ANAEROBIC AND AEROBIC DEGRADABILITY OF SURFACTANTS AND SURFACTANT-INK MIXTURES

By

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ANAEROBIC AND AEROBIC DEGRADABILITY

OF SURFACTANTS AND SURFACTANT-INK

MIXTURES

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CHAPTER I

INTRODUCTION

According to Karsa and Porter (1995), mankind has used soaps for thousands of years. These soaps, the earliest manufactured surfactants, were sodium salts of saturated and unsaturated fatty acids. Surfactants, which are also known as "surface active agents", are organic compounds which have the ability to lower the interfacial tension between phases of a mixture, thereby facilitating mixing (Karsa and Porter, 1995). The surfactants are molecules in which there is a hydrophobic and hydrophilic functional group. The surfactants are usually classified based on the charge characteristics of the hydrophilic part of the molecule. Anionic surfactants have negatively charged hydrophiles; non-ionic surfactants have uncharged hydrophiles; cationic surfactants have positively charged hydrophiles; and amphoterics have both positively and negatively charged hydrophiles.

According to Karsa and Porter (1995), surfactants have been used for home uses such as personal hygiene, washing and cleaning and for various other purposes such as industrial cleaning, emulsifiers, and as paint additives and oil field chemicals. Due to such wide usage, environmental concerns have increased regarding the levels of surfactants released to the environment. Biodegradability of surfactants is an important factor in assessing such environmental risk, as it may play a key role in the levels of surfactants in the environment. It is important that a surfactant released to the environment is biodegradable to prevent the possibility of future harm due to build-up in the environment.

Therefore, tests are frequently performed to determine the degradability of surfactants, thereby reducing the possibility of environmental burden. On a small scale, bench scale tests can be used to determine a surfactant's potential for biodegradation. This information can then help to determine the surfactant's fate in the environment or in various treatment systems.

The surfactants used in the current study are used to remove inks from printed plastic sheeting, rendering the plastic sheeting more available for reuse and recycling. Teeters et al. (1997) have demonstrated the use of various surfactants in the deinking of plastic packaging films and developed a standard method for the deinking process. This process results in the production of an aqueous waste stream, which has to be treated prior to reuse or release to the environment. This waste stream contains the dissolved surfactant, the ink residues removed from the plastic, as well as pH buffers (Teeters et al., 1997). In this study, standard, mixed culture biological waste treatment processes, including aerobic oxidation and anaerobic digestion, were tested for their ability to handle these wastes.

For the anaerobic study, a mixed bacterial system under anaerobic conditions was used for assessing the anaerobic degradability of surfactants and surfactant-ink mixtures. Anaerobic bench scale reactors were setup to compare the gas production for controls (which contained just bacterial culture and a specific amount of glucose) and for the samples (which contained the bacterial culture, the same amount of glucose and a specific concentration of the surfactant or surfactant-ink mixture). The gas production was monitored as evidence of biologic activity. The surfactant and surfactant-ink mixtures

were said to be anaerobically degradable if they produced more gas production compared to the controls.

For the aerobic study, the aerobic toxicity and potential biodegradability of the surfactant and surfactant-ink mixtures was determined by setting bench scale aerobic reactors. The dissolved oxygen (D.O.) drop and the biochemical oxygen demand (BOD) were determined for the base reactors (fed no surfactant) and the test samples (fed a seed culture and a specific concentration of surfactant or surfactant-ink mixture). The toxicity of the surfactants and surfactant-ink mixtures were inferred from the ultimate BOD values measured and comparison of these values to their theoretical BOD values, and to the ultimate BOD's of the control reactors.

Objectives

The overall objectives of this study were to:

a) Determine anaerobic degradability and toxicity of surfactants and surfactant-ink mixtures by comparing the gas production of these with the controls.

b) Determine aerobic toxicity and potential degradability of surfactants and surfactant-ink mixtures by their ultimate BOD values.

CHAPTER II

BACKGROUND AND LITERATURE REVIEW

Before the tests on aerobic and anaerobic degradability of surfactants were started, it was necessary to know about surfactants, their properties and uses, the environmental fate of surfactants and the importance of biodegradation of surfactants. This chapter addresses these topics, and reviews current studies, especially those concerning biodegradation of cationic surfactants.

Background

The word surfactant is derived from the term "surface active agent". Surfactants modify the surface properties of aqueous solutions by concentrating at the surface. The surfactant consists of a strongly hydrophilic group (water-liking) and a strong hydrophobic group (water-repelling) linked together in a single molecule. Soaps, the earliest known manufactured surfactants, were sodium salts of fatty acids formed from the alkaline hydrolysis of animal and plant fats and oils (Karsa and Porter, 1995). Table 1 lists a number of important applications of surfactants. Table 2 gives the different types of surfactants and their characteristics.

The extensive use of surfactants, as seen in Table 1, frequently results in enormous surfactant discharges. Understanding surfactant biodegradation is important for defining levels of surfactants in the environment and assessing environmental damage. The hydrophilic group of surfactants frequently contains medium-to-long alkyl chains, which are potentially excellent substrates for chemotrophic growth (Karsa and Porter, 1995).

Table 1: Applications of surfactants (Karsa and Porter, 1995)

Processes	Emulsification, Wetting, Dispersing, Foaming and Solubilizing
Operations and Products	Detergents, Cosmetics, Pharmaceuticals, Mining, Petroleum, Paints, Plastics, Food, Pulp and Paper, Agriculture and Leather

Table 2. Types of surfactants and their characteristics (Karsa and Porter, 1995)

Type	Characteristics
Cationic surfactant	Molecules with at least one hydrophobic group attached to a hydrophilic group carrying a positive charge
Anionic surfactant	Molecules with at least one hydrophobic group attached to a hydrophilic group carrying a negative charge
Non-ionic surfactant	Covalent compounds having hydrophilic and hydrophobic group and do not ionize when dissolved in water
Amphoteric surfactant	Surfactants with ionic charge and exhibit anionic and cationic characteristics based on the type of medium (acidic or alkaline)

Biodegradation as a treatment process prior to discharge also offers the advantage of not creating a residual waste stream that would require further treatment.

Biodegradation

Biodegradation is defined as the destruction of chemicals by the biologic activity of living organisms (Swisher, 1970). There are two main types of biodegradation defined for surfactants.

(1) Primary biodegradation:

The primary biodegradation of surfactants is said to have occurred when the molecule is oxidized or altered by metabolic activity to such an extent that it has lost its surfactancy properties (Karsa and Porter, 1995).

(2) Ultimate biodegradation:

The ultimate biodegradation of surfactants is said to have occurred when the molecule is completely degraded to carbon dioxide, water, mineral salts and biomass and is also known as mineralization (Karsa and Porter, 1995).

(3) Ready biodegradation:

Ready biodegradability of surfactants is said to have occurred when the molecule reaches 60 % of the theoretical ultimate BOD value (Karsa and Porter, 1995).

Mechanisms of biodegradation of surfactants

The mechanisms used by bacteria for the biodegradation of chemicals are usually enzyme-catalyzed, and most of these chemical transformation's net effect is oxidation (Karsa and Porter, 1995). The different types of oxidation that occur during degradation of surfactants are:

(1) ω-Oxidation:

The bacterial attack on the surfactant by the oxidation of the terminal carbon atoms of the hydrophobic part of the non-ionic surfactants to aldehydes and fatty acids is known as ω-Oxidation (Karsa and Porter, 1995). Surfactants with linear hydrocarbons can easily get degraded by this mechanism.

(2) β-Oxidation:

The next step in the oxidation of the fatty acids is called β-Oxidation. In this reaction, the carbon atoms are separated from the hydrocarbon chain by the co-enzyme, acetyl co-enzyme A. This process is continuous. After each β-Oxidation there is a free acetyl co-enzyme A formed which re-enters the cycle and results in complete oxidation (Karsa and Porter, 1995).

(3) α-Oxidation:

The branched hydrocarbons that are not totally oxidized by β-Oxidation need an alternative procedure known as α-Oxidation. In this process the carbon atom is oxidized to a ketonic group (Karsa and Porter, 1995).

(4) ω-hydrophile pathway:

The hydrophilic parts of the surfactants are degraded by the ω-hydrophile pathway (Karsa and Porter, 1995). The hydrophilic parts of non-ionic surfactants are reported to be completely mineralized.

Literature Review

In addition to the applications of surfactants seen in Table 1, surfactants are used for desorption of aromatic hydrocarbons from soil, deinking plastic packages in industries and many other uses (Figueroa et al., 1997). Surfactants have been proposed for use in remediation of hydrophobic compounds and non-aqueous-phase liquids in the saturated zone (Fountain et al., 1991) and in the vadose zone (Clarke et al., 1991) of the subsurface. Surfactants also have been proposed for use in above-ground surfactant washing on excavated soils and in-situ injection and recovery on intact soils (Figueroa et al., 1997). In such uses, waste streams are generated consisting of surfactant and solubilized contaminant, which then have to meet applicable environmental regulations to be discharged to the environment. In the current study, the surfactant-ink mixture samples tested are the waste streams generated when deinking the plastic packaging films during recycling of the plastic wrapping (Teeters et al., 1997).

Recycling of surfactant solutions that have been used to remove hydrophobic organic contaminants may be a desirable treatment option (Figueroa et al., 1997). According to Ellis et al. (1985), recycling techniques such as hydrolysis, ultrafiltration, foam fractionation and adsorption have met with limited success. Underwood et al. (1995) used a countercurrent solvent extraction system to remove hydrophobic moieties

from anionic surfactant solutions and the test generated a high residual waste, which required further treatment. Biodegradation in turn offers the advantage of simultaneously treating both the solubilizing agent and the contaminant without creating a residual waste stream that would require further treatment. Studies done by Abdul and Ang (1994) and Fountain et al. (1991) show that the surfactant is observed to have reached the primary biodegradation levels in biodegradation studies of hazardous waste/ surfactant mixtures, that is, the surfactant has been observed to lose its surfactancy properties. A study done by Abdul and Ang (1994) used 0.75 % (weight/weight) aqueous surfactant (Witconol SN-70) for in situ washing of polychlorinated biphenyls (PCBs) and oils at a contaminated field site. The resulting leachate from this test was then discharged into on-site bioreactors to biodegrade the oil and the surfactant. The biotreated leachate consisted of PCB whose concentration was well below the method detection limit of 0.5 mg/L. An -HPLC equipped with a 125 mm × 4 mm Lichrosorb RP-18 column was used to determine the concentration of surfactant in the biotreated leachate. The surfactant was not detected in the leachate and was found to have lost its surfactancy property. This leachate was then discharged to a nearby wastewater treatment facility. Studies by Fountain et al. (1991) involved the use of various known biodegradable surfactants for in situ extraction of dense organic pollutants from a contaminated aquifer. Five percent (weight/weight) aqueous solutions of biodegradable surfactants were used for this purpose. Air stripping was used to separate the organic compounds from the surfactants. Clean water was then pumped through the aquifer to remove the surfactants. As the surfactants used were biodegradable, these were disposed as normal wastewater at the conclusion of this process.

Factors that control surfactant biodegradability

Surfactant structure

Tests carried out on surfactants in activated–sludge systems have shown that linear hydrophobic surfactants are completely degraded whereas branched and aromatic hydrophobic surfactants are not. Adams et al. (1996) tested the biodegradability of many polyethoxylated surfactants around 0.01 % (weight/weight) and found complete degradation of linear polyethoxylates and incomplete degradation of aromatic and branched ethoxylates. At low concentrations (0.001% to 0.005% (weight/ weight)), complete disappearance of linear alcohol ethoxylates (LAEs) and partial disappearance of alkyl phenol ethoxylates (APEs) have been observed in batch studies and continuous activated-sludge systems (Ball et al., 1989; Larson and Games, 1981; and Swisher, 1987). Figueroa et al. (1997) tested the biodegradation of two polyethoxylated non-ionic surfactants, Neodol 91-8 and Makon 12, in sequencing batch reactors. The concentrations tested were 0.01, 0.025, and 0.05% (weight/weight). Neodol 91-8 is a non-aromatic compound and therefore was seen to be more rapidly and completely degraded than the Makon 12, which is an aromatic compound.

Critical Micelle Concentration

Surfactants have a phenomenal characteristic of aggregating into larger, oriented groups called micelles (Swisher, 1970). Up to a certain concentration of surfactant, which is called the Critical Micelle Concentration (CMC), the surfactant is present in the form of individual molecules or ions. Beyond this concentration, any added surfactant forms

micelles in solution instead of forming individual molecules (Swisher, 1970). The CMC is the key factor controlling substrate availability (Laha and Luthy, 1992), inhibition of bacterial growth (Tiehm, 1994) and interference with biochemical functions (Tanford and Reynolds, 1976). Rouse et al. (1994) evaluated ethoxylated alkylsulfate surfactants for use in subsurface remediation and determined a relationship between CMC and biodegradation for each of the surfactants tested. The concentrations above CMC's of the surfactants were found to be toxic to the culture (Rouse et al., 1994). Liu et al. (1995) studied the biodegradation of naphthalene in aqueous non-ionic surfactant systems. Two non-ionic surfactants, Brij 30 (alkyl ethoxylate) and Triton X-100 (alkyl phenol ethoxylate) were used in this study. The results showed that surfactant concentrations above the CMC were not found to be toxic to the naphthalene-degrading bacteria and the presence of micelles did not inhibit biodegradation of naphthalene.

Importance of biodegradability of cationic surfactants

Cationic surfactants may degrade by a variety of mechanisms. One of the process investigated is photodegradation of cationic surfactants. Ruiz Cruz (1981) showed that photodegradation by sunlight resulted in the formation of recalcitrant products. Krzeminski et al. (1973) compared photodegradation and biological degradation. Photodegradation was found to be slow compared with biological degradation. According to Karsa and Porter (1995), light degrades the cationic surfactants to a certain extent only. It was concluded that biodegradation is a better process compared to photodegradation in preventing the accumulation of cationic surfactants in the environment (Karsa and Porter, 1995).

One of the surfactants tested in the current study is a cationic surfactant, CTAB (CetylTrimethyl Ammonium Bromide). Table 3 gives different methods that have been used to test the biodegradation of CTAB. It is clearly seen from Table 3 that CTAB has not been shown to be aerobically biodegradable (Swisher, 1970). From Table 3, Continuous-flow activated sludge system (CAS) tests (Swisher, 1987) show that CTAB is found to be easily aerobically biodegradable by these tests. Information on the anaerobic biodegradation of CTAB is scarce (Karsa and Porter, 1995). An anaerobic screening test was carried out by Battersby and Wilson (1989), where 200 mg/L (0.55 mM) of CTAB was anaerobically digested and was tested for its biodegradability by measurement of the total net gas production (NGP). CTAB was found to inhibit the methane production of the sludge used as the inoculum. Positive NGP was observed only after the first two weeks. No NGP for the first two weeks suggested that some adaptation of sludge with CTAB or a detoxification process had occurred.

Anaerobic test methodology

The anaerobic test methodology used in the current study followed the protocol developed by Young and Khandaker (1995). Young and Khandaker (1995) used this anaerobic treatability screening protocol to determine the feasibility of using anaerobic processes for treating specific industrial wastes, including those from food processing, chemical production, petroleum, and landfill leachate. The anaerobic treatability protocol consists of operating small laboratory test reactors for sufficient periods of time. Treatability was judged based on assessment of the rate and extent of biodegradation, identification of the presence of toxic substances, and dilution effects (Young and

Khandaker, 1995). A good agreement of gas production from test reactors with that from a control reactor indicated a good potential for using anaerobic processes to treat these wastewaters. The following section discusses more about the Young and Khandaker (1995) testing protocol further.

The testing protocol developed by Young and Khandaker (1995) consisted of two phases. Phase I consists of conducting tests using laboratory-scale batch serum bottles, while Phase II involved the use of semi-continuous bench scale reactors. Batch serum tests provided an indication of the response of the test culture to a single batch dose of a test waste (Young and Khandaker, 1995). Waste samples and control substrates mixed in a nutrient/mineral/buffer (NMB) medium, as given in Table 5, were injected in the batch serum reactors containing the culture. The gas produced upon degradation was then measured by manual means or by using an anaerobic respirometer. Phase II bench-scale semi-continuous reactor tests were conducted to determine responses of test cultures to long-term feeding of taste wastes. Various parameters measured in these experiments include pH, chemical oxygen demand (COD), sulfate content, and total volatile suspended solids. Typical results of the laboratory analysis of wastewater from a food processing industry as measured by Young and Khandaker (1995) are shown in Table 4. Master Culture Reactors (MCRs) were used to provide seed cultures having identifiable and repeatable characteristics. These MCRs typically were maintained in 8-12 L glass vessels and were operated under the following conditions (Young and Khandaker, 1995):

1. Feedstock solution of 20,000 mg/L ethanol COD in NMB medium,
2. Temperature of 35°C,
3. Hydraulic and solids retention times of 20 days, and

Table 3. Biodegradation of CTAB surfactant (Swisher, 1970; and Swisher, 1987)

Method	Time	Percent biodegraded
BOD	5 days	0
CAS*	8 hours	100
BOD	5 days	0
CAS*	-	98-99
CAS*	-	91-98

* - Continuous-flow activated sludge system

Table 4. Results of laboratory analysis of wastewater from a Food Processing Industry (Young and Khandaker, 1995)

Test parameter	Value
COD	26,235 mg/L
pH	7
TSS	278 mg/L
VSS	224 mg/L
Sulfate	1300 mg/L
Organic constituents	Sugars, alcohols, organic acids and proteins

Table 5. Formulation of Nutrient/Mineral/Buffer Stock solution (Young, 1995)

1. Mineral Base I

   The following chemicals were added to 800 ml of distilled water and diluted to 1 L.

$CoCl_2.6H_2O$	0.25 g	$NaMoO_4.2H_2O$	0.025 g
$FeCl_2.4H_2O$	2.0 g	$NiCl_2.6H_2O$	0.025 g
$MnCl_2.4H_2O$	0.05 g	Na_2SeO_4	0.025 g
H_3BO_3	0.025 g	$CuCl_2$	0.025 g
$ZnCl_2$	0.025 g		

2. Mineral Base II

   The following chemicals were added to 800 ml of distilled water and diluted to 1 L.

$CaCl_2$	38 g
$MgCl_2.6H_2O$	50 g

3. Nutrient Base

   The following chemicals were added to 800 ml of distilled water and diluted to 1 L.

KH_2PO_4	135 g
K_2HPO_4	175 g
NH_4Cl	53 g
Na_2SO_4	15 g

4. Buffer Base

   60 g of $NaHCO_3$ was added to 800 ml of distilled water and diluted to 1 L.

Nutrient / Mineral / Buffer Stock Solution

100 ml each of Mineral Base I, Mineral Base II and Nutrient Base plus 1 L of Buffer Base were added to 8 L of distilled water and diluted to 10 L.

4. A COD loading rate of 1.0 g/L-d.

The test protocol as seen provides a very convenient and low-cost means of determining the feasibility of using anaerobic processes for treating wastes.

Summary

This chapter has given an introduction about surfactants, their uses, the importance of surfactant biodegradability, its mechanism and current studies done on surfactants. The chapter emphasizes the importance of surfactant properties such as surfactant structure and Critical Micelle Concentration (CMC) on its degradation. The chapter also reviews the current studies done on biodegradation of cationic surfactants. These studies indicate the potential for surfactant degradation but also demonstrate the limited information available on anyone, specific surfactant. Clearly, additional studies are needed to predict the environmental fate of the surfactants of interest.

CHAPTER III

MATERIALS AND METHODS

This chapter discusses the various materials and methods that were used in this study. The methods include anaerobic tests, aerobic tests and the various analytical methods used in the study.

Materials

Chemicals

The various chemicals needed include the chemicals to prepare the Nutrient / Mineral / Buffer (NMB) stock solution and are listed in Table 5. These chemicals were purchased from Fisher Chemicals (Pittsburgh, PA). Deionized water was used for the NMB stock solution and feedstock preparations. Milli-Q water ($\geq 18\Omega$.cm) produced by a Milli-Q purification system (Millipore Co., Molsheim, France) via deionization and reverse osmosis was used for all chemical analyses, standard preparation and sample treatment. All other reagents needed for analysis were also purchased from Fisher Chemicals and were used as delivered.

Tested Samples

The tested samples include aqueous solutions of three pure surfactants, namely, Varonic T-205 (Witco Corporation, NY) , Witconol SN-70 (Witco Corporation, NY) and CTAB (Sigma Chemical, St. Louis, MO) and three surfactant-ink mixtures, namely, Varonic T-

205-ink mixture (Water-based ink and T-205), Witconol SN-70- ink mixture (Water-based ink and SN-70) and CTAB-ink mixture (Solvent-based ink and CTAB). Table 6 gives the properties of these surfactants tested. Table 7 gives the tested surfactant's type and chemical composition. The surfactant-ink mixtures were effluents of bench-scale de-inking studies performed at the Institute for Applied Surfactant Research (IASR), University of Oklahoma, Norman. The surfactant-ink mixtures consisted of the 5 mM of surfactant, ink residues (removed from plastic by the surfactant) and phosphate buffers. The concentrations of ink in the surfactant-ink mixtures were not known. The pH for the Varonic T-205-ink mixture and Witconol SN-70-ink mixture was 10 and for the CTAB-ink mixture was 12 when received from IASR.

Methods

Anaerobic Tests

The anaerobic tests were performed as screening tests to determine the anaerobic degradability of the surfactants and the surfactant-ink mixtures. The tests followed the Phase I testing protocol developed by Young and Khandaker (1995). Anaerobic degradability under methanogenic conditions was determined by measurement of the volume of cumulative gas (CH_4 and CO_2) production and comparison to controls. Such gas production measurement has been proposed to be used as convenient screening test for assessing anaerobic degradation potential of organic chemicals under methanogenic conditions (Gledhill, 1979; and Shelton and Tiedje, 1984). Specific anaerobic tests performed are described below.

Table 6. Properties of the surfactants tested

Surfactant	Chemical formula	Molecular weight, gm/mole	Density (measured), gm/L	CMC, mM (Gecol et al., 1996)
CTAB	$C_{16}H_{33}N(CH_3)_3Br$	364.5	-	0.98
SN-70	Proprietary chemical	387	1128.4	-
T-205	Proprietary chemical	500	1014	-

Note: "-" indicates data are not available

Table 7. Surfactant's type and chemical composition

Surfactant	Type	Chemical composition
CTAB	Cationic surfactant	Hexadecyl trimethyl ammonium bromide
SN-70	Nonionic surfactant	Ethoxylated alcohol with ethoxylation number* of 5
T-205	Nonionic surfactant	Ethoxylated primary tallow amine with ethoxylation number* of 5

* Note: Ethoxylation number denotes the number of ethylene oxide units

Setting up of the Master Culture Reactor

A reactor called the Master Culture Reactor (MCR) was setup and was subjected to anaerobic conditions. Samples were then taken from this MCR to act as controls and to serve as the seed culture for testing the anaerobic degradability of the surfactants. A Nutrient / Mineral / Buffer (NMB) stock solution was prepared according to Young and Khandaker (1995). Table 5 gives the formulation of the NMB medium. To the prepared 10 L NMB stock solution, 1 L of anaerobic digester sample obtained from the Stillwater Wastewater Treatment Plant (Stillwater, OK) was added to seed the reactor. The pH of the master culture was maintained around 7 by adding buffer solution as needed (100 g/L dipotassium hydrogen phosphate solution). The master culture was kept mixed so that the culture withdrawn for tests would be well mixed and a representative sample. To make sure that glucose was the primary substrate for gas production in the reactors, samples from the MCR were setup without glucose in them and the gas production was monitored (using 5 cc syringes) for a week. There was no gas production noticed in the reactors.

Feedstock formulation

A feedstock solution of 100 g/L COD glucose in 10 L NMB medium was prepared. A glucose COD of 1 g/L was added to the Master Culture Reactor every day for one week and was added on alternate days after that.

Setup of batch serum reactors

Tables 8 and 9 give the details of the tests performed on surfactants and surfactant-ink mixtures, respectively. The tests were carried out at room temperature (25°C). The batch serum bottles were sealed with Teflon-lined rubber septa and standard seals (Supelco Corporation, Bellefonte, PA). The concentrations of surfactants tested covered a range above and below the concentrations expected in the effluents of the de-inking studies performed at the IASR, University of Oklahoma, Norman. In this way, the concentration at which inhibition of gas production occurs could be determined. Glucose was used as the substrate and was used to initiate the anaerobic reaction. As shown, glucose was occasionally re-spiked in some of the reactors as a means of testing for acclimation by the cultures to the presence of the surfactant. The bottles were well mixed and the gas production was measured with the use of 5 cc syringes.

Aerobic Tests

Aerobic tests served as toxicity screening tests for determining aerobic toxicity and/ or potential biodegradability of surfactant and surfactant-ink mixtures. Bench-scale BOD bottles were setup to test the aerobic toxicity of surfactants and surfactant-ink mixtures. The same surfactants and surfactant-ink mixtures tested for anaerobic tests were used for aerobic tests. The effects of increasing concentrations of surfactants and surfactant-ink mixtures on their toxicity were studied. Table 10 lists the variables tested for the various surfactants and surfactant-ink mixtures. The surfactants concentrations selected were around their presumed toxicity threshold concentrations.

