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DEVELOPMENT OF ENRICHMENT CULTURES FOR ANAEROBIC REDUCTIVE DECHLORINATION OF TETRACHLOROETHENE UNDER LOW pH CONDITIONS

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DEVELOPMENT OF ENRICHMENT CULTURES FOR ANAEROBIC REDUCTIVE DECHLORINATION OF TETRACHLOROETHENE UNDER LOW pH CONDITIONS

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ABSTRACT

Bioremediation is an attractive remediation strategy for groundwater contaminated with tetrachloroethene (PCE) since it can result in complete reductive dechlorination to non-hazardous ethene, often at a lower cost than other treatment methods. The optimum pH for microbes that chlororespire chlorinated ethenes is in the range of 6.5-7.5. However, the groundwater at many locations is outside this range, and typically on the low side. Addition of a base to increase the pH is problematic due to the difficulty of achieving homogenous distribution and the potential for clogging caused by precipitation. The objectives of this thesis were 1) to develop anaerobic enrichment cultures that are capable of chlororespiring chlorinated ethenes at a pH of 5.5; 2) to compare the use of phosphate versus 2-(N-morpholino) ethanesulfonic acid (MES) as buffering agents for the enrichment cultures, with the intent of maintaining a stable pH in the vicinity of 5.5; and 3) to compare the use of lactate, hydrogen and emulsified vegetable oil as electron donors for the low pH enrichment cultures to support PCE dechlorination to ethene

Development of enrichment cultures began with construction of microcosms using soil and groundwater samples from two locations in which there was field evidence for dechlorination activity at a pH below 6. In addition, enrichment cultures that were started in a previous project, using inoculum from a third hazardous waste site, were continued for this research. Combinations of enrichment cultures from two of the sites were also evaluated.

Several enrichment cultures were successfully developed with the capacity to dechlorinate PCE to ethene and/or ethane at a pH of 5.5. The most effective enrichment culture was created by combining enrichments from two of the hazardous waste sites. Phosphate buffered medium was effective for maintaining enrichment cultures at a pH close to 5.5; MES did not provide any better control of the pH. Lactate and hydrogen were effective electron donors for the low pH enrichment cultures, although lactate is more acceptable for practical application. Use of emulsified vegetable was discontinued after it failed to show any advantages in microcosms from one of the sites. Further development of the enrichment cultures will be necessary prior to evaluating their potential for bioaugmentation of chloroethene-contaminated groundwater with a pH below 6.

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SYMBOLS AND ABBREVIATIONS

cDCE	*cis*-1,2-Dichloroethene
COD	Chemical Oxygen Demand
EOS	Emulsified Vegetable Oil
GC	Gas Chromatograph
MES	2-(N-morpholino) ethanesulfonic acid (MES)
MSM	Mineral Salts Medium
PCE	Tetrachloroethene
SRS	Savannah River Site
TCE	Trichloroethene
VC	Vinyl Chloride
VOCs	Volatile Organic Compounds

CHAPTER ONE

1.0 INTRODUCTION

Tetrachloroethene (PCE) is a common groundwater contaminant (10). PCE and trichloroethene (TCE) are suspected carcinogens and ranked 31 and 16, respectively, on the EPA 2005 Comprehensive Environmental Response, Compensation, and Liability Act priority list for hazardous substances (24). PCE and TCE are common industrial solvents and degreasing agents. Both PCE and TCE are have an EPA maximum contaminant level for drinking water of 5 µg/L (http://water.epa.gov/drink/contaminants/index.cfm#List). *cis*-1,2-Dichloroethene (cDCE) is mainly formed from reductive dechlorination of TCE. Under anaerobic conditions, sequential reductive dechlorination of PCE, TCE and cDCE can result in accumulation of vinyl chloride (VC), which is the only known human carcinogen among the chlorinated ethenes and has a maximum contaminant level of 2 µg/L (http://water.epa.gov/drink/contaminants/index.cfm#List). Many National Priorities list sites are reported to contain VC (http://www.atsdr.cdc.gov/ToxProfiles/tp20.pdf). Therefore, methods are needed to remove chlorinated ethenes from the environment, especially from groundwater.

1.1 Bioremediation of Chlorinated Ethenes in Groundwater

Chemical and physical methods for remediating chlorinated ethenes in the subsurface include chemical oxidation, permeable reactive barriers, soil vapor extraction, and electrical resistance heating. Bioremediation is an attractive alternative since it can result in complete reductive dechlorination to non-hazardous ethene, often at a lower cost (10).

Reductive dechlorination of chlorinated ethenes can occur under specific anaerobic conditions (8). If the correct microbes are present along with an adequate supply of electron donor and appropriate geochemical conditions, chlorinated ethenes can be used as growth-supporting terminal electron acceptors via organohalide respiration. This process is also known by other names, including chlororespiration (22) . Geochemical conditions of concern include the presence of competing electron acceptors, including nitrate, Fe(III), Mn(IV) and sulfate; the presence of inhibitory compounds (e.g., 1,1,1-trichloroethane or chloroform); E_h; pH; and the availability of sufficient alkalinity to buffer against decreases in pH caused by release of HCl during dechlorination and accumulation of organic acids during fermentation of the electron donor.

The reductive dechlorination process occurs at a low redox potential, which typically means less than -110 mV. The redox potential is a measure of the tendency of a chemical species to acquire or lose electrons. In laboratory studies such as the one described in this thesis, resazurin is often used as a redox indicator (1 mg/L). Resazurin is pink at an E_h above -110 mV and clear at an E_h below -110 mV (19).

Hydrogen is widely regarded as the universal electron donor for reductive dechlorination of chlorinated ethenes (26) . Hydrogen is usually provided by fermentation of organic substrates such as lactate, emulsified vegetable oil (EOS), ethanol, or molasses. Gerritse et al. (9) ranked the rates of PCE dechlorination observed with different substrates as follows: lactate > ethanol > H_2. Adding insufficient electron donor does not adequately promote the process, whereas adding electron donor in excess may stimulate unwanted competitive processes, such as methane production. Under high hydrogen and

acetate concentrations, methanogens easily compete with *Dehalococcoides* for hydrogen and the dechlorination process may be arrested (http://www.drycleancoalition.org/ download/enhanced_reductive_dechlor.pdf).

The rate of dechlorination usually decreases as each chlorine atom is removed (7). Gerritse et al. (10) reported the following maximum rates of dechlorination in batch enrichments: PCE to TCE, 341 µmol/L·d; TCE to cDCE, 159 µmol/L·d; cDCE to VC, 99 µmol/L·d; and *trans*-DCE to VC, 22 µmol/L·d. More than 90% PCE was converted into DCE with a transient accumulation of TCE. Among the three isomers of DCE (*cis*-, *trans*- and 1,1-DCE), cDCE was the predominant product (5). However, *Dehalococcoides* sp. strain MB reportedly dechlorinates TCE mainly to *trans*-1,2-DCE rather than cDCE (3). Although ethene is most typically the terminal non-hazardous product, it can be reduced further to ethane under methanogenic conditions; this process is attributed to the cometabolic activity of methanogens (20).

1.2 Microbes that Chlororespire PCE

Many types of bacteria have been identified that are able to chlororespire PCE and TCE to cDCE, but no further. Damborský (4) summarized several strains of bacteria that can degrade PCE, including *Desulfitobacterium dehalogenans* JW/IU-DC1, *D. multivorans* and *D. chloroethenica* TT4B.

Dehalococcoides are the only bacteria known that are capable of respiring cDCE, although recently, Rouzeau-Szynalski et al. (30) provided evidence from an enrichment culture that *Desulfitobacterium* spp. are able to respire cDCE to VC. Some strains of *Dehalococcoides* are able to metabolically reduce VC to ethene, which is the most critical

step in the reduction process, due to the higher toxicity associated with VC. He et al. (12) demonstrated that *Dehalococcoides* sp. strains BAV-1 respires VC and cDCE, while PCE and TCE are co-metabolized during growth on cDCE and VC. Some strains of *Dehalococcoides* carry out reduction of VC to ethene cometabolically, i.e., they do not gain growth-linked energy from the transformation and it is considerably slower. A few stains of *Dehalococcoides* can use PCE or TCE as terminal electron acceptors. In many mixed culture environments, including groundwater, complete dechlorination of PCE to ethene involves a mixture of non- *Dehalococcoides* and *Dehalococcoides* with varying metabolic capabilities.

1.3 Effect of pH on Chlororespiration of Chlorinated Ethenes

In general, the optimum pH for microbes that chlororespire chlorinated ethene is in the range of 6.5-7.5. However, the groundwater at many locations is outside this range, and typically on the low side (34). Moreover, the pH of groundwater may change over time. Hill and Neal (16) reported that the pH in upper River Severn groundwater ranged from 4 to 7 within one year.

Even in aquifers that are circumneutral, bioremediation may act to depress the pH outside the neutral range. For each chlorine atom removed, one mole of HCl is released and will reduce alkalinity accordingly. Unless the aquifer is adequately buffered, release of HCl will cause the pH to decrease, which is especially problematic in the vicinity of source zones, where the highest concentrations of chlorinated ethenes are located. Even in a well-buffered aquifer, significant levels of reductive dechlorination will depress the pH outside the neutral range. Furthermore, fermentation of electron donors yields

organic acids that may also depress the pH.

Table 1.1 summarizes the effect of pH on 16 pure cultures, including five strains of *Dehalococcoides*. None exhibit dechlorination activity at pH 5.5. The activity of *Dehalococcoides* is strongly influenced by pH, with a several fold decrease below 6.5 (43). The optimum pH level for *Desulfitobacterium* sp. strain Y51 is 6.5- 7.5 (40). Its activity (reduction of PCE to cDCE) is significantly inhibited at pH 6, and stops completely at pH 5. *Sulfurospirillum multivorans* (4) *and Desulfuromonas michiganensis* (39) only exhibit activity (reduction of PCE to cDCE) at pH 7.0 to 7.5. The activity of *Geobacter lovleyi* SZ (reduction of PCE to cDCE) was tested from pH 5.5 to 8.0 (37), however, it was active only at pH levels between of 6.5 and 7.2. The dechlorination ability of *Desulfitobacterium* sp. PCE-1 was tested between pH 6.0 and 9.0, although it was only active between 6.5 and 8.0 (9).

Table 1.2 summarizes the effect of pH on several commercial bioaugmentation cultures, including KB-1, SDC-9, Bio-Dechlor Inoculum, the Pinellas culture, and a culture marketed by Bioremediation Consulting, Inc. The activity of KB-1 was tested from pH 5.0 to 10.0. However, it was active only at pH levels between of 6.0 and 8.3 (31). However, no reports were found in the literature for dechlorination activity below 6. For SDC-9, SHAW, Inc. reports that dechlorination can be accomplished within a pH range of 6.1-7.4 (41). Schaefer et al. (32) reported the poor performance of PCE dechlorination during in situ bioremediation under low pH conditions (approximately 5.5) with SDC-9. Ritalahti et al. (29) reported Bio-Dechlor Inoculum® can be used to accomplish PCE dechlorination; however, the effect of pH levels was not described. Ellis

et al. (6) reported that in situ complete PCE dechlorination can be achieved at pH levels from 6.0-6.5. Harkness et al. (11) reported complete dechlorination by the Pinellas culture at a neutral pH; the effect of lower pH levels was not described. Bioremediation Consulting, Inc. offers a bioaugmentation culture that they claim is effective to a pH of 5.6 (http://www.bcilabs.com/news.html). Nevertheless, nothing was found in the literature in which this or any other low pH tolerant culture has been evaluated in situ for bioaugmentation.

To help reduce the potential for aquifers becoming acidic, some vendors (e.g., EOS) offer amendments such as emulsified vegetable oil in combination with a buffer (e.g., AquaBupH). McCarty et al. (27) have suggested that formate be given more consideration as an electron donor, since it has less impact on pH than other, more conventional electron donors.

When the groundwater pH is already below 6, the options available to implement bioremediation of chlorinated ethenes are limited. Addition of a base has been evaluated, including NaOH and carbonates. One of the problems with this approach is the difficulty of achieving homogenous distribution; the area around the injection zone may see the pH rise too high, while areas further away may not receive sufficient base. Also, problems have been reported with clogging due to precipitation, especially around the injection wells (36). Lastly, large-scale adjustment of pH may make bioremediation less cost competitive than other remediation approaches.

An alternative approach is to use an enrichment culture that remains active at a pH of 5.5 or lower. There are some examples from contaminated sites that affirm the

possibility of achieving dechlorination of chlorinated ethenes to ethene at pH levels below 6. For example, at the Savannah River Site (SRS), a plume of TCE discharges to a wetland in which the pH is consistently below 6, yet complete dechlorination occurs; the electron donor consists of the large amount of organic material in the wetland. For a site in North Carolina (hereafter referred to as NC/HH), Zawtocki and Bramblett (44) reported on field data indicating ethene formation from PCE at pH levels ranging from 3.8-6.7.

Using samples from a site in North Carolina (hereafter referred to as NC/FRX) and the wetland area mentioned above at SRS, Hickey (15) initiated development of a low pH tolerant enrichment culture for reductive dechlorination of chlorinated ethenes. Although Hickey (15) observed dechlorination activity at a pH below 6, his work did not yield an active enrichment culture that could reliably reduce PCE to ethene at a pH of 5.5.

1.4 Objectives

The primary objective of this thesis was to complete the development of an anaerobic enrichment culture that is capable of chlororespiring chlorinated ethenes at a pH of 5.5. The research is both an extension of the work started by Hickey (15) and an expansion. The extension is based on the use of several of the most promising enrichment cultures developed by Hickey (15); the expansion is based on newly prepared microcosms, some of which were started with samples from the NC/HH site and some from SRS; enrichment cultures were then developed with inocula from the microcosms. In addition, various combinations of the most promising enrichment cultures were evaluated.

A secondary objective of the thesis was to compare the use of phosphate versus MES as buffering agent for the enrichment culture, with the intent of maintaining a stable pH in the vicinity of 5.5.

Please note that tables and figures are presented at the end of the thesis.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Chemicals and Media

Ethene (99.999%) was obtained from National Specialty Gases. Methane (99.999%) and ethane (99.995%) were obtained from Matheson. VC (>99.5%) was obtained from Fluka, PCE (99%) was obtained from Arcos Organics, TCE (99%) from Fisher Scientific and cDCE (99%) from TCI America. High purity hydrogen (99.99%) was obtained from Airgas National Welders. Sodium lactate syrup (containing 58.8-61.2% sodium lactate; specific gravity=1.31) was obtained from EM Science. Lactic acid (85%) was obtained from Fisher Scientific. EOS®598B42 was obtained from EOS Remediation, LLC. All other chemicals used were reagent grade unless otherwise indicated.

Two types of media were used for the enrichment cultures, differing primarily by the type of buffer (Table 2.1). The mineral salts medium (MSM) is the same composition used by Hickey (15), who identified this particular medium composition as MSM-1; it is buffered with phosphate. The other medium is buffered by 2-(N-morpholino) ethanesulfonic acid (MES); the amount of MES used was based on a pH 5.5 medium described by Howieson (18). After preparing the media, phosphoric acid (1 M, $pK_1 = 2.1$, $pK_2 = 7.2$) or a phosphate buffer solution (3 M K_2HPO_4) was used to adjust the pH to 5.5. Adjustments were made in an anaerobic chamber while the media was continuously mixed on a stir plate. The pH probe was allowed to equilibrate for ~1 min before a reading was taken. Further details of the protocol for preparing media are provided in Appendix A.

2.2 Soil and Groundwater Samples

Microcosm cultures were prepared with soil and groundwater from two sites. One is the NC/HH site, the other from the Twin Lakes area at SRS. A description of both sites is given below, along with information about the locations from which samples were collected.

The NC/HH site is contaminated primarily with PCE (44). The source area is located in the vicinity of a former textile mill, with PCE concentrations above 1500 ppb. The pH level of the groundwater is predominantly below 6. TCE, cDCE, VC and ethene are also present. After injection of Hydrogen Release Compound®, 99% of the PCE and 89% of the TCE were removed and transformed to VC and ethene. The field data suggest that the site has bacteria tolerant of low pH with an ability to dechlorinate PCE to ethene. Biostimulation supported the dechlorination process. Recent monitoring data suggest that *Dehalococcoides* are present. Approximately 10 L of groundwater and 5 kg of soil was shipped from the site by overnight carrier to Clemson University and was received in November, 2011. Samples were stored at 4°C prior to preparation of the microcosms (section 2.3.1).

The C-area burning rubble pit at SRS is the source of a plume of TCE. The contamination area extends 1220 meters and enters the seep line in wetlands along Twin Lakes. The pH of groundwater in the Twin Lakes area is in the range of 5 to 6. Groundwater samples were taken from wells 48B and 52B on October 3, 2011, by Mr. Mark Amidon. Equal volumes were combined to construct microcosms. Samples were stored at 4°C prior to preparation of the microcosms (section 2.3.2).

2.3 Experimental Approach

To accomplish the primary objective of this thesis, four types of microcosms and enrichment cultures were developed:

1) Microcosms and enrichment cultures developed with samples from the NC/HH site;

2) Microcosms and enrichment cultures developed with samples from the Twin Lakes site at SRS;

3) Hickey (15) developed microcosms and enrichment cultures from the NC/FRX site. A subset of these enrichment cultures was maintained and further enriched as part of this thesis; and

4) Several samples of the NC/HH enrichments and the SRS microcosms or enrichment cultures were combined, with the intent of finding the most active enrichment culture for dechlorination at pH ~5.5.

Most of the microcosms consisted of 50 mL of groundwater and 20 g (wet) of soil in 160 mL serum bottles, capped with Teflon-faced red rubber septa, as previously described (15). Several microcosms were prepared in 2.6 L glass bottles, which were sealed with Teflon-faced septa inside a screw cap (surrounded by an o-ring to keep the septum centered in the cap); one exception was a single 0.7 L bottle, which will be noted below. The serum bottles were more convenient to manage, while the larger bottles afforded an opportunity to scale up the culture. All of the microcosms and enrichment cultures were incubated quiescently in an inverted position, inside boxes (to exclude light), and at room temperature.

A description of the four types of microcosms and/or enrichment cultures outlined above follows. In order to keep track of the numerous bottles, a nomenclature system was used that includes the following abbreviations:

S = serum bottle (160 mL)

B = big bottle (typically 2.6 L)

AC = autoclaved

UN = unamended

GW = groundwater

EOS = emulsified vegetable oil

The nomenclature of most of the enrichment cultures includes the names of the microcosms or prior enrichments used to create them.

2.3.1 NC/HH Microcosms

Table 2.2 summarizes the experimental design for the NC/HH microcosms. An explanation for the various treatments follows. Triplicate microcosms were prepared for each treatment.

Treatments #1 (NC-AC-S) and #2 (NC-AC-B) served as autoclaved controls in serum bottles and 2.6 L bottles, respectively. Treatments #3 (NC-UN-S) and #4 (NC-UN-B) served as unamended live microcosms in serum bottles and 2.6 L bottles. Treatments #5 (NC-lactate-S) and #6 (NC-lactate-B) were live microcosms that were initially amended with lactate in serum bottles and 2.6 L bottles; later in the incubation period, hydrogen was added instead of lactate. Treatment #7 (NC-EOS-S) represented

live microcosms amended with EOS; there was not enough soil and groundwater to prepare the corresponding set in 2.6 L bottles.

Prior to setting up the microcosms, the groundwater was evaluated for the concentration of volatile organic compounds (VOCs). PCE and TCE were present at approximately 50 and 20 μg/L, respectively. Since higher concentrations were preferred at the start of the incubation period, PCE was added (see below).

The soil sample cores were unwrapped and representative sections were combined in a clean, sterile plastic container and homogenized with a sterile spoon. The composited sample and groundwater were stored at 4°C. They were subsequently moved to the anaerobic chamber and allowed to warm to room temperature overnight. The chamber contains an atmosphere of approximately 98.5% N_2 and 1.5% H_2. The serum bottles were prepared by adding 20±0.2 g soil and 50±0.2 mL of groundwater. The 2.6 L bottles received proportionally higher amounts: 288±0.2 g soil and 878±0.2 mL of groundwater. They were then sealed and removed from the chamber. Treatment #1, #3, #5 and #7 received 1 mL of PCE saturated water; treatment #2, #4 and #6 received 2 μL of neat PCE.

For treatments #5, 6 and 7, the amount of electron donor added was based on 100 times the stoichiometric amount needed to reduce PCE to ethene. The 100-fold excess ensured an adequate margin of safety, for factors such as competing electron acceptors and the need to establish low redox conditions. Thus, the total electron donor demand for the serum bottles was:

(0.009 μmol PCE/mL saturated water)*(1 mL saturated water/bottle)*
(8 meq/mmol)* (100-fold safety factor) = 0.72 meq/bottle

In terms of chemical oxygen demand (COD), 0.72 meq/bottle is equivalent to 5.76 mg COD/bottle, or 115 mg/L COD. The COD of the lactate was based on its known composition ($C_3H_5O_3^-$) while the COD of emulsified vegetable oil was based on its estimated composition ($C_8H_{16}O$). A stock solution of lactate was used to deliver 0.72 meq/bottle. A 1:10 dilution of EOS®598B42 was used to deliver the same amount of electron donor as emulsified vegetable oil. Several of the treatments were switched from lactate to hydrogen as the electron donor. In those cases, the amount of hydrogen added was calculated without the safety factor, since hydrogen is directly available as the electron donor. Details of the calculations used to determine the quantities of electron donor added are provided in Appendix B.

2.3.2 NC/HH Enrichment Cultures

The experimental design for the NC/HH enrichment cultures is summarized in Table 2.3 and Figure 2.1 shows the sequence by which the various enrichment cultures were developed. MES-buffered medium was used for several of the treatments to determine if it offers advantages with respect to maintaining more stability in the vicinity of pH 5.5. Two of the microcosm treatments were enriched by transferring them into site groundwater since, as the results will show, the groundwater significantly facilitated reductive dechlorination at pH ~5.5. All of the enrichment cultures were prepared in 160 mL serum bottles.

Only two of the NC/HH microcosm treatments were used as inoculum: unamended (small bottles, NC-UN-S) and lactate amended (NC-lactate-B), since these exhibited the most active level of reductive dechlorination to ethene at a low pH. All of

the enrichments were prepared with a 10% inoculum from the completely mixed microcosms, so that some of the soil present was transferred to the enrichment bottles. Lactate or hydrogen was used as the electron donor. The name of the enrichment bottles communicated the inoculum source, the media type, and the electron donor.

The enrichment bottles were prepared in an anaerobic chamber; the medium was added to the serum bottles followed by the inoculum. Transfers were made when PCE in the parent bottles were close to or below the detection limit. Once removed from the anaerobic chamber, PCE saturated water was added (0.5 mL/bottle). The bottles were put on a shaker table for at least one hour and then analyzed for VOCs.

2.3.3 NC/FRX Enrichment Cultures

The experimental design for the NC/FRX enrichment cultures is summarized in Table 2.4 and Figure 2.2 shows the sequence by which the various NC/FRX enrichment cultures were developed. Hickey (15) developed the microcosms (A1, B1, C1, D1) and the first set of enrichment cultures, shown as white boxes in Figure 2.2. The enrichment cultures developed for this thesis are shown as yellow and green boxes; results will be presented for the cultures shown in yellow boxes, which were the most promising with respect to dechlorination activity at pH ~5.5.

Enrichment culture RS6.0-3B was prepared in a 2.6 L bottle, while the four transfers from it were to serum bottles. Two of the serum bottles received phosphate-buffered MSM, the other two received MES-buffered medium. Within each pair, one bottle received lactate and/or lactic acid as the electron donor, the other received hydrogen. As described above, the bottles were prepared in an anaerobic chamber.

2.3.4 SRS Microcosms and Enrichment Cultures

The experimental design for the SRS microcosms and enrichment cultures is summarized in Tables 2.5 and 2.6. Figure 2.3 shows the sequence by which the various microcosms and enrichment cultures were developed. With only one exception, all of the microcosms and enrichments received cDCE and/or VC; only one treatment received PCE. Two sets of microcosms were prepared, each with a "fresh" source of soil and groundwater from the Twin Lakes wetland. Set I was prepared as part of the research for this thesis and the results will be presented; Set II was prepared by Hickey (15) (the white boxes for Set II in Figure 2.3) and those results will not be repeated. Each treatment for Set I was prepared in triplicate. Two of the microcosm treatments for Set I (GW-cDCE+VC-S and MM-cDCE+VC-S) were prepared in serum bottles and received the standard amounts of soil (20±0.2 g) and groundwater or medium (50±0.2 mL). The other treatments (GW-cDCE+VC-B and MM-cDCE+VC-B) were prepared in 2.6 L bottles; they received the same initial quantities of soil (20±0.2 g) and twice the amount of liquid (100±0.2 mL of groundwater or MSM) as in the serum bottles. The intent with these larger bottles was to first establish reductive dechlorination activity, and then gradually dilute the soil concentration by adding more groundwater or MSM. In this respect, the larger bottles served as both microcosms and enrichment cultures.

The initial concentration of cDCE and VC in the groundwater was sufficiently low (<75 μg/L) that it was necessary to add cDCE and VC at time zero. The initial pH of the groundwater was ~5.6, which is consistent with field measurements from the Twin Lakes wetland. The amount of electron donor needed for the microcosms was estimated

as described above, using 4 meq/mmol for cDCE and 2 meq/mmol for VC. Calculations for the electron donor supply and demand are provided in Appendix B.

From the Set I microcosms, only one enrichment was prepared, using MES-buffered medium in a 2.0 L bottle (MES-cDCE+VC-S2-B). The other bottle shown on the same row in Figure 2.3 (MM-PCE-S) was not an enrichment, since 100% of the inoculum was from the "parent" bottle (i.e., MM-cDCE+VC-B); the purpose of this bottle was to determine if the SRS microcosms could transform PCE (since they had previously been given only cDCE and VC).

Two enrichment cultures were developed from the Set II microcosms (Figure 2.3 and Table 2.6). GW-VC-4B was prepared in a 0.7 L bottle, while GW-cDCE+VC-B4 was prepared in a 2.6 L bottle; it was inoculated with 240 mL from four microcosm bottles and was then gradually diluted with MSM. Lactate or lactic acid was added as the electron donor for both bottles.

2.3.5 Combined Cultures

As the results will show, several of the enrichment cultures from each source (i.e., NC/HH, NC/FRX, and SRS) exhibited some potential for reductive dechlorination of chlorinated ethenes at low pH. However, some enrichments appeared to work fastest in terms of PCE and TCE reduction to cDCE, while others were faster in terms of cDCE and VC reduction to ethene. With the goal of developing the most efficient overall enrichment culture, three sets of samples from the NC/HH and the SRS enrichment cultures were combined.

The experimental design for the Set I combined cultures is summarized in Table 2.7 and Figure 2.4 shows the sequence by which the various enrichment cultures were combined. The same SRS enrichment culture was used (GW-cDCE+VC-B), coming from bottles #2 and 3. Treatments #1-3 were prepared in serum bottles, while #4 was prepared in a 2.6 L bottle. Lactate and/or lactic acid were used as the electron donor.

The experimental design for the Set II combined cultures is summarized in Table 2.8 and Figure 2.5 shows the sequence by which the various enrichment cultures were combined. The same SRS enrichment culture was used (GW-VC-4B). All treatments were prepared in serum bottles and lactate and/or lactic acid was used as the electron donor.

The experimental design for the Set III combined cultures is summarized in Table 2.9 and Figure 2.6 shows the sequence by which the various enrichment cultures were combined. The same SRS enrichment culture was used (MM-cDCE+VC-B), coming from bottles #1 and 3. Treatments #1-5 were prepared in serum bottles, while #6 was prepared in a 2.6 L bottle. Lactate and/or lactic acid were used as the electron donor.

2.4 pH Measurement and Adjustment

Before sampling for pH measurements, the solids in microcosms were allowed to settle overnight to ensure that only a liquid sample was withdrawn. For enrichment cultures, the concentration of solids was much lower and did not interfere with the measurement, therefore bottles were shaken vigorously before removing a homogenous sample. Samples (0.2 mL) were transferred to 1.5 mL conically shaped plastic micro tubes with snap caps; this was the minimum volume of liquid that can fully immerse the

pH probe in the micro tube. The pH probe was calibrated at 7.0 and 4.0 before measurement.

The target pH for all of the experiments was 5.5±0.05. When the pH rose above 5.5, it was lowered with phosphoric acid (1 M), or by adding lactic acid instead of lactate, or a combination of the two. Occasionally, when the pH fell below 5.45, it was increased back to ~5.5 using 8 M NaOH, or lactate was used in place of lactic acid, or both. To minimize the risk of making an excessive number changes to the pH (e.g., by making adjustments too frequently), bottles were allowed to incubate several days after adding an acid or base and the pH was measured again. If the pH was still outside the desired range (i.e., 5.45-5.55), another adjustment was made and the bottle was incubated several more days before the next measurement.

2.5 Analysis of Volatile Organic Compounds

The concentration 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. The carrier gas was nitrogen (~30 mL/min).

Results for VOCs are presented in terms of μmoles per bottle, which allows for a direct assessment of the stoichiometry of daughter product accumulation. The GC response to a headspace sample was calibrated to give the total mass of the compound (*M*) in that bottle. The response factors measured by Hickey (15) were used and are

listed in Appendix C. Response factors are specific to the ratio of the headspace to the liquid. For example, with microcosms prepared in serum bottles, the liquid volume was 50 mL and the headspace was 99 mL; the balance of the total volume (160 mL) was occupied by soil. For enrichment cultures, the volume of the liquid was 100 mL and the headspace was 60 mL (the volume of solids was minimal). Each case required a different response factor. When enrichment cultures were prepared in larger bottles, the same ratio of headspace to liquid was used (i.e., 0.60), such that the same response factor was applicable when multiplied by the ratio of the total volume of the larger bottle to the total volume of the smaller bottle (e.g., 2550 mL/160 mL = 15.94):

$$RF_B = RF_S \frac{V_B}{V_S} \qquad 2.1$$

where RF_B = response factor for the 2.6 L ("big") 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.

Several of the 2.6 L bottles were prepared with liquid and headspace volumes that were different from the ratios used to determine response factors. In these cases, the response factors were estimated by calculation (Appendix D).

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

$$C_l = \frac{M}{V_l + H_C V_g} \qquad 2.2$$

where C_l = concentration in the aqueous phase (μM); M = total mass present (μmol/bottle); V_l = volume of the liquid in the bottle; V_g = volume of the headspace in the

bottle; and H_c = Henry's constant (dimensionless) at 23°C. The Henry's Law constants reported by Hickey (15) were used.

CHAPTER THREE

3.0 RESULTS

Results are presented first for the microcosms and enrichment cultures derived from the NC/HH site. These are followed by results for the enrichment cultures developed from the NC/FRX site and then the SRS microcosm and enrichment cultures. Finally, results for the three sets of combined cultures are presented. Results are shown for each treatment highlighted in a yellow box in Figures 2.1-2.6. When replicate bottles were monitored, the results for only one are shown in this chapter; results for the other bottles are provided in Appendix E. Each section is started with a summary of the average pH level over the full incubation period for each of the live treatments, followed by the daughter product distribution in all treatments (i.e., including the autoclaved controls).

For all microcosms and enrichment cultures, the chlorinated ethenes, methane and ethane values are given in μmol per bottle. This allows for direct stoichiometric comparisons of the reductive dechlorination products, since the process yields one mole of product per mole of parent compound.

3.1 NC/HH Microcosms and Enrichment Cultures

3.1.1 NC/HH Microcosms

Results for average pH levels in the live NC/HH microcosms are summarized in Figure 3.1. The initial pH level in the microcosms was 5.8 or above, which required adjustment with phosphoric acid (1 M). Thereafter, the average pH remained 5.5.

Figure 3.2 shows the average distribution of dechlorination products in the NC/HH microcosms for the entire incubation period, which was calculated based on the total amount of PCE added and the amount of VOCs present at the last sampling point. Losses represent the difference between the total PCE consumed and the sum of daughter products at the final sampling point. Two of the treatments yielded ethene as the predominant product: NC-UN-S and NC-Lactate-B. It is unclear why the corresponding treatments in the other size bottles (i.e., NC-UN-B and NC-Lactate-S) did not behave similarly.

Average results for the autoclaved controls are shown in Figure 3.3. These demonstrate that physical losses (i.e., via diffusion and adsorption) in the serum bottles and large bottles were minor relative to the live treatments (see below) over the 199 days of incubation. Results for a representative unamended serum bottle (NC-UN-S1) are shown in Figure 3.4. Each dose of PCE was approximately 1.5 μmol/bottle, which is equivalent to 2.2 mg/L. Once PCE dechlorination started, repeat additions were consumed at an increasing rate, with cDCE and then VC accumulating. Starting around day 60, the accumulated VC was reduced to ethene. After the last addition of PCE, ethene accumulated more quickly. These results are consistent with field observations (44) and indicate that the groundwater contains electron donor. Results for a representative unamended 2.6 L bottle (NC-UN-B2) are shown in Figure 3.5. Like its smaller counterpart, PCE was readily reduced to cDCE; however, the bigger bottles experienced a "cDCE stall," i.e., little or no further dechlorination occurred.

Results for a lactate-amended serum bottle (NC-Lactate-S3) are shown in Figure 3.6. In this case, dechlorination stalled at VC. The electron donor was switched from lactate to hydrogen to try to induce VC reduction, without effect. In the other two replicates, dechlorination stalled at VC, which was a puzzling outcome, since complete PCE dechlorination occurred in the unamended serum bottles (Figure 3.4), and hydrogen is widely recognized as the universal electron donor. A representative result for one of the lactate-amended 2.6 L bottles is shown in Figure 3.7. In this case, complete dechlorination to ethene occurred. However, it is worth noting that a higher rate of VC reduction to ethene started after hydrogen additions were stopped. In this respect, the larger microcosm behaved more like the unamended serum bottles (Figure 3.4).

Figure 3.8 provides a representative result for an EOS-amended microcosm. Although PCE reduction to cDCE resembled the other treatments, no significant further reduction occurred, even after a dose of hydrogen was provided on day 133.

3.1.2 NC/HH Enrichment Cultures

Since the unamended serum bottles and the lactate/H_2 amended 2.6 L bottles exhibited the greatest extent of ethene formation, they were used as inoculum to develop enrichment cultures, using both phosphate-buffered and MES buffered media, as well as groundwater (Figure 2.1).

Results for average pH levels in the live NC/HH enrichment cultures are summarized in Figure 3.9. The average for each treatment was approximately 5.5, with similar magnitudes of standard deviation. Phosphoric acid (1 M) and varying levels of lactate and lactic acid were used to control the pH.

Figure 3.10 shows the average distribution of dechlorination products in the NC/HH enrichment cultures for the entire incubation period, as described above. All the bottles reduced PCE to cDCE; most reduced at least some of the cDCE to VC. The bottle with the highest output of ethene was NC-UN2-MSM-H_2.

Results for two of the four NC-UN-S bottles are presented in Figures 3.11 (NC-UN1-MSM-H_2) and 3.12 (NC-UN2-MSM-H_2); both achieved complete reduction of PCE to ethene at an average pH around 5.5. The other two bottles in this category (NC-UN-S) accumulated cDCE and exhibited only partial reduction to VC.

Results for one of the four NC-UN-MES bottles (NC-UN2-MES-H_2) are presented in Figure 3.13. The stall on cDCE was extended, although at ~day 80 reduction to VC started and at ~day 90 ethene started to accumulate. There was no significant accumulation of ethene in the other bottles.

Results for one of the two NC-UN-GW bottles is shown in Figure 3.14. At the time that monitoring was stopped, the cDCE that had accumulated was later reduced to VC; ethene had not yet started to be produced. Figure 3.15 provides a representative result for one of the three NC-lactate-MSM-S bottles. PCE was quickly converted to cDCE which was in the process of a slower reduction to VC; at the time that monitoring stopped, ethene had not accumulated. Results for one of the three NC-lactate-MES-S bottles is shown in Figure 3.16. The single dose of PCE added was quickly reduced to TCE and then cDCE, which was more slowly reduced to VC; ethene did not accumulate during the 97 days of incubation.

3.2 NC/FRX Enrichment Cultures

Results for average pH levels in the NC/FRX enrichment cultures are summarized in Figure 3.17. The average for each treatment was approximately 5.5, with similar magnitudes of standard deviation. Figure 3.18 shows the average distribution of dechlorination products in the NC/FRX enrichment cultures for the entire incubation period; ethene was the predominant product in all five bottles. Results for the single RS6.0-3B bottle are presented in Figure 3.19. After a long acclimation period, PCE dechlorination started and relatively soon thereafter, ethene became the predominant product.

Two types of further enrichments were prepared from RS6.0-3B (Figure 2.2); one using phosphate-buffered medium and the other MES-buffered. Figures 3.20 and 3.21 show the results for the MSM-buffered bottles, one of which was provided with hydrogen and the other with lactate. Figures 3.22 and 3.23 present the companion results for the MES-buffered medium. All of the bottles reduced PCE to ethene at an average pH around 5.5. In the two bottles with hydrogen added (Figures 3.20 and 3.22), reduction of VC to ethene occurred at a notably higher rate at the end of the incubation period.

3.3 SRS Microcosms and Enrichments Cultures

3.3.1 SRS Microcosms, Set 1

Results for average pH levels in the Set I SRS microcosms and enrichment cultures are summarized in Figure 3.24. The average for each treatment was approximately 5.5. Figure 3.25 shows the average distribution of dechlorination products for the Set I microcosms; ethene and ethane were predominant in most of the bottles.

Note that most of the Set I and II SRS treatments received cDCE and/or VC, but not PCE or TCE. The exception was treatment MM-PCE-S, which was used to evaluate if PCE could be dechlorinated as well as cDCE and VC.

Results for one of the three GW-cDCE+VC-S serum bottle microcosms are presented in Figure 3.26; these were prepared with soil and groundwater. The first three additions of cDCE and VC were reduced to ethene through day 85, with lactate added as the electron donor. At that point, an attempt was made to dilute the soil by adding 50 mL of groundwater. For reasons that are not yet known, dechlorination activity stopped thereafter in all three bottles. A companion set of 2.6 L bottles (GW-cDCE+VC-B) was also monitored; results for one of the triplicates is shown in Figure 3.27. Although it took longer for the first dose of VC and cDCE to be reduced, the rate improved with two subsequent additions even though groundwater was added (on days 149 and 195) to dilute the soil.

For the Set I SRS microcosms, two other treatments were prepared as described above, although phosphate buffered MSM was used in place of groundwater. The intent was to hasten acclimation of the microbes to a defined medium, so that enrichment would not rely on site specific groundwater. Results for one of the three MM-cDCE+VC-S serum bottle microcosms are presented in Figure 3.28. The use of medium in place of groundwater appeared to have a stimulatory effect on the rate of cDCE and VC reduction to ethene. On day 87, more medium was added in order to further dilute the soil. In this microcosm, dechlorination continued, albeit at a somewhat slower rate; in the replicate bottles, however, dechlorination activity ceased after adding more medium. Somewhat

better results were obtained with the 2.6 L bottles. As shown in Figure 3.29, the second, third and fourth additions of cDCE and VC were accompanied by additions of medium, yet the culture continue to dechlorinate at a high rate, and ethane became increasingly the terminal reduction product. Similar results were obtained for one of the other triplicates, while activity stalled in the third bottle after the first addition of medium.

3.3.2 SRS Enrichment Cultures, Set I

Only one enrichment culture was developed from the Set I SRS microcosms, using one of the MM-cDCE+VC-S bottles as inoculum. The average pH was approximately 5.5 (Figure 3.24). MES-buffered medium was used in place of the phosphate-buffered medium. In spite of the robust activity in the parent microcosm prior to addition of more medium (Figure 3.28), there was no activity in the enrichment bottles; a representative result is shown in Figure 3.30. It was unclear if this was related to the change in medium or further dilution of the SRS soil.

Strictly speaking, the serum bottles labeled MM-PCE-S on Figure 2.3 are not enrichments, since they received 100% of the contents from their parent bottles (MM-cDCE+VC-B). Nevertheless, the results are presented in this section since they represent a departure from the original SRS Set I microcosms. For this treatment, PCE was added in place of cDCE and VC. As shown in Figure 3.31, microbes in the soil from the Twin Lakes wetland at SRS do have the capacity to reduce PCE and cDCE. When monitoring of these bottles was stopped, VC had started to accumulate, which is consistent with the behavior of the other microcosms (Figures 3.26-3.29), all of which achieved reduction of cDCE to ethene and/or ethane. Thus, although not demonstrated in a single treatment, it

appears likely that the SRS soil has a consortium capable of completely dechlorinating PCE.

3.3.3 SRS Enrichment Cultures, Set II

The Set II enrichment cultures were prepared with inoculum from microcosms started by Hickey (15) (Figure 2.3). The average pH in both treatments was at 5.5 (Figure 3.24). One treatment (GW-VC-4B) was prepared in 0.7 L bottle and the other (GW-cDCE+VC-B4) was prepared in 2.6 L bottle and both were diluted with groundwater. Results for bottle GW-VC-4B are shown in Figure 3.32. The VC was consumed, but at a comparatively slow rate, so no further attempts to enrich were made. Results for bottle GW-cDCE+VC-B4 are shown in Figure 3.33. Reduction of cDCE and VC was also sluggish, and further attempts to dilute the soil via gradual dilution with phosphate-buffered MSM were not pursued.

3.4 Combined Cultures

3.4.1 Combined Cultures, Set I

As mentioned previously, the intent of combining microcosms and/or enrichment cultures was to develop a consortium with the best properties of the parent bottles, to achieve a high rate of PCE reduction to ethene at a pH of 5.5. For Set I, this entailed a 50/50 mixture of one of the SRS microcosm treatments (GW-cDCE+VC-B) and several of the NC/HH microcosms and enrichment cultures (Figure 2.4 and Table 2.7). Results for average pH levels in the Set I combined cultures are summarized in Figure 3.34. The average for each treatment was approximately 5.5. Figure 3.35 shows the average

distribution of dechlorination products; ethene and ethane were predominant in all of the bottles, which was an encouraging development.

Representative results for the three treatments prepared in serum bottles are shown in Figures 3.36-3.38. PCE was rapidly consumed and converted to ethene and ethane, with only transient increases in cDCE and VC. A single 2.6 L bottle was prepared as treatment #4 and its performance is shown in Figure 3.39. Although it has not been incubated as long, the initial results are encouraging, with nearly complete removal of the first addition of PCE in 22 days, and corresponding increases in ethene and ethane. As with many of these cultures, it is essential to prevent the pH from rising above the target of 5.5. Overall, the results show promise for creation of a combined culture.

3.4.2 Combined Cultures, Set II

Set II was created with a 50/50 mixture of one of the SRS enrichment cultures (GW-VC-4B) and several of the NC/HH microcosms and enrichment cultures (Figure 2.5 and Table 2.8). Results for average pH levels in the Set II combined cultures are summarized in Figure 3.40. The average for each treatment was approximately 5.5. Figure 3.41 shows the average distribution of dechlorination products; ethene and ethane were predominant in two of the treatments, while cDCE and VC persisted in the other two.

Representative results for the four treatments (all prepared in serum bottles) are shown in Figures 3.42-3.45. Treatments 1 and 2 both had relatively high rates of PCE and VC consumption; VC was carried over with the SRS enrichment culture. Ethane

accumulation was especially notable with treatment 1 (Figure 3.42). In contrast, VC accumulated in treatments 3 and 4.

3.4.3 Combined Cultures, Set III

Set III was created with a 50/50 mixture of one of the SRS microcosms that was subjected to dilution with phosphate-buffered medium (MM-cDCE+VC-B) and several of the NC/HH microcosms and enrichment cultures (Figure 2.6 and Table 2.9). Results for average pH levels in the Set III combined cultures are summarized in Figure 3.46. The average for each treatment was approximately 5.5. Figure 3.47 shows the average distribution of dechlorination products; ethene and ethane were the predominant products in all of the bottles.

Representative results for the five treatments prepared in serum bottles are shown in Figures 3.48-3.52. Each of them behaved similarly; PCE was rapidly consumed and converted to ethene and ethane, with only transient increases in cDCE and VC. A single 2.6 L bottle was prepared as treatment #6 and its performance is shown in Figure 3.53. Although it has not been incubated as long, the initial results are encouraging, with nearly complete removal of the first addition of PCE in 14 days, and corresponding increases in ethene and ethane. Overall, the results for the Set III combined cultures show promise for further enrichment.

CHAPTER FOUR

4.0 DISCUSSION

The results of this research demonstrate that an enrichment culture can be developed that reductively dechlorinates PCE to ethene and ethane at a pH of 5.5. A total of 46 treatments were evaluated. The most promising enrichment culture obtained contains a mixture of microbes from two locations, NC/HH and the Twin Lakes wetland at SRS. A comparison of ethene + ethane production rates is presented below, along with an evaluation of the phosphate-buffered and MES-buffered media, and the various types of electron donors that were used.

To compare the effectiveness of the various enrichment cultures that were developed, the rate of ethene + ethane formation was calculated by dividing the net amount of both products present at the end of the incubation period (μmol/bottle) by the volume of the culture (L/bottle) and the length of the incubation period (d). This approach was taken since conversion of the chlorinated ethenes to nonchlorinated products is the rate limiting step in the overall dechlorination process. It was necessary to include ethane since it was a predominant product in several of the enrichment cultures. Figure 4.1 presents the rates. The highest rate was observed (1.8 μM/d) in one of the 2.6 L combined cultures from Set III (MM-cDCE+VC-B1+NC-UN-B3). It is a combination of one of the enrichments developed in phosphate-buffered medium from SRS in a 2.6 L bottle (MM-cDCE+VC-B1; Table 2.9, Figure 3.29) and one of the unamended 2.6 L NC/HH microcosms (NC-UN-B3; Table 2.9, Figure 3.5 shows a replicate bottle). As shown in Figure 3.53, PCE was consumed without a lag, as was a residual level of cDCE

that came with the inoculum. Most of the PCE went directly to ethene and ethane, with only a minor rise in VC before it too was reduced. The rate of ethene + ethane formation was approximately twice as high as the next three highest cultures (GW-cDCE+VC-B2+NC-UN-B2; GW-cDCE+VC-B3+NC-UN2-GW-1; and MM-cDCE+VC-B3+NC-lactate-S1).

This accomplishment needs to be viewed in the context of what is achievable at a neutral pH. For example, the MicroCED culture was started from microcosms consisting of soil and groundwater from the Twin Lakes area at SRS (the same as the SRS microcosms developed for this research), although in the MicroCED case the enrichment process was performed in a phosphate-buffered mineral medium in the circumneutral range. For approximately five years, the culture has received repeated additions of ~40 mg/L TCE and 15 mg/L PCE. The rate of ethene formation for the MicroCED culture (ethane is insignificant) is more than ten times higher (~30 μM/d). Nevertheless, it remains to be seen if the newly developed low pH enrichment culture can approach this rate with further enrichment and exposure to higher concentrations of PCE and TCE.

The fact that PCE was completely dechlorinated by many of the low pH enrichment cultures developed during this study indirectly implies that *Dehalococcoides* were present that can tolerate a pH of ~5.5. However, this finding appears to be in conflict with the effect of pH on *Dehalococcoides* (Table 1.1), since none have been reported that are active at a pH below 6.0. One explanation may be that different strains of *Dehalococcoides* exist in nature with different tolerances to pH, and strains that grow in the pH range of 6.5-7.5 are the only ones that have been isolated so far. This is likely a

consequence of researchers defaulting to the use of pH 7 media, but also the likelihood that the kinetics of complete dechlorination are better at neutral pH, making isolation of an already challenging microbe that much easier. In support of this hypothesis, Bratt et al. (2) used restriction digest analysis of microcosms from the Twin Lakes area to evaluate the types of *Dehalococcoides* that are present. Although strong genotypic similarity between Bachman *Dehalococcoides* 16S rRNA gene sequence was found, variant genotypes were also recovered, suggesting the presence of novel *Dehalococcoides*.

One of the challenges with developing enrichment cultures during this research was keeping the pH close to 5.5. Initially, the only MSM used was phosphate buffered. Although effective, it was necessary to frequently monitor the pH, since there was a tendency for it to rise over time. It was also necessary not to overcompensate when adding an acid (either lactic or phosphoric) to reduce the pH. Since MES has a lower effective pH range (5.5-6.7) when compared to the pK_2 for phosphate (5.8-8.0) (http://www.sigmaaldrich.com/life-science/metabolomics/bioultra-reagents/biological-buffers.html), its use as a buffer was evaluated. Eighteen serum bottles containing enrichment cultures were compared, differing only in the type of buffer. Table 4.1 shows that the magnitude of change in pH during one cycle of PCE addition and consumption did not differ between the two buffering systems. Over the full period of incubation, the average pH in the nine bottles with MSM (5.47±0.03) was not different from the ones with MES (5.47±0.03) (Students *t*-test, α=0.05). Furthermore, there were no appreciable differences in the performance of these bottles with respect to the rate and extent of PCE dechlorination. It was, therefore, concluded that MES offers no advantage over the

phosphate-buffered MSM. This is fortuitous, since phosphate is less costly and would pose no regulatory concerns if it was added along with the culture during in situ bioaugmentation.

During the course of this research, three electron donors were used: lactate, hydrogen, and emulsified vegetable oil. In general, lactate proved to be effective and was also preferred from the perspective that it could be used to adjust pH either up (when added as lactate) or down (when added as lactic acid). Also, in situ injection of hydrogen is currently impractical, and direct hydrogen additions tended to promote methanogenesis. Excessive growth of methanogens can lead to what has been called a "spiral to failure," e.g., use of the hydrogen for methanogenesis leads to growth of more methanogens, leading to more consumption of hydrogen for a purpose other than dechlorination. EOS was evaluated with the NC/HH microcosms and was less effective than lactate or hydrogen, so its use was discontinued. Lactate was also the electron donor used for the most effective combined enrichment culture, MM-cDCE+VC-B1+NC-UN-B3 (Figure 4.1). It is noteworthy that the unamended NC/HH microcosms in serum bottles exhibited complete dechlorination of PCE to ethene at a pH that was approximately 5.5 (Figure 3.4). This indicated that the groundwater has an ample supply of electron donor, although its origin is not entirely known. The NC/HH enrichment cultures developed with phosphate-buffered medium (MSM) performed similarly well with lactate (Figure 3.11) or hydrogen (Figure 3.12).

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Based on the results of this research, the following conclusions were reached:

1. Several enrichment cultures were developed with the capacity to dechlorinate PCE to ethene at a pH of 5.5. The most effective enrichment culture was created by combining enrichments from the NC/HH site and enrichments from the SRS site. Further enrichment will be needed before this culture can be considered "soil free" and scaled up for use as a bioaugmentation culture.
2. With careful monitoring, a phosphate buffered medium is effective for maintaining the enrichment culture at a pH of 5.5. Adjustments to the pH can be made using lactate, lactic acid, and phosphoric acid. MES did not provide any better control of the pH; it is also more costly than phosphate, and may invoke some regulatory concern if the culture is ever to be used for bioaugmentation.
3. Lactate and hydrogen are effective electron donors for the low pH enrichment cultures, although lactate is more acceptable for practical application. Use of EOS was discontinued after it failed to show any advantages in the NC/HH microcosms.

5.2 Recommendations

Additional research is recommended on the following topics:

1. Further enrichment of the most promising combined enrichment cultures is necessary to guarantee that the cultures can be maintained in the laboratory in a

sediment-free medium. Acclimation of the enrichment cultures to higher concentrations of PCE and TCE may also result in transformation rates that are closer to what has been observed at a neutral pH.

2. Once the culture is further enriched, it should be evaluated for its bioaugmentation potential, first in microcosms and then in the field. A successful deployment in the field would improve the prospects for bioremediation at sites with low pH that cannot readily be adjusted to the pH range required for most commercially available bioaugmentation cultures.
3. The *Dehalococcoides* present in the low pH enrichment culture should be identified to determine if they are distinct in comparison to the *Dehalococcoides* that have been isolated from neutral pH environments. Then it will be possible to more rigorously test the effect of pH on the kinetics of dechlorination.

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 dehalogenans JW/IU-DC1	PCE --> cDCE	6.0-9.0	7.5[b]	(4)
Desulfitobacterium sp. PCE-1	PCE --> cDCE	6.0-9.0	6.5-8.0[c]	(9)
Desulfitobacterium sp. PCE-S	PCE --> cDCE	4.4-9.0	7.2[d]	(21)
Desulfitobacterium sp. strain Y51	PCE --> cDCE	4.0 to 10	6.0-9.5	(40)
Dehalobacter restrictus PER-K23	PCE --> cDCE	6.5-8.0	6.5-8.0[e]	(17)
Dehalobacter restrictus TEA	PCE --> cDCE	None Given	-	(42)
Sulfurospirillum multivorans (formerly Dehalospirillum)	PCE --> cDCE	6.0-8.0	7.0-7.5[b]	(4)
Desulfuromonas chloroethenica TT4B	PCE --> cDCE	6.5-7.4	7.4[b]	(4)
Desulfuromonas michiganensis	PCE --> cDCE	6.8-8.0	7.0-7.5[b]	(39)
Strain MS-1	PCE --> cDCE	7.0	Not Given	(35)
Geobacter lovleyi SZ	PCE --> cDCE	5.5-8.0	6.5-7.2[b]	(37)
Dehalococcoides ethenogenes strain 195	PCE --> VC	7.0	Not Given	(25)
Dehalococcoides strain BAV-1	cDCE --> ethene	7.2	Not Given	(12)
Dehalococcoides strain FL2	TCE --> VC	7.2	Not Given	(13)
Dehalococcoides strain GT	TCE --> ethene	7.2-7.3	Not Given	(38)
Dehalococcoides strain VS	TCE --> ethene	7.2	Not Given	(28)

[a] Adapted from Hickey (15).
[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.

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]	(31)
KB-1	PCE --> ethene	7.0	Not Given	(23)
KB-1	No activity	6.5-6.9	Not Given	(14)
KB-1	PCE --> ethene	None Given	-	(33)
SDC-9	PCE --> ethene	4.9-5.8	Not Given[c]	(32)
SDC-9	PCE --> ethene	5.0-9.5	(6.1-7.4)[d]	(41)
BioDechlor	PCE --> ethene	None Given	-	(29)
BioDechlor	PCE --> ethene	7.1-7.3	7.1-7.3	(1)
Pinellas	TCE --> cDCE	6.0-6.5	Not Given	(6)
BCI	PCE --> ethene	Not Given	≥5.6	(http://www.bcilabs.com/news.html)

[a] Adapted from Hickey (15).
[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.

Table 2.1 Components in MSM and MES media.

Compound	Concentration (mg/L)	
	MSM	MES
K_2HPO_4	525	591
NH_4Cl	535	535
$CaCl_2 \cdot 2H_2O$	47	47
$FeCl_2 \cdot H_2O$	163	163
H_3BO_3	0.6	0.6
$ZnSO_4 \cdot 7H_2O$	0.42	0.42
$NiCl_2 \cdot 6H_2O$	1.5	1.5
$MnCl_2 \cdot 4H_2O$	2.0	2.0
$CuCl_2 \cdot 2H_2O$	0.20	0.20
$CoCl_2 \cdot 6H_2O$	3.0	3.0
Na_2SeO_3	0.04	0.04
$Al_2(SO_4)_3 \cdot 16H_2O$	0.20	0.20
HCl	8.8	8.8
$MgSO_4 \cdot 7H_2O$	125	125
$Na_2S \cdot 9H_2O$	240	240
Yeast Extract	50	50
Resazarin	1	1
MES	0	2990

Table 2.2 Experimental design for the NC/HH microcosms.

