TREATMENT OF DYE WASTEWATERS IN THE ANAEROBIC BAFFLED REACTOR AND CHARACTERISATION OF THE ASSOCIATED MICROBIAL POPULATIONS
APPENDICES

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Table of Contents

A-1 ANALYTICAL METHODS

A1.1 ANAEROBIC MEDIUM FOR BATCH ASSAYS A1-1

A1.2 SUGAR/PROTEIN FEED A1-3

A1.3 ANALYTICAL METHODS A1-3

A1.3.1 pH Measurement A1-3

A1.3.2 COD Measurement A1-3

A1.3.3 Total Organic Carbon A1-4

A1.3.4 VFA Measurement A1-4

A1.3.5 Biogas Production A1-5

A1.3.6 Biogas Composition A1-6

A1.3.7 Total Solids (TS) and Volatile Solids (VS) A1-6

A1.3.8 Colour Measurement A1-6

A1.4 FLUORESCENT *IN SITU* HYBRIDISATION A1-6

A1.4.1 Sample Fixation A1-6

A1.4.2 Hybridisation A1-7

A1.4.3 Washing and mounting A1-7

A1.5 16S DNA CLONE LIBRARY CONSTRUCTION A1-8

A1.5.1 Extraction and purification of total sample DNA A1-8

A1.5.2 Amplification, cloning and sequencing of archaeal 16S rDNA A1-9

A1.5.3 Sequence analysis A1-9

A-2 CALIBRATIONS

A2.1 CHEMICAL OXYGEN DEMAND A2-1

A2.2 BIOGAS A2-4

A2.2.1 Methane A2-4

A2.2.2 Carbon Dioxide A2-5

A2.2.3 Nitrogen A2-6

A2.3 COLOUR A2-7

A2.3.1 Tartrazine A2-7

A2.3.2 CI Reactive Red 141 A2-8

A2.3.3 Industrial Food Dye Wastewater A2-9

A2.4 VOLATILE FATTY ACIDS A2-9

A2.4.1 Acetic Acid A2-9

A2.4.2 Propionic Acid A2-10

A2.4.3 Iso-butyric Acid A2-11

A2.4.4 Butyric Acid A2-11

A2.4.5 Iso-valeric Acid A2-12

A2.4.6 Valeric Acid A2-13

A-3 FOOD DYES
A3.1 ANAEROBIC TOXICITY ASSAYS A3-1
A3.1.1 Hypotheses and Objectives A3-1
A3.1.2 Materials and Methods A3-2
A3.1.3 Results A3-4
A3.1.4 Discussion A3-5
A3.1.5 Conclusions A3-7
A3.1.6 Biogas Production Plots A3-8
A3.2 BIODEGRADABILITY ASSAYS A3-24
A3.2.1 Hypotheses and Objectives A3-24
A3.2.2 Materials and Methods A3-24
A3.2.3 Results A3-26
A3.2.4 Discussion A3-28
A3.2.5 Conclusions A3-33
A3.2.6 Biogas Production Plots A3-34

A-4 TEXTILE DYES
A4.1 ANAEROBIC TOXICITY ASSAYS A4-1
A4.1.1 Hypotheses and Objectives A4-1
A4.1.2 Materials and Methods A4-1
A4.1.3 Results A4-2
A4.1.4 Discussion A4-5
A4.1.5 Conclusions A4-5
A4.1.6 Biogas Production Plots A4-6
A4.2 BIODEGRADABILITY ASSAYS A4-11
A4.2.1 Hypotheses and Objectives A4-11
A4.2.2 Materials and Methods A4-11
A4.2.3 Results A4-13
A4.2.4 Discussion A4-14
A4.2.5 Conclusions A4-16
A4.2.6 Biogas Production Plots A4-17
A4.3 COD BALANCE A4-24

A-5 ANALYTICAL RESULTS
A5.1 SUCROSE LABILE FEED A5-2
A5.2 CI REACTIVE RED 141

A-6 LIST OF PUBLICATIONS A5-18

List of Figures

Figure number	Title	Page
Figure A1.1	Plot showing the absorbance (610 nm) measurements for two sets of KHP standards, the one containing CI Reactive Red 141, to show that the dye had no effect of the COD absorbance measurements.	A1-4
Figure A1.2	Schematic diagram of the gas measuring device.	A1-5
Figure A2.1	COD calibration plots.	A2-2
Figure A2.2	Comparison of the COD absorbance measurements of KHP standard solutions and glucose standard solutions.	A2-3
Figure A2.3	Methane calibration curves.	A2-4
Figure A2.4	Carbon dioxide calibration curves.	A2-5
Figure A2.5	Nitrogen calibration curves.	A2-6
Figure A2.6	Tartrazine calibration curve.	A2-7
Figure A2.7	Wavelengths scan of CI Reactive Red 141, showing the maximum absorbance at 545 nm.	A2-8
Figure A2.8	CI Reactive Red 141 calibration curve.	A2-8
Figure A2.9	Industrial dye wastewater calibration curve.	A2-90
Figure A2.10	Acetic acid calibration curve.	A2-10
Figure A2.11	Propionic acid calibration curve.	A2-10
Figure A2.12	Iso-butyric acid calibration curve.	A2-11
Figure A2.13	Butyric acid calibration curve.	A2-12
Figure A2.14	Iso-valeric acid calibration curve.	A2-12
Figure A2.15	Valeric acid calibration curve.	A2-13
Figure A3.1	Plots of biogas production during the anaerobic toxicity assay with Sunset Yellow Supra (CI Food Yellow 3).	A3-8
Figure A3.2	Plots of biogas production during the anaerobic toxicity assay with Carmoisine Supra (CI Food Red 3).	A3-9
Figure A3.3	Plots of biogas production during the anaerobic toxicity assay with Brown FK Standard (CI Food Brown 1).	A3-10
Figure A3.4	Plots of biogas production during the anaerobic toxicity assay with Allura Red AC Supra (CI Food Red 17).	A3-11
Figure A3.5	Plots of biogas production during the anaerobic toxicity assay with Red 2G Supra (CI Food Red 10).	A3-12
Figure A3.6	Plots of biogas production during the anaerobic toxicity assay with Tartrazine Supra (CI Food Yellow 4).	A3-13
Figure A3.7	Plots of biogas production during the anaerobic toxicity assay with Ponceau 4R Supra (CI Food Red 7).	A3-14
Figure A3.8	Plots of biogas production during the anaerobic toxicity assay with Black PN Extra (CI Food Black 1).	A3-15
Figure A3.9	Plots of biogas production during the anaerobic toxicity assay with Green S Supra (CI Food Green 4).	A3-16
Figure A3.10	Plots of biogas production during the anaerobic toxicity assay with Patent Blue V Supra (CI Food Blue 3).	A3-17
Figure A3.11	Plots of biogas production during the anaerobic toxicity assay with Brilliant Blue Supra (CI Food Blue 2).	A3-18

Figure A3.12	Plots of biogas production during the anaerobic toxicity assay with Quinoline Yellow Extra (CI Food Yellow 13).	A3-19
Figure A3.13	Plots of biogas production during the anaerobic toxicity assay with Erythrosine Supra (CI Food Red 14).	A3-20
Figure A3.14	Plots of biogas production during the anaerobic toxicity assay with Indigo Carmine Supra (CI Food Blue 1).	A3-21
Figure A3.15	Plots of biogas production during the anaerobic toxicity assay with Food Dye untreated final effluent.	A3-22
Figure A3.16	Plots of biogas production during the anaerobic toxicity assay with Food Dye treated final effluent.	A3-23
Figure A4.1	Plots showing the methanogenic activity, as a fraction of that in the control, for each investigated dye concentration and the best-fit lines through the experimental data points, used to determine the IC_{50} concentration for each dye.	A4-3
Figure A4.2	Gas production plots for the methanogenic toxicity assays, showing the cumulative biogas production for each concentration of the investigated textile reactive dyes, relative to the gas production measured in the controls (black line).	A4-4
Figure A4.3	Plots of biogas production during the anaerobic toxicity assay with CI Reactive Red 141.	A4-6
Figure A4.4	Plots of biogas production during the anaerobic toxicity assay with CI Reactive Blue 171.	A4-7
Figure A4.5	Plots of biogas production during the anaerobic toxicity assay with CI Reactive Yellow 84.	A4-8
Figure A4.6	Plots of biogas production during the anaerobic toxicity assay with CI Reactive Green 19.	A4-9
Figure A4.7	Plots of biogas production during the anaerobic toxicity assay with the mixture of textile reactive dyes.	A4-10

List of Tables

Table A1.1	Components of the anaerobic nutrient medium.	A1-2
Table A1.2	Components of the sugar/protein solution.	A1-3
Table A1.3	Sequences and the formamide concentration of the oligonucleotide probes used in this investigation.	A1-8
Table A2.1	Absorbance data for COD calibration 1.	A2-1
Table A2.2	Absorbance data for COD calibration 2.	A2-1
Table A2.3	Absorbance data for COD calibration 3.	A2-1
Table A2.4	Absorbance data for the COD calibration test comparing the COD of KHP and glucose solutions.	A2-3
Table A2.5	Methane calibration data.	A2-4
Table A2.6	Carbon dioxide calibration data.	A2-5
Table A2.7	Nitrogen calibration data.	A2-6
Table A2.8	Tartrazine calibration data.	A2-7
Table A2.9	CI Reactive Red 141 calibration data.	A2-8
Table A2.10	Industrial dye wastewater calibration data.	A2-9
Table A2.11	Acetic acid calibration data.	A2-9
Table A2.12	Propionic acid calibration data.	A2-10
Table A2.13	Iso-butyric acid calibration data.	A2-11
Table A2.14	Butyric acid calibration data.	A2-11
Table A2.15	Iso-valeric acid calibration data.	A2-12
Table A2.16	Valeric acid calibration data.	A2-13
Table A3.1	List of food dyes investigated for inhibition of methanogenic activity.	A3-2
Table A3.2	Calculated methanogenic IC_{50} values for the investigated food dyes.	A3-4
Table A3.3	Bioassay conditions to assess the anaerobic biodegradability of a range of food dyes.	A3-25
Table A3.4	Maximum wavelengths of the investigated food dyes.	A3-27
Table A3.5	Results of the food dye anaerobic biodegradability assays (60 d).	A3-28
Table A3.6	Results for the controls in the biodegradability assay.	A3-34
Table A3.7	Results of the biodegradability assay with CI Food Yellow 3.	A3-35
Table A3.8	Results of the biodegradability assay with CI Food Red 3.	A3-36
Table A3.9	Results of the biodegradability assay with CI Food Brown 1.	A3-37
Table A3.10	Results of the biodegradability assay with CI Food Red 17.	A3-38
Table A3.11	Results of the biodegradability assay with CI Food Red 10.	A3-39
Table A3.12	Results of the biodegradability assay with CI Food Yellow 4.	A3-40
Table A3.13	Results of the biodegradability assay with CI Food Red 7.	A3-41
Table A3.14	Results of the biodegradability assay with CI Food Black 1.	A3-42
Table A3.15	Results of the biodegradability assay with CI Food Green 4.	A3-43
Table A3.16	Results of the biodegradability assay with CI Food Blue 5.	A3-44
Table A3.17	Results of the biodegradability assay with CI Food Blue 2.	A3-45

Table A3.18	Results of the biodegradability assay with CI Food Yellow 13.	A3-46
Table A3.19	Results of the biodegradability assay with CI Food Red 14.	A3-47
Table A3.20	Results of the biodegradability assay with CI Food Blue 1.	A3-48
Table A3.21	Results of the biodegradability assay with the Untreated Food Dye Effluent (20 % v/v).	A3-49
Table A3.22	Results of the biodegradability assay with the Untreated Food Dye Effluent (100 % v/v).	A3-50
Table A3.23	Results of the biodegradability assay with the Treated Food Dye Effluent (20 % v/v).	A3-51
Table A4.1	Details of the textile dye serum bottle tests.	A4-1
Table A4.2	Calculated methanogenic IC_{50} values for the investigated textile dyes.	A4-3
Table A4.3	Bioassay conditions to assess anaerobic biodegradability of a range of textile dyes.	A4-12
Table A4.4	Maximum wavelengths of the investigated textile dyes.	A4-13
Table A4.5	Results of the textile dye anaerobic biodegradability assays (60 d).	A4-14
Table A4.6	Results for the controls in the textile dye biodegradability assay.	A4-17
Table A4.7	Results of the biodegradability assay with CI Red 141 (with nutrient medium).	A4-18
Table A4.8	Results of the biodegradability assay with CI Red 141 (without nutrient medium).	A4-19
Table A4.9	Results of the biodegradability assay with CI Green 19.	A4-20
Table A4.10	Results of the biodegradability assay with CI Blue 171.	A4-21
Table A4.11	Results of the biodegradability assay with CI Yellow 84.	A4-22
Table A4.12	Results of the biodegradability assay with textile dye mixture.	A4-23
Table A4.13	COD balance data for the laboratory-scale ABR treating a synthetic CI Reactive Red 141 waste stream.	A4-24
Table A5.1	pH data for the baseline study with sucrose (Section 4.2.1).	A5-2
Table A5.2	Total solids data for the baseline study with sucrose (Section 4.2.2).	A5-3
Table A5.3	Total solids data for the baseline study with sucrose (Section 4.2.2).	A5-4
Table A5.4	Volatile solids data for the baseline study with sucrose (Section 4.2.2).	A5-5
Table A5.5	Volatile solids data for the baseline study with sucrose (Section 4.2.2).	A5-6
Table A5.6	COD data for the baseline study with sucrose (Section 4.2.3).	A5-7
Table A5.7	COD data for the baseline study with sucrose (Section 4.2.3).	A5-8
Table A5.8	COD data for the baseline study with sucrose (Section 4.2.3).	A5-9
Table A5.9	Compartment 1 biogas data for the baseline study with sucrose (Section 4.2.5).	A5-10
Table A5.10	Compartment 2 biogas data for the baseline study with sucrose (Section 4.2.5).	A5-11
Table A5.11	Compartment 3 biogas data for the baseline study with sucrose (Section 4.2.5).	A5-12
Table A5.12	Compartment 4 biogas data for the baseline study with sucrose (Section 4.2.5).	A5-13
Table A5.13	Compartment 5 biogas data for the baseline study with sucrose (Section 4.2.5).	A5-14
Table A5.14	Compartment 6 biogas data for the baseline study with sucrose (Section 4.2.5).	A5-15

Table A5.15	Compartment 7 biogas data for the baseline study with sucrose (Section 4.2.5).	A5-16
Table A5.16	Compartment 8 biogas data for the baseline study with sucrose (Section 4.2.5).	A5-17
Table A5.17	pH data for the CI Reactive Red 141 investigation (Section 5.4.5).	A5-18
Table A5.18	Total solids data for the CI Reactive Red 141 investigation (Section 5.4.6).	A5-19
Table A5.19	Volatile solids data for the CI Reactive Red 141 investigation (Section 5.4.6).	A5-19
Table A5.20	COD data for the CI Reactive Red 141 investigation (Section 5.4.7).	A5-20
Table A5.21	COD data for the CI Reactive Red 141 investigation (Section 5.4.7).	A5-21
Table A5.22	Compartment 1 biogas data for the CI Reactive Red 141 investigation (Section 5.4.10).	A5-22
Table A5.23	Compartment 2 biogas data for the CI Reactive Red 141 investigation (Section 5.4.10).	A5-23
Table A5.24	Compartment 3 biogas data for the CI Reactive Red 141 investigation (Section 5.4.10).	A5-24
Table A5.25	Compartment 4 biogas data for the CI Reactive Red 141 investigation (Section 5.4.10).	A5-25
Table A5.26	Compartment 5 biogas data for the CI Reactive Red 141 investigation (Section 5.4.10).	A5-26
Table A5.27	Compartment 6 biogas data for the CI Reactive Red 141 investigation (Section 5.4.10).	A5-27
Table A5.28	Compartment 7 biogas data for the CI Reactive Red 141 investigation (Section 5.4.10).	A5-28
Table A5.29	Compartment 8 biogas data for the CI Reactive Red 141 investigation (Section 5.4.10).	A5-29

A-1

Analytical Methods

A1.1 ANAEROBIC MEDIUM FOR BATCH ASSAYS

Preparation of 1 L of anaerobic medium: 10 mL of stock solution A1 (**Table A1.1**) was mixed with 10 mL of solution A2 and 1 mL of solution A3 and diluted with 967 mL of deionised water. The medium was boiled in an Erlenmeyer flask with a glass watch on top to prevent evaporation. The solution was cooled to room temperature whilst being purged with a gas mixture of 70 % N_2, 30 % CO_2. The solution was transferred into an anaerobic bottle with the appropriate amount of A6 (0.44 g of NH_4HCO_3 and 3.73 g of $NaHCO_3$), whilst still flushing with the gas mixture.

The bottle was sealed with a rubber stopper and aluminium cap, and a slight overpressure (0.5 bar).

The following were added to the solution, with a syringe, through the rubber stopper:

1 mL A4

10 mL A5

1 mL A7

1 mL A8

TABLE A1.1 : Components of the anaerobic nutrient medium.

Stock	Components	Concentration in stock	Final conc. in medium
A1	K_2HPO_4	65.3 g/L	0.653 g/L
A2	$NaH_2PO_4.2H_2O$	19.5 g/L	0.195 g/L
A3	Resazurin	0.5 g/L	0.5 mg/L
A4	**Trace Element Solution**	**mg/L**	
	EDTA	500	0.5 mg/L
	$FeCl_2.4H_2O$	2 000	2.0 mg/L
	$MnCl_2.4H_2O$	100	1.0 mg/L
	$CoCl_2.6H_2O$	190	0.19 mg/L
	$ZnCl_2$	70	0.07 mg/L
	$CuCl_2$	2	0.002 mg/L
	$AlCl_3.6H_2O$	10	0.01 mg/L
	H_3BO_3	6	0.006 mg/L
	Na_2MoO_4	36	0.036 mg/L
	$NiCl_2.6H_2O$	24	0.024 mg/L
	+ 1 mL conc. HCl		
A5	**Vitamin Solution**	**mg/L**	
	Biotin (vitamin H)	2	20 µg/L
	p-aminobenzoate (Na salt)	5	50 µg/L
	Pantothenate (Na salt)	5	50 µg/L
	Folic acid (dihydrate)	2	20 µg/L
	Lipoic acid (thioctic acid)	5	50 µg/L
	Pyridoxine (vitamin B_6)	10	100 µg/L
	Nicotinamide	5	50 µg/L
	Thiamine HCl (vitamin B_1)	5	50 µg/L
	Riboflavine (vitamin B_2)	5	50 µg/L
	Cyanocobalamine (B_{12})	0.1	2 µg/L
A6	NH_4HCO_3		0.4439 g/L
	$NaHCO_3$		3.730 g/L
A7	$Na_2S.9H_2O$	1 000 x	1.00 mM
A8	$CaCl_2.2H_2O$	1 000 x	0.110 g/L
	$MgCl_2.4H_2O$		0.101 g/L

A1.2 SUGAR/PROTEIN FEED

The components listed in **Table A1.2** were diluted in deionised water to make up 5 L of 40 g COD/L feed solution.

TABLE A1.2: Components of the sugar/protein feed solution.

Component	Amount added (g)	Purpose
Sugar	133.35	Carbohydrate
Peptone	40.00	Protein
Meat extract	13.35	Protein
K_2HPO_4	4.00	Macro-nutrient
$NaHCO_3$	162.50	Buffer
$CoCl_2.6H_2O$	0.119	Micro-nutrient
$FeCl_2.4H_2O$	0.785	Micro-nutrient
$MnCl_2.4H_2O$	0.0375	Micro-nutrient
$Na_2MoO_4.2H_2O$	0.0375	Micro-nutrient
$NiCl_2.6H_2O$	0.045	Micro-nutrient

A1.3 ANALYTICAL METHODS

A1.3.1 pH Measurement

pH was measured using a calibrated pH meter (Jenway 3020 and Metrohm 744). Values obtained were accurate to within ± 0.2.

A1.3.2 COD Measurement

The measurement of COD was based on the closed reflux, colorimetric method described in section 5220-D of Standard Methods (American Public Health Association, 1989). Reflux tubes were used. To each tube, 0.4 g $HgSO_4$, 2 mL 0.0147 M $K_2Cr_2O_7$ (Merck) and 1 mL sample were added. Sulphuric acid reagent (3 mL – containing 2.5 % w/w silver sulphate) was added. The tubes were tightly sealed and mixed. The mixtures were refluxed in a Hach COD reflux reactor (Model 45600) at 150 °C for 2 h. After cooling, the samples were analysed on a Pharmacia Biotech Ultrospec 2000 UV-VIS scanning spectrophotometer at a wavelength of 610 nm. Potassium hydrogen phthalate (KPH) was used to prepare standard solutions (for a calibration curve) with 17 g KPH/L being equivalent to 20 g COD/L. The calibration curves are given in **Appendix 2**.

To determine whether the reactive dye CI Reactive Red 141 would affect the COD measurements, using the colorimetric method, a test was conducted using two sets of KHP standards, the one containing no dye and the other containing 100 mg/L CI Reactive Red 141. The absorbance (610 nm) of each was determined and compared to assess whether the presence of the dye in the COD samples affected the COD absorbance measurements. **Figure A1.1** shows that the dye had no effect on the COD measurements, thus the colorimetric COD method was used in the investigation. These tests also proved that the COD of the dye was negligible, therefore, the composition of the sugar/protein feed was not altered to maintain the constant feed COD of 4 g COD/L.

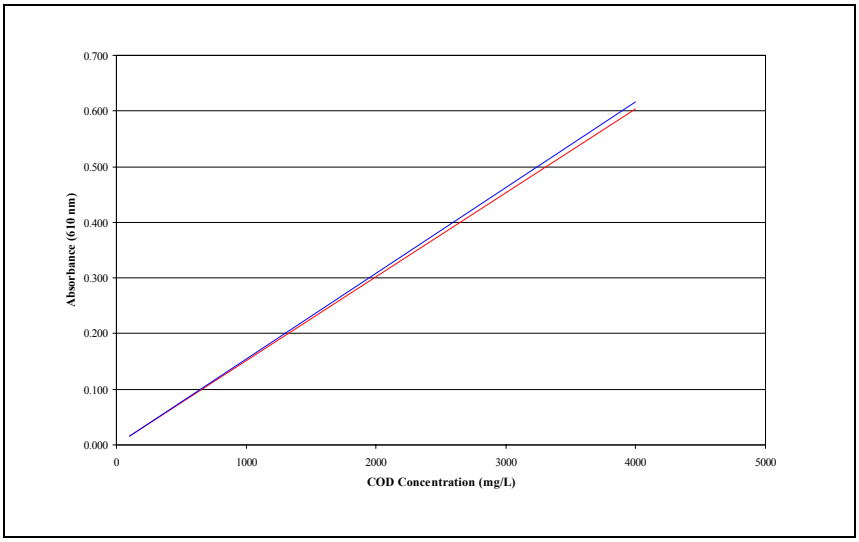


FIGURE A1.1 : Plot showing the absorbance (610 nm) measurements for two sets of KHP standards, the one containing CI Reactive Red 141, to show that the dye had no effect of the COD absorbance measurements.

A1.3.3 Total Organic Carbon

Total organic carbon measurement was based on quantitative infrared analysis performed with a Total Organic Carbon Analyser (Shimadzu, Model TOC-5050). TOC concentrations were obtained by subtracting the inorganic carbon (IC) concentration from the total carbon (TC) concentration. Standards for the TOC were prepared using potassium biphthalate ($C_6H_4(COOK)(COOH)$). Standards for IC were prepared using anhydrous sodium carbonate (Na_2CO_3).

A1.3.4 VFA Measurement

Method A (Imperial College, London) : Volatile fatty acids (acetic, propionic, butyric and valeric) were measured on a Shimadzu SCL-10A high-pressure liquid chromatograph (HPLC) with autosampler. Component separation was possible by an ion-exchange resin column (Biorad – Aminex). The carrier solvent was 0.01N H_2SO_4 at a flow rate of 0.5 mL/min. The oven temperature was set at 35 °C. Detection

of the separated components was by a Shimadzu UV-VIS detector at 210 nm, and a refractive index detector. Standard VFA solutions were made up containing 100, 200, 500, 1 000 and 2 000 mg/L of each acid (**Chapter 3**).

Method B (University of Natal, Durban) : The volatile fatty acids were measured on a Varian 3300 gas chromatograph (GC) with a flame ionisation detector (FID) and Quadrex 007-FFAP fused silica capillary column. Nitrogen was used as the carrier gas. The column temperature was ramped from 85 °C to 150 °C, the detector and injector temperatures were both set at 200 °C. The samples were prepared by centrifugation (10 000 rpm for 15 min) and the supernatants were filtered with 0.45 µm cellulose acetate filters. Sample (1 mL) was acidified with 32 % HCl, in a glass vial. The fatty acids were extracted into ether (3 mL) and a 1 µL sample, from the ether layer, was injected into the GC for analysis (**Chapters 4** and **5**).

A1.3.5 Biogas Production

In previous experiments it was found that measurement of gas production by water displacement was inaccurate and thus a COD balance, to assess the accuracy of the system and analytical measurements, could not be carried out. A system was devised in an attempt to obtain more accurate measurement of gas production within each compartment of the ABR. This was applied in the CI Reactive Red 141 study (**Chapter 5**). A gas-measuring device was attached to the gas outlet of each compartment. The device (**Figure A1.2**) consisted of a plastic U-tube (diameter of 45 mm), containing an earth probe and a minimum and maximum level sensor. The U-tube was filled with acidified water to the level of the minimum level probe. When gas was produced in the compartment, it entered the U-tube and the pressure of the gas caused the liquid level to rise. When the liquid came into contact with the maximum level sensor, a solenoid valve was opened, which vented the gas from the U-tube and the liquid level returned to the minimum level. When the solenoid valve was opened, a signal was passed to an analogue recorder which registered a count. The volume of gas produced with each count, was calculated as 15.9 mL, from the diameter of the U-tube and the distance between the minimum and maximum level probes.

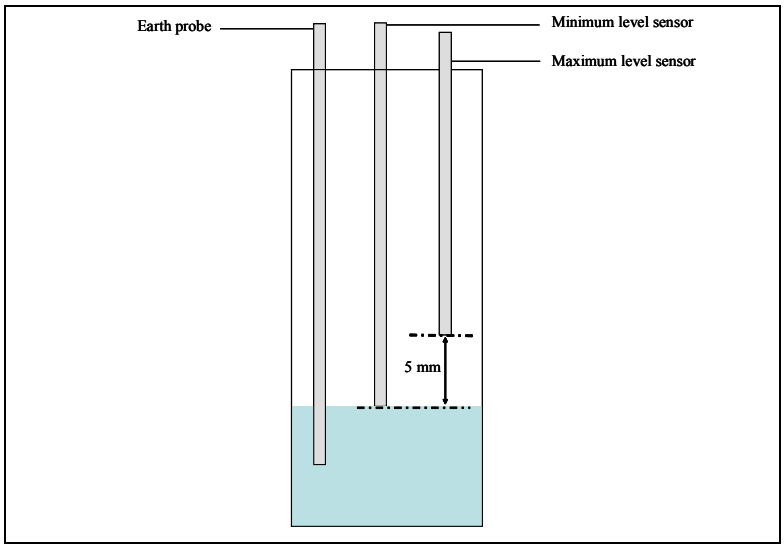


FIGURE A1.2 : Schematic diagram of the gas measuring device.

A1.3.6 Biogas Composition

Method A (Imperial College, London) : The composition of biogas (methane, carbon dioxide and nitrogen) was determined using a Shimadzu GC-TCD fitted with a Poropak N column (1 500 mm x 6.35 mm). Carrier gas was helium set at a flow rate of 50 mL/min, column temperature was 28 °C. The peak area was integrated by a Shimadzu Chromatopak C-R6A. Calibration gases were accurate to ± 5 %. Biogas samples of 1 mL were injected.

Method B (University of Natal, Durban) : The composition of biogas (methane, carbon dioxide and nitrogen) was determined using a GowMac 350 GC-TCD fitted with a Poropak N column (1 500 mm x 6.35 mm). Helium was used as the carrier gas, at a flow rate of 50 mL/min. The column temperature was 28 °C, detector temperature 38 °C and injector temperature 128 °C. Calibration gases were accurate to ± 5 %. Biogas samples of 0.2 mL were injected.

A1.3.7 Total Solids (TS) and Volatile Solids (VS)

The measurement of total solids (TS) and volatile solids (VS) was adapted from the procedure described in Section 2540-B and 2540-E of Standard Methods (American Public Health Association, 1989). A 10 mL sample was added to a pre-weighed crucible and the liquid fraction evaporated in an oven at 105 °C until the weight stabilised (approximately 24 h). The resultant weight was recorded and the crucible then placed in a furnace at 550 °C for 1 h. The final weight was then recorded and the calculations performed as given in Standard Methods (American Public Health Association, 1989).

A1.3.8 Colour Measurement

Samples (1.5 mL) were sealed in plastic Eppendorf tubes and centrifuged (4 000 rpm) for 5 min. The supernatants were then filtered through PVDF or glass fibre (0.45 µm) filters, whilst being kept anaerobic by bubbling oxygen-free nitrogen gas through the solution. The absorbance was measured immediately on the UV-VIS Spectrophotometer, in quartz microcuvettes (vol = 1.5 mL; path length = 10 mm) at the required maximum wavelength.

A1.4 FLUORESCENT *IN SITU* HYBRIDISATION

A1.4.1 Sample Fixation

Paraformaldehyde fixative (100 mL) was prepared by adding paraformaldehyde (4 g) to 65 mL distilled water at 65 °C. One drop of 2M NaOH solution was added and the solution was stirred rapidly until nearly clarified and then removed from the heat source. 3X PBS (33 mL) (phosphate buffered saline) was added and the pH adjusted to 7.2. The solution was rapidly cooled down to 4 °C.

Samples (0.5 mL) were taken from each compartment of the ABR and placed in labelled Eppendorf tubes. Paraformaldehyde (1.5 mL) was added to each of the samples and fixed overnight at 4 °C. The

cells were pelleted by centrifugation (13 000 rpm for 5 min) and the supernatant, containing the fixative, removed. The cells were washed in 1 mL 1X PBS, by centrifugation and then re-suspended in 1X PBS and absolute ethanol to give 10^8 to 10^9 cells/mL. The samples were then stored at –20 °C until required for probing. Unfixed samples to be used for DNA extraction and further analysis were stored at –20˚C until required.

A1.4.2 Hybridisation

Fixed cell samples (3 µL) were applied to teflon coated multi-well glass slides, allowed to air dry and dehydrated by serial immersion of the slide in 50, 80 and 98 % ethanol (3 min each). The purpose of the ethanol wash was to remove any cell debris from the slides. The hybridisation buffer was prepared containing 0.9 M NaCl, 20 mM Tris-HCl (pH 7.2), 0.01 % sodium dodecyl sulphate and appropriate amounts of formamide, for the required stringency of a particular probe. The slides were air dried, and 8 µL of hybridisation buffer was added to each well. The remainder of the buffer was used to humidify the hybridisation tube. The oligonucleotide probe (25 to 50 nt) was added (1 µL) to each well, to give a final concentration of 5 ng/µL. The slide was placed in the hybridisation tube, in the hybridisation oven at 46 °C for 2 h. Details of the oligonucleotide probes used in this project are given in **Table A1.3**.

A1.4.3 Washing and mounting

After the hybridisation, the slides were washed in a wash buffer containing a specific amount of NaCl, dependent on the stringency of the hybridisation. The wash buffer was warmed to 48 °C in a water bath. The slides were washed for 15 min, then rinsed in distilled water, air dried and mounted with Vectashield Mounting Medium containing DAPI (4',6-diamidino-2-phenylindole) (Vector Laboratories, Peterborough).

Cells were visualised using a Zeiss (Jena, Germany) Axioskop epifluorescence microscope equipped with a 50 W high-pressure bulb equipped for epifluorescence. Images were obtained either by photography using a Nikon N90 camera mounted on the epifluorescence microscope using Fuji Superia ISO 800 film with exposure times between 4 and 6 s or using the image analysis software. Counting of cells was performed by sampling at least 20 randomly selected fields with usually at least 1 500 cells counted for each probing event.

TABLE A1.3: Sequences and the formamide concentration of the oligonucleotide probes used in this investigation.

Probe	5´-Sequence- 3´	Formamide concentration (%)
ARC915	GTGCTCCCCCGCCAATTCCT	20
EUB338	GCTGCCTCCCGTAGGAGT	20
MX825	TCGCACCGTGGCCGACACCTAGC	20
MS5	GGCCACGGTGCGACCGTTGTCG	35
MS821	CGCCATGCCTGACACCTAGCGAGC	20
MB4	TTTATGCGTAAAATGGATT	35
MG1200	CGGATAATTCGGGGCATGCTG	20
ALF1b	CGTTCGGYTCTGAGCCAG	20
BET42a	GCCTTCCCACTTCGTTT	35
GAM42a	GCCTTCCCACATCGTTT	35
SRB385	CGGCGTCGCTGCGTCAGG	20
CF319a	TGGTCCGTGTCTCAGTAC	35
BAC303	CCAATGTGGGGGACCTT	0
HGC69a	TATAGTTACCACCGCCGT	25
LGC354a	TGGAAGATTCCCTACTGC*	20
LGC354b	CGGAAGATTCCCTACTGC	20
LGC354c	CCGAAGATTCCCTACTGC	20
DSV698	GTTCCTCCAGATATCTACGG	20
DSB985	CACAGGATGTCAAACCCAG	0

A1.5 16S DNA CLONE LIBRARY CONSTRUCTION

A1.5.1 Extraction and purification of total sample DNA

The frozen sludge sample was thawed and 500 µL was combined with an equal volume of buffer containing 200 mM NaCl, 200 mM Tris-HCl, 2 mM sodium citrate and 10 mM $CaCl_2$ adjusted to pH 8 with HCl. Lysozyme was added, to give a final concentration of 5 mg/mL, followed by gentle mixing and incubation at 37 ˚C for 40 min. Amounts of SDS and proteinase K were added to final concentrations of 0.3 % and 2 mg/mL respectively, and after further gentle mixing the samples were incubated at 50 ˚C for 30 min. A physical lysis step followed where SDS was added to a final concentration of 5 % together with an equal volume of phenol-chloroform-isoamylalcohol (24:24:1) and ca. 300 µL volume of acid-

washed 0.1 mm diameter zirconia glass beads (Stratech, Bedfordshire). Samples were shaken on a Mini Bead-Beater (BioSpec Products, Bartlesville) for 2 min on the low setting. Samples were then centrifuged for 3 min at 12 000 rpm and 4 ˚C to pellet the beads with the supernatant transferred to a new tube. Samples were then extracted with an equal volume of phenol-chloroform-isoamylalcohol (24:24:1) by thorough mixing and centrifugation for 3 min at 12 000 rpm. Nucleic acids were precipitated by the addition of an equal volume of isopropanol and ca. 0.1× volume of 3 M sodium acetate (pH 5.2) with samples placed on ice for 30 min then centrifuged for 20 min at 12 000 rpm and 4 ˚C. Pellets were rinsed with 500 µL of chilled 70 % ethanol and then air dried for 30 min before being resuspended in 50 µL of TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8). The extracted DNA was purified using the Wizard DNA Clean-Up System (Promega, Madison) and eluted in 50 µL of TE buffer

A1.5.2 Amplification, cloning and sequencing of archaeal 16S rDNA

PCR amplification of archaeal 16S rDNA was performed using the archaeal-specific primer 1Af (forward) (5'-TCYGKTTGATCCYGSCRGAG-3' (Munson, Nedwell et al., 1997) and the universal primer 1492r (5'-TACGGYTACCTTGTTACGACTT-3'). PCR reactions of total volume 100 µL contained 10 µL of 10× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleoside triphosphate, 0.2 µg of each primer, 0.1 to 0.2 µg of DNA template, 2 U of BioTaq polymerase (Bioline, London). Thermal cycling was performed with a hot start of 96 ˚C for 5 min before the addition of polymerase, followed by 28 cycles of 52 ˚C for 1 min, 72 ˚C for 2 min and 94 ˚C for 1 min with a final extension at 72 ˚C for 5 min in a thermal cycler (Hybaid, Teddington). The reaction products were visualised using 1 % agarose gel electrophoresis and then purified using Wizard PCR-Prep DNA purification system (Promega, Madison). Purified PCR product was inserted into the TA cloning vector (pCR2.1) using the TOPO-TA Cloning Kit (Invitrogen Corporation, San Diego) and then transformed into provided competent *Escherichia coli* cells. Plasmid inserts were amplified by PCR using the M13 primer set (Invitrogen Corporation), and full-sized-insert clones were screened using the two restriction endonucleases *Hha*I and *Hae*III (New England Biolabs) and visualised using 2.5 % agarose gel electrophoresis. Clones possessing different restriction profiles were selected for sequence analysis. Plasmid preparations from selected clones for DNA sequencing was performed using the Flexi-Prep Kit (Pharmacia). Automated DNA sequencing was performed on ABI Model 377 sequencer (Applied Biosystems) using the M13 primer set.

A1.5.3 Sequence analysis

Sequence data were aligned and analysed using the program package ARB (available at http://www.mikro.biologie.tu-muenchen.de/) by comparing clone sequences with the database released with ARB and the RDP_SSU database (Maidak, Cole et al., 1999). The program Check_Chimera was used to screen clone sequence data for the presence of chimeras (Maidak, Cole et al., 1999). Other sequences were obtained from GenBank for inclusion in analyses. Phylogenetic trees were constructed using distance matrix calculations employing the Jukes-Cantor correction and neighbour joining

functions available within the ARB program. Bootstrapping (100 samplings) was used to test the stability of branching patterns within the phylogenetic trees.

A-2

Calibrations

A2.1 CHEMICAL OXYGEN DEMAND

Potassium hydrogen phthalate (KPH) was used to prepare the standard solutions with 17 g KPH/L being equivalent to 20 g COD/L.

TABLE A2.1 : Absorbance data for COD calibration 1.

Conc (mg/L)	A610nm			
	1	2	3	Mean
0	0.000	0.000	0.000	0.000
200	0.054	0.042	0.042	0.046
500	0.093	0.091	0.096	0.093
1000	0.170	0.179	0.172	0.174
2000	0.318	0.336	0.338	0.331
3000	0.502	0.481	0.498	0.494

TABLE A2.2 : Absorbance data for COD calibration 2.

Conc (mg/L)	A610nm			
	1	2	3	Mean
0	0.000	0.000	0.000	0.000
100	0.048	0.049	0.057	0.051
200	0.063	0.069	0.068	0.067
500	0.114	0.117	0.114	0.115
1000	0.202	0.189	0.216	0.202
2000	0.338	0.367	0.335	0.347
3000	0.488	0.511	0.499	0.499

TABLE A2.3 : Absorbance data for COD calibration 3.

Conc (mg/L)	A610nm						
	1	Corrected	2	Corrected	3	Corrected	Mean
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100	0.048	0.013	0.049	0.014	0.057	0.022	0.016
200	0.063	0.028	0.069	0.034	0.068	0.033	0.032
500	0.114	0.079	0.117	0.082	0.114	0.079	0.080
1000	0.202	0.167	0.189	0.154	0.216	0.181	0.167
2000	0.338	0.303	0.367	0.332	0.335	0.300	0.312
3000	0.488	0.453	0.511	0.476	0.499	0.464	0.464

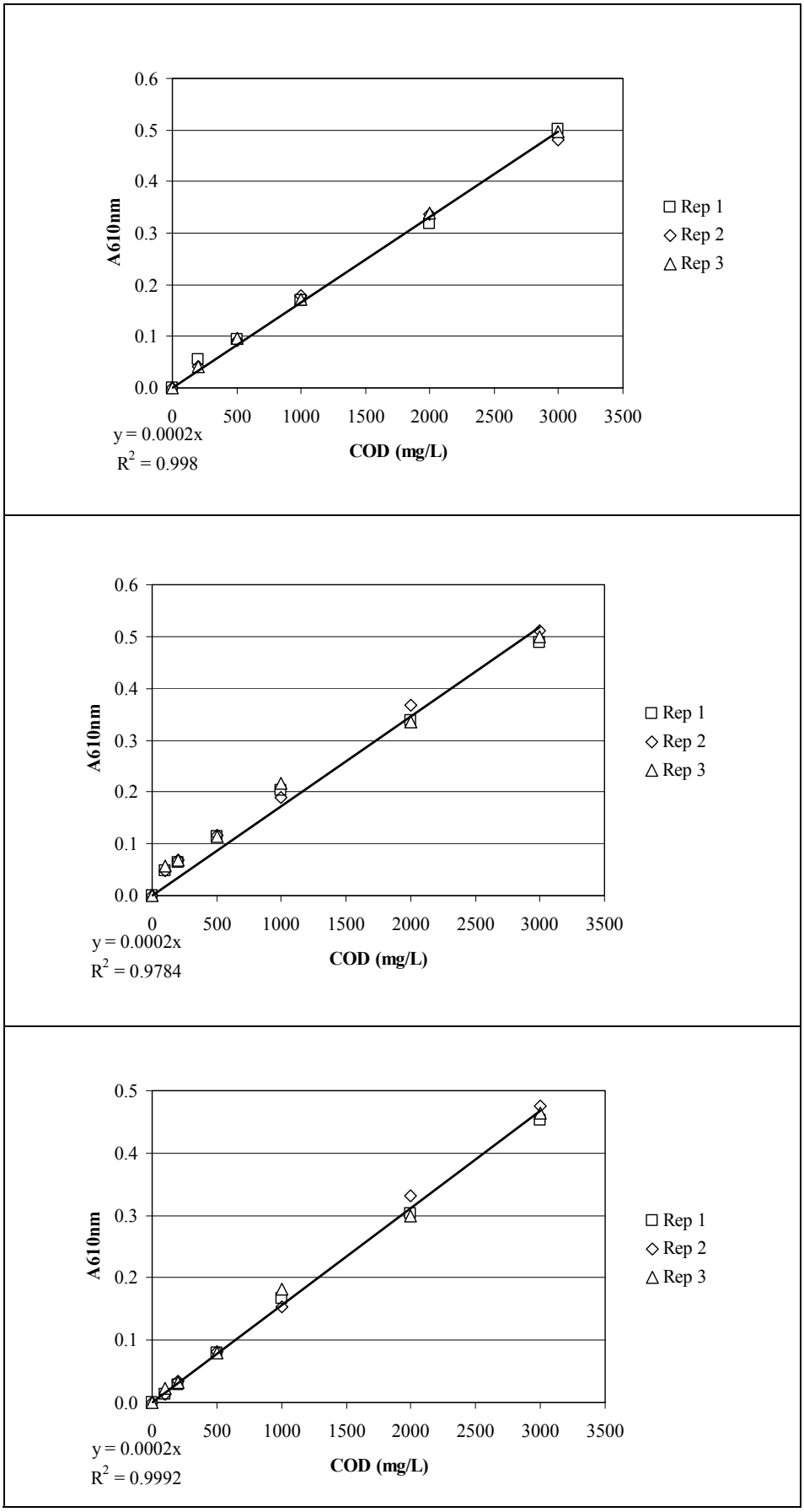


FIGURE A2.1 : COD calibration plots.

The measured absorbance of the COD standard solutions was compared with the absorbance of glucose solutions, made up to known COD concentrations, to assess the accuracy of the COD method.

TABLE A2.4 : Absorbance data for the COD calibration test comparing the COD of KHP and glucose solutions.

Conc (ppm)	A610nm						
	1	Corrected	2	Corrected	3	Corrected	Mean
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100	0.044	0.009	0.072	0.037	0.059	0.024	0.023
200	0.109	0.074	0.076	0.041	0.068	0.033	0.049
500	0.126	0.091	0.144	0.109	0.146	0.111	0.104
1000	0.156	0.121	0.196	0.161	0.206	0.171	0.151
2000	0.335	0.300	0.331	0.296	0.335	0.300	0.299
3000	0.501	0.466	0.512	0.477	0.472	0.437	0.460

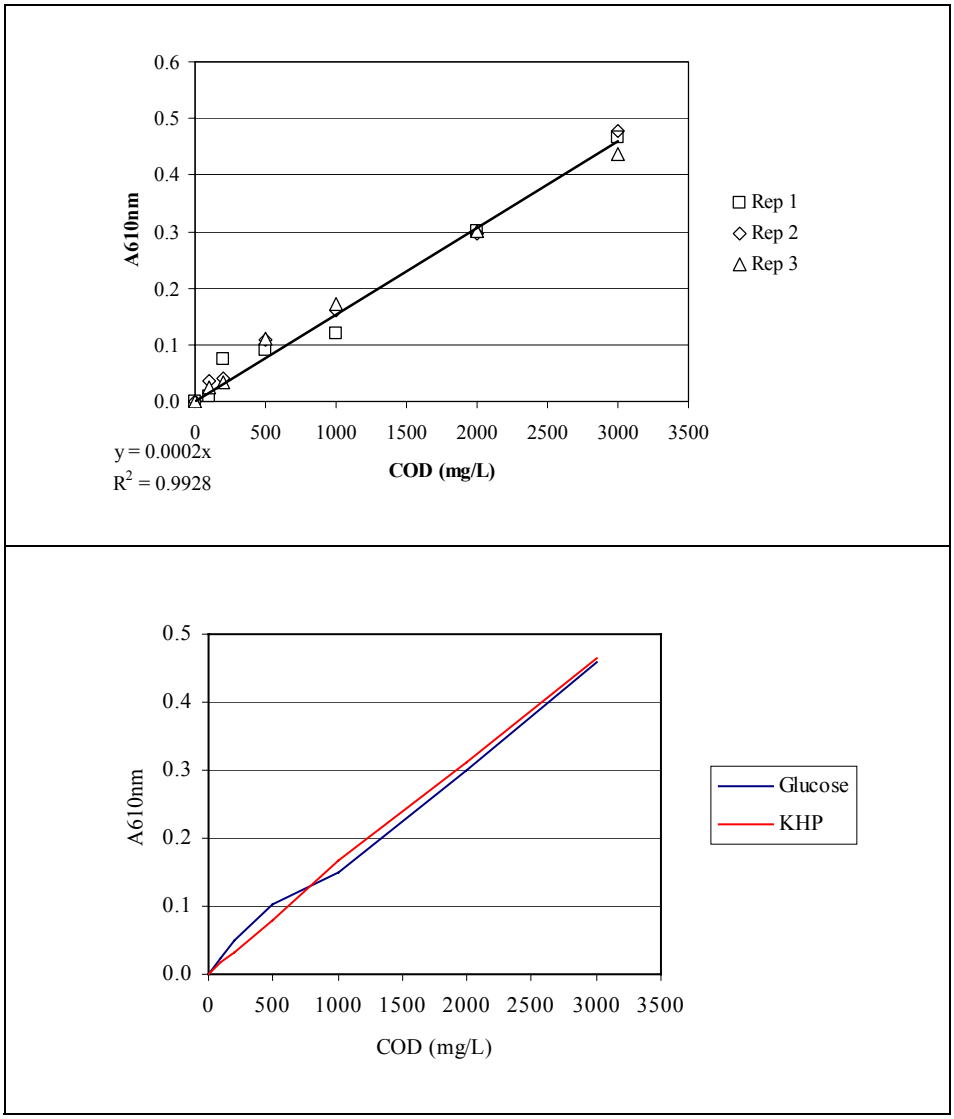


FIGURE A2.2: Comparison of the COD absorbance measurements of KHP standard solutions and glucose standard solutions.

A2.2 BIOGAS

A2.2.1 Methane

TABLE A2.5 : Methane calibration data.

Volume (µL)	Moles	Rep 1	Rep 2	Mean
0	0	0	0	0
20	8.18E-07	3829	3134	3481.5
40	1.64E-06	9914	10952	10433
60	2.45E-06	18993	19448	19220.5
80	3.27E-06	30114	29610	29862
100	4.09E-06	40834	40998	40916

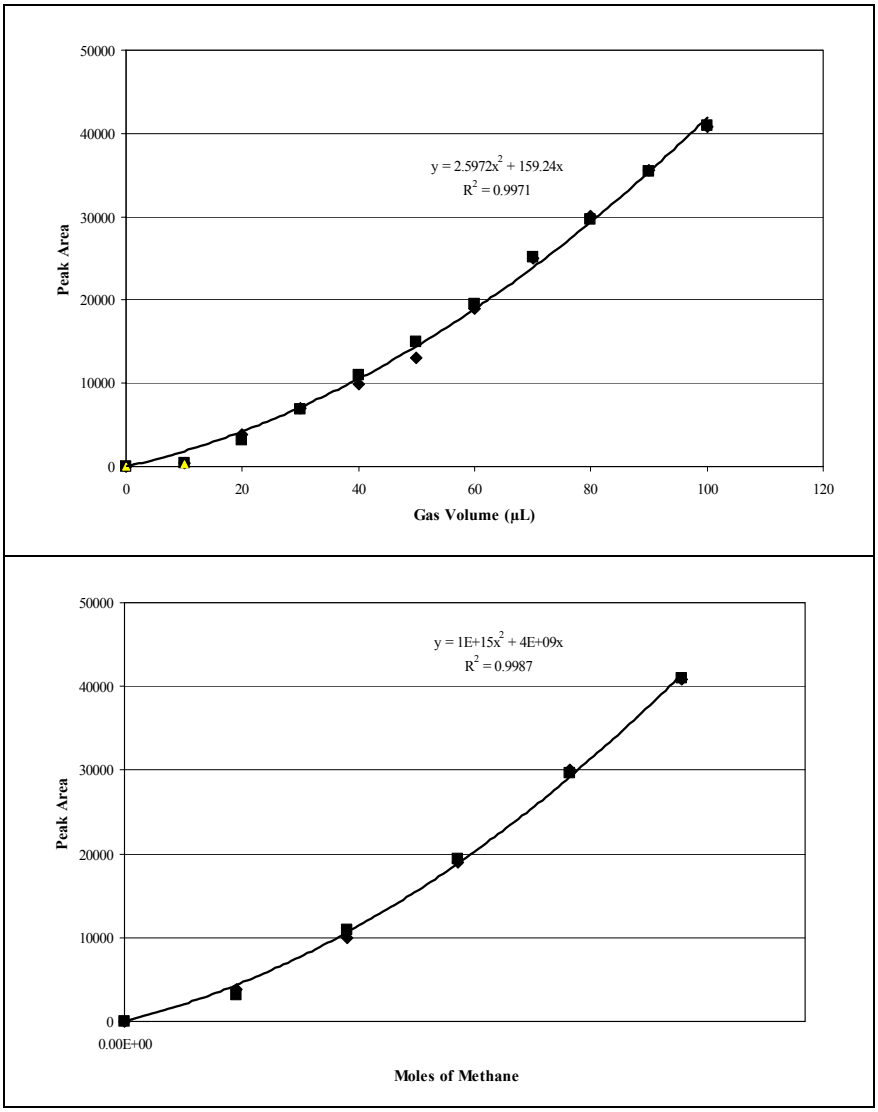


FIGURE A2.3 : Methane calibration curves.