Table 8. Details of anaerobic tests performed on surfactants

Type	Test number*	Conc. tested	Days tested	Glucose added
CTAB surfactant	Test # 1	0.05 mM – 1.0 mM	0 – 35 days	300 mg/ L on Day 0 and 300 mg/L on Day 7.
	Test # 2	0.05 mM – 1.0 mM	0 – 80 days	600 mg/L on Day 0 and 200 mg/L on Day 22.
	Test # 3	0.05 mM – 1.0 mM	80 – 145 days	300 mg/L on Day 80.
	Test # 4	0.05 mM – 1.0 mM	0 – 45 days	1000 mg/L on Day 0.
SN-70 surfactant	Test # 5	2.09 mM – 41.8 mM	0 – 35 days	300 mg/ L on Day 0 and 300 mg/L on Day 7.
	Test # 6	2.09 mM – 41.8 mM	0 – 80 days	600 mg/L on Day 0 and 200 mg/L on Day 22.
	Test # 7	0.05 mM – 1.0 mM	0 – 80 days	300 mg/L on Day 0 and 200 mg/L on Day 22.
T-205 surfactant	Test # 8	1.45 mM – 29.0 mM	0 – 35 days	300 mg/ L on Day 0 and 300 mg/L on Day 7.
	Test # 9	1.45 mM – 29.0 mM	0 – 80 days	600 mg/L on Day 0 and 200 mg/L on Day 22.
	Test # 10	1.45 mM – 29.0 mM	0 – 80 days	300 mg/L on Day 0 and 200 mg/L on Day 22.

* - For each of the tests performed, batch serum bottles with the culture and same amount of glucose as the tests were set up as the controls.

Table 9. Details of anaerobic tests performed on surfactant-ink mixtures

Type	Test number*	Conc. tested	Days tested	Glucose added
CTAB surfactant-ink mixture	Test # 11	0.07 mM – 1.43 mM	0 – 65 days	200 mg/ L on Day 0 and 200 mg/L on Day 18.
	Test # 12	0.07 mM – 1.43 mM	65 - 135 days	200 mg/L on Day 65.
	Test # 13	0.05 mM – 1.0 mM	0 – 45 days	1000 mg/L on Day 0.
SN-70 surfactant-ink mixture	Test # 14	0.07 mM -- 1.43 mM	0 – 65 days	200 mg/ L on Day 0 and 200 mg/L on Day 18.
	Test # 15	0.07 mM – 1.43 mM	65 - 135 days	200 mg/L on Day 65
T-205 surfactant-ink mixture	Test # 16	0.07 mM – 1.43 mM	0 – 65 days	200 mg/ L on Day 0 and 200 mg/L on Day 18.
	Test # 17	0.07 mM – 1.43 mM	65 - 135 days	200 mg/L on Day 65

* - For each of the tests performed, batch serum bottles with the culture and same amount of glucose as the tests were set up as the controls.

Table 10. Details of the aerobic tests performed on surfactants and surfactant-ink mixtures

Type	Test number*	Conc. Tested	Seed added
CTAB surfactant	Test # 1	1.18 uM	5 ml
	Test # 2	0.01, 0.1 and 1.0 uM	3 ml
SN-70 surfactant	Test # 3	4.87 uM	5 ml
	Test # 4	1.0, 10 and 100 uM	3 ml
T-205 surfactant	Test # 5	1.35 uM	5 ml
	Test # 6	0.01, 0.1 and 1.0 uM	3 ml
CTAB surfactant-ink mixture	Test # 7	0.33 uM	5 ml
	Test # 8	0.01, 0.1 and 1.0 uM	3 ml
SN-70 surfactant-ink mixture	Test # 9	0.33 uM	5 ml
	Test # 10	0.01, 0.1, 1.0 uM	3 ml
T-205 surfactant-ink mixture	Test # 11	0.83 uM	5 ml
	Test # 12	0.01, 0.1 and 1.0 uM	3 ml

* - For the tests performed, seed controls were setup with distilled water and with the same amount of seed as the test samples.

BOD bottles of 300 ml were used for tests. A seed control was also setup with just the seed and distilled water to act as the base reactor. The seed was obtained from the Stillwater Wastewater Treatment Plant (Stillwater, OK). The samples and the controls were incubated in the 20°C incubator. The dissolved oxygen (D.O.) was then measured for all the samples and controls. The measurement was done until the DO's measured were constant values. The BOD's were then calculated for the samples and the variation of BOD's over time was plotted. The measured ultimate BOD's of the samples were then compared with the theoretical BOD values and the results interpreted. Negative or near-zero values of BOD of the samples indicated their toxic nature on the culture. BOD values of samples much higher than the controls indicated their potential aerobic biodegradability.

Analytical Methods

pH

A glass combination electrode in conjunction with an Accumet model 900pH meter from Fisher Scientific Co. (Pittsburgh, PA), was used for pH measurement. Standard buffer solutions from Hach Co. (Loveland, CO) with pH values of 4.0, 7.0 and 10.0 were used for calibrating the pH meter.

COD

Hach COD Digestion solution (0 – 1500 ppm range) and Hach High Range Plus COD reagent (0 – 15000 ppm range) containing $K_2Cr_2O_7$ in vials (16 mm) were used to digest

the tested samples. 0.2 mL of sample was digested in the High Range Plus COD reagents (0 - 15000 ppm) and 2 mL of sample was digested in the Digestion solution (0 – 1500 ppm range). A Hach COD reactor was used to heat the samples, at 250°C for 2 hours. When the vials came to room temperature, a Hach DR/3000 Spectrophotometer was used to measure the COD's. The spectrophotometer was calibrated at a wavelength of 620 nm. All the Hach products used were bought from Hach Company (Loveland, CO).

Dissolved Oxygen (D.O.)

The D.O. was measured using a YSI 5000 Dissolved Oxygen Meter (YSI Incorporated, Yellow Springs, Ohio). The YSI 5000 D.O. meter was initially calibrated to the oxygen solubility in distilled water, exposed to water-saturated air, at atmospheric pressure and room temperature. The D.O. measurement was useful in aerobic studies, performed on surfactant and surfactant-ink mixtures. The D.O. measurement by the membrane electrode method is based on the rate of diffusion of molecular oxygen across the membrane. A YSI Model 5905/5010 BOD probe (Yellow Springs, OH) was used for measuring dissolved oxygen. The self-stirring probe included an easily replaceable membrane cap and a refurbishable electrode system. When the probe was not in use, the probe was stored in a BOD bottle containing at least 1 inch of water.

CHAPTER IV

RESULTS AND DISCUSSION

This chapter discusses the results of the experiments described in the previous chapter, namely, the anaerobic and aerobic tests.

Anaerobic test results

Anaerobic tests involved measuring the gas production in the test samples and the controls. The gas production was measured by using 5 cc syringes. The cumulative gas production was then calculated and plotted against time for the test samples and the controls. The plotted data were mostly the average of the duplicates and the average variation was within 2 ml. The test samples were then said to be toxic if the cumulative gas production in them was less than the cumulative gas production in the controls (after accounting for the average variation (± 2 ml) in them). Because all reactors were fed identical amounts of glucose, which is readily fermentable, any gas production above the control's would be an indication of possible fermentation of the surfactant and/ or ink. Table 11 correlates the different tests (as shown in Tables 8 and 9) with their respective figures and tables. As noted, the raw data tables appear in the appendix. Analysis done on the control's gas production for various tests performed is discussed in the next section. In the later sections, the anaerobic test results for the surfactants and surfactant-ink mixtures are discussed.

Analysis of control data

The analysis of control data was necessary to check if the anaerobic tests performed were behaving in a normal and predictable manner. The analysis comprised checking if the cumulative gas production in the controls matched well with their theoretical gas productions. The theoretical gas production was determined by calculating the maximum volume of gas that could be produced by the fermentation of the glucose (added to the controls). The theoretical gas production was calculated using the method described by Sawyer et al. (1994). An example of the calculation of the theoretical gas production for a control setup for Test # 1 is given in Appendix C. The anaerobic tests were performed in batches, referred to here as test groups. For example, in Table 12, Tests # 1, 5, and 8 were performed together as a batch. Table 12 gives the list of test groups. Unique controls (at least two controls) were setup for each of these test groups. The average of measured gas production in the controls (for each of the test groups) is listed in Table 12. As can be seen, the percentage gas production (theoretical vs. measured) of the controls for all the test groups are very reasonable (within 20% of expected value) with the exception of the test groups (Tests # 3, 7, and 10) and (Tests # 11, 14, and 16). Even these two groups could be said to have behaved relatively well. For these two test groups, a smaller amount of glucose (300 mg/L for the test group (Tests # 3, 7, and 10)) was added to the controls when compared to the glucose added (600 mg/L for the test group (Tests # 1, 5, and 8)) to the controls of other test groups. The second reason is that it can be seen from Table 12 that the absolute differences between the measured gas productions and the theoretical gas productions for the controls of the two test groups are relatively small (approx. 6 ml).

Table 11. Respective figures and tables for the various anaerobic tests on surfactants and surfactant-ink mixtures

Type	Test number	Figure number	Table number
CTAB surfactant	Test # 1	Figure 1	Table A-1
	Test # 2	Figure 2	Table A-2
	Test # 3	Figure 3	Table A-3
	Test # 4	Figure 4	Table A-4
SN-70 surfactant	Test # 5	Figure 5	Table A-5
	Test # 6	Figure 6	Table A-6
	Test # 7	Figure 7	Table A-7
T-205 surfactant	Test # 8	Figure 8	Table A-8
	Test # 9	Figure 9	Table A-9
	Test # 10	Figure 10	Table A-10
CTAB-ink mixture	Test # 11	Figure 11, 12	Table A-11
	Test # 12	Figure 13	Table A-12
	Test # 13	Figure 14	Table A-13
SN-70-ink mixture	Test # 14	Figure 15, 16	Table A-14
	Test # 15	Figure 17	Table A-15
T-205-ink mixture	Test # 16	Figure 18, 19	Table A-16
	Test # 17	Figure 20	Table A-17

Table 12. Comparison of theoretical gas production with measured gas production for the controls setup for the different test groups

Test group	Theoretical gas production for the control, setup for the test group (25°C), ml	Measured cumulative gas production in the control, setup for the test group, ml	Percentage gas produced (%)
Tests # 1,5,8	26.6	24.8	93
Tests # 2,6,9	35.5	29.9	84
Tests # 3,7,10	13.3	7.6	57
Tests # 4, 13	44.3	41.0	93
Tests # 11,14,16	17.7	11.4	64
Tests # 12,15,17	8.9	7.0	79

One of the possible factors that could have affected the gas production somewhat is the temperature. The room temperature while these two tests were performed was closer to 18°C, which is less than the normal room temperature (25°C) and therefore could have affected the reaction kinetics. The other reason could be that the glucose added to the controls could have been slightly less than what was thought to be added to the controls, resulting in a lower gas production. Overall, all the control tests performed could be said to have behaved in a reasonable and predictable manner (within 20% of theoretical predictions).

Surfactant results

CTAB cationic surfactant

Four set of experiments were done with the CTAB surfactant. Table 11 gives the details of each of these tests performed. The result of Test # 1 is shown in Figure 1. The maximum cumulative gas production was observed in CTAB (0.05 mM) and the least cumulative gas production was observed in CTAB (1.0 mM), but compared to the blanks (or controls) all the CTAB samples produced significantly less cumulative gas. The results indicate the possibility of toxicity of CTAB at concentrations of 0.1 mM - 1.0 mM. The degree of toxicity therefore appears to be correlated with the initial CTAB concentration. The observation that the cumulative gas produced by CTAB (0.5 mM) was greater than the CTAB (0.1 mM) could be just an anomaly. As can be seen in Figure 1, the CTAB (0.05 mM) and CTAB (0.1 mM) started producing greater gas volumes after 14 days, suggesting the possibility of gradual acclimation of culture to the surfactant

samples. Test # 2 was carried out to double check for this lag period occurring in the CTAB samples. Test # 2 was comprised of a new set of samples and controls. Test # 2 results, seen in Figure 2, indicate the same trends as Test # 1. It is also seen in Figure 2 that the CTAB samples (0.05 mM, 0.1 mM and 1.0 mM) started producing more gas after a lag period of 27 days which again suggests the possibility of acclimation of the culture to the surfactant samples. Again in this case, the CTAB at concentration's ranging from 0.5 mM - 1.0 mM, was found toxic to the culture.

Check for the possibility of acclimation of the CTAB samples

Test # 3 was a continuation to Test # 2 and its result is shown in Figure 3. Test # 3 used the same culture as in Test # 2 and was re-spiked with 300 mg/L of glucose on the 80th day. Test # 3 was conducted to test the effects of acclimation in the Test # 2 experiment. It is seen that CTAB (0.05 mM) had produced more cumulative gas compared to the blanks, suggesting that acclimation was complete at concentrations of 0.05 mM. The lag periods for the CTAB (0.05 mM) and CTAB (0.1 mM) decreased considerably in Test # 3 compared to Test # 2, as seen from the comparison of figures for these tests, clearly indicating a sign of acclimation. Again, the CTAB is seen to be toxic at the higher concentration range of 0.5 – 1.0 mM. Even though Test # 3 behaved in a reasonable manner, as seen from Table 12, the percentage gas production for the control for Test # 3 was comparatively lower than other tests like Test # 1. However, the absolute difference between the measured gas production and the theoretical gas production controls is small (within 6 ml).

Check for initial and final COD's in the CTAB samples

Test # 4 was performed to check the initial and final COD's in the CTAB samples. Its gas production results are shown in Figure 4. New test samples and controls were setup for this test. The samples were initially acclimated with 100 mg/L of glucose for 30 days and later 1.0 g/L of glucose was added. Therefore, the lag period for the test samples in Test # 4 is found to be less than Test # 2 and Test # 1. As is seen from Test # 1 and Test # 2, CTAB (0.05 mM) has produced the maximum gas production, but is still less than the gas production in the controls. At higher concentrations, CTAB (0.5 mM – 1.0 mM), CTAB is again found to be toxic. Table 13 compares the initial and final COD's for Test # 4. In Table 13, the expected final COD values (assuming 100% oxidation of added glucose and CTAB) for the controls (1123 mg/L) match very well with the measured final COD values (1150 mg/L), indicating that the COD tests were behaving in a reasonable fashion. Table 13 indicates that the COD lost is very small at CTAB concentrations of 0.5 mM – 1.0 mM, which clearly indicates the toxicity of CTAB at these concentrations. From Table 13, for CTAB (0.05 mM), the measured final COD value (1160 mg/L) matches very well with the expected final COD value (1157 mg/L). This strongly indicates that not only is the glucose degrading, but CTAB is also likely to be degrading in the samples. Therefore, the evidence from COD result matches very well with the cumulative gas production test results. Table 14 compares the mass of COD lost and the cumulative gas production for the test samples for Test # 4.

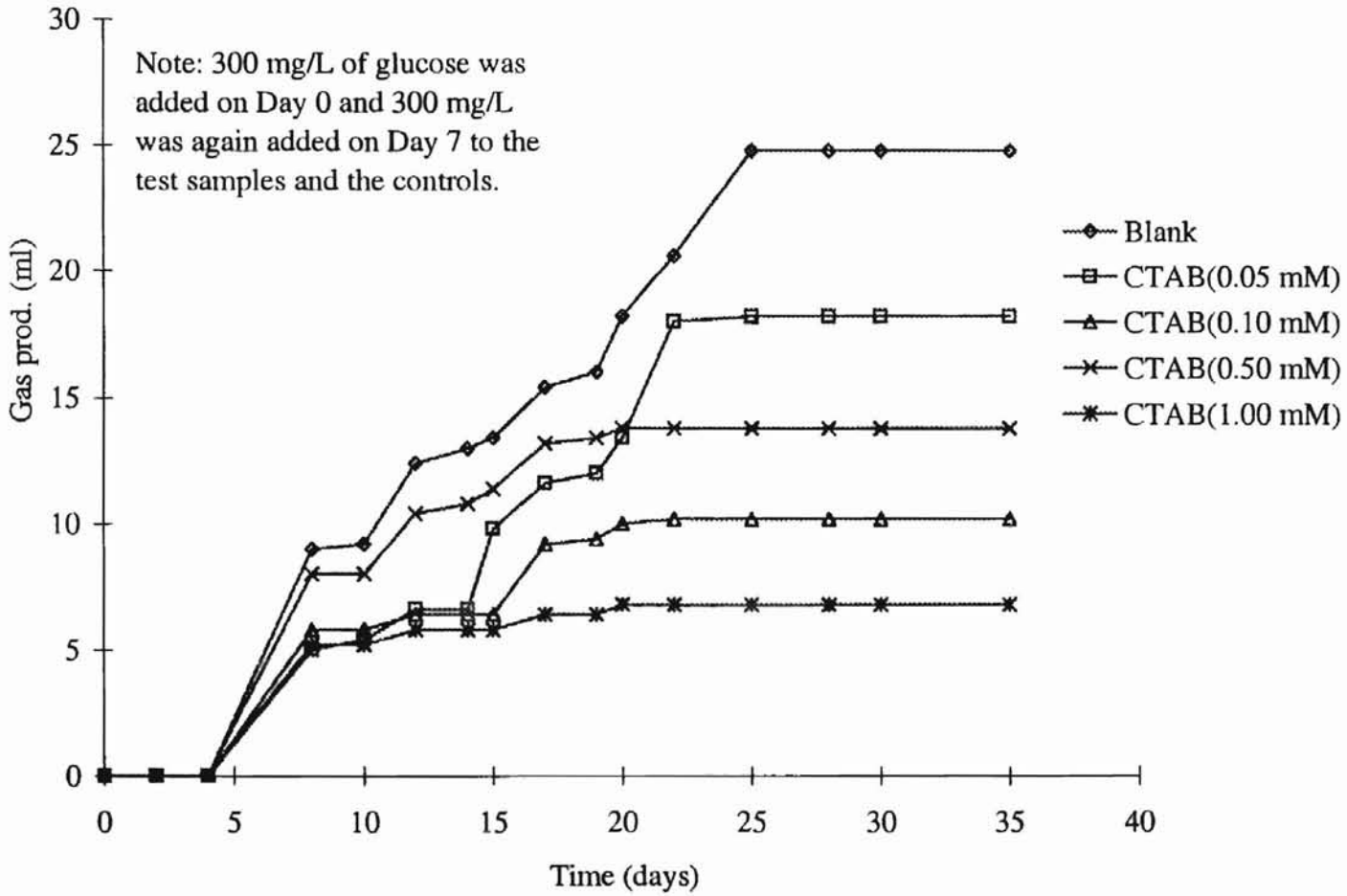

Figure 1. Comparison of the anaerobic biodegradation of different concentrations of CTAB surfactant - Test # 1

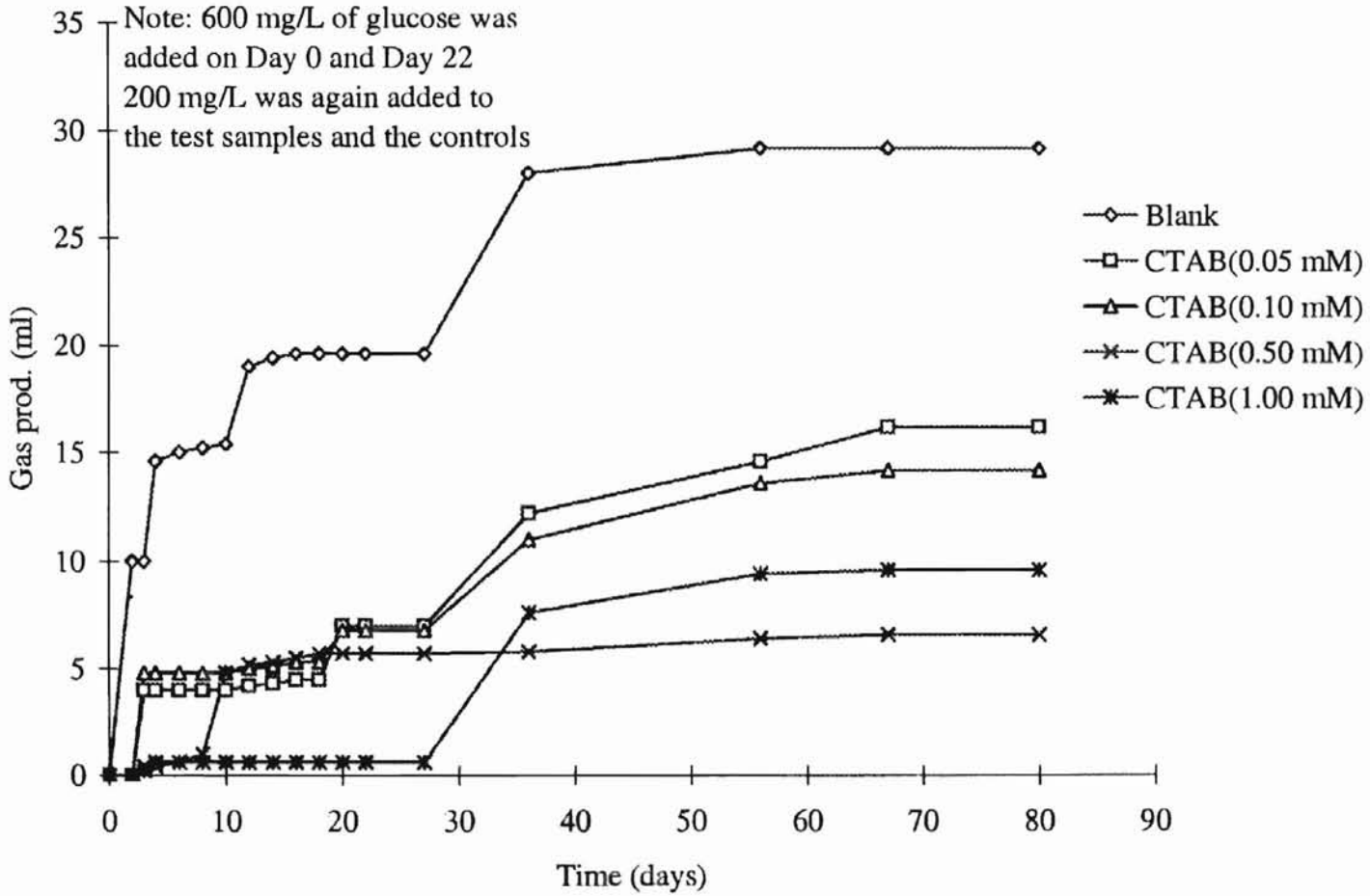

Figure 2. Comparison of the anaerobic biodegradation of different concentrations of CTAB surfactant - Test # 2

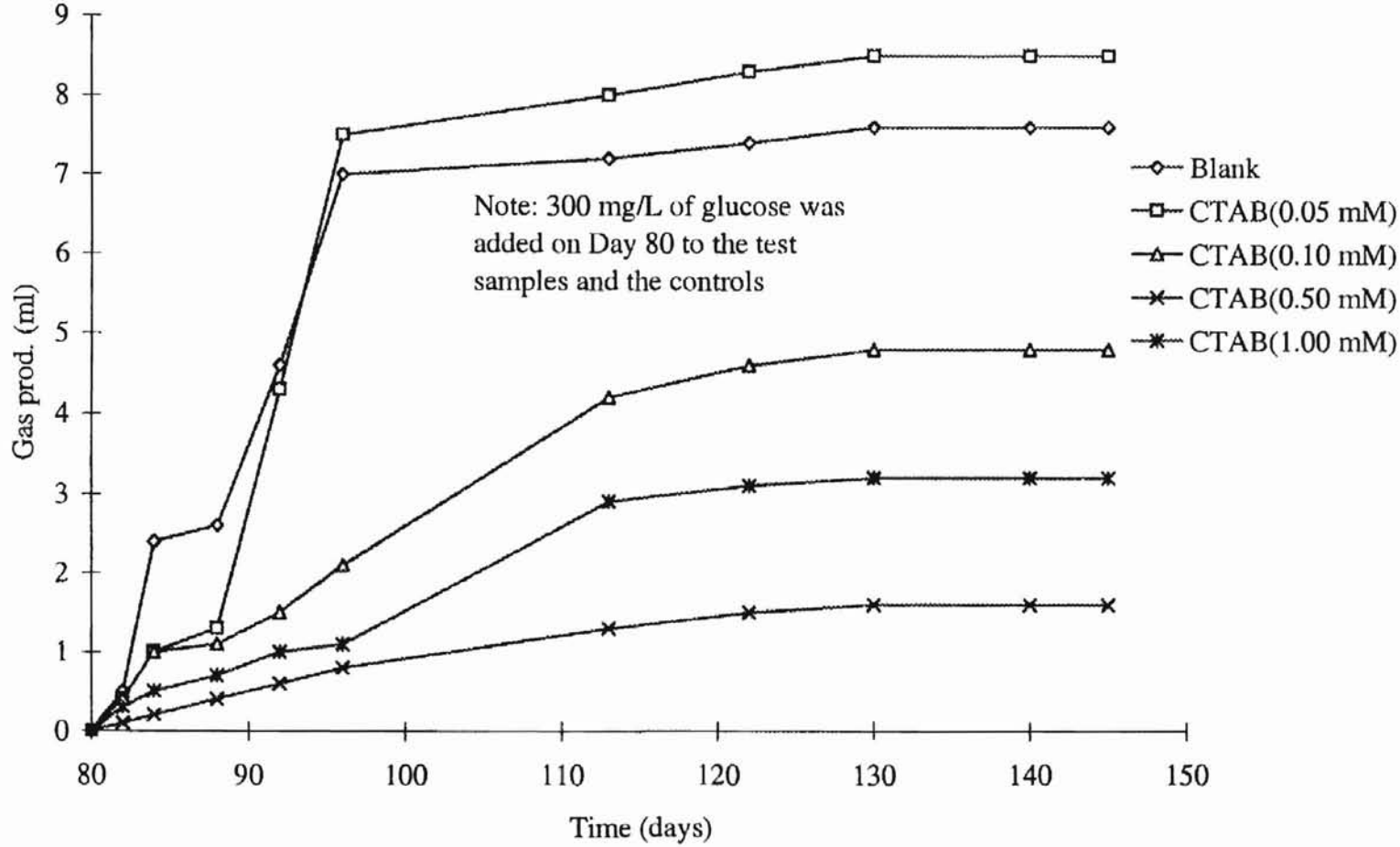

Figure 3. Comparison of the anaerobic biodegradation of different concentrations of CTAB surfactant - Test # 3

