CDM

Technical Memorandum

To: *John Scott, P.E., BRC*
Robert Scott, BRC
Joseph Weidmann, P.G., Haley and Aldrich

From: *Patrick Evans, Ph.D., CDM*
Ryan A. Wymore, P.E., CDM
Ian Lo, CDM

Cc: *Kent Sorenson, Ph.D., P.E., CDM*
Ravi Subramanian, P.E., CDM

Date: *May 25, 2007*

Subject: *FINAL - Results of Electron Donor Laboratory Treatability Study*
Remediation of Volatile Organic Compounds
Former C-6 Facility, Los Angeles, California

1.0 Introduction

Camp Dresser & McKee Inc (CDM) has prepared this Memorandum summarizing the laboratory treatability study conducted to evaluate electron donors and bioaugmentation cultures for remediation of volatile organic compound (VOC)-impacted groundwater at the Former C-6 Facility (Site). The primary contaminant of concern at this Site is trichloroethene (TCE) with 1,1-dichloroethene (1,1-DCE) as a secondary contaminant of concern. The study was conducted in accordance with the CDM documents titled "Pre-Remediation Workplan (CDM, February 14, 2006) which was approved by the Los Angeles Regional Water Quality Control Board (RWQCB) on February 23, 2006 and "Pre-Remediation Implementation Work Plan (CDM, August 7, 2006)

The objectives of this study were as follows:

- Determine the efficiency of select electron donors and bioaugmentation cultures in achieving complete reductive dechlorination of TCE to ethene;
- Determine the potentially toxic effects of chloroform and 1,1,1-trichloroethane on reductive dechlorination of TCE; and

- Evaluate the life-time and shelf life of each of the electron donors.
- Evaluate factors affecting the selection including any mixing, filtration, or other pretreatment requirements.
- Evaluate donor stability and potential for precipitation or fermentation under ambient storage conditions.

Electron donors for this treatability study were selected based on the results of a background "paper" evaluation conducted by CDM (CDM, October 10, 2006) and included sodium citrate, whey, sodium lactate, proprietary JRW mother liquor, Newman Zone, and whey plus proprietary JRW nutrient. Except for Newman Zone, all the electron donors are fast-release electron donors. Newman Zone is a slow release electron donor and slowly ferments to molecular hydrogen. JRW mother liquor is a fermentation product and JRW nutrient is a proprietary nutrient; they were provided by JRW Bioremediation LLC of Lenexa, Kansas.

Two bioaugmentation cultures were also selected for this treatability study based on the CDM background "paper" evaluation. The Sirem KB-1™ culture was obtained from SiREM laboratory. The KB-1™ culture is a natural dechlorinating microbial consortium that contains phylogenetic relatives of *Dehalococcoides* ethenogenes (Major *et al.*, 2002). The Shaw SDC-9™ is also a natural dechlorinating culture, and was obtained from Shaw Environmental, Inc. This culture was enriched by Shaw from a Navy site to treat chlorinated solvent-contaminated aquifers. The SDC-9™ culture contains *Dehalococcoides* spp. (DHC) bacteria that degrade chlorinated solvents to ethene (www.shawgrp.com/bioaugmentation/technologybioaugmentation.aspx).

The following sections provide: a description of the treatability study methods; the results of the study along with a detailed discussion of the data; conclusions developed from the study; recommendations for donor and bioaugmentation culture selection and further action, as appropriate; and a rough order of magnitude (ROM) economic analysis of capital and electron donor usage costs specific to the recommended donors.

2.0 Treatability Study Methodologies

2.1 Setup

The treatability study was conducted in CDM's environmental treatability laboratory in Bellevue, Washington. Groundwater samples for microcosm setup were collected by Avocet Environmental, Inc. (Avocet) on October 23rd, 2006 from the on-Site C-Sand monitoring well IRZCMW003 located at the Former Building 2 area of the Site. Two soils samples were also collected by Avocet on October 24th and 25th from the B-sand and C-sand aquifers, respectively, for microcosm setup during installation of well MWC024 at the Former Building 2 area of the Site. Two 12-inch-long samples were collected from each of three intervals, two

in the B-Sand (64.5 to 66.5 feet below ground surface [bgs] and 68 to 70 feet bgs) and one in the C-Sand (114.5 to 116.5 feet bgs). Detailed information regarding sample collection and handling procedures are included in the document titled "Well Completion Report, Boeing Realty Corporation, Former C-6 Facility, 1451 & 1452 Knox Street" (Avocet, February 6, 2007).

Table 1 presents the microcosm set-up. These microcosms were set up between October 25 and 30, 2006. Microcosms were constructed in duplicate in serum bottles in an anaerobic chamber with a nitrogen atmosphere. Each microcosm contained 50 grams (g) of soil sample (homogenized mixture of B-Sand and C-Sand soil) and 150 milliliters (mL) of groundwater sample. Microcosms were sealed with thick butyl rubber stoppers and aluminum crimp tops. Electron donors were added to microcosms as described in Table 1. The bottles were then removed from the anaerobic chamber. Any residual oxygen in the microcosms was allowed to be depleted by aerobes in the soil prior to the addition of bioaugmentation cultures as described in Table 1. Microcosms were then pressurized with ultra high purity nitrogen to 5 pounds per square inch (psi). Each microcosm was spiked with approximately 6,000 micrograms per liter (µg/L) of TCE, 450 µg/L of chloroform (CF), and 100 µg/L of 1,1,1-trichloroethane (TCA). Following baseline sampling and analysis, microcosms were re-spiked with 4,300 µg/L of TCE, 190 µg/L of CF, and 66 µg/L of TCA because observed concentrations were less than intended. CF and TCA were added to the microcosms to test for their potential toxicity towards bioaugmentation cultures because these contaminants are present at certain areas of the Site where bioaugmentation is being considered. Microcosms were stored upside down in the dark at room temperature (16°C to 22°C).

2.2 Laboratory Sampling and Analysis

A total of seven sampling events were conducted between November 1, 2006 and March 13, 2007. Sampling frequency was on a biweekly basis for the first four sampling events, and decreased to a monthly basis for the last three sampling events. For each sampling event, the following analyses were performed:

- pH – measured with conventional pH meter.
- Sulfate (SO_4) – measured with Hach DR/2010 Spectrophotometer (Method 8051)
- Chemical Oxygen Demand (COD) – measured with Hach DR/2010 Spectrophotometer (Method 8000)
- VOCs – Purge and trap sampling and analysis by gas chromatography with flame ionization detection.
- Methane, Ethane, and Ethene – Headspace sampling and analysis by gas chromatography with flame ionization detection.

The initial VOC analysis was conducted by OnSite Environmental of Redmond, Washington because of equipment failure associated with the CDM GC. Carbon dioxide (CO_2) and volatile fatty acids (VFAs) analyses were also performed at the end of the study. CO_2 was sampled in the headspace and measured with gas chromatography with thermal conductivity detection. Liquid samples were sent to Microseeps, Inc. for VFA analysis.

3.0 Treatability Study Results and Discussion

3.1 TCE Biodegradation

Figures 1 to 4 show TCE biodegradation in the microcosms over time. Figure 1 shows the sterile and non-sterile control microcosms with no added electron donor or bioaugmentation culture. Figures 2, 3, and 4 show microcosms with various electron donors with no bioaugmentation, with Sirem KB-1™ culture, and with Shaw SDC-9™ culture, respectively. The control microcosms confirm that significant TCE degradation was not observed without electron donor addition. The increase in TCE concentration on Day 16 was due to the second addition of VOCs. The gradual decline in TCE concentrations was attributable to abiotic losses and/or analytical variability. Table 2 summarizes all findings.

3.2 Electron Donor Effects

Table 2 shows that 10 microcosms transformed TCE to vinyl chloride (VC) in 31-49 days, which is the fastest of all microcosms. Nine of these microcosms contained whey or lactate. It is therefore concluded that whey and lactate had the fastest response times with respect to transformation to VC. Microcosms with whey, lactate, and Newman Zone were the most effective with respect to complete reductive dechlorination of TCE to ethene – eight out of the nine microcosms demonstrating complete reductive dechlorination contained whey, lactate, or Newman Zone. Sodium lactate promoted the fastest TCE to ethene conversion in 49-65 (bottle 17A) and 65-93 (bottle 17B) days. Sodium lactate appears to be the fastest electron donor in driving complete TCE to ethene dechlorination, although two Newman Zone and three whey microcosms also achieved dechlorination to ethene in 65-93 days.

3.3 Bioaugmentation Culture Effects

Eight out of 12 microcosms inoculated with Shaw SDC-9™ demonstrated complete TCE dechlorination to ethene in less than 134 days. Seven out of the eight Shaw SDC-9 ™ microcosms containing whey, lactate, or Newman Zone demonstrated complete reductive dechlorination to ethene. On the other hand, only one out of twelve microcosms inoculated with Sirem KB-1 ™ bioaugmentation culture demonstrated complete reduction of TCE to ethene in the same amount of time.

None of the microcosms without bioaugmentation achieved complete TCE to ethene conversion. Conversion to VC was observed in 11 out of 12 of these microcosms. This result is consistent with groundwater data for previously biostimulated locations at the Site, which

showed that dechlorination of TCE may have stalled at VC. This suggests that bioaugmentation is a requirement for complete reduction of TCE to ethene within a reasonable time.

3.4 Gas Production

Headspace analysis was used to estimate gas production in the microcosms (Figures 9 to12). Additional CO_2 and methane analyses were performed on selected microcosms (Table 3) on day 144, subsequent to the last sampling event. Results show minimal (i.e., less than 0.5% methane in the headspace) methane production in most microcosms (i.e., both unbioaugmented and bioaugmented) and minimal CO_2 production in all microcosms. In Shaw culture microcosms amended with whey, sodium lactate, or Newman Zone, relatively greater methane production was observed (i.e., 2 to 5% methane in the headspace). In one Sirem culture microcosm amended with whey plus nutrient, relatively greater methane production was observed (i.e., 3 to 4% methane in the headspace. Thus gas production with the Shaw culture and lactate or whey does not appear to be significant. Gas production with the Shaw culture and Newman Zone may be significant.

3.5 CF and TCA Toxicity Effects

Previous unpublished data provided by Shaw have shown that the SDC-9™ culture is capable of chloroform and TCA degradation. In this study, chloroform was degraded to below detection limit in microcosms bioaugmented with Shaw SDC-9™ culture and amended with sodium lactate and Newman Zone (Table 2). TCE was also degraded completely to ethene in these microcosms. During the final sampling event, chloroform was present in microcosms containing Shaw culture and whey plus nutrient. These microcosms also achieved complete TCE to ethene conversion. This demonstrates that in addition to Shaw's finding that the SDC-9™ culture is capable of chloroform degradation, it is also capable of achieving complete TCE degradation in the presence of chloroform. In contrast, during the final sampling event in microcosms with Sirem KB-1 ™ culture, chloroform was present in all but one bottle. Notably, this lone microcosm with no chloroform was also the only microcosm that was able to achieve complete reductive dechlorination of TCE to ethene. Based on these observations, it is apparent that the Shaw SDC-9 ™ culture was relatively more resistant than the Sirem KB-1 ™ culture to the toxicity or other form of inhibition associated with the added chloroform, either through its ability to transform CF, or its ability to reductively dechlorinate TCE to ethene in its presence.

3.6 Electron Donor Longevity

COD levels remained relatively constant throughout the study, even as TCE was being completely reduced (Figures 5 to 8). Microcosm gas production (Figures 9 to 12) showed insignificant methane production except in a few microcosms as noted in Section 3.4. Based on these observations, CDM's hypothesis for the lingering high levels of COD was that the

carbon sources added to the microcosms were not being converted completely into methane and carbon dioxide (CO_2); rather, they were being partially fermented to VFAs. This partial degradation could provide sufficient electrons to promote reductive dechlorination but may not lead to significant COD reduction. To evaluate this hypothesis, 10 microcosm bottles were selected for CO_2 and VFA analysis subsequent to the last sampling round on day 144. These results are presented in Table 3. Table 3 demonstrates that the electron donors were partially fermented to various VFAs, and that by taking VFAs and CO_2 into account, the COD recovery is close to 100% for all microcosms except those with Newman Zone. Newman Zone is known to be adsorbed to soil, so low COD recovery was expected. Total organic carbon (TOC) recovery of close to 100% for all microcosms except Newman Zone further validated the hypothesis. The following paragraphs discuss the longevity of sodium lactate, Newman Zone, and whey:

Sodium lactate

Based on the VFA analysis (Table 3), five out of six lactate-amended microcosms contained significant amount of propionic acid five months after the donors were injected. In the remaining microcosm (17A), acetic acid was the major VFA instead of propionic acid. Combined with the fact that microcosm 17A achieved complete TCE to ethene dechlorination in 49 to 65 days, it was estimated that sodium lactate could potentially have a longevity in the aquifer of at least two months and possibly longer than five months, which is longer than what is typically observed for lactate. These longevity estimates are believed to be due to the persistence of the propionate and acetate that are derived from the lactate. That being said, it is possible that propionate and acetate would be degraded more quickly in the field than was seen in the lab study. Because of this, the longevity of lactate would have to be validated during the pilot study.

Newman Zone

Since Newman Zone is an emulsified oil and it is adsorbed in the soil, a carbon balance could not be accurately performed. However, based on its usage at other sites, the life span of Newman Zone in soil is typically estimated to be one to two years.

Whey

Analyses for VFAs were not performed for microcosms amended with whey because whey is not a pure compound and electron balance calculations using whey would have been overly complicated for the scope of this study. However, since the COD changes over time of whey were similar to those of sodium lactate, it is assumed that the life span of whey is similar to that of sodium lactate. However, previous CDM experience suggests that whey may have a longer life span than sodium lactate.

3.7 Electron Donor Solubility and Shelf Life

The longevities and shelf life of sodium lactate, whey, and Newman Zone were evaluated by placing solutions containing these materials in bottles exposed to air and allowing them to stand at room temperature during the treatability study. Shelf life and other properties of each electron donors are presented in Table 4. These data indicate that sodium citrate, sodium lactate, and Newman Zone were stable and the other electron donors were perishable (i.e. biofilms growing in solution). However, it should be noted that the whey used in this study (and the whey that would be used in the field) is delivered to the site in powdered form rather than as a solution. The powdered form of whey has a shelf life of up to 2 years based on CDM experience.

