



## Regular article

# The feasibility of citric acid as external carbon source for biological phosphorus removal in a sequencing batch biofilm reactor (SBBR)

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## ABSTRACT

The aim of this study was to evaluate the feasibility of citric acid as external carbon source for biological phosphorus removal. Citric acid was the only source of organic carbon and biomass occurred only as a biofilm. The effect of varying the duration of the mixing and aeration phases on the process of dephosphatation and consumption of organic substrate was examined. The operation consisted of filling (0.5 h), mixing (2.75–8.25 h), aeration (2.75–8.25 h) and draw (0.5 h) phases. Total phosphorus and nitrogen influent concentrations were 7.02 mg PL<sup>-1</sup> and 2.05 mg NL<sup>-1</sup>, respectively. Enhanced intracellular accumulation of polyphosphates (poly-P) and their release in reactors with immobilized biomass (SBBR) was stated. The highest rates of phosphates release ( $0.059 \pm 0.004 \text{ g P-PO}_4^{3-} \text{ m}^{-2} \text{ h}^{-1}$ ) and phosphates uptake ( $0.028 \pm 0.002 \text{ g P-PO}_4^{3-} \text{ m}^{-2} \text{ h}^{-1}$ ) were observed for mixing to aeration phase duration ratio of 1:3. The percentage of phosphorus in dry matter of biofilm reached 4.6%.

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## 1. Introduction

Rotating biological contactors (RBC) as well as activated sludge sequencing batch reactors (SBR) have been designed for organic compounds removal [1]. These types of treatment technology are also increasingly required to be more effective in removing nitrogen and phosphorus in response to the tighter water quality objectives.

Expectations regarding increasing quality of wastewaters discharged to the natural environment have urged the need for increasing the effectiveness of nitrogen and phosphorus removal in technological solutions of this type. In some studies the classic sequencing batch reactors are shown to be superior to the continuous-flow systems (flexibility in respect of flow and load of contaminants) [2,3], and reactors with biofilm are superior to the suspended activated sludge systems (lesser susceptibility to changes in the load of contaminants, lesser biomass growth, higher biomass concentration, lower volume of reactors) [4–6], sequencing batch biofilm reactors (SBBR) are considered as a novel treatment approach.

Phosphorus removal in wastewater biological treatment systems requires the incorporation of the phosphorus into the biomass and then the removal of the excess biomass.

The mean content of phosphorus reaches 2–3% d.m. of bacterial cells [7,8]. However, some microorganisms are capable of its increased uptake and intracellular accumulation. The mechanism of enhanced biological phosphorus removal (EBPR) has applied for years been in technologies developed for nutrients removal from wastewater treatment [1].

Traditional heterotrophic denitrification and biological dephosphatation are, to a large extent, dependent on the quantity and type of biodegradable forms of organic carbon in respect of both total duration of reactor cycle and duration of its particular phases [5,9–12]. With the appropriately duration of the anaerobic phase and the presence of sufficient organic substrates, the dephosphatation may be only slightly delayed or even occur simultaneously with denitrification. The problem of the insufficient quantity of the organic compounds may be solved by applying an external source of organic compounds. The impact of different types of organic compounds on the effectiveness of nitrogen compounds removal or the capability of activated sludge for enhanced intracellular accumulation of polyphosphates has already been extensively investigated [9,13–15]. One of the most popular and the best explored sources of organic carbon in reactors with activated sludge is acetic acid. However, volatile fatty acids (VFA) [16–18], and substrates like glucose, methanol, ethanol [14,19–22] as well as selected types of industrial wastewaters [23,24] or waste products [25–27] may serve as good

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sources of substrates. The beneficial effects of other compounds, like citric acid continue to be investigated [28,29].

Citric acid is an important intermediate product of the tricarboxylic acid cycle (TCA). Under aerobic conditions, its metabolism causes an increase in ATP content in bacterial cells [28]. A high content of ATP may enhance the synthesis of polyphosphates (poly-P) in the aerobic phase and, consequently, result in a greater release of phosphates in the anoxic and anaerobic phases. The ratio of the aeration phase to the mixing phase seems to be of key significance in the processes of enhanced intracellular accumulation of polyphosphates with the share of citric acid in SBBR.

