) anaerobic digestion system. Results showed that 60 and 41% degradation of CTC and OTC in the acidogenic stage while in methanogenic stage, 76 and 78% of CTC and OTC degradation had occurred. Therefore, among all available treatment technologies, anaerobic digestion is primarily used to avoid discharge of antibiotic containing manure into environment as it produces biogas and also reduce the antibiotic load in animal manure to be used as fertilizer (Kasumba et al., 2020).

2.4 Anaerobic digestion

Anaerobic digestion (AD) converts biodegradable organic waste including crop residues and manures into biogas and digestate (Nghiem et al., 2017) by a serial multi stage biological process in the absence of oxygen (Wu et al., 2019).

2.4.1 Purpose of biogas from anaerobic digestion

The main hindrances in sustainable development of developing countries are energy security and shortage (Yasar et al., 2017). The fourth largest energy source is biogas which is the product of anaerobic digestion providing nearly 14% primary energy demand globally (Amir et al., 2020; Zuberi et al., 2013). This biogas is a mix of carbon dioxide and methane along with other trace gases (Rodríguez-Navas et al., 2013).

The basic purpose of biogas generation is to secure the environment by treating waste or manure and to overcome the increased prices of fuel. Another purpose is to cover and meet the gap of energy supply and demand. Phenomenon like natural hazards, deforestation and soil erosion may less likely to occur due to less use of fuel wood especially in rural areas. Livestock manure being readily available in rural areas can be the best reason for adoption and development of biogas technology (Rahman et al., 2000).

2.4.2 Process of anaerobic digestion

Anaerobic digestion is a four stage process including hydrolysis, acidogenesis, acetogenesis and methanogenesis. The two critical stages of AD process are hydrolysis and methanogenesis. The microorganisms involved in functionality of stages are particular in nature and they also have synergistic relation among stages. The resultant product of one stage is basically the substrate used for next step. The slight negative change in one step leads to system failure by disrupting the fast growing acidogens and highly sensitive methanogens (Wu et al., 2019). Mechanism of action for anaerobic digestion process is shown in Figure 2.4.

2.4.2.1 Hydrolysis

The first stage involves the breakdown of complex hydrocarbons into soluble compounds which basically includes the conversion of polymers like lipids, carbohydrates and proteins into monosaccharides i.e., fatty acids, sugars and amino acids respectively. It is multistage step intervened by extracellular enzymes which are either in solution or to microbial cells.

2.4.2.2 Acidogenesis

In this stage, the hydrolytic products are converted into organic acids, ammonia, CO_2, hydrogen sulphide (H_2S), low alcohols and volatile fatty acids (VFAs). The concentration of hydrogen produced at this stage affects the final product after digestion and the resulting organic matters such as VFAs are not suited for direct conversion to methane by the methanogens.

2.4.2.3 Acetogenesis

The end products of this stage are acetate, H_2 and CO_2 produced from conversion of VFAs. The major portion of CH_4 is about 65-95% produced from acetic acid.

2.4.2.4 Methanogenesis

Acetotrophic, hydrogenotrophic and methylotrophic group of methanogens are responsible for the generation of methane but major portion of methane is formed by acetotrophic methanogens by converting acetate into CH_4 and CO_2 (Sarker et al., 2019).

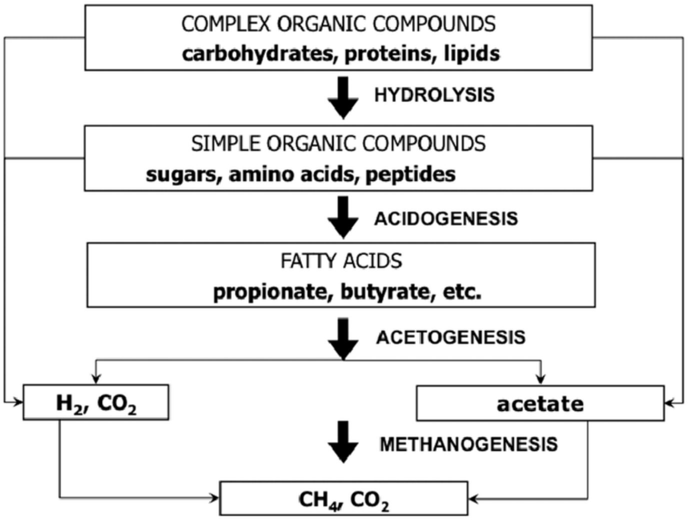


Figure 2.4 Mechanism of anaerobic digestion process

2.5 Factors affecting anaerobic digestion

In order to get maximum benefit from the anaerobic digestion, it is better to understand the fundamental process parameters. There are several parameters like pH, temperature, amount of VFAs, carbon to nitrogen (C/N) ratio, OLR and hydraulic retention time (HRT) that effect and control the performance of anaerobic digestion system. The change from their optimum level can even cease the digestion process (Neshat et al., 2017).

2.5.1 pH

pH should be well established as it's an important parameter for maximum CH_4 production. Optimum pH range is 7.0 to 7.2 but can vary from 6.5 to 8.2 (Carotenuto et al., 2020). Acidogens prefer an acidic environment with pH of 6.2 while methanogens boom in pH levels 6.8 to 7.2 (Sakeus, 2016). To maintain optimum pH level, acidic and basic and basic diluted solutions such as chemicals like such as hydrochloric acid (HCl) and sodium bicarbonate ($NaHCO_3$), lime (CaO) and sodium carbonate (Na_2CO_3) can be used (Raposo et al., 2012).

2.5.2 Temperature

The utmost important factor that affects the performance of anaerobic digestion and the microbial community is temperature (Sun et al., 2019; Zhang et al., 2018a). The AD process is usually conducted under three temperatures, classified as psychrophilic (<25°C), mesophilic (30–40 °C) or thermophilic (50–60 °C) temperatures but the degradation rate efficiency and more methane output is achieved through thermophilic conditions. Due to less cost, energy consumption, and more stability of process, mesophilic temperature is preferable (Westerholm & Schnürer, 2019). Based on universal agreement in literature, mesophilic temperature is most favorable and used condition in anaerobic digestion for methane production (Carotenuto et al., 2020).

2.5.3 Substrate

The AD performance is dependent on many factors, among them the most important factor is substrate type (Zhang et al., 2019; Lu, et al., 2019) The role of substrate types and substrate microbial community on the fate of antibiotic resistance genes during anaerobic digestion. Substrate composition and concentration are an important factor for the stability of AD process. Volumetric methane yield can be increased by increased substrate concentration (Zhang et al., 2014) but it can cease the AD system due to buildup of VFAs, total ammonia (TAN) and free ammonia (FAN) (Ziganshina et al., 2017).

2.5.4 Mixing

For stability of anaerobic digester, mixing is required to keep the feed homogeneity with the microbial community (Lindmark et al., 2014). The benefit of mixing is to combine bacteria with feed therefore prevents scum formation and temperature fluctuation in digester. As microorganisms are sensitive so intense mixing can disrupt them while too slow mixing can cause improper mixing and short circuiting (Abbasi et al., 2012).

2.5.5 Inoculum

The right choice of inoculum along with temperature is a must for the anaerobic digester start up. The increased inoculum can cease the anaerobic process while too little can lead to partial degradation of feedstock and results in problems like inhibition or slower methane production and VFA accumulation **(**Sarker et al., 2019).