All electron donors were completely soluble except whey and JRW nutrient. Previous studies indicated that a 10% whey solution contained about 23,000 mg/L total suspended solids (MacBeth et al., 2006). While this concentration is relatively high, CDM experience has been that injection of such whey solutions containing suspended solids has not resulted in well or aquifer plugging. Additionally, the solids may promote longevity of the whey. JRW nutrient at 1 grams per liter (g/L) did not go into solution even with vigorous mixing and heating, indicating limited solubility compared to other electron donors.

3.8 Supplemental Data

Supplemental data is provided in Appendix A (Figures 1A through 8A) and consist of pH and sulfate data for the various microcosms.

4.0 Economic Analysis

Based on the lab study results, both lactate and whey are expected to perform well in the field. While injection of whey does involve more manual labor and more equipment (e.g., handling and mixing equipment) than lactate, it offers the potential for significant cost savings over lactate. This cost savings is due to the lower material costs and more electron donor content for whey compared to lactate. Table 5 shows the results of a budgetary economic analysis of annual whey and lactate usage costs for a 50 gallon per minute (gpm) biorecirculation system assuming an injection frequency of 10% (approximately 17 hours a week) . For this scenario, using whey would cost approximately 50% less than using lactate ($688,000 compared to $1,350,000), even considering the additional labor costs to manually operate the whey mixing equipment. The mass rate difference between whey and lactate shown in Table 5 is solely attributable to lactate being a 60% solution and present as the sodium salt (96 lb/lb-mole) rather than as lactic acid (77 lb/lb-mole). These costs includes material costs and labor costs specific only to donor handling and do not include the capital costs for whey handling and mixing equipment. However assuming estimated capital costs of $100,000 to $200,000 (based on past experience) for whey mixing and handling equipment, the costs are still 33% less than lactate for this scenario. These savings would increase

substantially as the scale of the application increases. As stated in Table 5, the cost of whey ($0.87/lb) is highly volatile; changes in whey costs will affect this cost estimate but are unlikely to change the conclusion that whey is more cost-effective than lactate.

In addition to the above economic advantages, it has been shown at other sites that whey is able to enhance the dissolution of contaminants from the residual source at a faster rate than lactate (MacBeth et al., 2006). This can result in a shorter remediation timeframes.

5.0 Conclusions

The following conclusions were developed based on the results of this study:

- Whey, sodium lactate, and Newman Zone show the most promise as potential electron donors for use at the Site. Complete reductive dechlorination of TCE to ethene was observed in SDC-9™-bioaugmented microcosms with these donors.

- Sodium lactate was the fastest in achieving complete reductive dechlorination of TCE to ethene. It potentially has a life span in the Site aquifer of at least two months and possibly greater than five months, based on its fermentation to acetate and propionate. It had a shelf life of greater than five months.

- Whey exhibited the fastest response time in terms of TCE to VC conversion. It was capable of driving complete TCE to ethene dechlorination, although not as fast as lactate. It is expected to have an aquifer life span similar to that of sodium lactate. Whey was not completely soluble and filtration may be required. However previous CDM experience with whey indicated that filtration is not required. Therefore CDM recommends that whey be injected unfiltered and the potential effects of plugging being monitored during the pilot test. If plugging becomes an issue, filtration of whey or use of lactate can be implemented across the site. Once in solution, whey is perishable and had a shelf life of less than two months based on CDM experience. Powdered whey, however, has a shelf-life of two years based on CDM experience, as previously indicated in Section 3.7.

- Newman Zone performed consistently. It was capable of driving complete TCE to ethene dechlorination and typically has a long aquifer life span of one to two years and possibly longer. It had a shelf life of greater than five months.

- The addition of JRW nutrient may have slightly improved the performance of microcosms with whey as electron donor. It is, however, poorly soluble.

- The indigenous microbial community (i.e., microcosms without bioaugmentation) drove dechlorination of TCE to VC, but not to ethene. This is consistent with field observations in biostimulated areas at the Site and provides further evidence that bioaugmentation will be required to achieve complete dechlorination to ethene in a reasonable timeframe.

- Shaw SDC-9™ culture had the ability to utilize a wide range of electron donors and achieve complete reductive dechlorination of TCE to ethene. Based on the data collected during this study, it was relatively resistant to chloroform and even appears to be capable of chloroform transformation. This is consistent with data provided by Shaw from their own studies (Unpublished data provided to CDM by Dr. Robert Steffan of Shaw).

- Sirem KB-1™ culture appeared to be sensitive to chloroform. It could potentially achieve complete reductive dechlorination of TCE to ethene with no chloroform present.

- Partial fermentation of electron donors was observed in all microcosms selected for VFA analysis. It can therefore be concluded that, although COD analyses showed almost constant values throughout the study, electron donor utilization was in fact occurring. VFA analysis provided good COD and TOC recoveries and was an adequate tool for electron donor concentration assessment.

  As indicated in Section 3.6, whey was not selected for VFA analysis because of impurities in whey that would have overly complicated electron balance calculations.

- Gas production in most of the microcosms was less than expected. This suggests that gas blockage during electron donor injection in the field is not likely to be a significant issue with these electron donors. It should be noted, however, that Newman Zone produced the greatest amount of methane compared to other electron donors.

6.0 Recommendations

The following recommendations were developed based on the results of this study:

- Based on the lab study results and the economic analysis presented here, it is recommended that whey be used initially for the pilot study, and lactate be used if logistical issues (i.e. mixing, storage, handling, etc) or technical performance issues (e.g., solids plugging of injection wells) are encountered with whey.

- Newman Zone is recommended for slug injections if planned at the Site. Methane production should be taken into account and at the minimum monitored in the field when using Newman Zone.

- Sodium citrate and JRW mother liquor were inconsistent performers and are not recommended.

- Shaw SDC-9™ culture is the recommended choice for bioaugmentation.

- Sirem KB-1™ culture is not recommended for bioaugmentation at this site.

- JRW nutrient should not be used unless it can be modified by the manufacturer to increase its solubility.

7.0 References

Major DW *et al*. 2002. Field Demonstration of Successful Bioaugmentation To Achieve Dechlorination of Tetrachloroethene To Ethene. *Environ Sci Technol*. **36**(23):5106-5116.

Macbeth, T.W., L. Nelson, J.S. Rothermel, R.A. Wymore, and K.S. Sorenson Jr. 2006. Evaluation of Whey for Bioremediation of Trichloroethene Source Zones. *Bioremediation J.* **10**(3) :115-128.

8.0 Limitations

It should be noted that many of the conclusions from this study (especially comparisons of electron donors and cultures) are site-specific because they depend on the indigenous microbial community, groundwater geochemistry, soil chemistry, and contaminant mixture. Therefore some of the findings of this treatability study will not be transferable to other sites.

9.0 Attachments

Tables

Table 1 – Treatability Study Design
Table 2 – Summary of Results
Table 3 – COD and TOC Summary
Table 4 – Shelf Life of Electron Donors
Table 5 – Budgetary Economic Analysis of Whey and Lactate Costs

Figures

Figure 1 – TCE Degradation in Control Microcosms
Figure 2 – TCE Degradation in Microcosms with No Bioaugmentation
Figure 3 – TCE Degradation in Microcosms with Sirem KB-1 Bioaugmentation Culture
Figure 4 – TCE Degradation in Microcosms with Shaw SDC-9 Bioaugmentation Culture
Figure 5 – COD- Control Microcosms
Figure 6 – COD- Microcosms with no Bioaugmentation
Figure 7 – COD- Microcosms with Sirem KB-1 Bioaugmentation Culture
Figure 8 – COD- Microcosms with Shaw SDC-9 Bioaugmentation Culture
Figure 9 – Headspace Gas Production in Control Microcosms
Figure 10– Headspace Gas Production in Microcosms with no Bioaugmentation
Figure 11– Headspace Gas Production in Microcosms Bioaugmented with Sirem KB-1 Culture
Figure 12– Headspace Gas Production in Microcosms Bioaugmented with Shaw SDC-9 Culture

Appendix A – Supplemental Data

Figure 1A – pH- Control Microcosms
Figure 2A – pH- Microcosms with no Bioaugmentation
Figure 3A – pH- Microcosms with Sirem KB-1 Bioaugmentation Culture
Figure 4A – pH- Microcosms with Shaw SDC-9 Bioaugmentation Culture
Figure 5A – SO4- Control Microcosms
Figure 6A – SO4- Microcosms with no Bioaugmentation
Figure 7A – SO4- Microcosms with Sirem KB-1 Bioaugmentation Culture
Figure 8A – SO4- Microcosms with Shaw SDC-9 Bioaugmentation Culture

Table 1 - Treatability Study Design

Bottle[a,b]	Culture[c]	Sterile[d]	Donor	Donor Concentration (g/L)	Initial Target Concentrations		
					TCE (µg/L)	CF (µg/L)	TCA (µg/L)
1	None	Yes	None	NA	6000	450	100
2	None	No	None	NA	6000	450	100
3	None	No	Sodium citrate	1	6000	450	100
4	None	No	Whey powder	1	6000	450	100
5	None	No	Sodium lactate	1	6000	450	100
6	None	No	JRW mother liquor[e]	1	6000	450	100
7	None	No	Newman Zone	1	6000	450	100
8	None	No	Whey + JRW nutrient[f]	1 + 0.1	6000	450	100
9	Sirem KB-1	No	Sodium citrate	1	6000	450	100
10	Sirem KB-1	No	Whey powder	1	6000	450	100
11	Sirem KB-1	No	Sodium lactate	1	6000	450	100
12	Sirem KB-1	No	JRW mother liquor[e]	1	6000	450	100
13	Sirem KB-1	No	Newman Zone	1	6000	450	100
14	Sirem KB-1	No	Whey + JRW nutrient[f]	1 + 0.1	6000	450	100
15	Shaw SDC-9	No	Sodium citrate	1	6000	450	100
16	Shaw SDC-9	No	Whey powder	1	6000	450	100
17	Shaw SDC-9	No	Sodium lactate	1	6000	450	100
18	Shaw SDC-9	No	JRW mother liquor[e]	1	6000	450	100
19	Shaw SDC-9	No	Newman Zone	1	6000	450	100
20	Shaw SDC-9	No	Whey + JRW nutrient[f]	1 + 0.1	6000	450	100

footnotes:

[a]Each bottle was setup in duplicate and labelled A and B

[b]Each bottle contains 50 grams of soil and 150 ml of groundwater from the C-6 Facility.

[c]Microcosms were inoculated with 1.5ml of Bioaugmentation culture estimated to contain 10^8 cells / ml.

[d]Sterilization was conducted by autoclaving at 20 psig for 20 minutes two days in succession.

[e]JRW mother liquor is a fermentation product provided by JRW.

[f]JRW nutrient is a proprietary nutrient provided by JRW.

Abbreviations:

NA = Not applicable

TCE = Trichloroethene

CF = Chloroform

TCA = 1,1,1-Trichloroethane

g/L = grams per liter

µg/L = micrograms per liter

ml = milliliter

Table 2 - Summary of Results

Bottle[a]	Culture	Donor	Duplicate	Observed time for reductive dechlorination of TCE (Days):			Final Concentrations		
				to cis-1,2-DCE	to Vinyl Chloride	to Ethene	TCE (µg/L)	CF (µg/L)	TCA (µg/L)
1	None	None	A	*>134*	*>134*	*>134*	6155	363	*<80*
			B	*>134*	*>134*	*>134*	5687	253	*<80*
2	None	None	A	*>134*	*>134*	*>134*	582	279	*<80*
			B	*>134*	*>134*	*>134*	2808	652	*<80*
3	None	Sodium citrate	A	**31**	**134**	*>134*	*<50*	315	*<80*
			B	**31**	*>134*	*>134*	*<50*	916	*<80*
4	None	Whey powder	A	**16**	**49**	*>134*	*<50*	239	*<80*
			B	**16**	**65**	*>134*	*<50*	254	*<80*
5	None	Sodium lactate	A	**31**	**93**	*>134*	*<50*	275	*<80*
			B	**31**	**93**	*>134*	*<50*	283	*<80*
6	None	JRW mother liquor	A	**31**	**93**	*>134*	*<50*	286	*<80*
			B	**31**	**93**	*>134*	*<50*	306	*<80*
7	None	Newman Zone	A	**31**	**134**	*>134*	*<50*	195	*<80*
			B	**31**	**93**	*>134*	*<50*	171	*<80*
8	None	Whey + JRW nutrient	A	**16**	**49**	*>134*	*<50*	308	*<80*
			B	**16**	**49**	*>134*	*<50*	299	*<80*
9	Sirem KB-1	Sodium citrate	A	**31**	**134**	*>134*	*<50*	359	*<80*
			B	**31**	**134**	*>134*	*<50*	340	*<80*
10	Sirem KB-1	Whey powder	A	**31**	**49**	*>134*	*<50*	265	*<80*
			B	**16**	**49**	*>134*	*<50*	283	*<80*
11	Sirem KB-1	Sodium lactate	A	**16**	**65**	*>134*	*<50*	300	*<80*
			B	**16**	**65**	*>134*	*<50*	292	*<80*
12	Sirem KB-1	JRW mother liquor	A	**31**	**65**	*>134*	*<50*	310	*<80*
			B	**16**	**65**	*>134*	*<50*	84	*<80*
13	Sirem KB-1	Newman Zone	A	**16**	**93**	*>134*	*<50*	209	*<80*
			B	**16**	**93**	*>134*	*<50*	180	*<80*
14	Sirem KB-1	Whey + JRW nutrient	A	**31**	**49**	**93**	<50	*<50*	*<80*
			B	**31**	**49**	*>134*	*<50*	278	*<80*
15	Shaw SDC-9	Sodium citrate	A	**16**	**93**	*>134*	*<50*	317	*<80*
			B	**16**	**93**	*>134*	*<50*	400	*<80*
16	Shaw SDC-9	Whey powder	A	**16**	**65**	*>134*	*<50*	329	*<80*
			B	**16**	**65**	**93**	<50	*<50*	*<80*
17	Shaw SDC-9	Sodium lactate	A	**16**	**49**	**65**	<50	*<50*	*<80*
			B	**16**	**49**	**93**	<50	*<50*	*<80*
18	Shaw SDC-9	JRW mother liquor	A	**31**	**49**	**134**	<50	292	*<80*
			B	**31**	*>134*	*>134*	*<50*	940	*<80*
19	Shaw SDC-9	Newman Zone	A	**16**	**93**	**93**	<50	*<50*	*<80*
			B	**16**	**93**	**93**	<50	*<50*	*<80*
20	Shaw SDC-9	Whey + JRW nutrient	A	**16**	**65**	**93**	<50	210	*<80*
			B	**16**	**65**	**134**	<50	250	*<80*

footnotes:

[a]Each bottle was setup in duplicate and labelled A and B

Abbreviations:

TCE = Trichloroethene

cis-1,2-DCE = cis-1,2-dichloroethene

CF = Chloroform

TCA = Trichloroethane

Table 3 - COD and TOC Summary

					Methane Production	CO_2 Production[b]			Volatile Fatty Acids Production[c]					COD						TOC		
Bottle	Donor	Culture	Rep	Initial Donor	Methane	$CO_{2,aq}$	$CO_{2,g}$	Total CO_2	Acetic	Butyric	Lactic	Propionic	Pyruvic	Initial Observed COD	Final Observed COD	Observed COD % Recovery	Intial Calculated COD[d]	Final Calculated COD[e]	Calculated COD % Recovery	Initial Calculated TOC[f]	Final Calculated TOC[g]	TOC % Recovery
				mmol[a]	mmol[a]	mmol[a]			mmol[a]					mmol[a]	mmol[a]	%	mmol[a]	mmol[a]	%	mmol[a]	mmol[a]	%
5	Sodium lactate	None	*A*	1.339	0.0036	0.2649	0.0517	0.317	0.699	0.003	0	0.729	0	4.69	4.34	92%	4.02	3.97	99%	4.02	3.92	98%
5	Sodium lactate	None	*B*	1.339	0.0064	0.4861	0.0917	0.578	0.724	0.005	0	0.729	0	4.73	4.44	94%	4.02	4.02	100%	4.02	4.24	106%
11	Sodium lactate	Sirem KB-1	*A*	1.339	0.0064	0.3683	0.0771	0.445	0.624	0.004	0	0.709	0	4.45	4.45	100%	4.02	3.75	93%	4.02	3.84	96%
11	Sodium lactate	Sirem KB-1	*B*	1.339	0.0067	0.3883	0.0758	0.464	0.624	0.004	0	0.709	0	4.41	4.39	99%	4.02	3.75	93%	4.02	3.86	96%
17	Sodium lactate	Shaw SDC-9	*A*	1.339	0.1988	0.3750	0.0897	0.465	1.524	0.007	0	0.172	0	5.02	4.66	93%	4.02	3.69	92%	4.02	4.26	106%
17	Sodium lactate	Shaw SDC-9	*B*	1.339	0.0131	0.3906	0.0860	0.477	0.949	0.008	0	0.729	0	4.95	5.20	105%	4.02	4.49	112%	4.02	4.61	115%
15	Sodium citrate	Shaw SDC-9	*A*	0.510	0.0067	0.4810	0.0729	0.554	0.999	0.016	0	0.004	0	2.40	2.63	110%	2.30	2.10	91%	3.06	2.64	86%
15	Sodium citrate	Shaw SDC-9	*B*	0.510	0.0068	0.4190	0.0623	0.481	1.049	0.005	0	0.002	0	2.45	2.48	101%	2.30	2.13	93%	3.06	2.61	85%
19	Newman Zone	Shaw SDC-9	*A*	0.170	0.5035	0.5686	0.0894	0.658	0.092	0.073	0	0.006	0	1.02	1.58	155%	6.28	0.57	9%	9.67	1.66	17%
19	Newman Zone	Shaw SDC-9	*B*	0.170	0.4977	0.4264	0.0722	0.499	0.072	0	0.233	0.007	0	0.94	2.34	250%	6.28	0.87	14%	9.67	1.86	19%

footnote:

[a]All concentrations are presented in mmol / microcosm bottle

[b]CO2 concentrations are calculated based on Headspace CO2 concentrations

[c]Odor and visual observations were performed on the stock solutions that have been stored on shelf for the indicated length of time.

[d]Initial calculated COD was calculated based on initial donor COD concentrations

[e]Final calculated COD was calculated based on CH4, CO2, and volatile fatty acids COD calculations

[f]Initial calculated TOC was calculated based on initial donor TOC concentration

[g]Final calculated TOC was calculated based on CH4, CO2, and volatile fatty acids TOC calculations

Abbreviations:

$CO_{2,aq}$ = Carbon Dioxide in the aqueous phase (including bicarbonate and carbonic acid)

$CO_{2,g}$ = Carbon Dioxide in the gas phase

COD = Chemical Oxygen Demand

TOC = Total Organic Carbon

mmol = milimoles (per microcosm bottle)

Table 4 - Shelf Life of Electron Donors

Donor / Nutrient	Stock Solution Concentration (g/L)	Solubility[a]	Time on Shelf	Observed Odor Changes[b]	Observed Visual Changes[b]
Sodium Citrate	100	5	5 months	None	None
Whey	100	3	1.5 months	None	Contains floating film (fungus)
Sodium Lactate	100	5	5 months	None	None
JRW Mother Liquor	400	5	5 months	None	Contains floating film, sediments
Newman Zone	500	5	5 months	None	None
JRW Nutrient	1	1	5 months	Stronger, sweeter, and more acidic odor	Contains floating film

footnote:

[a]Solubility observation at stock solution concentration:
1 - Insoluble
2 - Slightly Soluble
3 - Soluble with solids settling
4 - Soluble
5 - Soluble with little mixing

[b]Odor and visual observations were performed on the stock solutions that have been stored on shelf for the indicated length of time and compared to initial observations when the stock solutions were freshly made

Abbreviations:
g/L = grams per liter

TABLE 5
BUDGETARY ECONOMIC ANALYSIS OF WHEY AND LACTATE COSTS

Electron Donor Information	50 gpm Biorecirculation System
Sodium Lactate	
60% NaLac flowrate (lb/min)	26.2
60% NaLac weekly Usage (lbs)	26395
60% NaLac Flowrate (gpm)	2.34
Number of 45,000 lb (4,000 gal) tankers: # per wk	0.6
Tank volume (gal) for 1 wk supply	2346
Labor costs/week (specific to lactate equipment) - ASSUMED NONE	$ -
cost per lb (includes est freight and taxes)	$0.98
Approx cost per year	$1,350,000
Whey Powder	
Whey Powder flowrate (lb/min)	12.49
Whey Powder weekly Usage (lbs)	12589
Whey Powder 2,000 lb Totes/wk	6
Whey Powder storage space for 1 wk supply (sq ft)	101
cost per lb (includes estimated freight and taxes)	$ 0.87
Labor Costs/week (specific to whey handling equipment)	$ 2,250.00
Approx cost per year	$688,000

Key Assumptions:

An injection of 30,000 ppm donor 10% of the time (17 hrs/week) was assumed, which is essentially equivalent to injecting 3 ppm all the time.

Cost is +30% and -20 % accuracy

Electron donor material and transportation costs are HIGHLY volatile for Whey, so these prices are to be considered ROM only

Cost includes material, estimated transportation costs for bulk delivery, and estimated taxes of 8.25%

No other costs are included

For Whey, a powder feed system will be needed consisting of a vibrating bulk bag unloader system, a metered screw drive, small washdown hopper, and an injection eductor