Citric acid is a solid, non-toxic, naturally occurring stable substance. It is produced on the industrial scale; its global production in 2007 was over 1.6 million tonnes [30]. The market price of technical citric acid is similar to the price of acetic acid, which is lower than the price of such substrates as glucose, propanol or ethanol [31]. It is easily available as well as conveniently transported and stored. Results of studies conducted so far [28,29] as well as practical and economic concerns indicate that citric acid may be a technologically-attractive source of organic carbon in the bio-processes of nutrients removal.

Citric acid may find application not only in large wastewater treatment plants but also in small installations or industrial wastewater treatment plants, where the use of substrates exhibiting toxic properties (like methanol) may be impracticable. Systems based on a biofilm and sequencing batch reactors with an external source of organic carbon may prove efficient in this application.

The aim of this study was to determine the possibility of applying citric acid for enhanced biological phosphorus removal with the use of a biofilm in a sequencing batch reactor. The rate of phosphates release and uptake in an SBBR from denitrified wastewaters (low nitrate concentration) was determined. The study analyzed the effect of the duration of mixing and aeration phases on the process of dephosphatation and consumption of organic substrate. It included the period of system adaptation and exact study that was aimed at determining the rate of phosphates uptake and release in different variants of reactors work. A 2% solution of citric acid was used as the only source of organic carbon in the reactors.

## 2. Methods

Experiments were carried out with three bioreactors made of plexiglas and each having an active volume of 5 L. The filling of each bioreactor was made of a set of 8 completely immersed disks with diameter of 0.1 m each, made of acid-proof steel, coupled co-axially and rotated by an electric engine (60 rpm). The distance between the disks was 2 cm. The disks were set to an angle of 30° in respect of the vertical axis of the reactor, which ensured the mixing of the entire volume of the reactor and facilitated the sloughing of excess biomass (Fig. 1). The active surface of the reactor reached 25 m<sup>2</sup> m<sup>-3</sup>. The bioreactor design allowed biomass retention only in the form of the biofilm that was covering bioreactor disks with the discharge of the whole volume of treated wastewaters together with sloughed biofilm. The reactors were aerated with aerating pumps (air flow: 150 L h<sup>-1</sup>), which ensured the concentration of dissolved oxygen at 4.1 mg O<sub>2</sub> L<sup>-1</sup>. The reactors were operated at room temperature (20–22 °C).

Experiments were conducted with synthetic wastewaters that were fed to the three reactors, which allowed the comparison of processes running under various conditions. Wastewaters were prepared using: KH<sub>2</sub>PO<sub>4</sub>, NaNO<sub>3</sub>, MgSO<sub>4</sub>·7H<sub>2</sub>O, KCl, CaCl<sub>2</sub>, enriched broth and tap water. Synthetic wastewaters were left for 24 h to settle. Thus prepared wastewater simulated the effluent from a municipal wastewater treatment plant in which organic matter and nitrogen were removed in the process of denitrification. The



Fig. 1. The SBBR disc media.

applied wastewater originated from the terminal stage of denitrification characterized by small amounts of oxidized forms of nitrogen, whereas NH<sub>3</sub> was practically absent. Such effluents are characterized by the lack of organic compounds necessary for biological dephosphatation. Therefore, an external source of organic carbon needs to be applied. Owing to this, dephosphatating bacteria would not have to compete with denitrifying bacteria for citric acid. The appropriate quantity of organic carbon was ensured using a 2% solution of citric acid. It was applied in a single dose at the beginning of reactor cycle and constituted the sole source of organic carbon supplied to the reactors. The acid's dose was calculated based on literature data according to which 40 mg COD mg<sup>-1</sup> P is necessary for successful removal of phosphorus [9]. The empirically determined ratio of COD (chemical oxygen demand, with the dichromate method) to TOC (total organic carbon, with the oxidizing combustion–chemiluminescence method) for citric acid reached 2:1. It enabled the determination of the initial TOC value in wastewaters at 140 mg C L<sup>-3</sup>. The organic loading rates (OLRs) at the beginning of the cycle reached 11.2 g C m<sup>-2</sup> d<sup>-1</sup> (280 g C m<sup>-3</sup> d<sup>-1</sup>), it corresponding 22.3 g COD m<sup>-2</sup> d<sup>-1</sup> (560 g COD m<sup>-3</sup> d<sup>-1</sup>). Values of physicochemical parameters of crude wastewaters before citric acid addition are presented in Table 1.