Treatment	Description	Soil (g)	GW (mL)	Electron donor	PCE Added
#1	NC-AC-S	20	50	N/A[a]	saturated water
#2	NC-AC-B	288	878	N/A	neat
#3	NC-UN-S	20	50	N/A	saturated water
#4	NC-UN-B	288	878	N/A	neat
#5	NC-lactate-S	20	50	Lactate/H_2	saturated water
#6	NC-lactate-B	288	878	Lactate/H_2	neat
#7	NC-EOS-S	20	50	EOS/H_2	saturated water

[a] Not applicable.

Table 2.3 Experimental design for the NC/HH enrichment cultures.

Treatment #	Description	Medium	Number of Bottles
1	NC-UN1-MSM-H_2	MSM	1
2	NC-UN1-MSM-lactate	MSM	1
3	NC-UN1-MES-H_2	MES	1
4	NC-UN1-MES-lactate	MES	1
5	NC-UN2-MSM-H_2	MSM	1
6	NC-UN2-MSM-lactate	MSM	1
7	NC-UN2-MES-H_2	MES	1
8	NC-UN2-MES-lactate	MES	1
9	NC-UN1-GW	GW	1
10	NC-UN2-GW	GW	1
11	NC-lactate-MSM-S	MSM	3
12	NC-lactate-MES-S	MES	3

Table 2.4 Experimental design for the NC/FRX enrichment cultures.

Treatment	Description	Inoculum source	Media	PCE Added	Number of bottles
#1	RS6.03-B	110 mL RS6.0-3	1544 mL MSM	neat	1
#2	RS6.03-B-MSM-H_2	10 mL RS6.0-3B	90 mL MSM	saturated water	1
#3	RS6.03-B-MSM-lactate	10 mL RS6.0-3B	90 mL MSM	saturated water	1
#4	RS6.03-B-MES-H_2	10 mL RS6.0-3B	90 mL MES	saturated water	1
#5	RS6.03-B-MES-lactate	10 mL RS6.0-3B	90 mL MES	saturated water	1

Table 2.5 Experimental design for the SRS microcosms and enrichment cultures, Set I.

Treatment	Description	Inoculum Sources	Electron Acceptor Added
#1	GW-cDCE+VC-S	20 g soil+50 mL GW	cDCE saturated water + VC gas
#2	GW-cDCE+VC-B	20 g soil+100 mL GW	cDCE saturated water + VC gas
#3	MM-cDCE+VC-S	20 g soil+50 mL MSM	cDCE saturated water + VC gas
#4	MM-cDCE+VC-B	20 g soil+100 mL MSM	cDCE saturated water + VC gas
#5	MES-cDCE+VC-S2-B	100 mL MM-cDCE+VC-S2	cDCE saturated water + VC gas
#6	MM-PCE-S	100 mL MM-cDCE+VC-1	PCE saturated water

Table 2.6 Experimental design for the SRS enrichment cultures, Set II.

Treatment	Description	Inoculum sources	Electron Acceptor Added
#1	GW-VC-4B	100 mL GW-VC-4	cDCE saturated water + VC gas
#2	GW-cDCE+VC-B4	60 mL GW-cDCE-3 + 60 mL GW-cDCE-4+ 60 mL MM-cDCE-2 + 60 mL MM-cDCE-4	cDCE saturated water + VC gas

Table 2.7 Experimental design for the combined cultures, Set I.

Treatment	Description	Inoculum source	Number of bottles
#1	GW-cDCE+VC-B3+NC-UN2-GW	50 mL GW-cDCE+VC-B3 + 50 mL NC-UN2-GW	2
#2	GW-cDCE+VC-B3+NC-UN2-MSM-lactate	50 mL GW-cDCE+VC-B3 + 50 mL NC-UN2-MSM-lactate	2
#3	GW-cDCE+VC-B3+NC-lactate-MSM-S1	50 mL GW-cDCE+VC-B3 + 50 mL NC-lactate-MSM-S1	2
#4	GW-cDCE+VC-B2+NC-UN-B2	1360 mL GW-cDCE+VC-B2 + 100 mL NC-UN-B2	1

Table 2.8 Experimental design for the combined cultures, Set II.

Treatment	Description	Inoculum Source	Number of Bottles
#1	GW-VC-4B+NC-lactate-S3	50 mL GW-VC-4B + 50 mL NC-lactate-S3	1
#2	GW-VC-4B+NC-UN1-GW	50 mL GW-VC-4B + 50 mL NC-UN1-GW	2
#3	GW-VC-4B+NC-lactate-MES-S2	50 mL GW-VC-4B + 50 mL NC-lactate-MES-S2	2
#4	GW-VC-4B+NC-lactate-MES-S3	50 mL GW-VC-4B + 50 mL NC-lactate-MES-S3	2

Table 2.9 Experimental design for the combined cultures, Set III.

Treatment	Description	Inoculum source	Number of bottles
#1	MM-cDCE+VC-B3+NC-lactate-S1	50 mL MM-cDCE+VC-B3 + 50 mL NC-lactate-S1	1
#2	MM-cDCE+VC-B3+NC-EOS-S1	50 mL MM-cDCE+VC-B3 + 50 mL NC-EOS-S1	1
#3	MM-cDCE+VC-B3+NC-UN1-MSM-lactate	50 mL MM-cDCE+VC-B3 + 50 mL NC-UN1-MSM-lactate	2
#4	MM-cDCE+VC-B3+NC-lactate-MSM-S2	50 mL MM-cDCE+VC-B3 + 50 mL NC-lactate-MSM-S2	2
#5	MM-cDCE+VC-B3+NC-lactate-MSM-S3	50 mL MM-cDCE+VC-B3 + 50 mL NC-lactate-MSM-S3	2
#6	MM-cDCE+VC-B1+NC-UN-B3	1360 mL MM-cDCE+VC-B1 + 100 mL NC-UN-B3	1

Table 4.1 Change in pH range during each cycle of PCE consumption in enrichment cultures with MES and phosphate-buffered media.

Enrichment Cultures in MES-Buffered Medium			Enrichment Cultures in Phosphate-Buffered Medium		
Bottle #	Figure #	pH Range per Cycle[a]	Bottle #	Figure #	pH Range per Cycle[a]
NC-UN1-MES-H_2	E.13	0.11	NC-UN1-MSM-H_2	3.11	0.07
NC-UN1-MES-lactate	E.14	0.09	NC-UN1-MSM-lactate	E.11	0.10
NC-UN2-MES-H_2	3.13	0.09	NC-UN2-MSM-H_2	3.12	0.09
NC-UN2-MES-lactate	E.15	0.09	NC-UN2-MSM-lactate	E.12	0.09
NC-lactate-MES-S1	E.19	0.11	NC-lactate-MSM-S1	E.17	0.10
NC-lactate-MES-S2	3.16	0.10	NC-lactate-MSM-S2	E.18	0.07
NC-lactate-MES-S3	E.20	0.12	NC-lactate-MSM-S3	3.15	0.11
RS6.0-3B-MES-H_2	3.22	0.08	RS6.0-3B-MSM-H_2	3.20	0.10
RS6.0-3B-MES-lactate	3.23	0.08	RS6.0-3B-MSM-lactate	3.21	0.09
Average		**0.10**	**Average**		**0.09**

[a]Range refers to the maximum minus the minimum for each cycle of PCE addition and consumption.

FIGURES

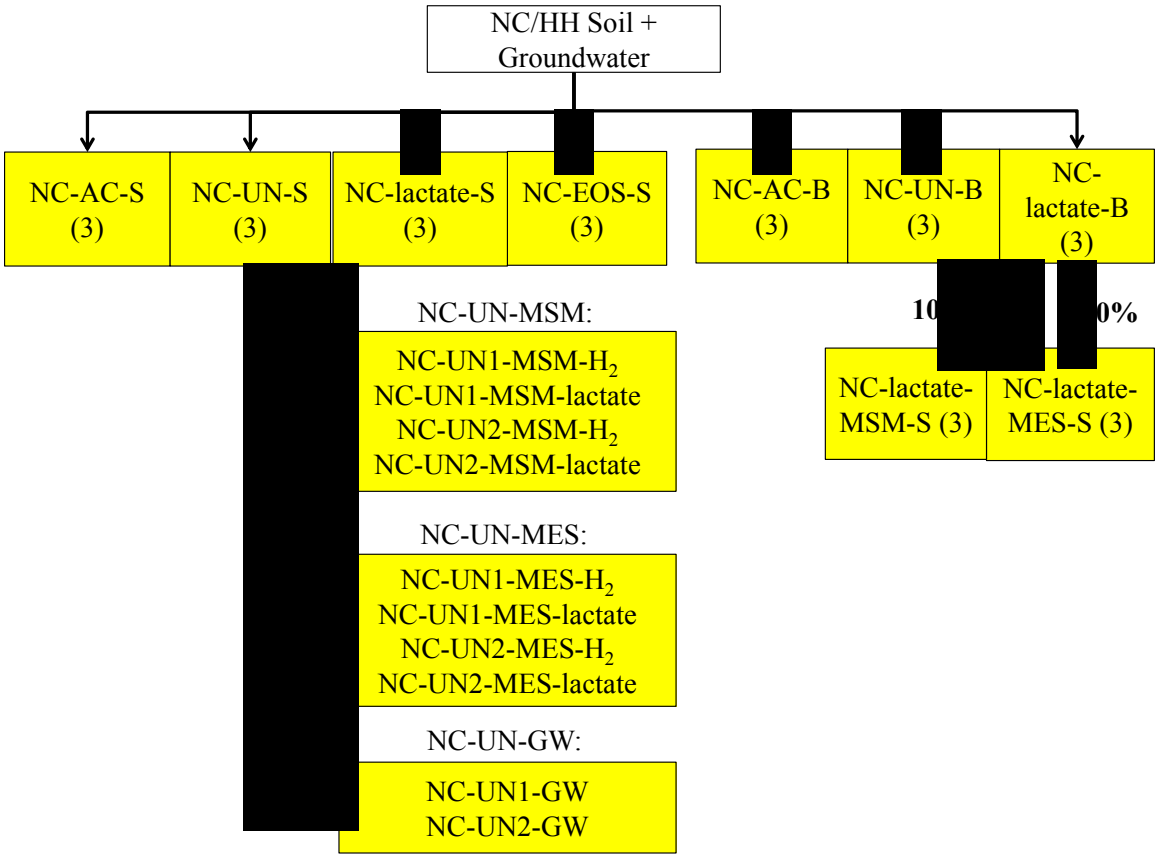

Figure 2.1 Sequence in the development of the NC/HH microcosms and enrichment cultures. Percentages next to an arrow indicate the inoculum volume. Values in parenthesis indicate the number of replicates.

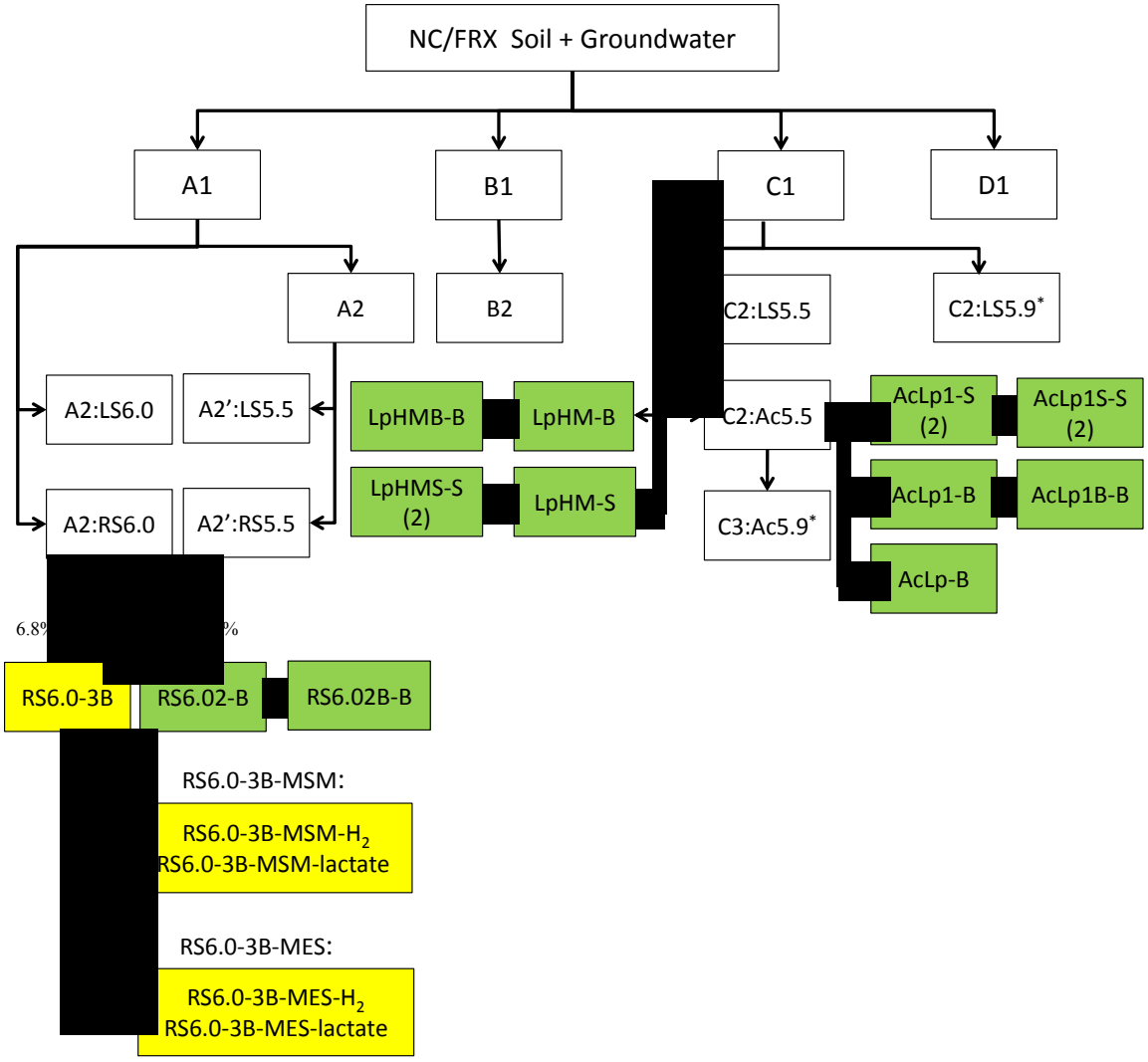

Figure 2.2 Sequence in the development of the NC/FRX enrichment cultures. White boxes represent microcosms and enrichment cultures originally developed by Hickey (15); green boxes were developed as part of this thesis but the results are not shown since the bottles did not exhibit significant dechlorination activity at low pH; yellow boxes were developed as part of this thesis and the results are presented in Chapter 3. Percentages next to an arrow indicate the inoculum volume.

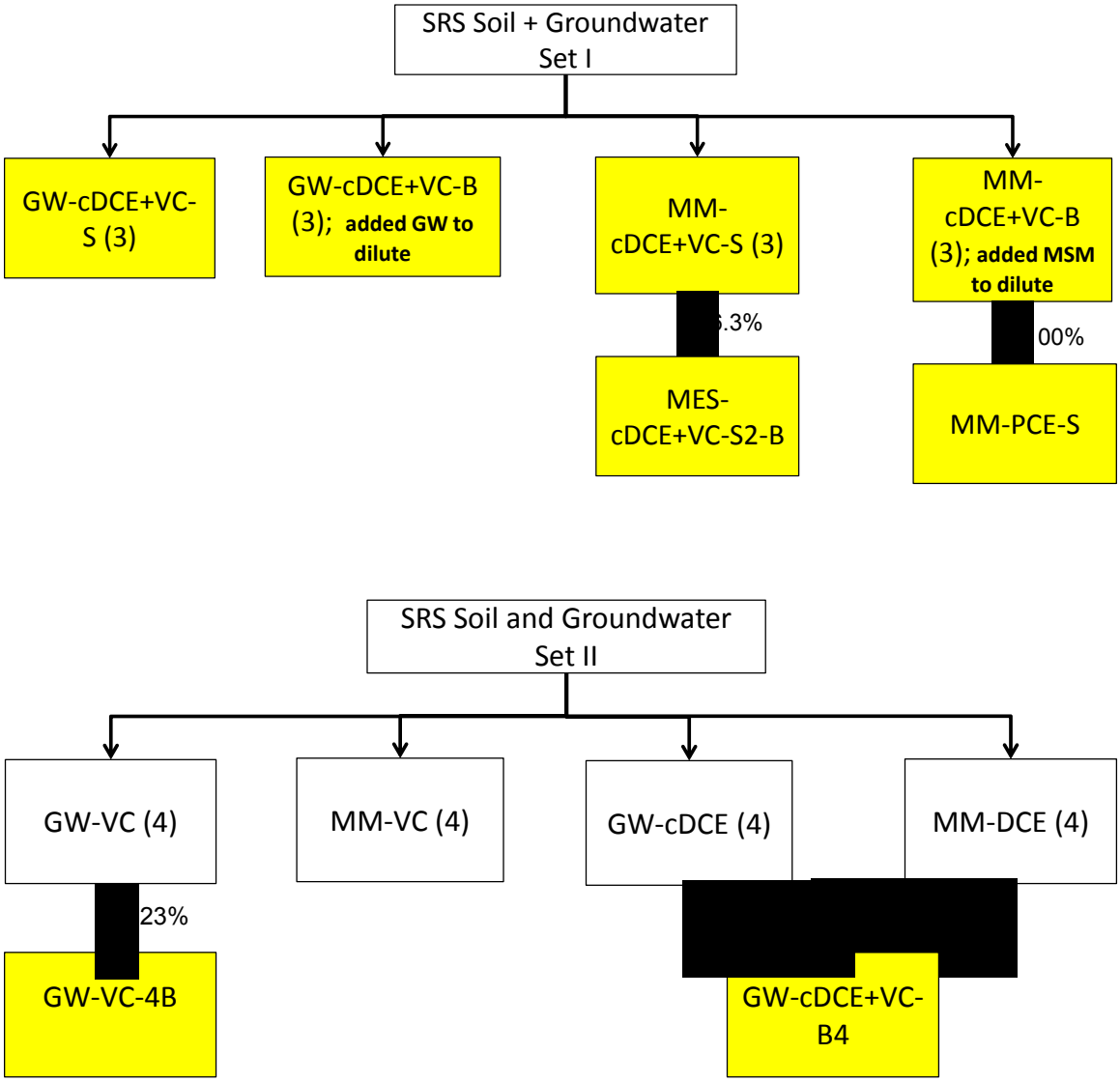

Figure 2.3 Sequence in the development of SRS microcosms and enrichment cultures. Set I was developed as part of this thesis and the results are described in Chapter 3; the microcosms for Set II (white boxes) were prepared by Hickey (15) and the results will not be repeated. The two enrichment cultures developed for Set II are part of this thesis and the results are described in Chapter 3. Percentages next to an arrow indicate the inoculum volume. GD*= gradual dilution, described in the text. Values in parenthesis indicate the number of replicates.

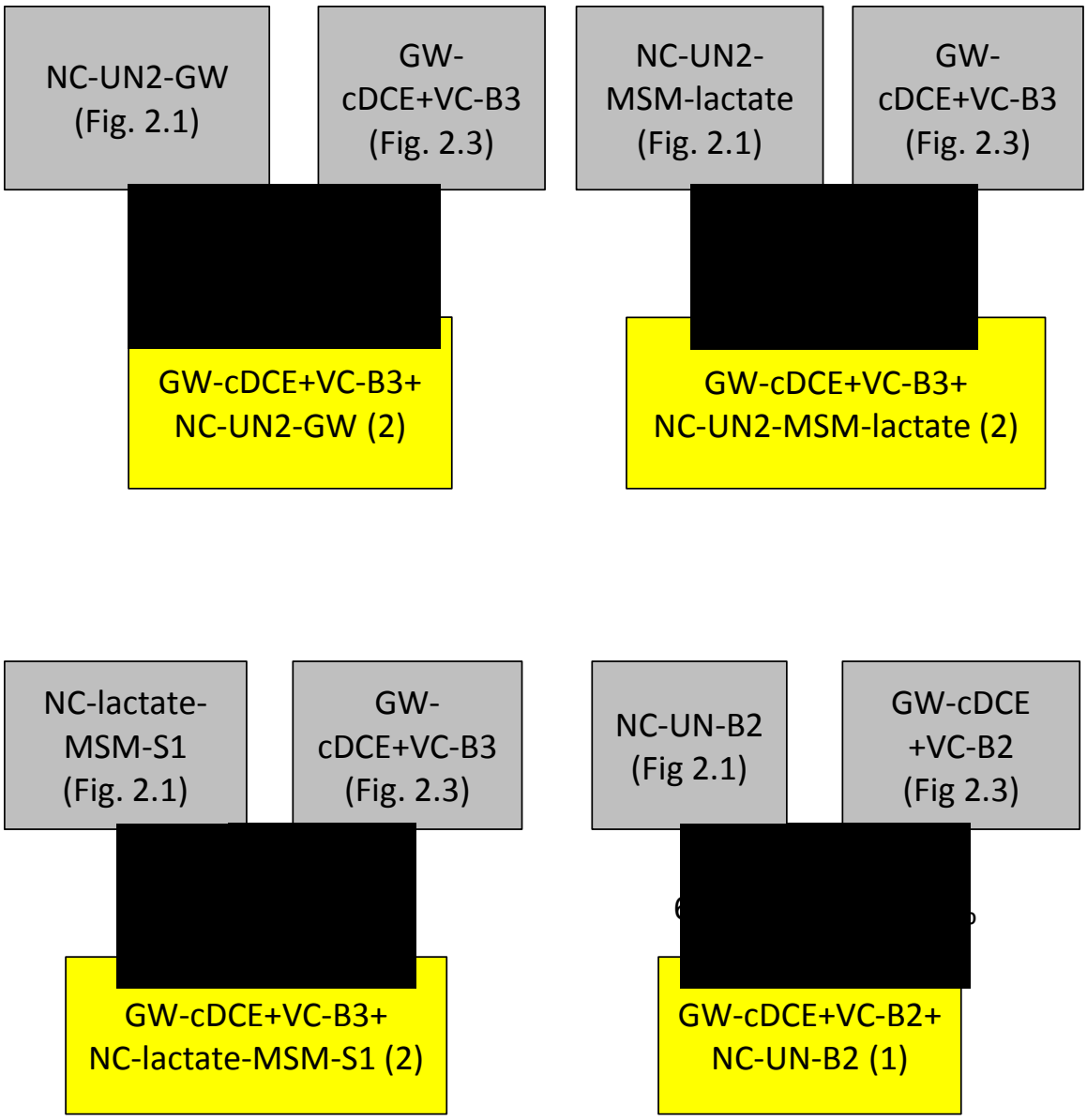

Figure 2.4 Sequence in the development of Set I enrichment cultures created by combining NC/HH microcosms and enrichment cultures (grey boxes on the left side of each pair) with SRS enrichment cultures (grey boxes on the right side of each pair). Percentages next to an arrow indicate the inoculum volume. Values in parenthesis indicate the number of replicates.

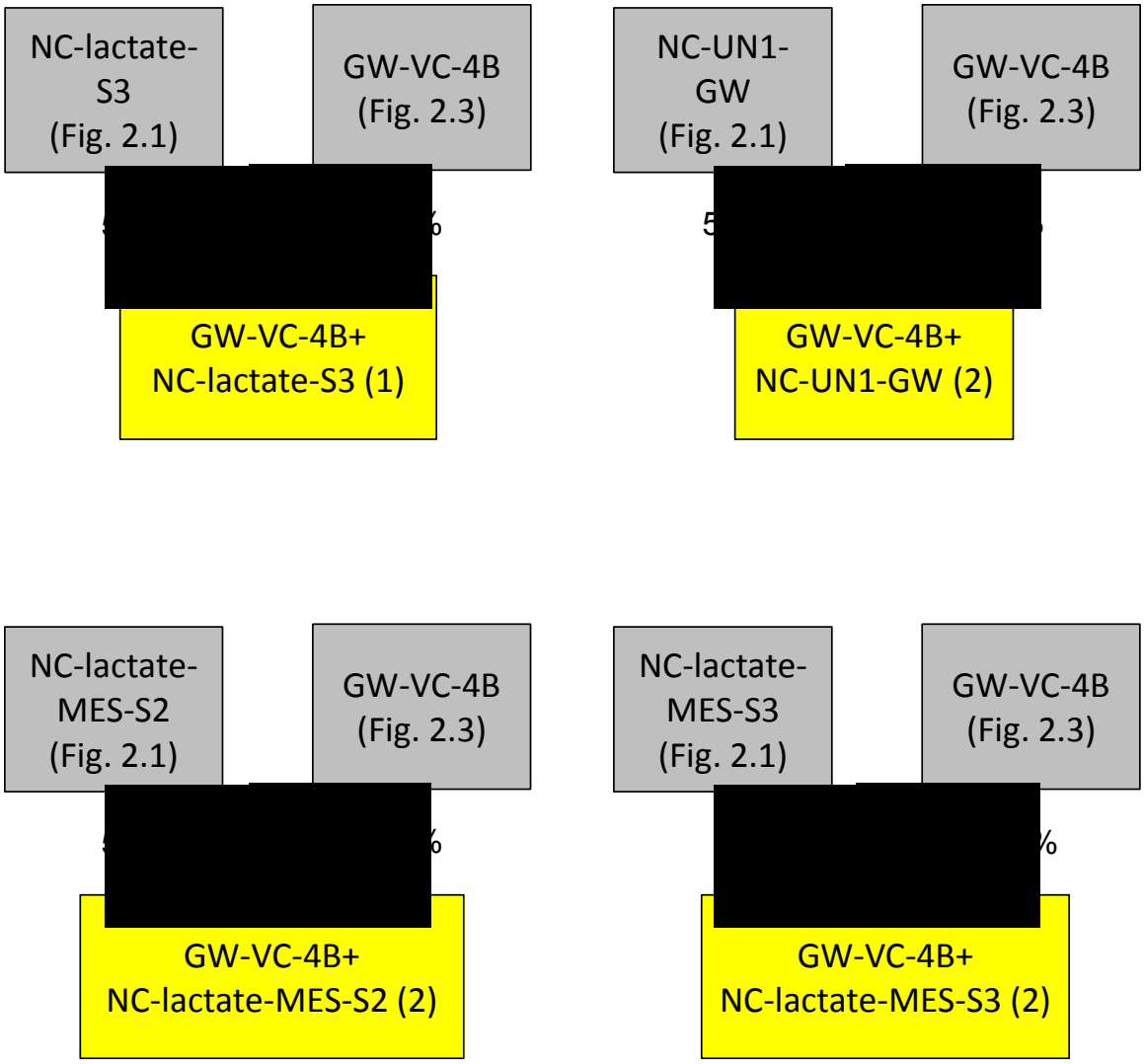

Figure 2.5 Sequence in the development of Set II enrichment cultures created by combining NC/HH microcosms and enrichment cultures (grey boxes on the left side of each pair) with SRS enrichment cultures (grey boxes on the right side of each pair). Percentages next to an arrow indicate the inoculum volume. Values in parenthesis indicate the number of replicates.

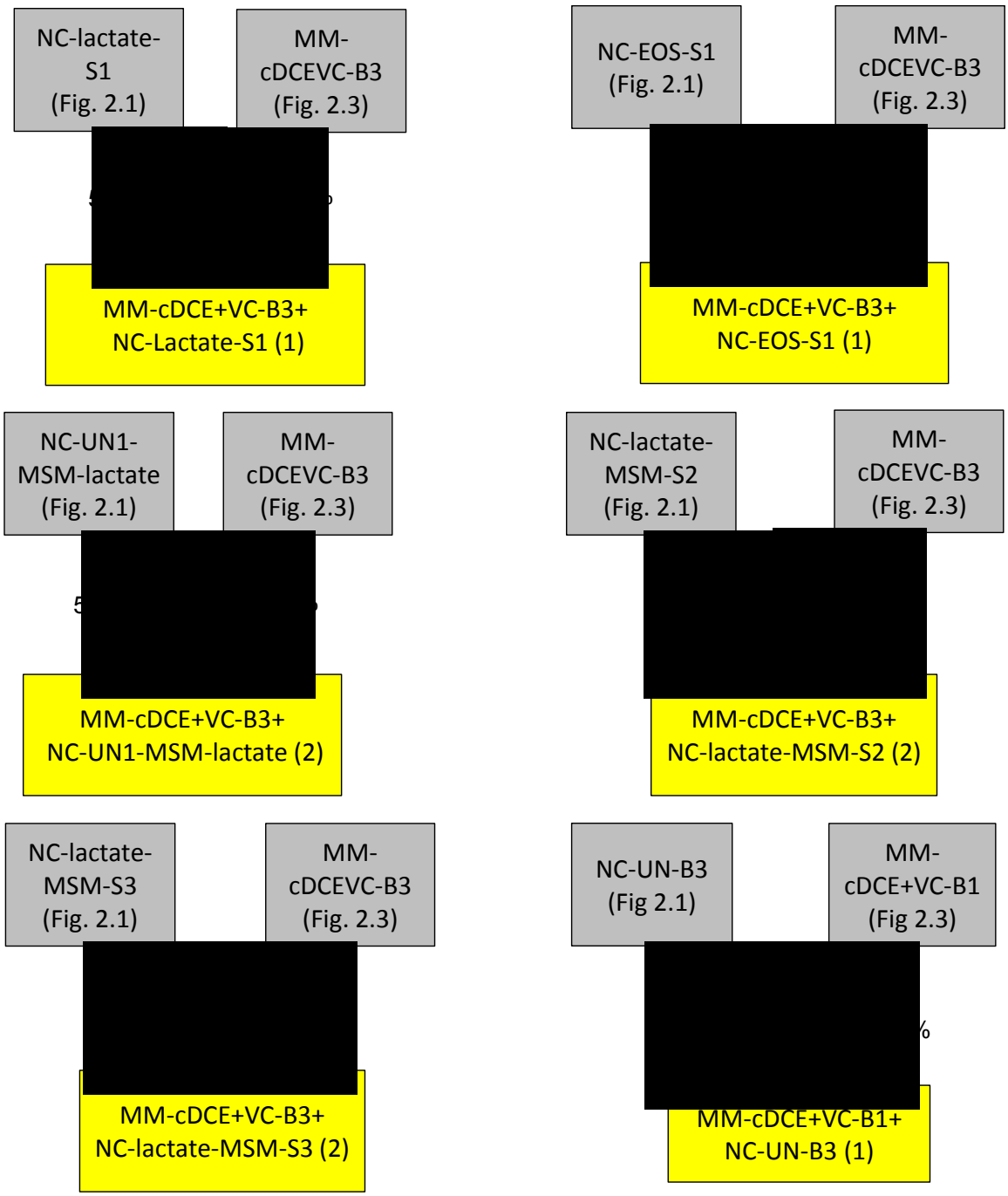

Figure 2.6 Sequence in the development of Set III enrichment cultures created by combining NC/HH microcosms and enrichment cultures (grey boxes on the left side of each pair) with SRS enrichment cultures (grey boxes on the right side of each pair). Percentages next to an arrow indicate the inoculum volume. Values in parenthesis indicate the number of replicates.

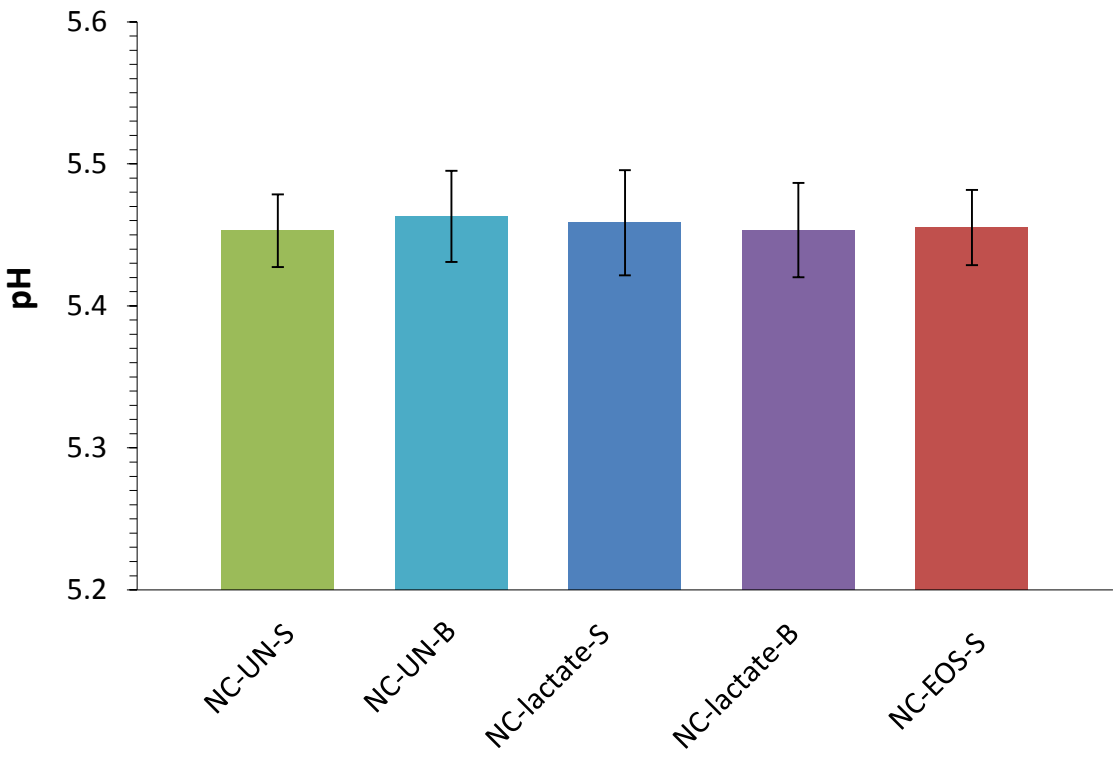

Figure 3.1 Average pH level for the NC/HH microcosms; error bars represent one standard deviation for triple bottles.

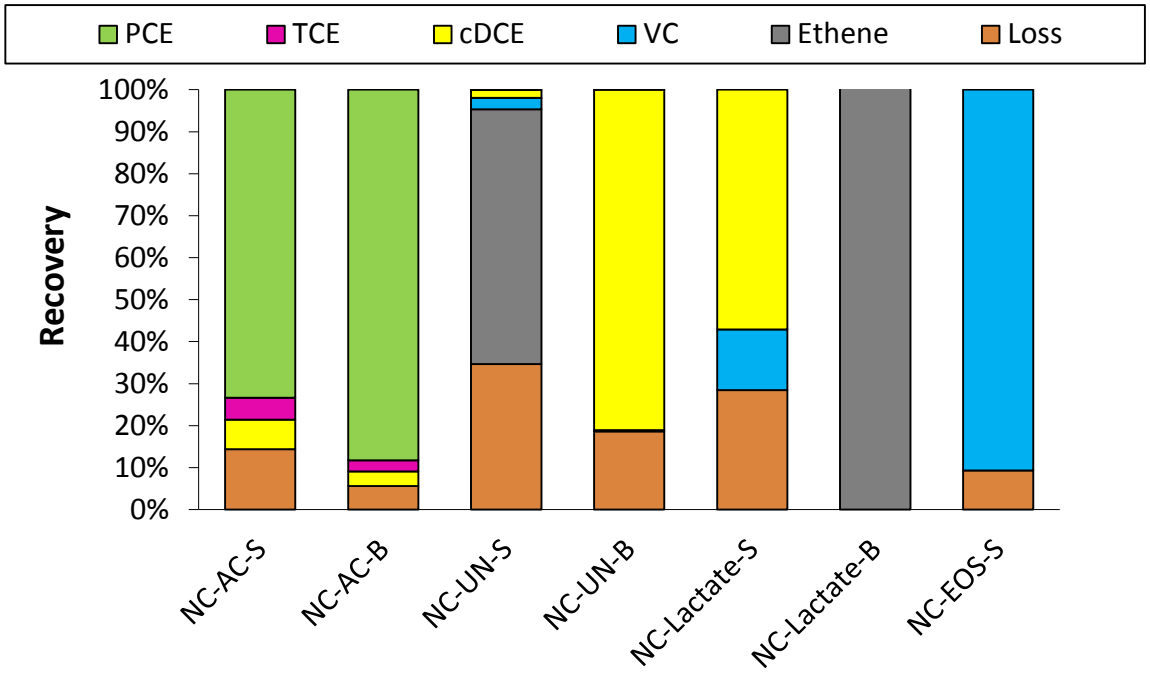

Figure 3.2 Average distribution of dechlorination products for the NC/HH microcosms, based on the total amount of PCE added and the amount of VOCs present at the final sampling point.

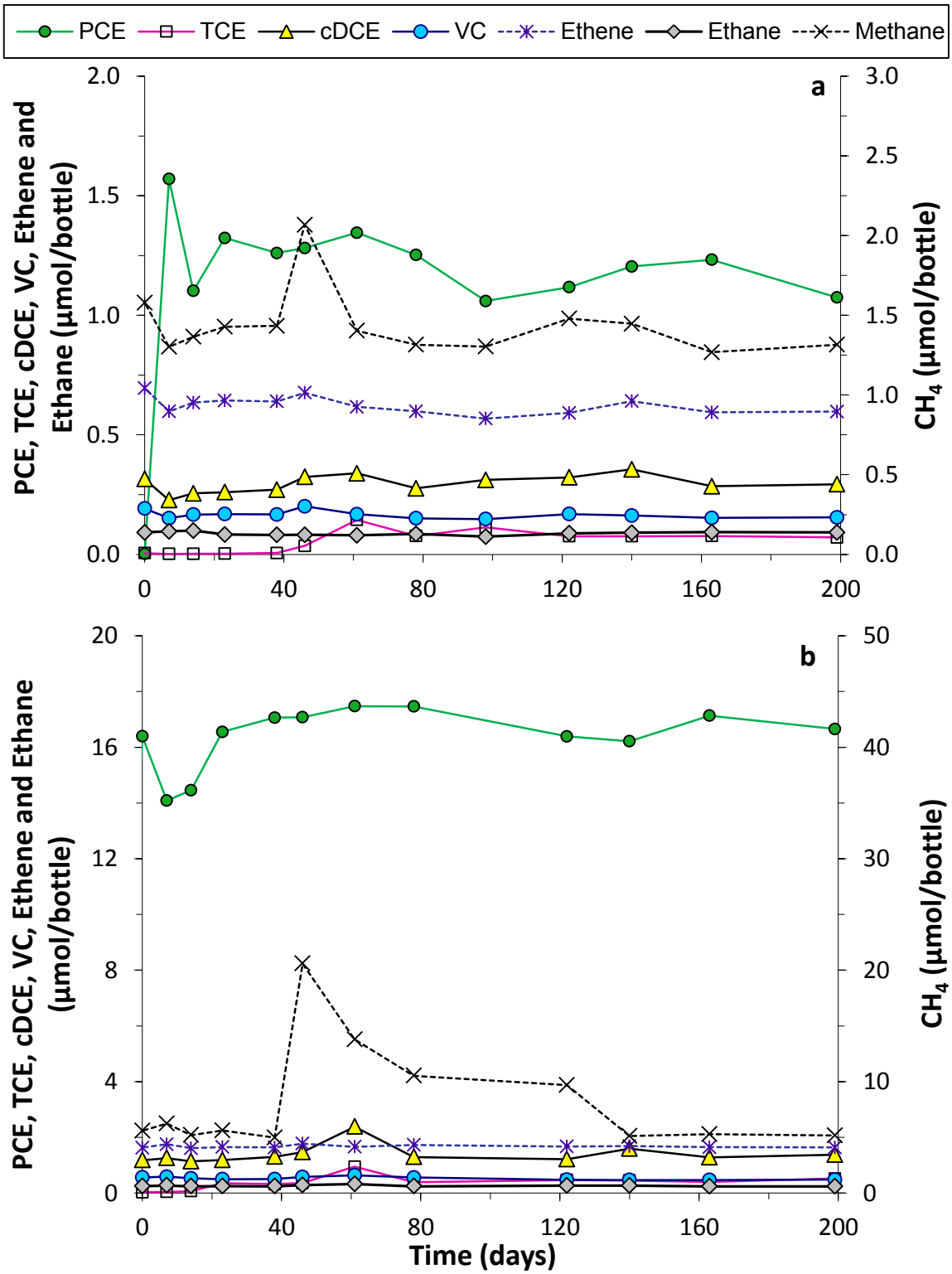

Figure 3.3 Average results for triplicate NC/HH autoclaved microcosms (NC-AC) for **a)** serum bottles and **b)** 2.6 L bottles.

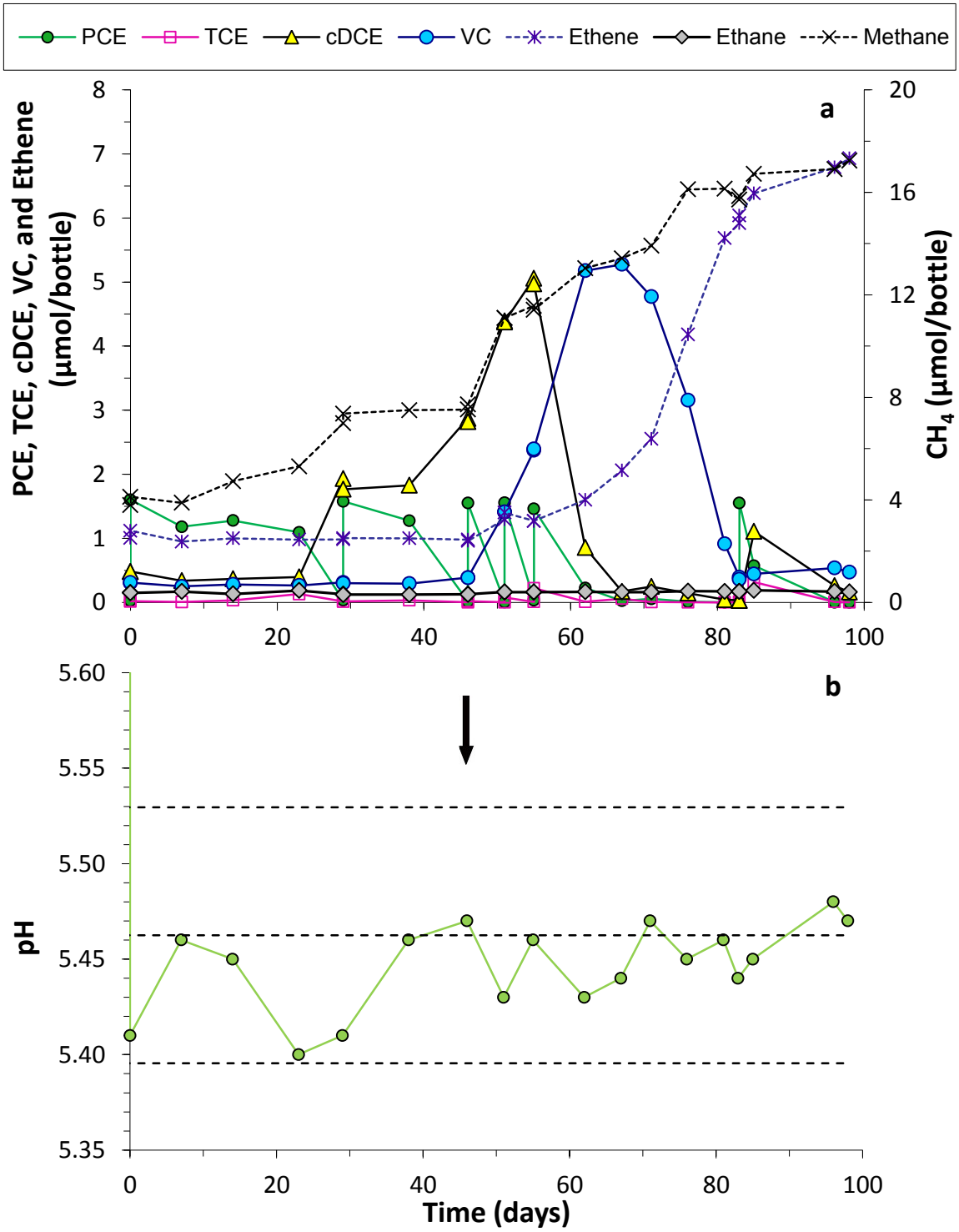

Figure 3.4 Results for an unamended NC/HH microcosm, serum bottle #1 (NC-UN-S1) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; the arrow indicates addition of phosphoric acid.

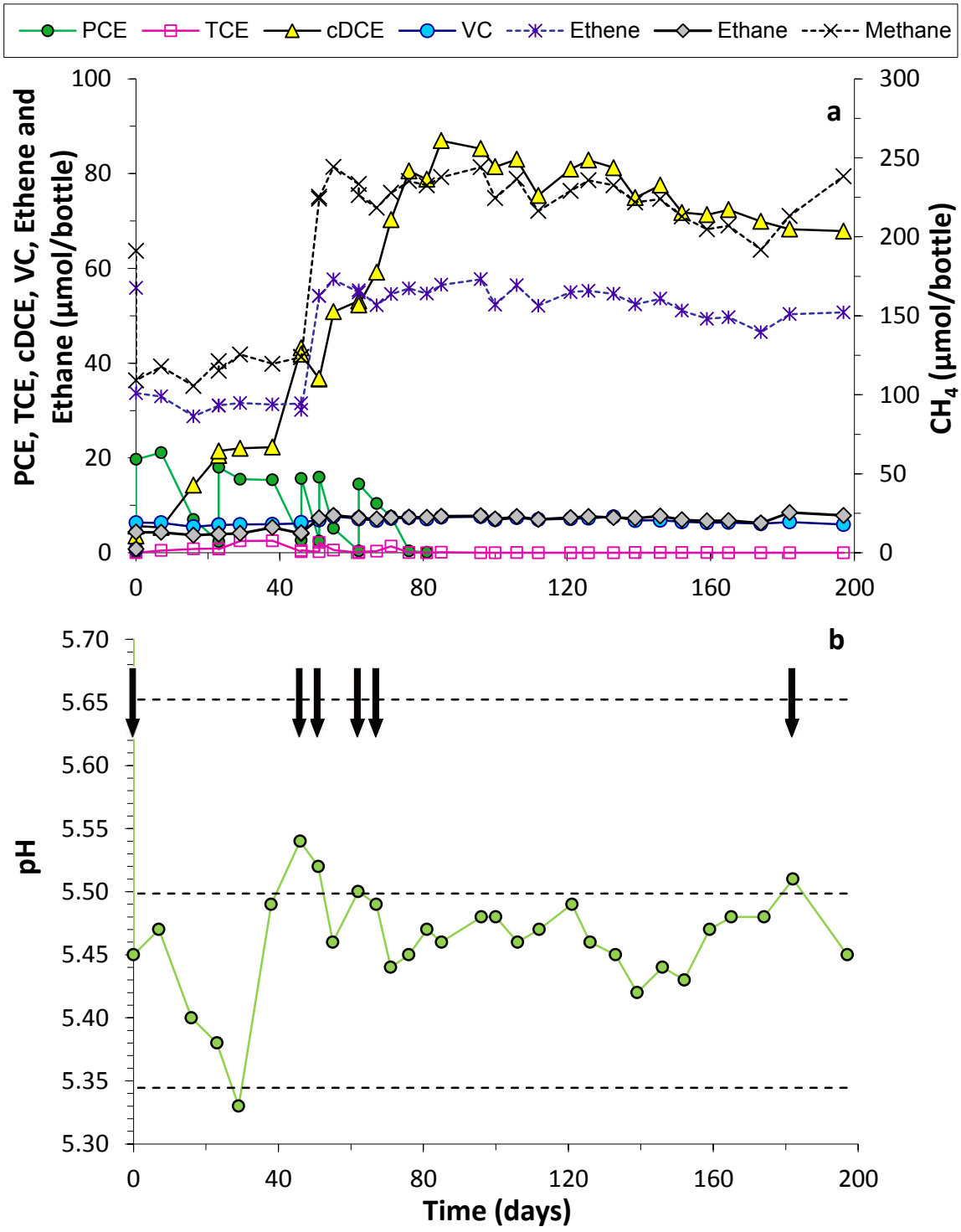

Figure 3.5 Results for an unamended NC/HH microcosm, 2.6 L bottle #2 (NC-UN-B2) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

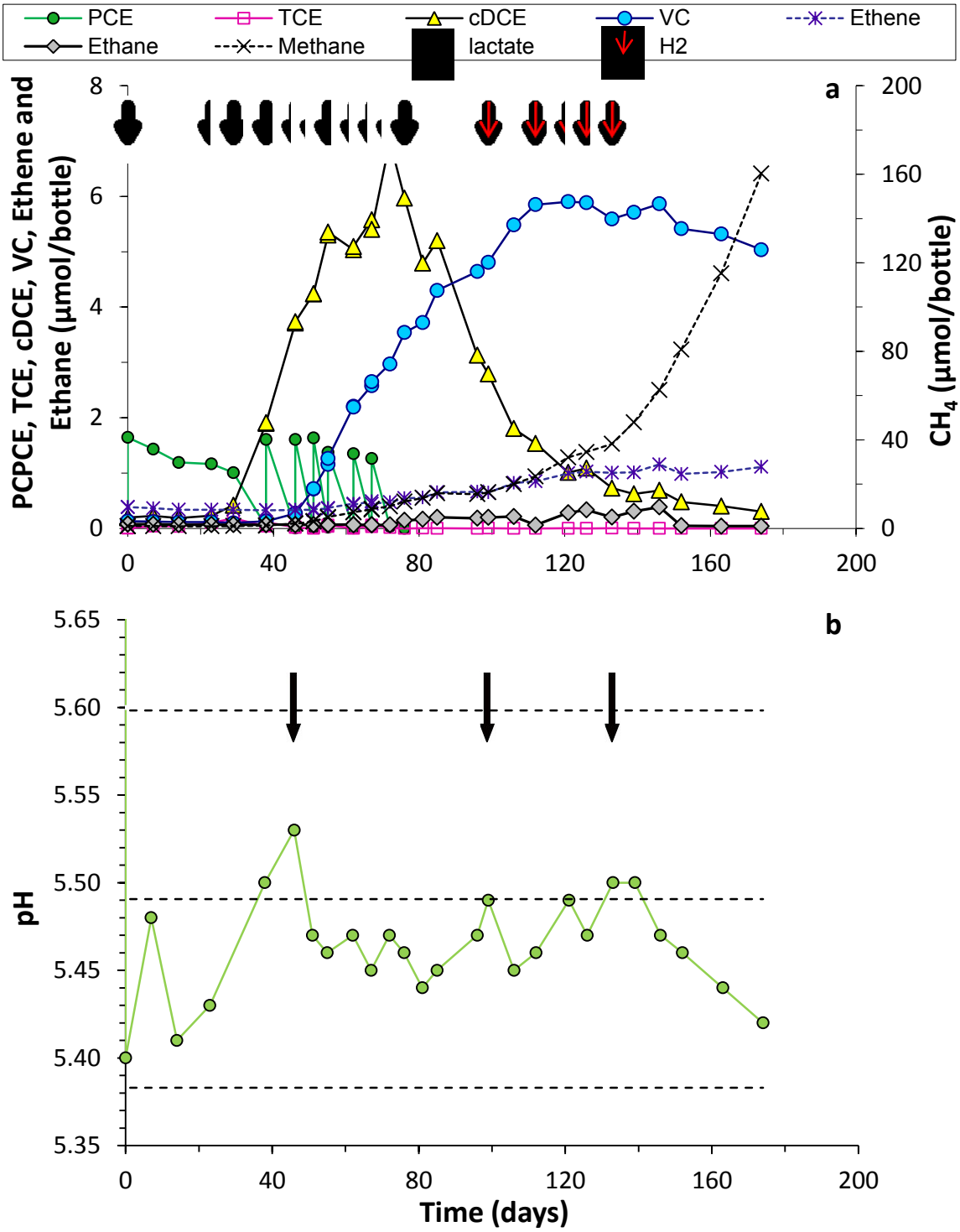

Figure 3.6 Results for a lactate-amended NC/HH microcosm, serum bottle #3 (NC-lactate-S3) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid. Hydrogen replaced lactate as the electron donor starting on day 99.

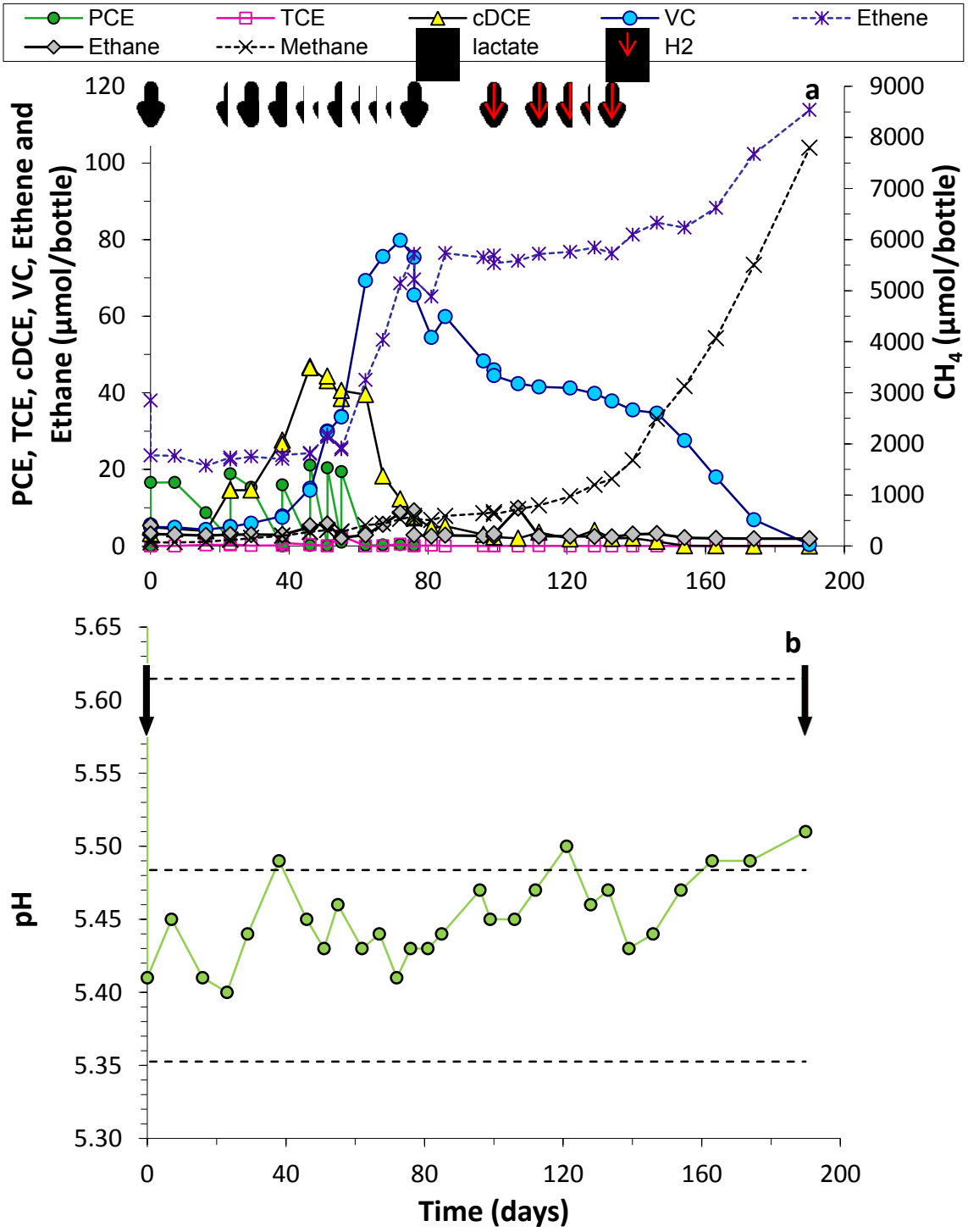

Figure 3.7 Results for a lactate-amended NC/HH microcosm, 2.6 L bottle #2 (NC-lactate-B2) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid. Hydrogen replaced lactate as the electron donor starting on day 99.