A2.2.2 Carbon Dioxide

TABLE A2.6 : Carbon dioxide calibration data.

Volume (µL)	Moles	Rep 1	Rep 2	Mean
0	0	0	0	0
20	8.18E-07	2762	2744	2753
40	1.64E-06	14422	17443	15932.5
60	2.45E-06	24693	23704	24198.5
80	3.27E-06	38869	39937	39403
100	4.09E-06	54404	46312	50358

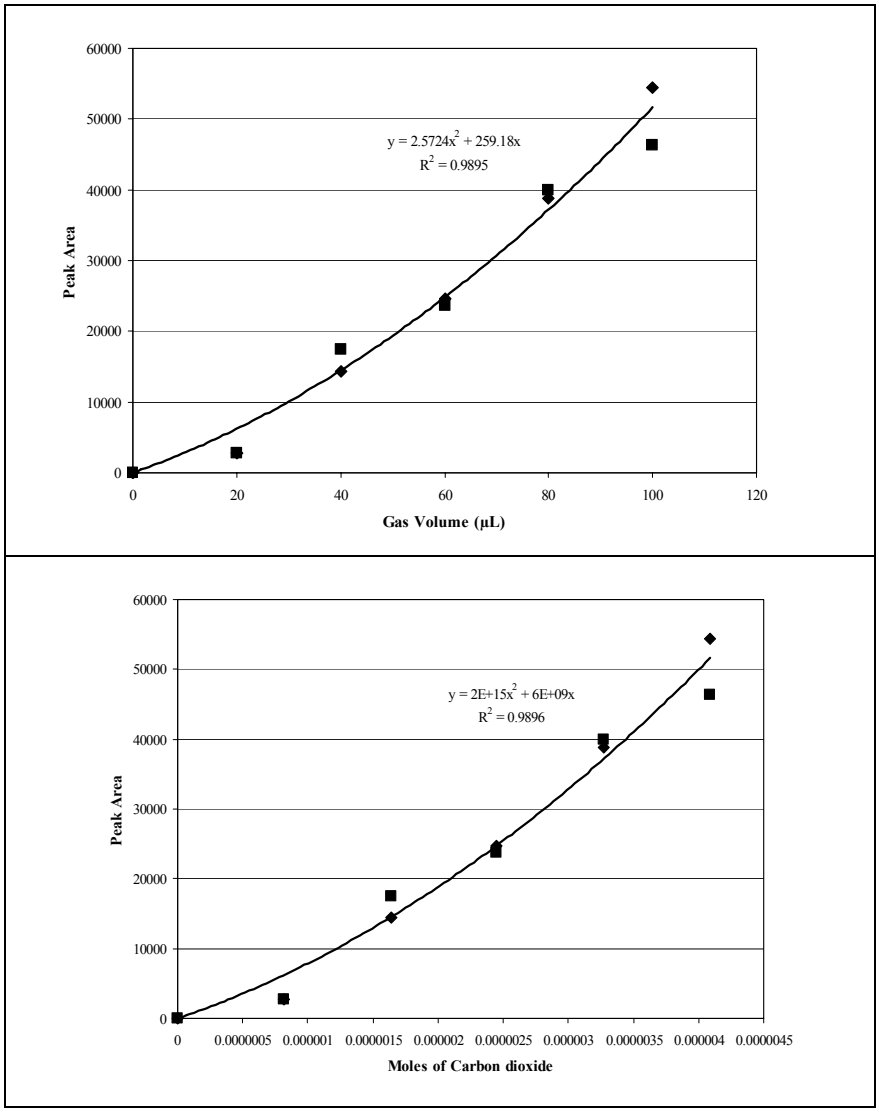


FIGURE A2.4 : Carbon dioxide calibration curves.

A2.2.3 Nitrogen

TABLE A2.7 : Nitrogen calibration data.

Volume (µL)	Moles	Rep 1	Rep 2	Mean
0	0	0	0	0
20	8.18E-07	6813	5498	6156
40	1.64E-06	9114	11031	10073
60	2.45E-06	20611	23751	22181
80	3.27E-06	39330	38231	39208
100	4.09E-06	49221	50450	49836

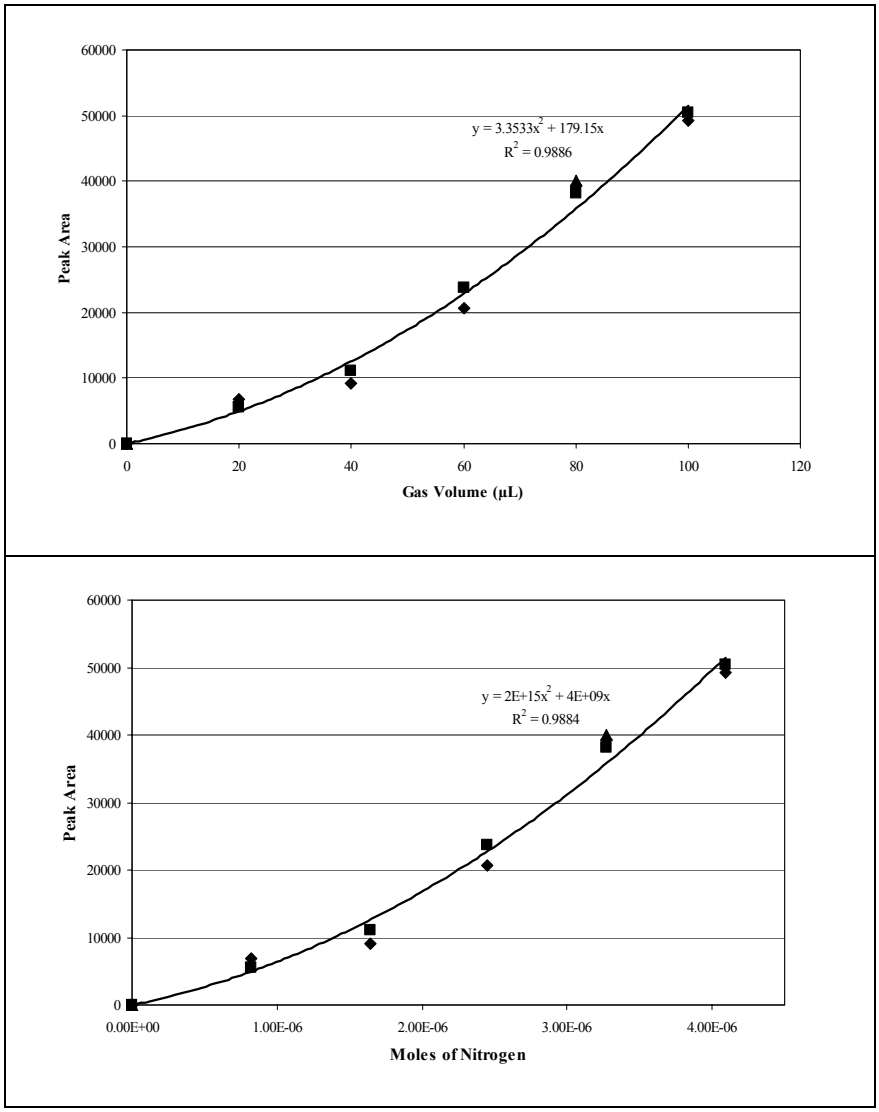


FIGURE A2.5 : Nitrogen calibration curves.

A2.3 COLOUR

A2.3.1 Tartrazine

TABLE A2.8 : Tartrazine calibration data.

Dye concentration mg/L	Absorbance 430nm
0	0
10	0.026
20	0.068
50	0.280
100	0.573
250	1.758
500	3.092

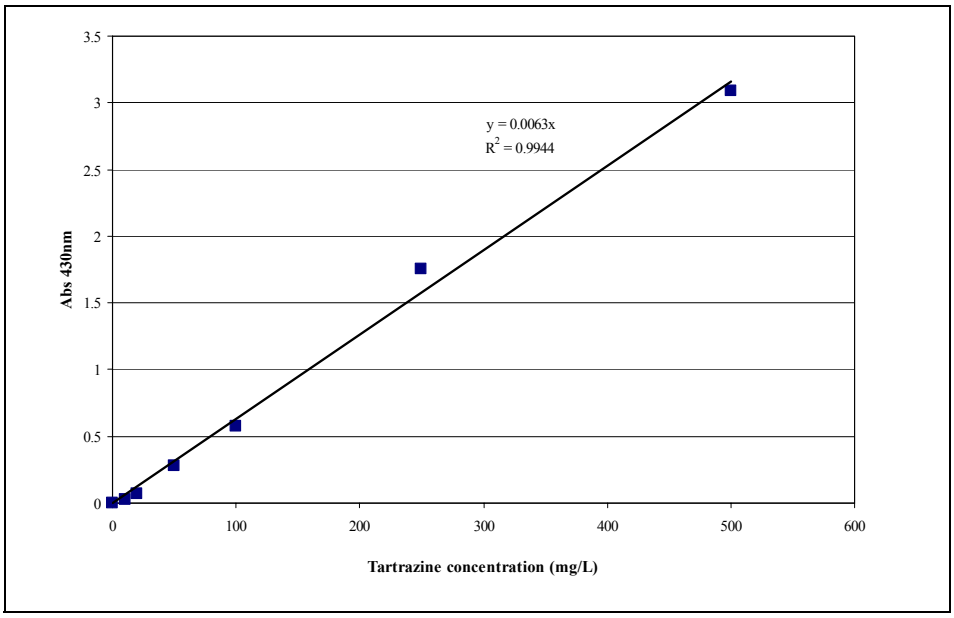


FIGURE A2.6 : Tartrazine calibration curve.

A2.3.2 CI Reactive Red 141

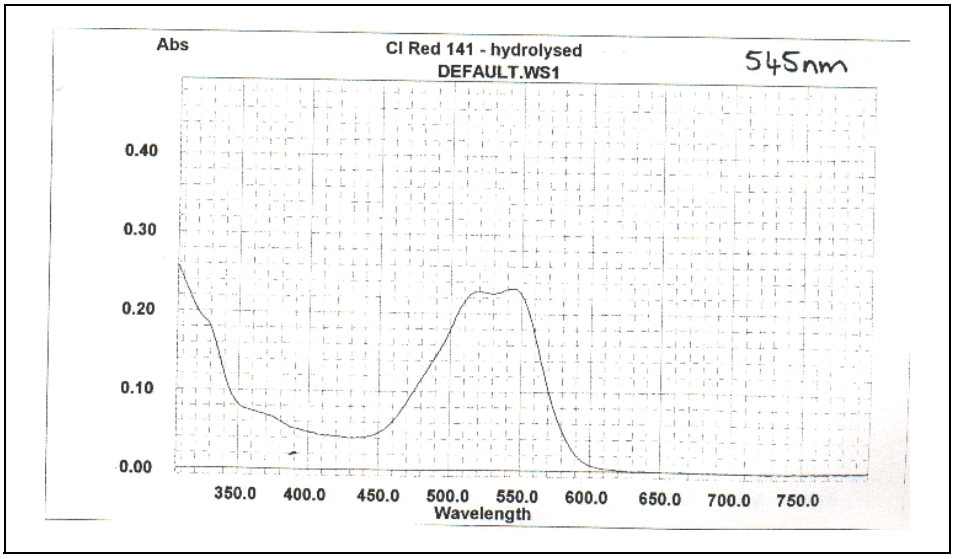


FIGURE A2.7 : Wavelength scan of CI Reactive Red 141, showing the maximum absorbance at 545 nm.

TABLE A2.9 : CI Reactive Red 141 calibration data.

Dye concentration	Abs 545nm			
mg/L	1	2	3	Mean
0	0.000	0.000	0.000	0.000
10	0.208	0.203	0.208	0.206
20	0.377	0.376	0.377	0.377
50	0.888	0.889	0.884	0.887
100	1.742	1.745	1.744	1.744
150	2.442	2.444	2.447	2.444
250	2.976	2.976	2.978	2.977
500	> max	> max	> max	> max

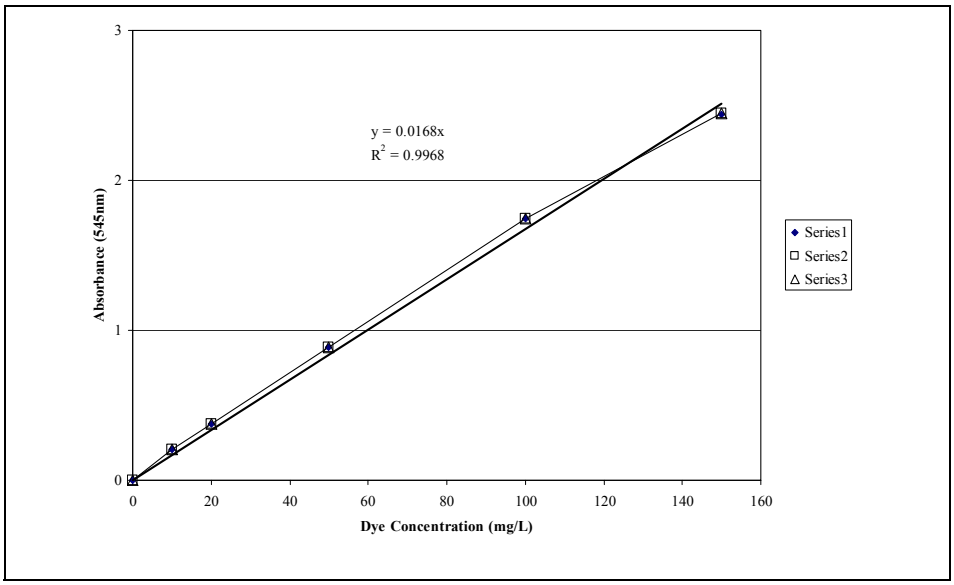


FIGURE A2.8 : CI Reactive Red 141 calibration curve.

A2.3.3 Industrial Food Dye Wastewater

TABLE A2:10 : Industrial dye wastewater calibration data.

Wastewater concentration % (v/v)	Absorbance 500nm
0	0
5	0.240
10	0.349
20	0.665
40	1.412
50	2.087
100	3.703

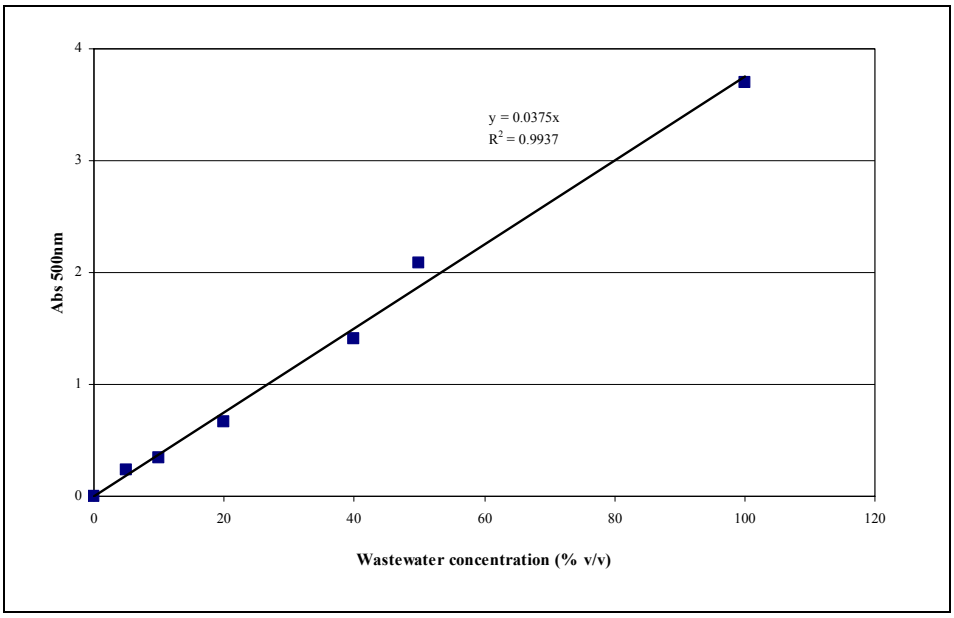


FIGURE A2.9 : Industrial dye wastewater calibration curve.

A2.4 VOLATILE FATTY ACIDS

A2.4.1 Acetic Acid

TABLE A2.11 : Acetic acid calibration data.

Concentration	Acetic			
mg/L	Rep 1	Rep 2	Rep 3	Mean
100	1116	966	1200	1094
200	1416	1608		1512
500	5532	4997	6377	5635
1000				
2000	25771	21587	22844	23401

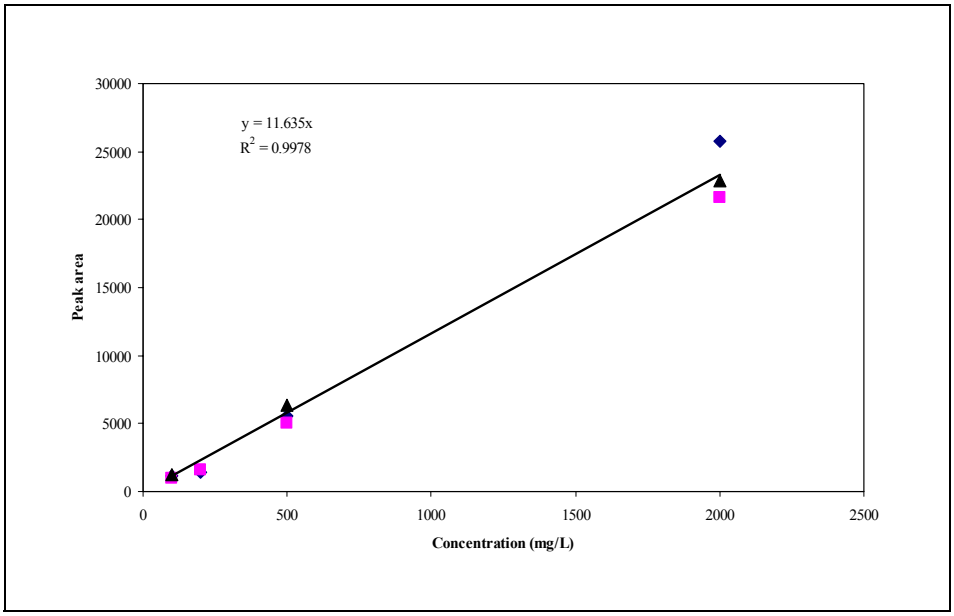


FIGURE A2.10 : Acetic acid calibration curve.

A2.4.2 Propionic Acid

TABLE A2.12 : Propionic acid calibration data.

Concentration	Propionic			
mg/L	Rep 1	Rep 2	Rep 3	Mean
100	996	1037	1552	1195
200	1917	2429		2173
500	7906	8074	8891	8290
1000	19258	19147	20344	19583
2000				

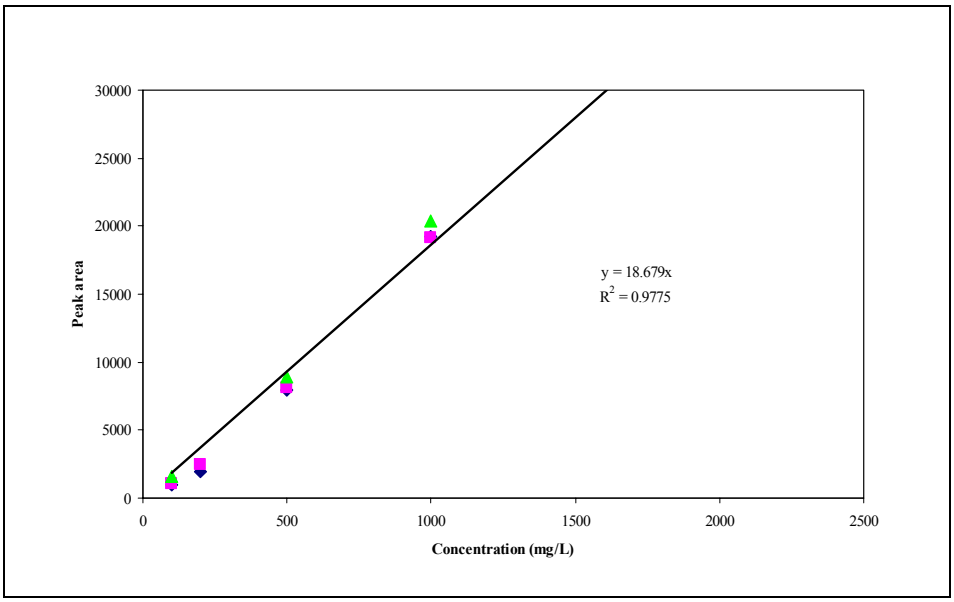


FIGURE A2.11: Propionic acid calibration curve.

A2.4.3 Iso-Butyric Acid

TABLE A2.13 : Iso-butyric acid calibration data.

Concentration	Iso-butyric			
mg/L	Rep 1	Rep 2	Rep 3	Mean
100	2858	3347		3103
200	7575	8324		7950
500	18497	23638	26324	22820
1000				
2000	67188	75979	76702	73290

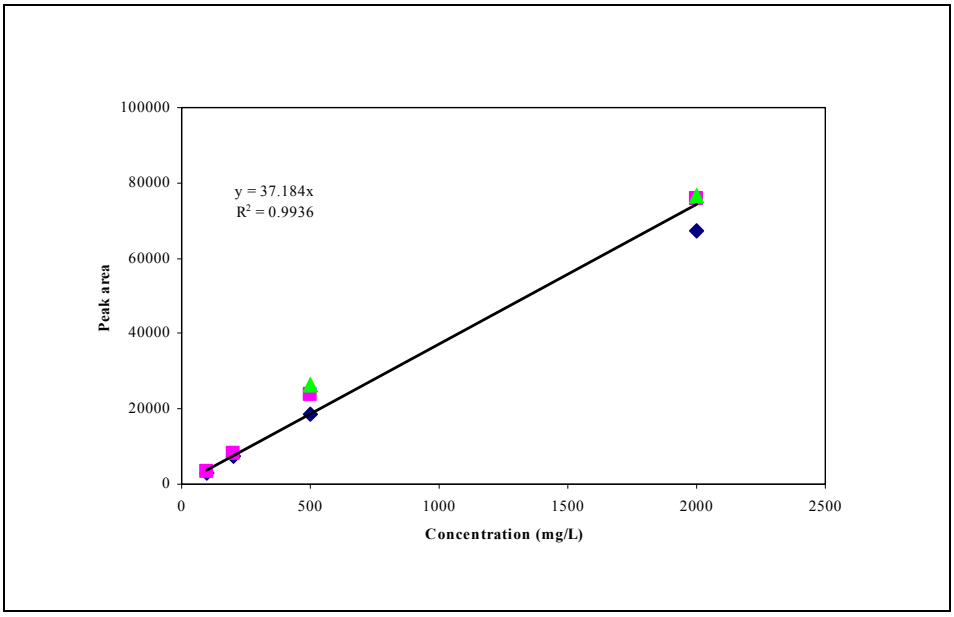


FIGURE A2.12 : Iso-butyric acid calibration curve.

A2.4.4 Butyric Acid

TABLE A2.14 : Butyric acid calibration data.

Concentration	Butyric			
mg/L	Rep 1	Rep 2	Rep 3	Mean
100	1783	2156		1970
200	5886	6064		5975
500	16918	20704	20846	19489
1000				
2000	62710	70843	70057	67870

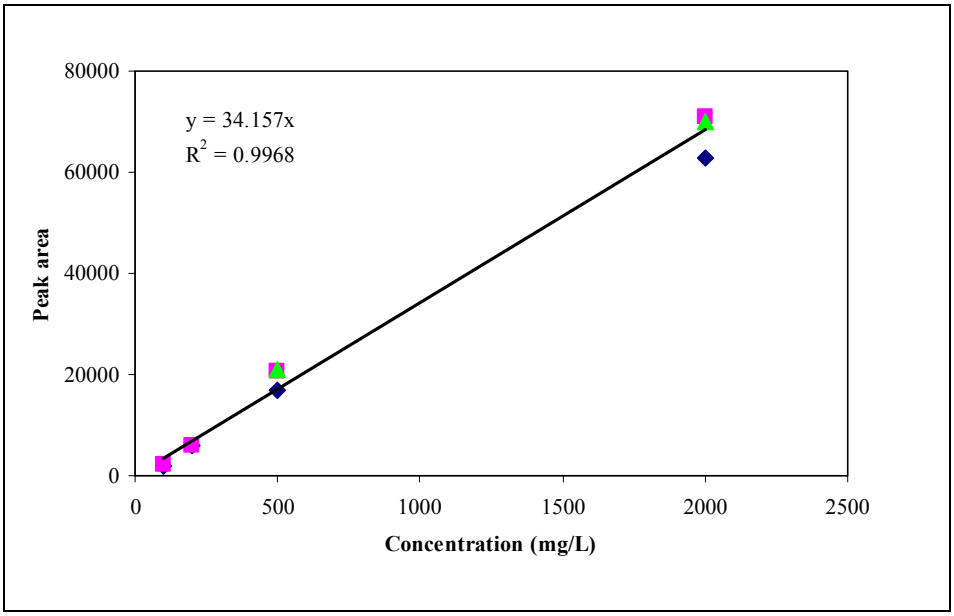


FIGURE A2.13 : Butyric acid calibration curve.

A2.4.5 Iso-Valeric Acid

TABLE A2.15 : Iso-valeric acid calibration data.

Concentration	Iso-valeric			
mg/L	Rep 1	Rep 2	Rep 3	Mean
100	2425	2939		2682
200	7645	8838		8242
500	20776	25419	26296	24164
1000				
2000	75238	85376	84475	81696

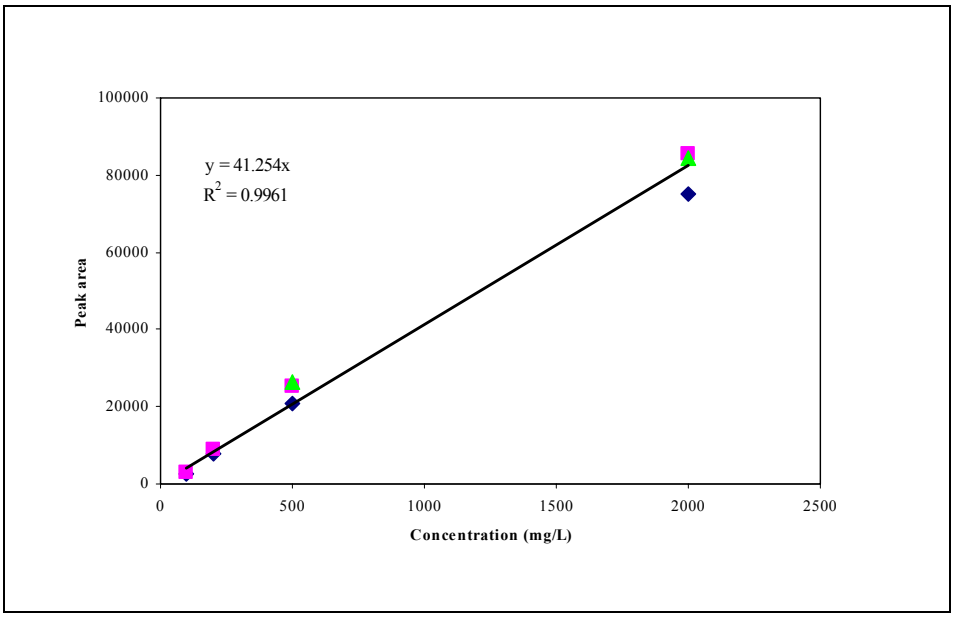


FIGURE A2.14 : Iso-valeric acid calibration curve.

A2.4.6 Valeric Acid

TABLE A2.16 : Valeric acid calibration data.

Concentration	Valeric			
mg/L	Rep 1	Rep 2	Rep 3	Mean
100	1440	1707		1574
200	8514	7086		7800
500	19682	24965	23643	22763
1000				
2000	75312	81228	78749	78430

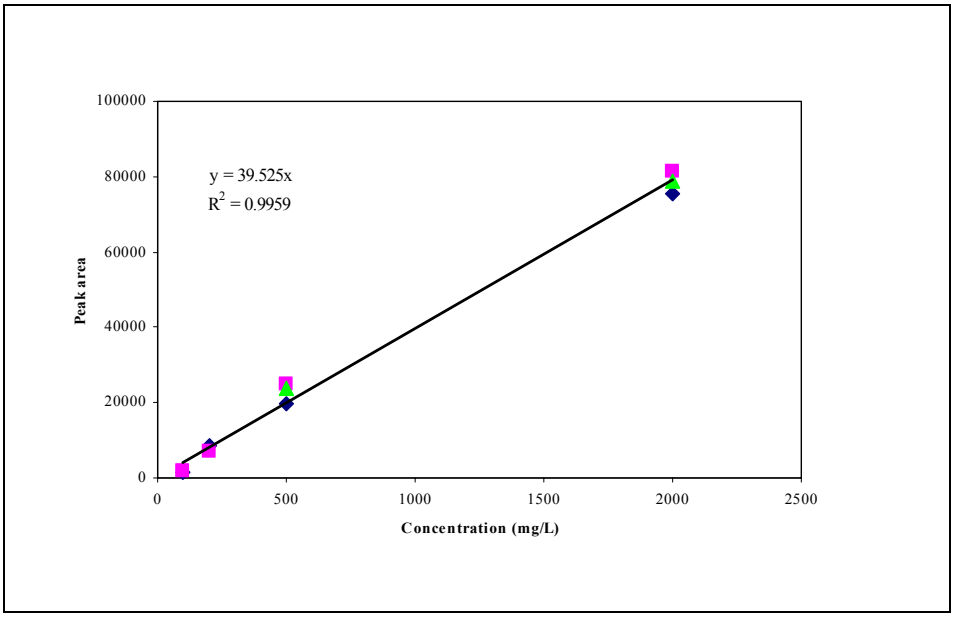


FIGURE A2.15: Valeric acid calibration curve.

A-3

Food Dyes

Anaerobic toxicity assays were set up in serum bottles, according to the method of Owen *et al*., 1979). The results of the anaerobic toxicity assays were used to guide the set-up of the biodegradability assays. Although these assays provided valuable information in that the concentration at which each dye became inhibitory to the anaerobic biomass, and the ultimate anaerobic biodegradability of the dyes was determined, these results could not be directly applied to the ABR. The reason for this is that the serum bottle test approximated a CSTR with a mixed anaerobic sludge, whereas in the ABR, the biomass within each compartment differs as does the substrate entering each compartment.

The details of these experiments, the results and discussion are described below.

A3.1 ANAEROBIC TOXICITY ASSAYS

Toxicity monitoring is useful for determining, in advance, potential toxic or inhibitory effects of an industrial effluent. Source identification and control of toxicants is the most effective management strategy (Willetts, 1999). Anaerobic toxicity assays can be used to determine the IC_{50} value and thus quantitatively describe the inhibitory effect of a given compound on the anaerobic biomass. The method followed in this study was that described by Owen *et al*. (1979). Bioassay techniques for measuring the presence or absence of inhibitory substances are effective since they are simple to set up, several substances can be tested simultaneously, they are inexpensive, and do not require knowledge of specific inhibitory substances (Owen *et al*., 1979). In these batch bioassays, anaerobic toxicity was determined as the adverse effect of a dye on the predominant methanogens. Methanogenic activity was stimulated, at the start of the test, by the addition of a *spike* containing the methanogenic precursors, acetate and propionate. The methanogenic metabolism of the acetate-propionate solution was monitored by total gas production, in the controls. Inhibition due to dye addition was determined as a decreased rate of gas production, relative to the controls. The first week of incubation was critical as these data reflected the true, unadapted response of the microorganisms to the dyes.

A3.1.1 Hypotheses and Objectives

Dye compounds and their degradation products can be toxic to humans, animals and microorganisms. Bioassay techniques for measuring the presence or absence of inhibitory substances are an effective indication of the effect that these substances would have on an anaerobic system.

The objective of this phase of the study was to assess the toxicity of a range of food dyes to the methanogens in anaerobic digester sludge.

A3.1.2 Materials and Methods

A food dye manufacturer provided samples of 15 food dyes, of varying chemical classes. The dyes are listed in **Table A3.1**, with both the commercial and Colour Index names. The classification into chemical class is dependent on the structure of the dye molecule and, most importantly, the chromophore type.

A stock solution (10 % w/v) of each dye was made up. The experiments were performed in 160 mL glass serum bottles, which were sealed with butyl rubber septa and aluminium crimp seals. A defined nutrient medium containing trace elements, minerals and vitamins was prepared according to Owen *et al*. (1979), with some modifications. The method for the preparation of the stock solutions and the nutrient medium are presented in **Appendix 1.**

TABLE A3.1 : List of food dyes investigated for inhibition of methanogenic activity.

Commercial Dye	Colour Index Classification	Dye Class
Sunset Yellow Supra	CI Food Yellow 3	Monoazo
Amaranth Supra	CI Food Red 9	Monoazo
Carmoisine Supra	CI Food Red 3	Monoazo
Brown FK Standard	CI Food Brown 1	Monoazo
Allura Red AC Supra	CI Food Red 17	Monoazo
Red 2G Supra	CI Food Red 10	Monoazo
Tartrazine Supra	CI Food Yellow 4	Monoazo
Ponceau 4R Supra	CI Food Red 7	Monoazo
Black PN Extra	CI Food Black 1	Disazo
Green S Supra	CI Food Green 4	Triarylmethane
Patent Blue V Supra	CI Food Blue 5	Triarylmethane
Brilliant Blue Supra	CI Food Blue 2	Triarylmethane
Quinoline Yellow Extra	CI Food Yellow 13	Quinoline
Erythrosine Supra	CI Food Red 14	Xanthene
Indigo Carmine Supra	CI Food Blue 1	Indigoid

The objective of a toxicity assay is to determine the concentration at which a substance becomes inhibitory to the biomass, thus, the serum bottles were dosed with a range of dye concentrations, to provide a range from non-inhibitory to toxic. The dye concentrations investigated, for each dye, were: 50 mg/L; 250 mg/L; 1 g/L; 5 g/L; 10 g/L and 20 g/L. Each concentration was repeated in triplicate.

The assay bottles were overgassed with a gas mixture containing 70 % N_2, 30 % CO_2 at a flow rate of 0.5 mL/min for 15 min. A 20 % (v/v) inoculum was used in each serum bottle, which was equivalent to

20 mL of anaerobic digester sludge (suspended; from Mogden Sewage Works) in a total working volume of 100 mL. The biomass was mixed with 30 mL of the nutrient medium. The dye stock solution was diluted to the required concentration, with deionised water, to make up a volume of 48 mL. The bottles were overgassed and sealed. After equilibration for 1 h, at the incubation temperature of 35 °C, the gas volumes were zeroed to ambient pressure with a glass syringe. The acetate-propionate solution (2mL, to give 75 mg acetate and 26.5 mg propionate in 100 mL working volume) was added to each bottle by means of a 2 mL glass syringe and hypodermic needle.

The anaerobic toxicity assays were also run with two industrial effluents. These were sampled at the food dye manufacturing factory. The company employed a chemical treatment of the final effluent (reducing agent, sodium dithionite), in an attempt to remove some of the colour. Effluent samples before and after chemical treatment were taken. A range of effluent concentrations was investigated: 20; 40; 60; 80 and 100 % (v/v) of the effluent, diluted in deionised water. These serum bottles were set up in the same manner as for the dye tests.

The controls, or blanks, contained only the inoculum sludge, the anaerobic nutrient medium and the acetate-propionate solution. The methanogenic metabolism of the acetate-propionate solution was monitored by total gas production, in the controls. Inhibition due to the addition of a dye was determined as a decreased rate of gas production, relative to the controls.

Gas volume measurement during incubation was performed with a graduated glass syringe (20 mL). The syringe was initially flushed with the 70 % N_2, 30 % CO_2 gas mixture and lubricated with deionised water. The syringe needle was inserted through the rubber septum into the headspace. Readings were taken at the incubation temperature and the syringe was held horizontal for measurement. Volume determinations were made by allowing the syringe plunger to move and equilibrate between the bottle and the atmospheric pressure. Readings were verified by drawing the plunger past the equilibrium point and releasing to ensure that the plunger returned to the original equilibration volume. Gas was either re-injected into the bottle, with minimal loss, or wasted with concurrent determination of the biogas composition.

The dye concentration at which 50 % of the methanogenic population was inhibited (IC_{50}) was calculated for each dye. Total biogas was measured during the incubation period. The methane composition of the biogas was determined, thus the methane fraction of the biogas was known. At the commencement of the tests, the total solids (TS) and volatile solids (VS) of the inoculum sludge were measured (**Appendix 1**). The methanogenic activity could thus be calculated (mL CH_4/g VS) for each dye concentration, and calculated as a fraction of the methanogenic activity in the controls. The activity at each concentration was plotted and a best-fit line was plotted through the data points. From the equation of the line, the dye concentration at which 50 % of the methanogenic biomass was inhibited (IC_{50}) could be calculated. These results are given below.

A3.1.3 Results

The gas production results, for each dye and wastewater investigated, are given in **Section A3.1.6**. The symbols represent the replicate samples, and the line through the data points is the calculated mean biogas production. The apparent change in the gradient of this line is due to a change in the values on the x-axis since some tests were run for a slightly longer time period than others. The gas production rate in the controls, represented by the black line, is, however, the same in all samples and figures. For each concentration, the gas production curve is shown relative to the gas production rate of the controls. A decrease in biogas production (line below that of the control) indicated inhibition of the methanogens due to addition of the dye.

The methanogenic activity for each dye concentration was calculated from the measured biogas volume and the analysed methane fraction. This activity was calculated as a percentage of the activity in the controls; no inhibition would have an activity of 100 %. From these data, the dye concentration at which 50 % of the methanogenic population was inhibited (IC_{50}) was calculated for each dye. These values are given in **Table A3.2**.

TABLE A3.2: Calculated methanogenic IC_{50} values for the investigated food dyes.

Dye	Molec. mass (g/mol)	Methanogenic IC_{50}
CI Food Yellow 3	452.2	19.6 g/L
CI Food Red 3	502.4	0.25 g/L
CI Food Brown 1	848.8	2.48 g/L
CI Food Red 17	496.0	> 20 g/L
CI Food Red 10	509.0	> 20 g/L
CI Food Yellow 4	534.4	14.3 g/L
CI Food Red 7	604.3	> 20 g/L
CI Food Black 1	871.7	> 20 g/L
CI Food Green 4	530.0	19.5 g/L
CI Food Blue 5	663.0	2.15 g/L
CI Food Blue 2	794.9	5.55 g/L
CI Food Yellow 13	401.2	8.38 g/L
CI Food Red 14	879.9	0.2 mg/L
CI Food Blue 1	468.3	14.03 g/L
Untreated Food Dye Effluent	-	22.5 % (v/v)
Treated Food Dye Effluent	-	15.9 % (v/v)

A3.1.4 Discussion

The presence of toxic compounds during the anaerobic degradation process can inhibit the normal sequence of anaerobic metabolic reactions, thereby causing inefficient treatment and possibly even failure (Razo-Flores *et al*., 1997). Of all the classes of organisms involved in anaerobic degradation, the methanogens are reported to be the most sensitive and slowest growing and thus a toxic shock to them is most detrimental (Kugelman and Chin, 1971; Speece, 1983; Razo-Flores *et al*., 1997). Inhibition of methanogenesis is generally indicated by reduced methane production and increased concentration of VFAs. The literature has indicated that dye compounds and their degradation products can be toxic. Donlon *et al*., (1997) found that, in batch toxicity assays, azo dye compounds were many times more toxic to methanogenic activity than their cleavage products (aromatic amines).

The reduction potential within each compartment was not measured because it was not possible to do so without exposing the anaerobic environment to oxygen contamination. Chung and Stevens (1993) found that the measurement of reduction potentials for aromatic compounds was not useful for estimating their rates of disappearance in anaerobic environments.

CI Food Red 9 was not included in the study as it was insoluble in both water and ethanol. The defined nutrient medium (**Appendix 1**) contained nutrients and vitamins required by anaerobic cultures. Resazurin was added to detect oxygen contamination. Sodium sulphide was included to provide a reducing environment and sodium bicarbonate ($NaHCO_3$) to provide buffering, for alkalinity control.

Table A3.2 gives the molecular mass of each dye molecule, to provide an indication of the variability in dyes that are available commercially and also because the size of the dye molecule would affect its ability to permeate the microbial cells. The molecular masses ranged between 400 and 900 g/mole. According to the literature, the average dye concentration, in an effluent, is ca. 1 mg/L. Dye concentrations usually investigated in laboratory studies range between 1 and 250 mg/L. Thus, it was believed that the wide concentration range used in these toxicity assays (50 mg/L to 20 g/L) should incorporate concentrations at which each dye was both non-inhibitory and inhibitory to the methanogens.

A wide range of toxicity data were obtained from these tests (**Table A3.2**), with IC_{50} values ranging from > 20 g/L (highest dye concentration investigated) to as low as 0.2 mg/L. It was surprising to find that some of the dyes, currently used to colour foodstuffs, were toxic to the methanogens at concentrations < 1 mg/L. These dyes could be problematic in the anaerobic treatment of dye wastewaters since they could cause inhibition of the methanogens present in the treatment system, resulting in reactor failure and inefficient treatment.

The gas production plots shown in **Section A3.1.6** suggest, in some cases, that the IC_{50} value would differ from those values given in **Table A3.2**. This discrepancy can be attributed to the fact that the calculated IC_{50} values are based entirely on methanogenic inhibition, whereas the plots show total biogas production measured over the incubation period. The fraction of methane in the biogas was calculated from the biogas composition analyses. The addition of the acetate-propionate solution made these tests specific for

methanogenic inhibition since the added VFAs are precursors to methanogenesis. To determine the inhibition to the acidogenic species in the digester sludge inoculum, a substrate such as glucose could be added and biogas production monitored relative to the controls.

No inhibition was observed in several of the dyes, including CI Food Yellow 3, Red 17, Red 10, Red 7, Black 1 and Green 4. Addition of these dyes did not cause 50 % inhibition at concentrations as high 20 g/L, and since it is unlikely that they would be present in higher concentrations in a wastewater, it was concluded that they would not have an inhibitory effect on an anaerobic treatment system. However, some of the investigated dyes did show toxicity to the methanogens. The two most toxic dyes were CI Food Red 3 (IC_{50} of 0.25 g/L) and CI Food Red 14 (IC_{50} of 0.2 mg/L). Although the lowest dye concentration investigated was 50 mg/L, this concentration of 0.2 mg/L could be calculated from the toxicity equation. These dyes could be problematic in anaerobic treatment if they were present in concentrations greater than the calculated IC_{50} concentrations. This could easily occur with wastage or washing procedures at the factory resulting in a large volume of the dye in the final effluent. Further tests would have to be conducted to determine whether the methanogenic biomass could acclimate to these inhibitory dyes.

The monoazo dye, tartrazine (CI Food Yellow 4) was of interest due to the large volume produced by the food dye manufacturer. The anaerobic toxicity assay showed the dye to be relatively non-inhibitory with an IC_{50} concentration of 14.3 g/L. These results were promising as they indicated that anaerobic treatment was a possibility for tartrazine waste streams. Results from the tests with the real industrial wastewaters showed that, after chemical treatment, the effluent became more inhibitory to the methanogenic biomass. Overall, the tests showed the food dye effluent to be inhibitory to the methanogens with IC_{50} values of 22.5 % and 15.9 % (v/v), for the untreated and chemically treated effluents, respectively. The increased inhibition after chemical treatment could be due to the presence of toxic aromatic amines that have been found to be released, from the combined form, by reduction with dithionite (Prival *et al*., 1993).

In similar batch toxicity assays, Razo-Flores *et al*. (1997) found selected azo dye compounds to be toxic towards methanogenic activity in anaerobic granular sludge. Considering the ability of anaerobic microorganisms to reduce and decolourise azo compounds, acclimation of the methanogens to the azo dyes is likely during anaerobic treatment. The objective of these experiments was to provide an initial indication of the characteristics of the food dyes, and to provide toxicity data on which further biodegradability tests could be based. Biodegradability tests would provide information on microbial metabolism of the dyes and acclimation of the microorganisms to the inhibitory dyes.

Acclimation may provide a solution to certain toxicity problems. The magnitude of the toxic or inhibitory effect to biomass caused by a substance can often be reduced significantly if the concentration of that substance is increased slowly over a given period, a process defined as acclimation (Speece, 1996). The same concentration of the toxicant which is inhibitory to an unacclimated biomass, causing complete termination of activity, may show no reduction in activity to a properly acclimated biomass. Through acclimation, the threshold toxicity concentrations of certain toxicants can be increased as much as ten-fold (Speece, 1996). Acclimation involves an adjustment of the biological population to the adverse

effects of the toxin, e.g., existing microorganisms may rearrange their metabolic resources to overcome the metabolic block produced by the substance. In addition, selection of those microorganisms that are innately more tolerant to the toxin occurs over time.

A3.1.5 Conclusions

1. The toxicity assays were specific to the methanogenic populations of the anaerobic digester sludge.

2. From the 15 investigated food dyes, a wide range of toxicity data were obtained with IC_{50} values ranging from > 20 g/L to 0.2 mg/L.

3. The two most inhibitory dyes were CI Food Red 3 (IC_{50} of 0.25 g/L) and CI Food Red 14 (IC_{50} of 0.2 mg/L).

4. The IC_{50} concentration of tartrazine was 14.3 g/L.

5. The food dye effluent was relatively inhibitory to the methanogens with IC_{50} values of 22.5 % and 15.9 % (v/v), for the untreated and chemically treated effluents, respectively.

6. These tests were efficient in determining the concentration at which each dye became inhibitory to the anaerobic biomass.

7. Further tests should be conducted to determine whether the methanogenic biomass could utilise the dyes as a substrate and acclimate to the inhibitory dyes.

A3.1.6 Biogas Production Plots

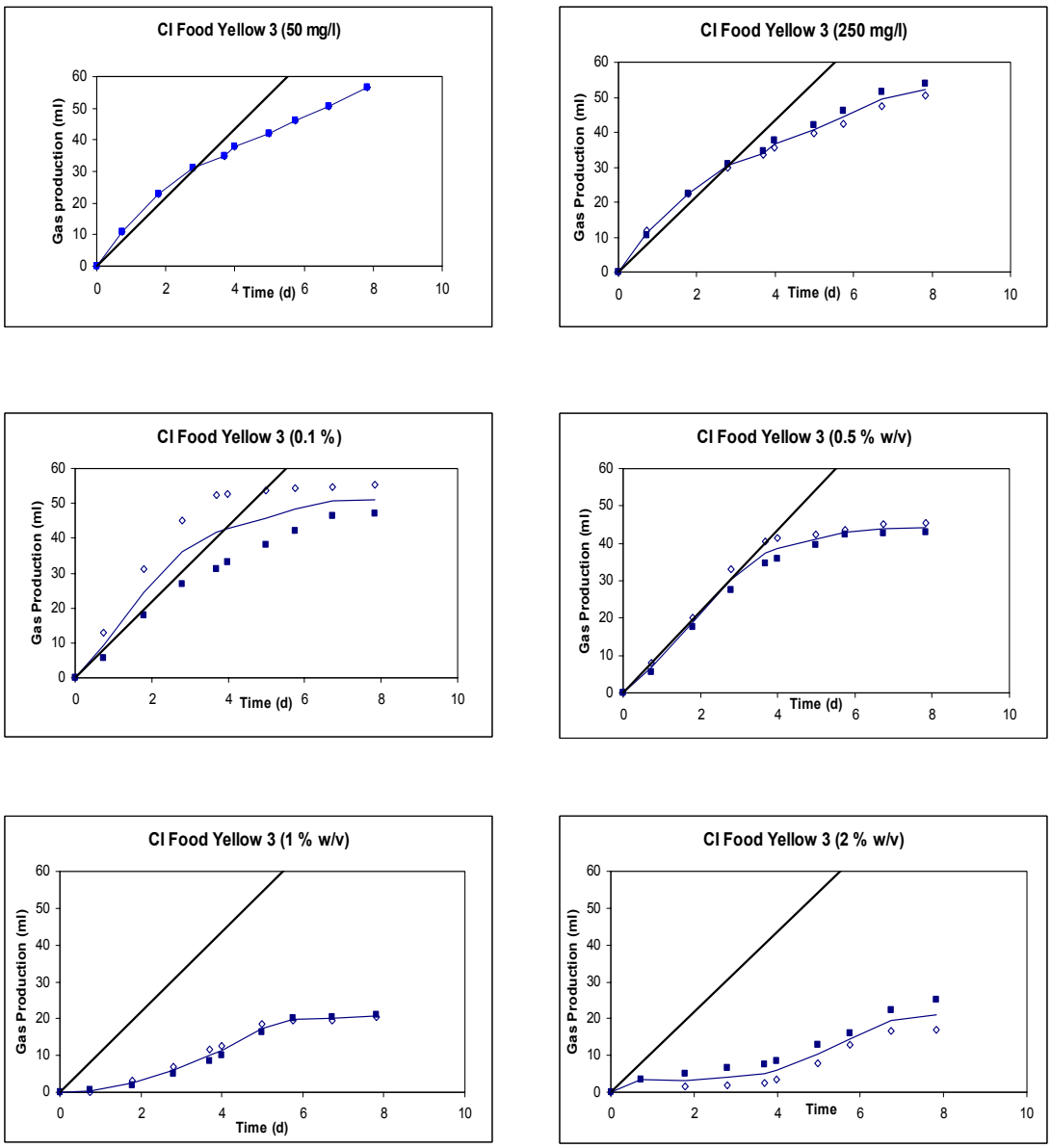


FIGURE A3.1 : Plots of biogas production during the anaerobic toxicity assay with Sunset Yellow Supra (CI Food Yellow 3).

