

KAUNAS UNIVERSITY OF TECHNOLOGY
THE FACULTY OF CHEMICAL TECHNOLOGY

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**APPLICATION OF BIOGENIC IRON OXIDES FOR PHOSPHATE
REMOVAL FROM WATER**

Master's Degree Final Project

Supervisor

Assoc. prof. dr. Dalia Jankūnaitė

KAUNAS, 2016

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Environmental engineering (code 621H17001)

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SUMMARY

Excess release of phosphate into the surface water even at very low concentrations could still lead to eutrophication. Phosphorus is an essential nutrient in agriculture, phosphate rock is a finite resource and significantly increasing demand for it is observed. As a consequence, more sustainable use of phosphorus and development of technologies to recover it from waste streams are encouraged. Unfortunately, current methods used for phosphorus removal such as dosing metal salts cannot meet the environmental standards and to recover phosphorus from formed products is not economical. Stricter environmental regulations promote the search for more efficient removal of phosphate in wastewater treatment plants (WWTPs) and reversible adsorption could be a solution. Successful application of chemically formed iron oxides (ChFeO) adsorbents for phosphate removal is attributed to a good adsorption capacity and high selectivity towards phosphate. Adsorption capacity can be improved by increasing surface area. It could be done by coating nanoparticles on supporting material, but this is hard to implement. Therefore, their application has some problems, which could be solved by using biogenic iron oxides (BioFeO). BioFeO are known for their wide occurrence, good phosphate adsorption capacity and that they occur as nanoparticles. This encouraged the study to investigate the applicability of BioFeO on phosphate removal from water and compare this with ChFeO.

Microbial mats of *Leptothrix* sp. were collected in iron-rich seepage areas for phosphate removal by batch adsorption experiments. For experiment also used microbial mats of *Gallionella* sp. formed in the laboratory. For comparison with ChFeO an engineered adsorbent (GEH) was used. Characterization of adsorbents was done using microwave digestion analysis, scanning electron microscopy with energy dispersive X-ray spectroscopy, light microscope and X-ray diffraction analysis. Adsorbents efficiency for phosphate removal was carried out using the kinetic and isotherm studies.

Non-washed microbial iron oxyhydroxides of *Leptothrix* sp. and GEH have reached equilibrium in four days. Unwashed microbial mats of *Leptothrix* sp. have lower affinity, but 27% higher phosphate

removal capacity in comparison to GEH. The high adsorption capacity is related to the combination of phosphate removal by different mechanisms – adsorption on BioFeO and surface precipitation with soluble iron. In order to avoid iron release, the microbial mats were washed with MQ (deionized) water. Washed microbial iron oxyhydroxides of *Leptothrix* sp. had much slower kinetics, equilibrium was not reached in seven days. MQ-washed microbial iron oxyhydroxides of *Gallionella* sp. and *Leptothrix* sp. have lower affinity and respectively 1.2 and 2.1 times lower adsorption capacity than GEH. Stalks of *Gallionella* sp. and *Leptothrix* sp. have different structures, but it does not have an influence on affinity and adsorption capacity. Iron content plays key-role on phosphate adsorption. Microbial iron oxyhydroxides can contain up to 50% of organic matter (OM), while synthetic FeO are pure iron oxyhydroxides. Microbial mats of *Leptothrix* sp. have restrictions for its application as adsorbents for phosphorus removal in WWTP, but iron-oxidizing bacteria (FeOB) is relevant for various research disciplines – for controlling phosphorus, heavy metals and other substances in the natural and engineered systems. In order to be able to make predictions about the bioavailability and transport of nutrients and contaminants in natural systems and also about their behavior in engineered systems a detailed knowledge is required about the influence of exopolymeric substances of BioFeO on interaction with Fe (e.g. type of bond and strength), which determines sorption mechanisms.

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SANTRAUKA

Fosfatų patekimas į paviršinius vandenis net labai mažomis koncentracijomis gali sukelti eutrofikaciją. Fosforo nepakeičiamumas žemės ūkyje, fosfatų uolienų išteklių ribotumas bei vis didėjantis fosforo poreikis skatina tvarų fosforo vartojimą bei fosforo atgavimo technologijų vystymą. Deja dabartiniai fosfatų šalinimo metodai tokie kaip metalų druskų dozavimas negali pasiekti aplinkosauginių reikalavimų, o fosforo atgavimas iš šio proceso metu susidariusių produktų nėra ekonomiškai. Taigi vis griežtėjantys aplinkosauginiai reikalavimai skatina ieškoti efektyvesnių fosfatų šalinimo iš nuotekų metodų. Grįžtamoji adsorbcija galėtų būti šių problemų sprendimu. Gana sėkmingas cheminių geležies oksidų (ChFeO) pagrindu suformuotų adsorbentų naudojimas yra pagrįstas gera adsorbcine geba bei dideliu atrankumu fosfatų atžvilgiu. Adsorbcinė geba gali būti pagerinta padidinus paviršiaus plotą, tai gali būti įgyvendinama atraminę medžiagą padengiant geležies nanodalelėmis, tačiau tai pasiekti yra gana sudėtinga. Minėtą problemą galėtų padėti išspręsti biogeninių geležies oksidų (BioFeO) naudojimas. BioFeO yra žinomi dėl plataus jų paplitimo, gerų fosfatų adsorbcinių savybių bei tai, jog jie paprastai yra aptinkami kaip nanodalelės. Visa tai paskatino ištirti BioFeO taikymo fosfatų šalinimui iš vandens galimybes bei palyginti su ChFeO. **Tyrimo objektas:** biogeniniai geležies oksidai. **Uždaviniai:** geležį oksiduojančių bakterijų plėvelės ant atraminės medžiagos formavimas siekiant sukurti BioFeO pagrindu suformuotus adsorbentus; įvertinti fosfatų surišimo kinetikos skirtumus tarp BioFeO ir ChFeO; ištirti fosfatų surišimo savybių tokių kaip adsorbcinės gebos bei giminingumo skirtumus tarp BioFeO ir ChFeO; identifikuoti morfologinius bei cheminės sudėties skirtumus tarp BioFeO ir ChFeO. **Tyrimo hipotezės:** BioFeO turi didesnį giminingumą fosfatų atžvilgiu ir didesnę adsorbcinę gebą lyginant su ChFeO; geresnės adsorbcinės savybės yra susijusios su unikalia BioFeO struktūra. **Tyrimų vieta:** visi eksperimentai atlikti 2015-2016 metais išvykus pagal Erasmus+ praktikos mobilumo programą į Europos vandens pažangiųjų ir darniųjų tyrimų centrą – Wetsus, įsikūrusį Olandijoje.

Leptothrix sp. bakterijų geležies oksidų nuosėdos naudotos laboratoriniams adsorbciniams fosfatų šalinimui iš vandens tyrimams buvo surinktos 2015 metų gruodžio 2016 metų vasario mėnesių laikotarpiu geležingose šiaurės Olandijos šlapžemėse. Eksperimentams taip pat buvo naudojamos *Gallionella* sp. bakterijų geležies oksidai suformuoti laboratorijoje. Palyginimui su cheminiais geležies oksidais buvo naudojamas inžinerinis adsorbentas (granuluotas geležies hidroksidas GEH). Bendra biogeninių geležies oksidų kietųjų bei organinių megžiagų koncentracijos nustatytos gravimetriniu metodu. Elementinė *Leptothrix* sp. ir *Gallionella* sp. bakterijų geležies oksidų sudėtis ištirta mėginį skaidant azoto rūgštyje bei nustatyta naudojant indukuotos plazmos masių spektrometriją. Morfologijos, struktūros ir elementinio pasiskirstymo analizės atliktos naudojant šviesos mikroskopą bei skenuojantį elektroninį mikroskopą sujungtą su dispersiniu rentgeno spindulių spektroskopiniu detektoriumi. Geležies oksidų tipas nustatytas naudojant rentgeno spindulių difraktometrą. Adsorbcijos eksperimentams naudoti nedžiovinti *Leptothrix* sp. bakterijų biogeniniai geležies oksihidroksidai, tačiau adsorbcinė geba išreikšta mgP/g sausos masės. Adsorbentų fosfatų šalinimo efektyvumas atliktas vykdant kinetikos ir izotermų eksperimentus. Kinetikos eksperimentų duomenys analizuoti naudojant nelinijinį kinetikos modelį (pseudo antros eilės). Izotermų eksperimentų duomenys analizuoti naudojant Langmuir ir Freundlich modelius. Po izotermų eksperimentų skystyje esančiame virš biogeninių geležies oksidų buvo analizuojamos tirpios geležies ir kitų metalų, fosfatų, nitritų, nitratų, sulfatų, bendros ir ištirpusios organinės anglies bei huminių rūgščių koncentracijos.

Neplauti *Leptothrix* sp. bakterijų biogeniniai geležies oksihidroksidai ir GEH pusiausvyrą pasiekė po keturių dienų. Neplauti *Leptothrix* sp. bakterijų biogeniniai geležies oksihidroksidai turi silpnesnį giminingumą fosfatams, tačiau 27% didesnę adsorbcinę gebą lyginant su GEH. Didelė adsorbcinė geba yra susijusi su kombinuotu fosfatų šalinimu skirtingais mechanizmais – adsorbcija ant biogeninių geležies oksidų ir nuosėdų susiformavimas bei iškritimas su tirpia geležimi. Siekiant išvengti tirpios geležies ir to pasekoje vykstančio nuosėdų formavimosi vykdant adsorbcijos eksperimentus buvo nuspręsta biogeninius geležies oksidus praplauti su MQ (dejonizuotu) vandeniu. Biogeniniai geležies oksidai buvo plaunami nusodinant, tai yra užpilant nuosėdas MQ (dejonizuotu) vandeniu ir po 24 valandų virš nuosėdų esantį skystį nupilant bei patikrinant jame ištirpusios geležies koncentraciją su FerroVer milteliais. Šis žingsnis buvo kartojamas tol, kol skystyje esančiame virš biogeninių geležies oksidų tirpios geležies koncentracija buvo arti nuliui. Kinetikos eksperimentai su praplautais *Leptothrix* sp. bakterijų biogeniniais geležies oksihidroksidais parodė, jog pusiausvyros nepasiekė per septynias dienas. MQ vandeniu praplauti *Leptothrix* sp. ir *Gallionella* sp. bakterijų biogeniniai geležies oksihidroksidai turėjo silpnesnį giminingumą fosfatams ir atitinkamai 1.2 ir 2.1 karto mažesnę

adsorbicinę gebą lyginant su GEH. *Leptothrix* sp. ir *Gallionella* sp. bakterijų biogeniniai geležies oksihidroksidai turi skirtingas struktūras, tačiau jos nedaro jokios įtakos giminingumui fosfatams bei adsorbicinei gebai. Geležies koncentracija vaidina pagrindinį vaidmenį fosfatų adsorbicijoje. Biogeniniuose geležies oksihidroksiduose organinių medžiagų koncentracija gali siekti iki 50%, kai tuo tarpu sintetiniai (cheminiai) geležies oksidai yra grynai geležies oksihidroksidai. *Leptothrix* sp. bakterijų biogeninių geležies oksihidroksidų kaip adsorbentų naudojimas nuotekų valymo valyklose turi apribojimų. Fosfatų šalinimas naudojant adsorbentus juos dalinai išsodinant su tirpia geležimi nėra tinkamas metodas siekiant adsorbentus pakartotinai naudoti bei atgauti kuo grynesnį fosforą. Tačiau geležį oksiduojančios bakterijos yra aktualios įvairioms mokslinių tyrimų sritims – kontroliuojant fosforo, sunkiųjų metalų bei kitų medžiagų, esančių natūraliose bei inžinerinėse sistemose, koncentracijas. Siekiant prognozuoti maistinių medžiagų ir teršalų biologinį prieinamumą bei pernašą natūraliose ir inžinerinėse sistemose, yra reikalingos išsamios žinios apie biogeninių geležies oksidų egzopolimerinių medžiagų sąveikos su geležimi (pavyzdžiui, jungties ir surišimo stiprumo tipas), kuri daro įtaką sorbcijos mechanizmams.

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LIST OF ABBREVIATIONS

BioFeO – biogenic iron oxides

ChFeO – chemical iron oxides

DO – dissolved oxygen

DOC – dissolved organic carbon

d.s. – dried solids

FeOB – iron oxidizing bacterias

Fig. – figure

GEH – commercial adsorbent (granulated ferric hydroxide)

GLSF – glass foam particles

HA – humic acids

IC – ion chromatography

ICP-MS – inductively coupled plasma mass spectrometry

OM – organic matter

Q_{\max} – maximum phosphate adsorption, parameter of Langmuir model

RMSE – Root-mean-square error

SEM-EDX – Scanning electron microscopy with energy dispersive X-ray spectroscopy

TOC – total organic carbon

TS – total solids

WTP – water treatment plant

WWTP – wastewater treatment plant

XRD – X-ray diffraction analysis

INTRODUCTION

Phosphate concentrations less than 50 $\mu\text{g/l}$ has been established as the high-quality criteria for the treated wastewater from municipalities and industries (UK environmental standards and conditions, 2008). Excess release of phosphate into the surface water even at very low concentrations could still stimulate algal growth and may lead to eutrophication, it depends on the sensitivity of aquatic ecosystem and many other factors (Oguz, Gurses & Yalcin, 2003). As a consequence, current discharge limits have been reviewed and stricter standards proposed by UK Technical Advisory Group on the Water Framework Directive. Therefore, there is a demand for more efficient removal of phosphate in wastewater treatment plants (WWTPs). Additionally, for various reasons, a more sustainable use of phosphorus and development of technologies to recover phosphorus from waste streams are encouraged: P is an essential nutrient in agriculture (Wang, Tay, Tay & Hung, 2010), phosphate rock is a finite resource and significantly increasing demand for it is observed, dependence on imports – Morocco and W. Sahara phosphorite deposits constitute 74 % of global resources (USA Geological Survey, 2016; EUC Consultative Communication, 2013; Tenkorang & Lowenberg, 2009). Unfortunately, current methods used for phosphorus removal such as dosing coagulants cannot meet the environmental standards and to recover phosphorus from formed products is not economical, but reversible adsorption could be a solution.

