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DEVELOPMENT AND EVALUATION OF AN ENRICHMENT CULTURE FOR REDUCTIVE DECHLORINATION OF TETRACHLOROETHENE UNDER LOW pH CONDITIONS

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DEVELOPMENT AND EVALUATION OF AN ENRICHMENT CULTURE FOR REDUCTIVE DECHLORINATION OF TETRACHLOROETHENE UNDER LOW pH CONDITIONS

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ABSTRACT

Perchloroethene (PCE) is a pollutant of major environmental concern at hazardous waste sites worldwide. PCE and trichloroethene (TCE) are suspected carcinogens and are ranked 16th and 31st, respectively, on the Environmental Protection Agency's priority list for hazardous substances, developed under the Comprehensive Environmental Response, Compensation, and Liability Act. As a consequence of the widespread use of chlorinated solvents (including PCE and TCE) for dry cleaning, chemical feedstocks, metal degreasing and other purposes, chloroethenes are widely distributed in the environment. Many soils and groundwater throughout the world are contaminated by chloroethenes. Therefore, further improvements are needed in clean-up methods.

Bioaugmentation has been used extensively to treat aquifers contaminated with chlorinated ethenes at sites that lack the microbes needed to accomplish reductive dechlorination at a reasonable rate. However, a major limitation to bioaugmentation has been aquifer pH. *Dehalococcoides* are required for achieving complete dechlorination to ethene, yet their reported pH optimum is approximately 6.5 to 7.5. To account for this in aquifers with a lower pH level, buffers have been added prior to injection of culture. However, buffer addition can lead to clogging by precipitates, the chemical costs can be substantial, and achieving homogenous distribution is very challenging. One alternative is to use bioaugmentation cultures that are able to function at lower pH levels. The observation of complete dechlorination of PCE and TCE at some sites with pH levels below 6 suggest this should be achievable. However, very limited information is

available on bioaugmentation cultures that are capable of complete dechlorination of PCE and TCE at low pH levels.

The objectives of this thesis were 1) to further develop an enrichment culture capable of anaerobic reductive dechlorination of PCE to ethene at a pH level of 5.5 or lower, in a large enough quantity to be used in a field demonstration (e.g., in a 19.6 L canister); 2) to evaluate the effect of solid support materials (perlite and sand) on the rate of ethene accumulation at pH 5.5 or lower; 3) to evaluate bioaugmentation with a low pH enrichment culture in groundwater that is poorly buffered; and 4) to test the effect of pH levels below 5.5 (e.g., 5.35 and 5.30) on the rate of reductive dechlorination of PCE, including the rate of ethene accumulation.

The starting point for this research was an enrichment culture that showed promise at pH levels below 6. The culture was further enriched over approximately two years of incubation and multiple transfers in mineral salts medium. The volume of culture was scaled up from serum bottles to 2.6 L bottles and then to 19.6 L canisters, creating enough culture to be used in a pilot test at a hazardous waste site in which the aquifer pH is below 6. Consistent reductive dechlorination of PCE to ethene was achieved with the culture at a pH level of approximately 5.5. The highest rate of ethene accumulation was 3.8 μM/d.

Supporting material was unnecessary for growth of this low pH enrichment culture. Perlite slightly reduced the lag time needed for the onset of PCE dechlorination and ethene accumulation, but once dechlorination activity was established, perlite did not improve the process. Likewise, sand offered no advantages for growth of the low pH

enrichment culture. This is fortuitous, since the presence of solids would hinder application of the culture in the field.

Having established consistent operation of the culture at pH 5.5, an experiment was performed to evaluate the effect of lower pH levels. The lowest pH evaluated was approximately 5.3. The culture continued to dechlorinate PCE to ethene; however, the rates were noticeably slower. Improvements in rate may be achievable at the lower pH levels with further incubation of the culture.

A microcosm experiment was performed with soil and groundwater from a site in which the pH is consistently below 6. Reductive dechlorination of PCE was observed in the treatment that was bioaugmented with the low pH enrichment culture developed during this research; no dechlorination occurred in the unamended treatment or the treatment that received only lactate or lactic acid. Thus far, the main dechlorination product in the bioaugmented treatment is cDCE; VC has started to accumulate. Although preliminary, these results indicate the low pH enrichment culture shows potential for use in bioaugmentation of low pH sites, without the need for chemical adjustment of the pH.

The enrichment culture was inefficient in terms of its use of lactate or lactic acid for reductive dechlorination; only ~1-2% of the electron equivalents were used for this purpose. The majority of electron donor use was for methanogenesis. Decreases in methanogenesis may be achievable by increasing the concentration of PCE added to a level that is inhibitory to methanogens.

The results of this study indicate that bioaugmentation of aquifers that have a pH below 6 may be a feasible remediation strategy for treating PCE and TCE. A field trial with the enrichment culture developed during this research is recommended.

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ABBREVIATIONS

cDCE	*cis*-1,2-Dichloroethene
GC	Gas Chromatograph
MSM	Mineral Salts Medium
PCE	Tetrachloroethene
TCE	Trichloroethene
VC	Vinyl Chloride
VOCs	Volatile Organic Compounds

CHAPTER ONE

1.0 INTRODUCTION

Perchloroethene (PCE) is a pollutant of major environmental concern at hazardous waste sites worldwide (1). PCE and trichloroethene (TCE) are suspected carcinogens and are ranked 16th and 31st, respectively, on the Environmental Protection Agency's priority list for hazardous substances (2), developed under the Comprehensive Environmental Response, Compensation, and Liability Act. As a consequence of the widespread use of chlorinated solvents (including PCE and TCE) for dry cleaning, chemical feedstocks, metal degreasing and other purposes (3), chloroethenes are widely distributed in the environment. Many soils and groundwater throughout the world are contaminated by chloroethenes (4, 5). Therefore, further improvements are needed in clean-up methods. The following sections focus on the use of bioremediation, with a particular emphasis on the effect of pH on this process.

1.1 Reductive Dechlorination of Chlorinated Ethenes

Chemical and physical methods for remediating chlorinated ethenes include chemical oxidation, permeable reactive barriers, soil vapor extraction, and electrical resistance heating. Compared to biological processes, these approaches are generally more costly and leave residuals that are still above clean-up goals. Bioremediation can result in complete reductive dechlorination of PCE and TCE to ethene, and it is comparatively inexpensive to implement (1). With bioremediation, PCE and TCE can be converted to harmless products, like ethene or ethane, instead of transferring them from

one part of the environment to another, as is often the case with pump-and-treat systems that include air stripping and/or activated carbon (1).

Under anaerobic conditions, sequential reductive dechlorination converts PCE to TCE, *cis*-1,2-dichloroethene (cDCE) or *trans*-1,2-dichlolroethene, vinyl chloride (VC) and ethene. In some instances, ethene is further reduced to ethane, a process that has been associated with the cometabolic activity of methanogens (6). During reductive dechlorination, each chlorine is replaced by hydrogen (6). Reductive dechlorination may occur cometabolically or metabolically, as a respiratory process. The respiratory process is carried out by organochlorine-reducing bacteria (7). Potentially competing electron acceptors include sulfate, Fe(III), Mn(IV) and nitrate (7). The presence of 1,1,1-trichloroethane or chloroform inhibits the reductive dechlorination process (8).

Hydrogen is generally considered to be the universal electron donor for organochlorine-reducing bacteria. However, direct injection of hydrogen into contaminated groundwater is not generally considered feasible. Instead, soluble substrates are added whose fermentation yields hydrogen. Many substrates have been evaluated, including methanol, fatty acids (including vegetable oils), toluene (9), hydrogen release compound, and lactate (10). The effectiveness of electron donors has been ranked by Gerritse et al. (11) as follows: lactate > ethanol > H_2. Chen (8) reported that lactate is more effective in comparison to emulsified vegetable oil and hydrogen.

The rate of reductive dechlorination typically decreases as the number of chlorines decreases, often making reduction of VC to ethene the rate limiting step (12).

Maximum dechlorination rates for PCE to TCE, TCE to cDCE and cDCE to VC reported by Gerritse et al. (1) are 341, 159 and 99 μM/d, respectively.

Redox potential (E_h) is a measure of the tendency of a chemical species to acquire or lose electrons. In laboratory studies, resazurin is often used as an indicator for redox potential. When the E_h is above -110 mV, resazurin is pink; below -110 mV, the color is clear. The typical redox potential for reductive dechlorination is below -110 mV, as is sulfate reduction and methanogenesis.

1.2 Microbes that Dechlorinate PCE

Many types of organochlorine-reducing bacteria have been described that are capable of respiring PCE and TCE to cDCE. Damborský (13) reviewed many of these, including *Desulfitobacterium dehalogenans* JW/IU-DC1, which dechlorinates PCE to TCE and trace levels of cDCE; *Desulfitobacterium* sp. PCE-S, which dechlorinates PCE to cDCE; *Desulfomonile tiedjei* DCB-1, which dechlorinates PCE to TCE and cDCE; *Dehalobacter restrictus* PER-K23, which dechlorinates PCE to TCE and cDCE. *Dehalococcoides mccartyi* and *Desulfitobacterium* ssp. are the only bacteria known that can respire cDCE to VC (14). *Dehalococcoides mccartyi* is the only microbe identified thus far with the ability to use VC as a TEA, with ethene as the product.

Different strains of *Dehalococcoides mccartyi* have different abilities to use PCE, TCE, DCE isomers, and VC as terminal electron acceptors. For example, BAV1 (15) is able to respire cDCE to VC and VC to ethene. In contrast, *Dehalococcoides mccartyi* strain 195 can dechlorinate PCE to VC metabolically, but reduction of VC to ethene is a comparatively slow cometabolic process. In groundwater, complete reductive

dechlorination of PCE to ethene typically involves a mixture of *Dehalococcoides* and non-*Dehalococcoides* organochlorine-reducing bacteria with different metabolic capabilities. Two recently isolated *Dehalococcoides* strains (11a and 11a5) with dissimilar functional abilities are described by Lee et al. (16). Strain 11a reductively dechlorinates TCE, 1,1-DCE, cDCE, *trans*-1,2-dichlolroethene, and VC to ethene, while strain 11a5 dechlorinates TCE and all three DCE isomers only to VC.

The genomes of several *Dehalococcoides mccartyi* have been sequenced, including strains DCMB5 and BTF08, which were enriched from a contaminated site in Germany (17). The genome of strain BTF08 is the first identified that contains all three enzymes necessary to couple the complete reductive dechlorination of PCE to ethene to growth. The genes encoding TCE and VC reductive dehalogenases, *tceA* and *vcrA*, are located within mobile genetic elements, suggesting their recent horizontal acquisition. The genome of strain DCMB5 contains 23 reductive dehalogenase genes, including one for reductive dechlorination of chlorobenzene to benzene.

1.3 Effect of pH on Reductive Dechlorination of Chlorinated Ethenes

Reductive dechlorination is now a commonly used treatment approach at circumneutral pH (~6.5-7.5). In this pH range, PCE can be completely dechlorinated to ethene and/or ethane. Below pH 6, dechlorination is often incomplete and the rate of activity declines significantly. However, the groundwater at many sites is outside the circumneutral range, and often below pH 6 (18). For example, Hill and Neal (19) reported that the groundwater pH for the upper River Severn area fluctuates from 4 to 7 annually.

Bioremediation processes contribute to decreases in alkalinity and potentially decreases in groundwater pH (10). Each chlorine atom removed via reductive dechlorination results in the release of HCl, a strong acid. Most types of electron donors added undergo fermentation that results in an increase in organic acids that also contribute to alkalinity consumption. Use of formate as an electron donor mitigates this impact (20), although formate is not a commonly used substrate due to its rapid use and comparatively higher cost.

Table 1.1 presents a summary of 18 pure cultures of organochlorine-reducing bacteria, most of which can only dechlorinate PCE to cDCE under near neutral pH conditions (~6.0-7.5). *Sulfurospirillum multivorans* (21) is an exception; it can transform PCE to cDCE at pH 5.5. The optimal pH range for pure cultures of *Dehalococcoides*, which are required for complete dechlorination of chlorinated ethenes, is 7.0-7.3 (15, 22, 23). Ethene accumulation by these cultures has not been reported a pH of 5.5 and below.

Table 1.2 summarizes a number of the mixed cultures that have been evaluated at various pH levels, including several commercial bioaugmentation cultures: KB-1 (8), SDC-9 (24), BioDechlor (25), BDI (21), and BCI (http://www.bcilabs.com/news.html). Of these, only the BCI culture is reported to have significant dechlorination potential at a pH below 6; however, documentation of this activity does not appear to be available in the peer-reviewed literature. SiREM reports that the activity of KB-1 is slow or incomplete in the pH range of 5.0-6.0 and 8.5-10; the optimal range cited is 6.8-7.5. A pH level above 6 is considered necessary for dechlorination to ethene (file:///C:/Users/David/Downloads/ SiREM%20Bioaugmentation.pdf). While evaluating

the use of minerals to serve as natural buffering agents, Lacroix et al. (26) developed several laboratory enrichment cultures, one of which (AQ-1) exhibited ethene accumulation at a pH as low as 5.5; additional information about the culture is not available.

Yang (21) reported the occurrence of PCE reduction to ethene at a pH below 5.5 in microcosms prepared with sediment samples from the Neckar River. However, after three transfers of the culture in MSM), reductive dechlorination to ethene stopped at pH 5.5±0.2. Yang concluded that soil or sediment can help dechlorination under low pH conditions. All of his research was conducted with samples in 160 mL of serum bottles.

In the Freedman lab, Hickey (27) used samples from North Carolina and a wetland area at the Savannah River Site to develop an enrichment culture that achieved dechlorination of PCE to cDCE and from cDCE to ethene mainly at a pH close to 6. However, the groundwater at many locations has a pH level below 6 (18), so further development of the enrichment culture was warranted. Hickey's cultures formed the starting point for another M.S. student in the Freedman lab, Chen, who developed several enrichment cultures with the capability to dechlorinate PCE to ethene at a pH of 5.4 to 5.5. Chen also determined that a phosphate buffered mineral salts medium (MSM) with lactate or hydrogen added as electron donors was effective for these enrichment cultures. However, Chen had difficulty in maintaining a stable pH over long incubation periods. Also, the largest amount of culture he developed was in 2.6 L bottles, which is not sufficient for use in a field demonstration. Chen's cultures also contained an appreciable level of sediment. Therefore, a larger amount of this kind of enrichment culture is

needed; better control of the pH is needed; the culture needs to be developed in without sediment present; and it is necessary to test how the enrichment culture will perform at pH levels below 5.5.

Although previous research has made some strides in developing enrichment cultures that function at a pH below 6, considerable challenges remain. These include determining if a solid support surface is needed to allow growth to occur, how low of a pH is feasible before activity ceases, and if an enrichment culture can be used to accomplish bioaugmentation of a low pH groundwater.

Adding buffer to an aquifer is another approach to addressing the problem posed by pH levels outside of the reportedly optimum range of 6.5 to 7.5. However, buffer addition presents some substantial challenges. Parker and Birk (28) reported that an aquifer may become clogged due to precipitation by adding buffer, and that buffers such as calcium carbonate are low in solubility. Perhaps most importantly, it is difficult to achieve a homogeneous distribution of buffer, especially in aquifers with heterogeneous permeability. Areas near the injection point may have pH levels well above circumneutral, while areas beyond the injection point may not receive adequate buffer. Sodium hydroxide and lime are recommended by the Environmental Protection Agency for pH adjustment of ground water (http://www.epa.gov/oust/cat/insitbio.htm). However, due to high cost, pH adjustment is not applicable for most sites. Unpredicted environmental impacts may also arise. For example, rapid changes of more than 1 or 2 units can inhibit microbial activity and damage the microbial consortium in the aquifer. Moreover, in situ adjustment of pH may lead to excessive accumulation of solids and

subsequent loss of hydraulic control (29). Therefore, the strategy of using a bioaugmentation culture adapted to low pH conditions warrants further investigation.

1.4 Bioaugmentation

Bioaugmentation is the process of adding microbes with specific metabolic capabilities to an environment where they are absent or present in such low numbers that there is no evidence of biodegradation activity (10). Several factors should be considered before implementing bioaugmentation, including pH, the availability of nutrients, temperature, the concentration of the target pollutant, and the presence of inhibitory co-contaminants such as heavy metals, chloroform, or chlorinated ethanes (30). The nutrients needed for growth of *Dehalococcoides* include Fe, Mg, P, N and trace metals; the pH for reductive dechlorination is approximately 5.5 to 8.0; the highest allowable temperature is 45°C; the inhibitory concentration of PCE is around 90 mg/L; and the sulfate concentration should not exceed 1000 mg/L, since sulfate-reducing bacteria will compete with *Dehalococcoides* for electron donor and sulfides are inhibitory (30). Although not always essential, laboratory scale testing is advisable to help ensure that bioaugmentation will be successful in the field.

The concentration of *Dehalococcoides* is another important factor for bioremediation. The recommended initial density of *Dehalococcoides* is 10^7 cells/L (31). For use in the field, enrichment cultures are often grown in stainless steel canisters with a volume of ~20 L; these are small enough to be shipped but large enough to provide sufficient inoculum for use in the field (31). One of the goals for this research was

development of a sediment-free, low pH enrichment culture in at least one 19.6 L canister that can be deployed in a field scale pilot test.

1.5 Objectives

The overall objectives of this research are:

1. To further develop an enrichment culture capable of anaerobic reductive dechlorination of PCE to ethene at a pH level of 5.5 or lower, in a large enough quantity to be used in a field demonstration (e.g., in a 19.6 L canister);
2. To evaluate the effect of solid support materials (perlite and sand) on the rate of ethene accumulation at pH 5.5 or lower;
3. To evaluate bioaugmentation with a low pH enrichment culture in groundwater that is poorly buffered; and
4. To test the effect of pH levels below 5.5 (e.g., 5.35 and 5.3) on the rate of reductive dechlorination of PCE, including the rate of ethene accumulation.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Chemicals and Medium

High purity hydrogen (99.99%) was obtained from Airgas National Welders. Methane (99.9%) and ethane (99.9%) were obtained from Matheson. Ethene (99.99%) was obtained from National Specialty Gases. VC (>95.5%) was obtained from Fluka, cDCE (99%) from TCI America, TCE (99%) from Fisher Scientific and PCE (99%) from Arcos Organics. A gas mixture used in preparation of anaerobic medium (70% N_2/30% CO_2) was obtained from Matheson. Sodium lactate (60% of syrup) was from EM Science and lactic acid (85%) from Fisher Scientific. Miracle-Gro perlite was obtained from Walmart and fine sand from Fisher Scientific. Phosphoric acid was from Fisher Scientific and sodium hydroxide (99-100%) was from EMD.

The anaerobic mineral medium used in this research is described by Chen (8), with the following modification. In order to efficiently control the pH at 5.4 to 5.5, exclusive use of K_2HPO_4 was changed to a mixture of K_2HPO_4 (52.5 g/L) and KH_2PO_4 (40.8 g/L), in a ratio of 2% and 98% by volume. The medium was prepared by combining the stock solutions for the phosphate buffers, salts, trace metals, and resazurin, then autoclaving for 2 hours and cooling for 12 hours. Yeast extract was then added and the medium was transferred to the anaerobic chamber. Next, 240 mg/L of $Na_2S \cdot 9H_2O$ and 144.8 mg/L of $FeCl_2 \cdot H_2O$ was added and continuously mixed on a stir plate. Within several minutes, the medium changed from pink to clear. The pH of the medium was

adjusted to 5.5±0.1 using 1 M H_3PO_4 or NaOH. Details of the protocol for preparing anaerobic mineral medium are provided in Appendix A.

2.2 Sources for the Enrichment Cultures

The research presented in this thesis started with enrichment cultures and microcosms developed by Chen (8). They consisted of 21 serum bottles (160 mL) and three 2.6 L bottles. Chen repeatedly spiked the cultures with electron donors (lactic acid, sodium lactate, or hydrogen) and PCE. The target pH was 5.5.

For the 160 mL serum bottles, eight contained 50 mL of groundwater and 20 g of soil; 13 of the serum bottles contained of 50 mL of medium and 20 g of soil. All the serum bottles were capped with Teflon-faced red rubber septa and sealed with aluminum crimp caps. All were capable of completely dechlorinating PCE (up to 12 mg/L) to ethene or ethane. Ethene or ethane accumulation rates varied from 0.1 to 1.8 µM/d. Stock solution of lactic acid (280 g/L) or sodium lactate (225.5 g/L) were used to add electron donor to most of the bottles; a few received hydrogen gas (99.99%). The initial amount of donor added was typically 100 times the stoichiometric amount needed for reduction of PCE to ethene, assuming complete oxidation of the lactate or lactic acid. Calculations for the initial amount of electron donor added are provided in Appendix B. The pH was maintained at ~5.4-5.5 by adding 1 M H_3PO_4 or NaOH.

The 2.6 L bottles contained 1.5 L of liquid. Two of these consisted of a mixture of various enrichment cultures developed by Chen. The bottles were capped with Teflon-faced rubber septa and sealed with plastic cap. PCE served as the electron acceptor. All of the bottles exhibited complete reductive dechlorination of PCE to ethene or ethane.

The PCE concentration ranged from 1.2 to 16 mg/L. Ethene or ethane accumulation rates varied from 0.1 to 0.4 μM/d. Lactic acid (280 g/L) or sodium lactate (225.5 g/L) was added as the electron donor (Appendix B). The initial amount of donor added was typically 100 times the stoichiometric amount needed for reduction of PCE to ethene, assuming complete oxidation of the lactate or lactic acid. The pH was maintained at ~5.4-5.5 by adding 1 M H_3PO_4 or NaOH.

2.3 Enrichment Culture Development

The microcosms and enrichment cultures developed by Chen (8) were further enriched by transfers (typically 5-10% v/v) in an anaerobic chamber to pH-adjusted medium. Bottles were then sealed with Teflon-faced septa, removed from the anaerobic chamber, and sparged with 70% N_2/30% CO_2 for 3 min. Lactic acid or sodium lactate was then added; subsequent additions were also made to maintain dechlorination activity. The decision on whether to add lactic acid or lactate was based on the pH. When the pH was above 5.6, lactic acid was added; when it was between 5.45 and 5.60, a mixture of the two was added; below 5.45, only lactate was added. After making the addition, the bottles were shaken vigorously and the solids were then allowed to settle for 30 to 40 min; then, a new sample was withdrawn, and the pH was measured again. If it was outside the range of 5.40-5.50, a base (1 M NaOH) or acid (1 M H_3PO_4) was added to bring the pH into the desired range. Most of the time it was not necessary to add either base or acid; lactic acid and lactate provide adequate pH adjustment.

Neat PCE was added to the 2.6 L bottles while PCE saturated water (~150 mg/L) was added to the serum bottles. To achieve equilibration, the bottles were placed on a

rotating shaker table for at least 1 hour after adding the PCE. After several days of incubation, headspace samples were monitored by GC (see below). When PCE was completely consumed, more was added. The bottles were incubated quiescently, in an inverted position and inside a cardboard box to avoid exposure to light.

One of the goals for the enrichment process was to gradually increase the total volume of enrichment culture available, first by transfer of serum bottles to 2.6 L bottles and then to the 19.6 L canisters. Details on which bottles were used for the transfers are presented in Appendix C.

Because of the size of the canisters, it was impractical to fill them inside the anaerobic chamber. Working with them on the bench top required that the cultures and medium be handled in such a way as to minimize exposure to air. The procedure for transferring medium into the canister is shown in Figure 2.1. Medium (18 L) was prepared on the bench top in a glass carboy (20 L). The empty canister was sparged for at least 30 min with 70% N_2/30% CO_2. Next, the medium was siphoned into the canister until the desired mass was reached (15.8 kg); the canister was set on a scale to determine the gain in mass. A stream of 70% N_2/30% CO_2 was passed over the medium to make sure a vacuum did not develop in the glass carboy; the sparging exhaust was routed to a fume hood, since the headspace of the medium contained hydrogen sulfide. The headspace of the canister was also vented to a fume hood.

Nest, the bottles used as inoculum were transferred to the anaerobic chamber and combined in a 4 L aspirator bottle, outfitted with the necessary tubing and a rubber stopper. The procedure for transferring inoculum into the canister is shown in Figure 2.2.

The inoculum was discharged by gravity from the aspirator bottle into the canister, so that the total mass added was ~18.8 kg. Finally, the pH was measured and adjusted as needed to 5.5 by adding 1 M of H_3PO_4 or NaOH. The initial neat PCE amount added was 10 µL, which resulted in an aqueous phase concentration of 1.2 mg/L. To achieve equilibration, the canister was placed in a wood cradle, attached on a rotating shaker table for 6 hour. The canister was held in place on the shaker table by placing it on a wooden cradle that was bolted to the shaker; the canister was held in place with elastic straps.

On occasion, liquid from the canisters was removed and fresh medium was added. The procedure for removing liquid from the canister is shown in Figure 2.3. The canister was placed upright on a lab bench and liquid was removed by opening one of the valves on the side. The liquid was discharged by gravity to a glass bottle outfitted with a two-holed stopper. The glass bottle was first made anaerobic by passing it through the anaerobic chamber and sealing off the latex tubing with clamps. Tubing from the canister was connected to one of the ports on the bottle; the other port vented to a fume hood. The headspace of the canister was sparged with the 70% N_2/30% CO_2 gas mix to avoid pulling a vacuum as the liquid was withdrawn. The glass bottle was on a scale that was used to determine when enough liquid was removed. To add fresh medium, the same procedure described in Figure 2.1 was used.

2.4 Experimental Design for Evaluating Support Material

During development of the low pH enrichment culture, variable performance suggested that the culture may require the presence of a support material in order to tolerate the low pH conditions. Perlite and sand were selected as support materials for

evaluation. Both were pretreated before use. Perlite was dried at 105°C for 1 hour and then crushed using a mortar and pestle. The perlite then was sieved to 425 µm using a number 40 sieve. Sand was also sieved to 425 µm.

To evaluate the effect of perlite, four serum bottles were prepared in the anaerobic chamber. Two received 2.5 g of sieved perlite, two received no support material. Each bottle received 25 mL of enrichment culture (from the same source: serum bottle MM-B3+NC-lactate-MSM-S3-1; Appendix D, Figure D.1a) and 75 mL of fresh medium. Lactic acid and sodium lactate were added as the electron donors; the pH was maintained at 5.4-5.5.

To evaluate the effect of sand, two 2.6 L bottles were prepared from the same source of inoculum (RS-6.0-3B; Appendix D, Figure D.2b). One received 10 g of sand, the other received no support material. Both bottles (identified as RS-6.0-3B and RS-6.0-3B-2) received 750 mL of enrichment culture and 750 mL of fresh medium. Lactic acid and sodium lactate was added as electron donors; the pH was maintained at 5.4-5.5.

All of the bottles were monitored for PCE and its daughter products. The rate of ethene accumulation was the main metric used to determine if a support material is or is not needed.

2.5 Experimental Design for Evaluating Bioaugmentation

The effectiveness of the enrichment culture for bioaugmentation of low pH groundwater and soil was evaluated. Four treatments were prepared (each in triplicate): unamended microcosms, biostimulated microcosms, bioaugmented microcosms, and water controls. The microcosms were prepared in 160 mL serum bottles. The unamended,

biostimulated, and bioaugmented treatments received 20 g of soil, 50 mL of groundwater, and 1 mg/L resazurin. The soil and groundwater came from the P-Area at the Savannah River Site, which is contaminated with PCE and has a pH below 6. Attempts to remediate a test zone using a pH 7 bioaugmentation culture have been unsuccessful.

After adding soil and groundwater to the serum bottles in the anaerobic chamber, they were incubated for several days until the color of the groundwater changed from pink to clear, indicating the establishment of low redox conditions (i.e., below -110 mV). Next, the pH of the groundwater was adjusted by adding 1 M H_3PO_4 or NaOH. The bottles were allowed to incubate for one week while the pH was checked several times, to ensure it remained within the range of 5.4 to 5.5. Next, 5 mL of PCE-saturated water was added, resulting in an aqueous phase concentration of 12 mg/L. Sodium lactate or lactic acid was added as the electron donor. The low pH enrichment culture from three samples (Appendix C, Figure C.8) were used for bioaugmentation.

Water controls were prepared with 50 mL of DDI water, glass beads that displaced the same volume as 20 g of soil (~11 mL), and the same amount of PCE as the microcosms.

2.6 Experimental Design for Evaluating pH Levels Below 5.5

After developing a stable enrichment culture at pH 5.5, an experiment was performed to evaluate the performance of the enrichment at lower pH levels. Using 1 L of culture from one of the canisters, nine serum bottles with were prepared, each containing 100 mL of culture (Figure 2.4). Three treatments of triplicate bottles were used. Initially, all of the bottles were maintained at a pH of ~5.4-5.5. The pH of one

treatments was maintained at this level. For the second treatment, the pH was lowered to ~5.35 by adding 1 M H_3PO_4, and was maintained at this level. The pH in the third treatment was gradually lowered, first to ~5.35 and then (once stable activity was observed) further to ~5.30, also by adding H_3PO_4. When the pH dropped below the target by more than 0.05 units, it was increased by adding 1 M NaOH.

2.7 Analytical Methods

2.7.1 Volatile Organic Compound (VOC)Analysis

The amount of PCE, TCE, cDCE, VC, ethene, ethane and methane in microcosms and enrichment cultures were measured with a gas chromatograph (GC). Headspace samples (0.5 mL) were removed with a syringe (Precisions Scientific, series A-2) and injected into a GC (Hewlett Packard 5890 Series II). The column was packed with 1% SP-1000 on 60/80 Carbopack B. All of the VOCs were quantified with a flame ionization detector (8).

Initially, the GC response factors measured by Chen (8) were used while the instrument was connected to the same integrator used by Chen (Appendix D). When the integrator was replaced with digital data acquisition software (Unichrom), a new set of standards was prepared in serum bottles. The same response factors were applied to the 2.6 L bottles, since they had the same ratio of liquid to headspace as in the serum bottles. For serum bottles the volume of the liquid was 100 mL and the headspace was 60 mL. For 2.6 L bottles, the same ratio of headspace to liquid was used (i.e., 0.60), so that the same response factor was applicable when multiplied by the ratio of the total volume of the large bottle to the total volume of the smaller bottle (e.g., 2550 mL/160 mL = 15.94):

$$RF_B = RF_S \frac{V_B}{V_S}$$

where RF_B = response factor for the 2.6 L bottle; RF_S = response factor for the serum bottle; V_B = volume of the 2.6 L bottle; V_S = volume of the serum bottle.

For the canister (19.6 L), the volume of the liquid was 18 L and volume of headspace was 1.6 L.

Assuming that the headspace and aqueous phase were in equilibrium, the total mass present was converted to an aqueous phase concentration as follows:

$$C_l = \frac{M}{V_l + H_c V_g}$$

Where C_l is aqueous concentration, M is total mass present, V_l *i*s the aqueous volume in the bottle, V_g is headspace volume in the bottle, and H_c is Henry's law constant (27). More details are shown in Appendix D.

2.7.2 pH Measurement and Adjustment

A SympHony pH meter and SympHony probe (model #14002-766) were used for measuring pH. Before measurement, the pH probe was calibrated at 7.0 and 4.0. The calibration solutions were from VWR. Samples were removed from bottles with a syringe and needle. To avoid clogging the needle, the samples were allowed to settle and only clarified liquid was withdrawn. The solids content in the canisters was sufficiently low that this was not a concern when they were sampled.

For serum bottles and the 2.6 L bottles, a 1 mL syringe with a side pore needle was used to remove the sample and 0.2-0.3 mL of the liquid was discharged to a 1.5 mL conically shaped plastic micro tube with a snap cap. The pH probe was then placed into

the tube, allowed to equilibrate for approximately 30 s, and the pH was recorded. For the canisters, 2-3 mL of liquid was removed via the mini-nert valve using a 10 mL syringe and side port needle; the liquid was discharged to a 10 mL glass bottle, where the pH was measured, also after allowing approximately 30 s for equilibration.

CHAPTER THREE

3.0 RESULTS

3.1 Evaluation of Support Material

Experiments were performed to evaluate the effect of adding perlite and sand on the reductive dechlorination activity of the low pH enrichment culture. Figure 3.1 shows the results for one of the serum bottles with perlite added (MM-B3 + NC-Perlite-2). PCE started degrading within the first week, and ethene accumulation started the following week. After one month, VC and ethene were the main products. Initially, the amount of PCE added was 0.5 µmol per bottle (0.6 mg/L when taking partitioning between the headspace and liquid into account), and was then gradually increased to 3.0 µmol per bottle (3.6 mg/L). Methane production was consistent from the start. Approximately 88% of the electron equivalents of lactate and lactic acid added was converted to methane, versus only 0.8% for reductive dechlorination. Maintaining the pH was challenging since perlite provides some buffering capacity. Nevertheless, the average pH was maintained at 5.50 (±0.08). Results for the duplicate serum bottle (MM-B3+NC-Perlite-1) are shown in Appendix E (Figure E.2).

Results for one of the serum bottles (MM-B3+NC-Control-2) without perlite added are shown in Figure 3.2. PCE started degrading the first week and ethene started to accumulate by the third week. By day 40, cDCE decreased and VC and ethene were the only products. As in the bottles with perlite, the initial amount of PCE added was 0.5 µmol per bottle (0.6 mg/L) and subsequent amounts added were gradually increased to 3.0 µmol per bottle (3.6 mg/L). Approximately 97% of the electron equivalents of lactate

and lactic acid added was converted to methane, versus only 0.9% for reductive dechlorination. The average pH was 5.50 (±0.08), equivalent to the pH in the bottles with perlite added. Results for the duplicate serum bottle (MM-B3+NC-Control-1) was shown in Appendix E (Figure E.3)

Cumulative PCE consumption and ethene accumulation for the treatments with and without perlite added are shown in Figure 3.3. Once ethene formation was underway in the treatment without perlite, the rate of accumulation was similar (0.7 µM/d with perlite added, 0.6 µM/d without perlite added). After 74 days of incubation, ethene represented approximately 42% of the PCE consumed. These results suggest that perlite reduced the time needed for the onset of ethene formation, but once underway, the presence of perlite offered no advantages. This is fortuitous, since it would be more difficult to bioaugment with a culture that needed perlite to survive.

Results for a 2.6 L bottle (RS-6.0-3B-2) with sand added are shown in Figure 3.4. PCE began degrading immediately and cDCE accumulated to 125 µmol per bottle (7.5 mg/L when taking partitioning between the headspace and liquid into account) through day 254, at which time cDCE decreased while VC increased to 71 µmol per bottle (1.7 mg/L when taking partitioning between the headspace and liquid into account). VC then declined and ethene started accumulating at a relatively high rate (up to 1.2 µM/d) between days 241 and 310 day. Approximately 77% of the electron equivalents of lactate and lactic acid added was converted to methane, versus only 0.7% for reductive dechlorination. The average pH was maintained at 5.50 (±0.06).

Results for the 2.6 bottle (RS-6.0-3B) without sand added are shown in Figure 3.5. Over the first 168 days, PCE was dechlorinated to cDCE, VC and a lower level of TCE. During this time no ethene accumulation occurred. On day 168, 50% of the culture was used to prepare canister A (see below) and the volume removed was replaced with MSM; this required opening the bottle in the glove box, which explains the vertical drop in amounts on day 168. Thereafter, VC increased to 29.4 µmol per bottle (0.7 mg/L when taking partitioning between the headspace and liquid into account), cDCE decreased and ethene started to accumulate. Between days 205 and 304, the ethene accumulation rate was 1.2 µM/d, which is similar to the bottle with sand added. Nearly all of the electron equivalents of lactate and lactic acid added was converted to methane, versus only 0.8% for reductive dechlorination.The average pH was maintained at 5.49 (±0.06).

Cumulative PCE consumption and ethene accumulation for the treatments with and without sand added are shown in Figure 3.6. Cumulative PCE consumption was similar until day 225, when the rate without sand added increased. Ethene accumulation started sooner in the treatment without sand added (possibly due to addition of fresh MSM on day 168), but once ethene production was underway, the rates were similar. After 310 days of incubation, ethene represented approximately 49-57% of the PCE consumed. These results indicate that adding sand offered no advantages. This is fortuitous, since it would be more difficult to bioaugment with a culture that needed sand to survive.

3.2 2.6 L Sediment-free Enrichments

Once it was established that a solid surface was not required for maintaining the low pH enrichment culture, further enrichment was pursued by developing additional sediment-free culture in 2.6 L bottles. During the course of this research, 11 2.6 L bottles were developed and maintained. In this section, the performance of four is described, as these exhibited the highest rates of dechlorination activity and had the least amount of solids from the original microcosms. Also, two of the four bottles (#1 and #2) served as inoculum for the canisters.

3.2.1 Bottle #1

Bottle #1 (Figure 3.7) was created by combining one microcosom (Figure E.5), one enrichment culture (Figure E.6), and MSM. PCE degraded slowly at first, although this was accompanied by VC and ethene accumulation. As the amount of PCE added was increased, cDCE and VC accumulated. cDCE started decreasing at day 83, but VC continued to accumulate to 51.8 µmol per bottle (1.2 mg/L when taking partitioning between the headspace and liquid into account). After 105 days of incubation, VC and ethene were the only products. Between days 82 and 105, the maximum ethene accumulation rate was 1.4 µM/d. Nearly all of the electron equivalents of lactate and lactic acid added were converted to methane, versus only 0.5% for reductive dechlorination. The average pH was maintained at 5.52 (±0.10). At day 105, bottle #1 was sacrificed to start canister A.

3.2.2 Bottle #2

Bottle #2 (Figure 3.8) was created by combining two microcosms that exhibited

high rates of PCE dechlorination (Figures E.7, E.8), along with MSM. TCE was initially the only daughter product, but it began to decline at day 46 and cDCE increased. VC started to accumulate after day 80. Ethene accumulation did not appear until VC reached 24 µmol per bottle (0.6 mg/L when taking partitioning between the headspace and liquid into account). After 162 days of incubation, PCE was dechlorinated primarily to VC and ethene. Between days 136 and 162, the maximum ethene accumulation rate reached 0.7 µM/d. Approximately 64% of the electron equivalents of lactate and lactic acid added was converted to methane, versus only 0.5% for reductive dechlorination. The average pH was maintained at 5.50 (±0.10). Between days 116 and 120, the pH fell below 5.40, and at day 123, TCE appeared again. On day 162, 50% of the culture was used to prepare canister A (see below) and the volume removed was replaced with MSM; this required opening the bottle in the glove box, which explains the vertical drop in amounts on day 162.