2.5.6 Hydraulic retention time

The hydraulic retention time (HRT) is the time period during which the substrate deteriorate completely. It varies from longer to shorter HRTs but an average HRT for mesophilic condition to degrade waste is 15 to 30 days. Methane production can be ceased with less growth of methanogens due to low HRT therefore, longer HRT at low temperature is beneficial for anaerobic digestion (Yao et al., 2020). HRT is very important in terms of determining the sum of volatile solids and organic matter provided for digestion (Odey et al., 2016). HRT is mainly important as it can affect the conversion of volatile solids (VS) into biogas (Shi et al., 2017). The longer HRT results in AD system failure as microbial population disrupted along with frequent washout of organic matter due to unwarranted feeding of AD system (Sakeus, 2016).

2.5.7 Organic loading rate

The organic loading rate abbreviated as OLR which is the amount of substrate fed to an anaerobic digester per unit volume per day and it can be expressed as OLR = C/HRT where C is the feed concentration in g VS/L, and HRT is the hydraulic retention time (Sarker et al., 2019). More OLR than system's capacity results in more acetate production leading to less methane yield while less OLR do not provide enough substrate to anaerobic microbes results in low biogas production. The OLR less than 2.0 kg VS/m^3/day is used to operate small scale anaerobic digesters (Kinyua et al., 2016).

2.5.8 Total solids

The feedstock in an anaerobic digester is either wet or dry depending on the amount of total solids (TS) content in it. If the TS% is less than 15% than is it known as wet anaerobic digestion and if TS% exceeds 15%, it is considered solid-state or dry anaerobic digestion (André et al., 2018). The high TS% usually results in reduced CH_4 production as less water in substrate to be used as medium to disseminate microorganisms and nutrients (Riya et al., 2018).

2.5.9 Carbon to nitrogen ratio

The essential nutrients for microbial growth are carbon (C) and nitrogen (N). Energy source and synthesis of protein and nucleic acids are the main uses of carbon and nitrogen respectively (Lin et al., 2018). Its ratio is basically an indicator of nutrients availability in form of carbon and nitrogen in substrate. Therefore, the optimum range for C/N ratio is between 20 and 30. Feedstock with low C/N ratio results in high production of VFAs and TAN. Increased TAN and VFAs concentration can cease the anaerobic digestion system due to excessive buildup of these two. On the contrary, the digestion process and microbial growth can be slow down due to inadequate nitrogen content (Carotenuto et al., 2020; Yao et al., 2020).

2.5.10 Volatile fatty acids

In an aerobic digestion, the intermediate products for methane production are VFAs. The key products of digestion process are acetic, butyric and propionic acid which are performance parameters of anaerobic digestion. Though, the excess of these acids results in lowering the pH to less than 6 of an anaerobic digestion system. Consequently, lower pH outcome includes the inactivity of methanogens along with the production of useless and unwanted products. A stable anaerobic digestion system has VFAs' concentration in range of 50-250 mg. L^{-1} while 1500-2500 mg. L^{-1} VFAs can inhibit the anaerobic digester functionality (Neshat et al., 2017).

2.5.11 Particle size

The size of a substrate plays a key role in biodegradation results. Particle size, its size reduction process and surface area are very important to mention as they not only effect but also determine the initial step of degradation in the anaerobic digestion process. The large particle size of feedstock may clog the digester making it difficult for microorganisms to continue digestion therefore size should be limited. If the feedstock has less degradation characteristic than it can be utilized by reducing the particle size and increasing the surface area. Although the suggested particle size is ≤10 mm but if it's difficult to reduce size then material should be cut, wrecked or processed to achieve desirable size (Raposo et al., 2012).

2.5.12 Alkalinity

The proteins in substrate of anaerobic digester are basically nitrogenous material which degraded and produced ammonia. The high ammonia concentration produced due to high protein content in feedstock is problematic. The high concentration of ammonia production results in inhibitory effect on methanogens (Jiang et al., 2019).

2.5.13 Inhibitors of anaerobic digestion

Livestock manure contains harmful substances such as antibiotics (tetracyclines, flouroquinolones, sulfonamides and ionophores), pathogens (rotavirus, escherichia coli and salmonella) and heavy metals (arsenic, copper and zinc). These toxic compounds containing manure can cause health problems and pollutes the environment when they directly applied on land (Zubair et al., 2020). Some of the existing and emerging inhibitors and their inhibitory concentrations are given in Table 2.6.

The presence of inhibitors is mainly linked with their presence, fate and concentration in all types of substrate. The substrate driven toxicants includes waste from slaughterhouse, ammonia nitrogen, H_2S, long-chain fatty acids (LCFAs) and liquid and sulfur rich substrates i.e., food waste, sludge, wastewater, microalgae which can negatively affect the methanogens during AD (Cai et al., 2021). Arikan et al., (2006) reported that 27% biogas production reduced compared to control due to presence of 3.1 mg OTC L^{-1}. Ke et al., (2014) also reported the inhibitory effect of OTC concentrations (20, 50 and 80 mg L^{-1}) on anaerobic digestion of cattle manure which reduced the biogas production 44, 65 and 78% compared to control.

Different inhibitors have different inhibitory concentrations in digestion process. They effect the anaerobic digestion process once their concentration exceeds the anaerobic microbe's threshold.

Table 2.6 Inhibitors of anaerobic digestion process

Inhibitors	**Effect on anaerobic digestion**	**Inhibitory concentration (mg L^{-1})**	**Source**
Volatile fatty acids	Reduce pH and inhibit methanogens	1500	Abou Khalil et al., (2017)
Sulfides	Inhibits methanogenic activity	200	
Ammonia	Inhibits methanogenic activity	3000	
Aromatic compounds	Inhibits methanogenic activity	100	
Chlorinated hydrocarbons	Toxic to methanogens	1	
Nitrate and nitrite	Inhibit methanogens	15	
Nanoparticles	Inhibits microbial activities	varies	
Antibiotics	Inhibits microbial activities	minute and varies	

2.6 Types of anaerobic digesters

Anaerobic digesters are of various kinds differing in design and operation, low-rate to Chinese and Indian fixed dome or floating digesters, respectively. AD reactors also exist as stirred to balloon digesters and even heated anaerobic digesters in temperate areas of the industrialized world (Sakeus, 2016).

2.6.1 Batch reactors

Batch reactors are quite simple reactors which require less operational cost, easy to operate and maintain (Sakeus, 2016), ability to strongly resist several substrates, thereby making it most favorable Ad reactor to be used in developing countries. In batch digestion, the digesters are fed once with substrate, inoculum and water at the start with the addition of a buffer solution to encounter pH changes during the digestion and then closed for the whole retention time. The gas production is low at the start and gradually increased to a maximum and then decreased to a constant. Batch digestion gives high degradation of substrates than continuous digestion as the substrate spend more time in digester than in continuous digestion (Carrere et al., 2016).

2.6.2 Continuous reactors

Continuous digesters functioning includes the continuous addition of feedstock and discharging of the digestate. In continuous digestion, substrate is fed 1-8 times on daily basis depends upon the type of substrate. The freshly fed substrate takes the place of the old fed material thus maintaining the constant digester volume so relatively less time is available for the substrate to biodegrade as compared to batch digesters. Continuous feed digesters provide constant gas production as compared to batch digestion (Wei et al., 2018).

2.7 Anaerobic digestion of cattle manure

The most general and excessively available resource to be used in AD process is dairy cow manure. Being rich in microorganisms and micro and macro nutrients accompanied by development in livestock production, this manure can produce biogas (McVoitte & Clark, 2019) as shown in Table 2.7.