The SBBRs have been operated for 2.5 months to establish a stable biofilm on disks. In this state, biofilm detachment in each cycle became constant. Then, the study was begun that lasted for 28 cycles. Based on results from this period, mean values of physicochemical parameters were determined.

Activated sludge from the dephosphatation chamber of the Municipal Wastewater Treatment Plant in Olsztyn served as the

**Table 1**  
Physicochemical characteristic of synthetic wastewater.

Parameter	Unit	Value
Temperature	°C	22
Redox potential	mV	171
pH	–	7.60
Total organic carbon (TOC)	mg C L <sup>-1</sup>	<0.1
Total nitrogen (TN)	mg N L <sup>-1</sup>	2.05
Nitrate nitrogen	mg N–NO <sub>3</sub> L <sup>-1</sup>	2.00
Nitrite nitrogen	mg N–NO <sub>2</sub> L <sup>-1</sup>	<0.01
Ammonium nitrogen	mg N–NH <sub>4</sub> L <sup>-1</sup>	<0.01
Total phosphorus (TP)	mg P L <sup>-1</sup>	7.02
Orthophosphate	mg P–PO <sub>4</sub> L <sup>-1</sup>	7.00

**Table 2**  
Mixing and aeration phases duration.

Variant number	Mixing [h]	Aeration [h]
1	2.75	8.25
2	5.5	5.5
3	8.25	2.75

inoculum for system adaptation. The working cycle of a single bioreactor lasted 12 h and consisted of the following phases: filling up (0.5 h), mixing (2.75–8.25 h), aeration (2.75–8.25 h), and discharge (0.5 h) of the whole volume of wastewaters. It corresponded to the mixing to aeration phase ratio of 1:3, 1:1, and 3:1 (Table 2).

Each time, 5 L of wastewaters were fed to the bioreactors. Samples were collected every 2.75 h from the beginning of the mixing phase and then filtered in a medium filter.

The following physicochemical parameters were controlled in the reactor:

- pH value, temperature using a CP-105 waterproof pH meter by Elmetron, redox potential using a pH211 Microprocessor by Hanna instruments, and dissolved oxygen using Oxi 330i/set by WTW.

Filtered wastewaters were analyzed for:

- total organic carbon (TOC) using Total Organic Carbon Analyzer TOC-L CPH/CPN by Shimadzu Corporation with the “oxidizing combustion-infrared analysis” method
- total nitrogen using Total Organic Carbon Analyzer TOC-L CPH/CPN with TNM-L device by Shimadzu Corporation for total nitrogen assay with the “oxidizing combustion–chemiluminescence method”
- orthophosphates using a flow injection analyzer FIA Modula by MLE (ISO 15681-1), with tin(II) chloride method
- total phosphorus using a UV–VIS 5000 DR spectrophotometer by HACH Lange with HACH Lange LCK 348–350 method
- chemical oxygen demand with dichromate method [PN74/C-04578/03],