Figure 1 - TCE Degradation in Control Microcosms

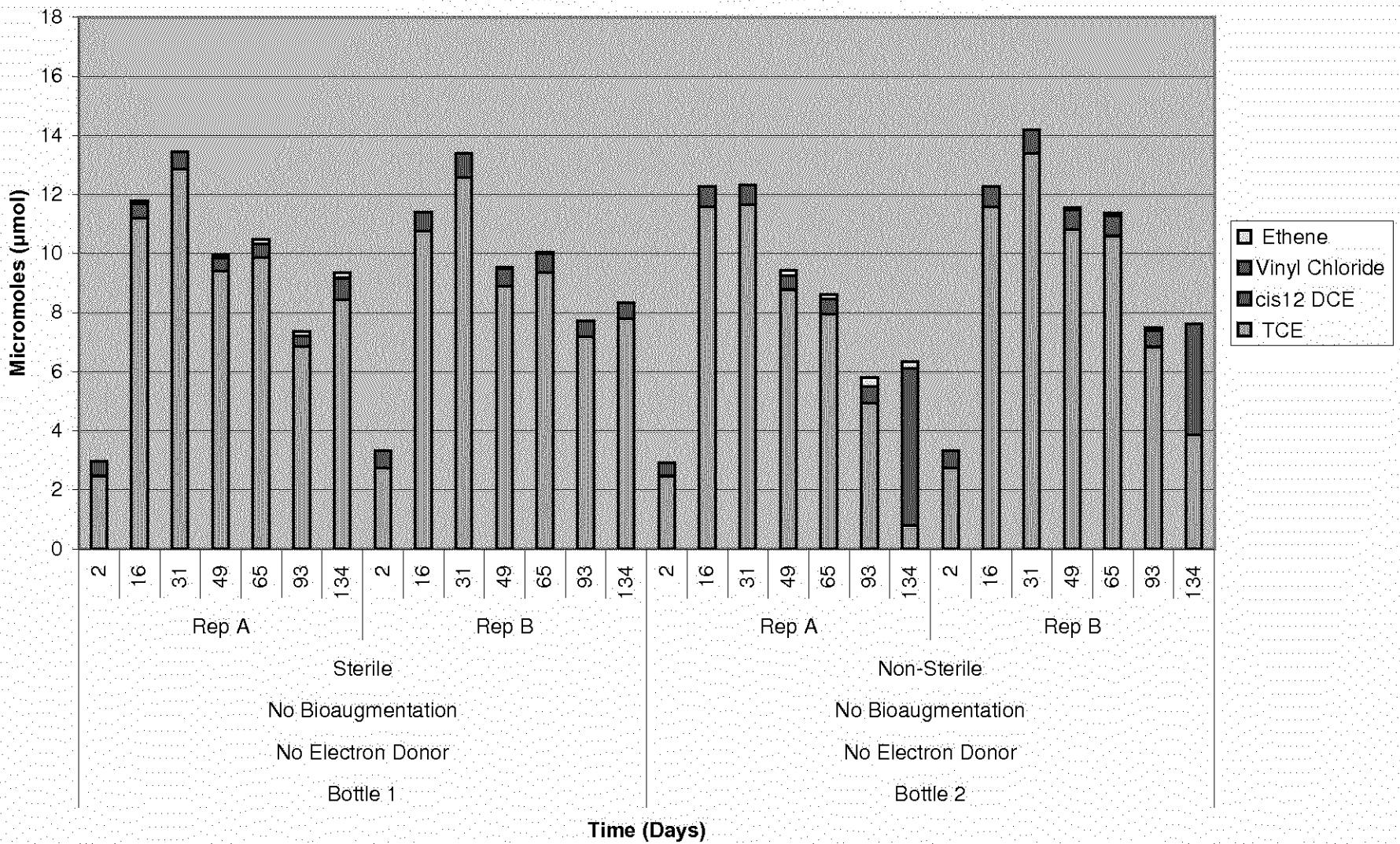

Figure 2 - TCE Degradation in Microcosms with No Bioaugmentation

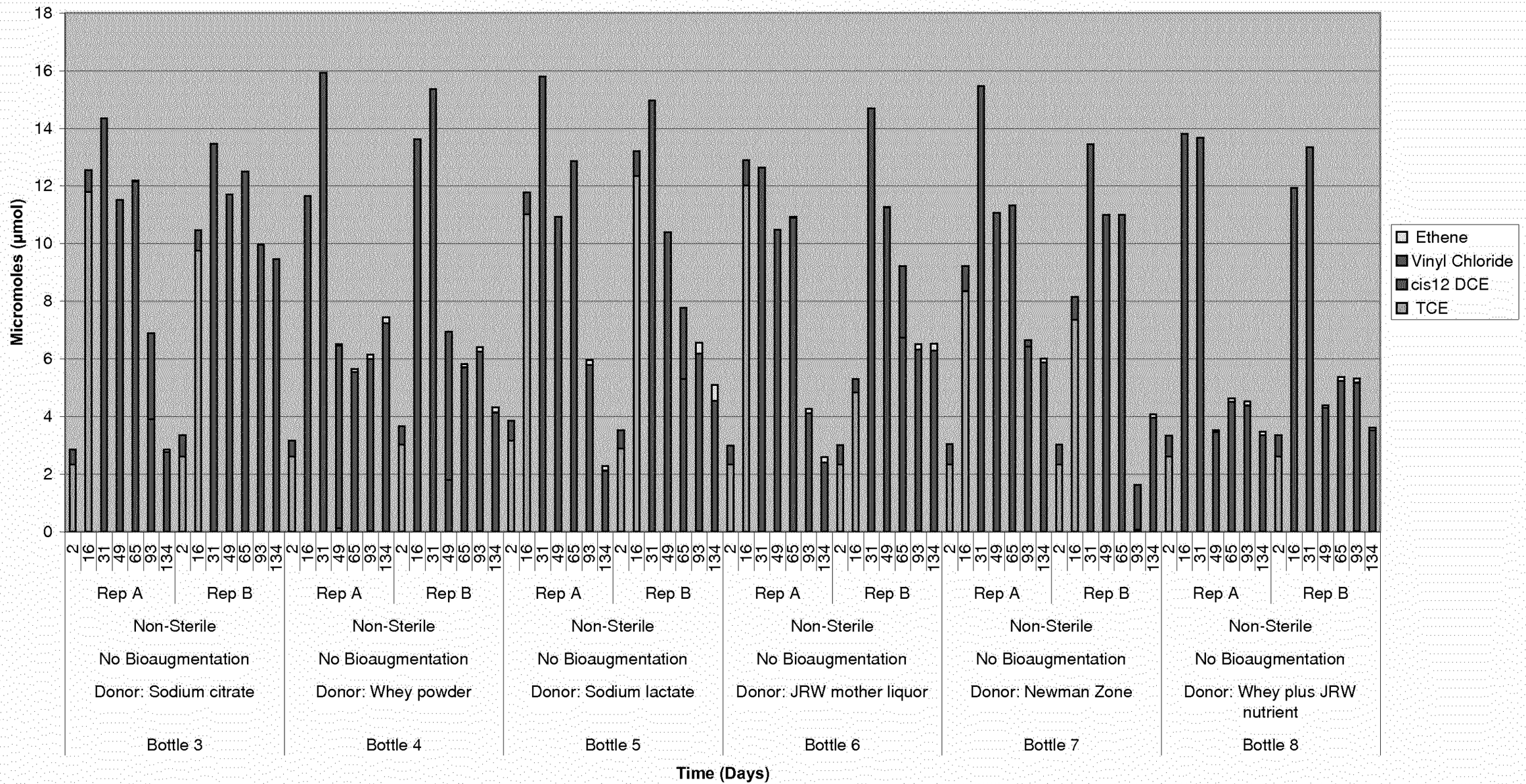

Figure 3 - TCE Degradation in Microcosms with Sirem KB-1 Bioaugmentation Culture

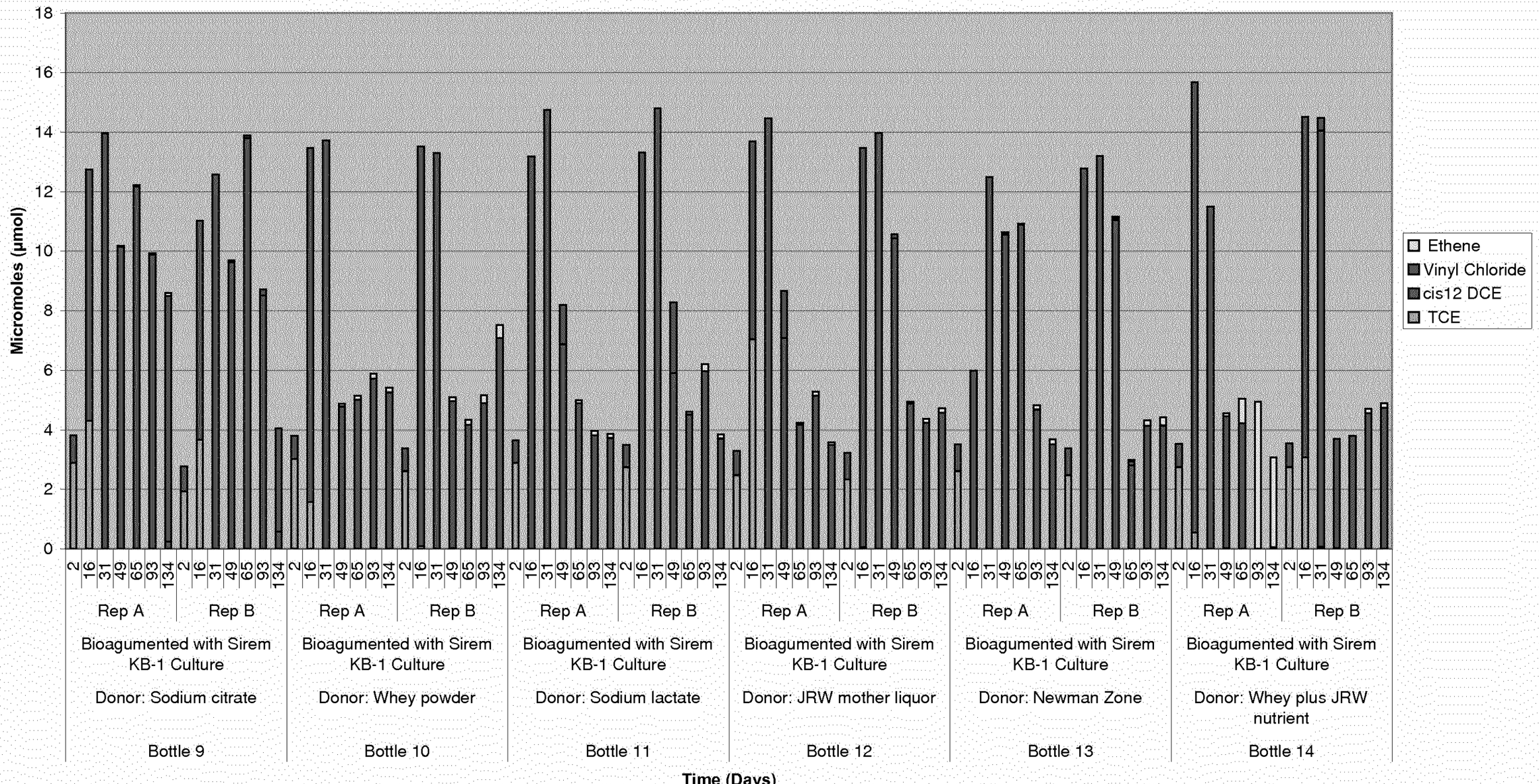

Figure 4 - TCE Degradation in Microcosms with Shaw SDC-9 Bioaugmentation Culture

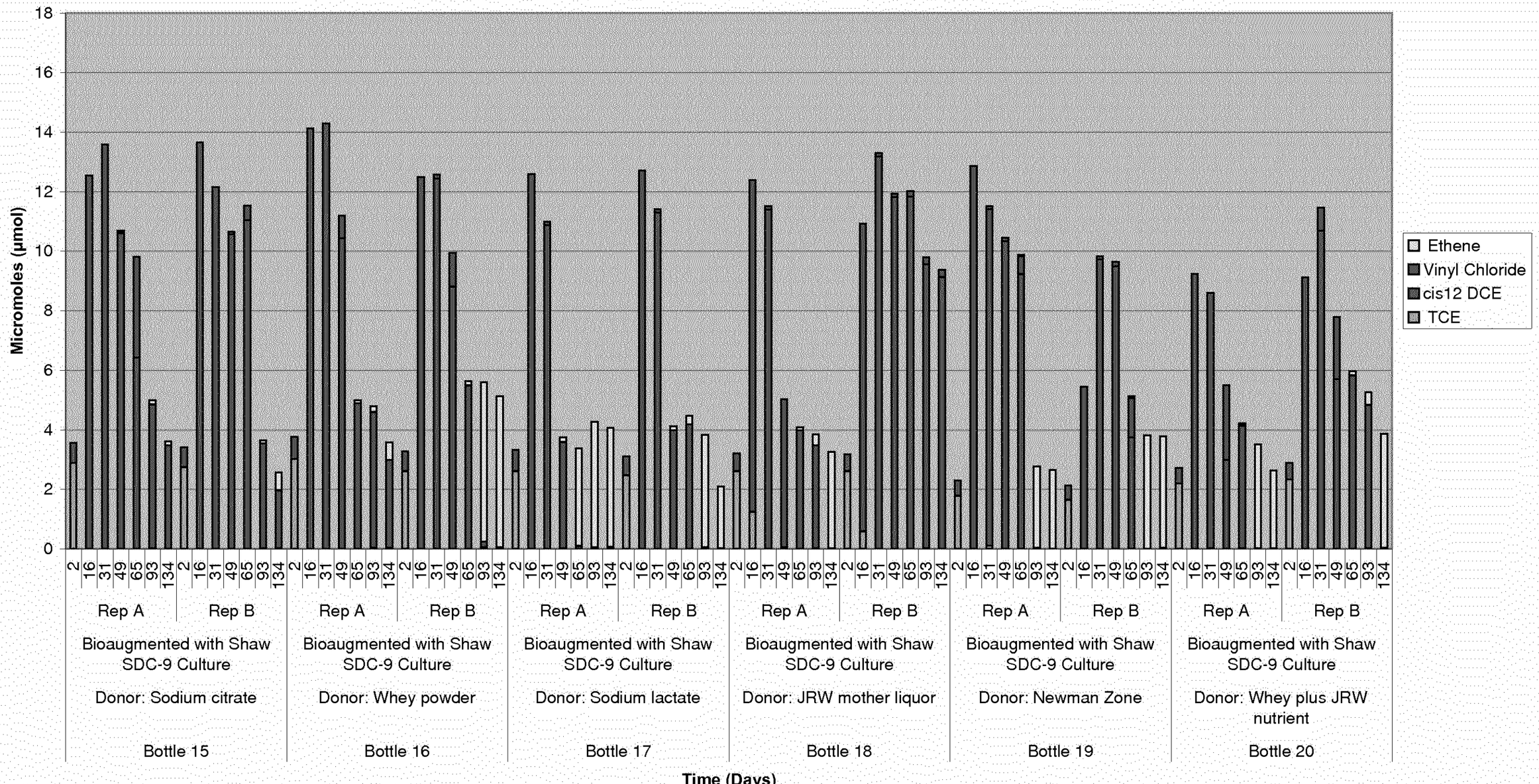