Table 2.7 Biogas/methane production from anaerobic digestion of cattle manure

Substrate	**Reactor type**	**Experimental conditions**	**Findings**	**Reference**
Cow manure	Batch	Temperature=35°C HRT=90 days	Maximum methane yield was 35,967 mL/kg VS on 65th day	Jafari-Sejahrood et al., (2019)
Cow dung	Semi continuous	Temperature=29°C HRT=55 days	Highest biogas yield of 77 L kg/VS	Haryanto, (2018)
Dairy manure	Batch	Temperature=37°C HRT=45 days	Methane potential was 204 mL/gVS with VS removal of 59%	Kafle & Chen, (2016)
Dairy manure	Batch	Temperature=37°C HRT=80 days	Maximum methane yield of 270 CH_4/g VS	Li et al., (2015)

The problems associated with the expansion of large and confined livestock feeding set-ups can be dealt with alternative solution such as AD of animal manure (Ileleji et al., 2020). AD of manure will not only produce biogas rich in methane and also tackle the problem of water and air pollution triggered by livestock manure (Kafle & Chen, 2016).

Table 2.8 Inhibitory effect of oxytetracycline on anaerobic digestion of cattle manure

Substrate	**Feed (TS%)**	**Reactor Type**	**Temperature (°C)**	**HRT (d)**	**OTC concentration (mg L^{-1})**	**Findings**	**Reference**
Dairy manure	-	Batch	37	45	40, 80, 160, 320, 640 1280	Methane yields were reduced by 10, 13, 26, 24, 32 and 32%	Andriamanohiarisoamanana et al., (2020)
Dairy manure	14	Batch	37 ± 1	60	3 and 3.11	38 and 48% reduced biogas as compared to control	Akyol et al., (2016)b
Cow manure	18	Batch	37 ± 1	60	50, 100 200	Biogas production was reduced by 41, 57 and 61% Biogas production was highly correlated with methanobacteriales	Coban et al., (2016)
Cow manure	25	Batch	37	50	20, 50 80	Biogas decreased by 44, 65 and 78% with increase in OTC concentration	Ke et al., (2014)
Dairy manure	-	Batch	55	16	30, 60 90	CFZ concentrations showed no inhibition while OTC decreased methane production by 21, 30, and 31%	Beneragama et al., (2013)
Cow manure	5	Batch	37	30	1-3.3	50-60% decrease inhibition in biogas yield	Ince et al., (2013)

2.8 Effect of oxytetracycline on anaerobic digestion of manure

Manure containing antibiotic for biogas production can enter the VAs in anaerobic digestion process. Veterinary antibiotics like tertacyclines or sulfonamides can remarkably reduce the biogas yield even in low concentration i.e. 10 mg L^{-1} (Spielmeyer et al., 2014). The well-established technology for treatment of livestock manure is anaerobic digestion (Arikan et al., 2006). The inhibitory effect of oxytetracycline on anaerobic digestion of cattle manure is illustrated in Table 2.8. Almost 70% of Oxytetracycline leaves the animal body without ample metabolism by means of urine and feces. Therefore, the presence of OTC in feedstock used for AD will upset and even cease the whole process by inhibiting microorganisms (Turker et al., 2018). That's why, the presence of OTC in manure can have an inhibitory effect on the microorganisms involved in AD for biogas production as a renewable energy source (Akyol et al., 2016a).

Chapter 3

Materials and Methods

This chapter describes the experimental framework adopted during the conducted research work. The work was divided into three phases. In first phase, inoculum was collected and anaerobically digested. In second phase, substrate was collected, the experimental conditions were executed and then lab-scale anaerobic digestion set up was developed. Finally, in last phase, kinetic models were applied to see which model gave the best fit results for experimental data. All the methodologies followed throughout the study are described here in detail.

3.1 Substrate and inoculum preparation

Cattle manure (CM) used as an inoculum and substrate in the study was collected from cattle shed located in the vicinity of H-13 sector, Islamabad. The cattle manure to be used as inoculum was incubated at 37 °C under anaerobic conditions for 15 days in a water bath (Memmert, Germany) under regular monitoring and degassing. Once inoculum was ready, the fresh cattle manure to be used as substrate was collected from the same source and used for experimental purpose.

3.2 Sample preparation for anaerobic digestion

3.2.1 Preparation of stock and standard solutions

Oxytetracycline hydrochloride (CAS no. 2058-46-0) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The stock solution of 100 mg OTC L^{-1} was prepared by dissolving 100 mg of OTC in 1000 L distilled water. The OTC working standard solutions of 0.12, 0.3, 0.6, 1.2, 3, 6, and 12 mg L^{-1} were prepared by diluting the stock solution in distilled water.

3.2.2 Sample spiking

Predetermined amount of inoculum, substrate and OTC standard solutions were placed in serum bottles. The samples were spiked with different OTC concentrations as shown in

Table 3.1. For control, inoculum and substrate was added i.e. no OTC was added while for different OTC concentrations, different OTC in mg L^{-1} were added. For instance, as per the working volume of reactors used in this study, 0.6 mg L^{-1} OTC spiked reactors, 0.135 mg of OTC was added while 2.7 mg OTC was added to make it 12 mg L^{-1} OTC concentration.

Table 3.1 Spiking of anaerobic reactors

OTC Concentration mg kg^{-1} TS	**Weight of OTC required mg**	**mg L^{-1}**
10	0.027	0.12
25	0.0675	0.3
50	0.135	0.6
100	0.27	1.2
250	0.675	3
500	1.35	6
1000	2.7	12

3.3 Analytical methods

3.3.1 Total solids, volatile solids and moisture content

The total solids and volatile solids of inoculum and substrate were determined in triplicate according to standard method in APHA (APHA, 2017). 20 g sample was taken into pre-weighed evaporating dish which was dried in oven at 105°C for 30 minutes. The sample containing evaporating dish was placed in oven for 24 hours at 105°C. It was cooled to ambient temperature in desiccator and weighed on analytical balance. Finally, the total solids percentage (%TS) was calculated by using equation 3.1

$$TS\% = \frac{A-B}{C-B}\ x\ 100 \qquad (3.1)$$

Where,

A = weight of dried residue and evaporating dish after 105°C

B = weight of evaporating dish

C = weight of wet sample and evaporating dish

Moisture content is the weight loss at 105°C. The volatile solids (%VS) were determined by using dried residues of sample in evaporating dish whose TS% was determined by the method described above. The dish was then placed into muffle furnace at 550°C for 1 hour. The loss in weight after ignition was the measure of VS content and was calculated by using equation 3.2

$$\text{VS (\%TS)} = \frac{A-D}{A-B}\ x\ 100 \qquad (3.2)$$

Where,

D = weight of evaporating dish and residue after ignition at 550°C

To assess reactor's stability before and after anaerobic digestion, samples were analyzed for TS%, VS (%TS), TA and VFA according to APHA standard methods (APHA, 2017). The pH was measured using pH meter (HANA, model 8521, U.S.A). Effect of OTC concentrations on VS removal was also measured by determining VS removal% using equation 3.3

$$\text{VS removal (\%)} = \frac{Initial\ gVS\ of\ substrate - Final\ gVS\ of\ substrate}{Initial\ gVS\ of\ substrate} \qquad (3.3)$$

Where,

Final gVS of substrate = Final gVS – Final gVS of inoculum

3.3.2 Total organic carbon and total kjeldahl nitrogen

Total organic carbon (TOC) was calculated using equation 3.4 developed by Adams et al., 1951

$$\text{Organic carbon (\%)} = \frac{\text{VS (\%TS)}}{1.8} \qquad (3.4)$$

Total kjeldahl nitrogen (TKN) was performed for inoculum and control in triplicates according to the method mentioned in APHA (APHA, 2017). The average values of samples were used in calculations.

3.3.3 Volatile Fatty Acids and Alkalinity

The total alkalinity (TA) and volatile fatty acids (VFA) were determined through titration method (APHA, 2017). The samples were run in triplicate and mean values were used in calculations.

