POTENTIAL APPLICATION AND PERFORMANCE OF METHANOTROPHIC MIXED CULTURE UNDER MAIN STREAM CONDITION IN WASTEWATER TREATMENT PLANT

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ABSTRACT

Wastewater treatment plants (WWTP) produce significant amount of biogas during sludge digestion in anerobic digestion (AD). Biogas is mainly methane gas which is a potent greenhouse gas (GHG) causing global warming. Direct use of biogas as fuel is an unattractive option due to its low efficiency, storage and transportation difficulty. There is an attractive option to convert methane gas biologically into more desirable forms like methanol and bio polymer. It has been proven through researches that the bio-conversion of methane can be done with the help of a biocatalyst called methanotrophs with higher efficiency and requires no or less energy input. The application of methanotrophs into WWTP has been challenged by its capability to grow under main stream conditions with Chemical Oxygen Demand (COD) along with diverse microbial community.

In this study the growing capability of methanotrophic mixed culture was examined under varying COD in synthetic feed. The experiment was done in repeated batch for fifty cycles to obtain the stable behavior of the microbial consortium. In addition, the nitrogen utilization mechanism was investigated to figure out whether the nitrogen is used only for cell synthesis or cell synthesis along with nitrification/ denitrification. The results show that the best microbial performance was attained at COD of 360 mg/L. The average Specific Growth Rate (day^{-1}) was 0.49±0.05, average Biomass Yield (mg-VSS/mg-CH_4) was 0.49±0.04 and the average methane uptake rate (mg/hr) was 2.50±0.36. In addition, average COD consumption was 89±5 % at COD 540mg/L and the average ammonia consumption was around 99.72±0.48 %. The percentage of ammonia utilized for cell synthesis was 61-72% and 28-39% of ammonia was converted to a gaseous form of nitrogen. These results demonstrate the feasibility of integrating methanotrophic cultures within the mainstream of existing WWTPs.

DEDICATION

To my beloved late father Md. Abdul Mazid, who is a great inspiration to me to move forward.

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AD : Anaerobic Digester ... 1

AME-D : Aerobic Methane Oxidation Coupled to Denitrification ... 28

AMO : Ammonia Monooxygenase ... 27

AOB : Ammonia Oxidizing Bacteria ... 2

AS : Activated Sludge ... 3

BNR : Biological Nutrient Removal ... 31

BOD : Biochemical Oxygen Demand ... 3

CBB : Calvin-Benson-Bassaham ... 16

CBOD : Carbonacious Biochemical Oxygen Demand ... 35

CHP : Combined Heat and Power ... 6

CNG : Compressed Natural Gas ... 3

COD : Chemical Oxygen Demand ... ii

DBD : Dielectric Barrier Discharge ... 2

DCW : Dry Cell Weight ... 50

DME : Di methylether ... 6

EDTA : Ethylenediaminetetraacetic Acid ... 22

FaDH : Formaldehayde Dehydrogenase ... 17

FDH : Formate Dehydrogenase ... 18

GC : Gas Chromotograph ... 39

GHG : Green House Gas ... 1

GTL : Gas to Liquid ... 8

HCCI : Homogeneous Charge Compression Ignition ... 7

IC : Ion Chromatograph ... 49

ICM : Intracytoplasmic Membrane ... 16

LNG : Liquified Natural Gas ... 3

MCFC : Molten Carbonate Fuel Cell ... 13

MDH : Methanol Dehydrogense ... 21

MFC : Microbial Fuel Cell ... 13

MMO : Methane Monooxygenase ... 2

MSM : Mineral Salts Medium 36
NAD : Nicotinamide Adenine Dinucleotide 18
NG : Natural Gas 8
NOB : Nitrite Oxidising Bacteria 29
OD : Optical Density : 37
ORC : Organic Rankine Cycle 14
ORFC : Oxidative Reforming Fuel Cell 13
PEMFC : Proton Exchange Membrane Fuel Cell 12
PHA : Poly Hydroxyalkanoates 23
PHB : Poly Hydroxybutyrate 4
pMMO : Particulate Methane Monooxygenase 16
PQQ : Pyrroloquinoline Quinone 17
RAS : Return Activated Sludge 4
RNA : Ryboneuclic Acid 15
RuMP : Ribulose Monophosphate 16
sMMO : Soluble Methane Monooxygenase 16
SNG: Substitute Natural Gas 8
SNR : Simultaneous Nitrification and Denitrification 29
SOFC : Solid Oxide Fuel Cell 12
TRL : Technology Readiness Levels 13
TSS : Total Suspended Solid 35
VSS : Volatile Suspended Solid 43
WAS : Waste Activated Sludge 4
WW : Wastewater 4
WWTP : Wastewater Treatment Plant 1
Y : Observed Growth Yield 41

CHAPTER 1: Introduction

1.1 Background

Methane (CH_4) is one of the major greenhouse gases (GHG) that are causing global warming directly and indirectly (Cicerone and Oremland, 1988). Methane has a life span of 12 years in the atmosphere and it has 21 to 28 times greater global warming potential than carbon dioxide (CO_2) (Donner and Ramanathan, 1980). In addition, methane is responsible for 14 % of global GHG emission. Among them, 60% of global methane originates from anthropogenic sources, whereas, wastewater treatment plants (WWTPs) contribute towards 5% of the global methane emissions (Czepiel et al., 1993). Global methane emission is expected to increase by 20% over the next two decades (US EPA, 2016). WWTPs produce significant amount of methane, for instance, each year 3.9 billion tones of biomethane are produced from wastewater treatment facilities in North America (AlSayed et al., 2018c). Typically, biogas from anaerobic digesters (AD) contains 60-70% methane with some impurities such as H_2S and NH_3. Biogas can be used as a fuel after being purified and converted into compressed natural gas or liquefied natural gas. Such processes are not cost effective as it requires high energy inputs and capital costs (Ge et al., 2014). Besides, due to the applied high pressure and methane explosive nature, it is unsafe to be stored, transferred and distributed (Ge et al., 2014). For this reason, it is necessary to develop a sustainable and cost-effective technology to mitigate AD-driven biogas with sustainable value-added product recovery.

The unreactive nature of methane is due to the energy of 438.8 kJ/mol required to break the C-H bond (Park and Lee, 2013). There are several thermochemical techniques with high temperature, pressure and catalyst to utilize methane gas (Park and Lee, 2013) which require intense energy use and expensive chemicals. However, this process is always involved with the generation of synthetic gas such as carbon monoxide, which is harmful to the environment. Moreover, there is another process called non-thermal dielectric barrier discharge (DBD) plasma chemical process which requires high voltage and electrodes (Park and Lee, 2013). Hence, both thermochemical and plasma chemical processes are costly, less efficient and not environmentally sustainable.

In contrast, biochemical process of methane utilization is a promising option as it can be operated in moderate condition with high conversion efficiency. In this process, microorganisms act as the biocatalyst which have wide biodiversity and can adapt to various environments (Ge et al., 2014). Two distinct groups of microorganisms: ammonia oxidizing bacteria (AOBs) and methane oxidizing bacteria (methanotrophs) have the ability to consume atmospheric methane gas (Hanson and Hanson, 1996). Between these groups of microorganism, methanotrophs are more advantageous due to their fast growing ability and utilizing methane as their sole carbon and energy source (Hanson and Hanson, 1996). Unlike thermochemical process, methanotrophs can activate C-H bond in methane by the methane monooxygenase (MMO) enzyme under ambient condition (Culpepper and Rosenzweig, 2012). In addition to methane mitigation, methanotrophs has attracted attention due to its wide range of biotechnological applications for value added products and processes such as methanol, biopolymers, Single-Cell Protein, Ecotine, Biodiesel, Microbial Fuel Cells, denitrification, etc (Strong et al., 2015).

1.2 Research Rationale

Methane is the second most important anthropogenic source of greenhouse gas having 28 times greater global warming potential than carbon dioxide over 100 years (Stocker et al. .). Since pre-industrial age, atmospheric methane concentration had increased from 0.7 ppmv to 1.7 ppmv in 1994 (El-Fadel and Massoud, 2001). Among all the anthropogenic sources WWTPs contribute about 4-5% or 20-25 Tg of CH_4 year^{-1} to the global methane emission (Czepiel et al., 1993; El-

Fadel and Massoud, 2001). It was estimated that methane emission for municipal wastewater in typical WWTPs using activated sludge (AS) method varies from 0.039 to 0.309 kg yr^{-1} per inhabitant (Yver-Kwok et al., 2013). With the increase of population it is predicted that the anthropogenic methane emission will increase by 28 percent from 2005 to 2030 (Höglund-Isaksson, 2012). Thus, it is important to find appropriate technology to mitigate increasing methane emissions from WWTPs.

In addition, conventional AS system is criticized for intensive energy requirement where most of energy (55-57%) is required for biological treatments specially for aeration (Mamais et al., 2015; Massara et al., 2017; Robert Smith, 1978). AS process is designed to remove organic matter (BOD) by microorganism from wastewater. In this process, aerobic microorganism degrade organic waste and covert ammonia into nitrate (Ahansazan et al., 2014). For nitrogen removal additional treatment processes are incorporated which requires additional energy (Massara et al., 2017). With the increase of population, the energy demand is going to be increased over time. To keep up with the increasing energy demand it several initiatives were taken to recover energy from biogas generated from the WWTP by heat production, electricity generation by gas turbine or converting into liquid fuel like LNG or CNG. These initiatives have some limitations which are putting obstacles to methane mitigation. The major problems with biogas are: it has impurities which costs a lot to purify, it has low electricity conversion efficiency, due to its low boiling point (-164℃) it difficult to store or transport(Ge et al., 2014). To address these issues biogas can be utilized biologically by methanotrophic bacteria with some value added resource recovery (Strong et al., 2015). However, the challenge with the biological methane recovery is the integration of the system in to WWTP.

To incorporate methanotrophs in WWTPs several studies have been done. Methanotrophs have been successfully grown directly from the seed from return activated sludge (RAS) and waste activated sludge (WAS) (AlSayed et al., 2018a, 2018c). In addition, some study showed that methanotrophs can grow in wastewater effluent and AD centrate instead of synthetic liquid feed (Fergala et al., 2018b). Moreover, for gas feed, biogas from AD was successfully applied to methanotrophs for methanol production (Patel et al., 2016a). There is another important thing to consider in WWPs, which is the high amount of COD presence in WW. So far, no study has been done to report the behavior of methanotrophs in real WW.

1.3 Research Objective

The aim of this research is to examine the growth capability of methanotrophic-heterotrophic mixed culture maintained under wastewater mainstream conditions in fed-batch mode. Specific objectives are as follows:

- To enrich methanotrophic mixed culture from WWTP
- To grow the enriched culture in fed-batch with ammonia and COD to reflect the main stream wastewater condition
- To observe growth parameters, methane, COD and nitrogen removal continuously in repeated batch until the culture reaches to a stable condition
- To investigate the nitrogen utilization pathway for determining the possibility of nitrification/ denitrification by the culture
- To apply the enriched culture for methanol and polyhydroxybutyrate (PHB) production

1.4 Thesis Layout

This thesis consists of six chapters. The **first chapter** is the Introduction where justification for current research including methane gas issues, methanotrophs application and their integration in WWTP, nitrogen removal issues in WWTP are provided. In the **second chapter,** the literature review on biogas utilization techniques, methanotrophs, methane metabolism in methanotrophs, methanol production, PHB production, nitrification-denitrification by methanotrophs are included. In the **third chapter,** methanotrophs growth performance and nitrogen removal activity under COD condition is provided. In the **fourth chapter,** two batch experiments for methanol and PHB production with COD are provided. Finally, the conclusion and future work are presented in the chapter 2

CHAPTER 2: Literature Review

2.1 Methane Mitigation and Utilization Techniques

Wastewater and landfill are responsible for 90% of methane emissions from the waste sector which is about 18% of global anthropogenic emissions in 2004. During conventional wastewater treatment processes biogas (CH_4 + CO_2) is being produced through anaerobic digestion (Bogner et al., 2008). The methane containing biogas produced from anaerobic digestion is a significant source of energy (24 MJ/m^3). To recover the energy in biogas several alternatives have been applied such as combustion in boilers for steam generation and heat generation for electricity and heat recovery (Monteith et al., 2005). There are various commercially feasible AD generated biogas utilization methods which include: a) electricity generation through combined heat and power (CHP) or fuel cells, b) multiple uses in industry through electricity, heat steam and cooling, c) injection into national gas grids, d) transportable fuels, e) chemical production, f) energy storage, g) cooking and lightning in rural areas, h) biohydrogen, etc.

2.1.1 Gas Engines and Turbines

Biogas power plants mostly use internal combustion gas engines to produce heat and electricity. Biogas is used in other engines such as:

1) Dual fuel engines where biogas is co-fired with a small proportion of bio-diesel, bio-ethanol or bio-DME (Barik and Murugan, 2014).

2) Micro-gas turbines which require purified compressed biogas and have low combustion temperatures to produce 28% and 56% of electrical and thermal conversion efficiency, respectively (Pöschl et al., 2010).

3) Stirling engines are non-fuel specific external combustion engines that require less maintenance (Praetorius et al., 2009) which have 20 to 45% electricity producing efficiency.

4) Combined heat and power (CHP) is a common biogas utilizing technique in Germany. In this method produced electricity is fed in to national grid and heat is transmitted towards a district heating network. Heat also can be used for AD operation and sterilization of feedstock (Pöschl et al., 2010).

5) Homogeneous charge compression ignition (HCCI) is a process of engine operation which have alternate combustion pathway for biogas. HCCI engines has less impact of CO_2 in biogas. As a result, HCCI engines have higher thermal efficiency (50%) close to diesel engine (Saxena and Bedoya, 2013). For small scale power generation, biogas fueled HCCI engine is a promising option.

6) Flameless combustion is a unique technique for combustion of different fuels. It is a mild combustion technique which is applied to alleviate unwanted emissions and improve fuel conversion efficiency (Budzianowski, 2016). Use of biogas in flameless combustion is novel but researchers found that biogas can be used effectively with this technology and it was found that electricity conversion efficiency was 53% and CHP efficiency was 82% (Hosseini and Wahid, 2013).

2.1.2 Biofuel conversion

After removing the impurities in biogas, it can be converted to various biofuels which are comparable with petroleum such as biomethane, biosyngas and biohydrogen, biomethanol and bio-DME (Budzianowski and Budzianowska, 2015).

2.1.2.1 Biomethane

Biomethane is a clean bio synthetic natural gas (bio-SNG) which can be directly injected into natural gas grid for commercial use (Budzianowski, 2016). Biomethane can be obtained from biogas by separating CO_2 or converting CO_2 into CH_4 by pressurized catalytic methanation. In Methanation of biogas CO_2 is hydrogenated at 20 bar pressure with heat exchanger connected to it to remove heat produced form exothermic reaction. Hydrogen gas can be obtained through electrolysis of water with surplus renewable energy (Jürgensen et al., 2014). There are several techniques such as absorption, membrane filtration, biological method and hybrid method to remove impurities from biogas to produce clean biomethane.

Moreover, Biomethane can be physically converted to various forms as liquefied natural gas (LNG), compressed natural gas (CNG) and gas to liquid (GTL). LNG is liquefied natural gas at -161℃ and 1 atm pressure. Liquefaction make 600 time reduction of gas volume which make it easy to store in an atmospheric tank and transport to the market easily (Hasan et al., 2009). In CNG, natural gas is compressed with a pressure of 1500 to 4000 psi to store it in a pressure vessel. CNG requires less volume than LNG and does not require a pipe line to transport. CNG is a simple way to utilize natural gas commercially (Young and Hanrahan, 2009). On the other hand, GTL is a technology to convert natural gas into synthetic liquid called “syncrude”. In this process NG is converted to syn-gas ($CO+H_2$) which is then converted to a liquid mixture of hydrocarbons (syncrude) by a Fischer–Tropsch reactor with cobalt and iron as a catalyst for selling into market (LAAN and BEENACKERS, 1999).

2.1.2.2 Biosyngas and biohydrogen

Biosyngas is biohydrogen rich gas which is obtained through reformation of biogas into H_2 and CO. Biosyngas can be used in methanol, DME and higher hydrocarbon production. Biogas is being purified for reformation as it has some impurities. For production of H_2 and CO there are three principle processes: 1) steam reforming, 2) carbon dioxide (or dry) reforming, and 3) partial oxidation (Lunsford, 2000). The reformation is done in an arc gliding reactor with plasma and catalyst. In the arc gliding reactor process, biogas is converted to hydrogen enriched gas comprising 41% of hydrogen gas which have 35% thermal efficiency (Yang et al., 2009). There is an alternate method called spark-shade plasma method by Zhu et al. In reformation of biogas, the catalyst plays an important role. It was found from researches that cobalt-nickel catalyst give the best performance for biogas reformation (Xu et al., 2009). In addition, tri-reforming sorption enhanced reforming are the other options for biogas reformation (Izquierdo et al., 2013).

2.1.2.3 Biomethanol and bioDME

Biomethanol is a promising sustainable fuel alternative considering its ease to storage and transport. Biogas can be converted to biomethane with the aid of catalysts but for development of suitable catalyst for direct conversion numerous researches have done. There are homogeneous, heterogeneous and enzymatic catalysts which were applied to methane gas for methanol production (Gunsalus et al., 2017). Both homogeneous and heterogenous systems have advantages and disadvantages. Homogeneous catalysts are able to break strong C-H bonds at low temperature. In this process mercury or platinum based catalysts are used with a strong oxidant like sulfuric acid or trifluoroacetic acid which are corrosive and less sustainable than O_2 (Periana et al., 1998). On the other hand, heterogeneous system is based on molybdenum, iron, or copper as catalyst where O_2 is used as oxidant. Molybdenum oxides is widely used with higher selection of methanol as well as stability of the product. Moreover, Iron zeolites and copper zeolites have higher methanol productivity with N_2O and O_2 as oxidant (Wang et al., 2017).

It was reported that natural gas is commercially converted to methanol by a Cu-Zn catalyst and geothermal energy ("CRI - Carbon Recycling International," n.d.). However, conversion of biogas into biomethanol has some challenges as it has some impurities. Considering the efficiency and economy of the conversion it was suggested that biogas can be co-synthesized into biomethanol along with one or two carbon hydrocarbons. This process consists of several parallel steps like reformation, bio-Methanol synthesis, bio-DME synthesis, biohydrocarbon synthesis(optional) and fractionations (Corradini and Mccormick, 2010). There is another process for methanol synthesis with metgas, Metgas is a mixture of H_2 and CO with a 2:1 ratio which is used in methanol synthesis with NiO/MgO as catalyst at 5-30 atm pressure and 800-950℃ temperature (Olah et al., 2013).

2.1.2.4 Ethylene

Methane can be converted to ethylene by oxidative coupling of methane (Holmen, 2009). In this process methane is coupled with O_2 to form ethylene with water, ethane and other higher order hydrocarbons. The reaction is catalyzed by different heterogeneous catalysts based on Li/MgO, Fe_2O_3, and La_2O_3 at 700℃ temperature. This process generates a significant amount of ethane, C3 products, CO, and CO_2 along with ethylene. There is an alternative way to produce ethylene by heating methane in absence of O_2 which generate ethane and H_2 but no oxygenates are formed (Holmen, 2009). Ethane can be upgraded to higher value ethylene by oxidative dehydrogenation where higher temperature >500℃ and tantalum hydride catalyst supported on SiO_2 is applied. In this process along with ethylene other higher hydrocarbons can be produces as by-product. The problem of this process is formation of hydrogen-deficient CH_x fragments and selection of catalyst with appropriate temperature.

2.1.2.5 Aromatics/ Higher hydrocarbon

Benzene is a precursor of range of aromatic compounds. Usually benzene is derived from petroleum-based compounds. However, there is a process called methane dehydroaromatization which was discovered in 1993 (Wang et al., 1993). In this process methane is converted into benzene and H_2 gas with no oxygenate including CO and CO_2. As a result, H_2 can be used directly in fuel cells. For the reaction, 900℃ temperature and zeolite-based catalyst such as Mo-ZSM-5 is used. The catalyst activates carbon bond and helps to form aromatic compounds. As the thermodynamically favorable condition of benzene is similar to naphthalene, naphthalene is formed as by-product in this process (Tang et al., 2014). The disadvantage of this process is that it requires high temperature to make the reaction happen. In addition, significant amount of coke is formed which makes the catalyst to be regenerated with $H_{2.}$ Further research is required to apply this method efficiently.

There is another process to synthesize higher hydrocarbons from biogas using Al-Co-Ni-Cr based catalysts (Gunnerman and Gunnerman, 2009). In this process biogas is fed into the first reactor where the catalyst is immersed into a liquid phase. The product gas is vaporised and drawn from the reactor and condensed and then fed into a second reactor. The condensate liquid is separated, and non-condensable gases are recycled back to first reactor. In this process the main synthesized products are paraffins (25-35%), naphthenes (58-63%) and aromatics (6-17%). The major issue with this process is the energy efficiency needed to consider while upscaling the system.

2.1.3 Fuel Cells

Fuel cells are innovative technologies for converting chemical energy of fuel into electricity with higher efficiency and less harmful emissions (Perry Murray et al., 1999). Biogas can be applied in fuel cells as fuel but since biogas has impurities, it is required to condition it before using it in fuel cells. There are various fuel cells where biogas can be applied for power generation.

2.1.3.1 SOFCs

Solid oxide fuel cell (SOFC) produce electricity by oxidizing fuel where solid oxide or ceramic construction materials are used. This process requires high operating temperature and long starting time which make it less operationally flexible. However, this process is resistant to biogas fuel impurities (Shiratori et al., 2008).   It was found that at 1000℃ SOFC can tolerate biogas with up to 1 ppm H_2S.

The problem of SOFC is excessive deposition of carbon in to the reactor. It was claimed that biogas diluted with air fed in to SOFC can prevent carbon deposition without compromising cell voltage (Shiratori et al., 2010). Moreover, in a study three differently conditioned biogas systems were applied to SOFC in order to prevent carbon deposition (Farhad et al., 2010). It was found from the study that partial oxidation was the best approach with 80.5% CHP conversion efficiency. In another study it was suggested that prior to SOFC application, biogas can be enriched with hydrogen gas which can lower carbon deposition (Lanzini and Leone, 2010). This study also claimed that at certain threshold of hydrogen level carbon deposition stops and for sustainable power generation hydrogen can be obtained through electrolysis of water.

2.1.3.2 PEMFCs

Unlike SOFC, proton exchange membrane fuel cell (PEMFC) requires low temperature (50-100℃) and uses a special polymer electrolyte membrane. Biogas is less suitable for PEMFC as it requires clean fuel. There are several techniques developed by scientists to utilize biogas into PEMFCs. In an experiment, biogas was reformed under steam to produce hydrogen with 50%

purity and the reformed gas was efficiently applied to PEMFC with stable performance (Schmersahl et al., 2007, p.). In another study high temperature PEMFC (HT-PEMFC) with a modular stack was suggested (Birth et al., 2014). In this process, biogas is reformed to yield hydrogen gas for downstream oxidation. The electrical efficiency of HT-PEMFC system was found to be 40%. There is another process called oxidative reforming fuel cell (ORFC) system where biogas is converted to a mixture of CO_2 and H_2 (Budzianowski, 2010). The gas mixture is separated to form hydrogen enriched gas which is finally sent to PEMFC for electricity generation. The calculated electrical efficiency is 56% which is higher than gas turbine cycles. The problem of this system is that it has low Technology Readiness Levels (TRL) to commercialize.

2.1.3.3 MCFCs

Molten carbonate fuel cell (MCFC) is a high-temperature fuel cell that uses electrolyte of molten carbonate salt mixture suspended in a porous ceramic matrix. MCFC operates at higher temperature (600-700°C) which enables use of cheaper catalysts based on Ni (Milewski et al., 2014). Biogas can be used in MCFC as it requires CO as fuel for this type of cells. However, H_2S required to be filtered for MCFC use. A combined heat, hydrogen and power (CHHP) was developed with MCFC system for sustainable biogas utilization (Hamad et al., 2014). In this system electricity can be use for local use, heat for biogas digester and hydrogen can be transported to the market.

2.1.3.4 MFCs

Microbial fuel cell (MFC) is technology to produce direct electricity or energy carriers from various organic substrate. In a study MFC was used to treat primary digested sludge where both biogas and electricity production were observed (Ge et al., 2013). Moreover, it was reported in several studies that methane gas can be used as an energy source to drive MFC for direct electricity production (Chen et al., 2018). However, the MFC system needs further research to minimize capital cost and stable performance in order to scale up for industrial use.

2.1.3.5 ORCs

Organic Rankine Cycle (ORC) is technique to utilize organic energy in liquid phase by lowering boiling point usually less than water steam. Using ORC the best energy conversion from biogas was achieved at 1.5 to 2 compression ratio with air temperature 335 to 340K (Di Maria et al., 2014). Biogas to electricity conversion efficiency was 20% which was lower than gas turbine efficiency. In order to utilize biogas for ORC it is required more investment and further research be conducted to increase energy conversion efficiency.

2.1.3.6 Hybrid energy system

Hybrid energy system is a combination of two or more energy systems to maximize fuel to energy conversion efficiency. For biogas SOFC-GT hybrid system was suggested in the literature. In this system SOFC provides high fuel to electricity conversion ratio and GT ensures maximum fuel utilization and exploits thermal energy derived from high temperature by SOFC. By optimizing biogas fed SOFC-GT hybrid system 65% of biogas to energy conversion was achieved (Wongchanapai et al., 2013). Moreover, it was feasible to produce 55% electrical efficiency and more than 80% CHP efficiency. The problem of hybrid systems are that they are expensive and not feasible for small scale biogas plant.

2.1.4 Biological methane mitigation

Compared to physical and chemical processes, biological methane utilization processes require less energy inputs. Various methanotrophic bacteria can utilize methane as their sole carbon and energy source. Methanotrophs can oxidize methane under both aerobic and anaerobic conditions.

Aerobic methanotrophs use oxygen as electron donor to oxidize methane terminally into CO_2 with methanol, formaldehyde and formate as intermediates (Hanson and Hanson, 1996). Based on the metabolic pathways, methanotrophs have various engineering application with methane utilization. In the next section background of methanotrophs, methane utilization mechanism and various applications will be discussed.

2.2 Methanotrophs

Methanotrophs are the organisms that can utilize single carbon source organic compound (C1) such as methane and methanol (Brock Biology of Microorganisms, 14th Edition, n.d.). Methanotrophs were first discovered in 1906 by Söhngen and later it was successfully isolated and classified in 1970 (Whittenbury et al., 1970). Methanotrophs utilize methane both` aerobically and anaerobically as a sole carbon and energy source. They are gram-negative bacteria that belong to *Proteobacteria* under the group of prokaryotes (Hanson and Hanson, 1996). Methanotrophs naturally exist in diverse environments such as: soils, peat bogs, wetlands, sediments, lakes, waste waters, fresh waters, and marine waters (Hanson and Hanson, 1996). Generally aerobic methanotrophs oxidizes methane in to carbon dioxide through a series of intermediates including methanol (CH_3OH), formaldehyde and formate (Hanson and Hanson, 1996).

2.2.1 Methanotrophs classification

Methanotrophs can be classified as aerobic and anaerobic methanotrophs depending on their terminal electron acceptor. In addition, based on the carbon assimilation pathways, cell morphology, membrane arrangement, 16S RNA sequences, and other metabolic characteristics methanotrophs are classified as Type I, Type II and Type III (Hanson and Hanson, 1996; Knief, 2015).

Type I methanotrophs are Gamma subdivision of Proteobacteria which have Methylococcaceae and Methylothermaceae families. Type Imethanotrophs can be found in various environment such as soils, landfills, sewage and activated sludge, denitrification reactors, anaerobic digesters (Bowman, 2014; Ho et al., 2013). Type I methanotrophs have well developed intracytoplasmic membrane (ICM) which enables them to produce mostly particulate methane monooxygenase (pMMO) (Bowman, 2014). For carbon assimilation they use formaldehyde (CHOH) trough ribulose monophosphate (RuMP) cycle (Bowman, 2006). Moreover type I methanotrophs have high growth rate and have higher methane oxidation efficiency (Fergala et al., 2018a). *Methylobacter, Methylomicrobium, Methylocaldum, Methylococcus, Methyloglobulus, Methylomonas, Methylosphaera*, etc. are some strains of type I methanotrophs (Knief, 2015).

On the other hand, type II methanotrophs are Alpha subdivision of Proteobacteria phylum can be found in soil, freshwater sediments, landfills, sewage sludge (Bowman, 2006; Ho et al., 2013). Type II methanotrophs have the ICM aligned to the cell periphery which have the ability to express soluble methane monooxygenase (sMMO) (Semrau et al., 2010). For cell synthesis they assimilate carbon from formate(CHOOH) through Serine cycle (Bowman, 2006). Unlike type I methanotrophs they are slow to grow and have the unique ability to accumulate biopolymer under nutrient limited condition (Bowman, 2006; Henckel et al., 2000). *Methylosinus*, *Methylocystis* are the strains of type II methanotrophs.

Type III methanotrophs are Verrucomicrobia can be found in hot acidic geothermal environment (Knief, 2015). Verrucomicrobia do not have ICM system but they possess pMMO with unidentified location inside their cell (van Teeseling et al., 2014). They assimilate carbon from Carbon dioxide via Calvin-Benson-Bassaham (CBB) cycle. *Methylacidiphilum* and

Methylacidimicrobium genus are the examples of type III methanotrophs . (AlSayed et al., 2018d; Knief, 2015).

2.2.2 Methane assimilation by methanotrophs

In methane oxidation by methanotrophs, methane is terminally oxidized in to carbon dioxide through series of intermediates with the aid of various enzyme secreted from the bacteria (Hanson and Hanson, 1996). Initially, methane is oxidized in to methanol by MMO enzyme which reduces the oxygen molecule into two monovalent oxygen atoms. One oxygen atom breaks the C-H bond in methane forming methanol. While the other oxygen atom is converted to water. MMO enzyme has two forms: sMMO and pMMO. sMMO uses nicotinamide adenine dinucleotide NAD(P)H as electron donor generated from formaldehyde and formate oxidation (Hanson and Hanson, 1996). The electron donor for pMMO is not clear but is assumed that pMMO has various electron source such as ubiquinol ($Q8H_2$) (Kalyuzhnaya et al., 2015). sMMO expressing cells have higher efficiency in methane oxidation and pMMO containing cells have broad substrate range (Chistoserdova et al., 2005; Kalyuzhnaya et al., 2015).

$$CH_4 + \mathrm{O}_2 + 2\mathrm{e}- + 2\mathrm{H}+ \rightarrow CH_3\mathrm{OH} + H_2\mathrm{O}$$

Methanol is further oxidized into formaldehyde through quinoprotein methnol dehydrogenase (MDH) (Chistoserdova et al., 2005). During this reaction pyrroloquinoline quinone (PQQ) is reduce to PQQH2 which later act as a electron donor for pMMO or other electron acceptor (Hanson and Hanson, 1996). Formaldehyde is an important intermediate in methanotrophs metabolism as part of it is terminally oxidized into carbon dioxide and part of it is assimilated for cell synthesis through RuMP or Serine pathway (Hanson and Hanson, 1996). Formaldehyde is oxidized into formate by formaldehayde dehydrogenase (FaDH) which is either nicotinamide adenine dinucleotide (NAD)-linked or PQQ containing cytochrome-linked enzyme. Formate is finally oxidized into carbon dioxide which is catalyzed by NAD dependant enzyme formate dehydrogenase (FDH) (Smith et al., 2010).

Methanol is further oxidized in to formaldehyde. Subsequently, part of formaldehyde is assimilated for new cell production and part of formaldehyde is further oxidized into formate by NAD (P) linked aldehyde dehydrogenase. Finally, part of formate can be assimilated for cell synthesis, whereas the remaining part is oxidized into CO_2 (Hanson and Hanson, 1996; Hwang et al., 2018).

2.2.3 Factors affecting methanotrophs growth

The growth rate varies for different types of methanotrophs. Type I methanotrophs are the fastest and type III are the slowest to grow (van Teeseling et al., 2014). Several factors affect the growth of methanotrophs such as: pH, temperature, substrates, methane to oxygen ratio, methane solubility, nitrogen source, copper, etc.