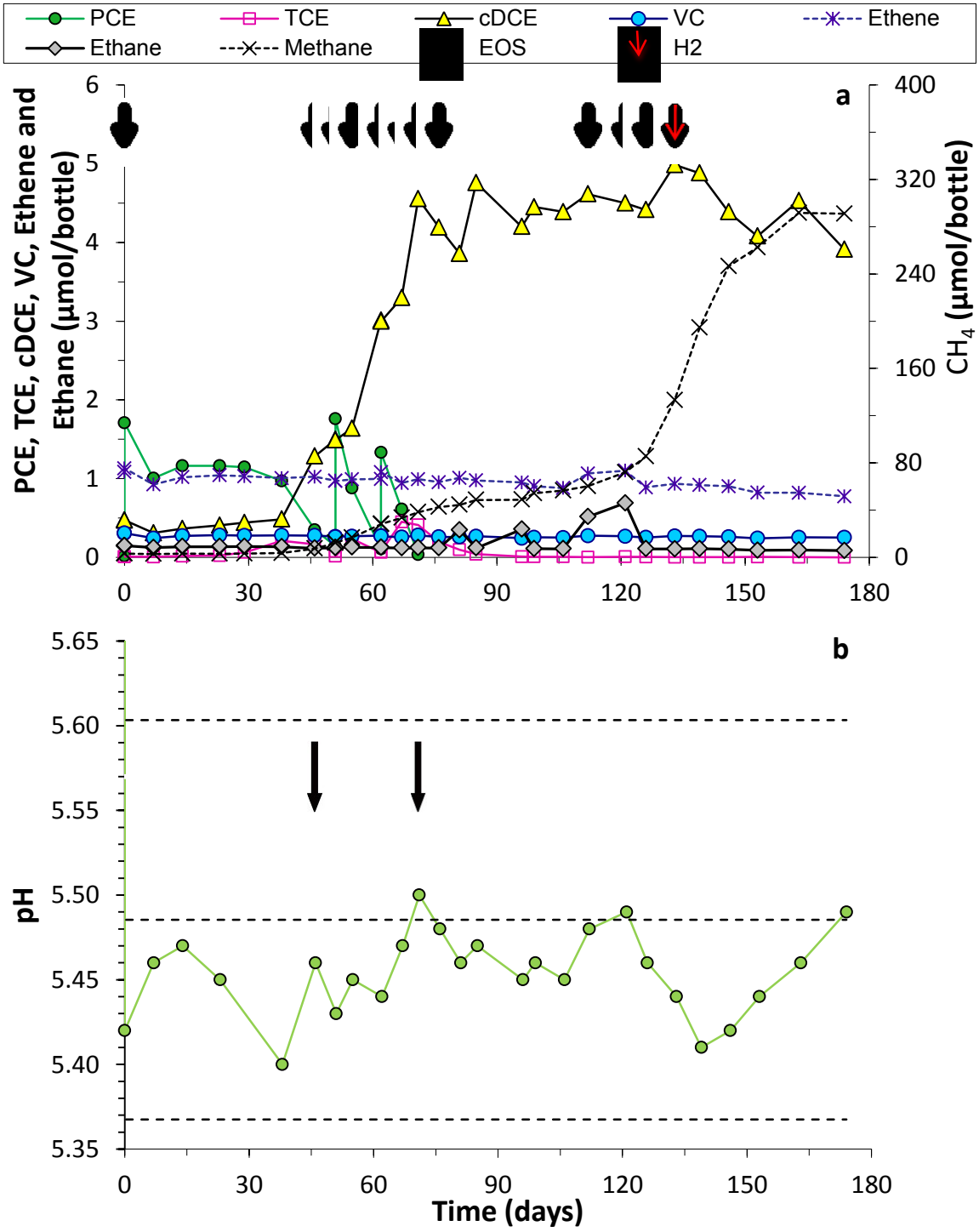

Figure 3.8 Results for an EOS-amended NC/HH microcosm, serum bottle #1 (NC-EOS-S1) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid. Hydrogen replaced EOS as the electron donor starting on day 133.

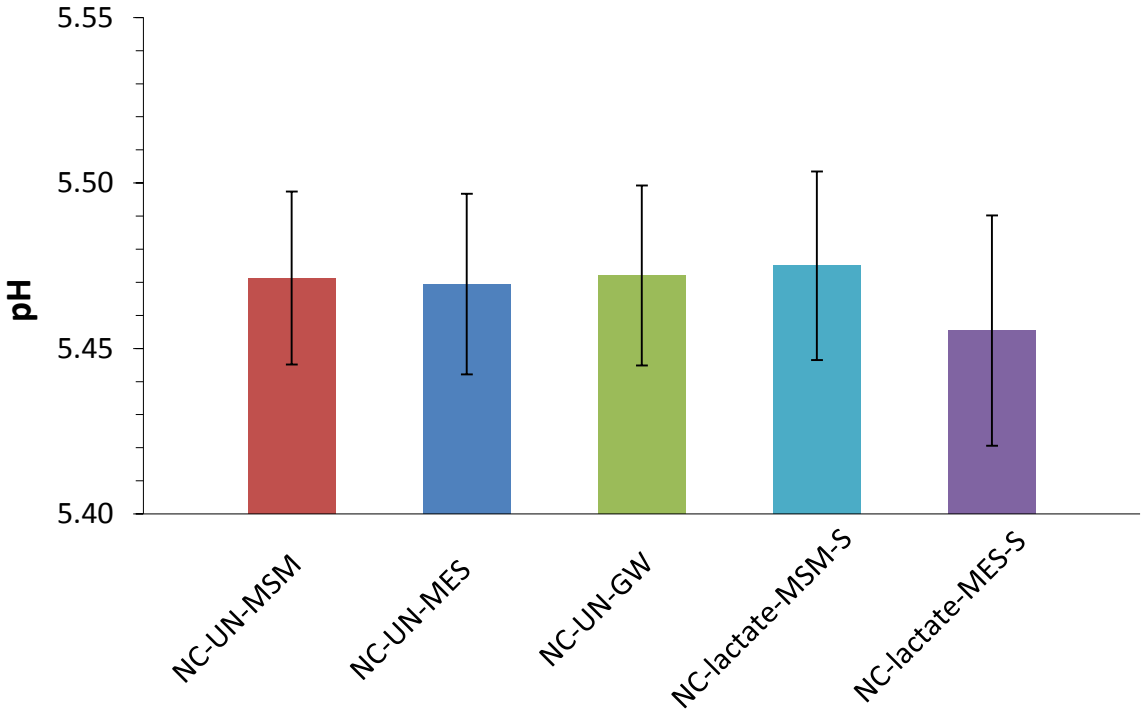

Figure 3.9 Average pH levels for the NC/HH enrichment cultures; error bars represent one standard deviation for triple bottles.

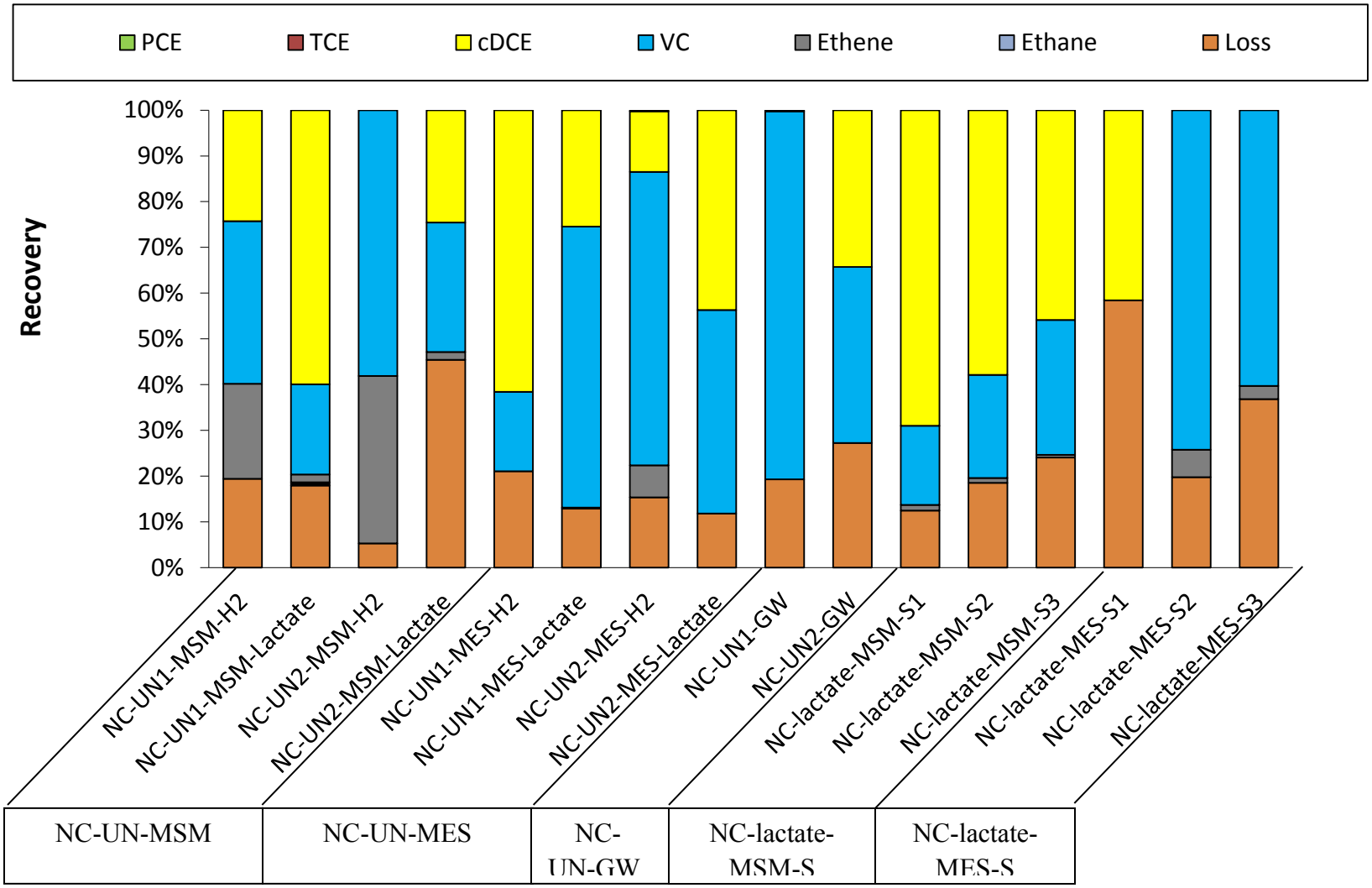

Figure 3.10 Average distribution of dechlorination products for the NC/HH enrichment cultures, based on the total amount of PCE added and the amount of VOCs present at the final sampling point. Groupings below the bottle names correspond to the treatments shown in Figure 3.9.

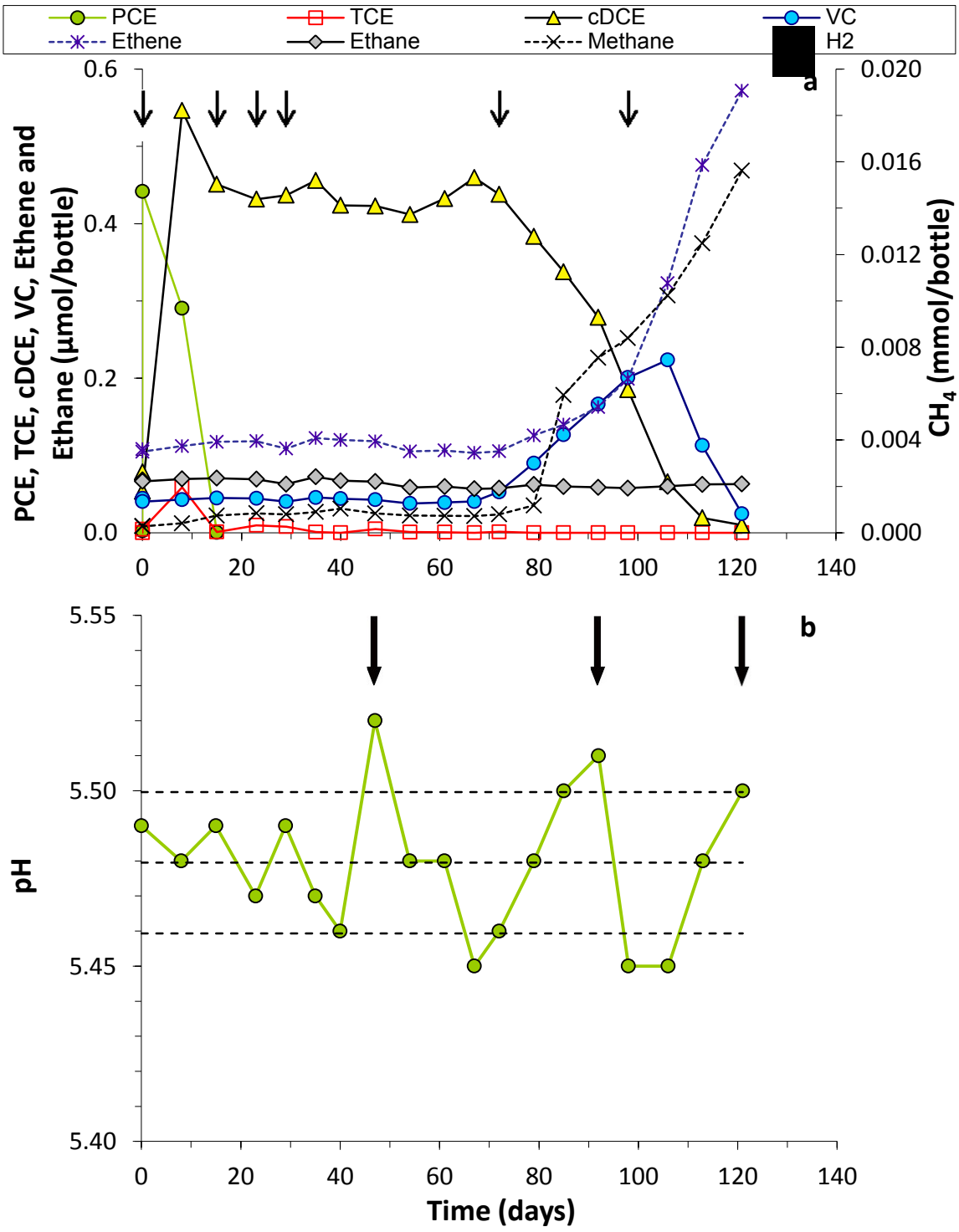

Figure 3.11 Results for a H_2-amended NC/HH enrichment culture developed with MSM (NC-UN1-MSM-H_2) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; the arrow indicates addition of phosphoric acid.

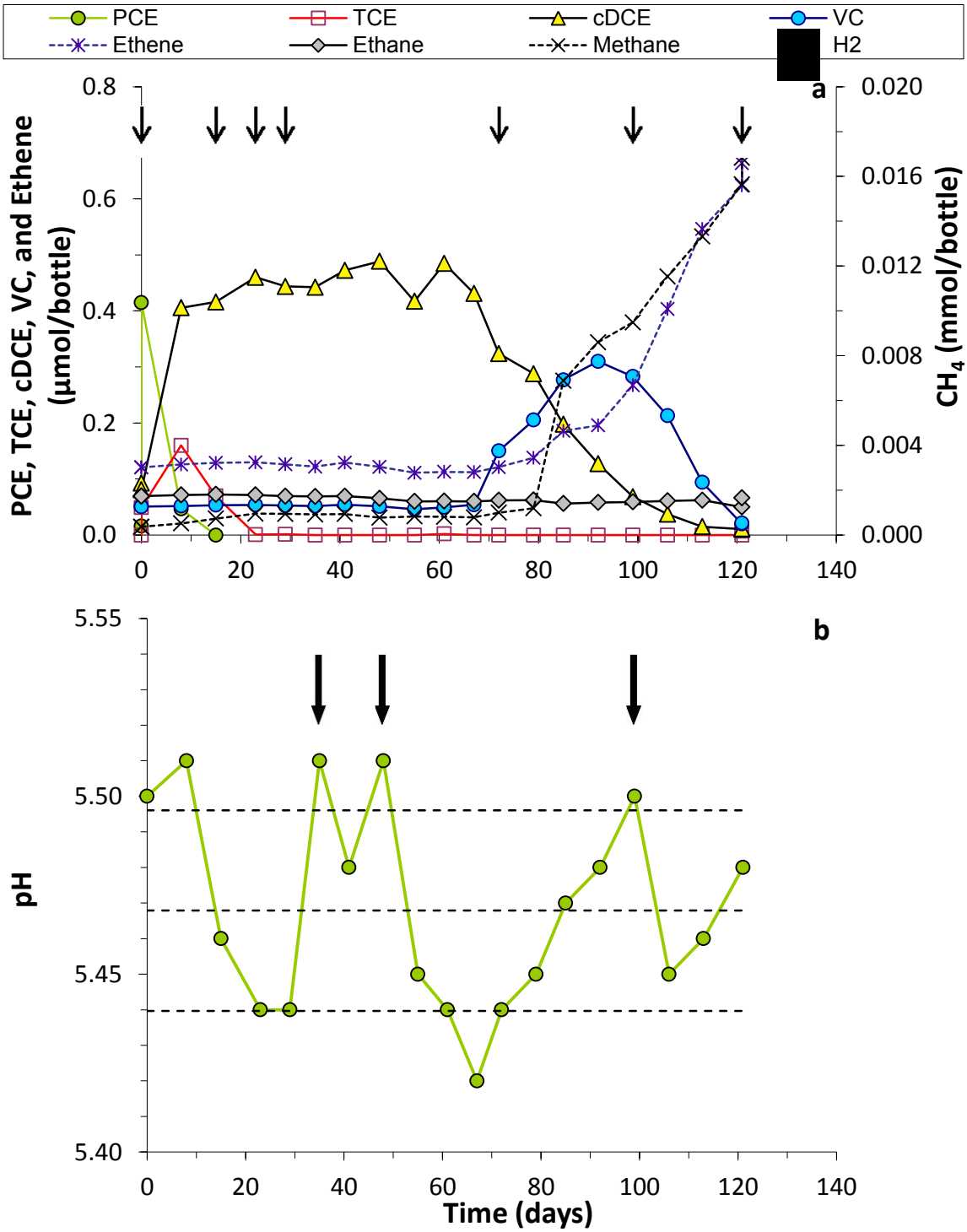

Figure 3.12 Results for a H_2-amended NC/HH enrichment culture developed with MSM (NC-UN2-MSM-H_2) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; the arrow indicates addition of phosphoric acid.

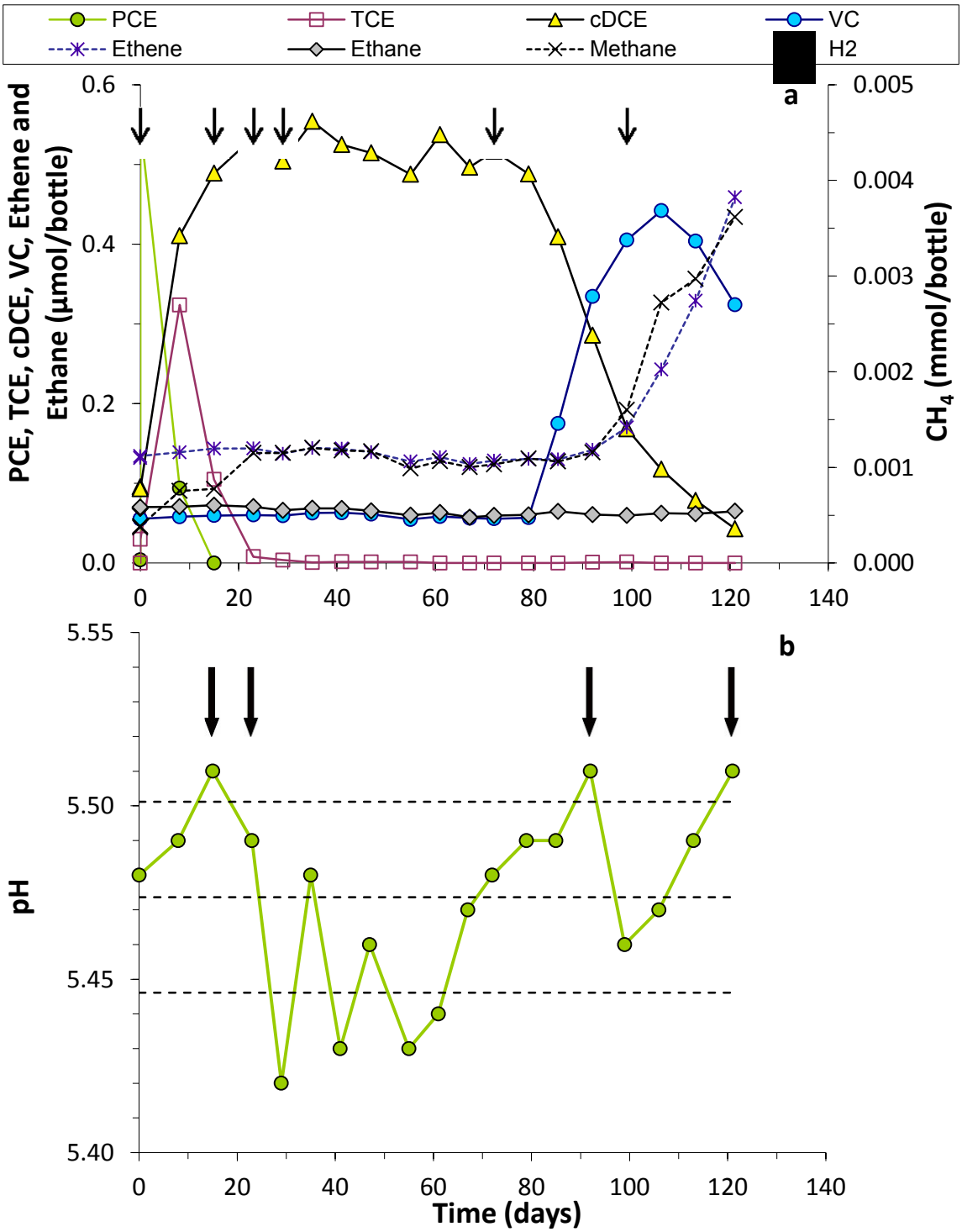

Figure 3.13 Results for a H_2-amended NC/HH enrichment culture developed with MES (NC-UN2-MES-H_2) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; the arrow indicates addition of phosphoric acid.

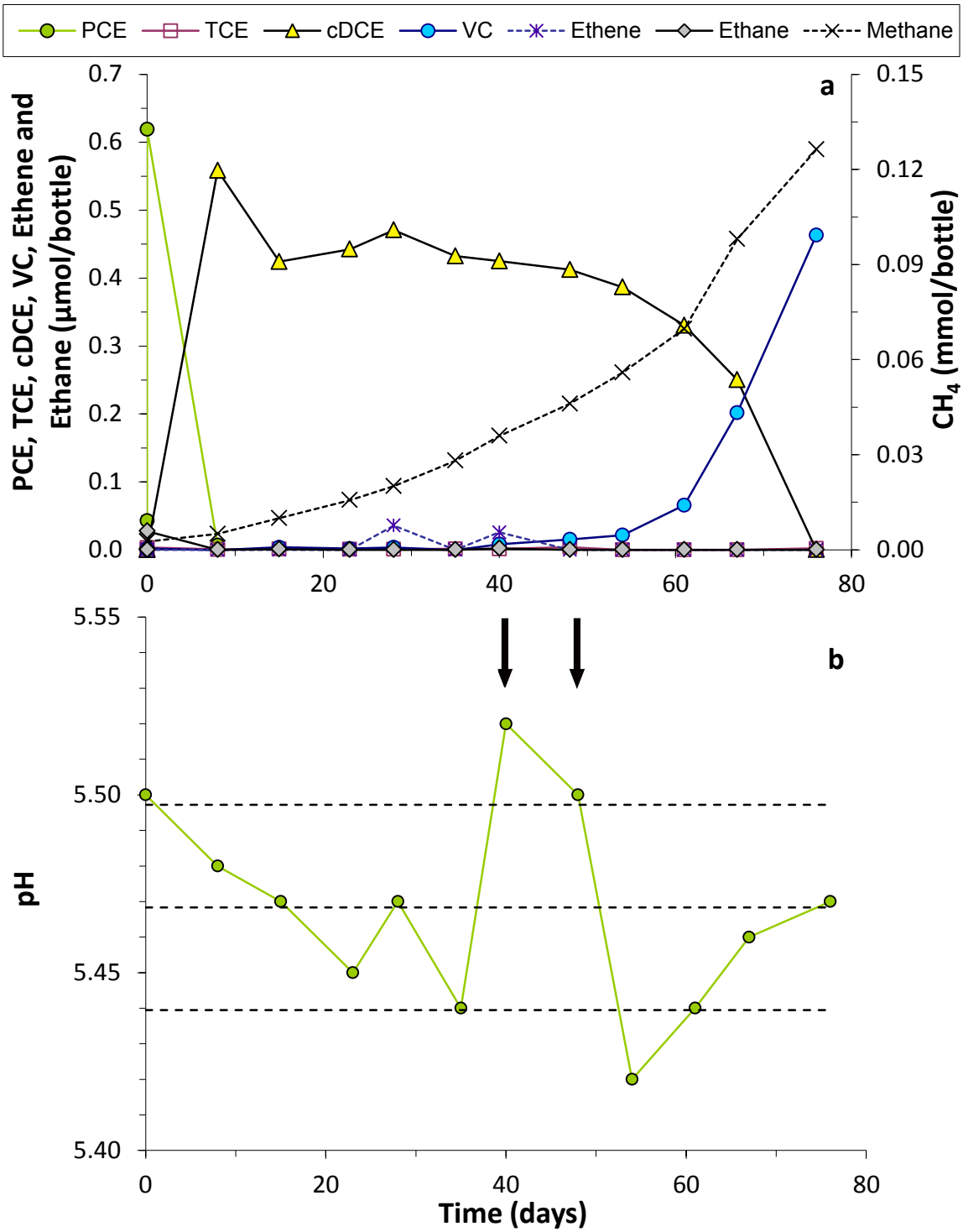

Figure 3.14 Results for an unamended NC/HH enrichment culture developed with groundwater, (NC-UN1-GW) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; the arrow indicates addition of phosphoric acid.

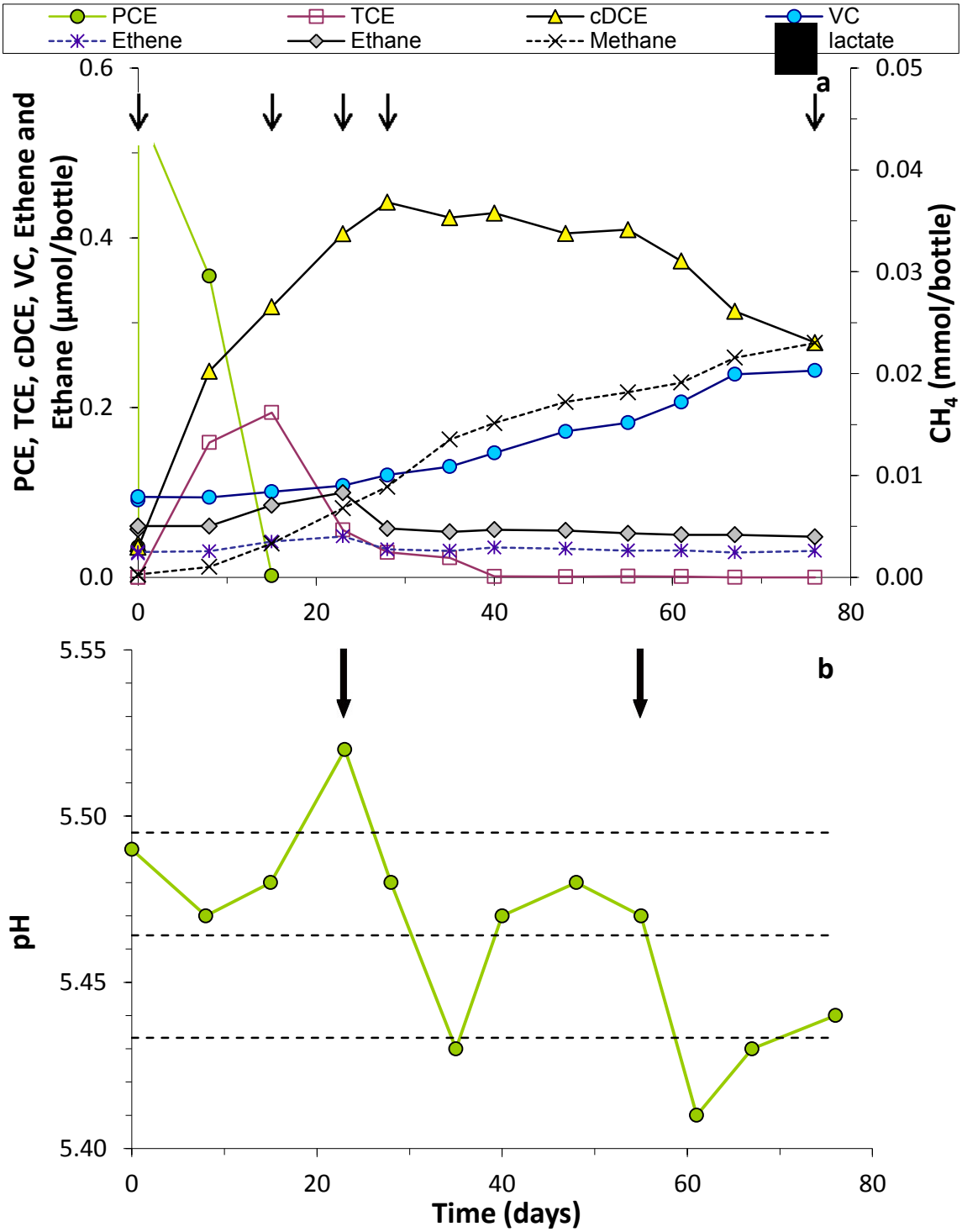

Figure 3.15 Results for a lactate-amended NC/HH enrichment culture developed with MSM (NC-lactate-MSM-S3) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; the arrow indicates addition of phosphoric acid.

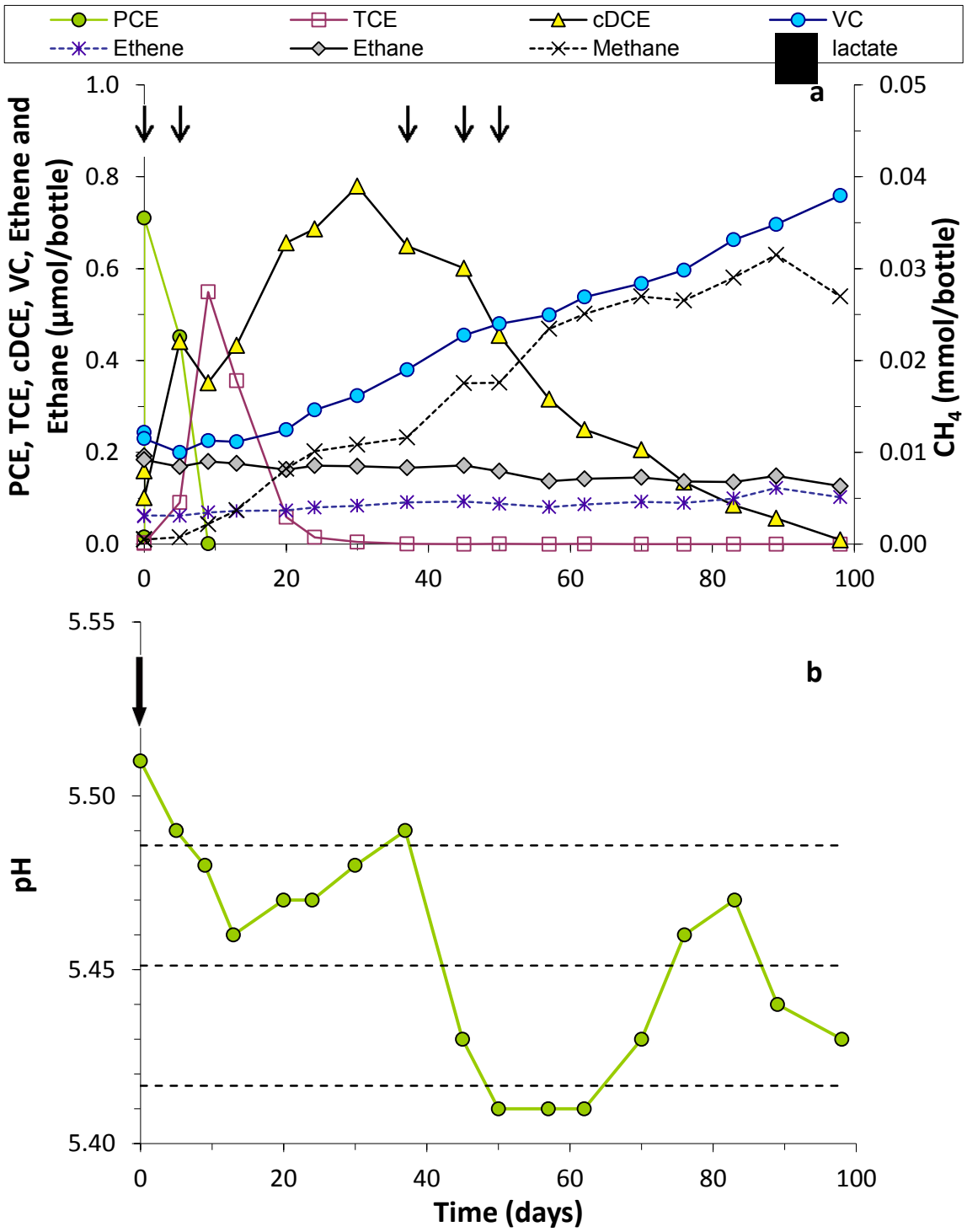

Figure 3.16 Results for a lactate-amended NC/HH enrichment culture developed with MES (NC-lactate-MES-S2) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; the arrow indicates addition of phosphoric acid.

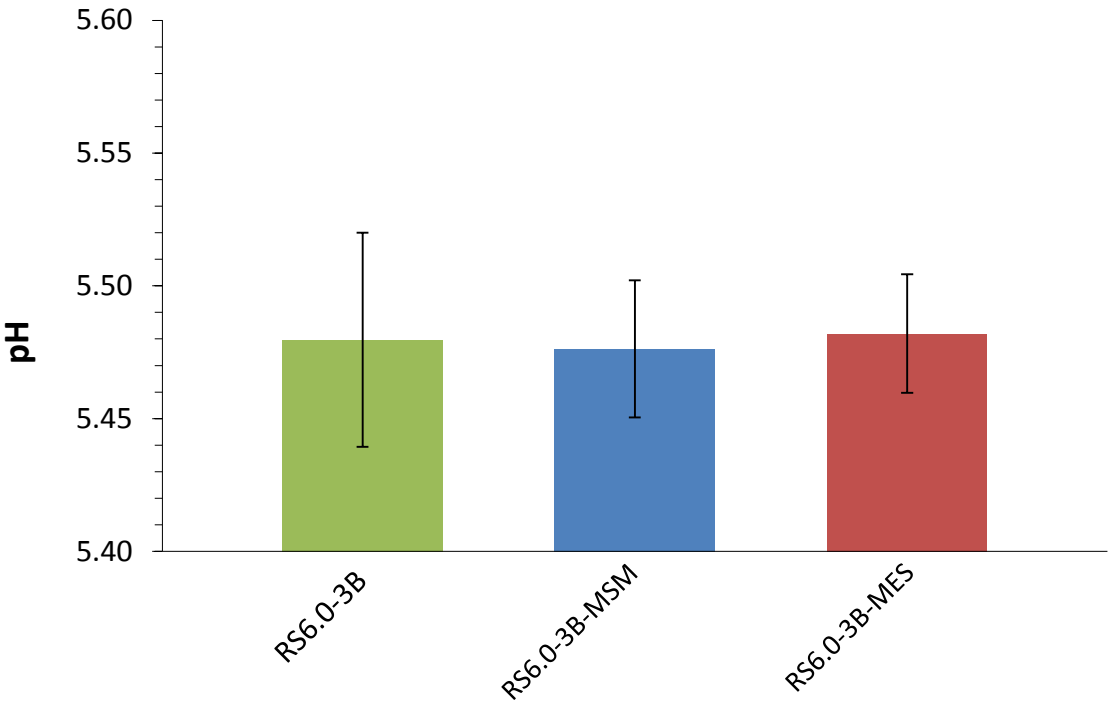

Figure 3.17 Average pH levels for NC/FRX enrichment cultures; error bars represent one standard deviation for triple bottles.

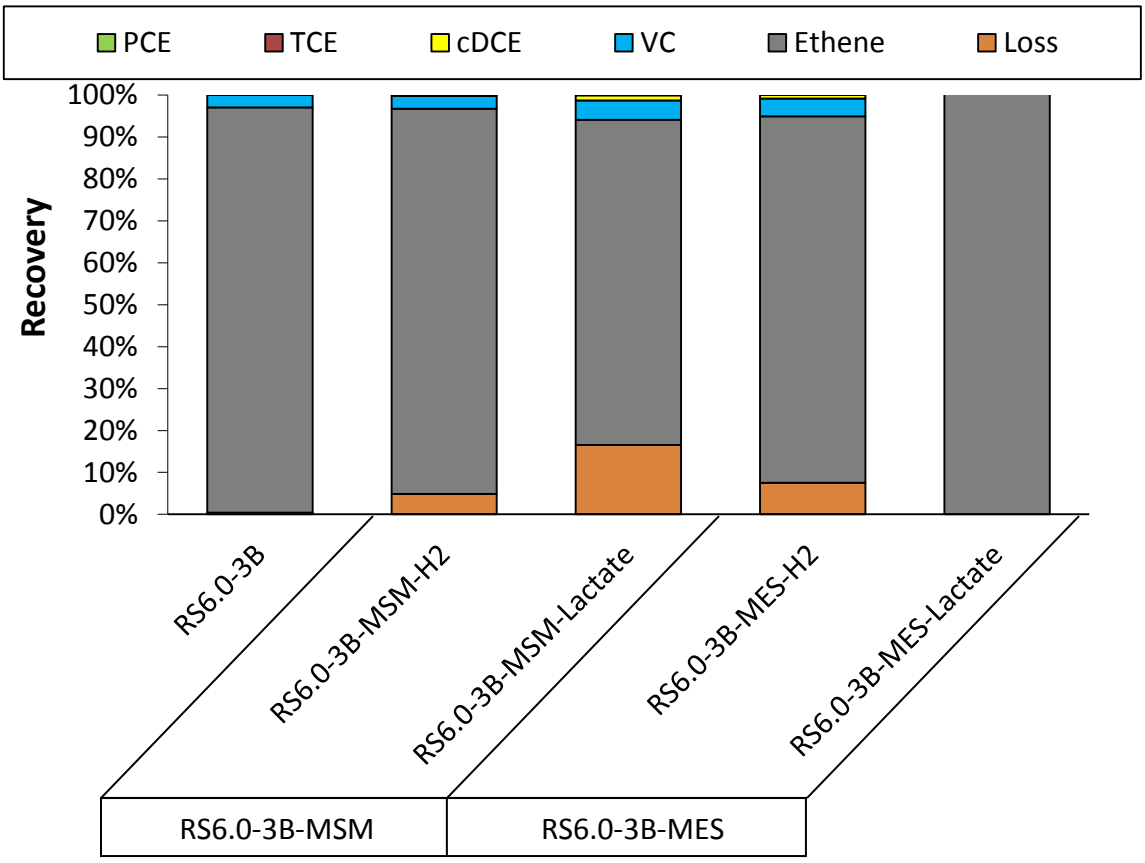

Figure 3.18 Average distribution of dechlorination products for the NC/FRX enrichment cultures, based on the total amount of PCE added and the amount present at the final sampling point. Groupings below the bottle names correspond to the treatments shown in Figure 3.17.

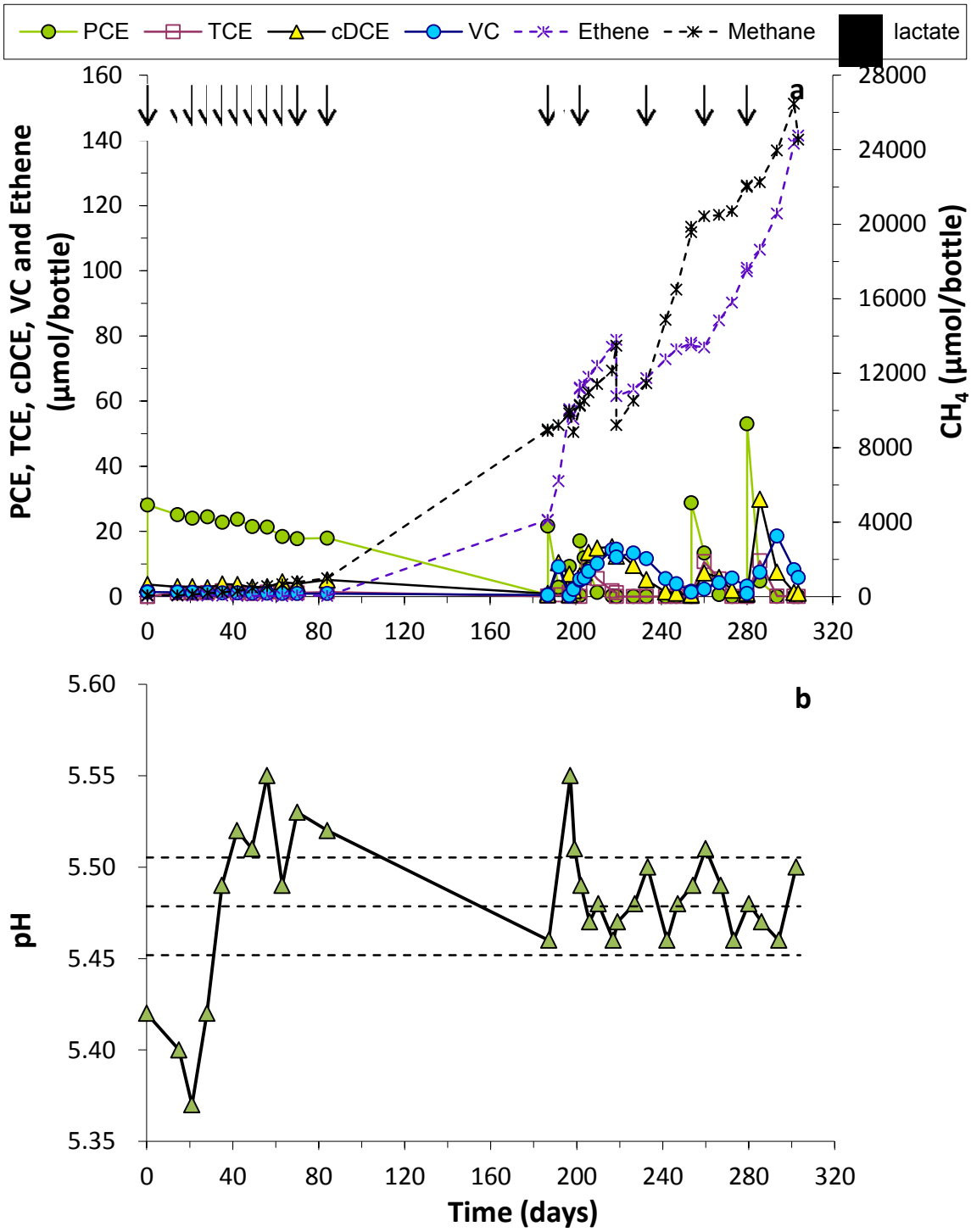

Figure 3.19 Results for a lactate-amended NC/FRX enrichment culture developed with MSM (RS6.0-3B) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; the arrow indicates addition of phosphoric acid.

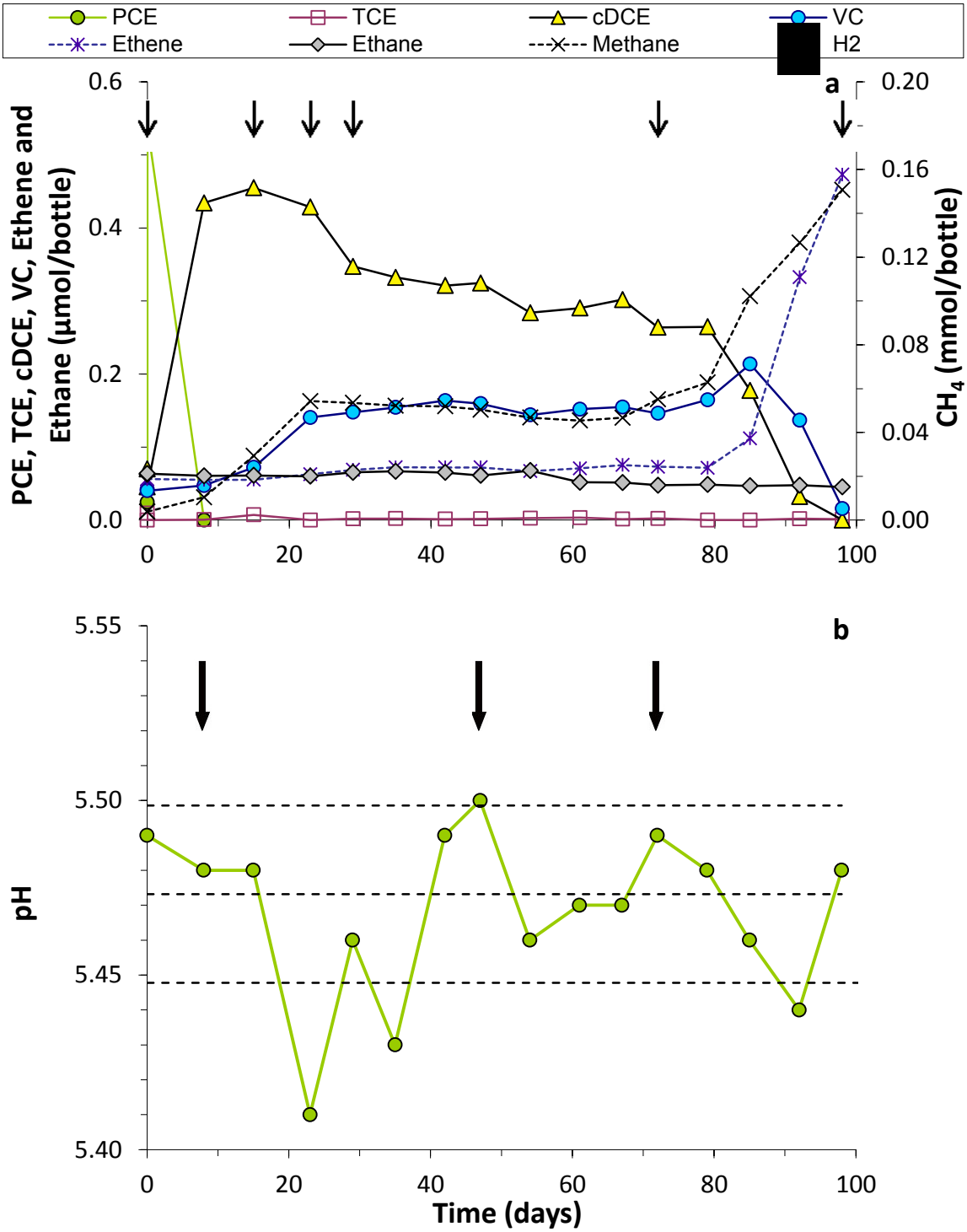

Figure 3.20 Results for a H_2-amended NC/FRX enrichment culture developed with MSM (RS6.0-3B-MSM-H_2) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; the arrow indicates addition of phosphoric acid.

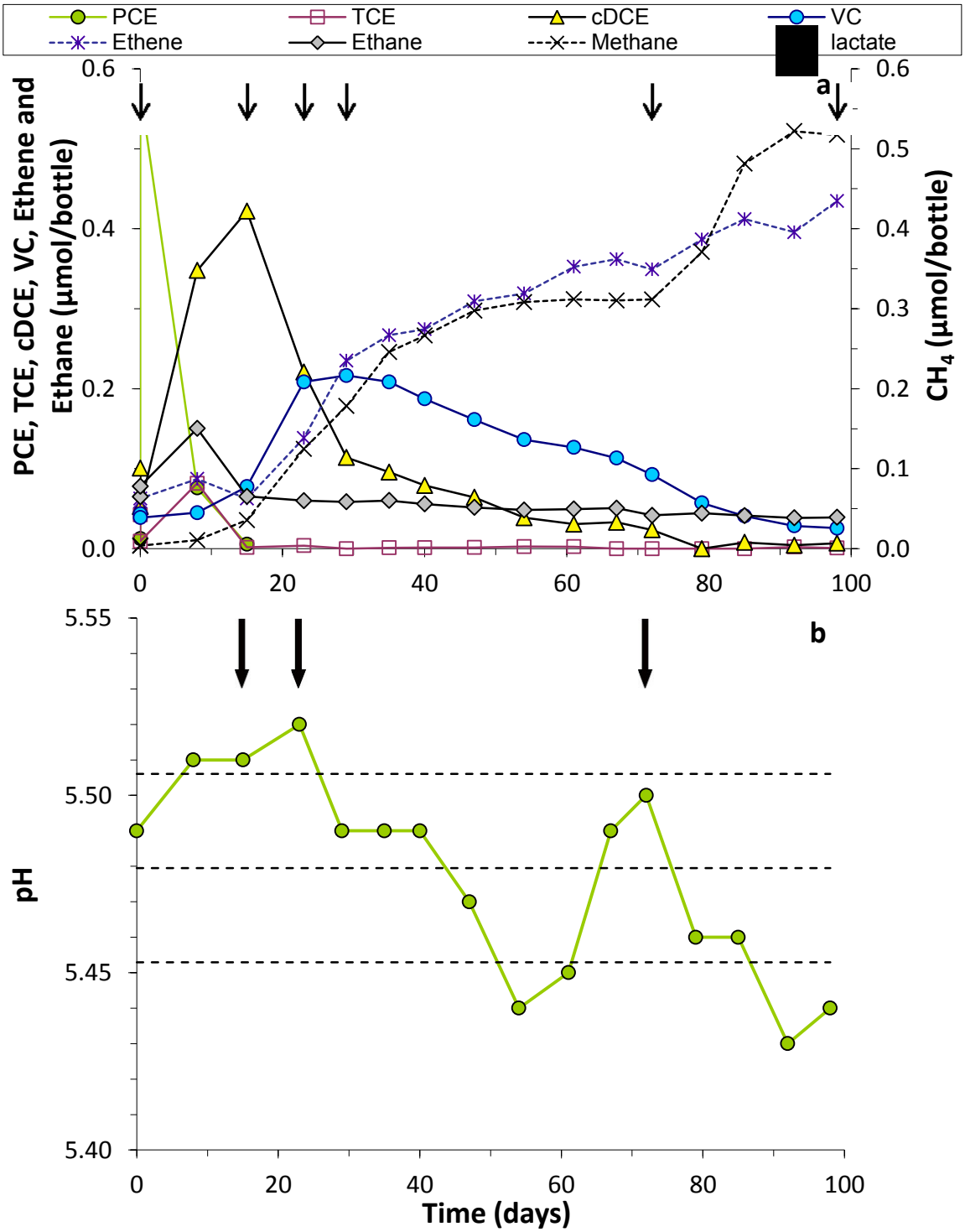

Figure 3.21 Results for a lactate-amended NC/FRX enrichment culture developed with MSM (RS6.0-3B-MSM-lactate) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; the arrow indicates addition of phosphoric acid.

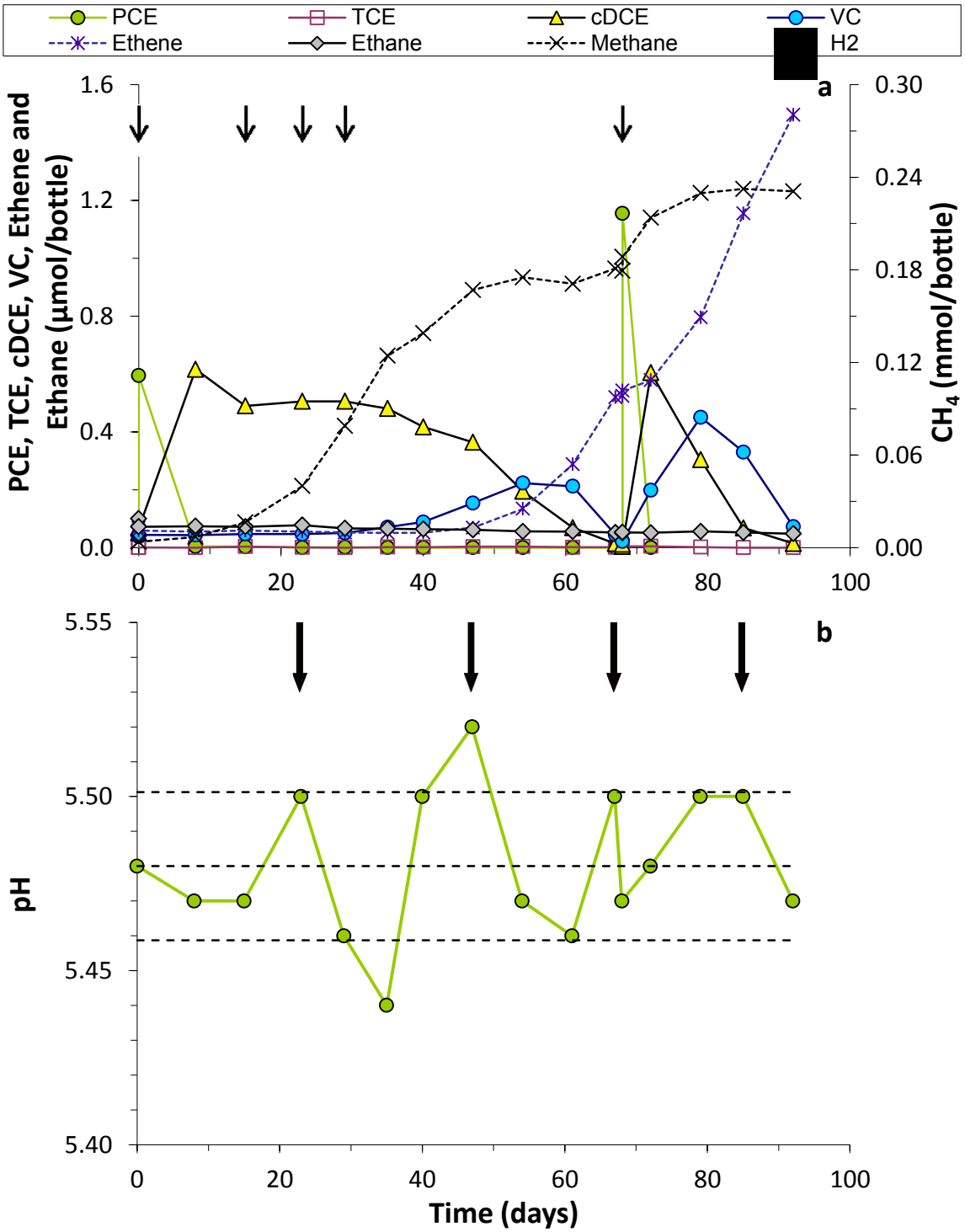

Figure 3.22 Results for a H_2-amended NC/FRX enrichment culture developed with MES (RS6.0-3B-MES-H_2) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; the arrow indicates addition of phosphoric acid.

