Evaluation of pre-treatment technologies for phosphorous removal from drinking water to mitigate membrane biofouling

M Frolova, K Tihomirova, L Mežule, J Rubulis, K Gruškeviča and T Juhna
Riga Technical University, Faculty of Civil Engineering, Research Centre for Civil Engineering, Water Research Laboratory, Kipsalas Str. 6a-263, Riga, Latvia
E-mail: Marta.Frolova@rtu.lv

Abstract. Membranes are widely used for the treatment of various solutions. However, membrane fouling remains the limiting factor for their usage, setting biofouling as the most severe type of it. Therefore, the production of biologically stable water prior to membranes is important. Since lack of phosphorus may hinder the growth of microorganisms, the aim of this research is to evaluate the effect of microbially available phosphorus (MAP) removal via affordable water pre-treatment methods (adsorption, biofiltration, electrocoagulation) on bacterial growth. Four cylindrical reactors were installed at an artificially recharged groundwater station. Further temperature influence and carbon limitation were tested for biofiltration technology. The amount of MAP and total cell count was measured by flow cytometry. The results showed that at lower temperatures electrocoagulation performed the best, resulting in complete MAP removal (detection limit $6.27 \times 10^{-3} \mu g P l^{-1}$). Sorbent demonstrated MAP removal of 70-90%. Biomass did not have any noteworthy results at $+8^\circ C$, however, at $+19^\circ C$ MAP removal of around 80% was achieved. Main conclusions obtained within this study are: (i) tested technologies effectively eliminate MAP levels; (ii) temperature has a significant effect on MAP removal in a bioreactor, (iii) multi-barrier approach might be necessary for better P limitation that might prolong operating time of a membrane.

1. Introduction
Membrane fouling is a limiting factor to their usage, leading to increased energy consumption and decreased treated water production. The most severe type of fouling is attributed to biofouling, as even almost complete microorganism removal is not sufficient due to their ability to multiply over time [1].

To contribute to microbial growth, a certain amount of nutrients is required. It is stated that optimal molar ratio of C:N:P is 100:10:1, respectively [2]. Furthermore, phosphorus (P) is considered to be the limiting nutrient in water with high humic content or in boreal regions, e.g., northern Europe, Russia, and North America [3], as natural waters have high organic carbon content. Van Nevel et al. [4] suggest that either extensive carbon (C) or P limitation can be used in water treatment design in order to obtain a drinking water, which is not susceptible to unwanted bacterial growth. As organic membranes might be a source of C themselves, that would make P a limiting nutrient, as C would always be in excess.

In order to remove P, a feed water pre-treatment might be implemented. Furthermore, a pre-treatment method needs to be affordable, so it could eliminate water production costs and also be used in developing countries. Chemical coagulation is one of the most cost-effective membrane pre-treatment techniques [5]. However, electrocoagulation might achieve better results [6]. It is a technology that
combines conventional coagulation and flocculation and electrochemistry. If compared to conventional chemical coagulation, electrocoagulation produces lower amounts of sludge, reduces the direct handling of corrosive chemicals, does not consume the natural buffering capacity (alkalinity) of the water and can be easily employed in portable water purification units [7–9]. Gamage et al. [9] suggest the use of aluminium electrocoagulation pre-treatment as an efficient membrane fouling reduction tool. Lacasa et al. [10] state that electrocoagulation is able to remove P below the detection limit of ionic chromatography (0.1 mg l\(^{-1}\)). Vasudevan et al. [11] have achieved the reduction of P by 99% that results in PO\(_4\)-P decrease down to 1 mg l\(^{-1}\). The removal of microbially available phosphorus (MAP) by conventional coagulation technology reaches 28-76% [12], depending on the season. However, the information about MAP removal by this technology is very limited, thus making it impossible for the results to be compared.