**Table 3**  
pH and redox potential value changes during single SBBR cycle.

	Time [h]				
	0.5	3.25	6	8.75	11.5
Variant	pH				
Mixing/aeration–1/3	5.5	6.29–6.52	8.31–8.33	8.32–8.34	8.24–8.37
Mixing/aeration–1/1	5.5	6.08–6.30	6.48–6.67	8.03–8.18	8.11–8.36
Mixing/aeration–3/1	5.5	6.21–6.36	6.51–6.68	6.65–6.81	8.25–8.32
Variant	Redox potential [mV]				
Mixing/aeration–1/3	160 ± 10	–130 ± 10	92 ± 2	94 ± 3	97 ± 4
Mixing/aeration–1/1	160 ± 10	–120 ± 6	–148 ± 8	–16 ± 12	48 ± 10
Mixing/aeration–3/1	160 ± 10	–151 ± 3	–147 ± 16	–171 ± 5	52 ± 19

Furthermore, gravimetric method was used to determine:

- total suspended matter [PN-72/C-04559/02], dry matter (acc. PN-EN 12880:2004); organic fraction (losses at dry matter roasting) (acc. PN-EN 12879:2004); and mineral fraction (residue after roasting) (acc. PN-EN 12879:2004).

The presence of polyphosphate in bacterial cells was analyzed by the Neisser staining method [32]. The stained cells were observed using a microscope BX51 (Olympus) with an immersion objective and a CCD camera (Olympus; magnification 1000×). Image was analyzed by a computer program Cell F (Olympus).

### 3. Results and discussion

This study determined the feasibility of using citric acid as external carbon source for biological phosphorus removal in the three variants of bioreactors mixing and aeration phases duration under the presence of only biofilm microorganisms. The quantity of suspended biomass was minimized by decanting the sloughed biomass. The biofilm thickness ranged from 1 to 5 mm during the study (Fig. 1).

Under anaerobic conditions with a lack of electron acceptors in the form of nitrates, sulfates or carbonates, the consumption of organic compounds may be low [8]. In the presented experiments, the consumption of citric acid in the mixing phase was decreasing along with a diminishing redox potential (ORP), which changed from (+)171 ± 9 mV at the beginning of the cycle to (–)130 ± 10 mV at the end of the mixing phase in Variant 1, or to (–)148 ± 8 mV in Variant 2 and to (–)171 ± 5 mV in Variant 3 (Table 3). In the aeration phase the redox potential was increasing and reached the values of 97 ± 4 mV, 48 ± 10 mV and 52 ± 19 mV at the end of the cycle in Variant 1–3, respectively, and the organic carbon uptake was much higher. The 8.25-h period of aeration applied in Variant 1 ensured its total consumption (Fig. 2). In variants 2 and 3, the concentration of TOC in the effluent reached 9.82 ± 6.06 and 40.35 ± 9.95 mg C L<sup>-1</sup>, respectively.

The introduction of a citric acid solution to the system contributed to a decrease in wastewaters pH value from pH 7.60 to pH 5.5 (Table 3). The consumption of citric acid by microorganisms and CO<sub>2</sub> expelling as a result of aeration was accompanied by an increasing pH value, which in the treated wastewaters accounted for pH 8.24–8.37 in Variant 1, for pH 8.11–8.36 in Variant 2, and for pH 8.25–8.32 in Variant 3 (Table 3). A low pH value at the beginning of the cycle could suppress the activity of PAO (*Polyphosphate Accumulating Bacteria*) [33], however considering the course of phosphate release and uptake profiles it may be concluded that its effect was not as significant as that of the ratio of mixing and aeration phases duration. Investigations carried out using reactors with suspended biomass demonstrate that, apart from PAOs, also the population of glycogen accumulating organisms (GAOs) may develop in the reactor. Unlike PAOs, the GAOs derive energy for the

