Characterization of organics for anaerobic digestion by modelling augmented biochemical methane potential test results

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MASTER'S DISSERTATION

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Abstract

This study aims to establish a dynamic model for the simulation of biochemical methane potential (BMP) test results, as well as to determine the additional measurements required in the BMP assay procedure for the model to estimate the composition of a homogenous biodegradable particulate organic sample. This research would aid the modelling of anaerobic digester behaviour to predict and prevent inhibition and failure.

Anaerobic digestion is a mechanism of biological processes which breaks down biodegradable material to biogas (primarily methane and carbon dioxide) and residual sludge in the absence of oxygen. The process was originally used to treat biological wastes, but is now being used to treat various forms of organic waste, such as food and agricultural industry by-products, municipal solid waste, etc. Anaerobic digestion is also a net energy-producing process as it forms the energy laden gas, methane (CH_4). The biological processes are, however, sensitive to organic characteristics and inhibitory toxins, among others, which can cause inefficient reactor systems. Currently, only expensive or complex monitoring technologies can provide adequate parameter concentrations to determine when unstable digester conditions are imminent.

The BMP test measures the biodegradability (biodegradable fraction of organics) of a substrate by comparing the methane production of a control sample (only containing anaerobic digestion seed inoculum) to that of a test sample (containing organic substrate and anaerobic digestion seed inoculum). The difference in biogas produced between the test and control samples is an indication of the potential methane that can be produced through the anaerobic digestion of the organic substrate. The BMP test is the preferred test to determine the feasibility and efficiency of the anaerobic digestion of organic wastes from various industries (e.g. agricultural and food) as it is relatively inexpensive in comparison to larger studies (Moody *et al.*, 2009). The simplicity of the BMP test and its established reputation makes it a suitable test to develop for the purpose of improving the reliability of anaerobic digestion. The simplicity of the BMP test is particularly important for this research to be applicable to countries and areas where technical skills and funding are scarce.

With the extension and modelling of the simple and affordable BMP test the factors which influence anaerobic digester behaviour, such as the influent organic composition and biodegradable fraction of the organics, may be estimated. Determining these influential factors allows for their use as inputs in an anaerobic digestion model. Since the outputs of an anaerobic

digester are entirely defined by the input an anaerobic digestion model supplied with accurate inputs can be used for predicting anaerobic digester behaviour in order to implement suitable control measures prior to the formation of unstable digester conditions.

In the model the organic composition of the biodegradable organics are expressed as x, y, z, a, b and c in $C_xH_yO_zN_aP_bS_c$. Because the outputs of an anaerobic digester are entirely defined by the input, the primary objective of this thesis was to investigate if the x, y, z, a and b (c is not included in the scope, although the model offers the option of including it) values could be determined from a BMP test, augmented with additional measurements of $H_2CO_3^*$ alkalinity, pH, free and saline ammonia (FSA) and ortho-phosphate. In order to achieve this objective the unbiodegradable fraction (sums to 1 with the biodegradable fraction) of the organics, the active fraction of the seed inoculum and the hydrolysis kinetic constants of the organics required calibration to the specific substrate and inoculum. Therefore, the list of additional measurements considered by the scope of this study was extended to further include the CH_4 and CO_2 gas production rates, chemical oxygen demand (COD) of the solubles and the partial pressure of CO_2 (pCO_2).

The scope of the study initially only included a theoretical modelling component, due to the reconstruction of the water quality laboratory at the University of Cape Town. However, the University of Padova kindly provided a basic set of BMP results for vinasse and cheese whey substrates recorded in 2012. Although the data did not include any additional measurements which this research aimed to investigate, the data was nevertheless useful for a basic testing of the BMP model.

The anaerobic digestion unit of the Plant Wide Model South Africa (PWM_SA_AD), developed by the University of Cape Town (UCT) and the University of KwaZulu-Natal (UKZN), was selected as a base model for the BMP model (PWM_SA_AD_BMP) developed in this thesis. The major changes included the conversion to a batch test system and the inclusion of fractionation parameters to control the fraction of unbiodegradable particulate organics (UPO), in the biomass inoculum and the organic feed, entering the test such that the fraction could be determined with the aid of the parameter estimation tool in the WEST modelling platform. The parameter estimation tool uses multiple model simulations to calibrate a set of user-selected model parameters against a set of BMP (or BSP) data. The BMP model was verified by checking that the COD, mass (C, H, O, N, P and S) and charge balance to 100.000% at every time step of the simulation.

The calibration concluded that the critical measurements in the BMP test, which were used as provided variable values and were required for the determination of the objective parameters (x, y, z, a, and b), included the CH_4 and CO_2 gas production rates, ortho-phosphate, FSA, soluble COD and pCO_2. However, the accuracy of the objective parameters were highly influenced by the accuracy of the provided variable values, therefore it was recommended that the full set of nine sensitive variables should be used in the PWM_SA_AD_BMP model parameter estimation. This set of nine variable values include the six mentioned above and the pH, $H_2CO_3^*$ alkalinity and volatile fatty acid (VFA) concentration.

Similar calibration tests were performed using sulphidogenic bioprocesses instead of the methanogenic bioprocesses which take place in the BMP test. This biochemical sulphide potential batch test (called BSP) produces hydrogen sulphide (H_2S/HS^-) from sulphate (sulphidogenesis) instead of methane from VFA and CO_2 (methanogenesis). The BSP test has the benefit of an aqueous sulphide and sulphate concentration measurement which can be measured with better accuracy than a gaseous methane and CO_2 production in the BMP test. Therefore, the pCO_2 and CH_4 and CO_2 production rates in the provided variable values were replaced with aqueous sulphide and sulphate concentrations. The BSP modelling produced the same accuracy levels as the BMP calibration tests. Hence, a hypothesis was presented which proposed that improved accuracy in the objective parameters can be achieved through improving the accuracy of the provided variable values by using aqueous sulphide and sulphate concentrations rather than gaseous methane and CO_2 production.

The testing of the PWM_SA_AD_BMP model with the vinasse and whey BMP test data provided by the University of Padova produced parameter estimations of reasonable accuracy considering that the calibration used only CH_4 and biogas accumulation data (the other seven variables were not measured). The testing also reinforced the requirement of further measurements in the BMP assay protocol to determine the composition constants, as only the y (H) and z (O) elemental compositions were accurately determined from the gas data.

In conclusion, the objective of creating a dynamic BMP model was achieved, but the level of success with which the PWM_SA_AD_BMP model can determine the objective parameters defining the composition of the biodegradable organics remains to be proven with actual augmented BMP test data. Using augmented BSP test measurements is preferable as the aqueous concentrations measured in the BSP test can be measured with better accuracy than gas production rates of the BMP test. The PWM_SA_AD_BMP model already allows for the

modelling of the BSP test because the sulphidogenic bioproesses have been included in the PWM_SA_AD model.

It is recommended that the BSP test proposed in this study be conducted on a selection of substrates and compared with BMP tests of the same substrates to confirm the hypothesized improvement in accuracy of a substrate's biodegradable fraction and specific methane potential. Furthermore, these tests should include the nine variable measurements proposed above in order to test the accuracy of the PWM_SA_AD_BMP model to its full potential.

Acknowledgements

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Abbreviations

ASM2: Activated sludge model 2
ATR-FTIR: Attenuated total reflectance Fourier transform infrared
BEPR: Biological excess phosphorus removal
BMP: Biological methane potential
BPO: Biodegradable particulate organics
BSO: Biodegradable soluble organics
COD: Chemical oxygen demand
FSA: Free and saline ammonia
HPLC: High-performance liquid chromatography
HRT: Hydraulic retention time
IDS: Inorganic dissolved solids
ISS: Inorganic settleable solids
IWA: International Water Association
IWAADM1: IWA Anaerobic Digestion Model 1
ND: Nitrification-Denitrification
OFMSW: Organic fraction of municipal solid waste
OHO: Ordinary heterotrophic organism
OLR: Organic loading rate
PAO: Polyphosphate accumulating organisms
PHA: Polyhydroxyalkanoate
PP: Polyphosphate
PWM_SA: Plant wide model South Africa
SCFA: Short chain fatty acid
SMPR: Specific methane production rate
SRT: Sludge retention time
TKN: Total Kjeldahl nitrogen
TDS: Total dissolved solids
UASB: Upflow anaerobic sludge blanket
UCT: University of Cape Town
UCTSDM1: UCT
PWM_SA: Plant Wide Model of South Africa
PWM_SA_AD: anaerobic digestion unit of PWM_SA
PWM_SA_AD_BMP: Batch test adjusted PWM_SA_AD for BMP test modelling
UKZN: University of KwaZulu-Natal
UPO: Unbiodegradable particulate organics
USO: Unbiodegradable soluble organics
VFA: Volatile fatty acid
VSS: Volatile suspended solids
WEST: Software by mikebydhi.com for dynamic modelling and simulation of wastewater treatment plants
WRC: Water Research Commission

Nomenclature

(H^+)	Hydrogen ion activity (mol/l)
$X_{1,2}$	5-point titration equation placeholder
$Y_{A1,2}$	5-point titration equation placeholder
$Y_{N1,2}$	5-point titration equation placeholder
$Y_{P1,2}$	5-point titration equation placeholder
A_1	5-point titration equation placeholder
A_2	5-point titration equation placeholder
B_1	5-point titration equation placeholder
B_2	5-point titration equation placeholder
Z_1	5-point titration equation placeholder
Z_2	5-point titration equation placeholder
A_T	Total acetate species
b_AC	Model decay rate constant for methanogenic acetogens
b_ACS	Model decay rate constant for sulphidogenic acetogens
b_AD	Model decay rate constant for acidogens
b_AM	Model decay rate constant for acetoclastic methanogens
b_AS	Model decay rate constant for acetoclastic sulphidogens
b_HM	Model decay rate constant for hydrogenotrophic methanogens
b_HS	Model decay rate constant for hydrogenotrophic sulphidogens
b_OHO_AD	Model decay rate constant for OHO
b_PAO_AD	Model decay rate constant for PAO
C	Carbon
C_a	Normality of strong acid (mol/l)
CH_4	Methane
CH4_rate	Model variable for methane production rate
CO_2	Carbon dioxide
CO2_rate	Model variable for carbon dioxide production rate
COD_per_mol_[Component]	Model COD mass per mole for each component
COD_s	Model variable for soluble COD concentration
C_T	Total carbonate species
e_s	Standard error
f_AC	Model methanogenic acetogen fraction of live organisms
f_ACS	Model sulphidogenic acetogen fraction of live organisms
f_AD	Model acidogenic fraction of live organisms
f_AM	Model acetoclastic methanogen fraction of live organisms
f_AS	Model acetoclastic sulphidogen fraction of live organisms
f_Biomass	Model live organism fraction of biodegradable portion of dead biomass
f_HM	Model hydrogenotrophic methanogen fraction of live organisms
f_HS	Model hydrogenotrophic sulphidogen fraction of live organisms
f_U_Inf	Model unbiodegradable particulate fraction of organic feed VSS
f_U_Org	Model unbiodegradable endogenous residue fraction of inoculum seed sludge VSS
f_XU_Bio_lysis	Model fraction of inert COD generated in methanogenic biomass decay with death regeneration model

f_XU_Bio_lysis_s Model fraction of inert COD generated in sulphidogenic biomass decay with death regeneration model

f_c TOC/VSS mass ratio

f_{cv} COD/VSS mass ratio

f_m Monovalent activity co-efficient

f_n organic N/VSS mass ratio

f_p organic P/VSS mass ratio

G_CH4 Methane model component

G_CO2 Carbon dioxide model component

gam_bp Model electrons per mole biodegradable particulate dead biomass

gam_bps Model electrons per mole biodegradable particulate influent/feed

gam_e Model electrons per mole endogenous residue

gam_f Model electrons per mole fermentable biodegradable soluble

gam_o Model electrons per mole organisms

G_{ij} Model generated variable values

H Hydrogen

H2CO3Alk Model variable for $H_2CO_3^*$ alkalinity concentration

H_2O Water model component

HAc Acetic acid (associated form of acetate)

HPr Propionic acid (associated form of propionate)

i Each time step

i_Ca_PP_mol_perP Model Ca/P ratio of polyphosphate

i_H_Org_mol_perC Model H/C ratio of organisms

i_H_SF_mol_perC Model H/C ratio of fermentable biodegradable soluble organics

i_H_SU_mol_perC Model H/C ratio of unbiodegradable soluble organics

i_H_XBInf Abbreviation for i_H_XBInf_mol_perC

i_H_XBInf_mol_perC Model H/C ratio of biodegradable particulate influent/feed

i_H_XBOrg Abbreviation for i_H_XBOrg_mol_perC

i_H_XBOrg_mol_perC Model H/C ratio of biodegradable particulate dead biomass

i_H_XUInf_mol_perC Model H/C ratio of unbiodegradable particulate influent/feed

i_H_XUOrg_mol_perC Model H/C ratio of unbiodegradable particulate endogenous residue

i_K_PP_mol_perP Model K/P ratio of polyphosphate

i_Mg_PP_mol_perP Model Mg/P ratio of polyphosphate

i_N_Org_mol_perC Model N/C ratio of organisms

i_N_SF_mol_perC Model N/C ratio of fermentable biodegradable soluble organics

i_N_SU_mol_perC Model N/C ratio of unbiodegradable soluble organics

i_N_XBInf Abbreviation for i_N_XBInf_mol_perC

i_N_XBInf_mol_perC Model N/C ratio of biodegradable particulate influent/feed

i_N_XBOrg Abbreviation for i_N_XBOrg_mol_perC

i_N_XBOrg_mol_perC Model N/C ratio of biodegradable particulate dead biomass

i_N_XUInf_mol_perC Model N/C ratio of unbiodegradable particulate influent/feed

i_N_XUOrg_mol_perC Model N/C ratio of unbiodegradable particulate endogenous residue

i_O_Org_mol_perC Model O/C ratio of organisms

i_O_SF_mol_perC Model O/C ratio of fermentable biodegradable soluble organics

i_O_SU_mol_perC Model O/C ratio of unbiodegradable soluble organics

i_O_XBInf Abbreviation for i_O_XBInf_mol_perC

i_O_XBInf_mol_perC Model O/C ratio of biodegradable particulate influent/feed

i_O_XBOrg Abbreviation for i_O_XBOrg_mol_perC

i_O_XBOrg_mol_perC Model O/C ratio of biodegradable particulate dead biomass

i_O_XUInf_mol_perC Model O/C ratio of unbiodegradable particulate influent/feed

i_O_XUOrg_mol_perC Model O/C ratio of unbiodegradable particulate endogenous residue

i_P_Org_mol_perC Model P/C ratio of organisms

i_P_SF_mol_perC Model P/C ratio of fermentable biodegradable soluble organics

i_P_SU_mol_perC Model P/C ratio of unbiodegradable soluble organics

i_P_XBInf Abbreviation for i_P_XBInf_mol_perC

i_P_XBInf_mol_perC Model P/C ratio of biodegradable particulate influent/feed

i_P_XBOrg Abbreviation for i_P_XBOrg_mol_perC

i_P_XBOrg_mol_perC Model P/C ratio of biodegradable particulate dead biomass

i_P_XUInf_mol_perC Model P/C ratio of unbiodegradable particulate influent/feed

i_P_XUOrg_mol_perC Model P/C ratio of unbiodegradable particulate endogenous residue

i_S_Org_mol_perC Model S/C ratio of organisms

i_S_SF_mol_perC Model S/C ratio of fermentable biodegradable soluble organics

i_S_SU_mol_perC Model S/C ratio of unbiodegradable soluble organics

i_S_XBInf_mol_perC Model S/C ratio of biodegradable particulate influent/feed

i_S_XBOrg_mol_perC Model S/C ratio of biodegradable particulate dead biomass

i_S_XUInf_mol_perC Model S/C ratio of unbiodegradable particulate influent/feed

i_S_XUOrg_mol_perC Model S/C ratio of unbiodegradable particulate endogenous residue

ISS_BM Model ISS to biomass for OHO and PAO

j Each output variable

K Objective function value

K_CO2 Model rate constant for CO_2 exchange

K_I_H_AM Model H^+ inhibition for acetoclastic methanogens

K_I_H_HM Model H^+ inhibition for hydrogenotrophic methanogens

K_I_H2 Model inhibition coefficient for H_2 in acidogenesis

K_I_HS_ACS Model H_2S inhibition for sulphidogenic acetogens

K_I_HS_AS Model H_2S inhibition for acetoclastic sulphidogens

K_I_HS_HS Model H_2S inhibition for hydrogenotrophic sulphidogens

K'_{aa1} First apparent dissociation constant for acetate system

K'_{aa2} Second apparent dissociation constant for acetate system

K'_{ac1} First apparent dissociation constant for carbonate system

K'_{ac2} Second apparent dissociation constant for the carbonate system

K'_{an1} First apparent dissociation constant for ammonium system

K'_{an2} Second apparent dissociation constant for ammonium system

K'_{ap1} First apparent dissociation constant for phosphate system

K'_{ap2} Second apparent dissociation constant for phosphate system

K'_{w} Apparent ionic product constant for water $(mol/l)^2$

kdis_cal Model dissolution of calcite

kdis_cap Model dissolution of calcium phosphate

kdis_mag Model dissolution of magnesite

kdis_mgkp Model dissolution of K-struvite

kdis_newb Model dissolution of newberyite

kdis_stru Model dissolution of struvite

kH_F_AD_hyd Model hydrolysis rate constant for fermentable BSO

kH_PHA_AD_hyd Model hydrolysis rate constant for poly-hydroxy-alkanoate

kH_PP_AD_hyd Model hydrolysis rate constant for polyphosphate

K_M Monod kinetics hydrolysis rate constant

kM_BInf Abbreviation for kM_BInf_AD_hyd

kM_BInf_AD_hyd Model Monod hydrolysis kinetic rate constant for biodegradable particulate influent/feed

kM_BOrg Abbreviation for kM_BOrg_AD_hyd

kM_BOrg_AD_hyd Model Monod hydrolysis kinetic rate constant for biodegradable particulate dead biomass

kM_fPP_PAO_PHAstor Model maximum rate for polyphosphate release with anaerobic poly-hydroxy-alkanoate storage

Kn_ACS Model half saturation coefficient for sulphidogenic acetogens

Kn_AS Model half saturation coefficient for acetoclastic sulphidogens

Kn_HS Model half saturation coefficient for hydrogenotrophic sulphidogens

K_S Monod kinetics half saturation coefficient

KS_AC Model Monod half saturation coefficient for methanogenic acetogens

KS_ACS Model Monod half saturation coefficient for sulphidogenic acetogens

KS_AD Model Monod half saturation coefficient for acidogens

KS_AM Model Monod half saturation coefficient for acetoclastic methanogens

KS_AS Model Monod half saturation coefficient for acetoclastic sulphidogens

KS_BInf	Abbreviation for KS_BInf_AD_hyd
KS_BInf_AD_hyd	Model Monod half saturation coefficient for biodegradable particulate influent/feed
KS_BOrg	Abbreviation for KS_BOrg_AD_hyd
KS_BOrg_AD_hyd	Model Monod half saturation coefficient for biodegradable particulate dead biomass
KS_HM	Model Monod half saturation coefficient for hydrogenotrophic methanogens
KS_HS	Model Monod half saturation coefficient for hydrogenotrophic sulphidogens
M_{ij}	Measured variable values
MM_C	Model molar mass of carbon
MM_H	Model molar mass of hydrogen
MM_N	Model molar mass of nitrogen
MM_O	Model molar mass of oxygen
MM_P	Model molar mass of phosphorus
MM_S	Model molar mass of sulphur
mu_AC	Model max specific growth rate for methanogenic acetogens
mu_ACS	Model max specific growth rate for sulphidogenic acetogens
mu_AD	Model max specific growth rate for acidogens
mu_AM	Model max specific growth rate for acetoclastic methanogens
mu_AS	Model max specific growth rate for acetoclastic sulphidogens
mu_HM	Model max specific growth rate for hydrogenotrophic methanogens
mu_HS	Model max specific growth rate for hydrogenotrophic sulphidogens
MW_[Component]	Model molar mass for each component
N	Nitrogen
N_i	Total number of time steps at which variable values were measured
NmlAccCH4	Model variable for accumulated normalized millilitres of CH_4
NmlAccCO2	Model variable for accumulated normalized millilitres of CO_2
N_p	Number of parameters selected for estimation
N_T	Total ammonia species
N_v	Total number of output variables used in the parameter estimation
O	Oxygen
OrthoP	Model variable for ortho-phosphate concentration
P	Phosphorus
p_co2	Model variable for partial pressure of carbon dioxide
p_H_s	Model variable for pH
P_{act}	Actual parameter value
pCO_2	Partial pressure of carbon dioxide
P_{est}	Estimated parameter value
pH_2	Partial pressure of hydrogen
$pK^{`}_w$	Negative log of $K^{`}_w$
P_T	Total phosphate species
S	Sulphur
S_Ca	Calcium model component
S_Cl	Chloride model component
S_CO3	Carbonate model component
S_F	Fermentable biodegradable soluble organics model component
S_Glu	Glucose model component
S_H	Hydrogen ion model component
S_H2	Dissolved hydrogen model component
S_HS	Sulphide model component
S_K	Potassium model component
S_Mg	Magnesium model component
S_N2	Dissolved nitrogen model component

S_Na	Sodium model component
S_NH	Ammonium model component
S_NO	Nitrate model component
S_O	Dissolved oxygen model component
S_PO4	Phosphate model component
S_Pr	Propionate model component
S_SO4	Sulphate model component
S_U	Unbiodegradable soluble organics model component
S_VFA	Acetate model component
V_gas	Model volume of headspace in the reactor
V_liq	Model volume of liquid in the reactor at the start of the simulation
V_s	Sample volume prior to titration (l)
$V_{x1,2}$	Volume of strong acid added from pH_1 to pH_2 (l)
X_AC	Methanogenic acetogens model component
X_ACP	Amorphous calcium phosphate model component
X_ACS	Sulphidogenic acetogens model component
X_AD	Acidogens (for sulphidogenesis and methanogenesis) model component
X_ADO	Autotrophic denitrifying organisms model component
X_AM	Acetoclastic methanogens model component
X_ANO	Autotrophic nitrifying organisms model component
X_AS	Acetoclastic sulphidogens model component
X_B_Inf	Influent/feed biodegradable particulate organics model component
X_B_Org	Biodegradable particulate organics from decayed biomass model component
X_Cal	Calcite model component
X_HM	Hydrogenotrophic methanogens model component
X_HS	Hydrogenotrophic sulphidogens model component
X_ISS	Influent inorganic settleable solids model component
X_Mag	Magnesite model component
X_Newb	Newberyite model component
X_OHO	Ordinary heterotrophic organisms (OHO) model component
X_PAO	Phosphate accumulating organisms (PAO) model component
X_PAO_PP	Polyphosphate model component
X_PAO_Stor	Poly-hydroxy-alkanoate model component
X_Str_K	K-struvite model component
X_Str_NH4	Struvite model component
X_U_inf	Unbiodegradable particulate organics from decayed biomass model component
X_U_Org	Endogenous residue model component
Y_AC	Model methanogenic acetogenesis yield
Y_ACETATE	Model fraction of electrons passed to acetate in sulphidogenic acetogenesis
Y_ACS	Model sulphidogenic acetogenesis yield
Y_AD	Model low H_2 acidogenesis yield
Y_AH	Model high H_2 acidogenesis yield
Y_AM	Model acetoclatic methanogenesis yield
Y_AS	Model acetoclatic sulphidogenesis yield

Y_f_PP_VFA	Model fraction of P released from PP per VFA used in PHA storage
Y_H2	Model fraction of electrons passed to hydrogen gas in sulphidogenic acetogenesis
Y_H2S	Model fraction of electrons passed to HS^- in sulphidogenic acetogenesis
Y_HM	Model hydrogenotrophic methanogenesis yield
Y_HS	Model hydrogenotrophic sulphidogenesis yield
δ	Percentage error
$\Delta MAlk_{1,2}H_2$	Change in mass of H_2 alkalinity from pH_1 to pH_2 (mol)
$\Delta MAlk_{1,2}H_2CO_3^*$	Change in mass of $H_2CO_3^*$ alkalinity from pH_1 to pH_2 (mol)
$\Delta MAlk_{1,2}H_2PO_4^-$	Change in mass of $H_2PO_4^-$ alkalinity from pH_1 to pH_2 (mol)
$\Delta MAlk_{1,2}HAc$	Change in mass of HAc alkalinity from pH_1 to pH_2 (mol)
$\Delta MAlk_{1,2}NH_4$	Change in mass of NH_4 alkalinity from pH_1 to pH_2 (mol)

1 Introduction

1.1 Background

Anaerobic digestion is a mechanism of biological processes which breaks down biodegradable material to biogas (primarily methane and carbon dioxide) and residual sludge in the absence of oxygen. The process was originally used to treat biological wastes, but is now being used to treat various forms of organic waste, such as food and agricultural industry by-products, municipal solid waste, etc. anaerobic digestion is also a net energy-producing process as it forms the energy-laden gas, methane. The biological processes are, however, sensitive to pH and inhibitory toxins, for example, which can cause inefficient reactor systems. Currently, only expensive, complex or time-consuming monitoring technologies can provide adequate parameter concentrations to determine when unstable digester conditions are imminent. A simple and affordable test is required to provide sufficient data for anaerobic digester simulation to predict reactor failure so that it can be prevented.

The biological methane potential (BMP) test may potentially be a solution. It is used to measure the potential yield of biogas (mostly methane and carbon dioxide) from the digestion of organic material. The BMP test follows a simple procedure that could, with the aid of a dynamic anaerobic digestion model, provide data beyond the ultimate methane production of anaerobic digestion. Determining the organic composition and biodegradable fraction of an organic waste, for example, opens opportunities for determining the organics composition, which influences the operation and stability of anaerobic digesters.

The popularity of the anaerobic digestion process has resulted in many facilities being funded and built, which have capacity in excess of their waste sludge supply from the wastewater treatment plant. This provides opportunity to import the organic fraction of municipal solid waste (OFMSW) to add to the digester. This will reduce the volumes of solid waste that go to landfills and the release of methane and carbon dioxide into the atmosphere. Instead, the biogas can be captured for energy generation. The impact of the imported OFMSW or other concentrated organic wastes on the digester, however, needs to be determined in order to prevent an overload of organics or digester failure. The characterization of the imported organics is required in an anaerobic digestion model for the prediction of system behaviour because the anaerobic digester aqueous concentrations (and pH) are entirely dependent on the influent biodegradable organics composition. The BMP test is already broadly used to

determine the efficiency (methane production, hydrolysis rate and biodegradable fraction) of organics for inclusion in anaerobic digestion. This research aims to show that by adding a few basic measurements to the BMP test, the composition of the organics can also be determined with the BMP procedure, which adds much valuable information to determine the impact of co-digestion of organic wastes on a digester.

1.2 Purpose

The purpose of this study is to build a dynamic model for the simulation of BMP test results, as well as to determine the additional measurements required in the BMP assay procedure for the model to function in reverse (i.e. find inputs from outputs). This would ideally provide an estimation of the composition of a biodegradable particulate or soluble organic (BPO or BSO) portion of an organic sample from BMP results and would aid the modelling of digester behaviour to predict and prevent inhibition and failure. The additional measurements selected should be comparable to the rest of the BMP test procedure in terms of simplicity and cost so that this research can aid all those already using the BMP test.

1.3 Scope and limitations

The scope of the study initially only included a theoretical modelling component, due to the reconstruction of the water quality laboratory at the University of Cape Town. However, the University of Padova kindly provided a basic set of BMP results for vinasse and cheese whey substrates recorded in 2012. Although the data did not include any additional measurements which this research aimed to investigate, the data was nevertheless useful for a basic calibration of the BMP model. The use of existing data also removes the probability of obtaining experimentally biased data towards a desired result.

1.4 Plan of development

This dissertation starts with a review and discussion of the literature which is relevant to the study and motivates the choice of research topic and objectives. This is followed by the development of the model, including a model description, key alteration to the base model and the procedure for verification by mass balance. Appendix A accompanies this chapter to

include the full list of model components, parameters and stoichiometry. The project setup chapter 4 is provided as a detailed methodology or user guide for reproducing the modelling environment in WEST. The project setup also discusses the procedures required to process data prior to modelling and after completing the modelling process. In the project sensitivity and calibration chapter 5 the selection of additional measurements to be included in the BMP test procedure is motivated and the PWM_SA_AD_BMP model's settings are calibrated. Chapter 6 discusses the methods and results of the model testing with the vinasse and whey data provided by the University of Padova. Finally, the conclusions and recommendations chapter 7 expands on the possibilities of the investigation towards future insights.

2 Literature review

Anaerobic digestion is a mechanism of biological processes which breaks down biodegradable material to biogas (primarily methane and carbon dioxide) and residual sludge in the absence of oxygen. The process was originally used to treat biological wastes, but is now being used to treat various forms of organic waste, such as food and agricultural industry by-products, municipal solid waste, etc. Anaerobic digestion is also a net energy-producing process as it produces the energy-laden gas, methane. The biological processes are, however, sensitive to pH and inhibitory toxins, for example, which can cause inefficient reactor systems. Currently, only expensive or complex monitoring technologies, such as gas chromatography, high-performance liquid chromatography (HPLC) or attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy (see Section 2.2.3), can provide adequate parameter concentrations to determine when unstable digester conditions are imminent. This review discusses the supporting literature behind the extension and modelling of the biochemical methane potential (BMP) test as a simple and affordable alternative for estimating influential anaerobic digester behavioural factors such as the influent organic composition and unbiodegradable fraction.

2.1 Biochemical methane potential (BMP) test

The BMP test measures the biodegradability (biodegradable fraction of organics) of a substrate by comparing the methane production of a control sample (only containing anaerobic digestion seed inoculum) to that of a test sample (containing organic waste and anaerobic digestion seed inoculum). The difference in biogas produced between the test and control samples is an indication of the potential methane that can be produced through the anaerobic digestion of the organic waste. The BMP test is the preferred test to determine the feasibility and efficiency of the anaerobic digestion of organic wastes from various industries (e.g. agricultural and food) as it is relatively inexpensive in comparison to larger studies (Moody *et al.*, 2009). It also provides an estimation of the proficiency of co-digesting wastes for optimal energy recovery. Due to the variability in organic content of waste products fed to digesters, the BMP test serves as a far more accurate tool for assessing the anaerobic digester parameters than estimations in literature (Moody *et al.*, 2009).

2.1.1 BMP test procedure

The BMP assay process involves the incubation of triplicate test and control samples between 100 ml and 2 litres, depending on substrate consistency, at a constant temperature for a period of up to 60 days, depending on the biodegradability of the organics (Angelidaki *et al.*, 2009). The biogas produced by each of the test and control samples can be measured in terms of the volume (keeping pressure constant) with a graduated piston or liquid displacement equipment (yellow liquid in Fig. 2.1) or in terms of the pressure (keeping volume constant) with a differential manometer or pressure transducer (Esposito *et al.*, 2012). The volumetric methods of determining biogas production allow the percentage methane content of the biogas to be determined using a gas chromatograph or by direct measurement of methane remaining after bubbling the biogas through an alkali solution such as sodium hydroxide (pink liquid in Fig. 2.1) to remove the carbon dioxide. This is, however, just one of the methods for the BMP test procedure and measuring the total volume of biogas before determining the methane content would provide more data about the organic content. A full description of the appropriate protocol for BMP assays is provided in Angelidaki *et al.* (2009). The simplicity of the BMP test procedure allows for the inclusion of other measurements as done by Raposo *et al.* (2006). They included daily measurements of the concentrations of each volatile fatty acid, pH and alkalinity (partial and total). The results demonstrate the reduction of the specific methane production rate (SMPR in ml CH_4/g VSS inoculum/day) during an accumulation in volatile fatty acid (VFA) concentration.

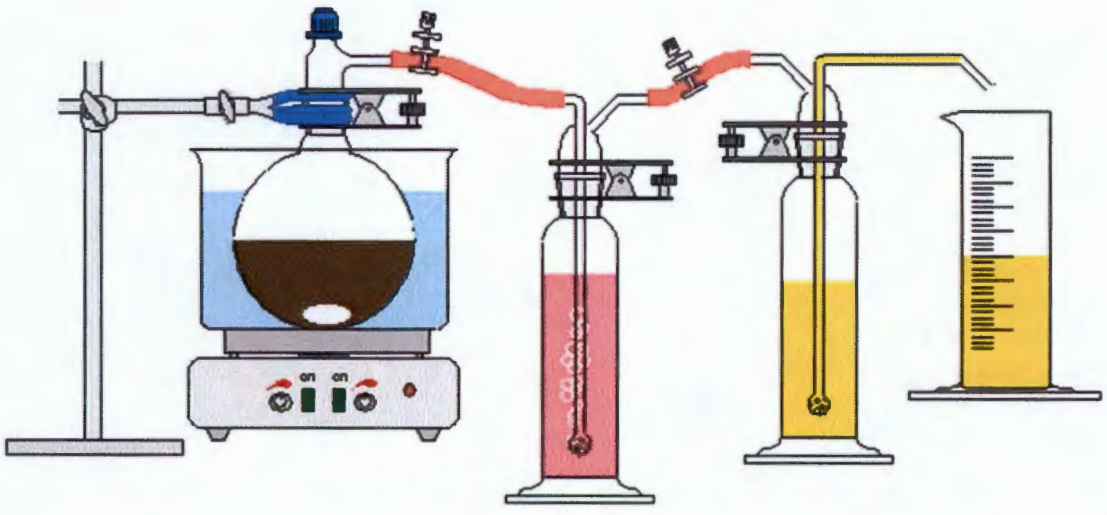

Figure 2.1: A BMP assay setup employing the Mariotte displacement principle for quantifying methane production (Madsen, 2008)

2.1.2 BMP applications

Owen *et al.* (1979) were the first to present the BMP test methods in order to assess the possibility of treating wastewater using anaerobic digestion. The study aimed to utilize the BMP test in a manner which assessed the performance of the anaerobic digestion process in terms of ultimate methane production. Subsequently, Speece (1996) presented a variety of applications of the BMP test, such as: establishing the wastewater organic concentration which can be turned into methane, evaluating the possible performance of an anaerobic system fed by a certain wastewater, measuring the remaining organic material which is able to undergo further anaerobic digestion, and checking for non-biodegradable remnants. Speece (1996) and Lin *et al.* (1999) also made use of BMP assays to relate the methane production to organic digestion through stoichiometric conversions which can be shown to be 350 ml of methane per gram of degraded COD (chemical oxygen demand) (22.4 l/mol CH_4 ÷ 64 gCOD/mol CH_4 × 1000 ml/l). The COD of a substrate is its electron donating capacity expressed as the equivalent amount of oxygen consumed by the oxidation.

Owen *et al.* (1979) also revealed that the biodegradability and methane production of municipal waste activated sludge could be improved and the volatile suspended solids (VSS) could be decreased using alkaline pretreatment. Many other studies have subsequently investigated methods of improving the digestibility of organic waste and thus the effectiveness of the anaerobic digestion process. Mata-Alvarez *et al.* (2000) provide a useful summary of some of these many studies as well as the scale, temperature, reactor type and substrate used in each, which is included in Table 2.1. Recent research (Jensen *et al.*, 2011) has made use of a linear hydrolysis rate and fraction of organics which degrade, calculated from BMP data, to calibrate full scale anaerobic digestion models (see Section 2.1.4 below).

Table 2.1: Studies concerning the performance of digestion of solid wastes presented at the II International Symposium on Anaerobic Digestion of Solid Waste (1999)

Substrate	Scale	Reactor type	Temperature	Reference
Slaughterhouse and catering	Pilot	-	Mesophilic	Membrez *et al.* (1999)
Poultry mortalities	Lab	Two-phase (Leach bed + UASB)	Mesophilic	Chen (1999)
OFMSW[1] in Bamako (Mali)	Pilot	(Leach bed + UASB)	Psychrophilic	Ouedraogo (1999)
Sewage sludge	Lab	Two-phase	Mesophilic	García-Heras (1999)
Mycelium waste (India)		Non-stirred digester	Psychrophilic	Yeole & Ranade (1999)
OFMSW	Lab	One and two stages	Psychro- and Mesophilic	Wang & Banks (1999)
Coffee pulp	Lab	Batch	Psychrophilic	Valdés *et al.* (1999)
Fish farming sludge	Lab	Batch	Mesophilic	Gebauer (1999)
OFMSW	Pilot	Two-phase	Thermophilic	Madokoro *et al.* (1999)
Food Wastes	Lab	Leach Bed	Mesophilic	Paik *et al.* (1999)
OFMSW	Lab	CSTR	Mesophilic	Houbron *et al.* (1999)
Coffee pulp	Pilot	Plug flow	Mesophilic	Farinet & Pommares (1999)
OFMSW/ Coffee pulp	Pilot	Two-phase	-	Edelmann *et al.* (1999)

2.1.3 BMP limitations

The simplicity of the BMP test has made it a broadly used tool in the anaerobic digestion industry to estimate the efficiency of anaerobic digestion of an organic waste. Despite this, comparing results from the test proves difficult without a common protocol. This is largely due to the use of many different instruments including monitoring systems, headspace volumes and pressures, as well as under various environmental conditions such as mixture ratios of inoculum to nutrients, stirring intensity, substrate characteristics, temperature and pH (Angelidaki *et al.*, 2009); the effects of which are discussed in Section 2.2.2.1. Furthermore, the units of measurement often vary between studies. The BMP protocol described in Section 2.1.1 above (Angelidaki *et al.*, 2009) was created in an attempt to minimize these differences.

2.1.4 BMP modelling

Despite the conservative results for hydrolysis rate and degradable fraction produced by the BMP test (Jensen *et al.*, 2011; Batstone *et al.*, 2009), it is considered an effective measure of the efficiency of anaerobically digesting an organic sample. Concerns of the use of the test for anaerobic digestion efficiency include the time-consuming (up to 60 day) test procedures, substantial capital costs and different test conditions from full-scale digesters. A model which predicts BMP results could reduce the duration and start-up costs associated with BMP assays. Of the attempts to model BMP test results, the majority determine a first order model of ultimate methane production by calculating a linear hydrolysis rate (e.g. Lin *et al.*, 1999; Bilgili *et al.*, 2009; Esposito *et al.*, 2012 *inter alia*), with some including a degradable fraction of the substrate as a model parameter (e.g. Batstone *et al.*, 2009; Jensen *et al.*, 2011). Appels *et al.* (2011) have developed the most comprehensive BMP model which considers 19 composition variables measured in triplicate for each of 29 sludge samples from different wastewater treatment plants across Belgium. The composition variables include *inter alia* total and soluble: COD, proteins and carbohydrates; as well as sulphur, phosphorus, pH and individual fatty acid concentrations. Appels *et al.* (2011) conclude that their linear model containing 10 of the variables with regression coefficients calibrated to the 29 sludges can estimate the ultimate methane production of a random sludge sample to within 1.15% of the measured value. The dynamics of BMP tests, however, have not been modelled using non-linear kinetics, but the similarity of anaerobic digestion could provide the background required for the development of a dynamic BMP test model.

2.2 Anaerobic digestion

Anaerobic digestion is one of the earliest discovered biological waste treatment processes. The first digester was reportedly built by a leper colony in 1859 in Bombay, India (Meynell, 1976). Anaerobic digestion was first seen in England in 1895 where biogas from a sewage treatment plant was used to power street lamps in Exeter (Lusk, 1998). Significant research into microbiology and hence anaerobic micro-organisms and methane production can be found since the 1930s. Recently, there has been a noticeable boost in interest in anaerobic digestion and numerous studies are focusing on improving the efficiency and operational control of the anaerobic digestion process (Moody *et al.*, 2009). The heightened interest is largely due to the increase in demand for renewable energy production, with the methane produced from the

anaerobic digestion of organic matter being a source of energy that has the potential to reduce the dependence on fossil fuels. Anaerobic digestion systems were primarily used to remove organics and reduce the odour of waste flows in municipal wastewater treatment plants, but the application of anaerobic digestion has spread beyond wastewater treatment plant organic removal for sewage sludge stabilization. There are over 35 different industries using anaerobic digestion to produce biogas, reduce odours and decrease the cost of sludge disposal to a wastewater treatment plant. Businesses that are applying anaerobic digestion as a pretreatment for energy recovery are essentially expanding the treatment capacity of local wastewater treatment plants. Some of these industries include food processing facilities; animal ranches; fibre, pharmaceutical and chemical producers; landfill sites and farms, including dairy and maize; amongst others (Mata-Alvarez *et al.*, 2000, Sötemann et al, 2005b).