In total at least 16 chemical iron oxides (ChFeO) are known (Cornell & Schwertmann, 2003). In nature, ChFeO formation is more likely to occur in aerobic, alkaline conditions, but the process is retarded by low water temperature (de Vet, Dinkla, Rietveld & van Loosdrecht, 2011; Sogaard, Peskir & Medenwaldtet, 2000). In sediment and soil systems iron oxides play an important role in retaining phosphorus by adsorbing it (Cornell & Schwertmann, 2003). ChFeO have been used as P adsorbents as an effluent polishing step for surface waters, municipal and industrial wastewater treatment and can be more efficient than traditional phosphate removal methods using coagulants (Chitrakar, et al. 2006; Genz, Kornmuller & Jekel, 2004; Oguz, 2004; Luengo, Brigante & Avena, 2007; Maor, Semiat & Shemer, 2011). Successful application of ChFeO is attributed to a good adsorption capacity and affinity, high selectivity towards phosphate and relatively low cost (Loganathan, Vigneswaran, Kandasamy & Bolan, 2014). Adsorption capacity can be improved by increasing surface area. It could be done by coating nanoparticles on supporting material (e.g. activated carbon), but this requires additional treatment (Maor, et al., 2011).

Microbial activity is an integral part of iron cycling (Posth, et al., 2014). Biogenic iron oxides (BioFeO) are formed (as intra or extracellular precipitates) for reasons which vary from energy production, protection of cell structure to cellular orientation purposes. Extracellular BioFeO can be formed by acidophilic

and neutrophilic iron oxidizing bacteria (FeOB) such as *Gallionella* sp. or *Leptothrix* sp. (Fortin & Langley, 2005). BioFeO may differ from their chemical formed counterparts in their morphologies and chemical composition (presence of organic matter and adsorbed heavy metals), which in turn affect their binding properties for phosphate. BioFeO have attracted the attention of many researchers. BioFeO are widespread and usually occur as nanoparticles and they have a strongly reactive surface, which should have a positive effect on phosphate adsorption capacity and strength (Fortin & Langley, 2005). First adsorption performance evaluation of BioFeO showed high adsorption capacity value (39.9 mgP/g adsorbent) compared with other determined adsorption capacity values of ChFeO (Rentz, Turner & Ullman, 2009). Additionally, in a study about an assessment of the microbially available phosphorus for nitrifiers, it was shown that in the presence of BioFeO nitrifying bacteria could not grow. Whereas in the presence of ChFeO they could grow, which indicates a higher affinity to phosphorus of formed BioFeO in comparison with ChFeO (de Vet, van Loosdrecht & Rietveld, 2012). Wide occurrence and especially good P adsorption characteristics (high adsorption capacity and possible good affinity) are the main reasons for studying the interaction of phosphorus with BioFeO (Rentz, et al., 2009; Emerson & de Vet, 2015).

Research hypothesis:

- BioFeO have higher affinity to phosphate and higher phosphate adsorption capacity in comparison to ChFeO.
- Better performance of phosphate adsorption by BioFeO are related to their unique structure.

The aim of this thesis is to investigate the applicability of BioFeO on phosphate removal from water and compare this with ChFeO.

The object of research: biogenic iron oxides (BioFeO).

Objectives:

1. Immobilize FeOB on supporting material in order to form BioFeO based adsorbents.
2. Investigate the differences in phosphate binding kinetics between BioFeO and ChFeO.
3. Explore the differences in phosphate binding properties (capacity, affinity) between BioFeO and ChFeO.
4. Identify differences in morphology and chemical composition between BioFeO and ChFeO.

Research place: experiments were conducted according to the Erasmus+ practice mobility programme in 2015-2016 in European centre of excellence for sustainable water technology – Wetsus, which is located in the Netherlands.

1. LITERATURE OVERVIEW

1.1. Demand of phosphorus recovery technologies

1.1.1. The importance and usage of phosphorus

Due to the high reactivity of elemental phosphorus, it is not found as an elemental form in nature – it is often allocated in minerals, mainly as phosphate (such as apatite, phosphorite, amblygonite, vivianite, xenotime and others) and the biggest reservoir for phosphate are apatite minerals. Phosphorus is a nonmetal, it is important to all life forms (animals, plants, fungi and procaryotes) and it is one of the most abundant minerals in the human body. Together with calcium it is, as apatites, part of bones and teeth, where 85-90 % of whole phosphorus in the body is found. Overall ~700 g of phosphorus can be found in adult human. It is also found in proteins and fats in the different tissues (phospholipids in cell membranes). Phosphorus and its compounds are essential for maintaining vital functions supplies energy (ATP) and affect the transfer of genetic information (DNA). Disruption of phosphorus metabolism increases the probability of rickets and vascular atherosclerosis development, fatigue, mental and physical disability (Cisse & Mrabet, 2004; Matulis & Janickis, 2006).

Phosphorus is one of the main macronutrients. In combination with nitrogen and potassium it is necessary for plants, therefore, these elements form the basis for fertilizers. The usage of fertilizers is necessary for the efficient production of quality food and their deficiency can lead to reduced yield (Wang, et al., 2010).

Global sedimentary phosphate rock resources are much larger than igneous and phosphorus mining from it consists of more than 80%. The phosphate rock is used in a variety of products, mainly for the production of fertilizers (see Fig. 1). (Everard, 2001; Cisse & Mrabet, 2004).

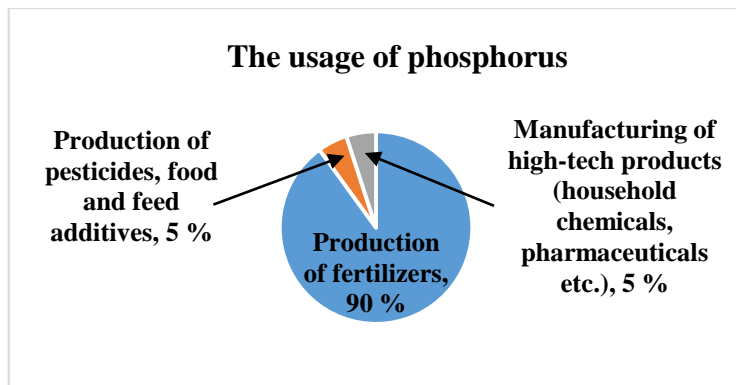


Fig. 1. The usage of phosphorus in various industrial sectors (data from (Cisse & Mrabet, 2004))

1.1.2. Resources of phosphorus, future needs and potential of recovery technologies

A rapid growth in the extraction of phosphate rock has been observed from 1995 until today. USA, China, Morocco and the Western Sahara are extracting the largest amounts of phosphate minerals in the world. USA was the leader in the extraction of phosphate rock until 2006, but it was replaced by China (see Table 1). Extraction of phosphate minerals in China was 3.6 times higher than in USA in 2015. During the last 20 years extraction of phosphate rock in USA decreased by 36.6%, while China's increased by 79%. According to the data collected by USA Geological Survey, the extraction of world's phosphate rock was ~ 223 million tons in 2015; whereas just little more than 131 million tons were extracted in 1995. Hence, over 20 years phosphorus extraction has increased by 41.2 %. The assessment of phosphorus reserves (2015) shows that 72.5 % of the world's resources are concentrated in Morocco and West Sahara.

Table 1. The annual extent of phosphorus extraction in the world and estimated reserves of 2015 (in 1000 metric tons) (data from the USA Geological Survey (2016))

Country	1995	2000	2005	2010	2015 ¹	2015 reserves ¹
USA	43.500	38.600	36.300	25.800	27.600	1.100.000
Brazil	3.530	4.900	6.100	5.700	6.700	320.000
China	21.000	19.400	30.400	68.000	100.000	3.700.000
N. Africa ²	7.410	8.340	10.730	15.400	10.700	3.500.000
Morocco and W. Sahara	20.200	21.600	25.200	25.800	30.000	50.000.000
W. Africa ³	3.600	3.170	2.740	1.800	2.000	80.000
S. Africa	2.790	2.800	2.580	2.500	2.200	1.500.000
Russia	8.800	11.100	11.000	11.000	12.500	1.300.000
Australia	–	–	2.050	2.600	2.600	1.000.000
The Arabian peninsula⁴	9.047	11.790	12.630	12.140	15.050	4.620.000
Other countries:	11.292	11.300	8.700	10.641	13.650	2.200.000
World total:	131.169	133.000	148.430	181.381	223.000	69.000.000

¹Estimated.

²N. Africa: the total of Tunisia, Egypt and Algeria.

³W. Africa: the total of Senegal and Togo.

⁴The Arabian peninsula: the total of Saudi Arabia, Israel, Syria, Iraq and Jordan.

A large amount of phosphorus is found in the crust of the Earth. The economical extractable reserves of phosphorite are concentrated in particular countries, and all of them (except Finland) are

located outside the EU. As a consequence, the European continent is the most dependent on the import of phosphate rock. Moreover, a lot of workplaces have been lost, because the production of final products (fertilizers and phosphorus-containing products) moved to phosphorus supplying countries. The import from Morocco dominates in the world trade, other countries supplying EU – Tunisia and Syria (EUC Consultative Communication, 2013). If the extraction rates of phosphate rock of 2015 remain the same in the future, the current reserves will be sufficient for 309 years (excluding the newly discovered resources) (see Table 1). Unfortunately, according to the data provided in Table 1, the consumption of phosphorus will increase in the future. The cost of phosphate rock increases because of the larger need for phosphorus. However, phosphorus has the important characteristic – it can be recycled an unlimited number of times, but it cannot be substituted. Some processes can be optimized; regulations restrict the usage of phosphate and other phosphorus compounds in consumer detergents. Household detergents may be produced as phosphate-free detergents (EUC Regulation on detergents, 2004). However, it is vital for agriculture (Cisse & Mrabet, 2004), phosphorus is used for animal feed, fertilizer and it can not be replaced by any other materials.

According to the forecasts of FAO, global demand of fertilizer will constantly increase and it is indicated that annual demand of phosphate fertilizer might increase up to 52.9 millions of tons by 2030. The tendency to use crops for biofuel cultivation is globally increasing, which promotes the use of fertilizers as well. It was estimated that phosphate fertilizer consumed for biofuel production was 870.000 tons in 2007-2008; therefore, it was suggested to better evaluate the price and benefits of energy at all stages (Tenkorang & Lowenberg, 2009).

Figure 2 represents the simplified scheme of phosphorus cycle in the society, the consumption of products containing phosphorus leads to huge waste streams rich in phosphorus resulting in the formation of wastewater and sludge. Bounded to the solid particles and dissolved phosphorus makes approximately 11% of total phosphorus in the influent to the wastewater treatment and it is removed with the sludge in the primary settling tank. During biological treatment, including secondary settling tank, 20-30% phosphorus can be removed, consequently, the largest part of phosphorus is removed with sludge. Phosphorus is flowed out and lost from improperly treated sewage, landfills and unsustainably fertilized fields. It happens because of inappropriate and ineffective management and use of biodegradable waste, wastewater, sludge and manure. All these factors lead to extremely undesirable consequence – eutrophication of water bodies. The analysis of phosphorus, which was conducted in France showed that 50% of the total used P is lost: ~ 20% with the wastewater; ~ 20% is

lost due to soil erosion and leaching; 10% with food or other biological wastes (Everard, 2001; EUC Consultative Communication, 2013).

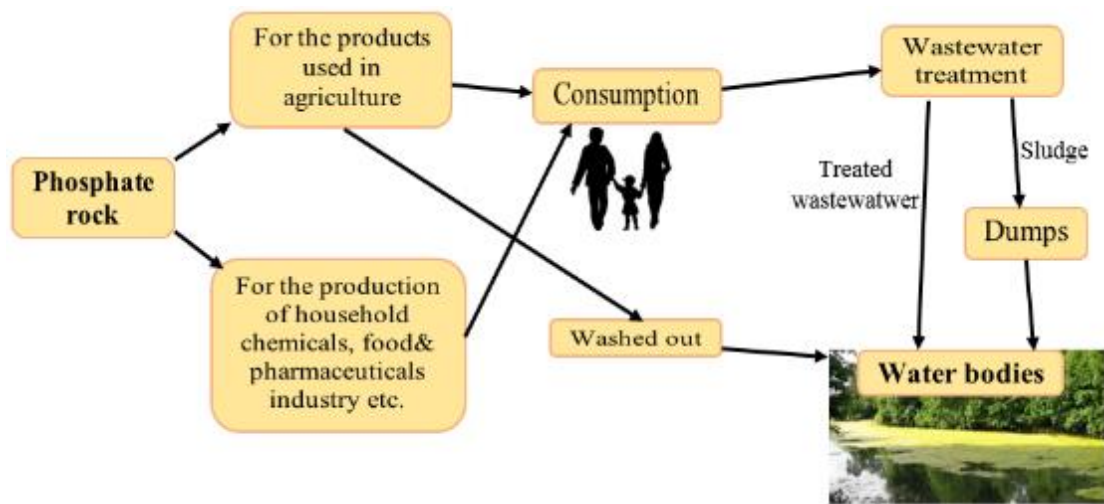


Fig. 2. Simplified scheme of phosphorus cycle in the society (modified according to Everard, 2001)

The excess of phosphorus compounds – one of the most important factors in promoting eutrophication in sensitive water systems – and increased concentrations of nitrogen act synergistically. Consequences of eutrophication process induce changes in biodiversity of water ecosystems, disruption of ecosystem trophic relationships, reduction of water quality for human needs. Hypoxic conditions and increased toxicity (because of during algae decomposition excreted metabolites, such as H_2S) are typical for eutrophication. Ecosystems of standing water (such as ponds, quarries, lakes) are the most sensitive to eutrophication. To change the situation, the removal of the pollution has to be applied for a long period of time, because phosphorus has already been accumulated in the sediments and after disturbance of it eutrophication process is repeated (Smith, Joye & Howarth, 2006; Schindler, 2006). For this reason, it is necessary to control the release of phosphate into the environment in order to protect water bodies is mandatory to follow the Wastewater Management Regulation (No. D1-236, 2006). Second appendix part B of this Regulation determines maximum allowable concentration and environmental quality standards to other controlled substances in sewage in Lithuania. It is determined that the maximum allowable concentration of total phosphorus into the environment can not exceed 2 mg/l. Such restrictions are not strict enough, because it is known that even lower concentrations of phosphorus may lead to eutrophication (Smith, et al., 2006; Oguz, et al., 2003). Therefore, more effective phosphorus removal from wastewater is necessary for controlling negative impact on the environment.

