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Effect of Iron Hydroxide on Phosphate Removal during Anaerobic Digestion of Activated Sludge

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Abstract—The addition of iron (III) hydroxide during methanogcnic digestion of activated sludge by anaerobic sludge displaying an iron-reducing activity resulted in a microbial reduction of iron (III) with the formation of iron (II). capable of precipitating phosphates. The feasibility of eliminating 66.6 to 99.6% of the dissolved phosphate at initial concentrations of 1000 to 3500 mg PO_4 /! by adding 6420 mg/l iron (III) hydroxide into a reactor for anaerobic fermentation of activated sludge was analyzed. The optimal ratio of iron (III) added to dissolved phosphate removed (mg) providing a 95% removal amounted to 2 : 1. These results may be used in new technology for anaerobic wastewater treatment with phosphate removal.

The average concentration of total phosphorus (the sum of inorganic and organic phosphorus) in household wastewater amounts to 10-20 mg/l; the major part (50-70%) of the phosphorus is present in a form of orthophosphate [I]. An increased concentration of phosphate in the treated wastewater entering a natural aquatic system may cause its eutrophication, resulting in a decrease in the concentration of dissolved oxygen and quality of the freshwater. Consequently, phosphate should be removed to the highest possible degree during the biological treatment of wastewater.

Most frequently, phosphate is removed from wastewater by chemical methods, for example, precipitation as calcium, iron, and aluminum salts [2J. The concentration of phosphate may also be decreased by microbiological methods due to its accumulation as polyphosphate granules by microbial cells [3]. However, further anaerobic digestion of activated sludge enriched with polyphosphates results in the release of soluble phosphate from microbial cells.

Theoretically, it is possible to use iron-reducing bacteria to eliminate phosphorus from wastewater. Iron-reducing bacteria are capable of using Fe(III) as an acceptor of electrons and reduce it to Fe(II) during oxidation of organic substrates under anaerobic conditions [4]. The potential feasibility of the technological application of iron-reducing bacteria for the biodegradation of aromatic hydrocarbons and their derivatives was demonstrated [5-7].

Iron-reducing bacteria may exist under the conditions observed in anaerobic reactors. For example, wastewater of Danish wastewater treatment facilities, whose technological scheme included phosphate removal, contained iron in considerable amounts [8]. This data suggested to the authors that iron-reducing bacteria might be a constant component of microbial communities in the wastewater treatment facilities that used anaerobic treatment of activated sludge [6]. Later, the presence of these bacteria in an amount exceeding 3% of the total microbial population in activated sludge of a wastewater treatment facility that used chemical phosphate removal by precipitation with iron sulfate was demonstrated [9].

A positive effect of iron addition on the methanogenic fermentation of the wastewater containing fats and products of their degradation, long-chain fatty acids, was reported [10]. The inclusion of iron into an integrated anaerobic-aerobic wastewater treatment results in the reduction of Fe(III) to Fe(II) under anaerobic conditions: at the aerobic stage, Fe(II) oxidizes to $Fe(OH)_4$ and precipitates the ammonium formed during the anaerobic activated sludge digestion. Consequently ammonium passes into an iron-containing sediment, which may be used as a fertilizer [11-13]. As demonstrated in these works, iron reduction and oxidation increase the efficiency of biological treatment and prevent the secondary pollution of the environment with ammonium and products of its oxidation.

Microbial reduction of iron (III) by iron-reducing bacteria and the co-precipitation of phosphates by the iron (II) ions formed may be the background for a new method for phosphate removal from wastewater or activated sludge digested anaerobically. Positively or negatively charged iron (П) hydroxides formed after iron (III) reduction are also capable of precipitating phosphates, according to the following equations:

$$+ CH_20 + H_20 - 4Fe^{2+} + C0_2 + 4H^+ \quad (1)$$

$$.r,Fe^{2+} + x, H, 0 - *, Fe(OH)'i$$
$$" \quad * \quad (2)$$
$$+ A-^4Fe(OH,)i + *^5Fe(OM);l + x^bU\backslash$$

$$(4-A,)Fe^{>} + (4-JC,)HP04 - (4-x,)FeHP0^4l, \quad (3)$$

$$.v,Fe(OHri+.v^3H^2P0^4 -*.v^3FeHP0^4l+.v^{:}(H^20. \quad (4)$$

where x,, x^2. x^3, x^4, x^y and $*^6$ are stoichiometrical coefficients of terms of the reaction equation.