Furthermore, interest in anaerobic digestion has stemmed from the concern over methane release from landfill sites and its contribution towards the negative impact of greenhouse gas emissions (each methane molecule contributes 22 times the effect of a carbon dioxide molecule). Although there are energy benefits from producing biogas, some landfill sites have opted for aerobic (intermittent aeration, Raga & Cossu, 2013) treatment, which produces less methane, as a lower risk alternative to anaerobic digestion. Besides this, the substantial capital cost involved and the sensitivity of anaerobic digesters to system conditions also make attentive designing and optimization planning essential. Despite these drawbacks, Batstone *et al.* (2002) consider that the low volumes of sludge produced and the capacity for high organic loads, which are characteristic of anaerobic digesters, promote adoption of anaerobic digestion in comparison to other biological organic removal operations.

Many cities are addressing the methane emissions at landfills through municipal solid waste separation in order to digest organic waste (OFMSW) before it is released onto landfill sites. Local authorities are also implementing co-digestion practices where external organic wastes are combined with the waste activated sludge fed to wastewater treatment plant anaerobic digesters (provided there is spare capacity). These imported organics in turn enhance the methane production in the digesters due to their high organic content. However, the potential impact of imported organics on the anaerobic digester operation needs to be quantified in order to allow for adequate feeding and control procedures to be implemented. This requirement is largely brought about by the sensitivity of anaerobic digestion systems to fluctuations in organic loading, organic composition and environmental variables (Labatut & Gooch, 2012).

2.2.1 Microbiological processes in anaerobic digestion

Anaerobic digestion is considered a series of biological processes which almost entirely converts biodegradable organic material into methane, carbon dioxide and new biomass. It is, however, important to differentiate between the total COD flowing into the digester and the biodegradable organics (substrate) because of the substantial fraction of anaerobically unbiodegradable content in the inflow COD (Ikumi *et al.*, 2014; Batstone *et al.*, 2002; Gossett & Belser, 1982). Only the biodegradable substrate, which consists of carbohydrates, proteins and lipids, then undergoes the biological processes as shown in Figure 2.2.

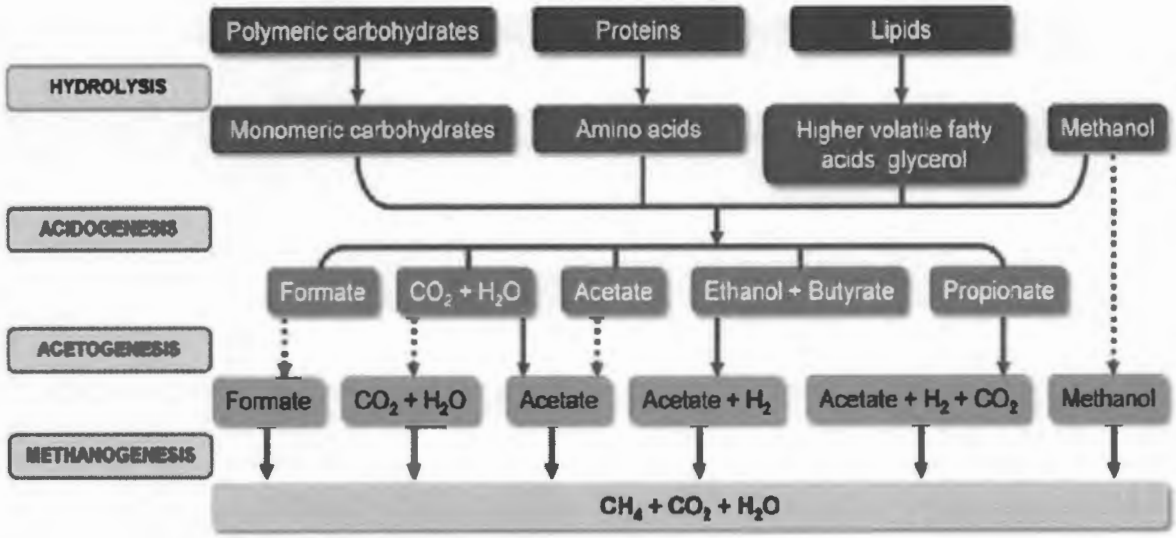

Figure 2.2: Anaerobic digestion biological processes (Source: Del Risco, 2011)

Sötemann *et al.* (2005a, b), Massé & Droste (2000), Moosbrugger *et al.* (1993a), Sam-Soon *et al.* (1991) and Mosey (1983) conceptualize anaerobic digestion as consisting of four bioprocesses: hydrolysis/acidogenesis, acetogenesis, acetoclastic methanogenesis and hydrogenotrophic methanogenesis. Batstone *et al.* (2002), Costello *et al.* (1991), Gujer & Zehnder (1983) and Gaudy & Gaudy (1980) amongst others, however, apply the more common system representation using six bioprocesses, where the hydrolysis/acidogenesis process is subdivided into a process for each of the constituents of organics, namely carbohydrates, proteins and lipids. The components hydrolyze to form soluble sugars, amino acids and fatty acids respectively, but these are considered intermediate products as the acidogens transform

them to short chain fatty acids (SCFA), carbon dioxide and hydrogen. Although this division provides a form of characterization of the organic waste, the composition and metabolism of proteins and lipids are not well understood (McInerney, 1988), measurements of the concentrations of the constituents are difficult to obtain routinely and the end products of hydrolysis/acidogenesis are essentially all the same (SCFA, CO_2, H_2 and ammonia), regardless of the subdivision. Thus, Sötemann *et al.* (2005b) consider it reasonable to model a single hydrolysis/acidogenesis process and instead establish the organics characterization in a generic form, $C_xH_yO_zN_a$ (McCarty, 1974, 1975; Ekama, 2009) for five different physically identifiable (measureable) organic groups: VFA, biodegradable soluble organics (BSO), biodegradable particulate organics (BPO), unbiodegradable soluble organics (USO) and unbiodegradable particulate organics (UPO). Figure 2.3 illustrates the division of these organic groups and the detailed explanation of this characterization method is provided by Wentzel & Ekama (2008).

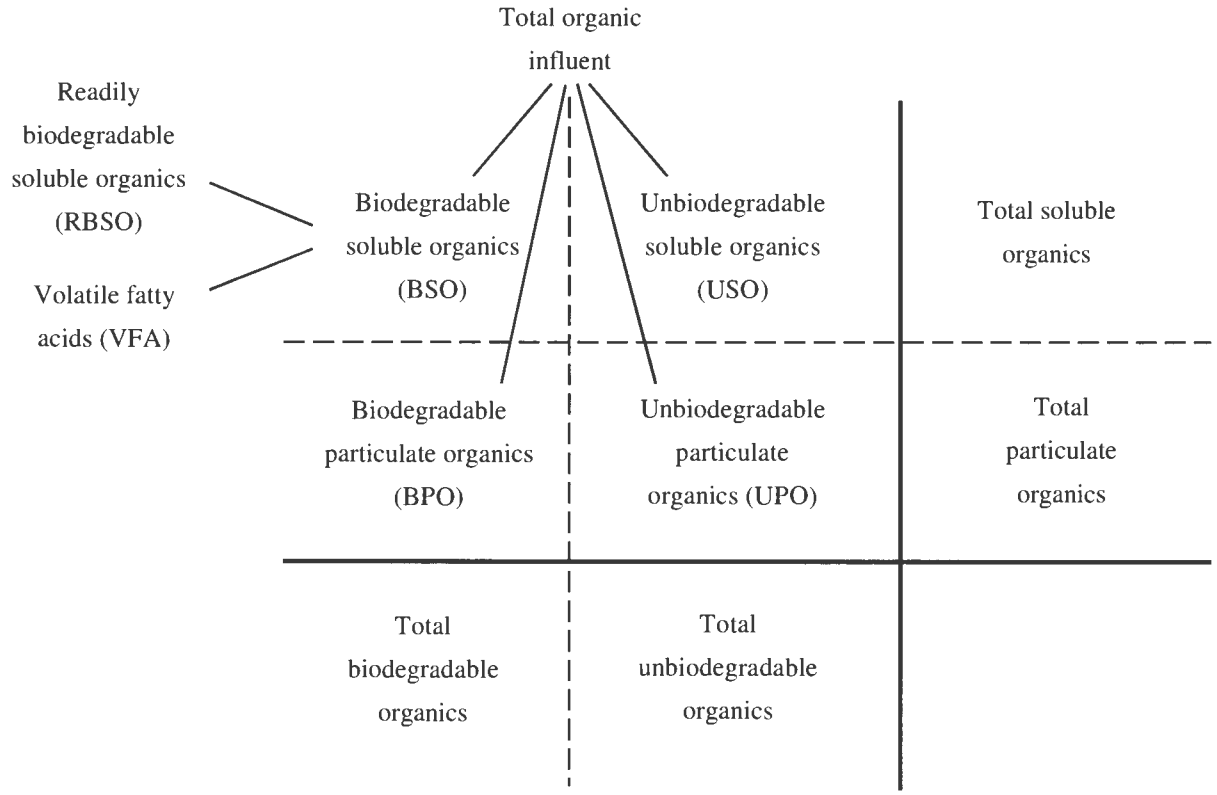

Figure 2.3: Characterization of organic influent into physically identifiable groups based on Wentzel & Ekama (2008)

2.2.1.1 Hydrolysis/acidogenesis

In the first step of the model of Sötemann *et al.* (2005b) the complex organics (BSO, BPO) are hydrolyzed to form the intermediate product, glucose. The use of glucose as the intermediate

product can be justified through the well-known and adequately documented pathways for the metabolism of glucose (Mosey, 1983). The acidogenic micro-organisms immediately break down the glucose via acidogenesis into hydrogen, carbon dioxide and SCFA (acetate and propionate) through a process commonly known as fermentation. Butyrate and other higher chain fatty acids only occur in negligible quantities (Sötemann *et al.*, 2005a, b; Sam-soon *et al.*, 1991). The trace concentrations of butyrate and other higher chain fatty acids are the result of acetate utilization being the rate-limiting reaction for complete degradation of butyrate to methane and carbon dioxide (Ahring & Westermann, 1987). Acidogenesis is, however, dependent on the partial pressure of hydrogen (pH_2). Under low pH_2 ($< \pm 10^{-3,7}$ atm.) conditions only acetate and hydrogen are formed. However, under high pH_2 ($> \pm 10^{-3,7}$ atm.) conditions the acidogens form not only acetate and hydrogen but also the intermediate product propionate and trace quantities of butyrate and other higher chain fatty acids (Sotemann *et al.*, 2005b; Moosbrugger *et al.*, 1993a; Sam-soon *et al.*, 1991). With complex organics the hydrolysis/acidogenesis process involving the breakdown of BPO is the slowest and most energy-consuming process in the anaerobic digestion system and is often regarded as the rate-limiting process in stable reactors (Sötemann *et al.*, 2005a, b; Vavilin *et al.*, 2004; Batstone *et al.*, 2002; Massé & Droste, 2000; Mata-Alvarez *et al.*, 2000; McCarty, 1974).

2.2.1.2 Acetogenesis

Acetate is not only produced through fermentation but also by acetogenic micro-organisms. The acetogens convert the propionate (and other longer carbon chain VFAs) into acetate, hydrogen and carbon dioxide. This process is also influenced by the pH_2. Propionate (and trace quantities of butyrate) conversion only takes place when $pH_2 < \pm 10^{-4,1}$ atm. as higher pH_2 inhibits the acetogenic process almost entirely (Sam-soon *et al.*, 1991). The inhibition causes an accumulation of longer carbon chain VFAs in the form of propionate (and trace quantities of butyrate) and may be accompanied by a reduction in pH, depending on the buffer capacity of the system (Moosbrugger *et al.*, 1993a). Although the removal of excess hydrogen is recommended to prevent pH_2 inhibition, it is not necessarily imperative as the hydrogenotrophic methanogens consume hydrogen.

2.2.1.3 Acetoclastic methanogenesis

The acetoclastic methanogens produce methane and carbon dioxide from acetate. The methane produced in this process constitutes approximately 75% of the total methane accumulation (Massé & Droste, 2000), but is dependent on the extent of the formation of higher chain fatty acids such as propionate and butyrate in high pH_2 (> ± $10^{-3,7}$ atm.) conditions. This methanogenic process (and also the hydrogenotrophic methanogenic process – see below) is highly sensitive to pH. Thus the possibility of SCFA accumulation, with resulting pH reduction, poses a great risk to the methanogen activity. Fortunately, the acetogenesis and acetoclastic methanogenesis processes which utilize the SCFAs are significantly faster than the hydrolysis/acidogenesis process under stable reactor conditions with complex organic substrate. In unstable, low pH conditions, however, Mosey (1983) confirms that the acetoclastic methanogenesis process is significantly rate limited. The acetoclastic methanogen growth rate is at a maximum at a pH of 7.0 but their activity decreases sharply when the pH falls below 6.6 (Moosbrugger *et al.*, 1993a). A neutral pH is, thus, essential for a stable and efficient methane production rate in a reactor. However, methanogens are also vulnerable to other inhibitors such as toxins (see Section 2.2.2.3) entering with the influent.

2.2.1.4 Hydrogenotrophic methanogenesis

The hydrogenotrophic methanogens reduce carbon dioxide and hydrogen to produce water and methane, which constitutes approximately 25% of the total methane production, again dependent on the extent of the formation of higher chain fatty acids. The hydrogenotrophic process aids the acidogenic and acetogenic processes by utilizing hydrogen to form a low pH_2 state. The hydrogenotrophic methanogenic micro-organisms are also highly sensitive to pH fluctuations and toxins. In the presence of an inhibiting substance the pH_2 will rise, causing the production and accumulation of propionate, an accompanying decrease in the methanogenic growth rate and the development of unstable conditions in an exponentially escalating fashion.

2.2.2 Anaerobic digestion performance

There are many factors which can influence the performance of anaerobic digestion by either enhancing or inhibiting processes in terms of the methane production rate, specific growth rate of organisms, degradation rate or substrate utilization. A major concern with anaerobic digestion performance is the frequency of process inhibition and failure. Inhibition refers to a

detrimental modification of a micro-organism group's biological processes or prevention of organism growth (Chen *et al.*, 2008) and is usually indicated by a decrease in the rate of methane production or an accumulation of VFAs. Failure refers to a point where the performance (methane production rate) of the anaerobic digestion process has decreased to the extent that complete shutdown (stopping influent feed) is the only option for revival (i.e. the inactivity of at least one of the bioprocesses). Indicators, however, only illustrate reactor upset conditions and little confirmation is found in the literature for concentration levels which indicate inhibition. These variations are largely due to the acclimation, antagonism and synergism of micro-organisms in the metabolic processes (Chen *et al.*, 2008). Acclimation refers to a micro-organism's ability of overcoming the toxic interference through restructuring of its metabolic pathways as toxin concentrations slowly increase. Antagonism and synergism is the reduction and enhancement, respectively, of the effect of a toxin due to the presence of another substance.

The investigation of failure mechanism pathways can aid in the determination of the origin of anaerobic digestion inhibition. Sötemann *et al.* (2005a) report three general causes of unstable reactors, namely inhibiting substances, large temperature fluctuations and organic over loading conditions. Björnsson *et al.* (2000) reinforce this by reporting performance indicators to be case specific with the process configuration (e.g. organic loading rate, temperature range and mixing regime) and the characterization of the organic waste as governing factors in the determination of process sensitivity. This section describes the relevant effects of the system configuration considerations, organic waste load characteristics and inhibitory substances on the behaviour of anaerobic digesters.

2.2.2.1 Process configuration

The configuration of anaerobic digesters is controlled by many factors including the reactor type, volume and shape, head space volume, effluent standards, etc. Most of these factors are site specific because many different anaerobic digestion solutions exist to create client specific solutions; however, those discussed below play a large role in the stability of the anaerobic digestion processes.

Mixing conditions: The mixing which takes place in an anaerobic digester is generally defined by the reactor type. However, this investigation is specifically concerned with the BMP test, which is considered to be a continuously stirred batch digester. Although mixing is a disputed topic, researchers agree that it is important in the digestion of solid waste. Mixing

enables adequate contact between influent substrate and micro-organism, assists with the disintegration of solids into smaller particles, prevents thermal pockets and reduces particulate sedimentation and scum formation. Vigorous, continuous mixing was reported to be "inhibitory" at high organic loading rates and attributed to the disruption of interdependent micro-organism relationships which require proximity and spatial juxtapositioning (Stroot *et al.*, 2001; Van Zyl *et al.*, 2008) to function co-operatively. Minimal stirring provided optimum methane production rates and accumulated gas concentrations.

Inoculum to substrate (I/S) ratio: The hydrolysis process has broadly been shown to be the rate-limiting step in anaerobic digestion, but the rate of the process is dependent on the concentration of active acidogenic organisms which produce enzymes that assist with the hydrolysis of the substrate. Jensen *et al.* (2011, 2009), Esposito *et al.* (2012) and Mourino *et al.* (2001) showed that if the I/S ratio is inadequately low (below 0.5 gVS inoculum/gVS substrate), the hydrolysis rate and methanogenic activity would be reduced as a result of instabilities such as high organic load (COD) and an accumulation of VFAs. The studies refer to BMP tests specifically, where it was shown that microbial limited samples produced inaccurate results for system parameters such as the degraded fraction of the substrate and hydrolysis rate. However, increasing the I/S ratio above 1.6 gVS inoculum/gVS substrate was also shown to decrease the biogas yield, but as a result of the increasing fraction of biogas being produced from the degradation of biomass in the test sample, hence a decrease in the biogas yield from substrate degradable organics (Esposito *et al.*, 2012; Raposo *et al.*, 2006). This is particularly applicable to BMP tests where it is important to ensure that sufficient inoculum is provided so as not to hinder hydrolysis and methanogenesis, but also not an oversupply of inoculum which could cause an underestimate of the methane production. This is particularly relevant to applications of BMP data because the results are most commonly used for estimating the efficiency as the methane production rate per unit of solids degraded of a substrate and a significant under estimation of this value can result in an undersized digester.

Retention time and organic loading: For flow through anaerobic digesters, the hydraulic retention time (HRT) is a measure which describes the average period that the dissolved substrate remains in the mixed digester. The organic loading rate (OLR) is the amount of organic matter treated per unit volume of reactor in a period of time. It is inversely proportional to the HRT; the longer the substrate remains in the reactor, the lower the OLR. The feed concentration also affects the OLR on a directly proportional basis. A low HRT or high OLR (for the same feed concentration) is preferred as it increases the volumetric biogas production

rate (l gas/m^3 reaction/d) and reduces the required reactor size, minimizing the capital cost. For the continuously stirred flow through anaerobic digester, the HRT is equal to sludge retention time (SRT) because the liquor is completely mixed and the digester sludge flow is also the hydraulic flow. If the SRT is less than the growth rate of the slowest growing micro-organism, the micro-organisms will be removed from the reactor with the effluent; causing system failure. Other concerns of low HRTs and high OLRs include the reduction in percentage organic removal and the higher risk of developing unstable conditions. Mosey (1983) reinforces this by stating that a sudden increase in the OLR is expected to cause an accumulation of VFAs, because the acetogenic growth rate is not only slower than that of the acidogens, but also needs time to increase from the higher VFA production. This "backlog" in VFA can cause the pH to decrease, slowing the methanogens even further.

The bioprocesses of anaerobic digestion have been reasonably well-understood for the past two decades and during this time research attention focused on intensifying the anaerobic digestion process by separating HRT and SRT with sludge/biomass retention using thickening techniques to remove supernatant and increase the digester solids concentration. Earlier research on biomass retention anaerobic digestion is the clarigester, which has a settling zone above the anaerobic digester. Later systems achieved this with (i) pelletization in upflow anaerobic sludge blanket (UASB) systems (Sam-Soon *et al.*, 1987; Moosbrugger *et al.*, 1993a; Wentzel *et al.*, 1994), (ii) membranes (Van Zyl *et al.*, 2008) and (iii) fixed media contact digestion (Dupla *et al.*, 2004). These have been particularly successful with dissolved organic wastes and OLR of up to 30 kgCOD/m^3/d have been achieved (Van Zyl *et al.*, 2008).

Temperature: The temperature of an anaerobic digestion system is a key parameter in determining the rate of substrate degradation as the level of hydrolytic and methanogenic activity is highly influenced by temperature fluctuations. Generally, either mesophilic (20-45°C) or thermophilic (>45°C) temperature ranges are applied, with optimum performance at 35°C and 55°C respectively (Mata-Alvarez, 2003). Although mesophilic conditions require longer retention times, the robust micro-organisms provide stability by allowing wider temperature fluctuations and environmental changes. The thermophilic micro-organisms are more susceptible to toxins and small temperature changes (±1 °C), but boast much higher methane production and kinetic rates, as well as pathogen inactivation. The major disadvantage of thermophilic conditions is the considerable loss of net energy production due to the extra heating requirements. Often thermophilic anaerobic digesters fail due to an inability to keep the temperature constant at 54–55 °C during an anaerobic digester upset, causing decreased gas

production. Pitt and Ekama (1996) argue that for a reliable and stable thermophilic anaerobic digester operation, two heating sources are required so that when gas production decreases, a constant temperature can be maintained with the second heat source – they demonstrated this with the high rate thermophilic dual (aerobic-anaerobic) digester.

2.2.2.2 Substrate characteristics

The characteristics of the solid waste fed to a digester have an impact on the success (or not) of the anaerobic digester and the behavioural response of the system. The important substrate characteristics highlighted in the literature include the particle size, biodegradability, organic composition, and carbon to nitrogen (C/N) ratio.

Particle size: Particulate material in this context is defined as the solids which cannot pass a 0.45 micron filter, hence that which passes through the filter is considered soluble. Influent biodegradable particulate organics (BPO) undergo hydrolysis, which breaks down the waste to allow fermentation, whereas biodegradable soluble organics (BSO) are already in readily biodegradable form. Hence, the particle size influences the HRT and SRT. In a flow through anaerobic digester (HRT = SRT), the BPO will require a longer HRT for digestion than the BSO, but in solids retention anaerobic digesters both HRT and SRT can be optimized for high removals of both. A smaller particle size organic acidifies faster, due to the greater surface area with which the organisms have contact (Hartmann & Ahring, 2006), which may result in a faster fermentation process than methanogenesis process, causing an accumulation of VFA. Thus, the OLR, HRT and SRT are important factors to consider along with the particulate nature of the waste to ensure stability in the reactor design configuration.

Biodegradability: The biodegradable fraction of organic waste is important to consider as only this portion of the organics undergo anaerobic digestion. The unbiodegradable constituents are unchanged in the influent COD (Batstone *et al.*, 2002; Gossett & Belser, 1982; Sötemann *et al.*, 2005b) and are considered passive in the overall COD balance.

Organic composition: The composition of the organics has mostly been denoted in two different ways, both of which were discussed with the biological processes of anaerobic digestion. The first entails determining the concentrations of the protein, carbohydrate and lipid constituents. Although this method is required for many anaerobic digestion models like International Water Association Anaerobic Digestion Model 1 (IWAADM1), these quantities are not routinely measured. Thus, the second method, which characterizes the organics in term

of the carbon (C), hydrogen (H), oxygen (O), nitrogen (N) and phosphorous (P) constituents as well as the COD and biodegradable fraction, is a preferable method because it aligns with some routine measurements made on wastewater and anaerobic digestion influents (COD, VSS, TKN, total phosphate). The C, H, O, N, P and S components in the biodegradable organics (x, y, z, a, b and c in $C_xH_yO_zN_aP_bS_c$) are released to the aqueous and gas phases of the anaerobic digester and establish the anaerobic digester pH. So if the x, y, z, a, b and c values of the biodegradable organics are known they provide quantitative estimates of the weak acid/base species concentrations in the reactor, which govern the anaerobic digester physiochemical processes and pH.

The elemental composition method assumes that each organic group contains mostly C, H, O, N and P (and S for organics containing high levels of sulphur) and is quantified by the values of x, y, z, a, b and c in $C_xH_yO_zN_aP_bS_c$. These composition quantities can also be expressed per unit of a selected base element, like if x = 1 and the other values are scaled by a factor of x to give $C_1H_{y/x}O_{z/x}N_{a/x}P_{b/x}S_{c/x}$. Any of the x, y, z, a, b and c can be used as a base and enables comparison between organics which have been expressed with different elemental composition bases.

C/N ratio: From the stoichiometry of anaerobic digestion, Ekama (2009) and Harding *et al.* (2011) show that at steady state the COD of degraded organics equal the COD of the methane produced and biomass grown (which constitutes less than 5% of the COD degraded organics). The C in the degraded organics exits the anaerobic digester as C in methane, C in CO_2 gas and C in dissolved CO_2 i.e. HCO_3^-. The N content of the degraded organics is released as NH_3, which due to the pH being in the range of 7 to 7.5, picks up an H^+ from the aqueous phase according to $NH_3 + H_2O + CO_2 \rightarrow NH_4^+ + HCO_3^-$. So the HCO_3^- (dissolved CO_2) or alkalinity in the aqueous phase comes from the N content of the degraded organics fed to the anaerobic digester. The higher the N (or alkalinity) content of the organics, the more alkalinity is transferred to the aqueous phase upon degradation (Harding *et al.*, 2011). So the gaseous CO_2 that is produced is the remainder of the C of the degraded organics after the C has been used for methane formation and dissolved C (HCO_3^-) from the N (and alkalinity) content has been formed. The gaseous CO_2 and CH_4 set the partial pressure of the CO_2 (pCO_2) in the head space, which together with the alkalinity (HCO_3^-) set the anaerobic digester pH. So the pH of the anaerobic digester at steady state is entirely defined by the composition of the biodegradable organics.

For organics with a very high C/N ratio the low availability of N may limit the micro-organism growth rates which result in a low gas production rate and poor degradation of particulates. In such cases N in the form of urea (or proteinaceous organics) can be dosed to provide sufficient N for growth (van Zyl *et al.*, 2008). A low C/N ratio, however, is also dangerous as an accumulation of free ammonia (NH_3) can result from a high alkalinity and pH, which can reach toxic levels (Hartmann & Ahring, 2006). Kayhanian & Hardy (1994) found that a C/N ratio of 25-30 was optimum. Interestingly, Wett *et al.* (2012) have found that at high free NH_3, acetclastic methanogens are inhibited, but a different organism group not sensitive to the high NH_3 develops. These organisms, called acetate oxidizers (Acox), oxidize acetate to H_2, which is then utilized by the hydrogenotrophic methanogens.

2.2.2.3 Inhibitory substances

Toxins which cause inhibition either enter the digester as a component of the feed or form as a by-product of the metabolic processes in the reactor. Noted toxins include ammonia; sulphide; light metal ions such as sodium, potassium, magnesium, calcium and aluminium; heavy metals such as chromium, iron, cobalt, copper, zinc, cadmium and nickel; and a variety of organic micropollutants. Details of these toxins beyond those relevant to the organic composition (mentioned in 2.2.2.2) can be found in a detailed literary discussion by Chen *et al.* (2008).

2.2.3 Monitoring performance indicators

In order to prevent the inhibition and other negative impacts caused by the factors which influence the behaviour of anaerobic digestion processes, sensitive parameters require monitoring. According to the literature, the VFA concentration, alkalinity and pH as well as hydrogen, methane and carbon dioxide gas production rates are some of the key indicators to monitor for digester imbalance (Björnsson *et al.*, 2000). Generally, multiple parameters are monitored as they provide complementary results (Hickey *et al.*, 1991).

This section discusses the behaviour of various indicators, such as pH_2, VFA concentration, pH and buffer capacity, and methods of monitoring them. The methods for measuring many of these parameters are, however, complex, costly or time-consuming. For example, the VFA concentration can be measured in a variety of ways, including by straight distillation, steam distillation, gas chromatography, high-performance liquid chromatography (HPLC) or attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy.

These methods are in order of increasing accuracy, with the gas chromatography, HPLC and ATR-FTIR spectroscopy procedures providing exact concentrations of each acid.

HPLC involves the pumping of an aqueous sample through a column of solid adsorbent material, which interacts differently with each constituent in the sample to produce different flow rates resulting in constituent separation. ATR-FTIR spectroscopy uses the reflection of infrared light on the internal surface of a prism to create an evanescent wave only penetrating 1 μm of the sample, which is reflected back to a detector. An ATR-FTIR spectrometer is advantageous due to its application as a probe, which can be fitted in a tank or flow to provide online (continuous and current) measurements of constituents. The chromatography and spectroscopy methods, however, require the use of costly equipment and software and for chromatography, skilled analysts. In contrast, steam distillation is simple, yet laborious. And while straight distillation is reasonably rapid and follows a basic technique, the empirical nature of the results lack the accuracy required to identify acute sensitivity.

In response to this need for a simple and affordable method of measuring the VFA concentration, which has been shown to be one of the key parameters in estimating whether digester failure is imminent, Moosbrugger *et al.* (1993b) developed a five-pH point titration method for determining the VFA concentration, total carbonate species (C_T) and $H_2CO_3^*$ (dissolved CO_2) alkalinity of aqueous solutions which contain other weak acid/base systems where the first pH point is the in-situ pH in the anaerobic digester. The procedure involves equating two alkalinity mass balance equations, one in terms of volume of acid titrated and the other in terms of species concentration. The result is an equation (Eqn. 2.1) with only three unknowns, i.e. the inorganic carbon aqueous concentration (C_T), VFA concentration (A_T) which includes the longer chain fatty acids such as propionic and butyric acid as they have similar pK values to acetic acid, and the volume of standard strong acid titrated (V_e) as unknowns. However, the method requires the temperature and TDS to be known to adjust the various equilibrium constants as well as the concentrations of other weak acid/base species in the system, such as ammonium (N_T), phosphate (P_T) and sulphide (S_T) and adequate precautions have been taken to minimize CO_2 loss during titration. The equation (Eqn. 2.1) should only require three pH points to solve, but this proved inaccurate as a result of sensitivity to slight errors in pH measurement. Four pH points at approximately symmetrical positions about peaks in buffer capacity (at pK'_a values) of C_T and A_T were found to provide the best results as the error in pH measurements (ΔpH) could also be calculated and used to adjust the pH values for better accuracy in the concentrations.

$$\Delta MAlk_{1,2}H_2CO_3^* + \Delta MAlk_{1,2}HAc + \Delta MAlk_{1,2}NH_4^+ + \Delta MAlk_{1,2}H_2PO_4^- + \Delta MAlk_{1,2}H_2 =$$
$$V_sC_TX_{1,2} + V_sA_TY_{A1,2} + V_sN_TY_{N1,2} + V_sP_TY_{P1,2} + V_sZ_1 - (V_s + V_{x1,2})Z_2 = C_aV_{x1,2} \quad (2.1)$$

where $\Delta MAlk_{1,2}$ = change in mass of reference species alkalinity from pH_1 to pH_2 (mol)

V_s = sample volume prior to titration (l)

$V_{x1,2}$ = volume of strong acid added from pH_1 to pH_2 (l)

$X_{1,2} = \frac{1}{A_1} + \frac{2}{B_1} - \frac{1}{A_2} - \frac{2}{B_2}$ where $A = \left[\frac{(H^+)}{K'_{ac1}} + 1 + \frac{K'_{ac2}}{(H^+)}\right]$ and $B = \left[\frac{(H^+)^2}{K'_{ac1}K'_{ac2}} + \frac{(H^+)}{K'_{ac2}} + 1\right]$

$Y_{1,2} = \frac{K'_a}{(H^+)_1 + K'_a} - \frac{K'_a}{(H^+)_2 + K'_a}$

$Z = 10^{pH - pK'_w} - \frac{10^{-pH}}{f_m}$

C_a = normality of strong acid (mol/l)

(H^+) = hydrogen ion activity (mol/l)

$pK'_w = -\log K'_w$

K'_w = apparent ionic product constant for water $(mol/l)^2$

K'_{ac1} = first ($_1$; $_2$ for second) apparent dissociation constant for the carbonate ($_c$; $_a$ for acetate; $_n$ for ammonium; $_p$ for phosphate) system

f_m = monovalent activity co-efficient (determined from μ in the Davies equation, see Appendix 1 of Loewenthal *et al.*, 1989)

For the other weak acid/base species in the system, Moosbrugger *et al.* (1993b) considered the inclusion of the ammonium, phosphate and sulphide weak acid/base species, but other species for which total species concentrations are known can be included similarly. Moosbrugger *et al.* (1993b) showed that the inclusion of P_T and S_T in the calculation (when present) are very important because an error in the measurement of the total phosphate and sulphide weak acid/base species has a large influence on the calculation of C_T, hence also the $H_2CO_3^*$ alkalinity, but insignificant effect on A_T. This is due to the phosphate and sulphide weak acid/base systems' pK values (~7.0) being close to that of the inorganic carbon system (~6.3), but far from the A_T system's pK value (~4.8). The total ammonium weak acid/base species (N_T) is not as influential because its pK value is high (~9.1) and so its exclusion from the calculation (of up to 500mgN/l) has insignificant effects on both C_T and A_T, thus it does not necessarily require measurement (Poinapen *et al.*, 2009). The fundamental calculations

described here have been coded into a computer programme available from the Water Research Commission (wrc.org.za) for automatic calculation, which reduces the test procedure to a simple titration. However, this programme only runs on Windows XP (32 bit). A new Microsoft Excel/Visual Basic for Applications (VBA) based programme which runs on Windows 7 and higher is available from its author, Chris Brouckaert (brouckae@ukzn.ac.za).

A detailed description of each method of measuring *inter alia* the VFA concentration, alkalinity and pH of an aqueous solution is given in *Standard Methods for the Examination of Water and Wastewater* (Clesceri *et al.*, 1998). These performance indicators are monitored regularly so that corrective actions can be applied before digester failure occurs. A calibrated model prediction of the behaviour of an anaerobic digester is preferable over this responsive assessment and would allow the operator to make informed decisions about what to feed and/or dose the reactor to prevent (rather than treat) any changes in these performance indicators. Hence, the focus of this research is on improving an anaerobic digestion model in order to establish the composition of an organic feed which, as described in Section 2.2.2.2, influences anaerobic digester behaviour. The stable operating ranges of each performance indicator are described below so that the results from an anaerobic digestion model can be put into context.

2.2.3.1 Hydrogen partial pressure

Massé & Droste (2000) reported that low levels of dissolved hydrogen produce better substrate utilization results. In order to prevent hydrogen inhibition of both the acidogens and acetogens, excess hydrogen needs to be removed from the system. pH_2 levels of greater than $\pm 10^{-3,7}$ atm. and $\pm 10^{-4,1}$ atm. inhibit the acidogenic and acetogenic processes respectively. Fortunately, the hydrogenotrophic methanogens use hydrogen to reduce carbon dioxide to methane, lowering the pH_2 levels, under stable digester conditions. In the case of hydrogenotrophic methanogen inhibition, caused by toxins or pH fluctuations for example, rising pH_2 levels could indicate imminent unstable conditions and the need for hydrogen removal to be applied. Although pH_2 is a reliable process behaviour indicator, the minimal traces of pH_2 found in anaerobic digestion and the specialized equipment required to measure it makes it impractical to monitor (Labatut & Gooch, 2012).

2.2.3.2 VFA concentration

VFAs are produced by acidogens and acetogens and are consumed by acetoclastic methanogens. Methanogenic micro-organisms, however, are prone to inhibition from toxins or fluctuations in pH levels. Methanogenic inhibition results in the accumulation of VFA and an accompanied decrease in pH, causing further activity reduction. Labatut & Gooch (2012) consider VFA concentration levels of greater than 1,5–2 g/L to be inhibitory, though also mention that a rapid increase in VFA concentration is a better indicator than a specific value.

2.2.3.3 pH

The pH of a digester is an important indicator of the performance of the anaerobic digestion processes. As already mentioned, the methanogenic activity is at an optimum level at a pH of 7,0 with values lower than 6,6 or higher than 7,6 hindering the processes considerably. Besides this, the reactor pH also dictates the relative species concentrations of weak acid/base systems. The speciation of the weak acid/base systems in conjunction with the species concentrations can influence the behaviour of anaerobic digesters. For example, free ammonia (NH_3) forms at high FSA concentration and pH (pK'_a = 9.245, at 25°C, Loewenthal *et al.*, 1989) and can be toxic in high concentrations; inhibiting the methanogens. The use of pH as a system indicator is, however, highly dependent on the buffer capacity of the digester and the influent substrate as described in Section 2.2.2.2. A manure feed, for example, has a higher buffer capacity than MSW and could mask the change in pH caused by an accumulation of VFAs.

A reactor's pH is generally controlled through the dosing of chemicals such as lime. However, Gerardi (2003) suggests that once a pH of 6,4 has been reached from the addition of lime, the pH should be further increased by bicarbonate salts, such as potassium- or sodium-bicarbonate, as the methanogens require the bicarbonate alkalinity for their bioprocesses. Capri & Marais (1974) and Van Zyl *et al.* (2008) discuss lime or NaOH dosing to anaerobic digesters for pH control when the organics contain too little alkalinity (low N content of biodegradable organics).

2.2.3.4 Buffer capacity

The buffering capacity of a reactor is a measure of the anaerobic digester's capacity to absorb acids or bases for a unit pH change and is proportional to the dissolved inorganic carbon (C_T), phosphate (P_T) and sulphide (S_T) species through their pK values at 6.3, 7.0 and 7.0

respectively. Generally the higher the alkalinity present in the system, the higher the buffer capacity. The main source of alkalinity in methanogenic anaerobic digestion is provided by the bicarbonate ion (HCO_3^-), which as explained in Section 2.2.2.2, comes from the N content of the degraded organics.

When the degraded organics comprise a significant P content, the released P does not affect the alkalinity, only the species that represent it – the P is released as H_3PO_4 and thus loses H^+ to form $H_2PO_4^-$ and HPO_4^{2-}. The released H^+ reacts with HCO_3^- to form H_2O and gaseous CO_2 which increases the pCO_2 of the gaseous phase. Thus the anaerobic digester pH is established by both the two-phase inorganic carbon system and the single-phase phosphate systems and will be lower than for organics with an insignificant P content.

A high buffer capacity maintains a digester's pH at a steady neutral level, which protects the system pH against changes in VFA concentration. Due to the high buffer capacity provided by a manure feed, it is often used in co-digestion with readily degradable industrial wastes of low N (alkalinity) content. The buffer capacity does not, however, prevent inhibition caused by toxic levels of VFAs. Thus the pH maintenance provided by a high alkalinity may mask anaerobic digestion inhibition in cases where VFA concentration is not monitored.

2.3 Anaerobic digestion modelling

Although this thesis is concerned with the modelling of the BMP test, an anaerobic digestion model can form a sound basis for the BMP test model development as the BMP test is essentially a miniature anaerobic digestion batch digester. The advantage of a BMP test model is that the data is more readily accessible as assays are affordable and quick in comparison to laboratory scale digesters with lengthy test schedules.

Anaerobic digestion is now extensively used, but Sötemann *et al.* (2005b) believe that the setup and procedure for planning and control of anaerobic digesters still largely relies on experimental studies. Thus, a mathematical model of the anaerobic digestion of organic waste is an important and helpful system evaluation tool for providing quantitative process responses and outputs. The predictions from a model allow a framework to be established which can determine the most efficient operating variables.