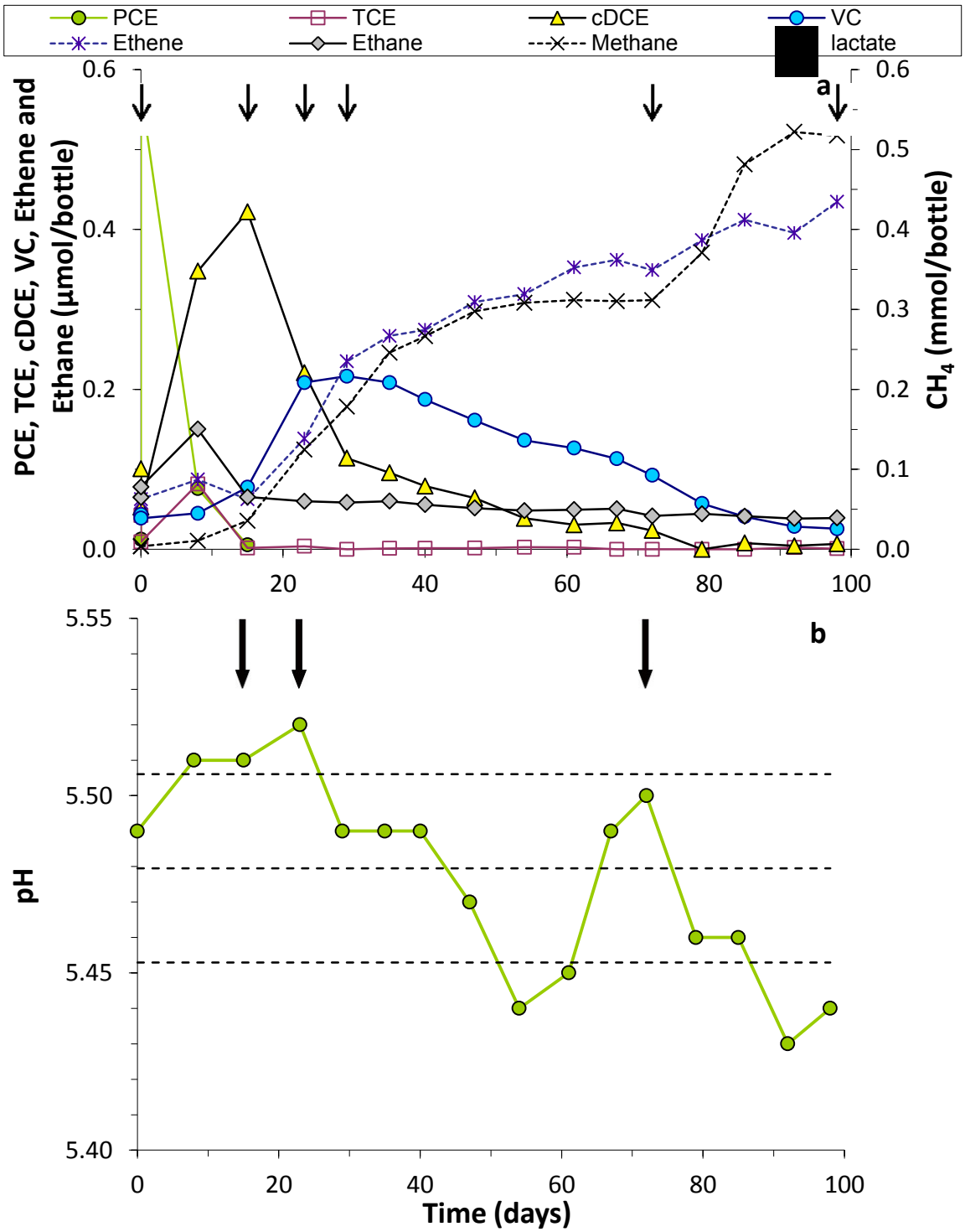

Figure 3.23 Results for a lactate-amended NC/FRX enrichment culture developed with MES (RS6.0-3B-MES-lactate) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; the arrow indicates addition of phosphoric acid.

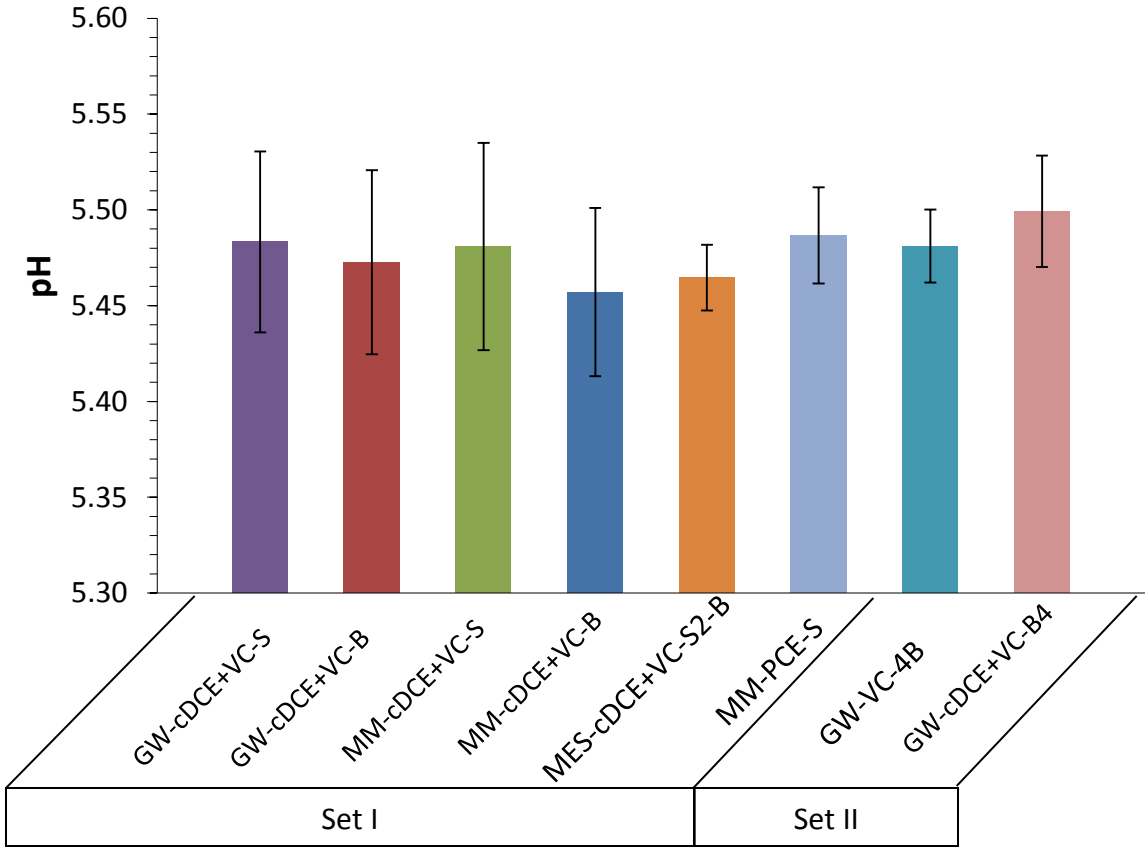

Figure 3.24 Average pH level for SRS microcosm and enrichment cultures; error bars represent one standard deviation for triple bottles.

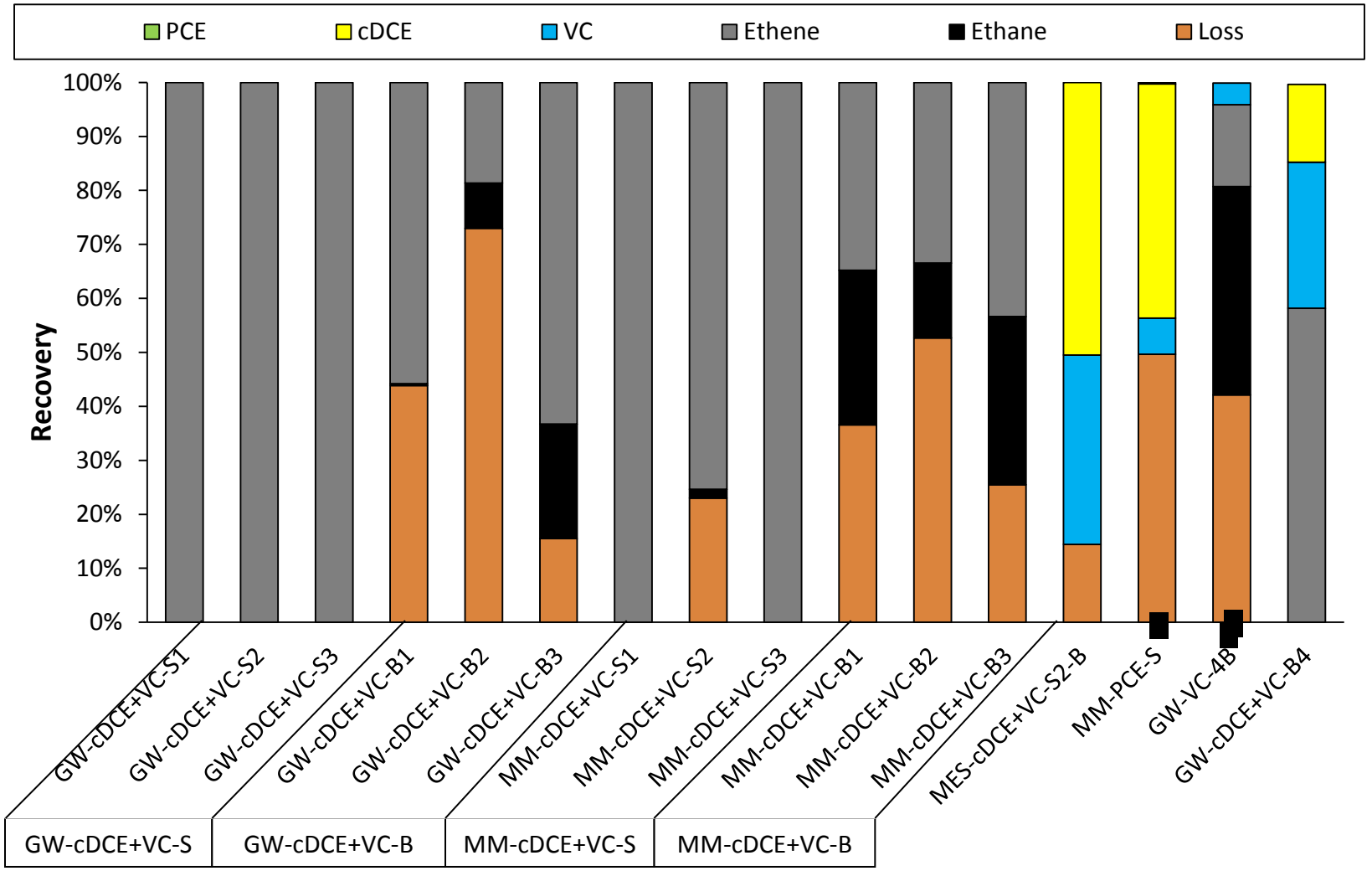

Figure 3.25 Average distribution of dechlorination products for the SRS microcosms and enrichment cultures, based on the total amount of PCE added and the amount present at the final sampling point; * = fed with PCE; ** = fed with VC only. Groupings below the bottle names correspond to the treatments shown in Figure 3.24.

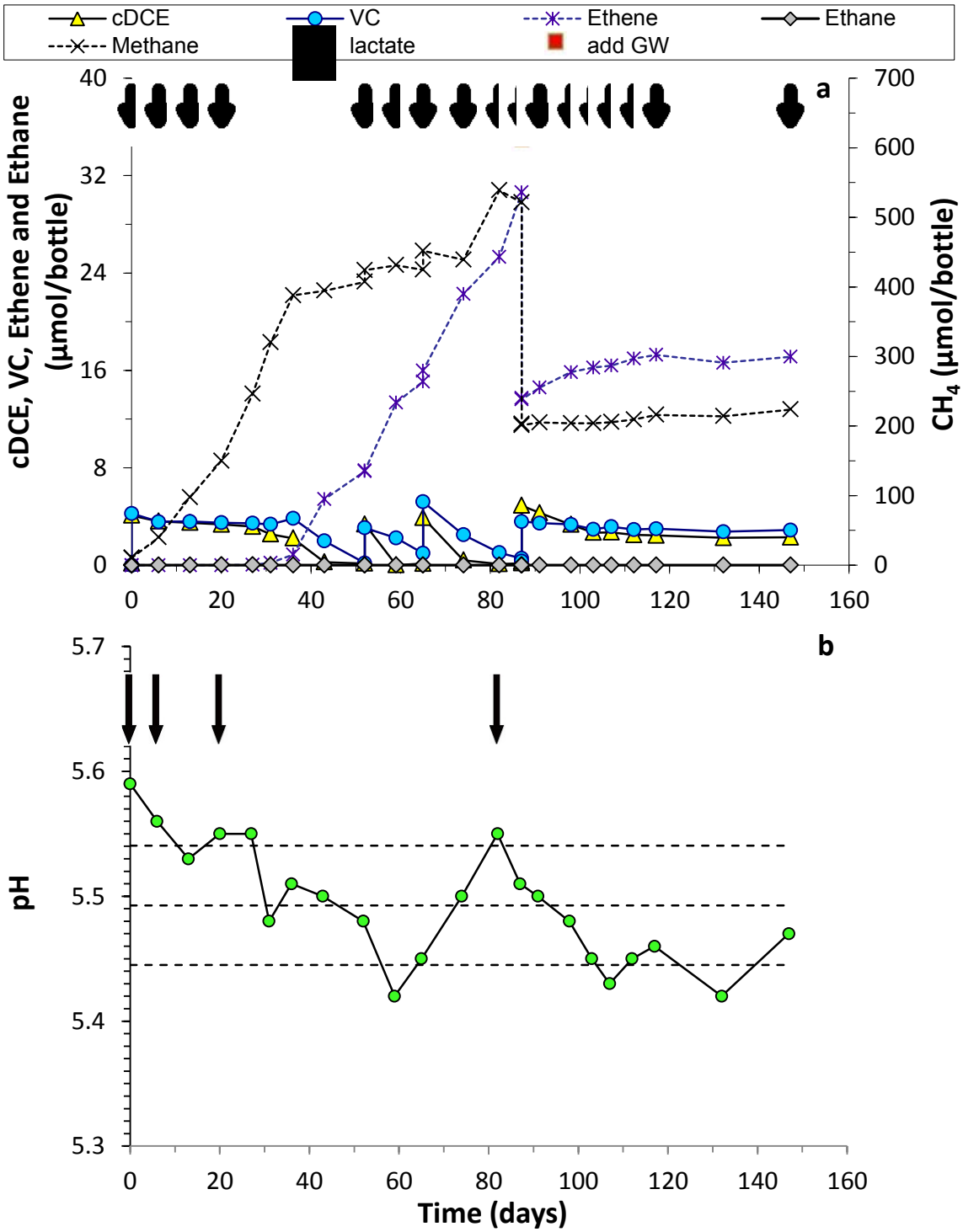

Figure 3.26 Results for a lactate-amended SRS microcosm developed with groundwater, serum bottle #1 (GW-cDCE+VC-S1) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

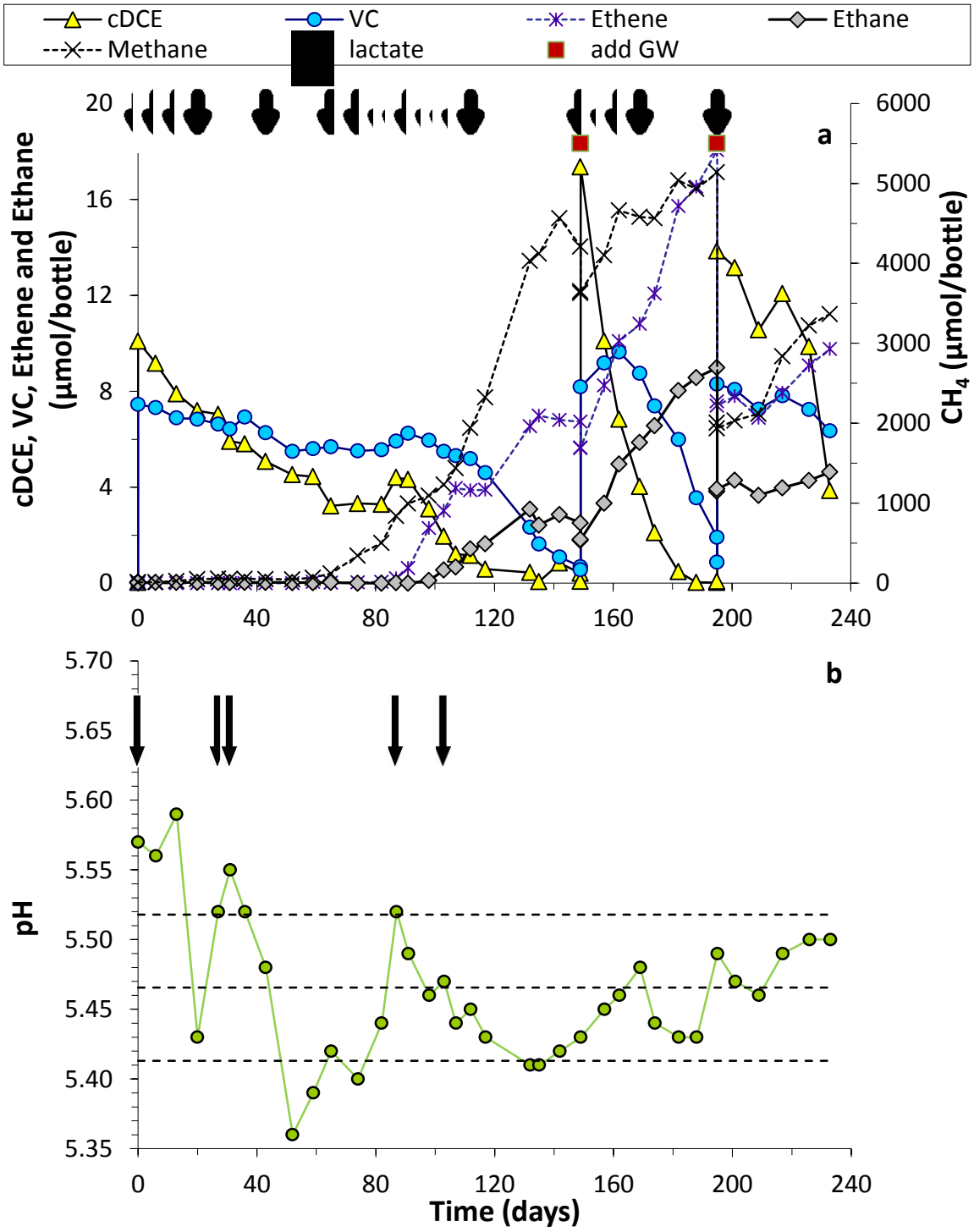

Figure 3.27 Results for a lactate-amended SRS microcosm developed with groundwater, 2.6 L bottle #2 (GW-cDCE+VC-B2) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

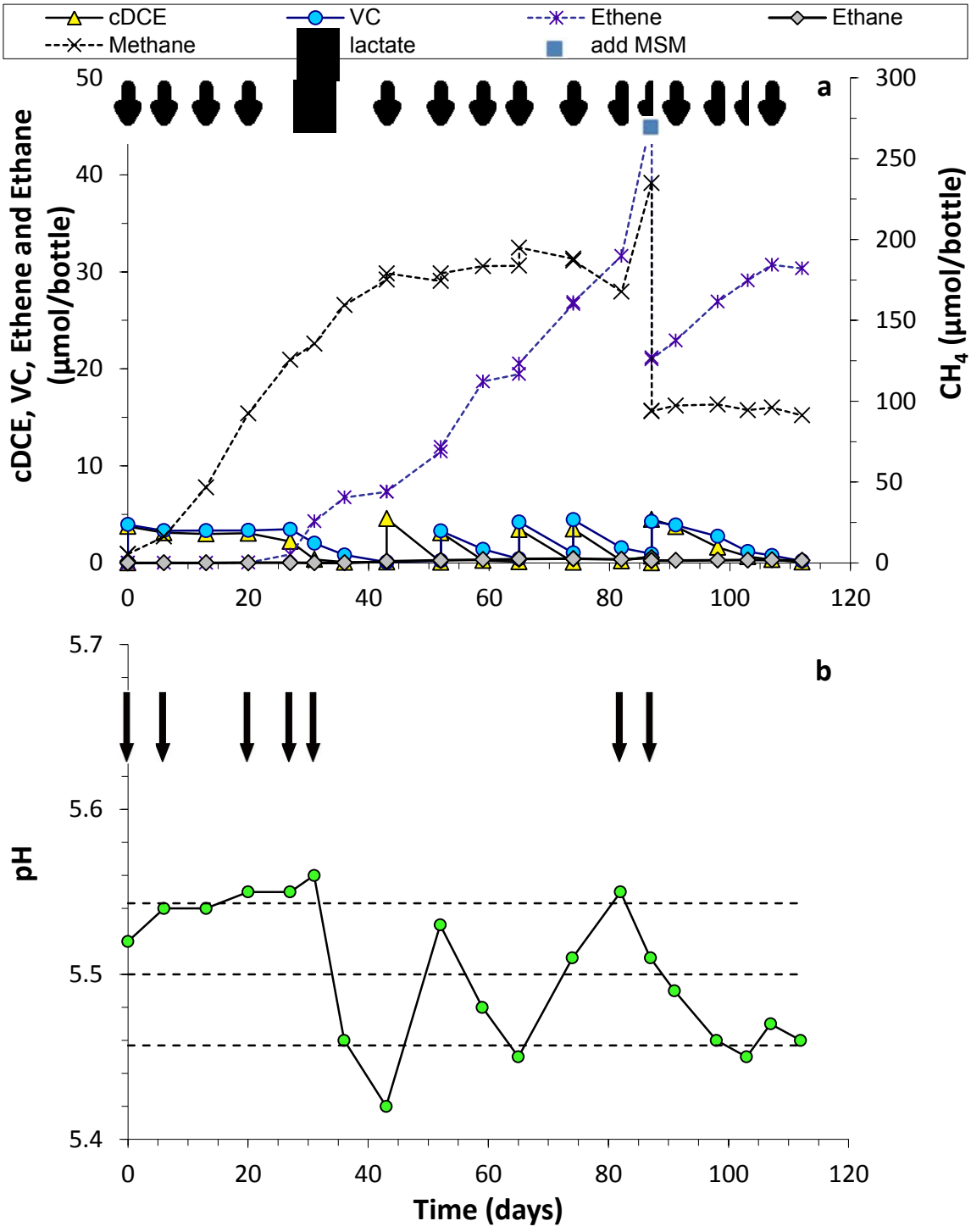

Figure 3.28 Results for a lactate-amended SRS microcosm developed with MSM, serum bottle #2 (MM-cDCE+VC-S2) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

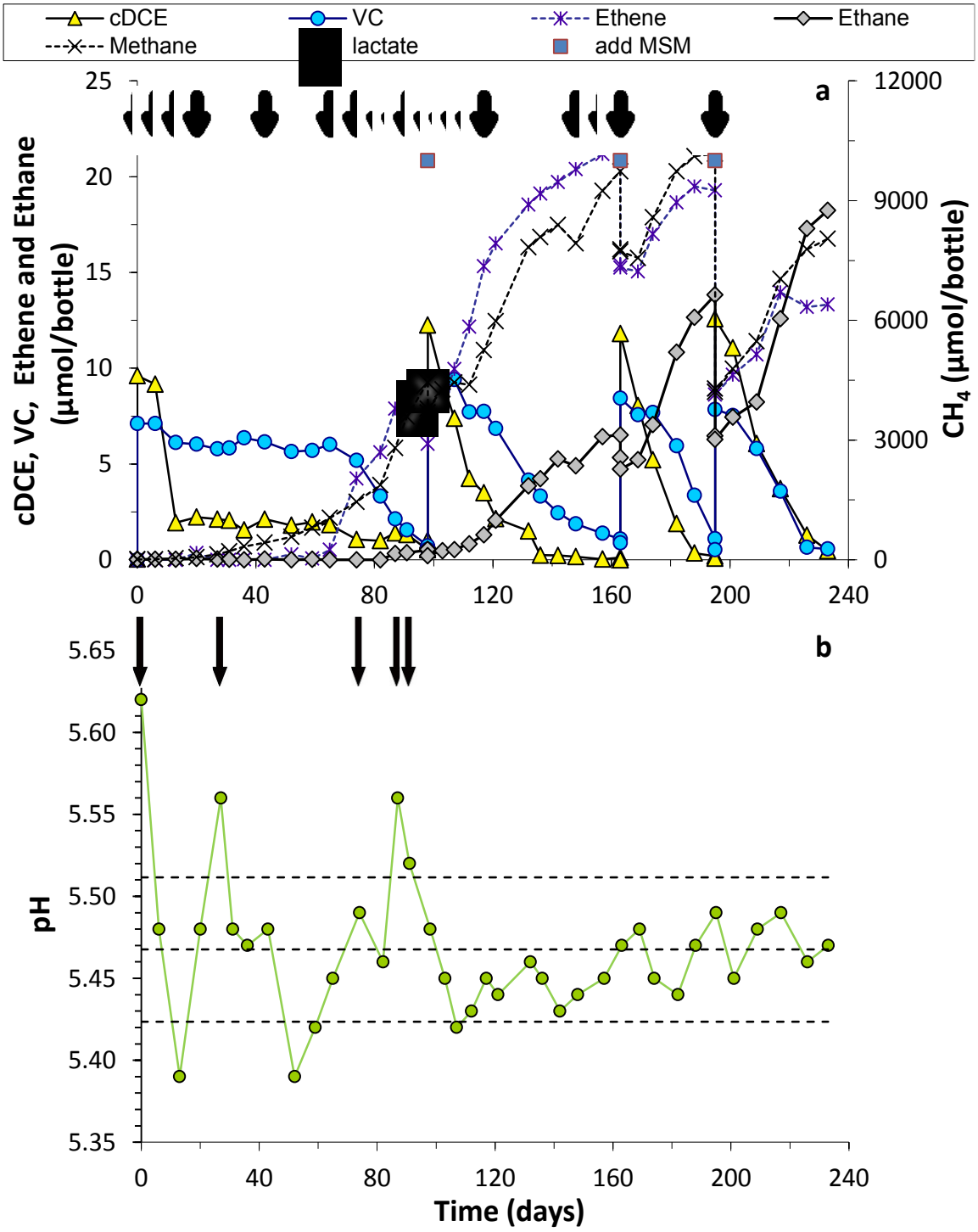

Figure 3.29 Results for a lactate-amended SRS microcosm developed with MSM, 2.6 L bottle #1 (MM-cDCE+VC-B1) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

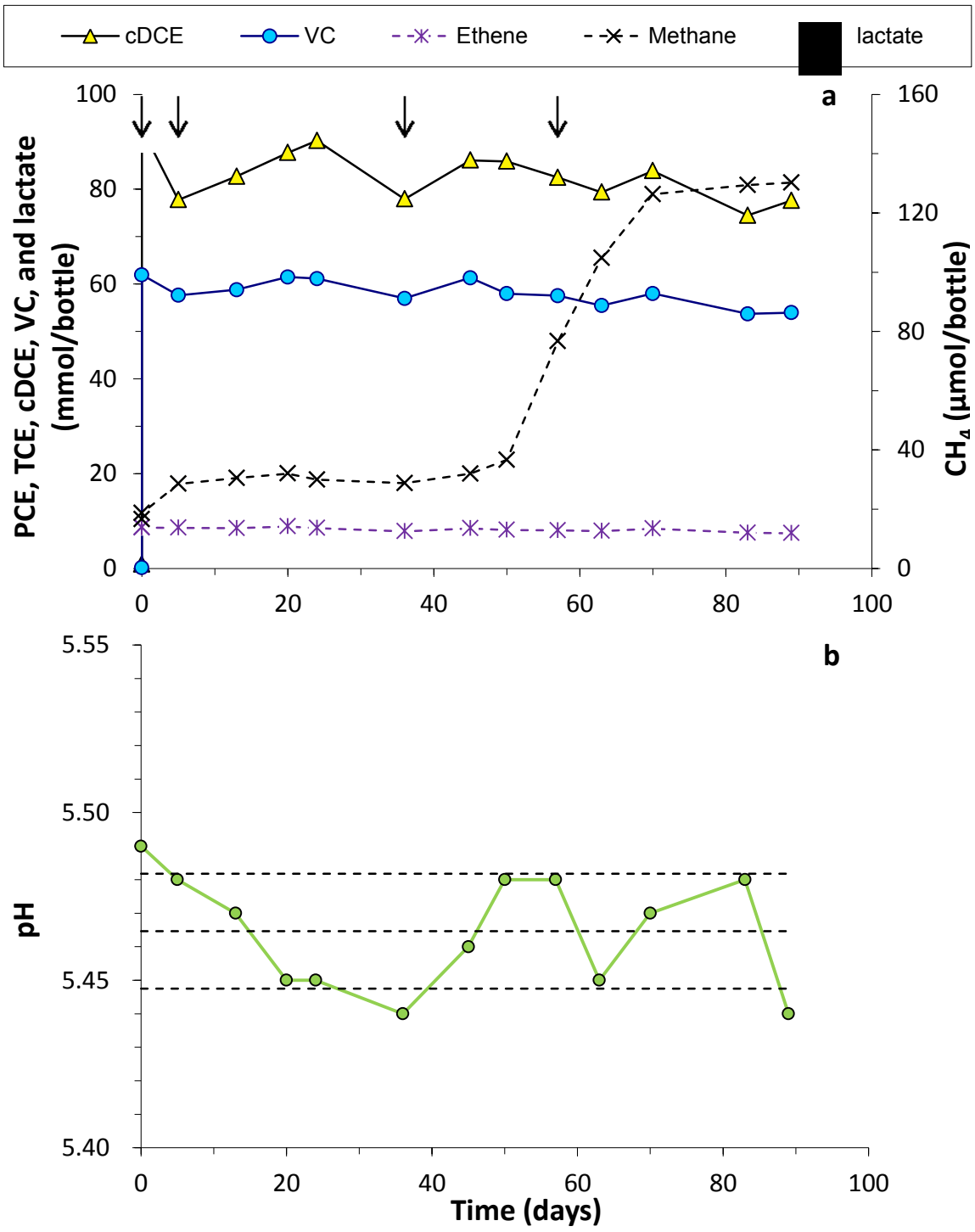

Figure 3.30 Results for a lactate-amended SRS enrichment culture, Set I, developed with MES, 2.6 L bottle #2 (MES-cDCE+VC-S2-B) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

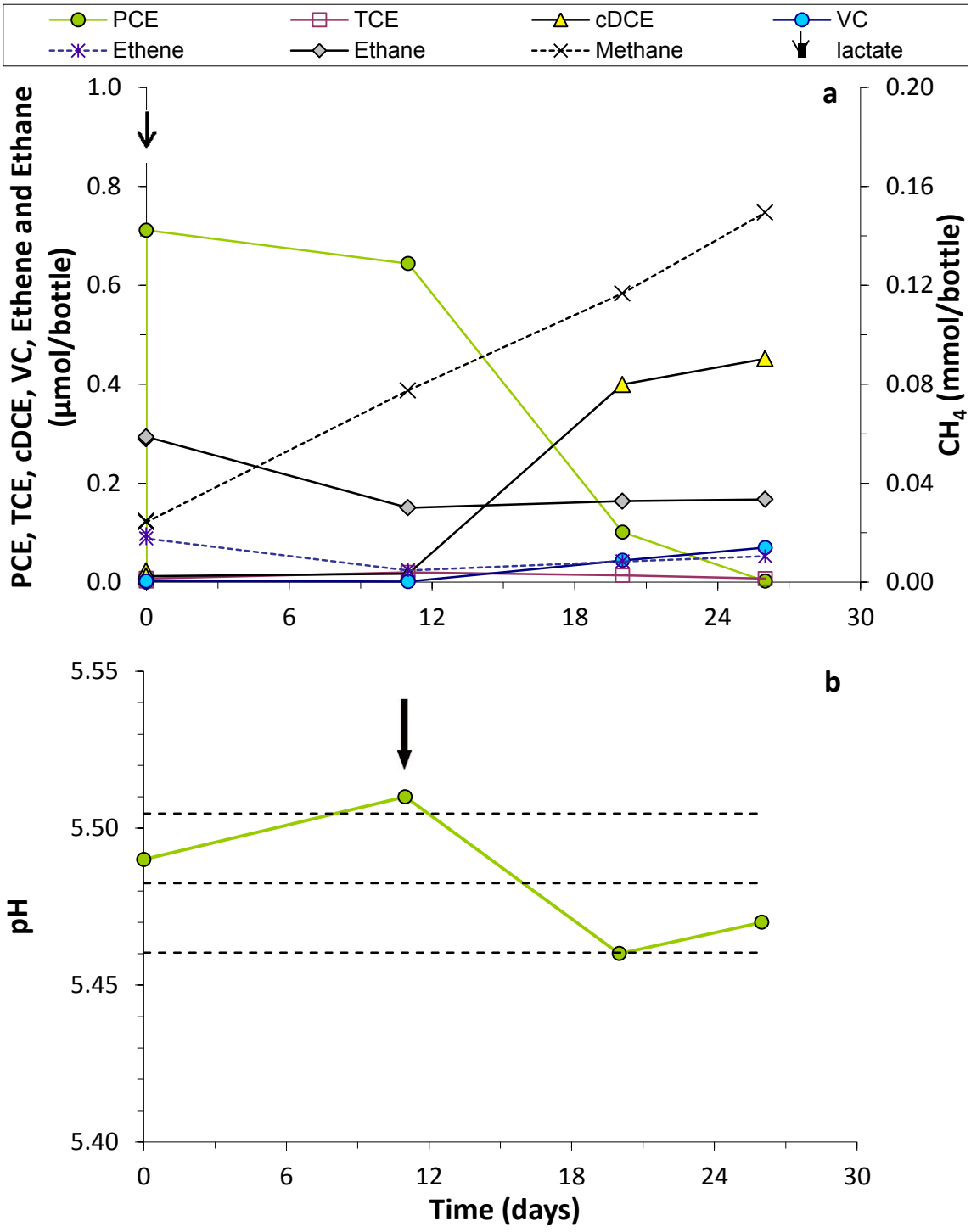

Figure 3.31 Results for a lactate-amended SRS microcosm developed with MSM and fed with PCE, serum bottle #1 (MM-PCE-S) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

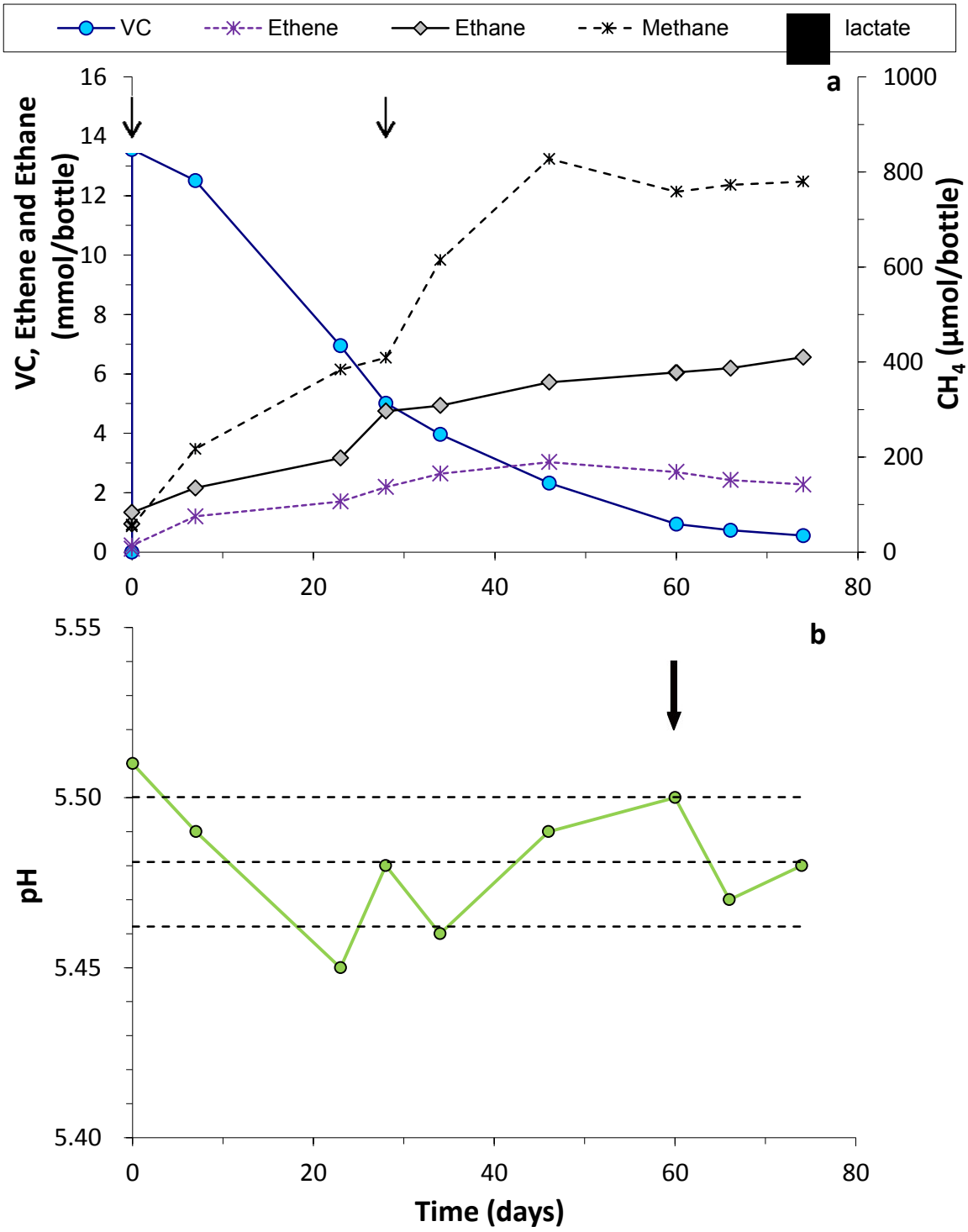

Figure 3.32 Results for a lactate-amended SRS enrichment culture, Set II, developed with groundwater, 2.6 L bottle #1 (GW-VC-4B) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

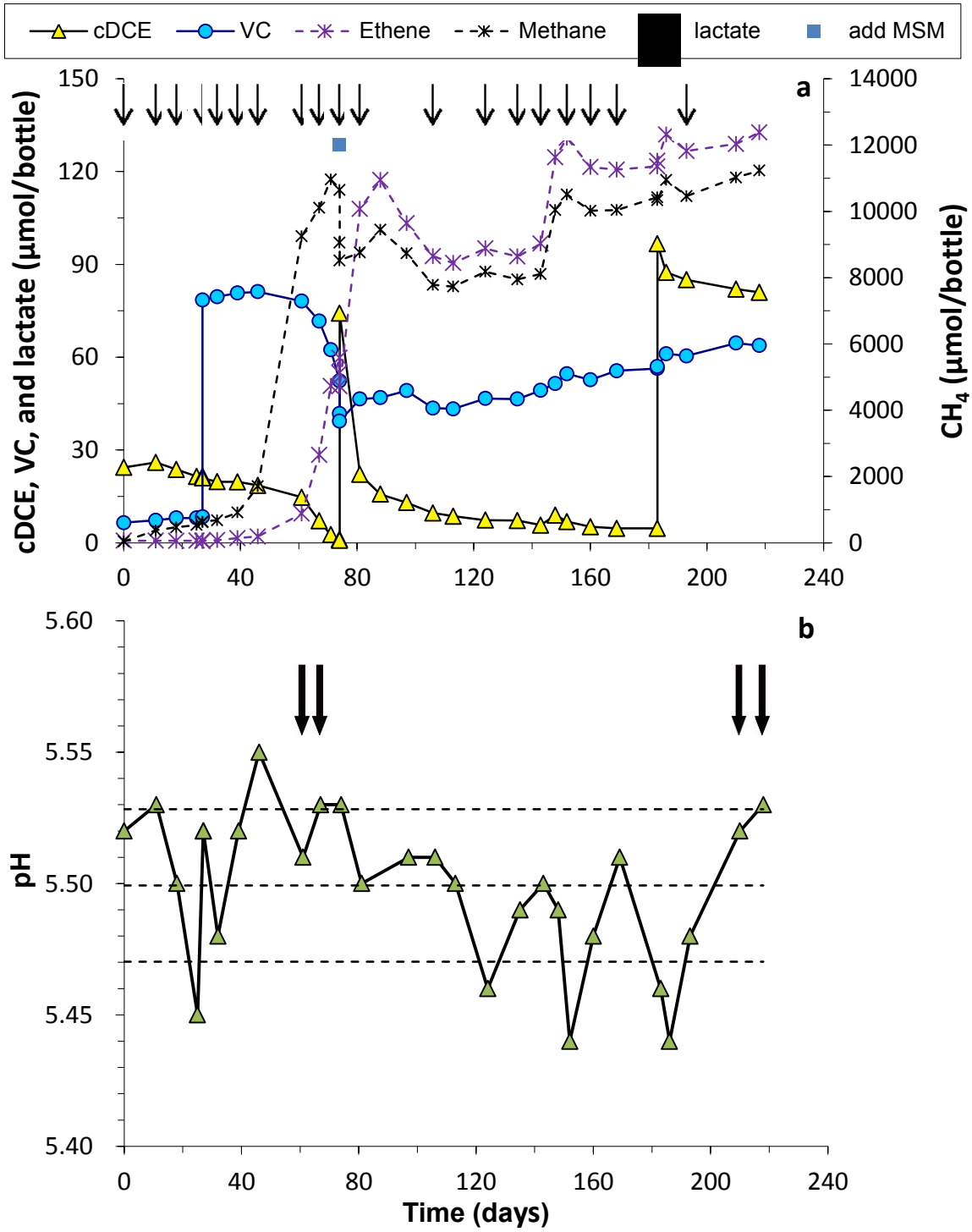

Figure 3.33 Results for a lactate-amended SRS enrichment culture, Set II, gradually diluted with MSM, 2.6 L bottle #1 (GW-cDCE+VC-B4) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

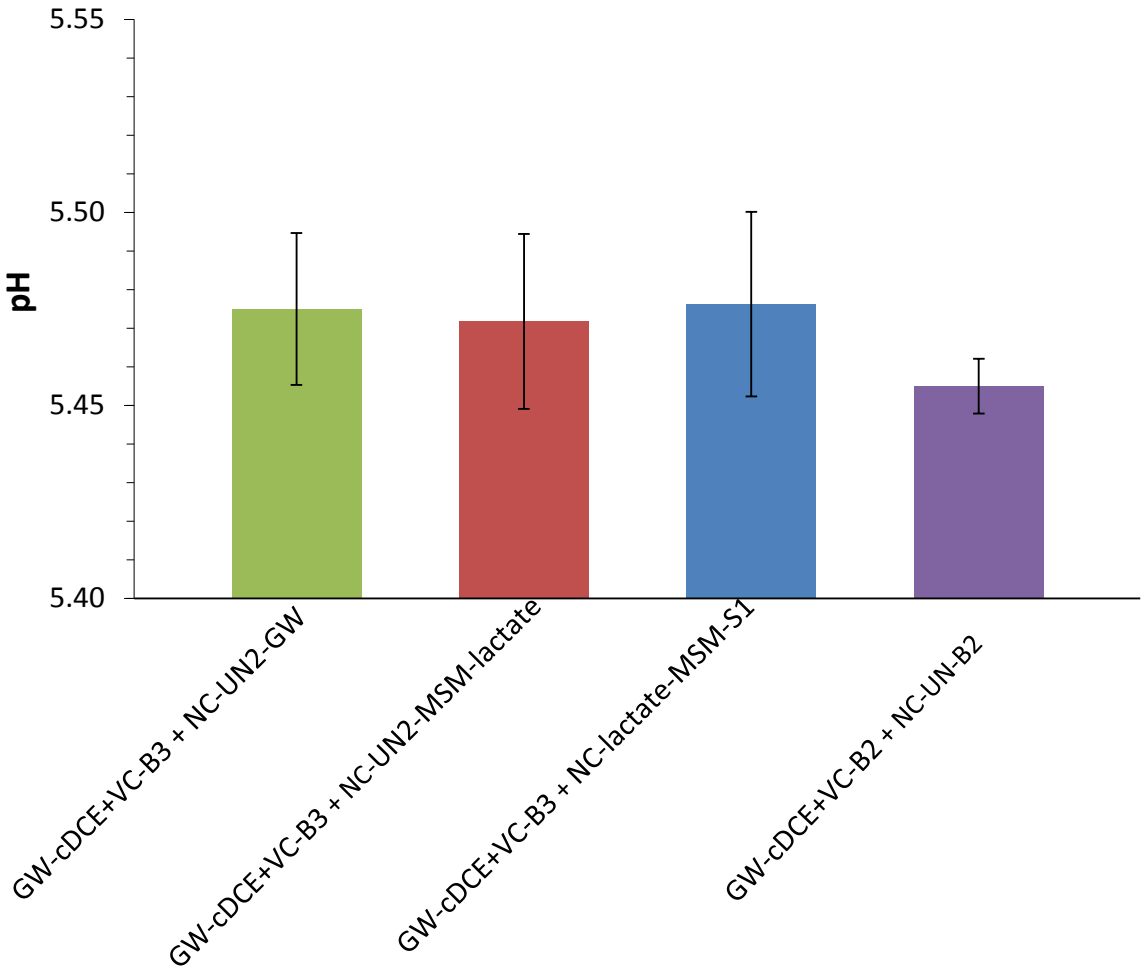

Figure 3.34 Average pH levels for the Set I combined cultures; error bars represent one standard deviation for triple bottles.

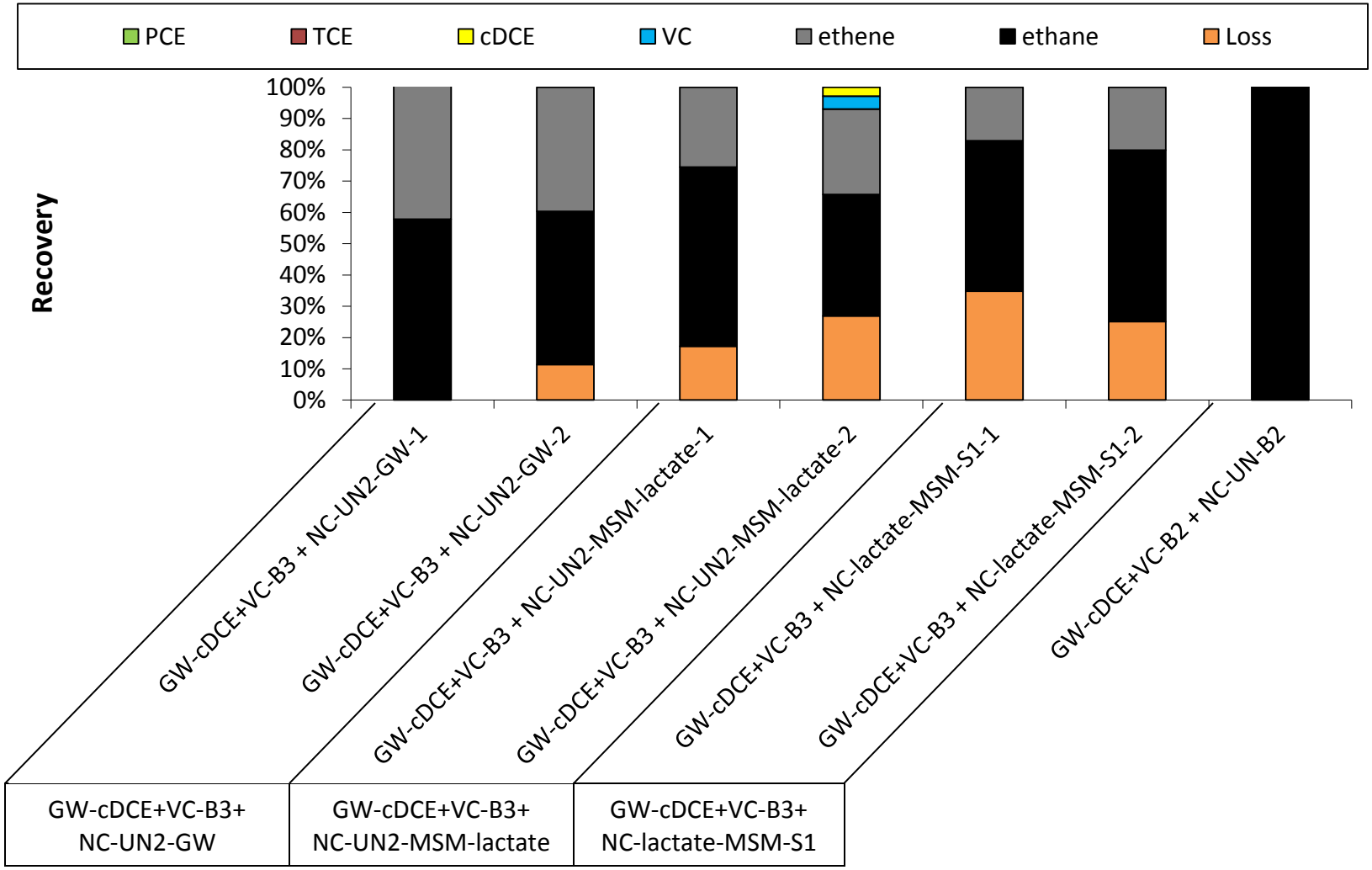

Figure 3.35 Average distribution of dechlorination products for the combined cultures, Set I, based on the total amount of PCE added and the amount present at the final sampling point. Groupings below the bottle names correspond to the treatments shown in Figure 3.34.

