

Review

Wastewater engineering applications of BioIronTech process based on the biogeochemical cycle of iron bioreduction and (bio)oxidation

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Abstract: Bioreduction of Fe(III) and biooxidation of Fe(II) can be used in wastewater engineering as an innovative biotechnology BioIronTech, which is protected for commercial applications by US patent 7393452 and Singapore patent 106658 “Compositions and methods for the treatment of wastewater and other waste”. The BioIronTech process comprises the following steps: 1) anoxic bacterial reduction of Fe(III), for example in iron ore powder; 2) surface renovation of iron ore particles due to the formation of dissolved Fe²⁺ ions; 3) precipitation of insoluble ferrous salts of inorganic anions (phosphate) or organic anions (phenols and organic acids); 4) (bio)oxidation of ferrous compounds with the formation of negatively, positively, or neutrally charged ferric hydroxides, which are good adsorbents of many pollutants; 5) disposal or thermal regeneration of ferric (hydr)oxide. Different organic substances can be used as electron donors in bioreduction of Fe(III). Ferrous ions and fresh ferrous or ferric hydroxides that are produced after iron bioreduction and (bio)oxidation adsorb and precipitate different negatively charged molecules, for example chlorinated compounds of sucralose production wastewater or other halogenated organics, as well as phenols, organic acids, phosphate, and sulphide. Reject water (return liquor) from the stage of sewage sludge dewatering on municipal wastewater treatment plants represents from 10 to 50% of phosphorus load when being recycled to the aeration tank. BioIronTech process can remove/recover more than 90% of phosphorus from this reject water thus replacing the conventional process of phosphate precipitation by ferric/ferrous salts, which are 20–100 times more expensive than iron ore, which is

used in BioIronTech process. BioIronTech process can remarkably improve the aerobic and anaerobic treatments of municipal and industrial wastewaters, especially anaerobic digestion of lipid- and sulphate-containing food-processing wastewater. It can also remove the recalcitrant compounds from industrial wastewater, enhance sustainability and quality of water resources, restore eutrophicated lakes due to removal of phosphate, ammonium, and pesticides from water, and recover ammonium and phosphate from municipal and food-processing wastes.

Keywords: BioIronTech process; iron ore bioreduction; recalcitrant compounds; sucralose; phosphate recovery; ammonium recovery

1. Introduction

Biogeochemical cycling of iron—which is the fourth most abundant element on Earth’s crust after oxygen, silicon, and aluminum—has a great importance for the functioning of ecosystems. There are also diverse interactions between biochemical cycles of iron and other elements, first of all carbon, nitrogen, and phosphorus [1,2,3]. The most important reactions of the biogeochemical cycle of iron are bioreduction of Fe^{3+} and biooxidation of Fe^{2+} . These reactions can be used for the numerous environmental and geotechnical engineering applications. The commercial importance of these applications is protected by US patent 7393452 and Singapore patent 106658 “Compositions and methods for the treatment of wastewater and other waste”. The major claim of these patents is a combination of anaerobic and aerobic processes where a valency of a redox mediator, mainly metal cation, is cyclically changed. In the BioIronTech process, which is based on these patents, a valency of Fe^{3+} in iron ore, used as a cheap electron acceptor, is changed due to anaerobic microbial reduction to Fe^{2+} , which is oxidized chemically or microbiologically under aerobic/microaerophilic conditions to Fe^{3+} using oxygen as an electron acceptor. Produced Fe^{3+} could be further either hydrolyzed and precipitated as ferric hydroxides or reacts with organic and inorganic substances forming insoluble compounds [4,5].

There are hundreds of potential practical applications of BioIronTech process in wastewater engineering. The aim of this paper is to show how the results of the study of the biogeochemical cycle of iron in the form of BioIronTech process can be used in wastewater treatment. Biogeochemical cycling of iron includes chemical or biological oxidation of Fe^{2+} to Fe^{3+} and bioreduction of Fe^{3+} to Fe^{2+} . Major natural depots of Fe(III) in nature are iron ores, iron hydroxides of wetland deposits, and iron-containing minerals of clay. Bioreduction of Fe(III) is performed anaerobically due to either non-specific reduction by hydrogen that is produced by fermenting bacteria or using specific reduction by iron-reducing bacteria. Different organic compounds, mainly organic acids, can be used as electron donors. Produced ferrous ions are involved in hydrolysis or forming chelates with organic acids but some portion of ferrous ions is remaining soluble under anaerobic conditions even at a neutral pH. Iron-reducing bacteria are present in anaerobic digester of organic wastes. However, reduction of insoluble iron compounds does not affect methanogenesis [19,20], probably, because iron-reducing bacteria oxidize fatty acids, which are the final products of acidogenic fermentation that are not used in methanogenesis. Chelates of ferrous ions with soil humic acids or organic acids that are produced during ferric bioreduction can be oxidized by neutrophilic iron-oxidizing bacteria or can be used as electron donors in bacterial anoxygenic photosynthesis [1,2,3].

The numerous applications of BioIronTech process are based on the interactions between the biogeochemical cycles of iron, carbon, phosphate, nitrogen, and sulphur, are shown in this paper.

2. Methods

Major reagent for BioIronTech process is hematite, Fe_2O_3 , of iron ore (Figure 1).