Resources of phosphorus minerals are not infinite and renewable. Consequently, they will be depleted sooner or later and if humanity wants to follow the principles of sustainable development it has to solve the problem. All used measures at ES and national level were basically just handling of disposal waste and water pollution by phosphorus problems so far. Also the encourage to use less products containing phosphorus and improve extraction and usage efficiency (designed balanced animal feed, feed and fertilizers with enzymes, stricter requirements for detergents etc.). Wastewater and sludge formed during wastewater treatment, animal manure and biodegradable waste should not be seen as a waste, which needs to be disposed. It should be seen as a raw material, which with phosphorus recovery technologies can be used for making high-quality phosphorus products. Such technologies would also contribute to solving problems of eutrophication of water ecosystems, management of generated waste and the increasing need for phosphorus and intensive extraction of phosphate resource. According to the Directive of Urban Wastewater Treatment, it is mandatory to remove phosphorus from wastewater, but is not required to remove it in a way that it would be suitable for reuse. Hence, the problem of phosphorus in wastewater is usually "solved" with chemical and biological methods by transferring phosphorus to the sewage sludge (de-Bashan & Bashan, 2004). However there is no international strategy for promoting the development of phosphorus recovery technologies. Therefore, it is necessary to identify market and problems of recycled phosphorus and implementing existing technologies, because it is claimed that Europe has already a lot of knowledge and experience of phosphorus recovery technologies, but in order to solve particular problems, international cooperation is required (EUC Consultative Communication, 2013).

Initiatives that are directly related to phosphorus efficiency and recovery, where have been sporadic and they were often not taken into consideration in policy-making. On the other hand, there were ambitions to change such situation. An exclusive and inspiring situation is observed in the Netherlands. This country is considered as one of the main leaders and initiators of the development and application of phosphorus recovery technologies. The Nutrient Platform was created, which induces inter-sectoral network unifying Dutch organizations. The main objective is to create the conditions necessary for the transition to a sustainable nutrient use. Value chain agreement of phosphate was reached in 2011 under which interested parties: more than 20 dutch companies, research centers and non-governmental organizations committed to establish a sustainable phosphorus market, in which reusable phosphorus flows would be integrated into production processes in order to increase usage of the secondary (recycled) phosphates (The Nutrient Platform, 2016).

Leaders from few European countries (scientific centers, non-governmental organizations, business sector and delegates of government) together with the European Commission have decided to organize the first European conference on sustainable use of phosphorus, which took place in 2013. The nutrients platform of the Netherlands has contributed to this initiative as well. During the conference, more than 150 companies and organizations have signed a declaration demonstrating the need to continue cooperation and to start building the platform for sustainable phosphorus (The European Sustainable Phosphorus Platform, 2016). After this conference, the European Commission for the first time at the European Union level during consultative communique has raised issues related to the sustainable use of phosphorus and decided to discuss current situation and measures that could be taken. Europe can not be self-sufficient in phosphorus without recovery technologies. EU dependence on phosphorus imports was ~ 92% in 2011, consequently the incentives to strengthen neighborhoods and other bilateral agreements are obvious. This opinion was even more reinforced after the events of 2008, when the price of phosphate has increased by 700% in more than one year, resulting in the growth of the price. Such aspects as phosphorus price, the aim to increase its accessibility, the unsustainable use and recycling of resources and with it related environmental pollution have been identified as the main reasons leading to the formation of phosphorus policy. During this communique, the most important objectives of EU phosphorus policy were formed to increase the self-sufficiency within the EU and to mitigate the impact of excessive use of phosphorus. Issues, discussed in the paper, are related to the sustainable use of phosphorus including more efficient extraction, processing, use and retention in agriculture, implemented EU initiatives, practices and action programs (EUC Consultative Communication, 2013).

It can be claimed that the Netherlands is one of the main countries contributing to the forming of phosphorus policy and market of recycled phosphorus at EU level. Changes are very slow, because lack of legal regulations and stronger financial incentives are missing, the need to organize a worldwide conference and initiation of Convention for sustainable use of phosphorus are one of the future visions (Visions for sustainable phosphorus tomorrow, 2014).

1.2. Technologies of phosphorus removal and recovery from wastewater

A number of researches were provided concerning the sustainable use of phosphorus and many technologies of phosphorus removal and recovery are designed in order to enable the recovery of phosphorus from the waste streams such as wastewater, biodegradable materials, animal manure and urine. Some of the technologies were improved during the last years. They implemented pilot projects

and few of them were applied in practice and are carried out on a commercial scale operation in the Western and Northern Europe. Technologies of phosphorus recovery from municipal and industrial wastewater can be applied to various stages of wastewater treatment and can be recovered from the liquid phase (efficiency 40-50%) and from sewage sludge or its ash (efficiency 90%). Polyphosphate accumulated by bacteria during biological P removal can partly be released under anaerobic conditions and recovered. The recovery of phosphorus from the liquid phase is economically useful when wastewater contains not less than 50-60 mgPO₄-P/l. Phosphorus concentration in the sewage treatment plant with a biological phosphorus removal and anaerobic digestion can range 75-300 mg/l (Kalmykova & Fedje, 2013; Gorazda, et al., 2016).

Phosphorus removal by traditional biological treatment processes is not very effective due to the lack of carbon sources and it is also very complicated process (He, Wang & Song, 2016). For this reason, additional treatment is required in order to remove phosphate at low concentrations chemical precipitation methods – dosing coagulants (ferrous or ferric salts, Al₂(SO₄)₃). Applying this treatment phosphorus from wastewater is transferred to the sludge (de Bashan & Bashan, 2004). However, the application of coagulants has drawbacks. In case that strongly bounded compound is formed the recovery of phosphorus is commercially not easy and requires large amounts of chemicals (Donnert & Salecker, 1999).

Phosphorus removal by adding metal salts can achieve 80-95% of total P. This method is not very effective, because to remove phosphate to low concentrations is hard. As a consequence, residual effluent concentration of 1 mgP/l total P can be reached (Maor, et al., 2011). The most primitive method to reuse phosphorus is to spread properly treated sewage sludge directly in the field. LAND 20-2001 Regulation determines the rules on the use of sewage sludge as fertilizer in agriculture, for fertilizing energy crops and the remediation of affected areas in Lithuania.

A large amount of advanced phosphorus recovery technologies target on sludge liquor, which is formed by draining sludge from anaerobic digesters. Calcium or magnesium salts are added in a special precipitation/crystallization tank, respectively, calcium and magnesium phosphate products are obtained. The latter is known as struvite (MgNH₄PO₄·6H₂O). Formation of struvite deposits in the equipment of wastewater treatment plants with anaerobic sludge treatment was initially defined as a problem. However, the potential of struvite as a fertilizer required to do more research on method development. Nowadays, this process is applied to a commercial scale as a successful technology for recovering phosphorus. Usually, Mg concentrations in wastewater treatment facilities are the limiting factor, therefore, it is increased by adding MgO or MgCl₂ (Barat, Bouzas, Marti, Ferrer & Seco,

2009). Recovered phosphate is allowed to use and to be sold as a fertilizer, if the substance is indicated in appendix A of the Regulation of fertilizers. While struvite is not officially indicated as a fertilizer at EU level, except in few European countries (The European Parliament and the Council of the European Union 2003), according to economic aspects, it is predicted that struvite will compete with phosphorus fertilizers in the future.

The principles of phosphorus recovery technologies from sludge are very similar to the method discussed above; it requires larger quantities of reagents, additionally, flocculants are used, so there is less cost-effective process. Phosphorus is recovered applying the extraction using sulfuric acid and the final product is struvite as in the previous methods. Due to the simple and well-established process, struvite is currently the main phosphate recovery technology. On the other hand phosphorus recovery using precipitation is not the final goal (de-Bashan & Bashan, 2004).

Technologies for phosphorus recovery from ash are very different. It is quite difficult and complex process, which requires higher energy and investment costs. There are two main methods to recover phosphorus from sludge ash: wet chemical (when phosphorus is extracted with acid or solvent) and dry thermal (by melting ash) (Schipper, et al., 2001). Incineration of sewage sludge reduces volume, removes organic matter, destroys pathogens. Created product does not have a high nutritious value, because ashes contain a large amount of heavy metals and bioavailability of P is relatively low. The usage of this product in agriculture is strict, hence, additional treatment steps such as thermochemical, electrothermal processes were created, which require the addition of magnesium or calcium chlorides. The product of thermochemical methods is calcinated phosphate, which is already more accessible to plants. They are provided with other nutrients and pellets are produced. The product of electrothermal processes is white phosphorus (P_4), which can be used for producing high-quality phosphoric acid used in detergents and other products (Adam, Peplinski, Michaelis, Kley & Simon, 2009).

Traditional methods for phosphate removal from wastewater by dosing metal salts cannot meet the requirements for environmental quality and to recover phosphorus from formed products is not economical. Physical methods such as reverse osmosis and electrodialysis are very effective techniques at low phosphorus concentrations, but these methods are very expensive as well (Loganathan, et al., 2014). Application of adsorption and desorption processes is considered as cost-effective technology that can be used both for drinking water treatment and industrial wastewater treatment as a polishing step (Genz, et al., 2004; Chitrakar, et al., 2006). Phosphorus might be removed using various mineral, biological and synthetic origin adsorbents: widely used granular or

powdered activated carbon adsorbents (specific surface area of up to 2000 m²/g) such as zeolite, sand, orange waste, mineral clay, fly ash and slag, juniper fibers, nano compounds, polymers, oxides and hydroxides of aluminum (Tanada, et al., 2003), calcium, magnesium and iron are used (Rentz, et al., 2009). Phosphorus recovery technologies can be applied in various stages of wastewater treatment (for liquid, sludge, ash), but it has an influence on the regeneration efficiency, process complexity and cost. It helps to create products that can be used as raw material for the production of phosphate fertilizers and high-quality products.

1.3. Phosphorus removal using adsorption

1.3.1. Principles of adsorption

Adsorption principle is based on the interaction at interfaces, typically the use of a solid surface (metal, biopolymers, glass, clay etc.) to bind compounds from a gaseous or liquid phase, when adsorbate is attached to a solid or liquid surface (adsorbent). Adsorption is a well established and powerful technique for separation processes of gaseous and liquid mixtures via molecular sieves, chemical analyses (various types of chromatography), and flotation processes. This is done for removal of impurities or for recovery of chemicals. There are several types of adsorption depending on the strengths of the interaction between adsorbent and adsorptive. Adsorbate can be adsorbed using physical (processes are controlled by relatively weak Van-der-Waals forces and electrostatic forces) or chemical (processes are controlled strong hydrogen bonding and mutual hydrophobic interactions) forces (Cornell & Schwertmann, 2003).

Adsorption efficiency depends on many physical and chemical factors: affinity between adsorbent and adsorbate, adsorbent surface area and concentration, initial concentration of adsorbate, interaction with the origin of adsorbed material (chemical, biological, type of iron oxides), contact time, impurities, pH, temperature, pressure and redox conditions (Tanada, et al., 2003; Chitrakar, et al., 2006; Maor, et al., 2011). Adsorbents are chosen from materials having porous structure and high surface area, which results in higher adsorption capacity. Activated carbon is the most widely used adsorbent especially for the removal of trace organic compounds from an aqueous solution because of its chemical (eg. surface groups) and physical properties (eg. pore size distribution and surface area). Surface area and optimum pore structure are important factors for adsorbate (Maor, et al., 2011; Xu & Teja, 2008; Cornell & Schwertmann, 2003).

Studies of adsorption equilibrium are important for determining the effectiveness of adsorption. Kinetics experiments show the time when equilibrium is reached. Adsorption is usually

described through isotherms, functions which connect the amount of adsorbate on the adsorbent with its pressure (if gas) or concentration (if liquid). Isotherms are run under equilibrium conditions. Isotherms provide important information about adsorption process – adsorption capacity at low concentration of adsorbate (affinity between adsorbent and adsorbate) and adsorption capacity at saturation stage (maximum adsorption capacity) (see Fig. 3) (Cornell & Schwertmann, 2003; Loganathan, et al., 2014).

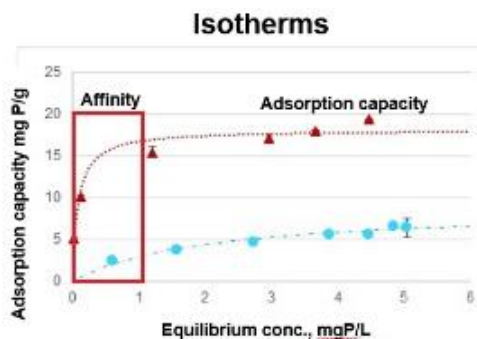


Fig. 3. An example of graph showing phosphate adsorption after isotherm experiments

Particular models describe the process of adsorption. Langmuir adsorption model shows that all adsorption occurs through the same mechanism and adsorption capacity is strictly limited to only one monolayer, adsorbed molecules do not interact. Freundlich adsorption isotherm is described by adsorption where the adsorbate forms multilayers (Cornell & Schwertmann, 2003; Loganathan, et al., 2014).

1.3.2. Phosphorus adsorption using chemical iron oxides

Adsorption methods can be more effective and efficient to recover phosphorus than traditional phosphorus removal methods at low concentrations (Chitrakar, et al., 2006; Genz, et al., 2004; Oguz, et al., 2004; Luengo, et al., 2007; Maor, et al., 2011). Wastes, containing iron compounds or specially designed substrates of synthetic oxides and hydroxides of iron, can be successfully used for phosphorus removal. It is attributed to a good adsorption capacity and relatively low cost (Chitrakar, et al., 2006; Maor, et al., 2011; Loganathan, et al., 2014). According to Chitrakar et al. study (2006), it is also explained by good binding affinity and high selectivity towards phosphate, because during research no influence of NO_3^- , Cl^- , SO_4^{2-} , Ca^{2+} ions on phosphate adsorption was observed. Because of selectivity, purer products can be recycled. The usage of adsorbents is especially relevant when membranes of reverse osmosis are applied for wastewater desalination processes. Phosphate ions leads to the clogging of the membranes through the precipitation of calcium phosphate salt, but by adsorption of phosphate fouling can be prevented (Greenberg, Hasson & Semiat, 2005).

