OPTIMIZATION OF ANAEROBIC CO-DIGESTION OF SEWAGE SLUDGE USING BIO-CHEMICAL SUBSTRATES

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Supervisor: . . . . . Date: . . . . . .

ABSTRACT

The anaerobic process is increasingly becoming a subject for many as it reduces greenhouse gas emissions and recovers carbon dioxide energy as methane. Even though these benefits are attainable, proper control and design of the process variables has to be done in order to optimize the system productivity and improve stability. The aim of this research was to optimize methane and biogas yields on the anaerobic co-digestion of sewage sludge using bio-chemical substrates as co-substrates. The first objective was to find the bio-chemical substrate that will generate the highest biogas and methane yields. The anaerobic digestion of these substrates was operated using 6 L digesters at 37.5℃. The substrate which generated the highest biogas and methane yield in the first batch experiment was then used for the second batch test. The objective was to optimize the anaerobic conditions (substrate to inoculum ratio, co-substrate concentration and temperature) in-order to optimize the biogas and methane yields. The second batch test was achieved using the conventional One-Factor-At-A-Time (OFAT) and the Design of Experiment (DOE) methods.

Final analysis showed that the bio-chemical substrates could be substrates of interest to biogas generators. Amongst the substrates tested in the first batch experiment glycerol (Oleo-Chemical Product waste) generated the highest methane and biogas yields of 0.71 and 0.93 L. (g volatile solids added)$^{-1}$, respectively. It was believed that glycerol contains significant amount of other organic substances such as lipids that have higher energy content than the other bio-chemical substrates, thus generating larger biogas and methane yields. Moreover, digestion of sewage sludge alone produced biogas yields of 0.19 L /g VS and 0.33 L/g COD, and methane yields of 0.16 L/g VS and 0.28 L/g COD. Generally, co-digestion yields were higher than digestion yields of sewage alone.

Using the OFAT method the results of the second batch test on glycerol demonstrated highest amounts of volatile solids (VS) reduction, chemical oxygen demand (COD) reduction, biogas yield and methane yield of 99.7%, 100%, 0.94 L (g VS added)$^{-1}$ and 0.75 L (g VS added)$^{-1}$ at a temperature, substrate to inoculum ratio and glycerol volume of 50℃, 1 (on VS basis) and 10 mL, respectively. Above 22 mL and substrate to inoculum ratio of 1, the process was inhibited.

The DOE results suggested that the highest methane and biogas yields were 0.75 and 0.94 L (g VS added)$^{-1}$, respectively. These results were similar to the OFAT results, thus the DOE software may be used to define the biogas and methane yields equations for glycerol.

In conclusion, anaerobic co-digestion of bio-chemical substrates as co-substrates on sewage sludge was successfully applied to optimize methane and biogas yields.

DECLARATION

The Registrar (Academic)
Durban University of Technology

1. Declaration by Student

I, Nhlanganiso Madondo, hereby declare that unless indicated, this thesis or dissertation titled '*Optimization of Anaerobic Co-digestion of Sewage Sludge Using Bio-chemical Substrates'* is the result of my personal research and has not been submitted in part or in whole for any other degree at another Institution.

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(Signature) (Date)

2. Declaration by Supervisor

I, Dr Manimagalay Chetty, as the student supervisor have proposed for the thesis titled '*Optimization of Anaerobic Co-digestion of Sewage Sludge Using Bio-chemical Substrates'* to be submitted for examination.

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(Signature) (Date)

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GLOSSARY

- ***Acclimation***: The adaptation of a bacterial centre of population to decompose an intrinsically *recalcitrant* substance throughout before being exposed to that substance.
- ***Anaerobic digestion***: The bacterial *deterioration* of an organic substance in an oxygen free environment.
- ***Analysis of Variance (ANOVA)***: a technique in which the deviation in a number of observations is divided into dissimilar components.
- ***Archaeas:*** These are microorganisms that are similar to bacteria in simplicity of structure and size. They however comprise of an ancient group transitional between the eukaryotes and bacteria.
- ***Biogas***: A gaseous fuel, mostly methane and carbon dioxide, formed during the *deterioration* of an organic substance in anaerobic digestion.
- ***Chemical Oxygen Demand (COD)***: A method that indirectly determines the overall amount of dissolved substance present in an effluent.
- ***Co-digestion***: The process of adding a secondary substrate in anaerobic digestion, with the aim of improving the digestibility of organic matter and improving the biogas formed.
- ***Confidence interval*:** It is a measure of the degree of confidence in the range so defined that there is a specific probability that the true value of the variable lies within that range.
- ***Degradation or deterioration***: The property of a material, principally reliant on the structure of the molecule, that refers to its vulnerability to undertake a biologically mediated break down into component elements.
- ***Degree of freedom***: It is an independent variable in a frequency distribution or statistical measure.
- ***Digestion:*** The biological break down or softening of a substance through expose to bacteria, enzymes, water, or heat.
- ***Effluent or wastewater***: A waste stream, containing organic and inorganic matters, which flows from a manufacturing process.
- ***Facultative microorganisms***: Microorganisms that consume oxygen.
- ***Gibbs free energy change (ΔG^o):*** It is a thermodynamic quantity that measures the difference between the enthalpy and the product of the absolute temperature and the

entropy. A negative value means that the system will release energy, whereas a positive value means that the system requires an external energy for the reaction to take place.

- ***Headspace:*** The space in an enclosed container that is occupied by the gas phase i.e. the space that is not occupied by the liquid phase.
- ***Homo-acetogens:*** They are a set of anaerobic prokaryotes that take place in the acetogenesis stage which can, just like methanogens, use carbon dioxide as an electron receiver.
- ***Inhibition or toxicity***: The process of hindering, prohibiting, or restraining the growth of microorganisms, as well as the inhibition of the activity of enzyme within the microorganisms.
- ***Inoculation:*** The process of introducing bacteria or cells into a culture medium.
- ***Labile***: Easily broken down or degradable.
- ***Mesophiles:*** Microorganisms that grow extremely well at moderate temperatures ($30 - 40℃$).
- ***Metabolic Rate:*** The rate at which the chemical or biological reactions in living cells metabolizes.
- ***Pathogen***: is either a virus or bacterium that can cause disease.
- ***Predicted Residual Error Sum of Squares (PRESS)***: Measures the difference between estimated and actual values.
- ***Recalcitrant***: Obstinately uncooperative to bacterial deterioration.
- ***Sludge***: The universal word used to the accumulated solids separated from wastewater. A bulky fraction of the sludge matter in an anaerobic digester contains of microorganisms which are accountable for its degradation.
- ***Standard deviation***: A statistical quantity that is calculated in-order to show the degree of deviation for a group as a whole.
- ***Substrate***: A surface or substance in a biochemical reaction on which micro-organism grows, feeds, or lives.
- ***Syntrophic reaction or interspecies H_2 transfer***: Is a metabolic reaction whereby the H_2 formed by the syntrophic acetogenic microorganisms is continuously removed by the H_2-consuming methanogenesis microorganisms.

- ***Thermophiles:*** Microorganisms that grow extremely well at high temperatures (50 – 60℃).
- ***Variance***: It is a description of how the data is scattered or spread.

NOMENCLATURE

ANOVA	Analysis of Variance
AP	Adequate Precision
APHA	American Public Health Association
ATW	Amanzimtoti Treatment Works
BBD	Box-Behnken Design
B-B	Sectional view for the digester assembly drawing
β	Beta, which is the (r x 1) vector of regression coefficients
$\hat{\beta}$	Least squares term for the regression coefficient
β_i	i^{th} coefficient estimate
β_j	The j^{th} coefficient of the model
$\beta_{j,-i}$	The j^{th} coefficient when the i^{th} experimental run is removed
β_l	l^{th} regression coefficient, where l = 1, 2, 3,....., p
$\hat{\beta}_l$	l^{th} regression coefficient in scalar notation
$\beta_{rl}x_r x_l$	Second-order cross product terms
$\beta_{rr}x_r^2$	Second-order quadratic terms
β_0	Regression coefficient at r = 0 i.e. at the intercept
$\hat{\beta}_0$	Regression coefficient at r = 0 in scalar notation
β_1	The coefficient estimate for temperature (A)
β_2	The coefficient estimate for the substrate to inoculum ratio (B)
β_3	The coefficient estimate for the volume of glycerol in the digester (C)
β_{11}	The coefficient estimate for A^2
β_{12}	The coefficient estimate for AB

β_{13}	The coefficient estimate for AC
β_{22}	The coefficient estimate for B^2
β_{23}	The coefficient estimate for BC
β_{33}	The coefficient estimate for C^2
CCD	Central Composite Design
COD	Chemical Oxygen Demand
C	Chemical element for carbon
C-C	Sectional view for the adaptor
CI	Confidence Interval
CO	Carbon monoxide
CO_2	Carbon dioxide
C.V.	Coefficient of variation
$CaCO_3$	Calcium carbonate
C_{A0}	Initial concentration of A, *kmol/ m^3*
CH_3CH_2COOH	Propionic acid
$CH_3(CH_2)_2COOH$	Butyrate acid or i-valerate or n-valerate
$CH_3CH_2CH_2CH_2COOH$	Pentanoic acid or Valeric acid
$CH_3(CH_2)_4COOH$	Caproate
CH_3CH_2OH	Ethanol
$CH_3CHOHCOOH$	Lactic acid
CH_3COOH	Acetic acid or acetate
CH_3NH_3Cl	Methylamine
CH_3OH	Methanol
$(CH_3)_2S$	Methyl mercaptan
CH_4	Methane
$C_nH_{n-2}O_{n-1}$	Carbohydrates
Cor. Total	Totals of all data corrected for the mean
C_p	Number of centre points
$Cr_2O_7^{2-}$	Dichromate ion

C_{T0}	Initial concentration of the mixture, *kmol/ m^3*
C_v	Coded input variables
$C_3H_8O_3$	Glycerol
$C_5H_7NO_2$	Ethyl cyanoacetate (Protein)
$C_6H_{10}O_5$	Cellulose (Carbohydrate)
$C_6H_{12}O_6$	Glucose
$C_{10}H_{20}O_6N_2$	Proteins
$C_{54}H_{106}O_6$	Lipids
$C_{57}H_{104}O_6$	Lipids
c_{jj}	The j[th] diagonal element of $(X'X)^{-1}$
DFBETAS	Difference in Betas (β)
DFFITS	Difference in Fit Statistic
DM	Doehlert Design
DOE	Design of experiment
DUT	Durban University of Technology
DWAF	Department of Water Affairs and Forestry
D	Reactor diameter, *m*
D-D	Sectional view for the bottom plate
Deg C	Degrees Celsius (℃)
D_i	Cook's distance
d	Gas collector diameter, *m*
df	Degrees of freedom
df_{CT}	Degree of freedom for cor. Total
df_{den}	Degree of freedom for the denominator
df_E	Degree of freedom for residual
df_{LOF}	Degree of freedom for the lack-of-fit
df_{num}	Degree of freedom for the numerator
df_{PE}	Degree of freedom for the pure error
df_R	Degree of freedom for regression

EVOP	Evolutionary operation
E-E	Sectional view for the feed cap
e	(N_e x 1) vector of random errors
e_i	Residual from the least squares fit
e_r	r^{th} experimental error term, where r = 1, 2, 3,....., N_e
F	The f-critical value, obtained from Table B1
F-F	Sectional view for the feed pipe
2Fl	Two-factor interaction
$F_{(df_R, df_E.1-\alpha)}$	The F-distribution at df_R, df_{PE} and $1-\alpha$ (obtained from Table B1)
F_{LOF}	F-value for lack-of-fit
F_R	F-value for regression
F^{-1}	The inverse of the F-critical value, $F^{-1} = \frac{1}{F}$
f	Response variable f
G	Reactor height, m
GC 101	Gas collector
G-G	Sectional view for the glass plate
ΔG^o	Gibbs free energy of Reaction
g	Glycerol fraction
glyc.	Glycerol
HCOOH	Formic acid
HBu	Butyrate
HCL	Hydrochloric acid
$\boldsymbol{H}$	Hat matrix, defined as $\boldsymbol{H} = \boldsymbol{X}(\boldsymbol{X}'\boldsymbol{X})^{-1}\boldsymbol{X}'$
H-H	Sectional view for the inside pipe
HNO_3	Nitric acid
HPr	Propionate
HVa	Valerate
HCO_3^-	Bicarbonate ion

H^+	Hydrogen ion
H_2	Hydrogen molecule
H_2CO_3	Carbonic acid
H_2O	Water molecule
H_2S	Hydrogen sulphide
H_2SO_4	Sulphuric acid
h	Gas collector height, *m*
h_{ii}	Diagonal element of the hat matrix (**H**).
I	Inerts
ID	Identity
IOP	Industrial Oleo-chemical products
ISO	International Standards Organization
i	Number of moles of carbon
j	Number of moles of oxygen
$KMnO_4$	Potassium permanganate
k	Number of moles of hydrogen
LCFA	Long chain fatty acids
L_{CD}	Limits for the Cook's distance
L_{DBETAS}	The DFBETAS limits
L_{DFFITS}	The DFFITS range
L_E	Limits for the residuals
Lev_{ave}	The average leverage of a design
L_L	Limits for leverage
l	Lipids fraction
MN	Mean
MS	Mean square
MSR	Magnetic stirrer
M	Average molar mass of the mixture, *kg/ kmol*
M-M	Sectional view for the outside pipe

MS_E	Mean square for error or residuals
MS_{LOF}	Mean square for lack-of-fit
MS_{PE}	Mean square for pure error
MS_R	Mean square for regression
M_A	Molar mass of acetate, *kg/ kmol*
M_c	Molar mass of carbon dioxide, *kg/kmol*
M_H	Molar mass of acetate hydrogen, *kg/ kmol*
M_m	Molar mass of methane, *kg/ kmol*
M_w	Molar mass of water, *kg/ kmol*
M_N	Molar mass of nitrogen, *kg/ kmol*
M_o	Molar mass of oxygen, *kg/ kmol*
m	Methanol fraction
m_A	Mass of acetate, *g*
max	Maximum
m_b	Mass of biogas, *g*
m_c	Moisture content in the sample, *g/g*
m_{CO2}	Mass of carbon dioxide generated, *g*
$m_{CO2\ dissolved}$	Mass of carbon dioxide dissolved in water, *g*
m_G	Mass of glycerol, *g*
m_H	Mass of hydrogen, *g*
m_{H2O}	Mass of water vapour generated, *g*
$m_{H2O\ before}$	Mass of water in the gas collector before digestion, *g*
$m_{H2O\ after}$	Mass of water in the gas collector after digestion, *g*

m_I	Mass of inoculum, *g*
m_{losses}	Mass losses in the system, *g*
m_N	Mass of nitrogen, *g*
$m_{methane}$	Mass of methane generated, *g*
$m_{Total\ fed}$	Total mass fed to the digester, *g*
$m_{Total\ output}$	Total output mass, *g*
m_w	Mass of water, *g*
$NaHCO_3$	Sodium bicarbonate
NH_3	Ammonia free
NH_4^-	Ammonium ion
NH_4Cl	Ammonium chloride
N	Nitrogen
N_A	Final moles of acetate, *kmol*
N_{A0}	Initial moles of acetate, *kmol*
N_B	Final moles of carbon dioxide, *kmol*
N_C	Final moles of methane, *kmol*
N_e	Number of experimental points or runs
N_f	Number of points in the factorial section of the design
N_I	Final moles of inerts, *kmol*
N_{I0}	Initial moles of the inert, *kmol*
N_s	The total number of star points in the system
N_T	Moles produced, *kmol*
N_{T0}	Initial moles, *kmol*
n	Number of variables

n_d	Distinct design points
$n_{glycerol}$	Number of moles of glycerol fed, *kmol*
n_s	Standard mole; $n_s = 1\ kmol$
OFAT	One-Factor-At-A-Time
OH^-	Hydroxyl ion
O_2	Oxygen
P&ID	Process and instrumentation diagram
PI	Predicted interval
PRESS	Predicted residual error sum of squares
PVC	Poly vinyl chloride
P-P	Sectional view for the first top plate
Pred.	Predicted
Prob.	Probability
P	Final or system pressure, *atm*
P_s	Standard Pressure; $P_s = 1\ atm$
P_0	Initial pressure, *atm*
p	Regressor or number of model coefficients
ρ_A	Density of A, kg/m^3
ρ_G	Density of inoculum, kg/m^3
pHI	pH indicator
ρ_I	Density of inoculum, kg/m^3
ρ_s	Density of the sample, kg/m^3
ρ_w	Density of water at 37.5℃
R 101	Reactor or digester
R-R	Sectional view for the second top plate
RSM	Response surface methodology

R-squared	Denotes the R^2 value
R	Universal gas constant
R_p	The number of times the experimentation is repeated
r_i^2	The square of the internally studentized residual at the ith case
SA	South Africa
SD	Standard deviation
SE	Standard error
SOS	Sum of squares
STP	Standard temperature and pressure
SOS_E	Sum of squares for error or residuals
SOS_{LOF}	Sum of squares for lack-of-fit
SOS_{model}	Model sum of squares
SOS_{PE}	Sum of squares for pure error
SOS_R	Sum of squares for regression
SOS_T	Sum of squares for the total
s_{-i}	Standard error or deviation if the ith term is removed from the design
TI	Temperature indicator
TS	Total solids
T	Final temperature or system temperature, *K*
T_R	Ratio of maximum to minimum response
T_s	Standard temperature; $T_s = 273\ K$
T_0	Initial temperature, *K*
t	t-value
t_i	The ith t-value

$t_{(tail,df)}$	The t-value obtained from Table B2 (see Appendix B) at $tail = \frac{\propto}{2N_e}$ and $df = N_e - p - 1$
UKZN	University of KwaZulu Natal
u	Stoichiometric coefficient of water
VFA	Volatile fatty acids
VIF	Variance inflation factor
VS	Volatile solids
V	Reactor volume
V_{air}	Volume of air initially in the system
V_b	Biogas volume, mL
$V_{biogas\ yield}$	Theoretical biogas yield, *L biogas / g VS*
V_C	Gas collector volume, *L*
V_{CH_4}	Volume of methane produced, *L*
V_D	Design volume of the fluid, m^3
V_G	Volume of glycerol, m^3
V_I	Volume of inoculum, m^3
V_L	Liquid volume, *L*
V_{N_2}	Volume of nitrogen in the system
V_s	Standard Volume; $V_s = 22.4\ m^3$
V_{STP}	Biogas volume in STP, *L*
V_T	Tube connector inside volume
V_V	Headspace volume, *L*
V_w	Volume of water, *L*
V_{yield}	Theoretical volumetric yield, *L/ g VS*

V_0	Initial volume of the fluid, m^3
v	Stoichiometric coefficient of methane
WD 101	Water displacement container
w	Stoichiometric coefficient of carbon dioxide
w_{afb}	Weight of crucible + sample at 550℃, g
w_{ah}	Weight of crucible + sample at 105℃, g
w_{bh}	Weight of crucible + sample before heating, g
w_{co}	Weight of the crucible, g
w_m	Weight of moisture, g
w_s	Weight of the sample, g
$\mathbf{X}$	(N_e x r) matrix of levels of independent input variables
$\mathbf{X'}$	Inverse matrix of $\mathbf{X}$
X_1	The temperature variable (A)
X_2	The substrate to inoculum ratio variable (B)
X_3	The variable for volume of glycerol in the digester (C)
x	Independent input variables from 1 to p
x_{A0}	Mass fraction of acetate, *g/g*
x_{H0}	Mass fraction of hydrogen, *g/g*
x_p	The term of the p^{th} input variable, where p = 1, 2, 3...N
x_{N0}	Mass fraction of nitrogen, *g/g*
x_p^0	Real input variable at the centre of the experimental domain

x_{rl}	Represents the l^{th} level of independent variable and r^{th} examination, where l = 1, 2, 3,....., p and r = 1, 2, 3,....., N_e
x_{w0}	Mass fraction of water, *g/g*
Δx_p	Step of change in real input variable
y	Response variable from 1 to N_e
$\hat{y}$	Response variable in terms of least squares
y_{A0}	Molar fraction of acetate, *v/v*
y_c	Molar fraction of carbon dioxide, *v/v*
$\bar{y}_c$	Average of the C_p experimental observations at the centre
y_{H0}	Molar fraction of hydrogen, *v/v*
y_{I0}	Molar fraction of inerts, *v/v*
y_m	Molar fraction of methane, *v/v*
$\hat{y}_{max}$	Maximum response
$\hat{y}_{min}$	Minimum response
y_n	Molar fraction of nitrogen, *v/v*
y_o	Molar fraction of oxygen, *v/v*
y_r	Single response variable, where r = 1, 2, 3,....., N_e
$\hat{y}_r$	Response variable in scalar notation
y_w	Molar fraction of water, *v/v*
Z	Conversion of acetate (A)
z	Experimental levels from 1 to p

$\propto$	Alpha value, which is the percentage of the confidence interval
λ	Lambda value
μ	Efficiency of one experimental design
σ^2	Standard error term
$\emptyset$	Angle of rotation, *degrees*

1. CHAPTER 1: INTRODUCTION

1.1. Anaerobic Process

Water serves as a fundamental constituent of all breathing organisms and in its absence, life would not exist, particularly when approximately 60 percent of man's body is made up of water (Huber, 2010). The planet we live in is usually known as the water planet because 75% of the earth's surface is covered with water. Water may appear plentiful, however, not all of it is easily accessible and available for use, since 97.2% of the earth's water is salted. Of the 2.8% remaining, 69% is frozen as ice in Polar Regions, and the remaining 31% is liquid fresh water (Brito, 1999). Thus, roughly 0.9% of the entire earth's water is fresh water. About 97 percent of this fresh water is hidden in underground water aquifers, with the remaining 3% being found in surface water; for example lakes, dams and rivers.

One of the biggest problems to be dealt with currently is the development of a pioneering plan to avoid severe water scarcity, and to fulfil the ever increasing water demand (Strauss, 2006). Water scarcity is recognized as the chief reason for applying innovative scientific knowledge to the use and re-use of water together with recycling and reproduction. South Africa (SA) is one of the most populated countries in Africa, currently number five in Africa, with a population of approximately 56.52 million people (Statistics of South Africa, 2017). The population has increased by nearly 14.62 million people from 1994 to 2015. The fast growth in population together with rapid economic growth has resulted in the production of large quantities of wastewaters with high organic matters (Saleh and Mahmood, 2004), thus causing tremendous risks to the animals, humans, and environment (Ahring, 2003). The increase in population has not only affected the water sector, but also resulted in high electricity demand; Eskom, SA's main electricity supplier, is failing to generate enough electricity to meet with the country's needs. Moreover, Eskom is faced with another crisis: about 90 percent of Eskom's electricity comes from coal (Republic of South Africa, 2009), which after combustion produces greenhouse gases that pollute the atmosphere and lead to global climate change. Anaerobic digestion is the key to reducing contaminants in the ever increasing wastewaters generated in SA, and providing an alternative source of electricity that is more eco-friendly, thereby bailing both the water sector and Eskom.

Anaerobic digestion has been exploited for the treatment of contaminated domestic and industrial effluents for more than a hundred years. Even though anaerobic digestion is an old process, the knowledge for biogas generation is still growing and a long way from being optimized (Lu, 2006). With stability the main driving force in achieving process optimization, factors affecting the anaerobic process; such as temperature, substrate to inoculum ratio and co-substrate concentration, if improperly designed and controlled can start a series reaction of products that can result in process inhibition, thus shifting away from process optimization. Proper control and design of the process's variables have to be carried out in order to improve stability, avoid process upsets, and attain process optimization.

Anaerobic co-digestion is increasingly becoming a subject for many due to the number of benefits it has, compared to conventional digestion, such as higher degradability and higher biogas productions. Some researchers have proven that combinations of industrial, municipal and agricultural wastewaters can be degraded effectively and economically together (Khalid *et al.*, 2011). Even though co-digestion is aimed at improving the amount of biogas produced, the amount produced will differ from co-substrate to co-substrate. This is because the rate of degradation is influenced by the complexity (degree of crystallinity), chemical structure and molar mass of the substrate. Furthermore, these factors together with the type of inoculum and the operating conditions will affect the period needed for acclimation (Dlamini, 2009).

Glycerol is a secondary product of biodiesel manufacture, and is often regarded as an industrial waste stream (Athanasoulia *et al.*, 2014). Currently, there is an excessive supply of glycerol, which has led to its low price. Motivated by the high availability and low price of glycerol, many studies have been done on glycerol as a co-substrate in anaerobic co-digestion (Wohlgemut, 2009; Foucault, 2011). Anaerobic digestion of other industrial waste streams; such as fruit juice, soft drink and oleo-chemical product have also been investigated by many researchers (El-Kamah *et al.*, 2010; Nweke *et al.*, 2015; Sarah, 2005). However, there is little knowledge concerning co-digestion of municipal sewage sludge together with these industrial waste streams.

1.2. Research Aim and Objectives

The main aim of this research is to produce biogas by optimizing the anaerobic co-digestion of sewage sludge using industrial bio-chemical substrates.

In order to achieve the main aim of this thesis, the following objectives have to be done:

a) To investigate the effect of various industrial bio-chemical substrates on the digestion process at a mesophilic temperature of 37.5℃.
b) To optimise operating conditions to maximize the methane generation in the biogas produced.
c) To determine the methane yield and biogas yield model equations.

1.3. Project Plan

The project plan for this investigation is depicted in Figure 1.1:

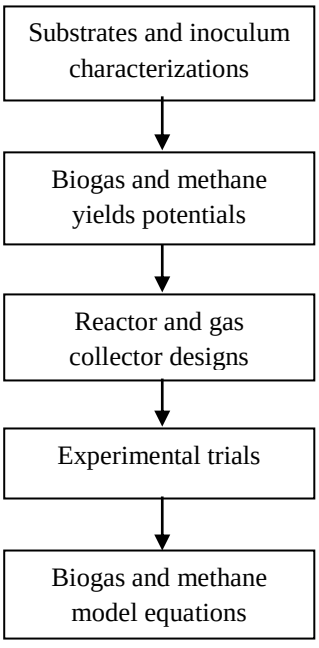

Figure 1.1: Project plan.

1.4. Thesis Outline

The thesis consists of seven sections:

- *Chapter 1*: The Introduction includes, water and wastewater, anaerobic process, research aim and objectives, project plan, and thesis outline.

- *Chapter 2*: This chapter contains water and wastewater in South Africa, factors influencing water supply and demand, wastewater disposal and treatment methods, anaerobic digestion, mechanisms found in anaerobic digestion, factors affecting anaerobic digestion, sewage sludge as a substrate in anaerobic digestion, anaerobic co-digestion, and design of experiments.

- *Chapter 3*: Materials and methods are presented.

- *Chapter 4*: Results and discussion are contained in this chapter.

- *Chapter 5*: This chapter includes conclusions and recommendations.

- *References*: Lists of references are listed.

- *Appendices*: This section shows samples of calculation and drawings.

2. CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

The focus of this thesis is to optimize the anaerobic co-digestion of sewage sludge using bio-chemical substrates. To get a good and proper understanding of water, wastewater, the anaerobic process and design of experiments, it is important to look into the details of water and wastewater in South Africa, factors influencing water supply and demand, wastewater disposal and treatment methods, anaerobic digestion, mechanisms found in anaerobic digestion, factors affecting anaerobic digestion, sewage sludge as a substrate in anaerobic digestion, and anaerobic co-digestion. This will be accomplished through a review of literature on these topics.

2.2. Water and Wastewater in South Africa

About 2 million tons of agricultural, industrial and sewage sludge waste are disposed of daily into seawater, landfill or surface water. In SA, approximately 72% of agricultural, 11% of industrial and 17% of domestic wastewaters are discharged into the sea, landfills, as well as surface water (Metcalf-Wallach, 2008). Wastewaters generally consist of contaminated pollutants, which if untreated, would cause pollution and lead to the spread of waterborne illnesses.

The issue of water quality in SA is due to water contamination as well as the waterborne illness-causing bacteria (pathogens) that live in surface waters. Every day humans are forced to siphon water from unclean dams or polluted rivers, promoting un-healthiness and illnesses. It is essential to realize that consuming polluted water, together with lack of cleanliness, are the major supporters to the projected 4 billion incidents of diarrhoeal diseases per annum, resulting in 1.5 million life losses (Jain, 2009). About 88 percent of diseases in the developing countries are due to the consumption of polluted water. In SA, 11.7 percent of schools and 15 percent of clinics lack safe, clean water (DWAF, 2002).

2.3. Factors influencing Water Supply and Demand

SA has a low average rainfall of 450 mm per year in comparison to the world's average of 860 mm (Republic of South Africa, 2017). Furthermore, the country has a small number of reservoirs; most of them being undersized, and situated in inaccessible non-urban places. Water is therefore a scarce resource in SA, therefore correct protective standards need to be in position to guarantee sustainability to protect the rivers, dams and the sea from pollution. Other than scarcity, there are other driving forces for water saving:

- Water costs, economic factors: Wastewater disposal expenses are motivating industries to try and find ways of saving water and reduce wastewater disposal, thus reducing the overall expenses.
- Population growth: The worldwide water utilization is doubling every 20 years, two times the rate at which the population grows. Moreover, it is projected that in 11 years, not less than 3 billion human beings will be staying in places where fresh water is inaccessible (GCC, 2013).
- Governance regulations: government regulations can demand and specify the amount and at times quality of effluent disposed by an industry.
- Weather conditions and changes: The change in weather has an effect on the water cycle, and thus the amount of water available for humans and nature. Earth's changes in temperature affects rainfall patterns. Higher temperature results in higher water evaporation, and hence drought conditions prevail (Trenberth, 2011).
- Land use or infrastructure: Land use and management exercises have an effect on the amount and the characteristics of drainage water and, consecutively, the water financial plan, water chemical properties and biological variations of bacteria in the water that receives it. Unsatisfactory operation and maintenance of effluent processing infrastructure adds to the major concerns that SA is faced with (Mema, 2009).

2.4. Wastewater Disposal and Treatment Methods

Because of the ecological issues due to wastewater over the past years, management of wastewater has turned out to be a great issue worldwide (Ingenieurs, 2009). As a result of the scarcity of fresh water, together with water costs, population growth, governance regulations

and land use, it is essential to protect water resources from all types of pollutants. Some researchers in the area of wastewater management have examined a variety of wastewater disposal and treatment methods; such as Bolin *et al.* (2009); Beam (2011) and El-Kamah *et al.* (2010)**.** The following are some of the well-known wastewater treatment and disposal methods:

- Direct application on farmland,
- Discharging into the sea,
- Distributing on wild land,
- Landfill,
- Incineration,
- Aerobic treatment or composting, and
- Anaerobic digestion.

The first three methods are very old disposal methods, and as a result of their toxicity to humans and the environment, they have been banned in certain places (Lu, 2006). Landfill is almost all characterized with the contamination of groundwater by waste-derived waters. Another major problem with landfills is the greenhouse gas emissions that are released into the atmosphere, which then affect humans and the greenhouse system. Due to a vast increase in population, the use of land is also becoming costly. Incineration involves the burning of wastes, thereby producing volatile organic products, carbon monoxide and NOX (Parawira, 2004). One of the major drawbacks of incineration is the need for an external energy, which is very costly as waste contains large amounts of water. Aerobic treatment or composting has to do with the deterioration of organic substance by bacteria in the presence of oxygen (Parawira, 2004). The problem with aerobic treatment is the high operational cost together with high energy cost that is required for oxygenation.

Of all the disposal and management methods, anaerobic digestion is the most efficient process (Lu, 2006). Anaerobic digestion: reduces greenhouse gas emissions, recovers carbon dioxide energy as methane, accommodates high loading rates and removes pathogens. Furthermore, the digestate from anaerobic processes may be used for fertilization, which relieves valuable nutrients to the soil. Because of these advantages, anaerobic digestion is therefore worth further investigation.

2.5. Anaerobic Digestion

Anaerobic digestion may be defined as the normal deterioration of the organic material by microorganisms in the absence of oxygen with the formation of biogas (Ahring, 2003). Biogas is chiefly made up of methane and carbon dioxide, but also has small amounts of hydrogen sulphide, oxygen, nitrogen, hydrogen and water. The generation of biogas is of high importance as it may be used to replace fossil fuels for the generation of electrical energy. In a normal anaerobic digestion process approximately 30 to 60 percent of the organic materials are transformed into biogas (Beam, 2011). Water is then removed from the remaining 40 to 70 percent of the organic materials. The de-watered solids, which have reduced contaminants, are then discharged to a disposal site.

The treatment of anaerobic digestion is a useful process for dozens of waste products; such as municipal, industrial and agricultural wastes. In the absence of oxygen, the substrate is deteriorated to $CH_4$and CO_2 with a low concentration of microbes. Under anaerobic conditions, the process is capable of converting about 90 percent of the energy stored in the organic substance to CH_4 (Ramsamy *et al.*, 2002).

SA has approximately 300 anaerobic digesters that are currently functioning (Biogas National Conference, 2013). Despite their presence, their exploit for electricity generation has not been widely used as the country's economic electricity power source is coal. However, SA's attention in renewable energy systems is stimulated by the need for cleaner energy sources and the failure of the national electricity supplier in meeting the country's demand. Stafford *et al.* (2013) did a study on energy recovery from South African wastewaters. Results show that approximately 3 200 to 9 000 MWth of energy can be recovered, which is more than 7% of the country's present electrical power supply. If implemented, 7% of the country's present electrical power supply could be saved.

2.6. Mechanisms found in Anaerobic Digestion

Anaerobic digestion is normally a process that relates to both physic-chemical and microbiological mechanisms, where the output of one step is used as the feed stock for the

next step. The steps involved in the degradation process, in their order, are: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Amongst these steps, hydrolysis is the only extracellular step (physic-chemical mechanism), with the others being intracellular (biological mechanism). A summary of the anaerobic microbiological process, including COD distribution in each stage, is depicted in Figure 2.1.

Equations 2.1 to 2.3 below show the overall anaerobic microbiological conversions:

The conversion of carbohydrates:

$$2\ C_nH_{n-2}O_{n-1} + 2n\ H_2O \rightarrow CH_4 + CO_2 \qquad (2.1)$$

The conversion of proteins:

$$5\ C_{10}H_{20}O_6N_2 + 6H_2O \rightarrow 11\ CH_4 + 9\ CO_2 + 4\ NH_3 \qquad (2.2)$$

The conversion of lipids (fats and vegetable oil):

$$C_{54}H_{106}O_6 + 28\ H_2O \rightarrow 40\ CH_4 + 17\ CO_2 \qquad (2.3)$$

Detailed explanation for each stage of anaerobic digestion will be given in the following sub-sections. The Gibbs free energy change (ΔG^{o}) is a thermodynamic parameter that measures the difference between the enthalpy and the product of the entropy and the absolute temperature. A positive Gibbs free energy change means that the system requires an external energy for the reaction to take place, whereas a negative Gibbs free energy change means that the system will release energy. If the system temperature is constant, the Gibbs free energy change for a chemical reaction (ΔG^{o}) is given by Equation 2.4:

$$\Delta G^0 = RT\ln\left[\frac{\text{Concentration of products}}{\text{Concentration of reactants}}\right] \qquad (2.4)$$

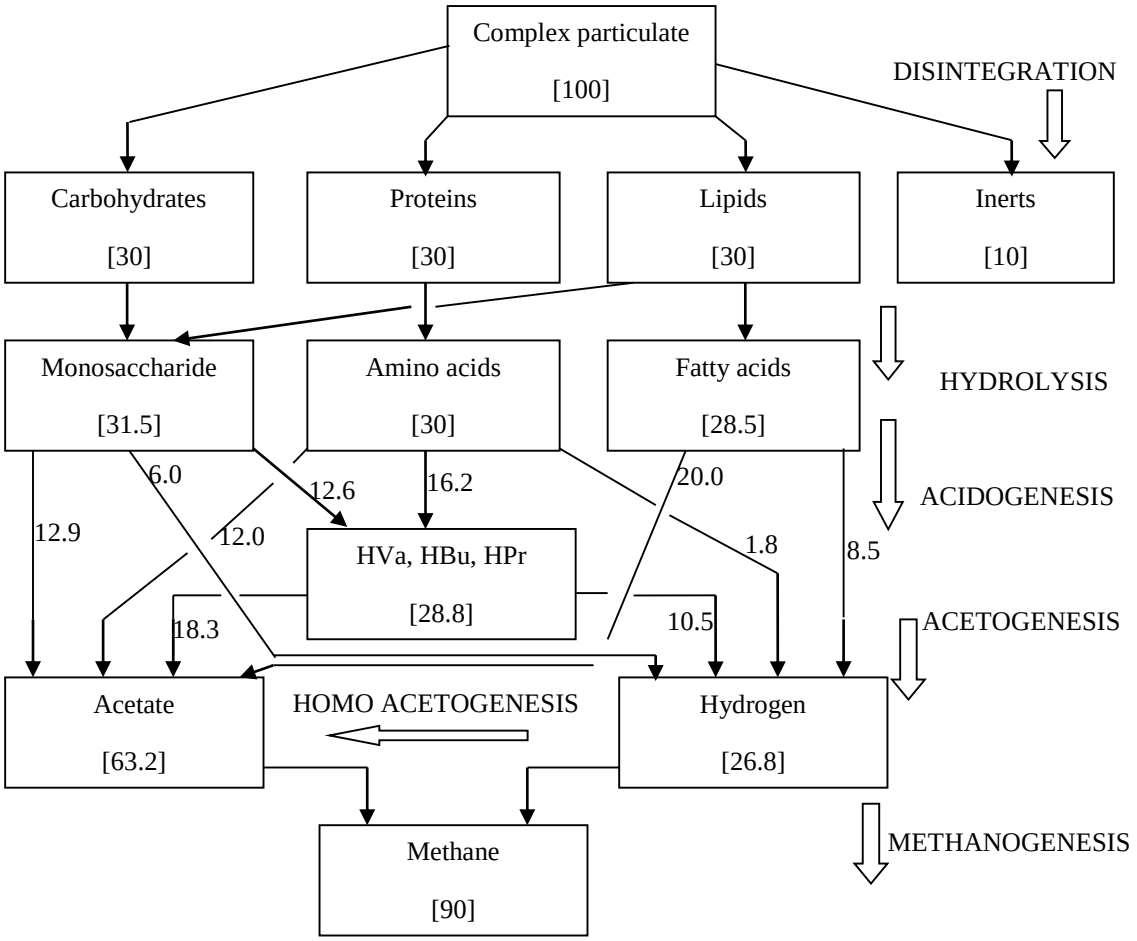

Figure 2.1: The anaerobic microbiological process-diagram. Units COD% (Dlamini, 2009).

From Equation 2.4, overproduction of a reaction in comparison to its reactants will result in ΔG^{o} increasing (i.e. $\Delta G^{o} > 0$), and thus inhibits the process. On the other hand, a negative ΔG^{o} implies that the reaction has less reactants in comparison to its products, and thus the process will not be inhibited. The stages involved in the anaerobic process have to be in equilibrium so as to make certain that sufficient products produced in each stage are available for use as a feed stream to the next stage with no overproduction. This is extremely important as the bioconversions in anaerobic digestion adhere to thermodynamic equilibria.

2.6.1. Hydrolysis

Wastewater, e.g. sewage and piggery waste, usually contains large-complex and insoluble substances; such as lipids, proteins and carbohydrates. Due to their complex nature, these organic substances cannot be consumed immediately by anaerobic microorganisms, and therefore have to be degraded to smaller molecules of a size sufficient to be absorbed by bacteria. This is achieved in the first step of anaerobic process known as hydrolysis, where carbohydrates, lipids and proteins are initially dissolved and then degraded by extracellular enzymes into sugar, long-chain fatty acids, and amino acids, respectively (Angelidaki and Sanders, 2004**).** The extracellular enzymes that are responsible for the metabolisms are cellulases, lipases, and proteases, with their formation being partially managed by the feed organic substance and product percentages in the reactor substance.

Microorganisms make the most of carbon (in carbon containing substrate) as an energy source to control mechanism and for the formation of other cellular substances**.** Different kinds of carbon source support different classes of microorganisms. Proteins, carbohydrates and lipids have different structures, and therefore do not contain the same number of carbons. Of all these organic substances, lipids are the main source of carbon, and hence are expected to generate the highest yield of methane. The molecular formulae and methane yields for various bio-chemical substances are reported in Table 2.1:

The values were calculated on the assumption that all organic substances are exclusively metabolized to methane. As indicated in Table 2.1 the highest methane yield (1.014 L/g Volatile Solids) and percentage (70 to 75%) is obtained from lipids.

Table 2.1: Methane yields of different substrates.

Organic substance	Molecular formula	Yield of Methane (Litre per gram VS) [a]	Methane percentage in biogas (%) [b]
Carbohydrates	$(C_6H_{10}O_5)n$	0.415	50 – 55
Proteins [c]	$C_5H_7NO_2$	0.496	68 – 73
Lipids (fats)	$C_{57}H_{104}O_6$	1.014	70 – 75

[a] The values are expressed in standard temperature and pressure of 273 K and 101 kPa, respectively (Angelidaki and Sanders, 2004)

[b] (Petersson and Wellinger, 2009)

[c] Nitrogen is metabolized to ammonia

The hydrolytic rate is significantly influenced by the complexity (degree of crystallinity), chemical structure and molar mass of the substrate. The hydrolysis stage is usually the rate-limiting stage when large-complex substances are degraded, since it determines the greatest amount of substrate presented for the following stage, and thus will have an effect on the rate of movement of the whole anaerobic system. Several manufacturing processes master the rate limitation by using pre-treatment methods to try and improve the hydrolysis, thus reducing the overall digestion period and increasing the productivity of methane (Verma, 2002). Hydrolysis pre-treatment methods are either chemical and/or thermal. The mostly used chemical methods are that of ammonia, acid or lime addition, whereas thermal pre-treatment methods involve hot water or steam**.**

2.6.2. Acidogenesis

Acidogenesis, otherwise called the initiation stage of fermentation, carries on the deterioration of the hydrolysis products, and generates various organic compounds as well as carbon dioxide, hydrogen and ammonia. The bacteria responsible for acidogenesis are acid-forming bacteria. The acidogenesis step is vigorous and usually the most rapid stage in the entire digestion system. Organic compounds formed in this process stage are predominantly acetate, alcohols, aldehydes and volatile fatty acids**.** Alcohols and volatile fatty acids are known as intermediate or transitional products, as the main product that is required in this process stage is acetate. Equations 2.5 to 2.11 below highlights some of the reactions involved in the acidogenesis step:

Glucose fermentation:

$C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3COO^- + 2HCO_3^- + 4H^+ + 4H_2$ $\Delta G^0 = -206.3\ \text{kJ.mol}^{-1}$ (2.5)

$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2CH_2CH_2COO^- + 2HCO_3^- + 3H^+ + 2H_2$ $\Delta G^0 = -254.8\text{kJ.mol}^{-1}$ (2.6)

$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2COO^- + 2HCO_3^- + 3H^+ + 2H_2$ $\Delta G^0 = -254.8\ \text{kJ.mol}^{-1}$(2.7)

Amino acids fermentation:

$\text{Glutamate} + 3H_2O \rightarrow 2CH_3COO^- + HCO_3^- + H^+ + NH_4^- + H_2$ $\Delta G^0 = -33.9\ \text{kJ.mol}^{-1}$ (2.8)

$2\ \text{Glycine} + 4H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + NH_4^- + H_2$ $\Delta G^0 = -51.5\ \text{kJ.mol}^{-1}$ (2.9)

$\text{Alanine} + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + NH_4^- + 2H_2$ $\Delta G^0 = +7.5\ \text{kJ.mol}^{-1}$ (2.10)

$\text{Glycine} + 2H_2 \rightarrow 2CH_3COO^- + 2NH_4^-$ $\Delta G^0 = -38.9\ \text{kJ.mol}^{-1}$(2.11)

The Gibbs free energy of change values are in standard temperature and pressure (273 K and 101 kPa) and at pH 7 (Lily, 2010).

Generally from the above equations, most of the products formed in the acidogenesis step are organic compounds and hydrogen (H_2). In a properly-controlled digester, approximately 70 to 80 percent of the products from the hydrolysis step will be converted directly to methanogenic products (acetate, carbon dioxide and hydrogen) and the other 20 to 30 percent will be converted into transitional products (Lu, 2006). The generation of such a large amount of methanogenesis products in acidogenesis is an advantage as it provides the methanogenesis step with enough substrates. Even though this is true there are some negative effects caused by the abundance of acetate and hydrogen:

- The abundance of hydrogen inhibits the process, resulting in the Gibbs free energy being positive. In such circumstances, the fermentative microorganisms will control the metabolic reaction in order that they can even attain a fraction of energy, and then approximately 50 to 70% of the substrates produced in the hydrolysis stage will be converted into the transitional products.
- Acetate is an acid. Its abundance in the system lowers the process pH. However, the ammonium ion (NH_4^-) formed by amino acids raises the pH of the system, thus stabilizing the anaerobic process.

2.6.3. Acetogenesis

For the reason that the volatile fatty acids have to be furthermore converted to methanogenesis products, the acetogenesis stage is vital for the effective formation of methanogens products (Lu, 2006). The acetogenesis step is normally the fastest step in anaerobic digestion process. Equations 2.12 to 2.19 below highlight some of the acetogenesis mechanisms:

Propionic acid breakdown:

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + 3H_2 + CO_2 \qquad \Delta G^0 = +76.1\ kJ.mol^{-1} \quad (2.12)$$

Lactic acid breakdown:

$$CH_3CHOHCOOH + H_2O \rightarrow CH_3COOH + 2H_2 + CO_2 \qquad \Delta G^0 = +21.4\ kJ.mol^{-1} \quad (2.13)$$

Butyric acid breakdown:

$$CH_3(CH_2)_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2 \qquad \Delta G^0 = +43.6\ kJ.mol^{-1} \quad (2.14)$$

i-valerate breakdown:

$$CH_3(CH_2)_2COOH + 2H_2O + CO_2 \rightarrow 3CH_3COOH + H_2 \qquad \Delta G^0 = +20.2\ kJ.mol^{-1} \quad (2.15)$$

n-valerate breakdown:

$$CH_3(CH_2)_2COOH + 2H_2O \rightarrow CH_3COOH + CH_3CH_2COOH + 2H_2 \qquad \Delta G^0 = +48.4\ kJ.mol^{-1} \quad (2.16)$$

Caproate breakdown:

$$CH_3(CH_2)_4COOH + 6H_2O \rightarrow 2CH_3COOH + 8H_2 + 2CO_2 \qquad \Delta G^0 = +48.4\ kJ.mol^{-1} \quad (2.17)$$

Ethanol breakdown:

$$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^- + 2H_2 \qquad \Delta G^0 = +9.7\ kJ.mol^{-1} \quad (2.18)$$

Hydrogen (Homo-acetogenic):

$$4H_2 + 2CO_2 \rightarrow CH_3COO^- + H^+ + 2H_2O \qquad \Delta G^0 = -95.0\ kJ.mol^{-1} \quad (2.19)$$

The Gibbs free energy of change values are in standard temperature and pressure (273K and 101kPa) and at pH 7 (Lily, 2010; Madigan and Martinko, 2006; and Reaume, 2009).

All of the above equations, except for the homo-acetogenic equation, have positive Gibbs free energy change values (i.e. endothermic) under normal atmospheric surroundings (1 atm, pH 7 and 25℃). Reactions with a Gibbs free energy change greater than zero are only achievable in syntrophic situations, which in anaerobic digestions are usually between acetogenic and hydrogenotrophic methanogenic microorganisms. Syntrophy involves the deterioration of a substrate by two different organic matters, preserve energy undertaking it and that neither between the two is capable of deteriorating the substance by itself. A syntrophic mechanism requires the formation of hydrogen by one associate related to hydrogen utilization by the other, hence also known as interspecies hydrogen transfer. Syntrophy makes certain that high hydrogen levels do not frequently occur in the system (Sutaryo, 2012). Figure 2.2 represents the syntrophic between acetogenesis (butyrate and propionate) and methanogenic microorganisms.

As indicated in Figure 2.2, there is a maximum value established by the acetogenic microorganisms, and a minimum value by the methanogenesis microorganisms of syntrophic transfer of butyrate and propionate to methane. The hydrogen partial pressure should be maintained between 10^{-4} and 10^{-6} atm to permit oxidation of propionate and butyrate. The hydrogen restriction range is the shaded region in Figure 2.2, and is defined as the "hydrogen region". Any reactions falling outside the hydrogen region will inhibit the autotrophic methanogenesis or acetogenesis, and this will result in overall failure of the anaerobic process.

The theory of syntrophy in the anaerobic digestion has been widely observed between acetogens and methanogens, however there has been a debate about the syntrophic nature of acetogens and homo-acetogens. A study done by Wang *et al.* (2013) showed that 29% of the acetate was produced from syntrophic of acetogens and homo-acetogens.

2.6.4. Methanogenesis

Methanogenesis is the final step in the anaerobic process, where transitional products formed from other anaerobic steps are converted by methanogenesis microorganisms to biogas, which is dominated by methane (60%) and carbon dioxide (40%).

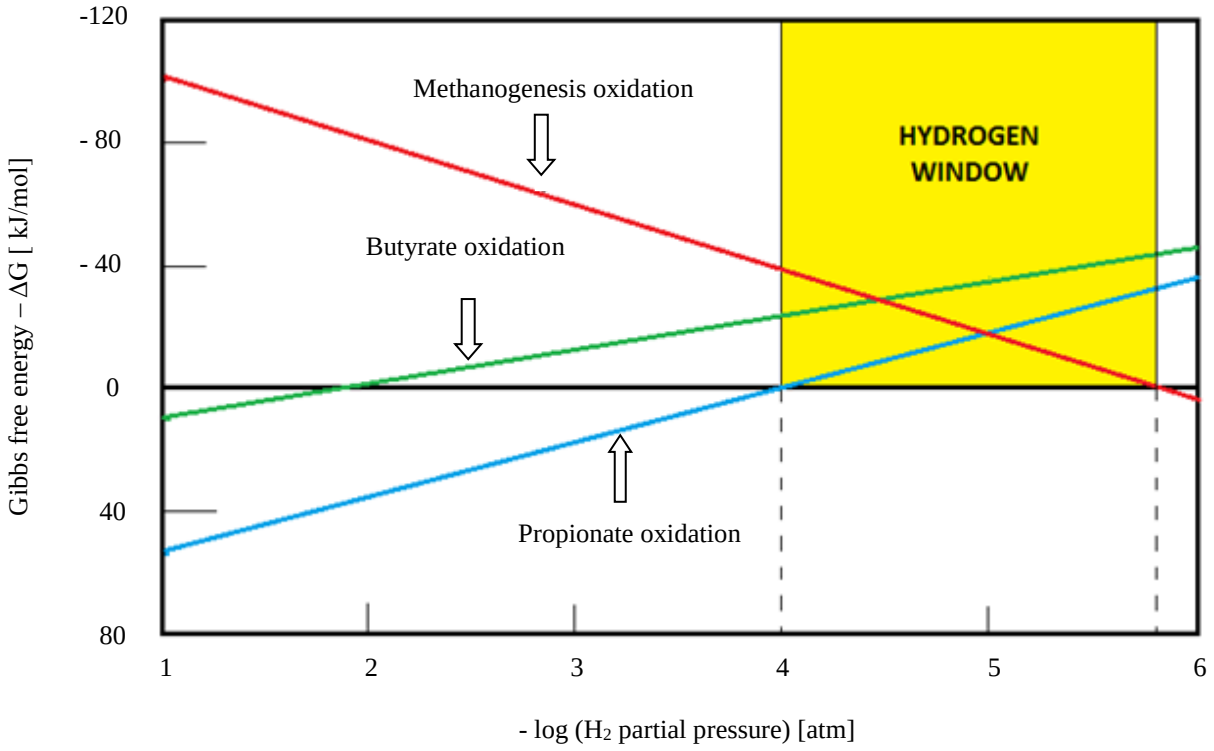

Figure 2.2: Syntrophic between acetogenesis (butyrate and propionate) and methanogenic microorganisms (Lu, 2006).

The methanogenesis stage is accomplished by the methane-producing bacteria, known as *Archaeas*, which are an extremely dedicated family of obligate anaerobic bacteria. The *Archaeas* consume several substances containing carbon monoxide, carbon dioxide, formic acid, acetic acid, methanol, methylamines and methyl mercaptan. *Archaeas* are obligates anaerobes since they cannot survive or grow in an oxygenated environment. On the other hand, the acid producers are principally facultative microorganisms that will protect the methanogenesis microorganisms by consuming any dissolved oxygen.

The methanogenesis microorganisms can generate methane in any of the following three pathways: hydrogenotrophic methanogens, acetoclastic cleavage of acetic acid and methylotrophic methanogens. Equations 2.20 to 2.27 below show these pathways:

Reduction of carbon monoxide:

$$4CO + 2H_2O \rightarrow CH_4 + 3CO_2 \qquad \Delta G^0 = -210.0\ \text{kJ.mol}^{-1} \quad (2.20)$$

Hydrogenotrophic/reduction of carbon dioxide:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad \Delta G^0 = -131.0\ \text{kJ.mol}^{-1} \quad (2.21)$$

Formic acid conversion:

$$4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O \qquad \Delta G^0 = -304.0\ \text{kJ.mol}^{-1} \quad (2.22)$$

Acetoclastic cleavage of acetic acid:

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad \Delta G^0 = -31.0\ \text{kJ.mol}^{-1} \quad (2.23)$$

Methanol conversion:

$$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O \qquad \Delta G^0 = -319.0\ \text{kJ.mol}^{-1} \quad (2.24)$$

$$CH_3OH + H_2 \rightarrow CH_4 + 2H_2O \qquad \Delta G^0 = -113.0\ \text{kJ.mol}^{-1} \quad (2.25)$$

Methylamine conversion:

$$4CH_3NH_3Cl + 2H_2O \rightarrow CO_2 + 4NH_4Cl + 3CH_4 \qquad \Delta G^0 = -230.0\ \text{kJ.mol}^{-1} \quad (2.26)$$

Methyl mercaptan conversion:

$$4(CH_3)_2S + 3H_2O \rightarrow H_2S + HCO_3^- + H^+ + 3CH_4 \qquad \Delta G^0 = -221.4\ \text{kJ.mol}^{-1} \quad (2.27)$$

The Gibbs free energy of change values are in standard temperature and pressure (273 K *and* 101 kPa) *and at pH* 7 *(Lily, 2010; Florencio, 1994).*

All of the methanogenesis pathways take place simultaneously in the methanogenesis step. The amount of methane generated via the methyltrophilic methanogens is infinitesimal when compared to that generated through the hydrogenotrophic and acetoclastic pathways, and is usually neglected. About 72% of methanogenesis microorganisms use the acetoclastic pathway to generate methane, with the remaining being that from the hydrogenotrophic step (Hufnagel, 2014). The limited amount of hydrogen produced in the process results in the acetoclastic reaction generating more methane than the reduction of carbon dioxide reaction.

2.7. Factors affecting Anaerobic Digestion

There are various dependent variables that have an effect on anaerobic digestion. These dependent variables, also known as process control indicators, assess the performance of the anaerobic process and provide a notification of upcoming process failure so that protective measures can be carried out timeously. Parameters that affect anaerobic digestion are: temperature, pH, alkalinity, total solids content, feed-to-inoculum ratio, metabolism time, toxicity accommodation, nutrients and carbon to nitrogen ratio. Proper control and design of the process's variables has to be done in order to optimize the system productivity, improve invariability, and avoid process upsets (Labatut and Gooch, 2012).

2.7.1. Temperature

Anaerobic digestion is a highly temperature dependent process like all biological systems. In anaerobic digestion, temperature can have an effect on the rate of microbial activity, growth, survival, and the concentration of pollutants in biogas. Furthermore, the amount of water in the final product is affected by the temperature, as the amount of water in the biogas is directly proportional to the process temperature (Beam, 2011).

Methane–producing microorganisms are temperature sensitive. Even the slightest changes in the surrounding conditions greatly affect the methanogenesis microorganisms**.** On the other hand, hydrolysis and acidogenesis are barely influenced by temperature. Haslaniza *et al.* (2010) studied how temperature affect the hydrolysis stage. The study was conducted at 30, 45 and 60℃. In their investigation the effect of temperature showed no significant improvement on the degree of hydrolysis.

The anaerobic process may be performed in either mesophilic ($30-40$℃) or thermophilic ($50-60$℃) temperature ranges. Although anaerobic digestion can be carried out in any of the two ranges there are optimum values in each temperature range. As observed from the biphasic temperature diagram depicted below (Figure 2.3), the optimum thermophilic and mesophilic temperatures are 35℃ and 55℃, respectively.

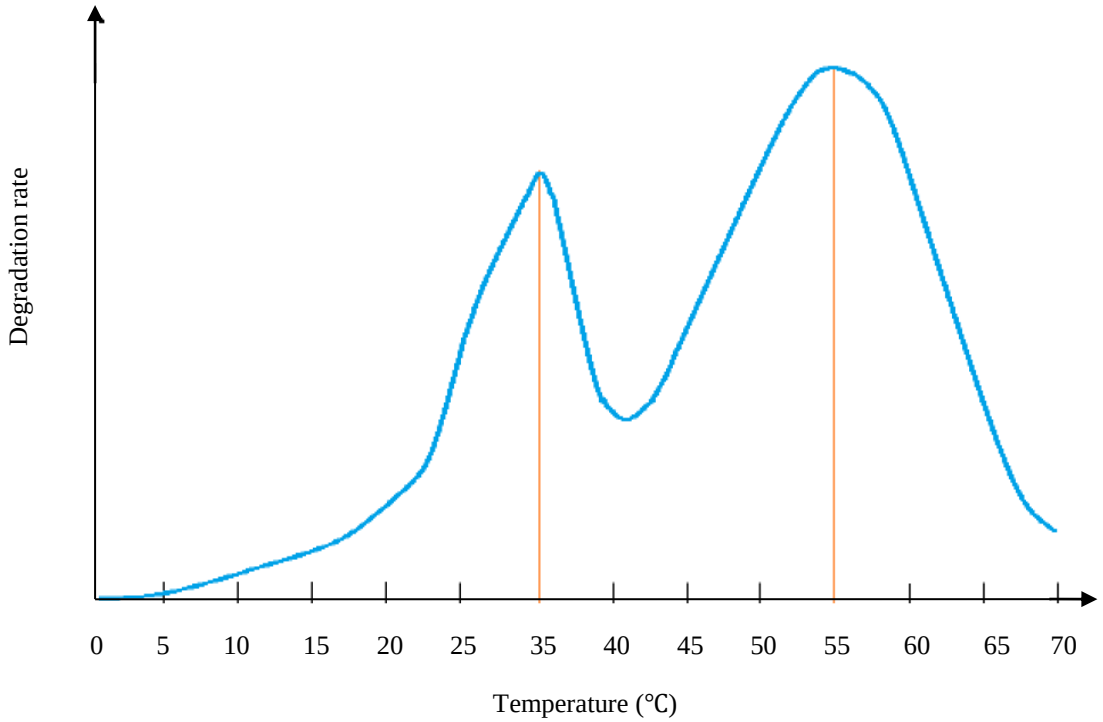

Figure 2.3: Biphasic temperature diagram (Juanga, 2005).

Knowing the best possible anaerobic temperature range is of supreme significance since both mesophilic and thermophilic temperatures present benefits and disadvantages. Table 2.2 represents comparisons between mesophilic and thermophilic temperature ranges.

Table 2.2: Mesophilic and thermophilic temperature ranges (Beam, 2011 and Nguyen, 2012).

Type	Mesophilic (30 – 40℃)	Thermophilic (50 – 60℃)
Retention time	High	Low
Odour possibilities	Low	High
Operational cost	Low	High
Heat required	Low	High
Stability	High	Low
Volatile fatty acid accumulations	Low	High
Organic matter reduction	Low	High
Growth rate of methanogenesis bacteria	Low	High
Microbial death rate	Low	High
Devastation of pathogens	Low	High
Metabolic rate	Low	High

Even though metabolism takes place much faster at thermophilic ranges, the additional energy required and process instability are likely to cancel out the benefits gained. The stability showed by mesophilic ranges balances the movements of all metabolic collections, and hence prevents accumulation of the transitional products in the digester. Digesters that are highly responsive to temperature variations; such as those exposed to cold environments, improper lagging, or small-scale anaerobic reactors, are better operated in mesophilic temperatures so as to reduce process failure (Ingenieurs, 2009). Moreover, with South Africa facing the electricity crisis, it is better to operate the digesters at mesophilic temperatures rather than thermophilic temperatures.

2.7.2. pH

pH is vital in anaerobic digestion as a whole, as it affects the growth and activity of bacteria. Even though this is true, the effect of pH differs from stage to stage. Hydrolysis and acidogenesis are less affected by pH, whereas acetogenesis and methanogenesis are very sensitive. Methanogenesis microorganisms are essentially slow growers, and are considered to be the most pH sensitive microorganisms of them all (Mateescu and Constantntinescu, 2011).

Methanogenesis microorganisms grow well at a pH between 6.3 and 7.8 i.e. a pH near the neutral range (Dlamini, 2009)**.** The rate at which methane is formed decreases below 6.3, thus reducing the overall production of biogas. Additional acids also build up quite rapidly at a pH less than 6.3, thus inhibiting the overall anaerobic process. A pH value greater than 7.8, on the other hand, delays the methanogenic rate, thus inhibiting the formation of the product gas. The overall anaerobic process fails beyond the methanogenesis limits. Due to the high impact that methanogenesis microorganisms have on the generation of biogas, the pH of the system is usually operated so as to favour them i.e. at neutral pH conditions.

2.7.3. Total alkalinity

Total alkalinity, which in anaerobic digestion is reported as $CaCO_3$ content, measures the buffering capacity of a digester solution. It also represents the ability of the reactor to

neutralise acids produced in the digestion process. This technique is by far the more reliable technique of measuring reactor imbalance when compared with pH measurement, as the generation of fatty acids will lower the total alkalinity drastically before the pH declines (Ward *et al.*, 2008).

Alkalinity is imperative for the treatment of wastewaters and waters. Alkalinity of wastewater and water is mostly a dependent of hydroxide, bicarbonate, and carbonates constituents. Total alkalinity may at times contain silicates, phosphates and borates. However, the constituents that largely affect the digester alkalinity are the equilibrium between bicarbonate and carbon dioxide, and that between ammonium and ammonia (Beam, 2011).

In the liquid phase, alkalinity is predominantly available in the form of bicarbonates, whereas, in the gas phase it is predominantly available in the form of carbon dioxide. Carbon dioxide is liberated during the deterioration of organic matters and both carbon dioxide and ammonia are formed during the decomposition of proteins and amino acids. The formation of ammonia brings about the generation of ammonium, which provides the most important source of hydroxide required to react with carbon dioxide to produce bicarbonate. The generation of carbon dioxide leads to the formation of carbonate, bicarbonate, and carbonic acid (Beam, 2011). Equations 2.28, 2.29 and 2.30 below show the ammonia to ammonium, and carbon dioxide to bicarbonate reactions:

Ammonia to ammonium:

$$NH_3 + H_2O = OH^- + NH_4^- \quad (2.28)$$

Carbon dioxide to bicarbonate:

$$CO_2 + H_2O = HCO_3^- + H^+ \quad (2.29)$$

$$OH^- + H_2CO_3 = HCO_3^- + H_2O \quad (2.30)$$

As much as alkalinity is required in the anaerobic process, there is a limit to the extent required. The optimum alkalinity range is between 1 000 and 5 000 $mg.L^{-1}$ as $CaCO_3$ in the liquid phase, and 25-45% CO_2 (biogas volume) in the gas phase, as indicated in Figure 2.4. The optimum alkalinity range aids in maintaining the pH at a constant level in the range 6.8 to 7.6, and also helps in buffering the effect caused by volatile fatty acids. Working within the alkalinity range ensures good digestion as well as formation of a high quality biogas

(Beam, 2011). Figure 2.4 shows limits of normal anaerobic treatment for optimized pH and alkalinity:

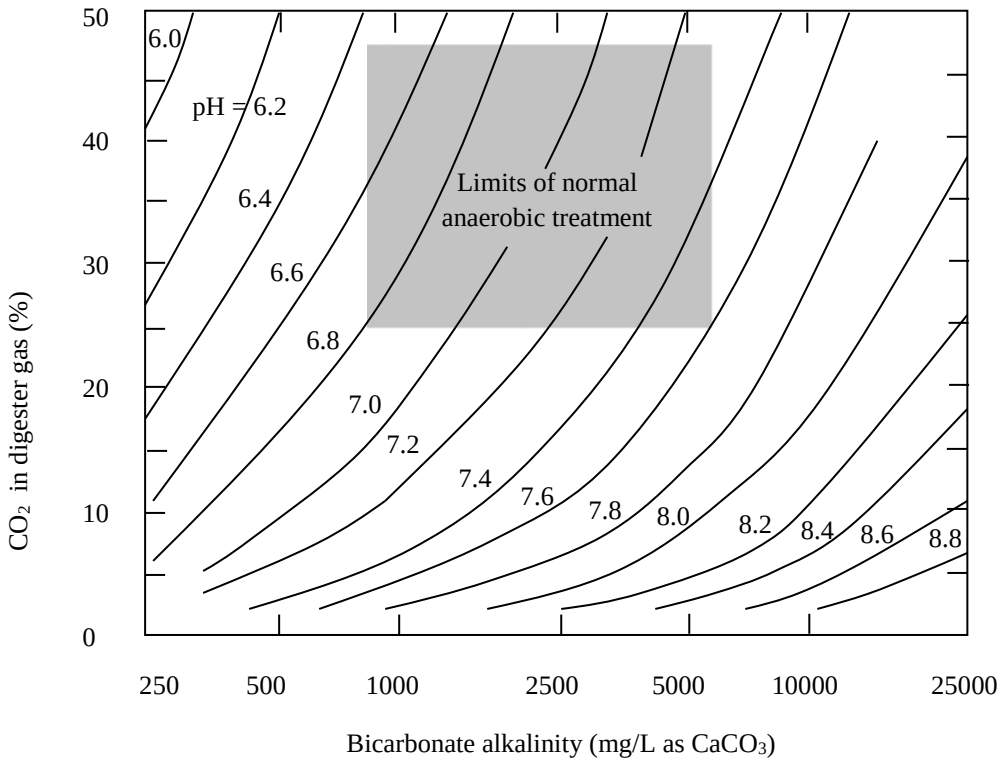

Figure 2.4: Limits of normal anaerobic treatment for optimized pH and alkalinity (Beam, 2011).

2.7.4. Total solids content

Total solids content may be defined as a measure of the total amount of substance left behind after all the moisture has been evaporated. There are mainly three categorisations of total solids content in anaerobic digestion:

- low or wet solids processes when <10% total solids ,
- medium or semi-dry solids are between 10 and 20%, and
- high or dry solids systems are between 20 and 40%.

The total solid content and the initial concentration of the substrate in the bio-digester can extensively influence how the digestion process performs and the quantity of methane formed. Dry anaerobic processes make the solution in the digester more compact, and provide high loading rates. The treatment step is easier and cheaper. The compactness and high loading rate improves the degree of stabilisation, which allows the dry process to

generate more biogas than a wet process. However, too dry conditions (over 40%) will limit the bacterial activity as microorganisms can only utilize organic molecules that are dissolved in water. Although dry conditions offer more advantages than wet conditions dry digester processes are mostly used with vegetable or municipal solid wastes rather than with manures (Ward *et al*., 2008). Wet processes, on the other hand, are usually used with manures.

2.7.5. Inoculation

Inoculum is introduced into the digester to give a lively source of microorganisms during start-up (Foucault, 2011). The use of an inoculum in a bio-chemical process is extremely important as it will speed up the process, improve stability and increase the formation of biogas. At first the substrate is used as an inoculum in a digester for days or even months to grow microorganisms and populate as much as possible. However, once the first run of the anaerobic process has been completed, the residual liquid from the process may be used as the inoculum for other anaerobic processes as it contains extremely energetic microorganisms which will drastically decrease the quantity of inoculum required in large scale reactors, and subsequently, the proportional reactor capacity.

Various inoculums are used in anaerobic processes essentially with the main purpose of promoting the degradation of the organic matter and improving the generation of biogas. To attain these benefits, the type and quality of inoculums should be known and taken into consideration before starting the anaerobic process. Digested manure and sewage sludge are the most frequently used inoculum sources because of their high propensity for biogas production and their high content of methanogens (Mateescu and Constantntinescu, 2011).

A comparative evaluation of cow manure against sewage sludge as inocula was done by Mateescu and Constantntinescu (2011). The main aim of the study was to determine which inoculum will generate more methanogenic microorganisms. The investigational outcomes demonstrated a high methanogens loading of the sewage sludge when compared to the cow manure. Conversely, cow manure results showed a higher amount of acidogens as compared to sewage sludge, which can be inhibitory to the growth of methanogenesis microorganisms. It was then concluded that sewage sludge is more appropriate to be used as inocula in anaerobic processes than cow manure. Even though sewage sludge looks to be the most

promising inoculum, naturally chosen strains or a mixture of artificial strains of bacteria may also be used.

Besides the nature of the inoculum, the amount of inoculum to be added in a digester is also important, and is often expressed as inoculum-to-feed ratio. Small amount of inoculum is usually required since the inoculum also plays a part in the generation of biogas, and as a result, its concentration might distort the results if biogas formation from inoculum is quite high in comparison to the feed substrate. Conversely, a small amount of inoculum might result in the anaerobic process being overloaded with acidification (Angelidaki and Sanders, 2004). Therefore, an optimum feed-to-inoculum ratio has to be known so as to attain all inoculum benefits.

A bio-chemical methane potential test was performed by Feng *et al.* (2013) to study the impact of substrate to inoculum ratios on vinegar. An optimum biogas yield was obtained at a feed to inoculum ratio of 1:1. Behera *et al.* (2010) investigated the formation of methane from food waste leachate with different feed to inoculum ratios. The highest biogas yield was obtained at a feed to inoculum ratio of 1:1. Fernandez and Encina (2009) did an investigation on the effect of feed to inoculum ratio on swine slurry. In their investigation, a faster degradation was seen with a ratio of 1:1. Conversely, a decline in methane yield was observed when the feed to inoculum ratio was increased further away from this ratio. Bueno *et al.* (2005) conducted an investigation on the effect of inoculum source in a biogas generation technique. In their investigation, the feed to inoculum ratio of 1:1 increased the digestibility of the process.

2.8. Sewage Sludge as a Substrate in Anaerobic Digestion

Globally, the anaerobic degradation of sewage sludge is possibly the largely significant anaerobic digestion process (Braun and Wellinger, 2009). The reason for its worldwide use in anaerobic digestion is that it is an extremely energetic substance which immediately leads to deterioration under anaerobic conditions. Furthermore, it has a low operational cost, it is appropriate to enhance the volume of water removal, and has organic matters containing energy.

Sewage sludge is an unavoidable and undesired secondary-product from effluent processing industries, the function of which is to purify effluent. It is produced by clarification both prior to and subsequently to the biological-processing stages, called primary sludge and secondary sludge, respectively. In other effluent processing industries, tertiary sludge may be generated. The volume of tertiary sludge is comparatively infinitesimal and is usually neglected.

Primary sludge also known as raw sludge is not of a uniform class due to several granular components. The solids fraction in raw sludge is approximately 5-10%, of which 70% is of biological material (Lu, 2006). Secondary sludge, also known as biological sludge, is composed of waste activated sludge (WAS) which is a product obtained from the aeration processing unit. The percentage of the biological sludge solids is approximately 1-6%, wherein the organic percentage is approximately 70%. Biological sludge is usually stable, and thus will produce a small amount of biogas if used in anaerobic digestion.

Effluent usually has dissimilar organics, which makes the changes in size allocation and fraction of raw sludge to be extremely complicated when compared to that of biological sludge. Once raw sludge, with distinct particular qualities is used in anaerobic digestion, the operation of the digester will possibly vary, particularly when the digester used is a continuous stirred tank reactor.

2.9. Anaerobic Co-digestion

Even though anaerobic degradation of sewage sludge is possibly the largest significant anaerobic digestion process, sewage sludge usually has low organic matter content, and hence low biogas yields are produced. The amount of organic matter and the nutrient balance can be enhanced by co-digesting a recalcitrant substrate, such as sewage sludge with a labile substrate or co-substrate - a process known as anaerobic co-digestion. By doing so, higher biogas yields are achieved together with higher process stability (Rao and Baral, 2011). Other than these advantages, anaerobic co-digestion has other benefits, such as: dilution of toxic compounds, and cost efficiency since one plant is used to treat more than one waste.

The advantages of increasing methane and biogas generation by co-digestion could result in financial advantages, which are significant enough to cancel out the operational and capital

expenses of the power generation equipment (Wohlgemut, 2009). Adding an easily biodegradable or labile organic substance will raise the reactor volume, for the reason that a biodegradable substance can prevail over some inhibiting pathways, which then leads to better removal efficiencies.

Anaerobic combination of Elephant grass – AB (co-substrate) and maize leaves – BC (primary substrate) was studied by Olugbemide *et al.* (2012) in an anaerobic reactor at psychrophilic temperature of 29+3 ℃. Exceptional consideration was paid to antagonistic and synergistic influence of co-substrates on the generation of biogas so as to find the optimum combination. Five distinct anaerobic combinations were arranged based on blend quantities in reactors numbered 1 to 5. Reactor 1 was fed with only maize leaves, which operated as base. The combinations of other reactors were: Reactor 2 was fed with 10% AB and 90% BC, Reactor 3 was fed with 20% AB and 80% BC, Reactor 4 was fed with 30% AB and 70% BC, and Reactor 5 was fed with 40% AB and 60% BC. At the end of the digestion process, Reactor 1 generated biogas volume of 520 ml. Reactors 5 and 2 had positive influences on biogas generation with biogas volumes of 610 ml and 870 ml, respectively. But, Reactors 4 and 3 had negative impacts on biogas volume generating 400 ml and 460 ml, respectively. Reactor 5 revealed 67.3% greater biogas generation than anaerobic digestion of Reactor 1 (only maize leaves).

Zhang (2010) did a study on the anaerobic combination of whey and municipal sewage sludge using the continuous stirred tank reactor, with height of 0.5 m and radius of 0.1 m. Biogas production for anaerobic digestion was compared to that of anaerobic co-digestion. The results demonstrated higher biogas generation for co-digestion than municipal sewage sludge alone, with biogas productions of 3.2 ± 0.1 L/d and 1.7 ± 0.1 L/d, respectively.

Anaerobic co-digestion of food waste and raw sludge was done by Prabhu and Mutnuri (2016) to find out the optimum food waste to sludge ratio. The experiments were conducted at ratios of 1:1, 1.5:1, 2:1, 1:1.5, and 1:2. The optimum biogas production of 0.823 L g/VS was found at food waste to raw sludge ratio of 1:2. Moreover, the VS reduction at this ratio was 84.8%.

Gomez *et al.* (2006) did a study on the mesophilic batch co-digestion of fruit and vegetable waste together with primary sludge. In their study the maximum methane yield was 600 L /kg

VS, with the biogas production rate of 4.40 L/d. Martin-Gonzalez *et al.* (2010) did an investigation on the mixing of fat, oil and grease waste with municipal solid wastes. The highest methane yield was 350 L/kg VS, and the highest biogas production rate was 13.6. An anaerobic investigation by Purcell and Stentiford (2000) was conducted on the combination of biodegradable superstore disposals and sewage sludge. Experiments were conducted in completely stirred digesters, at 35 °C. An overall solids destruction of 28% was observed for the control cells. The co-digestion cells, however, attained drastically higher destruction of 54%. The degradability of volatile solids in the co-digester was much higher than the control units, i.e. a solid destruction 63% for co-digestion and 37% for digestion.

Kanchanasuta *et al.* (2017) studied the effect of crude glycerol on palm oil decanter cake at glycerol concentrations of 0.25 to 2% (on weight basis). The experiment was conducted in 100 mL serum bottle at a thermophilic temperature of 55℃. The optimum hydrogen potential yield of 0.871 L (g TS removed)$^{-1}$ was achieved at glycerol concentration of 2%.

Leandro *et al.* (2016) conducted a study on the effect of crude glycerol on swine manure in biogas generation. The glycerol concentrations were 0, 5, 10, 20, 30 and 40% (on weight basis). The optimum biogas generation was found at a glycerol concentration of 5%. Above 5%, the process experienced inhibition.

Athanasoulia *et al*. (2014) investigated the influence of crude glycerol on sewage sludge. In their investigation glycerol concentrations of 0, 2, 3, and 4% (v/v) were used. The system could not function properly at 4% glycerol because of overloading. Foucault (2011) investigated the anaerobic co-digestion of chicken effluent using commercial and industrial crude glycerols as co-substrates. The process was operated at mesophilic temperature of 35°C. The results showed higher yields for co-digestion compared to the digestion of chicken effluent alone, with an average of 0.555 and 0.540 L per gram VS fed, respectively. The results further demonstrated that the methane yields for commercial and industrial glycerols were 0.702 and 0.375 L (gram volatile solids fed)$^{-1}$, respectively. These results showed that commercial glycerol had the highest methane yield as compared to industrial glycerol. Commercial glycerol had higher impurities (mainly lipids) than industrial glycerol which was the reason for it to have the highest yield.

Wohlegemut (2009) did an investigation on co-digestion of manure with glycerol at different co-substrate concentrations. He found out that the optimum methane yield was achieved at 1% glycerol (wt%). Poor digestion and COD overloading were observed beyond this value. Kullavanijaya and Thongduang (2012) did an investigation on the anaerobic combination of cassava effluent and biodiesel waste. The most favourable biodiesel waste concentration which generated the greatest biogas yield was recommended to be 2.0% (v/v). Above this value, digestion was poor as represented by a fall in pH, together with lower methane fraction, and high acid build up.

2.10. Design of Experiments (DOE)

In the view of the fact that the objective of the investigation is to acquire data, efficiency in research may well be defined as the amount of valuable data acquired per unit cost. One method for improving this research efficiency is that of the statistically designed experimentation (Pavelic and Saxena, 1969). This logical, efficient method speeds up the solution of the investigational projects by allowing predictions to be done before all experimental trials are completed. This technique also shows the relative significance of process factors as well as their interactions, something not usual achievable with other methods (Pavelic and Saxena, 1969).

2.10.1. Response surface methodology (RSM)

There are various kinds of experimentations used in real-life problems and circumstances. When treatments are from a continuous variety of values, then the actual correlation between input *x*'s and response variables *y* may possibly be unknown or perhaps very complicated (Bradley 2007; Carley *et al.*, 2004). The response variable may be approximated using the following equation, which is well known as the Response Surface Methodology (RSM):

$$y = f(x_1, x_2, \ldots, x_p) + e \qquad (2.31)$$

The *y* value is the response or dependent variable, and the *x* values are independent input variables from *1* to *p*. The *e* term is the total experimental error term, and denotes any error

on the response that is measured and also other kind of deviations not included in the RMS equation. The error term *e* is understood to comprise of a normal distribution with variance s^2 and zero mean (Carley *et al*., 2004).

The RSM is a combination of both statistical and mathematical methods, and is of great use for the analysis of problems and modelling wherein a dependent or response variable is affected by many input variables and the main aim of the problem is the optimization of the response variable. There are three kinds of RSMs: the first-order, the second-order, and three-level fractional factorial (Bradley, 2007). Since the actual response function *f* is usually not known, the experimenter has to formulate a correct estimation for the response function *f*. The experimental researcher normally begins with a low-order polynomial in particular small area. The estimating response surface is said to be a first-order model if the response surface generated is expressed by a straight line of input variables. However, if the estimated response surface has a parabolic curvature, then a polynomial (for example, a second-order model) has to be implemented.

2.10.2. First-order model

Perhaps the most well-known first-order model is the two-level fractional or full factorial, wherein all variables are examined at two levels. The first order-model is very useful when the aim of the experiment is to see which variable is of high importance, and possible, when dealing with a lot of variables. A first-order model is a multiple-regression model. A multiple-regression model is a mathematical model which has three or more independent variable. A first-order model with a single response variable *y*, *p* regressor variables and N_e experimental runs may possibly be represented by the following expression:

$$y_r = \beta_0 + \beta_1 x_{r1} + \beta_2 x_{r2} + \beta_3 x_{r3} \ldots . + \beta_p x_p + e_r = \beta_0 + \sum_{l=1}^{p} \beta_l \, x_{rl} + e_r \qquad (2.32)$$

where: *r* = 1, 2, 3,....., N_e, and

l = 1, 2, 3,....., *p*.

The above first order expression holds for $p < N_e$. The response y is a dependant of the independent variables $x_1, x_2, x_3 \ldots, x_p$, indicated as *f*, as well as the experimental error term e_r. The β_l term is called the regression coefficient, and computes the estimated change in

output y over a change in x_r (Bradley, 2007). The term x_{rl} represents the l^{th} level of independent variable and r^{th} examination.

2.10.3. Second-order model

The second-order model is very helpful in estimating a section of the whole response surface that has a curvature. This type of model has all of the first-order model terms, as well as all cross product terms and all quadratic terms. The second-order model may be approximated using the following expression:

$$y_r = \beta_0 + \sum_{l=1}^{p} \beta_l x_l + \sum_{r=1}^{N_e} \beta_{rr} x_r^2 + \sum \sum_{l>r} \beta_{rl} x_r x_l + \varepsilon = \beta_0 + x_r' \beta + x_r' \beta x_r + \varepsilon_{rl} \quad (2.33)$$

where: $\beta = [\beta_1, \beta_2, \beta_3, \ldots., \beta_p]$,

$x_r = [x_{1r}, x_{2r}, x_{3r}, \ldots, x_{rp}]$,

$\beta_{rl} x_r x_l$ are cross product terms, and

$\beta_{rr} x_r^2$ are quadratic terms.

The second-order is useful when the experimental aim is to optimize the experimentation runs. The second-order model is also very flexible, since it estimates the response surface in the neighbourhood and can take a wide range of functional forms (Bradley, 2007). Moreover, there is a substantial realistic practice demonstrating that second-order models are very successful in dealing with real-life response surface problems (Carley *et al.*, 2004). For these reasons, the second-order model is often a useful approximation of the actual response surface as compared to the first-order model. The most well-known second-order models are: the Central Composite Designs (CCD), the Doehlert matrix design (DM), and the Box and Behnken Designs (BBD).

2.10.4. Comparison between CCD, DM and BBD

The comparison amongst the 2^{nd}-order designs cited have shown that DM and BBDs are more efficient than CCDs, taking into consideration that the efficiency of one experimental design (μ) is given by the following equation:

$$\mu = p/N_e \quad (2.34)$$

where: p – is the number of coefficients of the model estimated.

Table 2.3 below shows a comparison of the efficiency of CCD, BBD, and DM designs:

Table 2.3: Efficiency comparison of DM, BBD and CCD (Ferreira *et al.*, 2004).

Variables (n)	**Number of coefficients (p)**	**Number of experiments (N_e)**			**Efficiency = p/N_e**		
		DM	**BBD**	**CCD**	**DM**	**BBD**	**CCD**
2	6	7	-	9	0.86	-	0.67
3	10	13	13	15	0.77	0.77	0.67
4	15	21	25	25	0.71	0.60	0.60
5	21	31	41	43	0.68	0.61	0.49
6	28	43	61	77	0.65	0.46	0.36
7	36	57	85	143	0.63	0.42	0.25
8	45	73	113	273	0.62	0.40	0.16

In general, whatever the number of variables (n) is, the DM design is the most efficient design system followed by the BBD. The DMs are also more efficient in mapping space: adjoining hexagons can fill a space efficiently and fully, because the hexagons fill space with no overlap. Furthermore, the DM has the ability for sequentially, where experiments can be used again when the limits have not been correctly selected initially (Ferreira *et al*., 2004). Despite these advantages, the uniform shell designs of DM have not been broadly used because their design variables are treated unequally.

3. CHAPTER 3: MATERIALS AND METHODS

3.1. Introduction

The methodology for the characterization of the streams and co-digestion to produce biogas is presented. This chapter is subdivided in five sub-sections: feed substrates and inoculum, equipment set-up, digestion experiments, scope of the analyses, and sampling analysis. A series of experiments were carried out on glycerol, industrial oleo-chemical products (IOP), fruit juice, and soft drinks wastewaters taken from local industries.

3.2. Feed Substrates and Inoculum

3.2.1. Primary substrate

The primary substrate that was used was sewage sludge, which was obtained from Amanzimtoti Treatment Works (ATW). About 50 L of sewage sludge sample was collected. The collected sample was then characterized for COD, pH, alkalinity, volatile fatty acids (VFA), density, volatile solids (VS) and total solids (TS). After characterization, about 25 L of the sample was immediately used in the digesters for the experimentation, and the remaining 25 L stored in the refrigerator (14 ^{0}C) for later use.

3.2.2. Secondary substrates

The secondary substrates that were used were:

a) **Industrial oleo-chemical product** (IOP), which is wastewater from Industrial Oleo-chemical Products in Jacobs, Durban.
b) **Glycerol** was also obtained from Industrial Oleo-chemical Products in Jacobs, which is a secondary product from the biodiesel manufacture. The company uses vegetable and sunflower oils wastes as feedstock for the biodiesel production. Glycerol contains approximately 33% pure glycerol, 42% water, and 25% methanol.

c) **Fruit juice** and **soft drink wastes** from ABI Company in Phoenix, Durban.

Approximately 500 mL of each of the secondary substrates was collected and sent to ATW. The samples were then characterized for pH, VFA, alkalinity, density, COD, TS and VS. The samples were then kept in containers and immediately used in digesters or stored in the refrigerator (14 ^{0}C) until the digesters were operated.

3.2.3. Inoculum

At ATW, primary sludge is co-digested with waste activated sludge in two digesters. The digestate from one of the co-digesters was used as inoculum for this investigation. Approximately 50 L of inoculum sludge was taken and analysed for pH, alkalinity, VFA, COD, density, TS and VS. After characterization, approximately 25 L of the sample was immediately used in digesters for the first digestion trial, with the remaining 25 L stored in the refrigerator (14 ^{0}C) for use in the second trial.

3.3. Equipment Set-up

Six 6 L thermal double wall polyvinyl chloride (PVC) digesters were used for the experiments, each having a working volume of 4.8 L. The total volume of the digester (6 L) was designed based on the working volume (4.8 L) as illustrated in Appendix C1. Assembly drawing and first angle drawings for the main body of the digester are shown in Appendix D. Figure 3.1 below shows the digester set-up at Durban University of Technology (DUT). Each digester had an inside diameter and height of 16 cm and 32 cm, respectively. The digesters were fabricated with clear PVC pipe to allow for the viewing of the substrates inside. Two 16 cm PVC discs were used for the bottom and top caps of the digester. The top cap, which was removable, was connected to the digester body by four bolts and nuts. Each digester top cover had three holes drilled:

a) The first hole consisted of a feeding pipe, which was used for liquid feeding and sampling.

b) The second hole was used for gas sampling.
c) The third hole consisted of a tubing connector (inside diameter of 0.6 cm), which was used to transport biogas to the gas collector.