However, these reactions may decrease the pH due to the release of protons into the medium; this may negatively affect the anaerobic digestion of wastewater or activated sludge. Therefore, the addition of iron during anaerobic digestion requires either automated maintenance of the pH or the use of a pH buffer. The removal of phosphate by particles of insoluble iron (Ш) or iron (II) hydroxides is less efficient, compared to iron (II) ions. Consequently, the conditions for this process should decrease the formation of iron (II) hydroxide from iron (II) ions by either a decrease in the pH or the formation of soluble complexes between organic acids and iron (II).

Anaerobic digestion of the activated sludge formed during aerobic treatment of municipal wastewater was modeled experimentally.

The goal of this work was to study the effect of iron (III) hydroxide on the removal of phosphates during anaerobic digestion of activated sludge.

MATERIALS AND METHODS

The experiments were performed in 100-ml and 12-1 reactors. Anaerobic sludge from treatment plants for industrial and household wastewaters or accumulation culture was used as a source of iron-reducing bacteria.

The accumulation culture of iron-reducing bacteria was obtained using a mineral medium containing 2.5 g/1 $NaHC0_3$, 1.5 g/1 NH_4CI, 0.6 g/l NaH_2P0_4. 0.1 g/l KCI, and 0.05% yeast extract. Iron citrate (20 raM) was added as a source of iron; ethanol (1 g/l), as a source of carbon. Trace elements (10 ml) and vitamins (10 ml) were added to 1 1 of medium [14,15]. The solution of trace elements comprised 1.5 g/1 nitrilotriacetic acid, 3.0 g/l $MgS0_4$ • 7 H,0, 0.5 g/1 $MnS0_4$ • 2HA 1.0 g/1 NaCl, 0.1 e/1 $FeS0_4$ • 7 H,0, 0.18 g/l $CoS0_4$ - 7H.O, 0.1 g/I CaCI, • 2H,0, 0.18 g/l $ZnS0_4$ • 7H,0, 0.01 gЛ $CuS0_4$ • 5РШ, 0.02 g/1, KA1($S0_4$), • I2BA 0.01 g/\ H,BO„ 0.01 g/1 Na,$Mo0_4$ • 2HД 0.025 g/1 $NiCl_2$ • $6H_20$, 6.0003 g/l Na_2Se0_3 • 5II?0, and distilled water to 1000 ml. When preparing this solution, nitrilotriacetic acid was dissolved first, the pH was adjusted to 6.5 with I N KOH, the rest of the mineral components were added, and the pH was adjusted to 7.0. The solution of vitamins contained 2.0 mg/1 biotin. 2.0mg/l folic acid, 10.0 mg/l pyridoxine-HCl, 5.0 mg/1 thiamine-HCl • $2H_20$. 5.0 mg/1 nicotinic acid, 5.0 mg/1 calcium D-pantothenate, 0.1 mg/I vitamin $B_{,2}$, 50 mg/1 *p*-aminobenzoic acid, 5.0 mg/1 lipoic acid, and distilled water to 1000 ml. Cycloheximide (Sigma, USA) was added to a final concentration of 0.01%, to prevent growth of microbial fungi in the accumulation culture. Anaerobic sludge was activated, kept for 3 days in ethanol-containing medium, and used as an inoculum (5 vol %) to obtain accumulation culture on the medium with iron citrate.

Enrichment cultures of iron-reducing bacteria were used when comparing iron hydroxide and iron-containing clay as inexpensive sources of iron (III). Iron hydroxide was obtained through the slow neutralization of 250 mM of an $FeCl_3$ solution in 2 M NaOH; the precipitate formed was rinsed six times with distilled water until chlorine and sodium were removed completely [15]. The clay used in the experiments contained 6.9% iron. An iron hydroxide suspension was added to a final iron (III) concentration of 3360 mg/1 (60 mM); 25% clay suspension, of 4480 mg/1 (80 mM). An accumulation culture of iron-reducing bacteria (5 vol %) was added as inoculum. The culture was grown in 100-ml glass reactors plugged with rubber stoppers in a shaker at 150 rpm at 25°C in darkness. A syringe was used for sampling.