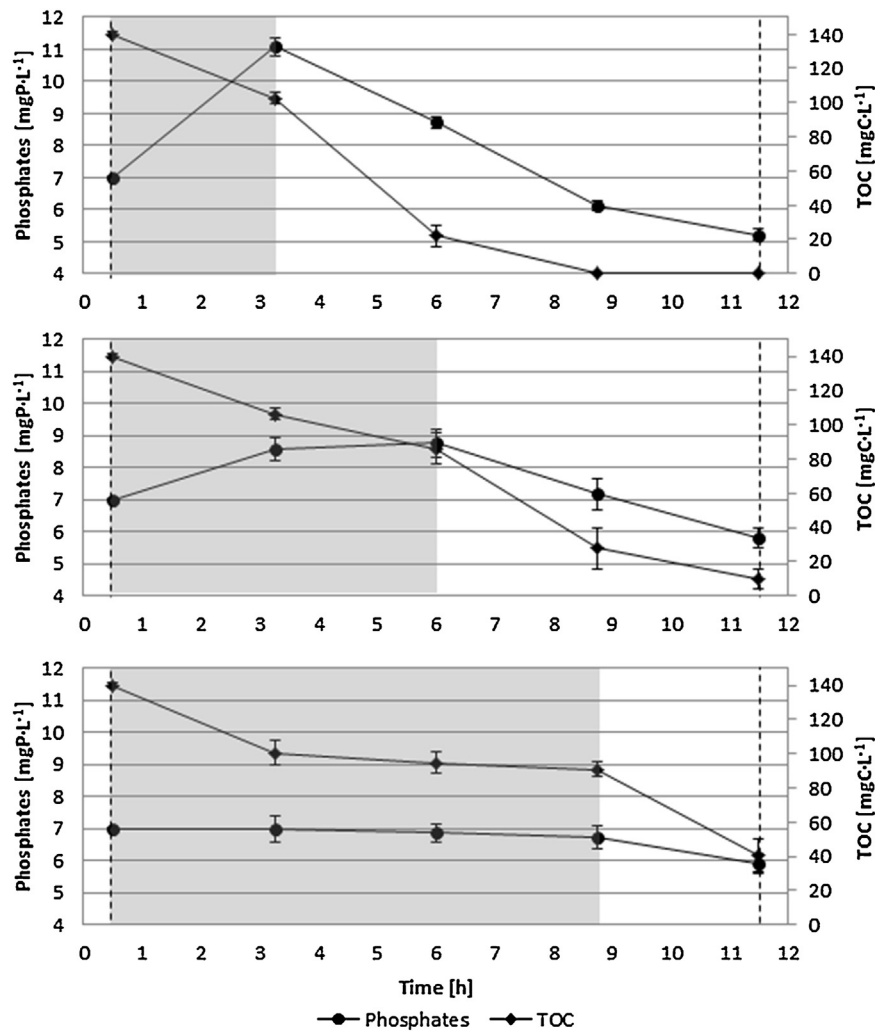


Fig. 2. Changes of phosphates and TOC concentrations during the SBBR process cycle.

accumulation of intracellular polyhydroxy acids (PHAs) from glycolysis and not from the hydrolysis of polyphosphates, whereas at the aerobic stage they compete with PAOs for organic substrate. As a consequence, the development of GAOs causes a decrease in biodephosphatation effectiveness [34]. In order to promote PAOs, the pH value should be maintained above 7.25 under anaerobic conditions and above 7.0 at the aerobic stage [34,35]. In this study, the ratio of mixing to aeration stages affected the rate of the increase in pH. In the first variant with the shortest mixing stage, pH below 7.25 was maintained for a considerably shorter period of time than in the two other variants, and the quantity of liberated and absorbed phosphates was the highest. This would confirm the effect of pH on the activity of PAOs and GAOs. It indicates that the feasibility of using citric acid to aid biological dephosphatation might be dependent on the reactor operation strategy.

Under anaerobic conditions, the PAOs uptake organic matter from treated wastewater for the synthesis of polyhydroxy acids (PHAs), and derive energy indispensable for their synthesis from degradation of intracellularly-accumulated poly-P. This results in an increased concentration of phosphates in treated wastewater. Under aerobic conditions, the PHAs constitute the source of carbon and energy for the growth and development of microorganisms. As a result of their degradation, energy is retained in the cell owing to re-synthesis of poly-P (Fig. 3). Effective dephosphatation proceeds when the quantity of phosphates released under anaerobic conditions is lower than their quantity uptaken under aerobic conditions.

