Addis Ababa University
Addis Ababa Institute of Technology
School of Chemical and Bio Engineering

STUDIES ON NITRIFICATION-DENITRIFICATION AND SLUDGE GRANULATION PROCESSES IN SEQUENCING BATCH AIRLIFT REACTOR FOR THE REMOVAL OF NITROGEN FROM TANNERY WASTEWATER

Aysanew Gorems Melesse

PhD Dissertation

In partial fulfillment of the requirements for the attainment of the degree of Doctor of Philosophy in Chemical Engineering (Environmental Engineering)

Addis Ababa University
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A Ph.D. Dissertation Submitted to the School of Chemical and Bio Engineering, Addis Ababa Institute of Technology, Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy (Ph.D) in Chemical Engineering (Environmental Engineering).

Submitted to: School of Chemical and Bio Engineering

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DECLARATION

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This is to certify that the dissertation prepared by Aysanew Gorems Melesse, titled "**STUDIES ON NITRIFICATION-DENITRIFICATION AND SLUDGE GRANULATION PROCESSES IN SEQUENCING BATCH AIRLIFT REACTOR FOR THE REMOVAL OF NITROGEN FROM TANNERY WASTEWATER**" and submitted in fulfillment of the requirements for the Degree of Doctor of Philosophy (Chemical Engineering/Environmental Engineering stream) complies with the Regulations of the University and meets the accepted standards with respect to originality and Quality.

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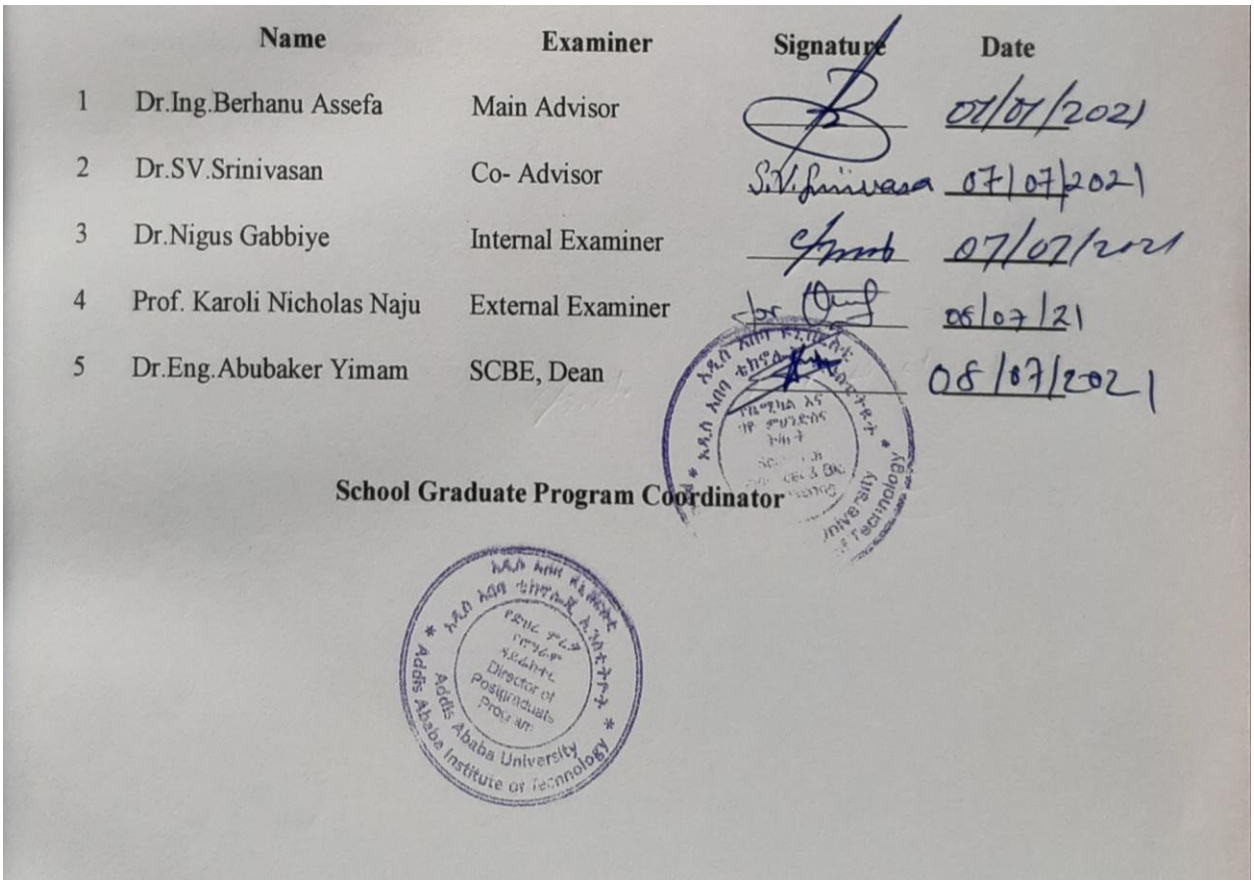

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ABBREVIATION AND ACRONYMS

AD	Anaerobic Digester
AmoA	Gene related to ammonium oxidizing bacteria
AOB	Ammonium oxidizing Bacteria
BOD	Biochemical Oxygen demand
CETP	Common effluent treatment plant
DNA	Deoxy ribonucleic acid
DO	Dissolved Oxygen
EPS	Exo-cellular polymeric substance
HRT	Hydraulic retention time
MLSS	Mixed Liquor Suspended Solid
MLVSS	Mixed Liquor Volatile Suspended Solid
N_2O	Nitrous oxide
NGS	New Generation Sequencing
NH_4-N	Ammonia Nitrogen
nirK	Gene related to nitrate reducing bacteria
NLR	Nitrogen loading rate
NO	Nitric oxide
NO_2-N	Nitrite Nitrogen
NO_3-N	Nitrate Nitrogen
NOB	Nitrite Oxidizing Bacteria
NO_x-N	Nitrite and nitrated nitrogen

OLR	Organic loading rate
Org-N	Organic nitrogen
ORP	Oxidation reduction potential
OTU	Operational taxonomic unit
PCR	Polymerized chain reaction
PLA46amx820	Gene related to anammox bacteria
PLC	Programmable Logic Controller
PN	Partial Nitrification
RDP	Ribosomal database project
RNA	Ribonucleic acid
rPCR	Real time Polymerized chain reaction
SBAR	Sequencing Batch Airlift reactor
SBR	Sequencing Batch Reactor
sCOD	Soluble Chemical Oxygen demand
SEM	Scanning Electron microscopy
SRT	Sludge Retention time
SVI	Sludge volume index
t_C	Total Cycle time
TCOD	Total Chemical oxygen demand
TDS	Total dissolved solids
TKN	Total Kjeldhal Nitrogen
TN	Total Nitrogen
TS	Total Solid

ABSTRACT

Tannery wastewater is characterized by high organic carbon and nitrogenous compounds due to the raw hides/skins and chemicals used in making leather. The present study employed sequencing batch airlift reactor (SBAR) system to develop the simultaneous removal of nitrogen and organic carbon from synthetic tannery wastewater. The reactor (working volume of 5 L) was run for 250 d at different operational conditions by automated control of the SBR cycles, pH, DO and temperature and constant 50% volume exchange ratio. The performance of the reactor was evaluated in terms of TCOD, sCOD, TKN, NH_4-N, NO_3-N, NO_2-N, DO and pH profile. The reactor was initially run at SRT of 7 d, MLSS of 3.5-4 g/L, pH in the range of 7.25-7.3 and DO of 2 mg/L for 129 days. The startup of nitrification process took about 30 days. During this time, the reactor showed almost complete organic carbon removal and unstable partial nitrification to nitrite with very less conversion to nitrate. Later the reactor operation was changed to different operating conditions: constant SRT of 20 d, resulting in MLSS of 10-11 g/L, DO of 1 mg/L and pH in the range of 7.25-7.3 and different cycle times of 18, 12, 10 and 8 h. The reactor was run for a minimum of two weeks for each cycle times. The result showed stable nitrification and denitrification with 97% removal in terms of COD and a 94% removal in terms of ammonical nitrogen. The total nitrogen removal was observed to increase as the cycle time increases. The total nitrogen removal efficiencies for 18, 12, 10 and 8 h cycle times were, 69, 58, 53 and 43 % respectively. The COD, DO and nitrogen species profiles showed that the removal of nitrogen was observed during the feeding and initial phase of the aeration due to denitrification. It was also observed that the remaining nitrogen in the effluent was more in the form of nitrate at higher cycle times and in the form of nitrite for lower cycle times. Kinetics study also showed that maximum removal of COD and ammonical nitrogen in 8 h cycle time under controlled condition. In addition,

cycle time control to change the characteristics of the effluent from complete nitrification to partial nitrification which is one of the important steps to link the reactor with anammox reactor system where in the presence of nitrite, ammonical nitrogen is anaerobically oxidized to nitrogen gas by anammox bacteria. Whole metagenome-16S rRNA sequencing of the bacteria population diversity of initial seed and reactor sludge after 200 d showed that the dominant bacteria were proteobacteria and the reactor sludge showed higher nitrifies and anammox population compared to seed sludge. Further experiments were conducted to change the flocculent sludge to granular sludge and improve the overall efficiency of the reactor. A COD loading rate of 6.3 kg/m^3.d and nitrogen loading rate of 0.425kg/m^3.d were first applied to the reactor for 105 days by using simulated tannery wastewater. The flocculent sludge was transformed to granular sludge when the operating settling time was reduced sequentially. The granules formed were having a final MLSS of 12.3 g/L, biomass density of 6.1 gVSS/L granule, settling velocity of 30-40 m/h, SVI of 16-20 ml/g TS and size distribution in the range 1.8-3.2 mm. Evaluation of the performance of the reactor showed a very good quality effluent with average COD, NH_4-N and TN removal of 97.7±1.5 (%), 87.1±11.8 and 74.43±11.7 (%) respectively. The effect of settling time on the existence and relative abundance of selected bacteria gene involved in nitrogen cycle is analyzed by PCR and qPCR. The result confirmed the existence of AOB-amoA, nirK and PLA46amx820 gene. The qPCR result also confirmed that the relative abundance of AOB-amoA and PLA46amx820 was higher in the reactor compared to the control.

Biological transformation of nitrogen results in generation of sludge which needs sustainable solution. Anaerobic digestion of these sludge yield energy and reduce the sludge volume significantly. The digestion of excess sludge from the SBAR reactor in this particular study yield biogas yield of 193.2 ml/g VSS and the digester reached steady state in about 30 days. However,

the supernatant after anaerobic digestion contains significantly high concentration of ammonia (i.e., 1097 mg/l) which again need sustainable solution. Partial nitrification (PN) of the supernatant after anaerobic digestion is a sustainable alternative to remove the nitrogen either through partial nitrification-denitrification route or through partial nitrification-anammox route. Though the PN-denitrification route may result in generation of unwanted N_2O gas. Therefore, the PN-anammox route is more sustainable. For the PN-anammox route, the NH_4-N in the wastewater need to be partially nitrified to NO_2-N in the ratio of 1:1. As a result, the SBAR reactor was operated at SRT of 7 days, DO of 1 mg/L, pH of 8, at ambient temperature and cycle time of 8 h to start up the PN process and the reactor was started up in 30 days with the required NO_2-N: NH_4-N ratio for anammox reactor feed. Moreover, the effect of various operational factors on the response variable (NO_2N: NH_4-N) was studied by considering seven operational factors (i.e. pH, DO, temperature, Cycle time, C/N, MLSS and aeration strategy (intermittent and continuous)). Fractional factorial design (Placket-Burman) was used to study the effect of the seven operational factors. The study showed that the individual factors considered for the study are not significant but the interactions between the factors are more significant. The results from experimental runs showed that it is possible to reach a stable partial nitrification with high pH (7.60), low C/N (0.5), high cycle time (10 h), low DO concentration (1 mg/L), low MLSS/MLVSS (3500 mg/L), high temperature (32ºC) and intermittent aeration.

Key words: Tannery wastewater, Nitrogen, SBAR, Cycle time, Aerobic granules, PN

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CHAPTER 1

1. INTRODUCTION

1.1. Background of the study

Nowadays the world is facing a steady increase in population and drinking water demand as well as increase in number of industries and industrial clusters. Therefore, a higher and higher pressure is applied on the surrounding environment and ecosystem, which has been affected by innumerable cases of pollution. In order to prevent further degradation there is a strong need for sustainable technologies, cleaner production and wastewater treatment. These important concepts should also be applied to effluent streams with unacceptable level of nitrogen [1, 2].

The tanning industry is among the many industries in Ethiopia contributing significantly to the economy and job creation. The country has 53.4 million cattle, 25.5 million sheep and 22.7 million goats. These numbers illustrate a considerable potential for the leather industry in the country[3]. With an expected off-take rate of 33%, 35% and 7% for sheep, goats and cattle, respectively, Ethiopia is capable of supplying 16–18 million hides and skins per annum[3, 4]. The sheep skins have a fine grain and compact structure and goat skins are well known for their quality and international acceptance for producing various leather products. The industry is the first from the manufacturing sectors interms of export earnings and just next to coffee from all the exports. However, leather industry especially the tanning industry is associated with a great social stigma in view of the pollution emanated from the process.

Leather making mainly involves wet process from which a large volume of liquid waste is continuously generated. The wastewater is generated from two main sections: beam house and tanning[5]. In the beam house process, highly loaded effluents are generated, containing organic

matter, suspended solids (as a result of unhairing and cleaning the collagen matrix), sulphides (used in the unhairing) and chlorides (from the salt present in the hides/skins). At this stage, bactericides (to prevent degradation of the hide/skins) and surfactants (to improve soaking) are also used. In the tanning process, the main pollutant is chromium, although other compounds such as tannins may also be used in the process, due to the variety of chemicals added at different stages of processing of hides and skins, the wastewater has complex characteristics[6, 7].

Tannery wastewater is having high organic carbon (measured as COD) and nitrogenous compounds due to the raw material (raw hides/skins) and chemicals used in making leather. The major forms of nitrogen in tannery wastewater are organic nitrogen (ORG-N). They originated from the breakdown of nitrogenous compounds from processed hides and skins and ammonical nitrogen (NH_4-N) originated from ammonium salts used in deliming operation [6, 8]. The common nitrogen compounds present in wastewaters are Ammonium ions (NH_4^+), Nitrite ions (NO_2^-) and Nitrate ions (NO_3^-). The discharge of too high nitrogen concentrations into the receiving waters without proper treatment causes dissolved oxygen depletion, toxicity, eutrophication, methemoglobinemia, deterioration of aesthetic quality, and odour from decomposing algae [9].

Currently, there are more than 30 tanning industries in Ethiopia, concentrated mainly in the southern central part of the country. The operation of these tanners are required to comply with the provisional discharge standard set by Federal EPA[10]. As a result, most of the tanneries have built at least primary treatment system. Yet most of the tanneries does not comply with the standards and have caused severe environmental pollution due to the disposal of untreated effluent on land and in water bodies. The effluents, which are discharged without any pretreatment, contaminate surface as well as sub-surface water and soils [11, 12]. There is urgent need for the treatment of tannery effluent prior to their disposal. The conventional tannery wastewater treatment involves physico-chemical treatment to remove suspended and colloidal solids followed

by biological treatment to remove soluble organics and nutrients [13-15]. For the removal of nitrogen along with organic carbon, the design and operation of the activated sludge system becomes more complex, expensive and process required large reactor volumes, sludge bulking issues and are susceptible to shock loadings [16]. The other alternative attached growth biological system generally faces blockage, mass transfer limitation and scale formation on the media especially due to the presence of hardness in tannery wastewater [17]. As the discharge limits imposed by regulators are becoming more stringent, a more flexible and less costly treatment option is required.

Sequencing Batch Reactor (SBR) technology has proved to be an effective system and a viable alternative to continuous flow systems when nutrient removal in addition to carbon removal is required [12, 18, 19]. Cyclic concentration gradients, to which biomass is exposed in SBR reactor, permit selection and enrichment of particular microbial species more capable of carrying out biological processes in the presence of inhibiting substances and high load feed [20, 21]. Besides, proper control of the SBR cycles, SRT, pH, DO, ORP, conductivity and temperature influence the microbial populations and thereby the accompanying biological reactions responsible for the removal of organic carbon and nitrogen. Therefore, the operation of SBR allows more flexibility of treatment and process selection to get optimized operating condition depending on the nature of the wastewater. The nature of SBR presents the following additional advantages: ability to incorporate aerobic, anoxic phases in a single reactor (if desired); sequencing batch closely resembles plug flow operation during the fill cycle; near ideal quiescent settling conditions and no separate clarifiers required [21].

In general, SBRs involve the mixing and aeration of reactor mixture to improve the mass transfer of oxygen, food and nutrient essential for microbial growth. Mixing can be provided mechanically or through aeration by creating pressure difference in the reactor. Mechanical mixing is usually

provided along with aeration but air mixing can be used for both aeration and mixing. In most cases, processes and equipment involving aeration are the major consumers of energy. It has been estimated that up to 60 percent of a plant's power consumption can be attributed to aerobic reactors in the activated sludge process of secondary treatment. Depending on the design, air mixing have more aeration efficiency and consumes less energy[22].

Sequencing Batch Airlift Reactor (SBAR) is one type of SBR where mixing and aeriation is provided by compressed air supply. These reactors are different from the normal SBRs that only compressed air is used for both mixing and aeration without the need for mechanical mixing[23]. The SBAR are usually helpful for selection of robust bacteria and formation of granules due to the potential high rate of mixing and aeration provided compared with normal SBRs[24]. The mixing and aeration rate of SBAR depend on the pneumatic mixing system and H/D of the reactor[25].In this study a draft tube pneumatic mixing system is employeed for mixing and aeration.

This study aims to investigate the suitability of SBAR for tannery wastewater treatment and the various operating conditions and process alternatives possible to treat wastewater from tannery.

1.2. Problem Statement

The tanning process involves an important consumption of water and generates a complex pollution consisting of a mixture of organic and inorganic substances rather difficult to treat. Untreated tannery wastewaters contain high levels of organic materials and nitrogenous compounds as a result of the raw materials used in making leather. The predominant forms of nitrogen in tannery wastewater are organic nitrogen (mainly proteins) and ammonia obtained from hides and skins degradation and chemicals used. The presence of nitrogen in wastewater discharge can be undesirable because it has ecological impacts and can affect public health.

Methemoglobinemia, eutrophication and depletion of dissolved oxygen in aquatic ecosystems are the major impacts.

Due to the increasing environmental concern, tanneries are required to comply with stringent environmental standards. Discharge limit compliance is the major challenge for tanning industries and is currently requiring sustainable solution. In this regard, physico-chemical treatment processes such as screening, coagulation, flocculation and sedimentation can help to remove suspended solids and colloidal solids[26, 27]. Furthermore, biological processes are the cheapest among other alternative treatment technologies and has the ability to decrease the organic and nutrient constituent and treat the tannery wastewater[15] . However, the design and operation of biological nutrient removal could be relatively complex and costly exercise. Therefore, researchers are working on various reactor configurations and operational conditions.

The modified activated sludge process (Nitrification-Denitrification) is the most widely used biological nitrogen removal method in tanning industries. However, this conventional biological nitrogen removal process is effective only for treating wastewaters with relatively low nitrogen concentration (total nitrogen concentration less than 100 mg/l and require a high C/N ratio (>5)[28]. Due to presence of high total nitrogen concentration in tannery wastewater, it is difficult or prohibitively expensive for tanneries to comply with nitrogen standards as it requires high energy and additional carbon source and this makes tanning industries less competitive.

Conventional activated sludge treatment methods in general are now becoming less competitive after the discovery of SBR reactors due to their flexibility, biomass selection potential, improving the settling property of sludge (granulation) and flexibility to induce alternative processes within one reactor (nitrification, denitrification and anammox processes). This technology provides various advantages to the overall treatment efficiency. It is by far more efficient, less energy intensive and requires less footprint and less costly[29]. Moreover, the search for more suitable

and energy efficient SBR reactor type to select robust bacteria and to improve energy efficiency are being worked out. Sequencing batch airlift reactor (SBAR) is an alternative SBR where mixing and aeration are integrated to influence the hydrodynamic condition in the reactor and to save energy.

Though much progress has been done in the conventional activated sludge process and sequential batch systems in tannery wastewater, information on the performance of SBAR for simultaneous removal of organic carbon and nitrogen from tannery wastewater; and cultivating aerobic granules in wastewater having complex composition like tannery wastewater is very limited.

Finding optimal operating conditions of SBR to induce the development of granules containing all the required bacteria population responsible for the removal of both organic carbon and nitrogen from wastewater stream needs to be investigated for tannery wastewater treatment. The challenge is to obtain good operating condition where proper granulation is taking place in the reactor containing all the bacteria population required for the removal of organic carbon and nitrogen.

The investigation of SBAR for the treatment of tannery wastewater in general and nutrient removal in particular is not fully worked out. To the knowledge of the author, there is no scientific paper published in this area. And this technology can provide sustainable alternative solution for tanning industries to comply with discharge standards.

1.3. Objective

1.3.1. General Objective

The main objective of this research work is to investigate the nitrification-denitrification and sludge granulation performance of sequencing batch airlift reactor for the treatment of tannery wastewater.

1.3.2. Specific Objectives

- To study the nitrification - denitrification performance of the sequencing batch airlift reactor for the treatment of synthetic tannery wastewater
- To investigate the change in microbial dynamics as a result of the sequencing batch airlift reactor operation
- To study the start-up of aerobic granules formation in sequencing batch airlift reactor treating synthetic tannery wastewater
- To characterize aerobic granules formed and to investigate the existence of selected bacteria gene and their gene expression level.
- To study the bio-methanatioin potential of excess sludge, partial nitrification of anaerobic digester supernatant in sequencing batch airlift reactor and to investigate the effect of operational factors on partial nitrification.

1.4. Research Application

The research can find application in the following areas:

- For designers and operators of SBAR, the study provide alternative operating conditions whereby more efficient and economic treatment can be done
- For tanning industries, the experimental studies provide valuable information for economic and sustainable treatment of tannery wastewater
- For other industries with wastewater characteristics and C/N ratio, similar to tannery wastewater
- The study could be important for a number of researchers working in the area of environmental science and wastewater treatment especially working on nutrient removal.

1.5. Research Scope

The scope of this study is limited to the application of SBAR to study the different alternative conditions suitable for simultaneous removal of organic carbon and nitrogen from tannery wastewater. For uniformity of data and interpretation, the real tannery wastewater is repeatedly characterized and simulated with synthetic tannery wastewater for the entire study.

1.6. Outline of the Research Approach

The research follows classical approach and is outlined as follows:

Chapters 1 provides general information on the study area, the research need, research objective, research application, assumptions, limitations and scope.

Chapter 2 details literature review on the following topics: biological nitrogen removal and process variables, nitrification and denitrifications, biological nitrogen removal process technologies, microbial transformation of nitrogen, nitrification process, nitrifying bacteria, environmental factors influencing nitrification, denitrification, denitrifying bacterial, environmental factors influencing denitrification, SBR technology (operational characteristics in SBR processes, SBAR), aerobic granulation in SBAR (mechanism of aerobic granulation, process configuration and operational condition affecting granulation), anaerobic digestion of excess biological sludge, partial nitrification of AD supernatant and Molecular study (NGS, PCR, qPCR)

Chapter 3 deals with materials and methods used to conduct the study. The chapter provides approaches used in this study: characterization of tannery wastewater samples, design and construction of lab scale SBAR, Startup monitoring and evaluation of the SBAR system under various operational conditions.

Chapter 4 presents study results and discussion. The study outputs described in this section include: performance studies of the SBAR with synthetic tannery wastewater, performance studies of the

granular SBAR and characteristics of granules formed, performance of the AD for excess sludge digestion and characteristics of AD supernatant and partial nitrification of AD supernatant. Chapter 5 summarizes conclusions drawn from this study and gives recommendations for future works.

CHAPTER 2

2. LITERATURE REVIEW

2.1. General

The treatment of tannery wastewater is challenging due to the complex nature of its composition; it is a mixture of biogenic matter of hides/skins, inorganic chemicals and a large variety of organic pollutants with large molecular weights and complex structure[15]. There are three different kinds of treatment techniques. These include primary physico-chemical, secondary biological and advanced tertiary treatments [27, 30].

Physico-chemical treatment of tannery effluents consist of coagulation, flocculation, sedimentation, filtration, air stripping, chemical precipitation, adsorption, ion exchange, electrochemical (electro-oxidation), and chemical oxidation [31-33].

Coagulation-flocculation has been applied to leather tanning wastewater to collect colloidal solids and to form a flocs that can easily settle down a settling tank. These processes reduces organic load and suspended solids as well as remove toxic substances [26, 27, 34, 35]. In addition, chromium can be reduced by chemical precipitation, adsorption, and ion exchange [31, 36, 37].

The removal of nitrogen and organic matter from tannery wastewater can be accomplished using physical, chemical or a combination of both. Organic matter can be reduced either by adding powder activated carbon directly into biological reactor or by advance oxidation processes (AOPs) such as UV, ozone (O_3), photocatalytic oxidation and their combination and Fenton reagent[32, 38, 39]. The available physico-chemical methods for ammonia removal include adsorption, chemical precipitation, membrane filtration, reverse osmosis, ion exchange, air stripping, breakpoint chlorination and electrochemical oxidation [33, 40-43]

All the processes which can be used for removal of both organic matter and nitrogen are simple in principle; however, they are expensive (high operating and maintenance cost, and consumption of chemicals) and also produces harmful products. Now a day, there is a growing interest in the development of new technologies and procedures for the purification of this waste. Among these procedures, biological methods have been recognized as a viable possibility for the degradation of these wastewaters[44].

With regard to nitrogen, biological nitrogen removal processes are more environmentally friendly than other technologies. Biological nitrogen removal is essentially a two-step process, nitrification followed by denitrification. Nitrification is the biological oxidation of ammonium to nitrite/nitrate by authrophic nitrifying organisms under aerobic conditions. Denitrification is the dissimilatory reduction of nitrate to molecular nitrogen by heterotrophic organisms using nitrate as an electron acceptor and degradable carbon as energy source under anoxic conditions. While the organics in the wastewater provide carbon substrate for denitrification, such a system is also important for the protection of the sensitive nitrifying organisms from shock by toxicants present in tannery effluents, which helps to achieve high degrees of nitrification.

2.2. Biological Nitrogen removal and process variables

2.2.1. Nitrogen: Environmental and wastewater Concern

The discharge of nitrogen compounds could cause environmental impact on the surrounding ecosystem and receiving water bodies or watersheds.

The commonly nitrogen compounds present in a wastewater treatment plant which may adversely impact the receiving water are Ammonium ions (NH_4^+), Nitrite ions (NO_2^-) and Nitrate ions (NO_3)

The main risks related to the presence of these compounds in high concentrations above the water quality standard or guidance values may cause:

- Dissolved Oxygen (O_2) depletion
- Toxicity
- Eutrophication
- Methemoglobinemia
- Deterioration of Water aesthetic quality and odours from decomposing algae.

Table 2-1. Pollution concerns related to excess of NH_4^+, NO_2^- and NO_3^- [9].

Nitrogen Ion	Pollution concerns
NH_4^+	Overabundant growth of aquatic plants
	Dissolved Oxygen depletion
	Toxicity as NH_3
NO_2^-	Overabundant growth of aquatic plants
	Dissolved Oxygen depletion
	Toxicity
	Methemoglobinemia
NO_3^-	Overabundant growth of aquatic plants
	Toxicity
	Methemoglobinemia

All three nitrogenous ions can be toxicity to aquatic life, especially fish. Ammonium ions and nitrite ions are extremely toxic, and nitrite ions are the most toxic of the three nitrogenous ions[9]. Although ammonium ions are preferred nitrogen nutrient for most organisms, ammonium ions are converted to ammonia with increasing pH (Figure 2-1). The ammonia at an elevated pH is toxic to aquatic life [45].

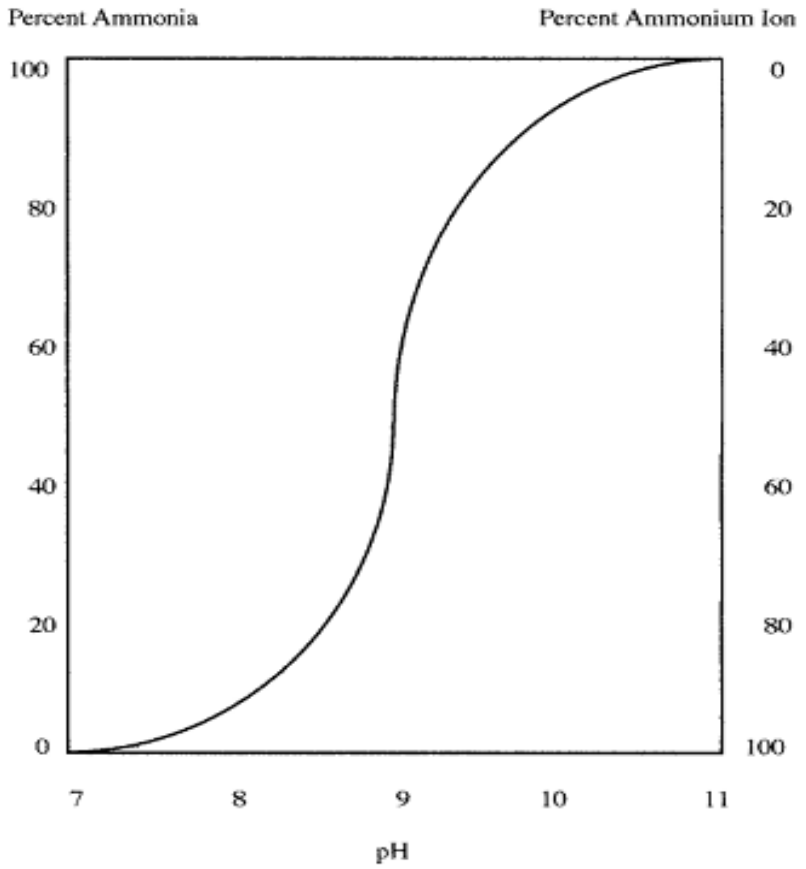


Figure 2-1 Conversion of ammonia and ammonium ions at different pH(Source[9])

The relative quantities of ammonia and ammonium ions in water are determined by the pH of the water. As the pH of the water decreases, ammonium ions are favored. As the pH of the water increases, ammonia is favored. At a pH value of 9.4 or higher, ammonia is strongly favored [9]. Ammonium ions are oxidized to nitrite ions by bacteria and nitrite ions are then oxidized to nitrates ions. Both these two reactions (nitrification) require dissolved oxygen. Thus, these processes deplete and reduce the oxygen within the water.

Besides this, these three ions represent forms of nitrogen nutrients which aquatic plants (i.e. algae) can use for their growth. With their death, the dead plants will induce an increment of organic matter to be decomposed by bacteria, which will lead to a further reduction of dissolved oxygen.

Nutrient enrichment can lead to localized eutrophication, which in turn is associated with more frequent or sever algal blooms[46, 47] with losses in ecological, commercial, recreational and aesthetic value of these water and changes in species composition and diversity of plant and animal community.

Eutrophication is defined as "the enrichment of water by nutrients, especially compounds of nitrogen and or phosphorus, causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concern".

Although nitrogen is an essential nutrient for biological growth and aquatic ecosystem integrity, it becomes a pollutant if its amount is beyond the natural capacity of the system to assimilate or flush the excess. This is particularly true for water bodies characterized by a low turnover rate (i.e. lagoon, lakes and rivers). Human activities are responsible for increasing and accelerating the natural process of eutrophication in the surrounding watersheds [48].

Direct consequence of this enhanced growth in a lake are a limited amount of light reaching the lower regions (leading to a loss of submerged aquatic vegetation), color, odor (associated with the growth and death of aquatic plants) and, above all, low level of dissolved oxygen at the bottom (i.e. hypoliminion), which in very eutrophic lakes with high concentration of organic matter could lead to the reduction of sulphate to hydrogen sulphide (H_2S). H_2S is very toxic for aquatic organisms. Recently it has been found that some cyanobacterial toxins have become widely recognized as a human health problem arising as a consequence of eutrophication[49]. Besides this, cyanobacteria ("blue –green algae") are nitrogen fixing bacteria which increase ammonium concentration in the aquatic ecosystem.

The simplest and most common individual parameters used to assess water eutrophication are total nitrogen and total phosphorus. Some advanced and comprehensive index such as total nutrient status index can be used [50].

The total nitrogen (TN) is the sum of dissolved inorganic nitrogen (i.e. nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), ammonium nitrogen (NH_4^+-N) and organic nitrogen (e.g. urea, peptides, proteins). Total Kjeldhal-Nitrogen (TKN) is used to estimate the sum of organic N and NH_3.

The nitrogenous ions can be toxic to aquatic life, especially to fish [9]. Nitrite and nitrate are extremely toxic, and nitrite ions are the most toxic of the three nitrogenous ions form. Ammonium ions, actually, are one of the most preferred nitrogen nutrient for most organisms, but they can be converted to ammonia with increasing pH of water (above 9.9), which can be toxic for aquatic life at concentration as low as 0.025 mg/l NH_3. High temperature and low salinity (freshwater) are other parameters that can contribute to a higher unionized-ammonia concentration in the water.

The toxic effects of nitrate exposure result from the conversion of nitrate to nitrite. Methemoglobinemia is a well-recognized hazard for ingestion of nitrates and nitrites [10]; Nitrates are reduced to nitrites in the digestive system and, combining with the hemoglobin of the blood results in stoppage of oxygen transport mechanism. Infants younger than 4 months of age who are fed with water from rural domestic wells are at highest risks of developing health effects from nitrate exposure [51].

2.2.2. Nitrification and denitrification

2.2.2.1. Biological nitrogen removal processes/technologies

Over the last 10 decades, many different technologies for wastewater treatment have been developed, all of which include biological treatment technologies at some stage, with the aim of

mitigating the destructive effect of man's wastes on nature. Increased eutrophication problem in major seawater bodies in Europe in the 1980s led to the development of improved technologies for biological wastewater treatment. Subsequently, increased demands on wastewater treatment efficiencies required a better understanding of the biological processes that were the basis of its treatment. Since then much treatment system has been developed and the development is still in progress[52]. In the field of wastewater treatment, it is generally accepted and proven that biological treatment is less energy intensive, cheaper and environmentally friendly. For biological treatment, wastewater is a medium for organisms, which work best under constant and steady conditions. An optimum environment with nutrients of known composition are rarely available in a given wastewater environment. The main problem with tannery wastewater treatment therefore arises from the variability of its composition with respect to amount, nutrients, toxicants and or inhibitors.

Depending on the characteristics of the waste streams and the effluent treatment objectives, various process configuration for biological nitrogen removal have evolved over the past twenty years[41, 53]. The main processes for biological nitrogen removal are based on how the microbial biomass is maintained in the system, as a fixed film or in a suspension, usually as an activated sludge. In a fixed biofilm process, the microorganisms are attached to a support and are exposed to the wastewater to be treated [54]. Trickling filters, submerged or expanded bed aerobic filters and rotating biological contactors have also been used for carbon removal and/or nitrification[41, 55] [56-58]. In trickling filter, the microorganisms are immobilized on a support medium such as rocks, wood slats or plastic sheets, over which wastewater are trickled. In rotating biological contactors (RBC), packed circular disks submerged in wastewater are rotated slowly to allow bacterial growth on the surface of the disk to form a slime layer. The disks contact wastewater and

air as they rotate. In submerged up-flow packed bed reactors, stones, gravels or plastic materials are used where microbial growth can be attached to media in a packed bed[59]. Wastewater is made to flow to the reactor and air or oxygen is introduced into the wastewater.

The activated sludge process is the most commonly applied biological process for the treatment of both municipal and industrial wastewaters. The majority of suspended growth biological nitrogen removal system in operation today are activated sludge processes with aerobic zones for carbon removal and nitrification, and anoxic zones for denitrification. In processes such as modified Ludzack Ettinger Process (pre-denitirfication zone upstream of the nitrification zone), nitrified mixed liquor is recycled from the aerobic zone to the anoxic zone, where it is denitrified using the incoming wastewater as a carbon source. High degrees of total nitrogen removal have been reported using these systems from both municipal [60] and industrial wastewater[61]. In processes with a post-denitrification zone, the denitrification rates are significantly lower.

The pre-denitrification–nitrification process is particularly suitable for the treatment of industrial wastewaters laden with toxic substances such as those from tanneries containing high concentrations of degradable organic carbon, nitrogen and ammonia. This process allows the optimal use of the incoming wastewater as carbon source for denitrification, protects the sensitive nitrifying organisms from toxic shocks, and helps to achieve high degrees of nitrification. The process relies on a dense microbial population mixed in suspensions with the wastewater under aerobic conditions.

The current biological treatment of tannery wastewater is being carried out using continuous activated sludge system[14]. However, for the removal of nitrogen along with organic carbon, the design and operation of the activated sludge system becomes more complex, expensive and process required large reactor volumes, sludge bulking issues and are susceptible to shock loadings

[16]. The other alternative attached growth biological system generally faces blockage, mass transfer limitation and scale formation on the media especially due to the presence of hardness in tannery wastewater [17].

The sequencing batch reactor (SBR) system has been demonstrated as the most flexible and cost effective biological treatment system. It involves a series of time oriented operations i.e. fill, react, settle, decant and idle each lasting for a defined period during which wastewater is treated [29]. The operation of SBR under selection pressure (low settling time and high shear force condition) can also help to form aerobic granules with excellent settling and treatment efficiency. Aerobic granules are formed by self-immobilization of microbial aggregates and have a strong and compact structure, excellent settleability and biomass retention [62]. It is reported that the aerobic granules has been effective for the organic removal and nutrients from domestic, industrial and toxic effluents with high loading rates [63].

2.2.2.2. Microbial Transformation of Nitrogen

Nitrogen is the most abundant element in the atmosphere and the fourth most common element found in cell as building block of proteins and nucleic acids. Nitrogen can be found in the environment under several forms as shown in Table 2-2.

Table 2-2.Forms of nitrogen in the environment

Unoxidized form	Oxidized form
Nitrogen gas (N_2)	Nitrite (NO_2^-)
Ammonia (NH_4^+,NH_3)	Nitrate (NO_3^-)
Organic Nitrogen (urea, amino acids,	Nitrous Oxide (N_2O)
Peptides, proteins, etc.)	Nitric Oxide (NO)
	Nitrogen Dioxide (NO_2)

The nitrogen cycle is a complex biogeochemical cycle in which nitrogen is converted from its inert atmosphere molecular form (N_2) into a form that can be used in biological processes. The classical nitrogen cycle includes:

- Nitrogen fixation: Conversion of inert form N_2 to an organic (or fixed) form which Organisms can use. Nitrogen fixation is mostly carried out by biological process (e.g. nitrogen-fixing bacteria such as Rhizobium or Azotobacter and Cyanobacteria). A small amount of nitrogen is 'fixed' through high-energy natural events such as lightning and forest fires. Nitrogen can also be fixed through man-made processes (e.g. ammonia and nitrogen rich fertilizers, explosives or combustion of fossil fuels which release NO_x)
- Nitrification: Conversion of ammonium (NH_4^+) into nitrite (NO_2^-) and then into nitrate (NO_3^-), which is the form that plants take up mostly. It is carried out by nitrifying bacteria under aerobic conditions.
- Assimilation: Uptake of nitrogen compounds (i.e. nitrate, nitrite, and ammonium) from soils by plants which used them for the formation of proteins.

- Ammonification (or mineralization): Conversion of organic nitrogen to ammonium-nitrogen. It is carried out by microorganisms (decomposers) which produces ammonium (NH_4^+) from dead organic matter (plants and animals tissue) and animal fecal matter.
- Denitrification: Conversion of Nitrate (NO_3^-) back to gaseous nitrogen (N_2) and, to a lesser extent, nitrous oxide gas, which is a strong greenhouse gas. It is carried out anaerobically by denitrifying bacteria. Through Denitrification process, nitrogen is removed from ecosystem and it is a way to contrast the increased nitrogen fixation.
- Dissimilatory Nitrate Reduction to Ammonia (DNRA): It is a form of anaerobic respiration process where nitrate (NO_3^-) is used as electron acceptor instead of oxygen and it is recycled to ammonium (NH_4^+). In contrast to Denitrification, this process doesn't remove the nitrogen from the habitat, but it remains available to primary producers. An example of dissimilatory nitrate reducer is Escherichia Coli.