Figure 5 - COD - Control Microcosms

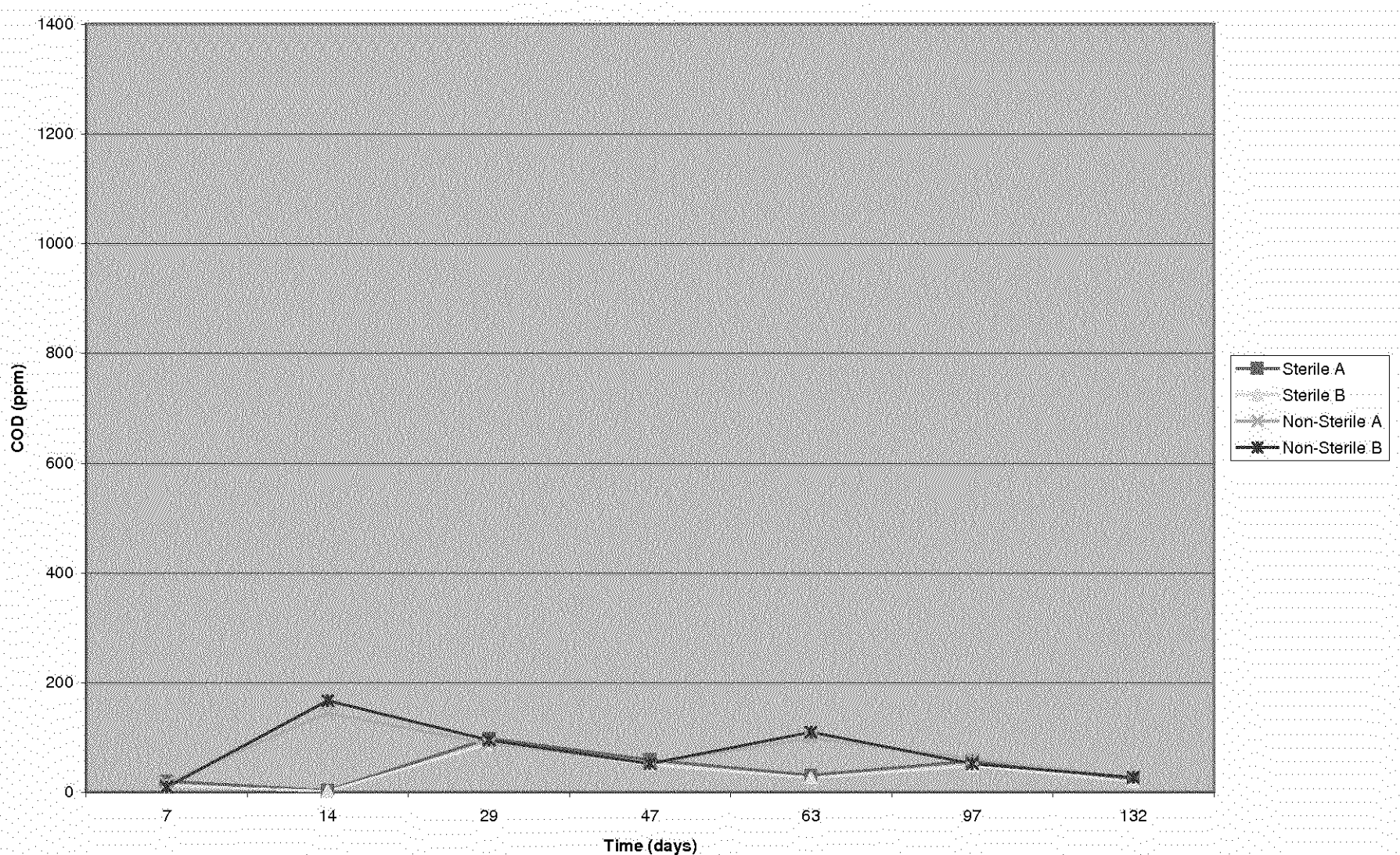

Figure 6 - COD - Microcosms with no Bioaugmentation

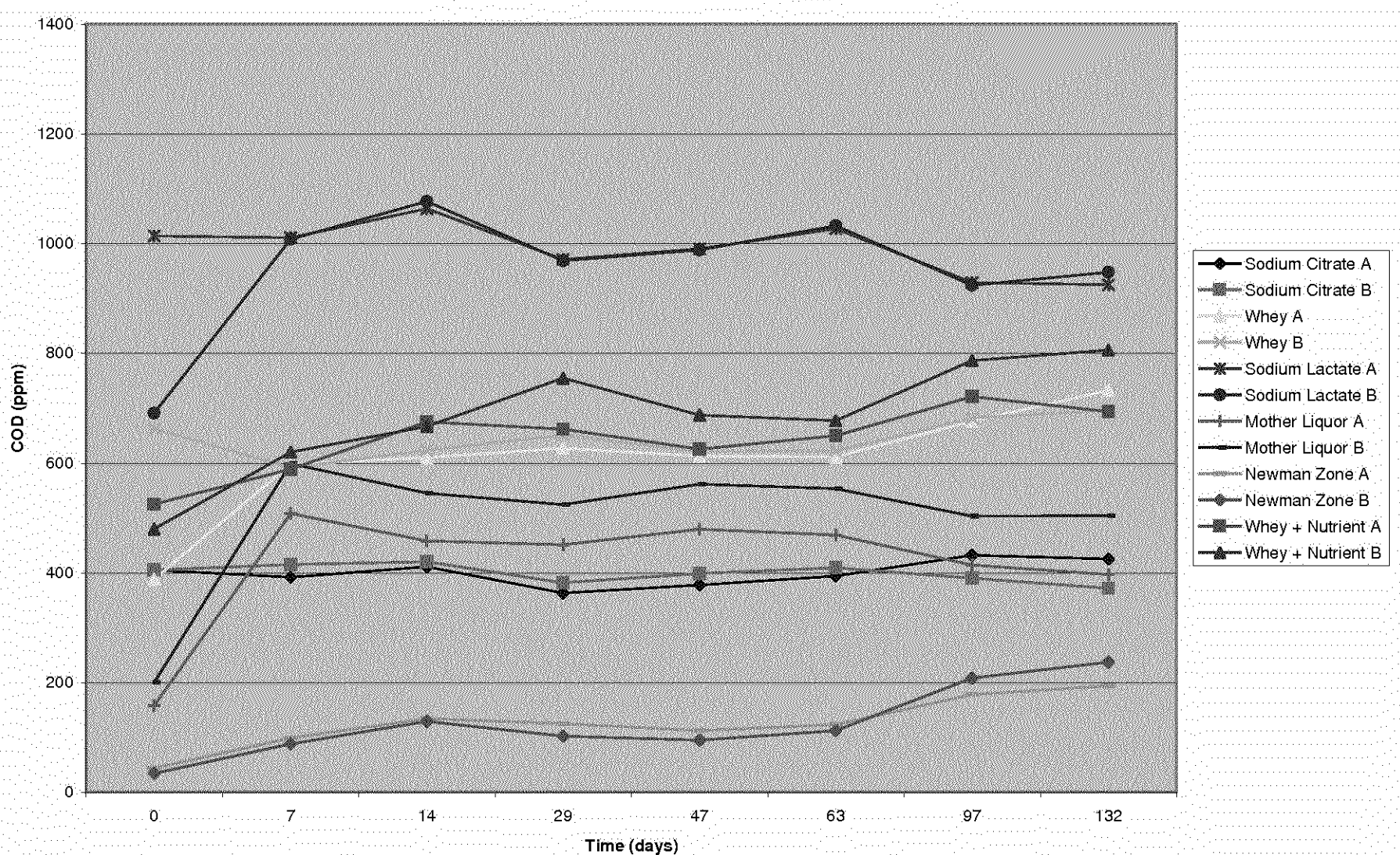

Figure 7 - COD - Microcosms with Sirem KB-1 Bioaugmentation Culture

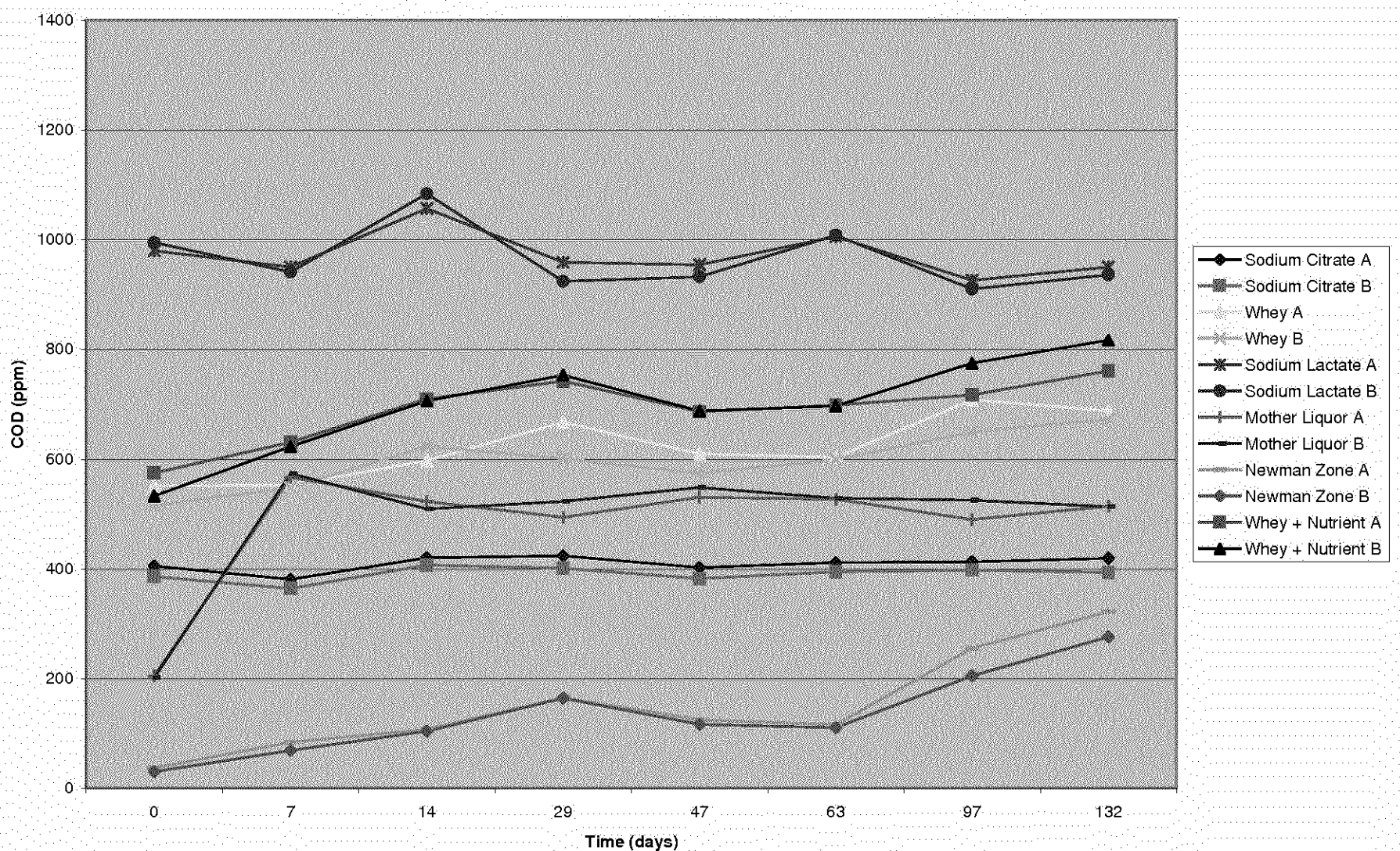

Figure 8 - COD - Microcosms with Shaw SDC-9 Bioaugmentation Culture

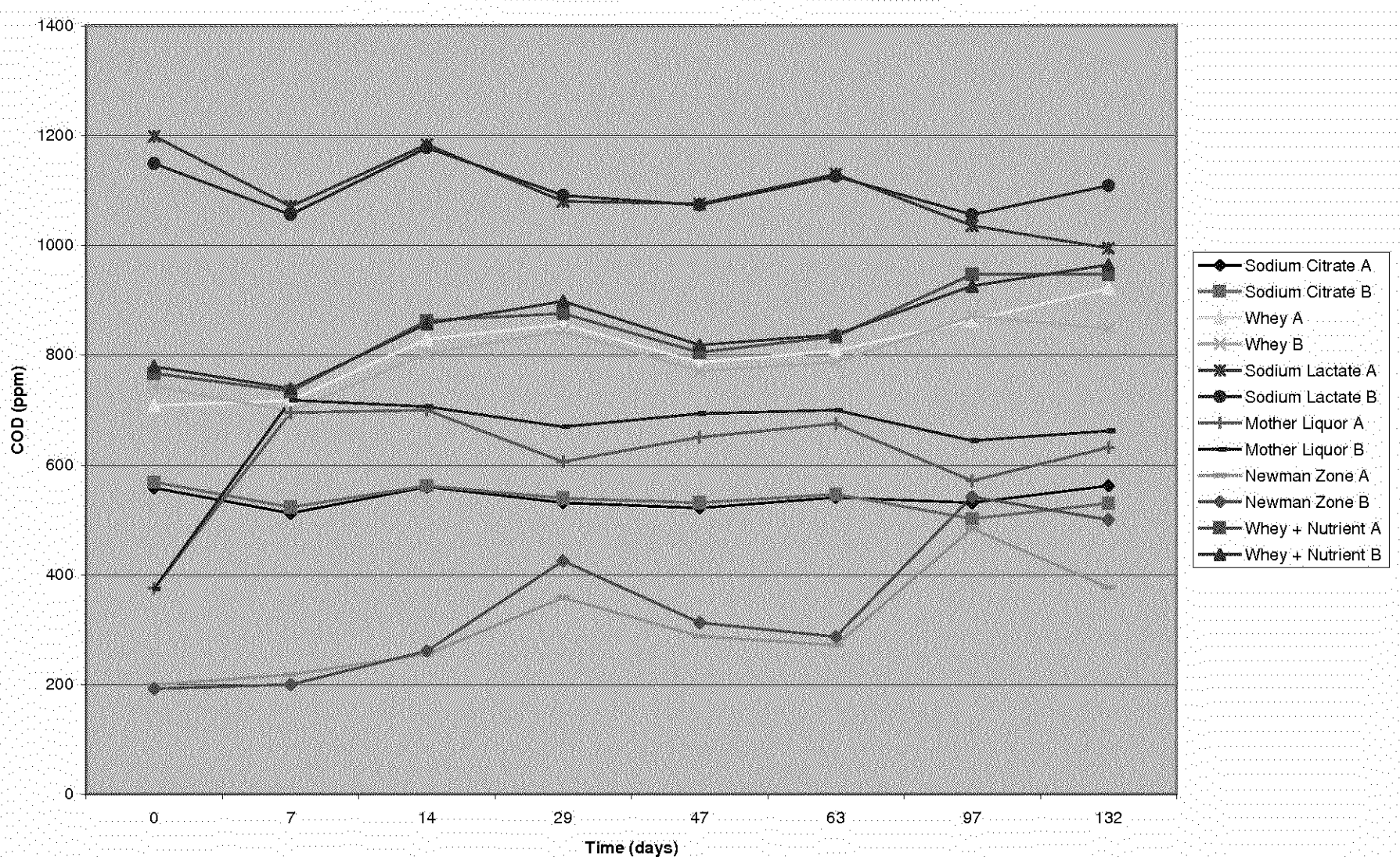