3.2.3 Bottle #3

Bottle #3 was created in two stages. First, two microcosms that exhibited high rates of PCE dechlorination were combined (Figures E.9, E.10), along with MSM, in a 2.6 L bottle. Second, after establishing dechlorination activity, the contents were diluted 1:1 with MSM. PCE dechlorination after this dilution step is shown in Figure 3.9. Initially, cDCE and VC were the main products. Ethene accumulation accelerated around day 100, corresponding to a decrease in cDCE; VC began decreasing around day 130. Between days 129 and 158, the ethene accumulation rate reached 3.0 µM/d. Approximately 59% of the electron equivalents of lactate and lactic acid added was

converted to methane, versus only 1.3% for reductive dechlorination. The average pH was maintained at 5.53 (±0.09).

3.2.4 Bottle #4

Bottle #4 was created by making repeated dilutions of the culture in a 2.6 L bottle (Figures E.6 and E.12). The performance of bottle #4 was similar to the others, with transient accumulation of cDCE (up to 89 µmol per bottle, 5.3 mg/L when taking partitioning between the headspace and liquid into account), and VC (up to 116 µmol per bottle, or 1.3 mg/L when taking partitioning into account) (Figure 3.10). A sharp increase in ethene corresponded to a rapid decrease in VC. Between days 140 and 166, the ethene accumulation rate was 3.8 µM/d. Approximately 59% of the electron equivalents of lactate and lactic acid added was converted to methane, versus only 1.3% for reductive dechlorination. During the 157 days of incubation time, the average pH was maintained at 5.51 (±0.07).

3.3 Canisters

Once a sufficient amount of enrichment culture was available, a portion was used to inoculate two canisters (A and B). Growing the enrichment in canisters will provide enough culture for a field trial at some point in the future. The performance of the enrichments in the canisters thus far is described below.

3.3.1 Canister A

Canister A was started by combining 6 L of enrichment culture and 12 L of MSM. The sources of inoculum included several of the 2.6 L bottles described above as well as others described in Appendix E (Figures E.4, E.6 and E.11). The performance of canister

A is shown in Figure 3.11. Repeated additions of 100 µmol of PCE (resulting in an aqueous phase concentration of 0.9 mg/L) were dechlorinated, with relatively low transient accumulation of cDCE and VC. Ethene was the predominant product. The overall ethene accumulation rate was 0.7 µM/day; the maximum rate was 1.6 µM/d, between days 42 and 57. Approximately 87% of the electron equivalents of lactate and lactic acid added was converted to methane, versus only 0.9% for reductive dechlorination.The average pH was maintained at 5.48±0.04.

3.3.2 Canister B

Canister B was started before canister A, by combining 2.2 L of enrichment culture plus the contents of several microcosms and 15.8 L of MSM. The performance of the microcosms is described in Appendix E (Figures E.13 to E.20). Use of microcosms as a source of inoculum turned out to be problematic, since the soil periodically clogged the valves on the canister and attempts to unclog them may have resulted in some oxygen contamination. Nevertheless, sufficient culture was delivered to initiate PCE dechlorination. The performance of canister B is shown in Figure 3.12. For the first 271 days, cDCE was the predominant product, reaching a maximum of 3.7 mmol per canister (20 mg/L when taking partitioning between the headspace and liquid into account). As cDCE declined, VC and ethene increased. The rate of VC accumulation then increased, with VC reaching 2.9 mmol per canister (9.0 mg/L when taking partitioning between the headspace and liquid into account). Between day 349 and 474, the ethene accumulation rate was 0.8 µM/d. Approximately 42% of the electron equivalents of lactate and lactic acid added was converted to methane, versus only 0.01% for reductive dechlorination.

The average pH was maintained at 5.49 (±0.06). Methanogenesis was sufficient to build up pressure in the headspace of the canister; for this reason the headspace was vented on three occasions to relieve the pressure. This explains the vertical drop in VOCs on days 93, 149 and 349.

3.4 Bioaugmentation

Three treatments were prepared to evaluate the effectiveness of the enrichment culture for bioaugmentation: unamended, biostimulated and bioaugmented. Figure 3.13 shows one of the triplicate unamended microcosms (#3); results for the other microcosms are presented in Appendix E (Figures E.21 and E.22). The initial amount of PCE added was 7.2 µmol/bottle, or 8.6 mg/L when taking partitioning into account. After 120 days incubation, there was no evidence of PCE dechlorination. The pH was maintained at 5.48 (±0.05). It was necessary to make repeated additions of phosphoric acid to keep the pH from drifting upward.

Figure 3.14 shows one of the triplicate biostimulated microcosms; results for the other microcosms are presented in Appendix E (Figures E.23 and E.24). There was no evidence of reductive dechlorination of PCE, although methane started to accumulate after day 78. Lactate additions coincided with additions made to the bioaugmented microcosms (see below). The average pH was maintained at 5.51 (±0.07).

Figure 3.15 shows one of the triplicate bioaugmented microcosms; results for the other microcosms are presented in Appendix E (Figures E.25 and E.26). The enrichment culture was added at the start and on day 34 (0.5 mL per addition). By day 78, the PCE was consumed and resulted in primarily cDCE; VC increased at a slow rate. Thus far,

only a trace amount of ethene has accumulated. Further incubation is needed to establish if bioaugmenatation will result in complete dechlorination. Methanogenesis was also notable in these microcosms. The average pH was maintained at 5.50 (±0.05). Fewer additions of phosphoric acid were required compared to the other treatments.

3.5 Evaluation of pH Levels Below 5.5

Once successful maintenance of the enrichment culture was established at a pH of approximately 5.5, an experiment was performed to determine the response of the culture to lower pH levels. Three treatments were prepared, consisting of triplicate serum bottles with 100 mL of enrichment culture. The pH in one treatment was maintained at approximately 5.5; the pH in the other treatments was gradually lowered (by adding H_3PO_4) to an average of 5.36 and 5.31, respectively. The performance of the individual bottles is provided in Appendix E (Figure E.27-E.35). Figure 3.16 shows the cumulative amount of PCE consumed and ethene formed over 74 days of incubation. Although the rates were higher at pH 5.5, the results indicate that the culture remained active at the lower pH levels. Ethene accumulation was slowest during the period when the pH was being lowered (days 18-31) and then began to recover. Improvements in rate may be achievable with additional acclimation. The percentage of PCE recovered as ethene was 99%, 60% and 78% for the pH levels at 5.47, 5.36 and 5.31, respectively. Approximately 41, 45, and 45% of the electron equivalents of lactate and lactic acid added was converted to methane, versus only 0.7, 0.6, and 0.5% for reductive dechlorination, at pH levels of 5.47, 5.36 and 5.31, respectively.

CHAPTER FOUR

4.0 DISCUSSION

Many sites that are contaminated with chlorinated ethenes have pH levels below 6. Current practice with bioaugmentation requires that the pH level be raised above 6 in order to achieve complete dechlorination to ethene. However, chemical adjustment of aquifer pH can result in clogging, may not yield homogenous adjustment, and can be costly. Having access to a bioaugmentation culture that functions at a pH level below 6 would obviate the need for pH adjustment. Observations of complete dechlorination at several sites in North and South Carolina that have pH levels below 6 indicate that activity in the lower pH range is possible. The results of this study yielded an enrichment culture that achieves consistent levels of complete dechlorination at a pH level of 5.5. A microcosm test with groundwater and soil at this pH level suggests the culture has potential application in the field, although additional monitoring of the bioaugmentation microcosms will be needed to confirm complete dechlorination.

Earlier work with the enrichment culture by Chen (8) suggested that dechlorination activity slowed down with increasing dilution using mineral medium. One possible explanation for this was that the culture needed the presence of solids to somehow compensate for the low pH conditions. A similar conclusion was reached by Yang (21), who observed that dechlorination of PCE at pH levels below 6 was inhibited after three transfers in medium; he was unable to develop an enrichment culture. Other studies involving growth of microbes at low pH have also been conducted in the presence of a solid support. For example, Cox et al. (32) demonstrated biodegradation of styrene

in a fluidized bed reactor at a pH of 2.7 to 5.7 using perlite as the packing material. Woertz et al. (33) reported that *Chadophialophora* can grow on porous perlite granules (1 g for a 20 mL vial and sevied to 3.45-4.75 mm average diameter) in a pH range of 2.5-4.0. Thaiyalnayaki and Sowmeyan (34) demonstrated that perlite was the most suitable carrier compared with other material (e.g., thermo cool, plastic beads, cork and teak wood) when operating a lab-scale anaerobic fluidized bed reactor in a pH range of 4.3-5.3, because perlite has a largest surface area for supporting growth. Like other minerals, perlite is advantageous because it helps to prevent acidification (26). This was evident in the treatments tested with perlite; the pH tended to increase more quickly above 5.5 in the presence of perlite. Nevertheless, when dechlorination activity was compared in the presence and absence of perlite and sand, the rates of PCE dechlorination and ethene accumulation at pH 5.5 were similar, indicating that a support material was not necessary for sustained maintenance and enrichment of the low pH culture. All subsequent enrichment of the culture was performed in the absence of added particulates.

Perhaps the most critical metric for the performance of a bioaugmentation culture for chlorinated ethenes is the rate of ethene accumulation. The simplest way to calculate these rates is the amount of ethene formed divided by the volume of culture and the time required. On this basis, the highest rates observed during this study were 3.8 µM/d in one of the 2.6 L bottles and 1.6 µM/d in one of the canisters. This is an improvement over the highest rate of 1.8 µM/d reported by Chen (8), who worked with the predecessors to the culture developed in this study. Also, the culture developed in this study is essentially sediment-free, while most of what Chen worked with included more soil from the

original microcosms. Nevertheless, the rates are still low in comparison to an enrichment culture developed under neutral pH conditions, which has an ethene accumulation rate of ~30 µM/d (8). Caution should be used in making such comparisons, since the concentration of microbes, and in particular *Dehalococcoides*, is not factored in. It may well be that the rates are equivalent when normalized for biomass. The yield for *Dehalococcoides* may be lower at the lower pH level, so that lower densities may be present at the lower pH. Additional studies are needed to determine this. On a related note, growth of the enrichment culture seemed to be inhibited at PCE levels above 15 mg/L (data not shown), which may have limited the density of *Dehalococcoides* achieved. Further efforts to acclimate the low pH enrichment culture to higher PCE concentrations is warranted.

One of the distinguishing features of bioaugmentation cultures is the kinetics of reduction among the daughter products. When dechlorination slows down between daughter products, the potential for accumulation of lesser chlorinated compounds occurs. This is problematic, since the lesser chlorinated daughter products do not adsorb to soil as strongly (indicated by lower soil-water-partition coefficients); consequently, the daughter products are not retarded as much and therefore tend to be transported at a faster rate in groundwater. This is especially a concern with VC, which has the lowest soil-water-partition coefficient but also has the most stringent clean-up requirement, based on having a lower maximum contaminant level than any of the other chlorinated ethenes (http://water.epa.gov/drink/contaminants/basicinformation/vinyl-chloride.cfm).

The enrichment culture developed in this study tended to accumulate notable levels of cDCE and VC before the onset of ethene accumulation. This was also evident in the results thus far for the microcosm evaluation of bioaugmentation. It is unclear if the kinetics of cDCE to VC and VC to ethene can be improved with further enrichment. The challenges presented by transient accumulation of daughter products, and VC in particular, must be considered if the culture is ever deployed in the field. The comparatively high rate of PCE reduction to cDCE suggests that these steps are carried out by microbes other than *Dehalococcoides*, such as *Desulfitobacterium*, *Dehalobacter*, *Sulfurospirillum*, *Desulfuromonas*, and *Geobacter*. There is a need for characterization of the different types of dechlorinators present in the enrichment culture.

The pH level of 5.5 selected as the target for this study was somewhat arbitrary. Some contaminated aquifers has an even lower pH level. For this reason, an experiment was performed to determine the activity of the culture at pH levels of 5.35 and 5.30. Ethene accumulation rates decreased more than the PCE dechlorination rate, suggesting that microbes other than *Dehalococcoides* are responsible for PCE dechlorination to cDCE in this enrichment. Additional operation of the culture at the lower pH levels is needed to determine if the ethene accumulation rate can be improved.

Methanogenesis was a dominant process in the low pH enrichment culture. Consistently more than one half of the electron donor added was consumed for this purpose, and only about 1-2% for reductive dechlorination. The balance of donor is likely attributable to organic acids such as propionate, which tends to accumulate during batch additions of lactate. However, organic acids were not quantified. Methanogenesis

is an unproductive use for electron donor; a small amount of methane output can be beneficial due to release of growth factors by methanogens, but the levels seen with this enrichment culture go way beyond that benefit. Similarly low levels of electron donor use have been reported. For example, the percent utilization for dechlorination reported by Azizian et al. (35) was 6.5% by using lactate as electron donor and TCE as electron accepter under neutral pH condition. One strategy to limit methanogens in an enrichment culture is to increase the chlorinated ethene concentration so that it becomes inhibitory. As mentioned above, the highest concentration of PCE added that the enrichment culture tolerated was 15 mg/L; higher levels resulted in a decrease in ethene accumulation. However, the increases in PCE may have been too abrupt; a more gradual increase may make it possible to reach a concentration that inhibits methanogens. This, combined with routine removal of culture and replacement with fresh medium will gradually wash out the majority of the methanogens.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1. The low pH dechlorination culture initiated by Hickey (27) and Chen (8) was further enriched over approximately two years of incubation and multiple transfers in MSM. The volume of culture was scaled up from serum bottles to 2.6 L bottles and then to 19.6 L canisters, creating enough culture to be used in a pilot test at a hazardous waste site in which the aquifer pH is below 6. Consistent reductive dechlorination of PCE to ethene was achieved with the culture at a pH level of approximately 5.5. The highest rate of ethene accumulation was 3.8 μM/d.
2. Supporting material was unnecessary for growth of this low pH enrichment culture. Perlite slightly reduced the lag time needed for the onset of PCE dechlorination and ethene accumulation, but once dechlorination activity was established, perlite did not improve the process. Likewise, sand offered no advantages for growth of the low pH enrichment culture. This is fortuitous, since the presence of solids would hinder application of the culture in the field.
3. Having established consistent operation of the culture at pH 5.5, an experiment was performed to evaluate the effect of lower pH levels. The lowest pH evaluated was approximately 5.3. The culture continued to dechlorinate PCE to ethene; however, the rates were noticeably slower. Improvements in rate may be achievable at the lower pH levels with further incubation of the culture.

4. A microcosm experiment was performed with soil and groundwater from a site in which the pH is consistently below 6. Reductive dechlorination of PCE was observed in the treatment that was bioaugmented with the low pH enrichment culture; no dechlorination occurred in the unamended treatment or the treatment that received only lactate or lactic acid. Thus far, the main dechlorination product in the bioaugmented treatment is cDCE; VC has started to accumulate. Although preliminary, these results indicate the low pH enrichment culture shows potential for use in bioaugmentation of low pH sites, without the need for chemical adjustment of the pH.
5. The enrichment culture was inefficient in terms of its use of lactate or lactic acid for reductive dechlorination; only ~1-2% of the electron equivalents were used for this purpose. The majority of electron donor use was for methanogenesis. Decreases in methanogenesis may be achievable by increasing the concentration of PCE added to a level that is inhibitory to methanogens.

5.2 Recommendations

1. Further monitoring of the bioaugmentation test is needed to determine if complete dechlorination to ethene can be achieved at a pH of ~5.5. The bioaugmentation test should be expanded to include a treatment in which the culture added has been routinely maintained at a circumneutral pH.
2. There is a need for characterization of the different types of dechlorinators present in the enrichment culture. The tendency of the culture to accumulate cDCE well before the appearance of VC or ethene suggests that microbes other than *Dehalococcoides*

are responsible for PCE reduction to cDCE; this needs to be evaluated, along with characterization of the type of *Dehalococcoides* present.

3. The experiment to evaluate the performance of the culture at pH levels below 5.5 should be continued, to determine if further incubation will result in improvements in the rates of dechlorination, especially the critical step of VC to ethene.
4. If the microcosm evaluation of the culture is positive (based on complete dechlorination of the PCE to ethene), the canisters should be used to test the culture at the field scale.

TABLES

Table 1.1 Summary of studies on the effect of pH on pure cultures.[a]

Microbe or Culture	Type	pH Range Tested	Active pH Range	Reference
Desulfitobacterium dehalogenase JW/IU-DC1	PCE --> cDCE	6.0-9.0	7.5[b]	(13)
Desulfitobacterium sp. PCE-1	PCE --> cDCE	6.0-9.0	6.5-8.0[c]	(11)
Desulfitobacterium sp. PCE-S	PCE --> cDCE	4.4-9.0	7.2[d]	(36)
Desulfitobacterium sp. strain *Y51*	PCE --> cDCE	4.0 to 10	6.0-9.5	(37)
Dehalobacter restricts PER-K23	PCE --> cDCE	6.5-8.0	6.5-8.0[e]	(38)
Dehalobacter restricts TEA	PCE --> cDCE	None Given	-	(39)
*Sulfurospirillum multiverses (*formerly *Dehalospirillum)*	PCE --> cDCE	6.0-8.0	7.0-7.5[b]	(13)
Desulfuromonas chloroethenica TT4B	PCE --> cDCE	6.5-7.4	7.4[b]	(13)
Desulfuromonas michiganensis	PCE --> cDCE	6.8-8.0	7.0-7.5[b]	(40)
Strain MS-1	PCE --> cDCE	7.0	Not Given	(41)
Geobacter lovleyi SZ	PCE --> cDCE	5.5-8.0	6.5-7.2[b]	(42)
Desulfuromonas michiganensis strain BB1	PCE --> cDCE	5.5-8.0	6.0-7.2[f]	(21)
Sulfurospirillum multivorans	PCE --> cDCE	5.5-8.0	5.5-7.2[f]	(21)
Dehalococcoides mccartyi strain 195	PCE --> VC	7.0	Not Given	(2)
Dehalococcoides strain BAV-1	cDCE --> ethene	7.2	Not Given	(15)
Dehalococcoides strain FL2	TCE --> VC	7.2	Not Given	(43)
Dehalococcoides strain GT	TCE --> ethene	7.2-7.3	Not Given	(22)
Dehalococcoides strain VS	TCE --> ethene	7.2	Not Given	(23)

[a] Adapted from Chen (8).
[b] Active pH range = stated optimum pH range.
[c] Active pH range = range in which growth rate $\geqslant$ ~50% of the maximum growth rate.
[d] Active pH range = optimum, no data given.
[e] Optimum listed as 6.8-7.6.
[f] M.S. thesis; not peer-reviewed.

Table 1.2 Summary of studies on the effect of pH on bioaugmentation cultures. [a]

Bioaugmentation Culture	Type	pH Range Tested	Active pH Range	Reference
KB-1	PCE --> ethene	5.0-10.0	6.0-8.3[b]	(8)
KB-1	PCE --> ethene	7.0	Not Given	(44)
KB-1	No activity	6.5-6.9	Not Given	(45)
KB-1	PCE --> ethene	None Given	-	(46)
SDC-9	PCE --> ethene	4.9-5.8	Not Given[c]	(47)
SDC-9	PCE --> ethene	5.0-9.5	(6.1-7.4)[d]	(24)
BioDechlor	PCE --> ethene	None Given	-	(48)
BioDechlor	PCE --> ethene	7.1-7.3	7.1-7.3	(25)
Pinellas	TCE --> cDCE	6.0-6.5	Not Given	(49)
BCI	PCE --> ethene	Not Given	≥5.6	-[e]
BDI	PCE --> ethene	5.5-8.0	7.2[f]	(21)
Neckar River Samples	PCE --> ethene	5.5-7.2	5.5-7.2[f]	(21)
SL2-PCEa	PCE --> ethene	5.0-7.5	6.5-7.0	(26)
PL2-PCEb	PCE --> cDCE	5.0-7.5	5.5-7.5	(26)
AQ-1	cDCE --> ethene	5.0-7.5	>5.5	(26)
AQ-5	PCE --> ethene	5.0-7.5	6.0-7.5	(26)
PM	PCE --> ethene	5.0-7.5	>5.5	(26)

[a] Adapted from Chen (8).

[b] Listed as optimum; reference not publically available.

[c] No success in range tested.

[d] Active Range = range in which pH was kept for successful bioaugmentation of PCE to ethene.

[e] http://www.bcilabs.com/news.html

[f] M.S. thesis; not peer-reviewed.

FIGURES

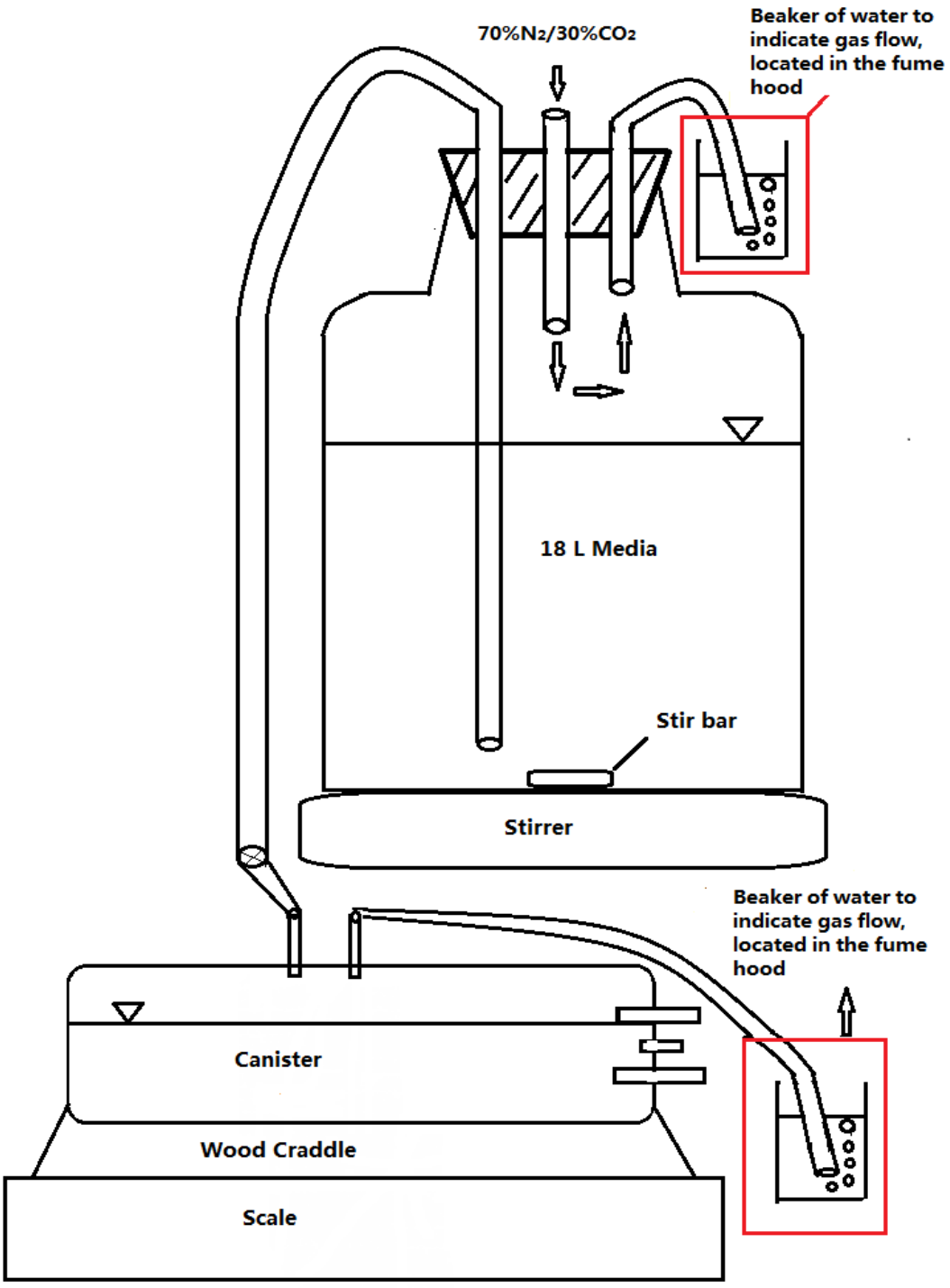

Figure 2.1: Showed the transfer medium to canister.

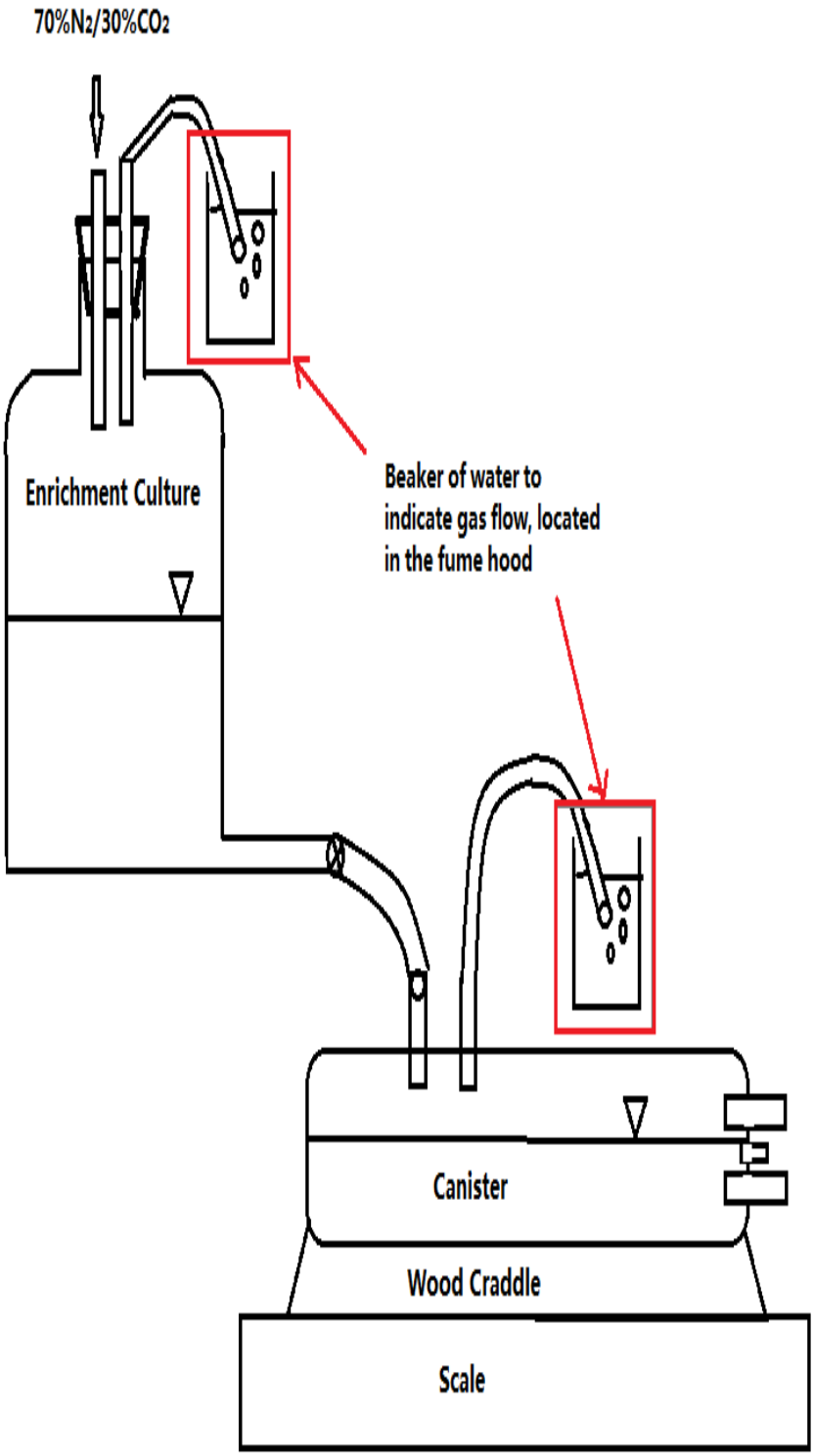

Figure 2.2: Showed the transfer enrichment to canister

]

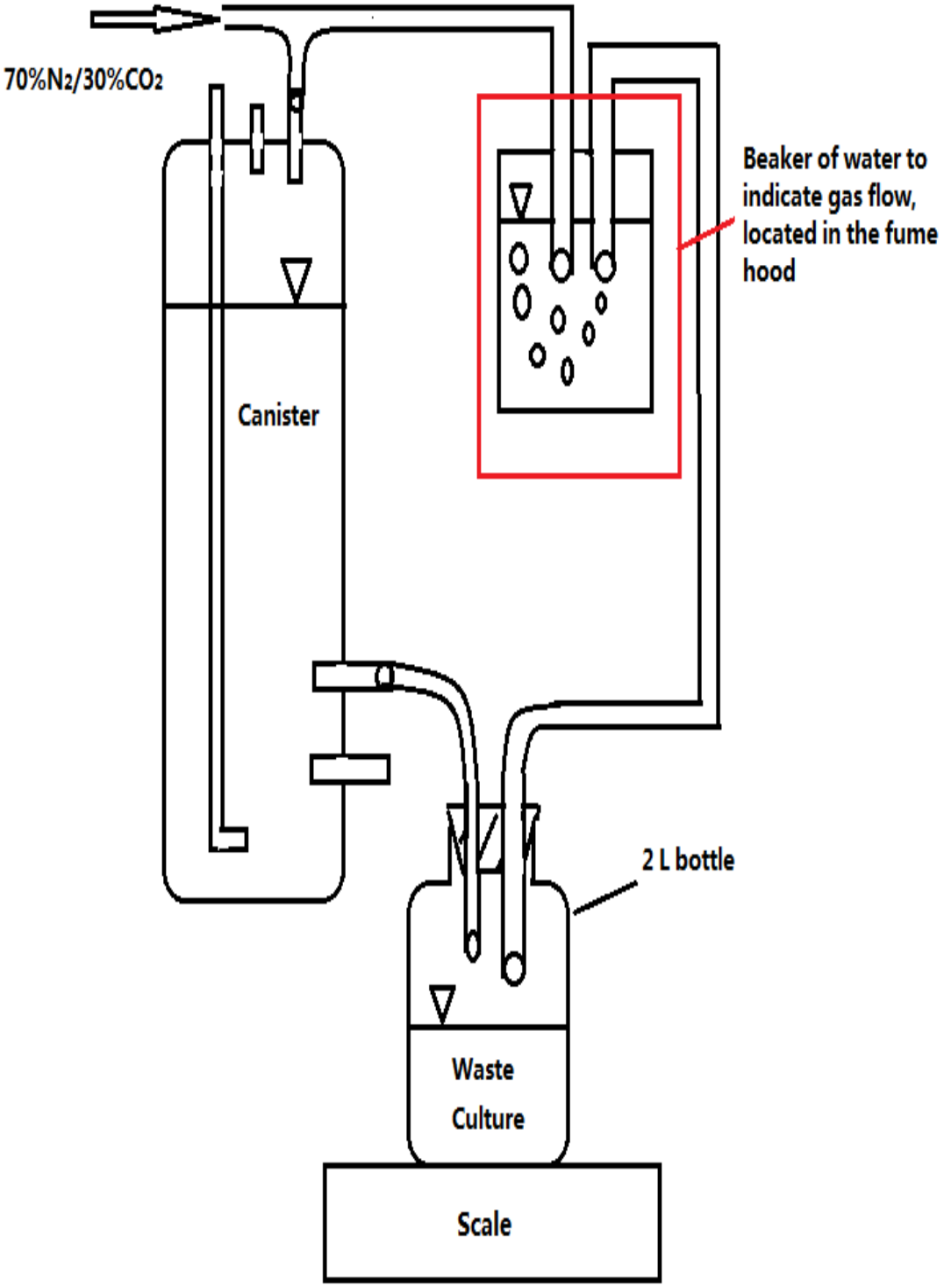

Figure 2.3: Showed the wasting enrichment culture from canister

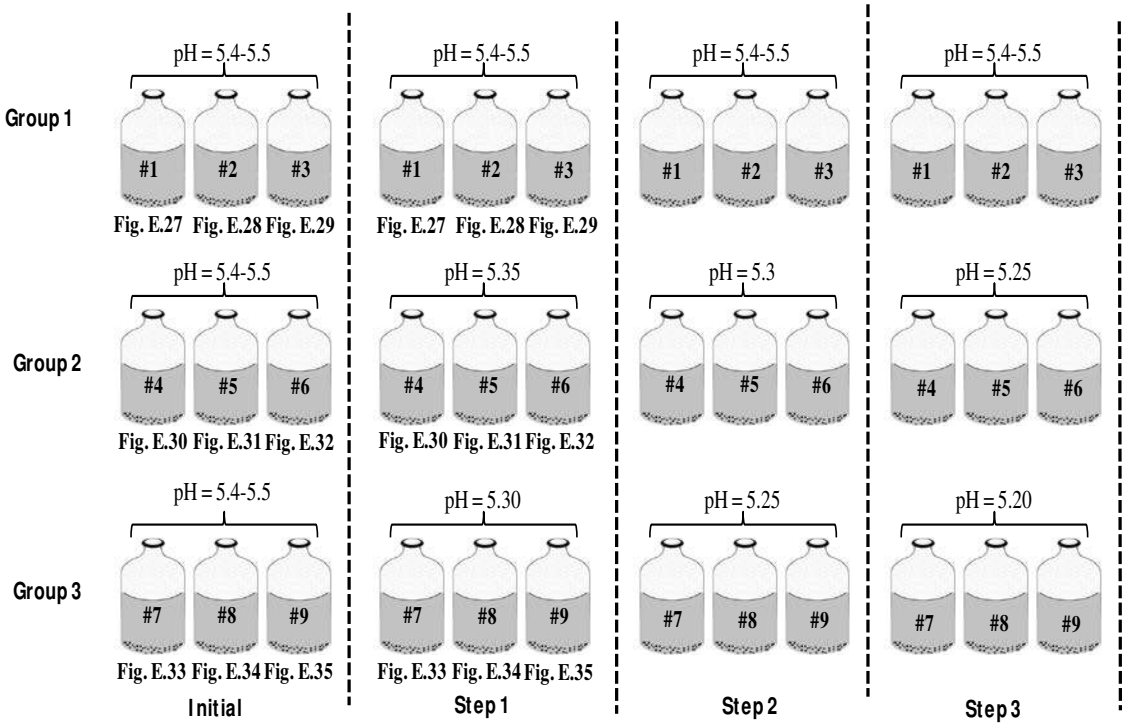

Figure 2.4: Experimental design to evaluate reductive dechlorination of PCE at pH levels of 5.5 and lower. Group 1 was maintained at pH 5.4-5.5. Group 2 started at pH 5.4-5.5 and the pH was gradually lowered. The same applies to Group 3, although the target pH was lower. Figures below each bottle indicate where the results may be found. Time did not permit advancing to setps 2 and 3 for Groups 2 and 3.

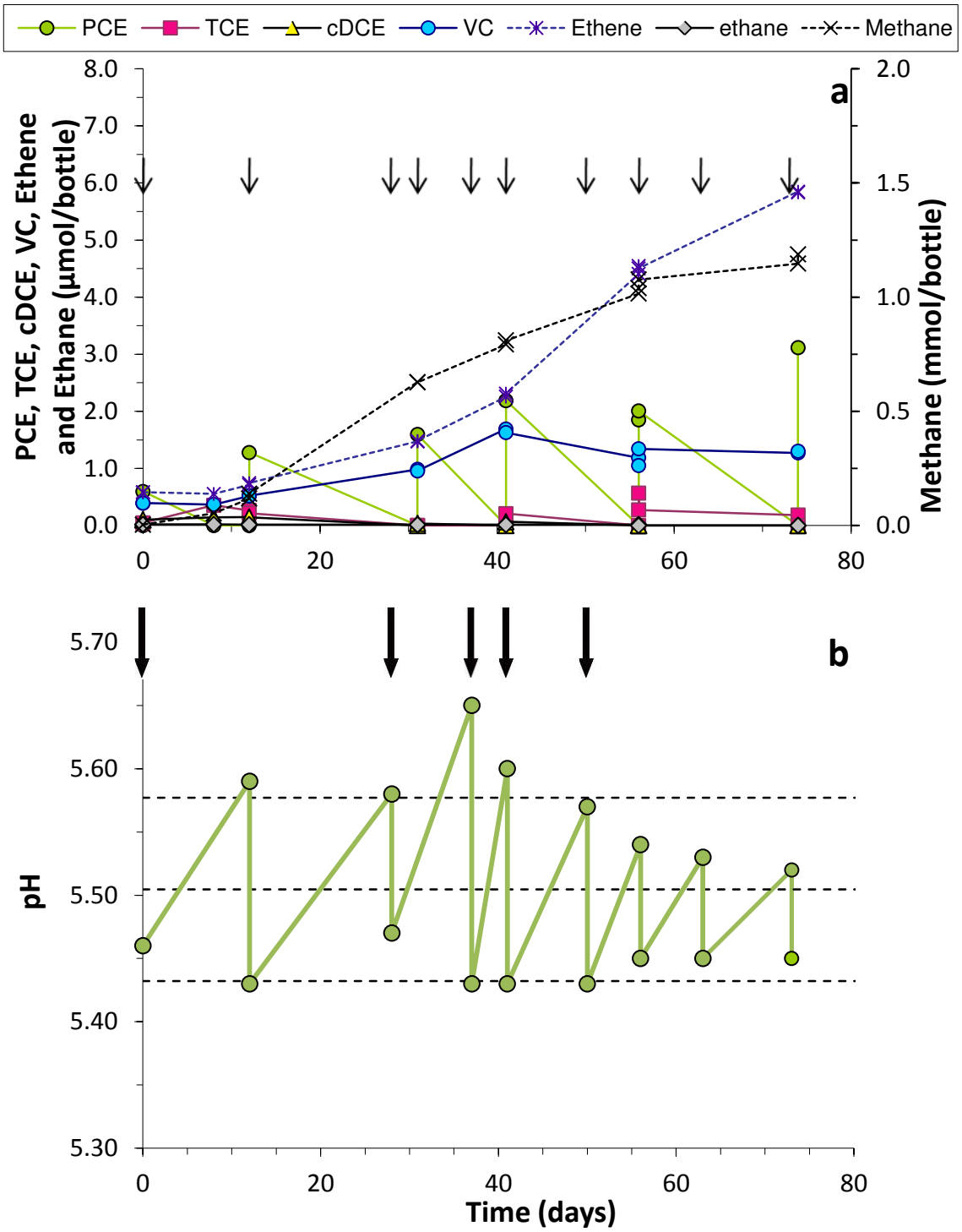

Figure 3.1: Lactate-amended enrichment culture with perlite added (serum bottle MM-B3+NC-perlite-2) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate addition of 1 M phosphoric acid.