Tabic I. Changes in iron (II) concentration (mg/1) in control and experiment

Time, days	Total Fe(II)		Dissolved Fe(II)		Precipitated Fe(II)	
	control	experiment	control	experiment	control	experiment
0	289.9	302.6	2.2	2.8	287.7	289.8
3	265.8	1176.8	1.9	1.8	263.9	1175.0
6	271.6	1507.1	1.3	1.4	270.2	1505.6
9	306.2	1572.5	1.2	6.5	305.0	1566.0
12	332.2	1685.2	3.0	8.8	329.2	1676.4
15	284.1	1466.6	5.5	3.4	278.5	1463.2
18	335.1	1390.5	1.4	2.0	333.7	1388.5
21	271.6	1324.1	1.3	2.9	270.2	1321.2

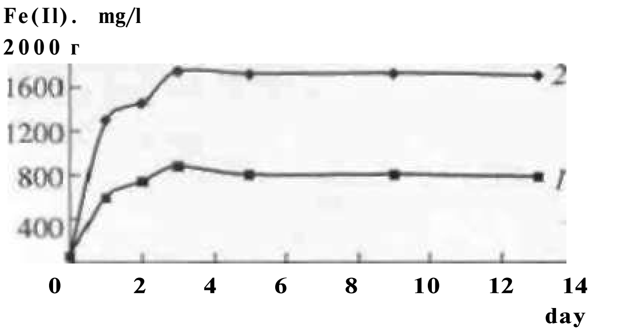
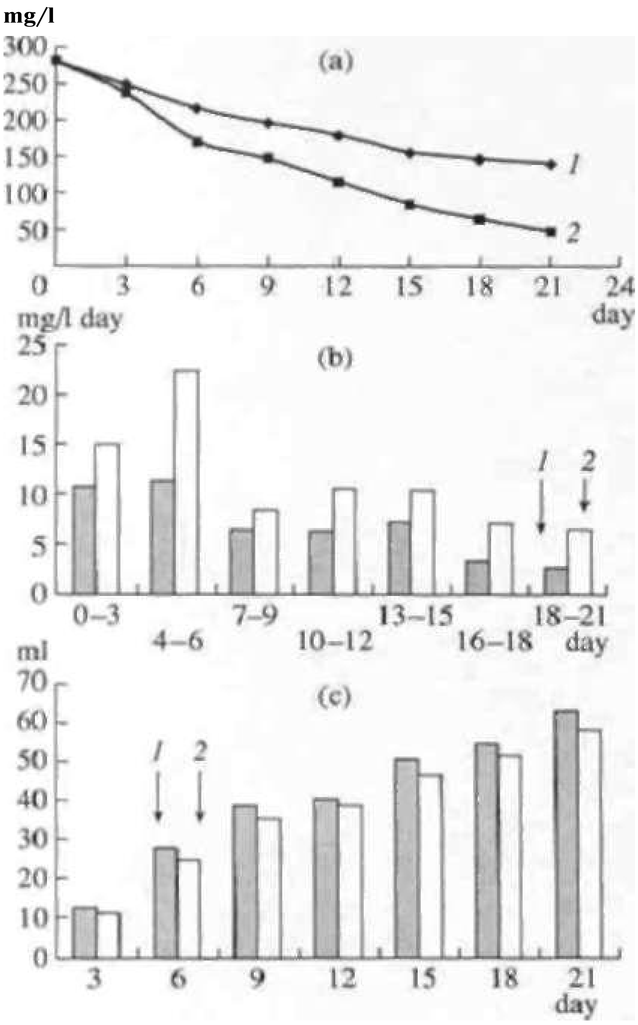


Fig. 1. Reduction of Fc(II) by an accumulation culture of iron-reducing bacteria using (/) iron-containing clay and *(2)* iron hydroxide.



Fiji- 2- (a) Content of total organic carbon, (b) removal rate of total organic carbon, and (c) formation of biogas in < /) control and *(2)* experiment.