Figure 9 - Headspace Gas Production in Control Micorocosms

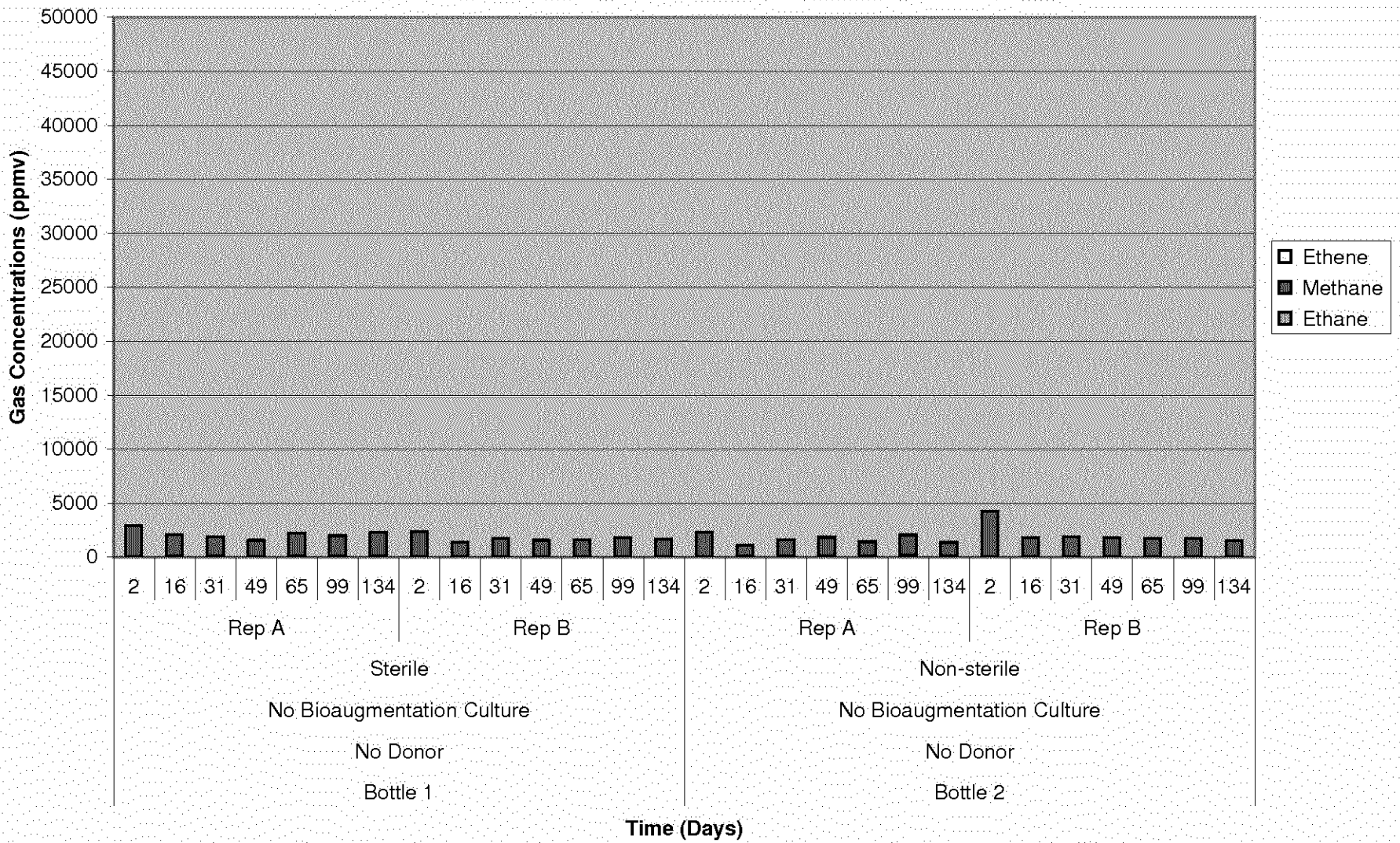

Figure 10 - Headspace Gas Production in Microcosms with No Bioaugmentation

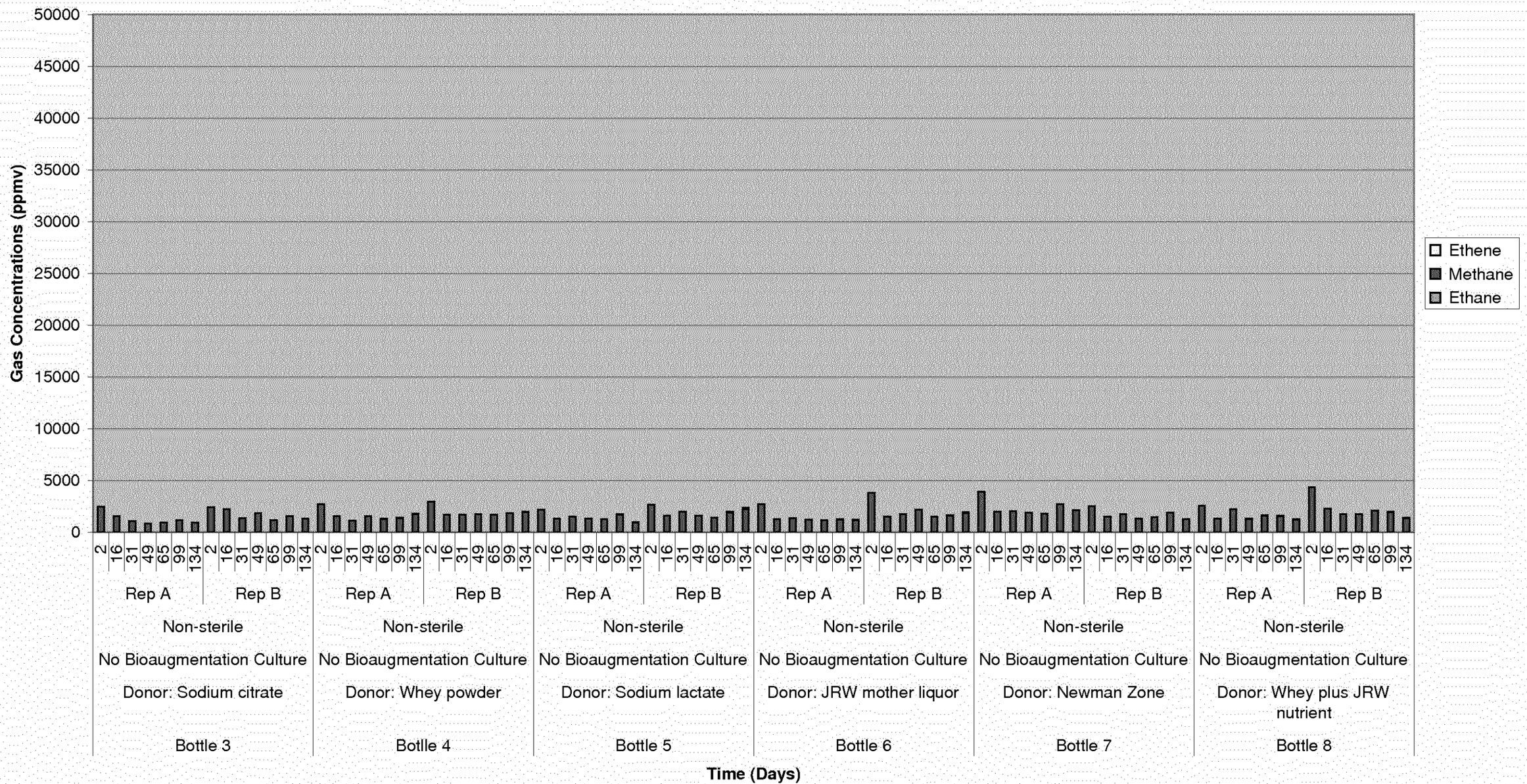

Figure 11 - Headspace Gas Production in Microcosms Bioaugmented with Sirem KB-1 Culture

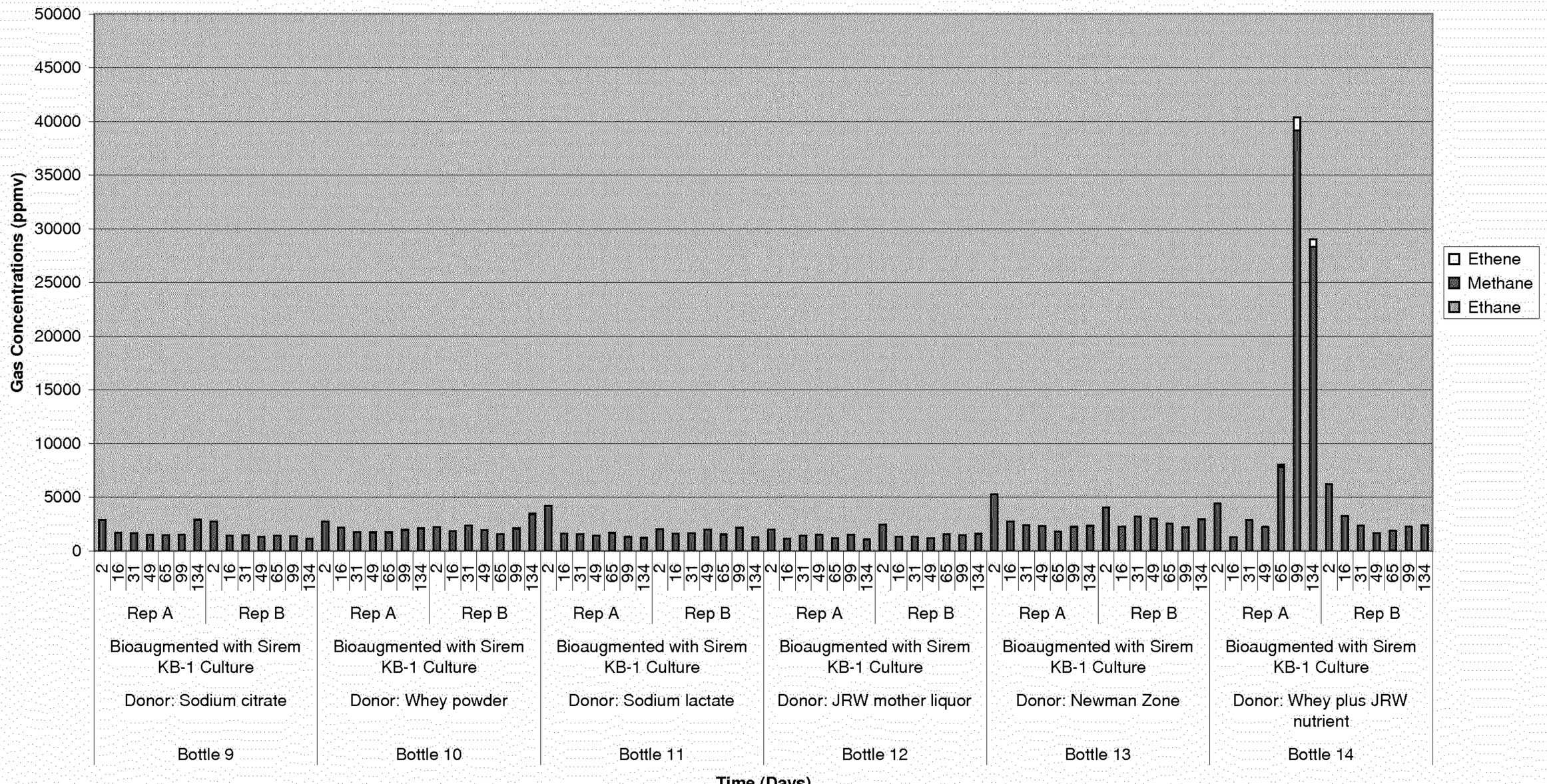

Figure 12 - Headspace Gas Production in Microcosms Bioaugmented with Shaw SDC-9 Culture