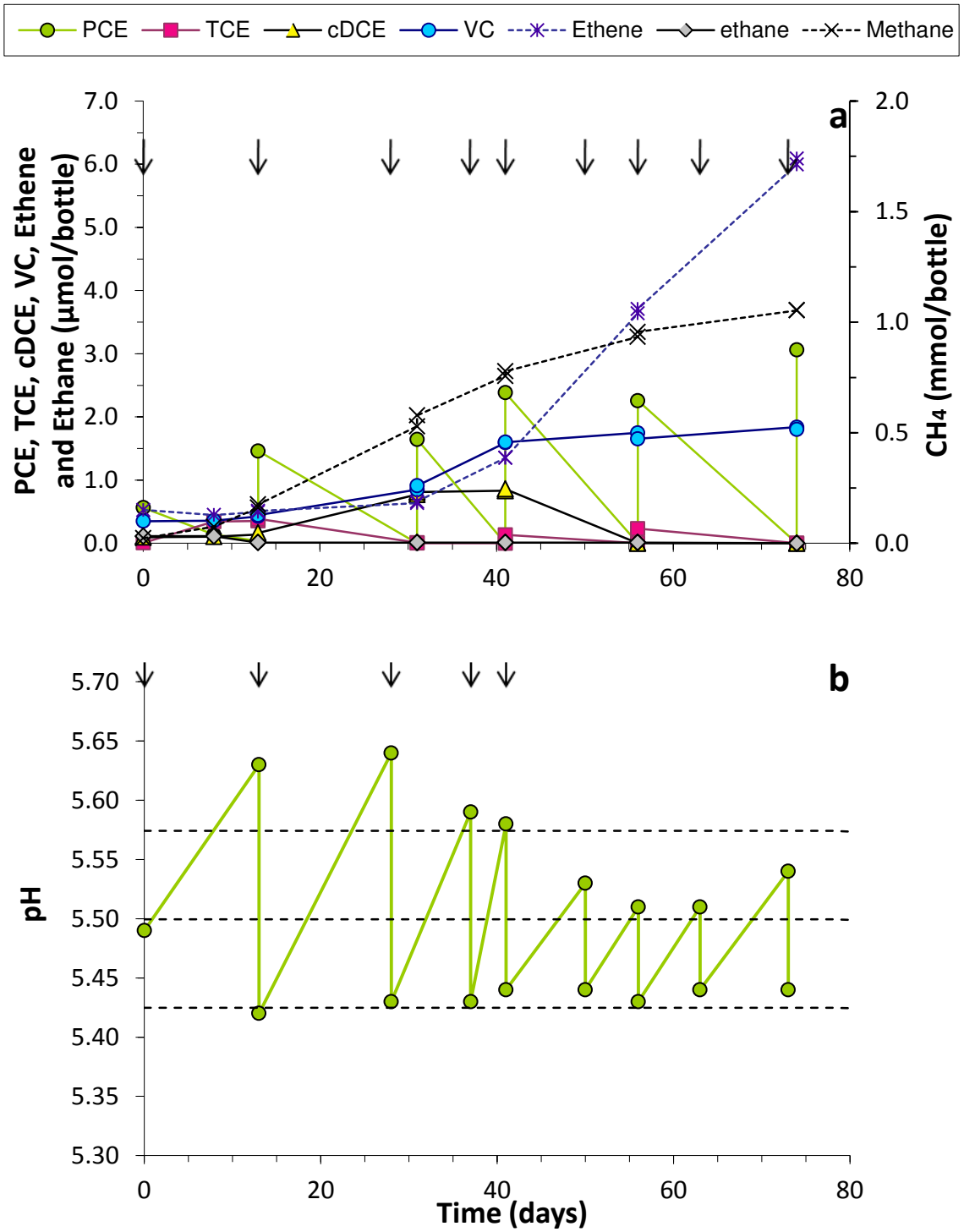

Figure 3.2: Lactate-amended enrichment culture (serum bottle MM-B3+NC-control-2) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; dashed horizontal lines represent the average ± one standard deviation; arrows indicate addition of 1 M phosphoric acid.

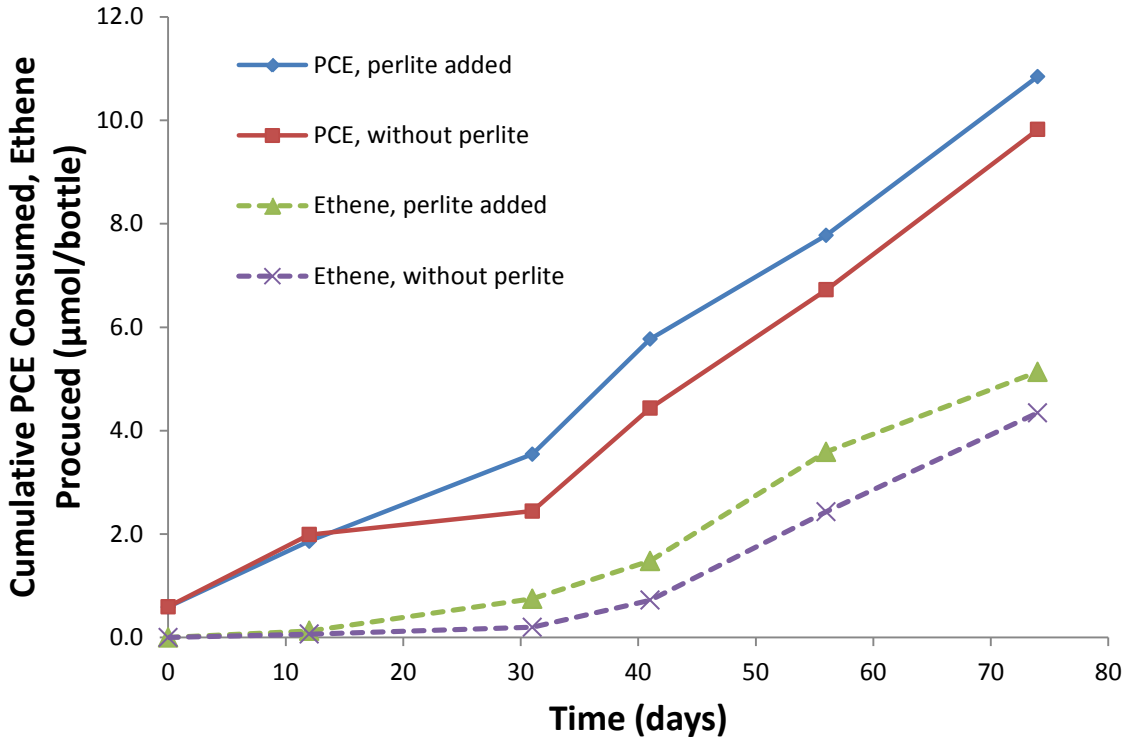

Figure 3.3: Average cumulative PCE addition and ethene production for two enrichment cultures (serum bottles MM-B3 + NC-Perlite-1 &MM-B3 + NC-Perlite-2) with perlite added and two (serum bottles MM-B3 + NC-Control-1&MM-B3 + NC-Control-2) without perlite.

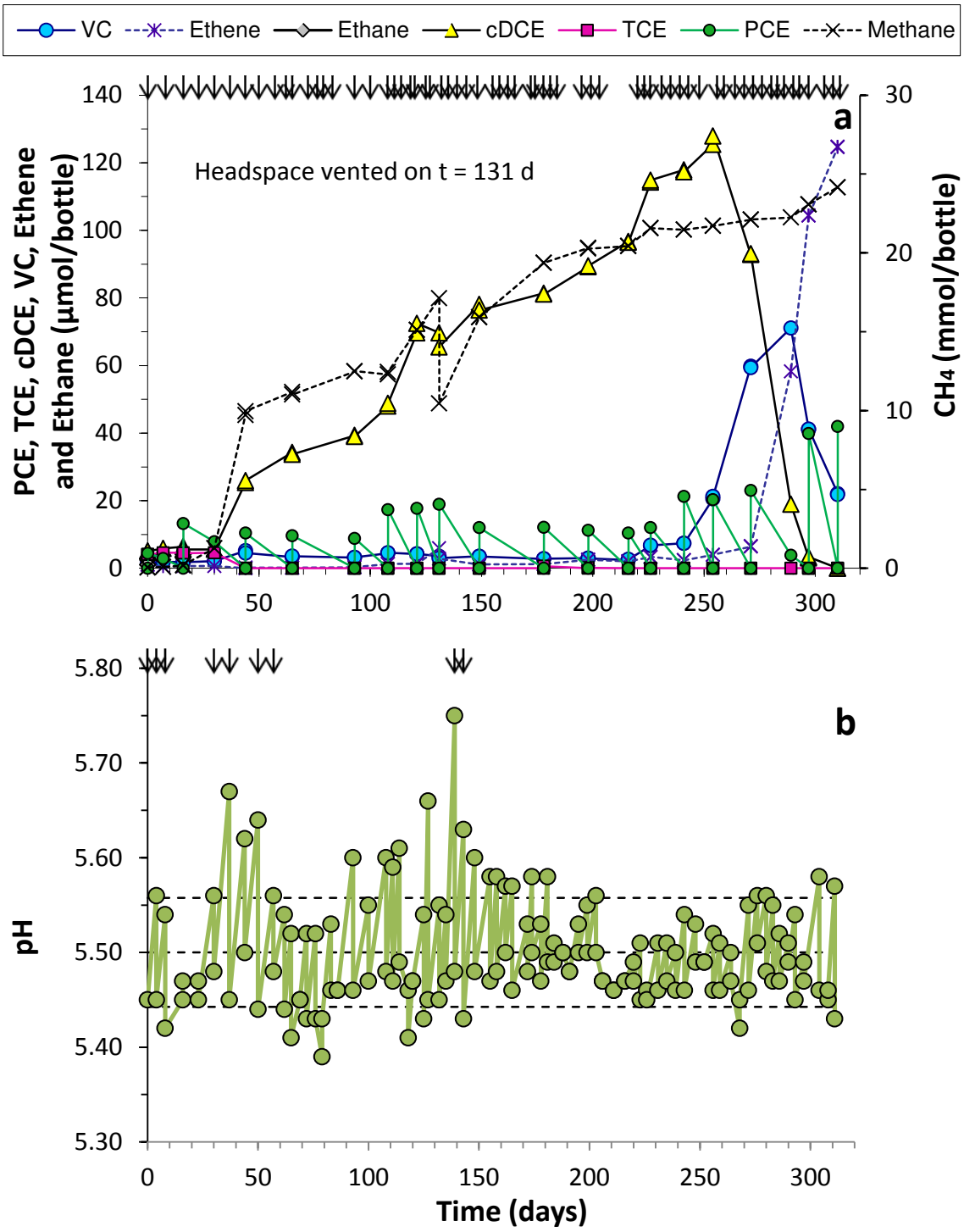

Figure 3.4: Lactate-amended enrichment culture with sand added in a 2.6 L bottle (RS-6.0-3B-2) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH, dashed horizontal lines represent the average ± one standard deviation; arrows indicate addition of 1 M phosphoric acid.

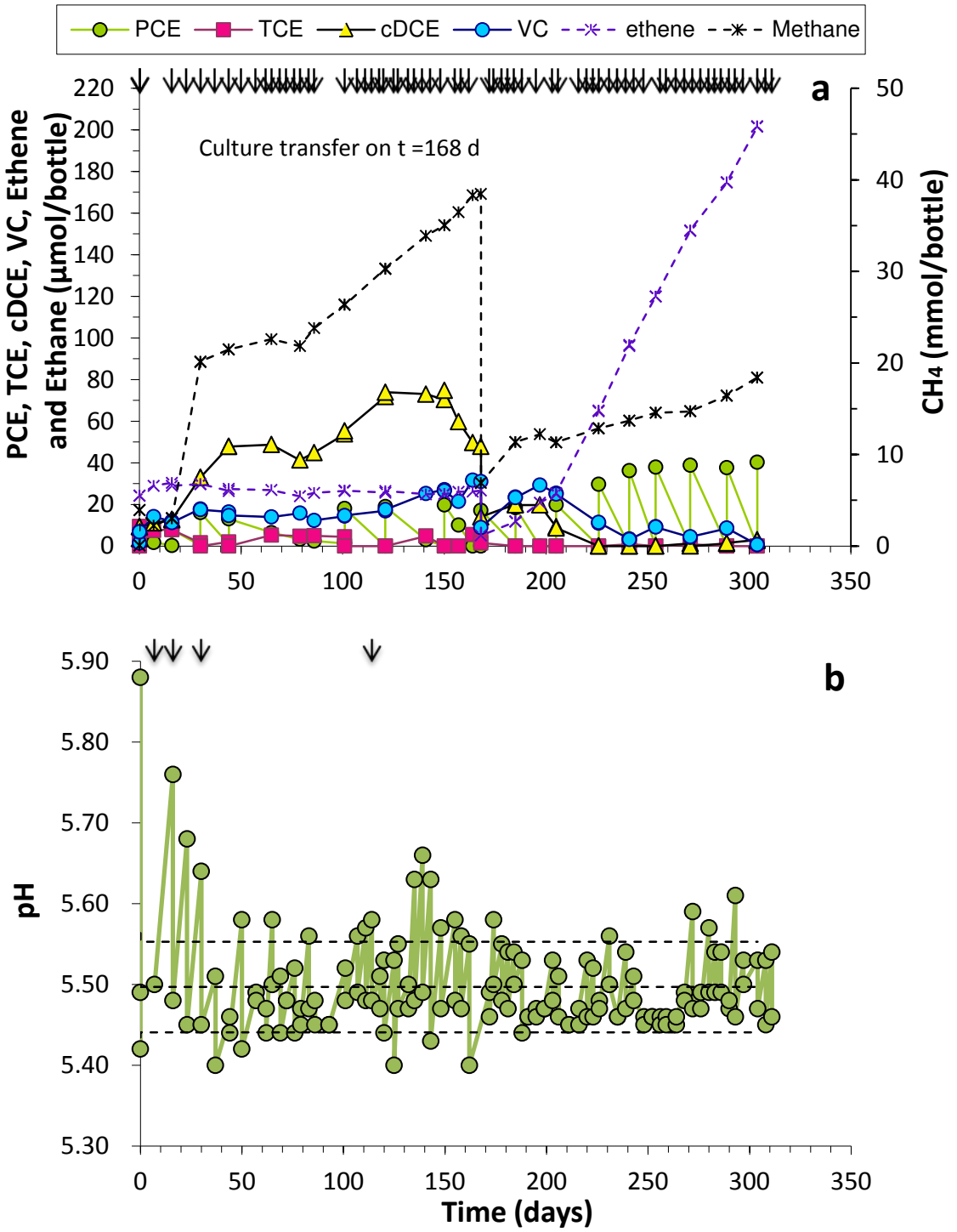

Figure 3.5: Lactate-amended enrichment culture in a 2.6 L bottle (RS-6.0-3B) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1 M phosphoric acid.

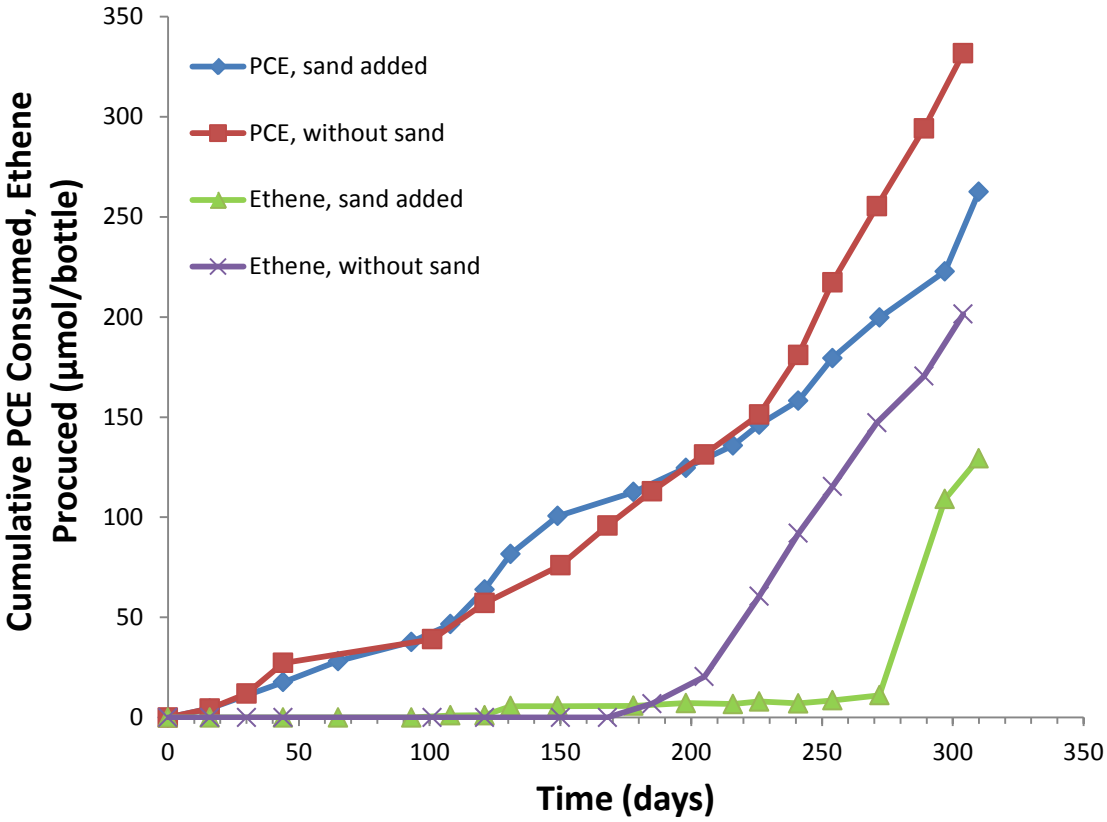

Figure 3.6: Cumulative PCE addition and ethene production for two enrichment cultures (RS-6.0-3B-2) with sand added and (RS-6.0-3B) without sand.

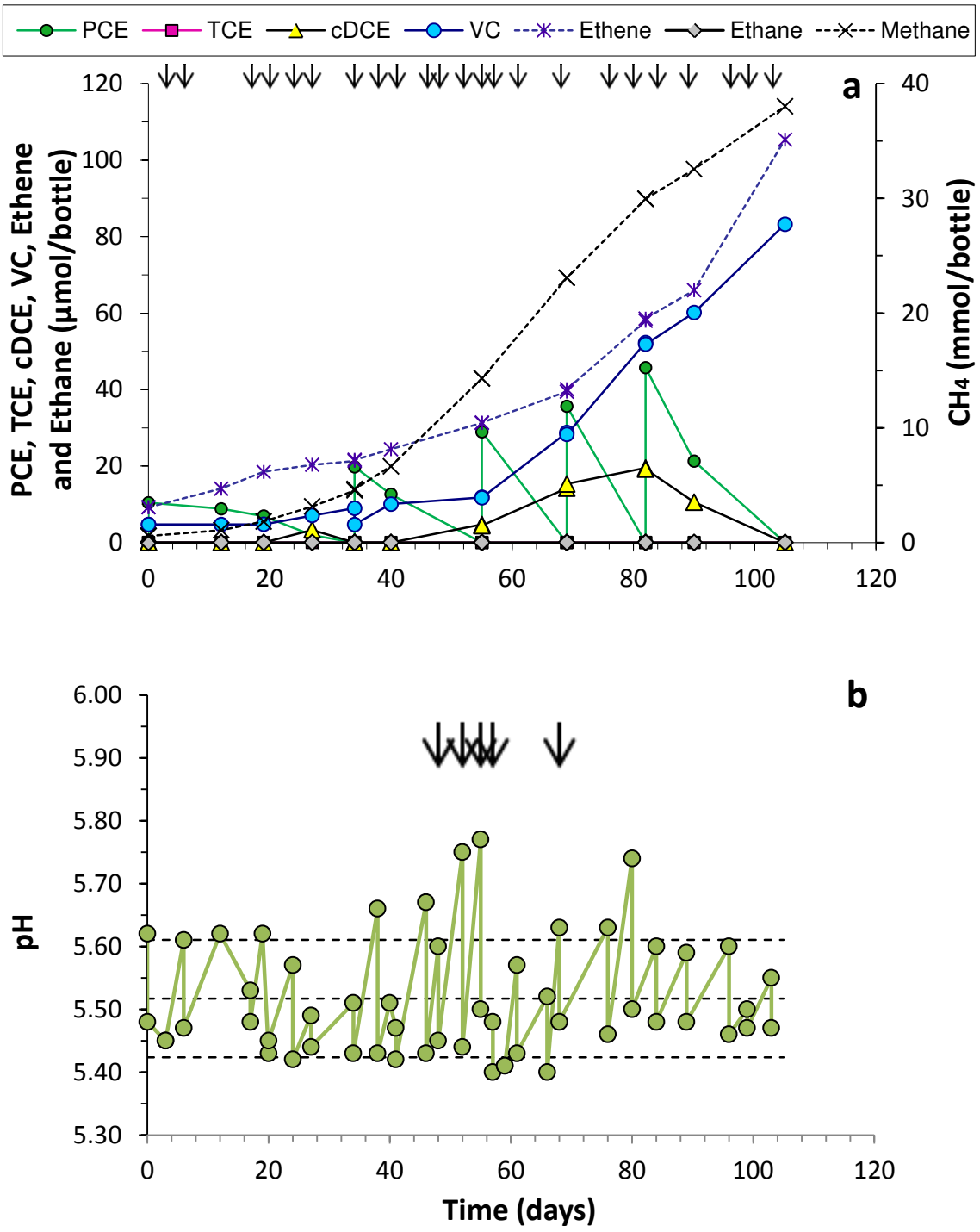

Figure 3.7: Lactate-amended enrichment culture in 2.6 L bottle#1 (GW-VCcDCE-B2+NC-UN-B2-3) for **a)** VOCs; arrows indicate addition of lactate or lactic acid; and **b)** pH; dashed horizontal lines represent the average ± one standard deviation; arrows indicate addition of 1 M phosphoric acid.

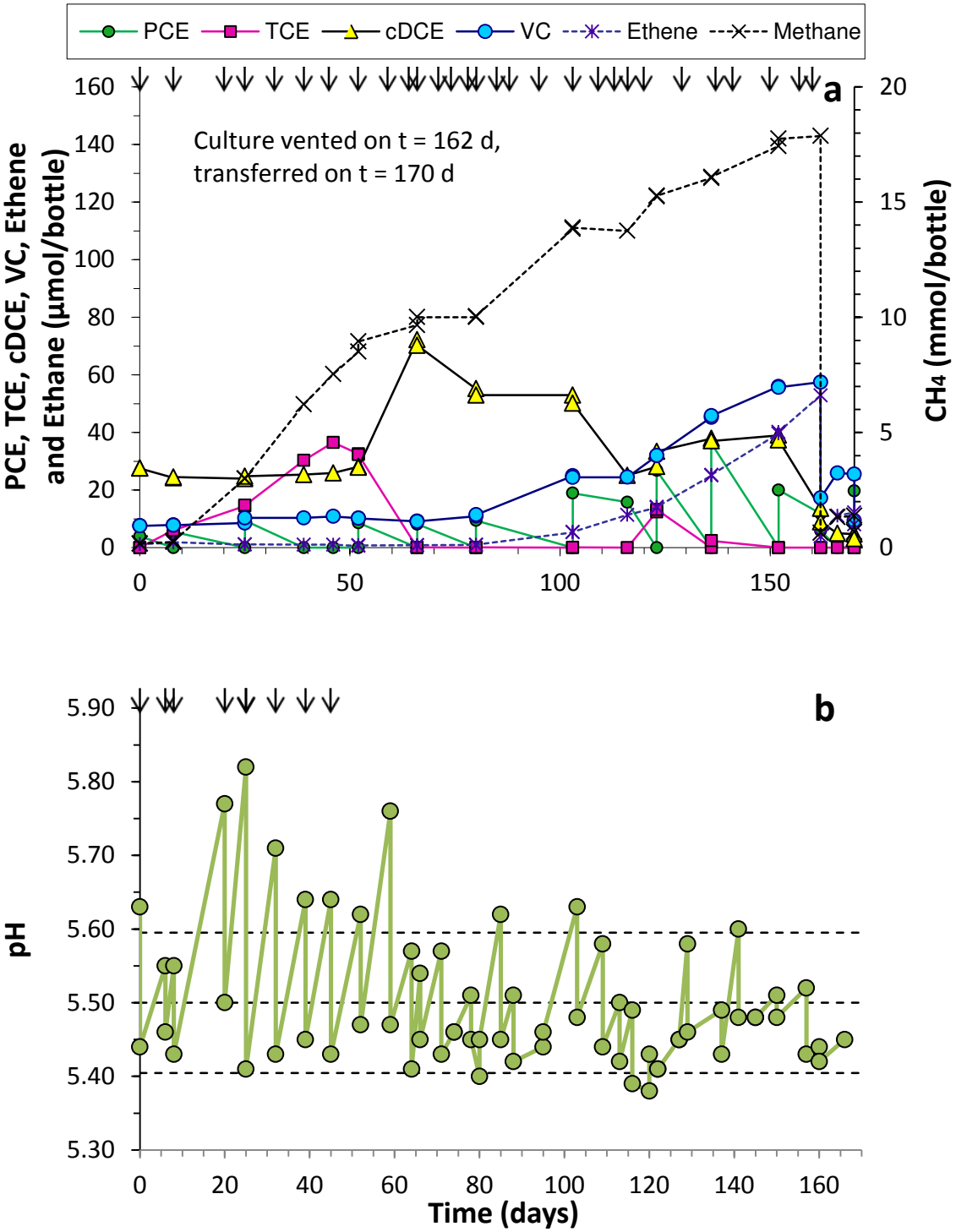

Figure 3.8: Lactate-amended enrichment culture in 2.6 L bottle #2 (MM-B3+NC+GW-4B+UN) for **a)** VOCs; arrows indicate addition of lactate or lactic acid; and **b)** pH; dashed horizontal lines represent the average ± one standard deviation; arrows indicate addition of 1 M phosphoric acid.

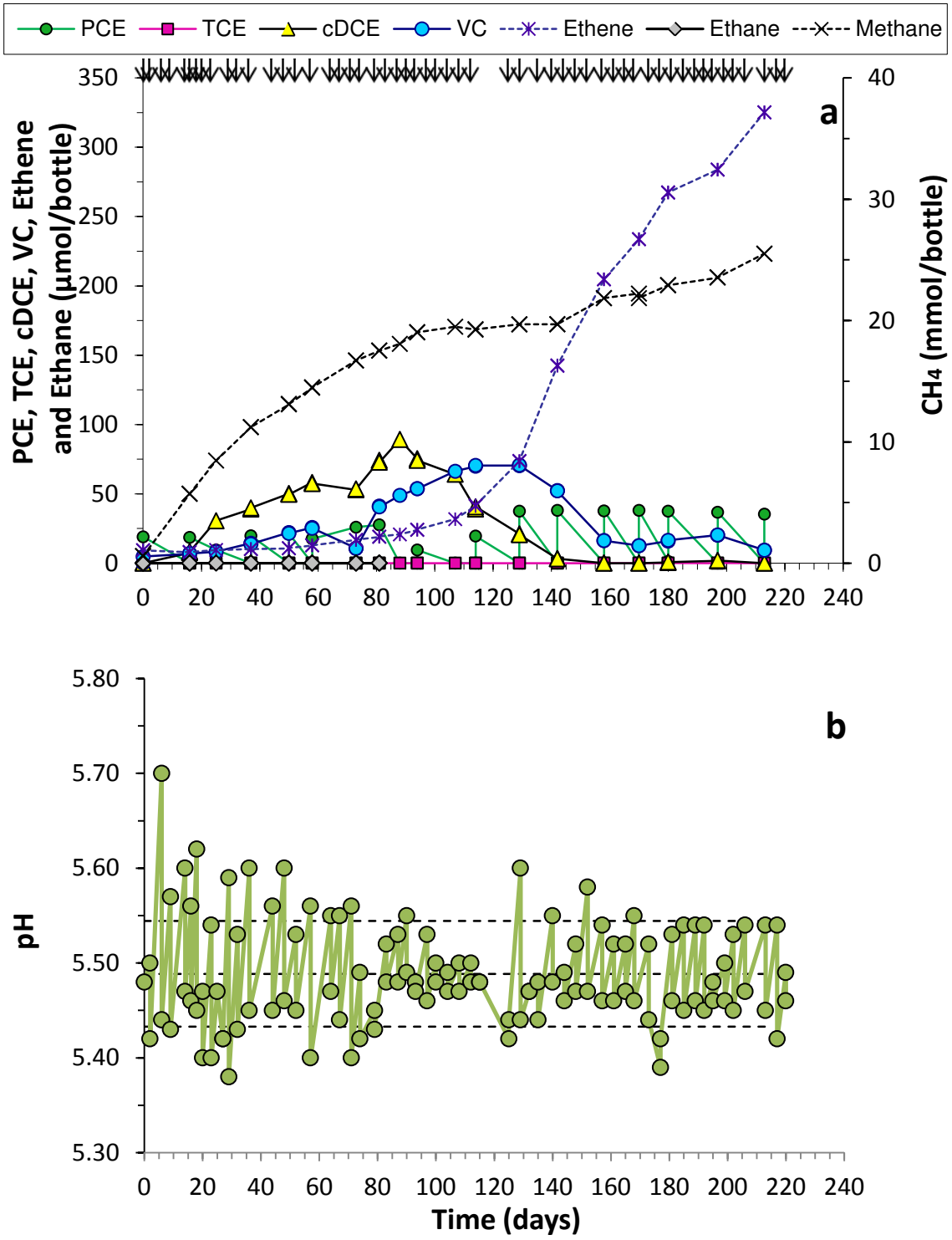

Figure 3.9: Lactate-amended enrichment culture in 2.6 L bottle #3 (GW-MM-B3-UN-MSM-1-2) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; dashed horizontal lines represent the average ± one standard deviation.

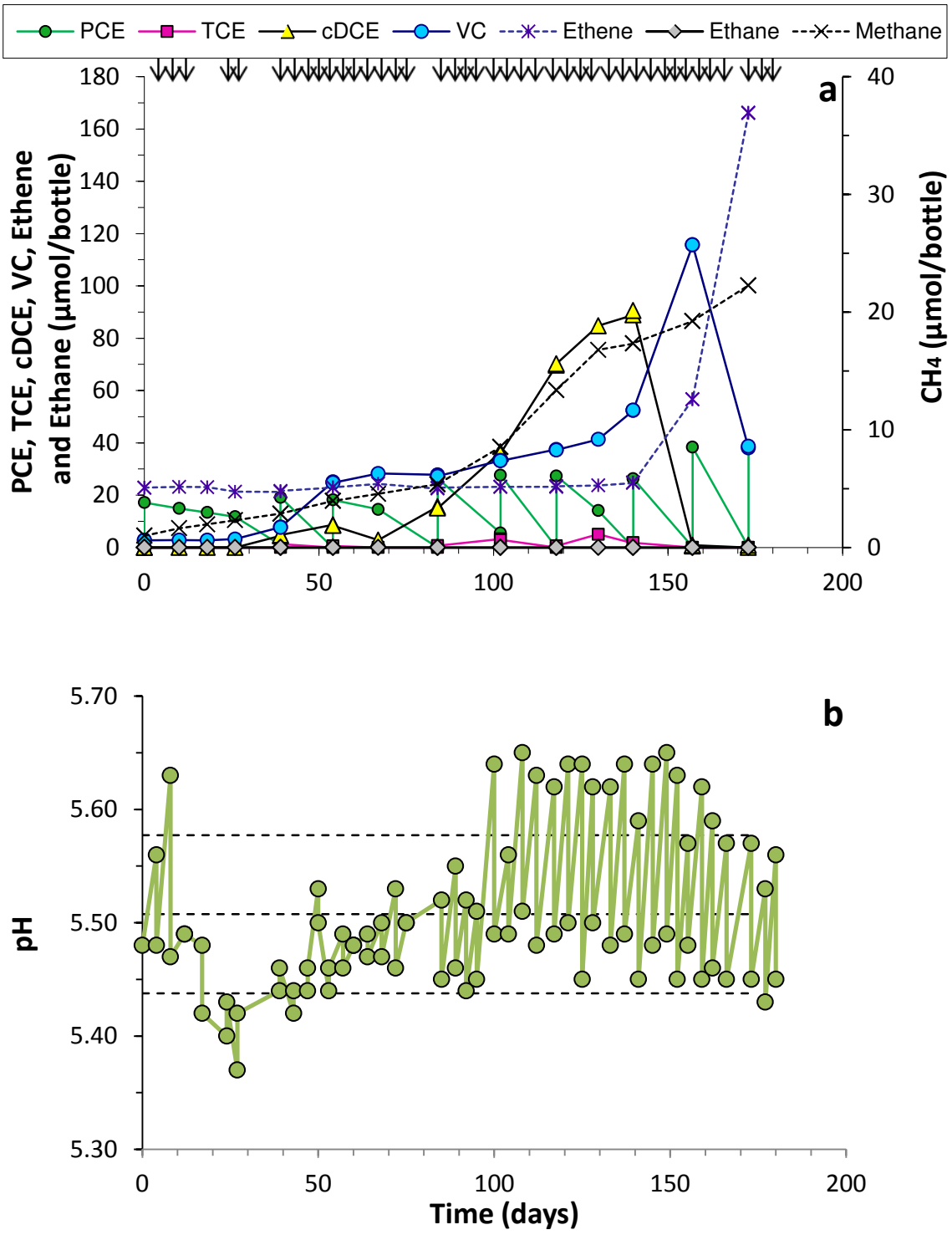

Figure 3.10: Lactate-amended enrichment culture in 2.6 L bottle#4 (GW-VCcDCE-B2 + NC-UN-B2-4) for **a)** VOCs; arrows indicate addition of lactate or lactic acid; and **b)** pH; dashed horizontal lines represent the average ± one standard deviation.

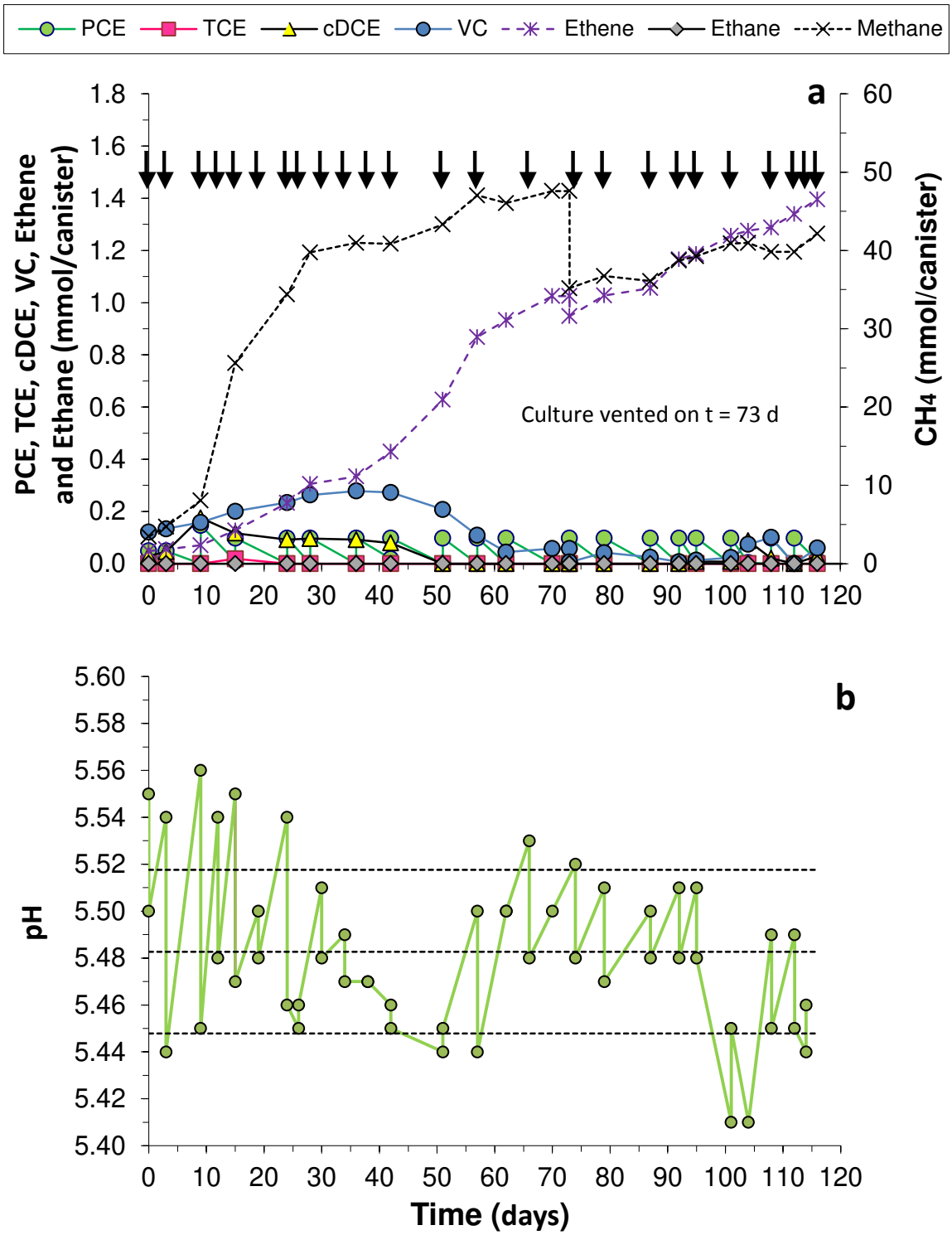

Figure 3.11: Lactate-amended enrichment culture in 21 L canister A for **a)** VOCs; arrows indicate addition of lactate or lactic acid; and **b)** pH; dashed horizontal lines represent the average ± one standard deviation.