Another option might be the use of biological processes. The capital and operating costs of biological treatment technologies are 5-20 and 3-20 times cheaper than chemical processes, respectively [13], making such technologies more affordable. Moreover, biological processes additionally save on sludge handling, since there is no need for chemical removal. Pramanik [14] has shown that microorganisms are able to remove readily biodegradable organic matter, which limits the growth of biofilm on the membrane surface. However, biodegradation of organic molecules depends on their molecular size [15]. Hallé et al. [16] have found that biofilter with a longer contact time can reduce both the hydraulically reversible and irreversible fouling. Concerning P removal, most studies focus on wastewater, where microbial biofilters are used, or urban stormwater runoff treatment, where plant-based biofilters are applied. The use of biofilters for drinking water production and microbially available P limitation still lacks attention.

As another promising low-cost solution, biosorption might be applied. It is a process, where contaminants are adsorbed on materials of biological origin, such as agricultural or industrial waste, microbial biomass and their derivatives for the treatment of aqueous solutions [17]. The mechanisms involved in the biosorption processes are following: physisorption, chemisorption, ion exchange and microprecipitation [18]. Most studies are dedicated to metal ions removal by biosorption [17,19]. Nutrients limitation by biosorption is mostly attributed to waste water studies; however, there are some studies available in respect to stormwater treatment from such nutrients as phosphorus and nitrogen, reporting 70–90% reduction in total dissolved P [20].

The aim of this study is to evaluate the effect of MAP removal by three affordable water pre-treatment methods, which incorporate such processes as biosorption, biofiltration and electrocoagulation.

2. Materials and methods

2.1. Experimental setup

A set of two experiments was performed to determine the MAP removal efficiencies for different pre-treatment methods. At first, pre-treatment methods were tested for MAP removal at natural groundwater temperature in a plug-flow configuration. The experiment was performed directly at a groundwater extraction site. Further, the influence of temperature and possible limitation of C rather than P were tested at elevated temperature in a recirculation mode (figure 1). To ensure constant temperature for recirculation mode, experiment was performed in a climatic chamber.

Plug-flow configuration setup consisted of four cylinders (custom-made from polyvinylchloride (PVC) pipes and metal fittings with following dimensions: diameter=7.5cm, length=50cm). The volume of each reactor was from 1.3 to 1.8 liters, depending on the treatment technology. The water flow through reactors was set on 65 ml min\(^{-1}\) (peristaltic pump MasterFlex 77202-50, Cole-Parmer Instrument Co., USA), thus, the contact time in each reactor was around 20 minutes. The experiment was carried out for 17 days (408 hours) and water samples were taken three times per week, thus the MAP removal effectiveness from each reactor was measured nine times.
Three of the reactors were filled with tested materials and one was left without any material to assure that there would be no MAP background coming from the materials used. The MAP concentration from effluent of the empty reactor in the results section is denoted as “0-out”. The results of empty column are compared with influent by T-test and statistically significant difference is established.

Biosorption reactor was filled with commercially produced plastic biomass carriers Bioflow 9 (RVT Process Equipment, Germany). Prior to the experiment, carriers were covered with ferric oxides, naturally produced during iron removal process from groundwater. Results of biosorption technology are further marked as “Sorbent-out”.

Biofiltration reactor was filled with same plastic carriers Bioflow 9, covered with biomass. The biomass on the carriers was grown for one month, using river and drinking water mixture [21]. Briefly, 1/3 of river water from the Daugava River filtered through 1.2µm pore filter (Millipore, Germany) was mixed with 2/3 of tap water coming from Riga city water supply. The water mixture was filled into sterile glass bottles and biomass carriers were placed into the bottles covered with aluminium foil to avoid algae growth. The bottles were placed on the orbital shaker and water was changed every week. Plastic carriers were filled into the reactor shortly before experiment. MAP concentration in the effluent of this reactor is further denoted as “Biomass-out”.

Electrocoagulation reactor was equipped with four 60/60 grade aluminium electrodes that were cylindrical in shape with a diameter of 2cm. A direct current of 2.5A was ensured with laboratory power supply unit (EA-PS 2084-10B, Elektro-Automatik, Germany). The distance between electrodes was 10cm and the current was applied in monopolar-parallel connection. A surface area of all electrodes was 622cm², thus providing current density of 4mA/cm². The results are further marked as “EC-out”.

Recirculation mode configuration setup consisted of similar two reactors as described above. Both columns were filled with biomass carries, the only difference from previous description here was that previously used amount of filter media was divided evenly for two columns, resulting in a slightly larger water volume in each reactor. The water flow through each column was 60ml min⁻¹ and contact time was around 25min. A total duration of experiment was 45.5 hours. During this time, six samples were taken from each column and MAP was analysed.