35 ± 5.0 mm 17.0 ± 3.0 mm 7.6 ± 1.9 mm 2.4 ± 0.4 mm 0.6 ± 0.1 mm



Figure 1. Hematite particles of iron ore with different sizes used in laboratory experiments and industrial scale tests.

The samples of water and wastewater were tested for Total Phosphorus (TP), Total Nitrogen (TN), Total Ferrous (TF), Total Organic Carbon (TOC), ammonium, nitrite, and nitrate concentrations, pH and Oxidation-Reduction Potential (ORP), Dissolved Oxygen Concentration (DOC), temperature, Total Suspended Solids (TSS), color and turbidity. The description of the analysis methods used is given in Table 1.

Table 1. Methods used in the research (APHA, 2005).

Parameters tested	Preservation/Pretreatment steps	Methods used	Instruments used
Total phosphorus (TP)	Samples preserved at -20 °C; Persulfate digestion method	4500-P E. Ascorbic acid method	Spectrophotometer DU 640B (Beckman Coulter, USA)
Total nitrogen (TN)	Samples preserved at -20 °C; 4500-N C. Persulfate digestion method	4500- NO_3^- F. Automated cadmium reduction method	Flow Injection Analyzer, QuickChem 8000 (LaChat Instruments, USA)
Total ferrous (Fe^{2+}) concentration	Samples were acidified at point of sampling by addition of 2 mL of concentrated HCl per 100 mL sample	3500-Fe B. Phenanthroline method	Spectrophotometer, UV-1201V (Shimadzu, Kyoto, Japan)
Total Dissolved Ferrous (Fe^{2+})	Samples were filtered and then acidified at point of sampling	3500-Fe B. Phenanthroline method	Spectrophotometer, UV-1201V (Shimadzu, Kyoto, Japan)
Total suspended solid (TSS)	Samples were refrigerated at 4 °C and tested within 7 days after sampling	2540 D. Total Suspended Solids dried at 103 – 105 °C	Oven
Turbidity	Samples were tested at point of sampling	—	In-situ turbidimeter

Parameters tested	Preservation/Pretreatment steps	Methods used	Instruments used
Total organic carbon (TOC)	Samples were refrigerated at 4 °C and tested within 7 days after sampling	5310 B. Combustion-infrared method	TOC analyzer C-VCSH(Shimadzu, Kyoto, Japan)
Ammonium concentration, NH ₄ ⁺	Samples were filtered, refrigerated at -20 °C and tested within 2 days	4500-NH ₃ H. Flow Injection Analysis	Flow Injection Analyzer, QuickChem 8000 (LaChat Instruments, USA)
Nitrate (NO ₃ ⁻) and nitrite (NO ₂ ⁻) concentration	Samples were filtered, refrigerated at -20 °C and tested within 2 days	4500-NO ₃ ⁻ F. Automated cadmium reduction method	Flow Injection Analyzer QuickChem 8000 (LaChat Instruments, USA)
Colour	In-situ determination	In-situ determination	Multiprobe YSI556 (Yellow Springs, OH, USA)
pH			
Redox potential			
Temperature			

Other specific methods are described in the related references below. All analyses have been made in triplicates. The statistical and correlation analysis of the results were performed with Microsoft Excel programs. Values of different parameters were expressed as the mean \pm standard deviation.

3. Results

3.1. Application of BioIronTech process for methanogenic fermentation of fat-containing wastewater

Lipids are one of the major organic pollutants in municipal and food-processing wastewater. Wastewaters produced from edible oil refinery, fish processing, slaughterhouse, wool scouring and dairy products industry contain a high concentration of lipids. Physico-chemical treatment can remove 90% of lipids but final biological treatment is necessary because of remaining emulsified and/or colloidal lipids. Fats are hydrolyzed to long-chain fatty acids (LCFA) and glycerol during anaerobic digestion but LCFA are inhibitors of both acidogenic fermentation and methanogenesis mainly because of their surface activity causing damage of cell membranes. Addition of dissolved ferrous/ferric salts diminished the inhibitory effect of LCFA because of the precipitation of LCFA as iron salt. Iron (II) was used to reduce the inhibition caused by long-chain fatty acids to prokaryotes involved in anaerobic digestion [6,7]. Degradation of stearic acid, one of model compound of LCFA, was improved for 10 days in the presence of divalent iron by 150% (Figure 2).

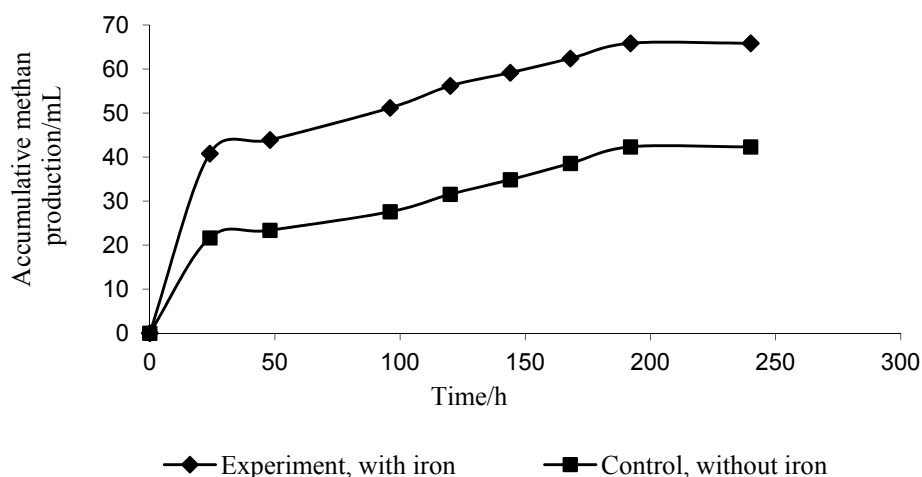


Figure 2. Accumulative methane production during fermentation of stearic acid.