3.3.4 Biogas and methane yield measurement

Daily biogas production was determined using the water displacement method (Maryam et al., 2021). The daily biogas data was converted and results were reported at normalized temperature (0°C) and pressure (760 mmHg) using Equation 3.5 (Dinuccio et al., 2010).

$$V_{TP} = \left(V_b \ x \ 273 \ x \ (760 - p_w)\right) / \left((273 + T) \ x \ 760\right) \qquad (3.5)$$

Where,

V_{TP} = dry biogas volume at normal temperature and pressure (NmL)

p_w = water vapor pressure based on temperature of ambient place (mmHg)

V_b = recorded volume of the biogas (mL)

T = ambient temperature (°C)

Methane content of biogas samples was also analyzed periodically using Gas Chromatograph (Shimadzu, GC 2010 plus, Japan) and multiplied with biogas volume to calculate the volume of methane produced from the reactors.

3.4 Experimental conditions and set up for anaerobic digestion

The anaerobic digestion was conducted in triplicate using 300 mL serum bottles which were used as reactors with 75% working volume (225 mL) and 25% headspace left for biogas accumulation. The predetermined amount of inoculum and substrate were added in reactors in ratio of 1:1 on g VS basis at organic loading of 10 g/VS L. The OTC and distilled water were added as per required concentration. The experiment was carried out in three groups (1) Blank group (only inoculum), (2) Control group (inoculum and CM without OTC) and (3) treatment group (inoculum, CM and treatments, i.e., seven different concentrations of OTC includes 0.12, 0.3, 0.6, 1.2, 3, 6 and 12 mg L^{-1}). The initial pH of all reactors was adjusted at 7.0 + 0.1 using 1M sodium bicarbonate ($NaHCO_3$). The reactors were capped with rubber septum and crimped with aluminum seals. After sealing, the nitrogen gas (N_2) was flushed through the headspace of reactors for 2 minutes to remove traces of oxygen and ensure anaerobic condition. The reactors were then placed at 37°C for 45 days in an incubator (Velp Scientifica- FOC 120E Cooled Incubator, Italy). The reactors were mixed twice a day manually for 2 to 3 minutes to ensure homogenization of reactor content. The blank group was prepared to measure its biogas volume which was later deducted from biogas volume of all reactors to determine the biogas produced from the CM only.

3.5 Statistical analysis

All experiments were conducted in triplicates and results are reported in mean values. The standard deviation is also mentioned along with their values in respective graphs and tables.

3.5.1 ANOVA

The difference in pH, VFA/TA, VS removal and cumulative biogas yield of control and OTC spiked reactors were determined by one-way analysis of variance (ANOVA) using SPSS 23 with probability less than 0.05 ($p < 0.05$). The plotting of all the data was done using Origin Pro 2021.

3.5.2 Kinetic models

Cumulative biogas yield obtained from the experimental data was fitted with two non-linear regression kinetic models, modified Gompertz (Equation (3.6)) and logistics function (Equation (3.7)) model. These models were selected to accurately analyze the metabolic pathways and kinetics of biogas production involved in AD of CM containing different OTC concentrations (Pramanik et al., 2019). Both kinetic models differ in their point of inflection, developed assuming that bacterial growth was the rate limiting step in

AD (Ware & Power, 2017) and are used to simulate biogas production of anaerobic digestion process (Rajput et al., 2018). Therefore, both kinetic models were used to determine cumulative biogas yield potential, lag phase duration and maximum biogas production.

Modified Gompertz model:

$$\mathrm{M_{pt}} = M_b . exp\left\{-exp\left[\frac{R_m \cdot e}{M_b}(\lambda - \mathrm{t}) + 1\right]\right\} \qquad (3.6)$$

Logistic function model:

$$\mathrm{M_{pt}} = \frac{Mb}{1+exp\left\{\frac{4*\mathrm{Rm}*\lambda-\mathrm{t}}{Mb}+2\right\}} \qquad (3.7)$$

Where,

$\mathrm{M_{pt}}$ = predicted biogas yield (NmL/g VS) at a given time t (days)

$\mathrm{M_b}$ = cumulative biogas production potential (NmL/g VS)

$\mathrm{R_m}$ = maximum specific biogas production rate (NmL/g VS/d)

E = 2.7183 used as Euler's function

λ= lag phase (days)

The kinetic model parameters ($\mathrm{M_b}$, $\mathrm{R_m}$ and λ) were analyzed using IBM SPSS statistics 23.

3.5.3 Model evaluation

To determine the best fit model among modified Gompertz and logistic function model, the six statistical criteria were used and compared. The criteria include coefficient of determination (R^2), root mean square error (RMSE (3.8)), Akaike information criteria (AIC (3.9)), delta Akaike information criteria (ΔAIC (3.10)), Bayesian information criteria (BIC (3.11)) and delta Bayesian information criteria (ΔBIC (3.12)).

$$\mathrm{RMSE} = \frac{\sqrt{\Sigma_{\mathrm{t}}\left(\mathrm{M_{pt}}-\mathrm{M_{expt}}\right)^2}}{N} \qquad (3.8)$$

$$\mathrm{AIC} = N\ln\left(\frac{RSS}{N}\right) + 2K + \frac{2K\,(K+1)}{(N-K-1)} \qquad (3.9)$$

ΔAIC = AIC of particular model – AIC of best model (3.10)

$$\text{BIC} = N \ln\left(\frac{RSS}{N}\right) + Kln(N) \tag{3.11}$$

ΔBIC = BIC of particular model – BIC of best model (3.12)

Where M_{pt} and M_{expt} are the predicted and experimental biogas volume, respectively. N and K are the number of experimental data points and fitted model parameters, respectively whereas RSS represents the residual sum of squares.

Chapter 4

Results and Discussion

4.1 Characterization of inoculum and substrate

The initial characteristics of feedstock play a crucial role in design, set up, biogas/methane production, process stability and performance of AD process. In start of experiment, the pH of inoculum and substrate was 6.4 and 6.9, respectively. The characteristics of inoculum and substrate are mentioned in Table 4.1. Coban et al., (2016) reported that before the anaerobic digestion of cow manure, the concentration of TS, VS, C/N ratio and TKN were 18%, 83%, 4/16 and 12,000 mg kg^{-1}, respectively.

Table 4.1 Characterization of inoculum and substrate

Parameters	**Units**	**Inoculum**	**Substrate**
pH	-	6.4±0.05	6.9±0.05
Total Solids (TS)	%	12±0.1	18±0.1
Volatile Solids (VS)	%TS	85±0.1	82±0.12
Moisture Content (MC)	%	88±0.12	82±0.1
Total Organic Carbon (TOC)	%	47±0.06	46±0.06
Ammonia Nitrogen	mg L^{-1}	67±1.5	89±0.4
Organic Nitrogen	mg L^{-1}	168±1.1	378±0.9
Total Kjeldahl Nitrogen	mg L^{-1}	235±1.4	467±1.1

4.2 Effect of oxytetracycline concentrations on stability parameters of reactors

The commonly used indicators for stability of anaerobic digestion process are pH and VFAs. As the increase and decrease in stability parameters values shows threshold of AD process, therefore, these parameters are monitored alongside to monitor the anaerobic digesters performance (Andriamanohiarisoamanana et al., 2020). The accumulation of VFAs reduce the pH which ultimately decrease or even cease the AD process. In such cases, alkalinity plays its role to neutralize the VFA concentrations thereby causing offset in pH changes. In order to have a proper overview on anaerobic digestion stability; volatile fatty acids, total alkalinity and pH must be monitored (Lukitawesa et al., 2020; Telesphore & Issah, 2020).