In this traditional version of the N-cycle, ammonium oxidation was assumed to take place only under aerobic conditions and the possibility of an anaerobic ammonium oxidation was not contemplated. Recently it was discovered that ammonium can also be oxidized under anaerobic conditions. This new discovery created a 'short-cut' in the traditional nitrogen cycle (Figure 2-2) and was called (Anaerobic AMMonium Oxidation-ANAMMOX), which is described below

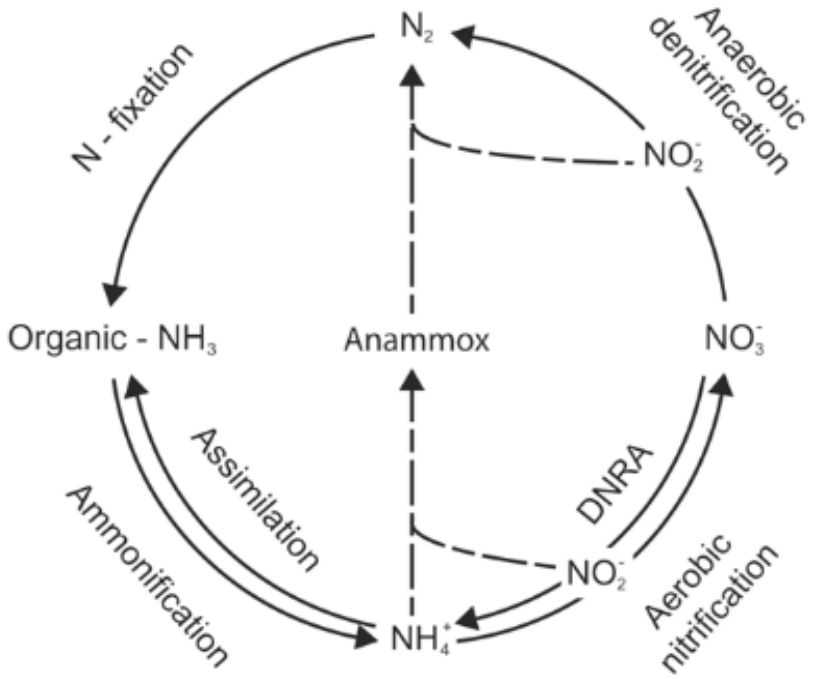


Figure 2-2. Updated nitrogen cycle following the discovery of ANAMMOX (Source http://aem.asm.org/cgi/content)

2.2.2.3. Nitrification process

Nitrification is a biological process whereby free ammonium is oxidized to nitrite and then to nitrate. It is mediated by autotrophic organisms (nitrifying bacteria) which obtain their energy requirement (catabolism) for biomass synthesis from inorganic nitrogen compounds, oxidizing ammonium to nitrite and to nitrate, and their carbon requirement (anabolism) from dissolved CO_2 [9].

Nitrification is therefore made up of two sequential steps:

1. Ammonium is oxidized to nitrite (NO_2^-) by nitrosomonas spp. bacteria

   $NH_4^+ + 1.5O_2 \longrightarrow NO_2^- + H_2O + 2H^+ + 2e-$ (Eq.2-1)

   These bacteria are called ammonium oxidizing bacteria (AOB)

2. Nitrite is converted to nitrate by Nitrobacter spp. bacteria:

$NO_2^- + H_2O \longrightarrow NO_3^- + 2H^+ + 2e^-$ (Eq.2-2)

These bacteria are also called Nitrite oxidizing bacteria (NOB)

The stoichiometric oxygen requirement for these reaction is: $1.5 * \frac{32}{14} = \frac{3.43\, mgO2}{mg\ N}$ for ammonium oxidation and: $0.5 * \frac{32}{14} = \frac{1.14\, mgO2}{mg\ N}$ for nitrite oxidation. The first reaction consumes alkalinity (about 7.1g of alkalinity as $CaCO_3$ for each gram of N-NH_4^+ oxidized)

The most commonly recognized genus of bacteria that carries out ammonium oxidation is Nitrosomonas, however, Nitrosococcus, Nitrosopira, Nitrosovibrio and Nitrosolobus are also able to oxidize ammonium to nitrite. The major bacteria group responsible for nitrite oxidation is Nitrobacter genus but several other genera such as Nitrospira, Nitrospina, Nitrococcys, and Nitrocystis are known to be involved [64].

2.2.2.4.Nitrifying Bacteria

As mentioned by Gerardi [9], recent molecular techniques have discovered that there are several genera of nitrifying organisms (i.e. Protozoa, Acitomycetes, Algae, fungi, and other bacteria such as Pseudomonas, Bacillus, Vibro, Proteus and Arthrobacter), but however, most of nitrification is carried out by Nitrosomonas spp. and Nitrobacter spp., whose rate of nitrification is often 1000 to 10000 times greater than the nitrification achieved by other organisms[9].

2.2.2.5. Environmental factors affecting nitrifying bacteria

The main factors which might influence the kinetics of nitrification are:

1. **pH:** Two reactions mentioned above (Eq. 2-1 and 2-2) produce H^+ and therefore lower the pH; the optimum pH for Nitrosomonas and Nitrobacter is between 7.2 and 8.5. At pH of 6.0 normally the nitrification stops. The pH also controls the concentration of free ammonia (NH_3) and nitrous acid (HNO_2) which are strong inhibitor of bacterial activity. Free ammonia can inhibit Nitrosomonas at concentration as low as 10 mg/l and Nitrobacter at

concentration as low as 0.1 mg/L. Free nitrous acid inhibits them at concentration as low as 1 mg/l and Nitrobacter at concentration as low as 0.1 mg/l. [9]. The pH can control these two equilibria:

$NH_4^+ \longleftrightarrow H^+ + NH_3$ and $NO_2^- + H^+ \longleftrightarrow HNO_2^-$

2. **DO:** Dissolved oxygen is an important parameter for nitrifiers growth. The DO concentration should be kept above 2-3 mg/l in order to not unduly depress the rate of removal. DO between 0.5-2.5 mg/l may limit the nitrification [65] in suspended or attached growth system under steady state conditions, depending on the degree of diffusion resistance, especially in attached biomass growth systems.
3. **Temperature:** Too low temperature (range below 10-15^{0}C) as well as sudden changes in temperature can decrease the removal rate. Nitrification reaches a maximum rate at temperatures between 30 and 35^{0}C)
4. **Heavy metals and organic compounds:** Some heavy metals (Zn^{2+}, Cd^{2+}, Cr^{+3}, Pb^{+2}, Ni^{+2}) may exert their inhibitory action from concentration of 1 mg/l. Active carbon or acclimatization of biomass can reduce the inhibitory action of many compounds.

2.2.2.6.Denitrification

Denitrification is a biological process whereby nitrate is reduced to nitrite and then to nitrogen gas. Heterotrophic microorganisms (i.e. denitrifying bacteria) which uses organic matter as carbon (anabolism) and energy source (catabolism) mediate it. These results in a much higher biomass growth compared with autotrophic bacteria (5-fold higher according to [4].

Among denitrifying bacteria, the most common are *Achromobater, Pseudomonas, Micrococcus, Bacillus* and *Alcaligens*. Other bacteria such as *Aerobacter, Proteus, Flavobacterium* are only able to convert NO_3^- to NO_2^-.

Denitrifying bacteria are facultative organisms that can use either dissolved oxygen or nitrates as source for metabolism and oxidation of organic matter. In the case of simultaneous presence of dissolved oxygen and nitrates, denitrifying bacteria use preferentially oxygen because the energy generated per unit weight of organic matter metabolized is higher.

Therefore, it is important to keep dissolved oxygen as low as possible (less than 0.5 mg/l), at least in the micro aerobic environment surrounding the bacteria. Under anoxic conditions, denitrification reactions can be simplified as the sum of Denitrification (2-3) and Denitrification (2-4):

$$NO_3^- + 1/3CH_3OH \longrightarrow NO_2^- + 1/3CO_2 + 2/3H_2O \quad \text{(Eq.2-3)}$$

$$NO_2^- + 0.5CH_3OH \longrightarrow 0.5N_2 + 0.5CO_2 + 0.5H_2O + OH^- \quad \text{(Eq.2-4)}$$

Since nitrogen gas has low water solubility, it is released into the atmosphere without any environmental concern. The second reaction occurs through the formation of nitrogen oxides (NO and N_2O) which was subsequently reduced to nitrogen gas. A carbon source (shown in the above equation as methanol, CH_3OH) is required for denitrification to occur. Organic matter may be in the form of raw wastewater or external carbon source (e.g. ethanol, molasses, distillery stillage, buttermilk, methanol or acetate). Methanol has a high toxicity in humans; therefore, the use of another carbon source would be preferable. When these sources are not present in the water, bacteria may depend on internal (endogenous) carbon reserves, but the nitrate removal may be lower. Removal of nitrogen is also partly due to the synthesis of new biomass and thus of organic nitrogen. This amount is about 4% of the total nitrogen removed.

2.2.2.7. Partial Nitrification-Denitrification

In conventional denitrification, the wastewater C/N ratio is very important for effective nitrogen removal [66]. For wastewater having low C/N ratio, an external carbon source is usually provided [67]. Short-cut partial nitrification-denitrification is possible where the ammonium nitrogen is first

converted to nitrite (Eq.2- 5) and then denitrified in the presence of organic carbon (Eq.2-6). This process helps significant saving in energy and cost of treatment [68].

$$NH_4^+ + 1.5O_2 \xrightarrow{AOB} NO_2^- + H_2O + 2H^+;\ \Delta G° = -275\ kJ/M \qquad (Eq.2-5)$$

$$NO_2^- + 0.24\ NH_4^+ + H^+ + 0.975\ CH_3COO^- \xrightarrow{HDB} 0.24C_5H_7O_2N + 0.015CO_2 + 0.5\ N_2 + 1.235H_2O \qquad (Eq.2-6)$$

2.2.2.8.Environmental factors affecting denitrification

The main factors which affect the efficiency of denitrification are:

1. ***DO***: As dissolved oxygen increases, denitrification rates decreases, therefore anoxic condition should be maintained.
2. ***Presence of organic matter***: The source of available carbon can influence the denitrification rate. The highest rate can be achieved by adding an easily biodegradable and assimilated carbon source, but this may imply costs for its purchase. The highest removal rates occur with the use of effluent from distillery and food industries.
3. ***pH and alkalinity***: The optimum pH is between 7.5-9.1, but denitrification can occur also at pH between 6 and 7.5. Alkalinity is produced during the process (about 3-3.5g of alkalinity as $CaCO_3$ for each gram of NO_3^- reduced).
4. ***Temperature***: It affects the growth rate of denitrifying organisms, greater growth rate occurs at higher temperatures. Denitrificaiton can occur between 5 and 30^0C
5. ***Heavy metals and Organic compounds***. Denitrifying organisms are generally less sensitive to toxic chemicals than nitrifiers, and recover from toxic shock loads quicker than nitrifiers.

2.3. Sequencing Batch Reactor

A Sequencing Batch Reactor (SBR) is a wastewater treatment system that is operated in a sequence of several cyclic stages[41]. Every operational cycle involves five consecutive time oriented steps of Fill, React, Settle, Decant and Idle (Figure 2-3). These steps can be altered for operational applications [69]. During the Fill stage, part of the liquid volume of the reactor is replaced with fresh wastewater. Treatment takes place during the React stage, which can consist of aerobic, anaerobic or a combination of aerobic, anoxic, and anaerobic conditions, depending on the goals of the system design. Excess biomass is removed during the react stage. After biomass wasting and settling, clarified wastewater is removed from the reactor in the decant stage and the cycle is repeated. The cycle can be of any duration but it is the total (sum of) time for fill, react, settle and decant stages required for treatment.

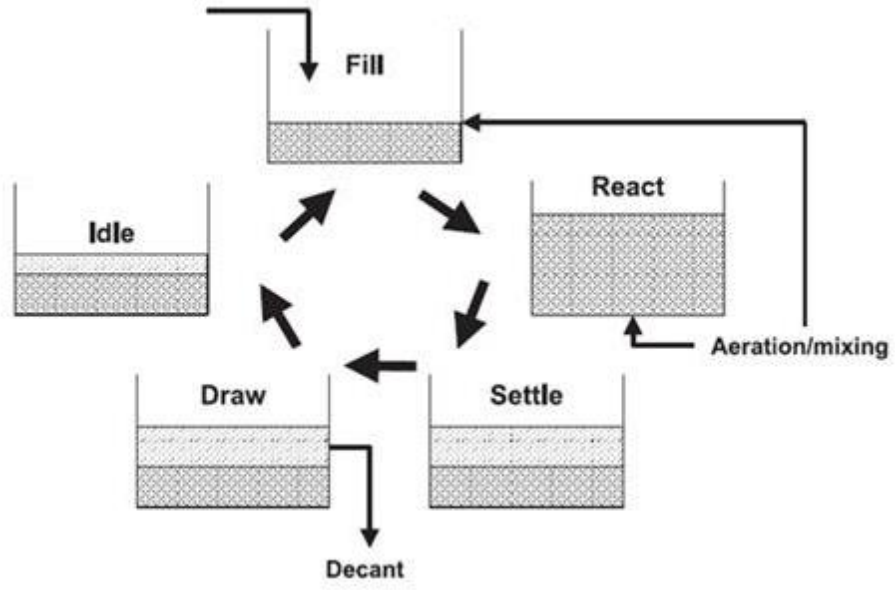


Figure 2-3. Typical Cycles of SBR (Source [70])

Sequencing batch reactor (SBR) operations have been used by many investigators for organic (COD and BOD) and phosphate removal [12, 14, 29] and nitrogen remove by nitrification and denitrification in addition to COD and phosphate removal [71, 72].

Many applications of the SBR have been reported on different literature. Several researchers have reported the success of SBR to remove nitrogen, organic matter and phosphorous from domestic wastewater [73-75], tannery wastewater [8, 12, 14, 18, 19, 76] and swine wastewater [77-79]. Sequencing batch reactor is also efficient for the treatment of textile wastewater [80, 81] and abattoir wastewater [82].

2.3.1. Advantages of SBRs

The SBR has gained popularity during the 20th century mainly due to its operating advantages and flexibility to treat different kinds of wastewater [83, 84].

The operational flexibility of SBR allows the control of filamentous bacteria through feast/famine cycles. A high substrate concentration may be imposed by a static fill operation and the react phase may be followed by an extended phase of starvation which, in turn, promotes the enrichment of flock-forming bacteria and the accumulation of exopolymers [12, 85]

The operation conditions (alternating high/low substrate concentration) induce the selection of robust bacteria[70]. The sludge adaptation to variations in the oxygen and substrate concentrations, in the course of a cycle and on a long-term basis, renders it capable of maintaining good performance under shock loads [23, 86, 87]

The SBR system provides the flexibility needed to treat a variable wastewater (load and composition) by simply adjusting the cycle time (e.g. using the time set aside for the idle phase), the duration of each phase or the mixing /aeration pattern during each cycle [29, 70, 84]. The ability to hold contaminants until they have been completely degraded makes the system excellent for the treatment of hazardous compounds [84].

The capacity to adjust the energy input and the fraction of volume used according to the influent loading can result in a reduction in operational costs. In addition, less space is required as all operations occur in one basin [41].

2.3.2. Operational Characteristics in SBR process

The SBR operate in a repeated cycle sequentially. A cycle is a group of operations or phases comprising between the beginning (fill) and the end (draw and idle) of a wastewater treatment. These cycles are defined by five phases: fill, react, settle, draw and idle. The total cycle time (t_C) is the sum of all theses phase as presented in equation 2-7. Sometimes idle phase is not necessary and it is omitted.

$$t_C = t_F + t_R + t_S + t_D + t_I \qquad (Eq. 2-7)$$

Where, t_C total cycle time, h t_R: react time, h t_S: settle time, h t_F: fill time, h t_D: draw time, h t_I: Idle time, h

In addition, the condition applied during react phase can be different depending on the performance desired (organic matter, nitrogen or phosphorus removal), So aerobic, anoxic or anaerobic reaction time can be found in the react time.

$$t_R = t_{AE} + t_{AX} + t_{AN} \qquad (Eq. 2-8)$$

where,

t_{AN}: anaerobic react time, h t_{AE}: aerobic react time, h t_{AX}: anoxic react time, h

The number of cycle (NC) per day is determined through the total cycle time (t_C)

$$NC = {}^{24}/_{t_C} \qquad (Eq. 2-9)$$

Throughout the cycle, SBR can operate with different volumes due to the filling and draw phases. The volume exchange ratio (VER) can be defined as the ratio the volume of reactor filled and discharged at the end of every cycle (V_F) with total working volume (V_T).

$$VER = {}^{V_F}/_{V_T} \qquad (Eq. 2-10)$$

Where: V_T: total reactor volume (L), V_F: Filling volume (L)

The definition of hydraulic retention time (HRT) for SBR is based on the equation of continuous system.

$$HRT = {}^{V_T}/_{Q} \qquad (Eq. 2-11)$$

where: HRT: hydraulic retention time, h; Q: daily wastewater flow rate, L/d

The flow (Q) in SBR is defined by the product of filling volume (V_F) and number of cycle per day (NC). Therefore, the HRT can be expressed as follow,

$$HRT = \frac{t_C}{{}^{V_F}/_{V_T} * 24} \qquad (Eq. 2-12)$$

Where, t_C: total cycle time, h; V_F/V_T: exchange ratio.

The solid retention time (SRT) determines the amount of biomass in the SBR, there by determining its overall average performance. Thus, solid retention time (SRT) is expressed as equation 2-13 assuming that biomass concentration inside the reactor (X) is practically constant during the whole cycle.

$$SRT = \frac{V_T Xr}{Q_W X_W} \qquad (Eq. 2-13)$$

Where, SRT = Solid retention time (d), Q_W = waste flow rate (mg/L)

X_W: Waste biomass concentration (mg/L), V_T= total reactor volume (L)

Xr: Biomass concentration inside the reactor with full filling (mg/L)

The other important operational parameters of SBR are the organic loading rate (OLR) and nitrogen loading rate (NLR) that determine the amount of organic matter and nitrogen loaded to the reactor per day. The OLR and NLR are calculated according to (Eq.2-14) and (Eq.2-15)

$$OLR = \frac{Q * Co}{V_T} = \frac{Co}{HRT} \qquad (Eq. 2-14)$$

$$NLR = \frac{QNo}{V_T} = \frac{No}{HRT} \qquad (Eq. 2-15)$$

where V_T is the volume of the reactor (L); Q is the flow rate (L/d); Co is feed COD (g/L); No is feed TKN (g/L)

2.3.3. Sequencing Batch Airlift Reactor

The SBAR reactor are used for granules formation because high aeration rate is required for granulation. Aeration rate plays two important roles in a bubble column or an airlift reactor: first it imposes the hydrodynamic conditions and secondly it controls the oxygen transfer. Both these effects should be considered for improving the granular sludge process[88].

On the one hand it is known that high mixing reduces the resistance to the substrate transfer in the liquid boundary layer at the granule surface. Renewing liquid layer at the biofilm surface should encourage substrate transfer and hence smoothen and densified aggregates. Moreover, high shear force is considered to induce erosion of aggregates, the surface detachment leading to a smooth surface. It is also reported that more exopolymeric substances are secreted by bacteria under high shear force. For these reasons, high shear force was believed to favor the formation of dense aerobic granules[89].

Secondly, high aeration rate imposes an elevated dissolved oxygen (DO) concentration. This significant role of DO was recently pointed out by McSwain and Irvine[90]. These authors showed

that granules could not form at DO concentration less than 5 mg/L in SBR with a static fill. However, it seemed that high DO concentration is not necessarily a decisive condition on the formation of aerobic granules [91]. For example, aerobic granules have been formed in SBR at DO concentration as low as 0.7–1.0 mg/L [92].

2.4. Aerobic Granulation

2.4.1. Definition of Aerobic Granular Sludge

Aerobic granules are dense spherical self-immobilized aggregates of microorganisms with a strong compact structure and excellent settling ability. They have a well-defined appearance and are visible as separate entities larger than 0.1mm in diameter after settling. However, a general definition of aerobic granular sludge had not been made until the first aerobic granular workshop was held in Munich, Germany in 2004 [93] and aerobic granule was defined as granules making up aerobic granular activated sludge which are to be understood as aggregates of microbial origin, significantly faster than activated sludge flocs.

In the second aerobic granular workshop in 2006, a further explanation of the definition was discussed, regarding aggregates of microbial origin, no coagulation under reduced hydrodynamic shear, settling faster than activated sludge flocs, minimum size and method of harvesting [94]. When an aggregate fulfils all characteristics as described above, it can be called aerobic granular sludge. This simplifies the interpretation of experimental results and clarifies when discussing about aerobic granular sludge, activated sludge or biofilms. Figure 2-4 shows the image of a typical aerobic granular sludge (source [95])

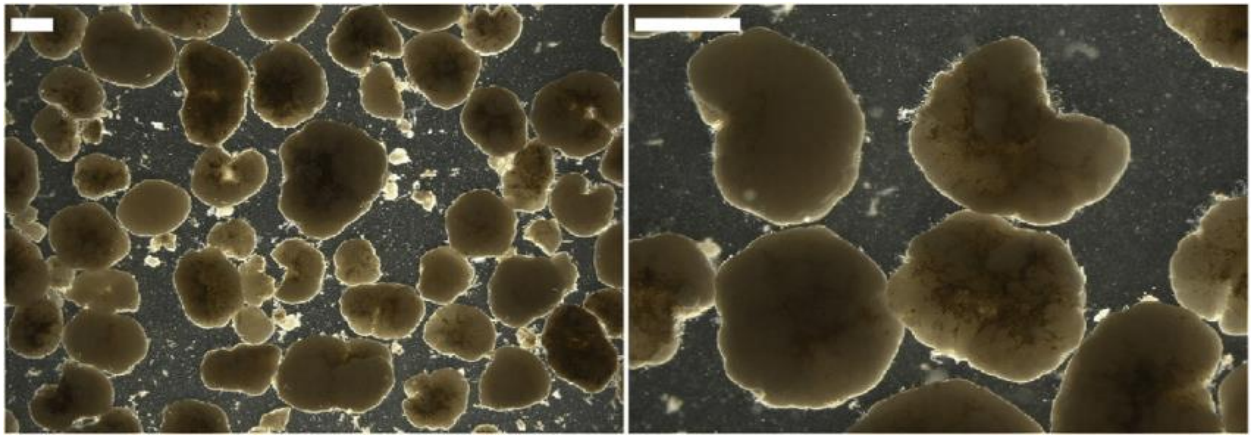


Figure 2-4. Images of aerobic granular sludge (Source[95])

2.4.2. Factors Affecting Aerobic Granule Formation

Granulation is affected by a number of operational parameters, such as substrate composition, organic loading rate, feeding strategy, reactor design, settling time, exchange ratio, and aeration intensity (hydrodynamic shear force). It seems that there is a relatively small operational "window" for the successful cultivation of aerobic granules. Under favorable conditions, the much desired super granules could be formed.

2.4.2.1. Substrate Composition and Organic Loading Rate

Aerobic granules have been developed with a wide variety of wastewater. Generally, aerobic granulation is independent of substrate type. However, the morphology and microstructure of aerobic granules are dependent highly on the composition of the wastewater they are grown on. As an example, the structural instability of aerobic granule occurred due to filamentous growth in the treatment of dairy wastewater [96]. The outgrowth of filamentous organisms was related to the fact that in the diary wastewater, easily biodegradable substances were slowly released at a low concentration attributed to slow hydrolysis of the initial polymeric substances.

It is recognized that carbohydrates in wastewater and a low DO level lead to flocculation, due to the favored growth of filamentous microorganisms [97, 98]. The glucose-grown aerobic granules

exhibit a filamentous structure, while acetate grown aerobic granules have a non-filamentous and very compact bacterial structure in which a rod-like species predominate [99]. At high potential growth rate on certain substrates, it is more difficult to cultivate aerobic granules. It is easier to obtain compact granule structure on methanol than on acetate, because of the growth rate of the microorganisms on methanol is lower than on acetate. The only observed exception was the growth of granular sludge on glucose. Usually, microorganisms have a high growth rate on glucose, but in biofilm and granule systems, populations are found to have a low growth rate on glucose and therefore dense and smooth structure are formed [93]. Aerobic nitrifying granules can be cultivated with an inorganic carbon source [100, 101]. The nitrifying granules show excellent nitrification ability. The accumulated evidence suggested that aerobic granules can form across a very wide range of organic loading rate (OLR) from 0.4-15 kg/m^3.d this indicates that the formation of aerobic granules in SBR is substrate concentration independent. However, it has been reported that kinetic behavior and morphology of aerobic granules are also related to the applied substrate loading [102, 103]. The mean size of aerobic granules increase from 1.6 to 1.9 mm with an increasing OLR from 3 to 9 kg COD/ m^3.d in 30 d [91]. Furthermore, OLR could affect the stability of aerobic granules. Zheng et al.[103]reported that bacteria-dominated aerobic granules with a mean diameter of 1 mm could be cultivated in SBR at a high OLR of 6 kg COD/m^3 /day in 30 d. However, under such high loading conditions, the bacteria-dominated granules were not stable and readily transited into large-sized filamentous ones. The instability of aerobic granules may be attributed to the mass transfer limitation and possible presence of anaerobes in the large-sized aerobic granules

2.4.2.2. Reactor Configuration

Reactor configuration influence the flow pattern of liquid and microbial aggregates in reactors [24, 104]. Until now, all aerobic granules are cultivated in pneumatically agitated reactors, including

bubble column and airlift reactor with sequencing batch operation. An important operation factor in aerobic granulation and reactor operation is superficial air velocity. It exerts an influence on aerobic granules through oxygen supply and hydrodynamic shear tress. In a column-type upflow reactor, higher ratio of reactor height to diameter (H/D) can ensure a longer circular flow trajectory, which in turn provides a more effective hydraulic attrition to aerobic granules. However, grow out more readily with filamentous and/or finger-type structure. In this case, no granules would be formed [93].

Shear stress provided by aeration rate depends on reactor configuration as well as reactor scale. Beun et al. [23] reported that much more dense granules with a smaller diameter were obtained in an airlift reactor at the same substrate loading rate compared to a bubble column. To reduce aeration rate to a reasonable value [105], a novel airlift loop reactor with divided draft tubes for aerobic granulation was designed.

2.4.2.3. Aeration Intensity

The positive role of a high hydrodynamic shear force provided a great aeration rate in the stable operation of biofilm and aerobic granule systems has been widely recognized[104]. Chen et al. [106] found that at shear forces of 2.4 and 3.2 cm/s, granules could maintain a robust and stable structure. Granules developed in low shear forces of 0.8 and 1.6 cm/s deteriorated to large-sized filamentous ones and resulted in operation instability. Granules cultivated under high shear forces of 2.4 and 3.2 cm/s stabilized with clear morphology, dense and compact structure, and resulted in good performance during 120 day operation. In most of studies on aerobic granulation, the upflow air superficial velocity in reactors is much higher than 1.2 m/s. However, a high aeration rate means a high energy consumption.

Adav et.al. [87] reported that the production of extracellular polysaccharides was closely associated with the shear force and the stability of aerobic granules. The extracellular polysaccharides content increases with the increasing shear force estimated in terms of superficial upflow air velocity. Thus, a high shear force stimulates bacteria to secrete more extracellular polysaccharides.

2.4.2.4. Cycle time

The cyclic operation of SBR consists of influent filling, aeration, settling and effluent removal. The settling time and exchange ratio of liquid volumes at the end of each cycle presets the main screening step to remove non-granular biomass from the reactor. A shorter cycle time results in a shorter hydraulic retention time (HRT), which provides a stronger selective pressure. Sludge loss is observed through hydraulic washout at a short cycle time because bacterial growth is unstable to compensate[107]. Liu et al [105] reported the influence of cycle time on the kinetic behavior of aerobic granules. The observed specific biomass growth rate of aerobic granules decreased from 0.226 to 0.031/d, while the observed biomass growth yield of granular sludge decrease from 0.316-0.063 g VSS g/COD when the cycle time was increased from 1.5 to 8 h.

The settling time acts as a major hydraulic selection pressure on microbial community in SBRs. A short settling time preferentially selects for the growth of rapid settling bacteria and the sludge with a poor settleability is washed out. It is recognized that the selection pressure imposed by short settling time should be more important in fully aerobic granule systems, but in anaerobic-aerobic alternative system with phosphate accumulating organisms (PAOs), the settling time seems to be less important because of the inherent tendency to PAOs to aggregate [93], meanwhile Meyer et al. [108] cultivated aerobic granules containing glycogen accumulating organisms at a settling time of 25 min.

2.4.2.5. Feeding Strategy

The unique feature of SBR over a continuous-flow activated sludge reactor is its cycle operation, which in turn results in a periodical starvation phase during the operation. It is proposed that such a periodical starvation would be somehow important to the aerobic granulation [99]. Although starvation is proposed not to be a prerequisite for aerobic granulation [105], the increase in hydrophobicity on carbon-starvation has been reported [109].

McSwain et al. enhanced aerobic granulation by intermittent feeding. In fact, pulse feeding to the SBR contributes to compact aerobic granules. Liu et al. observed that the aerobic granulation process was initiated by starvation and cooperated by shear force and anaerobic metabolism. A shorter starvation time resulted in a faster granulation[110]

2.4.2.6. Dissolved Oxygen, Temperature and pH

Aerobic granules are successfully cultivated at a DO concentration above 2 mg/L [110, 111]. However, Peng et al. [112] observed that small granules (diameter of 0.3-0.5 mm) were agglomerated into big flocs during settling in SBR at DO of 1 mg/ L. Mosquera-corral et al. [113] reported that reducing the oxygen saturation to 40 % caused deterioration, a decrease density and finally breaking of the granules. Based on the literature available, DO concentration is not a dominating factor for aerobic granulation.

The biological process rates depend on temperature. Most of the studies on aerobic granular sludge were carried out at room temperature (20-25^{0}C). In an investigation of the effect of temperature change on the conversion processes and the stability of aerobic granular sludge, de Kruek et al. [93] found that temperature change could affect the performance of an aerobic granular sludge reactors to a large extent. The startup of a reactor at low temperature led to the presence of organic COD in the aeration phase, deterioration of granule stability, and even biomass washout. Once a

reactor was started up at a higher temperature, it was possible to operate a stable aerobic granular sludge system at a lower temperature. Thus, they concluded that start-up should take place preferentially during warm summer periods, and that decrease temperatures during winter periods should not be a problem for granule stability and pollutant removal in a granular sludge system[93].

In microbial growth, pH is an important environmental factor. However, information regarding the effect of pH on species selection and aerobic granulation is still limited. Yang et al. [114] evaluated the effect of feeding alkalinity and pH on the formation on aerobic sludge granules. In SBR with a low alkalinity of 28.7 mg $CaCO_3$/L in the influent and a reactor pH of 3.0, rapid formation of fungi-dominating granules was achieved in 1 week. In another SBR with a high alkalinity of 301 mg $CaCO_3$ /L and a reactor pH of 8.1, formation of bacteria-dominating granules was achieved after 4 weeks of operation. These results suggest that microbial communities and structural features of aerobic granules could be formed through controlling the feeding alkalinity and reactor pH.

The review above showed that aerobic granulation is a very complex phenomenon with numerous internal interactions among process variables. All of them have significant effects on the overall reactor performance. Thus, cultivating aerobic granules with wastewaters with a complex composition, e.g. synthetic tannery wastewater, need to be further explored.

In addition, it is reported that the aerobic granules has been effective for the organic removal and nutrients from domestic, industrial and toxic effluents with high loading rates [63]. However, low nutrient removal efficiency is reported during the initial period of start-up mainly as a result of the harsh wash-out condition [115]. Operation at low sludge retention time (SRT) also resulted in wash-out of excessive biomass, a condition unfavorable for slow growing Ammonia and Nitrite Oxidizing Bacteria (AOB and NOB), which subsequently resulted in reduced nitrogen removal

efficiency. Modeling and experimental studies have shown that stepwise decline of settling time is a suitable method for improving biomass release during initial starting period [116, 117], however only a few research works have experimentally demonstrated the effect on granulation and nitrogen removal efficiency. Therefore, in this particular research, the effect of stepwise reduction of settling time on granule formation and nitrogen removal from synthetic tannery wastewater will be investigated.

2.5. Molecular methods for microbial community studies in wastewater

2.5.1. Overview

Traditionally, identification of microorganisms required the isolation of pure cultures and the identification of physiological and biochemical traits. These microbial consortia have mostly been analyzed by culture-dependent methods such as viable plate count or most probable numbers (MPN) techniques[118]. These techniques, apart from being laborious and slow, have the drawback of "enrichment bias" and lack sufficient sensitivity and selectivity for poorly or slowly growing microorganism [119] [120]. Moreover, questions like micro-scale distribution and in-situ activity of microorganisms are difficult to address by classical methods [121]. Often the most important microbial populations such as nitrifires and denitrifiers are difficult to cultivate. Culture-dependent methods underestimate the true picture of the composition of microorganisms in complex microbial communities[120].

During the last decade, advances in molecular biology and phylogeny analysis techniques have provided new insights into microbial ecology [122-125]. Molecular approaches developed to study microbial communities are based on the detection and analysis of the small subunit ribosomal RNA (rRNA) molecules and genes (16S rRNA) molecules for prokaryotes and (18S rRNA) for eukaryotes[126]. Today a combination of direct retrieval of rRNA sequence by the polymerase

chain reaction (PCR) and fluorescent probing in-situ enables the identification and phylogenetic characterization of microorganisms without cultivation [127]. For the monitoring of microbial community dynamics, these technical tools can be grouped into two categories; molecular probes, which are used more efficiently when there is previous knowledge of the targeted microbial populations, and DNA fragment analysis, which does not necessitate prior knowledge of microbial community structure before its study.

2.5.2. DNA fingerprinting

A variety of DNA fingerprinting techniques can be used to rapidly differentiate closely related environmental strains. DNA fingerprinting techniques typically are used not to specifically identify environmental isolates, but rather to demonstrate small genetic differences or similarities for population genetics studies, or for diversity studies within closely related species or strains.

The oldest of these approaches, restriction fragment length polymorphism (RFLP) analysis[128, 129], uses restriction endonuclease to digest purified DNA from individual strains in order to identify polymorphisms within individual genes. Polymorphisms or differences within a specific gene, may result in a different number of sites that are recognized by the restriction endonuclease used in each digestion. Such differences can be caused by single base pair changes or by large changes such as insertions, deletions, and rearrangements. Fragments within the digested DNA are separated by agarose gel electrophoresis, transferred to a DNA binding membrane, and detected by hybridization within a labelled probe specific for gene(s) of interest. Difference or similarities in the numbers and sizes of fragments from each isolate can then be identified.

Polymerase Chain reaction (PCR): PCR is widely used in microbial biotechnology [129]. In random amplified polymorphic DNA (RAPD) analysis [130-132], arbitrary primers (AP-PCR) produce a pattern that can be used to differentiate between closely related bacterial isolates. Unlike

RFLP analysis, which is designed to identify differences within specific gene, AP-PCR techniques screen for differences between entire genomes. AP-PCR is much faster than RFLP analysis because it is PCR based and no digestion with restriction endonuclease or hybridization is required. PCR amplified rDNA can also be analyzed by amplified fragment length polymorphism (AFLP) [133] to determine group and species specific patterns.

2.5.3. 16S rDNA sequencing

DNA sequencing and analysis of rRNA gene (16S rDNA) have revolutionized bacteria systematics. The most precise method of determining the phylogenetic affiliations of an isolate is sequencing of individual 16S rDNA molecules. The 16S rRNA genes code for the rRNA molecules needed for protein synthesis. The characterization of rRNA gene (rDNA) is a very powerful and frequently used means of accurately characterized environmental isolates to phylogenetic levels ranging from domain (Bacteria, Archaea and Eukaruya) [134] to a more specific phylogenetic placement. In comparison to phenotyping, 16S rDNA sequence analysis provides greater amount of genomic data that are directly linked to evolution and that can be used for phylogenetic comparison to all bacteria [135].

16S rDNA sequence have been instrumental in classifying bacteria and in assigning their taxonomic position in phylogenetic trees. The entire 16S rDNA from a mixed population can be amplified by PCR and then cloned in a vector suitable sequencing. In this way, organisms can be identified in a mixed population, even if the organism is non-cultivable. The selection of PCR primers determines which rRNA gen will be amplified. By combining non-specific PCR priers with cloning and sequence analysis techniques, it is possible to get information about the microbial sludge composition. For example, by applying PCR in combination with rRNA sequencing, a whole range of nitrifying-denitrifying microorganism in activated sludge of an industrial WWTP

was identified [136]. If selective primers are used, it is possible to amplify rRNA genes from specific group of microorganism present in the sludge. PCR amplification with methanogenic specific primers in combination with cloning and RFLP analysis was used to identify the methanogenic population in anaerobic sludge[137] and primers selective for ammonium oxidizing bacteria are also known[138].

The advantage of 16S rDNA sequencing is unambiguous identification of the organism in the community. The disadvantage of this protocol is that it is time-consuming and technically demanding.

2.5.4. Next Generation Sequencing (NGS)

Microbes are the most abundant biological entities found in the biosphere. Identification and measurement of microorganisms in the biosphere cannot be readily achieved due to limitations in culturing methods. A non-culture based approach, called “metagenomics”, was developed that enabled researchers to comprehensively analyze microbial communities in different ecosystems[139]. Metagenomic studies can be grouped into four categories based on different screening methods: (a) shotgun analysis using mass genome sequencing; (b) genomic activity-driven studies designed to search for specific microbial functions; (c) genomic sequence studies using phylogenetic or functional gene expression analysis; and (d) next generation sequencing technologies for determining whole gene content in environmental samples[140-142]. These four methods can be sub-classified under unselective (shotgun analysis and next generation sequencing) and targeted (activity-driven and sequence-driven studies) metagenomics [140, 141, 143]. Some studies have used an unselective metagenomic approach extensively because of its cost-effectiveness and simplicity in DNA sequencing [144].

The use of high throughput next generation technologies has allowed more comprehensive analysis than traditional Sanger sequencing[145]. For example, the identification, abundance and diversity of bacteria nitrifiers and denitrifieres and their functional gens responsible for nitrogen removal from tannery wastewaters was done using high throughput sequencing [120].

NGS technique was used for evaluation of the bacteria diversity change as a result of the process changes employed during different experiment conditions of this research work.

CHAPTER 3

3. MATERIALS AND METHODS

3.1. Wastewater sample and seed sludge

Primary treated tannery wastewater sample was brought from tannery common effluent treatment plant (CETP) located in Tamil Nadu, India. Aerobic sludge (5 L) from aeration tank and anoxic sludge (5 L) from anoxic tank of the tannery CETP (A mixed culture of heterotrophic and nitrifying bacteria) were brought to the lab. Settled and washed with tap water and phosphate buffer and arbitrarily mixed in 3:1 volumetric ratio. A volume of 1.5 L of the mixed sludge was used as seed sludge for start-up of the reactor.

3.2. Characterization of wastewater sample

The wastewater sample was characterized for basic parameters (Table 3-1) following standard methods for wastewater analysis [146]

3.3. Preparation of synthetic tannery wastewater

Synthetic wastewater with C/N ratio similar to tannery wastewater was prepared based on the characteristics of real wastewater (Table 3-1) and the literature [147, 148]. Commercial milk powder (Nestle Everyday) was used as sole source of organic carbon and Org-N whereas ammonium sulphate was used as source of NH_4-N. The composition of the synthetic wastewater used was : milk powder-2500 mg/L; $(NH_4)2SO_4$ -1378 mg/L; $MgSO_4.7H_2O$ - 25 mg/L; $FeSO_4.7H_2O$ - 20 mg/L; KH_2PO_4 - 88 mg/L; K_2HPO_4 - 90 mg/L; Na_2CO_3 - 66 mg/L; $NaHCO_3$- 105 mg/L; $CaCl_2.H_2O$ – 30 mg/L; and trace element solution (1ml/L) [19]. Ten replica analyses were done for COD, soluble sCOD, TKN and NH_4-N. The average equivalent total COD, soluble sCOD, TKN and NH_4-N of the synthetic wastewater were 5250±750, 3300±300, 425±25 and 270±20 mg/L, respectively.

Table 3-1. Characteristics of combined tannery wastewater

S/N	Parameters	Values[a]
1	pH	7-9
2	Suspended solids (mg/L)	2500-3500
3	COD (mg/L)	3500-6500
4	BOD_5, 20 ^oC	1400-3000
5	Sulphate (mg/L)	1500-3000
6	Sulphide (mg/L)	20-40
7	TKN (mg/L)	400-550
8	NH_4-N (mg/L)	100-400
9	Chromium (III) mg/L	50-70
10	Chloride (mg/L)	5000-8500
11	TDS (mg/L)	12000-20000

3.4. Nitrification-Denitrification study in SBAR using synthetic tannery

3.4.1. Experimental Setup of SBAR reactor

Laboratory scale sequential batch airlift reactor (SBAR) shown in Figure 3-1 (a & b) was used for the study. The reactor was constructed using plexiglass of working volume 5 L (diameter - 10 cm, total height - 80 cm, effective height - 64 cm, a head space - 16 cm and central draft tube having a depth of 60.5 cm). Air was supplied at a flow rate of 3.5 L/min from the blower controlled by airflow controller. The blower was connected to the draft tube in the reactor through plastic pipe. The draft tube was made of steel and had perforated openings at the bottom of the reactor where the compressed air was released to aerate and mix the reactor mixture. The reactor was equipped with wastewater collection tank, influent peristaltic pump, alkali feed pump, acid feed pump, pH,

DO and temperature sensors, air blower, central draft tube, solenoid valve, effluent collection tank and drainage port. The operation of the reactor was controlled by PLC controller. The controller was linked with input measurements from pH, DO and temperature sensors and timer.