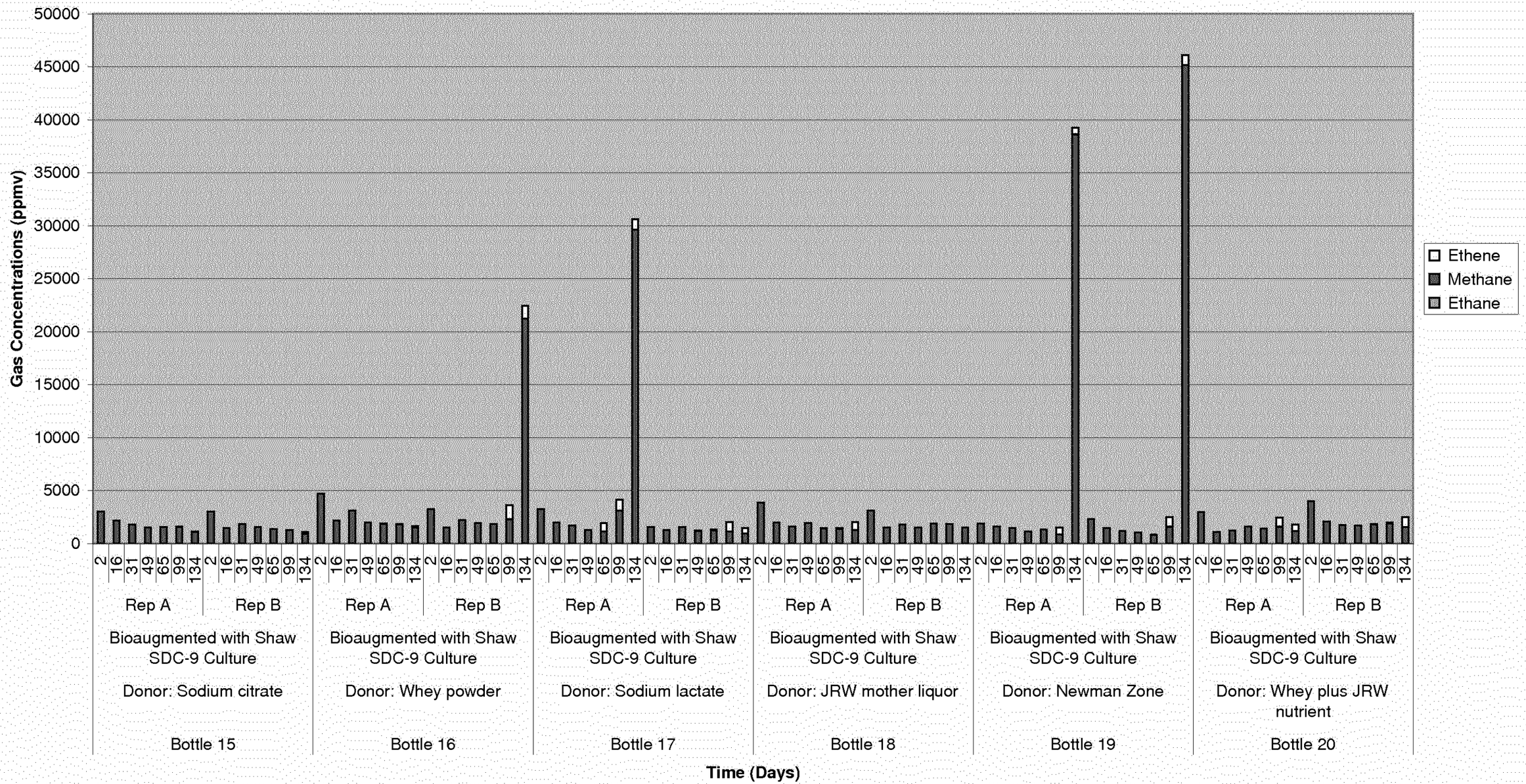

Figure 1A - pH - Control Microcosms

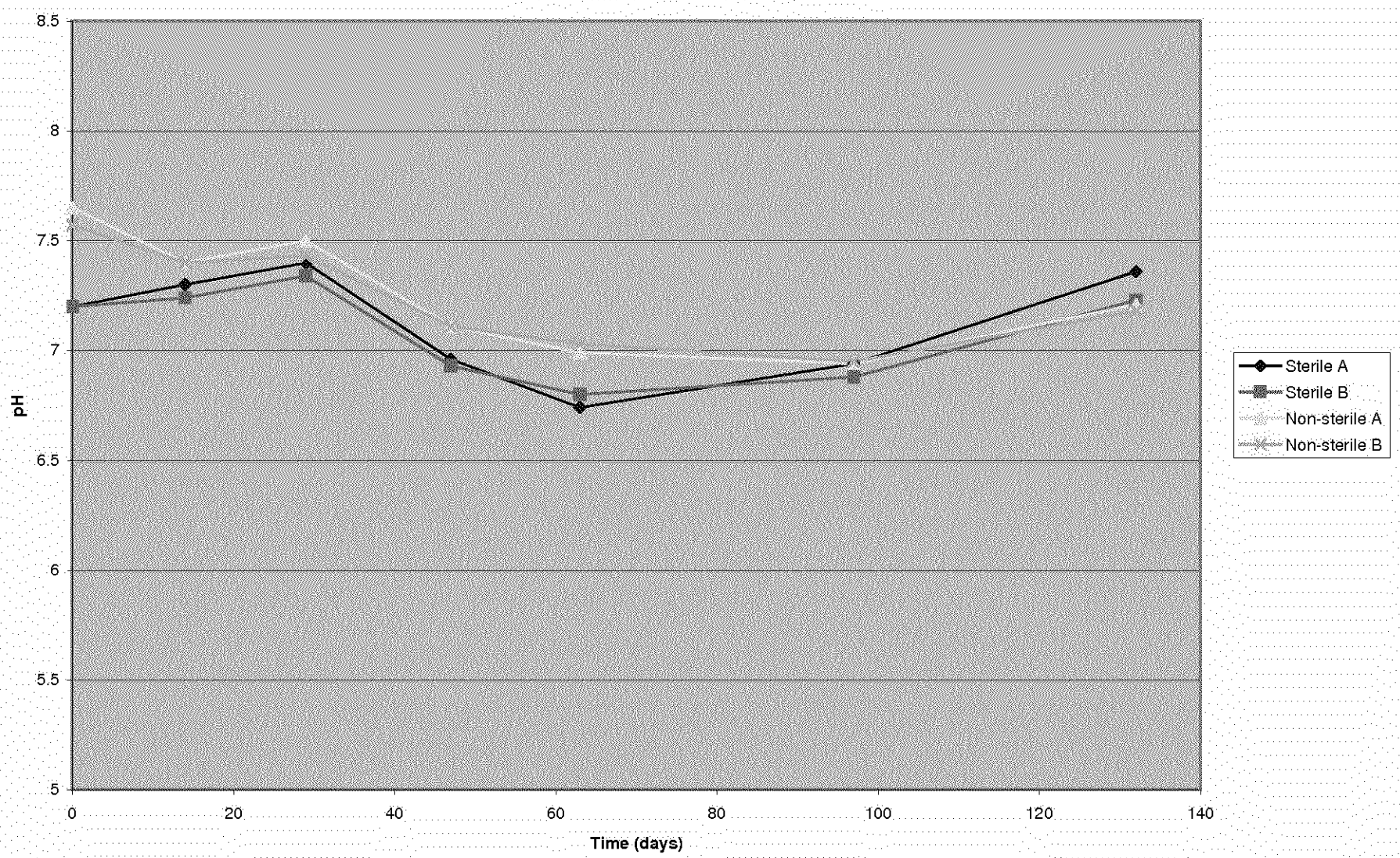

Figure 2A - pH - Microcosms with no Bioaugmentation

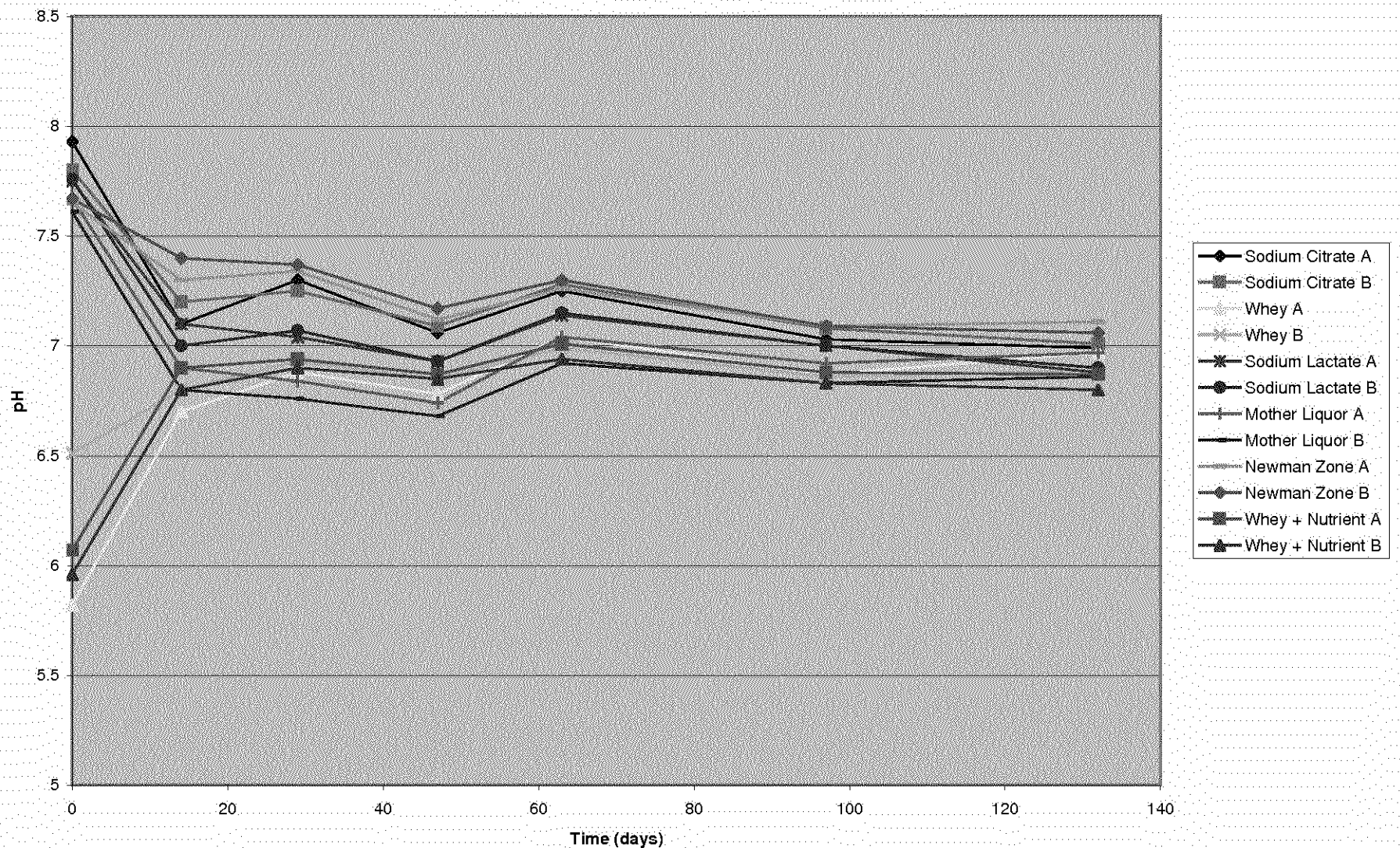

Figure 3A - pH - Microcosms with Sirem KB-1 Bioaugmentation Culture

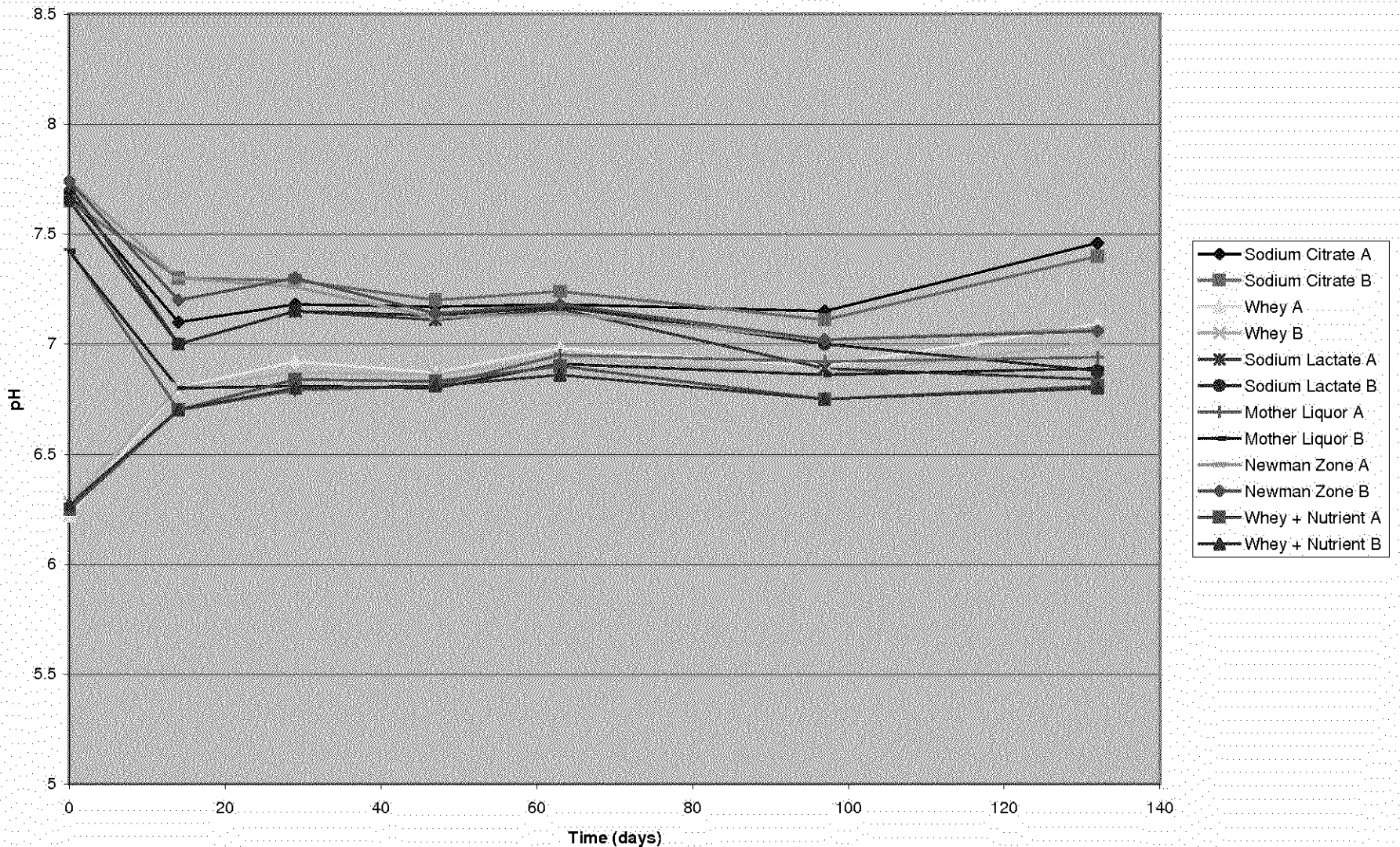

Figure 4A - pH - Microcosms with Shaw SDC-9 Bioaugmentation Culture