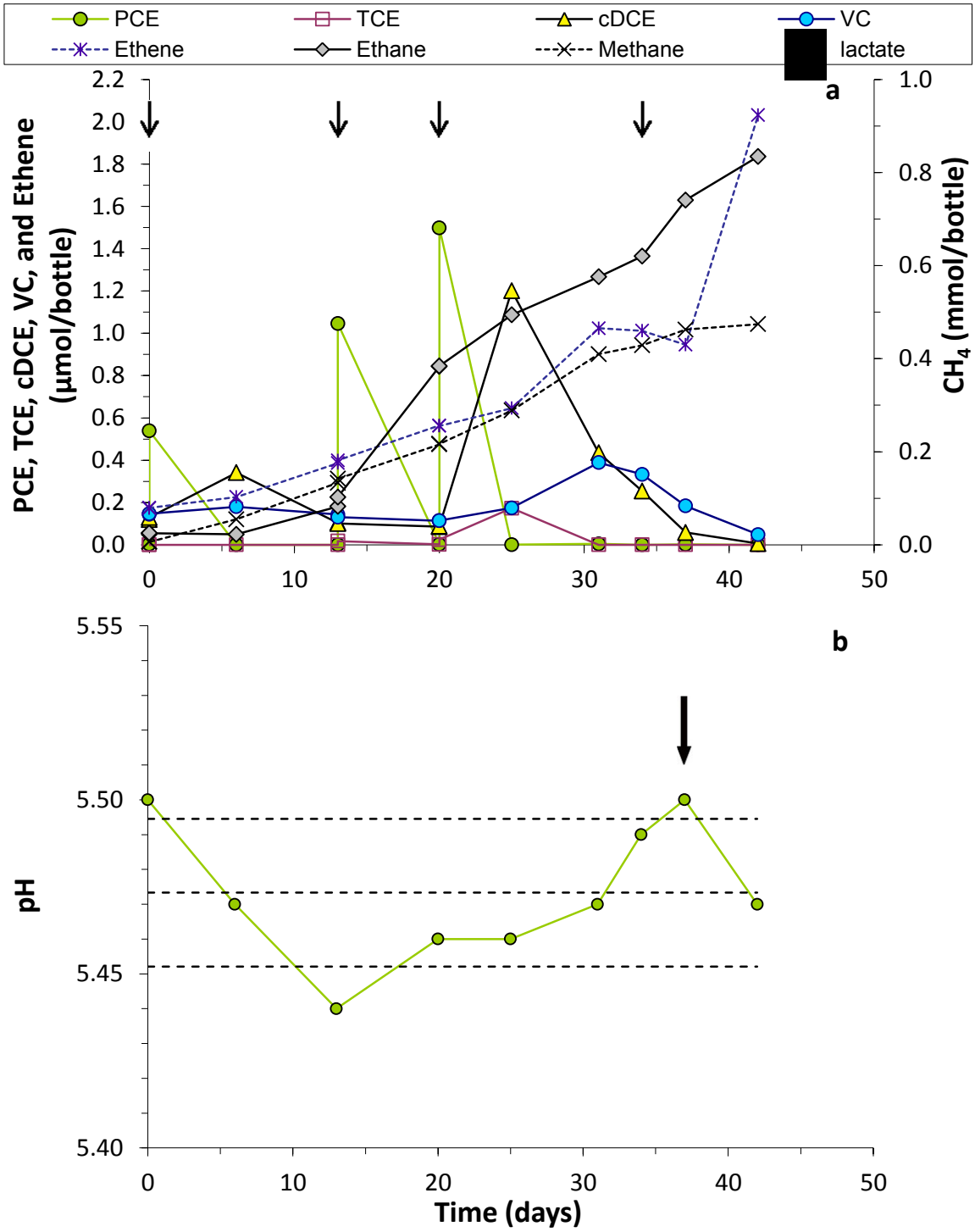

Figure 3.36 Results for a lactate-amended combined enrichment culture, Set I, serum bottle #1 (GW-cDCE+VC-B3+NC-UN2-GW-1) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

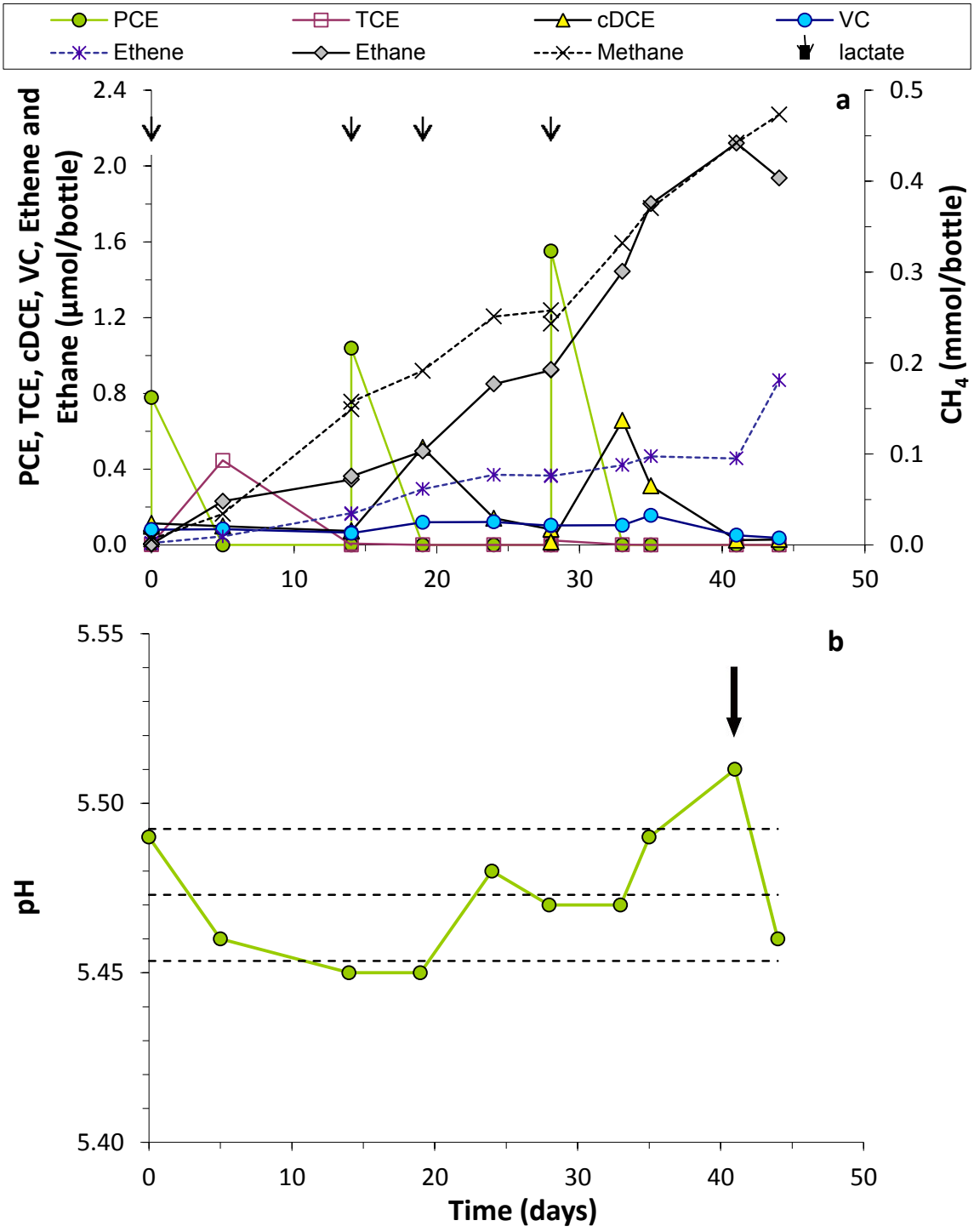

Figure 3.37 Results for a lactate-amended combined enrichment culture, Set I, serum bottle #1 (GW-cDCE+VC-B3+NC-UN2-MSM-lactate-1) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

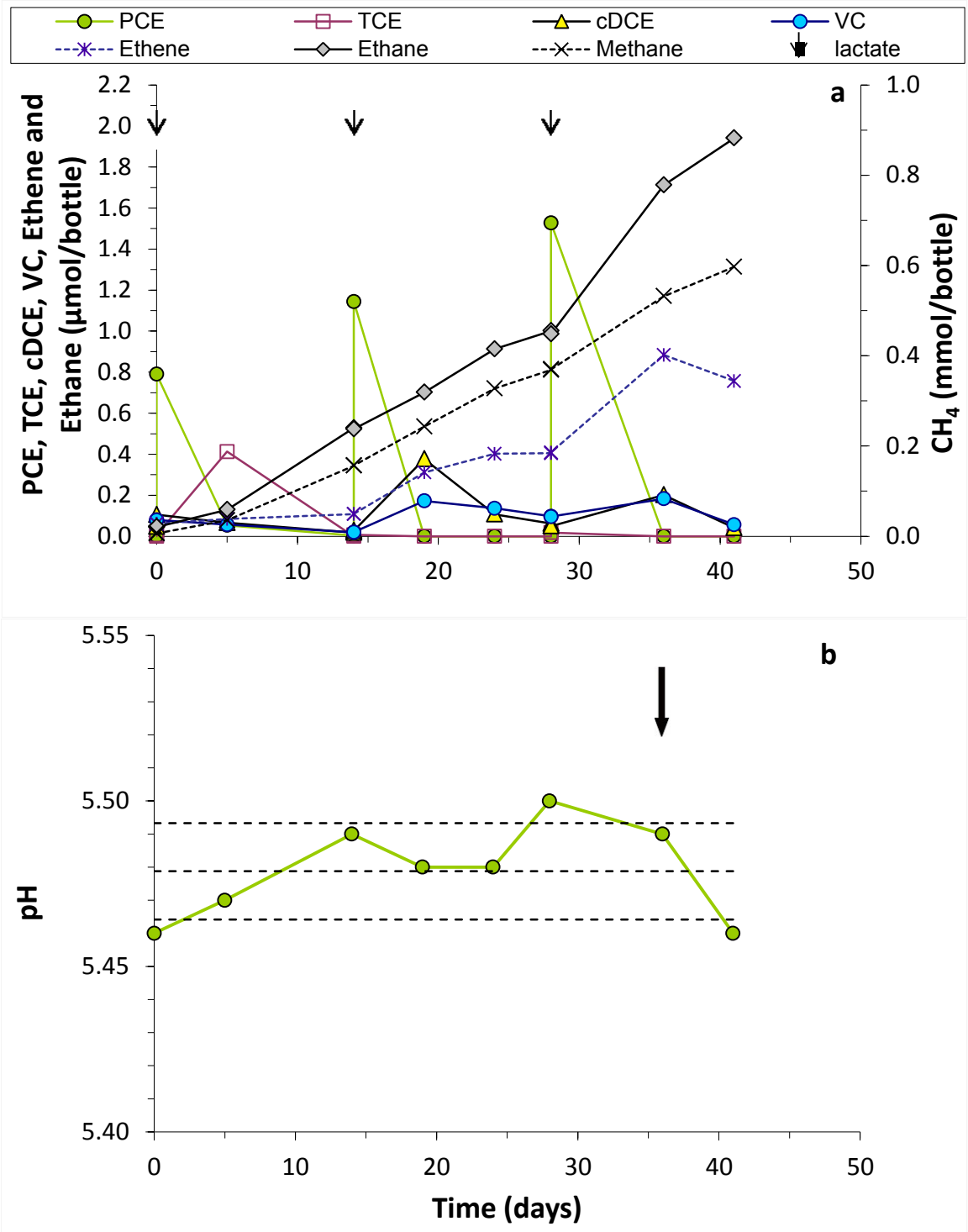

Figure 3.38 Results for a lactate-amended combined enrichment culture, Set I, serum bottle #2 (GW-cDCE+VC-B3+NC-lactate-MSM-S1-2) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

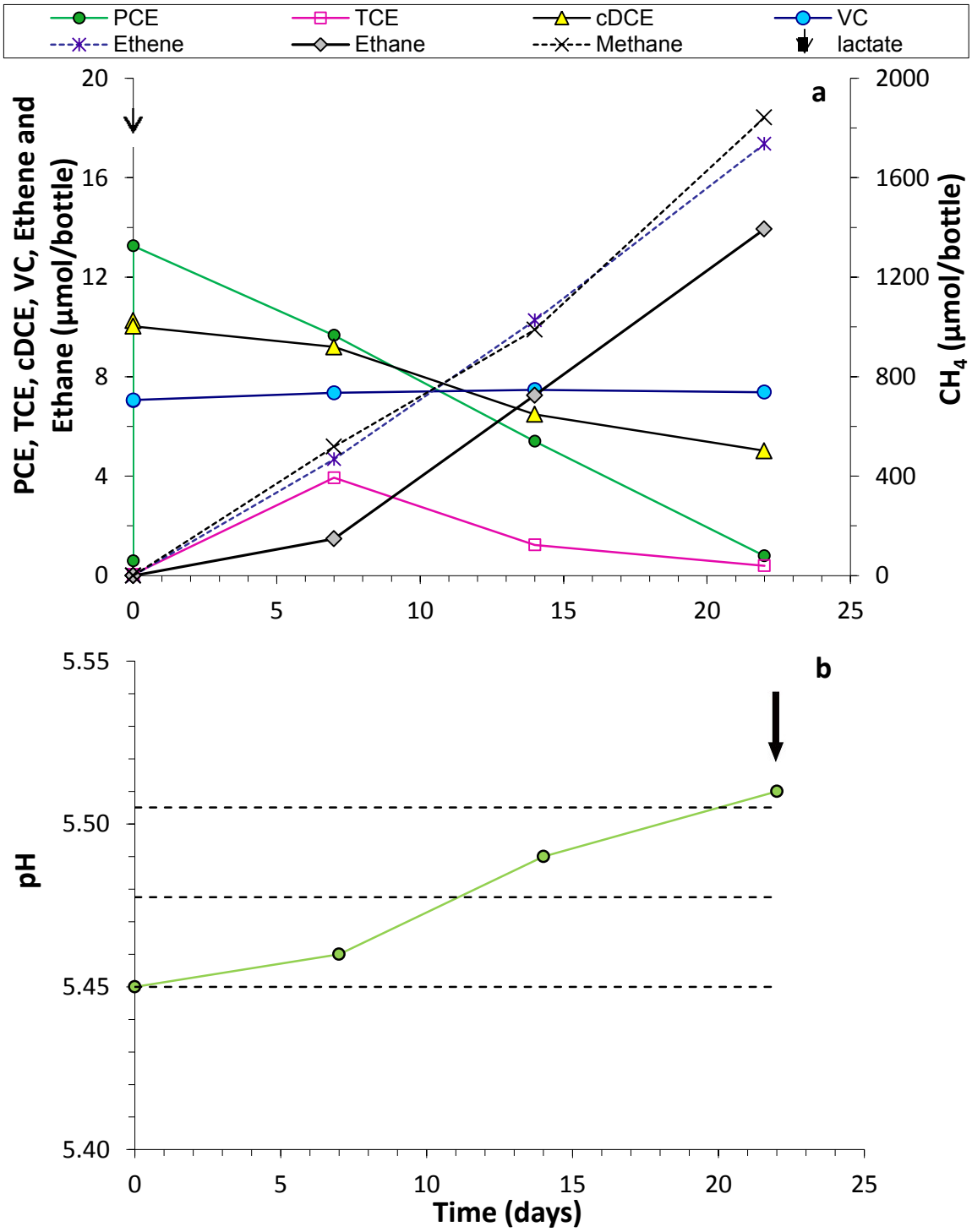

Figure 3.39 Results for a lactate-amended combined enrichment culture, Set I, serum bottle #2 (GW-cDCE+VC-B2+NC-UN-B2) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

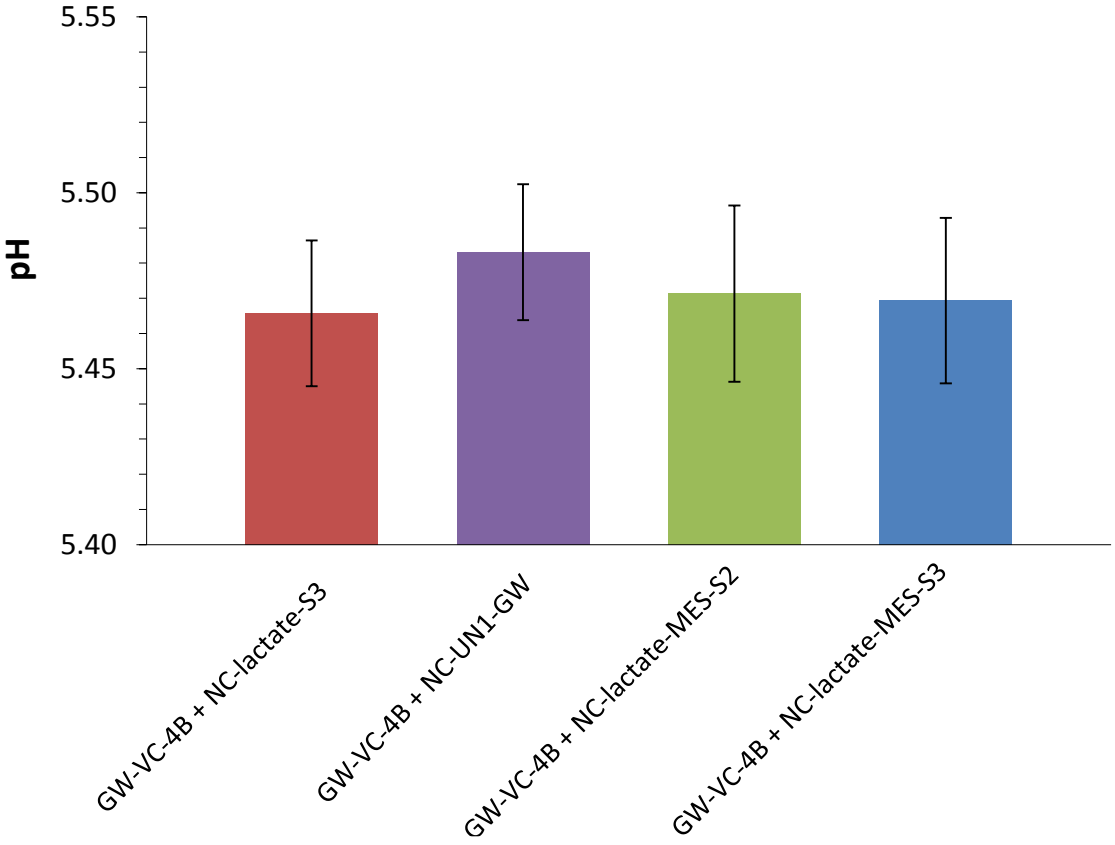

Figure 3.40 Average pH levels for the combined cultures, Set II; error bars represent one standard deviation for triple bottles.

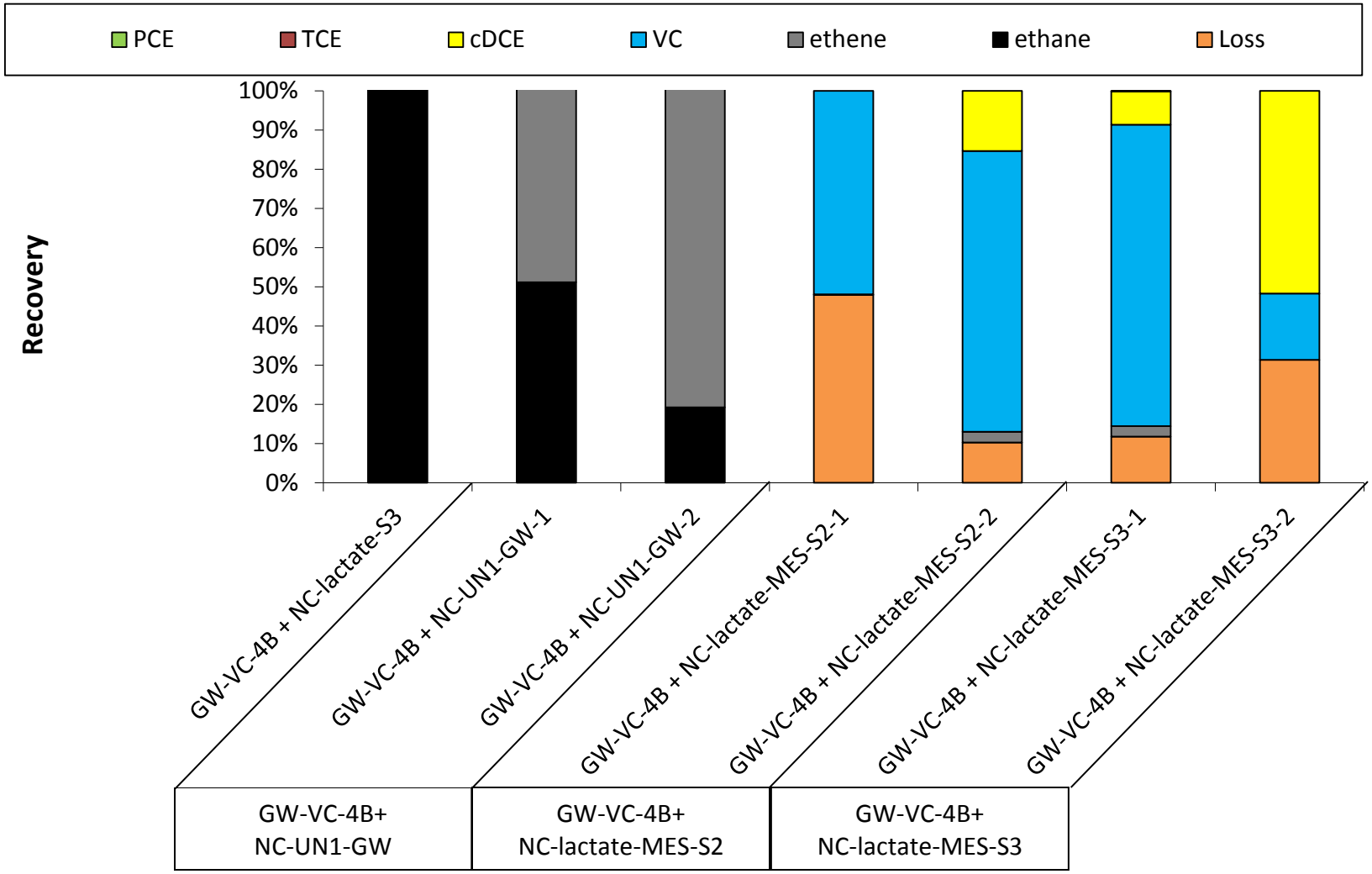

Figure 3.41 Average distribution of dechlorination products for the combined cultures, Set II, based on the total amount of PCE added and the amount present at the final sampling point. Groupings below the bottle names correspond to the treatments shown in Figure 3.40.

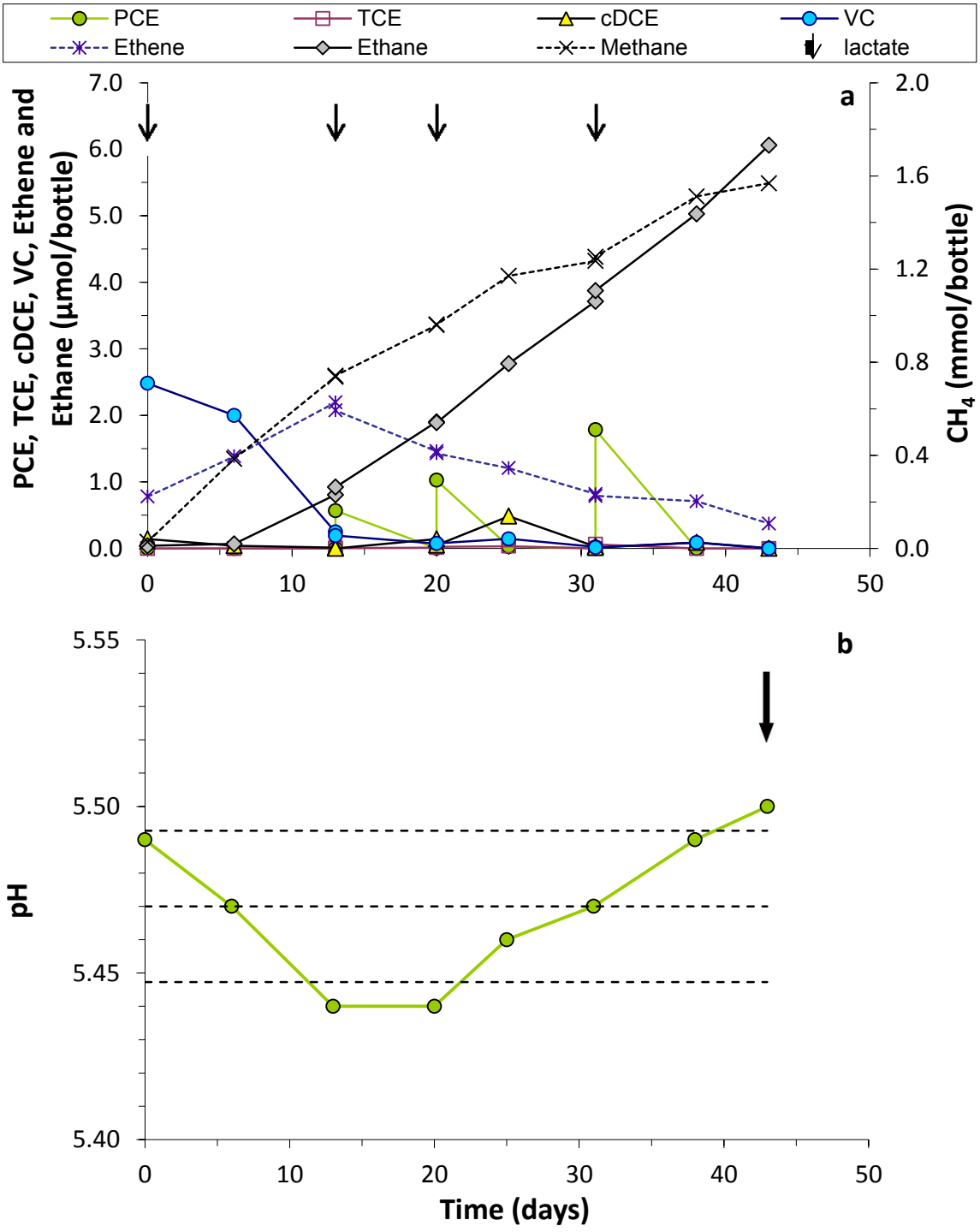

Figure 3.42 Results for a lactate-amended combined enrichment culture, Set II, serum bottle #1 (GW-VC-4B+NC-lactate-S3) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

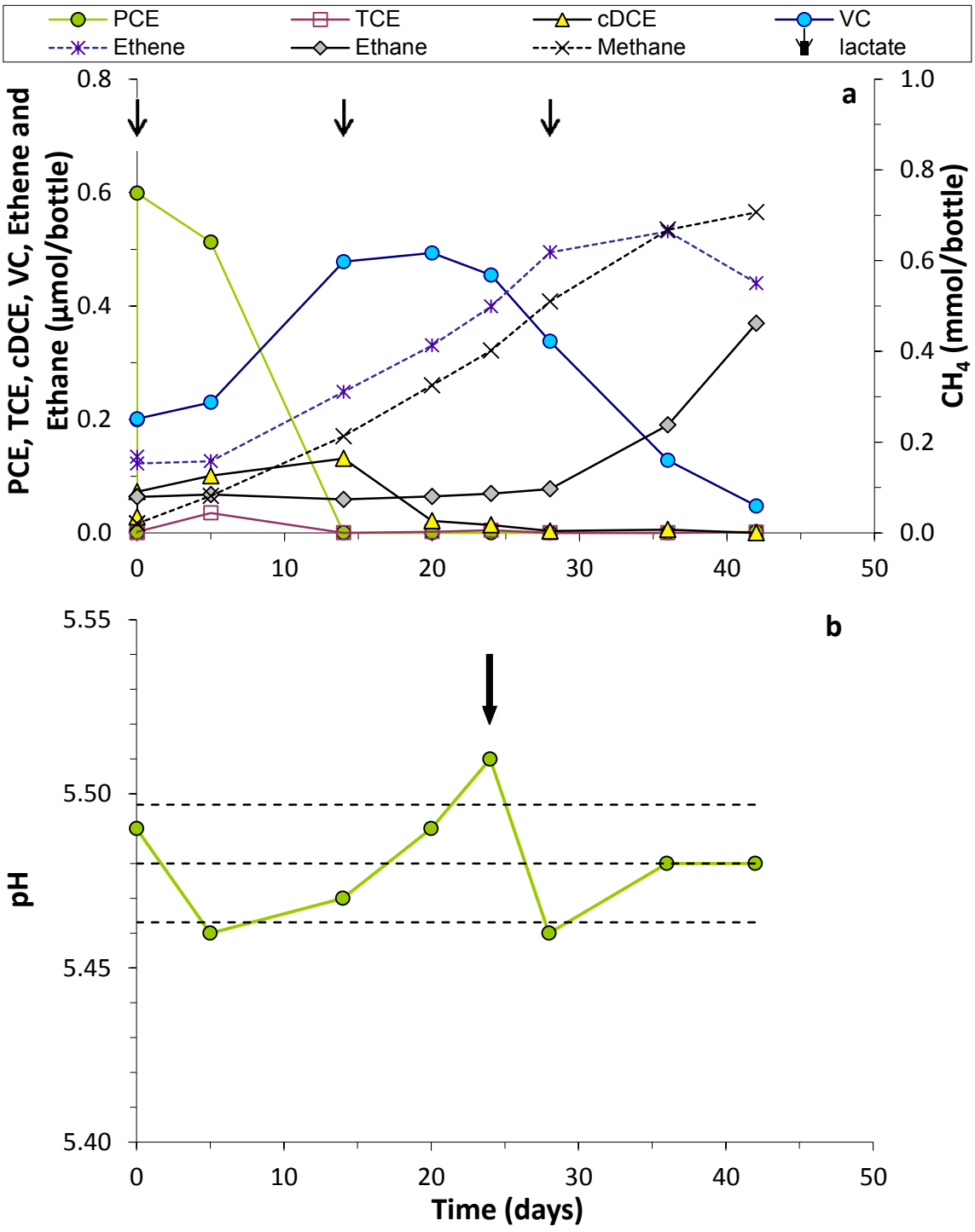

Figure 3.43 Results for a lactate-amended combined enrichment culture, Set II, serum bottle #1 (GW-VC-4B+NC-UN1-GW-1) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

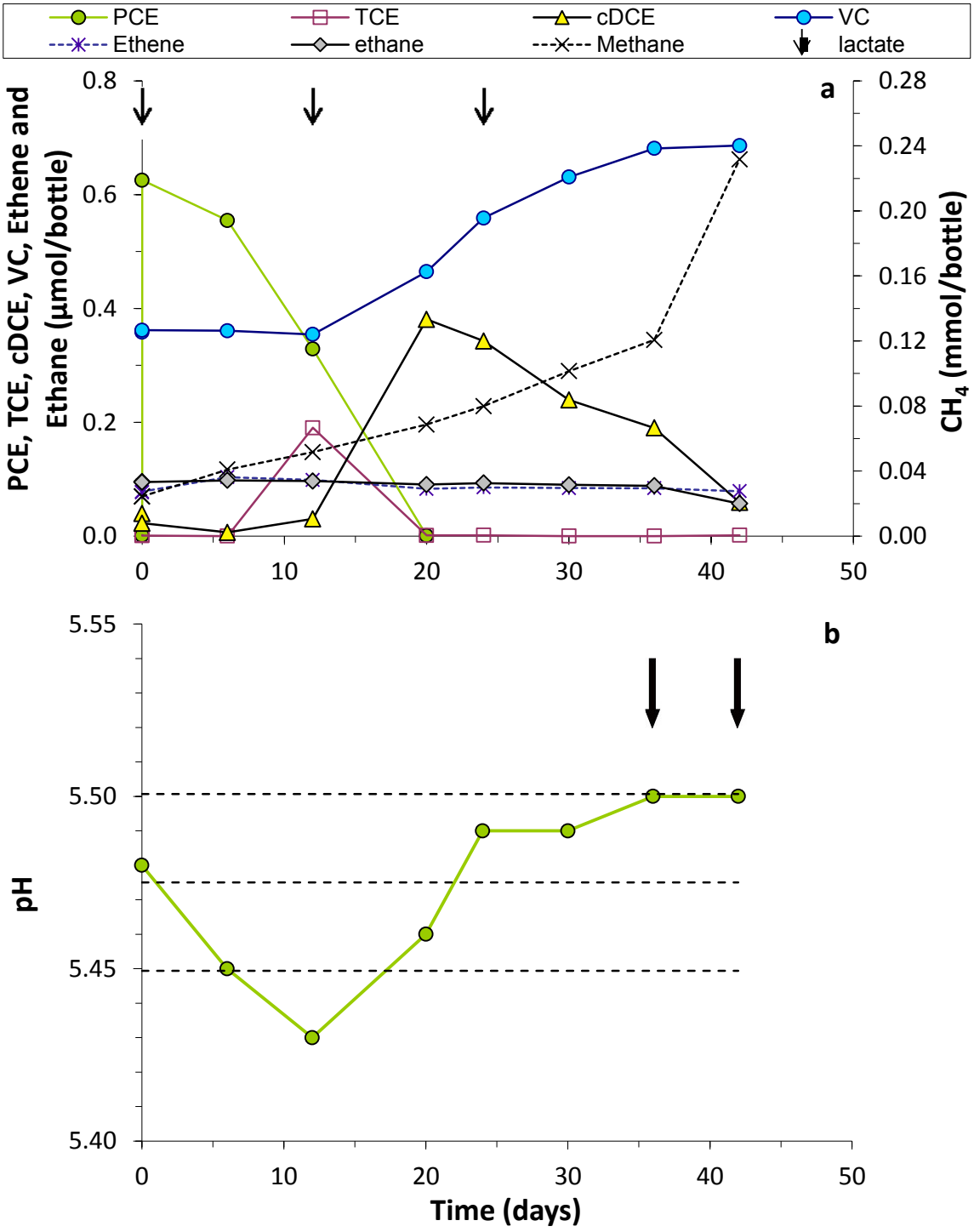

Figure 3.44 Results for a lactate-amended combined enrichment culture, Set II, serum bottle #1 (GW-VC-4B+NC-lactate-MES-S2-1) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

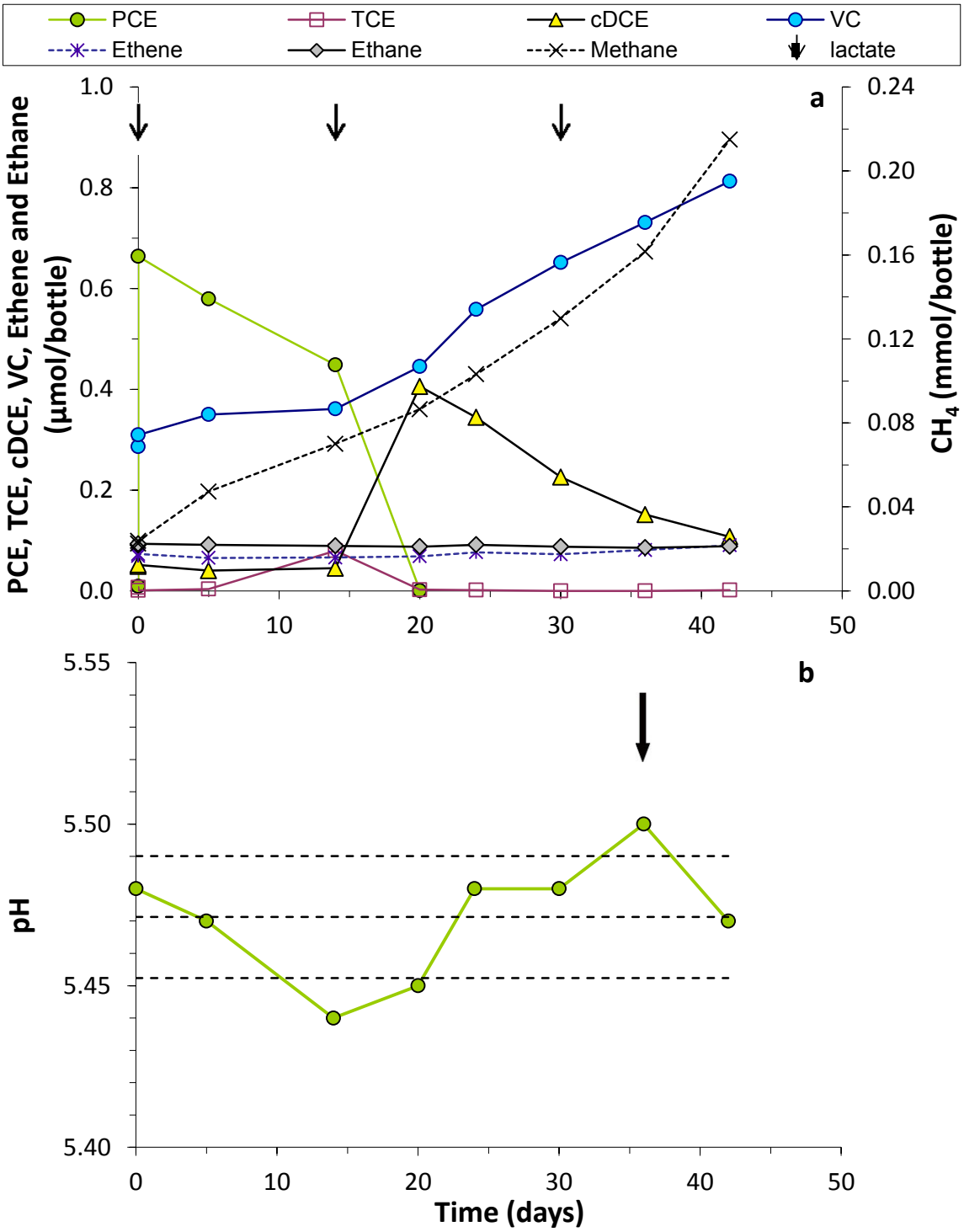

Figure 3.45 Results for a lactate-amended combined enrichment culture, Set II, serum bottle #1 (GW-VC-4B+NC-lactate-MES-S3-1) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

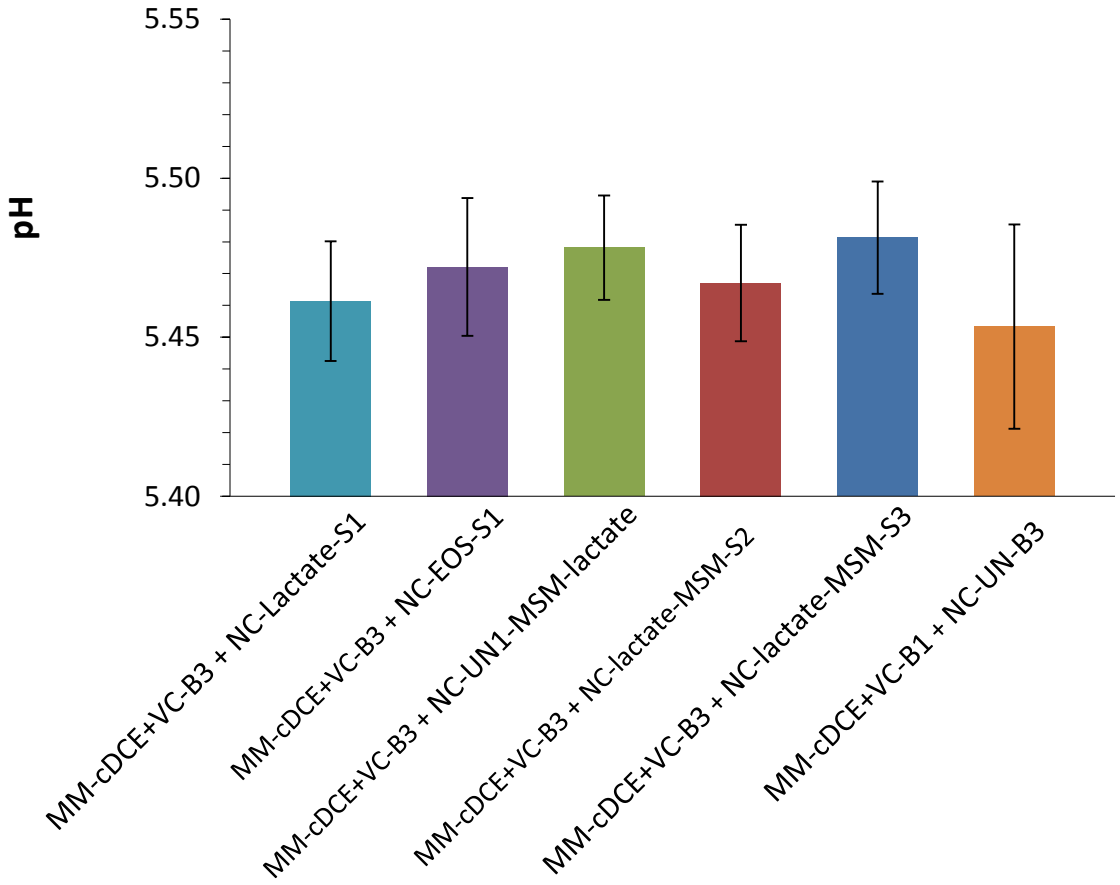

Figure 3.46 Average pH level for combined cultures, Set III; error bars represent one standard deviation for triple bottles.

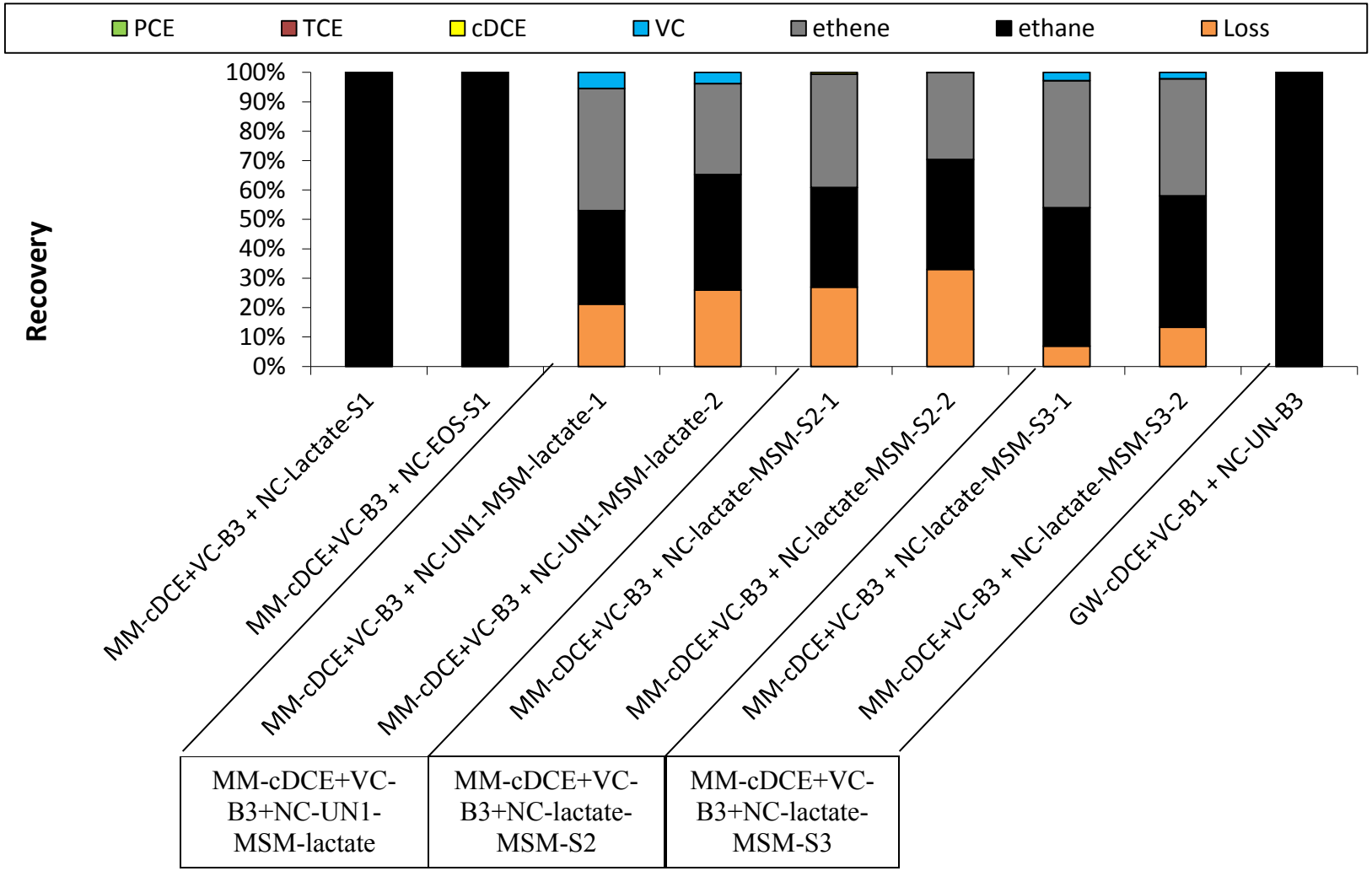

Figure 3.47 Average distribution of dechlorination products for the combined cultures, Set III, based on the total amount of PCE added and the amount present at the final sampling point. Groupings below the bottle names correspond to the treatments shown in Figure 3.46.

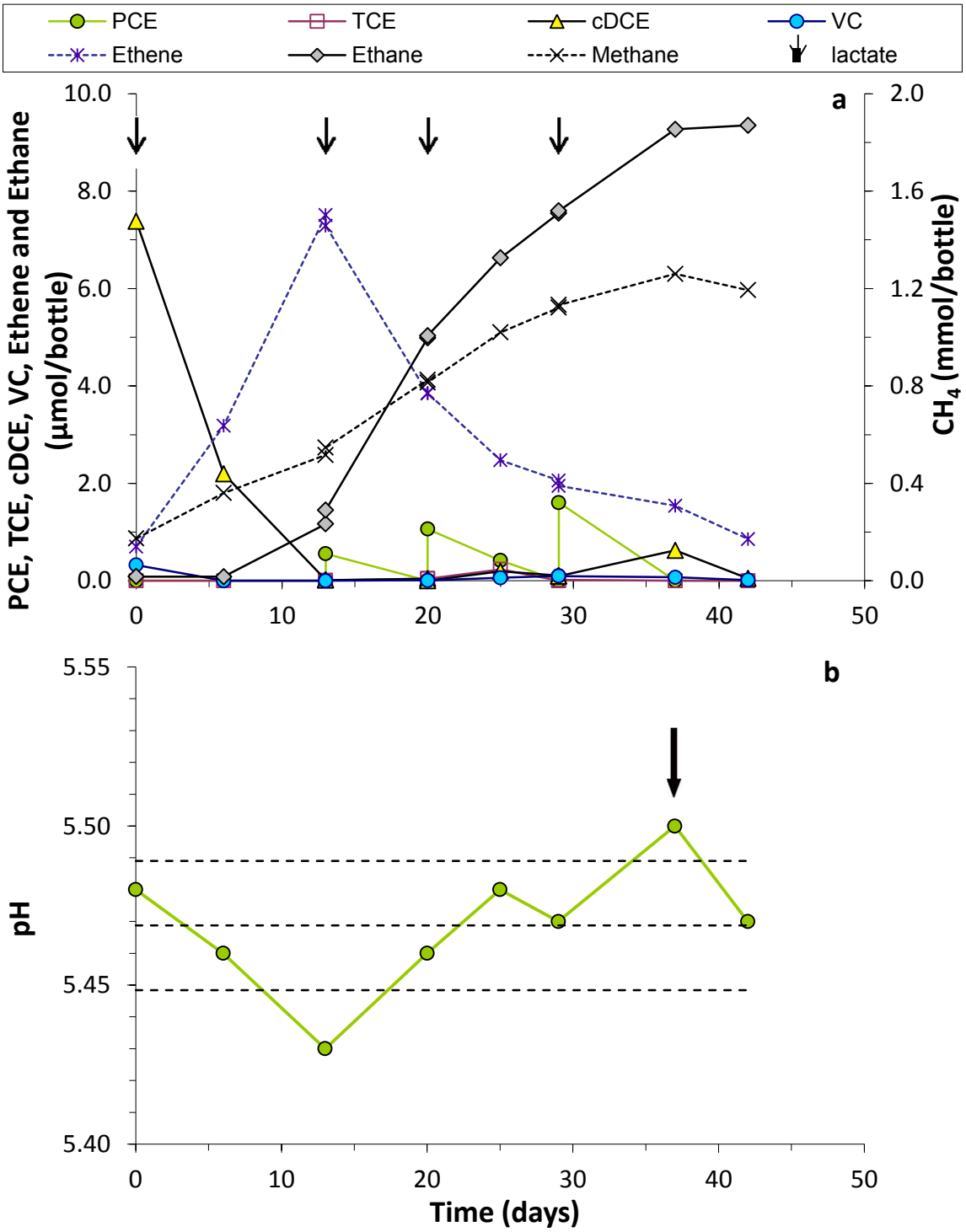

Figure 3.48 Results for a lactate-amended combined enrichment culture, Set III, serum bottle #1 (MM-cDCE+VC-B3+NC-lactate-S1) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

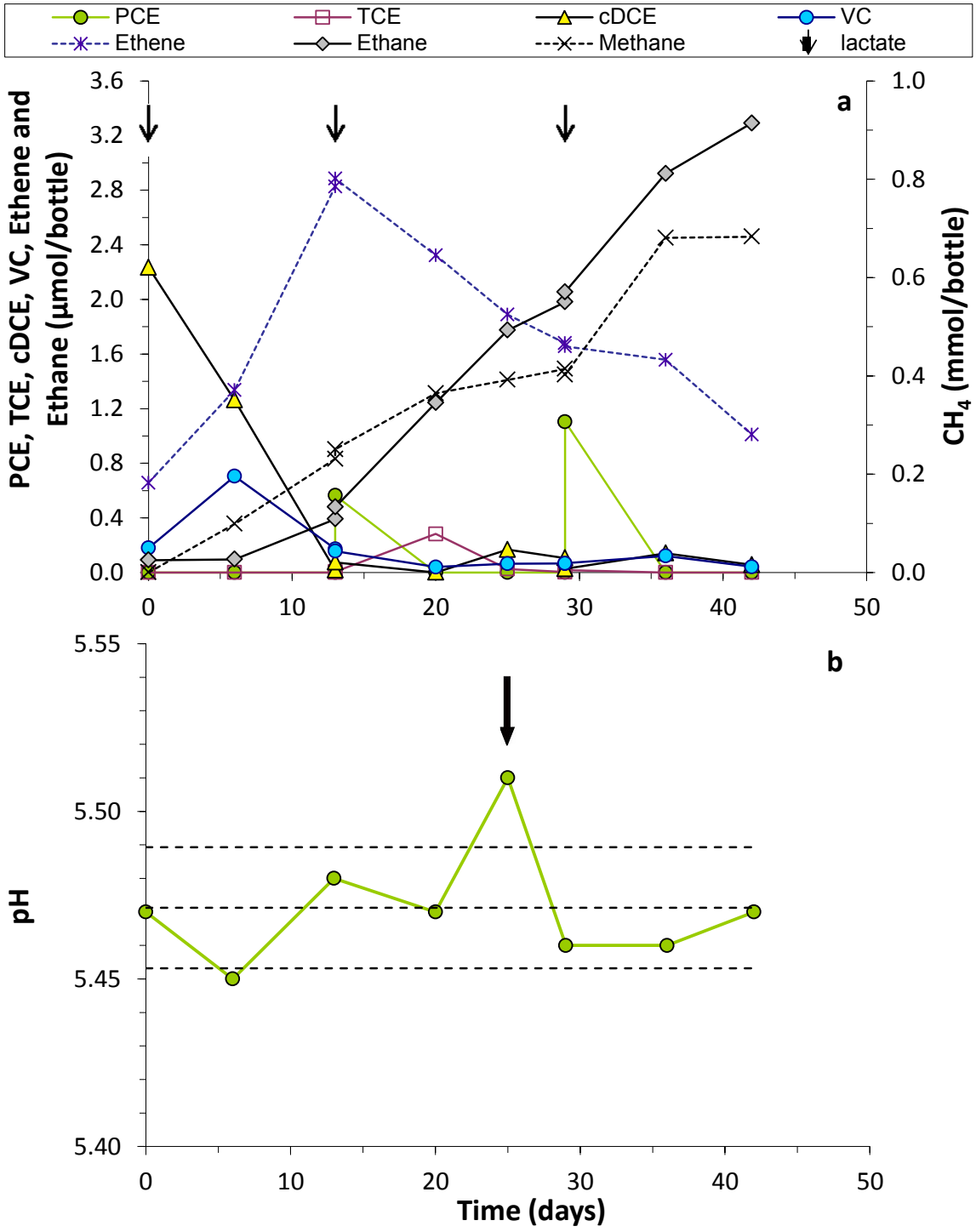

Figure 3.49 Results for a lactate-amended combined enrichment culture, Set III, serum bottle #1 (MM-cDCE+VC-B3+NC-EOS-S1) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

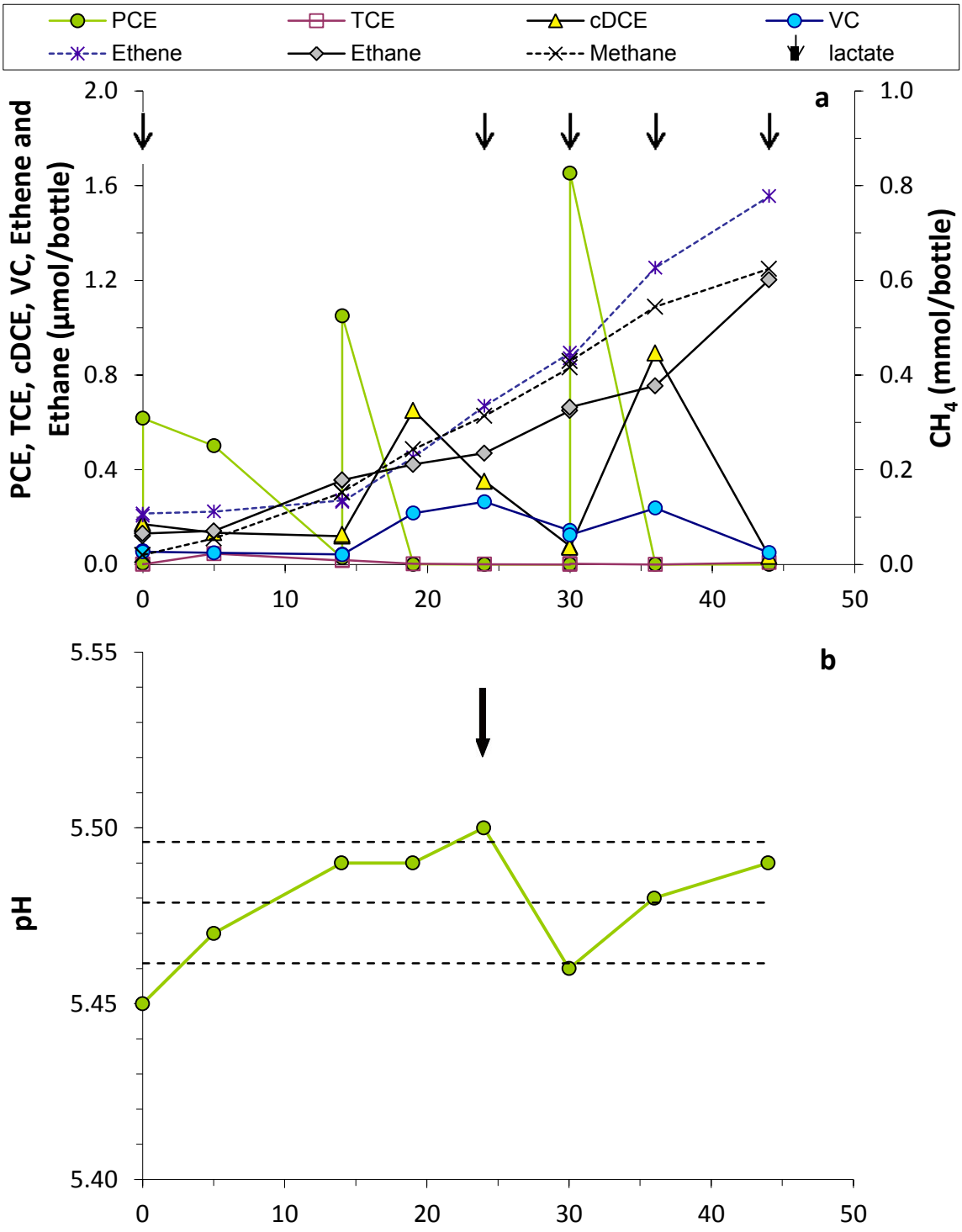

Figure 3.50 Results for a lactate-amended combined enrichment culture, Set III, serum bottle #1 (MM-cDCE+VC-B3+NC-UN1-MSM-lactate-1) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