These holes were silicone gel to prevent gas leaks or exchange and introduction of air to the digester.

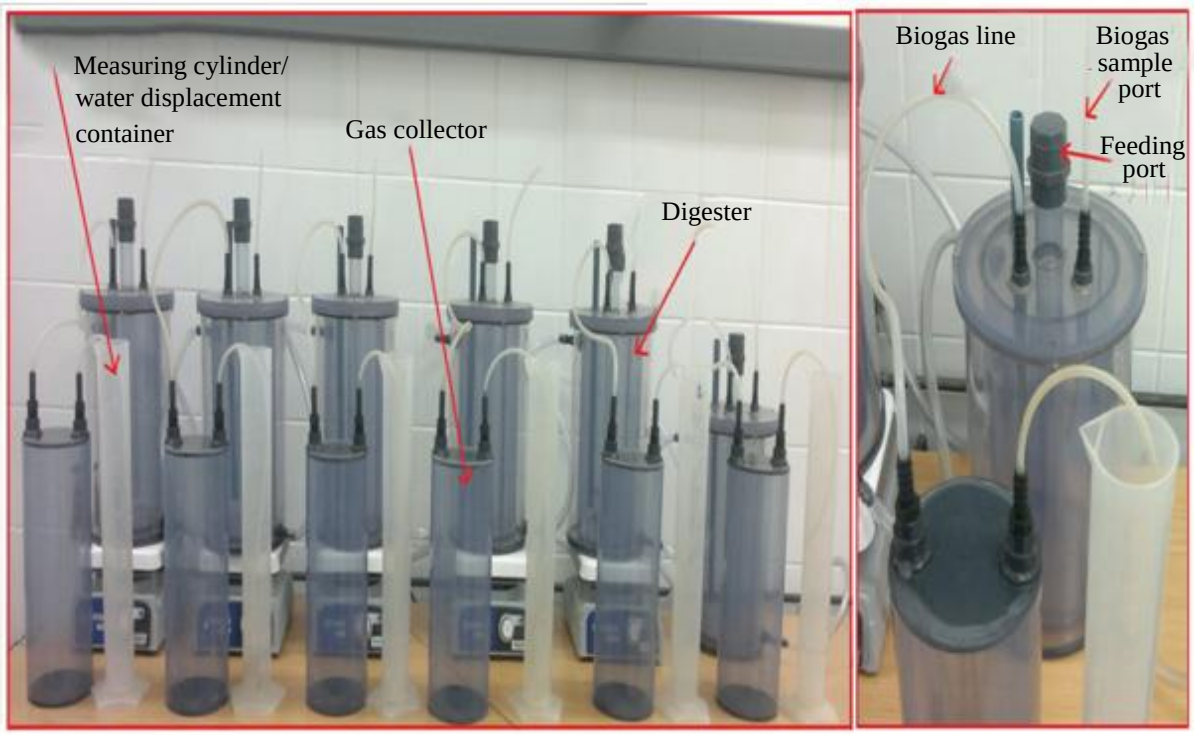

Figure 3.1: Digester set-up (left) and the digester top view (right).

The gas collectors were 6 L PVC containers of 16.0 cm inside diameter and about 32.0 cm long, with two 16.0 cm PVC discs used for the bottom and top caps of each digester (see Appendix C2 for gas collector design). The top cap of each of the digesters had two ports drilled. One of the ports consisted of a tubing connector (0.6 cm inside diameter) and was used to allow biogas coming from the digester. The second port also consisted of a tubing connector (0.6 cm inside diameter), which was used to transfer the displaced water to the water displacement container. The water displacement containers were 8 L PVC containers with inside diameter and height of 20 cm and 32 cm, respectively. The top part of the displacement containers was open to atmosphere. Figure 3.2 shows the Process and Instrumentation Diagram (P&ID) for the anaerobic process. Table 3.1 below shows the unit and stream descriptions.

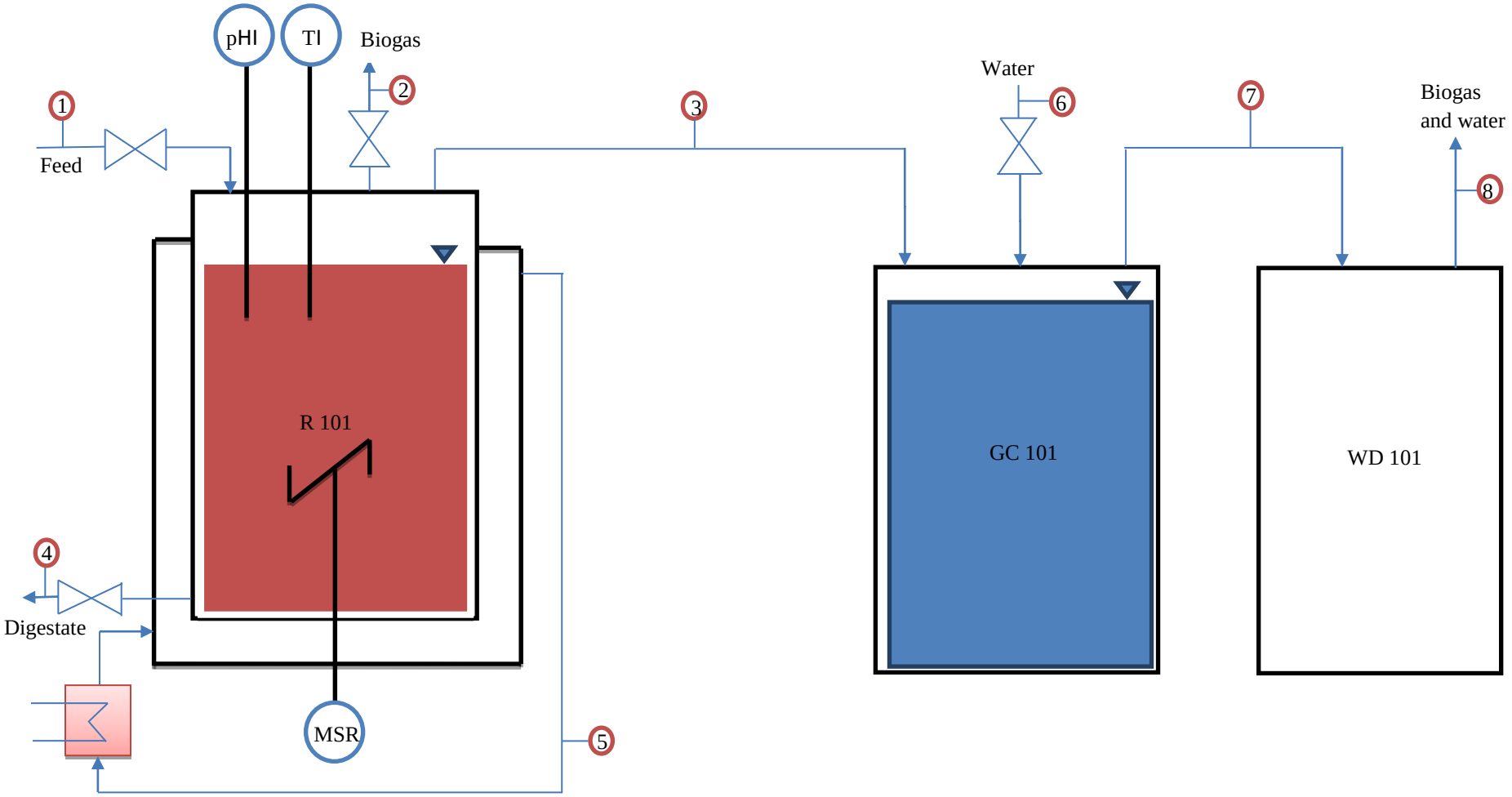

Figure 3.2: P&ID for a single digester.

Table 3.1: Unit and stream descriptions.

Unit name or stream number	Description
1	Feeding port – influent
2	Biogas sample port
3	Biogas line to the gas collector
4	Digestate outlet line
5	Circulating water line to and from the water bath
6	Water inlet
7	Water-biogas line to the water displacement container
8	Water-biogas exit
GC 101	Gas collector
MSR	Magnetic stirrer
pHI	pH indicator
R 101	Reactor or digester
TI	Temperature indicator
WD 101	Water displacement container

The six digesters were kept at a constant temperature (either psychrophilic, mesophilic or thermophilic) by two circulating water baths. The first three digesters were controlled by one water bath, and the remaining three digesters temperature were controlled by the second water bath.

Mixing in anaerobic digestion is beneficial because it removes scum and prevents the generation of layers with varying temperatures. Furthermore, it gives good interaction between the energetic biomass and the sludge. The substrate inside the five digesters was agitated by magnetic stirrers.

3.4. Digestion Experiments

In order to achieve the investigation's two objectives, two batch trials were carried out. The first batch trial was used to investigate the effect of soft drink wastewater, fruit juice wastewater, IOP wastewater and glycerol on the anaerobic process at 37.5 °C. The main aim was to find the substrate that will generate the highest biogas and methane yield. About 20

mL of the secondary substrate was added to the digesters by a manual pipette. The prepared samples were then mixed with 2.4 L of inoculum and 2.4 L of water. An additional digester (control) was fed with 2.4 L of inoculum and 2.4 L of sewage sludge.

The second batch trial was carried out to optimize operating conditions to maximize the methane yield in the biogas produced. This was achieved by testing the effect of temperature, substrate to inoculum ratio and secondary substrate concentration on the secondary substrate with the highest methane yield in the first trial. The digesters were tested at psychrophilic, mesophilic and thermophilic temperatures i.e. between 25℃ and 50℃. According to Luostarinen, Luste and Sillanpaa (2009), the optimum substrate to inoculum ratio (VS substrate/ VS inoculum) is 1. The investigation was therefore tested between 0.5 and 1.5. The optimum range of glycerol concentration is between 1g/L (10 mL glycerol) and 5 g/L (22 mL glycerol) of digester (Foucault, 2011). The digester is usually inhibited if the glycerol concentration is above 5 g/L (22 mL glycerol); therefore the secondary substrate volume was tested between 10 ml and 34 ml. The reason for going beyond 22 ml was to find out whether the process will be inhibited or not. The type of DOE used for these three variables was the Box-Behnken Design (BBD) with three centre points. The number of experimental runs was 15 (refer to appendix E for calculation of the number of experimental runs). Table 3.2 and Table 3.3 present the un-coded and coded feed combinations for a BBD for a there variable system, respectively:

3.5. Batch Time

3.5.1. First batch trial

Amongst the substrates tested in the first batch trial, glycerol was the most complex as it contains lipids, and thus will likely have the highest batch time than the other substrates. The batch time for the first batch trial was done based on glycerol. Foucault (2011) studied the anaerobic treatment of chicken wastewater on crude glycerol. Approximately 20 mL of crude glycerol, 2.5 L of wastewater and 2.3 L of inoculum were fed to a 4.8 L digester. The batch digesters were operated at a mesophilic temperature of 35℃. The digester ceased to generate biogas on day 20. The first objective of this investigation was to operate at 37.5℃.

Table 3.2: Un-coded feed combinations for a BBD for a three-variable system.

Std	Run	Temperature (℃)	Inoculum / substrate ratio (g VS/ g VS)	Secondary substrate volume (ml)
14	1	37.5	1.0	22
9	2	37.5	0.5	10
4	3	50.0	1.5	22
1	4	25.0	0.5	22
8	5	50.0	1.0	34
15	6	37.5	1.0	22
2	7	50.0	0.5	22
12	8	37.5	1.5	34
7	9	25.0	1.0	34
11	10	37.5	0.5	34
3	11	25.0	1.5	22
6	12	50.0	1.0	10
5	13	25.0	1.0	10
13	14	37.5	1.0	22
10	15	37.5	1.5	10

Table 3.3: Coded factor levels for a three-variable BBD system.

Std	Run	A: Temperature (℃)	B: Substrate / inoculum ratio (g VS/ g VS)	C: Secondary substrate volume (ml)
14	1	0	0	0
9	2	0	-1	-1
4	3	1	1	0
1	4	-1	-1	0
8	5	1	0	1
15	6	0	0	0
2	7	1	-1	0
12	8	0	1	1
7	9	-1	0	1
11	10	0	-1	1
3	11	-1	1	0
6	12	1	0	-1
5	13	-1	0	-1
13	14	0	0	0
10	15	0	1	-1

The feed volumes used were almost the same as those of Foucault (2011), with substrate volume of 20 mL, 2.4 L of water and 2.4 L of inoculum. The batch time for the first batch trial was chosen to be 20 days.

3.5.2. Second batch trial

Zeng *et al.* (2010) investigated the effect of inoculum to substrate ratio on methane yield. A mixture of lake water and algae bloom was mixed with dairy cattle manure at a substrate to inoculum ratio of 0.5, 1.0 and 2.0 (on VS basis). Biogas production ceased production on day 30. The batch time for the batch trial, where the effect of substrate to inoculum ratio was investigated, was set for 30 days.

Amongst the substrates tested in the first batch test glycerol possible had lipids. The batch time for the optimization of the concentration of the secondary substrate was based on glycerol. The effect of glycerol concentration on anaerobic digestion was studied by Foucault (2011). The glycerol concentrations were 4.6, 5.0 and 10.0 g/L. The production of biogas ceased on day 18 for the 4.6 and 5.0 g/L digesters, however the 10.0 g/L digester did not stabilise. The batch time for the second batch trial on the effect of the secondary substrate concentration on anaerobic digestion was for that reason assumed to be above 18 so as to allow the 9.0 g/L sample to cease biogas production. A batch time of 30 days was adopted.

Pandey and Soupir (2012) studied the impacts of temperature on biogas production in dairy manure. The study was carried out 52.5, 37 and 25℃, and the batch time was 76, 40 and 76, respectively. The batch time for the second batch trial to investigate the effect of temperature was assumed to be close to that of the 25 ℃ digester. A batch time of 70 days was selected.

3.6. Characterization

Since the amount of organic substance present in a substrate is proportional to the amount of biogas generated, the characterization of the amount of organic substance present in the

substrate is of high importance. Two types of organic substance representatives were characterized in the liquid substrates prior to performing the experiments: COD and volatile solids. While analysing for the volatile solids content the total solids content was indirectly analysed. This was useful in determining the amount of inorganic substance present in the substrates. The amount of organic substance degraded and digestion process effectiveness can be measured by the reduction in the volatile solids content or COD of the feed substrate and the digestate. To measure these useful parameters, liquid samples were collected on day one and on the last day of each trial for the determination of COD and volatile solids content. Additional liquid samples were collected on day one and on the last day of each trial, which was useful in measuring indicators of process stability; such as alkalinity, volatile fatty acids and pH. To improve the accuracy, samples were gathered in duplicates, with about 80 mL of samples collected. Additional daily liquid samples were collected to measure the pH. Prior to analyses, the samples were well mixed by shaking so as to obtain uniform sampling. After analysing for pH, the samples were fed back into the digesters via the feeding port so as to maintain a constant digester volume. The pH was measured and recorded during the experiments. Raw data for pH, VFA and alkalinity is shown in Appendix H1. Average values were taken for the results section, which are show in Appendix H2.

Effective anaerobic digestion of a substrate cannot take place without generation of the acceptable biogas volume. The conversion of organic material to biogas is an important anaerobic process variable. Measurements of the daily volumes of biogas generated were taken from the digesters. The biogas volumes were indirectly measured as the amount of water displaced in the 6 L liquid displacement collector. Daily gas samples were taken for the analyses of biogas composition. Gas sampling was done via a biogas sampling port positioned on top of the digester. A syringe was inserted in the gas sampling port to obtain biogas. About 70 mL of biogas was sampled which was analysed without delay for gas composition. The system was blacked out so as to not generate hydrogen sulphide (H_2S). Raw data for biogas compositions is shown in Appendix J1. Average values were taken for the results section, which are show in Appendix J2. The volumes of biogas were recorded on a daily basis, and were then converted to standard temperature and pressure. Raw data for biogas production is shown in Appendix F1. Average values were taken for the results section, which are show in Appendix F2.

3.7. Analysis of Substrates

3.7.1. Total and volatile solids

Standard methods were used for the evaluation of TS and VS contents, as proposed by APHA (2005). The following procedure was used for the TS and VS contents:

a) Each sample was well mixed by shaking.
b) Then, 30 mL of sample was dispensed into a crucible. Samples were analysed in duplicates so as to improve accuracy. The crucibles were kept in a drying oven at 105℃ all night for the determination of total solids.
c) The dried samples were then weighed.
d) The crucibles were then partly cooled in atmosphere until nearly all of the heat was removed.
e) The partly cooled crucibles were then transferred to a desiccator for final cooling to atmospheric conditions. The desiccator was used to cool and further dry the samples.
f) The dried samples were then inserted in a furnace at 550℃ for about 2 hours for the determination of the volatile solids.
g) The samples from the furnace were then weighed using a Sartorius TE1502S balance with a resolution of 0.1 mg (See Figure 3.3).

Figure 3.3: Weighing balance (Sartorius TE1502S).

The equations that were used for the determination of total and volatile solids content are illustrated in Appendix G2. Moisture content, total solids and volatile solids analysis were performed and results were interpreted using relevant equations. Feed characterization of all substrates that were used in the first digestion trial is shown in Appendix G1. The weights were measured in duplicate so as to improve accuracy.

3.7.2. COD

Chemical oxygen demand (COD) is an alternative technique of determining the dissolved substance in a solution, and is usually a constraint in anaerobic digestion. Of all COD measuring techniques that use other oxidants (for example, potassium permanganate - $KMnO_4$), the dichromate reflux technique is preferred as it is applicable to a number of wastes, and it is easy to use. It is also preferred for its greater oxidizing capability. The COD test determines the quantity of oxidant (dichromate ion, $Cr_2O_7^{2-}$) that reacts in a solution after deteriorating in a COD reactor, and is represented in milligrams oxygen equivalent per litre (mg O_2/ L).

Reactor digestion method 8000 (HACH®, Loveland, Colorado) was used for the COD test:

a) Approximately 25 mL of liquid was sampled from each digester.
b) The sample was then well mixed by shaking so as to ensure a homogeneous sample-solution.
c) Then, 0.2 mL was drawn out of the sample, which was then transferred into a COD vial by a pipette.
d) An additional 0.2 mL of distilled water was transferred into a COD vial by a pipette, which was then used as a base.
e) The vials were then tightly closed, and then turned upside down several times.
f) As soon as the solution inside the vials was well mixed, the vials were placed into a (Model 200) COD reactor (HACH®, Loveland, Colorado) for heating at 150℃ over a period of two hours.
g) The COD reactor was switched off and the vials were allowed to cool for about 25 minutes.

h) The vials were then removed from the COD reactor and allowed further cooling to room temperature.
i) The cooled vials were then wiped with a cloth.
j) The vials were then measured for absorbance by a (Model 3900) spectrometer/colorimeter (HACH®, Loveland, Colorado). Since the precipitated solids hinders with absorbance measurements, samples were permitted to gravitate prior to COD reading.

Figure 3.4 and Figure 3.5 show the digestion vials, and (Model 200) COD reactor and (Model 3900) spectrometer, respectively. The spectrometer that was used could read COD content as high as 1 600 mg / L. The inoculum vial was light green, whereas the raw sludge vial was dark green. The vials for the glycerol, IOP, fruit juice and soft drink samples were very dark (black), which meant that the vials had a higher COD content in comparison with the dichromate reagent. Readings could not be taken for the dark vials. The dark samples were diluted with deionised water at a substrate to deionised water ratio of 1 mL: 9 mL, and then analysed. Raw data for VS and COD is shown in Appendix I1, and was taken in duplicates. Average values were taken for the results section, which are show in Appendix I2.

Figure 3.4: Digestion solution (vials) for COD 20 – 1500 ppm range.

Figure 3.5: HACH® (Model 200) COD reactor (left) and HACH® (Model 3900) spectrometer (right).

3.7.3. Alkalinity, pH and VFA

A (Model 840) titration manager (TitraLab®, Villeurbanne, France) was used for the determination of pH, alkalinity and VFA. Figure 3.6 shows the Tim 840 titration manager:

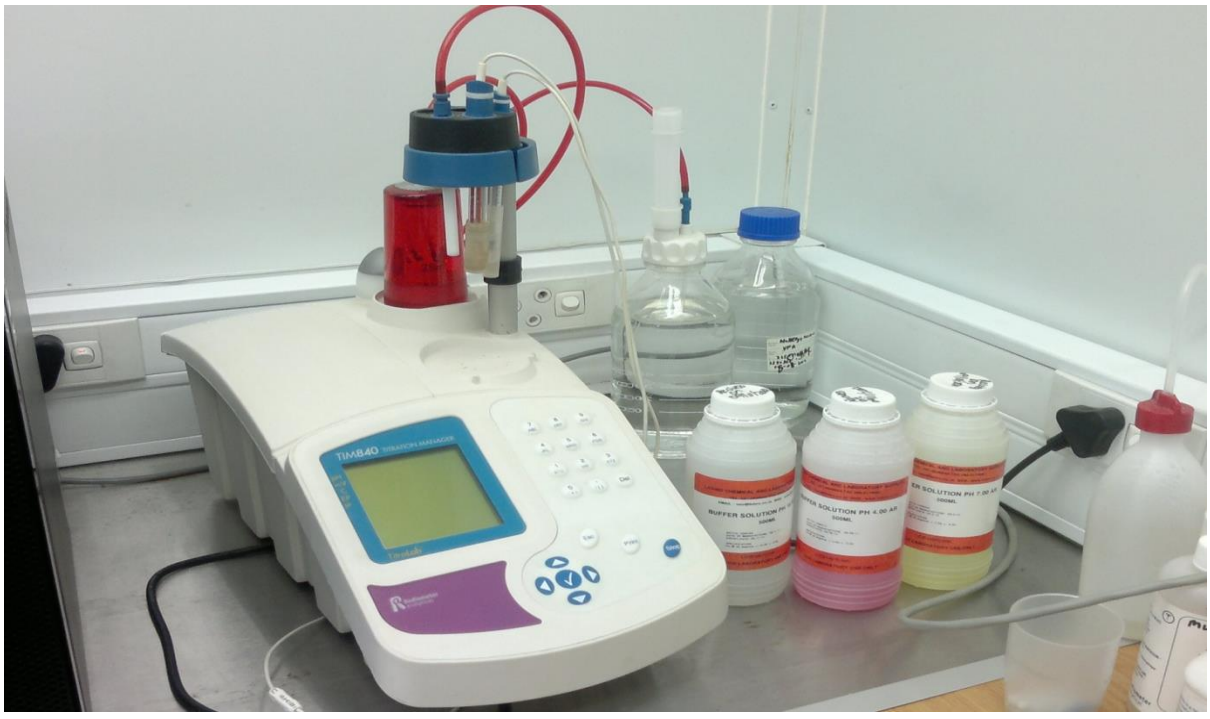

Figure 3.6: TitraLab® (Model 840) titration manager.

The following procedure was used for pH and alkalinity analyses:

a) Before analyses, the titration manager was calibrated at pH 7 (using a pH 7 buffer solution) and at pH 4 (using a pH 4 buffer solution).
b) After calibration the samples were analysed for pH and alkalinity.
c) The titration manager was linked to a computer that has a 5-point titration software. A pH versus the volume of the acid added graph was automatically recorded on the software as the titration manager was titrating for the determination of alkalinity.
d) The graph was then exported to a spreadsheet where the initial pH, volume before and after dilution, and the acid volumes at pH of 6.75, 5.95, 5.18 and 4.29 were recorded to the 5-point titration software.
e) The solution temperature as recorded on the titration manager together with the acid normality (0.1) was then inserted on the 5-point titration software for the determination of VFA.

3.7.4. Biogas analysis

Gas samples were analysed by a (Model 426) gas extraction monitor (Gas Data®, Coventry, United Kingdom) for the determination of fractions of methane, oxygen, ammonia, hydrogen and carbon dioxide gases. Figure 3.7 shows the (Model 426) gas extraction monitor. The reason for using the extraction monitor instead of other equipments is that it is portable and there is no need for additional equipment to transport the gas from the digester to the gas analyzer as is the case with a gas chromatography.

The following procedure was used for biogas analysis:

a) The tubing of the gas extraction monitor was inserted into the digester biogas line for biogas collection.
b) The ‘OK’ button was then pressed, and then the gas extraction monitor displayed the fractions of methane, oxygen, ammonia, hydrogen and carbon dioxide gases.

Figure 3.7: Gas Data® (Model 426) gas extraction monitor.

4. CHAPTER 4: RESULTS AND DISCUSSIONS

4.1. Introduction

This section of the investigation displays and discusses the outcomes of comprehensive trials related with enhancing the formation of biogas produced by means of the anaerobic digestion of bio-chemical substrates. Trials have been carried out with each test to improve the understanding of anaerobic systems so as to establish a correlation between a change in the rate of formation with the corresponding process variable adjustment. The theoretical proposed suggestion of this investigation is that optimizing the anaerobic process variables will result in an improvement in the yields of methane and biogas.

The discussion section begins by showing characterization of inoculum, and primary and secondary substrates. This is followed by comparison of various industrial waste-streams on the anaerobic process; this sub-section is aimed at achieving the first objective of the investigation which discusses the effect of fruit juice waste, soft drink waste, IOP waste and glycerol as digestive media for the anaerobic process at a mesophilic temperature of 37.5℃. This is followed by testing the effect of variables on the digestion process by means of the one-factor-at-a-time method (OFAT) with the main aim of finding the optimum conditions for the anaerobic process. Finally, the variables were optimized using Design Expects Software to determine the biogas and methane yields model equations.

4.2. Characterization of Inoculum, and Primary and Secondary Substrates

The purpose of characterization of inoculum, and primary and secondary substrates was to determine the constituents available in the substrates prior to the experiments as they affect the quality and growth of the anaerobic process, and hence the amount of biogas generated. Characterizations of the inoculum and substrates are shown in Table 4.1. A sample of

calculations for the characteristics of the inoculum and substrates before digestion is shown in Appendix G2.

Table 4.1: Characterizations of the inoculum and substrates before digestion.

Parameter	Units	Glycerol	IOP	Fruits juice	Inoculum	Raw sludge	Soft drink
pH[a]	-	7.7	7.6	7.6	7.2	7.4	7.4
COD[b]	mg/L	1 175 040	82 080	75 120	833	4 500	49 920
Density	kg/m^3	1 080	1 046	1 043	1 032	1 049	1 042
TS	%	78.3	11.2	5.4	2.5	3.9	4.9
VS	%	70.5	10.4	4.8	1.2	2.4	4.8
VS/TS	-	0.90	0.93	0.89	0.49	0.62	0.98

[a] Data was taken from Appendix H2.
[b] Data was taken from Appendix I2.

According to Ross *et al.* (1992), the theoretical TS range for raw sludge is 1 to 4% TS. The experimental TS percentage for raw sludge was 3.9%, which is within the theoretical range.

Moreover, the soft drink sample had the highest VS/TS ratio of 0.98, which implies that it contained more organic substances than insoluble substances. The substrate with the lowest VS/TS value was inoculum, with a VS/TS value of 0.49. This means that inoculum has more microorganisms than organic matter, and thus proving that it will be valuable as an inoculum rather than a substrate.

4.3. Comparison of Various Industrial Substrates

4.3.1. Feed characterization

The digesters were fed with 20 mL of each substrate together with 2.4 L of water and 2.4 L of inoculum. Table 4.2 shows the characteristics of each substrate mixture. A sample of calculations for the characteristics of the mixture before digestion is shown in Appendix G3. An additional digester (control) was fed with 2.4 L of inoculum and 2.4 L of sewage sludge. This digester was used for comparison of anaerobic digestion and anaerobic co-digestion. For this, comparisons were only made for biogas and methane yields, and organic removals.

Table 4.2: Characteristics of each substrate mixture before digestion.

	Co-substrate				Feed concentration in reactor	
Digester ID	**Volume (mL)**	**VS (g)**	**COD (mg/L)**	**COD (mg)**	**VS loading (g VS. L $^{-1}$)**	**COD loading (g COD. L $^{-1}$)**
Glycerol	20	15.5	1 175 040	23 501	3.23	4.90
IOP	20	1.4	82 080	1 642	0.29	0.34
Fruit juice	20	1.3	75 120	1 502	0.27	0.31
Soft drink	20	1.0	49 920	998	0.21	0.21

4.3.2. pH, VFA and alkalinity

The purpose of monitoring the process pH was to observe the health of the digestion process. The pH profiles for soft drinks, IOP, fruit juice and glycerol for the first digestion process are depicted in Figure 4.1. Raw data for pH, VFA and alkalinity is shown in Appendix H1, and was taken in duplicates. Average values were taken for the results section, which are show in Appendix H2.

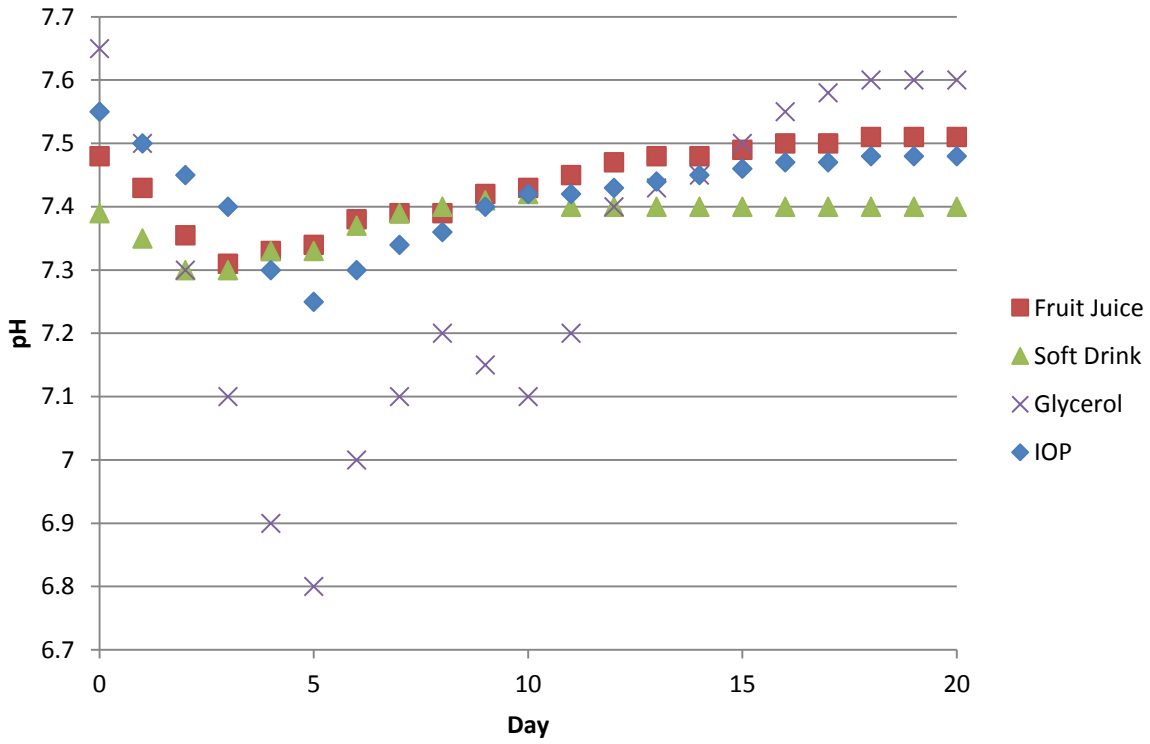

Figure 4.1: pH profile.

In the hydrolysis stage (day 1 to 5) the pH of all digesters dropped gradually because of the high generation of acid-forming microbes than methane-forming microbes. The drop in pH was not the same. As a result of the high VS loading of 3.23 g VS / L in glycerol, more VFAs were probable generated which made its pH drop last longer. On day 5, the pH of all substrates increased as the acids were transformed to gas i.e. the rate at which the methane-forming microorganisms were generated was higher than the rate at which acid-forming microorganisms were generated. In the final stages of the digestion process the pH of all substrates became stable as biogas generation ceased.

Furthermore, the system pH for the entire duration of the process was always within the required theoretical range of 6.3 and 7.8 (see Sub-section 2.7.2.), and hence there was no need for the addition of an acidic or basic buffer solution to control pH.

Figure 4.2 and Figure 4.3 depicts the level amount of total VFA and alkalinity values after digestion, respectively:

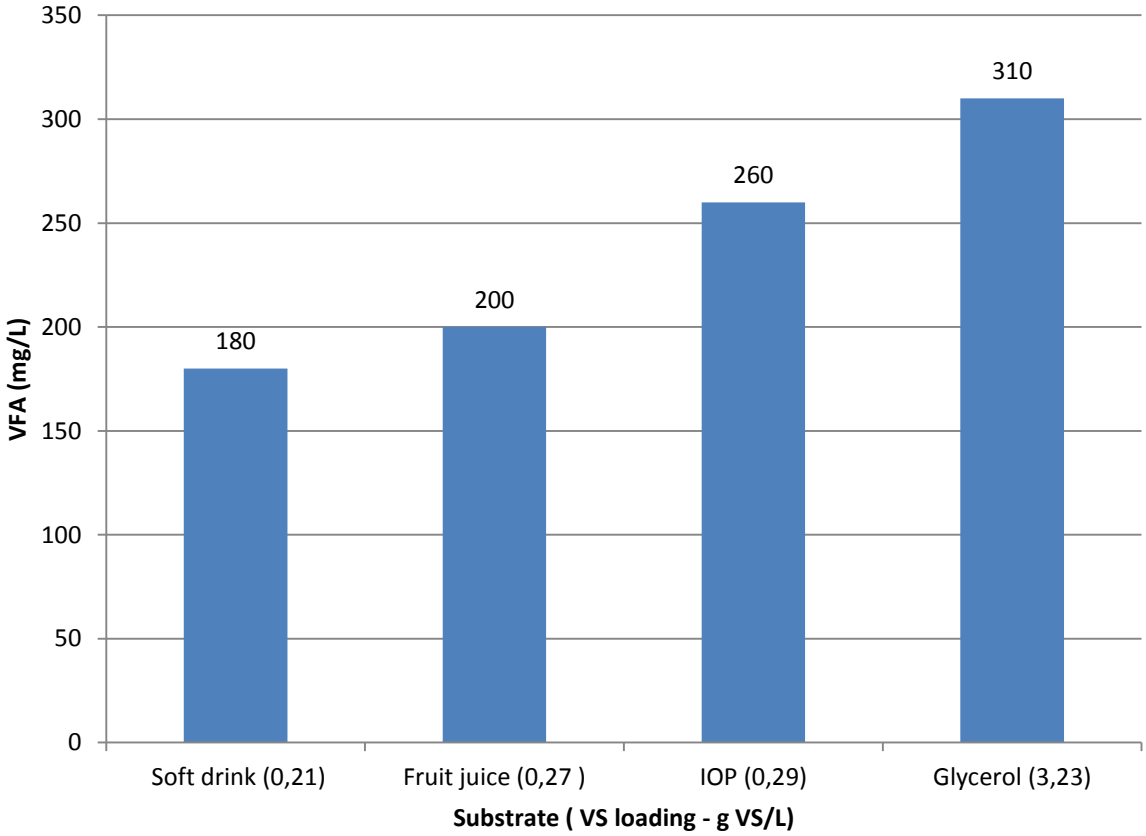

Figure 4.2: VFA graph for all substrates.

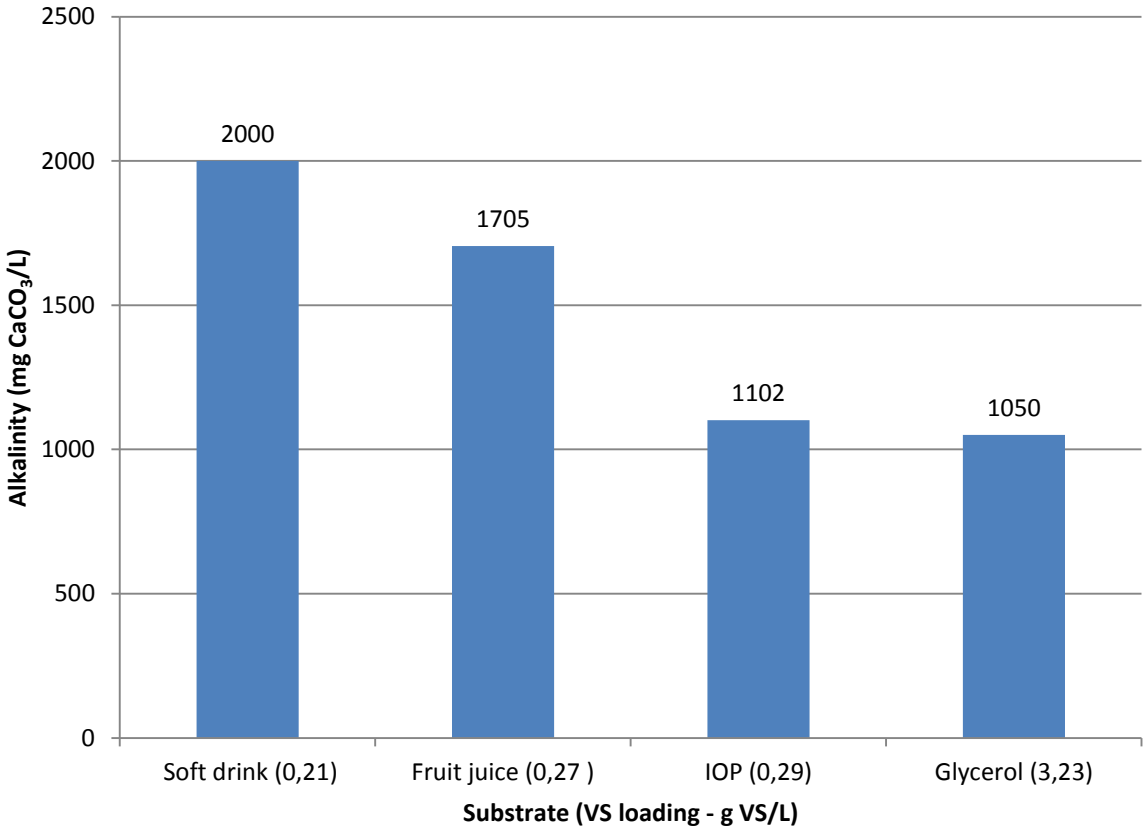

Figure 4.3: Alkalinity graph for all substrates.

Glycerol had the highest VFA of 310 mg/L (Figure 4.2) and the lowest alkalinity of 1 050 mg $CaCO_3$/L (Figure 4.3). An overload in the feed can be attributed to the accumulation of VFAs, which may possibly decrease the alkalinity (Ross *et al.*, 1992). Since glycerol had the highest VS loading of 3.23 g VS/L, this led to it generating the highest VFA. The digester of glycerol was therefore unable to neutralise acids formed, and thus produced the lowest alkalinity.

Another stability indicator of the digestion process that was measured was the VFA to alkalinity ratio, which is depicted in Figure 4.4. A sample of calculations for the VFA to alkalinity ratio is shown in Appendix H3. In order for the digester to stabilize, the rate at which the VFA are generated should not be far above the rate at which the alkalinity is neutralising the VFA. According to Callaghan *et al.* (2002), the anaerobic digestion process will be stable at VFA to alkalinity ratio below 0.4 – this line is indicated in Figure 4.4 as the stable baseline. Above the stable baseline the VFA accumulates quite more rapid, and as a

result, causes overload in the system. The VFA to alkalinity values for all substrates were less than 0.4, which demonstrated high stability in the digesters.

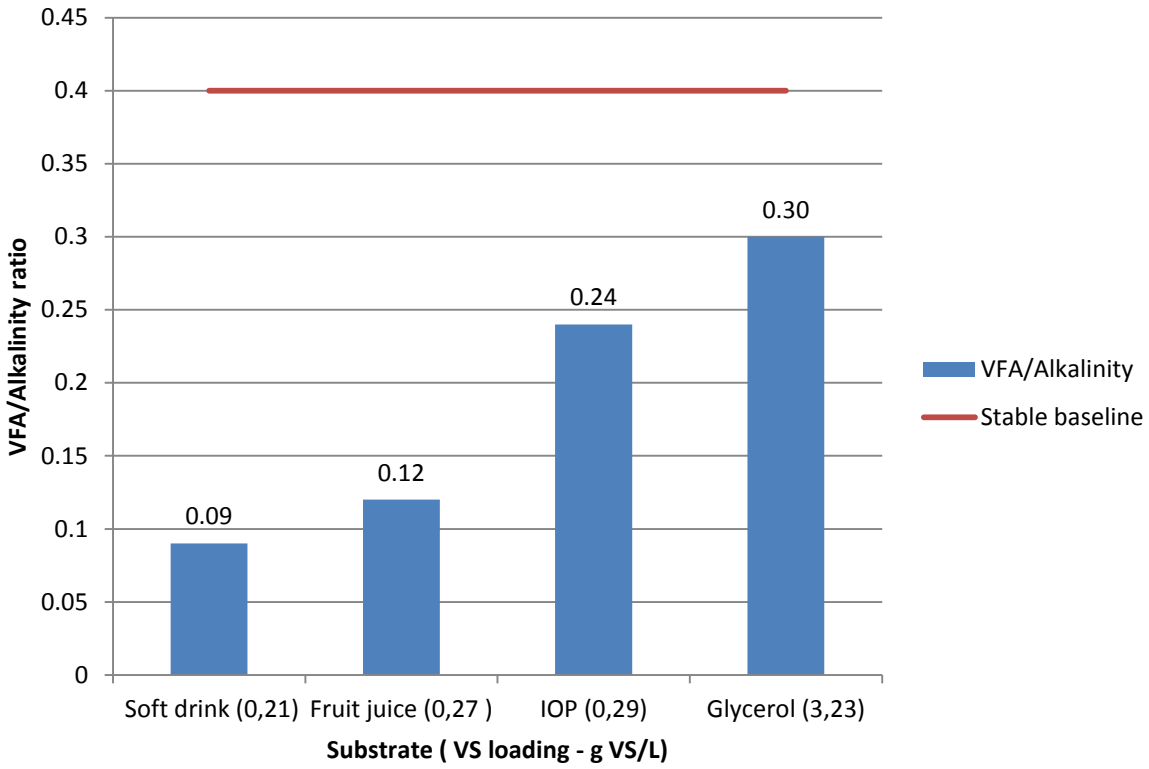

Figure 4.4: VFA to alkalinity ratio for all substrates.

Moreover, glycerol generated the highest VFA to alkalinity ratio of 0.3. It is the high organic loading in glycerol (3.23 g VS/L) that made it generate a very large VFA content in comparison to its alkalinity in such a way that its VFA to alkalinity ratio became the highest.

The VFA to alkalinity ratio for soft drink (0.2 g VS/L), fruit juice (0.27 g VS/L), IOP (0.29 g VS/L) and glycerol (3.23 g VS/L) were 0.09, 0.12, 0.24 and 0.30, respectively. The results demonstrated that higher organic loading produces higher VFA to alkalinity ratio. The same conclusion was made by Feng *et al.* (2013) when they did a study on the effect of feed to inoculum ratios on vinegar residue, and found that the VFA to alkalinity ratios were 0.1, 0.25, 0.3, 0.5 and 0.8 for feed to inoculum ratios of 1, 2, 4, 5 and 6, respectively.

4.3.3. Accumulative biogas production

Accumulative biogas productions for IOP, fruit juice and soft drinks wastes, and glycerol are shown in Figure 4.5 and Figure 4.6, respectively. Glycerol had significantly higher values than soft, drink and IOP, and was therefore plotted on a different graph. Raw data for biogas production is shown in Appendix F1, and was taken in duplicates. Average values were taken for the results section, which are show in Appendix F2. Biogas productions were then converted to standard temperature and pressure (see Appendix F3 for a sample of calculations).

Soft drink wastewater is the most readily degradable substrates amongst the substrates that were tested, and as a result, generated the highest biogas production in the hydrolysis stage (before day 5). Moreover, soft drink waste had the highest VS/TS ratio of 0.99, hence had less inorganic matters than inorganic matters, and therefore was less inhibited by inorganic matters in the hydrolysis stage. Soft drink had low VS and COD contents initially, which made it to produce the lowest biogas production of 411 mL at the end of the digestion process.

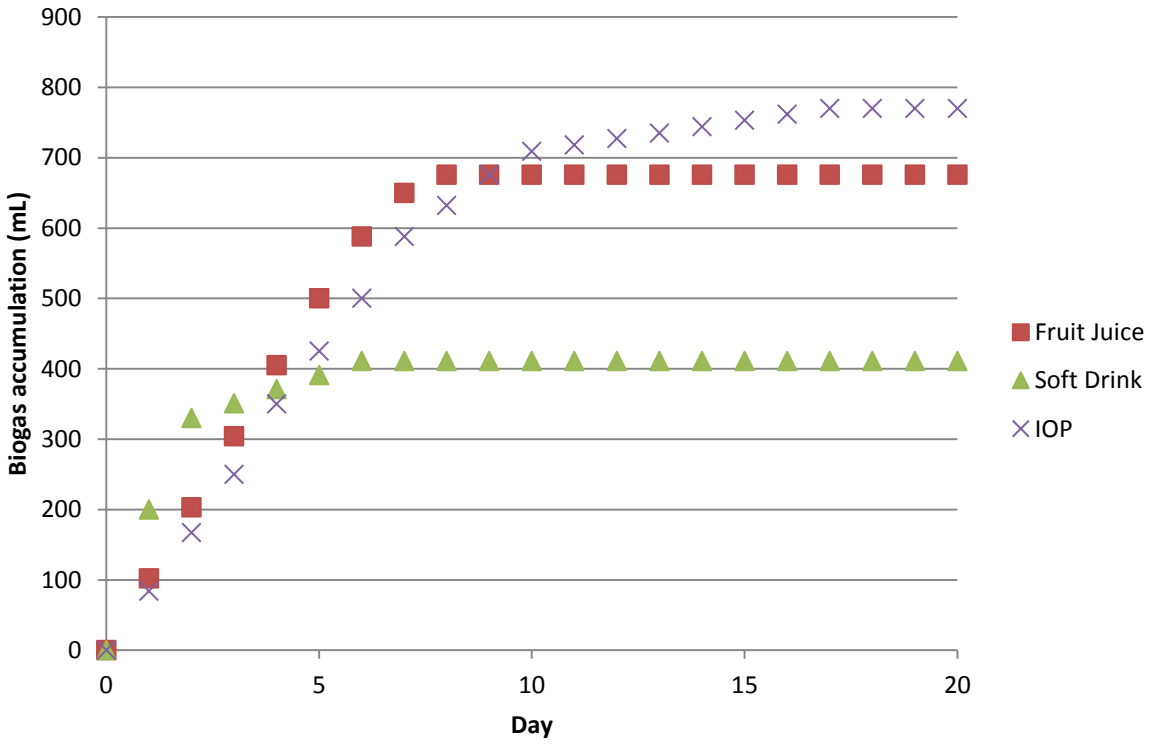

Figure 4.5: Accumulative biogas production for soft drink, fruit juice and IOP.

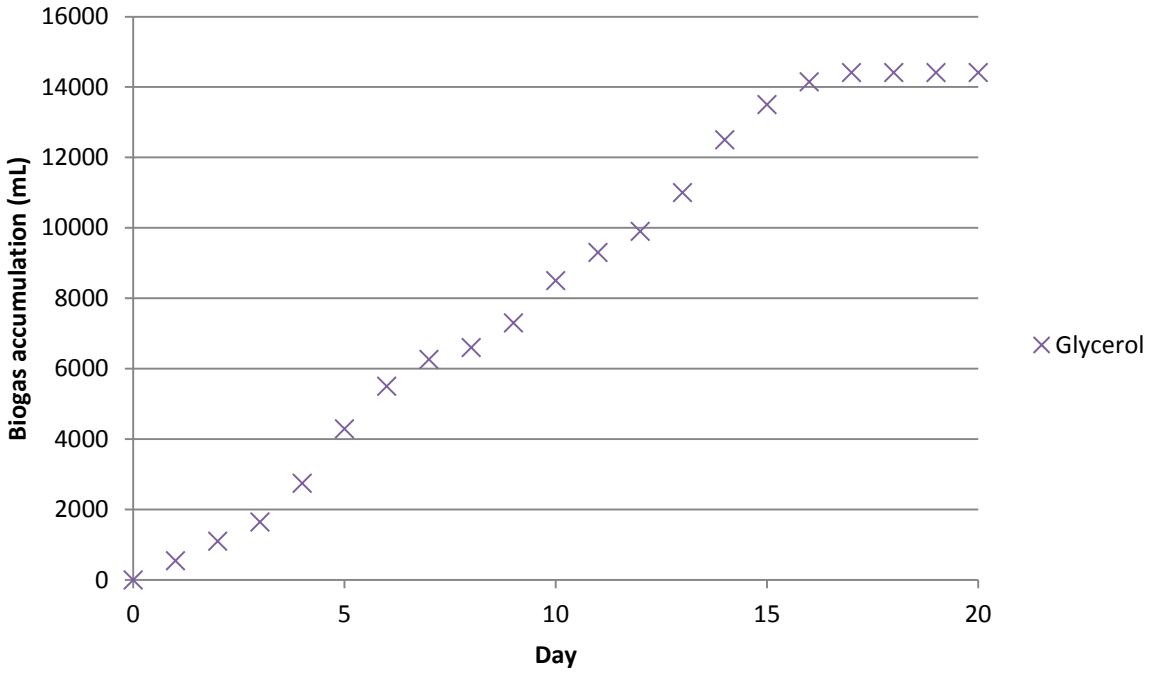

Figure 4.6: Accumulative biogas production for glycerol.

Glycerol has higher VFAs, triglyceride and lipids emulsions which have a larger molecular structure and are more complex than the other substrates. Hence, the presence of these components made glycerol to degrade very slowly in the anaerobic process, especially in the hydrolysis stage or initial stage of the anaerobic process. As soon as the complex-outer structure of glycerol was broken (day 4), the biogas generation increased rapidly. Furthermore, the pH of glycerol rose after day 5 (see Figure 4.1 above), which made a healthier environment for the growth of methanogens, and thus improve the biogas production.

4.3.4. Methane percentages and accumulation in biogas

Methane percentages for the first digestion trial are shown in Figure 4.7. Raw data for biogas compositions is shown in Appendix J1, and was taken in duplicates. Average values were taken for the results section, which are show in Appendix J2.

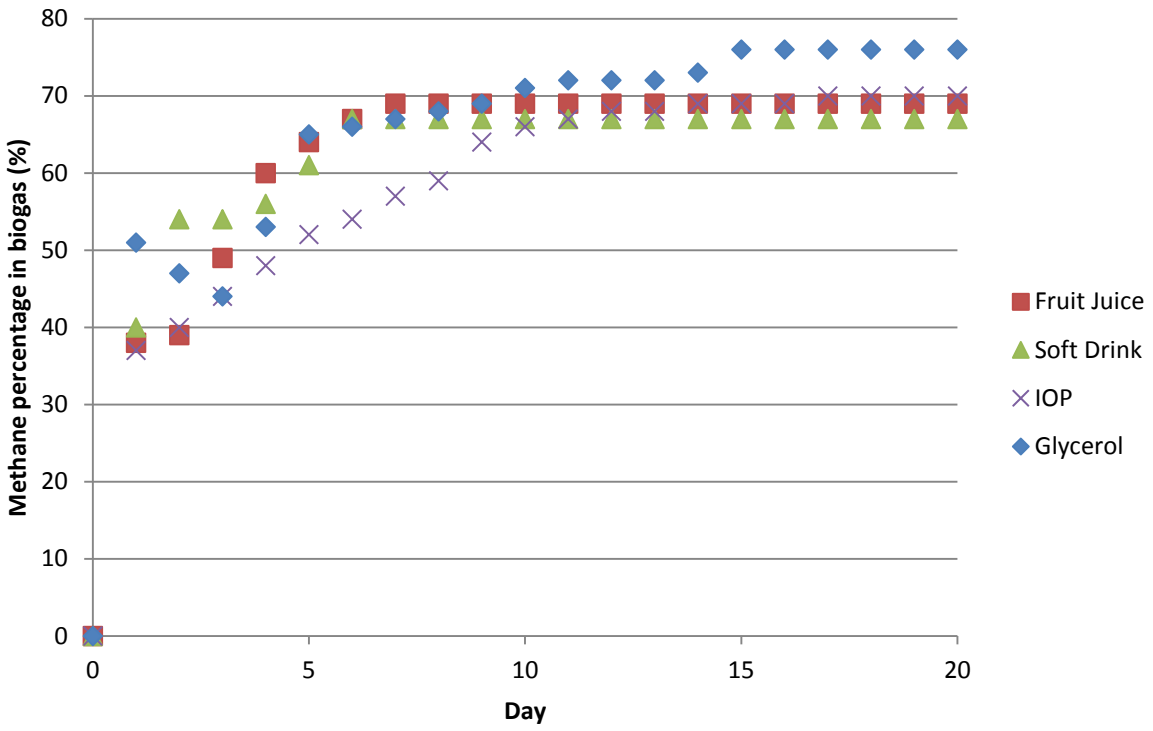

Figure 4.7: Methane percentage in biogas for the substrates.

The percentage of methane produced by glycerol decreased between day 1 and 3 due to the accumulation of VFAs in the process, which inhibited the growth of methanogens. The methane percentage began to increase from day 1 due to low organic loadings for the other substrates. For all digesters, methane percentages increased in the final stages of digestion as methanogens were generating methane (methanogenesis stage). The percentages of methane stabilized in the last stages of the digestion process as methane generation ceased.

In addition, the biogas content and production are strongly affected by the extent of the organic loading (Wohlgemut, 2009). Substrates with high organic loading or high strength produce the highest methane percentage at the end of the anaerobic process (Labatut and Gooch, 2012). This is why glycerol generated the highest methane percentage at the end of the digestion process. Moreover, the theoretical composition for fats that was suggested by Sacks (1997) was 76%, which was the case in this investigation.

Figure 4.8 and Figure 4.9 depict methane generated for soft drink, IOP and soft drink, and glycerol for the first digestion trial, respectively. Glycerol had significantly higher values than soft, drink and IOP, and was therefore plotted on a different graph.

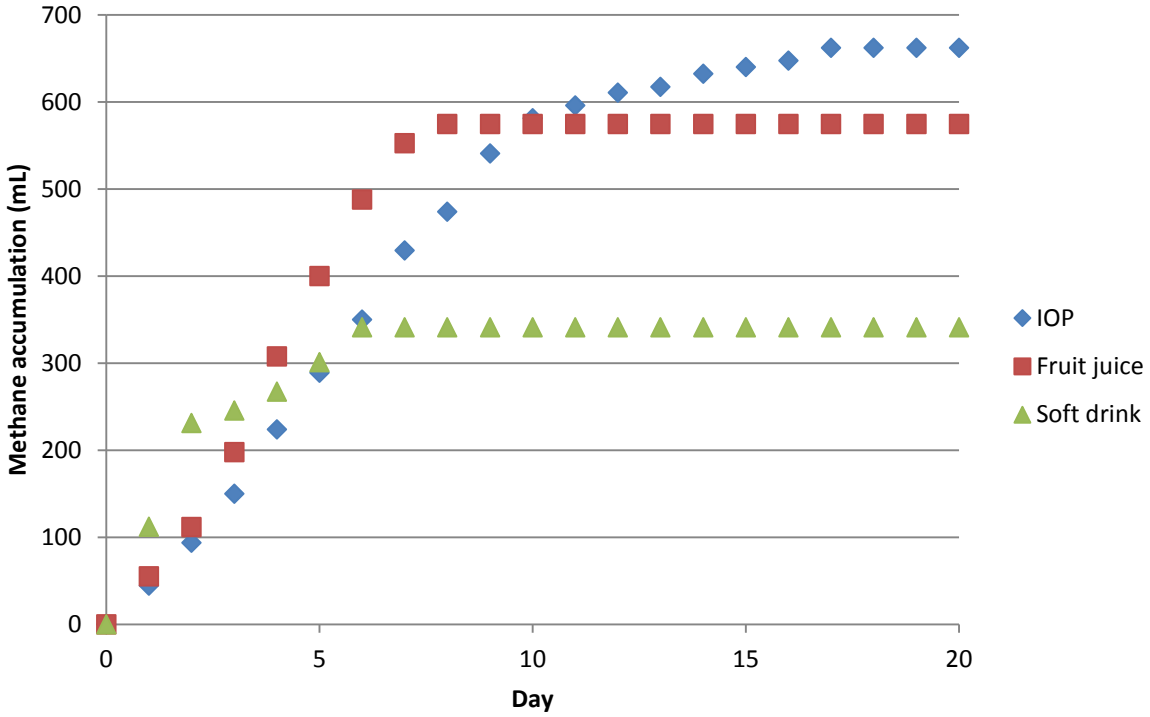

Figure 4.8: Methane accumulation for soft drink, fruit juice and IOP.

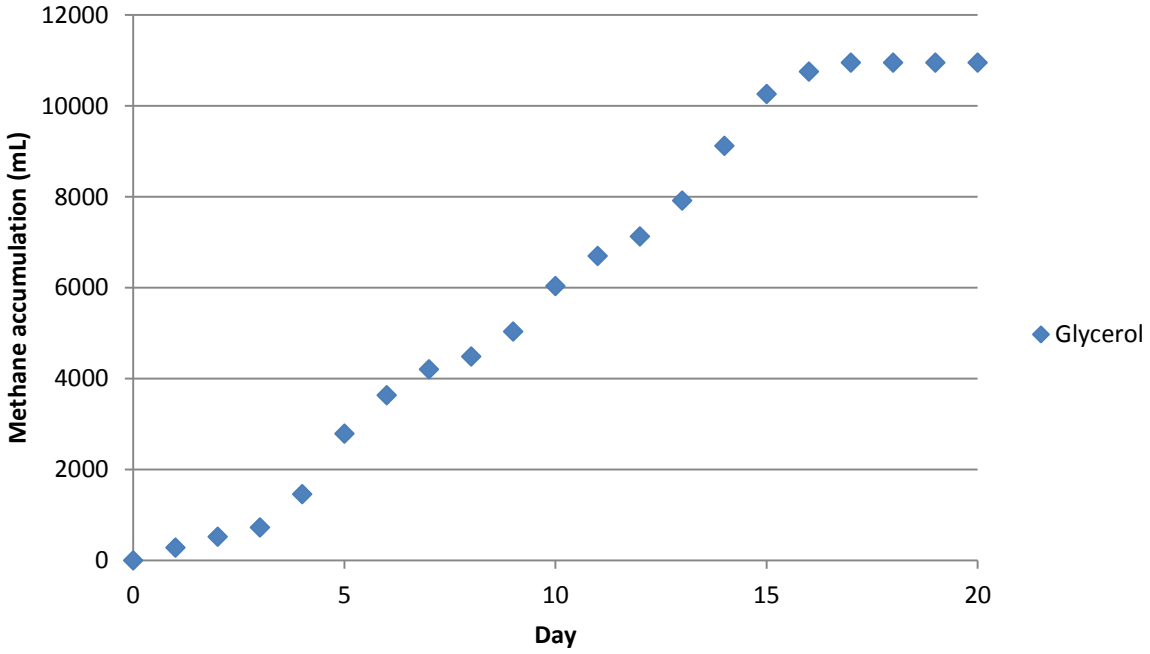

Figure 4.9: Methane accumulation for glycerol.

The trends for accumulative methane production were similar to those for accumulative biogas production that was shown in Figure 4.5 and Figure 4.6.

4.3.5. Carbon dioxide content in biogas

Figure 4.10 shows the percentage of carbon dioxide (CO_2) that was generated in biogas for all substrates in the first digestion trial. The high VFA content in glycerol in the early stages of the digestion process resulted in a shift to higher levels of CO_2, as it was not utilized fast enough by the H_2-utilizing methanogenesis microorganisms. The CO_2 percentage in biogas decreased in the last stages of the digestion process as biogas generation ceased.

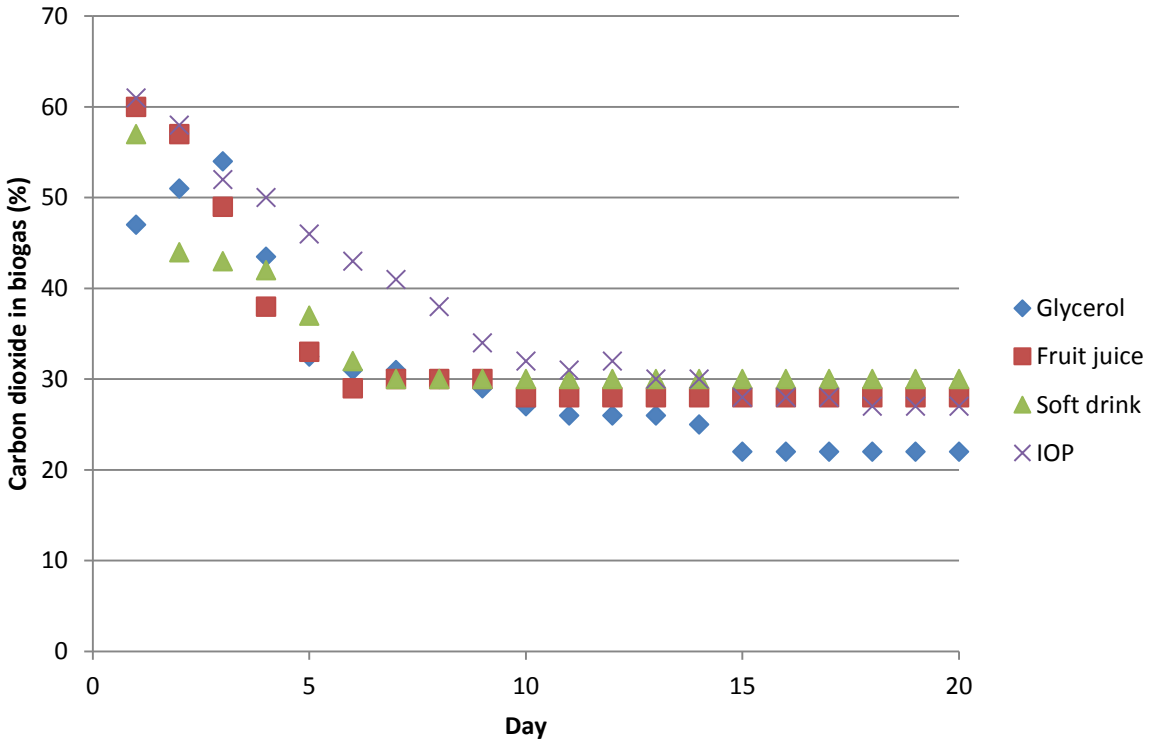

Figure 4.10: Carbon dioxide content in biogas.

4.3.6. Methane to carbon dioxide ratio for glycerol

Carbon dioxide is soluble in H_2O (1.14 g carbon dioxide / kg of H_2O at 37.5℃ and 1 atm), furthermore it reacts with H_2O to generate carbonic acid (H_2CO_3) which can then undergo

dissociation to generate bicarbonate (HCO_3^-), and then carbonate (CO_3^{2-}) as described by the following reaction:

$$CO_2 + H_2O = H_2CO_3 = HCO_3^- + H^+ = CO_3^{2-} + H^+ \quad (4.1)$$

The substrates that were used had a high water content (see Table G2), and thus carbon dioxide in biogas reacted with water (Equation 4.1). The content of biogas that was obtained using the (Model 426) gas analyzer was lower in carbon dioxide than what was generated by the system. Methane, on the other hand, is not highly soluble in H_2O (0.035 g methane / kg of H_2O at 1 atm and 25℃), thus the composition that was obtained from the gas analyzer may be assumed to be its true value.

Moreover, the biogas obtained in anaerobic digestion processes usually contain 60% methane and 40% carbon dioxide, thus the methane to carbon dioxide ratio will be closer to 60/40 = 1.5 (PBW, 2016). If the substrate is composed of C, H and O, the methane to carbon dioxide ratio is obtained from the Buswell and Neave (1930) equation. Using the Buswell and Neave (1930) equation, the methane to carbon dioxide ratio for glycerol was calculated to be 1.4 (see Appendix K1 for calculation). Figure 4.11 shows the experimental and theoretical methane to carbon dioxide ratios for glycerol (see Appendix J3 for sample of calculations for experimental methane to carbon dioxide ratio).

In the beginning of the digestion process (day 1 to 4) the methane to carbon dioxide ratio was below 1.4, and in the last days of the digestion process (after day 4) the methane to carbon dioxide ratio was usually greater than 1.4. According to Equation 2.12, 2.13 and 2.17 (see the Section 2.2.3.), four (4) moles of CO_2 are generated in the acetogenesis stage, and also according to Equation 2.15 and 2.19, three moles of CO_2 are consumed in the acetogenesis stage. Thus, the acetogenesis stage will generate more CO_2 than the rate of consumption. This is the reason why the methane to carbon dioxide ratio in the initial stages of the digestion process (day 1 to 3) decreased; the rate at which CO_2 was generated was higher than that of methane. The methane to carbon dioxide ratio increased in the last stages of the digestion

process as the process reached the methanogenesis stage where methanogenesis microorganisms were producing more methane than carbon dioxide.

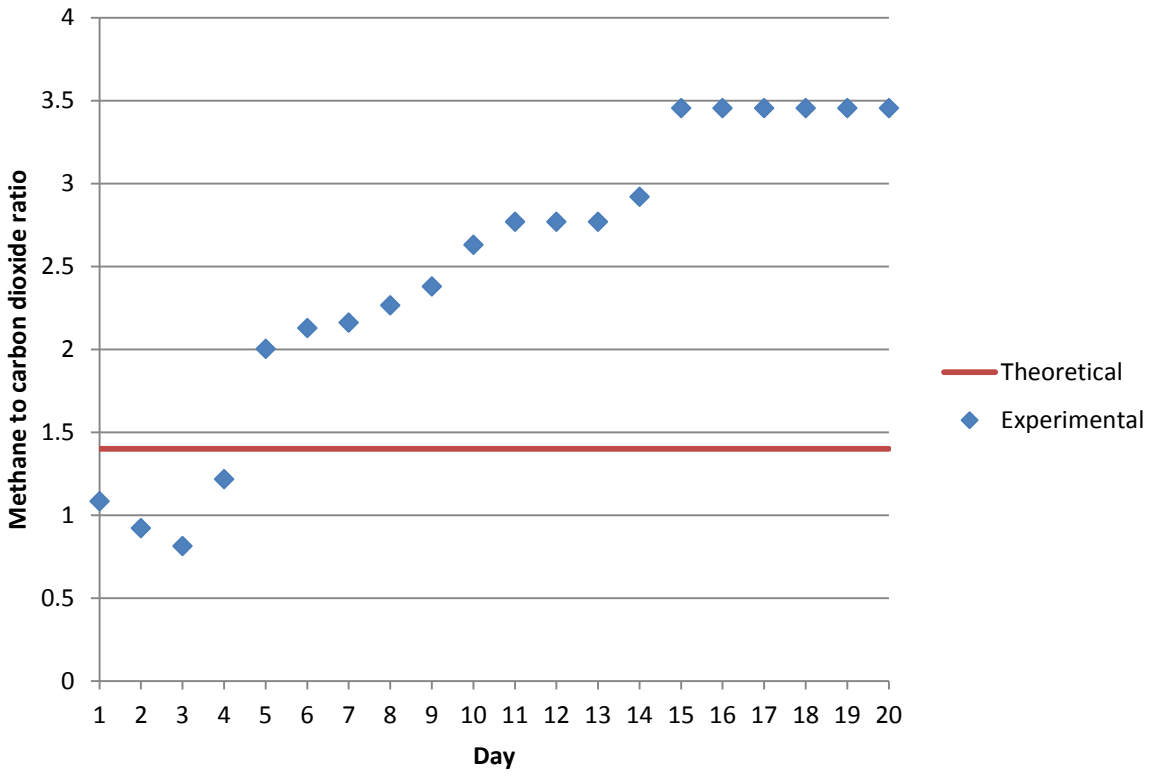

Figure 4.11: Methane to carbon dioxide ratio for glycerol.

4.3.7. Nitrogen and oxygen contents in biogas

Figure 4.12 and Figure 4.13 show the oxygen (O_2) and nitrogen (N_2) contents in biogas for the first trial, respectively. The nitrogen and oxygen present in the digesters were from air that entered the system while sampling for pH and during digester start-up, as any generation of nitrogen and oxygen by bacterial populations would be negligible (Ward *et al*., 2008). The gas analyzer (Model 426) does not analyse for nitrogen (N_2), but can analyze for oxygen (O_2). The volume of nitrogen in the system was assumed to be constant. It was also assumed that biogas is a mixture of CH_4, N_2, O_2, H_2, H_2O and CO_2 (H_2S was approximately zero since the system was blacked out). The content of nitrogen in biogas was then computed using this information (see Appendix J4 for sample of calculations for nitrogen volume and content).

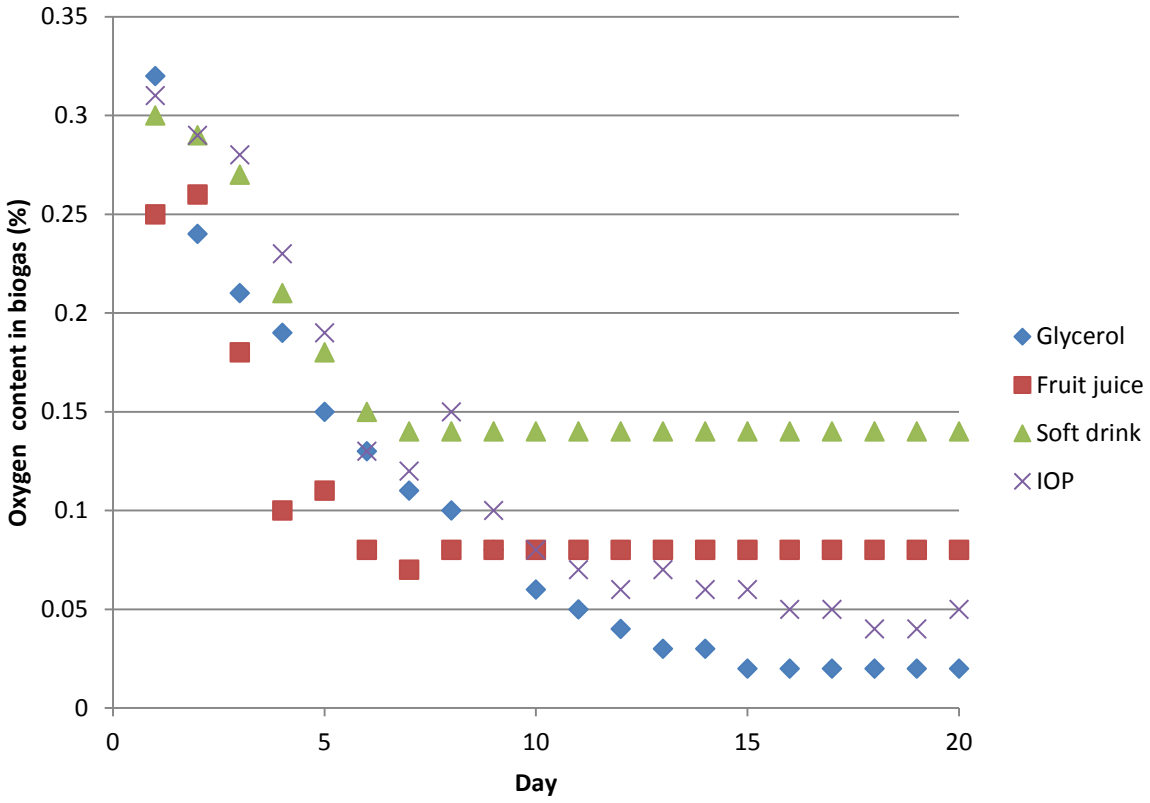

Figure 4.12: Oxygen content in biogas for the first digestion trial.

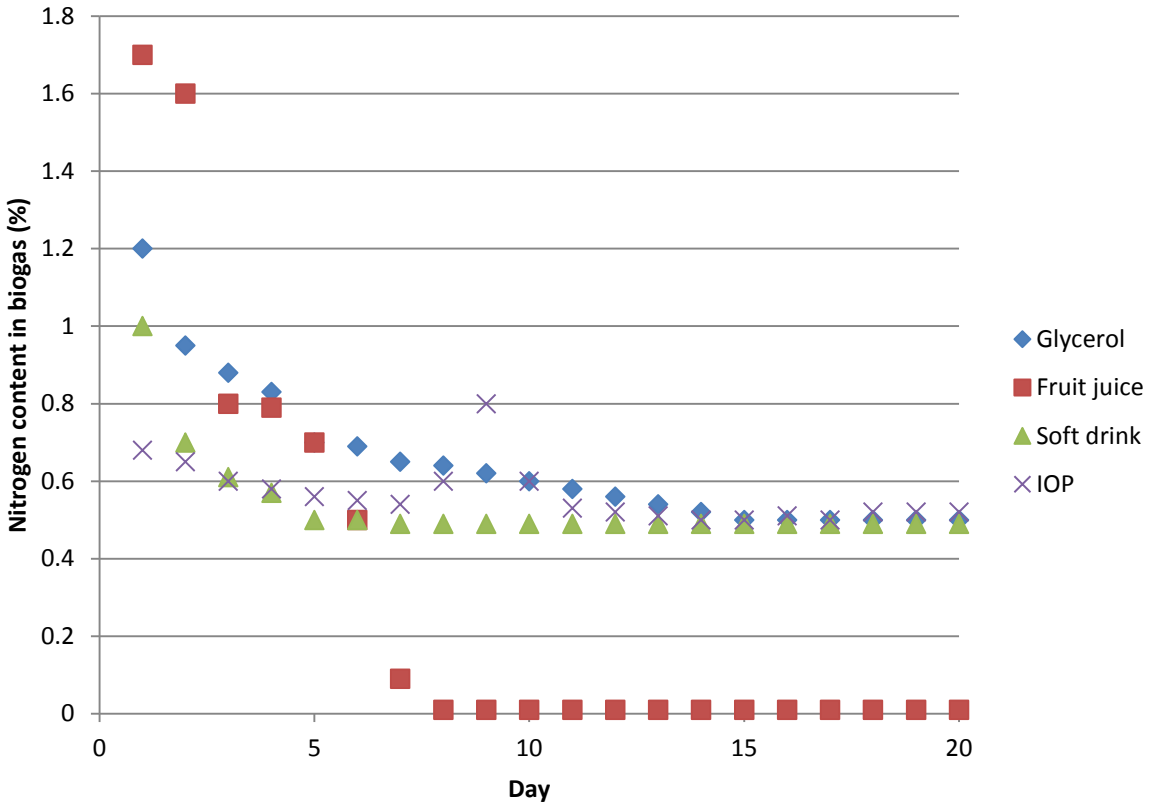

Figure 4.13: Nitrogen content in biogas for the first digestion trial.

Moreover, the percentage of nitrogen and oxygen slowly decreased, demonstrating air originally in the digester gas lines and headspace was being flushed out of the system by the biogas formation. Also, fruit juice experienced a major drop in nitrogen content than the other substrates (from day 1 to day 8). This was because fruit juice generated the highest amounts of methane between day 1 and day 8 than the other substrates (see Figure 4.8).

In addition, IOP had slight offset values of both oxygen and nitrogen contents in biogas on day 8 and day 9. This was possible due to large air entering the system on these days in comparison to other days.

Figure 4.14 shows the nitrogen to oxygen ratio for glycerol. A sample of calculations for the experimental nitrogen to oxygen ratio is shown in Appendix J4. The nitrogen to oxygen ratio in biogas samples was approximately equal to the air ratio of 79/21 = 3.76 in the early stages of the digestion process. As the time went on the nitrogen to oxygen ratio increased far above the theoretical value of 3.76, which was an indication that some oxygen was consumed by facultative microorganisms.

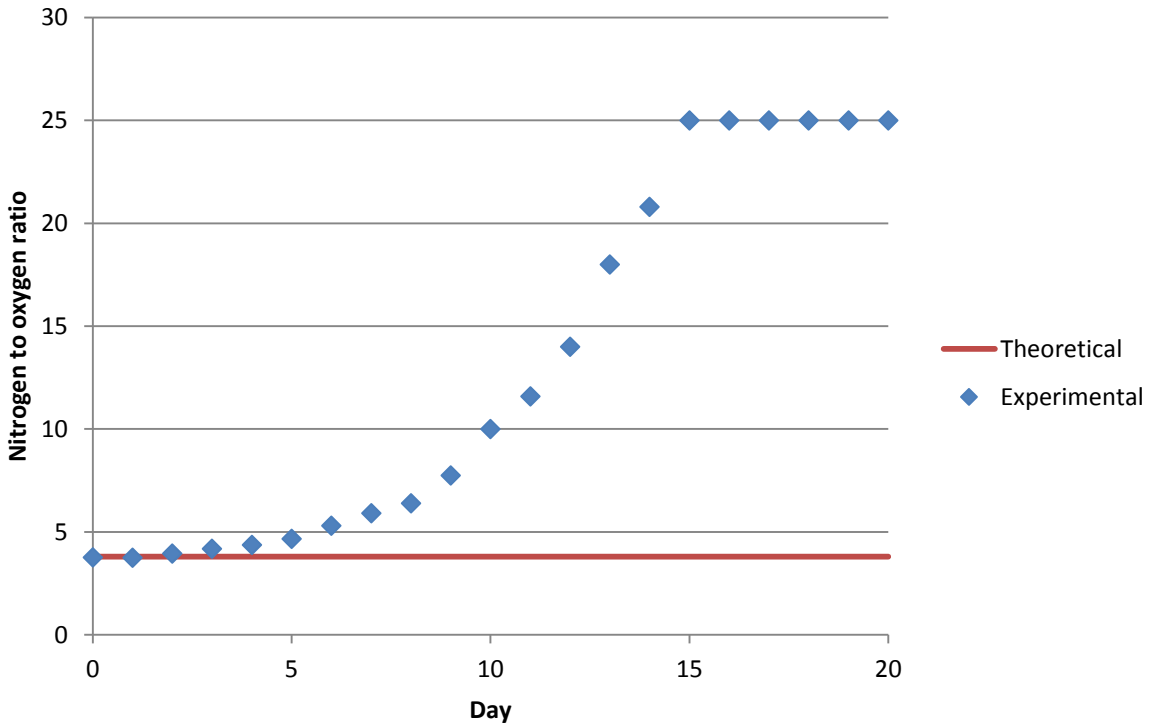

Figure 4.14: Nitrogen to oxygen ratio for glycerol.

4.3.8. Biogas and methane yields

Feed concentrations for the first digestion trial are shown in Table 4.3:

Table 4.3: Feed characteristics of all substrates for the first digestion trial.

	Co-substrate				Feed concentration in reactor	
Digester ID	**Volume (mL)**	**VS (g)**	**COD (mg/L)**	**COD (mg)**	**VS (g VS.L $^{-1}$)**	**COD (g COD.L^{-1})**
Glycerol	20	15.5	1 175 040	23 501	3.23	4.90
IOP	20	1.4	82 080	1 642	0.29	0.34
Fruit juice	20	1.3	75 120	1 502	0.27	0.31
Soft drink	20	1.0	49 920	998	0.21	0.21

Figure 4.15 and Figure 4.16 show the effects of VS and COD loading on biogas production, respectively:

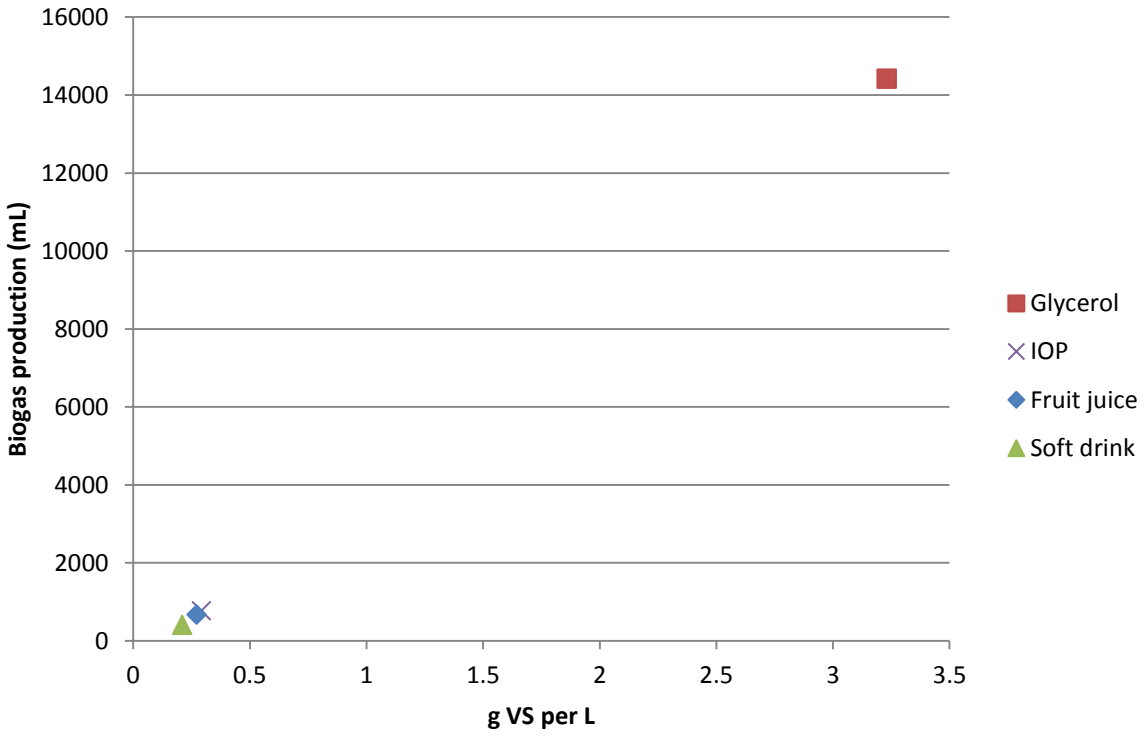

Figure 4.15: Effect of VS loading on biogas production.

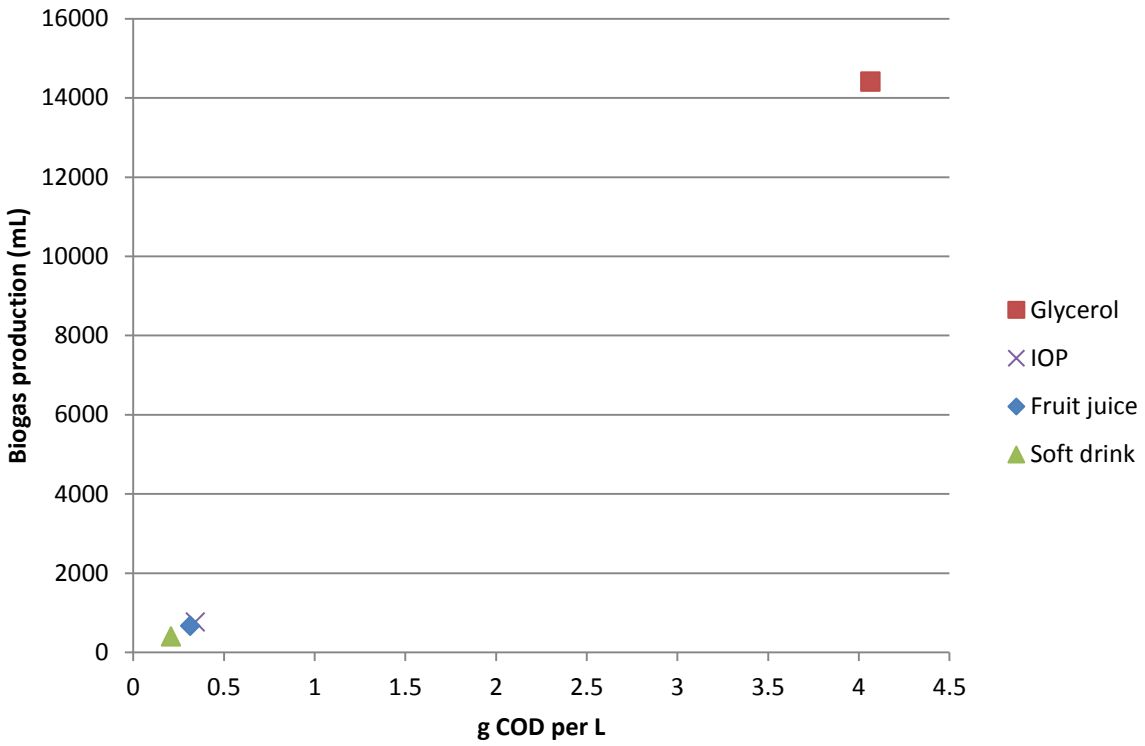

Figure 4.16: Effect of COD loading on biogas production.

For the substrates tested, glycerol generated the highest biogas production of 14 415 mL. It is the high VS and COD loadings in glycerol (3.23 g VS/L and 4.89 g COD/L, respectively) that made it produce the highest amount of biogas.

Furthermore, the biogas production for soft drink, fruit juice, IOP and glycerol were 411, 676, 770 and 14 415 mL, respectively. Generally, an increase in VS and COD loadings led to an increase in biogas production. The same observation was made by Foucault (2011) when he did a study on the co-digestion of glycerol on chicken processing wastewater. The biogas production for VS loading of 4.6, 4.7, 5.1, 8.4, 10.6, 11.6 and 12.6 g VS/L were 14 000, 15 000, 18 000, 25 000, 26 000 and 30 000 mL, respectively.

Figure 4.17 and Figure 4.18 show biogas and methane yields (on VS and COD basis) for the first digestion trial, respectively. A sample of calculations for calculation of biogas and methane yields is shown in Appendix L.