In the activated sludge, the synthesis of poly-P is also possible with a single-stage oxic process without PHA accumulation, and EBPR could be realized in net phosphate removal [36].

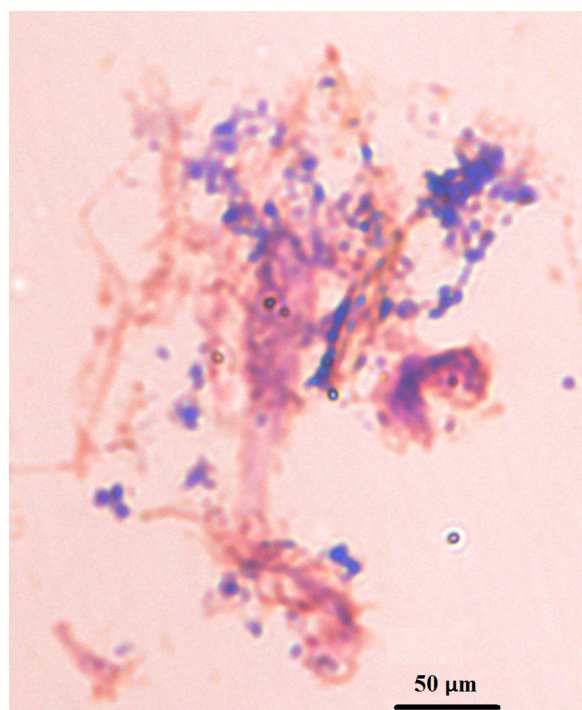
Oxygen concentration in the mixing phase was less than  $0.3 \text{ mg O}_2 \text{ L}^{-1}$  in each experiment, whereas at the end of the aeration phase it reached  $4.1 \pm 0.4 \text{ mg O}_2 \text{ L}^{-1}$ . The highest rate of phosphates release ( $59 \pm 4 \text{ mg P-PO}_4^{3-} \text{ m}^{-2} \text{ h}^{-1}$ ) and phosphates uptake ( $28 \pm 2 \text{ mg P-PO}_4^{3-} \text{ m}^{-2} \text{ h}^{-1}$ ) was noted in Variant 1 (Table 4). Elongation of the mixing phase reduced the rate of both phosphates release (to  $13 \pm 3 \text{ mg P-PO}_4^{3-} \text{ m}^{-2} \text{ h}^{-1}$ ) and uptake (to  $21 \pm 2 \text{ mg P-PO}_4^{3-} \text{ m}^{-2} \text{ h}^{-1}$ ) (Variant 2). In Variant 3—with the longest mixing phase, no release of phosphates was observed, and even though a reduction was observed in phosphates concentration in wastewaters in the entire cycle (Fig. 2).

The highest concentration of phosphates at the end of the mixing phase was determined in Variant 1 ( $11.07 \pm 0.28 \text{ mg P-PO}_4^{3-} \text{ L}^{-1}$ ). In the same variant, the lowest concentration of phosphates was recorded at the end of the aeration phase

Table 4  
Phosphate release and uptake rates.

Variant	Rate of phosphate release [ $\text{mg P m}^{-2} \text{ h}^{-1}$ ]	Rate of phosphate uptake [ $\text{mg P m}^{-2} \text{ h}^{-1}$ ]
Mixing/aeration—1/3	$59 \pm 4$	$28 \pm 2$
Mixing/aeration—1/1	$13 \pm 3$	$21 \pm 2$
Mixing/aeration—3/1	$-1 \pm 2$	$11 \pm 4$





**Fig. 3.** Polyphosphate staining by Neisser staining method in bacterial cells of the biofilm in Variant 1, magnification 1000 $\times$  (poly-P were stained on the lilac color). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