2.2.4 pH

Most of the methanotrophs grows in the pH range of 5.5 to 8 but there are some genus like verrucomicrobial methanotrophs that can grow in pH 1.5 to 3.5 and methylomicrobim species can grow in pH 8 to 10 (Bowman, 2006; van Teeseling et al., 2014). Methanotroph do no require sodium chloride for growth but they can tolerate up to 7 mg/L of NaCl (van der Ha et al., 2010).

2.2.5 Temperature

Methanotrophs mostly grow in the temperature rage of 20 to 30°C however, most type I and type III methanotrophs prefers higher temperature. For example type I methylococcus genus can grow optimally between 42 to 55 °C (Bowman, 2006). On the other hand, all type II methanotrophs can survive at lower temperature range 4 to 10 °C (Bowman, 2006).

2.2.6 Substrate

Methanotrophs usually take methane as their substrate but they can utilize other C1 compounds such as methanol, formate and methylamines as substrate (Bowman, 2014; Hanson and Hanson, 1996). Interestingly it was discovered that some type II methanotrophs like methylocella species can consume multiple carbon source such as acetate, ethanol, malate, succinate, etc. Moreover, methylocella silverstris can grow faster on acetate than methane (Semrau et al., 2011).

2.2.7 Methane to oxygen ratio

Methane to oxygen ratio is not a decisive parameter for growing specific type of methanotrophs. Type II methanotrophs can form stable slow growing community at above 1% methane concentration (Semrau et al., 2010). In addition, they can also dominate at methane concentration less than 0.06%. However, some studies reported that type II methanotrophs prefers to grow in high methane to low oxygen condition. On the other hand, type I methanotrophs usually dominates in the enrichment process as they are faster growing than type II (Semrau et al., 2010). So, both type of methanotrophs has the ability to grow in different methane and oxygen concentration.

2.2.8 Nitrogen source

All type II methanotrophs and some type I methanotrophs can fix atmospheric nitrogen via oxygen sensitive nitrogenase (Bowman, 2006). In addition, type II methanotrophs dominate in N-limited condition whereas type I methanotrophs prefer higher nitrogen condition. Mostly, methanotrophs like to grow on inorganic nitrogen, nitrate, ammonia, etc. Methanotrophs can tolerate up to certain concentration of ammonia as high ammonia content can inhibit the growth of methanotrophs.

Ammonia competes for MMO enzyme and produce toxic hydroxylamine or nitrite. In contrast, nitrate support higher growth rate for both type of methanotrophs. Some study show that ammonia has less toxic effect on type II methanotrophs which help them to form stable community. So, nitrate can be selected as nitrogen source for enrichment of type I methanotrophs and ammonia or nitrogen limited condition can be selected for type II methanotrophs.

2.2.9 Copper

Copper can control the form of MMO enzyme in pMMO or sMMO depending on the concentration level of copper (Semrau et al., 2010). However, copper is not a decisive parameter to enrich specific type of methanotrophs as both type I and type II can express pMMO enzyme (Cantera et al., 2016). Some studies showed that with the addition of copper had significantly increased the methane uptake rate for methanotrophic mixed culture (van der Ha et al., 2010). However high copper concentration can inhibit the growth of methanotrophs due to its toxicity.

2.3 Application of methanotrophs in WWTPs

Methanotrophs are promising, advantageous microorganism that they can be applied to produce product such as bio fuel, bio polymer, etc. They can be applied in certain bio process such as methane mitigation, contaminant bioremediation, denitrification, electricity generation in microbial fuel cells, etc (Strong et al., 2015). Methanotrophs have many aspects in WWTPs among them methanol, biopolymer production and nitrification/denitrification by methanotrophs will be discussed.

2.3.1 Methanol production by methanotrophs

Due to storage, transportation difficulties and low electricity conversion efficiency (25-45%), methane is not a good option for direct use a fuel (Bachmann, n.d.). In contrast, methanol has higher transportability, security, high energy content with higher efficiency making it a lucrative option as fuel. In addition, methanol is used as external carbon source for denitrification process in WWTPs (Strong et al., 2015). So, methanol can be considered as sustainable alternative option for methane. Methanotrophs oxidizes methane into methanol which is further oxidized consecutively to form carbon dioxide with formaldehyde and formate as intermediates. Methanol oxidation is catalyzed by methanol dehydrogense (MDH), formaldehyde dehydrogenase (FaDH) and formate dehydrogenase (FDH) subsequently (Sheets et al., 2017). To get methanol, the oxidation of methanol to formaldehyde is prevented by inhibiting the activity of (methanol dehydrogenase) MDH enzyme by sodium chloride, phosphate, etc (Hwang et al., 2014). Inhibition of MDH activity results in a shortage of electrons which is required for cellular energy and continuous methane oxidation. As a result, external electron source (formate) is added to complete the reaction. The reported methanol production from pure culture is 0.6-1120 mg/L with conversion efficiency 27-80% (Ge et al., 2014). In addition, methanol production from mixed culture achieved was 240~485 mg/L (AlSayed et al., 2018d).

Factors affecting methanol production:

Methanol production is affected by several factors such as: head space gaseous composition, MDH inhibitors and biomass density (Patel et al., 2016b). It is important to know how these factors affect methanol productivity to scale up the methanol production in a industrial scale.

Nutrients

Copper is an important nutrient for methanol production as copper support the expression of pMMO enzyme. With the addition of 5 μM of copper ion significantly increases methanol production but more than 10 μM inhibits the growth and methanol production. In addition, it is reported that addition of iron also increases the methanol productivity (Sheets et al., 2016).

Gas mixing ratio

Theoretically to produce one mole methanol it requires one mole methane and one mole oxygen gas. However, for longer incubation period more methane gas increases methanol production. Moreover, with increased methane concentration methane uptake rate increases.

MDH inhibition

Methanol in methanotrophs is oxidized by PQQ linked MDH enzyme (Hanson and Hanson, 1996). MDH inhibition is crucial part to prevent methanol oxidation. For MDH inhibition several chemicals such as Phosphate, NaCl, Cyclopropanol, EDTA, $MgCl_2$ and NH_4Cl are used separately or in combination (Sheets et al., 2016; Yoo et al., 2015). The problem with MDH inhibitors is they inhibit MDH activity and MMO activity as well (Ge et al., 2014). As a result, methanotrophs growth and methane uptake is negatively affected by MDH inhibitors. So, it is very important to choose suitable inhibitor to maximize methanol production. Phosphate is commonly used inhibitor and can be used with other inhibitor such as NaCl, EDTA, $MgCl_2$. It was found that with increased concentration of phosphate along with $MgCl_2$ increases methanol production as $mgCl_2$ support sMMO activity and cellular growth (Duan et al., 2011). In addition, NH_4Cl and NaCl have high methane to methanol conversion efficiency but they reduce the MMO activity (Yoo et al., 2015).

External electron donor

In methane oxidation, to produce one mole methanol 2 moles of electrons are required which usually come from afterward oxidation of methanol. Due to application of MDH inhibitor external electron source is required. Formate and formaldehyde can used as electron donor, but formaldehyde has toxic effect on methanotrophs. So, formate is a suitable source for external electrons(Hwang et al., 2015). The problem with formaldehyde is the cost to apply it in a industrial scale.

Biomass density

With the increase of biomass density from low to high increase methanol production to a certain limit, after that increase in cell density negatively affect methanol production (Lee et al., 2004). However, some study showed that with increased MDH inhibitor methanol production was increased at higher concentration of biomass. In addition, increasing methane concentration with increased biomass density also resulted higher methanol production (Lee et al., 2004).

Biopolymer production by methanotrophs

Bioplastic or biopolymer is a green alternative to conventional plastic products as biopolymer is produced naturally and degradable after some time. Most of the bioplastics belongs to the family of Polyhydroxyalkanoates (PHA) where polyhydroxybutyrate (PHB) is a member among them.

PHA accumulating methanotrophs can accumulate PHB using methane gas under nutrient limiting condition (Strong et al., 2015). Under aerobic condition, methanotrophs without essential nutrients such as nitrogen, phosphorus are forced to store PHB inside their cell for surviving (Karthikeyan et al., 2015).

2.3.2 PHB production mechanism in methanotrophs

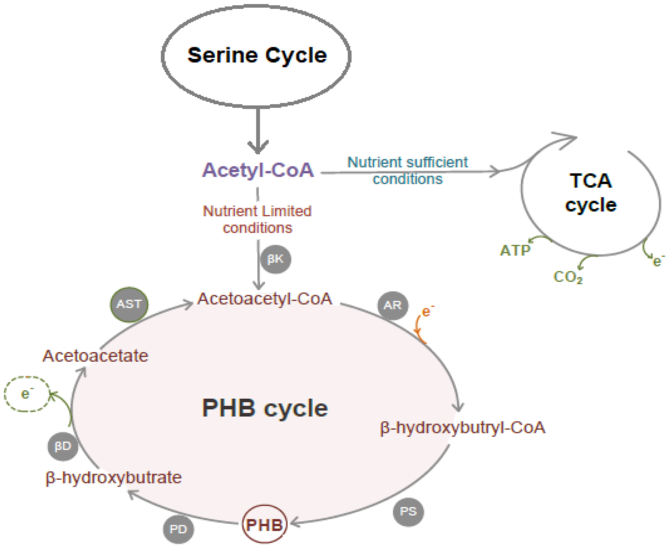


Figure 2.1 PHB production pathway in Methanotrophs. (Adapted from AlSayed et al., 2018b)

In PHB production acetyl-CoA is the main intermediate which is produced through serine pathway (Babel, 1992). This means PHB production might be exclusive to type II methanotrophs. Normally, in balanced condition with all nutrient, acetyl-CoA goes towards TCA cycle to fulfill energy needs. While in nutrients limiting condition, the bacteria adopt survival strategy by switching towards PHB cycle for cell maintenance. In PHB cycle, at first, acetyl-CoA is converted to Acetoacetyl-CoA which is reduced to β-hydroxybutryl-CoA with the help of β-ketothiolase and Acetoacetyl-CoA reductase enzyme. β-hydroxybutryl-CoA is then converted by a polymerase, PHB synthetase, to form PHB. PHB granules is depolymerized to Hydroxybutrate monomers by PHB depolymerize enzyme. After this step, β-hydroxybutyrate dehydrogenase enzyme converts Hydroxybutrate monemers into acetoacetate which is finally converted in to Acetoacetyl-CoA by Acetoacetate succinyl-CoA transferase enzyme to complete the whole cycle (Karthikeyan et al., 2015).

Factors affecting PHB production:

PHB accumulation is mainly done by type II methanotrophs. For PHB production, growth parameters should be selected to favor the growth of type II methanotrophs. Here the factors for type II selection and PHB accumulation will be discussed.

Nitrogen source

It has been proven through different studies that nitrogen limited condition is a reliable parameter for long term PHB productivity. Methanotrophs can grow on inorganic nitrogen such as ammonia, nitrate and nitrogen as their nitrogen source. However, as discussed earlier, for PHB accumulation selection of type II methanotrophs is an important factor. From studies it was found that strains of methanotrophs methylocystis parvus OBBP and methylocystis GB25 (in mixed culture) grown on ammonia can produce more PHB than grown on nitrate (Rostkowski et al., 2013; Wendlandt et al., 2001). Previous studies also suggest that methanotrophs grown on nitrate have higher biomass density but produce less PHB due to invasion of type I methanotrophs. On the other hand, higher PHB production was observed with ammonia but less biomass density results. Considering these factors, a strategy was developed to grow type II methanotrophic mixed culture from activate sludge with ammonia as nitrogen source then increase the biomass density using nitrate as nitrogen source. This technique yielded about 40% nitrogen which could be increased through optimization of nitrogen concentration (Criddle and Sundstrom, 2015).

Phosphorus

Phosphorus is important for Type II methanotrophs selection. Phosphorus concentration up to 2-25 mmol helps to maintain sMMO activity which is expressed through type II methanotrophs. However, for PHB accumulation phosphorus limited condition is required. It was observed that in phosphorus limited condition 46% PHB accumulated by methylocystis GB25 starin(Wendlandt et al., 2001).

Copper

The effect of copper on PHB accumulation has contradictory studies. Some study showed that reducing copper from 15 to 5μM increased PHB accumulation from 18% to 49% (Sundstrom and Criddle, 2015) . However, in another study it was found that addition of 5μM copper increased PHB accumulation from 25% at without copper to 51% (Zhang et al., 2017).

Other nutrients

It was found that iron concentration from 40 to 80 μM have positive effect on sMMO activity (Park et al., 1991). In addition, sodium, potassium, magnesium and mercury have inhibitory effect on PHB production (Wendlandt et al., 2005).

Temperature & pH

In some studies it was found that temperature more than 30℃ reduce the activity of sMMO and more than 45℃ reduce the activity of pMMO (Park et al., 1991). pH in the range of 6 to 7 is suitable for methanotrophs growth however, pH 5 can be used to promote the growth of type II methanotrophs (Pieja et al., 2011).

Methane and oxygen

At high level of oxygen cause increase in oxidation of methanol to formaldehyde produce inhibitory effect on metabolic activity of methanotrophs (Costa et al., 2001). In addition, with low methane concentration where ammonia is a nitrogen source increase hydroxylamine production causing both ammonia and hydroxylamine toxicity on biomass. In toxic condition bacteria are forcing to store PHB inside their cell to survive under harsh condition.

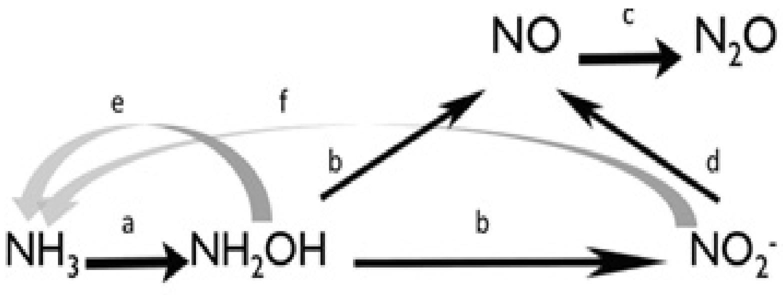
2.3.3 Nitrification and denitrification by methanotrophs

It has been discovered that several strains of methanotrophs can nitrify and denitrify using nitrogen compounds. In this section the mechanism for nitrification and denitrification and the enzymes involved will be discussed.

2.3.3.1 Nitrification by methanotrophs using ammonia as nitrogen source

MMO enzyme in methanotrophs have similar properties as Ammonia monooxygenase (AMO) enzyme that come from ammonia oxidizing bacteria (AOB). Due to this unique ability methanotrophs are able to oxidize ammonia through MMO enzyme. In this process ammonia competes with methane for MMO enzyme for oxidation (He et al., 2017).

In the first step ammonia is oxidised into hydroxylamine intermediate by MMO enzyme. Hydroxylamine is highly toxic for methanotrophs which make the methanotrophs to take detoxification strategies. One strategy is to oxidize hydroxylamine in to nitrite by hydroxylamine oxidoreductase (HAO) enzyme and, another strategy is to reduction of hydroxylamine back to ammonia by hydroxylamine reductase (Stein and Klotz, 2011).



a→ methanemono-oxygenase;
b → HAO and unknown enzymes;
c → NorB nitric oxide reductase and unknown enzymes;
d → NirK, NirS and HaoA' nitrite reductases and unknown enzymes;
e → hydroxylamine reductase;
f → NirB assimilatory nitrite reductase

Figure 2.2: nitrogen catabolic pathways in aerobic methanotrophic bacteria

Nitrite is also toxic for which methanotrophs take detoxification strategy by reducing it to nitric oxide by NirK, NirS and HaoA nitrite reductases and unknown enzymes or reduce back to ammonia by NirB assimilatory nitrite reductase (Stein and Klotz, 2011). Some methanotrophs were found to resist the toxicity of ammonia, hydroxylamine and nitrite. It was found that some strains M. album from type I and Methylocystis sp. And M. sporium from type II were able to produce nitrite from ammonia (Nyerges and Stein, 2009). In another study 14 genotypically different methanotrophs strains were tested to study their nitrogen fixation metabolism and tolerance against ammonia, hydroxylamine and nitrite. It was found that most of the strains can tolerate up to 40 mM of NH_4Cl, 2 mM of $NaNO_2$ and 1 mM hydroxylamine. In addition, all type I strains produced N_2O from hydroxylamine oxidation and all type II strains produced N_2O from nitrite oxidation (Hoefman et al., 2014).

2.3.3.2 Denitrification by methanotrophs

Methanotrophic can fix nitrogen through denitrification directly or indirectly. Aerobic methane oxidation coupled to denitrification (AME-D) is a widely discussed process where methanotrophs indirectly helps denitrification. In AME-D methanotrophs oxidize methane aerobically and produce organic compounds such as methanol, acetate, citrate and proteins which are utilized by the denitrifiers in WWTP as electron donor for denitrification (Modin et al., 2007). During this process the oxygen level is kept at a minimum to keep aerobic methanotrophs active along with no inhibitory effect on the denitrifiers. There is another indirect process called simultaneous nitrification and denitrification process (SNR) where methanotrophs, autotrophic nitrifiers and dentrifiers live together in a same bioreactor under same operating condition (Lee et al., 2001).

Recently it has been discovered that methanotrophs can denitrify by themselves under anoxic condition. Denitrification in methanotrophs can be explained as energy conservation strategy for respiration during oxygen limited condition and switching their electron acceptor from oxygen to nitrate or nitrite (Kits et al., 2015). Denitrification in methanotrophs is mainly partial denitrification because most of the strains tested were able to produce nitrous oxide terminally. For example, *Methylomonas denitrificans* FJG1 can do partial denitrification along with methane oxidation while producing nitrous oxide as terminal gas (Zhu et al., 2017). However, only one strain was found that was able to perform complete denitrification. Mythalocystis sp. SC2 strain was found to have nitrous oxide reductase (NOR) to convert nitrous oxide into nitrogen gas (Dam et al., 2013).

2.3.4 Nitrogen removal practice in WWTPs

Two approaches are followed in WWTPs for nitrogen removal: conventional nitrification-denitrification process and shortcut nitrogen removal process. Nitrification-denitrification process is most common in conventional WWTPs. Nitrification is a two-step process where ammonium is oxidised first to nitrite (NO_2^-) by ammonium oxidising bacteria (AOB) and then to nitrate (NO_3^-) by nitrite oxidising bacteria (NOB) under aerobic condition (Daims et al., 2016). In denitrification, under oxygen limited condition, denitrifying organisms reduce the nitrate terminally into nitrogen gas (Kraft et al., 2011). Aeration is needed for nitrifying bacteria what makes it energy intensive process. Whereas, the denitrifiers use organic substances such as methanol as a carbon source which requires the addition of external carbon (Zhu et al., 2017). On the other hand, the process of shortcut nitrogen removal was adopted which minimize or eliminate aeration and external carbon addition in the treatment process. There are several shortcut nitrogen removal processes such as: Nitritation-denitritation process, Deammonification and ammonia oxidation process by anaerobic ammonia oxidizing bacteria (Anammox). In nitritation-denitritation process nitrite oxidation to nitrate is bypassed to lower the oxygen requirement in the bioreactor. The limitation of this process is to inhibit the activity of NOBs to prevent further oxidation of nitrite into nitrate (Al-Omari et al., 2015). In Deammonification ammonium is oxidised directly to nitrogen gas by annamox bacteria without the need for carbon and in the absence of oxygen. The challenge for deammonification is that annamox is slow growing and it requires nitrite as oxidant which come through partial nitrification of ammonia by AOBs. Deammonification is more suitable to side stream treatment where ammonium concentration is high (Fernández et al., 2016). Collectively, both conventional and shortcut nitrogen removal processes expose different challenges that hindering it from being adopted in WWTP. Those challenges include the addition of external carbon source, extra energy input, controlling bacterial activity while nitritition, nitrogen removal in high COD environment.

2.3.5 Integration of methanotrophs in WWTPs

The best way to integrate methanotrophs in WWTP is to grow them within the system. WWTPs provide most of the elements required for methanotrophs cultivation. First of all, the seed required for methanotrophs cultivation can be obtained from waste activated sludge (WAS) or return activated sludge (RAS) during typical wastewater treatment processes. Secondly, for liquid feed WW effluent containing low level N, C and P can be used instead of synthetic feed. In addition, AD centrate contains high concentrated nutrients which can be used as feed by dilution.

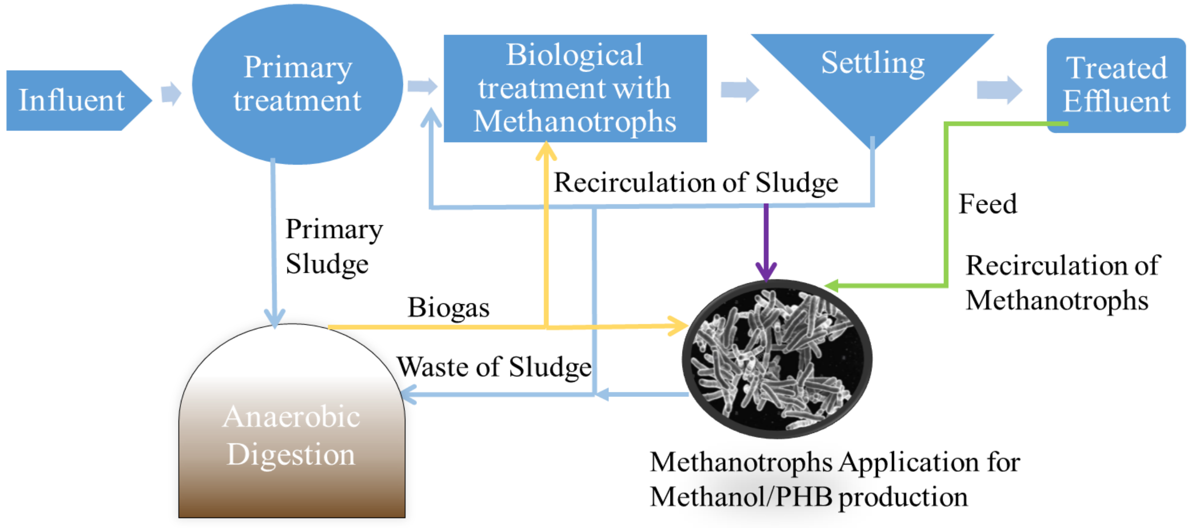


Figure 2.3: Integration of Methanotrophs in to WWTP

Moreover, methanotrophs can be grown with mainstream WW influent as feed containing COD, ammonia and phosphorus. Thirdly, the biogas generated from the anaerobic digester (AD) can be used as feed for methanotrophs growth and also can be used as external carbon source for denitrification process in Biological Nutrient Removal (BNR) system. Using the biogas methanotrophs will grow in the reactor and produce organic compounds to be used by the denitrifiers. Fourthly, Methanotrophs that are cultivated from the process can be utilized for methanol production or PHB production. PHB enriched methanotrophs can be utilized either in a BNR reactor for denitrification or can be used as external electron source instead of costly formate in methanol production.

So, integration of methanotrophs is beneficial as it can produce valuable products such as PHB and Methanol, and it can assist nitrification and denitrification directly or indirectly. These modifications can make onto WWTPs energy self-sufficient by saving and producing fuel. In addition, as this process will increase nitrogen removal efficiency from main stream wastewater which will make the process more sustainable, environment friendly and safe to public health.

CHAPTER 3: Methanotrophic-heterotrophic mixed culture performance under varying chemical oxygen demand (COD)

3.1 Introduction

Global methane emission is expected to increase by 20% over the next two decades (US EPA, 2016). WTPs produce significant amount of methane, for instance, each year 3.9 billion tones of biomethane are produced from wastewater treatment facilities in North America (AlSayed et al., 2018c). Typically, biogas from anaerobic digesters (AD) contains 60-70% methane with some impurities such as H_2S and NH_3. Biogas can be used as a fuel after being purified and converted into compressed natural gas or liquefied natural gas. Such processes are not cost effective as it requires high energy inputs and capital costs (Ge et al., 2014). Besides, due to the applied high pressure and methane explosive nature, it is unsafe to be stored, transferred and distributed (Ge et al., 2014). For this reason, it is necessary to develop a sustainable and cost-effective technology to convert AD-driven biogas into more sustainable value-added product.

There are several thermochemical techniques with high temperature, pressure and catalyst to utilize methane gas (Park and Lee, 2013) which require intense energy use and expensive chemicals. However, this process always involved with generation of synthetic gas such as carbon monoxide which is harmful to the environment. Moreover, there is another process called non-thermal dielectric barrier discharge (DBD) plasma chemical process which requires high voltage and electrodes (Park and Lee, 2013). Hence, both thermochemical and plasma chemical process is costly, less efficient and not environmentally sustainable.

3.2 Biochemical conversion of methane by methanotrophs

In contrast to physical and chemical processes, biochemical process of methane utilization is a promising option as it can be operated in moderate condition with high conversion efficiency. In this process, microorganisms act as the biocatalyst which have wide biodiversity and can adapt to various environment (Ge et al., 2014). Methanotrophs can grow on methane as their sole carbon and energy in which C-H bond with is activated by the methane monooxygenase (MMO) enzyme under ambient condition (Culpepper and Rosenzweig, 2012).

3.3 Nitrogen issues in WWTPs

Widely used conventional Activated Sludge (AS) system is criticized for intensive energy requirement where most of energy (55%) is required for biological treatment only (Mamais et al., 2015; Massara et al., 2017). AS process is designed to remove organic matter (BOD) by microorganism from wastewater. In this process in presence of oxygen microorganism utilize organic waste and also covert ammonia into nitrate (Ahansazan et al., 2014). For nitrogen removal additional treatment processes are incorporated which required additional energy (Massara et al., 2017).

Ammonia (NH_3-N) is the major form of nitrogen that present in typical wastewater. Typical municipal wastewater influent contains 20~60 mg/l total N including 60% NH_3-N and 300~500 mg/l COD (Chai et al., 2015; Gilbert et al., 2014; Hanson and Lee, 1971). Considering domestic wastewater dominance most of the treatment plant around the world tend to treat it biologically by activated sludge method (Krishna Reddy et al., 2017). The total nitrogen removal rate is around 50% during the biological treatment in conventional activated sludge process through cellular assimilation (Liu et al., 2018). As a result, treated wastewater effluent may contain up to 15~30 mg-N/L directly in to water bodies which are mainly in the form of nitrate (Tchobanoglous et al., 1991). WTPs in GTA area mainly remove CBOD, Phosphorus, TSS and E. Coli (City of Toronto, 2017). Nitrogen is considered as one of the major contributors to poor water quality and its (Purwono et al., 2017). Nitrogen presence provide the needed nutrients the growth of algae causing algal blooms, which depletes the dissolved oxygen in the water bodies needed for the aquatic. Such phenomenon is called eutrophication. Moreover, nitrogen presence in drinking water in excessive concentrations may cause serious public health issues (Obaja et al., 2003; Ward et al., 2018).

3.4 Potential application of Methanotrophs in WWTPs

In WWTP methanol can be produced from biogas generated from anaerobic diester. In biogas the presence of carbon dioxide can help MDH inhibition. The reported maximum methanol production from mixed culture achieved was 240~485 mg/L (AlSayed et al., 2018d). In addition, Methanotrophs without essential nutrients such as nitrogen, phosphorus can convert methane into PHB and store them inside their cell (Karthikeyan et al., 2015). PHB is extracted from the bacterial cell processed to produce polymer to be used in industries. The advantage of PHB is that it is biodegradable after certain period of use (Rostkowski et al., 2012). The reported PHB accumulation percentage ranges between 40 to 60% of their cellular weight (Fergala et al., 2018b; Khosravi-Darani et al., 2013). PHB can be used as electron donor in methanol production. Moreover, produced methanol can aid denitrification activity by providing carbon to the denitrifiers. From recent discovery, methanotrophic bacteria can do nitrification and partial denitrification along with methane oxidation while producing nitrous oxide as terminal gas (Zhu et al., 2017).

PHB is an eco-friendly bio-polymer and can be used as electron donor in methanol production. Methanol can be use as bio-fuel or external carbon source for denitrification to turn the WWTPs in to energy self-sufficient one. In addition, this process will increase nitrogen removal efficiency from main stream wastewater which is environment friendly and safe to public health.

3.5 Scope of Works

It is very important to integrate methanotrophs in to WWTP process to mitigate AD generated biogas and recover valuable resource from it.The aim of this chapter is to report the performance of maintaining methanotrophic-heterotrophic culture maintained under wastewater mainstream conditions in fed-batch mode. Furthermore, the culture capacity to remove nitrogen either for cellular assimilation or nitrification-denitrification was explored. Thereafter, the active culture with same condition was used in batch experiment for methanol and Poly Hydroxyburate (PHB) production.

3.6 Materials and methods

3.6.1 Operational condition

For enrichment and growth cycles, mineral salts medium (MSM) (Bowman, 2006) was used where the composition is as follows (in mg/L): $MgSO_4 \cdot 7H_2O$, 1000; $CaCl_2 \cdot 2H_2O$, 200; KH_2PO_4, 272; K_2HPO_4, 610; Fe-EDTA, 4 and 1 mL/L trace metal solution. The chemical composition of trace metal solution is (mg/L): $ZnSO_4 \cdot 7H_2O$, 10; $MnCl_2 \cdot 4H_2O$, 3; H_3BO_3, 30; $Na_2MoO_4 \cdot 2H_2O$, 3; $FeSO_4 \cdot 7H_2O$, 200; $NiCl_2 \cdot 6H_2O$, 2 and $CoCl_2 \cdot 6H_2O$, 20. In addition, for all experiments copper sulfate ($CuSO_4 \cdot 5H_2O$) concentration was made 10 μmol from a stock solution. Nitrate (in the form of $NaNO_3$) and ammonium (in the form of NH_4Cl) were used for enrichment and growth phase.

For all experiments' incubation was done using 250-mL serum bottles (Wheaton, Mealville, NJ, USA) capped with butyl-rubber stoppers. Liquid volume was 50 mL, and the headspace volume was 200 mL. The headspace was evacuated by a suction pump for 5 minutes and then it was filled with oxygen and methane or helium gas (to maintain same pressure) with purity >99% (Praxair Technology, Inc., Danbury, CT, USA). Methane and oxygen were added with a volumetric ratio of 1:1. The bottles filled with liquid and gas were placed horizontally on a MaxQ™ 4000 Benchtop Orbital Shakers (Thermo Fisher Scientific Inc., Waltham, MA, USA) at 165 rpm and the incubation temperature was 30°C. Gas and liquid sample measurements were carried out at the start and end of each cycle. For gas samples oxygen, nitrogen, methane and carbon dioxide were measured and for liquid samples optical density ($_{OD600}$), COD concentration as mg/L, ammonium, nitrate, nitrite concentration as mg-N/L were measured.

3.6.2 Methanotrophs mixed culture enrichment

In this stage methanotrophic mixed culture was enriched from the seed for the growth phase. The seed for cultivating methanotrophs cultures was collected from waste activated sludge from Humber wastewater treatment plant (Toronto, Canada). Fresh waste activated sludge from Humber wastewater treatment plant (Toronto, Canada) were used as a seed for type I methanotrophs enrichment. The sludge was filtered through 100-µm cell strainer to remove large particles. The filtered sludge was centrifuged and re-suspended in 50 ml MSM with 10 µM copper sulfate and 10 mM of sodium nitrate. Initial optical density (OD_{600}) were kept to 0.5 ± 0.1 for four bottles. The headspace was filled with 200 mL of O_2 and 200 mL of CH_4 every 24 hours. In addition, the culture medium was changed with fresh medium by centrifuging (4200 rpm) for 20 mins every two days. After four days, cultures started to shift to a pinkish color known for type I methanotrophs. The enrichment was continued for fourteen days to get type I methanotrophs with stable growth rate and gas consumption. Before starting of growth phase with ammonium and sodium acetate, the number of bottles increased to nine and liquid medium was changed every day with initial OD_{600} of 1 ± 0.1 to ensure the exponential phase. Samples are required for microbial analysis to confirm type I methanotrophs existence/dominance.