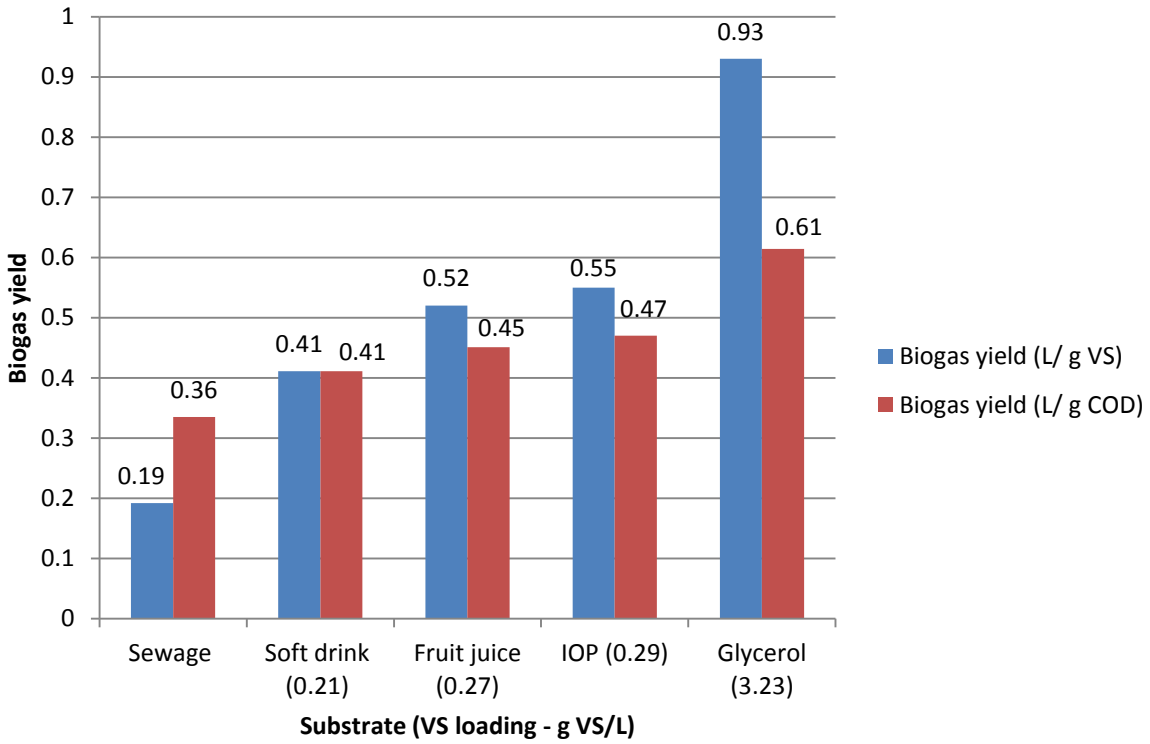

Figure 4.17: Biogas yields on COD and VS for all substrates.

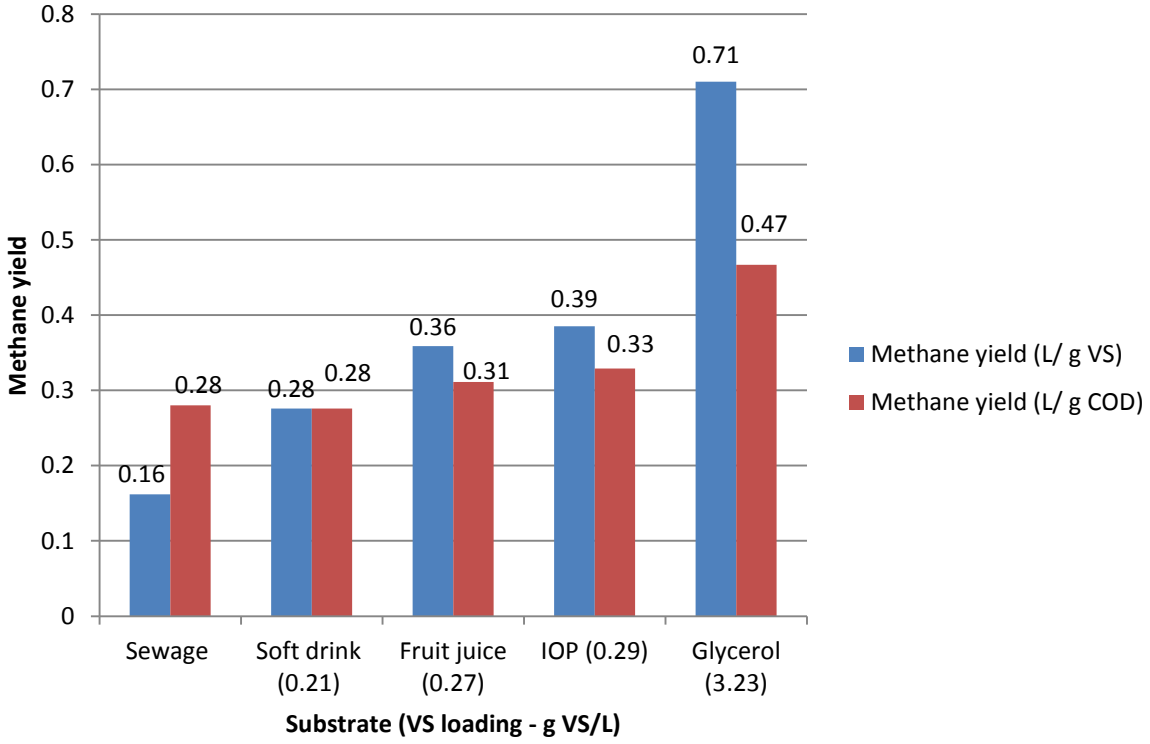

Figure 4.18: Methane yields on COD and VS basis for all substrates.

The highest biogas and methane yields, both on the basis of VS and COD, were produced by glycerol. This implies that glycerol had significant amounts of other organic substances, for example triglyceride or lipid emulsions, which have higher energy content.

In addition, digestion of sewage sludge alone produced biogas yields of 0.19 L /g VS and 0.33 L/g COD, and methane yields of 0.16 L/g VS and 0.28 L/g COD. Generally, co-digestion yields were higher than digestion yields of sewage alone.

Moreover, the biogas yields for co-digestion were 0.41, 0.52, 0.55 and 0.93 L $(g\ VS)^{-1}$ for soft drink (0.21 g VS/L), fruit juice (0.27 g VS/L), IOP (0.29 g VS/L) and glycerol (3.23 g VS/L), respectively. The methane yields were 0.28, 0.36, 0.39 and 0.71 L $(g\ VS)^{-1}$ for soft drink (0.21 g VS/L), fruit juice (0.27 g VS/L), IOP (0.29 g VS/L) and glycerol (3.23 g VS/L), respectively. Generally, an increase in VS loading lead to an increase in both the methane and biogas yields. The same observation was obtained by Foucault (2011) when he obtained methane yields of 0.399 and 0.621 L $(g\ VS)^{-1}$ for VS loading of 1.8 and 2.0 g VS/L, respectively.

Figure 4.19 and Figure 4.20 shows comparisons between the theoretical and the experimental biogas yields obtained from the first digestion trial on VS and COD basis, respectively. Theoretical methane yields versus experimental methane yields on VS basis are shown in Figure 4.21. The theoretical values for all substrates were taken as follows:

- The theoretical biogas yields on VS and COD basis, and the theoretical methane yield on VS basis for glycerol were found from the Buswell and Neave (1930) equation, and were 1.16 L $(g\ VS)^{-1}$, 0.58 L $(g\ COD)^{-1}$, and 0.70 L $(g\ VS)^{-1}$ respectively (see Appendix K2 for calculation).
- The theoretical biogas yield was taken from Braun and Welling (2009), which was 0.25 L $(g\ VS)^{-1}$. Assuming a methane percentage of 60% implies that the methane theoretical yield is 0.15 L $(g\ VS)^{-1}$.

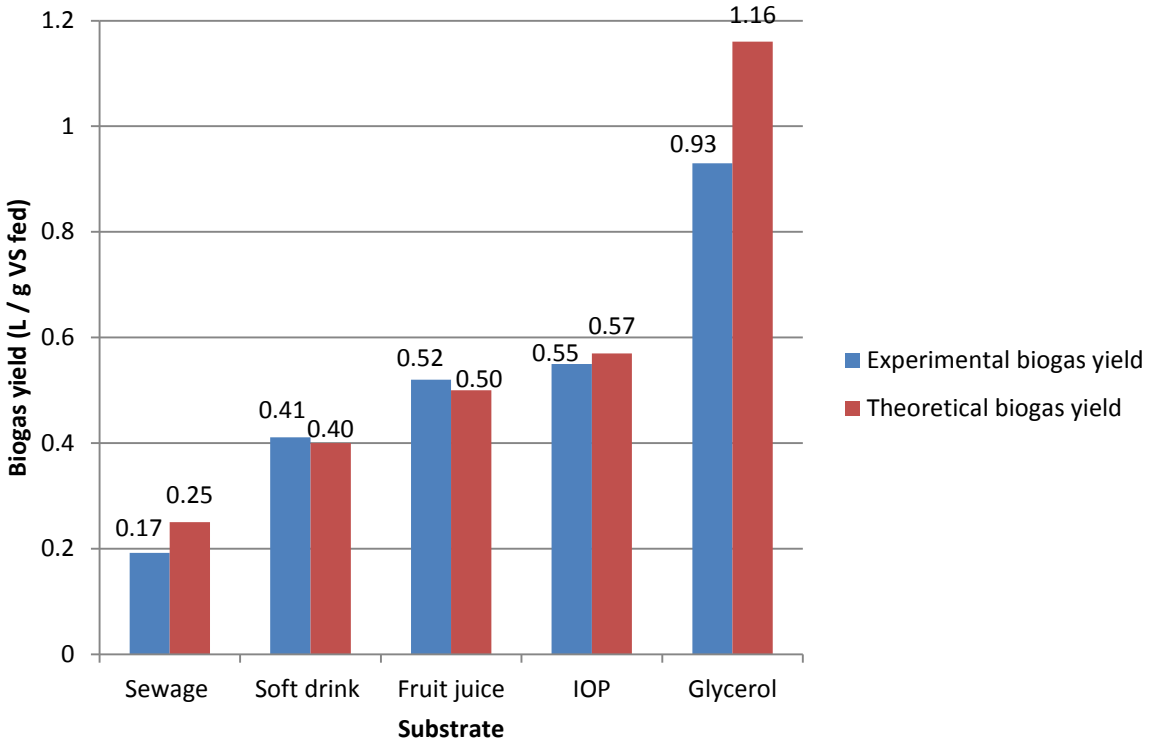

Figure 4.19: Comparison between theoretical and experimental biogas yields on VS basis.

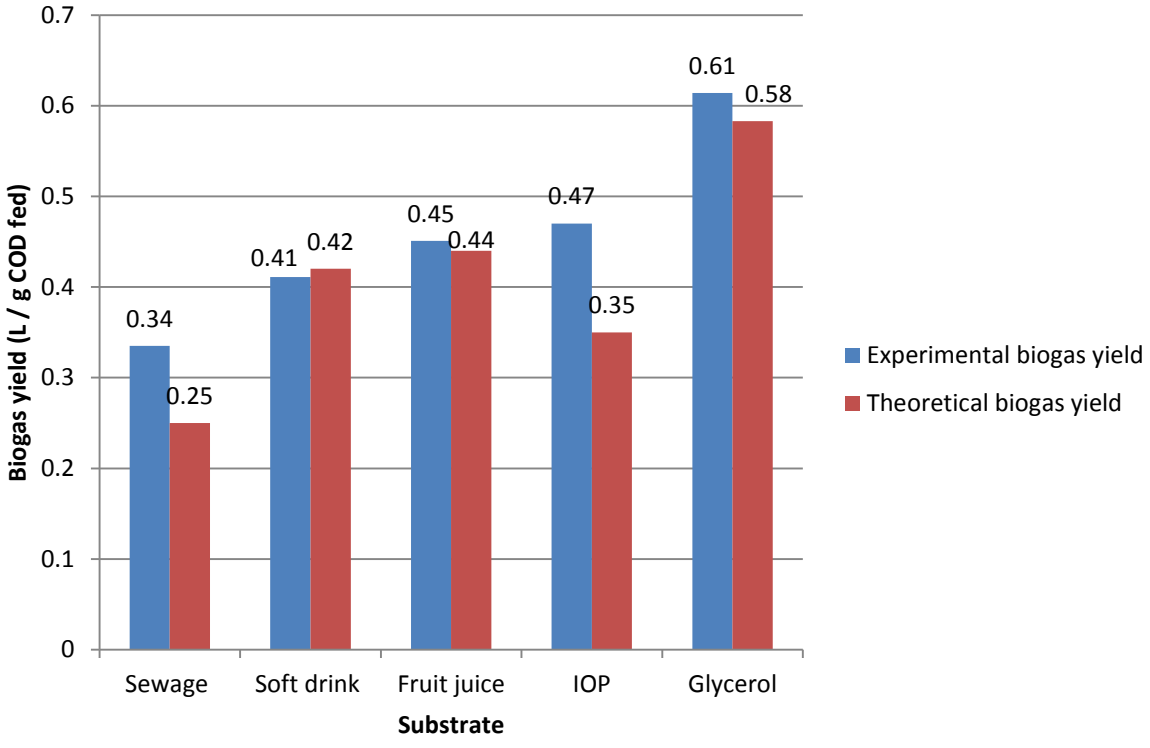

Figure 4.20: Comparison between theoretical and experimental biogas yields on COD basis.

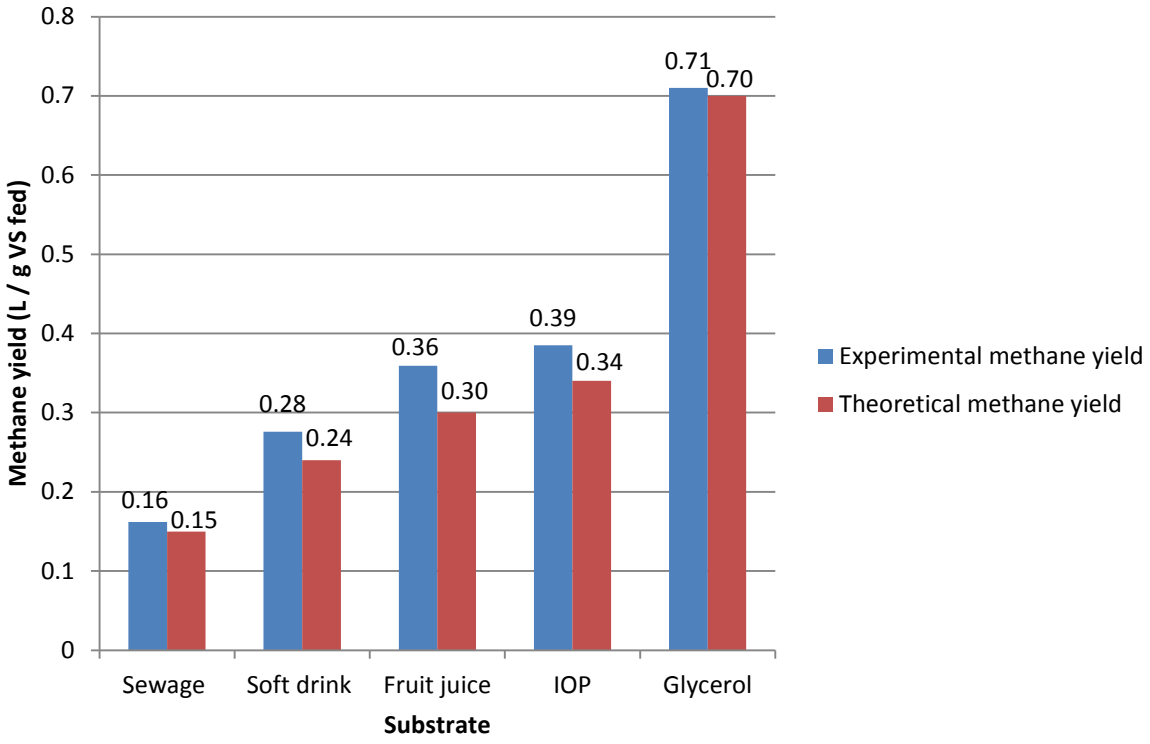

Figure 4.21: Comparison between theoretical and experimental methane yields on VS basis.

- IOP is an oil plant product. An investigation that was done by Kabouris *et al.* (2008) resulted in a methane yield of 0.34 L (g VS)$^{-1}$ for oil, fat and grease. According to PBW (2016), methane percentage in biogas is approximately 60%. Assuming this value, the theoretical biogas yield for IOP is therefore 0.34/0.60 = 0.57 L (g VS)$^{-1}$. A study that was done by Kubaska *et al.* (2010) on sunflower oil gave a biogas yield of 0.35 L (g COD)$^{-1}$. This value was then assumed as the theoretical biogas yield for IOP on a COD basis.
- Soft drink and fruit juice wastes are wastes obtained from food industries. The range of biogas yield for waste obtained from food industry is 400 to 600 L (g VS)$^{-1}$ (Braun and Welling, 2009). The organic loading of fruit juice obtained from this investigation was higher than that of soft drink, and thus fruit juice is expected to have a higher biogas yield than the soft drink. The theoretical biogas yields for fruit juice and soft drink were then assumed to be 0.50 and 0.40 L (g VS)$^{-1}$, respectively. If a methane percentage in biogas of 60% is assumed, the methane yields for fruit juice and soft drink are 0.30 and 0.24 (g VS)$^{-1}$, respectively. On a COD basis, the theoretical biogas

yields for fruit juice and soft drink were taken from studies from Khalid (2011) and Kubaska *et al.* (2010), and were found to be 0.44 and 0.42 L (g COD)$^{-1}$, respectively.

It is evident from Figure 4.19, Figure 4.20 and Figure 4.21 that the experimental values for each substrate were almost the same as its theoretical values.

4.3.9. Percentage of organic matter removed

The percentage of the organic content removed, based on VS and COD, are depicted in Figure 4.22. A sample of calculations for organic content removed is shown in Appendix I3.

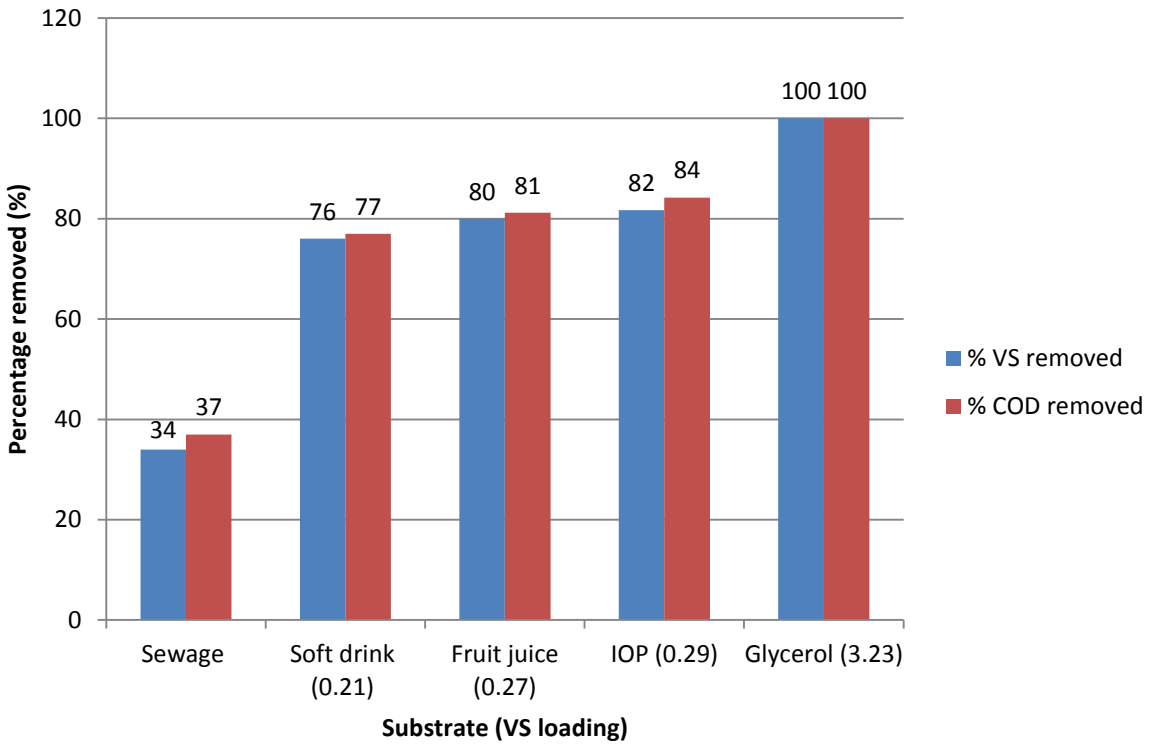

Figure 4.22: Percentage VS and COD removed for various substrates.

Co-digestion removals were higher than digestion removals of sewage alone, with VS and COD removals of 34 and 33%, respectively.

Moreover, glycerol had the highest removal efficiencies than the other substrates, with VS and COD efficiencies of 100%. Percentage VS removals for VS loadings of 0.21 (soft drink), 0.27 (fruit juice), 0.29 (IOP) and 3.23 (glycerol) g VS/L were 76, 80, 82 and 100%, whereas the percentage COD removals were 77, 81, 84 and 100%, respectively. The results suggested that an increase in organic loading increased the percentage organic matter removed. The same conclusion was made by Trnovec and Britz (1998) when he obtained COD removals of 70.0, 79.0 and 90.0% for VS loadings of 4.0, 7.0 and 11.0 g COD/L, respectively.

4.3.10. Material balance across the digesters for the first digestion trial

The material balance across the glycerol, fruit juice, soft drink and IOP digesters is shown in Table 4.4. The table shows the feed values (input) and the output values. The sample of calculations for the material balance across a digester is shown in Appendix O.

The main reason for doing material balance across the digesters was to determine the losses in each digester. If the losses in each digester was above 0.1 g, then the amount of loss will be added to the original volume of biogas generated by the system. It was assumed that some of biogas was vented to atmosphere while sampling for pH. To take into account the loss in inventory, a material balance across the digesters had to be done. The losses for glycerol, fruit juice, soft drink and IOP were 0.0007, 0.0047, 0.0047 and 0.0090 g, respectively. These losses were significantly small (less than 0.1 g).

Furthermore, it was assumed that some of the CO_2 was dissolved in H_2O, and thus had to be considered in the amount of biogas produced. The amount of CO_2 dissolved in H_2O for glycerol, IOP, fruit juice and soft drink were 16.40, 0.88, 0.77 and 0.47 g, with biogas volume produced of 14.50, 0.77, 0.68 and 0.41 L, respectively. In conclusion, the amount of CO_2 dissolved in H_2O increased with increase in biogas volume produced. Moreover, glycerol had the highest CO_2 dissolved in H_2O of 16.4 g possible due to the presence of lipids producing the highest biogas volume (14.50 L).

Table 4.4: Material balance across the digesters (first digestion trial).

	INPUT					OUTPUT											
Digester ID	**Water (g)**	**Sewage sludge (g)**	**Inoculum (g)**	**co-substrate (g)**	**Total(g)**	**Biogas volume (L)**	**Methane content (v/v)**	**Carbon dioxide content (v/v)**	**Moisture content in biogas (v/v)**	**Methane (g)**	**Carbon dioxide (g)**	**Moisture (g)**	**digestate (g)**	**water in the displacement before (g)**	**water in the displacement after (g)**	**Carbon dioxide dissolved in H_2O (g)**	**Total(g)**	**Losses (g)**
Glycerol	2400	0	2476	21.6	4898	14.50	76	22	1	7.00	5.60	0.10	4869	14416	14432	16.40	4898	0.0007
Fruit juice	2400	0	2476	20.9	4897	0.68	69	28	1	0.30	0.33	0.01	4896	676	676.8	0.77	4897	0.0079
Soft drink	2400	0	2476	20.8	4897	0.41	67	30	1	0.18	0.22	0.01	4896	411	411.5	0.47	4897	0.0079
IOP	2400	0	2476	20.9	4897	0.77	70	27	1	0.34	0.37	0.01	4895	770	770.9	0.88	4897	0.0090

4.4. Effect of Variables on the Anaerobic Process using the OFAT Method

The OFAT method was used in this sub-section to optimize the process variables in order to optimize biogas and methane yields. The first variable that was optimized was substrate to inoculum ratio, followed by volume concentration, and finally temperature. The substrate that was used for testing the effect of these variables on the anaerobic process was glycerol as it generated the highest amount of biogas and methane yields in the first digestion trial.

4.4.1. Effect of substrate to inoculum ratio on the anaerobic process

In order to see the effect of substrate to inoculum ratio on the anaerobic process using the OFAT method, the temperature and volume concentration were kept constant at 37.5℃ and 10 mL, respectively. The inoculum was added so as to maintain a digester volume of 4.8 L.

The feed characteristics of glycerol for various substrate to inoculum ratios are shown in Table 4.5. A sample of calculations for the feed characteristics of glycerol for various substrate to inoculum ratios is shown in Appendix G4.

Table 4.5: Feed characteristics of glycerol for various substrate to inoculum ratios.

	Glycerol				Raw sludge			Feed concentration in the digester	
Digester ID	**Volume**	**VS**	**COD**	**Concentration in the reactor**	**Volume**	**VS**	**COD**	**VS**	**COD**
	mL	**g**	**mg**	**g/L**	**mL**	**g**	**mg**	**g/L**	**g/L**
S:I = 0.5	10	7.8	11750	1	1 100	30.0	4950	7.9	3.5
S:I = 1.0	10	7.8	11750	1	1 500	41.7	6750	10.3	3.9
S:I = 1.5	10	7.8	11750	1	1 900	53.4	8550	12.8	4.2

The cumulative biogas production of glycerol for various substrate to inoculum ratios is illustrated in Figure 4.23:

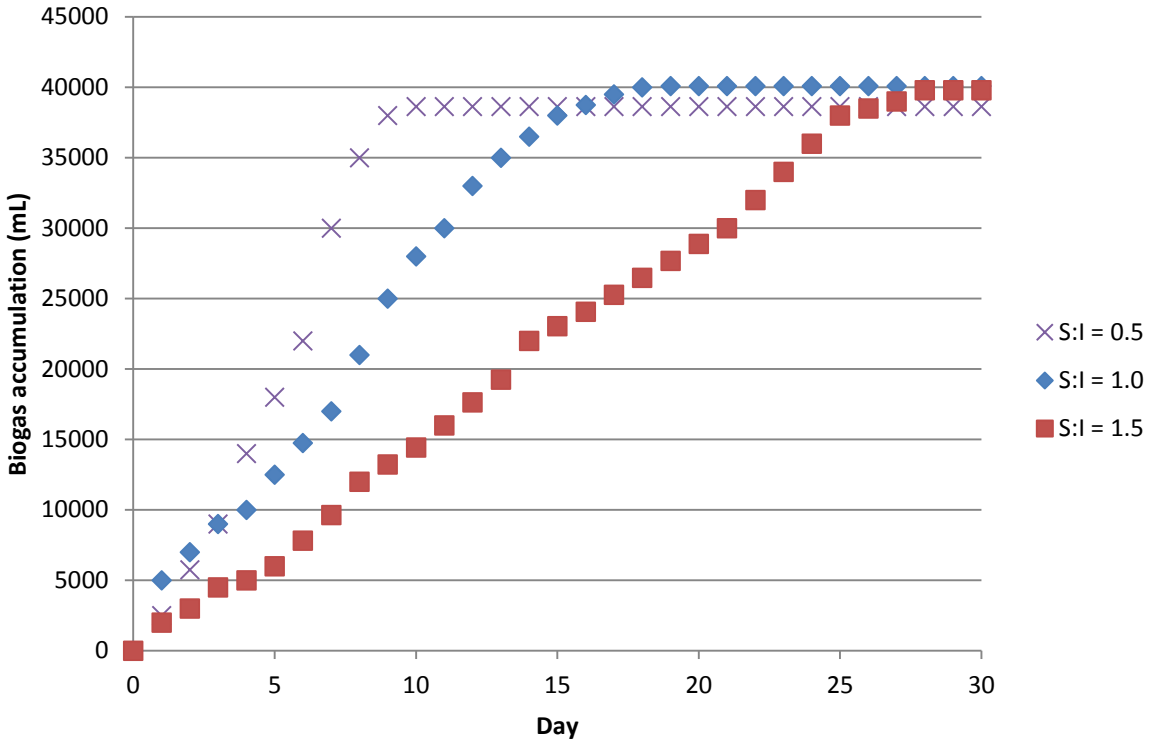

Figure 4.23: Cumulative biogas production at various substrate to inoculum ratios.

The stabilization time increased with increase in substrate to inoculum ratio; the digester that was operated at a substrate to inoculum ratio of 0.5 stabilized faster than the one that was operated at a substrate to inoculum ratio of 1 and 1.5, with the biogas peaks being on the 10th day, 18th day and 28th day, respectively. It was the low VS loading in the digester operated at a substrate to inoculum ratio of 0.5 that made it reach the peak of biogas generation early.

Figure 4.24 depicts the effect of VS concentration on biogas production. The lowest and highest biogas productions were 38 632 and 40 095 mL at substrate to inoculum ratios of 0.5 and 1, respectively. The low VS loading in the digester with low VS loading resulted in it the low biogas production. At a ratio of 1.5 there was a high loading content, which resulted in the overall biogas generation rate decreasing to 39 780 mL. The end result suggested that the substrate to inoculum ratio of 1 resulted in the optimum biogas production.

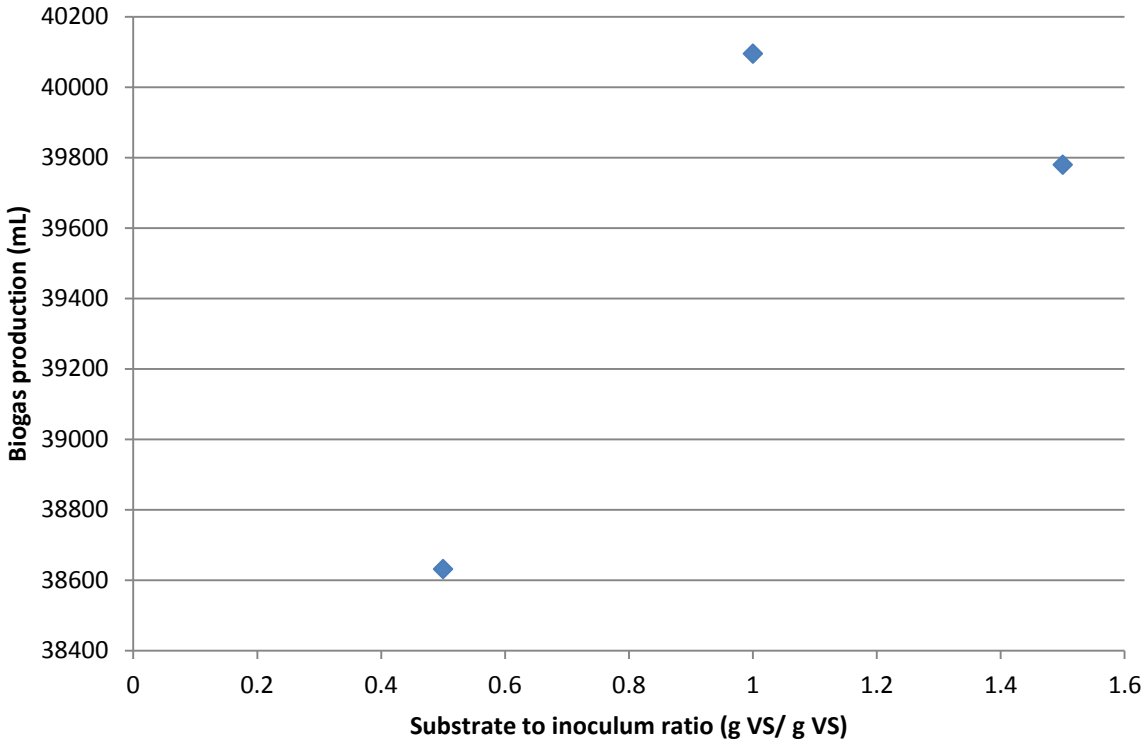

Figure 4.24: Effect of substrate to inoculum ratio on biogas production.

Table 4.6 shows the pH values at different substrate to inoculum ratios. Generally, an increase in substrate to inoculum ratio was accompanied by an increase in final pH, as well as an increase in pH rise.

Table 4.6: Effect of substrate to inoculum ratio on pH.

Substrate to inoculum ratio (g VS / g VS)	Initial pH	Final pH	pH rise (%)
0.5	7.1	7.5	5.6
1.0	7.2	7.7	6.9
1.5	7.3	7.9	8.2

Figure 4.25 shows the biogas yields of glycerol at various substrate to inoculum ratios:

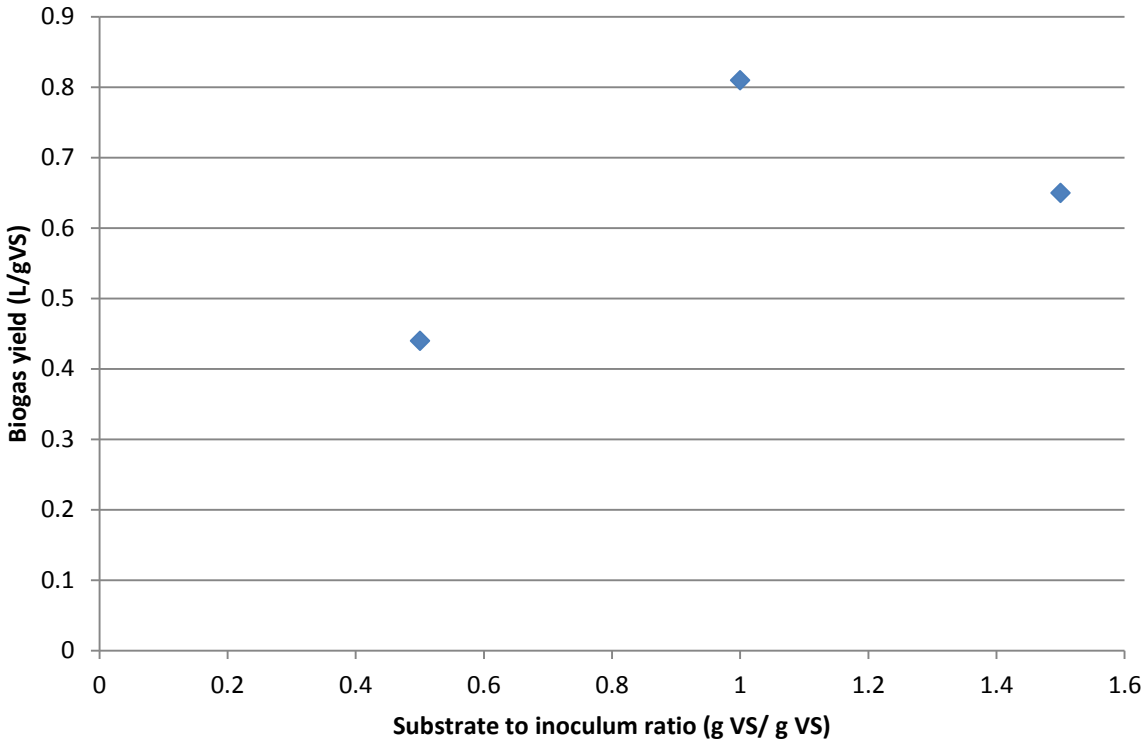

Figure 4.25: Effect of substrate to inoculum ratio on biogas yields.

The biogas yields for substrate to inoculum ratios of 0.5, 1.0 and 1.5 were 0.44, 0.81 and 0.65 L / (g VS), respectively. The results suggest that an increase in VS loading was accompanied by a decrease in biogas yield, with the substrate to inoculum ratio of 1 producing the highest biogas yield. The same conclusion was made by Feng *et al.* (2013) when they did a study on the effect of substrate to inoculum ratio of 1, 2, 3, 4, 5 and 6, and found that the optimum biogas yield of 0.46 L / (g VS) was found at the substrate to inoculum ratio of 1.

Figure 4.26 depicts the effect of substrate to inoculum ratio on VS and COD removal efficiencies for glycerol. The percentage VS removals for substrates to inoculum ratio of 0.5, 1.0 and 1.5 were 82, 98 and 99%, whereas the percentage COD removals were 86, 99 and 100%, respectively. The end results suggest that an increase in the substrate to inoculum ratio was followed by an increase in both the VS and COD efficiencies. This relationship was also evident in the investigation that was done by Chen *et al.* (2008), where the VS removal efficiencies for 1.67, 2.22 and 3.71 g/L were 60%, 95% and 100%.

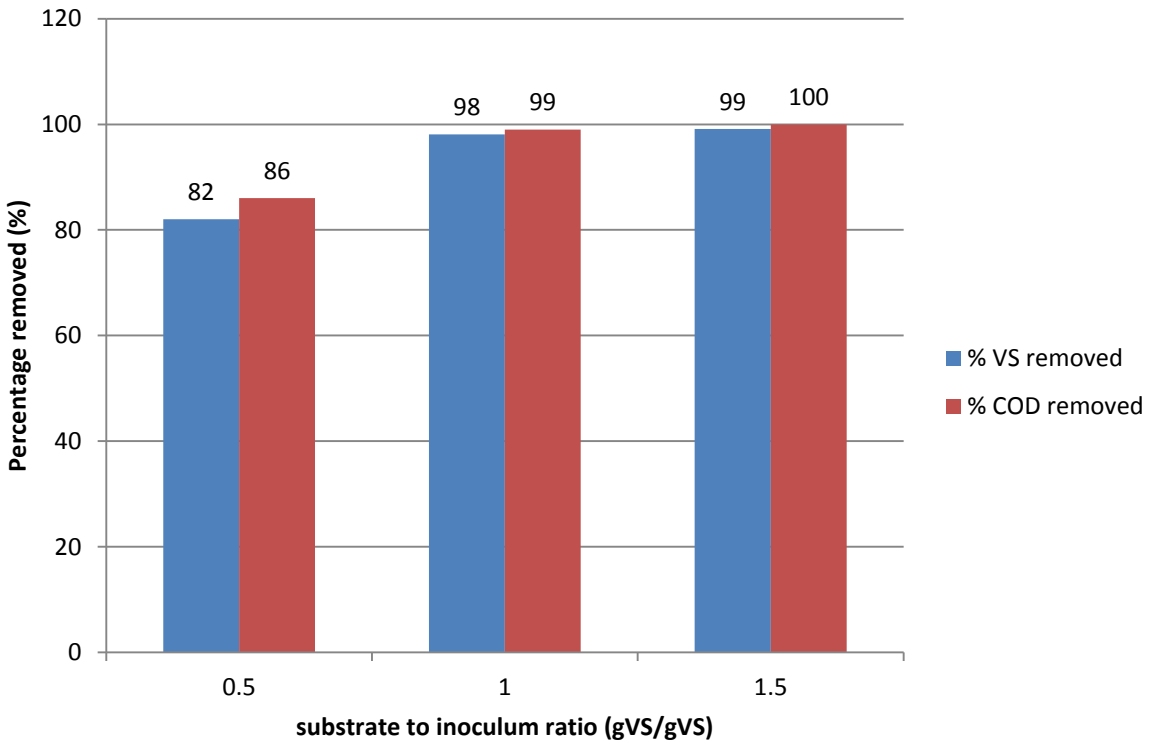

Figure 4.26: Effect of substrate to inoculum ratio on removal efficiencies.

The above results suggested that the optimum substrate to inoculum ratio is 1.1. With one variable optimized, the next step looks at the second variable, which is the glycerol volume concentration.

4.4.2. Effect of glycerol volume concentration on the anaerobic process

So as to see the impact of glycerol volume concentration on the digestion process using the OFAT method, the substrate to inoculum ratio and temperature were kept constant at 1.0 and 37.5℃, respectively. The inoculum was added so as to maintain a digester volume of 4.8 L. Table 4.7 shows the feed characteristics of glycerol at various concentrations in the digester. A sample of calculations for the feed characteristics of glycerol at various concentrations is shown in Appendix G5.

Table 4.7: Feed characteristics of glycerol at various glycerol concentrations in the digester.

	Glycerol			Raw sludge		Feed concentration in the digester
Digester ID	**Volume**	**VS**	**Concentration in the reactor**	**Volume**	**VS**	**VS**
	mL	**g**	**g glycerol/L**	**mL**	**g**	**g/L**
1 g glyc per L	10	7.8	1	1500	41.7	10.3
5 g glyc per L	22	17.1	5	1500	41.7	12.3
9 g glyc per L	34	26.4	9	1500	41.7	14.2

The cumulative biogas production of glycerol at various concentrations in the digester is illustrated in Figure 4.27:

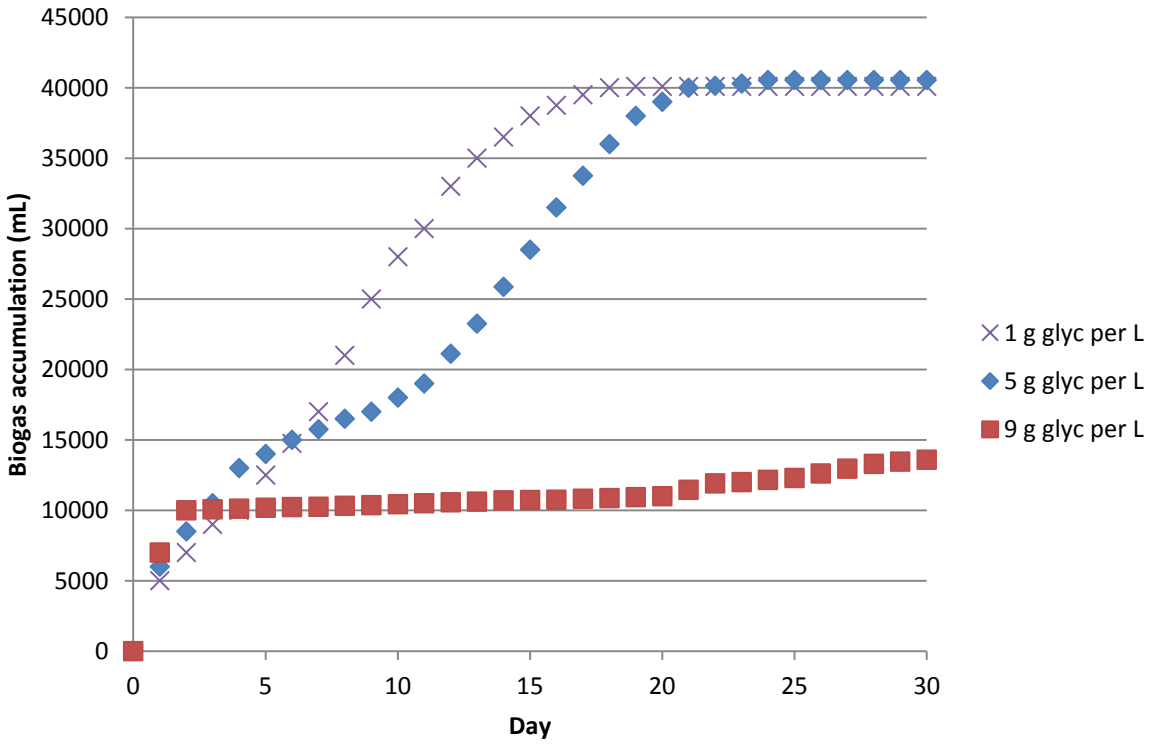

Figure 4.27: Accumulative biogas production at various glycerol concentrations.

In the early stages of the process, the generation of gas increased as the concentration of glycerol in the digester increased. The increase in the rate of production could be as a result of the degradation of the readily biodegradable soluble organic matters in the glycerol sample, but because of the sustained high VS loading in the digester with 9 g glycerol concentration per litre, VFAs accumulated in the system, and as a result, the overall system failed to stabilize. The digesters with 1 and 5 g glycerol concentration per litre were able to stabilize in the last stages of the digestion process. The same conclusion was made by Foucault (2011) after conducting a study on crude glycerol where the system only stabilized at glycerol concentration between 2.3 and 5 g glycerol per litre; the system was overloaded at 10 g glycerol per litre, and in the end did not stabilize.

Moreover, the stabilization time increased with an increase in g glycerol per litre (loading); the digester that was operated at 1 g glycerol per litre stabilized faster than the one that was operated at 5 g glycerol per litre, with the biogas peaks being on the 18^{th} day and 22^{th} day, respectively. It was the low glycerol concentration in the digester operated at 1 g glycerol per litre that caused it to reach the peak of biogas generation early. The digester that was operated at 9 g glycerol per litre was not operated long enough to reach stabilization.

Figure 4.28 depicts the effect of VS concentration on biogas production. The biogas production was the same for the 1 and 5 g glycerol concentrations, however, it dropped quite significantly for the 9 g glycerol concentration due to high VS loading of 14.2 g VS/L. For glycerol, the optimum biogas volume was between 1 and 5 g glycerol/L. The same observation was made by Holm-Nielsen *et al.* (2008) where the optimum biogas volume was between 3.0 and 5.0 g glycerol/L; the digester experienced overloading above 5 g glycerol/L.

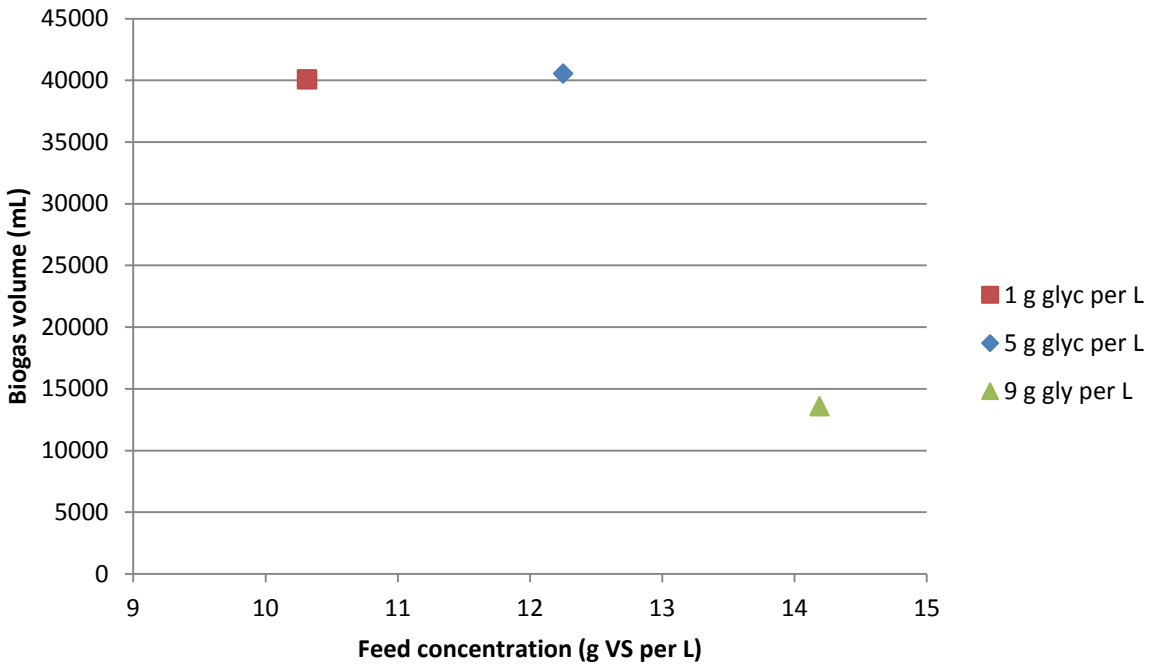

Figure 4.28: Effect of feed concentration (g VS/L) on biogas production.

Table 4.8 shows the effect of glycerol concentration on pH. For all digesters, the final pH was higher than the initial pH. Because of the low initial pH in the digester with 9 g glycerol concentration, the lowest final pH was observed in this digester.

Furthermore, an increase in glycerol concentration was accompanied by an increase in pH rise, with 9 g glycerol / L having the highest pH rise of 39.1%.

Table 4.8: Effect of glycerol concentration on pH.

Glycerol concentration (g glycerol / L)	Initial pH	Final pH	pH rise (%)
1	7.2	7.7	6.9
5	7.3	7.9	8.2
9	4.6	6.4	39.1

Figure 4.29 shows the biogas yields of glycerol. The biogas yields for feed concentrations of 10.31, 12.25 and 14.19 g VS/L were 0.81, 0.69 and 0.20 L / g VS, respectively. The results suggest that an increase in VS loading was accompanied by a decrease in biogas yield, with 0.5% concentration of glycerol in the digester producing the highest biogas yield. These results agree with the results that were obtained by Holm-Nielsen *et al.* (2008).

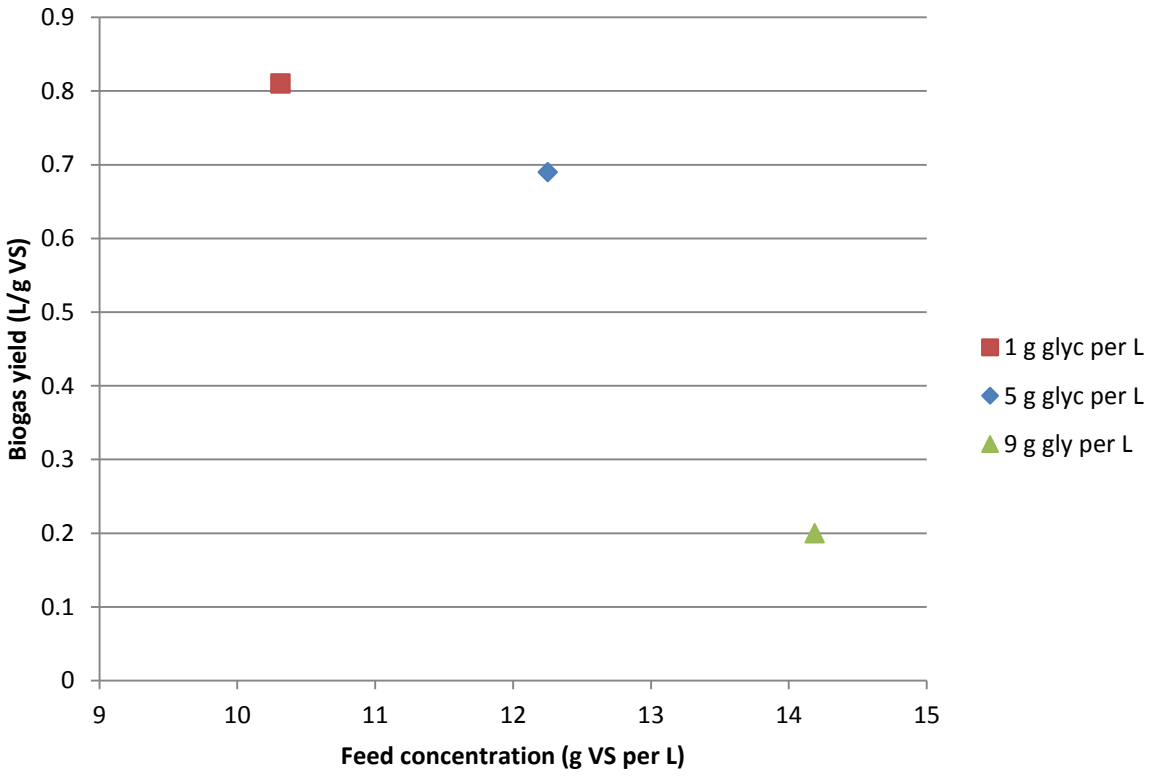

Figure 4.29: Effect of glycerol concentration in the digester on biogas yields.

Figure 4.30 shows VS and COD removal efficiencies for the glycerol samples. The removal efficiencies were almost the same for the glycerol concentration between 1 and 5. Because of the high loading in the digester with 9 g glycerol concentration, the digestion process was inhibited, and as a result, VS and COD efficiencies were very low.

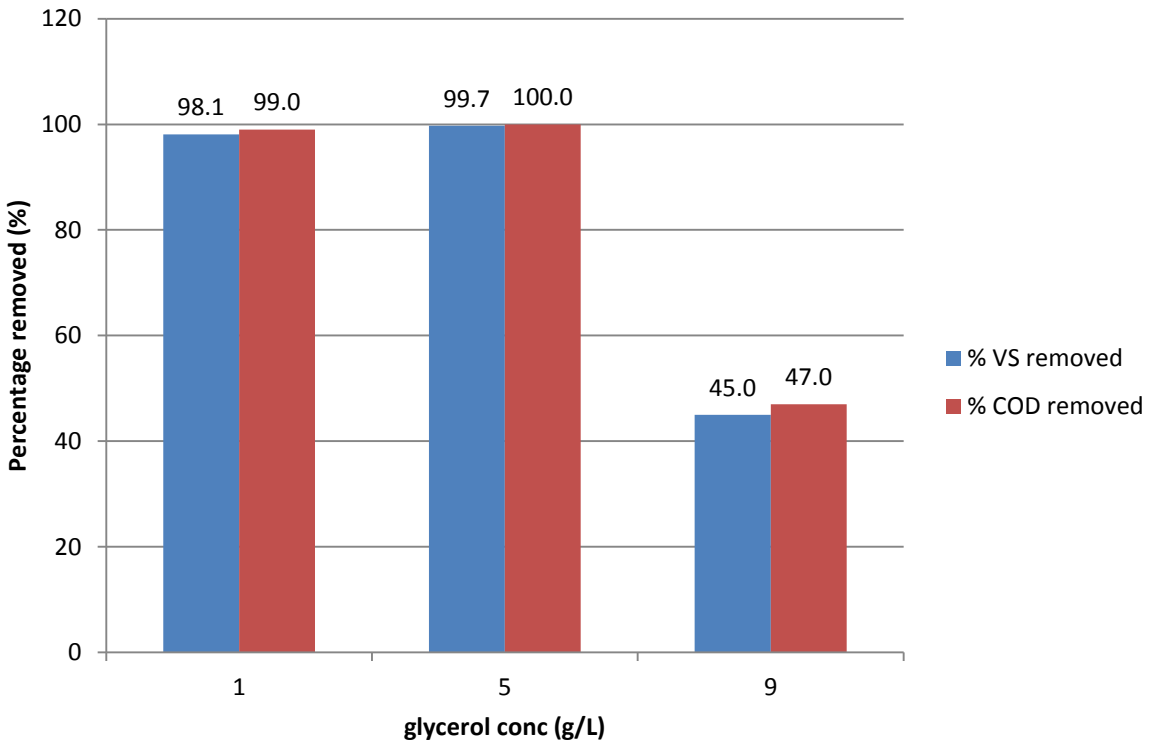

Figure 4.30: Percentage VS and COD removed.

The above results suggest that the digestion process can be operated between 10 mL and 22 mL (1 and 5 g glycerol concentration in the digester, respectively). However because of the high biogas yield in the digester with 10 mL, it was regarded as the optimum volume concentration. With two variables optimized, the next step was to look at the last variable, which was the temperature.

4.4.3. Effect of temperature on the anaerobic process

So as to observe the effect of temperature on anaerobic digestion using the OFAT method, the substrate to inoculum ratio and volume concentration were kept constant at 1.0 and 10 mL, respectively. The feed characteristics of glycerol at the selected temperatures are shown in Table 4.9:

Table 4.9: Feed characteristics of glycerol at various temperatures.

	Glycerol				Raw sludge			Feed concentration in the digester	
Digester ID	**Volume**	**VS**	**COD**	**Concentration in the reactor**	**Volume**	**VS**	**COD**	**VS**	**COD**
	mL	**g**	**mg**	**g glycerol/L**	**mL**	**g**	**mg**	**g/L**	**g/L**
25 deg C	10	7.80	11750	1	1500	41.7	6750	10.3	3.9
37.5 deg C	10	7.80	11750	1	1500	41.7	6750	10.3	3.9
50 deg C	10	7.80	11750	1	1500	41.7	6750	10.3	3.9

The cumulative biogas production of glycerol at 25℃, 37.5℃ and 50℃ is depicted in Figure 4.31:

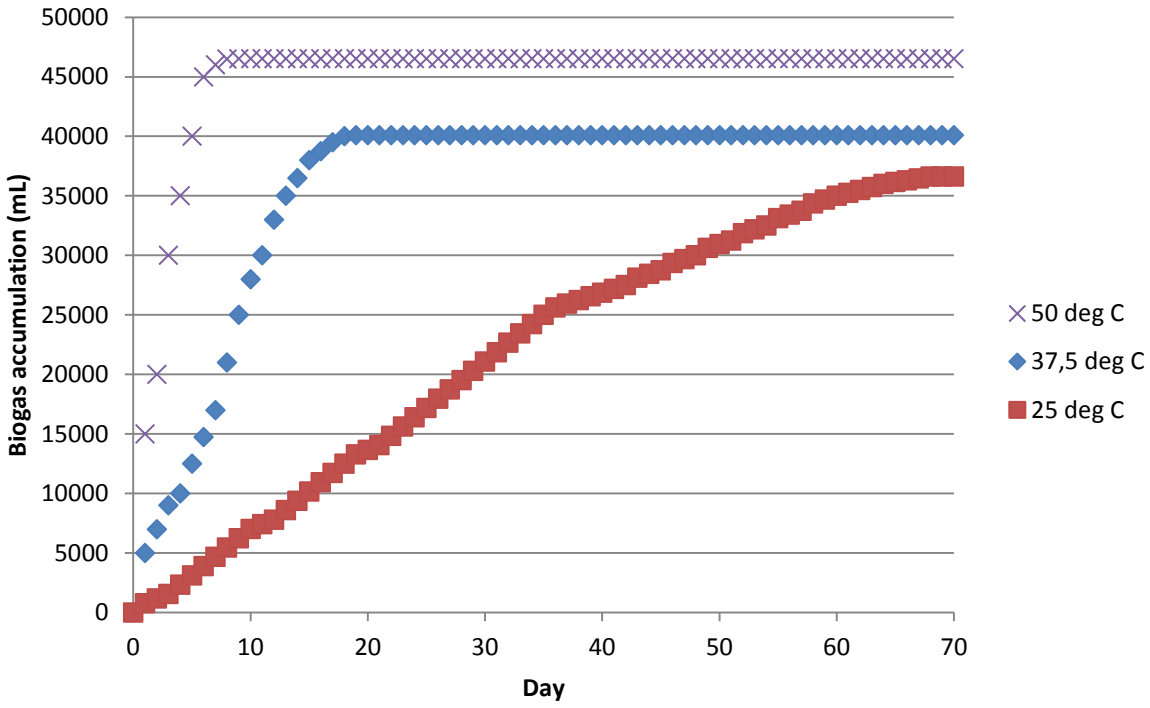

Figure 4.31: The cumulative biogas production at various temperatures.

The rate of generation of the digestion process increased with an increase in temperature, with the thermophilic temperature digester (50℃) showing the highest rate of generation. Thus, the activity of bacteria increased with an increase in temperature, which biodegraded the thermophilic substrate faster (as early as in the hydrolysis stage). The same conclusion

was made by Zhang *et al.* (2006) when they conducted an investigation on the impact of temperature at 25℃, 35℃, 45℃ and 55℃ on biogas formation, and found that the rate of generation was highest at 55℃.

Moreover, the biogas peaks were on the 10th day, 18th day and 60th day, respectively. The digester that was operated at a thermophilic temperature of 50℃ stabilized faster than the ones that was operated at a mesophilic temperature of 37.5℃, which stabilized faster than that at 25℃. It was the high rate of degradation in the thermophilic digester that resulted in the amount of biogas peaking on day 10. The final results suggest that an increase in temperature is accompanied by the decrease in biogas peak days. These results agree with Pandey and Soupir (2012) when they obtained biogas peaks on day 61, day 25 and day 15 for digesters at 25, 37 and 52.5 ℃, respectively.

Figure 4.32 depicts the effect of temperature on biogas generation:

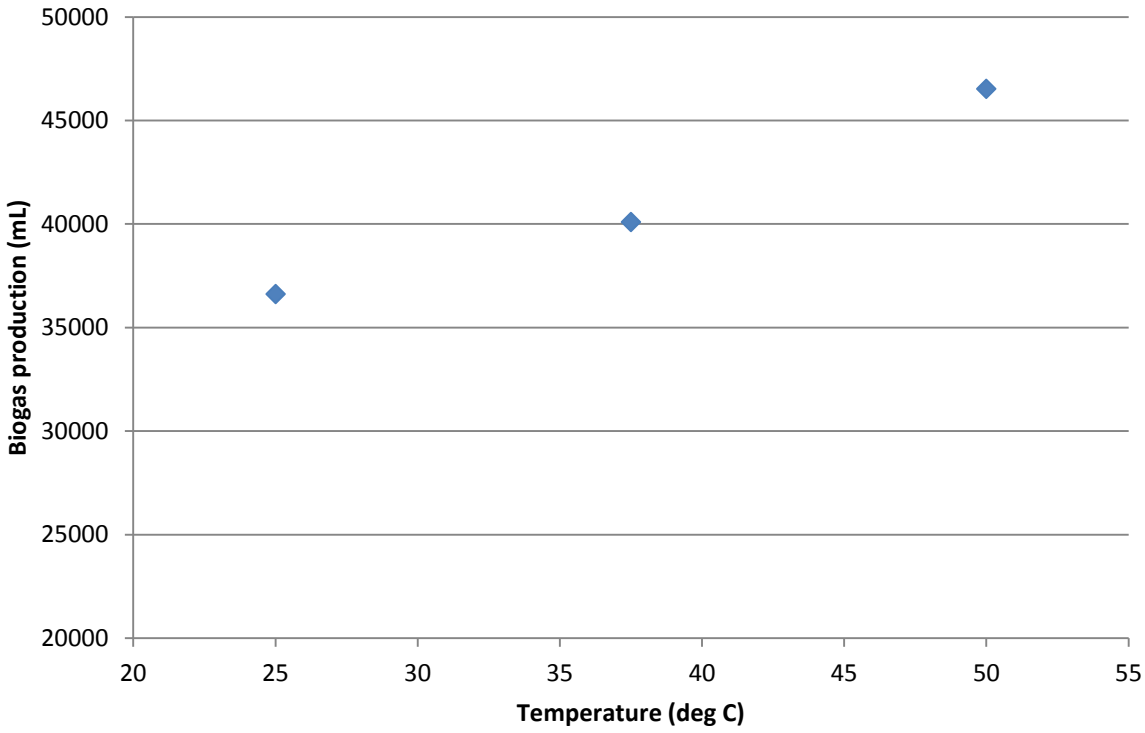

Figure 4.32: Effect of temperature on biogas generation.

The biogas productions were 36 630, 40 095 and 46 530 mL for 25, 37.5 and 50℃, respectively. Generally, the generation of biogas increased with an increase in temperature. This result was also evident in the study that was done by Zhang *et al.* (2006) where an investigation on effect of temperature at 25℃, 35℃, 45℃ and 55℃ on the biogas production rate, resulted in biogas productions of 5 500, 2 000, 2 600 and 2 700 mL/d, respectively. Moreover, the thermophilic digester (50℃) generated the highest amount of biogas (46 530 mL).

The effect of temperature on pH is shown in Table 4.10. The result suggested that an increase in temperature lead to a drop in pH, with the digester that was performed at a thermophilic temperature of 50℃ having the lowest final pH of 7.1. This could be due to high VFAs accumulated in the system. Zhao (2011) studied the effect of temperature on biogas production, and found that an increase in temperature lead to a decrease in pH.

Table 4.10: Effect of temperature on pH.

Temperature (℃)	Initial pH	Final pH
25	7.2	7.6
37.5	7.2	7.7
50	7.2	7.1

The effect of temperature on the biogas yields of glycerol trials is shown in Figure 4.33. The biogas yields for 25, 37.5 and 50℃ were 0.74, 0.81 and 0.94 L/g VS, respectively. The results suggest that an increase in temperature was accompanied by an increase in biogas yield, with the thermophilic temperature of 50℃ generating the highest biogas yield. The same conclusion was made by Zhang *et al*. (2006) when they conducted an investigation on the impact of temperature at 25℃, 35℃, 45℃ and 55℃ on biogas formation, and found biogas yields of 48.6, 113.2, 152.2 and 159.7 mL (g TS)$^{-1}$, respectively.

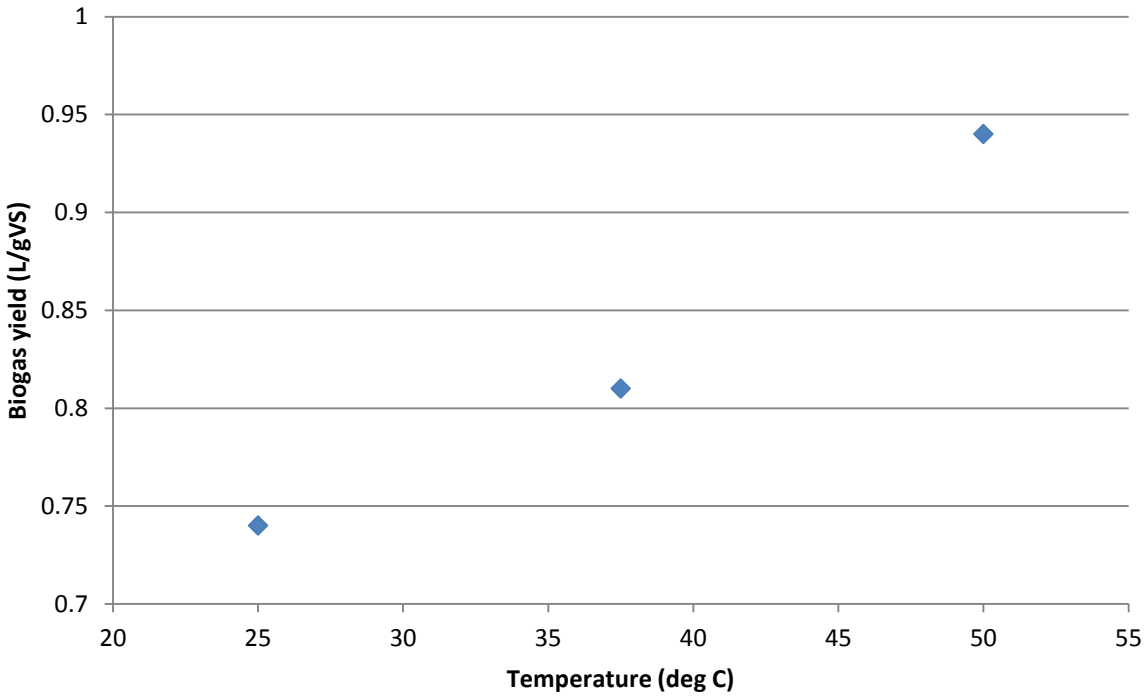

Figure 4.33: Effect of temperature on biogas yields.

The influence of temperature on methane percentage is shown in Figure 4.34:

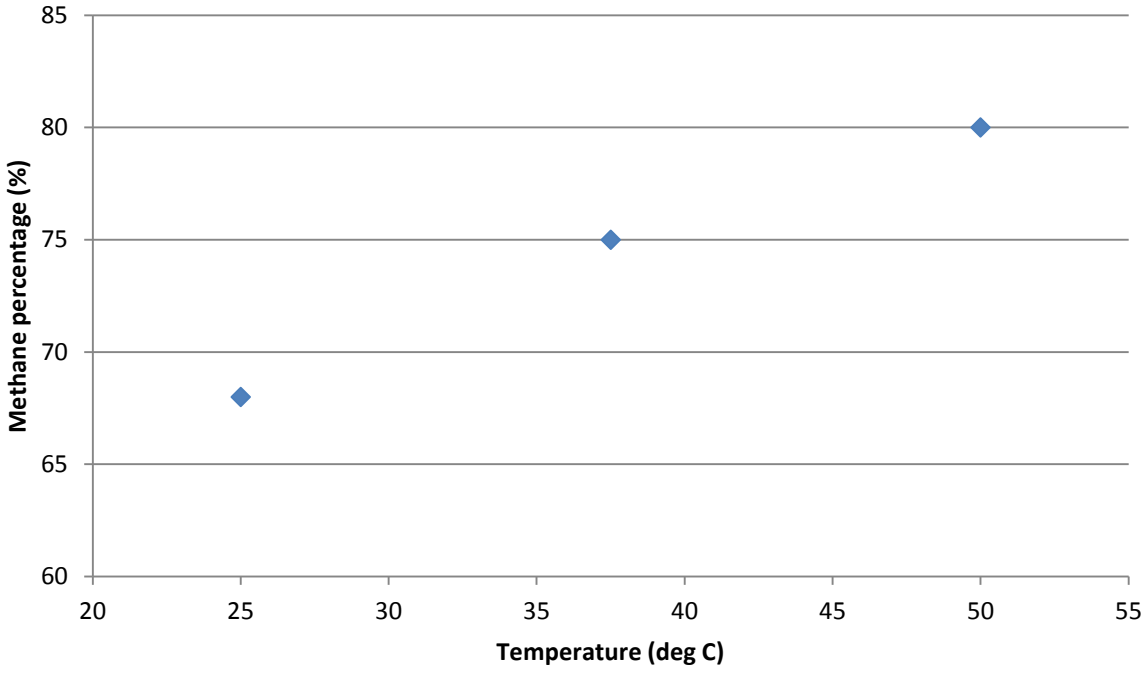

Figure 4.34: Effect of temperature on methane percentage.

The methane percentages for the digesters at 25, 37.5 and 50℃ were 68, 75 and 80%, respectively. The result suggested that an increase in temperature results in an increase in methane percentage. Zhang *et al*. (2006) reached the same conclusion during an investigation on the impact of temperature at 25℃, 35℃, 45℃ and 55℃ on biogas formation, and found methane percentages of 65.2, 66.4, 66.6 and 70.0%, respectively. Pandey and Soupir (2012) conducted an investigation on the effect of temperature on dairy manure at 37℃ and 52.5℃, and found methane percentages of 55 and 70%, respectively. They also did an investigation at 25℃, but the biogas production was too low for the methane content to be measured.

Figure 4.35 shows VS and COD removal efficiencies for the glycerol samples at various temperatures:

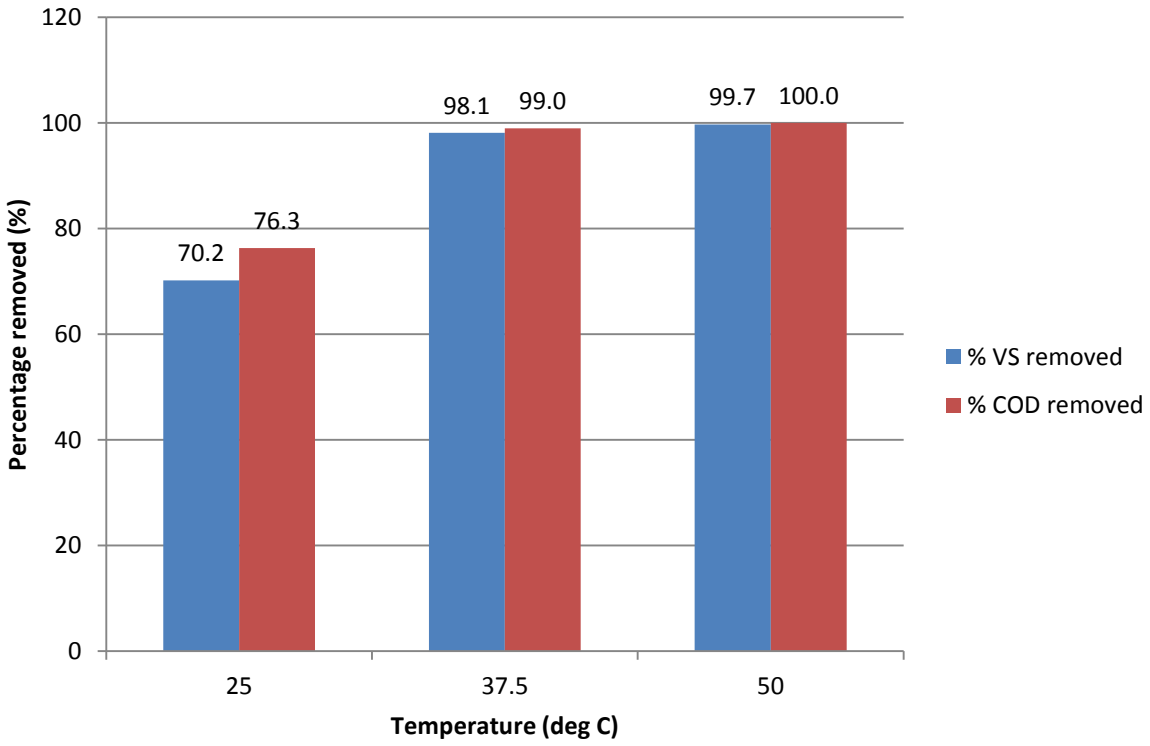

Figure 4.35: Percentage VS and COD removed at various temperatures.

The percentage VS removals for the digesters at 25, 37.5 and 50℃ were 70.2, 98.1 and 99.7%, whereas the percentage COD removals were 76.3, 99 and 100%, respectively. The

results suggest that an increase in temperature was followed by an increase in both the VS and COD efficiencies. This relationship was also evident in the investigation by Bello-Mendoza and Castillo-Rivera (1998), where the removal efficiencies for the 13 and 35℃ were 61% and 93.4%, respectively. Zhang *et al.* (2006) found 31.4, 44.0 and 57.1% COD reductions and 35.5, 53.2 and 59.8% VS reductions when they did an investigation of anaerobic treatment of waste from municipal solid at 25℃, 35℃ and 55℃, respectively.

4.4.4. Material balance across the digesters

The material balance for OFAT digesters is shown in Table 4.11. The table shows the feed values (input) and the output values. The sample of calculations for the material balance for digesters is shown in Appendix O. The main reason for doing material balance across the digesters was to determine the losses in each digester. If the losses in each digester was above 0.1 g, then the amount of loss will be added to the original volume of biogas generated by the system.

It was assumed that some of biogas was vented to atmosphere while sampling for pH. To take into account the loss in inventory, a material balance across the digesters had to be done. The losses for S:I = 0.5, S:I = 1.0, S:I = 1.5, 1 g glycerol/L, 5 g glycerol/L, 9 g glycerol/L, 25 deg C, 37.5 deg C and 50 deg C were 0.0336, 0.0005, 0.0005, 0.0092, 0.001, 0.0083, 0.0005 and 0.0084 g, respectively. These losses were assumed to have vented to atmosphere during sampling. The S:I = 0.5 digester had the highest losses of 0.0336 g. Nevertheless, the losses were all below 0.1 g, and thus can be neglected.

Furthermore, the amount of CO_2 dissolved in H_2O for 9 g glycerol/L, 25 deg C, S:I = 0.5, S:I = 1.5, S:I = 1.0, 37.5 deg C, 1 g glycerol/L, 5 g glycerol/L, and 50 g glycerol/L were 13.6, 36.6, 38.6, 39.8, 40.1, 40.1, 40.1, 40.6 and 46.5 g, with biogas volume produced of 15.5, 41.8, 44, 45.4, 45.7, 45.7, 45.7, 46.2 and 53 L, respectively. Generally, the amount of CO_2 dissolved in H_2O increased with increase in biogas volume produced. The 50 deg C digester had the highest CO_2 dissolved in H_2O of 53 g possible due to the high temperature producing the highest biogas volume (53 L).

Table 4.11: Material balance across the OFAT digesters.

	INPUT					OUTPUT												
Digester ID	**Water (g)**	**Sewage sludge (g)**	**Inoculum (g)**	**co-substrate (g)**	**Total(g)**	**Biogas volume (L)**	**Methane content (v/v)**	**Carbon dioxide content (v/v)**	**Moisture content in biogas (v/v)**	**Methane (g)**	**Carbon dioxide (g)**	**Moisture (g)**	**digestate (g)**	**water in the displacement before (g)**	**water in the displacement after (g)**	**Carbon dioxide dissolved in H_2O (g)**	**Total(g)**	**Losses (g)**
S:I = 0.5	0	1153.9	3817	10.8	4983	38.6	69	28	1	17.0	19.0	0.28	4902	38632	38676	44.0	4982	0.0336
S:I = 1.0	0	1573.5	3405	10.8	4989	40.1	75	22	1	19.2	15.5	0.29	4908	40095	40140	45.7	4989	0.0005
S:I = 1.5	0	1993.1	2992	10.8	4996	39.8	70	27	1	17.8	18.9	0.29	4914	39780	39825	45.4	4996	0.0005
1 g glyc/L	0	1573.5	3405	10.8	4989	40.1	75	22	1	19.2	15.5	0.29	4908	40095	40141	45.7	4989	0.0005
5 g glyc/L	0	1573.5	3405	23.8	5002	40.6	76	21	1	19.7	14.9	0.29	4920	40550	40596	46.2	5002	0.0092
9 g glyc/L	0	1573.5	3405	36.7	5015	13.6	77	20	1	6.7	4.8	0.10	4988	13600	13616	15.5	5015	0.0010
25 deg C	0	1573.5	3405	10.8	4989	36.6	68	29	1	15.9	18.7	0.26	4912	36630	36672	41.8	4989	0.0083
37.5 deg C	0	1573.5	3405	10.8	4989	40.1	75	22	1	19.2	15.5	0.29	4908	40095	40140	45.7	4989	0.0005
50 deg C	0	1573.5	3405	10.8	4989	46.5	80	17	1	23.8	13.9	0.33	4898	46530	46583	53.0	4989	0.0084

4.5. Design of Experiments - ANOVA

4.5.1. ANOVA

The ANOVA table helps the reader observe the chosen effects together with their coefficients. Table 4.12 and Table 4.13 show ANOVA outputs for biogas and methane yields, respectively (see Appendix M2 for a sample of calculations for ANOVA table). The model F-values of 11.72 and 13.19 imply that the models are significant (as indicated in the tables) for biogas and methane yields, respectively. The tables also have p-values that give the probabilities for the Model. Thus, there is only a 0.72% and 0.55% chance or probability that F-values this big could occur as a result of noise for biogas and methane yields, respectively – these values are indicated on the model F-values.

Table 4.12: ANOVA output for the biogas yields– partial sum of squares.

Source	Sum of Squares	df	Mean Square	F-value	p-value Prob > F	
Model:	0.87	9	0.097	11.72	0.0072	Significant
A-Temperature	*0.02*	*1*	*0.020*	*2.42*	*0.1802*	
B-Substrate to inoculum ratio	7.2×10^{-3}	*1*	7.2×10^{-3}	*0.87*	*0.3931*	
C-Glycerol volume	*0.64*	*1*	*0.64*	*77.39*	*0.0003*	
AB	*0.00*	*1*	*0.00*	*0.00*	*1.0000*	
AC	2.5×10^{-3}	*1*	2.5×10^{-3}	*0.30*	*0.6057*	
BC	*0.010*	*1*	*0.010*	*1.21*	*0.3211*	
A^2	2.3×10^{-3}	*1*	2.3×10^{-3}	*0.28*	*0.6195*	
B^2	*0.078*	*1*	*0.078*	*9.41*	*0.0279*	
C^2	*0.12*	*1*	*0.12*	*14.50*	*0.0125*	
Residual:	0.041	5	8.3×10^{-3}			
Lack of Fit	*0.041*	*3*	*0.014*			
Pure Error	*0.000*	*2*	*0.000*			
Cor Total	0.91	14				

Table 4.13: ANOVA output for the methane yields – partial sum of squares.

Source	Sum of Squares	df	Mean Square	F-value	p-value Prob > F	
Model:	0.53	9	0.059	13.19	0.0055	Significant
A-Temperature	*0.036*	*1*	*0.036*	*8.15*	*0.0357*	
B-Substrate to inoculum ratio	4.5×10^{-3}	*1*	4.5×10^{-3}	*1.01*	*0.3614*	
C-Glycerol volume	*0.35*	*1*	*0.35*	*77.90*	*0.0003*	
AB	*0.000*	*1*	*0.000*	*0.000*	*1.0000*	
AC	6.4×10^{-3}	*1*	6.4×10^{-3}	*1.43*	*0.2854*	
BC	5.6×10^{-3}	*1*	5.6×10^{-3}	*1.26*	*0.3132*	
A^2	5.8×10^{-6}	*1*	5.8×10^{-3}	1.3×10^{-3}	*0.9727*	
B^2	*0.057*	*1*	*0.057*	*12.64*	*0.0163*	
C^2	*0.082*	*1*	*0.082*	*18.26*	*0.0079*	
Residual:	0.022	5	4.5×10^{-3}			
Lack of Fit	*0.022*	*3*	7.5×10^{-3}			
Pure Error	*0.000*	*2*	*0.000*			
Cor Total	0.55	14				

By default, the Design Expert software takes into consideration p-values of less than or equal to 0.05 to be important, whereas values greater than 0.1 are not significant. For biogas yield, the p-values for the model terms C, B^2, and C^2 fall below 0.05, whereas the model terms A, C, B^2, and C^2 are significant for methane yield.

Table 4.14 and Table 4.15 show collection of summary tables for biogas yields and methane yield models, respectively. A sample of calculations for the collection of summary table is shown in Appendix M3.

Table 4.14: Collection of summary for biogas yield.

Source	Value
Standard Deviation	0.09
Mean	0.49
C.V. %	18.54
PRESS	0.66
R-Squared	0.96
Adjusted R-Squared	0.87
Predicted R-Squared	0.28
Adequate Precision	11.19

Table 4.15: Collection of summary for methane yield.

Source	Value
Standard Deviation	0.07
Mean	0.37
C.V. %	17.89
PRESS	0.36
R-Squared	0.96
Adjusted R-Squared	0.89
Predicted R-Squared	0.35
Adequate Precision	12.11

The "Predicted R-Squared" of 0.28 and 0.35 for biogas and methane yields are not nearly equal to the "Adjusted R-Squared" of 0.87 and 0.89 as one might normally expect; i.e. for both biogas and methane yields, the difference was above 0.5. This suggests that there is a huge block effect or an error with the data inserted or model chosen. Things to take into consideration are outliers, transformation for response, and model reduction. "Adequate Precision" computes the ratio between signal and noise. Design-Expert Software recommends a ratio above 4. In this investigation, the "Adequate Precisions" for biogas and methane yields were 11.19 and 12.11, respectively, which indicate that the signal was adequate. These models may be exploited for the navigation of the design space.

Table 4.16 and Table 4.17 show coefficient estimates for the biogas and methane yield models, respectively. A sample of calculations for the coefficient estimate table is show in Appendix M4.

Table 4.16: Coefficient estimate for biogas yield.

Factor	Coefficient estimate	df	Standard error	95% CI		VIF
				Low	High	
Intercept	0.650	1	0.052	0.520	0.780	
A-Temperature	0.050	1	0.032	-0.033	0.130	1.00
B-Substrate to inoculum ratio	0.030	1	0.032	-0.053	0.110	1.00
C-Glycerol volume	-0.280	1	0.032	-0.370	-0.200	1.00
AB	1.405×10^{-17}	1	0.045	-0.120	0.120	1.00
AC	-0.025	1	0.045	-0.140	0.092	1.00
BC	-0.050	1	0.045	-0.170	0.067	1.00
A^2	0.025	1	0.047	-0.097	0.150	1.01
B^2	-0.150	1	0.047	-0.270	-0.023	1.01
C^2	-0.180	1	0.047	-0.300	-0.058	1.01

Table 4.17: Coefficient estimate for methane yield.

Factor	Coefficient estimate	df	Standard error	95% CI		VIF
				Low	High	
Intercept	0.520	1	0.039	0.420	0.620	
A-Temperature	0.067	1	0.024	6.7×10^{-3}	0.130	1.00
B-Substrate to inoculum ratio	0.024	1	0.024	-0.037	0.085	1.00
C-Glycerol volume	-0.210	1	0.024	-0.270	-0.150	1.00
AB	1.280×10^{-19}	1	0.033	-0.086	0.086	1.00
AC	-0.040	1	0.033	-0.130	0.046	1.00
BC	-0.038	1	0.033	-0.120	0.048	1.00
A^2	-1.250×10^{-3}	1	0.035	-0.091	0.088	1.01
B^2	-0.120	1	0.035	-0.210	-0.034	1.01
C^2	-0.150	1	0.035	-0.240	-0.059	1.01

The equations for biogas and methane yields in terms of coded factors are (see Appendix M5 for a sample of calculations for the model equations in terms of coded factors):

$$Biogas\ yield = 0.650 + 0.050 \times A + 0.030 \times B - 0.280 \times C + 1.405 \times 10^{-17} \times AB - 0.025 \times AC - 0.050 \times BC + 0.025 \times A^2 - 0.150 \times B^2 - 0.180 \times C^2 \quad (4.2)$$

$$Methane\ yield = 0.520 + 0.067 \times A + 0.024 \times B - 0.210 \times C + 1.280 \times 10^{-19} AB - 0.040 \times AC - 0.038 \times BC - 1.250 \times A^2 - 0.120 \times B^2 - 0.150 \times C^2 \quad (4.3)$$

Equation 4.2 and 4.3 may possibly be used to make estimations about the coded factors for biogas and methane yields for given levels of each independent process variable.

Final equations for biogas and methane yields in terms of actual factors are (see Appendix M5 for sample of calculations of conversion from coded to actual factors):

$$Biogas\ yield = -0.3467 - 4.33333 \times 10^{-3} \times (temperature) + 1.40333 \times (substrate\ to\ inoculum\ ratio) + 0.046257 \times (glycerol\ volume) + 2.25 \times 10^{-18} \times (temperature) \times (substrate\ to\ inoculum\ ratio) - 1.66667 \times 10^{-4} \times (temperature) \times (glycerol\ volume) - 8.33333 \times 10^{-3} \times (substrate\ to\ inoculum\ ratio) \times (glycerol\ volume) + 1.6 \times 10^{-4} \times (temperature)^2 - 0.6 \times (substrate\ to\ inoculum\ ratio)^2 - 1.25 \times 10^{-3} \times (glycerol\ volume)^2 \quad (4.4)$$

$$Methane\ yield = -0.71101 + 0.011867 \times (temperature) + 1.175 \times (substrate\ to\ inoculum\ ratio) + 0.044306 \times (glycerol\ volume) + 4.39493 \times 10^{-17} \times (temperature) \times (substrate\ to\ inoculum\ ratio) - 2.66667 \times 10^{-4} \times (temperature) \times (glycerol\ volume) - 6.25 \times 10^{-3} \times (substrate\ to\ inoculum\ ratio) \times (glycerol\ volume) - 8.0 \times 10^{-6} \times (temperature)^2 - 0.495 \times (substrate\ to\ inoculum\ ratio)^2 - 1.03299 \times 10^{-3} \times (glycerol\ volume)^2 \quad (4.5)$$

Equation 4.4 and 4.5 may possibly be used to make estimations about the actual factors for biogas and methane yields for given levels of each independent process variable.

The rest of the DOE parameters are shown in Appendix P

4.5.2. Material balance across the system

The material balance for digesters 1 to 15 is shown in Table 4.18. The table shows the feed values (input) and the output values. The sample of calculations for the material balance is shown in Appendix O. The main reason for doing material balance across the digesters was to determine the losses in each digester. If the losses in each digester was above 0.1 g, then the amount of loss will be added to the original volume of biogas generated by the system.

It was assumed that some of biogas was vented to atmosphere while sampling for pH. To take into account the loss in inventory, a material balance across the digesters had to be done. The losses for digesters 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 were 0.0003, 0.005, 0.0048, 0.0094, 0.0009, 0.0015, 0.0647, 0.0065, 0.0022, 0.0051, 0.0045, 0.0067, 0.0009, 0.0109 and 0.0209 g, respectively. These losses were assumed to have vented to atmosphere during sampling. Digester 7 had the highest losses of 0.0647 g. Nevertheless, the losses were less than 0.1 g, and thus there was no need to include any in the original volume of biogas generated by the system.