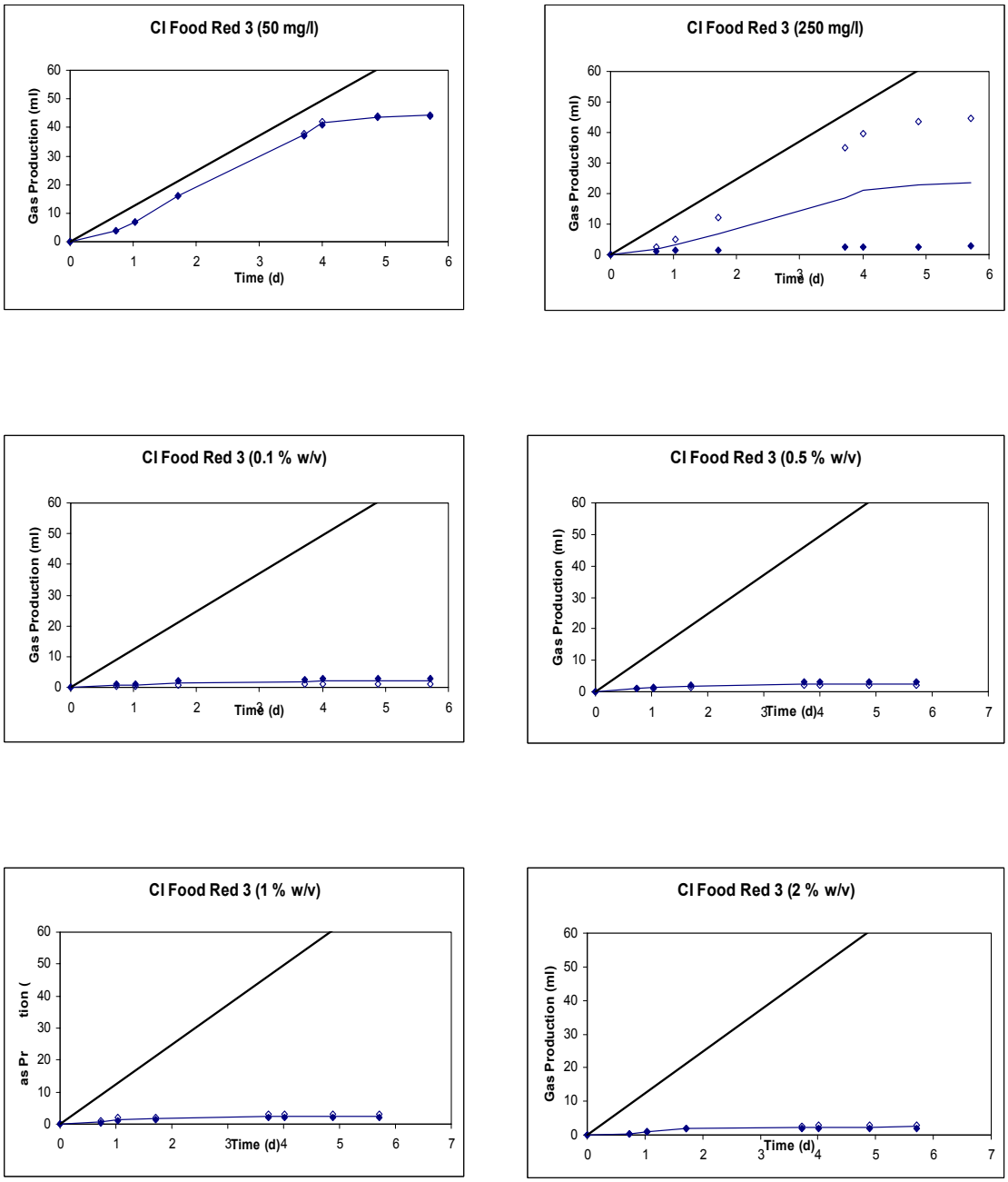


FIGURE A3.2 : Plots of biogas production during the anaerobic toxicity assay with Carmoisine Supra (CI Food Red 3).

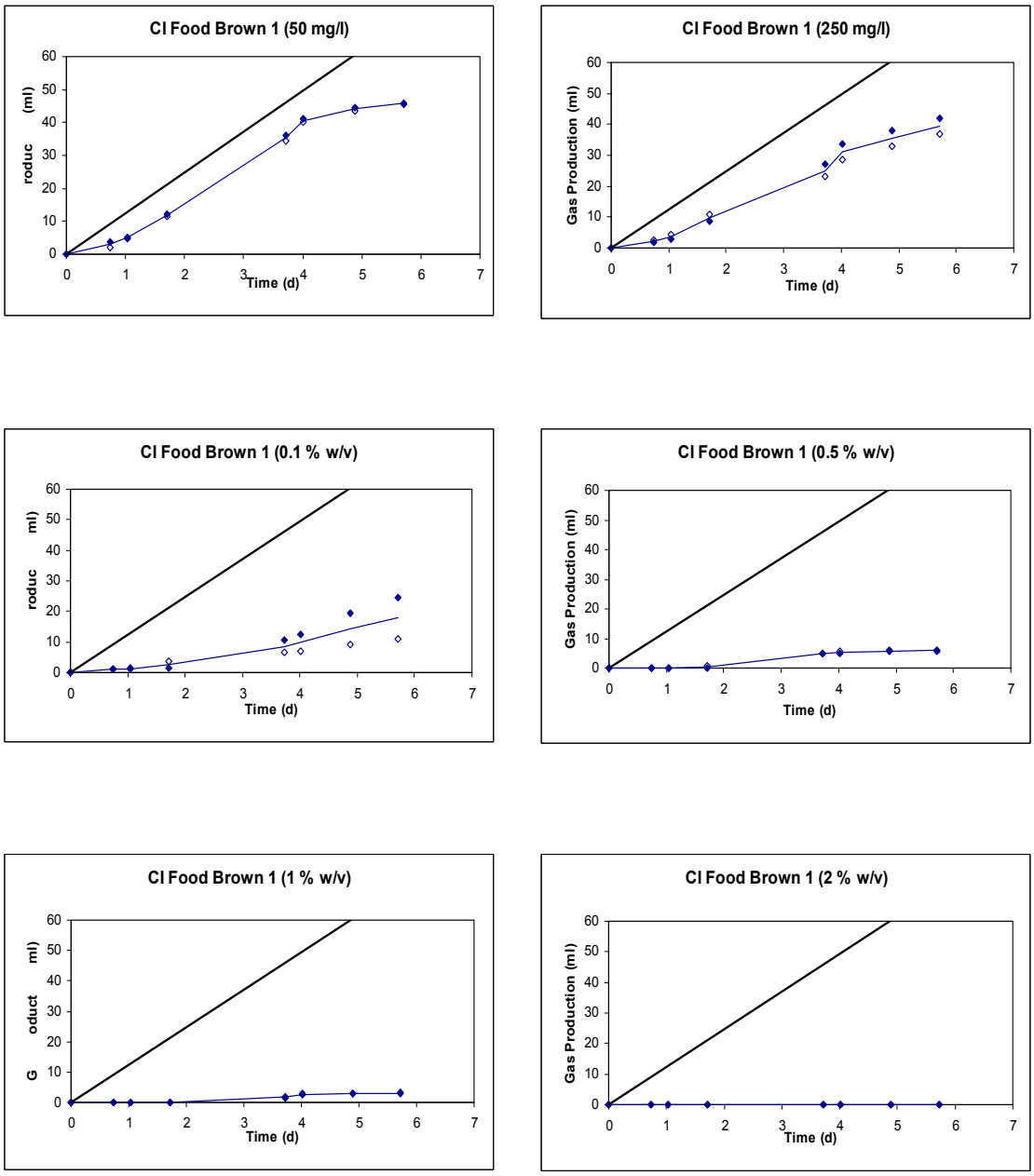


FIGURE A3.3 : Plots of biogas production during the anaerobic toxicity assay with Brown FK Standard (CI Food Brown 1).

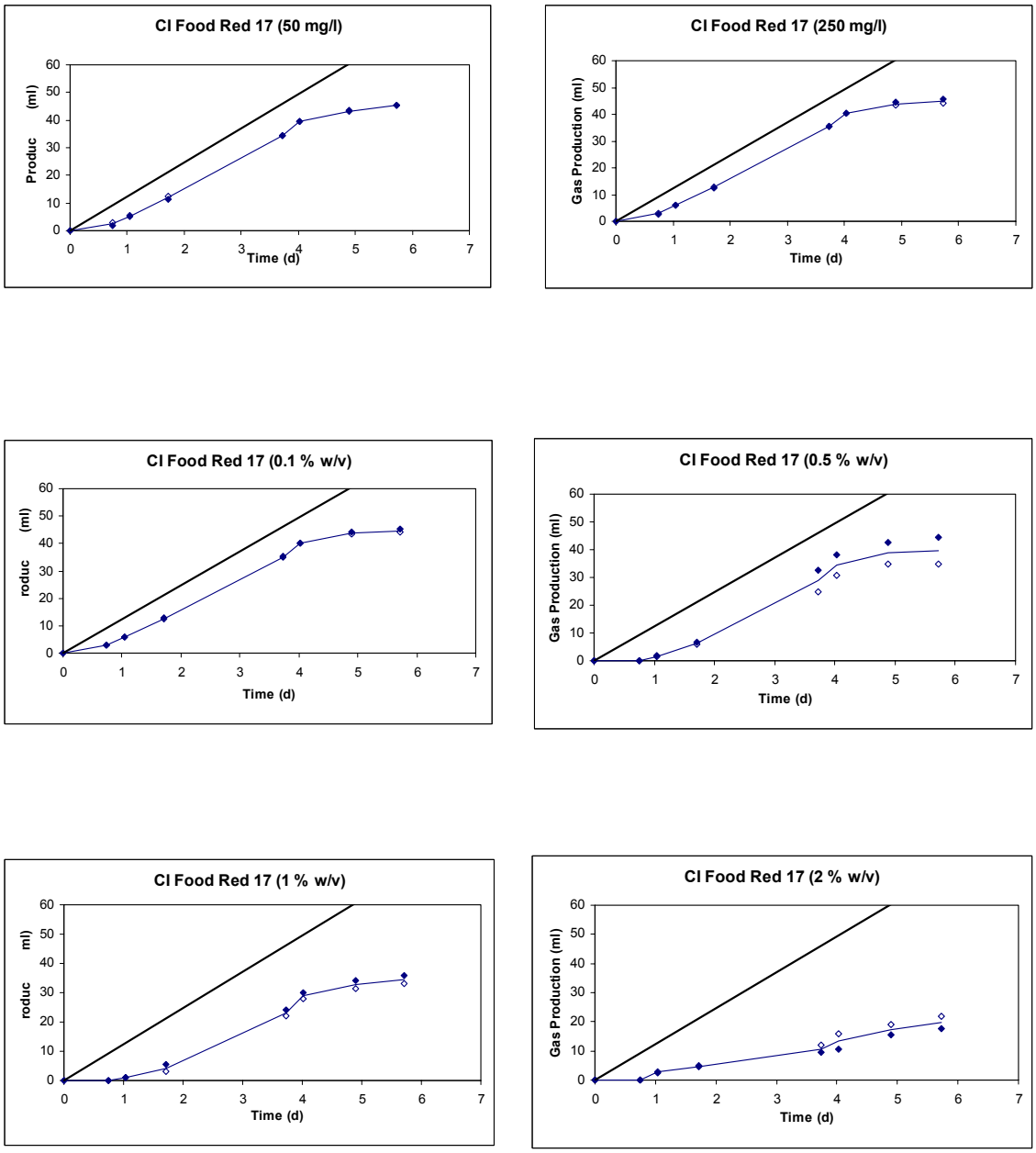


FIGURE A3.4 : Plots of biogas production during the anaerobic toxicity assay with Allura Red AC Supra (CI Food Red 17).

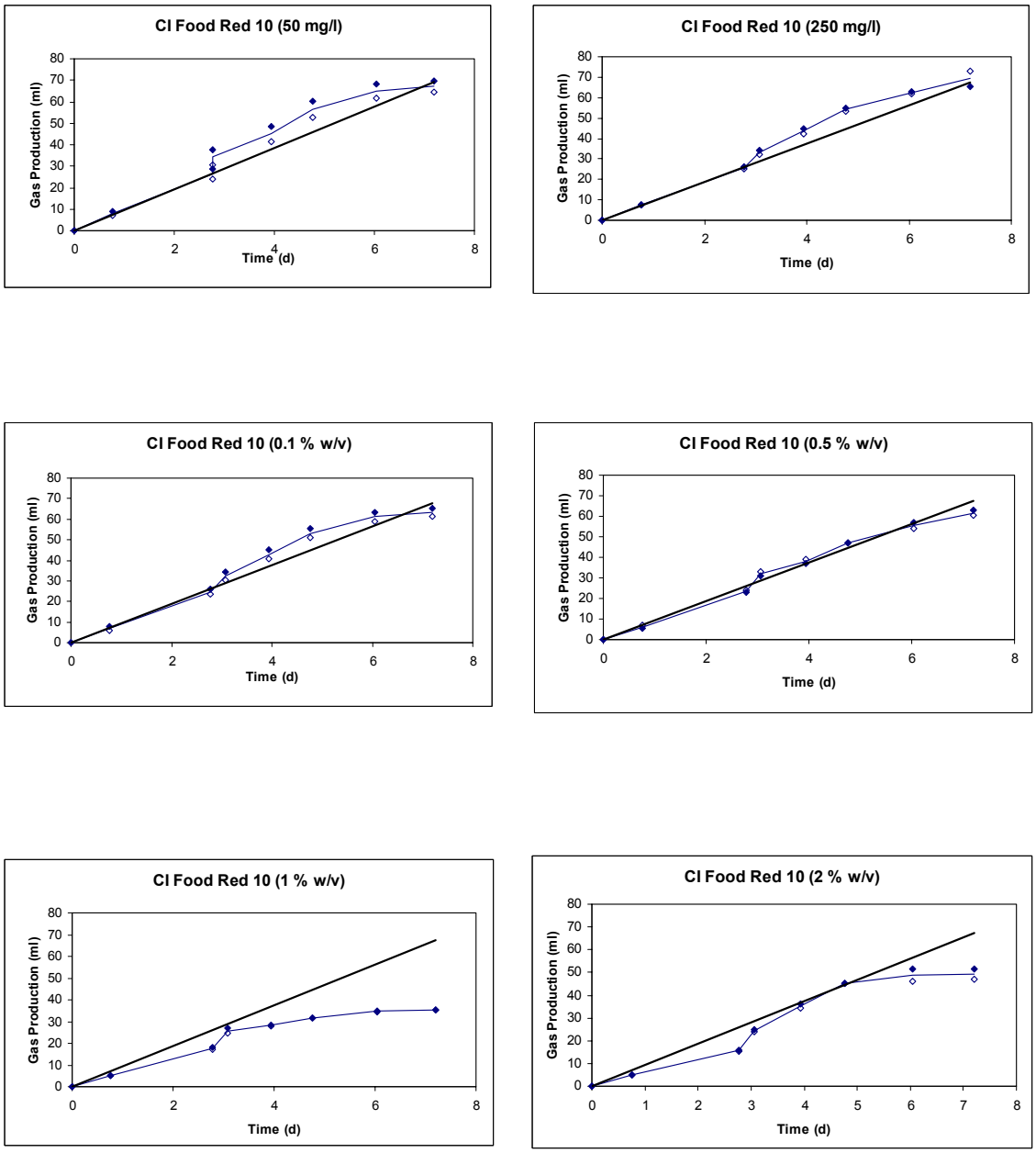


FIGURE A3.5 : Plots of biogas production during the anaerobic toxicity assay with Red 2G Supra (CI Food Red 10).

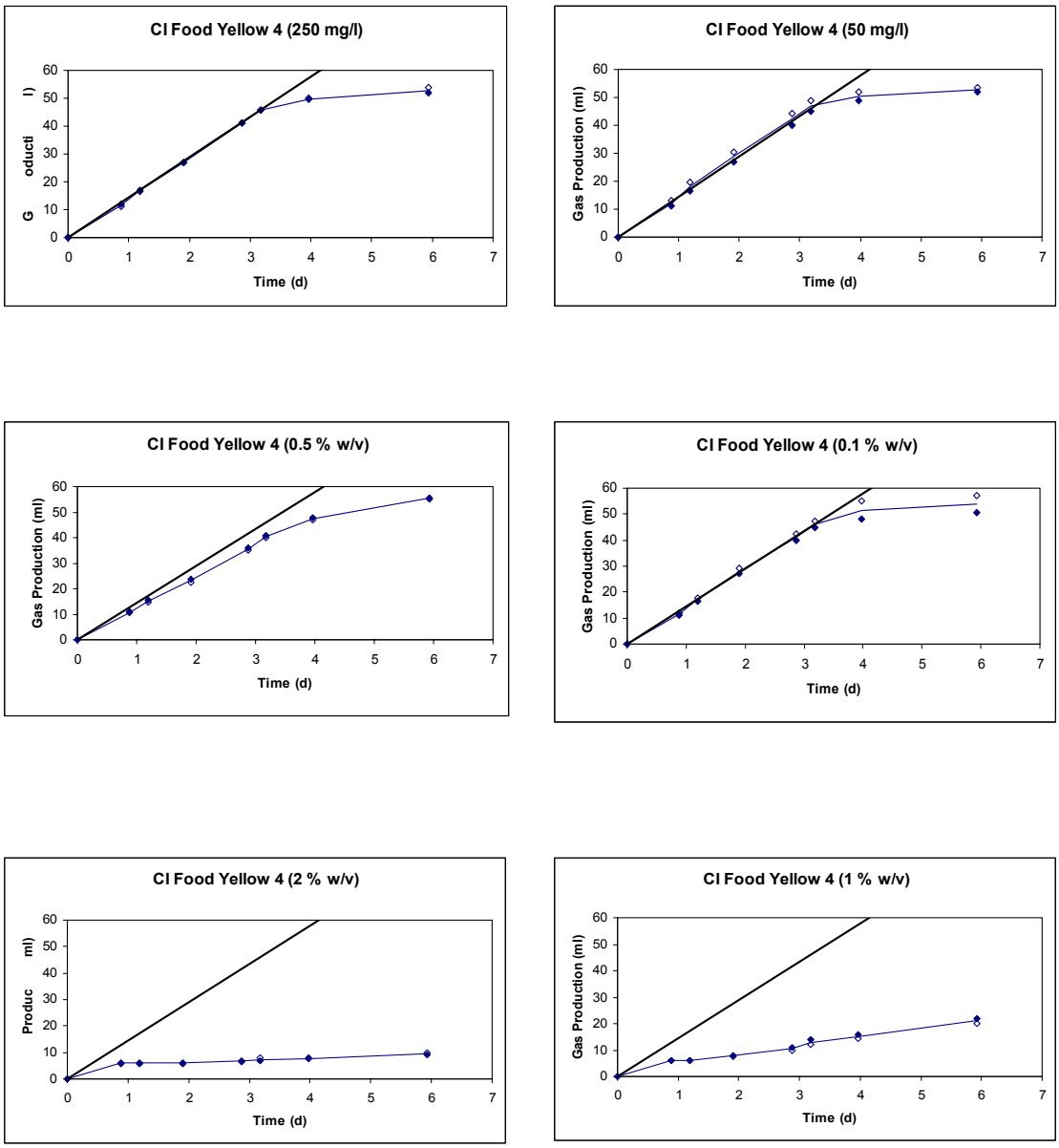


FIGURE A3.6 : Plots of biogas production during the anaerobic toxicity assay with Tartrazine Supra (CI Food Yellow 4).

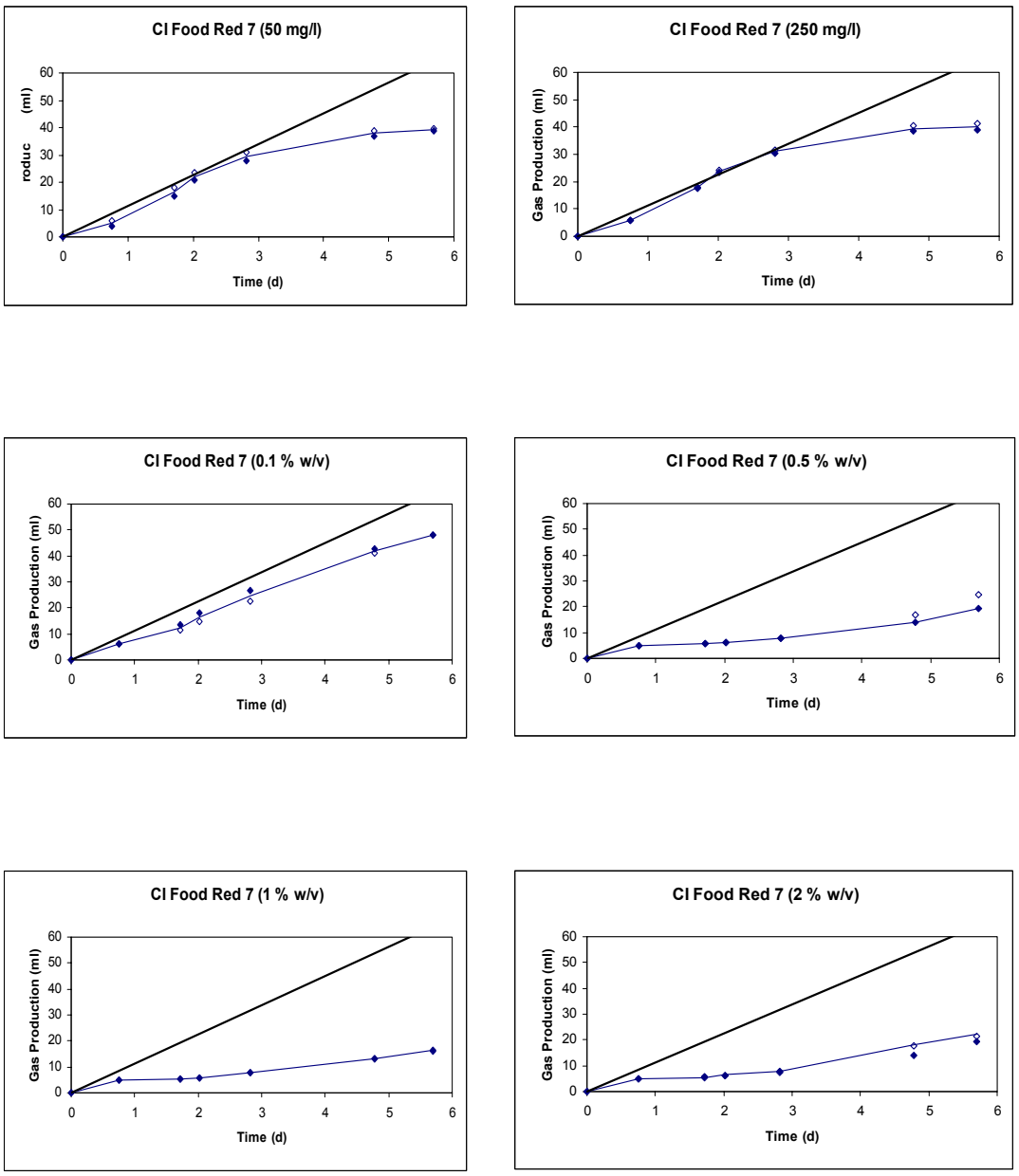


FIGURE A3.7 : Plots of biogas production during the anaerobic toxicity assay with Ponceau 4R Supra (CI Food Red 7).

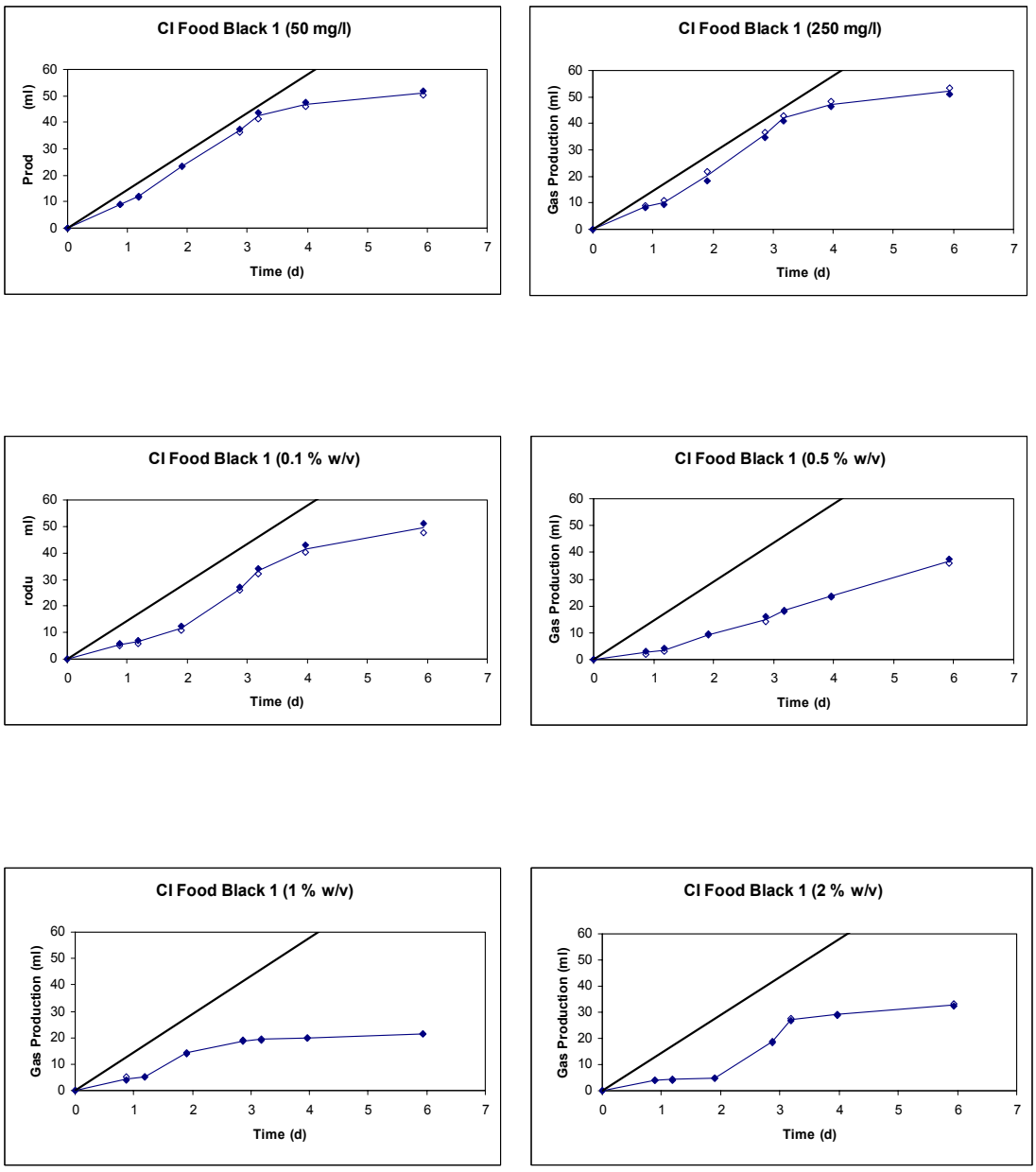


FIGURE A3.8 : Plots of biogas production during the anaerobic toxicity assay with Black PN Extra (CI Food Black 1).

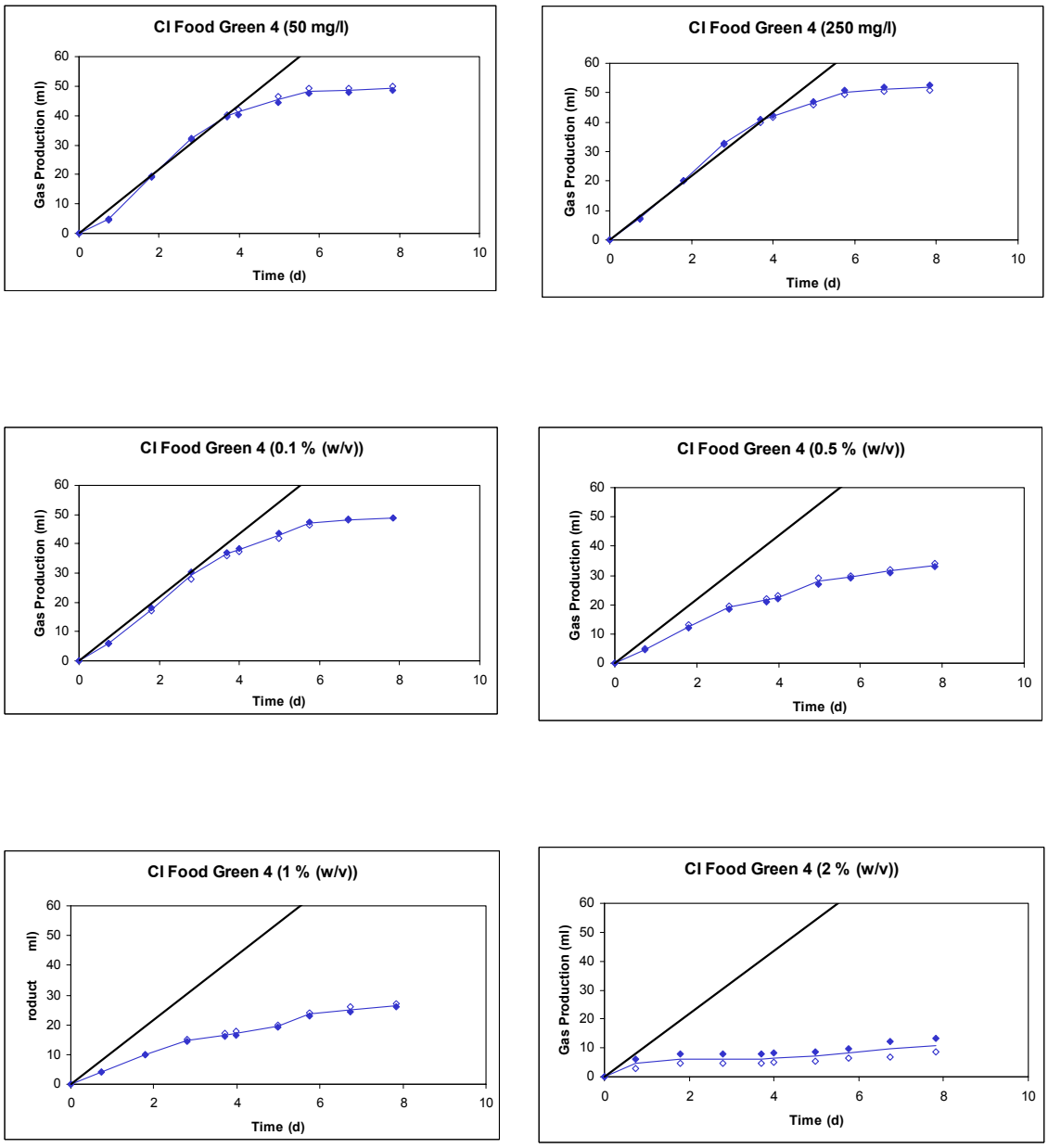


FIGURE A3.9 : Plots of biogas production during the anaerobic toxicity assay with Green S Supra (CI Food Green 4).

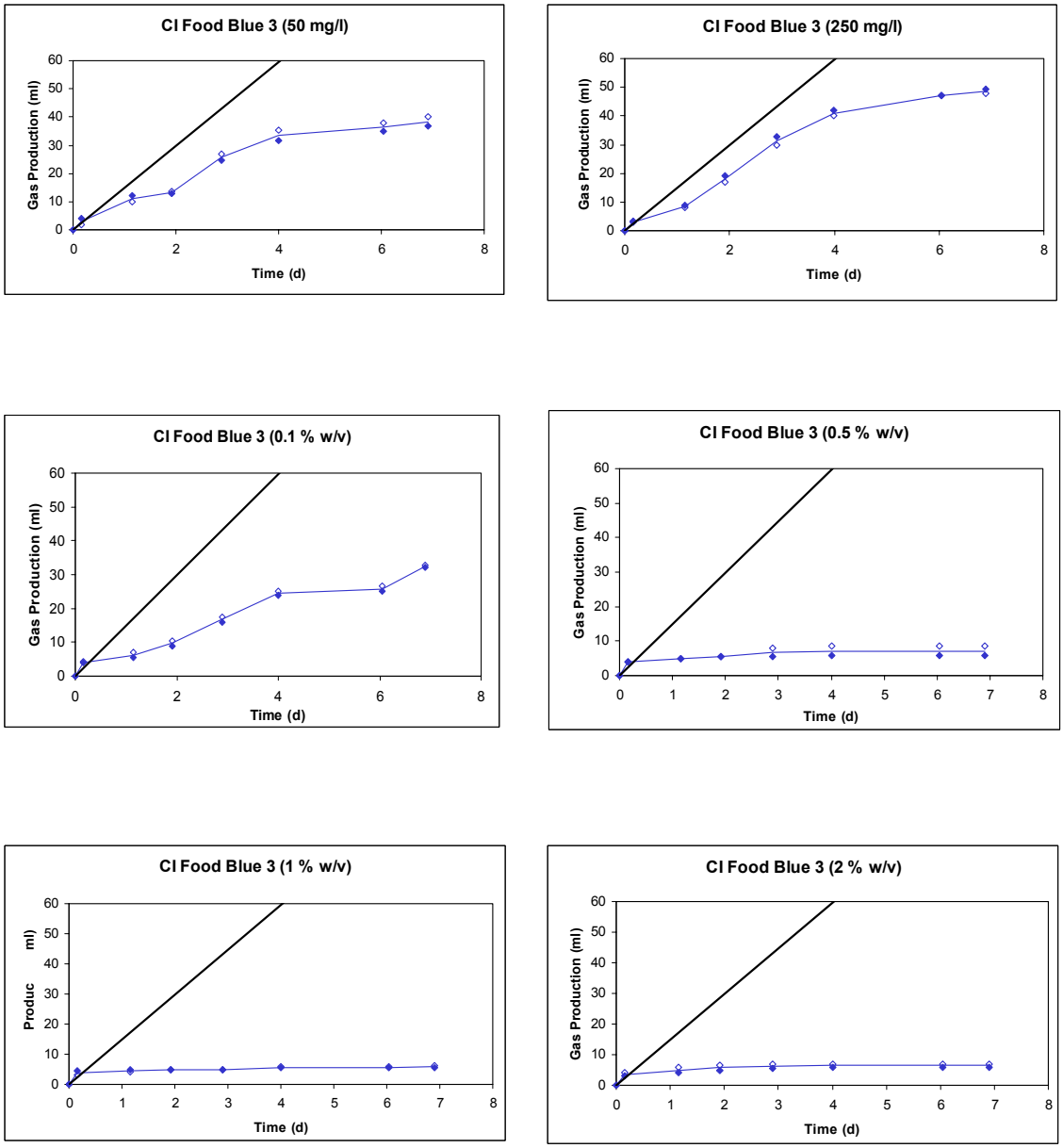


FIGURE A3.10 : Plots of biogas production during the anaerobic toxicity assay with Patent Blue V Supra (CI Food Blue 3).

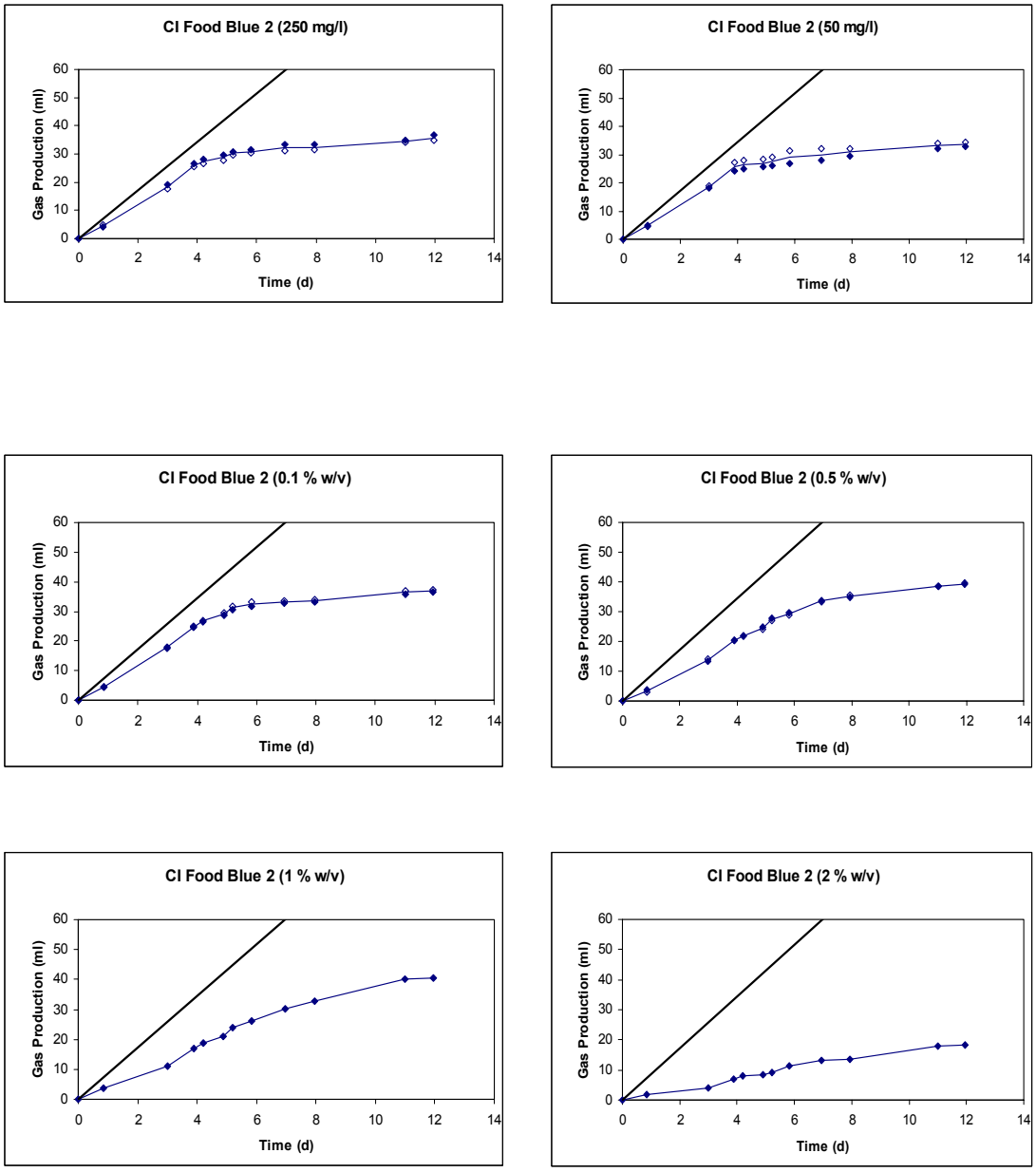
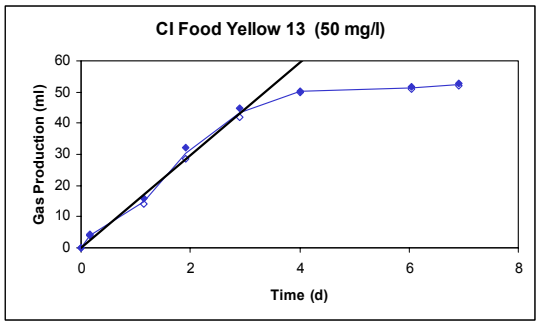
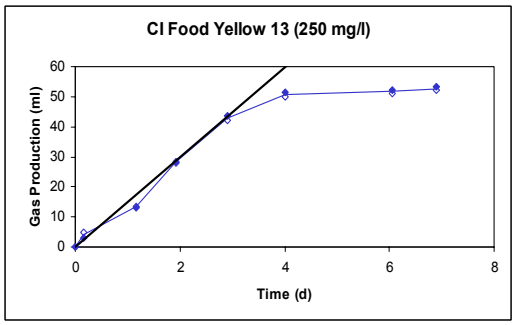
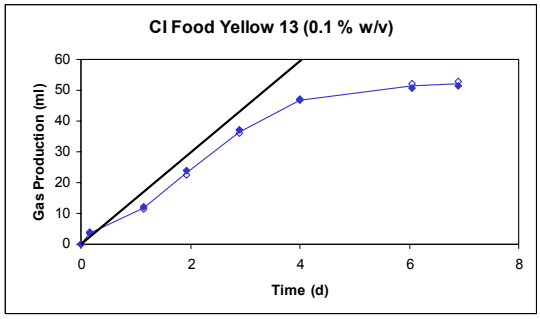
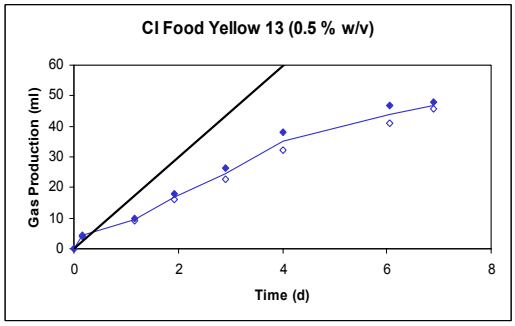


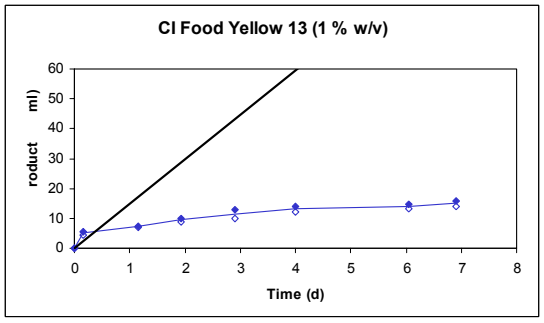
FIGURE A3.11 : Plots of biogas production during the anaerobic toxicity assay with Brilliant Blue Supra (CI Food Blue 2).











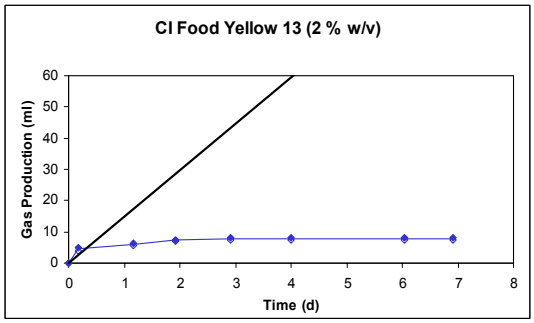


FIGURE A3.12 : Plots of biogas production during the anaerobic toxicity assay with Quinoline Yellow Extra (CI Food Yellow 13).

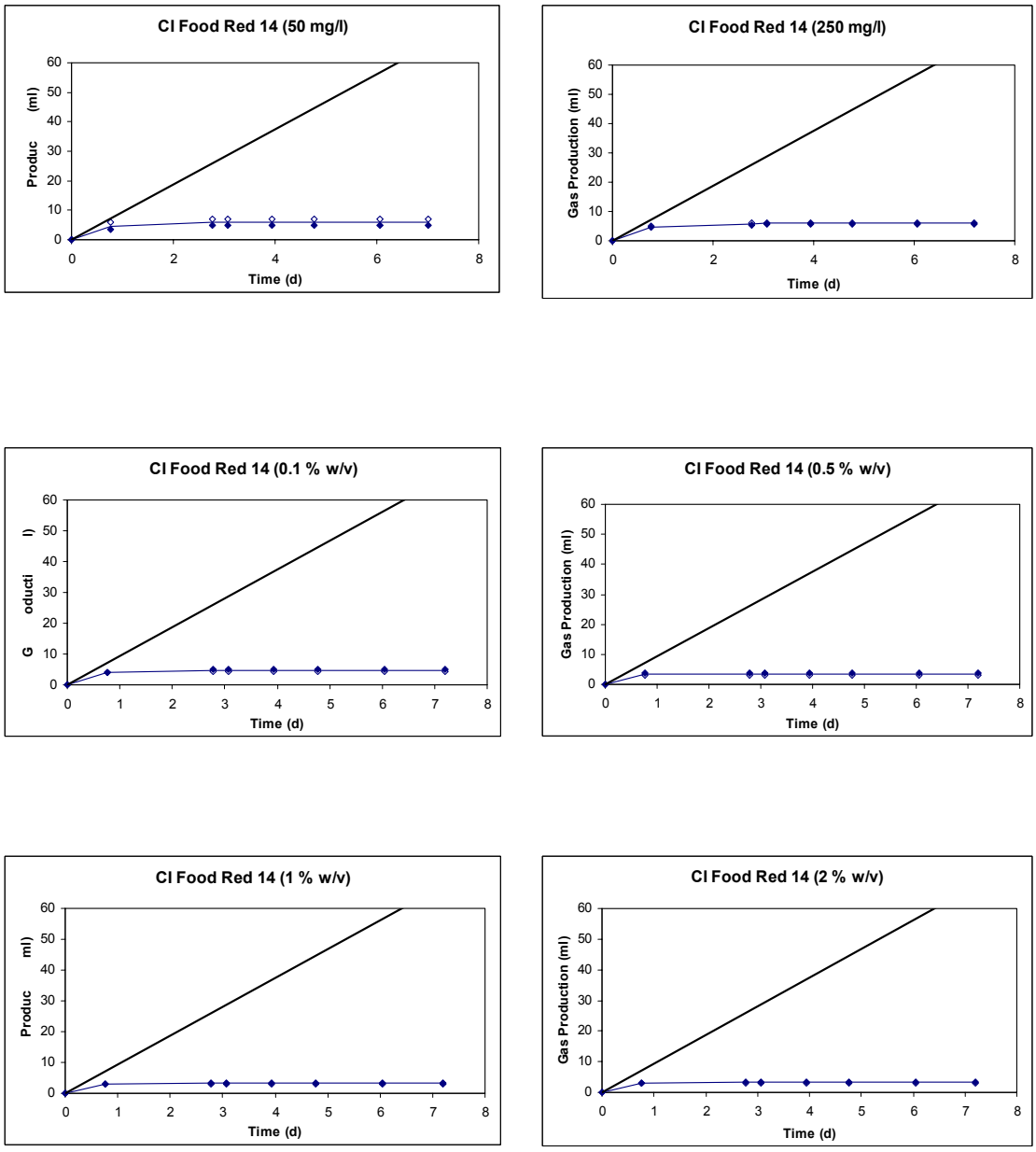


FIGURE A3.13 : Plots of biogas production during the anaerobic toxicity assay with Erythrosine Supra (CI Food Red 14).

