

# Effect of pH on phosphine production and the fate of phosphorus during anaerobic process with granular sludge

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## Abstract

The effect of pH on phosphine formation during anaerobic cultivation of granular sludge was investigated. The sludge was taken from full-scale anaerobic reactors treating brewery wastewater. Acetate and phosphate were used as the carbon source and phosphorus source respectively. After 10 days cultivation in the dark, results showed that acidic conditions were more favorable for free phosphine production. At pH 5, the optimum concentration 86.42 ng PH<sub>3</sub>m<sup>-3</sup> of free phosphine was obtained. The level at pH 7 was reduced to 18.53 ng PH<sub>3</sub>m<sup>-3</sup>, about 1/5 of the maximum. The maximum concentration of matrix-bound phosphine of 3.30 ng PH<sub>3</sub>kg<sup>-1</sup> wet sludge was achieved at pH 6. More than 83% of the total phosphine was matrix-bound phosphine, which accounted for 0.003–0.009‰ of the phosphate removal, while free phosphine comprised 0.00002–0.001‰ of the phosphate removal. Most of the phosphorus removal from solution was turned into chemical precipitation or was adsorbed by sludge. The mechanism of the phosphate reduction-step in the formation of phosphine production is still unknown. The promotion of phosphine formation by low pH is compatible with an acidic bio-corrosion mechanism of metal particles in the sludge or of metal phosphides which form phosphine at low pH.

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## 1. Introduction

Understanding the mechanisms of phosphine gas (PH<sub>3</sub>) formation in the volatile cycling of phosphorus could be beneficial for many areas like understanding lake eutrophication.

Phosphine gas has been found worldwide in the earth's atmosphere even at remote locations at the 1 ng m<sup>-3</sup> range (Glindemann et al., 1996), exploring also a diurnal night-time maximum of phosphine in the upper troposphere (Glindemann et al., 2003), in surface air of the Northern Sea (Gassmann et al., 1996) and in the urban air of Beijing at the 100 ngm<sup>-3</sup> range (Liu et al., 1999). The sources of phosphine which drive this gaseous link of the phosphorus cycle could be industrial (WHO, 1988) or environmental formation mechanisms.

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Gassmann and Glindemann (1993) coined the terms “free phosphine” and “matrix-bound phosphine”, after they found that the chemical digestion of sediments, sludges and other condensed samples with acids or bases causes the liberation of phosphine gas from an unknown precursor, which was termed matrix-bound phosphine. This concept was later found to be useful for many media.

The mechanism of anaerobic biological phosphine production has been proposed and researched for more than one hundred years (Roels and Verstraete, 2001), yet is still a controversy. Rutishauser and Bachofen (1999) reported phosphine production from sewage sludge cultures is a reaction microbiologically mediated. On the other hand, Glinderman et al. (1998) proved the existence of a “biologically mediated” formation mechanism of phosphine by bio-corrosion in iron which is rich of phosphorus. Corrosion of iron and phosphine production is accelerated at low pH in the presence of organic acids and of sulfide as exudates of bacterial metabolism. This mechanism is not based on microbial phosphate reduction, though; it refers to chemical reduction of phosphate in iron ore during metallurgical iron production. This mechanism was used to explain the finding of free phosphine (formed from highly reactive phosphides in iron) as well as of matrix-bound phosphine (formed from poorly reactive phosphides in iron). This mechanism is also able to explain why phosphine is formed under bio-anaerobic conditions, because, under aerobic conditions, the oxidation product phosphate will be the product of iron and iron phosphide corrosion. Morton et al. (2003) have analyzed pure iron phosphide ( $\text{Fe}_3\text{P}$ ), which is occurring as an important phosphorus species in iron metal, and showed that it is chemically more inert during neutral pH, but forms phosphine by acidic digestion. The yield of phosphine was low in these experiments with pure phosphide crystals.

Many factors affect the formation, transportation and stability of phosphine. Soil plays an important role in the stability of phosphine. When exposed to soil samples, phosphine disappeared according to exponential relationship (Eismann et al., 1997a). In the presence of light and oxygen, phosphine can be degraded quickly (Han et al., 2000). An increased concentration of phosphine has been detected as a variety of organic or inorganic substances (glucose, formate, phosphate and sulfide etc.) were added during anaerobic cultivation; the addition of Fe(III) can accelerate the removal of phosphine (Eismann et al., 1997b). Higher phosphate concentrations accelerate phosphine formation (Han et al., 2000). However, limited research has been conducted to investigate the effects of fermentation conditions and environmental conditions on the formation of phosphine. In addition, the effect of pH and temperature on phosphine formation during anaerobic process has never been investigated.