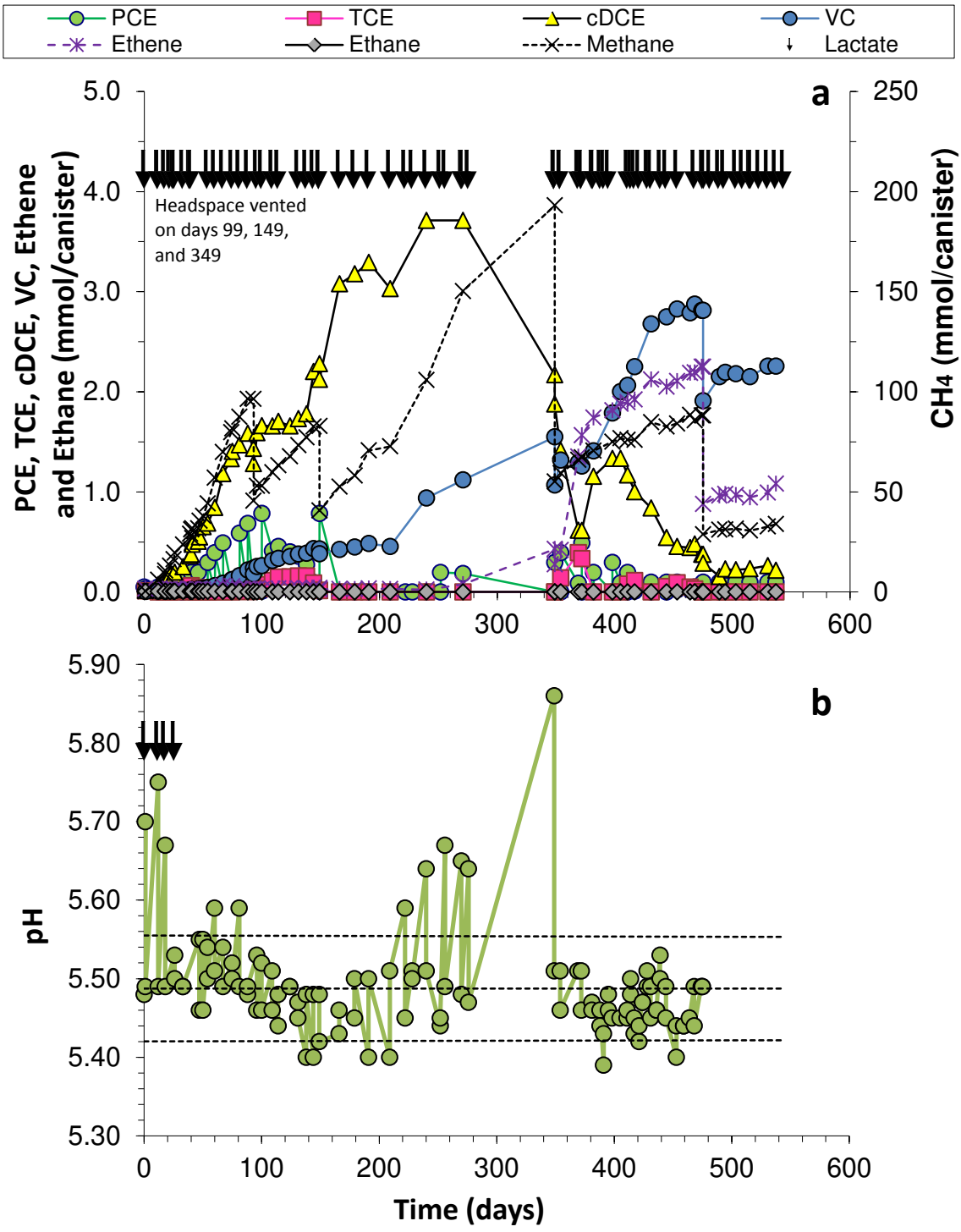

Figure 3.12: Lactate-amended enrichment culture in 21 L canister B for **a)** VOCs; arrows indicate addition of lactate or lactic acid; and **b)** pH; dashed horizontal lines represent the average ± one standard deviation; arrows indicate addition of 1 M phosphoric acid.

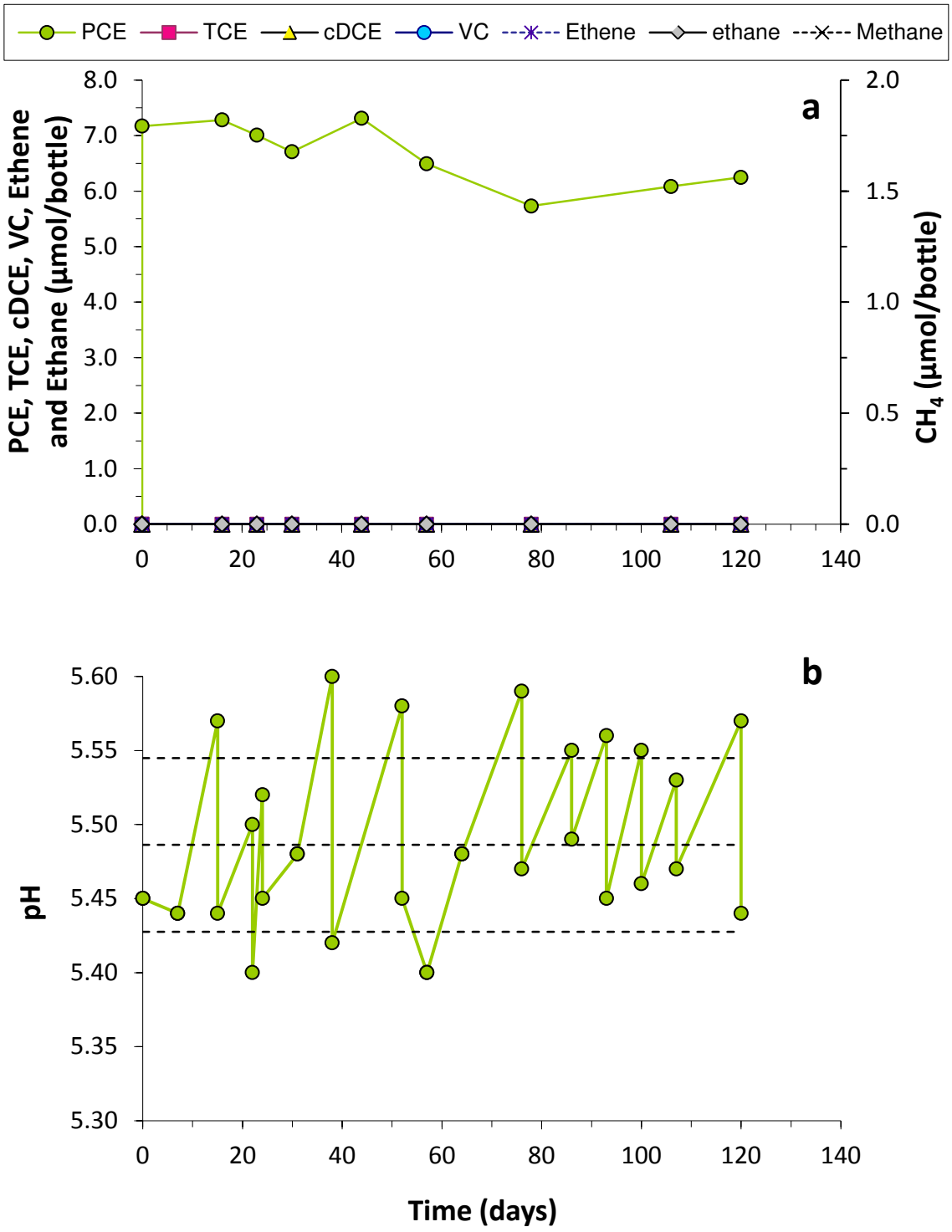

Figure 3.13: Unamended microcosm #3, part of the bioaugmentation experiment for **a)** VOCs; arrows indicate addition of lactate; and **b)** pH; dashed horizontal lines represent the average ± one standard deviation; arrows indicate addition of 1 M phosphoric acid.

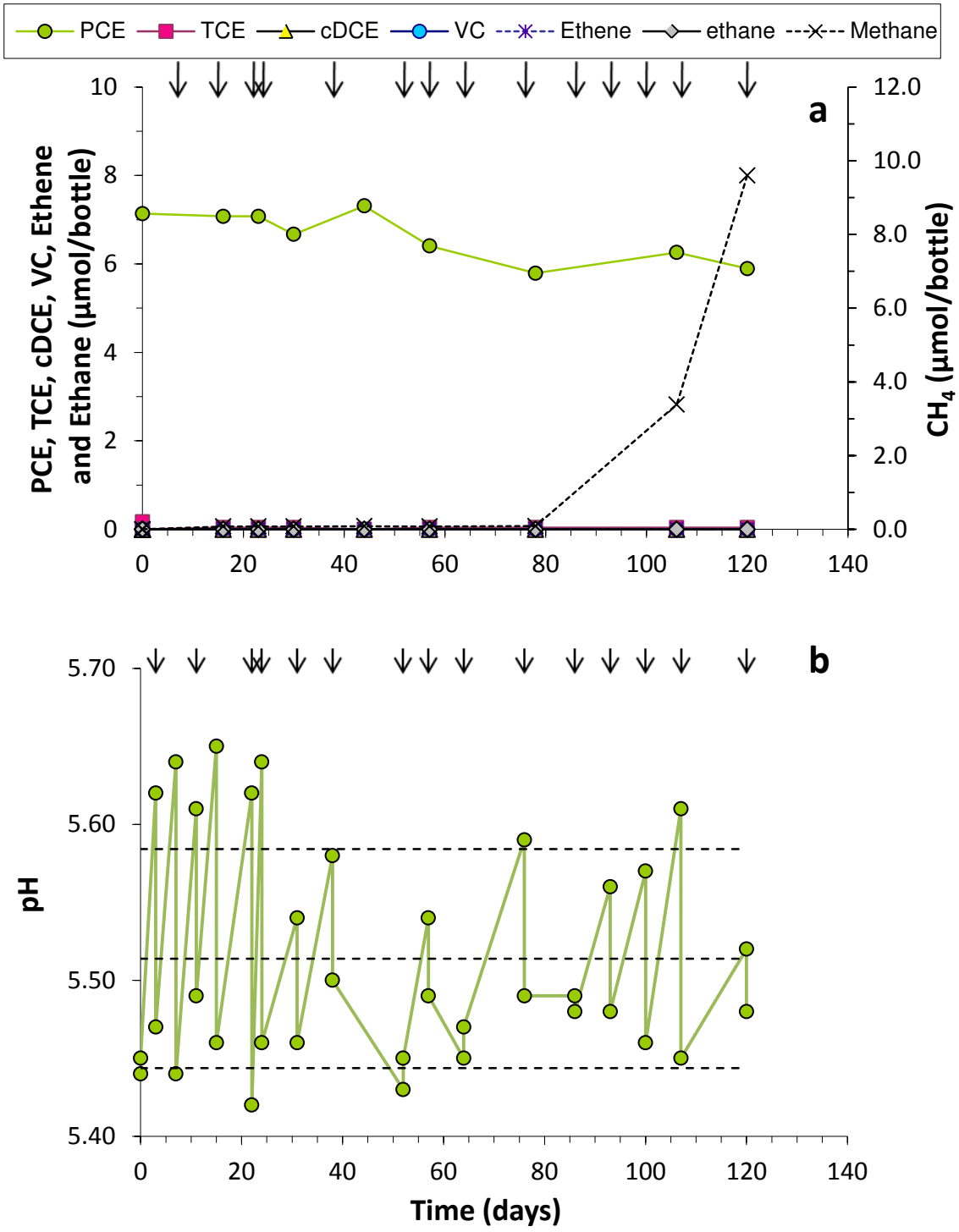

Figure 3.14: Biostimulation microcosm #1, part of the bioaugmentation experiment for **a)** VOCs; arrows indicate addition of lactate; and **b)** pH; dashed horizontal lines represent the average ± one standard deviation; arrows indicate addition of 1 M phosphoric acid.

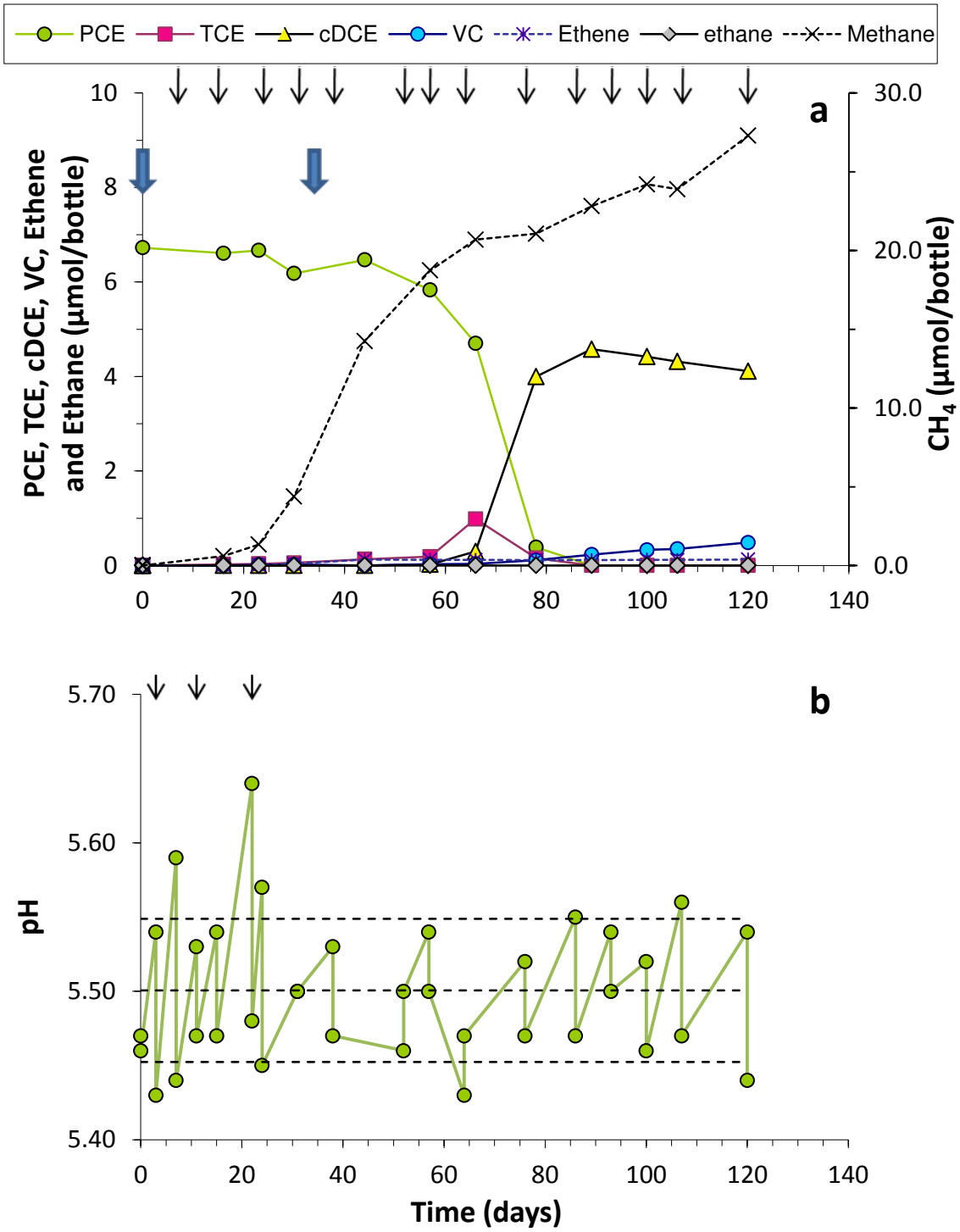

Figure 3.15: Bioaugmentation microcosm #2, part of the bioaugmentation experiment for **a)** VOCs; arrows indicate addition of lactate and large blue arrows indicate when culture was added; and **b)** pH; dashed horizontal lines represent the average ± one standard deviation; arrows indicate addition of 1 M phosphoric acid.

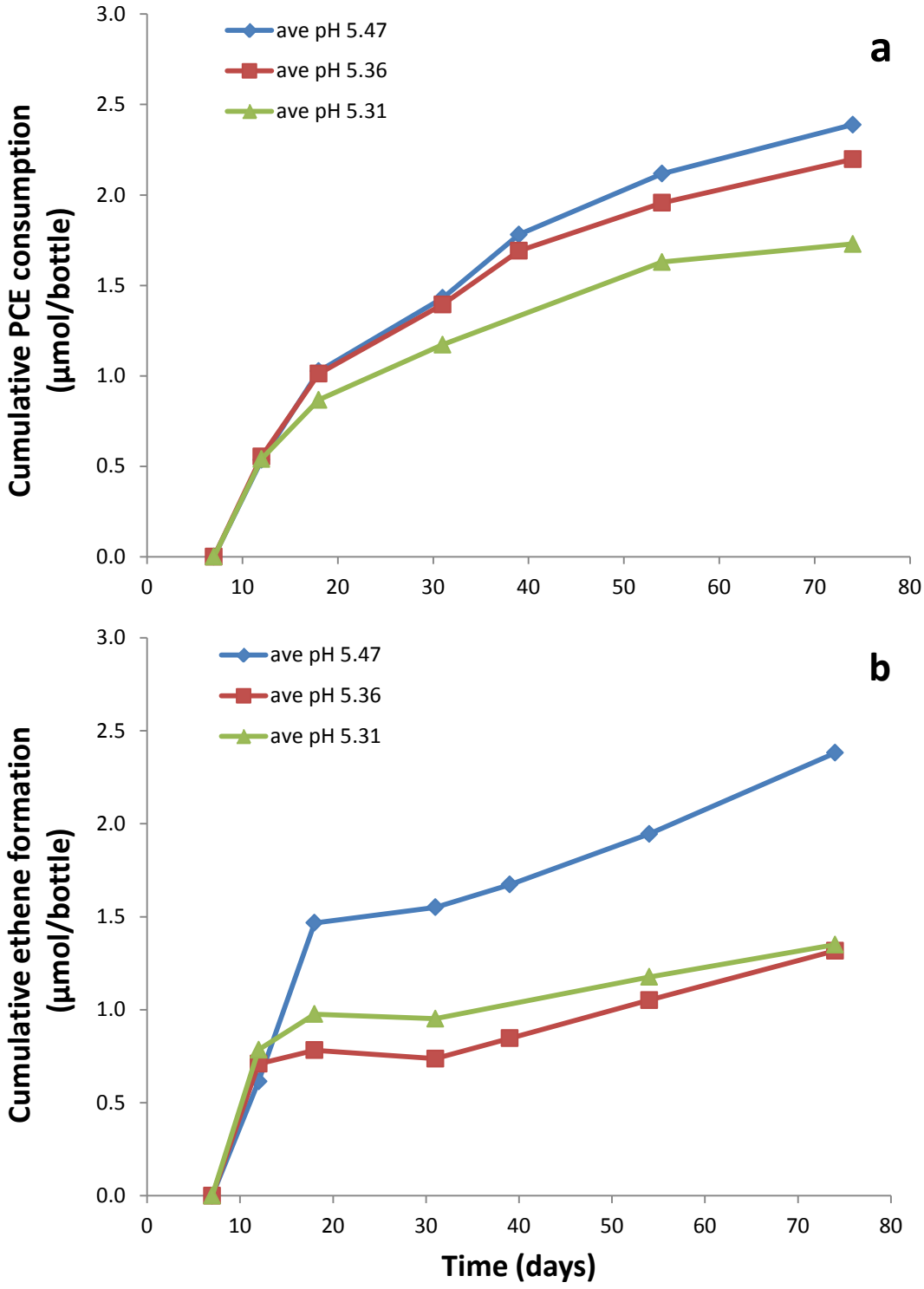

Figure 3.16: Average results for evaluation of pH levels at and below ~5.5; **a)** cumulative PCE consumed; and **b)** ethene production.

APPENDICES

Appendix A

Modified MSM Preparation

Reagents and stock solutions needed for the medium:

- Phosphate solution I
In a 100 mL volumetric flask add 5.25 g K_2HPO_4. Fill to 100 mL with DDI water.

- Phosphate solution II
In a 100 mL volumetric flask add 4.08 g KH_2PO_4. Fill to 100 mL with DDI water.

- Salt solution
In a 100 mL volumetric flask add:
5.35 g NH_4Cl
0.46976 g $CaCl_2 \cdot 2H_2O$
0.17787 g $FeCl_2 \cdot H_2O$
Fill to 100 mL with DDI water.

- Trace metals solution
In a 100 mL volumetric flask add:
0.03 g H_3BO_3
0.0211 g $ZnSO_4 \cdot 7H_2O$
0.075 g $NiCl_2 \cdot 6H_2O$
0.1 g $MnCl_2 \cdot 4H_2O$
0.01 g $CuCl_2 \cdot 2H_2O$
0.15 g $CoCl_2 \cdot 6H_2O$
0.002 g Na_2SeO_3
0.01 g $Al_2(SO_4)_3 \cdot 16H_2O$
1 mL HCl, 37%.
Fill to 100 mL with DDI water.

- Magnesium sulfate solution
In a 100 mL volumetric flask add 6.25 g $MgSO_4 \cdot 7H_2O$. Fill to 100 mL with DDI water.

- Redox solution
In a 10 mL volumetric flask add 0.01 g resazurin. Fill to 10 mL with DDI water.

- Yeast extract solution
In a 100 mL volumetric flask add 0.5 g yeast extract. Fill to 100 mL with DDI water.

- Ferrous sulfide
For 1 L of media, weigh into separate glass vials:

0.24 g of $Na_2S \cdot 9H_2O$
0.1448 g $FeCl_2 \cdot H_2O$

Media Preparation

1) In a 1 L bottle add:
    0.2 mL phosphate solution I
    9.8 mL phosphate solution II
    10 mL salt solution
    2 mL trace metals solution
    2 mL magnesium sulfate solution
    1 mL redox solution
    965 mL DDI water

2) Autoclave the above solution and allow to cool.

3) Add: 10 mL filter sterilized yeast extract

4) Transfer the bottle to the glove box along with the vials of sodium sulfide and ferrous chloride and 10 mL of sterile DDI water. When the O_2 reaches zero, add the 0.24 g of $Na_2S \cdot 9H_2O$ and rinse the vial with ~5 mL of sterile DDI water. Wait until the media turns from pink to clear.

5) Then add the 0.1448 g $FeCl_2 \cdot H2O$. Rinse the vial with ~5 mL of sterile DDI water.

6) After dispensing the media, remove bottles from the glove box and purge the headspace with oxygen-free gas containing 70% N_2 and 30% CO_2 .

7) Titrate media to desired pH using ~1 M H_3PO_4 .

Appendix B

Calculation of Electron Donor Needed for Reductive Dechlorination

Approaches for making sodium lactate solution:

70g of 60% sodium lactate mixed with 100 mL DDI water, the total volume of mixing solution was 150 mL.

So, concentration of sodium lactate $= \frac{70\ \text{g}}{0.15\ \text{L}} \times 60\% = 280\ \text{g/L} = 280\ \text{mg/mL}$

Suppose for 1 mmol of lactate can offer 12 meq of electron.

The electron of lactate for 1 mL of sodium lactate can offer was:

$$280\ \text{mg/mL} \times 1\ \text{mL} \times \frac{1\ \text{mmol}}{112\ \text{mg}} \times \frac{12\ \text{meq}}{\text{mmol}} = 30\ \text{meq}$$

Approaches for making lactic acid solution:

21 mL of lactic acid (85%) mixed with 79 mL of DDI water, the total volume of mixing solution was 97 mL.

So, concentration of lactic acid $= \frac{21\ \text{mL} \times 1.206\ \text{g/mL} \times 85\%}{0.097\ \text{L}} = 225.5\ \text{g/L} = 225.5\ \text{mg/mL}$

The electron of lactate for 1 mL of lactic acid can offer was:

$$225.5\ \text{mg/mL} \times 1\ \text{mL} \times \frac{1\ \text{mmol}}{90\ \text{mg}} \times \frac{12\ \text{meq}}{\text{mmol}} = 29.5\ \text{meq} \approx 30\ \text{meq}$$

For hydrogen gas, the electron for 1 mL of hydrogen can offer was:

$$1\ \text{mL} \times \frac{1\ \text{mmol}}{2\ \text{mg}} \times \frac{2\ \text{meq}}{\text{mmol}} = 1\ \text{meq}$$

The amount of electron required for 1 mL of saturate PCE water:

$$\frac{150\ \text{mg PCE}}{\text{L}} \times \frac{1\ \text{mmol}}{166\ \text{mg}} \times \frac{8\ \text{meq}}{\text{mmol}} \times \frac{0.001\ \text{L}}{\text{bottle}} = 0.0072\ \text{meq/bottle}$$

The amount of electron required for 1 μL of neat PCE water:

$$1\ \mu\text{L} \times \frac{1.6\ \text{mg}}{\mu\text{L}} \times \frac{1\ \text{mmol}}{166\ \text{mg}} \times \frac{8\ \text{meq}}{\text{mmol}} = 0.08\ \text{meq/bottle}$$

The amount of electron required for 1 mL of saturate TCE water:

$$\frac{1100\ \text{mg TCE}}{\text{L}} \times \frac{1\ \text{mmol}}{131\ \text{mg}} \times \frac{6\ \text{meq}}{\text{mmol}} \times \frac{0.001\ \text{L}}{\text{bottle}} = 0.05\ \text{meq/bottle}$$

The amount of electron required for 1 μL of neat TCE water:

$$1\ \mu\text{L} \times \frac{1.5\ \text{mg}}{\mu\text{L}} \times \frac{1\ \text{mmol}}{131\ \text{mg}} \times \frac{6\ \text{meq}}{\text{mmol}} = 0.07\ \text{meq/bottle}$$

The amount of electron required for 1 mL of saturate cDCE water:

$$\frac{3500\ \text{mg TCE}}{\text{L}} \times \frac{1\ \text{mmol}}{97\ \text{mg}} \times \frac{4\ \text{meq}}{\text{mmol}} \times \frac{0.001\ \text{L}}{\text{bottle}} = 0.10\ \text{meq/bottle}$$

The amount of electron required for 1 μL of neat cDCE water:

$$1\ \mu\text{L} \times \frac{1.3\ \text{mg}}{\mu\text{L}} \times \frac{1\ \text{mmol}}{97\ \text{mg}} \times \frac{4\ \text{meq}}{\text{mmol}} = 0.05\ \text{meq/bottle}$$

The amount of electron required for 1 mL of VC gas:

$$\frac{1\ \text{mmol VC}}{24.36\ \text{mL}} \times \frac{2\ \text{meq}}{\text{mmol}} \times \frac{1\ \text{mL}}{\text{bottle}} = 0.082\ \text{meq/bottle}$$

The electron required for methanogensis to produce 1 mmol of methane is 8 meq.

Considering the competitive growth in the environment like methanogens and sulfidogens will consume electron donor for growth, the amount of electron donor will be enough to achieve 100 times the stoichiometric reduction of PCE to ethene.

Appendix C

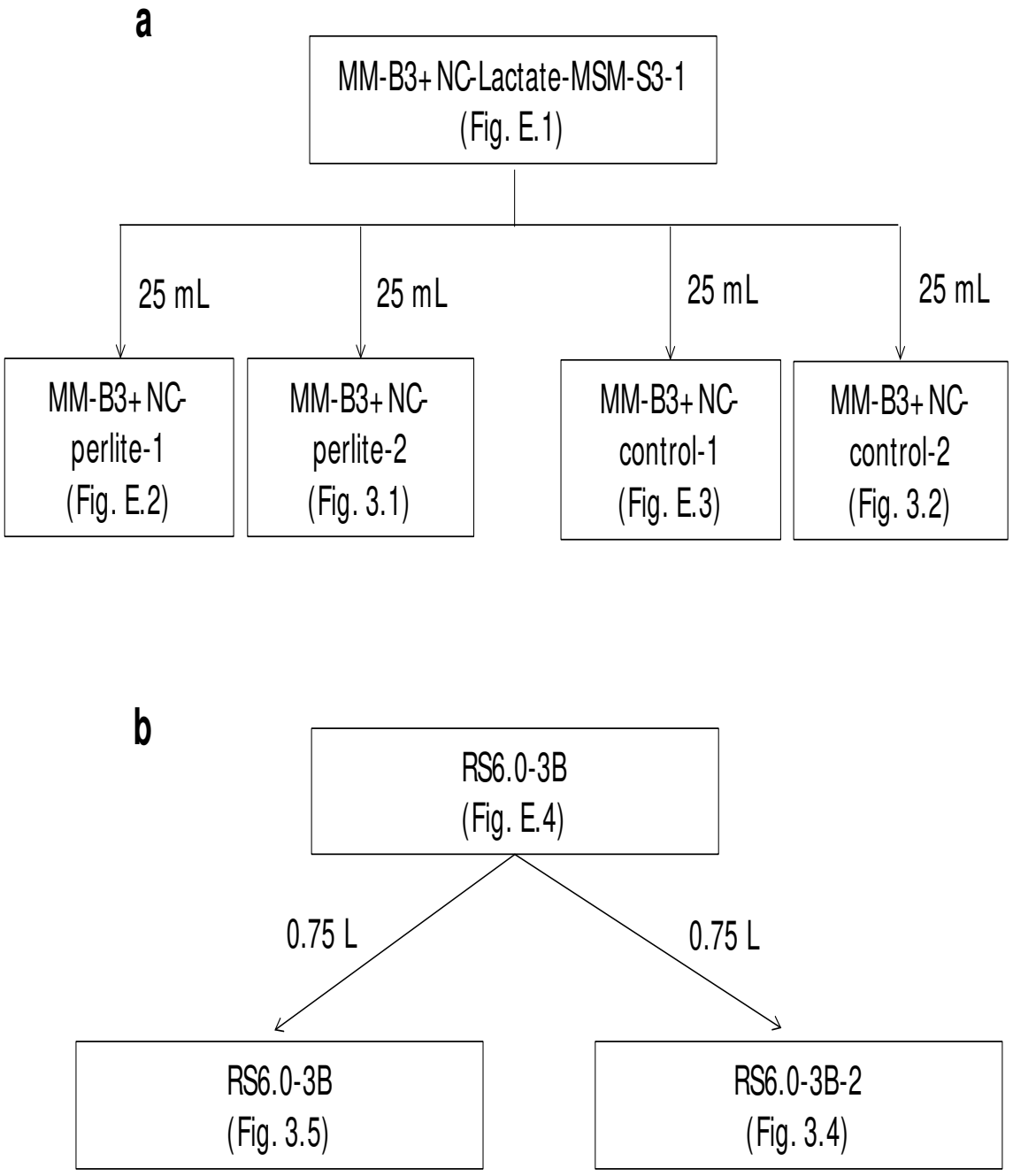

Figure C.1: Sequence for evaluation of support material **a)** for perlite evaluation; and **b)** for sand evaluation. Numbers next to arrows indicate the inoculum volume transfered.

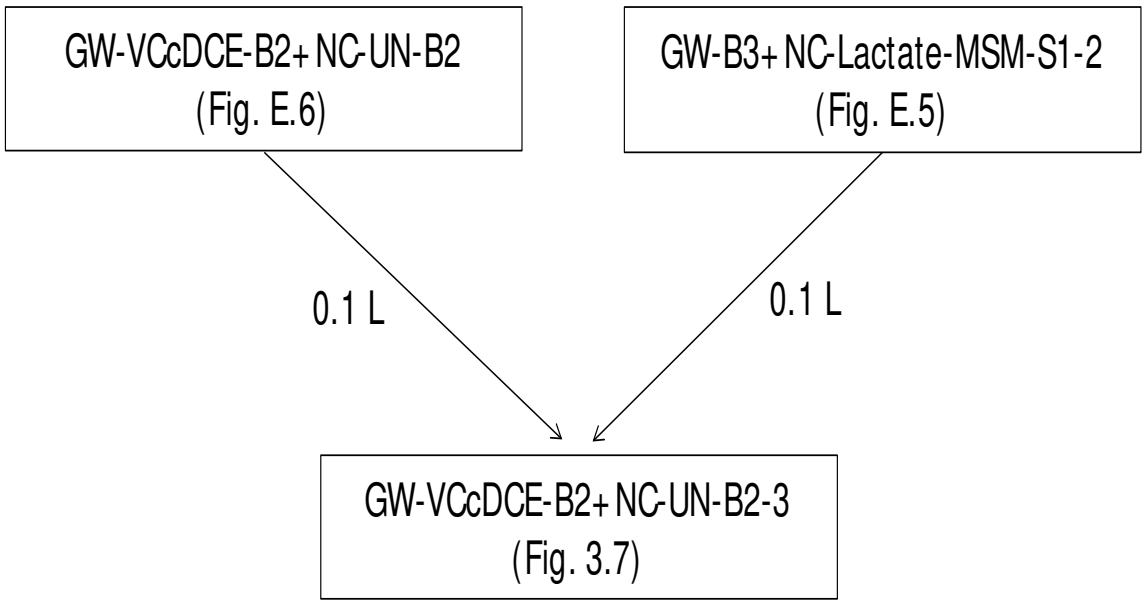

Figure C.2: Sequence in development of sediment-free enrichment cultures for 2.6 L bottle #1 (GW-VCcDCE-B2+NC-UN-B2-3). Numbers next to arrows indicate the inoculum volume transfered.

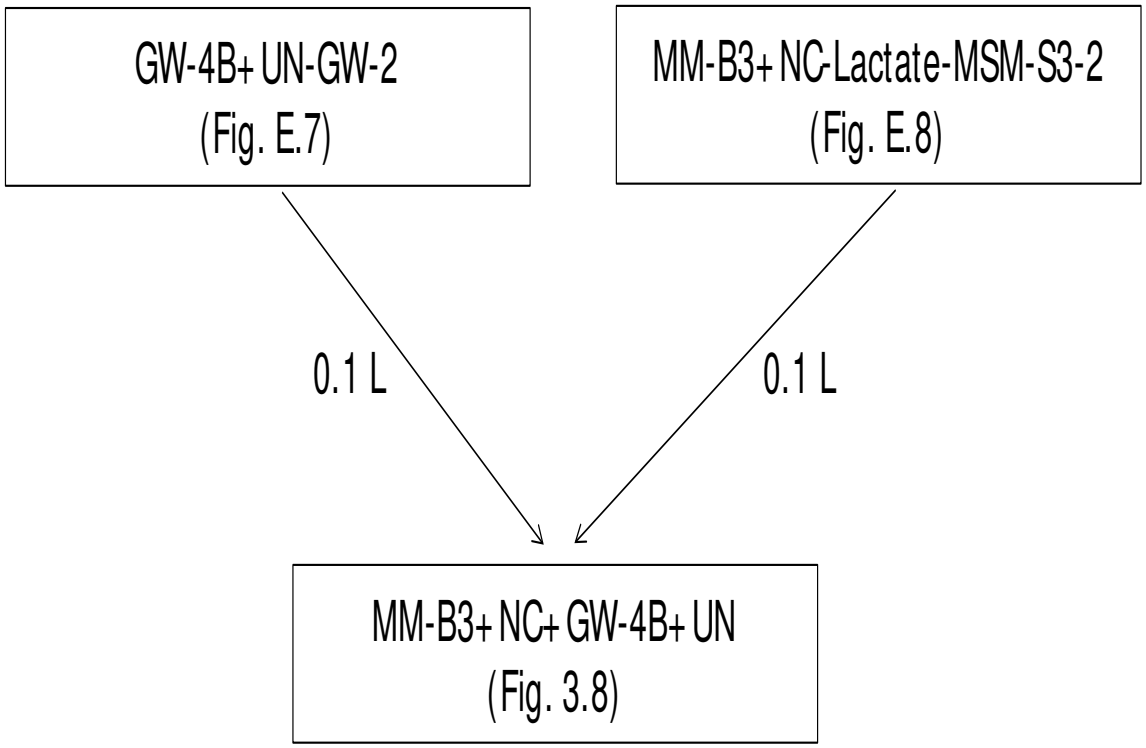

Figure C.3: Sequence in development of sediment-free enrichment cultures for 2.6 L bottle #2 (MM-B3+NC+GW-4B+UN). Numbers next to arrows indicate the inoculum volume transfered.

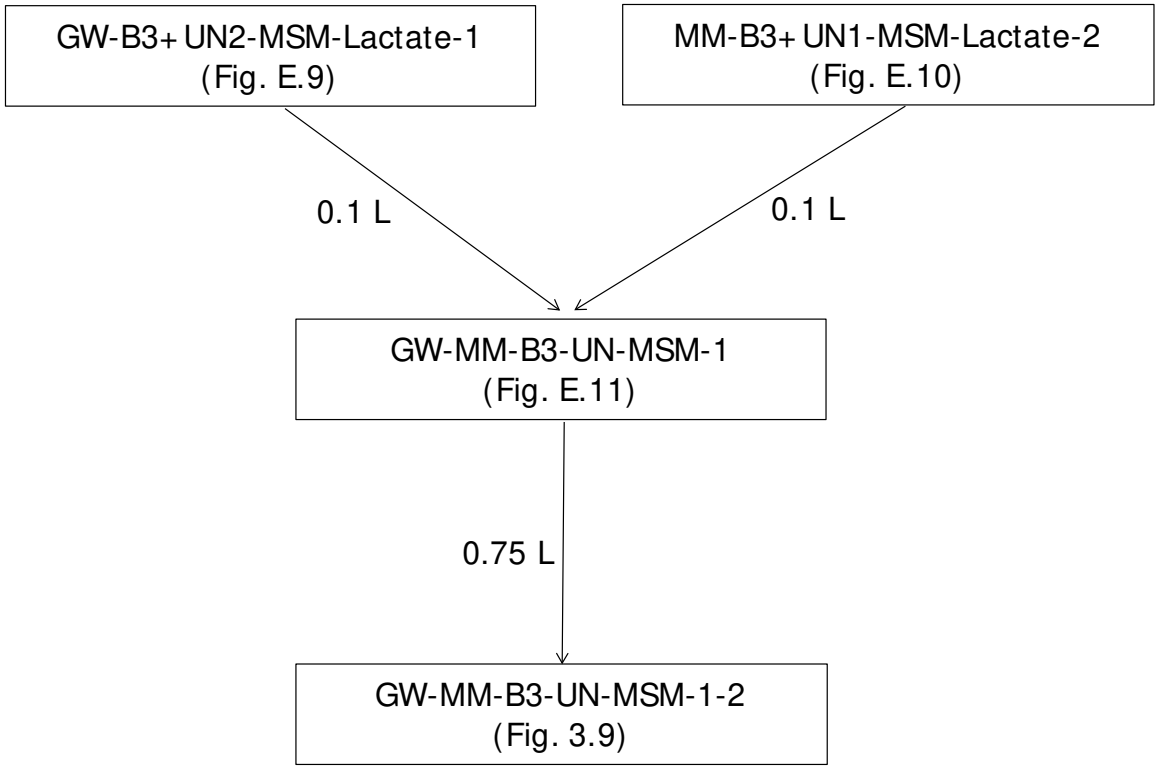

Figure C.4: Sequence in development of sediment-free enrichment culture for 2.6 L bottle #3 (GW-MM-B3-UN-MSM-1-2). Numbers next to arrows indicate the inoculum volume transfered.