Phosphate is adsorbed on surface of iron oxides active sites by ligand exchange with hydroxyl groups and bounded chemically (see Fig. 4) (Cornell & Schwertmann, 2003; Loganathan, et al., 2014). The simplest way to use adsorbed phosphorus is by spreading used adsorbents on the soil. More advanced technologies for adsorbent and phosphate recovery include thermal, chemical (acids, bases) and biological (by iron or sulphate reducing bacteria) methods.

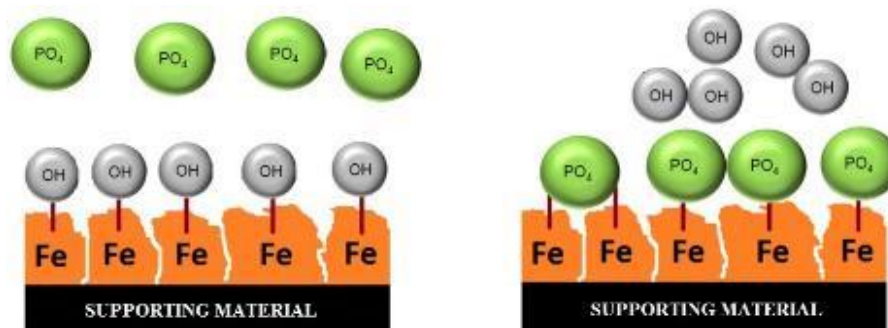


Fig. 4. Simplified scheme of phosphate adsorption process by ligand exchange with hydroxyl groups

Zelmanov and Semiat study (2014) have shown that after the application of nanoparticles suspension of iron oxides/hydroxides for phosphate removal, residual phosphorus concentration was less than 0.1 mg/l. Phosphorus recovery efficiency of 95-98% was reached by obtaining concentrated phosphate solution, which can be processed by forming crystals. An average size of particles was 50 nm, phosphate solution (3.3 mgP/l, pH = 8.4-8.5) with properties close to the typical municipal wastewater characteristics (1-5 mgP/l, pH 8 to 8.5) were used for the experiment. The study revealed that obtained adsorption capacity (27.25 mgP/g) is similar to other adsorbents performance: the granulated iron hydroxide (24.9 mgP/g at pH = 5.5, particle size 0.063 mm, surface area 280 m²/g) (Genz, et al., 2004) and synthetic goethite and akaganeite – 28 mgP/g (Liu, et al., 2007). These adsorbents have very high usage potential, because adsorption capacity of adsorbents such as activated red clay is just 0.026 mgP/g (pH=7, particle size 0.14 mm, surface area 14.1 m²/g) (Liu, et al., 2007). Adsorption efficiency may be improved by increasing the surface area of adsorbent. Nanoparticles have very high surface area, but its application requires additional treatment step – nano or ultrafiltration. Therefore, it is putting efforts in coating FeO nanoparticles on supporting material (backbone) with high surface area (e.g. activated carbon), which could be a solution (Maor, et al., 2011; Xu & Teja, 2008). But iron clusters on supporting material are one of the main issues why this technique is not very effective.

1.3.3. Phosphorus removal using biogenic iron oxides

Iron is the fourth most abundant element in the Earth's crust and microorganisms influence iron cycle in sediments of marine and freshwater ecosystems, groundwater, soil, wetlands and mine drainage systems. Figure 5 shows, an image of biogenic iron oxyhydroxides sampled at an iron-rich seepage areas in the Netherlands. They are formed inside the bacterial cells or on the outer part of it during dissimilatory iron oxidation, protection of cell structure and orientation purposes. Biogenic minerals are formed because of direct metabolic activity of bacteria or ongoing passive adsorption reactions (Fortin & Langley, 2005; Frankel & Bazylinski, 2003; Kikuchi, Makita, Takai, Yamaguchi & Takahashi, 2014; Emerson & Moyer, 2002).



Fig. 5. Photos of gelatinous microbial iron oxyhydroxides from wetland

Metabolic activity of acidophilic (*Acidithiobacillus* sp.) and neutrophilic (*Gallionella* sp., *Leptothrix* sp.) iron oxidizing bacteria under aerobic conditions promotes ferrous iron oxidation and formation of BioFeO as an extracellular product resulting on the wall of bacteria or next to it. Which can also result in passive adsorption reactions of iron associated with the charge of the cell. Surface activity of bacteria depends on pH and generally provides a negative charge to the cell wall, as a consequence soluble iron is bound and precipitates of iron oxide are formed (Frankel & Bazylinski, 2003; Emerson & Moyer, 2002; Chatellier, Fortin, West & Leppard, 2001). The chemical and microbial oxidation of soluble Fe^{2+} (ferrous iron) to Fe^{3+} (ferric iron), which results in insoluble oxyhydroxides, depends on the pH and the O_2 concentration (Fortin & Langley, 2005; Morgan & Lahav, 2007; Frankel & Bazylinski, 2003; Duckworth, Holmstrom, Pena & Sposito, 2009). The chemical oxidation of Fe^{2+} under acid sulfate conditions (e.g. in mine drainage environments and some hot springs) is very slow (Ferris, 2005). Chemical oxidation takes place at higher pH and O_2 concentration, therefore, especially neutrophils have to compete with the rapid

abiotic oxidation of Fe^{2+} (Sogaard, et al., 2000; de Vet, et al., 2011; Emerson & de Vet, 2015). Extracellular BioFeO of neutrophils have different structures. *Gallionella* sp. produces oxyhydroxide as twisted stalks, *Leptothrix* sp. as hollow microtubes. *Mariprofundus* sp., *Rhodobacter* sp. and other species also produce extracellular BioFeO. The BioFeO is usually characterized as amorphous or poorly crystalline and they vary in chemical and mineral composition. The most common extracellular BioFeO are hematite and magnetite, oxyhydroxides – goethite, ferrihydrite, lepidocrocite and akageneite. Such iron oxides usually occur as small crystals (2-500 nm) (see Fig. 6) and contain impurities (Si, PO_4 , SO_4 , Mn, Al, Cu, Zn) (Fortin & Langley, 2005; Frankel & Bazylinski, 2003; Kikuchi, et al., 2014; Emerson & Moyer, 2002; Fleming, Cetinic, Chan & King, 2014; Suzuki, et al., 2011; Posth, Canfield & Kappler, 2014).

BioFeO are produced also by microbial reduction of ferric iron oxide. Magnetotactic bacteria form intracellular iron compounds (magnetite minerals) by reducing of ferrihydrite under anaerobic conditions. The most popular crystals of magnetosomes (magnetite minerals) are magnetite and greigite (Fe_3S_4) with lengths ranging from 35 to 200 nm. Mackinawite, pyrite and various iron hydroxides are also found as an intracellular product, but not origin of magnetotactic bacteria (Fortin & Langley, 2005).

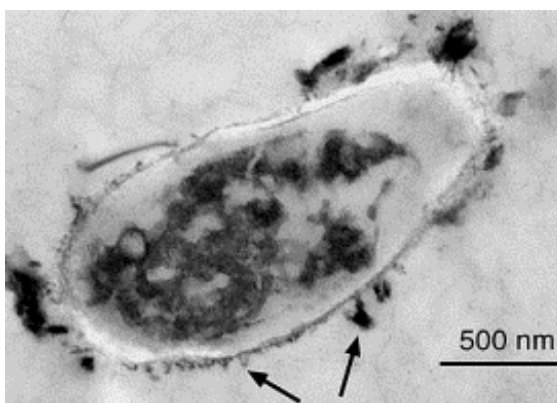


Fig. 6. TEM image of Fe-rich minerals (indicated by arrows) on the bacetrium cell wall (Fortin & Langley, 2005)

Microbial iron oxyhydroxides have attracted the attention of many researchers as very promising and environmentally friendly future biotechnologies. Biogenic iron oxides have not only large surface area but also strongly active surface, which results in effective binding of metals and metalloids (Fe, Mn, Pb, Cd, As, U, Sr etc.), organic xenobiotics and important nutrients – phosphorus. As a consequence, these bacterias have a high potential for technical applications such as treatment of groundwater, development of adsorbents, soils and aquatic ecosystems remediation, air purification (Fortin & Langley, 2005; Rentz, et al., 2009; Frankel & Bazylinski, 2003; Emerson & Moyer, 2002; Suzuki, et al., 2011; Posth, Canfield &

Kappler, 2014; Emerson & de Vet, 2015; Ferris, 2005; Heim, et al., 2015; Cornell & Schwertmann, 2003; Chan, Fakra, Edwards, Emerson & Banfield, 2009; Bai, Yang, Liang & Qu, 2016; Safarik, et al., 2013; Pokhrel & Viraraghavan, 2009; Natarajan, 2013; Ahoranta, Kokko, Papirio, Ozkaya & Puhakka, 2016; Katsoyiannis & Zouboulis, 2006), application in electronics and energetics (Xin, et al., 2009).

The Rentz, et al. study (2009) showed maximum adsorption capacity of biogenic iron oxides – 39.9 mgP/g and this is a high value compared with other iron-based adsorbents. Loosely flocculent oxyhydroxides were collected from a freshwater wetland and consisted of *Leptothrix ochracea* stalks (Rentz, et al., 2009). Phosphate in full-scale groundwater trickling filters is mainly removed chemically with iron by precipitation and adsorption on iron (oxy)hydroxides. During research of P limitation for nitrification of anaerobic groundwater rich in Fe, NH₄ and phosphate it was observed that phosphate concentrations were significantly decreased and nitrification was restricted. This reduction may be related to the growth of the FeOB – *Gallionella* sp., which was found growing in the filter with incomplete nitrification. In another filter where no FeOB observed nitrifying bacteria could grow. It could indicate a higher affinity to phosphorus of formed BioFeO in comparison with ChFeO (de Vet, et al., 2012). Good adsorption characteristics of BioFeO can provide an effective and efficient means to decrease phosphate to very low concentrations, also, possible regeneration opportunities (biological and chemical methods, membrane filtration) are the main reasons why engineering application of this approach should be investigated further.

BioFeO formed in the laboratory would be a purer and better material than samples from environment for adsorption studies because of easier comparison with chemically formed FeO. *Gallionella* sp. and *Leptothrix* sp. are dominant species in groundwater. *Gallionella* sp. is strictly lithotrophic, therefore, its growth can be seen as direct proof of biological iron oxidation (de Vet, et al. 2011; Katsoyiannis & Zouboulis 2006). Immobilization of iron and manganese oxidizing bacteria in groundwater sand filters has been shown to be very efficient in removing not only iron and manganese but also arsenic (Katsoyiannis, Zikoudi & Hug, 2008; Pokhrel & Viraraghavan, 2009; Katsoyiannis, Zouboulis, Althoff & Bartel, 2002). Development of an adsorbent by a uniform distribution and thin layer of BioFeO nanoparticles onto porous supporting material could make phosphate removal, recovery and reusability of adsorbent more simple, cheaper and more efficient. There would be no need of ultra/microfiltration systems or centrifugation for particles separation. Adsorbent would be continuously produced in WTP, because it is a part of iron removal from natural groundwater. Phosphate adsorption rate and capacity would be also improved due to easier access to active sites.

2. MATERIALS AND METHODS

2.1. Experimental set-up for formation of adsorbents coated with biogenic iron oxides

For formation of a uniform and thin biofilm of iron oxidizing bacteria (*Gallionella* sp.) on supporting material an upflow system was designed (see Fig. 7). Treated groundwater was continuously run through 2 separate columns. Treated groundwater is water after iron removal from raw groundwater, which can be done by biological and chemical oxidation. Ferrous iron (Iron(II) sulphate heptahydrate, concentration 3.3 mg/l Fe) was injected. Macronutrients (18.5 µg/l PO₄-P, 52.7 µg/l NH₄-N) and trace elements Zn, Co, Cu and Mo (0.03 µgZn/l ZnSO₄·7H₂O-Zn, 0.04 µgCo/l CoCl₂·6H₂O-Co, 0.01 µgCu/l CuSO₄·5H₂O-Cu, 0.03 µgMo/l Na₂MoO₄·2H₂O-Mo) were added to the treated groundwater in order to prevent bacterial growth limitation (concentrations refer to the final concentrations in the influent of column).

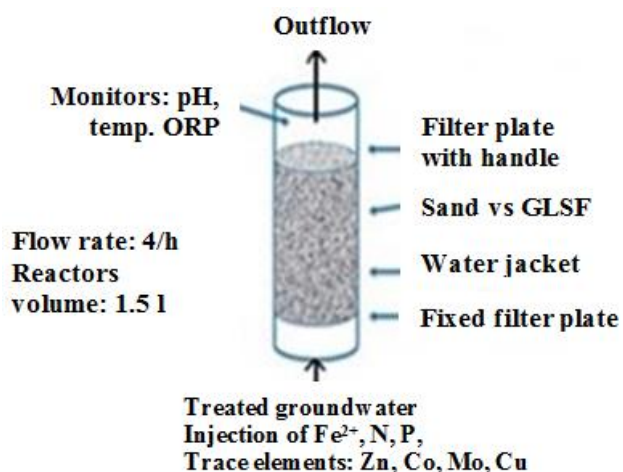


Fig. 7. Scheme of a filter column filled to form BioFeO on sand and glass foam and the photo of the set-up

The set-up consists of two columns: one filled with sand of diameter of $1 \cdot 10^{-3}$ - $2 \cdot 10^{-3}$ m and the other filled with glass foam (GLSF) particles (diameter $\leq 1 \cdot 10^{-2}$ m). GLSF is porous (see Fig. 8) and very light material, has a high strength and low thermal conductivity (Qu, et al., 2016).