The first dynamic model for anaerobic digestion was created by Andrews (1969), but was limited by the assumption of a constant pH. The incorporation of 2-phase (aqueous-gas) physicochemical relationships by Andrews and Graef (1971) resolved this restriction. A single group of micro-organisms (acetoclastic methanogens) were modeled and assumed to be the rate-limiters. Methanogen inhibition by VFA accumulation was incorporated in conjunction with Monod (1942) kinetics. Hill and Barth (1977) extended the work of Andrews and Graef (1971) for the anaerobic digestion of animal manure by including a second micro-organism group (acidogens) which hydrolyzed the particulate organics. They also included methanogen inhibition by free ammonia (NH_3). A comprehensive model incorporating four micro-organism populations (acidogens, acetogens, acetoclastic methanogens and hydrogenotrophic methanogens) was outlined by Mosey (1983) and formed the basis for advanced model development, such as the model including six micro-organism groups by Costello *et al.* (1991). These models predict changes in the anaerobic digester behaviour indicators (VFA concentration, pH, pH_2 and methane/biogas production) as a function of time.

Many researchers have investigated anaerobic digestion model developments, but the most accessible and most broadly applicable anaerobic digestion models available include: the International Water Association Anaerobic Digestion Model 1 (IWAADM1) (Batstone *et al.*, 2002), University of Cape Town Sludge Digestion Model 1 (UCTSDM1[†]) (Sötemann *et al.*, 2005b) and the anaerobic digestion unit of the Plant Wide Model of South Africa (PWM_SA) (Brouckaert *et al.*, 2010; and Ikumi *et al.*, 2014). The anaerobic digestion unit of the PWM_SA model can be used independently from the other units in the wastewater treatment plant-wide model and will be referred to as the PWM_SA_AD model, for simplicity and reference acknowledgement purposes. The PWM_SA_AD model is based on UCTSDM1 with the addition of *inter alia* the inorganic suspended solids (ISS) model developed by Ekama & Wentzel (2004), the aqueous equilibrium ionic speciation model created by Brouckaert *et al.* (2010) and the precipitation of numerous minerals. All three models have a similar conceptual description of the biological process systems; however, some significant differences in *inter alia* numeric stability, pH calculation, inhibitory substances and organic characterization exist. Table 2.2 summarizes these model differences by ranking each as an advantage or disadvantage.

[†] Sötemann *et al.* (2005b) called this UCTADM1. Because it is quite different, and not a derivative of IWAADM1 and calibrated only for AD of sewage sludges, it has been renamed UCTSDM1 to distinguish from IWAADM1.

IWAADM1 and PWM_SA_AD separate the ionic speciation reactions in the aqueous phase from the biological and interphase reactions, because the algebraic equilibrium relationships of the weak acid/base species chemical reactions are orders of magnitude faster and can be considered to be in a state of chemical equilibrium at all times. This provides these models with numerical stability and fast simulation times. UCTSDM1, however, does not separate these calculations and suffers from slow simulations due to the stiffness of the matrix of equations.

In IWAADM1, the pH is calculated using algebraic equilibrium relationships of weak acid/base chemical reactions and the conservation of charge balance across the system. This method requires every ionic species concentration in the influent to be measured in order to determine the initial charge. To reduce the number of influent concentrations that require measurement, the UCTSDM1 and PWM_SA_AD models, calculate the pH using "charge accounting" method. This method measures the 14 total ionic species concentrations which are most commonly found in wastewaters (see Table 3.2) as well as the pH, $H_2CO_3^*$ alkalinity, ionic strength (total dissolved solids, TDS or conductivity) and temperature. NaCl is added to the 14 total ionic species concentrations to achieve the measured ionic strength determined from the conductivity. The pH and temperature are used to divide each total ionic species concentration into the contributing species. The charge state defined by these initial species is used as a reference state and any changes in C, H, O, N, P, S and charge caused by the bioprocesses are tracked, and used to calculate the pH at the next time step.

Table 2.2: Comparison of dynamic anaerobic digestion models

Model	Advantages	Disadvantages
IWAADM1	▪ Separates fast ionic equilibrium reactions from slow bioprocesses by external algebraic equations for pH determination – model is not "stiff" and simulations are fast ▪ All bioprocess' kinetics calibrated	▪ Substrate is difficult to characterise – carbohydrates, lipids and proteins are not routinely measured ▪ Charge balance used – all aqueous ions need to be accounted for ▪ Effect of high pH_2 on acidogenic growth rate not included
UCTSDM1	▪ Substrate is characterised using typical wastewater measurements ▪ "Proton accounting" method used – does not require all aqueous ions to be accounted for ▪ Potentially can model digester failure due to inclusion of effect of high pH_2 on acidogenic growth	▪ Internal calculation of aqueous speciation and pH with differential equations – model stiffness, numerical instability and slow run times ▪ Calibrated only for the hydrolysis process

Model	Advantages	Disadvantages
	▪ Includes ion pairing effect on pH ▪ Includes elemental mass balance for COD and CHON	
PWM_SA_AD	▪ Substrate is characterised using typical wastewater measurements ▪ Fast and slow processes are separated – faster runtimes and prevents solver instability ▪ Three phase and thus can model gas and precipitation reactions ▪ Potentially can model digester failure due to inclusion of effect of high pH_2 on acidogenic growth ▪ Includes pK value correction and ion pairing to deal with high ionic strength wastewaters ▪ Includes elemental mass balance for COD and CHONP	▪ Only calibrated for the hydrolysis step – bioprocesses following hydrolysis have not been calibrated

IWAADM1 is the only one of the three models that does not include the inhibition of acidogens by high hydrogen partial pressure (pH_2). Therefore, IWAADM1 is unable to model the instability or failure of an anaerobic digester due to pH_2 fluctuations, where as UCTSDM1 and PWM_SA_AD make provision for this inhibition.

The differences in organic characterization have been described with the biological processes of anaerobic digestion (see Section 2.2.1). The UCTSDM1 and PWM_SA_AD generic elemental characterization ($C_xH_yO_zN_a$ and $C_xH_yO_zN_aP_bS_c$ respectively) was deemed preferable to the IWAADM1 proteins, carbohydrates and lipids characterization, because the concentrations of proteins, carbohydrates and lipids are not routinely measured.

The PWM_SA_AD model will be used as a basis for the modelling component of this dissertation. The PWM_SA_AD model is fully integrated into the WEST modelling platform library (mikebydhi.com) and its hydrolysis kinetic rate constants have been calibrated to primary sludge, waste activated sludge and the co-digestion of these sludges. It includes the following features and extensions from the UCTSDM1 model:

- Plant wide and includes activated sludge, anaerobic digestion, anoxic-aerobic digestion, nitrification-denitrification (ND) and biological excess phosphorus removal (BEPR) based on IWA Activated Sludge Model 2 (ASM2)

- Full elemental mass balances (C, H, O, N, P, S, COD and charge)
- Wastewater characterization based on COD, ammonia, phosphate and sulphate with six organics groups (HPr, HAc, BSO, BPO, UPO, USO) which each have compositions (x, y, z, a, b, c) of the form $C_xH_yO_zN_aP_bS_c$
- Additional components for the soluble and particulate biodegradable and unbiodegradable organics fed to the anaerobic digester from external sources or alternative sources within the wastewater treatment plant
- Polyphosphate hydrolysis kinetics for the digestion of waste activated sludge from BEPR systems
- Three phase (aqueous-gas-solid) so includes gas exchange and precipitation of $MgNH_4PO_4.6H_2O$ (struvite), $MgKPO_4.6H_2O$ (K-struvite) and $Ca_3(PO_4)_2$ (calcium phosphate)
- Separation of the differential kinetic equations (DE) and the 'instantaneous' algebraic equations (AE) for aqueous equilibrium and ion-paring reactions to reduce the model stiffness of the system's differential equations for numerical stability and faster run times
- Bioprocess stoichiometry based on organics composition parameters allows the various organic compositions to be entered as x, y, z, a, b and c values (in $C_xH_yO_zN_aP_bS_c$)
- Pre-processor and post-processor routines which transform measured influent parameters such as FSA, ortho-phosphate, $H_2CO_3^*$ alkalinity, VFA, pH and TDS (conductivity) to model components and correct equilibrium, Henry's law and solubility products for ionic strength and transforming output model components back to predicted 'measured' concentrations for comparison with the actual measured concentrations.

These advancements (Brouckaert *et al.*, 2010; Ikumi *et al.*, 2014) have made the PWM_SA_AD model the most comprehensive dynamic anaerobic digestion model. A full description of the features, capabilities and limitations of this model are listed by Ikumi *et al.* (2014) and are included as a base model description in Section 3.3.

2.4 Key questions

The following are the key questions that have formulated from gaps in the literature and that require further investigation:

- Can a dynamic BMP test model be developed to predict the BMP test outputs, such as methane and carbon dioxide production rates and the biodegradable fraction of the substrate?
- Can a dynamic BMP test model be developed to predict the BMP test inputs, such as organic substrate composition and kinetic rate constants?
- Which additional output measurements are most sensitive to the BMP test inputs and can aid a BMP test model in predicting the BMP test inputs?
- Can the selected sensitive measurements be conducted in areas with poor funding and technical experience levels, to make the research applicable to developing countries?
- How does the accuracy of the additional measurements influence the success of a BMP test model?

These key questions will guide this study in the creation of a dynamic BMP test model.

2.5 Closure

The literature discussed above provides a suitable background for the proposed investigation, as well as highlighting the areas of opportunity for further study. The knowledge gaps have been identified and these include the need for a simple and affordable test, such as the BMP (or BSP) test, for the prediction of anaerobic digester behaviour when fed organics of a specific composition, in order to prevent inhibition and failure, because the anaerobic digester behaviour is entirely governed by substrate characteristics. In conclusion, the motivation to create a dynamic BMP test model for determining the organic waste composition characteristics has been clearly described and addresses the research gaps in the development of the anaerobic digestion field.

3 Model development

A few BMP assay models have briefly been introduced in journal papers (Esposito *et al.*, 2012; Appels *et al.*, 2011; Jensen *et al.*, 2011; Batstone *et al.*, 2009; Bilgili *et al.*, 2009; Lin *et al.*, 1999), none of which are used for determining the composition of the organic substrate feed (see Section 2.1.4). As the BMP test is intended to simulate the anaerobic digestion treatment of a particular waste sample but under batch conditions, the BMP test model must include the same anaerobic digestion model used to simulate the real anaerobic digester. This section motivates the selection of the PWM_SA_AD model as the base model for both the BMP test and the real anaerobic digester and describes how the PWM_ SA_AD model functions. Furthermore, the alterations required to the PWM_SA_AD model to constrain it to function under batch test conditions are discussed. The PWM_SA_AD for batch test BMP conditions is called PWM_SA_AD_BMP model. It needs to be emphasized here that all the bioprocesses in PWM_SA_AD and PWM_SA_AD_BMP are identical; only the system constraints under which the bioprocesses function are different viz. batch for BMP and completely mixed sludge retention (SRT > HRT) or flow through (SRT = HRT) for real anaerobic digesters. Appendix B gives further details of both the PWM_SA_AD and PWM_SA_AD_BMP model components, parameters and stoichiometric bioprocess equations. To conclude this section, both real anaerobic digesters and BMP models were verified by mass balance continuity checks on C, H, O, N, P, S, COD and charge.

3.1 Model terminology

The model terminology used throughout this dissertation will refer to parameters, components, species and variables which require definition for clarity and the parameter nomenclature proposed by Corominas *et al.* (2010) is used:

Components: the compounds, compound groups and total species groups which take part in the stoichiometry of the anaerobic digestion processes. These are not values but rather a set of governing names to which a variable can apply. For example: H_2O, total soluble inorganic carbonate species, acidogens, acetogens, UPO from biomass decay (or endogenous residue), influent BPO, etc. (denoted as H2O, S_CO3, X_AD, X_AC, X_U_Org, X_B_Inf respectively in PWM_SA_AD and PWM_SA_AD_BMP – see Table 3.1 for a complete defined list).

Table 3.1: PWM_SA_AD and PWM_SA_AD_BMP model components

	Component name	Empirical formula	Notation
	Water	H_2O	H_2O
Total dissolved ionic concentrations	Hydrogen ion	H^+	S_H
	Sodium	Na^+	S_Na
	Potassium	K^+	S_K
	Calcium	Ca^{2+}	S_Ca
	Magnesium	Mg^{2+}	S_Mg
	Ammonium	NH_4^+	S_NH
	Chloride	Cl^-	S_Cl
	Acetate	CH_3COO^-	S_VFA
	Propionate	$CH_3CH_2COO^-$	S_Pr
	Carbonate	CO_3^{2-}	S_CO3
	Sulphate	SO_4^{2-}	S_SO4
	Phosphate	PO_4^{3-}	S_PO4
	Nitrate	NO_3^-	S_NO
	Sulphide	HS^-	S_HS
Soluble organics	Dissolved hydrogen	H_2	S_H2
	Dissolved oxygen	O_2	S_O
	Dissolved nitrogen	N_2	S_N2
	Glucose	$C_6H_{12}O_6$	S_Glu
	Unbiodegradable soluble organics	$CH_{Yu}O_{Zu}N_{Au}P_{Bu}S_{Cu}$*	S_U
	Fermentable biodegradable soluble organics	$CH_{Yf}O_{Zf}N_{Af}P_{Bf}S_{Cf}$*	S_F
Particulates	Unbiodegradable particulate organics from decayed biomass	$CH_{Yup}O_{Zup}N_{Aup}P_{Bup}S_{Cup}$*	X_U_inf
	Biodegradable particulate organics from decayed biomass	$CH_{Ybp}O_{zbp}N_{Abp}P_{Bbp}S_{Cbp}$*	X_B_Org
	Influent/feed biodegradable particulate organics	$CH_{Ybps}O_{Zbps}N_{Abps}P_{Bbps}S_{Cbps}$*	X_B_Inf
	Polyphosphate (PP)	$K_{kp}Mg_{mp}Ca_{cp}PO_3$**	X_PAO_PP
	Poly-hydroxy-alkanoate (PHA)	$C_4H_6O_2$	X_PAO_Stor
	Struvite	$MgNH_4PO_4.6H_2O$	X_Str_NH4
	Amorphous calcium phosphate (ACP)	$Ca_3(PO_4)_2$	X_ACP
	K-struvite	$MgKPO_4.6H_2O$	X_Str_K
	Calcite	$CaCO_3$	X_Cal
	Magnesite	$MgCO_3$	X_Mag
	Newberyite	$MgHPO_4$	X_Newb
	Influent inorganic settleable solids		X_ISS
Gas	Carbon dioxide	CO_2	G_CO2
	Methane	CH_4	G_CH4

	Component name	Empirical formula	Notation
Microorganism biomass	Ordinary heterotrophic organisms (OHO)	$CH_{Yo}O_{Zo}N_{Ao}P_{Bo}S_{Co}$*	X_OHO
	Phosphate accumulating organisms (PAO)	$CH_{Yo}O_{Zo}N_{Ao}P_{Bo}S_{Co}$*	X_PAO
	Autotrophic nitrifying organisms	$CH_{Yo}O_{Zo}N_{Ao}P_{Bo}S_{Co}$*	X_ANO
	Autotrophic denitrifying organisms	$CH_{Yo}O_{Zo}N_{Ao}P_{Bo}S_{Co}$*	X_ADO
	Acidogens (for sulphidogenesis and methanogenesis)	$CH_{Yo}O_{Zo}N_{Ao}P_{Bo}S_{Co}$*	X_AD
	Methanogenic acetogens	$CH_{Yo}O_{Zo}N_{Ao}P_{Bo}S_{Co}$*	X_AC
	Acetoclastic methanogens	$CH_{Yo}O_{Zo}N_{Ao}P_{Bo}S_{Co}$*	X_AM
	Hydrogenotrophic methanogens	$CH_{Yo}O_{Zo}N_{Ao}P_{Bo}S_{Co}$*	X_HM
	Endogenous residue	$CH_{Ye}O_{Ze}N_{Ae}P_{Be}S_{Ce}$*	X_U_Org
	Sulphidogenic acetogens	$CH_{Yo}O_{Zo}N_{Ao}P_{Bo}S_{Co}$*	X_ACS
	Acetoclastic sulphidogens	$CH_{Yo}O_{Zo}N_{Ao}P_{Bo}S_{Co}$*	X_AS
	Hydrogenotrophic sulphidogens	$CH_{Yo}O_{Zo}N_{Ao}P_{Bo}S_{Co}$*	X_HS

* These elemental compositions have been represented per unit carbon (C) i.e. the x, y, z, a, b and c values in $C_xH_yO_zN_aP_bS_c$ have each been divided by x to give Y = y/x, Z = z/x, A = a/x, B = b/x and C = c/x. The Y, Z, A, B and C values are given the new standardized modelling notation (Corominas *et al.*, 2010) of i_H_Org_mol_perC, i_O_Org_mol_perC, i_N_Org_mol_perC, i_P_Org_mol_perC and i_S_Org_mol_perC respectively for organisms. The "Org" is replaced by "XUOrg", "XBOrg", "XUInf", "XBInf", "SF" or "SU" for the respective components. See Table 3.3 for a complete list.

** The polyphosphate composition is given per unit phosphorus (P) with kp = i_K_PP_mol_perP, mp = i_Mg_PP_mol_perP and cp = i_Ca_PP_mol_perP in the new standardized modelling notation (Corominas *et al.*, 2010).

Species: the various ionic species which contribute to a total ionic species group component concentration. For example, the total soluble inorganic carbonate species component (S_CO3) concentration includes the concentrations of CO_3^{2-}, HCO_3^-, H_2CO_3 plus various other carbonate ion pair complexes present in the solution, such as $MgCO_3$ and $CaHCO_3^+$. Table 3.2 provides a list of the ionic species and complexes that contribute to each total soluble ionic component concentration. The list does not include insignificant species concentrations which burden the model with additional computation for insignificant improvement in ionic component representation (Brouckaert *et al.*, 2010).

Speciation: the detailed distribution of the total ionic component concentrations between the ionic species. The ionic speciation reactions in the aqueous phase are orders of magnitude faster than the biological and interphase (aqueous-gas, aqueous-solid) reactions, and can be considered to be in a state of chemical equilibrium at all times. Thus the model calculations are divided into differential mass balance and algebraic equilibrium speciation calculations. The differential mass balances determine the species composition in terms of total ionic component concentrations, as if each species group solely consisted of the species given in the "Component empirical formula" column in Table 3.2. This is followed by an algebraic equilibrium speciation calculation which determines the individual ionic species concentrations

at each time step. See Section 3.3 for further details pertaining to the speciation routine requirements, inputs and outputs.

Table 3.2: Ionic species and complexes contributing to dissolved ionic components

Dissolved ionic component	Component empirical formula	Contributing ionic species and complexes
S_H	H^+	H^+, OH^-, HCO_3^-, H_2CO_3, NH_3, HPO_4^{2-}, $H_2PO_4^-$, H_3PO_4, HAc, HPr, $CaOH^+$, $MgOH^+$, $NaHCO_3$, $NaHPO_4^-$, $CaHCO_3^+$, $CaHPO_4$, $MgHCO_3^+$, $MgHPO_4$, $MgH_2PO_4^+$, H_2S, S^{2-}
S_Na	Na^+	Na^+, $NaHPO_4^-$, $NaCO_3^-$, $NaHCO_3$, NaAc, $NaSO_4^-$, NaHS
S_K	K^+	K^+
S_Ca	Ca^{2+}	Ca^{2+}, $CaCO_3$, $CaHCO_3^+$, $CaPO_4^-$, $CaHPO_4$, $CaSO_4$, $CaOH^+$, $CaAc^+$, $CaPr^+$
S_Mg	Mg^{2+}	Mg^{2+}, $MgCO_3$, $MgHCO_3^+$, $MgPO_4^-$, $MgHPO_4$, $MgH_2PO_4^+$, $MgSO_4$, $MgOH^+$, $MgAc^+$, $MgPr^+$
S_NH	NH_4^+	NH_4^+, NH_3, $NH_4SO_4^-$
S_Cl	Cl^-	Cl^-
S_VFA	CH_3COO^- (or Ac^-)	Ac^-, HAc, $CaAc^+$, NaAc, $MgAc^+$
S_Pr	$CH_3CH_2COO^-$ (or Pr^-)	Pr^-, HPr, $CaPr^+$, $MgPr^+$
S_CO3	CO_3^{2-}	CO_3^{2-}, HCO_3^-, H_2CO_3, $CaCO_3$, $MgCO_3$, $CaHCO_3^+$, $MgHCO_3^+$, $NaCO_3^-$, $NaHCO_3$
S_SO4	SO_4^{2-}	SO_4^{2-}, $CaSO_4$, $MgSO_4$, $NH_4SO_4^-$, $NaSO_4^-$
S_PO4	PO_4^{3-}	PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, H_3PO_4, $MgPO_4^-$, $CaPO_4^-$, $MgHPO_4$, $CaHPO_4$, $NaHPO_4^-$, $MgH_2PO_4^+$
S_NO	NO_3^-	NO_3^-
S_HS	HS^-	HS^-, H_2S, NaHS

Variables: values which change at every calculated time step through integration or by an equation which incorporates an integrated variable directly or indirectly (in a sub-equation). A time step is the duration between consecutive calculations, where time continues independent of the values calculated. An array of variables can describe a type of quantity such as concentration or mass for each component. For example: concentration of each component (C[component name]), hydrolysis kinetic variables which are dependent on the concentration of acidogens, and anaerobic digester liquor characteristics such as COD, FSA, total Kjeldahl nitrogen, ortho-phosphate, total phosphate, etc.

In order to explain the details of the model alterations (Section 3.5) and modelling procedures (Chapter 4) the variable terminology has been illustrated to enable distinction. A variable refers to the name or definition of the measurement (e.g. COD, FSA, total Kjeldahl nitrogen, ortho-phosphate, total phosphate, etc.), which holds a different value at each time step. A "set of variables" or just "variables" refer to multiple concentrations or measurements,

each having their associated value at each time step. Variables have initial values, variable values and end point values. Initial values are those measured at time zero. The initial values of the masses of each componenet (M[component name]) and reactor liquid volume (V_liq) are required to calculate the initial concentration values in PWM_SA_AD model. These initial concentrations are used to calculate the change in each aqueous component's concentration and the gas volumes produced during the first time step via stoichiometric equations. These values are production rates and are accumulated by adding the production rate to the previous time step aqueous concentration or cumulative gas volume. Figure 3.1 illustrates this difference between cumulative variable values and production rate variable values for the methane produced during a BMP test. The term "variable values" refers to the set of measured or model generated values at each time step of a variable. Note that variables include both concentrations and production rates. For example, if the cumulative methane production variable in Figure 3.1 were measured daily for the 30 day test shown, the set of variable values would contain 31 values since there are 31 time steps. The first value of the set of variable values, measured at zero days, is the initial cumulative methane production value and the last value of the set of variable values, measured at 30 days, is the end point cumulative methane production value. The initial or starting point value of the cumulative and production rate of methane is zero, but this is not the case for all variables. For example, the initial VFA concentration will affect the set of variable values produced by each variable which is dependent on the VFA concentration.

Figure 3.1 also illustrates the importance of using production rate variable values for model accuracy calculations because the cumulation of production rate variable values dampens the gradient (rate of change) of the variable values and makes the errors between the modelled and measured variable values less evident.

Parameters: the model constants which are collected from literature, measured as a characteristic of a component or determined through calibration of the model to measured variable values of a set of components. For example: hydrolysis kinetic constants, elemental composition values (y/x, z/x, a/x and b/x in $C_1H_{y/x}O_{z/x}N_{a/x}P_{b/x}$) for each organic group, organism growth and decay rate constants, and the volume of headspace in the reactor. Table 3.3 lists and describes these parameters and provides the default value and units of each within the PWM_SA_AD and PWM_SA_AD_BMP models for the modelling of the anaerobic digestion of sewage sludges. The values and units of these parameters can be altered within the WEST user interface prior to a simulation, but remain constant for the duration of each simulation.

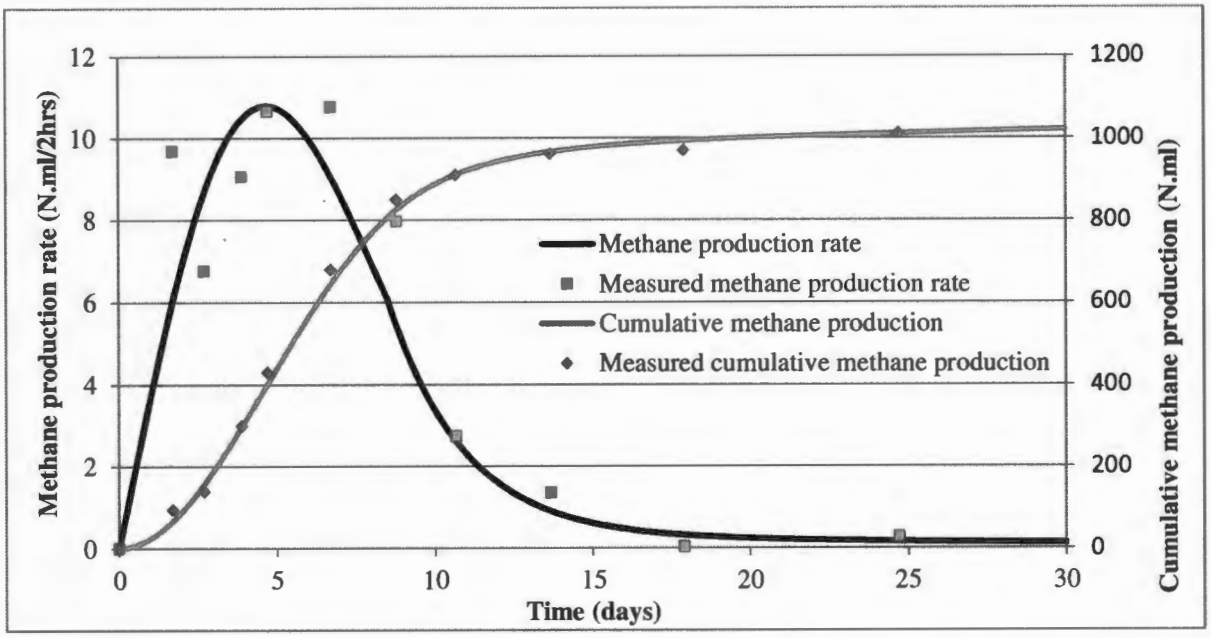

Figure 3.1: Example of BMP test methane production variable values

Table 3.3: PWM_SA_AD and PWM_SA_AD_BMP model parameters

Parameter	Default value#	Units	Description
System parameters:			
Temperature	35	°C	System temperature
V_liq	3400	m^3	Volume of liquid in the reactor at the start of the simulation
V_gas	300	m^3	Volume of headspace in the reactor
MW_[Component]		g/mol	Molar mass for each component
COD_per_mol_[Component]		gCOD/mol	COD mass per mole for each component
MM_C	12.011	g/mol	Molar mass of carbon
MM_H	1.0079	g/mol	Molar mass of hydrogen
MM_O	15.999	g/mol	Molar mass of oxygen
MM_N	14.007	g/mol	Molar mass of nitrogen
MM_P	30.974	g/mol	Molar mass of phosphorus
MM_S	30.974	g/mol	Molar mass of sulphur
Elemental composition parameters:			
i_H_SU_mol_perC	1.646	mol/mol	H/C: unbiodegradable soluble organics (USO)
i_H_SF_mol_perC	2.01	mol/mol	H/C: fermentable biodegradable soluble organics (FBSO)
i_H_XUOrg_mol_perC	1.482	mol/mol	H/C: unbiodegradable particulate endogenous residue (X_U_Org)
i_H_XUInf_mol_perC	1.482	mol/mol	H/C: unbiodegradable particulate influent/feed (X_U_Inf)

Parameter	Default value[#]	Units	Description
i_H_XBOrg_mol_perC	1.463[†]	mol/mol	H/C: biodegradable particulate dead biomass (X_B_Org)
i_H_XBInf_mol_perC	2.19	mol/mol	H/C: biodegradable particulate influent/feed (X_B_Inf)
i_H_Org_mol_perC	1.463	mol/mol	H/C: organisms
i_O_SU_mol_perC	0.593	mol/mol	O/C: unbiodegradable soluble organics (USO)
i_O_SF_mol_perC	0.592	mol/mol	O/C: fermentable biodegradable soluble organics (FBSO)
i_O_XUOrg_mol_perC	0.472	mol/mol	O/C: unbiodegradable particulate endogenous residue (X_U_Org)
i_O_XUInf_mol_perC	0.472	mol/mol	O/C: unbiodegradable particulate influent/feed (X_U_Inf)
i_O_XBOrg_mol_perC	0.355[†]	mol/mol	O/C: biodegradable particulate dead biomass (X_B_Org)
i_O_XBInf_mol_perC	0.653	mol/mol	O/C: biodegradable particulate influent/feed (X_B_Inf)
i_O_Org_mol_perC	0.355	mol/mol	O/C: organisms
i_N_SU_mol_perC	0.062	mol/mol	N/C: unbiodegradable soluble organics (USO)
i_N_SF_mol_perC	0.119	mol/mol	N/C: fermentable biodegradable soluble organics (FBSO)
i_N_XUOrg_mol_perC	0.113	mol/mol	N/C: unbiodegradable particulate endogenous residue (X_U_Org)
i_N_XUInf_mol_perC	0.113	mol/mol	N/C: unbiodegradable particulate influent/feed (X_U_Inf)
i_N_XBOrg_mol_perC	0.229[†]	mol/mol	N/C: biodegradable particulate dead biomass (X_B_Org)
i_N_XBInf_mol_perC	0.0643	mol/mol	N/C: biodegradable particulate influent/feed (X_B_Inf)
i_N_Org_mol_perC	0.229	mol/mol	N/C: organisms
i_P_SU_mol_perC	0.02	mol/mol	P/C: unbiodegradable soluble organics (USO)
i_P_SF_mol_perC	0.012	mol/mol	P/C: fermentable biodegradable soluble organics (FBSO)
i_P_XUOrg_mol_perC	0.022	mol/mol	P/C: unbiodegradable particulate endogenous residue (X_U_Org)
i_P_XUInf_mol_perC	0.022	mol/mol	P/C: unbiodegradable particulate influent/feed (X_U_Inf)
i_P_XBOrg_mol_perC	0.031[†]	mol/mol	P/C: biodegradable particulate dead biomass (X_B_Org)
i_P_XBInf_mol_perC	0.0097	mol/mol	P/C: biodegradable particulate influent/feed (X_B_Inf)
i_P_Org_mol_perC	0.031	mol/mol	P/C: organisms
i_S_SU_mol_perC	0	mol/mol	S/C: unbiodegradable soluble organics (USO)
i_S_SF_mol_perC	0	mol/mol	S/C: fermentable biodegradable soluble organics (FBSO)
i_S_XUOrg_mol_perC	0	mol/mol	S/C: unbiodegradable particulate endogenous residue (X_U_Org)
i_S_XUInf_mol_perC	0	mol/mol	S/C: unbiodegradable particulate influent/feed (X_U_Inf)
i_S_XBOrg_mol_perC	0[†]	mol/mol	S/C: biodegradable particulate dead biomass (X_B_Org)
i_S_XBInf_mol_perC	0	mol/mol	S/C: biodegradable particulate influent/feed (X_B_Inf)
i_S_Org_mol_perC	0	mol/mol	S/C: organisms
i_Mg_PP_mol_perP	0.297	mol/mol	Mg/P: polyphosphate
i_K_PP_mol_perP	0.312	mol/mol	K/P: polyphosphate
i_Ca_PP_mol_perP	0.053	mol/mol	Ca/P: polyphosphate
Kinetic rate constants:			
kH_F_AD_hyd	10	1/d	Hydrolysis rate constant for FBSO
kH_PP_AD_hyd	1.0	1/d	Hydrolysis rate constant for polyphosphate (PP)
kH_PHA_AD_hyd	5	1/d	Hydrolysis rate constant for poly-hydroxy-alkanoate (PHA)

Parameter	Default value[#]	Units	Description
kM_fPP_PAO_PHAstor	0.3	1/d	Maximum rate for PP release with anaerobic PHA storage
kM_BOrg_AD_hyd	1.95	1/d	Monod hydrolysis kinetic rate constant for X_B_Org
kM_BInf_AD_hyd	2.004	1/d	Monod hydrolysis kinetic rate constant for X_B_Inf
KS_BOrg_AD_hyd	10.37	gCOD/g COD	Monod half saturation coefficient for X_B_Org
KS_BInf_AD_hyd	10.124	gCOD/g COD	Monod half saturation coefficient for X_B_Inf
KS_AD	0.78	g/m^3	Monod half saturation coefficient for acidogens
KS_AC	0.089	g/m^3	Monod half saturation coefficient for methanogenic acetogens
KS_AM	0.013	g/m^3	Monod half saturation coefficient for acetoclastic methanogens
KS_HM	0.156	g/m^3	Monod half saturation coefficient for hydrogenotrophic methanogens
KS_ACS	2.631	g/m^3	Monod half saturation coefficient for sulphidogenic acetogens
KS_AS	0.3747	g/m^3	Monod half saturation coefficient for acetoclastic sulphidogens
KS_HS	0.004375	g/m^3	Monod half saturation coefficient for hydrogenotrophic sulphidogens
Kn_ACS	0.07703	g/m^3	Half saturation coefficient for sulphidogenic acetogens
Kn_AS	0.1999	g/m^3	Half saturation coefficient for acetoclastic sulphidogens
Kn_HS	0.1999	g/m^3	Half saturation coefficient for hydrogenotrophic sulphidogens
mu_AD	0.8	1/d	Max specific growth rate for acidogens
mu_AC	1.15	1/d	Max specific growth rate for methanogenic acetogens
mu_AM	4.39	1/d	Max specific growth rate for acetoclastic methanogens
mu_HM	1.2	1/d	Max specific growth rate for hydrogenotrophic methanogens
mu_ACS	0.583	1/d	Max specific growth rate for sulphidogenic acetogens
mu_AS	0.612	1/d	Max specific growth rate for acetoclastic sulphidogens
mu_HS	2.8	1/d	Max specific growth rate for hydrogenotrophic sulphidogens
b_OHO_AD	20	1/d	Decay rate constant for OHO
b_PAO_AD	20	1/d	Decay rate constant for PAO
b_AD	0.041	1/d	Decay rate constant for acidogens
b_AC	0.015	1/d	Decay rate constant for methanogenic acetogens
b_AM	0.037	1/d	Decay rate constant for acetoclastic methanogens
b_HM	0.01	1/d	Decay rate constant for hydrogenotrophic methanogens
b_ACS	0.0185	1/d	Decay rate constant for sulphidogenic acetogens
b_AS	0.0275	1/d	Decay rate constant for acetoclastic sulphidogens
b_HS	0.06	1/d	Decay rate constant for hydrogenotrophic sulphidogens
K_CO2	0.1	1/d	Rate constant for CO_2 exchange
kdis_stru	300	1/d	Dissolution of struvite

Parameter	Default value[#]	Units	Description
kdis_cap	150	1/d	Dissolution of calcium phosphate
kdis_mgkp	100	1/d	Dissolution of K-struvite
kdis_cal	0.5	1/d	Dissolution of calcite
kdis_mag	50	1/d	Dissolution of magnesite
kdis_newb	0.05	1/d	Dissolution of newberyite
K_I_H2	1.25	g/m^3	Inhibition coefficient for H_2 in acidogenesis
K_I_H_AM	1.15E–6	mol/kg	H^+ inhibition for acetoclastic methanogens
K_I_H_HM	0.00053	mol/kg	H^+ inhibition for hydrogenotrophic methanogens
K_I_HS_ACS	0.185	mol/kg	H_2S inhibition for sulphidogenic acetogens
K_I_HS_AS	0.164	mol/kg	H_2S inhibition for acetoclastic sulphidogens
K_I_HS_HS	0.55	mol/kg	H_2S inhibition for hydrogenotrophic sulphidogens
Stoichiometric parameters:			
ISS_BM	0.15	g/ gCOD	ISS to biomass for OHO and PAO
f_XU_Bio_lysis	0.08	gCOD/g COD	Fraction of inert COD generated in methanogenic biomass decay with death regeneration model
f_XU_Bio_lysis_s	0.08	gCOD/g COD	Fraction of inert COD generated in sulphidogenic biomass decay with death regeneration model
Y_f_PP_VFA	0.5	gCOD/g COD	Fraction of P released from PP per VFA used in PHA storage
Y_AD	0.0895	gCOD/g COD	Low H_2 acidogenesis yield
Y_AH	0.0895	gCOD/g COD	High H_2 acidogenesis yield
Y_AC	0.039714	gCOD/g COD	Methanogenic acetogenesis yield
Y_AM	0.03925	gCOD/g COD	Acetoclatic methanogenesis yield
Y_HM	0.04	gCOD/g COD	Hydrogenotrophic methanogenesis yield
Y_ACS	0.038229	gCOD/g COD	Sulphidogenic acetogenesis yield
Y_AS	0.046725	gCOD/g COD	Acetoclatic sulphidogenesis yield
Y_HS	0.0707	gCOD/g COD	Hydrogenotrophic sulphidogenesis yield
Y_ACETATE	0.548491	gCOD/g COD	Fraction of electrons passed to acetate in sulphidogenic acetogenesis
Y_H2S	0.411369	gCOD/g COD	Fraction of electrons passed to HS^- in sulphidogenic acetogenesis
Y_H2	0.001911	gCOD/g COD	Fraction of electrons passed to hydrogen gas in sulphidogenic acetogenesis
gam_o	4.221	e^-/mol	Electrons per mole organisms 4 + i_H_Org_mol_perC – 2*i_O_Org_mol_perC – 3*i_N_Org_mol_perC + 5*i_P_Org_mol_perC

Parameter	Default value[#]	Units	Description
			+ 6*i_S_Org_mol_perC
gam_f	4.529	e^-/mol	Electrons per mole fermentable biodegradable soluble 4 + i_H_SF_mol_perC – 2*i_O_SF_mol_perC – 3*i_N_SF_mol_perC + 5*i_P_SF_mol_perC + 6*i_S_SF_mol_perC
gam_e	4.309	e^-/mol	Electrons per mole endogenous residue 4 + i_H_XUOrg_mol_perC – 2*i_O_XUOrg_mol_perC – 3*i_N_XUOrg_mol_perC + 5*i_P_XUOrg_mol_perC + 6*i_S_XUOrg_mol_perC
gam_bp	4.221	e^-/mol	Electrons per mole biodegradable particulate dead biomass 4 + i_H_XBOrg_mol_perC – 2*i_O_XBOrg_mol_perC – 3*i_N_XBOrg_mol_perC + 5*i_P_XBOrg_mol_perC + 6*i_S_XBOrg_mol_perC
gam_bps	4.7396	e^-/mol	Electrons per mole biodegradable particulate influent/feed 4 + i_H_XBInf_mol_perC – 2*i_O_XBInf_mol_perC – 3*i_N_XBInf_mol_perC + 5*i_P_XBInf_mol_perC + 6*i_S_XBInf_mol_perC
Additional influent fractionation parameters in PWM_SA_AD_BMP:			
f_U_Inf	0.15	-	Unbiodegradable particulate fraction of organic feed VSS
f_U_Org	0.95	-	Unbiodegradable endogenous residue fraction of inoculum seed sludge VSS
f_Biomass	0.113	-	Live organism fraction of biodegradable portion of dead biomass
f_AD	0.66505[‡]	-	Acidogenic fraction of live organisms
f_AC	0.00284[‡]	-	Methanogenic acetogen fraction of live organisms
f_AM	0.19535[‡]	-	Acetoclastic methanogen fraction of live organisms
f_HM	0.13676[‡]	-	Hydrogenotrophic methanogen fraction of live organisms
f_ACS	0.00284[‡]	-	Sulphidogenic acetogen fraction of live organisms
f_AS	0.19535[‡]	-	Acetoclastic sulphidogen fraction of live organisms
f_HS	0.13676[‡]	-	Hydrogenotrophic sulphidogen fraction of live organisms

[#] The elemental composition values (i_H, i_O, i_N, i_P, i_S), kinetic constants (kS, kM, mu, kH, b), reactor volume (V_liq, V_gas) and PWM_SA_AD_BMP initial fractionation parameters are different for each reactor and organic feed. The values given here are the default values provided in PWM_SA_AD for a primary sludge influent (Ikumi *et al.*, 2014).