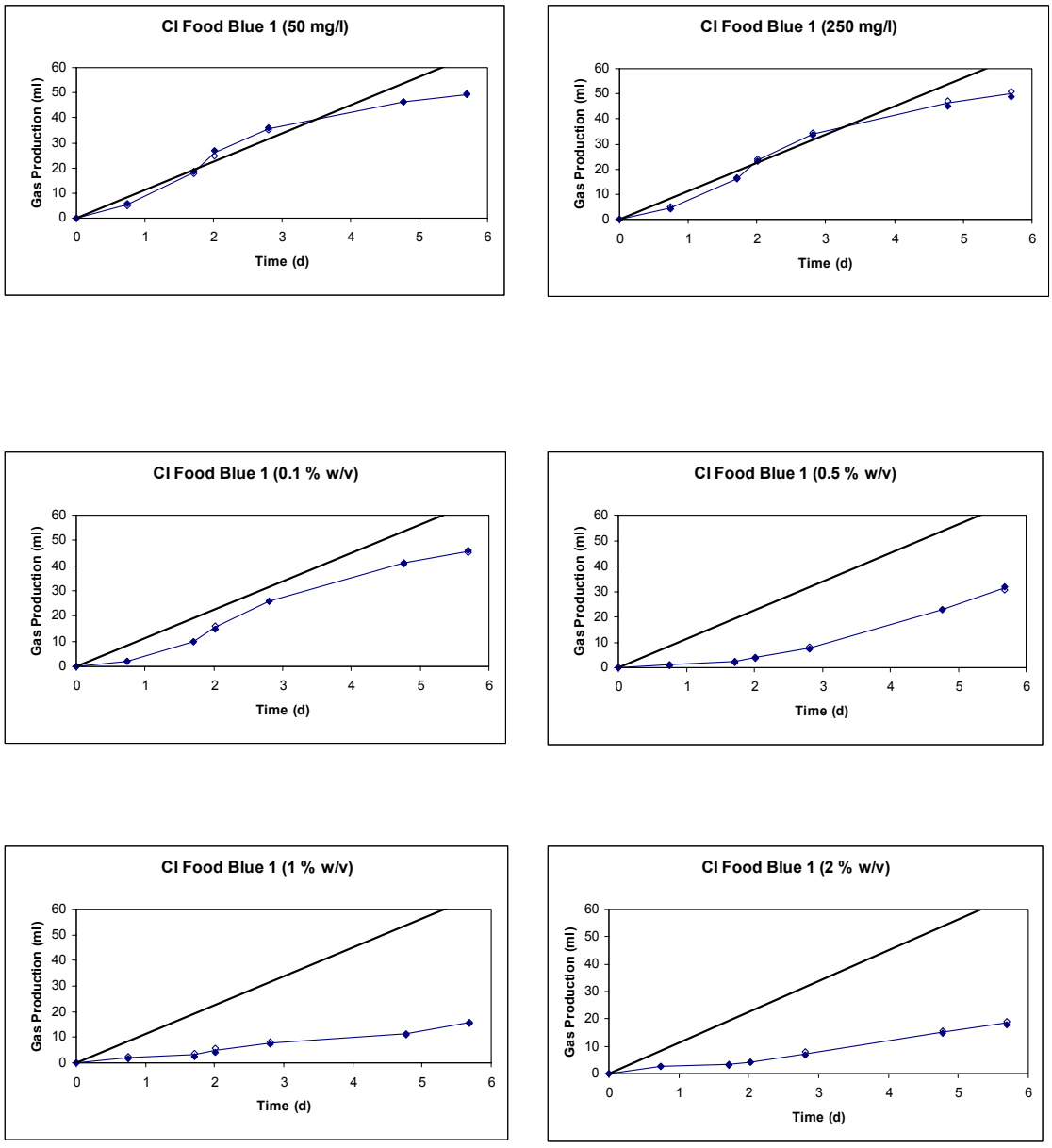
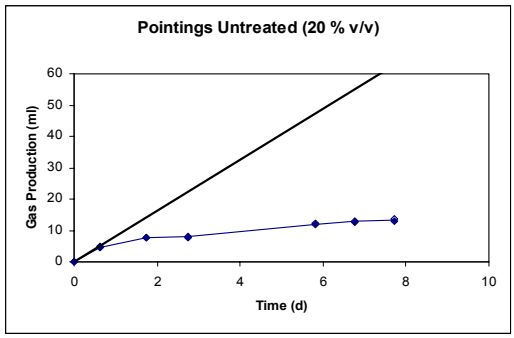
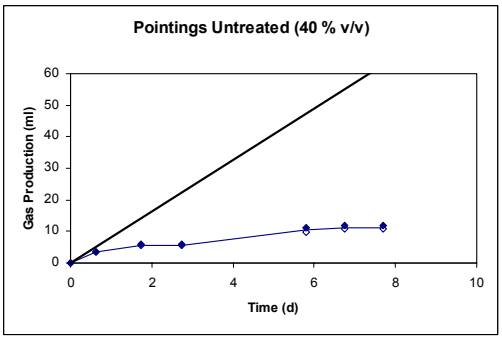
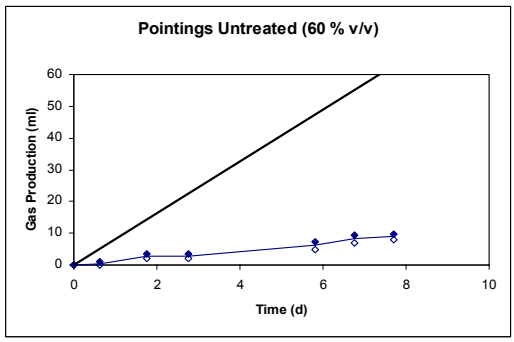
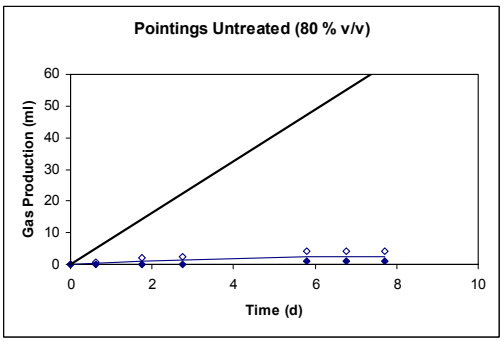


FIGURE A3.14 : Plots of biogas production during the anaerobic toxicity assay with Indigo Carmine Supra (CI Food Blue 1).









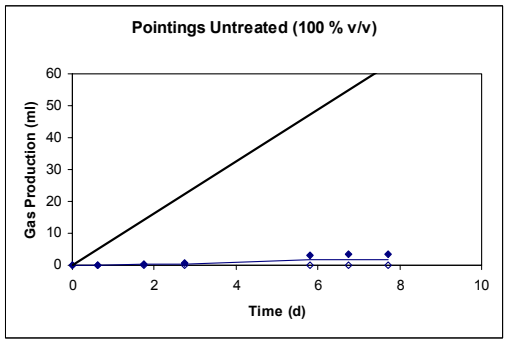
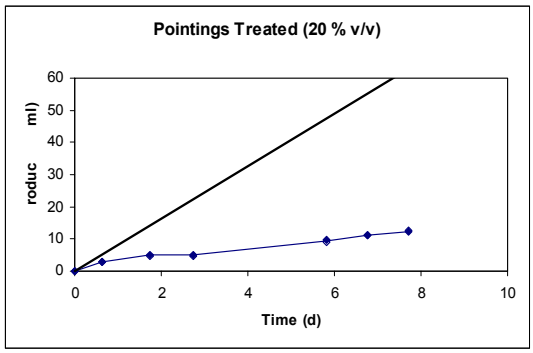
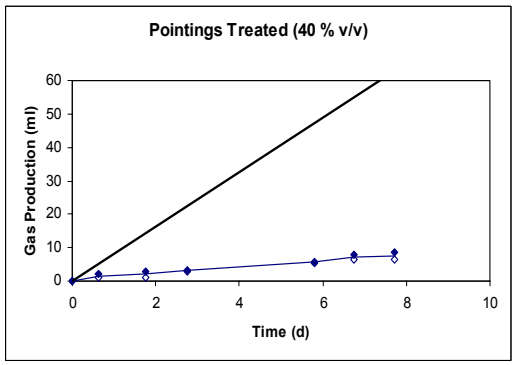
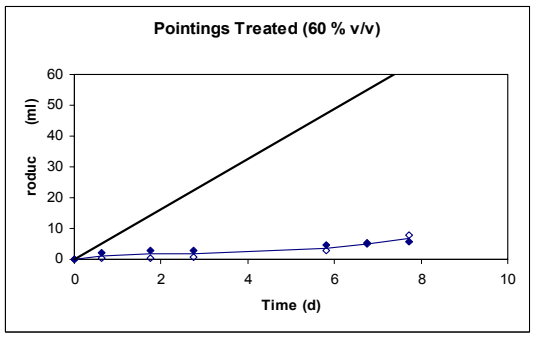
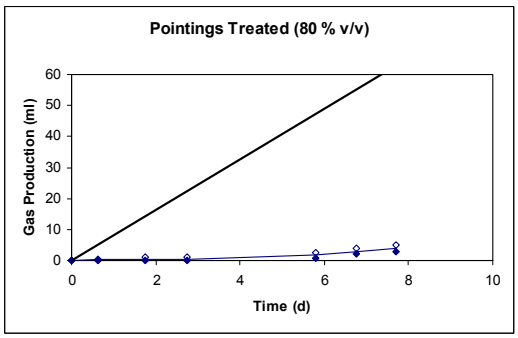


FIGURE A3.15 : Plots of biogas production during the anaerobic toxicity assay with the Food Dye untreated final effluent.









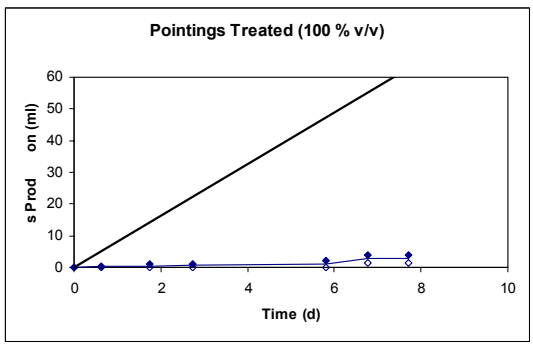


FIGURE A3.16 : Plots of biogas production during the anaerobic toxicity assay with Food Dye treated final effluent.

A3.2 BIODEGRADABILITY ASSAYS

Laboratory-scale models attempt to simulate the conditions prevailing in the whole or part of the natural environment under study (Atlas and Bartha, 1993). Batch biodegradability assays can function as preliminary screening tests to assess the anaerobic degradability of a particular substrate. It is critical that these tests are conducted prior to operation of a continuous reactor in order to evaluate the efficiency of the degradation process and to assess volumes and concentrations of the substrate that can be treated effectively, i.e. without causing reactor failure.

The results of the anaerobic toxicity assays were used to guide the set-up of the biodegradability assays. To prevent inhibition of the methanogenic biomass, dye concentrations lower than the measured IC_{50} concentrations were added to the assay bottles. Biodegradability of the dyes was determined by monitoring the cumulative biogas production during anaerobic incubation, according to the method of Owen *et al*. (1979).

A3.2.1 Hypotheses and Objectives

It was hypothesised that biodegradability assays would provide information on microbial metabolism of the dyes and acclimation of the anaerobic microorganisms to the inhibitory dyes.

Therefore, the objectives of the investigation were to:

1. Assess the anaerobic biodegradability of the food dyes by the microbial populations present in the anaerobic digester sludge.

2. Determine the anaerobic biodegradability of each dye.

3. Determine the methanogenic utilisation of the dye as a substrate.

A3.2.2 Materials and Methods

The same dyes were investigated as in the anaerobic toxicity assays (**Table A3.1**). The anaerobic degradability of the food dye manufacturing effluent was also evaluated. The nutrient medium was prepared as described in **Appendix 1**. The inoculum sludge was obtained from an operating anaerobic digester at the Mogden Sewage Works. The TS and VS of the inoculum sludge were measured.

The concentration of dye added to each assay bottle was calculated according to the theoretical COD of the dye, the theoretical gas production (to produce ca. 100 mL biogas), according to the Tarvin and Buswell (1934) equation, and the measured IC_{50} concentrations determined in the anaerobic toxicity assays. The theory behind these methods is described in detail in **Section A3.2.4**. The investigated concentration of each dye is given in **Table A3.3**.

TABLE A3.3: Bioassay conditions to assess the anaerobic biodegradability of a range of food dyes.

Dye	Chemical Formula	Methanogenic IC_{50}	Theoretical COD (g COD/g dye)	Assay Dye Conc. (g/L)
CI Food Yellow 3	$C_{16}H_{10}N_2O_7Na_2S_2$	19.6 g/L	0.96	1.25
CI Food Red 3	$C_{20}H_{12}N_2O_7Na_2S_2$	0.25 g/L	1.15	0.1
CI Food Brown 1	$C_{31}H_{27}N_{10}O_9Na_3S_3$	2.48 g/L	0.97	0.24
CI Food Red 17	$C_{18}H_{14}N_2O_8Na_2S_2$	> 20 g/L	1.48	0.7
CI Food Red 10	$C_{18}H_{13}N_3O_8Na_2S_2$	> 20 g/L	1.46	1.26
CI Food Yellow 4	$C_{16}H_9N_4O_9Na_3S_2$	14.3 g/L	0.644	1.5
CI Food Red 7	$C_{20}H_{11}N_2O_{10}Na_3S_3$	> 20 g/L	0.86	1.0
CI Food Black 1	$C_{28}H_{21}N_5O_{14}Na_2S_4$	> 20 g/L	0.826	0.28
CI Food Green 4	$C_{23}H_{27}N_2O_7Na_3S_2$	19.5 g/L	1.5	1.0
CI Food Blue 5	$C_{27}H_{31}N_2O_6NaS_2$	2.15 g/L	1.14	0.6
CI Food Blue 2	$C_{37}H_{36}N_2O_9Na_2S_3$	5.55 g/L	1.61	1.85
CI Food Yellow 13	$C_{18}H_{11}NO_6S_2$	8.38 g/L	1.35	1.0
CI Food Red 14	$C_{20}H_6O_5Na_2I_4$	0.2 mg/L	0.69	0.1 mg/L
CI Food Blue 1	$C_{16}H_{10}N_2O_8Na_2S_2$	14.03 g/L	0.89	0.52

The investigated concentrations for the treated and untreated industrial effluents were 20 % (v/v) and 100 % (v/v). The bottles were prepared in the same manner as for the anaerobic toxicity assays. Each sample was run in triplicate. Smaller serum bottles (100 mL) were used, with a working volume of 50 mL. A 10 % (v/v) inoculum was added to each bottle. The dye stock solutions were diluted, in the anaerobic medium, to a total volume of 40 mL. No additional carbon source or acetate-propionate solution was added. The serum bottles were equilibrated and then incubated in a waterbath, at a constant temperature of 35 °C. The bottles were shaken manually to facilitate contact between the microorganisms and the substrate.

Three sets of controls were set up, each in triplicate. The first set contained only the inoculum sludge and the nutrient medium, to account for gas production due to degradation of residual organic molecules in the inoculum sludge and any gas production associated with the nutrient medium. The second set of controls contained the nutrient medium and the assay concentration of each dye, to identify any decolourisation caused by reducing agents in the defined medium. In order to assess whether the dyes were adsorbed to the butyl rubber septa, the third set of controls contained only the dye solutions in sealed serum bottles.

Biogas production and composition were measured according to the methods described for the anaerobic toxicity assays. Biogas composition was determined whenever gas was wasted.

On the first day of incubation, samples (3 mL) were withdrawn from each bottle. The samples were centrifuged (4 000 rpm) and the supernatants filtered (0.45 µm). The COD and colour of each sample was measured, according to the methods outlined in **Appendix 1**. These are referred to as the *initial*, or starting measurements. The same parameters were measured after 60 d of incubation, to assess the reduction in both COD and colour. A spectrum scan (200 to 800 nm) was run on each dye to determine its maximum wavelength. The measured wavelengths were verified against the wavelengths given for the dyes in the Colour Index. The absorbance of each dye was measured at its specific maximum wavelength.

A3.2.3 Results

Measurements were taken and results calculated after 60 d of incubation at 35 °C. The results of the biodegradability assays are presented in **Section A3.2.6**. Each figure shows the measured biogas production, relative to the biogas produced in the controls containing the nutrient medium and the inoculum sludge. The corrected gas production is also plotted, for each dye. Here the amount of gas produced due to degradation of the dye alone is shown by subtraction of the control biogas from that measured in the samples. The symbols represent the triplicate samples, and the line through the data points, is the calculated mean biogas production. For each concentration, the gas production curve is shown relative to the gas production rate of the controls (solid black line). Each figure in **Section A3.2.6** summarises the biodegradability results for that particular dye or industrial effluent. These include the dye concentration added to each serum bottle, the theoretical COD of the dye and the theoretical COD of the assay, calculated from the theoretical COD of the dye and the amount of dye added.

The initial biogas production rate (mL/d) was the rate measured on day 2 of incubation. This provided an indication of degradability of the dye by the unacclimated microorganisms; the lower the gas production rate, the greater the inhibition. An extended lag period was observed with some of the assays, during which time the microorganisms acclimated to the dye, resulting in biogas production. The biogas production rate of the acclimated biomass is given for these bioassays. Acclimation occurs due to one or more characteristics of mixed microbial cultures. Bacteria are conservative; although a bacterium may possess the genetic information necessary to produce enzymes to degrade a particular new compound, it will not spend the energy and synthesise the necessary enzymes unless the compound is present. This lagging response to a new compound is called induction (Athanasopoulos, 1991). Population dynamics affect the removal of a new compound in mixed cultures. If a particular strain of bacteria acclimates, and the co-existing populations do not acclimate to the compound, the acclimated strain has a competitive advantage. Food is available to a strain which can increase its relative predominance in the total population (Athanasopoulos, 1991).

The volume of biogas produced was measured throughout the test period and the cumulative volume is given. Biogas composition was determined (**Appendix 1**) whenever gas was wasted from a serum bottle and after 60 d of incubation. The total volume of methane gas produced during the 60 d incubation period was determined. This was corrected for the amount of methane produced in the controls, to give the net methane production due to degradation of the added substrate (dye or industrial effluent). The COD

equivalent of the methane produced was calculated from the known conversion of 1 g COD being equal to 0.395 L CH_4 at 35 °C (Speece, 1996).

The amount of methanogenic activity in each serum bottle was estimated by calculating the fraction of dye COD converted to methane COD. The *theoretical* utilisation was based on the theoretical COD of the dye. The *actual* utilisation used the measured COD at the start of incubation. These values provide an indication of the extent of methanogenic utilisation of the dyes as substrates.

The COD balance was calculated from the measured COD values. *CODin* represents the initial COD measurement; *CODout* is the sum of the final soluble COD measurement and the COD transformed into methane. The measured reduction in COD is given as a percentage.

The maximum wavelength for each dye and industrial wastewater was determined by a spectrum scan on the UV-VIS spectrophotometer. These are given in **Table A3.4**. Colour reduction (%) was determined by the change in absorbance (at the maximum wavelength) from the initial starting colour, to the colour after 60 d of incubation. Decolourisation was corrected for by the controls to assess the amount of decolourisation due to reduction by reducing agents in the nutrient medium and adsorption of the dye to the butyl rubber stoppers. These values were negligible.

TABLE A3.4: Maximum wavelengths of the investigated food dyes.

Dye	Wavelength (nm)	Dye	Wavelength (nm)
CI Food Yellow 3	480	CI Food Green 4	634
CI Food Red 3	515	CI Food Blue 5	630
CI Food Brown 1	430	CI Food Blue 2	630
CI Food Red 17	500	CI Food Yellow 13	410
CI Food Red 10	520	CI Food Red 14	520
CI Food Yellow 4	430	CI Food Blue 1	610
CI Food Red 7	510	Food Dye Untreated	500
CI Food Black 1	570	Food Dye Treated Effluent	500

The results of the biodegradability bioassays are summarised in **Table A3.5**.

TABLE A3.5: Results of the food dye anaerobic biodegradability assays (60 d).

Dye	Methanogenic Utilisation (%)	COD Reduction (%)	Colour Reduction (%)
CI Food Yellow 3	0.0	64.3	78.5
CI Food Red 3	0.0	68.6	69.4
CI Food Brown 1	0.0	66.0	72.0
CI Food Red 17	0.9	55.5	90.0
CI Food Red 10	1.3	52.5	89.8
CI Food Yellow 4	0.84	48.2	94.4
CI Food Red 7	1.64	48.5	86.9
CI Food Black 1	1.9	55.0	74.2
CI Food Green 4	0.0	28.6	94.9
CI Food Blue 5	0.0	61.8	33.6
CI Food Blue 2	2.08	54.0	0.0
CI Food Yellow 13	0.0	57.4	0.0
CI Food Red 14	0.0	41.3	0.8
CI Food Blue 1	2.6	34.0	16.6
Food Dye Untreated (20 %)	2.2	37.0	65.8
Food Dye Untreated (100 %)	0.43	64.0	79.8
Food Dye Treated (20 %)	1.96	54.0	31.0
Food Dye Treated (100 %)	0.0	61.0	36.6

A3.2.4 Discussion

In an anaerobic system, an azo dye is not biodegraded by the microorganisms, instead the dye acts as an oxidising agent for the reduced flavin nucleotides of the microbial electron transport chain and is reduced and decolourised concurrently with re-oxidation of the reduced flavin nucleotides. Therefore, a source of reduction, resulting from the degradation of a suitable carbon source, is essential to ensure decolourisation and maintain the anaerobic population in the treatment system (Haug *et al*., 1991; Carliell *et al*., 1996). In these biodegradability assays the added substrate, i.e. the food dye or the industrial effluent was added as the sole substrate; no additional carbon source was added. The objective of these tests was to evaluate whether the anaerobic microbial populations would be able to utilise the added dye as a sole substrate. Experimental work has shown that an additional carbon source, such as glucose or VFA mixture enhances decolourisation (Razo-Flores *et al*., 1997; Carliell *et al*., 1995). However, Razo-

Flores *et al*. (1997) also found that a pharmaceutical azo dye, azodisalicylate, was completely decolourised and mineralised to CH_4 without the supplementation of an additional carbon source, at dye loading rates up to 225 mg/L.d. Additionally, it was found that a cleavage product of the azo dye Mordant Orange 1, namely 5-aminosalicylic acid (5-ASA) was biodegradable in methanogenic consortia under the operational conditions of a continuous anaerobic reactor (Donlon *et al*., 1997). These results indicated that some azo dyes could be mineralised in anaerobic environments in contrast to the common assumption that they are only biotransformed to mutagenic and carcinogenic aromatic amines.

Three sets of controls were set up for these bioassays. The function of the controls containing only the inoculum sludge and the anaerobic nutrient medium was to determine the amount of gas produced due to the microbial degradation of residual organic molecules in the inoculum or gas production associated with the nutrient medium. The measured gas volumes, for the experimental bottles, were corrected by subtracting the volume of gas produced in the controls to quantify the gas produced as a result of the degradation of the dye alone.

The controls containing the anaerobic medium and the assay concentration of each dye functioned to evaluate decolourisation due to the reducing agent, sodium sulphide, in the medium. Decolourisation may be attributed to adsorption and not necessarily degradation of the dye. To determine whether dyes were adsorbed to the butyl rubber septa, controls were set up containing only the dye solution in sealed serum bottles. Decolourisation due to adsorption or reduction of the azo bond was found to be negligible.

Appropriate sample size and liquid to headspace volume ratios are important for the precision and accuracy of the results. Smaller serum bottles were used because of availability. The working volume was scaled proportionally. The estimated degradable COD was kept at ca. 2 g/L, based on the method of Owen *et al*. (1979), to satisfy the carbon to nitrogen ratio required for anaerobic microorganisms with the defined nutrient medium. The concentration of dye added to each bottle was based on the production of a volume of biogas that could be measured accurately (ca. 60 to 100 mL), without exceeding a load of 2 g COD and not exceeding the dye IC_{50} concentration.

The dye structure or chemical formula of each dye was known, thus the theoretical COD could be calculated per g of dye. The theoretical gas production, assuming complete mineralisation to methane and carbon dioxide, was calculated according to the Tarvin and Buswell (1934) equation:

$$CnHaOb + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 \qquad [A3.1]$$

The amount of dye required to produce ca. 100 mL of biogas was calculated. The theoretical COD of the calculated mass of dye was calculated, according to the equation:

$$CnHaObNc + d\mathrm{Cr_2O_7^{2-}} + (8d + c)H^+ \rightarrow nCO_2 + \frac{a + 8d - 3c}{2}H_2O + cNH_4^+ + 2dCr_3^+ \qquad [A3.2]$$

where $d = 2n/3 + a/6 - b/3 - c/2$

The theoretical COD (3*d*/2) is calculated based on the amount of oxygen required to oxidise the molecule (Speece, 1996). The amount of dye added to each serum bottle was corrected if the theoretical COD was greater than 2 g and if the calculated dye concentration (based on the production of 100 mL biogas) was greater than the IC_{50} concentration, determined during the anaerobic toxicity assays. The dye concentration added to each assay bottle is shown in **Table A3.3**.

Gas production is indicative of metabolic activity, thus the shape of the gas production curve indicates the ease and the degree of degradability of a substrate. Biogas production was monitored throughout the incubation period. Determination of the biogas composition gave the fraction of methane in the total biogas. The volume of methane could then be calculated to give an indication of the extent of methanogenic activity within the serum bottles. It is known that in an anaerobic environment, COD is not destroyed, but transformed. Thus, a methane balance can be used to evaluate the methanogenic activity within a batch culture by calculating the amount of COD converted to methane. These values were calculated for each assay to assess the extent of methanogenic activity. The amount of methane produced was corrected for the volume of methane produced in the controls, such that the equivalent methane COD was attributed to degradation, or utilisation, of only the dye. From the results, it can be seen that generally, the methanogenic activity was low, suggesting that these dyes were not readily utilised by methanogenic populations.

There is a degree of inaccuracy associated with the data presented for the COD balances, resulting in the poor balances attained. The discrepancy lies with the measured COD values, relative to the theoretical values. For each assay, the theoretical COD of the dye added was calculated, from the mass of dye added. The COD of the nutrient medium was assumed to be negligible (measured at 36.7 mg/L). However, as shown in **Section A3.2.6**, the COD values, measured at the start of the incubation period, do not correlate with the theoretical values; they are generally larger. The final soluble COD was measured and the COD equivalent of the methane produced was calculated to give the final *CODout*.

Soluble COD was measured since it was assumed that biomass production would be negligible. Also, COD measurement of solids is inaccurate unless the samples are properly homogenised. The presence of a larger floc in a sample would greatly influence the measured COD. The total COD (soluble and insoluble) of each bottle was measured to assess the influence on the COD balances (data not shown). The samples were homogenised by passing them through a 0.6 mm syringe needle. The measured values were much greater than the measured initial COD values and, therefore, did not provide a solution to the poor COD balances. The loss of COD may be attributed to adsorption of the dye (and its associated COD) to the biomass.

In terms of effluent discharge, decolourisation is critical. Colour removal can be achieved by physical, chemical or biological means. Colour reduction in these bioassays could have been due to the reducing environment within the serum bottles or by adsorption to the biomass. The aim was to achieve biological decolourisation, i.e. utilisation of the dye by the microorganisms resulting in breakdown of the dye molecules and removal of colour. Decolourisation was of particular interest for samples with low IC_{50} concentrations because biodegradation was not expected but reduction, or breakage, of the dye

molecules, could occur resulting in decolourisation. Decolourisation in these assays could have been caused by either adsorption or degradation, or both.

Very little gas production was observed in the CI Food Yellow 3 bioassays (**Table A3.7**). The gas production approximated that of the controls, suggesting that gas production was due to degradation of residual organic molecules in the inoculum sludge or associated with the nutrient medium. The plot of the corrected gas production was negative at points, showing that gas production was lower than that in the controls, indicating inhibition due to addition of the dye. This was not expected since the IC_{50} concentration was calculated at 19.6 g/L. This inhibition value was specific for the methanogens, therefore, the dye could be inhibitory to the other microbial populations present in the digested sludge. However, there was no methanogenic activity either. These results suggest that the dye would be unsuitable for anaerobic degradation. Reduction in COD and colour were relatively high at 65 % and 78.5 %, respectively. These reductions were obviously not due to microbial activity and are, therefore, attributed to adsorption to the biomass.

Gas production in the CI Food Red 3 bottles was lower than in the controls (**Table A3.8**), which suggested that the dye was inhibitory to the anaerobic microorganisms. This correlated with the results of the anaerobic toxicity assays, where the IC_{50} concentration was low at 0.25 g/L. No methanogenic activity was present in the bottles. This dye would, therefore, not be suited to anaerobic treatment. Again, the COD and colour reductions were relatively high at 68.4 % and 68.6 %, respectively, which could have been due to adsorption to the biomass. Similar results were obtained for CI Food Brown 1 (**Table A3.9**), which also had a low IC_{50} concentration at 2.48 g/L. This resulted in inhibition of the microbial populations, including the methanogens which showed no activity. COD reduction was 66 % and there was a 72 % reduction in colour.

Biogas production was greater than in the controls, for CI Food Red 17 (**Table A3.10**). The methanogenic IC_{50} concentration was calculated at > 20 g/L, however, in these assays, methanogenic activity only attributed to 0.9 % of the utilisation of the dye. From this it can be deduced that, although the dye is not inhibitory to the methanogens, it is not readily utilised. This could be overcome by addition of a carbon source, which may result in co-metabolism of the dye. The bioassay COD was reduced by 55.5 % and the colour by 90 %. This could have been achieved by other bacterial populations, which is suggested by the volumes of biogas produced. Similar results were obtained for CI Food Red 10 (**Table A3.11**), which also had an IC_{50} concentration of > 20 g/L. Methanogenic utilisation accounted for 1.3 % of the dye. COD reduction was 52.5 % and colour was reduced by 89.8 %. CI Food Yellow 4 (**Table A3.12**) had an IC_{50} concentration of 14.3 g/L, and was, therefore, assumed not to be inhibitory to the methanogens. Methanogenic activity was recorded and accounted for 0.8 % of the degradation of the dye. Biogas production was greater than in the controls, suggesting metabolism of the dye by other microbial populations, also resulting in the reduction of the initial COD by 48.2 %. The initial yellow colour of the dye was reduced by 94.4 % but a change in colour to purple/maroon was observed. This could be problematic in treatment of the dye. The change in colour could be attributed to degradation

products bonding to form a different dye structure; or else it could have been due to oxidation of the degradation products, during gas measurement and sampling.

CI Food Red 7 (**Table A3.13**) had an IC_{50} concentration of > 20 g/L. Biogas production, during the incubation period, was greater than that in the controls, suggesting utilisation of the dye. Methanogenic metabolism contributed to 1.64 % of the degradation of the dye. COD reduction was measured at 48.5 % and colour reduction at 86.9 %. Thus, anaerobic treatment of this dye would be efficient, but it requires the co-operation of several bacterial populations and degradation would be enhanced with the addition of a carbon substrate. A similar deduction could be drawn for CI Food Black 1 (**Table A3.14**), with 1.9 % methanogenic utilisation of the dye. This case illustrates the discrepancy between the theoretical and measured COD values. Based on the theoretical dye COD, the methanogenic utilisation of the dye would have been 11.6 %. However, the initial measured COD was that much greater to reduce the methanogenic efficiency to 1.9 %.

Biogas production was lower than the controls in the CI Food Green 4 bioassays (**Table A3.15**). This was not expected since the methanogenic IC_{50} concentration was 19.6 g/L. The dye could have been inhibitory to other microorganisms in the biomass, or it could be that it was not readily utilised. Colour reduction was high at 94.9 %. No reduction in colour was observed in the colour controls, therefore, it is assumed that decolourisation was due to adsorption to the biomass.

Inhibition was observed in the CI Food Blue 5 assays (**Table A3.16**), where the biogas production was lower than in the controls. This verified the anaerobic toxicity assays results, which calculated the IC_{50} concentration at 2.15 g/L. The dye concentration added to the bioassays was, however, lower than this concentration. There was no methanogenic activity. Reduction in COD (61.8 %) could have been due to adsorption of the dye to the biomass (33.6 % reduction in colour). Biogas production in the CI Food Blue 2 bioasssays (**Table A3.17**) was greater than in the controls except that no reduction in colour was achieved. This was of interest because the food dye manufacturer could not achieve chemical decolourisation of the dye either. The gas production and reduction in COD (54 %) could be due to degradation of readily available side groups on the dye molecules, without actual break down of the dye molecule itself.

Biogas production was the same as that in the controls, for CI Food Yellow 13 (**Table A3.18**), up to day 10, after which biogas production increased. This would suggest acclimation of the microorganisms to the dye. There was no methanogenic utilisation of the dye. There was also no decolourisation.

Biogas production was greater than in the controls for CI Food Red 14 (**Table A3.19**), which was unexpected, due to the high toxicity data recorded in the anaerobic toxicity assays. The dye concentration added was lower than the IC_{50} concentration of 0.2 mg/L. However, there was still no methanogenic utilisation of the dye. The biogas production was due to activity by other microbial populations. Colour reduction was low at 0.8 %.

The untreated food dye effluent (**Tables A3.21** and **A3.22**) was more degradable at the lower concentration of 20 % (v/v) than at 100 % (v/v). Biogas production was greater than in the controls for the 20 % concentration. There was no gas production in the 100 % concentration until acclimation was achieved by ca. day 35. The biogas production rate was then 0.1 mL/d. Methanogenic utilisation of the dye was greater (2.2 %) in the lower wastewater concentrations than in the undiluted wastewater (0.43 % methanogenic utilisation). Methanogenic degradation of the chemically treated food dye effluent (**Tables A3.23** and **A3.24**) was lower, 1.96 % (for the 20 % concentration) than in the untreated samples. Similar COD and colour reductions were achieved. There was no methanogenic utilisation of the undiluted wastewater.

These bioassays provided a more thorough understanding of the dye characteristics and degradation potential. This knowledge can then be used to predict the optimal treatment option. It must be noted, however, that these bioassays investigated the final products of the industry, i.e. the dyes. The factory effluent would contain concentrations of the dye precursors, from the synthesis processes. These could have a severe effect on the treatment process as several aromatic amines have been shown to be toxic or inhibitory.

The results presented for these biodegradability assays showed that the dyes were not readily utilised as a sole methanogenic substrate, however, degradation of the dyes could be enhanced by co-metabolism with another substrate.

A3.2.5 Conclusions

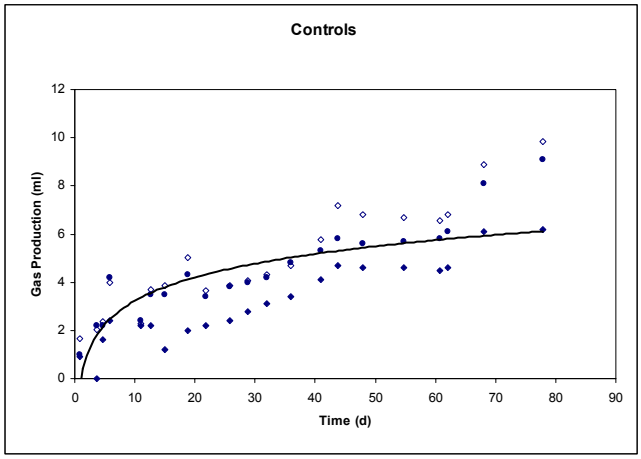
1. Although the bioassays showed efficient COD reduction and decolourisation, generally, the methanogenic activity was low, suggesting that the dyes are not readily utilised by methanogenic populations.

2. Supplementation of an additional carbon source could improve the methanogenic utilisation of the dyes.

3. The bioassays provided a more thorough understanding of the dye characteristics and degradation potential.

A3.2.6 Biogas Production Plots

TABLE A3.6 : Results for the controls in the biodegradability assay.

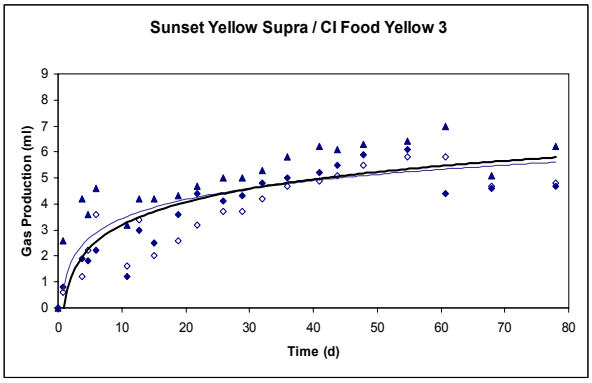


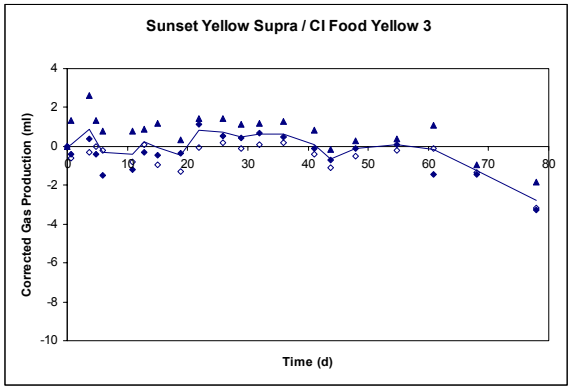
Initial biogas production rate :	1.60 mL/d
Acclimated biogas production rate (60 d) :	-
Total gas production (37 °C) :	7.0 mL
CH_4 fraction :	0.055
CH_4 production :	0.385 mL
CH_4 – COD :	0.970 mg

COD balance

CODin : 16.735 mg (in 50 mL)	COD out :	10.078 mg (in 50 mL)
	CH_4 – COD :	0.970 mg
	Total CODout :	11.048 mg
Balance :	**66 %**	
COD reduction :	34 %	

TABLE A3.7 : Results of the biodegradability assay with CI Food Yellow 3.





Biodegradability :

Dye concentration :	1.25 g/L
Theoretical dye COD :	0.96 g COD/g dye
Theoretical Assay COD (in 50 mL)	60 mg
Initial biogas production rate :	1.88 mL/d
Acclimated biogas production rate (60 d) :	-
Total gas production (37 °C) :	6.3 mL
CH_4 fraction :	0.045
CH_4 production :	0.2835 mL
Net CH_4 production :	0 mL
CH_4 – COD :	0.1133 mg

Methanogenic activity

Theoretical utilisation :	0 %
Actual utilisation :	0 %

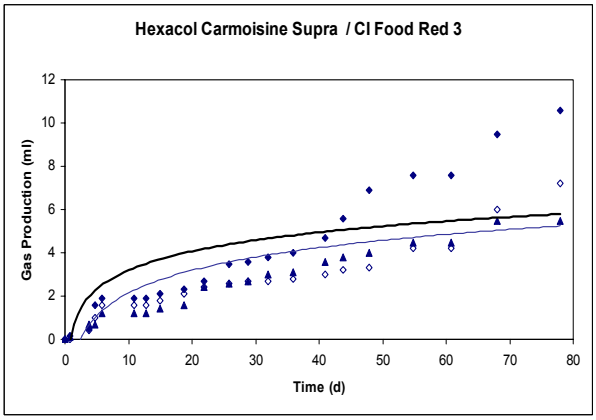
COD balance

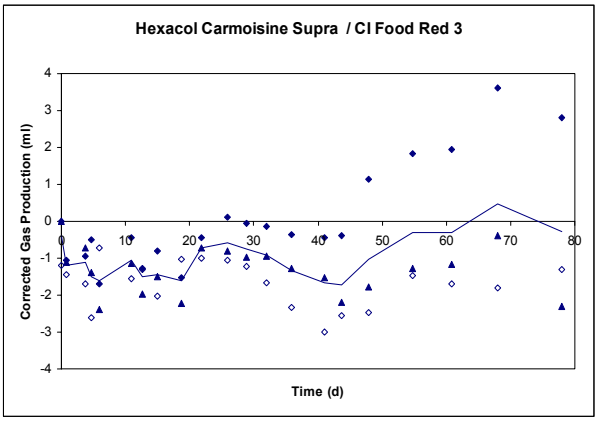
CODin : 127.6 mg (in 50 mL)	COD out :	45.4 mg (in 50 mL)
	CH_4 – COD :	0.1133 mg
	Total CODout :	45.51 mg
Balance :	**35.7 %**	
COD reduction :	64.3 %	

Colour reduction

Measured colour reduction :	78.5 % (480 nm) - Orange to brown

TABLE A3.8 *:* Results of the biodegradability assay with CI Food Red 3.





Biodegradability :

Dye concentration :	0.1 g/L
Theoretical dye COD :	1.15 g COD/g dye
Theoretical Assay COD (in 50 mL) :	5.75 mg COD
Initial biogas production rate :	0.12 mL/d
Acclimated biogas production rate (60 d) :	-
Total gas production (37 °C) :	4.07 mL
CH_4 fraction :	0.05
CH_4 production :	0.2035 mL
Net CH_4 production :	0 mL
CH_4 – COD :	0.51 mg

Methanogenic activity

Theoretical utilisation :	0 %
Actual utilisation :	0 %

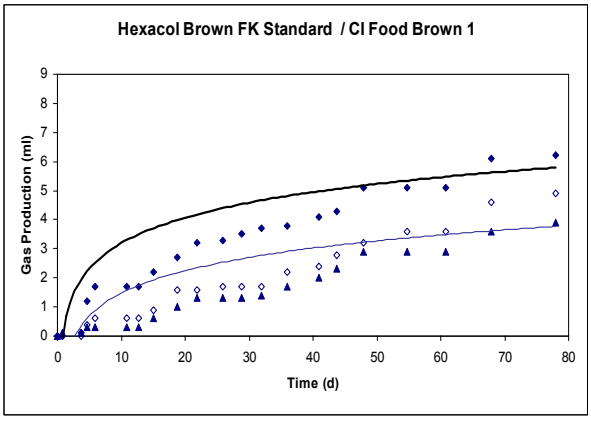
COD balance

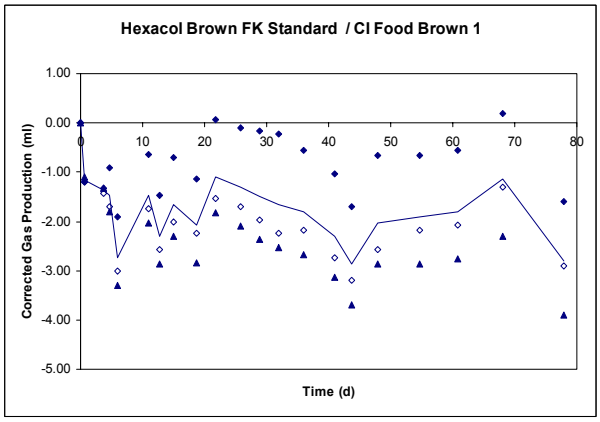
CODin : 67.6 mg (in 50 mL)	COD out :	20.7 mg (in 50 mL)
	CH_4 – COD :	0.51 mg
	Total CODout :	21.2 mg
Balance :	**31.4 %**	
COD reduction :	68.6 %	

Colour reduction

Measured colour reduction :	69.4 % (515 nm)

TABLE A3.9 *:* Results of the biodegradability assay with CI Food Brown 1.





Biodegradability :

Dye concentration :	0.24 g/L
Theoretical dye COD :	0.97 g COD/g dye
Theoretical Assay COD (in 50 mL) :	11.64 mg COD
Initial biogas production rate :	0.05 mL/d
Acclimated biogas production rate (60 d) :	-
Total gas production (37 °C) :	3.3 mL
CH_4 fraction :	0.047
CH_4 production :	0.156 mL
Net CH_4 production :	0 mL
CH_4 – COD :	0.39 mg

Methanogenic activity

Theoretical utilisation :	0 %
Actual utilisation :	0 %

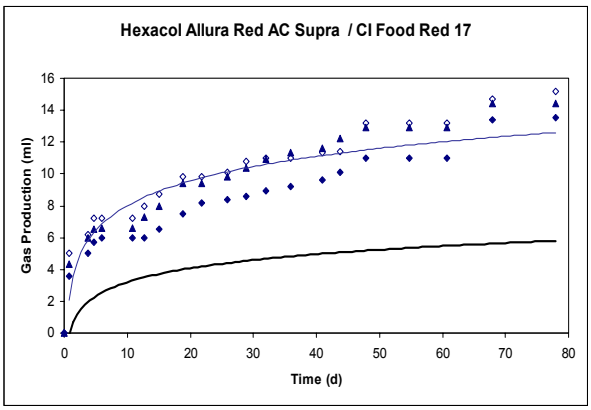
COD balance

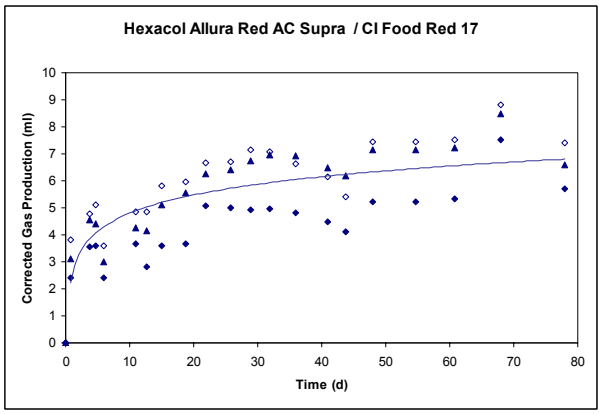
CODin : 60.86 mg (in 50 mL)	COD out : 20.3 mg (in 50 mL)
	CH_4 – COD : 0.39 mg
	Total CODout : 20.69 mg
Balance :	**34 %**
COD reduction :	66 %

Colour reduction

Measured colour reduction :	72 % (430 nm) - Orange/brown to brown

TABLE A3.10 : Results of the biodegradability assay with CI Food Red 17.





Biodegradability :

Dye concentration :	0.7 g/L
Theoretical dye COD :	1.48 g COD/g dye
Theoretical Assay COD (in 50 mL) :	51.8 mg COD
Initial biogas production rate :	6.06 mL/d
Acclimated biogas production rate (60 d) :	-
Total gas production (37 °C) :	11.7 mL
CH_4 fraction :	0.066
CH_4 production :	0.774 mL
Net CH_4 production :	0.389 mL
CH_4 – COD :	0.980 mg

Methanogenic activity

Theoretical utilisation :	1.9 %
Actual utilisation :	0.9 %

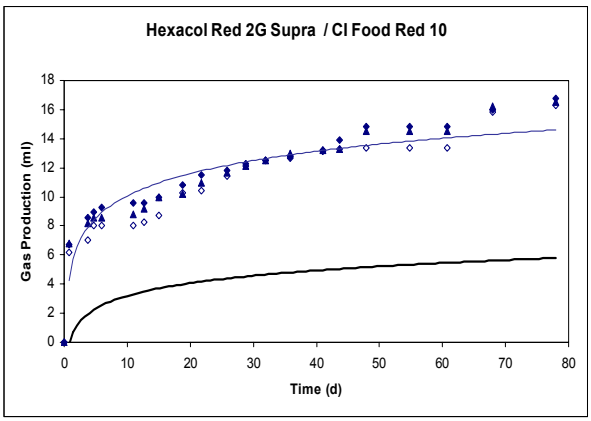
COD balance

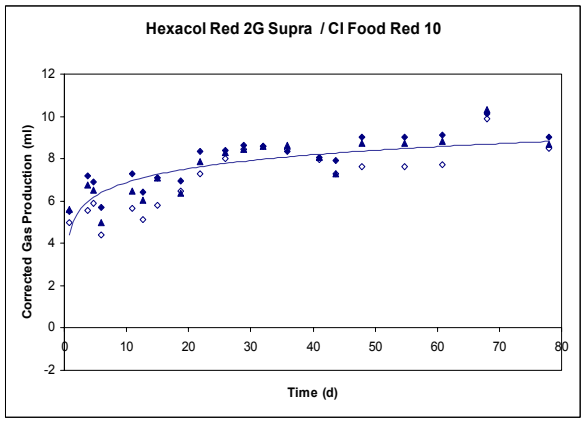
CODin : 107.8 mg (in 50 mL)	COD out :	46.9 mg (in 50 mL)
	CH_4 – COD :	0.980 mg
	Total CODout :	47.92 mg
Balance :	**44.5 %**	
COD reduction :	55.5 %	

Colour reduction

Measured colour reduction :	90 % (500 nm) - Red to brown

TABLE A3.11 : Results of the biodegradability assay with CI Food Red 10.





Biodegradability :

Dye concentration .:	1.26 g/L
Theoretical dye COD :	1.46 g COD/g dye
Theoretical Assay COD (in 50 mL) :	91.98 mg COD
Initial biogas production rate :	9.26 mL/d
Acclimated biogas production rate (60 d) :	-
Total gas production (37 °C) :	14.1 mL
CH_4 fraction :	0.068
CH_4 production :	0.956 mL
Net CH_4 production :	0.571 mL
CH_4 – COD :	1.439 mg

Methanogenic activity

Theoretical utilisation :	1.56 %
Actual utilisation :	1.3 %

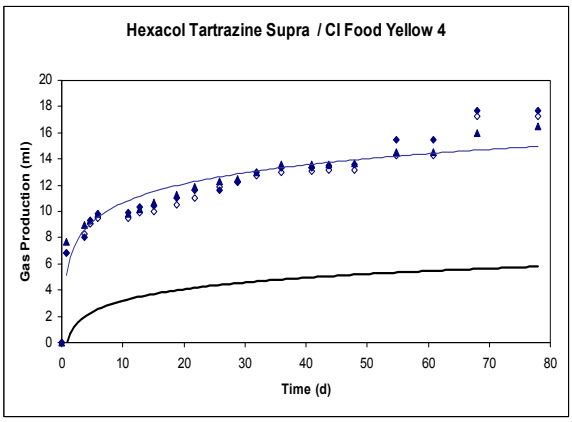
COD balance

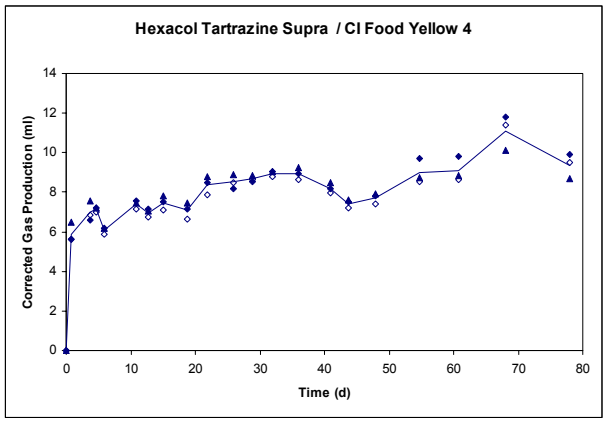
CODin : 101.1 mg (in 50 mL)	COD out :	46.6 mg (in 50 mL)
	CH_4 – COD :	1.439 mg
	Total CODout :	48.0 mg
Balance :	**47.5 %**	
COD reduction :	52.5 %	

Colour reduction

Measured colour reduction :	89.8 % (520 nm) - Red to brown

TABLE A3.12 : Results of the biodegradability assay with CI Food Yellow 4.