3.6.3 Growth Phase

Enriched cultures were cultivated in four sets of experiment (table 1) in 50ml MSM with ammonium and COD. It was decided to maintain the ratio (w/w) of COD: NH_4-N at around 6 for all sets of experiment. Sodium acetate was used to provide COD to liquid medium. For ammonium-nitrogen (NH_4-N) concentration (mg/L) 30, 60 and 90 corresponding COD concentrations were (mg/L) 180, 360 and 540 respectively. 200 mL of O_2 and 200 mL of CH_4 were used for all four sets. For each set, bottles were triplicated for data consistency. The experiment was run in fed-batch for 50 consecutive cycles with 24 hr cycle duration. For first ten days all measurements were taken then for 17 days there were no measurement but for final 12 days all measurements were taken again. In each cycle initial OD_{600} was made to 2 ± 0.1, liquid medium was transferred with fresh medium and headspace was replaced with appropriate gas volume.

Table 3.1: Experimental conditions for fed-batch experiments

Fed-Batch tests	OD_{600}	NH_4^+ (mg N/L)	COD (mg /L)	COD:N	CH_4 (ml)	O_2 (ml)
Control 1	2.0	60	0	-	200	200
A	2.0	60	360	6:1	200	200
B	2.0	30	180	6:1	200	200
C	2.0	90	540	6:1	200	200

3.6.4 Analytical methods

Gas samples were collected from serum bottles using an air tight syringe. With the same syringe, gas samples were injected in to a gas chromatography (SRI instrumentation, Torrance, USA) machine to analyse the gases. The GC is equipped with thermal conductivity detector (TCD), and molecular sieve column. The GC is connected to helium and hydrogen gas cylinder as carrier gas. The flow rate for helium and hydrogen gas were 15 ml/min and 20 ml/min respectively. GC analyses the gas through the column and gives peak of oxygen, nitrogen and methane consecutively at different time. With the peak area, gas concentration can be determined from previous calibration. In the program the temperature was set as: injector-80°C; Oven- 80°C; FID-300°C; TCD-155°C.

For optical density measurement, DR 3900 Benchtop Spectrophotometer (HACH Company, Loveland, Colorado, USA) was used. To obtain the dry cell weight (DCW), a previously developed correlation equation between OD_{600} and DCW was used (equation 1, alsayed et al, 2018).

DCW (mg) = OD_{600} / 0.0021 **X** liquid volume (L)--------------------------------(1)

To measure the NH_4^+, NO_3^-, NO_2^- ion chromatograph (IC) was used. COD and total nitrogen were measured based on optical measurement by, HACH methods and testing kits.

3.7 Results and Discussion

3.7.1 Influence of varying COD on methanotrophic microbial activity

The effect of COD on methanotrophic microbial activities tell us the possibility of growing methanotrophs under COD environment. The previously reported performance for methanotrophic activity was done in the presence of methanotrophs that favors the dominance and growth of methanotrophs. However, in real application in wastewater treatment plants, COD is expected with different concentrations based on the location of the methanotrophic reactor. The behavior of methanotrophs in COD environment with other microorganisms will be interesting.

In this section, biomass microbial activities such as specific growth rate or μ (day^{-1}), observed growth yield or Y (mg-VSS/mg-CH_4), methane uptake rate (mg/hr), CH_4 consumption, O_2 consumption and COD consumption are reported from fifty cycles of operation under different COD concentrations. The calculations for these parameters are included in Appendix 1 and Appendix 2. Sodium acetate is used as COD source with concentration varying from 0 to 540 mg/L. Ammonia is used as nitrogen source with concentration varying from 30 to 90 mg/L with same initial biomass density of OD_{600} 2.0±0.2 (952 mg/L). To reflect the main stream condition COD/N ratio is maintained as 6:1. For example, for 360 mg/L sodium acetate as COD concentration, nitrogen concentration is 60 mg/L as ammonia nitrogen and initial biomass density is OD_{600} 2.08 ± 0.13. From previous studies (Ahmed AlSayed et al. 2018), optimum growth condition is maintained to achieve maximum biomass growth. Volume of added methane is 200 ml and volume of added oxygen is 200 ml to maintain 1:1 methane/oxygen ratio(v/v). As mentioned earlier, all measurements had been taken for 10 cycles at the beginning and 12 cycles at the end and no measurements were taken for 28 cycles at the middle.

Specific growth rate μ and biomass yield Y:

As there are 22 cycles (10+12 cycles) data for different COD conditions, t-test were performed for data comparison between two conditions. In this study the data sets with varying COD were compared with data set with no COD. During t-test it was considered that all data were unequally variable. For t-test if p value is less than 0.05 then it can be considered that the data sets are different from each other. On the other hand, if p value is greater than 0.05 then the data sets are considered not significantly different.

For first 10 cycles specific growth rate μ, biomass yield Y and methane uptake rate increased from no COD condition with increasing COD (Figure 1a). At COD 0 mg/L and NH4-N 60 mg /L average specific growth rate, μ and biomass yield, Y were 0.28±0.25 d^{-1}, 0.30±0.25 mg-VSS/mg-CH_4 and 2.7±0.6 mg-CH_4 /hr respectively. Maximum specific growth rate was 0.41±0.15 d^{-1} at COD 540 mg/L which is 1.46-fold (p=0.002) higher than no COD condition. The maximum value of observed yield and methane uptake rate were also found at COD 540 mg/L. The values are 0.36±0.14 mg-VSS/mg-CH_4 and 3.2±0.5 mg-CH_4 /hr respectively and they are 1.2-fold (p=0.002) and 1.18-fold (p=0.01) higher than no COD condition. The higher standard deviation was observed for the first 10 cycles due to the fluctuation of growth parameters at the beginning. After then the experiment kept running without any measurement till 36th cycle. All measurements were taken again from 37th till 50th cycle. For final 12 cycles, at no COD condition average specific growth rate and μ and biomass yield Y were 0.39±0.13 d^{-1} and 0.41±0.12 mg-VSS/mg-CH_4 (Figure 1b) which are 1.39 (p=0.04) times and 1.36 (p=0.009) times higher than the respective values for first 10 cycles without COD condition (Figure 1b). Here less standard deviation than the beginning was observed confirming the stable performance of the culture. The maximum μ and Y were found at COD 360 mg/L were 0.49±0.12 d^{-1} and 0.49±0.11 mg-VSS/mg-CH_4 which are 1.25 (p=0.0008) times and 1.2 (p=0.0001) times higher than no COD condition. The values decreased marginally by 6 percent and 8 percent respectively at COD 540 mg/L. Which indicated that growth parameters increased till COD 360 mg/L after that growth may become stable or decline with increasing COD. To confirm this further experiment is required to perform with higher COD concentration for longer period.

Methane uptake rate

Another growth parameter, average methane uptake rate for first 10 cycles was found 2.7±0.3 mg-CH_4 /hr at no COD condition, which is 22% less ($p=0.01<0.05$) than the value at COD 540 mg/L. Here, p value is less than 0.05 signifies the difference in methane uptake rates for two COD conditions. For the final 12 cycles, methane uptake rate was 2.5±0.3 mg/hr, which remained unchanged ($p=0.88>0.05$) for increasing COD values. P value greater than 0.05 indicates no significant difference between two conditions. From this data it is evident that after 36 cycles methane uptake rate became stable and did not change with COD influence. This indicates that the COD has no significant influence on the methane utilization rate or methanotrophic activity of the consortium. Moreover, till certain level, COD had positive effect on the growth of the microbial community.

Comparison with literature

As mentioned earlier after day 36 most of the growth parameters were more stable than the beginning. Average values for all growth parameter from day 37 to day 50 are presented in table 1 to compare with literature values. Without COD the average specific growth rate was found 14 percent higher than the reported value. At no COD condition μ was 0.39±0.04d^{-1} where in literature (table 3.2) it is 0.344±0.06 for Type I methanotrophic mixed culture at OD 2.06 (AlSayed et al., 2018a). This confirms the best performance of the methanotrophic mixed culture at normal condition. The maximum μ at COD 360 mg/L is 1.44 times higher than the reported value by AlSayed et al. The Biomass Yield (mg-VSS/mg-CH4) for without COD is 0.41±.05 which is 33 percent higher than literature value of 0.33±0.04 for type I methanotrophs at OD 2.06 (AlSayed et al., 2018a). Average methane uptake rate (mg-CH_4/hr) were 2.50±0.52, 2.53±0.60, 2.50±0.36 and 2.48 ±0.49 for 0, 180, 360 and 540 mg/L COD concentration which is to comparable to the literature value of 2.73mg-CH_4/hr (AlSayed et al 2018). It was observed that the growth parameters showed increased values compared to reported values with the addition of COD. This might be due to the presence of other heterotrophic culture as COD favours the growth of heterotrophs. Overall, the incorporation of COD increased the specific growth rate, biomass yield and without affecting the methane utilization rate.

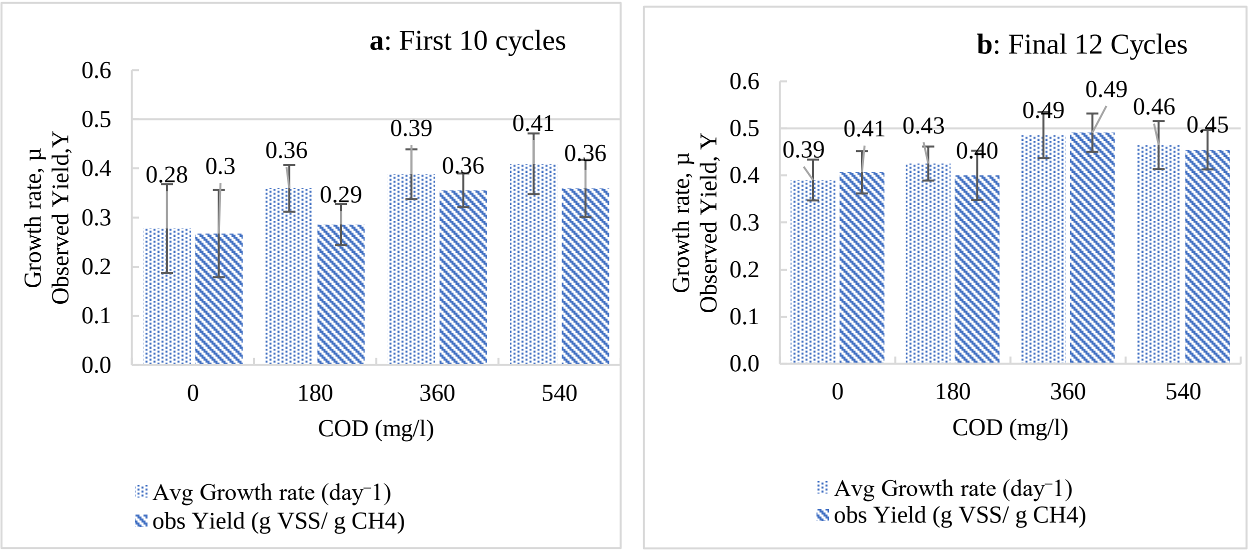


Figure 3.1: Average Growth rate (d^{-1}), Methane Uptake rate (mg-CH_4/hr) and Growth yield (g DCW/gCH_4) at COD 0 mg/L, 180 mg/L, 360 mg/L and 540 mg/L for a) first 10 cycles b) final 12 cycles

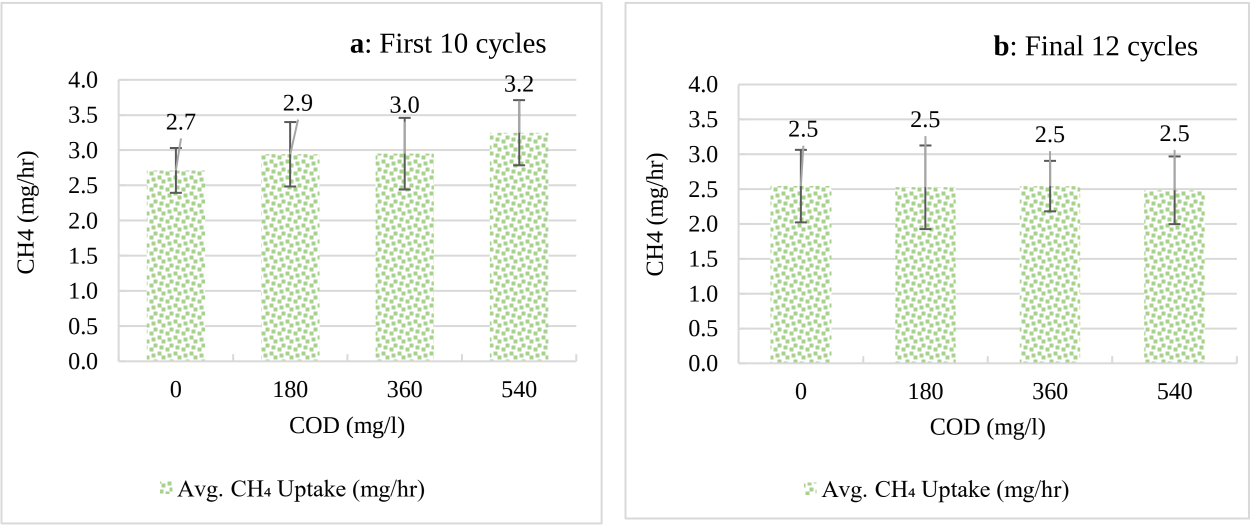


Figure 3. 2: Methane Uptake rate (mg-CH4/hr) at COD: 0 mg/L, 180 mg/L, 360 mg/L and 540 mg/L for a) first 10 cycles b) final 12 cycles

Table 3.2: Comparison of different growth parameters at different COD and ammonia concentration

Parameter	COD 0 (mg/l)	COD 180 (mg/l)	COD 360 (mg/l)	COD 540 (mg/l)	Literature Value (for mixed culture without COD)
Specific Growth Rate (day^{-1})	0.39 ±0.04	0.43 ±0.04	0.49 ±0.05	0.46 ±0.05	0.344±0.06 (Type I, at OD 2.06) AlSayed et al 2018
Methane Uptake Rate (mg/hr)	2.50 ±0.52	2.53 ±0.60	2.50 ±0.36	2.48 ±0.49	2.73 (Type I, at OD 2.06) AlSayed et al 2018
Biomass Yield (mg-VSS/mg-CH4)	0.41 ±0.05	0.40 ±0.05	0.49 ±0.04	0.45 ±0.04	0.33±0.04 (type I, at OD 2.06) AlSayed et al 2018

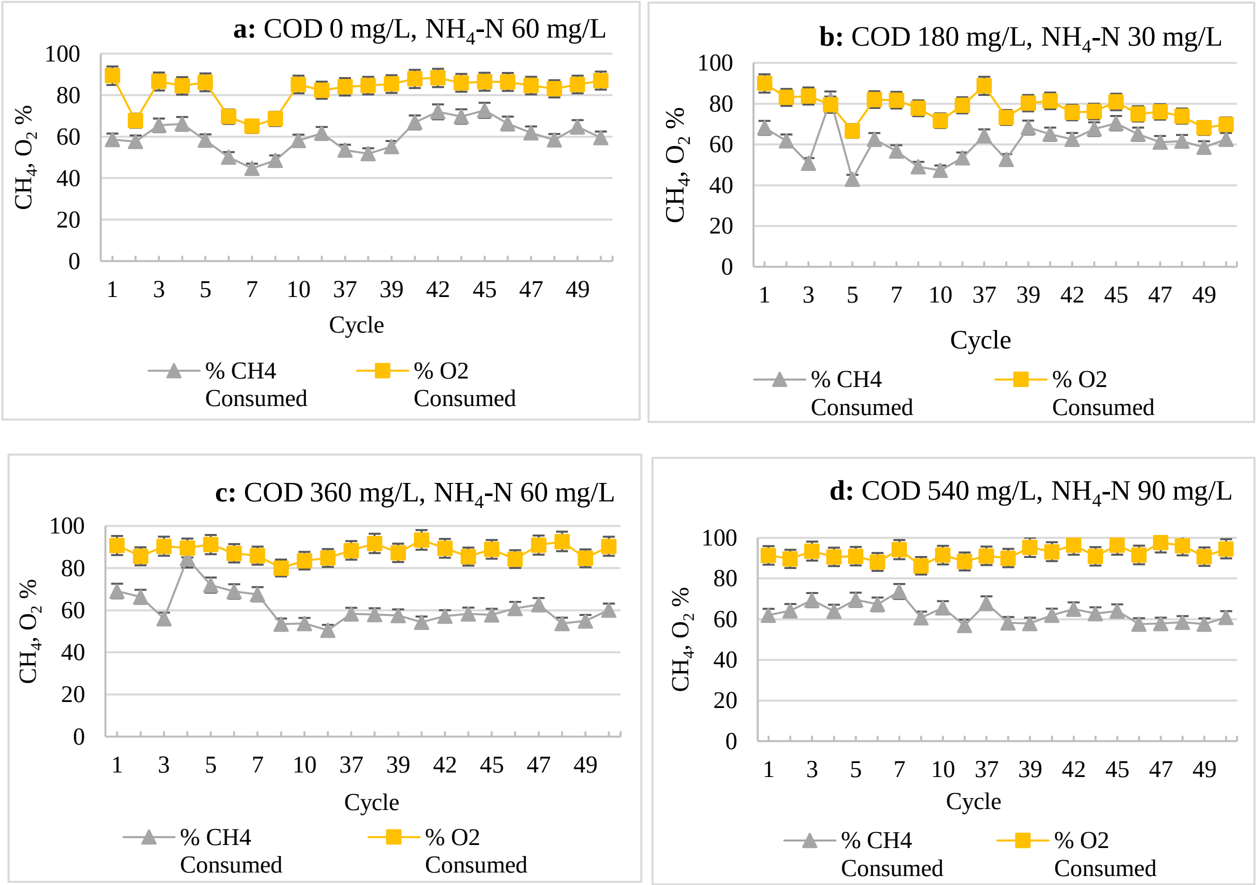


Figure 3. 3: Activity of biomass in terms of % CH_4 Consumption and % O_2 Consumption for a) COD 0 mg/L and ammonia-N 60 mg/L (control), b) COD 180 mg/L and ammonia-N 30 mg/L, c) COD 360 mg/L and ammonia-N 60 mg/L, d) COD 540 mg/L and ammonia-N 90 mg/L

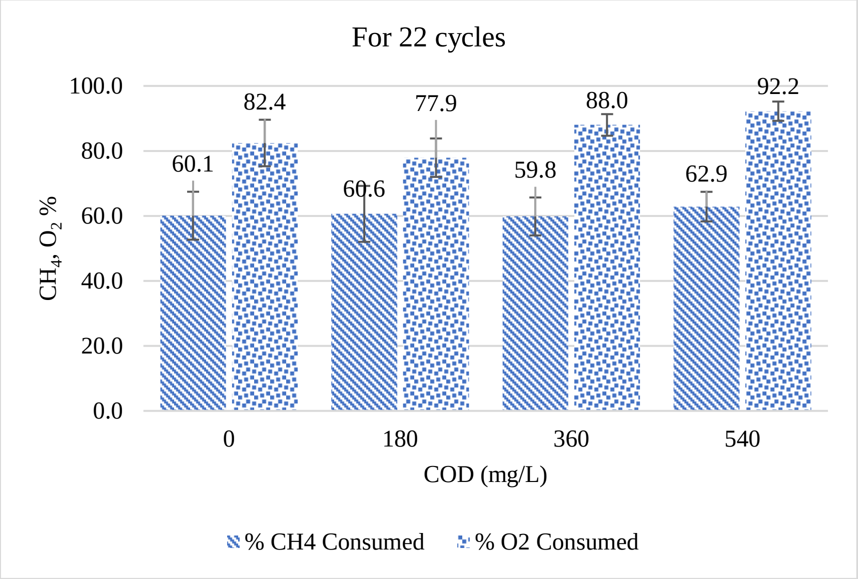


Figure 3. 4: average percentage of CH_4 and O_2 consumption for different COD

3.7.2 Methane, Oxygen and COD consumption by the microbial community

From methane and oxygen consumption data methanotrophic activity can be further confirmed. Moreover, COD consumption data ensures the growth of microorganisms other than methanotrophs. The calculation for all consumption data is attached in appendix I and II. The activity of the biomass monitored for different COD condition is compared without COD condition to reflect the influence of COD on the behaviour of the microbial community. For all COD condition methane and oxygen consumption was calculated from analytical data. Initially total methane and oxygen consumption percentage varied a little but after 10 cycles it became more consistent (Figure 3.3). For no COD condition, methane consumption varied from 45 to 73 percent and oxygen consumption varied from 65 to 88 percent (Figure 3.3 a). With COD 180 mg/L, methane and oxygen consumption became more stable after 36 cycles (Figure 3.3 b). From the beginning methane and oxygen consumption percentage varied from 43 to 70 percent and 67 to 89 percent respectively. With COD 360 mg/L there was fluctuation in methane consumption at the beginning while oxygen consumption was more stable throughout the cycles (Figure 3.3 c). Methane consumption also varied from 81 to 72 percent while oxygen consumption varied less from 84 to 93 percent. Interestingly, for COD 540 mg/L methane and oxygen consumption percentage were more stable from the beginning compared to other conditions (Figure 3.3 d). The values ranged from 57 to 69 percent and 86 to 98 percent respectively.

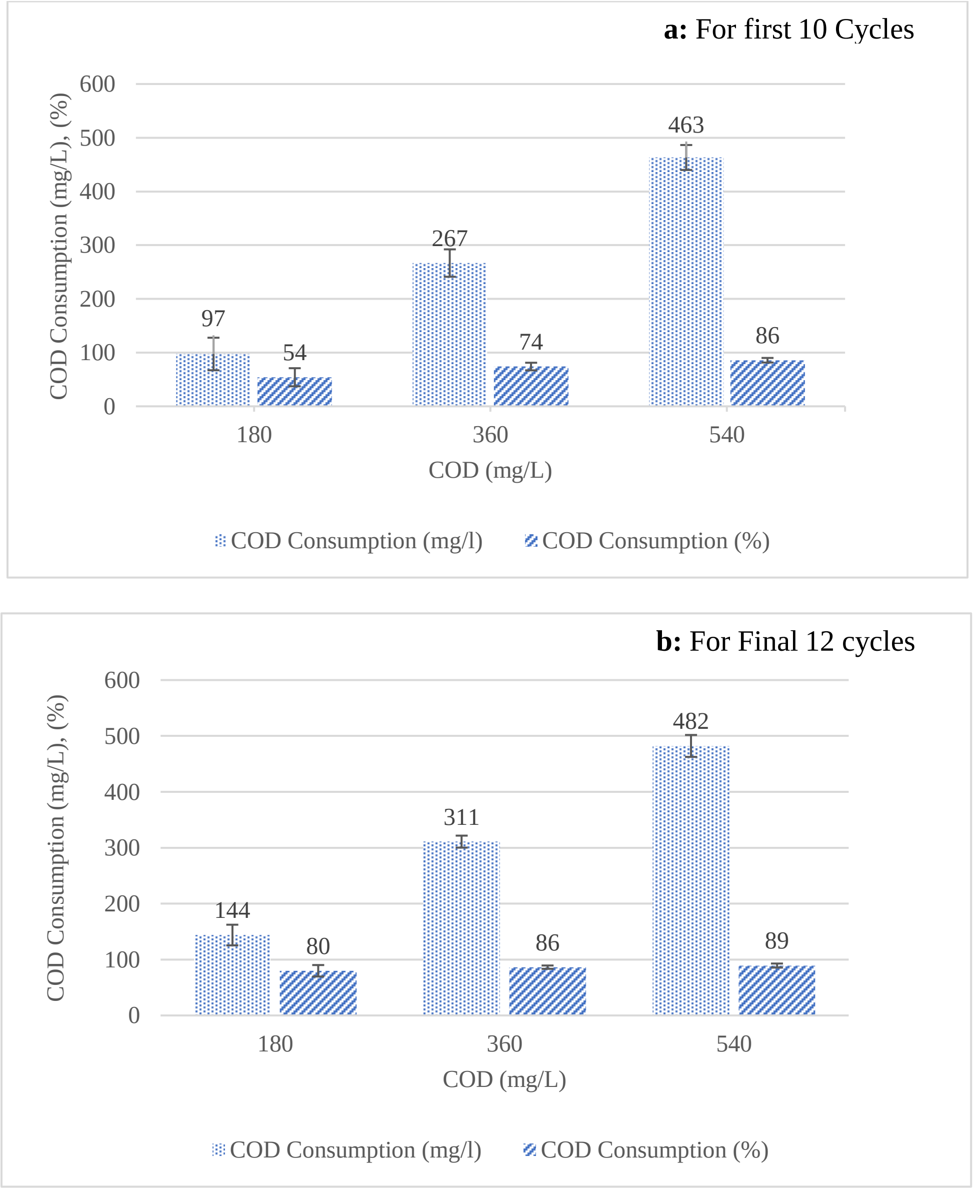


Figure 3. 5: Average COD Consumption as mg/L and percentage at COD 0 mg/L, 180 mg/L, 360 mg/L and 540 mg/L for a) first 10 cycles b) final 12 cycles

From the average values of 22 cycles data (figure 3.4) it was found that methane consumption was 60.1±10.1 percent for no COD condition. The value varied a little by increasing marginally by 5% at COD 540 mg/L. For other COD conditions the change in methane consumption was not significant. The oxygen consumption for no COD condition was 82.4±8.5 percent which was increased by maximum 12 percent at COD level 540 mg/L. Increasing COD had increased the oxygen consumption percentage. However, the oxygen consumption at COD 180 mg/L is 77.9±4 percent which is 2.5 percent less than no COD condition. This is might be the reason of low ammonia concentration for this condition. From the above data it is evident that the methanotrophic activity remains constant with COD incorporation but increasing oxygen consumption suggests the activity of heterotrophic bacteria with increasing COD concentration.

COD consumption data are presented in two stages for first 10 cycles and then final 12 cycles due to data fluctuations at the beginning (Figure 3.5). For the first 10 cycles COD consumption increased with the increase of COD concentration. The maximum of 86±4 percent COD consumption was found at 540 mg/L COD. At COD 180 mg/L, 97±30 mg/L (54%) of COD consumed while the consumption was 267±26mg/l (74%) out of 360mg/L. The consumption further increased to 463 mg/L (86%) with higher COD of 540 mg/L. Similar trend but increased consumption was observed for the final 12 cycles with less standard deviation. Highest COD consumption was 482±20 mg/L (89%) at COD 540 mg/L and lowest was observed 144±18mg/L (80%) at 180 mg/L of COD. In this period the difference in COD consumption percentage from COD 180 mg/L to 540 mg/L is 8%which signifies the sustainability for longer period. The increase in COD consumption with inlet COD increase signifies that the activity or growth of heterotrophs is happening. As previously mentioned with the increase of COD, methane uptake rate was stable which meant methanotrophic bacterial activity was not changing but at the same time the COD uptake rate was increasing with COD increase which means there is certainly some microorganisms other than methanotrophs present.

3.7.3 Nitrogen Removal

To apply methanotrophs in nitrogen removal process in WWTP, it is necessary to confirm their nitrogen removing capability. Some studies reported that methanotrophs can partially denitrify under hypoxic condition (Stein and Klotz, 2011). For nitrogen removal, nitrification and denitrification are the major steps in conventional WWTPs. So, it is important to find out the nitrification and denitrification activities to confirm the possibility nitrogen removal by methanotrophs.

Table 3.3: Average Ammonia consumption, nitrate, nitrite concentration at different COD level

COD (mg/L)	NH_4-N In (mg/L)	NH_4-N Out (mg/L)	% NH_4-N Consumed	NO_3^- (mg/L)	NO_2^- (mg/L)
0	61.6±10	14.6±19	79.6±24	0.59±0.4	0
180	34.3±6.7	0.11±0.15	99.7±0.5	0.16±0.19	0
360	60.8±10.7	0.16±0.29	99.72±0.48	0.27±0.23	0
540	91.5±5.6	2.3±3.4	97.45±3.5	0.12±0.1	0

To monitor the nitrogen removal, ammonia nitrogen, nitrate and nitrite were measured at the end of each cycle using Ion Chromatograph (IC). All calculation is attached in appendix II. The set without COD showed fluctuations at the beginning but from cycle 38 it showed consistent results. The average percentage of ammonia consumption was 79.6±24 percent (Table 2). Microbial community with COD showed consistent removal of ammonia from the beginning. For COD 180 and 360 mg/L the ammonia consumption was 99.7±0.5 percent and 99.72±0.48 percent and for COD 90 mg/L the value was 97.45±3.5 percent. So, from these data it can be said that the addition of COD had not much effect on ammonia consumption. There is little bit less ammonia consumption at COD 90 mg/L which might be the toxic effect of hydroxylamine from ammonia. In addition, there was an insignificant amount of nitrate and no nitrite found at the end of each cycle. This confirms that the nitrogen might be used for cell synthesis or might be converted to gas by denitrification. To confirm this, it is required to take samples at short interval to see any nitrate or nitrite is present or not, also to measure the nitrogen and nitric oxide gas to see if there is any denitrification. In addition, from the nitrogen balance it can be said that how much nitrogen is used for cell synthesis and how much is converted to gas.

3.7.4 Nitrogen Balance

The purpose of nitrogen balance is to determine how much nitrogen is going inside the system and how much nitrogen is left after the experiment. In addition, to determine the percentage of nitrogen inside the biomass and finally to determine how much nitrogen is converted to gas.

3.7.4.1 Methods

For nitrogen balance samples including biomass and liquid feed were collected at the end of each cycle and stored in a fridge. Then total nitrogen test was performed for selected samples using HACH vials (Method 10071). From the test total nitrogen (out) for each sample was measured and then total nitrogen (in) was calculated by back calculation. For this inorganic nitrogen (NH_4, NO_3, NO_2) in the liquid sample was also measured by ion chromatograph (IC). Then organic nitrogen was calculated by subtracting inorganic nitrogen from total nitrogen. Then percentage of nitrogen in biomass was calculated by dividing the organic nitrogen by dry cell weight (DCW). After then, total nitrogen (in) using nitrogen percentage in cell, DCW (in) and inorganic nitrogen (in). Finally, the amount of nitrogen converted to gas was calculated from in and out total nitrogen data.