(a)

Time control

(b)

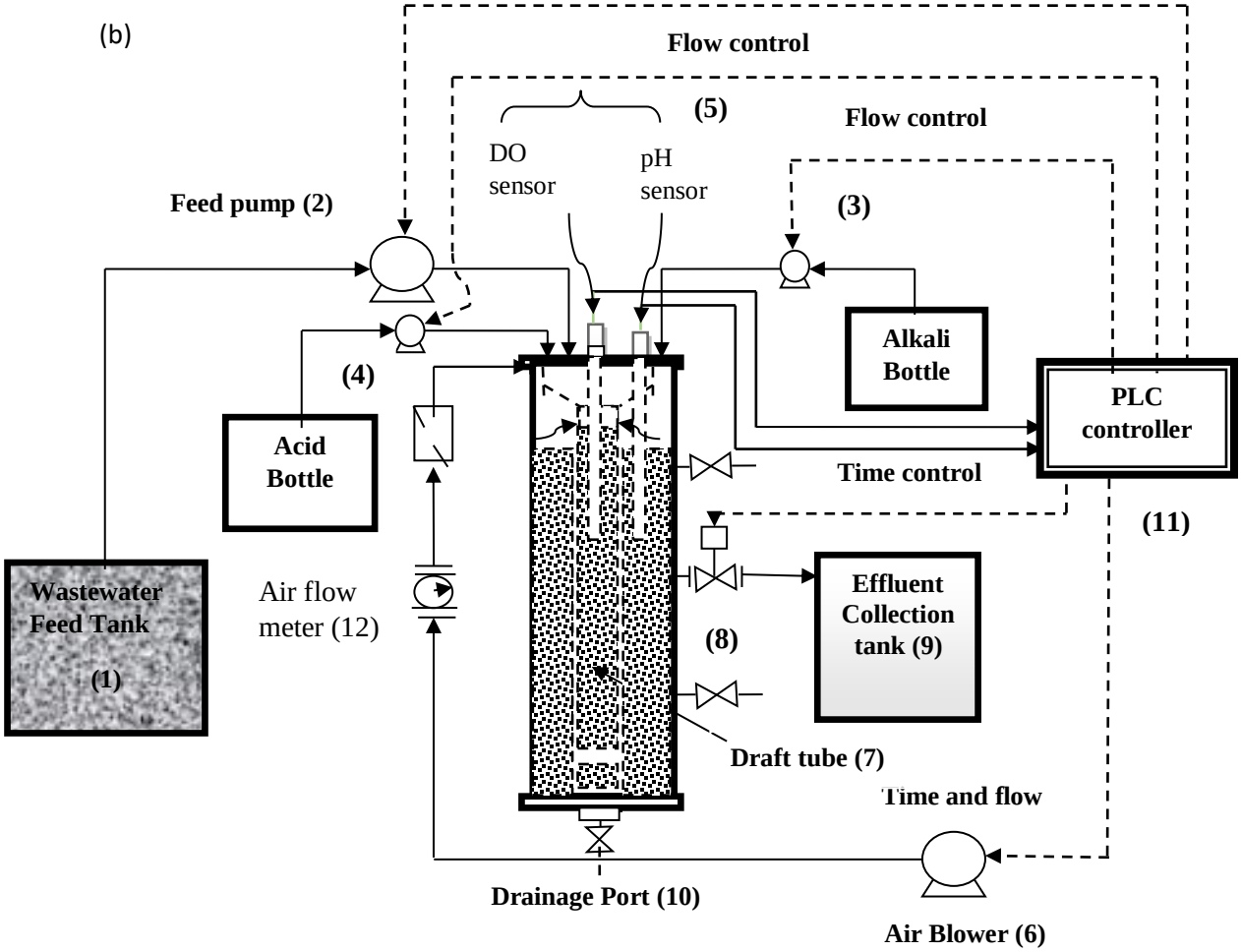


Figure **3-1** (a) Experimental Set up (b) Schematic diagram of SBAR used **in** the study

3.4.2. Operation of the reactor

The reactor was inoculated with seed sludge and filled with synthetic wastewater for working volume of 5 L, aeration was immediately started and at the end of the aeration period, the reactor mixture was allowed to settle and 2.5 L of the supernatant decanted at the end of the cycle. The reactor was run in semi-continuous SBR mode for more than 250 days a different total cycle time $t_C{}'$s of 18, 12, 10 and 8 h, and constant feed composition. The reactor was operated at a constant 50% volume exchange ratio (VER). During the first 129 days, the reactor was operated at an SRT of 7 d and t_C of 18 h (day 1 to day 61) and 12 h (day 61 to day 129), respectively, maintaining MLSS of 3.5 to 4 g/L. The MLSS was measured every two days and calculated amount of the excess sludge was withdrawn from the reactor.

The reactor was operated at constant SRT of 20 d and MLSS of 10 to 11 g/L during the remaining period of 121 days. The operating conditions of the reactor are shown in Table 3-2. In addition, optimal values were taken by referring to literatures [41, 67, 149, 150] as biological nitrogen removal is highly affected by operating temperature, pH and DO. The reactor was operated at room temperature (29-31°C) and at a relatively alkaline pH [151]. The pH was monitored and controlled online throughout the operating days and 0.25M $NaHCO_3$ and 0.25M HCl were used to maintain the reactor pH in the range 7.25 to 7.3. The DO was controlled at 1-2 mg/L through an on-off control of the air blower.

The t_C was also controlled by setting sequentially the time for feeding, aeration, settling and decanting. For all the t_C's, the feeding time was 9 min, settling 30 min and decanting 6 min. The different t_C's were carried out with different period of aeration time. A total t_C of 18, 12, 10, and 8 h were considered to study the effect of t_C on the performance of the reactor.

Table 3-2. Operation conditions of the reactor

S/N	Phase	Days	t_C (h)	DO (mg/L)	SRT (d)	Process
1	Phase I (1-61)	61	18	2	7	Start-up, nitrite accumulation and denitrification via nitrite
2	Phase II (61-129)	68	12	2	7	Nitrite accumulation and denitrification via nitrite
3	Phase III (129-152)	23	18	1	20	Stable nitrification and denitrification
4	Phase IV (152-164)	12	10	1	20	Stable nitrification and denitrification
5	Phase V (164-186)	22	12	1	20	Stable nitrification and denitrification
6	Phase VI (186-250)	64	8	1	20	Stable nitrification and denitrification

* Feed composition was constant (TCOD= 5250±750, TKN 350±13, NH_4-N, 282±8 mg/L) where values are averages for 10 samples)

*The pH of the reactor was maintained in the range (7.25-7.3) throughout the operating period by addition of 0.25M $NaHCO_3$ and 0.25M HCl

*The airflow was maintained 3.5 L/min throughout the operating period

3.4.3. Monitoring and performance evaluation of the reactor

Initial mixed liquor suspended solid concentrations (filtered) from the reactor and effluent samples (filtered) at the end of the SBR cycle were taken and analyzed immediately or preserved in refrigerator at 4°C till the analyses performed. The performance was evaluated in terms of COD, NH_4-N, NO_3-N and NO_2-N profiles and removal efficiencies.

All the physico-chemical analyses were done as per Standard Methods[146]. Online pH measurement and input for controller were made by gel filled autoclavable pH sensor (Brodley-James, USA) whereas online DO measurement was done with SS polarographic DO sensor (Brodley-James, USA). Ammonia was determined by titrimetric method following distillation of samples. TKN was determined by Kjeldhal method by digesting the sample, ammonia distillation and titration. COD was determined by closed reflux method using the COD digester (Merk, Germany). NO_3-N and NO_2-N were determined colorometrically using double beam spectrophotometer (U-2900/2910, Hitachi, Japan).

The total nitrogen removal efficiency was calculated as the percent difference between the initial pollutant concentrations in mixed liquor after 5 min of aeration and pollutant concentrations in effluent samples as given in Eq.3-1 and Eq.3-2. Removal is happening during the initial anoxic feeding and 5 min of aeration, this can be justified as the NO_x-N profiles show near zero value at the start of the CT.

$$TN - RE(\%) = \frac{[\mathrm{TIN}]-[\mathrm{TEN}]*100}{[\mathrm{TIN}]} \quad \text{(Eq.3-1)}$$

$$= \frac{Initial\ ([NH_4-N]+[NO_2-N]+[NO_3-N])-Effluent([NH_4-N]+[NO_2-N]+[NO_3-N])}{Initial\ ([NH_4-N]+[NO_2-N]+[NO_3-N])} \quad \text{(Eq.3-2)}$$

The COD and ammonia removal efficiencies were also calculated in the same way by taking the analytical values for initial and final concentrations in wastewater samples.

3.4.4. Kinetics Study

When the reactor showed stable performance, sampling was done weekly to study the kinetics by analyzing parameters for TCOD, sCOD, BOD, TKN, NO_3-N, NO_2-N, alkalinity, pH, DO, and MLSS/MLVSS for all t_C.

3.4.5. Microbial community analysis by illuminia MiSeq sequencing

3.4.5.1. DNA extraction

Sludge biomass samples were collected from tannery CETP and the SBAR reactor and stored at -20 °C. The samples were sent to Eurofins Genomics Bioinformatics Pvt.Ltd (Bangalore, India) for high-throughput sequencing on an Illumina MiSeq platform. Genomic DNA was isolated from the samples using Nucleo Spin Soil DNA Kit (Takara bio, USA) and the quality of the extracted DNA was assessed by resolving 2 µl of extracted genomic DNA on 0.8% Agarose gel at 120 V for approximately 60 min or till the samples reached 3/4th of the gel Figure 3-2. Also the quality of the DNA was checked quantitatively by taking 1 µl of each DNA and loading in nano drop for determining A260/280 nm wavelength.

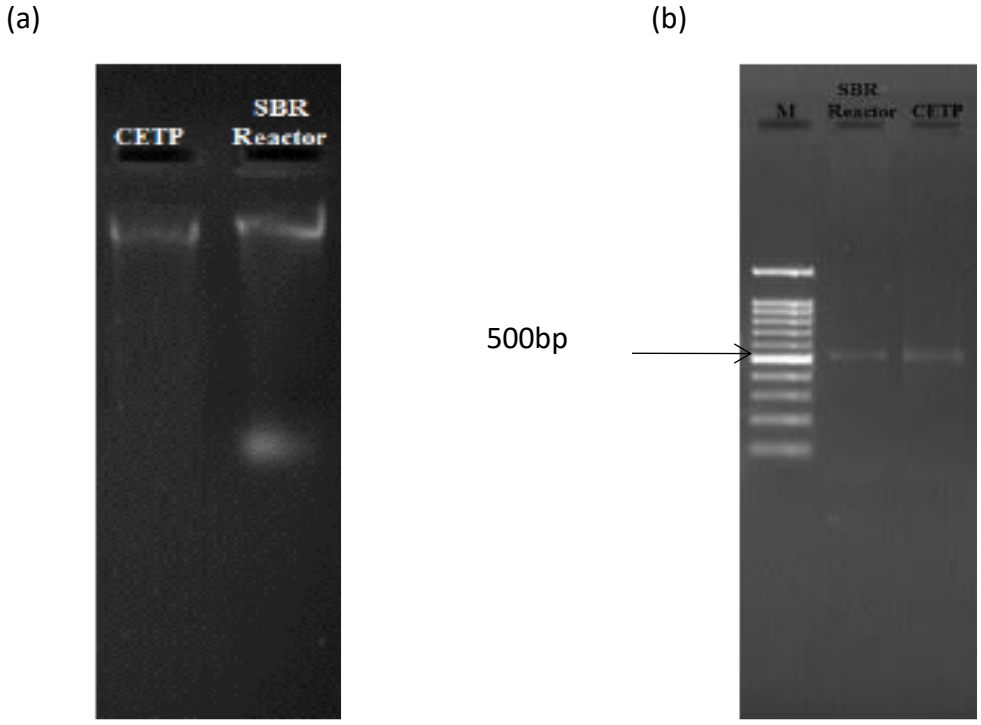


Figure 3-2(a) Quality check of DNA samples on 0.8% Agarose gel (b) First Amplicons on 1.2% Agarose gel

3.4.5.2. Library preparation and Miseq sequencing

The amplicon libraries (2*300 Miseq libraries) were prepared using Nextera XT Index Kit (Illumina inc.) as per the 16S metagenomic sequencing library preparation protocol (Part # 15044223 Rev. B). Primers for the amplification of the 16S rDNA gene specific for bacteria were designed at Eurofins Genomics Bioinformatics Lab, India. The forward (GCCTACGGGNGGCWGCAG) and reverse (ACTACHVGGGTATCTAATCC) primers were used to amplify the DNA. Amplification of the 16S rDNA gene were done targeting V3- V4 region specific for bacteria. In this analysis, 3 µl of PCR product was resolved on 1.2% Agarose gel at 120 V for approximately 60 min or till the samples reached 3/4th of the gel Figure 3-2 (b). The amplicons with the illumina adaptors were amplified by using i5 and i7 primers that add multiplexing index sequences as well as common adapters required for cluster generation (P5 and P7) as per the standard illumina protocol. The amplicon library was purified by 1X Ampure XP beads and quantified using Qubit fluorometer.

The amplified libraries were analyzed in 4200 Tape Station System (Agilent Technologies) using High Sensitivity D1000 Screen tape as per manufacturer instructions. After obtaining the mean peak size from Tape Station profile, libraries were loaded onto MiSeq at appropriate concentration (10-20pM) for cluster generation and sequencing. Paired-end sequencing allows the template fragments to be sequenced in both the forward and reverse directions on Miseq. The kit reagents were used in binding of samples to complementary adapter oligos on paired-end flow cell. The adapters were designed to allow selective cleavage of the forward strands after re-synthesis of the reverse strand during sequencing. The copied reverse strand was then used to sequence from the opposite end of the fragment.

3.4.5.3. Post processing of sequence data

The raw Fastq data were filtered by QIIME (Version 1.9.1), a comprehensive software comprising of tools and algorithms such as Fast Tree for heuristic based maximum-likelihood phylogeny inference [152], the ribosomal database project (RDP) classifier for the assignment of taxonomic data using a naïve Bayesian classifier[153] [154], and others. This allows QIIME, which continues to undergo development, to easily and relatively quickly adapt novel stand-alone tools, and thus improve in step with advances in the field of microbial community ecology.

All the sequences from all the samples were clustered into Operational Taxonomic Units (OTUs) based on their sequence similarity. OTUs are clusters of sequences, frequently intended to represent some degree of taxonomic relatedness done using UCLUST at 97% sequence similarity and each resulting cluster typically represents a species. Since each OTU may be made up of many sequences, a representative sequence was picked for that OTU for downstream analysis [155].

The data were further interpreted by using community diversity index (Shannon alpha diversity index) and rarefaction curve. A heat map analysis of the most abundant organisms at class level was conducted in each sample.

3.5. Aerobic granulation in SBAR using synthetic tannery wastewater

3.5.1. Operating condition of the reactor

The SBAR reactor described in Figure 3-1, was used for aerobic granulation study with the aim of improving the settling properties of the sludge without compromising the nitrification-denitrification performance of the SBAR reactor. The reactor operating condition are summarized in Table 3-3.

The reactor was operated to start up the granular sludge system by feeding synthetic tannery wastewater and an evaluation of the granulation and reactor performance during the experimental

period was studied. Flocculent sludge (8.6 g/L) from the lab scale SBAR reactor, which was operated for more than 6 months, which showed efficient performance towards removal of organic carbon and nitrogen simultaneously, was used as initial inoculum. The sludge retention time (SRT) was maintained at 20 d throughout the operating days.

The reactor was operated at a constant volume exchange ratio of 50% for a period of 105 days. The reactor was operated in 10 hour cycles with consisting of 9 min feeding, 555-582 min aeration, 3-30 min settling and 6 min effluent removal from the rector. The settling time was reduced gradually from 30 min to 3 min for 60 days and operated at 3 min settling during the rest of the reactor operation.

Operating temperature, pH and DO, suitable for biological nitrogen removal was selected based on literatures [151]. Operation temperature of the reactor maintained at ambient temperature (29-31°C) and pH was monitored and controlled online throughout the operating days in the range (7.25-7.30) by adding 0.25M $NaHCO_3$ and 0.25M HCl. The DO was maintained between 1-2 mg/L through the control of the air blower.

Table 3-3.Operating conditions of the SBAR reactor for aerobic granulation study

Process parameter	Condition
Feed medium	Synthetic medium
Reactor working volume	5 L
Effective liquid depth	64 cm
Total cycle time (h)	10 h
Settling time	3-30 min
Volume exchange ratio	50%
HRT	20 h
OLR	6.3 ± 0.9 kg/m^3.d
NLR	0.425 ± 0.03 kg/m^3.d
SRT	20 d
pH	7.25 - 7.30
Air flow rate	3.5 L/min
DO set value	1-2 mg/L
Temperature	29 - 31.5°C
Total number of operating days	105 d

Before the start of this experiment, the reactor operation was at stable performance in terms of COD and nitrogen removal. The feed synthetic wastewater with Carbon to Nitrogen ratio was simulated based on the characteristics of tannery wastewater collected from existing tannery wastewater treatment plant. The synthetic wastewater was prepared based on the characteristics of real tannery wastewater [156].

The average applied organic loading rate (OLR) and nitrogen loading rate in the reactor were 6.3 ± 0.9 kg COD/m^3.d and 0.425 ± 0.03 kg NH_4-N/ m^3.d respectively which resulted in COD: N ratio of 12.4:1. The reactor volume exchange ratio (VER) was retained at 50% and the total cycle time including feeding, aeration, settling and decanting was maintained at 10 h, which means the hydraulic retention time (HRT) was 20 h.

3.5.2. Monitoring the aerobic granulation and performance of the reactor

Granule formation was monitored using Sludge Volume Index (SVI), sieve analysis and light microscopy. The SVI30 was measured for a settling period of 30 min according to the Standard Methods. Sludge samples were also sieved using 0.212 mm sieve and the MLSS and the mass retained on the sieve was measured to estimate the percentage of granules from the total sludge. Biomass with diameter greater than 0.212 mm was considered to be granular [157]. The morphology of granular sludge was analyzed by a light microscope (Olympus CH20i). The microstructure of the granules was visualized by scanning electron microscopy (Jeol 1300 LV-SEM). Before sample observation, the granules sample was taken from the reactor and mildly washed using phosphate buffer. The washed granules were settled and treated with a series of fixation and dehydration processes (4% paraformaldehyde for fixing the sample, 40, 60, 80 and 100% ethanol for dehydration). The dehydrated granule sample was further dried using freeze dryer then the sample was ready for observation in SEM [99]. The biomass density (biomass concentration in the granules) was measured by first taking 100 ml of granules samples and filtering it using 45µm filter. The filtered granules were then added into 20 ml of demineralized water to estimate the granules volume by volumetric displacement method. The dry weight of the granules samples was estimated by oven drying for 24 h at 105^{o}C. Based on the volume and dry weight of the granule, the density of biomass was then estimated [24]. The settling velocity of the granules was analysed by monitoring the time consumed for an individual granule to settle in a 40 cm long cylinder. The settling velocity of the granules could then be calculated by dividing the length of the cylinder by the time taken by the granule to reach the bottom of the cylinder.

The relationship between COD degradation and Extracellular Polymeric Substances (EPS) formation with respect to time was analyzed for one complete cycle time. Samples were withdrawn

from the reactor at various intervals and EPS was extracted from the bacterial biomass. Biomass was removed by centrifugation 10,000g for 30 min. (Eppendrof 5804 R). The supernatant was precipitated overnight at 4°C with two volumes of ice cold 95% ethanol. The resulting precipitate was removed by centrifugation at 12,000 g for 30 min at 4°C. After centrifugation, the extracted EPS (Pellet) was lyophilized [158]. Further biochemical characterization of lyophilized EPS was estimated by total carbohydrates and proteins where glucose was used as the standard for carbohydrates and Bovine serum albumin for proteins [159, 160].

To evaluate the performance of the granular SBAR reactor, initial pollutant concentrations in mixed liquor of reactor samples were collected 5 min after addition of fresh feed and start of aeration and final pollutant concentrations in effluent samples were withdrawn at the end of the SBR cycles and estimated either immediately or preserved in a refrigerator at 4°C till the analyses were performed. The reactor outcome was evaluated in terms of pollutants concentrations i.e., sCOD, NH_4-N, NO_3-N and NO_2-N profiles and removal efficiencies. The Mixed Liquor Suspended Solids (MLSS), Mixed Liquor Volatile Suspended Solids (MLVSS) and Sludge Volume Index (SVI) of the sludge were measured for every sequential operating settling time considered for the study to investigate the effect of the pressure applied. The analytical procedures as per Standard Methods [161] were used for analysis of all physico-chemical parameters.

Online pH measurement and input for controller were made using gel filled autoclavable pH sensor (Brodley-James, USA) and online DO measurement was done using SS polarographic DO sensor (Brodley-James, USA). Ammoniacal nitrogen (NH_4-N) was determined by titrimetiric method following distillation of samples. TKN was determined by Kjeldhal method by digesting the sample, ammonia distillation and titration. COD was determined by closed reflux method using the COD digester (Merk, Germany). Nitrate nitrogen (NO_3-N) and nitrite nitrogen (NO_2-N) were

determined calorimetrically using double beam spectrophotometer (U-2900/2910, Hitachi, Japan). The total nitrogen (TN) was estimated as the sum of NH_4-N, NO_2-N and NO_3-N concentrations as described in section 3.4.3.

The removal efficiencies of the reactor for COD, NH_4-N and TN was estimated by calculating the percentage difference between the initial concentrations of the respective parameter in mixed liquor sample, after 5 minute of aeration and final concentrations in the effluent sample after completion of the cycle time (i.e., treated wastewater discharged from the SBAR reactor).

3.5.3. Calculation of biomass yield

The biomass yield of stable granules were estimated by calculating the observed and theoretical kinetic parameters [162].

3.5.4. DNA Extraction and confirmation of selected gene

This study was carried out to confirm the presence of nitrifiers, denitrifiers and anammox specific genes responsible in the nitrogen removal cycle. Biomass sample was collected from the reactor and genomic DNA was extracted using Nucleospin soil (Macherey – Nagel), (as per the manufacturer's procedure). The quality of the DNA was checked at 260/280nm by nano drop method (Table 3-4).

Table 3-4. DNA concentration of the test samples at 260/280nm by Nano drop method.

S.No	Sample Name	DNA concentration ng/µL (nanodrop)	260/280nm
01	Control	148.4	1.82
02	SBR	102.8	1.81

For further amplification of isolated DNA, PCR was carried out with the total reaction volume of 10µL where the 5µL master mix (NEB) consists of 0.9 µL of DNA template, 0.9 µL of Primers (Forward and reverse) and 3.2 µL of nuclease free water (Thermo – Fisher scientific) obtained

using the thermal cycler (Agilent sure Cycler 8800). After completing 35 cycles, PCR amplified samples were loaded in agarose gel and Electrophoresis was used for the detection of amplified genes and documented (Syngene G: BOX F3). The targeted genes, primer pairs, references and annealing temperature used are shown in Tables 3-5 and 3-6.

3.5.5. Quantitative Polymerized chain reaction (qPCR)

The qPCR study was carried out to quantify the relative abundance of specific genes responsible for nitrogen removal. Aerobic sludge from the existing tannery wastewater treatment plant was used as a seed inoculum (control) and the SBAR sample inoculum was collected from the SBAR reactor. The effect of the test samples on its gene expression was tested by using quantitative real time PCR. The control and SBAR samples were analyzed for the gene expression.

The isolated DNA samples were subjected to gene expression and quantitative PCR (qPCR) analysis was carried out with a reaction volume of 20 µl consisting 1µl of DNA (20 ng) and 10µl of SYBR Green Supermix (Bio Rad, USA). The qPCR reaction was done for 35 cycles followed by denaturation at 95ºC for 30 sec, annealing 52 ºC - 58 ºC (gradient) for 30 sec extension for 72 ºC for 15 sec using Bio-Rad CFX96 system. The gene expression levels of targeted gene amoA and PLA46+AMX820 were normalized to the level of housekeeping gene (16s) which used as internal control for normalization. The gene expression levels were analyzed based on Comparative Δ Delta Ct (cycle threshold) method. The Ct values of the test samples were estimated and the data was expressed in terms of fold change over Control sample.

The relative quantification and comparison of targeted gene was made by measuring the number of cycles essential for the fluorescent signals to cross the threshold level called the Ct (cycle threshold) level. The Ct levels in the sample is inversely proportional to the targeted nucleic acid (i.e. higher Ct values indicate lower quantity of targeted nucleic acid and vice versa)

Table 3-5. List of targeted primers, annealing temperature and references used for the PCR study

Targets	Primer	Sequence	Annealing Temperature (^{o}C)	fragment size (bp)	References
Planctomycetes	Pla46(F)	GGATTAGGCATGCAAGTC	53.7	840	[163-166]
Brocadia	Amx820®	AAAACCCCTCTACTTAGTGCCC	60.3		
AOB-*amoA*	amoA-1F	GGGGTTTCTACTGGTGGT	56	491	[167]
	amoA-2R	CCCCTCGGCAAAGCCTTCTTC	63.7		
nirS	cd3Af	GTSAACGTSAAGGARACSGG	67.8	425	[168, 169]
	R3cd	GASTTCGGRTGSGTCTTGA	64		
nirK	F1aCu	ATCATGGTSCTGCCGCG	60.5	473	[170]
	R3Cu	GCCTCGATCAGRTTGTGGTT	59.8		
nosZ	nosZ2F	CGCRACGGCAASAAGGTSMSSGT	>75	261	[171]
	nosZ2R	CAKRTGCAKSGCRTGGCAGAA	68		
Bacterial	341f	CCTACGGGAGGCAGCAG		174	[172].
16S rRNA	515r	AATCCGCGGCTGGCA			

Table 3-6. List of the primers used for the qPCR study

Gene	Forward primer	Reverse primer
16s (Standard)	AGAGTTTGATCCTGGCTCAG	CCGTCAATTCMTTTRAGTTT
amoA	GGGGTTTCTACTGGTGGT	CCCCTCGGCAAAGCCTTCTTC
PLA46+AMX820	GGATTAGGCATGCAAGTC	AAAACCCCTCTACTTAGTGCCC

3.6. Partial nitrification of anaerobic digester supernatant

3.6.1. Anaerobic digestion of excess sludge

The general mass balance for nitrogen in the biological treatment process indicate that some amount of the nitrogen is used in the cell building process. Therefore, the biomass produced in the course of biological process can generate significant quantity of nitrogen during cell decay.

In view of effectively generating the digester supernatant containing significant amount of NH_4^+-N, the following schematic diagram of the preliminary processes were used, Figure 3-3.

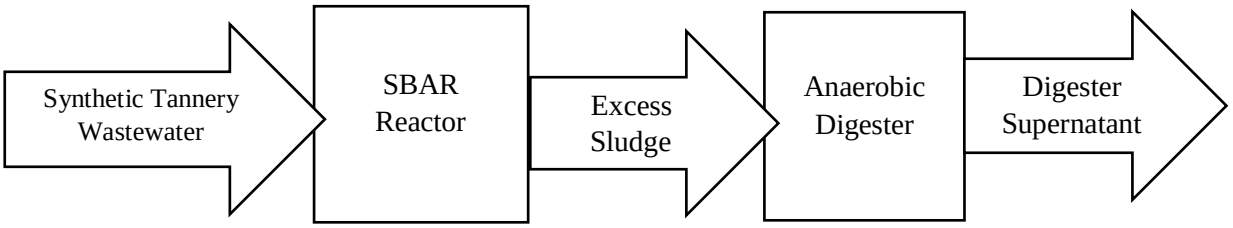


Figure 3-3.Schematic flow diagram for generation of digester supernatant

3.6.1.1. Characterization of raw sludge

Initially the aerobic sludge from the lab scale SBAR running for more than 1 year treating synthetic tannery wastewater for removal of organic carbon and nitrogen was taken. The physic-chemical characteristics of the raw sludge obtained from the aerobic SBAR were assessed.

3.6.1.2. Experimental Set up of the digester

The raw excess sludge was inoculated with anaerobic seed sludge from Koyembedu biogas plant (located in Chennai, India) and the digester was set up based on VDI-4601 German standard method. Similarly a control anaerobic digestor containing only the seed sludge was kept and monitored along with the experimental digestors. The digestion was carried for 35 days and the gas pressure of both the experimental and control bottles were monitored using gas pressure manometer. At the end of the experiment, digester supernatant was collected.

3.6.1.3. Characterization of digester supernatant

The digester supernatant obtained from the anaerobic digester was characterized as per standard method for analysis of wastewater samples[173]; and several essential characteristics, such as ammonia, Total Nitrogen, Total Kjeldahl Nitrogen, COD, and alkalinity were analyzed.

3.6.2. Partial nitrification of anaerobic digester supernatant

3.6.2.1. Experimental Objective

The objective of this experiment was to study the effect of different operational factors on partial nitrification of anaerobic digester supernatant in SBAR with the aim of getting effluent suitable for Anammox reactor feed.

3.6.2.2. Experimental Set up

The treatment of the anaerobic digester supernatant was carried out at lab-scale where the partial nitrification was developed in the SBAR. The temperature was maintained by means of a thermostatic bath and pH was monitored using a field measurement system connected to a pH/T probe. Temperature and pH profiles were monitored and these data were then exported and

represented in each cycle. A closed intermittent-flow respirometer was used to characterize the system. Dissolved oxygen was measured using a portable oxygen meter. Air was supplied through aquarium air pumps, a flow meter, and porous stones, which provided fine bubbles. Biomass suspension and mixing were achieved through uninterrupted aeration during the reaction phases.

3.6.2.3. Operational condition and design of experiment

Seven operational factors (pH, DO, Temperature, Cycle time, MLSS, C/N and aeration strategy) which significantly affect the NO_2-N: NH_4-N ratio were considered for the study, Table 3-7. Plackett-Burman DOE was used to find out which of these factors have the most significant effect in achieving the required NO_2-N: NH_4-N ratio. A total of 12 runs based on the design were operated for a minimum of three days per run, Table 3-8. Data were collected and the results were interpreted using Minitab software to analyze the effect of the factors affecting the NO_2-N/NH_4-N ratio.

Table 3-7.Operational factors affecting NO_2:NH_4 ratio and range of values taken

Parameters	Negative(-)	Positive(+)
pH	6.5	8
DO(mg/L)	1	4
Temperature(^oC)	20	32
Cycle time(h)	5	10
MLSS (mg/L)	3500	10000
C/N ratio	0.5	3
Aeration strategy	Intermittent/30min-off/2h-on	Continuous

Table 3-8 Placket-Burman Design of Experiment

Run	pH	DO	Temp(^oC)	CT	MLSS/VSS	C/N	Aeration strategy
1	+	+	+	-	-	+	+
2	-	-	-	-	+	+	+
3	-	+	-	+	+	+	-
4	-	-	-	-	-	-	-
5	+	+	-	+	-	-	-
6	+	+	-	-	+	-	+
7	+	-	+	-	+	-	-
8	+	-	-	+	-	+	+
9	-	+	+	-	-	+	-
10	-	-	+	+	-	-	+
11	+	-	+	+	+	+	-
12	-	+	+	+	+	-	+

3.6.2.4. Analytical Method

The analyses of alkalinity, chemical oxygen demand (COD), TKN, ammonia nitrogen, nitrites and nitrates were performed following the methods reported in the standard methods for the examination of water and wastewater[173].

CHAPTER 4

4. RESULTS AND DISCUSSION

4.1. Performance of SBAR reactor with synthetic tannery wastewater

4.1.1. Operation of the reactor during Phase I and II

During the first seven days of startup period, the SBAR reactor was operated with HRT of 36 h by recycling back treated effluent and by changing the feed wastewater every three days. Recycling was done to properly acclimatize the seed sludge to the reactor condition and to prevent washout of seed sludge from the reactor. The recycling was interrupted after a period of one week and the reactor was operated with the operating conditions given in Table 3-2.

The operating conditions in Phase I and II were designed to study the startup of the reactor and the effect of HRT for constant low SRT (7days) on the characteristics of nitrification and denitrification processes. Even if the wastewater under consideration has high COD/N ratio, studies showed that the high COD/N ratio resulted in high growth of heterotrophs but has minor effect on the growth of nitrifiers [174].The engineered system (SBAR) helped to manipulate the growth of these two important groups of bacteria involved in simultaneous removal of organic carbon and nitrogen.

The operation of the SBAR reactor involved the control of pH, DO, HRT, SRT and temperature. The control of the above parameters in the reactor have been reported to selectively inhibit NOB's or washout NOB's from the system [68, 175]. Jubany et.al demonstrated that total and stable washout of NOB's occurs at pH of 8.3 and DO of 1.2-1.9 mg/L in a nitrifying continuous activated system [176]. The operating conditions in Phase I was set at t_C of 18 h, SRT of 7 d and controlled low dissolved oxygen concentration. The maximum DO maintained (1 mg/L) was taken by

considering the feed COD/N ratio and the economy of aeration. The pH range (7.25-7.3) was also taken to help the start up faster. Analysis of the daily effluent profile for COD and NH_4-N, Figure 4-1, showed that simultaneous removal of organic carbon and nitrogen. In phase II, the reactor was operated with increased loading rate keeping all other parameters same as phase I.

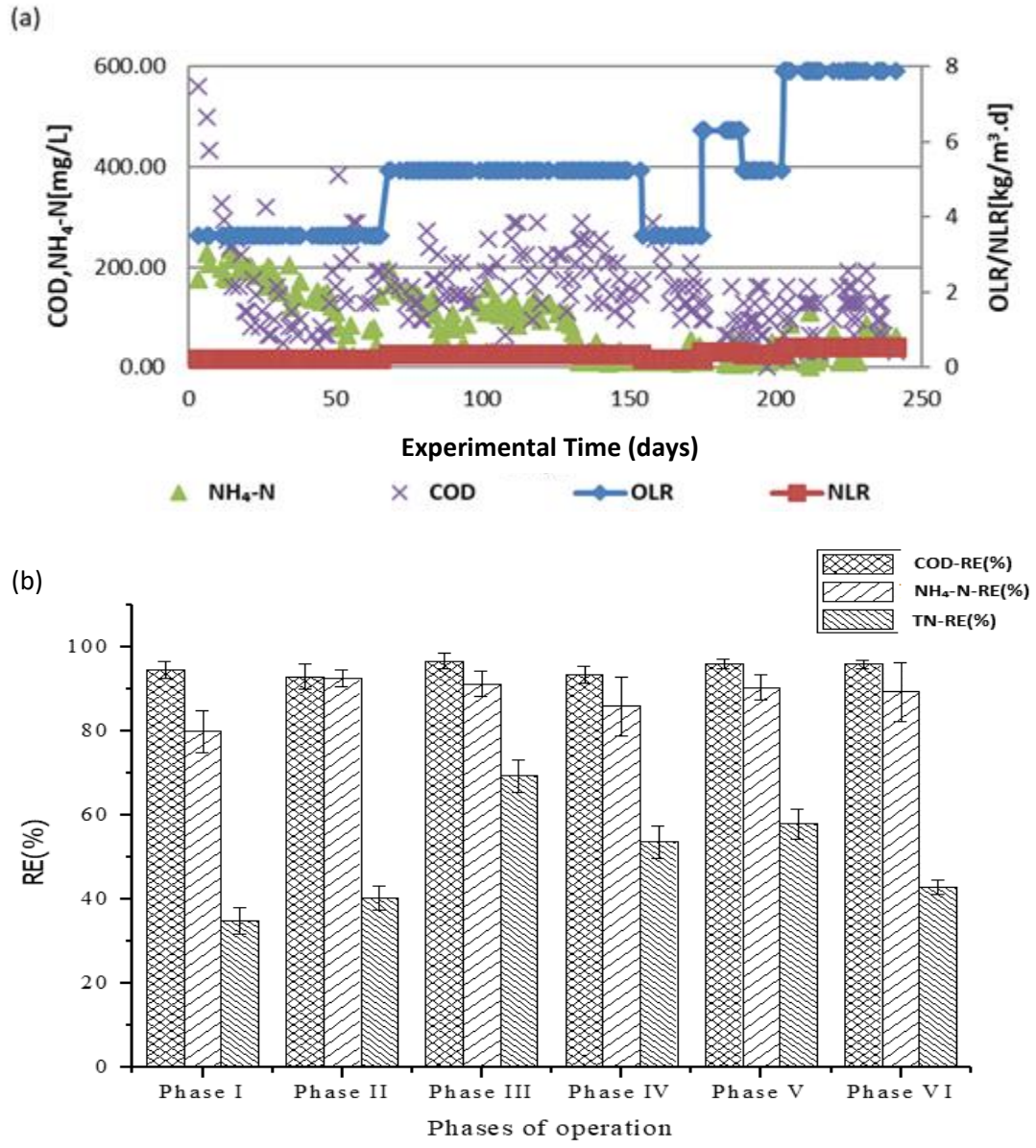


Figure 4-1 Daily operational history of the SBAR and its performance (a) Daily profile of final treated effluent COD and NH_4-N under varying OLR and NLR (b) Average removal efficiencies (RE-(%)) at steady state for NH_4-N, COD and TN at each phases of operation.

Figure 4-2 (a) shows the NH_4-N, NO_2-N and NO_3-N (NO_x-N) profile at steady state operation. This profile clearly shows the ammonium conversion in one complete t_C (18 h). The profile shows increasing NO_x-N concentrations, but the increases were very high and insignificant for NO_2-N and NO_3-N respectively, while Figure 4-2 (b) shows the profile including NH_4-N and COD. This profiles shows decrease in both COD and NH_4-N and increase in NO_x-N as a result of the ammonium oxidation. The removal pattern of nitrogen species follow the typical pattern for incomplete nitrification reported [177]. The graphs clearly showed nitrite accumulation and very less conversion to nitrate. This indicates relatively higher startup period required for NOB compared to AOB and heterotrophs [12] and the relatively higher pH and low SRT maintained favor AOB more than NOB. The SRT was maintained at 7 d days resulting in MLSS of 3500-4000 mg/L. During partial nitrification, nitrogen and organic carbon removal was achieved. Denitrification seemed to play a role in the organic carbon removal mainly during the anoxic feeding and initial period of aeration. During this phase of operation, the reactor showed 94.6±2% COD removal, 80±5% NH_4-N oxidation to nitrite and 35±9.2% TN removal in steady state.

Phase II (day 61to day 129) showed similar trend of phase I, but there was aeration problem after the end of phase I and the reactor had taken some days to restore its nitrification efficiency back. During this phase, the reactor again showed a good partial nitrification to nitrite. The COD, NH_4-N and TN removal efficiencies were 93±3, 92.7±2 and 40.4±3%, respectively.