4.2.1 pH

The pH of control and different concentrations of OTC are given in Figure 4.1. After anaerobic digestion of cattle manure, the pH of all reactors was increased from 7 to 7.4. The control has highest pH i.e., 7.4. Arikan et al., (2006) and Beneragama et al., (2013) also found that pH was increased after anaerobic digestion. The increase in pH after anaerobic digestion might due to the continuous stirring which results in the dissolution of cattle manure (Zhang et al., 2018). It also shows the balance of organic acid concentration between fermentative bacteria's and methanogens. The significant difference ($p < 0.05$) on the pH of OTC spiked reactors at concentrations of 0.6, 1.2, 3, 6 and 12 mg L^{-1} was observed in comparison to control.

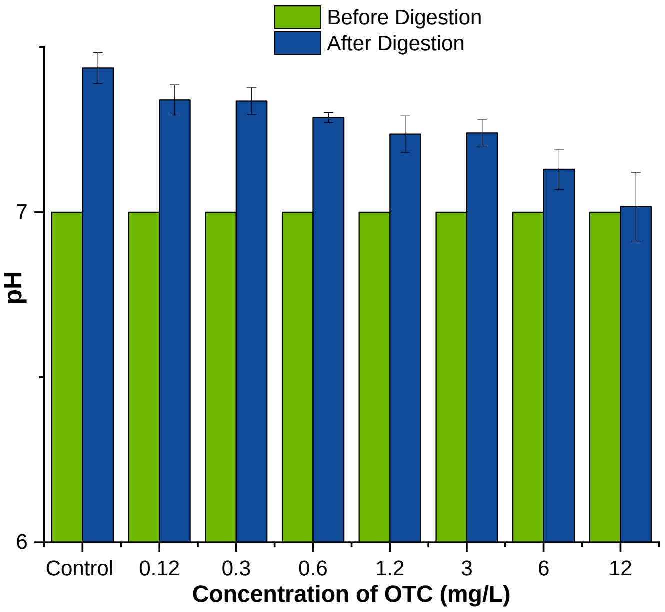


Figure 4.1 Effect of oxytetracycline concentrations on pH of cattle manure

4.2.2 VFA/TA ratio

Figure 4.2 shows the VFA/TA ratio of different concentrations of OTC compared to control. The VFA/TA ratio of reactors increased with the increase in concentrations of OTC. The control has 0.2 VFA/TA which increased to 0.44 in 1.2 mg L^{-1} reactor and further increased to 0.58 in 12 mg L^{-1} OTC spiked reactor. Compared to control, the concentrations of OTC at 0.12 and 0.3 mg L^{-1} showed no significance difference ($p > 0.05$) while remaining concentrations from 0.6 to 12 mg L^{-1} showed significant difference ($p < 0.05$) in VFA/TA. Beneragama et al., (2013) reported that the reactors containing OTC (30, 60 and 90 mg L^{-1}) exhibited increase in VFA/TA as compared to control. The reason might be that the less VFA/TA results in more biogas production as more volatile fatty acids were consumed to produce biogas while more VFA/TA ratio shows less biogas production.

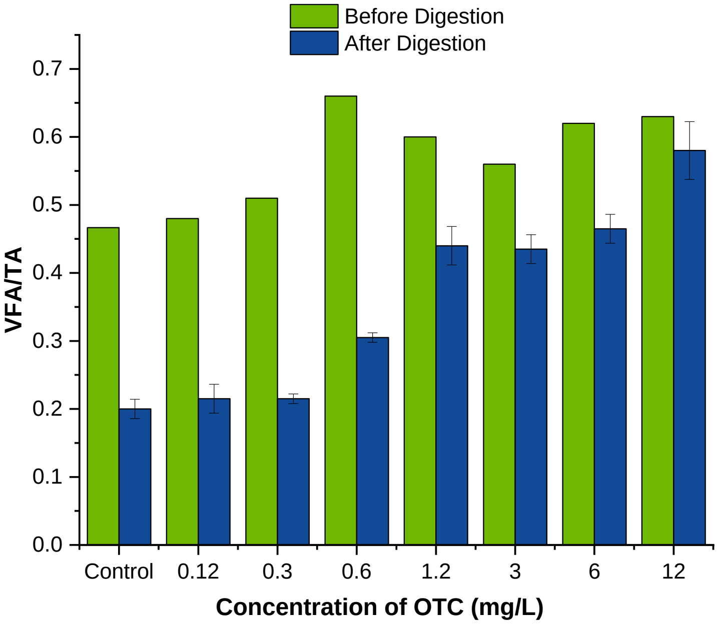


Figure 4.2 Effect of oxytetracycline concentrations on VFA/TA of cattle manure

4.3 Effect of oxytetracycline concentrations on anaerobic digestion performance

The effect of OTC concentrations i.e. 0.12, 0.3, 0.6, 1.2, 3, 6 and 12 mg L^{-1} on anaerobic digestion of cattle manure were studied in terms of daily biogas production (NmL), cumulative biogas yield in NmL/g VS, methane content (%) and cumulative methane yield in NmL/CH_4g VS.

4.3.1 Effect of oxytetracycline concentrations on daily and cumulative biogas yield of cattle manure

Figure 4.3 illustrates the daily biogas production for control and OTC concentrations during 45 days of anaerobic digestion. Daily biogas production started from the first day of experiment but rapid rise in biogas production observed in second week. Overall, the well prepared inoculum results in short lag phase. The highest daily biogas (Dm) was 41 NmL for control and Dm for 0.12, 0.3, 0.6, 1.2, 3, 6 and 12 mg L^{-1} was 55, 38, 43, 35, 37, 35 and 38 NmL. Although the Dm of control was less than 0.12 mg OTC L^{-1}, the cumulative biogas yield of control was higher in comparison to OTC spiked reactors. The reason could be that the microbial community in control group might have taken some time to acclimatized and then produced constantly higher daily biogas (Coban et al., 2016).

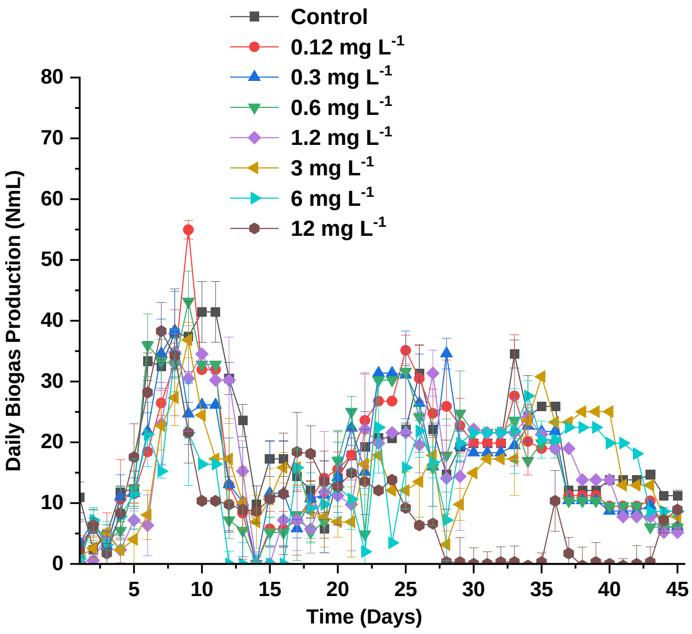


Figure 4.3. Effect of oxytetracycline concentrations on daily biogas production of cattle manure

The cumulative biogas yields of cattle manure reactors spiked with different OTC concentrations were affected as presented in Figure 4.4. The cumulative biogas yield of

CM decreased with increase in OTC concentrations compared to the control. The control has highest cumulative biogas yield of 393 NmL/g VS while the reactors spiked with OTC concentrations from 0.12 to 12 mg L^{-1} in this study have shown reduction in cumulative biogas yield from 345 to 177 NmL/g VS. Anova was applied to see the statistical significance between different treatments and control group. The results showed no statistical difference ($p > 0.05$) in biogas yield of reactors spiked with OTC concentrations of 0.12, 0.3, 0.6 and 1.2 mg L^{-1} in contrast to control. In case of high OTC concentrations (3, 6 and 12 mg L^{-1}), each showing a significantly higher difference ($p < 0.05$) in biogas yields in comparison to control.