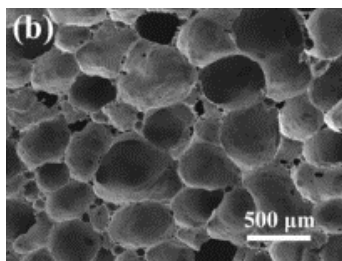


Fig. 8. SEM image of the glass foam sintered at 700 °C (Qu, et al., 2016)

Table 2 shows the characteristics of influent. The volume of one reactor 1.5 l, initial flow rate 7.958 l/h was controlled by pump. The backwash of the filters was conducted once per week by removing the supporting material and washing it externally with fixed volume of 3 l drinking water to prevent clogging. The set-up was designed according to the reference system described in de Vet, et al. (2011) study. Water samples were taken before and after columns and their chemical composition were analyzed by using inductively coupled plasma mass spectrometry (ICP-MS), ion chromatography (IC), total organic carbon (TOC) analysis and ferrozine method for Fe²⁺/Fe³⁺ determination (Viollier, Inglett, Hunter, Roychoudhury & van Cappellen, 2000).

Table 2. Characteristics of treated groundwater

Measurement, units	Value (average of the measurements)
Temperature ¹ , °C	15 ± 0.6
pH ¹	7.2 ± 0.2
Dissolved O ₂ concentration ² , mg/l	8.2 ± 0.6
Redox potential ¹ , mV	-280.2 ± 50
Conductivity, mS/cm	3.6 ± 0.3

¹ Continuously every 5 seconds measured values

² Measurements by optical dissolved oxygen meter

2.2. Biogenic iron oxides from other sources

2.2.1. Microbial mats of *Leptothrix* sp.

Iron-rich seepage areas in the north of the Netherlands were chosen for the collection of biogenic iron oxides. Deposits collected between December 2015 and February 2016 from groundwater seeps in Earnewald, Beetsterzwaag and Lettelbert areas were used for adsorption experiments. Microbial mats (loosely flocculent biogenic oxyhydroxides) were collected using sterile 100 ml plastic syringes and concentrated by settling, decanting the supernatant of the bottle and filling

it with more biogenic iron deposits. During sampling temperature, pH, dissolved oxygen and Redox potential for water overlaying microbial mats were measured. Before experiments samples were kept at 4 °C. Water samples were taken (filtered over 0.45 µm polycarbonate membranes) for chemical composition analyses, which was performed by using ICP-MS, IC, LC-OCD. Filtered samples for total iron determination were acidified in the field with HNO₃ (300 µl per 10 ml sample). The sampling procedure was adjusted and performed according to the reference article (Rentz, et al., 2009).

2.2.2. Microbial mats of *Gallionella* sp.

One sample contained stalks of *Gallionella* sp. was provided by scientist Weren de Vet from his set-up designed for biological iron oxidation by *Gallionella* sp. in drinking water production (de Vet, et al., 2011).

2.3. Chemical iron oxides

Granulated ferric hydroxide (GEH) is an engineered adsorbent, which has a crystalline structure. For experiments was used grinded GEH with the size of granules ≤ 150 µm.

2.4. Characterization of BioFeO and ChFeO

Total solids (EN 12880:2000) and organic matter (EN 12879:2000) of backwash water of the set-up and collected deposits of *Leptothrix* sp. were determined by the gravimetric method using four replicates. To determine the elemental composition of the material formed in the set-up, deposits of backwash water and biogenic iron oxides after air-drying were digested in the microwave (10 ml HNO₃ on 0.5 g material for 45 minutes at ~180°C) and determined by ICP-MS.

The morphological, structural and elemental distribution analysis of solids in backwash water of the set-up and deposits of biogenic iron oxides were examined by scanning electron microscope with energy dispersive X-ray spectroscopy (JEOL JSM-6480 LV) (SEM-EDX), X-ray diffraction analysis (XRD) and light microscopy. Samples for SEM-EDX analysis were prepared according to the modified method described in Heim, et al. (2015). Fresh samples for SEM-EDX analysis reserved in 2.5 % glutaraldehyde and stored at 4 °C. Prior to analyses, the samples were rinsed with PBS and dehydrated in an ethanol series 15, 30, 50, 70 % ethanol (30 min each), followed by 90, 100 % (1 h each). Final drying with 100 % ethanol was performed by mounting the sample on a stub, after drying the sample were kept in a desiccator, before analysis coated with gold. Samples for XRD analysis

were air-dried. Samples for XRD analyses were filled in glass capillaries and sealed first with modelling clay and then superglue. Right before analyses, glass capillaries were sealed using a burner. The measurements were done on a PANalytical X'Pert PRO diffractometer with Cu-K α radiation (5-80 °2 θ , step size 0.008°).

2.5. Phosphate adsorption experiments

Washing is an important step to avoid iron release during adsorption experiments. Adsorbents produced in the set-up and deposits of *Gallionella* sp. were washed with MQ water and vacuum dried at 25 °C. To prevent changes of iron oxides it is recommended to use drying temperature below 40°C (Schwertmann & Cornell, 2000). Deposits of *Leptothrix* sp. were washed with MQ water and for adsorption experiments non-dried microbial mats were used. The uptakes of phosphate were calculated from the decreases of phosphate concentrations relative to those of the initial concentration. Phosphate adsorption capacity is calculated using equation 1 and expressed mgP/g of dried solids.

$$q_t = \frac{(C_0 - C_t)V}{m}; \quad (1)$$

where q_t – is the adsorption at time t (min), mgP/g;

C_0 – concentration of phosphate in the initial solution, mgP/L;

C_t – concentration of phosphate at time t (min), mgP/L;

V – is the volume of solution, L;

m – is the weight of the sample added to the solution, g.

All the adsorption studies were carried out by a batch method in duplicates at room temperature. 5 mgP/l concentration solution with pH 6.5±0.2 used for phosphate adsorption experiments were prepared by addition of KH₂PO₄ salt in MQ water.

The rates of phosphate adsorption from a KH₂PO₄ solution were determined by manually stirring (once per day) 0.1 g of adsorbent in a 0.2 l volume of solution for **kinetics experiments**, a total volume of 1.5 ml of supernatant was sampled with a syringe after 1, 2, 3, 4 and 7 days of reaction time.

For **isotherm experiments** different concentrations of adsorbent (2 g/l, 1 g/l, 0.5 g/l, 0.25 g/l, 0.125 g/l) were used in a 0.1 l volume of solution. A total volume of 1.5 ml of supernatant was sampled with a syringe after 4 days of reaction time.

The sampled suspension was filtered through a 0.45 μm polycarbonate membrane filter. The phosphate concentrations in the supernatant solutions were determined by IC, the supernatant was also analyzed by ICP-MS and TOC.

The experimental kinetic data were fitted with the non-linear form of kinetic model (pseudo-second-order) (see (2) eq.).

$$q_t = \frac{k * q_e^2 * t}{1 + (k * q_e * t)}; \quad (2)$$

where q_t – is the adsorption at time t (min), mgP/g;

k – second-order rate constants of adsorption, mgP/g min;

q_e – is the adsorption capacity of the final concentration, mgP/g.

Langmuir (see (3) eq.) and Freundlich (see (4) eq.) isotherms were used to describe batch data. Root-mean-square error (RMSE) analysis was used to compare the fit of isotherms models.

$$Q_e = \frac{Q_{\max} * K_1 * C_e}{1 + K_1 * C_e}; \quad (3)$$

where Q_e – adsorption capacity at equilibrium, mgP/g;

Q_{\max} – maximum adsorption capacity, mgP/g;

K_1 – equilibrium constant for the Langmuir adsorption, L/mgP;

C_e – concentration at equilibrium, mgP/L.

$$Q = K_f * C^{1/n}; \quad (4)$$

where Q – adsorbed phosphorus concentration, mgP/g;

K_f – the Freundlich adsorption affinity constant, L/mg P;

C – aqueous equilibrium concentration, mgP/L;

n – Freundlich adsorption intensity constant.

3. RESULTS AND DISCUSSION

3.1. Phosphate adsorption performance of adsorbents formed in the set-up

The first aim of this thesis was to immobilize BioFeO on supporting material and study their interaction with phosphate. The primary design of the set-up was based on usage of anoxic groundwater as a source of Fe^{2+} and FeOB connected with sprayers in order to ensure oxygen demand for bacterias; but the cultivation of bacterias was unsuccessful. It might be related with pre-oxidation of groundwater because of aeration of groundwater with sprayers. Hanert (2006) and de Vet, et al. (2011) concluded that the stability of ferrous iron in the presence of oxygen is a key-factor for *Gallionella* sp. existence. Pre-oxidation resulted as chemical oxidation (even at very low levels) inhibits biological oxidation. Also it was difficult to control the residence time and as a consequence, sprayers were removed. Subsequently, the set-up was modified into an upflow system. Anoxic raw groundwater was mixed with treated/aerated groundwater, which consisted 8 % of total inflow (concentrations refer to the final concentrations in the influent of column: $1\pm 0.3 \text{ mgO}_2/\text{l}$, $3.5\pm 0.3 \text{ mgFe}^{2+}/\text{l}$). When iron-oxidizing bacteria takes over chemical ferrous oxidation complete ferrous oxidation should be observed within one week. Therefore, biological ferrous oxidation was evaluated by the effectiveness of ferrous oxidation and by checking deposits of backwash water with light microscopy. After efforts in decreasing temperature (from 17°C to 15°C) and pH by HCl injection (pH was decreased from 7.2 to 6.5) of influent positive effect on bacterial growth in reactors was observed, but it was insignificant (see Fig. 9b). According to the literature, to cultivate FeOB is complicated because of difficulties in establishing the narrow anoxic-oxic interface required for the growth of these organisms (Kikuchi, et al., 2014). There has been a study on evaluation of biological and chemical iron oxidation in groundwater filtration systems at different pH and it provided conclusion that in the oxygen-saturated treated groundwater water at circumneutral pH biological iron oxidation was the dominant process (de Vet, et al., 2011). As a consequence, very slow bacterial growth could be related to the same groundwater pre-oxidation problem (the stability of Fe^{2+}). Measured redox potential was fluctuating from -355.9 mV to -249.2 mV during 24 hours. But sometimes more drastic changes were observed when redox potential from -355.9 mV has increased up to -40.9 mV in 2-5 hours. In order to avoid the pre-oxidation and to form purer biogenic precipitates the set-up was again modified. Instead of using oxygen free groundwater containing Fe^{2+} , the set-up was connected to aerated groundwater and spiked with ferrous iron, additionally, macronutrients were added (detailed description of the system in chapter 2.1.). The change of ferrous iron source has not had an influence on bacterial growth during the first week, but after addition of trace elements significant increase was observed within one week (see Fig.

9b). This showed that *Gallionella* sp. require not only inorganic carbon, macronutrients, stable ferrous iron, optimal temperature and pH, but also trace elements all together are very important factors for *Gallionella* sp. growth.

Accordingly, trace elements could be also the reason for an unsuccessful cultivation of bacterias in the primary system connected to the raw groundwater, but this hypothesis was not checked. WTP where biological iron removal occur there is no additional dosing of trace elements, because groundwater contains trace elements (Katsoyiannis, et al., 2008; Pokhrel & Viraraghavan, 2009; Katsoyiannis, et al., 2002).

Faster cultivation of bacterias was always observed in the column filled with GLSF than sand. It might be explained by surface roughness/irregularities of supporting material, which improves attachment of bacterial cells to surfaces prior to biofilm formation (Alnnasouri, Lemaitre, Gentric, Dagot & Pons, 2011). FeOB contribute to clogging of wells, pipes, draining and water distribution systems (Emerson & de Vet, 2015). Therefore, from a practical point of view, the biofouling can be prevented by limiting macronutrients or trace elements.

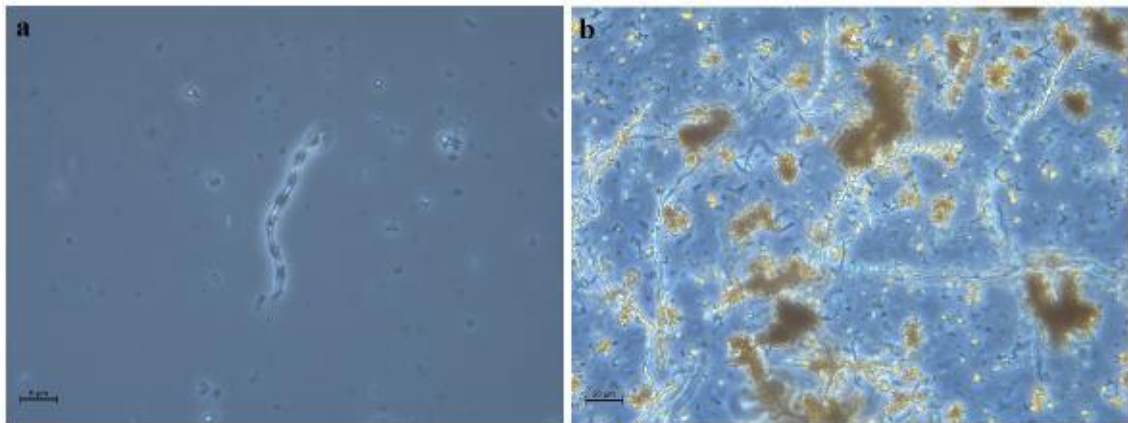


Fig. 9. Images of light microscopy. Backwash water of glass foam column: a) in the upwards flow system connected to the raw groundwater after 38 days (bar equals 8 μm); b) after one week in the system connected to treated groundwater and spiked with ferrous iron with the addition of macronutrients and trace elements (bar equals 20 μm)

Experiments with adsorbents created in the set-up connected to treated groundwater spiked with ferrous iron and with the addition of macronutrients and trace elements showed that kinetics were slow, equilibrium was not reached even in 7 days. The adsorption capacity of phosphate was insignificant – 0.1 mgP/g of sand and 0.8 mgP/g of GLSF, it could be explained by a low content of iron 0.9 %, 5.6 % respectively and a very low ratio of BioFeO (see Fig. 10). According to the literature, sand has low

adsorption capacity of phosphate. The adsorption capacity of naturally iron oxide coated sand is 0.88 mgP/g, while synthetically iron oxide coated sand has a bit higher adsorption capacity – 1.5 mgP/g (Boujelben, et al., 2008). On the other hand adsorption capacity of phosphate with iron oxide coated sand is higher in comparison with other materials such as red clay, which has the adsorption capacity of 0.026 mgP/g (Liu, et al., 2007).