[†] The biodegradable particulate dead biomass elemental composition values were linked (set equal) to the organism elemental composition values in PWM_SA_AD_BMP so that both compositions would vary together during the parameter estimation of the organism composition.

[‡] For methanogenesis f_AD + f_AC + f_AM + f_HM = 1, while the sulphidogenic bioprocesses are turned off. For sulphidogenesis f_AD + f_ACS + f_AS + f_HS = 1, while the methanogenic bioprocesses are turned off.

Parameter estimation: a set of model simulations which calibrates a set of user-selected model parameters. It uses variable values measured at each time step of a set of variables (selected by the user) and compares them to the corresponding variable values generated by the model. The

set of selected parameter values are changed slightly for every simulation until the error between the measured variable values and the model generated variable values is at a minimum. Section 4.4 provides a comprehensive description of the parameter estimation tool.

Objective parameters: a set of user-selected model parameters selected for calibration during the parameter estimation simulation. To maintain parameter estimation accuracy, the number of parameters in the objective set must not exceed the number of variables for which variable values are provided in the parameter estimation simulation.

3.2 Base model selection motivation

The three phase PWM_SA_AD model, created in the modelling platform WEST by mikebydhi (www.mikebydhi.com), was selected as the base anaerobic digestion model. The technical features and capabilities which qualify the PWM_SA_AD model to serve as a base model for a dynamic BMP model are outlined in Section 2.4 and described in detail in this section. Further motivations for this selection range from the ease of access to the software and assistance from the developer to the methods with which organic compositions have been represented in the model.

A key motivation was that the WEST software was already used at UCT at the time of commencement of research. Although many of the models which were considered are available in WEST, only PWM_SA_AD would be accompanied by developer assistance as the creators of the model are researchers at UCT. This type of technical support was considered to be an invaluable aid. Also parameter estimation, the statistical procedure for finding the best model parameters (unbiodegradable fraction of influent organics, organics composition and kinetic rate constants) is an embedded feature of WEST.

The model structures were also an important feature as the objectives of this research, such as the prediction of the elemental composition of the BPO substrate require the model to have certain capabilities. PWM_SA_AD does not fractionate the organic substrates into proteins, lipids and carbohydrates before elemental fractionation, as is the case of IWAADM1. The double fractionation would result in a tripling of the number of parameters that would need to be found in order to determine the composition of the BPO substrate.

Although the PWM_SA_AD model is best suited to be a base model for the development of a BMP model, some features may limit the practicality of the model. This was to be

expected as no base model is perfect; otherwise there would be no need for a new BMP batch test model.

3.3 Base model description and features

The PWM_SA_AD model's key extensions to the UCTSDM1 (Sötemann *et al.*, 2005b) on which it was based, were described in Section 2.4. This section further describes the PWM_SA_AD model features and capabilities included by Ikumi *et al.* (2014):

- The model calculations are divided into differential mass balance (DEs) and algebraic equilibrium speciation (AEs) calculations in order to model the very rapid ionic dissociation and ion pairing equilibrium reactions separately from the slower biological and physical processes. Table 3.2 lists the 44 species that were selected to represent the distribution of the mixed weak acid/base system species and ion pairs that can form from the 14 ionic components. The 14 ionic components represent the total concentrations of the various weak acid/base systems and are applied in the model material balance calculations (stoichiometry provided in Appendix A).
- The path followed by the measured wastewater characteristics used as model inputs to reach the ionic speciation routine and the post-processor includes:
    - Measured influent wastewater concentrations, including ortho-phosphate, free and saline ammonia (FSA), magnesium (Mg^{2+}), potassium (K^{+}), calcium (Ca^{2+}), pH, $H_2CO_3^*$ alkalinity and acetate (VFA) (plus propionate, nitrate, sulphate and sulphide, if significant); as well as ionic strength (total dissolved solids or TDS) and temperature are entered into the PWM_SA_AD pre-processor.
    - The pre-processor adds NaCl to achieve the measured ionic strength (TDS). It then calculates the ionic activity coefficients from the temperature and divides the total measured concentrations into the species that contribute to each weak acid/base system component at the measured influent pH.
    - The charge represented by this ionic state becomes the reference charge state of the model and any changes in charges due to the biological and physical processes are compared to this reference state.

- The initial concentrations of the ionic components comprise all the contributing species concentrations (see Table 3.2). These components are involved in the model material balance calculations at each time step.
- The speciation routine runs once at each time step. The speciation divides the ionic components into their respective contributing species using the reference charge state and pH of the previous step. The charge represented by the ionic state at each time step is recalculated and compared with the (moving) reference charge state. Any difference between the charges between time steps can be attributed to a change in pH and the model pH value is adjusted accordingly for the next step. Therefore, the model's pH is calculated via charge "accounting" at each time step.
- The post-processor does the reverse of the pre-processor by combining the various contributing species concentrations to obtain the measured wastewater concentrations and pH at the effluent ionic strength (TDS).

- Figure 3.2 provides a process scheme which outlines how the key components are involved in the physical (hydrolysis, precipitation) and biological (acidogenesis, acetogenesis, acetoclastic methanogenesis, hydrogenotrophic methanogenesis) processes.
- The influent BPO undergoes hydrolysis to glucose and then acidogenesis of glucose by acidogens to form acetate and hydrogen which are utilized by the acetoclastic and hydrogenotrophic methanogens for growth. The end products include biomass, CH_4, CO_2 (dissolved HCO_3^- and gaseous CO_2), NH_4^+ and water. The extent to which the BPO are hydrolyzed is dependent on the calibrated hydrolysis rate of the organic type in the feed and the length of time that the organics spend in the anaerobic digester (i.e. sludge age, SRT). Hydrolysis is the rate limiting step and controls the subsequent rates, which are potentially faster so there is no accumulation of glucose, acetate, propionate and hydrogen intermediates under normal conditions.
- The model calibration to determine the hydrolysis rate constants was focused on achieving similar trends to experimentally measured results for primary sludge (PS), waste activated sludge and blends of primary and waste activated sludge (WAS) in order to improve general applicability of the model, rather than fitting the model to each anaerobic digester sludge retention time and sludge type (for better correlation). The results were also compared with those of the two phase UCTSDM1 model (Sötemann *et al.*, 2005b) using the same primary sludge anaerobic digestion results of Izzett *et al.*

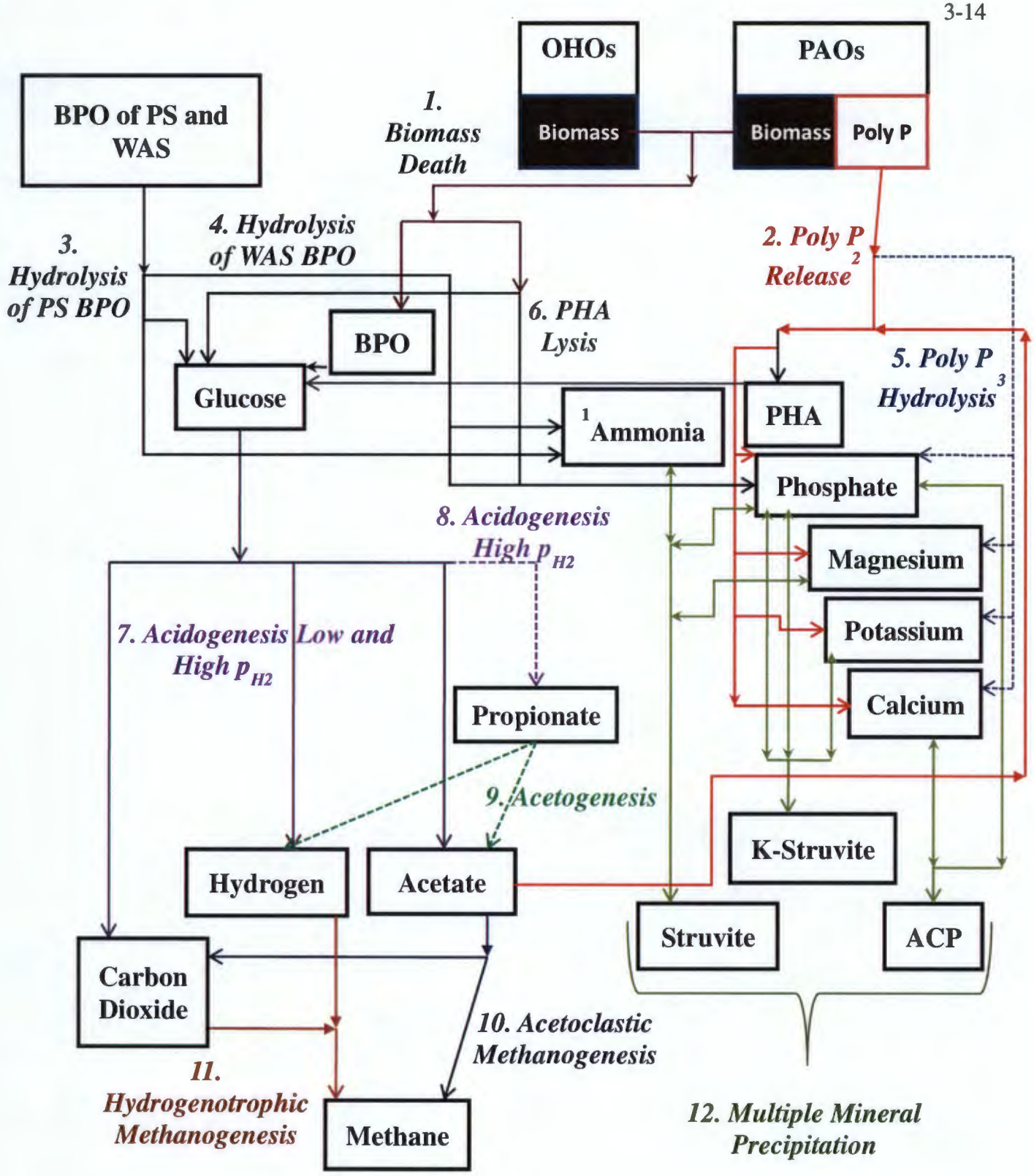

Figure 3.2: PWM_SA_AD and UCTBMP process scheme.

Note that (1) ammonia is released in the NH_3 form and picks up a proton from H_2CO_3 to form NH_4^+, (2) process 2 is for PP release with the uptake of acetate and (3) process 5 is for the PP hydrolysis with the death of the PAOs, (4) ER stands for the endogenous residue of biomass. Process 12 only shows for P precipitates, but other precipitates (i.e. newberyite, calcite and magnesite, which are less likely to form) are also included in the model. (Source: Ikumi *et al.*, 2014)

(1992), which were used to validate the UCTSDM1 model. Identical results were obtained by Ikumi *et al.* (2014).

- The hydrolysis rate of the influent BPO dictates the rate of BPO bound N and P release as FSA and ortho-phosphate to the aqueous phase of the anaerobic digester. With an increase in anaerobic digester sludge retention time, the COD removal and associated with FSA and ortho-phosphate release increases due to the longer period of time available for the BPO to be hydrolyzed and the associated organically bound N and P released as FSA and ortho-phosphate into the anaerobic digester liquor.

- To give an insight to what in effect the gas exchange kinetics and speciation routine are doing in the model to determine the pH the following simplpified in principle explanation is given, but it must be remembered the sequence of steps given below are done in a single "charge accounting" calculation at each time step of the simulation. The production of methane results in the transfer of COD from degraded influent BPO. The amount of methane COD produced is dependent on the COD of the BPO degraded because only a very small amount (2–5%) of COD is used to produce the anaerobic digester biomass. The C of the degraded BPO which is not included in CH_4 (or in the very small amount of biomass formed) becomes CO_2 either dissolved HCO_3^- or gas. Methane generally all escapes as gas as soon as it is formed by the biological reactions because it is largely insoluble at pressures close to atmospheric pressure. The mole fraction of the CO_2 gas [CO_2 / (CO_2 + CH_4)] sets the partial pressure of the gas phase (pCO_2), which together with the total alkalinity, comprising all the weak acid base systems and ion pairs, sets the pH of the anaerobic digestion system.

- Organically bound N is released via the hydrolysis of BPO as non-ionic NH_3 which is alkalinity because the NH_3 consumes an H^+ from the aqueous system to form the model's ammonia weak acid/base system reference species NH_4^+ in the pH range 7 to 8. In an anaerobic digester pH range of between 7 and 8, if the inorganic carbon system dominates the H^+, then the NH_3 reacts with the dissolved CO_2 (H_2CO_3*) to form HCO_3^- according to $NH_3 + H_2O + CO_2 \rightarrow NH_4^+ + HCO_3^-$. This consumption of H^+ within the system increases the alkalinity by the concentration of NH_3 released. The total alkalinity of the system actually does not change in the sense that the N content of the fed BPO is alkalinity in the influent fed to the anaerobic digester, and this alkalinity is transferred from the organically bound N in the BPO to the aqueous phase in this instance the inorganic carbon system. This is the main generation of H_2CO_3* alkalinity in the aqueous

phase of an anaerobic digester treating primary sludge or waste activated sludge that has a low P content and comes from the intrinsic alkalinity (N content) of the influent BPO. In systems which treat P-rich organics and contain polyphosphate (PP) (like BEPR waste activated sludge) the aqueous H_2CO_3* alkalinity also depends on PP and cell bound P release (Harding *et al.*, 2011).

- The organically bound P in the PAO (and OHO) biomass in waste activated sludge is released as H_3PO_4. H_3PO_4 is the reference species for the ortho-phosphate weak acid/base system so there is no change in total alkalinity with this P release. In an anaerobic digester pH range of between 7 and 8, the H_3PO_4 reacts with HCO_3^- to form $H_2PO_4^-$ or HPO_4^{2-}, with the HCO_3^- forming H_2O and gaseous CO_2. So the aqueous total alkalinity remains constant, but the species that represent it change to both HCO_3^- of the inorganic carbon system and the $H_2PO_4^-$ and HPO_4^{2-} species of the ortho-phosphate system. The "extra" gaseous CO_2 exits the anaerobic digester as gas and increases the pCO_2. Because the CH_4 gas production remains unchanged as it is fixed by the COD of the biodegraded organics, the anaerobic digester pH decreases due to the higher pCO_2 and lower H_2CO_3* alkalinity and the anaerobic digester pH is now governed by the inorganic carbon and ortho-phosphate systems.

- For anaerobic digestion of waste activated sludge, initially PP release and polyhydroxyalkanoate (PHA) storage by polyphosphate accumulating organisms (PAOs) takes place and includes an uptake of acetate. This results in an increase in aqueous alkalinity because the PP is released as $H_2PO_4^-$ (and HPO_4^{2-}) according to $MePO_3 + H_2O \rightarrow H_2PO_4^- + Me^+$ where Me represents a combination of metals, magnesium (Mg), potassium (K) and calcium (Ca). The $H_2PO_4^-$ increases the aqueous total alkalinity. Because the ortho-phosphate system has a pK value near 7.0 some of the $H_2PO_4^-$ species become HPO_4^{2-} species by reacting with HCO_3^- to form HPO_4^{2-}, H_2O and CO_2. In this H^+ exchange again the total alkalinity remains constant but alkalinity is transferred from the HCO_3^- of the inorganic carbon system to the HPO_4^{2-} of the ortho-phosphate system. The CO_2 that exits the digester as gas is increased by this exchange and so the pCO_2 of the anaerobic digester gas increases. Again the increase in pCO_2 and decrease in H_2CO_3* alkalinity decreases the anaerobic digester pH, but now the pH is governed by both the inorganic carbon and ortho-phosphate systems (Harding *et al.*, 2011).

- The high-energy PP in the PAOs is not a cell bound part of the biomass and it is released much faster during anaerobic digestion than the organically bound P of biomass. This P

release is not related to the hydrolysis/acidogenesis kinetics of the PAO (and OHO) biomass. Without PP, the hydrolysis rate of PAO and OHO biomass is the same so that the hydrolysis kinetics of BEPR system waste activated sludge is the same as that of the waste activated sludge from fully aerobic or N removal activated sludge system. Because the scope of the experimental investigation of Ikumi *et al.* (2014) did not allow a distinction to be made in compositions of BPO material from OHOs and PAOs, they were modelled to have the same composition except that PAOs included a PP content.

- The rapid release of PP and associated Mg^{2+} and the slow release of biomass N and P generate high concentrations of P, NH_4^+ and Mg^{2+} species in the anaerobic digester liquor, which promotes struvite and other mineral precipitation. The precipitation of struvite decreases the total alkalinity by triple the concentration of struvite precipitated. This results in a change in the speciation of the inorganic carbon system, which increases pCO_2 (because more gaseous CO_2 is released during the re-speciation) and decreases anaerobic digester pH (Loewenthal *et al.*, 1994). If the total alkalinity of the waste activated sludge is low due to a low N content in the organics and the P concentration is high, the precipitation of struvite decreases alkalinity and pH. However, the digester would not be at risk of failure because precipitation requires a pH at the upper end of the range for stable anaerobic digester operation, i.e. above 7.5 (van Rensburg *et al.*, 2005).
- The PAOs cannot grow in anaerobic digesters as they require oxygen as the terminal electron acceptor. Therefore, PAO organics hydrolyze in the anaerobic digestion model, but at a rate faster than their endogenous respiration rate. Their PHA and any remaining stored PP is released, which also contributes to alkalinity in the form of $H_2PO_4^-$.
- Due to the acetoclastic methanogens only utilising the associated form of VFA (HAc), all dissociated VFA fed to the anaerobic digester, increases the aqueous alkalinity through the uptake of protons according to $Ac^- + H_2O + CO_2 \rightarrow HAc + HCO_3^-$.
- Therefore, aqueous alkalinity is increased only by the release of N and PP and the utilization of dissociated VFA. The aqueous influent alkalinity plus these three intrinsic alkalinities contained in the influent organics transferred to the aqueous phase in the anaerobic digester establish the total alkalinity in the anaerobic digester. Thus the generated aqueous total alkalinity and digester pCO_2, which together set the anaerobic digester pH, are completely defined by the composition of the organics digested and the type of bioprocess, which in this case is methanogenesis, which by itself does not

generate alkalinity like sulphate reduction does (Poinapen and Ekama, 2010). The model is based on equilibrium conditions between the dissolved CO_2 (H_2CO_3*) and headspace CO_2 concentrations. This equilibrium condition would need to apply in order to match predicted and measured pH, but poor mixing of the anaerobic digester could decrease the gaseous CO_2 discharge to the headspace (hence lower pCO_2), and decrease pH.

- The influent inorganic settleable (fixed) solids (ISS) is deemed not to take part in any reactions and is simply enmeshed in the sludge mass, increasing with a longer anaerobic digester sludge retention time as presented by Sötemann *et al.* (2006) and Ekama *et al.* (2006). The ISS model by Ekama and Wentzel (2004) includes the uptake of some influent inorganic dissolved solids (IDS) in the growth of OHO and PAO biomass in the activated sludge reactor, which contributes to the mixed liquor ISS concentration when VSS samples are dried (or as precipitates form in the anaerobic digester). The total ISS in an anaerobic digester with a waste activated sludge feed from an NDBEPR system comprises the ISS content of the OHO, PAO and ANO biomass (i.e. ISS_BM = 0.15 mgISS/mg biomass), the PP stored by PAO (3.23 mgISS/mgP), all precipitates formed (struvite, amorphous calcium phosphate (ACP), and K-struvite ($MgKPO_4$)) and the influent ISS that is enmeshed with sludge. Significant phosphorus mineral precipitation occurs in anaerobic digestion systems fed concentrated waste activated sludge from an NDBEPR system (~10gTSS/l), which increases as the P removal of the parent NDBEPR system and concentration of the feed waste activated sludge increases. In contrast, the ISS in an anaerobic digester which is fed primary sludge (which does not contain OHO, PAO and ANO) would consist only of influent ISS that is enmeshed with a primary sludge feed. Other anaerobic digester feeds are generally organic and so do not significally contribute to the inorganics of the AD.
- A BMP (or BSP) test on P rich BEPR waste activated sludge would be a demanding test for PWM_SA_AD model, but outside the scope of this project. In fact, none of the P related processes described above are relevant to organics feed characterization because their P content is very low.

3.4 Base model scope and limitations

The PWM_SA_AD model was selected as the most suitable model base for the BMP model; however any choice will be synonymous with exclusivity. The most serious limitation of selecting the PWM_SA_AD model was the model's focus on the treatment of sewage sludges. This project aims to aid anaerobic digestion facilities with predicting the impact of including imported OFMSW and/or other organic waste streams such as whey or vinasse into an under-capacity digester, thus the sewage sludge calibration of the base model may not accurately represent other compositions and degradation rates of these other waste organics. The model can be re-calibrated to suit different substrates, provided the data is available to do so. Unfortunately, time series BMP test data augmented with changes in soluble COD, VFA, FSA, ortho-phosphate and H_2CO_3 alkalinity, and pH which would be required for calibration, were not available and could not be measured due to the reconstruction of the UCT water quality laboratory. Taking literature values for kinetic rates is generally accepted as a second-rate alternative to measured data calibration; however, kinetic rates are substrate composition specific and thus would be required for each substrate tested anyway. This limitation extends the purpose of this study to include not only the estimation of BPO substrate composition values (x, y, z, a, b and c in $C_xH_yO_zN_aP_bS_c$), but also their kinetic hydrolysis rates, with the use of the parameter estimation tool in WEST.

Although the way in which PWM_SA_AD uses a single elemental composition for the influent BPOs of the substrate is beneficial in reducing the number of parameters that need to be found, it assumes that the substrate is homogenous with a single hydrolysis rate. In reality the organics may comprise several groups that have different compositions and hydrolyze at different rates. This may be considered the subject for future extensions of the PWM_SA_AD_BMP model, but the scope of this investigation only considers homogenous substrates as a basic starting point in the development of a BMP model. With successful results in determining the composition of homogenous BPOs, further research would need to be conducted into extending the model to two or three sub-fractions of BPOs.

Ghoor (2014) has extended the PWM_SA_AD model to include the biological sulphate reduction bioprocess stoichiometry and kinetics, as well as S content parameters for each organic group (c in $C_xH_yO_zN_aP_bS_c$). The sulphidogenesis and methanogenesis bioprocesses co-exist within the PWM_SA_AD model base but have not been modelled to interact yet. Thus, either methanogenesis or sulphidogenesis bioprocesses can be activated, while the other set remain within the model but are set to an inactive state. The hydrolysis and acidogensis

processes are common in both the methanogenic and sulphidogenic systems and therefore are always active. This allows the model to be used for biological sulphate reduction batch tests (called BSP), where hydrogen sulphide (H_2S/HS^-) is produced from sulphate (sulphidogenesis) instead of methane from CO_2 (methanogenesis). A BSP test is an innovation, based on the BMP test, to monitor sulphidogenic anaerobic digester behaviour. Theoreticaly at least a BSP test is the same as a BMP test except that the COD of the degraded organics transform sulphate to sulphide instead of to methane. The BSP test measures *inter alia* the aqueous sulphide and sulphate concentration of a biological sulphate reduction batch test containing inoculum biomass and organic feed for comparison with a control batch test that only contains inoculum biomass. The difference in the COD of the sulphide production, which can be confirmed with changes in sulphate, between the test and control is caused by the degradation of the biodegradable organics in the organic feed. This difference in COD is equal to the biodegradable COD of the organic feed utilized if the BSP test is done to completion, or until negligible sulphide production and sulphate reduction are taking place. Hence, the biodegradable (or unbiodegradable = 1 – biodegradable) fraction of the organic feed can be determined.

The BSP test has the benefit of aqueous sulphide and sulphate concentration measurements which can be measured with better accuracy than a gaseous methane and CO_2 production rate in the BMP test. This is because sulphide is very soluble and all the C from the degraded organics remains dissolved as HCO_3^-; a feature of BSR which Poinapen and Ekama (2010) call carbon deficiency. By maintaining a zero volume headspace, no H_2S or other gas is produced in the test because a plunger, as shown in Figure 3.3, keeps the headspace sealed as it pushes a mixed liquor sample out of the batch test for the measuring of aqueous concentrations. Although the modelling of the BSP test is not the focus of this thesis, the simplicity with which the sulphidogenic system can be activated in the PWM_SA_AD model provides the opportunity to include the modelling of the BSP test briefly in this study (see Section 7).

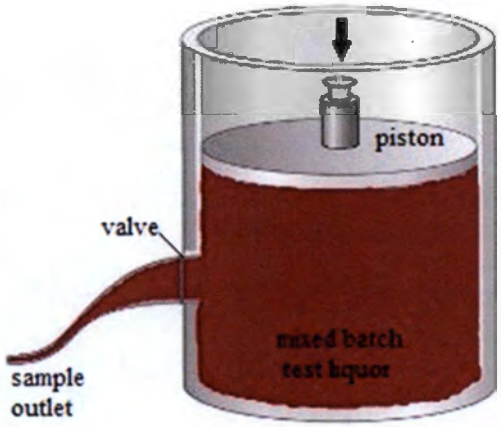

Figure 3.3: Biological sulphide reduction batch test apparatus for zero headspace

Although the scope of this thesis does not include the determination of the S content of the BPO feed, the inclusion of the S content parameters for each organic group within the model allows for c (in $C_xH_yO_zN_aP_bS_c$) to be determined, if it is significant.

It is important to note that this thesis focuses on the modelling of BMP tests on wastewater BPO and organic industrial and agricultural by-products. Unlike waste activated sludge, these substrates do not include PP and PAOs. Therefore, the complex PP and PAO relationships described in Section 3.3 do not play a role in the modelling in this study. The bioprocesses which relate to the growth and hydrolysis of PAOs and the release and hydrolysis of PP remain within the model, but are inactive as no PAOs or PP are included in the influent.

Smaller limitations such as conversion from a flow through system, a constant volume batch system and dealing with the extensive component list of PWM_SA_AD are addressed in the next section which describes the changes that were made to PWM_SA_AD to form PWM_SA_AD_BMP.

3.5 Key alterations

The BMP test is representative of anaerobic digestion, but differs in a few critical ways such as the closed batch test nature of the BMP test rather than a flow through system used in anaerobic digesters. This section outlines how the selected model was altered to form PWM_SA_AD_BMP by accommodating these differences, as well as including additional parameters and their role in the model.

3.5.1 Conversion to batch test system

The selected anaerobic digestion model, PWM_SA_AD, is part of a plant-wide wastewater treatment model PWM_SA. The anaerobic digestion part can be used in a stand-alone capacity as PWM_SA_AD (because the activated sludge and other unit operation processes are dormant), which then requires inflow and initial digester characterization data which in the PWM_SA setup is provided by the other units of the treatment plant. The PWM_SA_AD model also assumes an outflow volume such that a constant volume remains in the digester. Retaining the gas-flow sensors to monitor the production of biogas and methane and by setting the inflow to zero and providing the initial masses of components in the digester at the start of the simulation batch test conditions are established. However, this approach alone does not allow for the intrinsic fractionation of initial concentrations which enables the determination of BPO composition parameters (x, y, z, a and b in $C_xH_yO_zN_aP_b$) using the parameter estimation tool of WEST. For example, the fraction of VSS which is unbiodegradable cannot readily be measured but with a kinetic model and measured data, the value can be calculated by varying the fraction until the model output matches the measured data as accurately as possible (Sötemann *et al.*, 2005b). By setting the model's liquid outflow to zero and modifying the model to allow for a small increase in volume, the initial digester concentrations were added as a small amount of additional water containing the biomass inoculum and BPO substrate of the BMP test entering the digester as an instantaneous (1 min) inflow. The result is a model which functions as a closed batch test system identical to the BMP test with a basis for parameter control of initial concentrations. As in the actual BMP test procedure a Test (inoculum and organics) and a Control (inoculum only) are run and the difference in methane production is due to the organics. In the Control, the parameter estimation uses the measured variable values from the Control test data to find the "endogenous" rate, unbiodegradable fraction and biomass composition, which are then used as known parameters for the Test. The measured variable values from the Test data and the parameters determined from the Control are then used to find, by the parameter estimation procedure, the hydrolysis rate, unbiodegradable fraction and composition (x, y, z, a, b, c in $C_xH_yO_zN_aP_bS_c$) of the organics.

Anaerobic bioprocesses consume water which means from a mass balance perspective, the anaerobic digestion products which include H and O (all except CH_4) obtain this H and O not only from the organics but also from water. This water consumption has to be monitored in the model to check the H and O mass balances of the bioprocesses. If the H and O mass

balances are checked including the H and O of the water volume of the batch, errors in the bioprocess H and O mass balances will not be found because they are overwhelmed by the relatively vast mass of H and O in the water volume compared with that in the organic and biomass (inoculum) inputs. The PWM_SA model keeps track of H and O uptake and release as H_2O in the bioprocess stoichiometry mass balance continuity checks to avoid this.

3.5.2 Parameter fractionation of influent

DHI's WEST provides a user friendly interface which allows for the fractionation of non-standard influents into model components using parameters. However, the parameters created within the inflow unit operation (or terminal) cannot be calculated via the parameter estimation on the digester unit operation module. Therefore, the parameters were created intrinsically, reducing the number of inflow concentrations that need to be predetermined. Table 3.4 lists the components which have intrinsic parameter fractionations and shows that the total VSS of the biomass and separately the total VSS of the substrate are entered for each of the respective components which make up these totals. The fractionation parameters described in Table 3.5 divide the total VSS between the components. Figure 3.4 illustrates the fractionation of the biomass inoculum and separately the organic substrate for a methanogenic system. The sulphidogenic system fractionation is illustrated in Figure 3.5. In the PWM_SA_AD model either methanogenic or sulphidogenic bioprocesses are activated; they must not run simultaneously because their interaction has not been coded yet.

Table 3.4: Intrinsic fractionation of the influent VSS concentrations

Components	Description	Influent concentration	Intrinsic fractionation equation
X_U_Org	Unbiodegradable portion of decayed biomass	Inoculum VSS	(f_U_Org) [Inoculum VSS]
X_B_Org	Biodegradable portion of decayed biomass	Inoculum VSS	(1 – f_Biomass) (1 – f_U_Org) [Inoculum VSS]
X_AD	Live acidogenic organisms	Inoculum VSS	(f_AD) (f_Biomass) (1 – f_U_Org) [Inoculum VSS]
X_AC	Live methanogenic acetogen organisms	Inoculum VSS	(f_AC) (f_Biomass) (1 – f_U_Org) [Inoculum VSS]
X_AM	Live acetoclastic methanogen organisms	Inoculum VSS	(f_AM) (f_Biomass) (1 – f_U_Org) [Inoculum VSS]
X_HM	Live hydrogenotrophic methanogen organisms	Inoculum VSS	(f_HM) (f_Biomass) (1 – f_U_Org) [Inoculum VSS]
X_ACS	Live sulphidogenic acetogen organisms	Inoculum VSS	(f_ACS) (f_Biomass) (1 – f_U_Org) [Inoculum VSS]
X_AS	Live acetoclastic sulphidogen organisms	Inoculum VSS	(f_AS) (f_Biomass) (1 – f_U_Org) [Inoculum VSS]
X_HS	Live hydrogenotrophic sulphidogen organisms	Inoculum VSS	(f_HS) (f_Biomass) (1 – f_U_Org) [Inoculum VSS]
X_U_Inf	Unbiodegradable organic substrate	Substrate VSS	(f_U_Inf) [Substrate VSS]
X_B_Inf	Biodegradable organic substrate	Substrate VSS	(1 – f_U_Inf) [Substrate VSS]

Table 3.5: Intrinsic fractionation parameters

Fractionation Parameters	Definition
f_U_Org	Unbiodegradable fraction of total inoculum VSS
f_Biomass	Live organism fraction of biodegradable inoculum
f_AD	Acidogen fraction of live organism inoculum
f_AC	Methanogenic acetogen fraction of live organism inoculum
f_AM	Acetoclastic methanogen fraction of live organism inoculum
f_HM	Hydrogenotrophic methanogen fraction of live organism inoculum
f_ACS	Sulphidogenic acetogen fraction of live organism inoculum
f_AS	Acetoclastic sulphidogen fraction of live organism inoculum
f_HS	Hydrogenotrophic sulphidogen fraction of live organism inoculum
f_U_Inf	Unbiodegradable fraction of total substrate VSS

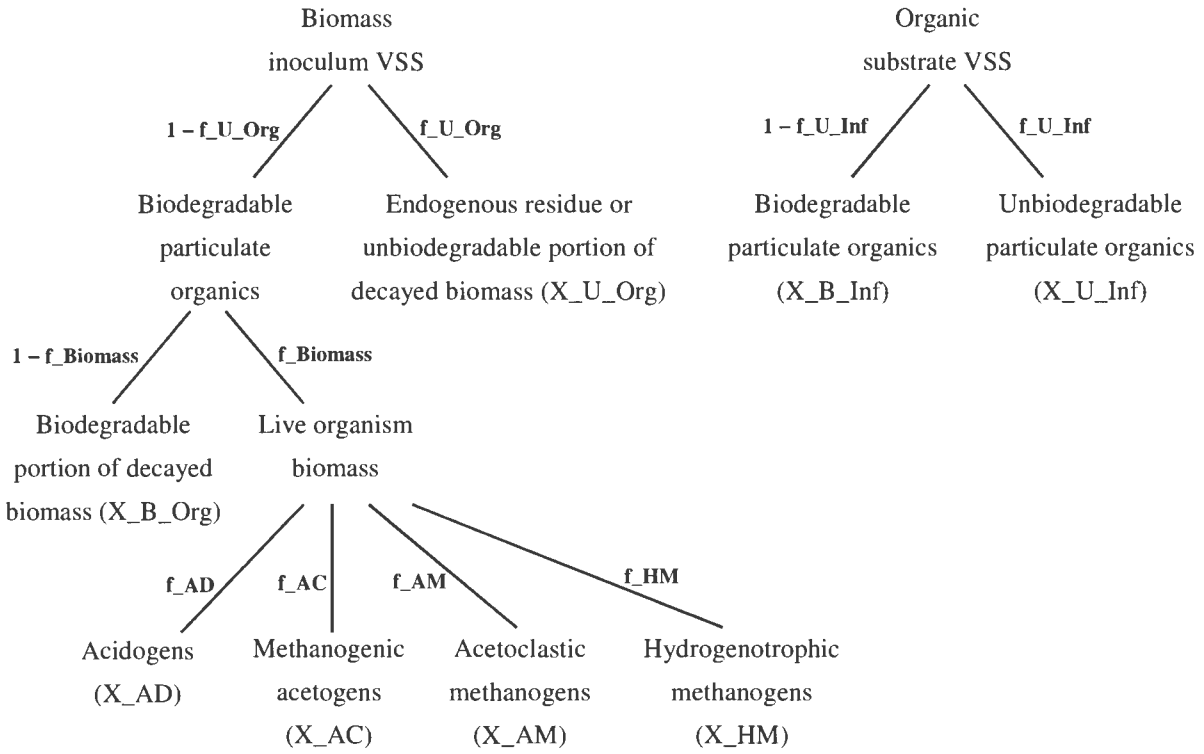

Figure 3.5: Fractionation of biomass inoculum and organic substrate for methanogenic system

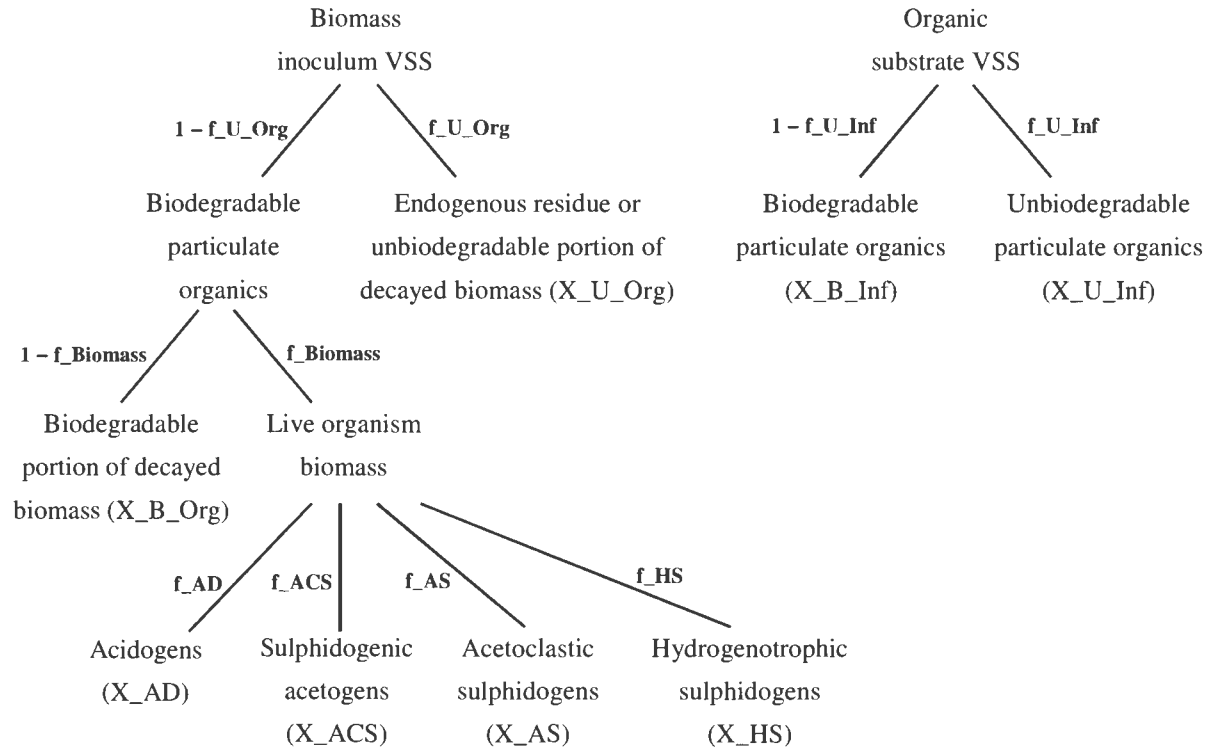

Figure 3.4: Fractionation of biomass inoculum and organic substrate for sulphidogenic system

The unbiodegradable fraction of the inoculum (f_U_Org) and substrate (f_U_Inf) can be estimated from the ultimate methane production result provided by the BMP test and the measured COD/VSS ratio by assuming that all the biodegradable COD has been converted to methane by the end of the BMP test. This is a reasonable initial estimate to start the simulation as only a small fraction of the biodegradable COD is converted to biomass. The fraction of biodegradable biomass that is alive at the start of the BMP test (f_Biomass) was initially assumed to be equal to the combined yield value of the different organism groups given as 0.113 by Sötemann *et al.* (2005a). These fractions can then be calibrated by including them in the parameter estimation for the Control (inoculum only) simulation.

For a substrate which contains a high concentration of VFAs or a low buffer capacity (e.g. low organic N content), a buffering solution is added to the BMP test to ensure the pH in the test stays within a neutral range. This buffering solution is included with the instantaneous inflow, but requires prior fractionation into the PWM_SA_AD_BMP components as their speciation is dependent on the starting pH of the test. This fractionation could also have been incorporated into the model influent setup described for VSS, but due to the numerous buffers used in BMP tests (no standard has been set) this would be a very laborious task, so instead an explanation of the easier pre-processing procedure is given in Chapter 4.