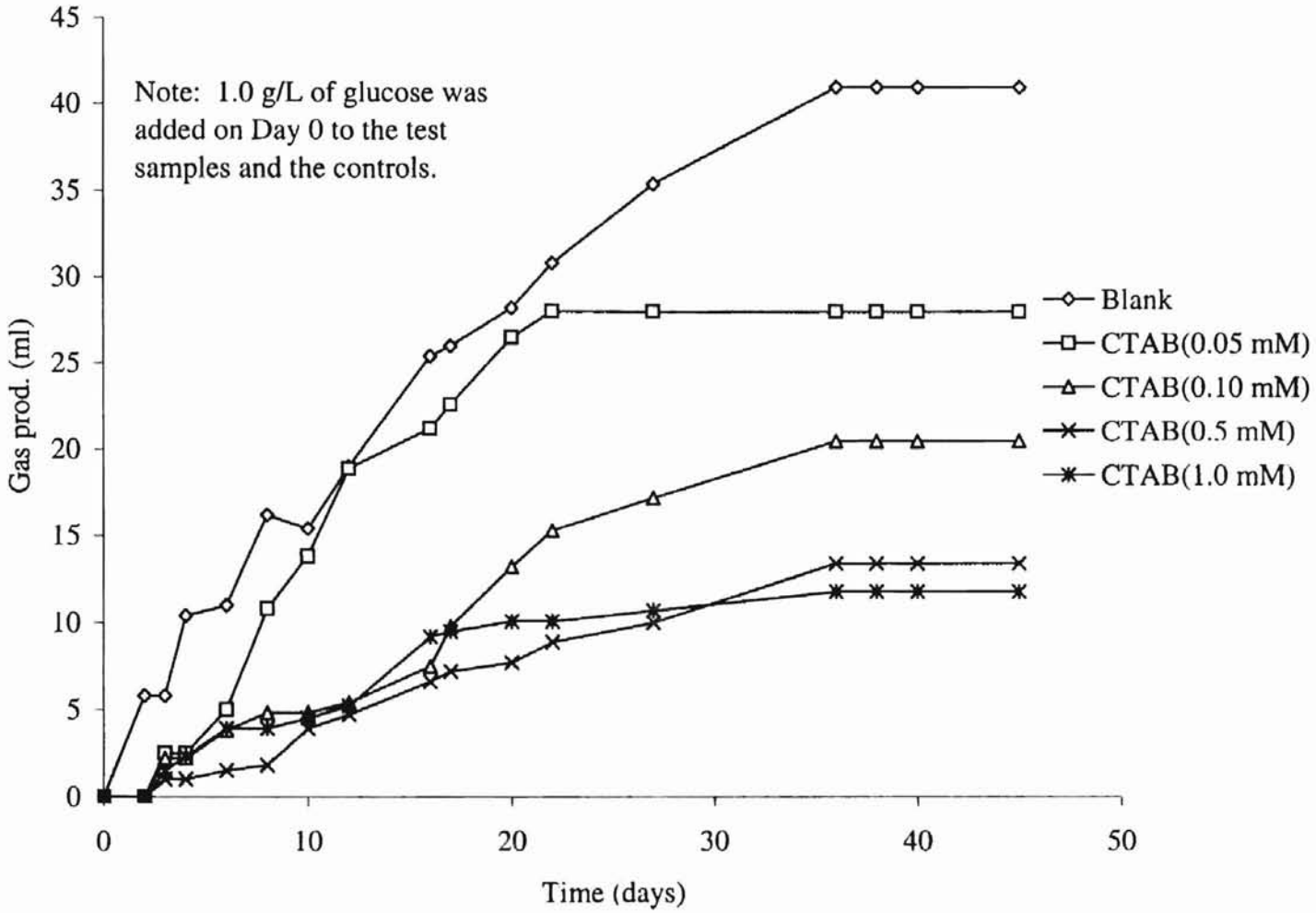

Figure 4. Comparison of the anaerobic biodegradation of different concentrations of CTAB surfactant - Test # 4

Table 13. Comparison of initial and final COD's for Test # 4 and Test # 13

Test Sample	Initial COD (mg/L) (1)	Expected COD drop (mg/L)* (2)	Expected final COD (mg/L) (3) = (1) – (2)	Measured final COD (mg/L) (4)
Blank (Test Control)	2300	1177	1123	1150
CTAB				
0.05 mM	2380	1223	1157	1160
0.1 mM	2450	1269	1181	1700
0.5 mM	2750	1637	1113	2480
1.0 mM	3200	2097	1103	2900
CTAB-ink mixture				
0.05 mM	2500	1223	1277	1080
0.5 mM	3550	1637	1913	2770
1.0 mM	4200	2097	2103	4000

* Note:

1) Expected COD drop includes 1177 mg/L glucose COD (1100 mg/L glucose) and the respective CTAB COD's added to the test samples
2) CTAB (0.05 mM) corresponds to 46 mg/L CTAB COD

Table 14. Comparison of mass COD lost with the cumulative gas production for Test # 4 and Test # 13

Test Sample	Mass COD lost, mg	Cumulative gas production, mL	Ratio (mL gas/ mg COD) *
Blank	70	41.0	0.58
CTAB			
0.05 mM	56.7	28.0	0.49
0.1 mM	42.0	20.5	0.49
0.5 mM	15.4	13.4	0.87
1.0 mM	10.5	11.8	1.12
CTAB-ink mixture			
0.05 mM	92.4	38.5	0.42
0.5 mM	54.6	19.9	0.36
1.0 mM	14.0	7.8	0.56

* Note: The theoretical (mL/mg COD) for the test control at 25°C is 0.63

From Table 14, it is seen that the theoretical mL gas/mg COD (at 25 °C) for the control (0.63) matches well with the measured ml gas/ mg COD of all the test samples and controls, which again clearly indicates that the Test # 4 was behaving in a reasonable and predictable manner.

Summary of CTAB anaerobic tests

From the four sets of tests performed on CTAB, it was found that toxicity in the range of concentrations 0.5 mM – 1.0 mM occurred. As seen from Test # 3, CTAB (0.05 mM) has produced more gas when compared to the controls (likely due to acclimation of culture to the CTAB). COD tests performed in Test # 4 confirm the degradability results of CTAB (0.05 mM). From Table 13, the expected final COD for CTAB (0.05 mM) is 1157 mg/L which matches very well with the measured final COD for CTAB (0.05 mM) (1160 mg/L).

SN-70 and T-205 nonionic surfactants

Tests # 5, 6 and 7 correspond to the SN-70 anaerobic tests and their results are shown in Figures 5, 6 and 7 respectively. Test # 5 was performed to test the SN-70 anaerobic biodegradation in the concentration range 2.09 mM – 41.8 mM. As can be seen from Figure 5, the SN-70 samples have not produced any significant gas compared to the controls. SN-70 is therefore found to be toxic to the culture. Test # 6 was performed to recheck for the anaerobic toxicity of SN-70 at the same concentration range. New samples and controls were setup for Test # 6. The results of Test # 6, as seen from Figure 6, again indicates that SN-70 is anaerobically toxic to the culture in the concentration

range 2.09 mM – 41.8 mM. Test # 7 was performed to determine the SN-70 degradability at a lower concentration range of 0.05 mM – 1.0 mM. The results of Test # 7, as seen in Figure 7, indicate that SN-70 is anaerobically toxic to the culture at these concentrations (0.05 mM – 1.0 mM) as well. Even though the results of Test # 7 are reasonable as mentioned previously, the percentage gas produced in the control for the Test # 7 was somewhat low at 57 % (refer Table 12). Reasons for this have been discussed.

The gas production for Tests # 8, 9, and 10 correspond to T-205 anaerobic tests and their results shown in Figures 8, 9 and 10 respectively. Similar to SN-70, T-205 too is found to be toxic to the anaerobic culture. Test # 8 was to test T-205 anaerobic biodegradation in the concentration range 1.45 mM – 29.0 mM. In Figure 8, the cumulative gas produced in the T-205 test samples is insignificant when compared to the test controls. Test # 9 was performed to check the anaerobic toxicity of T-205 surfactant. New samples and controls were setup with the same concentrations 1.45 mM – 29.0 mM. T-205 is again found to be toxic, as can be seen in Figure 9. Test # 10 was performed to determine the degradability of T-205 in the concentration range (0.05 mM – 1.0 mM). Figure 10 indicates that T-205 (0.05 mM) is found to have produced gas comparable with the control. But at other concentrations (0.1 mM – 1.0 mM), T-205 is found to be toxic to the culture. It should be noted that, in Table 12, for Test # 10, the control had produced a percentage gas of 57 % but the absolute difference between the measured gas production and the theoretical gas production controls is small.

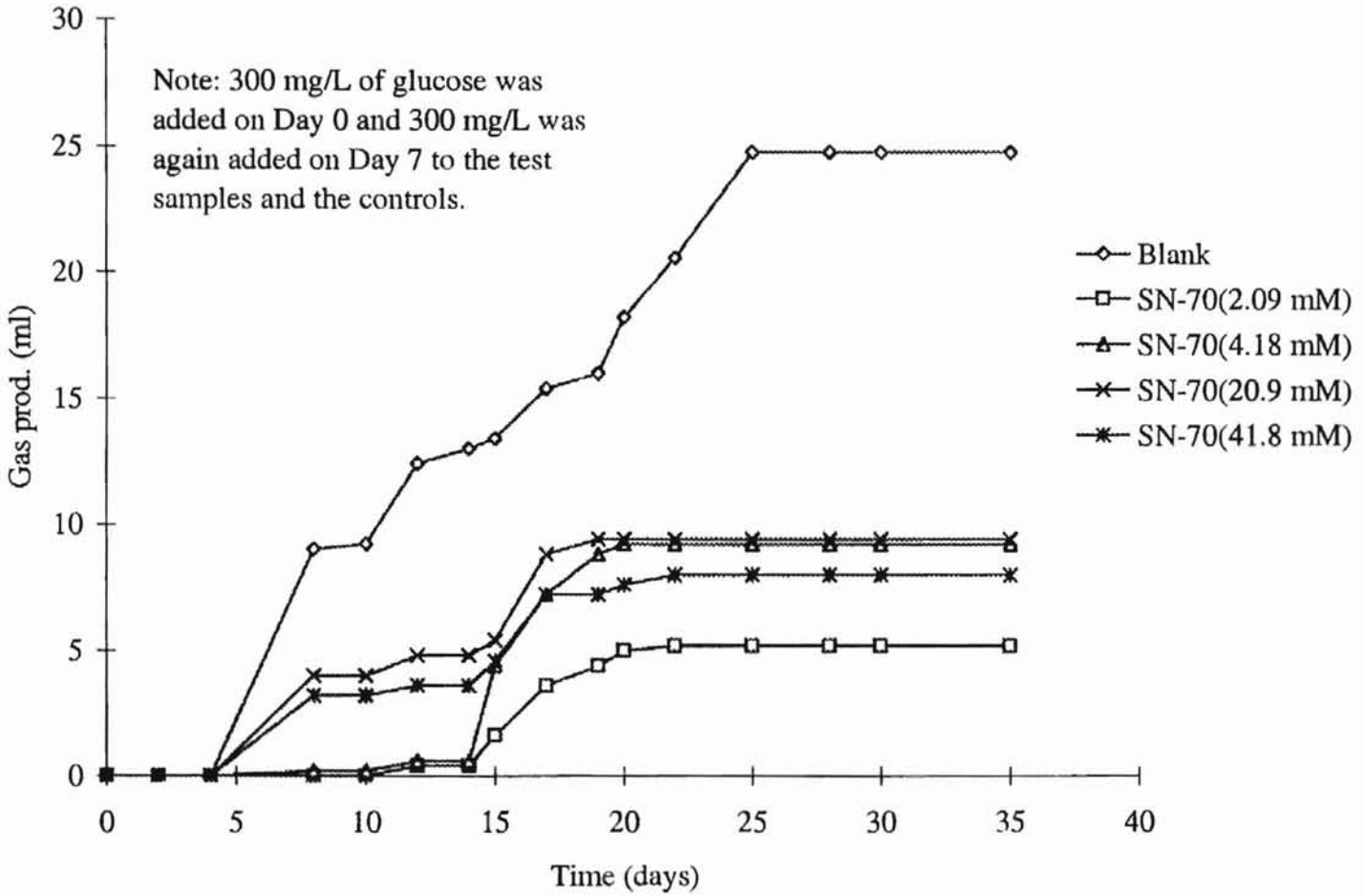

Figure 5. Comparison of the anaerobic biodegradation of different concentrations of SN-70 surfactant - Test # 5

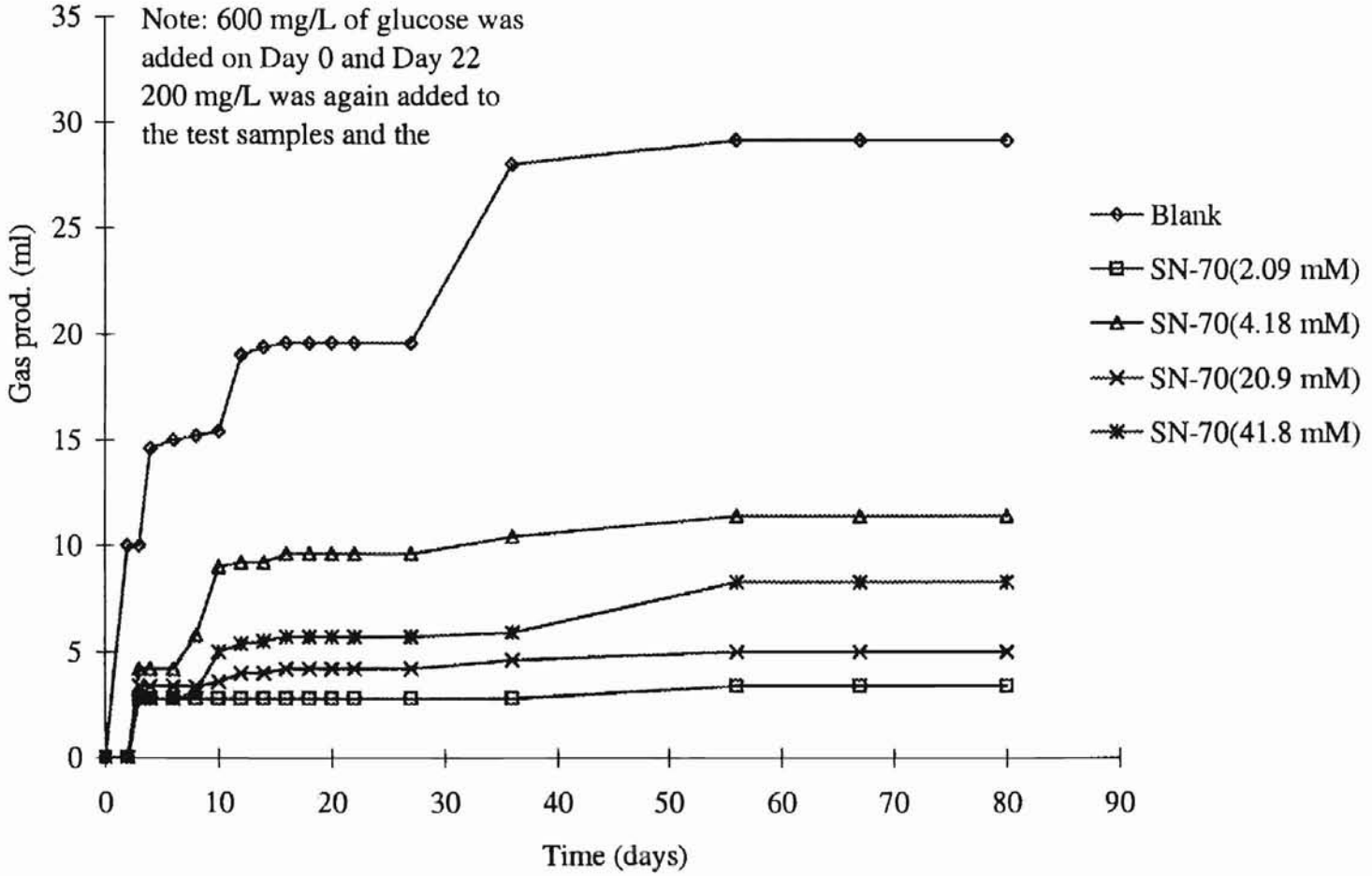

Figure 6. Comparison of the anaerobic biodegradation of different concentrations of SN-70 surfactant - Test # 6

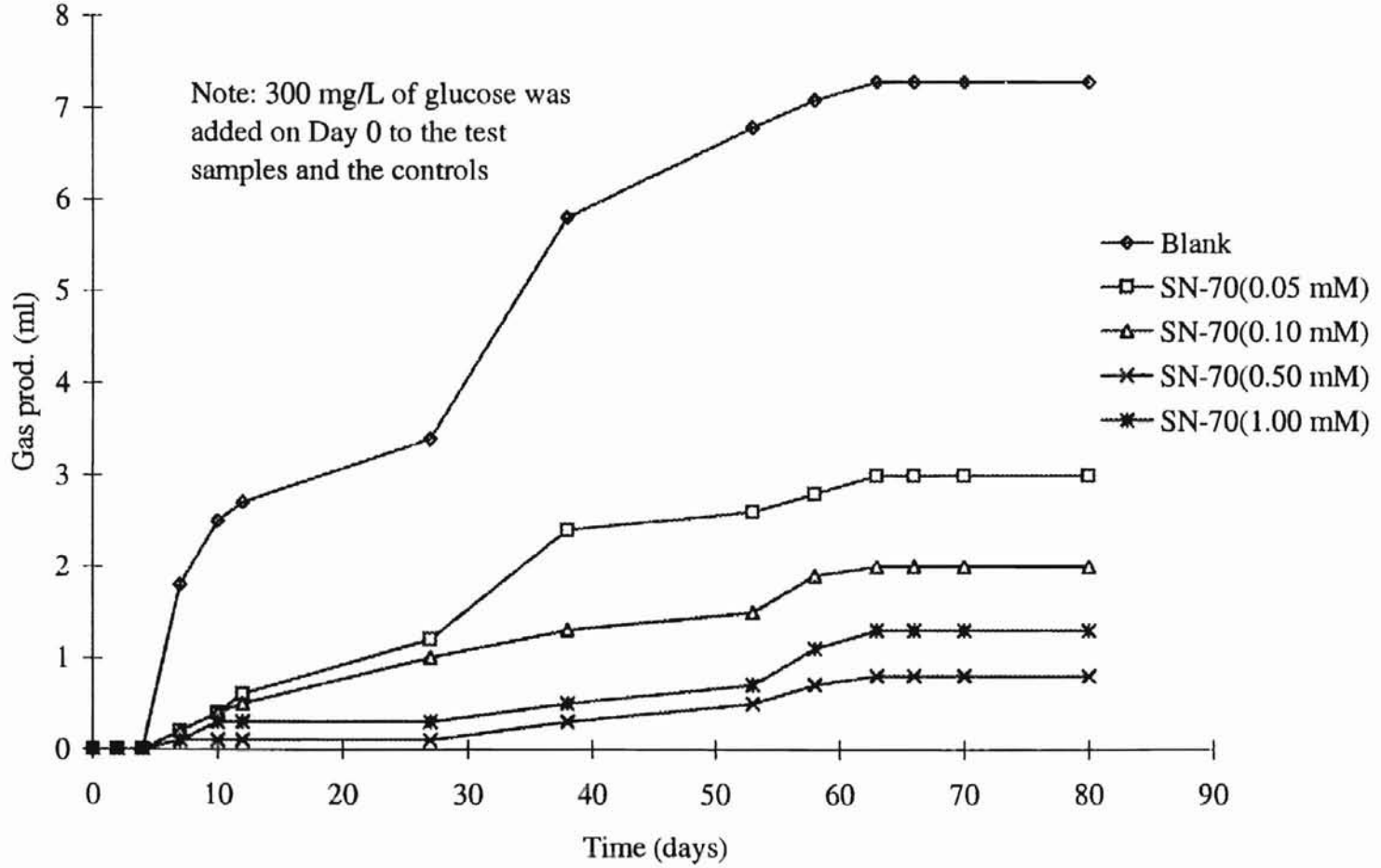

Figure 7. Comparison of the anaerobic biodegradation of different concentrations of SN-70 surfactant - Test # 7

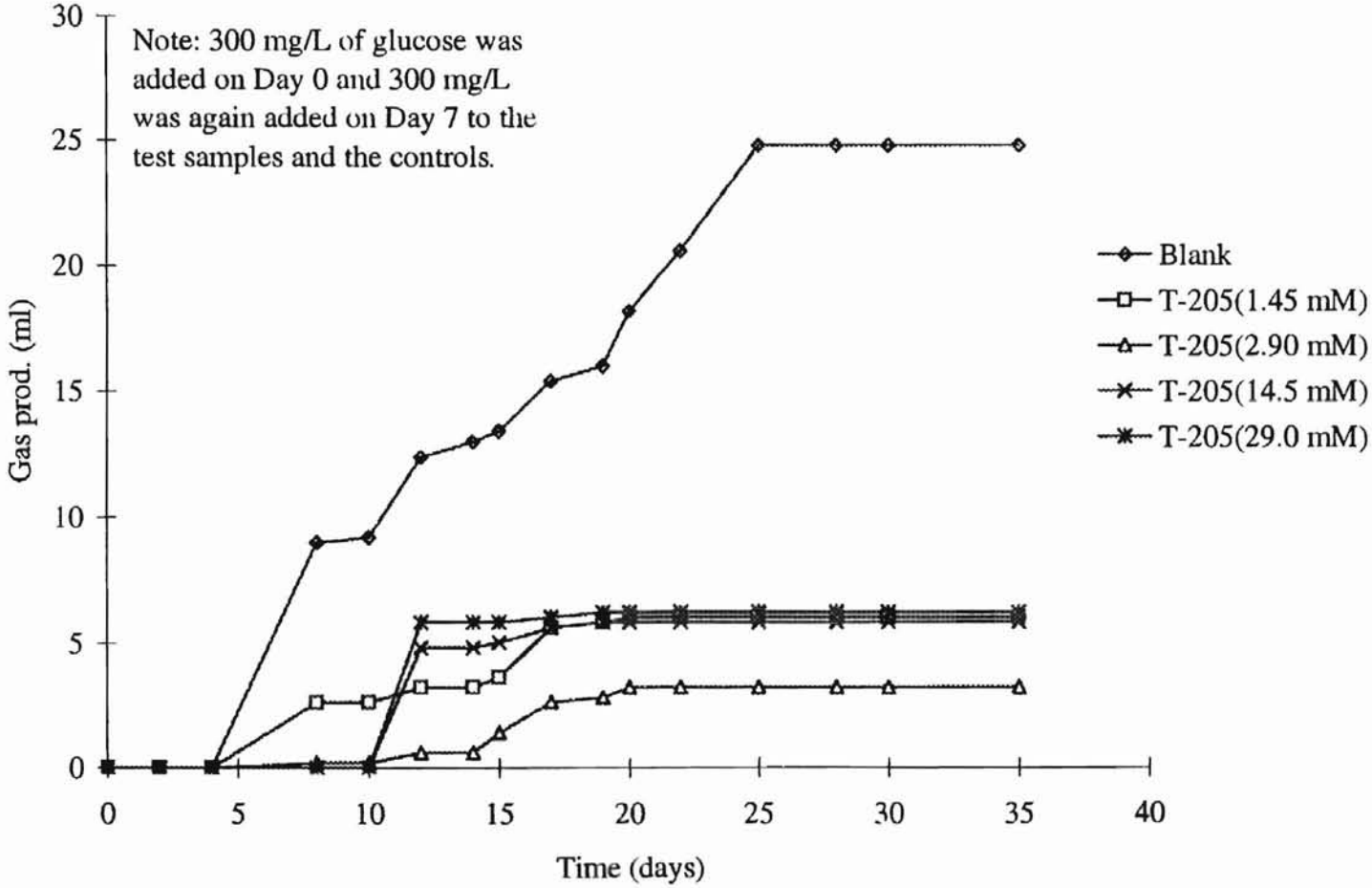

Figure 8. Comparison of the anaerobic biodegradation of different concentrations of T-205 surfactant - Test # 8