Furthermore, to investigate the fate of phosphorus during anaerobic processes, chemical extraction methods are used to identify various phosphorus species in granular sludge. Sequential chemical phosphorus extraction (fractionation) methods have been widely utilized over the past two decades to quantify various forms of phosphorus in sediment (Baldwin, 1998). Uhlmann et al. (1990) modified the procedure of Psenner et al. (1988) and applied it to activated sludge from waste treatment plants for biological phosphate removal.

The aim of this research was two fold: firstly, to investigate the effects of the initial solution pH on phosphine formation during anaerobic digestion; secondly, to evaluate the behavior of phosphate during anaerobic wastewater treatment, including phosphate removal, transportation, distribution of phosphorus between sludge and headspace. In this paper, the method developed by Uhlmann et al. (1990) was used for normal phosphate species characterization and quantification.

## 2. Materials and methods

### 2.1. Materials

The granular sludge for inoculation came from the anaerobic reactor of Wuxi Lion Nathan Taihushui Brewery Co. Ltd. in Jiangsu (China). The granules were black, and of 0.1–2 mm diameter. The Suspended Solid (SS) and Volatile Suspended Solid (VSS) content of the granular sludge were 57.7 and 46.2  $\text{g l}^{-1}$  respectively. The culture medium contained ( $\text{g l}^{-1}$ ): acetate, 2.34;  $\text{KH}_2\text{PO}_4$ , 1.76;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.03;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.03;  $\text{FeCl}_2$ , 0.01;  $\text{NH}_4\text{Cl}$ , 0.06. The salts were dissolved in deionized water and the substrate had not been sterilized before tests. A sodium bicarbonate buffer (6 mM) was used.

### 2.2. Batch experiments

Batch experiments were performed in 600 ml serum bottles in duplicate. 100 ml of granular sludge and 174 ml of culture medium were added into serum bottles. Solutions of 1 M NaOH or 0.5 M HCl were used to adjust the pH to 4, 5, 6, 7, 8 and 9. Then samples of solution were taken for analyzing soluble reactive phosphate and we defined this concentration as initial phosphate concentration. After being flushed with high purity  $\text{N}_2$ , the bottles were sealed anaerobically and incubated in the dark at 15 °C for 10 d. The deviation between the bottles was less than  $\pm 10\%$ . The bottles were vigorously shaken for at least 3 min before gas samples were taken to equilibrate the gas concentration in liquid and gas, to prevent supersaturation of phosphine in the liquid phase. Gas production was measured by volume dis-

placement of a gas tight 60 ml plastic syringe. Because some cultures produced low gas volume, 100 ml N<sub>2</sub> were injected into the bottle and then 100 ml gas was taken out after mixture. Both the primary gas production and 100 ml gas displacement were injected into GC column for free phosphine detection. The detected concentration of free phosphine was corrected for the actual injected volume. After measurements these samples were discarded to prevent any possible errors introduced by sampling procedures, such as gas leakage during sampling.

### 2.3. Chromatographic detection of free phosphine and matrix-bound phosphine

Phosphine was analyzed by a HP 4890 GC with an NPD (Nitrogen-phosphorus detector). The instrument was equipped with a cryogenic trapping system using liquid nitrogen. To eliminate the interference of acidic gases such as hydrogen sulfide, gas samples were treated with sodium hydroxide before injection. Chromatographic separation was performed on a PLOT-Al<sub>2</sub>O<sub>3</sub>/Na<sub>2</sub>SO<sub>4</sub> column (25 m × 0.53 mm I.D. × 10 μm). The column temperature was 40 °C, and the detector temperature was 300 °C. The flow-rates of the detector gases were 7 ml min<sup>-1</sup> for H<sub>2</sub>, 95 ml min<sup>-1</sup> for air and 25 ml min<sup>-1</sup> for N<sub>2</sub>, as the make-up gas.

For matrix-bound phosphine analysis, 5 ml of 2 M sodium hydroxide solution and 1 g of wet granular sludge were mixed and boiled, and the produced gas was analyzed according to the method (Gassmann, 1994). The granular sludge samples were analyzed within 1 h. The working standard was 10.2 ppm phosphine diluted in nitrogen gas.