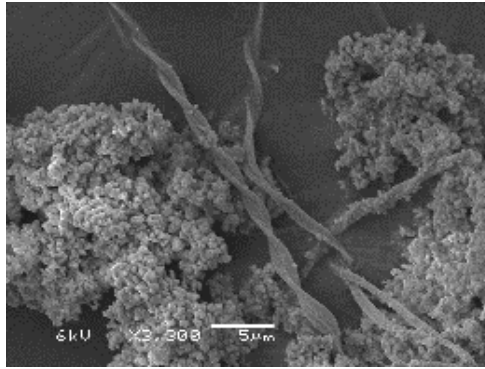


Fig. 10. SEM image of *Gallionella* sp. stalks in backwash water of glass foam column in the upwards flow system connected to treated groundwater and spiked with ferrous iron, scale bar equals 5 μm

3.2. Characterization of *Leptothrix* sp. and *Gallionella* sp. microbial mats

Observation of thin, breakable, blue film on the water overlying flocculent orange iron oxyhydroxides helped to find microbial mats in the fields (see Fig. 11). The blue film of iron oxides is formed as a result of microbial Fe^{2+} ions oxidation (Reina, et al., 2015). The origin of gelatinous iron oxyhydroxides was also confirmed with light microscope – all three samples from different places were microbial origination. Since it was an environmental sample, it contained impurities and some stalks of *Gallionella* sp. were observed as well, but mainly it contained deposits of *Leptothrix* sp. Therefore, all samples were defined as microbial mats of *Leptothrix* sp. with E, B or L letter indicating sampling place.



Fig. 11. Photo of iron oxides film on the water overlying microbial mats

During sampling of microbial mats of *Leptothrix* sp. pH, oxygen and temperature were measured in water overlaying microbial mats (see Table 3). All sampling locations had neutral pH, temperature was in the 4.3-7.9 °C range. Dissolved oxygen concentration was in the 4.5-5.5 mg/l range, what confirms that FeOB can grow not only in low oxygen waters. Different iron oxides vary in chemical composition and morphology, which affects their adsorption (Cornell & Schwertmann, 2003; Loganathan, et al., 2014). Such iron oxides usually contain impurities – adsorbed Si, PO₄, SO₄, Mn, Al, OM (Fortin & Langley, 2005). *Leptothrix* sp. samples consisted of 23.6 % of iron, which corresponds to the values (23.1-34.4 %) reported by Rentz et al. (2009), who also performed phosphate adsorption experiments with deposits of *Leptothrix* sp. collected from freshwater wetland. Deposits of *Gallionella* sp. have a higher percentage of iron in its stalks (39.1 ± 1.3 % of Fe). It could be explained by the fact that *Gallionella* sp. is an iron-oxidizing and strictly chemolithotrophic bacterium, while *Leptothrix* sp. gain energy by oxidizing not only Fe²⁺ but also Mn²⁺, therefore the products are ferric hydroxide and manganese oxide (Katsoyiannis & Zouboulis, 2006). For adsorption experiments with ChFeO commercial adsorbent GEH was used, which is pure akageneite and contains 56,6 % iron.

Table 3. Characteristics of microbial mats of *Leptothrix* sp. from different locations

	<i>Leptothrix</i> sp. E	<i>Leptothrix</i> sp. A	<i>Leptothrix</i> sp. L
pH (n=5)¹	6.9 ± 0.2	6.9 ± 0.1	7.0 ± 0.4
Oxygen, mg/l (n=5)¹	4.9 ± 0.3	4.5 ± 0.2	5.5 ± 0.3
Temperature, °C (n=5)¹	6.2 ± 0.2	7.9 ± 0.2	4.3 ± 0.4
Fe content, mgFe/g (n=3)²	231.2 ± 6.1	252.9 ± 3.4	222.9 ± 0.4
OM, % (n=4)²	49.7 ± 0.3	34.1 ± 0.3	47.4 ± 1.6

¹Measured in water overlaying microbial mats.

²Concentrations of MQ-washed microbial mats.

Deposits of *Leptothrix* sp. also contained a high percentage of organic matter 44.7 ± 6.3 % (n=36). Microbial mats of *Gallionella* sp. contained 5-20 % OM. BioFeO could be defined as complex Fe-organic matter aggregates, which consist of iron (oxyhydr)oxides, exopolysaccharides, organic metabolites, microbial exudates and cell detritus (Kikuchi, et al., 2014; Posth, et al., 2014; Suzuki, et al., 2011; Fleming, et al., 2014; Fortin & Langley, 2005). The autotrophic stalk-forming *Gallionella* sp. uses CO₂ for organic compounds (biomass) production (de Vet, et al., 2011). The sheath-forming *Leptothrix* sp. requires organic carbon compounds as carbon source, therefore, sheaths contain huge amounts of organic matter (mainly polysaccharides) as a result of bacterial growth (Katsoyiannis &

Zouboulis, 2006; Chan, et al., 2009). As a consequence, *Gallionella* sp. occurs in waters with low organic carbon and steep redoxclines. *Leptothrix* sp. is more abundant in waters that contain high concentrations of complex organic carbon (e.g. bogs, fens, tile drains and certain chalybeate springs), Fe and Mn and gentle redoxclines. Finally, these findings identify a largely unexplored relationship between FeOB and organic carbon (Fleming, et al., 2014). Organic compounds have an influence on the initial nucleation of BioFeO, organic molecules play a key-role in preventing the encrustation of the cell (Posth et al., 2014). SEM-EDX elemental analysis also confirmed that BioFeO are strongly interrelated with OM, because C, Fe and O were the main components.

Neutrophilic iron oxidizing bacteria produce extracellular BioFeO, which have different structures. *Leptothrix* sp. is a sheathed filamentous bacterium and form oxyhydroxides as a hollow microtubes with the diameter of 1 μm and by up to 200 μm in length (see Fig. 12). *Gallionella* sp. produce iron oxides, which have the shape of twisted stalks with the diameter of 2 μm and by up to 50 μm in length. According to the literature, BioFeO is usually characterized as amorphous or poorly crystalline (Fortin & Langley, 2005; Frankel & Bazylinski, 2003; Kikuchi, et al., 2014; Emerson & Moyer, 2002; Suzuki, et al., 2011), XRD analysis have shown that both bacteria produced mainly amorphous iron oxyhydroxides.

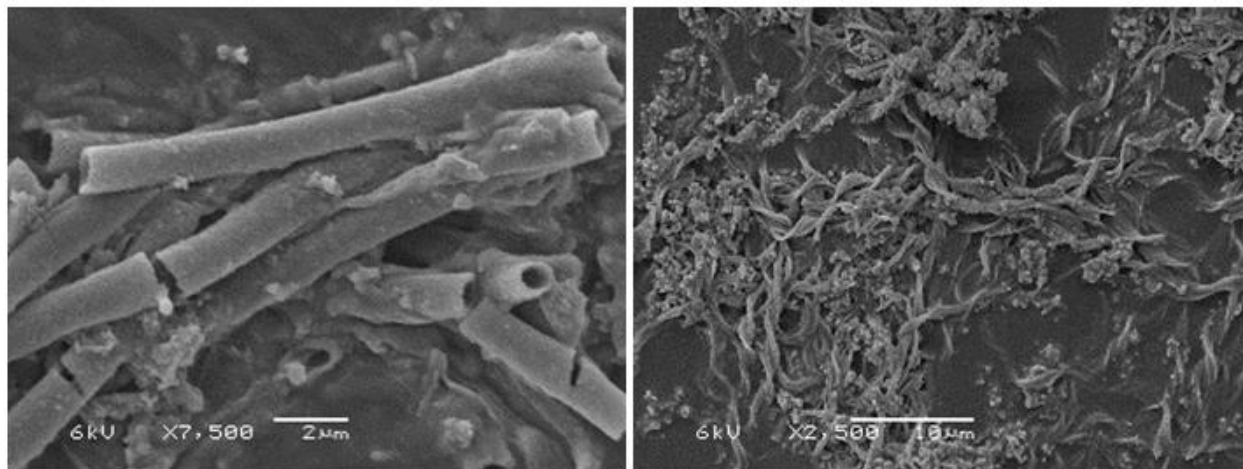


Fig. 12. SEM image of *Leptothrix* sp. sheaths (sample from Earnewald) on the left and an image of *Gallionella* sp. stalks, sample from Weren's upflow oxidation column

3.3. Phosphate adsorption kinetics and isotherms of BioFeO and ChFeO

Kinetics and isotherms experiments were performed with non-dried deposits of *Leptothrix* sp., in order to avoid disruption of the cells, which could damage BioFeO and for better comparison with Rentz et al. (2009) research. Phosphate adsorption kinetics was described by non-linear kinetic model

(pseudo-second-order), because, according to the literature, concerning the kinetics of phosphate on iron-based adsorbents, this model usually fits data the best (Maor, et al., 2011; Mezenner & Bensmaili, 2009). Langmuir (see (3) eq.) and Freundlich (see (4) eq.) equations were used to describe batch data of phosphate adsorption isotherms of BioFeO and GEH. Data fitted well with both models (RMSE<2.1), slightly better fit was with Freundlich equation. All of the isotherm constants for adsorption of phosphate by BioFeO and GEH are summarized in Appendix. Phosphate adsorption mechanism is based on ligand exchange with hydroxyl groups, when adsorbate is adsorbed chemically on surface active sites by forming innersphere complex (Loganathan, et al., 2014; Xue, Hou & Zhu, 2009; Zhang, Liu, Liu, & Qu, 2009; Yan, et al., 2010). Langmuir model represents chemisorption, therefore, for further discussion parameters of Langmuir isotherm will be taken into consideration. The Langmuir model states that there are specific (active) sites on the adsorbent where the adsorbate (e.g. phosphate) binds. The amount of phosphate, which is bound at equilibrium, correlates to the concentration of unoccupied active sites and phosphate at equilibrium. The term of adsorption affinity is defined as the constant that relates adsorption capacity at very low phosphate concentration. This term is determined by the slope of the Langmuir adsorption curve as the concentration of phosphate tends to zero. As $C_e \rightarrow 0$ (and provided K_L is finite) in the Langmuir equation (see (3) eq.) and the equation becomes $Q_e = Q_{max} * K_L * C_e$. The term, $Q_{max} * K_L$, represents the slope of the initial part of the isotherm curve and denotes the adsorption affinity.

Phosphate adsorption with BioFeO is much slower comparing to other iron-rich adsorbents (Chitrakar, et al., 2006; Daou, et al., 2007). According to the Rentz et al. (2009) study, non-dried deposits of *Leptothrix* sp. have reached equilibrium in one day. In case of this research, the kinetics were even slower. Experiments with non-washed non-dried microbial mats of *Leptothrix* sp. E and grinded GEH have shown that adsorption equilibrium was reached in four days (see Fig. 13). Kinetics with MQ-washed microbial mats of *Leptothrix* sp. E were much slower and equilibrium was not reached in seven days. All the kinetics parameters are summarized in table 4. From an application point of view, it is not practical to run adsorption for more than a week, as a consequence, it was decided to perform all isotherm experiments for 4 days.

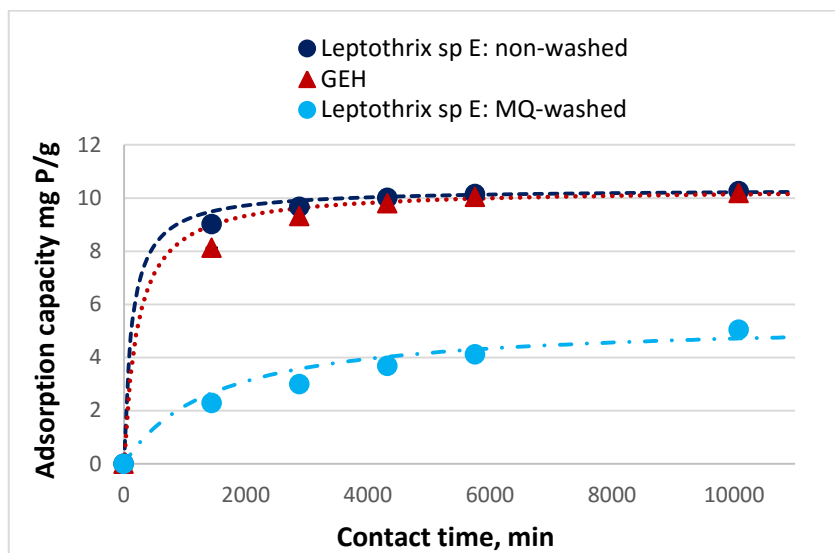


Fig. 13. The pseudo-second-order fitting of phosphate adsorption kinetics of *Leptothrix sp. E* and GEH during 7 days, mgP/g of dried solids

Table 4. Kinetic parameters for the adsorption of phosphate by BioFeO and GEH

Sample	C_0^1 , mgP/l	q_e , mgP/g	k , mgP/g min
<i>Leptothrix sp. E</i> non-washed	5	10.3 ± 0.0	$7.5 \times 10^{-4} \pm 1.1 \times 10^{-4}$
<i>Leptothrix sp. E</i> + MQ	5	5.4 ± 0.1	$1.3 \times 10^{-4} \pm 1.7 \times 10^{-5}$
GEH grinded, $\leq 150 \mu\text{m}$	5	10.4 ± 0.0	$4.3 \times 10^{-4} \pm 2.1 \times 10^{-6}$

¹Is the initial phosphate concentration

Figure 14 represents isotherms of microbial mats of *Leptothrix sp. E* with different treatment, where linear represent Langmuir model fitting and symbols – experimental values. Maximum phosphate adsorption (Q_{max}) of non-washed *Leptothrix sp. E* was 24.7 ± 0.2 mgP/g dried solids and it is in the 10.8-39.9 mgP/g dried solids range reported by Rentz et al. (2009). It would confirm the observation of Rentz et al. (2009), that BioFeO have high potential of usage as adsorbents for phosphate removal, but during experiment Fe release into solution was observed. In order to avoid the release of soluble Fe, biogenic iron oxides were washing with settling. The supernatant was decanted after 24 hours and total aqueous iron concentration was checked with FerroVer powder pillow, the step was repeated until aqueous iron concentration was close to zero. Microbial mats of *Leptothrix sp. E* were washed with MQ water and with SALINE solution (100 mM NaCl). SALINE solution was also used for isotherm experiments to check if disruption of cells, which is in close contact with the

structure of iron oxyhydroxides (Rentz, et al., 2009), affects adsorption. Previous research, which has evaluated the adsorption capacity of BioFeO (Rentz, et al., 2009), washed microbial mats with the SALINE solution just once, but our study has shown that washing of BioFeO (to remove soluble iron) is very time-consuming process. Soluble iron concentrations in the supernatant after decanting were up to 10.6 mg/l and 19.4 mg/l when microbial mats of *Leptothrix* sp. E were washed, respectively, with MQ water and with SALINE solution. As a consequence, the total iron content in deposits of *Leptothrix* sp. E was lower by 11.5% in comparison with deposits washed with MQ water. Observation of more intensive iron dissolution when microbial mats were washed with SALINE solution could be related with the effect of ionic strength of the NaCl solution. Chloride ions have high diffusivity, strong anionic nature and very high solubility of chloride salt (Asaduzzaman, Mustafa & Islam, 2011).