When studying the effect of iron reduction by iron-reducing bacteria on phosphate removal during the digestion of excess activated sludge; activated sludge, anaerobic sludge (biomass from an industrial anaerobic reactor), and distilled water at a ratio of 3 : 2 : 1 were added to the reactor. A suspension of iron hydroxide, $Fe(OH)_3$, was used as a source of iron (ГО). In the experiment, the suspension was added instead of distilled water. An Na_3HPO_4 solution (50 g phosphate/1) was used to obtain various concentrations of dissolved phosphate (1000 to 3500 mg *PO]~* per 1 I). Activated sludge with various concentrations of dissolved phosphate was digested anaerobically without (control) and with iron (III) hydroxide (experiment). The removal of phosphate was studied over 11 days. The concentration of total organic carbon was assayed by the conventional method in an Analyser TOC-V (Shimadzu, Japan) with automated sampling [16]; the formation of biogas, volumetrically according to the volume of displaced water. The concentrations of the total and dissolved iron (II) were determined by the phenanthroline method [16]. The content of dissolved iron (II) was determined in suspension upon filtration through a membrane with 0.2-цт pores. The solution assayed was immediately mixed with phenanthroline solution and acetate-ammonium buffer at a volume ratio of 2 : 1 : 5. To determine the total iron (11), it was first extracted from the sample for 30 min by adding 1 N ЫС1 (1:1). The content of total iron (II) was determined upon filtration though a membrane with 0.2-fim pores. To construct the calibration curve, serial dilutions of a standard iron (II) solution with a concentration of 1000 mg/l. prepared by dissolving 0.355 g FeCb • $4H_2O$ in 100 ml of 1 N HCI (1 : t), was used. The concentrations of the total and dissolved phosphate were determined colorimetrically according to the intensity of the yellow color of the solution, resulting from the formation of vanadomolibdophosphoric acid, in a LTV-120IV (Shimadzu Corporation. Japan) spectrophotometer at 510 nm [16]. The content of dry substances in suspensions was determined by drying to a constant weight at 103°C-105°C; the content of organic substances was calculated according to the loss in weight upon incineration of the sample at 550°C[16].

RESULTS AND DISCUSSION

Assays of the anaerobic sludge from an industrial and household wastewater treatment facility demonstrated the presence ofa considerable amount of iron. A suspension of the anaerobic sludge displayed the following properties: pFI 7.3; content of dry substances. 21.7 ± 0.8 g/1; content of organic substances, 72.1 ± 0.5 g/1; content of total iron (II), 1.59 ± 0.26 g/l; and content of dissolved iron (II), 0.77 ± 0.01 g/1. A high iron content in anaerobic sludge may result from its presence in industrial wastewater as well as from the accumulation of precipitated iron during anaerobic digestion of activated sludge, containing 0.21 ± 0.01 g/1 (mean value + standard deviation; $n = 3$) total iron. The presence of iron in anaerobic sludge provides the conditions for growth of iron-reducing bacteria in the microbial consortium. Therefore, anaerobic sludge was chosen as the inoculum for obtaining an accumulation culture of iron-reducing bacteria.

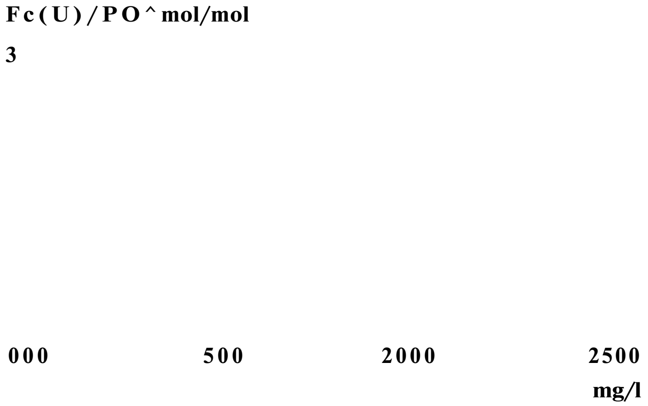


Fig. 3. Ratios of reduced iron (П) and bound phosphate (mol/mol) at various amounts of dissolved phosphate (mg/l).

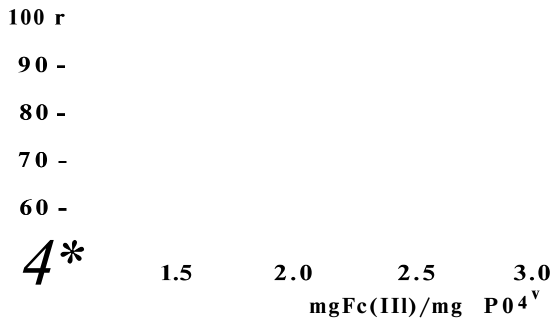


Fig. 4. Dependence of the amount of removed dissolved phosphate (% of the initial content) on the ratio of added iron (II) to removed dissolved phosphate (mg Fe(III) to mg PO_4^{3-}). Arrow indicates the optimal ratio of added iron (II) to removed dissolved phosphate.