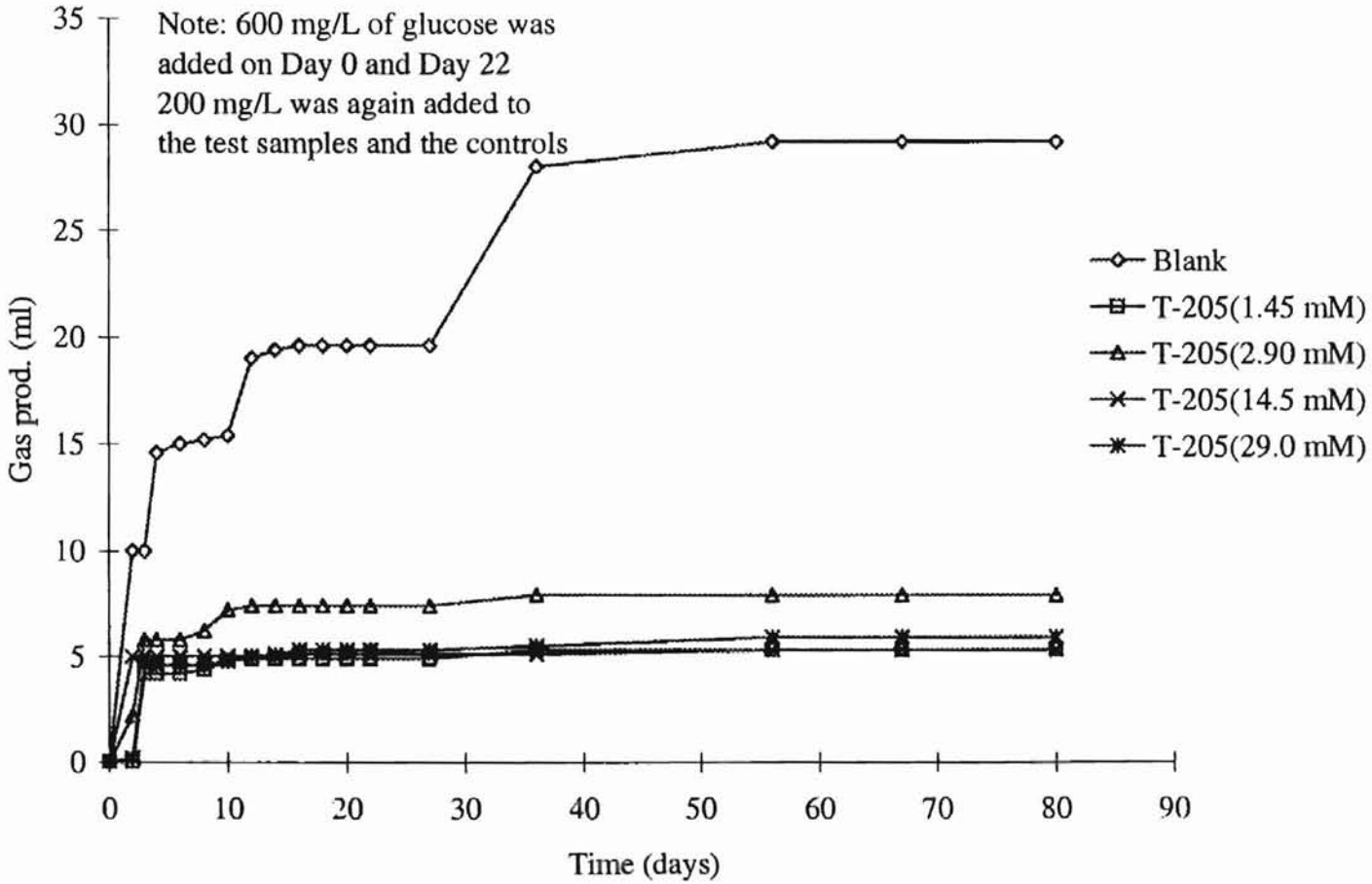

Figure 9. Comparison of the anaerobic biodegradation of different concentrations of T-205 surfactant - Test # 9

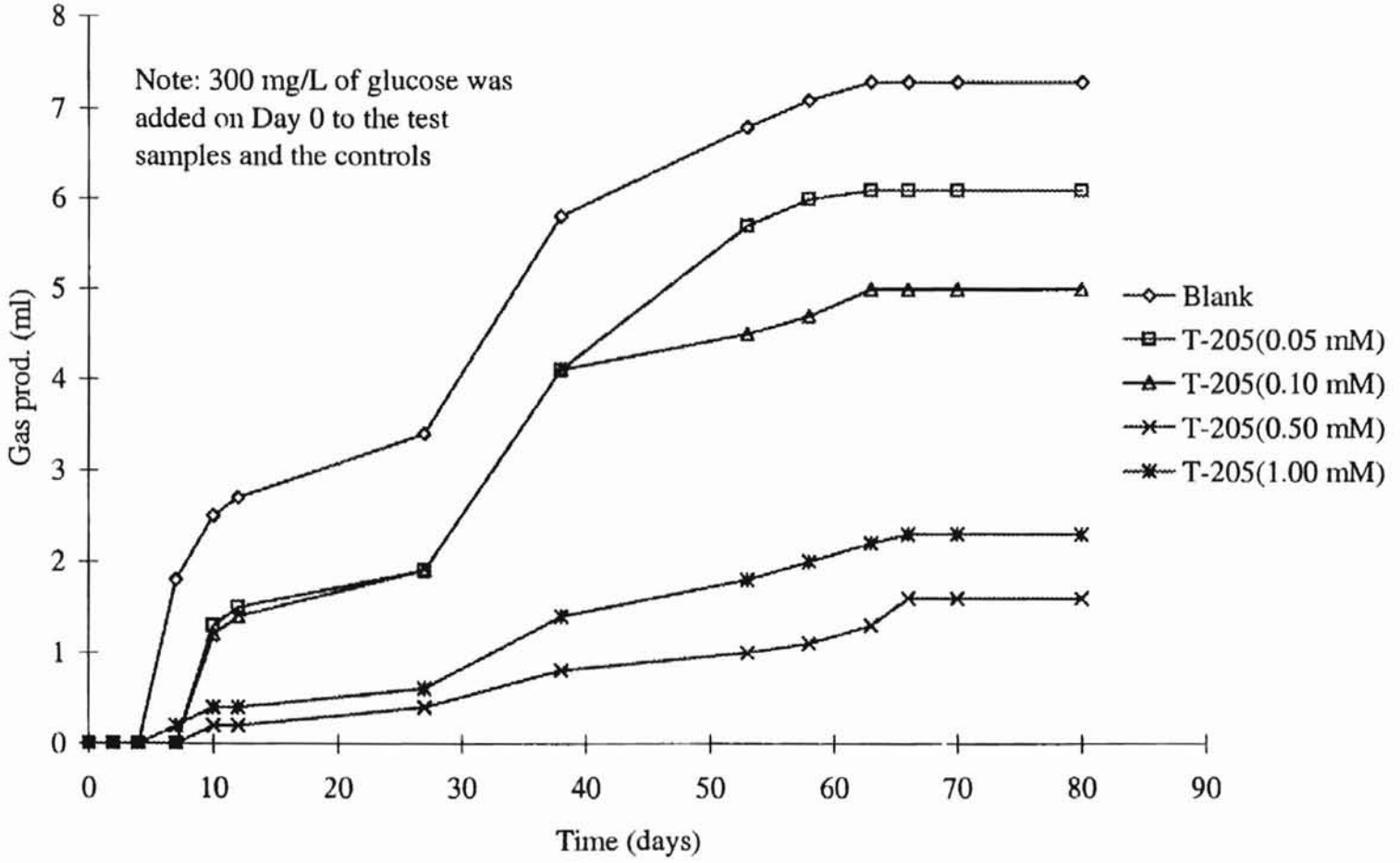

Figure 10. Comparison of the anaerobic biodegradation of different concentrations of T-205 surfactant - Test # 10

Summary of SN-70 and T-205 anaerobic tests

From Test # 5 and Test # 6, SN-70 is found to be toxic to the anaerobic culture in the range of concentrations (2.09 mM – 41.8 mM). From Test # 7, SN-70 is toxic to the culture even in the range of concentrations (0.05 mM – 1.0 mM).

From Test # 8 and Test # 9, T-205 is found to be toxic in the range of concentrations (1.45 mM – 29.0 mM). From Test # 10, T-205 is toxic to the culture in the range of (0.1 mM – 1.0 mM). T-205 (0.05 mM) is seen to have produced comparable gas as the controls. Tests can be performed in the future for testing anaerobic degradability of T-205 surfactant around 0.05 mM concentration.

Surfactant-ink mixture results

Surfactant-ink mixtures tested were products of the bench scale studies performed at the Institute for Applied Surfactant Research (IASR), University of Oklahoma, Norman. The surfactant-ink mixtures consisted of the 5 mM of surfactant, ink residues (removed from plastic by the surfactant) and phosphate buffers. The phosphate buffers present in the original samples were in lower concentration than buffers added to the samples and to the controls in the nutrient solutions. The pH of the final mixtures in the test samples was around 6.5. The succeeding sections discuss the anaerobic test results of surfactant-ink mixtures.

CTAB-ink mixture

Test # 11 corresponds to CTAB-ink mixture anaerobic tests and its results are shown in Figure 11 and Figure 12. Figure 11 and Figure 12 represent the results for duplicates samples run for Test # 11. It is seen in Figure 11 and Figure 12 that at low concentrations (0.07 – 0.36 mM), the anaerobic degradation of CTAB-ink mixture has produced more cumulative gas compared to the controls. One of the reasons for this result could be due to adsorption. Specifically, the CTAB could be adsorbed to the ink particles, thereby resulting in a lesser CTAB concentration in solution and thus the degradability result. As in the case of CTAB surfactant alone, these ink-mixtures too suggest the possibility of acclimation of the culture to these organics. The role of ink in the anaerobic degradation of the samples is an issue that has to be investigated and is one of the future scopes for research. It is noted that, in Table 12, for Test # 11, the percentage gas produced in the control is somewhat low at 64%. In general, however, results of Test # 11 are found to be predictable and reasonable.

Check for the possibility of acclimation of CTAB-ink mixtures

Test # 12 is a continuation of Test # 11. Test # 12 used the same test samples used by Test # 11. The samples were re-spiked with 200 mg/L of glucose on the 65th day. Test # 12 was performed to check for the possible acclimation to the CTAB-ink mixtures and its result is shown in Figure 13. It is seen that the CTAB-ink mixtures with concentrations of 0.07 mM, 0.36 mM and 1.43 mM have produced a greater amount of cumulative gas

compared to the blanks. As can be seen in Figure 13, CTAB-ink has started producing significant gas after a lag period of 17 days, indicating the possibility of acclimation.

Check for the initial and final COD's of CTAB-ink mixtures

Test # 13 was carried out to investigate the fate of COD of CTAB-ink mixtures. Its result is shown in Figure 14. New samples were setup for Test # 13. It can be seen that the CTAB-ink (0.05 mM) has produced nearly the same cumulative gas as controls, indicating no toxicity and anaerobic degradation of the CTAB-ink mixture at 0.05 mM. Also, it is seen that CTAB-ink mixture is not anaerobically degradable at higher concentrations (0.5 mM – 1.0 mM). The CTAB-ink was initially acclimated with 100 mg/L of glucose for 30 days and then 1.0 g/L of glucose was added to check for gas production. Thus, the samples started producing gas from the first day of Test # 13 (no lag period). Table 13 gives the gas production in mL gas per mass COD lost in gm for CTAB-ink mixture samples and the controls. In Table 13, the expected final COD value for the control matches very well with the measured final COD value, indicating that the COD tests were behaving in a predictable and reasonable manner. The COD lost in CTAB-ink mixture at 0.05 mM (2500 mg/L to 1080 mg/L) is more than the COD lost in the controls (2300 mg/L to 1150 mg/L) as seen in Table 13. This indicates that the CTAB-ink mixture is likely to be anaerobically degradable at 0.05 mM. This is consistent with the earlier, pure surfactant studies. The measured final COD was less than the expected final COD value, which indicates that perhaps not only glucose and CTAB are degrading, but also, there is a possibility of ink degrading in the mixtures.

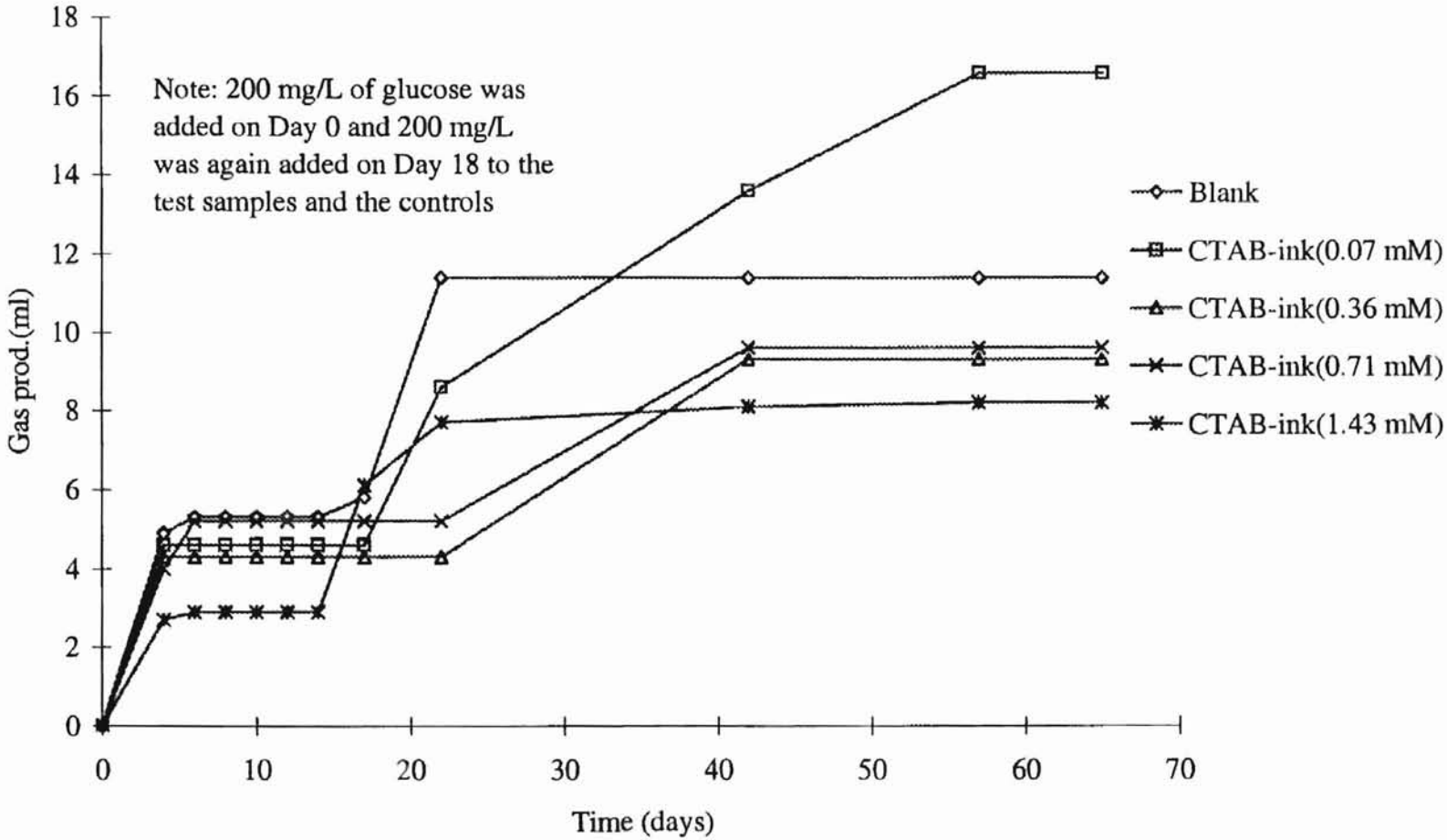

Figure 11. Comparison of the anaerobic biodegradation of different concentrations of CTAB-ink mixture - Test # 11 (first set)

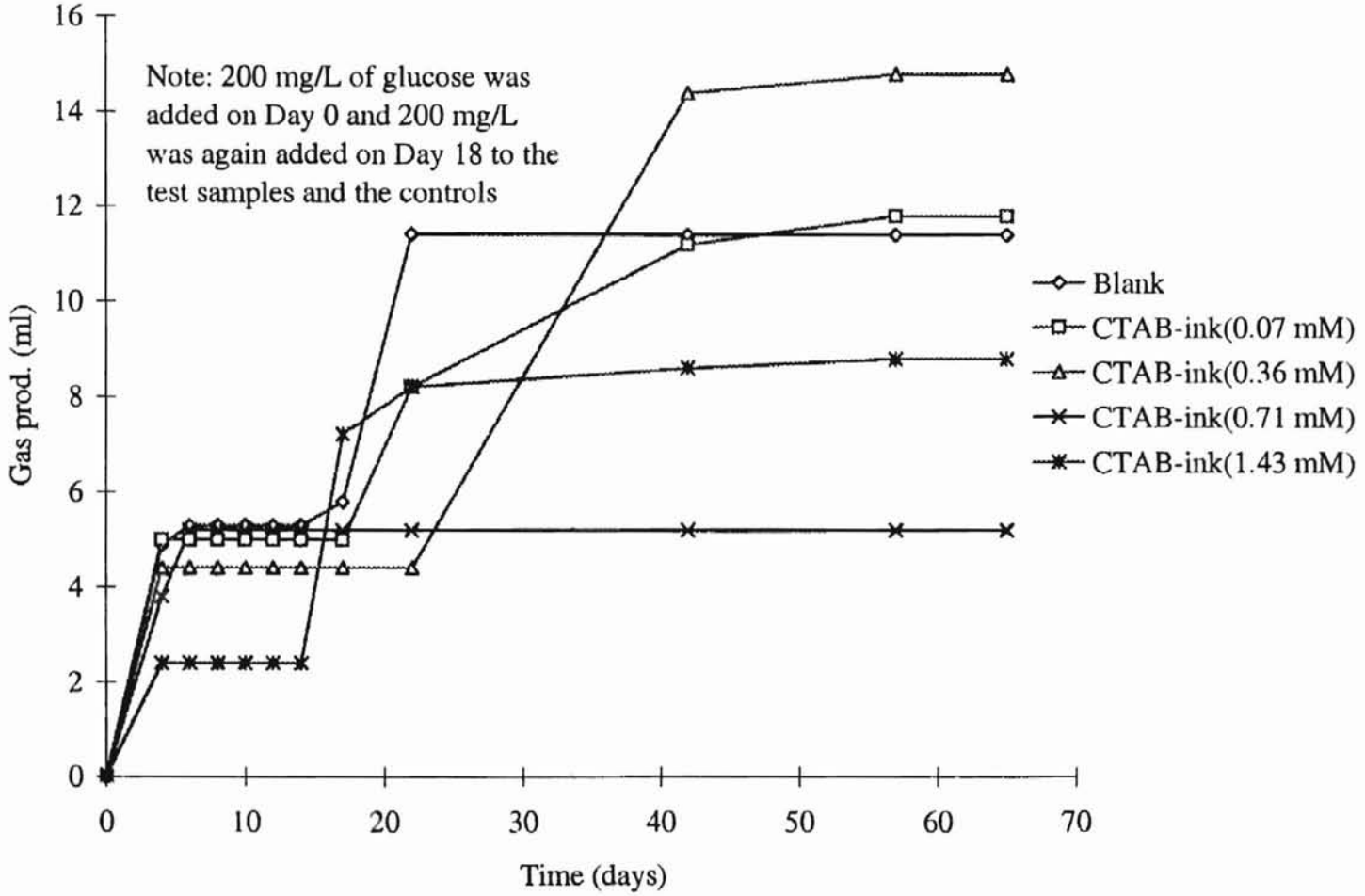

Figure 12. Comparison of the anaerobic biodegradation of different concentrations of CTAB-ink mixture - Test # 11 (second set)

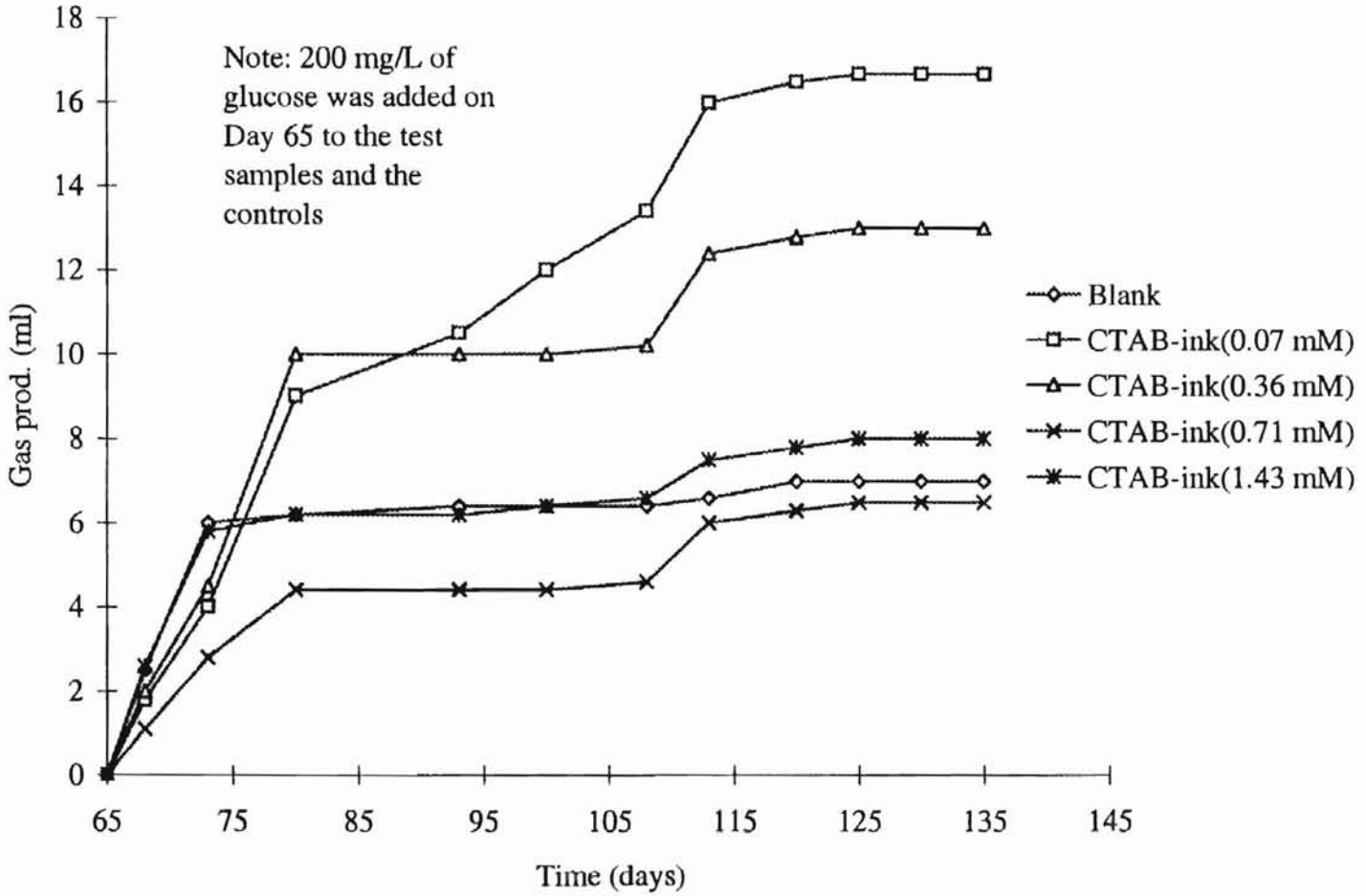

Figure 13. Comparison of the anaerobic biodegradation of different concentrations of CTAB-ink mixture - Test # 12

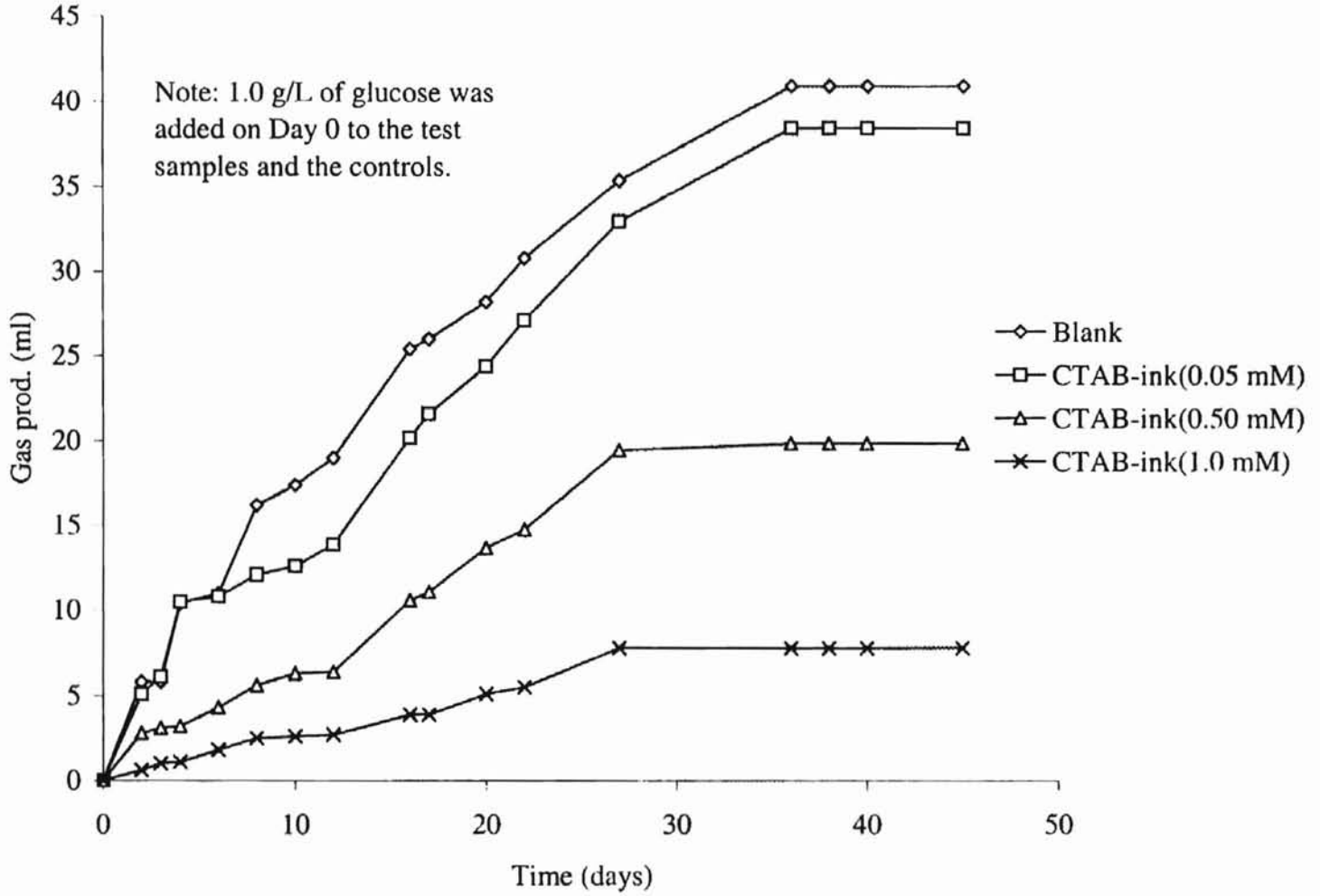

Figure 14. Comparison of the anaerobic biodegradation of different concentrations of CTAB-ink mixture - Test # 13

Future studies will be needed to confirm this. At higher concentrations (0.5 mM – 1.0 mM), the COD lost is less, which indicates the toxic effect of the CTAB-ink mixture on the culture at these concentrations. From Table 14, it can seen that the theoretical ml gas /mg COD (at 25° C) for the control matches very well with all the test samples and control. The COD results also match well with the cumulative gas production results for Test # 13.