( $5.22 \pm 0.17 \text{ mg P-PO}_4^{3-} \text{ L}^{-1}$ ). In the second variant, phosphates concentration after mixing and aeration phases reached  $8.76 \pm 0.45$  and  $5.81 \pm 0.32 \text{ mg P-PO}_4^{3-} \text{ L}^{-1}$ , respectively. In turn, in Variant 3 the effluent concentration of phosphates reached  $5.94 \pm 0.28 \text{ mg P-PO}_4^{3-} \text{ L}^{-1}$  (Fig. 2). The effluent concentration of total phosphorus was  $5.32 \pm 0.15$ ,  $5.89 \pm 0.32$  and  $6.09 \pm 0.25 \text{ mg PL}^{-1}$  in Variants 1–3, respectively. It corresponded to the load of removed phosphorus reaching 130, 90 and  $70 \text{ mg P m}^{-2} \text{ d}^{-1}$  in Variants 1, 2 and 3, respectively.

Some of the P was used for biomass growth. According to Grady [1] about 0.017 mg of phosphorus is required for each mg of heterotrophic biomass COD formed. One of the causes of such an effectiveness of phosphorus removal in the present study could be its sorption by the biofilm. However, if the sorption process had been responsible for phosphorus removal, shortly after formation of the biofilm it would be subject to saturation and the effectiveness of phosphorus removal via sorption would not be high. In addition, in case of Variants 1 and 2 of this study, a tangible increase of phosphate concentration was noted in wastewater in the anaerobic phase and phosphate uptake under aerobic conditions (Fig. 2). This process occurs at enhanced intracellular accumulation of poly-P observed during the study (Fig. 3), and not during sorption. For this reason, it appears that sorption was not a predominating process responsible for the effectiveness of phosphorus removal. The aerobic metabolism of citric acid contributes to an increased ATP content in bacterial cells. The longer aeration phase in Variant 1 could result in a higher ATP content, compared to Variants 2 and 3. The higher ATP content means that a greater quantity of polyphosphates synthesized in the aerobic phase and its greater release in the anoxic and anaerobic phases. This was the likely reason behind the different profiles achieved for phosphate release and uptake depending on the ratio of aeration and mixing phases.

Glucose, acetic acid and peptone were applied by Li et al. [37] as external sources of carbon in a SBBR. The highest release of phosphate by biofilm occurred within the first 3 h of the anaerobic phase.

In the successive hours of the mixing phase, the concentration of phosphate in wastewaters slightly increased. According to authors, rates of phosphate release and uptake depended on the applied load of organic compounds and type of substrate. The highest rates were noted for acetic acid and the lowest one for peptone. These authors stated also that the quantity of phosphate released in the anaerobic phase had a direct impact on the final effectiveness of dephosphatation, however they did not refer the rate of dephosphatation to the surface of the bioreactor media.

The final rate of phosphate removal is not always influenced by the volume of phosphate released in the anaerobic phase. Janczukowicz et al. [38] investigated the rate of phosphate release and uptake in activated sludge originating from an SBR reactor with VFA (acetic, propionic, butyric, isobutyric, valeric and isovaleric) used as the external sources of carbon. They showed the highest rate of phosphate release ( $3.85 \text{ mg P-PO}_4^{3-} \text{ L}^{-1} \text{ h}^{-1}$ ) and uptake ( $5.19 \text{ mg P-PO}_4^{3-} \text{ L}^{-1} \text{ h}^{-1}$ ) in denitrified wastewaters for acetic acid, and the lowest rate of phosphate release ( $0.85 \text{ mg P-PO}_4^{3-} \text{ L}^{-1} \text{ h}^{-1}$ ) and uptake ( $2.86 \text{ mg P-PO}_4^{3-} \text{ L}^{-1} \text{ h}^{-1}$ )—for isovaleric acid. The highest rates of phosphate release and uptake in the presence of acetic acid resulted from the highest effectiveness of dephosphatation process (65.25%), however such a correlation was not observed for other fatty acids used as carbon sources.