Furthermore, the amount of CO_2 dissolved in H_2O for digesters 11, 12, 7, 8, 9, 1, 2, 3, 5, 4, 10, 13, 14, 15 and 6 were 6.9, 7.5, 8.1, 16.3, 20.8, 28.9, 31.9, 42.6, 44.5, 46.8, 47.4, 54.3, 54.3, 54.3 and 56.5 g, with biogas volume produced of 6, 6.6, 7.1, 14.3, 18.3, 25.4, 27.9, 37.4, 38.9, 41.1, 41.6, 47.7, 47.7, 47.7 and 49.5 L, respectively. In conclusion, the amount of CO_2 dissolved in H_2O increased with increase in biogas volume produced. Digester 6 had the highest CO_2 dissolved in H_2O of 53 g possible due to the high loading producing the highest biogas volume of 49.5 L.

Table 4.18: Material balance across the system (digesters 1 to 15).

	INPUT					OUTPUT												
Digester ID	**Water (g)**	**Sewage sludge (g)**	**Inoculum (g)**	**co-substrate (g)**	**Total(g)**	**Biogas volume (L)**	**Methane content (v/v)**	**Carbon dioxide content (v/v)**	**Moisture content in biogas (v/v)**	**Methane (g)**	**Carbon dioxide (g)**	**Moisture (g)**	**digestate (g)**	**water in the displacement before (g)**	**water in the displacement after (g)**	**Carbon dioxide dissolved in H_2O (g)**	**Total(g)**	**Losses (g)**
1	0	1153.9	3817	23.8	4995	25.4	67	30	1	10.9	13.4	0.18	4942	25400	25429	28.9	4995	0.0003
2	0	1153.9	3817	23.8	4995	27.9	69	28	1	12.3	13.8	0.20	4937	27940	27972	31.9	4995	0.0050
3	0	1993.1	2992	23.8	5009	37.4	68	29	1	16.2	19.1	0.27	4931	37383	37426	42.6	5009	0.0048
4	0	1993.1	2992	23.8	5009	41.1	69	28	1	18.1	20.2	0.30	4923	41048	41095	46.8	5009	0.0094
5	0	1573.5	3405	10.8	4989	38.9	77	20	1	19.2	13.7	0.28	4911	38998	39042	44.5	4989	0.0009
6	0	1573.5	3405	10.8	4989	49.5	80	17	1	25.3	14.8	0.36	4892	49538	49595	56.5	4989	0.0015
7	0	1573.5	3405	36.7	5015	7.1	61	36	1	2.8	4.5	0.05	4999	7130	7138	8.1	5015	0.0647
8	0	1573.5	3405	36.7	5015	14.3	60	37	1	5.5	9.3	0.10	4984	14260	14276	16.3	5015	0.0065
9	0	1153.9	3817	10.8	4982	18.3	65	32	1	7.6	10.3	0.13	4943	18260	18281	20.8	4982	0.0022

(Continued)

Table 4.18 (Continued).

	INPUT					OUTPUT												
Digester ID	**Water (g)**	**Sewage sludge (g)**	**Inoculum (g)**	**co-substrate (g)**	**Total(g)**	**Biogas volume (L)**	**Methane content (v/v)**	**Carbon dioxide content (v/v)**	**Moisture content in biogas (v/v)**	**Methane (g)**	**Carbon dioxide (g)**	**Moisture (g)**	**digestate (g)**	**water in the displacement before (g)**	**water in the displacement after (g)**	**Carbon dioxide dissolved in H_2O (g)**	**Total(g)**	**Losses (g)**
10	0	1993.1	2992	10.8	4996	41.6	70	27	1	18.6	19.7	0.30	4910	41600	41647	47.4	4996	0.0051
11	0	1153.9	3817	36.7	5008	6.0	61	36	1	2.3	3.8	0.04	4995	6010	6016.9	6.9	5008	0.0045
12	0	1993.1	2992	36.7	5022	6.6	60	37	1	2.5	4.3	0.05	5007	6611	6618.5	7.5	5022	0.0067
13	0	1573.5	3405	23.8	5002	47.7	74	23	1	22.5	19.3	0.34	4905	47645	47699.3	54.3	5002	0.0009
14	0	1573.5	3405	23.8	5002	47.7	74	23	1	22.5	19.3	0.34	4905	47645	47699.3	54.3	5002	0.0109
15	0	1573.5	3405	23.8	5002	47.7	74	23	1	22.5	19.3	0.34	4905	47645	47699.3	54.3	5002	0.0209

5. CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1. Introduction

This chapter makes logical decisions or forms judgements about the optimization of anaerobic co-digestion of sewage sludge using bio-chemical substrates. Furthermore, suggestions or proposals are made on future anaerobic process investigations.

5.2. Conclusions

In agreement with previous studies on bio-chemical substrates, this investigation showed that soft drink waste, fruit juice waste, IOP waste and glycerol could be substrates of interest to biogas producers. The results of the first batch test confirmed that glycerol generates the highest amounts of biogas and methane yields. Glycerol had methane and biogas yields of 0.71 and 0.93 L (g VS added)$^{-1}$, respectively. Soft drink produced the lowest methane and biogas yields of 0.28 and 0.41 L (g VS added)$^{-1}$, respectively. Methane and biogas yields of glycerol were 2.6 and 2.3 times that of soft drink, respectively. It is believed that glycerol contained considerable amount of other organic substance, for example fatty acids and lipids, which have a higher energy content than the other bio-chemical waste streams, thus generating more biogas and methane yields. Glycerol had the highest VS and COD loadings of 3.23 g VS/L and 4.90 g COD/L, respectively. The high VS and COD loadings contributed to the highest methane composition of 76%, VS removal of 100% and COD removal of 100%.

Moreover, co-digestion removals were higher than digestion removals of sewage alone, with VS and COD removals of 34 and 33%, respectively. Furthermore, digestion of sewage sludge alone produced biogas yields of 0.19 L /g VS and 0.33 L/g COD, and methane yields of 0.16 L/g VS and 0.28 L/g COD. Generally, co-digestion yields were higher than digestion yields of sewage alone.

Using the OFAT method, the results of the second batch test on glycerol demonstrated an optimum biogas production of 46 530 mL, the highest VS reduction of 99.7%, highest COD reduction of 100%, highest methane percentage of 80%, highest methane yield of 0.75 L (g VS added)$^{-1}$ and highest biogas yield of 0.94 L (g VS added)$^{-1}$ at a temperature, substrate to inoculum ratio and glycerol volume of 50℃, 1 (on VS basis) and 10 mL, respectively. The end results suggest that biogas production increases with increase in temperature, and decreases with increase in glycerol concentration or substrate to inoculum ratio.

Moreover, co-digestion trials with high organic loadings (above substrate to inoculum ratio of 1 and 22 mL) resulted in severe inhibition. High organic loadings digesters degraded rapidly and generated large amounts of fatty acids that resulted in acidification, and hence inhibited the generation of biogas. The inhibition of the bacterial populations was somehow reversible, although it took some days for the digesters to recover biogas generation.

Using the Design Expert software (Box–Behnken design method) the results of the DOE suggested that the highest methane and biogas yields were 0.75 and 0.94 L (g VS added)$^{-1}$, respectively. The biogas and methane yields obtained using the Design Expert software were comparatively the same as those that were obtained using the OFAT method. Using the DOE software, Design Expert, the glycerol models for biogas and methane yields were:

$$Biogas\ yield = -0.3467 - 4.33333 \times 10^{-3} \times A + 1.40333 \times B + 0.046257 \times C + 2.25 \times 10^{-18} \times AB - 1.66667 \times 10^{-4} \times AC - 8.33333 \times 10^{-3} \times BC + 1.6 \times 10^{-4} \times A^2 - 0.6 \times B^2 - 1.25 \times 10^{-3} \times C^2 \quad (5.1)$$

$$Methane\ yield = -0.71101 + 0.011867 \times A + 1.175 \times B + 0.044306 \times C + 4.39493 \times 10^{-17} \times AB - 2.66667 \times 10^{-4} \times AC - 6.25 \times 10^{-3} \times BC - 8.0 \times 10^{-6} \times A^2 - 0.495 \times B^2 - 1.03299 \times 10^{-3} \times C^2 \quad (5.2)$$

where: A is the temperature variable, B is the substrate to inoculum ratio variable, and C is the volume of glycerol in the digester variable.

Generally, bio-chemical substrate generators may finally benefit by selling the waste to biogas generators as a secondary substrate for the anaerobic process. In actual fact, bio-chemical substrate generators can get energy (methane) from waste using their own digester, although they require a primary substrate to provide nutrients for the anaerobic digestion process. Bio-chemical substrate generators would not only acquire income from the bio-chemical waste or low-value glycerol, but also improve their environmentally friendly image if waste is used in the digesters.

5.3. Recommendations

Future anaerobic digestion investigations should look at the quality of glycerol so as to enhance the generation of methane. Glycerol should be analysed for the compositions of methanol, glycerol and lipids so as to obtain the true theoretical methane yield. The Buswell and Neave (1930) equation may be used for the determination of the theoretical methane yield of glycerol that has organic contaminants.

Moreover, the anaerobic co-digestion of the bio-chemical substrates have to be studied in continuous anaerobic digestion digesters to ensure the balance of nutrient, as well as micronutrients and nitrogen, is adequate to maintain the generation of biogas.

In addition, the digesters should be built-in with automated pH and temperature detectors, and the gas lines should be coupled to gas flow-meters. The pH probe, temperature probe and the gas flow should then be processed electronically by an analogue to digital converter linked to a computer. The use of automation will provide the following advantages:

- It will ensure that there are no loses due to sampling for pH.
- It will ensure that the true pH value is obtained as sampling exposes the sample to the surrounding temperature, and thus affect the pH value.
- It will ensure that no carbon dioxide is absorbed in water as the gas flow-meter does not require water to measure gas volume. The true value will be obtained from the flow-meter.

- On a full-scale the digesters will require tonnes of water for the water displacement system. The use of automation will minimize water usage, and hence reduce the cost.

In future, acidified solution such as NaCl with high ionic concentration may possibly be used in the water displacement system because it is able to maintain the volume and composition of the biogas. The acidified solution will reduce the amount of CO_2 dissolved in H_2O.

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APPENDIX A – Letter to confirm the editing process

Dr. P. Moodley

EDITING SERVICES

Cell: 084 711 3864

Telephone: 031 373 6870 email: padhmam@dut.ac.za

Date: 23 May 2017

Re: Editing services rendered – MADONDO, N.I. Thesis entitled: "Optimization of anaerobic co-digestion of sewage sludge using bio-chemical substrates"

The following areas were considered during editing and proof reading of this thesis:

- Spelling
- Punctuation
- Grammar
- Sentence Structure
- Word reduction
- Cross reference citations
- Reference check
- Formatting basics
- Presentation style
- Other mechanics of the English language.

Corrections were made using track changes which have been clearly outlined for your reference.

Should you have further queries in this regard, please contact me via email or telephonically.

Yours Faithfully

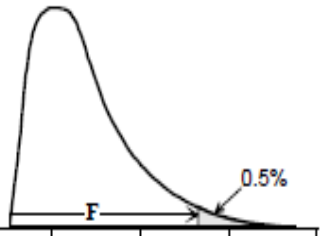
Dr. P. Moodley

(PhD Communications (UNIZUL), Master of Arts (UNIZUL), Honors Communication Culture and Media Studies- Cum Laude (UKZN), Bachelor of Arts (UNISA), Senior Lecturer, Department of Marketing and Retail ManagementFaculty of Management Sciences, Durban University of Technology

APPENDIX B – F and t distribution graphs

B1 - Percentage points of F-distribution

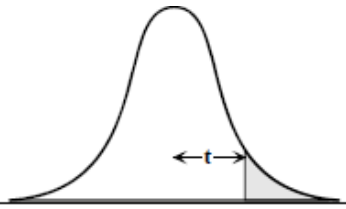
Table B1: Percentage points of the F-distribution (Handbook for Experimenters, 2014).



df_{num} / df_{den}	1	2	3	4	5	6	7	8	9	10	15	20
1	16210.7	19999.5	21614.7	22499.6	23055.8	23437.1	23714.6	23925.6	24091.0	24224.5	24630.2	24836.0
2	198.50	199.00	199.17	199.25	199.30	199.33	199.36	199.37	199.39	199.40	199.43	199.45
3	55.552	49.799	47.467	46.195	45.392	44.838	44.434	44.126	43.882	43.686	43.085	42.778
4	31.333	26.284	24.259	23.155	22.456	21.975	21.622	21.352	21.139	20.967	20.438	20.167
5	22.785	18.314	16.530	15.556	14.940	14.513	14.200	13.961	13.772	13.618	13.146	12.903
6	18.635	14.544	12.917	12.028	11.464	11.073	10.786	10.566	10.391	10.250	9.814	9.589
7	16.236	12.404	10.882	10.050	9.522	9.155	8.885	8.678	8.514	8.380	7.968	7.754
8	14.688	11.042	9.596	8.805	8.302	7.952	7.694	7.496	7.339	7.211	6.814	6.608
9	13.614	10.107	8.717	7.956	7.471	7.134	6.885	6.693	6.541	6.417	6.032	5.832
10	12.826	9.427	8.081	7.343	6.872	6.545	6.302	6.116	5.968	5.847	5.471	5.274
11	12.226	8.912	7.600	6.881	6.422	6.102	5.865	5.682	5.537	5.418	5.049	4.855
12	11.754	8.510	7.226	6.521	6.071	5.757	5.525	5.345	5.202	5.085	4.721	4.530
13	11.374	8.186	6.926	6.233	5.791	5.482	5.253	5.076	4.935	4.820	4.460	4.270
14	11.060	7.922	6.680	5.998	5.562	5.257	5.031	4.857	4.717	4.603	4.247	4.059
15	10.798	7.701	6.476	5.803	5.372	5.071	4.847	4.674	4.536	4.424	4.070	3.883
16	10.575	7.514	6.303	5.638	5.212	4.913	4.692	4.521	4.384	4.272	3.920	3.734
17	10.384	7.354	6.156	5.497	5.075	4.779	4.559	4.389	4.254	4.142	3.793	3.607
18	10.218	7.215	6.028	5.375	4.956	4.663	4.445	4.276	4.141	4.030	3.683	3.498
19	10.073	7.093	5.916	5.268	4.853	4.561	4.345	4.177	4.043	3.933	3.587	3.402
20	9.944	6.986	5.818	5.174	4.762	4.472	4.257	4.090	3.956	3.847	3.502	3.318
21	9.830	6.891	5.730	5.091	4.681	4.393	4.179	4.013	3.880	3.771	3.427	3.243
22	9.727	6.806	5.652	5.017	4.609	4.322	4.109	3.944	3.812	3.703	3.360	3.176
23	9.635	6.730	5.582	4.950	4.544	4.259	4.047	3.882	3.750	3.642	3.300	3.116
24	9.551	6.661	5.519	4.890	4.486	4.202	3.991	3.826	3.695	3.587	3.246	3.062
25	9.475	6.598	5.462	4.835	4.433	4.150	3.939	3.776	3.645	3.537	3.196	3.013

B2 - One-tailed / two-tailed t-table

Table B2: One-tailed / two-tailed t-table (Handbook for Experimenters, 2014).



	tail area probability									
1-tail	**0.40**	**0.25**	**0.10**	**0.05**	**0.025**	**0.01**	**0.005**	**0.0025**	**0.001**	**0.0005**
2-tail	**0.80**	**0.50**	**0.20**	**0.10**	**0.050**	**0.02**	**0.010**	**0.0050**	**0.002**	**0.0010**
df=1	0.325	1.000	3.078	6.314	12.706	31.821	63.657	127.32	318.31	636.62
2	0.289	0.816	1.886	2.920	4.303	6.965	9.925	14.089	22.326	31.598
3	0.277	0.765	1.638	2.353	3.182	4.541	5.841	7.453	10.213	12.924
4	0.271	0.741	1.533	2.132	2.776	3.747	4.604	5.598	7.173	8.610
5	0.267	0.727	1.476	2.015	2.571	3.365	4.032	4.773	5.893	6.869
6	0.265	0.718	1.440	1.943	2.447	3.143	3.707	4.317	5.208	5.959
7	0.263	0.711	1.415	1.895	2.365	2.998	3.499	4.029	4.785	5.408
8	0.262	0.706	1.397	1.860	2.306	2.896	3.355	3.833	4.501	5.041
9	0.261	0.703	1.383	1.833	2.262	2.821	3.250	3.690	4.297	4.781
10	0.260	0.700	1.372	1.812	2.228	2.764	3.169	3.581	4.144	4.587
11	0.260	0.697	1.363	1.796	2.201	2.718	3.106	3.497	4.025	4.437
12	0.259	0.695	1.356	1.782	2.179	2.681	3.055	3.428	3.930	4.318
13	0.259	0.694	1.350	1.771	2.160	2.650	3.012	3.372	3.852	4.221
14	0.258	0.692	1.345	1.761	2.145	2.624	2.977	3.326	3.787	4.140
15	0.258	0.691	1.341	1.753	2.131	2.602	2.947	3.286	3.733	4.073
16	0.258	0.690	1.337	1.746	2.120	2.583	2.921	3.252	3.686	4.015
17	0.257	0.689	1.333	1.740	2.110	2.567	2.898	3.222	3.646	3.965
18	0.257	0.688	1.330	1.734	2.101	2.552	2.878	3.197	3.610	3.922
19	0.257	0.688	1.328	1.729	2.093	2.539	2.861	3.174	3.579	3.883
20	0.257	0.687	1.325	1.725	2.086	2.528	2.845	3.153	3.552	3.850
21	0.257	0.686	1.323	1.721	2.080	2.518	2.831	3.135	3.527	3.819
22	0.256	0.686	1.321	1.717	2.074	2.508	2.819	3.119	3.505	3.792
23	0.256	0.685	1.319	1.714	2.069	2.500	2.807	3.104	3.485	3.767
24	0.256	0.685	1.318	1.711	2.064	2.492	2.797	3.091	3.467	3.745
25	0.256	0.684	1.316	1.708	2.060	2.485	2.787	3.078	3.450	3.725
26	0.256	0.684	1.315	1.706	2.056	2.479	2.779	3.067	3.435	3.707
27	0.256	0.684	1.314	1.703	2.052	2.473	2.771	3.057	3.421	3.690
28	0.256	0.683	1.313	1.701	2.048	2.467	2.763	3.047	3.408	3.674
29	0.256	0.683	1.311	1.699	2.045	2.462	2.756	3.038	3.396	3.659
30	0.256	0.683	1.310	1.697	2.042	2.457	2.750	3.030	3.385	3.646
40	0.255	0.681	1.303	1.684	2.021	2.423	2.704	2.971	3.307	3.551
60	0.254	0.679	1.296	1.671	2.000	2.390	2.660	2.915	3.232	3.460
120	0.254	0.677	1.289	1.658	1.980	2.358	2.617	2.860	3.160	3.373
∞	0.253	0.674	1.282	1.645	1.960	2.326	2.576	2.807	3.090	3.291

APPENDIX C - Reactor and gas collector design

C1 – Reactor design

Method 1 – reaction method

The main aim of designing a reactor was to find its volume, and hence the reactor diameter and height. By so doing, the reactor capital cost will be minimized and the correct size reactor for the process can be used. Since about 72% of methanogenesis microorganisms use the acetoclastic pathway to generate methane (Hufnagel, 2014), the reactor design will be based on the acetoclastic pathway (Equation 2.23):

$$CH_3COOH(l) \rightarrow CO_2(g) + CH_4(g)$$

Let: CH_3COOH be *A*, CO_2 be *B* and CH_4 be *C*

Therefore, Equation 2.23 becomes:

$$A(l) \rightarrow B(g) + C(g) \qquad \text{(C1)}$$

Below is the stoichiometric table for the reactor (Table C1):

Table C1: Stoichiometric for the reaction with phase change.

Species	Symbol	Initial	Change	Product
CH_3COOH	*A*	N_{A0}	$-N_{A0}Z$	$N_A = N_{A0}(1-Z)$
CO_2	*B*	*0*	$N_{A0}Z$	$N_B = N_{A0}Z$
CH_4	*C*	*0*	$N_{A0}Z$	$N_C = N_{A0}Z$
Inerts	*I*	N_{I0}	*0*	$N_I = N_{I0}$
TOTAL		$N_{T0} = N_{I0} + N_{A0}$		$N_T = N_{I0} + N_{A0}(1+Z)$

where:

- N_{A0} – initial moles of A, kmol,
- N_{I0}– initial moles of the inert I, kmol,

- Z – conversion of A,
- N_A – final moles of A, kmol,
- N_B – final moles of B, kmol,
- N_C – final moles of C, kmol,
- N_I – final moles of I, kmol,
- N_T – moles produced, kmol, and
- N_{T0} – initial moles, kmol.

The amount of moles produced (N_T) are:

$$N_T = N_A + N_B + N_C + N_I = N_{I0} + N_{A0}(1 + Z) \quad \text{(C2)}$$

The experiments will be done in a batch system, and is neither a parallel or series system. The system volume for batch reactor (V_D) can be obtained by:

$$V_D = V_0 \frac{N_T}{N_{T0}} \frac{P_0}{P} \frac{T}{T_0} \quad \text{(C3)}$$

where:

- V_D – design/system volume of the fluid, m^3,
- V_0 – initial volume of the fluid, m^3,
- P_0 – initial pressure, atm,
- P – final/system pressure, atm,
- T – final/system temperature, K, and
- T_0 – initial temperature, K

Substituting Equation C2 into Equation C3, and using the fact that $N_{T0} = N_{I0} + N_{A0}$, implies that the reactor volume is:

$$V_D = V_0 \frac{[N_{I0} + N_{A0}(1+Z)]}{(N_{I0} + N_{A0})} \frac{P_0}{P} \frac{T}{T_0} \quad \text{(C4)}$$

The following can be said:

- Even though the process is going to be done at two temperature values (mesophilic or thermophilic temperature), the reactor temperature will be kept constant at a particular temperature i.e. isothermal process. Therefore, $\frac{T_0}{T} = 1$

- Assume an overall conversion of 1, which is the maximum conversion for irreversible, for safe design. Therefore, $Z = 1$.
- The initial feed volume will be $V_0 = 4.8L$.
- Let us assume that the change in pressure is small enough that we can neglect it. Therefore, $\frac{P_0}{P} = 1$

The initial acetate mole (N_{A0}), may be expressed as:

$$N_{A0} = C_{A0}V_0 = y_{A0}C_{T0}V_0 = \frac{\rho_A}{M}V_0 \quad \text{(C5)}$$

where:

- C_{A0} – initial concentration of A, kmol/ m^3,
- C_{T0} – initial concentration of the mixture, kmol/ m^3,
- ρ_A– density of A, kg/ m^3, and
- M– average molar mass in the reactor, kg/ kmol.

According to Foucault (2011):

- 63.2%, by weight, of solids in the organic matter are converted to acetate (x_{AC}),
- 26.8%, by weight, of solids in the organic matter are converted to hydrogen (x_{HC}),
- 1.0%, by weight, of solids in the organic matter is converted to nitrogen (x_{OC}).

Let's assume that the type of solid content is medium or semi-dry type, with a solid content of 20% (see Section 2.3.4.). Assume an initial total mass of 100kg (m_{T0}). Therefore, the mass of volatile solids (m_{VS}) and water (m_W) are 20kg and 80kg, respectively. Therefore, the mass of acetone entering the methanogenesis step (m_A) is:

$$m_A = \frac{x_{AC}}{100} \times m_{VS} \quad \text{(C6)}$$

$$= 0.632 \times 20$$

$$= 12.64 \text{ kg}$$

The mass of hydrogen (m_H) entering the methanogenesis step is:

$$m_H = \frac{x_{HC}}{100} \times m_{VS} \quad \text{(C7)}$$

$= 0.268 \times 20$

$= 5.36$ kg

The mass of nitrogen (m_N) entering the methanogenesis step is:

$$m_N = \frac{x_{NC}}{100} \times m_{VS} \quad \text{(C8)}$$

$= 0.010 \times 20$

$= 2$ kg

The mass fractions of species entering the methanogenesis step are:

- Mass fraction of acetone (x_{A0}) is $x_{A0} = \frac{12.64}{100} = 0.1264$,
- Mass fraction of hydrogen (x_{H0}) is $x_{H0} = \frac{5.36}{100} = 0.0536$,
- Mass fraction of nitrogen (x_{N0}) is $x_{N0} = \frac{2}{100} = 0.02$,
- Mass fraction of water (x_{w0}) is $x_{W0} = \frac{80}{100} = 0.80$.

The density of acetate at 37.5℃ is 1031.07 kg/m^3. The molar mass of acetone (M_A), hydrogen (M_H) and nitrogen (M_N) are $M_A = 60$ kg/kmol, $M_H = 2$ kg/kmol and $M_N = 28$ kg/kmol, respectively.

The average molecular mass in the reactor (M) is:

$$M = {}^{1}\Big/\left[\left(\frac{x_{A0}}{M_A}\right) + \left(\frac{x_{H0}}{M_H}\right) + \left(\frac{x_{N0}}{M_N}\right) + \left(\frac{x_{W0}}{M_{WN}}\right)\right] \quad \text{(C9)}$$

$$= {}^{1}\Big/\left[\left(\frac{0.126}{60}\right) + \left(\frac{0.0536}{2}\right) + \left(\frac{0.02}{28}\right) + \left(\frac{0.8}{18}\right)\right]$$

$= 13.50$ kg/kmol

The initial molar fractions of acetate (y_{A0}), hydrogen (y_{H0}) and inert (y_{I0}) are:

- $y_{A0} = \frac{m_{Ao} \times M_A}{m_{T0} \times M} = \frac{12.64 \times 60}{100 \times 13.5} = 0.56$,
- $y_{H0} = \frac{m_{Ho} \times M_H}{m_{T0} \times M} = \frac{5.36 \times 2}{100 \times 13.5} = 0.01$,
- $y_{I0} = 1 - 0.56 - 0.01 = 0.43$.

The inerts are water and nitrogen.

Substituting to Equation C5 implies that the initial acetate concentration (N_{A0}) is:

$$N_{A0} = \frac{1033.87}{14.50} \times \frac{4.8}{1000}$$

$$= 0.37 \text{ kmol}$$

The initial inert concentration (N_{I0}) is:

$$N_{I0} = \frac{y_{I0}}{y_{A0}} N_{A0} \qquad \text{(C10)}$$

$$= \frac{0.43}{0.56} \times 0.37$$

$$= 0.28 \text{ kmol}$$

Substituting to Equation C4 implies that the volume required for the system (V_D) is:

$$V_D = 4.8 \times \frac{[0.28 + 0.37(1 + 1)]}{(0.28 + 0.37)} \times 1 \times 1$$

$$= 7.52 \text{ L}$$

This of course is not necessarily the reactor volume, but rather the volume required for both the liquid and gas. In our investigation this is the volume of the reactor plus that of the tube connecting the reactor (V_T) plus that of the portion occupied by the gas in the gas collector (V_C). To get the reactor volume, the volumes of the tube connector and the gas space in the gas collector have to be subtracted from the design volume. Therefore, the reactor volume (V) is:

$$V = V_D - V_T - V_C \qquad \text{(C11)}$$

Assume that the gas collector and tube connector dimensions of this report are similar to that was designed by Foucault (2011) that is:

- Tube connector length is 200 cm,
- Tube connector diameter is 0.635 cm ≈ 0.64 cm,
- Gas collector height is 122 cm, and
- Gas collector diameter is 7.6 cm.

In addition to this, assume that the gas in the gas collector will occupy 20% of the total gas collector volume. Therefore, the reactor volume is (Equation C11):

$$V = 7.52 - \left[\frac{\pi}{4} \times \left(\frac{0.635}{100}\right)^2 \times \frac{200}{100}\right] - 0.20 \times \left[\frac{\pi}{4} \times \left(\frac{7.6}{100}\right)^2 \times \frac{122}{100}\right]$$

$$= 6.35 \approx 6 \text{ L}$$

Method 2 – ISO 11734 method

The reactor volume (V) can be computed as the sum of the headspace volume (V_V) and the liquid volume ($V_L = V_0 = 4.8$ L):

$$V = V_V + V_L \quad \text{(C12)}$$

ISO 11734 (1995) suggested that the headspace volume in anaerobic digestion should be 10% to 30% of the total volume. If we choose the maximum value 25%, the headspace volume is therefore:

$$V_V = 0.25V \quad \text{(C13)}$$

Substituting Equation C13 to Equation C12 implies that the reactor volume (V):

$$V = \frac{V_L}{(1 - 0.25)}$$

$$= \frac{4.8}{0.75}$$

$$= 6.40 \text{ L} \approx 6 \text{ L}$$

This volume is approximately the same as that from method 1.

According to Coker (2001), the aspect ratio (G: D), which is the ratio between the reactor height to its diameter, varies between 1.2 and 3.0. The writer further suggests that an aspect ratio of 2:1 elliptical heads can guarantee a rise in pressure resistance of the reactor**.** Therefore, an aspect ratio G: D of 2:1 is going to be used in this investigation. This means that the reactor height is:

$$G = 2D \quad \text{(C14)}$$

Using this proportion and the fact that the reactor is cylindrical, the reactor volume (V) may be computed as:

$$V = 6 \times 10^{-3}\ \text{m}^3 = \frac{\pi D^2}{4} G = \frac{\pi D^2}{4} \times 2D = \frac{\pi D^3}{2} \quad \text{(C15)}$$

Making D the subject of the formula in Equation C15 implies that the reactor diameter (D) is:

$$D = \left[\frac{6 \times 10^{-3} \times 2}{\pi}\right]^{\frac{1}{3}}$$

$$= 0.159\ \text{m}$$

$$= 15.9\ \text{cm}$$

Take a standard diameter of D = 16 cm

Substituting to Equation C14 implies that the reactor height (G) is:

$$G = 2 \times 16$$

$$= 32\ \text{cm}$$

C2 – Gas collector design

The gas collector height (h) was assumed to be 32 cm. The gas collector height to gas collector diameter ratio ($h{:}\,d$) was assumed to be 1:2. Assuming this value, the corrected gas collector diameter (d) becomes:

$$d = \frac{h}{2} \quad \text{(C16)}$$

$$= \frac{32}{2}$$

$$= 16.0\ \text{cm}$$

Therefore, the collector volume is

$$V_C = \frac{\pi(d/100)^2}{4} \times h \quad \text{(C17)}$$

$$= \frac{\pi(0.16)^2}{4} \times (0.32) \times 1000$$

$$= 6.43 \text{ L}$$

This was approximately equal to the designed reactor capacity of 6 L.

APPENDIX D – Reactor assembly and first angle views

This section gives the assembly and first angle drawings for all parts of the digester. Figure D1 below shows the full assembled drawing of the digester. Figure D2 shows the isometric and first angle drawing for the adapter. Figure D3 shows the isometric and first angle drawing for the bottom plate. Figure D4 shows the isometric and first angle drawing for the feed cap. Figure D5 shows the isometric and first angle drawing for the feed pipe. Figure D6 shows the isometric and first angle drawing for the glass plate. Figure D7 shows the isometric and first angle drawing for the inside pipe. Figure D8 shows the isometric and first angle drawing for the outside pipe. Figure D9 shows the isometric and first angle drawing for the first top plate. Figure D10 shows the isometric and first angle drawing for the second top plate. Figure D11 shows the isometric and first angle drawing for the thermometer. All measurements were in millimetres.

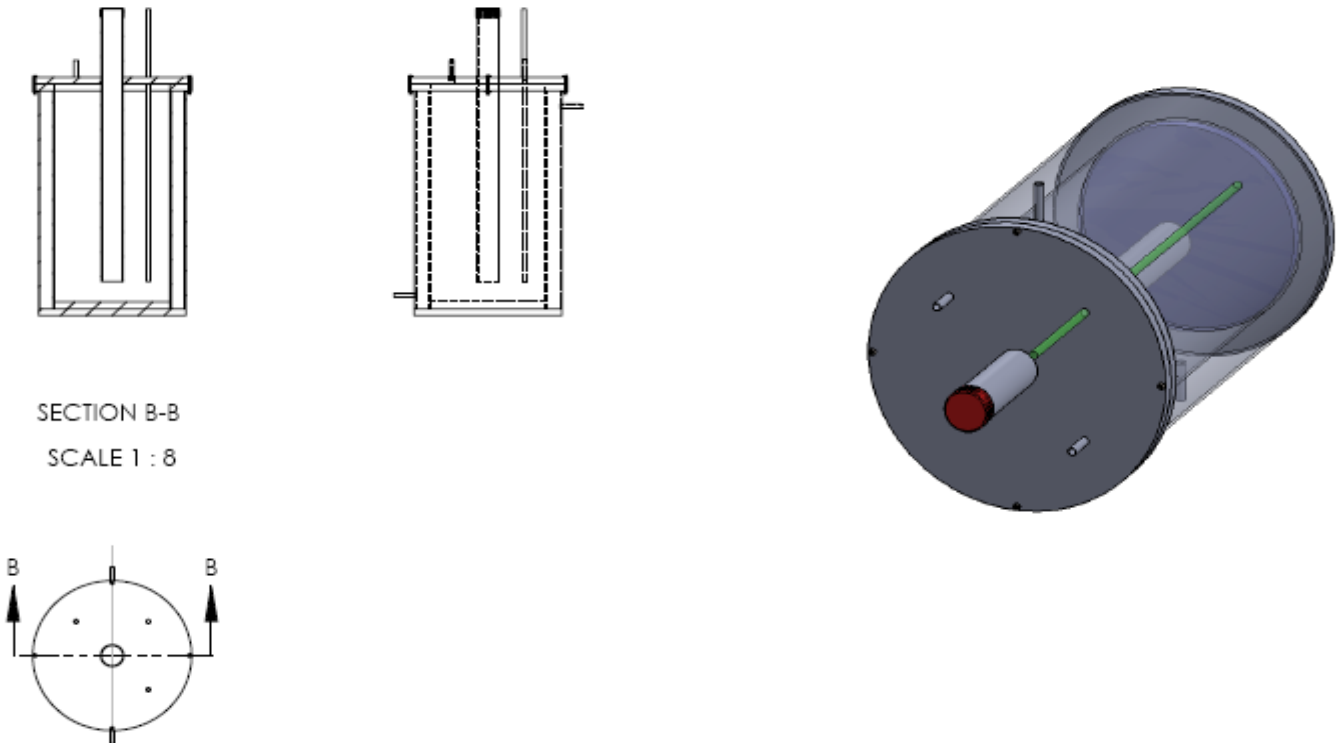


Figure D1: Digester assembly drawing.

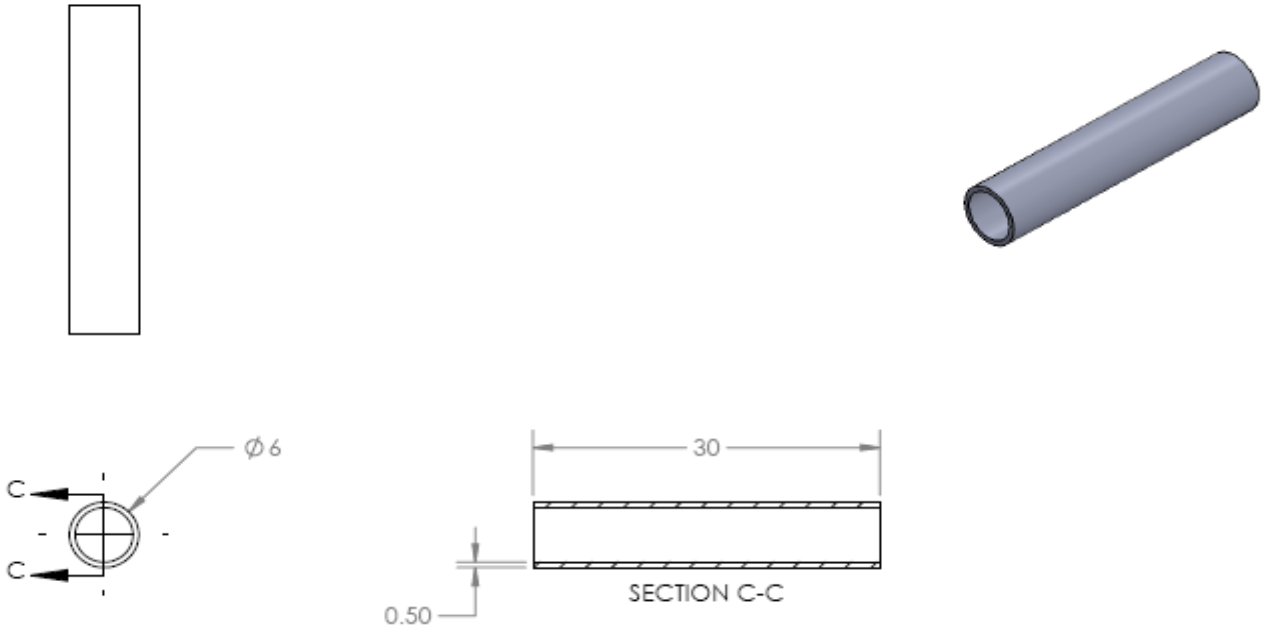


Figure D2: Adaptor first angle and isometric drawings.

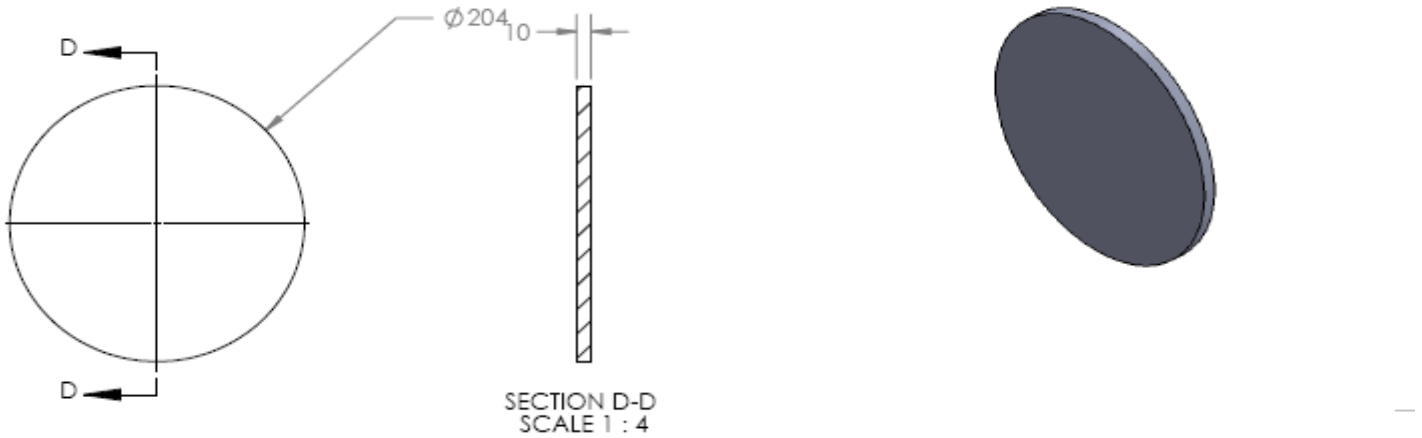


Figure D3: Bottom plate first angle and isometric drawings.

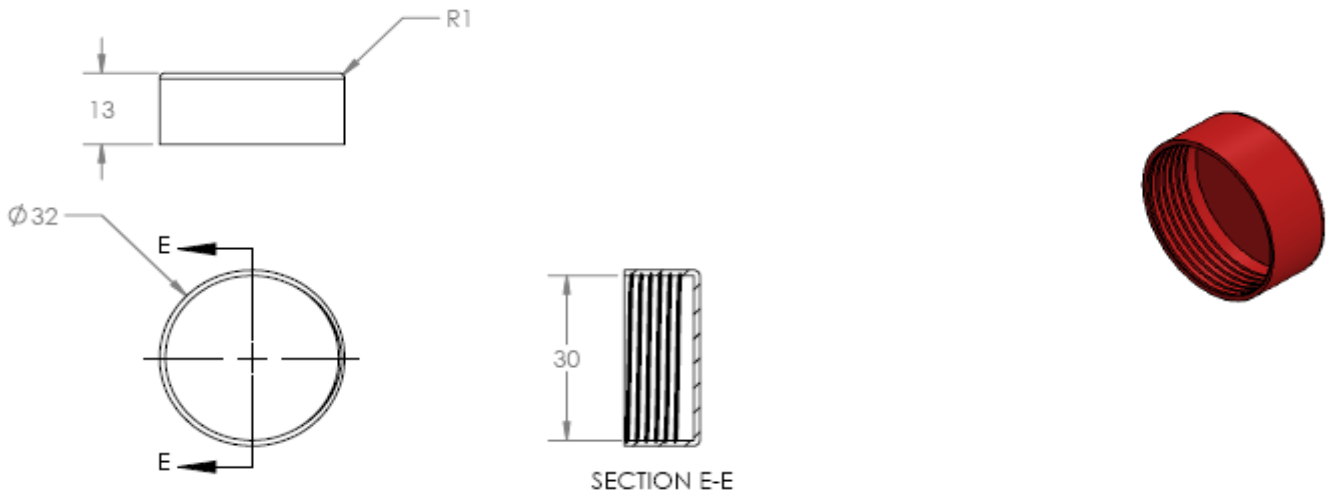


Figure D4: Feed cap first angle and isometric drawings.

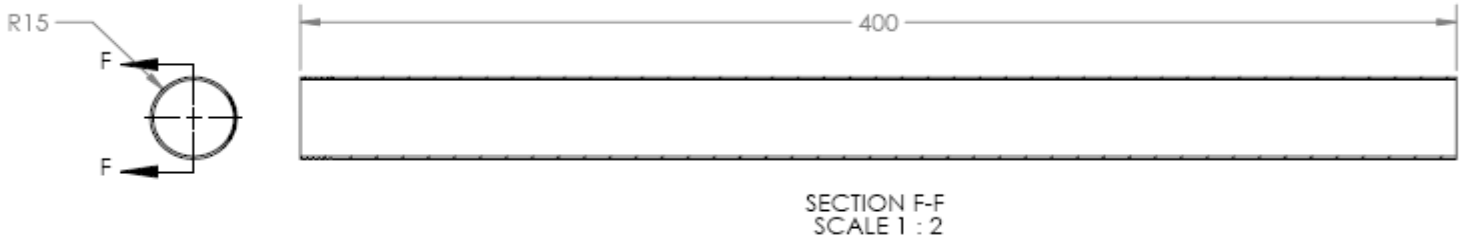


Figure D5: Feed pipe first angle and isometric drawings.

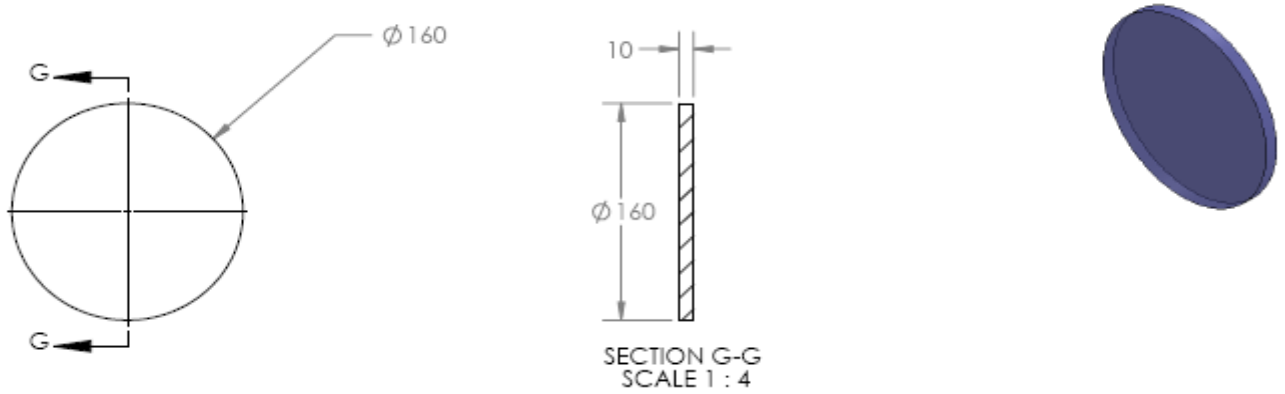


Figure D6: Glass plate first angle and isometric drawings.

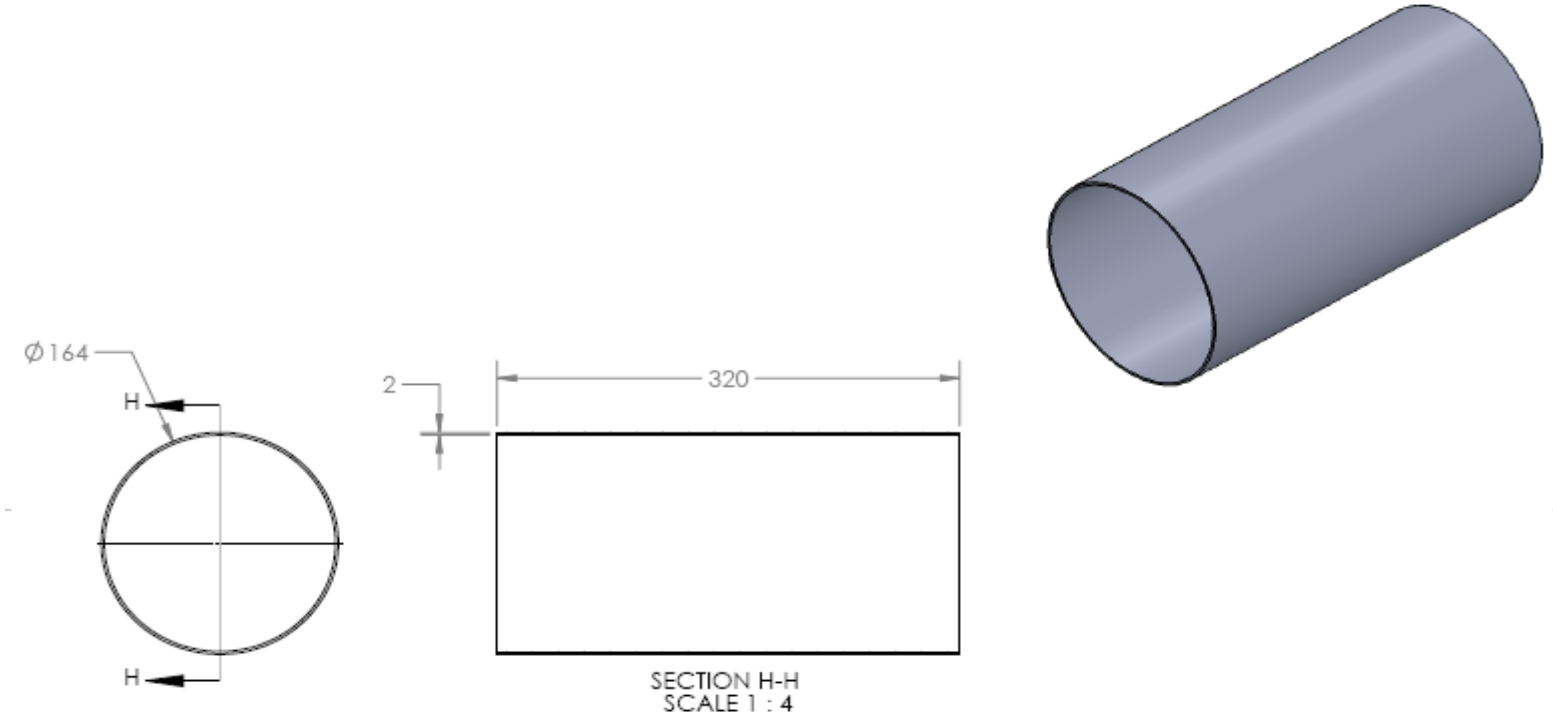


Figure D7: Inside pipe first angle and isometric drawings.

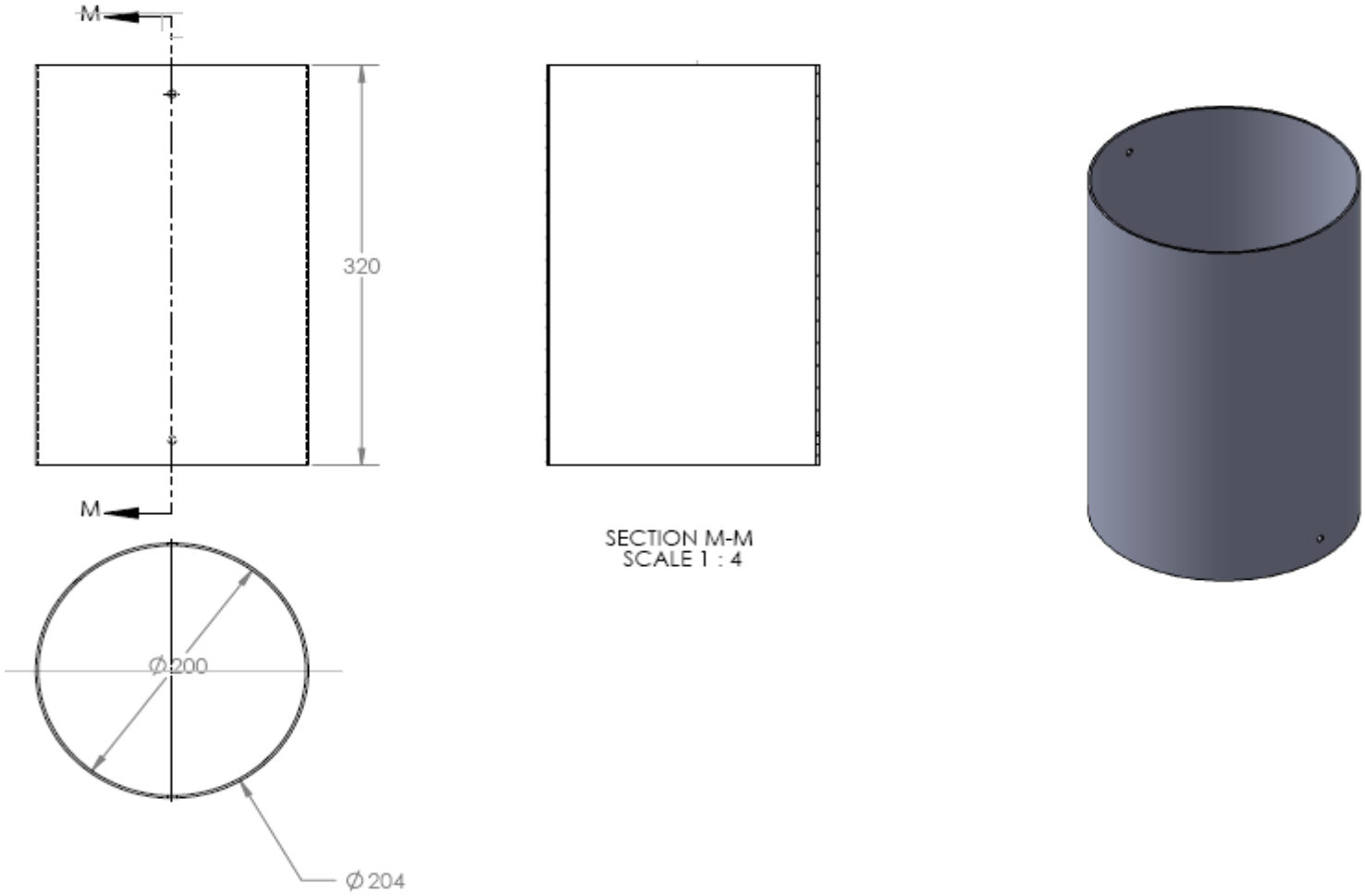


Figure D8: Outside pipe first angle and isometric drawings.

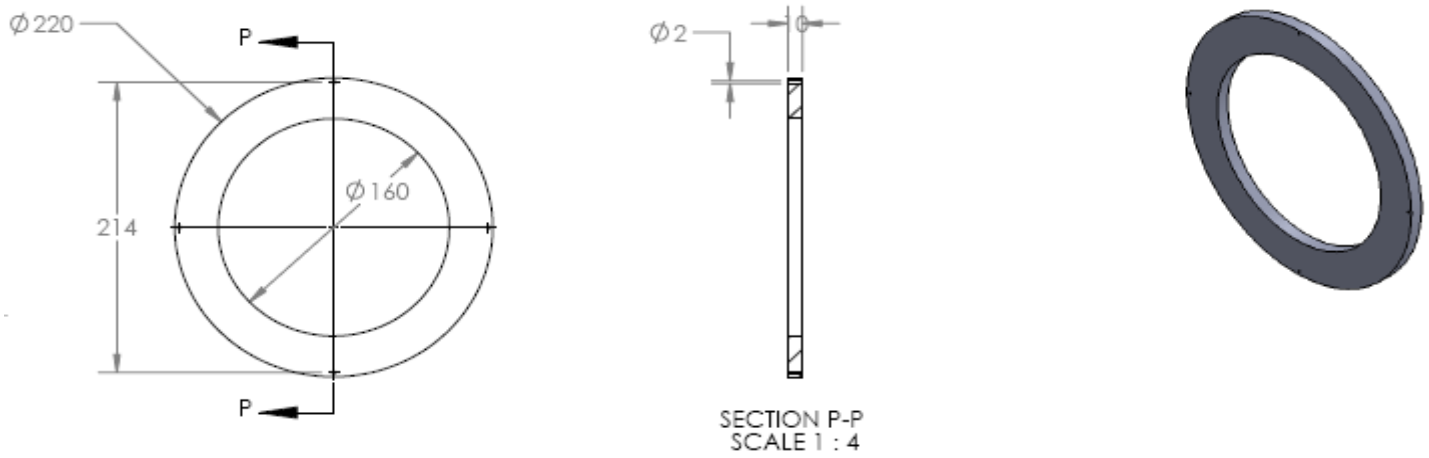


Figure D9: First top plate first angle and isometric drawings.

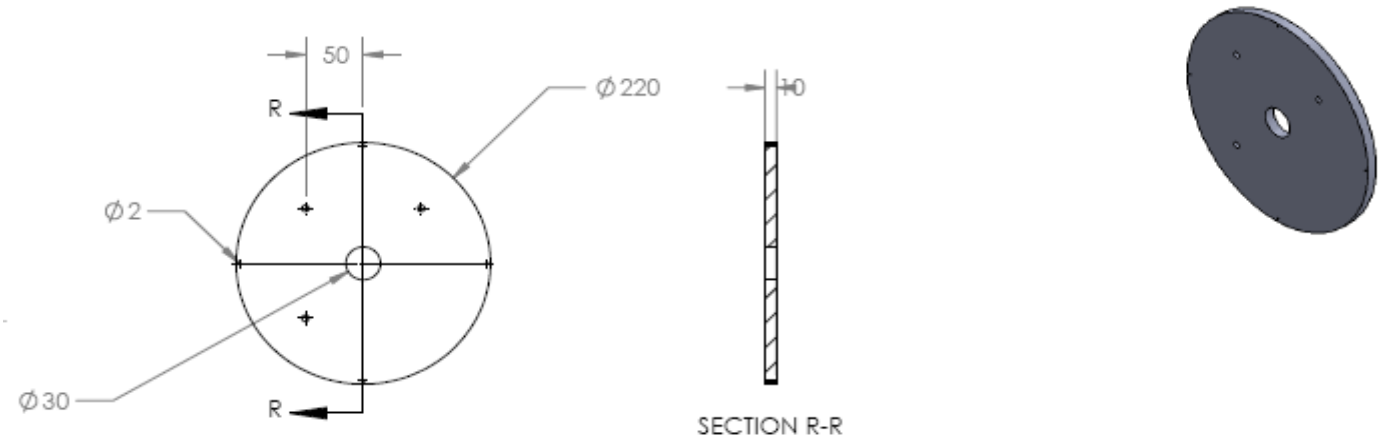


Figure D10: Second top plate first angle and isometric drawings.

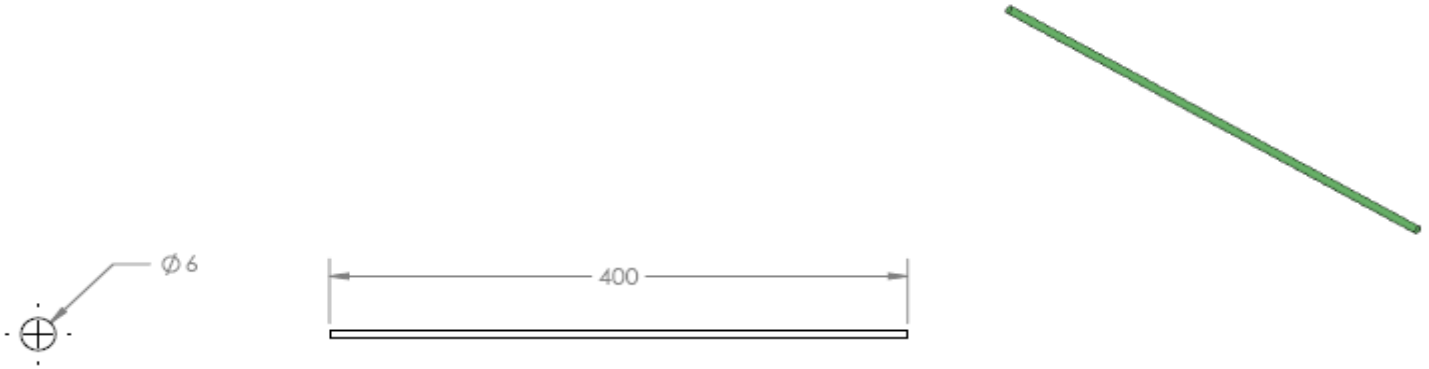


Figure D11: Thermometer first angle and isometric drawings.

APPENDIX E – Calculation of the number of experimental runs

The number of experimental points (N_e) for a BBD is defined by the expression:

$$N_e = 2n(n-1) + C_p$$

where n is the number of variables and C_p is the number of centre points.

In this investigation there are three variables i.e. n = 3. Taking three centre points i.e. C_p = 3, the number of experimental points (N_e) is:

$$N_e = 2 \times 3 \times (3-1) + 3$$

$$= 15$$

Three variables will be tested:

- Variable A – Temperature, which will be tested between [25℃,50℃].
- Variable B - The inoculum / substrate ratio (g VS inoculum/ g VS inoculum), which will be tested between [0.5, 1.5].
- Variable C - The secondary volume (ml) between, which will be tested between [10, 34].

APPENDIX F – Data for biogas production

F1 – Raw data

Table F1, Table F2 and Table F3 show the raw data for biogas productions for the first batch trial, second batch trial and digester 1 to 15 respectively.

Table F1: Biogas production for the first batch trial (raw data).

	Volume (mL)							
Day	**Glycerol**		**Fruit juice**		**Soft drink**		**IOP**	
	a	***b****	***a***	***b****	***a***	***b****	***a***	***b****
1	632	620	112	120	238	216	93	99
2	625	627	123	107	165	131	96	92
3	633	619	121	109	20	28	95	93
4	1248	1254	106	124	36	54	128	100
5	1601	1901	101	115	15	17	85	85
6	1390	1362	117	83	6	8	84	86
7	870	882	78	64	0	0	85	115
8	370	380	25	35	0	0	52	48
9	785	807	0	0	0	0	48	52
10	1380	1350	0	0	0	0	32	44
11	911	909	0	0	0	0	9	11
12	681	683	0	0	0	0	10	10
13	1212	1290	0	0	0	0	8	10
14	1702	1710	0	0	0	0	10	10
15	1128	1146	0	0	0	0	10	10
16	744	734	0	0	0	0	11	9
17	295	307	0	0	0	0	9	9
18	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0

(*) Duplicate values

Table F2: Biogas production for the second batch trial (raw data).

	Volume (mL)																	
Day	**S:I = 0.5**		**S:I = 1.0**		**S:I =1.5**		**1 g glyc per L**		**5 g glyc per L**		**9 g glyc per L**		**25 deg C**		**37.5 deg C**		**50 deg C**	
	a	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****
1	2300	2700	5050	4950	1900	2100	5050	4950	6500	5500	6950	7050	774	790	4398	5602	14500	15500
2	3100	3400	1995	2005	990	1010	1995	2005	2700	2300	2800	3200	379	401	1655	2345	4495	5505
3	3250	3250	1999	2001	1600	1400	2100	1900	1889	2111	18	22	382	400	2020	1980	9890	10110
4	4700	5300	995	1005	550	450	999	1001	2450	2550	36	50	772	790	940	1060	4600	5400
5	13995	14005	2450	2550	1020	980	2700	2300	800	1200	124	130	774	788	2248	2752	4679	5321
6	1995	2005	4450	4550	1804	1822	2300	2200	1095	905	25	35	770	792	2210	2290	4900	5100
7	2990	3010	3950	4050	1807	1817	2295	2205	730	770	28	32	787	777	2230	2270	750	1250
8	2050	1950	3800	3700	2360	2390	4050	3950	740	760	61	65	773	789	3675	4325	440	560
9	2994	3006	230	270	1209	1229	4010	3990	450	550	61	63	780	782	3875	4125	5	15
10	600	600	2900	3100	1208	1230	3100	2900	980	1020	62	64	772	790	2879	3121	25	15
11	0	0	1500	2500	1554	1570	1999	2001	985	1015	61	63	381	401	1879	2121	0	0
12	0	0	2800	3200	1620	1640	2950	3050	2110	2140	61	65	384	398	2880	3120	0	0
13	0	0	1900	2100	1620	1620	2010	1990	2105	2145	49	65	771	791	1848	2152	0	0
14	0	0	1430	1570	2700	2800	1498	1502	2550	2700	90	70	774	788	1313	1687	0	0
15	0	0	1440	1560	1027	1035	1490	1510	2470	2780	24	26	773	789	1473	1527	0	0
16	0	0	745	755	1024	1040	710	790	2990	3010	25	25	793	771	679	821	0	0
17	0	0	748	752	1201	1205	720	780	2225	2275	60	66	785	777	720	780	0	0
18	0	0	505	495	1199	1207	550	450	2270	2230	64	70	770	792	420	580	0	0
19	0	0	85	95	1202	1204	100	80	2098	1902	52	68	790	772	79	101	0	0
20	0	0	0	0	1203	1203	0	0	1015	985	50	70	377	405	0	0	0	0

(*) Duplicate values *(Continued)*

Table F2 (Continued).

	Volume (mL)																	
Day	**S:I = 0.5**		**S:I = 1.0**		**S:I =1.5**		**1 g glyc per L**		**5 g glyc per L**		**9 g glyc per L**		**25 deg C**		**37.5 deg C**		**50 deg C**	
	a	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****
21	0	0	0	0	1124	1126	0	0	1010	990	430	470	387	395	0	0	0	0
22	0	0	0	0	2010	1990	0	0	110	190	440	460	777	785	0	0	0	0
23	0	0	0	0	2000	2000	0	0	397	403	90	110	419	423	0	0	0	0
24	0	0	0	0	2000	2000	0	0	0	0	144	156	1084	1198	0	0	0	0
25	0	0	0	0	1999	2001	0	0	0	0	145	155	766	798	0	0	0	0
26	0	0	0	0	450	550	0	0	0	0	320	340	777	785	0	0	0	0
27	0	0	0	0	475	525	0	0	0	0	315	325	770	792	0	0	0	0
28	0	0	0	0	778	782	0	0	0	0	360	340	778	784	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	170	130	771	793	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	156	144	784	778	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0	776	786	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0	773	789	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0	789	779	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	810	750	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	764	796	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	615	645	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	295	325	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	315	305	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	295	325	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	307	333	0	0	0	0

(*) Duplicate values *(Continued)*

Table F2 (Continued).

	Volume (mL)																	
Day	**S:I = 0.5**		**S:I = 1.0**		**S:I =1.5**		**1 g glyc per L**		**5 g glyc per L**		**9 g glyc per L**		**25 deg C**		**37.5 deg C**		**50 deg C**	
	a	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****
41	0	0	0	0	0	0	0	0	0	0	0	0	295	325	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	275	345	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0	650	610	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0	315	305	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0	308	312	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0	660	600	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0	330	290	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0	318	302	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0	649	611	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0	308	312	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0	0	318	302	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0	635	625	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0	310	310	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0	318	302	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0	635	625	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0	318	302	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0	288	332	0	0	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0	635	625	0	0	0	0
59	0	0	0	0	0	0	0	0	0	0	0	0	312	308	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0	299	321	0	0	0	0

(*) Duplicate values

(Continued)

Table F2 (Continued).

	Volume (mL)																	
Day	**S:I = 0.5**		**S:I = 1.0**		**S:I =1.5**		**1 g glyc per L**		**5 g glyc per L**		**9 g glyc per L**		**25 deg C**		**37.5 deg C**		**50 deg C**	
	a	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****
61	0	0	0	0	0	0	0	0	0	0	0	0	260	240	0	0	0	0
62	0	0	0	0	0	0	0	0	0	0	0	0	265	235	0	0	0	0
63	0	0	0	0	0	0	0	0	0	0	0	0	259	241	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0	245	255	0	0	0	0
65	0	0	0	0	0	0	0	0	0	0	0	0	162	158	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	0	0	151	159	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0	159	157	0	0	0	0
68	0	0	0	0	0	0	0	0	0	0	0	0	158	156	0	0	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(*) Duplicate values

Table F3: Biogas production for digester 1 to 15 (raw data).

	Volume (mL) [a]	
Digester ID	***a***	***b****
1	28874	28900
2	31910	31550
3	42420	42650
4	46786	46700
5	44279	44201
6	56382	56210
7	8125	8025
8	16305	16221
9	20802	20822
10	47323	47299
11	7813	5835
12	7491	7521
13	54248	54250
14	54258	54240
15	54348	54150

[a] Data was taken on the last day of the digestion process

[*] Duplicate values

F2 – Average data

Table F4, Table F5 and Table F6 show the average biogas productions for the first batch trial, second batch trial and digester 1 to 15 respectively. The data was taken by taking the average values for the biogas productions (***a*** and ***b****) found in Appendix F1.

Table F4: Biogas production for the first batch trial.

	Volume (mL)			
Day	**Glycerol**	**Fruit juice**	**Soft drink**	**IOP**
1	626	116	227	96
2	626	115	148	94
3	626	115	24	94
4	1251	115	45	114
5	1751	108	16	85
6	1376	100	7	85
7	876	71	0	100
8	375	30	0	50
9	796	0	0	50
10	1365	0	0	38
11	910	0	0	10
12	682	0	0	10
13	1251	0	0	9
14	1706	0	0	10
15	1137	0	0	10
16	739	0	0	10
17	301	0	0	9
18	0	0	0	0
19	0	0	0	0
20	0	0	0	0

Table F5: Biogas production for the second batch trial.

	Volume (mL)								
Day	**S:I = 0.5**	**S:I = 1.0**	**S:I =1.5**	**1 g glyc per L**	**5 g glyc per L**	**9 g glyc per L**	**25 deg C**	**37.5 deg C**	**50 deg C**
1	2500	5000	2000	5000	6000	7000	782	5000	15000
2	3250	2000	1000	2000	2500	3000	390	2000	5000
3	3250	2000	1500	2000	2000	20	391	2000	10000
4	5000	1000	500	1000	2500	43	781	1000	5000
5	14000	2500	1000	2500	1000	127	781	2500	5000
6	2000	4500	1813	2250	1000	30	781	2250	5000
7	3000	4000	1812	2250	750	30	782	2250	1000
8	2000	3750	2375	4000	750	63	781	4000	500
9	3000	250	1219	4000	500	62	781	4000	10
10	600	3000	1219	3000	1000	63	781	3000	20
11	0	2000	1562	2000	1000	62	391	2000	0
12	0	3000	1630	3000	2125	63	391	3000	0
13	0	2000	1620	2000	2125	57	781	2000	0
14	0	1500	2750	1500	2625	80	781	1500	0
15	0	1500	1031	1500	2625	25	781	1500	0
16	0	750	1032	750	3000	25	782	750	0
17	0	750	1203	750	2250	63	781	750	0
18	0	500	1203	500	2250	67	781	500	0
19	0	90	1203	90	2000	60	781	90	0
20	0	0	1203	0	1000	60	391	0	0

(Continued)

Table F5 (Continued).

	Volume (mL)								
Day	**S:I = 0.5**	**S:I = 1.0**	**S:I =1.5**	**1 g glyc per L**	**5 g glyc per L**	**9 g glyc per L**	**25 deg C**	**37.5 deg C**	**50 deg C**
21	0	0	1125	0	1000	450	391	0	0
22	0	0	2000	0	150	450	781	0	0
23	0	0	2000	0	400	100	421	0	0
24	0	0	2000	0	0	150	1141	0	0
25	0	0	2000	0	0	150	782	0	0
26	0	0	500	0	0	330	781	0	0
27	0	0	500	0	0	320	781	0	0
28	0	0	780	0	0	350	781	0	0
29	0	0	0	0	0	150	782	0	0
30	0	0	0	0	0	150	781	0	0
31	0	0	0	0	0	0	781	0	0
32	0	0	0	0	0	0	781	0	0
33	0	0	0	0	0	0	784	0	0
34	0	0	0	0	0	0	780	0	0
35	0	0	0	0	0	0	780	0	0
36	0	0	0	0	0	0	630	0	0
37	0	0	0	0	0	0	310	0	0
38	0	0	0	0	0	0	310	0	0
39	0	0	0	0	0	0	310	0	0
40	0	0	0	0	0	0	320	0	0

(Continued)

Table F5 (Continued).

	Volume (mL)								
Day	**S:I = 0.5**	**S:I = 1.0**	**S:I =1.5**	**1 g glyc per L**	**5 g glyc per L**	**9 g glyc per L**	**25 deg C**	**37.5 deg C**	**50 deg C**
41	0	0	0	0	0	0	310	0	0
42	0	0	0	0	0	0	310	0	0
43	0	0	0	0	0	0	630	0	0
44	0	0	0	0	0	0	310	0	0
45	0	0	0	0	0	0	310	0	0
46	0	0	0	0	0	0	630	0	0
47	0	0	0	0	0	0	310	0	0
48	0	0	0	0	0	0	310	0	0
49	0	0	0	0	0	0	630	0	0
50	0	0	0	0	0	0	310	0	0
51	0	0	0	0	0	0	310	0	0
52	0	0	0	0	0	0	630	0	0
53	0	0	0	0	0	0	310	0	0
54	0	0	0	0	0	0	310	0	0
55	0	0	0	0	0	0	630	0	0
56	0	0	0	0	0	0	310	0	0
57	0	0	0	0	0	0	310	0	0
58	0	0	0	0	0	0	630	0	0
59	0	0	0	0	0	0	310	0	0
60	0	0	0	0	0	0	310	0	0

(Continued)

Table F5 (Continued).

	Volume (mL)								
Day	**S:I = 0.5**	**S:I = 1.0**	**S:I =1.5**	**1 g glyc per L**	**5 g glyc per L**	**9 g glyc per L**	**25 deg C**	**37.5 deg C**	**50 deg C**
61	0	0	0	0	0	0	250	0	0
62	0	0	0	0	0	0	250	0	0
63	0	0	0	0	0	0	250	0	0
64	0	0	0	0	0	0	250	0	0
65	0	0	0	0	0	0	160	0	0
66	0	0	0	0	0	0	155	0	0
67	0	0	0	0	0	0	158	0	0
68	0	0	0	0	0	0	157	0	0
69	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0

Table F6: Biogas production for digester 1 to 15.

Digester ID	Volume (mL) [a]
1	28 887
2	31 730
3	42 535
4	46 743
5	44 240
6	56 296
7	8 075
8	16 263
9	20 812
10	47 311
11	6 824
12	7 506
13	54 249
14	54 249
15	54 249

[a] Data was taken on the last day of the digestion process

F3 – Sample of calculations for standard temperature and pressure

This sample of calculations was done based on the glycerol-only digester for the first digestion trial on day 1 (see Table F4 in Appendix F2). Temperature in the system (T) was 37.5℃ (310.65 K). Volume of biogas on day 1 (V_b) was 626 mL. We will assume that the system pressure (P) is 1 atm.

The standard conditions for gases are as follows:

- Standard Volume (V_s) - $V_s = 22.4$ m^3,
- Standard Temperature (T_s) - $T_s = 273$ K,
- Standard Pressure (P_s) - $P_s = 1$ atm, and
- Standard mole (n_s) - $n_s = 1$ kmol.

Assuming that the biogas is an ideal gas, the biogas volume in standard temperature and pressure (V_{STP}) may be given by the following equation:

$$V_{STP} = V_b \times \frac{T_s}{T} \times \frac{P}{P_s} \quad \text{(F1)}$$

$$= 626 \text{ mL} \times \frac{273.15 \text{ K}}{310.65 \text{ K}} \times \frac{1 \text{ atm}}{1 \text{ atm}}$$

$$= 550 \text{ mL}$$

Table F7, Table F8 and Table F9 show biogas productions for the first batch trial, second batch trial and digester 1 to 15 in standard temperature and pressure (STP), respectively.

Table F7: Biogas production for the first batch trial (STP data).

	Volume (mL)			
Day	**Glycerol**	**Fruit juice**	**Soft drink**	**IOP**
1	550	102	200	84
2	550	101	130	83
3	550	101	21	83
4	1100	101	40	100
5	1540	95	14	75
6	1210	88	6	75
7	770	62	0	88
8	330	26	0	44
9	700	0	0	44
10	1200	0	0	33
11	800	0	0	9
12	600	0	0	9
13	1100	0	0	8
14	1500	0	0	9
15	1000	0	0	9
16	650	0	0	9
17	265	0	0	8
18	0	0	0	0
19	0	0	0	0
20	0	0	0	0

Table F8: Biogas production for the second batch trial (STP data).

	Volume (mL)								
Day	**S:I = 0.5**	**S:I = 1.0**	**S:I =1.5**	**1 g glyc per L**	**5 g glyc per L**	**9 g glyc per L**	**25 deg C**	**37.5 deg C**	**50 deg C**
1	2500	5000	2000	5000	6000	7000	782	5000	15000
2	3250	2000	1000	2000	2500	3000	390	2000	5000
3	3250	2000	1500	2000	2000	20	391	2000	10000
4	5000	1000	500	1000	2500	63	781	1000	5000
5	14000	2500	1000	2500	1000	127	781	2500	5000
6	2000	4500	1813	2250	1000	30	781	2250	5000
7	3000	4000	1812	2250	750	30	782	2250	1000
8	2000	3750	2375	4000	750	63	781	4000	500
9	3000	250	1219	4000	500	62	781	4000	10
10	600	3000	1219	3000	1000	63	781	3000	20
11	0	2000	1562	2000	1000	62	391	2000	0
12	0	3000	1630	3000	2125	63	391	3000	0
13	0	2000	1620	2000	2125	57	781	2000	0
14	0	1500	2750	1500	2625	80	781	1500	0
15	0	1500	1031	1500	2625	25	781	1500	0
16	0	750	1032	750	3000	25	782	750	0
17	0	750	1203	750	2250	63	781	750	0
18	0	500	1203	500	2250	67	781	500	0
19	0	90	1203	90	2000	60	781	90	0
20	0	0	1203	0	1000	60	391	0	0

(Continued)

Table F8 (Continued).

	Volume (mL)								
Day	**S:I = 0.5**	**S:I = 1.0**	**S:I =1.5**	**1 g glyc per L**	**5 g glyc per L**	**9 g glyc per L**	**25 deg C**	**37.5 deg C**	**50 deg C**
21	0	0	1125	0	1000	450	391	0	0
22	0	0	2000	0	150	450	781	0	0
23	0	0	2000	0	400	100	421	0	0
24	0	0	2000	0	0	150	1141	0	0
25	0	0	2000	0	0	150	782	0	0
26	0	0	500	0	0	330	781	0	0
27	0	0	500	0	0	320	781	0	0
28	0	0	780	0	0	350	781	0	0
29	0	0	0	0	0	150	782	0	0
30	0	0	0	0	0	150	781	0	0
31	0	0	0	0	0	0	781	0	0
32	0	0	0	0	0	0	781	0	0
33	0	0	0	0	0	0	784	0	0
34	0	0	0	0	0	0	780	0	0
35	0	0	0	0	0	0	780	0	0
36	0	0	0	0	0	0	630	0	0
37	0	0	0	0	0	0	310	0	0
38	0	0	0	0	0	0	310	0	0
39	0	0	0	0	0	0	310	0	0
40	0	0	0	0	0	0	320	0	0

(Continued)

Table F8 (Continued).

	Volume (mL)								
Day	**S:I = 0.5**	**S:I = 1.0**	**S:I =1.5**	**1 g glyc per L**	**5 g glyc per L**	**9 g glyc per L**	**25 deg C**	**37.5 deg C**	**50 deg C**
41	0	0	0	0	0	0	310	0	0
42	0	0	0	0	0	0	310	0	0
43	0	0	0	0	0	0	630	0	0
44	0	0	0	0	0	0	310	0	0
45	0	0	0	0	0	0	310	0	0
46	0	0	0	0	0	0	630	0	0
47	0	0	0	0	0	0	310	0	0
48	0	0	0	0	0	0	310	0	0
49	0	0	0	0	0	0	630	0	0
50	0	0	0	0	0	0	310	0	0
51	0	0	0	0	0	0	310	0	0
52	0	0	0	0	0	0	630	0	0
53	0	0	0	0	0	0	310	0	0
54	0	0	0	0	0	0	310	0	0
55	0	0	0	0	0	0	630	0	0
56	0	0	0	0	0	0	310	0	0
57	0	0	0	0	0	0	310	0	0
58	0	0	0	0	0	0	630	0	0
59	0	0	0	0	0	0	310	0	0
60	0	0	0	0	0	0	310	0	0

(Continued)

Table F8 (Continued).

	Volume (mL)								
Day	**S:I = 0.5**	**S:I = 1.0**	**S:I =1.5**	**1 g glyc per L**	**5 g glyc per L**	**9 g glyc per L**	**25 deg C**	**37.5 deg C**	**50 deg C**
61	0	0	0	0	0	0	250	0	0
62	0	0	0	0	0	0	250	0	0
63	0	0	0	0	0	0	250	0	0
64	0	0	0	0	0	0	250	0	0
65	0	0	0	0	0	0	160	0	0
66	0	0	0	0	0	0	155	0	0
67	0	0	0	0	0	0	158	0	0
68	0	0	0	0	0	0	157	0	0
69	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0

Table F9: Biogas production for digester 1 to 15 (STP data).

Digester ID	Volume (mL) [a]
1	25 400
2	27 900
3	37 400
4	41 100
5	38 900
6	49 500
7	7 100
8	14 300
9	18 300
10	41 600
11	6 000
12	6 600
13	47 700
14	47 700
15	47 700

[a] Data was taken on the last day of the digestion process

APPENDIX G – Feed characterization before co-digestion

G1 – Feed characterization raw data and average values

Table G1 and G2 below show raw data and average values for the feed characterization for the first digestion trial before co-digestion, respectively. Table G2 was taken by taking the average values for the data in Table G1.

G2 – Sample of calculations for the characterization before digestion

This sample of calculations is for the feed characterization of IOP in Table G1 (the 1st column - **1**).

Density

Weight of the 30 mL sample (w_s) is given by:

$$w_s = w_{bh} - w_{co} \quad \text{(G1)}$$

$$= 57.38 - 26.15 = 31.23 \text{ g}$$

where: w_{bh} - is the crucible weigh + 30 mL before heating, and

w_{co}- is the weight of the crucible only (g).

The density of the 30 mL sample (ρ_s) is therefore:

$$\rho_s = \frac{w_s}{30} \times 1000 \quad \text{(G2)}$$

$$= \frac{31.23}{30} \times 1000 = 1041 \text{ kg/m}^3$$

Table G1: Feed characterization of all substrates that were used for the first digestion trial before co-digestion (raw data).

	IOP		Fruits juice		Inoculum		Raw sludge		Soft drink	
	1	**1** [(a)]	**2**	**2** [(a)]	**3**	**3** [(a)]	**4**	**4** [(a)]	**5**	**5** [(a)]
B) Crucible only (g)	26.15	25.30	28.29	25.17	24.54	23.09	26.23	30.91	33.01	30.07
C) Crucible + 30 mL before heating (g)	57.38	56.84	59.70	56.32	55.44	54.09	57.74	62.34	64.47	61.11
A) Crucible + 30 mL after heating (g) @ 105 C	29.62	28.86	29.98	26.86	25.41	23.75	27.18	31.91	34.52	31.68
D) Crucible + 30 mL after furnace burning (g) @ 550 C	26.31	25.65	28.48	25.35	24.98	23.43	26.42	31.12	33.07	30.13
Weight of the 30mL (g)	31.23	31.54	31.41	31.15	30.90	31.00	31.51	31.43	31.46	31.04
Density of the 30mL (kg/m3)	1041	1051	1047	1038	1030	1033	1050	1048	1049	1035
Moisture (g)	27.76	27.98	29.72	29.46	30.03	30.34	30.56	30.43	29.95	29.43
Moisture (wt %)	88.9	88.7	94.6	94.6	97.2	97.9	97.0	96.8	95.2	94.8
TS (g)	2.20	2.29	1.69	1.69	0.87	0.66	1.38	1.31	2.50	2.51
TS (wt %)	11.10	11.30	5.38	5.43	2.80	2.14	3.00	3.18	4.80	5.20
VS (g)	2.04	2.13	1.50	1.51	0.43	0.33	0.85	0.85	2.49	2.56
VS (wt %) based on sample	10.6	10.2	4.79	4.85	1.38	1.05	2.4	2.5	4.6	5
VS (wt %) based on TS	92.5	92.6	88.9	89.4	49.2	49.2	61.2	61.2	99.0	99.8
VS/TS	0.93	0.93	0.89	0.89	0.49	0.49	0.61	0.61	0.99	0.99

[(a)] Duplicate values

Table G2: Feed characterization of all substrates that were used for the first digestion trial before co-digestion.

	IOP	Fruits juice	Inoculum	Raw sludge	Soft drink
Average moisture (%)	88.8	94.6	97.5	96.9	95
Average density (kg/m^3)	1046	1043	1032	1049	1042
Average TS (%)	11.2	5,4	2.5	3.9	4.9
Average VS based on the sample (%)	10.4	4,8	1.2	2.4	4.8
Average VS based on TS (%)	93.0	89.0	49.0	62.0	98.0
Average VS/TS	0.93	0.89	0.49	0.62	0.98

Moisture content

The moisture content in the sample (m_c) is given by the following equation:

$$m_c = \frac{w_m}{w_s} \times 100 = \frac{(w_{bh} - w_{ah})}{w_s} \times 100 \quad \text{(G3)}$$

$$= \frac{(57.38 - 29.62)}{31.23}$$

$$= \frac{29.03}{31.23} \times 100$$

$$= 88.9\%$$

where: w_m - is the weight of moisture (g), and

w_{ah} - is the crucible weight + 30 mL sample after heating at 105℃ (g).

Total and volatile solids contents

The total solids content (TS) is given by the following equation:

$$\text{TS} = \frac{(w_s - w_m)}{w_s} \times 100 \quad \text{(G4)}$$

$$= \frac{31.23 - 27.76}{31.23} \times 100$$

$$= 11.1\%$$

The total volatile solids (VS) are given by the following equation:

$$\text{VS} = \frac{(w_{ah} - w_{afb})}{w_s} \times 100 \quad \text{(G5)}$$

$$= \frac{29.62 - 26.31}{31.23} \times 100$$

$$= 10.6\%$$

where w_{afb} is the crucible weight + 30 mL sample after furnace burning at 550℃.

Total and volatile solids ratio

The total and volatile solids ratio $\left(\frac{\text{VS}}{\text{TS}}\right)$ is given by the following equation:

$$\frac{\text{VS}}{\text{TS}} = \frac{10.6}{11.1}$$

$$= 0.93$$

G3 – Sample of calculations for the feed characterization of the mixture before digestion

This sample of calculations is based on the glycerol only sample on Table 4.2.

VS in grams

Glycerol had a density of 1080 kg/m^3 and a VS% of 70.5% (see Table 4.1). The feed VS of glycerol is given by Equation G6:

$$\text{VS (g)} = \left(\frac{\text{density of glycerol}}{1000}\right) \times \left(\frac{\text{VS\%}}{100}\right) \times \text{volume of glycerol} \qquad \text{(G6)}$$

$$= \left(\frac{1080}{1000}\right) \times \left(\frac{70.5}{100}\right) \times 20$$

$$= 15.5 \text{ g}$$

COD in milligrams

Glycerol had a COD (mg/L) of 1175040 mg/L and a volume of 20 mL (see Table 4.1). The feed COD in mg is given by Equation G7:

$$\text{COD (mg)} = (\text{COD in milligrams per litre}) \times \left(\frac{\text{volume of glycerol}}{1000}\right) \qquad \text{(G7)}$$

$$= 1175040 \times \frac{20}{1000}$$

$$= 23501 \text{ mg}$$

Feed concentration in reactor on VS and COD basis

Glycerol had 15.5 g VS and 23501 g COD. The digester had a working volume of 4.8 L. The feed concentration of glycerol in the digester on VS basis is given by Equation G8:

$$\text{Feed concentration in reactor (g VS /L)} = \frac{\text{g VS}}{\text{reactor working volume}} \qquad \text{(G8)}$$

$$= \frac{15.5}{4.8}$$

$$= 3.23 \text{ g VS/L}$$

The feed concentration of glycerol in the digester on COD basis is given by Equation G9:

$$\text{Feed concentration in reactor (g COD /L)} = \frac{\text{g COD}}{\text{reactor working volume}} \qquad \text{(G9)}$$

$$= \frac{23501/1000}{4.8}$$

$$= 4.90 \text{ g VS/L}$$

G4 – Sample of calculations for the feed characterization at different substrate to inoculum ratios

This sample of calculations is based on the substrate to inoculum ratio of 1.0 on Table 4.5. The VS of glycerol, raw sludge and inoculum were 7.8, 41.7 and 49.5 g VS, respectively. The substrate to inoculum ratio is given by Equation G10:

$$\text{Substrate to inoculum ratio (g VS/g VS)} = \frac{\text{(VS of glycerol)+(VS of raw sludge)}}{\text{VS of inoculum}} \qquad \text{(G10)}$$

$$= \frac{7.8 + 41.7}{49.5}$$

$$= 1.0 \text{ g VS/g VS}$$

G5 – Sample of calculations for the feed characterization at various g glycerol concentrations in the reactor

This sample of calculations is based on the 5 g glycerol concentration in the reactor on Table 4.7. Glycerol had 70.5% of VS (Table 4.1) and 17.1 g VS. The glycerol concentration in the reactor is given by Equation G11:

$$\text{glycerol conc (g /L)} = \frac{\text{g VS of glycerol}}{\left(\frac{\text{\% VS of glycerol}}{100}\right) \times \text{working volume of the reactor}} \qquad \text{(G11)}$$

$$= \frac{17.1}{\left(\frac{70.5}{100}\right) \times 4.8}$$

$$= 5 \text{ g glycerol/L}$$

APPENDIX H – pH, VFA and alkalinity

H1 – Raw data

Table H1 and Table H2 show raw data for pH for the first and the second digestion trials, respectively. Table H3 shows raw data for VFA and Alkalinity for the first digestion trial.