### 2.4. Phosphorus fractionation of granular sludge

Sequential extraction steps were performed according to the method of Uhlmann et al. (1990) as shown in Table 1. About 1 g of granular sludge was placed in a 50 ml acid-washed centrifuge tube for fractionation. The supernatants of each extraction after centrifugation for 12 min at 4500 rpm were analyzed for molybdate

reactive phosphorus. All analyses were run in triplicate, and the results were given on a wet sludge weight basis.

## 3. Results and discussion

### 3.1. Effects of pH on phosphine production

Anaerobic granular sludge was cultured at 15 °C for 10 d with synthetic media containing 300 mg P l<sup>-1</sup> of phosphate as the sole phosphorus source and the initial pH of solution varied from 4 to 9. Fig. 1 presents the effects of pH on free phosphine production, the concentration of matrix-bound phosphine and biogas yield.

Acidic conditions were more favorable for the production of free phosphine. When pH was increased from 4 to 5, the concentration of free phosphine increased from 38.07 to a maximum of 86.42 ng PH<sub>3</sub> m<sup>-3</sup> and then dropped to 18.53 ng PH<sub>3</sub> m<sup>-3</sup> at neutral pH, almost 1/5 as much as the maximum. This value dramatically declined to 1.72 ng PH<sub>3</sub> m<sup>-3</sup> when the initial pH of the solution was 9. Biogas production, on the other hand, is greatest at neutral pH (Fig. 1c).

These results also confirmed the study of Jenkins et al. (2000), in which he claimed that fermentative bacteria, rather than methanoarchaea, participate in generating phosphine. Monoseptic cultures of certain mixed acid fermentors (*Escherichia coli*, *Salmonella gallinarum*, and *Salmonella arizonae*) and solvent fermentors (*Clostridium sporogenes*, *Clostridium acetobutyricum* and *Clostridium cochliarium*) also generated phosphine. In another aspect, low pH promoting phosphine formation is also compatible with an acidic bio-corrosion mechanism of phosphorus-bearing metal particles or metal phosphides in the sludge (Glinderman et al., 1998). The source of free phosphine in the headspace during anaerobic digestion is still a controversy and needs further research.

Matrix-bound phosphine was analyzed to understand the effect of initial pH on free phosphine production. Matrix-bound phosphine, is defined as the phosphine gas liberated from chemical digestion of a sample, and originates likely from the adsorbed phosphine, metalphosphine complexes or inorganic

Table 1  
Phosphorus extraction scheme and fractional composition of reactive phosphorus (25 °C)

Exactant	Volume (ml)	Time (h)	Fraction acronym	Main species
H <sub>2</sub> O	40	0.2	H <sub>2</sub> O-P	Easily extractable (washable) phosphorus
0.11 M NaHCO <sub>3</sub> + 0.11 M Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub>	25	0.5	BD-P	Phosphate bound to ferric hydroxide
1 M NaOH	25	18	NaOH-P	Phosphate bound to metal oxides and exchangeable against OH <sup>-</sup> ; Phosphorus compounds soluble in bases
0.5 M HCl	25	18	HCl-P	Phosphate bound to CaCO <sub>3</sub> , MgCO <sub>3</sub> , in apatite and possibly in struvite

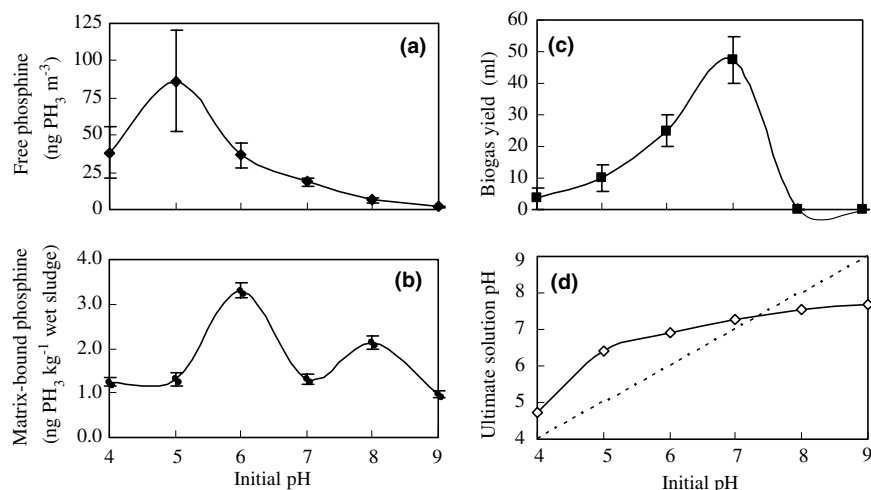


Fig. 1. (a) Effects of initial pH on free phosphine production (—◆—), (b) the concentration of matrix-bound phosphine (—●—), (c) biogas yield (—■—) and (d) the ultimate solution pH after cultivation (—○—) ultimate pH (dashed line means initial pH).