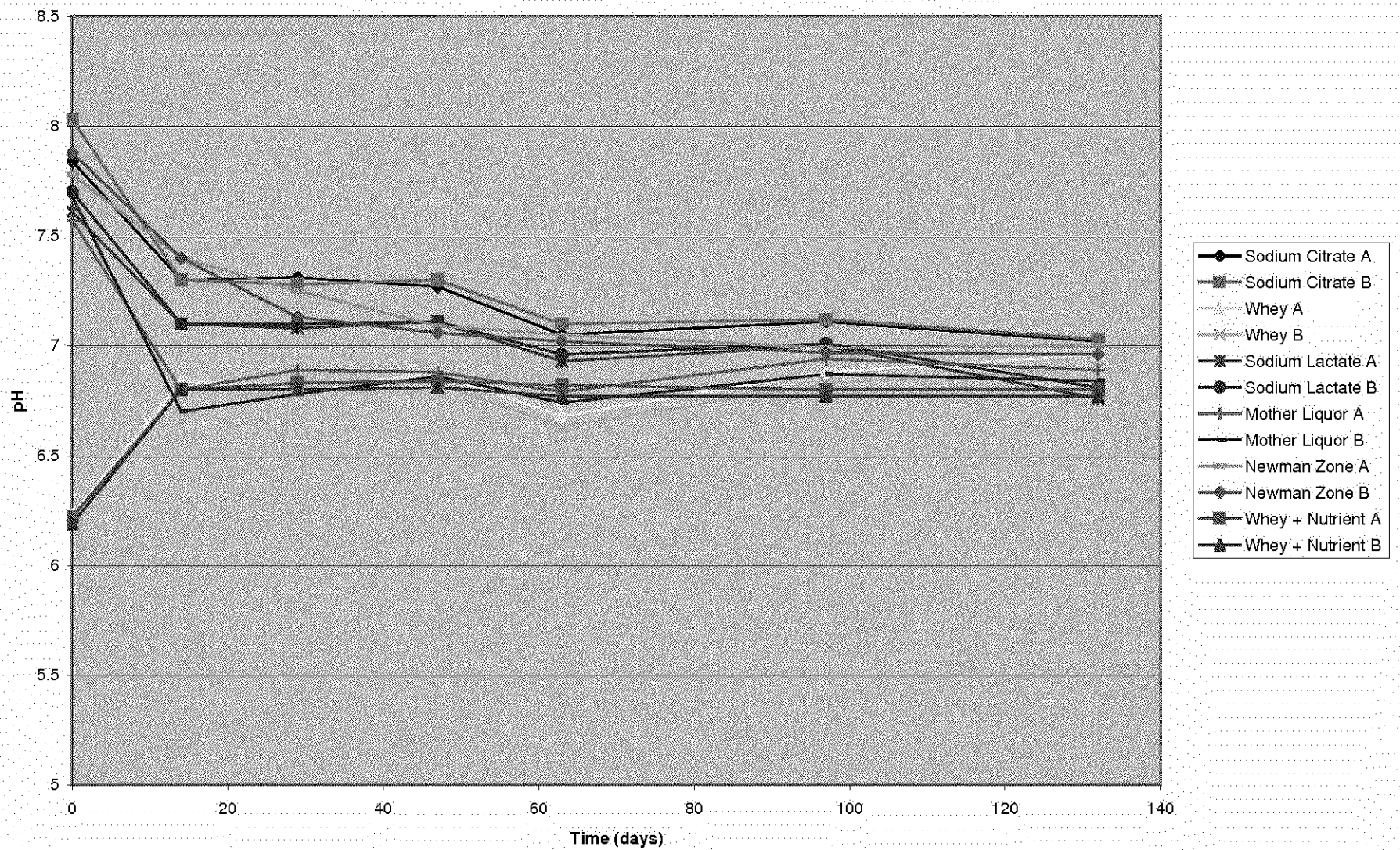

Figure 5A - SO4 - Control Microcosms

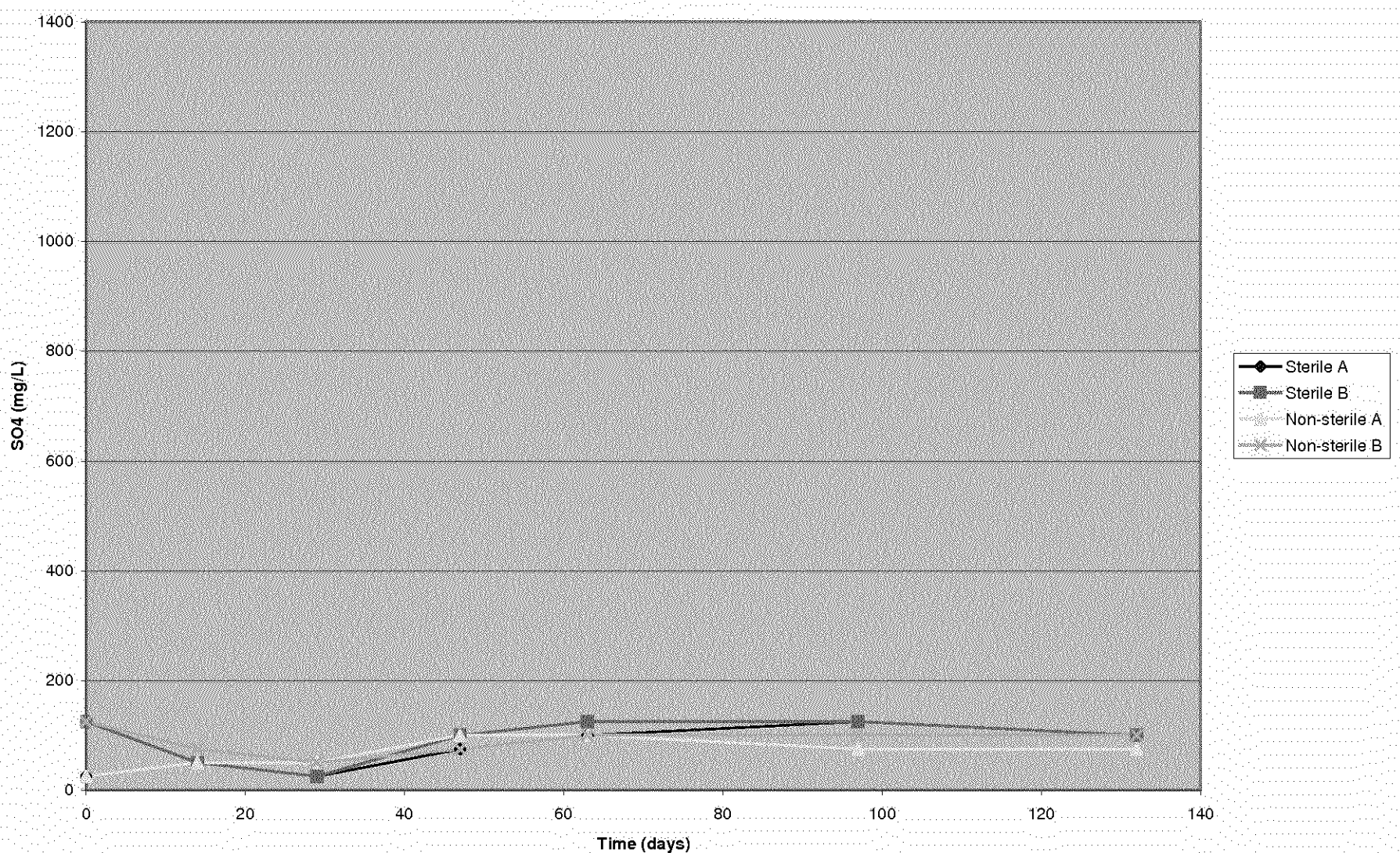

Figure 6A - SO4 - Microcosms with no Bioaugmentation

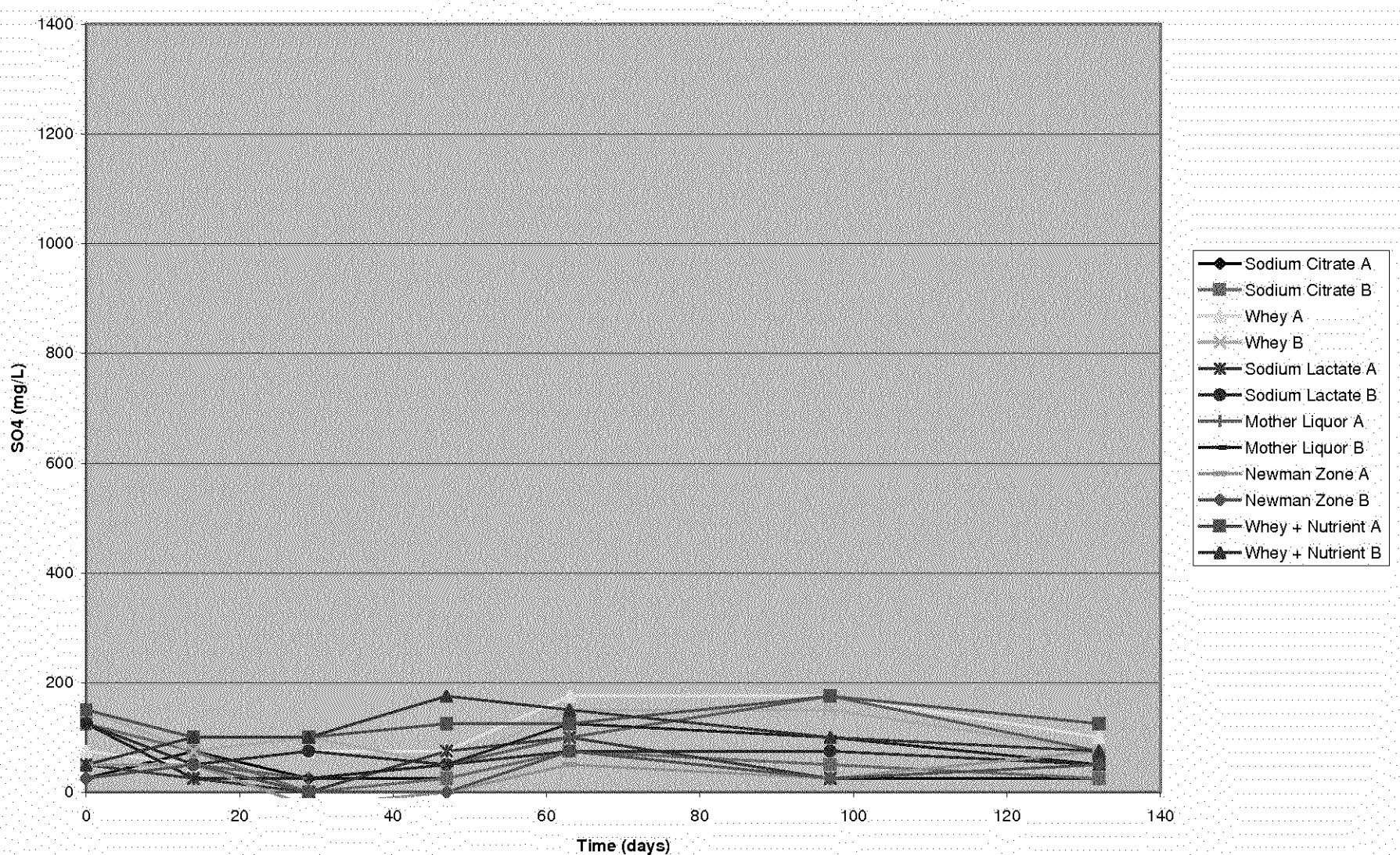

Figure 7A - SO4 - Microcosms with Sirem KB-1 Bioaugmentation Culture

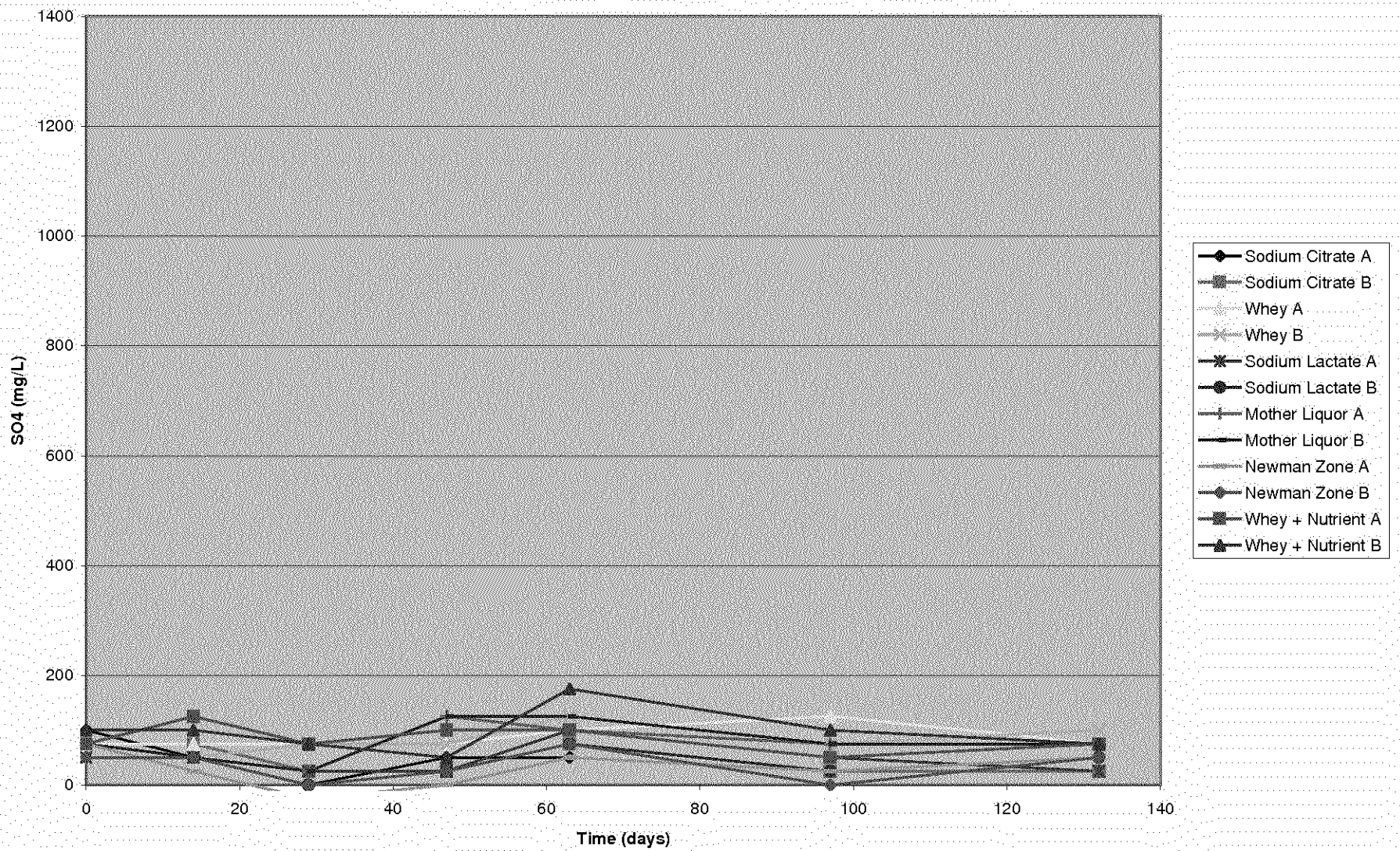
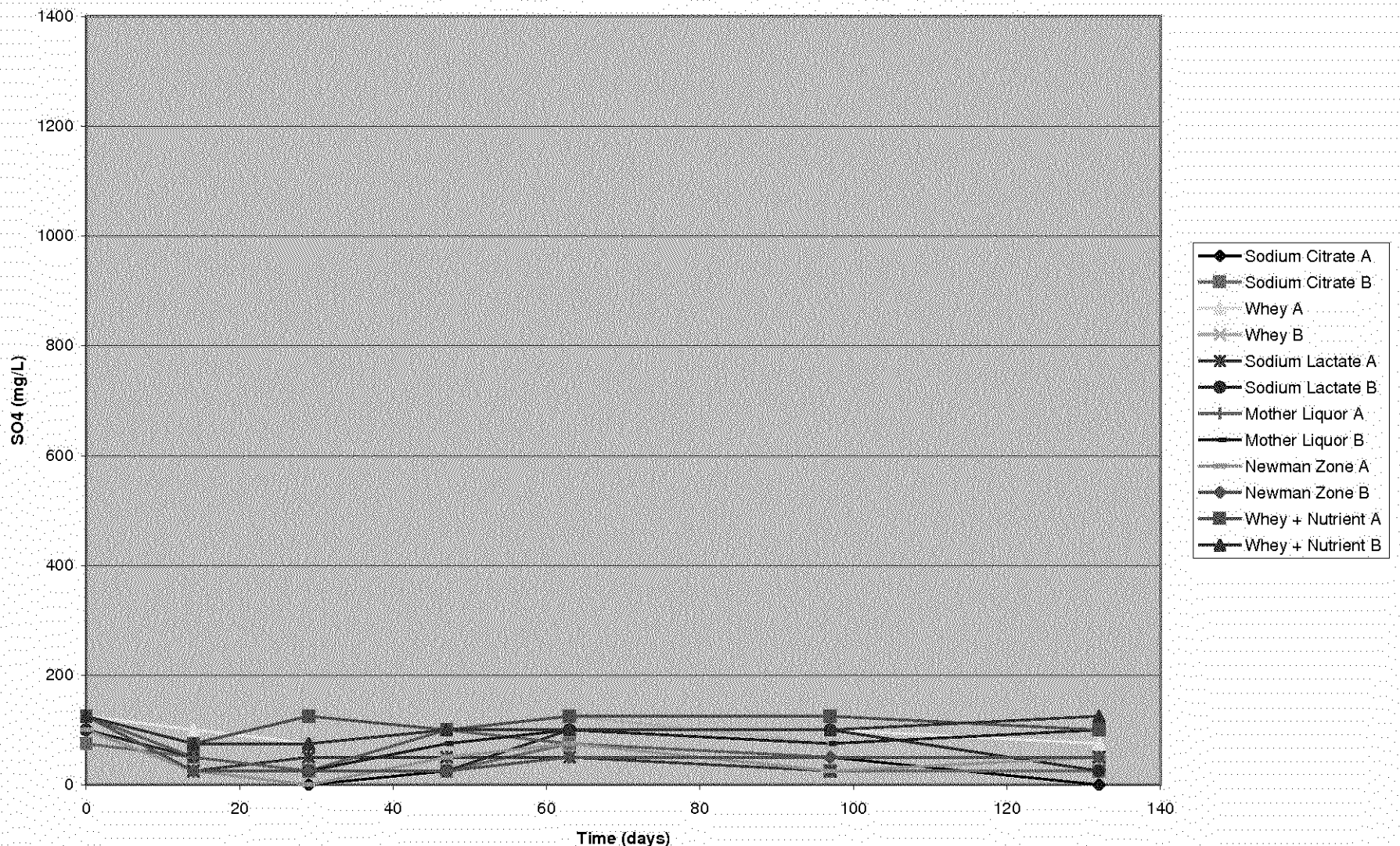
Figure 8A - SO4 - Microcosms with Shaw SDC-9 Bioaugmentation Culture