Inorganic Nitrogen (IN_{out}) = NH_4 (mg/L) + NO_3 (mg/L) + NO_2 (mg/L)

Organic Nitrogen (ON_{out})= TN_{out} (mg/L) - IN_{out} (mg/L)

% of N in Biomass= ON_{out} (mg/L)÷ DCW_{Out}(mg/L)

TN_{In}= NH_4-N_{In} + (DCW_{In} x % of N in biomass)

Nitrogen converted to gas (mg/L) = TN_{In} - TN_{out}

3.7.4.2 Result and discussion

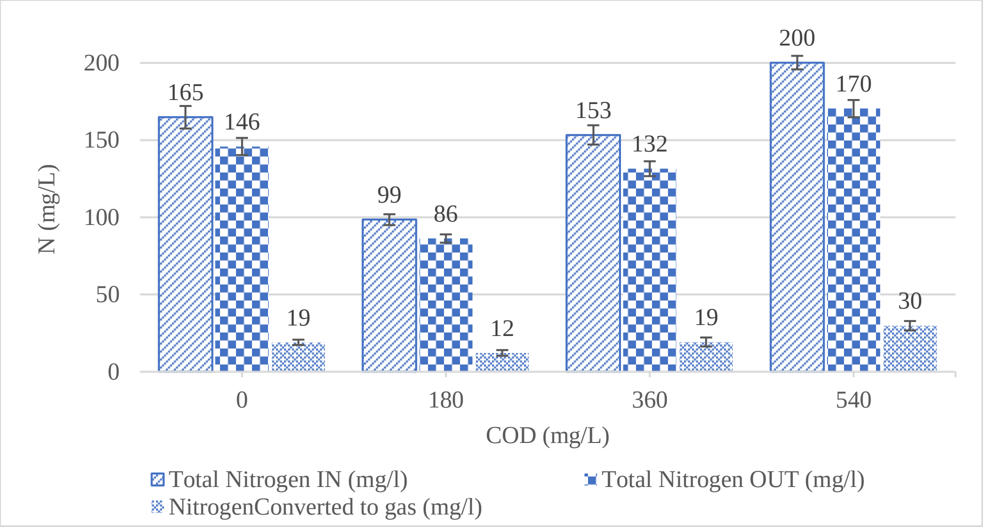


Figure 3. 6: Nitrogen balance data at different COD concentration showing In an Out of total nitrogen and amount of nitrogen converted to gas

Nitrogen converted to gas

From nitrogen balance data (Figure 3) for each condition the total nitrogen (out) was less than the total nitrogen (in). Which confirmed that some nitrogen is converted to gas. At COD 0 and 360 mg/L the NH_4-N was 60 mg/L, which shows almost similar nitrogen conversion to gas of 19 mg/L but the later one showed 1% higher nitrogen gas conversion. From COD 180 to 540 mg/L NH_4-N was increased form 30 to 90 mg/L where in and out data for total nitrogen were increased proportionately. In addition, the amount of nitrogen converted to gas was increased from 12 to 30 mg/L. The percentages of total nitrogen gas conversion were increased with increased COD concentration. The values were 11.5, 12.1, 12.4 and 15 percent for COD 0, 180, 360 and 540 mg/L respectively. So, COD along with NH_4-N concentration increase caused higher nitrogen gas conversion. During nitrogen balance, the percentage of nitrogen in biomass was calculated. It was observed that the percentage was varied from 8 to 11 percent (Figure 4). The lowest nitrogen in biomass was observed at NH_4-N 30 mg/L and the highest nitrogen content was observed at NH_4-N 90 mg/L. With same NH_4-N 60 mg/L at COD 0 and 360 mg/L shows the nitrogen percentage is 1 percent less with incorporation of COD. Theoretically, bacteria cell contains 12 percent of nitrogen considering the chemical formula $C_5H_7O_2N$. From the data it can be said that the nitrogen percentage at higher ammonia concentration is close to theoretical value.

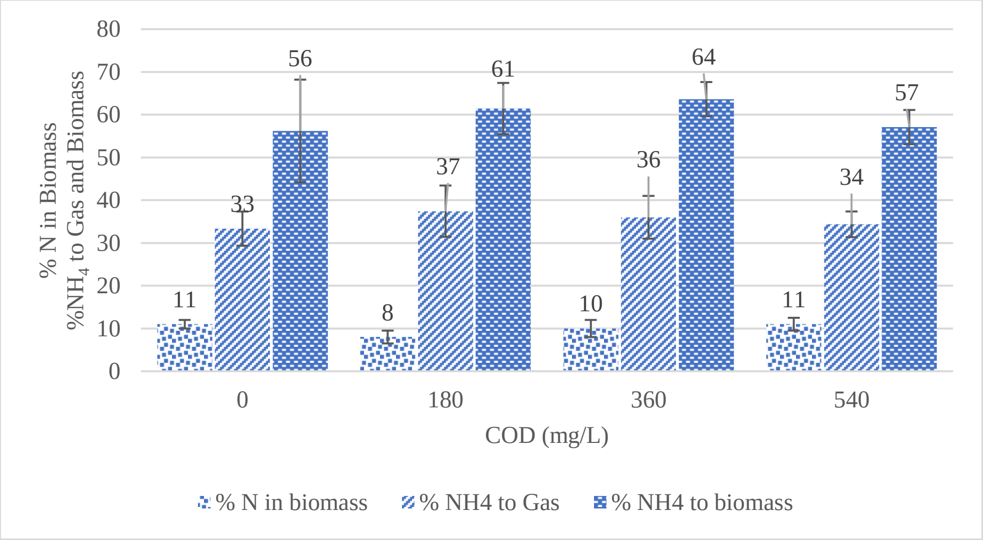


Figure 3. 7: Ammonia utilization pathway showing the percentage of ammonia (in) converted to gas, utilized for cell synthesis and percentage of nitrogen inside the biomass

Ammonia utilization pathway

From amount of total nitrogen converted to gas and organic nitrogen in biomass data how much ammonia nitrogen from feed was converted to nitrogen gas and utilized in the cell was calculated.

Table 3.4: Ammonia utilization pathways at different COD condition

COD (mg/L)	NH_4-N in (mg/L)	% NH_4-N to gas	% NH_4-N to Biomass	% NO_3-, NO_2-	% Unused NH_4
0	60	33.4	56.2	1.16	9.3
180	30	37.4	61.4	0.5	0.6
360	60	36.0	63.6	0.3	0.1
540	90	34.4	57.1	0.44	8.1

With the increase of ammonia concentration from 30 to 60 mg/L % NH_4-N to biomass conversion increased from 61.4 to 63.6 percent but NH_4-N at 90 mg/L the conversion percentage reduced by 3 percent. In addition, about 8 percent NH_4-N remained unused at this concentration. This can be explained as, at higher level of ammonia the toxic effect may inhibited the biomass growth. The maximum ammonia to gas conversion was observed at NH_4-N 30 mg/L which signify that low level of ammonia is favourable for gas conversion. There was 9.3 percent ammonia remained unused at no COD and NH_4-N 60 mg/L while with COD 360 and same ammonia the almost all ammonia was utilized by the bacteria and the nitrogen conversion was increased by 3 percent from 33.4 percent. This implies that COD addition has positive impact on the microbial community in nitrogen utilization and removal.

3.8 Conclusion

Addition of COD positively impacted the growth of methanotrophic mixed culture in terms of increased growth activities of growth rate and growth yield. Moreover, methane uptake rate by the consortium remained unchanged with addition of COD which signifies the stable and sustainable behavior of methanotrophs under COD condition. In addition, COD consumption by the consortium indicate the presence of other microorganism which also supports the fact of surviving of methanotrophs with other cultures. From nitrogen balance data, it was found that some part of consumed nitrogen is being converted towards gaseous form of nitrogen in addition to cell synthesis. This data supports the denitrification by methanotrophic mixed consortium in wastewater treatment process. Finally, methanotrophs can be grown in mainstream condition with production of value-added product and nitrogen removal from wastewater.

CHAPTER 4: Methanol and PHB production by Methanotrophic-heterotrophic mixed culture performance under different COD concentrations

4.1 Introduction

‘Methanotrophs’ have the ability to convert methane in to biofuel like methanol and biopolymer like PHB. Methanol has an higher energy density than methane and can be used as alternate fuel for cars and does not have safety issues with storage and transportation (Hwang et al., 2014). Similarly, PHB is a eco friendly biopolymer which is degradable and can be recycled and restocked to the environment.

Methanotrophs oxidize methane aerobically into methanol which is further oxidized terminally into carbon dioxide gas through a series of intermediated like formaldehyde and formate (Ge et al., 2014). Usually, methanol production is done by inhibiting the MDH enzyme which is responsible for methanol oxidation. For inhibition phosphate and sodium chloride are used and formate is used as external electron source for survival and metabolic activity of methanotrophs (Ge et al., 2014). On the other hand, certain type of methanotrophs can produce PHB as intracellular granules under nutrient limiting condition. In nutrient limiting condition such as without nitrogen, methanotrophs adopts survival strategy and switch themselves to PHB cycle to provide energy for cellular maintenance (Karthikeyan et al., 2015).

The integration of methanol and PHB production into WWTPs have many challenges like: grow methanotrophs inside the system, utilize methanotrophs for treatment processes and produce valuable product using the feed from mainstream WW. In chapter 3, it is shown that methanotrophs can grow in mainstream WW condition, but is it possible to use the same condition for application side? To answer this question two applications of methanotrophs were done by main stream WW condition. To do that like as growth phase in previous chapter, ammonia and COD with varying concentration were applied to methanol and PHB production consecutively.

4.2 Materials and Methods

4.2.1 Methanol production

After 50 cycles of methanotrophs growth phase with COD the same biomass was collected and enriched with same liquid and gas feed for methanol batch experiment. The enrichment continued until stable activity in terms of growth rate, methane and oxygen consumption was achieved. Methanol batch experiment was carried by 6 hours growth phase followed by 4 hours methanol phase with different liquid medium.

4.2.2 Growth phase

The enriched active biomass was used for growth phase with initial OD ~3.0 in 50 ml liquid medium where MSM, trace metal, copper, ammonium and COD were added. The ingredients for MSM were: $MgSO_4 \cdot 7H_2O$ (1000 mg/L), $CaCl_2 \cdot 2H_2O$ (200 mg/L), KH_2PO_4 (272 mg/L), K_2HPO_4 (610 mg/L), Fe-EDTA (4 mg/L) in addition to this 1 mL/L trace metal solution and 1.6 mg/L copper sulfate ($CuSO_4 \cdot 5H_2O$) were added. For the batch four different sets were used with different nitrogen and and COD concentration. For nitrogen source NH_4Cl was used and for COD sodium acetate (CH_3COONa) was used. For four sets to make 0, 180, 360, 540 mg/L COD solution, 0, 225, 400 and 600 mg/L CH_3COONa were added respectively. To make 30, 60 and 90 mg/L NH_4-N solution, 114.5, 229 and 343.5 mg/L NH_4Cl added respectively.

For each condition of experiment, triplicated 250 ml serum bottles were used for consistency in results. After suspension of the biomass in liquid medium, each bottle was evacuated for 5 minutes using a suction pump. Afterwards, the bottles were fed with 200 ml methane and 200 ml oxygen gas and all the bottles were incubated in a orbital shaker for 6 hr at a speed of 165 rpm and temperature of 25 to 30℃. At the end of growth phase all biomass in each bottle was collected after centrifuging them.

4.2.3 Methanol Phase

Biomass from the growth phase was used for methanol production. The liquid feed was changed for MSM with MDH inhibitor and electron donor. In 50 ml solution the following chemicals were used as inhibitors: MgCl2.6H2O (2033 mg/L), NaH2PO4 (2879.5 mg/L), Na2HPO4 (9311.5 mg/L), electron donor: Sodium Formate (8160 mg/L). In addition, 1ml/L trace metal from stock solution, 1.6 mg/L $CuSO_4 \cdot 5H_2O$ and ammonia and COD concentration were the same as growth phase. Same methane and oxygen gas were used as growth phase and the samples were incubated in the orbital shaker for 4 hours. Then all the samples were centrifuged to collect supernatant for methanol measurement. The supernatant was injected in a GC equipped with a MXT-WAX column where a flame ionization detector (FID) was used. The injector oven temperature was 30℃ and FID temperature was 300℃ and helium was used as the carrier gas.

4.2.4 PHB production

After methanol batch experiment all biomass were collected and enriched again with MSM, trace, copper, ammonia and COD. The enrichment continued in four different sets till the biomass activity became stable in terms of growth rate, methane and oxygen consumption.

Experimental condition for PHB

Enriched biomass was used for PHB batch experiment with initial OD 1.5~2.0 in four different sets. For liquid feed MSM, trace metal and copper were used same as before, but no nitrogen source was used to create N-limiting condition. In addition, to incorporate COD, 0, 225, 400 and

600 mg/L CH_3COONa were added in for different sets. At the beginning of the experiment 200 ml of oxygen and 200 ml of methane were injected to each serum bottle which were replaced with same gases after 24 hours. All the bottles fed with biomass were incubated in the orbital shaker at 165 rpm and 30℃ for 48 hours. After the experiment, the PHB enriched biomass were collected for measurement.

Analytical Measurements

The PHB was measured by the method developed by Braun egg, Sonnleitner and Lafferty (Braunegg et al., 1978). At the beginning of this process, 10−15 mg of biomass sample is collected in a test tube where 2 mL of acidified methanol (3% sulfuric acid) and 2 mL of chloroform are added to a glass vial. The mixture is then subjected to heat at 100 °C for 3.5 h and then left to cool down to room temperature. After then, 1 mL of deionized water is added to the mixture and vortexed for 1 min, and then kept for a while for phase separation. The lower organic phase is tested for PHB measurement using a gas

chromatograph equipped with a flame ionization detector (FID) and an MXT-wax column. The temperature condition is: 1 min 80 °C, 10 °C/min, 180 °C for 4 min. The results are calibrated with standard curves obtained using PHB standards (Sigma-Aldrich). Benzoic acid is used as an

internal standard to increase the accuracy.

4.3 Results and Discussion

4.3.1 Influence of COD on methane and oxygen consumption during methanol production

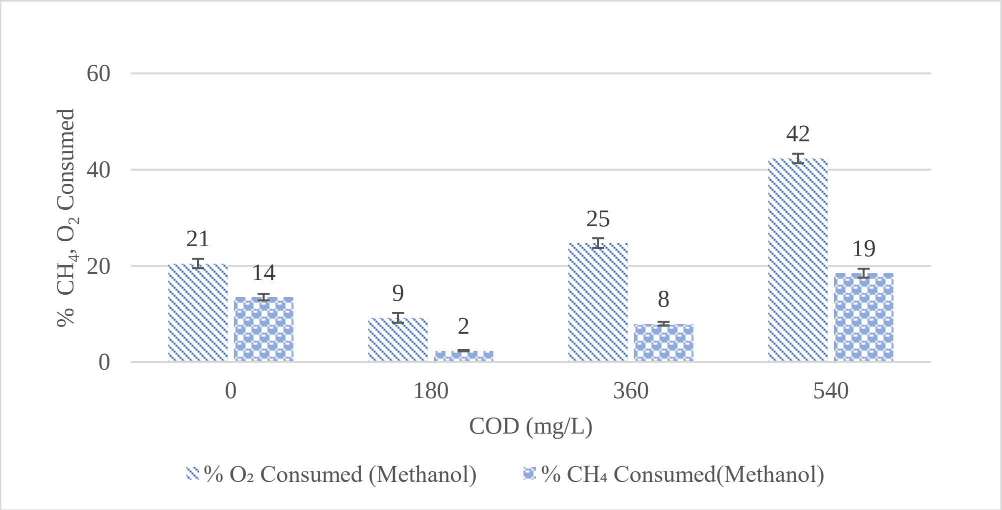


Figure 4.1: Percentage of CH_4 and O_2 Consumption during methanol production phase

During methanol production batch experiment low methane and oxygen consumption were observed (Figure 4.1) which is a usual case as MDH inhibitors inhibit both MDH and MMO activity as well (Ge et al., 2014). At no COD condition 21 % oxygen and 14% methane was consumed whereas with same ammonia and with 360 mg/L COD resulted increased oxygen consumption of 25% but decreased methane consumption of 8%. Here the addition of COD somehow inhibiting the methane consumption which means methanotrophic activity during methanol production might be hampered by COD addition. Moreover, With the increase of COD from 180 to 540 mg/L the oxygen consumption increased from 9% to 42 % and methane consumption increased from 2% to 19%. So, both oxygen and methane consumption increased with the increase of COD concentration. From this data it can be interpreted that increasing COD has a positive effect on methane and oxygen consumption, but more investigation is required to determine highest COD at which maximum methanotrophic activity will occur.

4.3.2 Influence of COD on methane and oxygen consumption during PHB production

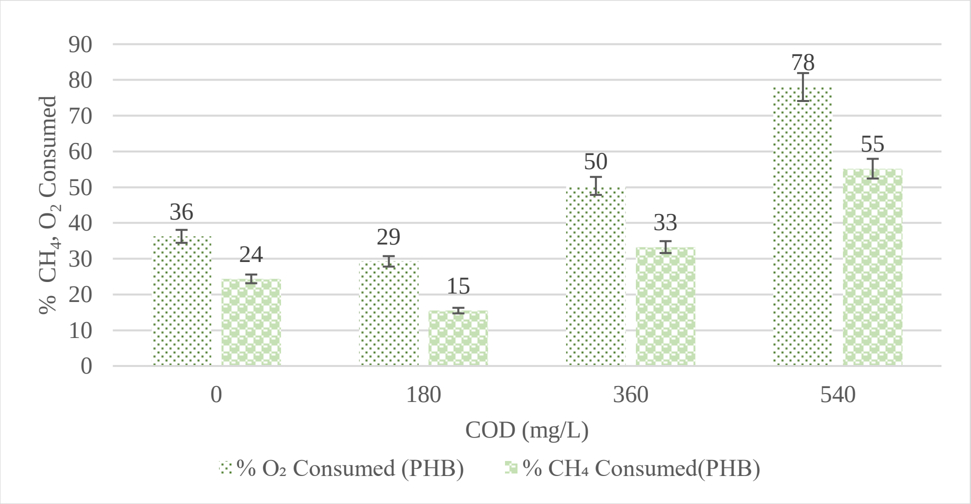


Figure 4. 2: Methane and Oxygen consumption Percentage during PHB production

During PHB production with N-limiting condition methane and oxygen consumption were higher than methanol production phase. Moreover, higher methane and oxygen consumption were observed with the addition of COD compared to no COD where ammonia nitrogen were 60 mg/L for both cases. Oxygen consumption increased from 29% to 78% and methane consumption increased from15% to 55% at COD increase from 180 mg/L to 540mg/L, respectively. So, in N-limiting condition COD has a positive effect on methanotrophic activity.

4.3.3 Influence of COD on methanol and PHB production

Under desirable condition, methanotrophs are able to produce methanol and PHB. However, it would be interesting to see the ability of methanotrophs to produce methanol and PHB under COD environment. In the batch experiment, methanol and PHB production were examined under varying COD.

Table 4. 1: Methanol and PHB production at different COD condition

COD (mg/L)	Methanol (mg/L)	PHB %
0	91±11	24±5
180	0	0
360	19±4	4±1
540	63±6	0

Without COD and with ammonia, methanol production is 91 mg/L (Table 4.1) which is 70 percent less than the reported methanol production with nitrate as nitrogen source (AlSayed et al., 2018d). No methanol was detected at COD 180 mg/L and NH4-N 30 mg/L while with the increase of COD to 360 mg/L and NH4-N 60 mg/L methanol production increases to 19±4 mg/L. With COD maximum methanol production was found 63±6 mg/L at COD 540 mg/L and NH4-N 60 mg/L.For PHB production at no COD condition PHB production is 24±5 percent which is 25 percent lower than the reported value for methanotrophic mixed culture(Fergala et al., 2018a). The lower production of PHB might be due to dominance of type I methanotrophs. PHB yield can be increased by providing proper condition to select type II methanotrophs. Unfortunately, with the addition of COD PHB production negatively impacted. Only 4 % of PHB production detected at COD 360 mg/L and NH_4-N 60 mg/L. To maximize the PHB production, better understanding of the behavior of PHB producing bacteria under COD condition is required.

CHAPTER 5: Conclusion and Future Work

5.1 Conclusion

There is no doubt for application for methanotrophs in methane mitigation and valuable resource recovery. It would be a revolution if this biotechnical application can be integrated in to WWTP. From the study, it is evident that methanotrophs can grow in mainstream condition, but it is necessary to decide where to put them in existing treatment processes like: to grow methanotrophs in the aerobic reactor with biogas generated from AD. The summary of the thesis findings are as follows:

- From 50 cycles activities of the biomass in terms of specific growth rate, growth yield and methane uptake rate in different COD condition, it is evident that methanotrophs can survive in COD environment with other microorganism like heterotrophic bacteria.
- From stoichiometric data, it is visible that the biomass activity became stable after 36 cycle of activity. Highest activity of the microbial community in terms of cellular growth and methane and COD removal was found at COD 360 mg/l. These findings signify that in COD condition, it takes about 36 days to the methanotrophic microbial consortium to become stable. Moreover, the best microbial performance at COD 360 mg/L confirms the applicability of methanotrophs in domestic WW effluent which contains about 300-500 mg/L of COD.
- Average maximum COD consumption was 89±5 percent at COD 540mg/L and the average for ammonia consumption was around 99.72±0.48 percent at COD 360 mg/L. As 60 percent of domestic sewage contains ammonia, methanotrophs are able to remove ammonia from domestic WW.

- Addition of COD is favourable for heterotrophic bacteria growth without affecting methanotrophic bacterial activity. From this finding, it can be said that methanotrophs are able to survive in diverse microbial community.

- COD consumption was increased proportionately with the increase of COD concentration till 540 mg/L means the activity of heterotrophic bacteria is positively affected in presence of methanotrophs and the COD removal capacity also increases with the influent COD increase.

- Addition of ammonia up to 60 mg/L did not affected the ammonia consumption while ammonia concentration at 90 mg/L the consumption was decreased by 2.3 percent. Due to the toxicity of ammonia, methanotrophic activity might be hampered at ammonia level of 90 mg/L. Usually, domestic WW contains 20 to 60 mg/L of total nitrogen which still remains suitable to methanotrophic bacterial growth

- From nitrogen balance, it was observed that 19 mg/L (12.5%) of nitrogen from 153 mg/L of total nitrogen was converted to gaseous nitrogen at NH_4-N and COD concentration 60 mg/L and 360 mg/L respectively. With the increase of ammonia up to 360 mg/L, the nitrogen gas conversion increases.

- Percentage of nitrogen inside biomass was found 11% at 90 mg/L of NH_4-N which is close to the theoretical value of 12% nitrogen content in biomass.

- 100% ammonia was utilized at 60 mg/L NH_4-N level from which 64% ammonia was converted to gaseous nitrogen and 36% was consumed into biomass.

- Maximum methanol production was found 63 mg/L at COD 540 mg/L which is 30% less than without COD condition and 70% less than reported value for type I methanotrophs.

Without Cod less methanol was observed than reported value, the reason might be the presence of both type I and II, as type I methanotrophs can yield more methanol. In COD much less methanol yield was observed which signify the negative impact of COD on methanol production.

- Only 4 % of PHB production detected at COD 360 mg/L, no PHB was detected in COD 180 and 540 mg/L. PHB production was significantly impacted by the addition of COD. To increase PHB yield, certain conditions require to increase the percentage of type II methanotrophs in the consortium.

5.2 Future Study

- In future, the culture can be grown in a continuous system with reduced SRT to produce more solids to reproduce biogas.
- For methanol production further study can be done with more ammonia and COD loading rate to maximize methanol yield.
- To maximize PHB production, it is required to study the behavior of PHB producing methanotrophs (type II) in mainstream condition. Moreover, to increase the number of PHB producing type II bacteria, various techniques such as optimizing ammonia concentration or by feast and famine approach can be applied during growth phase.
- After growing methanotrophs in mainstream condition, the biomass can be applied to methanol and PHB production with WW effluent or AD centrate as feed to minimize the COD effect.

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APPENDIX A

Data analysis of Growth Rate, Growth Yield, Methane Uptake Rate, Methane and Oxygen Consumption:

Cycle #	Day	sample	OD (in)	OD (out)	TSS (mg/L) inc	time(hr)	Growth rate (day-1)	Growth rate (day-1)	Gas volume (ml)	O2 Initial (mg/l)	N2 initial (mg/l)	CH4 initial (mg/l)	CO2 initial (mg/l)	Gas volume (ml)	O2 final (mg/l)	N2 final (mg/l)	CH4 final (mg/l)	CO2 final (mg/l)	O2 consumed (mg)	% O2 Consumed	CH4 consumed (mg)	% CH4 Consumed	CO2 Produc d (mg)	O2 (mg/hr	CH4 (mg/hr	obs Yield
1	23-Oct	1-1	2.185	2.11	-35.714	24	-0.70	-0.035	400	528.2	177.0	0.0	71.2	373.7	522.7	189.4	0.0	62.75	15.95	7.5	0.00	#DIV/0!	-5.03	0.66	0.00	#DIV/0
		1-2	2.11	2.08	-14.286	24	-0.29	-0.01	400	549.6	162.3	0.0	50.3	391.9	528.9	165.6	0.0	61.86	12.58	5.7	0.00	#DIV/0!	4.13	0.52	0.00	#DIV/0
		1-3	2.08	2.08	0.000	24	0.00	0.00	400	532.2	235.1	0.0	48.1	1028.5	145.5	91.4	0.0	81.44	63.26	29.7	0.00	#DIV/0!	64.53	2.64	0.00	#DIV/0
		2-1	1.99	2.77	371.429	24	6.55	0.33	400	552.2	265.6	240.3	44.9	258	300.7	411.7	185.3	261.2	143.31	64.9	48.28	50.2	49.4	5.97	2.01	7.69
		2-2	2.07	2.6	252.381	24	4.54	0.23	400	529.1	253.7	239.3	55.2	160	170.3	635.0	96.1	294.6	184.41	87.1	80.36	84.0	25.0	7.68	3.35	3.14
		2-3	2.17	2.76	280.952	24	4.79	0.24	400	310.8	139.4	142.1	77.9	118	88.9	473.2	159.7	435.2	113.85	91.6	38.02	66.9	20.1	4.74	1.58	7.39
		3-1	2.11	3.01	428.571	24	7.03	0.35	400	538.4	266.6	253.4	48.1	207	71.2	514.9	176.3	485.5	200.64	93.2	64.84	64.0	81.3	8.36	2.70	6.61
		3-2	2.13	3.08	452.381	24	7.29	0.36	400	556.0	264.3	246.8	53.0	1701	0.0	62.2	12.8	445.0	222.38	100.0	76.91	77.9	735.7	9.27	3.20	5.88
		3-3	2.11	2.94	395.238	24	6.57	0.33	400	543.9	212.9	284.6	57.4	190	90.1	447.4	206.3	398.7	200.42	92.1	74.57	65.5	52.9	8.35	3.11	5.30
		4-1	2.19	3.21	485.714	24	7.56	0.38	400	532.1	251.9	267.4	51.2	206	96.1	490.1	177.0	381.8	193.07	90.7	70.59	66.0	58.0	8.04	2.94	6.88
		4-2	2.02	3.06	495.238	24	8.19	0.41	400	533.5	259.3	262.0	53.4	215	103.7	483.3	173.5	355.1	191.13	89.6	67.58	64.5	54.8	7.96	2.82	7.33
		4-3	2.01	3.03	485.714	24	8.10	0.40	400	547.6	261.7	246.4	46.3	215	107.3	486.1	119.3	382.7	195.96	89.5	72.88	73.9	63.9	8.16	3.04	6.66
		5-1	2.06	2.99	442.857	24	7.37	0.37	400	536.1	229.0	278.6	0.0	198	76.1	463.7	211.8	435.7	199.42	93.0	69.61	62.5	86.1	8.31	2.90	6.36
		5-2	2.03	3.08	500.000	24	8.22	0.41	400	557.0	243.7	261.8	61.0	214	75.9	455.4	188.0	445.0	206.55	92.7	64.48	61.6	70.9	8.61	2.69	7.75
		5-3	2.04	2.97	442.857	24	7.43	0.37	400	486.8	402.2	202.8	0.0	251	90.3	641.9	123.4	403.2	172.07	88.4	50.20	61.9	101.1	7.17	2.09	8.82
2	24-Oct	1-1	2.05	2.14	42.857	19	1.09	0.05	400	595.6	208.9	0.0	61.9	396.5	541.4	210.7	0.0	89.00	23.54	9.9	0.00	#DIV/0!	10.55	1.24	0.00	#DIV/0
		1-2	1.92	2	38.095	19	1.03	0.05	400	576.8	245.4	0.0	0.0	405.8	532.8	241.9	0.0	171.77	14.55	6.3	0.00	#DIV/0!	69.70	0.77	0.00	#DIV/0
		1-3	1.84	2.05	100.000	19	2.73	0.14	400	558.3	290.4	0.0	0.0	397.1	519.1	292.5	0.0	135.73	17.14	7.7	0.00	#DIV/0!	53.90	0.90	0.00	#DIV/0
		2-1	2	2.67	319.048	19	7.25	0.36	400	567.1	209.6	266.4	56.1	277	321.6	302.3	240.4	314.2	137.64	60.7	39.89	37.4	64.7	7.24	2.10	8.00
		2-2	1.83	2.73	428.571	19	9.97	0.50	400	576.5	223.3	260.7	0.0	508	120.4	175.7	74.8	317.7	169.41	73.5	66.26	63.5	161.5	8.92	3.49	6.47
		2-3	2	2.84	400.000	19	8.77	0.44	400	547.8	308.6	228.6	0.0	259	262.8	475.9	170.0	312.4	150.95	68.9	47.35	51.8	81.0	7.94	2.49	8.45
		3-1	2.04	3.03	471.429	19	9.87	0.49	400	568.9	222.3	262.3	52.5	206	162.5	431.1	192.2	406.3	194.03	85.3	65.27	62.2	62.8	10.21	3.44	7.22
		3-2	2.27	3.27	476.190	19	9.12	0.46	400	592.4	194.3	277.5	0.0	190	178.1	409.8	186.6	377.4	203.19	85.7	75.61	68.1	71.6	10.69	3.98	6.30
		3-3	2.12	3.22	523.810	19	10.41	0.52	400	580.2	197.3	277.3	49.8	177	186.6	446.2	195.7	420.1	199.09	85.8	76.31	68.8	54.4	10.48	4.02	6.86
		4-1	2.11	2.88	366.667	19	7.80	0.39	400	575.8	202.6	277.3	0.0	219	228.2	369.5	221.6	372.9	180.27	78.3	62.31	56.2	81.8	9.49	3.28	5.88
		4-2	2.03	2.89	409.524	19	8.83	0.44	400	572.6	236.0	265.9	48.5	174	155.9	543.5	126.8	318.2	201.95	88.2	84.33	79.3	35.9	10.63	4.44	4.86
		4-3	2.1	2.85	357.143	19	7.66	0.38	400	540.7	308.7	239.4	0.0	238	156.6	518.8	131.0	321.3	179.01	82.8	64.58	67.4	76.5	9.42	3.40	5.53
		5-1	2.19	3.23	495.238	19	9.70	0.48	400	574.5	216.9	275.7	0.0	269	65.5	322.1	120.9	431.2	212.18	92.3	77.71	70.5	116.1	11.17	4.09	6.37
		5-2	2.14	3.44	619.048	19	11.77	0.59	400	561.3	253.0	262.3	0.0	213	112.7	474.2	173.7	418.7	200.45	89.3	67.86	64.7	89.4	10.55	3.57	9.12
		5-3	1.96	3.36	666.667	19	13.30	0.66	400	540.6	296.7	247.6	0.0	250	109.9	474.2	168.2	424.1	188.72	87.3	56.95	57.5	106.1	9.93	3.00	11.71
3	25-Oct	1-1	1.75	2	119.048	23	2.78	0.14	400	544.1	330.1	0.0	54.1	418.3	505.0	315.7	0.0	82.77	6.42	3.0	0.00	#DIV/0!	12.99	0.28	0.00	#DIV/0
		1-2	1.75	1.99	114.286	23	2.68	0.13	400	534.7	347.1	0.0	46.3	405.9	496.5	342.0	0.0	82.77	12.37	5.8	0.00	#DIV/0!	15.08	0.54	0.00	#DIV/0
		1-3	1.82	2.12	142.857	23	3.18	0.16	400	502.7	387.9	0.0	0.0	402.5	469.4	385.6	0.0	76.54	12.15	6.0	0.00	#DIV/0!	30.80	0.53	0.00	#DIV/0
		2-1	2.26	3.03	366.667	23	6.08	0.30	400	504.3	302.7	247.2	0.0	157	94.5	771.0	66.4	309.3	186.87	92.6	88.45	89.5	48.6	8.12	3.85	4.15
		2-2	2.36	3.2	400.000	23	6.31	0.32	400	509.4	258.2	278.4	46.7	218	95.9	473.8	217.6	363.1	182.85	89.7	63.90	57.4	60.5	7.95	2.78	6.26
		2-3	2.56	3.17	290.476	23	4.44	0.22	400	460.6	308.7	261.7	65.9	191	81.8	648.1	145.5	310.2	168.64	91.5	76.96	73.5	32.8	7.33	3.35	3.77
		3-1	2.39	3.34	452.381	23	6.92	0.35	400	474.4	377.7	229.5	0.0	242	82.0	625.2	154.8	341.8	169.94	89.6	54.41	59.3	82.6	7.39	2.37	8.31
		3-2	2.34	3.25	433.333	23	6.79	0.34	400	459.5	366.2	236.6	0.0	277	57.2	528.6	152.1	318.6	167.95	91.4	52.47	55.5	88.3	7.30	2.28	8.26
		3-3	2.47	3.35	419.048	23	6.31	0.32	400	478.6	319.1	259.1	0.0	236	79.5	540.3	204.2	368.5	172.67	90.2	55.40	53.5	87.0	7.51	2.41	7.56
		4-1	2.21	3	376.190	22.5	6.47	0.32	400	475.2	323.3	259.9	45.0	253	116.8	510.7	215.7	317.3	160.52	84.4	49.37	47.5	62.3	7.13	2.19	7.62
		4-2	2.32	3.13	385.714	22.5	6.34	0.32	400	473.5	337.5	254.9	0.0	253	111.7	533.5	206.3	313.7	161.13	85.1	49.74	48.8	79.4	7.16	2.21	7.75
		4-3	2.21	3.1	423.810	22.5	7.15	0.36	400	527.6	328.7	236.4	52.8	244	158.2	540.0	170.7	348.9	172.52	81.7	52.97	56.0	63.8	7.67	2.35	8.00
		5-1	2.36	3.26	428.571	22.5	6.83	0.34	400	507.8	330.8	236.7	0.0	207	71.3	640.2	120.9	353.8	188.38	92.7	69.70	73.6	73.1	8.37	3.10	6.15
		5-2	2.59	3.39	380.952	22.5	5.71	0.29	400	489.2	219.7	247.4	0.0	210	63.0	418.9	173.7	348.0	182.47	93.2	62.53	63.2	73.0	8.11	2.78	6.09
		5-3	2.62	3.44	390.476	22.5	5.77	0.29	400	562.0	187.0	295.2	46.3	178	70.8	420.0	190.0	347.1	212.18	94.4	84.22	71.3	43.3	9.43	3.74	4.64
4	26-Oct	1-1	1.81	2.02	100.000	20	2.63	0.13	400	559.0	400.3	0.0	49.0	411.5	500.2	389.1	0.0	84.55	17.75	7.9	0.00	#DIV/0!	15.21	0.89	0.00	#DIV/0
		1-2	1.91	1.27	-304.762	20	-9.66	-0.48	400	374.5	332.2	0.0	46.7	291.2	454.5	456.4	0.0	226.06	17.47	11.7	0.00	#DIV/0!	47.13	0.87	0.00	#DIV/0
		1-3	1.92	2	38.095	20	0.98	0.05	400	528.0	445.8	0.0	0.0	396.5	471.7	449.7	0.0	115.70	24.16	11.4	0.00	#DIV/0!	45.88	1.21	0.00	#DIV/0
		2-1	2.48	3.2	342.857	20	6.08	0.30	400	496.3	328.2	243.4	0.0	441	62.0	297.4	73.6	402.3	171.18	86.2	64.86	66.6	177.6	8.56	3.24	5.29
		2-2	2.56	3.25	328.571	20	5.70	0.29	400	526.2	313.0	234.3	0.0	768	35.0	163.1	81.4	151.3	183.58	87.2	31.20	33.3	116.2	9.18	1.56	10.53
		2-3	2.56	3.18	295.238	20	5.18	0.26	400	511.4	290.1	256.9	0.0	234	101.8	495.0	150.4	356.4	180.69	88.3	67.50	65.7	83.5	9.03	3.38	4.37
		3-1	2.2	3.08	419.048	20	8.00	0.40	400	721.6	306.3	126.5	0.0	263	63.7	466.8	23.1	318.6	271.92	94.2	44.56	88.0	83.6	13.60	2.23	9.40
		3-2	2.25	3.15	428.571	20	8.00	0.40	400	296.7	198.2	141.1	0.0	158	107.8	502.0	162.2	392.9	101.64	85.7	30.82	54.6	62.1	5.08	1.54	13.91
		3-3	2.19	3.03	400.000	20	7.72	0.39	400	495.4	348.0	228.3	0.0	297	74.9	468.7	58.6	285.2	175.93	88.8	73.90	80.9	84.7	8.80	3.70	5.41
		4-1	2.16	2.95	376.190	20	7.42	0.37	400	497.9	262.4	281.9	0.0	392	87.6	267.5	129.1	303.5	164.79	82.7	62.10	55.1	119.0	8.24	3.11	6.06
		4-2	2.2	2.93	347.619	20	6.83	0.34	400	627.1	341.2	166.6	0.0	316	237.5	431.9	39.5	312.8	175.79	70.1	54.16	81.3	98.8	8.79	2.71	6.42
		4-3	2.11	2.9	376.190	20	7.57	0.38	400	476.0	336.3	244.2	0.0	226	121.1	595.8	75.6	269.7	163.05	85.6	80.59	82.5	60.9	8.15	4.03	4.67
		5-1	2.48	3.38	428.571	20	7.37	0.37	400	517.0	277.2	259.1	0.0	212	92.9	523.9	179.0	380.9	187.14	90.5	65.74	63.4	80.6	9.36	3.29	6.52
		5-2	2.47	3.18	338.095	20	6.03	0.30	400	490.1	327.6	239.3	0.0	166	91.0	787.8	70.6	316.8	180.91	92.3	83.98	87.7	52.7	9.05	4.20	4.03
		5-3	2.55	3.45	428.571	20	7.20	0.36	400	481.4	356.9	230.3	0.0	264	79.7	541.7	124.6	363.1	171.59	89.1	59.27	64.3	95.7	8.58	2.96	7.23
	27-Oct	1-1	1.76	1.15	-290.476	23	-8.75	-0.44	400	598.1	391.3	0.0	46.3	417.3	523.6	375.1	0.0	85.44	20.74	8.7	0.00	#DIV/0!	17.14	0.90	0.00	#DIV/0
		1-2	1.17	0.96	-100.000	23	-4.12	-0.21	400	399.3	375.8	0.0	0.0	370.3	504.5	405.9	0.0	185.12	-27.09	-17.0	0.00	#DIV/0!	68.56	-1.18	0.00	#DIV/0
		1-3	1.8	1.19	-290.476	23	-8.52	-0.43	400	549.4	467.9	0.0	0.0	394.5	461.8	474.5	0.0	121.93	37.61	17.1	0.00	#DIV/0!	48.10	1.64	0.00	#DIV/0
		2-1	2.5	2.73	109.524	23	1.84	0.09	400	570.6	202.4	272.7	53.0	217	165.7	373.1	201.8	464.6	192.30	84.2	65.27	59.8	79.6	8.36	2.84	1.68