3.6 Mass balance for model verification

Mass balances for COD, C, H, O, N, P and charge were used to verify the operational credibility of the model. Completely mixed flow through systems compare the total influx of each element with their total out flux. More or less than exactly 100% (to five decimal places) exiting the system compared with that entering it indicates a gain or loss of mass and an error in the stoichiometry or set up of the system. Theoretically, the total flux of each of the elements must be conserved and thus can only be converted from one component to another. As the BMP assay is a batch test system, no liquid outflow exists to compare to the initial starting conditions. However with time series measured data or model generated values the total COD, C, H, O, N, P and charge can be determined at every time step and because the BMP test is a closed system, these total element masses must remain constant with time. These totals were included in the model and serve as a simple visual check that the model's stoichiometry balances so that no net losses or gains take place in the system.

As mentioned in Section 3.4.1, the inclusion of the H and O of water in the respective elemental mass balances will mask any fluctuations due to the overwhelming mass contribution of water. Accordingly, only the change in water contributed to H and O masses by the bioprocess stoichiometry were included in the respective mass balances. The change in water mass due to the anaerobic bioprocesses is of a similar order of magnitude to the other stoichiometric changes in H and O, thus the mass balance check excluding the actual water H and O will expose any discrepancies.

3.7 Closure

The final PWM_SA_AD_BMP model largely resembles the PWM_SA_AD model with respect to the core stoichiometric and kinetic structures. The defining stoichiometric matrix, kinetics, components and parameters of these models are provided in Appendix A: PWM_SA_AD and PWM_SA_AD_BMP model details.

4 Project setup

From the previous chapter, the word "model" refers to the set of relationships between components in terms of stoichiometry, kinetics, defining equations, etc. In this respect PWM_SA_AD and PWM_SA_AD_BMP are identical. A model can be used in various project configurations. The project configuration or setup refers to the type of reactors, inflow and outflow methods, treatment process, etc. which are included in the project. The initial conditions and unit specification parameters of each process and inflow, which includes *inter alia* the reactor size, inflow concentrations and hydrolysis kinetic constants, are also important features of the project setup. The details of the batch test project configuration used in this study are discussed in this chapter. It is only in this respect of project setup that PWM_SA_AD and PWM_SA_AD_BMP are different.

This chapter also serves as a user's guide for modelling BMP tests with the PWM_SA_AD_BMP model. Detailed explanations are provided of the pre- and post-processing of data that is required to model the BMP assay using the PWM_SA_AD_BMP model within WEST. By illustrating the setup procedure used for this study the user is guided through some of the features of WEST which allow the project setup to be altered to suit the requirements of the user. The flexibility of the configuration of a project is important in BMP test modelling because a standard protocol for the BMP assay has not yet widely been adopted. The project setup described below is an ideal situation, where all the required data for modelling the BMP test has been measured. This may not be the case if the facilities do not allow for augmented BMP test measurements. An example of an adapted project setup, for a case where limited data is available, is provided in Chapter 6.

The first three parts of this chapter focus on the layout of the project and the initial values that are required to generate BMP variable values (e.g. concentrations, gas production rates and cumulative gas production at each time step) using a simulation of the PWM_SA_AD_BMP model. These sections apply to both the modelling of the BMP test (the first objective of this dissertation) as well as the post-processing of the parameter estimation results (determining the accuracy of the objective parameters). A model simulation generates BMP variable values (concentrations, such as VFA, $H_2CO_3^*$ alkalinity, FSA, ortho-phosphate, as well as CH_4 and CO_2 production at each time step) from BMP starting point concentrations, organic group composition parameters, kinetic constants and reactor defining parameters. The parameter estimation simulation requirements differ from these and are discussed in Section 4.4. The

parameter estimation function uses BMP variable values to determine objective parameter values. Once the objective parameters are calculated, they are used to simulate BMP variable values, referred to as the post-processing step. These simulated BMP variable values are compared with the measured BMP variable values to determine the accuracy of the parameters and the model. The final section of this chapter explains how the model and parameter accuracy can be determined.

4.1 System layout

The layout of the system used in this study is shown in Figure 4.1. An influent municipal flow is connected to an anaerobic digester. The municipal influent element in the layout is simply a name for the icon used in the WEST platform and does not define the influent concentrations. The concentrations of each model component in the influent require manual specification (see Section 4.3), a procedure known as influent generation.

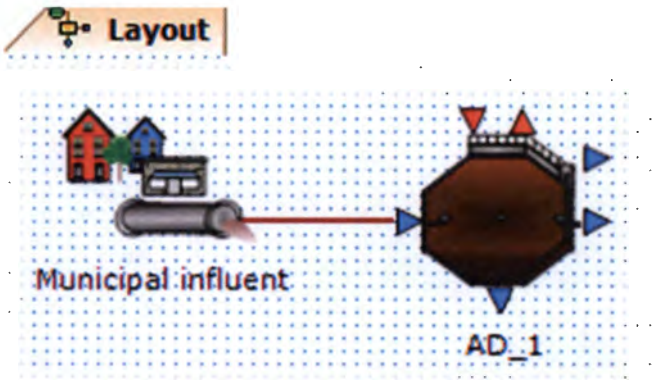

Figure 4.1: PWM_SA_AD_BMP system layout in WEST

The anaerobic digester volume can be set according to that of the apparatus used in the experiment from which data was sourced. The volume is divided into headspace (gas) volume and liquid volume. The PWM_SA_AD_BMP model was converted to a batch test model from the constant-volume, flow-through PWM_SA_AD model. The key motivation for this alteration is to allow for the parameter fractionation of the inoculum and feed, respectively, within the BMP test model. The details of the model alterations are provided in Chapter 3. The result is that the total liquid volume in the anaerobic digester includes the liquid volume specified in the anaerobic digester block details (AD_1) as well as the volume of water that is added instantaneously to the anaerobic digester via the "municipal influent" block (because the

PWM_SA_AD_BMP model has no outflow) to establish the initial component masses (or concentrations) in the anaerobic digester.

4.2 Initial conditions in anaerobic digester

In order to generate model outputs (called variable values), such as component concentrations at each time step, all the model parameters and initial component masses (used for the calculation of concentrations) in the reactor require quantification. These values are requirements for a model simulation, which generates BMP variable values (concentrations, such as VFA, $H_2CO_3^*$ alkalinity, FSA, ortho-phosphate, as well as CH_4 and CO_2 production at each time step) from BMP starting point concentrations, organic group compositions and kinetic constants. The parameter estimation simulation requirements differ from these and are discussed in Section 4.4. This section describes and motivates the methodology used to set up the anaerobic digester parameters and initial component masses in the reactor.

4.2.1 Initial component masses

In order to allow the respective inoculum and feed VSS to be fractionated into biomass, biodegradable and unbiodegradable portions (as per Figure 3.4 and 3.5), these concentrations are included in the "instantaneous" influent flow. If the anaerobic digestion "reactor" (or BMP test volume) is initially empty (contains no water), model errors occur because some of the model's defining equations include division by the masses of water, acidogens, methane or carbon dioxide. Therefore, these masses in the reactor cannot initially be zero. Consequently, the water included in a BMP sample is modelled as the initial reactor contents in order to define small mass values of water, acidogens, methane and carbon dioxide to prevent model errors. The water is anyway included in a BMP assay procedure to ensure equal volumes in the triplicate samples and optimal dilution of the feed so that overloading or potential inhibition of the biological organisms does not occur (Angelidaki *et al.*, 2009).

The volume of water included as the initial anaerobic digester water mass value does not need to be the exact volume of water added to each BMP sample because the remainder of the sample volume will flow into the completely mixed reactor via the "instantaneous" influent. Thus, it is recommended that a small fraction of the total sample volume is selected and used consistently throughout the modelling procedure to minimize the pre-processing of data. For

example, during this study an initial reactor volume of 100 ml of tap water was used, where the total liquid sample volume in the test was 500 ml. Therefore, the remaining 400 ml of water entered the reactor via the influent. The concentrations in the influent are adjusted to this influent volume of water so that the concentrations in the reactor, once the influent has flowed in, are consistent with those in the BMP sample at the start of the test. The determination of these influent concentrations is discussed in Section 4.3.

Angelidaki *et al.* (2009) recommends the use of distilled water in BMP samples to ensure that the addition of any minerals and nutrients in the BMP sample can be accurately documented. Tap water is often used instead as it is more readily available and already includes some of the nutrients required in a BMP assay. The use of tap water does not compromise the accuracy of the BMP test as long as the mineral and nutrient content of the tap water is known. The average characteristics of tap water are generally made available by the local municipality and include the alkalinity, calcium and magnesium content of the water. The total permanent hardness of tap water is another common characteristic provided, which describes the calcium and magnesium content of the water according to

$$\text{Hardness (g/L as } CaCO_3) = 2.5[Ca^{2+}] + 4.1[Mg^{2+}] \quad (4.1)$$

where $[Ca^{2+}]$ and $[Mg^{2+}]$ are the concentrations of calcium (g Ca^{2+}/L) and magnesium (g Mg^{2+}/L) ions respectively. If the hardness of the water is provided an accompanying measurement of either the calcium or magnesium content of the water would be required in order to determine the remaining unknown (either $[Ca^{2+}]$ or $[Mg^{2+}]$) from Equation 4.1. The concentrations of calcium and magnesium need to be converted to initial reactor masses by multiplying the concentrations by the initial reactor volume. For example, the calcium and magnesium concentrations in the tap water used in the data of this study were 70 and 22 mg/L as $CaCO_3$, respectively. So the initial mass of calcium, M[S_Ca] (using the PWM_SA_AD_BMP model notation), is calculated from

M[S_Ca] = Ca concentration × volume × equivalent weight of Ca

÷ equivalent weight of $CaCO_3$

= 70 mg/L as $CaCO_3$ × 0.1 L × 20.04 equiv. Ca/mol ÷ 50.045 equiv. $CaCO_3$/mol

= 2.80 mg Ca

Similarly the initial mass of magnesium, M[S_Mg], is

M[S_Mg] = Mg concentration × volume × equiv. weight of Mg ÷ equiv. weight of $CaCO_3$

= 22 mg/L as $CaCO_3$ × 0.1 L × 12.15 equiv. Mg/mol ÷ 50.045 equiv. $CaCO_3$/mol

= 0.53 mg Mg

The example above demonstrates the insignificant amounts of minerals contributed by tap water. Therefore, calculating the mineral contributions would only be required in cases where the tap water has a particularly high hardness concentration.

Assuming that the alkalinity of tap water is mostly represented by the inorganic carbonate system, the log species-pH diagram (or the associated equations) of the inorganic carbonate system (Loewenthal & Marais, 1976; Loewenthal *et al.*, 1989) can be used to determine the initial reactor masses of H^+ and CO_3^{2-} from the alkalinity of the tap water, the initial volume in the reactor and the initial pH of the sample. For example, the alkalinity of the tap water used in the data of this study was 230 mg/L as $CaCO_3$. This can be converted to mol/L via

Alkalinity (mol/L) = alkalinity ÷ equiv. weight of $CaCO_3$

= 0.23 g/L as $CaCO_3$ ÷ 50.045 equiv. $CaCO_3$/mol

= 0.0046 or $10^{-2.33724}$ mol/L

At the initial sample pH of 7.5 for the data in this study, the inorganic carbon species concentrations were calculated from

$$[H_2CO_3] = \frac{Alk - 10^{pH - pK'_w} + 10^{-pH}}{10^{pH - pK'_{ac1}} + 10^{2 + 2pH - pK'_{ac1} - pK'_{ac2}}} = 0.000252 \text{ mol/L} \quad (4.2)$$

$$[HCO_3^-] = \frac{Alk - 10^{pH - pK'_w} + 10^{-pH}}{1 + 10^{2 + pH - pK'_{ac2}}} = 0.003908 \text{ mol/L} \quad (4.3)$$

$$[CO_3^{2-}] = \frac{Alk - 10^{pH - pK'_w} + 10^{-pH}}{2 + 10^{pK'_{ac2} - pH}} = 0.000008 \text{ mol/L} \quad (4.4)$$

where [ ] represents concentration in mol/L, pK'_{ac1} and pK'_{ac2} are the negative logarithms of the apparent inorganic carbon system dissociation constants at 35°C of 6.3095 and 10.2516, respectively, and pK'_w is the negative logarithm of the apparent ion product constant for water at 35°C of 13.9128. With the carbonate concentrations known the initial masses of H^+ (M[S_H]) and C_T (M[S_CO3]), which is represented by CO_3^{2-} in PWM_SA_AD_BMP model,

are given by Equations 4.5 and 4.6 respectively. The portion of the total H^+ concentration determined from pH ($[H^+]$ in Equation 4.5) is equal to $10^{-pH}/f_m$ where f_m is the monovalent activity co-efficient. f_m can be calculated from the conductivity (TDS) of the solution via the Davies equation for low salinity waters (TDS $\leq$ 2500 mg/L) given in Appendix 1 of Loewenthal *et al.* (1989). Equation 4.5 assumes a value of 1 for f_m as no conductivity measurements were recorded. This assumption had an insignificant impact on M[S_H] because the value of $[H^+]$ was four orders of magnitude smaller than the other concentrations in Equation 4.5.

$$\begin{aligned} M[S\_H] &= \text{volume} \times ([H^+] + 2[H_2CO_3] + [HCO_3^-]) \times \text{molar mass of } H^+ \qquad (4.5) \\ &= 0.1\ L \times (10^{-7.5}/1 + 2 \times 0.000252 + 0.003908)\ mol/L \times 1.008\ g\ H^+/mol \\ &= 0.000445\ g\ H^+ \end{aligned}$$

$$\begin{aligned} M[S\_CO3] &= \text{volume} \times ([CO_3^{2-}] + [H_2CO_3] + [HCO_3^-]) \times \text{molar mass of } CO_3^{2-} \qquad (4.6) \\ &= 0.1\ L \times (0.000008 + 0.000252 + 0.003908)\ mol/L \times 60\ g\ CO_3^{2-}/mol \\ &= 0.025008\ g\ CO_3^{2-} \end{aligned}$$

The initial reactor masses are specified under the *Variables* tab of the *Block Details* panel, which appears when the anaerobic digester is selected from the layout, as shown in Figure 4.2. Ensure that the *Dynamic* tab is selected under the *Control Center* panel because the default is set to the *Steady State* tab. The model units for masses are grams.

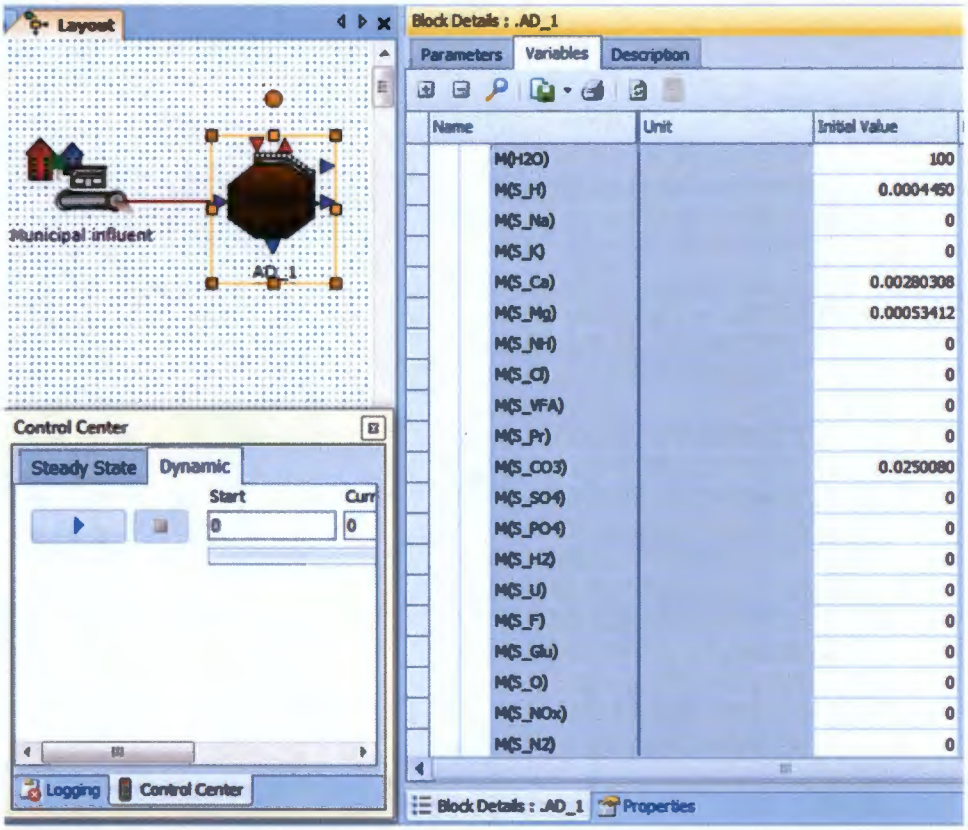

Figure 4.2: Specifying initial reactor masses in WEST

4.2.2 Initial parameter values

Section 4.4 will explain the project setup requirements to use the PWM_SA_AD_BMP model for the purpose of calculating a set of parameter values using parameter estimation. This section discusses the parameter requirements for project simulation which produces component concentrations (BMP results) at each time step. There are many parameters under the *Parameters* tab of the reactor's *Block Details* that require specification in order to simulate the model. However, the default values have been set to literature recommended values and can mostly be left unchanged. Those parameters that require measurement or calculation, as well as the additional influent fractionation parameters in the PWM_SA_AD_BMP model, are highlighted in this section to describe their purpose and a method for their calculation. The kinetic constants for the hydrolysis of the feed BPOs have been calibrated to a primary sludge feed for the original application of the PWM_SA_AD model and would require calibration to

each BMP test feed. Therefore, they have not been altered initially, as their calibration is discussed in Section 4.4 which describes the parameter estimation procedure.

4.2.2.1 BMP test sample headspace and volume

The volumes of liquid (V_liq) and gas (V_gas) are the first parameters on the list in WEST that require specification. V_liq is set to a small constant volume, with the remaining BMP test sample volume flowing in via the influent, as described in Section 4.2.1 for the initial mass of water. The volume of water, V_liq (m^3), and initial mass of water, M[H2O] (g), should correspond because the density of water is known for a given temperature and pressure (an approximation of 1000 kg/m^3 is suitable). The volume of headspace provided in the experimental setup should be specified for V_gas (m^3).

4.2.2.2 Influent flow fractionation parameters

Tables 3.1 and 3.2 (pg. 3-10) define the fractionation parameters and explain each of their functions by providing the dependent model equations. These fractions divide the measured VSS in the inoculum and feed, respectively, into their biodegradable and unbiodegradable constituents. The unbiodegradable fraction can be approximated by (i) continuing the BMP test until insignificant activity (methane production) is measured, (ii) calculating the COD of the total methane produced (64 g COD/ mol CH_4), (iii) subtracting the methane COD from the initial COD to determine the unbiodegradable COD, and (iv) dividing the unbiodegradable COD by the initial COD. For the inoculum's unbiodegradable fraction (f_U_Org) the inoculum only, reference BMP test is used. For the feed's unbiodegradable fraction (f_U_Inf) the initial and ultimate methane production COD of the inoculum only, Control BMP test is subtracted from that of the inoculum and feed sample BMP Test.

A portion of the biodegradable fraction of the inoculum is assigned to the components which represent the live organism groups, while the remaining dead biomass acts as feed for the live organisms. This live organism fraction of the biodegradable inoculum (f_Biomass) has been set to 0.113, the steady state biomass yield value developed by Sötemann *et al.* (2005a), as an initial estimate, but would require calibration. The measurement of biomass activity is possible using specialized equipment, but the purpose of this study includes the practicality of its application within an unspecialized laboratory.

The live biomass is divided into four micro-organism groups: acidogens, methanogenic acetogens, acetoclastic methanogens and hydrogenotrophic methanogens for methanogenesis or acidogens, sulphidogenic acetogens, acetoclastic sulphidogens and hydrogenotrophic sulphidogens for sulphidogenesis. Either methanogenic or sulphidogenic bioprocesses are active; they cannot run simultaneously or interact. The fractions of the live biomass concentration which belong to each micro-organism group can be estimated with the PWM_SA_AD model instead of using specialized equipment for measurement. BMP test description should include details of the digester from which the inoculum sample was taken as well as an ultimate methane potential (Control). If enough inoculum source digester information is provided to simulate a steady state anaerobic digester run with the PWM_SA_AD model, the approximate steady state concentrations of each organism group can be determined for the source sludge, which can be assumed to be the same as the inoculum sample micro-organism group concentrations.

The vinasse and whey BMP test data which was received from the University of Padova did not include sufficient source digester information to simulate the micro-organism group concentrations, but a typical digester was modelled to estimate the organism group fractions which were used throughout this study. The digester feed was a primary sludge derived from a typical municipal wastewater for dry weather given in the influent file for Benchmark Simulation Model 2 (BSM2; Pons MN, IWA, 2012). The steady state reactor had a volume of 6,8 l and was run at 35 °C in WEST using the PWM_SA_AD model. A sludge age of 20 days was maintained by using a 300 ml/d inflow and allowing an 800 ml headspace. The initial conditions in the reactor were kept as the default values provided by WEST for the PWM_SA_AD model. The reactor was simulated for 200 days to ensure that the concentrations were at steady state. Each organism group fraction of the total biomass concentration was then determined from the reactor concentrations at steady state. These fractions were deemed to be reasonable starting values because f_Biomass would be calibrated to the data or calculated in the parameter estimation anyway (see Section 4.4) and only the sub-fractionation into organism groups is being specified. The values of the live organism group fractions are given in Table 4.1. If these fractions are altered, ensure that they still add up to one.

Table 4.1: Live biomass fractionation parameters

Fractionation Parameters	Definition	Value
Methanogenesis:		
f_AD	Acidogenic fraction of live organism inoculum	0.66505
f_AC	Methanogenic acetogen fraction of live organism inoculum	0.00284
f_AM	Acetoclastic methanogen fraction of live organism inoculum	0.19535
f_HM	Hydrogenotrophic methanogen fraction of live organism inoculum	0.13676
	Total	1.00000
Sulphidogenesis*:		
f_AD	Acidogenic fraction of live organism inoculum	0.66505
f_ACS	Sulphidogenic acetogen fraction of live organism inoculum	0.00284
f_AS	Acetoclastic sulphidogen fraction of live organism inoculum	0.19535
f_HS	Hydrogenotrophic sulphidogen fraction of live organism inoculum	0.13676
	Total	1.00000
* Notice that the biomass fractions for the methanogenesis and sulphidogenesis are the same. This is because the yield coefficients and kinetic constants are assumed to be the same.		

4.2.2.3 Inoculum and feed elemental composition values

The elemental composition of an organic group (e.g. BPOs and UPOs) can be calculated from the mass ratios of the respective organic group (Harding, 2009). The mass ratios include: COD/VSS (f_{cv}), TOC/VSS (f_c), organic N/VSS (f_n) and organic P/VSS (f_p). Therefore the COD, TOC, organic N, organic P and VSS concentrations need to be measured, but determining the concentrations of each organic group requires some calculation to disaggregate the COD, TKN, total phosphate and VSS measurements into the values for the different organic groups.

Firstly, the filtered and unfiltered concentrations are required. The difference between the unfiltered and the filtered concentrations for each of the inoculum or feed samples are the concentrations of the total particulate organics (POs). The masses of COD, TOC, organic N, organic P and VSS in the POs are calculated from: concentration × volume. These steps are repeated with concentrations measured when the sample has been digested to its apparent end point (no biodegradable organics left over) or when no more significant activity is measured.

At the digestion end point the biodegradable organics would all have been degraded and only unbiodegradable particulate organics (UPO) remain. The UPO mass ratios can be determined from the digested sample masses and the BPO mass ratios can be determined from the differences between the masses of the POs (before digestion) and the UPOs (after digestion) masses. The mass ratios can be converted to elemental compositions using Equations 4.7 – 4.9 from Ekama and Brouckaert (2015).

$$C_XH_YO_ZN_AP_B = C_{f_c/12}\,H_{f_h/1}\,O_{f_o/16}\,N_{f_n/14}\,P_{f_p/31} \tag{4.7}$$

$$f_h = \frac{1}{9}\left(1 + f_{cv} - \frac{44}{12}f_c + \frac{10}{14}f_n - \frac{71}{31}f_p\right) \tag{4.8}$$

$$f_o = \frac{16}{18}\left(1 - \frac{1}{8}f_{cv} - \frac{8}{12}f_c - \frac{17}{14}f_n - \frac{26}{31}f_p\right) \tag{4.9}$$

The elemental compositions of the soluble organic groups do not require calculation as the scope of this study only considers particulate feeds and a granular inoculum. However, the same process can be used as for the POs except the filtered sample should be digested to completion in order to determine the unbiodegradable fraction and mass ratios.

If the mass ratios cannot be determined the elemental compositions of the POs would still require an initial estimation in order to proceed with their parameter estimation, of which further details are provided in Section 4.4.

4.3 Influent concentrations

In order to allow parameters to control the amount of biodegradable inoculum and feed that is present in the modelled BMP assay, the inoculum and feed concentrations are included in the influent flow. The parameter control is required in order to use the parameter estimation function to determine the unbiodegradable fractions of the inoculum and feed, respectively. The influent flow rate is set at a high enough value to ensure that the inflow time does not affect the model results. One minute was considered a small enough duration of influent flow as the impact of one minute in a 30 day BMP test is insignificant. The flow rate is determined from the volume of the BMP test less the initial reactor volume (V_liq) and the inflow time of one minute. For example, in this study the BMP assays have a volume of 500 ml less V_liq of

100 ml. Therefore 400 ml enters via the influent flow during one minute at a flow rate of 0.576 m^3/d.

Using the volume that enters via the influent, the concentration of each component can be calculated from the mass or concentration and volume included in the BMP test. This process is explained below for the nutrient and buffer solution, which is included in all the BMP tests; the Control BMP test sample, which only includes the inoculum sludge and serves as a reference/control test; and the BMP Test sample, which includes both the inoculum sludge and the organic feed. Once the concentrations are calculated they can be imported into WEST as a text file. An example of an influent text file is provided with the PWM_SA_AD_BMP model and Figure 4.3 gives a preview of the file.

File Edit Format View Help

```
%%BeginHeader
t                H2O      S_H      S_Na     S_K      S_Ca     S_Mg     S_NH     S_Cl     S_VFA    S_Pr
d                m3/d     mg/L     mg/L     mg/L     mg/L     mg/L     mg/L     mg/L     mg/L     mg/L
%%EndHeader
0                0.576    134.84   2804.90  1525.59  14.03    2.67     0.00     0.00     0.00     0.00
0.00069444       0        0        0        0        0        0        0        0        0        0
```

Figure 4.3: Sample of influent text file for PWM_SA_AD_BMP

Figure 4.4 shows the *Influent* settings panel that appears when double-clicking on the *Municipal influent* icon in the *Layout*. Under the *General* tab the *Standard* option of input components should be selected (Figure 4.4). In the next tab, *Data Import*, the influent text file can be selected from file by clicking on the green, plus-sign button (Figure 4.5). The *Simulation Input Interpolation* option in this tab should be deselected to prevent a linear decline to zero of the flow rate over the one minute inflow period. The last tab, *Generate and Review*, creates the inflows from the influent file. Ensure that the *Dynamic* tab is selected before clicking on *Generate*. These inflows should be saved using the available *save* and *save as* icons so that they can be reloaded in this tab for future simulations. This *Generate and Review* tab also allows for changes to be made to the influent flow rate and concentrations within the WEST user interface, rather than reloading and generating inflows from a new text file.

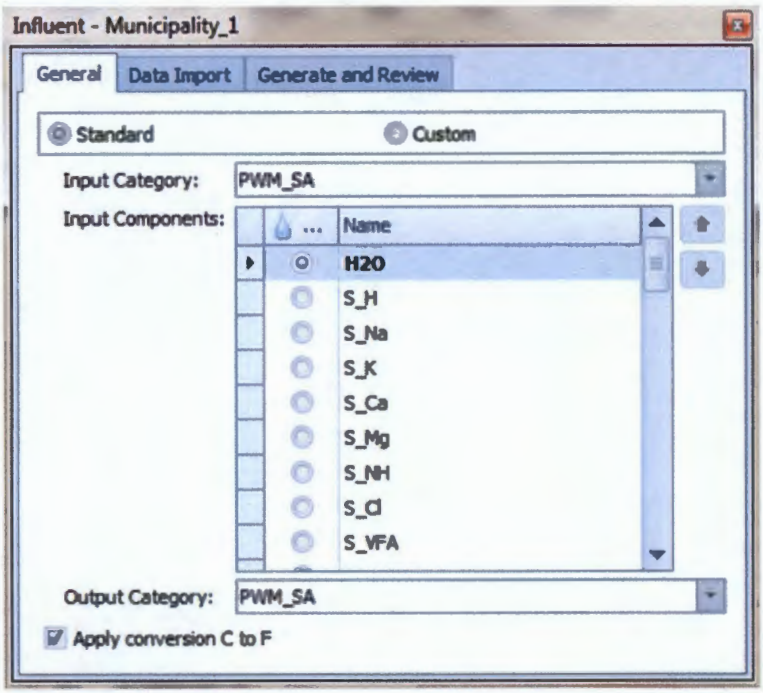

Figure 4.4: General tab of influent flow settings

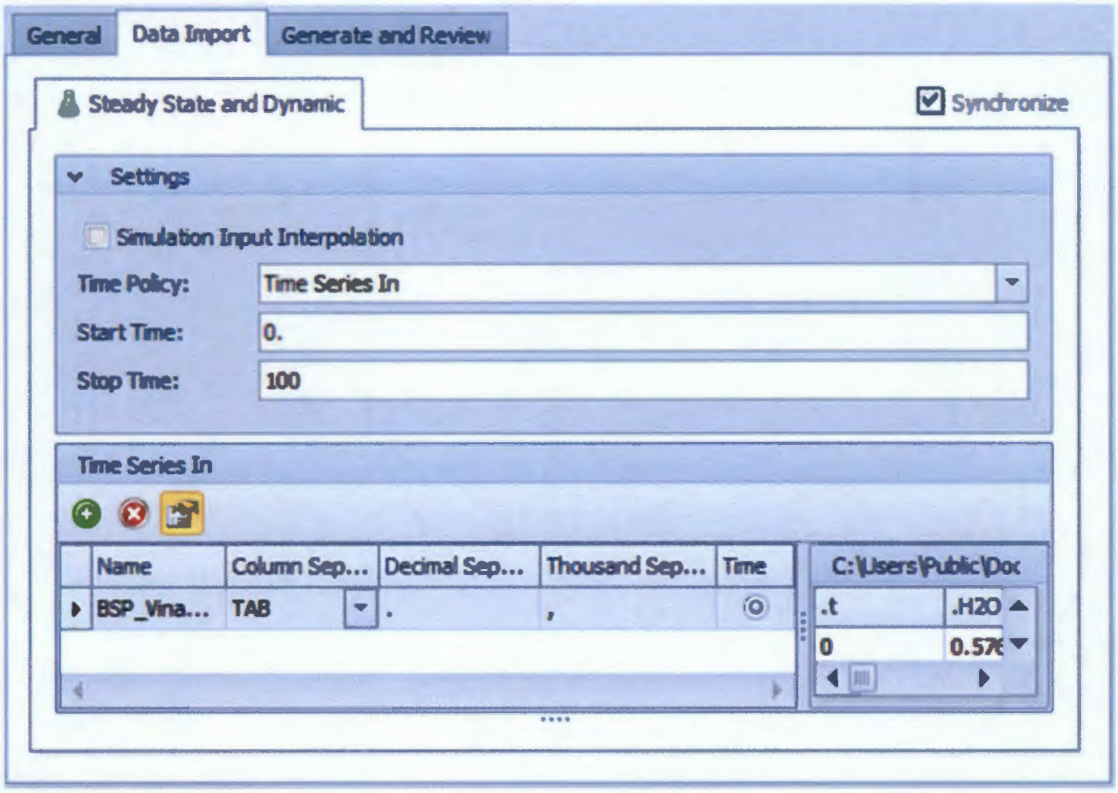

Figure 4.5: Data Import tab of influent flow settings

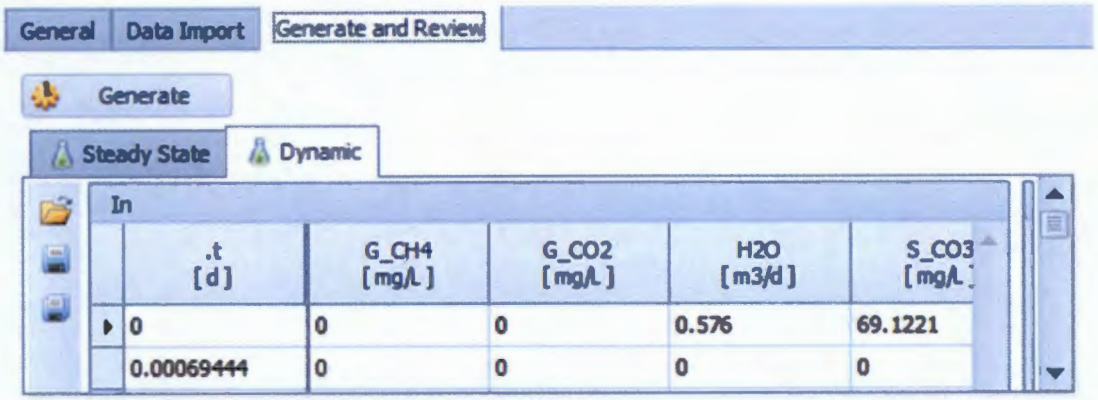

Figure 4.6: Generate and Review tab of influent flow settings

4.3.1 Nutrients and buffer solution

Nutrients are required for the optimal function of the micro-organisms present in the inoculum sludge and the buffer prevents acidification (low pH) during the assay. These solutions vary between feeds. For example, readily biodegradable feeds can cause a rapid drop in pH when degraded to VFAs during the acidogenesis process and therefore require a stronger buffer solution than complex, slowly biodegradable particulate feeds, which may need more nutrients due to the longer assay periods. The BMP test protocol presented by Angelidaki *et al.* (2009) provides details of a recommended buffer and nutrient solution, but the PWM_SA_AD_BMP model has no specified solution. The solution which is used will be included in the influent by dividing its contents among the components. For example, this study included a 200 ml buffer solution containing 10.62 g/L of KH_2PO_4 and 17.32 g/L of Na_2HPO_4 in every BMP test. These compounds in solution are divided into the soluble ionic components: potassium (K^+), hydrogen (H^+), phosphate (PO_4^{3-}) and sodium (Na^+). The corresponding model component names are S_K, S_H, S_PO4 and S_Na, respectively. The compound concentrations are converted to component concentrations by dividing by the compound molecular weight and multiplying by the component molecular weight and the number of moles of component per mole of compound. The concentrations were also adjusted for volume by multiplying by the buffer solution volume (200 ml) and dividing by the influent volume (400 ml) to ensure that the correct concentration of buffer enters the reactor with the influent flow. The same procedure can be followed for any nutrients added and a complete list of the PWM_SA_AD_BMP model components among which the compounds should be divided can be found in Section 3.1. These

component concentrations can then be included in the influent text file and imported into WEST as described above.

4.3.2 Inoculum only BMP test influent

The inoculum only Control BMP test is required as a reference test to indicate the amount of methane potential the inoculum sludge itself produces. This methane potential is subtracted from the inoculum and feed test methane potential to determine a feed's BMP. In the PWM_SA_AD_BMP model the inoculum only test is modelled to determine the unbiodegradable fraction of the inoculum (f_U_Org), the live organism fraction of the biodegradable inoculum (f_Biomass) and the elemental composition of the live inoculum. These parameters are required to provide a baseline in order to determine the elemental composition of the feed BPOs and the unbiodegradable fraction of the feed when the inoculum and feed test is modelled.

Section 4.2.2.2 set the values of the fractionation parameters; therefore the total inoculum concentration should be included for all the components which constitute the inoculum VSS: X_U_Org, X_B_Org, X_AD, X_AC, X_AM and X_HM (see Table 3.1). For example, this study uses 10 gVS of granular sludge which will flow in via the 400 ml influent volume. Therefore the concentration of each of the components above would be set relative to 25000 gVS/m^3 in the influent text file, which already contains the time, flow rate and nutrients and buffer solution concentrations.

4.3.3 Inoculum and feed BMP test influent

For the inoculum and feed test, the total feed concentration is included for all the feed components (X_U_Inf and X_B_Inf) in the same way as was done for the inoculum. Now the influent file will include the time, flow rate, nutrients, buffer, inoculum and feed concentrations for the modelling of the BMP test. For example, 2.5 gVS of feed was included in the BMP tests from which data was obtained and used for modelling in this study. This translates to 6250 gVS/m^3 in the 400 ml influent volume.

4.4 Parameter estimation

Thus far, the project which uses the PWM_SA_AD_BMP model has been set up to simulate *inter alia* the variables measured in a BMP test, provided the hydrolysis kinetic constants, organic group elemental compositions and initial component concentrations are known. As outlined in the purpose of this research, the next goal was to determine the unbiodegradable fraction of the organic feed, the elemental composition parameters of the BPO feed and the hydrolysis kinetic constants of the BPO feed from an augmented set of variable values measured in the BMP test. The parameter estimation tool in WEST was used to achieve this.

4.4.1 Parameter estimation explanation

Parameter estimation uses measured variable values at each time step of a set of variables (selected by the user) and compares them to the corresponding variable values generated by the model as it varies a set of selected input parameter values (usually kinetic constants but in this investigation also the composition parameters of the BPO) until the error between the provided variable values and the generated variable values is at a minimum. The best model input parameters to predict the provided output variable values have been determined when the error between the provided and generated output variable values is at a minimum. The variable values refer to BMP test aqueous (e.g. COD, $H_2CO_3^*$ alkalinity, VFA, ortho-phosphate, FSA, pH, etc.) and gaseous (CH_4 and CO_2 volumes, pCO_2, etc.) characteristics, some of which are already measured in the BMP test and others are considered for inclusion in the BMP test in the next chapter. The parameter estimation tool is simply selected under the *Project* tab at the top of the WEST window. The parameter estimation settings can also be found in the *Project* tab by selecting the *Analysis* icon. These settings are discussed and calibrated in the next chapter.

4.4.2 Parameters selected for estimation

A set of parameters which require estimation could be formulated from the purpose of this research, which aims to determine the biodegradable fraction of a PO feed and the elemental composition and hydrolysis kinetic constants of the BPO. This same set would arise from inspection of the parameters which could only be estimated during the initial parameter specification in Section 4.2.2. Hence, the aims of this study are aligned with the requirements of modelling anaerobic digestion and BMP tests. This set of parameters which requires

estimation is provided in Table 4.2 and are separated into two simulations, one for the Control and one for the Test BMP tests.

Table 4.2: Set of parameters selected for estimation

Name	Description	Units	Reference
Inoculum only simulation:			
i_H_XBOrg_mol_perC	y/x of biodegradable particulate dead biomass	-	i_H_XBOrg
i_O_XBOrg_mol_perC	z/x of biodegradable particulate dead biomass	-	i_O_XBOrg
i_N_XBOrg_mol_perC	a/x of biodegradable particulate dead biomass	-	i_N_XBOrg
i_P_XBOrg_mol_perC	b/x of biodegradable particulate dead biomass	-	i_P_XBOrg
f_U_Org	Unbiodegradable particulate fraction of inoculum	-	f_U_Org
f_Biomass	Live organism fraction of biodegradable inoculum		f_Biomass
kM_BOrg_AD_hyd	Monod hydrolysis rate constant for biodegradable dead biomass	1/d	kM_BOrg
KS_BOrg_AD_hyd	Monod half saturation coefficient for biodegradable dead biomass	gCOD/gCOD	KS_BOrg
Inoculum and feed simulation:			
i_H_XBInf_mol_perC	y/x of biodegradable particulate organic feed	-	i_H_XBInf
i_O_XBInf_mol_perC	z/x of biodegradable particulate organic feed	-	i_O_XBInf
i_N_XBInf_mol_perC	a/x of biodegradable particulate organic feed	-	i_N_XBInf
i_P_XBInf_mol_perC	b/x of biodegradable particulate organic feed	-	i_P_XBInf
f_U_Inf	Unbiodegradable particulate fraction of organic feed VSS	-	f_U_Inf
kM_BInf_AD_hyd	Monod hydrolysis rate constant for biodegradable feed	1/d	kM_BInf
KS_BInf_AD_hyd	Monod half saturation coefficient for biodegradable feed	gCOD/gCOD	KS_BInf
* For simplicity the parameters are referred to by the abbreviated form provided in the Reference column.			