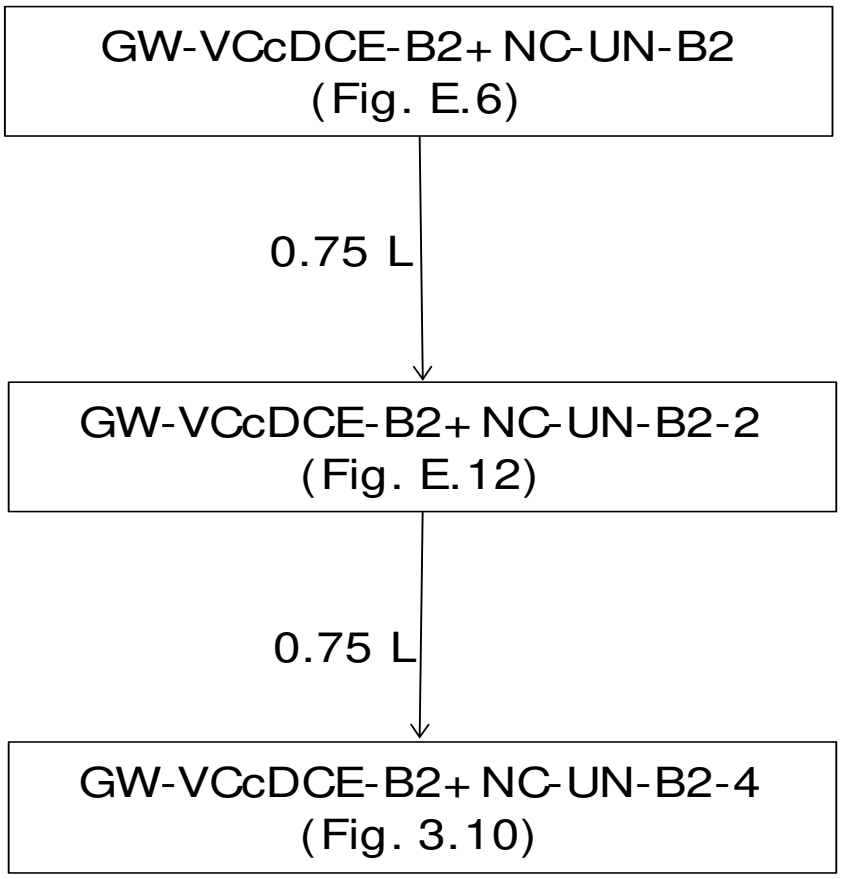

Figure C.5: Sequence in development of sediment-free enrichment culture for 2.6 L bottle #4 (GW-VCcDCE-B2+NC-UN-B2-4). Percentages next to arrow indicate the inoculum volume.

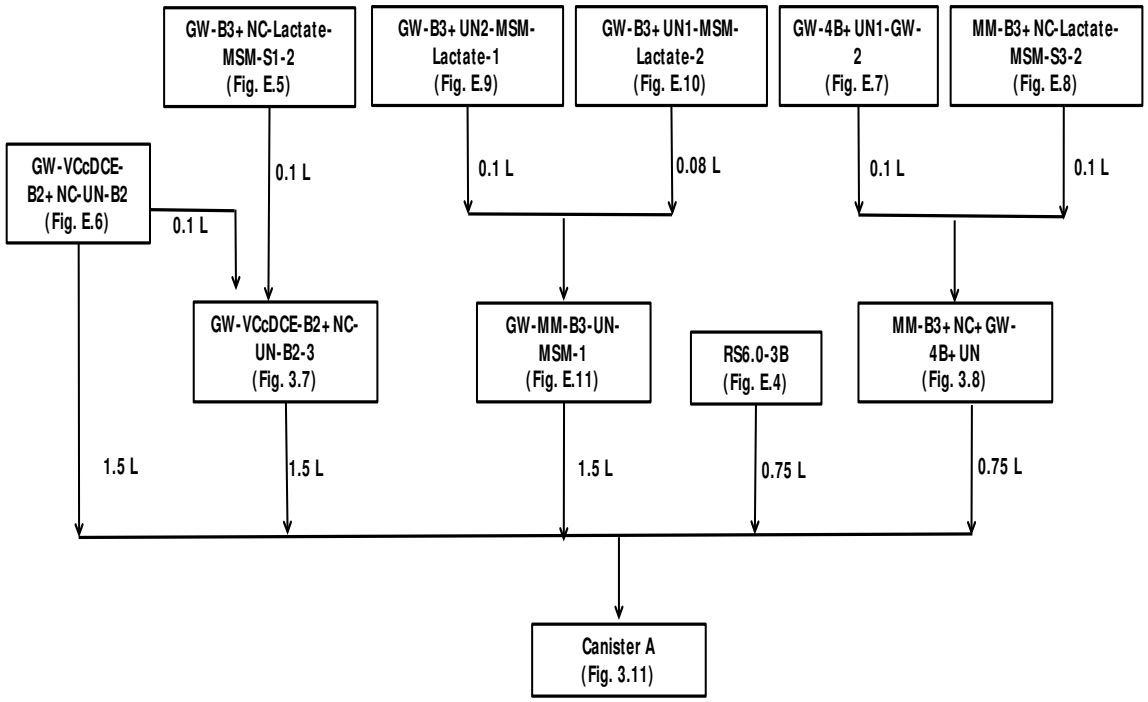

Figure C.6: Sequence in development of canister A. Numbers next to arrows indicate the inoculum volumes transfered.

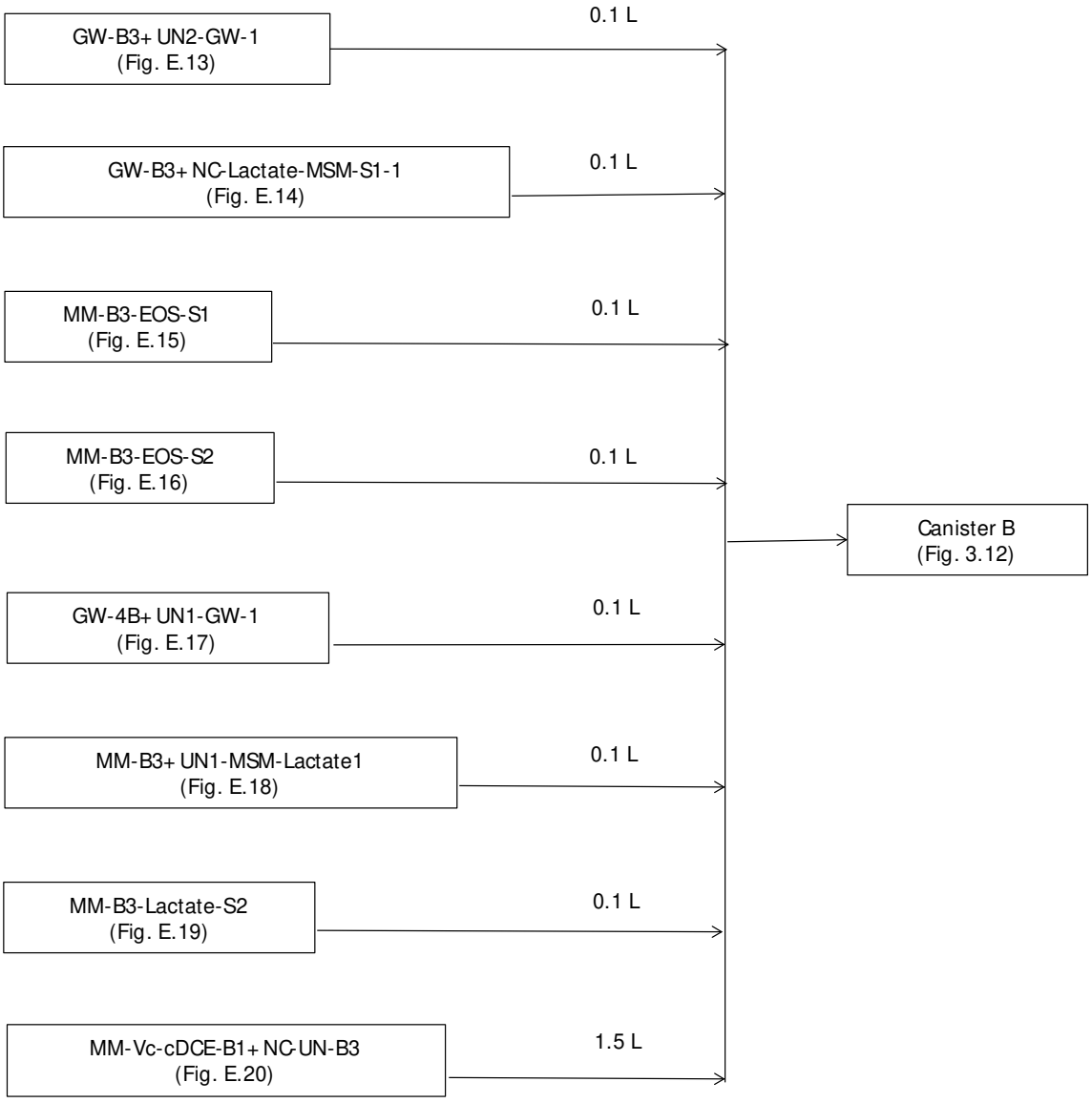

Figure C.7: Sequence in development of canister B. Numbers next to arrows indicate the inoculum volumes transferred.

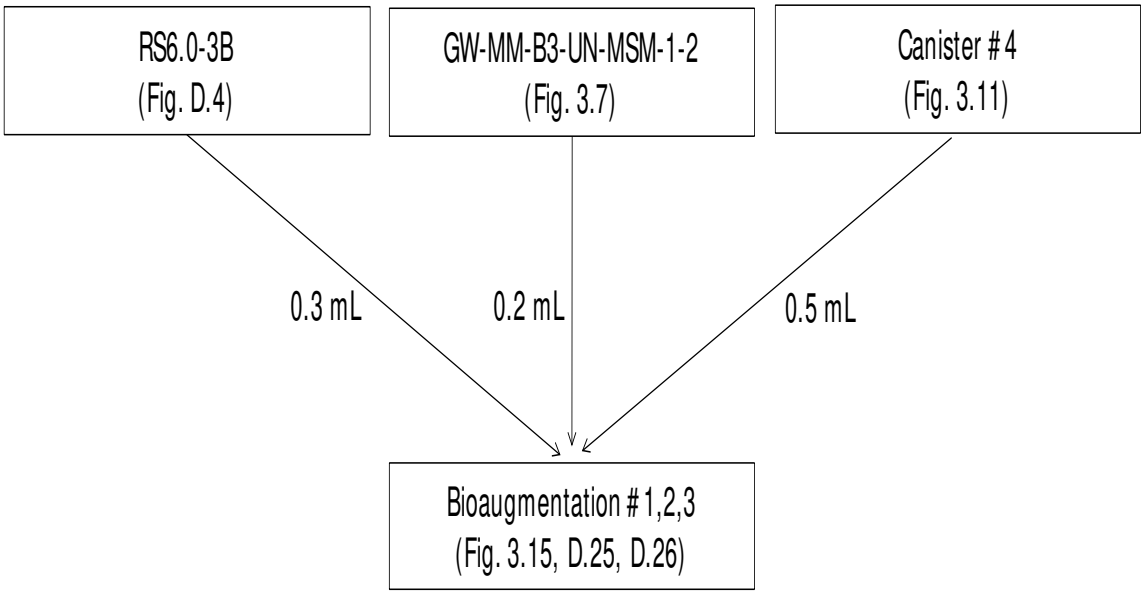

Figure C.8: Sources of inoculum for the bioaugmentation experiment.

Appendix D

Appendix D-1 GC Response Factors

Table D-1 GC Response Factors, HP chromatograms, 100 mL liquid and 60 mL headspace from Chen (8)

Compound	Response Factor (µmol/bottle/PA)	R^2
PCE	3.8801E-06	0.9999
TCE	6.2710E-06	0.9986
cDCE	1.3707E-05	0.9996
VC	2.3874E-06	0.9999
Ethane	1.1361E-06	0.9999
Ethene	1.2943E-06	0.9999
Methane	2.2780E-06	0.9999

Table D-2 GC Response Factors, UniChrom chromatograms, 100 mL liquid and 60 mL headspace

Compound	Response Factor (µmol/bottle/PA)	R^2
PCE	5.42E-02	0.9996
TCE	8.28E-02	0.9997
cDCE	1.91E-01	0.9995
VC	3.54E-02	0.9980
Ethane	1.86E-02	0.9994
Ethene	1.60E-02	0.9998
Methane	2.78E-02	0.9968

Table D-3 GC Response factors, UniChrom chromatograms, 18 L liquid and 1.6 L

Compound	Response Factor (mmol/Canister/PA)
PCE	8.100E-03
TCE	1.570E-02
cDCE	4.170E-02
VC	5.800E-03
Ethane	6.000E-04
Ethene	1.100E-03
Methane	1.200E-03

Note: These are based on a one-point calibration.

Table D-4 GC Response factors, UniChrom chromatograms, 50 mL liquid, 20 g soil and 99 mL of headspace

Compound	Response Factor (µmol/bottle/PA)	R^2
PCE	5.85E-02	0.9978
TCE	7.81E-02	0.9994
cDCE	1.14E-01	0.9988
VC	4.35E-02	0.9995
Ethane	2.92E-02	0.9995
Ethene	3.03E-02	0.9965
Methane	5.55E-02	0.9986

Appendix D-2 Calculation of GC Response Factors for 2.6 L Bottles

The GC response factor by using HP chromatograms was got from Chen, and all the assumptions, calculations and letters were based on Chen's thesis (8). For 160 mL of serum bottles, containing 100 mL liquid and 60 mL headspace. The total mass of chlorinated ethenes can be expressed as:

$$PA_s \times RF_s = C_{ls} \times V_{ls} + C_{gs} \times V_{gs}$$

And according to Henry's law: $C_{ls} = C_{gs}/H_c$

PA_s is the GC peak area for 160 mL serum bottles, dimensionless;

RF_s is the GC response factor for 160 mL serum bottles, μmol/bottle;

C_{ls} is the liquid concentration of 160 mL serum bottles, μM;

C_{gs} is the headspace concentration of 160 mL serum bottles, μM;

V_{ls} is the volume of liquid phase in 160 mL serum bottles, mL;

V_{gs} is the volume of headspace in 160 mL serum bottles, mL;

H_c is the Henry's law constant at 23°C, dimensionless.

So, PA_s can be rewrited as: $PA_s = \frac{C_{gs} \times (V_{ls}/H_c + V_{gs})}{RF_s}$

For 2.6 L bottles, the liquid volume is 1.5 L, assume when culture transfer from 160 mL serum bottle to 2.6 L bottle, the total mass of chlorinated ethenes is the same, which means:

$$PA_s \times RF_s = PA_B \times RF_B = the\ total\ amount\ of\ chlorinated\ ethenes$$

So, $RF_s \times \frac{C_{gs} \times (V_{ls}/H_c + V_{gs})}{RF_s} = RF_B \times \frac{C_{gB} \times (V_{lB}/H_c + V_{gB})}{RF_B}$

PA_B is the GC peak area for 2.6 L bottles, dimensionless;

RF_B is the GC response factor for 2.6 L bottles, μmol/bottle;

C_{lB} is the liquid concentration of 2.6 L bottles, μM;

C_{gB} is the headspace concentration of 2.6 L bottles, μM;

V_{lB} is the volume of liquid phase in 2.6 L bottles, L;

V_{gB} is the volume of headspace 2.6 L serum bottles, L;

H_c is the Henry's law constant at 23°C, dimensionless.

Then, equation can be rewrited as: $C_{gs} \times \left(V_{ls}/H_c + V_{gs}\right) = C_{gB} \times \left(V_{lB}/H_c + V_{gB}\right)$

Assume the concentration of methane, ethane, ethene and chlorinated ethenes is headspace is proportional to peak area, then,

$$\frac{C_{gs}}{C_{gB}} = \frac{PA_s}{PA_B} = \frac{V_{lB}/H_c + V_{gB}}{V_{ls}/H_c + V_{gs}}$$

And $\frac{RF_B}{RF_s} = \frac{PA_s}{PA_B}$

Therefore, $\frac{RF_B}{RF_s} = \frac{V_{lB}/H_c + V_{gB}}{V_{ls}/H_c + V_{gs}}$

$$RF_B = RF_s \times \frac{V_{lB}/H_c + V_{gB}}{V_{ls}/H_c + V_{gs}}$$

Suppose the ration of headspace to liquid space for 2.6 L bottle and 160 mL serum bottle are the same (~0.6), which means

$V_{gB} = a \times V_{gs}$

$V_{lB} = a \times V_{ls}$

$V_B = a \times V_s$

V_B is the volume of 2.6 L bottle;

V_s is the volume of 160 mL serum bottles.

a is the ratio of headspace to liquid space

So, $RF_B = RF_s \times \frac{(V_{ls}/H_c + V_{gs}) \times a}{V_{ls}/H_c + V_{gs}} = a \times RF_s = RF_s \times \frac{V_B}{V_s}$

Appendix E: Results for Replicate Bottles Presented in Chapter 3

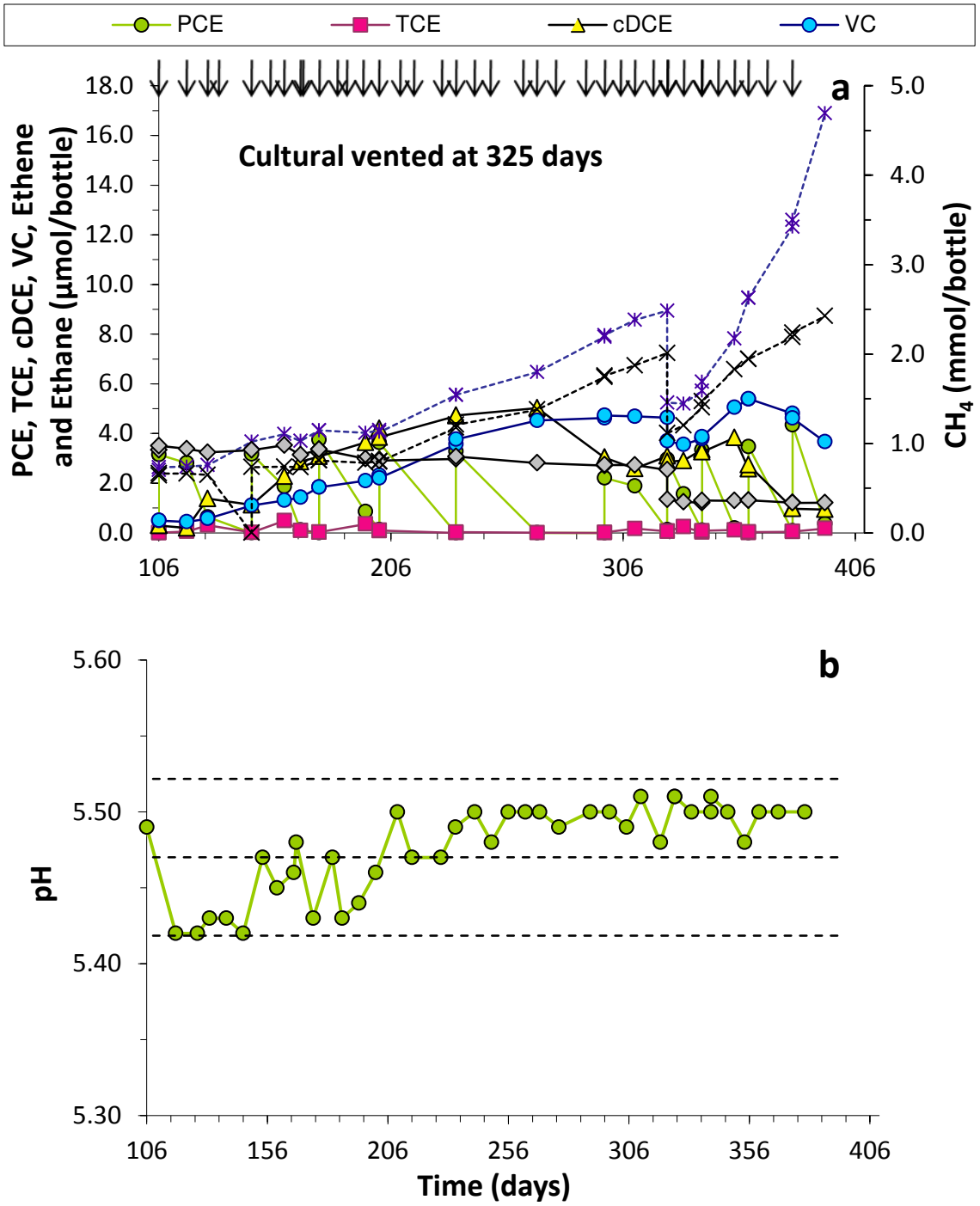

Figure E.1: Lactate-amended enrichment culture (serum bottle MM-B3 + NC-lactate-MSM-S3-1) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation.

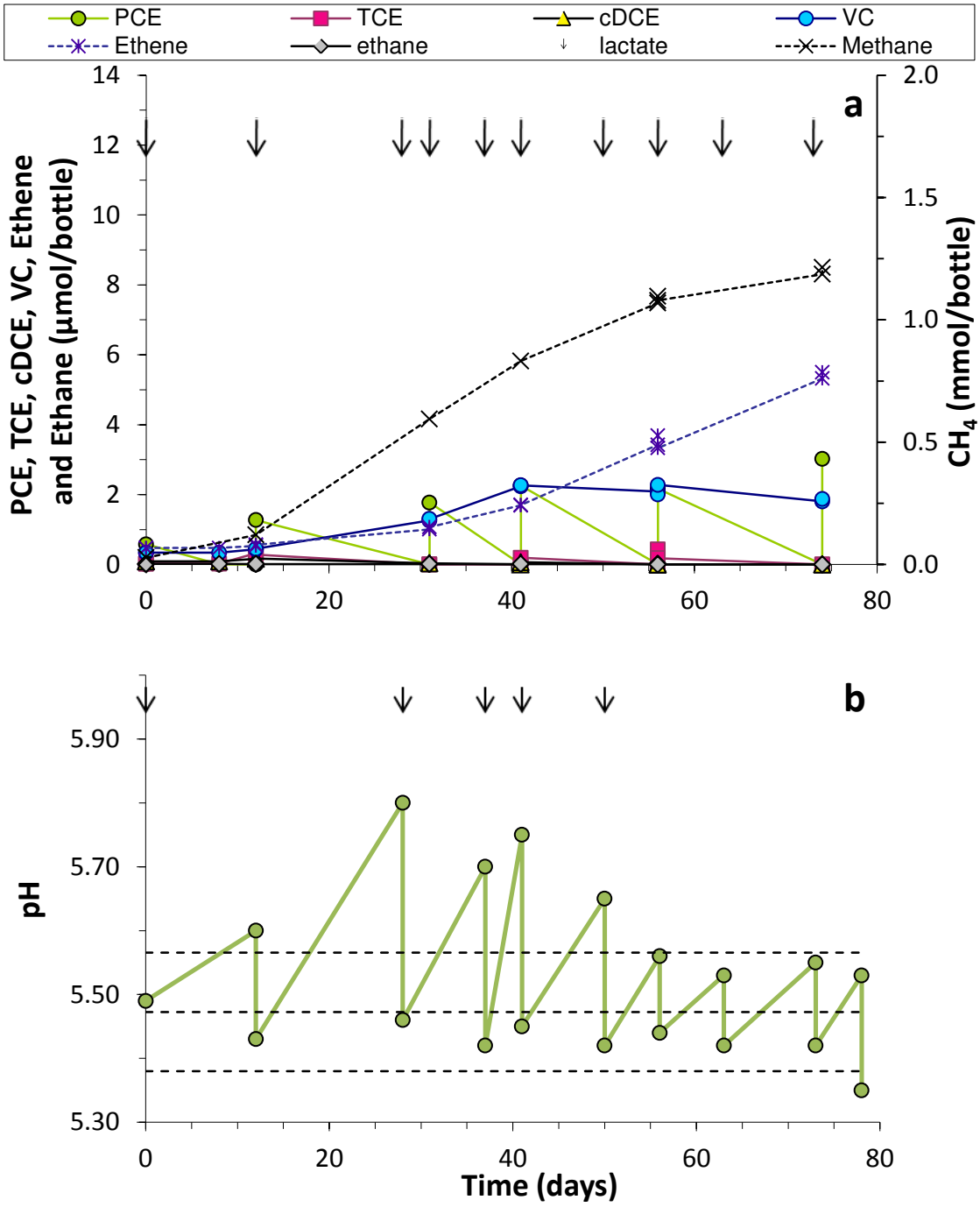

Figure E.2: Lactate-amended enrichment culture with perlite added (serum bottle MM-B3+NC-perlite-1) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate addition of 1 M phosphoric acid.

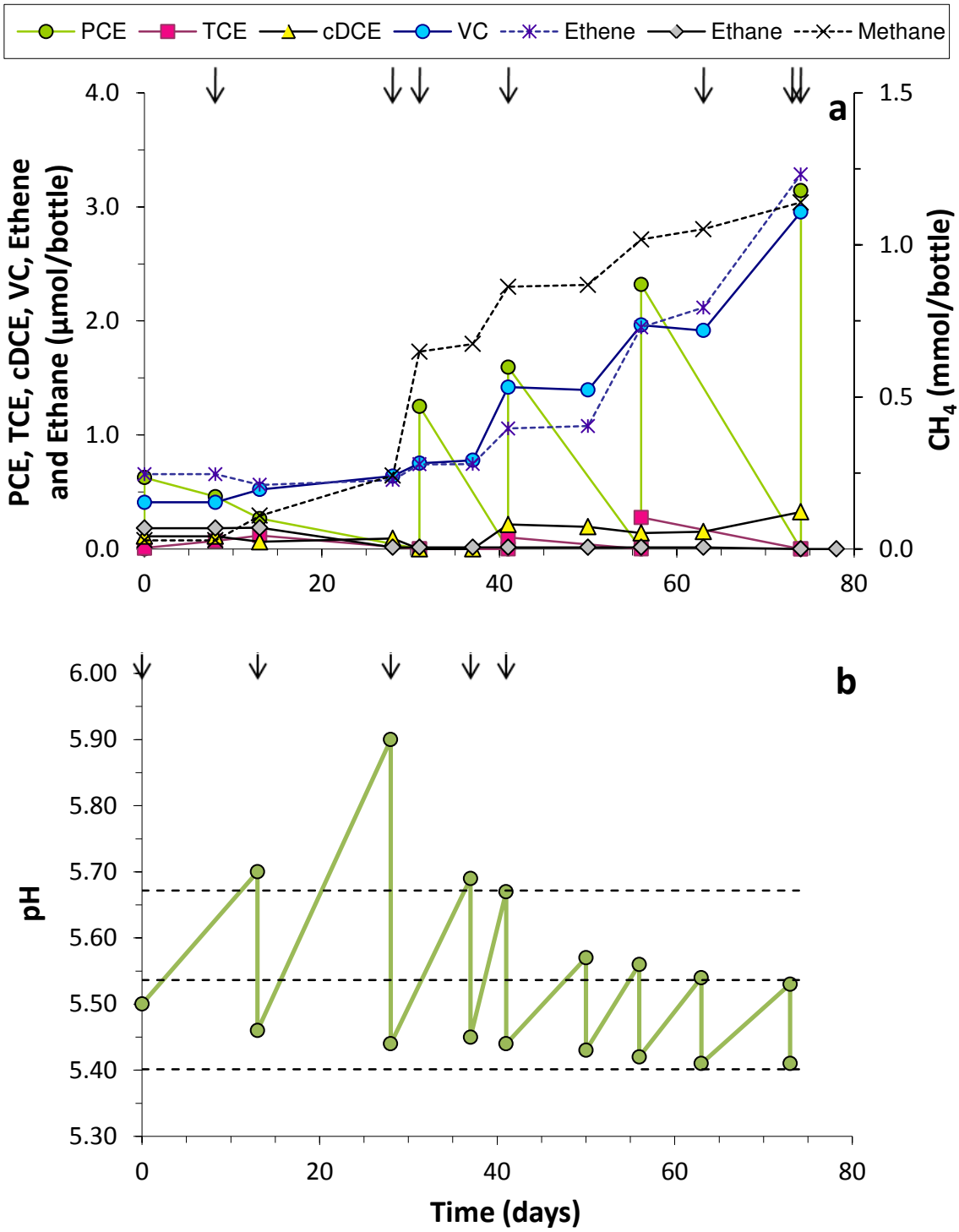

Figure E.3: Lactate-amended enrichment culture (serum bottle MM-B3+NC-control-1) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; dashed horizontal lines represent the average ± one standard deviation; arrows indicate addition of 1 M phosphoric acid.

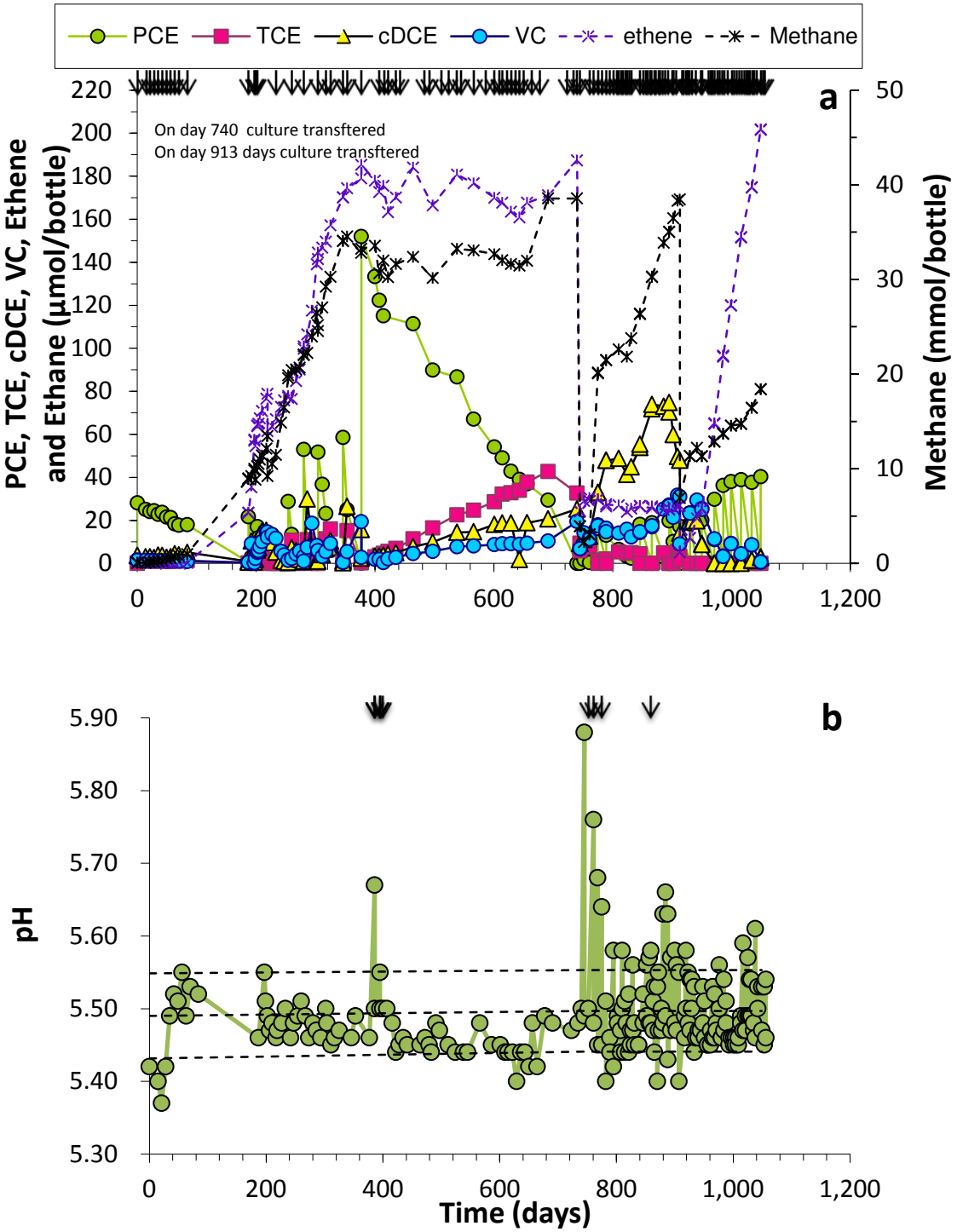

Figure E.4: Lactate-amended enrichment culture in a 2.6 L bottle (RS-6.0-3B) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1 M phosphoric acid.

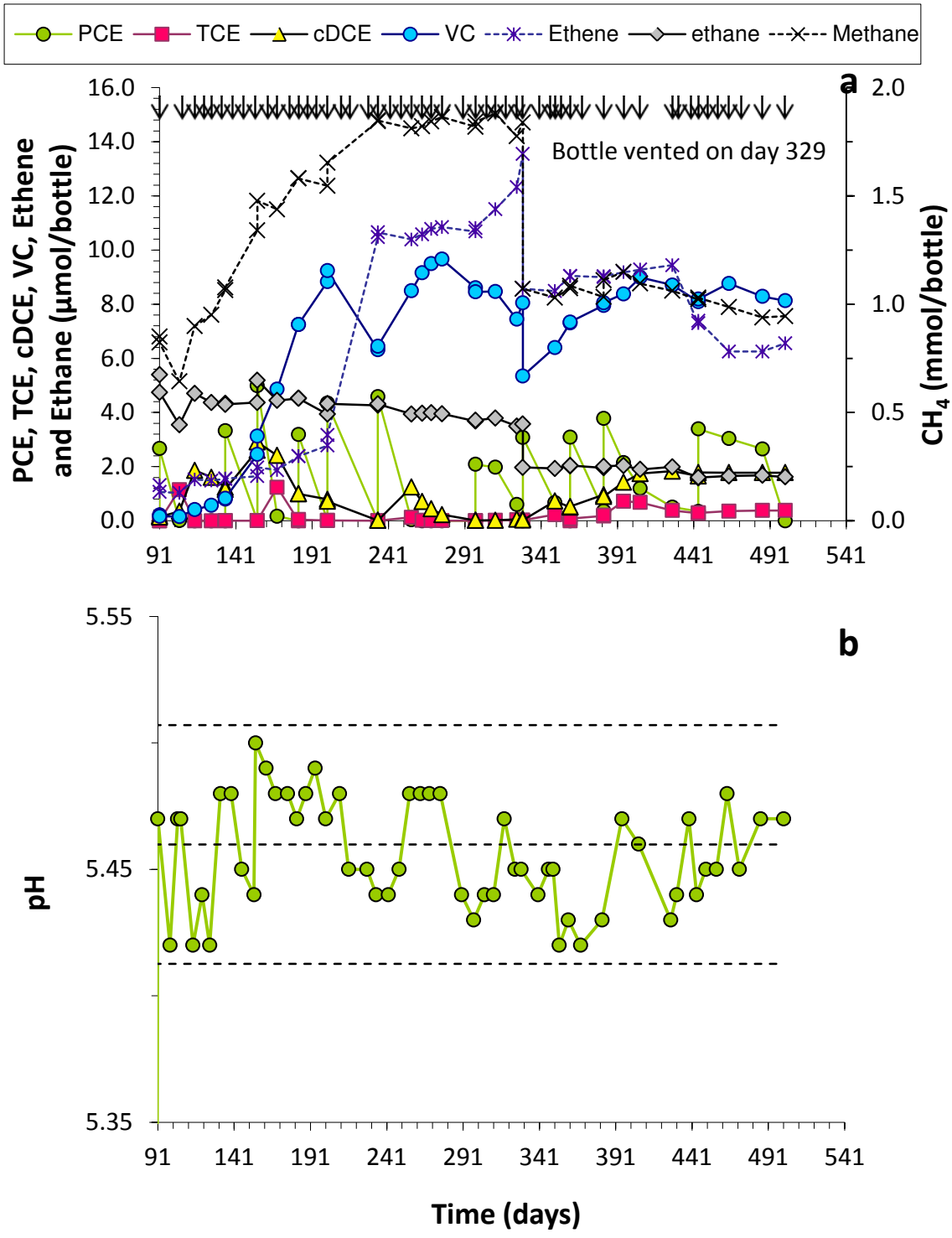

Figure E.5: Lactate-amended enrichment culture (serum bottle GW-B3 + NC-lactate-MSM-S1-2) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; dashed horizontal lines represent the average ± one standard deviation.