The methane production rate was higher in the presence of divalent iron (0.21 mL/L/h) as compared to control (0.17 mL/L/h) where iron salt addition was absent. Methane yield was 0.1 L/g COD in experiment and 0.08 L/g COD in control.

Iron ore or even iron-containing clay can be applied for anaerobic degradation of vegetable oil. The methane production was increased 1.5 times as compared to control receiving no clay. COD removal efficiency was 98%, 80% and 77%, when iron was added to ensure the ratio of 20, 40, and 80 mg COD/mg Fe, respectively. Acetic and propionic acids were accumulated in the methanogenic reactors and inhibited methanogenesis when either iron was not present or COD/Fe ratio was higher than 20. However, no accumulation of soluble acetic and propionic acids was observed when the mass ratio of COD/Fe was below 20. So, presence of iron (II) significantly improved the anaerobic digestion of lipids [6,7]. Iron (II) can be produced in the treatment system from hematite of iron ore (Fe_2O_3), ferric hydroxide and other iron-containing minerals. These results were confirmed by other researchers [8].

3.2. Application of BioIronTech process for methanogenic fermentation of sulphate-containing wastewater

Wastewaters of fish-processing and biotechnological plants, pulp mills, chemical plants have concentration of sulphate above 1000 mg/L, which inhibits methanogenesis due to the production of toxic H_2S , competition for electron donors (acetate and hydrogen) between sulphate-reducing bacteria and methanogens, and precipitation of trace elements by sulphide. There is no production of methane when concentration of free sulphide is above about 250 mg/L. Inhibition of methanogenesis is appeared when $\text{COD}/\text{SO}_4^{2-}$ ratio is below 10 [9].

Sulphate-reducing bacteria and iron-reducing bacteria are competitors for electron donors. Some sulphate-reducing bacteria are also able to reduce Fe(III). Therefore, an addition of ferric hydroxide and iron-reducing bacteria in anaerobic treatment of sulphate-containing wastewater inhibits sulphate bioreduction, production of sulphide, and increased removal of total organic carbon (TOC) and methane production [9,10]. Effect of ferric compound addition on anaerobic treatment of the model fish-processing wastewater depended on Fe(III) dosage, which can be determined as a molar ratio of

Fe(III)/SO₄²⁻ (Table 2). These data showed efficiency of BioIronTech process for anaerobic treatment of sulphate-containing wastewater.

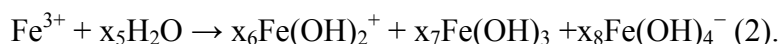
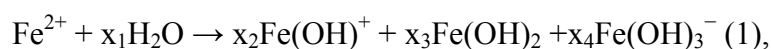
Table 2. The parameters of methanogenic fermentation during 10–14 days of the treatment [modified from 9,10].

Initial molar ratio of Fe ²⁺ /SO ₄ ²⁻ in the model wastewater of fish-processing plant	The rate of fermentation, g TOC/g VSS h	Final concentration of sulfide in wastewater, mg/L	Specific methane production rate, mL/gVSS day	Content of methane in biogas/% (v/v)
0.06	0.23	91	0.936	25
0.5	0.55	45	1.128	41
1.0	0.69	0	1.536	55
2.0	0.76	0	1.656	62

3.3. Application of BioIronTech process for removal of xenobiotics and recalcitrant compounds from industrial wastewaters

The removal of recalcitrant compounds from wastewater is due to (1) anoxic oxidation; (2) formation of Fe²⁺ and insoluble ferrous and ferric salts and chelates of organic acids and phenols; (3) sedimentation of polar recalcitrant molecules during coagulation of ferrous produced from iron ore.

Adsorption and precipitation of xenobiotics is due to formation of positively and negatively charged hydroxides of ferrous/ferric on anaerobic and aerobic stages of BioIronTech process:



BioIronTech process was used for the treatment of wastewater of the plant producing artificial sweetener sucralose. Sucralose (trichlorosucrose) is used in food and beverages since 1991 and now is permitted for use in over 60 countries. Sucralose is hardly biodegradable compound. It present in sewage treatment plant effluents and waterways throughout Europe and USA [11]. Sucralose and by-products of its synthesis are almost not degraded on existing biofacilities and on municipal wastewater treatment plants (MWWTP). Concentration of sucralose in effluent of MWWTP could be up to several thousand ng/L, so its concentration in surface waters in some countries is up to several hundred ng/L. Therefore, sucralose could be considered at present as a tracer of anthropogenic contamination of environment. It was shown the presence of sucralose even in North American coastal and open ocean waters [12].