The first parameter estimation simulation determines the live biomass elemental composition, biodegradable endogenous residue hydrolysis kinetic constants and particulate inoculum fractionation as the inoculum only sample data is provided. The results from the first parameter estimation are then used in a second parameter estimation provided with the inoculum and feed sample data to determine the feed BPO elemental composition and hydrolysis kinetic constants, and the unbiodegradable fraction of the feed.

4.4.3 Objective function

As already described, the parameter estimation aims to minimize the error between the provided output variable values and the model generated variable values. This error can be calculated using a variety of different statistical methods, where the chosen defining equation is called the objective function. In this study the mean least squared objective function is minimized to fit the model generated variables to the provided variable values. The objective function K is given as

$$K = \frac{1}{N_v} \sum_{j=1}^{N_v} \left\{ \frac{1}{N_i} \sum_{i=1}^{N_i} \left| M_{ij} - G_{ij} \right|^2 \right\} \tag{4.10}$$

where j denotes each output variable and i is each time step at which the measured (M_{ij}) and corresponding model generated (G_{ij}) variable values are compared. N_v is the total number of output variables used in the parameter estimation and N_i is the total number of time steps at which variable values were measured.

4.4.4 Post-processing via BMP result generation using estimated parameters

The results of the parameter estimation include the value of each parameter which minimizes the objective function. The inoculum only test is modelled first and the results are used in the inoculum and feed test parameter estimation run. The parameter estimation determined parameters are also used without the parameter estimation tool to generate variable values which can be compared to the measured variable values to determine the accuracy of the estimated parameters. The layout of the project remains the same in WEST to generate variable values. The parameter estimation tool can simply be removed under the *Project* tab. Another option is to create a new project without the addition of the parameter estimation tool. A new project is preferable as the removal of the parameter estimation tool results in the loss of the parameter estimation analysis settings and parameter selection and therefore the new project provides an independent assessment.

4.5 Result accuracy assessment methods

The input parameters associated with the minimum objective function found by the parameter estimation simulation require accuracy assessment criteria. The criteria used in this investigation include the percentage error of each of the estimated parameter values and Theil's Inequality Coefficient (TIC) is calculated as an indication of the goodness-of-fit of the model generated variable values.

Percentage error: The percentage error (δ) calculation requires the actual parameter value to be known (P_{act}) and determines the relative error between P_{act} and the corresponding estimated parameter value (P_{est}) in terms of a percentage for each parameter via

$$\delta = \left| \frac{P_{act} - P_{est}}{P_{act}} \right| \times 100 \tag{4.11}$$

Theil's Inequality Coefficient (TIC): This goodness-of-fit indicator compares the measured and model generated variable values. In order to determine TIC the variable values associated with the estimated parameters need to be generated via a PWM_SA_AD_BMP model simulation with known input parameters and initial conditions. With M_{ij} as the measured variable values and G_{ij} as the corresponding generated variable values, for each time step i and variable j, TIC can be calculated from

$$TIC = \frac{\sqrt{\frac{1}{N_i}\sum_{i=1}^{N_i}(M_{ij} - G_{ij})^2}}{\sqrt{\frac{1}{N_i}\sum_{i=1}^{N_i}(M_{ij})^2} + \sqrt{\frac{1}{N_i}\sum_{i=1}^{N_i}(G_{ij})^2}} \tag{4.12}$$

where N_i is the total number of time steps at which variable values were measured. A value less than 0.3 for TIC indicates that the measured variable values are well represented by the model generated variable values (i.e. a good fit).

4.6 Closure

This chapter has described the procedures involved in creating a project environment layout in WEST as well as outlined the settings and data required for project simulation and parameter estimation simulation. This chapter can be used as a methodology and a reference for setting up a project in WEST for the PWM_SA_AD_BMP model. It also serves as an important basis for

the next two chapters which refer to *inter alia* the set of parameters chosen in this chapter to be estimated.

5 Project sensitivity and calibration

The sensitivity of the variable values, which could be measured at each time step of the BMP test, to the elemental composition and hydrolysis kinetics of the BPO feed and the unbiodegradable fraction of the POs reported in literature is the focus of the first part of this chapter. This sensitivity discussion aims to determine the best set of variable measurements that should be included in the BMP test procedure. The remainder of the chapter assesses the impact of (i) uncertainty in measured variable values, (ii) errors in initial parameter values, (iii) changes in statistical parameter estimation settings and (iv) reducing the number of provided variable values on the accuracy of the parameter estimation simulation. The response of the parameter estimation to these impacts is referred to as the model calibration, which aims to determine and define the optimal PWM_SA_AD_BMP model parameter estimation procedure. The procedure followed to conduct the calibration is included in the calibration methodology section of this chapter. The optimal parameter estimation procedure and settings are presented alongside the discussion of results, which serves as a framework for the methodology presented in Chapter 6 where the PWM_SA_AD_BMP model is applied to non-augmented BMP data.

5.1 Measurable variable selection from literature

The choice of variable value measurements to use within the parameter estimation is a critical part of this investigation as they must be sensitive and effective to the organic feed parameters (composition and hydrolysis kinetic constants of the BPOs and unbiodegradable fraction) required as well as simple enough to be included in the existing BMP test methodology (see Section 2.1). Common, measurable anaerobic digester liquor characteristics considered here include total alkalinity, $H_2CO_3^*$ alkalinity, VFA, total Kjeldahl nitrogen, FSA, organic N, total organic carbon, unfiltered and filtered COD, total phosphate, ortho-phosphate, organic P and volatile settleable solids concentrations and pH. Furthermore, measurements already taken in the BMP test can also contribute to the parameter estimation and include CH_4 and CO_2 accumulation from which their production rates and pCO_2 can be determined. The sensitivity discussion below is followed by an assessment of the complexity of the measurement procedures for each of the sensitive variables to determine the optimal set of variables for use in the parameter estimation calibration.

5.1.1 Sensitivity of measurable variables

The sensitivity of a variable output to a model parameter refers to the effect that a change in a model parameter causes in the output variable and *vice versa*. Variables which are sensitive to a particular parameter are those that vary by equal or greater percentages than the percentage change applied to the reference parameter.

The total (unfiltered plus gaseous) COD, C. H, O, N and P masses should not change during the BMP test if a 100% mass balance is achieved. With the PWM_SA_AD_BMP model based on a 100% mass balance, the total masses (e.g. total phosphate, TKN and total COD) will be insensitive over time, but these total masses split between gaseous, aqueous and solid phase changes as the test progresses. The increases and decreases of these different phase concentrations during the BMP test may enable the unbiodegradable organic fraction, composition and hydrolysis kinetics of the BPOs in the organic feed to be determined.

Harding (2009) and Ekama and Brouckaert (2015) describe how the elemental composition of an organic group (e.g. BPOs and UPOs) can be calculated from the mass ratios of the organic group: COD/VSS (f_{cv}), TOC/VSS (f_c), organic N/VSS (f_n) and organic P/VSS (f_p). Furthermore, the COD, TOC, organic N, organic P and VSS concentrations, measured for the calculation of the mass ratios, can be used to determine initial masses (batch system) or mass fluxes (flow through system) via: initial mass = concentration × volume and mass flux = concentration × flow rate, respectively. The difference in initial masses (or mass fluxes) of the total particulate organics (POs) and UPOs gives the initial masses (or mass fluxes) of the BPOs from which the mass ratios and elemental composition of the BPOs can be calculated. The requirements for this calculation, which characterizes the BPOs, include the initial unbiodegradable fraction of the VSS (f_U_Inf in the PWM_SA_AD_BMP model), the elemental composition of the UPOs and the initial masses (or influent mass fluxes) of the POs, calculated from the initial masses (or influent flow rate) and concentrations listed above. As these initial PO concentrations are used to calculate the mass ratios of BPOs and hence the BPO elemental composition, they are sensitive to the composition of the BPOs. This conclusion was also demonstrated by Botha (2012).

Botha (2012) discussed the sensitivity of aqueous increases in FSA, ortho-phosphate, $H_2CO_3^*$ alkalinity and CH_4 and CO_2 gas production rates and pCO_2 to variations in the mass ratios (f_{cv}, f_c, f_n and f_p), the unbiodegradable fraction of VSS (in terms of COD as f_{Sup}) and the Monod hydrolysis kinetic constants (K_M and K_S). Different fractions of the total masses of N

(FSA instead of organic N) and P (ortho-phosphate instead of organic P) were used in this study compared with the characterization calculation above, but as described, where one fraction increases another decreases as the total mass stays constant. Although a steady state model was used for this sensitivity analysis, the relationships between the parameters and variable outputs will be similar to a dynamic model. Also useful here is the inter-relationship between the different anaerobic digestion variables described in Section 3.3. Consequently, the results from the study provide a valuable guide for the selection of sensitive variables. The relevant results of the sensitivity analysis (Botha, 2012) include:

- The unbiodegradable fraction (f_{Sup} in terms of COD here but f_U_Inf in terms of VSS in PWM_SA_AD_BMP) was inversely proportional on a one-to-one percentage basis to the production rates of CH_4 (Fig. 5.1), CO_2, ortho-phosphate, FSA and $H_2CO_3^*$ alkalinity. The pCO_2 did not correlate as strongly.
- An increase in the Monod hydrolysis kinetic rate constant (K_M here but kM_BInf_AD_hyd in PWM_SA_AD_BMP) resulted in a steepening of the production rate curves for CH_4, CO_2, ortho-phosphate, FSA and $H_2CO_3^*$ alkalinity (Fig. 5.2). This can also be explained as an initial positive correlation followed by a negative correlation.
- An increase in the Monod half saturation coefficient (K_S here but KS_BInf_AD_hyd in PWM_SA_AD_BMP) produced the

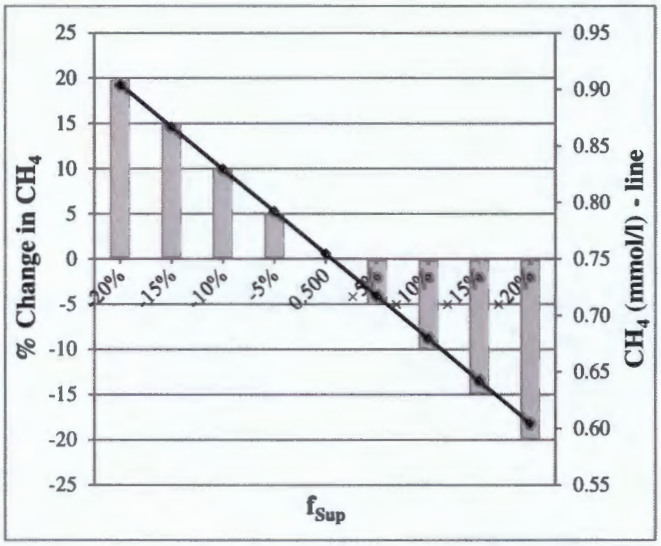

Figure 5.1: Negative correlation of f_{Sup} to CH_4 production rate at 1.833 days (Botha, 2012)

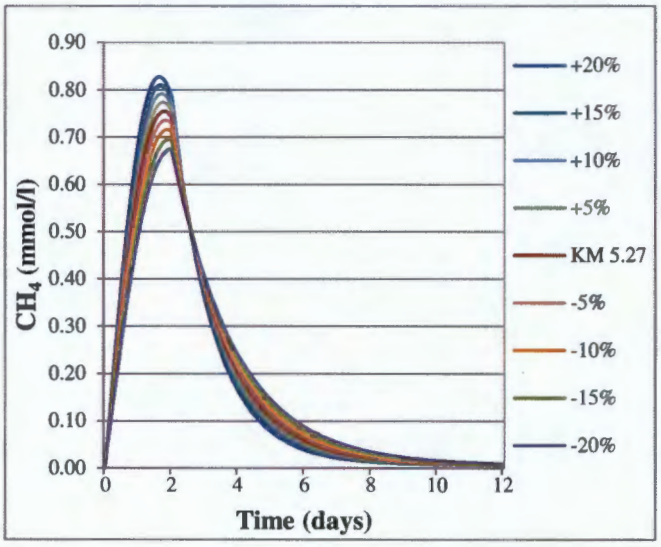

Figure 5.2: CH_4 production rate over time with variation of K_M (Botha, 2012)

opposite effects as K_M since the Monod hydrolysis kinetic rate is directly proportional to K_M and inversely proportional to K_S.

- The COD/VSS ratio of the biodegradable organics (f_{cv}) was directly proportional to the CH_4 production rate but inversely proportional to all the other outputs, with a particularly strong negative correlation to the pCO_2 (Fig. 5.3).
- The TOC/VSS ratio of the biodegradable organics (f_c) only affected the CO_2 production rate (Fig. 5.4) and pCO_2 with strong positive correlations for both, as would be expected.
- The N/VSS ratio of the biodegradable organics (f_n) displayed a negative correlation to the CO_2 production rate and pCO_2 as well as a strong positive correlation to FSA and $H_2CO_3^*$ alkalinity (Fig. 5.5).
- The P/VSS ratio of the biodegradable organics (f_p) only influenced the ortho-phosphate value significantly (Fig. 5.6), with minor positive and negative correlations with pCO_2 and $H_2CO_3^*$ alkalinity respectively.

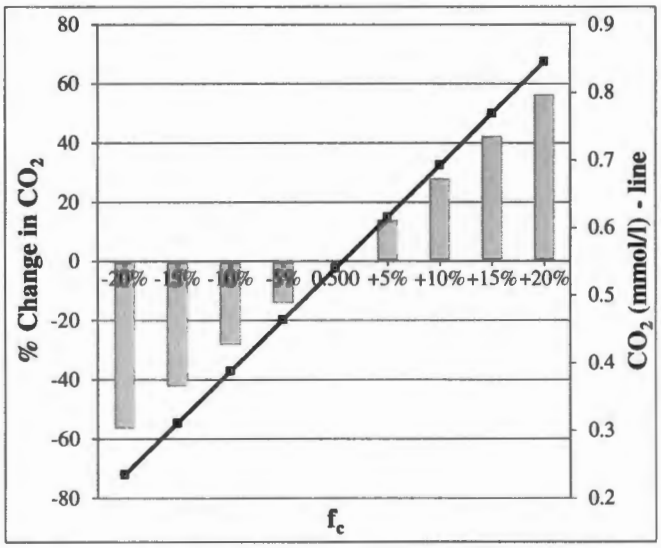

Figure 5.3: Positive correlation of f_c to CO_2 production rate at 1.75 days (Botha, 2012)

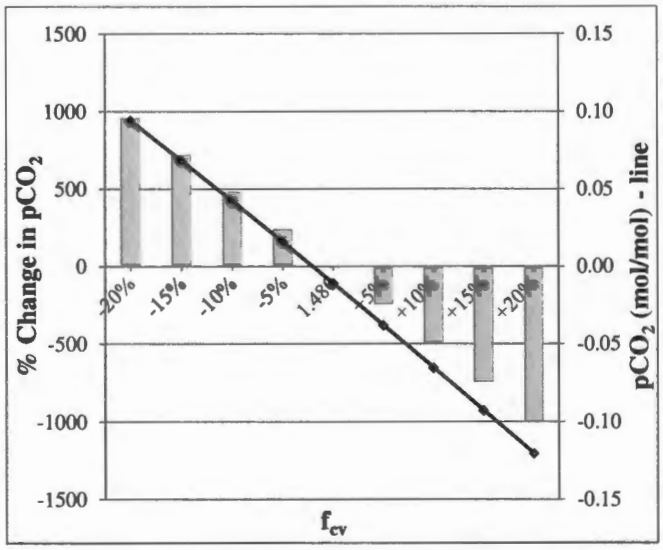

Figure 5.4: Strong negative correlation of f_{cv} to pCO_2 at 0.083 days (Botha, 2012)

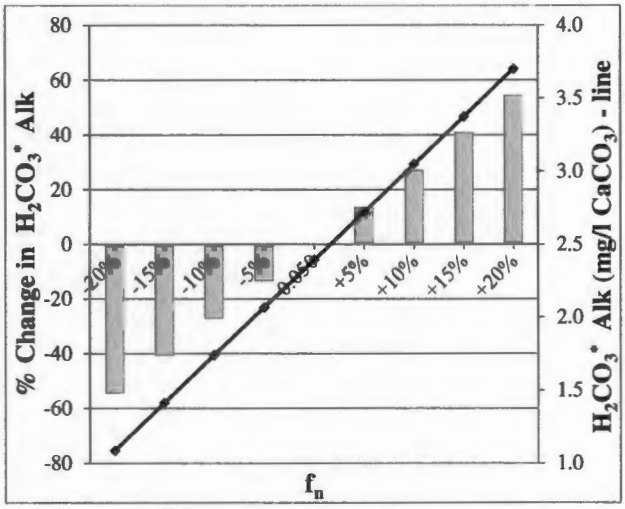

Figure 5.5: Positive correlation of f_n to $H_2CO_3^*$ alkalinity production rate at 1.75 days (Botha, 2012)

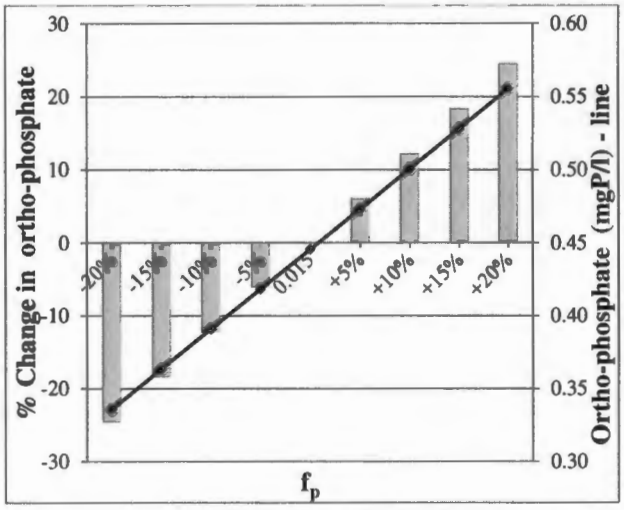

Figure 5.6: Positive correlation of f_p to ortho-phosphate production rate at 1.75 days (Botha, 2012)

The use of ortho-phosphate, FSA and CO_2 production rates as sensitive variables to the P, N and C content respectively of the organics is preferable to the use of organic P, N and C measurements because total organic carbon (TOC) is not a common measurement and organic P (or N) is obtained from the difference of two measurements: total P (or N) and ortho-phosphate (or FSA). Furthermore, Harding (2009) found that TOC measurements obtained from an external laboratory were too inconsistent and variable to use reliably.

The sensitivity analysis provided by Botha (2012) clearly indicates that the production rates of CH_4, CO_2, ortho-phosphate, FSA and $H_2CO_3^*$ alkalinity and the pCO_2 are sensitive to the composition (mass ratios) of the organics and should be tested in the PWM_SA_AD_BMP model parameter estimation for suitability of inclusion in the BMP test procedure. Further variables, such as the pH and VFA production rate which were not discussed by Botha (2012), should also be assessed. The VFA production rate can be expected to correlate well with the Monod hydrolysis kinetic constants (K_M and K_S). The pH, however, is linked to the $H_2CO_3^*$ alkalinity and the pCO_2, which in turn are dependent on the organic composition mass ratios and consequently may not add an extra independent output variable but rather a dependent one. Their inclusion during calibration of the PWM_SA_AD_BMP model parameter estimation procedure may provide further insight on their applicability. The outcome of this sensitivity analysis quantifies the sensitivity of the measurement parameters and is completely aligned with what one would expect from an evaluation of anaerobic digestion stoichiometry (see Section 3.3).

5.1.2 Practicality of measuring sensitive variables in the BMP test procedure

Many additional measurements have been selected as sensitive outputs for the determination of the BPO composition, kinetic constants and the unbiodegradable fraction of the PO, but are the measurement procedures involved practical to include in the simple, low cost BMP test? This section describes how the sensitive variables would commonly be measured and discusses their applicability to the goal. Besides the gas production rates and pCO_2, which are already measured in the BMP assay procedure, the following variables could also be measured:

pH: The pH of a sample can easily be measured in various ways including the affordable and rudimentary use of litmus paper and indicators which are compared to a colour scale as well as with the commonly used method of pH meter and probe.

VFA and $H_2CO_3^*$ alkalinity: The five-pH point titration method developed by Moosbrugger *et al.* (1993b) determines the concentrations of VFAs, total inorganic carbonate species (C_T) and $H_2CO_3^*$ alkalinity using straightforward titration procedure as presented in Section 2.2.3. The method is quick, cost efficient and reasonably accurate, but requires the concentrations of the total ammonium (FSA), phosphate (ortho-phosphate) and any other weak acid/base species (like sulphide) present.

COD: The method for measuring the COD of a solution involves the addition of potassium dichromate and sulphuric acid before boiling for two hours. A titration follows and the results are compared to the same test performed on distilled water. It only requires basic glassware and chemicals but can be time consuming to repeat often. The COD of the PO can be determined by subtracting the COD of a filtered sample (soluble COD) from the COD of an unfiltered sample.

FSA: This analysis involves a steam distillation after the pH of the sample has been raised by the addition of a strong base. The steam is condensed into boric acid and a pH indicator. Finally, a titration is performed using standardized sulphuric acid. Once again the method only involves basic glassware and chemicals but the time consuming process may be inconvenient and laborious if required on a daily basis. BMP assay measurements are commonly performed more frequently (at least daily) during the first week of the test as it is the most active period when the feed includes readily biodegradable organics (BSO) or BPO which hydrolyze within a few days.

Ortho-phosphate: A sample is mixed with ammonium molybdate and ammonium vanadate solutions so that the ortho-phosphate present in the sample can react with the

molybdates and vanadates, to form yellow ammonium phosphoric vanadomolybdate. The intensity of the yellow colour is proportional to the concentration of ortho-phosphate present in the sample up to 2000 mgP/l. A spectrophotometer is used to determine this colour intensity by measuring the amount of 380nm-wavelength light that is absorbed by the sample. The Beer-Lambert law is used to determine molar concentration of the ortho-phosphate from the absorbance. This method is generally known as the yellow method.

5.2 Model calibration methodology

This section provides a description of how parameter estimation is applied to the PWM_SA_AD_BMP model concept and includes assumptions, statistical parameter estimation methods and model accuracy calculations. The focus of this section is the methodology for testing the impact of parameter estimation configuration settings, initial parameter value accuracy and measured variable value uncertainty on the accuracy of the parameter estimation results.

5.2.1 PWM_SA_AD_BMP model parameter estimation requirements

In order to simulate the PWM_SA_AD_BMP model (i.e. generate variables values) or run the parameter estimation analysis (i.e. find best values of a set of parameters) the initial conditions in the BMP test are required. A list of the inoculum and organic feed characteristics which need to be measured for the calculation of these initial conditions as well as the procedure for these pre-processing calculations are provided in Chapter 4. This chapter of the study assumes that the requirements to run the parameter estimation analysis have been met in order to find an optimal parameter estimation procedure, whereas Chapter 6 includes the adapted parameter estimation methodology used for the non-augmented vinasse and whey BMP test data, respectively.

5.2.2 Control parameter estimation simulation

The calibration aims to assess the impacts of factors which influence the parameter estimation simulation accuracy; therefore a control simulation is required for comparison. Firstly, a set of provided variable values are generated by simulation to serve as the measured variable values

for the calibration tests and referred to as the provided variable values. Secondly, the provided variable values are used in the parameter estimation to determine the control simulation with which subsequent parameter estimation accuracies can be compared. Finally, Section 5.2.3 provides the methodology used for each calibration test.

5.2.2.1 Provided variable value simulation

This simulation assumes values for the input parameters and initial conditions required for the simulation of the PWM_SA_AD_BMP model. These input parameters were described and calculated in Section 4.2.1 from the non-augmented BMP test data provided by the University of Padova. The output variable values generated from this simulation represent the measured variable values referred to in Equations 4.10 – 4.12 and are referred to as the provided variable values in this chapter. Table 5.1 lists the input parameters which differ from the default values provided in Appendix A and initial conditions used for this control simulation.

Table 5.1: Input parameters and initial conditions for provided variable value simulation

Name	Description	Value	Units
Input parameters:			
V_liq	Initial volume of liquid in the reactor	0.0001	m^3
V_gas	Volume of the headspace in the reactor	0.0005	m^3
Initial masses in the digester:			
M[H2O]	Mass of water	100	g
M[S_H]	Mass of H^+ ions in the aqueous solution	0.000445	g
M[S_Ca]	Mass of calcium in the aqueous solution	0.00280	g
M[S_Mg]	Mass of magnesium in the aqueous solution	0.00053	g
M[S_CO3]	Mass of inorganic carbonate species as CO_3^{2-} in the aqueous solution	0.025008	g
M[X_AD]	Mass of live acidogens[a]	1E–6	g
M[G_CH4]	Mass of methane gas[b]	1E–6	g
M[G_CO2]	Mass of carbon dioxide gas[b]	1E–6	g
Influent concentrations for 1 minute of inflow:			
H2O	Water	0.576	m^3/d
S_H	H^+ ions in the aqueous solution	134.84	g/m^3
S_Na	Sodium in the aqueous solution	2804.90	g/m^3
S_K	Potassium in the aqueous solution	1525.59	g/m^3
S_Ca	Calcium in the aqueous solution	14.03	g/m^3
S_Mg	Magnesium in the aqueous solution	2.67	g/m^3

Name	Description	Value	Units
S_CO3	Inorganic carbonate species as CO_3^{2-} in the aqueous solution	69.12	g/m^3
S_PO4	Phosphate species as PO_4^{3-} in the aqueous solution	9499.92	g/m^3
X_U_Inf	Unbiodegradable particulate organic feed	6250.00	g/m^3
X_B_Org	Biodegradable particulate portion of decayed organisms	25000.00	g/m^3
X_AD	Acidogens	25000.00	g/m^3
X_AC	Acetogens	25000.00	g/m^3
X_AM	Acetoclastic methanogens	25000.00	g/m^3
X_HM	Hydrogenotrophic methanogens	25000.00	g/m^3
X_U_Org	Unbiodegradable particulate endogenous residue	25000.00	g/m^3
X_B_Inf	Biodegradable particulate organic feed	6250.00	g/m^3
X_ISS	Inorganic particulates	12826.60	g/m^3

[a] The equation for the hydrolysis kinetics of BPOs includes a division by the concentration of acidogens, thus an insignificantly small amount is provided as the initial value of acidogens in the digester because it cannot be zero.
[b] The equation for the partial pressure of carbon dioxide would divide by zero if these values were set to zero.

These initial conditions are the same as those used in the testing of the model using the vinasse and whey data and the method used for calculating them is provided in Chapter 6 as their specific values do not influence this parameter estimation procedure calibration chapter. A small amount (100 ml) of water is provided in the reactor initially with the constituents of the BMP test flowing into the reactor within the first minute of simulation. The BMP test was modelled in this way to allow the POs in the influent to be fractionated intrinsically by parameters within the model as explained in Section 3.5.2. Therefore, the values for the influent POs in Table 4.1 are those of the total inoculum (25000 g/m^3) and total PO feed (6250 g/m^3) which collects as 10 g and 2.5 g respectively in the 500 ml (400 ml influent and 100 ml initially in the reactor) total liquid volume of the BMP test.

No soluble organics are included in the initial conditions in order to reduce the number of parameters assumed in the PWM_SA_AD_BMP model. It is reasonable to assume that there are no soluble organics in either the inoculum or the organic feed if a granular sludge is used as inoculum, such as was used for the vinasse and whey BMP tests which are used in Chapter 6, and if the organic feed is particulate. For this particulate organic feed a homogenous BPO content was assumed so that all the BPOs in the organic feed hydrolyze at the same rate and can be described by a single elemental composition.

5.2.2.2 Control parameter estimation

The parameter estimation was run with the initial values of the parameters set to the actual assumed values and the provided variables values from the simulation above to determine whether the model is reversible, i.e. whether the parameter estimation simulation determines the same parameters from which the provided variable values were generated (Test 0). Table 5.2 lists the PWM_SA_AD_BMP model parameters that were selected to be estimated and PWM_SA_AD_BMP model variables that were provided for this control parameter estimation. The composition parameters of the BPO feed are represented using a C content of 1 as a base; i.e. in $C_xH_yO_zN_aP_b$ the x value has been set to 1 so that the composition is represented as $C_1H_{y/x}O_{z/x}N_{a/x}P_{b/x}$. The S content of the BPO feed was assumed to be negligible and was not included as a selected parameter in the BMP test modelling. Therefore, the S content (c/x or i_S_XBInf_mol_perC) remains set to the default value of zero (see Table 3.3).

Table 5.2: The 7 parameters and 9 variables for parameter estimation control

Parameters determined by parameter estimation (7):			
i_H_XBInf_mol_perC	y/x of biodegradable particulate influent/feed	-	i_H_XBInf
i_O_XBInf_mol_perC	z/x of biodegradable particulate influent/feed	-	i_O_XBInf
i_N_XBInf_mol_perC	a/x of biodegradable particulate influent/feed	-	i_N_XBInf
i_P_XBInf_mol_perC	b/x of biodegradable particulate influent/feed	-	i_P_XBInf
f_U_Inf	Unbiodegradable particulate fraction of organic feed VSS	-	f_U_Inf
kM_BInf_AD_hyd	Monod hydrolysis rate constant for X_B_Inf	1/d	kM_BInf
KS_BInf_AD_hyd	Monod half saturation coefficient for X_B_Inf	gCOD/gCOD	KS_BInf
Variables representing measurements used in parameter estimation (9):			
p_co2	Partial pressure of carbon dioxide	mol/mol	pCO_2
CH4_rate	Methane production rate	$g/m^3/d$	CH_4 rate
CO2_rate	Carbon dioxide production rate	$g/m^3/d$	CO_2 rate
H2CO3Alk	$H_2CO_3^*$ alkalinity concentration	g/m^3 as $CaCO_3$	$H_2CO_3^*$ alkalinity
VFA	Volatile fatty acid concentration	$gCOD/m^3$	VFA
FSA	Free and saline ammonia concentration	gN/m^3	FSA
OrthoP	Ortho-phosphate concentration	gP/m^3	Ortho-phosphate
COD_s	Soluble COD concentration	$gCOD/m^3$	Soluble COD
p_H_s	pH	-	pH
* For simplicity the parameters are referred to by the abbreviated form provided in the Reference column. The			

variables will be denoted by their commonly accepted abbreviation as already used throughout this dissertation.

The range within which each of the 7 parameters was allowed to vary was set to ±50% of the initial value and values of the 9 variables were provided daily for 4 days (i.e. 9×4). The objective function was set as described in Section 4.4.3 and the provided variable values were uploaded in the form of a Tornado encrypted text document as required by WEST. The parameter estimation was run and the estimated value of each parameter was compared to those assumed during the generation of the provided variable values using the percentage error (Eq. 4.11).

5.2.3 Procedure of calibration tests

The effects on the parameter estimation which were considered for investigation include the size of the range within which each of the 7 parameters is allowed to vary; the number of time steps (N_i) at which the variable values are provided; the number of parameters selected for estimation ($N_p \leq 7$) in comparison to the number of variables provided ($N_v \leq 9$); the accuracy of the initial value selected for each parameter and the uncertainty of the measured variable values. The procedures for determining the effect of changes in these factors are described in this section with the results of the investigation provided in Section 5.3.

5.2.3.1 Effect of initial parameter value error

In the control parameter estimation the initial parameter values were set equal to the assumed parameter values for the generation of the provided variable values (Test 0). However, this initial value would not be known when the model is used to find an unknown BPO feed composition. To test the impact of selecting an incorrect initial value for the parameters each initial parameter value was increased or decreased by the percentage value indicated in Table 5.3, with all other values kept the same as for the control parameter estimation (Tests 1-6).

Table 5.3: Initial parameter values for Tests 0-6

Test	Description	Initial parameter value (% change in brackets)						
0	Control	2.19	0.653	0.0643	0.0097	0.15	2.004	10.124
1	+5% in initial parameter values	2.2995 (+5%)	0.6857 (+5%)	0.0675 (+5%)	0.0102 (+5%)	0.1575 (+5%)	2.1042 (+5%)	10.6302 (+5%)
2	−5% in initial parameter values	2.0805 (−5%)	0.6204 (−5%)	0.0611 (−5%)	0.0092 (−5%)	0.1425 (−5%)	1.9038 (−5%)	9.6178 (−5%)
3	+10% in initial parameter values	2.4090 (+10%)	0.7183 (+10%)	0.0707 (+10%)	0.0107 (+10%)	0.1650 (+10%)	2.2044 (+10%)	11.1364 (+10%)
4	−10% in initial parameter values	1.9710 (−10%)	0.5877 (−10%)	0.0579 (−10%)	0.0087 (−10%)	0.1350 (−10%)	1.8036 (−10%)	9.1116 (−10%)
5	Initial parameter value changed by random value between ±5%	2.2776 (+4%)	0.6269 (−4%)	0.0675 (+5%)	0.0092 (−5%)	0.1425 (−5%)	1.9038 (−5%)	10.1240 (+0%)
6	Initial parameter value changed by random value between ±10%	2.2119 (+1%)	0.6008 (−8%)	0.0675 (+5%)	0.0105 (+8%)	0.1605 (+7%)	1.9038 (−5%)	9.7190 (−4%)

5.2.3.2 Effect of uncertainty in measured variable values

The measurements of the sensitive variables, as described in Section 5.1.2, can only be as accurate as the rating of the device or procedure used. Therefore, some experimental error may occur. The effect of this uncertainty in the measured variable values was investigated by applying a scattering of up to 5 and 10% respectively (Test 7, 8a & 8b). This was done by generating a random value between −5 and +5 (or −10 and +10) and applying that random value as a percentage increase or decrease to each value of the provided generated variable values. The parameter estimation was performed with this error applied to the provided variable values to determine the impact on the accuracy of the parameters estimated. All other values remained the same as for the control parameter estimation for comparison. Table 5.4 summarizes these tests.

Table 5.4: Tests 0, 7, 8a and 8b for variable value uncertainty

Test	Description
0	Control: no change to provided variable values
7	Provided variable values changed by random value between ±5%
8a	Provided variable values changed by random value between ±10%
8b	Provided variable values changed by random value between ±10%

Tests 8a and 8b were named in this way because their procedures were identical: a scattering of the provided variable values of a randomly generated value between −10 and +10. The only difference was the randomly generated percentage scatter values were regenerated for each test to check whether a particular random scattering was significantly better than another.

5.2.3.3 Effect of parameter range size

The size of the range within which each parameter was allowed to vary was initially set equal to the size of the parameter in the control by calculating an increase in each parameter value of 50% for the upper limit of the range and a decrease in each parameter value of 50% for the lower limit of the range. The range was decreased to ±25% (Test 9) and all the other values were kept the same as for Test 7 (Table 5.4). Test 7 was used for comparison as the reduction in parameter range size was expected to improve the accuracy of the parameter estimation; therefore a test with considerable error was required for comparison. Table 5.5 summarizes these tests.

Table 5.5: Parameter range sizes for Tests 7 and 9

Test	Description	Parameter range
7	Provided variable values changed by random value between ±5% with parameter range of ±50% of the initial value	±50%
9	Provided variable values changed by random value between ±5% with parameter range of ±25% of the initial value	±25%

5.2.3.4 Effect of number of time steps of provided variables

An increase in the number of time steps (N_i) at which the variable values were provided was expected to improve the accuracy of the estimated parameters. To test this hypothesis the variable values were provided daily for the longer period of 8 days for comparison with the

control parameter estimation setting of 4 days. This test (Test 10) was compared with Test 9 (Table 5.5) with only the number of time steps differing. Table 5.6 summarizes these tests.

Table 5.6: Number of time steps (N_i) of provided variable values for Tests 9 and 10

9	4 days of provided variable values changed by random value between ±5% with parameter range of ±25% of the initial value	4 days
10	8 days of provided variable values changed by random value between ±5% with parameter range of ±25% of the initial value	8 days

5.2.3.5 Effect of number of parameters and number of variables selected

The number of parameters selected for estimation (N_p) in comparison to the number of variables provided (N_v) was investigated to determine which variables influence the accuracy of the parameter estimation the most and if fewer variables can be used to determine the parameter values with reasonable accuracy (Tests 11-21). Test 6 (Table 5.3) was used for comparison because the use of accurate initial parameter values with the provided variable values in the parameter estimation results in the first simulation generated an insignificantly small objective function value ($K = 10^{-13.318}$); therefore any changes to the parameter estimation settings will not influence the accuracy of the parameter estimation as the correct solution was provided for the first simulation. The other effects have been assessed with uncertainty applied to the provided variable values to prevent this, but a random scatter of the provided variables values could have interfered with this test where the impact of excluding or including each variable in the parameter estimation was investigated. Table 5.7 indicates which variables of the set of nine variables used in the tests thus far and listed in Table 5.2, were excluded from each of Tests 11-18.

Table 5.7: Variables excluded from Tests 6, and 11-21

Test	Description	Excluded variable
6	Initial parameter value changed by random value between ±10%	None
11	Initial parameter value changed by random value between ±10% with pH excluded	pH
12	Initial parameter value changed by random value between ±10% with soluble COD excluded	Soluble COD
13	Initial parameter value changed by random value between ±10% with $H_2CO_3^*$ alkalinity excluded	$H_2CO_3^*$ alkalinity
14	Initial parameter value changed by random value between ±10% with pH and $H_2CO_3^*$ alkalinity excluded	pH, $H_2CO_3^*$ alkalinity
15	Initial parameter value changed by random value between ±10% with VFA excluded	VFA
16	Initial parameter value changed by random value between ±10% with pH, $H_2CO_3^*$ alkalinity and VFA excluded	pH, $H_2CO_3^*$ alkalinity, VFA
17	Initial parameter value changed by random value between ±10% with FSA excluded	FSA
18	Initial parameter value changed by random value between ±10% with ortho-phosphate excluded	Ortho-phosphate
19	Initial parameter value changed by random value between ±10% with pCO_2 excluded	pCO_2
20	Initial parameter value changed by random value between ±10% with CH_4 rate excluded	CH_4 rate
21	Initial parameter value changed by random value between ±10% with CO_2 rate excluded	CO_2 rate

5.3 Model calibration results and discussion

The results from the calibration tests described above have been summarized in Table 5.8 on the next page, which provides the percentage error of each parameter for each test as well as an average percentage error across the seven parameters. The subsections below discuss the results of each of the effects investigated in order to determine the optimum parameter estimation settings as well as the uncertainty associated with the parameter estimation procedure in the PWM_SA_AD_BMP model which is presented in the closure (Section 5.4).

5.3.1 Control parameter estimation

The results from the control parameter estimation show that the PWM_SA_AD_BMP model is reversible as the parameters were estimated exactly (Test 0). These results, however, require comparison with the results of the calibration tests in order to conclude on the ability of the PWM_SA_AD_BMP model to predict the selected parameters (Table 5.2) as the exact initial parameter values and 100% accurate measured variables values will not be available like for the control parameter estimation.

5.3.2 Effect of initial parameter value error

The results of Tests 1-6 (Table 5.8) show that an error in the initial parameter value does not significantly impact the accuracy of the results. The largest error in the estimated parameters in these six tests was the 0.0311% error of i_N_XBInf in Test 6. This is an important conclusion because the parameters which are being estimated have been selected in the parameter estimation because their values are not known and an estimated initial value in error of up to 10% has been shown here to have an insignificant effect on the accuracy of the estimated parameters.