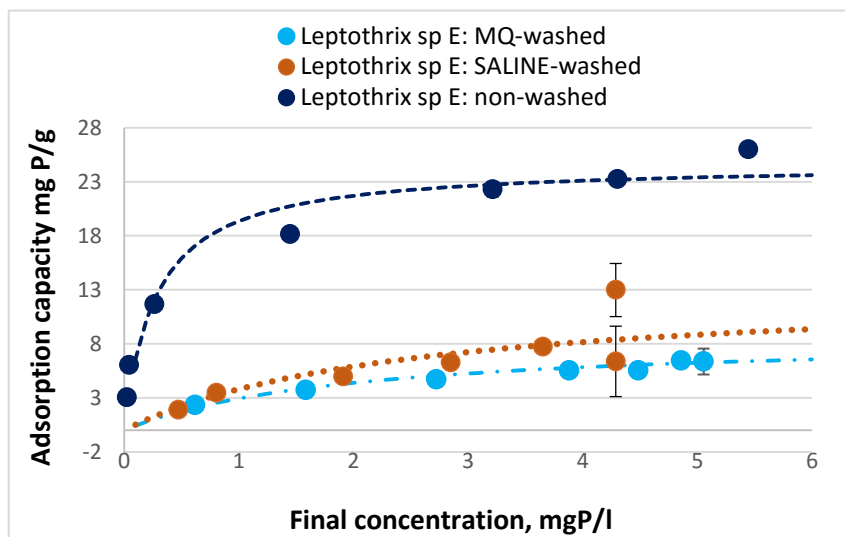


Fig. 14. Isotherms for phosphate adsorption to adsorbents after 4 days, mgP/g of dried solids

Q_{\max} (mgP/g dried solids) of non-washed *Leptothrix* sp. E was 1.9 and 2.8 times higher, respectively, with SALINE solution and MQ water washed microbial mats. Affinity was also better with non-washed microbial mats. Adsorption capacity was 34.1% higher, when microbial mats were washed with SALINE solution (13.2 ± 4.5 mgP/g d.s.) comparing with deposits washed with MQ water (8.7 ± 1.5 mgP/g d.s.). After repeating the isotherm experiments with washed microbial mats, the iron release was still observed and aqueous iron concentrations in the supernatant were up to 2.2 mg/l and 5.4 mg/l, respectively, with MQ water and with SALINE solution. It was decided for further adsorption experiments to use BioFeO washed with MQ water. Due to big Q_{\max} standard deviation of *Leptothrix* sp. washed with SALINE solution and more intensive dissolution of iron, which might cause higher adsorption.

Phosphate precipitates not only with soluble iron but also with calcium, zinc and aluminum (Koilaraj & Kannan, 2010; Khadhraoui, Watanabe & Kuroda, 2002). Precipitation with calcium, zinc or aluminum could not play a role in this case. As a consequence, it was concluded that determined high adsorption capacity of non-washed microbial mats of *Leptothrix* sp. E discussed above included the combination of phosphate removal from solution mechanisms – adsorption on BioFeO and surface precipitation with soluble iron (Loganathan, et al., 2014). When adsorption takes place, the phosphate adsorbed on the surface of the adsorbent can be released (recovered) and the adsorbent can be reused. But when precipitation takes place, a part of the adsorbent is lost and it cannot be reused. Furthermore, precipitation is not selective, therefore, the iron can precipitate not just with phosphate, but also with any other counterion of wastewater. Moreover, if precipitates are used to recover phosphate, it will have impurities and the recovered phosphate will not be pure. As a consequence, phosphate removal by precipitation is not convenient for the phosphorus recovery (Loganathan, et al., 2014; Li, 2000).

Figure 15 shows isotherms with MQ-washed microbial mats of *Leptothrix* sp. from iron-rich seepage areas in Earnewald, Beetsterzwaag and Lettelbert. All *Leptothrix* sp. samples shows the same pattern. Maximum phosphate adsorption of MQ-washed *Leptothrix* sp. deposits was in the 8.7-9.6 mgP/g dried solids range, which is approximately 2.7 times lower comparing with non-washed microbial mats (24.7 ± 0.2 mgP/g d.s.) and results of previous research, which have evaluated adsorption capacity of microbial mats of *Leptothrix* sp. (10.8-39.9 mgP/g d.s.) (Rentz, et al., 2009).

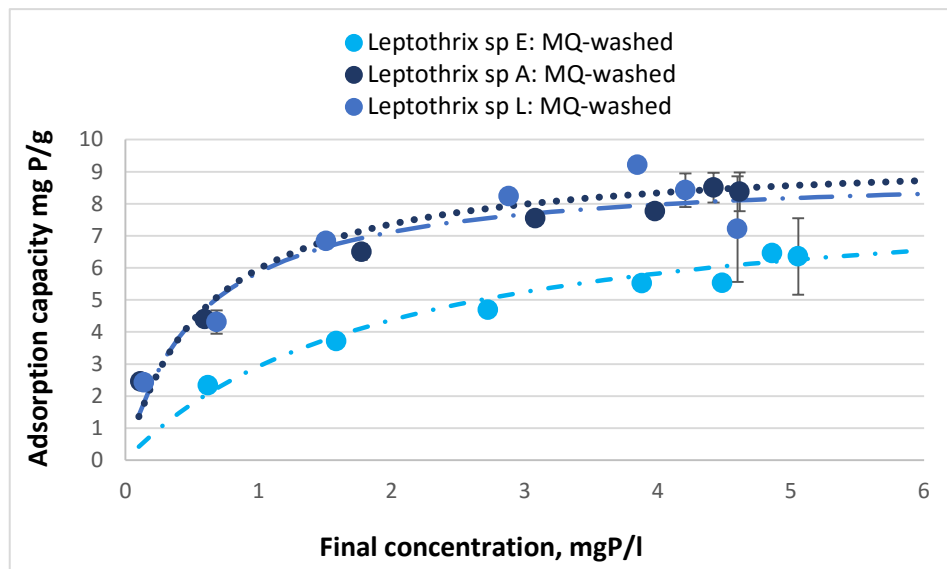


Fig. 15. Isotherms for phosphate adsorption to adsorbents after 4 days, mgP/g of dried solids

Figure 16 illustrates isotherms with MQ-washed microbial mats of *Leptothrix* sp. E and *Gallionella* sp. and GEH. Q_{\max} dried solids of GEH was 2.1 and 1.2 times higher comparing with *Leptothrix* sp. E and *Gallionella* sp. respectively and the affinity was better of GEH ($Q_{\max} * K_1$: 4.4-5.2 < 218.7). Experiments have shown that Q_{\max} phosphate adsorption capacity of *Gallionella* sp. stalks (14.7 ± 3.5 d.s.) is 40.8 % higher in comparison with *Leptothrix* sp. E sheaths (8.7 ± 1.5 d.s.). It is related to the higher content of iron in *Gallionella* sp. stalks, because Q_{\max} is 37.6 ± 9.0 mgP/g Fe for *Gallionella* sp. and 37.6 ± 6.3 mgP/g Fe for *Leptothrix* sp. E. Therefore, it was concluded that structure of biogenic iron oxides does not have a significant influence on affinity and adsorption capacity.

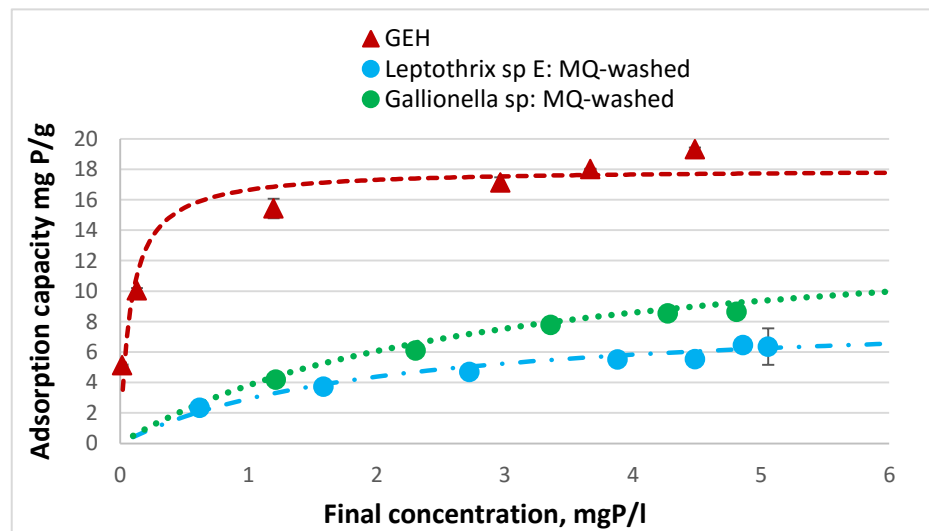


Fig. 16. Isotherms for phosphate adsorption to adsorbents after 4 days, mgP/g of dried solids

Goethite and hematite are one of the most abundant iron oxides in soils and sediments and has been used in various adsorption studies including studies of phosphate (Borggaard, et al., 2005). Microbial minerals widespread in natural systems are hematite and magnetite, oxyhydroxides – goethite, ferrihydrite, lepidocrocite and akageneite. BioFeO are usually characterized as amorphous or poorly crystalline (Fortin & Langley, 2005). Figure 17 shows phosphate adsorption capacities of different BioFeO and ChFeO. Maximum phosphate adsorption capacity of MQ-washed microbial mats of *Leptothrix* sp. and *Gallionella* sp. is in the 9.1-14.7 mgP/g dried solids range. Phosphate adsorption capacity of ChFeO such as akageneite (Kim, et al., 2011; Chitrakar, et al., 2006) also in this range, on the other hand Genz, et al. study (2004) shows much higher phosphate adsorption capacity for akageneite (23.3 mgP/g d.s.). According to the literature, phosphate adsorption capacity differs even within the same type of iron oxides and depends on the conditions of synthesis and

adsorption experiment (Wilfert, et al., 2015). High phosphate adsorption capacity of ferrihydrite is related to its amorphous nature, which provides higher surface site density leading to the higher adsorption. Well-crystalline iron (hydr)oxides such as hematite and goethite have much lower adsorption capacity in comparison to the amorphous oxides (Wang, et al., 2013).

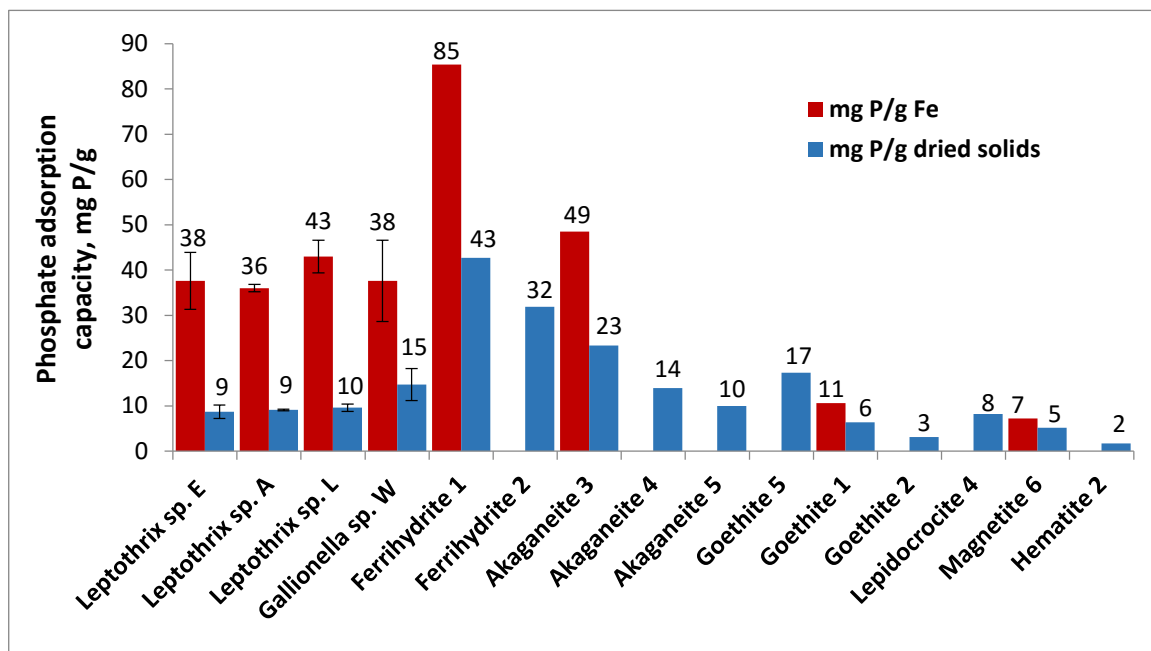


Fig. 17. Phosphate adsorption capacities of different BioFeO and ChFeO. First four adsorbents represent MQ washed BioFeO analyzed in this study. Values from: 1 – Borggaard, et al. (2005); 2 – Wang, et al. (2013); 3 – Genz, et al. (2004); 4 – Kim, et al. (2011); 5 – Chitrakar, et al. (2006); 6 – Daou, et al. (2007)

The phosphate adsorption capacity of BioFeO is in the 36.0-43.0 mgP/g Fe range, but it is hard to compare these values with the literature. Adsorption capacity is usually expressed in terms of adsorbent because it is an important parameter for designing filters. In order to evaluate the performance of iron-based adsorbents, adsorption efficiency normalized to iron content should be also included because iron content plays key-role in adsorption process (Rentz, et al., 2009).