As a feed, artificially recharged groundwater (Riga, Latvia) was used in both configurations. In the experiment operated in recirculation mode, feed water for one column was enriched with sodium acetate.
(CH$_3$COONa) as a carbon source to reach an additional concentration of 2mg C l$^{-1}$ (around 100 times more than P concentration in the feed water) to test possible C limitation. Additionally, it was performed under elevated temperature conditions to test the temperature limitation. Water quality was monitored at the common intake for all reactors in a plug-flow mode experiment and at the sampling point in a recirculation loop in the other mode (figure 1). The main parameters are summarised in table 1.

**Table 1.** Inlet water quality: a) operation in a plug-flow mode, b) operation in a recirculation mode.

| Parameter, unit | Average | Counts |
|-----------------|---------|--------|
|                 |         | a      | b      | a      | b      |
| $t_{room}$ (°C) | 10.9±0.8| 21.0   | 9      | 12     |
| $t_{water}$ (°C) | 8.1±0.1 | 19.3±0.4 | 5   | 12     |
| Turbidity (NTU) | 0.82±0.25 | -  | 5      | -      |
| pH              | 8.04±0.32 | 8.11±0.31 | 5   | 12     |
| ORP (mV)        | 41.4±50.3 | 68.5±51.6 | 5   | 12     |
| EVS (µS cm$^{-1}$) | 910±11.6 | 968±11.3 | 5   | 12     |
| Fe$_{tot}$ (mg l$^{-1}$) | 0.29±0.04 | -  | 2      | -      |
| Fe II (mg l$^{-1}$) | 0.07 | -  | 1      | -      |
| O$_2$(mg l$^{-1}$) | -       | 9.14±0.22 | -  | 12     |

2.2. MAP determination

2.2.1. Inoculum preparation. All glassware and plastic caps were washed in the dishwasher with phosphate-free detergents and further glassware was muffle d in the furnace at 500°C for three hours, while plastic caps were autoclaved at 121°C for 20min. *Pseudomonas brenneri* P17 (ATCC 49642) was used as inoculum. At first the cells were grown in liquid R2A medium. Afterwards they were washed and inoculated into 0.1µm filtered Evian (Danone, France) water with added CH$_3$COONa as a carbon source (final added concentration 1mg C l$^{-1}$). All samples were incubated at 30°C on an orbital shaker (150RPM) for 24hours.

2.2.2. Preparation of samples for MAP tests. Salts and acetate stock solutions were added to samples in excess to ensure that P would be the only limiting nutrient. Salts stock consisted of NH$_4$NO$_3$, MgSO$_4$·7H$_2$O, CaCl$_2$·2H$_2$O, KCl and NaCl suspension in deionized water, resulting in added concentrations of 250µg N l$^{-1}$, 10µg Mg l$^{-1}$, 27µg Ca l$^{-1}$, 53µg K l$^{-1}$ and 40µg Na l$^{-1}$ per sample. Acetate (CH$_3$COONa) stock solution was used as additional carbon source to reach a concentration of 2mg C l$^{-1}$ added into the sample. Afterwards samples were pasteurized in a heated water bath at 60°C for 50min, further cooled to the room temperature and inoculated with *P. brenneri* cells to reach the concentration of around 10$^3$ TCC ml$^{-1}$ (total cell count). Finally, the samples were kept in a shaker-incubator at 30°C and 150RPM. All samples were prepared in triplicates to ensure higher precision.