Cycle #	Day	sample	OD (in)	OD (out)	TSS (mg/L) inc	time(hr)	Growth rate (day-1)	Growth rate (day-1)	Gas volume (ml)	O_2 Initial (mg/l)	N_2 initial (mg/l)	CH_4 initial (mg/l)	CO_2 initial (mg/l)	Gas volume (ml)	O_2 final (mg/l)	N_2 final (mg/l)	CH_4 final (mg/l)	CO_2 final (mg/l)	O_2 consumed (mg)	% O_2 Consumed	CH_4 consumed (mg)	% CH_4 Consumed	CO_2 Produced (mg)	O_2 (mg/hr)	CH_4 (mg/hr)	obs Yield
5		2-3	2.55	2.94	185.714	23	2.97	0.15	400	519.1	275.8	249.4	82.8	222	85.8	496.2	166.8	460.1	188.57	90.8	62.66	62.8	69.2	8.20	2.72	2.96
		3-1	2.36	3.27	433.333	23	6.75	0.34	400	575.6	212.9	269.9	44.5	330	66.9	257.7	86.4	416.1	208.13	90.4	79.42	73.6	119.7	9.05	3.45	5.46
		3-2	2.22	3.23	480.952	23	7.74	0.39	400	551.0	231.8	268.4	49.4	566	31.0	163.8	51.7	398.3	202.86	92.0	78.09	72.7	205.7	8.82	3.40	6.16
		3-3	1.93	3.3	652.381	23	10.93	0.55	400	535.4	292.9	243.5	0.0	207	92.6	565.0	143.5	391.6	194.98	91.0	67.63	69.4	81.2	8.48	2.94	9.65
		4-1	1.93	2.84	433.333	23	7.96	0.40	400	559.7	218.3	274.3	0.0	260	273.6	336.3	241.9	304.8	152.83	68.3	46.91	42.8	79.1	6.64	2.04	9.24
		4-2	1.83	2.78	452.381	23	8.60	0.43	400	556.7	220.4	267.7	63.6	277	315.2	317.9	238.8	322.2	135.27	60.8	40.87	38.2	63.9	5.88	1.78	11.07
		4-3	2.1	2.97	414.286	23	7.16	0.36	400	536.0	288.1	243.3	52.5	260	240.5	443.8	195.1	302.2	151.92	70.9	46.65	47.9	57.5	6.61	2.03	8.88
		5-1	2.03	3.29	600.000	23	9.89	0.49	400	555.8	217.7	273.4	0.0	246	82.7	353.4	132.7	388.5	201.94	90.8	76.66	70.1	95.7	8.78	3.33	7.83
		5-2	2.02	3.17	547.619	23	9.25	0.46	400	580.7	160.0	297.3	0.0	231	66.2	276.5	146.7	417.0	216.97	93.4	84.95	71.4	96.5	9.43	3.69	6.45
		5-3	1.99	3.29	619.048	23	10.28	0.51	400	373.5	225.0	160.3	0.0	194	87.3	463.8	108.8	405.8	132.46	88.7	43.00	67.1	78.7	5.76	1.87	14.40
6	28-Oct	1-1	1.15	0.91	-114.286	20	-5.59	-0.28	400	619.0	429.7	0.0	50.7	395.7	591.2	434.3	0.0	66.31	13.63	5.5	0.00	#DIV/0!	5.95	0.68	0.00	#DIV/0!
		1-2	0.95	0.9	-23.810	20	-1.30	-0.06	400	611.0	453.9	0.0	62.7	396.3	579.5	458.1	0.0	81.88	14.73	6.0	0.00	#DIV/0!	7.35	0.74	0.00	#DIV/0!
		1-3	1.05	0.95	-47.619	20	-2.40	-0.12	400	582.9	490.7	0.0	0.0	396.7	552.1	494.8	0.0	85.00	14.13	6.1	0.00	#DIV/0!	33.72	0.71	0.00	#DIV/0!
		2-1	2.26	2.61	166.667	20	3.45	0.17	400	565.9	223.0	262.0	0.0	262	313.8	340.6	222.7	311.9	144.16	63.7	46.48	44.4	81.7	7.21	2.32	3.59
		2-2	2.2	2.71	242.857	20	4.99	0.25	400	561.8	239.5	260.4	0.0	252	278.4	380.8	210.4	311.5	154.66	68.8	51.21	49.2	78.4	7.73	2.56	4.74
		2-3	2.35	2.74	185.714	20	3.68	0.18	400	552.8	287.4	238.5	45.8	242	215.9	474.2	170.7	388.5	168.75	76.3	54.03	56.6	75.9	8.44	2.70	3.44
		3-1	2.26	3.27	480.952	20	8.77	0.44	400	575.3	212.9	269.3	0.0	190	152.8	449.1	181.1	410.3	201.13	87.4	73.39	68.1	77.8	10.06	3.67	6.55
		3-2	2.31	3.35	495.238	20	8.82	0.44	400	565.9	245.3	256.0	0.0	199	137.9	492.4	153.9	410.3	198.87	87.9	71.74	70.1	81.8	9.94	3.59	6.90
		3-3	2.37	3.35	466.667	20	8.22	0.41	400	539.0	328.0	218.5	0.0	222	137.7	590.7	123.9	413.0	185.00	85.8	59.88	68.5	91.7	9.25	2.99	7.79
		4-1	2.32	3.15	395.238	20	7.28	0.36	400	561.8	230.0	262.7	0.0	297	142.7	309.5	131.0	356.4	182.31	81.1	66.13	62.9	106.0	9.12	3.31	5.98
		4-2	2.35	3.13	371.429	20	6.83	0.34	400	562.5	245.8	255.4	0.0	215	193.3	458.2	180.9	395.2	183.52	81.6	63.33	62.0	84.8	9.18	3.17	5.87
		4-3	2.16	2.96	380.952	20	7.50	0.38	400	533.1	328.6	220.9	0.0	206	173.6	639.4	52.2	293.3	177.56	83.3	77.63	87.9	60.3	8.88	3.88	4.91
		5-1	2.39	3.16	366.667	20	6.66	0.33	400	539.7	292.7	234.4	0.0	222	123.9	527.2	139.7	369.4	188.35	87.3	62.71	66.9	82.0	9.42	3.14	5.85
		5-2	2.41	3.37	457.143	20	7.97	0.40	400	557.9	238.4	256.9	0.0	197	105.8	483.8	166.2	426.8	202.30	90.7	70.00	68.1	84.1	10.11	3.50	6.53
		5-3	2.26	3.27	480.952	20	8.77	0.44	400	535.4	298.3	230.7	0.0	260	111.2	459.1	118.1	449.9	185.27	86.5	61.57	66.7	117.0	9.26	3.08	7.81
7	29-Oct	1-1	0.88	0.67	-100.000	20.5	-6.34	-0.32	400	577.0	516.6	0.0	0.0	416.8	525.7	495.7	0.0	66.75	11.68	5.1	0.00	#DIV/0!	27.82	0.57	0.00	#DIV/0!
		1-2	0.8	0.57	-109.524	20.5	-7.86	-0.39	400	579.7	529.6	0.0	0.0	414.6	526.6	511.0	0.0	93.01	13.56	5.9	0.00	#DIV/0!	38.56	0.66	0.00	#DIV/0!
		1-3	0.9	0.64	-123.810	20.5	-7.91	-0.40	400	557.9	558.0	0.0	0.0	407.0	521.3	548.5	0.0	168.66	11.02	4.9	0.00	#DIV/0!	68.64	0.54	0.00	#DIV/0!
		2-1	1.98	2.56	276.190	20.5	5.98	0.30	400	584.6	205.1	269.5	0.0	599	98.6	137.0	68.2	342.2	174.82	74.8	66.96	62.1	204.9	8.53	3.27	4.12
		2-2	1.98	2.38	190.476	20.5	4.30	0.21	400	575.6	222.3	263.9	0.0	299	399.3	296.9	230.6	245.2	110.66	48.1	36.51	34.6	73.4	5.40	1.78	5.22
		2-3	2.06	2.33	128.571	20.5	2.88	0.14	400	554.7	273.6	244.6	0.0	298	380.0	367.5	205.5	242.5	108.74	49.0	36.67	37.5	72.2	5.30	1.79	3.51
		3-1	1.97	2.4	204.762	20.5	4.61	0.23	400	585.2	196.7	274.7	0.0	355	486.8	221.6	270.4	174.9	61.26	26.2	13.91	12.7	62.1	2.99	0.68	14.72
		3-2	2.36	3.41	500.000	20.5	8.52	0.43	400	580.6	217.3	266.9	0.0	199	146.4	436.4	165.6	400.5	203.07	87.4	73.76	69.1	79.8	9.91	3.60	6.78
		3-3	2.33	3.16	395.238	20.5	7.08	0.35	400	560.4	265.0	246.8	0.0	358	98.1	296.1	93.6	429.0	189.05	84.3	65.22	66.1	153.6	9.22	3.18	6.06
		4-1	2.19	2.88	328.571	20.5	6.37	0.32	400	571.9	203.1	278.9	0.0	230	149.6	353.9	145.5	386.3	194.42	85.0	78.15	70.1	88.7	9.48	3.81	4.20
		4-2	2.08	2.64	266.667	20.5	5.56	0.28	400	567.6	227.1	265.2	0.0	264	329.6	344.5	219.6	294.1	140.17	61.7	48.19	45.4	77.5	6.84	2.35	5.53
		4-3	2.15	2.84	328.571	20.5	6.48	0.32	400	543.7	300.0	233.8	0.0	236	198.6	507.6	178.7	367.6	170.55	78.4	51.27	54.8	86.9	8.32	2.50	6.41
		5-1	2.24	3.09	404.762	20.5	7.47	0.37	400	541.6	276.4	247.3	0.0	190	74.2	580.9	103.2	375.1	202.53	93.5	79.28	80.2	71.4	9.88	3.87	5.11
		5-2	2.28	3.29	480.952	20.5	8.49	0.42	400	581.4	194.7	277.4	0.0	264	85.7	295.4	91.9	430.3	209.99	90.3	86.74	78.2	113.5	10.24	4.23	5.55
		5-3	2.28	3.3	485.714	20.5	8.56	0.43	400	563.0	231.8	265.4	0.0	301	8.9	308.0	93.1	431.7	222.55	98.8	78.14	73.6	130.0	10.86	3.81	6.22
8	30-Oct	1-1	0.84	0.78	-28.571	18	-1.98	-0.10	400	632.1	506.9	0.0	0.0	407.1	588.2	498.1	0.0	#VALUE!	13.39	5.3	0.00	#DIV/0!	#VALUE!	0.74	0.00	#DIV/0!
		1-2	0.73	0.71	-9.524	18	-0.74	-0.04	400	627.1	239.7	0.0	0.0	193.7	572.6	495.0	0.0	#VALUE!	139.95	55.8	0.00	#DIV/0!	#VALUE!	7.78	0.00	#DIV/0!
		1-3	0.82	0.89	33.333	18	2.18	0.11	400	605.2	553.0	0.0	0.0	431.7	556.0	512.4	0.0	#VALUE!	2.06	0.9	0.00	#DIV/0!	#VALUE!	0.11	0.00	#DIV/0!
		2-1	2.06	2.8	352.381	18	8.12	0.41	400	494.5	271.3	271.8	0.0	260	132.0	417.5	220.8	#VALUE!	163.49	82.6	51.32	47.2	#VALUE!	9.08	2.85	6.87
		2-2	1.99	2.7	338.095	18	8.07	0.40	400	557.6	229.5	267.6	0.0	219	177.5	418.7	203.2	#VALUE!	184.11	82.6	62.51	58.4	#VALUE!	10.23	3.47	5.41
		2-3	1.94	2.45	242.857	18	6.20	0.31	400	501.8	313.0	234.8	0.0	295	267.6	423.8	190.9	#VALUE!	121.64	60.6	37.54	40.0	#VALUE!	6.76	2.09	6.47
		3-1	1.75	3	595.238	18	14.04	0.70	400	523.4	243.3	275.3	0.0	219	195.1	445.2	211.3	#VALUE!	166.71	79.6	63.93	58.1	#VALUE!	9.26	3.55	9.31
		3-2	2.3	3.05	357.143	18	7.48	0.37	400	495.8	298.6	246.7	0.0	263	170.5	454.6	205.2	#VALUE!	153.52	77.4	44.77	45.4	#VALUE!	8.53	2.49	7.98
		3-3	2.42	3.17	357.143	18	7.16	0.36	400	507.8	312.9	239.8	0.0	240	143.2	521.9	173.1	#VALUE!	168.79	83.1	54.40	56.7	#VALUE!	9.38	3.02	6.56
		4-1	2.35	2.92	271.429	18	5.77	0.29	400	526.2	226.4	277.9	0.0	206	227.4	439.9	194.4	#VALUE!	163.67	77.8	71.14	64.0	#VALUE!	9.09	3.95	3.82
		4-2	1.98	2.76	371.429	18	8.78	0.44	400	521.3	254.0	265.7	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		4-3	2.17	2.52	166.667	18	3.98	0.20	400	497.2	283.9	257.3	0.0	303	314.7	375.1	224.2	#VALUE!	103.58	52.1	35.02	34.0	#VALUE!	5.75	1.95	4.76
		5-1	2.23	3.2	461.905	18	9.53	0.48	400	515.8	251.7	268.9	0.0	208	131.9	485.0	198.2	#VALUE!	178.93	86.7	66.40	61.7	#VALUE!	9.94	3.69	6.96
		5-2	2.38	3.29	433.333	18	8.56	0.43	400	505.7	261.2	267.2	0.0	212	122.5	492.5	199.6	409.0	176.29	87.2	64.55	60.4	86.8	9.79	3.59	6.71
		5-3	2.4	3.29	423.810	18	8.34	0.42	400	489.9	327.5	235.4	0.0	234	126.6	559.0	161.3	409.8	166.32	84.9	56.37	59.9	96.0	9.24	3.13	7.52
10	01-Nov	1-1	0.83	0.88	23.810	23	1.22	0.06	400	369.4	433.3	0.0	0.0	407.8	331.9	425.0	0.0	101.91	12.43	8.4	0.00	#DIV/0!	41.55	0.54	0.00	#DIV/0!
		1-2	0.7	0.68	-9.524	23	-0.60	-0.03	400	405.0	238.6	0.0	0.0	391.7	304.6	243.6	0.0	65.86	42.66	26.3	0.00	#DIV/0!	25.80	1.85	0.00	#DIV/0!
		1-3	0.86	0.84	-9.524	23	-0.49	-0.02	400	521.6	309.3	0.0	0.0	345.8	483.9	357.7	0.0	53.85	41.29	19.8	0.00	#DIV/0!	18.62	1.80	0.00	#DIV/0!
		2-1	2.16	2.92	361.905	23	6.24	0.31	400	509.6	282.9	238.6	0.0	787	43.0	143.8	43.7	384.9	169.98	83.4	61.06	64.0	303.0	7.39	2.65	5.93
		2-2	2.39	2.98	280.952	23	4.59	0.23	400	449.6	376.6	220.7	0.0	474	42.3	317.5	74.5	381.4	159.76	88.8	52.94	60.0	180.9	6.95	2.30	5.31
		2-3	2.18	2.87	328.571	23	5.70	0.29	400	475.2	295.0	254.9	0.0	705	45.3	167.3	72.0	365.3	158.14	83.2	51.17	50.2	257.7	6.88	2.22	6.42
		3-1	2.21	3.25	495.238	23	7.95	0.40	400	516.0	244.0	260.2	0.0	474	54.0	205.8	90.3	400.5	180.78	87.6	61.23	58.8	190.0	7.86	2.66	8.09
		3-2	1.98	2.81	395.238	23	7.23	0.36	400	297.7	194.3	399.5	0.0	291	38.4	266.7	383.1	212.7	107.89	90.6	48.13	30.1	62.0	4.69	2.09	8.21
		3-3	2.22	2.7	228.571	23	4.07	0.20	400	478.3	347.9	219.5	0.0	270	195.4	514.9	167.4	315.5	138.49	72.4	42.58	48.5	85.3	6.02	1.85	5.37
		4-1	1.91	2.25	161.905	23	3.41	0.17	400	508.7	278.7	246.1	0.0	965	79.7	115.5	68.6	330.2	126.59	62.2	32.25	32.8	318.7	5.50	1.40	5.02

Cycle #	Day	sample	OD (in)	OD (out)	TSS (mg/L) inc	time(hr)	Growth rate (day-1)	Growth rate (day-1)	Gas volume (ml)	O2 Initial (mg/l)	N2 initial (mg/l)	CH4 initial (mg/l)	CO2 initial (mg/l)	Gas volume (ml)	O2 final (mg/l)	N2 final (mg/l)	CH4 final (mg/l)	CO2 final (mg/l)	O2 consumed (mg)	% O2 Consumed	CH4 consumed (mg)	% CH4 Consumed	CO2 Produced (mg)	O2 (mg/hr)	CH4 (mg/hr)	obs Yield
		4-2	2.07	2.29	104.762	23	2.11	0.11	400	535.2	224.1	271.1	0.0	377	189.9	237.6	151.2	273.7	142.43	66.5	51.38	47.4	103.3	6.19	2.23	2.04
		4-3	2.13	2.99	409.524	23	7.01	0.35	400	534.9	237.7	262.3	0.0	553	53.2	172.1	72.5	401.8	184.56	86.3	64.86	61.8	222.1	8.02	2.82	6.31
		5-1	2.33	3.3	461.905	23	7.19	0.36	400	525.0	193.1	281.1	0.0	514	32.2	150.4	59.9	417.0	193.45	92.1	81.65	72.6	214.2	8.41	3.55	5.66
		5-2	2.41	3.34	442.857	23	6.75	0.34	400	522.7	235.1	264.4	0.0	502	33.1	187.2	69.9	427.6	192.45	92.0	70.63	66.8	214.8	8.37	3.07	6.27
		5-3	2.37	3.26	423.810	23	6.60	0.33	400	506.6	276.5	246.8	0.0	222	88.7	498.4	190.0	417.4	182.95	90.3	56.56	57.3	92.6	7.95	2.46	7.49
11	02-Nov	1-1	0.81	0.78	-14.286	19	-0.95	-0.05	400	515.4	376.7	0.0	0.0	367.4	406.2	410.2	0.0	59.19	56.94	27.6	0.00	#DIV/0!	21.74	3.00	0.00	#DIV/0!
		1-2	0.7	0.71	4.762	19	0.36	0.02	400	488.0	422.8	0.0	0.0	372.8	485.9	453.6	0.0	109.03	14.05	7.2	0.00	#DIV/0!	40.65	0.74	0.00	#DIV/0!
		1-3	0.83	0.9	33.333	19	2.04	0.10	400	463.4	453.6	0.0	0.0	336.8	506.0	538.7	0.0	0.00	14.92	8.0	0.00	#DIV/0!	0.00	0.79	0.00	#DIV/0!
		2-1	2.02	2.92	428.571	19	9.21	0.46	400	492.8	277.9	263.3	0.0	323	102.7	344.0	118.1	287.0	163.93	83.2	67.18	63.8	92.8	8.63	3.54	6.38
		2-2	2.17	2.85	323.810	19	6.84	0.34	400	470.1	320.6	215.6	0.0	226	137.5	566.6	223.0	338.6	156.92	83.4	35.76	41.5	76.6	8.26	1.88	9.06
		2-3	2.06	2.58	247.619	19	5.66	0.28	400	425.5	324.0	233.4	0.0	1158	28.7	111.9	16.5	226.5	136.99	80.5	74.22	79.5	262.4	7.21	3.91	3.34
		3-1	2.13	3	414.286	19	8.57	0.43	400	459.8	322.0	259.0	0.0	212	117.5	606.2	245.0	299.5	158.97	86.4	51.53	49.7	63.6	8.37	2.71	8.04
		3-2	2.1	3.09	471.429	19	9.64	0.48	400	460.6	366.0	235.7	0.0	241	107.3	607.5	178.0	330.2	158.37	86.0	51.38	54.5	79.6	8.34	2.70	9.18
		3-3	1.92	2.94	485.714	19	10.60	0.53	400	499.3	261.7	310.5	0.0	215	167.6	486.9	304.6	313.3	163.70	82.0	58.72	47.3	67.3	8.62	3.09	8.27
		4-1	1.95	2.8	404.762	19	9.04	0.45	400	499.5	297.4	272.3	0.0	266	144.6	447.0	181.1	376.5	161.32	80.7	60.73	55.8	100.2	8.49	3.20	6.67
		4-2	1.88	2.72	400.000	19	9.23	0.46	400	459.7	357.6	260.6	0.0	203	190.5	705.7	269.8	292.8	145.27	79.0	49.56	47.5	59.3	7.65	2.61	8.07
		4-3	2.1	2.65	261.905	19	5.85	0.29	400	466.0	452.6	210.9	0.0	209	197.4	866.2	173.9	273.7	145.17	77.9	48.00	56.9	57.2	7.64	2.53	5.46
		5-1	2.16	3.11	452.381	19	9.11	0.46	400	451.7	386.1	256.3	0.0	249	97.4	619.4	275.9	327.1	156.41	86.6	33.73	32.9	81.6	8.23	1.78	13.41
		5-2	2.14	3.18	495.238	19	9.88	0.49	400	461.8	414.5	238.5	0.0	181	104.8	917.3	248.1	359.6	165.78	89.7	50.56	53.0	65.0	8.73	2.66	9.79
		5-3	2.24	3.28	495.238	19	9.52	0.48	400	453.8	460.6	223.2	0.0	202	100.7	912.9	190.8	323.1	161.21	88.8	50.76	56.9	65.2	8.48	2.67	9.76
37	28-Nov	1-1	1.3	1.58	133.333	22	4.24	0.21	400	549.1	398.7	0.0	0.0	409.2	442.5	389.8	0.0	73.43	38.55	17.6	0.00	#DIV/0!	30.05	1.75	0.00	#DIV/0!
		1-2	1.28	1.42	66.667	22	2.26	0.11	400	385.9	312.3	0.0	0.0	813.5	156.6	153.6	0.0	102.80	26.91	17.4	0.00	#DIV/0!	83.62	1.22	0.00	#DIV/0!
		1-3	1.2	1.29	42.857	22	1.58	0.08	400	536.0	463.1	0.0	0.0	#DIV/0!	0.0	0.0	0.0	92.56	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
		2-1	1.9	2.92	485.714	22.5	9.03	0.45	400	456.1	249.2	278.5	0.0	588	44.1	169.4	79.5	364.5	156.52	85.8	64.63	58.0	214.5	6.96	2.87	7.52
		2-2	1.98	2.98	476.190	22.5	8.60	0.43	400	451.0	361.6	218.1	0.0	#DIV/0!	0.0	0.0	0.0	267.4	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
		2-3	1.9	2.93	490.476	22.5	9.10	0.45	400	459.7	343.7	240.1	0.0	306	70.4	449.4	160.6	366.2	162.32	88.3	46.91	48.9	112.0	7.21	2.08	10.46
		3-1	1.9	3.16	600.000	22.5	10.62	0.53	400	488.9	248.5	280.3	0.0	648	32.9	153.4	57.7	339.1	174.22	89.1	74.74	66.7	219.7	7.74	3.32	8.03
		3-2	1.88	3.29	671.429	22.5	11.64	0.58	400	460.6	318.4	249.1	0.0	230	69.0	554.4	186.3	311.1	168.37	91.4	56.84	57.1	71.4	7.48	2.53	11.81
		3-3	2.04	3.2	552.381	22.5	9.45	0.47	400	467.1	311.4	246.5	0.0	666	42.8	186.9	72.6	313.7	158.30	84.7	50.25	51.0	209.1	7.04	2.23	10.99
		4-1	2.14	3.1	457.143	22.5	7.82	0.39	400	467.1	307.3	254.0	0.0	208	84.4	591.4	56.4	265.2	169.29	90.6	89.88	88.5	55.1	7.52	3.99	5.09
		4-2	1.96	2.96	476.190	22.5	8.67	0.43	400	447.5	355.2	233.9	0.0	#DIV/0!	0.0	0.0	0.0	255.9	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
		4-3	2.07	2.94	414.286	22.5	7.41	0.37	400	393.1	227.1	228.2	0.0	1092	18.9	83.2	14.5	264.8	136.56	86.8	75.40	82.6	289.2	6.07	3.35	5.49
		5-1	1.9	3.25	642.857	22.5	11.18	0.56	400	457.4	343.4	236.4	0.0	245	74.5	559.6	128.0	329.7	164.65	90.0	63.13	66.8	80.9	7.32	2.81	10.18
		5-2	1.88	3.2	628.571	22.5	11.09	0.55	400	456.0	334.6	235.6	0.0	579	31.7	231.1	50.6	350.2	164.03	89.9	64.92	68.9	202.8	7.29	2.89	9.68
		5-3	1.91	2.95	495.238	22.5	9.13	0.46	400	449.6	345.8	236.0	0.0	217	54.0	638.0	100.7	268.3	168.13	93.5	72.56	76.9	58.2	7.47	3.22	6.83
38	29-Nov	1-1	1.5	1.68	85.714	20.5	2.65	0.13	400	564.1	457.8	0.0	0.0	5839.3	18.2	31.4	0.0	59.63	119.18	52.8	0.00	#DIV/0!	348.20	5.81	0.00	#DIV/0!
		1-2	1.4	1.61	100.000	20.5	3.27	0.16	400	500.4	443.4	0.0	0.0	406.0	506.8	436.8	0.0	60.97	-5.60	-2.8	0.00	#DIV/0!	24.75	-0.27	0.00	#DIV/0!
		1-3	1.25	1.33	38.095	20.5	1.45	0.07	400	569.4	454.2	0.0	0.0	420.6	516.7	431.9	0.0	57.85	10.45	4.6	0.00	#DIV/0!	24.33	0.51	0.00	#DIV/0!
		2-1	1.68	2.53	404.762	21.5	9.02	0.45	400	490.5	367.2	209.6	0.0	398	59.5	369.2	110.4	328.4	172.52	87.9	39.91	47.6	130.7	8.02	1.86	10.14
		2-2	1.82	2.87	500.000	21.5	10.00	0.50	400	488.9	311.1	254.0	0.0	778	35.6	160.0	61.6	347.1	167.88	85.9	53.74	52.9	269.9	7.81	2.50	9.30
		2-3	2.02	2.8	371.429	21.5	7.23	0.36	400	310.3	225.4	158.7	0.0	330	45.3	273.0	86.5	361.8	109.15	87.9	34.88	55.0	119.5	5.08	1.62	10.65
		3-1	1.96	2.98	485.714	21.5	9.22	0.46	400	453.1	369.6	223.5	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		3-2	1.83	2.82	471.429	21.5	9.51	0.48	400	432.2	426.0	196.7	0.0	1698	0.0	100.4	17.7	266.6	172.89	100.0	48.66	61.9	452.5	8.04	2.26	9.69
		3-3	1.94	3.04	523.810	21.5	9.86	0.49	400	451.2	401.7	211.2	0.0	270	110.8	594.6	143.1	275.9	150.53	83.4	45.81	54.2	74.6	7.00	2.13	11.43
		4-1	1.83	2.95	533.333	22	10.22	0.51	400	443.0	379.0	223.5	0.0	405	113.5	373.9	121.9	282.6	131.22	74.0	40.00	44.7	114.6	5.96	1.82	13.33
		4-2	1.68	2.92	590.476	22	11.76	0.59	400	448.5	379.7	227.1	0.0	310	219.1	489.2	188.4	197.6	111.37	62.1	32.37	35.6	61.3	5.06	1.47	18.24
		4-3	1.68	2.84	552.381	22	11.20	0.56	400	429.8	350.7	223.0	0.0	245	115.1	571.6	81.4	222.5	143.67	83.6	69.21	77.6	54.6	6.53	3.15	7.98
		5-1	1.86	2.77	433.333	22	8.58	0.43	400	429.6	384.9	216.3	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		5-2	1.79	3.03	590.476	22	11.23	0.56	400	422.1	374.4	229.0	0.0	514	38.2	291.3	94.5	340.4	149.21	88.4	43.00	46.9	175.0	6.78	1.95	13.73
		5-3	1.8	2.84	495.238	22	9.78	0.49	400	452.2	333.6	242.4	0.0	471	31.7	283.5	86.3	332.4	165.98	91.8	56.37	58.1	156.5	7.54	2.56	8.79
39	30-Nov	1-1	1.51	1.76	119.048	20.5	3.58	0.18	400	174.7	234.1	0.0	54.7	176.3	476.5	531.2	0.0	61.41	-14.11	-20.2	0.00	#DIV/0!	-11.07	-0.69	0.00	#DIV/0!
		1-2	1.13	1.45	152.381	20.5	5.81	0.29	400	426.2	634.2	0.0	57.4	449.9	472.1	563.9	0.0	44.95	-41.87	-24.6	0.00	#DIV/0!	-2.74	-2.04	0.00	#DIV/0!
		1-3	1.2	1.63	204.762	20.5	7.12	0.36	400	340.5	422.8	0.0	0.0	296.5	465.5	570.4	0.0	105.02	-1.81	-1.3	0.00	#DIV/0!	31.14	-0.09	0.00	#DIV/0!
		2-1	2.07	3.02	452.381	20	8.96	0.45	400	376.1	337.7	192.5	0.0	220	64.4	614.3	162.3	357.8	136.28	90.6	41.31	53.7	78.7	6.81	2.07	10.95
		2-2	2.2	3.1	428.571	20	8.15	0.41	400	423.4	417.6	209.0	0.0	418	58.9	400.0	91.8	357.8	144.74	85.5	45.27	54.2	149.4	7.24	2.26	9.47
		2-3	2.22	3.13	433.333	20	8.16	0.41	400	436.7	389.5	222.8	0.0	255	61.8	610.5	148.0	352.9	158.90	91.0	51.34	57.6	90.0	7.95	2.57	8.44
		3-1	2.03	2.91	419.048	20	8.55	0.43	400	295.6	243.6	162.3	0.0	2210	#VALUE!	44.1	#VALUE!	297.7	#VALUE!	#VALUE!	#VALUE!	#VALUE!	657.8	#VALUE!	#VALUE!	#VALUE!
		3-2	2.07	3.28	576.190	20	10.86	0.54	400	356.7	362.2	168.5	0.0	229	76.1	631.8	140.9	313.7	125.21	87.8	35.11	52.1	71.9	6.26	1.76	16.41
		3-3	2.05	2.88	395.238	20	8.08	0.40	400	447.8	337.7	248.5	49.0	503	47.4	268.8	73.2	295.9	155.29	86.7	62.60	63.0	129.1	7.76	3.13	6.31
		4-1	1.97	3.02	500.000	20	10.10	0.51	400	423.6	430.6	206.3	0.0	214	112.7	804.6	71.0	250.1	145.29	85.8	67.34	81.6	53.5	7.26	3.37	7.42
		4-2	1.99	2.95	457.143	20	9.33	0.47	400	444.4	344.0	249.5	0.0	258	124.6	533.7	170.1	258.1	145.65	81.9	55.92	56.0	66.5	7.28	2.80	8.17
		4-3	2	2.9	428.571	20	8.82	0.44	400	428.0	406.3	314.9	0.0	265	173.8	613.1	156.1	222.1	125.12	73.1	84.59	67.2	58.9	6.26	4.23	5.07
		5-1	1.91	2.97	504.762	20	10.43	0.52	400	380.6	305.3	306.1	49.0	250	21.8	487.6	286.3	291.9	146.77	96.4	50.72	41.4	53.5	7.34	2.54	9.95
		5-2	2	3.08	514.286	20	10.20	0.51	400	424.1	405.4	221.9	0.0	240	39.8	675.5	155.2	319.5	160.08	94.4	51.50	58.0	76.7	8.00	2.57	9.99
		5-3	2	3.12	533.333	20	10.50	0.53	400	435.8	353.2	246.7	0.0	232	36.1	608.9	180.2	313.3	165.93	95.2	56.87	57.6	72.7	8.30	2.84	9.38