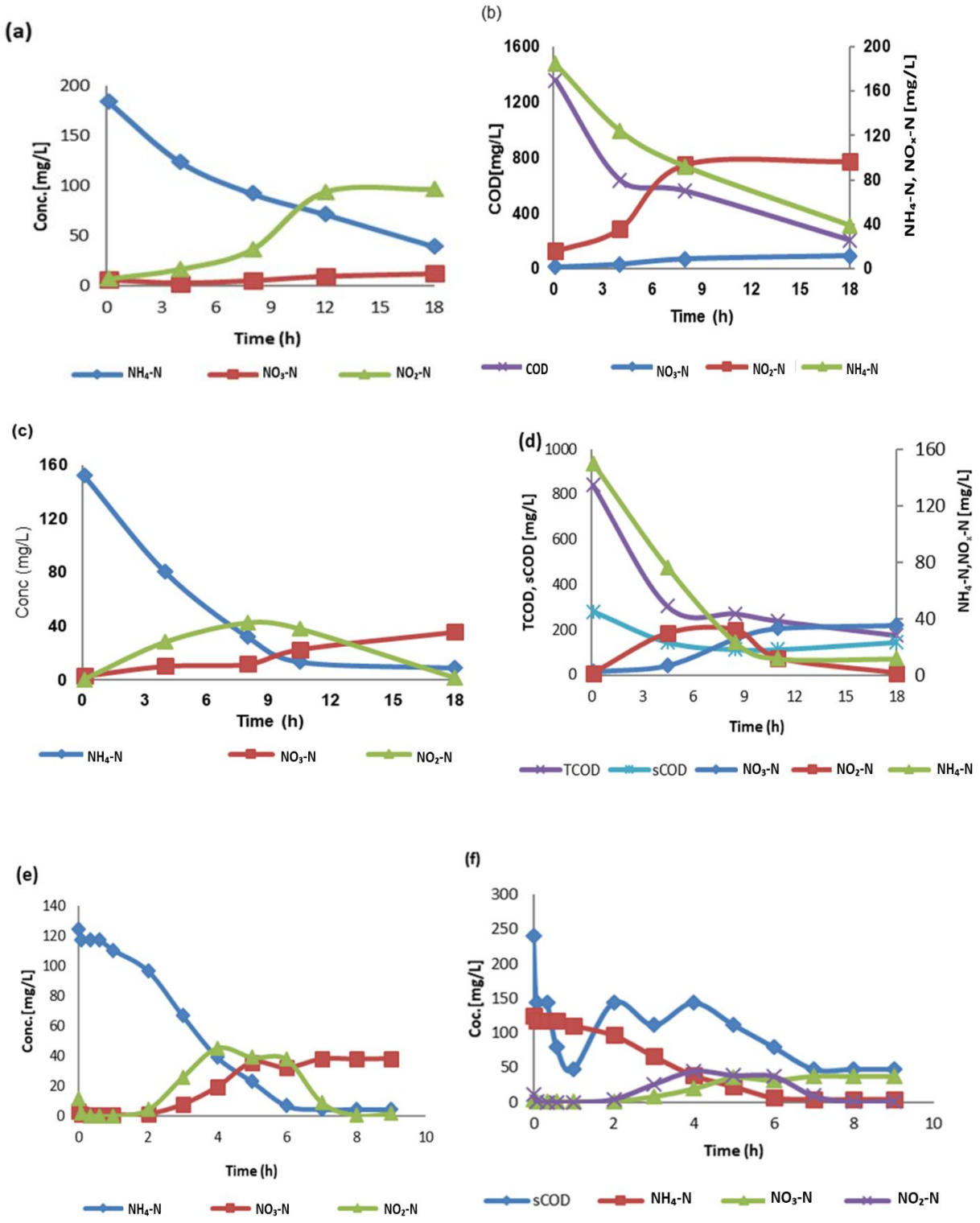
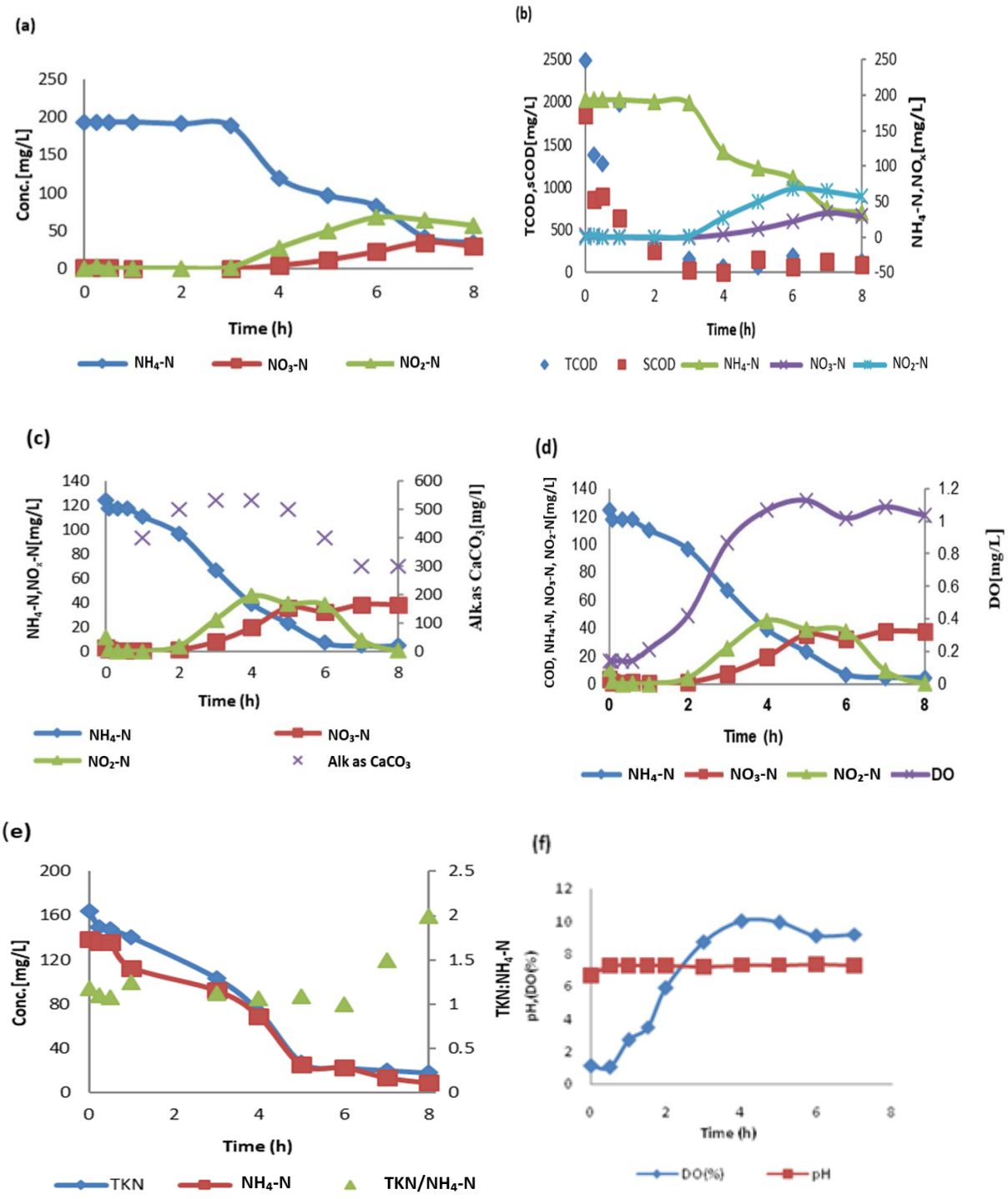


Figure 4-2. Typical profiles for NH_4-N, NO_x-N and COD over the operated cycle time: (a) Phase I and II, NH_4-N and NO_x-N profile (b) Phase I and II, NH_4-N, NO_x and COD profiles. (c) Phase (III), NH_4-N and NO_x-N profile (d) Phase III, NH_4-N, NO_x and COD profiles (e) Phase IV, NH_4-N and NO_x-N profiles (f) Phase IV, NH_4-N, NO_X and COD profiles

4.1.2. Operation of the reactor (Phase III to VI)

The effect of increasing the SRT on the removal efficiency and characteristics of the effluent was studied by maintaining the SRT at 20 d but by setting different values of total t_Cs (i.e. 18 h, 12 h, 10 h, and 8 h). During this period, the COD and NH_4-N removal efficiencies were also very high and complete oxidation of ammonium to nitrate was observed at the end of the t_C. This showed that higher SRTs favour the growth of both nitrifiers (AOBs and NOBs). Figure 4-2 (c) and (d) displays the NH_4-N, NO_x-N, and COD profiles for different operating days at 18 h t_C. The results clearly showed that the ammonium oxidation was taking place as in phase I and II but the NO_2-N and NO_3-N profiles were changed significantly and effluent had more of nitrate than nitrite. From Figure 4-2 (c) and (d), it was observed that the NH_4-N removal followed a logarithmic correlation while NO_2-N and NO_3-N conversion followed parabolic and linear correlations respectively. A similar kinetics study was conducted for 10 h t_C. The results are plotted in Figure 4-2 (e) and (f) for NH_4-N, NO_x-N and COD profiles on day 181. This profiles showed the optimized forms of Figure 4-2(c)) and (d) where extra aeration time were cut down to 10 h and still showed same removal performance. Figure 4-3 (a), (b), (c), and (d)) shows the NH_4-N, NO_x-N and COD profiles for 8 h complete t_C on 200th day. The profile again showed that under controlled condition, the CT could be further reduced to 8 h.



* (Note that 100% DO saturation is equal to 8 mg/L)

Figure 4-3.Typical profiles for NH_4-N, NOx-N and Alkalinity, pH and DO in phase VI over the operated cycle time: (a) NH_4-N and NOx-N profiles (b) NH_4-N and NOx-N, TCOD, sCOD profiles (c) NH_4-N and NOx-N and alkalinity profiles (d) NH_4-N and NOx-N and DO profiles (e) Variation of NH_4-N and TKN (f) Time variation of pH and DO

4.1.3. Removal efficiencies of SBAR and sludge characteristics

4.1.3.1. Organic carbon removal

The composition of the feed synthetic tannery wastewater was kept constant throughout the operation of the reactor. The average concentrations of feed TCOD, TKN and NH_4-N were 5250±750mg/L, 350±13 mg/L and 282±8mg/L respectively. In Phase I, the operating conditions were mainly maintained for biomass acclimatization and development of nitrifiying biomass. During the whole study, the daily COD and nitrogen removal efficiencies and effluent characteristics were evaluated. The organic loading rates (OLR) were increased during the course of operation by decreasing the HRT. High removal efficiencies were obtained in all the loading rates as observed from Figure 4-1 (b). At a maximum OLR of 7.875 kg COD/ m^3.d, the reactor showed very good removal in terms of COD. The results showed that the overall the COD removal was not affected by increasing the OLR of the system.

Since the VER was 50%, the theoretical initial COD was taken as average of the sum of effluent COD from the previous cycle and the feed COD. The actual initial COD in the mixed liquor was however lower than the theoretical value as there is rapid uptake of COD by cells due to starvation and absorption to cell surface. The daily COD removal efficiencies of the reactor, except for very few days of the initial periods of the reactor operation, were very high and showed 95±1.8% average COD removal efficiency. The average effluent total COD (TCOD) value was 158±31mg/L. These data confirmed that the reactor provided consistently high COD removal efficiency.

In phases I and II, the DO in the reactor was maintained 2 mg/L, the reactor showed an increase in the COD removal efficiency and reached a maximum in few days of operation. During the active partial nitrification to nitrite, the reactor showed very low effluent COD as nitrification and

denitrification played a role in COD removal. After day 129, the reactor DO was maintained at 1mg/L and SRT of 20 d, the change of DO did not affect the COD removal.

Variation of the reactor COD with respect to time in 8 h complete t_C operated at SRT of 20 d is shown in Figure 4-3 (b), and showed a sudden drop of COD during the first 5 min of aeration after feeding fresh feed. The COD removal rate was very high in the first two hours. Denitrification would also play a major role during the initial period of the reactor operation as DO demand would be very high. Thus, during this time, DO in the bulk liquid was made very low (0-0.4 mg/L). This condition creates favorable environment for denitrifiers to utilize the available NO_X and organic carbon for denitrification. In fact, the lactose in milk solution is complex substrate (higher molecular weight) and which must first be hydrolyzed by extracellular metabolism before utilization by the bacteria. Therefore, the oxidation rate of lactose would be less than simpler molecule such as glucose and acetate. The remaining COD in the effluent was mainly composed of soluble refractory substance from the feed synthetic tannery wastewater itself and from the refractory substances released from starvation by the microorganisms themselves in the sludge.

4.1.3.2. Ammonium removal by nitrification

The feed wastewater contains both ORG-N and NH_4-N. The ORG-N is readily converted to NH_4-N by ammonification process contributing to the total ammonia in the system [178]. Ammonium removal happened due to assimilation by biomass, due to nitrification by nitrifiers and anaerobic oxidation by anammox bacteria as the reactor was operated at low dissolved oxygen concentration. The assimilation of ammonium by hetrotrophs happens in preference to nitrification and anammox conversion. The addition of organic matter provokes the growth of hetrotrophs and inhibits ammonium oxidation. This could be due to the mass transfer limitation of ammonium from bulk liquid to the cells of nitrifieres as a result of the crowded cells of hetrotrophs [179].

Figure 4-3 (e) shows the NH_4-N and TKN profiles in the 8 h optimum t_C. The result showed that the TKN: NH_4-N ratio is greater than one during the initial 2 h; closer and maintained at one during the next 4 h; and become above one during the next two hours. This indicates that the initial organic nitrogen is getting converted to ammoniacal nitrogen during the initial 2 h. The effluent ratio signifies the discharge of biomass contributing to higher TKN.

Figure 4-2 (a) shows the typical ammonium conversion profile in phase I. This graph shows that ammonium was being oxidized mainly to nitrite and only small amount of nitrate at the end of the 18 h t_C. Figure 4-2 (c) shows the ammonium conversion profile for phase III; Phase IV- VI also have similar profile as phase III; Figure.4-2 (f) and Figure 4-3 (c) show the ammonium profile for phase IV and VI respectively. These profiles clearly showed how the SRT maintained in the reactor affects the nitrification efficiency and characteristics of nitrogen species present at the end of the SBR cycle time. As reported in literature, low SRT conditions reduced nitrification efficiency and increase N_2O emission rate in oxic reactors [180]. The nitrite accumulation rate (NAR) also increased with decrease in SRT [181]. Increased SRT, generally enhanced the nitrification efficiency and reduce nitrous oxide N_2O emission [182] Figure 4-3 (c) and (d) show the nitrification kinetics with alkalinity and DO profiles, respectively, when the reactor was operated at 20 d SRT and 8 h total t_C. The reactor was operated within the pH range of 7.25-7.30 by continuously feeding with 0.25 M $NaHCO_3$ and 0.25 M HCl and maximum DO value of 1 mg/L by automatic on-off control of the air pump.

Nitrogen loading rate (NLR) from 0.236 to 0.531 kg NH_4-N/ m^3.d was gradually increased, during the course of operation; (refer Figure 4-1 (a)). It was observed that the OLR and NLR variation had little impact on ammonium removal within the range of values studied. However, the results showed that NH_4-N removal was highly affected by the process aeration, DO and alkalinity as it

could be seen during phase II. After Phase II, the process was restored back to good performance. A total t_C of 8 h was found to be the optimum for effective removal of ammonium at 20 d SRT.

4.1.3.3. Total Nitrogen removal

Analysis of the removal kinetics in the reactor showed that nitrogen was simultaneously removed with organic carbon at the start of every cycle due to the instant very high COD exerted and the presence of NO_x from previous cycle. The result in Figure 4-3 (d) shows the DO profile in the reactor during the initial 2 h and DO was below 0.4 mg/L and simultaneous removal of nitrogen and organic carbon were found mainly to happen due to denitrification. This process significantly reduces the oxygen requirement for COD reduction in aerobic reactor as the NO_x-N was used instead of DO as terminal electron acceptor [183, 184]. The process of denitrification was supported by the increase in alkalinity and pH as shown in Figure 4-3 (c) and Figure 4-3 (f), respectively, during the start of aeration. Figure 4-3 (c) also shows the alkalinity profile where the alkalinity was increased to 500 mg/L as $CaCO_3$ in the first 2 h and later decreased to 300 mg/L as $CaCO_3$ at the end of the 8 h t_C. The increase in alkalinity at the start of the cycle could be due to denitrification while the decrease in alkalinity at the end of the cycle was due to nitrification [67].

It has been reported that the wastewater COD/N ratio highly affect the total nitrogen removal efficiency and lack of suitable organic substrate may result in poor denitrification [185]. Further it has been reported that 3.3–5.0 g of COD/NO_3-N ratio is required to achieve complete denitrification [186]. The production of some alkalinity was noticed during pre-denitrification which resulted in a net savings in alkalinity requirements for the nitrification reaction [187]. The organic matter was not the issue in the total nitrogen removal as the feed COD: TKN ratio was 15. The total nitrogen removal efficiency was affected by the nitrification efficiency, SRT and HRT maintained.

Figure 4-3 (c) and (d) also showed that ammonium oxidation started at the early stage where the DO was very less (DO<0.2 mg/L); where DO>0.4 mg/L, NO_x-N accumulation started after 2h of operation; and between DO 0.2 mg/L - 0.4 mg/L where any NO_x-N produced was denitrified. In every kinetic data, it has been observed that the NO_3-N was accumulated after the COD was removed and thereby favoring the C/N ratio for anammox to be active. This nitrate accumulation could be due to the action of Anammox bacteria. It has been reported by Anjali and Sabumon [148, 188] that anammox could be maintained in the presence of COD at lower C/N ratios.

Figure 4-4 (a) shows the total influent and effluent nitrogen profile with associated removal efficiency profile. The result showed that high nitrogen removal efficiency in phase (III-VI) and the average removal efficiency increased with increase in operating t_C at constant SRT of 20 d (Figure 4-4 (b)). At 20 d SRT, total nitrogen removal was 43.10±1.7%, 53.74±8.8%, 58.05±10.5% and 69.35±3.8% for t_C values of 8, 10, 12, 18h respectively. The total nitrogen removal showed a linear correlation with the t_C and removal increased with increasing t_C and removal was affected at very low t_C.

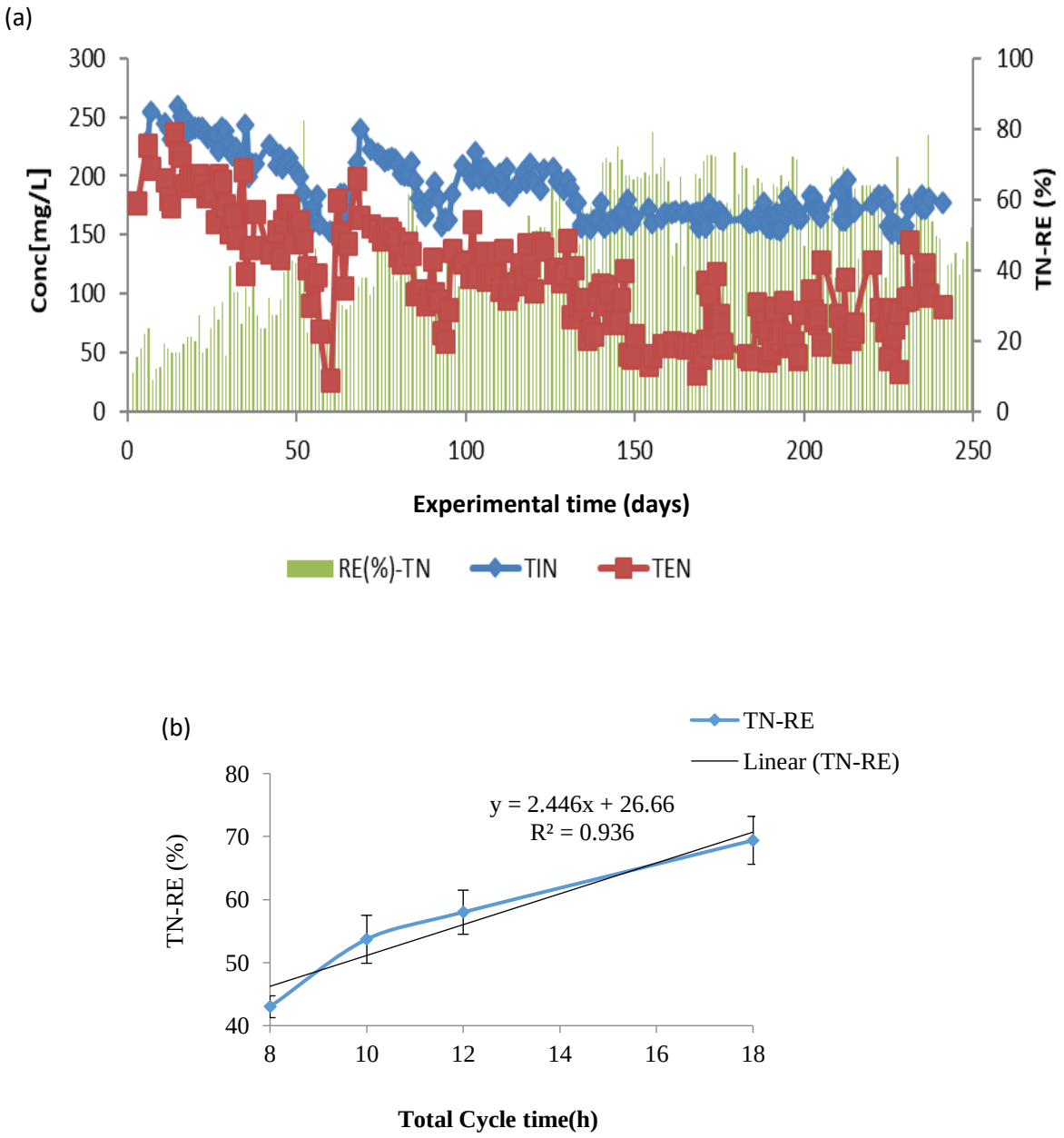


Figure 4-4. Daily nitrogen removal profile and respective cycle times average total nitrogen removal: (a) Daily initial total nitrogen and effluent total nitrogen with corresponding removal efficiency (b) Average removal efficiency for phase (III-VI) at different operating $\boldsymbol{t_C}$

4.1.4. Rate of Nitrification and Denitrification

Evaluation of the removal process showed that denitrification was happening during the anoxic feeding and initial 5 minutes of aeration period and nitrification was occurring at a later stage after the COD is depleted and the dissolved oxygen level was close to 0.5 mg/L. The initial NO_x-N profile in Figure 4-3 (b) is almost zero while the values rise up as time progresses and reach a higher value at the end of the aeration time which could be by the action of either NOBs or anammox; the same is denitrified at the start of consecutive cycles. It was observed that nitrification and denitrification rates were not uniform in one complete cycle; nitrification rate was increasing whereas the denitrification rate was decreasing. This is because COD was high at the start of every cycle and the starvation of heterotrophs exert high dissolved oxygen demand as a result the DO in the bulk liquid was very low. The COD and dissolved oxygen level at the start of every cycle is favorable for denitrifiers and they consume the available NO_x-N as terminal electron acceptor and reduce NO_x to nitrogen gas. When the organic level is depleted the nitrifiers gets enough oxygen for their metabolic activity and anammox harboured inside the flocs gets opportunity for the action.

4.1.5. Profiles of DO, pH and alkalinity

Typical profiles of DO and pH, in the reactor in cycle (phase VI), are given in Figure 4-3 (f). These variations correspond well with the change of organic carbon and nitrogen compositions. During the filling period, the organic carbon oxidation rate was very high and large quantity of oxygen was required. Even though the air supply rate was as high as 3.5 $L.min^{-1}$., it was still much less than the oxygen utilization rate of the heterotrophic organisms, which caused DO to decline to a very low level in the reactor (nearly zero). Correspondingly, ORP in the reactor was also at a very low level, which favored denitrification. Therefore, denitrification took place together with aerobic

organic carbon oxidation during the filling period. Since denitrification is an alkalinity-producing process, the pH in the reactor increased at the same time. After the end of filling, DO in the reactor started to increase slowly and reached the set point (1 mg/L) in 4 h and was then maintained. ORP also increased gradually with the reaction time (result not shown). Nitrification started after 2 h from the start of aeration which led to the decline of pH in the reactor and alkali consumption. However, since the SBAR is pH controlled, the drop in pH is compensated by continuous addition of alkali. Figure 4-3 (c) also shows the alkalinity profile where the alkalinity was increased to 500 mg/L as $CaCO_3$ in the first 2 h and later decreased to 300 mg/L as $CaCO_3$ at the end of the 8 h cycle time. The increase in alkalinity at the start of the cycle was due to denitrification while the decrease in alkalinity at the end of the cycle was due to nitrification.

4.1.6. Sludge production

The excess sludge was discharged in two ways, either at the end of the reaction time or at the beginning of the filling time. In phase I and II, 500 mL of mixed liquor was removed from the reactor per day and in phase (III-VI), 250 mL sludge was removed. The average MLSS in the reactor was maintained at 3.5 - 4 g/L for phase I and II and 10-11 g/L in Phase (III-VI). The average sludge production rate were 0.151g MLVSS/g COD.d for phase I and II compared to 0.126 g MLVSS/g .d for phase (III-VI). Microorganisms utilize oxygen as an electron acceptor under high DO, but switch to nitrate under low DO. It has been theoretically and experimentally proven that the biomass growth rate with nitrate respiration is much lower than that with oxygen respiration. Therefore, the sludge production rate under high DO is certainly higher than that under low DO. Lower sludge production means lower sludge disposal cost, which is another benefit of the low DO process.

4.1.7. Sludge Activity

The sludge possessed very good settling property and high activity in both organic carbon oxidation and ammonium nitrification, even though the DO in the reactor was operated at low level. For organic carbon, COD utilization rate increased with organic loading rates. For ammonium nitrification, the oxidation rates decreased as usual with the increased organic loading rates. This phenomenon is usually explained by the competition for oxygen between heterotrophic bacteria and nitrifiying bacteria. Similarly, the process could be well explained in our case as the COD in the reactor was very high level as shown in Figure 4-3 (b). Since the organic loading was very high during the initial period of the cycle, the COD utilization rate was very high and as a result the DO demand was very high. Competition for oxygen between the two groups of bacteria was so high as a result there was practically no ammonium oxidation during the initial aeration phase. Therefore, it is possible to say that the decrease of nitrification activity during this period was due to competition for oxygen. In addition, there is a possibility of decrease of the fraction of nitrifying bacteria in the mixture. Compared with organic carbon oxidation bacteria, nitrifying bacteria grow more slowly [189] when organic loading rates were increased, the percentage of nitrifying oxidizers in the sludge would certainly decline and this would result in a lower nitrification activity.

Nitrification is affected by DO concentration [190] and nitrite oxidizers are strongly inhibited by low DO in a continuous system with no sludge return [179]. The effect of low dissolved oxygen concentration and organic loading rate using the substrate acetate and glucose has been studied [179, 191]. However, the substrate and the loading rates for both nitrogen and organic are different and much higher in this experiment. The experimental data showed that nitrifying bacteria could accommodate low DO concentration and relatively high organic loading rate and performed well.

4.2. Microbial abundance and diversity

Microbial community analysis of the initial seed CETP sludge and the reactor sludge sample taken at the end of phase VI was done by whole metagenom 16s rRNA sequencing using illuminia platform. Genomic DNA isolated from these samples were having concentrations of 680.2ng/µl and 1028.8ng/µl for CETP and SBAR samples respectively. The quality was checked for both samples by nano drop measurement. The nanodrop reading of the isolated DNA showed an ODA260/280 value of 1.85 and 1.88 for SBAR and CETP samples respectively. The corresponding ODA260/230 was 1.77 and 1.84 respectively. So in all cases, the isolated DNA pass the quality check (QC).

The QC passed DNA samples were used for first amplicon generation and targeting V3-V4 region with specific primer set followed by library preparation using Nextera XT Index Kit (Illumina inc.) preparation kit. The mean of the library fragment size distribution was 622bp and 618bp for CETP and SBAR samples respectively. The library was sequenced on MiSeq using 2 x 300bp chemistry to generate ~1,00,000 reads/sample.

The raw sequence data was processed using OTU' and resulted in 529 OTU's (183Mb) for SBAR sample and 845 OTU's (135Mbs) for CETP sludge sample. These data were quantified and interpreted to see the relative abundance of the bacteria population for the inoculum and reactor sludge samples at different taxonomic levels. The study gives an understanding of the bacteria population diversity and the change as a result of the process condition and control. The results of these studies were analyzed at different taxonomic levels in terms of relative abundance of bacteria groups, the study in detail summarizes on dominant bacteria group at different levels and shown in Table 4-1 and Table 4-2. Table 4-1 shows the first most dominant bacteria groups of the inoculum and SBAR sludge sample respectively at the respective taxonomic levels. Table 4-2

shows the comparative diversity of a nitrifier (*Nitrosomonadaceae family*) and anammox bacteria (*Planctomycetaceae family)* for the two samples. The result at phylum level showed that the dominant bacteria in both samples were *proteobaceria (>80%)* as shown in Table 4-1. Besides, denitrifier populations such as the genera of *Pseudomonas*, *Thauera*, *Paracoccus*, *Hyphomicrobium*, and *Azoarcus*, were identified, these findings were comparable with reported results[120]. It is also interesting to observe that the SBAR sludge had more nitrifiers than CETP sludge (1.35% as compared to 0.005% in inoculum/CETP sample). However compared to the other bacteria population, the relative diversity of nitrifiers is less yet giving very good removal efficiency. More importantly, the SBAR reactor sample showed higher *planctomycetes* group (0.5% as compared to 0.026% in inoculum/CETP sludge sample) as shown in Table 4-2, which shows the process condition in the SBAR was also favorable for growth of anammox bacteria population. These kinds of bacteria consortium also play role in the total nitrogen removal process under anaerobic condition when both ammonium and nitrite species are available in the reactor. In addition, significant population of *bacteriodetes* were found in both samples. *Chlorobi* (which includes green sulphur bacteria (*Chlorobiaceae*) and *Ignavibacteriaceae*) were also detected in SBAR sludge samples. The observed Shannon alpha diversity index was 5.23 and 4.42 for CETP and SBAR samples respectively, signifying that the process control in SBAR caused selection of certain bacteria.

Table 4-1.Comparison of dominant bacteria groups between inoculum/CETP and SBAR sludge samples

Sample	Inoculum/ CETP Sludge	SBAR Reactor Sludge
Phylum	*Proteobacteria* (89.22%)	*Proteobacteria* (83.96%)
Class	*Gamma proteobacteria* (72.29%)	*Gamma proteobacteria* (64.67%)
Order	*Aeromonadales* (34.22%)	*Xanthomonadales* (64.03%)
Family	*Aeromonadaceae*34.22%)	*Xanthomonadaceae*(64.03)
Genus	Unclassified-genus from *Aeromonadaceae* family (34.19%)	*Thermomonas* (21.82%)
Species	Unclassified-species from *Aeromonadaceae* family (34.19%)	Unclassified-species from *Thermomonas* family (21.79)

Table 4-2 Comparative diversity of Nitrifiers and Anammox bacteria

Kingdom	Phylum	Class	Order	Family	Genus	Species	CETP	SBAR
k__Bacteria	p__*Proteobacteria*	c__*Betaproteobacteria*	o__*Nitrosomonadales*	f__*Nitrosomonadaceae*	Unclassified	Unclassified	0.005 (%)	1.35 (%)
k__Bacteria	p__*Planctomycetes*	c__*Planctomycetia*	o__*Planctomycetales*	f__*Planctomycetaceae*	g__*Planctomyces*	Unclassified	0.026 (%)	0.5 (%)

4.3. Aerobic granulation and performance of the granular SBAR

4.3.1. Granule formation

The reactor operating conditions were maintained as described in Table 3-3. The reactor was operated to study the granule formation and performance using synthetic tannery wastewater. The parameter selected to induce granulation process was stepwise reduction of the settling time**.** Figure 4-5 (a) describes the operating days versus the operated settling time in the reactor and the figure also clearly shows the stepwise declining of settling time from 30 min to 3 min. The reactor sludge was initially flocculent which showed excellent removal for organic carbon and nitrogen. The granulation strategy followed was to maintain the simultaneous removal of organic carbon and nitrogen by preventing the excessive washout of slow growing nitrifiers from the system and improve the biomass density and settleability of the sludge. The transition from flocculent to granular sludge was achieved in few days maintaining the required hydrodynamic pressure needed to form granules. The granule formation was monitored by visual observation, light microscopy and measurement of the SVI and settling velocity of the sludge granules at different operating days. The results clearly showed that the formation of aerobic granules was achieved with size in the range of 1.8-3.2 mm at SVI of 16-20 mL/g, granular biomass density of 6.1 g/L and settling velocity in the range of 30 - 40 m/h, without affecting the nitrification efficiency. The performance of the reactor in terms of COD and nitrogen removal was monitored and the results demonstrated that the selected parameter has resulted in formation of granules without affecting the removal efficiency of the system.

To prevent severe washout and at the same time to induce granulation, an optimized strategy was followed by controlling the biomass discharge ratio and reducing the settling time to a lower value until the end of the experiment. The biomass loss during the first 30 days of operation was not significant indicating that the seed biomass had a good settling property. The average suspended solids concentration in the effluent during this period was only 0.102 g/L as shown in Figure 4-5 (b). After the 30^{th} day, the settling time was further reduced sequentially from 10 min down to 5

min until the 40th day where there was significant biomass loss Figure 4-5 (b). The TSS in the effluent increased and reached to 1.024 g/L on the 33rd day. At the end of the 40th day, it was observed that the MLSS has further increased to 8,853 mg/L. The settling time was then further reduced to 3 min at the end of 60th day and maintained until the end of the experiment. However, the MLSS was not much affected and the biomass in the reactor has increased to 10,571 mg/L and showed excellent settling property until the end of the experiment. The average TSS in the effluent, after the settling time was decreased to 3 min, was only 0.025 g/L signifying a higher quality effluent compared to that with flocculent sludge.

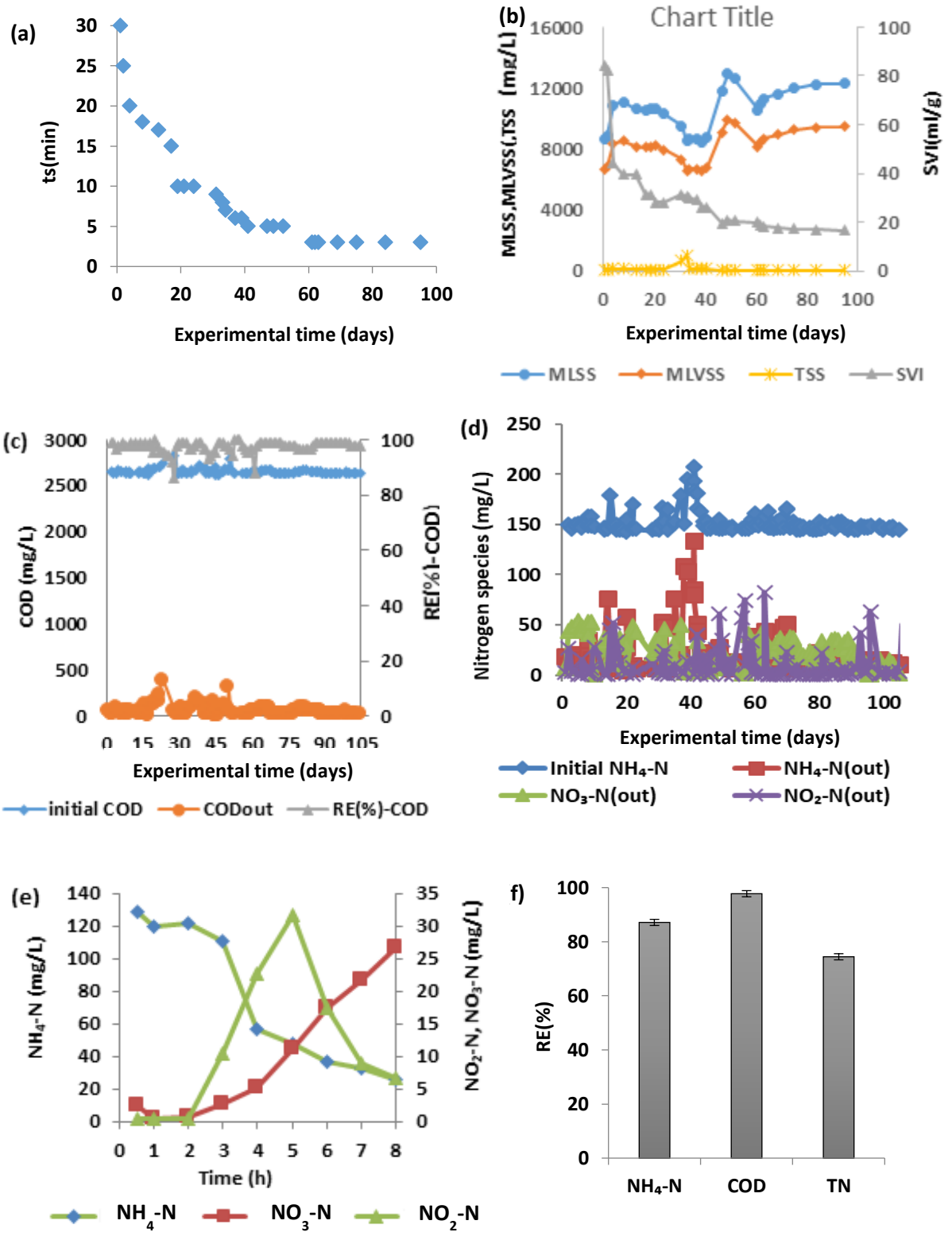


Figure 4-5 Operated settling time of the SBAR and performance of the reactor during granulation process: (a) Profile of settling time maintained in the SBAR reactor over the operating days (b) Reactor MLSS, effluent TSS and SVI of the sludge profile (c) Initial mixed liquor and treated effluent COD and the corresponding removal efficiency (d) Profile of initial mixed liquor NH_4-N and effluent NH_4-N, NO_2-N and NO_3-N over operating days (e) Typical profile of nitrogen species in one complete cycle time (f) Average removal efficiency for COD, NH_4-N and total nitrogen (TN).

The SVI profile in Figure 4-5 (b) also showed that it is significantly reduced from the start of the experiment and reached to 16 mL/g TSS at the end of the experiment. In the meantime, the MLSS increased after the 40th day and reached a maximum of 12,918 mg/L on 49th day and thereafter it was maintained in the same range till the end of 95th day. The SVI result confirmed the formation of granules, as the SVI value kept on decreasing as the MLSS was increasing.

Granulation was observed without severe washout, the TSS of treated effluent was seen increasing between the 30th and 40th day and it was found to be at a minimum during the remaining experimental period as shown in Figure 4-5(b). The sludge discharge ratio (the ratio of biomass escaping the effluent discharge) during the startup of the granulation process was in the range 0.01-0.05, which is in agreement with the reported result of the modeling and simulation study [116].

Morphological changes of the sludge and the formation of granules were observed by light microscopy and SEM at different operating days of the reactor (Figure 4-6). Granules were observed at the 10th day of the experiment when the settling time was reduced to 10 min are shown in Figure 4-6 (b) and Figure 4-6 (f). Additional sampling were carried out on 30th and 90th day of the experiment to study the morphology changes and the images taken at different periods of experiments are shown in Figure 4-6(c), Figure 4-6(d), Figure 4-6(e) and Figure 4-6 (g) respectively. The image showed more dominant granules on the 30th day than the 10th day and granules dominance increase even more on the 90th day. The images clearly showed the changes as a result of the applied pressure (sequentially reducing settling time) on the appearance and maturation of granules. The results indicated that the strategy followed helped to achieve granules formation from flocculent sludge without seriously affecting the removal efficiency.

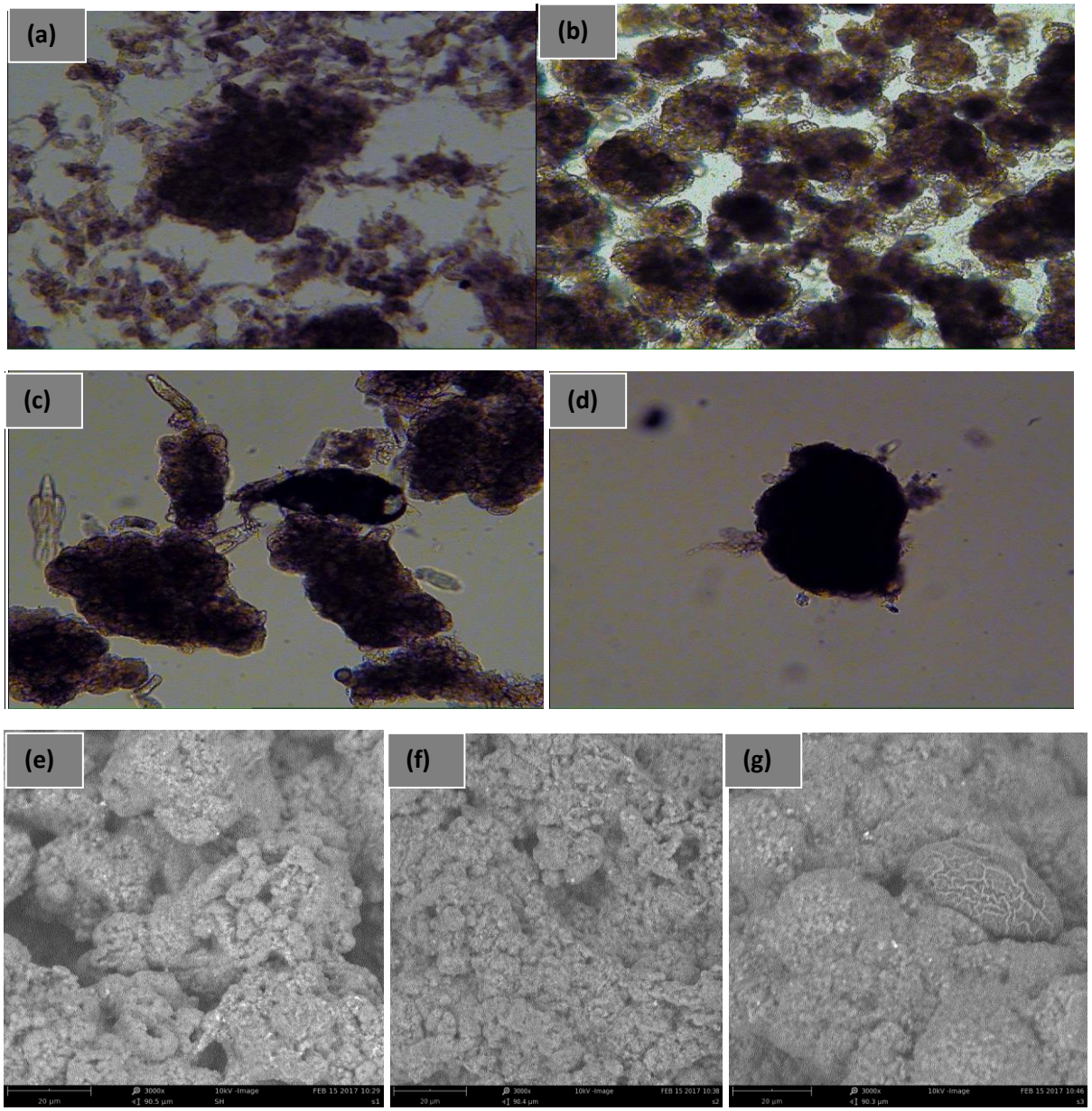


Figure 4-6 Microscopic images (10X magnification) and SEM images (3000X magnification) of sludge samples: a) Microscopic image on first day; (b) Microscopic image on 10th day; (c) Microscopic image on 30th day; (d) Microscopic image on 90th day (e) SEM image on first day (f) SEM image 10^{th} day (g) SEM image 90^{th} day.