2.2.3. Measurements. Total cell count (TCC) was measured using flow cytometer (FCM) PartecCyFlow® SL (Partec, Germany) by method described previously [22,23]. The CyFlow SL instrument has a quantification limit of 1000 cells ml$^{-1}$ [22]. Briefly, 1ml of sample was heated for at least 3min at 35°C. Afterwards, 10µl of SYBR Green I (Invitrogen, Switzerland) and 10µl of EDTA solution was added and left to stain for 10min at 35°C in the dark before measurement. Prior to measurements, samples were diluted with 0.1µm filtered Evian in order not to exceed instrumental detection limit. Instrument settings were set as described by Nescerecka et al. [24] and further converted into MAP after reaching steady state of bacterial growth on day four or five. The conversion factor was
used to transform TCC into MAP, dividing TCC by 159494 [25], which means that 1µg of PO₄-P corresponds to 1.59x10⁸ cells of P. brenneri. Based on the added sodium acetate concentration, this conversion gives the absolute values for the range of 0–10µg MAP l⁻¹, as determined by Lethola et al. [26]. The detection limit is dependent on the precision of FCM and for the current calibration it is 6.27x10⁻³µg P l⁻¹.

3. Results and Discussion

3.1. Efficiency of MAP removal

A comparison of MAP removal technologies at natural groundwater temperature (figure 2) presented a complete MAP removal by electrocoagulation, starting from the second week of experiment.

At the first day, when the samples were taken directly after switching-on the equipment, an ‘empty’ column showed almost 80% (table 2) MAP removal, leading to some adsorption in the very beginning. On day 3 this column showed higher values if compared to the rest; that might indicate to some release of MAP accumulated on the first day. On day 9 a small adsorption might be distinguished that amounts to 20% MAP removal by column itself. Although there were some fluctuations within the ‘empty’ column, the release or adsorption of MAP by columns themselves could be assumed negligible, as results from both columns (“IN” and “0-out”) displayed no significant difference (p>0.2). Comparing “IN” results with those obtained from groundwaters in Finland, it can be concluded that 30% fluctuation in MAP results is insignificant [27].

![Figure 2. The amount of MAP entering and exiting pre-treatment reactors.](image)

Although an ‘empty’ column itself showed a rather high MAP adsorption directly after starting, all other techniques displayed a decreased efficiency during that time, indicating to inefficient operation during start-up. Notably, electrocoagulation showed relatively the highest removal during the start-up, as chemical methods do not require adaptation. Although electrocoagulation has short start-up time, it requires periodical replacement of electrodes, and it may cause the formation of some toxic chlorinated organic compounds in situ in case of the removal of organic compounds if chlorides are also present [8].

A fairly good MAP removal was achieved also by the column containing ferric-oxide coated biocarriers, having an average removal rate of around 80%. However, one of limiting factors of its usage is the need to replace it after sorption capacity would be reached. Furthermore, at the same conditions biomass column achieved the poorest performance, providing overall around 20% of MAP removal. The reasons for that might have been the different nutrient, rather than P, or temperature limitation. Therefore, an additional test was performed (section 3.2.).
Table 2. The elimination of MAP by pre-treatment methods (detection limit 6.27x10^{-3}\mu g P l^{-1}).

| Pre-treatment method/ Day of experiment | Elimination of MAP, % |
|----------------------------------------|-----------------------|
|                                        | 1  2    3    8    10   15   16   17 |
| Sorbent-out                            | 41 75 87 86  -1  72  81  81 |
| Biomass-out                            | 7 35 17 42  59  20  14  13 |
| EC-out                                 | 76 n/a n/a 98 100 100 100 100 |

3.2. Temperature and additional carbon influence on biological MAP removal

A two-day experiment was performed to test the effect of temperature and possible limitation of C on biomass growth, instead of P. The artificially recharged groundwater with and without additional carbon source was used as feed water in recirculation mode.

Results showed that there was no significant difference in terms of MAP removal between both columns – with or without added carbon source. That denied the possibility of C limitation, leaving P a limiting nutrient in the used groundwater.

Comparing gained results with biomass column from previous section (figure 3), a great difference can be seen. The water temperature of +8°C was not sufficient for biomass to perform efficiently. Higher temperature leads to around 80% MAP elimination. That might be linked to both, bacterial communities and time, as in lower temperatures microorganisms would grow slower than in greater and the microbial community of river water, that was grown on biocarriers, would have different species acclimatized to different temperatures. Moreover, it is stated that biological treatment methods provide a greater biopolymer removal at temperatures higher than 15°C due to higher enzyme activities and higher uptake rates of organics by the microorganisms [16,28]. Therefore, water temperatures above 15°C are more favourable for the use of biological technologies. Although biofiltration states to be a promising technology, it requires longer start-up time due to adaptation of pre-cultured microorganisms or in-situ microbial growth that can take up to several weeks or months. However, it is a promising technology to provide a particular nutrient limited water, as they would consume nutrients for their metabolism.