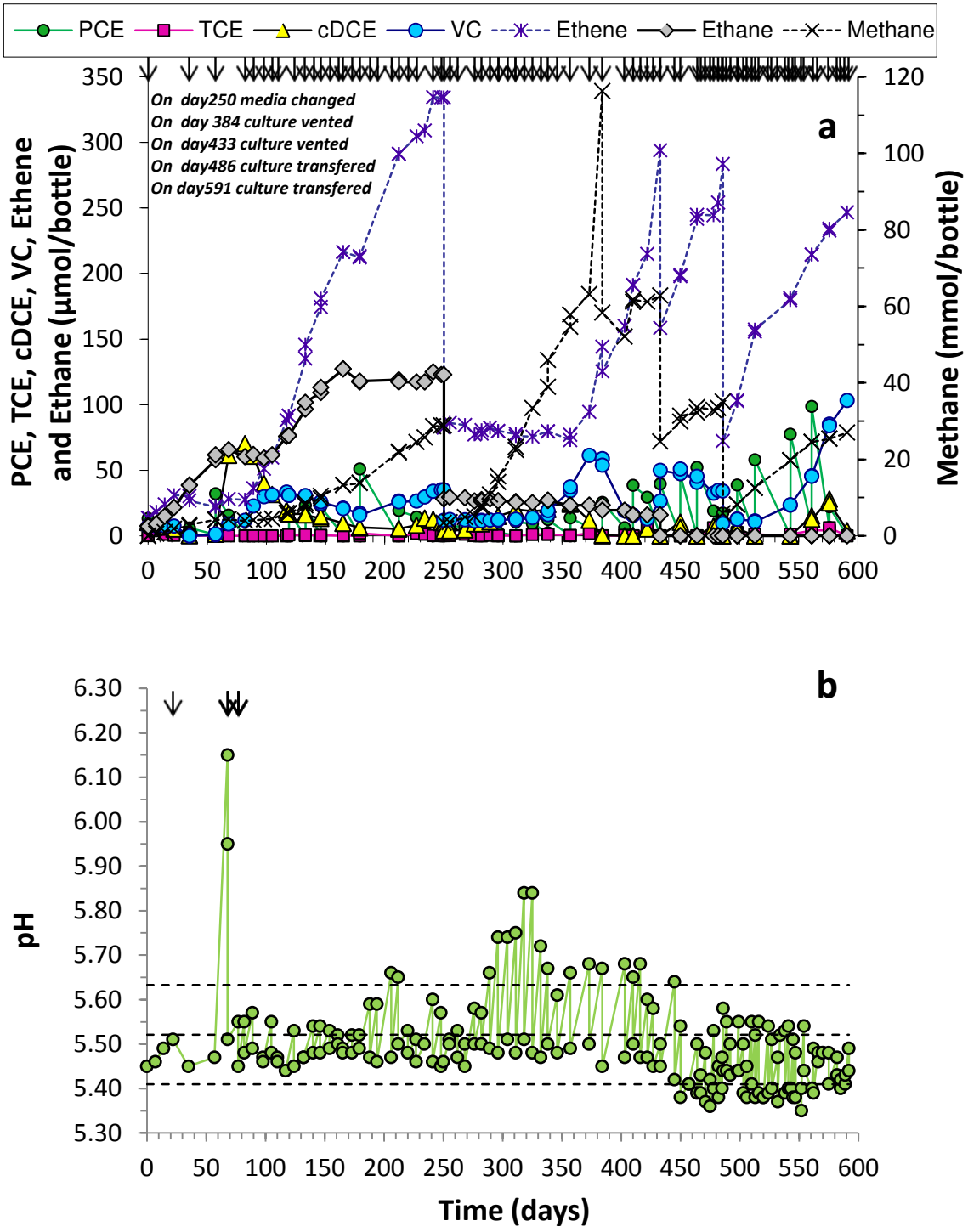

Figure E.6: Lactate-amended enrichment culture in a 2.6 L bottle (GW-VCcDCE-B2 + NC-UN-B2) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate addition of 1 M phosphoric acid.

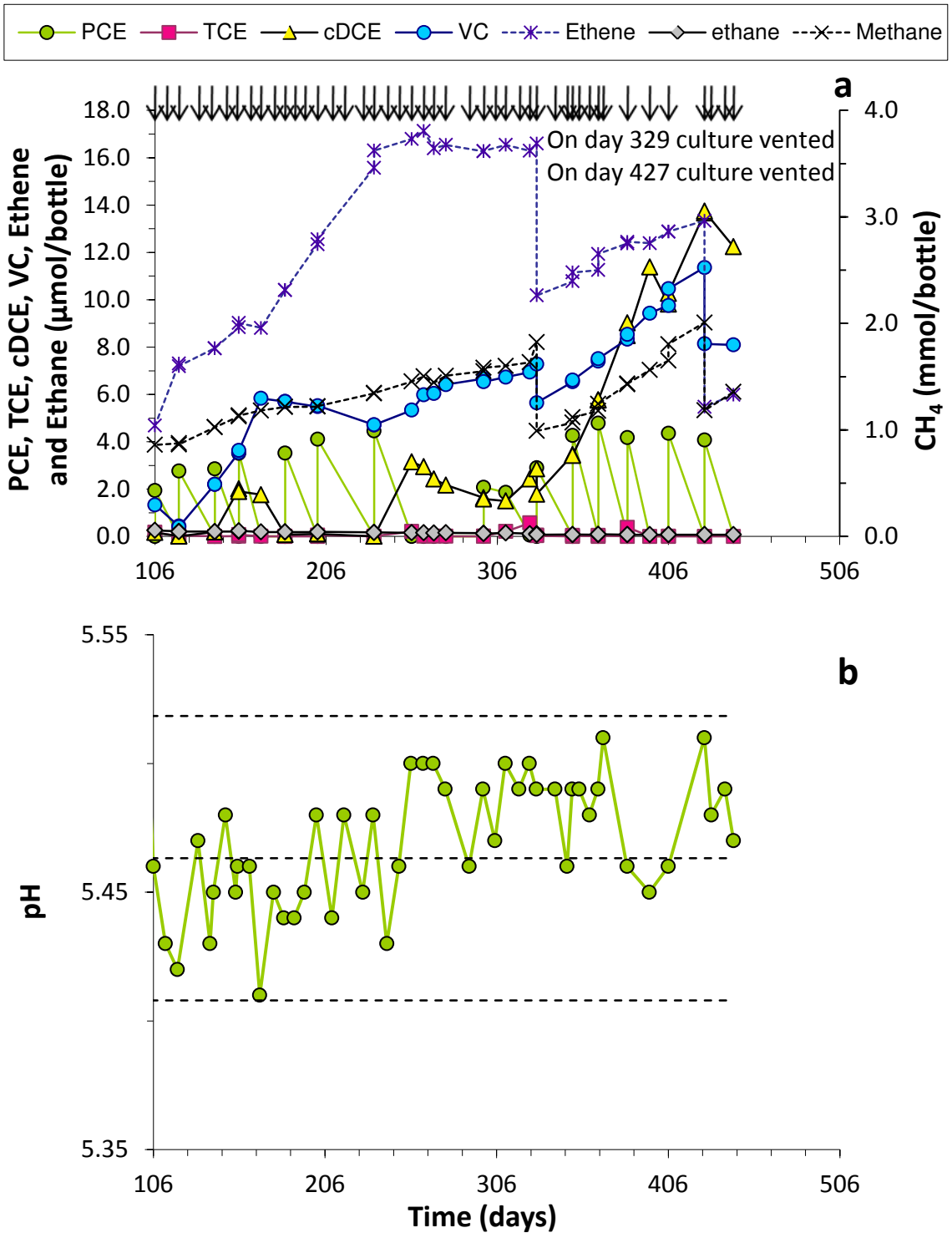

Figure E.7: Lactate-amended enrichment culture (serum bottle GW-4B + UN1-GW-2) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation.

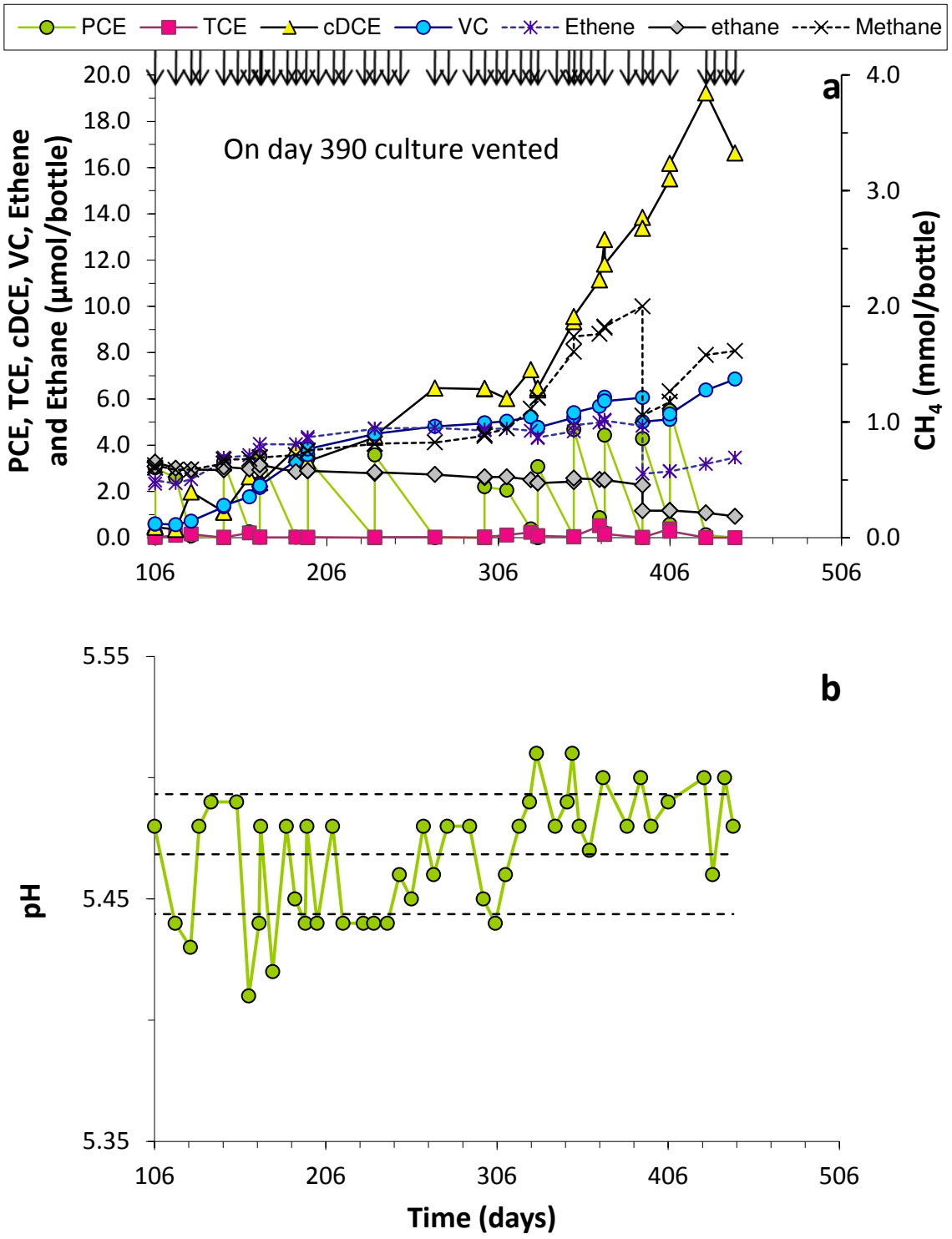

Figure E.8: Lactate-amended enrichment culture (serum bottle MM-B3 + NC-lactate-MSM-S3-2) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation.

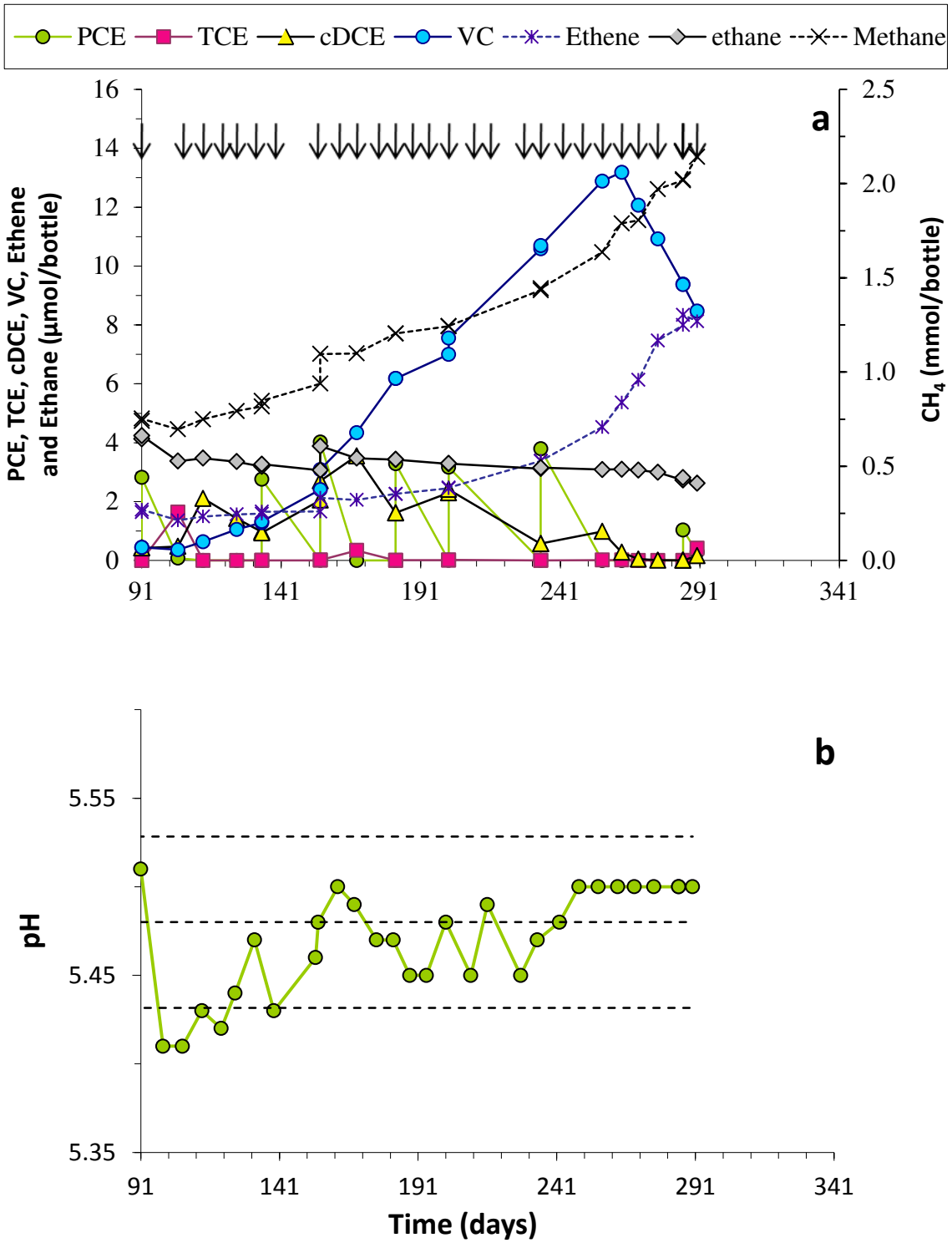

Figure E.9: Lactate-amended enrichment culture (serum bottle GW-B3 + UN2-MSM-lactate-1) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation.

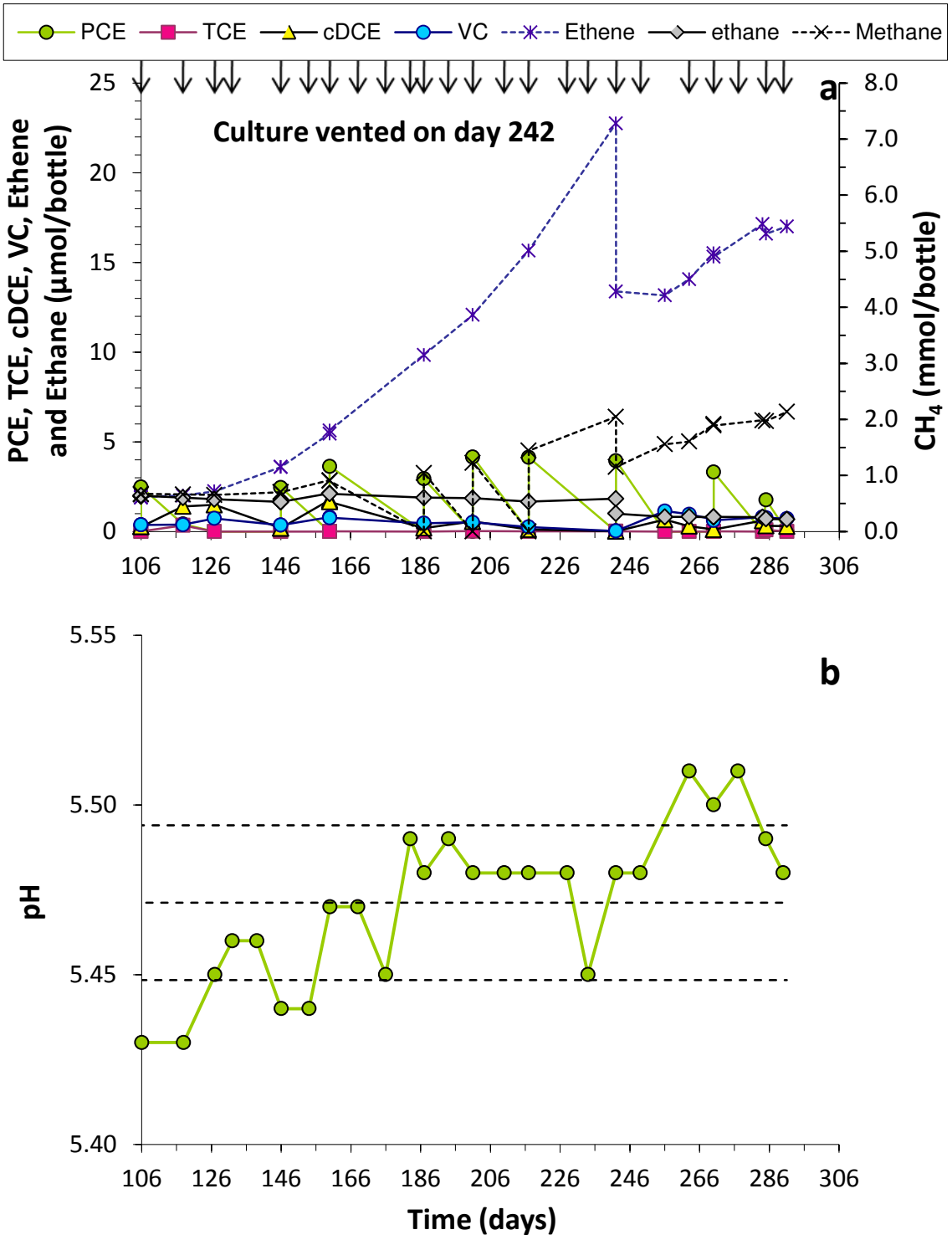

Figure E.10: Lactate-amended enrichment culture (serum bottle MM-B3 + UN1-MSM-Lactate2) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation.

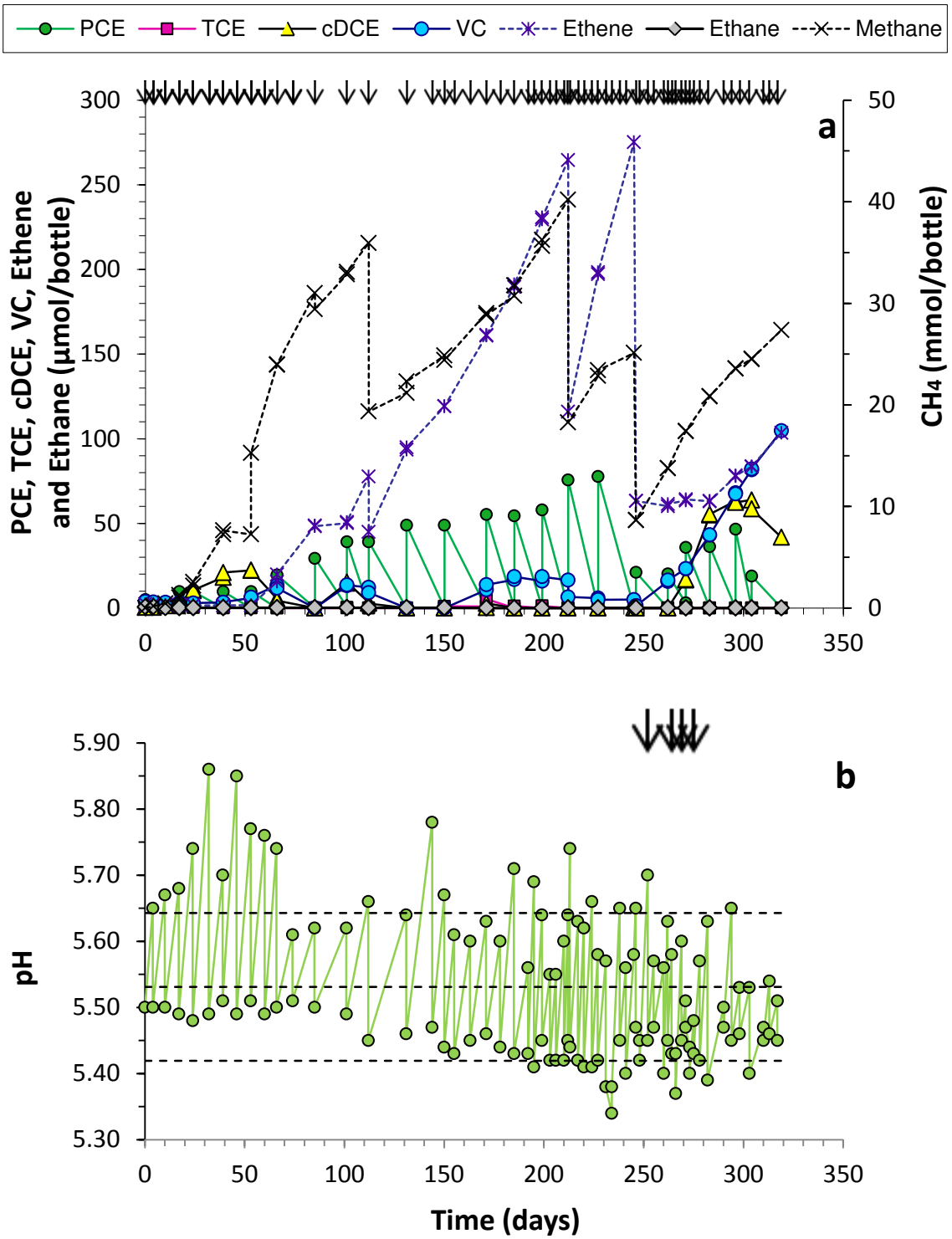

Figure E.11: Lactate-amended enrichment culture in a 2.6 L bottle (GW-MM-B3-UN-MSM-1) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate addition of 1 M phosphoric acid.

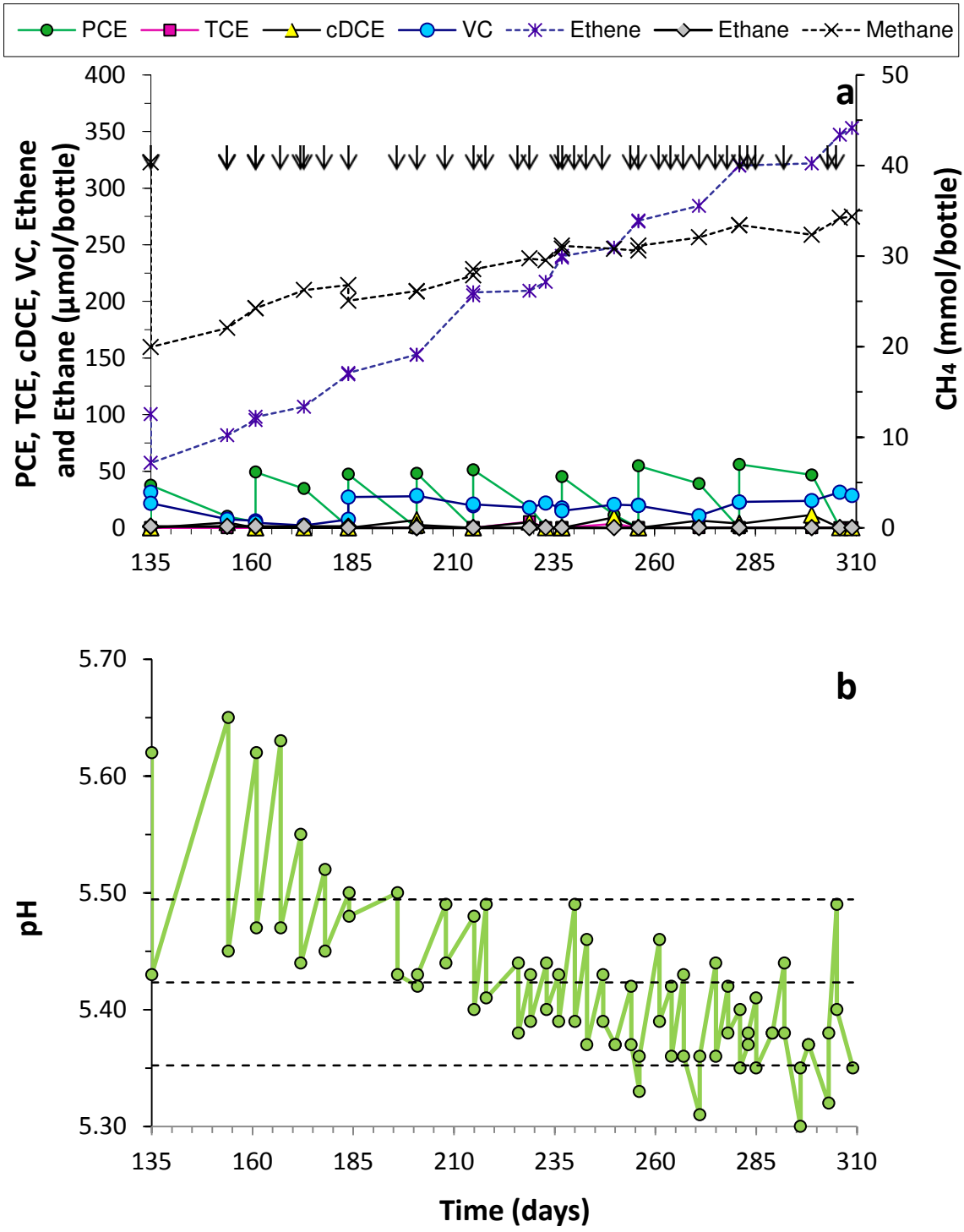

Figure E.12: Lactate-amended enrichment culture in a 2.6 L bottle (GW-VCcDCE-B2 + NC-UN-B2-2) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation.

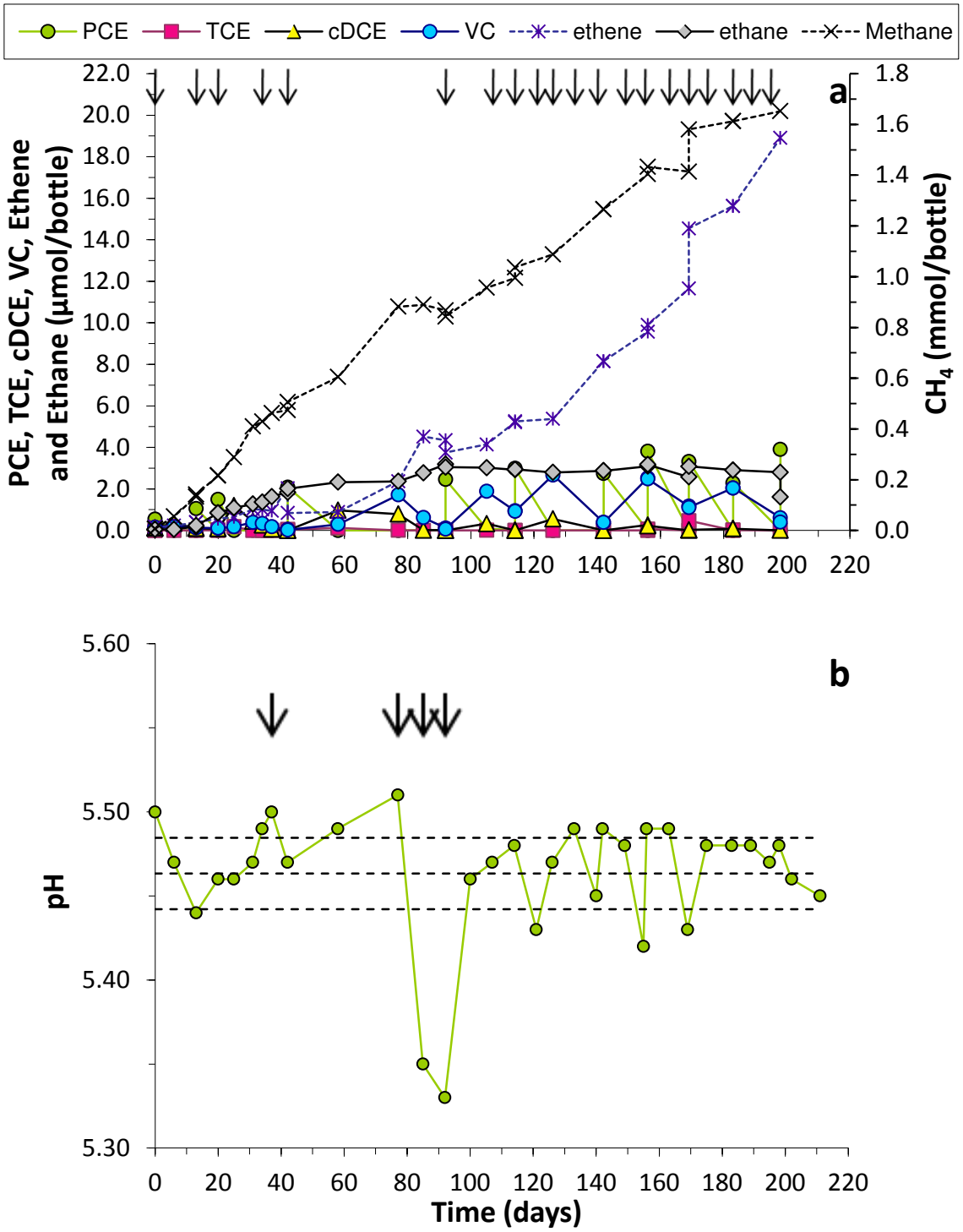

Figure E.13: Lactate-amended enrichment culture (serum bottle GW-B3 + UN2-GW-1) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1M phosphoric acid or sodium hydroxide.

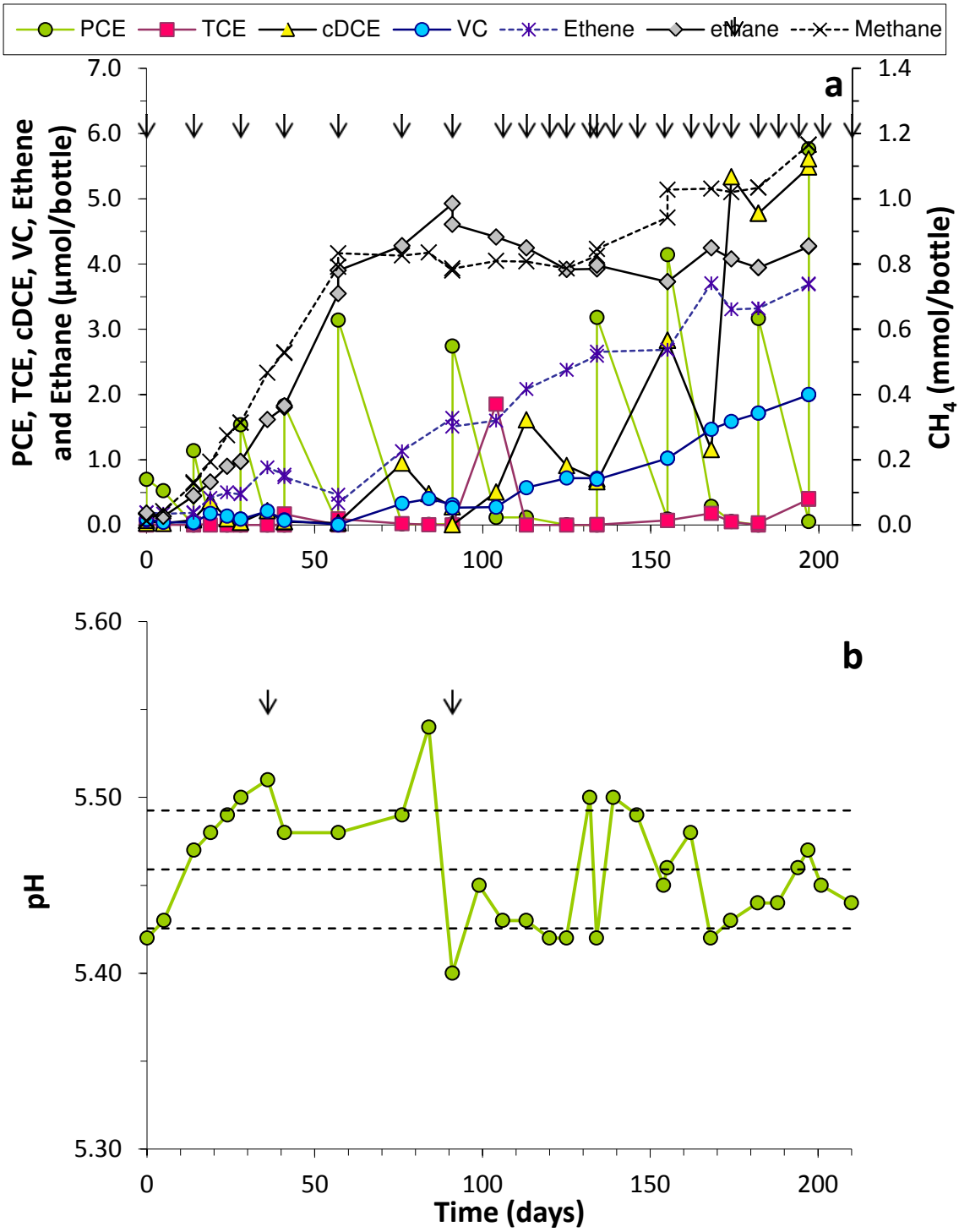

Figure E.14: Lactate-amended enrichment culture (serum bottle GW-B3 + NC-lactate-MSM-S1-1) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1M phosphoric acid or sodium hydroxide.

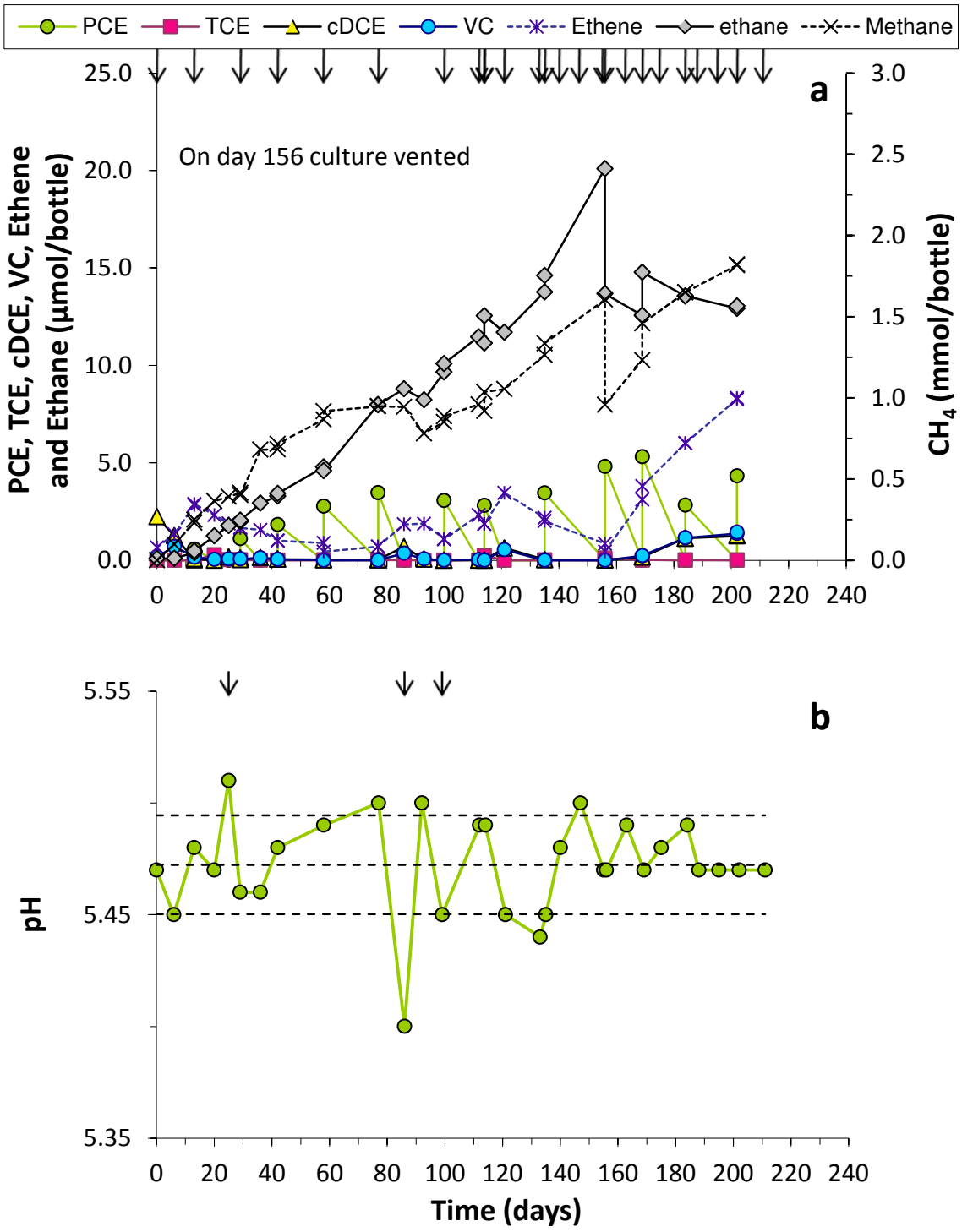

Figure E.15: Lactate-amended enrichment culture (serum bottle MM-B3-EOS-S1) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1M phosphoric acid or sodium hydroxide.