The wastewater of sucralose production contains the remainder of sucralose as well as chlorinated by-products of its synthesis. These organic substances are almost not biodegradable. Their biodegradability showed as a ratio of biological and chemical consumption of oxygen for oxidation (BOD/COD ratio) is 0.003. As shown in our studies, these compounds can be partially removed from wastewater using BioIronTech process. The major mechanism of recalcitrant organics removal from wastewater of sucralose is adsorption of negatively charged chlorinated organics on positively charged particles of iron (hydr)oxides. However, without BioIronTech process an

adsorption capacity of iron (hydr)oxide particles is low because of a low specific surface of the particles. However, iron-reducing bacteria in BioIronTech process renew permanently the surface of iron ore particles and produce from iron ore small particles of ferrous and ferric hydroxides with high specific surface adsorbing chlorinated organics. We showed that application of BioIronTech process with bioreduction of iron ore by added sucrose removed up to 70% of xenobiotics from the sucralose production wastewater and almost 100% of colored substances (parameter “True Color”). Therefore, BioIronTech process can significantly diminish huge quantity of granulated activated carbon that is used currently or is planned to be used for the removal of recalcitrant organics from industrial wastewater of sucralose production. This process can be designed even more effectively because iron (hydr)oxide can be regenerated by thermal treatment that will burn safely all adsorbed xenobiotics. A mechanism of the removal of xenobiotics from the wastewater of sucralose production plant is shown in Figure 3.

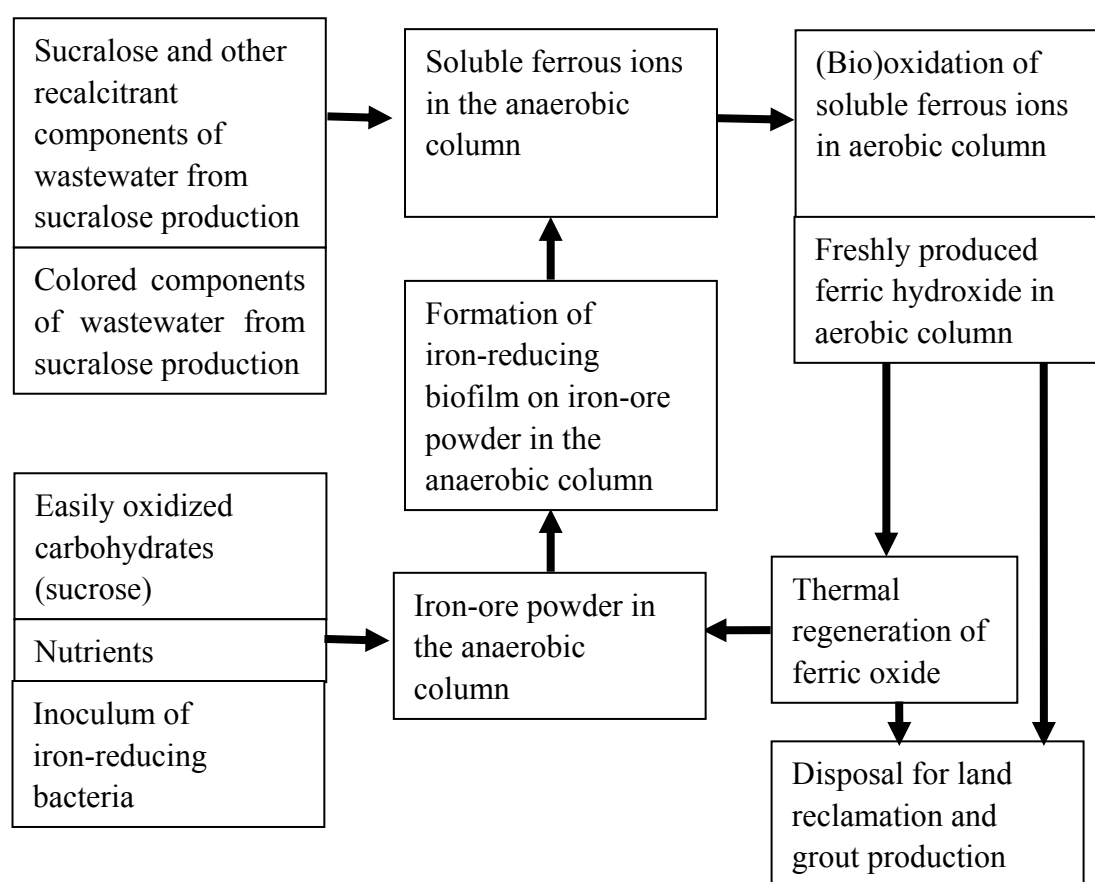


Figure 3. A mechanism of the removal of xenobiotics from the wastewater of sucralose production plant.