Figure 3. The amount of MAP after treatment of artificially recharged groundwater in recirculation mode by biological processes.

Legends: “with C, Tw.=19°C” – MAP concentration after biomass column in recirculation mode with additional carbon source, water temperature +19°C, “without C, Tw.=19°C” – MAP concentration after biomass column in recirculation mode, water temperature +19°C, “without C, Tw.=8°C” – MAP concentration after biomass column in flow-through mode, water temperature +8°C (results of previously stated “Biomass-out”). Values of MAP in the range of 0-10\mu g P l^{-1} can be interpreted in their absolute values, higher results are compared as the relative values towards each other.

3.3. Membrane biofouling mitigation

MAP limitation was used as a measure of membrane biofouling mitigation. Miettinen et al. [3] stated that the concentration of P needed to stimulate microbial growth was as low as 1\mu g P l^{-1}. All tested
methods showed MAP removal. Electrocoagulation generated the best results at lower water temperatures (up to 100% removal). Such removal corresponded to P elimination down to detection limits of measuring equipment [10] or almost complete removal [11] achieved by other researchers. The use of ferric oxide is also suitable at lower temperatures, reaching the reduction down to 2.5µg P l⁻¹. Temperature proved to be an important factor in the application of biomass as at +19°C the MAP removal was 80%, reaching reduction down to 1.7µg P l⁻¹. However, at +8°C it was on average only around 25%. Although all technologies showed reduction of MAP, only electrocoagulation achieved the results below 1µg P l⁻¹. However, the complete MAP removal by electrocoagulation might be slightly questionable, as the samples contained remaining aluminium flocs that could continue sorption not only of elements already contained in the samples but also of organisms added by inoculation [29].

4. Conclusions
Biosorption, biofiltration and electrocoagulation were examined on MAP removal from artificially recharged groundwater. Although the measurements were performed within MAP conversion range of 1–10µg P l⁻¹ and appeared to exceed it, they are still comparable on relative basis. However, the conversion range needs to be widened for further analysis of same water source.

Biosorption and electrocoagulation can be used as pre-treatment techniques in the cold-climate countries, leading to 70-80% and up to 100% MAP removal, respectively. Biofiltration is more suitable for warmer places, leading to 80% MAP removal at elevated temperature; however, a longer start-up time must be taken into account.

To ensure more efficient MAP elimination, a multi-barrier approach might be more effective and needs further evaluation.