Biodegradability :

Dye concentration :	1.5 g/L
Theoretical dye COD :	0.97 g COD/g dye
Theoretical Assay COD (in 50 mL) :	72.75 mg COD
Initial biogas production rate :	10.01 mL/d
Acclimated biogas production rate (60 d) :	-
Total gas production (37 °C) :	13.6 mL
CH_4 fraction :	0.06
CH_4 production :	0.816 mL
Net CH_4 production :	0.431 mL
CH_4 – COD :	1.09 mg

Methanogenic activity

Theoretical utilisation :	2.3 %
Actual utilisation :	0.84 %

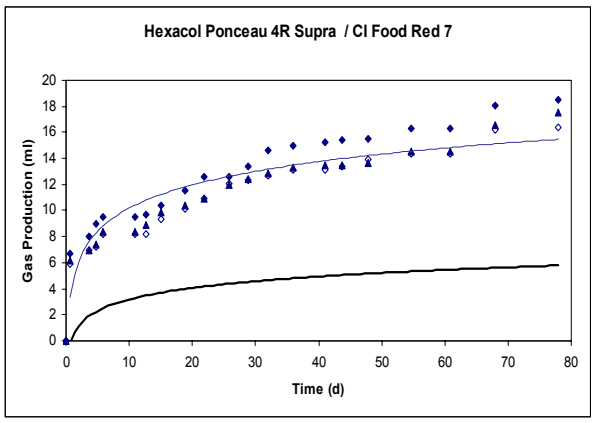
COD balance

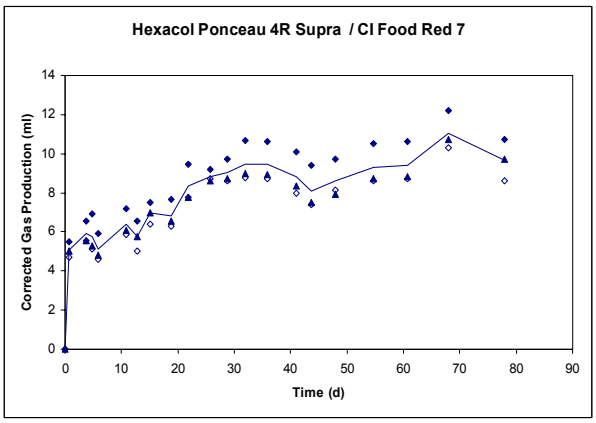
CODin : 131.36 mg (in 50 mL)	COD out :	66.9 mg (in 50 mL)
	CH_4 – COD :	1.09 mg
	Total CODout :	68.0 mg
Balance :	**51.8 %**	
COD reduction :	48.2 %	

Colour reduction

Measured colour reduction :	94.4 % (430 nm) - Yellow to purple

TABLE A3.13 : Results of the biodegradability assay with CI Food Red 7.





Biodegradability :

Dye concentration :	1.0 g/L
Theoretical dye COD :	0.86 g COD/g dye
Theoretical Assay COD (in 50 mL) :	43 mg COD
Initial biogas production rate :	8.84 mL/d
Acclimated biogas production rate :	-
Total gas production (37 °C) :	13.5 mL
CH_4 fraction :	0.074
CH_4 production :	0.998 mL
Net CH_4 production :	0.613 mL
CH_4 – COD :	1.54 mg

Methanogenic activity

Theoretical utilisation :	3.6 %
Actual utilisation :	1.64 %

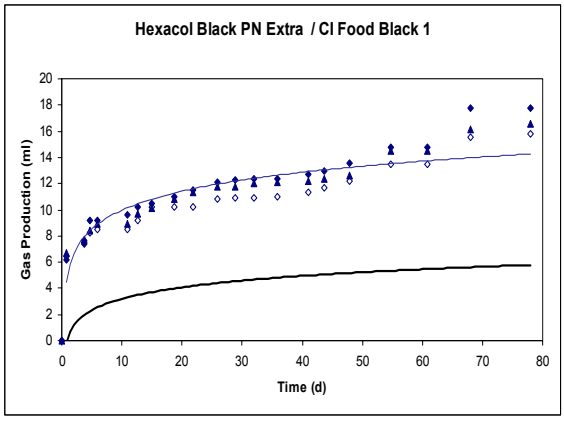
COD balance

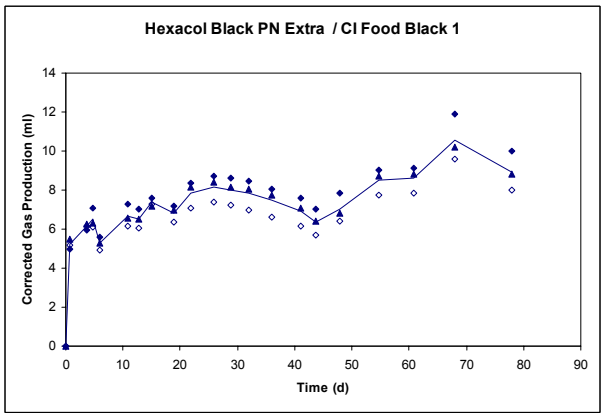
CODin : 93.72 mg (in 50 mL)	COD out :	46.8 mg (in 50 mL)
	CH_4 – COD :	1.54 mg
	Total CODout :	48.3 mg
Balance :	**52 %**	
COD reduction :	48.5 %	

Colour reduction

Measured colour reduction :	86.9 % (510 nm) - Red to brown

TABLE A3.14 *:* Results of the biodegradability assay with CI Food Black 1.





Biodegradability :

Dye concentration :	0.28 g/L
Theoretical dye COD :	0.826 g COD/g dye
Theoretical Assay COD (in 50 mL) :	11.56 mg COD
Initial biogas production rate :	9.07 mL/d
Acclimated biogas production rate (60 d) :	-
Total gas production (37 °C) :	13.3 mL
CH_4 fraction :	0.069
CH_4 production :	0.916 mL
Net CH_4 production :	0.531 mL
CH_4 – COD :	1.34 mg

Methanogenic activity

Theoretical utilisation :	11.6 %
Actual utilisation :	1.9 %

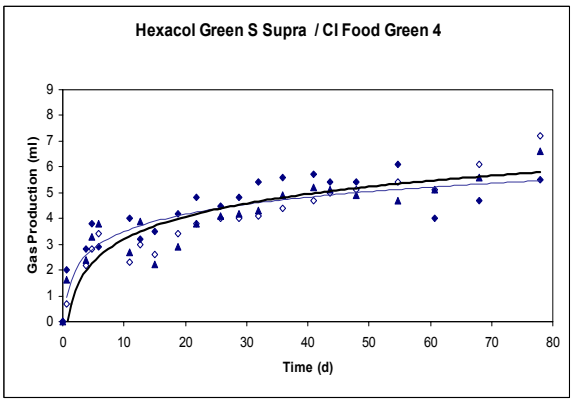
COD balance

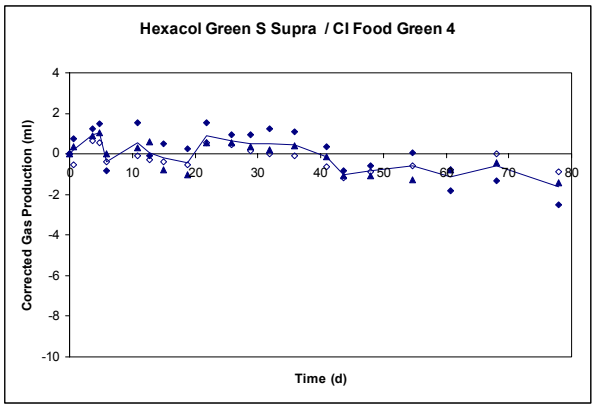
CODin : 69.6 mg (in 50 mL)	COD out :	30.09 mg (in 50 mL)
	CH_4 – COD :	1.34 mg
	Total CODout :	31.34 mg
Balance :	**45 %**	
COD reduction :	55 %	

Colour reduction

Measured colour reduction :	74.2 % (520 nm)

TABLE A3.15 *:* Results of the biodegradability assay with CI Food Green 4.





Biodegradability :

Dye concentration :	1.0 g/L
Theoretical dye COD :	1.5 g COD/g dye
Theoretical Assay COD (in 50 mL) :	75 mg COD
Initial biogas production rate :	2.02 mL/d
Acclimated biogas production rate (60 d) :	-
Total gas production (37 °C) :	5.5 mL
CH_4 fraction :	0.047
CH_4 production :	0.2585 mL
Net CH_4 production :	0 mL
CH_4 – COD :	0.6511 mg

Methanogenic activity

Theoretical utilisation :	0 %
Actual utilisation :	0 %

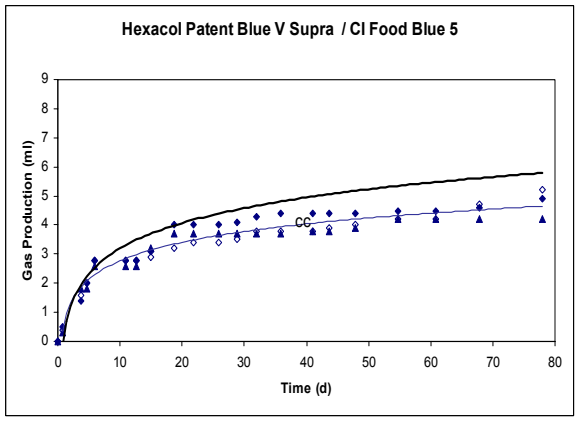
COD balance

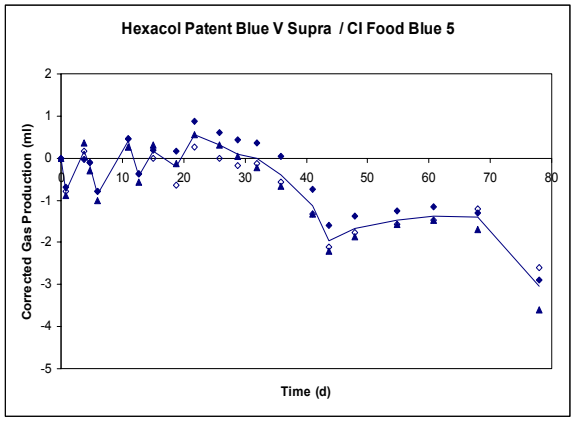
CODin : 118.1 mg (in 50 mL)	COD out :	83.7 mg (in 50 mL)
	CH_4 – COD :	0.6511 mg
	Total CODout :	84.35 mg
Balance :	**71.4 %**	
COD reduction :	28.6 %	

Colour reduction

Measured colour reduction :	94.9 % (630 nm) - Dark blue to light blue

TABLE A3.16 *:* Results of the biodegradability assay with CI Food Blue 5.





Biodegradability :

Dye concentration :	0.6 g/L
Theoretical dye COD :	2.1 g COD/g dye
Theoretical Assay COD (in 50 mL) :	63 mg COD
Initial biogas production rate :	0.56 mL/d
Acclimated biogas production rate (60 d) :	-
Total gas production (37 °C) :	1.77 mL
CH_4 fraction :	0.01
CH_4 production :	0.0197 mL
Net CH_4 production :	0 mL
CH_4 – COD :	0.05 mg

Methanogenic activity

Theoretical utilisation :	0 %
Actual utilisation :	0 %

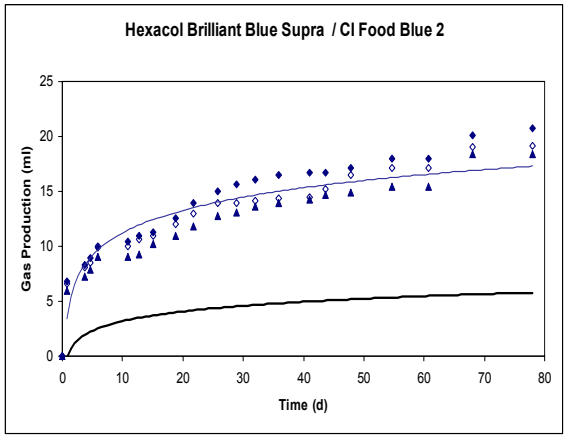
COD balance

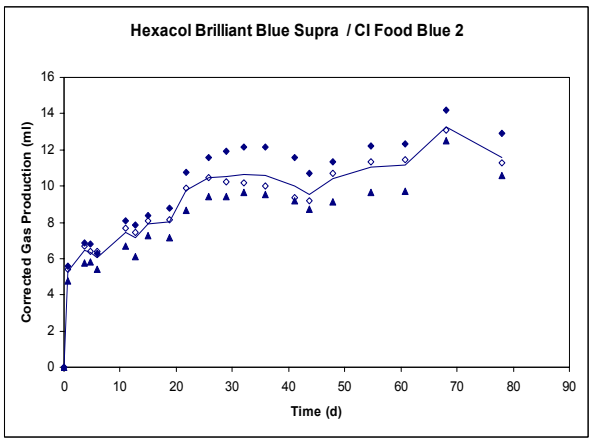
CODin : 188.9 mg (in 50 mL)	COD out :	72.2 mg (in 50 mL)
	CH_4 – COD :	0.05 mg
	Total CODout :	72.25 mg
Balance :	**38.2 %**	
COD reduction :	61.8 %	

Colour reduction

Measured colour reduction :	33.6 % (630 nm)

TABLE A3.17 *:* Results of the biodegradability assay with CI Food Blue 2.





Biodegradability :

Dye concentration :	1.15 g/L
Theoretical dye COD :	1.61 g COD/g dye
Theoretical Assay COD (in 50 mL) :	92.57 mg COD
Initial biogas production rate :	9.12 mL/d
Acclimated biogas production rate (60 d) :	-
Total gas production (37 °C) :	15.0 mL
CH_4 fraction :	0.087
CH_4 production :	1.31 mL
Net CH_4 production :	0.926 mL
CH_4 – COD :	2.33 mg

Methanogenic activity

Theoretical utilisation :	2.52 %
Actual utilisation :	2.08 %

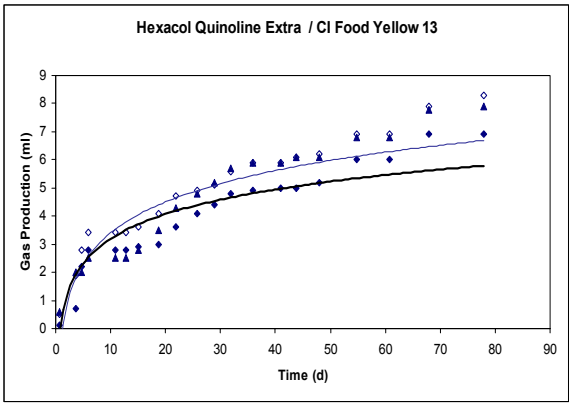
COD balance

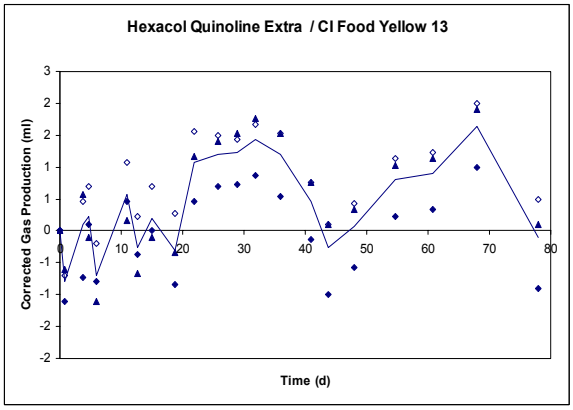
CODin : 112 mg (in 50 mL)	COD out :	48.8 mg (in 50 mL)
	CH_4 – COD :	2.33 mg
	Total CODout :	51.17mg
Balance :	**45.7%**	
COD reduction :	54 %	

Colour reduction

Measured colour reduction :	0 % (630 nm)

TABLE A3.18 *:* Results of the biodegradability assay with CI Food Yellow 13.





Biodegradability :

Dye concentration :	1.0 g/L
Theoretical dye COD :	1.35 g COD/g dye
Theoretical Assay COD (in 50 mL) :	67.5 mg COD
Initial biogas production rate :	0.56 mL/d
Acclimated biogas production rate (60 d) :	-
Total gas production (37 °C) :	5.37 mL
CH_4 fraction :	0.05
CH_4 production :	0.272 mL
Net CH_4 production :	0 mL
CH_4 – COD :	0.6851 mg

Methanogenic activity

Theoretical utilisation :	0 %
Actual utilisation :	0 %

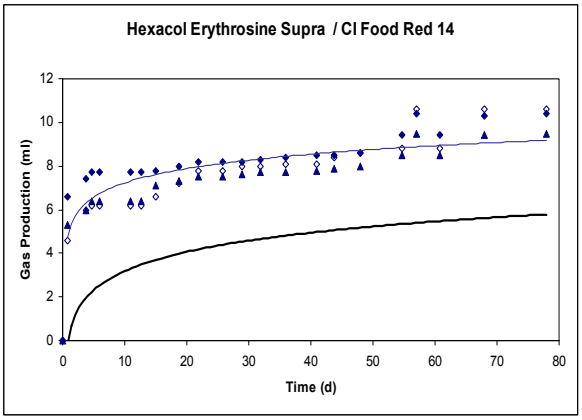
COD balance

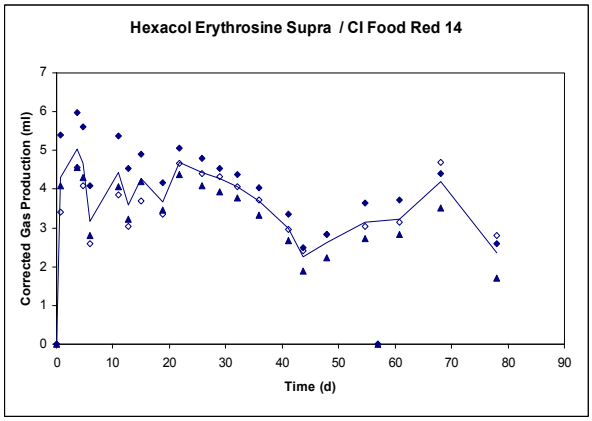
CODin : 106.5 mg (in 50 mL)	COD out :	44.7 mg (in 50 mL)
	CH_4 – COD :	0.6851 mg
	Total CODout :	45.39 mg
Balance :	**42.6 %**	
COD reduction :	57.4 %	

Colour reduction

Measured colour reduction :	0 % (410 nm)

TABLE A3.19 *:* Results of the biodegradability assay with CI Food Red 14.





Biodegradability :

Dye concentration :	0.0001 g/L
Theoretical dye COD :	0.69 g COD/g dye
Theoretical Assay COD (in 50 mL) :	0.00345 mg COD
Initial biogas production rate :	7.76 mL/d
Acclimated biogas production rate (60 d) :	-
Total gas production (37 °C) :	6.83 mL
CH_4 fraction :	0.02
CH_4 production :	0.145 mL
Net CH_4 production :	0 mL
CH_4 – COD :	0.365 mg

Methanogenic activity

Theoretical utilisation :	0 %
Actual utilisation :	0 %

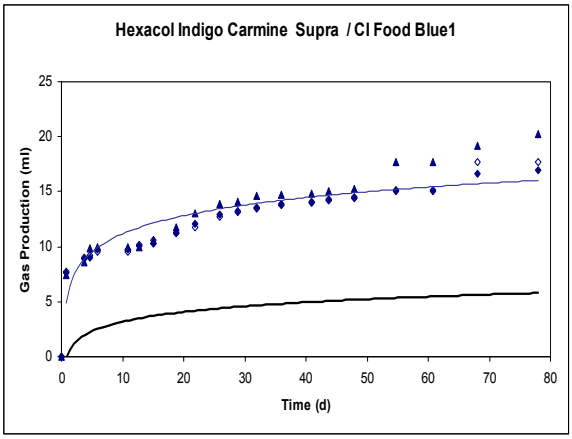
COD balance

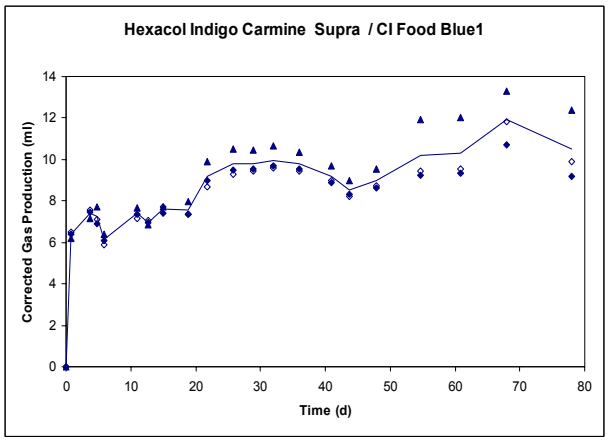
CODin : 51.9 mg (in 50 mL)	COD out : 30.12 mg (in 50 mL)
	CH_4 – COD : 0.365 mg
	Total CODout : 30.49 mg
Balance :	**59 %**
COD reduction :	41.3 %

Colour reduction

Measured colour reduction :	0.8 % (520 nm) - Pink to pink

TABLE A3.20 *:* Results of the biodegradability assay with CI Food Blue 1.





Biodegradability :

Dye concentration :	0.52 g/L
Theoretical dye COD :	0.89 g COD/g dye
Theoretical Assay COD (in 50 mL) :	23.14 mg COD
Initial biogas production rate :	10.67 mL/d
Acclimated biogas production rate (60 d) :	-
Total gas production (37 °C) :	14.3 mL
CH_4 fraction :	0.068
CH_4 production :	0.978 mL
Net CH_4 production :	0.583 mL
CH_4 – COD :	1.47 mg

Methanogenic activity

Theoretical utilisation :	6.4 %
Actual utilisation :	2.6 %

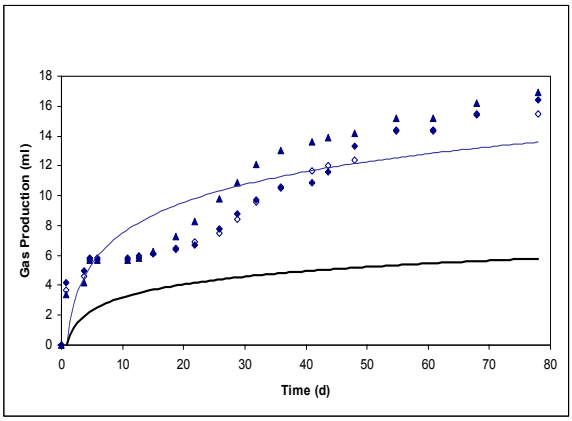
COD balance

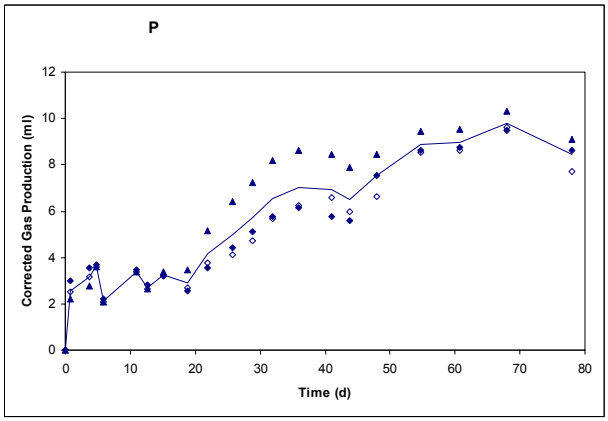
CODin : 57.23 mg (in 50 mL)	COD out :	36.03 mg (in 50 mL)
	CH_4 – COD :	1.47 mg
	Total CODout :	37.5 mg
Balance :	**66 %**	
COD reduction :	34 %	

Colour reduction

Measured colour reduction :	16.6 % (610 nm)

TABLE A3.21 *:* Results of the biodegradability assay with the Untreated Food Dye Effluent (20 % v/v).





Biodegradability :

Initial biogas production rate :	5.31 mL/d
Acclimated biogas production rate :	-
Total gas production (37 °C) :	13.3 mL
CH_4 fraction :	0.092
CH_4 production :	1.226 mL
Net CH_4 production :	0.841 mL
CH_4 – COD :	2.1 mg

Methanogenic activity

Actual utilisation : 2.2 %

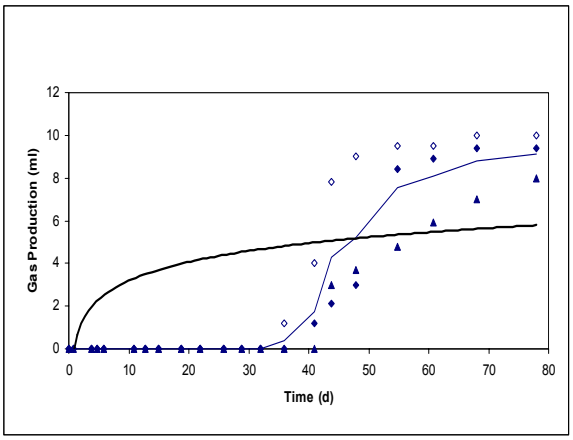
COD balance

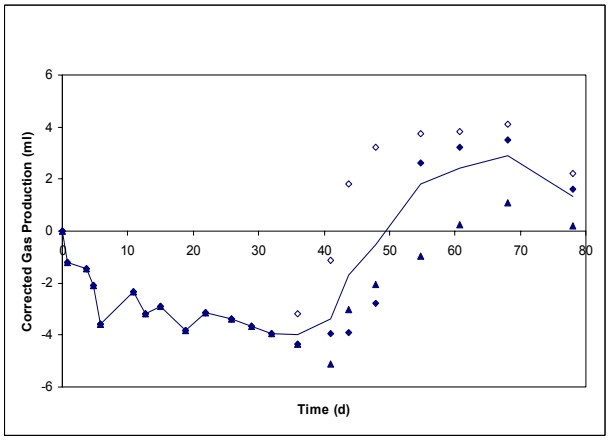
CODin : 93.9 mg (in 50 mL)	COD out :	56.8 mg (in 50 mL)
	CH_4 – COD :	2.1 mg
	Total CODout :	58.92 mg
Balance :	**62.7 %**	
COD reduction :	37 %	

Colour reduction

Measured colour reduction : 65.8 % (500 nm)

TABLE A3.22 *:* Results of the biodegradability assay with the Untreated Food Dye Effluent (100 % v/v).





Biodegradability :

Initial biogas production rate :	0 mL/d
Acclimated biogas production rate (40 d) :	0.1 mL/d
Total gas production (37 °C) :	7.4 mL
CH_4 fraction :	0.103
CH_4 production :	0.762 mL
Net CH_4 production :	0.377 mL
CH_4 – COD :	0.95 mg

Methanogenic activity

Actual utilisation : 0.43 %

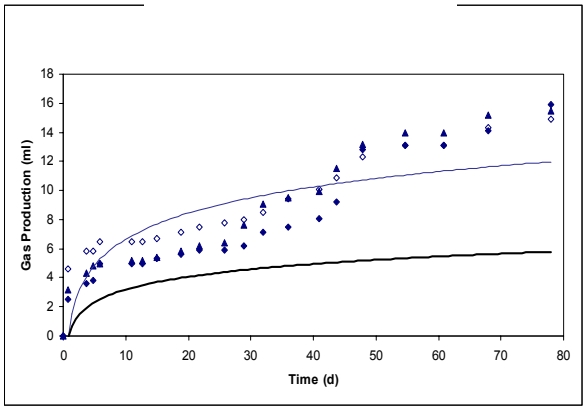
COD balance

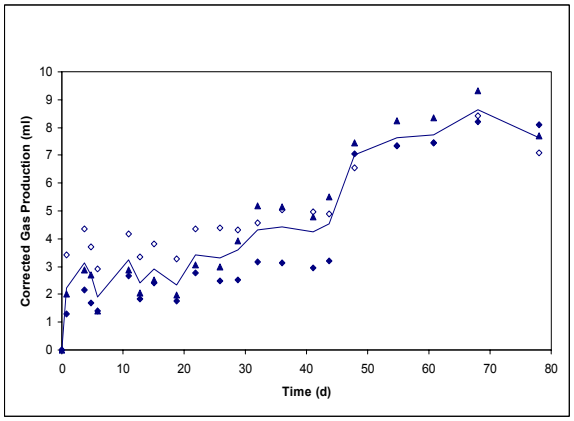
CODin : 220.7 mg (in 50 mL)	COD out :	78.6 mg (in 50 mL)
	CH_4 – COD :	0.95 mg
	Total CODout :	79.6 mg
Balance :	**36 %**	
COD reduction :	64 %	

Colour reduction

Measured colour reduction : 79.8 % (500 nm)

TABLE A3.23 *:* Results of the biodegradability assay with the Treated Food Dye Effluent (20 % v/v).





Biodegradability :

Initial biogas production rate :	4.84 mL/d
Acclimated biogas production rate (40 d) :	-
Total gas production (37 °C) :	12.77 mL
CH_4 fraction :	0.089
CH_4 production :	1.14 mL
Net CH_4 production :	0.755 mL
CH_4 – COD :	1.9 mg

Methanogenic activity

Actual utilisation : 1.96 %

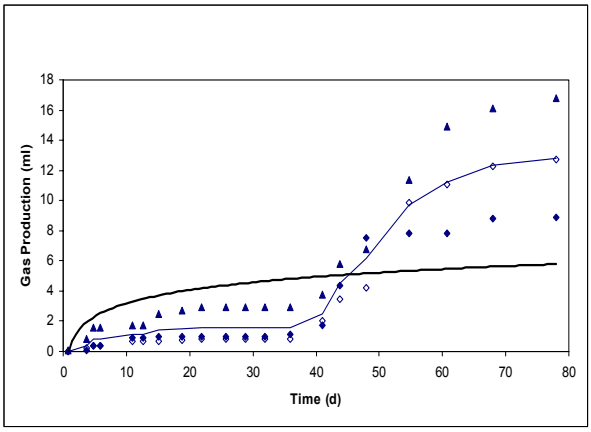
COD balance

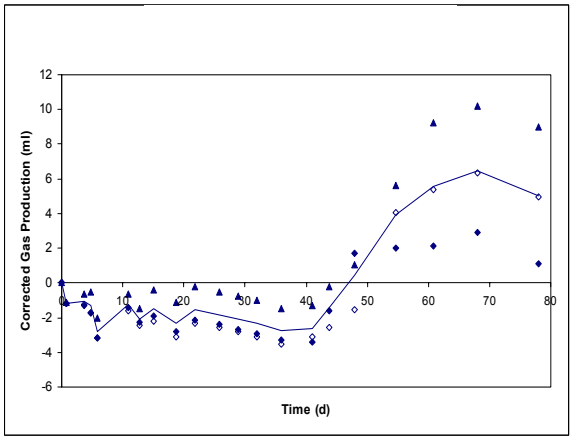
CODin : 96.8 mg (in 50 mL)	COD out :	42.3 mg (in 50 mL)
	CH_4 – COD :	1.9 mg
	Total CODout :	44.2 mg
Balance :	**46 %**	
COD reduction :	54 %	

Colour reduction

Measured colour reduction : 31 % (500 nm)

TABLE A3.24 *:* Results of the biodegradability assay with the Treated Food Dye Effluent (100 % v/v).





Biodegradability :

Initial biogas production rate :	0.05 mL/d
Acclimated biogas production rate (40 d) :	0.19 mL/d
Total gas production (37 °C) :	2.97 mL
CH_4 fraction :	0.103
CH_4 production :	0.306 mL
Net CH_4 production :	0 mL
CH_4 – COD :	0.77 mg

Methanogenic activity

Actual utilisation : 0 %

COD balance

CODin : 184.5 mg (in 50 mL)	COD out :	71.8 mg (in 50 mL)
	CH_4 – COD :	0.77 mg
	Total CODout :	72.6 mg
Balance :	**39 %**	
COD reduction :	61 %	

Colour reduction

Measured colour reduction : 36.6 % (500 nm)

A-4

Textile Dyes

A series of serum bottle tests as described in **Table A4.1**, and the details of each are given.

TABLE A4.1 : Details of the textile dye serum bottle tests.

Test	*Spike*	Substrate	Reference
Methanogenic toxicity	Acetate-propionate	Textile dyes	Section A4.1
Acidogenic toxicity	Glucose	Textile dyes	Section 5.2
Anaerobic biodegradability	-	Textile dyes	Section A4.2
Anaerobic biodegradability	-	Textile dye degradation products	Section 5.3

A4.1 ANAEROBIC TOXICITY ASSAYS

Inhibition is commonly reported in investigations describing the biological treatment of textile dye wastewaters. This inhibition may be related to the dyes themselves or to other components of the wastewater such as the various additives required in the dyeing process. This study was limited to the investigation of the inhibitory effects of the dyes.

A4.1.1 Hypotheses and Objectives

From a study of the literature, it was hypothesised that the reactive dye compounds, present in textile dye wastewaters, could exert an inhibitory effect on the anaerobic biomass involved in the treatment process Bioassay techniques are an effective indication of the effect that these substances would have on an anaerobic system.

The objective of this investigation was to assess the toxicity of a range of textile dyes to the methanogens in anaerobic digester sludge.

A4.1.2 Materials and Methods

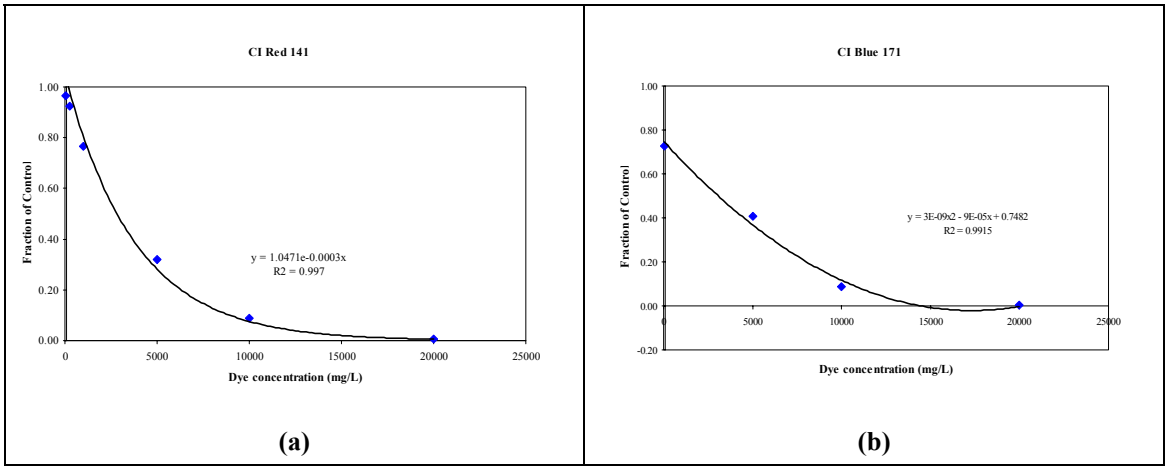
A stock solution (10 % w/v) of each dye (**Table 5.2**) was made up. The experiments were performed in 160 mL glass serum bottles, which were sealed with butyl rubber septa and aluminium crimp seals. A defined nutrient medium containing trace elements, minerals and vitamins was prepared according to Owen *et al*. (1979) (**Appendix 1).**

The bottles were prepared with 100 mL working volumes. The bottles were seeded with anaerobic digester sludge collected from the Umbilo Sewage Works (TS = 30.67 g/L; VS = 13.67 g/L). The dye concentrations investigated, for each dye, were: 50 mg/L; 250 mg/L; 1 g/L; 5 g/L; 10 g/L and 20 g/L. The anaerobic toxicity assays were also run with a mixture of the four dyes. Each concentration was repeated in triplicate. The acetate-propionate solution (2mL, to give 75 mg acetate and 26.5 mg propionate in 100 mL working volume) was added to each bottle after equilibration for 1 h, at the incubation temperature of 35 °C.

The controls, or blanks, contained only the inoculum sludge, the anaerobic nutrient medium and the acetate-propionate solution. The methanogenic metabolism of the acetate-propionate solution was monitored by total gas production, in the controls. Inhibition due to the addition of a dye was determined as a decreased rate of gas production, relative to the controls.

Gas volume and composition measurements were the same as described in **Section A3.1.2**. The methanogenic activity was calculated (mL CH_4/g VS) for each dye concentration, and calculated as a fraction of the methanogenic activity in the controls. The activity at each concentration was plotted and a best-fit line was fitted through the data points. From the equation of the line, the dye concentration at which 50 % of the methanogenic biomass was inhibited (IC_{50}) could be calculated. These results are given below.

A4.1.3 Results



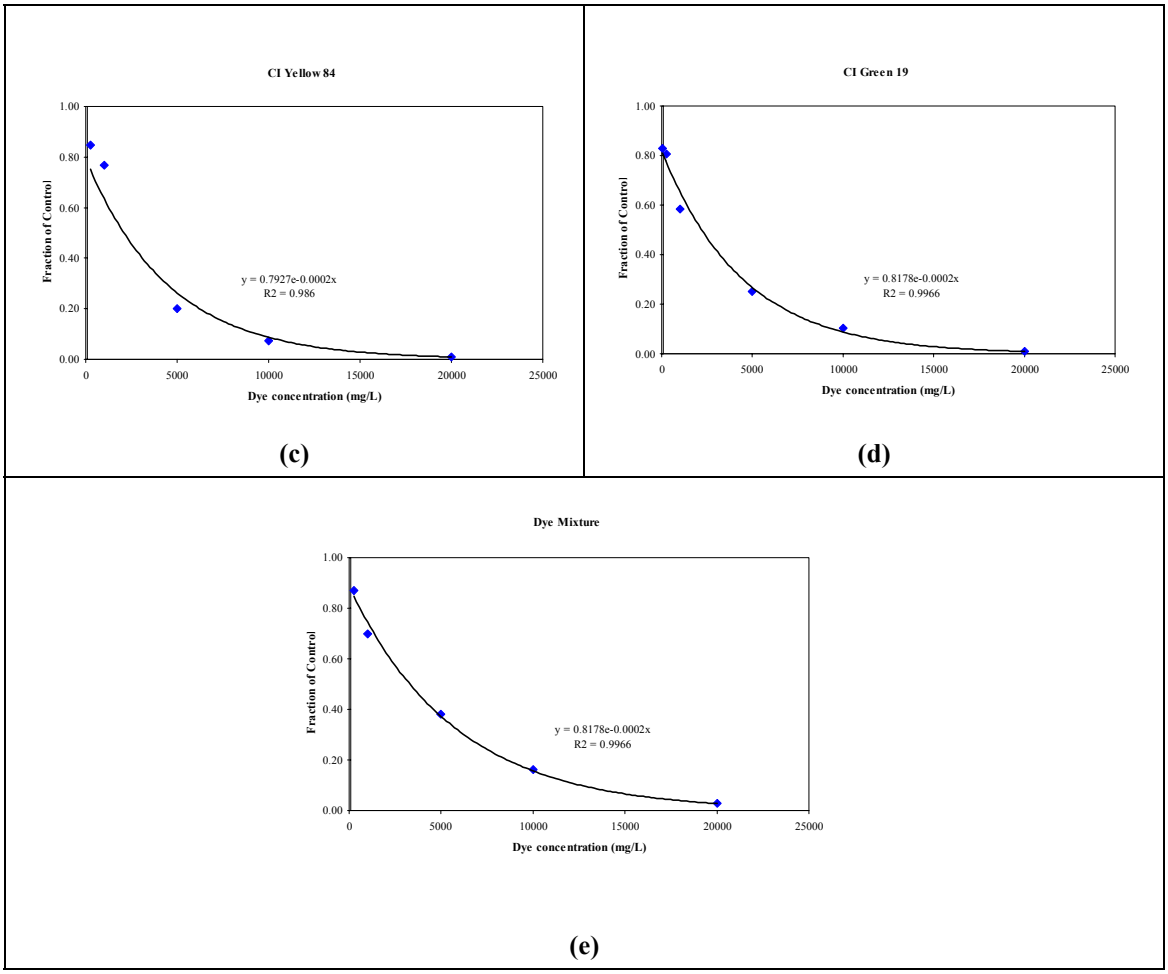


FIGURE A4.1 : Plots showing the methanogenic activity, as a fraction of that in the control, for each investigated dye concentration and the best-fit lines through the experimental data points, used to determine the IC_{50} concentration for each dye.

The gas production results for each dye are given in **Section A4.1.6**. The symbols represent the duplicate samples, and the line through the data points is the calculated mean biogas production. For each concentration, the gas p*roduction curve is shown relative to the gas production rate of the controls. A decrease in biogas production (line below that of the control) indicated inhibition of the methanogens due to addition of the dye. The calculated IC_{50} concentration for each dye is given in **Table A4.2**.

TABLE A4.2 : Calculated methanogenic IC_{50} values for the investigated textile dyes.

Dye	Methanogenic IC_{50} (g/L)
CI Red 141	2.46
CI Blue 171	3.07
CI Yellow 84	2.30
CI Green 19	2.46
Dye mixture	2.46

Figure A4.2 shows the cumulative biogas production for each concentration of the investigated textile reactive dyes, relative to the gas production measured in the controls.

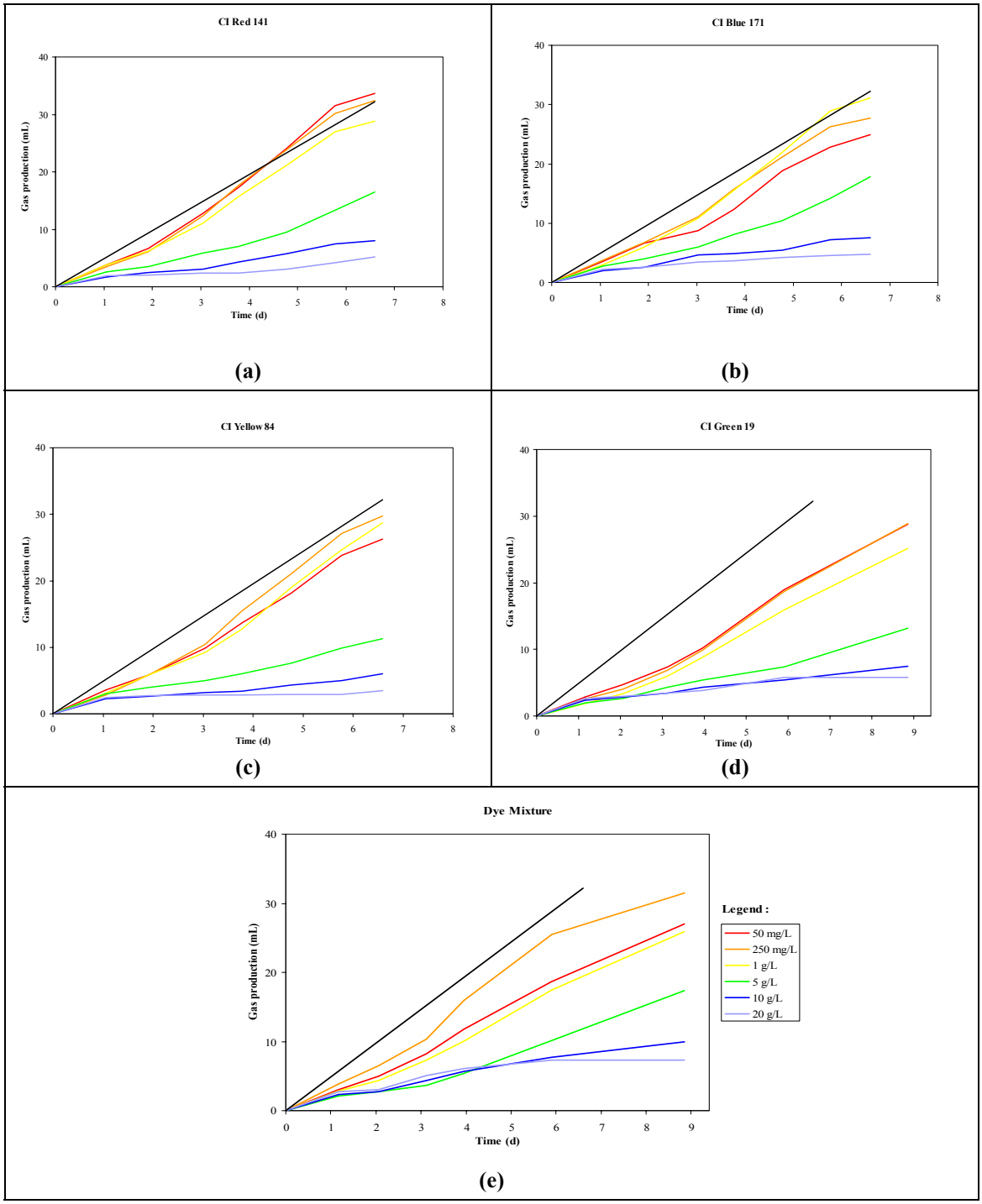


FIGURE A4.2 : Gas production plots for the methanogenic toxicity assays, showing the cumulative biogas production for each concentration of the investigated textile reactive dyes, relative to the gas production measured in the controls (black line).

A4.1.4 Discussion

The literature has indicated that dye compounds and their degradation products can be toxic. The results of these toxicity assays showed that the four investigated dyes and the dye mixture were inhibitory to the methanogens, with all of the IC_{50} concentrations < 3.1 g/L. This indicates that these dyes could be problematic in the anaerobic treatment of dye wastewaters since they could cause inhibition of the methanogens present in the treatment system, resulting in reactor failure and inefficient treatment. Although it is unlikely that a normal dyehouse effluent would have a dye concentration > 3.1 g/L, it is not impossible. This could easily occur with wastage or washing procedures, resulting in a large volume of the dye in the final effluent.

The gas production plots shown in **Section A4.1.6** show the total biogas production measured over the incubation period. The fraction of methane in the biogas was calculated from the biogas composition analyses. The addition of the acetate-propionate solution made these tests specific for methanogenic inhibition since the added VFAs are precursors to methanogenesis.

The gas production for all concentrations of the investigated dyes, and the dye mixture, was lower than the gas production in the controls, indicating that all of the dyes were inhibitory to the methanogens, resulting in reduced methanogenic activity within the serum bottles. There were two exceptions, in the 50 and 250 mg/L concentrations of CI Reactive Red 141, the gas production curve was initially lower than the controls, but increased to equal the gas production in the controls, by day 5 (**Figure A4.2 (a)**). This suggests acclimation of the biomass to the lower dye concentrations. In all cases, the degree of inhibition increased with increasing dye concentration. The most inhibitory dye was CI Reactive Yellow 84 (IC_{50} of 2.3 g/L).

Carliell *et al*. (1995) observed that CI Reactive Red 141 was inhibitory to methane production at concentrations over 100 mg/L using the toxicity assay methodology described by Owen *et al*. (1979). The results of this study indicate acclimation and methane production at a CI Reactive Red 141 concentration of 250 mg/L.

An interesting result was observed in the CI Reactive Yellow 84 assays (**Figure A4.2 (c)**); although equal volumes of biogas produced were measured for the first three days, after this the gas production in the 250 mg/L and 1 g/L assay bottles was marginally greater than in the 50 mg/L assay bottles. This would suggest acclimation and utilisation of the dye as a substrate, however, the gas production in all of these bottles was still lower than in the controls, indicating inhibition of the methanogens due to the addition of the dye. The same was observed in the dye mixture assays (**Figure 5.1 (e)**) where the gas production in the 250 mg/L assay bottles was greater than in the 50 mg/L bottles.

A4.1.5 Conclusions

1. The toxicity assays were specific to the methanogenic populations of the anaerobic digester sludge.

2. The results showed that the four investigated dyes, and the dye mixture, were inhibitory to the methanogens, with all of the IC_{50} concentrations < 3.1 g/L.