Cycle #	Day	sample	OD (in)	OD (out)	TSS (mg/L) inc	time(hr)	Growth rate (day-1)	Growth rate (day-1)	Gas volume (ml)	O2 Initial (mg/l)	N2 initial (mg/l)	CH4 initial (mg/l)	CO2 initial (mg/l)	Gas volume (ml)	O2 final (mg/l)	N2 final (mg/l)	CH4 final (mg/l)	CO2 final (mg/l)	O2 consumed (mg)	% O2 Consumed	CH4 consumed (mg)	% CH4 Consumed	CO2 Produced (mg)	O2 (mg/hr)	CH4 (mg/hr)	obs Yield
41	02-Dec	1-1	1.58	1.74	76.190	23.25	1.99	0.10	400	622.7	539.7	0.0	66.8	422.8	593.0	510.6	0.0	72.98	-1.63	-0.7	0.00	#DIV/0!	4.16	-0.07	0.00	#DIV/0!
		1-2	1.25	1.35	47.619	23.25	1.59	0.08	400	454.7	487.1	0.0	44.9	362.5	559.1	537.5	0.0	80.99	-20.80	-11.4	0.00	#DIV/0!	11.38	-0.89	0.00	#DIV/0!
		1-3	1.35	1.39	19.048	23.25	0.60	0.03	400	555.8	531.7	0.0	45.4	452.4	497.4	470.1	0.0	79.66	-2.70	-1.2	0.00	#DIV/0!	17.88	-0.12	0.00	#DIV/0!
		2-1	1.99	3.02	490.476	23.25	8.49	0.42	400	527.6	389.8	186.7	46.7	638	26.7	244.4	49.9	437.4	194.01	91.9	42.86	57.4	260.3	8.34	1.84	11.44
		2-2	2.1	2.91	385.714	23.25	6.68	0.33	400	286.9	153.2	132.5	0.0	#DIV/0!	0.0	0.0	0.0	249.6	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
		2-3	2.04	2.9	409.524	23.25	7.19	0.36	400	179.5	133.3	74.4	0.0	329	18.4	161.8	21.4	62.7	65.73	91.6	22.71	76.3	20.7	2.83	0.98	18.03
		3-1	2.01	3.24	585.714	23.5	9.57	0.48	400	501.3	375.3	208.1	0.0	5206	0.0	28.8	0.0	322.6	200.51	100.0	83.26	100.0	1679.5	8.53	3.54	7.03
		3-2	2	3.21	576.190	23.5	9.49	0.47	400	525.9	310.5	232.5	0.0	222	69.6	560.1	146.5	333.3	194.92	92.7	60.50	65.1	73.9	8.29	2.57	9.52
		3-3	2.06	3.01	452.381	23.5	7.65	0.38	400	440.9	372.0	192.2	0.0	287	77.2	518.6	151.3	353.3	154.22	87.4	33.45	43.5	101.4	6.56	1.42	13.52
		4-1	1.98	2.98	476.190	23.75	8.15	0.41	400	441.6	268.2	202.2	0.0	131	100.9	816.8	53.8	209.2	163.39	92.5	73.80	91.3	27.5	6.88	3.11	6.45
		4-2	1.95	2.97	485.714	23.75	8.38	0.42	400	516.7	323.1	225.9	0.0	324	80.9	399.4	103.4	358.7	180.49	87.3	56.92	63.0	116.1	7.60	2.40	8.53
		4-3	1.95	2.95	476.190	23.75	8.25	0.41	400	507.3	352.7	215.7	0.0	284	254.5	495.9	179.7	247.4	130.51	64.3	35.17	40.8	70.4	5.50	1.48	13.54
		5-1	2.04	3.24	571.429	24	9.09	0.45	400	502.5	363.0	209.6	0.0	288	54.7	504.1	136.0	428.1	185.25	92.2	44.64	53.3	123.3	7.72	1.86	12.80
		5-2	1.99	3.24	595.238	24	9.56	0.48	400	505.7	357.7	211.6	0.0	295	41.6	484.8	80.0	379.1	190.00	93.9	61.02	72.1	111.9	7.92	2.54	9.75
		5-3	2	3.26	600.000	24	9.58	0.48	400	483.9	321.6	203.1	0.0	285	44.4	451.8	111.9	372.5	180.92	93.5	49.38	60.8	106.0	7.54	2.06	12.15
42	03-Dec	1-1	1.59	1.7	52.381	21.75	1.48	0.07	400	670.1	535.1	0.0	64.5	421.4	604.3	507.9	0.0	98.35	13.41	5.0	0.00	#DIV/0!	15.63	0.62	0.00	#DIV/0!
		1-2	1.15	1.2	23.810	21.75	0.94	0.05	400	631.4	574.1	0.0	54.7	424.9	563.9	540.5	0.0	97.01	12.95	5.1	0.00	#DIV/0!	19.32	0.60	0.00	#DIV/0!
		1-3	1.2	1.31	52.381	21.75	1.93	0.10	400	668.0	536.9	0.0	49.4	410.6	620.4	523.0	0.0	93.45	12.47	4.7	0.00	#DIV/0!	18.61	0.57	0.00	#DIV/0!
		2-1	2.11	3	423.810	22	7.60	0.38	400	540.9	288.3	238.5	65.9	217	79.7	531.4	123.4	488.2	199.08	92.0	68.64	71.9	79.6	9.05	3.12	6.17
		2-2	1.96	3.01	500.000	22	9.22	0.46	400	565.9	221.2	268.4	53.4	687	26.7	128.8	46.6	438.8	207.99	91.9	75.37	70.2	280.1	9.45	3.43	6.63
		2-3	1.96	3.07	528.571	22	9.63	0.48	400	529.6	295.3	234.5	47.2	243	62.1	485.9	101.4	406.3	196.73	92.9	69.15	73.7	79.9	8.94	3.14	7.64
		3-1	2.08	3	438.095	22.25	7.81	0.39	400	463.9	370.7	223.4	0.0	723	30.4	205.0	41.4	306.2	163.54	88.1	59.41	66.5	221.5	7.35	2.67	7.37
		3-2	2.01	3.08	509.524	22.25	9.07	0.45	400	484.8	373.8	209.7	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	343.1	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		3-3	2.12	3.28	552.381	22.25	9.27	0.46	400	548.9	244.3	252.0	0.0	257	80.4	380.8	115.9	370.2	198.93	90.6	71.07	70.5	95.0	8.94	3.19	7.77
		4-1	2.03	2.97	447.619	22.5	8.02	0.40	400	541.4	268.5	249.6	0.0	397	148.0	270.8	107.9	227.4	157.88	72.9	57.02	57.1	90.2	7.02	2.53	7.85
		4-2	1.97	2.98	480.952	22.5	8.71	0.44	400	511.9	337.8	220.7	0.0	296	232.8	456.4	157.0	182.9	135.84	66.3	41.78	47.3	54.2	6.04	1.86	11.51
		4-3	1.99	3.02	490.476	22.5	8.77	0.44	400	510.6	328.4	227.3	0.0	185	135.9	709.0	83.3	290.6	179.07	87.7	75.50	83.0	53.8	7.96	3.36	6.50
		5-1	2.02	3.05	490.476	24	8.13	0.41	400	498.6	347.1	215.9	0.0	174	120.2	798.4	29.4	346.2	178.55	89.5	81.27	94.1	60.2	7.44	3.39	6.04
		5-2	1.93	3.04	528.571	24	8.93	0.45	400	517.4	288.3	240.1	0.0	2196	0.0	52.5	9.6	395.6	206.95	100.0	74.95	78.1	868.9	8.62	3.12	7.05
		5-3	1.99	2.95	457.143	24	7.77	0.39	400	487.8	366.1	209.9	0.0	1344	0.0	108.9	12.6	370.2	195.12	100.0	66.95	79.8	497.8	8.13	2.79	6.83
44	05-Dec	1-1	1.63	1.7	33.333	21.25	0.95	0.05	400	493.3	636.4	0.0	57.0	388.8	468.5	654.8	0.0	94.34	15.16	7.7	0.00	#DIV/0!	13.90	0.71	0.00	#DIV/0!
		1-2	1.05	1.1	23.810	21.25	1.05	0.05	400	470.1	676.8	0.0	48.5	398.4	456.8	679.4	0.0	170.88	6.03	3.2	0.00	#DIV/0!	48.68	0.28	0.00	#DIV/0!
	New feed	1-3	0.96	0.99	14.286	21.25	0.70	0.03	400	#VALUE!	#VALUE!	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		2-1	2.04	3	457.143	21.5	8.50	0.43	400	429.0	599.3	81.3	0.0	341	50.3	703.6	108.4	367.6	154.49	90.0	-4.40	-13.5	125.2	7.19	-0.20	-103.82
		2-2	2	2.98	466.667	21.5	8.79	0.44	400	395.8	448.6	198.2	0.0	673	28.7	266.7	51.0	341.8	139.02	87.8	44.95	56.7	229.9	6.47	2.09	10.38
		2-3	2.04	2.89	404.762	21.5	7.70	0.38	400	394.7	448.7	198.6	0.0	2509	0.0	71.5	9.6	290.6	157.88	100.0	55.39	69.7	729.0	7.34	2.58	7.31
		3-1	2.08	3.15	509.524	21.75	9.03	0.45	400	395.4	440.2	204.6	0.0	362	56.6	486.4	77.0	276.8	137.66	87.0	53.96	65.9	100.2	6.33	2.48	9.44
		3-2	2.05	3.11	504.762	21.75	9.07	0.45	400	392.8	483.1	182.5	0.0	501	50.6	385.4	63.7	291.0	131.72	83.8	41.04	56.2	145.9	6.06	1.89	12.30
		3-3	2.04	3.14	523.810	21.75	9.37	0.47	400	371.7	480.8	200.5	0.0	573	37.7	335.6	66.0	290.6	127.08	85.5	42.38	52.8	166.5	5.84	1.95	12.36
		4-1	1.98	2.87	423.810	22	8.01	0.40	400	376.3	484.0	197.9	0.0	7857	0.0	24.6	0.0	226.5	150.52	100.0	79.15	100.0	1779.6	6.84	3.60	5.35
		4-2	1.9	2.75	404.762	22	7.98	0.40	400	386.6	504.8	171.6	0.0	8435	0.0	23.9	10.8	188.2	154.63	100.0	-22.72	-33.1	1587.8	7.03	-1.03	-17.81
		4-3	1.98	2.89	433.333	22	8.15	0.41	400	#VALUE!	#VALUE!	#VALUE!	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		5-1	2.19	3.09	428.571	22.25	7.35	0.37	400	389.6	481.0	185.6	0.0	268	43.0	719.0	101.7	299.5	144.32	92.6	47.05	63.4	80.1	6.49	2.11	9.11
		5-2	2.07	2.91	400.000	22.25	7.28	0.36	400	#VALUE!	#VALUE!	#VALUE!	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		5-3	1.99	2.87	419.048	22.25	7.81	0.39	400	379.5	511.1	172.0	0.0	499	32.9	409.4	52.3	227.8	135.35	89.2	42.67	62.0	113.8	6.08	1.92	9.82
45	06-Dec	1-1	1.46	1.51	23.810	23.5	0.69	0.03	400	301.1	400.7	0.0	0.0	420.7	319.0	380.9	0.0	85.44	-13.76	-11.4	0.00	#DIV/0!	35.95	-0.59	0.00	#DIV/0!
		1-2	1	1.03	14.286	23.5	0.60	0.03	400	205.9	264.6	0.0	0.0	261.6	277.9	404.6	0.0	#VALUE!	9.65	11.7	0.00	#DIV/0!	#VALUE!	0.41	0.00	#DIV/0!
		1-3	1	1.06	28.571	23.5	1.19	0.06	400	557.9	665.0	0.0	0.0	435.8	542.7	610.4	0.0	64.53	-13.33	-6.0	0.00	#DIV/0!	28.12	-0.57	0.00	#DIV/0!
		2-1	2.1	2.92	390.476	23.5	6.67	0.33	400	226.2	162.3	109.4	0.0	108	447.1	598.5	22.7	#VALUE!	42.00	46.4	41.31	94.4	#VALUE!	1.79	1.76	9.45
		2-2	2.13	2.92	376.190	23.5	6.39	0.32	400	408.2	316.8	197.6	0.0	206	60.5	616.1	141.4	324.9	150.81	92.4	49.97	63.2	66.8	6.42	2.13	7.53
		2-3	1.91	2.63	342.857	23.5	6.48	0.32	400	345.5	219.5	172.5	0.0	258	27.8	340.6	47.8	280.4	131.04	94.8	56.69	82.1	72.3	5.58	2.41	6.05
		3-1	2	3.18	561.905	24	9.11	0.46	400	449.2	421.5	203.7	0.0	672	34.7	250.7	54.2	282.6	156.36	87.0	44.99	55.2	190.0	6.52	1.87	12.49
		3-2	1.92	3.13	576.190	24	9.58	0.48	400	459.3	424.2	196.5	0.0	242	64.3	700.1	113.1	363.6	168.15	91.5	51.16	65.1	88.1	7.01	2.13	11.26
		3-3	1.92	3.09	557.143	24	9.34	0.47	400	449.8	401.4	189.8	0.0	255	83.5	628.7	139.7	311.9	158.57	88.1	40.24	53.0	79.7	6.61	1.68	13.85
		4-1	1.97	2.88	433.333	23.5	7.66	0.38	400	402.5	325.4	188.2	0.0	387	43.7	336.1	46.1	193.6	144.07	89.5	57.44	76.3	74.9	6.13	2.44	7.54
		4-2	2.04	3.03	471.429	23.5	7.98	0.40	400	444.3	447.7	177.7	0.0	939	29.4	190.8	13.9	181.6	150.13	84.5	58.04	81.7	170.4	6.39	2.47	8.12
		4-3	2	3.02	485.714	23.5	8.30	0.42	400	449.2	426.9	196.7	0.0	321	83.5	532.1	129.2	214.5	152.88	85.1	37.24	47.3	68.8	6.51	1.58	13.04
		5-1	2.06	3.17	528.571	24	8.49	0.42	400	626.0	418.6	204.2	0.0	238	38.8	702.4	105.5	329.7	241.18	96.3	56.50	69.2	78.6	10.05	2.35	9.36
		5-2	1.86	3.08	580.952	24	9.88	0.49	400	460.7	361.6	217.4	0.0	230	32.7	628.0	143.7	275.0	176.75	95.9	53.84	61.9	63.3	7.36	2.24	10.79
		5-3	1.97	3.09	533.333	24	8.85	0.44	400	445.2	422.1	192.0	0.0	228	20.9	739.8	113.7	364.0	173.30	97.3	50.84	66.2	83.1	7.22	2.12	10.49
	07-Dec	1-1	1.2	1.37	80.952	19	3.34	0.17	400	480.4	712.2	0.0	46.3	443.0	476.1	643.0	0.0	88.11	-18.78	-9.8	0.00	#DIV/0!	20.52	-0.99	0.00	#DIV/0!
		1-2	0.96	1.01	23.810	19	1.28	0.06	400	533.7	695.4	0.0	44.5	417.5	489.2	666.3	0.0	101.91	9.22	4.3	0.00	#DIV/0!	24.74	0.49	0.00	#DIV/0!
		1-3	0.87	1.1	109.524	19	5.90	0.29	400	520.6	704.8	0.0	0.0	422.2	519.8	667.7	0.0	48.95	-11.27	-5.4	0.00	#DIV/0!	20.67	-0.59	0.00	#DIV/0!
		2-1	1.99	2.84	404.762	19.25	8.78	0.44	400	480.2	533.1	121.8	0.0	686	42.8	310.9	43.4	339.5	162.70	84.7	18.94	38.9	232.9	8.45	0.98	21.37
		2-2	2.02	2.91	423.810	19.25	9.00	0.45	400	445.5	433.7	201.2	0.0	296	63.7	586.2	98.0	339.1	159.34	89.4	51.47	63.9	100.4	8.28	2.67	8.23

Cycle #	Day	sample	OD (in)	OD (out)	TSS (mg/L) inc	time(hr)	Growth rate (day-1)	Growth rate (day-1)	Gas volume (ml)	O2 Initial (mg/l)	N2 initial (mg/l)	CH4 initial (mg/l)	CO2 initial (mg/l)	Gas volume (ml)	O2 final (mg/l)	N2 final (mg/l)	CH4 final (mg/l)	CO2 final (mg/l)	O2 consumed (mg)	% O2 Consumed	CH4 consumed (mg)	% CH4 Consumed	CO2 Produced (mg)	O2 (mg/hr)	CH4 (mg/hr)	obs Yield
46		2-3	1.94	2.9	457.143	19.25	9.89	0.49	400	432.9	436.2	200.0	0.0	231	48.3	755.6	13.1	277.2	162.02	93.6	76.96	96.2	64.0	8.42	4.00	5.94
		3-1	2.03	3.18	547.619	19.5	10.87	0.54	400	436.3	424.2	202.8	0.0	360	73.3	471.0	86.5	289.3	148.12	84.9	49.98	61.6	104.2	7.60	2.56	10.96
		3-2	2.02	3.11	519.048	19.5	10.46	0.52	400	443.4	409.8	213.8	0.0	403	65.5	406.8	73.6	291.0	150.97	85.1	55.88	65.3	117.3	7.74	2.87	9.29
		3-3	2.05	3.16	528.571	19.5	10.49	0.52	400	429.8	452.3	183.7	0.0	392	75.4	461.9	82.9	288.4	142.36	82.8	41.00	55.8	113.0	7.30	2.10	12.89
		4-1	2.05	2.77	342.857	19.75	7.26	0.36	400	431.5	450.1	192.1	0.0	636	82.0	283.1	54.2	227.0	120.49	69.8	42.34	55.1	144.3	6.10	2.14	8.10
		4-2	2.09	2.68	280.952	19.75	6.01	0.30	400	437.7	407.8	205.2	0.0	330	302.8	494.9	196.6	152.2	75.26	43.0	17.29	21.1	50.2	3.81	0.88	16.25
		4-3	2.06	2.89	395.238	19.75	8.15	0.41	400	#VALUE!	#VALUE!	#VALUE!	0.0	#VALUE!	67.1	276.4	49.5	245.2	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		5-1	1.97	3.07	523.810	20	10.48	0.52	400	429.6	429.2	195.8	0.0	856	22.3	200.6	33.7	313.3	152.74	88.9	49.46	63.1	268.1	7.64	2.47	10.59
		5-2	1.97	2.97	476.190	20	9.72	0.49	400	428.3	419.9	199.8	0.0	913	23.2	184.0	42.0	212.7	150.17	87.6	41.58	52.0	194.2	7.51	2.08	11.45
		5-3	1.95	3.19	590.476	20	11.58	0.58	400	#VALUE!	#VALUE!	#VALUE!	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
47	08-Dec	1-1	1.4	1.48	38.095	23.75	1.12	0.06	400	481.1	733.3	0.0	46.3	424.9	449.2	690.3	0.0	72.54	1.56	0.8	0.00	#DIV/0!	12.31	0.07	0.00	#DIV/0!
		1-2	1.06	1	-28.571	23.75	-1.18	-0.06	400	471.7	752.6	0.0	44.5	439.5	454.5	685.0	0.0	62.30	-11.08	-5.9	0.00	#DIV/0!	9.58	-0.47	0.00	#DIV/0!
		1-3	1.05	1.05	0.000	23.75	0.00	0.00	400	486.2	742.1	0.0	0.0	#VALUE!	#VALUE!	#VALUE!	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		2-1	2.02	2.83	385.714	23.5	6.82	0.34	400	391.9	485.5	197.3	0.0	272	62.1	714.3	107.2	293.3	139.86	89.2	49.79	63.1	79.7	5.95	2.12	7.75
		2-2	2.02	2.78	361.905	23.5	6.47	0.32	400	394.9	501.2	184.0	0.0	268	0.0	747.0	108.1	258.5	157.95	100.0	44.59	60.6	69.4	6.72	1.90	8.12
		2-3	1.9	2.75	404.762	23.5	7.47	0.37	400	409.4	474.7	178.4	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		3-1	1.94	3.05	528.571	23.5	9.09	0.45	400	406.4	476.7	186.9	0.0	257	48.7	742.6	117.2	220.3	150.06	92.3	44.64	59.7	56.6	6.39	1.90	11.84
		3-2	1.92	3.04	533.333	23.5	9.22	0.46	400	405.5	464.1	198.1	0.0	586	29.0	316.5	46.6	217.2	145.18	89.5	51.93	65.5	127.4	6.18	2.21	10.27
		3-3	1.96	3.04	514.286	23.5	8.82	0.44	400	396.1	509.0	174.0	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		4-1	1.81	2.73	438.095	23.75	8.19	0.41	400	394.5	528.8	165.9	0.0	1584	0.0	133.6	12.8	204.7	157.81	100.0	46.04	69.4	324.2	6.64	1.94	9.52
		4-2	1.81	2.77	457.143	23.75	8.47	0.42	400	399.8	484.0	184.0	0.0	439	87.6	440.9	79.1	162.4	121.46	75.9	38.86	52.8	71.3	5.11	1.64	11.77
		4-3	1.9	2.81	433.333	23.75	7.81	0.39	400	382.9	509.7	175.8	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		5-1	2.1	3	428.571	23.75	7.13	0.36	400	375.6	527.4	166.6	0.0	265	0.0	796.6	106.6	240.7	150.24	100.0	38.43	57.7	63.8	6.33	1.62	11.15
		5-2	2.02	2.79	366.667	23.75	6.47	0.32	400	390.3	500.6	177.1	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		5-3	1.94	3.04	523.810	23.75	8.93	0.45	400	378.4	530.6	163.7	0.0	325	21.6	653.7	84.6	302.6	144.36	95.4	37.99	58.0	98.3	6.08	1.60	13.79
48	09-Dec	1-1	1.35	1.42	33.333	23.5	1.03	0.05	400	525.0	347.8	0.0	50.7	435.2	483.0	319.6	0.0	101.02	-0.23	-0.1	0.00	#DIV/0!	23.67	-0.01	0.00	#DIV/0!
		1-2	0.96	0.99	14.286	23.5	0.63	0.03	400	522.2	401.8	0.0	46.3	516.0	479.5	311.5	0.0	89.45	-38.54	-18.5	0.00	#DIV/0!	27.64	-1.64	0.00	#DIV/0!
		1-3	0.82	0.92	47.619	23.5	2.35	0.12	400	#VALUE!	#VALUE!	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		2-1	2.03	2.97	447.619	23.5	7.68	0.38	400	516.3	239.0	278.7	0.0	235	166.4	407.5	156.9	342.7	167.50	81.1	74.68	67.0	80.4	7.13	3.18	5.99
		2-2	2.09	2.97	419.048	23.5	7.10	0.36	400	499.0	299.3	259.2	0.0	1029	0.0	116.3	50.6	320.4	199.59	100.0	51.55	49.7	329.7	8.49	2.19	8.13
		2-3	2.06	2.81	357.143	23.5	6.29	0.31	400	#VALUE!	#VALUE!	#VALUE!	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		3-1	2.07	3.19	533.333	23.5	8.70	0.43	400	502.1	274.8	267.9	0.0	232	71.7	474.3	205.9	290.1	184.25	91.7	59.45	55.5	67.2	7.84	2.53	8.97
		3-2	2.03	3.25	580.952	23.5	9.44	0.47	400	503.6	250.5	276.1	0.0	242	53.5	413.1	218.3	276.3	188.46	93.6	57.49	52.1	67.0	8.02	2.45	10.11
		3-3	2.09	3.18	519.048	23.5	8.45	0.42	400	502.0	275.1	268.8	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		4-1	1.97	3.06	519.048	23.5	8.85	0.44	400	506.6	253.7	279.1	0.0	223	103.4	455.1	209.7	265.7	179.58	88.6	64.90	58.1	59.2	7.64	2.76	8.00
		4-2	1.99	3.02	490.476	23.5	8.40	0.42	400	500.0	302.0	254.5	0.0	310	263.7	390.2	239.0	282.6	118.36	59.2	27.81	27.3	87.5	5.04	1.18	17.64
		4-3	2.01	3.05	495.238	23.5	8.40	0.42	400	#VALUE!	#VALUE!	#VALUE!	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		5-1	1.97	3.06	519.048	23.5	8.85	0.44	400	501.3	273.6	268.9	0.0	220	42.8	497.1	200.6	397.4	191.08	95.3	63.38	58.9	87.5	8.13	2.70	8.19
		5-2	1.95	3.06	528.571	23.5	9.05	0.45	400	508.0	255.4	278.7	0.0	193	31.9	529.6	181.3	353.8	197.05	97.0	76.53	68.6	68.2	8.39	3.26	6.91
		5-3	1.95	3.11	552.381	23.5	9.37	0.47	400	488.0	294.6	259.0	0.0	243	29.9	484.3	177.9	316.0	187.92	96.3	60.30	58.2	76.9	8.00	2.57	9.16
49	10-Dec	1-1	1.28	1.45	80.952	18.75	3.19	0.16	400	529.6	405.6	0.0	53.4	553.1	335.1	293.3	0.0	103.24	26.50	12.5	0.00	#DIV/0!	35.74	1.41	0.00	#DIV/0!
		1-2	0.85	0.97	57.143	18.75	3.38	0.17	400	516.5	397.7	0.0	73.4	645.3	265.3	246.5	0.0	141.51	35.38	17.1	0.00	#DIV/0!	61.95	1.89	0.00	#DIV/0!
		1-3	0.96	1.09	61.905	18.75	3.25	0.16	400	#VALUE!	#VALUE!	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		2-1	2.04	3.03	471.429	19	9.87	0.49	400	495.2	383.0	126.5	0.0	652	42.5	234.9	89.6	392.0	170.39	86.0	-7.81	-15.4	255.7	8.97	-0.41	-60.35
		2-2	2.05	2.96	433.333	19	9.18	0.46	400	484.3	295.8	264.2	0.0	1093	18.8	108.2	33.3	384.5	173.19	89.4	69.32	65.6	420.4	9.12	3.65	6.25
		2-3	2.02	2.96	447.619	19	9.54	0.48	400	490.6	286.4	268.1	0.0	211	83.9	542.9	183.6	353.8	178.55	91.0	68.48	63.9	74.7	9.40	3.60	6.54
		3-1	1.96	3.18	580.952	19.25	11.84	0.59	400	475.2	306.9	261.8	0.0	229	111.9	536.1	195.0	354.2	164.48	86.5	60.07	57.4	81.1	8.54	3.12	9.67
		3-2	1.91	3.2	614.286	19.25	12.59	0.63	400	460.9	351.0	240.4	0.0	1037	30.6	135.4	43.9	323.5	152.61	82.8	50.63	52.7	335.5	7.93	2.63	12.13
		3-3	1.97	3.18	576.190	19.25	11.72	0.59	400	#VALUE!	#VALUE!	#VALUE!	0.0	#VALUE!	73.3	317.5	98.5	326.6	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		4-1	2.07	3.03	457.143	19.5	9.27	0.46	400	466.9	337.1	248.2	0.0	275	136.1	490.7	172.5	295.9	149.37	80.0	51.88	52.3	81.3	7.66	2.66	8.81
		4-2	1.98	2.87	423.810	19.5	9.03	0.45	400	454.7	373.4	231.1	0.0	330	241.4	452.9	225.2	239.4	102.27	56.2	18.21	19.7	78.9	5.24	0.93	23.28
		4-3	1.99	2.91	438.095	19.5	9.24	0.46	400	#VALUE!	#VALUE!	#VALUE!	0.0	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
		5-1	2.02	2.96	447.619	20	9.06	0.45	400	464.8	323.5	256.1	0.0	970	24.2	133.4	25.5	380.9	162.40	87.3	77.75	75.9	369.5	8.12	3.89	5.76
		5-2	1.95	3.08	538.095	20	10.78	0.54	400	459.8	341.2	248.7	73.4	229	47.3	596.3	184.7	369.8	173.12	94.1	57.21	57.5	55.3	8.66	2.86	9.40
		5-3	2	3	476.190	20	9.60	0.48	400	#VALUE!	#VALUE!	#VALUE!	81.9	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
50	11-Dec	1-1	1.4	1.39	-4.762	20.67	-0.17	-0.01	400	558.4	429.9	0.0	0.0	415.0	513.3	414.4	0.0	88.11	10.35	4.6	0.00	#DIV/0!	36.57	0.50	0.00	#DIV/0!
		1-2	0.96	1.04	38.095	20.67	1.86	0.09	400	558.3	417.5	0.0	0.0	417.1	514.4	400.4	0.0	97.01	8.78	3.9	0.00	#DIV/0!	40.46	0.42	0.00	#DIV/0!
		1-3	1.08	0.89	-90.476	20.67	-4.48	-0.22	400	546.0	433.6	0.0	#VALUE!	406.3	514.0	426.9	#VALUE!	98.79	9.58	4.4	#VALUE!	#VALUE!	#VALUE!	0.46	#VALUE!	#VALUE!
		2-1	2.14	2.97	395.238	20.92	7.45	0.37	400	502.9	257.3	273.4	0.0	222	73.3	463.4	201.8	400.5	184.87	91.9	64.55	59.0	89.0	8.84	3.09	6.12
		2-2	1.97	2.93	457.143	20.92	8.99	0.45	400	504.6	268.2	269.5	0.0	221	72.7	485.1	195.3	401.8	185.76	92.0	64.60	59.9	88.9	8.88	3.09	7.08
		2-3	2.14	2.91	366.667	20.92	7.00	0.35	400	504.3	256.2	276.5	0.0	236	47.3	433.6	196.4	379.6	190.54	94.5	64.21	58.0	89.7	9.11	3.07	5.71
		3-1	1.98	3.11	538.095	21.2	10.05	0.50	400	503.7	271.6	271.3	0.0	207	98.6	526.0	200.7	325.3	181.13	89.9	67.07	61.8	67.2	8.54	3.16	8.02
		3-2	1.88	3.11	585.714	21.2	11.16	0.56	400	493.8	261.1	276.3	0.0	220	74.9	474.5	208.4	291.5	181.05	91.7	64.62	58.5	64.2	8.54	3.05	9.06
		3-3	1.9	3.11	576.190	21.2	10.94	0.55	400	489.4	288.7	255.0	0.0	224	91.7	516.3	200.3	314.6	175.26	89.5	57.21	56.1	70.4	8.27	2.70	10.07
		4-1	2.07	2.96	423.810	21.42	7.93	0.40	400	487.1	285.7	271.4	0.0	253	167.1	452.6	236.3	293.3	152.65	78.3	48.90	45.0	74.1	7.13	2.28	8.67