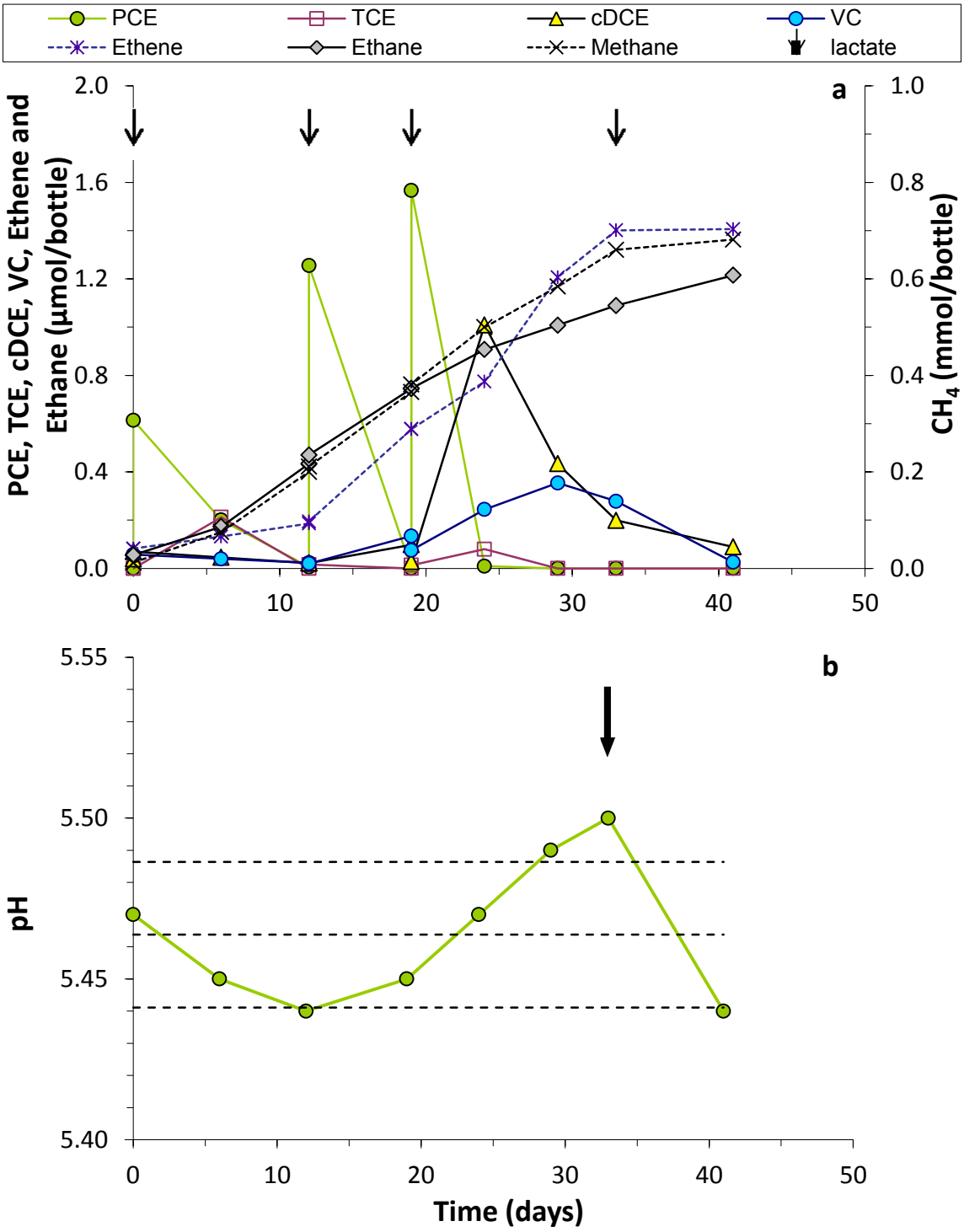

Figure 3.51 Results for a lactate-amended combined enrichment culture, Set III, serum bottle #1 (MM-cDCE+VC-B3+NC-lactate-MSM-S2-1) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

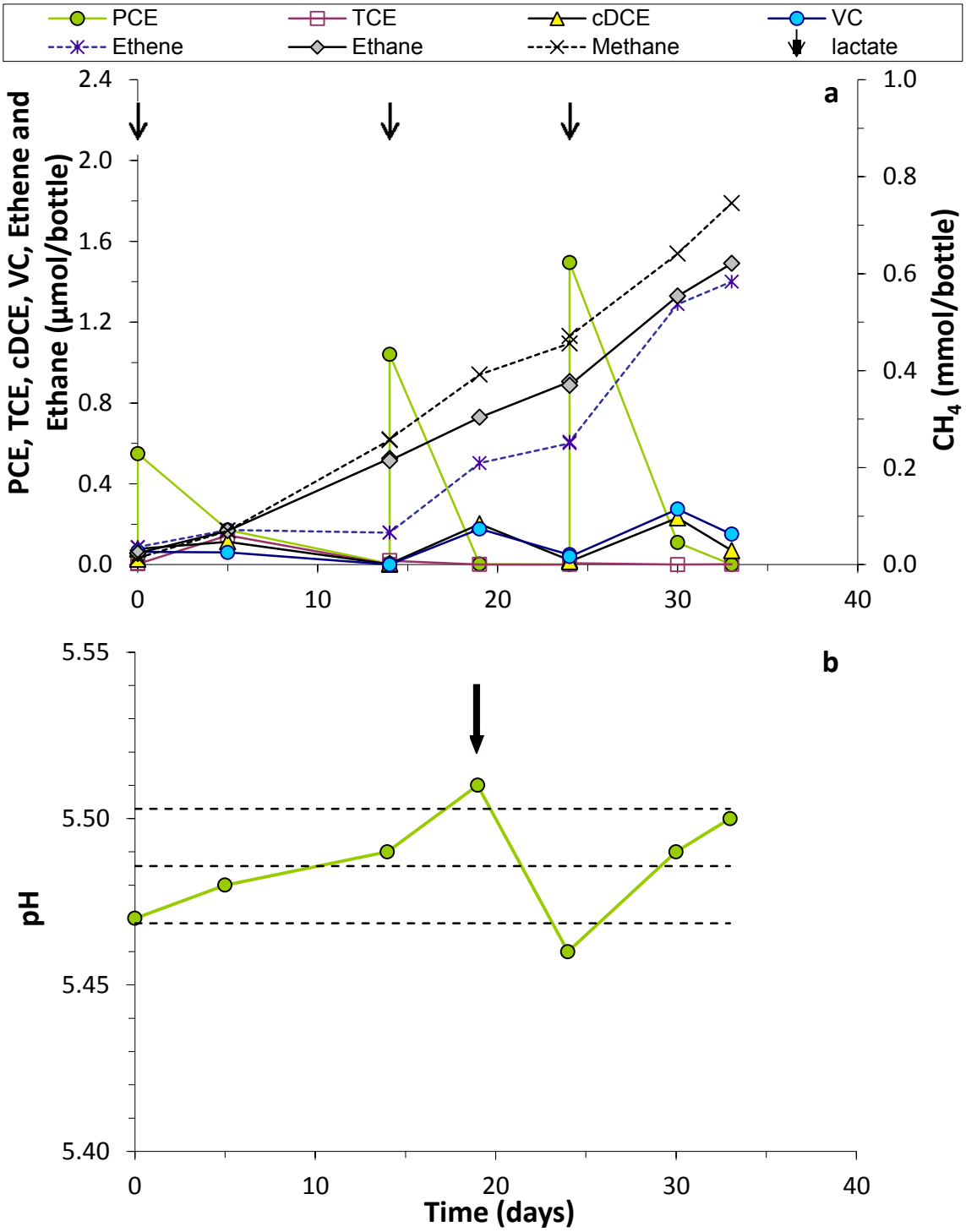

Figure 3.52 Results for a lactate-amended combined enrichment culture, Set III, serum bottle #1 (MM-cDCE+VC-B3+NC-lactate-MSM-S3-1) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

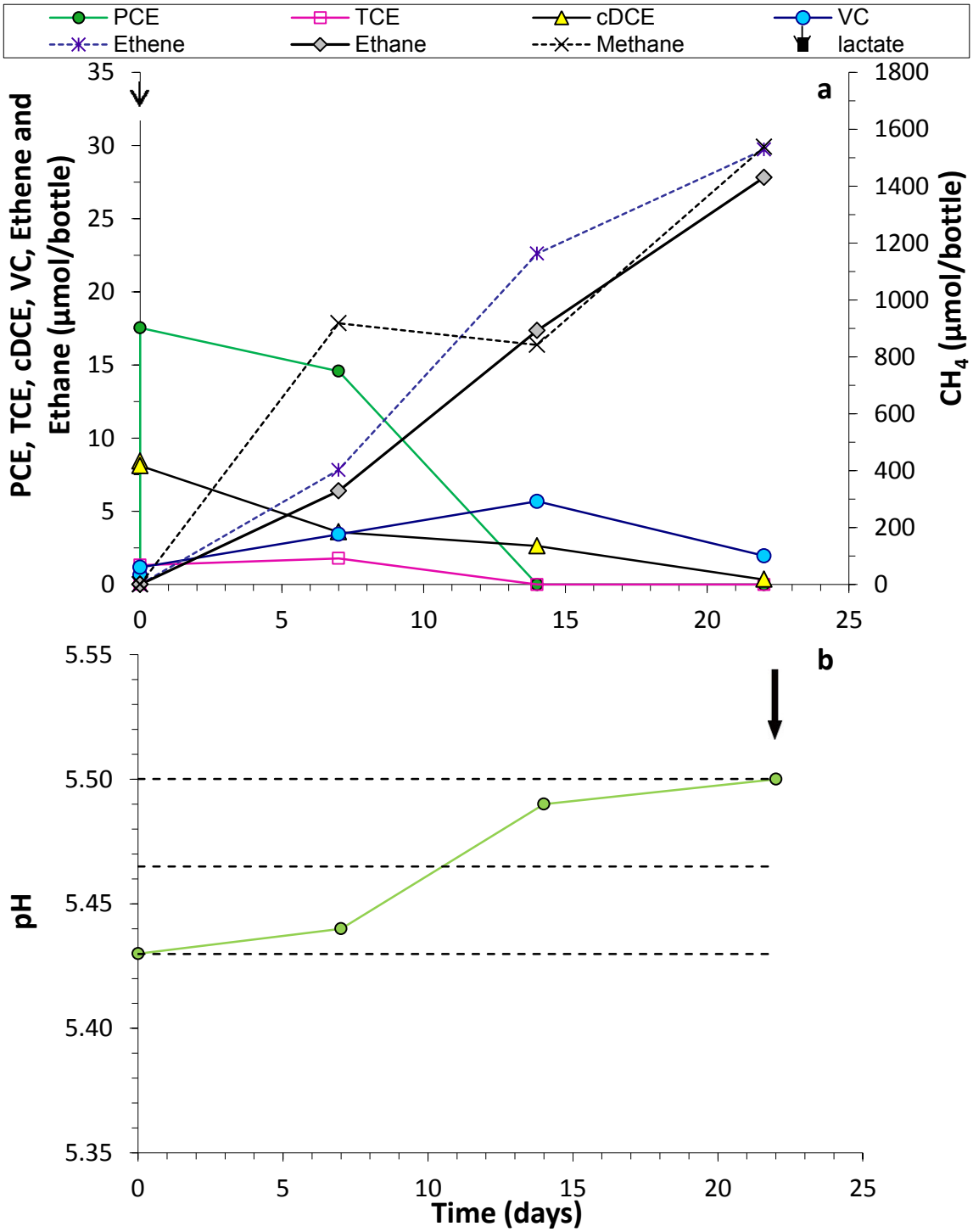

Figure 3.53 Results for a lactate-amended combined enrichment culture, Set III, serum bottle #1 (MM-cDCE+VC-B1+NC-UN-B3) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

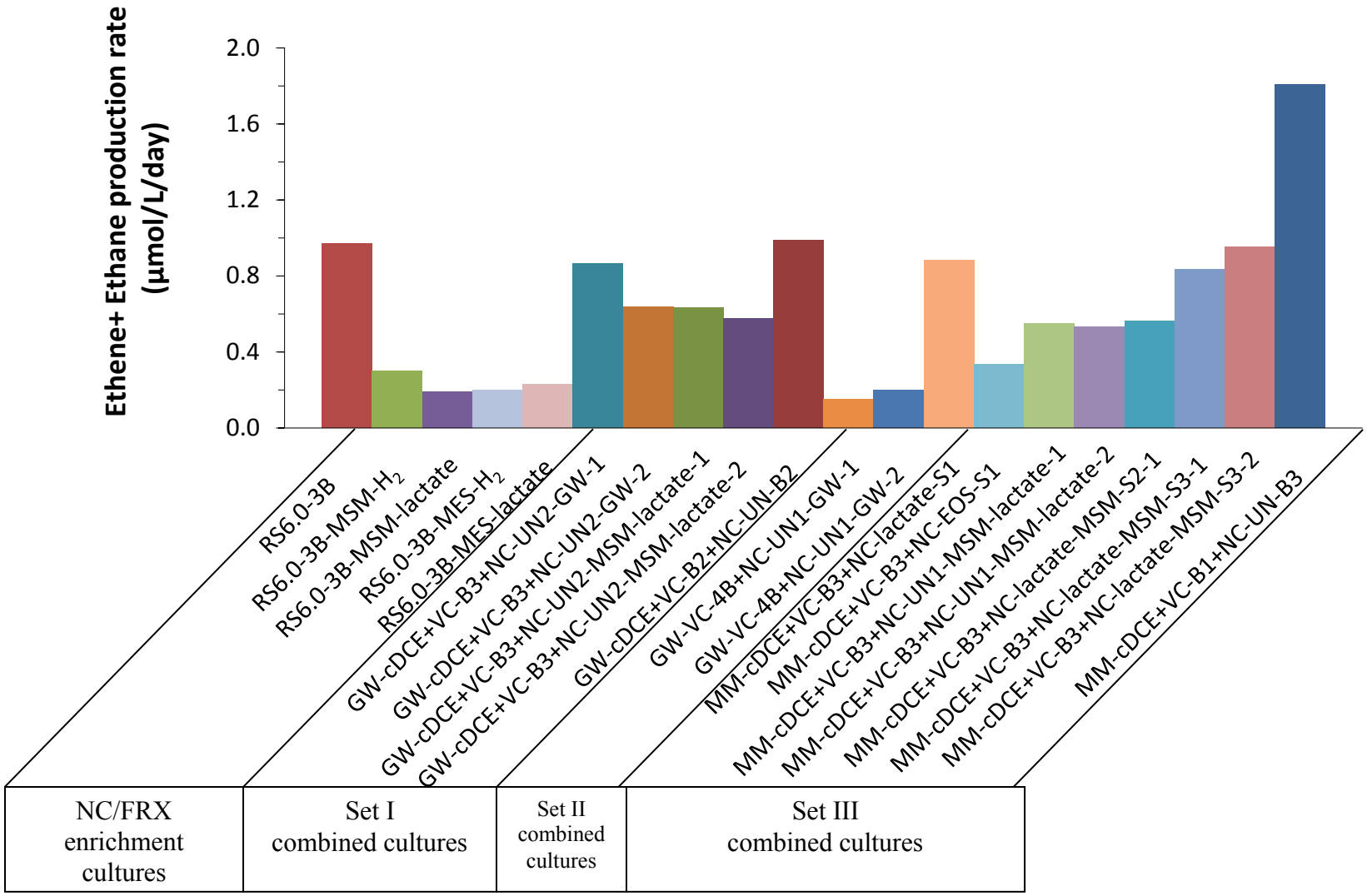

Figure 4.1 Comparison of ethene + ethane production rates.

Appendices

Appendix A

Appendix A-1: MSM Preparation

Reagents and stock solutions needed for media:

- Phosphate solution
In a 100 mL volumetric flask add 5.25 g K_2HPO_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:
   10 mL phosphate solution
   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 A-2: MES Preparation

Reagents and stock solutions needed for media:

- 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 0.625 g K_2HPO_4. Fill to 100 mL with DDI water.

- MES solution
In a 100 mL volumetric flask add 29.87 g MES. 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:
    10 mL phosphate solution I
    10 mL phosphate solution II
    10 mL MES solution
    10 mL salt solution
    2 mL trace metals solution
    2 mL magnesium sulfate solution
    1 mL redox solution
    945 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 or phosphate solution I.

Appendix B

Estimation of Amount of Electron Donor Needed for Microcosms

Milliequivalents Required

The amount of electron required for 0.5 mL PCE addition:

$$\frac{150\ mg\ PCE}{L} \times \frac{1\ mmol}{166\ mg} \times \frac{8\ meq}{mmol} \times \frac{0.0005\ L}{bottle} = 0.003614\ meq/\ bottle$$

The amount of electron required for 0.1 mL cDCE addition:

$$\frac{3500\ mg\ cDCE}{L} \times \frac{1\ mmol}{96.94\ mg} \times \frac{4\ meq}{mmol} \times \frac{0.0001\ L}{bottle} = 0.01444\ meq/\ bottle$$

The amount of electron required for 0.1 mL VC addition:

$$\frac{1\ mmol\ VC}{24.36\ mL} \times \frac{0.1\ mL}{bottle} \times \frac{2\ meq}{mmol} = 0.00821\ meq/\ bottle$$

Lactate (for stock solution, 34000 mg 60% syrup per 100 mL stock solution) required for 0.5 mL PCE saturated water

$$\frac{0.003614\ meq}{bottle} \times \frac{1\ mmol}{12\ meq} \times \frac{112\ mg\ NaLac}{mmol} \times \frac{1\ mg\ syrup}{0.6\ mg\ NaLac} \times \frac{1000\ \mu L}{mL} \times \frac{100\ mL\ stock\ solution}{34000\ mg\ syrup} \times 100\ = 16.5\ \mu L$$

EOS (for stock solution, 1:10 dilution of 50% EOS) required for 0.5 mL PCE saturated water

$$\frac{0.003614\ meq}{bottle} \times \frac{1\ mmol}{46\ meq} \times \frac{128\ mg}{1\ mmol} \times \frac{1\ mL}{1000\ mg} \times \frac{1\ mg\ EOS\ solution}{0.5\ mg\ EOS} \times \frac{10\ mg\ EOS\ stock\ solution}{1\ mg\ EOS\ solution} \times 100\ \times \frac{1000\ \mu L}{1\ mL}$$

$= 20\ \mu L$

H_2 required for 0.5 mL PCE saturated water

$$\frac{0.003614\ meq}{bottle} \times \frac{1\ mmol}{2\ meq} \times \frac{24.36\ mL}{1\ mmol\ H2} = 0.044\ mL$$

NOTE: This amount was rounded up to 0.05 mL, using a 1.0 mL syringe.

Lactate required for 0.1 mL cDCE saturated water

$$\frac{0.01444\ meq}{bottle} \times \frac{1\ mmol}{12\ meq} \times \frac{112\ mg\ NaLac}{mmol} \times \frac{1\ mg\ syrup}{0.6\ mg\ NaLac} \times \frac{1000\ \mu L}{mL} \times \frac{100\ mL\ stock\ solution}{34000\ mg\ syrup} \times 100 = 66.1\ \mu L$$

Lactate required for 0.1 mL VC gas

$$\frac{0.00821\ meq}{bottle} \times \frac{1\ mmol}{12\ meq} \times \frac{112\ mg\ NaLac}{mmol} \times \frac{1\ mg\ syrup}{0.6\ mg\ NaLac} \times \frac{1000\ \mu L}{mL} \times \frac{100\ mL\ stock\ solution}{34000\ mg\ syrup} \times 100\ = 37\ \mu L$$

Appendix C

GC Response Factors

Table C-1 GC Response Factors Set #2, 50 mL Liquid (15)

Compound	Response Factor (umol/bottle/PA)	R^2
PCE, run #1	2.2433E-06	0.9989
PCE, run #2	2.4528E-06	0.9986
PCE, ave	2.3481E-06	
TCE	4.3124E-06	0.9981
cDCE	7.5926E-06	0.9993
VC	2.6658E-06	0.9998
Ethene	1.8366E-06	0.9996
Methane	3.5741E-06	0.9994

Table C-2 GC Response Factors Set #3, 100 mL Liquid (15)

Compound	Response Factor (umol/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

Appendix D

Calculation for GC response factors for cultures developed in different sizes of bottles

The GC response factors for enrichment cultures with 100 mL liquid and 60 mL headspace and microcosm cultures with 20 g soil and 50 mL media in serum bottle were got from Hickey (15). The GC response factors for other volumes of liquid and headspace are calculated from the equation described below.

The original GC response factor is from Hickey's thesis with 100 mL liquid and 60 mL headspace in serum bottle.

$RF_S \times PA_S = C_{gs} \times V_{gs} + C_{ls} \times V_{ls}$

$C_{ls} = C_{gs}/H_c$

Where RF_S = response factor for GC for the 160 mL serum bottle (µmol/bottle); PA_S= peak area from GC for the 160 mL serum bottle (dimensionless); C_{gs} = concentration in the headspace in the 160 mL serum bottle (µM); V_{gs}= volume of the headspace in the 160 mL serum bottle (L); $C_{l\,s}$= concentration in the aqueous phase in the 160 mL serum bottle (µM); V_{ls}= volume of the aqueous phase in the 160 mL serum bottle (L); H_c = Henry's constant (dimensionless) at 23°C.

For enrichment cultures with 100 mL liquid and 60 mL headspace:

$RF_S \times PA_S = C_{gs} \times V_{gs} + C_{ls} \times V_{ls}$

$$PA_S = \frac{C_{gS} \times (V_{gS} + V_{lS}/H_c)}{RF_S}$$

For my cultures, if a microcosms culture in serum bottle was transferred to an enrichment culture in a big bottle, the total amounts of chlorinated ethenes, ethane and methane are still same. Therefore,

$RFs \times PA_S$= the total amounts of chlorinated ethenes $= RF_B \times PA_B$

$$\text{Therefore, } RF_S \times \frac{C_{gS} \times (V_{gS} + V_{lS}/H_c)}{RF_S} = RF_B \times \frac{C_{gB} \times (V_{gB} + V_{lB}/H_c)}{RF_B}$$

Where RF_B = response factor for GC for the big bottle (µmol/bottle); PA_B= peak area from GC for the big bottle (dimensionless); C_{gB} = concentration in the headspace in the big bottle (µM); V_{gB}= volume of the headspace in the big bottle (L); C_{lB} = concentration

in the aqueous phase in the big bottle (μM); V_l= volume of the aqueous phase in the big bottle (L); H_c = Henry's constant (dimensionless) at 23°C.

So, $C_{gS}\times(V_{gS}+V_{lS}/H_c)= C_{gB}\times(V_{gB}+V_{lB}/H_c)$

Therefore, $\frac{C_{gS}}{C_{gB}}=\frac{V_{gB}+V_{lB}/H_c}{V_{gS}+V_{lS}/H_c}$

Because the concentration of chlorinated ethenes, ethane and methane in headspace is proportional to the peak area, therefore,

$$\frac{C_{gS}}{C_{gB}}=\frac{V_{gB}+V_{lB}/H_c}{V_{gS}+V_{lS}/H_c}=\frac{PA_S}{PA_B}$$

Because, $\frac{PA_S}{PA_B}=\frac{RF_B}{RF_S}$,

Therefore, $\frac{RF_B}{RF_S}=\frac{V_{gB}+V_{lB}/H_c}{V_{gS}+V_{lS}/H_c}$

So, $RF_B=\frac{(V_{gB}+V_{lB}/H_c)\times RF_S}{V_{gS}+V_{lS}/H_c}$

The volume of the big bottle has the same ratio of headspace to liquid as serum bottle,

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

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

$V_B= a\times V_S$

where V_B= volume of the big bottle; V_S=volume of the serum bottle, therefore

$$RF_B=\frac{a(V_{gS}+V_{lS}/H_c)\times RF_S}{V_{gS}+V_{lS}/H_c}= a\times RF_S= RF_S\frac{V_B}{V_S}$$

Appendix E: Results for Replicate Bottles Pressented in Chpater 3

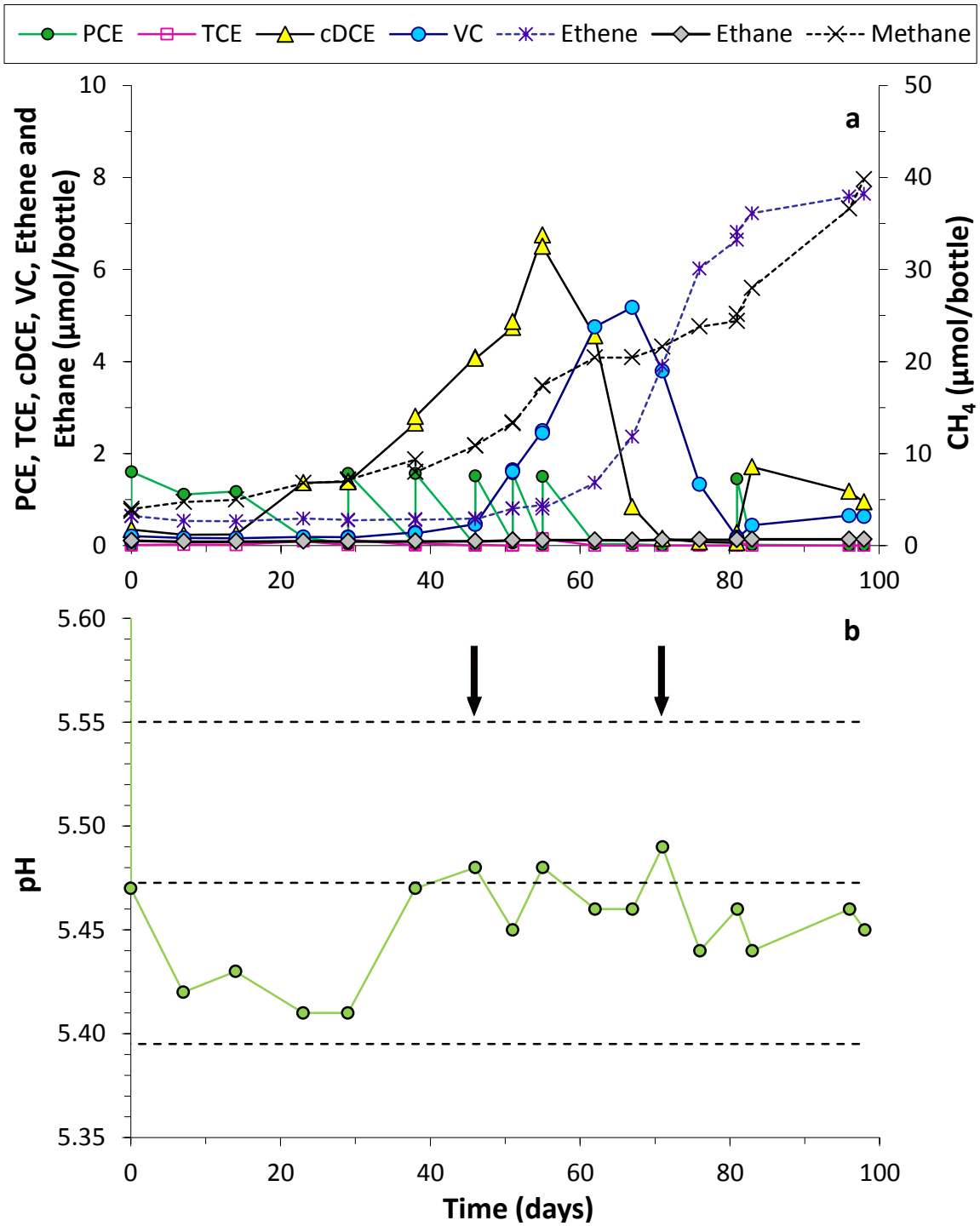

Figure E.1 Results for an unamended NC/HH microcosm, serum bottle #2 (NC-UN-S2) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

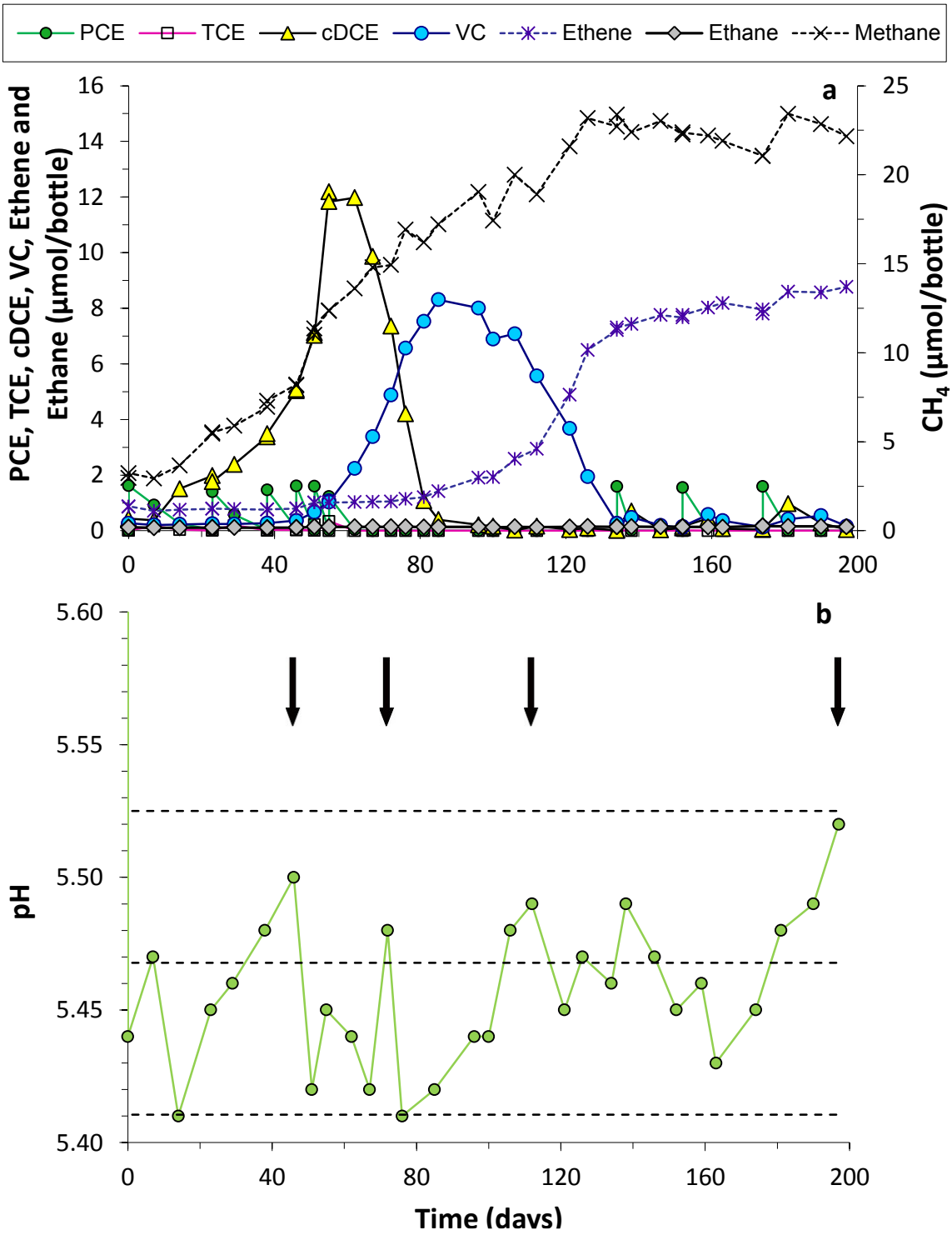

Figure E.2 Results for an unamended NC/HH microcosm, serum bottle #3 (NC-UN-S3) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

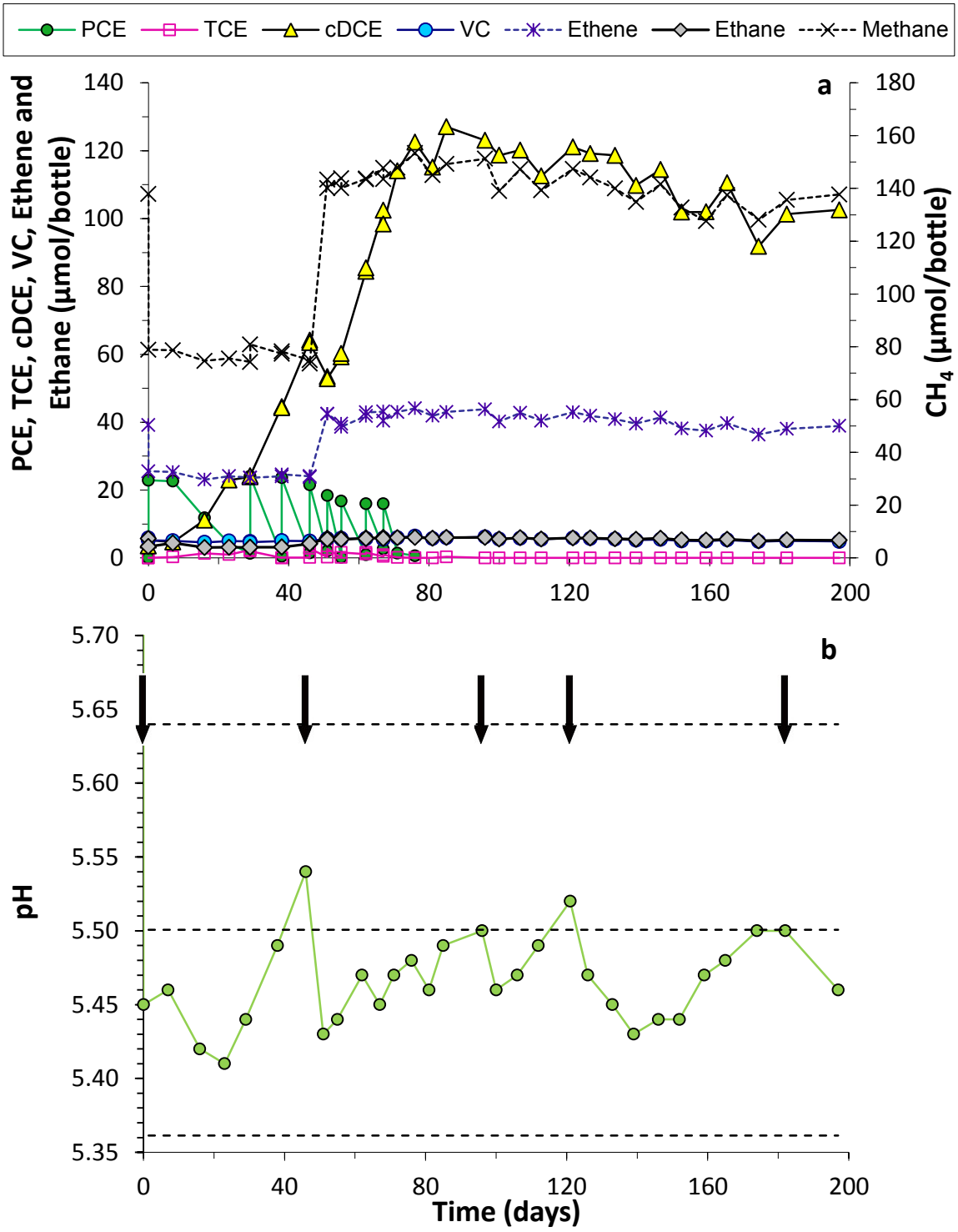

Figure E.3 Results for an unamended NC/HH microcosm, 2.6 L bottle #1 (NC-UN-B1) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

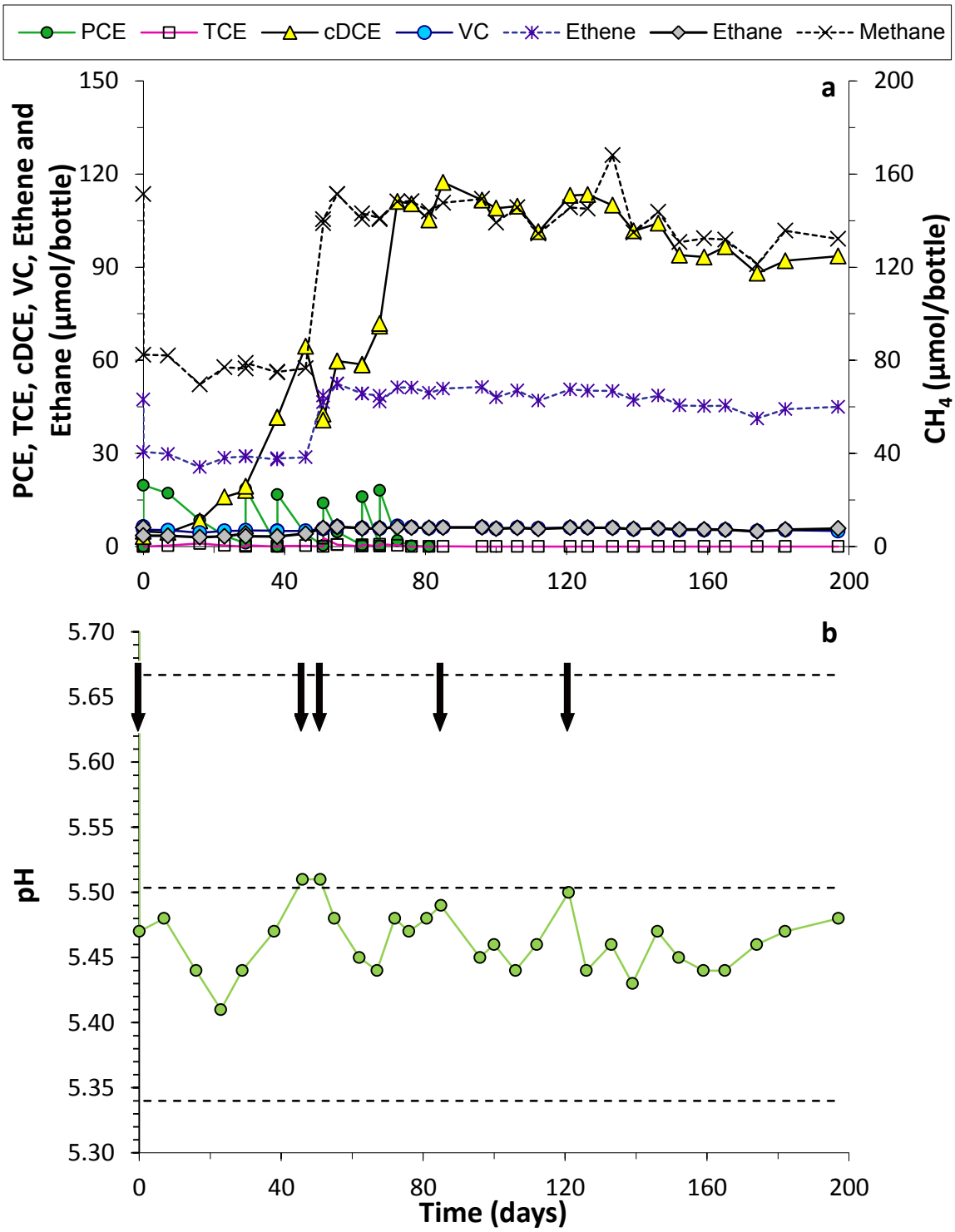

Figure E.4 Results for an unamended NC/HH microcosm, 2.6 L bottle #3 (NC-UN-B3) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid.

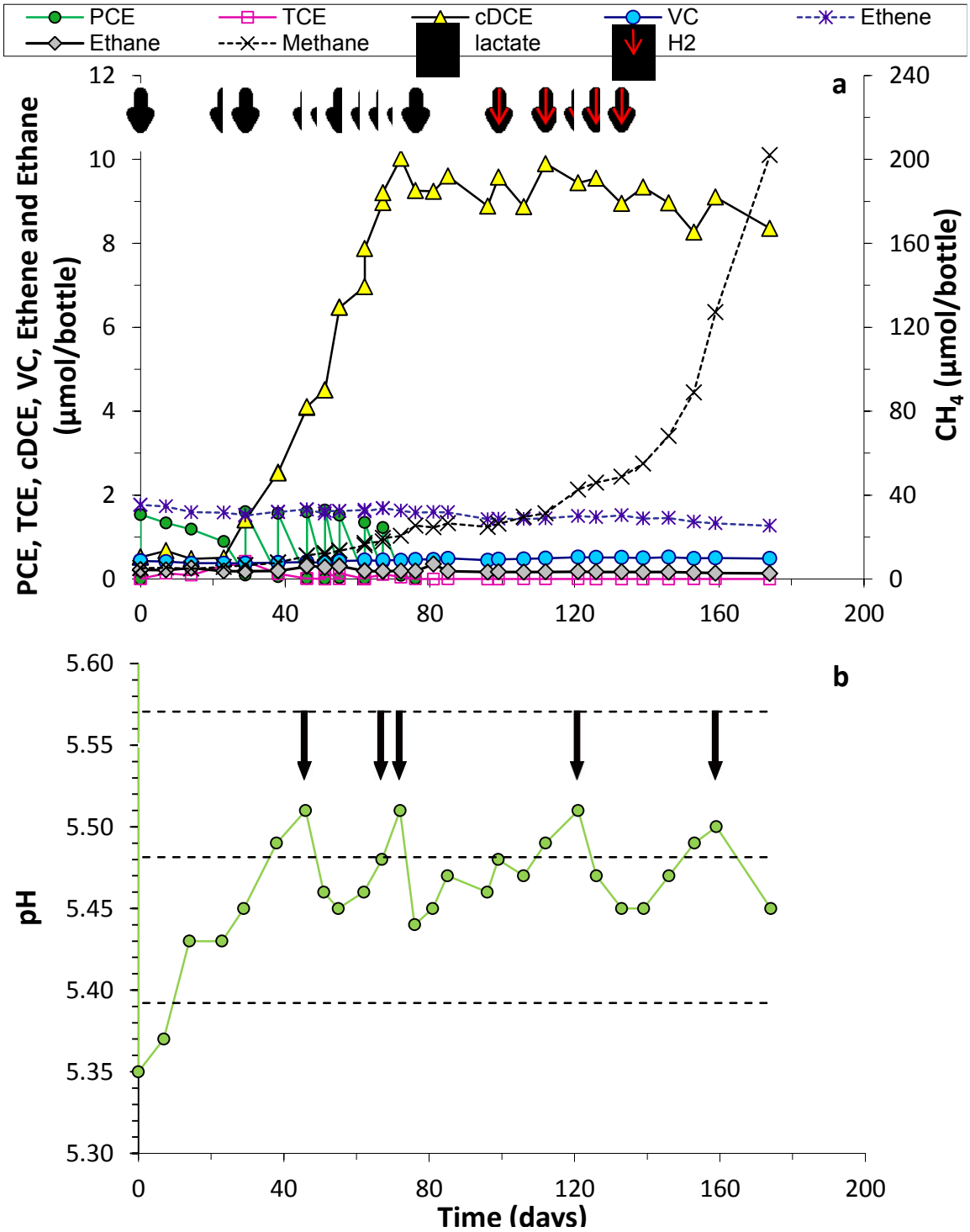

Figure E.5 Results for a lactate-amended NC/HH microcosm, serum bottle #1 (NC-lactate-S1) for **a)** VOCs and **b)** pH; the dashed horizontal lines represent the average ± standard deviation; arrows indicate addition of phosphoric acid. Hydrogen replaced lactate as the electron donor starting on day 99.

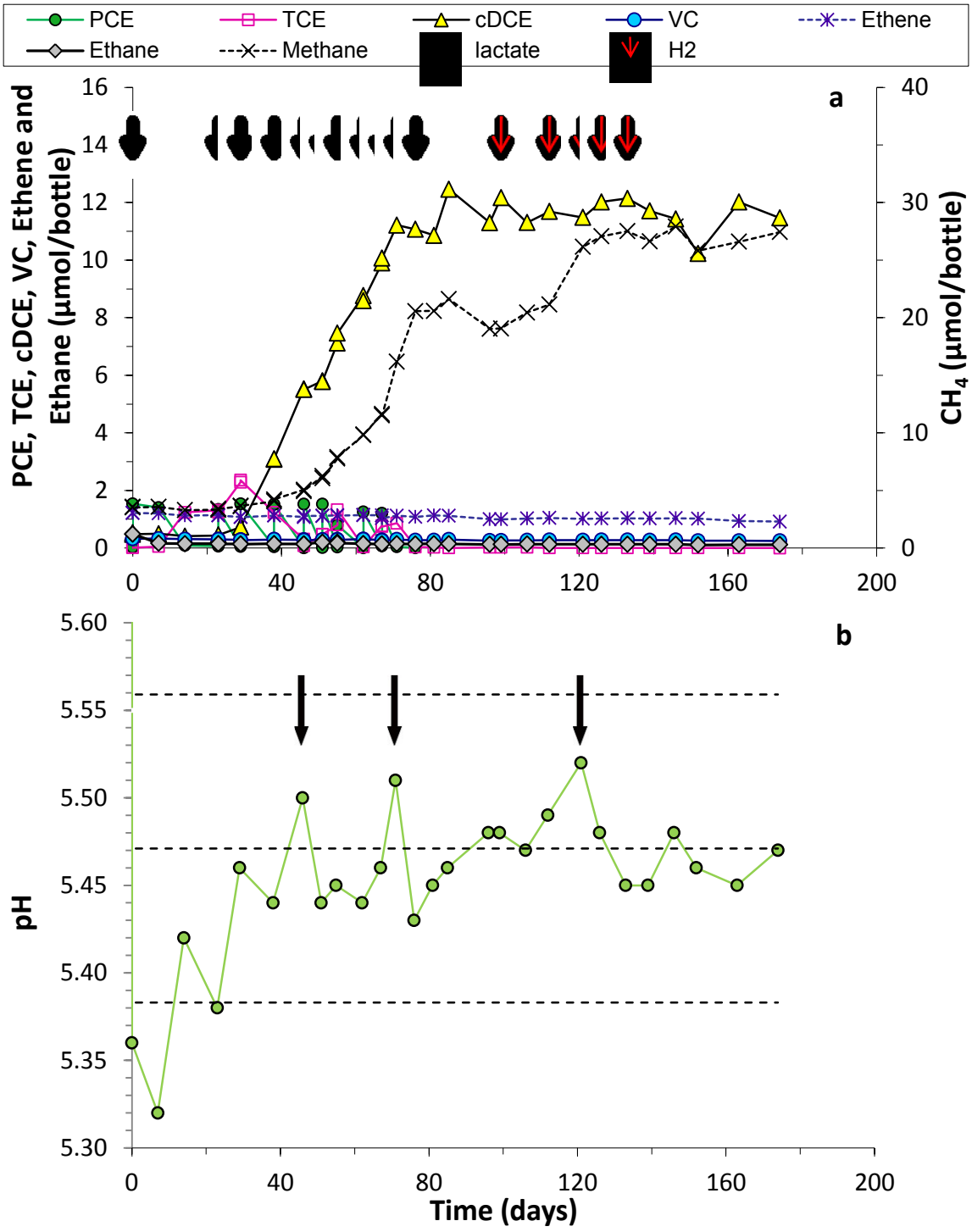

Figure E.6 Results for the NC/HH microcosms, serum bottle #2 (NC-lactate-S2) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid. Hydrogen replaced lactate as the electron donor starting on day 99.

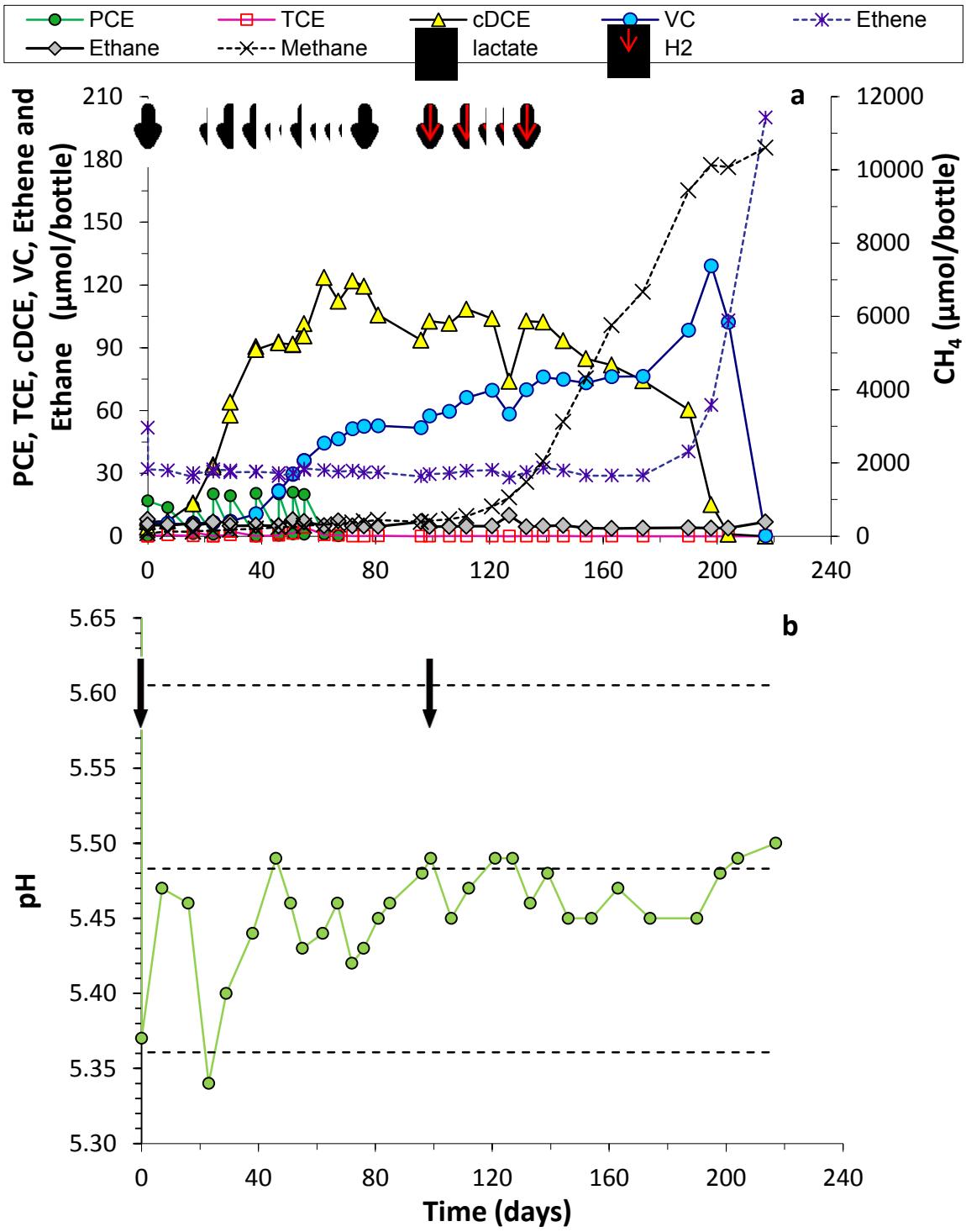

Figure E.7 Results for a lactate-amended NC/HH microcosm, 2.6 L bottle #1 (NC-lactate-B1) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid. Hydrogen replaced lactate as the electron donor starting on day 99.

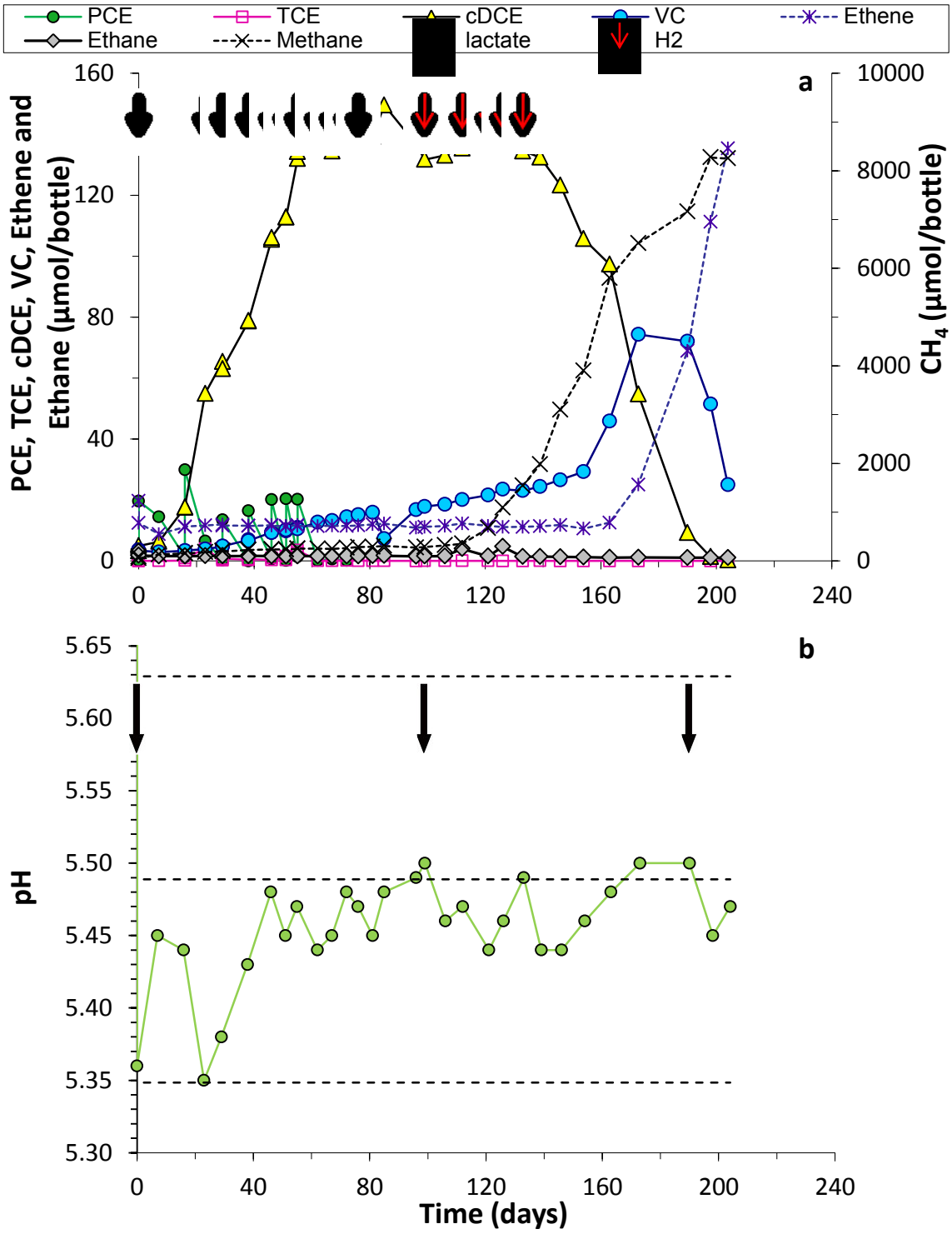

Figure E.8 Results for a lactate-amended NC/HH microcosm, 2.6 L bottle #3 (NC-lactate-B3) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid. Hydrogen replaced lactate as the electron donor starting on day 99.

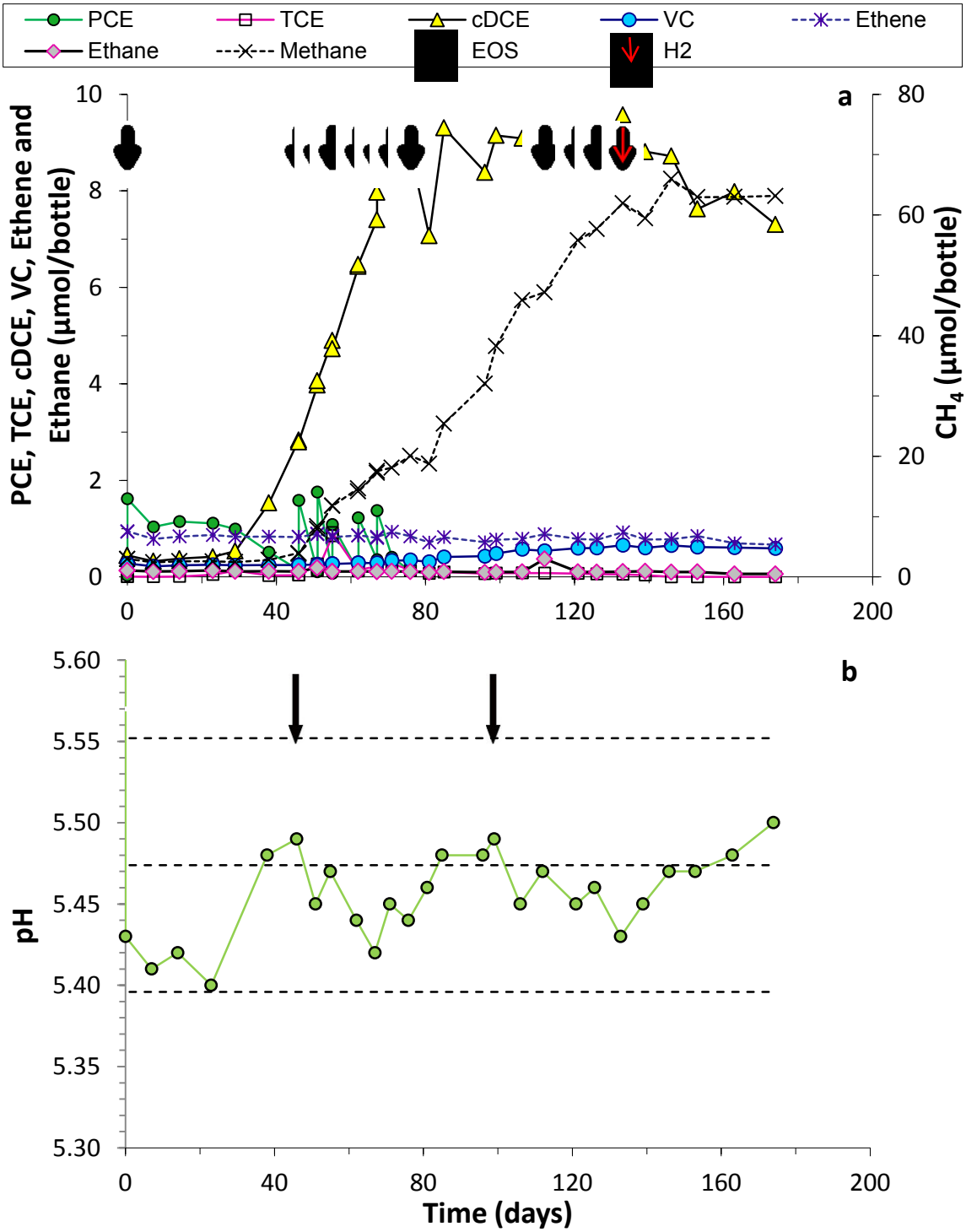

Figure E.9 Results for the NC/HH microcosms, serum bottle #2 (NC-EOS-S2) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid. Hydrogen replaced EOS as the electron donor starting on day 133.