Previous reported works have been dealing with granulation with constant low settling time from the start of the process and this resulted in significant loss of biomass from the reactor [192]. Recent studies suggested gradually decreasing the settling time to prevent severe washout of biomass during the startup of the granulation process [117]. Modeling studies suggested an optimized strategy for controlling severe washout of biomass and yet helping granule formation by controlling the biomass discharge rate from the reactor [116]. Earlier investigation reported

that gradually decreasing the settling time over a long time (95 days to reach 5 min) helped to improve the settling property of the sludge but failed to form granules [193]. However, in this study, the gradual decrease to 5 min settling time was achieved in 40 days and the granules formed had a settling velocity comparable to reported results. In another study, the settling time was reduced gradually and resulted in formation of granules and also demonstrated the rate of gradual decline in settling time intensely affects the nitrifiers community [117]. Therefore, reduction in settling time should be optimum to prevent severe washout and at the same time help the granule formation.

4.2. Biomass yield

Compared to activated sludge system, aerobic granular sludge system are characterized with low biomass sludge production rate. The low sludge production rate are the result of relatively high sludge age achieved by aerobic granular biomass[162]. The SBAR reactor was completely granular after the 60th day and the aerobic granular sludge reached steady state. Steady state was maintained at SRT of 20 days. The corresponding observed specific biomass growth rate (μ_{obs}), the observed biomass yield (Y_{obs}), observed substrate utilization rate of stable granules (q_{obs}) were found to be, 0.05/d, 0.017 mg VSS / mg COD, 0.335 mg COD / mg VSS /d respectively. The theoretical yield (Y) and the specific biomass decay rate values were calculated and found to be 0.145 mg VSS / mg COD and 0.05/d respectively. The theoretical yield is much higher than the observed yield which clearly indicate that the experimental granular sludge system produce significantly less sludge.

4.3.2. COD removal performance

The COD profile for initial mixed liquor in the SBAR reactor and effluent samples from SBAR reactor are shown in Figure 4-5 (c)**.** About 70% of the COD was removed during the initial 30 min of aeration. The COD removal efficiency (the percentage difference between inlet and outlet COD concentrations) was very high from the start of the study (98%) and the removal efficiency was not significantly affected by the gradual decrease in settling time until the 20th day. After the

20th day, the settling time was kept at 10 min until the 30th day which resulted in slight reduction of COD removal to 86% on the 23rd day. After the 23rd day, the COD removal efficiency was restored back to high level (>97%) and maintained until the end of the study. The reason for the very high COD removal efficiency even at the steepest and lowest settling time was due to the higher HRT maintained and the improvement of the COD removal efficiency of the sludge biomass due to aggregation and increase in density. Between the 30th and 40th day, the settling time was brought down to 5 min and as a result, there was a significant loss in biomass as shown in Fig. 2 (b). The overall average COD removal efficiency was 97.6±1.9% which resulted in the effluent COD value in the range of (32-128 mg/L).

4.3.3. Nitrogen removal performance

It has been well reported that NH_4-N is mainly removed by assimilation in cells and by autotrophic nitrification. Nitrification resulted in conversion of ammonium to nitrite and nitrate which are the result of the activity of AOB and NOB bacteria involved in the process. Compared to the COD removal efficiency, the ammonium removal efficiency was significantly affected due to the reduction in settling time but the stepwise reduction helped to retain the removal efficiency. At some particular days like 14th, 20th, 31st, (38th - 42nd), 69th and 70th day, the removal was affected as seen from Figure 4-5 (d). However, the calculated NH_4-N removal efficiency (the percentage difference between inlet and out let NH_4-N concentrations) reduction was not severe and it was easily recovered. Nitrification was observed throughout the operating days and as a result nitrate and nitrite was found in the treated effluent Figure 4-5 (d). The reactor showed an average NH_4-N removal efficiency of 87.1±1.2% resulting in effluent having an average NH_4-N, NO_3-N and NO_2-N concentrations of 20±2, 22.7±1.4 and 9.7±1.5 mg/L respectively. Subsequent to the reduction of settling time to 3 min on 60th day, the ammonium removal was improved and the reactor showed stable removal efficiency until the end of the experiment. Table 4-3 shows the comparison between the overall average ammonium, COD and total nitrogen removals after the 60th day. The nitrogen species profile can also be correlated with the activity of AOB and NOB;

the result showed that both bacteria were actively working but during the time where the settling time was sharply reduced the (30^{th} - 40^{th} day), the NOB activity was reduced due to biomass washout and the effluent showed more of NO_2-N than NO_3-N **(**Figure 4-5 (d)).

Table 4-3 Comparison of the average concentration of nitrogenous pollutants in treated effluent and their average removal efficiency

Operating Days	NH_4-N(out)	NO_3-N(out)	NO_2-N(out)	COD(out)	RE(%)-NH_4-N	RE(%)-COD	RE(%)-TN
Day (1-105)	20.0±2	22.7±1.4	9.7±1.5	63.3±5	87.1±1.2	97.6±0.2	74.4±1.1
Day (60-105)	14.8±1.3	20.5±1.6	6.6±2.1	50.9±3.5	90.1±0.8	98.1±0.1	79.0±1.4

***** All values except the percentage removal are in mg/L

Ammonium and nitrate removals showed a linear correlation while nitrite showed parabolic correlation Figure 4-5 (e). From the Figure 4-5 (e), it is observed that the NO_x profile for both NO_2-N and NO_3-N showed declining to zero value during the first 2 h of the reactor operation. At the same time, the NH_4-N profile showed declining trend. This phenomenon indicated that there is NH_4-N conversion during this time, and in parallel NO_X species removal also observed. The DO level during this time was in the range 0.1-0.2 mg/L, which is more suitable for denitrification and annamox process. Therefore, the simultaneous decline in the NH_4-N and NOx species is an indication that there is simultaneous nitrification and denitrification during this time of the reactor operation. Accumulation of NO_x-N started after 2 h when the COD got reduced. The NO_2-N removal picked up after an additional 3 h and declined during the next 3 h of operation; whereas NO_3-N curve showed a linear increase until the end of the cycle time.

The total nitrogen removal was determined based on the initial total nitrogen (TIN) and the effluent total nitrogen (TEN) which was found to be 74.4±1.1% (Figure 4-6 (f)). Higher total nitrogen removal efficiency was observed even when the settling time was reduced to 3 min after the 60^{th} day **(**Table 4-3**)**.

In addition to the above observations, EPS production has also been estimated in the current study, as it had significant role on the physiochemical properties of the microbial consortia to

form aggregates which includes surface charge ie., ionic properties, flocculation, settling and adsorption ability [194]. Though there are different schools of thought reported in literature regarding the EPS role in granule formation [195], it is evidently tacit that granule formation and its stability is predominantly due to the properties of EPS in which hydrophobicity and adhesive tendency plays a major role in aggregating cells together [196, 197]. The cyclic SBAR operation creates feast and famine periods which favours the secretion of EPS from the bacteria cells during famine period [198]. The hydrodynamic shear force created in the SBAR due to mixing could also contribute for the formation of EPS [104]. EPS formation along with the stepwise washout of poorly settling biomass from the reactor could be the main reason for the formation of granules in the SBAR.

Table 4-4 EPS formation and its carbohydrate and protein composition during the operating cycle time

Time (h)	COD (mg/L)	EPS (mg/g)	Carbohydrates (mg/g)	Protein (mg/g)
0	1565	423	181.6	604.3
2	470	418	73.0	469.9
4	320	441	118.9	662.0
6	250	495	139.6	631.5
8	96	523	127.9	651.7

Table 4-4 shows the COD degradation and EPS profiles over the course of cycle time in SBAR. The result clearly showed that about 70% of the COD is utilized during the first 2 h and the EPS starts to increase after 4 h, when the microorganisms get starved due to depletion of available organic matter in the reactor. It was reported that EPS could also serve as carbon source for energy production during the time of starvation of nutrient depletion[199] . During the treatment period, EPS production was mainly due to the cell lysis and secretions released by the bacteria. It was primarily composed of carbohydrates and proteins whereas humic substances are also one of the key component when biological wastewater treatment reactors are concerned because the

components of EPS mostly depend on the sludge origins and extraction methods [194, 200, 201]. In the present study, the observed levels of carbohydrate content in the range of 73-182 mg/g and protein content in the range of 470 – 662 mg/g is comparable with the results reported wherein protein content in the granular sludge is higher when compared to other components of EPS and polysaccharide content is comparatively lower [202]. This condition could be due to the enzymatic activities in the reactor for digestion of the macro molecules where nutrient assimilation takes place [203]. Also studies reported that sludge which has better settling ability possess tightly bound EPS and plays crucial role in the structural stability of aerobic granule [204] which is reflected in the current study with reduction in the settling time from 30 min to 3 min. This EPS makes the aerobic granular sludge with dense and strong microbial consortium and researcher reported that this aerobic granular sludge is suitable for treating the wastewater with high strength of organic load due to the higher biomass retention [205]. This could also be correlated with the current study where the high organic COD loading rate of 6.5 kg/m^3 was treated in SBAR.

4.4. Polymerized Chain Reaction (PCR) study

This study established the presence of selected bacteria genes involved in the nitrogen cycle during the enhanced nitrogen removal period of the SBAR operated at stable performance during the end of the experiment. The result of the PCR study after running the gel confirmed the existence of selected genes involved in the nitrogen cycle, as shown in Figure 4-7. Out of the six different genes selected for testing, three were present (AOB-amoA, nirK and PLA46+Amx820) and three were absent (AOA-amoA, nirS and nosZ) and the details are shown in Table 4-5. The results confirmed the presence of PLA46+Amx820 gene, where a clear band was seen at 820bp. The presence of the gene (PLA46+Amx820), which is related to anammox species, is an indication that under the operated process condition, anammox bacteria existed in the reactor. The gel picture Figure 4-7 also confirmed the presence of AOB-amoA gene with a very clear

band at 491bp. The AOB-amoA gene was found alongside a gene responsible in denitrification (nirK) at 473bp.

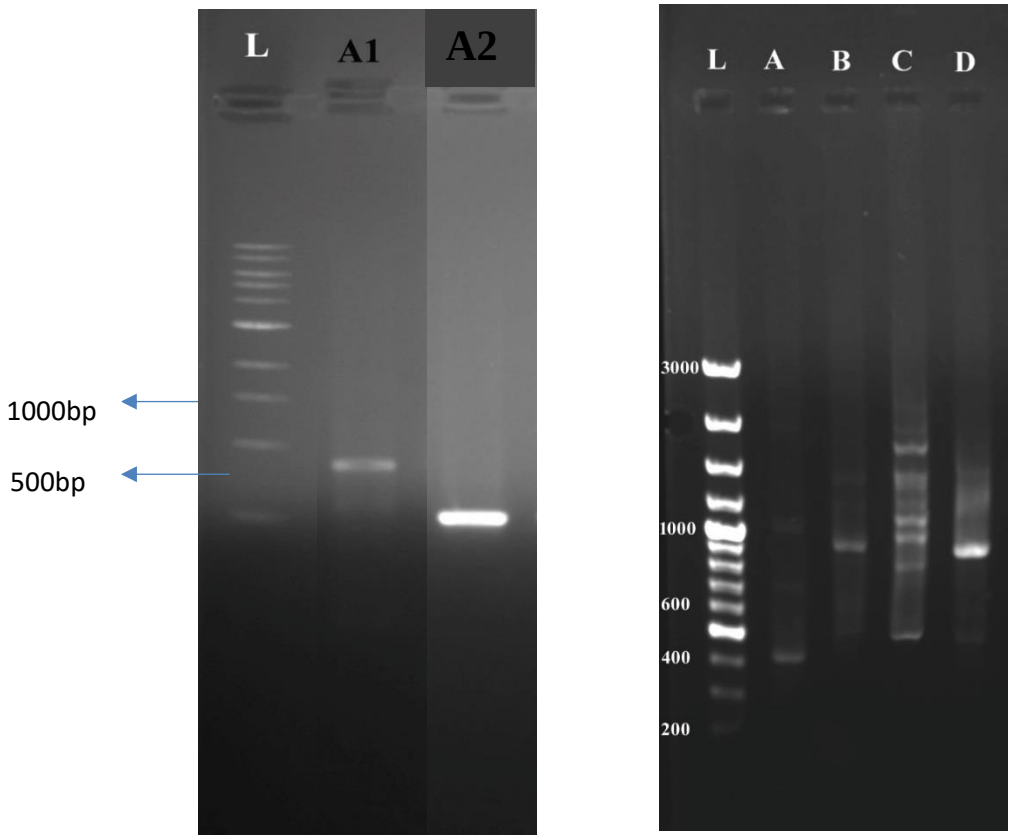


Figure 4-7 PCR amplification of bacteria gene DNA involved in nitrogen removal

Table 4-5 Result of the PCR study

Lane	Target	Sample	Fragment (bp)	Detection
A1	PLA46+Amx820	SBAR	820	Positive
A2	AOB-amoA	SBAR	491	Positive
A	AOA-amoA	SBAR	635	Negative
B	nirS	SBAR	425	Negative
C	nirK	SBAR	473	Positive
D	nosZ	SBAR	267	Negative

4.5. Gene expression study

This study was conducted for selected genes (AOB-amoA and PLA46+Amx820) which play a major role in the rate limiting step of nitrogen removal [206]. The targeted gene expression levels were normalized to the level of housekeeping gene (16s gene) as internal control. The Ct values of the test samples were calculated and the data was expressed in terms of fold change over control

sample. The normalized expression level of amoA in the control and SBR was 0.01343 and 0.12892 respectively with a P value < 0.05. Similarly normalized expression level of PLA46+AMX820 in the control and SBR was 0.00529 and 0.05830 respectively with P value < 0.05 as shown in Table 4-6. The results revealed that the test samples from the reactor showed upregulation of amoA gene by 9.6 folds and PLA46+AMX820 gene by 11.06 over control sample respectively (Table 4-6). The upregulation of targeted gene amoA and PLA46+AMX820 indicates the increase in population of ammonia oxidizers and anammox bacteria in the SBAR during the treatment of synthetic tannery wastewater. The result of the Ct value (Ct<29) indicates a strong positive reaction suggesting abundant nucleic acid in the sample [207].

Table 4-6.Normalized gen expression of qPCR analysis of the test samples over control sample

S. no	Samples	Ct value		Normalized Expression	Relative Normalized Expression (Fold change over control)	P value	Ct Value		Normalized Expression	Relative Normalized Expression (Fold change over control)	P value
		House keeping Gene 16s	Gene of Interest amoA				House keeping Gene 16s	Gene of Interest PLA46+ AMX820			
1	Control	16.52	22.74	0.01343	1.00	0.009038	15.32	22.89	0.00529	1.00	0.049134
2	SBAR	15.96	18.92	0.12892	9.6		18.94	23.04	0.05830	11.06	

4.6. Anaerobic digestion of excess sludge and partial nitrification of digester supernatant

During biological treatment of nitrogen, soluble nitrogen in the form of (NH_4-N) and organic nitrogen (Org-N) are transformed into various forms of nitrogen (NO_x-N, NO, N_2O and free N_2) and part of this will be used in building up the bacteria cells. Therefore, the excess sludge produced during biological wastewater treatment needs sustainable and cost effective treatment before disposal.

In wastewater treatment plants with anaerobic sludge digestion, 15–20% of the nitrogen load is recirculated to the main stream with the return liquors from dewatering. Separate treatment of this ammonium-rich digester supernatant significantly reduces the nitrogen load of the activated sludge system. Two biological applications are usually applied for nitrogen elimination: (i) classical autotrophic nitrification/heterotrophic denitrification and (ii) partial nitration/ autotrophic anaerobic ammonium oxidation (anammox). With both applications, 85–90% nitrogen removal can be achieved, but there are considerable differences in terms of sustainability and costs. The final gaseous products for heterotrophic denitrification are generally not measured and are assumed to be nitrogen gas (N_2). However, significant nitrous oxide (N_2O) production can occur at elevated nitrite concentrations in the reactor. Denitrification via nitrite instead of nitrate has been promoted in recent years in order to reduce the oxygen and the organic carbon requirements. Obviously this “achievement” turns out to be rather disadvantageous from an overall environmental point of view. On the other hand, no unfavorable intermediates are emitted during anaerobic ammonium oxidation. A cost estimate for both applications demonstrates that partial nitration/Anammox is also more economical than classical nitrification/denitrification. Therefore autotrophic nitrogen elimination should be used in future to treat ammonium-rich sludge liquors [208].

4.6.1. Characteristics of raw sludge

The physico-chemical characteristics of the raw sludge is depicted in Table 4-7. From table 4-7, it is understood that the raw sludge exhibit substantially high amounts of total solids, total volatile solids, Total Kjeldahl Nitrogen and total COD. Among these parameters, it is clearly understood that the TKN content in the sludge is responsible in generating highly significant ammonia content in the anaerobically digested supernatant.

Table 4-7.Characteristics of Raw Sludge from the SBAR.

S/No.	PARAMETERS	RESULTS*
1	Colour	Light-brownish
2	pH	6.4
3	TS	34.9 mg/g
4	VS	27.1 mg/g
5	TCOD	10.9 g/L
6	sCOD	0.8 g/L
7	TKN	5.4 g/L
8	Ammonia	0.06 g/L
9	Total phosphorus	0.8 g/L

*-average of three periodical results.

4.6.2. Performance of Anaerobic Digester

As far as the anaerobic digestion in the preliminary studies is concerned, the biogas production was daily monitored by using manometer (i.e.; 5442, NC2012-5416) Figure 4-8, and is shown in Figure 4-9.

Figure 4-8 Photograph of the Gas Flow Manometer

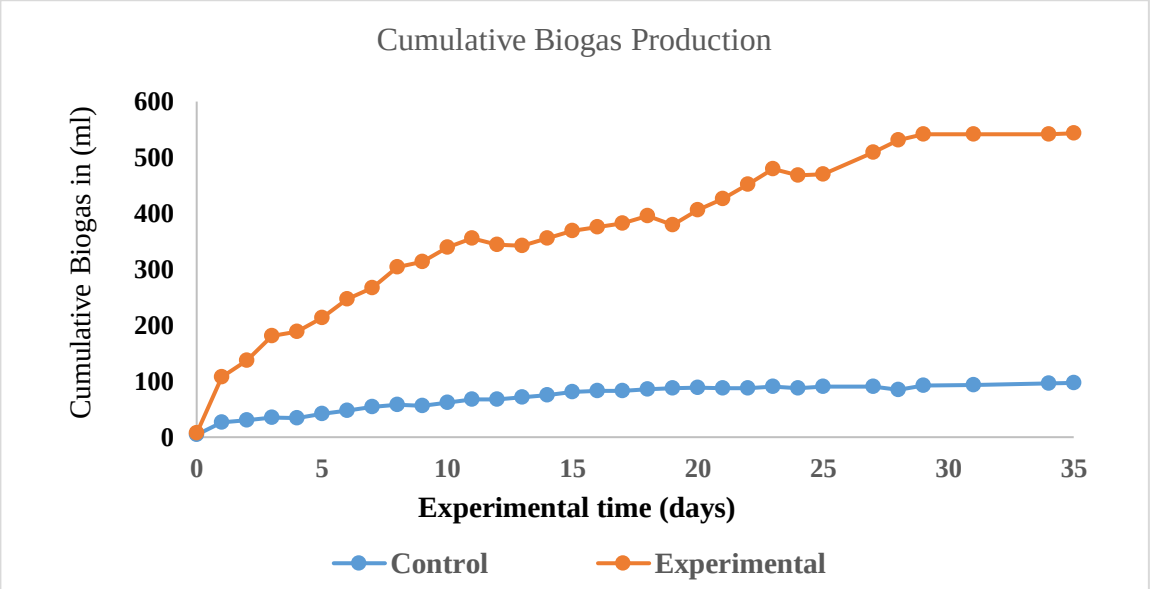


Figure 4-9 Cumulative Biogas Production for control and experimental anaerobic digestors

From Figure 4-9, it is can be seen that the biogas production is continuously increasing from the 1st day to 30th day (i.e., except the significant periodical reduction due to pop-out of the septum of the gas flow meter). But, beyond 30th day of operation of the reactor reached a steady-state. However, the reactor operation was practically stopped at the end of 35 d. The study showed that the specific biogas yield of the excess sludge was 193.2 ml/g of VS.

4.6.3. Characteristics of Digester Supernatant

Table 4-8 shows the characteristics of the digester supernatant. Comparing Table 4-7 and 4-8 it can be observed that the essential parameters required for understanding the need for autotrophic nitritation/Anammox process, particularly the NH_4^+-N increased from 64.4 to 1097 mg/l, TKN is decreased from 5404 to 1100 mg/l and total COD is decreased from 10,933 to 1840 mg/l.. Moreover the enhanced ammonia concentration from 64.4 to 1097 mg/l was directly attributed towards the effective conversion of TKN from 5404 to 1100mg/l (refer table 4-7 and 4-8). Therefore, it can be concluded that the digester supernatant contain substantial amount of NH_4^+-N (i.e., 1097 mg/l).

Table 4-8.Physico-chemical and Biochemical characteristics of anaerobic digester supernatant

S.No.	Parameter	Results*
1	Color	Brownish
2	pH	7.3
3	Total BOD, mg/l	80
4	Soluble BOD, mg/l	60
5	Total COD, mg/l	1840
6	Soluble COD, mg/l	1040
7	Alkalinity, mg/l as $CaCO_3$	7800
8	Ammonia, mg/l as NH_4-N	1097
9	TKN, mg/l	1100.2
10	Total nitrogen, mg/l	1104.0
11	Nitrite, mg/l as NO_2-N	1.9
12	Nitrate, mg/l as NO_3-N	5.01
13	Total phosphorus, mg/l	46.08

(*average of three periodical results)

4.6.4. Performance of the partial nitrification reactor

4.6.4. 1. Start-up of the *Partial Nitrification (*PN) reactor

Anaerobic digestion step helps in the reduction of the organic load and the coexistence of heterotrophic activity within PN reactors, which may negatively affect these autotrophic processes[209]. The nitrogen removal can be carried out by partial nitrification / denitrification or ANAMMOX system. Partial Nitrification of ammonium to nitrite is presented as a possible way to achieve an ANAMMOX influent of the required composition, where ANAMMOX (Anaerobic ammonium oxidation is autotrophic process of nitrogen removal in which ammonium is converted, under anaerobic conditions, directly into nitrogen gas with nitrite as an electron acceptor and in the absence of organic carbon sources[210, 211].

$$NH_4^+ + 1.5O_2 \xrightarrow{AOB} NO_2^- + H_2O + 2H^+;\ \Delta G° = -275\ kJ/M \qquad Eq.(4-1)$$

$$NH_4^+ + 1.31NO_2^- + 0.066HCO_3^- + 0.13H^+ \xrightarrow{Anammox\ bacteria} 1.02N_2 + 0.26\,NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O\ ;\ \Delta G° = -357\ kJ/M \qquad Eq.(4-2)$$

The PN reactor was operated at SRT of 7 days, Cycle time of 8 h, pH of 8, DO of 1 mg/L and temperature of 32^0C for 30 days. Figures 4-10 and Figure 4-11 respectively show the effluent profiles of the respective variables, such as NH_4^+-N, NO_2^--N, NO_3^--N, pH and alkalinity over the start-up period. It is evidenced from Figure 4-10 and 4-11 that the PN reactor was effectively started at the end of 30th day. Figure 4-12 and Figure 4-13 also show the NH_4-N and NOx profile at the end of the 30th day and the graphs signify the partial conversion of the influent NH_4-N into NO_2-N. Similarly, significant sequential reduction in the respective alkalinity and pH values could also substantiated the effective start-up of the PN reactor. Therefore, in view of the criteria discussed above, the start-up of the PN reactor was expected to be in accordance with the standard literatures available elsewhere[212].

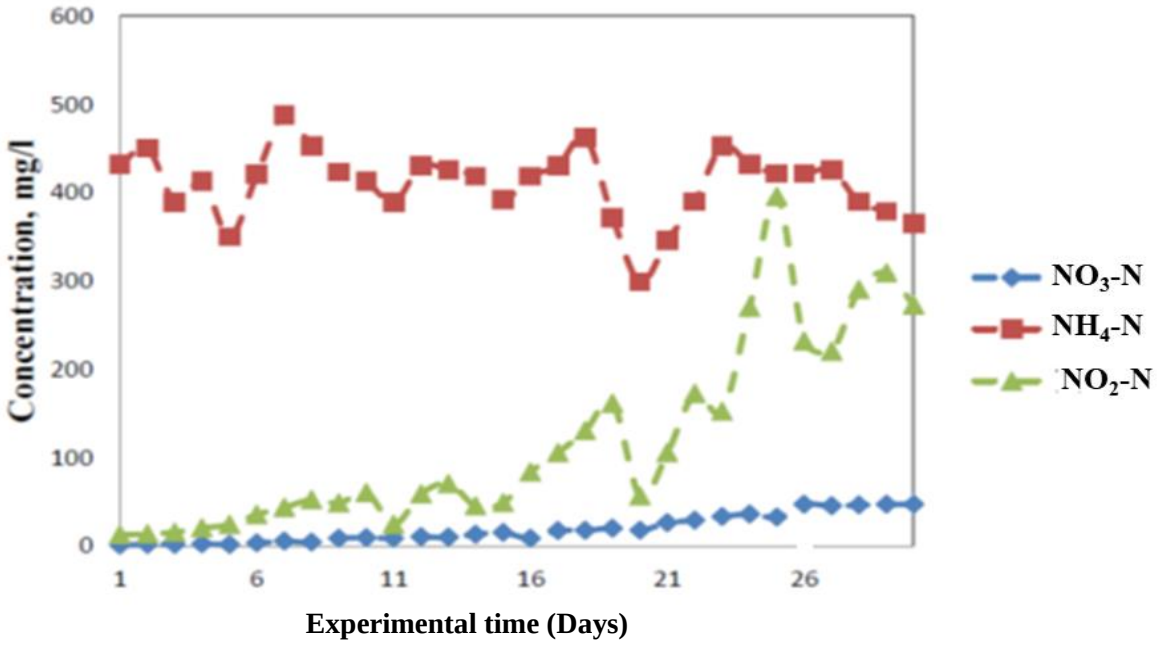


Figure 4.10 Effluent NH_4-N and NO_X-N profile of the PN reactor during the startup period

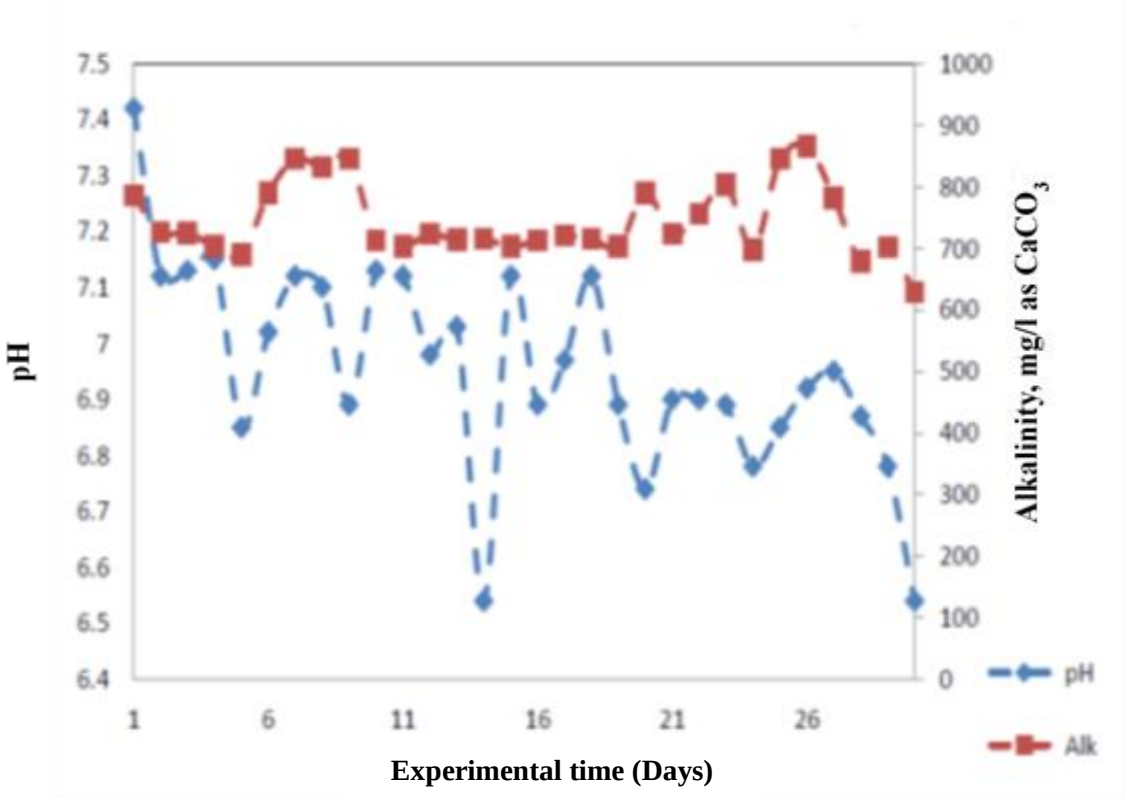


Figure 4-11 Effluent pH and Alkalinity profile during the startup of the PN reactor

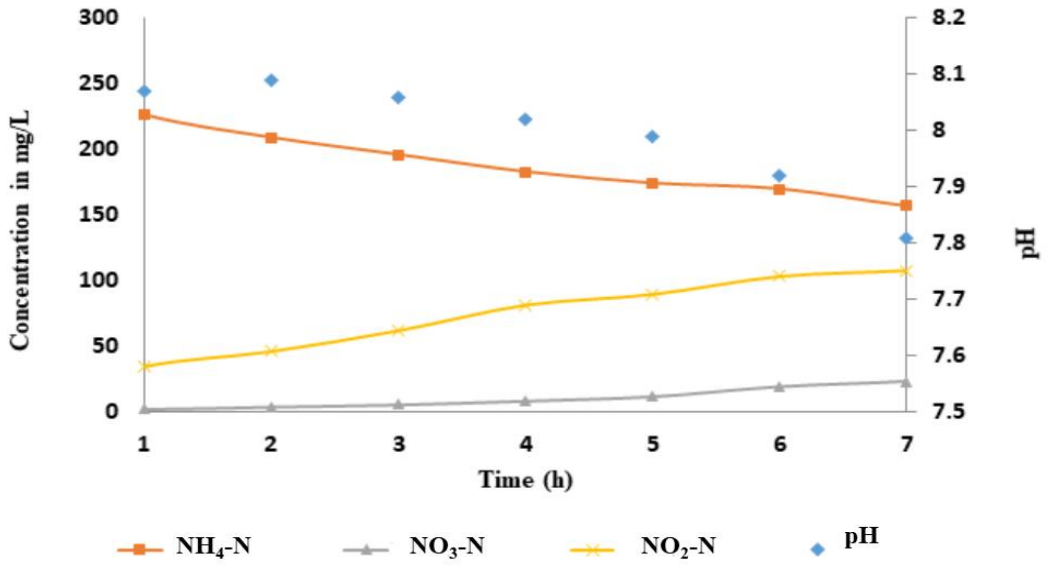


Figure 4-12.The reactor pH, NH_4-N, NO_2-N and NO_3-N profile over an operating cycle time

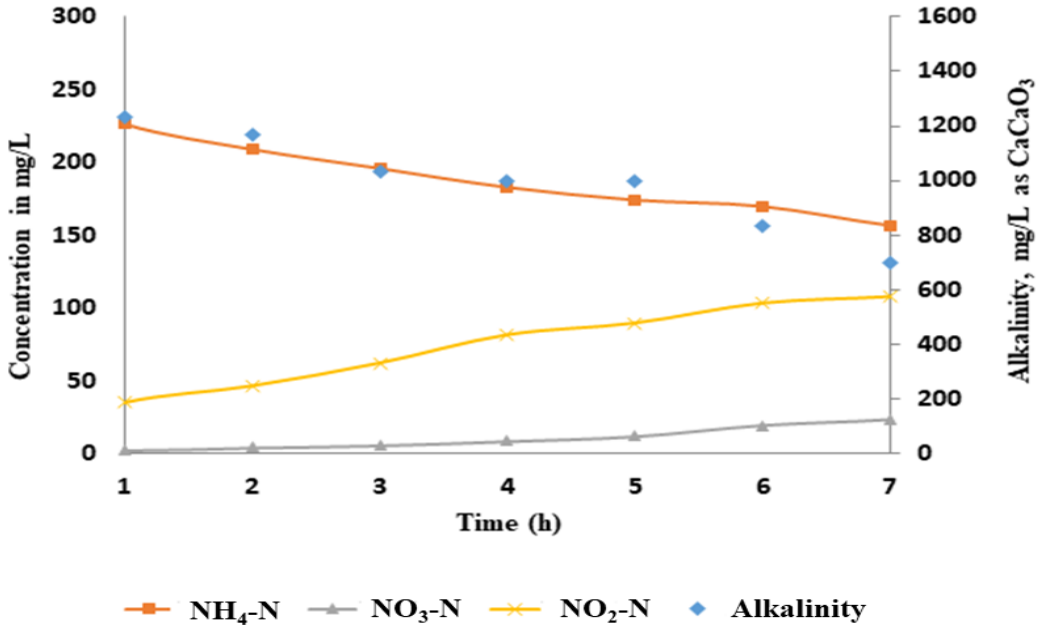


Figure 4-13. Alkalinity, NH_4-N, NO_2-N and NO_3-N profile over an operating cycle time in reactor

4.6.4.2. Performance of the PN reactor

The PN process is greatly influenced by essential parameters like with presence and/or absence of the external carbon source, variations in MLSS concentrations, variations in DO levels, variations in pH values, variations in residence times in each phase, appropriate influent loading rates of NH_4-N and NO_2-N and aeration strategy[213]. Placket-Burman Design of Experiment was used to identify which of these have most significant effect in the performance of the PN reactor. Table 4-9 shows the seven operation factors, design of the experiment and the response variable.

Table 4-9 Placket-Burman Design of experiment for selected operational factors along with the response variable

Run No.	pH	DO (mg/L)	Temperature (^oC)	Cycle time(h)	MLSS (mg/L)	C/N	Aeration strategy	NO_2-N/NH_4-N
1	7.6	4	32	10	10000	3	continuous	0.26
2	6.5	1	20	10	10000	0.5	continuous	0.53
3	6.5	4	32	5	10000	0.5	Intermittent	0.28
4	7.6	1	5	5	10000	3	Intermittent	0.075
5	7.6	1	32	10	3500	0.5	Intermittent	1.03
6	6.5	4	20	10	3500	3	Intermittent	0.776
7	6.5	1	32	5	3500	3	continuous	0.35
8	7.6	4	20	5	3500	0.5	continuous	0.34
9	7.6	4	32	10	10000	3	Intermittent	0.14
10	7.6	4	32	5	3500	0.5	Intermittent	0.84
11	6.5	1	20	10	3500	0.5	continuous	0.33
12	6.5	1	20	5	10000	3	continuous	0.16

The results from the Table 4-9 indicates the possible operational conditions to arrive at the rquried NO_2-N: NH_4-N: ratio suitable for anammox feed. The experimental result shows that it is possible to reach a stable partial nitrification in run 5 with high pH (7.60), low C/N (0.5), high cycle time (10 h), low DO concentration (1mg/L), low MLSS/MLVSS (3500) mg/L), high temperature (32ºC) and intermittent aeration.

Analysis of the data in Minitab 16 software provide the possible identification of the most important factors determining the ratio of NH_4-N and NO_2-N.

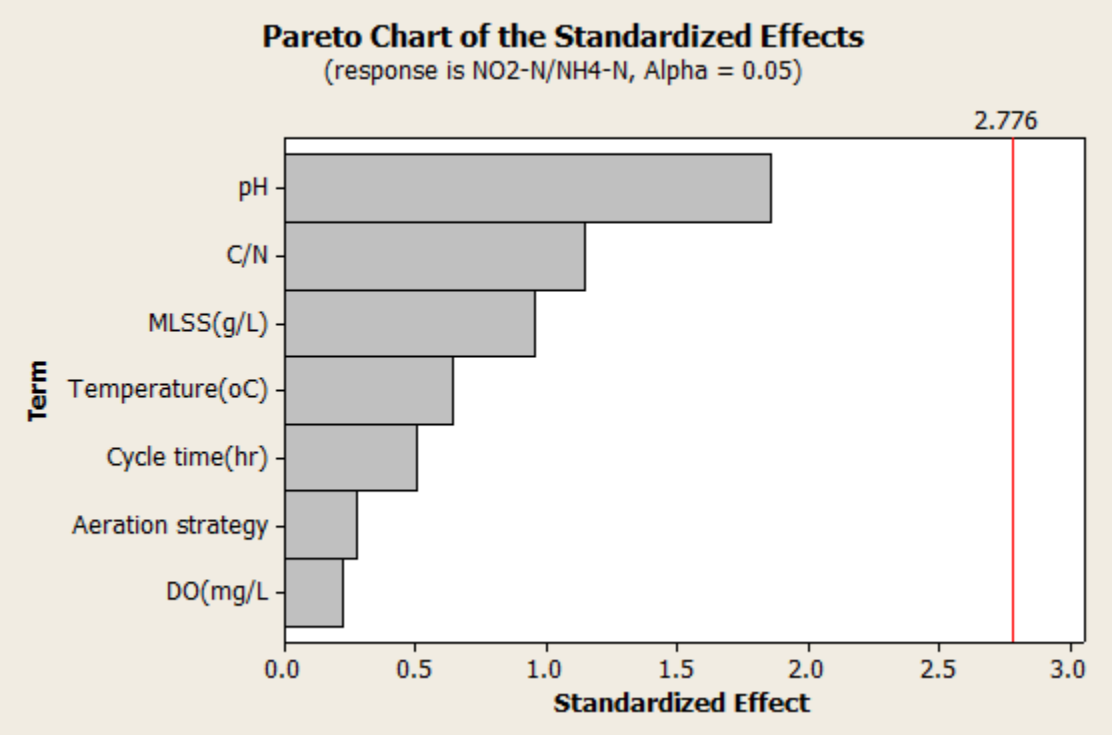


Figure 4-14 Pareto chart of the standardized effect for selected operational parameters

Figure 4-14, shows that pH and the feed C/N ratio affect the NO_2-N to NH_4-N ratio more significantly than DO and aeration strategy. This signifies that changes in the values of pH and C/N ratio are associated with changed in the response variable. The residual plots, Figure 4-15 and Figure 4-15 signify the normal plot of the standardized effect and how well the data are randomized

and fit to the model. From these figures it can be seen that the effects are not significant but the randomization and fitness to the model was good.