Summary of CTAB-ink mixture anaerobic tests

From Tests # 11 and 12, CTAB-ink (0.07 mM) was not toxic to the anaerobic culture and potential biodegradability was indicated (as its gas production exceeded that of the control). COD results from Test # 13 indicate a possible potential biodegradability of CTAB-ink (0.05 mM) (gas production in this reactor was very similar to that of the control). The expected final COD value for CTAB (0.05 mM) for Test # 4 (seen from Table 13) is 1277 mg/L. The final COD value measured for CTAB (0.05 mM) is 1080 mg/L. As the final COD values is less than expected COD value, it is possible that not only are glucose and CTAB degrading, ink could also be degrading in the test samples. Future tests are required to prove this. From Test # 12, CTAB-ink (0.36 mM) is found to be non-toxic to the anaerobic culture (perhaps due to acclimation of culture to the CTAB). From Tests # 11 and 13, CTAB-ink in the concentration range (0.50 mM – 1.43 mM) was found to be toxic to the culture because the cumulative gas production values in the CTAB-ink samples were found to be much lower than the controls.

SN-70-ink mixture and T-205-ink mixtures

Test # 14 (Figures 15 and 16) and Test # 15 (Figure 17) correspond to SN-70-ink mixture anaerobic tests. Test # 15 was a continuation of Test # 14. In Test # 15, the same samples from Test # 14 were re-spiked with 200 mg/L of glucose on the 65th day. As can be seen in Figures 15 and 16, it is seen that there is no specific trend in the variability of gas production for the samples for SN-70- ink. More tests on SN-70-ink in the range 0.05 mM – 1.0 mM are necessary to find out the range of concentrations where SN-70-ink is degradable and/or toxic. In Figure 17, SN-70-ink (0.07 mM – 1.43 mM) has produced gas in amounts comparable with the control. These results could be possibly attributed to the adsorption of SN-70 on ink particles. There is a possibility that different concentrations SN-70 can be adsorbed to the ink and therefore resulting in variable concentrations of SN-70 in solution.

Test # 16 (Figures 18 and 19) and Test # 17 (Figure 20) correspond to T-205-ink mixture anaerobic tests. Test # 17 used the same samples from Test # 16. Test # 16 comprised re-spiking Test # 16 samples with 200 mg/L of glucose on the 65th day. Similar conclusions as SN-70-ink mixture can be made on T-205-ink mixture which can be seen from Figure 18, Figure 19 and Figure 20. However, the T-205-ink (0.07 mM) is found to have produced gas comparable with the control and T-205-ink is found to be toxic at concentrations in the range 0.71 mM – 1.43 mM. Here again perhaps due to adsorption, at low concentrations T-205-ink (0.07 mM), the concentration of T-205 in solution could be much less than 0.05 mM. This may have reduced the toxicity effect of T-205 on the culture.

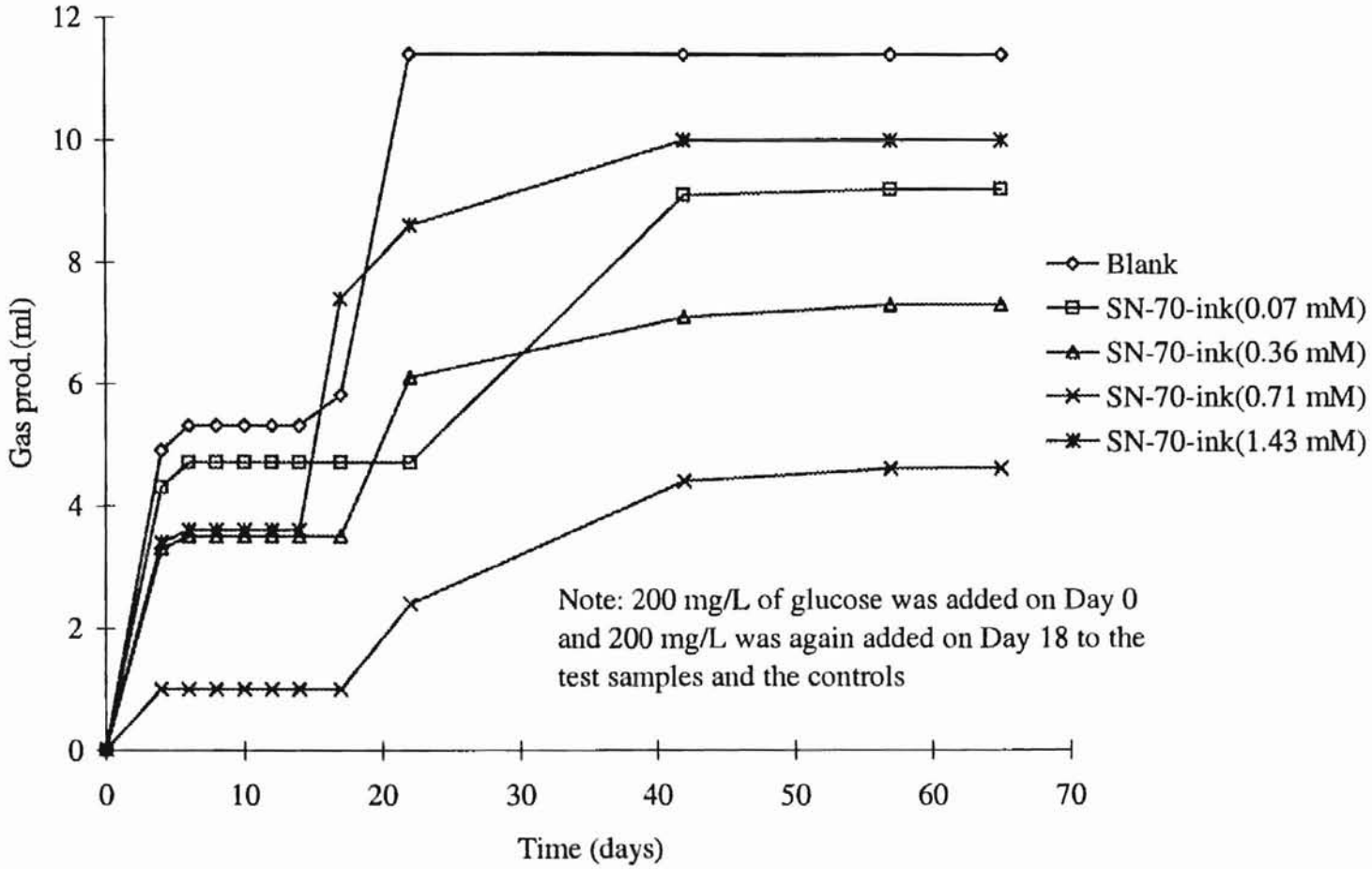

Figure 15. Comparison of the anaerobic biodegradation of different concentrations of SN-70-ink mixture - Test # 14 (first set)

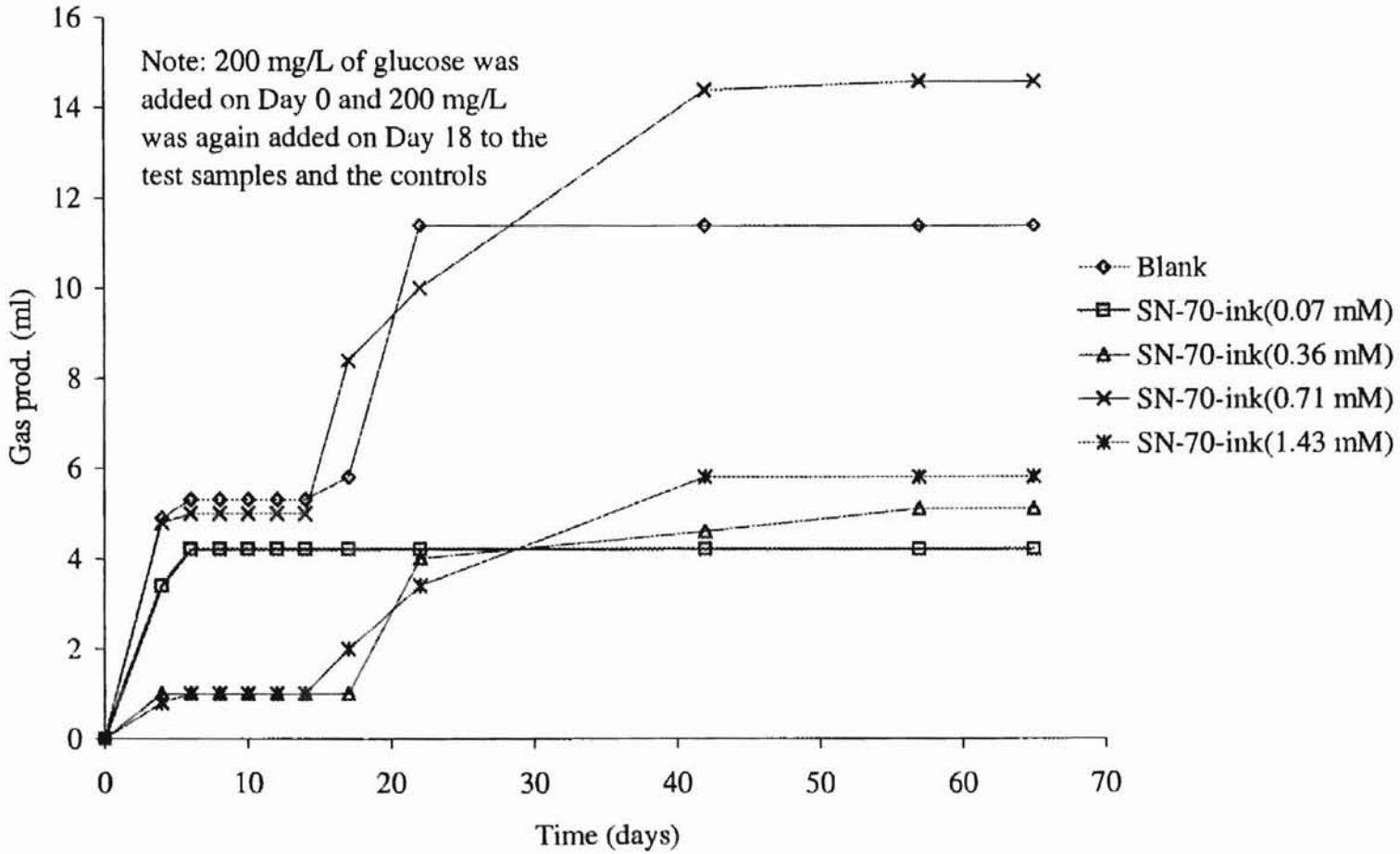

Figure 16. Comparison of the anaerobic biodegradation of different concentrations of SN-70-ink mixture - Test # 14 (second set)

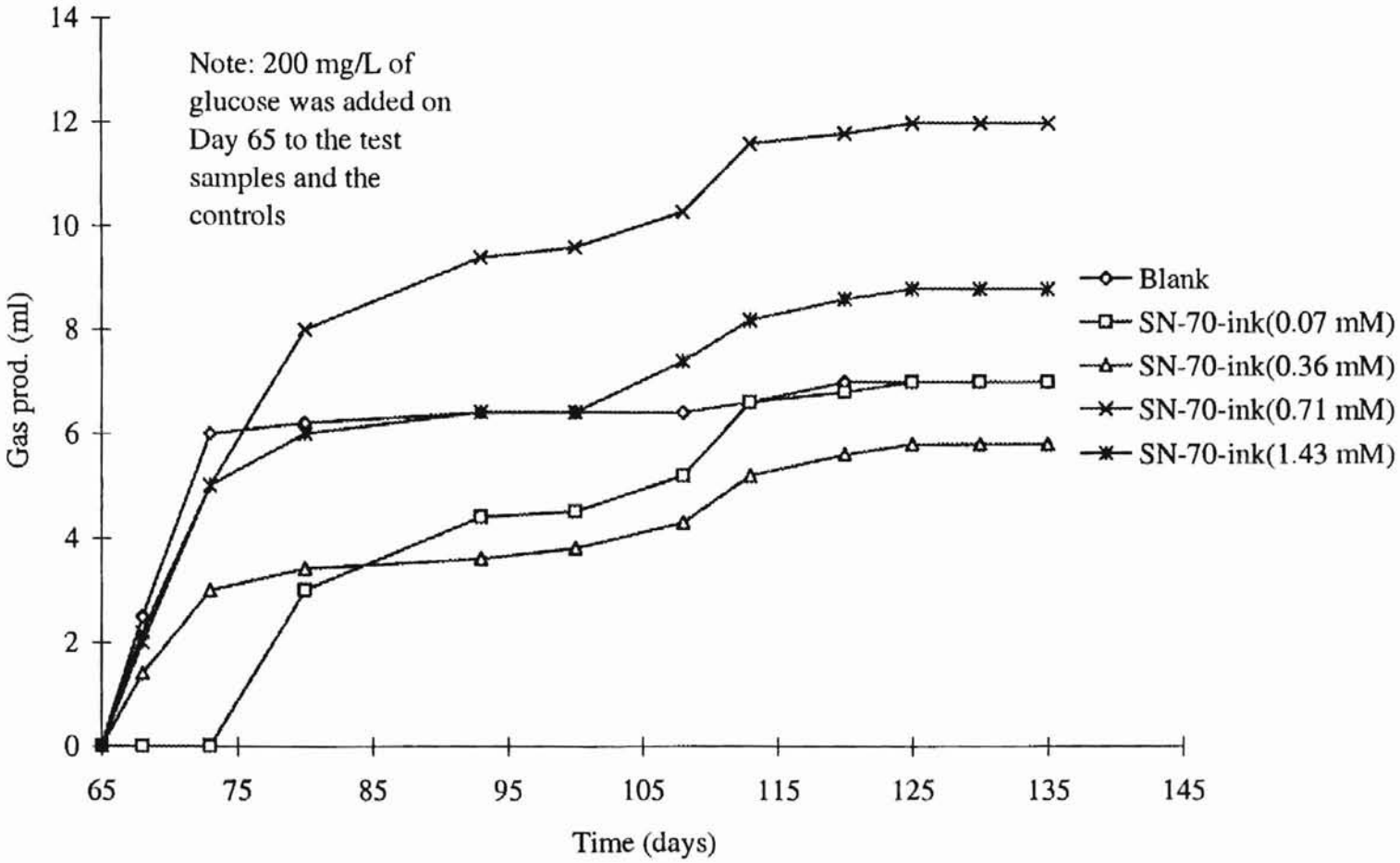

Figure 17. Comparison of the anaerobic biodegradation of different concentrations of SN-70-ink mixture - Test # 15

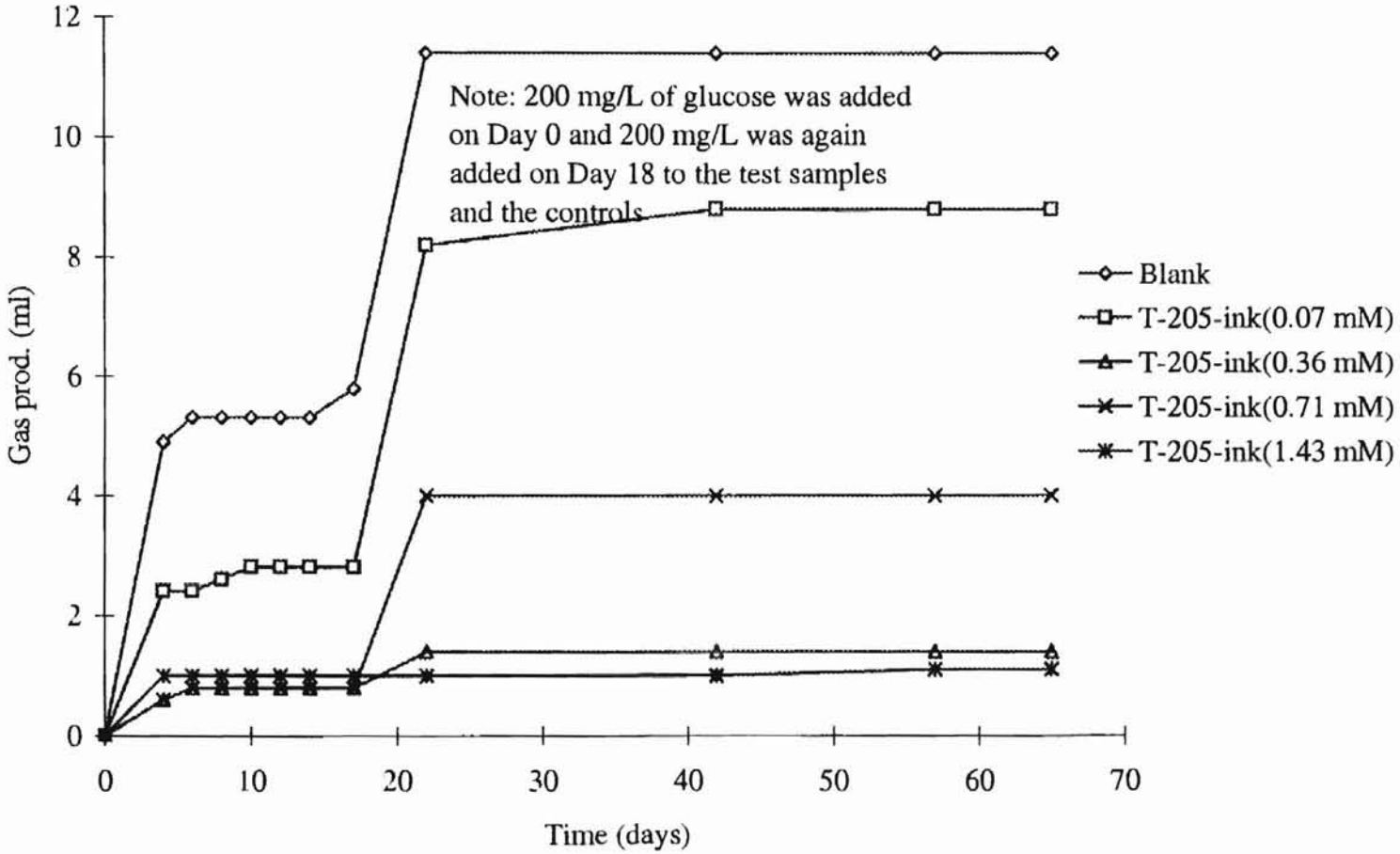

Figure 18. Comparison of the anaerobic biodegradation of different concentrations of T-205-ink mixture- Test # 16 (first set)

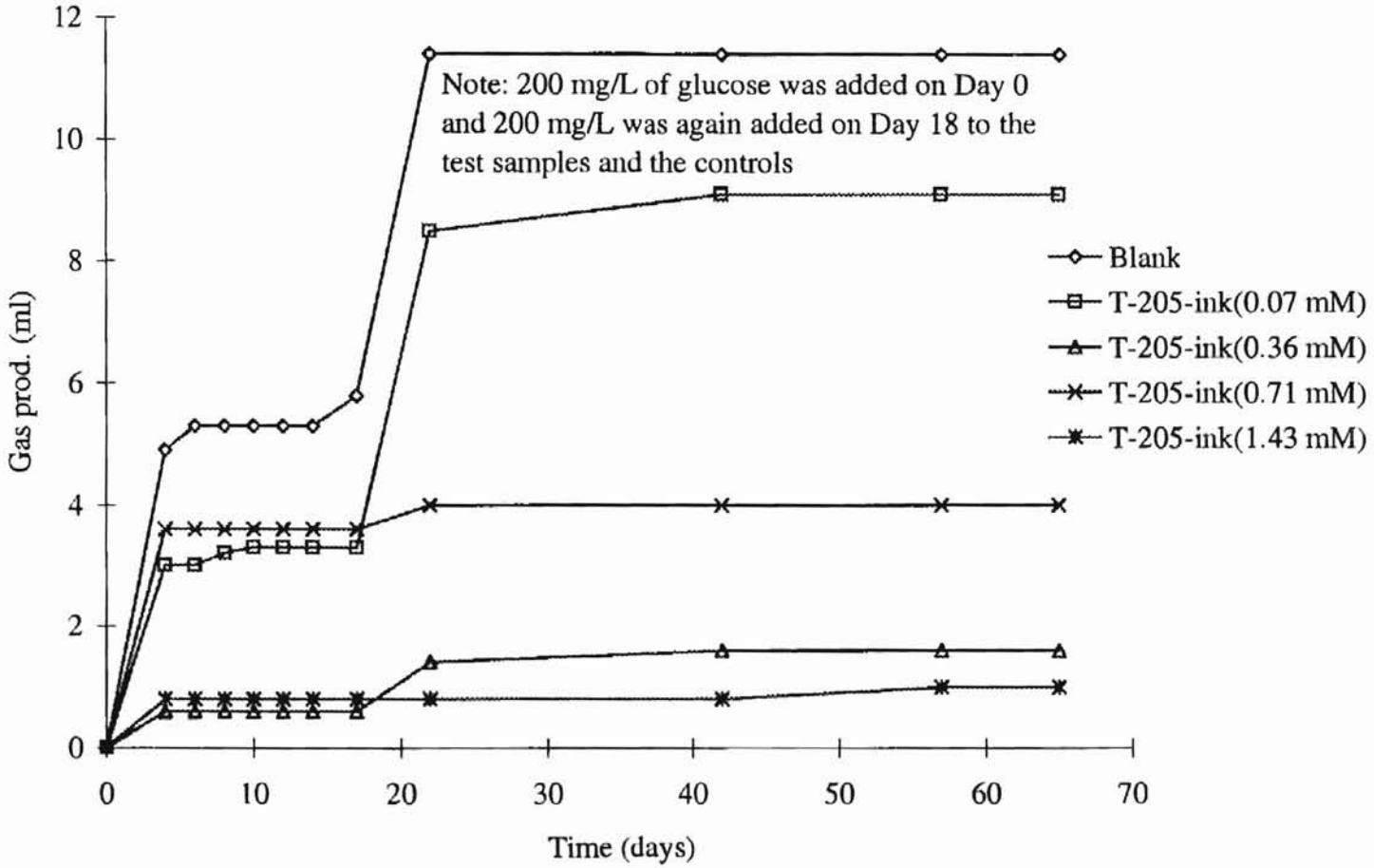

Figure 19. Comparison of the anaerobic biodegradation of different concentrations of T-205-ink mixture - Test # 16 (second set)

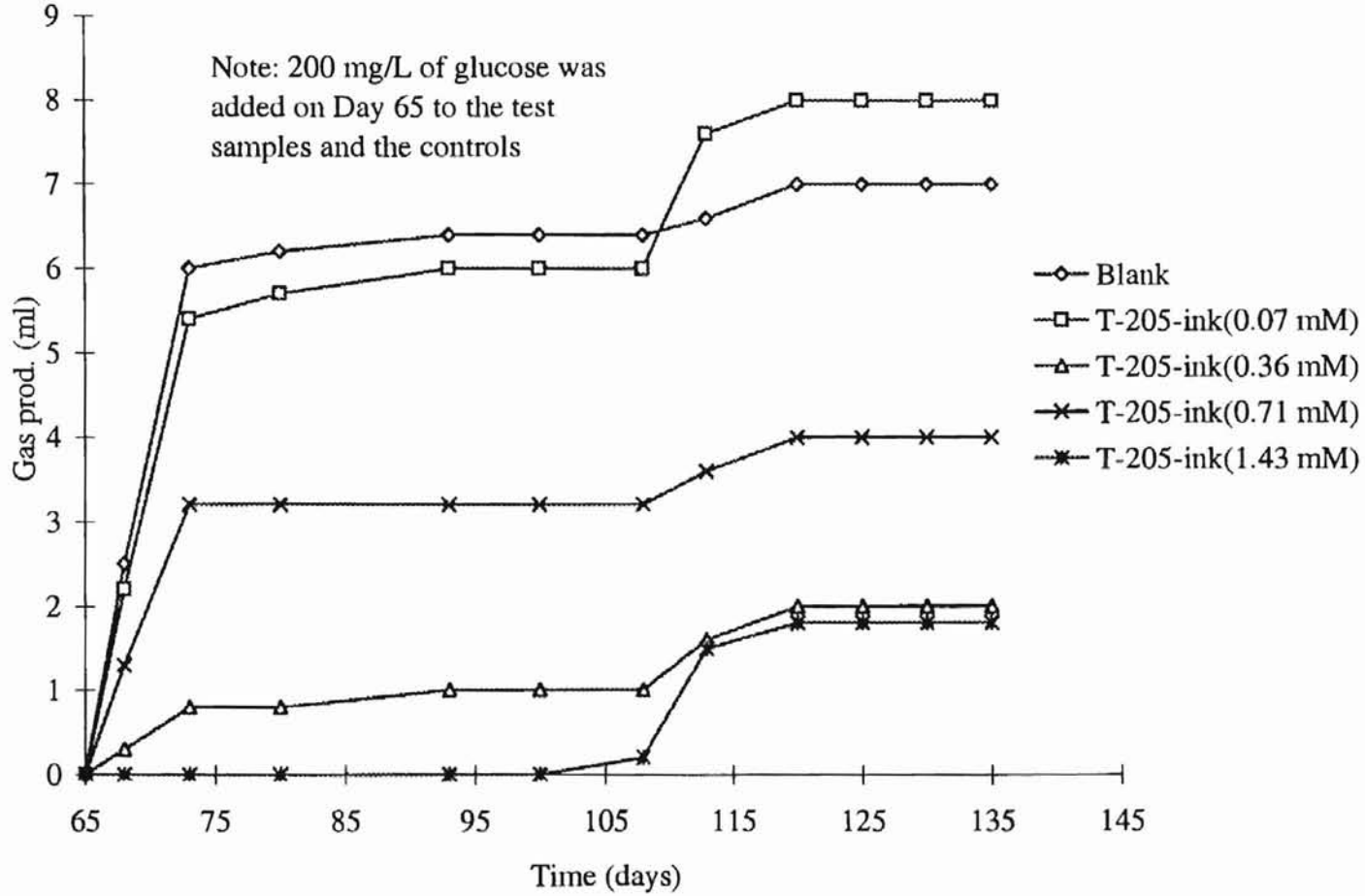

Figure 20. Comparison of the anerobic biodegradation of different concentrations of T-205-ink mixture - Test # 17

Summary of SN-70-ink and T-205-ink mixtures anaerobic tests

There was no apparent trend of the gas production for the SN-70-ink test samples. More tests are needed on SN-70-ink (0.05 mM – 1.0 mM) to determine accurately the SN-70-ink degradability and toxicity. From Test # 14, it is seen that there is no specific trend of gas production in the SN-70-ink samples. Test # 15 indicates that SN-70 (0.07 mM – 1.43 mM) is found to be non-toxic to the culture.