Fe(III) of hematite in iron ore is very cheap and industrially suitable an electron acceptor in anoxic biodegradation of xenobiotics [13,14]. For example, selected strain *Stenotrophomonas maltophilia* BK showed an ability to reduce Fe(III) using such xenobiotics as diphenylamine, m-cresol, 2,4-dichlorophenol and p-phenylphenol as sole sources of carbon under anaerobic conditions [13]. Natural estrogens such as estrone, 17 β -estradiol, estriol, and the synthetic component of contraceptive pills, 17 α -ethynylestradiol, enter the municipal wastewater treatment

plant *via* human excretions. A significant portion of these substances remains in wastewater effluent after aerobic treatment of sewage and in returned liquor (reject water) after anaerobic digestion of activated sludge. In this study, the effect of oxidant, Fe(III), and iron-reducing bacteria on the anaerobic degradation of estrogens in returned liquor was investigated. A facultative anaerobic strain of *Alcaligenes faecalis*, capable to degrade estrogens anaerobically with ferric hydroxide or iron ore as oxidants, was isolated and tested. Biodegradation of estrogens and accumulation of their metabolites were analysed using liquid chromatography-tandem mass spectrometry. Synthetic 17 α -ethynylestradiol remained resistant to anaerobic biodegradation by iron-reducing bacteria, while natural estrogens such as 17 β -estradiol, estriol and estrone were removed by 92, 60 and 27%, respectively. A large fraction of 17 β -estradiol was converted to estrone during the anaerobic treatment. The ability of facultative anaerobic iron-reducing bacteria to degrade estrogens can be used for the anaerobic removal of trace organics from return liquor in the municipal wastewater treatment plants [13,14].

3.4. Application of BioIronTech process for removal of phosphate from wastewater

Main point for precipitation of phosphate is production of Fe²⁺ ions (Figure 3). The rotating reactor is most suitable for the production of ferrous ions from hematite of iron ore (Table 3).

Table 3. The production of ferrous ions by iron-reducing bacteria using hematite of iron ore and reject water of the municipal wastewater treatment plant.

The sizes of iron ore particles/mm	Fe ²⁺ concentration after 15 days of batch cultivation/mg/L	
	in the rotating reactor	in the static reactor
0.6	52	115
2.4	209	48
7.6	480	27

The specific rate of Fe²⁺ production negatively correlated with the size of the iron ore particles in the static reactor, which is explained by the decrease of the specific surface of the particles with increase of their sizes. Meanwhile, there was the positive correlation between the specific rates of Fe²⁺ production with the size of the iron ore particles in the rotating reactor. This strange trend was explained by experimental data of the higher concentration of the fine iron ore particles produced from iron ore particles of big size in the rotating reactor.

The reduction of ferric compounds and the production of ferrous ions led to the precipitation of phosphate. Experimental mass ratio of added Fe(III) to removed dissolved phosphate was 2 in the anaerobic digester [15] and 4 during anaerobic treatment of effluent from the anaerobic digester [7,13]. The mass ratio of removed P to produced Fe(II) in the microbiological process was 0.17 g P/g Fe²⁺ [15] and 0.22 g P/g Fe²⁺ in the chemical precipitation of phosphate by ferric salts. Addition of ferric hydroxide to the reactor of methanogenic fermentation of activated sludge followed with microbial reduction of Fe (III) and the formation of ferrous ions, which precipitated phosphate. It was shown that 66.6–99.6% of dissolved phosphate with the initial concentration of 1000–3500 mg PO₄³⁻/L can be removed by the addition of ferric hydroxide in concentration of 6420 mg Fe (III)/L and anaerobic sludge with iron-reducing activity. Optimal ratio of added Fe(III): removed dissolved phosphate, ensured not less than 95% removal of phosphate was 2. These data could be used in a new technology

of anaerobic treatment of wastewater with simultaneously removal of phosphate. An alternative technology for the phosphate precipitation from wastewater can be the production of ferrous ions using the microbial reduction of iron ore. The cost of 1 ton of Fe in iron ore is approximately US\$100–150, which is much cheaper than commercial ferric or ferrous salts [16]. This biotechnology aiming to the microbial production of cheap ferrous reagent for phosphate precipitation was proposed, studied and patented [4,5,13,16,17].

3.5. Application of BioIronTech process for removal of phosphate from stormwater and the recovery of the eutrophicated ponds and lakes

Phosphate and ammonia are common pollutants of environment and their concentrations in the discharge streams are limited by 0.1–2 mg/L in almost all national environmental standards of Asian countries. Excessive phosphorus and ammonia concentrations in aquatic systems caused their eutrophication appeared in excessive growth of phototrophs, dissolved oxygen depletion, decline in recreational value of water, and appearance of foul taste of drinking water. Concentration of phosphate in the streams and collecting reservoirs is especially important because of two reasons: 1) phosphorus is triggering element in eutrophication of reservoirs, and 2) accumulation of phosphate in the bottom sediments can make pollution of reservoirs irreversible, or at least unrecoverable, for hundred years. The signs of eutrophication in the Singapore's reservoirs require urgent measures to avoid discharge of phosphate and ammonia into reservoirs. It is supposed that the main source of this pollution is urban runoff. Due to shortage of land it would be impossible to treat storm runoff in conventional artificial wetland. The enhanced engineered wetland design consists of two parts, which are a BioIronTech (BIT) reactor followed with the constructed wetland. The BIT reactor produces ferrous flux using naturally occurring local anaerobic iron-reducing bacteria to reduce iron ore and produce ferrous ions, which react with phosphate and precipitated in the deep pit before entrance to constructed wetland (Figure 4 and Figure 5). The anaerobic BioIronTech reactor for production of ferrous ions was installed as a biofilter inside the dry weather flow channel of drain (Figure 5). Effluent from anaerobic BioIronTech biofilter was mixed with the drain water (Figure 4) with the designed volume ratio of the mixing drain water and effluent of BioIronTech reactor 9:1. The goal of the construction of BioIronTech biofilter in the drain was precipitation of ferric phosphate at the settling zone at the end of the drain to prevent release of phosphate into the reservoir and mitigate eutrophication there. The length of BioIronTech biofilter, filled with iron ore, was 130 m. The monitoring of BioIronTech biofilter was continued for 8 months. Using the monitoring data, three months were considered as the maturation period needed for the formation of biofilm on iron ore particles.