Table 5.8: Calibration results as percentage errors in estimated parameters

Test	Description	Estimated parameter value (% error in round brackets) [K in square brackets]							
		KS_BInf	kM_BInf	f_U_Inf	i_P_XBInf	i_N_XBInf	i_O_XBInf	i_H_XBInf	Average
0	Control	10.12400 (0)	2.00400 (0)	0.15000 (0)	0.00970 (0)	0.06430 (0)	0.65300 (0)	2.19000 (0)	(0) [1E–53]
1	+5% in initial parameter values	10.12400 (0)	2.00400 (0)	0.15000 (0)	0.00970 (0)	0.06430 (0)	0.65302 (0.00306)	2.19000 (0)	(0.00044) [2E–12]
2	–5% in initial parameter values	10.12400 (0)	2.00401 (0.00050)	0.15000 (0)	0.00970 (0)	0.06430 (0)	0.65300 (0)	2.19001 (0.00046)	(0.00014) [3E–13]
3	+10% in initial parameter values	10.12400 (0)	2.00404 (0.00200)	0.15002 (0.01333)	0.00970 (0)	0.06431 (0.01555)	0.65301 (0.00153)	2.19000 (0)	(0.00463) [6.8E–4]
4	–10% decrease in initial parameter values	10.12413 (0.00128)	2.00406 (0.00299)	0.15000 (0)	0.00970 (0)	0.06430 (0)	0.65304 (0.00613)	2.19000 (0)	(0.00149) [4.9E–4]
5	Initial parameter value changed by random value between ±5%	10.12401 (0.00010)	2.00402 (0.00100)	0.15001 (0.00667)	0.00970 (0)	0.06431 (0.01555)	0.65303 (0.00459)	2.19000 (0)	(0.00399) [2.4E–8]
6	Initial parameter value changed by random value between ±10%	10.12405 (0.00049)	2.00407 (0.00349)	0.15000 (0)	0.00970 (0)	0.06432 (0.03110)	0.65302 (0.00306)	2.19001 (0.00046)	(0.00552) [3.9E–6]
7	Provided variable values changed by random value between ±5% with parameter range of ±50% of the initial value	13.10591 (29.4539)	2.50101 (24.8009)	0.14021 (6.5267)	0.00801 (17.4227)	0.08825 (37.2473)	0.78912 (20.8453)	1.95263 (10.8388)	(21.0194) [3.188]
8a	Provided variable values changed by random value between ±10%	8.01524 (20.8293)	1.80560 (9.9002)	0.19815 (32.1000)	0.00772 (20.4124)	0.09447 (46.9207)	0.51002 (21.8959)	1.78812 (18.3507)	(24.3442) [3.772]
8b	Provided variable values changed by random value between ±10%	15.01506 (48.3115)	2.30776 (15.1577)	0.17223 (14.8200)	0.01198 (23.5052)	0.08958 (39.3157)	0.71222 (9.0689)	1.46562 (33.0767)	(26.1794) [4.640]
9	4 days of provided variable values changed by random value between ±5% with parameter range of ±25% of the initial value	10.34681 (2.2008)	2.07160 (3.3733)	0.14786 (1.4267)	0.00935 (3.6082)	0.07084 (10.1711)	0.67433 (3.2665)	2.28019 (4.1183)	(4.0235) [0.324]
10	8 days of provided variable values changed by random value between ±5% with parameter range of ±25% of the initial value	12.19124 (20.4192)	2.24100 (11.8263)	0.13801 (7.9933)	0.00916 (5.5670)	0.07443 (15.7543)	0.66213 (1.3982)	2.37616 (8.5005)	(10.2084) [1.590]

Test	Description	Estimated parameter value (% error in round brackets) [K in square brackets]							
		KS_BInf	kM_BInf	f_U_Inf	i_P_XBInf	i_N_XBInf	i_O_XBInf	i_H_XBInf	Average
11	Initial parameter value changed by random value between ±10% with pH excluded	10.14513 (0.2087)	2.01019 (0.3089)	0.14881 (0.7933)	0.00968 (0.2062)	0.06452 (0.341)	0.65430 (0.1991)	2.19120 (0.0548)	(0.3019) [0.052]
12	Initial parameter value changed by random value between ±10% with soluble COD excluded	11.00140 (8.6665)	2.22204 (10.8802)	0.17244 (14.9600)	0.00994 (2.4742)	0.07833 (21.8196)	0.55943 (14.3292)	2.00105 (8.6279)	(11.6797) [1.095]
13	Initial parameter value changed by random value between ±10% with $H_2CO_3^*$ alkalinity excluded	10.08414 (0.3937)	2.01154 (0.3762)	0.14984 (0.1067)	0.00971 (0.1031)	0.06501 (1.1042)	0.65143 (0.2404)	2.18889 (0.0507)	(0.3393) [0.102]
14	Initial parameter value changed by random value between ±10% with pH and $H_2CO_3^*$ alkalinity excluded	10.16511 (0.4061)	2.01441 (0.5195)	0.15518 (3.4533)	0.00981 (1.1340)	0.06454 (0.3733)	0.65558 (0.3951)	2.18750 (0.1142)	(0.9136) [0.981]
15	Initial parameter value changed by random value between ±10% with VFA excluded	10.20608 (0.107)	2.01128 (0.3633)	0.15152 (1.0133)	0.0098 (1.030)	0.06407 (0.3577)	0.65173 (0.1945)	2.18611 (0.176)	(0.5640) [0.766]
16	Initial parameter value changed by random value between ±10% with pH, $H_2CO_3^*$ alkalinity and VFA	10.24705 (1.2154)	2.00915 (0.270)	0.14686 (2.0933)	0.00979 (0.9278)	0.0658 (2.3328)	0.64788 (0.7841)	2.18472 (0.2411)	(1.1217) [0.835]
17	Initial parameter value changed by random value between ±10% with FSA excluded	9.92473 (1.9683)	1.92587 (3.8986)	0.14032 (6.4507)	0.00975 (0.4845)	0.08075 (25.5832)	0.68637 (5.1103)	2.11902 (3.2410)	(6.6767) [1.019]
18	Initial parameter value changed by random value between ±10% with ortho-phosphate excluded	9.78605 (3.3381)	1.98327 (1.0345)	0.13838 (7.7493)	0.01071 (10.4124)	0.06115 (4.8989)	0.70409 (7.8239)	2.05332 (6.2409)	(5.9283) [1.180]
19	Initial parameter value changed by random value between ±10% with pCO_2 excluded	9.64538 (4.7276)	1.89090 (5.6437)	0.15978 (6.5187)	0.00962 (0.827)	0.06949 (8.0715)	0.62141 (4.8377)	1.98763 (9.2408)	(5.6950) [1.437]
20	Initial parameter value changed by random value between ±10% with CH_4 rate excluded	11.48714 (13.4644)	2.19383 (9.4727)	0.14428 (3.8125)	0.00982 (1.2371)	0.06811 (5.9238)	0.59873 (8.3109)	1.99780 (8.7763)	(7.2854) [2.120]
21	Initial parameter value changed by random value between ±10% with CO_2 rate excluded	10.92889 (7.9504)	2.14710 (7.1408)	0.13878 (7.4771)	0.00991 (2.1649)	0.06673 (3.7760)	0.69605 (6.5926)	2.10797 (3.7455)	(5.5496) [1.218]

5.3.3 Effect of uncertainty in measured variable values

The influence of experimental error on the accuracy of the estimated parameters was investigated in Tests 7, 8a and 8b. In Test 7, a random number between −5 and +5 was generated and applied as a percentage increase or decrease to each provided variable value to represent the uncertainty associated with experimental results. The results of Test 7 were very poor with an average error in the estimated parameters of 21.0194%. As described above, Tests 8a and 8b were named in this way because their procedures were identical: a scattering of the provided variable values of a randomly generated value between −10 and +10. The only difference was the randomly generated percentage scatter values were regenerated for each test to check whether a particular random scattering was significantly better than another. The results of Test 8a and 8b from Table 4.8 indicate that an increase in the scatter of the provided variable values from ±5% in Test 7 to ±10% in Test 8a and 8b only increased the average percentage error of the estimated parameters slightly to 24.3442% and 26.1794% respectively. The similarity between the results of Test 8a and 8b indicates that the scattering of the provided variable values was successfully random because one random scattering is not significantly better than the next random scattering.

The significant errors produced in the estimated parameter values in these calibration tests inspired the assessment of the next two calibration tests in the hope that a reduction in the parameter range size and an increase in the number of time steps at which variable values were provided would reduce the uncertainty in the estimated parameter values.

5.3.4 Effect of parameter range size

The size of the range within which each parameter was allowed to vary played a significant role in the accuracy of the estimated parameters. In Test 7 the range size was kept the same as for the control parameter estimation at ±50% of each parameter initial value. This test was accompanied by a scattering of ±5% of the provided variables values and estimated parameter values with a very large average error of 21.0194%. When the parameter range size was reduced to ±25% in Test 9, with all other test conditions identical to that of Test 7, the average error in the estimated parameters was reduced to 4.0235%. Therefore, with this smaller range of ±25% of each parameter's initial value, the PWM_SA_AD_BMP model parameter estimation has an approximately one-to-one proportionality between the uncertainty in the provided variable values and the uncertainty in the estimated parameters. This influence of the

parameter range size indicates that if an estimate for a parameter's initial values can be provided within a small confidence interval (i.e. with low uncertainty), the size of the range within which the parameter would be estimated could be narrowed down and produce results of equivalent accuracy to that of the measured variable values.

5.3.5 Effect of number of time steps of provided variables

The number of time steps at which the variable values were provided was increased from daily for 4 days in Test 9 to daily for 8 days in Test 10 to determine whether the accuracy of the estimated parameters would be influenced. As described above, the estimated parameters from Test 9 had the small average error of 4.0235%. With double the number of time steps of variable values provided, but all other values the same as for Test 9, the Test 10 results did not improve as expected and the average error in the estimated parameters increased to 10.2084%. Therefore, fewer and more accurate variable values are best for the estimation of the selected set of parameters. This is because for these particular BMP tests the feed biodegradable organics were virtually completely (99.999 %) utilized so the data from day 4 to 8 do not add any value from a kinetics point of view.

5.3.6 Effect of number of parameters and number of variables selected

The effects of removing provided variables from the parameter estimation were investigated to determine the significance of each variable on the selected set of parameters. Test 6 (average error of 0.00552%) was used for comparison in these tests as a scattering of the provided variable values would detract from the influence which each variable has on the accuracy of the estimated parameters. Therefore, the initial parameter values were increased or decreased by the same random percentage between −10% and +10% as that applied to the initial parameter values in Test 6 (see Table 5.3). These changes in initial parameter values has already been shown to have an insignificant effect on the accuracy of the estimated parameters, therefore any inaccuracy in the estimated parameter values from these calibration tests (Tests 11-21) can be associated with the exclusion of the provided variable(s) described in Table 5.7. From the results provided in Table 5.8 for Tests 11-21 the following conclusions were drawn:

- The pH, $H_2CO_3^*$ alkalinity and VFA values had little influence on the estimation of the parameter values. When these three variables were individually excluded from the

parameter estimation the average error in the estimated parameter values was only 0.3019%, 0.3393% and 0.5640% respectively. Test 16 excluded all three variables, but the accuracy of each of the estimated parameters was still above 97.5% with an average error across the set of parameters of only 1.1217%.

- The soluble COD values made a significant contribution to the accuracy of the estimated parameter values which can be seen from the 11.6797% average error in the estimated parameters for Test 12, for which the soluble COD was excluded from the variables for the parameter estimation.
- The remaining variables (FSA, ortho-phosphate, pCO_2, CH_4 rate and CO_2 rate) also made a significant contribution to the accuracy of the estimated parameter values, but did not influence the accuracy of the estimated parameters as much as the soluble COD. The average errors for the individual exclusion of FSA, ortho-phosphate, pCO_2, CH_4 rate and CO_2 rate were 6.6767%, 5.9283%, 5.6950%, 7.2854% and 5.5496% respectively.
- The exclusion of FSA and ortho-phosphate respectively also had a significant influence on the accuracy of the N and P contents respectively of the BPO feed (i_N_XBInf and i_P_XBInf). By removing the FSA variable from the parameter estimation in Test 17 the value of i_N_XBInf was over-estimated by 25.5832%. Similarly, excluding the ortho-phosphate variable in Test 18 resulted in an error in i_P_XBInf of 10.4124%.

5.4 Closure

This chapter selected nine variable values to use in the PWM_SA_AD_BMP model parameter estimation calibration tests. The calibration tests aimed to determine the optimal methodology to use for the parameter estimation. The variables were selected from literature for their sensitivity to the set of parameters and from the simplicity of their measurement procedures. The set of 7 parameters include the elemental composition values of the BPOs in the organic feed (i_H_XBInf, i_O_XBInf, i_N_XBInf, i_P_XBInf), the unbiodegradable fraction of the organic feed (f_U_Inf) and the Monod hydrolysis kinetic constants of the BPOs in the organic feed (kM_BInf and KS_BInf). The nine variables include the aqueous measurements of pH, $H_2CO_3^*$ alkalinity, ortho-phosphate, FSA, VFA and soluble COD concentrations as well as the gaseous measurements of the CO_2 and CH_4 gas production rates and pCO_2.

An estimate for the initial parameter values was found to be sufficient as an error of 10% in the initial parameter value only produced an average error of 0.0311% in the estimated parameter values. However, if this initial parameter value can be estimated within a narrow confidence interval so that the size of the range in which the parameter is allowed to vary can be reduced (from ±50% to ±25% of the initial parameter value) then the accuracy of the estimated parameter values, from measured variable values with an uncertainty of up to ±5%, can be improved (from an average error of 21.0194% to 4.0235%).

An increase in the number of time steps (from 4 to 8 days, daily) at which variable values were provided decreased the accuracy of the results (from an average error of 4.0235% to 10.2084%). The factor which had the largest impact on the accuracy of the estimated parameter values was the uncertainty in the variables values. An uncertainty of up to ±5% resulted in an average error in the estimated parameters of 21.0194% and an uncertainty of up to ±10% resulted in average errors of 24.3442% and 26.1794%. As mentioned above, a reduction in the size of the range in which the parameters are allowed to vary can reduce these errors considerably.

Of the nine variables, the ortho-phosphate, FSA and soluble COD values were highlighted as critical measurements because FSA and ortho-phosphate significantly influenced the accuracy of the N and P content of the BPO feed (i_N_XBInf and i_P_XBInf) respectively, while soluble COD had a significant influence on the average accuracy across all the parameters. Unfortunately the ortho-phosphate, FSA and soluble COD are the three least practical measurements to include in the BMP test due to their relatively time consuming protocols.

Finally, a set of six variables, which includes ortho-phosphate, FSA, soluble COD, CO_2 rate, CH_4 rate and pCO_2, was shown to be sufficient for estimating the set of seven parameters (i_H_XBInf, i_O_XBInf, i_N_XBInf, i_P_XBInf, f_U_Inf, kM_BInf and KS_BInf) assuming the variable values are 100% accurate. As the accuracy of the estimated parameters decreases with an increase in experimental error the use of the full set of nine sensitive variables in the PWM_SA_AD_BMP model parameter estimation is recommended.

6 Project testing with non-augmented BMP test data

Chapter 5 developed the optimum parameter estimation protocol to estimate the set of seven parameters (i_H_XBInf, i_O_XBInf, i_N_XBInf, i_P_XBInf, f_U_Inf, kM_BInf and KS_BInf), which describe the BPOs of the organic feed, as accurately as possible. This chapter aims to apply the developed protocol to measured variable values as opposed to the model generated variable values used in Chapter 5 for the model calibration tests. However, the lack of comprehensive experimental data (due to UCT water quality laboratory renovations) only allowed for limited model testing to be done. The limitations required numerous assumptions to be made which are described and addressed throughout the data pre-processing and parameter estimation methodology sections of this chapter. A future study with suitable experimental data could reduce the number of assumptions to determine the organic feed unbiodegradable fraction, feed BPO elemental composition and hydrolysis kinetic constants with better certainty. This chapter provides the steps that were used and results obtained from the testing of two organic sample data sets, vinasse and whey, for which non-augmented BMP test data was received from the University of Padova.

Since the required variable values, which were selected in Chapter 5, were not measured during the BMP tests, the parameter estimation procedure differs from the ideal parameter estimation protocol created in Chapter 4. However, the mass ratios of the POs of the respective organic feeds (vinasse and whey) were measured and can be used to calculate the composition of the POs of each feed (Harding, 2009). If the feed UPO mass fractions are known (e.g. measured after a long batch test so that all the BPOs have been degraded), the BPO mass ratios can be calculated from the mass flux differences between the POs and the UPOs as described in Section 4.2.2.3. Unfortunately the UPO mass ratios were not measured, therefore this chapter uses the measured PO mass ratios with assumed UPO mass ratios to calculate the BPO mass ratios and hence the BPO elemental composition. Using these compositions with the measured variable values allows for the kinetic constants to be calibrated to the organic feed. With the calibrated model the BPO elemental composition parameters are estimated using the parameter estimation and compared to those calculated from the PO mass ratios. The details of this process is clearly explained in this chapter.

6.1 Data background and pre-processing

This section provides a brief description of the feeds used in the BMP tests conducted by the University of Padova as well as the details of the data collection process.

6.1.1 Vinasse

Vinasse is a by-product of the sugar manufacturing process. Once the sugar crystals have been produced from sugarcane or sugar beet, the remaining molasses is fermented to produce ethanol, ascorbic acid and other products. The remaining material after the removal of the alcohol or acid is known as vinasse. The consistency of vinasse is similar to that of molasses, but the colour and total solids concentrations depend on the source. Cane-vinasse has a light brown hue and a total solids content of 2 - 4%, whereas beet-vinasse is black-red in colour with a solids concentration of up to 10% (Primary Information Services, India).

Due to the high biodegradable organic fraction (mostly carbohydrates) and moderate nutrient content (N and P) of vinasse it cannot directly be disposed. As a result, vinasse is commonly used for co-digestion in anaerobic digesters with low methane potential wastes to increase the methane production. The combination of N and P nutrients and high potassium levels in vinasse also allows for it to be used as a fertilizer.

6.1.2 Cheese whey

Whey, also known as milk serum, is the liquid left behind after milk has been curdled to form cheese and other dairy products. This by-product of cheese manufacturing is commonly used as a form of protein enrichment in processed foods. In liquid form, whey consists mostly of water and lactose. When dried, however, the whey powder can have up to 90% protein by weight.

6.1.3 Experimental procedure

The data used for the calibration of the PWM_SA_AD_BMP model was supplied by the University of Padova. Triplicate BMP assays were conducted during January and February of 2012 for each substrate: vinasse and cheese whey. For the blank test containing only inoculum, 10 grams of granular sludge was dissolved in tap water and digested in a 1 litre bottle where 500 ml of headspace was provided above the 500 ml total liquid volume. The substrate samples

included an extra 2.5 gVSS of vinasse (or whey), setting the inoculum to substrate ratio at 4:1. To ensure that the pH of the trials would stay within a neutral range for the duration of the experimental procedure (up to 60 days) a 200 ml buffer solution of potassium diphosphate (KH_2PO_4 of 10.62 g/l) and disodium phosphate (Na_2HPO_4 of 17.32 g/l) was included in the total liquid volume of 500 ml. The temperature was kept constant at 35°C and the bottles were routinely stirred. Measurements of the accumulation of CH_4 and biogas (CH_4 + CO_2) were initially taken daily, but this decreased to every second day after day five and the measurement frequency continued to decrease after day ten as the gas production rate also decreased. The vinasse tests were conducted for 64 days during which 15 data points were measured and for whey 13 data points over 34 days. The following initial characteristics of the two substrates were also measured: TSS, VSS, COD, TOC, TKN, FSA and total phosphate.

6.1.4 Model initial conditions

The initial conditions in the BMP test sample and the pre-processing of this data for inclusion in the PWM_SA_AD_BMP model is the same as that described in Chapter 4. The 500 ml liquid volume of the sample was spilt between a 100 ml initial water mass in the anaerobic digester and a 400 ml influent flow containing just inoculum for the first parameter estimation and both inoculum and feed for the second BMP test. The influent flow is used to allow for the intrinsic parameter fractionation of the particulate organics, which, as explained in Section 3, makes it possible to estimate the unbiodegradable fraction of the organic feed within the parameter estimation of the model.

6.1.5 Pre-processing of measured variable values for use in the model

The vinasse and whey BMP tests only measured standard BMP test variable values, thus the set of variables, different from the ideal set of 9 determined in Chapter 5, require definition. A BMP test measures the biogas and methane production over the period of the test in normalized millilitres. The CO_2 production can be calculated from the difference between the biogas and methane production. The PWM_SA_AD_BMP model provides two variables, NmlAccCH4 and NmlAccCO2, for the CH_4 and CO_2 productions, respectively, in normalized millilitres. The gas productions are used to calculate the gas production rates (Eqn. 6.1 and 6.2) and the CO_2 partial pressure (Eqn.6.3), which is used together with the NmlAccCH4 and NmlAccCO2 in

the parameter estimations. So a standard BMP test provides only three measured variables with time.

$$CH4\_rate_{\frac{i+(i-1)}{2}} = \frac{\left(CH_{4_i} - CH_{4_{i-1}}\right)}{\left(t_i - t_{i-1}\right)} \frac{MW_{CH_4}}{(0.5l)(24.0552)} \tag{6.1}$$

$$CO2\_rate_{\frac{i+(i-1)}{2}} = \frac{\left(CO_{2_i} - CO_{2_{i-1}}\right)}{\left(t_i - t_{i-1}\right)} \frac{MW_{CO_2}}{(0.5l)(24.0552)} \tag{6.2}$$

$$p\_co2_i = \frac{CO_{2_i}}{\left(CH_{4_i} + CO_{2_i}\right)} \tag{6.3}$$

6.2 Methodology

This section describes the procedures followed in testing the PWM_SA_AD_BMP model parameter estimation with the vinasse and whey data respectively. It should be noted that the data provided only included biogas and methane accumulation over time. Attempting a model calibration using only gas data is far from ideal as gas measurements are renowned for imprecision (wide scatter and inaccuracy (deviation from true value). Thus, the calibration is rather an attempt to show that the model can approximately reproduce BMP test results. See Chapter 5 for the measurements that are required to determine the elemental composition and kinetic rate constants of the BPOs of the feed as well as the unbiodegradable fraction of the particulate organics to a level of reasonable accuracy.

6.2.1 Calibration of inoculum hydrolysis kinetic constants and composition parameters

This is the first parameter estimation step of the testing procedure. It aims to estimate the composition of the live organisms, where all the groups of organisms (acidogens, acetogens, acetoclastic methanogens and hydrogenotrophic methanogens) have been assumed to have the same composition. This composition was also linked to the BPOs formed when the live biomass groups undergo endogenous respiration (X_B_Org) and calculated as

X_B_Org = (1 – f_U_Org) x (1 – f_Biomass) x (inoculum POs of 10 grams)

where f_U_Org is the fraction of the inoculum POs that is unbiodegradable and f_Biomass is the fraction of the inoculum BPOs that is live organisms, leaving the remaining fraction to be the X_B_Org. The linking of the compositions implies that as the composition of the live biomass is varied during the parameter estimation, so the X_B_Org composition also varies.
An estimate of f_U_Org was calculated by dividing the remaining COD, after removal of the ultimate CH_4 COD produced during the Control test, by the total COD of the sample (see Section 4.2.2.2). This was used as an initial value for the parameter estimation to narrow the range of the search.

With the organism group sub-fractions determined and an initial value of f_U_Org and f_Biomass estimated (in Section 4.2.2.2), the parameter estimation could be run. The parameters that were to be estimated included the Monod hydrolysis kinetic constants of the dead biodegradable organisms (kM_BOrg and KS_BOrg) and the composition parameters of the biodegradable inoculum (i_H_XBOrg, i_O_XBOrg, i_N_XBOrg and i_P_XBOrg), f_Biomass and f_U_Org. Table 4.2 provides definitions of each of these parameters. The provided variable values included the CH_4 and CO_2 production rates and their accumulated values as well as the pCO_2 of the inoculum only BMP test for vinasse and whey, respectively.

6.2.2 Calibration of feed BPO hydrolysis kinetic constants

The estimated parameters from the previous step were then used in another parameter estimation, where the same variables were provided as for the previous parameter estimation. The variable values, however, were now the average outputs from the triplicate inoculum and feed BMP test samples. The intention of this parameter estimation simulation was to determine the Monod hydrolysis kinetic constants of the feed BPOs (kM_BInf and KS_BInf) and the unbiodegradable fraction of the feed POs (f_U_Inf). The composition of the BPOs was calculated from the measured PO mass ratios, an assumed composition of the UPOs and an approximate f_U_Inf, determined in the same way that the f_U_Org value was calculated from the remaining COD.

6.2.3 Assessment of calibration

The success of the calibration of the PWM_SA_AD_BMP model can then be determined by comparing the model generated variable values, when all the calibrated parameters are used, to the variable values provided in the parameter estimation, which were determined from the

measured BMP test data. The accuracy of the time-series results were compared using a single and double standard error margin with the standard error (e_s) calculated as

$$e_s = \sum_{i=1}^{N_i} \frac{G_i - M_i}{N_i}$$

where G_i denotes the model generated variable values, M_i the measured variable values determined from the BMP test data and N_i the total number of data points. Of the measured data points, at least 68% should fall within ± one standard error of the modelled data and 95% within ± two standard errors of the modelled data for the model to be deemed reasonably accurate.

A further accuracy assessment was conducted using a third parameter estimation to determine the compositional parameters of the feed BPOs (i_H_XBInf, i_O_XBInf, i_N_XBInf and i_P_XBInf), which were previously calculated from the PO mass ratios and an assumed UPO composition. Since the model was calibrated to the vinasse and whey data, respectively, the model should be able to determine the feed BPOs composition accurately when provided with the calibrated parameters. The same variable values were provided as for the second parameter estimation (Section 6.2.2 above). The results were then compared to the calculated composition values by the percentage error function (Eqn. 4.11). This was done in order to allow for result comparison with the results shown at the end of Chapter 5, where it was concluded that augmented BMP test measurements are required to determine the feed BPO composition accurately from BMP tests.

6.3 Results and discussion

The results of the PWM_SA_AD_BMP model calibration to the vinasse and whey data can be analyzed from Figures 6.1 – 6.4. Each figure indicates the modelled results as a solid line with the measured data as points. Dotted lines indicate the single and double standard error ranges. The results did not meet the required standards of accuracy defined as 68% of the measured data falling within one standard error and 95% within two standard errors of the modelled data. These standard error results are provided in Table 6.1. The most successfully modelled output was that of the accumulated CH_4 production for the vinasse feed, where 92,5 % of the data fell within two standard errors.

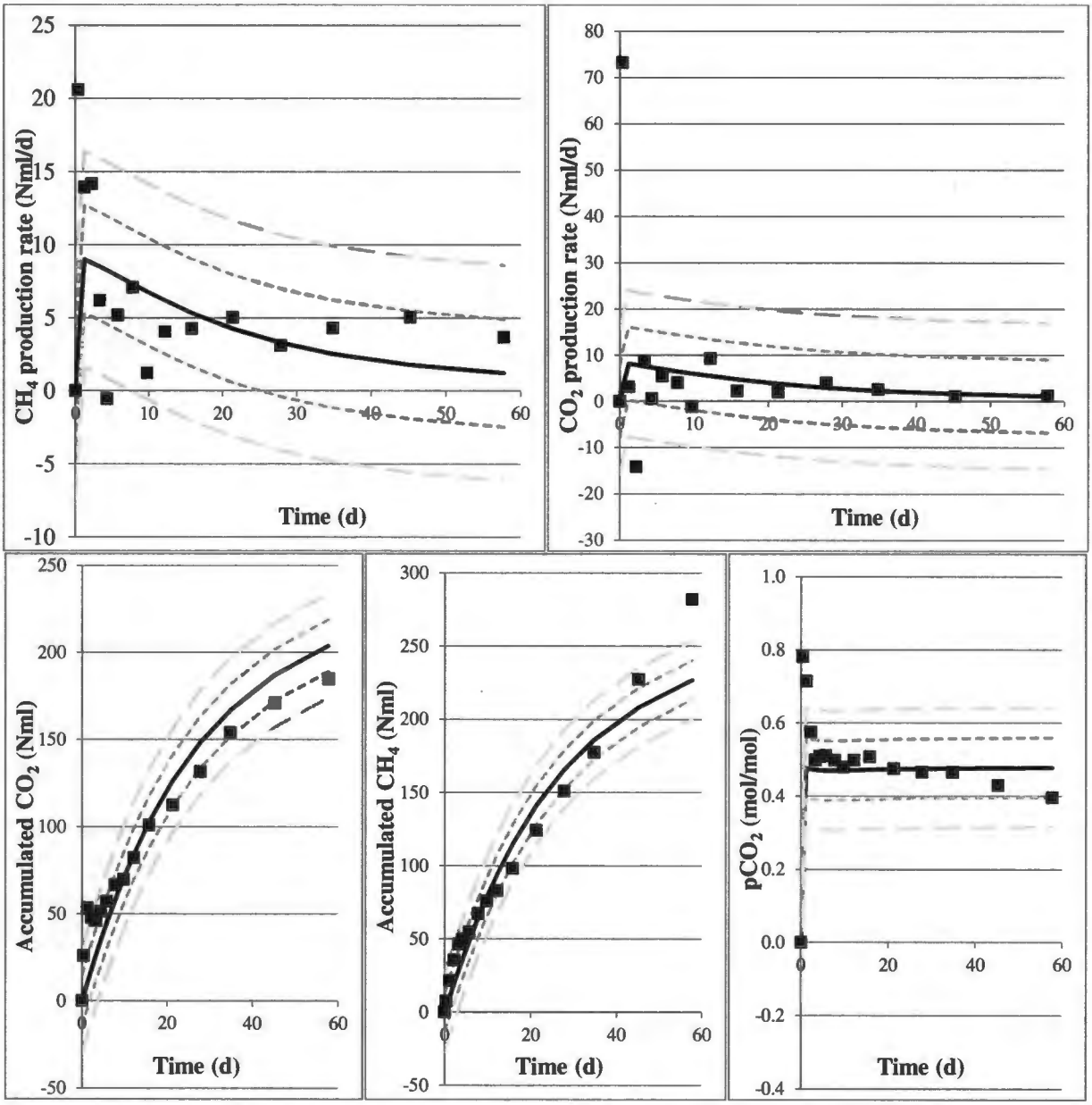

Figure 6.1: Measured (▪) and simulated (—) variable values for control test of the vinasse BMP test, with single (- - -) and double (— — —) standard error ranges

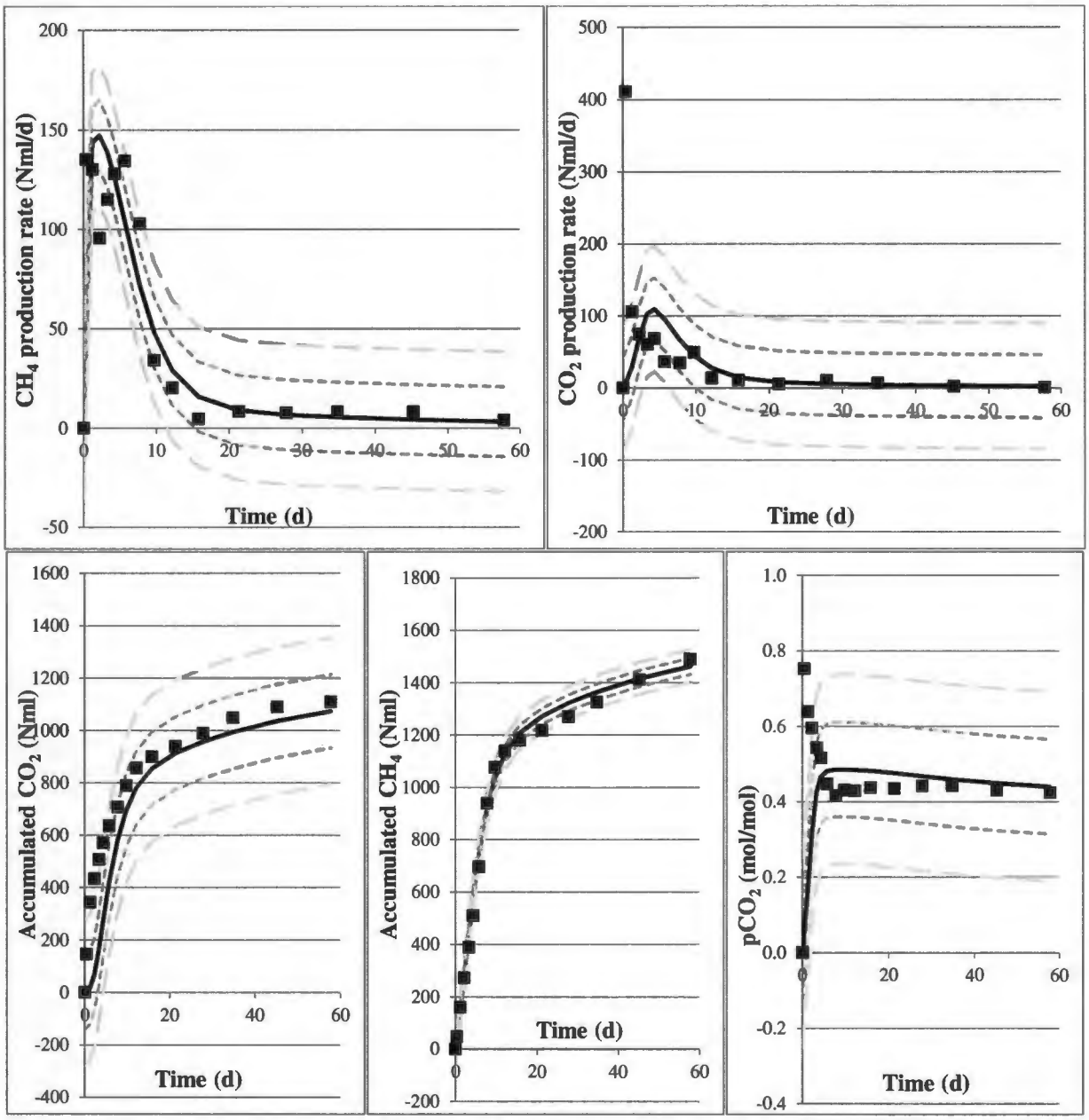

Figure 6.2: Measured (▪) and simulated (—) variable values for vinasse BMP test, with single (- - -) and double (— — —) standard error ranges

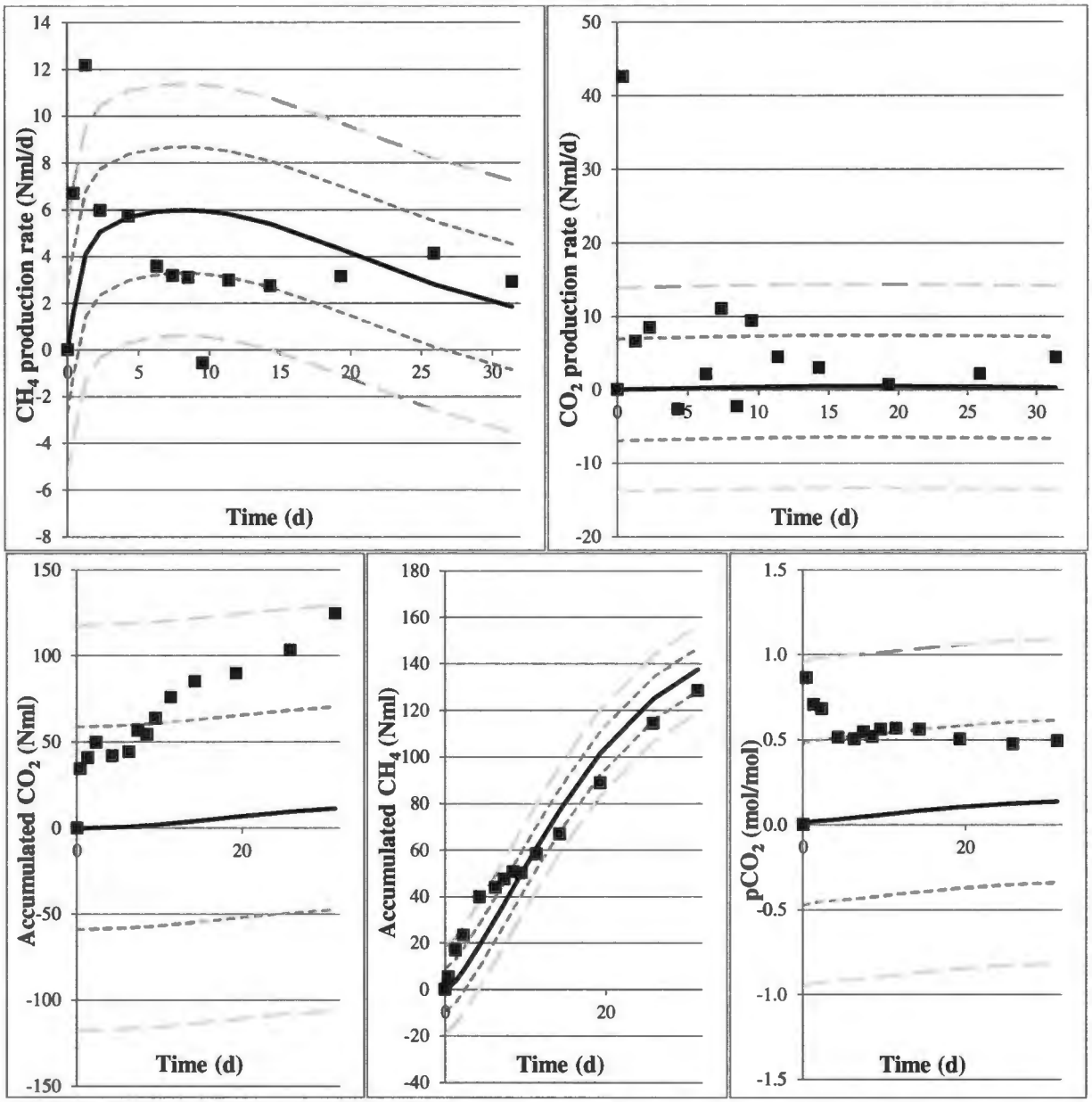

Figure 6.3: Measured (▪) and simulated (—) variable values for control (inoculum only) test of whey BMP test, with single (- - -) and double (— — —) standard error ranges

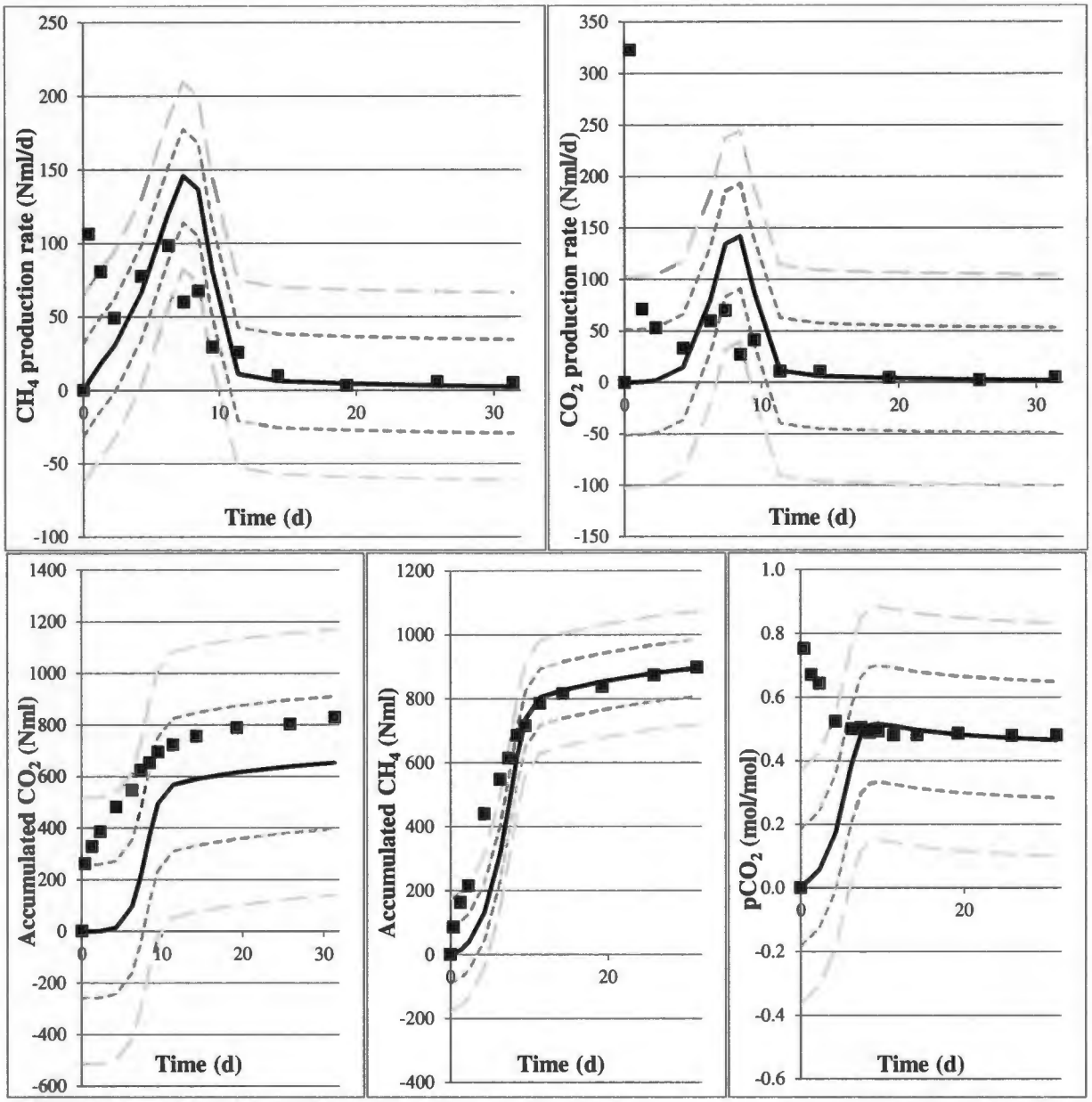

Figure 6.4: Measured (▪) and simulated (—) variable values for whey BMP test, with single (- - -) and double (— — —) standard error ranges

Table 6.1: Accuracy of BMP test calibration per variable value for vinasse and whey

	$AccCH_4$	$AccCO_2$	CH_4 rate	CO_2 rate	pCO_2
Inoculum of Vinasse					
% within double standard error	93.75	87.50	87.50	87.50	87.50
% within single standard error	56.25	50.00	68.75	87.50	75.00
Vinasse					
% within double standard error	93.75	87.50	87.50	87.50	87.50
% within single standard error	56.25	50.00	68.75	87.50	75.00
Inoculum of Whey					
% within double standard error	93.75	100.00	87.50	93.75	100.00
% within single standard error	50.00	62.50	62.50	75.00	50.00
Whey					
% within double standard error	87.50	100.00	81.25	87.50	81.25
% within single standard error	68.75	56.25	68.75	75.00	75.00

The second form of accuracy assessment included the prediction of the feed BPO composition parameters using the calibrated kinetic constants, biomass composition and fractionation parameters. The calibrated values as well as the predicted composition constants are given in Table 6.2 and indicate that the N and P composition cannot accurately be determined from only the gas production data provided by a non-augmented BMP test. The H and O compositions were estimated reasonably accurately with only a 1.92 and 2.93 percentage error, respectively, for vinasse and 2.12% and 1.31%, respectively, for whey.