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References
[1] Nguyen T, Roddick F A and Fan L 2012 Biofouling of water treatment membranes: A review of the underlying causes, monitoring techniques and control measures Membranes (Basel). 2 804–40
[2] Chandy J P and Angles M L 2001 Determination of nutrients limiting biofilm formation and the subsequent impact on disinfectant decay Water Res. 35 2677–82
[3] Miettinen I T, Vartiainen T and Martikainen P J 1997 Phosphorus and bacterial growth in drinking water Appl. Environ. Microbiol. 63 3242–5
[4] Van Nevel S, De Roy K and Boon N 2013 Bacterial invasion potential in water is determined by nutrient availability and the indigenous community FEMS Microbiol. Ecol. 85 593–603
[5] Arhin S G, Banadda N, Komakech A J, Kabenge I and Wanyama J 2016 Membrane fouling control in low pressure membranes: A review on pretreatment techniques for fouling abatement Environ. Eng. Res. 21 109–20
[6] Zaleschi L and Teodosiu C 2012 A comparative study of electrocoagulation and chemical coagulation processes applied for wastewater treatment
[7] Holt P K, Barton G W and Mitchell C A 2005 The future for electrocoagulation as a localised water treatment technology Chemosphere 59 355–67
[8] Mollah M Y A, Morkovsky P, Gomes J A G, Kesmez M, Parga J and Cocke D L 2004 Fundamentals, present and future perspectives of electrocoagulation J. Hazard. Mater. 114 199–210
[9] Gamage N P and Chellam S 2011 Aluminum electrocoagulation pretreatment reduces fouling
during surface water microfiltration. J. Memb. Sci. 379 97–105
[10] Lacasa E, Ca P, Sáez C, Fernández F J and Rodrigo M A 2011 Electrochemical phosphates removal using iron and aluminium electrodes 172 137–43
[11] Vasudevan S, Lakshmi J, Jayaraj J and Sozhan G 2009 Remediation of phosphate-contaminated water by electrocoagulation with aluminium, aluminium alloy and mild steel anodes 164 1480–6
[12] Jiang D, Chen Y and Ni G 2012 Phosphorus in drinking water and it’s removal in conventional treatment process 461 453–6
[13] Marco A, Esplugas S and Saum G 1997 How and why combine chemical and biological processes for wastewater treatment Water Sci. Technol. 35 321–7
[14] Pramanik B K 2015 Biological pre-treatment to enhance low pressure membrane filtration for wastewater reclamation (Malaysia: RMIT University)
[15] Leisinger T 1981 Microbial degradation of xenobiotics and recalcitrant compounds (London: Academic Press for the Swiss Academy of Sciences and the Swiss Society of Microbiology)
[16] Hallé C, Huck P M, Peldszus S, Haberkamp J and Jekel M 2009 Assessing the performance of biological filtration as pretreatment to low pressure membranes for drinking water Environ. Sci. Technol. 43 3878–84
[17] Gaur N, Flora G, Yadav M and Tiwari A 2014 A review with recent advancements on bioremediation-based abolition of heavy metals. Environ. Sci. Process. Impacts 16 180–93
[18] Robalda A, Naja G M and Klavins M 2016 Highlighting inconsistencies regarding metal biosorption J. Hazard. Mater. 304 553–6
[19] Ayangbenro A and Babalola O 2017 A new strategy for heavy metal polluted environments: A review of microbial biosorbents Int. J. Environ. Res. Public Health 14 94
[20] O’Reilly A M, Wanielista M P, Chang N Bin, Xuan Z and Harris W G 2012 Nutrient removal using biosorption activated media: Preliminary biogeochemical assessment of an innovative stormwater infiltration basin Sci. Total Environ. 432 227–42
[21] Tihomirova K 2011 Natural Organic Matter Removal from Water and Its Influence on the Water Quality in Distribution Network (Riga: RTU)
[22] Hammes F, Berney M, Wang Y, Vital M, Köster O and Egli T 2008 Flow-cytometric total bacterial cell counts as a descriptive microbiological parameter for drinking water treatment processes Water Res. 42 269–77
[23] Prest E I, Hammes F, Kötzsch S, van Loosdrecht M C M and Vrouwenvelder J S 2013 Monitoring microbiological changes in drinking water systems using a fast and reproducible flow cytometric method Water Res. 47 7131–42
[24] Neserecka A, Rubulis J, Vital M, Juhna T and Hammes F 2014 Biological instability in a chlorinated drinking water distribution network PLoS One 9 1–11
[25] Frolova M, Zemītis J, Tihomirova K, Mežule L, Rubulis J, Gruškeviča K and Juhna T 2017 Approximation of microbially available phosphorus (MAP) determination method by flow cytometry Environment.Technology.Resources. Proc. of the 11th Int. Scientific and Practical Conf. 1 89–92
[26] Lehtola M J, Miettinen I T, Vartiainen T and Martikainen P J 1999 A new sensitive bioassay for determination of microbially available phosphorus in water Appl. Environ. Microbiol. 65 5–8
[27] Lehtola M J, Miettinen I T, Vartiainen T and Martikainen P J 2002 Changes in content of microbially available phosphorus, assimilable organic carbon and microbial growth potential during drinking water treatment processes Water Res. 36 3681–90
[28] Zheng X, Ernst M and Jekel M 2010 Pilot-scale investigation on the removal of organic foulants in secondary effluent by slow sand filtration prior to ultrafiltration Water Res. 44 3203–13
[29] Tanneru C T, Rimer J D and Chellam S 2013 Sweep flocculation and adsorption of viruses on aluminum flocs during electrochemical treatment prior to surface water microfiltration Environ. Sci. Technol. 47 4612–8