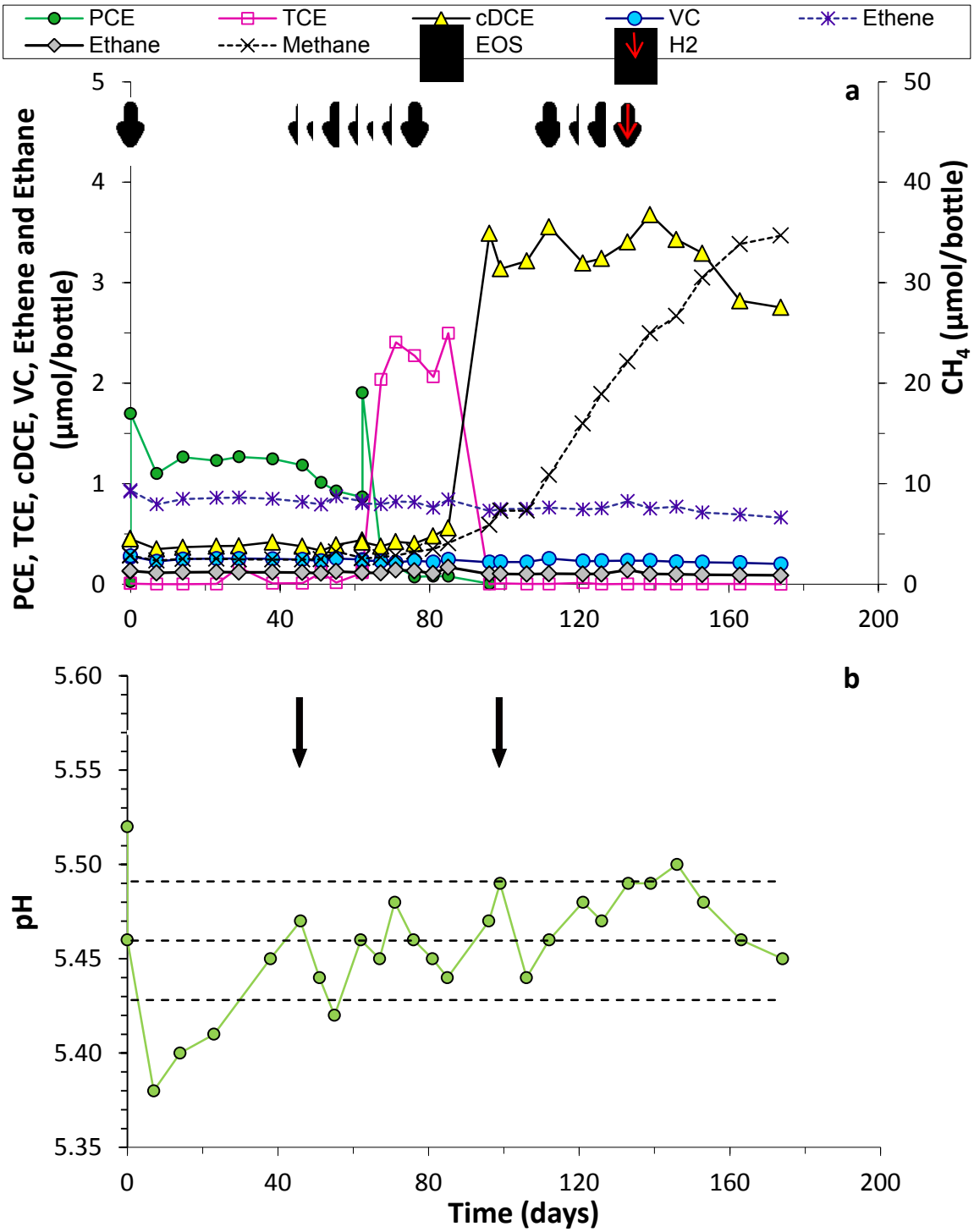

Figure E.10 Results for the NC/HH microcosms, serum bottle #3 (NC-EOS-S3) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid. Hydrogen replaced EOS as the electron donor starting on day 133.

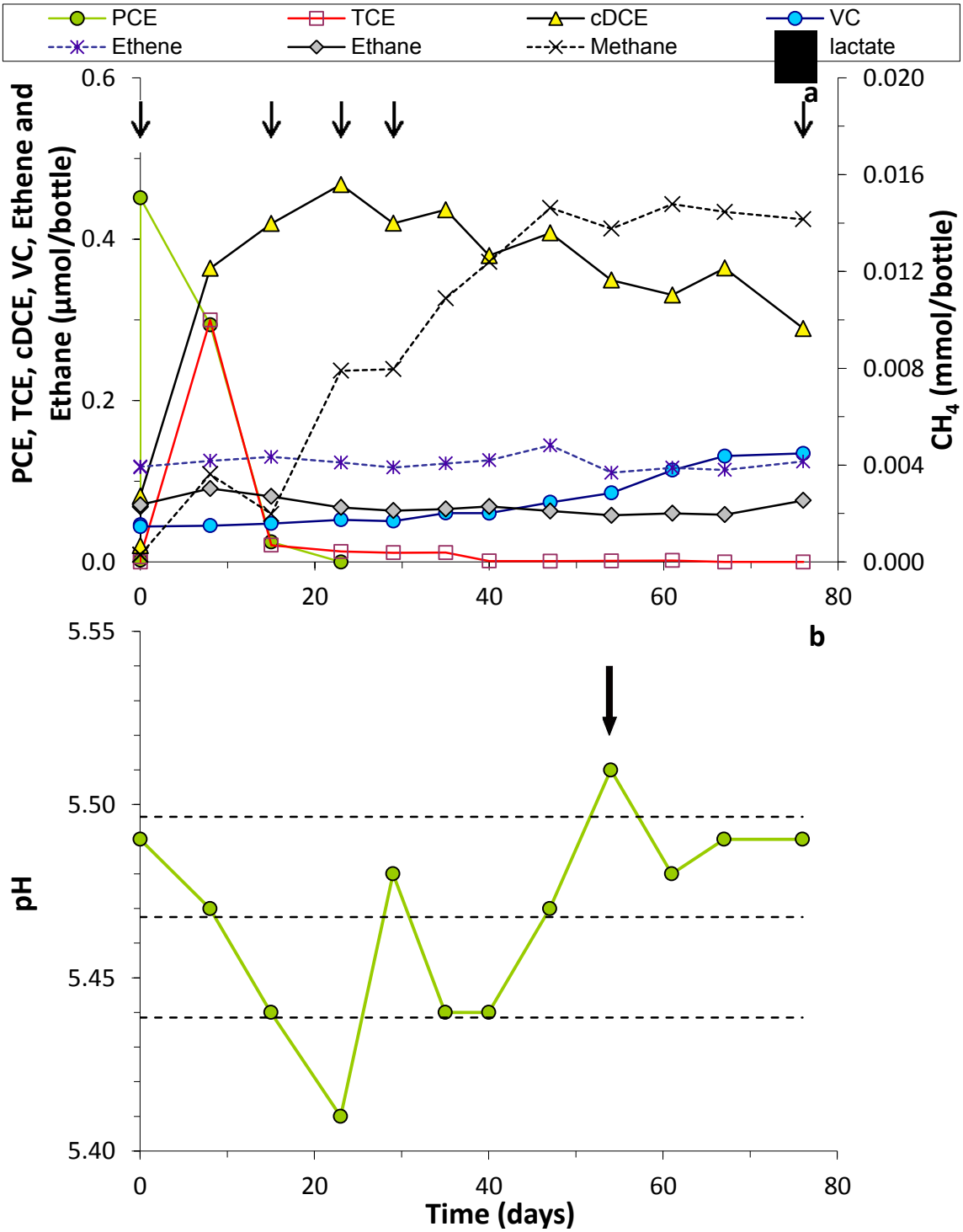

Figure E.11 Results for a lactate-amended NC/HH enrichment culture developed with MSM (NC-UN1-MSM-lactate) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

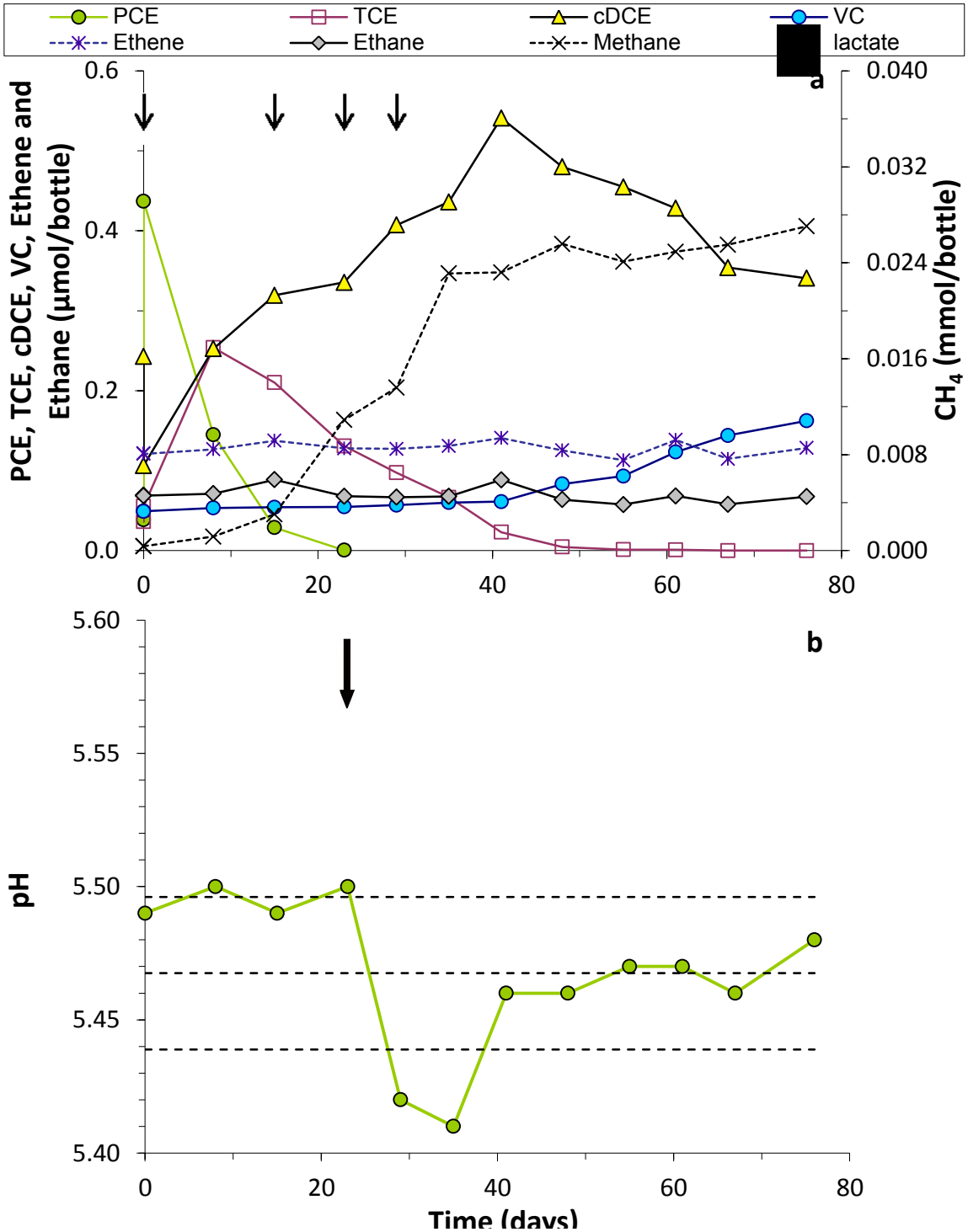

Figure E.12 Results for a lactate-amended NC/HH enrichment culture developed with MSM (NC-UN2-MSM-lactate) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

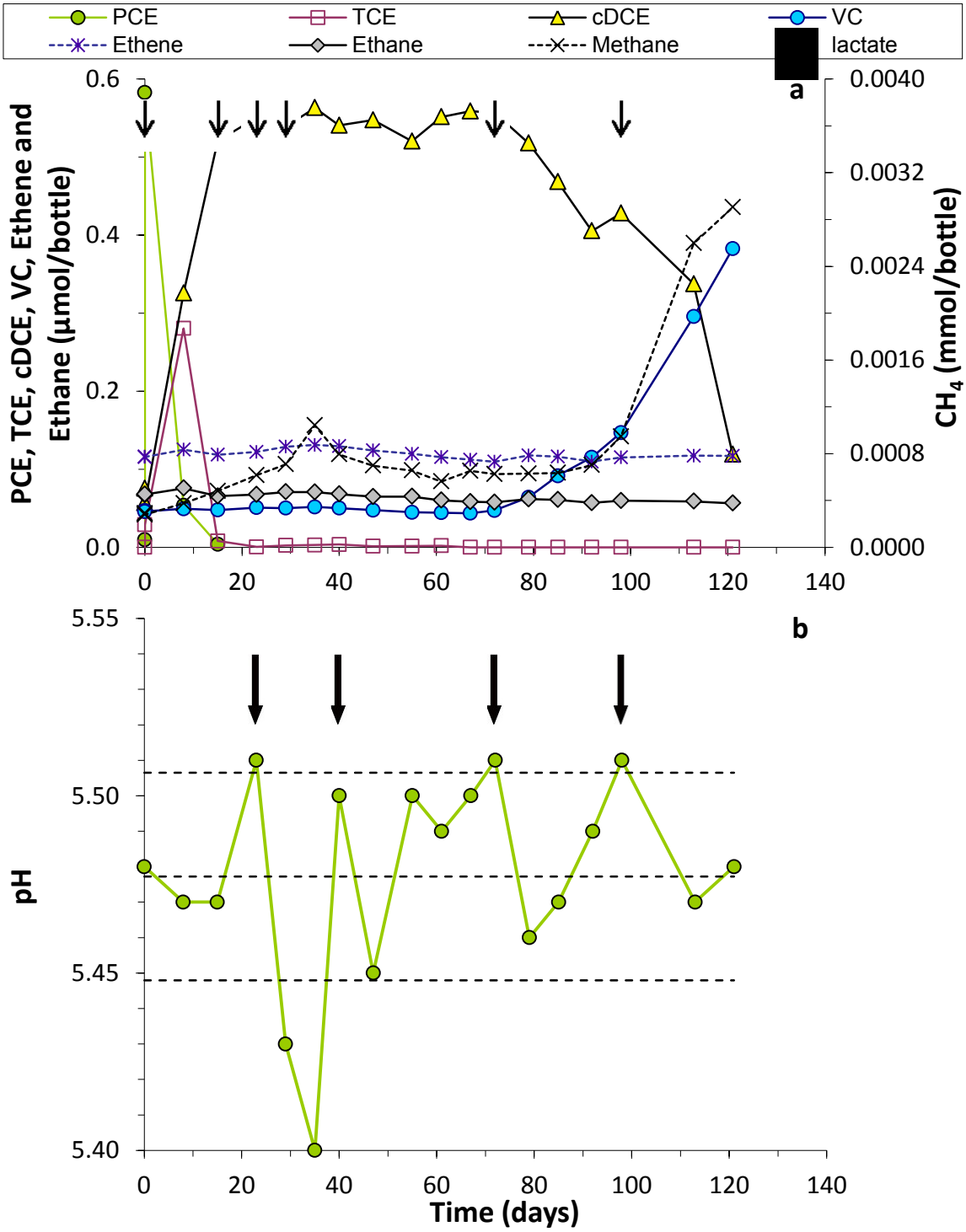

Figure E.13 Results for a lactate-amended NC/HH enrichment culture developed with MES (NC-UN1-MES-H_2) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

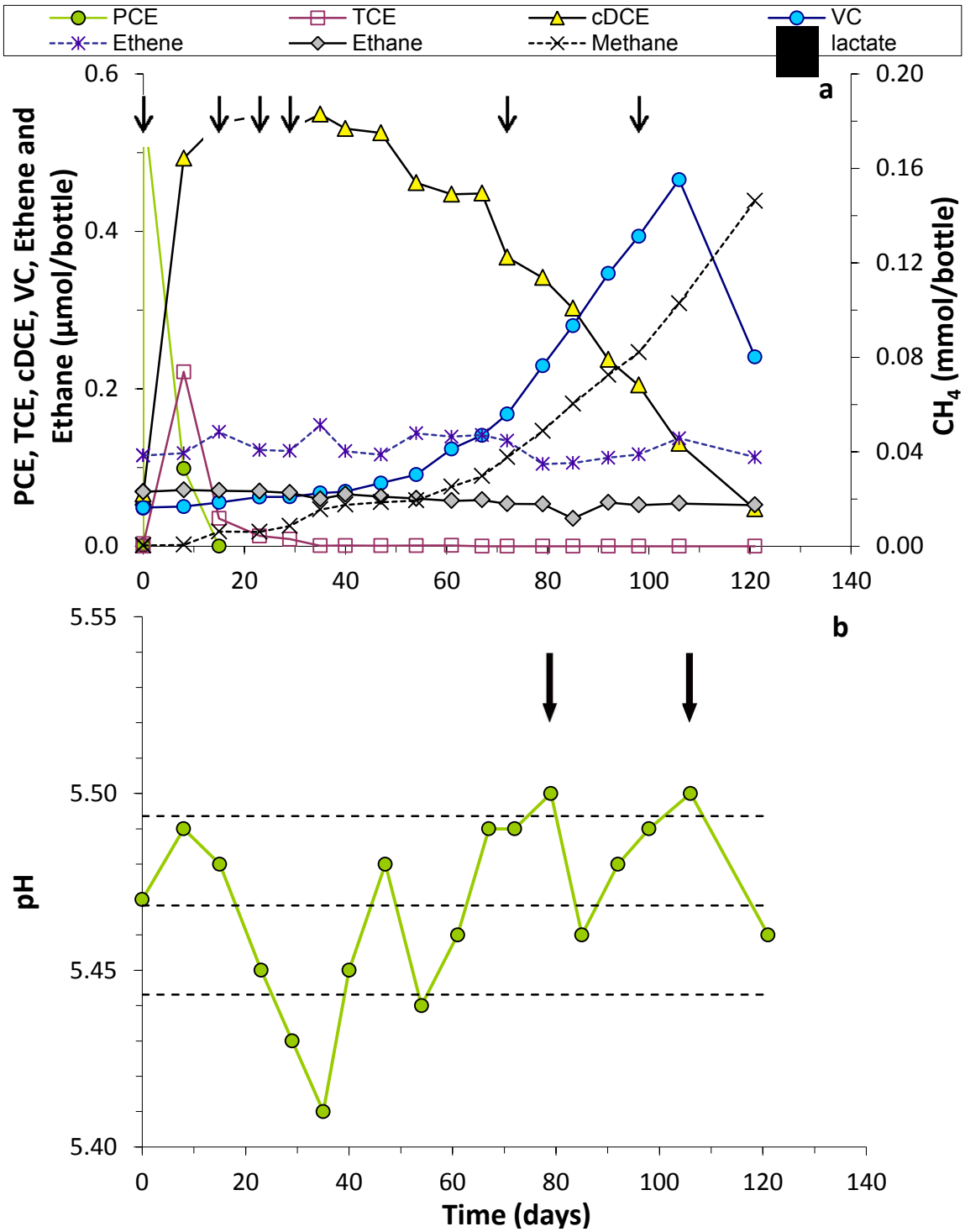

Figure E.14 Results for a lactate-amended NC/HH enrichment culture developed with MES (NC-UN1-MES-lactate) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

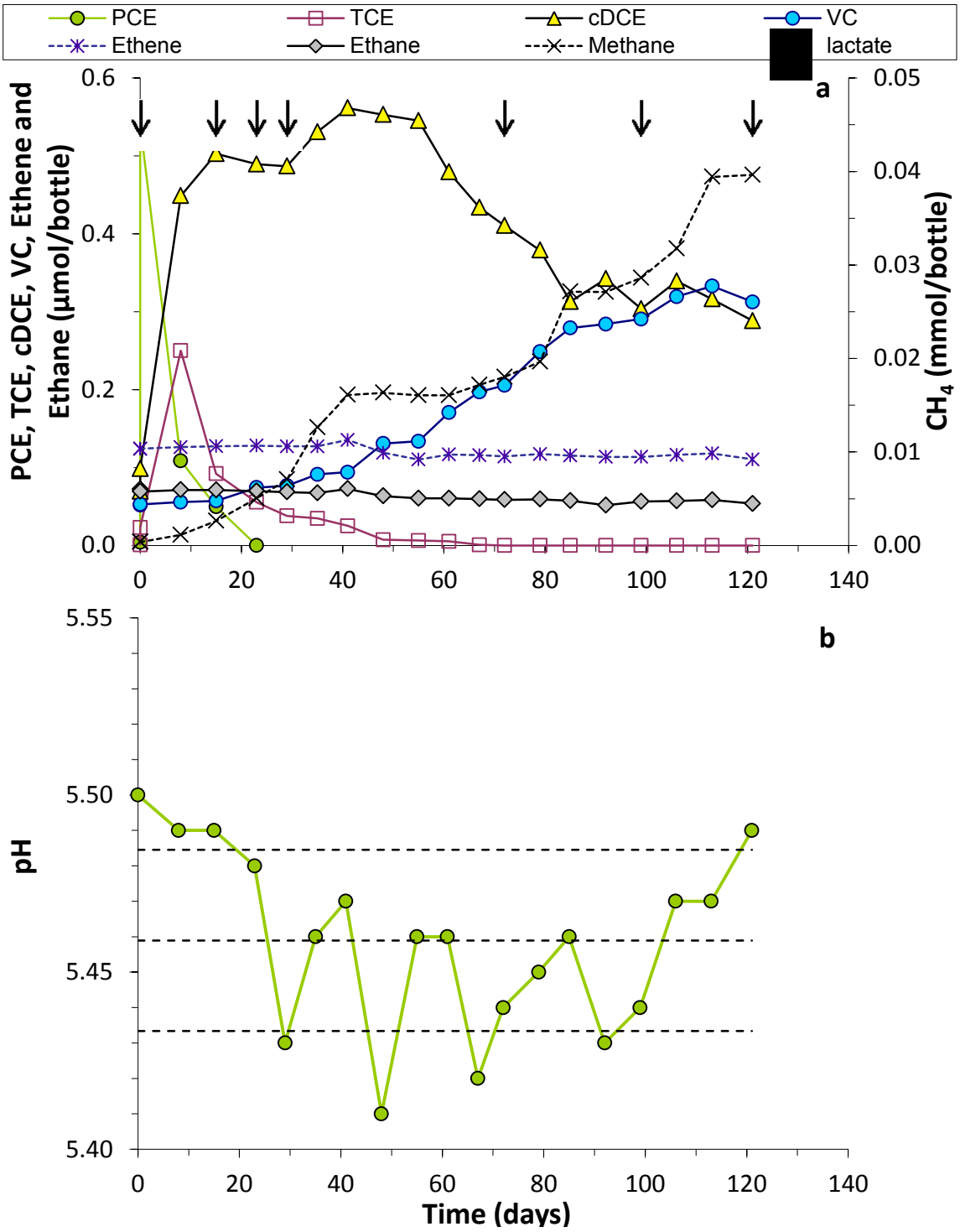

Figure E.15 Results for a lactate-amended NC/HH enrichment culture developed with MES (NC-UN2-MES-lactate) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

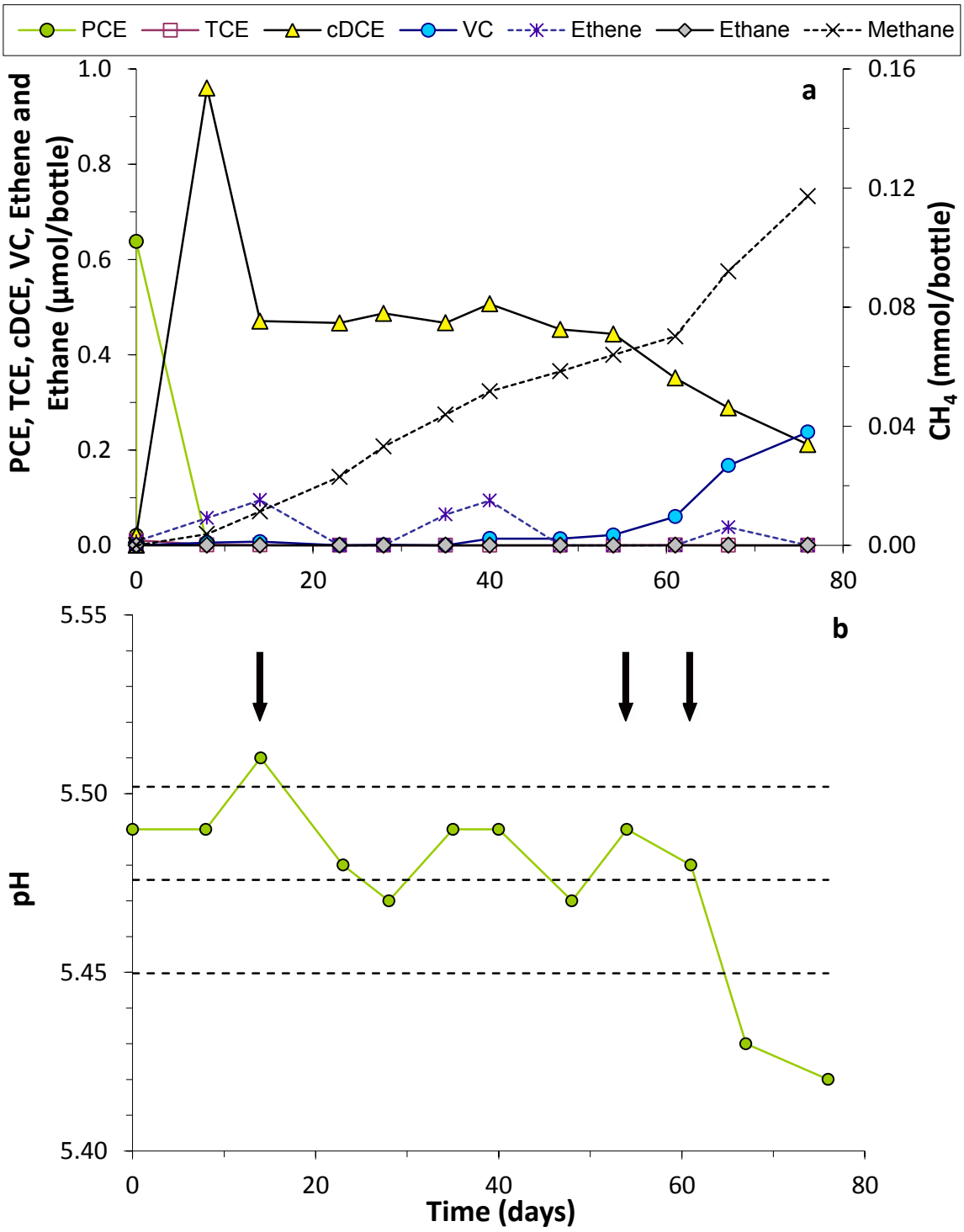

Figure E.16 Results for an unamended NC/HH enrichment culture developed with groundwater (NC-UN2-GW) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

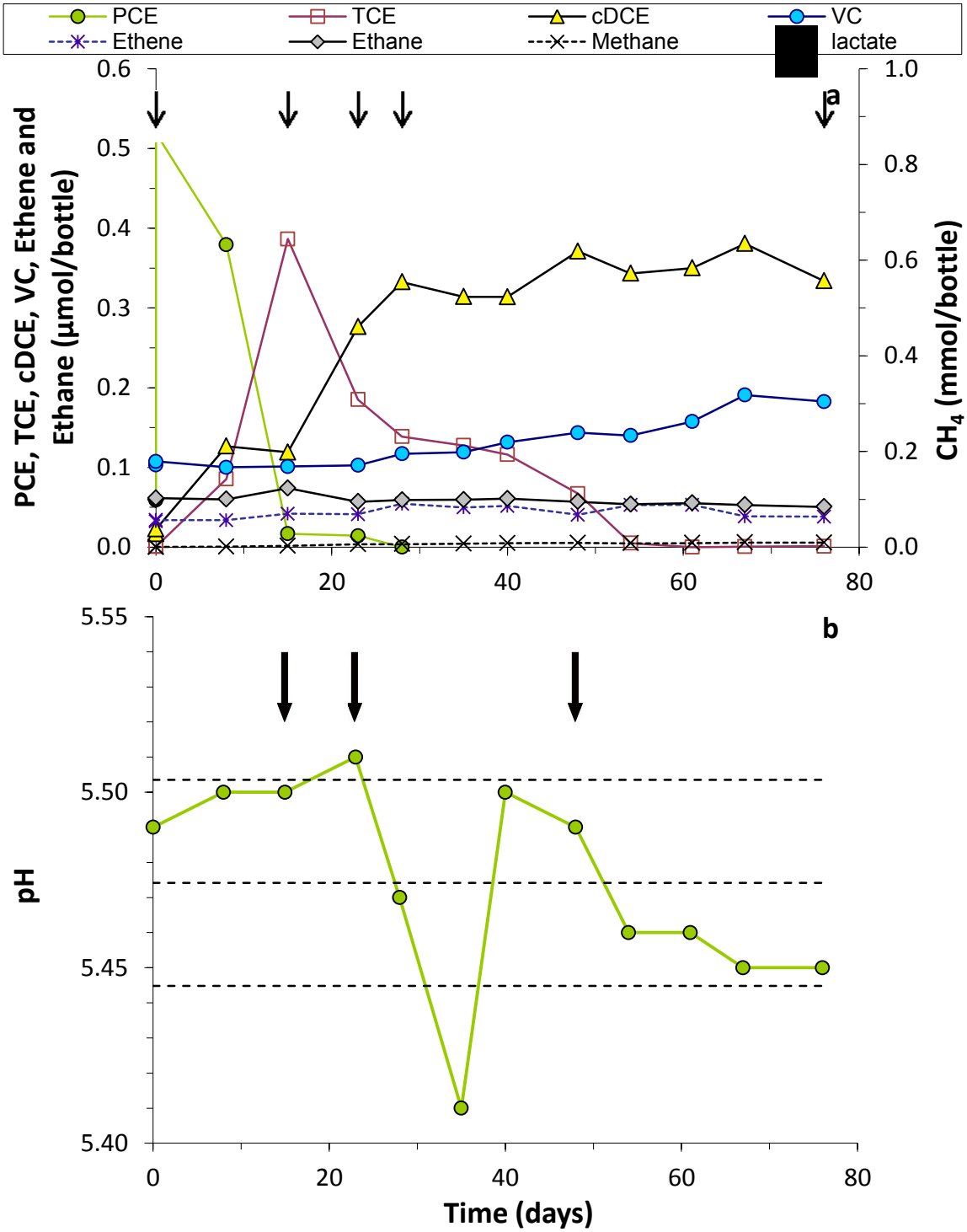

Figure E.17 Results for a lactate-amended NC/HH enrichment culture developed with MSM (NC-lactate-MSM-S1) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

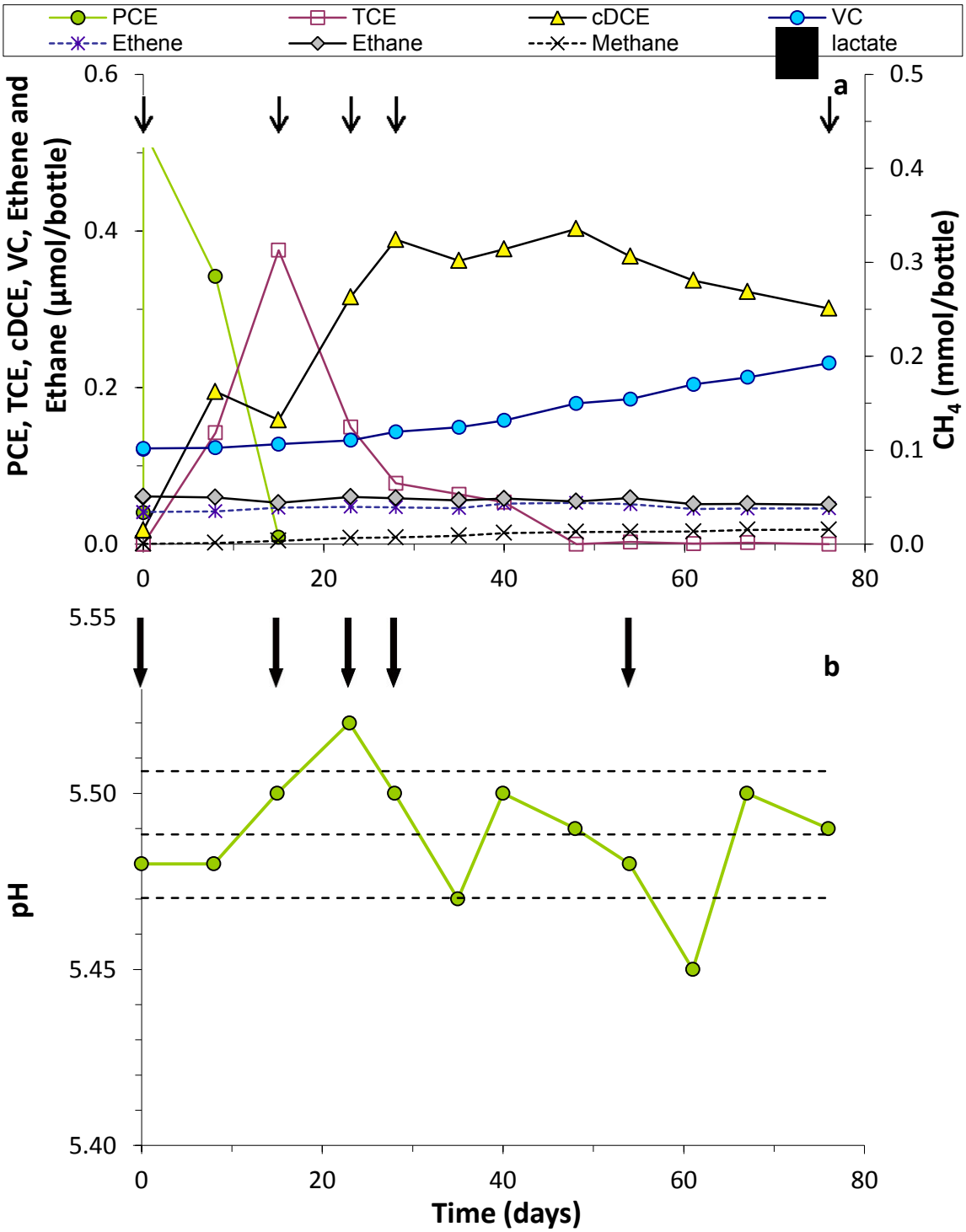

Figure E.18 Results for a lactate-amended NC/HH enrichment culture developed with MES (NC-lactate-MSM-S2) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

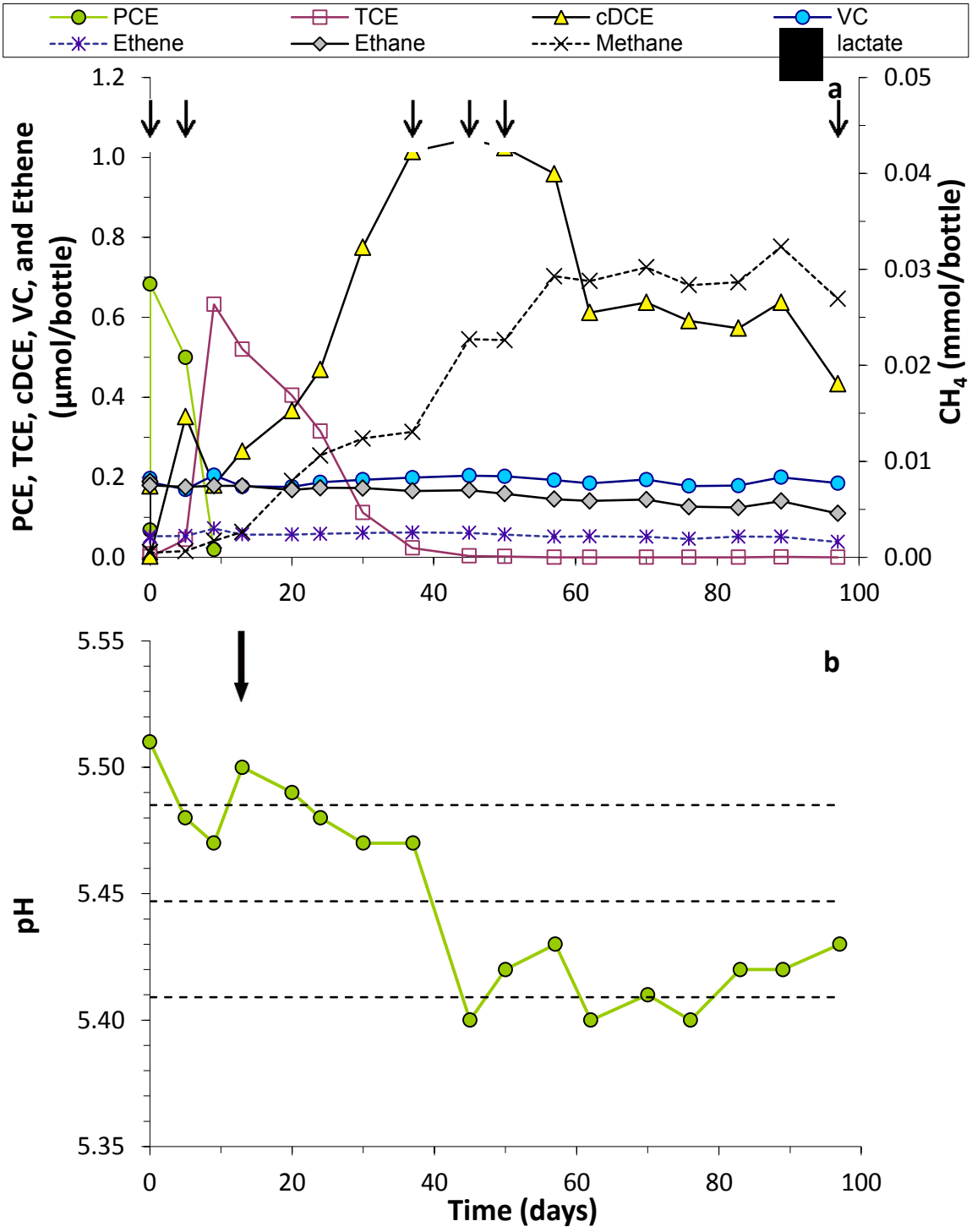

Figure E.19 Results for a lactate-amended NC/HH enrichment culture developed with MES (NC-lactate-MES-S1) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

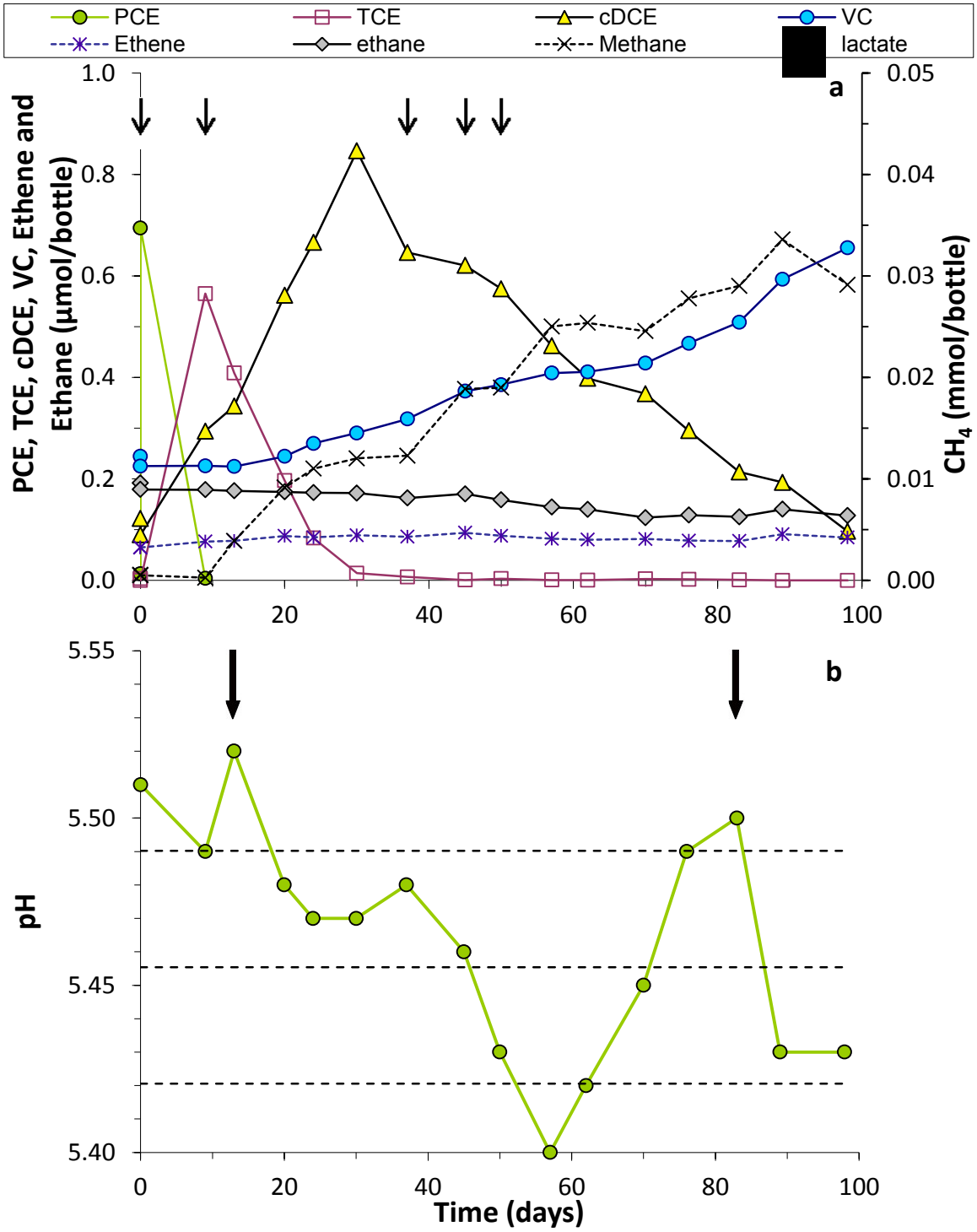

Figure E.20 Results for a lactate-amended NC/HH enrichment culture developed with MES (NC-lactate-MES-S3) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

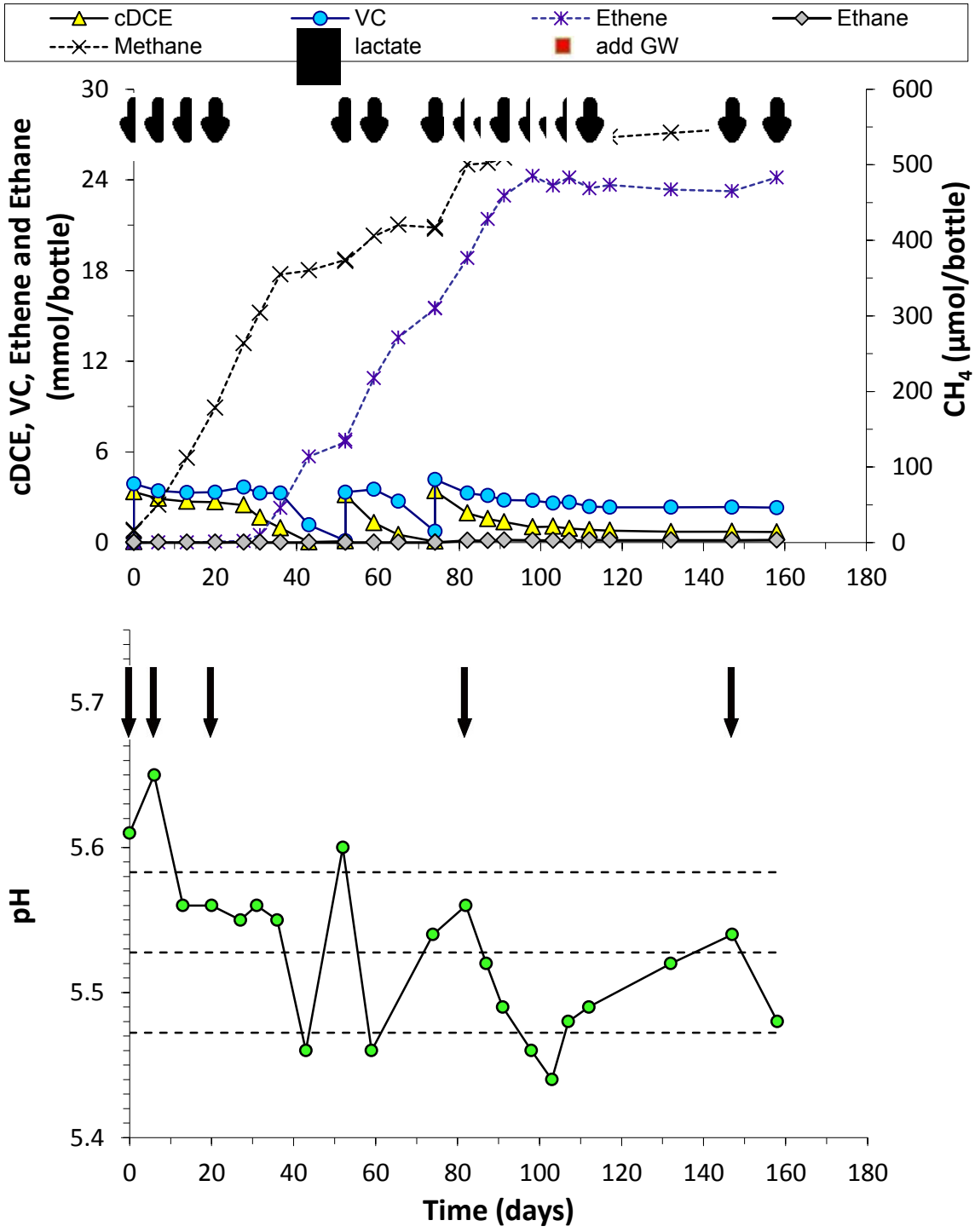

Figure E.21 Results for a lactate-amended SRS microcosm developed with groundwater, serum bottle #2 (GW-cDCE+VC-S2) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

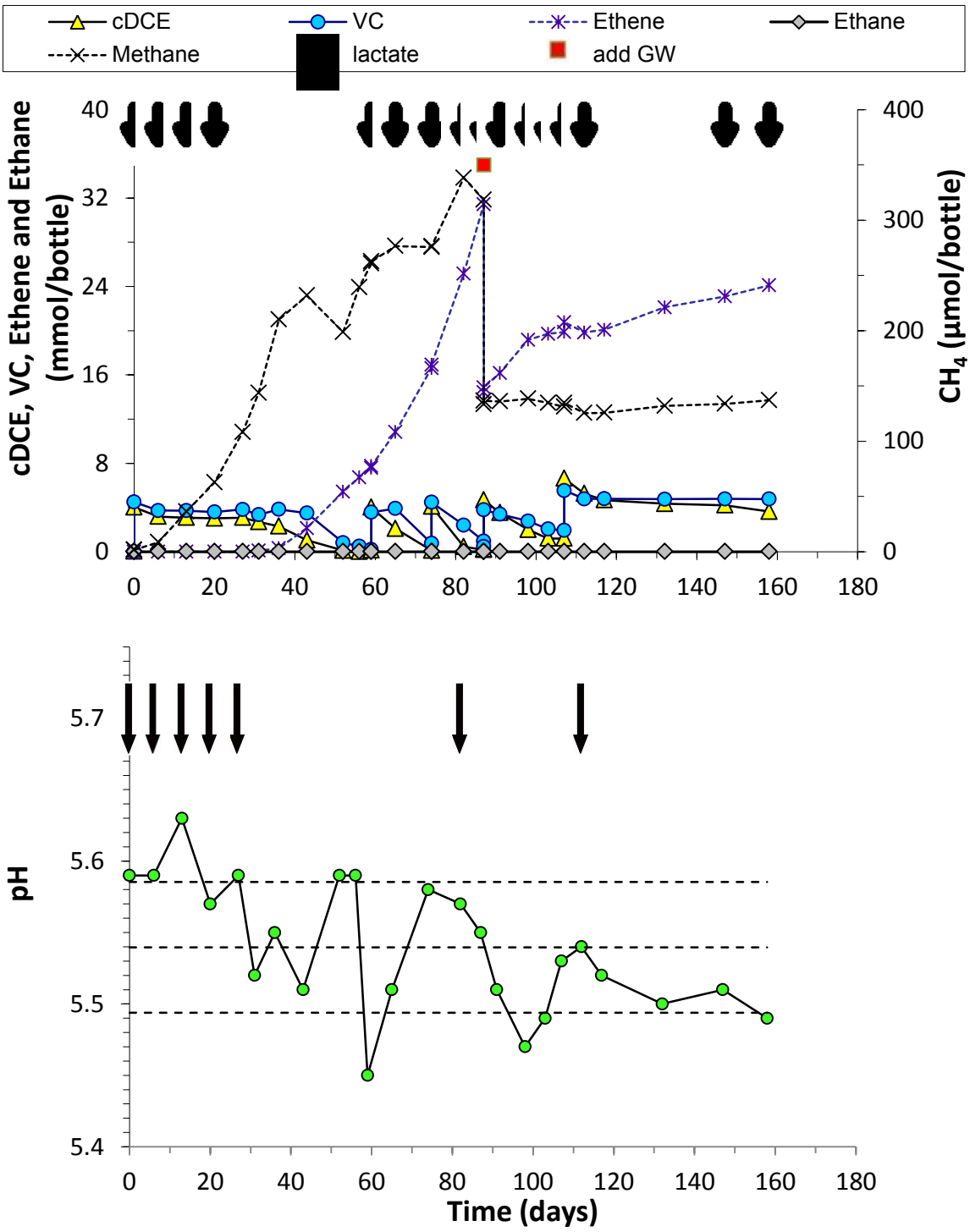

Figure E.22 Results for a lactate-amended SRS microcosm developed with groundwater, serum bottle #3 (GW-cDCE+VC-S3) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

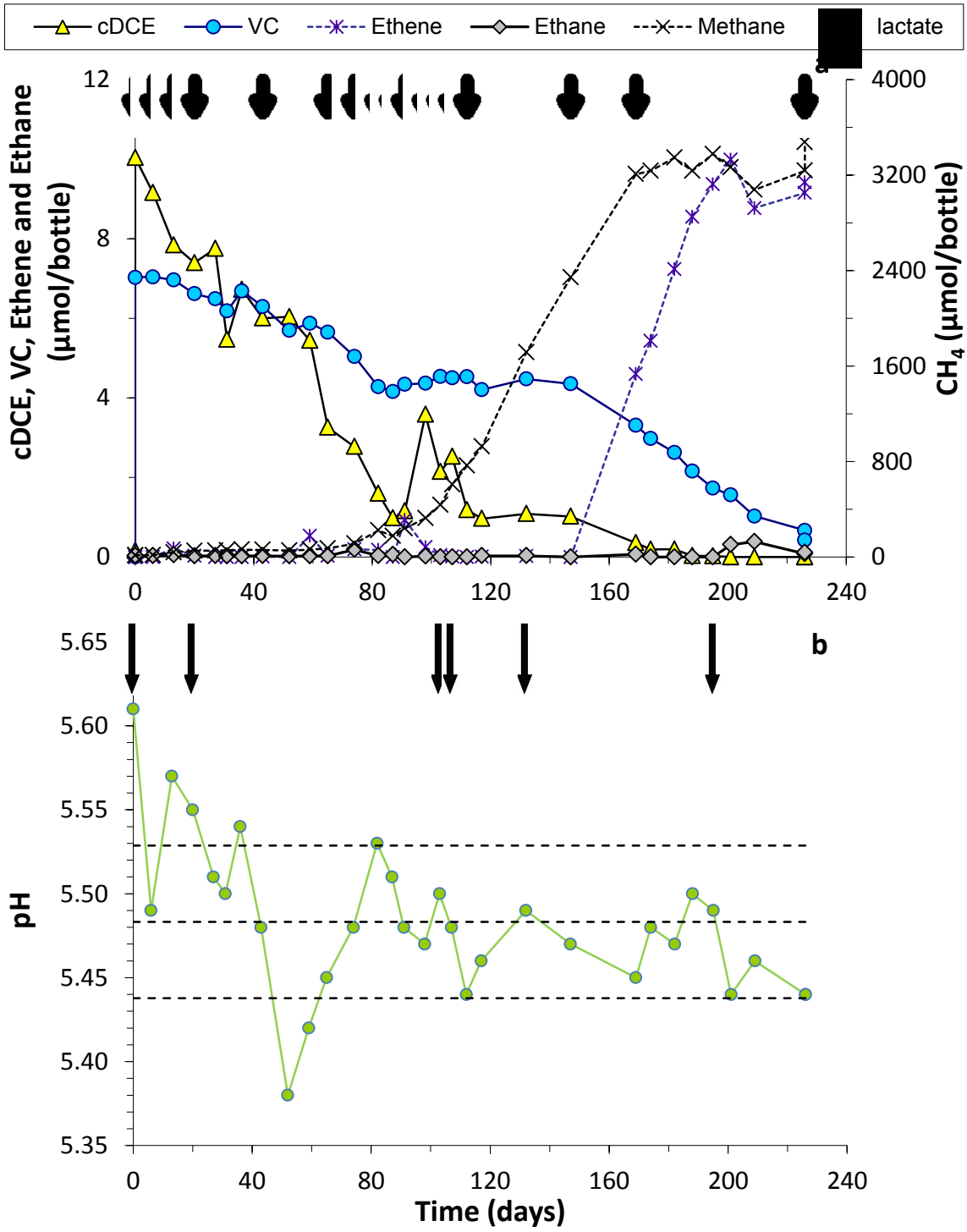

Figure E.23 Results for a lactate-amended SRS microcosm developed with groundwater, 2.6 L bottle #1 (GW-cDCE+VC-B1) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