From Tests # 16 and 17, T-205-ink (0.07 mM) is found to be non-toxic to the culture and T-205-ink in the range (0.36 mM – 1.43 mM) is found to be toxic to the anaerobic culture.

Aerobic test results

Aerobic studies done are basically screening tests for determining the biodegradability and/or toxicity of the surfactant and surfactant-ink mixtures. Aerobic studies were done by conducting bench-scale BOD tests. This including setting up 300 ml BOD bottles and measuring the DOs of the samples and the controls. The Biochemical Oxygen Demand (BOD) was then calculated and plotted against time. Table 15 correlates the various tests performed and the figures and tables of the results of surfactant and surfactant-ink mixtures. The results as seen from the figures are discussed in the succeeding sections. Table 16 shows the various concentrations tested on the various surfactants and surfactant-ink mixtures and their respective ultimate BOD values.

Analysis of data

The analysis of seed control data is necessary to check if the aerobic tests were behaving in a predictable and reliable manner. This analysis is done by comparing the BOD_{28}'s of the seed controls tested and checking if the BOD_{28}'s are consistent. Two sets of seed controls were setup for the aerobic study. One seed control set (atleast two controls) was performed for the Tests # 1, 3, 5, 7, 9, and 11 and another seed control set (atleast two controls) was performed for the Tests # 2, 4, 6, 8, 10, and 12. The average of BOD_{28}'s of the controls for the former seed control set was found to be 301.5 mg/L and the average of BOD_{28}'s of the controls for the latter seed control set was found to be 309.0 mg/L. As clearly seen, the average BOD_{28}'s of the seed controls for the two sets match very well.

Table 15. Respective figures and tables for the various aerobic tests on surfactants and surfactant-ink mixtures

Type	Test number	Figure number	Table number
CTAB surfactant	Test # 1	Figure 21	Table B-1
	Test # 2	Figure 22	Table B-2
SN-70 surfactant	Test # 3	Figure 21	Table B-1
	Test # 4	Figure 23	Table B-2
T-205 surfactant	Test # 5	Figure 21	Table B-1
	Test # 6	Figure 24	Table B-2
CTAB-ink mixture	Test # 7	Figure 21	Table B-1
	Test # 8	Figure 25	Table B-2
SN-70-ink mixture	Test # 9	Figure 21	Table B-1
	Test # 10	Figure 26	Table B-2
T-205-ink mixture	Test # 11	Figure 21	Table B-1
	Test # 12	Figure 27	Table B-2

Table 16. Experimental results of aerobic tests

Type	Conc. Tested (*)	BOD_{28} measured
CTAB		
Test # 1	1.18 uM	negative
Test # 2	0.01 uM	negative
	0.1 uM	zero
	1.0 uM	5.3 mg/L
SN-70		
Test # 3	4.87 uM	414 mg/L
Test # 4	1.0 uM	zero
	10.0 uM	62.1 mg/L
	100.0 uM	80.5 mg/L
T-205		
Test # 5	1.35 uM	negative
Test # 6	0.01 uM	negative
	0.1 uM	negative
	1.0 uM	32.3 mg/L
CTAB-ink mixture		
Test # 7	0.33 uM	negative
Test # 8	0.01 uM	negative
	0.1 uM	negative
	1.0 uM	80.0 mg/L
SN-70-ink mixture		
Test # 9	0.33 uM	28.5 mg/L
Test # 10	0.01 uM	negative
	0.1 uM	zero
	1.0 uM	62.6 mg/L
T-205-ink mixture		
Test # 11	0.33 uM	13.8 mg/L
Test # 12	0.01 uM	zero
	0.1 uM	zero
	1.0 uM	30.0 mg/L

* Note: For Test # 1, 3, 5, 7, 9, 11 –seed control's average BOD_{28} is 301.5 mg/L
For Test # 2, 4, 6, 8, 10, 12 – seed control's average BOD_{28} is 309.0 mg/L

These numbers are somewhat higher than the average BOD_{28} value routinely cited for the Stillwater Wastewater Treatment Plant, but are still reasonable. Therefore, the aerobic tests could be said to have behaved in a reasonable and predictable way.

The interpretations of the BOD curves shown in Figures # 21, 22, 23, 24, 25, 26 and 27 are as follows. The BOD_{28}'s of tested samples were corrected for the BOD of seed control. If the tested samples have positive BOD_{28} values, it means that the samples indicate traits of potential biodegradability. If the tested samples have zero BOD_{28} values, it indicates that the samples are neither toxic to the aerobic culture nor exhibit any potential biodegradability traits. If the tested samples are noted to have negative BOD_{28}'s, samples are concluded to be toxic to the aerobic culture

Surfactant results

CTAB cationic surfactant

In aerobic Test # 1, the DO drop in the CTAB sample (1.18 uM) was found to be less than the seed control and therefore the BOD value is negative, as seen in Table 16. In Table 16, for Test # 2, BOD_{28} of CTAB (1.0 uM) is 5.3 mg/L, BOD_{28} of CTAB (0.01 uM) is negative and BOD_{28} of CTAB (0.1 uM) is zero. Therefore, in Test # 2, BOD_{28} for CTAB samples were found to be negligible. Figure 22 depicts the BOD curve for the CTAB samples for Test # 2. From these tests, it can be concluded that CTAB surfactant (0.01 uM to 1.18 uM) is not aerobically degradable and is in fact toxic to the aerobic culture. These results match well with the results of aerobic CTAB studies cited in the literature review section.

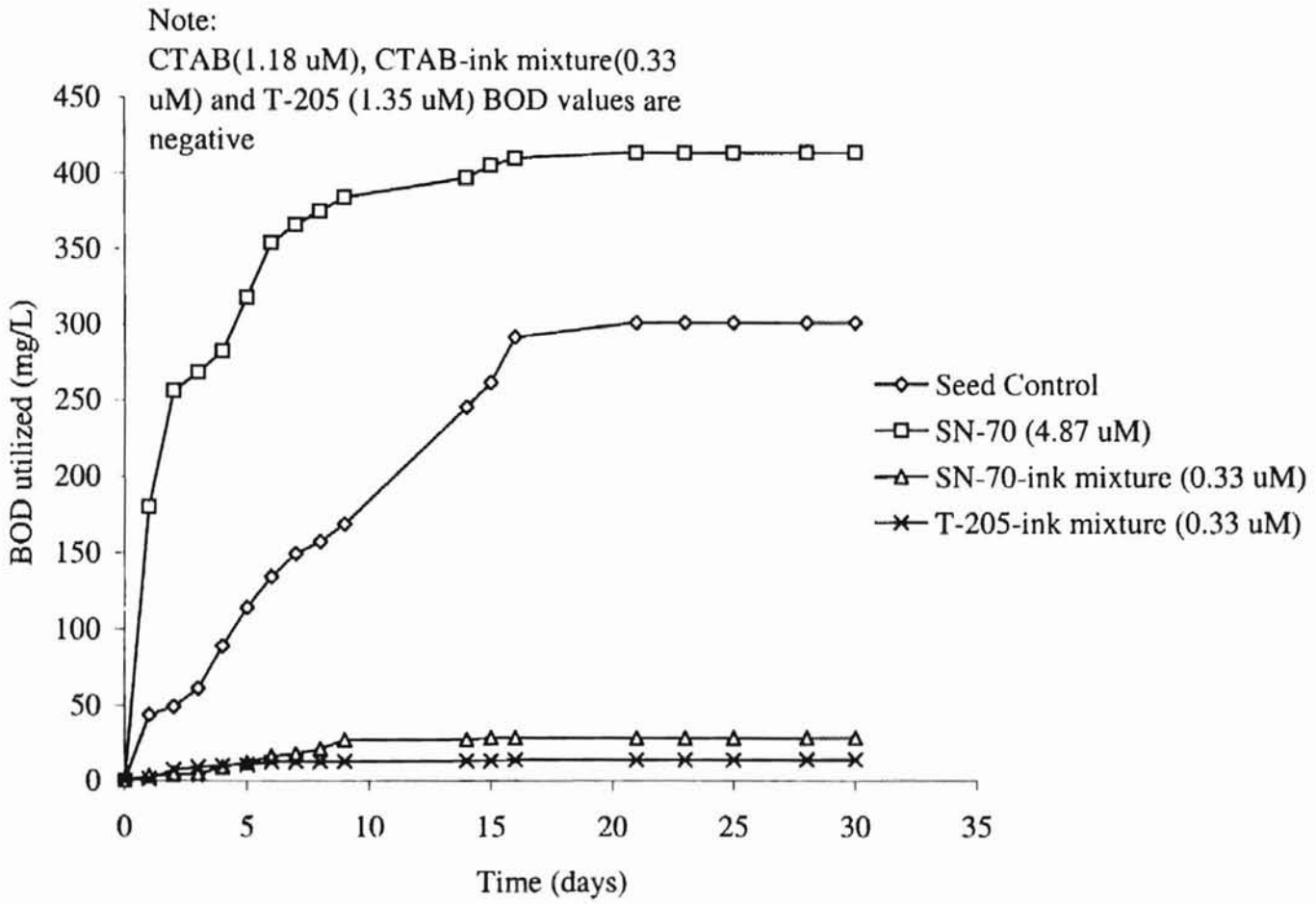

Figure 21. Comparison of the aerobic degradation of surfactant and surfactant-ink mixtures - Tests # 1, 3, 5, 7, 9, and 11