The reduction (%) in cumulative biogas yield is mainly associated with the effect of different OTC concentrations on cattle manure. As compared to control, the increase in OTC concentrations to 3, 6 and 12 mg L^{-1} in the cattle manure anaerobic digesters caused 25, 29 and 55% reduction in cumulative biogas yield. When OTC concentrations were 3.1 and 3.2 mg L^{-1}, 50 to 60% inhibition occurred in biogas production contrasted with control (Ince et al., 2013). Coban et al., (2016) reported that when OTC concentrations were 50, 100 and 200 mg L^{-1}, the reduction in biogas production was 41, 57 and 61% respectively.

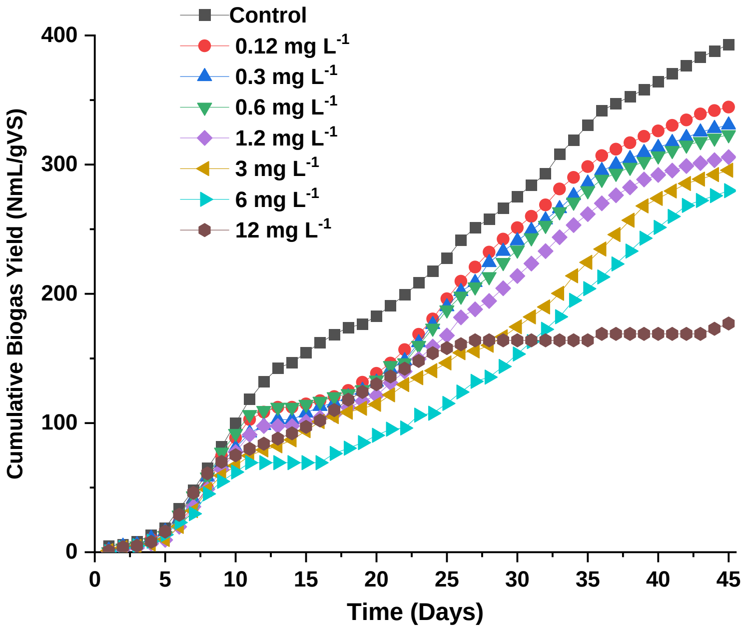


Figure 4.4. Effect of oxytetracycline concentrations on cumulative biogas yield of cattle manure

4.3.2 Effect of oxytetracycline concentrations on methane content (%) and cumulative methane yield of cattle manure

The methane content (%) in the produced biogas of different OTC spiked reactors is shown in Figure 4.5. The methane content of control increased from 62 to 69% after 15 days. As compared to control, the effect of OTC concentrations can be seen on high OTC containing reactors especially in 3 and 12 mg L^{-1} where methane content (%) decreased from 70 to 61% and 64 to 53%, respectively. The reactor spiked with 6 mg L^{-1} showed a different behavior than other treatments. Its methane content suppressed in start of digestion period showing inhibition in methane yield but there was sudden increase in methane yield which depicts that system recovered from high antibiotic concentration. When several antibiotics including OTC concentration was 500 mg L^{-1}, the digesters methane yield first suppressed then started producing methane yield (Zhi & Zhang, 2019).

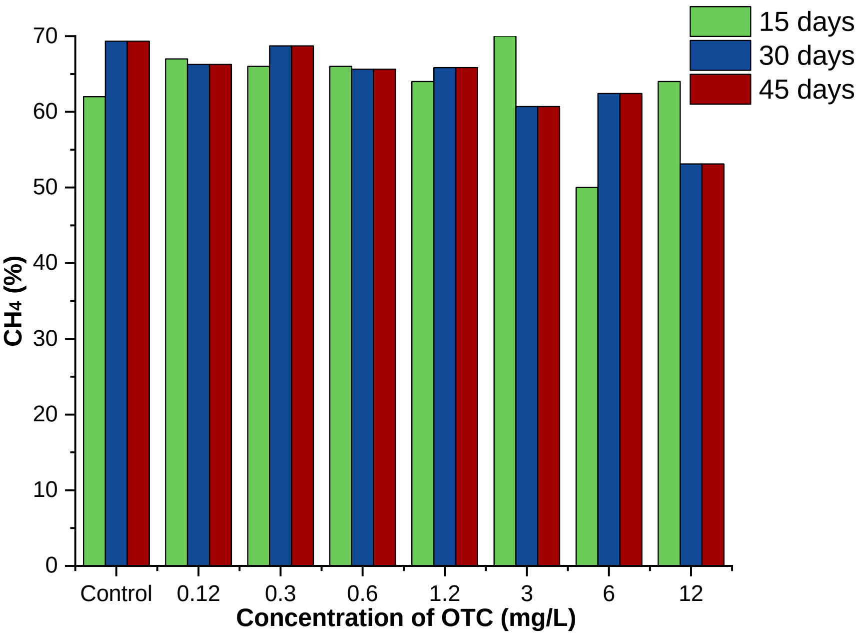


Figure 4.5. Effect of oxytetracycline concentrations on methane content (%) of cattle manure

The cumulative methane yield of cattle manure of OTC spiked reactors is presented in Figure 4.6. The highest cumulative methane yield was 271 $NmLCH_4$/g VS for control while the OTC spiked reactors from 0.12 to 12 mg L^{-1} have lower cumulative methane yields which reduced from 228 to 94 $NmLCH_4/g$ VS. This showed the effect of OTC concentrations increased on methane yields of reactors. Beneragama et al., (2013) authored that by using OTC concentrations from 30 to 90 mg L^{-1} in contrast to control, the methane yield reduced to 90 mL/g VS compared to control i.e. 150 mL/g VS which showed the significant but low inhibitory effect at specific OTC concentrations which did not affect the methanogenic activity. In this study, the increase in concentrations of OTC from 0.12 to 12 mg L^{-1} resulted in reduction of cumulative methane yields ($NmLCH_4/g$ VS) which showed evident effect of OTC concentrations on methane yields. Andriamanohiarisoamanana et al., (2020) reported the reduction (%) in methane yield decreased from 10 to 32% while Arikan et al., (2006) reported 27% inhibition in methane yield of calves' manure.