phosphide. Results show that the concentration of matrix-bound phosphine followed a wave-like trend with increasing initial solution pH (Fig. 1b). At pH 6 and 8, it reached two peaks respectively. A maximum of 3.30 ng PH<sub>3</sub> kg<sup>-1</sup> wet sludge was obtained at pH 6; at pH 8 the concentration was 2.04 ng PH<sub>3</sub> kg<sup>-1</sup> wet sludge. This value was kept at a range of 1.25–1.31 ng PH<sub>3</sub> kg<sup>-1</sup> wet sludge when pH was 4, 5 and 7. The lowest concentration of 0.98 ng PH<sub>3</sub> kg<sup>-1</sup> wet sludge was observed at pH 9. This result is different from the conclusion of Han et al. (2002), who claimed that neutral conditions (pH = 6.74) produced highest matrix-bound phosphine and the level in acidic soil (pH = 4.85) was the lowest. But in the anaerobic system reported by them, no media was added to the soil during cultivation, which means that little methanogenesis occurred. Whether large amount of biogas production will affect the concentration of matrix-bound phosphine is still a presumption,

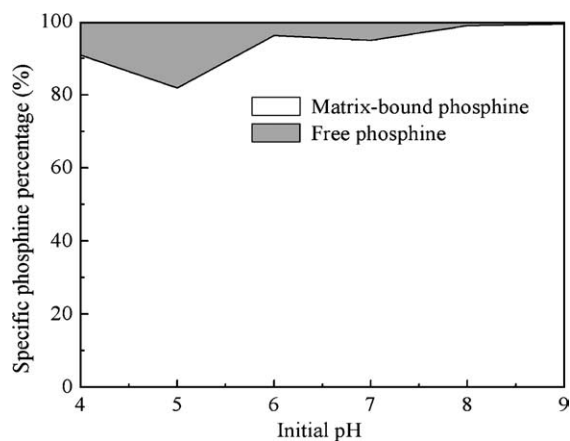


Fig. 2. Effects of initial solution pH on distribution of matrix-bound phosphine and free phosphine (filled area); matrix-bound phosphine (open area).

Table 2

Anaerobic phosphate removal and phosphine production (external phosphorus added 69.6 mg P per bottle)

Initial pH of solution	Initial phosphate conc. (mg P l <sup>-1</sup> )	Phosphate conc. after cultivation (mg P l <sup>-1</sup> )	Phosphate removal rate (%)	Mean phosphorus loss from solution (mg P)	Total phosphine (pg P)	The ratio of total matrix-bound phosphine to phosphate loss (‰)	The ratio of total free phosphine to phosphate loss (‰)
4	270 ± 23	120 ± 7	56	26.2	126 ± 14	0.004	0.0004
5	268 ± 7	115 ± 7	57	26.6	146 ± 25	0.004	0.0010
6	327 ± 19	137 ± 17	58	33.0	313 ± 17	0.009	0.0004
7	266 ± 24	133 ± 14	50	23.2	124 ± 12	0.005	0.0003
8	249 ± 11	86.3 ± 2	65	28.3	188 ± 14	0.006	0.0001
9	295 ± 3	106 ± 4	64	33.0	90.1 ± 8	0.003	0.00002

which needs more evidence to verify. When the cultivation ended, the pH of solution deviated to neutral conditions (Fig. 1d).

The distribution of phosphine between headspace and granular sludge is presented in Fig. 2. Results showed that matrix-bound phosphine comprised 83–99%, while free phosphine accounted for 1–17%. At pH 5, free phosphine reached the maximum level. Burford and Bremner (1972) found that, soil constituents sorbed phosphine and presumed that only when the rate of phosphine formation by microbial reduction of phosphate is larger than the rate of phosphine sorption by soil constituents can free phosphine be detected. Results presented in Fig. 2 are similar to data reported by Dévai et al. (1999), who claimed that about 85% of total phosphine was matrix-bound phosphine.

### 3.2. The behavior of phosphorus during anaerobic wastewater treatment

During anaerobic wastewater treatment, phosphate removal, translation and transformation were investigated. The initial phosphate concentration was in the range of 249–327 mg P l<sup>-1</sup>, i.e. there was a decrease of the phosphate concentration after the media (399 mg P l<sup>-1</sup>) were added to the sludge. After 10 d of cultivation, 50–65% phosphate was removed from the solution compared to the initial one, as is shown in Table 2, that is, 23–33 mg P was removed. No obvious relationship between the initial pH of solution and phosphate removal has been observed.