3. The CI Reactive Red 141 assays showed acclimation of the biomass to the dye, at the 50 and 250 mg/L concentrations.

4. For all dyes, the degree of inhibition increased with increasing dye concentration.

5. The most inhibitory dye was CI Reactive Yellow 84 (IC_{50} of 2.3 g/L).

A4.1.6 Biogas Production Plots

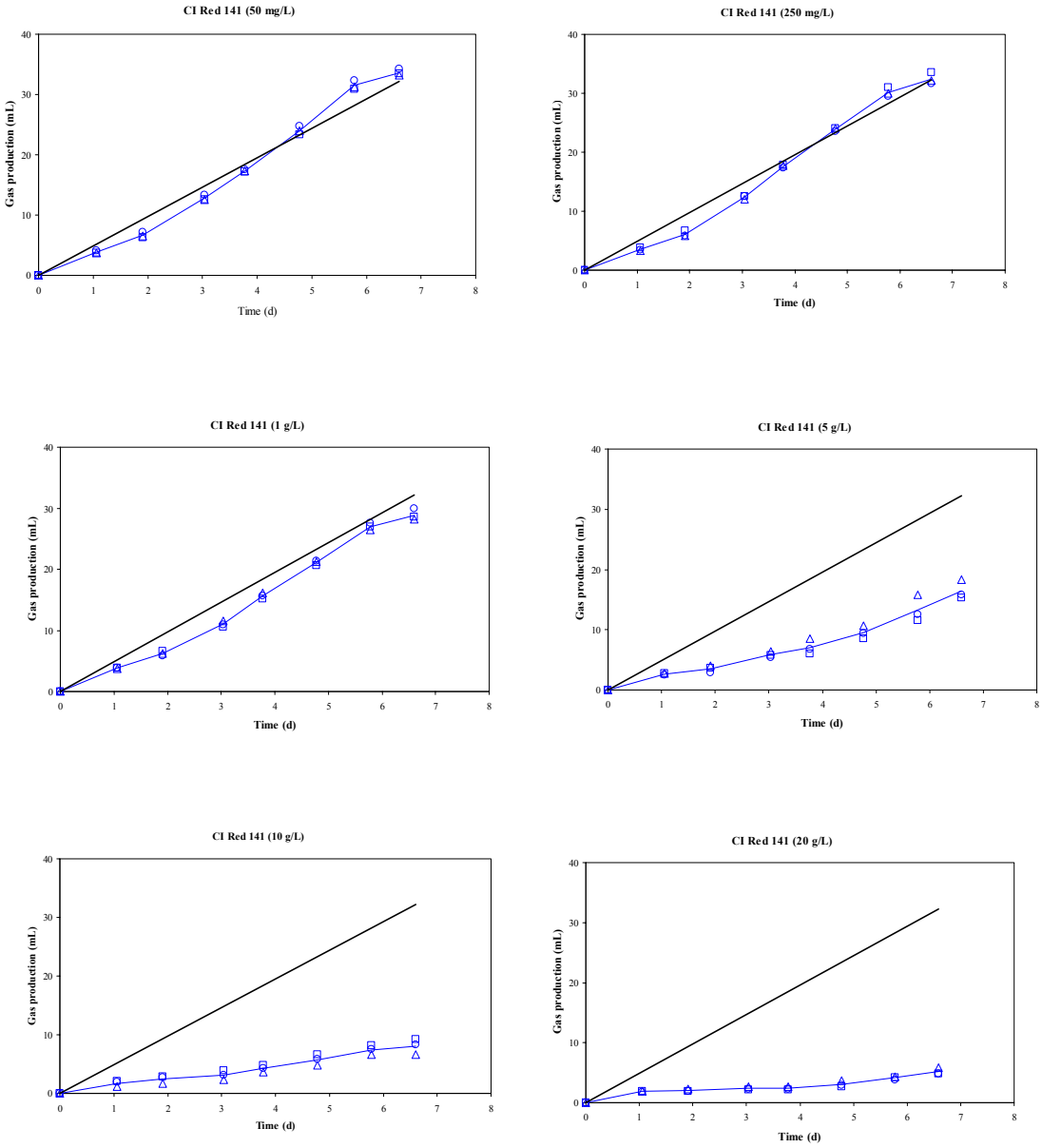


FIGURE A4.3 : Plots of biogas production during the anaerobic toxicity assay with CI Reactive Red 141.

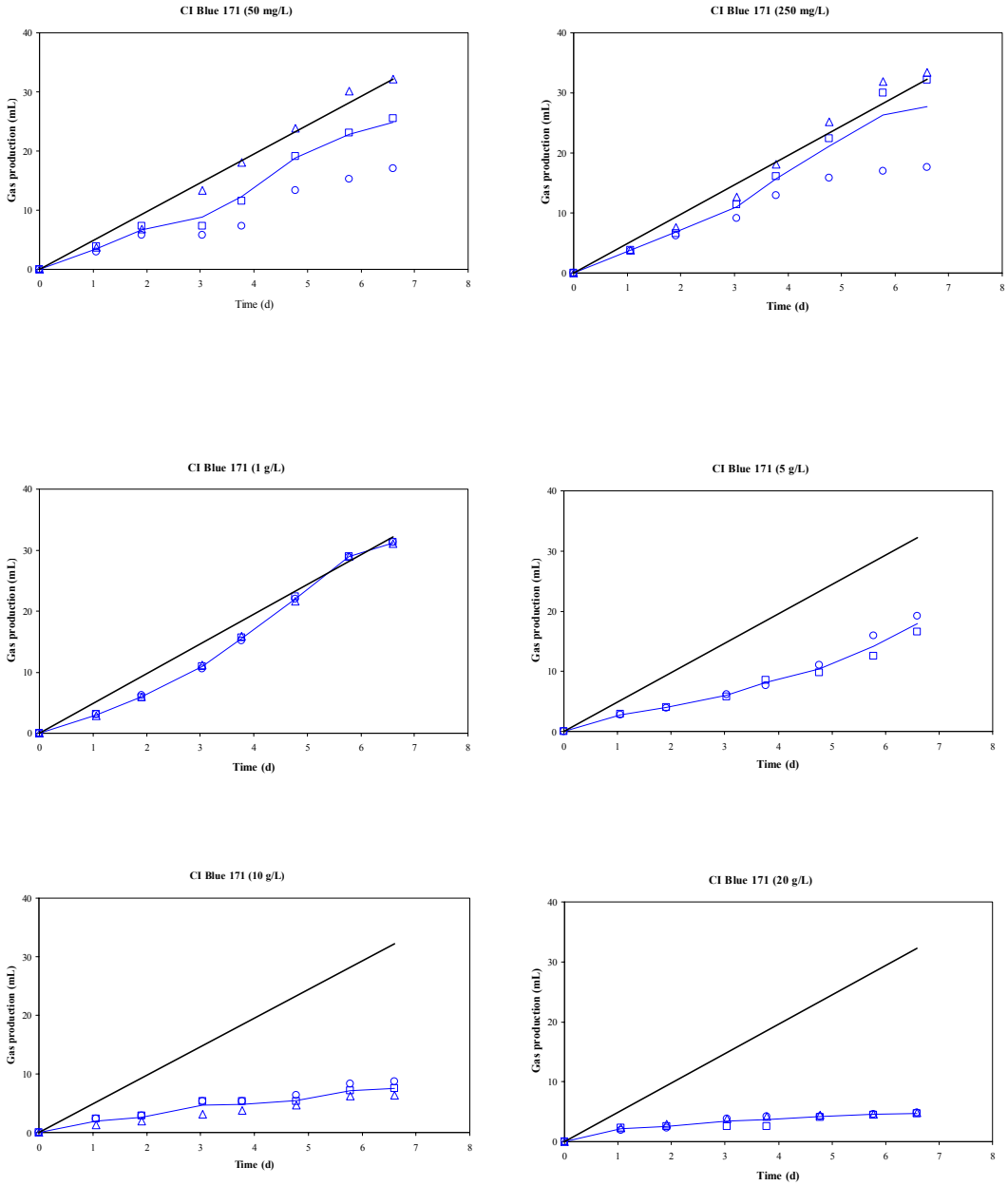


FIGURE A4.4 : Plots of biogas production during the anaerobic toxicity assay with CI Reactive Blue 171.

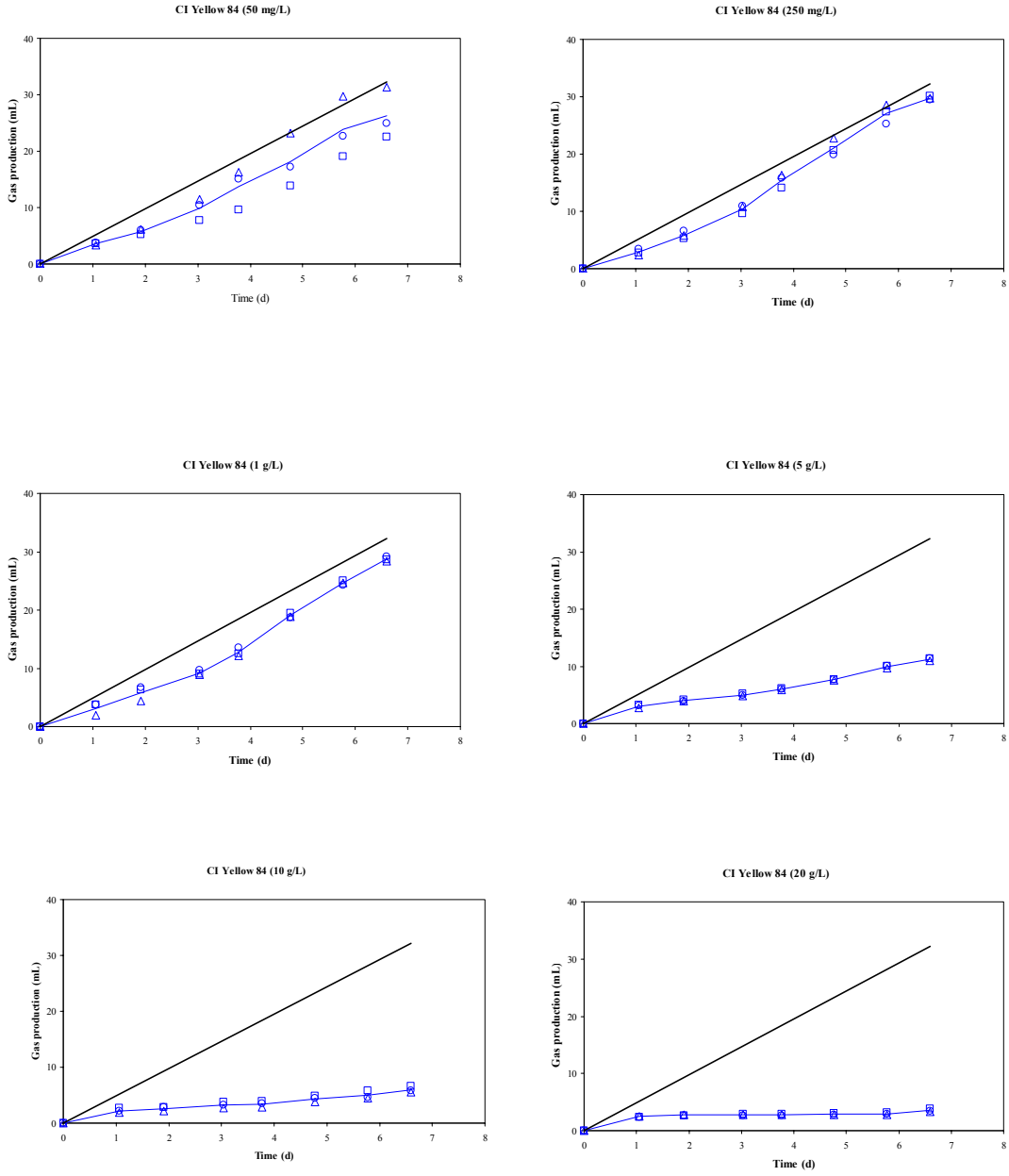


FIGURE A4.5 : Plots of biogas production during the anaerobic toxicity assay with CI Reactive Yellow 84.

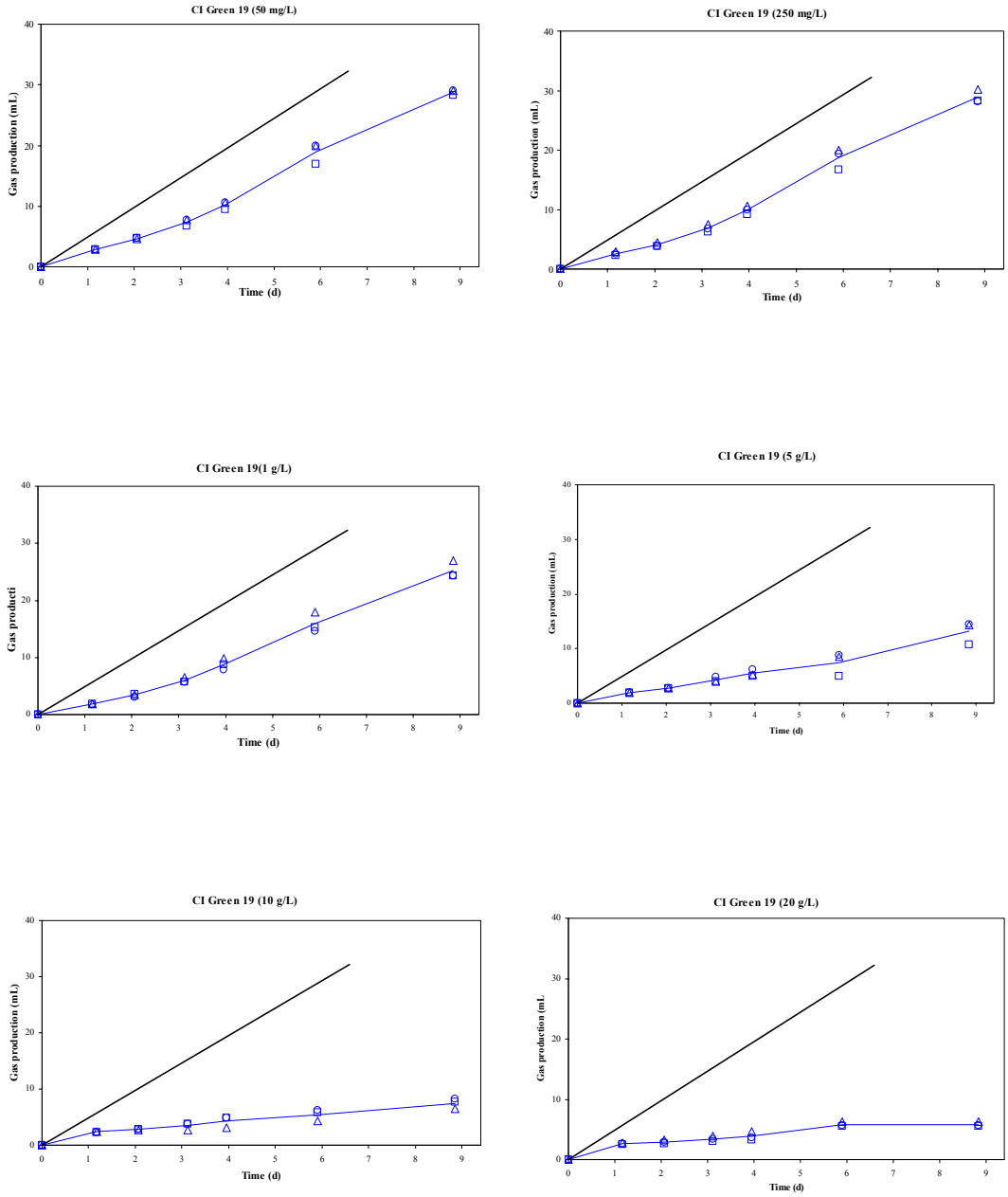


FIGURE A4.6 : Plots of biogas production during the anaerobic toxicity assay with CI Reactive Green 19.

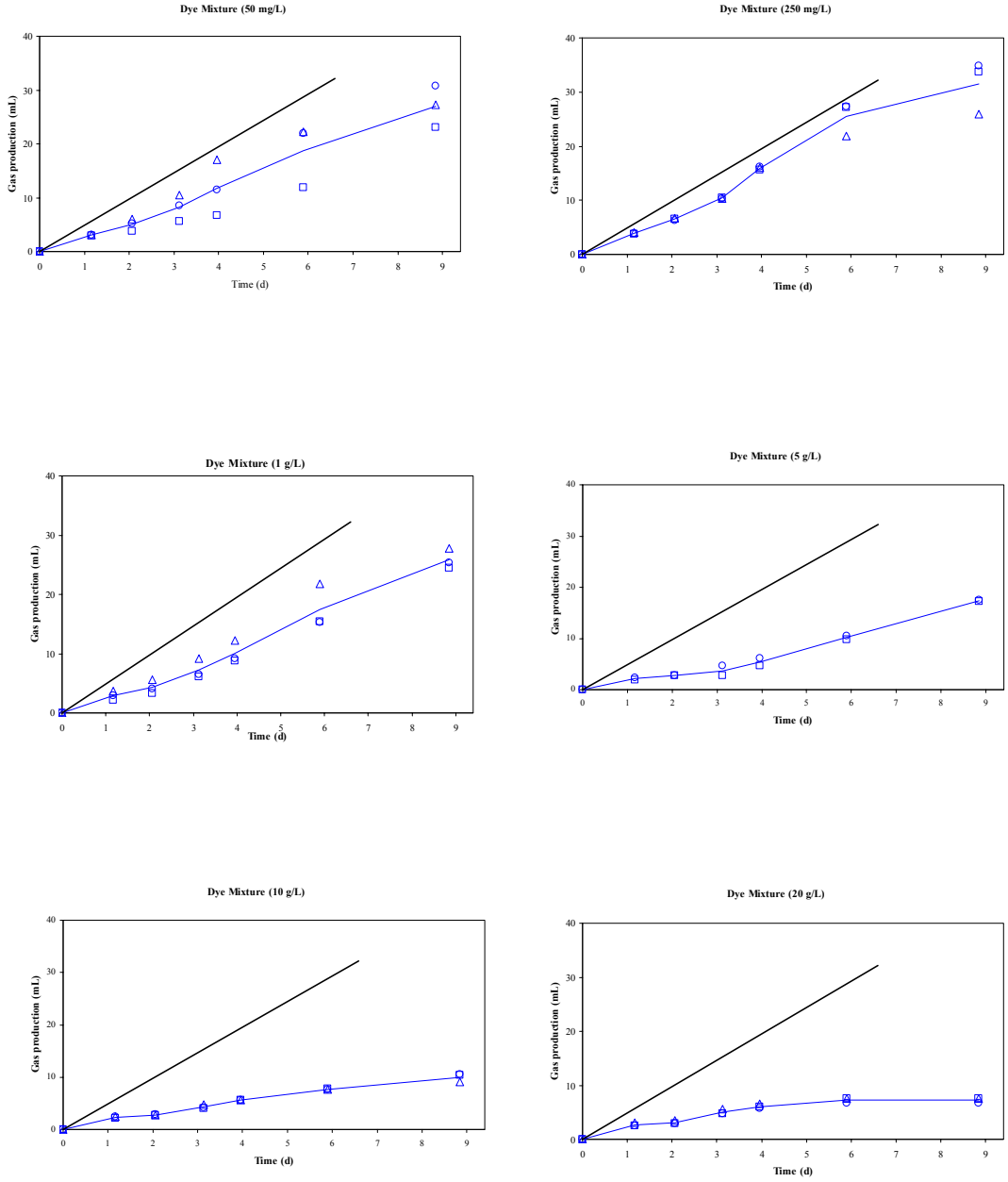


FIGURE A4.7 : Plots of biogas production during the anaerobic toxicity assay with the mixture of textile reactive dyes.

A4.2 BIODEGRADABILITY ASSAYS

The anaerobic biodegradability of the textile dyes was determined by monitoring the cumulative biogas production during anaerobic incubation, according to the method of Owen *et al*. (1979).

A4.2.1 Hypotheses and Objectives

It was hypothesised that biodegradability assays would provide information on microbial metabolism of the dyes and acclimation of the anaerobic microorganisms to the inhibitory dyes.

Therefore, the objectives of the investigation were to:

1. Assess the anaerobic biodegradability of the textile dyes by the microbial populations present in the anaerobic digester sludge.

2. Determine the anaerobic biodegradability of each dye.

3. Determine the methanogenic utilisation of the dye as a substrate.

A4.2.2 Materials and Methods

A preliminary control run (data not shown) investigated the difference in degradation potential of both un-hydrolysed and hydrolysed dyes. The dyes were hydrolysed, to imitate their form in a wastewater stream, by raising the pH to 11 with 0.2 M NaOH and heating at 80 °C for 2 h. There was a negligible difference in the results, thus un-hydrolysed dyes were used for the remainder of the study. Fontenot *et al*. (2001) also found no significant difference between the decolourisation reaction of a hydrolysed and unhydrolysed reactive dye.

The same four dyes were investigated as in the anaerobic toxicity assays (**Table 5.2**). The nutrient medium was prepared as described in **Appendix 1**. The inoculum sludge was obtained from an operating anaerobic digester at the Umbilo Sewage Works (TS = 18 g/L and VS = 12.7 g/L.). These assay bottles were set up slightly differently to the food dye biodegradability assays in that the concentration of dye added to each assay bottle was not calculated according to the theoretical COD of the dye and the theoretical gas production. The reason for this is that, despite continued attempts, knowledge of all of the dye structures was not known. The same volume of a 2 % (w/v) dye stock solution was added to each assay bottle, such that the dye concentration within each bottle was the same. The result was that the COD within the serum bottles was relatively low. In retrospect, this method was not optimal. The investigated concentration of each dye is given in **Table A4.3**. The dyes were diluted to the correct concentration with the nutrient medium. The dyes were mixed, in equal proportions, to form the dye mixture. The working volume in each bottle was 50 mL.

TABLE A4.3 : Bioassay conditions to assess anaerobic biodegradability of a range of textile dyes.

Dye	Methanogenic IC_{50} (g/L)	Theoretical COD (g COD/g dye)	Assay Dye Conc. (g/L)
CI Reactive Red 141	2.46	0.93	0.263
CI Reactive Blue 171	3.07	1.98	0.263
CI Reactive Green 19	1.16	1.98	0.263
CI Reactive Yellow 84	2.30	Unknown*	0.263
Dye Mixture	2.46	-	0.263 of each

* The chemical structure of this dye was unknown

No additional carbon source or acetate-propionate solution was added. The serum bottles were equilibrated and then incubated in a constant temperature room at 35 °C. The bottles were shaken manually to facilitate contact between the microorganisms and the substrate. The controls contained only the inoculum sludge and the nutrient medium, to account for gas production due to degradation of residual organic molecules in the inoculum sludge and any gas production associated with the nutrient medium.

Two variations of the CI Reactive Red 141 assays were set up; the one contained nutrient medium and the other did not. The purpose of this was to determine the difference in colour reduction in the presence and absence of the reducing agents in the anaerobic nutrient medium.

Biogas production and composition were measured according to the methods described in **Section A3.1.2**. Biogas composition was determined whenever gas was wasted.

On the first day of incubation, samples (2.5 mL) were withdrawn from each bottle. The samples were centrifuged (10 000 rpm) and the supernatants filtered (0.45 μm). The COD and colour of each sample was measured, according to the methods outlined in **Appendix 1**. These are referred to as the *initial*, or starting measurements. The same parameters were measured after 60 d of incubation, to assess the reduction in both COD and colour. The samples were kept and used in a subsequent experiment to determine the biodegradability of the dye degradation products (**Section 5.3**). The optimum wavelength of each dye was found by generating a spectrum of the absorbance of each dye over a range of UV wavelengths (200 to 800 nm). These wavelengths corresponded with those measured and used by Hansa (1999).

TABLE A4.4 : Maximum wavelengths of the investigated textile dyes.

Dye	Maximum wavelength (nm)
CI Reactive Red 141	545
CI Reactive Blue 171	600
CI Reactive Green 19	630
CI Reactive Yellow 84	405
Dye Mixture	550

A4.2.3 Results

Measurements were taken and results calculated after 60 d of incubation at 35 °C. The results of the biodegradability assays are presented in **Section A4.2.6**. Each table shows the measured biogas production, relative to the biogas produced in the controls containing the nutrient medium and the inoculum sludge. Each plot is divided into two by a dotted line; the data on the left of the line represent the results of the initial dye biodegradability assays and the data on the right of the dotted line represent the results of the degradation product assays (**Section 5.3**). The corrected gas production was also plotted for each dye. Here the amount of gas produced due to degradation of the dye alone is shown by subtraction of the control biogas from that measured in the samples. The symbols represent the triplicate samples, and the line through the data points, is the calculated mean biogas production. For each concentration, the gas production curve is shown relative to the gas production rate of the controls (solid black line). Each table in **Section A4.2.6** summarises the biodegradability results for that particular dye. These include the dye concentration added to each serum bottle, the theoretical COD of the dye and the theoretical COD of the assay, calculated from the theoretical COD of the dye and the amount of dye added.

The initial biogas production rate (mL/d) was the rate measured on day 2 of incubation. This provided an indication of degradability of the dye by the unacclimated microorganisms; the lower the gas production rate, the greater the inhibition. The volume of biogas produced was measured throughout the test period and the cumulative volume is given. Biogas composition was determined (**Appendix 1**) whenever gas was wasted from a serum bottle and after 60 d of incubation. The total volume of methane gas produced during the 60 d incubation period was determined. This was corrected for the amount of methane produced in the controls, to give the net methane production due to degradation of the added dye. The COD equivalent of the methane produced was calculated from the known conversion of 1 g COD being equal to 0.395 L CH_4 at 35 °C (Speece, 1996).

The amount of methanogenic activity in each serum bottle was estimated by calculating the fraction of dye COD converted to methane COD. The *theoretical* utilisation was based on the theoretical COD of the dye. The *actual* utilisation used the measured COD at the start of incubation. These values provide an

indication of the extent of methanogenic utilisation of the dyes as substrates. The COD balance was calculated.

Colour reduction (%) was determined by the change in absorbance (at the maximum wavelength) from the initial starting colour, to the colour after 60 d of incubation.

TABLE A4.5 : Results of the textile dye anaerobic biodegradability assays (60 d).

Dye	Methanogenic Activity (mL CH_4/g VS)	COD Reduction (%)	Colour Reduction (%)
CI Reactive Red 141 (with nutrient medium)	26.0	78	99
CI Reactive Red 141 (without nutrient medium)	26.0	92	99
CI Reactive Blue 171	21.8	87	94
CI Reactive Green 19	24.3	89	96
CI Reactive Yellow 84	19.2	90	97
Dye Mixture	16.8	79	93

A4.2.4 Discussion

The objective of these tests was to evaluate whether the anaerobic microbial populations would be able to utilise the added dye as a sole substrate. The measured gas volumes were corrected by subtracting the volume of gas produced in the controls to quantify the gas produced as a result of the degradation of the dye alone.

A dye concentration of 0.26 g/L was added to each set of bottles. The dye concentration was lower than the calculated IC_{50} value for all of the dyes. The dye concentrations added were relatively low, thus the COD load in each bottle was low which would explain the low metabolic activity observed.

Gas production is indicative of metabolic activity, thus the shape of the gas production curve indicates the degree of degradability of a substrate. Biogas production was monitored throughout the incubation period. Determination of the biogas composition gave the fraction of methane in the total biogas. The volume of methane could then be calculated to give an indication of the extent of methanogenic activity within the serum bottles. It is known that in an anaerobic environment, COD is not destroyed, but transformed. Thus, a methane balance can be used to evaluate the methanogenic activity within a batch culture by calculating the amount of COD converted to methane. These values were calculated for each assay (**Section A4.2.6**). The amount of methane produced was corrected for the volume of methane produced in the controls, such that the equivalent methane COD was attributed to degradation, or utilisation, of only the dye. From the results, it can be seen that the methanogenic activity was low, indicating that these dyes were not readily utilised by methanogenic populations.

As with the food dye assays, there was a degree of inaccuracy associated with the data presented for the COD balances, resulting in the poor balances attained. The reason is unclear. The loss of COD may be attributed to adsorption of the dye (and its associated COD) to the biomass. Spencer (1999) provided possible reasons for inaccurate results and balances in batch screening tests. These include: (i) the inability to accurately measure the COD removed or COD contribution of the inoculum sludge, (ii) the presence of toxins, (iii) the lack of trace metals, (iv) poor acclimation of the inoculum sludge to the substrate, (v) faults in the methodology, particularly in ensuring strict anaerobic conditions in all seed and substrate transfers, and (vi) gas leaks in the system and the inaccuracy of gas measurement because of the small volumes of gas being measured (Spencer, 1999).

The biogas production in the CI Reactive Red 141 bioassays, with nutrient medium (**Table A4.7**), was equal to or greater than the biogas production in the controls. The initial biogas production rate was 2.03 mL/d. For each time step, the standard deviation (σ) between the three replicates was calculated. The average standard deviation over the test period, to give an indication of the degree of variability between the replicate samples, was 0.25. A total volume of 3.1 mL of methane was produced (methanogenic activity = 26 mL CH_4/g VS), however, this was less than the amount produced in the controls (3.18 mL), thus the net methane production, or methane production due to degradation of the dye molecule, was zero. Reduction in COD and colour were relatively high at 78 % and 99 %, respectively.

In the CI Reactive Red 141 bioassays without the nutrient medium (**Table A4.8**) the biogas production was lower than in the controls and lower than in the assays with the nutrient medium. However, the methane production was greater, resulting in a net methane production of 0.1 mL (from degradation of the dye). The COD and colour removal were high at 92 % and 99 %, respectively. Thus, it could be deduced that the nutrient medium did not enhance colour reduction since the same colour reduction was achieved in both assays. The unexpected result was the increased COD reduction and methane production, relative to the assays with the nutrient medium. Lower metabolic activity would have been expected because of the absence of trace metals and nutrients in the batch culture. However, these results have suggested actual metabolism of the dye by the anaerobic biomass. The average standard deviation for these data points was 0.47.

The biogas production was low in the CI Reactive Blue 171 assays (**Table A4.10**). The total volume of methane produced was 2.8 mL, with a methanogenic activity of 21.8 mL CH_4/g VS. However, the net methane production, i.e. the methane produced due to degradation of the dye, was zero. The average standard deviation for these data points was 0.56. A COD reduction of 87 % was achieved and a colour reduction of 94 %. These results suggest that the acidogenic populations, which were shown not to be inhibited by the dye (**Section 5.2**), were responsible for the measured biogas production and the COD and colour reduction. The difference in biogas production between the assay samples and the controls was due to the inhibited, or reduced, methanogenic activity in the assay bottles due to addition of the dye.

Similar results were recorded for the CI Reactive Green 19 samples (**Table A4.9**), where the methanogenic activity was low; total volume of methane produced was 3.1 mL with a methanogenic activity of 24.3 mL CH_4/g VS. However, although the net methane production was zero, there was still

89 % reduction in COD and 96 % reduction in colour. The average standard deviation for these data was 0.41. These results also suggest inhibition of the methanogens in the anaerobic biomass but metabolism by the acidogens.

Similarly for CI Reactive Yellow 84 (**Table A4.11**), although the net methane production was zero, with a methanogenic activity of 19.2 mL CH_4/g VS, the COD was reduced by 90 % and the colour by 97 %. This metabolic activity was attributed to the acidogens. The average standard deviation was 0.52.

Willetts (1999) investigated the decolourisation rate reactions of mixtures of dyes. These results showed that the two dyes in a mixture affected one another in terms of the kinetics of their decolourisation. The more easily reduced dye of the pair was decolourised at the same rate as when it was present alone. Reduction of the second dye, however, showed a slower start. First order kinetics were thus no longer adhered to, indicating that the supply of reducing equivalents became rate-limiting. The decolourisation did, however, still reach completion. An increase in the decolourisation rate of the second dye was evident at the point that the first dye was completely reduced and therefore, no longer consuming the reducing equivalents (Willetts, 1999). No sequential degradation was observed in the biogas production plot for the textile dye mixture (**Table A4.12**). Methanogenic activity was inhibited in these assays with the 79 % COD reduction and 93 % colour reduction being attributed to the acidgenic bacteria.

The VFAs in each serum bottle were analysed after 60 d incubation. VFAs were only detected in six of the samples and all of the concentrations were ≤ 5 mg/L. The only VFAs detected were propionic, *i*-butyric and butyric acids. The production of VFAs was not expected since no additional carbon source was added to the assay bottles, i.e. the microorganisms only had the dye molecules to degrade. Methods for identifying the dye degradation products were not developed during this project. It is likely that the samples would have contained aromatic amines from the reduction of the azo bonds by the acidogenic bacteria.

The results presented for these biodegradability assays showed that the dyes were not readily utilised as a sole methanogenic substrate, however, the biogas production, COD and colour reduction suggests that the acidogenic populations were actively reducing the dyes. The methanogenic activity may have been enhanced by supplementation with an additional carbon source. Although, in similar tests conducted by Bell (1998), on CI Reactive Red 141, it was found that an increase in solids concentration and/or addition of a source of readily biodegradable COD (glucose) had little effect on the rate of decolourisation of CI Reactive Red 141 (Bell, 1998).

A4.2.5 Conclusions

1. Although the bioassays showed efficient COD reduction and decolourisation, the methanogenic activity was low, indicating that the dyes were not readily utilised by methanogenic populations.

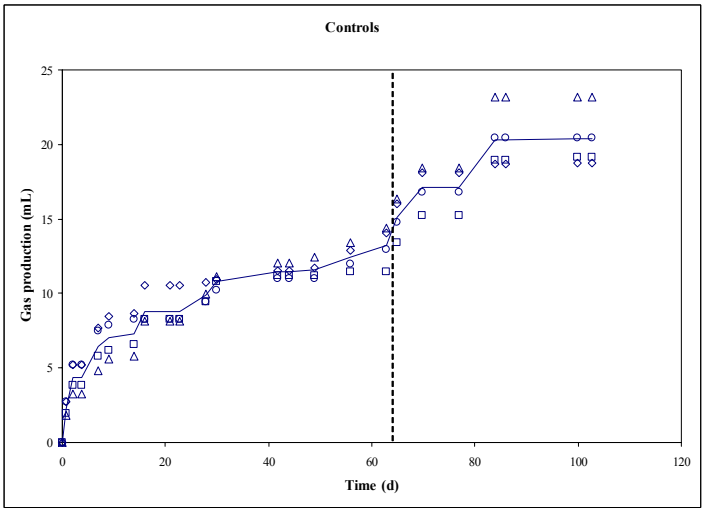
2. The acidogenic bacteria were responsible for the biogas production and the COD and colour reduction.

3. No significant VFA concentrations were detected; the samples would have contained aromatic amines from the reduction of the azo bonds by the acidogenic bacteria.

4. The bioassays provided a more thorough understanding of the dye characteristics and degradation potential.

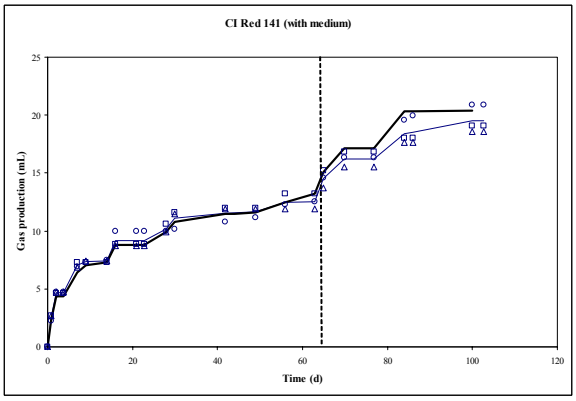
A4.2.6 Biogas Production Plots

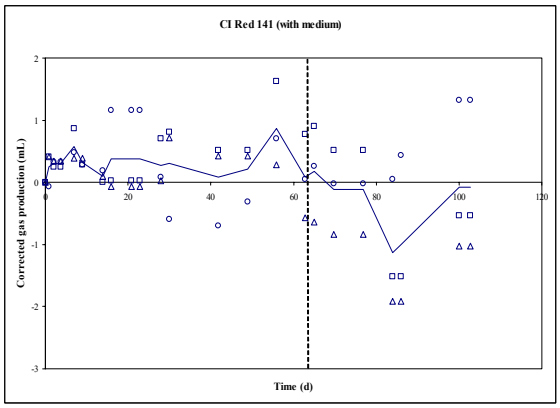
TABLE A4.6 : Results for the controls in the textile dye biodegradability assay.



Initial biogas production rate :	2.9 mL/d	
Acclimated biogas production rate (60 d) :	-	
		Degradation Products
Total gas production (37 °C) :	12.51 mL	7.87 mL
CH_4 production :	3.1836 mL	4.4392 mL
CH_4 – COD :	8.02 mg	11.2 mg

TABLE A4.7 *:* Results of the biodegradability assay with CI Red 141 (with nutrient medium).





Biodegradability :

Dye concentration :	0.26 g/L	
Theoretical dye COD :	0.93 g COD/g dye	
Theoretical Assay COD (in 47.5 mL) :	11.625 mg COD	
Initial biogas production rate :	2.03 mL/ d	**Degradation Products**
Total gas production (37 °C) :	12.61 mL	6.9 mL
CH_4 production :	3.0892 mL	4.0763 mL
Net CH_4 production :	0 mL	0 mL
CH_4 – COD :	7.78 mg	10.3 mg

Methanogenic activity

Methanogenic activity :	26 mL CH_4/g VSS	32.2 mL CH_4/g VSS

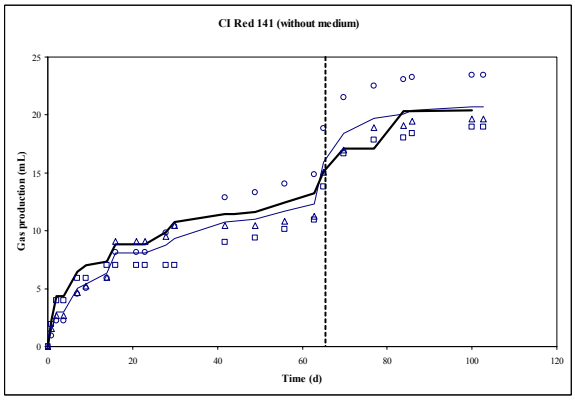
COD balance

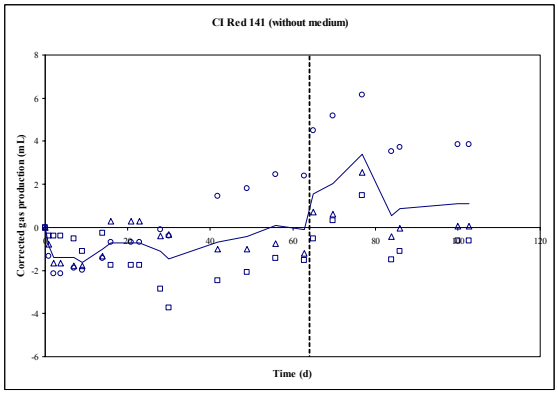
CODin : 117.2 mg (in 47.5 mL)	COD out :	26.3 mg (in 47.5 mL)
	CH_4 – COD :	7.78 mg
	Total CODout :	34.05 mg
Balance :	**29 %**	
COD reduction :	78 %	

Colour reduction

Measured colour reduction :	99 % (545 nm)

TABLE A4.8 *:* Results of the biodegradability assay with CI Red 141 (without nutrient medium).





Biodegradability :

Dye concentration :	0.26 g/L	
Theoretical dye COD :	0.93 g COD/g dye	
Theoretical Assay COD (in 47.5 mL) :	11.625 mg COD	
Initial biogas production rate :	1.91 mL/ d	**Degradation Products**
Total gas production (37 °C) :	12.34 mL	8.36 mL
CH_4 production :	3.2948 mL	4.6548 mL
Net CH_4 production :	0.1112 mL	0.2156 mL
CH_4 – COD :	8.299 mg	11.8 mg

Methanogenic activity

Methanogenic activity :	26 mL CH_4/g VSS	36.7 mL CH_4/g VSS

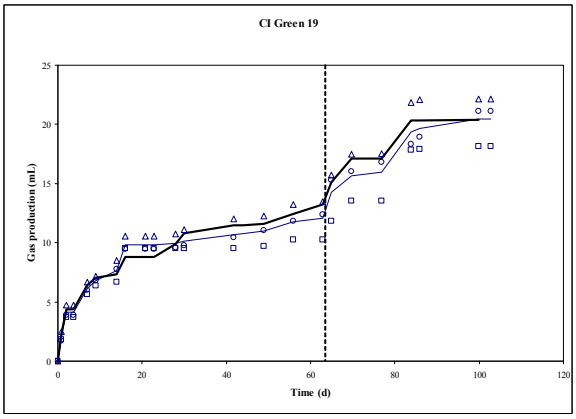
COD balance

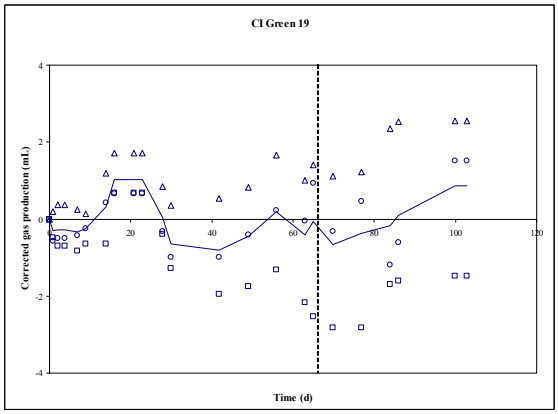
CODin : 117.6 mg (in 47.5 mL)	COD out :	9.5 mg (in 47.5 mL)
	CH_4 – COD :	8.299 mg
	Total CODout :	17.8 mg
Balance :	**15 %**	
COD reduction :	92 %	

Colour reduction

Measured colour reduction :	99 % (545 nm)

TABLE A4.9 *:* Results of the biodegradability assay with CI Green 19.





Biodegradability :

Dye concentration :	0.26 g/L	
Theoretical dye COD :	1.98 g COD/g dye	
Theoretical Assay COD (in 47.5 mL) :	24.75 mg COD	
Initial biogas production rate :	1.81 mL/ d	**Degradation Products**
Total gas production (37 °C) :	12.05 mL	8.41 mL
CH_4 production :	3.0754 mL	4.3090 mL
Net CH_4 production :	0 mL	0 mL
CH_4 – COD :	7.75 mg	10.9 mg

Methanogenic activity

Methanogenic activity :	24.3 mL CH_4/g VSS	34.0 mL CH_4/g VSS

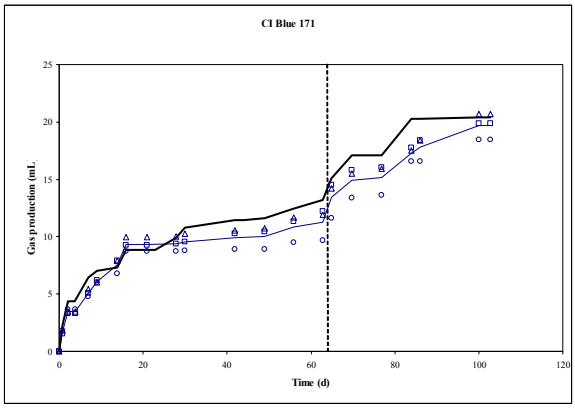
COD balance

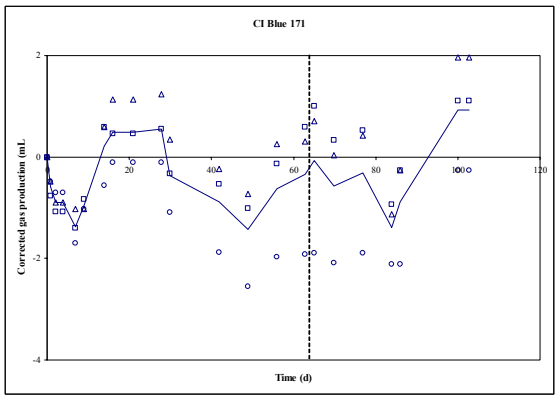
CODin : 118.7 mg (in 47.5 mL)	COD out :	13.2 mg (in 47.5 mL)
	CH_4 – COD :	7.75 mg
	Total CODout :	20.9 mg
Balance :	**18 %**	
COD reduction :	89 %	

Colour reduction

Measured colour reduction :	96 % (630 nm)

TABLE A4.10 *:* Results of the biodegradability assay with CI Blue 171.





Biodegradability :

Dye concentration :	0.26 g/L	
Theoretical dye COD :	1.98 g COD/g dye	
Theoretical Assay COD (in 47.5 mL) :	24.75 mg COD	
Initial biogas production rate :	1.53 mL/ d	**Degradation Products**
Total gas production (37 °C) :	11.26 mL	8.42 mL
CH_4 production :	2.7561 mL	4.2289 mL
Net CH_4 production :	0 mL	0 mL
CH_4 – COD :	6.94 mg	10.7 mg

Methanogenic activity

Methanogenic activity :	21.8 mL CH_4/g VSS	33.4 mL CH_4/g VSS

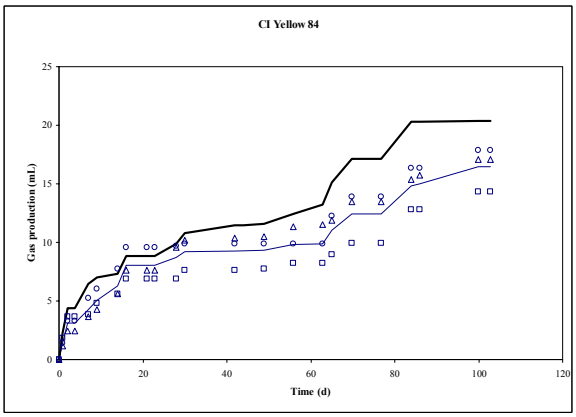
COD balance

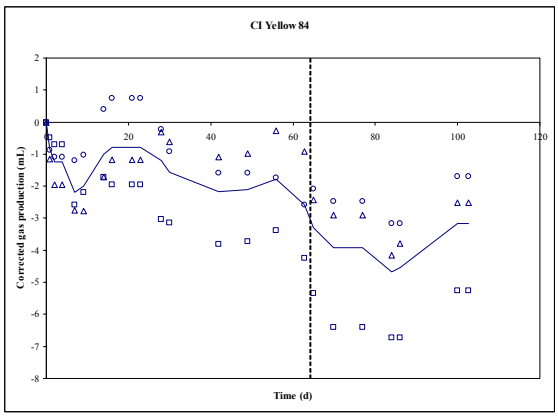
CODin : 117.3 mg (in 47.5 mL)	COD out : 15.4 mg (in 47.5 mL)
	CH_4 – COD : 6.94 mg
	Total CODout : 22.3 mg
Balance :	**19 %**
COD reduction :	87 %

Colour reduction

Measured colour reduction :	94 % (600 nm)

TABLE A4.11 *:* Results of the biodegradability assay with CI Yellow 84.





Biodegradability :

Dye concentration :	0.26 g/L	
Initial biogas production rate :	1.81 mL/ d	**Degradation Products**
Total gas production (37 °C) :	9.87 mL	8.02 mL
CH_4 production :	2.4270 mL	3.2713 mL
Net CH_4 production :	0 mL	0 mL
CH_4 – COD :	6.11 mg	8.3 mg

Methanogenic activity

Methanogenic activity :	19.2 mL CH_4/g VSS	25.8 mL CH_4/g VSS

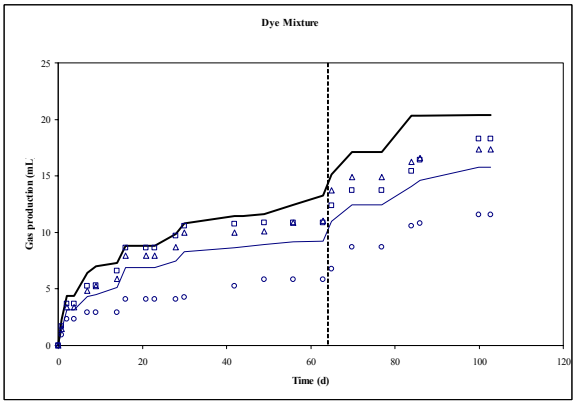
COD balance

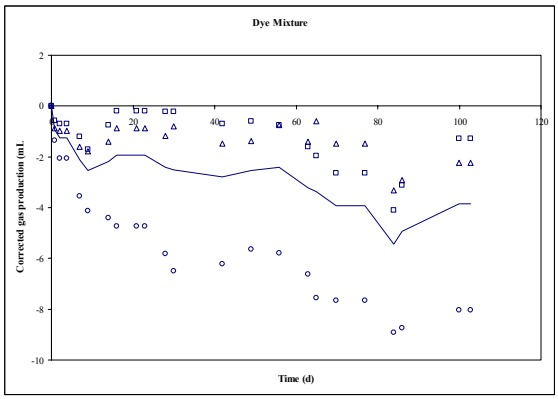
CODin : 116.4 mg (in 47.5 mL)	COD out : 11.3 mg (in 47.5 mL)
	CH_4 – COD : 6.11 mg
	Total CODout : 17.4 mg
Balance :	**15 %**
COD reduction :	90 %

Colour reduction

Measured colour reduction :	97 % (405 nm)

TABLE A4.12 *:* Results of the biodegradability assay with textile dye mixture.





Biodegradability :

Dye concentration :	0.26 g/L of each dye	
Initial biogas production rate :	1.72 mL/d	**Degradation Products**
Total gas production (37 °C) :	9.24 mL	9.07 mL
CH_4 production :	2.1287 mL	2.8337 mL
Net CH_4 production :	0 mL	0 mL
CH_4 – COD :	5.4 mg	7.2 mg

Methanogenic activity

Methanogenic activity :	16.8 mL CH_4/g VSS	22.4 mL CH_4/g VSS

COD balance

CODin : 121.9 mg (in 47.5 mL)	COD out :	25.4 mg (in 47.5 mL)
	CH_4 – COD :	5.4 mg
	Total CODout :	30.8 mg
Balance :	**25 %**	
COD reduction :	79 %	

Colour reduction

Measured colour reduction :	93 % (550 nm)

A4.3 COD BALANCE

From the biogas production data, a COD balance was calculated for each time step. The results are presented below.

TABLE A4.13 : COD balance data for the laboratory-scale ABR treating a synthetic CI Reactive Red 141 waste stream.

Total COD_{in} (g COD)	Total CH_4-COD (g COD)	Total Effluent-COD (g COD)	Total COD_{out} (g COD)	Balance (%)
51.477	0.000	5.599	5.599	**10.9**
54.566	0.000	4.435	4.435	**8.1**
54.500	0.000	1.976	1.976	**3.6**
114.294	0.000	1.331	1.331	**1.2**
112.143	94.369	4.395	98.764	**88.1**
117.842	0.090	2.943	3.033	**2.6**
151.511	18.117	1.590	19.706	**13.0**
353.526	299.457	14.636	314.093	**88.8**
478.518	212.894	1.129	214.022	**44.7**
194.665	0.093	2.039	2.133	**1.1**
157.594	0.069	2.137	2.206	**1.4**
199.181	0.070	4.193	4.264	**2.1**
194.181	0.401	6.048	6.449	**3.3**
177.085	0.098	13.548	13.646	**7.7**
420.941	1.263	3.629	4.891	**1.2**
191.923	0.125	4.493	4.618	**2.4**
95.731	0.134	5.242	5.376	**5.6**
141.604	0.137	8.047	8.184	**5.8**
311.731	0.237	5.184	5.421	**1.7**
159.805	0.099	1.440	1.539	**1.0**
26.081	0.055	14.429	14.483	**55.5**
87.299	0.000	1.365	1.365	**1.6**
87.990	0.136	2.650	2.785	**3.2**

A-5

Analytical Results

The analytical results, reported in Chapters 4 and 5 of the thesis, are presented below. Due to the volume of results, the VFA data has not been included in this appendix, but it saved on the disk which is attached to the back cover of the thesis.