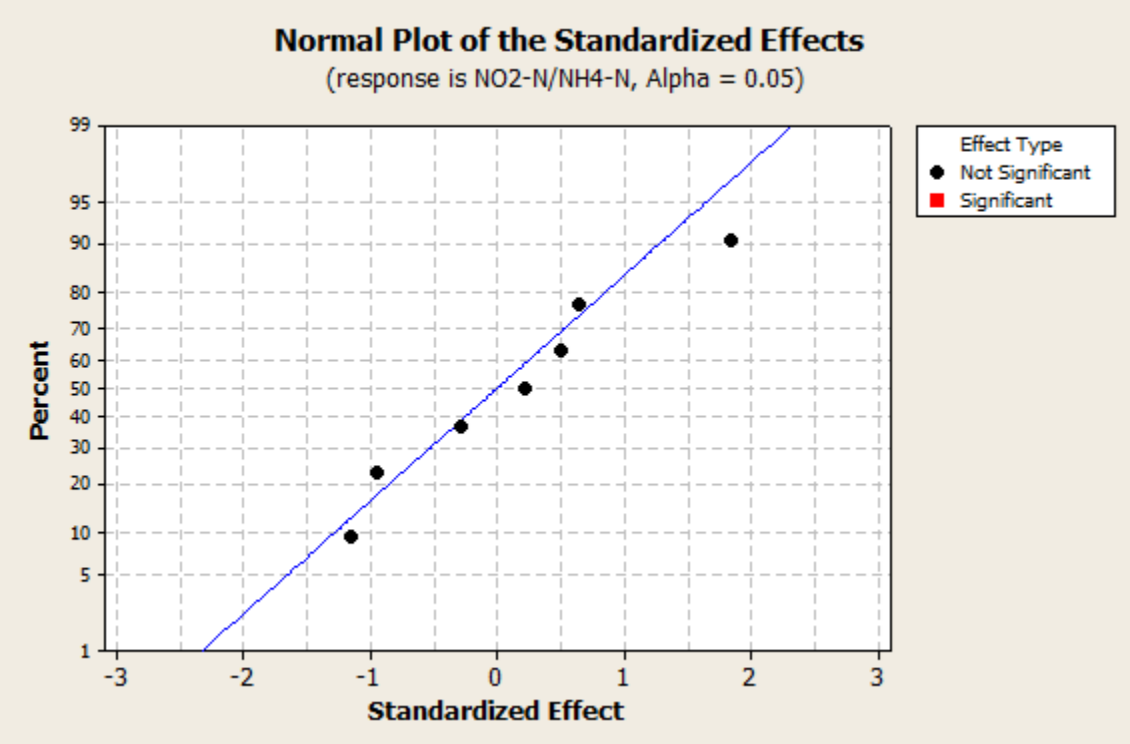


Figure 4-15.Normal plot of the standard effects

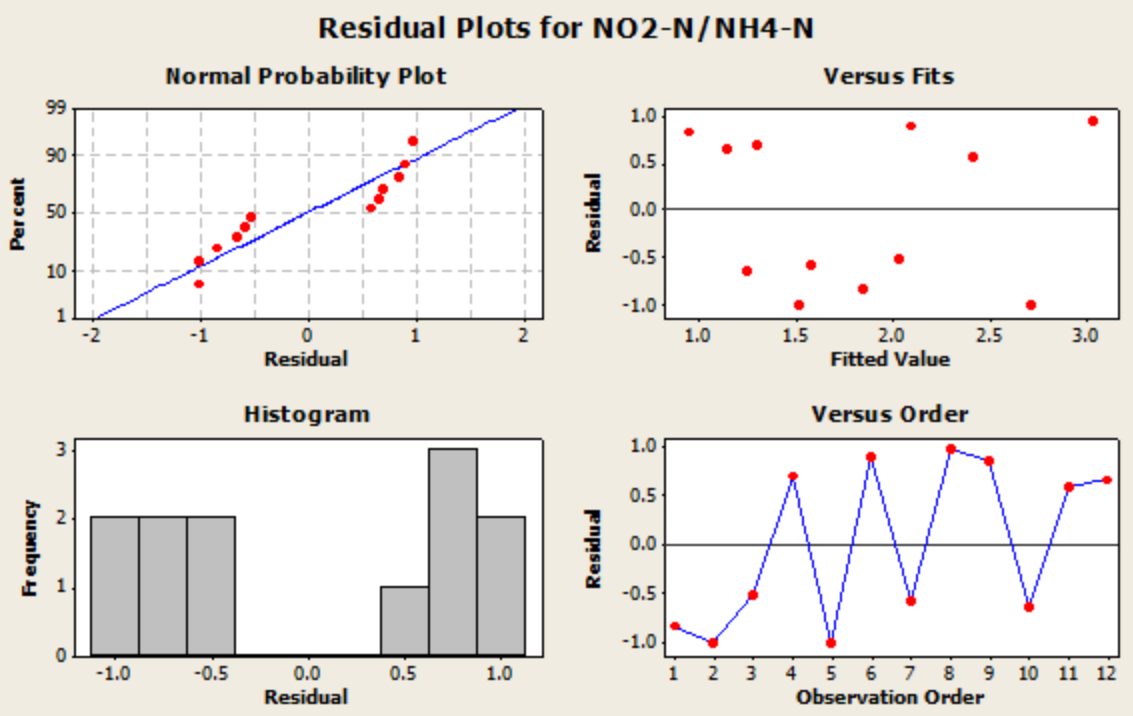


Figure 4-16 Residual plots for the response (NO_2-N/NH_4-N)

The main effect interaction plots, Figure 4-17 also shows the NO_2-N/NH_4-N increases with increasing pH, DO, Temperature and cycle time and decreases with the corresponding MLSS and C/N ratio operated.

Though Figure 4-15 shows that the effects are not significant, but the interaction with other model terms can be significant, see figure 4-18shows the interaction of the model parameters.

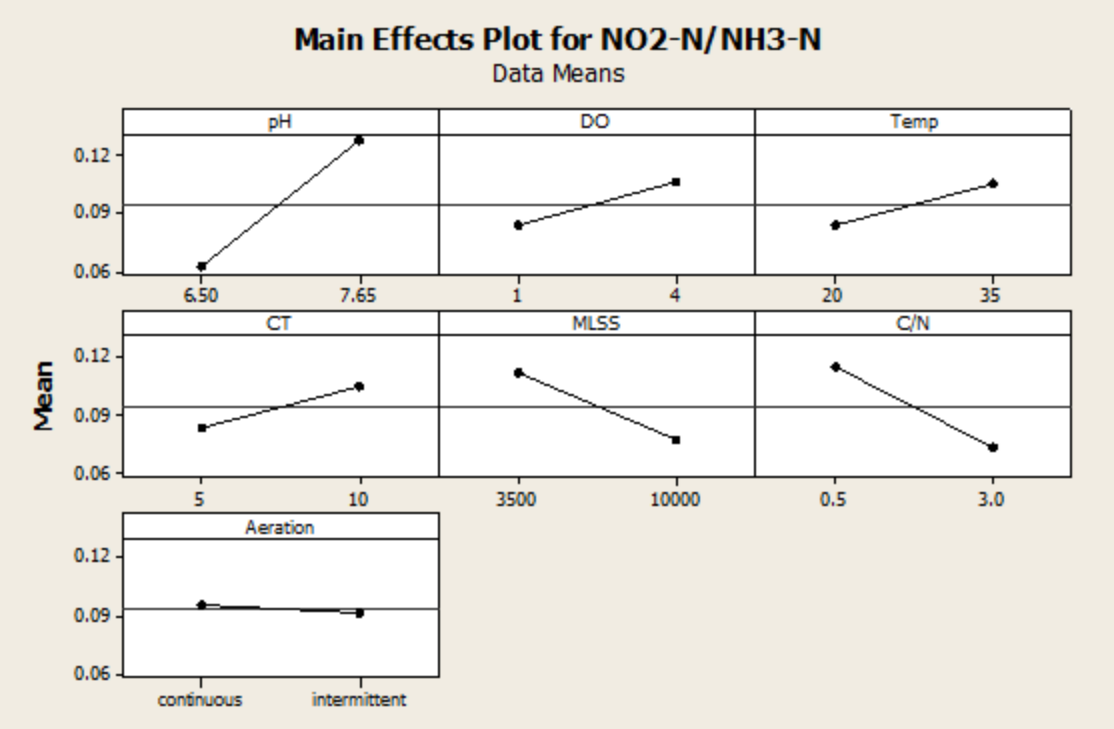


Figure 4-17 Main Interaction plot for the response (NO_2-N: NH_4-N)

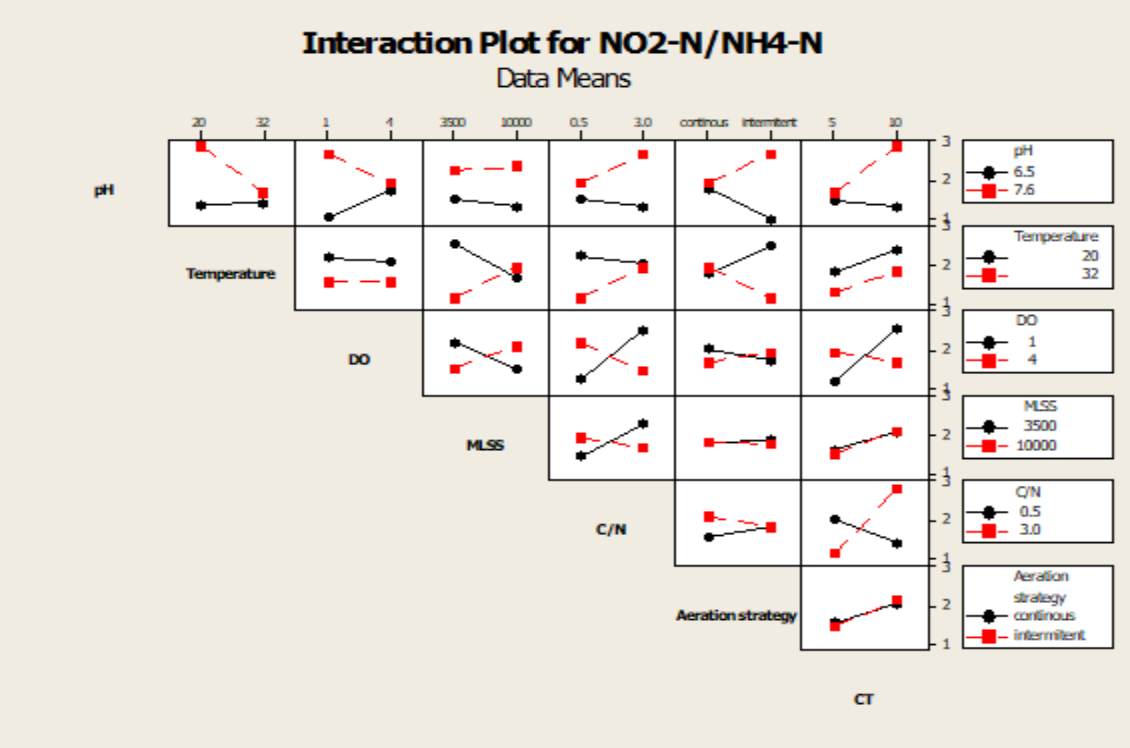


Figure 4-18 Interaction plot for the response (NO_2-N: NH_4-N)

CHAPTER 5

5. CONCLUSIONS AND RECOMMENDATION

5.1. Conclusion

From the operational performance study of the SBAR reactor, it was confirmed that operating SRT and t_C has a significant effect on the overall removal efficiency and the characteristics of final treated effluent. Operating at low SRT resulted in partial nitrification and denitrification was mainly happening via nitrite. Higher SRT maintained showed complete nitrification and partial nitrification was happening in short period time compared to low SRT maintained. Denitrification was happening during the anoxic feeding and initial period of aeration due to the very high dissolved oxygen demand where nitrate and/or nitrite from previous cycles can be used as alternative electron acceptor and denitrified. The optimum cycle time for (>90%) removal of both organic carbon and nitrogen was 8 h when the reactor was operated at 20 d SRT. The total nitrogen removal was also found to be dependent on the operating SRT and t_C. In general, the higher the cycle time, for constant SRT, the higher is the total nitrogen removal. At 20d SRT, total nitrogen removal was 43.10±1.7%, 53.74±8.8%, 58.05±10.5% and 69.35±3.8% for t_C values of 8, 10, 12, 18 h, respectively. The total nitrogen removal showed a linear correlation with the t_C and removal increased with increasing t_C and removal was affected at very low t_C. Microbial characterization study also revealed that there is a change in microbial diversity as a result of the process control parameters maintained in the reactor. The microbial diversity of the seed sludge and reactor sludge shows predominantly proteobacteria population, but with more nitrifires and anammox population in the reactor sample than in the seed sludge contributing to the high nitrogen removal efficiency of the SBAR.

During the aerobic granulation study in the SBAR, it was confirmed that reducing the settling time sequentially resulted in formation of aerobic granules with excellent settling properties without severely affecting the performance of the reactor. It was concluded that complete granulation was obtained at the end of 60^{th} day when the settling time was reduced to 3 min without affecting the nitrification efficiency. The observed characteristics of formed aerobic granules showed size, SVI and settling velocity in the range of 1.8-3.2 mm, 16-20 mL/g, settling velocity of 30 - 40 m/h and biomass density of 6.1 g/L respectively. From the study, it was also concluded that the overall average COD removal efficiency of 97.6±1.9% and the effluent COD value in the range of 32-128 mg/L could be achieved in SBAR. Further, an average NH_4-N removal efficiency of 87.1±1.2% and effluent with average NH_4-N, NO_3-N and NO_2-N concentrations of 20±2, 22.7±1.4 and 9.7±1.5 mg/L respectively could be obtained. The strategy followed also confirmed the retention of slow growing bacteria such as ammonia oxidizers and anammox bacteria in the reactor through performance and molecular studies.

Biological transformation of nitrogen results in significant generation of sludge which needs sustainable solution. Anaerobic digestion of these sludge yield energy and reduce the sludge volume significantly. The digestion of excess sludge from the SBAR reactor in this particular study yield biogas yield of 193.2 ml/g VSS and the digester reached steady state in about 30 days. However, the supernatant after anaerobic digestion contains significantly high concentration of ammonia (i.e., 1097 mg/l) which again need sustainable solution. Partial nitrification of the supernatant is a sustainable alternative to remove the nitrogen either through partial nitrification-denitrification route or through partial nitrification-anammox route. Though the PN-denitrification route may result in generation of unwanted N_2O gas. Therefore the PN-anammox route is more sustainable. For the PN-anammox route, the NH_4-N in the wastewater needs to be

partially nitrified to NO_2-N in the ratio of 1:1. As a result, the SBAR reactor was operated at SRT of 7 days, DO of 1 mg/L, pH of 8, temperature of 32°C and cycle time of 8 h to start up the PN process and the reactor was properly started up in 30 days with the required NO_2-N: NH_4-N ratio for anammox reactor feed. Moreover, the effect of various operational factors on the response variable (NO_2N: NH_4-N) was studied by considering seven operational factors (i.e. pH, DO, temperature, Cycle time, C/N, MLSS, aeration strategy (intermittent, Continuous)). Fractional factorial design (Placket-Burman) experimental design was used to study the effect of the seven operational factors. The study shows that the individual factors considered for the study are not significant but the interactions between the factors are more significant. The results from experimental runs showed that it is possible to reach a stable partial nitrification with high pH (7.60), low C/N (0.5), high cycle time (10 h), low DO concentration (1 mg/L), low MLSS/MLVSS (3500 mg/L), high temperature (32ºC) and intermittent aeration.

5.2. Recommendation

From the present study, it was confirmed that the SBAR reactor can be used to remove ammoniacal and organic nitrogen from simulated tannery wastewater and that the reactor can be fully granulated to improve the settling properties of the sludge and removal efficiency of the SBAR reactor. However, the following points which were not addressed in this research should be considered in the future to check its real application at large scale.

- The effect of residual pollutants from primary physico-chemical treatment of tannery wastewater such as sulfide and recalcitrant organic compounds shall be studied for practical application of the research findings.

- For practical application of the aerobic granular system, the effect of H/D of the SBAR reactor shall be studied by taking various H/D reactors.
- Though it takes long time to start up (more than 1 year), the start-up and operational linkage of anammox reactor with PN reactor shall be worked out in detail for the treatment of anaerobic sludge digester supernatant.

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APPENDIX I: Comparative microbial analysis between the SBAR sludge sample and the inoculum CETP sludge sample at different taxonomic levels

A. Phylum Level

Kingdom	Phylum	SBAR Reactor	CETP
k__Bacteria	p__Proteobacteria	83.96352	89.21909
k__Bacteria	p__Bacteroidetes	11.20865	6.058742
k__Bacteria	p__Chlorobi	1.358718	0.031876
k__Bacteria	p__Planctomycetes	1.204073	0.276821
k__Bacteria	p__TM7	0.767837	0.099544
k__Bacteria	p__Actinobacteria	0.520098	0.72253
k__Bacteria	p__Chloroflexi	0.35699	0.236556
k__Bacteria	p__Verrucomicrobia	0.298902	0.027962
k__Bacteria	p__[Thermi]	0.133102	1.531183
k__Bacteria	p__Acidobacteria	0.110405	0.021251
k__Bacteria	p__Firmicutes	0.047701	0.668844
k__Archaea	p__Euryarchaeota	0.00654	0.001678
k__Bacteria	p__Spirochaetes	0.006155	0.126946
k__Bacteria	p__Cyanobacteria	0.003847	0.005033
k__Bacteria	p__Synergistetes	0.003078	0.002796
k__Bacteria	p__Lentisphaerae	0.002693	0.003355
k__Bacteria	p__OP3	0.002308	0
k__Bacteria	p__GN02	0.001923	0
k__Bacteria	p__Gemmatimonadetes	0.000769	0.07326
k__Bacteria	p__NKB19	0.000769	0.045298
k__Bacteria	p__Tenericutes	0.000769	0.001678
k__Bacteria	p__Thermotogae	0.000769	0.002796
k__Bacteria	p__SBR1093	0.000385	0.113525
k__Bacteria	p__BRC1	0	0.019573
k__Bacteria	p__Chlamydiae	0	0.015659
k__Bacteria	p__Chrysiogenetes	0	0.001678
k__Bacteria	p__SR1	0	0.001118
k__Bacteria	p__WPS-2	0	0.085004
k__Bacteria	p__WS6	0	0.60621

B. Class Level

Kingdom	Phylum	Class	SBAR Reactor	CETP
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	64.66642	72.28995
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	13.54525	3.983984
k__Bacteria	p__Bacteroidetes	c__[Saprospirae]	8.193467	1.038498
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	5.05326	12.73991
k__Bacteria	p__Bacteroidetes	c__Sphingobacteriia	1.718016	0.074378
k__Bacteria	p__Chlorobi	c__OPB56	1.304477	0.002237
k__Bacteria	p__Planctomycetes	c__Planctomycetia	1.191763	0.224812
k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	0.762067	4.258008
k__Bacteria	p__TM7	c__TM7-3	0.748603	0.099544

k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	0.690515	0.134775
k__Bacteria	p__Actinobacteria	c__Actinobacteria	0.514712	0.674996
k__Bacteria	p__Bacteroidetes	c__Cytophagia	0.409308	0.10961
k__Bacteria	p__Chloroflexi	c__Thermomicrobia	0.242353	0.139249
k__Bacteria	p__Verrucomicrobia	c__Opitutae	0.150798	0
k__Bacteria	p__Verrucomicrobia	c__[Spartobacteria]	0.14195	0.002237
k__Bacteria	p__[Thermi]	c__Deinococci	0.133102	1.531183
k__Bacteria	p__Bacteroidetes	c__Bacteroidia	0.125793	0.572656
k__Bacteria	p__Chloroflexi	c__Anaerolineae	0.113868	0.050331
k__Bacteria	p__Acidobacteria	c__Solibacteres	0.103866	0.006711
k__Bacteria	p__Chlorobi	c__Ignavibacteria	0.054241	0.029639
k__Bacteria	p__Firmicutes	c__Clostridia	0.038469	0.517851
k__Bacteria	p__TM7	c__TM7-1	0.013464	0
k__Bacteria	p__Planctomycetes	c__BD7-11	0.011156	0
k__Bacteria	p__Firmicutes	c__Bacilli	0.009233	0.150993
k__Bacteria	p__Proteobacteria	c__TA18	0.008078	0
k__Bacteria	p__Verrucomicrobia	c__[Pedosphaerae]	0.006155	0.001118
k__Bacteria	p__TM7	Unclassified	0.00577	0
k__Archaea	p__Euryarchaeota	c__Methanomicrobia	0.00577	0.001678
k__Bacteria	p__Actinobacteria	c__Acidimicrobiia	0.005386	0.046416
k__Bacteria	p__Acidobacteria	c__Acidobacteria-6	0.003847	0.01454
k__Bacteria	p__Spirochaetes	c__[Leptospirae]	0.003078	0
k__Bacteria	p__Cyanobacteria	c__4C0d-2	0.003078	0
k__Bacteria	p__Spirochaetes	c__Spirochaetes	0.003078	0.012862
k__Bacteria	p__Synergistetes	c__Synergistia	0.003078	0.002796
k__Bacteria	p__Lentisphaerae	c__[Lentisphaeria]	0.002693	0.003355
k__Bacteria	p__Acidobacteria	c__iii1-8	0.002693	0
k__Bacteria	p__OP3	c__PBS-25	0.002308	0
k__Bacteria	p__GN02	Unclassified	0.001923	0
k__Bacteria	p__Planctomycetes	c__Phycisphaerae	0.001154	0.027402
k__Bacteria	p__NKB19	Unclassified	0.000769	0.045298
k__Bacteria	p__Chloroflexi	c__Ellin6529	0.000769	0
k__Bacteria	p__Gemmatimonadetes	c__Gemmatimonadetes	0.000769	0.001118
k__Archaea	p__Euryarchaeota	c__Methanobacteria	0.000769	0
k__Bacteria	p__Cyanobacteria	c__ML635J-21	0.000769	0.003915
k__Bacteria	p__Tenericutes	c__Mollicutes	0.000769	0.001678
k__Bacteria	p__Thermotogae	c__Thermotogae	0.000769	0.002796
k__Bacteria	p__SBR1093	c__VHS-B5-50	0.000385	0.113525
k__Bacteria	p__SR1	Unclassified	0	0.001118
k__Bacteria	p__WPS-2	Unclassified	0	0.085004
k__Bacteria	p__Bacteroidetes	c__[Rhodothermi]	0	0.005592
k__Bacteria	p__WS6	c__B142	0	0.60621
k__Bacteria	p__Chlamydiae	c__Chlamydiia	0	0.015659
k__Bacteria	p__Chrysiogenetes	c__Chrysiogenetes	0	0.001678
k__Bacteria	p__Proteobacteria	c__Epsilonproteobacteria	0	0.070463
k__Bacteria	p__Gemmatimonadetes	c__Gemm-2	0	0.069904
k__Bacteria	p__Gemmatimonadetes	c__Gemm-3	0	0.002237
k__Bacteria	p__Spirochaetes	c__MVP-15	0	0.114084
k__Bacteria	p__Planctomycetes	c__OM190	0	0.024606

k__Bacteria	p__Cyanobacteria	c__Oscillatoriophycideae	0	0.001118
k__Bacteria	p__BRC1	c__PRR-11	0	0.019573
k__Bacteria	p__Actinobacteria	c__Thermoleophilia	0	0.001118
k__Bacteria	p__Chloroflexi	c__TK17	0	0.046976
k__Bacteria	p__Verrucomicrobia	c__Verruco-5	0	0.003915
k__Bacteria	p__Verrucomicrobia	c__Verrucomicrobiae	0	0.020692

C. Order Level

Kingdom	Phylum	Class	Order	SBAR Reactor	CETP
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	64.02553	2.770446
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	8.327339	1.480293
k__Bacteria	p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	8.193467	1.038498
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	3.179061	0.70799
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	3.175983	10.49291
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	1.771872	0.181751
k__Bacteria	p__Bacteroidetes	c__Sphingobacteriia	o__Sphingobacteriales	1.718016	0.074378
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Nitrosomonadales	1.351793	0.04362
k__Bacteria	p__Chlorobi	c__OPB56	Unclassified	1.304477	0.002237
k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	0.762067	4.258008
k__Bacteria	p__TM7	c__TM7-3	Unclassified	0.747064	0.091714
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	0.569723	0.002796
k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	0.514712	0.674996
k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Pirellulales	0.514712	0.180633
k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Planctomycetales	0.49971	0.026284
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Rhodocyclales	0.448931	0.041943
k__Bacteria	p__Bacteroidetes	c__Cytophagia	o__Cytophagales	0.409308	0.10961
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	0.391997	20.12963
k__Bacteria	p__Chloroflexi	c__Thermomicrobia	o__JG30-KF-CM45	0.221196	0.003355
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Aeromonadales	0.220041	34.22121
k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Gemmatales	0.171571	0.017895
k__Bacteria	p__Verrucomicrobia	c__Opitutae	o__Opitutales	0.150798	0
k__Bacteria	p__Verrucomicrobia	c__[Spartobacteria]	o__[Chthoniobacterales]	0.14195	0.002237
k__Bacteria	p__[Thermi]	c__Deinococci	o__Deinococcales	0.133102	1.531183
k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	0.125793	0.572656
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	0.116945	1.417658
k__Bacteria	p__Acidobacteria	c__Solibacteres	o__Solibacterales	0.103866	0.006711
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Bdellovibrionales	0.097326	0.015659
k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Caldilineales	0.078476	0
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Ellin329	0.070398	0.000559
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__SC-I-84	0.055395	0
k__Bacteria	p__Chlorobi	c__Ignavibacteria	o__Ignavibacteriales	0.054241	0.029639
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	Unclassified	0.039623	0.124709
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	0.038469	0.517851
k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__SBR1031	0.033853	0.048653
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Caulobacterales	0.026928	0.055364
k__Bacteria	p__Chloroflexi	c__Thermomicrobia	o__AKYG1722	0.021158	0.135894
k__Bacteria	p__TM7	c__TM7-1	Unclassified	0.013464	0

k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Hydrogenophilales	0.013464	0
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__BD7-3	0.013079	0.003915
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__GMD14H09	0.012695	0.054805
k__Bacteria	p__Planctomycetes	c__BD7-11	Unclassified	0.011156	0
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Desulfobacterales	0.010771	0.025166
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Enterobacteriales	0.009233	10.52367
k__Bacteria	p__Proteobacteria	c__TA18	o__PHOS-HD29	0.008078	0
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Thiotrichales	0.008078	0.010066
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Legionellales	0.006924	0.011185
k__Bacteria	p__Firmicutes	c__Bacilli	o__Lactobacillales	0.00654	0.004474
k__Bacteria	p__Verrucomicrobia	c__[Pedosphaerae]	o__[Pedosphaerales]	0.006155	0.001118
k__Bacteria	p__TM7	Unclassified	Unclassified	0.00577	0
k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__B97	0.00577	0
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Neisseriales	0.00577	0.126946
k__Bacteria	p__Actinobacteria	c__Acidimicrobiia	o__Acidimicrobiales	0.005386	0.046416
k__Archaea	p__Euryarchaeota	c__Methanomicrobia	o__Methanosarcinales	0.005001	0.001118
k__Bacteria	p__Acidobacteria	c__Acidobacteria-6	o__iii1-15	0.003847	0.01454
k__Bacteria	p__Spirochaetes	c__[Leptospirae]	o__[Leptospirales]	0.003078	0
k__Bacteria	p__Cyanobacteria	c__4C0d-2	o__MLE1-12	0.003078	0
k__Bacteria	p__Spirochaetes	c__Spirochaetes	o__Spirochaetales	0.003078	0.012862
k__Bacteria	p__Synergistetes	c__Synergistia	o__Synergistales	0.003078	0.002796
k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	0.002693	0.146519
k__Bacteria	p__Acidobacteria	c__iii1-8	o__SJA-36	0.002693	0
k__Bacteria	p__Lentisphaerae	c__[Lentisphaeria]	o__Victivallales	0.002693	0.003355
k__Bacteria	p__OP3	c__PBS-25	Unclassified	0.002308	0
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Alteromonadales	0.002308	1.98584
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Chromatiales	0.002308	1.054715
k__Bacteria	p__GN02	Unclassified	Unclassified	0.001923	0
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__ASSO-13	0.001923	2.017717
k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Anaerolineales	0.001539	0
k__Bacteria	p__TM7	c__TM7-3	o__EW055	0.001539	0.007829
k__Bacteria	p__Planctomycetes	c__Phycisphaerae	o__WD2101	0.001154	0
k__Bacteria	p__NKB19	Unclassified	Unclassified	0.000769	0.045298
k__Bacteria	p__Chloroflexi	c__Ellin6529	Unclassified	0.000769	0
k__Bacteria	p__Cyanobacteria	c__ML635J-21	Unclassified	0.000769	0.003915
k__Bacteria	p__Tenericutes	c__Mollicutes	o__Acholeplasmatales	0.000769	0
k__Bacteria	p__Gemmatimonadetes	c__Gemmatimonadetes	o__Gemmatimonadales	0.000769	0.001118
k__Archaea	p__Euryarchaeota	c__Methanobacteria	o__Methanobacteriales	0.000769	0
k__Archaea	p__Euryarchaeota	c__Methanomicrobia	o__Methanocellales	0.000769	0.000559
k__Bacteria	p__Thermotogae	c__Thermotogae	o__Thermotogales	0.000769	0.002796
k__Bacteria	p__SBR1093	c__VHS-B5-50	Unclassified	0.000385	0.113525
k__Bacteria	p__SR1	Unclassified	Unclassified	0	0.001118
k__Bacteria	p__WPS-2	Unclassified	Unclassified	0	0.085004
k__Bacteria	p__WS6	c__B142	Unclassified	0	0.60621
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	Unclassified	0	0.016777
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	Unclassified	0	0.007829
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	Unclassified	0	0.016777
k__Bacteria	p__Gemmatimonadetes	c__Gemm-2	Unclassified	0	0.069904
k__Bacteria	p__Gemmatimonadetes	c__Gemm-3	Unclassified	0	0.002237

k__Bacteria	p__Tenericutes	c__Mollicutes	Unclassified	0	0.001678
k__Bacteria	p__BRC1	c__PRR-11	Unclassified	0	0.019573
k__Bacteria	p__Chloroflexi	c__TK17	Unclassified	0	0.046976
k__Bacteria	p__Verrucomicrobia	c__Verruco-5	Unclassified	0	0.003915
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__[Marinicellales]	0	0.009507
k__Bacteria	p__Bacteroidetes	c__[Rhodothermi]	o__[Rhodothermales]	0	0.005592
k__Bacteria	p__Proteobacteria	c__Epsilonproteobacteria	o__Campylobacterales	0	0.070463
k__Bacteria	p__Chlamydiae	c__Chlamydiia	o__Chlamydiales	0	0.015659
k__Bacteria	p__Chrysiogenetes	c__Chrysiogenetes	o__Chrysiogenales	0	0.001678
k__Bacteria	p__Planctomycetes	c__OM190	o__CL500-15	0	0.024606
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Desulfovibrionales	0	0.016218
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Desulfuromonadales	0	0.009507
k__Bacteria	p__Actinobacteria	c__Thermoleophilia	o__Gaiellales	0	0.001118
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__HTCC2188	0	0.261722
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Kiloniellales	0	0.002796
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__NB1-j	0	0.002796
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Oceanospirillales	0	0.709109
k__Bacteria	p__Cyanobacteria	c__Oscillatoriophycideae	o__Oscillatoriales	0	0.001118
k__Bacteria	p__Planctomycetes	c__Phycisphaerae	o__Phycisphaerales	0	0.027402
k__Bacteria	p__Spirochaetes	c__MVP-15	o__PL-11B10	0	0.114084
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rickettsiales	0	0.008948
k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__S0208	0	0.001678
k__Bacteria	p__Verrucomicrobia	c__Verrucomicrobiae	o__Verrucomicrobiales	0	0.020692
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Vibrionales	0	0.586077

D. Family Level

Kingdom	Phylum	Class	Order	Family	SBAR Reactor	CETP
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	64.02514	2.768209
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	8.326569	0.979219
k__Bacteria	p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Chitinophagaceae	8.193467	0.241589
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	2.920166	4.127147
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	1.770718	0.173922
k__Bacteria	p__Bacteroidetes	c__Sphingobacteriia	o__Sphingobacteriales	Unclassified	1.714939	0.029639
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Nitrosomonadales	f__Nitrosomonadaceae	1.351793	0.04362
k__Bacteria	p__Chlorobi	c__OPB56	Unclassified	Unclassified	1.304477	0.002237
k__Bacteria	p__TM7	c__TM7-3	Unclassified	Unclassified	0.747064	0.091714
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Phyllobacteriaceae	0.723983	0.0878
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Hyphomicrobiaceae	0.666279	0.418866
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	Unclassified	0.563952	0.001678
k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Pirellulales	f__Pirellulaceae	0.514712	0.180633
k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__[Weeksellaceae]	0.504711	2.205619
k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Planctomycetales	f__Planctomycetaceae	0.49971	0.026284
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Xanthobacteraceae	0.471627	0
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Rhodocyclales	f__Rhodocyclaceae	0.448931	0.041943
k__Bacteria	p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	0.408923	0.07997
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Rhizobiaceae	0.366223	0.0727
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	Unclassified	0.359683	0.112965
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	0.306596	12.40605
k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Flavobacteriaceae	0.256972	1.278409
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Alcaligenaceae	0.255048	0.013422
k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	Unclassified	0.240815	0.002237
k__Bacteria	p__Chloroflexi	c__Thermomicrobia	o__JG30-KF-CM45	Unclassified	0.221196	0.003355
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Aeromonadales	f__Aeromonadaceae	0.219657	34.21953
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Bradyrhizobiaceae	0.217733	0.009507
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Bartonellaceae	0.200038	0.002796
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Brucellaceae	0.173494	0.001678
k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Dermatophilaceae	0.161954	0
k__Bacteria	p__Verrucomicrobia	c__Opitutae	o__Opitutales	f__Opitutaceae	0.150798	0
k__Bacteria	p__Verrucomicrobia	c__[Spartobacteria]	o__[Chthoniobacterales]	f__[Chthoniobacteraceae]	0.14195	0.002237

k__Bacteria	p__[Thermi]	c__Deinococci	o__Deinococcales	f__Trueperaceae	0.133102	1.055275
k__Bacteria	p__Acidobacteria	c__Solibacteres	o__Solibacterales	f__Solibacteraceae	0.102327	0.006711
k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Gemmatales	f__Gemmataceae	0.094249	0.017336
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	0.085401	7.723582
k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Caldilineales	f__Caldilineaceae	0.078476	0
k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Gemmatales	f__Isosphaeraceae	0.077322	0.000559
k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__Prevotellaceae	0.071552	0.025725
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Ellin329	Unclassified	0.070398	0.000559
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Bdellovibrionales	f__Bacteriovoracaceae	0.069244	0.012862
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Rhodospirillaceae	0.069244	0.403767
k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Gordoniaceae	0.063089	0.000559
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__SC-I-84	Unclassified	0.055395	0
k__Bacteria	p__Chlorobi	c__Ignavibacteria	o__Ignavibacteriales	f__lheB3-7	0.052318	0.016218
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Acetobacteraceae	0.047317	0.000559
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	Unclassified	Unclassified	0.039623	0.124709
k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Nocardioidaceae	0.036161	0.002237
k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__SBR1031	f__A4b	0.033853	0.002796
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Bdellovibrionales	f__Bdellovibrionaceae	0.028082	0.002796
k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__BA008	0.025005	0
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Caulobacterales	f__Caulobacteraceae	0.021927	0.054805
k__Bacteria	p__Chloroflexi	c__Thermomicrobia	o__AKYG1722	Unclassified	0.021158	0.135894
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__[Acidaminobacteraceae]	0.014233	0.139809
k__Bacteria	p__TM7	c__TM7-1	Unclassified	Unclassified	0.013464	0
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Hydrogenophilales	f__Hydrogenophilaceae	0.013464	0
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__BD7-3	Unclassified	0.013079	0.003915
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__GMD14H09	Unclassified	0.012695	0.054805
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Veillonellaceae	0.012695	0.006152
k__Bacteria	p__Planctomycetes	c__BD7-11	Unclassified	Unclassified	0.011156	0
k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	Unclassified	0.011156	0.005033
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Desulfobacterales	f__Desulfobulbaceae	0.010771	0.005592
k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Tsukamurellaceae	0.010387	0
k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__Porphyromonadaceae	0.009617	0.022369
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Enterobacteriales	f__Enterobacteriaceae	0.009233	10.52367
k__Bacteria	p__Proteobacteria	c__TA18	o__PHOS-HD29	Unclassified	0.008078	0
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Thiotrichales	f__Piscirickettsiaceae	0.008078	0.010066

k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Legionellales	Unclassified	0.006924	0
k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__Rikenellaceae	0.00654	0.011744
k__Bacteria	p__Firmicutes	c__Bacilli	o__Lactobacillales	f__Streptococcaceae	0.00654	0.002796
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Lachnospiraceae	0.006155	0.006711
k__Bacteria	p__Verrucomicrobia	c__[Pedosphaerae]	o__[Pedosphaerales]	f__R4-41B	0.006155	0.001118
k__Bacteria	p__TM7	Unclassified	Unclassified	Unclassified	0.00577	0
k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__B97	Unclassified	0.00577	0
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	f__Haliangiaceae	0.00577	0
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Neisseriales	f__Neisseriaceae	0.00577	0.126946
k__Bacteria	p__Actinobacteria	c__Acidimicrobiia	o__Acidimicrobiales	f__Microthrixaceae	0.005386	0.005592
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Caulobacterales	Unclassified	0.005001	0.000559
k__Archaea	p__Euryarchaeota	c__Methanomicrobia	o__Methanosarcinales	f__Methanosaetaceae	0.005001	0.001118
k__Bacteria	p__Acidobacteria	c__Acidobacteria-6	o__iii1-15	Unclassified	0.003847	0.01454
k__Bacteria	p__Cyanobacteria	c__4C0d-2	o__MLE1-12	Unclassified	0.003078	0
k__Bacteria	p__Synergistetes	c__Synergistia	o__Synergistales	f__Dethiosulfovibrionaceae	0.003078	0.000559
k__Bacteria	p__Spirochaetes	c__[Leptospirae]	o__[Leptospirales]	f__Leptospiraceae	0.003078	0
k__Bacteria	p__Bacteroidetes	c__Sphingobacteriia	o__Sphingobacteriales	f__Sphingobacteriaceae	0.003078	0.044739
k__Bacteria	p__Spirochaetes	c__Spirochaetes	o__Spirochaetales	f__Spirochaetaceae	0.003078	0.012862
k__Bacteria	p__Acidobacteria	c__iii1-8	o__SJA-36	Unclassified	0.002693	0
k__Bacteria	p__Lentisphaerae	c__[Lentisphaeria]	o__Victivallales	f__Victivallaceae	0.002693	0.003355
k__Bacteria	p__OP3	c__PBS-25	Unclassified	Unclassified	0.002308	0
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Chromatiales	Unclassified	0.002308	0.205239
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Alteromonadales	f__[Chromatiaceae]	0.002308	1.715171
k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__Bacillaceae	0.002308	0.11688
k__Bacteria	p__GN02	Unclassified	Unclassified	Unclassified	0.001923	0
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__ASSO-13	Unclassified	0.001923	2.017717
k__Bacteria	p__Chlorobi	c__Ignavibacteria	o__Ignavibacteriales	f__Ignavibacteriaceae	0.001923	0
k__Bacteria	p__TM7	c__TM7-3	o__EW055	Unclassified	0.001539	0.007829
k__Bacteria	p__Acidobacteria	c__Solibacteres	o__Solibacterales	Unclassified	0.001539	0
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__[Mogibacteriaceae]	0.001539	0.00727
k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Anaerolineales	f__Anaerolinaceae	0.001539	0
k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Mycobacteriaceae	0.001539	0.003915
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	Unclassified	0.001154	0
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	Unclassified	0.001154	0.002237
k__Bacteria	p__Planctomycetes	c__Phycisphaerae	o__WD2101	Unclassified	0.001154	0