Cycle #	Day	sample	OD (in)	OD (out)	TSS (mg/L) inc	time(hr)	Growth rate (day-1)	Growth rate (day-1)	Gas volume (ml)	O2 Initial (mg/l)	N2 initial (mg/l)	CH4 initial (mg/l)	CO2 initial (mg/l)	Gas volume (ml)	O2 final (mg/l)	N2 final (mg/l)	CH4 final (mg/l)	CO2 final (mg/l)	O2 consumed (mg)	% O2 Consumed	CH4 consumed (mg	% CH4 Consumed	CO2 Produc d (mg)	O2 (mg/hr	CH4 (mg/hr	obs Yield
		4-2	1.62	2.48	409.524	21.42	9.40	0.47	400	487.5	301.3	261.5	0.0	314	331.3	384.0	242.2	111.7	91.00	46.7	28.62	27.4	35.1	4.25	1.34	14.31
		4-3	1.86	2.87	480.952	21.42	9.57	0.48	400	482.0	308.1	255.6	0.0	262	115.6	470.0	175.6	227.8	162.48	84.3	56.18	55.0	59.8	7.59	2.62	8.56
		5-1	1.89	2.97	514.286	21.42	9.96	0.50	400	467.3	350.8	237.1	0.0	263	37.3	533.7	197.0	319.1	177.09	94.7	43.04	45.4	83.9	8.27	2.01	11.95
		5-2	2.04	3.1	504.762	21.42	9.24	0.46	400	475.4	304.6	258.9	0.0	276	31.5	441.1	152.3	372.9	181.47	95.4	61.48	59.4	103.0	8.47	2.87	8.21
		5-3	1.97	3.04	509.524	21.42	9.57	0.48	400	477.5	298.9	263.9	0.0	208	57.9	574.8	190.9	373.8	178.98	93.7	65.87	62.4	77.7	8.36	3.08	7.74

APPENDIX B

Data Analysis for COD Consumption and Nitrogen Balance:

Cycle #	Day	sample	CO2 Produced (mg)	O2 (mg/hr)	CH4 (mg/hr)	obs Yield	COD (mg/L) IN	NH4- N (mg/L) IN (Cal)	COD: N	COD (mg/L) OUT	NH4- (mg/L) OUT (Cal)	NH4-N (mg/L) OUT	COD (mg/L) Consumed	% COD Consumed	NH4-N (mg/L) Consumed	NO3- (mg/L)	NO2- (mg/L)	NH4-N for Cell Synthesis 0.12 TSS	NH4-N for nitrification	Total Nitrogen OUT (mg/l)	Organic Nitrogen (mg/l)	N% for Cell Synthesis	Total Nitrogen IN (mg/l)	Nitrogen Converted to gas (mg/l)	NH4-N% converted to gas	NH4-N% converted to biomass
1	23-Oct	1-1	-5.03	0.66	0.00	#DIV/0!	411	53	7.7	75	14	11.14	336	82%	42.34	1.7	1.8	-4.3	46.6							
		1-2	4.13	0.52	0.00	#DIV/0!	411	53	7.7	65	39	30.25	346	84%	23.23	1.97	1.3	-1.7	24.9							
		1-3	64.53	2.64	0.00	#DIV/0!	411	53	7.7	58	53	41.71	353	86%	11.77	1.43	0.78	0.0	11.8							
		2-1	49.4	5.97	2.01	7.69	0	56	0.0	168	0	0.00	-168	#DIV/0!	55.72	0.18	0	44.6	11.1							
		2-2	25.0	7.68	3.35	3.14	0	56	0.0	168	0	0.00	-168	#DIV/0!	55.72	0	0	30.3	25.4							
		2-3	20.1	4.74	1.58	7.39	0	56	0.0	180	0	0.00	-180	#DIV/0!	55.72	0	0	33.7	22.0							
		3-1	81.3	8.36	2.70	6.61	404	53	7.6	193	0	0.00	211	52%	53.40	0	0	51.4	2.0							
		3-2	735.7	9.27	3.20	5.88	404	53	7.6	115	0	0.00	289	72%	53.40	0	0	54.3	-0.9							
		3-3	52.9	8.35	3.11	5.30	404	53	7.6	149	0	0.00	255	63%	53.40	0	0	47.4	6.0							
		4-1	58.0	8.04	2.94	6.88	225	30	7.5	141	0	0.00	84	37%	30.10	0	0	58.3	-28.2							
		4-2	54.8	7.96	2.82	7.33	225	30	7.5	154	0	0.00	71	32%	30.10	0	0	59.4	-29.3							
		4-3	63.9	8.16	3.04	6.66	225	30	7.5	159	0	0.00	66	29%	30.10	0.34	0	58.3	-28.2							
		5-1	86.1	8.31	2.90	6.36	626	76	8.2	92	0	0.00	534	85%	76.44	0.2	0	53.1	23.3							
		5-2	70.9	8.61	2.69	7.75	626	76	8.2	90	0	0.00	536	86%	76.44	0	0	60.0	16.4							
		5-3	101.1	7.17	2.09	8.82	626	76	8.2	158	0	0.00	468	75%	76.44	0	0	53.1	23.3							
2	24-Oct	1-1	10.55	1.24	0.00	#DIV/0!	411	53	7.7	56	62	48.05	355	86%	5.43	1.9	0	5.1	0.3							
		1-2	69.70	0.77	0.00	#DIV/0!	411	53	7.7	59	49	38.22	352	86%	15.26	1.6	0	4.6	10.7							
		1-3	53.90	0.90	0.00	#DIV/0!	411	53	7.7	123	52	40.73	288	70%	12.75	1.58	0	12.0	0.7							
		2-1	64.7	7.24	2.10	8.00	0	56	0.0	111	52	40.84	-111	#DIV/0!	14.88	0.2	0	38.3	-23.4							
		2-2	161.5	8.92	3.49	6.47	0	56	0.0	127	16	12.34	-127	#DIV/0!	43.38	0	0	51.4	-8.0							
		2-3	81.0	7.94	2.49	8.45	0	56	0.0	127	21	16.38	-127	#DIV/0!	39.34	0	0	48.0	-8.7							
		3-1	62.8	10.21	3.44	7.22	404	53	7.6	80	0	0.00	324	80%	53.40	0	0	56.6	-3.2							
		3-2	71.6	10.69	3.98	6.30	404	53	7.6	87	0	0.00	317	78%	53.40	0	0	57.1	-3.7							
		3-3	54.4	10.48	4.02	6.86	404	53	7.6	92	0	0.00	312	77%	53.40	0.26	0	62.9	-9.5							
		4-1	81.8	9.49	3.28	5.88	225	30	7.5	106	0	0.00	119	53%	30.10	0	0	44.0	-13.9							
		4-2	35.9	10.63	4.44	4.86	225	30	7.5	100	0	0.00	125	56%	30.10	0	0	49.1	-19.0							
		4-3	76.5	9.42	3.40	5.53	225	30	7.5	117	0	0.00	108	48%	30.10	0	0	42.9	-12.8							
		5-1	116.1	11.17	4.09	6.37	626	76	8.2	90	0	0.00	536	86%	76.44	0.1	0	59.4	17.0							
		5-2	89.4	10.55	3.57	9.12	626	76	8.2	101	0	0.00	525	84%	76.44	0	0	74.3	2.2							
		5-3	106.1	9.93	3.00	11.71	626	76	8.2	126	0	0.00	500	80%	76.44	0	0	80.0	-3.6							
3	25-Oct	1-1	12.99	0.28	0.00	#DIV/0!	411	53	7.7	0	62	48.38	411	100%	5.10	1.2	0	14.3	-9.2							
		1-2	15.08	0.54	0.00	#DIV/0!	411	53	7.7	0	46	36.04	411	100%	17.44	1.15	0	13.7	3.7							
		1-3	30.80	0.53	0.00	#DIV/0!	411	53	7.7	0	4	3.28	411	100%	50.20	1.29	0	17.1	33.1							
		2-1	48.6	8.12	3.85	4.15	0	56	0.0	49	62	48.59	-49	#DIV/0!	7.13	0	0	44.0	-36.9							
		2-2	60.5	7.95	2.78	6.26	0	56	0.0	39	6	4.94	-39	#DIV/0!	50.78	0	0	48.0	2.8							
		2-3	32.8	7.33	3.35	3.77	0	56	0.0	42	10	8.07	-42	#DIV/0!	47.65	0.17	0	34.9	12.8							
		3-1	82.6	7.39	2.37	8.31	404	53	7.6	76	13	10.12	328	81%	43.27	0.09	0	54.3	-11.0							
		3-2	88.3	7.30	2.28	8.26	404	53	7.6	67	0	0.00	337	83%	53.40	0.11	0	52.0	1.4							
		3-3	87.0	7.51	2.41	7.56	404	53	7.6	44	0	0.00	360	89%	53.40	0.11	0	50.3	3.1							
		4-1	62.3	7.13	2.19	7.62	225	30	7.5	45	0	0.00	180	80%	30.10	0.11	0	45.1	-15.0							
		4-2	79.4	7.16	2.21	7.75	225	30	7.5	57	0	0.00	168	75%	30.10	0	0	46.3	-16.2							
		4-3	63.8	7.67	2.35	8.00	225	30	7.5	30	0	0.00	195	87%	30.10	0	0	50.9	-20.8							
		5-1	73.1	8.37	3.10	6.15	626	76	8.2	15	0	0.00	611	98%	76.44	0.11	0	51.4	25.0							
		5-2	73.0	8.11	2.78	6.09	626	76	8.2	96	0	0.00	530	85%	76.44	0.19	0	45.7	30.7							
		5-3	43.3	9.43	3.74	4.64	626	76	8.2	55	0	0.00	571	91%	76.44	0.12	0	46.9	29.6							
4	26-Oct	1-1	15.21	0.89	0.00	#DIV/0!	411	53	7.7	22	63	49.47	389	95%	4.01	1.68	0	12.0	-8.0							
		1-2	47.13	0.87	0.00	#DIV/0!	411	53	7.7	52	67	52.20	359	87%	1.28	1.44	0	-36.6	37.9							
		1-3	45.88	1.21	0.00	#DIV/0!	411	53	7.7	30	65	50.34	381	93%	3.14	1.12	0	4.6	-1.4							
		2-1	177.6	8.56	3.24	5.29	0	56	0.0	69	16	12.56	-69	#DIV/0!	43.16	0	0	41.1	2.0							
		2-2	116.2	9.18	1.56	10.53	0	56	0.0	45	11	8.74	-45	#DIV/0!	46.98	0	0.12	39.4	7.6							
		2-3	83.5	9.03	3.38	4.37	0	56	0.0	50	13	10.05	-50	#DIV/0!	45.67	0	0	35.4	10.2							
		3-1	83.6	13.60	2.23	9.40	404	53	7.6	63	0	0.00	341	84%	53.40	0.14	0	50.3	3.1							
		3-2	62.1	5.08	1.54	13.91	404	53	7.6	60	0	0.00	344	85%	53.40	0.26	0	51.4	2.0							
		3-3	84.7	8.80	3.70	5.41	404	53	7.6	62	0	0.00	342	85%	53.40	0	0	48.0	5.4							
		4-1	119.0	8.24	3.11	6.06	225	30	7.5	65	0	0.00	160	71%	30.10	0.13	0	45.1	-15.0							
		4-2	98.8	8.79	2.71	6.42	225	30	7.5	60	0	0.00	165	73%	30.10	0.22	0	41.7	-11.6							
		4-3	60.9	8.15	4.03	4.67	225	30	7.5	83	0	0.00	142	63%	30.10	0.14	0	45.1	-15.0							
		5-1	80.6	9.36	3.29	6.52	626	76	8.2	76	0	0.00	550	88%	76.44	0.11	0	51.4	25.0							
		5-2	52.7	9.05	4.20	4.03	626	76	8.2	40	0	0.00	586	94%	76.44	0.11	0	40.6	35.9							

Cycle #	Day	sample	CO2 Produced (mg)	O2 (mg/hr)	CH4 (mg/hr)	obs Yield	COD (mg/L) IN	NH4-N (mg/L) IN (Cal)	COD:N	COD (mg/L) OUT	NH4- (mg/L) OUT (Cal)	NH4-N (mg/L) OUT	COD (mg/L) Consumed	% COD Consumed	NH4-N (mg/L) Consumed	NO3- (mg/L)	NO2- (mg/L)	NH4-N for Cell Synthesis 0.12 TSS	NH4-N for nitrification	Total Nitrogen OUT (mg/l)	Organic Nitrogen (mg/l)	N% for Cell Synthesis	Total Nitrogen IN (mg/l)	Nitrogen Converted to gas (mg/l)	NH4-N% converted to gas	NH4-N% converted to biomass
		5-3	95.7	8.58	2.96	7.23	626	76	8.2	55	0	0.00	571	91%	76.44	0.11	0	51.4	25.0							
5	27-Oct	1-1	17.14	0.90	0.00	#DIV/0!	411	53	7.7	47	77	60.17	364	89%	-6.69	1.47	0	-34.9	28.2							
		1-2	68.56	-1.18	0.00	#DIV/0!	411	53	7.7	25	53	41.28	386	94%	12.20	1.41	0	-12.0	24.2							
		1-3	48.10	1.64	0.00	#DIV/0!	411	53	7.7	40	49	38.00	371	90%	15.48	1.17	0	-34.9	50.3							
		2-1	79.6	8.36	2.84	1.68	0	56	0.0	233	19	14.85	-233	#DIV/0!	40.87	0.18	0	13.1	27.7							
		2-2	47.0	5.70	3.15	2.37	0	56	0.0	38	13	9.94	-38	#DIV/0!	45.78	0.08	0	20.6	25.2							
		2-3	69.2	8.20	2.72	2.96	0	56	0.0	201	14	10.81	-201	#DIV/0!	44.91	0	0	22.3	22.6							
		3-1	119.7	9.05	3.45	5.46	404	53	7.6	100	0	0.00	304	75%	53.40	0.11	0	52.0	1.4							
		3-2	205.7	8.82	3.40	6.16	404	53	7.6	55	0	0.00	349	86%	53.40	0.08	0	57.7	-4.3							
		3-3	81.2	8.48	2.94	9.65	404	53	7.6	30	0	0.00	374	93%	53.40	0.09	0	78.3	-24.9							
		4-1	79.1	6.64	2.04	9.24	225	30	7.5	94	0	0.00	131	58%	30.10	0	0	52.0	-21.9							
		4-2	63.9	5.88	1.78	11.07	225	30	7.5	71	0	0.00	154	68%	30.10	0	0	54.3	-24.2							
		4-3	57.5	6.61	2.03	8.88	225	30	7.5	76	0	0.00	149	66%	30.10	0.1	0	49.7	-19.6							
		5-1	95.7	8.78	3.33	7.83	626	76	8.2	59	0	0.00	567	91%	76.44	0.12	0	72.0	4.4							
		5-2	96.5	9.43	3.69	6.45	626	76	8.2	40	0	0.00	586	94%	76.44	0.11	0	65.7	10.7							
		5-3	78.7	5.76	1.87	14.40	626	76	8.2	55	0	0.00	571	91%	76.44	0.16	0	74.3	2.2							
6	28-Oct	1-1	5.95	0.68	0.00	#DIV/0!	372	57	6.6	35	98	76.55	337	91%	-19.95	1.2	0	-13.7	-6.2	-						
		1-2	7.35	0.74	0.00	#DIV/0!	372	57	6.6	28	69	54.16	344	92%	2.44	1.14	0	-2.9	5.3	-						
		1-3	33.72	0.71	0.00	#DIV/0!	372	57	6.6	33	87	67.49	339	91%	-10.89	1.17	0	-5.7	-5.2	-						
		2-1	81.7	7.21	2.32	3.59	60	54	1.1	218	77	10.00	-158	-263%	44.00	0.73	0	20.0	24.0	155	144.3	12%	178.9	24	44.3	35.8
		2-2	78.4	7.73	2.56	4.74	60	54	1.1	218	61	5.00	-158	-263%	49.00	0.7	0	29.1	19.9	150	144.3	11%	171.1	21	39.2	50.3
		2-3	75.9	8.44	2.70	3.44	60	54	1.1	215	68	15.00	-155	-258%	39.00	0.17	0	22.3	16.7	156	140.8	11%	174.8	19	34.8	37.1
		3-1	77.8	10.06	3.67	6.55	372	62	6.0	94	0	0.00	278	75%	62.00	0.17	0	57.7	4.3	-					0.0	
		3-2	81.8	9.94	3.59	6.90	372	62	6.0	76	0	0.00	296	80%	62.00	0.155	0	59.4	2.6	130	129.8	8%	151.5	22	34.7	65.0
		3-3	91.7	9.25	2.99	7.79	372	62	6.0	84	0	0.00	288	77%	62.00	0.09	0	56.0	6.0	130	129.9	8%	153.9	24	38.6	61.3
		4-1	106.0	9.12	3.31	5.98	176	33	5.3	126	0	0.00	50	28%	33.00	0.18	0	47.4	-14.4	90	89.8	6%	99.2	9	27.7	71.7
		4-2	84.8	9.18	3.17	5.87	176	33	5.3	190	0	0.00	-14	-8%	33.00	0	0	44.6	-11.6	85	85.0	6%	96.8	12	35.8	64.2
		4-3	60.3	8.88	3.88	4.91	176	33	5.3	77	0	0.00	99	56%	33.00	0.22	0	45.7	-12.7	88	87.8	6%	97.1	9	27.4	71.9
		5-1	82.0	9.42	3.14	5.85	562	85	6.6	47	24	18.78	515	92%	66.22	0.142	0	44.0	22.2	164	145.1	10%	194.7	31	36.1	41.6
		5-2	84.1	10.11	3.50	6.53	562	85	6.6	75	0	0.00	487	87%	85.00	0.39	0	54.9	30.1	184	183.6	11%	216.3	32	38.0	61.5
		5-3	117.0	9.26	3.08	7.81	562	85	6.6	69	0	0.00	493	88%	85.00	0.15	0	57.7	27.3	176	175.9	11%	206.5	31	35.9	63.9
7	29-Oct	1-1	27.82	0.57	0.00	#DIV/0!	372	57	6.6	16	93	72.19	356	96%	-15.59	0.932	0	-12.0	-3.6							
		1-2	38.56	0.66	0.00	#DIV/0!	372	57	6.6	16	91	70.65	356	96%	-14.05	0.932	0	-13.1	-0.9							
		1-3	68.64	0.54	0.00	#DIV/0!	372	57	6.6	19	78	60.82	353	95%	-4.22	0.934	0	-14.9	10.6							
		2-1	204.9	8.53	3.27	4.12	60	57	1.1	465	74	12.00	-405	-675%	44.60	0.792	0	33.1	11.5							
		2-2	73.4	5.40	1.78	5.22	60	57	1.1	518	84	5.00	-458	-763%	51.60	0.904	0	22.9	28.7							
		2-3	72.2	5.30	1.79	3.51	60	57	1.1	582	86	3.00	-522	-870%	53.60	0.72	0	15.4	38.2							
		3-1	62.1	2.99	0.68	14.72	372	57	6.6	98	73	57.00	274	74%	-0.40	0.48	0	24.6	-25.0							
		3-2	79.8	9.91	3.60	6.78	372	57	6.6	80	0	0.00	292	78%	56.60	0.1	0	60.0	-3.4							
		3-3	153.6	9.22	3.18	6.06	372	57	6.6	123	0	0.00	249	67%	56.60	0.1	0	47.4	9.2							
		4-1	88.7	9.48	3.81	4.20	176	33	5.3	371	0	0.00	-195	-111%	33.00	0	0	39.4	-6.4							
		4-2	77.5	6.84	2.35	5.53	176	33	5.3	529	0	0.00	-353	-201%	33.00	0.12	0	32.0	1.0							
		4-3	86.9	8.32	2.50	6.41	176	33	5.3	523	0	0.00	-347	-197%	33.00	0.11	0	39.4	-6.4							
		5-1	71.4	9.88	3.87	5.11	562	85	6.6	34	42	32.98	528	94%	52.02	0.105	0	48.6	3.5							
		5-2	113.5	10.24	4.23	5.55	562	85	6.6	46	14	10.75	516	92%	74.25	0.094	0	57.7	16.5							
		5-3	130.0	10.86	3.81	6.22	562	85	6.6	56	18	14.31	506	90%	70.69	0.113	0	58.3	12.4							
8	30-Oct	1-1	#VALUE!	0.74	0.00	#DIV/0!	372	57	6.6	14	95	74.27	358	96%	-17.67	0.68	0	-3.4	-14.2	-						
		1-2	#VALUE!	7.78	0.00	#DIV/0!	372	57	6.6	11	88	68.25	361	97%	-11.65	0.64	0	-1.1	-10.5	-						
		1-3	#VALUE!	0.11	0.00	#DIV/0!	372	57	6.6	13	97	75.39	359	97%	-18.79	0.72	0	4.0	-22.8	-						
		2-1	#VALUE!	9.08	2.85	6.87	60	57	1.1	84	48	10.00	-24	-40%	46.60	0.405	0	42.3	4.3	139	128.6	10%	151.2	12	21.6	60.0
		2-2	#VALUE!	10.23	3.47	5.41	60	57	1.1	342	55	5.00	-282	-470%	51.60	0.71	0	40.6	11.0	140	134.3	10%	155.6	16	27.5	62.4
		2-3	#VALUE!	6.76	2.09	6.47	60	57	1.1	544	82	3.00	-484	-807%	53.60	0.893	0	29.1	24.5	144	140.1	12%	167.5	24	41.6	51.5
		3-1	#VALUE!	9.26	3.55	9.31	372	57	6.6	35	0	0.00	337	91%	56.60	0.152	0	71.4	-14.8	-						
		3-2	#VALUE!	8.53	2.49	7.98	372	57	6.6	197	0	0.00	175	47%	56.60	0	0	42.9	13.7	132	132.0	9%	156.1	24	42.7	57.3
		3-3	#VALUE!	9.38	3.02	6.56	372	57	6.6	77	0	0.00	295	79%	56.60	0	0	42.9	13.7	144	144.0	10%	166.5	23	39.8	60.2
		4-1	#VALUE!	9.09	3.95	3.82	176	33	5.3	172	0	0.00	4	2%	33.00	0.092	0	32.6	0.4	95	94.9	7%	109.4	14	43.6	56.1
		4-2	#VALUE!	#VALUE!	#VALUE!	#VALUE!	176	33	5.3	76	0	0.00	100	57%	33.00	0.083	0	44.6	-11.6	76	75.9	6%	87.5	11	34.7	65.0
		4-3	#VALUE!	5.75	1.95	4.76	176	33	5.3	488	0	0.00	-312	-177%	33.00	2.5	0	20.0	13.0	95	92.5	8%	112.7	18	53.5	38.9
		5-1	#VALUE!	9.94	3.69	6.96	562	85	6.6	27	20	15.46	535	95%	69.54	0.115	0	55.4	14.1	177	161.4	11%	197.5	20	24.1	57.6

Cycle #	Day	sample	CO2 Produced (mg)	O2 (mg/hr)	CH4 (mg/hr)	obs Yield	COD (mg/L) IN	NH4-N (mg/L) IN (Cal)	COD:N	COD (mg/L) OUT	NH4-N (mg/L) OUT (Cal)	NH4-N (mg/L) OUT	COD (mg/L) Consumed	% COD Consumed	NH4-N (mg/L) Consumed	NO3- (mg/L)	NO2- (mg/L)	NH4-N for Cell Synthesis 0.12 TSS	NH4-N for nitrification	Total Nitrogen OUT (mg/l)	Organic Nitrogen(mg/l)	N% for Cell Synthesis	Total Nitrogen IN (mg/l)	Nitrogen Converted to gas (mg/l)	NH4-N% converted to gas	NH4-N% converted to biomass
		5-2	86.8	9.79	3.59	6.71	562	85	6.6	28	12	9.31	534	95%	75.69	0	0	52.0	23.7	170	160.7	10%	201.2	31	36.8	52.3
		5-3	96.0	9.24	3.13	7.52	562	85	6.6	35	22	17.17	527	94%	67.83	0.103	0	50.9	17.0	175	157.7	10%	200.1	25	29.5	50.2
10	01-Nov	1-1	41.55	0.54	0.00	#DIV/0!	372	57	6.6	22	#VALUE!	#VALUE!	350	94%	#VALUE!	-	0	2.9	#VALUE!							
		1-2	25.80	1.85	0.00	#DIV/0!	372	57	6.6	16	#VALUE!	#VALUE!	356	96%	#VALUE!	-	0	-1.1	#VALUE!							
		1-3	18.62	1.80	0.00	#DIV/0!	372	57	6.6	18	#VALUE!	#VALUE!	354	95%	#VALUE!	-	0	-1.1	#VALUE!							
		2-1	303.0	7.39	2.65	5.93	60	57	1.1	81	#VALUE!	#VALUE!	-21	-35%	#VALUE!	-	0	43.4	#VALUE!							
		2-2	180.9	6.95	2.30	5.31	60	57	1.1	103	#VALUE!	#VALUE!	-43	-72%	#VALUE!	-	0	33.7	#VALUE!							
		2-3	257.7	6.88	2.22	6.42	60	57	1.1	66	#VALUE!	#VALUE!	-6	-10%	#VALUE!	-	0	39.4	#VALUE!							
		3-1	190.0	7.86	2.66	8.09	372	57	6.6	70	#VALUE!	#VALUE!	302	81%	#VALUE!	-	0	59.4	#VALUE!							
		3-2	62.0	4.69	2.09	8.21	372	57	6.6	58	#VALUE!	#VALUE!	314	84%	#VALUE!	-	0	47.4	#VALUE!							
		3-3	85.3	6.02	1.85	5.37	372	57	6.6	428	#VALUE!	#VALUE!	-56	-15%	#VALUE!	-	0	27.4	#VALUE!							
		4-1	318.7	5.50	1.40	5.02	176	33	5.3	564	#VALUE!	#VALUE!	-388	-220%	#VALUE!	-	0	19.4	#VALUE!							
		4-2	103.3	6.19	2.23	2.04	176	33	5.3	517	#VALUE!	#VALUE!	-341	-194%	#VALUE!	-	0	12.6	#VALUE!							
		4-3	222.1	8.02	2.82	6.31	176	33	5.3	103	#VALUE!	#VALUE!	73	41%	#VALUE!	-	0	49.1	#VALUE!							
		5-1	214.2	8.41	3.55	5.66	562	85	6.6	39	#VALUE!	#VALUE!	523	93%	#VALUE!	-	0	55.4	#VALUE!							
		5-2	214.8	8.37	3.07	6.27	562	85	6.6	42	#VALUE!	#VALUE!	520	93%	#VALUE!	-	0	53.1	#VALUE!							
		5-3	92.6	7.95	2.46	7.49	562	85	6.6	38	#VALUE!	#VALUE!	524	93%	#VALUE!	-	0	50.9	#VALUE!							
11	02-Nov	1-1	21.74	3.00	0.00	#DIV/0!	372	57	6.6	21	#VALUE!	#VALUE!	351	94%	#VALUE!	-	0	-1.7	#VALUE!							
		1-2	40.65	0.74	0.00	#DIV/0!	372	57	6.6	20	#VALUE!	#VALUE!	352	95%	#VALUE!	-	0	0.6	#VALUE!							
		1-3	0.00	0.79	0.00	#DIV/0!	372	57	6.6	20	#VALUE!	#VALUE!	352	95%	#VALUE!	-	0	4.0	#VALUE!							
		2-1	92.8	8.63	3.54	6.38	60	57	1.1	62	#VALUE!	#VALUE!	-2	-3%	#VALUE!	-	0	51.4	#VALUE!							
		2-2	76.6	8.26	1.88	9.06	60	57	1.1	102	#VALUE!	#VALUE!	-42	-70%	#VALUE!	-	0	38.9	#VALUE!							
		2-3	262.4	7.21	3.91	3.34	60	57	1.1	50	#VALUE!	#VALUE!	10	17%	#VALUE!	-	0	29.7	#VALUE!							
		3-1	63.6	8.37	2.71	8.04	372	57	6.6	188	#VALUE!	#VALUE!	184	49%	#VALUE!	-	0	49.7	#VALUE!							
		3-2	79.6	8.34	2.70	9.18	372	57	6.6	76	#VALUE!	#VALUE!	296	80%	#VALUE!	-	0	56.6	#VALUE!							
		3-3	67.3	8.62	3.09	8.27	372	57	6.6	74	#VALUE!	#VALUE!	298	80%	#VALUE!	-	0	58.3	#VALUE!							
		4-1	100.2	8.49	3.20	6.67	176	33	5.3	102	#VALUE!	#VALUE!	74	42%	#VALUE!	-	0	48.6	#VALUE!							
		4-2	59.3	7.65	2.61	8.07	176	33	5.3	118	#VALUE!	#VALUE!	58	33%	#VALUE!	-	0	48.0	#VALUE!							
		4-3	57.2	7.64	2.53	5.46	176	33	5.3	503	#VALUE!	#VALUE!	-327	-186%	#VALUE!	-	0	31.4	#VALUE!							
		5-1	81.6	8.23	1.78	13.41	562	85	6.6	29	#VALUE!	#VALUE!	533	95%	#VALUE!	-	0	54.3	#VALUE!							
		5-2	65.0	8.73	2.66	9.79	562	85	6.6	31	#VALUE!	#VALUE!	531	94%	#VALUE!	-	0	59.4	#VALUE!							
		5-3	65.2	8.48	2.67	9.76	562	85	6.6	43	#VALUE!	#VALUE!	519	92%	#VALUE!	-	0	59.4	#VALUE!							