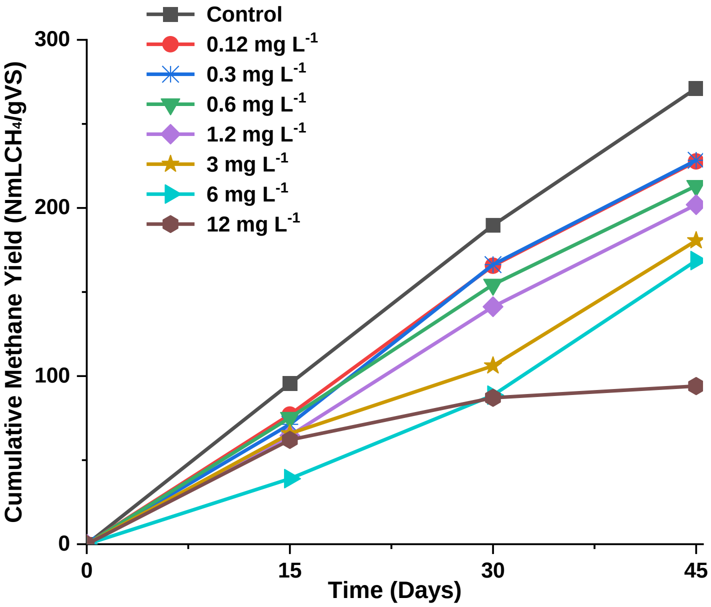


Figure 4.6. Effect of oxytetracycline concentrations on cumulative methane yield of cattle manure

4.3.3 Volatile solids removal (%)

The effect of different OTC concentrations and control on volatile solids (VS) removal can be seen in Figure 4.7. The graph shows a decreasing trend in VS removal with increase in OTC concentrations. The control showed a highest VS removal of 39%. The VS removal for 1.2 and 12 mg L^{-1} were 23% and 13% compared to control. In contrast to control, all OTC spiked reactors have significant difference ($p < 0.05$) which reveals the effect on biogas production with spiked OTC concentrations. The results of VS removal are in accordance with the biogas and methane yields of this study. Beneragama et al., (2013) reported that the VS consumption of control was 24% and decreased by 18, 17 and 19% with the 30, 60 and 90 mg L^{-1} OTC spiked concentrations, respectively which supports the methane production behavior.

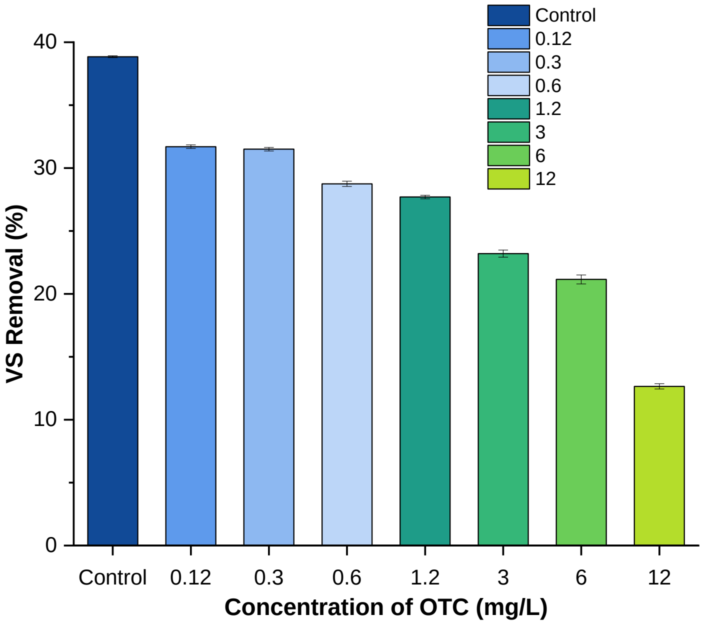
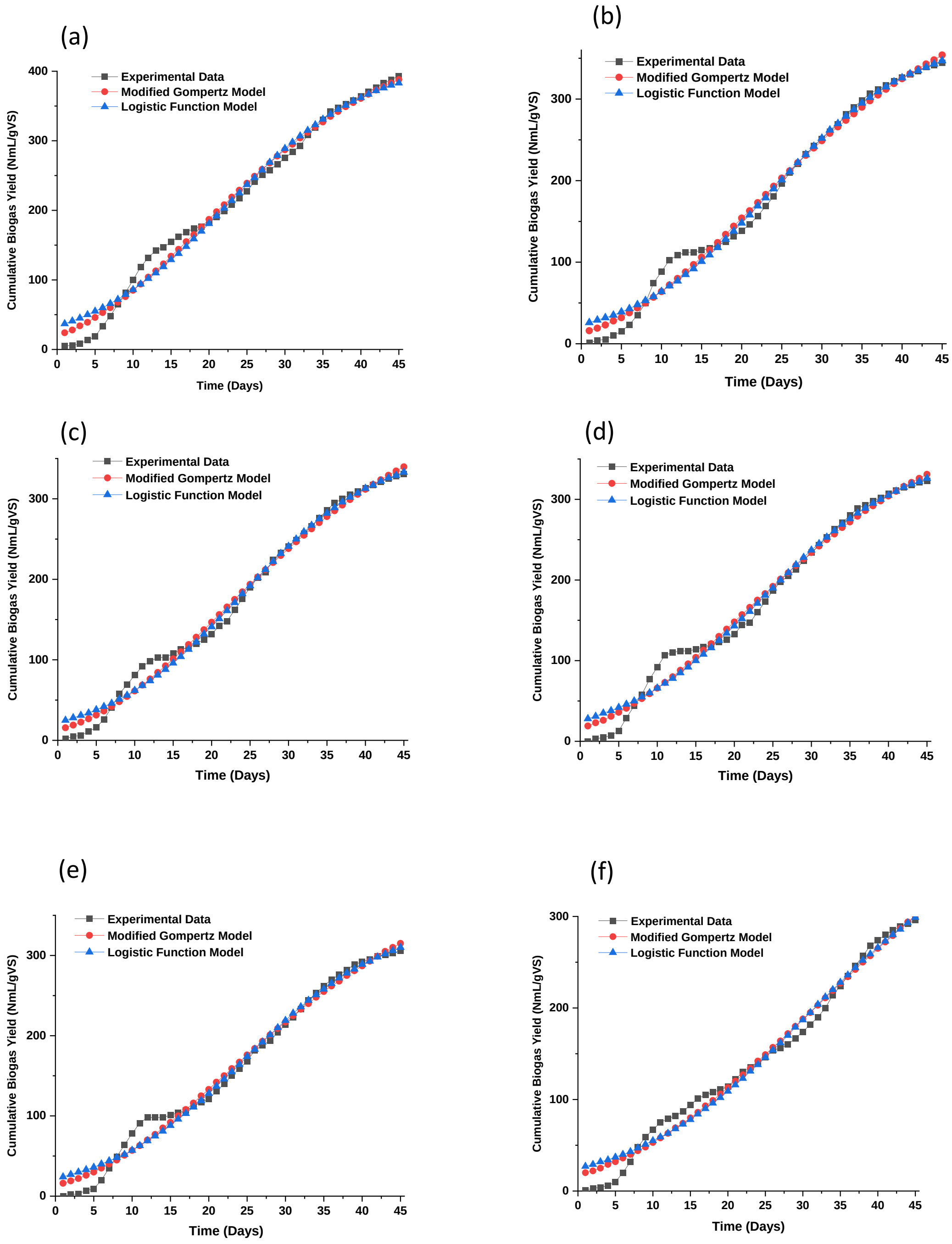


Figure 4.7 Effect of oxytetracyline concentrations on VS removal (%) of cattle manure

4.4 Comparison and selection of kinetic models

Mathematical kinetic analysis of anaerobic digestion plays an important role in monitoring and simulating the performance of AD process (Pramanik et al., 2019). Kinetic study is a cost effective and fast method to accurately predict and evaluate the mechanism and behavior of AD process. Therefore, robust model provides better estimation and prediction of kinetic model parameters necessary for design and smooth run of AD process (El-Mashad, 2013). The experimental data and model predicted curves of cumulative biogas yield from anaerobic digestion of CM spiked with seven OTC concentrations and control group can be seen in Figure 4.8.