Table H1: pH for the first digestion trial (raw data).

	pH							
Day	**Glycerol**		**Fruit juice**		**Soft drink**		**IOP**	
	a	***b****	***a***	***b****	***a***	***b****	***a***	***b****
1	7.63	7.67	7.50	7.46	7.38	7.40	7.60	7.50
2	7.52	7.48	7.45	7.41	7.35	7.35	7.50	7.50
3	7.41	7.19	7.40	7.32	7.30	7.30	7.40	7.50
4	6.90	7.30	7.30	7.32	7.31	7.29	7.30	7.50
5	7.00	6.80	7.31	7.35	7.32	7.34	7.40	7.20
6	6.70	6.90	7.39	7.29	7.33	7.33	7.26	7.24
7	7.10	6.90	7.40	7.36	7.38	7.36	7.31	7.29
8	7.40	6.80	7.41	7.37	7.38	7.40	7.35	7.33
9	7.30	7.10	7.35	7.43	7.40	7.40	7.39	7.33
10	7.20	7.10	7.41	7.43	7.40	7.42	7.41	7.39
11	7.20	7.00	7.44	7.42	7.41	7.43	7.42	7.42
12	7.10	7.30	7.46	7.44	7.40	7.40	7.43	7.41
13	7.30	7.50	7.48	7.46	7.40	7.40	7.44	7.42
14	7.50	7.36	7.48	7.48	7.40	7.40	7.44	7.44
15	7.40	7.50	7.48	7.48	7.40	7.40	7.46	7.44
16	7.53	7.47	7.48	7.50	7.40	7.40	7.47	7.45
17	7.59	7.51	7.50	7.50	7.40	7.40	7.47	7.47
18	7.60	7.56	7.50	7.50	7.40	7.40	7.48	7.46
19	7.60	7.60	7.50	7.52	7.40	7.40	7.48	7.48
20	7.61	7.61	7.51	7.51	7.40	7.40	7.48	7.48

(*) Duplicate values

Table H2: pH for the second batch trial (raw data).

	pH [a]			
Day	**In**		**Out**	
	a	***b****	***a***	***b****
S:I = 0.5	7.2	7.0	7.5	7.5
S:I = 1.0	7.1	7.3	7.6	7.8
S:I = 1.5	7.4	7.2	7.8	8.0
1 g glyc per L	7.2	7.2	7.6	7.8
5 g glyc per L	7.2	7.4	7.8	8.0
9 g glyc per L	4.5	4.7	6.4	6.4
25 deg C	7.2	7.2	7.7	7.5
37.5 deg C	7.2	7.2	7.8	7.6
50 deg C	7.2	7.2	7.1	7.1

[a] Data was taken on the first and last day of the digestion trial

[*] Duplicate values

Table H3: VFA and alkalinity for the first digestion trial.

	VFA [b]		Alkalinity [b]	
Digester ID	**mg/L**		**mg $CaCO_3$/L**	
	a	***b****	***a***	***b****
Glycerol	315	305	1070	1030
Fruit juice	195	205	1708	1702
Soft drink	183	177	1998	2002
IOP	262	258	1101	1103

[b] Data was taken at the end of the digestion trial

[*] Duplicate values

H2 – Average data

Table H4 and Table H5 show pH for the first and the second digestion trials, respectively. The data was taken by taking the average values for the raw data (***a*** and ***b****) found in Appendix H1. VFA and alkalinity for the first digestion trial are shown in Table H6. Data for VFA and alkalinity was taken on the first and last day of the digestion trial

Table H4: pH for the first digestion trial.

	pH			
Day	**Glycerol**	**Fruit juice**	**Soft drink**	**IOP**
0	7.65	7.48	7.35	7.55
1	7.50	7.43	7.35	7.50
2	7.30	7.36	7.30	7.45
3	7.10	7.31	7.30	7.40
4	6.90	7.33	7.33	7.30
5	6.80	7.34	7.33	7.25
6	7.00	7.38	7.37	7.30
7	7.10	7.39	7.39	7.34
8	7.20	7.39	7.40	7.36
9	7.15	7.42	7.41	7.40
10	7.10	7.43	7.42	7.42
11	7.20	7.45	7.40	7.42
12	7.40	7.47	7.40	7.43
13	7.43	7.48	7.40	7.44
14	7.45	7.48	7.40	7.45
15	7.50	7.49	7.40	7.46
16	7.55	7.50	7.40	7.47
17	7.58	7.50	7.40	7.47
18	7.60	7.51	7.40	7.48
19	7.61	7.51	7.40	7.48
20	7.62	7.51	7.40	7.48

Table H5: pH for the second digestion trial.

	pH	
Day	**In**	**Out**
S:I = 0.5	7.1	7.5
S:I = 1.0	7.2	7.7
S:I = 1.5	7.3	7.9
1 g glyc per L	7.2	7.7
5 g glyc per L	7.3	7.9
9 g glyc per L	4.6	6.4
25 deg C	7.2	7.6
37.5 deg C	7.2	7.7
50 deg C	7.2	7.1

Table H6: VFA and alkalinity for the first digestion trial.

	VFA [(b)]	**Alkalinity** [(b)]
Digester ID	**mg/L**	**mg $CaCO_3$/L**
Glycerol	310	1050
Fruit juice	200	1705
Soft drink	180	2000
IOP	260	1102

[(b)] Data was taken at the end of the digestion trial

H3 – Sample of calculations for VFA to alkalinity ratio

This sample of calculations is based on the VFA to alkalinity ratio of glycerol on Figure 4.4. Glycerol had VFA of 310 mg/L (see Figure 4.2) and alkalinity of 1050 mg/L (see Figure 4.4). The VFA to alkalinity ratio is given by Equation H1:

$$\text{VFA to alkalinity ratio } \left(\frac{\text{mg/L}}{\text{mg/L}}\right) = \frac{\text{VFA}}{\text{alkalinity}} \qquad \text{(H1)}$$

$$= \frac{310}{1050}$$

$$= 0.3$$

APPENDIX I – VS and COD

I1 – Raw data

Table I1 shows raw data for VS and COD. Data was not taken for digesters 1 to 15.

Table I1: VS and COD (raw data).

	VS (g)				COD (mg/L)			
Digester ID	**Fed**		**Out**		**Fed**		**Out**	
	a	***b****	***a***	***b****	***a***	***b****	***a***	***b****
Glycerol	15.0	16.0	0.00	0.00	1175080	1175000	0	0
Fruit juice	1.1	1.5	0.31	0.21	75130	75110	14126	14120
Soft drink	1.2	0.8	0.22	0.26	49938	49902	11464	11500
IOP	1.5	1.3	0.27	0.25	82087	82073	13029	12909
S:I = 0.5	36.6	39.0	6.70	6.90	16760	16840	2346	2358
S:I = 1.0	48.0	51.0	0.98	0.90	18730	18710	18707	18733
S:I = 1.5	61.5	60.9	0.52	0.58	20168	20152	0	0
1 g glyc per L	50.0	49.0	0.95	0.93	49470	49410	487	501
5 g glyc per L	58.6	59.0	0.21	0.15	59070	59010	0	0
9 g glyc per L	67.7	68.5	38.00	37.00	68190	68130	36120	36130
25 deg C	49.0	50	15.10	14.50	18725	18715	4429	4445
37.5 deg C	49.6	49.4	0.92	0.96	18721	18719	183	191
50 deg C	49.7	49.3	0.16	0.14	18719	18721	0	0

(*) Duplicate values

I2 – Average data

Table I2 shows raw data for VS and COD. The data was taken by taking the average values for the VS and COD (***a*** and ***b****) found in Table I1.

Table I2: VS and COD.

	VS (g)		COD (mg/L)	
Digester ID	**Fed**	**Out**	**Fed**	**Out**
Glycerol	15.50	0.00	1 175 040	0
Fruit juice	1.30	0.26	75 120	14 123
Soft drink	1.00	0.24	49 920	11 482
IOP	1.40	0.26	82 080	12 969
S:I = 0.5	37.80	6.80	16 800	2 352
S:I = 1.0	49.50	0.94	18 720	18 720
S:I = 1.5	61.20	0.55	20 160	0
1 g glyc per L	49.50	0.94	49 440	494
5 g glyc per L	58.80	0.18	59 040	0
9 g glyc per L	68.10	37.50	68 160	36 125
25 deg C	49.50	14.80	18 720	4 437
37.5 deg C	49.50	0.94	18 720	187
50 deg C	49.50	0.15	18 720	0

I3 – Sample of calculations for percentage of VS and COD removed

This sample of calculations was based on the glycerol percentage organic removed on Figure 4.22. Glycerol had 15.5 g VS fed (see Table 4.2) and 0 g VS at the end of the digestion process. On VS basis, the % VS removed is obtained by Equation I8:

$$\%\ \text{VS removed}\ (\%) = \frac{(\text{g VS fed}) - (\text{g VS out})}{\text{g VS fed}} \times 100\% \qquad \text{(I8)}$$

$$= \frac{15.5 - 0}{15.5} \times 100\%$$

$$= 100\%$$

Glycerol had 23 501 mg COD fed (see Table 4.2) and 0 mg COD at the end of the digestion process. On COD basis, the % COD removed is obtained by Equation I9:

$$\%\ \text{COD removed}\ (\%) = \frac{(\text{g COD fed}) - (\text{g COD out})}{\text{g COD fed}} \times 100\% \qquad \text{(I9)}$$

$$= \frac{23\ 501 - 0}{23501} \times 100\% = 100\%$$

APPENDIX J – Biogas compositions

J1 – Raw data

Table J1 and Table J2 show raw data for biogas compositions for the first and the second trials, respectively. Nitrogen content was calculated (see Appendix J4 for calculation).

Table J1: Biogas compositions for the first digestion trial (raw data).

		Volume percentage (%)											
Digester ID	**Day**	**CH_4**		**CO_2**		**O_2**		**H_2**		**N_2**		**H_2O**	
		a	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****
Glycerol	1	47	55	45	49	0.34	0.30	0.90	0.14	1.30	1.10	1	1
	2	49	45	47	55	0.18	0.30	0.20	0.18	0.90	1.00	1	1
	3	42	46	50	58	0.13	0.29	0.10	0.08	0.89	0.87	1	1
	4	51	55	43	44	0.20	0.18	1.10	1.86	0.80	0.86	1	1
	5	69	61	32	33	0.10	0.20	0.30	1.00	0.60	0.80	1	1
	6	72	60	30	32	0.16	0.10	1.00	1.36	0.50	0.88	1	1
	7	63	71	32	30	0.12	0.10	0.24	0.24	0.80	0.50	1	1
	8	66	70	31	29	0.08	0.12	0.26	0.26	0.80	0.48	1	1
	9	70	68	28	30	0.06	0.10	0.30	0.30	0.79	0.45	1	1
	10	73	69	26	28	0.07	0.05	0.34	0.34	0.50	0.70	1	1
	11	74	70	25	27	0.04	0.06	0.37	0.37	0.49	0.67	1	1
	12	74	70	26	26	0.04	0.04	0.40	0.40	0.50	0.62	1	1
	13	73	71	27	25	0.04	0.02	0.30	0.56	0.50	0.58	1	1
	14	74	72	24	26	0.02	0.04	0.45	0.45	0.49	0.55	1	1
	15	75	77	23	21	0.03	0.01	0.49	0.47	0.49	0.51	1	1
	16	76	76	22	22	0.03	0.01	0.49	0.47	0.49	0.51	1	1
	17	76	76	22	22	0.03	0.01	0.49	0.47	0.49	0.51	1	1
Fruit juice	1	38	36	58	62	0.23	0.27	2.00	1.40	0.05	0.05	1	1
	2	40	38	54	60	0.27	0.25	2.10	1.10	1.50	0.78	1	1
	3	48	50	47	51	0.16	0.20	0.70	0.90	0.02	0.02	1	1
	4	61	59	38	38	0.08	0.12	0.70	0.88	0.11	0.11	1	1
	5	65	63	35	31	0.12	0.10	0.60	0.80	0.90	1.48	1	1
	6	67	67	29	29	0.06	0.10	0.50	0.50	2.42	2.42	1	1
	7	69	69	30	30	0.08	0.06	0.08	0.10	0.16	0.16	1	1
	8	69	69	28	32	0.08	0.08	0.01	0.01	0.09	0.09	1	1

(*) Duplicate values *(Continued)*

Table J1 (Continued).

		Volume percentage (%)											
Digester ID	**Day**	**CH_4**		**CO_2**		**O_2**		**H_2**		**N_2**		**H_2O**	
		a	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****
Fruit juice	9	69	69	32	28	0.08	0.08	0.01	0.01	0.09	0.09	1	1
	10	69	69	28	28	0.08	0.08	0.01	0.01	1.91	1.91	1	1
Soft drink	1	40	40	54	60	0.29	0.31	1.50	0.50	0.80	0.60	1	1
	2	54	54	45	43	0.27	0.31	0.60	0.80	0.01	0.01	1	1
	3	54	54	44	42	0.29	0.25	0.58	0.64	1.10	1.14	1	1
	4	56	56	43	41	0.22	0.20	0.51	0.63	0.19	0.25	1	1
	5	61	61	39	35	0.16	0.20	0.50	0.50	0.14	0.50	1	1
	6	66	66	33	31	0.15	0.15	0.50	0.50	0.35	0.35	1	1
	7	67	67	30	30	0.14	0.14	0.49	0.49	1.00	1.74	1	1
	8	67	67	30	30	0.14	0.14	0.49	0.49	1.00	1.74	1	1
IOP	1	38	36	60	62	0.27	0.35	0.80	0.56	0.01	0.01	1	1
	2	37	43	56	60	0.33	0.25	0.60	0.70	0.06	0.06	1	1
	3	45	43	51	53	0.26	0.30	0.59	0.61	2.00	2.24	1	1
	4	49	47	51	49	0.26	0.20	0.50	0.66	0.16	0.22	1	1
	5	54	50	48	44	0.17	0.21	0.56	0.56	0.18	0.32	1	1
	6	55	53	44	42	0.11	0.15	0.54	0.56	1.00	1.64	1	1
	7	58	56	43	39	0.11	0.13	0.53	0.55	0.30	0.38	1	1
	8	60	58	39	37	0.16	0.14	0.50	0.70	1.10	1.40	1	1
	9	65	63	35	33	0.08	0.12	0.50	1.10	0.10	0.10	1	1
	10	67	65	31	33	0.06	0.10	0.51	0.69	0.30	0.34	1	1
	11	66	68	30	32	0.06	0.08	0.53	0.53	0.40	0.40	1	1
	12	66	68	32	32	0.05	0.07	0.51	0.53	0.60	0.56	1	1
	13	65	71	29	31	0.08	0.06	0.56	0.46	0.40	0.44	1	1
	14	66	70	31	29	0.07	0.05	0.53	0.47	0.41	0.47	1	1
	15	70	68	28	28	0.06	0.06	0.55	0.45	1.44	1.44	1	1
	16	70	68	29	27	0.05	0.05	0.45	0.57	1.44	1.44	1	1
	17	70	70	29	27	0.05	0.05	0.44	0.56	0.45	0.45	1	1
	18	70	70	27	27	0.04	0.04	0.44	0.60	1.44	1.44	1	1
	19	70	70	27	27	0.04	0.04	0.50	0.54	1.44	1.44	1	1
	20	70	70	27	27	0.06	0.04	0.50	0.54	1.43	1.43	1	1

(*) Duplicate values

Table J2: Biogas compositions for the second digestion trial (raw data).

	Volume percentage (%)											
Digester ID	**CH_4**		**CO_2**		**O_2**		**H_2**		**N_2**		**H_2O**	
	a	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****	***a***	***b****
S:I = 0.5	66	72	27	29	0.00	0.20	0.20	0.62	0.38	1.60	1	1
S:I = 1.0	74	76	19	25	0.13	0.11	0.11	0.65	0.55	1.50	1	1
S:I = 1.5	69	71	23	31	0.07	0.05	0.05	0.49	0.51	1.50	1	1
1 g glyc / L	74	76	23	21	0.08	0.12	0.12	0.48	0.52	1.39	1	1
5 g glyc / L	75	77	19	23	0.07	0.09	0.09	0.60	0.62	1.30	1	1
9 g glyc / L	78	76	21	19	0.08	0.10	0.10	0.61	0.63	1.28	1	1
25 deg C	65	71	28	30	0.13	0.11	0.11	0.60	0.62	1.30	1	1
37.5 deg C	74	76	18	26	0.06	0.10	0.10	0.59	0.61	1.50	1	1
50 deg C	80	80	16	18	0.07	0.03	0.03	0.50	0.56	1.50	1	1
1	66	68	31	29	0.18	0.20	0.80	0.60	1.10	1.12	1	1
2	68	70	29	27	0.12	0.14	0.70	0.60	1.20	1.24	1	1
3	67	69	27	31	0.17	0.13	0.66	0.68	1.20	1.16	1	1
4	68	70	29	27	0.08	0.10	0.61	0.59	1.30	1.32	1	1
5	78	76	21	19	0.00	0.10	0.59	0.57	1.30	1.44	1	1
6	80	80	16	18	0.03	0.01	0.10	0.12	1.88	1.86	1	1
7	57	65	37	35	0.11	0.09	0.74	0.66	1.50	0.90	1	1
8	57	63	39	35	0.09	0.09	0.62	0.60	1.31	1.29	1	1
9	64	66	33	31	0.09	0.11	0.68	0.70	1.30	1.12	1	1
10	73	67	28	26	0.07	0.03	0.58	0.60	1.30	1.42	1	1
11	62	60	38	34	0.15	0.13	0.64	0.68	1.12	1.28	1	1
12	61	59	39	35	0.12	0.10	0.70	0.72	1.10	1.26	1	1
13	75	73	21	25	0.08	0.10	0.60	0.56	1.34	1.32	1	1
14	73	75	24	22	0.10	0.10	0.61	0.63	1.33	1.23	1	1
15	74	74	25	21	0.10	0.10	0.61	0.63	1.32	1.24	1	1

(*) Duplicate values

J2 – Average data

Table J3 and J4 show biogas compositions for the first and second digestion trials, respectively. The data was taken by taking the average values for the biogas compositions (***a*** and ***b****) found in Appendix J1. There is error in the glycerol digester on day 1, 2 and 3, fruit juice digester on day 7, 8 and 9, and IOP digester on day 12. The error in the glycerol digester

on day 1, 2 and 3 could be due to false estimation of water content in the mixture. Water is not formed in the hydrolysis stage (day 1, 2 and 3), which means that the content of water should be less than 1. The error in the fruit juice digester on day 7, 8 and 9, and IOP digester on day 12 could be due to biogas extraction monitor displaying inaccurate values.

Table J3: Biogas compositions for the first digestion trial.

		Volume percentage (%)						
Digester ID	**Day**	**CH_4**	**CO_2**	**O_2**	**H_2**	**N_2**	**H_2O**	**Sum**
Glycerol	1	51	47	0.32	0.52	1.20	1	101.04
	2	47	51	0.24	0.19	0.95	1	100.38
	3	44	54	0.21	0.09	0.88	1	100.18
	4	53	43.5	0.19	1.48	0.83	1	100.00
	5	65	32.5	0.15	0.65	0.70	1	100.00
	6	66	31	0.13	1.18	0.69	1	100.00
	7	67	31	0.11	0.24	0.65	1	100.00
	8	68	30	0.10	0.26	0.64	1	100.00
	9	69	29	0.08	0.30	0.62	1	100.00
	10	71	27	0.06	0.34	0.60	1	100.00
	11	72	26	0.05	0.37	0.58	1	100.00
	12	72	26	0.04	0.40	0.56	1	100.00
	13	72	26	0.03	0.43	0.54	1	100.00
	14	73	25	0.03	0.45	0.52	1	100.00
	15	76	22	0.02	0.48	0.50	1	100.00
	16	76	22	0.02	0.48	0.50	1	100.00
	17	76	22	0.02	0.48	0.50	1	100.00
Fruit juice	1	37	60	0.25	0.05	1.70	1	100.00
	2	39	57	0.26	1.14	1.60	1	100.00
	3	49	49	0.18	0.02	0.80	1	100.00
	4	60	38	0.10	0.11	0.79	1	100.00
	5	64	33	0.11	1.19	0.70	1	100.00
	6	67	29	0.08	2.42	0.50	1	100.00
	7	69	30	0.07	0.01	0.09	1	100.32
	8	69	30	0.08	0.02	0.01	1	100.18
	9	69	30	0.08	0.02	0.01	1	100.18
	10	69	28	0.08	1.91	0.01	1	100.00

(Continued)

Table J3 (Continued).

		Volume percentage (%)						
Digester ID	**Day**	**CH_4**	**CO_2**	**O_2**	**H_2**	**N_2**	**H_2O**	**Sum**
Soft drink	1	40	57	0.30	0.70	1.00	1	100.00
	2	54	44	0.29	0.01	0.70	1	100.00
	3	54	43	0.27	1.12	0.61	1	100.00
	4	56	42	0.21	0.22	0.57	1	100.00
	5	61	37	0.18	0.32	0.50	1	100.00
	6	66	32	0.15	0.35	0.50	1	100.00
	7	67	30	0.14	1.37	0.49	1	100.00
	8	67	30	0.14	1.37	0.49	1	100.00
IOP	1	37	61	0.31	0.01	0.68	1	100.00
	2	40	58	0.29	0.06	0.65	1	100.00
	3	44	52	0.28	2.12	0.60	1	100.00
	4	48	50	0.23	0.19	0.58	1	100.00
	5	52	46	0.19	0.25	0.56	1	100.00
	6	54	43	0.13	1.32	0.55	1	100.00
	7	57	41	0.12	0.34	0.54	1	100.00
	8	59	38	0.15	1.25	0.60	1	100.00
	9	64	34	0.10	0.10	0.80	1	100.00
	10	66	32	0.08	0.32	0.60	1	100.00
	11	67	31	0.07	0.40	0.53	1	100.00
	12	67	32	0.06	0.58	0.52	1	101.16
	13	68	30	0.07	0.42	0.51	1	100.00
	14	68	30	0.06	0.44	0.50	1	100.00
	15	69	28	0.06	1.44	0.50	1	100.00
	16	69	28	0.05	1.44	0.51	1	100.00
	17	70	28	0.05	0.45	0.50	1	100.00
	18	70	27	0.04	1.44	0.52	1	100.00
	19	70	27	0.04	1.44	0.52	1	100.00
	20	70	27	0.05	1.43	0.52	1	100.00

Table J4: Biogas compositions for the second digestion trial.

	Volume percentage (%) [a]						
Digester ID	**CH_4**	**CO_2**	**O_2**	**H_2**	**N_2**	**H_2O**	**Sum**
S:I = 0.5	69	28	0.10	1.40	0.50	1	100
S:I = 1.0	75	22	0.12	1.28	0.60	1	100
S:I = 1.5	70	27	0.06	1.44	0.50	1	100
1 g glyc / L	75	22	0.10	1.40	0.50	1	100
5 g glyc / L	76	21	0.08	1.31	0.61	1	100
9 g glyc / L	77	20	0.09	1.29	0.62	1	100
25 deg C	68	29	0.12	1.27	0.61	1	100
37.5 deg C	75	22	0.08	1.32	0.60	1	100
50 deg C	80	17	0.05	1.42	0.53	1	100
1	67	30	0.19	1.11	0.70	1	100
2	69	28	0.13	1.22	0.65	1	100
3	68	29	0.15	1.18	0.67	1	100
4	69	28	0.09	1.31	0.60	1	100
5	77	20	0.05	1.37	0.58	1	100
6	80	17	0.02	1.87	0.11	1	100
7	61	36	0.10	1.20	0.70	1	100
8	60	37	0.09	1.30	0.61	1	100
9	65	32	0.10	1.21	0.69	1	100
10	70	27	0.05	1.36	0.59	1	100
11	61	36	0.14	1.20	0.66	1	100
12	60	37	0.11	1.18	0.71	1	100
13	74	23	0.09	1.33	0.58	1	100
14	74	23	0.10	1.28	0.62	1	100
15	74	23	0.10	1.28	0.62	1	100

[a] Data was taken on the last day of the digestion process

J3 – Sample of calculations for methane to carbon dioxide ratio

This sample of calculations is based on the glycerol methane to carbon dioxide ratio of 3.5 on day 20 of Figure 4.11. On day 20, glycerol had methane percentage of 76% (see Figure 4.7) and carbon dioxide percentage of 22% (see Figure 4.10). The methane to carbon dioxide ratio is given by Equation J1:

$$\text{methane to carbon dioxide ratio } \left(\frac{\%}{\%}\right) = \frac{\text{methane \%}}{\text{carbon dioxide \%}} \quad \text{(J1)}$$

$$= \frac{76}{22}$$

$$= 3.46$$

J4 – Sample of calculations for nitrogen content and volume in biogas, and nitrogen to oxygen ratio

This sample of calculations is based on glycerol on day 17 of Table J3.

Nitrogen volume in biogas

Since the nitrogen in the system is that of air, the volume of nitrogen initially in the system can be assumed to be 79%. The volume of air initially in the system (V_{air}) is equal to the headspace volume in the digester (V_V) plus the volume in the tube connecter (V_T):

$$V_{air} = V_V + V_T \quad \text{(J2)}$$

$$= \left[\frac{\pi}{4} \times \left(\frac{0.635}{100}\right)^2 \times \frac{200}{100}\right] + \left[0.23 \times 6 \times \frac{1}{1000}\right]$$

$$= 0{,}001443 \text{ m}^3$$

Therefore, the volume of nitrogen in the system (V_{N_2}) was:

$$V_{N_2} = 0.79 \times V_{air} \quad \text{(J3)}$$

$$= 0.79 \times 0{,}001443$$

$$= 0{,}00114 \text{ m}^3$$

Nitrogen content in biogas

The volume of nitrogen in the system was assumed to be constant for the whole digestion period. According to Beam (2011), the amount of water vapour in biogas is approximately 1% (y_w) i.e. $y_w = 0.01$ v/v. Assuming this value for the water content in biogas, and also assuming that the biogas that was obtained is a mixture of CH_4, N_2, O_2, H_2, H_2O and CO_2 (H_2S was approximately zero since the system was blacked out), the content of nitrogen in biogas (y_n) for the whole duration of the digestion process can be computed by the following equation:

$$y_n = 1 - 0.01 - y_o - y_{HO} - y_c - y_m = 0.99 - y_o - y_{H0} - y_c - y_m \quad \text{(J4)}$$

where: y_o is the molar fraction of oxygen, *v/v*;

y_{H0} is the molar fraction of hydrogen, *v/v*;

y_c is the molar fraction of carbon dioxide, *v/v*; and

y_m is the molar fraction of methane, *v/v*.

On day 17, glycerol had methane percentage of 76%, carbon dioxide percentage of 22%, hydrogen percentage of 0.48% and oxygen percentage of 0.02%. According to Equation J4, the content of nitrogen in biogas for glycerol is:

$y_n = 0.99 - y_o - y_{HO} - y_c - y_m$

$= 0.99 - 0.0002 - 0.0048 - 0.22 - 0.76$

$= 0.0050 = 0.5\%$

Nitrogen to oxygen ratio

The nitrogen to oxygen ratio for glycerol on day 17 is therefore:

$$nitrogen\ to\ oxygen\ ratio = \frac{0.5}{0.02} = 25$$

APPENDIX K – Theoretical methane to carbon dioxide ratio and biogas yield for glycerol

K1 – Theoretical methane to carbon dioxide ratio

The Buswell and Neave (1930) equation may be used for the determination of the mol or volume ratio (i.e. yield) of methane to carbon dioxide that is generated by the anaerobic digestion process. The ratio is obtained by assuming that the organic substance used as feed undergoes complete conversion. Equation K1 below shows the Buswell and Neave (1930) equation:

$$C_iH_jO_k + uH_2O \rightarrow vCH_4 + wCO_2 \quad \text{(K1)}$$

where: u, v and w are stoichiometric coefficients of water, methane and carbon dioxide, respectively, and

i, *j* and *k* are the number of moles of carbon (C), oxygen (O) and hydrogen (H) in $C_iH_jO_k$, respectively.

In the reaction described above, water (H_2O) acts as an oxidizing agent whereas C is reduced or oxidized to methane and carbon dioxide, respectively. Equation K1 does not take into consideration C remaining in recalcitrant organic substance or utilized in the production of cell matter. The equation also neglects the hydrogen (H_2) produced during anaerobic process.

The stoichiometric coefficients of water, carbon dioxide and methane obtained from elemental balances are given by Equations K2, K3 and K4, respectively:

$$u = i - \frac{j}{4} - \frac{k}{2} \quad \text{(K2)}$$

$$v = \frac{i}{2} - \frac{j}{8} - \frac{k}{4} \quad \text{(K3)}$$

$$w = \frac{i}{2} - \frac{j}{8} - \frac{k}{4} \quad \text{(K4)}$$

The Buswell equation (Equation K1) may then be expressed by the following equation:

$$C_iH_jO_k + \left(i - \frac{j}{4} - \frac{k}{2}\right)H_2O \rightarrow \left(\frac{i}{2} - \frac{j}{8} - \frac{k}{4}\right)CH_4 + \left(\frac{i}{2} - \frac{j}{8} - \frac{k}{4}\right)CO_2 \quad \text{(K5)}$$

The molecular formula for glycerol is $C_3H_8O_3$. The stoichiometric coefficients *i*, *j* and *k* for glycerol are 3, 8 and 3, respectively. Substituting these stoichiometric coefficients in Equation K5 means that the stoichiometric equation for conversion of glycerol ($C_3H_8O_3$), which is completely biodegradable, is:

$$C_3H_8O_3 + (-0.5)H_2O \rightarrow 1.75CH_4 + 1.25CO_2 \quad \text{(K6)}$$

The theoretical methane to carbon dioxide ratio for glycerol may be obtained by Equation K6, and is:

$$\text{Methane to carbon dioxide ratio} = \frac{1.75 \text{ mol } CH_4}{1.25 \text{ mol } CO_2} = 1.4$$

K2 – Theoretical biogas and methane yields for glycerol

The Buswell and Neave equation can be used to determine the theoretical yields for biogas and CH_4 from glycerol assuming ideal gas behaviour at standard temperature and pressure (STP) conditions (273.15 K and 1 atm, respectively). The theoretical volumetric yield of methane (V_{yield}) is:

$$V_{yield}(\text{L CH}_4/\text{ mol glycerol fed}) = \frac{V_{CH_4}}{n_{glycerol}} = \frac{nRT/_P}{n_{glycerol}} \quad \text{(K7)}$$

where: V_{CH_4} is the volume of methane produced,

$n_{glycerol}$ is the number of moles of glycerol fed,

R is the universal gas constant, 0.08206 L (atm.K)$^{-1}$,

T is the process temperature, K, and

P is the process pressure, atm.

The number of moles of methane generated from Equation K6 is 1.75 kmol. The process temperature, process pressure and universal gas constant are 37.5℃, 1 atm and 0.08206 L (atm.K)$^{-1}$, respectively. Therefore, Equation K7 becomes:

$$V_{yield} = \frac{[1.75 \times 0.082062 \times (37.5 + 273.15)]/_1}{1}$$

$$= 39.23 \text{ L CH}_4/\text{mol glycerol}$$

Let assume that VS weighs as glycerol i.e. 1 g VS = 1 g glycerol. The molecular mass of glycerol is 92 g/mol. Therefore, the theoretical volumetric yield of methane (V_{yield}) becomes:

$$V_{yield}(\text{L CH}_4/\text{ g VS fed}) = 39.23 \frac{\text{L CH}_4}{\text{mol glycerol}} \times \frac{\text{mol glycerol}}{92 \text{ g glycerol}} \times \frac{1 \text{ g glycerol}}{1 \text{ g VS}}$$

$$= 0.426 \text{ L CH}_4/\text{ g VS fed}$$

The theoretical yield can also be determined on COD basis. During COD analysis, organic matter consisting of only C, H, and O is fully oxidized to CO_2 and H_2O. We will assume that the organic matter does not contain N and S that may be oxidized to NH_3 or HNO_3 and H_2SO_4, respectively. The stoichiometric equation for complete oxidation of glycerol is given by:

$$2C_3H_8O_3 + 7O_2 \rightarrow 6CO_2 + 8H_2O \quad \text{(K8)}$$

The molecular masses for glycerol ($C_3H_8O_3$) and oxygen (O_2) are 92 g /mol and 32 g /mol, respectively. Using Equation K8, the theoretical COD yield for glycerol (V_{yield}) is:

$$V_{yield}(\text{g COD/ g VS fed}) = \frac{7 \text{ mol } \times 32 \frac{\text{g}}{\text{mol}} \text{COD}}{2 \text{ mol } \times 92 \frac{\text{g}}{\text{mol}} \text{glycerol}}$$

$$= 1.22 \text{ g COD/ g glycerol}$$

$$= 1.22 \text{ g COD/ g VS fed}$$

The theoretical methane yield of glycerol on COD basis is then:

$$V_{yield}(\text{L } CH_4\text{/ g COD fed}) = 0.426 \frac{\text{L } CH_4}{\text{g VS fed}} \times \frac{\text{g VS fed}}{1.22 \text{ g COD}}$$

$$= 0.350 \text{ L } CH_4\text{/ g COD fed}$$

The methane content in biogas (y_m) is approximately 60% (PBW, 2016). Therefore, the theoretical biogas yield of glycerol ($V_{biogas\ yield}$) is:

$$V_{biogas\ yield} = \frac{V_{yield}}{y_m} \quad \text{(K9)}$$

$$= 0.350 \text{ L} \frac{CH_4}{\text{g COD}} \times \frac{\text{biogas}}{0.60 \text{ } CH_4}$$

$$= 0.583 \text{ L biogas / g COD fed}$$

$$= 0.583 \frac{\text{L biogas}}{\text{g COD}} \times 1.22 \text{ g} \frac{\text{COD}}{\text{g VS}}$$

$$= 0.712 \frac{\text{L biogas}}{\text{g VS}}$$

Let us assume that the glycerol sample was a mixture of methanol, lipids and pure glycerol with mass fractions of *m*, *l* and *g*, respectively. The density that was obtained from the glycerol sample form the experiment was 1080 g/L. Pure glycerol has a density of 1260 g/L. Let us assume that the lipids were composed of triglycerides with a density of 915 g/L. The

content of methanol (m) was assumed to be 0.25, with the density of 791 g/L. The density of dissolved catalyst was assumed to be 82 g/L. The sum of the compositions is 1, and thus the following equation holds:

$$l + g = 1 - 0.25 = 0.75 \quad \text{(K10)}$$

The product of density and mass fraction equation yield:

$$915l + 1260g + (791 * 0.25) = 1080 - 82 \quad \text{(K11)}$$

Solving Equation K10 and Equation K11 simultaneously implies that:

$$g = 0.33$$

$$l = 0.42$$

Using the Buswell and Neave (1930) equation (Equation K5), the theoretical methane yields for lipids - $C_{57}H_{104}O_6$ (V_{ML}) and methanol (V_{MM}) are 1.014 and 0.525 L/g VS, respectively. The reactor was at 37.5 °C. Let us assume that P = 1 atm. Using Equation K6, the theoretical methane yield for pure glycerol (V_{MP}) is 0.426 L/g VS. The theoretical methane yield for the glycerol only sample (V_{MG}) may be given by the following equation:

$$V_{MG} = (V_{ML} \times l) + (V_{MP} \times g) + (V_{MM} \times m) \quad \text{(K12)}$$

$$= (1.014 \times 0.42) + (0.426 \times 0.33) + (0.525 \times 0.25)$$

$$= 0.70 \text{ L/gVS}$$

The theoretical biogas yield for glycerol (V_{BG}) is:

$$V_{BG} = \frac{V_{MG}}{y_m} \quad \text{(K13)}$$

$$= \frac{0.70 \frac{\text{L}}{\text{g}} \text{VS}}{0.60}$$

$$= 1.16 \text{ L/gVS}$$

APPENDIX L – Sample of calculations for biogas and methane yields

This sample of calculations is based on the glycerol biogas yield on Figure 4.17. Glycerol had 15.5 g VS (see Table 4.2), and generated a biogas accumulation of 14 415 mL (see Figure 4.6). On VS basis, the biogas yield is obtained by Equation L6:

$$\text{biogas yield (L/ g VS fed)} = \frac{\text{biogas accumulaton/1000}}{\text{g VS fed}} \quad \text{(L6)}$$

$$= \frac{14\,415/1000}{15.5\ \text{g VS fed}}$$

$$= 0.93\ \text{L/ g VS fed}$$

Glycerol had 23 501 mg COD (see Table 4.2). On COD basis, the biogas yield is obtained by Equation L7:

$$\text{biogas yield (L/ g COD fed)} = \frac{\text{biogas accumulaton}}{\text{mg COD fed}} \quad \text{(L7)}$$

$$= \frac{14\,415}{23\,501\ \text{mg COD fed}}$$

$$= 0.614\ \text{L/ g COD fed}$$

The calculation of methane yield is similar to that of biogas yield, except that in Equation L6 and Equation L7 methane values are used instead of biogas values.

APPENDIX M – DOE sample of calculations for biogas yield

This is the sample of calculations for the DOE section for biogas yield only. The calculation for methane is the same.

M1 – Transformation

This sample of calculations is based on Figure 4.48, which is the transformation figure for biogas yield. The response values for biogas yield ranges from 0.10 to 0.94 L / g VS. The maximum response ($\hat{y}_{max}$) is 0.94 L / g VS, whereas the minimum response ($\hat{y}_{min}$) is 0.10 L / g VS.

The ratio of maximum to minimum response (T_R) is obtained by the following equation:

$$T_R = \frac{\hat{y}_{max}}{\hat{y}_{min}}$$

$$= \frac{0.94}{0.10}$$

$$= 9.4$$

M2 – The ANOVA table

This sample of calculation is based on Table 4.12, which is the ANOVA table for biogas yields. The total sum of squares (SOS_T) is given by the following equation:

$$SOS_T = \sum_{i=1}^{N_e} y_i^2 - \frac{\left[\sum_{i=1}^{N_e} y_i\right]^2}{N_e} = y'y - \frac{\left[\sum_{i=1}^{N_e} y_i\right]^2}{N_e}$$

$$= 0.91$$

The error or residual sum of squares is given by the following equation:

$$SOS_E = y'y - \frac{\left[\sum_{i=1}^{N_e} y_i\right]^2}{N_e} - \left\{\hat{\beta}'X'y - \frac{\left[\sum_{i=1}^{N_e} y_i\right]^2}{N_e}\right\} = y'y - \hat{\beta}'X'y = \sum_{i=1}^{N_e}[y_i - \hat{y}_i]^2$$

$= 0.041$

The following equation is used to calculate the regression or model sum of squares (SOS_R):

$SOS_R = SOS_T - SOS_E$

$= 0.91 - 0.041$

$= 0.87$

The lack-of-fit sum of squares (SOS_{LOF}) may be obtained by the following equation:

$SOS_{LOF} = SOS_E - SOS_{PE}$

$= 0.041 - 0$

$= 0.041$

The number of variables is $n = 3$. The number of distinct points is $n_d = 7$. The number of centre points is $C_p = 3$. The number of factorial points (n_f) for a Box-Behnken method is given by the following equation:

$n_f = 2n(n-1)$

$= 2 \times 3 \times (3-1)$

$= 12$

The number of experimental points or observations (N_e) is given by:

$N_e = n_f - C_p$

$= 12 - 3$

$= 9$

The degree of freedom for regression (df_R) is obtained by the following equation:

$$df_R = N_e = 9$$

The degree of freedom for the lack-of-fit is given by the following equation:

$$df_{LOF} = n_d - n - 1$$

$$= 7 - 3 - 1$$

$$= 3$$

The degree of freedom for the pure error is given by the following equation:

$$df_{PE} = N_e - n_d$$

$$= 9 - 7$$

$$= 2$$

The degree of freedom for residual is given by the following equation:

$$df_E = df_{LOF} + df_{PE}$$

$$= 3 + 2$$

$$= 5$$

The degree of freedom for cor. total (df_{CT}) is given by:

$$df_{CT} = df_R + df_E$$

$$= 9 + 5$$

$$= 14$$

The mean square for regression (MS_R) given by:

$$MS_R = \frac{SOS_R}{df_R}$$

$$= \frac{0.87}{9}$$

$$= 0.97$$

The mean square for error (MS_E) given by:

$$MS_E = \frac{SOS_E}{df_E}$$

$$= \frac{0.041}{5}$$

$$= 8.3 \times 10^{-3}$$

The lack of fit mean square is given by:

$$MS_{LOF} = \frac{SOS_{LOF}}{df_{LOF}}$$

$$= \frac{0.041}{3}$$

$$= 0.014$$

The mean squares for the pure error SOS_{PE} is given by the following equation:

$$MS_{PE} = \frac{SOS_{PE}}{d_{PE}}$$

$$= \frac{0.000}{2}$$

$$= 0$$

The sample of calculations for the f-value and p-value were done for the model row. The F-value for regression (F_R) is given by:

$$F_R = \frac{MS_R}{MS_R}$$

$$= \frac{0.097}{8.3 \times 10^{-3}}$$

$= 11.72$

The DOE was measured at 95% confidence interval, thus $1 - \alpha = 1 - 0.95 = 0.05 = 0.5\%$. Therefore, the F-distribution $F_{(df_R, df_{PE}.1-\alpha)} = F_{(9,2.0.5\%)}$. From Table B1, the $F_{(9,2.0.5\%)}$ is 199.29. The p-value may then be obtained by the following equation:

$$p\ (value) = \frac{1}{F_{(df_R, df_{PE}.1-\alpha)}}$$

$$= \frac{1}{199.29}$$

$$= 0.0055$$

M3 – The collection of summary statistics table

This sample of calculations is based on Table 4.14, which is the collection of summary table for biogas yields. The residual mean square (MS_E) was 8.3×10^{-3}. The standard deviation (SD) is obtained by the following equation:

$$SD = \sqrt{MS_E}$$

$$= \sqrt{8.3 \times 10^{-3}}$$

$$= 0.09$$

The mean (MN) is:

$$MN = \frac{\sum_{i=1}^{N_e} y_i}{N_e}$$

$$= \frac{0.5+0.55+0.51+0.56+0.74+0.94+0.10+0.20+0.44+0.65+0.10+0.11+0.65+0.65+0.65}{15} = \frac{7.35}{15} = 0.49$$

The coefficient of variation (C.V. %) is obtained by the following equation:

$$C.V.\% = \frac{SD}{MN} \times 100\%$$

$$= \frac{0.09}{0.49} \times 100\%$$

$$= 18.54\%$$

The PRESS value is obtained by:

$$PRESS = \sum_{i=1}^{n} [y_i - \hat{\hat{y}}_i]^2 = \sum_{i=1}^{n} \left[\frac{e_i}{1 - h_{ii}}\right]^2$$

$$= 0.66$$

The R-squared value is obtained by:

$$R^2 = \frac{SOS_R}{SOS_T}$$

$$= 1 - \frac{SOS_E}{SOS_T}$$

$$= 1 - \frac{0.041}{0.91}$$

$$= 0.96$$

The *adjusted-R^2* term is given by:

$$adjusted\ R^2 = 1 - \frac{SOS_E}{(N_e - n - 1)} \div \frac{SOS_T}{(N_e - 1)}$$

$$= 1 - \left({}^{0.041}/_{5}\right) \div \left({}^{0.91}/_{14}\right)$$

$$= 0.87$$

The model and residual sum of squares SOS_{model} and SOS_E were 0.87 and 0.041, respectively. The PRESS value was 0.66. Therefore, the *predicted-R^2* term is:

$$predicted\ R^2 = 1 - \frac{PRESS}{(SOS_{model} + SOS_E)}$$

$$= 1 - \frac{0.66}{(0.87 + 0.041)}$$

$$= 0.28$$

There are nine (9) coefficients in the model, therefore $p = 9$. The maximum and minimum response values were 0.94 ($\hat{y}_{max}$) and 0.10 ($\hat{y}_{min}$), respectively. The adequate precision (AP) ratio is:

$$AP = \frac{\hat{y}_{max} - \hat{y}_{min}}{\sqrt{\left({pMS_E}/{N_e}\right)}}$$

$$= \frac{0.94 - 0.10}{\sqrt{\left(9 \times {8.3E-03}/{15}\right)}}$$

$$= 11.19$$

The AP ratio was above 4.0 implying satisfactory discrimination.

M4 – The coefficient estimate table

This sample of calculations is based on Table 4.16, which is the coefficient estimate table for biogas yields. Since the chosen model is a quadratic model (second-order model), the model equation describing the biogas yield is obtained by using Equation 2.33. There are three process variables for this model, thus Equation 2.33 becomes (quadratic equation):

$$y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \varepsilon \quad \text{(M1)}$$

where: X_1 is the temperature variable (A),

X_2 is the substrate to inoculum ratio variable (B),

X_3 is the volume of glycerol in the digester variable (C),

β_0 is the coefficient estimate for the intercept,

β_1 is the coefficient estimate for temperature (A),

β_2 is the coefficient estimate for the substrate to inoculum ratio (B),

β_3 is the coefficient estimate for the volume of glycerol in the digester (C),

β_{11} is the coefficient estimate for A^2,

β_{22} is the coefficient estimate for B^2,

β_{33} is the coefficient estimate for C^2,

β_{12} is the coefficient estimate for AB,

β_{13} is the coefficient estimate for AC, and

β_{23} is the coefficient estimate for BC.

The residual sum of squares SOS_E was obtained by ($N_e = 15$):

$$SOS_E = \sum_{i=1}^{N_e} [y_i - \hat{y}_i]^2$$

$$= \sum_{i=1}^{15} (y_l - b_0 - b_1X_1 - b_2X_2 - b_3X_3 - b_{11}X_1^2 - b_{22}X_2^2 - b_{33}X_3^2 - b_{12}X_1X_2 - b_{13}X_1X_3 - b_{23}X_2X_3) \quad \text{(M2)}$$

Taking each factor (variable) partial derivative SOS_E/b_i, and equating its result to zero, implies that:

$$N_e b_0 + b_1 \sum_{i=1}^{15} X_1 + b_2 \sum_{i=1}^{15} X_2 + b_3 \sum_{i=1}^{15} X_3 + b_{11} \sum_{i=1}^{15} X_1^2 + b_{22} \sum_{i=1}^{15} X_2^2 + b_{33} \sum_{i=1}^{15} X_3^2 + b_{12} \sum_{i=1}^{15} X_1X_2 + b_{13} \sum_{i=1}^{15} X_1X_3 + b_{23} \sum_{i=1}^{15} X_2X_3 = \sum_{l=1}^{15} y_i \quad \text{(M3)}$$

$$b_0 \sum_{i=1}^{15} X_1 + b_1 \sum_{i=1}^{15} X_1^2 + b_2 \sum_{i=1}^{15} X_1 X_2 + b_3 \sum_{i=1}^{15} X_1 X_3 + b_{11} \sum_{i=1}^{15} X_1^3 + b_{22} \sum_{i=1}^{15} X_1 X_2^2 + b_{33} \sum_{i=1}^{15} X_1 X_3^2 + b_{12} \sum_{i=1}^{15} X_1^2 X_2 + b_{13} \sum_{i=1}^{15} X_1^2 X_3 + b_{23} \sum_{i=1}^{15} X_1 X_2 X_3 = \sum_{l=1}^{15} X_1 y_i \quad \text{(M4)}$$

$$b_0 \sum_{i=1}^{15} X_2 + b_1 \sum_{i=1}^{15} X_1 X_2 + b_2 \sum_{i=1}^{15} X_2^2 + b_3 \sum_{i=1}^{15} X_2 X_3 + b_{11} \sum_{i=1}^{15} X_2 X_1^2 + b_{22} \sum_{i=1}^{15} X_2^3 + b_{33} \sum_{i=1}^{15} X_2 X_3^2 + b_{12} \sum_{i=1}^{15} X_1 X_2^2 + b_{13} \sum_{i=1}^{15} X_1 X_2 X_3 + b_{23} \sum_{i=1}^{15} X_2^2 X_3 = \sum_{l=1}^{15} X_2 y_i \quad \text{(M5)}$$

$$b_0 \sum_{i=1}^{15} X_3 + b_1 \sum_{i=1}^{15} X_1 X_3 + b_2 \sum_{i=1}^{15} X_2 X_3 + b_3 \sum_{i=1}^{15} X_3^2 + b_{11} \sum_{i=1}^{15} X_1^2 X_3 + b_{22} \sum_{i=1}^{15} X_2^2 X_3 + b_{33} \sum_{i=1}^{15} X_3^3 + b_{12} \sum_{i=1}^{15} X_1 X_2 X_3 + b_{13} \sum_{i=1}^{15} X_1 X_3^2 + b_{23} \sum_{i=1}^{15} X_2 X_3^2 = \sum_{l=1}^{15} X_3 y_i \quad \text{(M6)}$$

$$b_0 \sum_{i=1}^{15} X_1 X_3 + b_1 \sum_{i=1}^{15} X_1^2 X_3 + b_2 \sum_{i=1}^{15} X_1 X_2 X_3 + b_3 \sum_{i=1}^{15} X_1 X_3^2 + b_{11} \sum_{i=1}^{15} X_1^3 X_3 + b_{22} \sum_{i=1}^{15} X_1 X_2^2 X_3 + b_{33} \sum_{i=1}^{15} X_1 X_3^3 + b_{12} \sum_{i=1}^{15} X_1^2 X_2 X_3 + b_{13} \sum_{i=1}^{15} X_1^2 X_3^2 + b_{23} \sum_{i=1}^{15} X_1 X_2 X_3^2 = \sum_{l=1}^{15} X_1 X_3 y_i \quad \text{(M7)}$$

$$b_0 \sum_{i=1}^{15} X_2 X_3 + b_1 \sum_{i=1}^{15} X_1 X_2 X_3 + b_2 \sum_{i=1}^{15} X_2^2 X_3 + b_3 \sum_{i=1}^{15} X_2 X_3^2 + b_{11} \sum_{i=1}^{15} X_1^2 X_2 X_3 + b_{22} \sum_{i=1}^{15} X_2^3 X_3 + b_{33} \sum_{i=1}^{15} X_2 X_3^3 + b_{12} \sum_{i=1}^{15} X_1 X_2^2 X_3 + b_{13} \sum_{i=1}^{15} X_1 X_2 X_3^2 + b_{23} \sum_{i=1}^{15} X_2^2 X_3^2 = \sum_{l=1}^{15} X_2 X_3 y_i \quad \text{(M8)}$$

$$b_0 \sum_{i=1}^{15} X_1 X_2 + b_1 \sum_{i=1}^{15} X_1^2 X_2 + b_2 \sum_{i=1}^{15} X_1 X_2^2 + b_3 \sum_{i=1}^{15} X_1 X_2 X_3 + b_{11} \sum_{i=1}^{15} X_1^3 X_2 + b_{22} \sum_{i=1}^{15} X_1 X_2^3 + b_{33} \sum_{i=1}^{15} X_1 X_2 X_3^2 + b_{12} \sum_{i=1}^{15} X_1^2 X_2^2 + b_{13} \sum_{i=1}^{15} X_1^2 X_2 X_3 + b_{23} \sum_{i=1}^{15} X_1 X_2^2 X_3 = \sum_{l=1}^{15} X_1 X_2 y_i \quad \text{(M9)}$$

$$b_0 \sum_{i=1}^{15} X_1 X_3 + b_1 \sum_{i=1}^{15} X_1^2 X_3 + b_2 \sum_{i=1}^{15} X_1 X_2 X_3 + b_3 \sum_{i=1}^{15} X_1 X_3^2 + b_{11} \sum_{i=1}^{15} X_3 X_1^3 + b_{22} \sum_{i=1}^{15} X_1 X_3 X_2^2 + b_{33} \sum_{i=1}^{15} X_1 X_3^3 + b_{12} \sum_{i=1}^{15} X_1^2 X_2 X_3 + b_{13} \sum_{i=1}^{15} X_1^2 X_3^2 + b_{23} \sum_{i=1}^{15} X_1 X_2 X_3^2 = \sum_{l=1}^{15} X_1 X_3 y_i \quad \text{(M10)}$$

$$b_0 \sum_{i=1}^{15} X_1^2 + b_1 \sum_{i=1}^{15} X_1^3 + b_2 \sum_{i=1}^{15} X_1^2 X_2 + b_3 \sum_{i=1}^{15} X_1^2 X_3 + b_{11} \sum_{i=1}^{15} X_1^4 + b_{22} \sum_{i=1}^{15} X_1^2 X_2^2 + b_{33} \sum_{i=1}^{15} X_1^2 X_3^2 + b_{12} \sum_{i=1}^{15} X_1^3 X_2 + b_{13} \sum_{i=1}^{15} X_1^3 X_3 + b_{23} \sum_{i=1}^{15} X_1^2 X_2 X_3 = \sum_{l=1}^{15} X_1^2 y_i \quad \text{(M11)}$$

$$b_0 \sum_{i=1}^{15} X_1^2 X_2 + b_1 \sum_{i=1}^{15} X_1^3 X_2 + b_2 \sum_{i=1}^{15} X_1^2 X_2^2 + b_3 \sum_{i=1}^{15} X_1^2 X_3 X_2 + b_{11} \sum_{i=1}^{15} X_1^4 X_2 + b_{22} \sum_{i=1}^{15} X_1^2 X_2^3 + b_{33} \sum_{i=1}^{15} X_1^2 X_2 X_3^2 + b_{12} \sum_{i=1}^{15} X_1^3 X_2^2 + b_{13} \sum_{i=1}^{15} X_1^3 X_3 X_2 + b_{23} \sum_{i=1}^{15} X_1^2 X_2^2 X_3 = \sum_{l=1}^{15} X_1^2 X_2 y_i \quad \text{(M12)}$$

The sums of the values in Equation M3 to M12 are calculated from Table P1. For example, the sum of X_1 (temperature) is:

$$\sum_{i=1}^{15} X_1 = 25 + 50 + 25 + 50 + 25 + 50 + 25 + 50 + 37.5 + 37.5 + 37.5 + 37.5 + 37.5 + 37.5 + 37.5 = 562.5$$

The sum of values for other combinations is calculated by the same procedure. Substituting all the sums, and solving the Equations M3 to M12 simultaneously, means that the regression coefficients or coefficient estimates are as follows:

$\beta_0 = 0.650,$

$\beta_1 = 0.050,$

$\beta_2 = 0.030,$

$\beta_3 = -0.280,$

$\beta_{11} = 0.025,$

$\beta_{22} = -0.150,$

$\beta_{33} = -0.180,$

$\beta_{12} = 1.405 \times 10^{-17},$

$\beta_{13} = -0.025,$ and

$\beta_{23} = -0.050.$

For all coefficients in the coefficient estimate table, the degree of freedom is 1.0.

The sample of calculations for the standard error and 95% CI values was based on the intercept term. The standard error term for the intercept (σ^2) is by:

$$\sigma^2 = MS_E \times (X'X)^{-1}$$

$$= 0.097 \times (X'X)^{-1}$$

$$= 0.052$$

The 95% CI high was obtained by:

$$95\%\ CI\ high = \beta_i + 1.96\left(\sqrt{\frac{\sigma^2}{N_e}}\right)$$

$$= 0.650 + 1.96 \times \left(\sqrt{\frac{0.052}{15}}\right)$$

$$= 0.780$$

The 95% CI low was obtained by:

$$95\%\ CI\ low = \beta_i - 1.96\left(\sqrt{\frac{\sigma^2}{N_e}}\right)$$

$$= 0.650 - 1.96\left(\sqrt{\frac{0.052}{15}}\right)$$

$$= 0.520$$

Since each factor in the chosen model is independent to other factors, the VIF value is 1.0 all coefficients.

M5 – The model equation

Model equation in terms of coded factors

The biogas yield for glycerol was defined by Equation M1 in Appendix M4. Substituting the coefficient estimates that were obtained above to Equation M1, and neglecting the error term, implies that the biogas yield model in terms of coded factors is:

$$y_i = 0.65 + 0.050X_1 + 0.030X_2 - 0.280X_3 + 0.025X_1^2 - 0.150X_2^2 - 0.180X_3^2 + 1.405 \times 10^{-17}X_1X_2 - 0.025X_1X_3 - 0.050X_2X_3$$

$$= 0.650 + 0.050A + 0.030B - 0.280C + 0.025A^2 - 0.150B^2 - 0.180C^2 + 1.405 \times 10^{-17}AB - 0.025AC - 0.050BC$$

Model equation in terms of actual factors

Temperature ranges from 25 to 50℃ , with a change of 25℃ and an average value of 37.5℃. The coded value (C_v) for the temperature term (A) is 0.050. The actual temperature term (A) is therefore:

$$Actual\ A\ term = C_v \times \left[\frac{2 \times (A - 37.5)}{\Delta x_p}\right]$$

$$= 0.050 \times \left[\frac{2 \times (A - 37.5)}{25}\right]$$

$$= 0.050 \times \frac{2}{25} \times [A - 37.5]$$

$$= 4 \times 10^{-3}A - 0.15$$

Substrate to inoculum ratio ranges from 0.5 to 1.5 g VS/ g VS , with a change of 1.0 g VS/ g VS and an average value of 1.0 g VS/ g VS. The coded value (C_v) for the substrate to inoculum ratio term (B) is 0.030. The actual substrate to inoculum ratio term (B) is therefore:

$$Actual\ B\ term = C_v \times \left[\frac{2 \times (B - 1.0)}{\Delta x_p}\right]$$

$$= 0.030 \times \left[\frac{2 \times (B - 1.0)}{1}\right]$$

$$= 0.030 \times \frac{2}{1} \times [B - 1.0]$$

$$= 0.06B - 0.06$$

Secondary substrate volume ranges from 10 to 34 ml, with a change of 24 ml and an average value of 22 ml. The coded value for the secondary substrate volume term (C) is - 0.280. The actual secondary substrate volume term (C) is therefore:

$$Actual\ C\ term = C_v \times \left[\frac{2 \times (C - 22)}{\Delta x_p}\right]$$

$$= -0.280 \times \left[\frac{2 \times (C - 22)}{24}\right]$$

$$= -0.280 \times \frac{2}{24} \times [C - 22]$$

$$= -0.023333C + 0.513333$$

The coded value for the A^2 term is 0.025. The actual A^2 term is therefore:

$$Actual\ A^2\ term = 0.025 \times \left\{\left[\frac{2 \times (A - 37.5)}{25}\right] \times \left[\frac{2 \times (A - 37.5)}{25}\right]\right\}$$

$$= 0.025 \times \frac{2 \times 2}{25 \times 25} \times \{[A - 37.5] \times [A - 37.5]\}$$

$$= 1.6 \times 10^{-4} \times \{A^2 - 75A + 1406.25\}$$

$$= 1.6 \times 10^{-4}A^2 - 1.2 \times 10^{-2}A + 0.23$$

The coded value for the B^2 term is - 0.150. The actual B^2 term is therefore:

$$Actual\ B^2 term = -0.150 \times \left\{\left[\frac{2 \times (B - 1.0)}{1}\right] \times \left[\frac{2 \times (B - 1.0)}{1}\right]\right\}$$

$$= -0.150 \times \frac{2 \times 2}{1} \times \{[B - 1.0] \times [B - 1.0]\}$$

$$= -0.6 \times \{B^2 - 2B + 1\}$$

$$= -0.6B^2 + 1.2B - 0.6$$

The coded value for the C^2 term is - 0.180. The actual C^2 term is therefore:

$$Actual\ C^2term = -0.180 \times \left\{\left[\frac{2 \times (C - 22)}{24}\right] \times \left[\frac{2 \times (C - 22)}{24}\right]\right\}$$

$$= -0.180 \times \frac{2 \times 2}{24 \times 24} \times \{[C - 22] \times [C - 22]\}$$

$$= -1.25 \times 10^{-3} \times \{C^2 - 44C + 484\}$$

$$= -1.25 \times 10^{-3}C^2 + 0.055C - 0.61$$

The coded value for the AB term is $1.405E - 017$. The actual AB term is therefore:

$$Actual\ AB\ term = 1.405E - 017 \times \left\{\left[\frac{2 \times (A - 37.5)}{25}\right] \times \left[\frac{2 \times (B - 1.0)}{1}\right]\right\}$$

$$= 1.405E - 017 \times \frac{2 \times 2}{25} \times \{[A - 37.5] \times [B - 1.0]\}$$

$$= 2.25 \times 10^{-18} \times \{AB - A - 37.5B + 37.5\}$$

$$= 2.25 \times 10^{-18}AB - 2.25 \times 10^{-18}A - 8.43 \times 10^{-17}B + 8.43 \times 10^{-17}$$

The coded value for the AC term is -0.025. The actual AC term is therefore:

$$Actual\ AC\ term = -0.025 \times \left\{\left[\frac{2 \times (A - 37.5)}{25}\right] \times \left[\frac{2 \times (C - 22)}{24}\right]\right\}$$

$$= -0.025 \times \frac{2 \times 2}{25 \times 24} \times \{[A - 37.5] \times [C - 22]\}$$

$$= -1.67 \times 10^{-4} \times \{AC - 22A - 37.5C + 825\}$$

$$= -1.67 \times 10^{-4}AC + 3.67 \times 10^{-3}A + 6.26 \times 10^{-3}C - 0.14$$

The coded value for the BC term is -0.050. The actual BC term is therefore:

$$Actual\ BC\ term = -0.050 \times \left\{\left[\frac{2 \times (B - 1.0)}{1}\right] \times \left[\frac{2 \times (C - 22)}{24}\right]\right\}$$

$$= -0.050 \times \frac{2 \times 2}{24} \times \{[B - 1.0] \times [C - 22]\}$$

$$= -8.33 \times 10^{-3} \times \{BC - 22B - C + 22\}$$

$$= -8.33 \times 10^{-3}BC + 0.18B + 8.33 \times 10^{-3}C - 0.18$$

The biogas yield model in terms of actual factors is therefore:

$$\begin{aligned} y_i = {} & 4 \times 10^{-3}A - 0.15 + 0.06B - 0.06 - 0.023333C + 0.513333 + 1.6 \times 10^{-4}A^2 - 1.2 \\ & \times 10^{-2}A + 0.23 - 0.6B^2 + 1.2B - 0.6 - 1.25 \times 10^{-3}C^2 + 0.055C - 0.61 \\ & + 2.25 \times 10^{-18}AB - 2.25 \times 10^{-18}A - 8.43 \times 10^{-17}B + 8.43 \times 10^{-17} \\ & - 1.67 \times 10^{-4}AC + 3.67 \times 10^{-3}A + 6.26 \times 10^{-3}C - 0.14 - 8.33 \\ & \times 10^{-3}BC + 0.18B + 8.33 \times 10^{-3}C - 0.18 \end{aligned}$$

$$\begin{aligned} = {} & -0.9967 - 4.33333 \times 10^{-3} \times A + 1.40333 \times B + 0.046257 \times C + 2.25E \times 10^{-18} \\ & \times AB - 1.66667 \times 10^{-4} \times AC - 8.33333 \times 10^{-3} \times BC + 1.6 \times 10^{-4} \times A^2 \\ & - 0.6 \times B^2 - 1.25 \times 10^{-3} \times C^2 \end{aligned}$$

The coded value for the constant term is 0.65, where else the actual value for the constant term from the above equation is -0.9967. The biogas yield equation in terms of actual factors therefore becomes:

$$\begin{aligned} y_i = {} & (0.5 - 0.9967) - 4.33333 \times 10^{-3} \times A + 1.40333 \times B + 0.046257 \times C + 2.25 \\ & \times 10^{-18} \times AB - 1.66667 \times 10^{-4} \times AC - 8.33333 \times 10^{-3} \times BC + 1.6 \\ & \times 10^{-4} \times A^2 - 0.6 \times B^2 - 1.25 \times 10^{-3} \times C^2 \end{aligned}$$

$$\begin{aligned} = {} & -0.3467 - 4.33333 \times 10^{-3} \times A + 1.40333 \times B + 0.046257 \times C + 2.25 \times 10^{-18} \times AB \\ & - 1.66667 \times 10^{-4} \times AC - 8.33333 \times 10^{-3} \times BC + 1.6 \times 10^{-4} \times A^2 - 0.6 \\ & \times B^2 - 1.25 \times 10^{-3} \times C^2 \end{aligned}$$

$$= -0.3467 - 4.33333 \times 10^{-3} \times (temperature) + 1.40333 \times (substrate\ to\ inoculum\ ratio) + 0.046257 \times (glycerol\ volume) + 2.25 \times 10^{-18} \times (temperature) \times (substrate\ to\ inoculum\ ratio) - 1.66667 \times 10^{-4} \times (temperature) \times (glycerol\ volume) - 8.33333 \times 10^{-3} \times (substrate\ to\ inoculum\ ratio) \times (glycerol\ volume) + 1.6 \times 10^{-4} \times (temperature)^2 - 0.6 \times (substrate\ to\ inoculum\ ratio)^2 - 1.25 \times 10^{-3} \times (glycerol\ volume)^2$$

M6 – Residuals limits

Since the confidence interval was investigated at 95% CI, the alpha value was therefore $\propto = 100 - 95 = 5\%$. The tail value is therefore:

$$tail = \frac{\propto}{2N_e}$$

$$= \frac{\frac{5}{100}}{2 \times 15}$$

$$= 0.0017$$

The degree of freedom is therefore:

$$df = N_e - p - 1$$

$$= 15 - 9 - 1$$

$$= 5$$

From Table B2 (see Appendix B), the limits for the residuals (L_E) at $tail = 0.0017$ and $df = 5$ is:

$$L_E = \pm t_{(tail,df)} = \pm t_{(0.0017,5)}$$

$$= \pm 5.3$$

M7 – Cook's distance limits

The degree of freedom for the numerator (df_{num}) is:

$df_{num} = p = 9$

The degree of freedom for the denominator (df_{den}) is:

$df_{den} = N_e - p$

$= 15 - 9$

$= 3$

From Table B1 (refer to Appendix B), the F-value at 0.5%, $df_{num} = 9$ and $df_{den} = 3$ is 43.883. The minimum value is:

$L_{CD} = F^{-1} = \frac{1}{F}$

$= \frac{1}{43.88}$

$= 0.023$, which is approximately equal to zero

The Cook's minimum and maximum F-critical values are 0 and 1, respectively.

M8 – Leverage limits

The number of experimental replication for this experiment (R_p) is 1. The leverage limit for this experiment is therefore:

$L_L = \frac{1}{R_p}$

$= \frac{1}{1}$

$= 1$

The average leverage is therefore:

$Lev_{ave} = \frac{p}{N_e}$

$= \frac{9}{15}$

$= 0.6$

M9 – DFFITS limits

The range in this investigation is:

$L_{DFFITS} = \pm max\left(1{,}3 \times \sqrt{p, N_e}\right)$

$= \pm max\left(1{,}3 \times \sqrt{9{,}15}\right)$

$= \pm 2.449$

M10 – DBETAS limits

The DBETAS range in this investigation is:

$L_{DBETAS} = \pm \frac{3}{\sqrt{N_e}}$

$= \pm \frac{3}{\sqrt{15}}$

$= 0.7746$

APPENDIX N – Digestate and water displacement weights

N1 – Raw data

Table N1 shows the raw data for the digestate and water displacement.

Table N1: Weights for the digestate and water displacement (raw data).

Digester ID	digestate (g)		water in the displacement before (g)		water in the displacement after (g)	
	a	***b****	***a***	***b****	***a***	***b****
Glycerol	4868	4870	14407	14425	14419	14445
Fruit juice	4902	4890	667	685	676	678
Soft drink	4904	4888	421	401	407	416
IOP	4908	4882	775	765	774	768
S:I = 0.5	4902	4902	38619	38645	38682	38670
S:I = 1.0	4906	4910	40099	40091	40100	40180
S:I = 1.5	4918	4910	39765	39795	39949	39701
1 g glyc per L	4896	4920	40085	40105	40259	40023
5 g glyc per L	4910	4930	40555	40545	40660	40532
9 g glyc per L	4984	4992	13594	13606	13687	13545
25 deg C	4899	4925	36624	36636	36742	36602
37.5 deg C	4895	4921	40065	40125	40268	40012
50 deg C	4910	4886	46535	46525	46565	46601
1	4934	4950	25420	25380	25357	25501
2	4932	4942	27950	27930	28054	27890
3	4927	4935	37376	37390	37350	37502
4	4921	4925	41041	41055	41100	41090
5	4902	4920	39005	38991	39034	39050

(*) Duplicate values *(Continued)*

Table N1 (Continued).

Digester ID	digestate (g)		water in the displacement before (g)		water in the displacement after (g)	
	a	***b****	***a***	***b****	***a***	***b****
6	4899	4885	49574	49502	49688	49502
7	5013	4985	7140	7120	7136	7140
8	4983	4985	14261	14259	14272	14280
9	4984	4902	18330	18190	18273	18289
10	4919	4901	41610	41590	41614	41680
11	5488	4502	6019	6001	6014	6020
12	5004	5010	6622	6600	6585	6652
13	4905	4905	47634	47656	47699	47700
14	4900	4910	47640	47650	48386	47013
15	4902	4908	47635	47655	47699	47700

(*) Duplicate values

N2 – Average data

Table N2 shows the weights for the digestate and water displacement. The data was taken by taking the average values for the digestate and water displacement raw data (***a*** and ***b****) found in Appendix N1.

Table N2: Weights for the digestate and water displacement.

Digester ID	digestate (g)	water in the displacement before (g)	water in the displacement after (g)
Glycerol	4869	14416	14432
Fruit juice	4896	676	677
Soft drink	4896	411	412
IOP	4895	770	771
S:I = 0.5	4902	38632	38676
S:I = 1.0	4908	40095	40140
S:I = 1.5	4914	39780	39825
1 g glyc per L	4908	40095	40141
5 g glyc per L	4920	40550	40596
9 g glyc per L	4988	13600	13616
25 deg C	4912	36630	36672
37.5 deg C	4908	40095	40140
50 deg C	4898	46530	46583
1	4942	25400	25429
2	4937	27940	27972
3	4931	37383	37426
4	4923	41048	41095
5	4911	38998	39042
6	4892	49538	49595
7	4999	7130	7138
8	4984	14260	14276
9	4943	18260	18281
10	4910	41600	41647
11	4995	6010	6017
12	5007	6611	6619
13	4905	47645	47699
14	4905	47645	47699
15	4905	47645	47699

APPENDIX O – Material balance across a digester

The sample of calculations for glycerol will be used for the material balance. Density of water at 37.5℃ (ρ_w) is 1000 g/L. Volume of water that was added to the system (V_w) was 2.4 L. Mass of water fed into the system (m_w) is therefore:

$$m_w = \rho_w \times V_w \quad \text{(O1)}$$

$$= 1000 \frac{\text{g}}{\text{L}} \times 2.4\text{L}$$

$$= 2400 \text{ g}$$

Density of inoculum (ρ_I) is 1031.7 g/L. Volume of inoculum that was added to the system (V_I) was 2.4 L. Mass of inoculum fed into the system (m_I) is:

$$m_I = \rho_I \times V_I \quad \text{(O2)}$$

$$= 1031.7 \frac{\text{g}}{\text{L}} \times 2.4\text{L}$$

$$= 2476 \text{ g}$$

Density of glycerol (ρ_G) is 1080.1 g/L. Volume of glycerol that was added to the system (V_G) was 20 mL. Mass of co-substrate fed (glycerol) into the system (m_G) is therefore:

$$m_G = \rho_G \times V_G \quad \text{(O3)}$$

$$= 1080.1 \times \frac{20}{1000}$$

$$= 21.6 \text{ g}$$

Total mass fed to the digester ($m_{Total\ fed}$) is:

$$m_{Total\ fed} = m_w + m_I + m_G \quad \text{(O4)}$$

$$= 2400 + 2476 + 21.6$$

$$= 4897.682 \text{ g}$$

The biogas volume (V_b) that was generated by the digester was 14.45 L. The biogas compositions for methane (y_m) and carbon dioxide (y_c) were 76% and 22% (v/v), respectively. According to Beam (2011), the amount of water vapour in biogas (y_w) is approximately 1% (v/v). Therefore, let us assume that the water content in biogas is 1%.

Let us assume ideal gas behaviour, and that the system pressure (P) is 1 atm. The universal gas constant, R, is $0.08206\ \text{kmol.K/(kg.atm.L)}$. The mass of methane generated in the system ($\text{m}_{\text{methane}}$) is:

$$m_{methane} = \frac{M_m \times P \times V_b \times y_m}{R \times T} \quad \text{(O5)}$$

$$= \frac{16 \times 1 \times 14.45 \times 0.76}{0.08206 \times (37.5 + 273.15)}$$

$$= 7.00 \text{ g}$$

The mass of carbon dioxide generated in the system (m_{CO2}) is:

$$m_{CO2} = \frac{M_c \times P \times V_b \times y_c}{R \times T} \quad \text{(O6)}$$

$$= \frac{44 \times 1 \times 14.45 \times 0.22}{0.08206 \times (37.5 + 273.15)}$$

$$= 5.57 \text{ g}$$

The mass of water vapour generated in the system (m_{H2O}) is:

$$m_{H2O} = \frac{M_w \times P \times V_b \times y_w}{R \times T} \quad \text{(O7)}$$

$$= \frac{18 \times 1 \times 14.45 \times 0.01}{0.08206 \times (37.5 + 273.15)}$$

$$= 0.10 \text{ g}$$

The mass of water in the gas collector (displacement) before and after the digestion process ($m_{H2O\ before}$ and $m_{H2O\ after}$) were obtained using the mass scale, and were 14416 and 14432 g, respectively. The amount of carbon dioxide dissolved in water ($m_{CO2\ dissolved}$) is equal to

the difference between the mass of water in the gas collector before and after the digestion process:

$$m_{CO2\ dissolved} = m_{H2O\ after} - m_{H2O\ before} \quad \text{(O8)}$$

$$= 14432 - 14416$$

$$= 16.43 \text{ g}$$

The mass of the digestate (m_d) was obtained using the mass scale was 4869 g. The total output mass in the system is therefore ($m_{Total\ output}$):

$$m_{Total\ output} = m_{methane} + m_{CO2} + m_{H2O} + m_d + m_{CO2\ dissolved} \quad \text{(O9)}$$

$$= 7.00 + 5.57 + 0.10 + 4869 + 16.43$$

$$= 4897.681 \text{ g}$$

The amount of losses in the system (m_{losses}) is therefore:

$$m_{losses} = m_{Total\ fed} - m_{Total\ output} \quad \text{(O10)}$$

$$= 4897.682 - 4897.681$$

$$= 0.0007 \text{ g}$$

APPENDIX P – DOE

The Doehlert matrix design (DM) is the most efficient design system followed by the Box Behnken design (BBD). The DMs are also more efficient in mapping space: adjoining hexagons can fill a space efficiently and fully, because the hexagons fill space with no overlap. Furthermore, the DM has the ability for sequentially, where experiments can be used again when the limits have not been correctly selected initially (Ferreira *et al*., 2004). Despite these advantages, the uniform shell designs of DM have not been broadly used because their design variables are treated unequally. For this reason, the Box Behnken method was used to determine the response equations for glycerol.

P1 - Pre-analysis of effects via sorts and simple scatter plots

Table P1 shows the design layout in standard order for a Box-Behnken for all three variables used in this investigation together with biogas and methane yields for all combinations. The data was arranged randomly so that lurking variables that are function of time would not bias the overall results. Design-Expert software allows for data to be sorted for multiple variables to find effects on the overall responses. For instance, data can be sorted by any of the three variables and thereafter see the effect it has on the overall response. The impact of each variable on the response variable is found in the correlation graph (under Graph Columns) in Design-Expert Software.

Figure P1, Figure P2, Figure P3, Figure P4, Figure P5 and Figure P6 show the impact of temperature, volume of glycerol in the digester and substrate to inoculum ratio on biogas and methane yields, respectively. The correlation graph shows the response versus variable graph for all the data points that were entered in Design-Expert Software. With three points for each variable (-1, 0, 1), a linear plot is generated for each variable. Since this was a linear plot, the correlation was given by the slop or gradient or ‘skewness’ of the graph. In this case the glycerol volume graph has the highest skewness, with temperature and substrate to inoculum ratio having almost horizontal slope. Thus, amongst the variables that were measured the glycerol volume had the highest effect on both the biogas and methane yields.

Table P1: Design layout in standard order for a Box-Behnken for a three variable system – biogas and methane yields data entered.

Std	Run	Variable 1 A: Temperature (℃)	Variable 2 B: Substrate / inoculum ratio (g VS/ g VS)	Variable 3 C: volume of glycerol in the digester (ml)	Biogas yield (L / g VS)	Methane yield (L / g VS)
14	1	37.5	1.0	22	0.65	0.52
9	2	37.5	0.5	10	0.44	0.33
4	3	50.0	1.5	22	0.56	0.45
1	4	25.0	0.5	22	0.5	0.34
8	5	50.0	1.0	34	0.20	0.16
15	6	37.5	1.0	22	0.65	0.52
2	7	50.0	0.5	22	0.55	0.44
12	8	37.5	1.5	34	0.11	0.09
7	9	25.0	1.0	34	0.10	0.07
11	10	37.5	0.5	34	0.10	0.08
3	11	25.0	1.5	22	0.51	0.35
6	12	50.0	1.0	10	0.94	0.75
5	13	25.0	1.0	10	0.74	0.50
13	14	37.5	1.0	22	0.65	0.52
10	15	37.5	1.5	10	0.65	0.49

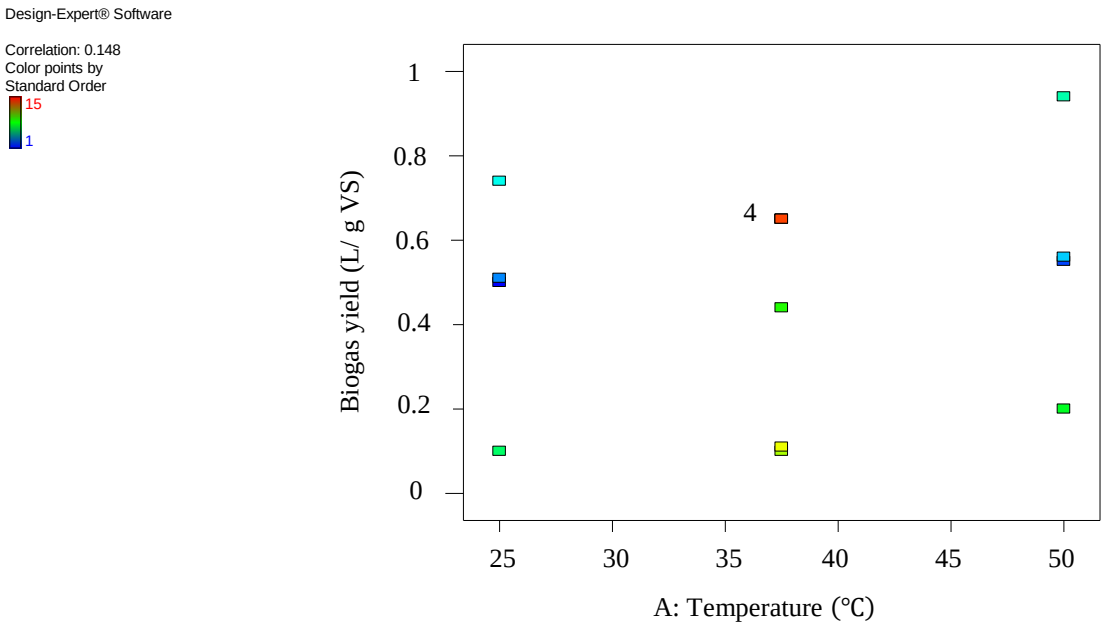


Figure P1: Effect of temperature on biogas yield.

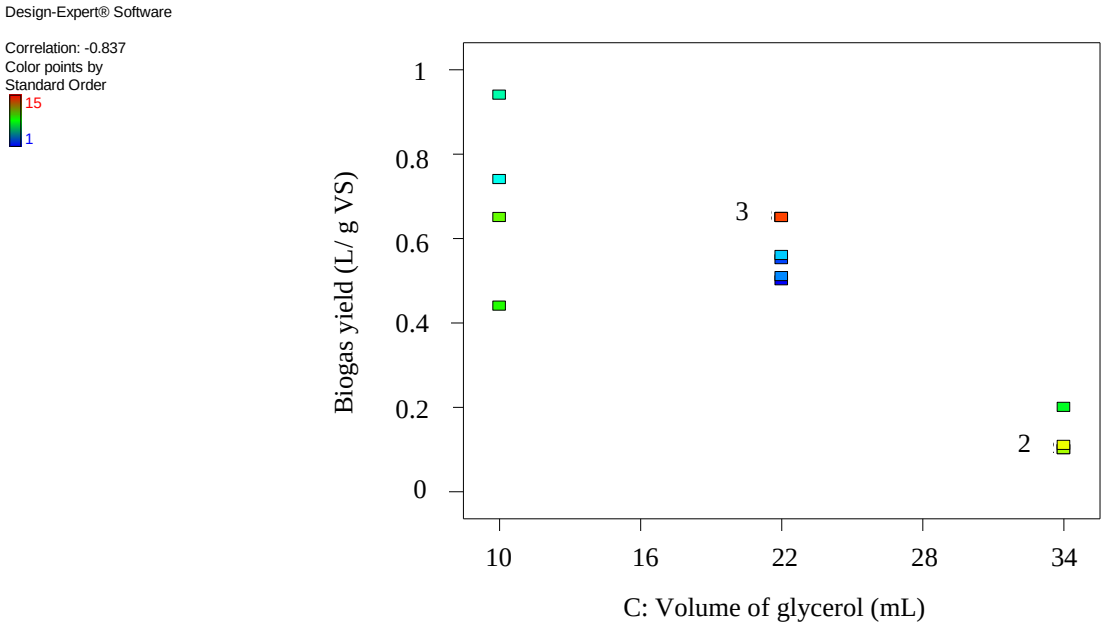


Figure P2: Effect of glycerol volume on biogas yield.

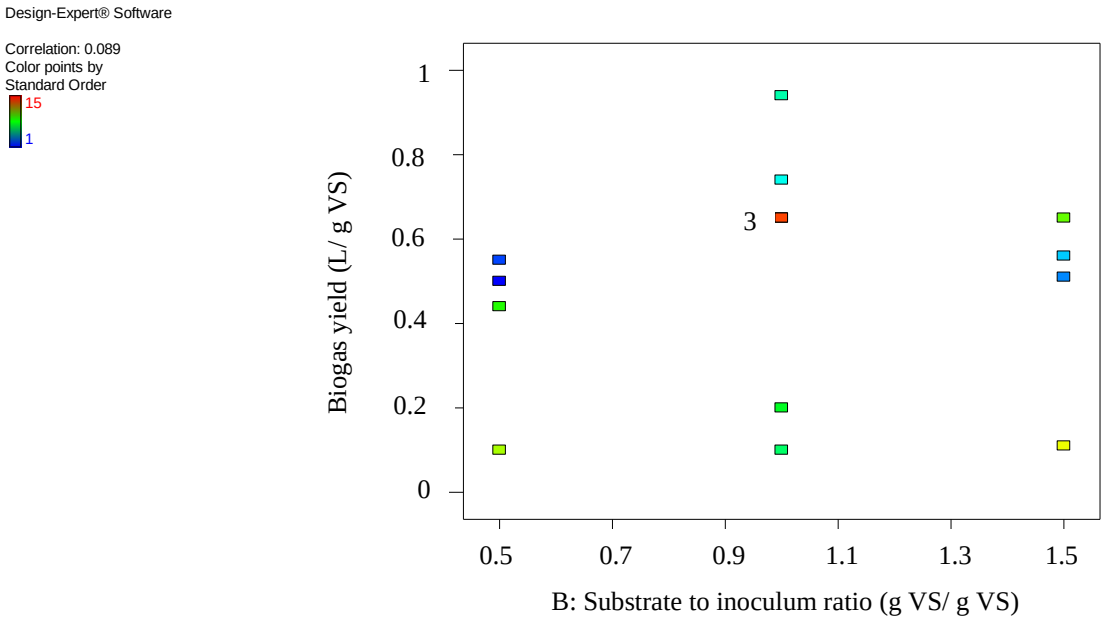


Figure P3: Effect of substrate to inoculum ratio on biogas yield.

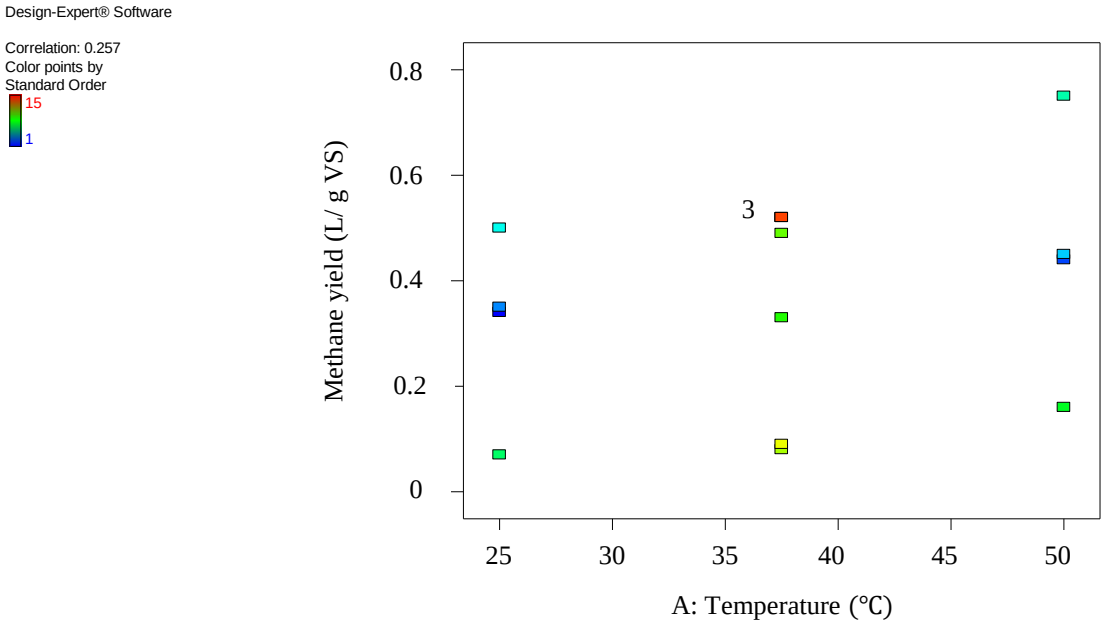


Figure P4: Effect of temperature on methane yield.