One of the key factors that affect the final effectiveness of phosphorus removal in SBBRs is the quantity of excess biomass discharged outside the system. The capability of oxygen and organic compounds to penetrate into the biofilm is of little significance in this case [39]. In presented experiment, the highest biomass growth ( $2920 \pm 630 \text{ mg d.m. m}^{-2} \text{ d}^{-1}$ ) was determined in the variant with the longest aerobic phase. It corresponded to the highest effectiveness of phosphate removal. The longer mixing phase resulted in diminished biomass growth that accounted for  $2210 \pm 550 \text{ mg d.m. m}^{-2} \text{ d}^{-1}$  and  $2000 \pm 470 \text{ mg d.m. m}^{-2} \text{ d}^{-1}$  in Variants 2 and 3, respectively. The lower volume of biomass discharged from the system resulted from smaller biomass growth observed under anaerobic compared to aerobic conditions [40].

Despite the occurrence of the mechanism of phosphate release and uptake, the removal of phosphorus was insignificant. The final effectiveness of biological removal of phosphorus depends on the quantity of excess biomass discharged from the system and on the percentage content of phosphorus in dry matter. In the presented study, the highest percentage of phosphorus in dry matter discharged from the reactor was determined in the first variant, i.e. it reached 4.6% and was higher compared to activated sludge SBR reactors with enhanced intracellular accumulation of polyphosphates (4% of biomass) [41]. In Variants 2 and 3 with a longer phase of mixing, phosphorus constituted 4.0% and 3.6% of dry matter, respectively.

Nitrogen also ensures microorganisms growth and development. Too high a concentration of nitrate may, however cause, lower phosphorus removal effectiveness, which has been confirmed in many experiments with activated sludge [29,42,43]. It results from the competition of denitrifying bacteria with bacteria characterized by enhanced accumulation of polyphosphate for organic compounds. In the conducted experiment, treated wastewaters were characterized by total nitrogen concentration of  $2.05 \text{ mg NL}^{-1}$ , which ensured bacteria growth and simultaneously minimized competition for organic substrate. In every variant, the concentration of total nitrogen at the end of the cycle was lower than  $1 \text{ mg NL}^{-1}$ .

Citric acid glucose, acetic acid, butyric acid and propionic acid were compared by Kargi et al. [28] as external sources of carbon in their study on the rate of phosphate release and uptake in activated sludge. In the case of a mixture of glucose and citric acid in the COD ratio of 1:1, the rate of phosphate removal reached

2.23 mg PL<sup>-1</sup> h<sup>-1</sup> whereas the rate of phosphate uptake reached 8.1 mg PL<sup>-1</sup> h<sup>-1</sup>. These were the highest values achieved in the study of these authors. Although it does not belong to the group of VFA, citric acid enables enhanced biological removal of phosphate not only suspended but also biofilm systems, which was observed in the reported study.

#### 4. Conclusions

This study showed enhanced intracellular accumulation of polyphosphate in the aeration phase and their release in the mixing phase in reactors with immobilized biomass (SBBR) and with citric acid as an external source of carbon. This phenomenon occurred in spite of the fact the citric acid does not belong to the so-called “volatile fatty acids”. Phosphorus removal efficiency and the rate of phosphate release and uptake in the presence of citric acid depended on the duration of cycle phases. The quantity of phosphate released in the presence of citric acid influenced the effectiveness of phosphorus removal. The highest phosphorus removal effectiveness and the highest values of phosphates release and absorption rates were noted in the SBBR with the shortest mixing stage and the longest aeration stage. Increasing the mixing stage resulted in a lower effectiveness of phosphorus removal and reduced the consumption of citric acid introduced to the reactor. The effectiveness of phosphorus removal in SBBR in the presence of citric acid was linked with the extent of biomass growth and the quantity of biofilm removed from the reactor after completion of a treatment cycle. Increasing the aeration stage facilitated higher biomass growth and, resulted in the, higher effectiveness of phosphorus removal. It additionally resulted in a higher rate of citric acid consumption and increased pH of wastewater. The effectiveness of citric acid as an external source of carbon in the process of biological dephosphatation depends on the assumed SBBR operational strategy.

Despite 4.6% content of phosphorus in dry matter, the phosphorus removed load was limited by a low quantity of biomass removed from the reactor.

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