A5.1 SUCROSE LABILE FEED

The following data correspond to the results presented in **Chapter 4**.

TABLE A5.1 : pH data for the baseline study with sucrose (Section 4.2.1).

	Compartment									
Elapsed Time (d)	**In**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Out**
1	8.4	6.83	6.96	6.98	6.98	7	7.02	7.01	7.01	7.02
3		6.92	7.34	7.34	7.23	7.16	7.13	7.06	7.07	7.11
10		6.61	7.1	7.12	7.18	7.29	7.18	7.32	7.32	7.3
16		6.56	7.05	7.18	7.29	7.37	7.46	7.3	7.44	7.5
35		7.02	7.3	7.29	7.28	7.32	7.3	7.35	7.37	7.52
37		6.99	7.44	7.41	7.33	7.32	7.3	7.31	7.37	7.39
42		6.63	7.22	7.24	7.24	7.25	7.28	7.33	7.36	7.22
44	6.76	6.92	7.41	7.41	7.37	7.28	7.26	7.27	7.24	7.35
49	6.75	6.81	7.33	7.41	7.32	7.27	7.3	7.26	7.33	7.36
54		7.19	7.3	7.41	7.43	7.41	7.45	7.45	7.46	7.64
58	7.27	6.70	7.12	7.24	7.19	7.10	7.13	7.16	7.17	7.00
63	7.84	6.98	7.00	7.04	7.05	7.04	7.06	7.04	7.08	7.15
75			7.71	7.66	7.67	7.59	7.66	7.60	7.49	7.37
78	7.77	6.46	6.82	7.10	7.30	7.39	7.33	7.42	7.45	7.35
82	7.29	6.29	6.68	6.90	7.11	7.34	7.40	7.41	7.46	7.30
84	9.02	6.17	6.72	6.78	7.04	7.33	7.47	7.46	7.41	7.38
89	9.23	6.29	6.99	7.19	7.35	7.43	7.48	7.57	7.53	7.34
91	9.07	6.45	7.05	7.37	7.38	7.44	7.49	7.42	7.41	7.31
95	9.29	6.39	6.51	6.77	7.08	7.27	7.40	7.52	7.44	7.70
98		6.16	7.12	7.40	7.53	7.45	7.42	7.38	7.35	7.28
103		6.56	6.98	7.07	7.15	7.20	7.30	7.29	7.37	7.26
114		6.54	6.84	7.17	7.40	7.24	7.30	7.34	7.34	7.23
118	9.59	6.30	6.83	6.98	7.03	7.05	7.18	7.27	7.36	7.45
120	9.27	6.44	7.00		7.34	7.35	7.33	7.37	7.40	7.38
125	7.04	6.46	6.65	7.06	7.16	7.32	7.36	7.31	7.34	7.35
128		6.32	6.59	6.95	7.01	7.15	7.12	7.25	7.11	7.18
138	9.01	6.66	6.97	7.20	7.43	7.53	7.69	7.72	7.67	7.67
141	9.09	6.55	6.84	7.01	7.32	7.36	7.37	7.29	7.28	7.24
145	9.07	6.28	6.55	6.65	6.82	6.98	7.05	7.15	7.17	7.27
148	8.68	6.41	6.75	7.13	7.33	7.48	7.47	7.47	7.51	7.69
156	9.33	6.34	7.00	7.38	7.42	7.60	7.66	7.72	7.58	7.82
168	8.06	6.63	6.86	7.30	7.36	7.36	7.39	7.35	7.35	7.51
175	9.11	6.50	6.92	7.19	7.26	7.31	7.27	7.31	7.27	7.40
200	8.13	6.53	6.90	7.08	7.15	7.13	7.10	7.11	7.10	7.24
216	8.78	6.27	6.73	7.14	7.21	7.23	7.23	7.25	7.20	7.25
223	9.81	6.26	6.95	7.32	7.41	7.54	7.44	7.47	7.53	7.53
230	8.88	5.71	6.62	7.17	7.20	7.34	7.31	7.29	7.40	7.31
235	9.11		5.75	6.48	6.66	6.61	6.78	6.81	6.87	6.89
244	9.68	6.28	6.04	6.37	6.56	6.83	6.88	6.96	7.06	7.27
252	9.62	6.53	6.56	7.26	7.36	7.33	7.32	7.31	7.30	7.47
259			6.79	7.15	7.24	7.21	7.22	7.08	7.09	9.50
266			7.06	7.09	7.12	7.32	7.48	7.27	7.26	7.29
273	8.75		7.02	7.13	7.09	7.26	7.41	7.32	7.30	7.26
280	7.92	6.48	6.93	7.08	7.06	7.02	6.99	7.09	7.04	9.08
284	9.69	6.29	6.05	6.42	6.61	6.92	7.07	7.20	7.26	7.32
295	9.62	6.56	6.86	6.97	7.05	7.15	7.23	7.48	7.49	9.05
302	9.54	6.32	6.72	6.89	7.01	7.17	7.18	7.39	7.54	9.02
326	9.55	6.35	7.14	7.41	7.36	6.86	6.75	7.18	6.71	6.79
330	9.62	6.35	6.25	6.22	6.22	7.28	6.29	7.21	6.40	6.47
337	9.24	6.02	6.58	6.84	7.00	6.99	6.97	7.00	6.90	6.89
341	8.91	6.43	6.93	7.17	7.14	7.07	7.05	7.18	7.16	7.22
344	8.31	6.58	6.71	7.11	7.12	7.09	7.01	7.20	7.26	7.20
347	9.30	6.59	6.58	7.01	7.03	7.01	7.03	7.23	7.08	7.33

TABLE A5.2 : Total solids data for the baseline study with sucrose (Section 4.2.2).

	Compartment						
Elapsed (d)	**1-Rep1**			**1-Rep 2**			
	Dish (g)	**Wt after (g)**	**TS (g/L)**	**Dish (g)**	**Wt after (g)**	**TS (g/L)**	**Mean (g/L)**
0							
1	57.04	57.22	18.00	55.88	56.12	24.00	21.00
10				56.97	57.26	29.00	29.00
35	55.83	55.94	11.00	55.18	55.26	8.00	9.50
37							
42	52.62	52.73	11.00				11.00
49	54.16	54.27	11.00	55.99	56.12	13.00	12.00
54	57.22	57.32	10.00	57.15	57.31	16.00	13.00
58	57.21	57.28	7.00	56.05	56.11	6.00	6.50
63	57.23	57.27	4.00	56.03	56.05	2.00	3.00
75							
78	57.18	57.29	11.00				11.00
82							
84							
89	53.88	54.02	14.00	54.18	54.30	12.00	13.00
91							
95							
98	57.25	57.30	5.00	55.97	56.10	13.00	9.00
114	57.22	57.41	19.00				19.00
118							
120							
125	56.03	56.16	13.00	54.19	54.40	21.00	17.00
141	52.65	52.81	16.00				16.00
145	54.19	54.24	5.00	53.88	54.00	12.00	8.50
148	54.17	54.37	20.00	52.64	52.77	13.00	16.50
156	55.95	56.26	31.00				31.00
168							
175	55.96	56.06	10.00				10.00
200	52.64	52.74	10.00				10.00
216	55.97	56.06	9.00				9.00
223	52.62	52.80	18.00				18.00
230	55.81	56.01	20.00				20.00
235	52.63	52.72	9.00				9.00
244	54.18	54.23	5.00				5.00
252	57.17	57.25	8.00				8.00
266	55.93	56.09	16.00				16.00
273	56.03	56.18	15.00				15.00
330	54.17	54.26	9.00				9.00
337	53.90	53.96	6.00				6.00
341	55.82	55.96	14.00				14.00

TABLE A5.3: Total solids data for the baseline study with sucrose (Section 4.2.2).

	Compartment						
Elapsed (d)	**Effluent-Rep1**			**Effluent-Rep 2**			
	Dish (g)	**Wt after (g)**	**TS (g/L)**	**Dish (g)**	**Wt after (g)**	**TS (g/L)**	**Mean (g/L)**
0							
1	57.24	57.26	2.00	53.89	53.91	2.00	2.00
10	57.13	57.18	5.00	56.01	56.11	10.00	7.50
35	52.61	52.68	7.00	57.17	57.23	6.00	6.50
37	55.84	56.00	16.00	55.18	55.33	15.00	15.50
42	53.89	53.98	9.00				9.00
49	57.17	57.20	3.00	57.22	57.25	3.00	3.00
54	54.16	54.25	9.00	56.04	56.10	6.00	7.50
58	55.86	55.94	8.00	57.27	57.36	9.00	8.50
63	57.18	57.20	2.00	55.83	55.84	1.00	1.50
75	57.19	57.20	1.00	55.84	55.86	2.00	1.50
78	60.48	60.59	11.00				11.00
82	54.17	54.31	14.00	57.22	57.36	14.00	14.00
84	53.86	53.94	8.00	56.00	56.08	8.00	8.00
89	52.62	52.79	17.00	56.02	56.19	17.00	17.00
91	53.89	54.04	15.00	56.04	56.17	13.00	14.00
95	57.18	57.22	4.00	55.84	55.87	3.00	3.50
98	54.19	54.23	4.00	56.04	56.08	4.00	4.00
114	56.01	56.12	11.00	55.84	55.94	10.00	10.50
118	52.63	52.67	4.00				4.00
120	56.02	56.04	2.00	55.83	55.86	3.00	2.50
125	53.88	53.92	4.00	52.63	52.68	5.00	4.50
141	54.19	54.21	2.00				2.00
145	57.22	57.23	1.00				1.00
148	56.02	56.07	5.00	55.82	55.84	2.00	3.50
156	56.89	56.91	2.00				2.00
168	54.18	54.20	2.00	53.88	53.91	3.00	2.50
175	56.02	56.04	2.00	55.82	55.85	3.00	2.50
200	53.89	53.91	2.00	54.18	54.20	2.00	2.00
216	55.83	55.85	2.00				2.00
223	57.17	57.22	5.00				5.00
230	55.96	55.99	3.00	60.47	60.50	3.00	3.00
235	53.88	53.88	0.00	57.18	57.18	0.00	0.00
244	55.82	55.84	2.00	60.48	60.50	2.00	2.00
252	29.97	29.98	1.00	52.64	52.64	0.00	0.50
266	55.68	55.69	1.00	65.26	65.28	2.00	1.50
273	55.93	55.93	0.00	55.67	55.68	1.00	0.50
330	55.82	55.82	0.00	53.90	53.90	0.00	0.00
337	52.65	52.65	0.00	54.19	54.20	1.00	0.50
341	53.89	53.91	2.00	54.19	54.21	2.00	2.00

TABLE A5.4 : Volatile solids data for the baseline study with sucrose (Section 4.2.2).

	Compartment						
Elapsed (d)	**1-Rep1**			**1-Rep 2**			
	Dish (g)	**Wt after (g)**	**TS (g/L)**	**Dish (g)**	**Wt after (g)**	**TS (g/L)**	**Mean (g/L)**
0							
1	57.22	57.06	16.00	56.12	55.93	19.00	17.50
10				57.26	57.21	5.00	5.00
35	55.94	55.87	7.00	55.26	55.22	4.00	5.50
37							
42	52.73	52.65	8.00				8.00
49	54.27	54.20	7.00	56.12	56.05	7.00	7.00
54	57.32	57.24	8.00	57.31	57.21	10.00	9.00
58	57.28	57.22	6.00	56.11	56.07	4.00	5.00
63	57.27	57.25	2.00	56.05	56.04	1.00	1.50
75							
78	57.29	57.21	8.00				8.00
82							
84							
89	54.02	53.92	10.00	54.30	54.23	7.00	8.50
91							
95							
98	57.30	57.21	9.00	56.10	55.96	14.00	11.50
114	57.41	57.26	15.00				15.00
118							
120							
125	56.16	56.04	12.00	54.40	54.22	18.00	15.00
141	52.81	52.71	10.00				10.00
145	54.24	54.20	4.00	54.00	53.91	9.00	6.50
148	54.37	54.22	15.00	52.77	52.68	9.00	12.00
156	56.26	56.03	23.00				23.00
168							
175	56.06	56.01	5.00				5.00
200	52.74	52.65	9.00				9.00
216	56.06	56.01	5.00				5.00
223	52.80	52.65	15.00				15.00
230	56.01	55.87	14.00				14.00
235	52.72	52.66	6.00				6.00
244	54.23	54.19	4.00				4.00
252							
266	56.09	56.01	8.00				8.00
273	56.18	56.11	7.00				7.00
330	54.26	54.20	6.00				6.00
337	53.96	53.91	5.00				5.00
341	55.96	55.85	11.00				11.00

TABLE A5.5: Volatile solids data for the baseline study with sucrose (Section 4.2.2).

	Compartment						
Elapsed (d)	**Effluent-Rep1**			**Effluent-Rep 2**			
	Dish (g)	**Wt after (g)**	**TS (g/L)**	**Dish (g)**	**Wt after (g)**	**TS (g/L)**	**Mean (g/L)**
0							
1	57.26	57.26	0.00	53.91	53.91	0.00	0.00
10	57.13	57.01	12.00	56.11	56.06	5.00	8.50
35	52.68	52.67	1.00	57.23	57.20	3.00	2.00
37	56.00	55.93	7.00	55.33	55.27	6.00	6.50
42	53.98	53.93	5.00				5.00
49	57.20	57.20	0.00	57.25	57.25	0.00	0.00
54	54.25	54.22	3.00	56.10	56.05	5.00	4.00
58	55.94	55.87	7.00	57.36	57.28	8.00	7.50
63	57.20	57.20	0.00	55.84	55.84	0.00	0.00
75	57.20	57.18	2.00	55.86	55.84	2.00	2.00
78	60.59	60.50	9.00				9.00
82	54.31	54.24	7.00	57.36	57.28	8.00	7.50
84	53.94	53.92	2.00	56.08	56.06	2.00	2.00
89	52.79	52.67	12.00	56.19	56.06	13.00	12.50
91	54.04	53.91	13.00	56.17	56.05	12.00	12.50
95	57.22	57.18	4.00	55.87	55.83	4.00	4.00
98	54.23	54.18	5.00	56.08	56.03	5.00	5.00
114	56.12	56.04	8.00	55.94	55.86	8.00	8.00
118	52.67	52.66	1.00				1.00
120	56.04	56.04	0.00	55.86	55.85	1.00	0.50
125	53.92	53.91	1.00	52.68	52.68	0.00	0.50
141	54.21	54.21	0.00				0.00
145	57.23	57.22	1.00				1.00
148	56.07	56.03	4.00	55.84	55.84	0.00	2.00
156	56.91	56.91	0.00				0.00
168	54.20	54.19	1.00	53.91	53.90	1.00	1.00
175	56.04	56.04	0.00	55.85	55.84	1.00	0.50
200	53.91	53.89	2.00	54.20	54.20	0.00	1.00
216	55.85	55.85	0.00				0.00
223	57.22	57.21	1.00				1.00
230	55.99	55.97	2.00	60.50	60.48	2.00	2.00
235	53.88	53.88	0.00	57.18	57.18	0.00	0.00
244	55.84	55.83	1.00	60.50	60.48	2.00	1.50
252							
266	55.69	55.68	1.00	65.28	65.26	2.00	1.50
273	55.93	55.93	0.00	55.68	55.67	1.00	0.50
330	55.82	55.82	0.00	53.90	53.90	0.00	0.00
337	52.65	52.65	0.00	54.20	54.19	1.00	0.50
341	53.91	53.91	0.00	54.22	54.21	1.00	0.50

TABLE A5.6 : COD data for the baseline study with sucrose (Section 4.2.3).

	Compartment								
	1			2			3		
Elapsed Time (d)	Rep 1 COD (mg/L)	Rep 2 COD (mg/L)	Mean (mg/L)	Rep 1 COD (mg/L)	Rep 2 COD (mg/L)	Mean (mg/L)	Rep 1 COD (mg/L)	Rep 2 COD (mg/L)	Mean (mg/L)
1	1853		1853	628		628	338		338
3	2185	2050	2118	720	830	775	285	325	305
10	2753	2733	2743	618	583	600	43	208	125
16	3065	3170	3118	1465	1585	1525	550	500	525
35	1670	1565	1618	250	265	258	270	290	280
37	1470	1465	1468	465	515	490	100	110	105
42	1540	1540	1540				230	500	365
44	1515	1465	1490	260	375	318	85	85	85
49	868	1278	1073	428	403	415	158	93	125
54	628	658	643	203	178	190	383		383
58	1043	1003	1023	818	823	820	148	103	125
63	568	588	578	43	53	48	58	63	60
75	3023	2573	2798	518	1003	760	468	413	440
78	2338	1838	2088	2003	1888	1945	1723	1588	1655
82	2520	2520	2520	2065	1935	2000	1405	1490	1448
84									
89	2293	2308	2300	1603	1578	1590	1113	1548	1330
91	2440	2600	2520	1725	1715	1720	990	1000	995
95	890	935	913	55	60	58		50	50
98	518	483	500	733	768	750	723	808	765
103	2015	1900	1958	1185	1180	1183	1065	1055	1060
114	2428	2513	2470	1823	1818	1820	1488	1198	1343
118	575	580	578	945	820	883	480	470	475
120	2885	2320	2603	1575	1615	1595	335	355	345
125	2330	1970	2150	1980	1905	1943	1100		1100
128	1728	1668	1698	1683	1713	1698	1188	1153	1170
138	2315	2310	2313	2090	1800	1945	1490	1540	1515
141	2770	3055	2913	2415	2440	2428	1700	1745	1723
145	1577.5	1502.5	1540	1312.5	1302.5	1307.5			
148	2978	2868	2923	2123	2323	2223	1573	1568	1570
156	2660	2545	2603	2075	1535	1805	1005	965	985
168									
175	1855	2095	1975	1240	1055	1148	715	740	728
200	1940	1700	1820	1110	1175	1143	230	315	273
216	2495	1370	1933	915	960	938	355	395	375
223	1450	1545	1498	975	975	975	430	455	443
230	488	558	523	458	428	443	338	363	350
235	1385	1245	1315	415	725	570	95	95	95
244	2165	2175	2170	1365	1375	1370	845	845	845
252	1870	1885	1878	1460	1475	1468	510	495	503
259									
266	2255	2225	2240	1155	1170	1163	980	1010	995
273	2776	2763	2770	1737	1718	1728	1363	1363	1363
280	1034	970	1002	150	189	170	641	628	634
284	1783	1770	1776	892	892	892	563	563	563
295				1060	1054	1057	512	525	518
302									
326	3550	3834	3692	3944	3789	3867	3467		3467
330	608	589	599	544	673	608	337	428	383
337									
341	1889	1857	1873	767	754	760	521	192	357

TABLE A5.7 : COD data for the baseline study with sucrose (Section 4.2.3).

	Compartment								
	4			5			6		
Elapsed Time (d)	Rep 1 COD (mg/L)	Rep 2 COD (mg/L)	Mean (mg/L)	Rep 1 COD (mg/L)	Rep 2 COD (mg/L)	Mean (mg/L)	Rep 1 COD (mg/L)	Rep 2 COD (mg/L)	Mean (mg/L)
1	253		253	358		358	358		358
3	270	280	275	265	265	265	230	250	240
10				183	318	250	158	138	148
16	210	140	175	290	270	280	330	330	330
35	220	200	210	90	205	148	260	235	248
37	45	35	40	200	195	198	180	185	183
42	110	130	120	110	125	118	265	200	233
44	105	105	105	120	115	118	155		155
49	163	83	123	78	133	105	68	143	105
54	133	133	133	68	43	55	98	93	95
58	58	93	75	178	83	130	53	88	70
63		13	13	68	33	50		53	53
75	388	498	443	328	333	330	388	393	390
78	998	1103	1050	278		278	773	833	803
82	1025	1130	1078	625	625	625	375	340	358
84									
89	1133	878	1005	618	623	620	463	448	455
91	855	765	810	580	555	568	385	375	380
95	10	20	15	60	30	45	105	155	130
98	413	463	438	373	398	385	283	258	270
103	910	880	895	780	780	780	615	545	580
114	958	1083	1020	858	808	833	638	698	668
118	135	175	155	75		75	130	115	123
120	705	690	698	360	360	360	335	285	310
125	840	835	838	420	530	475	315	395	355
128	1058	973	1015	778	623	700	563		563
138	970	990	980	805	835	820	705		705
141	1110	1210	1160	905	930	918	680	715	698
145	812.5	882.5	847.5	522.5	612.5	567.5	342.5	387.5	365
148	818	868	843	328	308	318	178	253	215
156	620	1125	873	305		305	645		645
168									
175	455	345	400	310	415	363	240	210	225
200	80	70	75	375	325	350	120	295	208
216	175	535	355	60	60	60	5	135	70
223	215	165	190	-5	40	18	100	65	83
230	238	208	223	248	113	180	68	53	60
235	745	350	548	545	500	523	135	165	150
244	765	770	768	960	965	963	580	570	575
252	415	405	410	410	420	415	475	505	490
259									
266	415	405	410	855	880	868	360	340	350
273	1228	1221	1225	1112	1125	1118	634	647	641
280	383	376	379	757	731	744	602	596	599
284	537	537	537	479	473	476	402	415	408
295	150	144	147	221	196	208	389	376	383
302									
326	1441	1525	1483	305	396	350	344	370	357
330	1434	1537	1486	273	447	360	202	183	192
337									
341	83	121	102	70	89	79	115	96	105

TABLE A5.8 : COD data for the baseline study with sucrose (Section 4.2.3).

	Compartment								
	7			8			Effluent		
Elapsed Time (d)	Rep 1 COD (mg/L)	Rep 2 COD (mg/L)	Mean (mg/L)	Rep 1 COD (mg/L)	Rep 2 COD (mg/L)	Mean (mg/L)	Rep 1 COD (mg/L)	Rep 2 COD (mg/L)	Mean (mg/L)
1	388		388	318		318	3018	3048	
3	150	165	158	165	210	188	145	175	160
10	98	98	98				143	158	150
16	350	205	278	220	255	238	190	195	193
35	175	70	123	180	135	158	160	200	180
37	130	145	138	110	60	85	290	135	213
42	300	85	193	115	90	103	85	100	93
44	315	115	215	200	120	160	75	85	80
49				38	123	80	43	43	43
54	68	133	100	43	63	53	263	133	198
58	63	108	85	88	78	83	88	58	73
63	68	33	50	28	43	35		58	58
75	373	358	365	418	288	353	328	333	330
78	368	393	380	98	133	115	273	483	378
82	110	130	120	90	105	98	125	90	108
84				58	63	60	138	103	120
89	183	258	220	418	273	345	183	113	148
91	120	155	138	110	125	118	120	185	153
95	180	100	140	165	200	183	95	470	283
98	218	208	213	218	183	200	123	153	138
103	735	445	590	375	360	368	630	535	583
114	583	593	588	658	468	563	458	448	453
118	190	220	205	210	180	195	230	200	215
120	375		375	320	310	315	330	380	355
125	370		370	400		400	300	360	330
128	553	518	535	473	463	468	458		573
138	770	835	803	830	905	868	960	950	955
141	745	730	738	720	685	703	835	735	785
145	257.5	277.5	267.5	382.5	352.5	367.5	302.5	267.5	285
148	158	118	138	108	98	103	123	108	115
156	335	190	263	190	130	160	185	135	160
168									
175	325	240	283	305	225	265	200		200
200	330	205	268	235	270	253	130	40	85
216	165	480	323	-30	60	15	270	70	170
223	285	265	275	365	575	470	260	350	305
230	608	208	408	138	153	145	123	173	148
235	120	200	160	85	150	118	110	40	75
244	605	595	600	620	620	620	500	490	495
252	915	895	905	35	50	43	120	95	108
259									450
266	190	185	188	30	25	28	115	120	118
273	267	292	279	434	428	431	73	73	420
280	531	557	544	486	460	473	376	363	299
284	241	260	250	196	189	192	499	492	315
295	963	944	954	189	157	173	776	802	300
302									
326	2	8	5	67	8	37	8	125	250
330	750	273	512	144	28	86	-11		200
337									185
341	225	199	212	186	128	157	689	115	155

TABLE A5.9 : Compartment 1 biogas data for the baseline study with sucrose (Section 4.2.5).

	Compartment 1									
			Methane					**CO_2**		**N_2**
Elapsed Time (d)	**Gas prodn (mL)**	**Cumul. gas (mL)**	**(area)**	**(%)**	**moles**	**(mg)**	**mg COD**	**(area)**	**(%)**	**(area)**
1	0.0	0.0		34.50					49.90	
3	0.0	0.0								
10	340.0	340.0								
16	0.0	340.0								
35	41.0	381.0								
37	105.0	486.0								
42	65.0	551.0								
44	115.0	666.0								
49	150.0	816.0								
54	14.0	830.0								
58	0.0	830.0								
63	0.0	830.0								
75	0.0	830.0								
78	0.0	830.0								
103	0	830.0	**21184**	31.46	3.0183E-06	0.048294	0.17	**41533**	61.68	**4621**
114	1040	1870.0	**30886**	23.86	3.9064E-06	0.062503	0.22	**75369**	58.23	**23181**
118	0	1870.0	**7348**	22.90	1.3686E-06	0.021899	0.08	**12985**	40.46	**11758**
120	0	1870.0	**28577**	33.18	3.7076E-06	0.059322	0.21	**42772**	49.66	**14773**
125	1080	2950.0	**53393**	37.25	5.5758E-06	0.089213	0.32	**86964**	60.67	**2994**
128	0	2950.0	**39856**	30.15	4.6223E-06	0.073958	0.26	**80799**	61.11	**11558**
138	0	2950.0	**28012**	23.63	3.6579E-06	0.058527	0.21	**75331**	63.56	**15177**
141	0	2950.0	**35602**	26.48	4.2930E-06	0.068688	0.24	**86395**	64.27	**12429**
145	0	2950.0	**18161**	24.41	2.7075E-06	0.043321	0.15	**35246**	47.37	**20999**
148	0	2950.0	**29392**	20.09	3.7785E-06	0.060457	0.21	**103349**	70.64	**13554**
156	0	2950.0	**28001**	20.49	3.6569E-06	0.058511	0.21	**87085**	63.74	**21542**
168	950	3900.0	**17748**	19.80	2.6634E-06	0.042616	0.15	**44182**	49.30	**27684**
175		3900.0	**9757**	16.33	1.7090E-06	0.027345	0.10	**19035**	31.87	**30944**
200		3900.0	**79237**		7.1234E-06	0.113975	0.40	**309**	0.30	**22465**
216		3900.0	**47994**	31.89	5.2106E-06	0.083371	0.30	**90485**	60.12	**12017**
223		3900.0	**71666**	44.62	6.6986E-06	0.107178	0.38	**78408**	48.82	**10524**
230		3900.0	**28568**	19.16	3.7068E-06	0.059309	0.21	**17525**	11.75	**103002**
235		3900.0	**12268**	12.64	2.0333E-06	0.032534	0.12	**11382**	11.72	**73435**
244		3900.0	**45741**	36.29	5.0527E-06	0.080844	0.29	**20585**	16.33	**59734**
252		3900.0	**39363**	35.37	4.5850E-06	0.073361	0.26	**67240**	60.41	**4697**
266		3900.0	**7278**	21.79	1.3582E-06	0.021732	0.08	**16497**	49.38	**9630**
280		3900.0	**30619**	32.75	3.8837E-06	0.062141	0.22	**58404**	62.46	**4479**
295		3900.0	**80087**	52.74	7.1699E-06	0.114718	0.41	**68700**	45.24	**3071**
326		3900.0	**10369**	48.04	1.7906E-06	0.02865	0.10	**11217**	51.96	**102954**
330		3900.0	**19180**		2.8145E-06	0.045033	0.16	**7576**	28.32	
337		3900.0	**56720**	42.12	5.7923E-06	0.092677	0.33	**73040**	54.24	**4901**
341		3900.0	**67907**	44.68	6.4798E-06	0.103677	0.37	**79295**	52.17	**4778**

TABLE A5.10 : Compartment 2 biogas data for the baseline study with sucrose (Section 4.2.5).

	Compartment 2									
			Methane					CO_2		N_2
Elapsed Time (d)	Gas prodn (mL)	Cumul. gas (mL)	(area)	(%)	moles	(mg)	mg COD	(area)	(%)	(area)
1	0.0	0.0		38.80					50.40	
3	1.5	1.5								
10	125.0	126.5								
16	0.0	126.5								
35	0.0	126.5								
37	0.0	126.5								
42	0.0	126.5								
44	20.5	147.0								
49	0.0	147.0								
54	0.0	147.0								
58	0.0	147.0								
63	0.0	147.0								
75	0.0	147.0								
78	0.0	147.0								
103		147.0	**13946**	13.19	2.24E-06	0.03578	0.13	**26862**	25.40	**64950**
114	4170.00	4317.0	**58568**	37.96	5.91E-06	0.09456	0.34	**93806**	60.80	**1918**
118	2310.00	6627.0	**36436**	50.10	4.36E-06	0.069743	0.25	**34679**	47.69	**1610**
120	0.00	6627.0	**38343**	42.73	4.51E-06	0.072114	0.26	**47788**	53.26	**3597**
125	2150.00	8777.0	**73949**	49.83	6.83E-06	0.109262	0.39	**71652**	48.28	**2812**
128	3180.00	11957.0	**57677**	45.98	5.85E-06	0.093656	0.33	**67770**	54.02	**0**
138	7420.00	19377.0	**60819**	53.11	6.05E-06	0.096816	0.34	**53201**	46.45	**505**
141	2060.00	21437.0	**49717**	39.15	5.33E-06	0.085267	0.30	**68781**	54.16	**8498**
145	2150.00	23587.0	**19466**	29.69	2.84E-06	0.045507	0.16	**24978**	38.10	**21121**
148	2030.00	25617.0	**70549**	49.31	6.63E-06	0.106147	0.38	**70498**	49.27	**2027**
156	1970.00	27587.0	**58035**	42.04	5.88E-06	0.09402	0.33	**74881**	54.24	**5143**
168	4110.00	31697.0	**19186**	28.42	2.82E-06	0.045043	0.16	**24871**	36.84	**23453**
175		31697.0	**13365**	29.27	2.17E-06	0.034674	0.12	**12857**	28.16	**19439**
200		31697.0	**82874**	50.67	7.32E-06	0.11713	0.42	**76819**	46.96	**3876**
216		31697.0	**68494**	43.70	6.51E-06	0.104229	0.37	**83551**	53.30	**4703**
223		31697.0	**81429**	53.02	7.24E-06	0.115884	0.41	**68815**	44.80	**3347**
230		31697.0	**89863**	67.60	7.69E-06	0.123013	0.44	**35817**	26.94	**7256**
235		31697.0	**15591**	22.43	2.43E-06	0.038819	0.14	**7741**	11.14	**46166**
244		31697.0	**52721**	39.52	5.53E-06	0.088501	0.31	**21536**	16.14	**59161**
252		31697.0	**48567**	44.62	5.25E-06	0.084005	0.30	**58832**	54.05	**1442**
266		31697.0	**67863**	41.87	6.48E-06	0.103635	0.37	**58372**	36.01	**35849**
280		31697.0	**44842**	45.09	4.99E-06	0.079819	0.28	**53604**	53.90	**1009**
295		31697.0	**82479**	56.88	7.3E-06	0.116791	0.41	**60626**	41.81	**1910**
326		31697.0	**27575**	41.09	3.62E-06	0.057907	0.21	**39533**	58.91	
330		31697.0	**52493**	63.64	5.52E-06	0.088259	0.31	**12965**	15.72	**17027**
337		31697.0	**67684**	51.11	6.47E-06	0.103466	0.37	**63312**	47.81	**1438**
341		31697.0	**74462**	52.69	6.86E-06	0.109726	0.39	**64782**	45.84	**2069**

TABLE A5.11 : Compartment 3 biogas data for the baseline study with sucrose (Section 4.2.5).

	Compartment 3									
			Methane					CO_2		N_2
Elapsed Time (d)	Gas prodn (mL)	Cumul. gas (mL)	(area)	(%)	moles	(mg)	mg COD	(area)	(%)	(area)
1	0.0	0.0		39.50					48.80	0.0
3	0.0	0.0								0.0
10	95.0	95.0								95.0
16	0.0	95.0								0.0
35	0.0	95.0								0.0
37	0.0	95.0								0.0
42	0.0	95.0								0.0
44	0.0	95.0								0.0
49	0.0	95.0								0.0
54	0.0	95.0								0.0
58	0.0	95.0								0.0
63	0.0	95.0								0.0
75	0.0	95.0								0.0
78	0.0	95.0								0.0
103		95.0	**54598**	44.90	5.65E-06	0.090479	0.32	**64005**	52.63	
114	3450.00	3545.0	**68678**	44.38	6.53E-06	0.104402	0.37	**83874**	54.20	3450.00
118	0.00	3545.0	**42928**	61.32	4.85E-06	0.077606	0.28	**25744**	36.77	0.00
120	0.00	3545.0	**48130**	55.78	5.22E-06	0.083522	0.30	**36540**	42.35	0.00
125	1135.00	4680.0	**93633**	62.57	7.88E-06	0.126095	0.45	**55466**	37.06	1135.00
128	1500.00	6180.0	**34548**	51.17	4.21E-06	0.067339	0.24	**28475**	42.18	1500.00
138	2540.00	8720.0	**73304**	62.51	6.79E-06	0.108676	0.39	**42200**	35.98	2540.00
141	1500.00	10220.0	**39737**	48.07	4.61E-06	0.073814	0.26	**37897**	45.85	1500.00
145	1200.00	11420.0	**20750**	41.29	2.97E-06	0.047599	0.17	**17910**	35.64	1200.00
148	1470.00	12890.0	**88797**	67.88	7.63E-06	0.12213	0.43	**42013**	32.12	1470.00
156	2130.00	15020.0	**118874**	58.47	9.08E-06	0.145358	0.52	**81072**	39.88	2130.00
168	2260.00	17280.0	**18940**	36.42	2.79E-06	0.044633	0.16	**19369**	37.24	2260.00
175		17280.0	**6877**	40.55	1.3E-06	0.020768	0.07	**3694**	21.78	
200		17280.0	**84468**	54.47	7.41E-06	0.118492	0.42	**68076**	43.90	
216		17280.0	**76586**	51.89	6.98E-06	0.111632	0.40	**69205**	46.89	
223		17280.0	**90746**	61.13	7.73E-06	0.12374	0.44	**55815**	37.60	
230		17280.0	**99061**	71.10	8.15E-06	0.13043	0.46	**37716**	27.07	
235		17280.0	**48202**	32.70	5.23E-06	0.083602	0.30	**16462**	11.17	
244		17280.0	**1463**	41.75	3.37E-07	0.005397	0.02	**376**	10.73	
252		17280.0	**56096**	49.32	5.75E-06	0.092035	0.33	**56366**	49.56	
266		17280.0	**76579**	50.35	6.98E-06	0.111625	0.40	**66770**	43.90	
280		17280.0	**50467**	33.98	5.38E-06	0.086083	0.31	**47499**	31.98	
295		17280.0	**88497**	60.77	7.62E-06	0.121881	0.43	**55682**	38.24	
326		17280.0	**25490**	32.34	3.43E-06	0.054888	0.19	**53338**	67.66	
330		17280.0	**53924**	64.19	5.61E-06	0.089773	0.32	**11065**	13.17	
337		17280.0	**79155**	59.35	7.12E-06	0.113903	0.40	**52609**	39.45	
341		17280.0	**79540**	55.70	7.14E-06	0.11424	0.40	**61615**	43.15	

TABLE A5.12 : Compartment 4 biogas data for the baseline study with sucrose (Section 4.2.5).

	Compartment 4									
			Methane					CO_2		N_2
Elapsed Time (d)	Gas prodn (mL)	Cumul. gas (mL)	(area)	(%)	moles	(mg)	mg COD	(area)	(%)	(area)
1	0.0	0.0		39.70					46.80	
3	0.0	0.0								
10	86.0	86.0								
16	0.0	86.0								
35	0.0	86.0								
37	0.0	86.0								
42	0.0	86.0								
44	0.0	86.0								
49	0.0	86.0								
54	0.0	86.0								
58	0.0	86.0								
63	0.0	86.0								
75	0.0	86.0								
78	0.0	86.0								
103		86.0	**83974**	53.53	7.38E-06	0.118071	0.42	**67887**	43.27	**5021**
114	1670.00	1756.0	**56222**	47.03	5.76E-06	0.092165	0.33	**62089**	51.94	**1222**
118	2350.00	4106.0	**14510**	41.13	2.3E-06	0.036837	0.13	**18487**	52.41	**2280**
120	0.00	4106.0	**50116**	58.31	5.36E-06	0.085702	0.30	**34396**	40.02	**1438**
125	1730.00	5836.0	**93535**	65.96	7.88E-06	0.126016	0.45	**46201**	32.58	**2060**
128	2250.00	8086.0	**83386**	62.16	7.35E-06	0.117569	0.42	**50107**	37.35	**664**
138	2215.00	10301.0	**75172**	70.62	6.9E-06	0.110366	0.39	**31274**	29.38	**0**
141	1110.00	11411.0	**36248**	49.89	4.34E-06	0.069506	0.25	**33364**	45.92	**3045**
145	0.00	11411.0	**89713**	59.15	7.68E-06	0.122889	0.44	**59431**	39.18	**2536**
148	0.00	11411.0	**97199**	71.56	8.06E-06	0.128956	0.46	**37331**	27.48	**1305**
156	0.00	11411.0	**47601**	58.02	5.18E-06	0.082934	0.29	**32359**	39.44	**2078**
168	0.00	11411.0	**25414**	40.84	3.42E-06	0.054775	0.19	**21815**	35.06	**15001**
175		11411.0	**86964**	68.13	7.54E-06	0.1206	0.43	**38926**	30.50	**1750**
200		11411.0	**92508**	56.79	7.82E-06	0.125182	0.44	**68692**	42.17	**1708**
216		11411.0	**74671**	51.89	6.87E-06	0.109915	0.39	**59279**	41.20	**9948**
223		11411.0	**85821**	62.82	7.48E-06	0.119638	0.42	**49114**	35.95	**1689**
230		11411.0	**99609**	70.11	8.18E-06	0.130862	0.46	**37667**	26.51	**4798**
235		11411.0	**50866**	38.68	5.41E-06	0.086515	0.31	**26648**	20.26	**53991**
244		11411.0			0	0	0.00			
252		11411.0	**73287**	52.23	6.79E-06	0.108661	0.39	**64738**	46.14	**2289**
266		11411.0	**92555**	52.88	7.83E-06	0.12522	0.44	**66879**	38.21	**15601**
280		11411.0	**87119**	55.58	7.55E-06	0.12073	0.43	**66953**	42.71	**2679**
295		11411.0	**91602**	63.36	7.78E-06	0.124442	0.44	**51563**	35.66	**1420**
326		11411.0	**24467**	32.38	3.34E-06	0.053367	0.19	**51103**	67.62	
330		11411.0	**50938**	65.29	5.41E-06	0.086592	0.31	**11019**	14.12	**16057**
337		11411.0	**79719**	58.97	7.15E-06	0.114397	0.41	**54332**	40.19	**1132**
341		11411.0	**79278**	56.98	7.13E-06	0.114011	0.40	**58069**	41.74	**1785**

TABLE A5.13 : Compartment 5 biogas data for the baseline study with sucrose (Section 4.2.5).

	Compartment 5									
			Methane					CO_2		N_2
Elapsed Time (d)	Gas prodn (mL)	Cumul. gas (mL)	(area)	(%)	moles	(mg)	mg COD	(area)	(%)	(area)
1	0.0	0.0		37.90					47.80	
3	0.0	0.0								
10	84.0	84.0								
16	0.0	84.0								
35	0.0	84.0								
37	0.0	84.0								
42	0.0	84.0								
44	0.0	84.0								
49	0.0	84.0								
54	0.0	84.0								
58	0.0	84.0								
63	0.0	84.0								
75	0.0	84.0								
78	0.0	84.0								
103		84.0	**13973**	27.62	2.24E-06	0.035831	0.13	**7031**	13.90	**29592**
114	225.00	309.0	**62675**	48.32	6.17E-06	0.098648	0.35	**63941**	49.29	**3103**
118	0.00	309.0	**46041**	84.56	5.07E-06	0.081183	0.29	**7420**	13.63	**984**
120	0.00	309.0	**54829**	58.80	5.67E-06	0.09072	0.32	**36577**	39.22	**1847**
125	1460.00	1769.0	**99045**	67.76	8.15E-06	0.130418	0.46	**45145**	30.89	**1980**
128	820.00	2589.0	**87069**	63.12	7.54E-06	0.120688	0.43	**49294**	35.74	**1572**
138	0.00	2589.0	**74732**	73.52	6.87E-06	0.10997	0.39	**26064**	25.64	**857**
141	275.00	2864.0	**24696**	53.81	3.36E-06	0.05371	0.19	**18225**	39.71	**2970**
145	2030.00	4894.0	**90885**	62.83	7.74E-06	0.123854	0.44	**52742**	36.46	**1035**
148	490.00	5384.0	**101972**	72.09	8.29E-06	0.132708	0.47	**37928**	26.81	**1555**
156	1945.00	7329.0	**70314**	69.67	6.62E-06	0.105929	0.38	**28716**	28.45	**1891**
168	3040.00	10369.0	**21128**	42.72	3.01E-06	0.048205	0.17	**12147**	24.56	**16178**
175		10369.0	**47203**	65.47	5.16E-06	0.08249	0.29	**21008**	29.14	**3888**
200		10369.0	**91173**	56.97	7.76E-06	0.124091	0.44	**66795**	41.74	**2067**
216		10369.0	**71947**	52.91	6.71E-06	0.107436	0.38	**55383**	40.73	**8661**
223		10369.0	**90222**	64.23	7.71E-06	0.123309	0.44	**47098**	33.53	**3157**
230		10369.0	**96415**	68.82	8.02E-06	0.128332	0.45	**37515**	26.78	**6173**
235		10369.0	**50352**	42.42	5.37E-06	0.085958	0.30	**20920**	17.63	**47417**
244		10369.0	**27997**	50.67	3.66E-06	0.058505	0.21	**6306**	11.41	**20947**
252		10369.0	**68726**	53.09	6.53E-06	0.104447	0.37	**58518**	45.20	**2211**
266		10369.0			0	0	0.00			
280		10369.0	**87535**	57.41	7.57E-06	0.121078	0.43	**63524**	41.66	**1415**
295		10369.0	**92120**	60.69	7.8E-06	0.124865	0.44	**58636**	38.63	**1033**
326		10369.0	**32072**	35.91	4.01E-06	0.064096	0.23	**57247**	64.09	
330		10369.0	**50779**	65.12	5.4E-06	0.086421	0.31	**16203**	20.78	**11000**
337		10369.0	**65231**	58.02	6.32E-06	0.101128	0.36	**45003**	40.03	**2191**
341		10369.0	**78452**	59.19	7.08E-06	0.113285	0.40	**52903**	39.91	**1196**

TABLE A5.14 : Compartment 6 biogas data for the baseline study with sucrose (Section 4.2.5).

	Compartment 6									
			Methane					CO_2		N_2
Elapsed Time (d)	Gas prodn (mL)	Cumul. gas (mL)	(area)	(%)	moles	(mg)	mg COD	(area)	(%)	(area)
1	0.0	0.0		37.70					46.10	
3	0.0	0.0								
10	140.0	140.0								
16	0.0	140.0								
35	0.0	140.0								
37	0.0	140.0								
42	0.0	140.0								
44	0.0	140.0								
49	0.0	140.0								
54	0.0	140.0								
58	0.0	140.0								
63	0.0	140.0								
75	0.0	140.0								
78	0.0	140.0								
103		140.0	**103724**	58.46	8.38E-06	0.134064	0.48	**39088**	22.03	**34605**
114	945.00	1085.0	**76009**	49.96	6.94E-06	0.111116	0.39	**73837**	48.53	**2298**
118	1790.00	2875.0	**14988**	19.65	2.36E-06	0.03772	0.13	**7499**	9.83	**53787**
120	0.00	2875.0	**54529**	62.91	5.65E-06	0.090407	0.32	**30212**	34.86	**1937**
125	0.00	2875.0	**98820**	66.27	8.14E-06	0.13024	0.46	**49417**	33.14	**884**
128	1105.00	3980.0	**90964**	65.25	7.74E-06	0.123919	0.44	**46412**	33.29	**2039**
138	1220.00	5200.0	**72716**	76.26	6.76E-06	0.10814	0.38	**22488**	23.58	**146**
141	0.00	5200.0	**12530**	61.22	2.07E-06	0.033051	0.12	**6768**	33.07	**1170**
145	0.00	5200.0	**95810**	64.51	7.99E-06	0.127848	0.45	**52367**	35.26	**336**
148	0.00	5200.0	**104487**	73.50	8.42E-06	0.134651	0.48	**35997**	25.32	**1684**
156	0.00	5200.0	**19300**	70.88	2.83E-06	0.045232	0.16	**6153**	22.60	**1776**
168	0.00	5200.0	**34553**	38.50	4.21E-06	0.067346	0.24	**17236**	19.21	**37955**
175		5200.0	**67998**	67.89	6.49E-06	0.103763	0.37	**30390**	30.34	**1769**
200		5200.0	**86761**	55.56	7.53E-06	0.12043	0.43	**66609**	42.66	**2778**
216		5200.0	**68050**	50.76	6.49E-06	0.103812	0.37	**46970**	35.03	**19052**
223		5200.0	**79899**	56.26	7.16E-06	0.114554	0.41	**52980**	37.30	**9144**
230		5200.0	**107641**	69.13	8.57E-06	0.137056	0.49	**45175**	29.01	**2882**
235		5200.0	**71993**	46.57	6.72E-06	0.107478	0.38	**68317**	44.20	**14268**
244		5200.0	**7424**	62.63	1.38E-06	0.022079	0.08	**2243**	18.92	**2186**
252		5200.0	**75892**	56.94	6.94E-06	0.111012	0.39	**56751**	42.58	**639**
266		5200.0	**61813**	40.57	6.11E-06	0.0978	0.35	**45532**	29.89	**45007**
280		5200.0	**88782**	59.33	7.63E-06	0.122117	0.43	**59634**	39.85	**1224**
295		5200.0	**96007**	63.22	8E-06	0.128006	0.45	**54280**	35.75	**1566**
326		5200.0	**86831**	61.08	7.53E-06	0.120488	0.43	**55323**	38.92	
330		5200.0	**65425**	77.41	6.33E-06	0.101315	0.36	**17016**	20.13	**2075**
337		5200.0	**70587**	58.57	6.64E-06	0.106182	0.38	**48987**	40.64	**951**
341		5200.0	**94255**	57.09	7.91E-06	0.126598	0.45	**67338**	40.78	**3512**

TABLE A5.15 : Compartment 7 biogas data for the baseline study with sucrose (Section 4.2.5).

	Compartment 7									
			Methane					CO_2		N_2
Elapsed Time (d)	Gas prodn (mL)	Cumul. gas (mL)	(area)	(%)	moles	(mg)	mg COD	(area)	(%)	(area)
1	0.0	0.0		33.30					38.60	
3	0.0	0.0								
10	80.0	80.0								
16	0.0	80.0								
35	0.0	80.0								
37	0.0	80.0								
42	0.0	80.0								
44	0.0	80.0								
49	0.0	80.0								
54	0.0	80.0								
58	0.0	80.0								
63	0.0	80.0								
75	0.0	80.0								
78	0.0	80.0								
103		80.0	**100677**	66.21	8.23E-06	0.131699	0.47	**36704**	24.14	**14679**
114	2145.00	2225.0	**78078**	51.53	7.06E-06	0.112955	0.40	**71705**	47.32	**1737**
118	2835.00	5060.0	**44189**	68.57	4.94E-06	0.079069	0.28	**18401**	28.55	**1852**
120	480.00	5540.0	**33801**	37.71	4.15E-06	0.066372	0.24	**18350**	20.47	**37486**
125	880.00	6420.0	**78187**	67.83	7.07E-06	0.113051	0.40	**36080**	31.30	**999**
128	1160.00	7580.0	**94110**	70.15	7.91E-06	0.126481	0.45	**39205**	29.22	**847**
138	990.00	8570.0	**55186**	73.13	5.69E-06	0.091092	0.32	**20026**	26.54	**254**
141	0.00	8570.0	**74400**	57.10	6.85E-06	0.10967	0.39	**51591**	39.59	**4306**
145	0.00	8570.0	**96535**	64.74	8.03E-06	0.128427	0.46	**51142**	34.30	**1427**
148	0.00	8570.0	**98501**	75.42	8.12E-06	0.129988	0.46	**30087**	23.04	**2012**
156	0.00	8570.0	**13818**	60.33	2.22E-06	0.035538	0.13	**4159**	18.16	**4926**
168	0.00	8570.0	**17514**	44.53	2.64E-06	0.042213	0.15	**5987**	15.22	**15834**
175		8570.0	**30260**	69.56	3.85E-06	0.061651	0.22	**13243**	30.44	**0**
200		8570.0	**83365**	56.87	7.35E-06	0.117551	0.42	**61108**	41.69	**2114**
216		8570.0	**38869**	46.50	4.55E-06	0.072759	0.26	**26105**	31.23	**18621**
223		8570.0	**89757**	61.48	7.68E-06	0.122925	0.44	**54070**	37.04	**2169**
230		8570.0	**95532**	61.45	7.98E-06	0.127625	0.45	**42779**	27.52	**17152**
235		8570.0	**57170**	48.29	5.82E-06	0.093138	0.33	**49520**	41.83	**11702**
244		8570.0	**46081**	56.58	5.08E-06	0.081229	0.29	**13257**	16.28	**22111**
252		8570.0	**74464**	55.62	6.86E-06	0.109728	0.39	**55932**	41.78	**3489**
266		8570.0	**67594**	35.24	6.46E-06	0.103381	0.37	**55124**	28.74	**69088**
280		8570.0	**94800**	59.55	7.94E-06	0.127037	0.45	**63286**	39.76	**1103**
295		8570.0	**87506**	62.13	7.57E-06	0.121054	0.43	**51503**	36.57	**1834**
326		8570.0	**41423**	54.07	4.74E-06	0.075835	0.27	**35192**	45.93	
330		8570.0	**4359**	49.68	8.91E-07	0.014259	0.05	**4415**	50.32	
337		8570.0	**79339**	61.70	7.13E-06	0.114064	0.40	**47348**	36.82	**1906**
341		8570.0	**85863**	58.69	7.48E-06	0.119674	0.42	**58278**	39.83	**2158**

TABLE A5.16 : Compartment 8 biogas data for the baseline study with sucrose (Section 4.2.5).