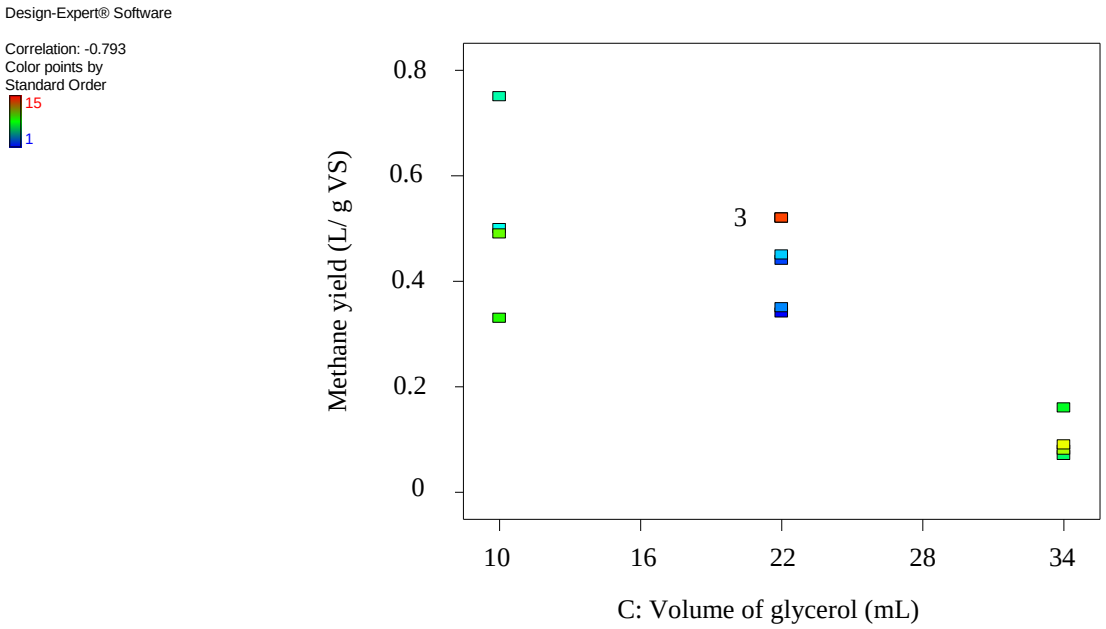


Figure P5: Effect of glycerol volume on methane yield.

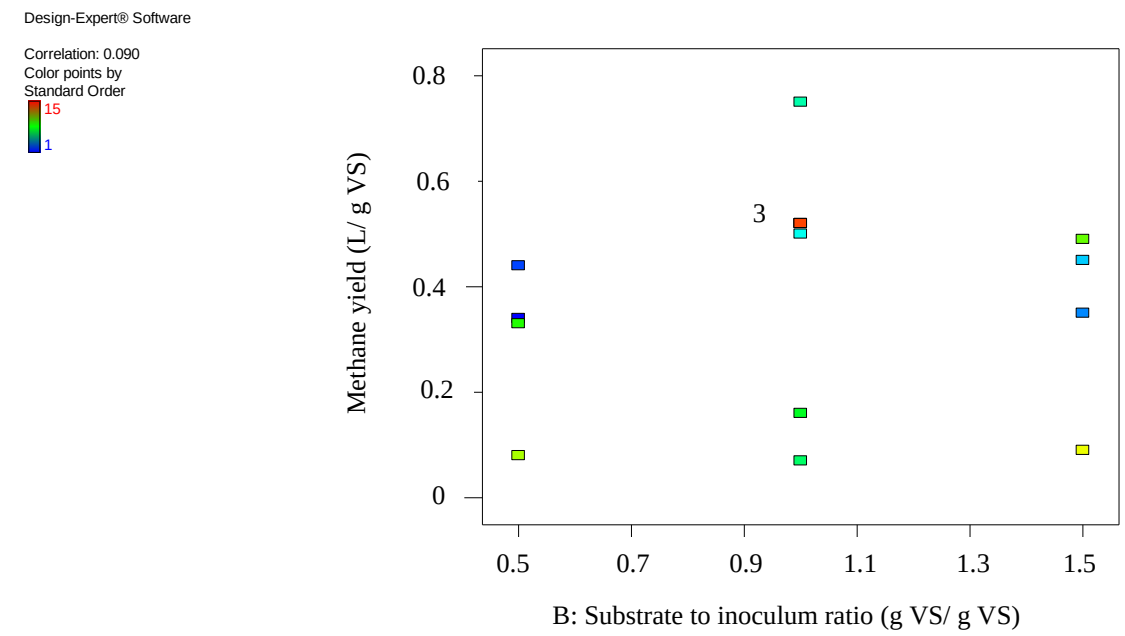


Figure P6: Effect of substrate to inoculum ratio on methane yield.

Another useful indicator of the effect of a variable on the response is the correlation grid box, which in Design-Expert Software is located at the intersection of the variables in the graph columns. Figure P7 and Figure P8 show the correlation of temperature to biogas and methane yields, respectively. The correlation of the glycerol volume in the digester and substrate to inoculum ratio on biogas and methane yields is depicted in Figure P9, Figure P10, Figure P11 and Figure P12, respectively.

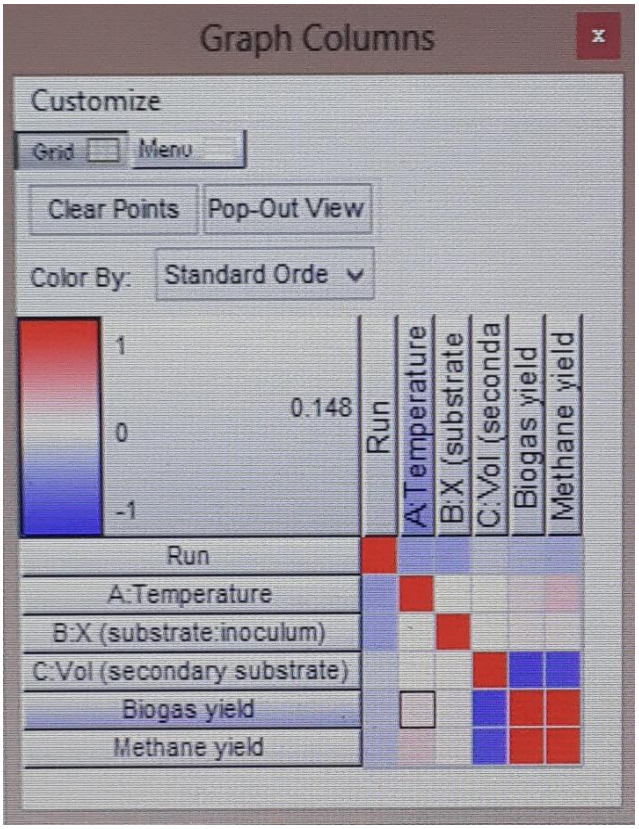


Figure P7: Correlation grid of the temperature factor on biogas yield.

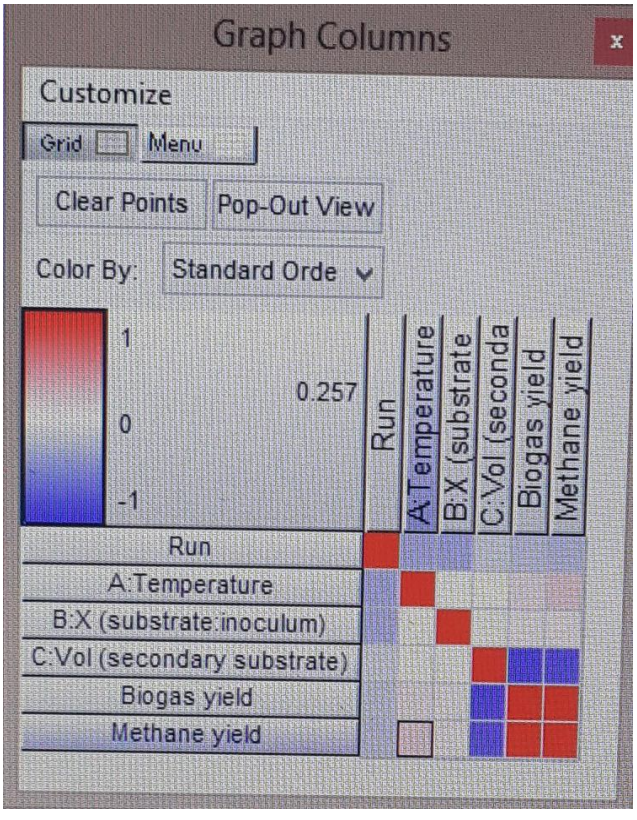


Figure P8: Correlation grid of the temperature factor on methane yield.

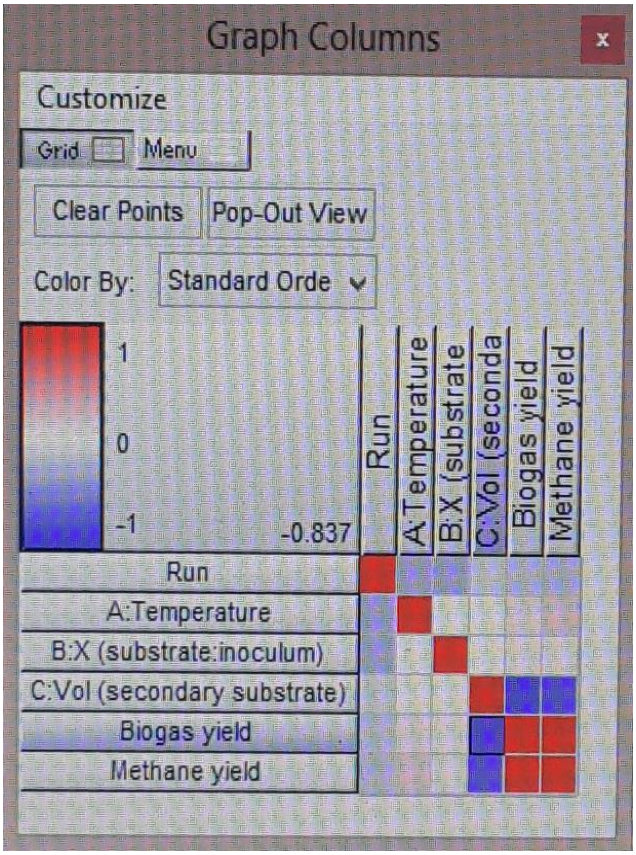


Figure P9: Correlation grid of the glycerol volume factor on biogas yield.

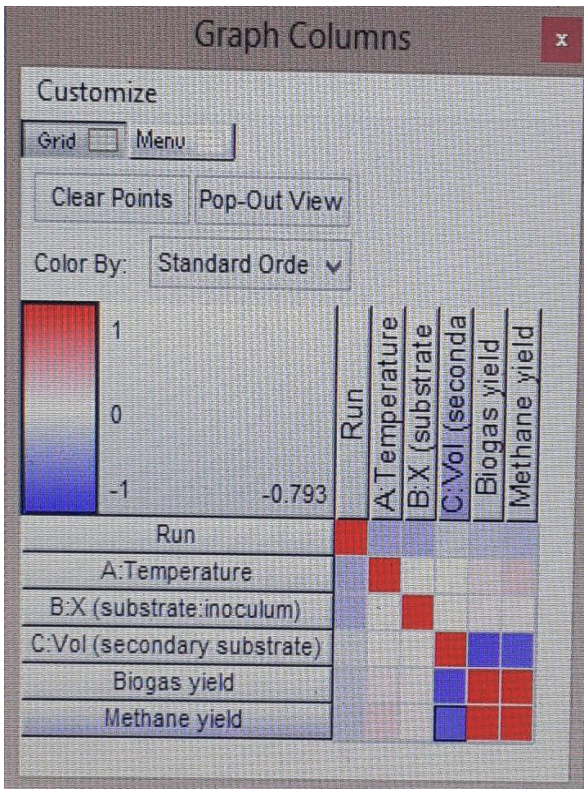


Figure P10: Correlation grid of the glycerol volume factor on methane yield.

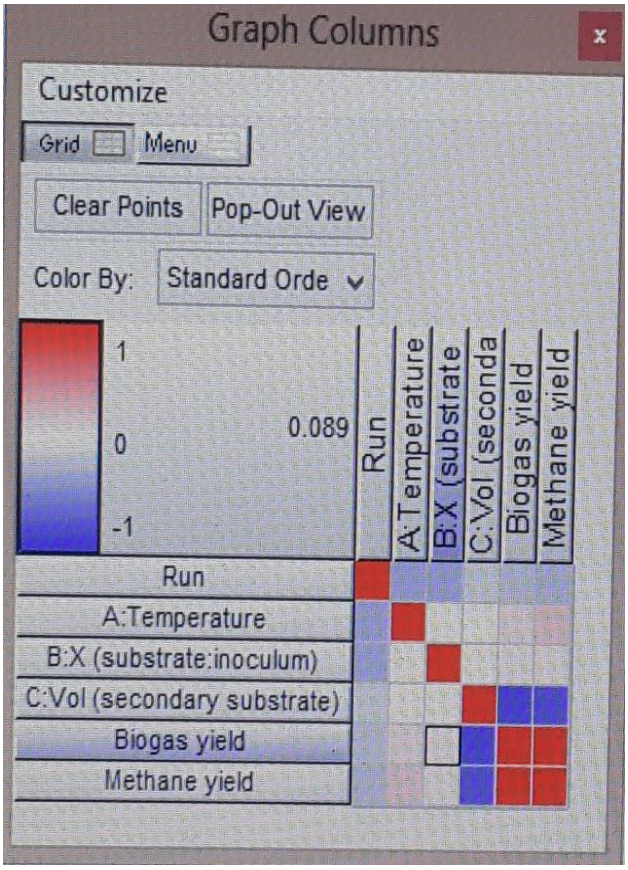


Figure P11: Correlation grid of the substrate to inoculum ratio on biogas yield.

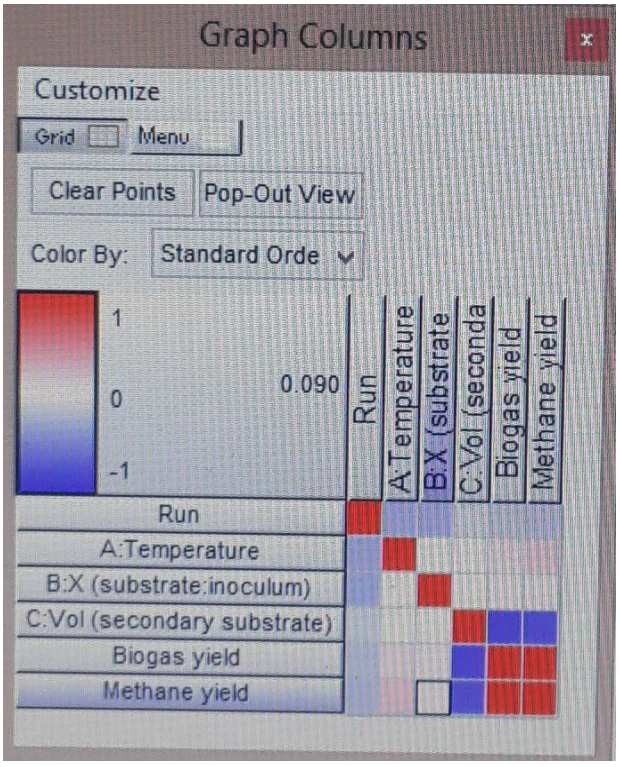


Figure P12: Correlation grid of the substrate to inoculum ratio on methane yield.

The correlation value (shown on the legend – left hand-side of the figures) shows how much each variable affects the response, ranging from negative 1 to positive 1. A correlation with a positive value implies that the response increases with an increase in variable, whereas a negative value implies that the response decreases with an increase in variable. In the case of this study, an increase either in temperature or substrate to inoculum ratio increased the biogas and methane yields, whereas an increase in glycerol volume led to a decline in both biogas and methane yields. The variable with the highest correlation (in value) on both biogas and methane yields was glycerol (Figure P9 and Figure P10), with correlations of -0.837 and -0.793 for biogas and methane yields, respectively. Thus, the glycerol volume had the highest effect on biogas and methane yields followed by temperature, and then finally substrate to inoculum ratio.

P2 - Transformation

Figure P13 and Figure P14 show the transformation graphs for biogas and methane yields, respectively. The response values for biogas yield and methane yield ranges from 0.10 to 0.94 L/g VS and 0.07 to 0.75 L/g VS, respectively. The ratio of maximum to minimum for biogas and methane yields are 9.4 and 9.375, respectively (see Appendix M1 for sample of calculations). These ratios are both below 10, thus there was no need for transformation in the responses of both biogas yield and methane yield.

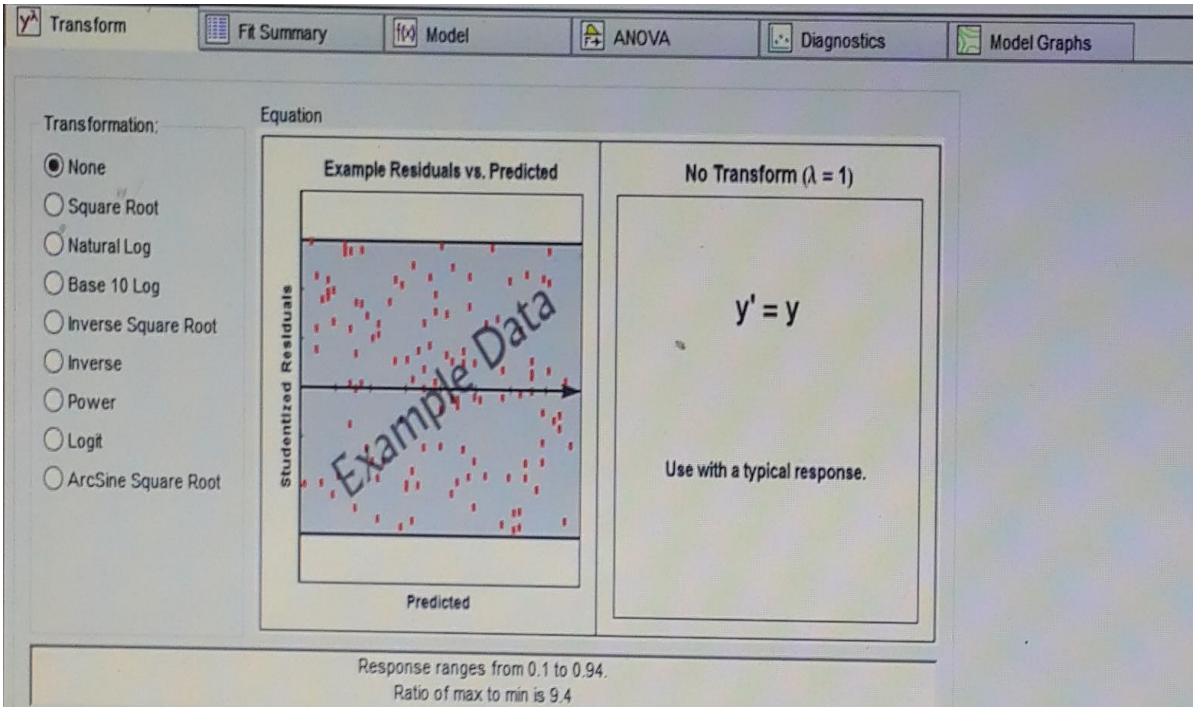


Figure P13: Transformation for the biogas yields.

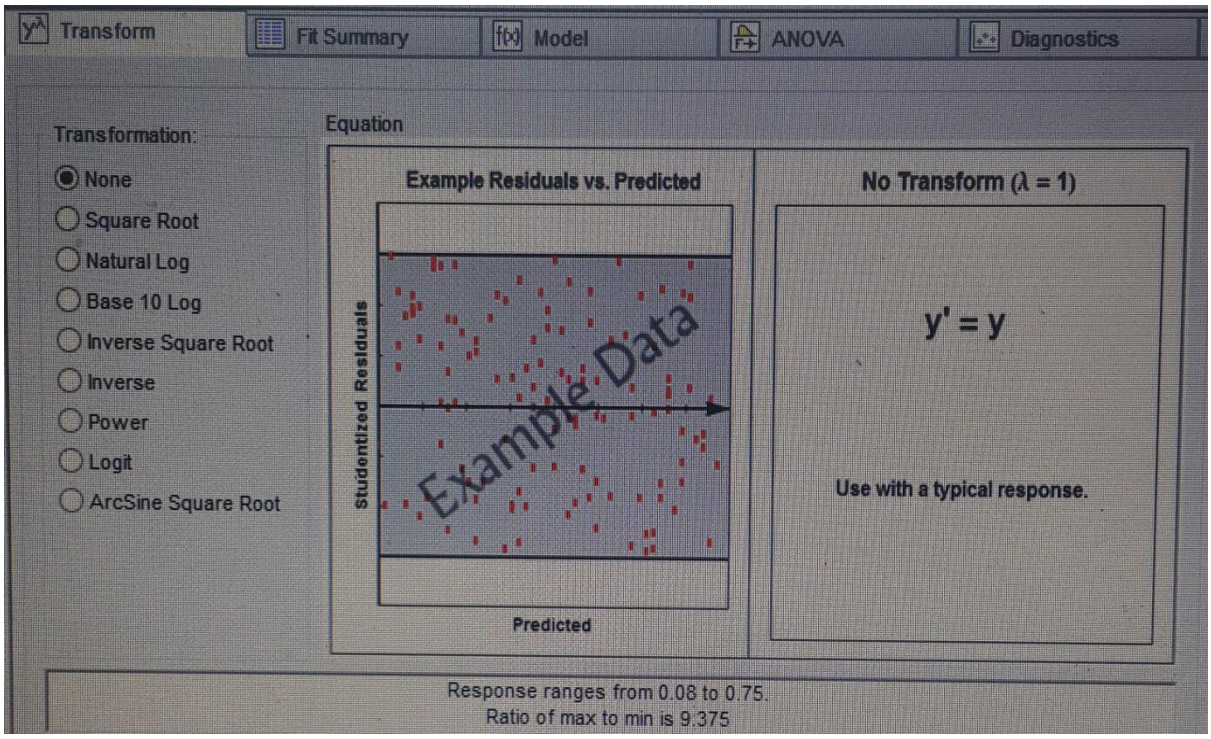


Figure P14: Transformation for the methane yields.

P3 - Fit summary

Fit summary gives the fit of points for all types of model or transforms, and the model with the best fit may be chosen. The models that were tested were linear, quadratic and cubic models. Fit summary, sequential model sum, lack of fit tests and model summary statistics for biogas and methane yields are shown in Table P2, Table P3, Table P4, Table P5, Table P6, Table P7, Table P8 and Table P9, respectively.

The model with the highest adjusted R-Squared, Predicted R-Squared, p-value and F-value is regarded as the best fit model. In this case, the quadratic had the highest R-Squared, Adjusted R-Squared, p-value and F-value. The linear model had the highest Predicted R-Squared and F-value. The suggested model can therefore either be linear or quadratic, however, the cubic model cannot be used as it is aliased. In order to make a nearly true representation for all the points, a quadratic model was chosen.

Table P2: Fit Summary for biogas yield.

Source	Sequential p-value	Adjusted R-Squared	Predicted R-Squared	
Linear	0.0018	0.66	0.51	Suggested
2Fl	0.9314	0.55	-0.01	
Quadratic	0.0250	0.87	0.28	Suggested
Cubic		1.00		Aliased

Table P3: Fit Summary for methane yield.

Source	Sequential p-value	Adjusted R-Squared	Predicted R-Squared	
Linear	0.0031	0.62	0.48	Suggested
2Fl	0.8861	0.52	0.02	
Quadratic	0.0160	0.89	0.35	Suggested
Cubic		1.00		Aliased

Table P4: Sequential Model Sum of Squares for biogas yield [Type 1].

Source	Sum of Squares	df	Mean Square	F -value	P-value Prob. > F	
Mean vs. Total	3.60	1	3.60			
Linear vs. Mean	0.67	3	0.22	9.92	0.0018	Suggested
2Fl vs. Linear	0.01	3	0.00	0.14	0.9314	
Quadratic vs. 2Fl	0.19	3	0.06	7.77	0.0249	Suggested
Cubic vs. Quadratic	0.04	3	0.01			Aliased
Residual	0.00	2	0.00			
Total	4.51	15	0.30			

Table P5: Sequential Model Sum of Squares for methane yield [Type 1].

Source	Sum of Squares	df	Mean Square	F -value	P-value Prob.> F	
Mean vs. Total	2.10	1	2.098			
Linear vs. Mean	0.39	3	0.130	8.70	0.003	Suggested
2Fl vs. Linear	0.01	3	0.004	0.21	0.886	
Quadratic vs. 2Fl	0.13	3	0.043	9.66	0.016	Suggested
Cubic vs. Quadratic	0.02	3	0.008			Aliased
Residual	0.00	2	0.000			
Total	2.65	15	0.176			

Table P6: Lack of fit tests for biogas yield.

Source	Sum of Squares	df	Mean Square
Linear	0.25	9	0.03
2Fl	0.23	6	0.04
Quadratic	0.04	3	0.01
Cubic	0.00	0	
Pure Error	0.00	2	0.00

Table P7: Lack of fit tests for methane yield.

Source	Sum of Squares	df	Mean Square
Linear	0.16	9	0.018
2Fl	0.15	6	0.025
Quadratic	0.02	3	0.008
Cubic	0.00	0	
Pure Error	0.00	2	0.000

Table P8: Model summary statistics for biogas yield.

Source	Standard Deviation	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.15	0.73	0.66	0.51	0.45	Suggested
2Fl	0.17	0.74	0.55	-0.01	0.92	
Quadratic	0.09	0.96	0.87	0.28	0.66	Suggested
Cubic	0.00	1.00	1.00		+	Aliased

Table P9: Model summary statistics for methane yield.

Source	Standard Deviation	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.12	0.70	0.62	0.48	0.29	Suggested
2Fl	0.14	0.73	0.52	0.02	0.54	
Quadratic	0.07	0.96	0.89	0.35	0.36	Suggested
Cubic	0.00	1.00	1.00		+	Aliased

P4 - Validation of the model – diagnostics test

The next step was to carry out some diagnostics to validate the model. The first graph to look at is the normal plot of residuals. Figure P15 and Figure P16 show the normal plots of residuals for biogas and methane yields, respectively. The data points on the graphs should be almost a straight line indicating a normality in the error term, which is the case for the biogas and methane yields. The straight line suggests that there is no need for a transformation of the biogas and methane yields responses.

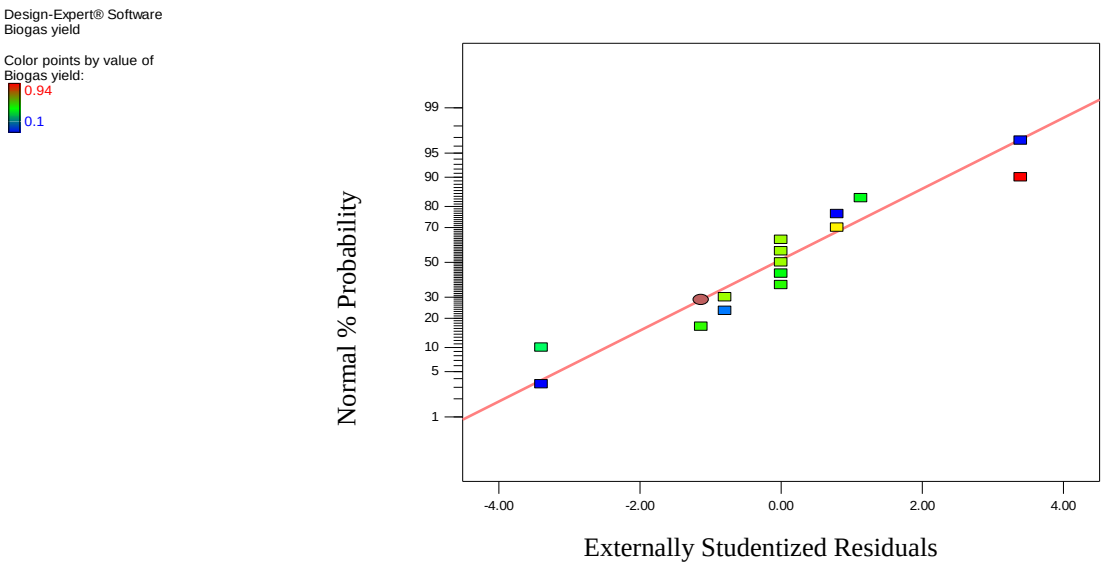


Figure P15: Normal plot of residuals for the biogas yields.

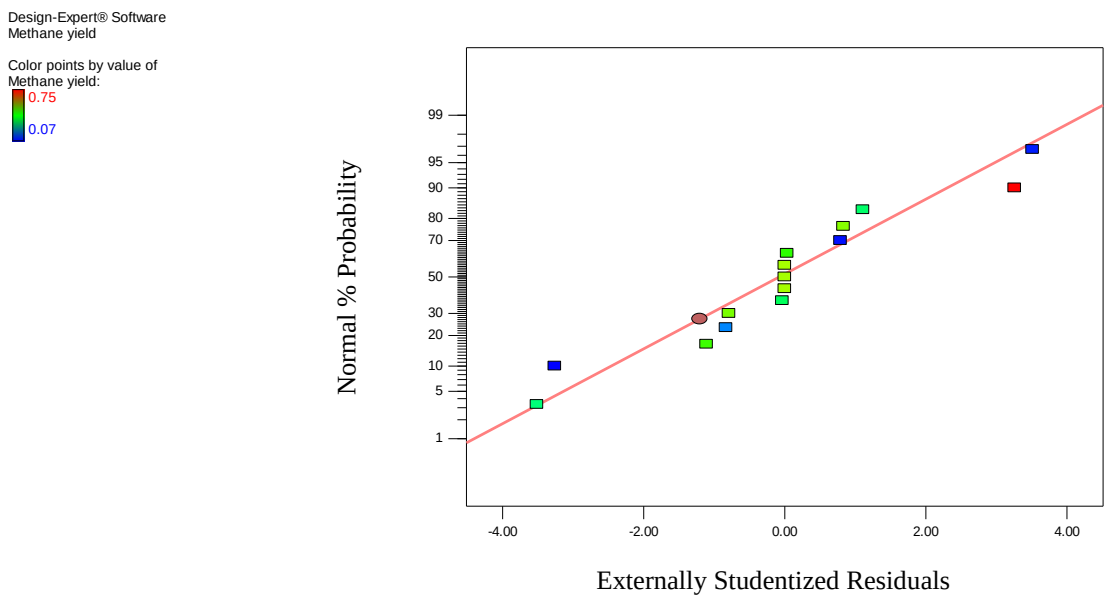


Figure P16: Normal plot of residuals for the methane yields.

The next useful graph in checking the diagnostics to validate the model is the residuals versus predicted graph. Residuals versus predicted biogas and methane yields are presented in Figure P17 and Figure P18, respectively. The magnitude of the residuals should not be dependent on its predicted value i.e. the manner in which the studentized residuals are distributed vertically should be almost similar across all levels of the predicted values. This was the case for both the biogas and the methane yields.

Moreover, the residuals limits are ± 5.3 (see Appendix M6 for calculation). In both graphs, there were no points that were outliers i.e. outside the residuals limits. This means that all observations were fit well by the model. There is also no need for response transformation as there are no outliers in both graphs.

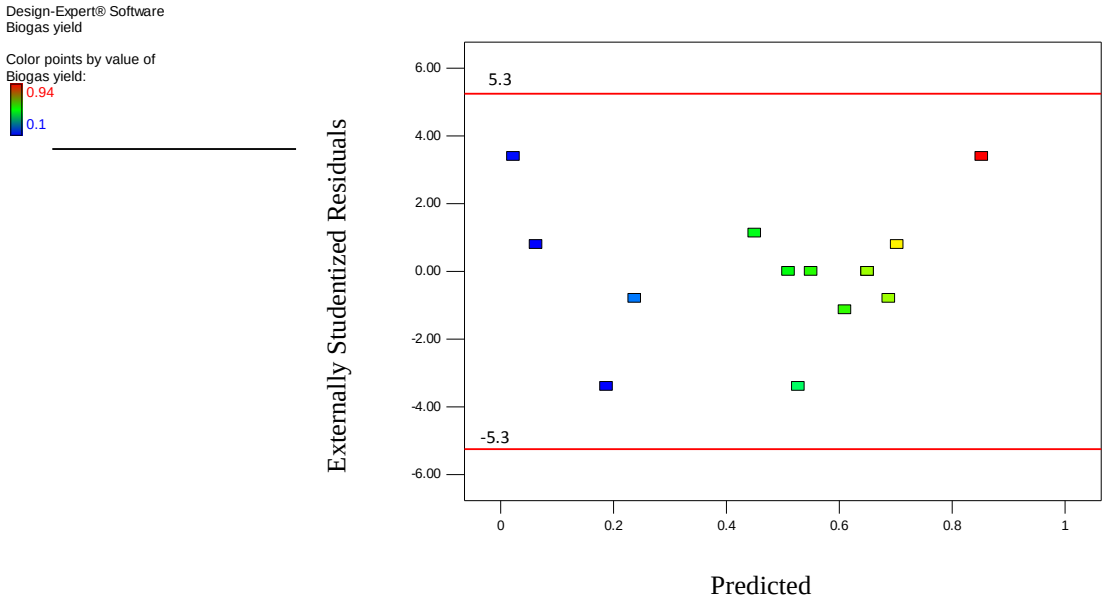


Figure P17: Residuals versus predicted for biogas yields.

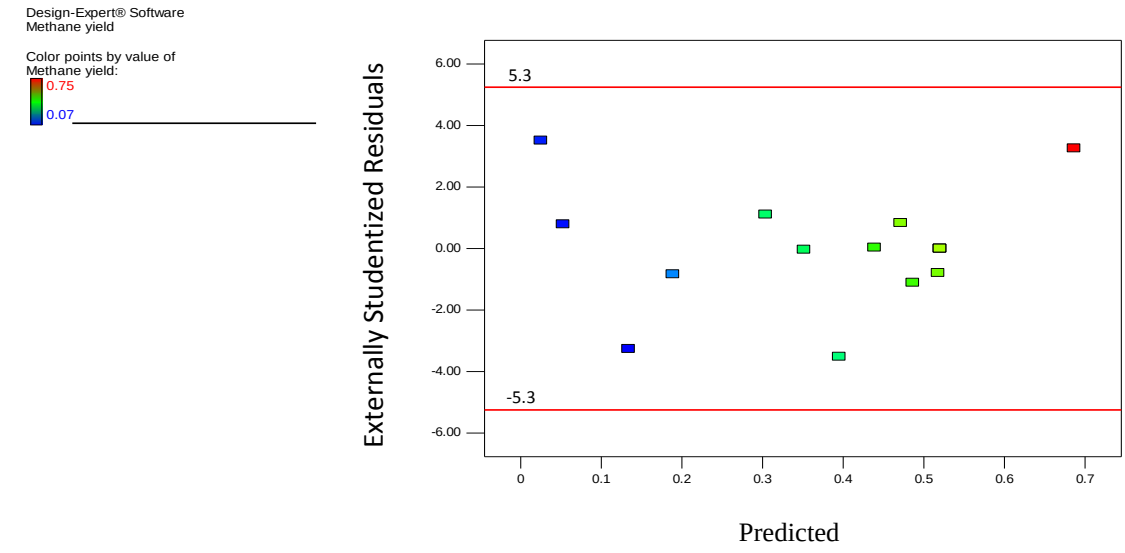


Figure P18: Residuals versus predicted for methane yields.

Another very useful diagnostics tool to look at is the "Residuals versus Run" plot. Figure P19 and Figure P20 show the residuals versus run plots for biogas and methane yields, respectively. The residuals limits are ± 5.3 (see Appendix M6 for calculation). None of the observational points were beyond the residuals limits, suggesting that no response transformation was required in any of the experimental runs for both biogas and methane yields. The graphs also show a random scatter, which suggest that there is no variable lurking in the background that is time-related.

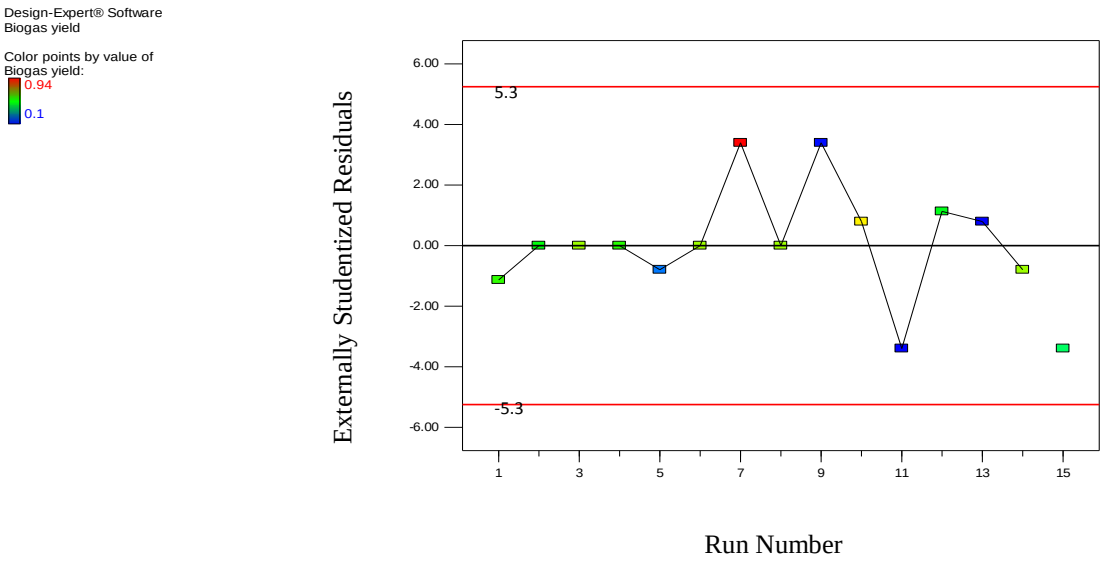


Figure P19: Residuals versus run plot for biogas yields.

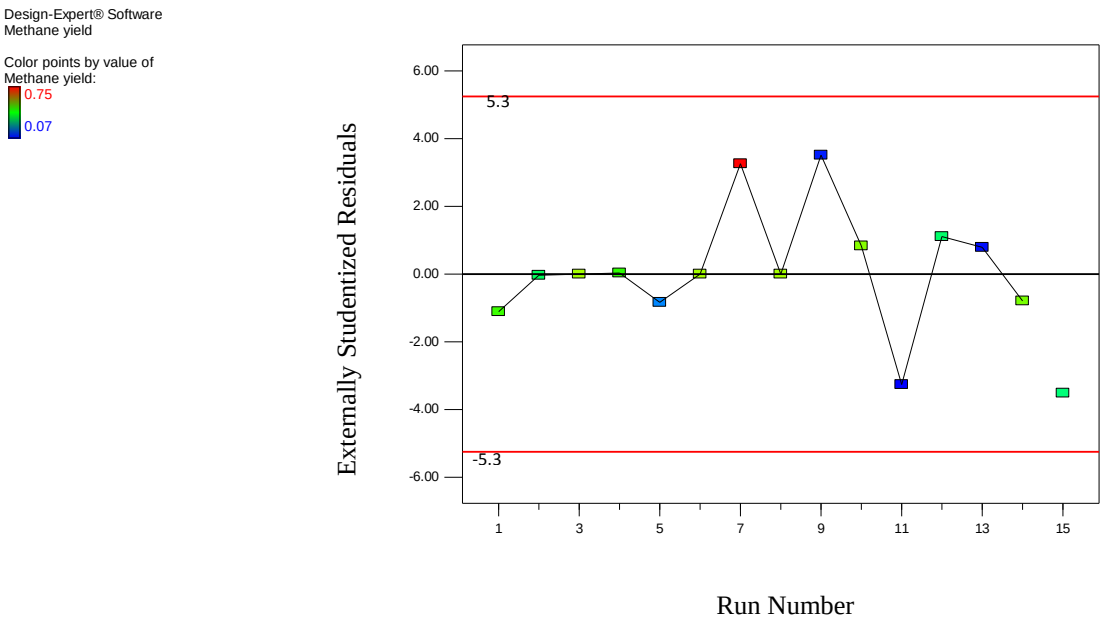


Figure P20: Residuals versus run plot for methane yields.

The next diagnostics graph to look at is the predicted versus actual graph. Predicted versus actual plots for biogas and methane yields are shown in Figure P21 and Figure P22, respectively. The points should be linearly distributed along the 45 degree line, indicating that the model has almost predicted the actual points. Both the biogas and methane yields had data points that are distributed along the 45 degree line.

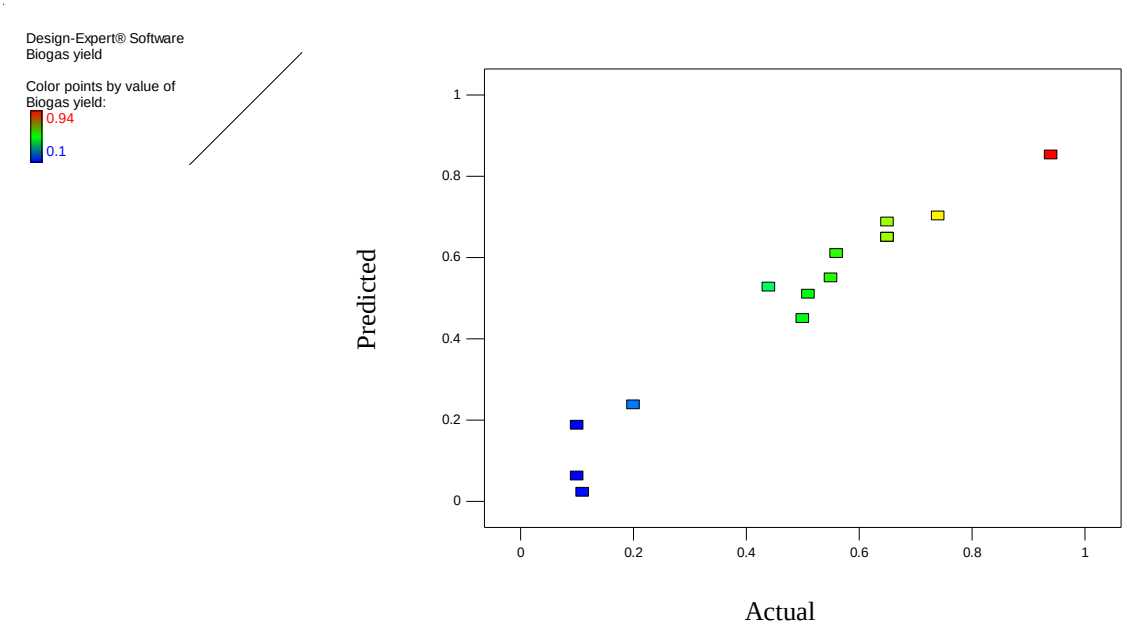


Figure P21: Predicted versus actual plot for biogas yields.

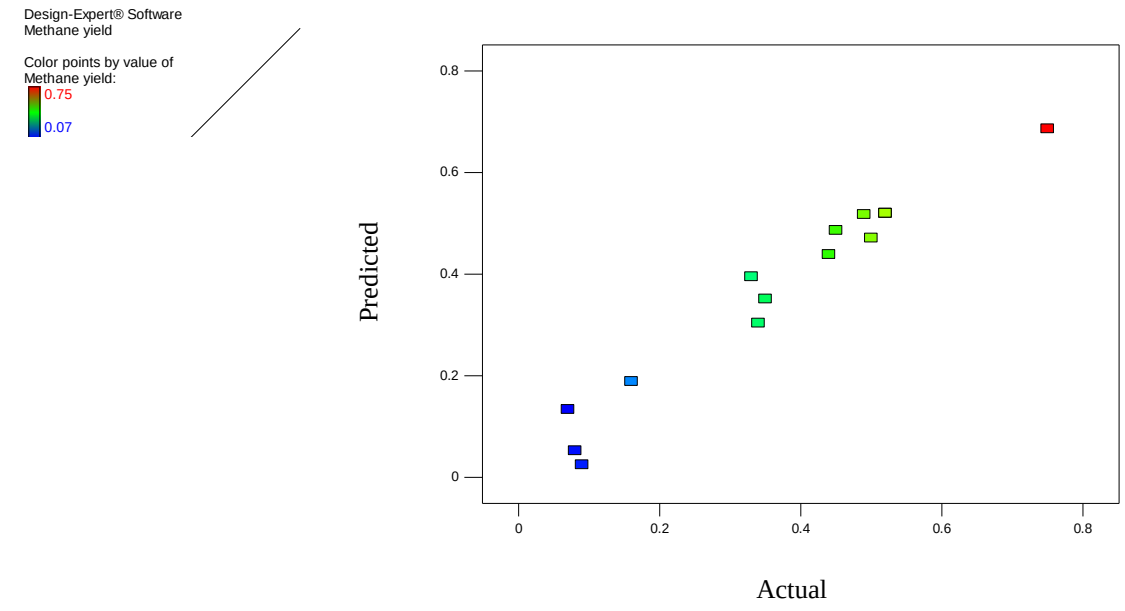


Figure P22: Predicted versus actual plot for methane yields.

Another tool that may be used to detect diagnosis in the model is the "Box Cox Plot for Power Transforms" graph. Figure P23 and Figure P24 show the Box Cox plots for power transformations for biogas and methane yields, respectively.

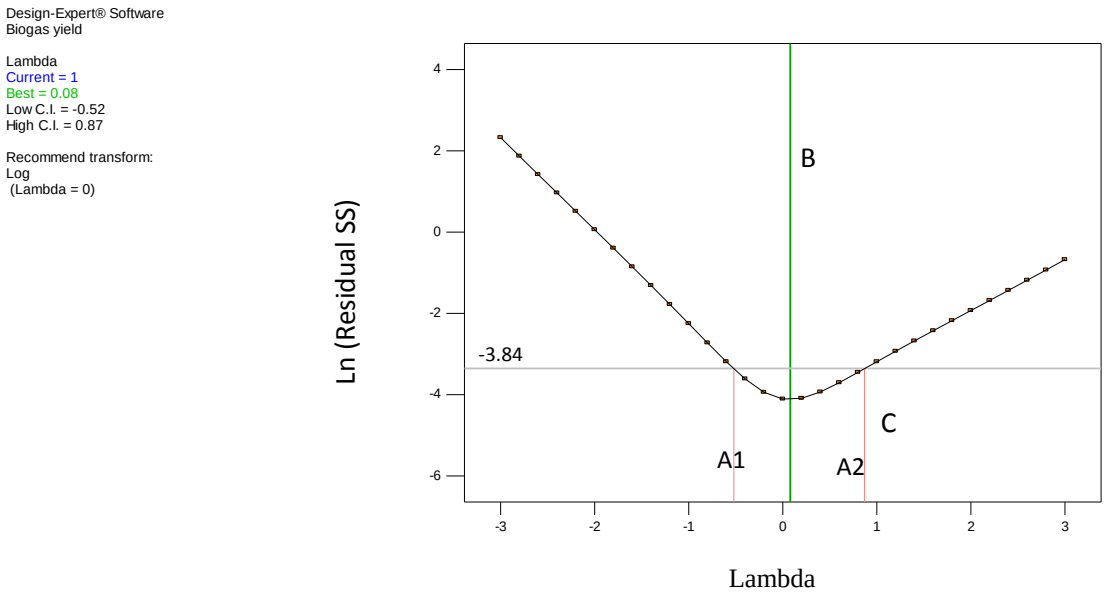


Figure P23: Box Cox plot for power transformation for biogas yields.

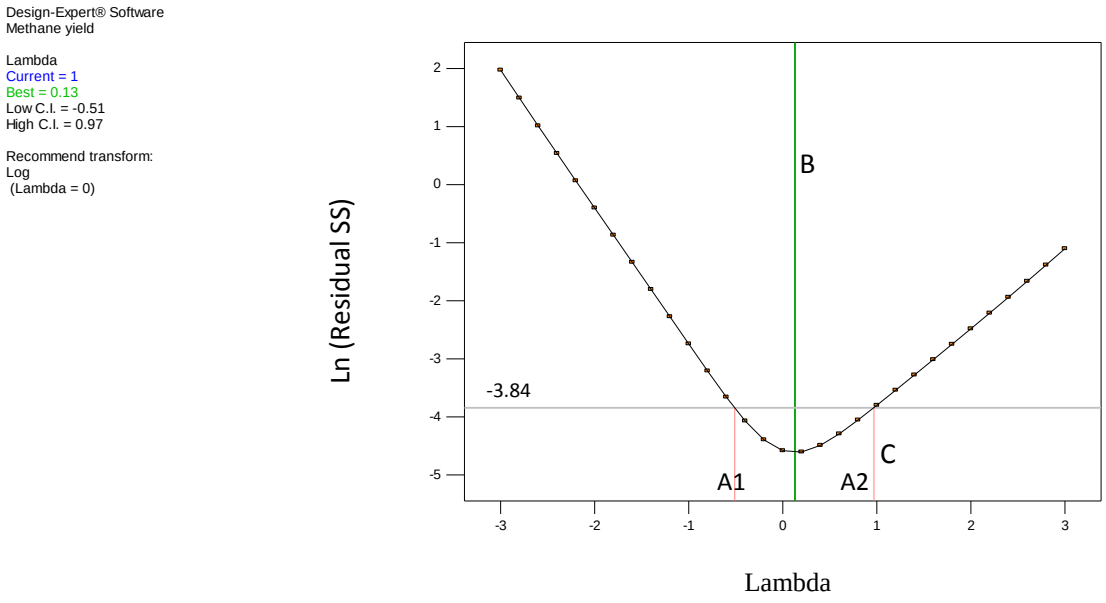


Figure P24: Box Cox plot for power transformation for methane yields.

The information on the left hand-side of the B-line show the transformation that was recommended by Design-Experts Software whereas the information on the right hand-side gives the current transformation that was selected using the information that was used in this investigation. The C-line indicates the current lambda (λ), which in our case is 1 for both biogas and methane yields. The B-line shows the greatest or most excellent lambda (λ), whereas the A1 and A2 lines denote the 95% confidence interval (95% C.I.) that surrounds the best λ. Design-Experts Software will recommend the standard transformation that falls closest to the best λ and that which is within the 95% C.I. If there is no standard transformation within the 95% C.I., then the Power transformation is recommended with the best λ value. In this case the C-lines for both biogas and methane yields lie outside the red lines, with best λ values of 0.08 and 0.13 as the best fit powers for the transformations, respectively. These λ values are close to the log transformation λ of zero. Therefore, the recommended transformation for both biogas and methane yields is the Log transformation, with λ value of zero.

The last tool that may be used in Design-Expert to detect diagnosis in the model is the "Residuals versus Block" graph. Residuals versus temperature, residuals versus substrate: inoculum ratio and residuals versus co-substrate volume plots for biogas and methane yields are depicted in Figure P25, Figure P26, Figure P27, Figure P28, Figure P29 and Figure P30, respectively:

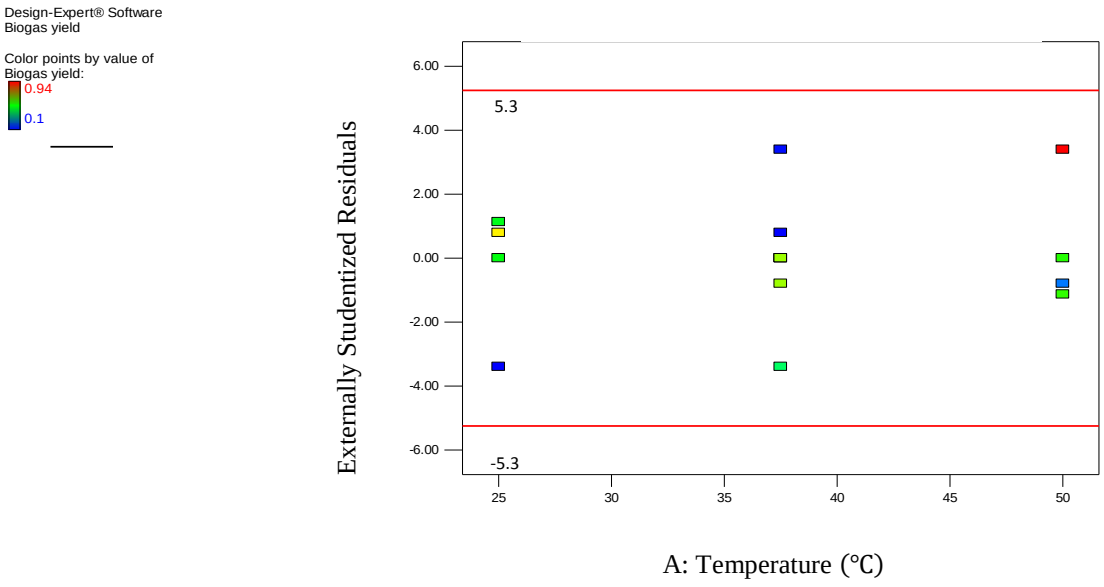


Figure P25: Residuals versus temperature plot for biogas yields.

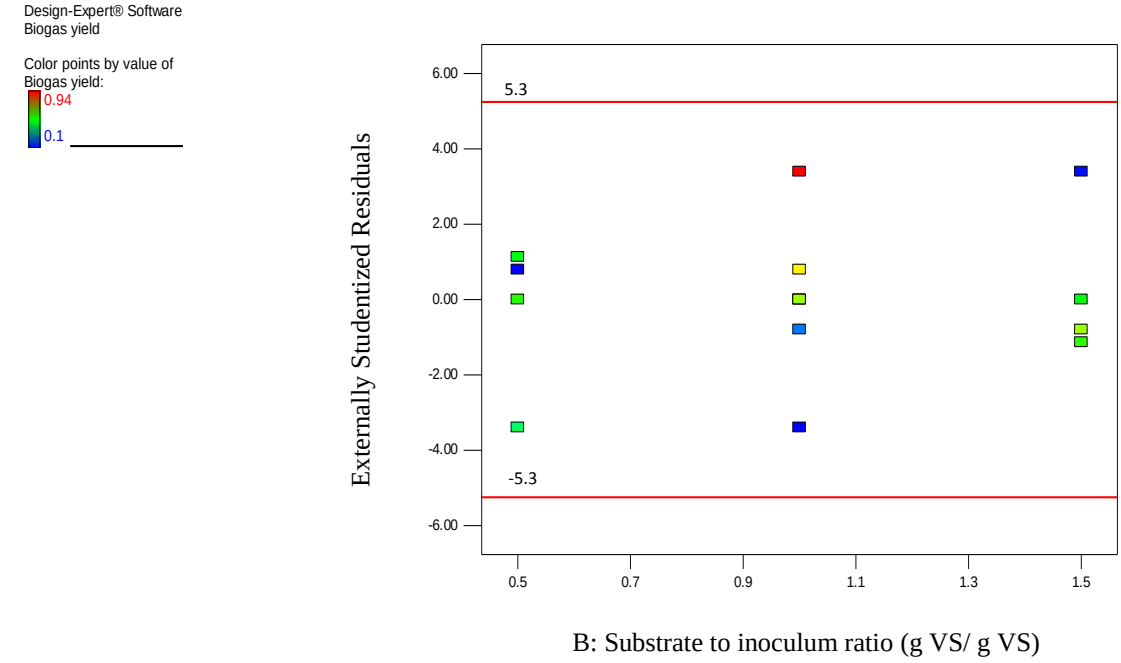


Figure P26: Residuals versus substrate to inoculum ratio plot for biogas yields.

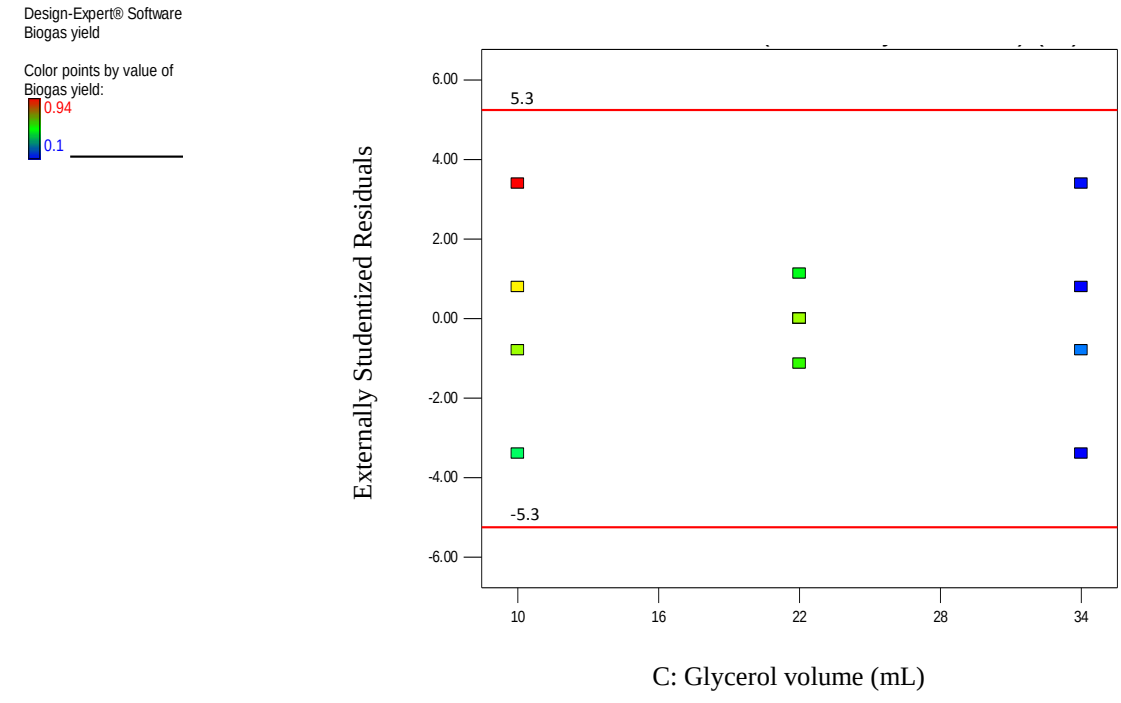


Figure P27: Residuals versus glycerol volume plot for biogas yields.

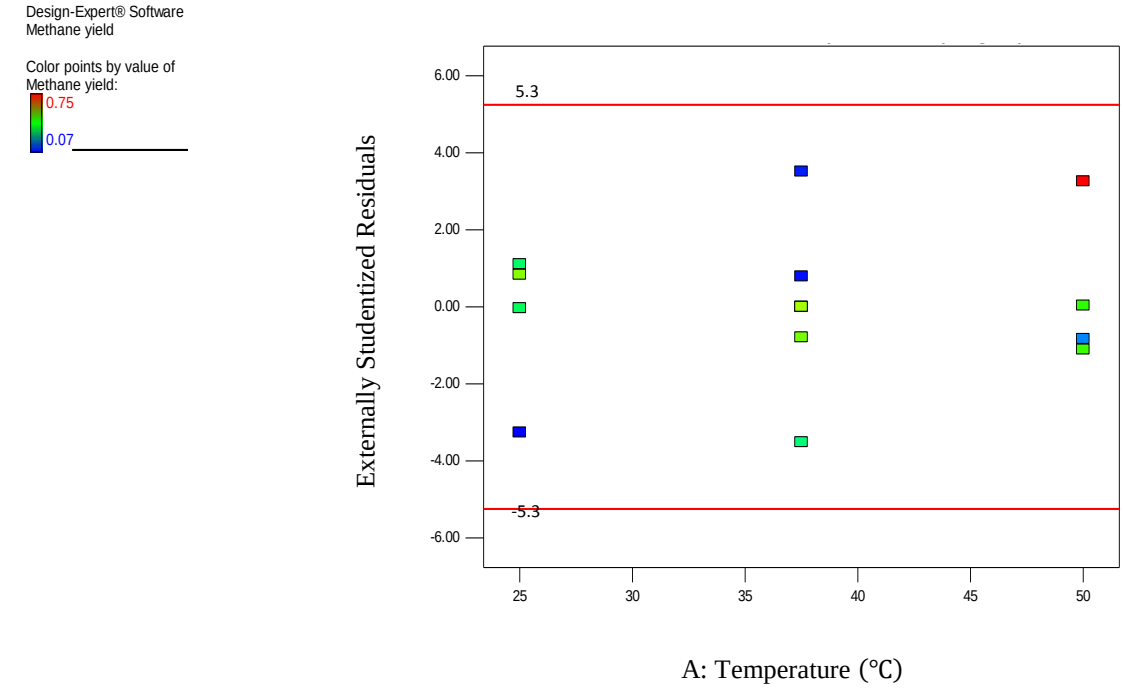


Figure P28: Residuals versus temperature plot for methane yields.

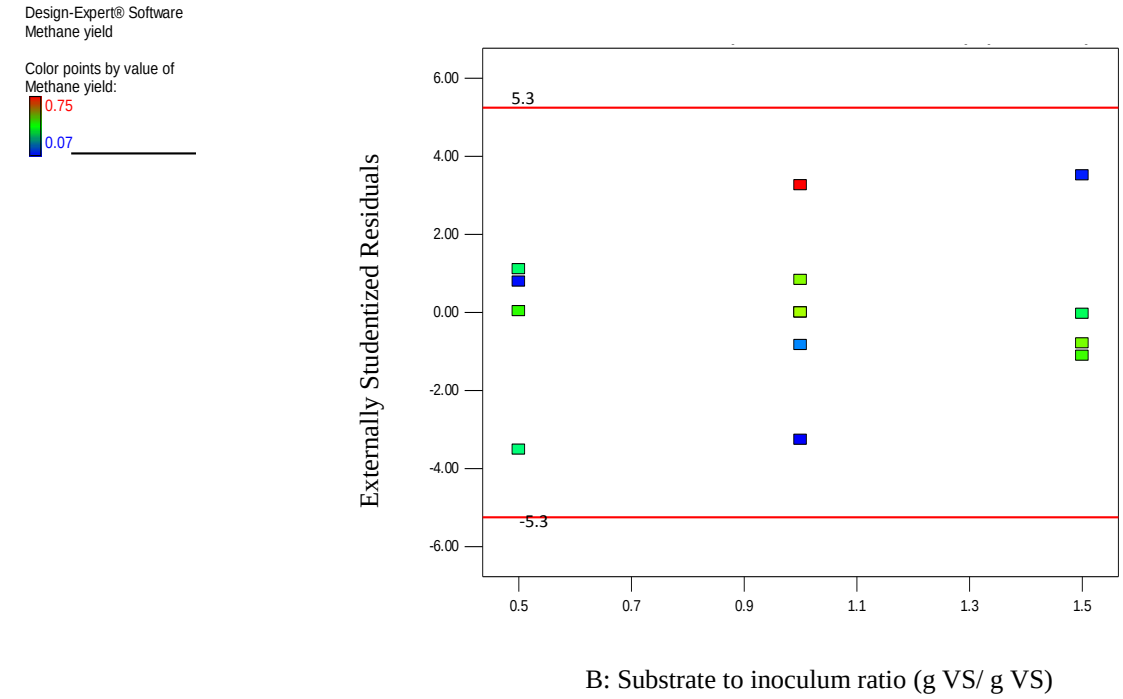


Figure P29: Residuals versus substrate to inoculum ratio plot for methane yields.

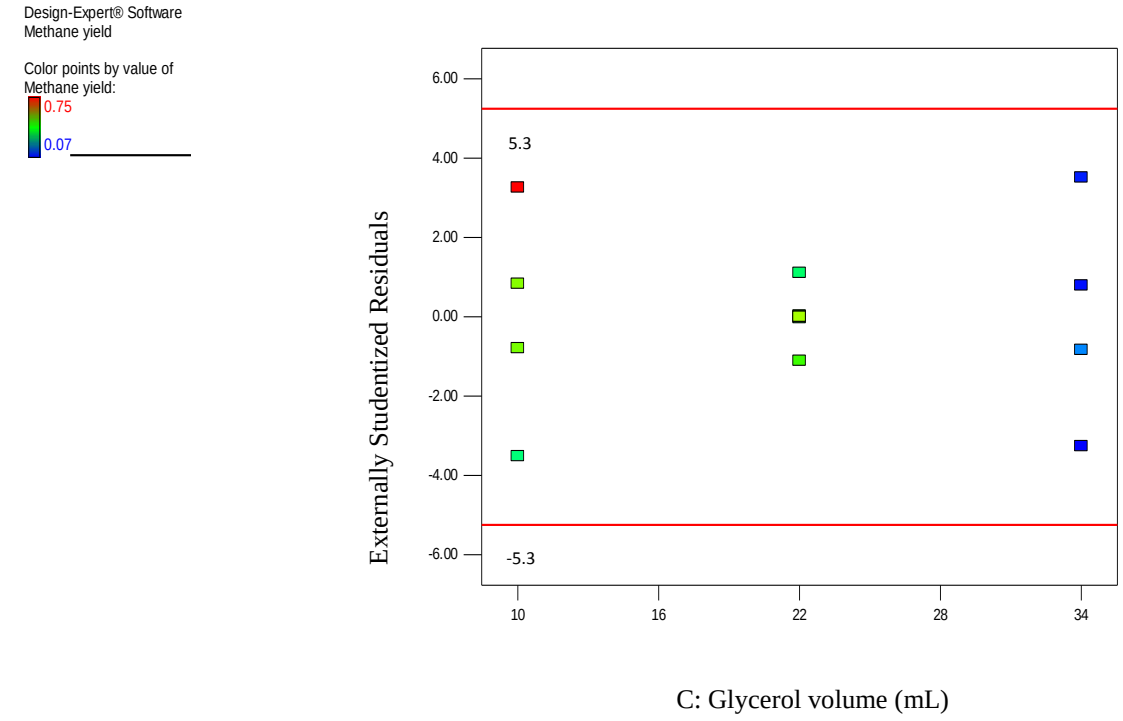


Figure P30: Residuals versus glycerol volume plot for methane yields.

The limits for the residuals were ± 5.3. At both ends of the bounds, the data points on the graphs should be separated by the externally studentized residuals line that is equal to zero. Furthermore, there should not be any effects outside the limits and also higher variations between the data points. In all figures the points are inside the limits, and there is no high variation in the data points.

The next tool to look at is the Cook's distance. Cook's distance versus run number for biogas and methane yields are shown in Figure P31 and Figure P32, respectively. The Cook's distance limits for this investigation ranges from 0 to 1 (see Appendix M7 for calculation). The Cook's distance for run 2, 8, 9 and 12 are outside the limits for both biogas and methane yields. These are the runs to investigate first if more than one outlier appears from other diagnostics graphs. However, the other diagnostics graphs showed observations within their limits, thus there is no need to further investigate these runs.

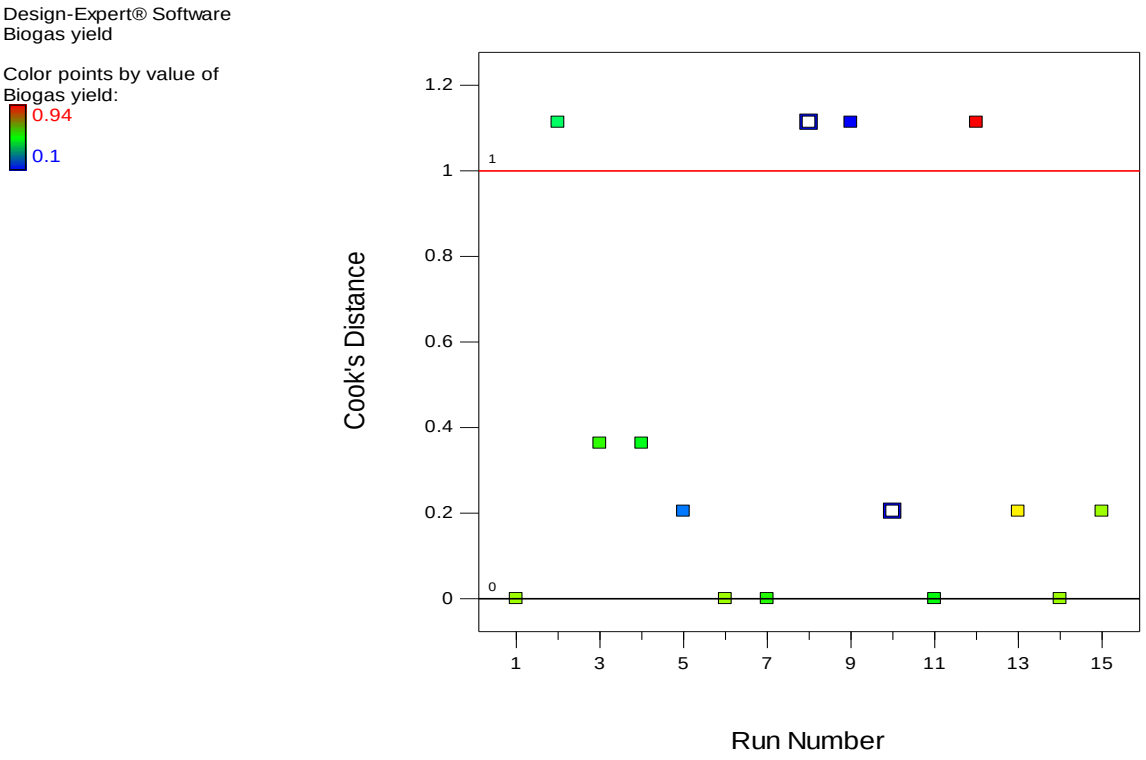


Figure P31: Cook's distance versus run number for biogas yield

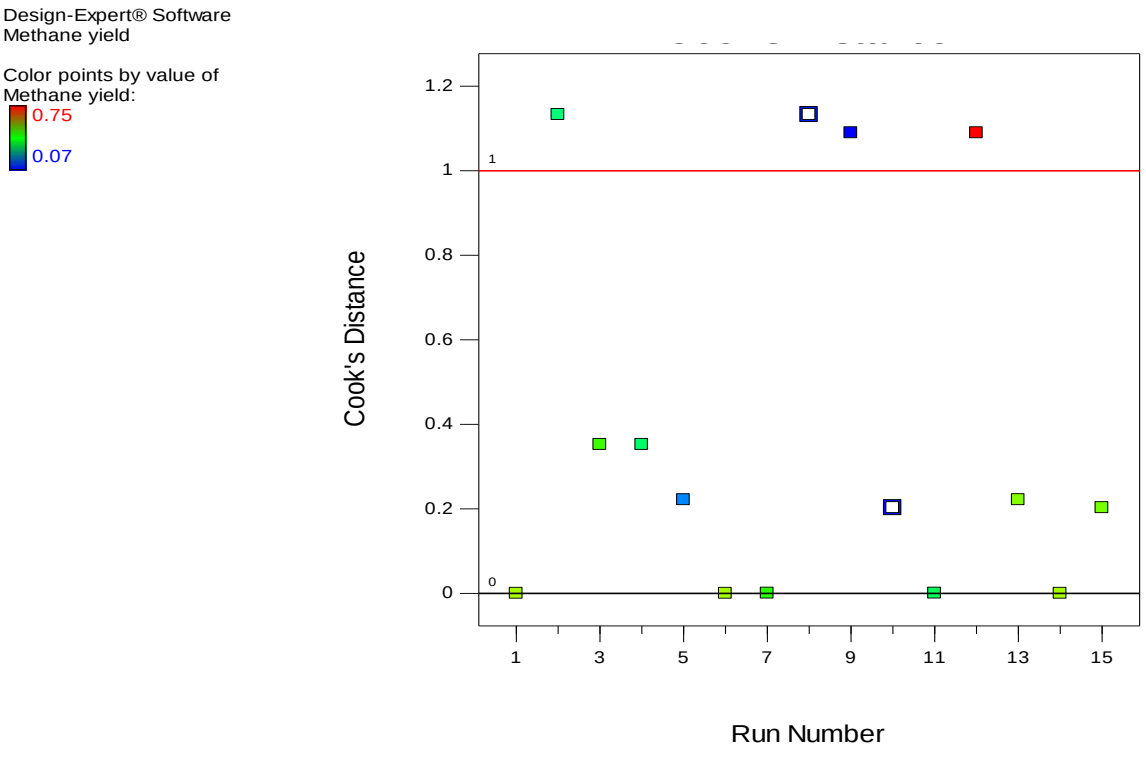


Figure P32: Cook's distance versus run number for methane yield

Another significant effect tool to look at is the leverage graph. The leverage versus run number for biogas and methane yields are shown in Figure P33 and Figure P34, respectively. The average leverage was 0.6 (see Appendix M8 for calculation). An observation with a leverage of 1 or above 2 times the average leverage is regarded as having a very high value. From both graphs, no leverage value is equal to 1 or above 2 times the average leverage (i.e. $2 \times 0.6 = 1.2$), which suggests that the leverage values were normal.

Moreover, the number of coefficient in the model (p) was 9. Both for biogas and methane yields, the leverages for runs 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13 and 15 were 0.68, whereas for runs 1, 6 and 14 were 0.28. According to Equation 2.89, the sum of the leverages for biogas should be equal to the number of coefficients, which was the case:

$$\sum Leverage = (12 \times 0.68) + (3 \times 0.28) = 9 = p$$

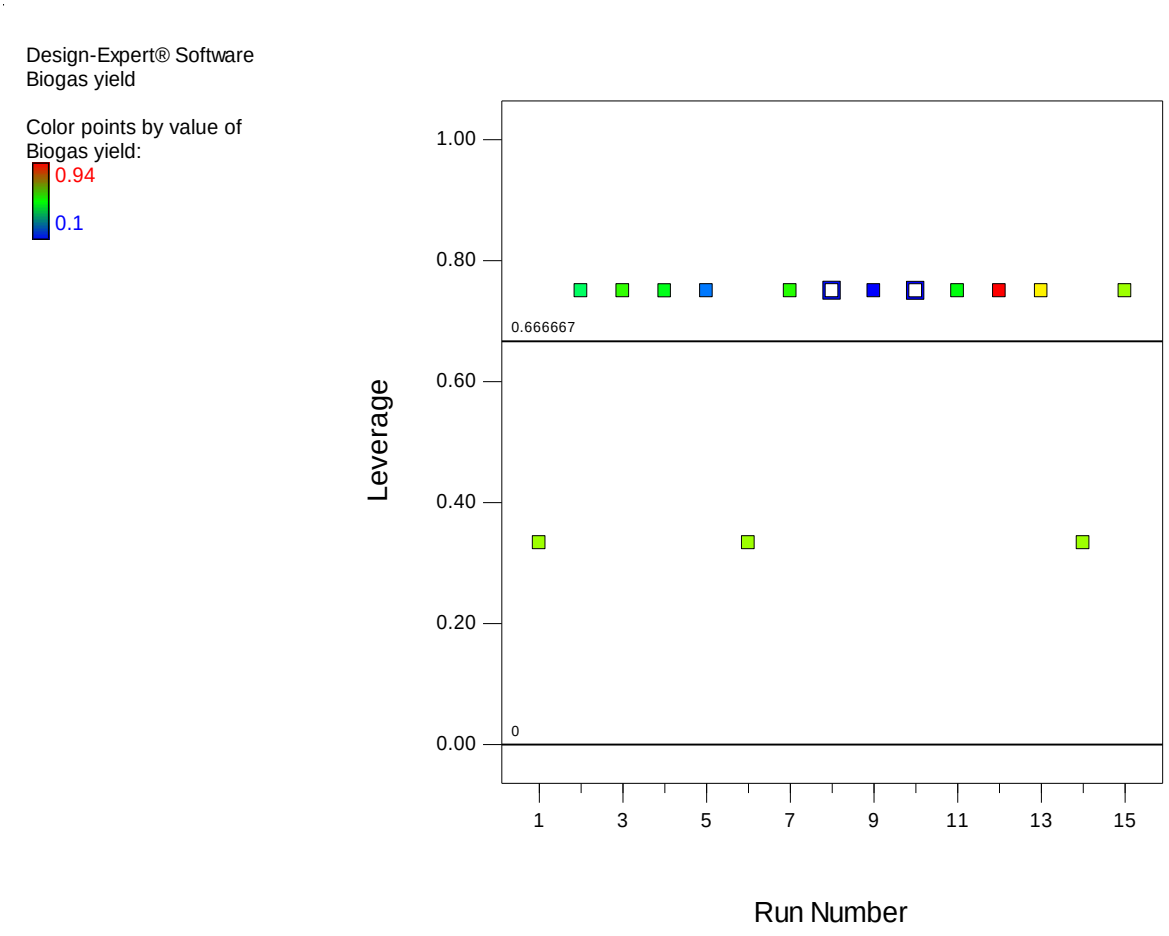


Figure P33: Leverage versus run number for biogas yield

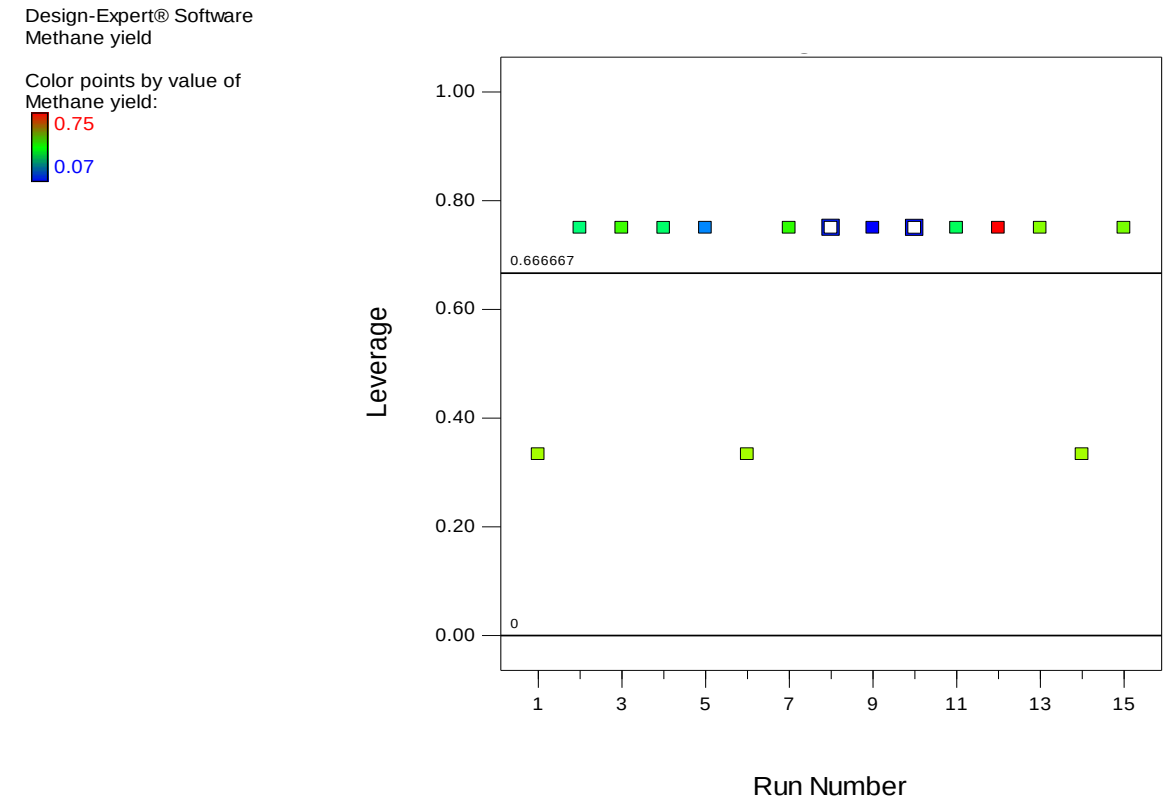


Figure P34: Leverage versus run number for methane yield

Another significant effect tool to look at is the DFFITS tool. Figure P35 and Figure P36 depict the DFFITS versus run number for biogas and methane yields, respectively. The DFFITS limits for both biogas and methane yields were ± 2.44949 (see Appendix M9 for calculation). Run 2, 8, 9 and 12 lie outside the DFFITS limits. The high value in these runs suggests that they are more influential in the fitted model than the others. These runs should therefore be investigated first if more than one outlier appears from other diagnostics graphs. However, most diagnostics graphs showed observations within their limits, thus there is no need to further investigate these runs.

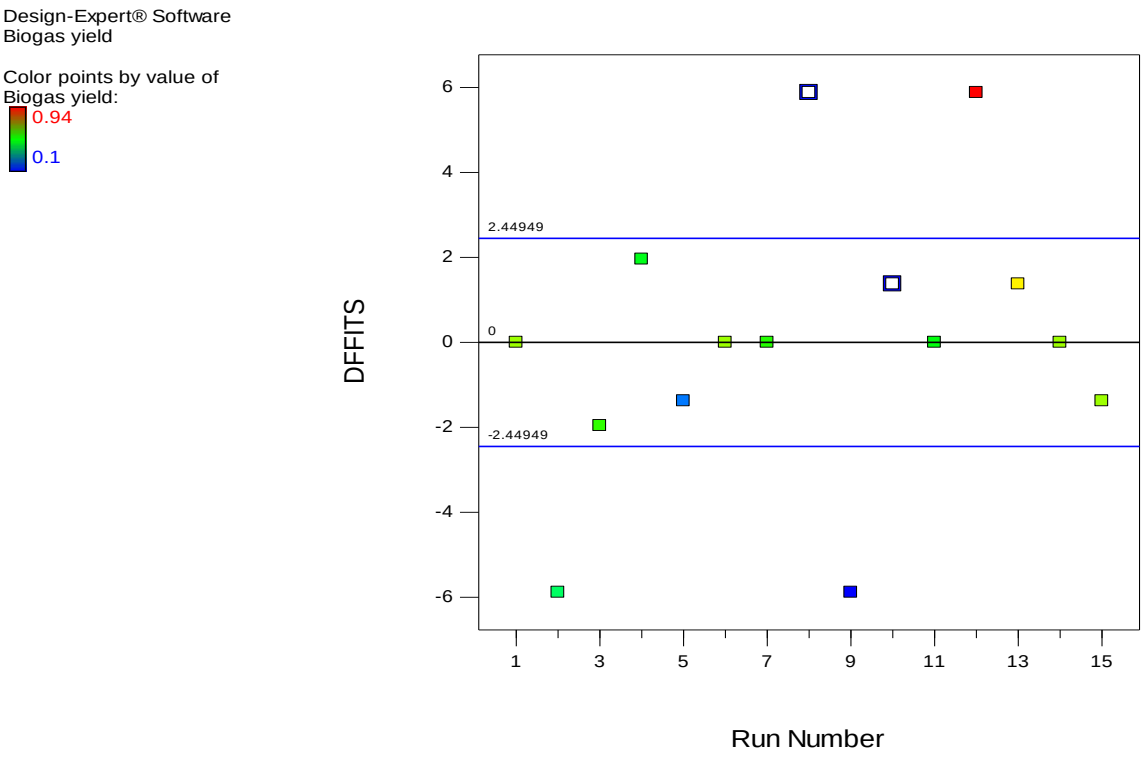


Figure P35: DFFITS versus run number for biogas yield

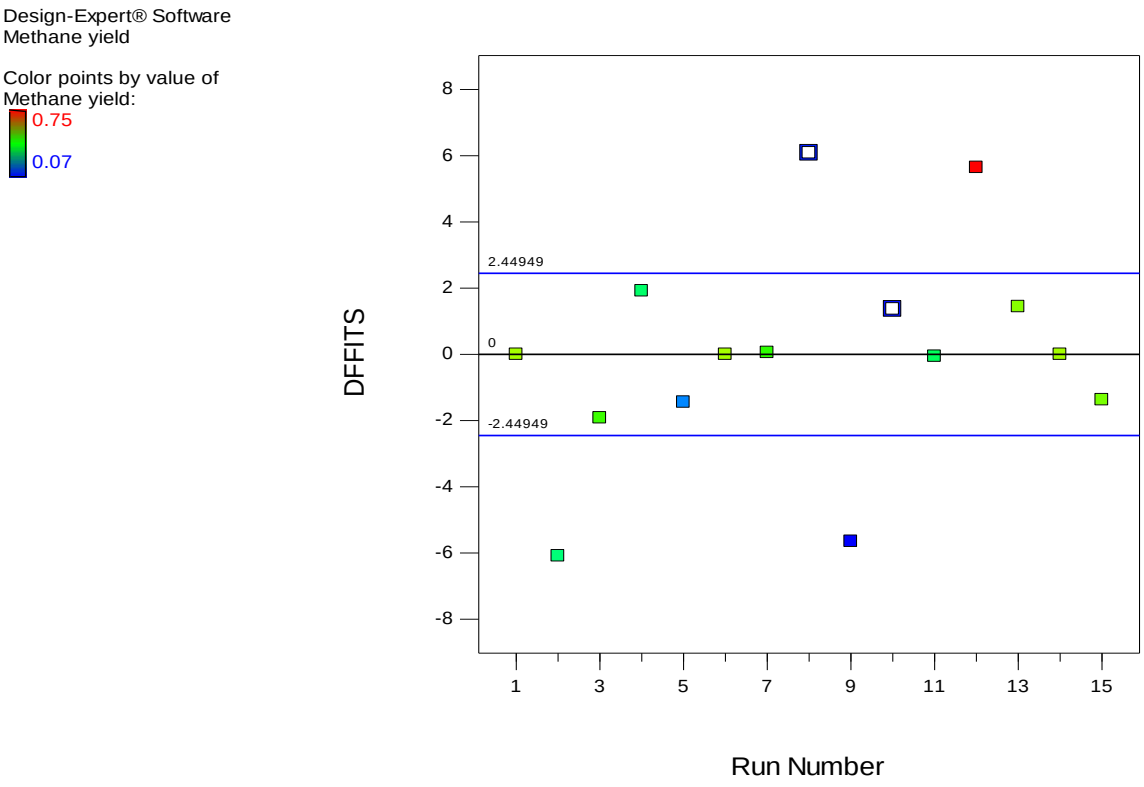


Figure P36: DFFITS versus run number for methane yield

The last significant effect tool to look at is the DBETAS tool. Figure P37, Figure P38, Figure P39, Figure P40, Figure P41, Figure P42, Figure P43, Figure P44, Figure P45, Figure P46, Figure P47, Figure P48, Figure P49, Figure P50, Figure P51, Figure P52, Figure P53, Figure P54, Figure P55, and Figure P56 show the DFBETAS versus run number for the intercept, A, B, C, AB, AC, BC, A^2, B^2 and C^2 for biogas and methane yields, respectively. The DBETAS limits were ± 0.774597 (see Appendix M10 for calculation). Run 9 and 12 are outside the DBETAS limits for A. Run 2 and 8 are outside the DBETAS limits for B and BC. Run 2, 8, 9 and 12 are outside the DBETAS limits for C, A^2, B^2 and C^2. Run 3 and 4 are outside the DBETAS limits for AB. Run 5 and 9 are outside the DBETAS limits for AC. The DFBETAS values outside the limits are indications that the coefficients for these terms are changing as a result of effect from the run.