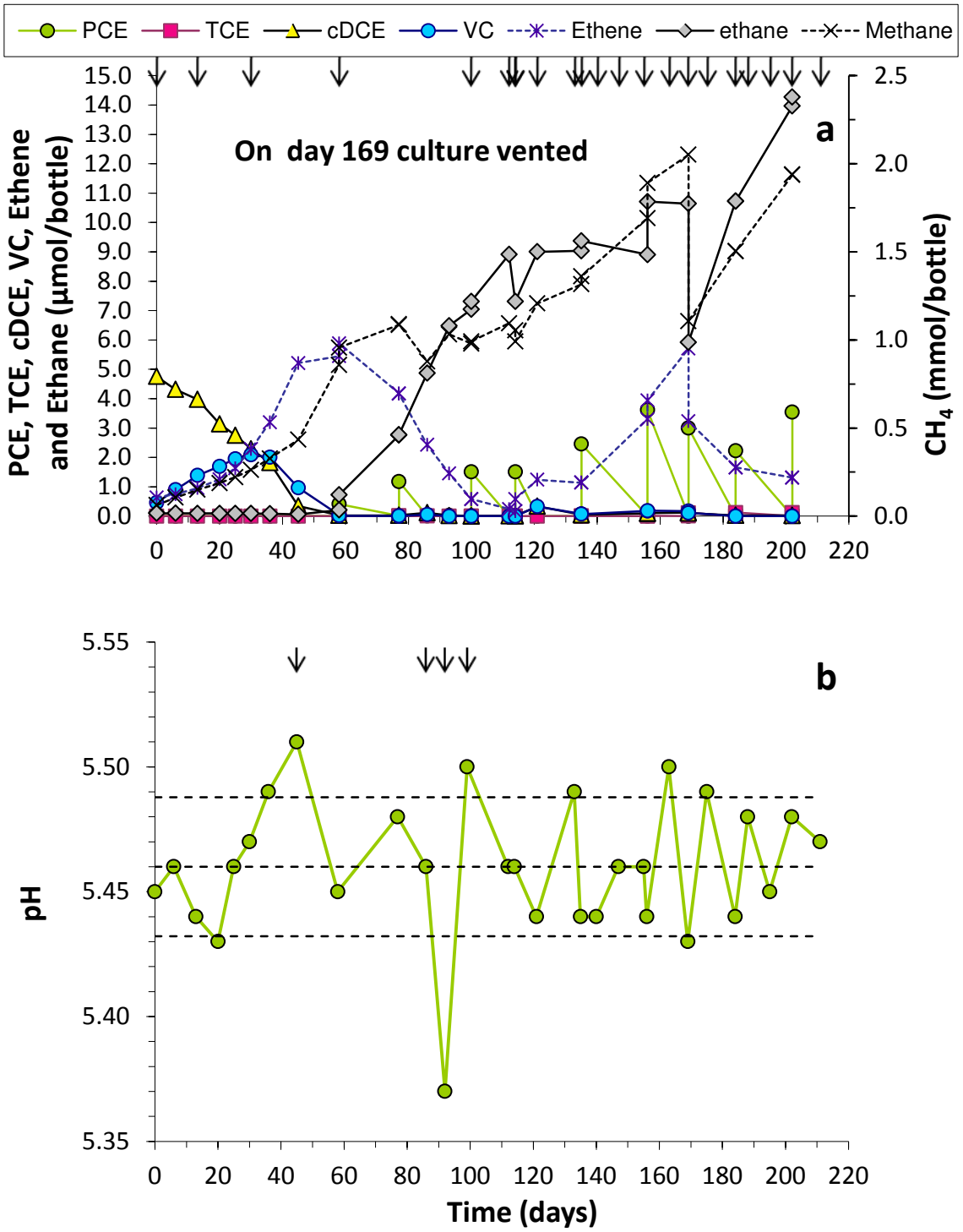

Figure E.16: Lactate-amended enrichment culture (serum bottle MM-B3-EOS-S2) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1M phosphoric acid or sodium hydroxide.

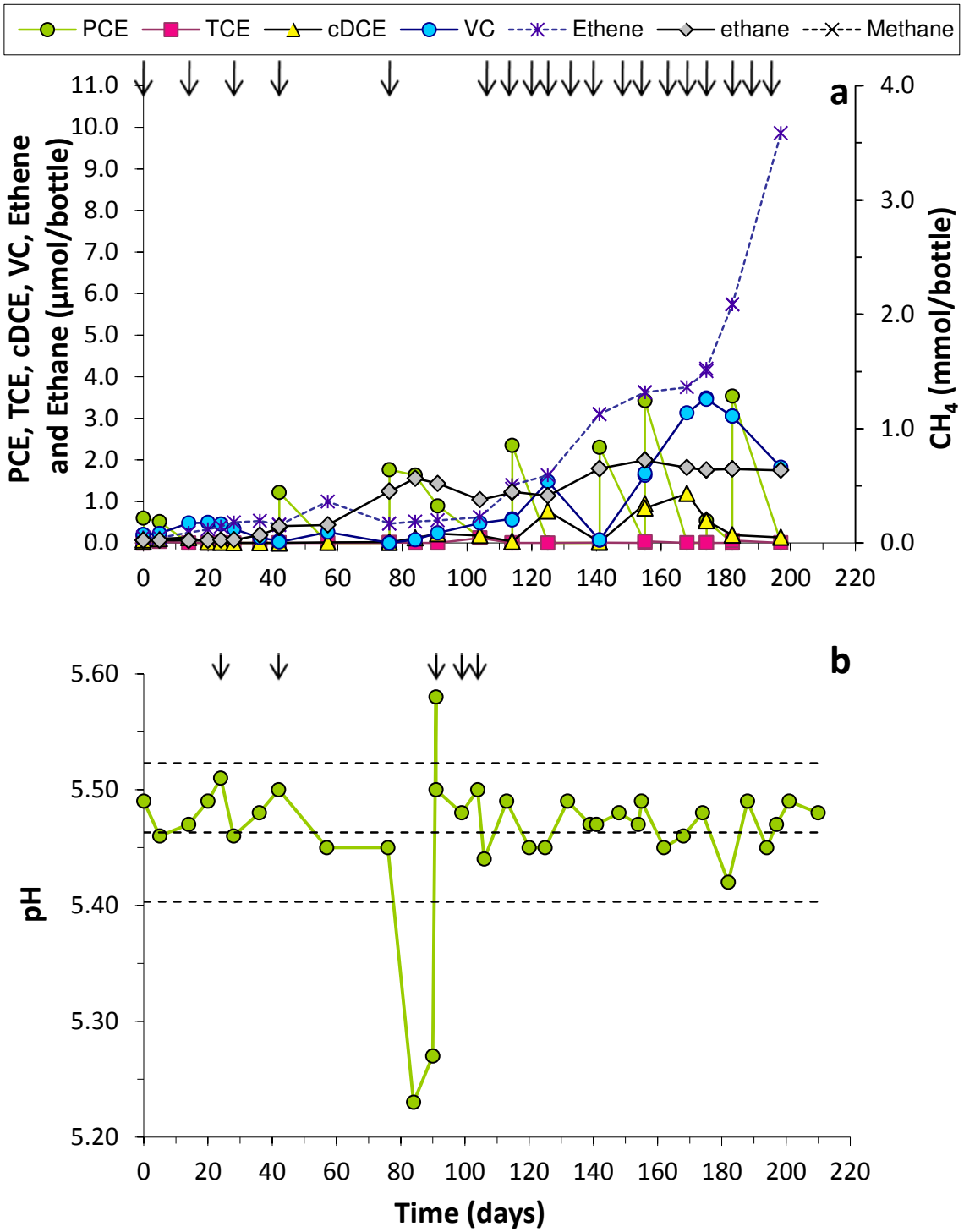

Figure E.17: Lactate-amended enrichment culture (serum bottle GW-4B + UN1-GW-1) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1M phosphoric acid or sodium hydroxide.

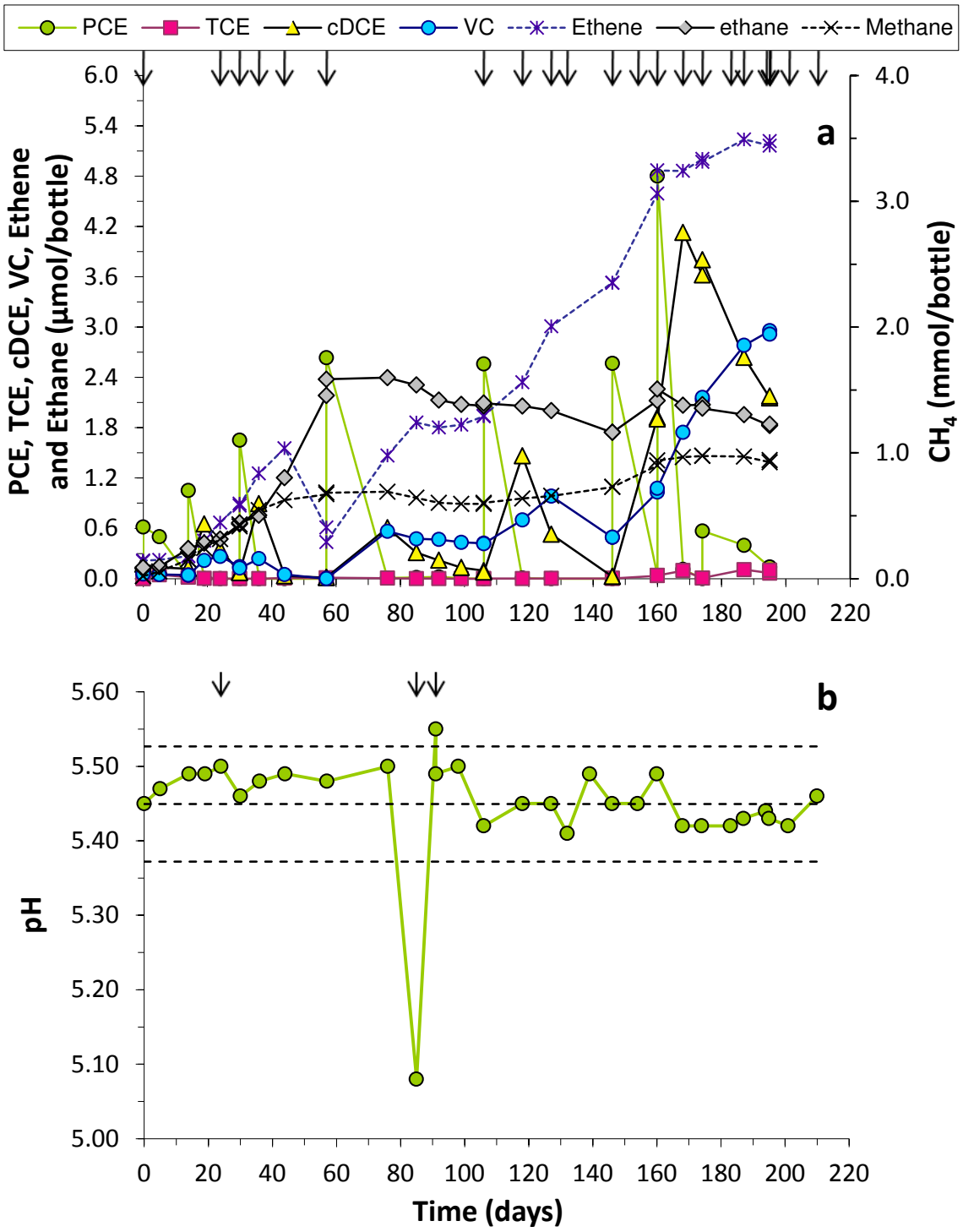

Figure E.18: Lactate-amended enrichment culture (serum bottle MM-B3+UN1-MSM-Lactate1) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1M phosphoric acid or sodium hydroxide.

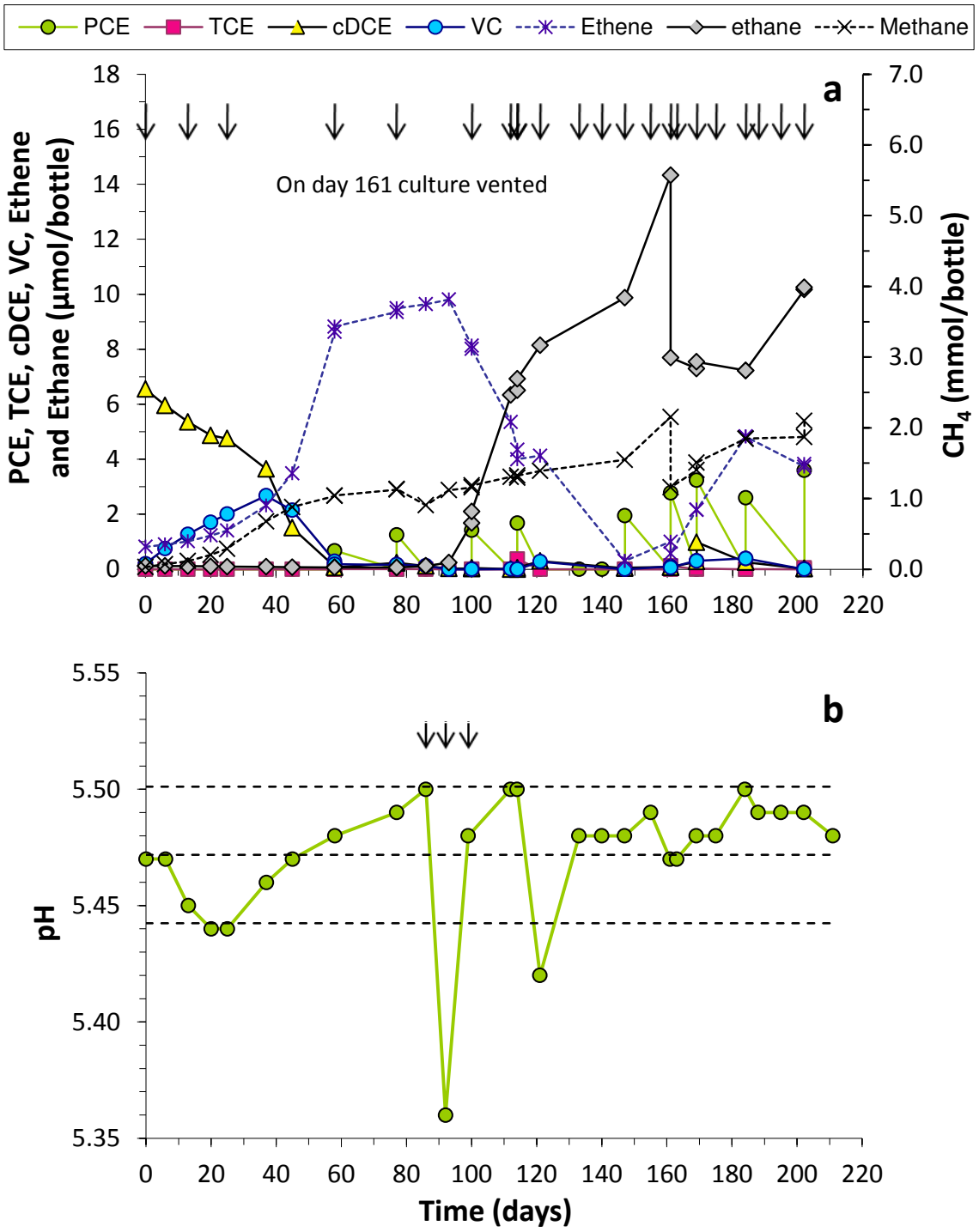

Figure E.19: Lactate-amended enrichment culture (serum bottle MM-B3-Lactate-S2) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1M phosphoric acid or sodium hydroxide.

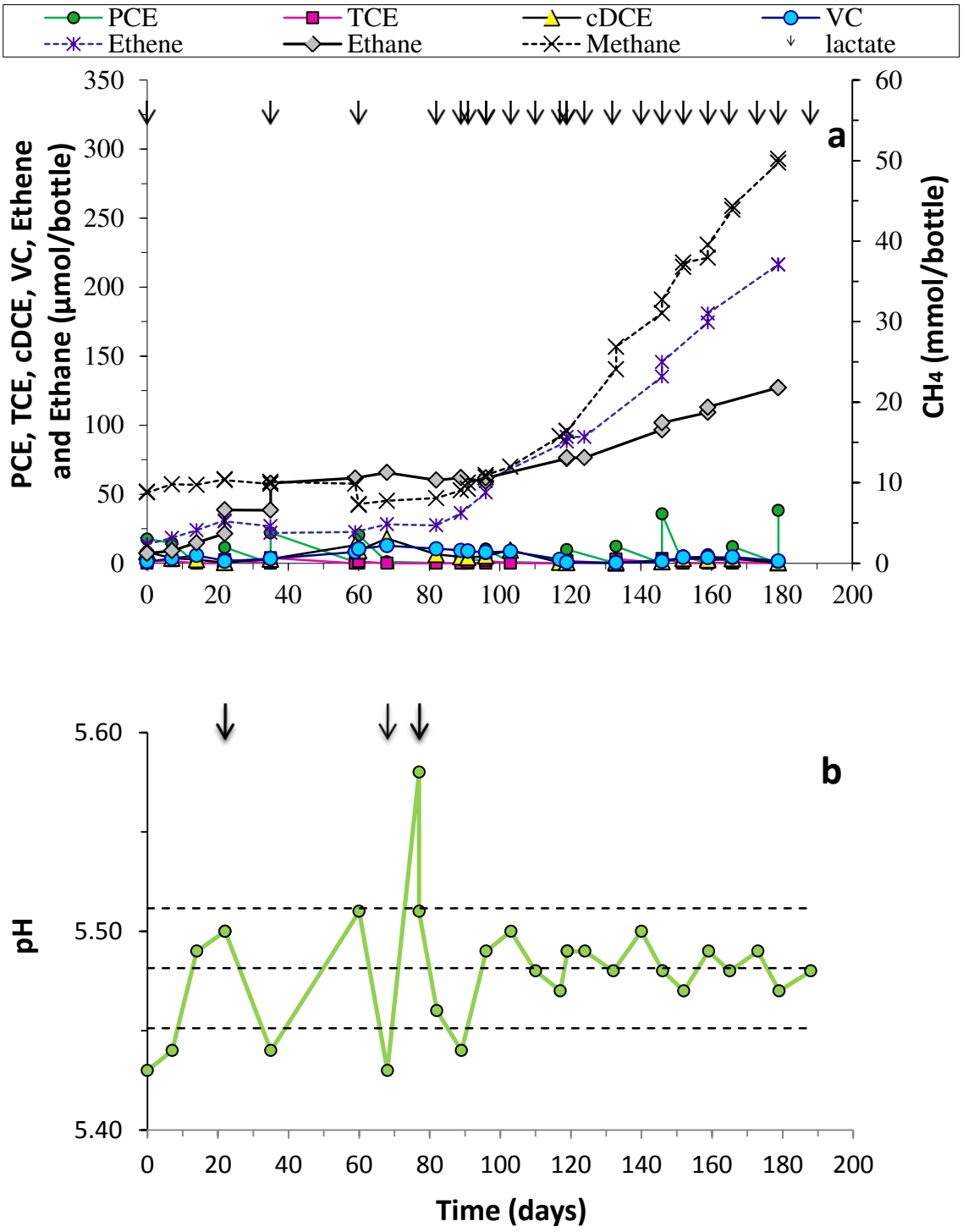

Figure E.20: Lactate-amended enrichment culture in a 2.6 L bottle (MM-VC-cDCE-B1 + NC-UN-B3) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid and **b)** pH,;the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1 M phosphoric acid.

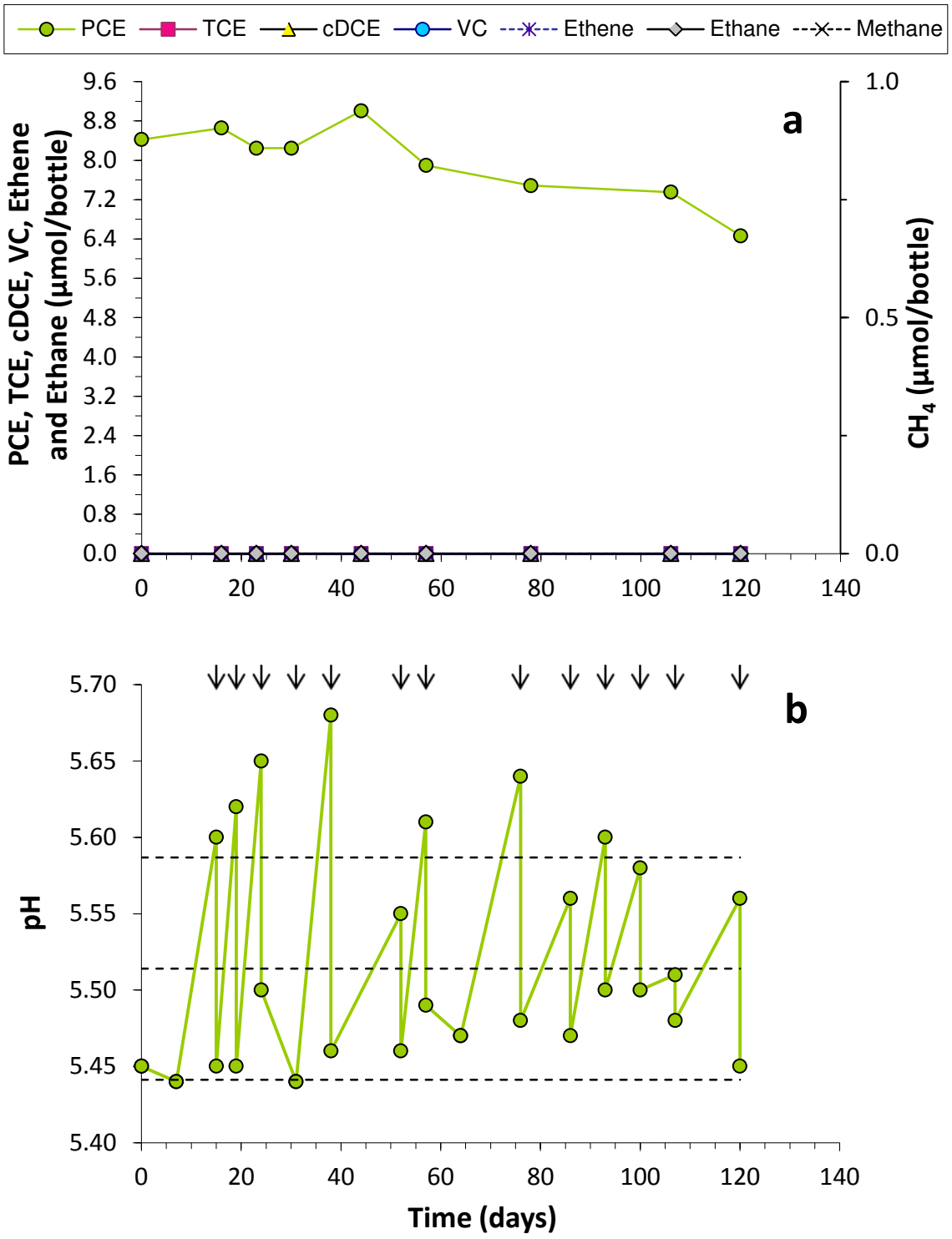

Figure E.21: Microcosm (unamended #1) for **a)** VOCs; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; the arrows indicate the addition of 1 M phosphoric acid.

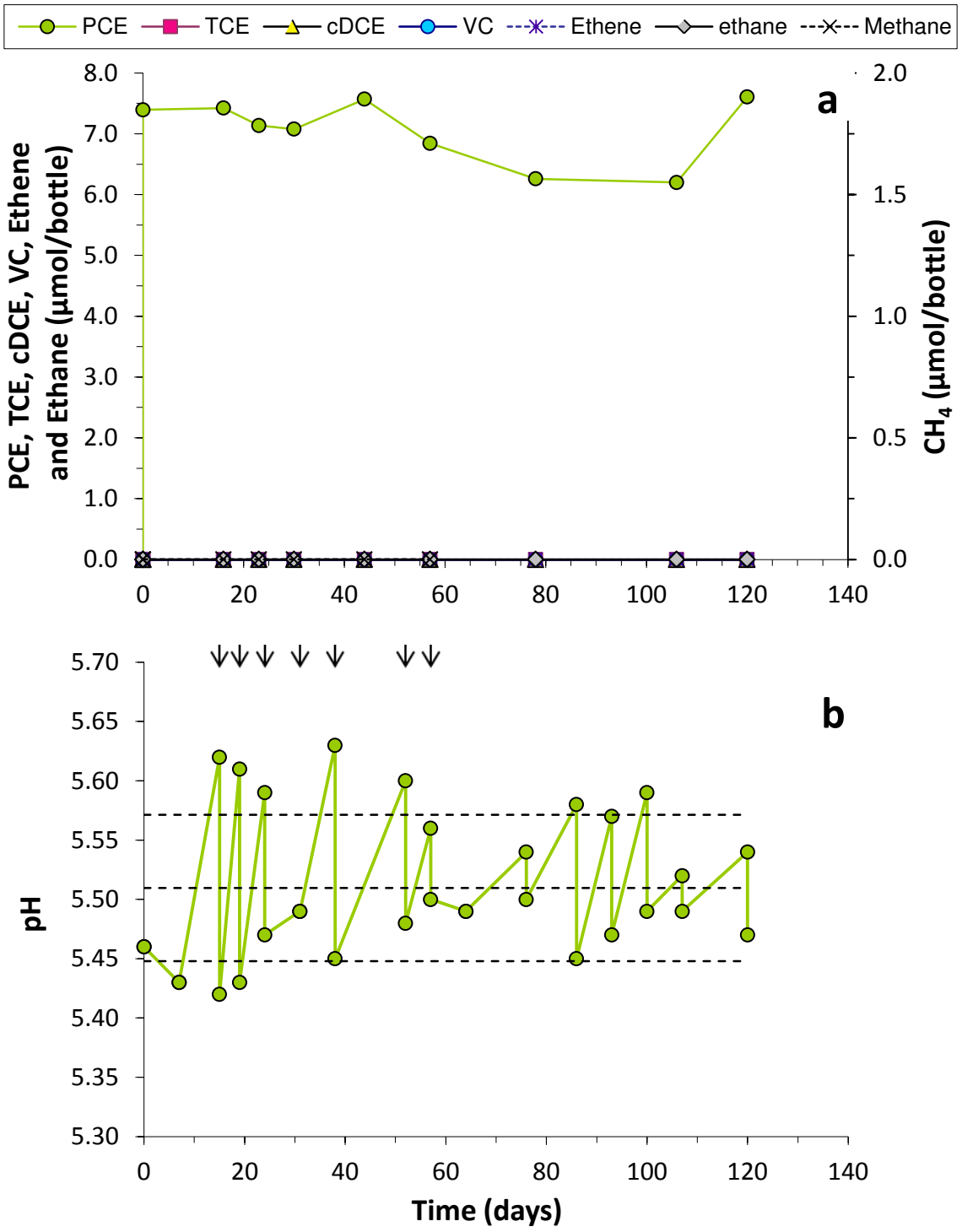

Figure E.22: Microcosm (unamended #2) for **a)** VOCs; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1 M phosphoric acid.

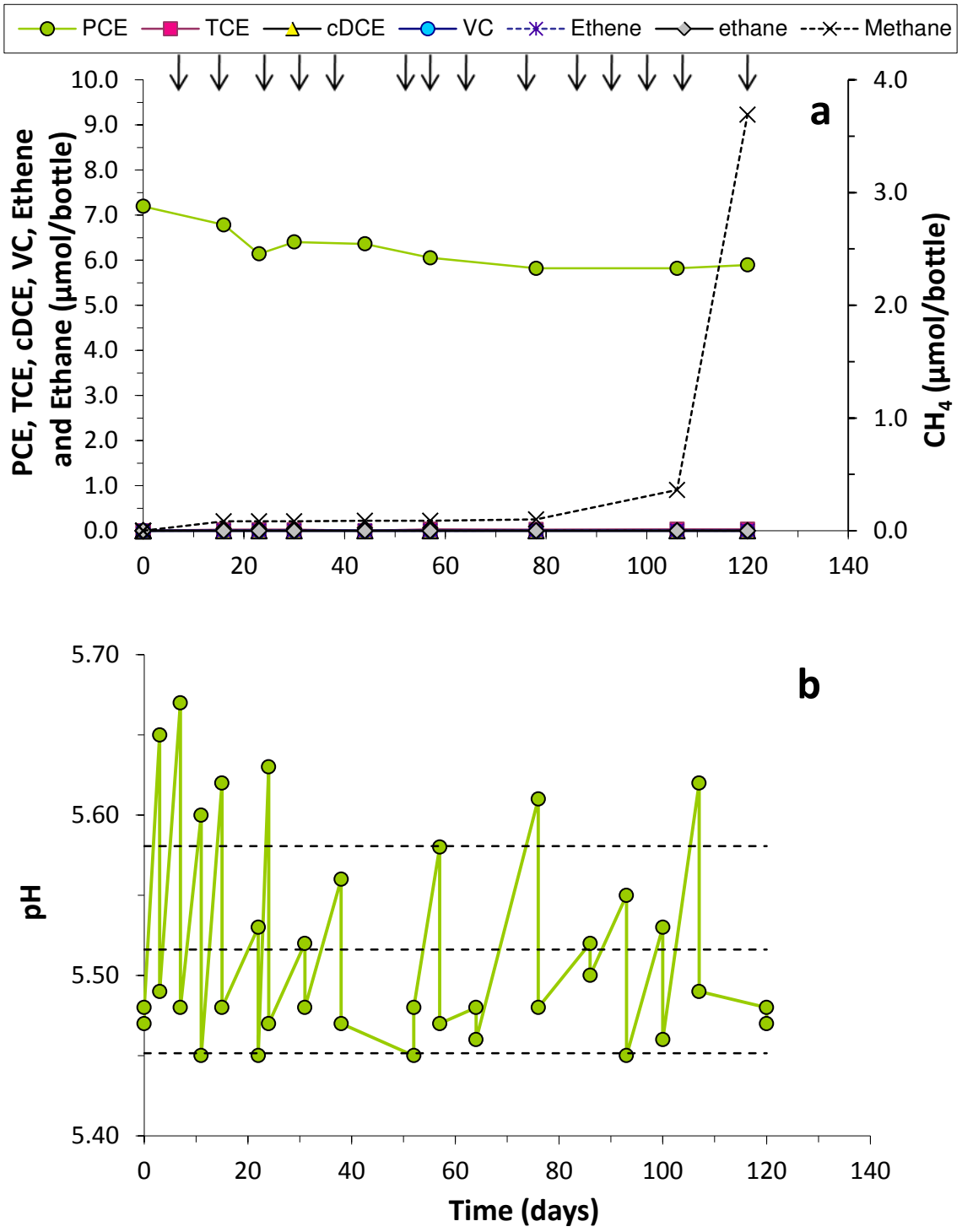

Figure E.23: Microcosm (biostinmulation #2) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation.

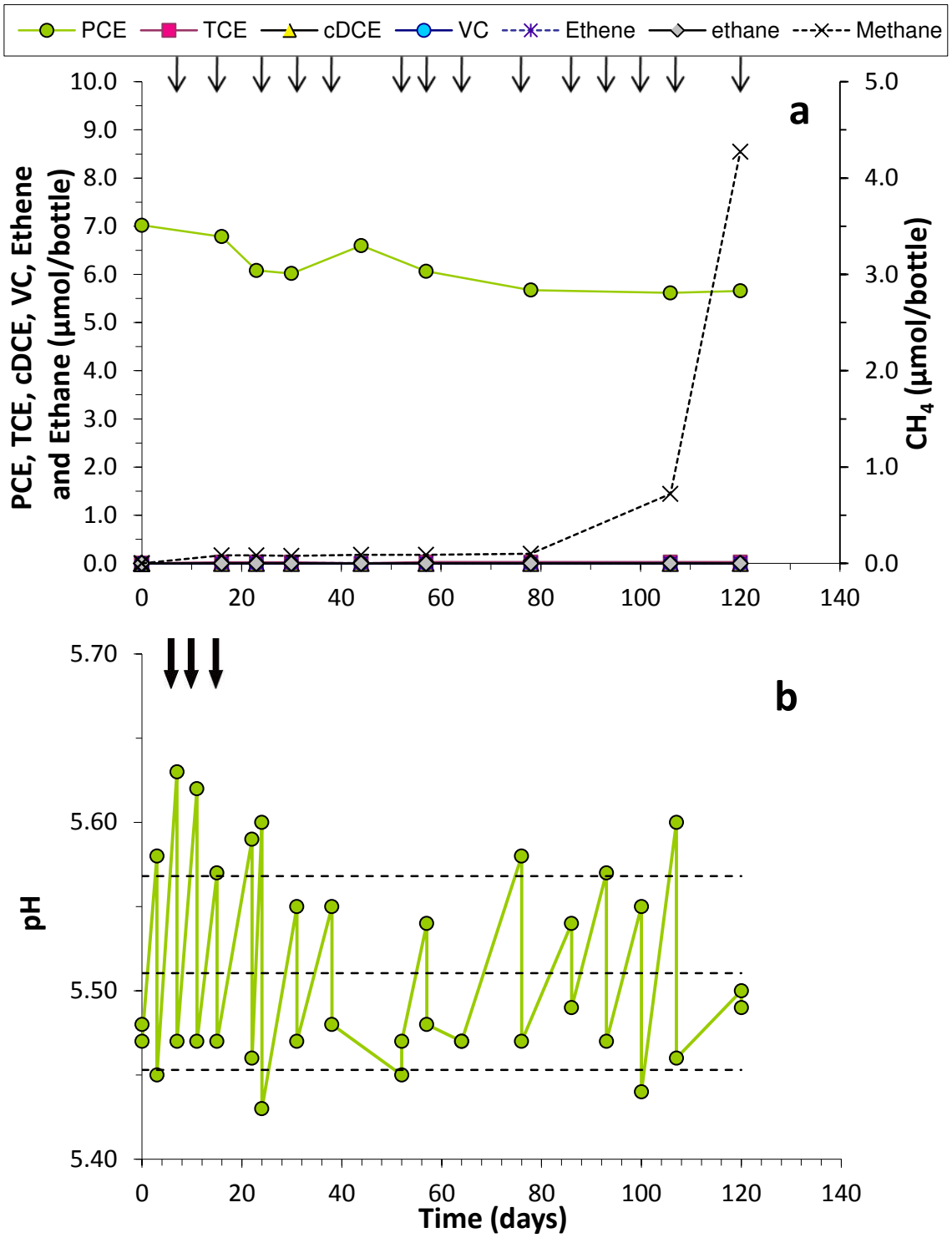

Figure E.24: Microcosm (biostinmulation #3) for **a)** VOCs; small arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1 M phosphoric acid.

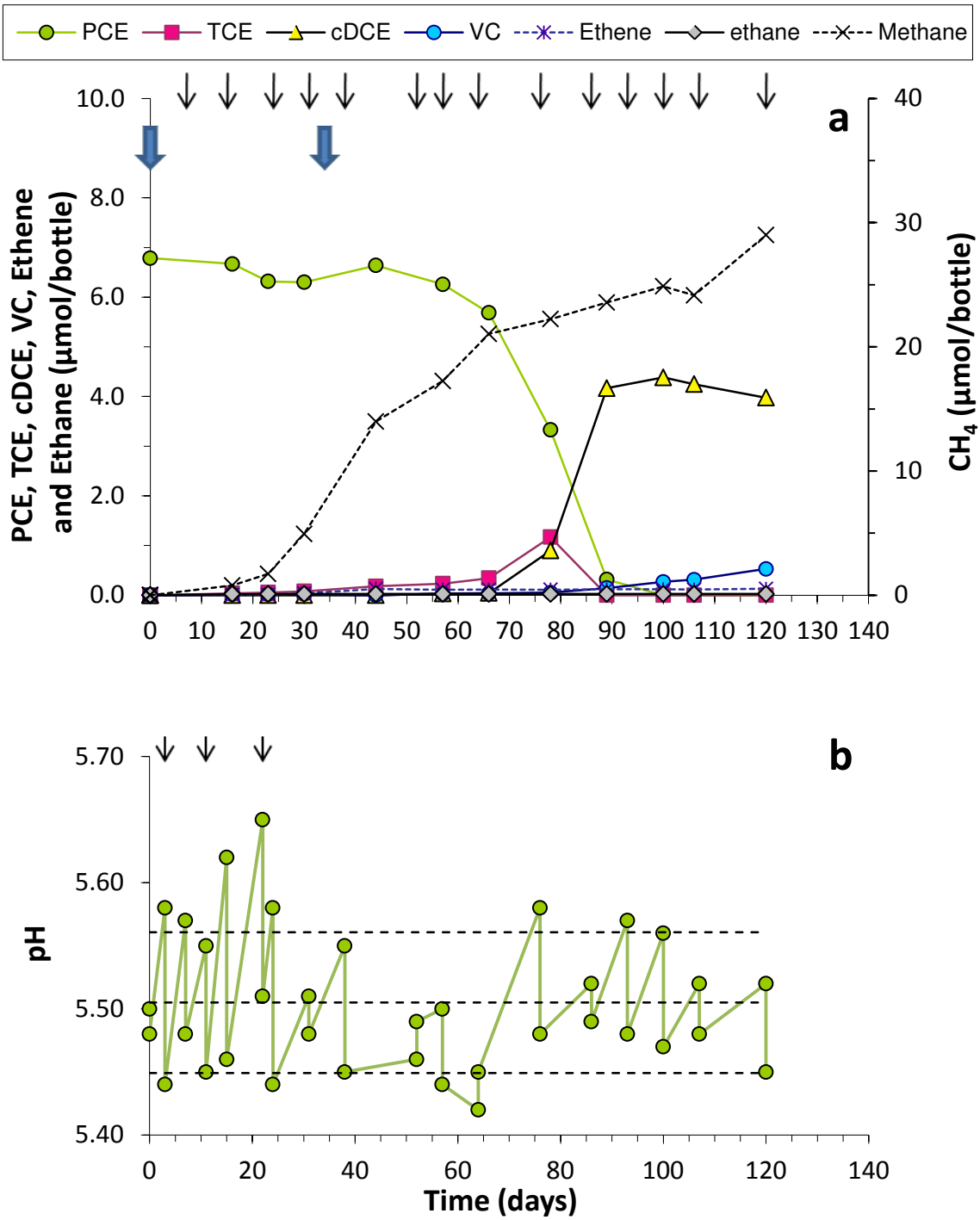

Figure E.25: Microcosm (bioaugmentation #1) for **a)** VOCs; small arrows indicate the addition of lactate or lactic acid and large ones indicate addition of the enrichment culture; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; the arrows indicate the addition of 1 M phosphoric acid.