Cycle #	Day	sample	CO2 Produced (mg)	O2 (mg/hr)	CH4 (mg/hr)	obs Yield	COD (mg/L) IN	NH4-N (mg/L) IN (Cal)	COD:N	COD (mg/L) OUT	NH4-N (mg/L) OUT (Cal)	NH4-N (mg/L) OUT	COD (mg/L) Consumed	% COD Consumed	NH4-N (mg/L) Consumed	NO3- (mg/L)	NO2- (mg/L)	NH4-N for Cell Synthesis 0.12 TSS	NH4-N for nitrification	Total Nitrogen OUT (mg/l)	Organic Nitrogen(mg/l)	N% for Cell Synthesis	Total Nitrogen IN (mg/l)	Nitrogen Converted to gas (mg/l)	NH4-N% converted to gas	NH4-N% converted to biomass
37	28-Nov	1-1	30.05	1.75	0.00	#DIV/0!	354	65	5.4	13	#VALUE!	#VALUE!	341	96%	#VALUE!	-	0	16.0	#VALUE!							
		1-2	83.62	1.22	0.00	#DIV/0!	354	65	5.4	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	8.0	#VALUE!							
		1-3	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	354	65	5.4	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	5.1	#VALUE!							
		2-1	214.5	6.96	2.87	7.52	10	56	0.2	25	#VALUE!	#VALUE!	-15	-150%	#VALUE!	-	0	58.3	#VALUE!							
		2-2	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	10	56	0.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	57.1	#VALUE!							
		2-3	112.0	7.21	2.08	10.46	10	56	0.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	58.9	#VALUE!							
		3-1	219.7	7.74	3.32	8.03	341	55	6.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	72.0	#VALUE!							
		3-2	71.4	7.48	2.53	11.81	341	55	6.2	35	#VALUE!	#VALUE!	306	90%	#VALUE!	-	0	80.6	#VALUE!							
		3-3	209.1	7.04	2.23	10.99	341	55	6.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	66.3	#VALUE!							
		4-1	55.1	7.52	3.99	5.09	174	22	7.8	44	#VALUE!	#VALUE!	130	75%	#VALUE!	-	0	54.9	#VALUE!							
		4-2	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	174	22	7.8	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	57.1	#VALUE!							
		4-3	289.2	6.07	3.35	5.49	174	22	7.8	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	49.7	#VALUE!							
		5-1	80.9	7.32	2.81	10.18	517	90	5.7	30	#VALUE!	#VALUE!	487	94%	#VALUE!	-	0	77.1	#VALUE!							
		5-2	202.8	7.29	2.89	9.68	517	90	5.7	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	75.4	#VALUE!							
		5-3	58.2	7.47	3.22	6.83	517	90	5.7	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	59.4	#VALUE!							
38	29-Nov	1-1	348.20	5.81	0.00	#DIV/0!	354	65	5.4	-			#VALUE!	#VALUE!	65.09	-	0	10.3	54.8							
		1-2	24.75	-0.27	0.00	#DIV/0!	354	65	5.4	11			343	97%	65.09	-	0	12.0	53.1							
		1-3	24.33	0.51	0.00	#DIV/0!	354	65	5.4	13		49.50	341	96%	15.59	-	0	4.6	11.0							
		2-1	130.7	8.02	1.86	10.14	10	56	0.2	43		0.02	-33	-330%	56.44	-	0	48.6	7.9							
		2-2	269.9	7.81	2.50	9.30	10	56	0.2	-			#VALUE!	#VALUE!	56.38	-	0	60.0	-3.6							
		2-3	119.5	5.08	1.62	10.65	10	56	0.2	39		0.08	-29	-290%	#REF!	-	0	44.6	#REF!							
		3-1	#VALUE!	#VALUE!	#VALUE!	#VALUE!	341	55	6.2	-			#VALUE!	#VALUE!	54.60	-	0	58.3	-3.7							
		3-2	452.5	8.04	2.26	9.69	341	55	6.2	28		0.18	313	92%	54.42	-	0	56.6	-2.2							
		3-3	74.6	7.00	2.13	11.43	341	55	6.2	32		0.20	309	91%	54.40	-	0	62.9	-8.5							
		4-1	114.6	5.96	1.82	13.33	174	22	7.8	63		0.75	111	64%	21.45	-	0	64.0	-42.6							
		4-2	61.3	5.06	1.47	18.24	174	22	7.8	54		0.02	120	69%	22.18	-	0	70.9	-48.7							
		4-3	54.6	6.53	3.15	7.98	174	22	7.8	-			#VALUE!	#VALUE!	22.20	-	0	66.3	-44.1							

Cycle #	Day	sample	CO2 Produced (mg)	O2 (mg/hr)	CH4 (mg/hr)	obs Yield	COD (mg/L) IN	NH4-N (mg/L) IN (Cal)	COD:N	COD (mg/L) OUT	NH4-N (mg/L) OUT (Cal)	NH4-N (mg/L) OUT	COD (mg/L) Consumed	% COD Consumed	NH4-N (mg/L) Consumed	NO3- (mg/L)	NO2- (mg/L)	NH4-N for Cell Synthesis 0.12 TSS	NH4-N for nitrification	Total Nitrogen OUT (mg/l)	Organic Nitrogen(mg/l)	N% for Cell Synthesis	Total Nitrogen IN (mg/l)	Nitrogen Converted to gas (mg/l)	NH4-N% converted to gas	NH4-N% converted to biomass
		5-1	#VALUE!	#VALUE!	#VALUE!	#VALUE!	517	90	5.7	-			#VALUE!	#VALUE!	90.30	-	0	52.0	38.3							
		5-2	175.0	6.78	1.95	13.73	517	90	5.7	45		1.33	472	91%	88.97	-	0	70.9	18.1							
		5-3	156.5	7.54	2.56	8.79	517	90	5.7	46		2.96	471	91%	87.34	-	0	59.4	27.9							
39	30-Nov	1-1	-11.07	-0.69	0.00	#DIV/0!	354	65	5.4	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	14.3	#VALUE!							
		1-2	-2.74	-2.04	0.00	#DIV/0!	354	65	5.4	7	#VALUE!	#VALUE!	347	98%	#VALUE!	-	0	18.3	#VALUE!							
		1-3	31.14	-0.09	0.00	#DIV/0!	354	65	5.4	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	24.6	#VALUE!							
		2-1	78.7	6.81	2.07	10.95	10	56	0.2	21	#VALUE!	#VALUE!	-11	-110%	#VALUE!	-	0	54.3	#VALUE!							
		2-2	149.4	7.24	2.26	9.47	10	56	0.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	51.4	#VALUE!							
		2-3	90.0	7.95	2.57	8.44	10	56	0.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	52.0	#VALUE!							
		3-1	657.8	#VALUE!	#VALUE!	#VALUE!	341	55	6.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	50.3	#VALUE!							
		3-2	71.9	6.26	1.76	16.41	341	55	6.2	39	#VALUE!	#VALUE!	302	89%	#VALUE!	-	0	69.1	#VALUE!							
		3-3	129.1	7.76	3.13	6.31	341	55	6.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	47.4	#VALUE!							
		4-1	53.5	7.26	3.37	7.42	174	22	7.8	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	60.0	#VALUE!							
		4-2	66.5	7.28	2.80	8.17	174	22	7.8	36	#VALUE!	#VALUE!	138	79%	#VALUE!	-	0	54.9	#VALUE!							
		4-3	58.9	6.26	4.23	5.07	174	22	7.8	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	51.4	#VALUE!							
		5-1	53.5	7.34	2.54	9.95	517	90	5.7	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	60.6	#VALUE!							
		5-2	76.7	8.00	2.57	9.99	517	90	5.7	42	#VALUE!	#VALUE!	475	92%	#VALUE!	-	0	61.7	#VALUE!							
		5-3	72.7	8.30	2.84	9.38	517	90	5.7	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	64.0	#VALUE!							
41	02-Dec	1-1	4.16	-0.07	0.00	#DIV/0!	354	65	5.4	-	#VALUE!	34.03	#VALUE!	#VALUE!	31.06	-	0	9.1	21.9							
		1-2	11.38	-0.89	0.00	#DIV/0!	354	65	5.4	-	#VALUE!		#VALUE!	#VALUE!	19.73	-	0	5.7	14.0							
		1-3	17.88	-0.12	0.00	#DIV/0!	354	65	5.4	6	#VALUE!	45.35	348	98%	#REF!	-	0	2.3	#REF!							
		2-1	260.3	8.34	1.84	11.44	10	56	0.2	14	#VALUE!	0.10	-4	-40%	56.36	-	0	58.9	-2.5							
		2-2	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	10	56	0.2	-	#VALUE!		#VALUE!	#VALUE!	56.33	-	0	46.3	10.0							
		2-3	20.7	2.83	0.98	18.03	10	56	0.2	-	#VALUE!	0.13	#VALUE!	#VALUE!	#REF!	-	0	49.1	#REF!							
		3-1	1679.5	8.53	3.54	7.03	341	55	6.2	-	#VALUE!		#VALUE!	#VALUE!	54.41	-	0	70.3	-15.9							
		3-2	73.9	8.29	2.57	9.52	341	55	6.2	51	#VALUE!	0.19	290	85%	54.42	-	0	69.1	-14.7							
		3-3	101.4	6.56	1.42	13.52	341	55	6.2	-	#VALUE!	0.18	#VALUE!	#VALUE!	#REF!	-	0	54.3	#REF!							
		4-1	27.5	6.88	3.11	6.45	174	22	7.8	-	#VALUE!		#VALUE!	#VALUE!	22.20	-	0	57.1	-34.9							
		4-2	116.1	7.60	2.40	8.53	174	22	7.8	43	#VALUE!	0.28	131	75%	21.92	-	0	58.3	-36.4							
		4-3	70.4	5.50	1.48	13.54	174	22	7.8	-	#VALUE!	0.21	#VALUE!	#VALUE!	21.99	-	0	57.1	-35.2							
		5-1	123.3	7.72	1.86	12.80	517	90	5.7	52	#VALUE!	0.39	465	90%	89.91	-	0	68.6	21.3							
		5-2	111.9	7.92	2.54	9.75	517	90	5.7	-	#VALUE!		#VALUE!	#VALUE!	89.94	-	0	71.4	18.5							
		5-3	106.0	7.54	2.06	12.15	517	90	5.7	-	#VALUE!	0.36	#VALUE!	#VALUE!	#REF!	-	0	72.0	#REF!							
	03-Dec	1-1	15.63	0.62	0.00	#DIV/0!	354	65	5.4	6	#VALUE!	#VALUE!	348	98%	#VALUE!	-	0	6.3	#VALUE!							

Cycle #	Day	sample	CO_2 Produced (mg)	O_2 (mg/hr)	CH_4 (mg/hr)	obs Yield	COD (mg/L) IN	NH_4-N (mg/L) IN (Cal)	COD:N	COD (mg/L) OUT	NH_4-N (mg/L) OUT (Cal)	NH_4-N (mg/L) OUT	COD (mg/L) Consumed	% COD Consumed	NH_4-N (mg/L) Consumed	NO_3^- (mg/L)	NO_2^- (mg/L)	NH_4-N for Cell Synthesis 0.12 TSS	NH_4-N for nitrification	Total Nitrogen OUT (mg/l)	Organic Nitrogen(mg/l)	N% for Cell Synthesis	Total Nitrogen IN (mg/l)	Nitrogen Converted to gas (mg/l)	NH_4-N% converted to gas	NH_4-N% converted to biomass
42		1-2	19.32	0.60	0.00	#DIV/0!	354	65	5.4	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	2.9	#VALUE!							
		1-3	18.61	0.57	0.00	#DIV/0!	354	65	5.4	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	6.3	#VALUE!							
		2-1	79.6	9.05	3.12	6.17	10	56	0.2	13	#VALUE!	#VALUE!	-3	-30%	#VALUE!	-	0	50.9	#VALUE!							
		2-2	280.1	9.45	3.43	6.63	10	56	0.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	60.0	#VALUE!							
		2-3	79.9	8.94	3.14	7.64	10	56	0.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	63.4	#VALUE!							
		3-1	221.5	7.35	2.67	7.37	341	55	6.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	52.6	#VALUE!							
		3-2	#VALUE!	#VALUE!	#VALUE!	#VALUE!	341	55	6.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	61.1	#VALUE!							
		3-3	95.0	8.94	3.19	7.77	341	55	6.2	41	#VALUE!	#VALUE!	300	88%	#VALUE!	-	0	66.3	#VALUE!							
		4-1	90.2	7.02	2.53	7.85	174	22	7.8	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	53.7	#VALUE!							
		4-2	54.2	6.04	1.86	11.51	174	22	7.8	33	#VALUE!	#VALUE!	141	81%	#VALUE!	-	0	57.7	#VALUE!							
		4-3	53.8	7.96	3.36	6.50	174	22	7.8	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	58.9	#VALUE!							
		5-1	60.2	7.44	3.39	6.04	517	90	5.7	46	#VALUE!	#VALUE!	471	91%	#VALUE!	-	0	58.9	#VALUE!							
		5-2	868.9	8.62	3.12	7.05	517	90	5.7	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	63.4	#VALUE!							
		5-3	497.8	8.13	2.79	6.83	517	90	5.7	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	54.9	#VALUE!							
44	05-Dec	1-1	13.90	0.71	0.00	#DIV/0!	363	58	6.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	4.0	#VALUE!							
		1-2	48.68	0.28	0.00	#DIV/0!	363	58	6.2	5	#VALUE!	#VALUE!	358	99%	#VALUE!	-	0	2.9	#VALUE!							
	New feed	1-3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	363	58	6.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	1.7	#VALUE!							
		2-1	125.2	7.19	-0.20	-103.82	3	50	0.1	17	#VALUE!	#VALUE!	-14	-467%	#VALUE!	-	0	54.9	#VALUE!							
		2-2	229.9	6.47	2.09	10.38	3	50	0.1	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	56.0	#VALUE!							
		2-3	729.0	7.34	2.58	7.31	3	50	0.1	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	48.6	#VALUE!							
		3-1	100.2	6.33	2.48	9.44	373	60	6.3	62	#VALUE!	#VALUE!	311	83%	#VALUE!	-	0	61.1	#VALUE!							
		3-2	145.9	6.06	1.89	12.30	373	60	6.3	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	60.6	#VALUE!							
		3-3	166.5	5.84	1.95	12.36	373	60	6.3	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	62.9	#VALUE!							
		4-1	1779.6	6.84	3.60	5.35	203	22	9.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	50.9	#VALUE!							
		4-2	1587.8	7.03	-1.03	-17.81	203	22	9.2	38	#VALUE!	#VALUE!	165	81%	#VALUE!	-	0	48.6	#VALUE!							

Cycle #	Day	sample	CO2 Produced (mg)	O2 (mg/hr)	CH4 (mg/hr)	obs Yield	COD (mg/L) IN	NH4-N (mg/L) IN (Cal)	COD:N	COD (mg/L) OUT	NH4-N (mg/L) OUT (Cal)	NH4-N (mg/L) OUT	COD (mg/L) Consumed	% COD Consumed	NH4-N (mg/L) Consumed	NO3- (mg/L)	NO2- (mg/L)	NH4-N for Cell Synthesis 0.12 TSS	NH4-N for nitrification	Total Nitrogen OUT (mg/l)	Organic Nitrogen(mg/l)	N% for Cell Synthesis	Total Nitrogen IN (mg/l)	Nitrogen Converted to gas (mg/l)	NH4-N% converted to gas	NH4-N% converted to biomass
		4-3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	203	22	9.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	52.0	#VALUE!							
		5-1	80.1	6.49	2.11	9.11	561	94	6.0	67	#VALUE!	#VALUE!	494	88%	#VALUE!	-	0	51.4	#VALUE!							
		5-2	#VALUE!	#VALUE!	#VALUE!	#VALUE!	561	94	6.0	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	48.0	#VALUE!							
		5-3	113.8	6.08	1.92	9.82	561	94	6.0	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	50.3	#VALUE!							
45	06-Dec	1-1	35.95	-0.59	0.00	#DIV/0!	363	58	6.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	2.9	#VALUE!							
		1-2	#VALUE!	0.41	0.00	#DIV/0!	363	58	6.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	1.7	#VALUE!							
		1-3	28.12	-0.57	0.00	#DIV/0!	363	58	6.2	4	#VALUE!	#VALUE!	359	99%	#VALUE!	-	0	3.4	#VALUE!							
		2-1	#VALUE!	1.79	1.76	9.45	3	50	0.1	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	46.9	#VALUE!							
		2-2	66.8	6.42	2.13	7.53	3	50	0.1	16	#VALUE!	#VALUE!	-13	-433%	#VALUE!	-	0	45.1	#VALUE!							
		2-3	72.3	5.58	2.41	6.05	3	50	0.1	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	41.1	#VALUE!							
		3-1	190.0	6.52	1.87	12.49	373	60	6.3	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	67.4	#VALUE!							
		3-2	88.1	7.01	2.13	11.26	373	60	6.3	57	#VALUE!	#VALUE!	316	85%	#VALUE!	-	0	69.1	#VALUE!							
		3-3	79.7	6.61	1.68	13.85	373	60	6.3	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	66.9	#VALUE!							
		4-1	74.9	6.13	2.44	7.54	203	22	9.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	52.0	#VALUE!							
		4-2	170.4	6.39	2.47	8.12	203	22	9.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	56.6	#VALUE!							
		4-3	68.8	6.51	1.58	13.04	203	22	9.2	37	#VALUE!	#VALUE!	166	82%	#VALUE!	-	0	58.3	#VALUE!							
		5-1	78.6	10.05	2.35	9.36	561	94	6.0	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	63.4	#VALUE!							
		5-2	63.3	7.36	2.24	10.79	561	94	6.0	56	#VALUE!	#VALUE!	505	90%	#VALUE!	-	0	69.7	#VALUE!							
		5-3	83.1	7.22	2.12	10.49	561	94	6.0	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	64.0	#VALUE!							
46	07-Dec	1-1	20.52	-0.99	0.00	#DIV/0!	363	58	6.2	8	49	38.60	355	98%	19.70	-	0	9.7	10.0							
		1-2	24.74	0.49	0.00	#DIV/0!	363	58	6.2	-	45	35.43	#VALUE!	#VALUE!	22.87	-	0	2.9	20.0							
		1-3	20.67	-0.59	0.00	#DIV/0!	363	58	6.2	-			#VALUE!	#VALUE!	58.30	-	0	13.1	45.2							
		2-1	232.9	8.45	0.98	21.37	3	62	0.0	-	0	0.05	#VALUE!	#VALUE!	61.95	0	0	48.6	13.4	145	144.9	11%	163.57	18.57	29.9	70.0
		2-2	100.4	8.28	2.67	8.23	3	62	0.0	16	0	0.03	-13	-433%	61.97	0	0	50.9	11.1	140	140.0	10%	159.16	19.16	30.9	69.0
		2-3	64.0	8.42	4.00	5.94	3	62	0.0	-			#VALUE!	#VALUE!	62.00	-	0	54.9	7.1	144						
		3-1	104.2	7.60	2.56	10.96	373	65	5.7	53	1	0.57	320	86%	64.43	0	0	65.7	-1.3	125	124.4	8%	144.43	19.43	29.9	69.2
		3-2	117.3	7.74	2.87	9.29	373	65	5.7	-	1	0.62	#VALUE!	#VALUE!	64.38	0	0	62.3	2.1	128	127.4	9%	147.73	19.73	30.4	68.7
		3-3	113.0	7.30	2.10	12.89	373	65	5.7	-			#VALUE!	#VALUE!	65.00	-	0	63.4	1.6							
		4-1	144.3	6.10	2.14	8.10	203	32	6.3	42	0	0.22	161	79%	31.78	0	0	41.1	-9.4	80	79.8	6%	91.04	11.04	34.5	64.8
		4-2	50.2	3.81	0.88	16.25	203	32	6.3	-			#VALUE!	#VALUE!	32.00	0	0	33.7	-1.7	85	85.0	7%	98.29	13.29	41.5	58.5
		4-3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	203	32	6.3	-	0	0.22	#VALUE!	#VALUE!	31.78	-	0	47.4	-15.7							
		5-1	268.1	7.64	2.47	10.59	561	90	6.2	77	2	1.26	484	86%	88.74	0	0	62.9	25.9	165	163.7	11%	195.07	30.07	33.4	65.2
		5-2	194.2	7.51	2.08	11.45	561	90	6.2	-	2	1.32	#VALUE!	#VALUE!	88.68	0	0	57.1	31.5	160	158.7	11%	195.25	35.25	39.2	59.4
		5-3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	561	90	6.2	-		0.00	#VALUE!	#VALUE!	90.00	-	0	70.9	19.1							
	08-Dec	1-1	12.31	0.07	0.00	#DIV/0!	363	58	6.2	8	#VALUE!	#VALUE!	355	98%	#VALUE!	-	0	4.6	#VALUE!							
		1-2	9.58	-0.47	0.00	#DIV/0!	363	58	6.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	-3.4	#VALUE!							

47		1-3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	363	58	6.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	0.0	#VALUE!
		2-1	79.7	5.95	2.12	7.75	3	50	0.1	22	#VALUE!	#VALUE!	-19	-633%	#VALUE!	-	0	46.3	#VALUE!
		2-2	69.4	6.72	1.90	8.12	3	50	0.1	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	43.4	#VALUE!
		2-3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	3	50	0.1	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	48.6	#VALUE!
		3-1	56.6	6.39	1.90	11.84	373	60	6.3	51	#VALUE!	#VALUE!	322	86%	#VALUE!	-	0	63.4	#VALUE!
		3-2	127.4	6.18	2.21	10.27	373	60	6.3	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	64.0	#VALUE!
		3-3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	373	60	6.3	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	61.7	#VALUE!
		4-1	324.2	6.64	1.94	9.52	203	22	9.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	52.6	#VALUE!
		4-2	71.3	5.11	1.64	11.77	203	22	9.2	35	#VALUE!	#VALUE!	168	83%	#VALUE!	-	0	54.9	#VALUE!
		4-3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	203	22	9.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	52.0	#VALUE!
		5-1	63.8	6.33	1.62	11.15	561	94	6.0	76	#VALUE!	#VALUE!	485	86%	#VALUE!	-	0	51.4	#VALUE!
		5-2	#VALUE!	#VALUE!	#VALUE!	#VALUE!	561	94	6.0	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	44.0	#VALUE!
		5-3	98.3	6.08	1.60	13.79	561	94	6.0	72	#VALUE!	#VALUE!	489	87%	#VALUE!	-	0	62.9	#VALUE!
48	09-Dec	1-1	23.67	-0.01	0.00	#DIV/0!	363	58	6.2	6	#VALUE!	#VALUE!	357	98%	#VALUE!	-	0	4.0	#VALUE!
		1-2	27.64	-1.64	0.00	#DIV/0!	363	58	6.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	1.7	#VALUE!
		1-3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	363	58	6.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	5.7	#VALUE!
		2-1	80.4	7.13	3.18	5.99	3	50	0.1	17	#VALUE!	#VALUE!	-14	-467%	#VALUE!	-	0	53.7	#VALUE!
		2-2	329.7	8.49	2.19	8.13	3	50	0.1	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	50.3	#VALUE!
		2-3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	3	50	0.1	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	42.9	#VALUE!
		3-1	67.2	7.84	2.53	8.97	373	60	6.3	58	#VALUE!	#VALUE!	315	84%	#VALUE!	-	0	64.0	#VALUE!
		3-2	67.0	8.02	2.45	10.11	373	60	6.3	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	69.7	#VALUE!
		3-3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	373	60	6.3	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	62.3	#VALUE!
		4-1	59.2	7.64	2.76	8.00	203	22	9.2	56	#VALUE!	#VALUE!	147	72%	#VALUE!	-	0	62.3	#VALUE!

Cycle #	Day	sample	CO2 Produced (mg)	O2 (mg/hr)	CH4 (mg/hr)	obs Yield	COD (mg/L) IN	NH4-N (mg/L) IN (Cal)	COD:N	COD (mg/L) OUT	NH4-(mg/L) OUT (Cal)	NH4-N (mg/L) OUT	COD (mg/L) Consumed	% COD Consumed	NH4-N (mg/L) Consumed	NO3- (mg/L)	NO2- (mg/L)	NH4-N for Cell Synthesis 0.12 TSS	NH4-N for nitrification	Total Nitrogen OUT (mg/l)	Organic Nitrogen(mg/l)	N% for Cell Synthesis	Total Nitrogen IN (mg/l)	Nitrogen Converted to gas (mg/l)	NH4-N% converte d to gas	NH4-N% converted to biomass
		4-2	87.5	5.04	1.18	17.64	203	22	9.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	58.9	#VALUE!							
		4-3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	203	22	9.2	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	59.4	#VALUE!							
		5-1	87.5	8.13	2.70	8.19	561	94	6.0	-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	-	0	62.3	#VALUE!							
		5-2	68.2	8.39	3.26	6.91	561	94	6.0	149	#VALUE!	#VALUE!	412	73%	#VALUE!	-	0	63.4	#VALUE!							
		5-3	76.9	8.00	2.57	9.16	561	94	6.0	81	#VALUE!	#VALUE!	480	86%	#VALUE!	-	0	66.3	#VALUE!							
49	10-Dec	1-1	35.74	1.41	0.00	#DIV/0!	363	58	6.2		#VALUE!	#VALUE!	363	100%	#VALUE!	-	0	9.7	#VALUE!							
		1-2	61.95	1.89	0.00	#DIV/0!	363	58	6.2		#VALUE!	#VALUE!	363	100%	#VALUE!	-	0	6.9	#VALUE!							
		1-3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	363	58	6.2		#VALUE!	#VALUE!	363	100%	#VALUE!	-	0	7.4	#VALUE!							
		2-1	255.7	8.97	-0.41	-60.35	3	50	0.1		#VALUE!	#VALUE!	3	100%	#VALUE!	-	0	56.6	#VALUE!							
		2-2	420.4	9.12	3.65	6.25	3	50	0.1		#VALUE!	#VALUE!	3	100%	#VALUE!	-	0	52.0	#VALUE!							
		2-3	74.7	9.40	3.60	6.54	3	50	0.1		#VALUE!	#VALUE!	3	100%	#VALUE!	-	0	53.7	#VALUE!							
		3-1	81.1	8.54	3.12	9.67	373	60	6.3		#VALUE!	#VALUE!	373	100%	#VALUE!	-	0	69.7	#VALUE!							
		3-2	335.5	7.93	2.63	12.13	373	60	6.3		#VALUE!	#VALUE!	373	100%	#VALUE!	-	0	73.7	#VALUE!							
		3-3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	373	60	6.3		#VALUE!	#VALUE!	373	100%	#VALUE!	-	0	69.1	#VALUE!							
		4-1	81.3	7.66	2.66	8.81	203	22	9.2		#VALUE!	#VALUE!	203	100%	#VALUE!	-	0	54.9	#VALUE!							
		4-2	78.9	5.24	0.93	23.28	203	22	9.2		#VALUE!	#VALUE!	203	100%	#VALUE!	-	0	50.9	#VALUE!							
		4-3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	203	22	9.2		#VALUE!	#VALUE!	203	100%	#VALUE!	-	0	52.6	#VALUE!							
		5-1	369.5	8.12	3.89	5.76	561	94	6.0		#VALUE!	#VALUE!	561	100%	#VALUE!	-	0	53.7	#VALUE!							
		5-2	55.3	8.66	2.86	9.40	561	94	6.0		#VALUE!	#VALUE!	561	100%	#VALUE!	-	0	64.6	#VALUE!							
		5-3	#VALUE!	#VALUE!	#VALUE!	#VALUE!	561	94	6.0		#VALUE!	#VALUE!	561	100%	#VALUE!	-	0	57.1	#VALUE!							
50	11-Dec	1-1	36.57	0.50	0.00	#DIV/0!	363	58	6.2		#VALUE!	23.53	363	100%	34.77	0	0	-0.6	35.3							
		1-2	40.46	0.42	0.00	#DIV/0!	363	58	6.2		#VALUE!	25.82	363	100%	32.48	1.6278	0	4.6	27.9							
		1-3	#VALUE!	0.46	#VALUE!	#VALUE!	363	58	6.2		#VALUE!	25.77	363	100%	32.53	0	0	-10.9	43.4							
		2-1	89.0	8.84	3.09	6.12	3	50	0.1		#VALUE!	10.01	3	100%	39.99	1.2585	0	47.4	-7.4	94.5	83	6%	110	15	31%	
		2-2	88.9	8.88	3.09	7.08	3	50	0.1		#VALUE!	25.08	3	100%	24.92	4.1549	0	54.9	-29.9							
		2-3	89.7	9.11	3.07	5.71	3	50	0.1		#VALUE!	19.19	3	100%	30.81	2.8207	0	44.0	-13.2	87.5	65	5%	98	11	21%	
		3-1	67.2	8.54	3.16	8.02	373	60	6.3		#VALUE!	0.99	373	100%	58.51	0.9502	0	64.6	-6.1	73.5	72	5%	105	32	53%	
		3-2	64.2	8.54	3.05	9.06	373	60	6.3		#VALUE!	0.18	373	100%	59.32	0.7744	0	70.3	-11.0	73.5	73	5%	103	30	50%	
		3-3	70.4	8.27	2.70	10.07	373	60	6.3		#VALUE!	1.18	373	100%	58.32	0	0	69.1	-10.8		-	#VALUE!	#VALUE!	#VALUE!	#VALUE!	
		4-1	74.1	7.13	2.28	8.67	203	22	9.2		#VALUE!	0.54	203	100%	21.46	0	0	50.9	-29.4	65	64	5%	67	2	9%	
		4-2	35.1	4.25	1.34	14.31	203	22	9.2		#VALUE!	0.38	203	100%	21.62	0	0	49.1	-27.5	60	60	5%	61	1	4%	
		4-3	59.8	7.59	2.62	8.56	203	22	9.2		#VALUE!	0.46	203	100%	21.54	0	0	57.7	-36.2							
		5-1	83.9	8.27	2.01	11.95	561	94	6.0		#VALUE!	28.29	561	100%	65.71	0	0	61.7	4.0	130	102	7%	159	29	31%	
		5-2	103.0	8.47	2.87	8.21	561	94	6.0		#VALUE!	29.11	561	100%	64.89	0	0	60.6	4.3							
		5-3	77.7	8.36	3.08	7.74	561	94	6.0		#VALUE!	28.47	561	100%	65.53	0.7377	0	61.1	4.4	140	111	8%	166	26	27%	