Table 6.2: Calculated, calibrated and predicted values for the vinasse and whey BMP tests

	Vinasse				Whey			
	Calculated	Calibrated	Predicted	% error	Calculated	Calibrated	Predicted	% error
i_H_XBOrg	-	1.2709	-	-	-	1.2889	-	-
i_O_XBOrg	-	0.5094	-	-	-	0.4299	-	-
i_N_XBOrg	-	0.3224	-	-	-	0.2233	-	-
i_P_XBOrg	-	0.0447	-	-	-	0.0208	-	-
kM_BOrg	-	16.559	-	-	-	12.252	-	-
KS_BOrg	-	0.574	-	-	-	1.661	-	-
f_Biomass	-	0.212	-	-	-	0.044	-	-
f_U_Org	-	0.888	-	-	-	0.966	-	-
kM_BInf	-	18.741	-	-	-	1.791	-	-
KS_BInf	-	5.451	-	-	-	4.000	-	-
f_U_Inf	-	0.141	-	-	-	0.0468	-	-
i_H_XBInf	0.9459	-	0.9274	1.96	2.1944	-	2.2411	2.12
i_O_XBInf	0.4883	-	0.4740	2.93	1.1651	-	1.1498	1.31
i_N_XBInf	0.0237	-	0.0346	46.34	0.0307	-	0.0389	26.72
i_P_XBInf	0.00466	-	0.00413	11.43	0.00643	-	0.00529	17.69

6.4 Closure

This chapter has shown that the non-augmented measurements from BMP tests are insufficient for determining all the objective parameters with reasonable accuracy. However, the accumulated CH_4 and biogas data can provide a good estimate of the H and O content (to within 2.12% and 2.93%, respectively) of the BPOs of an organic feed. These results are based on an adjusted modelling procedure, which uses the feed PO mass ratios (measured for the vinasse and whey data) and an assumed feed UPO composition to calculate the feed BPO composition. The feed BPO composition was then used to calibrate the feed BPO hydrolysis kinetic rate constants and the unbiodegradable fraction of the feed POs. This calibration aided the prediction of the feed BPO composition parameters.

7 Conclusions and recommendations

Once the PWM_SA_AD model was adjusted to accommodate a batch test with intrinsic parameter fractionation of the influent and the set of parameters requiring estimation was formulated from the purpose of this research, the set of provided variable values and parameter estimation settings were optimized in the calibration chapter (Section 4). The parameter set included *inter alia* the objective parameters, namely: the unbiodegradable fraction of a PO feed (f_U_Inf), the elemental composition (i_H_XBInf, i_O_XBInf, i_N_XBInf and i_P_XBInf) and Monod hydrolysis kinetic constants (kM_BInf and KS_BInf) of the BPO feed. The methodology required two parameter estimation simulations to determine these parameters. The first parameter estimation simulation determined the live biomass elemental composition (i_H_XBOrg, i_O_XBOrg, i_N_XBOrg and i_P_XBOrg), biodegradable endogenous residue Monod hydrolysis kinetic constants (kM_BOrg and KS_BOrg) and particulate inoculum fractionation (f_U_Org and f_Biomass) parameters when the inoculum only sample data served as the provided variable values. The results from the first parameter estimation were then used in a second parameter estimation which used provided variable values from the inoculum and feed sample data to determine the objective parameters.

The calibration concluded that the critical measurements, which were used as provided variable values and were required for the determination of the objective parameters, included the CH_4 and CO_2 production rates, ortho-phosphate, FSA, soluble COD and pCO_2. However, the accuracy of the objective parameters were highly influenced by the accuracy of the provided variable values, therefore it was recommended that the full set of nine sensitive variables should be used in the PWM_SA_AD_BMP model parameter estimation. This set of nine variable values include the six mentioned above and the pH, $H_2CO_3^*$ alkalinity and VFA.

Similar calibration tests were performed using sulphidogenic bioprocesses instead of the methanogenic bioprocesses which take place in the BMP test. This biological sulphate reduction batch test (called BSP) produces hydrogen sulphide (HS^-) from sulphate (sulphidogenesis) instead of methane from CO_2 (methanogenesis). The BSP test has the benefit of an aqueous H_2S/HS^- and SO_4^{2-} concentration measurement which can be measured with better accuracy than a gaseous methane and CO_2 volume in the BMP test. Therefore, the pCO_2 and CH_4 and CO_2 production rates in the provided variable values were replaced with SO_4^{2-} reduction and H_2S/HS^- production rate. The BSP modelling produced the same accuracy levels as the BMP calibration tests.

The testing of the PWM_SA_AD_BMP model with the vinasse and whey BMP test data provided by the University of Padova produced parameter estimations of reasonable accuracy considering that the calibration used only CH_4 and biogas accumulation data. The testing also reinforced the requirement of additional measurements in the BMP assay protocol to determine the composition constants, as only the H and O elemental compositions were accurately determined from the gas data.

Finally, the objective of creating a dynamic BMP model was achieved, but the level of success with which the PWM_SA_AD_BMP model can determine the objective parameters defining the composition of the biodegradable organics remains to be proven with actual augmented BMP test data. Using augmented BSP test measurements is preferable as the aqueous concentrations measured in the BSP test can be measured with better accuracy than gas production rates of the BMP test. In addition, the PWM_SA_AD_BMP model already allows for the modelling of the BSP test.

It is recommended that the BSP test proposed in this study be conducted on a selection of substrates and compared with BMP tests of the same substrates to confirm the hypothesized improvement in accuracy of a substrate's biodegradable fraction and specific methane potential. Furthermore, these tests should include the nine variable measurements proposed above in order to test the accuracy of the PWM_SA_AD_BMP model to its full potential.

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9 Appendix A: PWM_SA_AD and PWM_SA_AD_BMP model details

Table 9.1: PWM_SA_AD and PWM_SA_AD_BMP model parameters containing repetitive stoichiometry

Parameter	Units	Description
Stoi_H2O_decay	g/m^3	MW_H2O*(f_XU_Bio_lysis*(i_O_Org_mol_perC – 3 – 4*i_P_Org_mol_perC – 4*i_S_Org_mol_perC + (3 – i_O_XUOrg_mol_perC + 4*i_P_XUOrg_mol_perC + 4*i_S_XUOrg_mol_perC)*gam_o/gam_e) + (1 – f_XU_Bio_lysis)*(i_O_Org_mol_perC – 3 – 4*i_P_Org_mol_perC – 4*i_S_Org_mol_perC + (3 – i_O_XBOrg_mol_perC + 4*i_P_XBOrg_mol_perC + 4*i_S_XBOrg_mol_perC)*gam_o/gam_bp))
Stoi_H_decay	g/m^3	MW_S_H*(f_XU_Bio_lysis*(3*i_P_Org_mol_perC + 2*i_S_Org_mol_perC + 2 – i_N_Org_mol_perC + (i_N_XUOrg_mol_perC – 3*i_P_XUOrg_mol_perC – 2*i_S_XUOrg_mol_perC – 2)*gam_o/gam_e) + (1 – f_XU_Bio_lysis)*(3*i_P_Org_mol_perC + 2*i_S_Org_mol_perC + 2 – i_N_Org_mol_perC + (i_N_XBOrg_mol_perC – 3*i_P_XBOrg_mol_perC – 2*i_S_XBOrg_mol_perC – 2)*gam_o/gam_bp))
Stoi_CO3_decay	g/m^3	MW_S_CO3*(f_XU_Bio_lysis*(1 – gam_o/gam_e) + (1 – f_XU_Bio_lysis)*(1 – gam_o/gam_bp))
Stoi_NH4_decay	g/m^3	MW_S_NH*(f_XU_Bio_lysis*(i_N_Org_mol_perC – gam_o/gam_e* i_N_XUOrg_mol_perC) + (1 – f_XU_Bio_lysis)*(i_N_Org_mol_perC – gam_o/gam_bp*i_N_XBOrg_mol_perC))
Stoi_PO4_decay	g/m^3	MW_S_PO4*(f_XU_Bio_lysis*(i_P_Org_mol_perC – gam_o/gam_e*i_P_XUOrg_mol_perC) + (1 – f_XU_Bio_lysis)*(i_P_Org_mol_perC – gam_o/gam_bp* i_P_XBOrg_mol_perC))
Stoi_SO4_decay	g/m^3	MW_S_SO4*(f_XU_Bio_lysis*(i_S_Org_mol_perC – gam_o/gam_e*i_S_XUOrg_mol_perC) + (1 – f_XU_Bio_lysis)*(i_S_Org_mol_perC – gam_o/gam_bp* i_S_XBOrg_mol_perC))
Stoi_ER_decay	g/m^3	MW_X_U_Org*f_XU_Bio_lysis*gam_o/gam_e
Stoi_s_H2O_decay	g/m^3	MW_H2O*(f_XU_Bio_lysis_s*(i_O_Org_mol_perC – 3 – 4*i_P_Org_mol_perC – 4*i_S_Org_mol_perC + (3 – i_O_XUOrg_mol_perC + 4*i_P_XUOrg_mol_perC + 4*i_S_XUOrg_mol_perC)*gam_o/gam_e) + (1 – f_XU_Bio_lysis_s)*(i_O_Org_mol_perC – 3 – 4*i_P_Org_mol_perC – 4*i_S_Org_mol_perC + (3 – i_O_XBOrg_mol_perC + 4*i_P_XBOrg_mol_perC + 4*i_S_XBOrg_mol_perC)*gam_o/gam_bp))
Stoi_s_H_decay	g/m^3	MW_S_H*(f_XU_Bio_lysis_s*(3*i_P_Org_mol_perC + 2*i_S_Org_mol_perC + 2 – i_N_Org_mol_perC + (i_N_XUOrg_mol_perC – 3*i_P_XUOrg_mol_perC – 2*i_S_XUOrg_mol_perC – 2)*gam_o/gam_e) + (1 – f_XU_Bio_lysis_s)*(3*i_P_Org_mol_perC + 2*i_S_Org_mol_perC + 2 – i_N_Org_mol_perC

Parameter	Units	Description
		+ (i_N_XBOrg_mol_perC – 3*i_P_XBOrg_mol_perC – 2*i_S_XBOrg_mol_perC – 2)*gam_o/gam_bp))
Stoi_s_CO3_decay	g/m^3	MW_S_CO3*(f_XU_Bio_lysis_s*(1 – gam_o/gam_e) + (1 – f_XU_Bio_lysis_s)*(1 – gam_o/gam_bp))
Stoi_s_NH4_decay	g/m^3	MW_S_NH*(f_XU_Bio_lysis_s*(i_N_Org_mol_perC – gam_o/gam_e* i_N_XUOrg_mol_perC) + (1 – f_XU_Bio_lysis_s)*(i_N_Org_mol_perC – gam_o/gam_bp*i_N_XBOrg_mol_perC))
Stoi_s_PO4_decay	g/m^3	MW_S_PO4*(f_XU_Bio_lysis_s*(i_P_Org_mol_perC – gam_o/gam_e*i_P_XUOrg_mol_perC) + (1 – f_XU_Bio_lysis_s)*(i_P_Org_mol_perC – gam_o/gam_bp* i_P_XBOrg_mol_perC))
Stoi_s_SO4_decay	g/m^3	MW_S_SO4*(f_XU_Bio_lysis_s*(i_S_Org_mol_perC – gam_o/gam_e*i_S_XUOrg_mol_perC) + (1 – f_XU_Bio_lysis_s)*(i_S_Org_mol_perC – gam_o/gam_bp* i_S_XBOrg_mol_perC))
Stoi_s_ER_decay	g/m^3	MW_X_U_Org*f_XU_Bio_lysis_s*gam_o/gam_e

Table 9.2: PWM_SA_AD and PWM_SA_AD_BMP model stoichiometry

Hydrolysis of FSO	
Component	**Stoic. Equation**
H2O	MW_H2O * (i_O_SF_mol_perC – 3 – 4 * i_P_SF_mol_perC – 4 * i_S_SF_mol_perC + gam_f / 2)
S_H	MW_S_H * (3 * i_P_SF_mol_perC + 2 * i_S_SF_mol_perC + 2 – i_N_SF_mol_perC – gam_f / 2)
S_NH	MW_S_NH * i_N_SF_mol_perC
S_CO3	MW_S_CO3 * (1 – gam_f / 4)
S_PO4	MW_S_PO4 * i_P_SF_mol_perC
S_SO4	MW_S_SO4 * i_S_SF_mol_perC
S_F	– MW_S_F
S_Glu	MW_S_Glu * gam_f / 24
Process Rate	kH_F_AD_hyd * S_F / MW_S_F

Hydrolysis of BPO	
Component	**Stoic. Equation**
H2O	MW_H2O * (i_O_XBOrg_mol_perC – 3 – 4 * i_P_XBOrg_mol_perC – 4 * i_S_XBOrg_mol_perC + gam_bp / 2)
S_H	MW_S_H * (3 * i_P_XBOrg_mol_perC + 2 * i_S_XBOrg_mol_perC + 2 – i_N_XBOrg_mol_perC – gam_bp / 2)
S_NH	MW_S_NH * i_N_XBOrg_mol_perC
S_CO3	MW_S_CO3 * (1 – gam_bp / 4)
S_PO4	MW_S_PO4 * i_P_XBOrg_mol_perC
S_SO4	MW_S_SO4 * i_S_XBOrg_mol_perC
S_Glu	MW_S_Glu * gam_bp / 24
X_B_Org	– MW_X_B_Org
Process Rate	kM_BOrg_AD_hyd * (COD_X_B_Org / COD_X_AD) / (KS_BOrg_AD_hyd + (COD_X_B_Org / COD_X_AD)) * X_AD / MW_X_AD * gam_o/ gam_bp

Lysis of OHOs in AD	
Component	**Stoic. Equation**
H2O	Stoi_H2O_decay
S_H	Stoi_H_decay
S_NH	Stoi_NH4_decay
S_CO3	Stoi_CO3_decay
S_PO4	Stoi_PO4_decay
S_SO4	Stoi_SO4_decay
X_B_Org	MW_X_B_Org * (1 – f_XU_Bio_lysis) * gam_o / gam_bp
X_OHO	– MW_X_OHO
X_U_Org	Stoi_ER_decay
Process Rate	b_OHO_AD * X_OHO / MW_X_OHO

Lysis of PAOs in AD	
Component	**Stoic. Equation**
H2O	Stoi_H2O_decay
S_H	Stoi_H_decay
S_NH	Stoi_NH4_decay
S_CO3	Stoi_CO3_decay
S_PO4	Stoi_PO4_decay
S_SO4	Stoi_SO4_decay
X_B_Org	MW_X_B_Org * (1 – f_XU_Bio_lysis) * gam_o / gam_bp
X_PAO	– MW_X_PAO
X_U_Org	Stoi_ER_decay
Process Rate	b_PAO_AD * X_PAO / MW_X_PAO

PP Release in AD	
Component	**Stoic. Equation**
H2O	(4/9 – Y_f_PP_VFA) * MW_H2O
S_H	– (5/9 – 2*Y_f_PP_VFA) * MW_S_H
S_K	Y_f_PP_VFA * i_K_PP_mol_perP * MW_S_K
S_Ca	Y_f_PP_VFA * i_Ca_PP_mol_perP * MW_S_Ca
S_Mg	Y_f_PP_VFA * i_Mg_PP_mol_perP * MW_S_Mg
S_VFA	– MW_S_VFA
S_CO3	2 * MW_S_CO3 / 9
S_PO4	Y_f_PP_VFA * MW_S_PO4
X_PAO_PP	– Y_f_PP_VFA * MW_X_PAO_PP
X_PAO_Stor	4 * MW_X_PAO_Stor / 9
Process Rate	kM_fPP_PAO_PHAstor * X_PAO_PP / MW_X_PAO_PP

Hydrolysis of PP in AD	
Component	**Stoic. Equation**
H2O	– MW_H2O
S_H	2 * MW_S_H
S_K	i_K_PP_mol_perP * MW_S_K
S_Ca	i_Ca_PP_mol_perP * MW_S_Ca
S_Mg	i_Mg_PP_mol_perP * MW_S_Mg
S_PO4	MW_S_PO4
X_PAO_PP	– MW_X_PAO_PP
Process Rate	kH_PP_AD_hyd * X_PAO_PP / MW_X_PAO_PP

Hydrolysis of Influent (PS) BPO	
Component	**Stoic. Equation**
H2O	MW_H2O * (i_O_XBInf_mol_perC – 3 – 4 * i_P_XBInf_mol_perC – 4 * i_S_XBInf_mol_perC + gam_bps / 2)
S_H	MW_S_H * (3 * i_P_XBInf_mol_perC + 2 * i_S_XBInf_mol_perC + 2 – i_N_XBInf_mol_perC – gam_bps / 2)
S_NH	MW_S_NH * i_N_XBInf_mol_perC
S_CO3	MW_S_CO3 * (1 – gam_bps / 4)
S_PO4	MW_S_PO4 * i_P_XBInf_mol_perC
S_SO4	MW_S_SO4 * i_S_XBInf_mol_perC
S_Glu	MW_S_Glu * gam_bps / 24
X_B_Inf	– MW_X_B_Inf
Process Rate	kM_BInf_AD_hyd * (COD_X_B_Inf / COD_X_AD) / (KS_BInf_AD_hyd + (COD_X_B_Inf / COD_X_AD)) * X_AD / MW_X_AD * gam_o/ gam_bps

Hydrolysis of PHA in AD	
Component	**Stoic. Equation**
H2O	– 4 * MW_H2O / 3
S_H	– 4 * MW_S_H / 3
S_CO3	– 2 * MW_S_CO3 / 3
S_Glu	MW_S_Glu
X_PAO_Stor	– 4 * MW_X_PAO_Stor / 3
Process Rate	kH_PHA_AD_hyd * X_PAO_Stor / MW_X_PAO_Stor

Acidogenesis (Low pH)	
Component	**Stoic. Equation**
H2O	MW_H2O * (– 4 + ((72 – 24 * i_O_Org_mol_perC + 96 * i_P_Org_mol_perC + 96 * i_S_Org_mol_perC) / gam_o – 8) * Y_AD)
S_H	MW_S_H * (6 + (6 + (24 * i_N_Org_mol_perC – 72 * i_P_Org_mol_perC – 48 * i_S_Org_mol_perC – 48) / gam_o) * Y_AD)
S_NH	MW_S_NH * (– 24 * Y_AD / gam_o * i_N_Org_mol_perC)
S_VFA	MW_S_VFA * (2 – 2 * Y_AD)
S_CO3	MW_S_CO3 * (2 + (4 – 24 / gam_o) * Y_AD)
S_PO4	MW_S_PO4 * (– 24 * Y_AD / gam_o * i_P_Org_mol_perC)
S_SO4	MW_S_SO4 * (– 24 * Y_AD / gam_o * i_S_Org_mol_perC)
S_H2	MW_S_H2 * (4 – 4 * Y_AD)
S_Glu	– MW_S_Glu
X_AD	24 * Y_AD / gam_o * MW_X_AD
Process Rate	mu_AD * (S_Glu / MW_S_Glu) / (KS_AD + S_Glu / MW_S_Glu) * (1 – COD_S_H2 / (K_I_H2 + COD_S_H2)) * X_AD / MW_X_AD * gam_o / (Y_AD * 24)

Acidogenesis (high pH)	
Component	**Stoic. Equation**
H2O	MW_H2O * (– 1 + ((72 – 24 * i_O_Org_mol_perC + 96 * i_P_Org_mol_perC + 96 * i_S_Org_mol_perC) / gam_o – 11) * Y_AH)
S_H	MW_S_H * (4 + (8 + (24 * i_N_Org_mol_perC – 72 * i_P_Org_mol_perC – 48 * i_S_Org_mol_perC – 48) / gam_o) * Y_AH)
S_NH	MW_S_NH * (– 24 * Y_AH / gam_o * i_N_Org_mol_perC)
S_VFA	MW_S_VFA * (1 – Y_AH)
S_Pr	MW_S_Pr * (1 – Y_AH)
S_CO3	MW_S_CO3 * (Y_AH * (5 – 24 / gam_o) + 1)
S_PO4	MW_S_PO4 * (– 24 * Y_AH / gam_o * i_P_Org_mol_perC)
S_SO4	MW_S_SO4 * (– 24 * Y_AH / gam_o * i_S_Org_mol_perC)
S_H2	MW_S_H2 * (1 – Y_AH)
S_Glu	– MW_S_Glu
X_AD	24 * Y_AH / gam_o * MW_X_AD
Process Rate	mu_AD * (S_Glu / MW_S_Glu) / (KS_AD + S_Glu / MW_S_Glu) * (COD_S_H2 / (K_I_H2 + COD_S_H2)) * X_AD / MW_X_AD * gam_o / (Y_AH * 24)

Acidogen endogenous decay	
Component	**Stoic. Equation**
H2O	Stoi_H2O_decay
S_H	Stoi_H_decay
S_NH	Stoi_NH4_decay
S_CO3	Stoi_CO3_decay
S_PO4	Stoi_PO4_decay
S_SO4	Stoi_SO4_decay
X_B_Org	MW_X_B_Org * (1 – f_XU_Bio_lysis) * gam_o / gam_bp
X_AD	– MW_X_AD
X_U_Org	Stoi_ER_decay
Process Rate	b_AD * X_AD / MW_X_AD

Methanogenic acetogenesis	
Component	**Stoic. Equation**
H2O	MW_H2O * (– 3 + ((42 – 14 * i_O_Org_mol_perC + 56 * i_P_Org_mol_perC + 56 * i_S_Org_mol_perC) / gam_o – 4) * Y_AC)
S_H	MW_S_H * (2 + (3 + (14 * i_N_Org_mol_perC – 42 * i_P_Org_mol_perC – 28 * i_S_Org_mol_perC – 28) / gam_o) * Y_AC)
S_NH	MW_S_NH * (– 14 * Y_AC / gam_o * i_N_Org_mol_perC)
S_VFA	MW_S_VFA * (1 – Y_AC)
S_Pr	– MW_S_Pr
S_CO3	MW_S_CO3 * (1 + Y_AC * (2 – 14 / gam_o))
S_PO4	MW_S_PO4 * (– 14 * Y_AC / gam_o * i_P_Org_mol_perC)
S_SO4	MW_S_SO4 * (– 14 * Y_AC / gam_o * i_S_Org_mol_perC)
S_H2	MW_S_H2 * (3 – 3 * Y_AC)

Methanogenic acetogenesis	
Component	**Stoic. Equation**
X_AC	14 * Y_AC / gam_o * MW_X_AC
Process Rate	mu_AC * (S_Pr / MW_S_Pr) / (KS_AC + (S_Pr / MW_S_Pr)) * (1 – COD_S_H2 / (K_I_H2 + COD_S_H2)) * X_AC / MW_X_AC * gam_o / (Y_AC * 24)

Methanogenic acetogen endogenous decay	
Component	**Stoic. Equation**
H2O	Stoi_H2O_decay
S_H	Stoi_H_decay
S_NH	Stoi_NH4_decay
S_CO3	Stoi_CO3_decay
S_PO4	Stoi_PO4_decay
S_SO4	Stoi_SO4_decay
X_B_Org	MW_X_B_Org * (1 – f_XU_Bio_lysis) * gam_o / gam_bp
X_AC	– MW_X_AC
X_U_Org	Stoi_ER_decay
Process Rate	b_AC * X_AC / MW_X_AC

Sulphidogenic acetogenesis	
Component	**Stoic. Equation**
H2O	MW_H2O * (– 7 + 7 * Y_ACETATE + 7 * Y_H2S + (42 – 14 * i_O_Org_mol_perC + 56 * i_P_Org_mol_perC + 56 * i_S_Org_mol_perC) / gam_o * Y_ACS)
S_H	MW_S_H * (5 – 21 * Y_ACETATE / 4 – 7 * Y_H2S / 4 + (14 * i_N_Org_mol_perC – 42 * i_P_Org_mol_perC – 28 * i_S_Org_mol_perC – 28) / gam_o) * Y_ACS)
S_NH	MW_S_NH * (– 14 * Y_ACS / gam_o * i_N_Org_mol_perC)
S_VFA	MW_S_VFA * 7 * Y_ACETATE / 4)
S_Pr	– MW_S_Pr
S_CO3	MW_S_CO3 * (3 – 7 * Y_ACETATE / 2 – 14 * Y_ACS / gam_o)
S_PO4	MW_S_PO4 * (– 14 * Y_ACS / gam_o * i_P_Org_mol_perC)
S_SO4	MW_S_SO4 * (– 7 * Y_H2S / 4 – 14 * Y_ACS / gam_o * i_S_Org_mol_perC)
S_H2	MW_S_H2 * (7 * Y_H2)
S_HS	MW_S_HS * (7 * Y_H2S / 4)
X_ACS	14 * Y_ACS / gam_o * MW_X_ACS
Process Rate	mu_ACS * (S_Pr / MW_S_Pr) / (KS_ACS + (S_Pr / MW_S_Pr)) * (1 – COD_S_H2 / (K_I_H2 + COD_S_H2)) * (S_SO4 / MW_S_SO4) / (Kn_ACS + (S_SO4 / MW_S_SO4)) * X_ACS / MW_X_ACS * gam_o / (Y_ACS * 14)

Sulphidogenic acetogen endogenous decay	
Component	**Stoic. Equation**
H2O	Stoi_s_H2O_decay
S_H	Stoi_s_H_decay
S_NH	Stoi_s_NH4_decay

Sulphidogenic acetogen endogenous decay	
Component	**Stoic. Equation**
S_CO3	Stoi_s_CO3_decay
S_PO4	Stoi_s_PO4_decay
S_SO4	Stoi_s_SO4_decay
X_B_Org	MW_X_B_Org * (1 – f_XU_Bio_lysis) * gam_o / gam_bp
X_ACS	– MW_X_ACS
X_U_Org	Stoi_s_ER_decay
Process Rate	b_ACS * X_ACS / MW_X_ACS

Acetoclastic methanogenesis	
Component	**Stoic. Equation**
H2O	MW_H2O * (– 1 + (– 3 + (24 – 8 * i_O_Org_mol_perC + 32 * i_P_Org_mol_perC + 32 * i_S_Org_mol_perC) / gam_o) * Y_AM)
S_H	MW_S_H * (1 + Y_AM * (2 + (8 * i_N_Org_mol_perC – 24 * i_P_Org_mol_perC – 16 * i_S_Org_mol_perC – 16) / gam_o))
S_NH	MW_S_NH * (– 8 * Y_AM / gam_o * i_N_Org_mol_perC)
S_VFA	– MW_S_VFA
S_CO3	MW_S_CO3 * (1 + (1 – 8 / gam_o) * Y_AM)
S_PO4	MW_S_PO4 * (– 8 * Y_AM / gam_o * i_P_Org_mol_perC)
S_SO4	MW_S_SO4 * (– 8 * Y_AM / gam_o * i_S_Org_mol_perC)
X_AM	8 * Y_AM / gam_o * MW_X_AM
G_CH4	MW_G_CH4 * (1 – Y_AM)
Process Rate	mu_AM * (S_VFA / MW_S_VFA) / (KS_AM + S_VFA / MW_S_VFA) * K_I_H_AM / (K_I_H_AM + molality[H]) * X_AM / MW_X_AM * gam_o / (Y_AM * 8)

Acetoclastic methanogen endogenous decay	
Component	**Stoic. Equation**
H2O	Stoi_H2O_decay
S_H	Stoi_H_decay
S_NH	Stoi_NH4_decay
S_CO3	Stoi_CO3_decay
S_PO4	Stoi_PO4_decay
S_SO4	Stoi_SO4_decay
X_B_Org	MW_X_B_Org * (1 – f_XU_Bio_lysis) * gam_o / gam_bp
X_AM	– MW_X_AM
X_U_Org	Stoi_ER_decay
Process Rate	b_AM * X_AM / MW_X_AM

Acetoclastic sulphidogenesis	
Component	**Stoic. Equation**
H2O	MW_H2O * ((– 4 + (24 – 8 * i_O_Org_mol_perC + 32 * i_P_Org_mol_perC + 32 * i_S_Org_mol_perC) / gam_o) * Y_AS)
S_H	MW_S_H * (2 + Y_AS * (1 + (8 * i_N_Org_mol_perC – 24 * i_P_Org_mol_perC – 16 * i_S_Org_mol_perC – 16) / gam_o))
S_NH	MW_S_NH * (– 8 * Y_AS / gam_o * i_N_Org_mol_perC)
S_VFA	– MW_S_VFA
S_CO3	MW_S_CO3 * (2 – 8 * Y_AS / gam_o)
S_PO4	MW_S_PO4 * (– 8 * Y_AS / gam_o * i_P_Org_mol_perC)
S_SO4	MW_S_SO4 * (– 1 + Y_AS * (1 – (8 * i_S_Org_mol_perC / gam_o)
S_HS	MW_S_HS * (1 – Y_AS)
X_AS	8 * Y_AS / gam_o * MW_X_AS
Process Rate	mu_AS * (S_VFA / MW_S_VFA) / (KS_AS + S_VFA / MW_S_VFA) * (S_SO4 / MW_S_SO4) / (Kn_AS + (S_SO4 / MW_S_SO4)) * X_AS / MW_X_AS * gam_o / (Y_AS * 8)

Acetoclastic sulphidogen endogenous decay	
Component	**Stoic. Equation**
H2O	Stoi_s_H2O_decay
S_H	Stoi_s_H_decay
S_NH	Stoi_s_NH4_decay
S_CO3	Stoi_s_CO3_decay
S_PO4	Stoi_s_PO4_decay
S_SO4	Stoi_s_SO4_decay
X_B_Org	MW_X_B_Org * (1 – f_XU_Bio_lysis_s) * gam_o / gam_bp
X_AS	– MW_X_AS
X_U_Org	Stoi_s_ER_decay
Process Rate	b_AS * X_AS / MW_X_AS

Hydrogenotrophic methanogenesis	
Component	**Stoic. Equation**
H2O	MW_H2O * (0.75 + ((6 – 2 * i_O_Org_mol_perC + 8 * i_P_Org_mol_perC + 8 * i_S_Org_mol_perC) / gam_o – 0.75) * Y_HM)
S_H	MW_S_H * (– 0.5 + (0.5 + (2 * i_N_Org_mol_perC – 6 * i_P_Org_mol_perC – 4 * i_S_Org_mol_perC – 4) / gam_o) * Y_HM)
S_NH	MW_S_NH * (– 2 * Y_HM / gam_o * i_N_Org_mol_perC)
S_CO3	MW_S_CO3 * (– 0.25 + (0.25 – 2 / gam_o) * Y_HM)
S_PO4	MW_S_PO4 * (– 2 * Y_HM / gam_o * i_P_Org_mol_perC)
S_SO4	MW_S_SO4 * (– 2 * Y_HM / gam_o * i_S_Org_mol_perC)
S_H2	– MW_S_H2
X_HM	2 * Y_HM / gam_o * MW_X_HM
G_CH4	MW_G_CH4 * (0.25 – 0.25 * Y_HM)
Process Rate	mu_HM * (S_H2 / MW_S_H2) / (KS_HM + S_H2 / MW_S_H2) * K_I_H_HM / (K_I_H_HM + molality[H]) * X_HM / MW_X_HM * gam_o / (Y_HM * 2)

Hydrogenotrophic methanogen endogenous decay	
Component	**Stoic. Equation**
H2O	Stoi_H2O_decay
S_H	Stoi_H_decay
S_NH	Stoi_NH4_decay
S_CO3	Stoi_CO3_decay
S_PO4	Stoi_PO4_decay
S_SO4	Stoi_SO4_decay
X_B_Org	MW_X_B_Org * (1 – f_XU_Bio_lysis) * gam_o / gam_bp
X_HM	– MW_X_HM
X_U_Org	Stoi_ER_decay
Process Rate	b_HM * X_HM / MW_X_HM

Hydrogenotrophic sulphidogenesis	
Component	**Stoic. Equation**
H2O	MW_H2O * (1 + ((6 – 2 * i_O_Org_mol_perC + 8 * i_P_Org_mol_perC + 8 * i_S_Org_mol_perC) / gam_o – 1) * Y_HS)
S_H	MW_S_H * (– 0.25 + (0.25 + (2 * i_N_Org_mol_perC – 6 * i_P_Org_mol_perC – 4 * i_S_Org_mol_perC – 4) / gam_o) * Y_HS)
S_NH	MW_S_NH * (– 2 * Y_HS / gam_o * i_N_Org_mol_perC)
S_CO3	MW_S_CO3 * (– 2 * Y_HS / gam_o)
S_PO4	MW_S_PO4 * (– 2 * Y_HS / gam_o * i_P_Org_mol_perC)
S_SO4	MW_S_SO4 * (– 0.25 + Y_HS * (0.25 – 2 * i_S_Org_mol_perC / gam_o))
S_H2	– MW_S_H2
S_HS	MW_S_HS * (0.25 – 0.25 * Y_HS)
X_HS	2 * Y_HS / gam_o * MW_X_HS
Process Rate	mu_HS * (S_H2 / MW_S_H2) / (KS_HS + S_H2 / MW_S_H2) * (S_SO4 / MW_S_SO4) / (Kn_HS + (S_SO4 / MW_S_SO4)) * X_HS / MW_X_HS * gam_o / (Y_HS * 2)

Hydrogenotrophic sulphidogen endogenous decay	
Component	**Stoic. Equation**
H2O	Stoi_s_H2O_decay
S_H	Stoi_s_H_decay
S_NH	Stoi_s_NH4_decay
S_CO3	Stoi_s_CO3_decay
S_PO4	Stoi_s_PO4_decay
S_SO4	Stoi_s_SO4_decay
X_B_Org	MW_X_B_Org * (1 – f_XU_Bio_lysis_s) * gam_o / gam_bp
X_HS	– MW_X_HS
X_U_Org	Stoi_s_ER_decay
Process Rate	b_HS * X_HS / MW_X_HS

Evolution of CO_2 gas	
Component	**Stoic. Equation**
H2O	MW_H2O
S_H	– 2 * MW_S_H
S_CO3	– MW_S_CO3
G_CO2	MW_G_CO2
Process Rate	CO2_rate / MW_G_CO2

Precipitation (and dissolution) of NH_4–struvite ($MgNH_4PO_4$) in AD	
Component	**Stoic. Equation**
H2O	– MW_H2O * 6
S_Mg	– MW_S_Mg
S_NH	– MW_S_NH
S_PO4	– MW_S_PO4
X_Str_NH4	MW_X_Str_NH4
Process Rate	K_stru * Driver_Str

Precipitation (and dissolution) of ACP ($Ca_3(PO_4)_2$) in AD	
Component	**Stoic. Equation**
S_Ca	–3 * MW_S_Ca
S_PO4	–2 * MW_S_PO4
X_ACP	MW_X_ACP
Process Rate	K_cap * Driver_cap

Precipitation (and dissolution) of ($MgKPO_4$) in AD	
Component	**Stoic. Equation**
H2O	–MW_H2O * 6
S_K	–MW_S_K
S_Mg	–MW_S_Mg
S_PO4	–MW_S_PO4
X_Str_K	MW_X_Str_K
Process Rate	K_mgkp * Driver_mgkp

Precipitation (and dissolution) Calcite ($CaCO_3$) in AD	
Component	**Stoic. Equation**
S_Ca	–MW_S_Ca
S_CO3	–MW_S_CO3
X_Cal	MW_X_Cal
Process Rate	K_cal * Driver_cal

Precipitation (and dissolution) Magnetite in AD	
Component	**Stoic. Equation**
S_Mg	-MW_S_Mg
S_CO3	-MW_S_CO3
X_Mag	MW_X_Mag
Process Rate	K_mag * Driver_mag

Precipitation (and dissolution) Newberyite in AD	
Component	**Stoic. Equation**
H2O	-MW_H2O * 3
S_H	-MW_S_H
S_Mg	-MW_S_Mg
S_PO4	-MW_S_PO4
X_Newb	MW_X_Newb
Process Rate	K_newb * Driver_newb