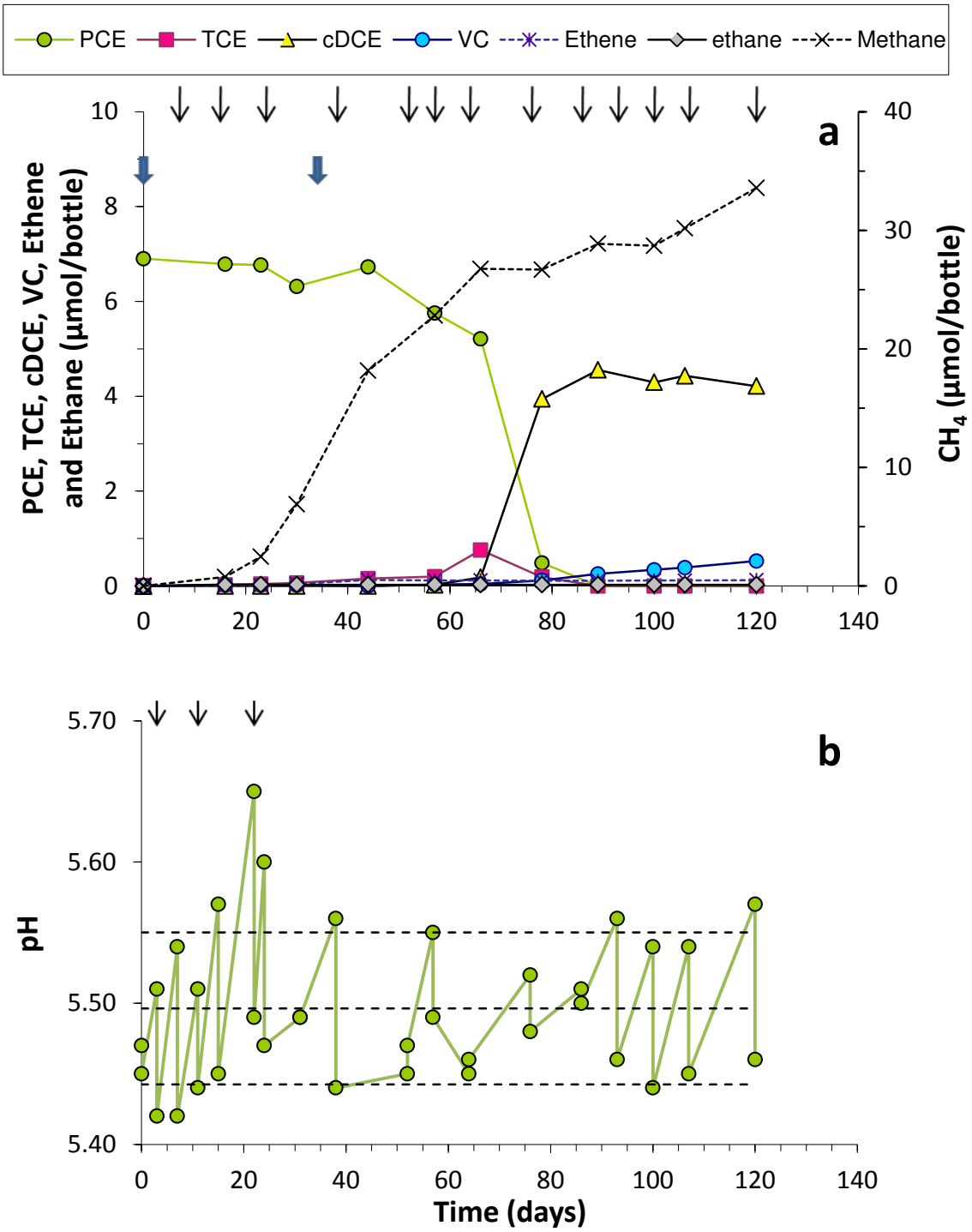

Figure E.26: Microcosm (bioaugmentation #3) for **a)** VOCs; small arrows indicate the addition of lactate or lactic acid and large ones indicate addition of the enrichment culture and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1 M phosphoric acid.

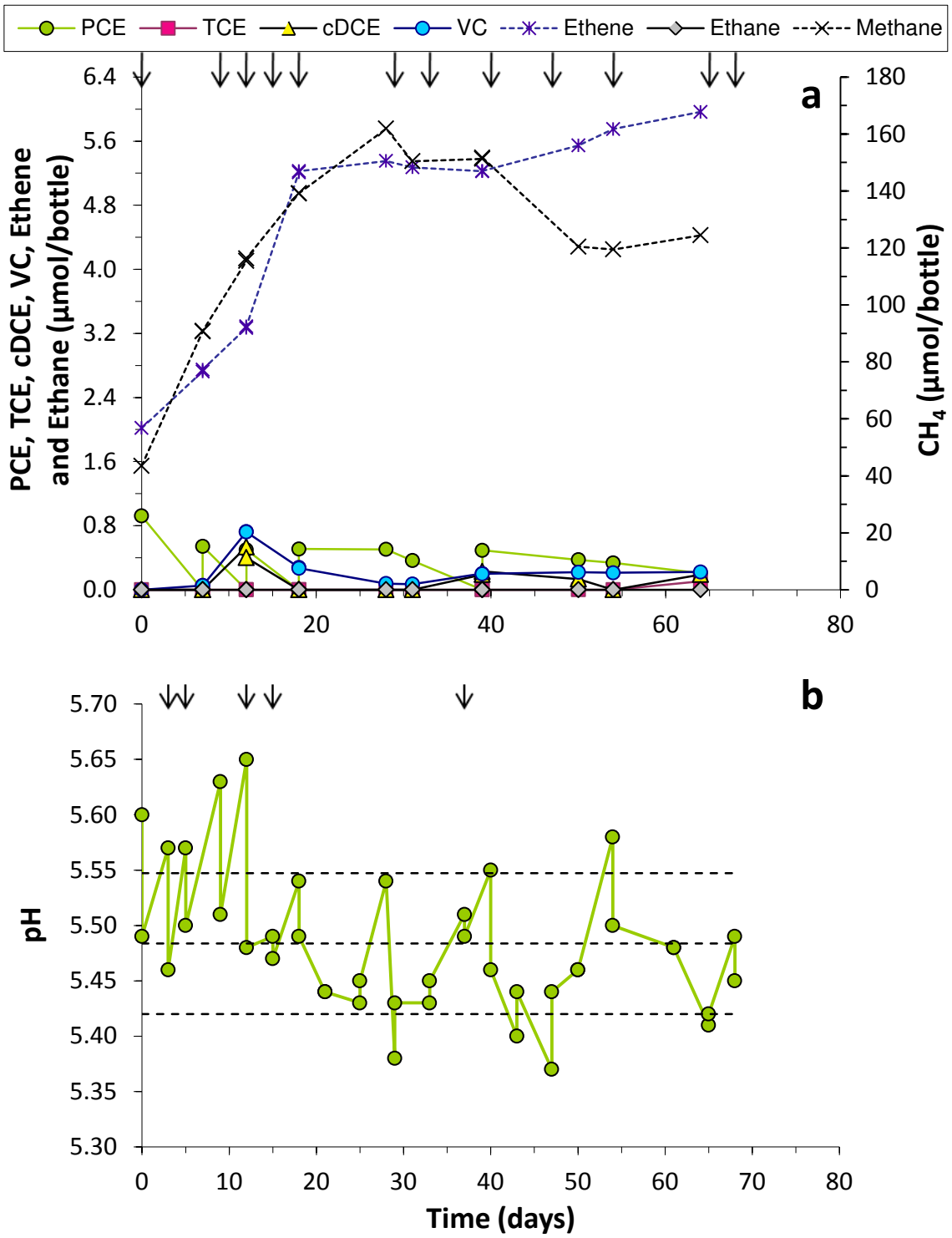

Figure E.27: Serum bottle (pH testing #1) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1 M phosphoric acid.

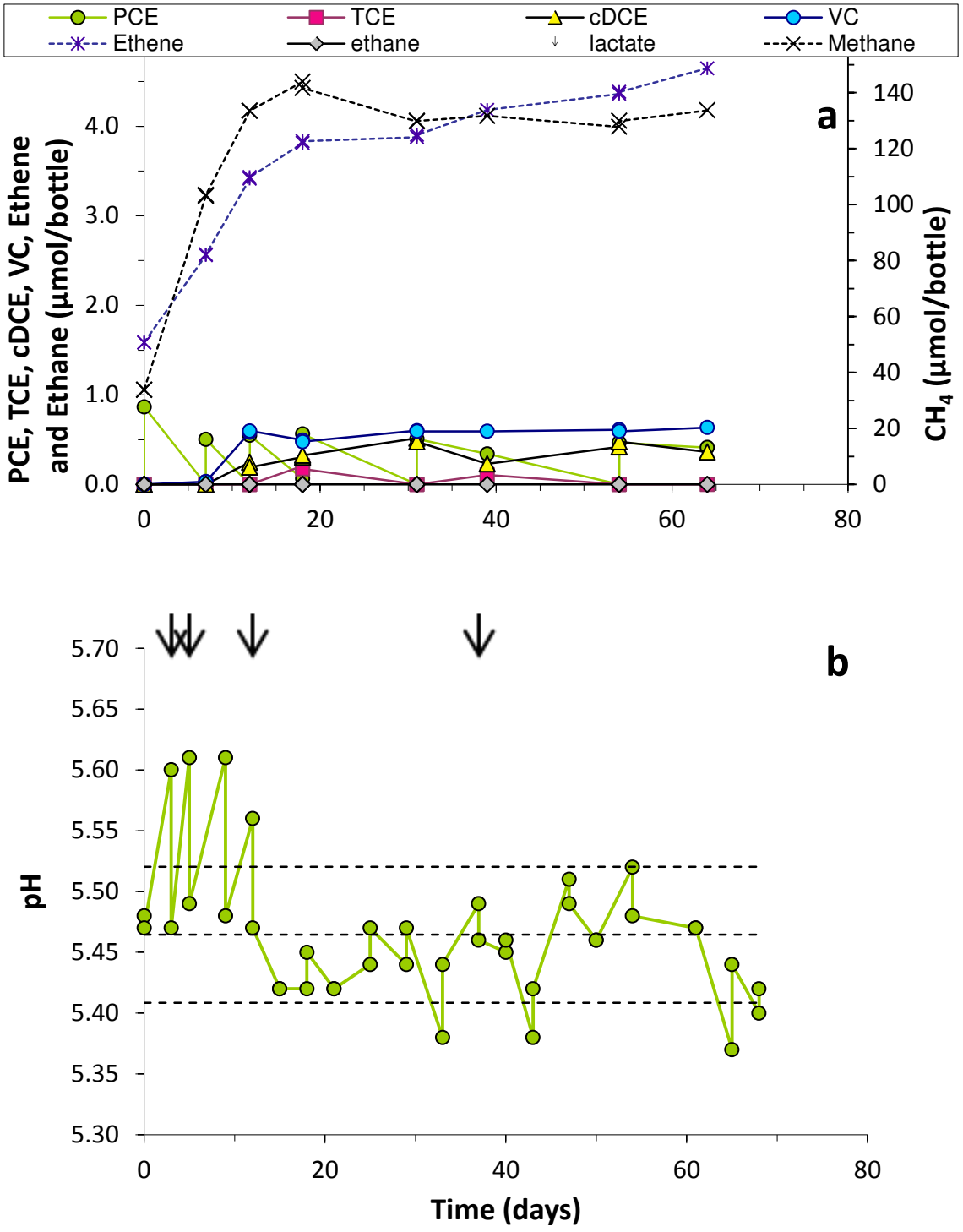

Figure E.28: Serum bottle (pH testing #2) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1 M phosphoric acid.

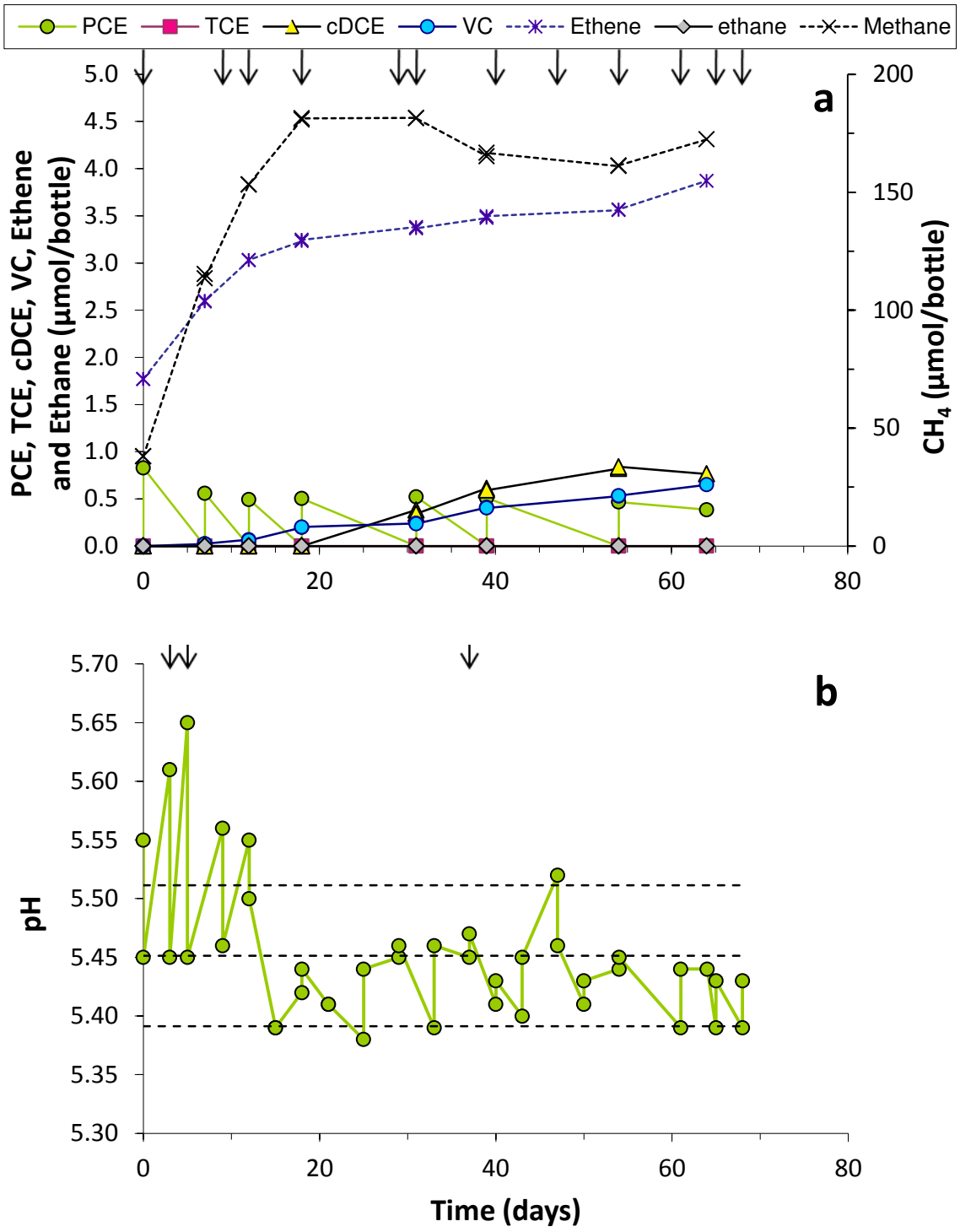

Figure E.29: Serum bottle (pH testing #3) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1 M phosphoric acid.

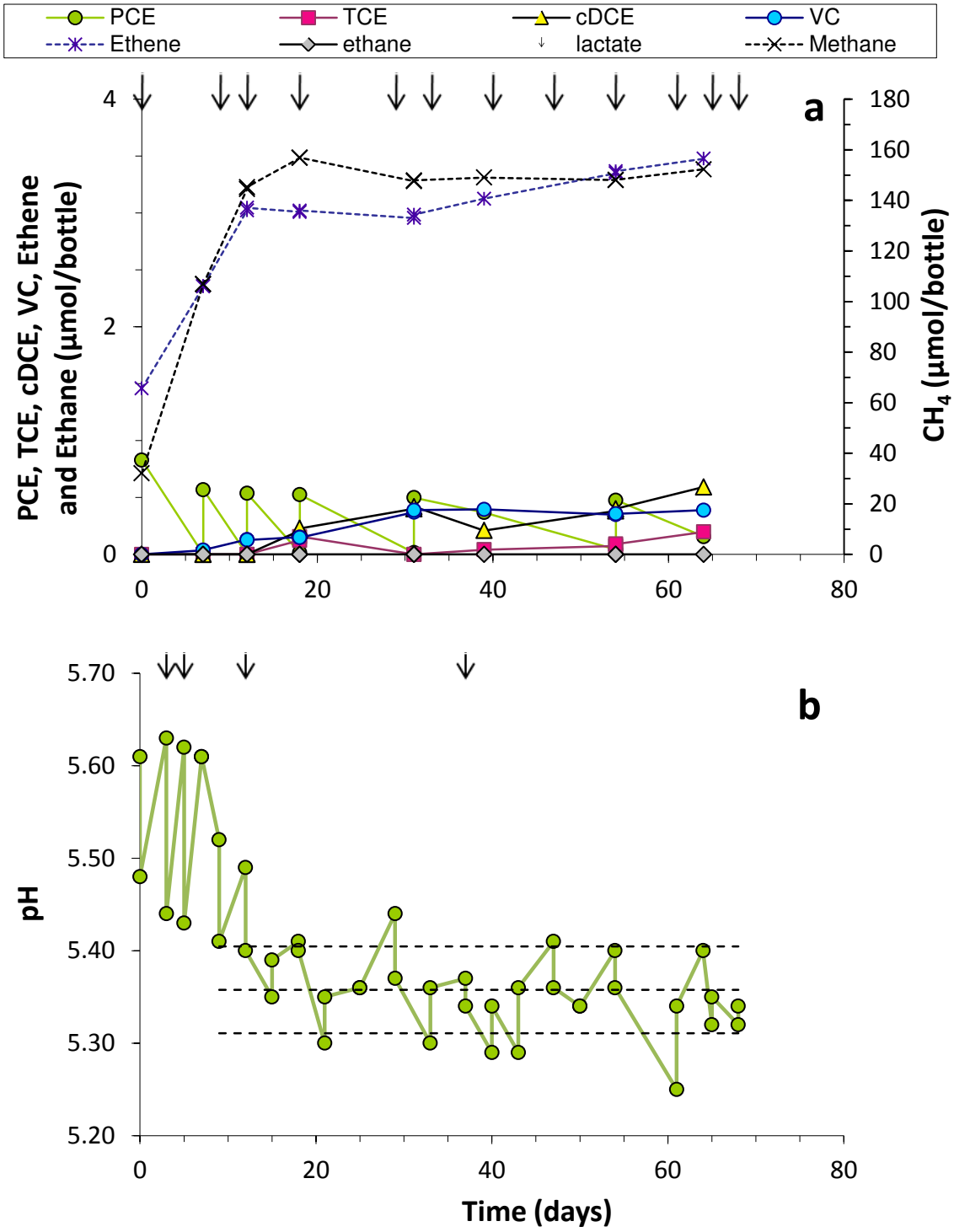

Figure E.30: Serum bottle (pH testing #4) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH, the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1 M phosphoric acid.

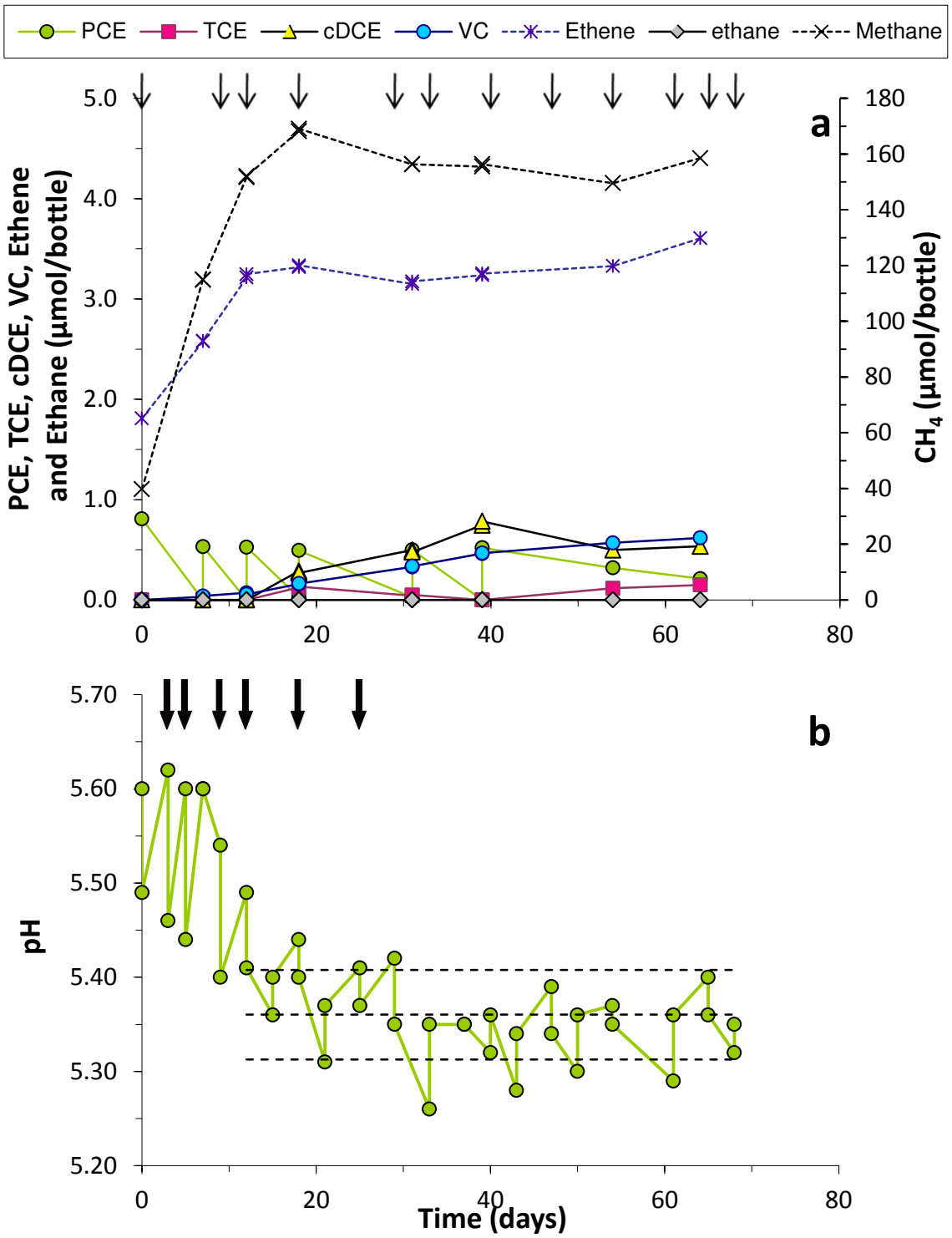

Figure E.31: Serum bottle (pH testing #5) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid; and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1 M phosphoric acid.

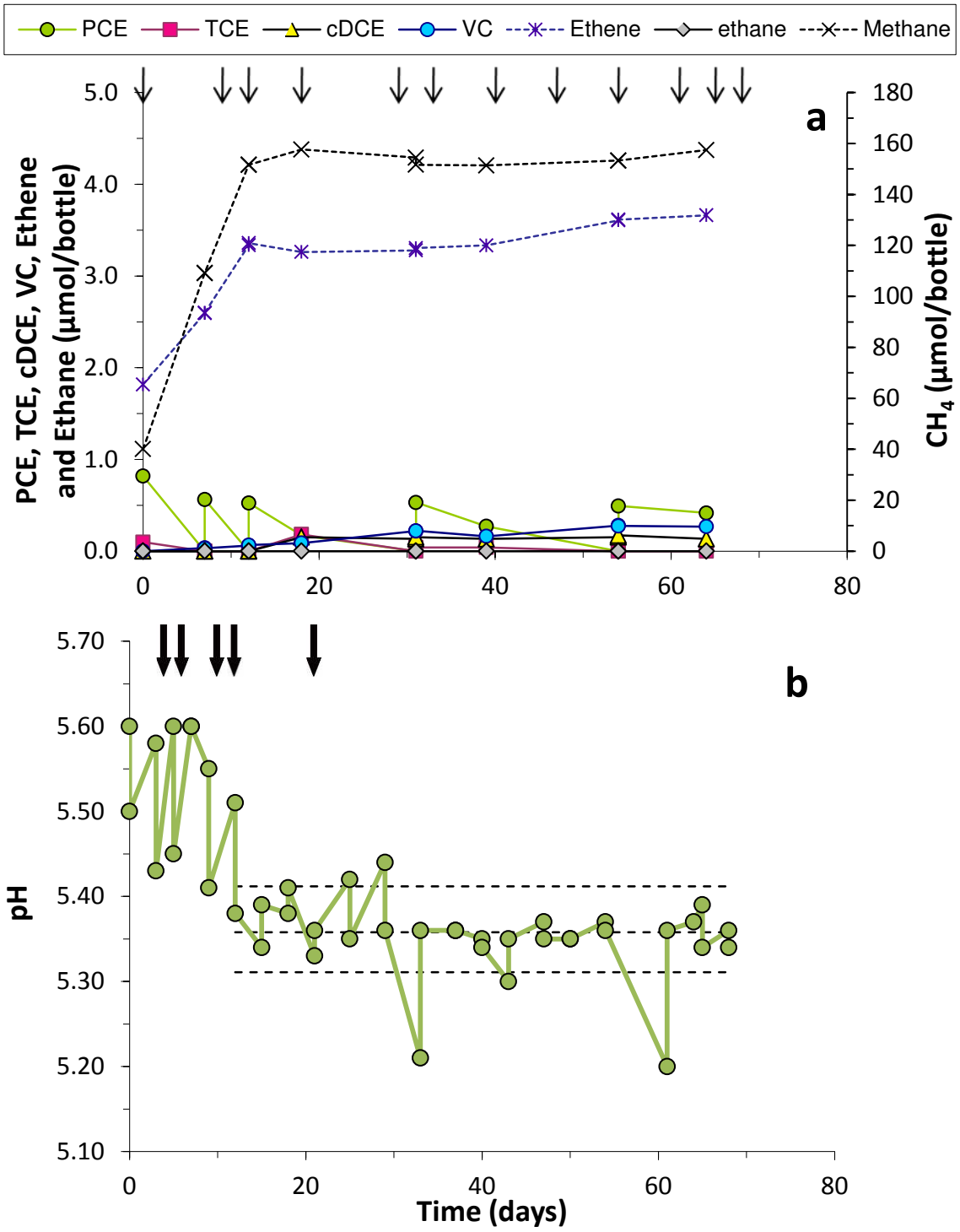

Figure E.32: Serum bottle (pH testing #6) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1 M phosphoric acid.

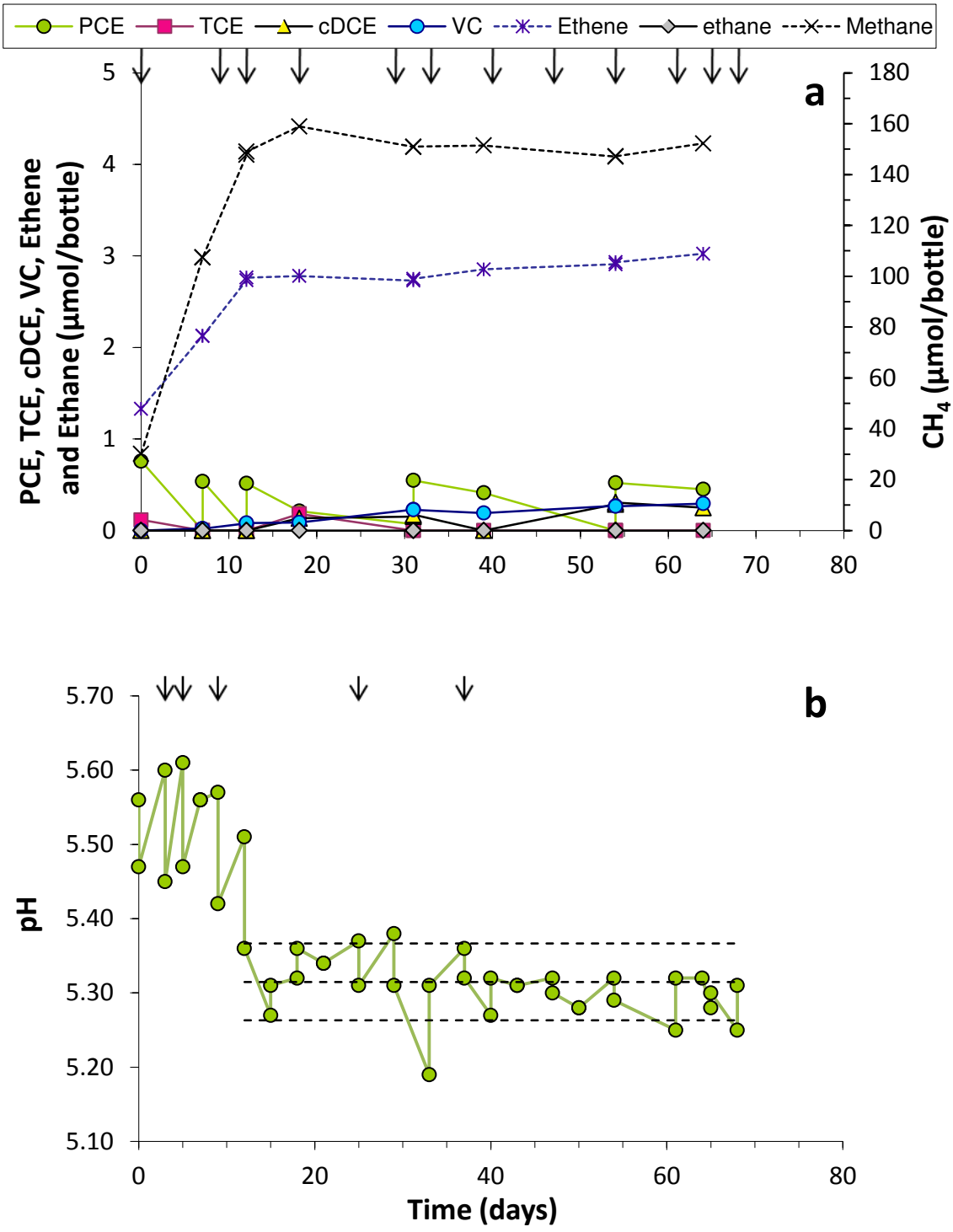

Figure E.33: Serum bottle (pH testing #7) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1 M phosphoric acid.

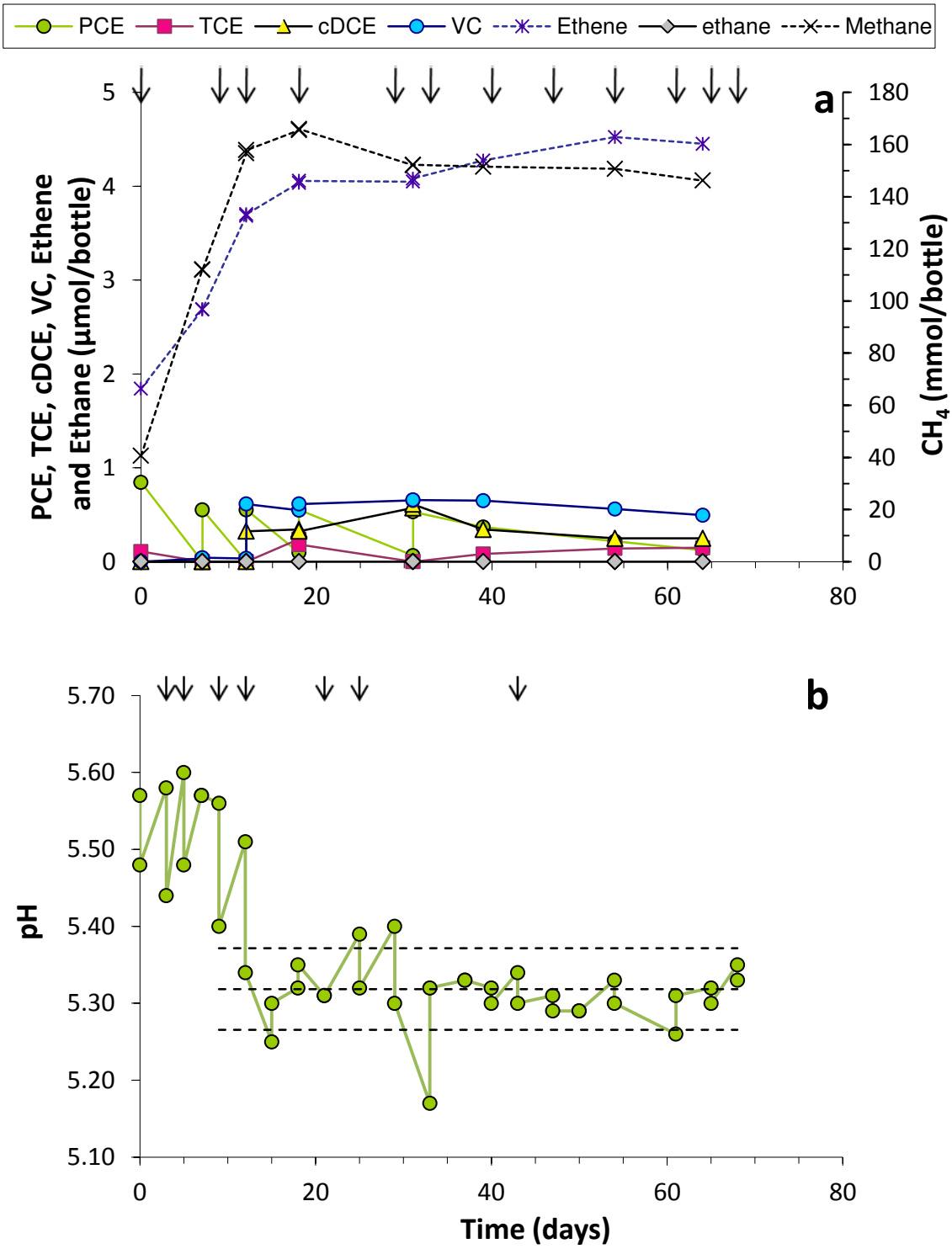

Figure E.34: Serum bottle (pH testing #8) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1 M phosphoric acid.

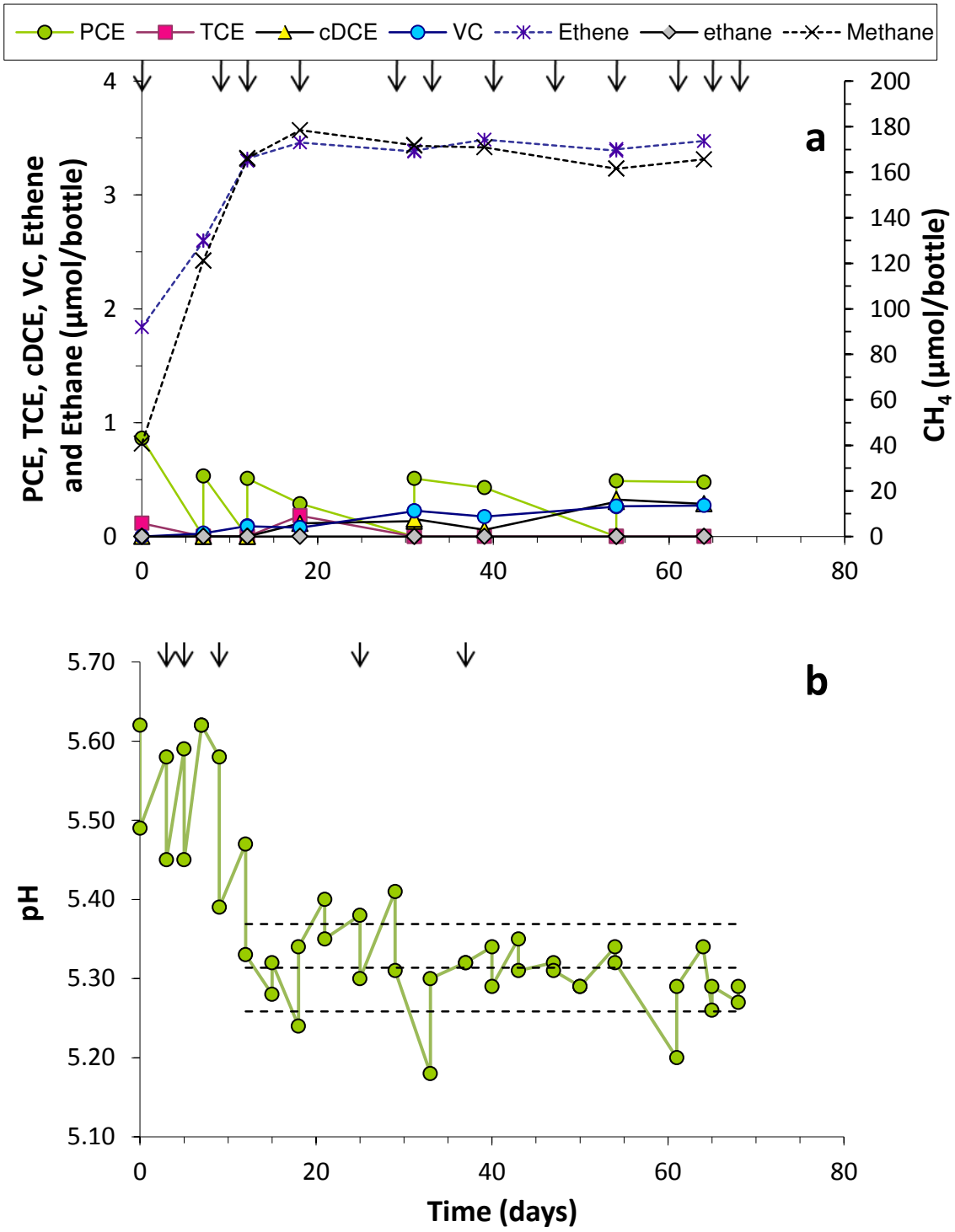

Figure E.35: Serum bottle (pH testing #9) for **a)** VOCs; arrows indicate the addition of lactate or lactic acid and **b)** pH; the dashed horizontal lines represent the average ± one standard deviation; arrows indicate the addition of 1 M phosphoric acid.

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