	Compartment 8									
			Methane					CO_2		N_2
Elapsed Time (d)	Gas prodn (mL)	Cumul. gas (mL)	(area)	(%)	moles	(mg)	mg COD	(area)	(%)	(area)
1	0.0	0.0		27.10					32.50	
3	0.0	0.0								
10	61.0	61.0								
16	0.0	61.0								
35	0.0	61.0								
37	0.0	61.0								
42	0.0	61.0								
44	0.0	61.0								
49	0.0	61.0								
54	0.0	61.0								
58	0.0	61.0								
63	0.0	61.0								
75	0.0	61.0								
78	0.0	61.0								
103		61.0								
114	2230.00	2291.0	**64908**	51.97	6.30108E-06	0.100817	0.36	**58143**	46.55	**1844**
118	2025.00	4316.0	**21389**	35.88	3.03875E-06	0.04862	0.17	**7900**	13.25	**30316**
120	1610.00	5926.0	**34359**	43.44	4.19346E-06	0.067095	0.24	**16344**	20.66	**28388**
125	670.00	6596.0	**81787**	69.88	7.26213E-06	0.116194	0.41	**33438**	28.57	**1822**
128	540.00	7136.0	**93873**	71.87	7.89308E-06	0.126289	0.45	**35280**	27.01	**1454**
138	1000.00	8136.0	**66453**	69.51	6.39363E-06	0.102298	0.36	**28430**	29.74	**719**
141	0.00	8136.0	**41348**	57.17	4.73409E-06	0.075745	0.27	**29153**	40.31	**1828**
145	0.00	8136.0	**100941**	64.41	8.24407E-06	0.131905	0.47	**49659**	31.68	**6128**
148	960.00	9096.0	**104257**	76.00	8.40466E-06	0.134475	0.48	**31391**	22.88	**1533**
156	1390.00	10486.0	**104108**	72.75	8.3975E-06	0.13436	0.48	**31723**	22.17	**7275**
168	320.00	10806.0	**22852**	42.07	3.18189E-06	0.05091	0.18	**8430**	15.52	**23034**
175		10806.0	**25780**	69.48	3.45711E-06	0.055314	0.20	**11323**	30.52	**0**
200		10806.0	**86587**	57.61	7.51772E-06	0.120284	0.43	**60964**	40.56	**2739**
216		10806.0	**5844**	47.34	1.13751E-06	0.0182	0.06	**4758**	38.54	**1744**
223		10806.0	**71429**	55.83	6.68499E-06	0.10696	0.38	**49891**	39.00	**6621**
230		10806.0	**93817**	66.58	7.89025E-06	0.126244	0.45	**39199**	27.82	**7886**
235		10806.0	**63417**	47.21	6.21079E-06	0.099373	0.35	**47950**	35.69	**22969**
244		10806.0	**6258**	8.70	1.20281E-06	0.019245	0.07	**1764**	2.45	**63883**
252		10806.0	**72412**	51.75	6.7414E-06	0.107862	0.38	**65831**	47.05	**1679**
266		10806.0	**59411**	66.31	5.9631E-06	0.09541	0.34	**26347**	29.40	**3843**
280		10806.0	**90881**	60.02	7.74069E-06	0.123851	0.44	**59341**	39.19	**1200**
295		10806.0	**91214**	62.21	7.75777E-06	0.124124	0.44	**53803**	36.70	**1601**
326		10806.0	**84154**	65.40	7.38904E-06	0.118225	0.42	**44531**	34.60	
330		10806.0	**6865**	69.59	1.29621E-06	0.020739	0.07	**3000**	30.41	
337		10806.0	**77535**	63.57	7.02967E-06	0.112475	0.40	**43326**	35.52	**1101**
341		10806.0	**85031**	60.23	7.43562E-06	0.11897	0.42	**54579**	38.66	**1570**

A5.2 CI REACTIVE RED 141

The following data correspond to the results presented in **Chapter 5**.

TABLE A5.17 : pH data for the CI Reactive Red 141 investigation (Section 5.4.5).

	Compartment									
Elapsed Time (d)	**In**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Out**
9	6.82	6.65	6.29	6.61	7.06	7.27	7.31	7.32	7.3	7.39
16	6.8	6.49	6.53	7.14	7.14	7.1	7.11	7.1	7.06	7.21
23	7.3		6.2	6.63	7.04	7.16	7.14	7.19	7.11	7.11
30	6.42	6.8	7.33	7.39	7.35	7.4	7.37	7.36	7.38	7.51
37	8.29	6.67	7.04	7.33	7.35	7.32	7.36	7.29	7.31	7.23
44	8.26	6.58	7.05	7.16	7.34	7.29	7.27	7.34	7.36	7.8
50	9.05	6.38	6.85	7.32	7.4	7.44	7.37	7.28	7.28	7.25
64	8.54	6.46	6.22	6.62	6.8	6.87	6.95	6.95	7.07	7.66
71	9.11	6.4	7.36	7.43	7.54	7.42	7.42	7.4	7.41	8.01
78	9.06	6.14	6.24	6.32	6.51	6.72	6.8	7	7.11	7.61
84	7.96		7.52	7.42	7.32	7.26	7.15	7.09	7.08	7.81
91	8.35	6.21	6.34	6.71	6.75	6.98	7.00	7.11	7.22	7.68
98	8.41	6.37	6.95	7.23	7.44	7.40	7.41	7.41	7.49	7.67
105	9.00	6.24	7.22	7.34	7.38	7.39	7.45	7.45	7.47	7.40
119	9.09	6.25	7.23	7.51	7.54	7.52	7.52	7.49	7.49	7.82
126	8.64	6.33	7.08	7.37	7.28	7.18	7.08	7.12	7.30	7.33
131	7.67	6.20	6.81	7.32	7.58	7.57	7.50	7.44	7.48	7.77
138	6.80	6.05	7.06	6.97	7.34	7.39	7.26	7.38	7.52	7.50
149	8.53	6.21	6.56	7.30	7.25	7.37	7.31	7.33	7.39	7.33
155		6.01	6.90	7.36	7.30	7.42	7.52	7.46	7.66	7.66
156	8.01	6.30	6.76	7.30	7.43	7.54	7.39	7.49	7.49	7.58
159	8.86	6.18	6.47	6.95	7.13	0.44	7.42	7.48	7.48	7.63
162	8.34	6.05	6.69	7.15	7.38	7.50	7.51	7.61	7.55	7.75
167	7.35	6.37	7.01	7.39	7.36	7.40	7.43	7.50	7.56	7.65

TABLE A5.18 : Total solids data for the CI Reactive Red 141 investigation (Section 5.4.6).

Elapsed (d)	Compartment 1			Effluent		
	Dish (g)	Wt after (g)	TS (g/L)	Dish (g)	Wt after (g)	TS (g/L)
9	35.30	35.34	4.00	55.79	55.82	3.00
16	37.78	37.87	9.00	57.17	57.21	4.00
23	35.29	35.41	12.00	55.79	55.82	3.00
37	73.82	74.00	18.00	39.58	39.61	3.00
50	53.14	54.22	108.00	55.78	55.80	2.00
64	42.74	42.91	17.00			
71	39.55	39.60	5.00	37.78	37.81	3.00
91	53.81	54.03	22.00	57.17	57.19	2.00
98	54.14	54.29	15.00	41.62	41.65	3.00
119	55.93	56.17	24.00	55.96	56.00	4.00
126	43.90	44.09	19.00	55.78	55.80	2.00
131	42.46	42.66	20.00			
138	55.93	56.06	13.00	55.78	55.83	5.00
149				55.96	55.97	1.00
155				55.78	55.80	2.00
156				53.81	53.84	3.00
159				57.17	57.23	6.00
167				57.14	57.17	3.00
169	57.18	57.40	22.00	41.27	41.30	3.00

TABLE A5.19 : Volatile solids data for the CI Reactive Red 141 investigation (Section 5.4.6).

Elapsed (d)	Compartment 1			Effluent		
	Dish (g)	Wt after (g)	TS (g/L)	Dish (g)	Wt after (g)	TS (g/L)
9	35.34	35.32	2.00	55.82	55.80	2.00
16	37.87	37.82	5.00	57.21	57.19	2.00
23	35.41	35.34	7.00	55.82	55.81	1.00
37	74.00	73.85	15.00	39.61	39.60	1.00
50	54.22	54.16	6.00	55.80	55.79	1.00
64	42.91	42.77	14.00			
71	39.60	39.58	2.00	37.81	37.80	1.00
91	54.03	53.84	19.00	57.19	57.19	0.00
98	54.29	54.16	13.00	41.65	41.64	1.00
119	56.17	55.97	20.00	56.00	55.99	1.00
126	44.09	43.95	14.00	55.80	55.79	1.00
131	42.66	42.51	15.00			
138	56.06	55.95	11.00	55.83	55.81	2.00
149				55.97	55.93	4.00
155				55.10	55.08	2.00
156				53.84	53.84	0.00
159				57.23	57.21	2.00
167				57.17	57.16	1.00
169	57.40	57.22	18.00	41.30	41.30	0.00

TABLE A5.20 : COD data for the CI Reactive Red 141 investigation (Section 5.4.7).

	Compartment								
	1			2			3		
Elapsed Time (d)	Rep 1 COD (mg/L)	Rep 2 COD (mg/L)	Mean (mg/L)	Rep 1 COD (mg/L)	Rep 2 COD (mg/L)	Mean (mg/L)	Rep 1 COD (mg/L)	Rep 2 COD (mg/L)	Mean (mg/L)
9	2605	2450	2528	2460	2400	2430	1965	2025	1995
16	2415	2420	2418	2580	2450	2515	1355	1335	1345
23	2785	2625	2705	2675	2670	2673	1910	1995	1953
30	2435	2450	2443	1110	1115	1113	540	455	498
37	2530	2270	2400	1425	1400	1413	480	535	508
44	2785	2680	2733	1280	1325	1303	640	850	745
50	2448	2218	2333	1388	1353	1370	673	633	653
71	2425	2490	2458	995	855	925	565	920	743
78	3380	2470	2925	935	935	935	340	235	288
84	2805	2785	2795	585	485	535	215	215	215
91	1225	1525	1375	485	850	668	255	150	203
98	1708	1868	1788	598	743	670	273	438	355
105	2855	2815	2835	1035	1020	1028	260	390	325
119	2100	2295	2198	1165	1245	1205	630	685	658
126	2913	2963	2938	1418	1533	1475	1348	598	973
131	1735	1680	1708	1570	990	1280	640	1025	833
138	1483	1273	1378	313	448	380	373	358	365
149	3098	2923	3010	2388	2263	2325	628	613	620
155	2150	2105	2128	1415	1285	1350	705	630	668
156	2845	2820	2833	2085	2035	2060	775	760	768
159	2920	2735	2828	2435	2475	2455	1685	1770	1728
162	2765	2630	2698	515	455	485	2040	2025	2033
167	2918	2933	2925	1428	1368	1398	828	753	790
	Compartment								
	4			5			6		
Elapsed Time (d)	Rep 1 COD (mg/L)	Rep 2 COD (mg/L)	Mean (mg/L)	Rep 1 COD (mg/L)	Rep 2 COD (mg/L)	Mean (mg/L)	Rep 1 COD (mg/L)	Rep 2 COD (mg/L)	Mean (mg/L)
9	1185	1365	1275	855	1130	993	610	705	658
16	935	1045	990	755	1240	998	225	275	250
23	1025	970	998	315	310	313	345	315	330
30	160	250	205	250	365	308	125	190	158
37	400	195	298	340	175	258	360	100	230
44	175	245	210	120	120	120	75		75
50	323	303	313	228	243	235	208	203	205
71	370	270	320	315	335	325	400	440	420
78	65	250	158	140	205	173	50	105	78
84	195	160	178	130	160	145	25	170	98
91	210	155	183	110	385	248	110	200	155
98	273	278	275	208	333	270	263	228	245
105	275	285	280	335	190	263	190	200	195
119	490	640	565	345	460	403	435	495	465
126	438	353	395	88	143	115	123	7	65
131	500	575	538	635	490	563	525	645	585
138	733	558	645	343	498	420	463	1108	785
149	888	778	833	398	428	413	368	518	443
155	480	470	475	605	595	600	450	380	415
156	855	1015	935	1525	640	1083	815	570	693
159	1645	1840	1743	1345	1320	1333	1420	1455	1438
162	1255	1120	1188	605	595	600	225	220	223
167	783	553	668	383	423	403	313	288	300

TABLE A5.21: COD data for the CI Reactive Red 141 investigation (Section 5.4.7).									
	Compartment								
	7			8			Effluent		
Elapsed Time (d)	Rep 1 COD (mg/L)	Rep 2 COD (mg/L)	Mean (mg/L)	Rep 1 COD (mg/L)	Rep 2 COD (mg/L)	Mean (mg/L)	Rep 1 COD (mg/L)	Rep 2 COD (mg/L)	Mean (mg/L)
9	605	490	548	440	350	395	255	285	270
16	155	230	193	195	115	155	270	280	275
23	100	85	93	415	405	410	75	170	123
30	80	100	90	135	130	133	75	90	83
37	100	95	98	195	100	148	315	230	273
44	50	60	55	335	100	218	290	75	183
50	308	323	315	178	158	168	123	108	115
71	370	320	345	350	390	370	320	285	303
78	55	40	48	55	155	105	75	65	70
84	90		90	170		170	110	185	148
91	335	120	228	135	145	140	145	120	133
98	273	263	268	198	183	190	283	238	260
105	210	285	248	340	315	328	375		375
119	375	365	370	430	330	380	520	320	420
126	148	13	80	258	153	205	133	318	225
131	400	605	503	455	460	458	410	370	390
138	398	543	470	443	378	410	293	358	325
149	268	353	310	268	258	263	348	288	318
155	445	430	438	410	520	465	350	400	375
156	425	445	435	510	445	478	550	700	625
159	1715	1560	1638	2135	3040	2588	2065	2110	2088
162	150	3220	1685	175	140	158	160	235	198
167	273	383	328	323	268	295	223	238	230

TABLE A5.22 : Compartment 1 biogas data for the CI Reactive Red 141 investigation (Section 5.4.10).

	Compartment 1									
			Methane					CO_2		N_2
Elapsed Time (d)	Gas prodn (mL)	Cumul. gas (mL)	(area)	(%)	moles	Volume (L)	g COD	(area)	(moles)	(moles)
9	0.00	0.00	**57860**	31.50	5.865E-06	0.0000	0.0000	**77706**	4.91E-06	3.63E-06
16	0.00	0.00	**6286**	4.65	1.207E-06	0.0000	0.0000	**30470**	2.68E-06	5.67E-06
23	0.00	0.00	**39676**	25.48	4.609E-06	0.0000	0.0000	**110925**	6.1E-06	6.9E-07
30	0.00	0.00	**8880**	6.91	1.589E-06	0.0000	0.0000	**30462**	2.68E-06	5.35E-06
37	0.00	0.00	**15171**	10.62	2.378E-06	0.0000	0.0000	**44303**	3.44E-06	5.13E-06
44	95.40	95.40	**16664**	12.88	2.546E-06	0.0237	0.0599	**28050**	2.53E-06	5.18E-06
50	15868.20	15963.60	**47567**	34.07	5.181E-06	7.1561	18.1167	**81344**	5.05E-06	1.26E-06
64	62487.00	78450.60	**103326**	60.98	8.36E-06	37.5062	94.9523	**44268**	3.44E-06	2.13E-06
71	524.70	78975.30	**45506**	25.55	5.036E-06	0.2101	0.5318	**124464**	6.53E-06	1.01E-06
78	15.90	78991.20	**42582**	28.22	4.825E-06	0.0061	0.0153	**55997**	4E-06	3.83E-06
84	31.80	79023.00	**52621**	37.68	5.525E-06	0.0143	0.0362	**36583**	3.03E-06	3.74E-06
91	0.00	79023.00	**12618**	8.32	2.077E-06	0.0000	0.0000	**12306**	1.4E-06	6.6E-06
98	47.70	79070.70	**60048**	36.97	6.003E-06	0.0218	0.0551	**80422**	5.02E-06	2.14E-06
105	0.00	79070.70	**51364**	29.15	5.441E-06	0.0000	0.0000	**115250**	6.24E-06	1.16E-06
119	174.90	79245.60	**55685**	40.73	5.726E-06	0.0857	0.2171	**68653**	4.55E-06	1.41E-06
126	31.80	79277.40	**40521**	20.80	4.672E-06	0.0121	0.0306	**152582**	7.36E-06	2.62E-07
131	31.80	79309.20	**85142**	61.36	7.442E-06	0.0200	0.0507	**47864**	3.62E-06	7.63E-07
138	15.90	79325.10	**55807**	37.61	5.733E-06	0.0073	0.0185	**71648**	4.67E-06	2.07E-06
149	31.80	79356.90	**53184**	35.92	5.562E-06	0.0156	0.0395	**93061**	5.48E-06	2.79E-07
155	15.90	79372.80	**75556**	55.72	6.919E-06	0.0095	0.0242	**55460**	3.98E-06	6.32E-07
156	0.00	79372.80	**49046**	33.76	5.283E-06	0.0000	0.0000	**92638**	5.47E-06	5.11E-07
159	0.00	79372.80	**39149**	25.66	4.569E-06	0.0000	0.0000	**108799**	6.03E-06	6.33E-07
162	15.90	79388.70	**30495**	18.29	3.873E-06	0.0054	0.0136	**128551**	6.66E-06	9.72E-07
167	31.80	79420.50	**43005**	36.25	4.856E-06	0.0155	0.0391	**72768**	4.72E-06	4.17E-07

TABLE A5.23 : Compartment 2 biogas data for the CI Reactive Red 141 investigation (Section 5.4.10).

	Compartment 2									
			Methane					CO_2		N_2
Elapsed Time (d)	Gas prodn (L)	Cumul. gas (L)	(area)	(%)	moles	Volume (L)	g COD	(area)	(moles)	(moles)
9	0.00	0.00	**81322**	45.06	7.24E-06	0.0000	0.0000	**87497**	5.28E-06	1.34E-06
16	0.00	0.00	**7822**	6.28	1.44E-06	0.0000	0.0000	**35759**	2.99E-06	5.04E-06
23	0.00	0.00	**52654**	32.67	5.53E-06	0.0000	0.0000	**105851**	5.93E-06	3.9E-07
30	0.00	0.00	**11798**	10.02	1.97E-06	0.0000	0.0000	**24689**	2.32E-06	5.05E-06
37	41.40	41.40	**26696**	19.94	3.54E-06	12.9800	32.8608	**45332**	3.49E-06	4.26E-06
44	0.02	41.42	**23764**	18.88	3.27E-06	0.0049	0.0123	**33950**	2.88E-06	4.53E-06
50	0.00	41.42	**60011**	42.17	6E-06	0.0000	0.0000	**64842**	4.39E-06	1.81E-06
64	56.11	97.53	**109717**	74.64	8.66E-06	39.1841	99.2002	**24716**	2.32E-06	1.42E-06
71	74.35	171.88	**81339**	43.24	7.24E-06	39.9333	101.0969	**104351**	5.88E-06	3.6E-07
78	0.00	171.88	**59245**	40.76	5.95E-06	0.0000	0.0000	**45324**	3.49E-06	3.26E-06
84	0.00	171.88	**51412**	36.99	5.44E-06	0.0000	0.0000	**34363**	2.91E-06	3.87E-06
91	0.06	171.94	**11123**	7.75	1.89E-06	0.0126	0.0318	**10005**	1.19E-06	6.47E-06
98	0.05	171.99	**86942**	51.41	7.54E-06	0.0270	0.0684	**74224**	4.77E-06	9.93E-07
105	0.00	171.99	**68788**	42.19	6.53E-06	0.0000	0.0000	**87529**	5.28E-06	8.68E-07
119	0.19	172.18	**62592**	46.58	6.16E-06	0.1014	0.2567	**62190**	4.27E-06	1.16E-06
126	0.02	172.20	**57521**	43.96	5.84E-06	0.0087	0.0221	**72620**	4.71E-06	1.14E-07
131	0.02	172.21	**77526**	64.65	7.03E-06	0.0105	0.0266	**39466**	3.19E-06	4.27E-07
138	0.02	172.23	**73043**	62.94	6.78E-06	0.0102	0.0258	**37720**	3.09E-06	7.12E-07
149	0.05	172.28	**60666**	41.38	6.04E-06	0.0253	0.0641	**84926**	5.19E-06	1.6E-07
155	0.02	172.29	**89684**	58.06	7.68E-06	0.0098	0.0249	**61162**	4.23E-06	5.15E-07
156	0.02	172.31	**60209**	40.62	6.01E-06	0.0083	0.0211	**86608**	5.25E-06	2.16E-07
159	0.00	172.31	**51052**	34.36	5.42E-06	0.0000	0.0000	**95068**	5.56E-06	3.67E-07
162	0.02	172.32	**52206**	33.65	5.5E-06	0.0076	0.0191	**100797**	5.76E-06	3.22E-07
167	0.02	172.34	**71158**	43.93	6.67E-06	0.0086	0.0218	**88637**	5.32E-06	3.28E-07

TABLE A5.24 : Compartment 3 biogas data for the CI Reactive Red 141 investigation (Section 5.4.10).

	Compartment 3									
			Methane					CO_2		N_2
Elapsed Time (d)	Gas prodn (mL)	Cumul. gas (mL)	(area)	(%)	moles	Volume (L)	g COD	(area)	(moles)	(moles)
9	0.00	0.00	**78940**	45.57	7.11E-06	0.0000	0.0000	**83073**	5.12E-06	1.3E-06
16	0.00	0.00	**28203**	24.13	3.67E-06	0.0000	0.0000	**39106**	3.17E-06	3.7E-06
23	0.00	0.00	**68793**	39.77	6.53E-06	0.0000	0.0000	**100800**	5.76E-06	4.88E-07
30	0.00	0.00	**16589**	14.68	2.54E-06	0.0000	0.0000	**22450**	2.17E-06	4.76E-06
37	906.30	906.30	**36725**	20.40	4.38E-06	0.2942	0.7447	**100058**	5.73E-06	3.39E-06
44	0.00	906.30	**29349**	23.17	3.77E-06	0.0000	0.0000	**36465**	3.03E-06	4.22E-06
50	0.00	906.30	**36725**	72.31	4.38E-06	0.0000	0.0000	**13181**	1.47E-06	1.41E-07
64	15.90	922.20	**102305**	79.96	8.31E-06	0.0121	0.0306	**22350**	2.16E-06	4.74E-07
71	2082.90	3005.10	**87441**	48.19	7.56E-06	1.1873	3.0058	**92449**	5.46E-06	2.42E-07
78	47.70	3052.80	**59694**	42.20	5.98E-06	0.0229	0.0580	**30696**	2.69E-06	3.77E-06
84	0.00	3052.80	**57866**	37.17	5.87E-06	0.0000	0.0000	**38351**	3.13E-06	4.16E-06
91	0.00	3052.80	**10893**	7.42	1.86E-06	0.0000	0.0000	**10194**	1.21E-06	6.57E-06
98	31.80	3084.60	**87281**	58.60	7.55E-06	0.0195	0.0494	**55991**	4E-06	7.56E-07
105	0.00	3084.60	**37753**	50.92	4.46E-06	0.0000	0.0000	**34591**	2.92E-06	2.74E-07
119	15.90	3100.50	**83957**	58.73	7.38E-06	0.0099	0.0250	**55444**	3.97E-06	5.07E-07
126	0.00	3100.50	**85112**	49.95	7.44E-06	0.0000	0.0000	**83708**	5.14E-06	2.41E-07
131	0.00	3100.50	**106585**	68.97	8.52E-06	0.0000	0.0000	**45602**	3.51E-06	3.52E-07
138	0.00	3100.50	**73590**	62.66	6.81E-06	0.0000	0.0000	**32278**	2.79E-06	1.33E-06
149	0.00	3100.50	**63632**	46.63	6.22E-06	0.0000	0.0000	**71366**	4.66E-06	2.27E-07
155	0.00	3100.50	**80572**	58.65	7.2E-06	0.0000	0.0000	**52614**	3.84E-06	5.84E-07
156	0.00	3100.50	**61383**	46.35	6.09E-06	0.0000	0.0000	**69893**	4.6E-06	1.81E-07
159	0.00	3100.50	**63461**	41.15	6.21E-06	0.0000	0.0000	**89205**	5.34E-06	2.41E-07
162	0.00	3100.50	**76885**	48.75	6.99E-06	0.0000	0.0000	**78467**	4.94E-06	3.54E-07
167	0.00	3100.50	**73161**	50.67	6.78E-06	0.0000	0.0000	**69513**	4.58E-06	2.61E-07

TABLE A5.25 : Compartment 4 biogas data for the CI Reactive Red 141 investigation (Section 5.4.10).										
	Compartment 4									
			Methane					**CO_2**		**N_2**
Elapsed Time (d)	**Gas prodn (L)**	**Cumul. gas (L)**	**(area)**	**(%)**	**moles**	**Volume (L)**	**g COD**	**(area)**	**(moles)**	**(moles)**
9	0	0	**92817**	53.30	7.84E-06	0.0000	0.0000	**73524**	4.75E-06	9.81E-07
16	0	0	**36868**	25.80	4.39E-06	0.0000	0.0000	**50037**	3.72E-06	4E-06
23	0	0	**78105**	45.91	7.06E-06	0.0000	0.0000	**87560**	5.28E-06	6.17E-07
30	0	0	**16082**	13.42	2.48E-06	0.0000	0.0000	**25080**	2.35E-06	4.95E-06
37	41.4036	41.4036	**38596**	32.21	4.53E-06	17.0093	43.0614	**44034**	3.43E-06	3.07E-06
44	0.0159	41.4195	**31080**	25.21	3.92E-06	0.0057	0.0144	**38352**	3.13E-06	3.9E-06
50	0	41.4195	**89231**	67.00	7.66E-06	0.0000	0.0000	**33027**	2.83E-06	1.28E-06
64	56.1111	97.5306	**121467**	75.55	9.2E-06	40.9229	103.6023	**36803**	3.04E-06	3.71E-07
71	74.3484	171.879	**83586**	48.89	7.36E-06	42.7342	108.1879	**85991**	5.23E-06	2.17E-07
78	0	171.879	**59830**	43.08	5.99E-06	0.0000	0.0000	**29796**	2.64E-06	3.69E-06
84	0	171.879	**44792**	33.70	4.99E-06	0.0000	0.0000	**32170**	2.78E-06	4E-06
91	0.0636	171.9426	**10893**	7.34	1.86E-06	0.0121	0.0307	**11187**	1.3E-06	6.59E-06
98	0.0477	171.9903	**95255**	68.33	7.96E-06	0.0332	0.0840	**43715**	3.41E-06	7.21E-08
105	0	171.9903	**100966**	66.46	8.25E-06	0.0000	0.0000	**49258**	3.68E-06	2.61E-07
119	0.1908	172.1811	**94252**	71.27	7.91E-06	0.1363	0.3451	**37524**	3.08E-06	7.73E-08
126	0.0159	172.197	**64765**	52.13	6.29E-06	0.0095	0.0240	**58661**	4.12E-06	1.31E-07
131	0.0159	172.2129	**103557**	71.24	8.37E-06	0.0112	0.0284	**40186**	3.23E-06	2.5E-07
138	0.0159	172.2288	**44949**	51.95	5E-06	0.0087	0.0221	**18895**	1.92E-06	2.19E-06
149	0.0477	172.2765	**66567**	49.02	6.4E-06	0.0276	0.0699	**68465**	4.54E-06	1.21E-07
155	0.0159	172.2924	**88781**	59.12	7.63E-06	0.0099	0.0250	**56915**	4.04E-06	6.2E-07
156	0.0159	172.3083	**62434**	51.39	6.15E-06	0.0094	0.0239	**58367**	4.11E-06	1.11E-07
159	0	172.3083	**64312**	44.16	6.27E-06	0.0000	0.0000	**79984**	5E-06	2.11E-07
162	0.0159	172.3242	**98738**	70.11	8.14E-06	0.0112	0.0284	**41460**	3.29E-06	1.03E-07
167	0.0159	172.3401	**94209**	69.20	7.91E-06	0.0111	0.0282	**41194**	3.28E-06	1.18E-07

TABLE A5.26 : Compartment 5 biogas data for the CI Reactive Red 141 investigation (Section 5.4.10).

	Compartment 5									
			Methane					CO_2		N_2
Elapsed Time (d)	Gas prodn (mL)	Cumul. gas (mL)	(area)	(%)	moles	Volume (L)	g COD	(area)	(moles)	(moles)
9	0.00	0.00	**103158**	61.55	8.35E-06	0.0000	0.0000	**59412**	4.15E-06	6.82E-07
16	0.00	0.00	**43292**	32.39	4.88E-06	0.0000	0.0000	**49520**	3.7E-06	3.26E-06
23	0.00	0.00	**96092**	57.67	8E-06	0.0000	0.0000	**66986**	4.48E-06	5.05E-07
30	0.00	0.00	**13697**	8.61	2.21E-06	0.0000	0.0000	**35610**	2.98E-06	6.06E-06
37	14564.40	14564.40	**42724**	34.12	4.84E-06	6.2127	15.7283	**51795**	3.81E-06	2.69E-06
44	0.00	14564.40	**32577**	27.67	4.05E-06	0.0000	0.0000	**37445**	3.08E-06	3.61E-06
50	0.00	14564.40	**57727**	40.89	5.86E-06	0.0000	0.0000	**28328**	2.55E-06	3.96E-06
64	1144.80	15709.20	**90817**	57.79	7.74E-06	0.6602	1.6714	**33732**	2.87E-06	2.81E-06
71	15.90	15725.10	**90095**	49.21	7.7E-06	0.0092	0.0232	**91692**	5.44E-06	2.05E-07
78	15.90	15741.00	**61611**	44.77	6.1E-06	0.0079	0.0201	**25252**	2.36E-06	3.76E-06
84	15.90	15756.90	**48465**	31.23	5.24E-06	0.0065	0.0164	**37341**	3.07E-06	4.58E-06
91	15.90	15772.80	**12487**	7.62	2.06E-06	0.0031	0.0078	**16329**	1.73E-06	6.85E-06
98	47.70	15820.50	**111259**	67.56	8.74E-06	0.0322	0.0815	**49994**	3.72E-06	4.92E-07
105	15.90	15836.40	**104753**	70.03	8.43E-06	0.0112	0.0284	**44353**	3.44E-06	7.88E-08
119	190.80	16027.20	**102244**	69.74	8.31E-06	0.1344	0.3402	**43953**	3.42E-06	6.68E-08
126	15.90	16043.10	**70103**	52.61	6.61E-06	0.0095	0.0242	**62470**	4.29E-06	1.09E-07
131	15.90	16059.00	**95650**	71.41	7.98E-06	0.0112	0.0284	**36362**	3.02E-06	2.93E-07
138	31.80	16090.80	**114249**	67.88	8.87E-06	0.0216	0.0546	**51148**	3.77E-06	4.25E-07
149	31.80	16122.60	**71244**	49.86	6.67E-06	0.0185	0.0469	**70684**	4.63E-06	1.5E-07
155	15.90	16138.50	**68191**	59.58	6.5E-06	0.0100	0.0253	**43211**	3.38E-06	4.44E-07
156	0.00	16138.50	**70973**	51.90	6.66E-06	0.0000	0.0000	**64798**	4.39E-06	1.57E-07
159	0.00	16138.50	**70082**	47.76	6.61E-06	0.0000	0.0000	**75555**	4.83E-06	1.75E-07
162	15.90	16154.40	**104217**	72.13	8.4E-06	0.0114	0.0288	**39309**	3.18E-06	1.51E-07
167	0.00	16154.40	**82209**	61.25	7.28E-06	0.0000	0.0000	**34044**	2.89E-06	1.85E-06

TABLE A5.27 : Compartment 6 biogas data for the CI Reactive Red 141 investigation (Section 5.4.10).

	Compartment 6									
			Methane					CO_2		N_2
Elapsed Time (d)	Gas prodn (mL)	Cumul. gas (mL)	(area)	(%)	moles	Volume (L)	g COD	(area)	(moles)	(moles)
9	0.00	0.00	**109650**	61.47	8.66E-06	0.0000	0.0000	**61912**	4.26E-06	8.8E-07
16	0.00	0.00	**46473**	34.35	5.1E-06	0.0000	0.0000	**51020**	3.77E-06	3.1E-06
23	0.00	0.00	**66410**	54.88	6.39E-06	0.0000	0.0000	**49132**	3.68E-06	7.32E-07
30	0.00	0.00	**11670**	7.36	1.96E-06	0.0000	0.0000	**37041**	3.06E-06	6.06E-06
37	47.70	47.70	**49626**	42.34	5.32E-06	0.0233	0.0589	**47913**	3.62E-06	1.98E-06
44	0.00	47.70	**38797**	29.26	4.54E-06	0.0000	0.0000	**42322**	3.34E-06	3.79E-06
50	0.00	47.70	**60088**	42.93	6.01E-06	0.0000	0.0000	**29592**	2.63E-06	3.73E-06
64	0.00	47.70	**103190**	65.67	8.35E-06	0.0000	0.0000	**39839**	3.21E-06	1.55E-06
71	31.80	79.50	**87948**	51.74	7.59E-06	0.0189	0.0477	**80979**	5.04E-06	1.69E-07
78	0.00	79.50	**94120**	69.56	7.91E-06	0.0000	0.0000	**23696**	2.25E-06	1.82E-06
84	15.90	95.40	**36762**	32.20	4.38E-06	0.0066	0.0166	**27767**	2.52E-06	3.7E-06
91	0.00	95.40	**80346**	52.36	7.18E-06	0.0000	0.0000	**32786**	2.82E-06	3.23E-06
98	15.90	111.30	**99609**	64.34	8.18E-06	0.0102	0.0259	**48434**	3.64E-06	8.74E-07
105	31.80	143.10	**92177**	70.76	7.81E-06	0.0227	0.0575	**38084**	3.11E-06	0
119	31.80	174.90	**94420**	67.99	7.92E-06	0.0220	0.0557	**43813**	3.41E-06	1.03E-07
126	15.90	190.80	**68356**	53.25	6.51E-06	0.0096	0.0243	**59392**	4.15E-06	9.84E-08
131	0.00	190.80	**96905**	71.21	8.05E-06	0.0000	0.0000	**36961**	3.05E-06	3.32E-07
138	15.90	206.70	**37384**	31.14	4.43E-06	0.0065	0.0164	**27186**	2.48E-06	3.98E-06
149	0.00	206.70	**53335**	38.19	5.57E-06	0.0000	0.0000	**24832**	2.33E-06	4.24E-06
155	0.00	206.70	**98328**	70.01	8.12E-06	0.0000	0.0000	**33065**	2.83E-06	1.1E-06
156	0.00	206.70	**49424**	38.28	5.31E-06	0.0000	0.0000	**28801**	2.58E-06	3.76E-06
159	0.00	206.70	**100254**	73.92	8.21E-06	0.0000	0.0000	**35371**	2.96E-06	0
162	15.90	222.60	**110853**	74.53	8.72E-06	0.0116	0.0294	**36624**	3.03E-06	1.97E-07
167	0.00	222.60	**99962**	71.63	8.2E-06	0.0000	0.0000	**34576**	2.92E-06	6.8E-07

TABLE A5.28 : Compartment 7 biogas data for the CI Reactive Red 141 investigation (Section 5.4.10).

	Compartment 7									
			Methane					CO_2		N_2
Elapsed Time (d)	Gas prodn (mL)	Cumul. gas (mL)	(area)	(%)	moles	Volume (L)	g COD	(area)	(moles)	(moles)
9	0.00	0.00	**88488**	60.65	7.62E-06	0.0000	0.0000	**49768**	3.71E-06	9.64E-07
16	0.00	0.00	**47368**	37.03	5.17E-06	0.0000	0.0000	**50463**	3.74E-06	2.66E-06
23	0.00	0.00	**47928**	52.28	5.21E-06	0.0000	0.0000	**39669**	3.2E-06	5.71E-07
30	0.00	0.00	**9583**	6.78	1.69E-06	0.0000	0.0000	**33428**	2.85E-06	5.67E-06
37	286.20	286.20	**36478**	29.77	4.36E-06	0.1126	0.2851	**36357**	3.02E-06	3.71E-06
44	15.90	302.10	**3644**	2.82	7.65E-07	0.0014	0.0034	**30255**	2.67E-06	5.56E-06
50	0.00	302.10	**3535**	2.33	7.45E-07	0.0000	0.0000	**13322**	1.49E-06	6.85E-06
64	0.00	302.10	**8250**	5.69	1.5E-06	0.0000	0.0000	**29740**	2.64E-06	5.97E-06
71	0.00	302.10	**71240**	43.03	6.67E-06	0.0000	0.0000	**60083**	4.18E-06	2.9E-06
78	0.00	302.10	**17711**	11.33	2.66E-06	0.0000	0.0000	**27222**	2.48E-06	6.11E-06
84	0.00	302.10	**40775**	26.81	4.69E-06	0.0000	0.0000	**47351**	3.59E-06	4.35E-06
91	15.90	318.00	**0**	0.00	0	0.0000	0.0000	**23010**	2.21E-06	7E-06
98	63.60	381.60	**17634**	10.92	2.65E-06	0.0143	0.0363	**41969**	3.32E-06	5.79E-06
105	15.90	397.50	**29438**	18.46	3.78E-06	0.0049	0.0123	**39935**	3.21E-06	5.38E-06
119	15.90	413.40	**75158**	54.42	6.9E-06	0.0089	0.0227	**43490**	3.4E-06	1.96E-06
126	0.00	413.40	**16219**	10.58	2.5E-06	0.0000	0.0000	**28691**	2.57E-06	6.01E-06
131	0.00	413.40	**33977**	20.99	4.16E-06	0.0000	0.0000	**41751**	3.31E-06	5.23E-06
138	0.00	413.40	**27148**	20.68	3.58E-06	0.0000	0.0000	**29513**	2.62E-06	4.79E-06
149	15.90	429.30	**40785**	30.01	4.69E-06	0.0064	0.0162	**26776**	2.45E-06	4.53E-06
155	0.00	429.30	**60171**	44.69	6.01E-06	0.0000	0.0000	**27456**	2.5E-06	3.57E-06
156	15.90	445.20	**15882**	11.76	2.46E-06	0.0038	0.0096	**25818**	2.39E-06	5.49E-06
159	0.00	445.20	**98369**	69.28	8.12E-06	0.0000	0.0000	**33108**	2.84E-06	1.24E-06
162	15.90	461.10	**46309**	30.99	5.09E-06	0.0064	0.0163	**34841**	2.94E-06	4.53E-06
167	0.00	461.10	**69232**	47.41	6.56E-06	0.0000	0.0000	**31351**	2.73E-06	3.5E-06

TABLE A5.29 : Compartment 8 biogas data for the CI Reactive Red 141 investigation (Section 5.4.10).

	Compartment 8									
			Methane					CO_2		N_2
Elapsed Time (d)	Gas prodn (mL)	Cumul. gas (mL)	(area)	(%)	moles	Volume (L)	g COD	(area)	(moles)	(moles)
9	0.00	0.00	**104404**	61.08	8.41E-06	0.0000	0.0000	**57653**	4.07E-06	1.09E-06
16	0.00	0.00	**44482**	35.41	4.96E-06	0.0000	0.0000	**48684**	3.66E-06	2.8E-06
23	0.00	0.00	**83117**	52.08	7.33E-06	0.0000	0.0000	**69538**	4.58E-06	8.92E-07
30	0.00	0.00	**9144**	7.03	1.63E-06	0.0000	0.0000	**30489**	2.68E-06	5.39E-06
37	1685.40	1685.40	**41788**	28.14	4.77E-06	0.6436	1.6295	**43300**	3.39E-06	4.33E-06
44	0.00	1685.40	**8779**	5.81	1.57E-06	0.0000	0.0000	**33188**	2.84E-06	6.04E-06
50	0.00	1685.40	**457**	0.76	1.11E-07	0.0000	0.0000	**8481**	1.05E-06	3.77E-06
64	0.00	1685.40	**17568**	9.95	2.64E-06	0.0000	0.0000	**40773**	3.26E-06	6.33E-06
71	0.00	1685.40	**71455**	41.33	6.69E-06	0.0000	0.0000	**61310**	4.24E-06	3.22E-06
78	0.00	1685.40	**21447**	16.36	3.04E-06	0.0000	0.0000	**28635**	2.57E-06	5.04E-06
84	0.00	1685.40	**19493**	15.47	2.85E-06	0.0000	0.0000	**29970**	2.65E-06	4.87E-06
91	0.00	1685.40	**0**	0.00	0	0.0000	0.0000	**32634**	2.81E-06	6.85E-06
98	0.00	1685.40	**23569**	13.70	3.25E-06	0.0000	0.0000	**45559**	3.5E-06	5.83E-06
105	0.00	1685.40	**90010**	59.00	7.7E-06	0.0000	0.0000	**44062**	3.43E-06	1.89E-06
119	0.00	1685.40	**88508**	60.92	7.62E-06	0.0000	0.0000	**49461**	3.69E-06	9.31E-07
126	0.00	1685.40	**27827**	25.55	3.64E-06	0.0000	0.0000	**3399**	4.87E-07	4.91E-06
131	0.00	1685.40	**71778**	55.92	6.71E-06	0.0000	0.0000	**47672**	3.61E-06	1.09E-06
138	0.00	1685.40	**13504**	42.16	2.18E-06	0.0000	0.0000	**9292**	1.13E-06	1.12E-06
149	0.00	1685.40	**20398**	13.82	2.94E-06	0.0000	0.0000	**32092**	2.78E-06	5.56E-06
155	0.00	1685.40	**58508**	35.89	5.91E-06	0.0000	0.0000	**31999**	2.77E-06	4.71E-06
156	0.00	1685.40	**14877**	10.27	2.34E-06	0.0000	0.0000	**26825**	2.46E-06	5.84E-06
159	0.00	1685.40	**54816**	40.81	5.67E-06	0.0000	0.0000	**32537**	2.8E-06	3.57E-06
162	0.00	1685.40	**42455**	29.20	4.82E-06	0.0000	0.0000	**32630**	2.81E-06	4.62E-06
167	0.00	1685.40	**68919**	47.10	6.54E-06	0.0000	0.0000	**30782**	2.7E-06	3.56E-06

A-6

List of Publications

1. **SACKS, J**., BUCKLEY, C.A. and STUCKEY, D.C. (1998). *Treatment of High -Strength or Toxic Organic Effluents in the Anaerobic Baffled Reactor (ABR).* WISA 98 Biennial Conference and Exhibition. Cape Town, South Africa.

2. **SACKS, J**. and BUCKLEY, C.A. (1999). *Anaerobic Treatment of Textile Size Effluent*. Water Science & Technology: **40**(1), pp. 177-182.

3. **BELL, J**., PLUMB, J., BUCKLEY, C. A., and STUCKEY, D. C. (2000). *Treatment and decolourisation of dyes in an anaerobic baffled reactor.* Journal of Environmental Engineering: **126** pp. 1026-1032.

4. **BELL, J**., BUCKLEY, C.A., STUCKEY, D., DAMA, P. and SENIOR, E. (2000). *The Anaerobic Baffled Reactor - Pre Scale-Up Laboratory Investigation.* BioY2K Combined Millennium Meeting. Rhodes University, Grahamstown, South Africa.

5. DAMA, P., **BELL, J**., BROUCKAERT, C.J., BUCKLEY, C.A. and STUCKEY, D.C. (2000). *The Design of an Anaerobic Baffled Reactor with the Aid of Computational Fluid Dynamics.* BioY2K Combined Millennium Meeting. Rhodes University, Grahamstown, South Africa.

6. MURUGAN, L.H., GOVENDER, M., **BELL, J**., BUCKLEY, C.A., ROZZI, I and FRESTEL, S. (2000). *Comparison of Methods for Measuring Methanogenic Activity.* BioY2K Combined Millennium Meeting. Rhodes University, Grahamstown, South Africa.

7. AMBROSIA, D.D., BUCKLEY, C.A., ROZZI, A., **BELL, J**. and NAIDOO, V. (2000). *Evaluation of the Effect of Microbial Population Composition on the Methanogenic Activity.* BioY2K Combined Millennium Meeting. Rhodes University, Grahamstown, South Africa

8. **BELL, J**., DAMA, P., BUCKLEY, C.A., STUCKEY, D. and SENIOR, E. (2000). *Pre scale-up laboratory investigation of the anaerobic baffled reactor.* The Water Institute of Southern Africa (WISA 2000) Biennial Conference and Exhibition. Sun City, South Africa.

9. **BELL, J**., BUCKLEY, C.A., STUCKEY, D. and PLUMB, J. (2000). *Degradation of food dyes in the anaerobic baffled reactor.* The Water Institute of Southern Africa (WISA 2000) Biennial Conference and Exhibition. Sun City, South Africa.

10. DAMA, P., **BELL, J**., BROUCKAERT, C.J., BUCKLEY, C.A. and STUCKEY, D.C. (2000). *Computational fluid dynamics : application to the design of the anaerobic baffled reactor.* The

Water Institute of Southern Africa (WISA 2000) Biennial Conference and Exhibition. Sun City, South Africa.

11. **BELL, J**., DAMA, P., BUCKLEY, C.A., STUCKEY, D.C. and SENIOR, E. (2000). *Treatment of Industrial Wastewater in the Anaerobic Baffled Reactor.* The South African Institution of Chemical Engineers (SAIChE 2000) 9th National Meeting. Secunda, Mpumalanga, South Africa.

12. DAMA, P., **BELL, J**., BROUCKAERT, C.J., BUCKLEY, C.A. and STUCKEY, D.C. (2000). *Hydrodynamic in an Anaerobic Baffled Reactor- Application of Computational Fluid Dynamics and Tracer Tests.* The South African Institution of Chemical Engineers (SAIChE 2000) 9th National Meeting. Secunda, Mpumalanga, South Africa.

13. NAIDOO, V., **BELL, J**., DU PREEZ, M., NDIMANDE, S., ODHAV, B. and BUCKLEY, C.A. (2000). *Co-digestion of High Strength / Toxic Organic Liquid Effluent in Anaerobic Digesters.* International Training Seminar on Control, Management and Treatment of Landfill Emissions. School of Civil Engineering, University of Natal, Durban, South Africa.

14. PLUMB J. J., **J. BELL**, and D. C. STUCKEY (2001). *Microbial populations associated with treatment of an industrial dye effluent in an anaerobic baffled reactor*. Appl. and Environ. Microbiol,: **67** (7), pp. 3226-3235.

15. **BELL, J**., DAMA, P., GOVENDER, K.M., BUCKLEY, C.A. and STUCKEY, D.C. (2001). *Performance characterisation and microbial populations associated with the start-up of a laboratory-scale and a pilot -scale anaerobic baffled reactor.* 9th World Congress on Anaerobic Digestion. Antwerp, Belgium.

16. DAMA, P., **BELL, J**., FOXON, K., NAIDOO, V., BROUCKAERT, C.J., BUCKLEY, C.A. and STUCKEY, D. (2001). *The Anaerobic Baffled Reactor for the Treatment of Domestic Wastewater in Dense Peri-urbain Communities.* 9th World Congress on Anaerobic Digestion. Antwerp, Belgium.

17. DAMA, P., GOVENDER, K., HUANG, J., FOXON, K., **BELL, J**., BROUCKAERT, C.J., BUCKLEY, C.A., NAIDOO, V. and STUCKEY, D. (2001). *Flow Patterns in an Anaerobic Baffled Reactor.* 9th World Congress on Anaerobic Digestion. Antwerp, Belgium.

18. DAMA, P., **BELL, J**., FOXON, K.M, BROUCKAERT, C.J., HUANG, T., BUCKLEY, C.A., NAIDOO, V., and STUCKEY, D. (2001). *Pilot-scale Study of an Anaerobic Baffled Reactor for the Treatment of Domestic Wastewater.* The International Water Association Conference on Water and Wastewater Management for Developing Countries. Kuala Lumpur, Malaysia. (***In Press: Wat Sci Tech***)