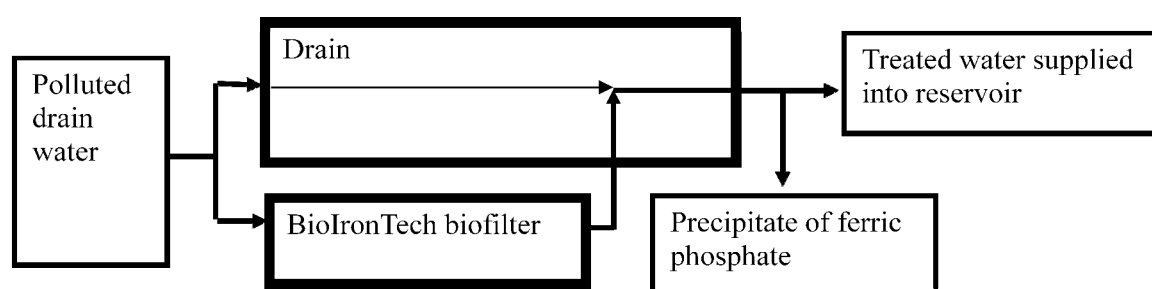


Figure 4. Flows in the continuous BioIronTech system for the treatment of polluted drain water.



Figure 5. Construction of BioIronTech biofilter in the drain of polluted stormwater.

These processes reduce the phosphate concentration in water, which enters reservoir. The average total phosphate concentration in upstream drain water was 0.24 ± 0.03 mg P/L but after BIT reactor average total phosphate concentration was 0.09 ± 0.02 mg P/L with removal efficiency by BIT reactor of 54%. The average total nitrogen concentration in upstream drain water was 0.93 ± 0.12 mg N/L and after BIT reactor an average total nitrogen concentration was 0.76 ± 0.08 mg N/L with removal efficiency of 22%. BioIronTech biotechnology requires approximately 5 times less area than constructed wetland with the same efficiency. Phosphate is recovered from wastewater by precipitation with ferrous ions produced by iron-reduced bacteria. Ammonia is recovered from polluted water probably by co-precipitation with negatively charged ferric hydroxides of cell sheathes of iron-oxidizing bacteria. The value-added by-product of BioIronTech process is fertilizer containing slow-releasing phosphate and ammonia. It is also important that the designed time of exploitation after construction is longer than 20 years. The BioIronTech biofilter of drain water is working in Singapore for last several years removing permanently about 54% of P, 22% of N, and 95% of As from collecting rain water.

Similar BioIronTech systems with the recycle of water *a priori* can be used for the recovery of the heavily eutrophicated and polluted ponds and lakes due to removal of P, N, As, other heavy metals, radionuclides, and pesticides from recycled water. There are known experimental data showing that ferric hydroxide produced by oxidation of ferrous ions is a good adsorbent of heavy metals and radionuclides containing in polluted water. For example, precipitation of freshly produced flocs of $\text{Fe}(\text{OH})_3$ obtained by oxidation of Fe^{2+} can remove using adsorption, inclusion, and occlusion processes up to 90% of gross-alfa and 70% of gross-beta activity of groundwater containing such radionuclides as ^{226}Ra , ^{228}Ra , and ^{238}U [18,19]. Adsorption of such heavy metals as As, Cd, Co, Ni, Pb and Zn on ferric hydroxide particles is also well studied [20].

3.6. Application of BioIronTech process for removal of ammonium from wastewater

It was shown that BioIronTech process can be used for the production of Fe^{2+} ions and their slow biooxidation in water by iron-oxidizing bacteria. This oxidation prevents nitrification due to the binding of ammonium ions with negatively charged ferric hydroxide, which was produced by neutrophilic iron-oxidizing bacteria under microaerophilic conditions [21,22]. The existence of ferric hydroxide as anions $\text{Fe}(\text{OH})_4^-$ at pH from 6 to 8 is well known. These anions could bind and precipitate

positively charged ammonium ions. The removal of ammonium from solution occurred during chemical oxidation of Fe(II) [21,22]. The molar ratio ammonium/iron in sediment was 0.77, while, theoretically, this ratio in $(\text{NH}_4)\text{Fe}(\text{OH})_4$ is 1. The difference is probably due to the big size of iron hydroxide particles. It is known precipitation of ammonia in struvite, magnesium ammonium phosphate hexahydrate ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$), but it is performed at the pH higher than 9. Another co-precipitation can be as ammonium jarosite $(\text{NH}_4)\text{Fe}_3(\text{SO}_4)_2(\text{OH})_6$ but according to the literature data it can be done at low pH. Co-precipitation of ammonia with negatively charged Fe(III) hydroxides formed by iron-oxidizing bacteria was proposed for recovery of ammonia in aerobic treatment of wastewater [23,24].