The total amount of matrix-bound phosphine in the granular sludge of each bottle was estimated to be in the range of 89.6–301 µg P, which accounted for 0.003–0.009‰ of phosphate removal. Yet free phosphine only comprised as less as 0.00002–0.001‰ of the phosphate removal. Total phosphine, which included free phosphine and matrix-bound phosphine, was in the range of 90.1–313 µg P (Table 2).

Results of phosphorus fractionation of granular sludge are shown in Table 3. The concentration of OH-exchangeable phosphorus and metal oxides-bound phosphate (NaOH-P) increased dramatically during cultivation, from 329 mg P kg<sup>-1</sup> wet sludge to 576–736 mg P kg<sup>-1</sup> wet sludge, i.e. 36–59% of the external phosphorus was turned into ion-bound phosphorus. The concentration of water-dissolved phosphate (H<sub>2</sub>O-P) was increased from 12.6 mg P kg<sup>-1</sup> wet sludge to 152–208 mg P kg<sup>-1</sup> wet sludge, which accounted for 20–28% of external phosphorus. This indicated that chemical precipitation and adsorption play a critical role in the removal of phosphorus from the solution. Moreover, the reductant soluble phosphate (phosphate bound to ferric hydroxide) also increased 9.2–12.5%, yet the concentration of calcium- and magnesium-bound phosphorus (HCl-P) changed little.

Table 3  
Phosphate fractions of inoculum granular sludge and granular sludge cultivated for 10 d

Initial pH of solution	Phosphate fraction of sludge (mg P kg <sup>-1</sup> wet sludge)				Mean increase of phosphate species after cultivation (mg P)				The ratio of phosphate species increase to additive phosphorus (%)			
	H <sub>2</sub> O-P	BD-P	NaOH-P	HCl-P	H <sub>2</sub> O-P	BD-P	NaOH-P	HCl-P	H <sub>2</sub> O-P	BD-P	NaOH-P	HCl-P
Inocula	12.6 ± 2.0	18.4 ± 1.3	329 ± 21	49.8 ± 2.7	—	—	—	—	—	—	—	—
4	152 ± 17	101 ± 0.4	736 ± 55	41.4 ± 2.5	13.9	8.30	40.7	-0.84	19.7	11.9	58.5	-1.21
5	170 ± 4	82.6 ± 7.3	581 ± 61	46.1 ± 4.6	15.7	6.43	25.2	-0.37	22.6	9.2	36.2	-0.53
6	209 ± 15	106 ± 8.1	735 ± 5	58.4 ± 4.6	19.6	8.73	40.6	0.86	28.2	12.5	58.3	1.24
7	182 ± 5	98.4 ± 8.2	614 ± 25	41.0 ± 2.9	16.9	8.00	30.7	-0.88	24.3	11.5	44.1	-1.26
8	175 ± 15	83.2 ± 2.7	576 ± 6	55.6 ± 12	16.2	6.49	24.7	0.59	23.3	9.3	35.5	0.85
9	180 ± 10	103 ± 6.7	656 ± 59	42.1 ± 4.2	16.7	8.50	32.7	-0.77	24.0	12.2	46.9	-1.11

#### 4. Conclusions

The influence of pH in the biogas process on free phosphine and matrix phosphine was tested and the importance of phosphine versus phosphate in phosphorus removal from sewage sludge was evaluated. Acidic conditions were more favorable for free phosphine production. At pH 6, the maximum concentration of matrix-bound phosphine of  $3.30 \text{ ng PH}_3 \text{ kg}^{-1}$  wet sludge was achieved. Phosphine was mainly present in matrix-bound form and only 1–17% of phosphine was free phosphine.

The total amount of matrix-bound phosphine in the granular sludge per bottle accounted for 0.003–0.009% of phosphate removal. Free phosphine comprised 0.00002–0.001% of the phosphate removal. Most of the phosphorus removal from solution was turned into chemical precipitation and adsorbed by sludge. It is not known at this time, if the produced phosphine originates from reduction of pentavalent phosphorus (any form of phosphate) or from the “microbially mediated” bio-corrosion mechanism (Glindermann et al., 1998) due to presence of phosphorus containing traces of iron or of iron phosphides in the sludge samples.

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