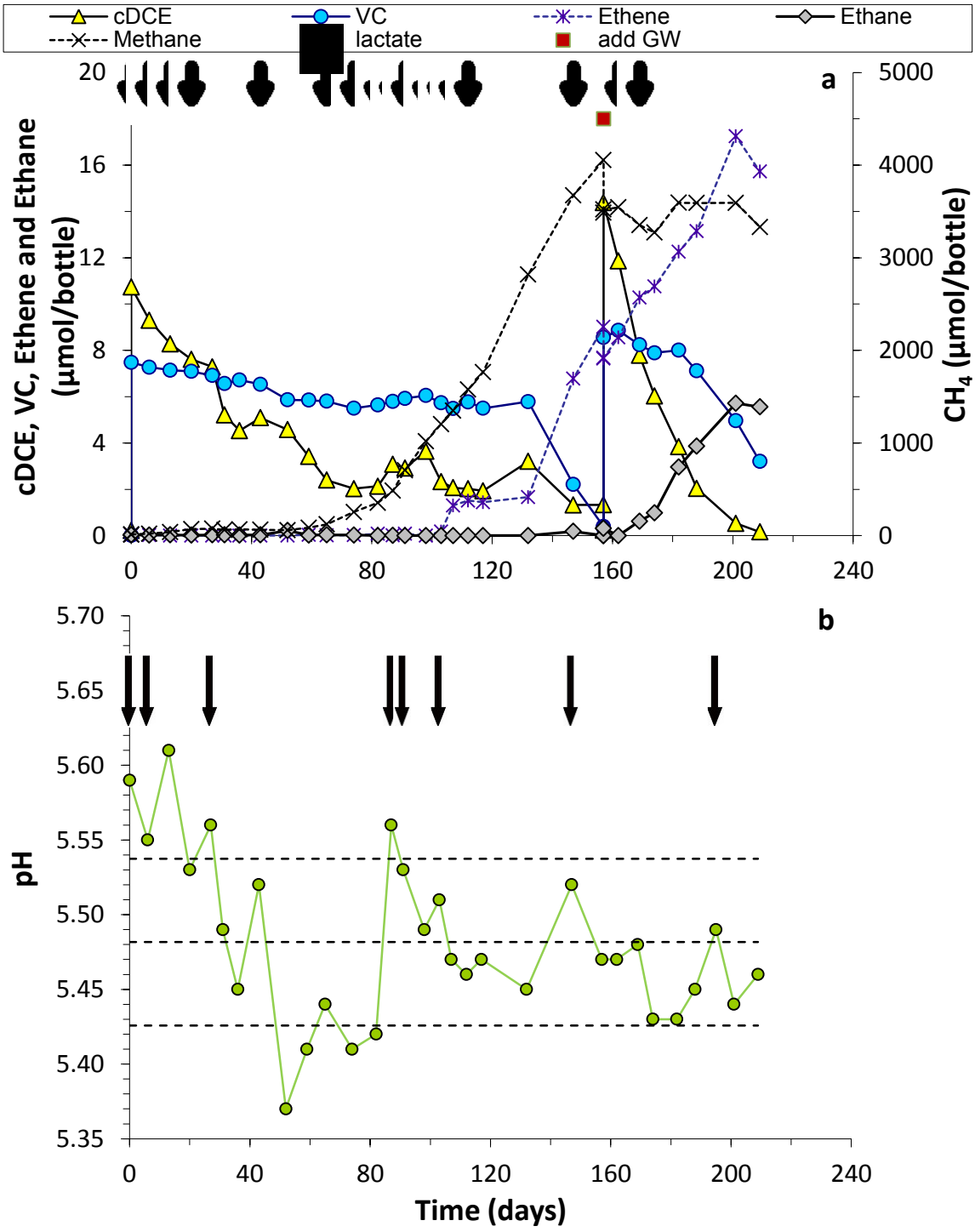

Figure E.24 Results for a lactate-amended SRS microcosm developed with groundwater, 2.6 L bottle #3 (GW-cDCE+VC-B3) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

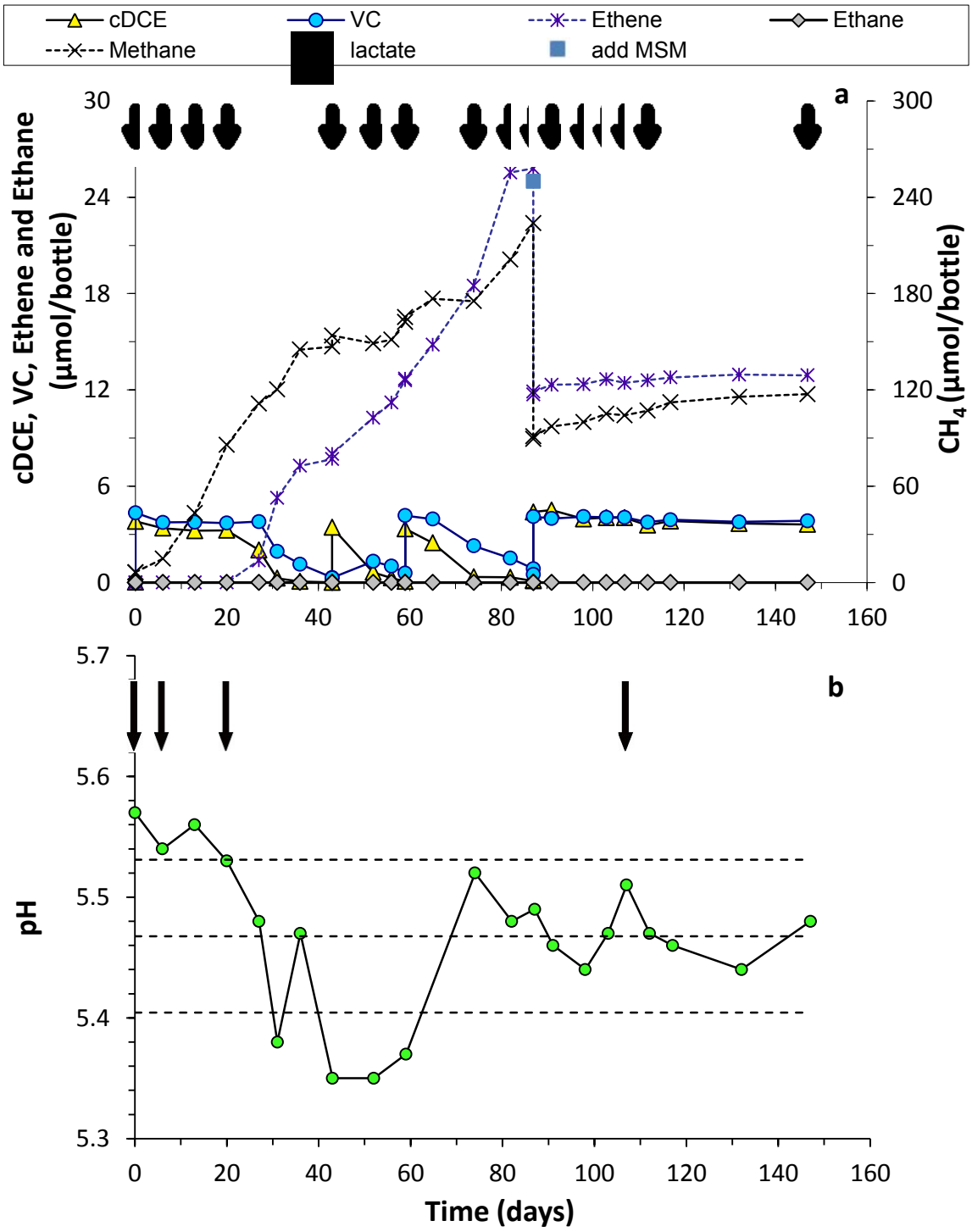

Figure E.25 Results for a lactate-amended SRS microcosm developed with MSM, serum bottle #1 (MM-cDCE+VC-S1) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

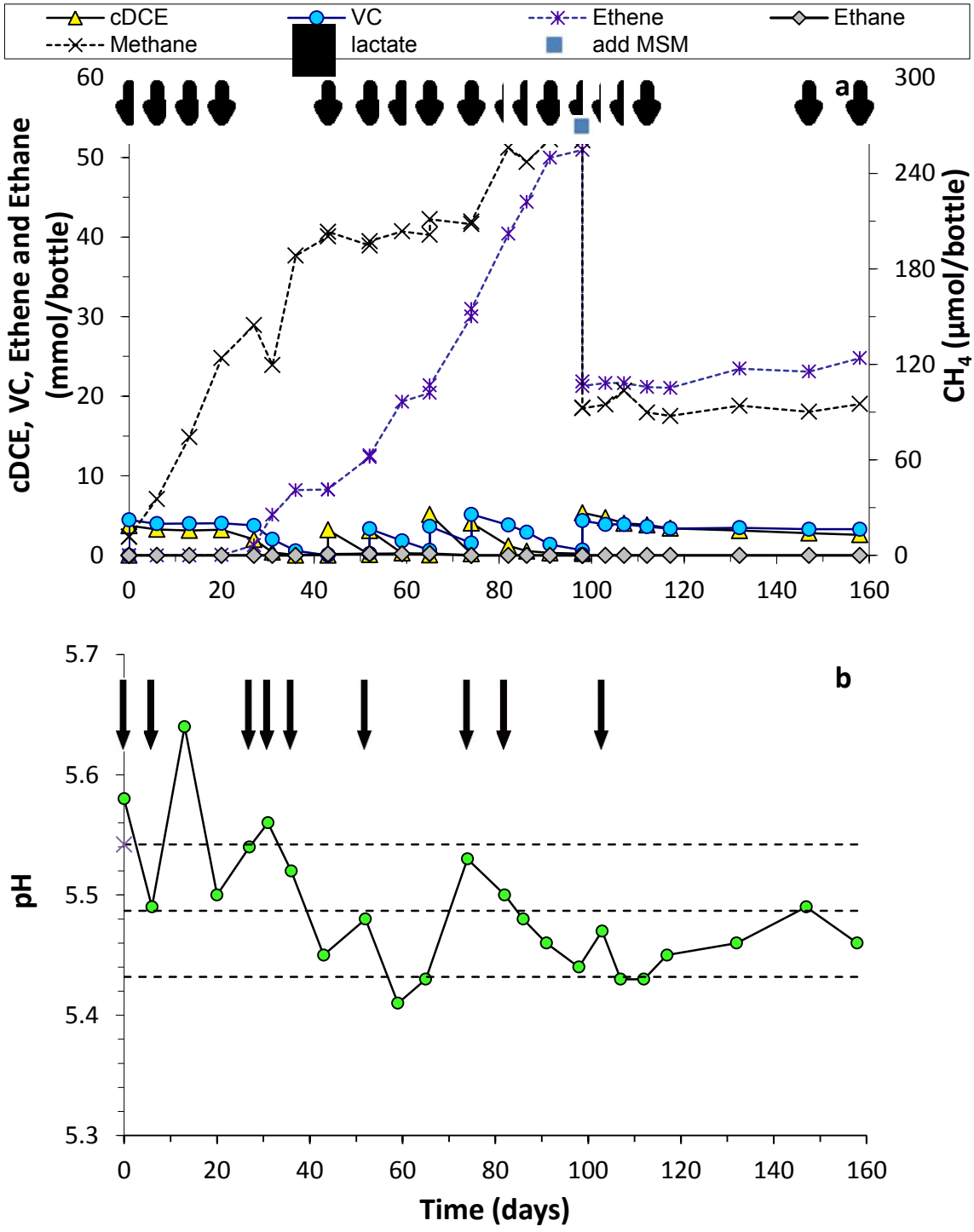

Figure E.26 Results for a lactate-amended SRS microcosm developed with MSM, serum bottle #3 (MM-cDCE+VC-S3) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

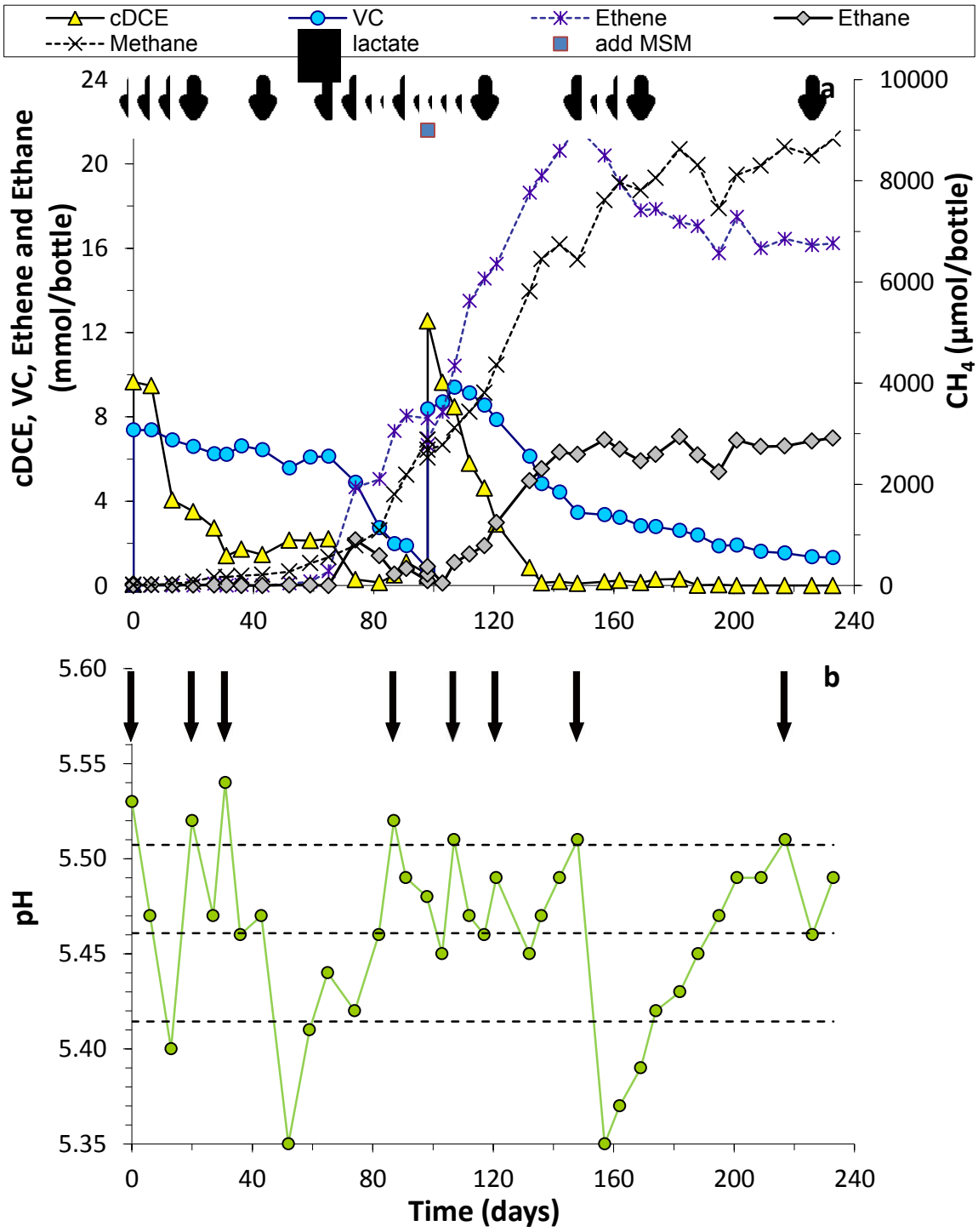

Figure E.27 Results for a lactate-amended SRS microcosm developed with MSM, 2.6 L bottle #2 (MM-cDCE+VC-B2) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

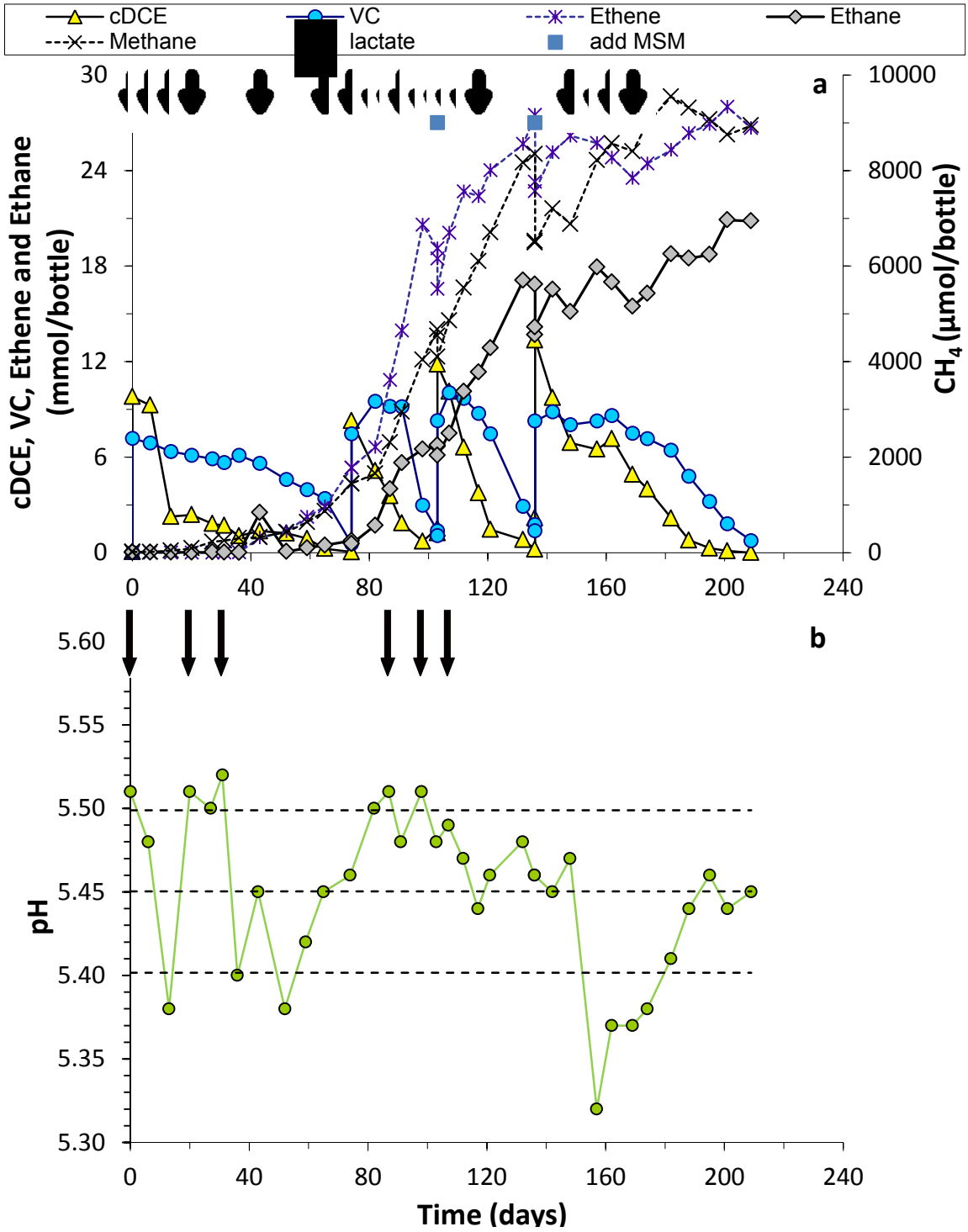

Figure E.28 Results for a lactate-amended SRS microcosm developed with MSM, 2.6 L bottle #3 (MM-cDCE+VC-B3) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

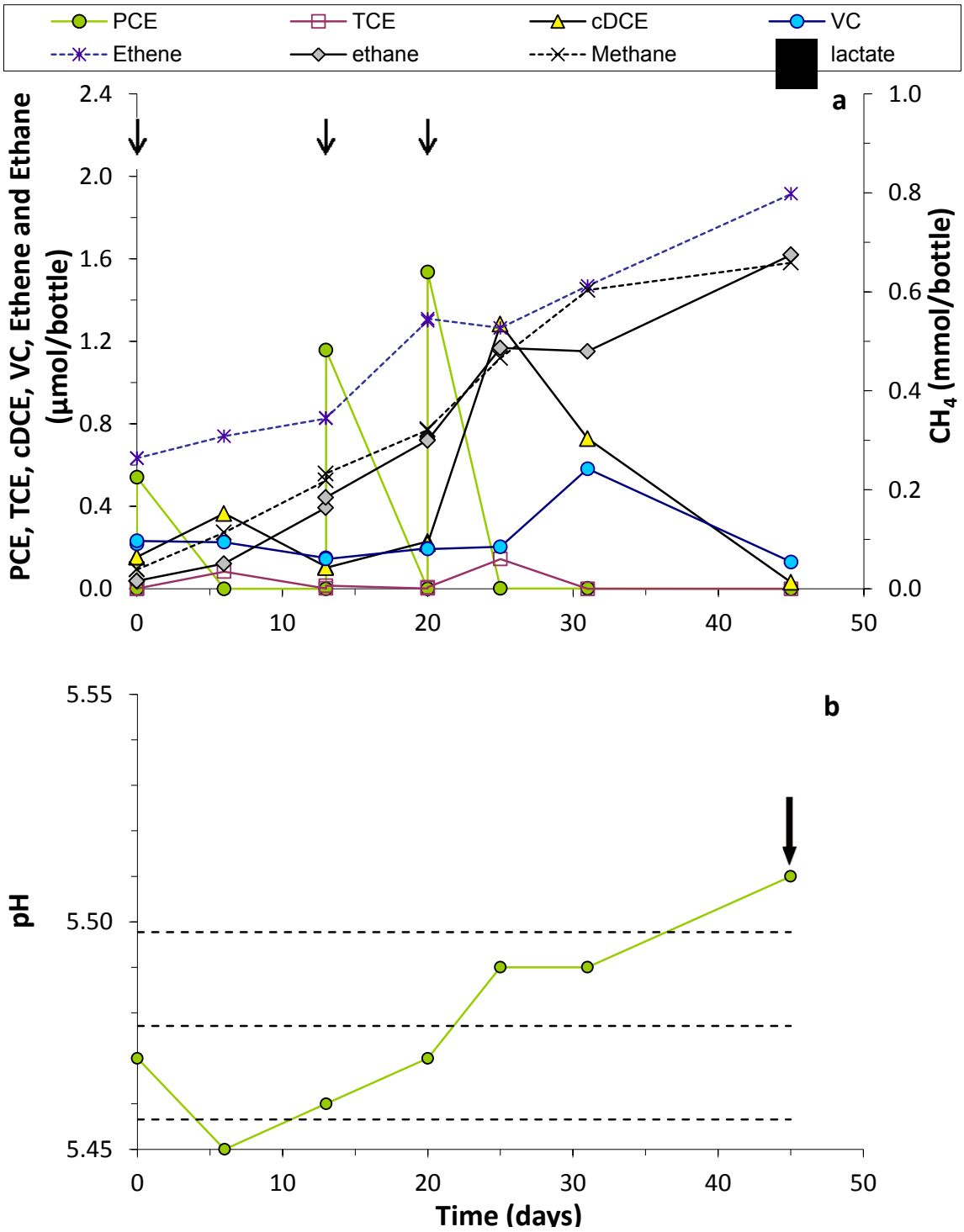

Figure E.29 Results for a lactate-amended combined enrichment culture, Set I, serum bottle #2 (GW-cDCE+VC-B3+NC-UN2-GW-2) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

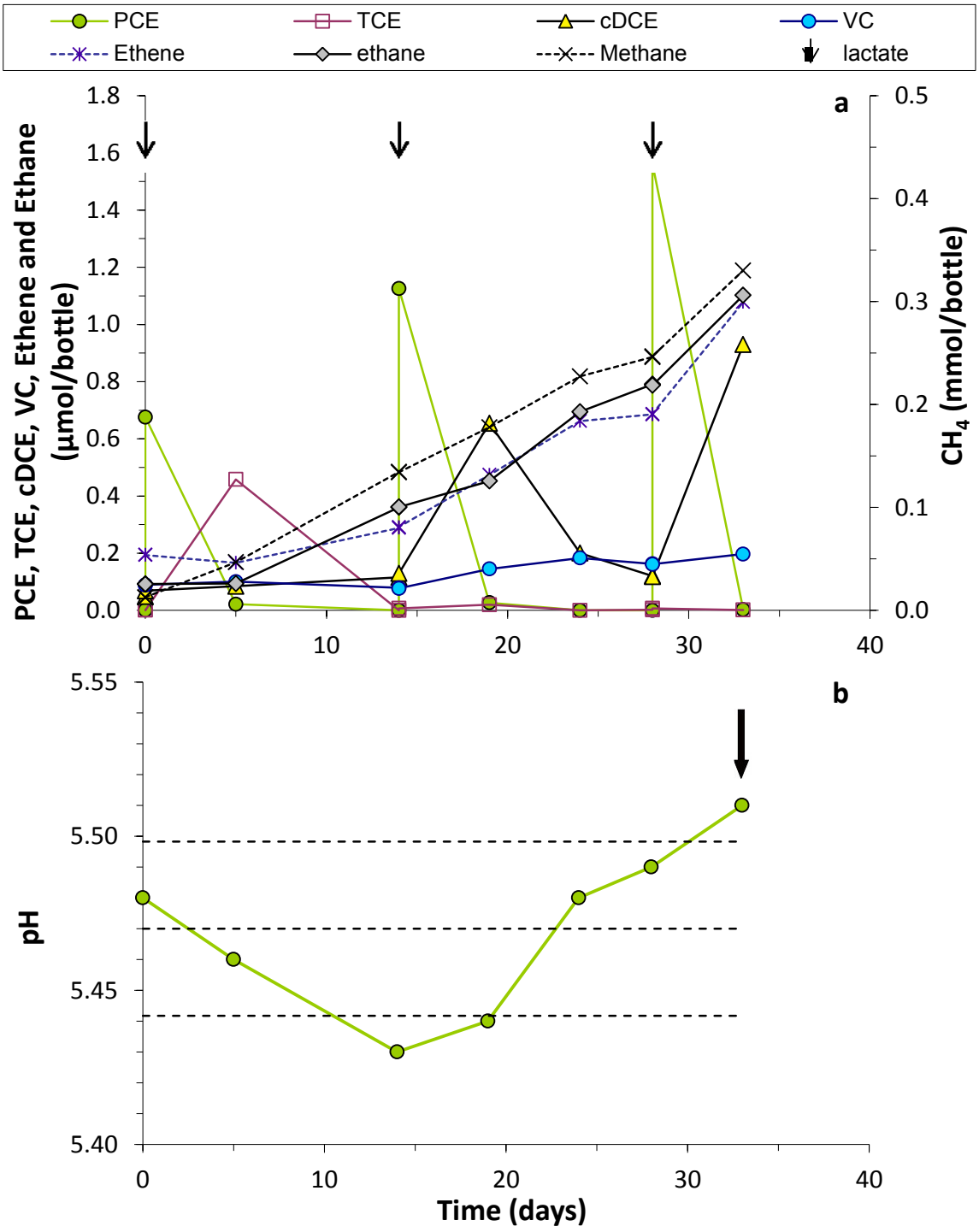

Figure E.30 Results for a lactate-amended combined enrichment culture, Set I, serum bottle #2 (GW-cDCE+VC-B3+NC-UN2-MSM-lactate-2) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

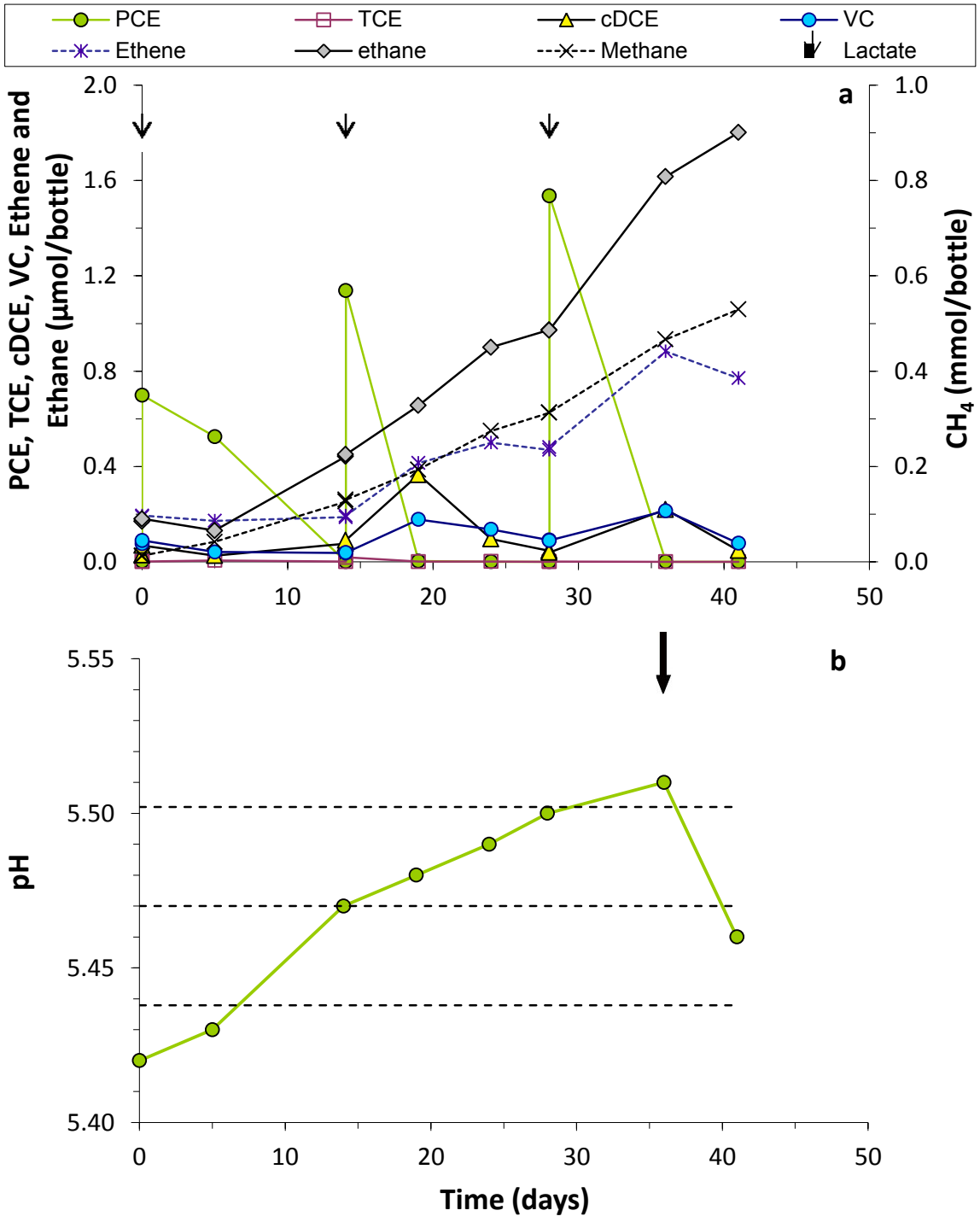

Figure E.31 Results for a lactate-amended combined enrichment culture, Set I, serum bottle #1 (GW-cDCE+VC-B3+NC-lactate-MSM-S1-1) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

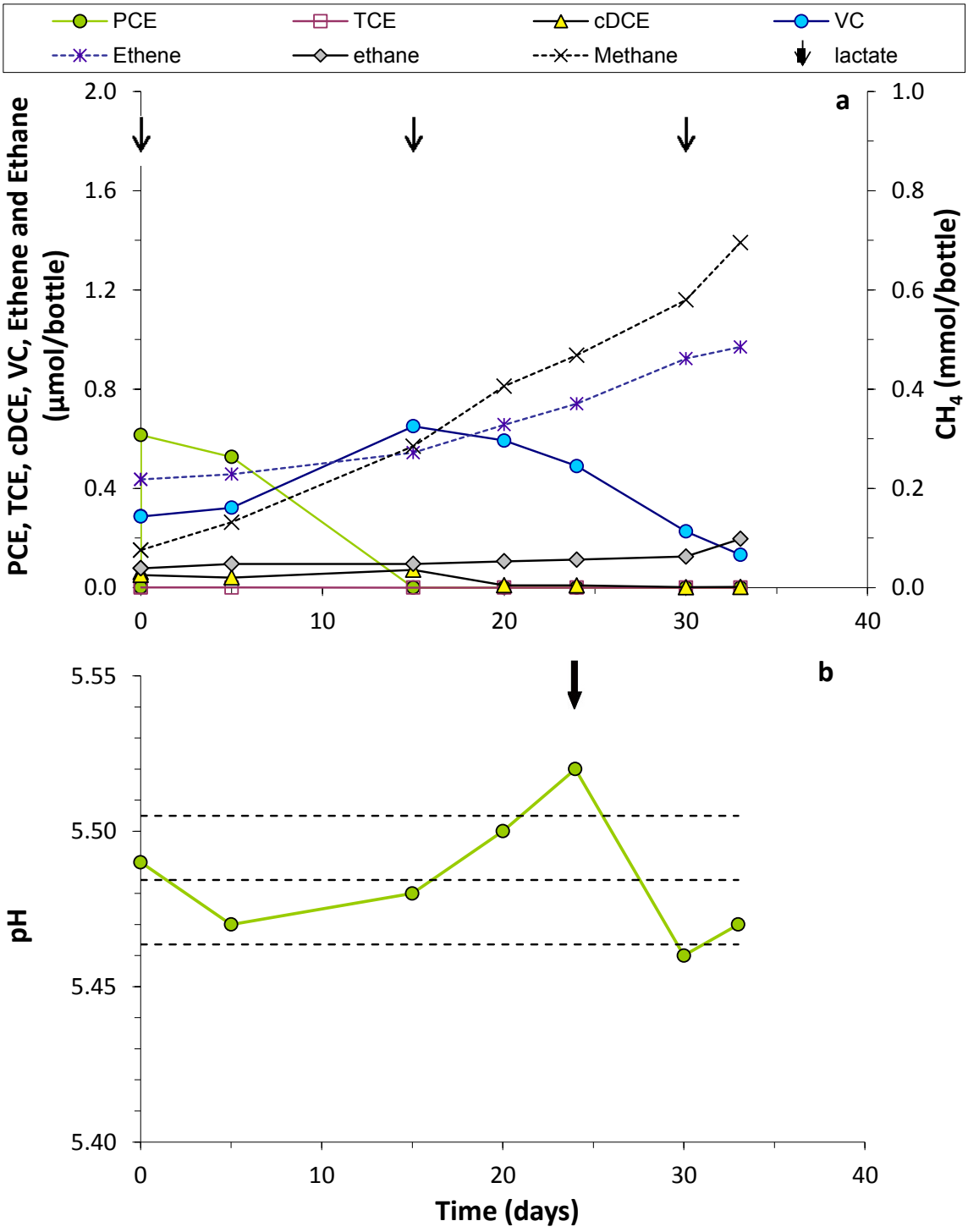

Figure E.32 Results for a lactate-amended combined enrichment culture, Set II, serum bottle #2 (GW-VC-4B+NC-UN1-GW-2) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

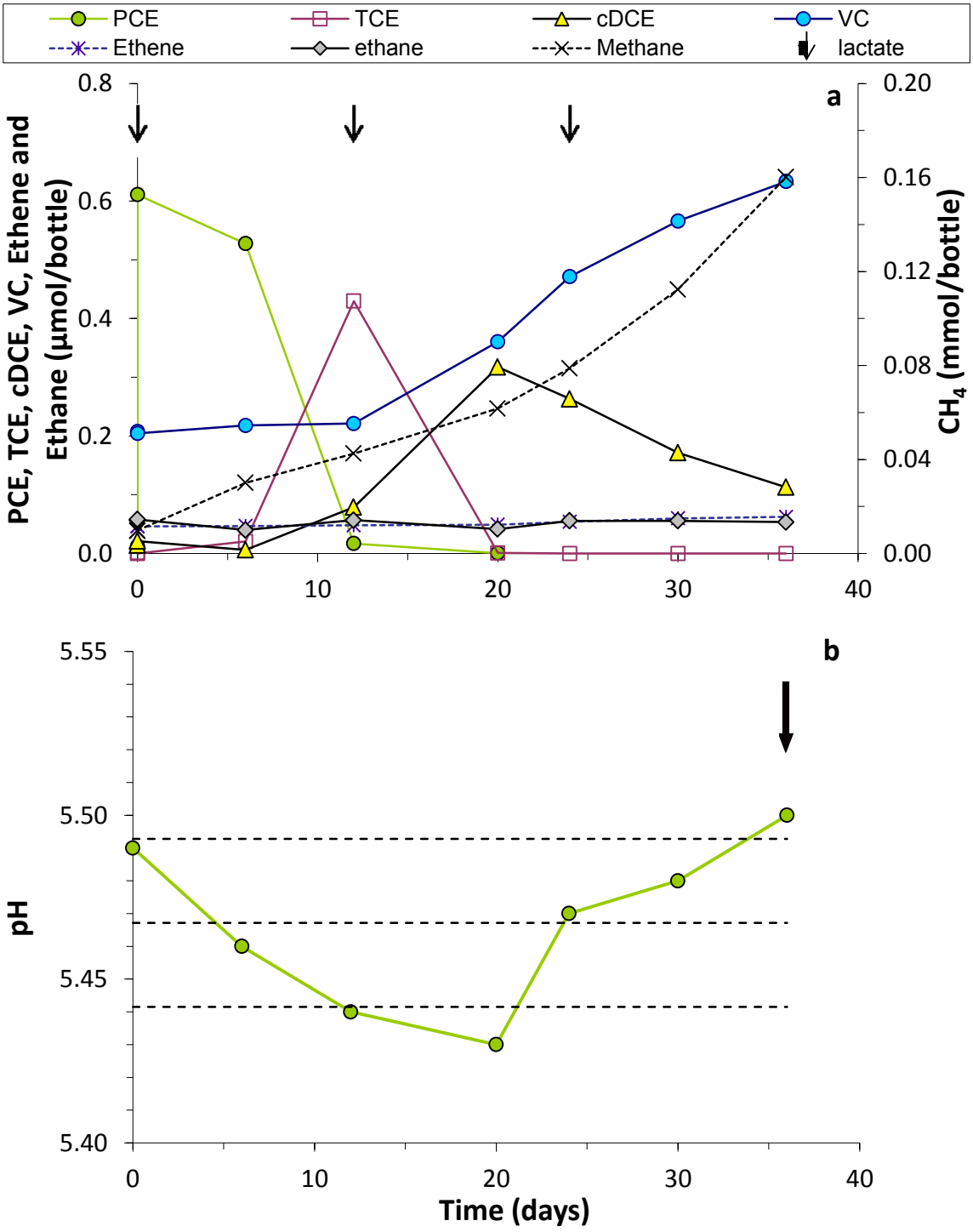

Figure E.33 Results for a lactate-amended combined enrichment culture, Set II, serum bottle #2 (GW-VC-4B+NC-lactate-MES-S2-2) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

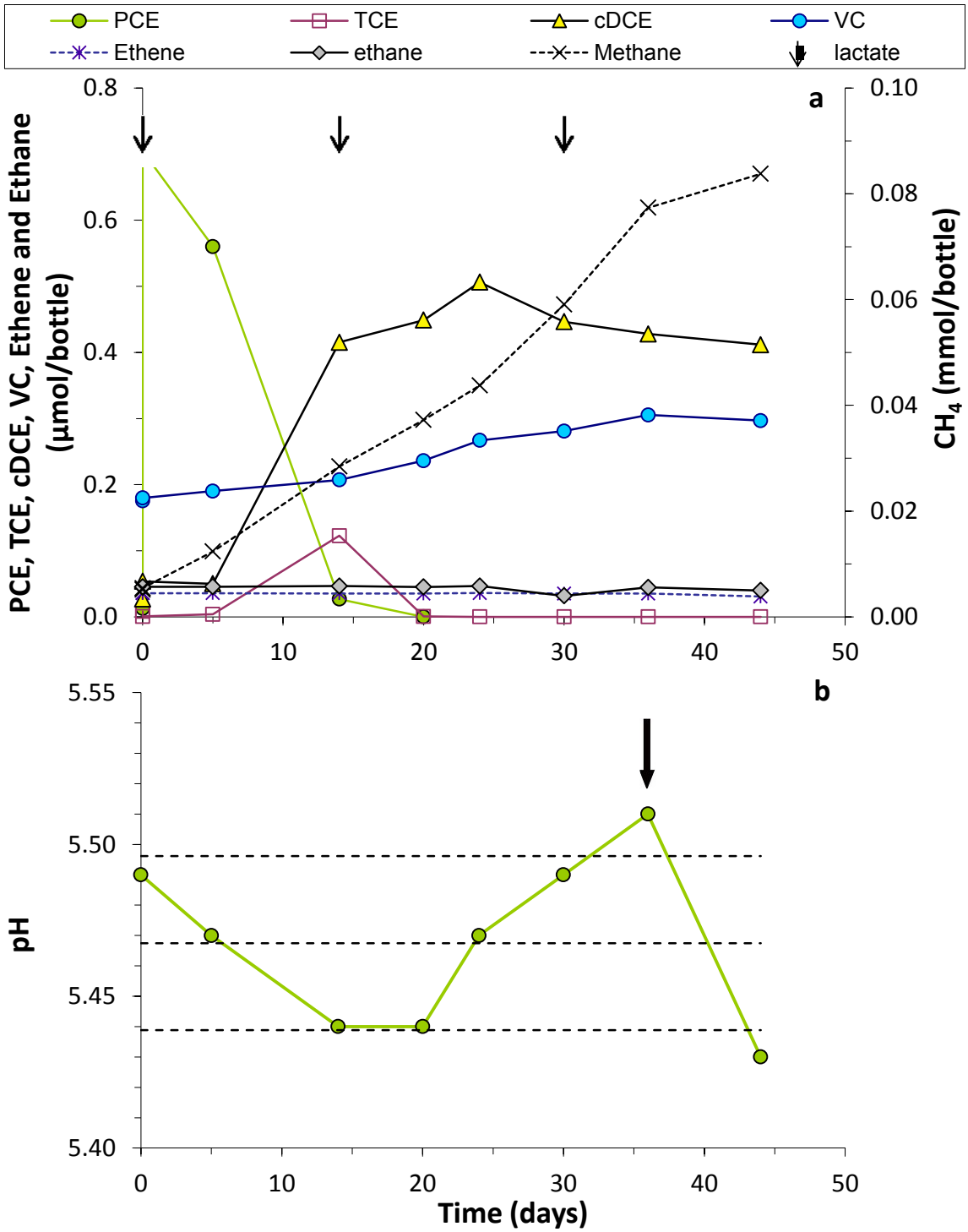

Figure E.34 Results for a lactate-amended combined enrichment culture, Set II, serum bottle #2 (GW-VC-4B+NC-lactate-MES-S3-2) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

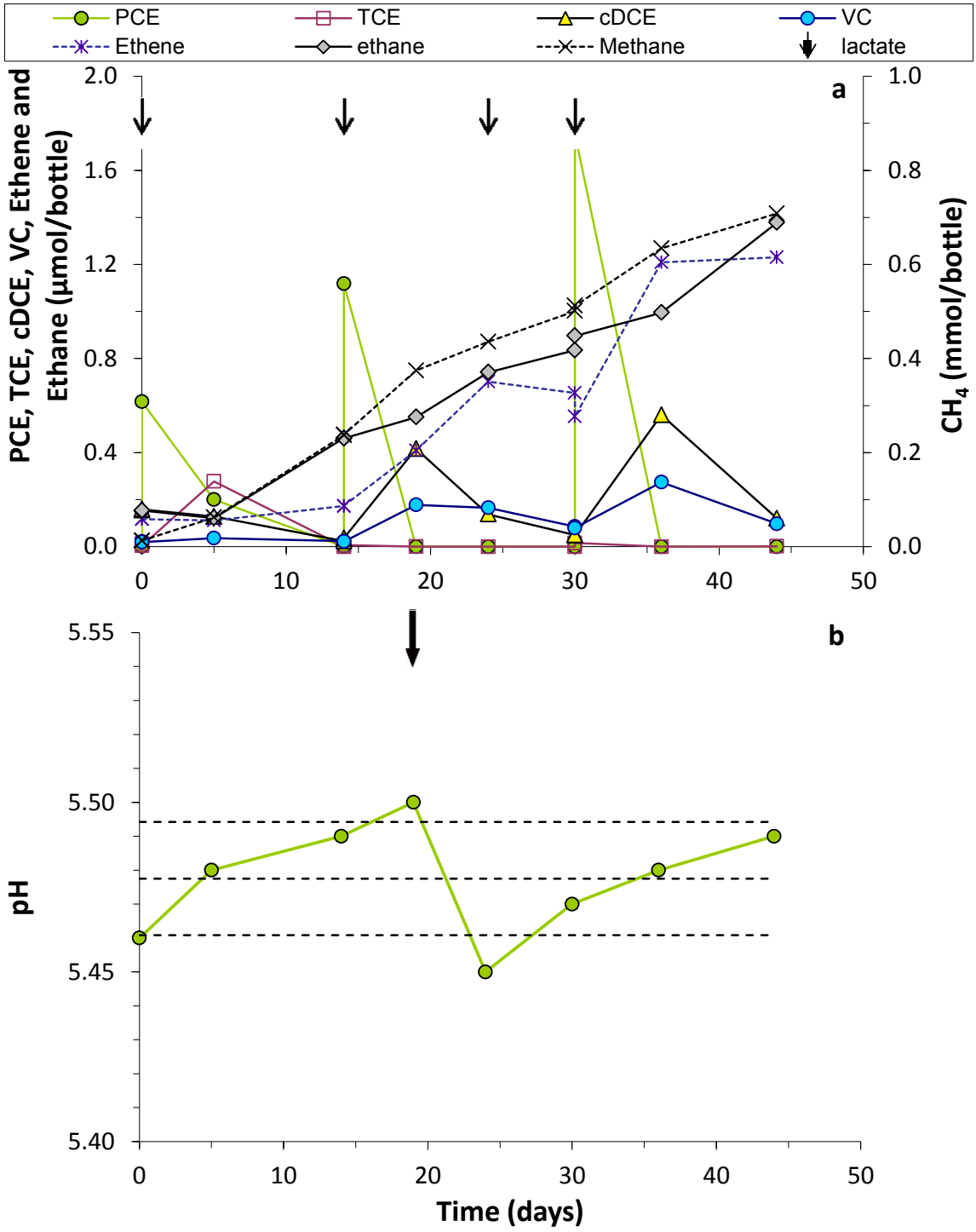

Figure E.35 Results for a lactate-amended combined enrichment culture, Set III, serum bottle #2 (MM-cDCE+VC-B3+NC-UN1-MSM-lactate-2) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

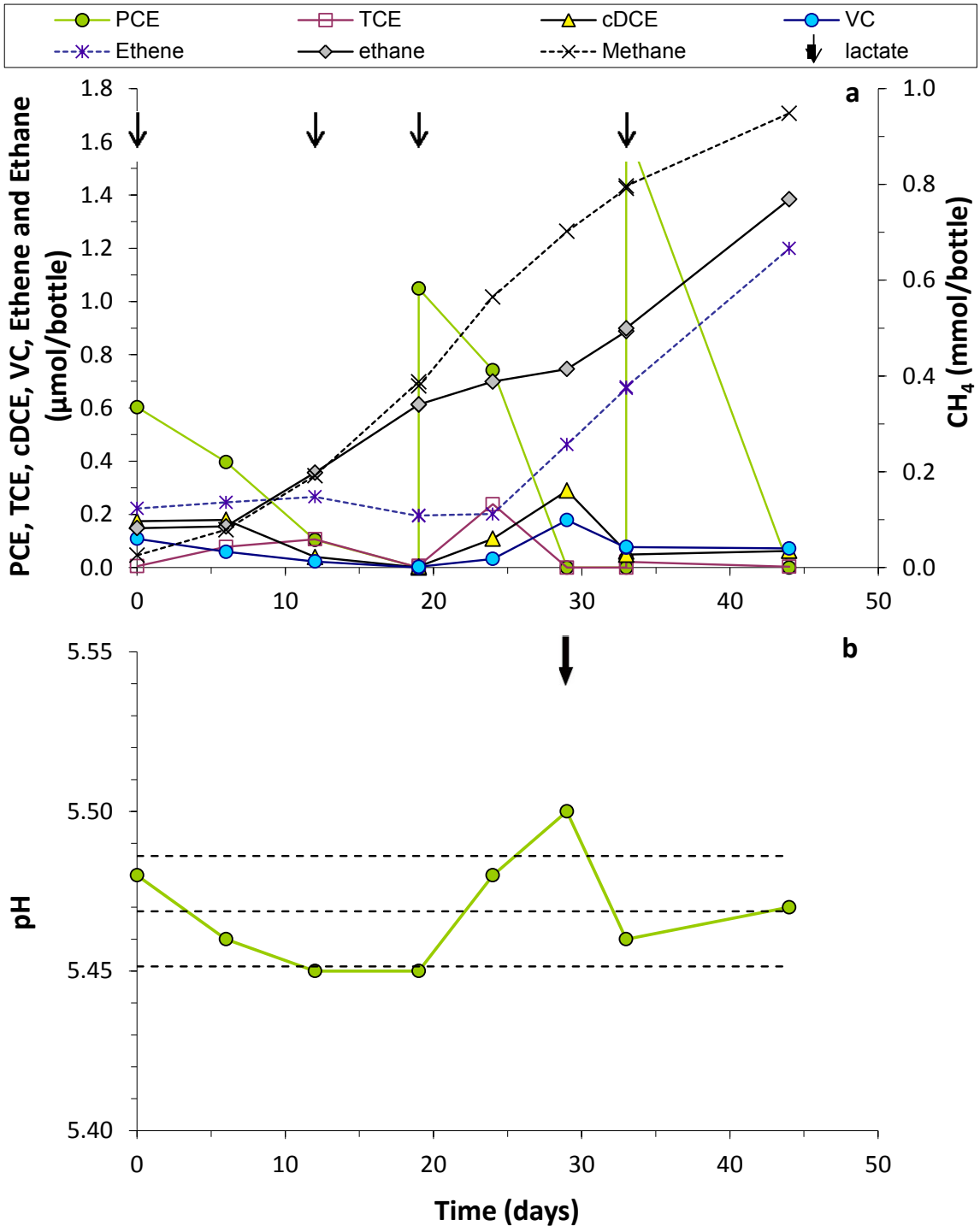

Figure E.36 Results for a lactate-amended combined enrichment culture, Set III, serum bottle #2 (MM-cDCE+VC-B3+NC-lactate-MSM-S2-2) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

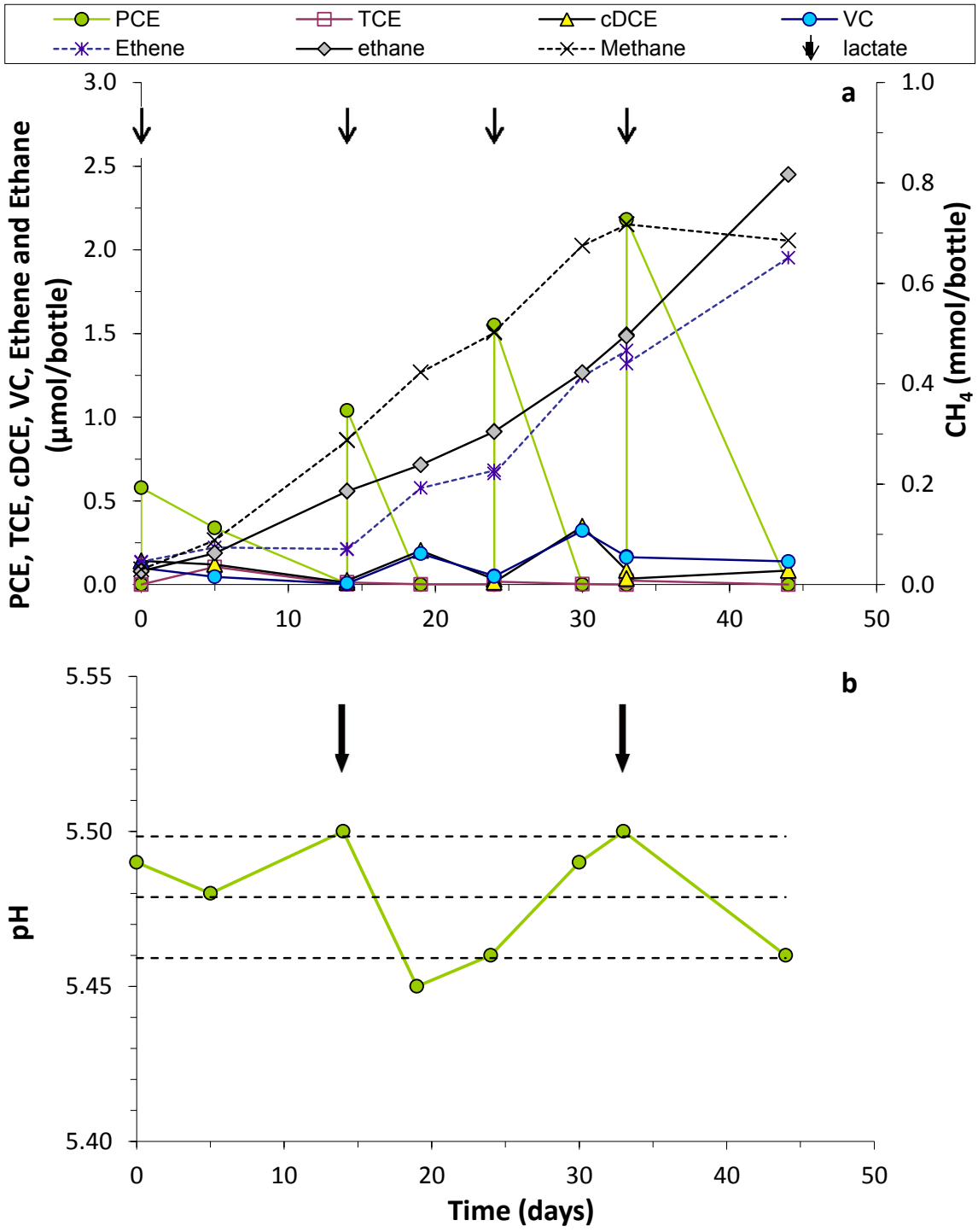

Figure E.37 Results for a lactate-amended combined enrichment culture, Set III, serum bottle #1 (MM-cDCE+VC-B3+NC-lactate-MSM-S3-1) for (a) VOCs and (b) pH; the dashed horizontal lines represent the average ± standard deviation. Arrows indicate addition of phosphoric acid.

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