When studying the effect of iron on the anaerobic digestion of fat-containing wastewater, it was proposed to use iron-containing clay as an inexpensive and readily available source of iron [9]. However, a comparison of iron reductions from iron-containing clay and from iron hydroxide by the accumulation culture of iron-reducing bacteria during a two-week incubation demonstrated that both the rate of iron (II) formation and its concentration were higher in the case of iron (III) hydroxide (Fig. 1). For example, the amounts of iron reduced were 57.1 and 21.5% of the total iron introduced while the maximal reduction rates equaled 51.9 ± 4.3 and 22.4 ± 1.8 mg/I h in the cases of $Fe(OH)_3$ and clay, respectively. Therefore, iron hydroxide was used in this work as the source of iron (III).

The iron-reducing activity of anaerobic sludge allowed us to use it as a source of iron-reducing bacteria. The concentration of total iron (II) remained constant, amounting to 295 ± 27 mg/l, during a 21-day anaerobic digestion of activated sludge by anaerobic sludge (Table 1). In the experimental variant with iron hydroxide, the concentration of iron (II) increased to 1685 mg/I on day 12 followed by a slow decrease to 1324 mg/I on day 21. Almost all iron (II) was in an undissolved form. The rate of iron (II) formation was highest at the beginning of the process (days 0-3) and amounted to 291 mg/l day decreasing then through 110 mg/l day (days 4-6) to 89 mg/I day (days 7-9; Table 1). The maximal amount of reduced iron accumulated by anaerobic sludge was similar to the amount accumulated by the accumulation culture of iron-reducing bacteria. However, in the former case, this level was reached on day 12 versus day 3 in the latter case. The pFI value remained stable during the anaerobic digestion, amounting to 6.9 ± 0.1 and 7.1 ± 0.1, respectively, in the control and experiment. The content of total organic carbon decreased at a higher rate and was more pronounced in the experiment with iron hydroxide compared with the control (Fig. 2). The difference in gas formation was insignificant; however, slightly lower in the experiment than in the control. The content of total phosphate in both the control and experiment remained constant and amounted to 165 ± 10 mg PO_4/I.

Table 2. Effect of iron on removal of dissolved phosphate during anaerobic digestion of activated sludge

Condition	Concentration of dissolved phosphate, mg/l		Removed dissolved phosphate	
	initial concentration, mg/l	final concentration, mg/l	mg/l	% of initial concentration
Without Fc(lll) (control)	971 ± 7	500 ± 0	471 ± 9	48.5
	1446±13	997 ± 2	448 ± 9	31.0
	1999± 13	1573 ± 10	426 ± 10	21.3
	3500 ± 24	3002 + 13	499 ± 8	14.2
Fe(III)	1071 ± 8	0.4 ± 0.0	1071 ± 9	100.0
	1471 ± 10	4.9 ± 0.3	1466± 10	99.7
	1971±13	183 ± 3	1788 ± 15	90.7
	3471 ± 18	1126± 10	2345±16	67.6

When adding iron hydroxide during anaerobic digestion of activated sludge with various concentrations of dissolved phosphate, it was demonstrated that the amount of removed phosphate in the control remained virtually constant (Tabic 2). However, the samples supplemented with iron hydroxide displayed a considerably higher percent of phosphate removal than the control. For example, removal of the dissolved phosphate present at an initial concentration of 1000 mg VOi~/\ amounted to 48.5% in the control and 100% in the experiment; at 1500 PO] /1, to 31.5 and 99.7%, respectively; at 2000 POj" /I, to 21.3 and 90.7%, respectively; and at 3500 PO^/L, to 14.2 and 67.6%, respectively.

The major form of phosphate at neutral pH is the ion HPC>4⁻ [17]. As anaerobic digestion was performed at neutral pH, it is likely that FeHP0[4] Will be the main phosphorus-conLaining compound, According to the results obtained, the maximal amount of reduced iron was 1685 fng/1; the maximal amount of dissolved phosphate removed, 2345 mg/l. The molar stoichiometric ratio of iron (II) to removed dissolved phosphate changed from 2,7 to 1.2 (Fig. 3), agreeing with the possibility of eliminating phosphate in the form of FeHPOj. A twofold excess of the added iron {Ш} over the removed dissolved phosphate is necessary to reach an removal of 95% (Fig. 4.),

Thus, the addition of iron hydroxide during the digestion of activated sludge by anaerobic sludge, displaying an iron-reducing activity, resulted in the formation of bivalent iron ions, capable of precipitating phosphates. This study forms the background for developing a new anaerobic technology for the treatment of the wastewater polluted with phosphates or the digestion of activated sludge with a high phosphate content. This technology should involve the use of anaerobic sludge with artificially increased iron-reducing activity and the addition of iron hydroxide when anaerobically digesting phosphate-containing substrates.

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