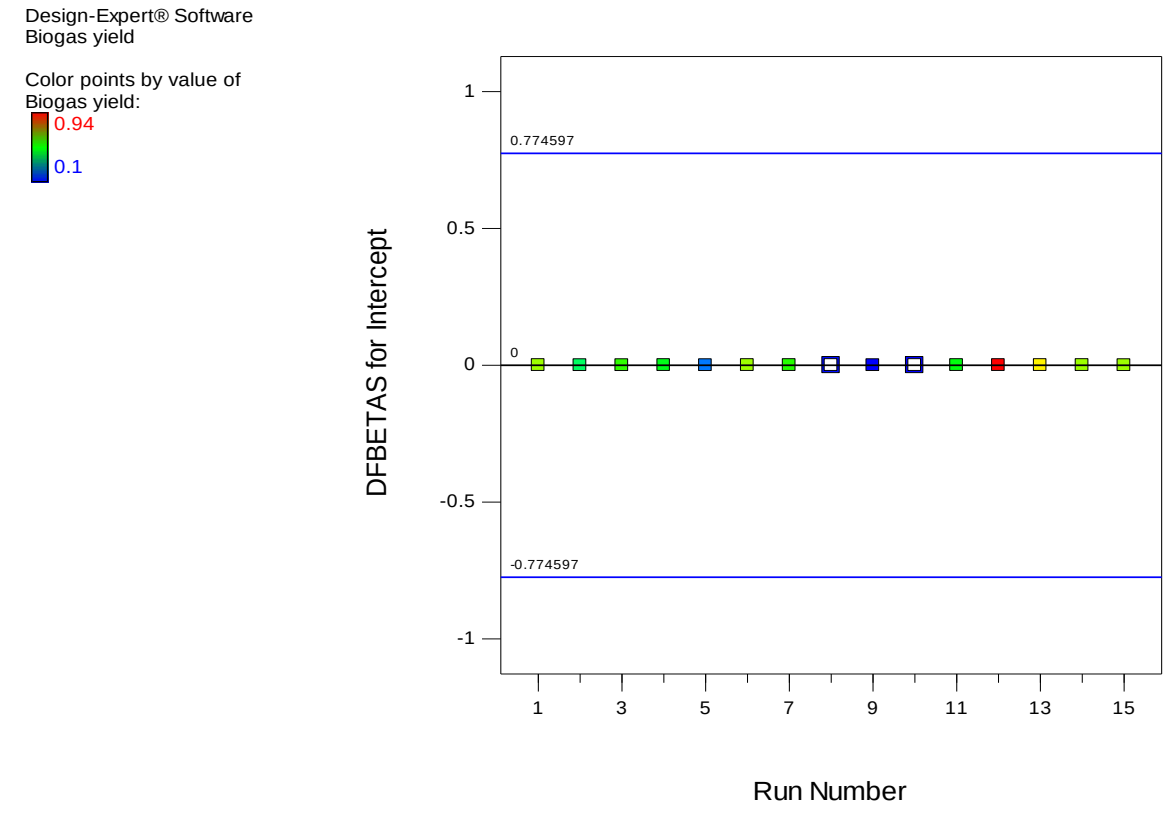


Figure P37: DFBETAS for intercept versus run number for biogas yield

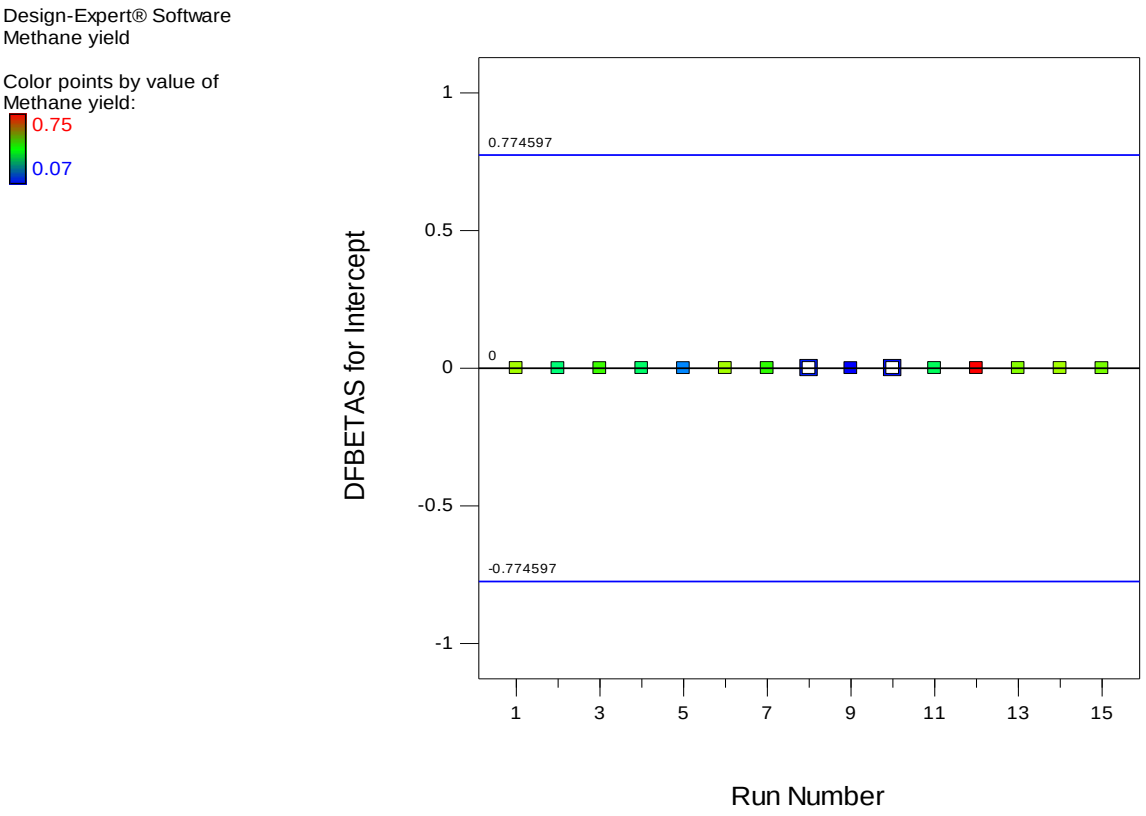


Figure P38: DFBETAS for intercept versus run number for methane yield

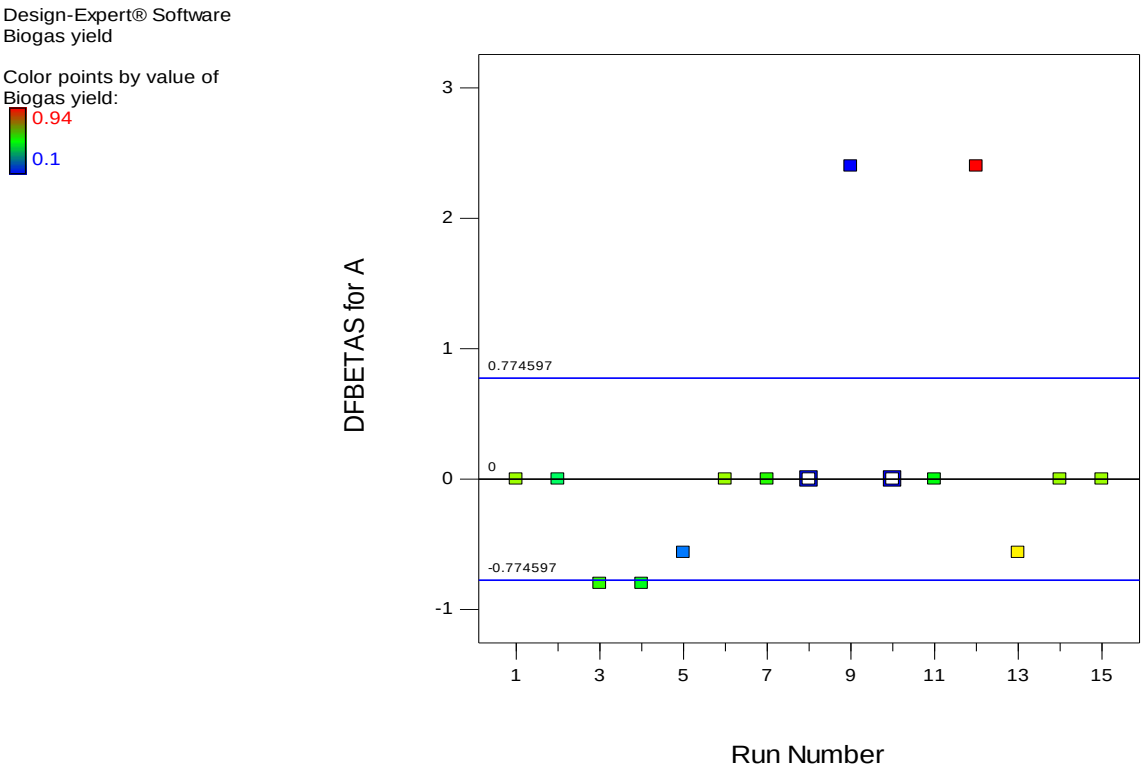


Figure P39: DFBETAS for A versus run number for biogas yield

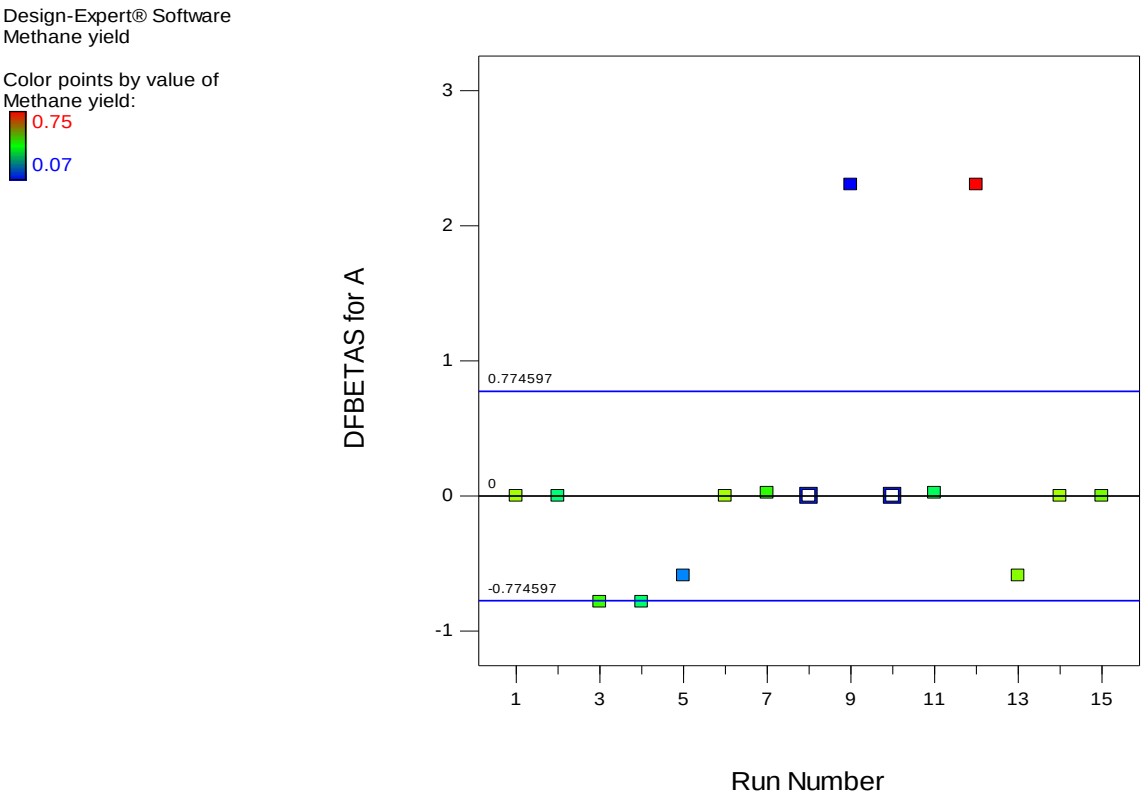


Figure P40: DFBETAS for A versus run number for methane yield

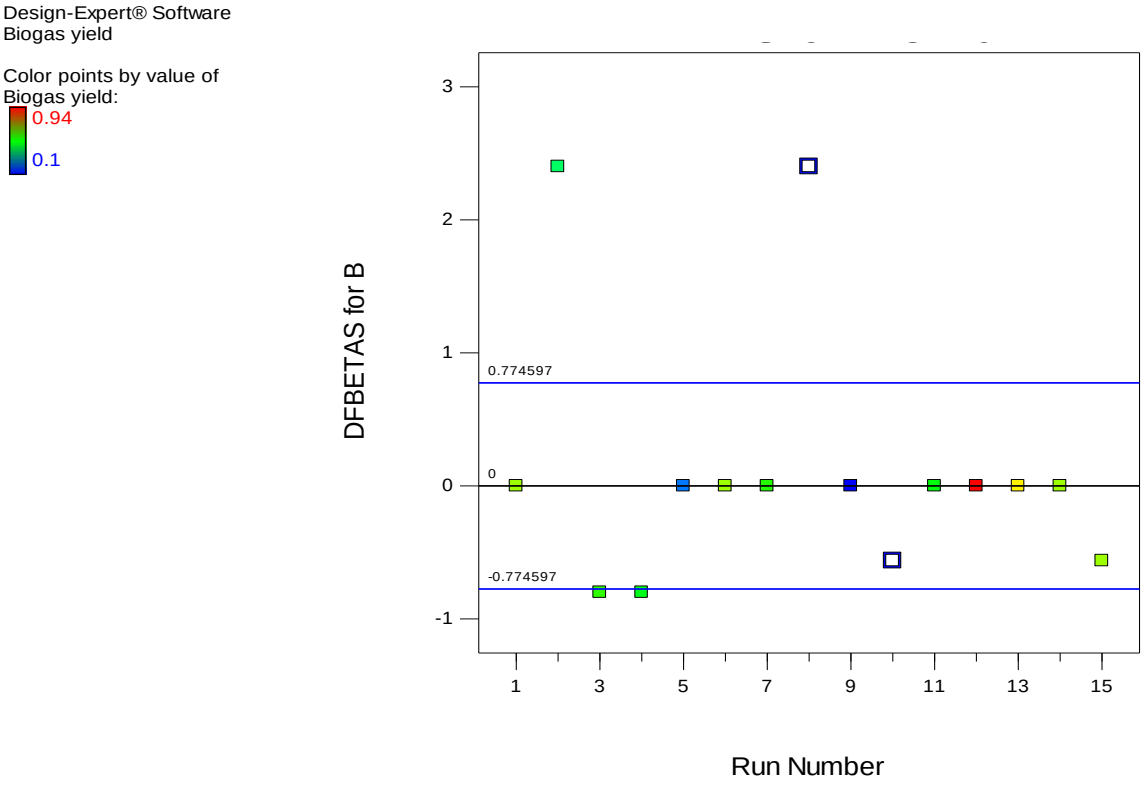


Figure P41: DFBETAS for B versus run number for biogas yield

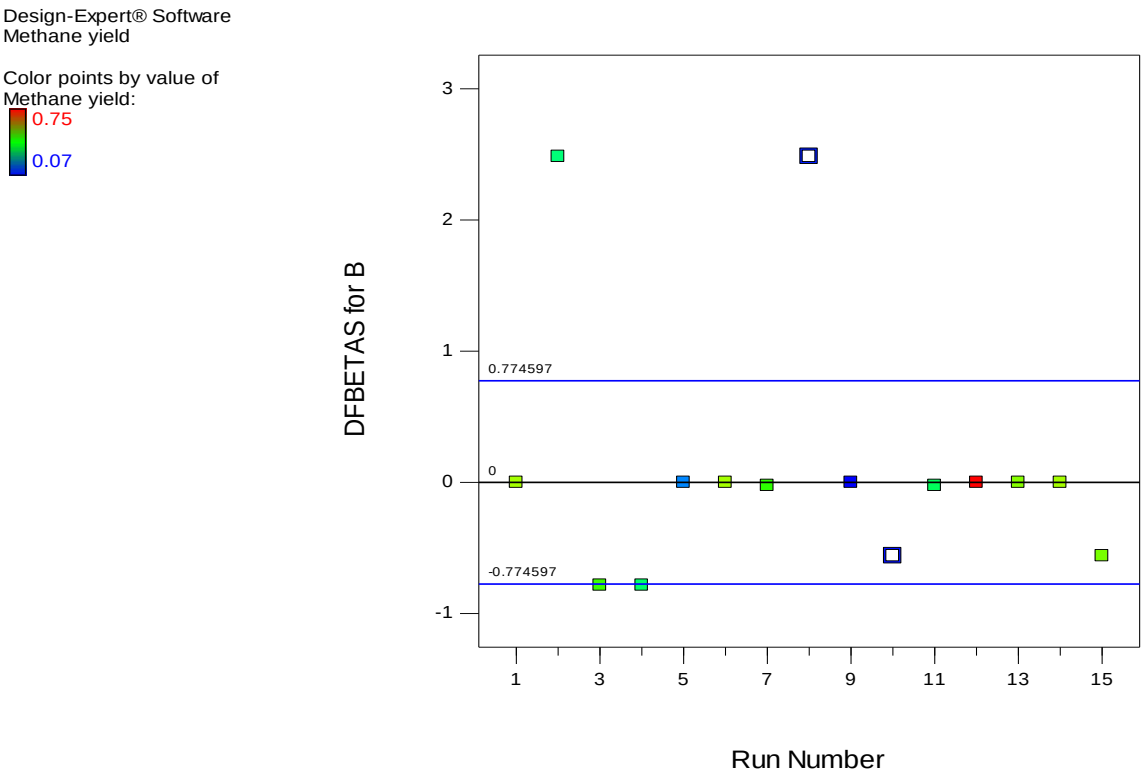


Figure P42: DFBETAS for B versus run number for methane yield

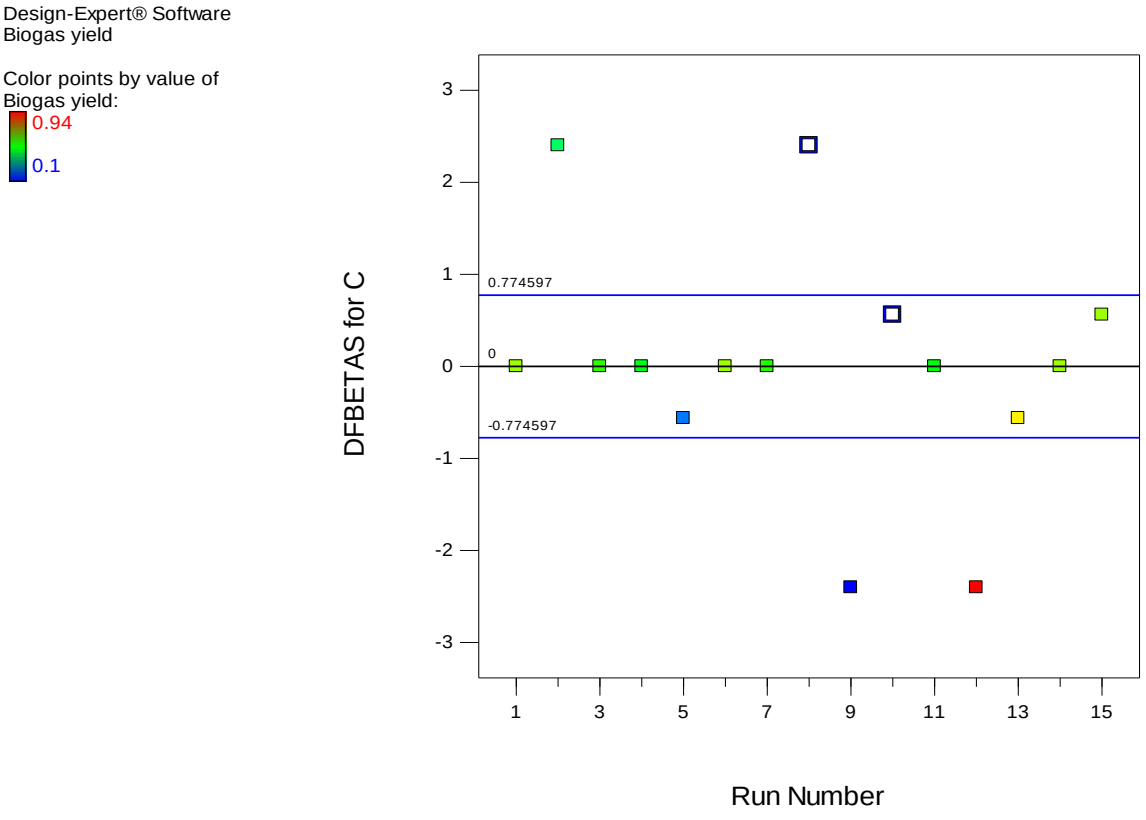


Figure P43: DFBETAS for C versus run number for biogas yield

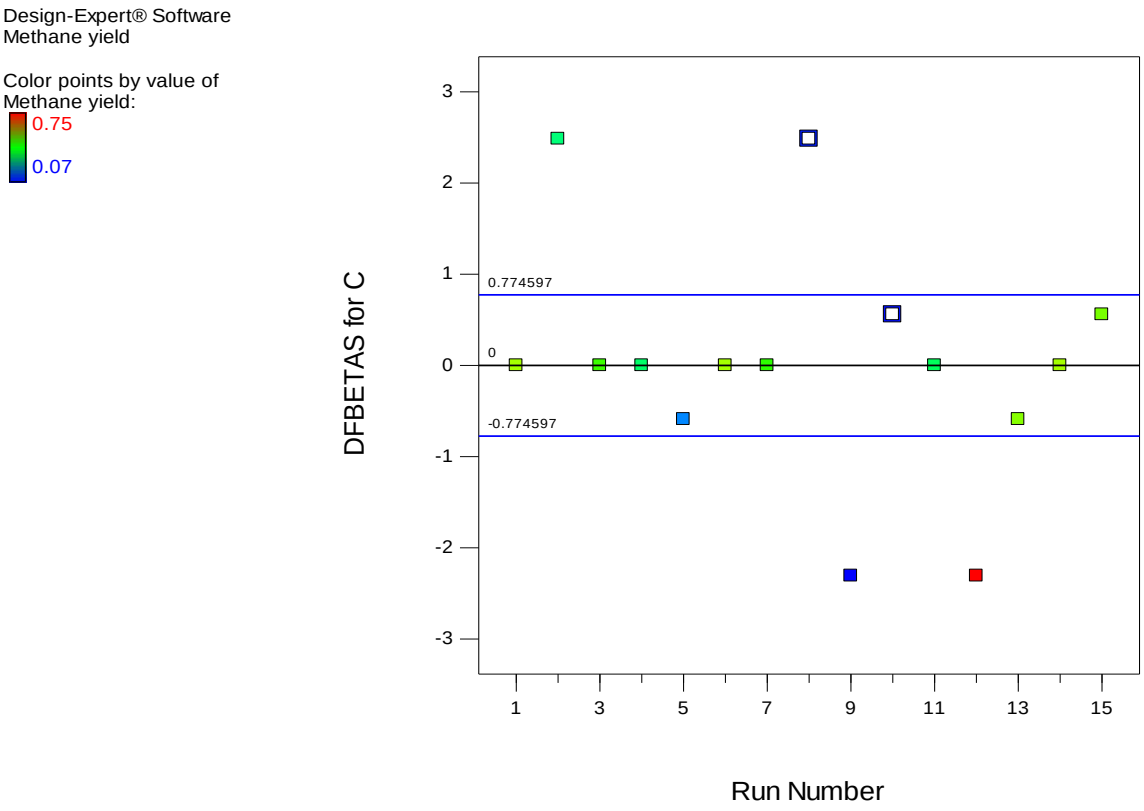


Figure P44: DFBETAS for C versus run number for methane yield

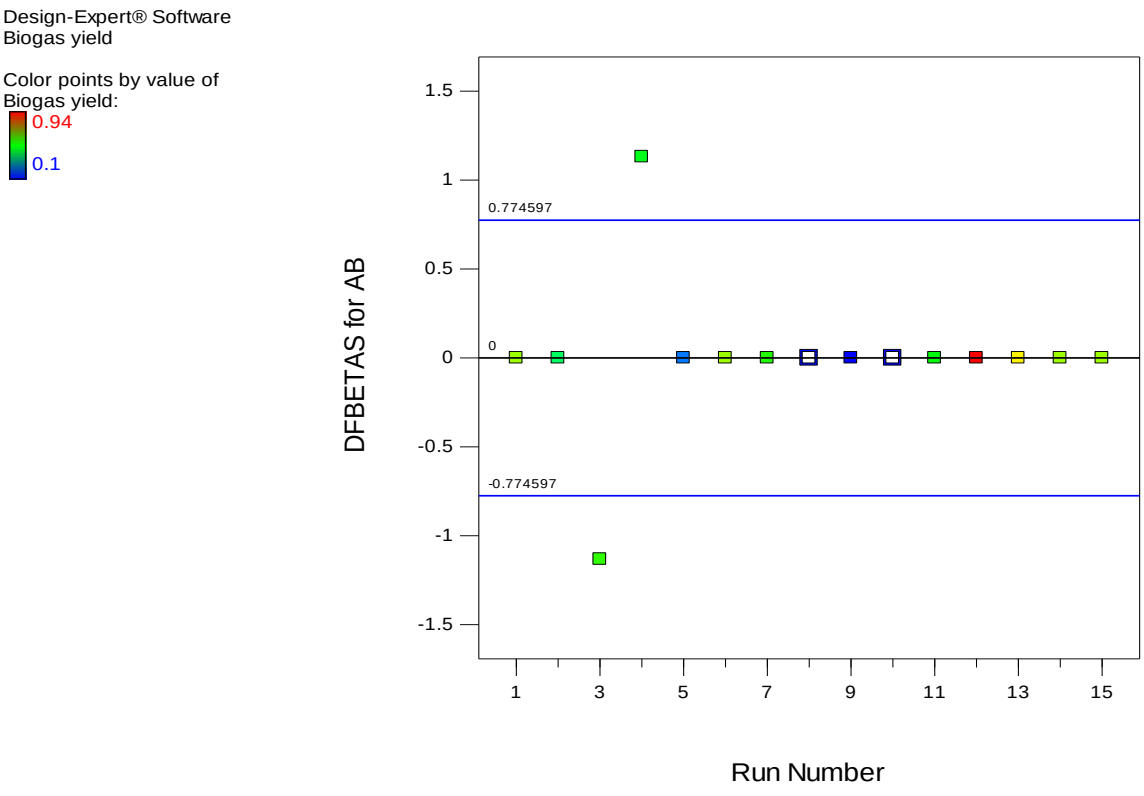


Figure P45: DFBETAS for AB versus run number for biogas yield

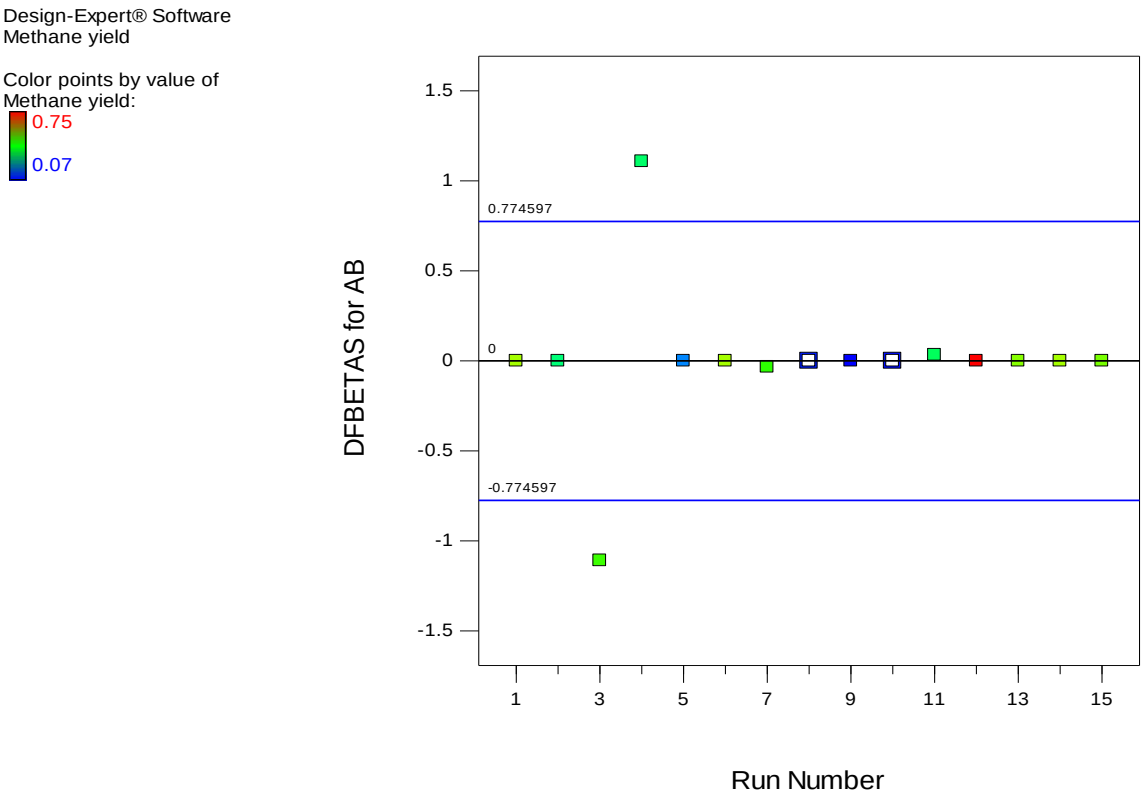


Figure P46: DFBETAS for AB versus run number for methane yield

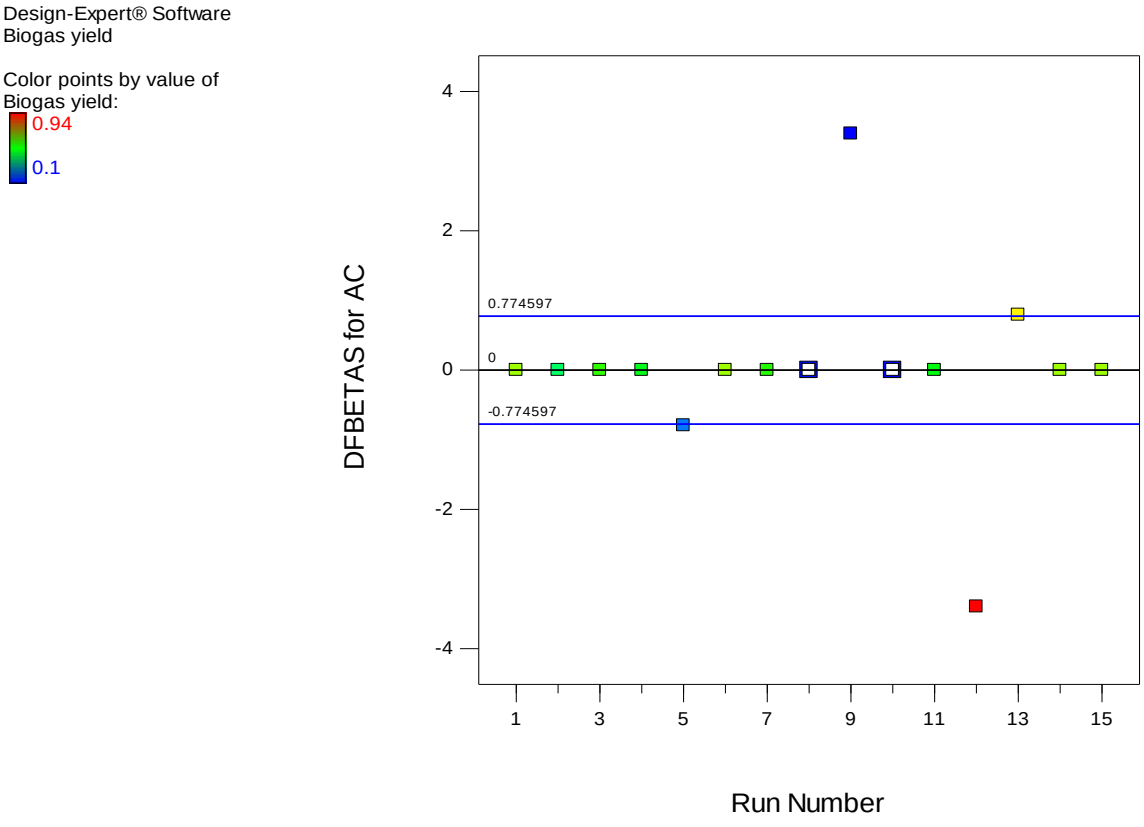


Figure P47: DFBETAS for AC versus run number for biogas yield

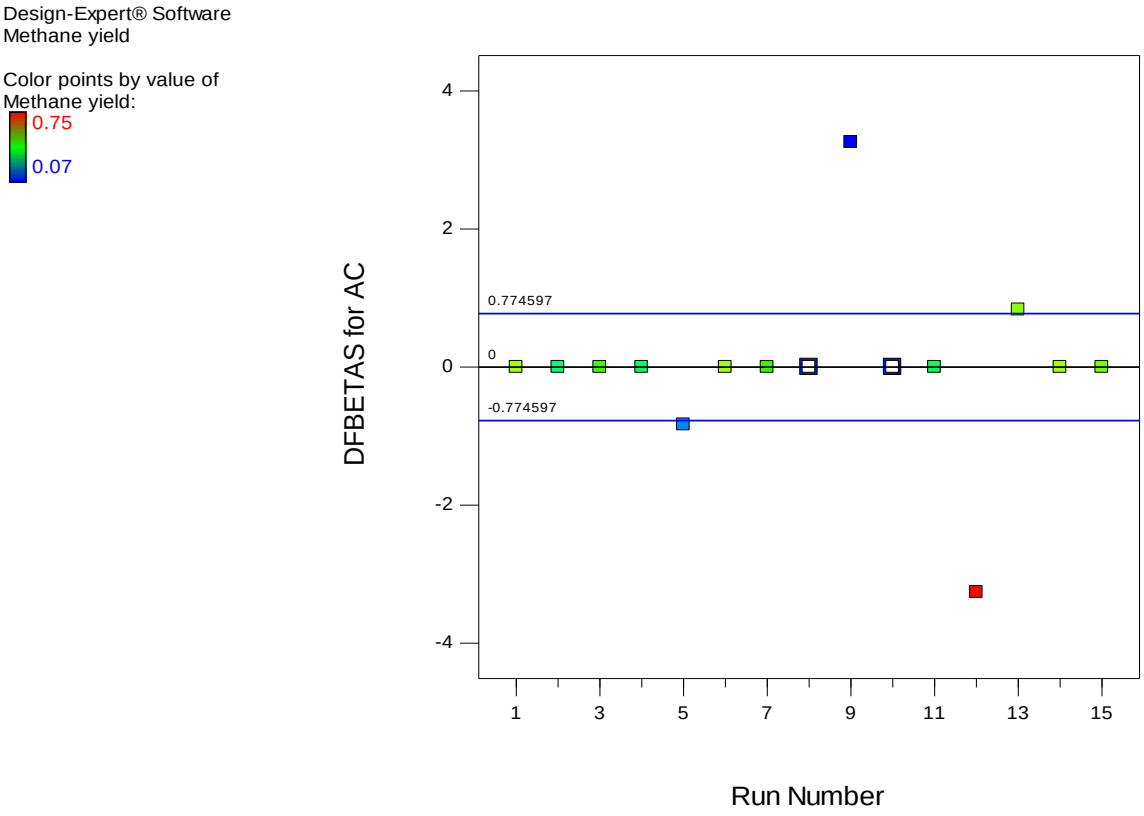


Figure P48: DFBETAS for AC versus run number for methane yield

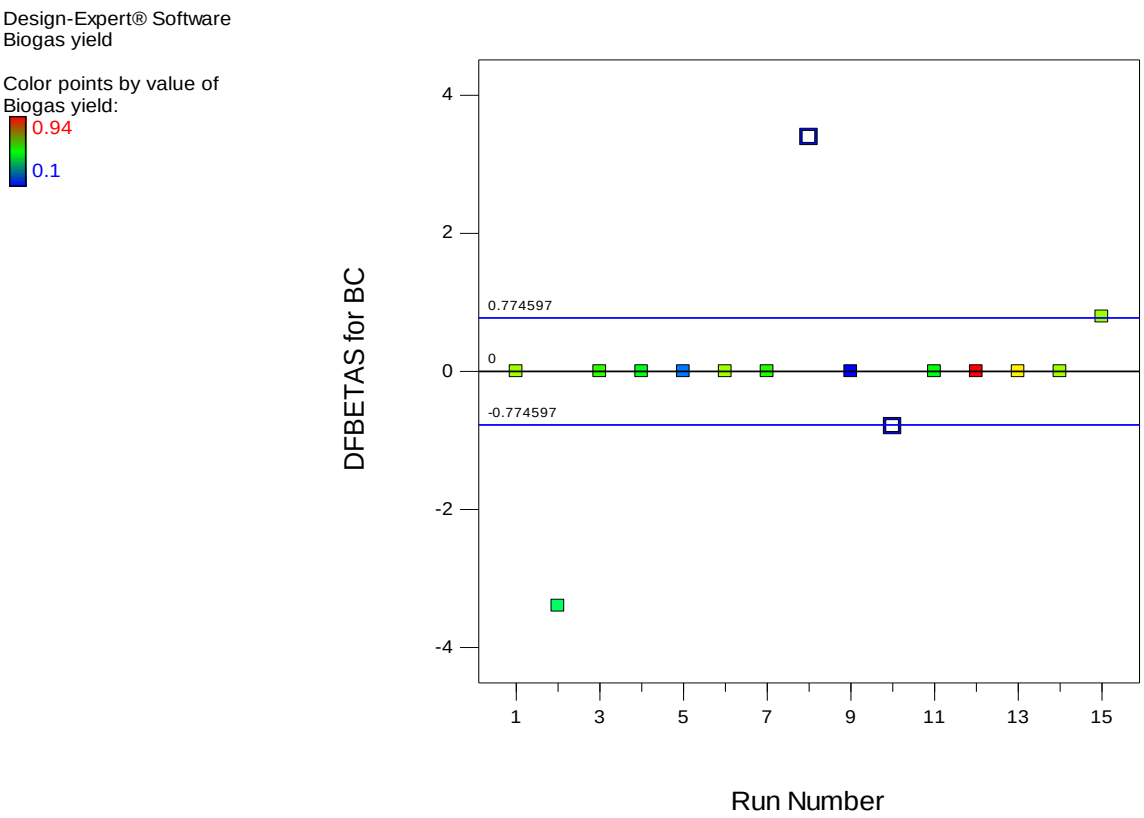


Figure P49: DFBETAS for BC versus run number for biogas yield

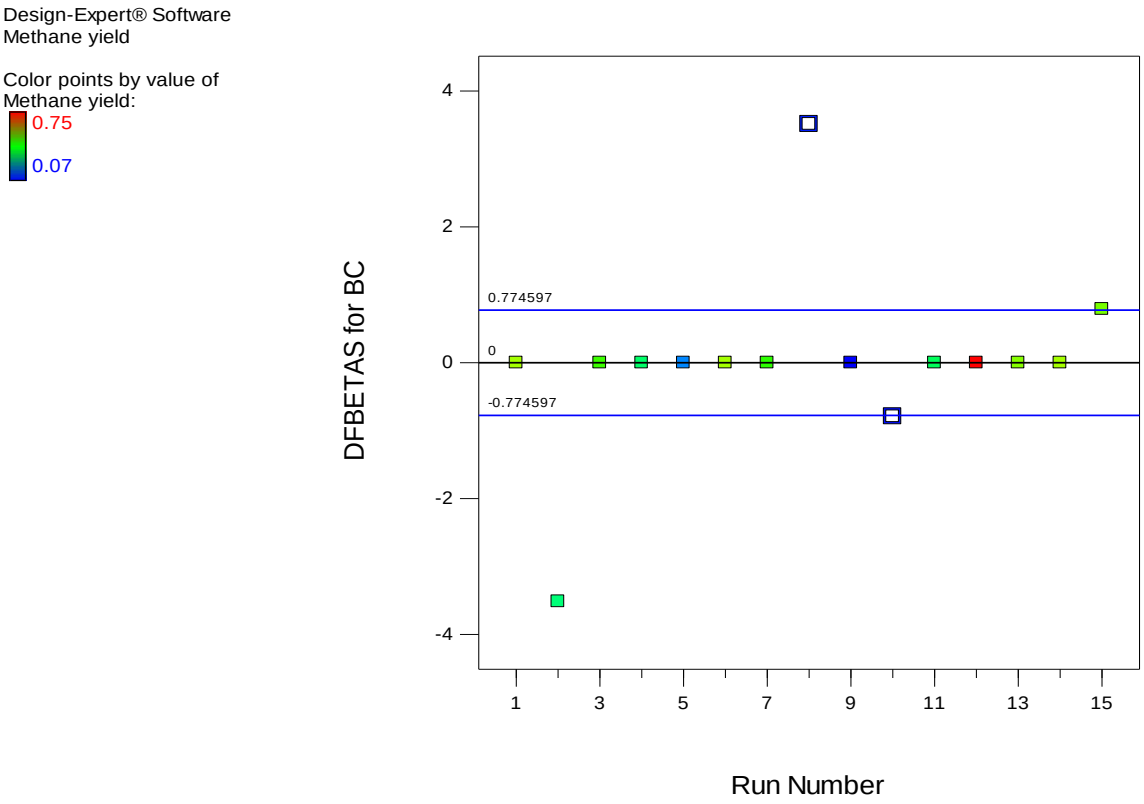


Figure P50: DFBETAS for BC versus run number for methane yield

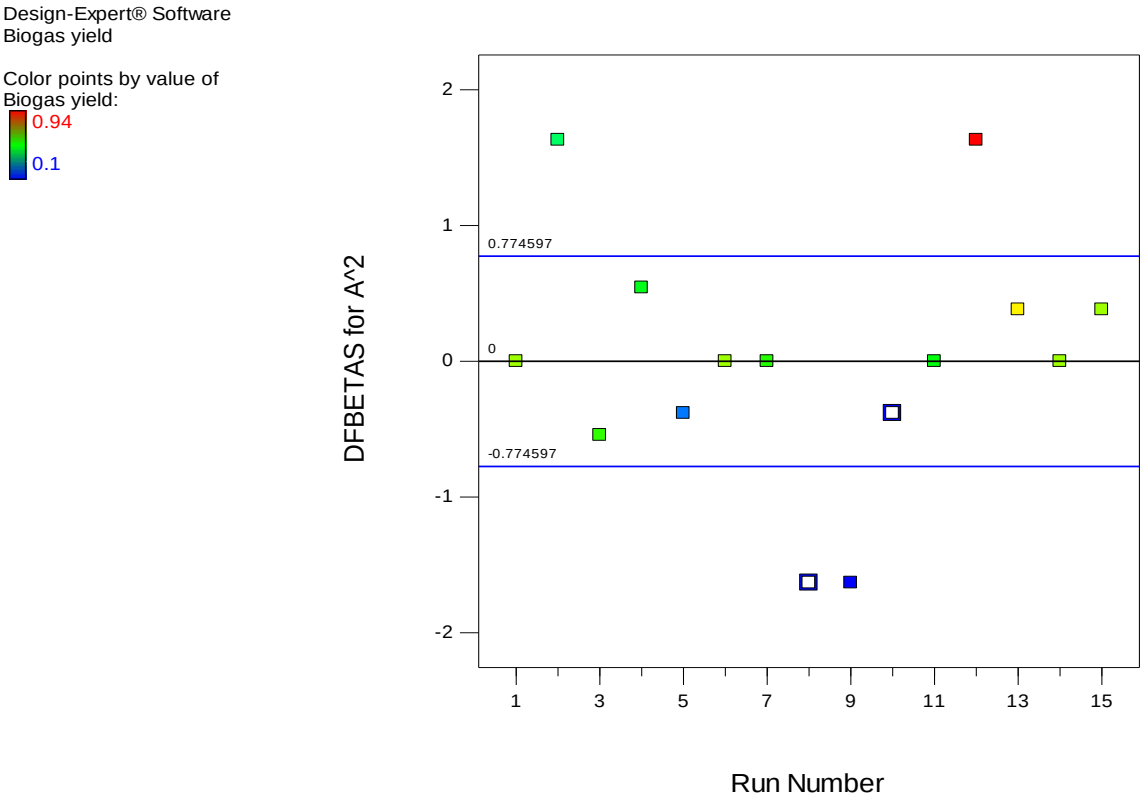


Figure P51: DFBETAS for A^2 versus run number for biogas yield

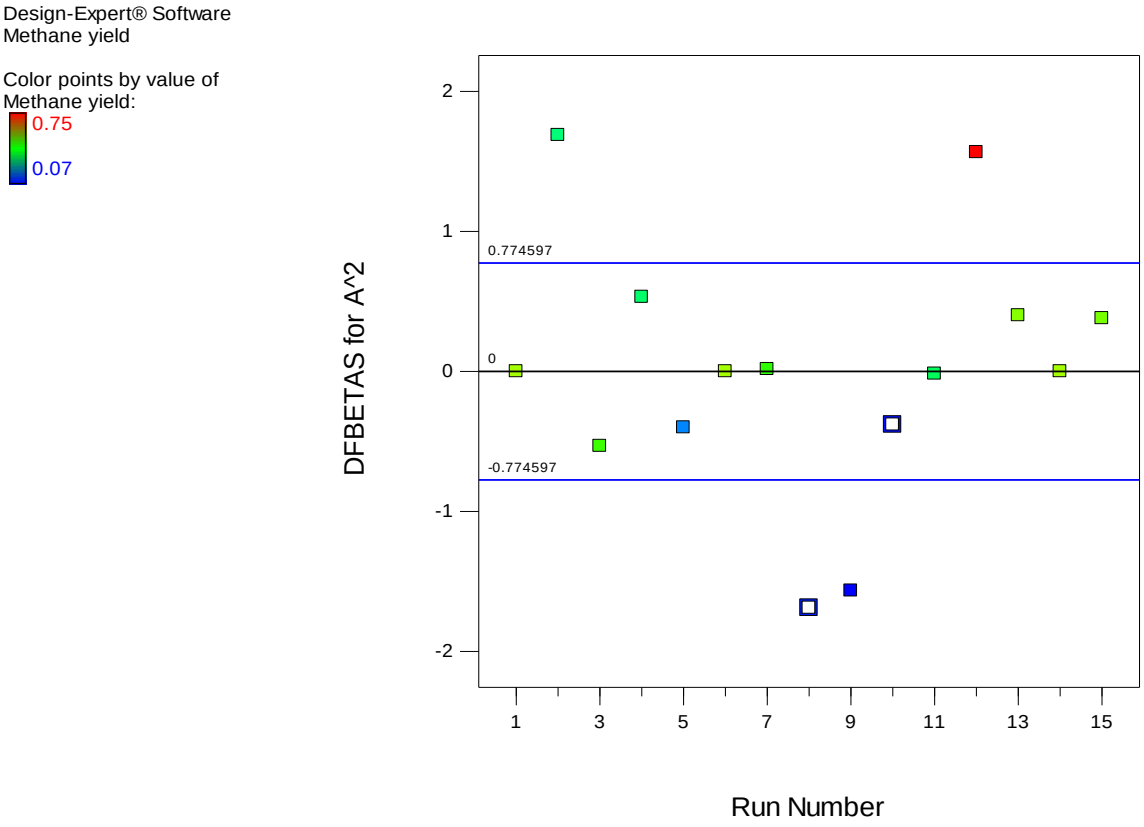


Figure P52: DFBETAS for A^2 versus run number for methane yield

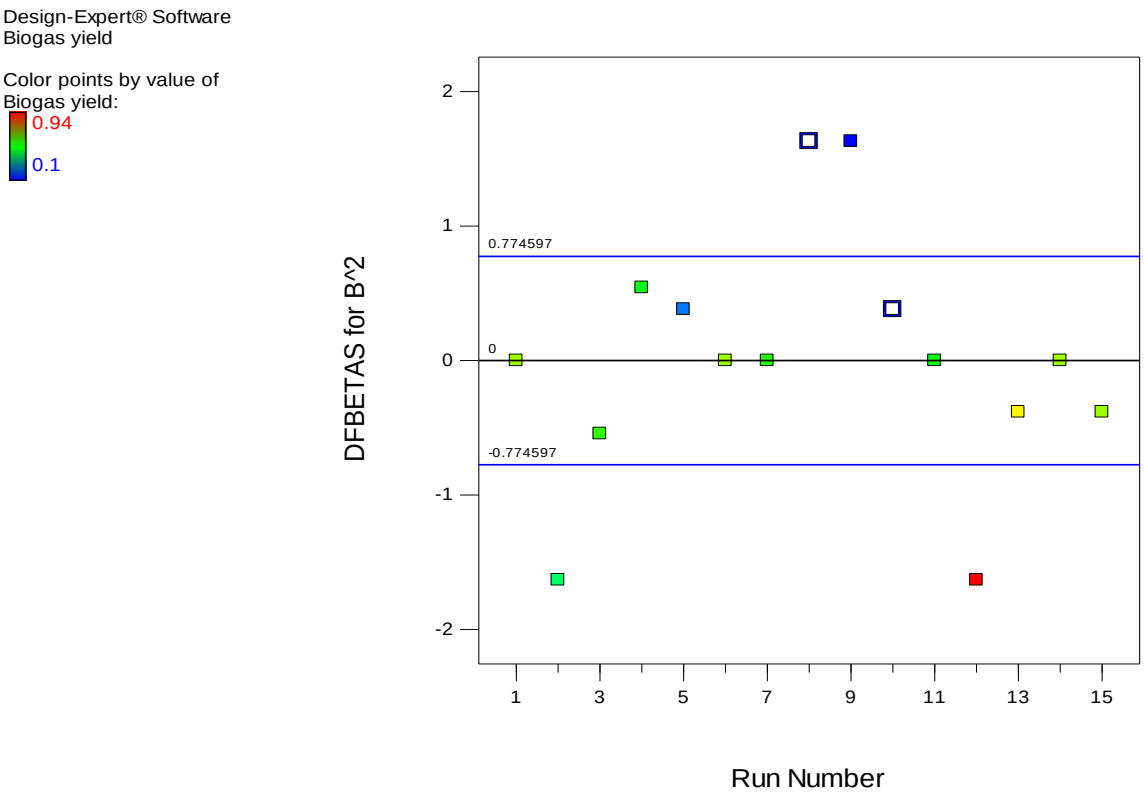


Figure P53: DFBETAS for B^2 versus run number for biogas yield

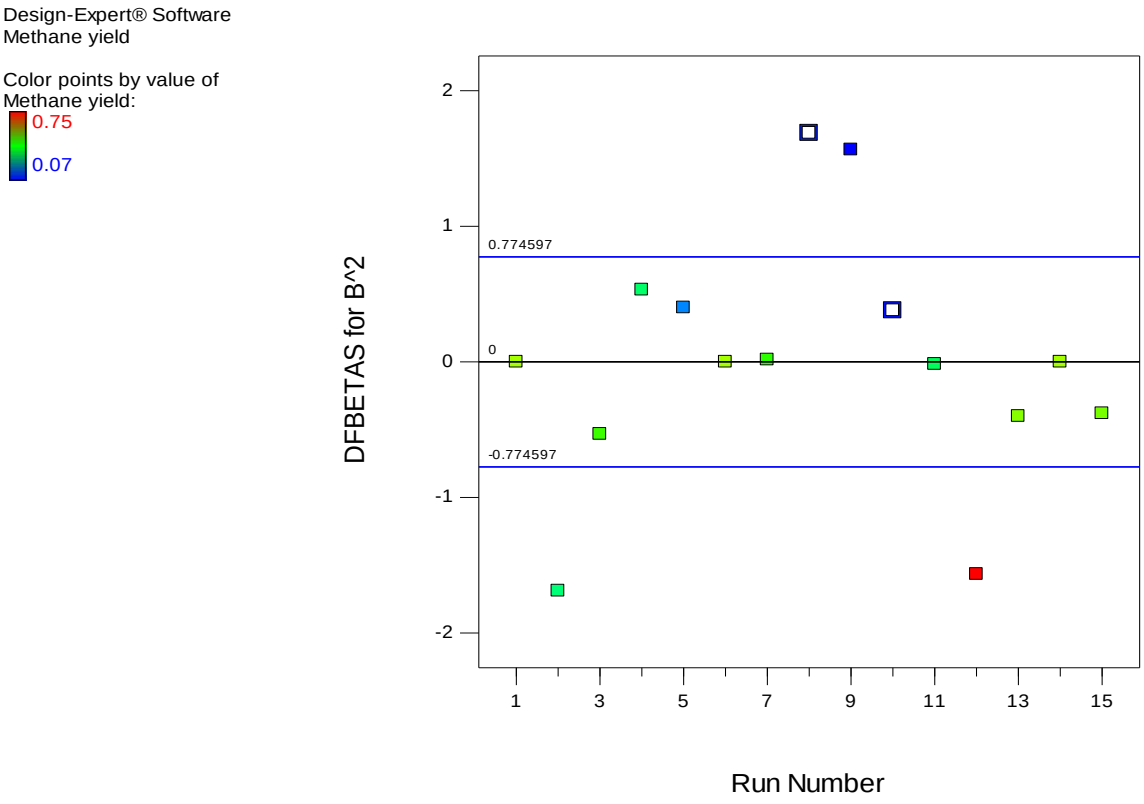


Figure P54: DFBETAS for B^2 versus run number for methane yield

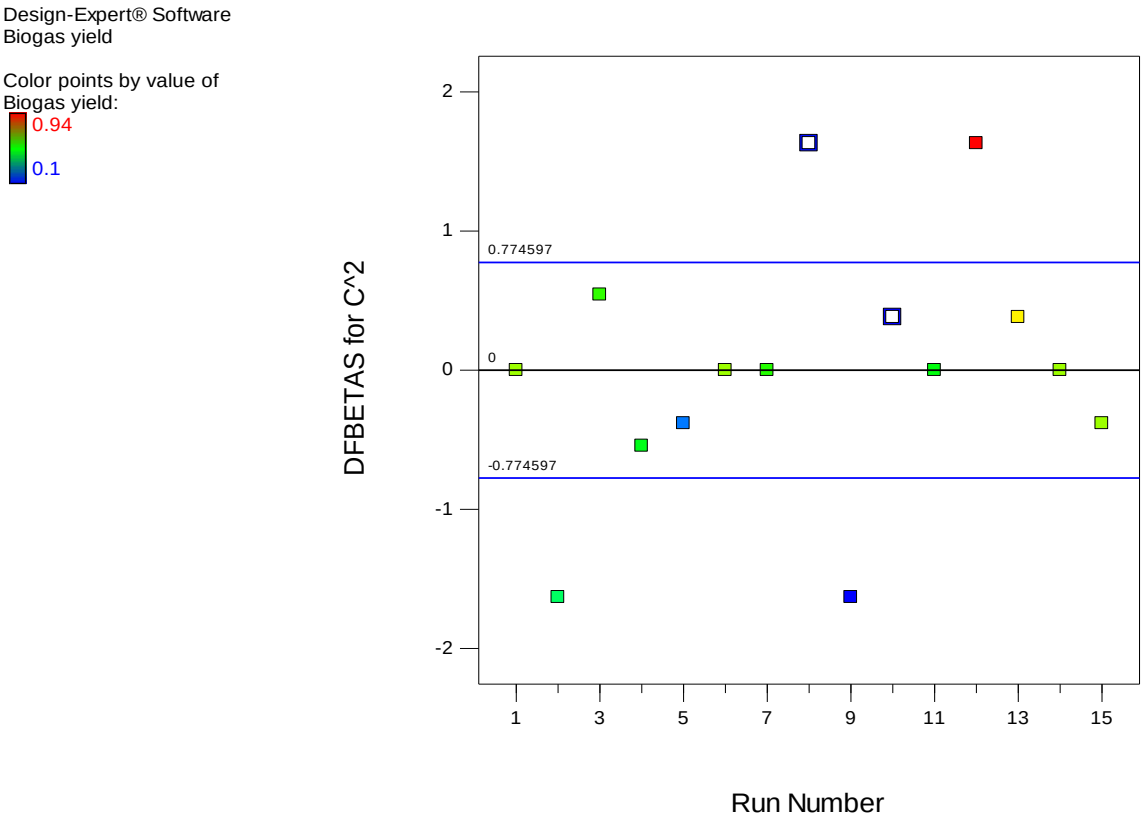


Figure P55: DFBETAS for C^2 versus run number for biogas yield

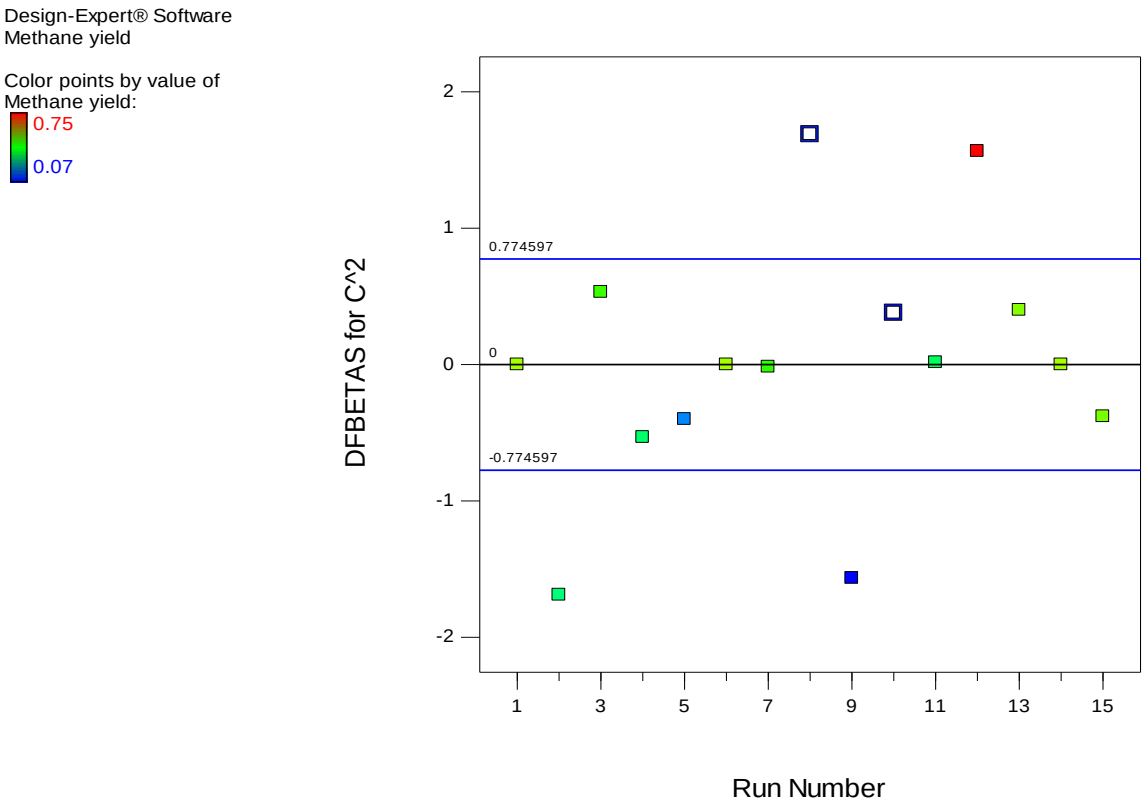


Figure P56: DFBETAS for C^2 versus run number for methane yield

The case-by-case diagnostic statistical reports for biogas and methane yields are shown in Table P10 and Table P11, respectively. The diagnostics report shows the numbers used to generate the diagnostic graphs. Values that are discrepant will be highlighted if the model is abnormal (those with asterisk *). In this case, run order 2, 8, 9 and 12 exceed limits.

Table P10: Diagnostics report for biogas yields.

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residual	Externally Studentized Residual	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
1	0.65	0.65	0.000	0.333	0.000	0.000	0.000	0.000	14
2	0.44	0.53	-0.088	0.750	-1.927	-3.395	1.114*	-5.881*	9
3	0.56	0.61	-0.050	0.750	-1.101	-1.131	0.364	-1.960	4
4	0.50	0.45	0.050	0.750	1.101	1.131	0.364	1.960	1
5	0.20	0.24	-0.038	0.750	-0.826	-0.795	0.205	-1.376	8
6	0.65	0.65	0.000	0.333	0.000	0.000	0.000	0.000	15
7	0.55	0.55	0.000	0.750	0.000	0.000	0.000	0.000	2
8	0.11	0.022	0.088	0.750	1.927	3.395	1.114*	5.881*	12
9	0.10	0.19	-0.088	0.750	-1.927	-3.395	1.114*	-5.881*	7
10	0.10	0.06	0.037	0.750	0.826	0.795	0.205	1.376	11
11	0.51	0.51	0.000	0.750	0.000	0.000	0.000	0.000	3
12	0.94	0.85	0.087	0.750	1.927	3.395	1.114*	5.881*	6
13	0.74	0.70	0.037	0.750	0.826	0.795	0.205	1.376	5
14	0.65	0.65	0.000	0.333	0.000	0.000	0.000	0.000	13
15	0.65	0.69	-0.038	0.750	-0.826	-0.795	0.205	-1.376	10

* Discrepant values as the model is abnormal

Table P11: Diagnostics report for methane yields.

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residual	Externally Studentized Residual	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
1	0.52	0.52	0.000	0.333	0.000	0.000	0.000	0.000	14
2	0.33	0.40	-0.065	0.750	-1.943	-3.514	1.133*	-6.086*	9
3	0.45	0.49	-0.036	0.750	-1.084	-1.108	0.352	-1.920	4
4	0.34	0.30	0.036	0.750	1.084	1.108	0.352	1.920	1
5	0.16	0.19	-0.029	0.750	-0.860	-0.833	0.222	-1.442	8
6	0.52	0.52	0.000	0.333	0.000	0.000	0.000	0.000	15
7	0.44	0.44	1.25×10^{-3}	0.750	0.037	0.033	0.000	0.058	2
8	0.09	0.025	0.065	0.750	1.943	3.514	1.133*	6.086*	12
9	0.07	0.13	-0.064	0.750	-1.906	-3.260	1.090*	-5.646*	7
10	0.08	0.053	0.027	0.750	0.822	0.791	0.203	1.370	11
11	0.35	0.35	-1.25×10^{-3}	0.750	-0.037	-0.033	0.000	-0.058	3
12	0.75	0.69	0.064	0.750	1.906	3.260	1.090*	5.646*	6
13	0.50	0.47	0.029	0.750	0.860	0.833	0.222	1.442	5
14	0.52	0.52	0.000	0.333	0.000	0.000	0.000	0.000	13
15	0.49	0.52	-0.028	0.750	-0.822	-0.791	0.203	-1.370	10

* Discrepant values as the model is abnormal.

P5 - Main effects and interaction of variables

In general, an effect or impact is the change in the output caused by changes in the variables. A linear effect is the average output value at the high setting of a variable less the average output value at the low setting of the variable. An interaction takes place when the output is different depending on the locations of two variables. Graphically, this occurs where the two curves meet. If the curves are parallel, then there is no interaction between the two variables.

Interaction of concentration and temperature for biogas and methane yields at substrate to inoculum ratios of 0.5, 1.0 and 1.5 are shown in Figure P57, Figure P58, Figure P59, Figure P60, Figure P61 and Figure P62, respectively. For substrate to inoculum of 0.5 the volume

concentration intersects the temperature at 31 and 48℃ for the biogas and at 32 and above 50℃ methane yields, respectively. This means that the impact of concentration is not as much of significant at mesophilic temperatures and also at thermophilic temperatures. Thus, the experimenters may operate either at mesophilic temperature and increase the volume concentration or at thermophilic temperatures and reduce the volume concentration of glycerol in the digester, to achieve higher biogas and methane yields.

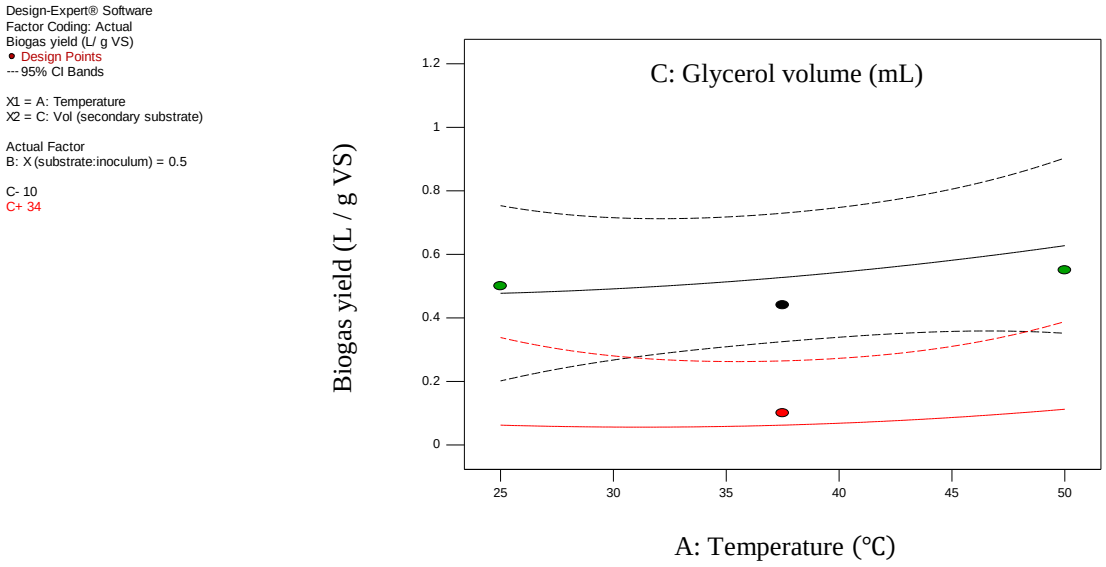


Figure P57: Interaction of glycerol volume and temperature for biogas yield at a substrate to inoculum ratio of 0.5.

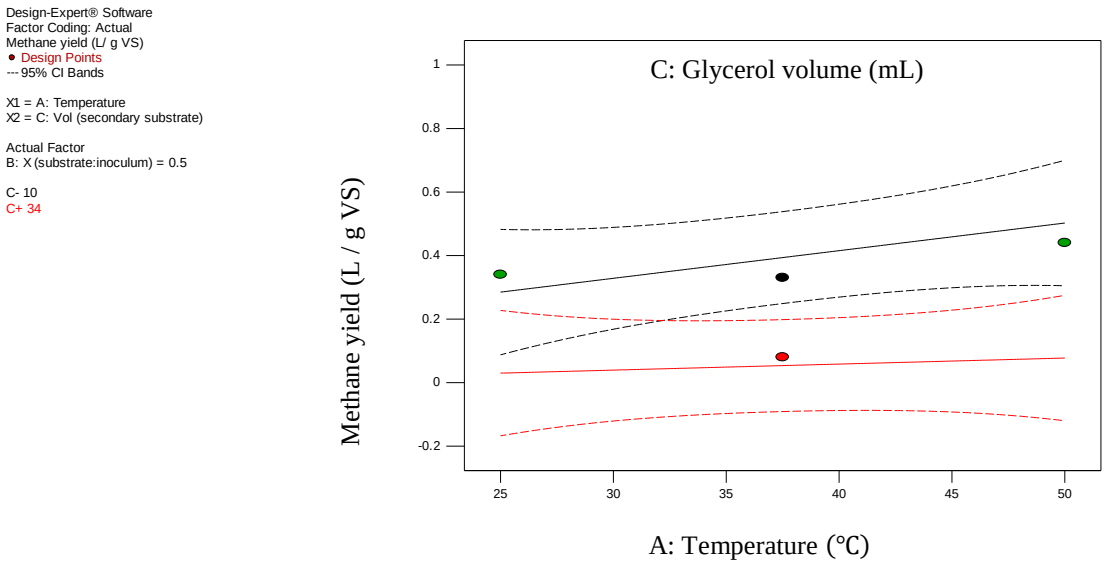


Figure P58: Interaction of glycerol volume and temperature for methane yield at a substrate to inoculum ratio of 0.5.

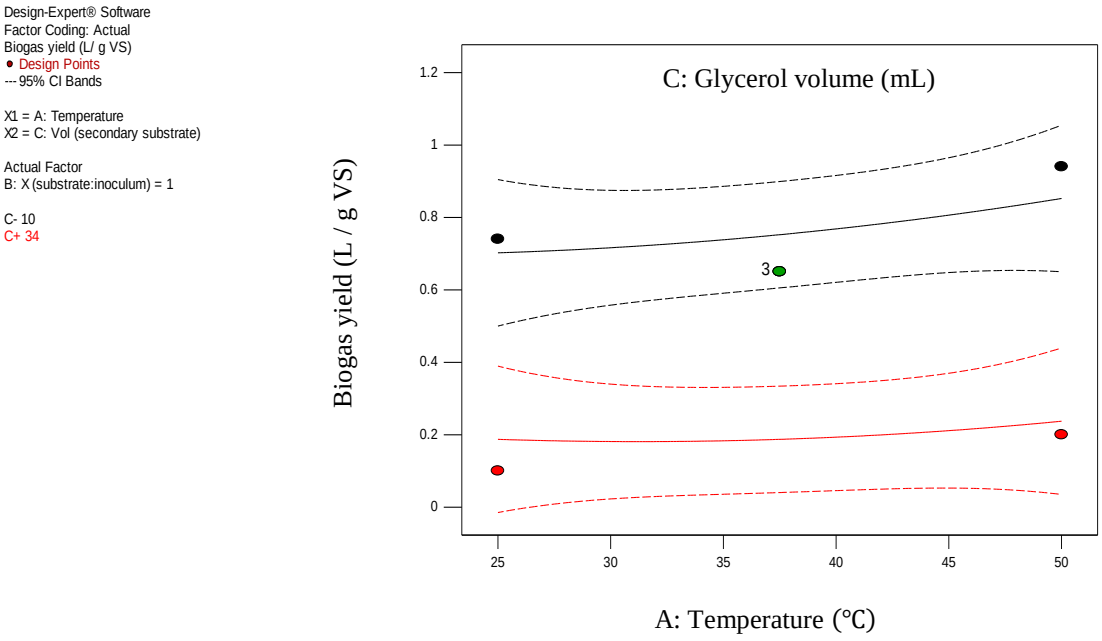


Figure P59: Interaction of glycerol volume and temperature for biogas yield at a substrate to inoculum ratio of 1.0.

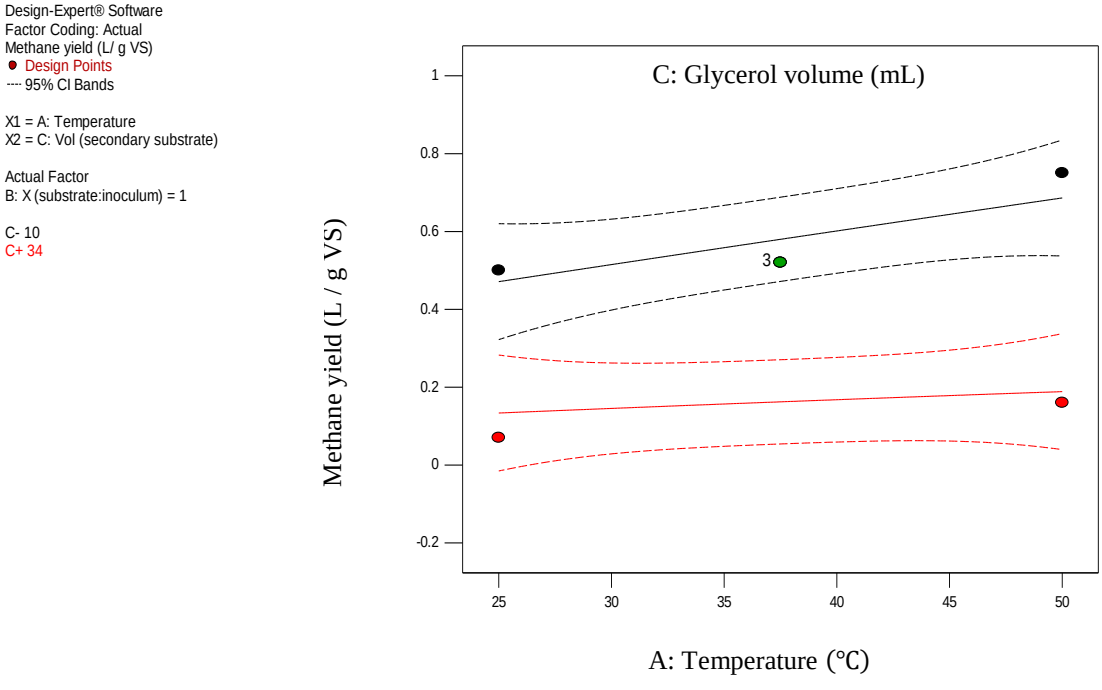


Figure P60: Interaction of glycerol volume and temperature for methane yield at a substrate to inoculum ratio of 1.0.

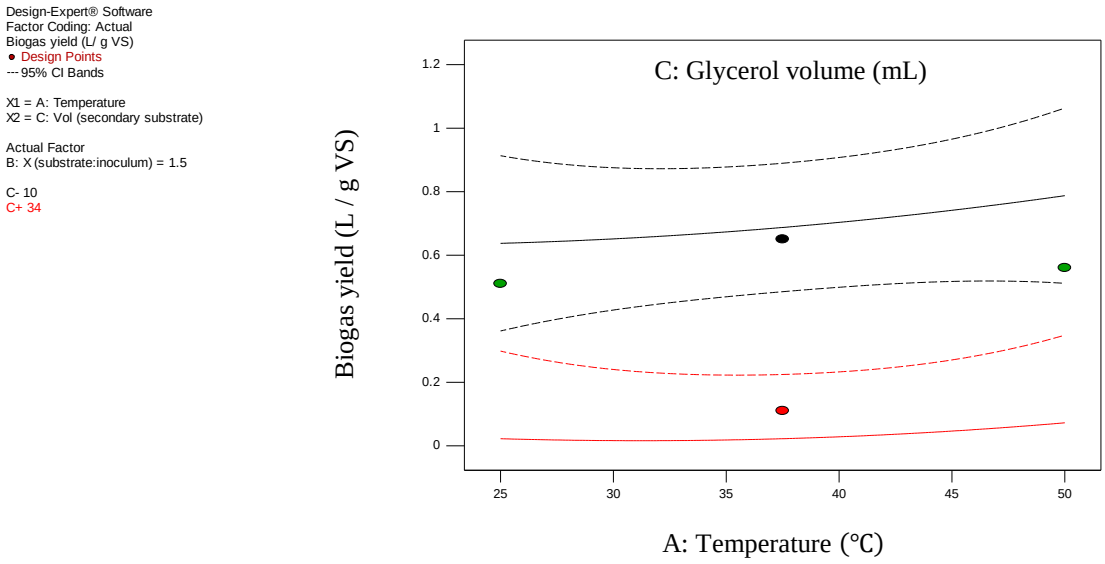


Figure P61: Interaction of glycerol volume and temperature for biogas yield at a substrate to inoculum ratio of 1.5.

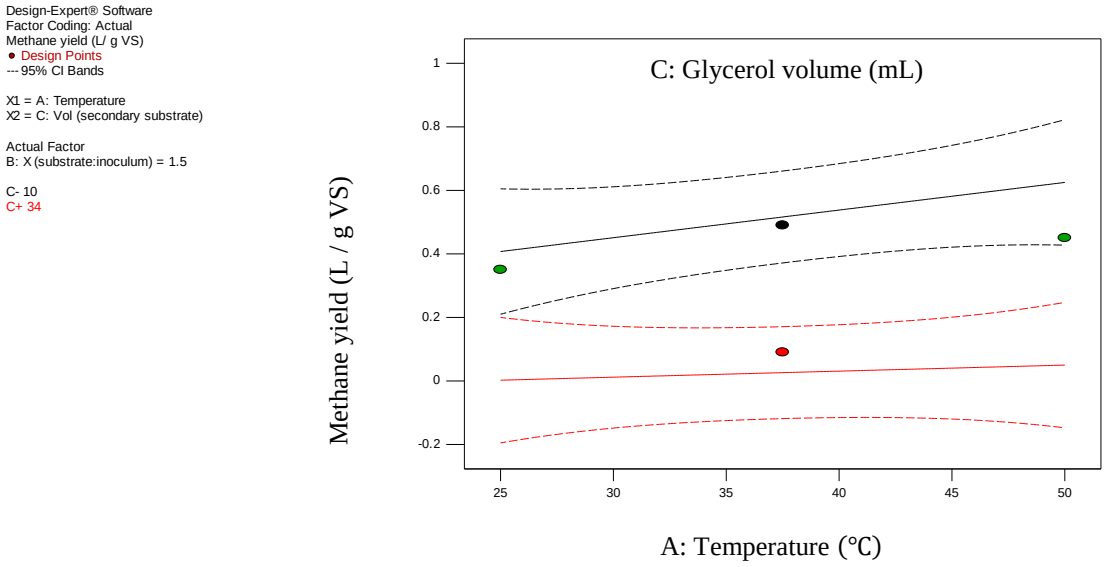


Figure P62: Interaction of glycerol volume and temperature for methane yield at a substrate to inoculum ratio of 1.5.

The interaction of substrate to inoculum ratio and temperature (AC) for biogas and methane yields at 10, 22 and 34 mL are shown in Figure P63, Figure P64, Figure P65, Figure P66, Figure P67 and Figure P68, respectively. For all glycerol volumes the curves are parallel, thus there were no interactions between the substrate to inoculum ratio and the temperature.

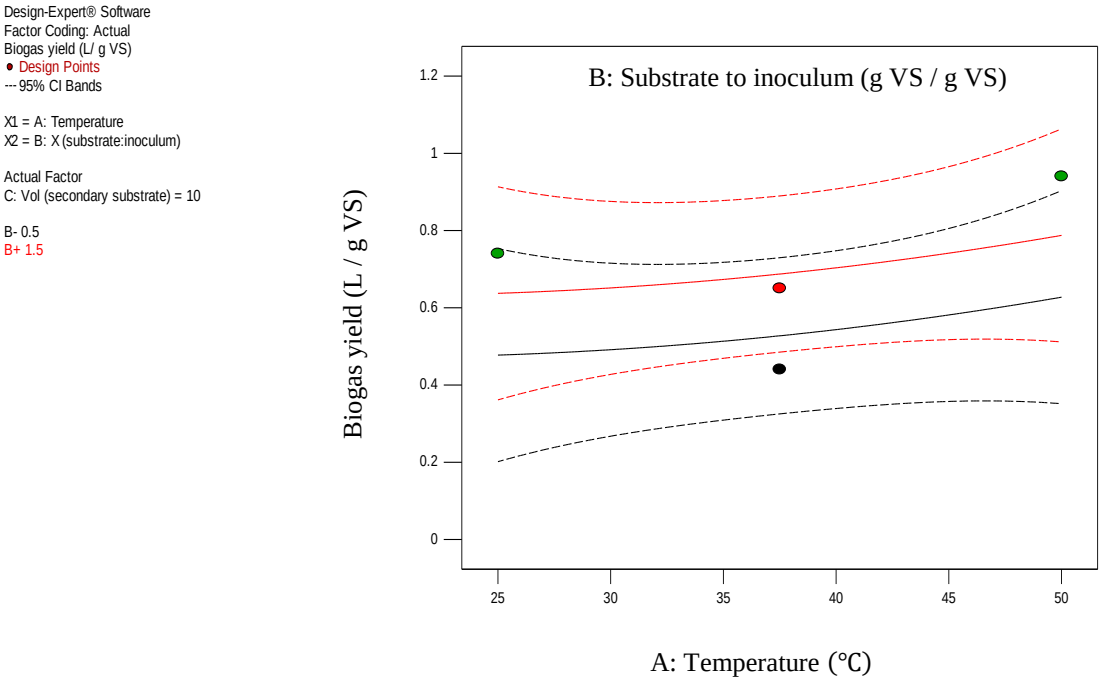


Figure P63: Interaction of substrate to inoculum ratio and temperature for biogas yield at a glycerol volume of 10 mL.

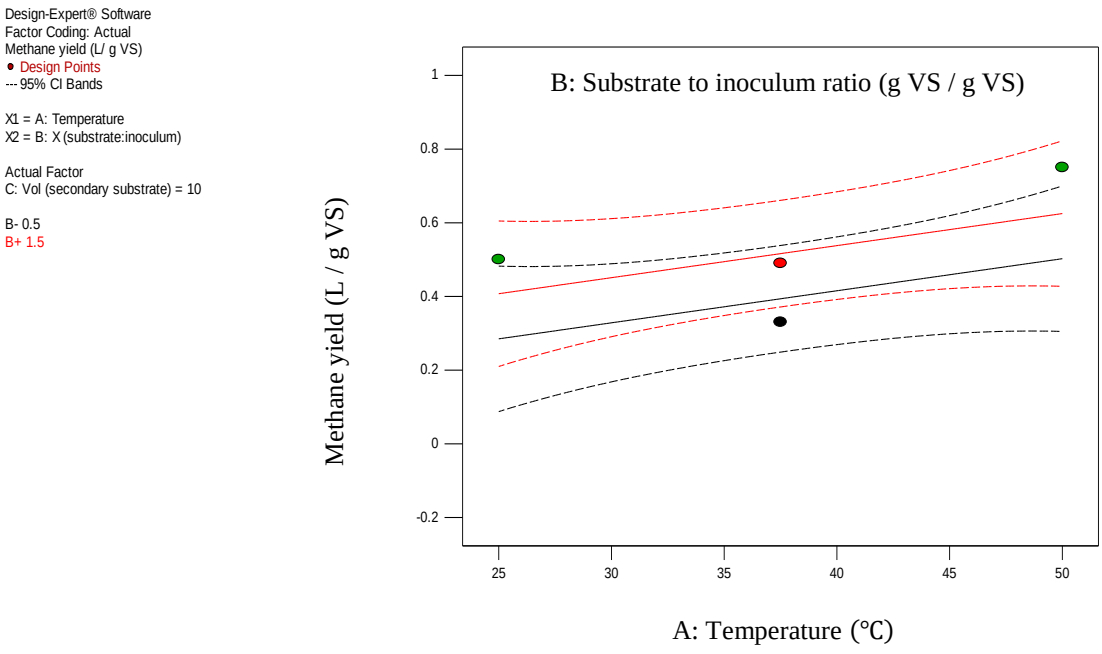


Figure P64: Interaction of substrate to inoculum ratio and temperature for methane yield at a glycerol volume of 10 mL.

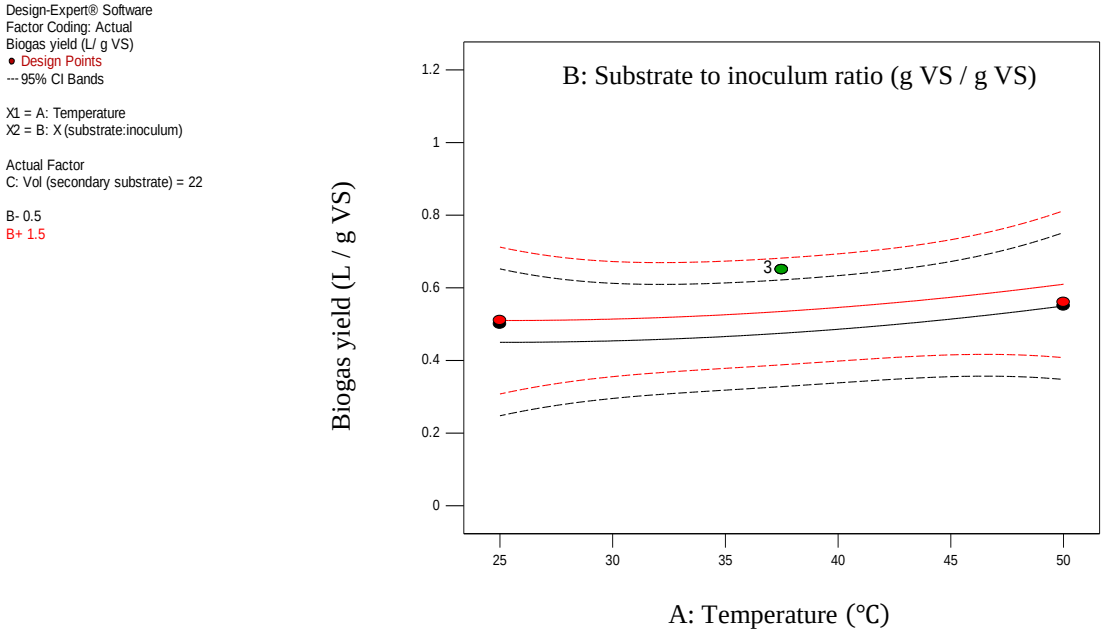


Figure P65: Interaction of substrate to inoculum ratio and temperature for biogas yield at a glycerol volume of 22 mL.

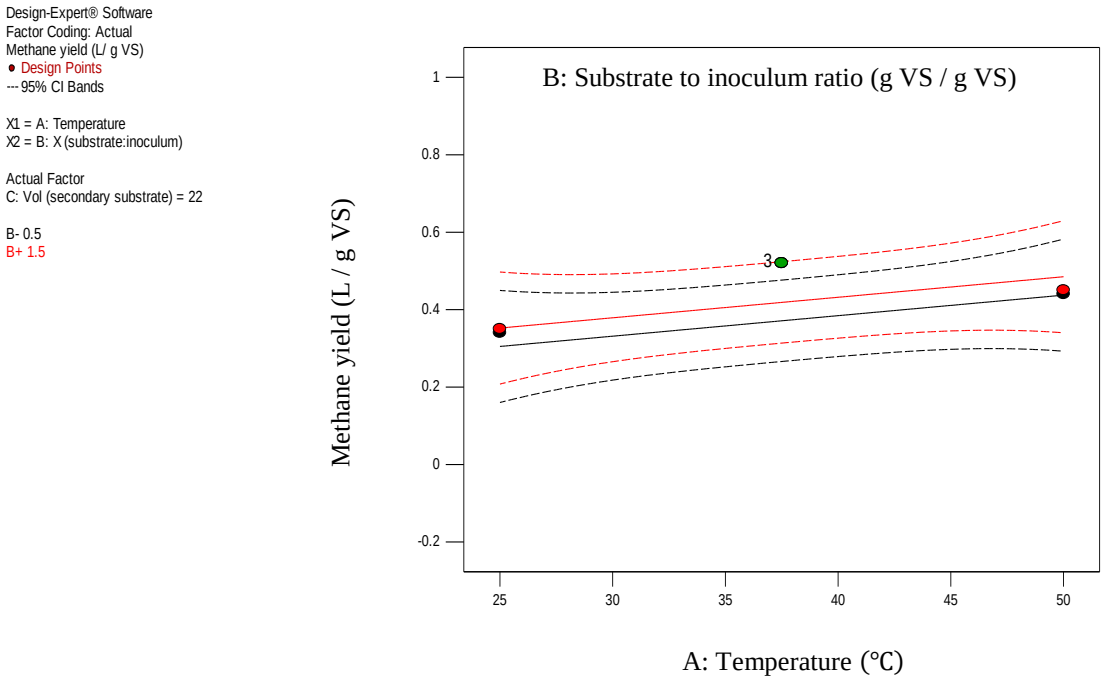


Figure P66: Interaction of substrate to inoculum ratio and temperature for methane yield at a glycerol volume of 22 mL.