According to the literature, phosphorus binding capacity in natural systems is up to 2.6 mgP/g soil, which is quite low (see Table 5). Especially deep sandy soils (< 0.03 mgP/g soil) have low phosphorus binding capacity while higher P adsorption capacity has soils and sediments richer in clay and iron and aluminum (hydr)oxides (Ritchie & Weaver, 1993). Phosphate adsorption capacity of microbial mats of *Leptothrix* sp. and *Gallionella* sp. is higher in comparison to the natural systems. Therefore, it is concluded that BioFeO play an important role in immobilizing phosphorus and

preventing its leakage from sediments of marine and freshwater ecosystems, groundwater, soil, wetlands and mine drainage systems, where FeOB occur.

Table 5. Phosphorus binding capacities in natural systems

Type of natural system	P binding capacity, mgP/g	Reference article
Deep sandy soils	< 0.03	Ritchie & Weaver, 1993
Loamy and clayey soils	0.15-0.8	
Red loamy soils	0.15-1.5	
Organic soils in wetlands	0.6	Leader, Dunne & Reddy, 2008
Ditch soils	0.5-0.6	Liu, et al., 2013
Paddy soils	0.2-1.9	Huang, Thompson & Zhang, 2014
Other soils	0.3-2.6	Pinto, et al., 2013
Lake sediments	0.03-1.2	Olila & Reddy, 1993

After isotherm experiments with MQ-washed microbial mats of *Leptothrix* sp. iron release into solution was observed. Dissolved Fe concentrations in the supernatant correlated well ($R^2=0.949-0.987$) with DOC, which indicates organic matter decomposition (see Fig. 18). Not all OM is mineralized, part of it is transformed into stable OM – humic compounds (fulvic and humic acids, humins), which is a portion of DOC (Stevenson, 1994). Organic-rich environments, where *Leptothrix* sp. normally occurs, such waters are rich in complex decaying OM. Humic substances can retain high concentrations of Fe(II) and Fe(III) in the solution despite circum-neutral pH or oxygen saturation (Dhungana & Crumbliss, 2005; Mayer & Jarrell, 2000). Chan et al. study (2009) have shown that microbial iron oxyhydroxides mainly contains acidic polysaccharides with carboxyl functional groups, which also strongly bind dissolved iron (Chan, et al., 2009). The oxidation of OM in marine sediments releases HCO_3^- , NH_4^+ , HPO_4^{2-} also Mn^{2+} , Fe^{2+} and other ions can be produced (Posth, et al., 2014), because of phosphate released from OM mineralization, as a consequence, the final adsorption capacity could be underestimated. HA-Fe complexes can bind phosphate (Mayer & Jarrell, 2000). Further experiments have shown that HA-Fe complexes formed during isotherm experiments with MQ-washed microbial mats of *Leptothrix* sp. have not participated in phosphate adsorption, because concentration of total phosphorus was the same as phosphate, but it could play a role in the adsorption performed with non-washed microbial mats. No iron release was observed after isotherm experiments with *Gallionella* sp. stalks, but it cannot indicate that *Leptothrix* sp. stalks are more

susceptible to decomposition because of samples differences. Samples with *Leptothrix* sp. sheats were fresh environmental samples while the sample with microbial mats of *Gallionella* sp. was from the cultivation in the experimental set-up and few years old, therefore, it can be stated that at least stalks of *Gallionella* sp. have a stable structure.

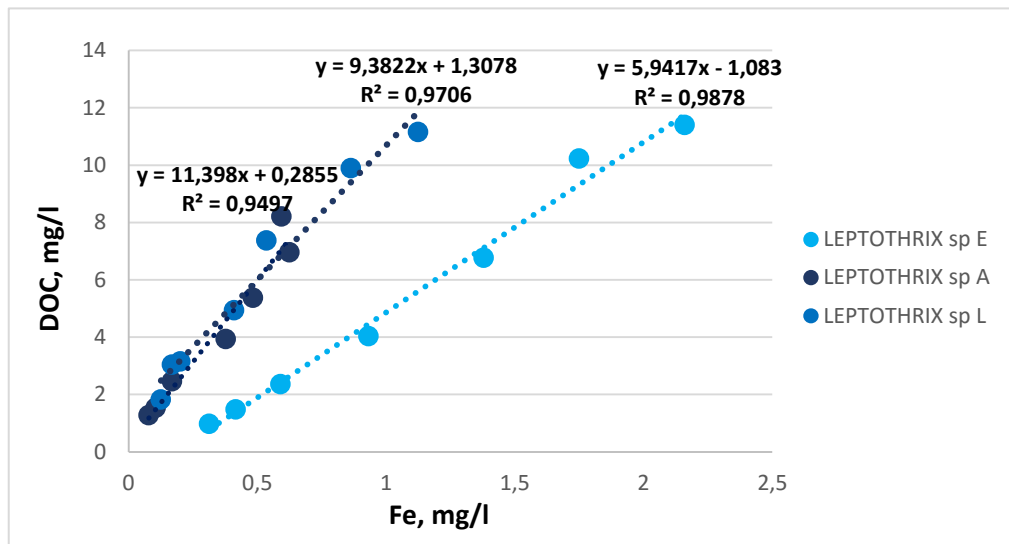


Fig. 18. Correlation of DOC and Fe concentrations in the supernatant after isotherms with microbial mats of *Leptothrix* sp.

Organic matter plays a significant role in nutrients and trace elements cycling in the environment (Stevenson, 1994). The previous study, which has shown that microbial mats of *Leptothrix* sp. have very high adsorption capacity for phosphate have not evaluated iron dissolution effect on adsorption and the role of organic matter, which can contain up to 50% of microbial iron oxyhydroxides. Wilfert et al. (2015) discussed complex effect of humic substances on iron and phosphorus interaction, which results in increase or decrease of phosphate adsorption capacity. Exopolymeric substances of BioFeO mainly possess carboxylic, phosphoric and amino groups. BioFeO play an important role in cycling nutrients, heavy metals, radionuclides and other contaminants in natural systems. In order to be able to make predictions about the bioavailability and transport of nutrients and contaminants in natural systems and also about their behavior in engineered systems a detailed knowledge is required about the influence of exopolymeric substances of BioFeO on interaction with Fe (e.g. type of bond and strength), which determines sorption mechanisms. Soluble Fe(II) and Fe(III) resulting from the BioFeO decomposition might precipitate with phosphate and result in high phosphate removal capacity, but to recover phosphorus from products formed during precipitation is not economically beneficial and low prospects for reusability (Loganathan, et al., 2014; Li, 2000). As

a consequence, it is concluded that microbial mats of *Leptothrix* sp. were overrated for its application as adsorbents for phosphorus removal in WWTP.

One of the engineering applications of BioFeO should be related to the drinking water preparation. Literature sources provide the information on chemical behavior of AsO_4 is very similar to phosphate, because of similar structure and reactivity and as a consequence, have similar adsorption mechanisms (Violante & Pigna, 2002; Antelo, Avena, Fiol, López & Arce, 2005). Arsenate is toxic and carcinogenic. For this reason, high concentrations in groundwaters affect a large number of people. This problem is worldwide, especially relevant for Southeast Asia (Bangladesh, Vietnam, Nepal, India, Bengal, Cambodia, Taiwan, Thailand), China, USA, Canada and for Europe as well (Romania, Hungary, Croatia, Greece, Spain, Finland, Germany) (Katsoyiannis, et al., 2008; Pokhrel & Viraraghavan, 2009; Katsoyiannis, et al., 2002; Smedley & Kinniburgh, 2002). Biological groundwater treatment combined with physicochemical methods could have many advantages: more efficient and faster removal of Fe, Mn, As, Cr, U and Se, lowered addition of chemicals, easier adsorbent regeneration (backwash) and lower operation of WTP costs (Katsoyiannis et al., 2002; Katsoyiannis & Zouboulis, 2006). The formation of BioFeO lowers As removal efficiency from groundwater possibly due to the repulsion of negatively charged biogenic iron minerals (Karlsson & Persson, 2012), while other studies show an increase in As removal efficiency because of microbial activity (Katsoyiannis, et al., 2008; Katsoyiannis et al., 2002). Controversial results show the necessity for more deep understanding of BioFeO biogeochemistry. Application of FeOB has high usage potential not only for groundwater (Katsoyiannis, et al., 2008; Pokhrel & Viraraghavan, 2009; Katsoyiannis, et al., 2002) but also for WWTP, mining effluent treatment (Ahoranta, Kokko, Papirio, Ozkaya & Puhakka, 2016) and surface water bioremediation (Bai, et al., 2016), because the mechanism of removal (adsorption or/and precipitation) is not very important factor until heavy metals, radionuclides and other contaminants are efficiently removed (excluding the cases when recovery is desired). But these applications require studies about the reactivity of functional groups, dissolution mechanisms and long-term stability properties of formed products. FeOB is also relevant for various research disciplines – for controlling nutrients, heavy metals and radionuclides in the natural and engineered systems (Emerson & de Vet, 2015). Therefore, it is believed that complex biogeochemistry of BioFeO will be a focus for future studies.

CONCLUSIONS

1. Formation of BioFeO adsorbents in the set-up could be restricted by groundwater pre-oxidation and/or by limiting macronutrients and trace elements. This information can be useful in solving biofouling problems.
2. MQ-washed microbial iron oxyhydroxides of *Leptothrix* sp. have much slower kinetics, equilibrium have not reached in seven days. Non-washed microbial iron oxyhydroxides of *Leptothrix* sp. and GEH have reached equilibrium in four days. Faster kinetics are related to the surface precipitation of phosphate with soluble iron.
3. Unwashed microbial mats of *Leptothrix* sp. have lower affinity, but 27.1% higher phosphate removal capacity in comparison to GEH. Determined high adsorption capacity is related to the combination of phosphate removal from solution mechanisms – adsorption on BioFeO and surface precipitation with soluble iron. MQ-washed microbial iron oxyhydroxides of *Gallionella* sp. and *Leptothrix* sp. have lower affinity and respectively 1.2 and 2.1 times lower adsorption capacity than GEH.
4. Stalks of *Gallionella* sp. and *Leptothrix* sp. have different structures, but it does not have an influence on affinity and adsorption capacity. Iron content plays key-role on phosphate adsorption. Microbial iron oxyhydroxides can contain up to 50% of OM, while synthetic FeO are pure iron oxyhydroxides.
5. Microbial mats of *Leptothrix* sp. have restrictions for its application as adsorbents for phosphorus removal in WWTP, but FeOB are relevant for various research disciplines – for controlling phosphorus, heavy metals and other substances in the natural and engineered systems. In order to be able to make predictions about the bioavailability and transport of nutrients and contaminants a detailed knowledge is required about the influence of exopolymeric substances of BioFeO on interaction with Fe (e.g. type of bond and strength), which determines sorption mechanisms.

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APPENDIX. ISOTHERMS CONSTANTS FOR THE ADSORPTION OF PHOSPHATE BY BioFeO AND GEH

Sample	Langmuir isotherms					Freundlich isotherms			
	Q_{\max} , mgP/g dried solids	Q_{\max} , mgP/g Fe	K_L , L/mgP	$Q_{\max} * K_L$, L/g	RMSE	K_f dried solids	K_{fFe}	n	RMSE
<i>Leptothrix sp.</i> , Rentz et al., 2009 non-dried	10.8-39.9	46.9-165.0	-	-	-	5.9-8.2	23.7-35.8	1.8-4.2	-
<i>Leptothrix sp. E</i> non-washed non-dried	24.7 ± 0.2	109.6 ± 1.0	3.6 ± 0.1	89.3 ± 3.7	1.7 ± 0.0	15.5 ± 0.0	68.7 ± 0.2	3.3 ± 0.0	1.1 ± 0.1
<i>Leptothrix sp. E</i> + MQ non-dried	8.7 ± 1.5	37.6 ± 6.3	0.5 ± 0.2	4.4 ± 1.6	0.4 ± 0.1	2.9 ± 0.1	12.7 ± 0.5	2.1 ± 0.3	0.3 ± 0.0
<i>Leptothrix sp. E</i> + SALINE non- dried	13.2 ± 4.5	64.6 ± 21.9	0.4 ± 0.2	5.3 ± 2.9	2.1 ± 0.2	3.1 ± 0.6	15.2 ± 2.7	1.3 ± 0.5	1.9 ± 0.0
<i>Leptothrix sp. A</i> + MQ non-dried	9.1 ± 0.2	36.0 ± 0.8	1.8 ± 0.2	16.6 ± 2.0	0.5 ± 0.0	5.2 ± 0.1	20.6 ± 0.3	3.1 ± 0.1	0.3 ± 0.1
<i>Leptothrix sp. L</i> + MQ non-dried	9.6 ± 0.8	43.0 ± 3.6	1.6 ± 0.5	15.8 ± 5.2	0.8 ± 0.3	5.4 ± 0.1	24.1 ± 0.6	3.1 ± 0.4	0.9 ± 0.4
<i>Gallionella sp.</i> W + MQ dried	14.7 ± 3.5	37.6 ± 9.0	0.4 ± 0.2	5.2 ± 2.5	0.4 ± 0.2	3.9 ± 0.3	10.0 ± 0.9	1.9 ± 0.4	0.4 ± 0.3
GEH grinded, ≤150 μm	18.0 ± 0.3	31.8 ± 0.5	12.1 ± 0.8	218.7 ± 15.1	1.3 ± 0.2	14.1 ± 0.1	24.9 ± 0.2	4.9 ± 0.0	0.7 ± 0.1

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