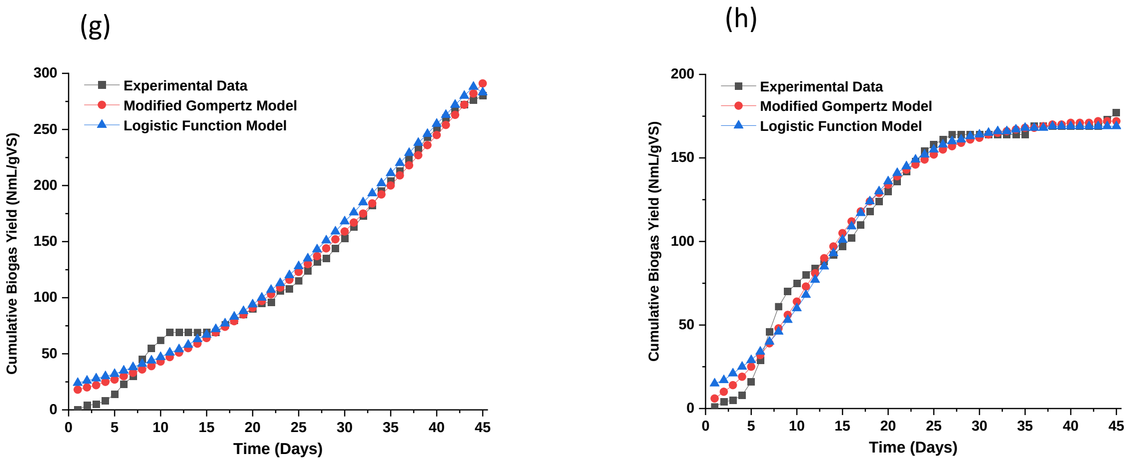


Figure 4.8. Experimental and predicted cumulative biogas yields at various concentrations of oxytetracycline for control (a), 0.12 mg L^{-1} (b), 0.3 mg L^{-1} (c), 0.6 mg L^{-1} (d), 1.2 mg L^{-1} (e), 3 mg L^{-1} (f), 6 mg L^{-1} (g) and 12 mg L^{-1} (h)

In this study, two kinetic models i.e. modified Gompertz and logistic function models were used to simulate the cumulative biogas yield from OTC spiked cattle manure during batch anaerobic digestion. The fitting results of kinetic parameters such as biogas production rate (R_m), maximum biogas production potential (M_b) and lag phase (λ) with experimental data can be seen in Table 4.2. The shorter lag phase of control showed earlier biogas production compared to OTC concentrations from 0.12 to 6 mg L^{-1}. The shorter λ in 12 mg L^{-1} OTC spiked reactor is due to high CH_4 % (64%) during first 15 days which started declining and reduced to 53% after 15 days and eventually stopped producing biogas due to high antibiotic concentration. Zhi & Zhang, (2019) reported that reactors spiked with 10, 100 and 500 mg L^{-1} OTC and other antibiotics during dry anaerobic digestion, the digesters containing 100 mg L^{-1} had lower λ values due to earlier methane production. When the data of two kinetic models was compared in this study, the modified Gompertz model showed the less lag phase (λ) and biogas production rate (Rm) as compared to logistic function model which are in accordance to the experimental data. According to results shown in Figure 4.8 and Table 4.2, it is quite evident that modified Gompertz model provides more robust estimation and best fitted with the experimental data. Rajput et al.,

(2018) and Zhi & Zhang, (2019) also found that the modified Gompertz model best fitted to the cumulative biogas and methane yields.

Table 4.2 Estimated parameters of kinetic models for validation of biogas yield data

Kinetic Models and Parameters		**Unit**	**Oxytetracycline (mg L^{-1})**							
			Control	**0.12**	**0.3**	**0.6**	**1.2**	**3**	**6**	**12**
Modified Gompertz Model										
Lag phase (λ)		Days	2.5	4.4	4.4	3.4	4.4	6	14.5	2.4
Maximum biogas production rate (Rm)		mL/gVS d^{-1}	10.7	9.8	9.4	8.9	8.5	7.8	12.7	8.5
Biogas yield	Predicted	mL/gVS	388	354	340	331	315	301	284	161
	Measured	mL/gVS	393	345	331	323	306	296	280	177
Logistic Function Model										
Lag phase (λ)		Days	3.9	6.3	6.3	5.2	6.3	7.6	14.3	2.8
Maximum biogas production rate (Rm)		mL/gVS d^{-1}	11.2	10.7	10.3	9.6	9.4	8.3	9.2	8.3
Biogas yield	Predicted	mL/gVS	383	347	333	326	310	299	283	163
	Measured	mL/gVS	393	345	331	323	306	296	280	177

The best fit model was selected among modified Gompertz and logistic function model based on six statistical indicators as shown in Table 4.3. The lower values of R^2, rMSPE, RSS, AIC, and BIC indicate a more appropriate model (Yang et al., 2016). In this study, modified Gompertz model showed R^2 ranged between 0.99 to 0.983 for all groups. The RMSE ranged between i.e. 6 to 14.2 and AIC and BIC ranged between 165-245.6 and 169.8-249.4, respectively.

Table 4.3 Comparison of kinetic model parameters using statistical criteria

Statistical criteria for model comparison	Oxytetracycline (mg L^{-1})							
	Control	**0.12**	**0.3**	**0.6**	**1.2**	**3**	**6**	**12**
Modified Gompertz Model								
R^2	0.986	0.987	0.99	0.983	0.986	0.984	0.987	0.989
RMSE	14.2	12.6	10.8	13.6	11.7	11.4	9.5	6
AIC	245.6	235.5	220.3	241.1	227.2	225.8	209.2	165.0
BIC	249.4	240.3	225.2	246	232	230.6	214.1	169.8
ΔAIC	0	0	0	0	0	0	0	0
ΔBIC	0	0	0	0	0	0	0	0
Logistic function Model								
R^2	0.978	0.984	0.988	0.979	0.983	0.979	0.986	0.983
RMSE	17.8	13.9	11.8	14.9	13	13.1	11.5	8
AIC	265.8	243.5	228.3	249.7	237.1	238	212.6	186.4
BIC	270.6	248.3	233.1	254.5	241.9	242.8	217.4	191.2
ΔAIC	21.1	8	7.9	8.5	9.8	12.2	1.8	2.2
ΔBIC	21.1	8	7.9	8.5	9.8	12.2	1.8	2.2

The highest R^2 given by logistic function model was 0.988. Compared to modified Gompertz model, logistic function RMSE ranged between 8 to 17.8 while AIC (186.4 – 265.8) and BIC (191.2 – 270.6) values were also high. The high R^2 of Gompertz model was well correlated with less RMSE, AIC, ΔAIC, BIC and ΔBIC values compared to logistic function model. Thus, it shows that modified Gompertz model is more robust model and can be applied for better estimation of biogas yield from anaerobic digestion of cattle manure which was consistent with the reported literatures (Pramanik et al., 2019; Nguyen et al., 2019).

Chapter 5

Conclusions and Recommendations

This study was designed to determine the effect of low OTC concentrations on batch mesophilic anaerobic digestion of cattle manure. Based on results, following conclusions are drawn and recommendations are also given in this chapter.

5.1 Conclusions

- Significant difference on cumulative biogas and methane yields of 3, 6 and 12 mg OTC L^{-1} was observed compared to control.

- Effect on stability parameters of reactors containing high OTC concentrations were significant.

- Volatile solids removal was highest for control i.e. 39% and decreased with increase in concentrations of OTC.

- The modified Gompertz model best fitted to the experimental data of all reactors based on high R^2 and low AIC, BIC, ΔAIC and ΔBIC values.

5.2 Recommendations

- Research on the degradation and removal of oxytetracycline by composting and anaerobic digestion is recommended.
- Effect of oxytetracycline concentrations on microbial communities need to be studied.
- Encourage prudent use practices among farmers and veterinarians.
- Co-digestion with agricultural crops, food waste and bio-solids should be done to increase biogas yield rather using cattle manure as sole substrate.

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