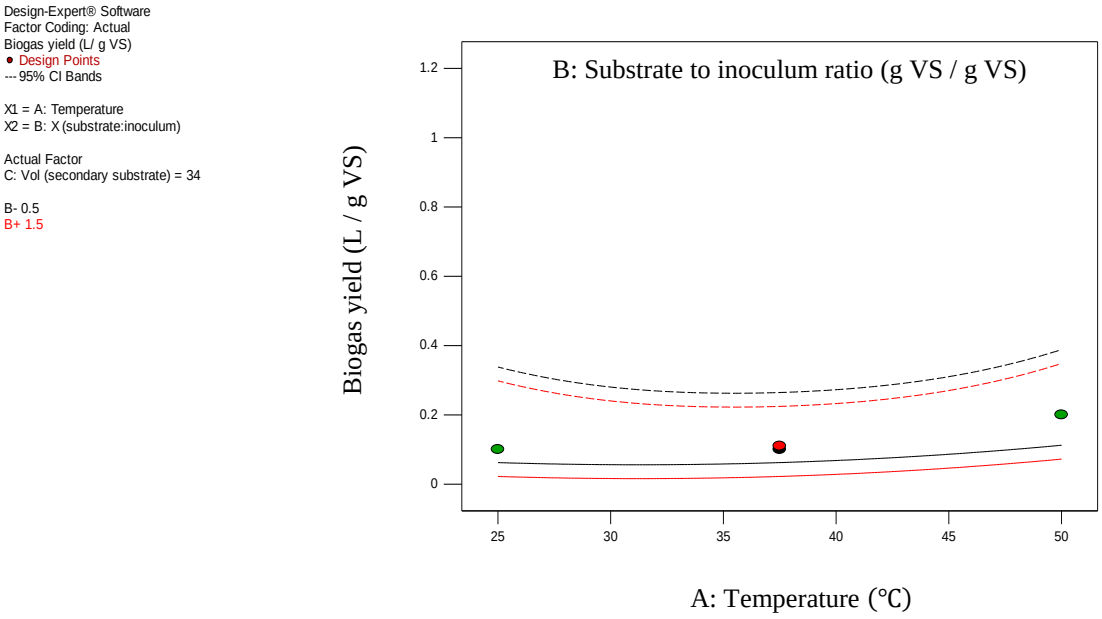


Figure P67: Interaction of substrate to inoculum ratio and temperature for biogas yield at a glycerol volume of 34 mL.

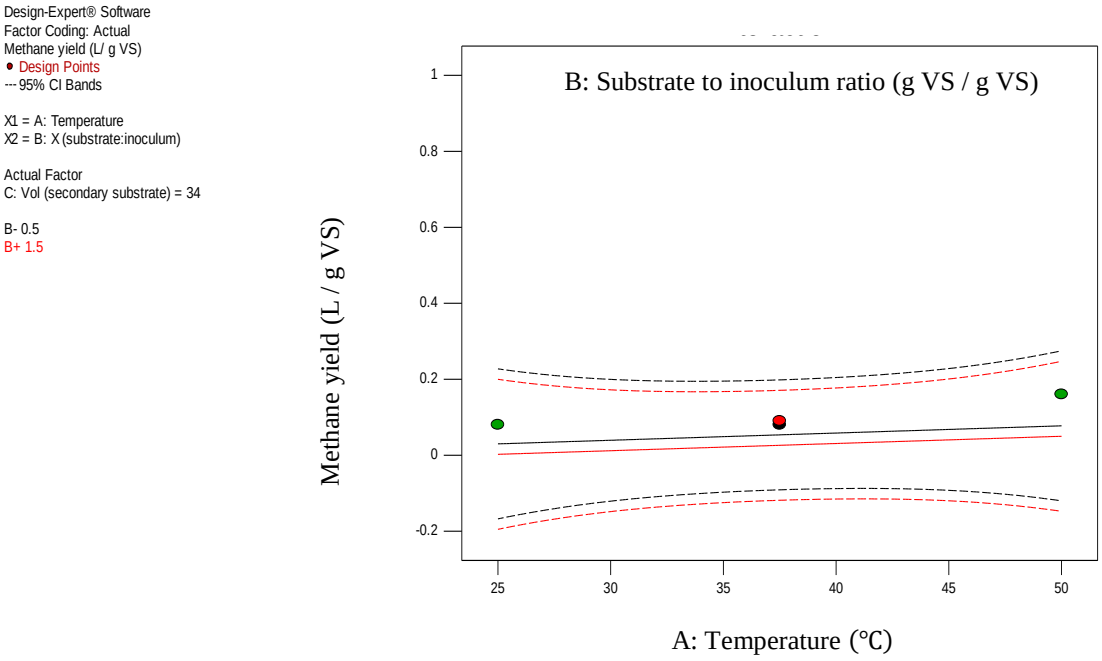


Figure P68: Interaction of substrate to inoculum ratio and temperature for methane yield at a glycerol volume of 34 mL.

The last significant interaction is that of substrate to inoculum ratio and glycerol volume (AC). Interaction of substrate to inoculum ratio and temperature for biogas and methane yields at temperatures of 25, 37.5 and 50℃ are shown in Figure P69, Figure P70, Figure P71, Figure P72, Figure P73 and Figure P74, respectively. For a temperature of 25℃ the glycerol volume intersects the substrate to inoculum ratio at 0.69 and 0.80 for the biogas and methane yields, respectively. But, in general, the manner in which the points are distributed on the right hand-side of the figures (where the substrate to inoculum ratio is high) was greater than the manner in which the points were distributed on the left hand-side of the figures (where the substrate to inoculum ratio is low). This implies that the effect of glycerol volume was less significant at low temperatures. Thus, the experimenters may operate at low substrate to inoculum ratio and increase the volume concentration of glycerol in the digester, at the same time achieving higher biogas and methane yields.

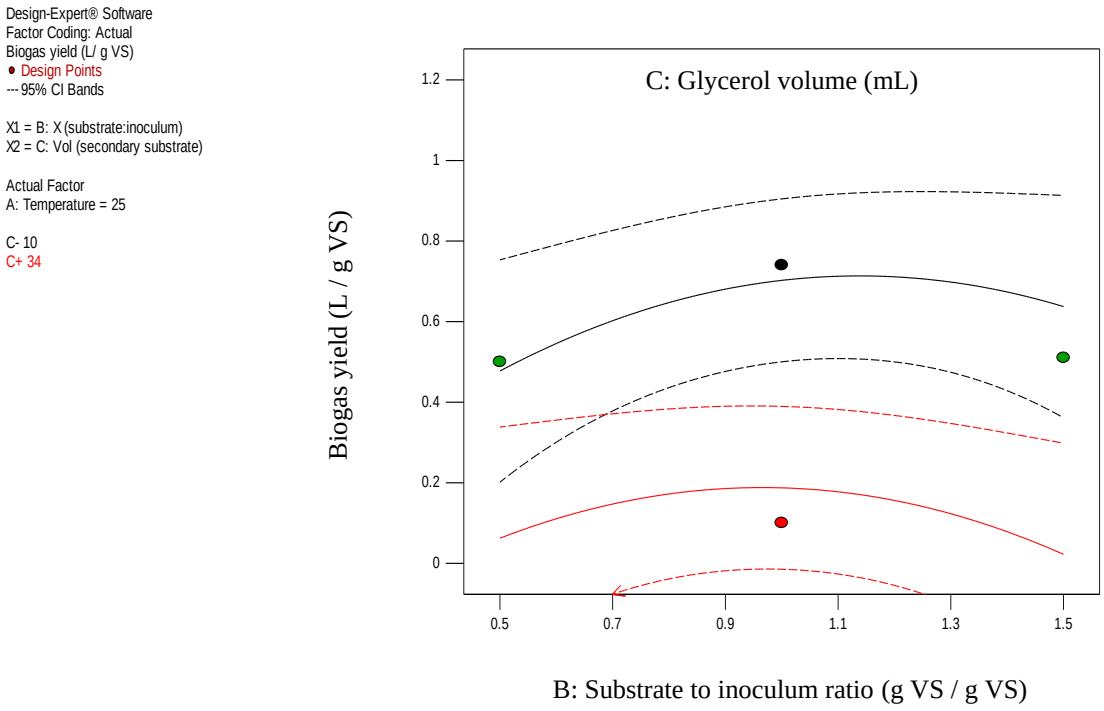


Figure P69: Interaction of substrate to inoculum ratio and glycerol volume for biogas yield at 25℃.

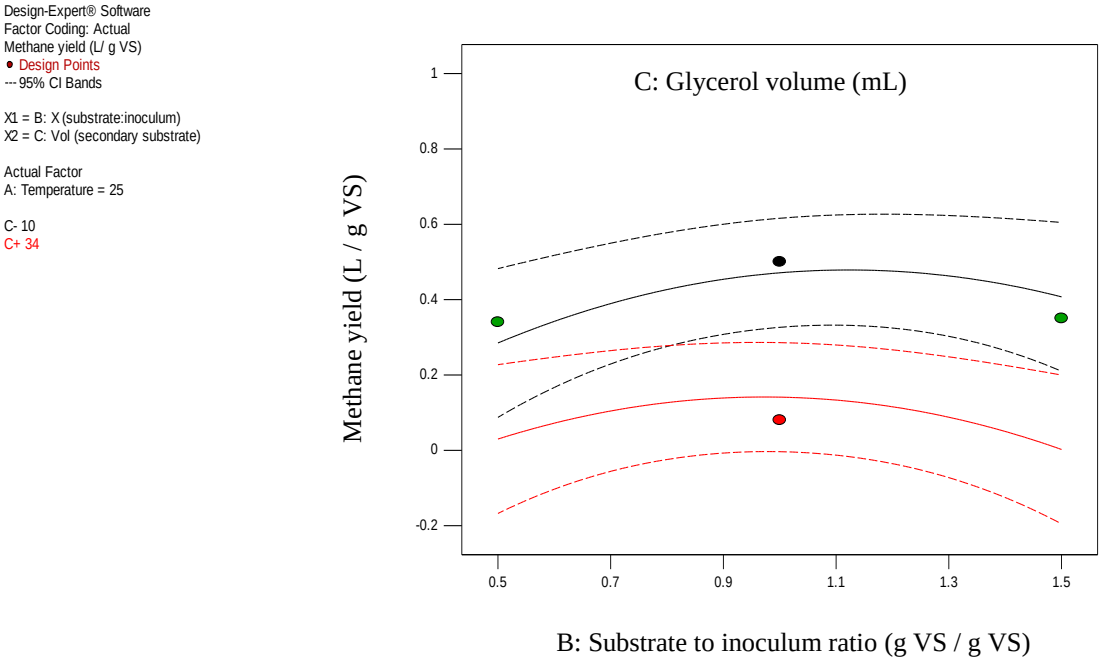


Figure P70: Interaction of substrate to inoculum ratio and glycerol volume for methane yield at 25℃.

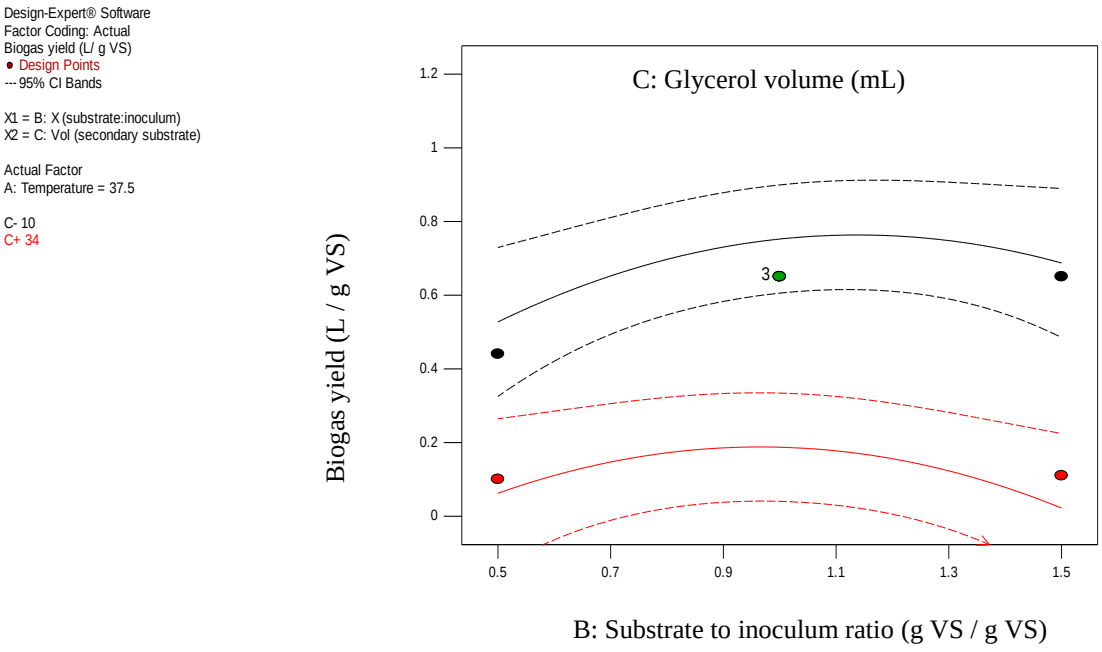


Figure P71: Interaction of substrate to inoculum ratio and glycerol volume for biogas yield at 37.5℃

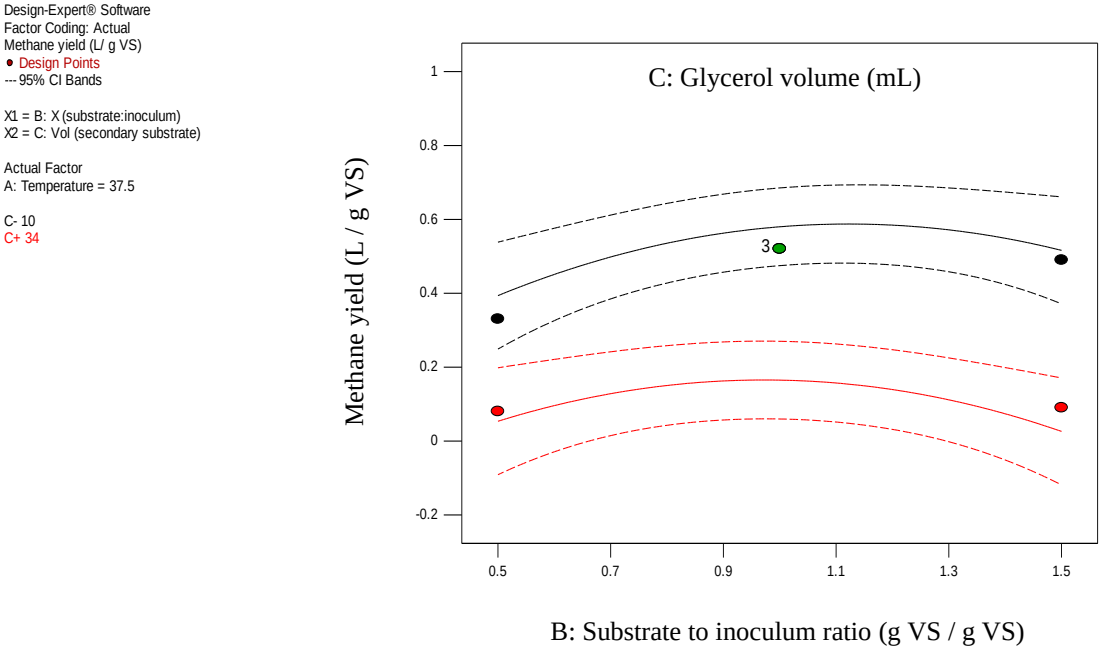


Figure P72: Interaction of substrate to inoculum ratio and glycerol volume for methane yield at 37.5℃.

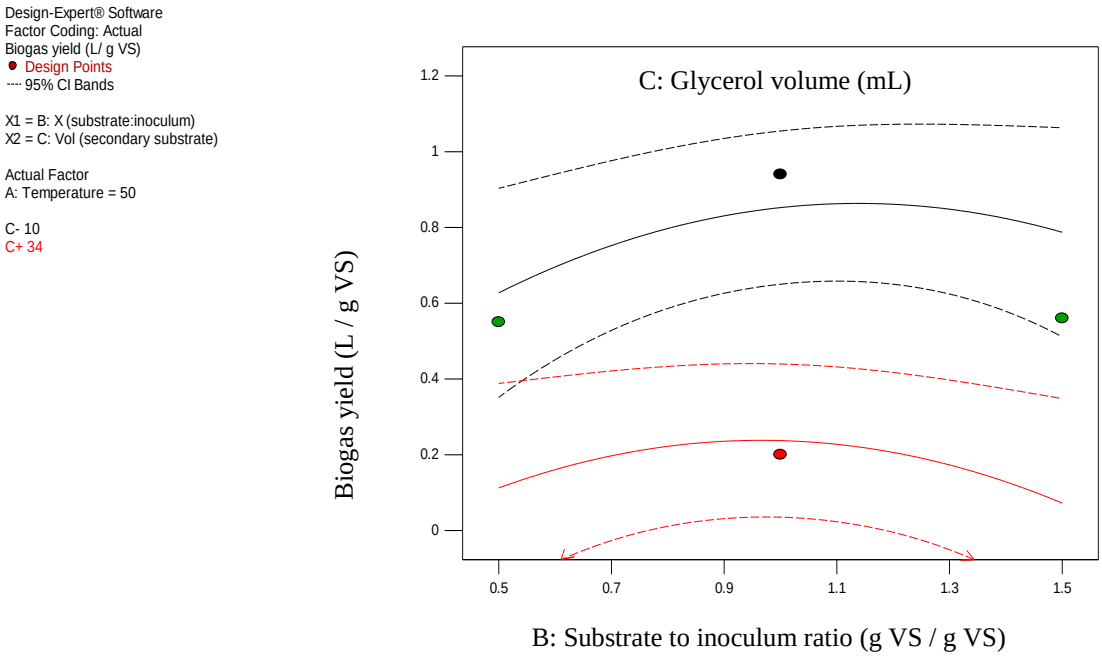


Figure P73: Interaction of substrate to inoculum ratio and glycerol volume for biogas yield at 50℃.

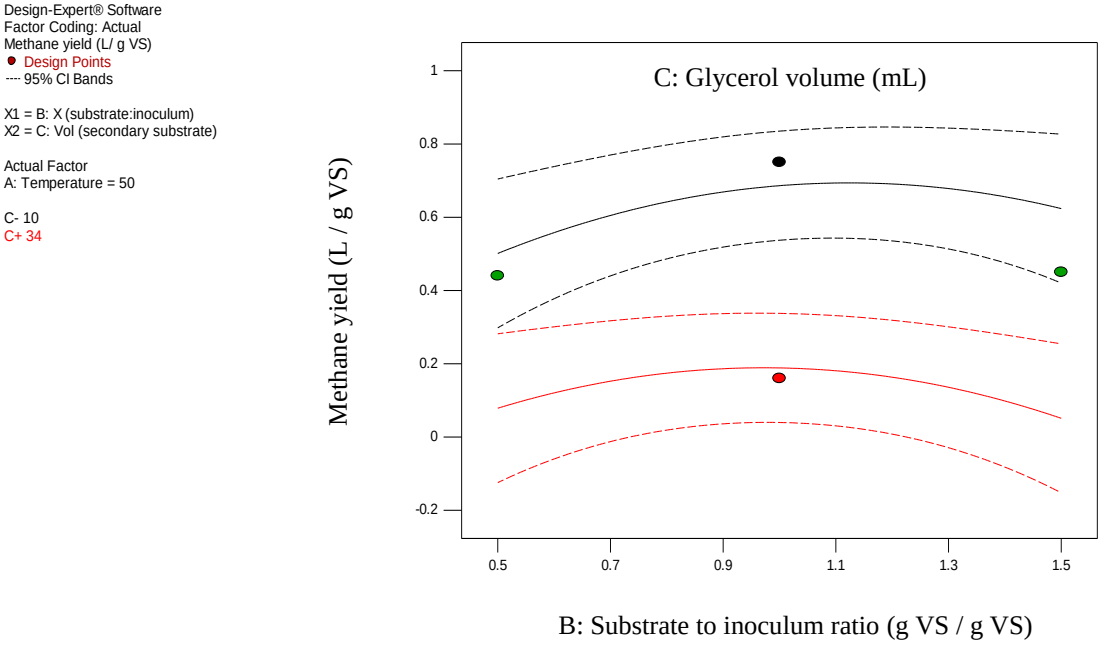


Figure P74: Interaction of substrate to inoculum ratio and glycerol volume for methane yield at 50℃.

P6 - Counter and 3D plots

Interaction graphs that were described above represent a second order response that is not linear. It may be useful to investigate three dimensional (3D) and counter diagrams of the interaction to have an understanding of non-linearity. Counter graphs for the AC, AB and BC interactions for biogas and methane yields at -1, 0 and +1 levels are shown in Figure P75, Figure P76, Figure P77, Figure P78, Figure P79, Figure P80, Figure P81, Figure P82, Figure P83, Figure P84, Figure P85, Figure P86, Figure P87, Figure P88, Figure P89, Figure P90, Figure P91 and Figure P92, respectively.

The figures display a wide range of colours – from red for higher response levels (biogas and methane yields) to cool blue for lower values. Thus, the highest biogas and methane yields are above 0.80 L / g VS and 0.60 L / g VS at 50℃, 1 g VS / g VS substrate to inoculum ratio and 10 mL, respectively.

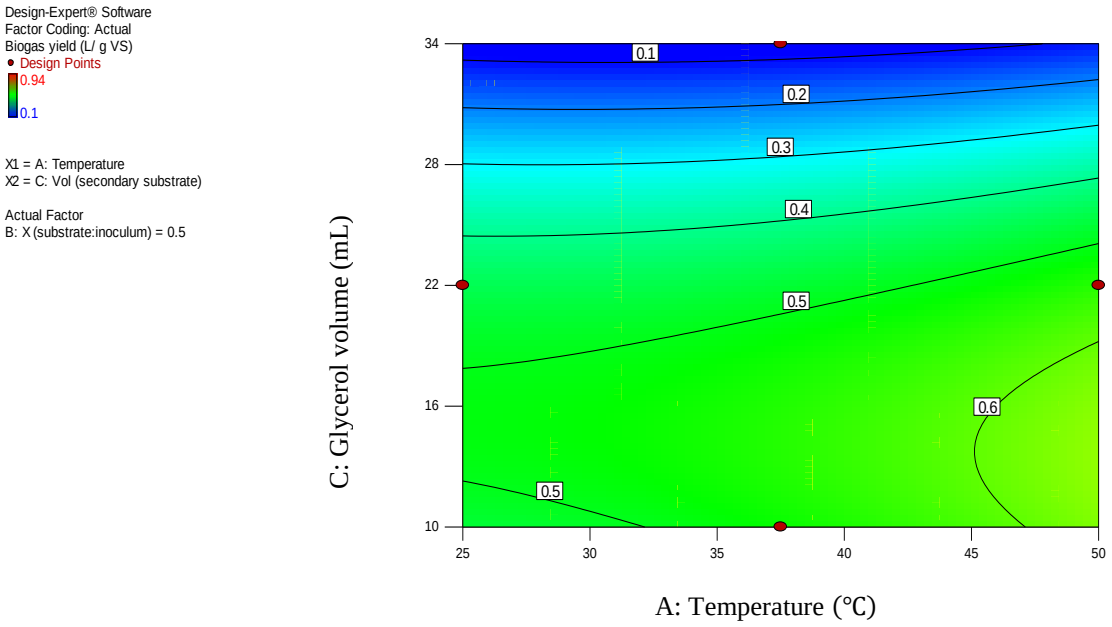


Figure P75: Counter graph for the AC interaction for biogas yield at a substrate to inoculum of 0.5.

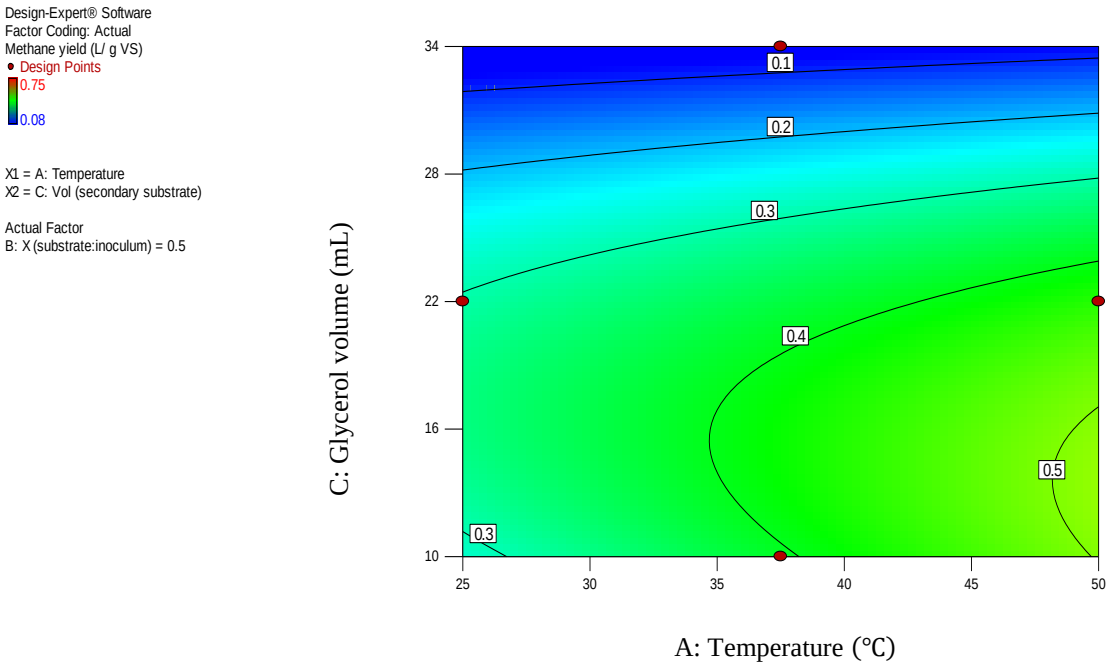


Figure P76: Counter graph for the AC interaction for methane yield at a substrate to inoculum ratio of 0.5.

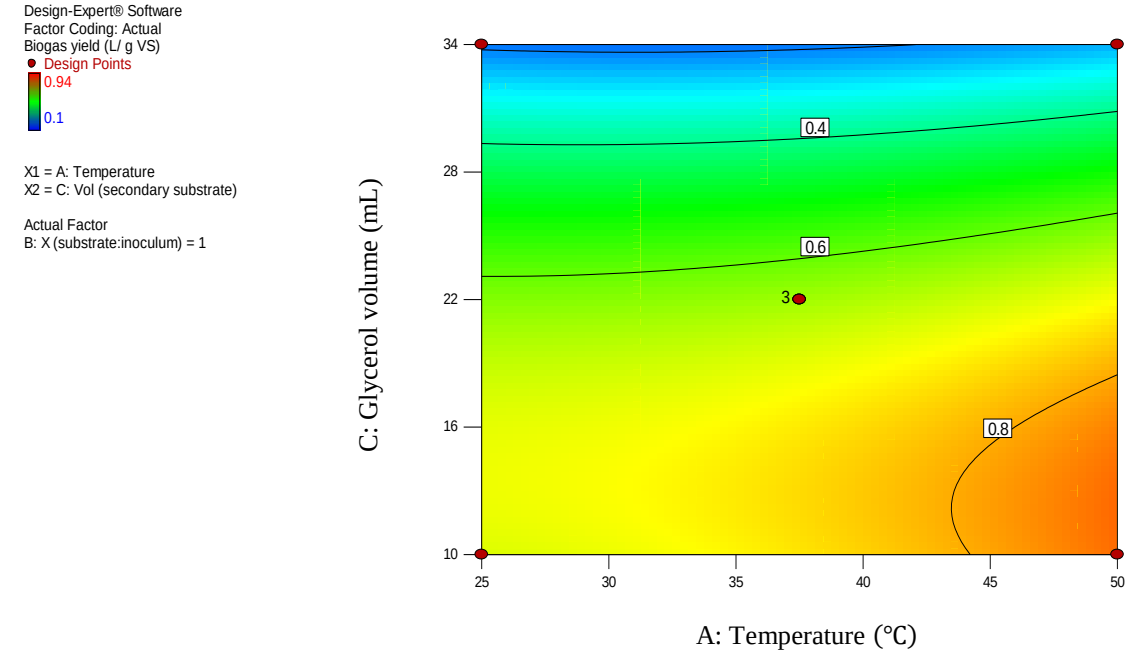


Figure P77: Counter graph for the AC interaction for biogas yield at a substrate to inoculum ratio of 1.0.

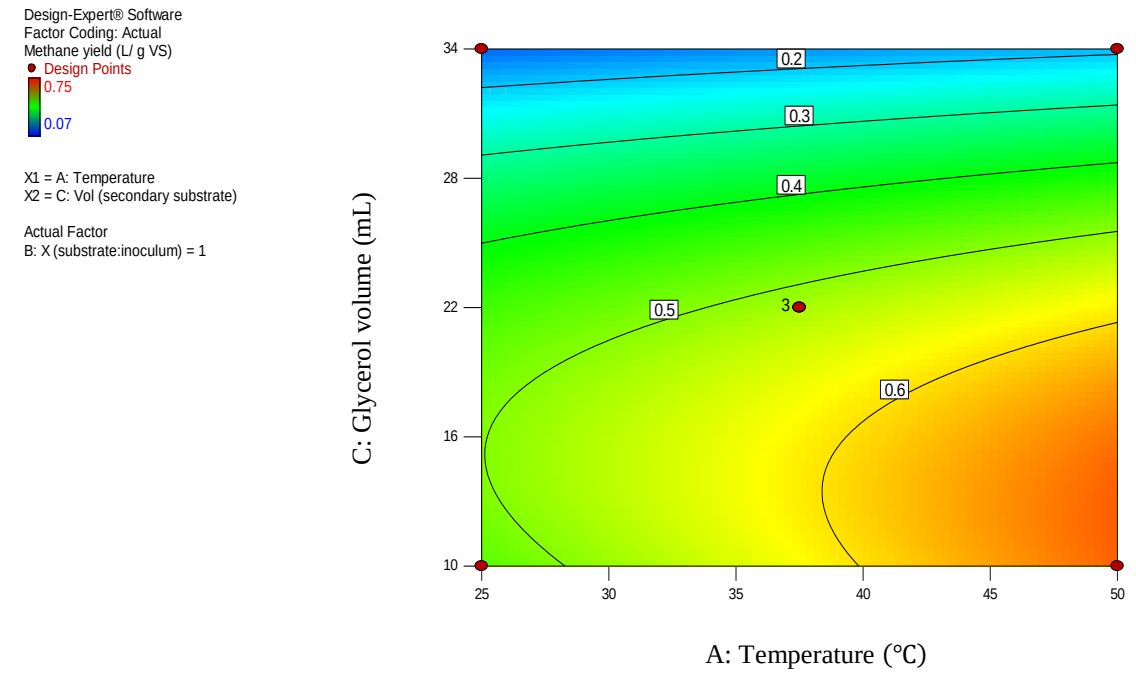


Figure P78: Counter graph for the AC interaction for methane yield at a substrate to inoculum ratio of 1.0.

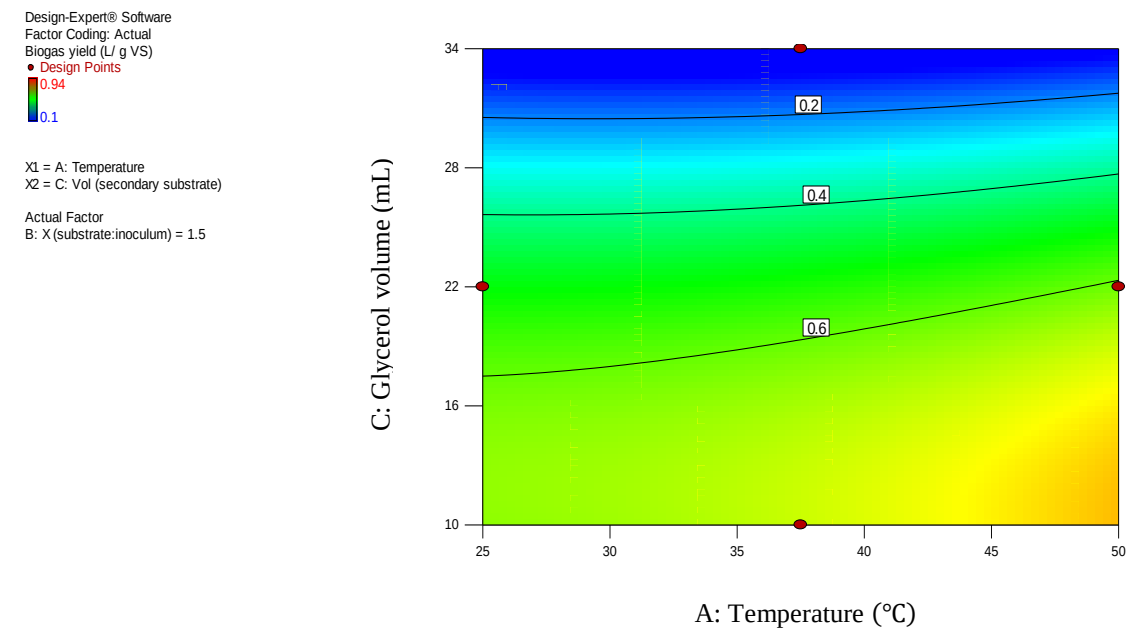


Figure P79: Counter graph for the AC interaction for biogas yield at a substrate to inoculum ratio of 1.5.

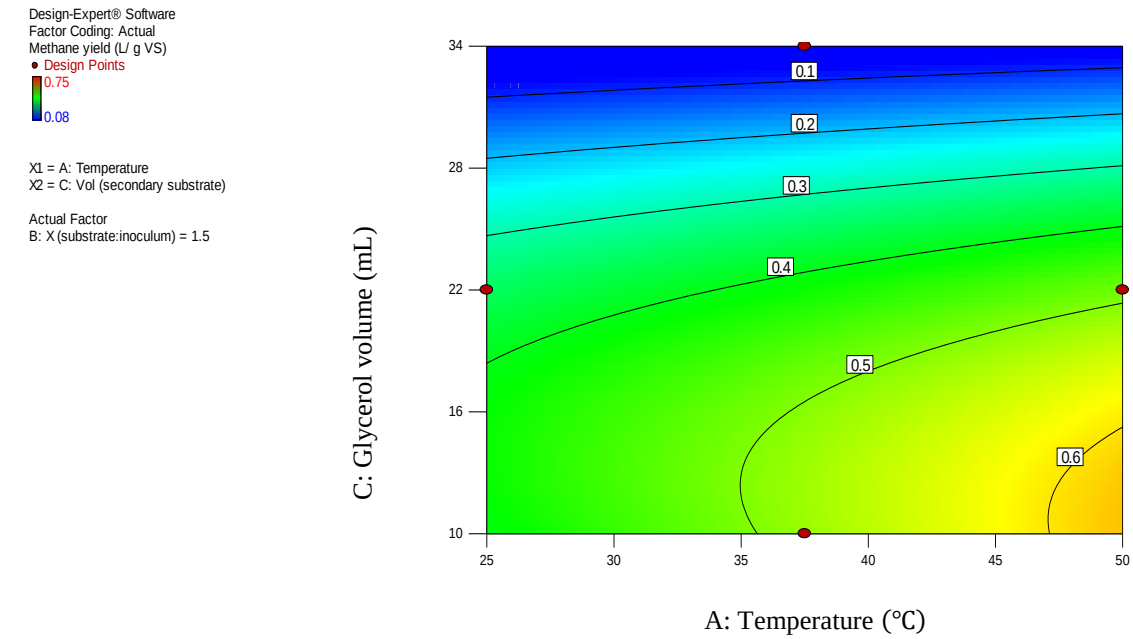


Figure P80: Counter graph for the AC interaction for methane yield at a substrate to inoculum ratio of 1.5.

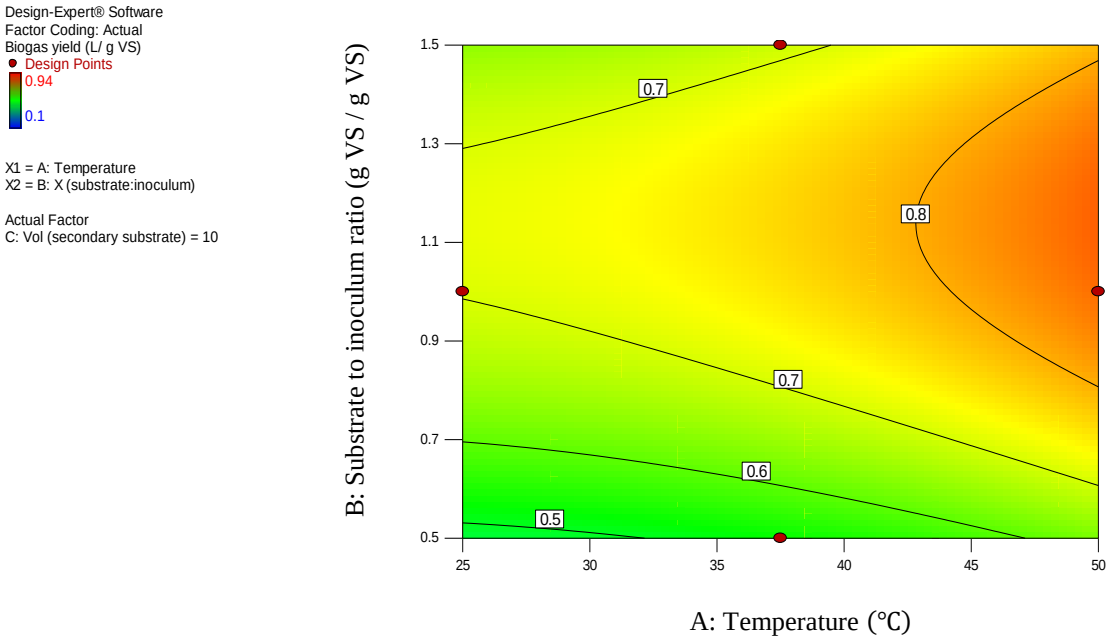


Figure P81: Counter graph for the AB interaction for biogas yield at a volume concentration of 10.

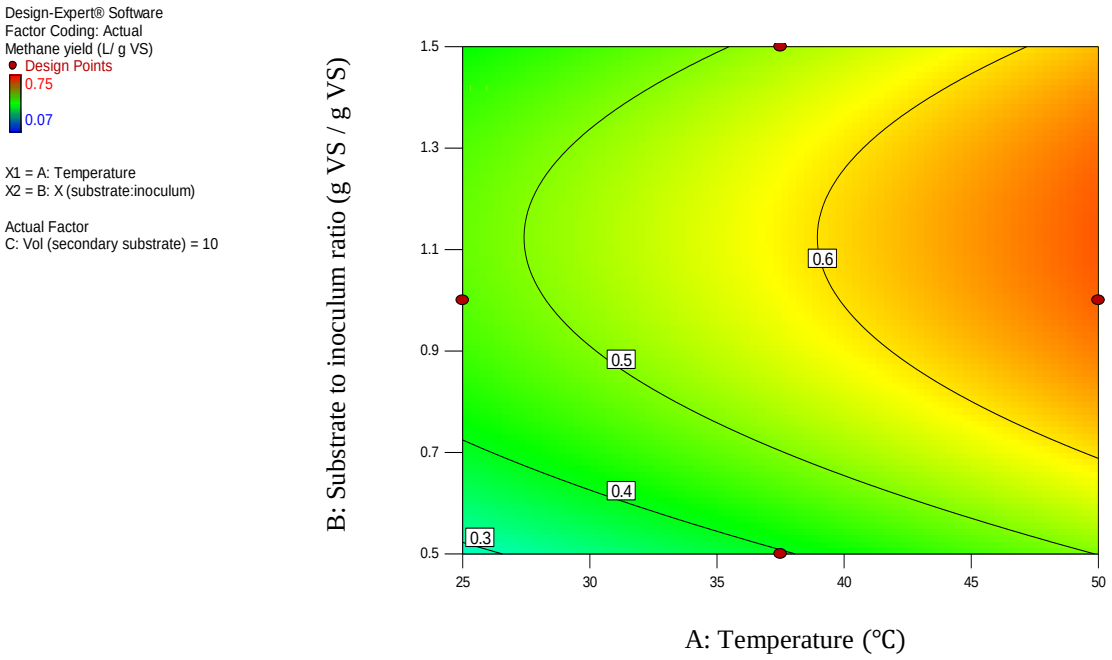


Figure P82: Counter graph for the AB interaction for methane yield at a volume concentration of 10.

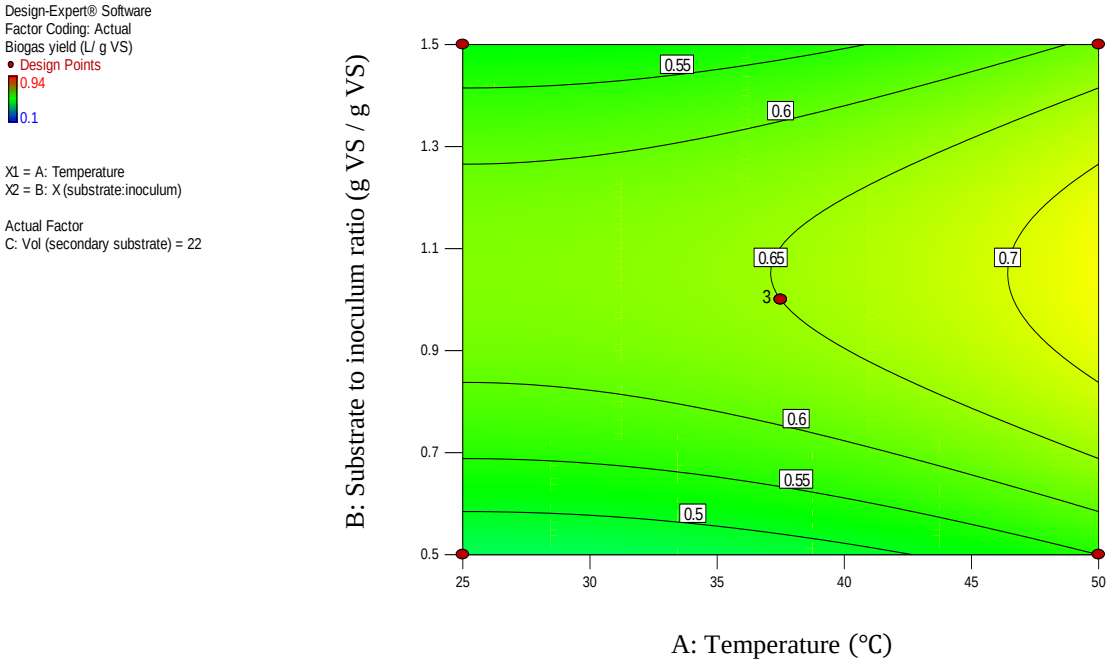


Figure P83: Counter graph for the AB interaction for biogas yield at volume concentration of 22.

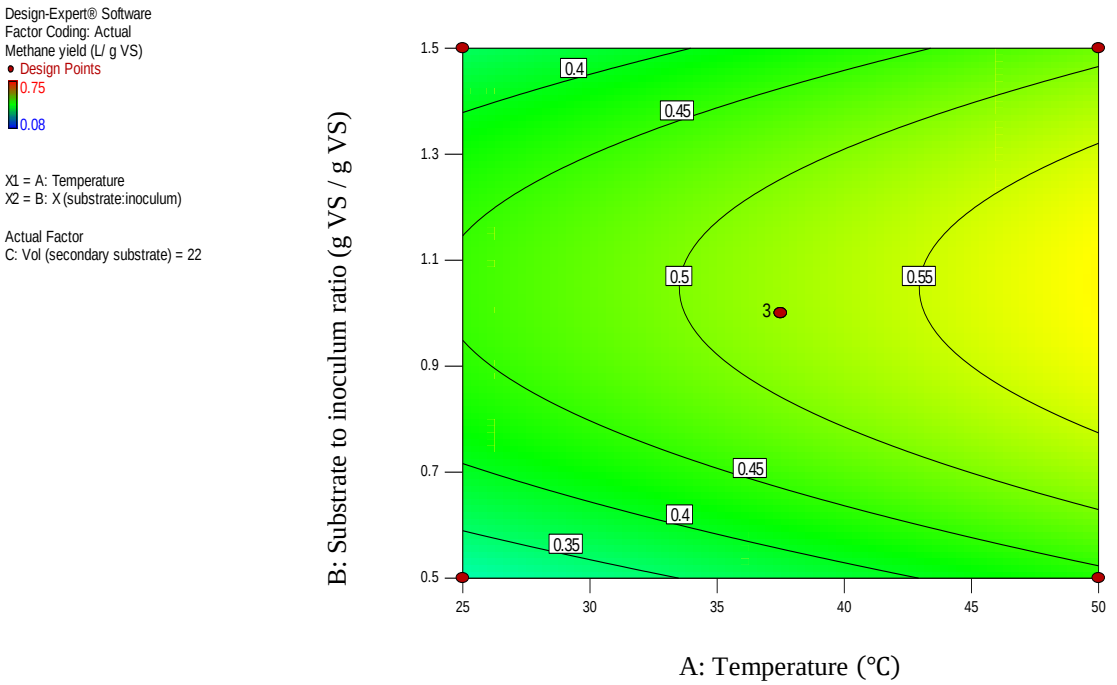


Figure P84: Counter graph for the AB interaction for methane yield at volume concentration of 22.

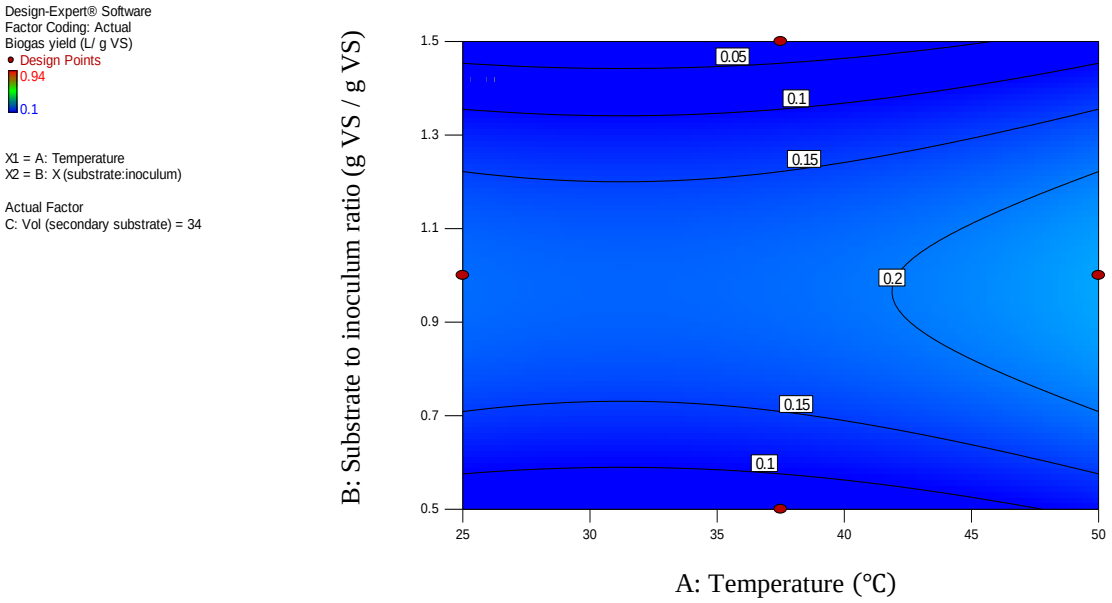


Figure P85: Counter graph for the AB interaction for biogas yield at volume concentration of 34.

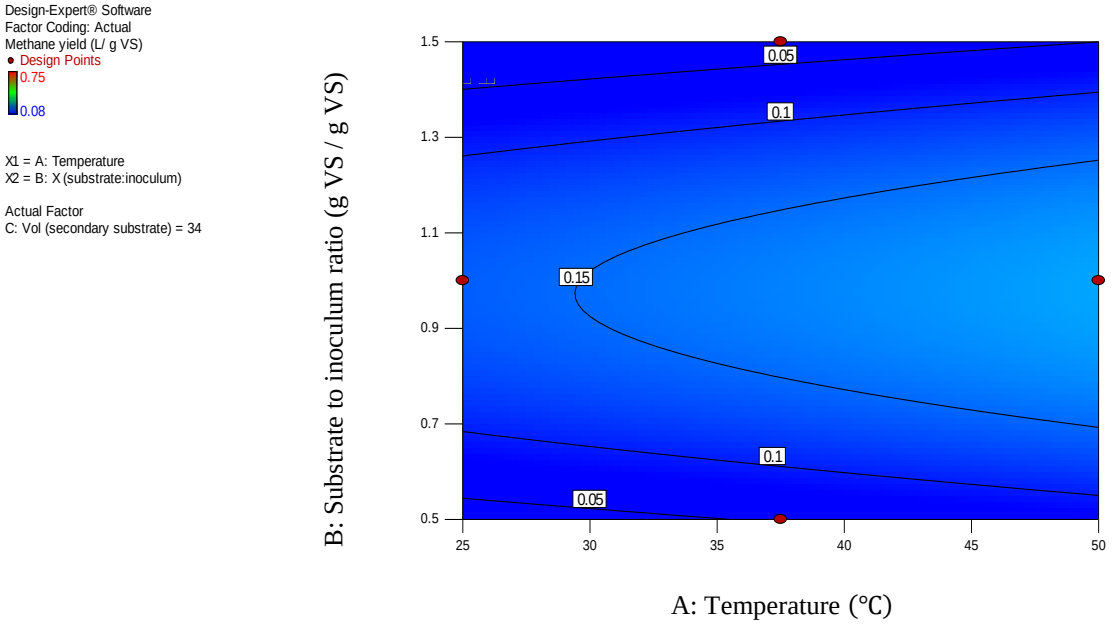


Figure P86: Counter graph for the AB interaction for methane yield at volume concentration of 34.

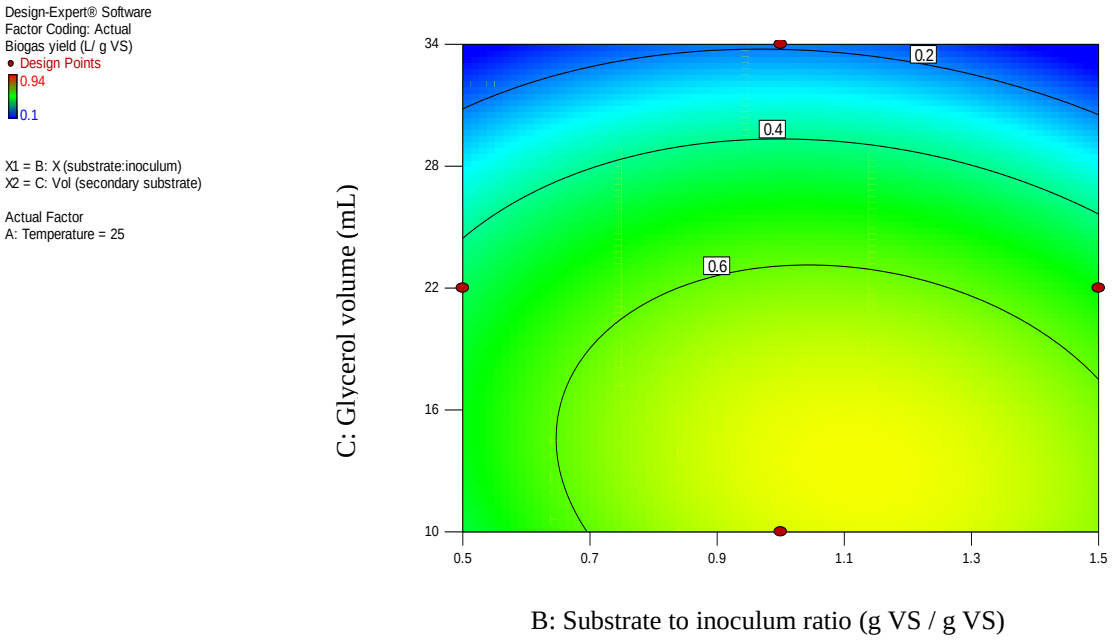


Figure P87: Counter graph for the CB interaction for biogas yield at 25℃.

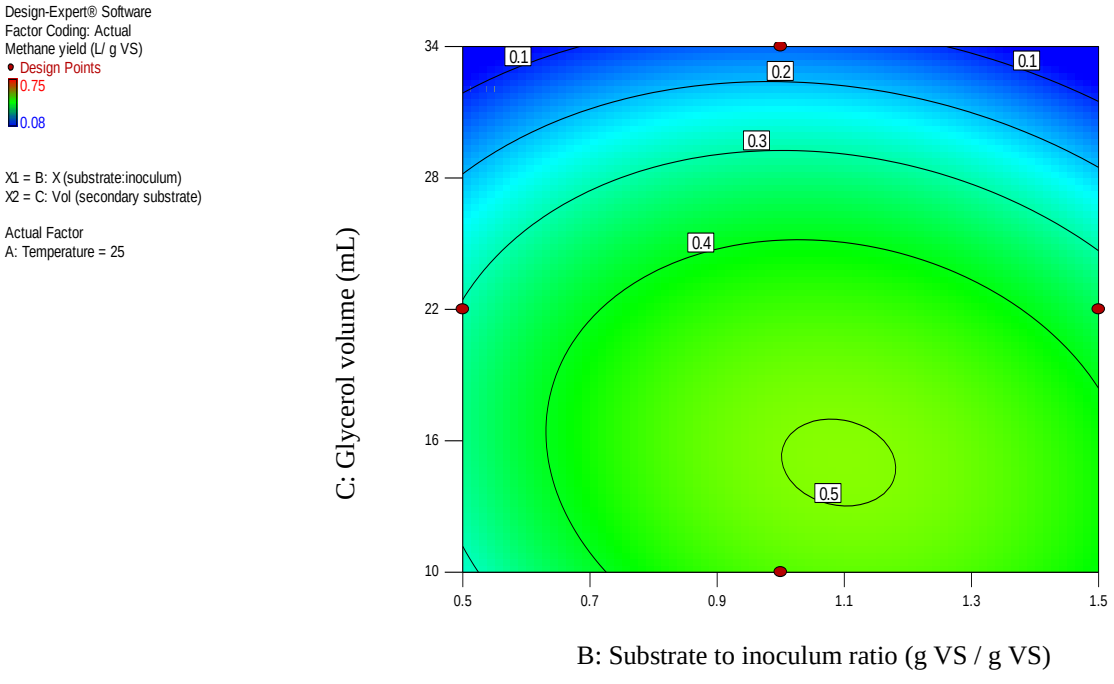


Figure P88: Counter graph for the CB interaction for methane yield at 25℃.

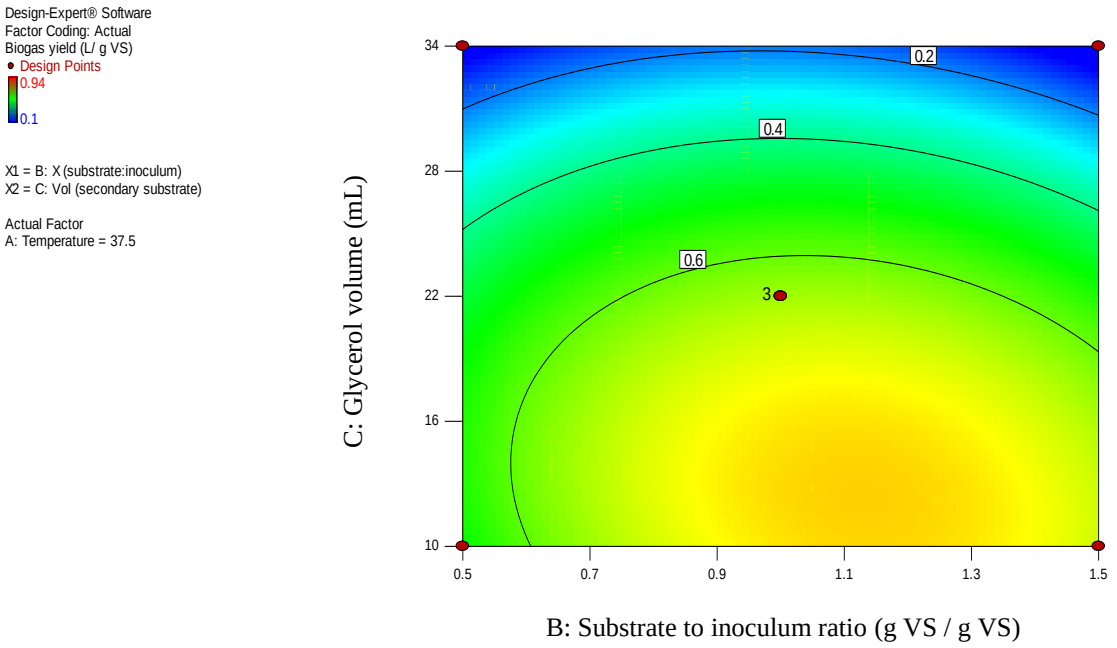


Figure P89: Counter graph for the CB interaction for biogas yield at 37.5℃.

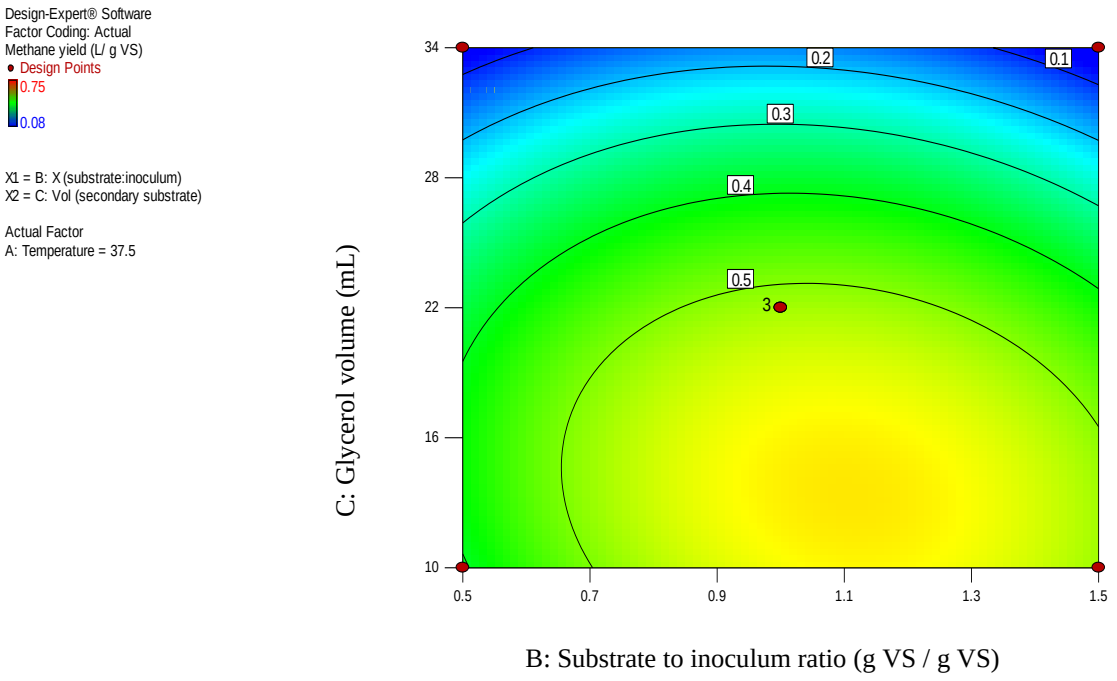


Figure P90: Counter graph for the CB interaction for methane yield at 37.5℃.

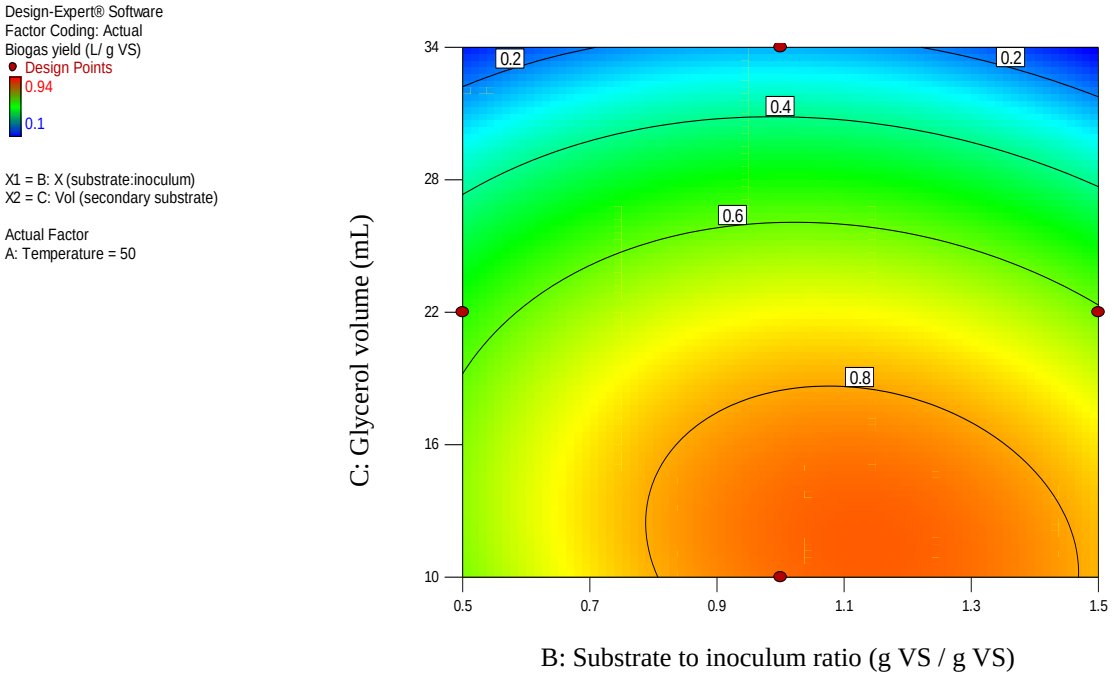


Figure P91: Counter graph for the CB interaction for biogas yield at 50℃.

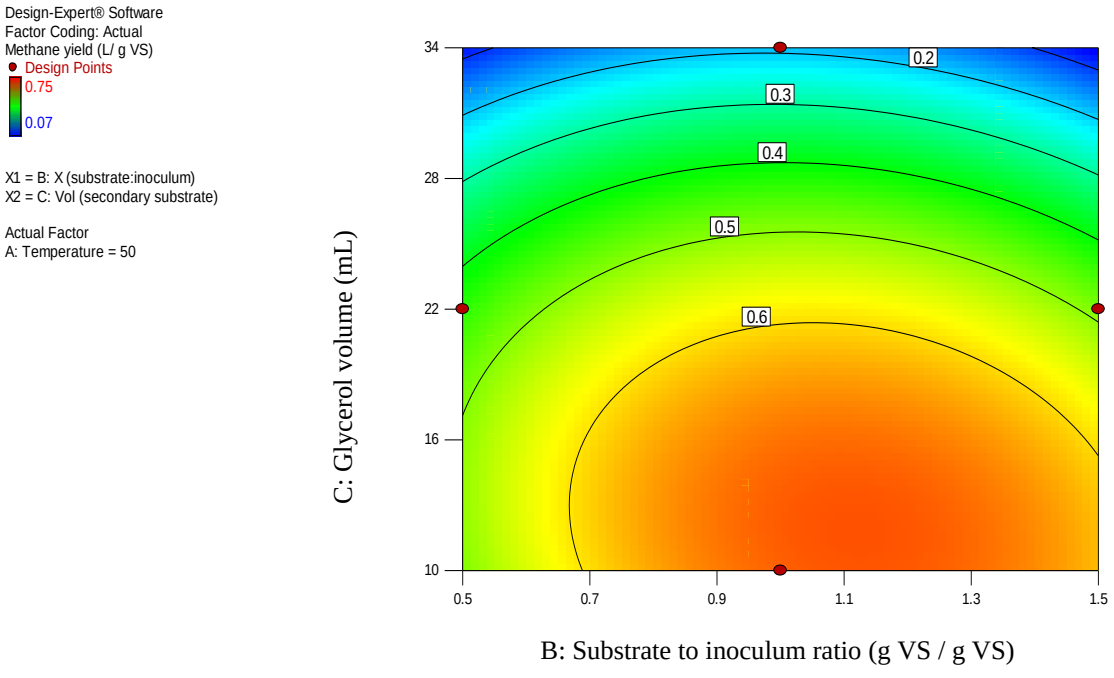


Figure P92: Counter graph for the CB interaction for methane yield at 50℃.

The counter graphs shown above only show two dimensional views of the results, which makes it difficult to see the exact optimum response. A three dimensional (3D) surface makes it easy to understand how things shape up from this investigation. Figure P93, Figure P94, Figure P95, Figure P96, Figure P97 and Figure P98 depict 3D graphs for the AC, AD and CD interactions for biogas and methane yields, respectively.

From the 3D graphs, the highest biogas and methane yields are roughly 0.94 and 0.75 L/g VS at 50℃, 1 g VS / g VS substrate to inoculum ratio and 10 mL, respectively.

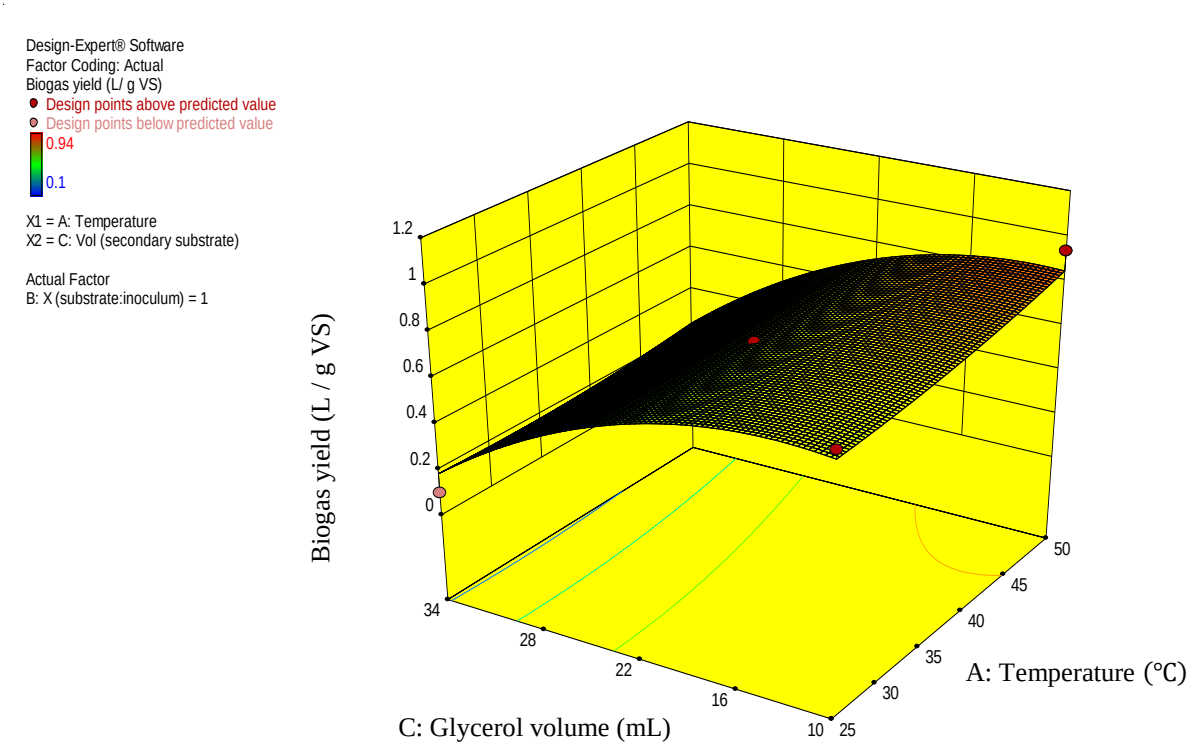


Figure P93: 3D graph for the AC interaction for biogas yields.

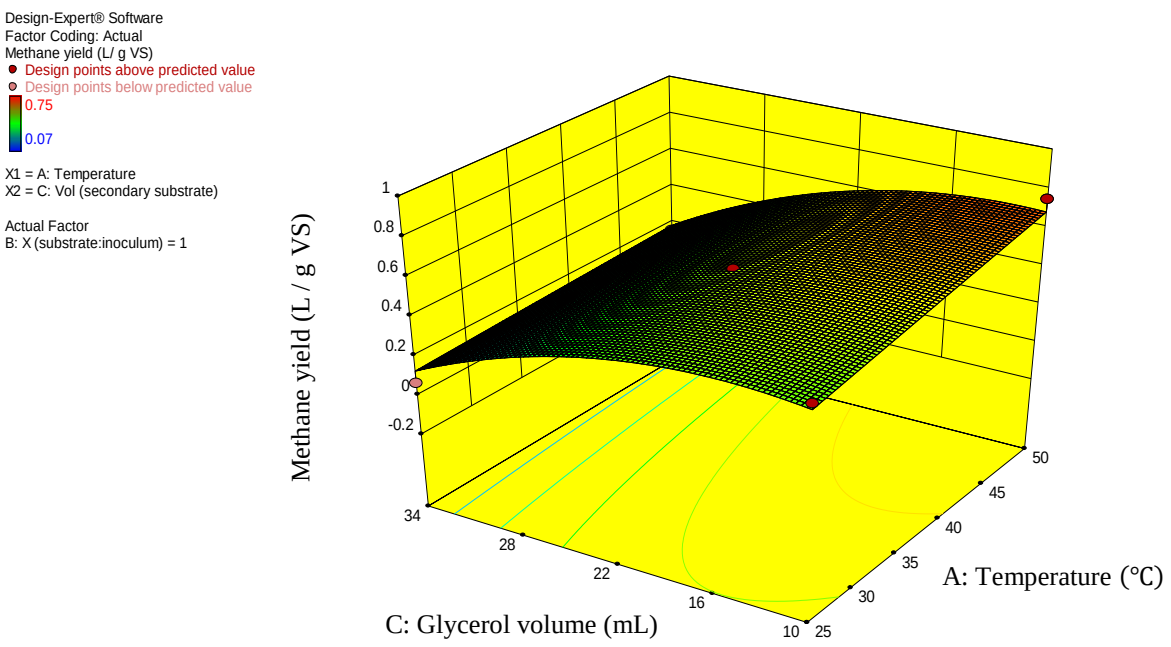


Figure P94: 3D graph for the AC interaction for methane yields.

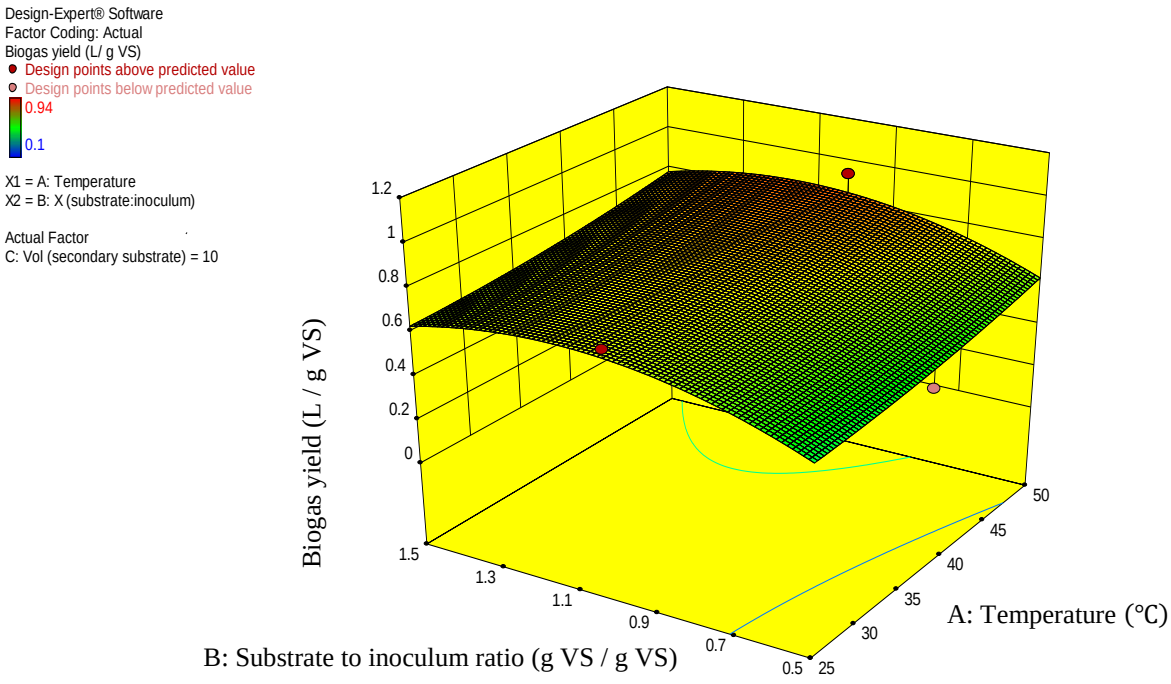


Figure P96: 3D graph for the AB interaction for biogas yields.

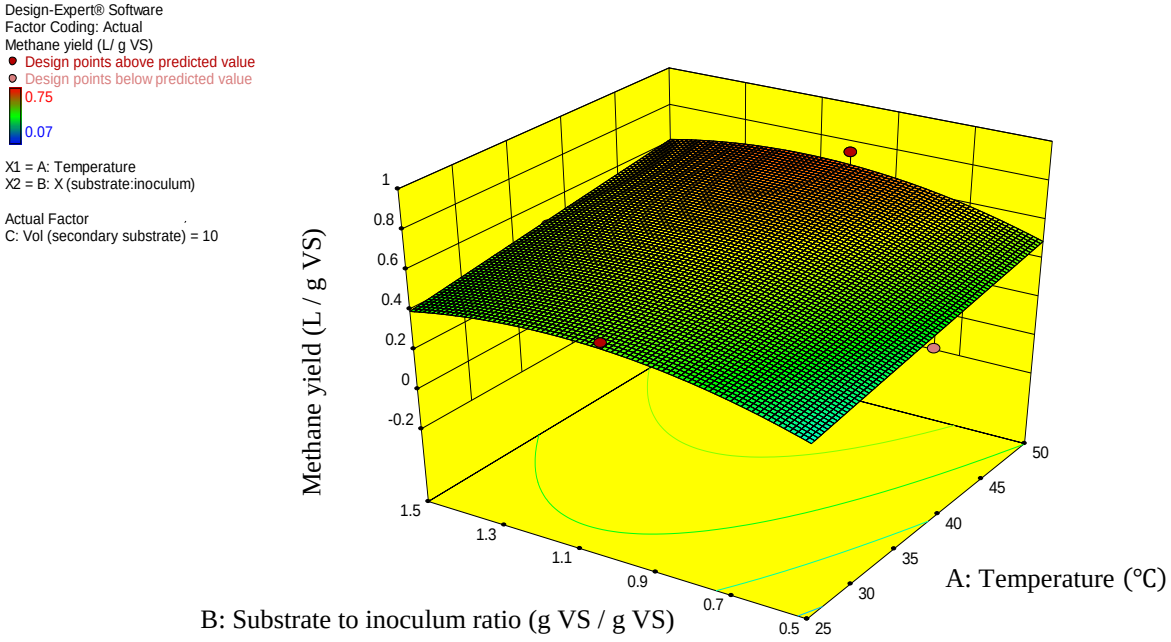


Figure P96: 3D graph for the AB interaction for methane yields.

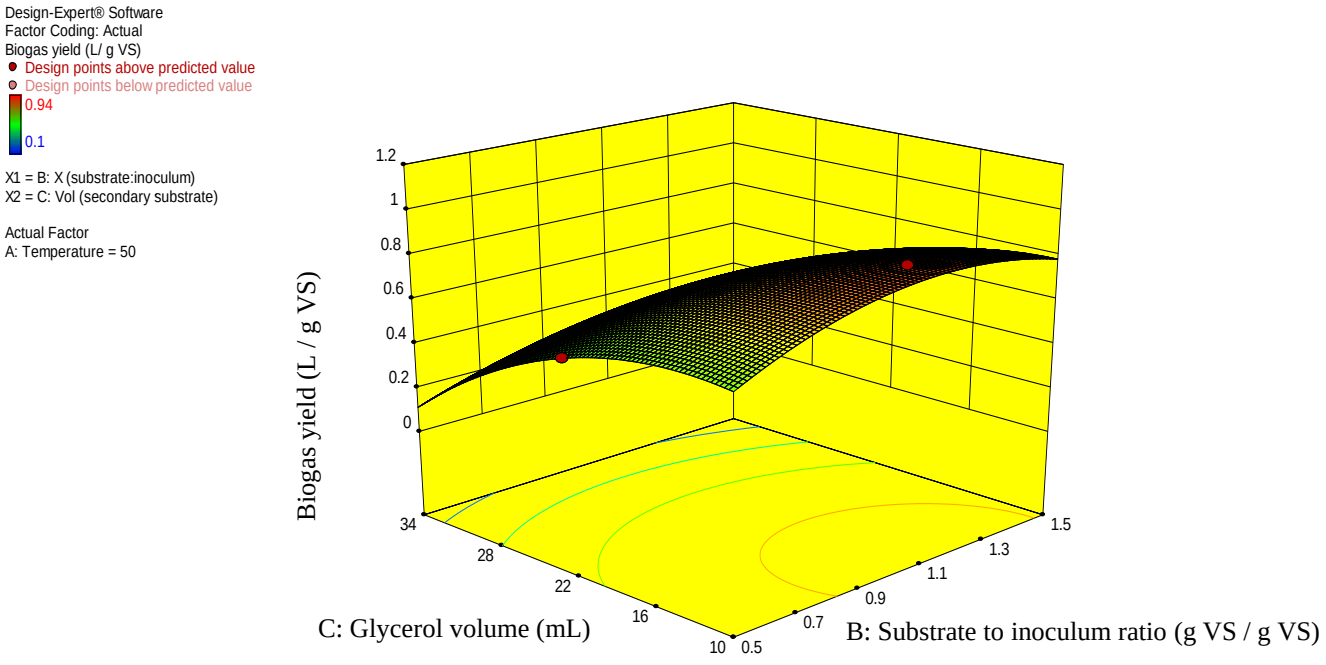


Figure P97: 3D graph for the CB interaction for biogas yields.

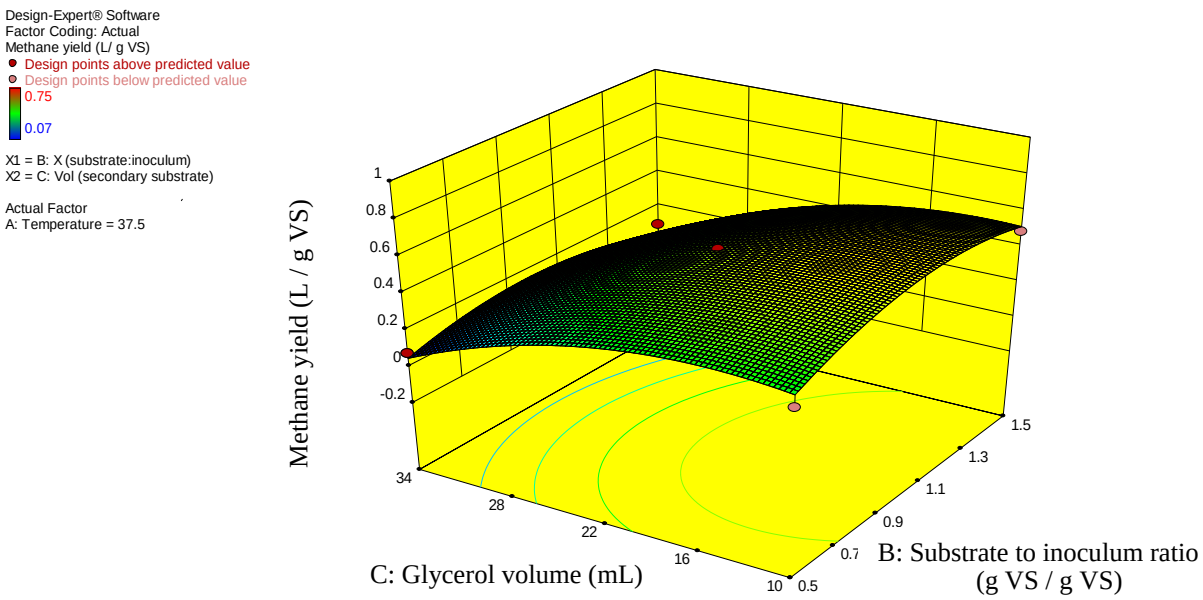


Figure P98: 3D graph for the CB interaction for methane yields.

P7 - Cube plot

The cube plot gives an estimated response as a function of the three variables (in a cube form) for the combinations of the -1 and +1 levels. Figure P99 and Figure P100 show the cube plots of all three variables for biogas and methane yields, respectively. These figures depict how three variables relate to affect the response variables i.e. the biogas and methane yields. The values depicted are values that are predicted by Design-Expert software, and as a result, allows plots to be constructed even if there was information that was not available with the actual data. The red dots are centre points at level zero.

Generally, the biogas and methane yields decreased with increase in volume concentration, with 10 mL being the optimum volume concentration. There was also an increase in biogas and methane yields with increase in temperature, with 50℃ being the optimum temperature. The biogas and methane yields are optimum at a substrate to inoculum ratio of 1, where the temperature was 50℃. The predicted optimum biogas and methane yields at 50℃, 1 g VS / g VS substrate to inoculum ratio and 10 mL (at C-, D+ and A+) were 0.94 and 0.75 L/g VS, respectively. These values are similar to those that were found using the OFAT method.

Design-Expert® Software
Factor Coding: Actual
Biogas yield (L/ g VS)

Biogas yield (L/ g VS) = 0.94
Std # 6 Run # 7

X1 = B: X (substrate:inoculum)
X2 = C: Vol (secondary substrate)
X3 = A: Temperature

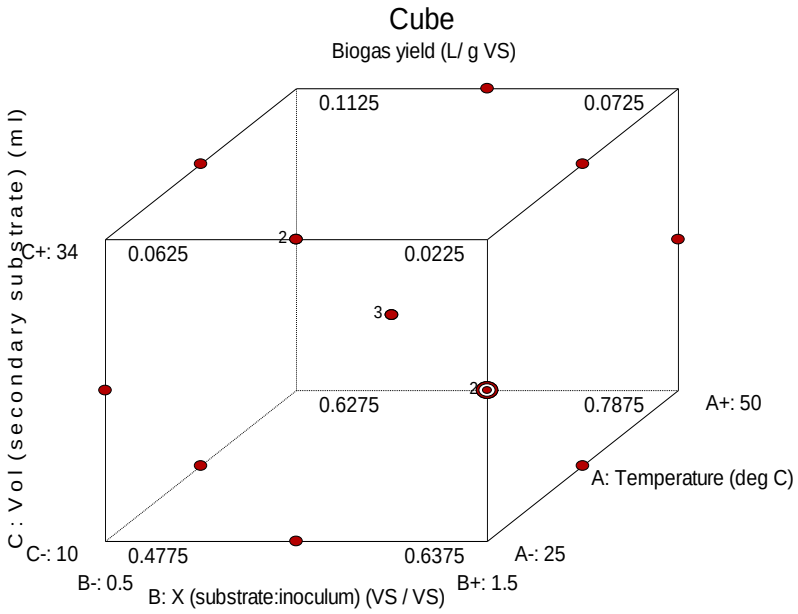


Figure P99: Cube plot of all three variables for biogas yields.

Design-Expert® Software
Factor Coding: Actual
Methane yield (L/ g VS)

Methane yield (L/ g VS) = 0.75
Std # 6 Run # 7

X1 = B: X (substrate:inoculum)
X2 = C: Vol (secondary substrate)
X3 = A: Temperature

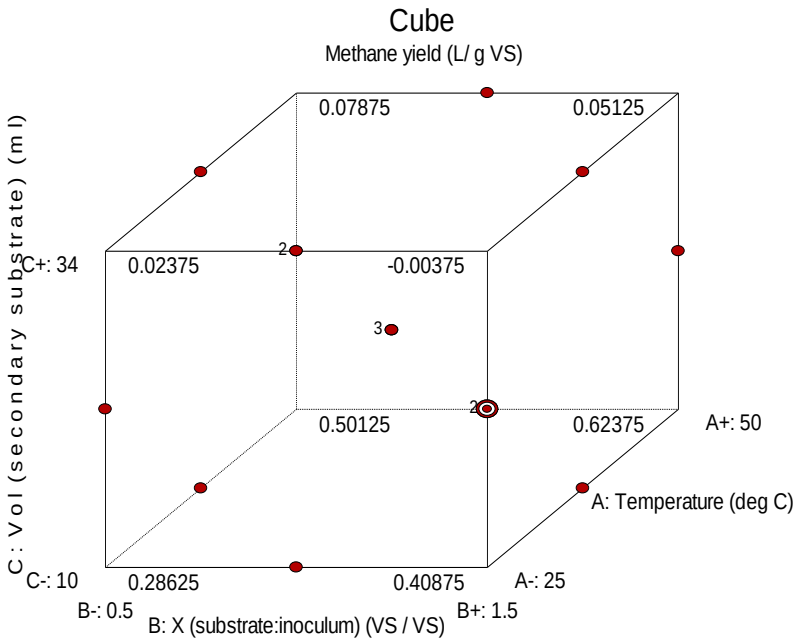


Figure P100: Cube plot of all three variables for methane yields.

P8 - Confirmation

The last aspect that was explored in Design-Expert was the confirmation report, which may be used to make response estimations for any combinations of process variables. Table P12 portrays the confirmation at best factor settings for biogas and methane yields. The software uses the model obtained from practical results to estimate the interval (PI) and mean for the number (n) of confirmation runs at the conditions that were used, which offer or give the highest biogas and methane yields with smallest concentration of glycerol. As see from the confirmation tables, the predicted mean values were similar to the predicted median, which means that the model can predict actual outcomes.

Table P12: Confirmation at best factor settings for biogas and methane yields.

Two-sided	**Confidence = 95%**			**n = 1**			
Factor	**Name**		**Level**	**Low Level**	**High Level**	**Standard Deviation**	**Coding**
A	Temperature		50.00	25.00	50.00	0.00	Actual
B	Substrate to inoculum ratio		1.00	0.50	1.50	0.00	Actual
C	Glycerol volume		10.00	10.00	34.00	0.00	Actual
Response	**Predicted Mean**	**Predicted Median**	**Standard Deviation**	**n**	**SE Pred.**	**95% PI low**	**95% PI high**
Biogas yield	0.85	0.85	0.091	1	0.12	0.54	1.16
Methane yield	0.69	0.69	0.067	1	0.09	0.46	0.91