Reject water, which is the liquid fraction produced after dewatering of anaerobically digested activated sludge on the municipal wastewater treatment plants (MWWTPs), contributes up to 80% of the nitrogen and phosphorus loads to the MWWTP. Therefore, it was proposed to combine the removal of nitrogen from reject water using the sequential biooxidation of NH_4^+ and bioreduction of NO_3^- with precipitation of phosphate by Fe^{2+} ions produced due to bioreduction of Fe^{3+} in iron ore. Bioreduction of NO_3^- decreased Fe^{3+} bioreduction rate in reject water from 37 to 21 mg $\text{Fe}^{2+}/\text{L d}$ due to competition between NO_3^- and Fe^{3+} for electron donors. Addition of acetate as the electron donor increased both bioreduction rates of Fe^{3+} and NO_3^- but acetate interfered with the competition between nitrate and phosphate anions reacting with ferrous cations thus decreasing efficiency of the phosphate removal from reject water. Therefore, the stages of denitrification and ferric bioreduction/phosphate precipitation must be performed sequentially [17,25]. Nitrate can be also removed by oxidation of Fe^{2+} , produced by iron-reducing bacteria [25].

4. Conclusion

Major advantage of BioIronTech process is that iron ore is a relatively cheap commodity so the large scale water or wastewater treatments will be much more economical technologies than those requiring supply of expensive chemical reagents or adsorbents. Second advantage of BioIronTech process is the diversity of the pollutants that can be removed from water or wastewater. The applicability of BioIronTech process is show in the Table 4.

It is important to note that BioIronTech process is based on activity of two physiological groups of bacteria: 1) anaerobic iron-reducing bacteria and 2) usually neutrophilic microaerophilic "iron-oxidizing" bacteria, which actually do not oxidize Fe^{2+} but just oxidize organic substances of chelating envelope of ferrous atom. A lot of pure cultures of iron-reducing bacteria can be isolated and used in BioIronTech process [4,5,26,27]. However, because of the diversity of temperature, pH, salinity, type of electrons donor, type of ferrous and ferric chelating substances, redox potential, etc. in the field, it could be more practical select the enrichment cultures of iron-reducing bacteria and to cultivate them for applications [26]. Also, if necessary, neutrophilic "iron-oxidizing" bacteria can be cultivated and used in BioIronTech applications as enrichment culture.

Table 4. Potential applications of BioIronTech process in wastewater treatment.

Industry, process	Pollutant(s), which may be removed most effectively using BioIronTech process
Anoxic treatment of reject water on municipal wastewater treatment plants	Ammonia, phosphate, sulfides
Anaerobic treatment of wastewater	Sulfides, phosphate, phenols, tannins, lipids, xenobiotics, sucralose
Landfill construction and landfill leachate treatment	Organic acids, ammonium, phosphate, sulfide, heavy metals, phenols, chlorophenols, pesticides, radionuclides
Chemical industry, industrial wastewater treatment	Organic acids, cyanide, thiocyanate, phosphate, sulfate, sulfide, heavy metals, phenols, chlorophenols, pesticides, radionuclides, sucralose and chlorinatedcarbohydrates
Pharmaceutical industry, wastewater treatment	Organic acids, cyanide, thiocyanate, phosphate, sulfate, sulfide, heavy metals, phenols, chlorophenols
Brewing industry and industrial biotechnology, wastewater treatment	Ammonium, phosphate, organic acids, sulfate, sulfides
Mining, drainage waters, polluted groundwater	Cyanide, heavy metals, sulfate, arsenic
Dairy industry, wastewater treatment	Lipids, proteins, ammonia, phosphate
Vegetable oil production, treatment of wastewater	Lipids
Fish or meat processing, canning industry, wastewater treatment	Lipids, proteins, sulfate, sulfides
Shrimp aquaculture(fresh and sea water)	Ammonium, phosphate, particles, arsenic, pesticides in recycling water
Agricultural drainage	Ammonium, phosphate, pesticides, heavy metals
Electroplating and electronics, wastewater treatment	Heavy metals, sulfates, cyanides
Nuclear power plants and nuclear processing facilities; radionuclides-polluted water	Radionuclides
Aerospace industry, drainage water treatment	toxic components of fuels

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Technology Thonburi, Thailand.

Conflict of Interest

Authors declare no conflicts of interest in this paper.