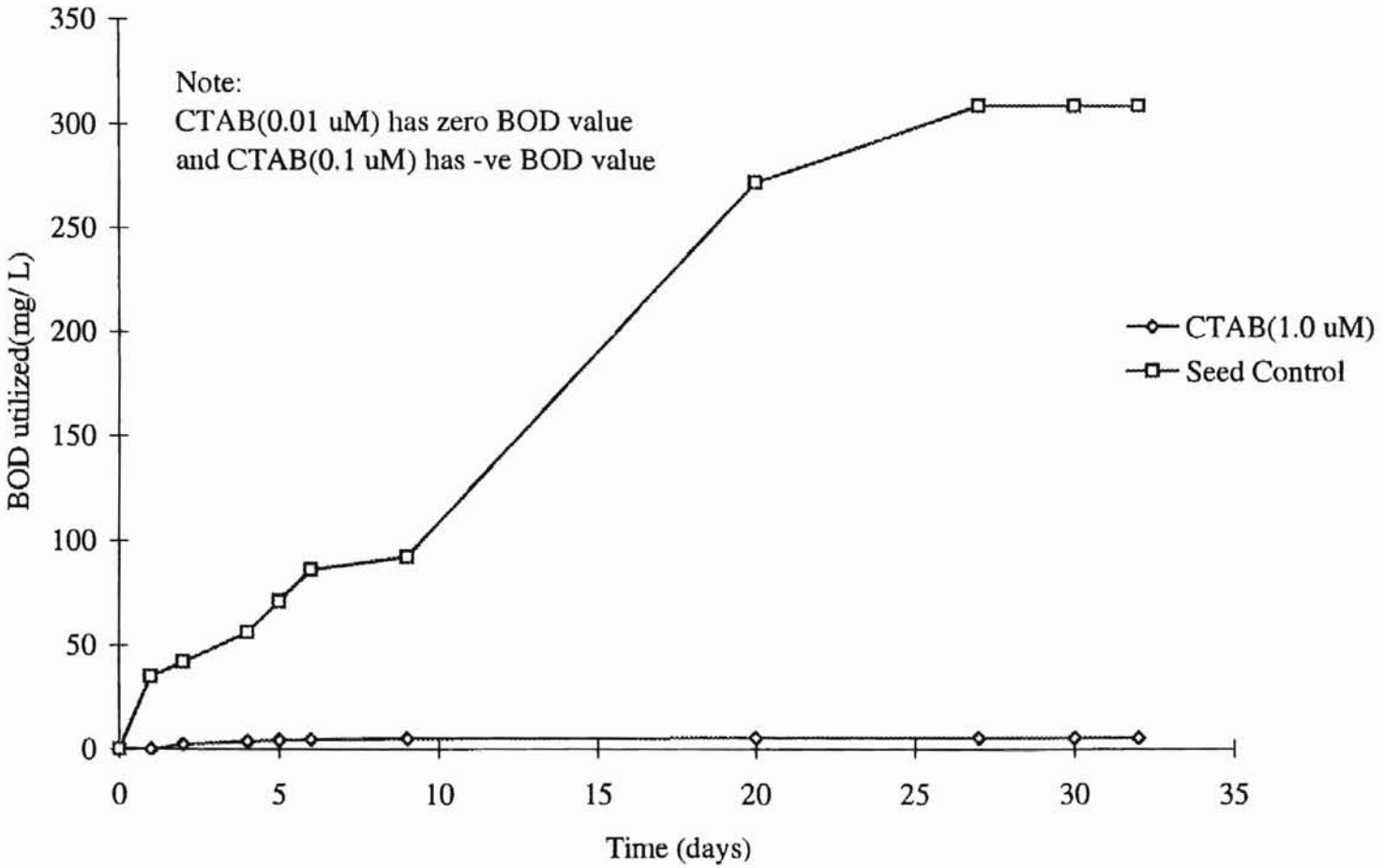

Figure 22. Aerobic biodegradation of CTAB surfactant - Test # 2

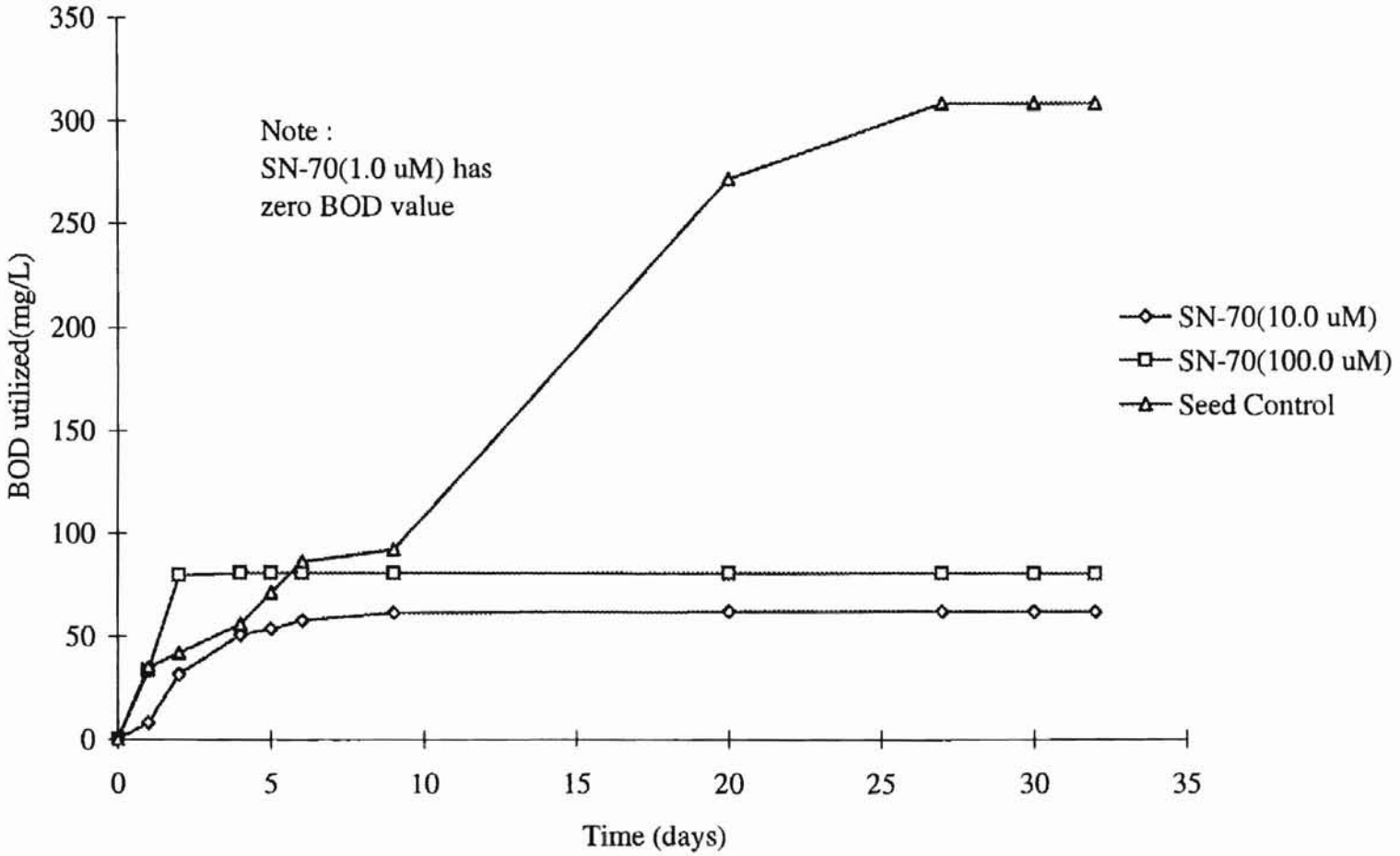

Figure 23. Aerobic biodegradation of SN-70 surfactant - Test # 4

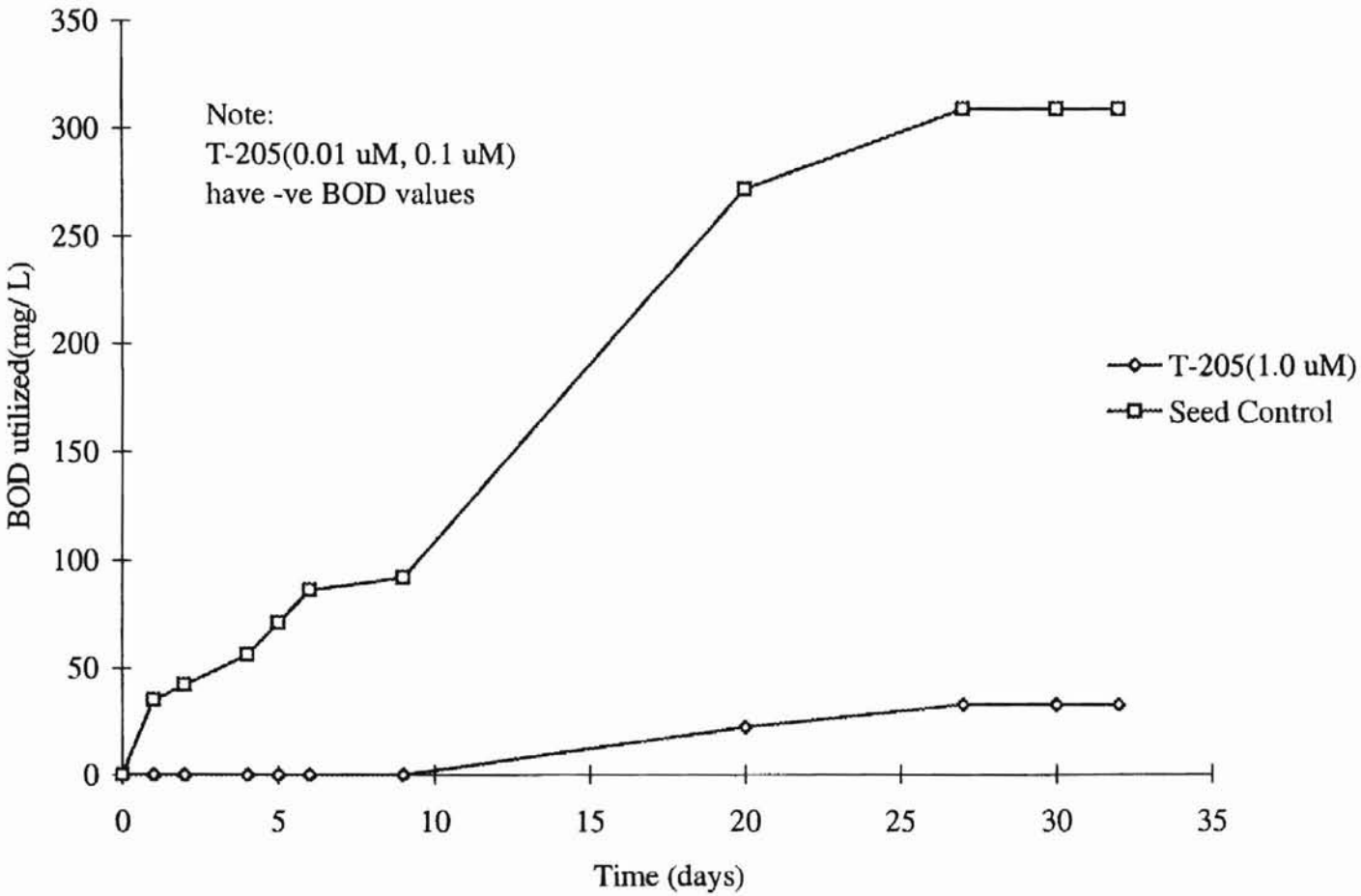

Figure 24. Aerobic biodegradation of T-205 surfactant - Test # 6

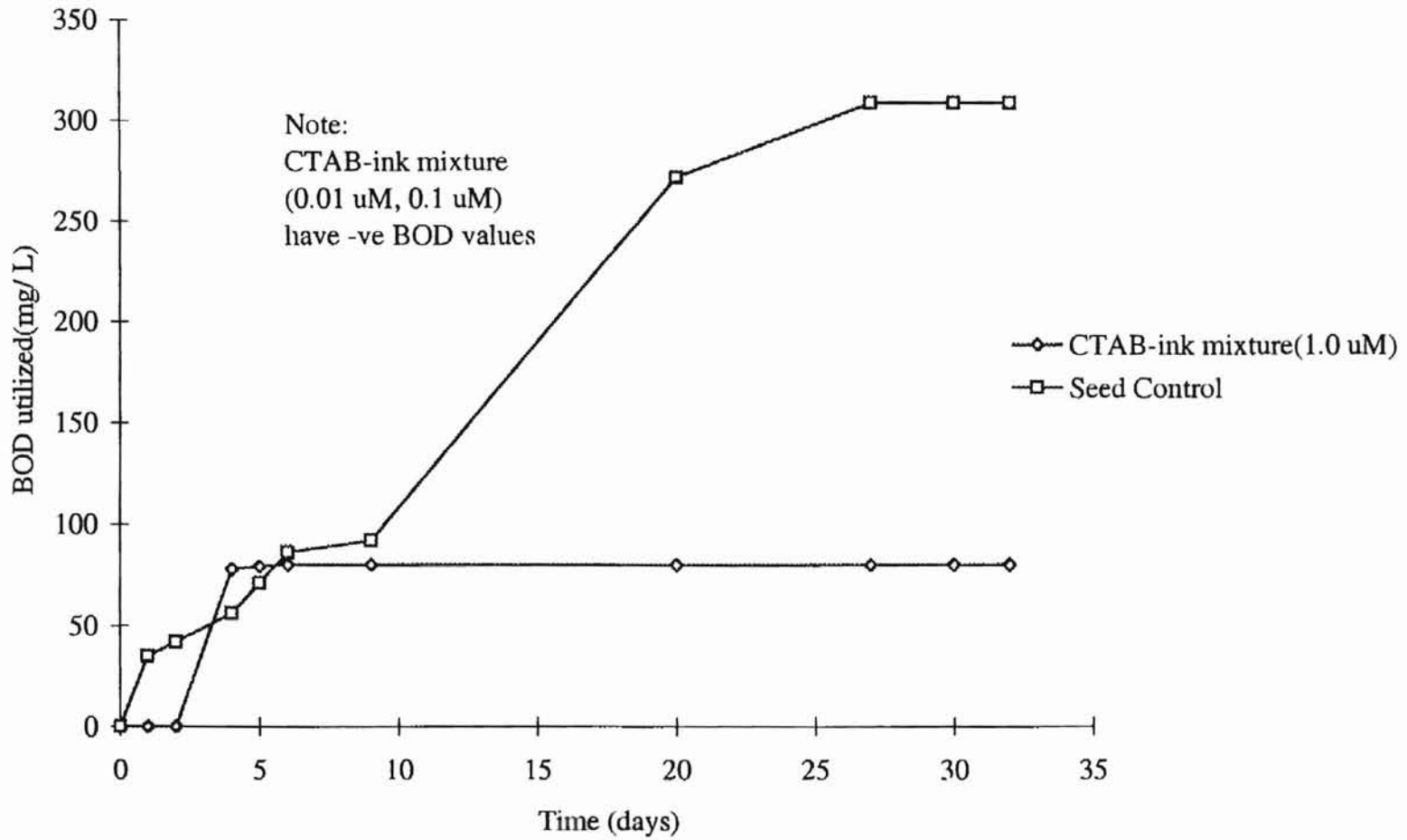

Figure 25. Aerobic biodegradation of CTAB-ink mixture - Test # 8

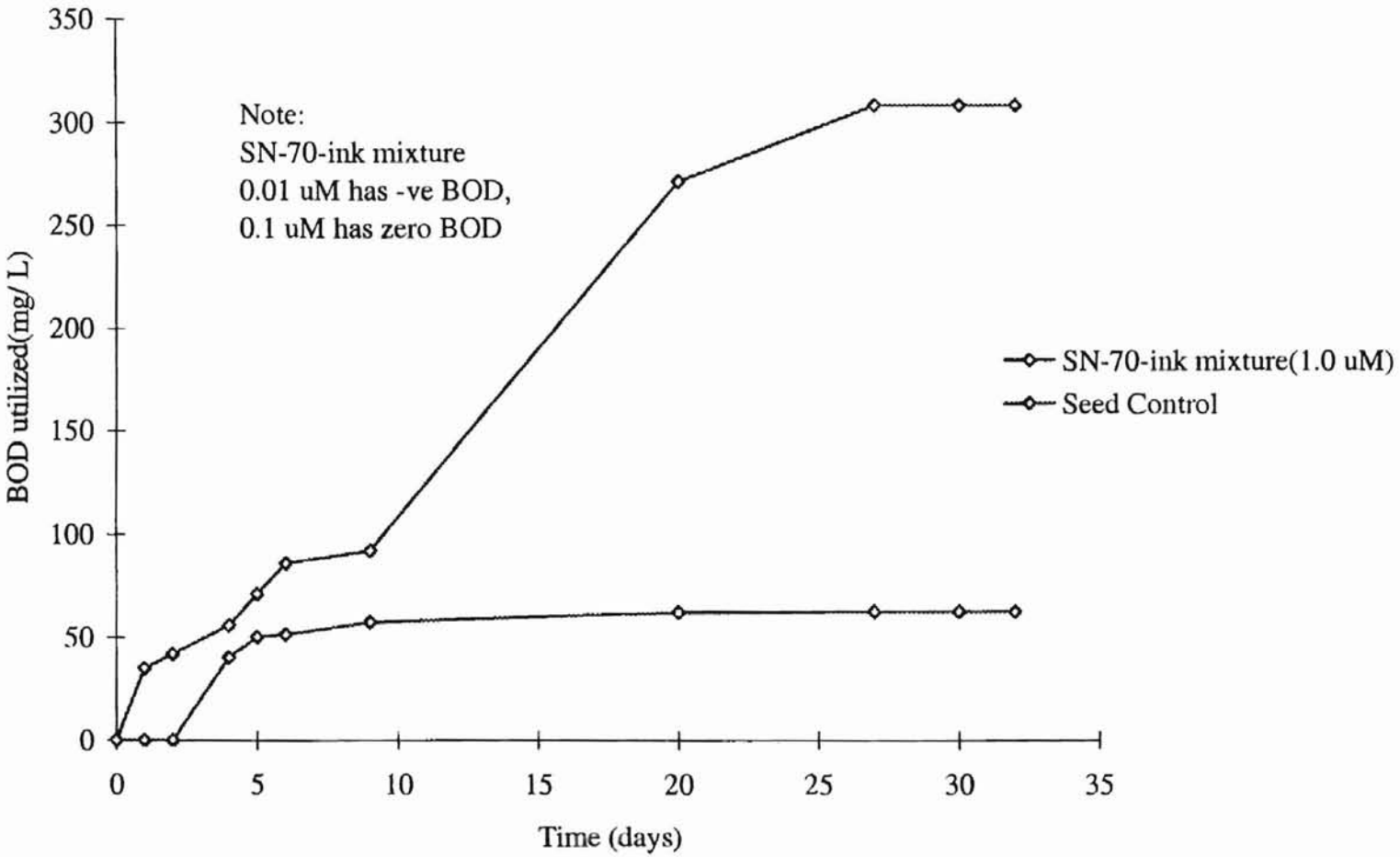

Figure 26. Aerobic biodegradation of SN-70 ink mixture - Test # 10

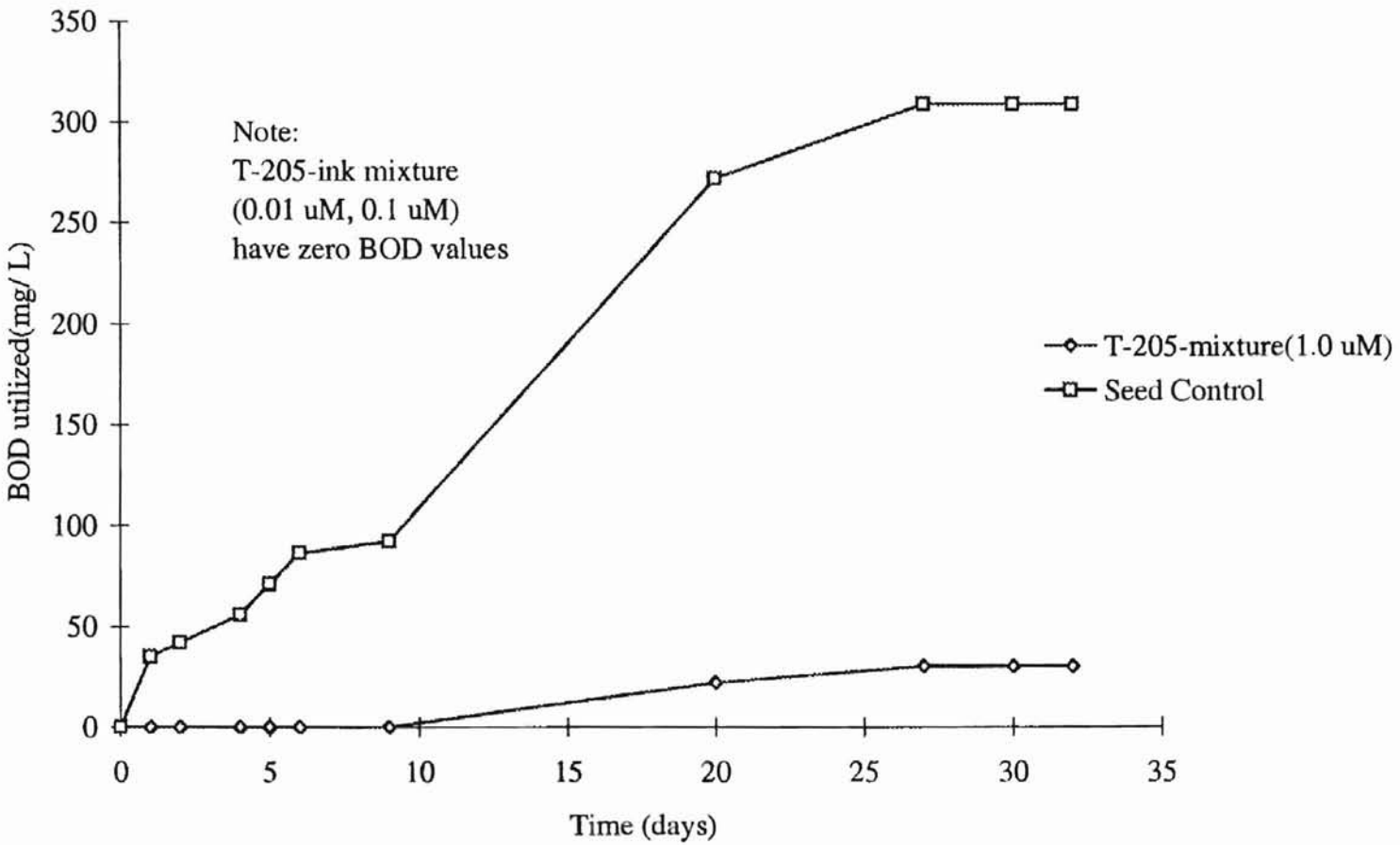

Figure 27. Aerobic biodegradation of T-205-ink mixture - Test # 12

SN-70 and T-205 non-ionic surfactants

Test # 3 and Test # 4 were performed to determine the aerobic toxicity and potential biodegradability of SN-70 surfactant. The results of these tests are shown in Figure 21 and Figure 23 respectively. In Table 16, for Test # 3, it is seen that the BOD_{28} in the SN-70 sample (4.87 uM) is 414 mg/ L. For Test # 3, the BOD_{28} for the seed control is found to be 301.5 mg/L. For Test # 4, the BOD_{28} of SN-70 (1.0 uM) is zero, BOD_{28} of SN-70 (10.0 uM) is 62.1 mg/ L and BOD_{28} of SN-70 (100.0 uM) is 80.5 mg/ L. For Test # 4, BOD_{28} for the seed control is found to be 309.0 mg/L. As can be seen, SN-70 has indicated a potential aerobic biodegradability in the range of concentrations (5.0 uM – 100.0 uM) tested. However, there is no specific trend obtained for the aerobic biodegradability of SN-70. Oxidation appears to be erratic and unpredictable.

Similarly, Test # 5 and Test # 6 correspond to T-205 aerobic biodegradability tests and their results are shown in Figure 21 and Figure 24 respectively. For Test # 5, the DO drop in the T-205 sample (1.35 uM) was found to be less than the seed control and therefore the BOD value was negative. T-205 (1.35 uM) is found to be toxic to the aerobic culture. For Test # 6, the BOD_{28} of T-205 (1.0 uM) was found to be 32.3 mg/L, BOD_{28} of T-205 (0.01 uM) and the BOD_{28} of T-205 (0.1 uM) were found to be negative. From Test # 6, it is seen that T-205 is toxic to the aerobic culture in the range of concentrations tested (0.01 uM – 0.1 uM).

Summary of SN-70 and T-205 aerobic tests

From Test # 3 and Test # 4, SN-70 is found to be potentially aerobically biodegradable in the concentration range 4.87 uM to 100.0 uM. There is no specific trend obtained for the

aerobic biodegradability of the SN-70 surfactant. In Test # 4, SN-70 (1.0 uM) was found to be neither toxic nor does indicate potential biodegradability. More tests are needed to better define these reactions and to test the variation of potential biodegradability. From Test # 5 and Test # 6, T-205 is found to be toxic in the range of concentrations tested (0.01 uM – 1.35 uM).

Surfactant-ink mixtures

CTAB-ink, SN-70-ink and T-205-ink mixtures

As seen from Table 15, Tests # 7 and 8 correspond to CTAB-ink mixture tests, Tests # 9 and 10 correspond to SN-70-ink mixture tests and Test # 11 and 12 correspond to T-205-ink tests. Results of Tests # 7, 9, and 11 are shown in Figure 21 and results of Tests # 8, 10, and 12 are shown in Figures # 25, 26, and 27 respectively.

In Test # 7, the DO drop in the CTAB-ink sample (0.33 uM) was found to be less than the seed control and therefore the BOD value was negative. In Table 16, it is seen for Test # 8 that the BOD_{28} of CTAB-ink (1.0 uM) is 80.0 mg/L, BOD_{28} of CTAB-ink 0.01 uM) and (0.1 uM) are negative. The result of Test # 8 is shown in Figure 25. CTAB-ink (1.0 uM) indicates potential biodegradability as seen from Test # 8. From Test # 7 and Test # 8, CTAB-ink is found to be toxic to aerobic culture in the range of concentrations tested (0.01 uM – 0.33 uM). It is clearly seen that CTAB-ink (1.0 uM) has produced contradictory results when compared with the CTAB-ink (0.01 uM) and CTAB-ink (0.33 uM). It is possible that the result of CTAB-ink (1.0 uM) could be wrong. Future studies need to be done to confirm this.

In SN-70-ink tests (Test # 9), the final BOD in the SN-70-ink sample (0.33 uM) was found to be 28.5 mg/ L. The BOD_{28} of seed control for Test # 9 is found to be 301.5 mg/L. From Table 16, for Test # 10, the BOD_{28} of SN-70-ink (1.0 uM) is 62.6 mg/ L, BOD_{28} of SN-70-ink (0.1 uM) is zero and the BOD_{28} of SN-70-ink (0.01 uM) is negative. The BOD_{28} of the seed control for Test # 10 is found to be 309.0 mg/L. Figure 26 shows the results corresponding to Test # 10. From Test # 10, SN-70-ink (0.01 uM) is found to be toxic to the aerobic culture and SN-70-ink (0.1 uM) is neither toxic nor does it indicate potential biodegradable traits. From Test # 9 and Test # 10, SN-70-ink, in the range of concentrations tested (0.33 uM – 1.0 uM), shows potential biodegradability. It is clearly evident that there is a contradiction between the result of SN-70-ink (0.01 uM) and results of SN-70-ink (0.1 uM – 1.0 uM). Future studies need to be done on SN-70-ink (≥ 0.1 uM) to determine accurately the SN-70-ink degradation.

Test # 11 and Test # 12 were performed for testing the aerobic biodegradability and/or toxicity of T-205-ink mixtures. In Test # 11, the final BOD of the T-205 sample (0.83 uM) was found to be 13.8 mg/ L. Figure 21 depicts the BOD curve for these samples. The result of Test # 12 is shown in Figure 27. From Table 14, it is seen for Test #12, BOD_{28} of T-205-ink (1.0 uM) is 30.0 mg/L, BOD_{28} of T-205-ink (0.01 uM) and BOD_{28} of T-205-ink (0.1 uM) are zero. In this case again, there is a contradiction because the T-205-ink (0.33 uM) and T-205 (1.0 uM) results do not match with the results of T-205-ink (0.01 uM – 0.1 uM). From Test # 12, T-205-ink (0.01 uM) and T-205-ink(0.1 uM) don't either indicate toxicity or traits of potential biodegradability. From Tests # 11 and 12, T-205-ink in the range of concentrations tested (0.33 uM – 1.0 uM) shows traits of potential biodegradability. Future studies need to test and recheck the T-205-ink

(0.33 uM – 1.0 uM) potential biodegradability.

Summary of ink-mixtures aerobic tests

From Tests # 7 and 8, CTAB-ink is toxic to the aerobic culture in the range of concentrations tested (0.01 uM – 0.33 uM). From Tests # 9 and 10, SN-70-ink (0.01 uM) is toxic to the aerobic culture. From Tests # 11 and 12, T-205-ink concentrations in the range 0.01 uM to 0.1 uM, is neither toxic to the aerobic culture nor is biodegradable. More tests in the future need to be performed on CTAB-ink (≥ 0.33 uM), SN-70-ink (≥ 0.1 uM) and T-205-ink (≥ 0.1 uM) to test their potential biodegradability. The role of ink must also be investigated to determine the aerobic degradability of CTAB-ink, SN-70-ink and T-205-ink mixtures.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Anaerobic degradability of surfactants and surfactant-ink mixtures were tested by comparing the cumulative gas production in the tested samples and controls. Initial and final COD's were also tested and compared for the experimental samples.

The predominant findings of the anaerobic studies are as follows:

* CTAB surfactant was found to be anaerobically degradable at concentrations of 0.05 mM. The degradability was seen after a lag period of 14 – 27 days. This observation was seen in all the tested CTAB samples. At higher concentrations, in the range 0.1 mM / and above, CTAB surfactant was found to be toxic to the anaerobic culture.

* Witconol SN-70 non-ionic surfactant was found to be toxic in the range of concentrations tested (0.05 mM – 41.8 mM).

* Varonic T-205 surfactant was found to be toxic to the anaerobic culture in all but the lowest concentrations tested (0.1 mM – 29.0 mM). T-205 (0.05 mM) produced comparable gas as the controls, indicating absence of toxicity.

* CTAB-ink (0.07 mM) produced more gas compared to the controls, indicating the potential for biodegradability. COD results indicate that

CTAB, glucose and ink appeared to be degrading in the CTAB-ink (0.07 mM) samples. CTAB-ink (0.36 mM) was found neither to be toxic to culture nor anaerobically biodegradable. CTAB-ink in the range of concentrations (0.50 mM – 1.43 mM) was generally found to be toxic to the anaerobic culture.

* Even though SN-70-ink samples (0.07 mM – 1.43 mM) produced more cumulative gas compared to the controls, there is no specific trend with varying concentrations. The presence of the ink residues resulted in a variable response of the surfactant. As such, it was not possible to assess with certainty the degradability of the SN-70-ink solutions.

* T-205-ink (0.07 mM) was found to be non-toxic to the culture and T-205-ink in the range of concentrations 0.36 mM – 1.43 mM was found to be toxic to the anaerobic culture.

Aerobic studies on surfactant and surfactant-ink mixtures included measuring the DO's of the experimental samples and controls, and the determination of the ultimate BOD.

The predominant findings of the aerobic study were:

* The CTAB surfactant at concentrations 0.01 uM to 1 uM was found to be toxic to the aerobic culture.

* Witconol SN-70 surfactant indicated minimal toxicity and potential aerobic biodegradability at concentrations 5 uM – 100 uM. However, there was no specific trend in BOD's for the various concentrations of SN-70.

* Varonic T-205 surfactant was found to be toxic to the aerobic culture at concentrations 0.01 uM – 0.1 uM. Tests at higher concentrations were ambiguous.

* The CTAB-ink solution was found to be toxic to the aerobic culture in the range of concentrations tested (0.01 uM – 0.33 uM). At a higher concentration CTAB-ink (1.0 uM), the results obtained were ambiguous.

* The results of aerobic tests performed on SN-70-ink and T-205-ink solutions were ambiguous and thus, it was not possible to determine the toxicity threshold for these mixtures.

Recommendations

This study has raised new concerns and questions and further studies are therefore recommended. They include the following:

* A study is needed to determine the role of ink in the anaerobic and aerobic degradability of surfactant-ink mixtures. One of the possible ways is to determine a suitable mechanism to separate surfactant and ink. Filtering is an option and tests can be done on filtered and unfiltered surfactant-ink mixtures and then the results then would make clear the role of ink.

* Further tests can be done on CTAB cationic surfactant at concentrations around 0.05 mM at which it was found anaerobically degradable. The operating temperature for the study was 25°C. Tests can be done at higher temperatures to interpret the effect of variation of temperature on CTAB biodegradability.

* T-205 has shown potential anaerobic biodegradability traits at a concentration of 0.05 mM. Anaerobic tests on T-205 ($\leq$ 0.05 mM) is necessary to investigate the potential biodegradability / toxicity of T-205 at low concentrations.

* Anaerobic tests need to be done on SN-70-ink samples at concentrations 0.07 mM – 1.43 mM to investigate the correct trend of gas production with varying SN-70-ink concentrations.

* Aerobic tests need to be done on SN-70 ($\geq$ 10 uM), T-205 ($\geq$ 1 uM), CTAB-ink ($\geq$ 1 uM), SN-70-ink ($\geq$ 0.33 uM), and T-205-ink ($\geq$ 0.33 uM) to test the potential biodegradability and / or toxicity of these surfactants and surfactant-ink mixtures and compare the results obtained with the results of this study.

* Tests on surfactants can be done around their Critical Micelle Concentrations to determine the effect of CMC on degradability.

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APPENDICES

APPENDIX A – Anaerobic study

Table A-1. CTAB – Test # 1

Days	Cumulative gas production, ml				
	Blank (0 mM CTAB)	CTAB (0.05mM)	CTAB (0.10 mM)	CTAB (0.50 mM)	CTAB (1.00 mM)
0	0	0	0	0	0
2	0	0	0	0	0
4	0	0	0	0	0
8	9	5	5.8	8	5.2
10	9.2	5.4	5.8	8	5.2
12	12.4	6.6	6.4	10.4	5.8
14	13	6.6	6.4	10.8	5.8
15	13.4	9.8	6.4	11.4	5.8
17	15.4	11.6	9.2	13.2	6.4
19	16	12	9.4	13.4	6.4
20	18.2	13.4	10	13.8	6.8
22	20.6	18	10.2	13.8	6.8
25	24.8	18.2	10.2	13.8	6.8
28	24.8	18.2	10.2	13.8	6.8
30	24.8	18.2	10.2	13.8	6.8
35	24.8	18.2	10.2	13.8	6.8

Table A-2. CTAB – Test # 2

Days	Cumulative gas production, ml				
	Blank (0 mM CTAB)	CTAB (0.05mM)	CTAB (0.10 mM)	CTAB (0.50 mM)	CTAB (1.00 mM)
0	0	0	0	0	0
2	10	0	0	0	0
3	10	4	4.8	0.4	0.2
4	14.6	4	4.8	0.4	0.6
6	15	4	4.8	0.6	0.6
8	15.2	4	4.8	1	0.6
10	15.4	4	4.8	4.8	0.6
12	19	4.2	5	5.2	0.6
14	19.4	4.3	5.1	5.3	0.6
16	19.6	4.5	5.3	5.5	0.6
18	19.6	4.5	5.3	5.7	0.6
20	19.6	7	6.8	5.7	0.6
22	19.6	7	6.8	5.7	0.6
27	19.6	7	6.8	5.7	0.6
36	28	12.2	11	5.8	7.6
56	29.2	14.6	13.6	6.4	9.4
67	29.2	16.2	14.2	6.6	9.6
80	29.2	16.2	14.2	6.6	9.6

Table A-3. CTAB – Test # 3

Days	Cumulative gas production, ml				
	Blank (0 mM CTAB)	CTAB (0.05 mM)	CTAB (0.10 mM)	CTAB (0.50 mM)	CTAB (1.0 mM)
80	0	0	0	0	0
82	0.5	0.4	0.4	0.1	0.3
84	2.4	1	1	0.2	0.5
88	2.6	1.3	1.1	0.4	0.7
92	4.6	4.3	1.5	0.6	1
96	7	7.5	2.1	0.8	1.1
113	7.2	8	4.2	1.3	2.9
122	7.4	8.3	4.6	1.5	3.1
130	7.6	8.5	4.8	1.6	3.2
140	7.6	8.5	4.8	1.6	3.2
145	7.6	8.5	4.8	1.6	3.2

Table A-4. CTAB – Test # 4

Days	Cumulative gas production, ml				
	Blank (0 mM CTAB)	CTAB (0.05mM)	CTAB (0.10 mM)	CTAB (0.50 mM)	CTAB (1.00 mM)
0	0	0	0	0	0
2	5.8	0	0	0	0
3	5.8	2.5	2.2	1.0	1.5
4	10.4	2.5	2.2	1.0	2.3
6	11.0	5.0	3.8	1.5	3.9
8	16.2	10.8	4.8	1.8	3.9
10	15.4	13.8	4.8	3.9	4.5
12	19.0	18.9	5.4	4.7	5.2
16	25.4	21.2	7.5	6.6	9.2
17	26.0	22.6	9.8	7.2	9.5
20	28.2	26.5	13.2	7.7	10.1
22	30.8	28.0	15.3	8.9	10.1
27	35.4	28.0	17.2	10.0	10.7
36	41.0	28.0	20.5	13.4	11.8
38	41.0	28.0	20.5	13.4	11.8
40	41.0	28.0	20.5	13.4	11.8
45	41.0	28.0	20.5	13.4	11.8