k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__SB-1	0.001154	0.040265
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Syntrophomonadaceae	0.001154	0
k__Bacteria	p__NKB19	Unclassified	Unclassified	Unclassified	0.000769	0.045298
k__Bacteria	p__Chloroflexi	c__Ellin6529	Unclassified	Unclassified	0.000769	0
k__Bacteria	p__Cyanobacteria	c__ML635J-21	Unclassified	Unclassified	0.000769	0.003915
k__Bacteria	p__Tenericutes	c__Mollicutes	o__Acholeplasmatales	f__Acholeplasmataceae	0.000769	0
k__Bacteria	p__Gemmatimonadetes	c__Gemmatimonadetes	o__Gemmatimonadales	f__Gemmatimonadaceae	0.000769	0.001118
k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__GZKB119	0.000769	0.450183
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Hyphomonadaceae	0.000769	0.501074
k__Archaea	p__Euryarchaeota	c__Methanobacteria	o__Methanobacteriales	f__Methanobacteriaceae	0.000769	0
k__Archaea	p__Euryarchaeota	c__Methanomicrobia	o__Methanocellales	f__Methanocellaceae	0.000769	0.000559
k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Microbacteriaceae	0.000769	0.654304
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Oxalobacteraceae	0.000769	6.317667
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Ruminococcaceae	0.000769	0.002237
k__Bacteria	p__Thermotogae	c__Thermotogae	o__Thermotogales	f__Thermotogaceae	0.000769	0.002796
k__Bacteria	p__SBR1093	c__VHS-B5-50	Unclassified	Unclassified	0.000385	0.113525
k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	Unclassified	0.000385	0.622987
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	Unclassified	0.000385	1.013332
k__Bacteria	p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cyclobacteriaceae	0.000385	0.021251
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Peptococcaceae	0.000385	0.01454
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Peptostreptococcaceae	0.000385	0.011185
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Sinobacteraceae	0.000385	0.002237
k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__Staphylococcaceae	0.000385	0.007829
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Aeromonadales	f__Succinivibrionaceae	0.000385	0.001678
k__Bacteria	p__SR1	Unclassified	Unclassified	Unclassified	0	0.001118
k__Bacteria	p__WPS-2	Unclassified	Unclassified	Unclassified	0	0.085004
k__Bacteria	p__WS6	c__B142	Unclassified	Unclassified	0	0.60621
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	Unclassified	Unclassified	0	0.016777
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	Unclassified	Unclassified	0	0.007829
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	Unclassified	Unclassified	0	0.016777
k__Bacteria	p__Gemmatimonadetes	c__Gemm-2	Unclassified	Unclassified	0	0.069904
k__Bacteria	p__Gemmatimonadetes	c__Gemm-3	Unclassified	Unclassified	0	0.002237
k__Bacteria	p__Tenericutes	c__Mollicutes	Unclassified	Unclassified	0	0.001678
k__Bacteria	p__BRC1	c__PRR-11	Unclassified	Unclassified	0	0.019573
k__Bacteria	p__Chloroflexi	c__TK17	Unclassified	Unclassified	0	0.046976

k__Bacteria	p__Verrucomicrobia	c__Verruco-5	Unclassified	Unclassified	0	0.003915
k__Bacteria	p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	Unclassified	0	0.153789
k__Bacteria	p__Actinobacteria	c__Acidimicrobiia	o__Acidimicrobiales	Unclassified	0	0.01454
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	Unclassified	0	0.034673
k__Bacteria	p__Planctomycetes	c__OM190	o__CL500-15	Unclassified	0	0.024606
k__Bacteria	p__Actinobacteria	c__Thermoleophilia	o__Gaiellales	Unclassified	0	0.001118
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__HTCC2188	Unclassified	0	0.258366
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Kiloniellales	Unclassified	0	0.002796
k__Bacteria	p__Planctomycetes	c__Phycisphaerae	o__Phycisphaerales	Unclassified	0	0.025725
k__Bacteria	p__Spirochaetes	c__MVP-15	o__PL-11B10	Unclassified	0	0.114084
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rickettsiales	Unclassified	0	0.007829
k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__S0208	Unclassified	0	0.001678
k__Bacteria	p__Bacteroidetes	c__[Rhodothermi]	o__[Rhodothermales]	f__[Balneolaceae]	0	0.005592
k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__[Exiguobacteraceae]	0	0.010625
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__[Marinicellales]	f__[Marinicellaceae]	0	0.009507
k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__[Paraprevotellaceae]	0	0.001118
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__[Tissierellaceae]	0	0.247741
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Oceanospirillales	f__Alcanivoracaceae	0	0.590551
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Alteromonadales	f__Alteromonadaceae	0	0.2483
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Aurantimonadaceae	0	0.001678
k__Bacteria	p__Actinobacteria	c__Acidimicrobiia	o__Acidimicrobiales	f__C111	0	0.026284
k__Bacteria	p__Proteobacteria	c__Epsilonproteobacteria	o__Campylobacterales	f__Campylobacteraceae	0	0.070463
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Chromatiales	f__Chromatiaceae	0	0.081089
k__Bacteria	p__Chrysiogenetes	c__Chrysiogenetes	o__Chrysiogenales	f__Chrysiogenaceae	0	0.001678
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Clostridiaceae	0	0.082207
k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Cryomorphaceae	0	0.150993
k__Bacteria	p__[Thermi]	c__Deinococci	o__Deinococcales	f__Deinococcaceae	0	0.475908
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Desulfobacterales	f__Desulfobacteraceae	0	0.019573
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Desulfovibrionales	f__Desulfomicrobiaceae	0	0.010066
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Desulfovibrionales	f__Desulfovibrionaceae	0	0.006152
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Desulfuromonadales	f__Desulfuromonadaceae	0	0.009507
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Chromatiales	f__Ectothiorhodospiraceae	0	0.768388
k__Bacteria	p__Firmicutes	c__Bacilli	o__Lactobacillales	f__Enterococcaceae	0	0.001678
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Erythrobacteraceae	0	0.005592
k__Bacteria	p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Flammeovirgaceae	0	0.008389

k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Legionellales	f__Francisellaceae	0	0.011185
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Oceanospirillales	f__Halomonadaceae	0	0.06599
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__HTCC2188	f__HTCC2089	0	0.003355
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Alteromonadales	f__HTCC2188	0	0.002237
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Alteromonadales	f__Idiomarinaceae	0	0.020132
k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Intrasporangiaceae	0	0.011744
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__NB1-j	f__JTB38	0	0.002796
k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__ML635J-40	0	0.001118
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Oceanospirillales	f__Oceanospirillaceae	0	0.052568
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	f__OM27	0	0.001118
k__Bacteria	p__Chlorobi	c__Ignavibacteria	o__Ignavibacteriales	f__OTUb7	0	0.013422
k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__p-2534-18B5	0	0.015099
k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__Paenibacillaceae	0	0.011185
k__Bacteria	p__Cyanobacteria	c__Oscillatoriophycideae	o__Oscillatoriales	f__Phormidiaceae	0	0.001118
k__Bacteria	p__Planctomycetes	c__Phycisphaerae	o__Phycisphaerales	f__Phycisphaeraceae	0	0.001678
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Vibrionales	f__Pseudoalteromonadaceae	0	0.536305
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rickettsiales	f__Rickettsiaceae	0	0.001118
k__Bacteria	p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Saprospiraceae	0	0.643119
k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__SBR1031	f__SHA-31	0	0.045857
k__Bacteria	p__Synergistetes	c__Synergistia	o__Synergistales	f__Synergistaceae	0	0.001118
k__Bacteria	p__Synergistetes	c__Synergistia	o__Synergistales	f__Thermovirgaceae	0	0.001118
k__Bacteria	p__Verrucomicrobia	c__Verrucomicrobiae	o__Verrucomicrobiales	f__Verrucomicrobiaceae	0	0.020692
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Vibrionales	f__Vibrionaceae	0	0.049772
k__Bacteria	p__Chlamydiae	c__Chlamydiia	o__Chlamydiales	f__Waddliaceae	0	0.015659

A. Genius Level

Kingdom	Phylum	Class	Order	Family	Genus	SBAR Reactor	CETP
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Thermomonas	21.82334363	0.04529796
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	Unclassified	16.43886733	2.178775948
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Dokdonella	16.14034953	0.026843236
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	Unclassified	8.255786667	0.673317824
k__Bacteria	p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Chitinophagaceae	Unclassified	8.186927536	0.102899069
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Lysobacter	6.340425696	0.109050644
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	Unclassified	2.769752761	3.382247674
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Pseudoxanthomonas	2.709356763	0.151552434

k__Bacteria	p__Bacteroidetes	c__Sphingobacteriia	o__Sphingobacteriales	Unclassified	Unclassified	1.714938585	0.029639406
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	g__Sphingopyxis	1.408342341	0.071022727
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Nitrosomonadales	f__Nitrosomonadaceae	Unclassified	1.351793223	0.005033107
k__Bacteria	p__Chlorobi	c__OPB56	Unclassified	Unclassified	Unclassified	1.304476613	0.002236936
k__Bacteria	p__TM7	c__TM7-3	Unclassified	Unclassified	Unclassified	0.74706387	0.091714388
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Phyllobacteriaceae	g__Mesorhizobium	0.595881532	0.022369363
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	Unclassified	Unclassified	0.563952437	0.001677702
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Arenimonas	0.553950552	0
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Hyphomicrobiaceae	g__Devosia	0.519328643	0.04250179
k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Planctomycetales	f__Planctomycetaceae	g__Planctomyces	0.499709561	0.026284001
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Xanthobacteraceae	g__Xanthobacter	0.470088594	0
k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__[Weeksellaceae]	g__Cloacibacterium	0.467395778	0.196850394
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Rhodocyclales	f__Rhodocyclaceae	g__Dok59	0.43085043	0
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	Unclassified	Unclassified	0.359683171	0.112965283
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Rhizobiaceae	Unclassified	0.351220038	0.050331067
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	Unclassified	0.304672804	0.084444345
k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Pirellulales	f__Pirellulaceae	Unclassified	0.27812934	0.180632606
k__Bacteria	p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	Unclassified	0.256971506	0.021810129
k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Flavobacteriaceae	g__Aequorivita	0.256971506	0.001118468
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Alcaligenaceae	g__Denitrobacter	0.250047124	0.001677702
k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	Unclassified	Unclassified	0.240814615	0.002236936
k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Pirellulales	f__Pirellulaceae	g__A17	0.236583048	0
k__Bacteria	p__Chloroflexi	c__Thermomicrobia	o__JG30-KF-CM45	Unclassified	Unclassified	0.221195533	0.003355404
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	Unclassified	0.221195533	0.19908733
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Aeromonadales	f__Aeromonadaceae	Unclassified	0.219656781	34.19380816
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Bartonellaceae	Unclassified	0.200037699	0.00279617
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Brucellaceae	g__Ochrobactrum	0.173494235	0.001677702
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Bradyrhizobiaceae	g__Bradyrhizobium	0.171570796	0.005033107
k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Dermatophilaceae	g__Piscicoccus	0.161953599	0
k__Bacteria	p__Verrucomicrobia	c__[Spartobacteria]	o__[Chthoniobacterales]	f__[Chthoniobacteraceae]	g__Candidatus Xiphinematobacter	0.141180453	0.002236936
k__Bacteria	p__[Thermi]	c__Deinococci	o__Deinococcales	f__Trueperaceae	g__B-42	0.133102008	1.055274696
k__Bacteria	p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	g__Runella	0.129255129	0.008388511
k__Bacteria	p__Verrucomicrobia	c__Opitutae	o__Opitutales	f__Opitutaceae	g__Opitutus	0.103096353	0
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Hyphomicrobiaceae	g__Rhodoplanes	0.103096353	0.001118468

k__Bacteria	p__Acidobacteria	c__Solibacteres	o__Solibacterales	f__Solibacteraceae	Unclassified	0.102326977	0.006710809
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	0.08540071	7.61341267
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Comamonas	0.084246646	0.163855583
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Phyllobacteriaceae	Unclassified	0.083861959	0.015658554
k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Caldilineales	f__Caldilineaceae	Unclassified	0.076168201	0
k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Gemmatales	f__Isosphaeraceae	Unclassified	0.073090698	0.000559234
k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__Prevotellaceae	g__Prevotella	0.071551946	0.025724767
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Ellin329	Unclassified	Unclassified	0.070397883	0.000559234
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Bdellovibrionales	f__Bacteriovoracaceae	Unclassified	0.069243819	0.012862384
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Rhodospirillaceae	Unclassified	0.068474443	0.066548855
k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Gordoniaceae	g__Gordonia	0.063088813	0.000559234
k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Gemmatales	f__Gemmataceae	Unclassified	0.060780686	0
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__SC-I-84	Unclassified	Unclassified	0.055395055	0
k__Bacteria	p__Chlorobi	c__Ignavibacteria	o__Ignavibacteriales	f__lheB3-7	Unclassified	0.052317552	0.016217788
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	g__Sphingomonas	0.049240049	0.00279617
k__Bacteria	p__Verrucomicrobia	c__Opitutae	o__Opitutales	f__Opitutaceae	Unclassified	0.047701298	0
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	g__Psychrobacter	0.047701298	3.552254832
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Acetobacteraceae	Unclassified	0.04731661	0.000559234
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Rhodobacter	0.04731661	0.016217788
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Bradyrhizobiaceae	Unclassified	0.043469731	0.003355404
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	Unclassified	Unclassified	Unclassified	0.039622852	0.124709198
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Limnohabitans	0.038468788	0.016777022
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Phyllobacteriaceae	g__Hoeflea	0.037699413	0
k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Nocardioidaceae	g__Propionicimonas	0.036160661	0
k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__SBR1031	f__A4b	Unclassified	0.033852534	0.00279617
k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Gemmatales	f__Gemmataceae	g__Gemmata	0.033467846	0.017336256
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	g__Acinetobacter	0.032313782	8.598783107
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Hyphomicrobiaceae	g__Hyphomicrobium	0.032313782	0.128623837
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Bdellovibrionales	f__Bdellovibrionaceae	g__Bdellovibrio	0.028082215	0.00279617
k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__BA008	Unclassified	0.025004712	0
k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__[Weeksellaceae]	g__Chryseobacterium	0.021927209	1.986399427
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Paracoccus	0.021927209	0.218101288
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Caulobacterales	f__Caulobacteraceae	g__Phenylobacterium	0.021542521	0.002236936
k__Bacteria	p__Chloroflexi	c__Thermomicrobia	o__AKYG1722	Unclassified	Unclassified	0.021157834	0.13589388
k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__[Weeksellaceae]	Unclassified	0.015387515	0.022369363

k__Bacteria	p__TM7	c__TM7-1	Unclassified	Unclassified	Unclassified	0.013464076	0
k__Bacteria	p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	g__Emticicia	0.013464076	0.006710809
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Hydrogenophilales	f__Hydrogenophilaceae	g__Thiobacillus	0.013464076	0
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__BD7-3	Unclassified	Unclassified	0.013079388	0.003914639
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__GMD14H09	Unclassified	Unclassified	0.0126947	0.054804939
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__[Acidaminobacteraceae]	g__Fusibacter	0.0126947	0.139808518
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Rhizobiaceae	g__Agrobacterium	0.011925324	0.021810129
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Hyphomicrobiaceae	Unclassified	0.011540637	0.062634216
k__Bacteria	p__Planctomycetes	c__BD7-11	Unclassified	Unclassified	Unclassified	0.011155949	0
k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	Unclassified	Unclassified	0.011155949	0.005033107
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Stenotrophomonas	0.010771261	0.044738726
k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Tsukamurellaceae	Unclassified	0.010386573	0
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Veillonellaceae	g__Selenomonas	0.009617197	0.002236936
k__Bacteria	p__Proteobacteria	c__TA18	o__PHOS-HD29	Unclassified	Unclassified	0.008078446	0
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Thiotrichales	f__Piscirickettsiaceae	Unclassified	0.008078446	0.010066213
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Hydrogenophaga	0.008078446	0.178954903
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Alicycliphilus	0.00730907	0.05760111
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Legionellales	Unclassified	Unclassified	0.006924382	0
k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__Porphyromonadaceae	Unclassified	0.006924382	0.018454724
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Rhodocyclales	f__Rhodocyclaceae	Unclassified	0.006539694	0.002236936
k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__Rikenellaceae	g__Blvii28	0.006539694	0.011743916
k__Bacteria	p__Verrucomicrobia	c__[Pedosphaerae]	o__[Pedosphaerales]	f__R4-41B	Unclassified	0.006155006	0.001118468
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Desulfobacterales	f__Desulfobulbaceae	g__Desulfobulbus	0.006155006	0.001677702
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	g__Sphingosinicella	0.006155006	0.007270043
k__Bacteria	p__TM7	Unclassified	Unclassified	Unclassified	Unclassified	0.005770318	0
k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__B97	Unclassified	Unclassified	0.005770318	0
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	f__Haliangiaceae	Unclassified	0.005770318	0
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Diaphorobacter	0.005770318	0.013980852
k__Bacteria	p__Firmicutes	c__Bacilli	o__Lactobacillales	f__Streptococcaceae	g__Streptococcus	0.005770318	0.00279617
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Enterobacteriales	f__Enterobacteriaceae	Unclassified	0.00538563	1.433316929
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Lachnospiraceae	Unclassified	0.00538563	0.006710809
k__Bacteria	p__Actinobacteria	c__Acidimicrobiia	o__Acidimicrobiales	f__Microthrixaceae	Unclassified	0.00538563	0.005592341
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Phyllobacteriaceae	g__Aminobacter	0.00538563	0.000559234
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	g__Enhydrobacter	0.00538563	0.055923407
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Caulobacterales	Unclassified	Unclassified	0.005000942	0.000559234

k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Rhodocyclales	f__Rhodocyclaceae	g__Azospira	0.005000942	0.001677702
k__Archaea	p__Euryarchaeota	c__Methanomicrobia	o__Methanosarcinales	f__Methanosaetaceae	g__Methanosaeta	0.005000942	0.001118468
k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Desulfobacterales	f__Desulfobulbaceae	Unclassified	0.004616255	0.003914639
k__Bacteria	p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	g__Dyadobacter	0.004616255	0.016217788
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Rhodanobacter	0.004616255	0
k__Bacteria	p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	g__Spirosoma	0.004616255	0.000559234
k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Gemmatales	f__Isosphaeraceae	g__Nostocoida	0.004231567	0
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Rhodocyclales	f__Rhodocyclaceae	g__Thauera	0.004231567	0.031876342
k__Bacteria	p__Acidobacteria	c__Acidobacteria-6	o__iii1-15	Unclassified	Unclassified	0.003846879	0.014540086
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Neisseriales	f__Neisseriaceae	Unclassified	0.003846879	0
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Enterobacteriales	f__Enterobacteriaceae	g__Klebsiella	0.003462191	9.028834109
k__Bacteria	p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Chitinophagaceae	g__Lacibacter	0.003462191	0.097865963
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Alcaligenaceae	g__Pigmentiphaga	0.003462191	0
k__Bacteria	p__Cyanobacteria	c__4C0d-2	o__MLE1-12	Unclassified	Unclassified	0.003077503	0
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Veillonellaceae	Unclassified	0.003077503	0.003914639
k__Bacteria	p__Synergistetes	c__Synergistia	o__Synergistales	f__Dethiosulfovibrionaceae	g__HA73	0.003077503	0.000559234
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Hylemonella	0.003077503	0.043061024
k__Bacteria	p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Chitinophagaceae	g__Niabella	0.003077503	0
k__Bacteria	p__Bacteroidetes	c__Sphingobacteriia	o__Sphingobacteriales	f__Sphingobacteriaceae	g__Pedobacter	0.003077503	0.007829277
k__Bacteria	p__Spirochaetes	c__Spirochaetes	o__Spirochaetales	f__Spirochaetaceae	g__Treponema	0.003077503	0.000559234
k__Bacteria	p__Acidobacteria	c__iii1-8	o__SJA-36	Unclassified	Unclassified	0.002692815	0
k__Bacteria	p__Lentisphaerae	c__[Lentisphaeria]	o__Victivallales	f__Victivallaceae	Unclassified	0.002692815	0.003355404
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Rhizobiaceae	g__Ensifer	0.002692815	0
k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__Porphyromonadaceae	g__Paludibacter	0.002692815	0.003914639
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k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Chromatiales	Unclassified	Unclassified	0.002308127	0.205238905
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Aquincola	0.002308127	0
k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__Bacillaceae	g__Bacillus	0.002308127	0.112965283
k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Caldilineales	f__Caldilineaceae	g__Caldilinea	0.002308127	0
k__Bacteria	p__Spirochaetes	c__[Leptospirae]	o__[Leptospirales]	f__Leptospiraceae	g__Leptospira	0.002308127	0
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Luteimonas	0.002308127	0.030757874
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k__Bacteria	p__GN02	Unclassified	Unclassified	Unclassified	Unclassified	0.001923439	0

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k__Bacteria	p__TM7	c__TM7-3	o__EW055	Unclassified	Unclassified	0.001538752	0.007829277
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k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Xanthobacteraceae	g__Azorhizobium	0.001538752	0
k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Mycobacteriaceae	g__Mycobacterium	0.001538752	0.003914639
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k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Rhodovulum	0.001154064	0.002236936
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k__Bacteria	p__Cyanobacteria	c__ML635J-21	Unclassified	Unclassified	Unclassified	0.000769376	0.003914639
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k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Alcaligenaceae	Unclassified	0.000769376	0.011743916
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k__Bacteria	p__Thermotogae	c__Thermotogae	o__Thermotogales	f__Thermotogaceae	g__AUTHM297	0.000769376	0
k__Bacteria	p__Verrucomicrobia	c__[Spartobacteria]	o__[Chthoniobacterales]	f__[Chthoniobacteraceae]	g__DA101	0.000769376	0
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Rhodocyclales	f__Rhodocyclaceae	g__Dechloromonas	0.000769376	0.005592341
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k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Lachnospiraceae	g__Lachnospira	0.000769376	0
k__Bacteria	p__Firmicutes	c__Bacilli	o__Lactobacillales	f__Streptococcaceae	g__Lactococcus	0.000769376	0
k__Bacteria	p__Spirochaetes	c__[Leptospirae]	o__[Leptospirales]	f__Leptospiraceae	g__Leptonema	0.000769376	0

k__Archaea	p__Euryarchaeota	c__Methanobacteria	o__Methanobacteriales	f__Methanobacteriaceae	g__Methanobacterium	0.000769376	0
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k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Thiomonas	0.000769376	0
k__Bacteria	p__SBR1093	c__VHS-B5-50	Unclassified	Unclassified	Unclassified	0.000384688	0.113524517
k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	Unclassified	Unclassified	0.000384688	0.622986757
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	Unclassified	Unclassified	0.000384688	1.01333214
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k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	Unclassified	Unclassified	Unclassified	0	0.016777022
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k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__ML635J-40	Unclassified	0	0.001118468
k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Nocardioidaceae	Unclassified	0	0.001118468
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k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	f__OM27	Unclassified	0	0.001118468

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k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Microbacteriaceae	g__Cryobacterium	0	0.003914639
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k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Oxalobacteraceae	g__Cupriavidus	0	0.001118468
k__Bacteria	p__[Thermi]	c__Deinococci	o__Deinococcales	f__Deinococcaceae	g__Deinococcus	0	0.475908196
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k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Desulfobacterales	f__Desulfobacteraceae	g__Desulfobotulus	0	0.001118468

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k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Desulfuromonadales	f__Desulfuromonadaceae	g__Desulfuromonas	0	0.002236936
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__[Tissierellaceae]	g__Dethiosulfatibacter	0	0.063752684
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Neisseriales	f__Neisseriaceae	g__Elbe	0	0.002236936
k__Bacteria	p__Firmicutes	c__Bacilli	o__Lactobacillales	f__Enterococcaceae	g__Enterococcus	0	0.001677702
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Enterobacteriales	f__Enterobacteriaceae	g__Erwinia	0	0.010066213
k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__[Exiguobacteraceae]	g__Exiguobacterium	0	0.010625447
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k__Bacteria	p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Chitinophagaceae	g__Flavisolibacter	0	0.001118468
k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Flavobacteriaceae	g__Flavobacterium	0	1.263309771
k__Bacteria	p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	g__Flectobacillus	0	0.026284001
k__Bacteria	p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Cryomorphaceae	g__Fluviicola	0	0.009506979
k__Bacteria	p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Saprospiraceae	g__Haliscomenobacter	0	0.004473873
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Oceanospirillales	f__Halomonadaceae	g__Halomonas	0	0.065989621
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Alteromonadales	f__HTCC2188	g__HTCC	0	0.002236936
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Alteromonadales	f__Idiomarinaceae	g__Idiomarina	0	0.00279617
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Rhodospirillaceae	g__Inquilinus	0	0.001118468
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Oxalobacteraceae	g__Janthinobacterium	0	0.069345025
k__Bacteria	p__Thermotogae	c__Thermotogae	o__Thermotogales	f__Thermotogaceae	g__Kosmotoga	0	0.00279617
k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Microbacteriaceae	g__Leucobacter	0	0.002236936
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Loktanella	0	0.007829277
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Erythrobacteraceae	g__Lutibacterium	0	0.001118468
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Rhodospirillaceae	g__Magnetospirillum	0	0.010625447
k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__Bacillaceae	g__Marinibacillus	0	0.00279617
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Alteromonadales	f__Alteromonadaceae	g__Marinimicrobium	0	0.00279617
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Alteromonadales	f__Alteromonadaceae	g__Marinobacter	0	0.245503758
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Caulobacterales	f__Caulobacteraceae	g__Mycoplana	0	0.00279617
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Peptococcaceae	g__Niigata-25	0	0.01230315
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Oceanospirillales	f__Oceanospirillaceae	g__Nitrincola	0	0.00279617
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Nitrosomonadales	f__Nitrosomonadaceae	g__Nitrosomonas	0	0.038587151
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Rhodospirillaceae	g__Novispirillum	0	0.006710809

k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Hyphomonadaceae	g__Oceanicaulis	0	0.221456693
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Oceanospirillales	f__Oceanospirillaceae	g__Oleibacter	0	0.005592341
k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__Paenibacillaceae	g__Paenibacillus	0	0.00279617
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Hyphomicrobiaceae	g__Parvibaculum	0	0.18119184
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Vibrionales	f__Vibrionaceae	g__Photobacterium	0	0.001118468
k__Bacteria	p__Cyanobacteria	c__Oscillatoriophycideae	o__Oscillatoriales	f__Phormidiaceae	g__Planktothrix	0	0.001118468
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Polaromonas	0	0.001118468
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Hyphomicrobiaceae	g__Polymorphum	0	0.001118468
k__Bacteria	p__Firmicutes	c__Bacilli	o__Bacillales	f__Bacillaceae	g__Pontibacillus	0	0.001118468
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Clostridiaceae	g__Proteiniclasticum	0	0.03019864
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Pseudofulvimonas	0	0.180632606
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Ramlibacter	0	0.031876342
k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Rhodobaca	0	0.011743916
k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Microbacteriaceae	g__Salinibacterium	0	0.197409628
k__Bacteria	p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Chitinophagaceae	g__Sediminibacterium	0	0.039705619
k__Bacteria	p__Bacteroidetes	c__Sphingobacteriia	o__Sphingobacteriales	f__Sphingobacteriaceae	g__Sphingobacterium	0	0.034113278
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Aeromonadales	f__Succinivibrionaceae	g__Succinivibrio	0	0.001118468
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Peptostreptococcaceae	g__Tepidibacter	0	0.001677702
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Chromatiales	f__Ectothiorhodospiraceae	g__Thioalkalivibrio	0	0.763913744
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Chromatiales	f__Chromatiaceae	g__Thiocapsa	0	0.077174302
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Clostridiaceae	g__Tindallia_Anoxynatronum	0	0.01789549
k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__[Tissierellaceae]	g__Tissierella_Soehngenia	0	0.178395669
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Enterobacteriales	f__Enterobacteriaceae	g__Trabulsiella	0	0.002236936
k__Bacteria	p__Synergistetes	c__Synergistia	o__Synergistales	f__Synergistaceae	g__vadinCA02	0	0.001118468
k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Variovorax	0	0.017336256
k__Bacteria	p__Verrucomicrobia	c__Verrucomicrobiae	o__Verrucomicrobiales	f__Verrucomicrobiaceae	g__Verrucomicrobium	0	0.020691661
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Vibrionales	f__Vibrionaceae	g__Vibrio	0	0.048653364
k__Bacteria	p__Chlamydiae	c__Chlamydiia	o__Chlamydiales	f__Waddliaceae	g__Waddlia	0	0.015658554
k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Aeromonadales	f__Aeromonadaceae	g__Zobellella	0	0.001118468

E. Species Level

Phylum	Class	Order	Family	Genus	Species	SBRReactor	CETP
p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	Unclassified	Unclassified	Unclassified	0	0.15378937
p__Actinobacteria	c__Acidimicrobiia	o__Acidimicrobiales	Unclassified	Unclassified	Unclassified	0	0.014540086

p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	Unclassified	Unclassified	Unclassified	0.240814615	0.002236936
p__Chloroflexi	c__Thermomicrobia	o__AKYG1722	Unclassified	Unclassified	Unclassified	0.021157834	0.13589388
p__Proteobacteria	c__Betaproteobacteria	o__ASSO-13	Unclassified	Unclassified	Unclassified	0.001923439	2.017716535
p__Planctomycetes	c__Planctomycetia	o__B97	Unclassified	Unclassified	Unclassified	0.005770318	0
p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	Unclassified	Unclassified	Unclassified	0.011155949	0.005033107
p__Proteobacteria	c__Alphaproteobacteria	o__BD7-3	Unclassified	Unclassified	Unclassified	0.013079388	0.003914639
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	Unclassified	Unclassified	Unclassified	0	0.034672513
p__Proteobacteria	c__Alphaproteobacteria	o__Caulobacterales	Unclassified	Unclassified	Unclassified	0.005000942	0.000559234
p__Proteobacteria	c__Gammaproteobacteria	o__Chromatiales	Unclassified	Unclassified	Unclassified	0.002308127	0.205238905
p__Planctomycetes	c__OM190	o__CL500-15	Unclassified	Unclassified	Unclassified	0	0.024606299
p__Firmicutes	c__Clostridia	o__Clostridiales	Unclassified	Unclassified	Unclassified	0.001154064	0
p__Proteobacteria	c__Alphaproteobacteria	o__Ellin329	Unclassified	Unclassified	Unclassified	0.070397883	0.000559234
p__TM7	c__TM7-3	o__EW055	Unclassified	Unclassified	Unclassified	0.001538752	0.007829277
p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	Unclassified	Unclassified	Unclassified	0.000384688	0.622986757
p__Actinobacteria	c__Thermoleophilia	o__Gaiellales	Unclassified	Unclassified	Unclassified	0	0.001118468
p__Proteobacteria	c__Deltaproteobacteria	o__GMD14H09	Unclassified	Unclassified	Unclassified	0.0126947	0.054804939
p__Proteobacteria	c__Gammaproteobacteria	o__HTCC2188	Unclassified	Unclassified	Unclassified	0	0.258366142
p__Acidobacteria	c__Acidobacteria-6	o__iii1-15	Unclassified	Unclassified	Unclassified	0.003846879	0.014540086
p__Chloroflexi	c__Thermomicrobia	o__JG30-KF-CM45	Unclassified	Unclassified	Unclassified	0.221195533	0.003355404
p__Proteobacteria	c__Alphaproteobacteria	o__Kiloniellales	Unclassified	Unclassified	Unclassified	0	0.00279617
p__Proteobacteria	c__Gammaproteobacteria	o__Legionellales	Unclassified	Unclassified	Unclassified	0.006924382	0
p__Cyanobacteria	c__4C0d-2	o__MLE1-12	Unclassified	Unclassified	Unclassified	0.003077503	0
p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	Unclassified	Unclassified	Unclassified	0.563952437	0.001677702
p__Proteobacteria	c__TA18	o__PHOS-HD29	Unclassified	Unclassified	Unclassified	0.008078446	0
p__Planctomycetes	c__Phycisphaerae	o__Phycisphaerales	Unclassified	Unclassified	Unclassified	0	0.025724767
p__Spirochaetes	c__MVP-15	o__PL-11B10	Unclassified	Unclassified	Unclassified	0	0.114083751
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	Unclassified	Unclassified	Unclassified	0.359683171	0.112965283
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	Unclassified	Unclassified	Unclassified	0.000384688	1.01333214
p__Proteobacteria	c__Alphaproteobacteria	o__Rickettsiales	Unclassified	Unclassified	Unclassified	0	0.007829277
p__Chloroflexi	c__Anaerolineae	o__S0208	Unclassified	Unclassified	Unclassified	0	0.001677702
p__Proteobacteria	c__Betaproteobacteria	o__SC-I-84	Unclassified	Unclassified	Unclassified	0.055395055	0
p__Acidobacteria	c__iii1-8	o__SJA-36	Unclassified	Unclassified	Unclassified	0.002692815	0
p__Acidobacteria	c__Solibacteres	o__Solibacterales	Unclassified	Unclassified	Unclassified	0.001538752	0
p__Bacteroidetes	c__Sphingobacteriia	o__Sphingobacteriales	Unclassified	Unclassified	Unclassified	1.714938585	0.029639406
p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	Unclassified	Unclassified	Unclassified	0.001154064	0.002236936

p__Planctomycetes	c__Phycisphaerae	o__WD2101	Unclassified	Unclassified	Unclassified	0.001154064	0
p__GN02	Unclassified	Unclassified	Unclassified	Unclassified	Unclassified	0.001923439	0
p__NKB19	Unclassified	Unclassified	Unclassified	Unclassified	Unclassified	0.000769376	0.04529796
p__SR1	Unclassified	Unclassified	Unclassified	Unclassified	Unclassified	0	0.001118468
p__TM7	Unclassified	Unclassified	Unclassified	Unclassified	Unclassified	0.005770318	0
p__WPS-2	Unclassified	Unclassified	Unclassified	Unclassified	Unclassified	0	0.085003579
p__Proteobacteria	c__Alphaproteobacteria	Unclassified	Unclassified	Unclassified	Unclassified	0.039622852	0.124709198
p__WS6	c__B142	Unclassified	Unclassified	Unclassified	Unclassified	0	0.606209735
p__Planctomycetes	c__BD7-11	Unclassified	Unclassified	Unclassified	Unclassified	0.011155949	0
p__Proteobacteria	c__Betaproteobacteria	Unclassified	Unclassified	Unclassified	Unclassified	0	0.016777022
p__Proteobacteria	c__Deltaproteobacteria	Unclassified	Unclassified	Unclassified	Unclassified	0	0.007829277
p__Chloroflexi	c__Ellin6529	Unclassified	Unclassified	Unclassified	Unclassified	0.000769376	0
p__Proteobacteria	c__Gammaproteobacteria	Unclassified	Unclassified	Unclassified	Unclassified	0	0.016777022
p__Gemmatimonadetes	c__Gemm-2	Unclassified	Unclassified	Unclassified	Unclassified	0	0.069904259
p__Gemmatimonadetes	c__Gemm-3	Unclassified	Unclassified	Unclassified	Unclassified	0	0.002236936
p__Cyanobacteria	c__ML635J-21	Unclassified	Unclassified	Unclassified	Unclassified	0.000769376	0.003914639
p__Tenericutes	c__Mollicutes	Unclassified	Unclassified	Unclassified	Unclassified	0	0.001677702
p__Chlorobi	c__OPB56	Unclassified	Unclassified	Unclassified	Unclassified	1.304476613	0.002236936
p__OP3	c__PBS-25	Unclassified	Unclassified	Unclassified	Unclassified	0.002308127	0
p__BRC1	c__PRR-11	Unclassified	Unclassified	Unclassified	Unclassified	0	0.019573193
p__Chloroflexi	c__TK17	Unclassified	Unclassified	Unclassified	Unclassified	0	0.046975662
p__TM7	c__TM7-1	Unclassified	Unclassified	Unclassified	Unclassified	0.013464076	0
p__TM7	c__TM7-3	Unclassified	Unclassified	Unclassified	Unclassified	0.74706387	0.091714388
p__Verrucomicrobia	c__Verruco-5	Unclassified	Unclassified	Unclassified	Unclassified	0	0.003914639
p__SBR1093	c__VHS-B5-50	Unclassified	Unclassified	Unclassified	Unclassified	0.000384688	0.113524517
p__Bacteroidetes	c__[Rhodothermi]	o__[Rhodothermales]	f__[Balneolaceae]	Unclassified	Unclassified	0	0.005592341
p__Proteobacteria	c__Gammaproteobacteria	o__[Marinicellales]	f__[Marinicellaceae]	Unclassified	Unclassified	0	0.009506979
p__Firmicutes	c__Clostridia	o__Clostridiales	f__[Mogibacteriaceae]	Unclassified	Unclassified	0.001538752	0
p__Firmicutes	c__Clostridia	o__Clostridiales	f__[Tissierellaceae]	Unclassified	Unclassified	0	0.005592341
p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__[Weeksellaceae]	Unclassified	Unclassified	0.015387515	0.022369363
p__Chloroflexi	c__Anaerolineae	o__SBR1031	f__A4b	Unclassified	Unclassified	0.033852534	0.00279617
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Acetobacteraceae	Unclassified	Unclassified	0.04731661	0.000559234
p__Tenericutes	c__Mollicutes	o__Acholeplasmatales	f__Acholeplasmataceae	Unclassified	Unclassified	0.000769376	0
p__Proteobacteria	c__Gammaproteobacteria	o__Aeromonadales	f__Aeromonadaceae	Unclassified	Unclassified	0.219656781	34.19380816
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Alcaligenaceae	Unclassified	Unclassified	0.000769376	0.011743916

p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Aurantimonadaceae	Unclassified	Unclassified	0	0.001677702
p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__BA008	Unclassified	Unclassified	0.025004712	0
p__Proteobacteria	c__Deltaproteobacteria	o__Bdellovibrionales	f__Bacteriovoracaceae	Unclassified	Unclassified	0.069243819	0.012862384
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Bartonellaceae	Unclassified	Unclassified	0.200037699	0.00279617
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Bradyrhizobiaceae	Unclassified	Unclassified	0.043469731	0.003355404
p__Actinobacteria	c__Acidimicrobiia	o__Acidimicrobiales	f__C111	Unclassified	Unclassified	0	0.026284001
p__Chloroflexi	c__Anaerolineae	o__Caldilineales	f__Caldilineaceae	Unclassified	Unclassified	0.076168201	0
p__Proteobacteria	c__Alphaproteobacteria	o__Caulobacterales	f__Caulobacteraceae	Unclassified	Unclassified	0.000384688	0.008947745
p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Chitinophagaceae	Unclassified	Unclassified	8.186927536	0.102899069
p__Proteobacteria	c__Gammaproteobacteria	o__Chromatiales	f__Chromatiaceae	Unclassified	Unclassified	0	0.003914639
p__Firmicutes	c__Clostridia	o__Clostridiales	f__Clostridiaceae	Unclassified	Unclassified	0	0.014540086
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	Unclassified	Unclassified	2.769752761	3.382247674
p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Cryomorphaceae	Unclassified	Unclassified	0	0.031876342
p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cyclobacteriaceae	Unclassified	Unclassified	0.000384688	0.021250895
p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	Unclassified	Unclassified	0.256971506	0.021810129
p__Proteobacteria	c__Deltaproteobacteria	o__Desulfobacterales	f__Desulfobacteraceae	Unclassified	Unclassified	0	0.001118468
p__Proteobacteria	c__Deltaproteobacteria	o__Desulfobacterales	f__Desulfobulbaceae	Unclassified	Unclassified	0.004616255	0.003914639
p__Proteobacteria	c__Deltaproteobacteria	o__Desulfuromonadales	f__Desulfuromonadaceae	Unclassified	Unclassified	0	0.007270043
p__Proteobacteria	c__Gammaproteobacteria	o__Chromatiales	f__Ectothiorhodospiraceae	Unclassified	Unclassified	0	0.004473873
p__Proteobacteria	c__Gammaproteobacteria	o__Enterobacteriales	f__Enterobacteriaceae	Unclassified	Unclassified	0.00538563	1.433316929
p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Erythrobacteraceae	Unclassified	Unclassified	0	0.004473873
p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Flammeovirgaceae	Unclassified	Unclassified	0	0.008388511
p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Flavobacteriaceae	Unclassified	Unclassified	0	0.012862384
p__Proteobacteria	c__Gammaproteobacteria	o__Legionellales	f__Francisellaceae	Unclassified	Unclassified	0	0.011184681
p__Planctomycetes	c__Planctomycetia	o__Gemmatales	f__Gemmataceae	Unclassified	Unclassified	0.060780686	0
p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__GZKB119	Unclassified	Unclassified	0.000769376	0.450183429
p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	f__Haliangiaceae	Unclassified	Unclassified	0.005770318	0
p__Proteobacteria	c__Gammaproteobacteria	o__HTCC2188	f__HTCC2089	Unclassified	Unclassified	0	0.003355404
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Hyphomicrobiaceae	Unclassified	Unclassified	0.011540637	0.062634216
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Hyphomonadaceae	Unclassified	Unclassified	0.000384688	0.002236936
p__Proteobacteria	c__Gammaproteobacteria	o__Alteromonadales	f__Idiomarinaceae	Unclassified	Unclassified	0	0.017336256
p__Chlorobi	c__Ignavibacteria	o__Ignavibacteriales	f__Ignavibacteriaceae	Unclassified	Unclassified	0.001923439	0
p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Intrasporangiaceae	Unclassified	Unclassified	0	0.011743916
p__Planctomycetes	c__Planctomycetia	o__Gemmatales	f__Isosphaeraceae	Unclassified	Unclassified	0.073090698	0.000559234
p__Proteobacteria	c__Deltaproteobacteria	o__NB1-j	f__JTB38	Unclassified	Unclassified	0	0.00279617