References

1. Ivanov V, Hung YT (2010) Applications of environmental biotechnology. In: *Handbook of Environmental Engineering*. Vol.10. Environmental Biotechnology, Totowa, NJ, USA: Humana Press, Inc. 2-18.
2. Ivanov V (2010) Microbiology of environmental engineering systems. In: *Handbook of Environmental Engineering*. Vol.10. Environmental Biotechnology, Humana Press, Inc. Totowa, NJ, USA: Humana Press, Inc. 19-80.
3. Ivanov V (2010) Environmental Microbiology for Engineers. CRC Press, Taylor & Francis Group, Boca Raton.402 p.
4. Tay JH, Tay STL, Ivanov V, et al. (2006) Compositions and methods for the treatment of wastewater and other waste. Patent of Singapore 106658. Date of grant 31 October 2006, date of filing 16 April 2002.
5. Tay JH, Tay STL, Ivanov V, et al. (2008) Compositions and methods for the treatment of wastewater and other waste.US Patent 7,393,452.Date of grant July 1, 2008 Date of filing 1 April 11, 2003.
6. Ivanov V, Stabnikova EV, Stabnikov VP, et al. (2002). Effects of iron compounds on the treatment of fat-containing wastewaters. *Appl Biochem & Microbiol* 38: 255-258.
7. Zubair A, Ivanov V, Hyun SH, et al. (2001) Effect of divalent iron on methanogenic fermentation of fat-containing wastewater. *Environ Eng Res* 6: 139-146.
8. Li Z, Wrenn BA, Venosa AD (2006) Effects of ferric hydroxide on methanogenesis from lipids and long-chain fatty acids in anaerobic digestion. *Water Environ Res* 78: 522-530.
9. Stabnikov VP, Ivanov VN.(2006) The effect of various iron hydroxide concentrations on the anaerobic fermentation of sulfate-containing model wastewater. *Appl Biochem & Microbiol* 42: 284-288.
10. Ivanov V, Tay STL, Wang JY, et al. (2004) Improvement of sludge quality by iron-reducing bacteria. *J Residuals Sci Tech* 1: 165-168.
11. Kessler R (2009) Sweeteners persist in waterways. *Environ Health Persp* 117: A438-A438.
12. Mead RN, Morgan JB, Avery GB, et al. (2009) Occurrence of the artificial sweetener sucralose in coastal and marine waters of the United States. *Mar Chem* 116: 13-17.
13. Ivanov V, Stabnikov V, Zhuang WQ, et al. (2005) Phosphate removal from return liquor of municipal wastewater treatment plant using iron-reducing bacteria. *J Appl Microbiol* 98: 1152-1161.
14. Ivanov V, Lim JJW, Stabnikova O, et al. (2010) Biodegradation of estrogens by facultative anaerobic iron-reducing bacteria. *Process Biochem* 45: 284-287.
15. Stabnikov V, Tay STL, Tay JH, et al. (2004) Effect of iron hydroxide on phosphate removal during anaerobic digestion of activated sludge. *Appl Biochem & Microbiol* 40: 376-380.
16. Ivanov V, Kuang SL, Guo CH, et al. (2009) The removal of phosphorus from reject water in a

- municipal wastewater treatment plant using iron ore. *J Chem Technol Biot* 84:78-82.
17. Guo CH, Stabnikov V, Ivanov V (2009) The removal of phosphate from wastewater using anoxic reduction of iron ore in the rotating reactor. *Biochem Eng J* 46: 223-226.
 18. Lumiste L, Munter R, Sutt J, et al. (2012) Removal of radionuclides from Estonian groundwater using aeration, oxidation, and filtration. *P Est Acad Sci* 61: 58-64.
 19. Munter R (2013) Technology for the removal of radionuclides from natural water and waste management: state of the art. *P Est Acad Sci* 62: 122-132.
 20. Chiang YW, Ghyselbrecht K, Santos RM, et al. (2012) Adsorption of multi-heavy metals onto water treatment residuals: Sorption capacities and applications. *Chem Eng J* 200: 405-415.
 21. Ivanov V, Sihanonth P, Menasveta P (1996) Multistage-ferrous- modified-biofiltration for removal of ammonia from aquacultural water. In: *Proceedings of the Asia-Pacific Conference on Sustainable Energy and Environmental Technology*. Singapore: World Scientific Publishing 57-63.
 22. Ivanov V, Stabnikova EV, Shirokih VO (1997) Influence of ferrous oxidation on the nitrification in aqueous and soil model ecosystems. *Mikrobiologia (Moscow)* 66: 428-433.
 23. Ivanov V, Wang JY, Stabnikova O, et al. (2004) Iron-mediated removal of ammonia from strong nitrogenous wastewater of food processing. *Water Sci Technol* 49: 421-431.
 24. Stabnikova O, Wang JY, Ivanov V (2010) Value-added biotechnological products from organic wastes. In: *Handbook of Environmental Engineering. Vol.10. Environmental Biotechnology*, Totowa, NJ, USA: Humana Press, Inc. 343-394.
 25. Guo CH, Stabnikov V, Ivanov V (2010) The removal of nitrogen and phosphorus from reject water of municipal wastewater treatment plant using ferric and nitrate bioreductions. *Bioresource Technol* 101: 3992-3999.
 26. Ivanov V, Stabnikov V, Hung YT (2012) Screening and selection of microorganisms for the environmental biotechnology process. In: *Handbook of Environment and Waste Management. Air and Water Pollution Control*. World Scientific Publishing Co., Inc., 1137-1149.
 27. Tay JH, Tay STL, Ivanov V, et al. (2004) Application of biotechnology for industrial waste treatment. In: *Handbook of Industrial Wastes Treatment*. 2nd edition, revised and expanded by Marcel Dekker, NY, 585-618.

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