Table A-5. SN-70 – Test # 5

Days	Cumulative gas production, ml				
	Blank (0 mM SN-70)	SN-70 (2.09 mM)	SN-70 (4.18 mM)	SN-70 (20.9 mM)	SN-70 (41.8 mM)
0	0	0	0	0	0
2	0	0	0	0	0
4	0	0	0	0	0
8	9	0	0.2	4	3.2
10	9.2	0	0.2	4	3.2
12	12.4	0.4	0.6	4.8	3.6
14	13	0.4	0.6	4.8	3.6
15	13.4	1.6	4.4	5.4	4.6
17	15.4	3.6	7.2	8.8	7.2
19	16	4.4	8.8	9.4	7.2
20	18.2	5	9.2	9.4	7.6
22	20.6	5.2	9.2	9.4	8
25	24.8	5.2	9.2	9.4	8
28	24.8	5.2	9.2	9.4	8
30	24.8	5.2	9.2	9.4	8
35	24.8	5.2	9.2	9.4	8

Table A-6. SN-70 – Test # 6

Days	Cumulative gas production, ml				
	Blank (0 mM SN-70)	SN-70 (2.09 mM)	SN-70 (4.18 mM)	SN-70 (20.9 mM)	SN-70 (41.8 mM)
0	0	0	0	0	0
2	10	0	0	0	0
3	10	2.8	4.2	3.4	2.8
4	14.6	2.8	4.2	3.4	2.8
6	15	2.8	4.2	3.4	2.8
8	15.2	2.8	5.8	3.4	3.1
10	15.4	2.8	9	3.6	5
12	19	2.8	9.2	4	5.4
14	19.4	2.8	9.2	4	5.5
16	19.6	2.8	9.6	4.2	5.7
18	19.6	2.8	9.6	4.2	5.7
20	19.6	2.8	9.6	4.2	5.7
22	19.6	2.8	9.6	4.2	5.7
27	19.6	2.8	9.6	4.2	5.7
36	28	2.8	10.4	4.6	5.9
56	29.2	3.4	11.4	5	8.3
67	29.2	3.4	11.4	5	8.3
80	29.2	3.4	11.4	5	8.3

Table A-7. SN-70 – Test # 7

Days	Cumulative gas production, ml				
	Blank (0 mM SN-70)	SN-70 (0.05 mM)	SN-70 (0.10 mM)	SN-70 (0.50 mM)	SN-70 (1.0 mM)
0	0	0	0	0	0
2	0	0	0	0	0
4	0	0	0	0	0
7	1.8	0.2	0.2	0.1	0.1
10	2.5	0.4	0.4	0.1	0.3
12	2.7	0.6	0.5	0.1	0.3
27	3.4	1.2	1	0.1	0.3
38	5.8	2.4	1.3	0.3	0.5
53	6.8	2.6	1.5	0.5	0.7
58	7.1	2.8	1.9	0.7	1.1
63	7.3	3	2	0.8	1.3
66	7.3	3	2	0.8	1.3
70	7.3	3	2	0.8	1.3
80	7.3	3	2	0.8	1.3

Table A-8. T-205 – Test # 8

Days	Cumulative gas production, ml				
	Blank (0 mM T-205)	T-205 (1.45 mM)	T-205 (2.90 mM)	T-205 (14.5 mM)	T-205 (29.0 mM)
0	0	0	0	0	0
2	0	0	0	0	0
4	0	0	0	0	0
8	9	2.6	0.2	0	0
10	9.2	2.6	0.2	0	0
12	12.4	3.2	0.6	4.8	5.8
14	13	3.2	0.6	4.8	5.8
15	13.4	3.6	1.4	5	5.8
17	15.4	5.6	2.6	5.6	6
19	16	5.8	2.8	5.8	6.2
20	18.2	6	3.2	5.8	6.2
22	20.6	6	3.2	5.8	6.2
25	24.8	6	3.2	5.8	6.2
28	24.8	6	3.2	5.8	6.2
30	24.8	6	3.2	5.8	6.2
35	24.8	6	3.2	5.8	6.2

Table A-9. T-205 – Test # 9

Days	Cumulative gas production, ml				
	Blank (0 mM T-205)	T-205 (1.45 mM)	T-205 (2.90 mM)	T-205 (14.5 mM)	T-205 (29.0 mM)
0	0	0	0	0	0
2	10	0	2.2	5	0.2
3	10	4.2	5.8	5	4.6
4	14.6	4.2	5.8	5	4.6
6	15	4.2	5.8	5	4.6
8	15.2	4.4	6.2	5	4.6
10	15.4	4.8	7.2	5	4.8
12	19	4.9	7.4	5	5
14	19.4	4.9	7.4	5	5.1
16	19.6	4.9	7.4	5.1	5.3
18	19.6	4.9	7.4	5.1	5.3
20	19.6	4.9	7.4	5.1	5.3
22	19.6	4.9	7.4	5.1	5.3
27	19.6	4.9	7.4	5.1	5.3
36	28	5.3	7.9	5.1	5.5
56	29.2	5.3	7.9	5.3	5.9
67	29.2	5.3	7.9	5.3	5.9
80	29.2	5.3	7.9	5.3	5.9

Table A-10. T-205 – Test # 10

Days	Cumulative gas production, ml				
	Blank (0 mM T-205)	T-205 (0.05 mM)	T-205 (0.10 mM)	T-205 (0.50 mM)	T-205 (1.0 mM)
0	0	0	0	0	0
2	0	0	0	0	0
4	0	0	0	0	0
7	1.8	0	0	0	0.2
10	2.5	1.3	1.2	0.2	0.4
12	2.7	1.5	1.4	0.2	0.4
27	3.4	1.9	1.9	0.4	0.6
38	5.8	4.1	4.1	0.8	1.4
53	6.8	5.7	4.5	1	1.8
58	7.1	6	4.7	1.1	2
63	7.3	6.1	5	1.3	2.2
66	7.3	6.1	5	1.6	2.3
70	7.3	6.1	5	1.6	2.3
80	7.3	6.1	5	1.6	2.3

Table A-11. CTAB-ink – Test # 11

Days	Cumulative gas production, ml									
	Blank (0 mM CTAB-ink)		CTAB-ink (0.07 mM)		CTAB-ink (0.36 mM)		CTAB-ink (0.71 mM)		CTAB-ink (1.43 mM)	
0	0	0	0	0	0	0	0	0	0	0
4	4.9	4.9	4.6	5	4.3	4.4	4	3.8	2.7	2.4
6	5.3	5.3	4.6	5	4.3	4.4	5.2	5.2	2.9	2.4
8	5.3	5.3	4.6	5	4.3	4.4	5.2	5.2	2.9	2.4
10	5.3	5.3	4.6	5	4.3	4.4	5.2	5.2	2.9	2.4
12	5.3	5.3	4.6	5	4.3	4.4	5.2	5.2	2.9	2.4
14	5.3	5.3	4.6	5	4.3	4.4	5.2	5.2	2.9	2.4
17	5.8	5.8	4.6	5	4.3	4.4	5.2	5.2	6.1	7.2
22	11.4	11.4	8.6	8.2	4.3	4.4	5.2	5.2	7.7	8.2
42	11.4	11.4	13.6	11.2	9.3	14.4	9.6	5.2	8.1	8.6
57	11.4	11.4	16.6	11.8	9.3	14.8	9.6	5.2	8.2	8.8
65	11.4	11.4	16.6	11.8	9.3	14.8	9.6	5.2	8.2	8.8

Table A-12. CTAB-ink– Test # 12

Days	Cumulative gas production, ml				
	Blank (0 mM CTAB-ink)	CTAB-ink (0.07 mM)	CTAB-ink (0.36 mM)	CTAB-ink (0.71mM)	CTAB-ink (1.43 mM)
65	0	0	0	0	0
68	2.5	1.8	2	1.1	2.6
73	6	4	4.5	2.8	5.8
80	6.2	9	10	4.4	6.2
93	6.4	10.5	10	4.4	6.2
100	6.4	12	10	4.4	6.4
108	6.4	13.4	10.2	4.6	6.6
113	6.6	16	12.4	6	7.5
120	7	16.5	12.8	6.3	7.8
125	7	16.7	13	6.5	8
130	7	16.7	13	6.5	8
135	7	16.7	13	6.5	8

Table A-13. CTAB-ink– Test # 13

Days	Cumulative gas production, ml			
	Blank (0 mM CTAB)	CTAB-ink (0.05 mM)	CTAB-ink (0.50 mM)	CTAB-ink (1.00 mM)
0	0	0	0	0
2	5.8	5.1	2.8	0.6
3	5.8	6.1	3.1	1.0
4	10.4	10.5	3.2	1.1
6	11.0	10.8	4.3	1.8
8	16.2	12.1	5.6	2.5
10	15.4	12.6	6.3	2.6
12	19.0	13.9	6.4	2.7
16	25.4	20.2	10.6	3.9
17	26.0	21.6	11.1	3.9
20	28.2	24.4	13.7	5.1
22	30.8	27.1	14.8	5.5
27	35.4	33.0	19.5	7.8
36	41.0	38.5	19.9	7.8
38	41.0	38.5	19.9	7.8
40	41.0	38.5	19.9	7.8
45	41.0	38.5	19.9	7.8

Table A-14. SN-70-ink – Test # 14

Days	Cumulative gas production, ml									
	Blank (0 mM SN-70-ink)		SN-70-ink (0.07 mM)		SN-70-ink (0.36 mM)		SN-70-ink (0.71 mM)		SN-70-ink (1.43 mM)	
0	0	0	0	0	0	0	0	0	0	0
4	4.9	4.9	4.3	3.4	3.3	1	1	4.8	3.4	0.8
6	5.3	5.3	4.7	4.2	3.5	1	1	5	3.6	1
8	5.3	5.3	4.7	4.2	3.5	1	1	5	3.6	1
10	5.3	5.3	4.7	4.2	3.5	1	1	5	3.6	1
12	5.3	5.3	4.7	4.2	3.5	1	1	5	3.6	1
14	5.3	5.3	4.7	4.2	3.5	1	1	5	3.6	1
17	5.8	5.8	4.7	4.2	3.5	1	1	8.4	7.4	2
22	11.4	11.4	4.7	4.2	6.1	4	2.4	10	8.6	3.4
42	11.4	11.4	9.1	4.2	7.1	4.6	4.4	14.4	10	5.8
57	11.4	11.4	9.2	4.2	7.3	5.1	4.6	14.6	10	5.8
65	11.4	11.4	9.2	4.2	7.3	5.1	4.6	14.6	10	5.8

Table A-15. SN-70-ink – Test # 15

Days	Cumulative gas production, ml				
	Blank (0 mM SN-70-ink)	SN-70-ink (0.07 mM)	SN-70-ink (0.36 mM)	SN-70-ink (0.71mM)	SN-70-ink (1.43 mM)
65	0	0	0	0	0
68	2.5	0	1.4	2.2	2
73	6	0	3	5	5
80	6.2	3	3.4	8	6
93	6.4	4.4	3.6	9.4	6.4
100	6.4	4.5	3.8	9.6	6.4
108	6.4	5.2	4.3	10.3	7.4
113	6.6	6.6	5.2	11.6	8.2
120	7	6.8	5.6	11.8	8.6
125	7	7	5.8	12	8.8
130	7	7	5.8	12	8.8
135	7	7	5.8	12	8.8

Table A-16. T-205-ink – Test # 16

Days	Cumulative gas production, ml									
	Blank (0 mM T-205-ink)		T-205-ink (0.07 mM)		T-205-ink (0.36 mM)		T-205-ink (0.71 mM)		T-205-ink (1.43 mM)	
0	0	0	0	0	0	0	0	0	0	0
4	4.9	4.9	2.4	3	0.6	0.6	0.6	3.6	1	0.8
6	5.3	5.3	2.4	3	0.8	0.6	0.8	3.6	1	0.8
8	5.3	5.3	2.6	3.2	0.8	0.6	0.8	3.6	1	0.8
10	5.3	5.3	2.8	3.3	0.8	0.6	0.8	3.6	1	0.8
12	5.3	5.3	2.8	3.3	0.8	0.6	0.8	3.6	1	0.8
14	5.3	5.3	2.8	3.3	0.8	0.6	0.8	3.6	1	0.8
17	5.8	5.8	2.8	3.3	0.8	0.6	0.8	3.6	1	0.8
22	11.4	11.4	8.2	8.5	1.4	1.4	4	4	1	0.8
42	11.4	11.4	8.8	9.1	1.4	1.6	4	4	1	0.8
57	11.4	11.4	8.8	9.1	1.4	1.6	4	4	1.1	1
65	11.4	11.4	8.8	9.1	1.4	1.6	4	4	1.1	1

Table A-17. T-205-ink – Test # 17

Days	Cumulative gas production, ml				
	Blank (0 mM T-205-ink)	T-205-ink (0.07 mM)	T-205-ink (0.36 mM)	T-205-ink (0.71mM)	T-205-ink (1.43 mM)
65	0	0	0	0	0
68	2.5	2.2	0.3	1.3	0
73	6	5.4	0.8	3.2	0
80	6.2	5.7	0.8	3.2	0
93	6.4	6	1	3.2	0
100	6.4	6	1	3.2	0
108	6.4	6	1	3.2	0.2
113	6.6	7.6	1.6	3.6	1.5
120	7	8	2	4	1.8
125	7	8	2	4	1.8
130	7	8	2	4	1.8
135	7	8	2	4	1.8

APPENDIX B – Aerobic study

Table B-1. Aerobic study - Tests # 1, 3, 5, 7, 9, and 11

i) Comparison of DO's of the surfactants during 0 – 3 days

Sample	Day 0 (mg/ L)	Day 1 (mg /L)	Day 2 (mg/ L)	Day 3 (mg/ L)
Seed Control	9.18	8.45	8.36	8.16
Surfactants				
CTAB (1.18 uM)	9.18	8.52	8.38	8.38
SN-70 (4.87 uM)	9.17	8.14	7.92	7.70
T-205 (1.35 uM)	9.18	8.49	8.32	8.20
Surfactant-ink mixtures				
CTAB-ink (0.33 uM)	9.18	8.58	8.56	8.51
SN-70-ink (0.33 uM)	9.18	8.43	8.33	8.13
T-205-ink (0.83 uM)	9.17	8.42	8.23	8.00

ii) Comparison of DO's of the surfactants during 4 – 7 days

Sample	Day 4 (mg/ L)	Day 5 (mg /L)	Day 6 (mg/ L)	Day 7 (mg/ L)
Seed Control	7.70	7.28	6.94	6.69
Surfactants				
CTAB (1.18 uM)	8.14	7.96	7.63	7.36
SN-70 (4.87 uM)	7.22	6.74	6.34	6.07
T-205 (1.35 uM)	7.85	7.57	7.19	6.92
Surfactant-ink mixtures				
CTAB-ink (0.33 uM)	8.27	7.93	7.35	7.06
SN-70-ink (0.33 uM)	7.64	7.20	6.83	6.57
T-205-ink (0.83 uM)	7.52	7.09	6.72	6.47

iii) Comparison of DO's of the surfactants during 8- 15 days

Sample	Day 8 (mg/ L)	Day 9 (mg /L)	Day 14 (mg/ L)	Day 15 (mg/ L)
Seed Control	6.56	6.37	5.09	4.82
Surfactants				
CTAB (1.18 uM)	7.06	6.76	6.68	6.56
SN-70 (4.87 uM)	5.93	5.72	4.42	4.14
T-205 (1.35 uM)	6.66	6.36	6.18	6.07
Surfactant-ink mixtures				
CTAB-ink (0.33 uM)	6.89	6.43	6.27	6.14
SN-70-ink (0.33 uM)	6.42	6.19	4.91	4.63
T-205-ink (0.83 uM)	6.34	6.15	4.86	4.59

iv) Comparison of DO's of the surfactants during 16 - 25 days

Sample	Day 16 (mg/ L)	Day 21 (mg /L)	Day 23 (mg/ L)	Day 25 (mg/ L)
Seed Control	4.32	4.16	4.16	4.16
Surfactants				
CTAB (1.18 uM)	6.43	6.05	6.05	6.05
SN-70 (4.87 uM)	3.63	3.46	3.46	3.46
T-205 (1.35 uM)	5.92	3.99	3.99	3.99
Surfactant-ink mixtures				
CTAB-ink (0.33 uM)	6.10	4.58	4.58	4.58
SN-70-ink (0.33 uM)	4.13	3.97	3.97	3.97
T-205-ink (0.83 uM)	4.09	3.93	3.93	3.93

Table B-2. Aerobic study – Tests # 2, 4, 6, 8, 10, and 12

i) Comparison of the DO's of the various surfactants during 0- 2 days

Sample	Day 0 (mg/ L)		Day 1 (mg/ L)		Day 2 (mg/ L)	
	(1)	(2)	(1)	(2)	(1)	(2)
Seed Control	8.41	8.43	8.06	8.13	7.99	8.07
Surfactants						
CTAB (0.01 uM)	8.43	8.43	8.11	8.17	8.04	8.04
CTAB (0.1 uM)	8.42	8.42	8.17	8.14	8.20	8.12
CTAB (1.0 uM)	8.35	8.34	8.06	8.08	7.71	7.73
SN-70 (1.0 uM)	8.52	8.49	8.18	8.13	8.18	8.13
SN-70 (10 uM)	8.54	8.53	7.37	7.37	4.98	4.86
SN-70 (100 uM)	8.53	8.57	4.82	4.90	0.14	0.14
T-205 (0.01 uM)	8.42	8.40	8.22	8.37	8.22	8.37
T-205 (0.1 uM)	8.42	8.41	8.39	8.43	8.39	8.43
T-205 (1.0 uM)	8.86	8.84	8.69	8.66	8.69	8.66
Surfactant-ink mixtures						
CTAB-ink (0.01 uM)	8.42	8.39	8.42	8.41	8.42	8.41
CTAB-ink (0.1 uM)	8.54	8.56	8.48	8.50	8.37	8.36
CTAB-ink (1.0 uM)	8.77	8.74	8.54	8.54	8.39	8.37
SN-70-ink (0.01 uM)	8.83	8.86	8.57	8.66	8.57	8.66
SN-70-ink (0.1 uM)	8.74	8.73	8.55	8.55	8.55	8.55
SN-70-ink (1.0 uM)	8.73	8.71	8.63	8.59	8.54	8.55
T-205-ink (0.01 uM)	8.81	8.83	8.73	8.71	8.73	8.71
T-205-ink (0.1 uM)	8.82	8.85	8.59	8.60	8.59	8.60
T-205-ink (1.0 uM)	8.87	8.89	8.54	8.57	8.54	8.57

ii) Comparison of the DO's of the various surfactants during 3 - 6 days

Sample	Day 4 (mg/ L)		Day 5 (mg/ L)		Day 6 (mg/ L)	
	(1)	(2)	(1)	(2)	(1)	(2)
Seed Control	7.85	7.91	7.65	7.69	7.55	7.65
Surfactants						
CTAB (0.01 uM)	7.94	7.94	7.75	7.73	7.68	7.64
CTAB (0.1 uM)	8.08	7.89	7.85	7.69	7.77	7.58
CTAB (1.0 uM)	7.40	7.52	7.15	7.28	7.01	7.14
SN-70 (1.0 uM)	7.76	7.88	7.75	7.63	7.74	7.58
SN-70 (10 uM)	2.92	2.91	2.30	2.28	1.91	1.90
SN-70 (100 uM)	0.14	0.14	0.14	0.14	0.14	0.14
T-205 (0.01 uM)	8.08	8.37	8.00	8.26	7.96	8.06
T-205 (0.1 uM)	8.19	8.25	7.98	8.06	7.84	7.95
T-205 (1.0 uM)	8.49	8.44	8.41	8.37	8.38	8.34
Surfactant-ink mixtures						
CTAB-ink (0.01 uM)	8.36	8.34	8.26	8.30	8.22	8.27
CTAB-ink (0.1 uM)	7.24	7.26	7.09	7.01	6.98	6.90
CTAB-ink (1.0 uM)	0.76	3.72	0.43	0.73	0.13	0.20
SN-70-ink (0.01 uM)	8.56	8.66	8.49	8.61	8.46	8.57
SN-70-ink (0.1 uM)	8.28	8.27	8.11	8.10	8.03	8.00
SN-70-ink (1.0 uM)	4.53	4.57	3.50	3.62	3.09	3.20
T-205-ink (0.01 uM)	8.58	8.60	8.47	8.54	8.43	8.50
T-205-ink (0.1 uM)	8.39	8.42	8.21	8.27	8.11	8.18
T-205-ink (1.0 uM)	8.46	8.56	8.37	8.48	8.33	8.43

iii) Comparison of the DO's of the various surfactants during 7- 27 days

Sample	Day 9 (mg/ L)		Day 20 (mg/ L)		Day 27 (mg/ L)	
	(1)	(2)	(1)	(2)	(1)	(2)
Seed Control	7.49	7.48	5.69	5.75	5.32	5.06
Surfactants						
CTAB (0.01 uM)	7.68	7.60	5.93	5.73	5.47	5.31
CTAB (0.1 uM)	7.77	7.57	6.45	6.10	6.15	5.78
CTAB (1.0 uM)	6.92	7.02	7.35	6.02	5.31	4.91
SN-70 (1.0 uM)	7.56	7.41	5.68	5.57	4.93	5.29
SN-70 (10 uM)	1.48	1.63	0.68	1.41	0.15	0.75
SN-70 (100 uM)	0.14	0.14	0.10	0.10	0.10	0.10
T-205 (0.01 uM)	7.91	7.95	6.51	6.42	5.98	5.50
T-205 (0.1 uM)	7.76	7.63	7.54	7.42	5.41	5.14
T-205 (1.0 uM)	8.36	8.27	4.73	4.23	3.47	2.52
Surfactant-ink mixtures						
CTAB-ink (0.01 uM)	8.14	8.03	7.20	7.33	5.45	6.51
CTAB-ink (0.1 uM)	6.83	6.53	6.41	6.51	5.53	5.77
CTAB-ink (1.0 uM)	0.13	0.16	0.13	0.15	0.13	0.15
SN-70-ink (0.01 uM)	8.33	8.47	6.41	6.71	5.82	6.24
SN-70-ink (0.1 uM)	7.83	7.78	6.19	5.51	5.71	5.01
SN-70-ink (1.0 uM)	2.56	2.41	1.13	0.18	1.26	0.14
T-205-ink (0.01 uM)	8.14	8.28	6.31	6.57	5.30	5.82
T-205-ink (0.1 uM)	7.99	7.97	6.65	7.31	5.03	5.72
T-205-ink (1.0 uM)	8.10	8.17	4.19	3.96	2.94	3.12

iv) Comparison of the DO's of the various surfactants during 28- 32 days

Sample	Day 30 (mg/ L)		Day 32 (mg/ L)	
	(1)	(2)	(1)	(2)
Seed Control	5.32	5.06	5.32	5.06
Surfactants				
CTAB (0.01 uM)	5.47	5.31	5.47	5.31
CTAB (0.1 uM)	6.15	5.78	6.15	5.78
CTAB (1.0 uM)	5.31	4.91	5.31	4.91
SN-70 (1.0 uM)	4.93	5.29	4.93	5.29
SN-70 (10 uM)	0.15	0.75	0.15	0.75
SN-70 (100 uM)	0.10	0.10	0.10	0.10
T-205 (0.01 uM)	5.98	5.50	5.98	5.50
T-205 (0.1 uM)	5.41	5.14	5.41	5.14
T-205 (1.0 uM)	3.47	2.52	3.47	2.52
Surfactant-ink mixtures				
CTAB-ink (0.01 uM)	5.45	6.51	5.45	6.51
CTAB-ink (0.1 uM)	5.53	5.77	5.53	5.77
CTAB-ink (1.0 uM)	0.13	0.15	0.13	0.15
SN-70-ink (0.01 uM)	5.82	6.24	5.82	6.24
SN-70-ink (0.1 uM)	5.71	5.01	5.71	5.01
SN-70-ink (1.0 uM)	1.26	0.14	1.26	0.14
T-205-ink (0.01 uM)	5.30	5.82	5.30	5.82
T-205-ink (0.1 uM)	5.03	5.72	5.03	5.72
T-205-ink (1.0 uM)	2.94	3.12	2.94	3.12

APPENDIX C - Check for the theoretical gas production of controls

Method to calculate theoretical gas production in controls for Test # 1 (Sawyer et al., 1994):

For anaerobic degradation,
the equation for fermentation of glucose is given by,

$R = 0.25\ CH_2O + 0.014\ HCO_3^- + 0.014\ NH_4^+$
$\rightarrow 0.014\ CO_2 + 0.056\ H_2O + 0.09\ CH_4 + 0.014\ C_5H_7O_2N$

$\Rightarrow$ 0.25 mol or 7.5 gm of CH_2O results in production of 0.104 mol or 2.33 litres of CO_2 and 0.09 mol or 0.02 litres (STP) of CH_4.

For Test # 1,

600 mg/ L of glucose was added to the 70 ml test controls (i.e. 0.042 gm) whose fermentation would result in production of

$\frac{0.042 \times 2.33}{7.5}$ = 13.05 ml of CO_2, and

$\frac{0.042 \times 2.02}{7.5}$ = 11.31 ml of CH_4

=> Theoretical gas production = 24.36 ml (0°C) = 26.6 ml (25°C)

The measured gas production in the control to which 600 mg/L glucose was added was 24.8 ml which compares well with the theoretical gas production (26.6 ml).

VITA

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