p__Firmicutes	c__Clostridia	o__Clostridiales	f__Lachnospiraceae	Unclassified	Unclassified	0.00538563	0.006710809
p__Chlorobi	c__Ignavibacteria	o__Ignavibacteriales	f__lheB3-7	Unclassified	Unclassified	0.052317552	0.016217788
p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Microbacteriaceae	Unclassified	Unclassified	0.000384688	0.432847173
p__Actinobacteria	c__Acidimicrobiia	o__Acidimicrobiales	f__Microthrixaceae	Unclassified	Unclassified	0.00538563	0.005592341
p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__ML635J-40	Unclassified	Unclassified	0	0.001118468
p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	Unclassified	Unclassified	0.221195533	0.19908733
p__Proteobacteria	c__Betaproteobacteria	o__Neisseriales	f__Neisseriaceae	Unclassified	Unclassified	0.003846879	0
p__Proteobacteria	c__Betaproteobacteria	o__Nitrosomonadales	f__Nitrosomonadaceae	Unclassified	Unclassified	1.351793223	0.005033107
p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Nocardioidaceae	Unclassified	Unclassified	0	0.001118468
p__Proteobacteria	c__Gammaproteobacteria	o__Oceanospirillales	f__Oceanospirillaceae	Unclassified	Unclassified	0	0.044179492
p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	f__OM27	Unclassified	Unclassified	0	0.001118468
p__Verrucomicrobia	c__Opitutae	o__Opitutales	f__Opitutaceae	Unclassified	Unclassified	0.047701298	0
p__Chlorobi	c__Ignavibacteria	o__Ignavibacteriales	f__OTUb7	Unclassified	Unclassified	0	0.013421618
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Oxalobacteraceae	Unclassified	Unclassified	0.000769376	6.24720383
p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__p-2534-18B5	Unclassified	Unclassified	0	0.01509932
p__Firmicutes	c__Clostridia	o__Clostridiales	f__Peptococcaceae	Unclassified	Unclassified	0.000384688	0.001118468
p__Firmicutes	c__Clostridia	o__Clostridiales	f__Peptostreptococcaceae	Unclassified	Unclassified	0.000384688	0.009506979
p__Planctomycetes	c__Phycisphaerae	o__Phycisphaerales	f__Phycisphaeraceae	Unclassified	Unclassified	0	0.001677702
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Phyllobacteriaceae	Unclassified	Unclassified	0.083861959	0.015658554
p__Planctomycetes	c__Planctomycetia	o__Pirellulales	f__Pirellulaceae	Unclassified	Unclassified	0.27812934	0.180632606
p__Proteobacteria	c__Gammaproteobacteria	o__Thiotrichales	f__Piscirickettsiaceae	Unclassified	Unclassified	0.008078446	0.010066213
p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__Porphyromonadaceae	Unclassified	Unclassified	0.006924382	0.018454724
p__Proteobacteria	c__Gammaproteobacteria	o__Vibrionales	f__Pseudoalteromonadaceae	Unclassified	Unclassified	0	0.536305476
p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	Unclassified	Unclassified	0	0.110169112
p__Verrucomicrobia	c__[Pedosphaerae]	o__[Pedosphaerales]	f__R4-41B	Unclassified	Unclassified	0.006155006	0.001118468
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Rhizobiaceae	Unclassified	Unclassified	0.351220038	0.050331067
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	Unclassified	Unclassified	8.255786667	0.673317824
p__Proteobacteria	c__Betaproteobacteria	o__Rhodocyclales	f__Rhodocyclaceae	Unclassified	Unclassified	0.006539694	0.002236936
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Rhodospirillaceae	Unclassified	Unclassified	0.068474443	0.066548855
p__Proteobacteria	c__Alphaproteobacteria	o__Rickettsiales	f__Rickettsiaceae	Unclassified	Unclassified	0	0.001118468
p__Firmicutes	c__Clostridia	o__Clostridiales	f__Ruminococcaceae	Unclassified	Unclassified	0.000769376	0.002236936
p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Saprospiraceae	Unclassified	Unclassified	0	0.638645311
p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__SB-1	Unclassified	Unclassified	0.001154064	0.040264853
p__Chloroflexi	c__Anaerolineae	o__SBR1031	f__SHA-31	Unclassified	Unclassified	0	0.045857194
p__Acidobacteria	c__Solibacteres	o__Solibacterales	f__Solibacteraceae	Unclassified	Unclassified	0.102326977	0.006710809

p__Bacteroidetes	c__Sphingobacteriia	o__Sphingobacteriales	f__Sphingobacteriaceae	Unclassified	Unclassified	0	0.00279617
p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	Unclassified	Unclassified	0.304672804	0.084444345
p__Spirochaetes	c__Spirochaetes	o__Spirochaetales	f__Spirochaetaceae	Unclassified	Unclassified	0	0.01230315
p__Synergistetes	c__Synergistia	o__Synergistales	f__Thermovirgaceae	Unclassified	Unclassified	0	0.001118468
p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Tsukamurellaceae	Unclassified	Unclassified	0.010386573	0
p__Firmicutes	c__Clostridia	o__Clostridiales	f__Veillonellaceae	Unclassified	Unclassified	0.003077503	0.003914639
p__Lentisphaerae	c__[Lentisphaeria]	o__Victivallales	f__Victivallaceae	Unclassified	Unclassified	0.002692815	0.003355404
p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	Unclassified	Unclassified	16.43886733	2.178775948
p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__[Paraprevotellaceae]	g__[Prevotella]	Unclassified	0	0.001118468
p__Planctomycetes	c__Planctomycetia	o__Pirellulales	f__Pirellulaceae	g__A17	Unclassified	0.236583048	0
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Alcaligenaceae	g__Achromobacter	Unclassified	0.000769376	0
p__Firmicutes	c__Clostridia	o__Clostridiales	f__[Acidaminobacteraceae]	g__Acidaminobacter	Unclassified	0.001538752	0
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Acidovorax	Unclassified	0	0.043061024
p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	g__Acinetobacter	Unclassified	0.031929094	4.469398712
p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Flavobacteriaceae	g__Aequorivita	Unclassified	0.256971506	0.001118468
p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Nocardioidaceae	g__Aeromicrobium	Unclassified	0	0.001118468
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Rhizobiaceae	g__Agrobacterium	Unclassified	0.011925324	0.021810129
p__Proteobacteria	c__Gammaproteobacteria	o__Oceanospirillales	f__Alcanivoracaceae	g__Alcanivorax	Unclassified	0	0.590551181
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Alicycliphilus	Unclassified	0.00730907	0.05760111
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Amaricoccus	Unclassified	0	0.044179492
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Phyllobacteriaceae	g__Aminobacter	Unclassified	0.00538563	0.000559234
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Anaerospora	Unclassified	0	0.002236936
p__Firmicutes	c__Clostridia	o__Clostridiales	f__[Mogibacteriaceae]	g__Anaerovorax	Unclassified	0	0.007270043
p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Aquimonas	Unclassified	0.001154064	0.000559234
p__Proteobacteria	c__Epsilonproteobacteria	o__Campylobacterales	f__Campylobacteraceae	g__Arcobacter	Unclassified	0	0.070463493
p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Flavobacteriaceae	g__Arenibacter	Unclassified	0	0.001118468
p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Arenimonas	Unclassified	0.553950552	0
p__Thermotogae	c__Thermotogae	o__Thermotogales	f__Thermotogaceae	g__AUTHM297	Unclassified	0.000769376	0
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Xanthobacteraceae	g__Azorhizobium	Unclassified	0.001538752	0
p__Proteobacteria	c__Betaproteobacteria	o__Rhodocyclales	f__Rhodocyclaceae	g__Azospira	Unclassified	0.005000942	0.001677702
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Rhodospirillaceae	g__Azospirillum	Unclassified	0.000384688	0.134216178
p__[Thermi]	c__Deinococci	o__Deinococcales	f__Trueperaceae	g__B-42	Unclassified	0.133102008	1.055274696
p__Firmicutes	c__Bacilli	o__Bacillales	f__Bacillaceae	g__Bacillus	Unclassified	0.002308127	0.099543665
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Bradyrhizobiaceae	g__Balneimonas	Unclassified	0	0.001118468
p__Proteobacteria	c__Deltaproteobacteria	o__Bdellovibrionales	f__Bdellovibrionaceae	g__Bdellovibrio	Unclassified	0.028082215	0.00279617

p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__Rikenellaceae	g__Blvii28	Unclassified	0.006539694	0.011743916
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Bradyrhizobiaceae	g__Bradyrhizobium	Unclassified	0.171570796	0.005033107
p__Chloroflexi	c__Anaerolineae	o__Caldilineales	f__Caldilineaceae	g__Caldilinea	Unclassified	0.002308127	0
p__Verrucomicrobia	c__[Spartobacteria]	o__[Chthoniobacterales]	f__[Chthoniobacteraceae]	g__Candidatus Xiphinematobacter	Unclassified	0.141180453	0.002236936
p__Proteobacteria	c__Alphaproteobacteria	o__Caulobacterales	f__Caulobacteraceae	g__Caulobacter	Unclassified	0	0.001677702
p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__[Weeksellaceae]	g__Chryseobacterium	Unclassified	0.021927209	1.986399427
p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__[Weeksellaceae]	g__Cloacibacterium	Unclassified	0.467395778	0.196850394
p__Firmicutes	c__Clostridia	o__Clostridiales	f__Clostridiaceae	g__Clostridium	Unclassified	0	0.014540086
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Comamonas	Unclassified	0.084246646	0.163855583
p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Microbacteriaceae	g__Cryocola	Unclassified	0	0.003355404
p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Cryomorphaceae	g__Cryomorpha	Unclassified	0	0.109609878
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Oxalobacteraceae	g__Cupriavidus	Unclassified	0	0.001118468
p__Verrucomicrobia	c__[Spartobacteria]	o__[Chthoniobacterales]	f__[Chthoniobacteraceae]	g__DA101	Unclassified	0.000769376	0
p__Proteobacteria	c__Betaproteobacteria	o__Rhodocyclales	f__Rhodocyclaceae	g__Dechloromonas	Unclassified	0.000769376	0.005592341
p__[Thermi]	c__Deinococci	o__Deinococcales	f__Deinococcaceae	g__Deinococcus	Unclassified	0	0.475908196
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Delftia	Unclassified	0.000384688	0.175599499
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Alcaligenaceae	g__Denitrobacter	Unclassified	0.250047124	0.001677702
p__Firmicutes	c__Clostridia	o__Clostridiales	f__Peptococcaceae	g__Desulfitobacter	Unclassified	0	0.001118468
p__Proteobacteria	c__Deltaproteobacteria	o__Desulfobacterales	f__Desulfobulbaceae	g__Desulfobulbus	Unclassified	0.003077503	0.001677702
p__Proteobacteria	c__Deltaproteobacteria	o__Desulfobacterales	f__Desulfobacteraceae	g__Desulfococcus	Unclassified	0	0.001118468
p__Proteobacteria	c__Deltaproteobacteria	o__Desulfovibrionales	f__Desulfomicrobiaceae	g__Desulfomicrobium	Unclassified	0	0.010066213
p__Proteobacteria	c__Deltaproteobacteria	o__Desulfobacterales	f__Desulfobacteraceae	g__Desulfotignum	Unclassified	0	0.010625447
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p__Proteobacteria	c__Deltaproteobacteria	o__Desulfuromonadales	f__Desulfuromonadaceae	g__Desulfuromonas	Unclassified	0	0.002236936
p__Firmicutes	c__Clostridia	o__Clostridiales	f__[Tissierellaceae]	g__Dethiosulfatibacter	Unclassified	0	0.063752684
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Hyphomicrobiaceae	g__Devosia	Unclassified	0.519328643	0.04250179
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Diaphorobacter	Unclassified	0.005770318	0.013980852
p__Proteobacteria	c__Betaproteobacteria	o__Rhodocyclales	f__Rhodocyclaceae	g__Dok59	Unclassified	0.43085043	0
p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Dokdonella	Unclassified	16.14034953	0.026843236
p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	g__Dyadobacter	Unclassified	0.004616255	0.016217788
p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	g__Emticicia	Unclassified	0.013464076	0.006710809
p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	g__Enhydrobacter	Unclassified	0.00538563	0.055923407
p__Firmicutes	c__Bacilli	o__Lactobacillales	f__Enterococcaceae	g__Enterococcus	Unclassified	0	0.001677702
p__Proteobacteria	c__Gammaproteobacteria	o__Enterobacteriales	f__Enterobacteriaceae	g__Erwinia	Unclassified	0	0.010066213
p__Firmicutes	c__Bacilli	o__Bacillales	f__[Exiguobacteraceae]	g__Exiguobacterium	Unclassified	0	0.010625447

p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Chitinophagaceae	g__Flavisolibacter	Unclassified	0	0.001118468
p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Flavobacteriaceae	g__Flavobacterium	Unclassified	0	0.642000716
p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	g__Flectobacillus	Unclassified	0	0.026284001
p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Cryomorphaceae	g__Fluviicola	Unclassified	0	0.009506979
p__Firmicutes	c__Clostridia	o__Clostridiales	f__[Acidaminobacteraceae]	g__Fusibacter	Unclassified	0.0126947	0.139808518
p__Planctomycetes	c__Planctomycetia	o__Gemmatales	f__Gemmataceae	g__Gemmata	Unclassified	0.013464076	0.017336256
p__Gemmatimonadetes	c__Gemmatimonadetes	o__Gemmatimonadales	f__Gemmatimonadaceae	g__Gemmatimonas	Unclassified	0.000769376	0.001118468
p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Gordoniaceae	g__Gordonia	Unclassified	0.063088813	0.000559234
p__Synergistetes	c__Synergistia	o__Synergistales	f__Dethiosulfovibrionaceae	g__HA73	Unclassified	0.003077503	0.000559234
p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Saprospiraceae	g__Haliscomenobacter	Unclassified	0	0.004473873
p__Proteobacteria	c__Gammaproteobacteria	o__Oceanospirillales	f__Halomonadaceae	g__Halomonas	Unclassified	0	0.064871152
p__Proteobacteria	c__Gammaproteobacteria	o__Alteromonadales	f__HTCC2188	g__HTCC	Unclassified	0	0.002236936
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Hydrogenophaga	Unclassified	0.008078446	0.178954903
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Hylemonella	Unclassified	0.003077503	0.043061024
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Hyphomicrobiaceae	g__Hyphomicrobium	Unclassified	0.0126947	0.071581961
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p__Proteobacteria	c__Gammaproteobacteria	o__Alteromonadales	f__Idiomarinaceae	g__Idiomarina	Unclassified	0	0.00279617
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Rhodospirillaceae	g__Inquilinus	Unclassified	0	0.001118468
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Oxalobacteraceae	g__Janthinobacterium	Unclassified	0	0.005033107
p__Proteobacteria	c__Gammaproteobacteria	o__Enterobacteriales	f__Enterobacteriaceae	g__Klebsiella	Unclassified	0.003462191	9.028834109
p__Firmicutes	c__Bacilli	o__Lactobacillales	f__Streptococcaceae	g__Lactococcus	Unclassified	0.000769376	0
p__Spirochaetes	c__[Leptospirae]	o__[Leptospirales]	f__Leptospiraceae	g__Leptonema	Unclassified	0.000769376	0
p__Spirochaetes	c__[Leptospirae]	o__[Leptospirales]	f__Leptospiraceae	g__Leptospira	Unclassified	0.002308127	0
p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Microbacteriaceae	g__Leucobacter	Unclassified	0	0.002236936
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Limnohabitans	Unclassified	0.038468788	0.016777022
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Loktanella	Unclassified	0	0.007829277
p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Luteimonas	Unclassified	0.002308127	0.030757874
p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Erythrobacteraceae	g__Lutibacterium	Unclassified	0	0.001118468
p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Lysobacter	Unclassified	0	0.038587151
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Rhodospirillaceae	g__Magnetospirillum	Unclassified	0	0.010625447
p__Firmicutes	c__Bacilli	o__Bacillales	f__Bacillaceae	g__Marinibacillus	Unclassified	0	0.00279617
p__Proteobacteria	c__Gammaproteobacteria	o__Alteromonadales	f__Alteromonadaceae	g__Marinimicrobium	Unclassified	0	0.00279617
p__Proteobacteria	c__Gammaproteobacteria	o__Alteromonadales	f__Alteromonadaceae	g__Marinobacter	Unclassified	0	0.245503758
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Phyllobacteriaceae	g__Mesorhizobium	Unclassified	0.595881532	0.022369363
p__Euryarchaeota	c__Methanomicrobia	o__Methanocellales	f__Methanocellaceae	g__Methanocella	Unclassified	0.000769376	0.000559234

p__Euryarchaeota	c__Methanomicrobia	o__Methanosarcinales	f__Methanosaetaceae	g__Methanosaeta	Unclassified	0.005000942	0.001118468
p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Microbacteriaceae	g__Microbacterium	Unclassified	0.000384688	0.014540086
p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Mycobacteriaceae	g__Mycobacterium	Unclassified	0.001538752	0.003914639
p__Proteobacteria	c__Alphaproteobacteria	o__Caulobacterales	f__Caulobacteraceae	g__Mycoplana	Unclassified	0	0.00279617
p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Chitinophagaceae	g__Niabella	Unclassified	0.003077503	0
p__Firmicutes	c__Clostridia	o__Clostridiales	f__Peptococcaceae	g__Niigata-25	Unclassified	0	0.01230315
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Phyllobacteriaceae	g__Nitratireductor	Unclassified	0.001154064	0.047534896
p__Proteobacteria	c__Gammaproteobacteria	o__Oceanospirillales	f__Oceanospirillaceae	g__Nitrincola	Unclassified	0	0.00279617
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Rhodospirillaceae	g__Novispirillum	Unclassified	0	0.006710809
p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	g__Novosphingobium	Unclassified	0.002308127	0.006710809
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Hyphomonadaceae	g__Oceanicaulis	Unclassified	0	0.221456693
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Brucellaceae	g__Ochrobactrum	Unclassified	0.173494235	0.000559234
p__Proteobacteria	c__Gammaproteobacteria	o__Oceanospirillales	f__Oceanospirillaceae	g__Oleibacter	Unclassified	0	0.005592341
p__Verrucomicrobia	c__Opitutae	o__Opitutales	f__Opitutaceae	g__Opitutus	Unclassified	0.103096353	0
p__Firmicutes	c__Bacilli	o__Bacillales	f__Paenibacillaceae	g__Paenibacillus	Unclassified	0	0.00279617
p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__Porphyromonadaceae	g__Paludibacter	Unclassified	0.002692815	0.003914639
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Paracoccus	Unclassified	0.016926267	0.164974052
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Hyphomicrobiaceae	g__Parvibaculum	Unclassified	0	0.18119184
p__Bacteroidetes	c__Sphingobacteriia	o__Sphingobacteriales	f__Sphingobacteriaceae	g__Pedobacter	Unclassified	0.003077503	0.007829277
p__Proteobacteria	c__Alphaproteobacteria	o__Caulobacterales	f__Caulobacteraceae	g__Phenylobacterium	Unclassified	0.021542521	0.002236936
p__Proteobacteria	c__Gammaproteobacteria	o__Vibrionales	f__Vibrionaceae	g__Photobacterium	Unclassified	0	0.001118468
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Alcaligenaceae	g__Pigmentiphaga	Unclassified	0.003462191	0
p__Planctomycetes	c__Planctomycetia	o__Planctomycetales	f__Planctomycetaceae	g__Planctomyces	Unclassified	0.499709561	0.026284001
p__Cyanobacteria	c__Oscillatoriophycideae	o__Oscillatoriales	f__Phormidiaceae	g__Planktothrix	Unclassified	0	0.001118468
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Polaromonas	Unclassified	0	0.001118468
p__Firmicutes	c__Bacilli	o__Bacillales	f__Bacillaceae	g__Pontibacillus	Unclassified	0	0.001118468
p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__Prevotellaceae	g__Prevotella	Unclassified	0.071551946	0.025724767
p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Nocardioidaceae	g__Propionicimonas	Unclassified	0.036160661	0
p__Proteobacteria	c__Betaproteobacteria	o__Rhodocyclales	f__Rhodocyclaceae	g__Propionivibrio	Unclassified	0.001154064	0
p__Firmicutes	c__Clostridia	o__Clostridiales	f__Clostridiaceae	g__Proteiniclasticum	Unclassified	0	0.03019864
p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	Unclassified	0.06539694	1.380189692
p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	g__Psychrobacter	Unclassified	0.047701298	3.551136364
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Ramlibacter	Unclassified	0	0.031876342
p__Proteobacteria	c__Gammaproteobacteria	o__Alteromonadales	f__[Chromatiaceae]	g__Rheinheimera	Unclassified	0	1.714052434
p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Rhodanobacter	Unclassified	0.004616255	0

p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Rhodobaca	Unclassified	0	0.011743916
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Rhodobacter	Unclassified	0.04731661	0.016217788
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Hyphomicrobiaceae	g__Rhodoplanes	Unclassified	0.103096353	0.001118468
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Rhodovulum	Unclassified	0.001154064	0.002236936
p__Proteobacteria	c__Gammaproteobacteria	o__Aeromonadales	f__Succinivibrionaceae	g__Ruminobacter	Unclassified	0.000384688	0.000559234
p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	g__Runella	Unclassified	0.129255129	0.008388511
p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Microbacteriaceae	g__Salinibacterium	Unclassified	0	0.197409628
p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Chitinophagaceae	g__Sediminibacterium	Unclassified	0	0.039705619
p__Firmicutes	c__Clostridia	o__Clostridiales	f__Veillonellaceae	g__Selenomonas	Unclassified	0.00538563	0
p__Chloroflexi	c__Anaerolineae	o__Anaerolineales	f__Anaerolinaceae	g__SHD-231	Unclassified	0.001538752	0
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Rhizobiaceae	g__Shinella	Unclassified	0.000384688	0.000559234
p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	g__Sphingomonas	Unclassified	0.0380841	0.002236936
p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	g__Sphingopyxis	Unclassified	1.408342341	0.071022727
p__Bacteroidetes	c__Cytophagia	o__Cytophagales	f__Cytophagaceae	g__Spirosoma	Unclassified	0.004616255	0.000559234
p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Stenotrophomonas	Unclassified	0.000384688	0.003355404
p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Sinobacteraceae	g__Steroidobacter	Unclassified	0.000384688	0.002236936
p__Firmicutes	c__Bacilli	o__Lactobacillales	f__Streptococcaceae	g__Streptococcus	Unclassified	0	0.001118468
p__Proteobacteria	c__Gammaproteobacteria	o__Aeromonadales	f__Succinivibrionaceae	g__Succinivibrio	Unclassified	0	0.001118468
p__Firmicutes	c__Clostridia	o__Clostridiales	f__Syntrophomonadaceae	g__Syntrophomonas	Unclassified	0.001154064	0
p__Firmicutes	c__Clostridia	o__Clostridiales	f__Peptostreptococcaceae	g__Tepidibacter	Unclassified	0	0.001677702
p__Proteobacteria	c__Betaproteobacteria	o__Rhodocyclales	f__Rhodocyclaceae	g__Thauera	Unclassified	0.004231567	0.031876342
p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Thermomonas	Unclassified	21.79026047	0.044738726
p__Proteobacteria	c__Gammaproteobacteria	o__Chromatiales	f__Ectothiorhodospiraceae	g__Thioalkalivibrio	Unclassified	0	0.626901396
p__Proteobacteria	c__Betaproteobacteria	o__Hydrogenophilales	f__Hydrogenophilaceae	g__Thiobacillus	Unclassified	0.013464076	0
p__Proteobacteria	c__Gammaproteobacteria	o__Chromatiales	f__Chromatiaceae	g__Thiocapsa	Unclassified	0	0.077174302
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Thiomonas	Unclassified	0.000769376	0
p__Firmicutes	c__Clostridia	o__Clostridiales	f__Clostridiaceae	g__Tindallia_Anoxynatronum	Unclassified	0	0.01789549
p__Firmicutes	c__Clostridia	o__Clostridiales	f__[Tissierellaceae]	g__Tissierella_Soehngenia	Unclassified	0	0.178395669
p__Proteobacteria	c__Gammaproteobacteria	o__Enterobacteriales	f__Enterobacteriaceae	g__Trabulsiella	Unclassified	0	0.002236936
p__Spirochaetes	c__Spirochaetes	o__Spirochaetales	f__Spirochaetaceae	g__Treponema	Unclassified	0.003077503	0.000559234
p__Synergistetes	c__Synergistia	o__Synergistales	f__Synergistaceae	g__vadinCA02	Unclassified	0	0.001118468
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Variovorax	Unclassified	0	0.016217788
p__Verrucomicrobia	c__Verrucomicrobiae	o__Verrucomicrobiales	f__Verrucomicrobiaceae	g__Verrucomicrobium	Unclassified	0	0.020691661
p__Proteobacteria	c__Gammaproteobacteria	o__Vibrionales	f__Vibrionaceae	g__Vibrio	Unclassified	0	0.021810129
p__Proteobacteria	c__Betaproteobacteria	o__Neisseriales	f__Neisseriaceae	g__Vogesella	Unclassified	0.001923439	0.124709198

p__Chlamydiae	c__Chlamydiia	o__Chlamydiales	f__Waddliaceae	g__Waddlia	Unclassified	0	0.015658554
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Xanthobacteraceae	g__Xanthobacter	Unclassified	0.442775754	0
p__Proteobacteria	c__Betaproteobacteria	o__Rhodocyclales	f__Rhodocyclaceae	g__Zoogloea	Unclassified	0.000384688	0.000559234
p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Stenotrophomonas	s__acidaminiphila	0.010386573	0.024606299
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Rhizobiaceae	g__Ensifer	s__adhaerens	0.002692815	0
p__Proteobacteria	c__Deltaproteobacteria	o__Desulfovibrionales	f__Desulfovibrionaceae	g__Desulfovibrio	s__alaskensis	0	0.001118468
p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	s__alcaligenes	0.004616255	0.362942913
p__Chrysiogenetes	c__Chrysiogenetes	o__Chrysiogenales	f__Chrysiogenaceae	g__Desulfurispirillum	s__alkaliphilum	0	0.001677702
p__Proteobacteria	c__Deltaproteobacteria	o__Desulfobacterales	f__Desulfobacteraceae	g__Desulfobotulus	s__alkaliphilus	0	0.001118468
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Paracoccus	s__aminovorans	0.001538752	0.052008769
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Xanthobacteraceae	g__Xanthobacter	s__autotrophicus	0.02731284	0
p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	s__balearica	0	0.008388511
p__Euryarchaeota	c__Methanobacteria	o__Methanobacteriales	f__Methanobacteriaceae	g__Methanobacterium	s__beijingense	0.000769376	0
p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Lysobacter	s__brunescens	6.340425696	0.070463493
p__Firmicutes	c__Clostridia	o__Clostridiales	f__Clostridiaceae	g__Clostridium	s__butyricum	0	0.001118468
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Bradyrhizobiaceae	g__Pseudomonas	s__carboxydohydrogena	0.002692815	0
p__Bacteroidetes	c__[Saprospirae]	o__[Saprospirales]	f__Chitinophagaceae	g__Lacibacter	s__cauensis	0.003462191	0.097865963
p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	g__Sphingomonas	s__changbaiensis	0.000769376	0
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Acidovorax	s__delafieldii	0	0.001677702
p__Proteobacteria	c__Gammaproteobacteria	o__Chromatiales	f__Ectothiorhodospiraceae	g__Thioalkalivibrio	s__denitrificans	0	0.137012348
p__Proteobacteria	c__Gammaproteobacteria	o__Aeromonadales	f__Aeromonadaceae	g__Zobellella	s__denitrificans	0	0.001118468
p__Proteobacteria	c__Alphaproteobacteria	o__Caulobacterales	f__Caulobacteraceae	g__Brevundimonas	s__diminuta	0	0.039146385
p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	s__fragi	0.015387515	5.320552971
p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Thermomonas	s__fusca	0.033083158	0.000559234
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Hyphomicrobiaceae	g__Filomicrobium	s__fusiforme	0	0.001677702
p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Pseudofulvimonas	s__gallinarii	0	0.180632606
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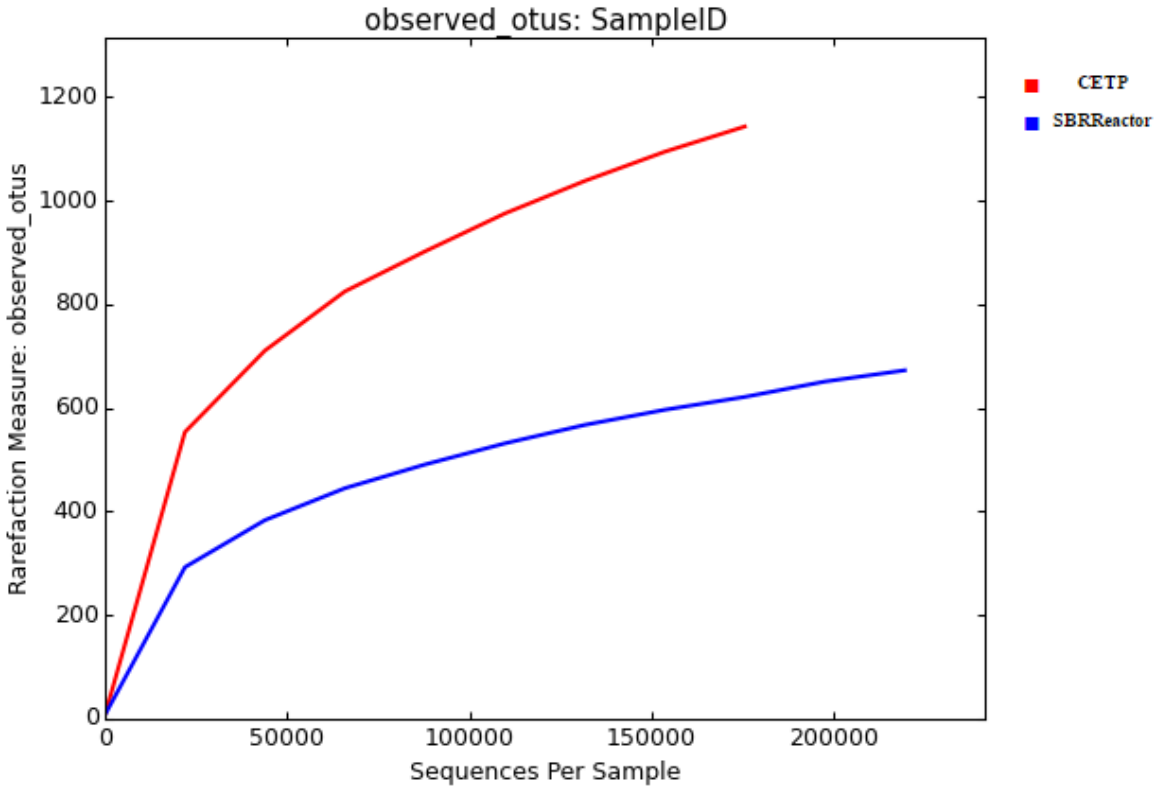
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Hyphomicrobiaceae	g__Polymorphum	s__gilvum	0	0.001118468
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Brucellaceae	g__Ochrobactrum	s__grignonense	0	0.001118468
p__Proteobacteria	c__Betaproteobacteria	o__Nitrosomonadales	f__Nitrosomonadaceae	g__Nitrosomonas	s__halophila	0	0.038587151
p__Proteobacteria	c__Gammaproteobacteria	o__Aeromonadales	f__Aeromonadaceae	g__Aeromonas	s__hydrophila	0	0.024606299
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Rhodospirillaceae	g__Oceanibaculum	s__indicum	0.000384688	0.184547244
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Albidovulum	s__inexpectatum	0.000384688	0.003355404
p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Dermatophilaceae	g__Piscicoccus	s__intestinalis	0.161953599	0
p__Proteobacteria	c__Betaproteobacteria	o__Neisseriales	f__Neisseriaceae	g__Elbe	s__Iso18	0	0.002236936

p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	g__Acinetobacter	s__johnsonii	0	0.394819256
p__Planctomycetes	c__Planctomycetia	o__Gemmatales	f__Isosphaeraceae	g__Nostocoida	s__limicola	0.004231567	0
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Oxalobacteraceae	g__Janthinobacterium	s__lividum	0	0.064311918
p__Firmicutes	c__Bacilli	o__Lactobacillales	f__Streptococcaceae	g__Streptococcus	s__luteciae	0.005770318	0.001677702
p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Stenotrophomonas	s__maltophilia	0	0.003914639
p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Paracoccus	s__marcusii	0.003462191	0.001118468
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Phyllobacteriaceae	g__Hoeflea	s__marina	0.037699413	0
p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	s__mendocina	0	0.001118468
p__Proteobacteria	c__Gammaproteobacteria	o__Vibrionales	f__Vibrionaceae	g__Vibrio	s__metschnikovii	0	0.026843236
p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Xanthomonadaceae	g__Pseudoxanthomonas	s__mexicana	2.709356763	0.151552434
p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	g__Sphingosinicella	s__microcystinivorans	0.006155006	0.007270043
p__Thermotogae	c__Thermotogae	o__Thermotogales	f__Thermotogaceae	g__Kosmotoga	s__mrcj	0	0.00279617
p__Proteobacteria	c__Deltaproteobacteria	o__Desulfobacterales	f__Desulfobacteraceae	g__Desulfococcus	s__multivorans	0	0.005592341
p__Bacteroidetes	c__Sphingobacteriia	o__Sphingobacteriales	f__Sphingobacteriaceae	g__Sphingobacterium	s__multivorum	0	0.034113278
p__Firmicutes	c__Bacilli	o__Bacillales	f__Bacillaceae	g__Bacillus	s__muralis	0	0.013421618
p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	g__Blastomonas	s__natatoria	0	0.001677702
p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	s__nitroreducens	0	0.53406854
p__Planctomycetes	c__Planctomycetia	o__Gemmatales	f__Gemmataceae	g__Gemmata	s__obscuriglobus	0.02000377	0
p__Proteobacteria	c__Gammaproteobacteria	o__Oceanospirillales	f__Halomonadaceae	g__Halomonas	s__pacifica	0	0.001118468
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Phyllobacteriaceae	g__Nitratireductor	s__pacificus	0	0.001677702
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Variovorax	s__paradoxus	0	0.001118468
p__Firmicutes	c__Clostridia	o__Clostridiales	f__Lachnospiraceae	g__Lachnospira	s__pectinoschiza	0.000769376	0
p__Firmicutes	c__Clostridia	o__Clostridiales	f__Clostridiaceae	g__Clostridium	s__perfringens	0	0.001118468
p__Proteobacteria	c__Gammaproteobacteria	o__Alteromonadales	f__[Chromatiaceae]	g__Rheinheimera	s__perlucida	0.002308127	0.001118468
p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	s__pseudoalcaligenes	0	0.001677702
p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Microbacteriaceae	g__Cryobacterium	s__psychrophilum	0	0.003914639
p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	g__Psychrobacter	s__pulmonis	0	0.001118468
p__Firmicutes	c__Bacilli	o__Bacillales	f__Paenibacillaceae	g__Brevibacillus	s__reuszeri	0	0.008388511
p__Proteobacteria	c__Deltaproteobacteria	o__Desulfobacterales	f__Desulfobulbaceae	g__Desulfobulbus	s__rhabdoformis	0.003077503	0
p__Firmicutes	c__Clostridia	o__Clostridiales	f__Veillonellaceae	g__Selenomonas	s__ruminantium	0.004231567	0.002236936
p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	g__Acinetobacter	s__schindleri	0.000384688	3.73456514
p__Firmicutes	c__Bacilli	o__Bacillales	f__Staphylococcaceae	g__Staphylococcus	s__sciuri	0.000384688	0.007829277
p__Proteobacteria	c__Gammaproteobacteria	o__Enterobacteriales	f__Enterobacteriaceae	g__Plesiomonas	s__shigelloides	0.000384688	0.049212598
p__Bacteroidetes	c__Flavobacteriia	o__Flavobacteriales	f__Flavobacteriaceae	g__Flavobacterium	s__succinicans	0	0.621309055
p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__Aquincola	s__tertiaricarbonis	0.002308127	0

p__Firmicutes	c__Clostridia	o__Clostridiales	f__Clostridiaceae	g__Alkaliphilus	s__transvaalensis	0	0.00279617
p__Proteobacteria	c__Gammaproteobacteria	o__Pseudomonadales	f__Pseudomonadaceae	g__Pseudomonas	s__umsongensis	0	0.004473873
p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	g__Sphingomonas	s__wittichii	0.010386573	0.000559234
p__Proteobacteria	c__Alphaproteobacteria	o__Rhizobiales	f__Hyphomicrobiaceae	g__Hyphomicrobium	s__zavarzinii	0.019619082	0.057041875
Phylum	**Class**	**Order**	**Family**	**Genus**	**Species**	**SBAR Reactor**	**CETP**
p__Proteobacteria	c__Betaproteobacteria	o__Nitrosomonadales	f__Nitrosomonadaceae	Unclassified	Unclassified	1.351793223	0.005033107
p__Planctomycetes	c__Planctomycetia	o__Planctomycetales	f__Planctomycetaceae	g__Planctomyces	Unclassified	0.499709561	0.026284001
p__Proteobacteria	c__Betaproteobacteria	o__Nitrosomonadales	f__Nitrosomonadaceae	g__Nitrosomonas	s__halophila	0	0.038587151

APPENDIX II: Rarefaction curve /Speciest richness as a function of number of sequence per sample



APPENDIX III: Calculation of the kinetic parameters of stable granules

The SRT of the reactor was calculated by the relation

$$\theta = \frac{X_{VSS}V_r}{(X_dV_d + X_eV_e)/_{tc}} \text{.....................} (1)$$

where θ is solid retention time (d), X_{VSS} the volatile solid concentration in reactor (g VSS l^{-1}), V_r the working volume of SBR reactor (l), X_d the biomass concentration of manually discharged mixture, V_d manually discharged mixture volume, X_e the effluent volatile solid concentrations (g VSS l^{-1}), V_e the effluent volume in SBR operating cycle (l) and tc is the cycle time of SBR Operation (d).

Since there was no manual sludge discharging during the reactor operation in this study, Eq. (1) can be simplified as Eq. (2).

$$\theta = \frac{X_{VSS}V_r}{X_eV_e/_{tc}} \text{..............................} (2)$$

The calculated SRT according to Eq. (2) was 20 days which is suitable for simultaneous COD removal and nitrification.

The kinetic parameters of stable granules were calculated according to the relation.

$$\theta = \frac{1}{\mu obs} \text{...............................} (3)$$

Where μ_{obs} is the observed specific biomass growth rate. The observed biomass yield (Y_{obs}) is the ratio of the biomass production rate to the substrate removal rate, which can be calculated from Eq. (4):

$$Y_{obs} = \frac{[(X_{VSS2} - X_{VSS1})V_r + X_eV_e)]/_{t_c}}{(C_i - C_e)V_e/_{t_c}} \text{..........} (4)$$

where Y_{obs} is the observed biomass yield (mg VSS mg^{-1}COD),

X_{VSS2} the volatile solid concentration at the end of cycle operation in SBR reactor (g VSS l^{-1}), X_{VSS1} the volatile solid concentration at the beginning of cycle operation in SBR reactor (g VSS l^{-1}), V_r the working volume of SBR reactor (l), tc the cycle time of SBR operation (d), X_e the effluent volatile solid concentrations (g VSS l^{-1}), V_e the effluent volume in SBR operating cycle (l), C_i the concentration of influent COD (mgCODl^{-1}) and C_e is the concentration of effluent COD (mgCODl^{-1}).

When reactor reaches the steady state and the biomass concentration in the reactor maintained at a constant value, Y_{obs} corresponds to the net sludge production and Eq. (4) can be simplified as Eq. (5):

$$Y_{obs} = \frac{X_e}{(C_i - C_e)} \ldots\ldots\ldots\ldots\ldots (5)$$

The theoretical yield coefficient (Y) is distinguished from

Y_{obs}. The difference is caused by different mechanisms, including

endogenous metabolism, death, and predation. It can be expressed by means of the specific biomass decay rate k_d. The values of both parameters (Y and kd) were calculated from the following equation:

$$\frac{1}{\theta} = Y q_{obs} - k_d \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

The observed specific substrate removal rate q_{obs} could be calculated by the following equation:

$$q_{obs} = \frac{(C_i - C_e)V_e / t_c}{X_{VSS} V_r} \ldots\ldots\ldots\ldots (7)$$

APPENDIX IV: International Conference Participation Certificate

APPENDIX V: National Conference Participation Certificate