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# Application of Anaerobic-Aerobic Combined Bioreactor in Phosphorus Removal

**Abstract:** A two-stage anaerobic-aerobic sequencing reactor system was developed in order to enhance the removal of biological phosphorus in the sequencing of combined reactors. Combining both aerobic and anaerobic designs in one reactor improved the efficiency and reduced the construction and operating costs. The combination of an upflow anaerobic fixed bed (UAFB) and a floating activated sludge aerobic bioreactor was designed with respective Kaldnes packing ratios of 90 and 30% for the anaerobic and aerobic sections. The controlled parameters were pH levels within a neutral range, a temperature of 37°C, mixed liquor suspended solids (MLSS) of 1220 and 1030 mg/L for the aerobic and anaerobic sections, respectively, and an attached growth that was equal of 743 and 1190 mg/L for the aerobic and anaerobic sections, respectively. Tests were conducted for three different initial phosphorus concentrations (12.8, 32.0, and 44.8 mg/L), two different volumes for each section, and four chemical oxygen demands (CODs) (500, 1000, 1200, and 1400 mg/L). The results demonstrated that, generally, the phosphorus removal in the anaerobic section fell significantly by increasing the inlet COD, and the maximum removal occurred at COD = 500 mg/L. More than 90% of the phosphorus was removed in the aerobic section at COD = 500 mg/L. In other words, the best performance of the reactor was when the ratio of the COD : N : P = 100 : 5 : 2, composition of phosphorus in industrial wastewater.

**Keywords:** phosphorus removal, hydraulic loading, anaerobic-aerobic combined bioreactor, kaldnes packing, municipal and industrial wastewater

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# 1. Introduction

Whether it is treated or not, the discharge of wastewater into surface water with nutrients can create a variety of issues, including alga development and the resulting drops in adequate oxygen concentrations in the water. Among the nutrients, phosphorus compounds have been recognized as a growth-limitation factors that aggravate the eutrophication phenomenon (nutritionalism) [1, 2]. Eutrophication refers to the excessive growth of algae and other aquatic plants due to high nutrient levels (including phosphorus and nitrogen). While nitrogen can also act as a limiting factor in some environments, phosphorus is often a key driver of eutrophication. Small increases in phosphorus concentrations can promote algal blooms, deplete oxygen levels, and harm other organisms; therefore, the presence of phosphorus compounds can aggravate eutrophication [3]. The presence of different phosphorus species in wastewater treatment systems (including inorganic phosphorus [IP], polyphosphate [poly-P], and organic phosphorus [OP]) poses challenges for effectively removing phosphorus [4]. Among the waste-activated sludges (WASs), polyphosphate represents 30–80% of the total phosphorus (TP), IP represents 10–30% of the TP, and OP represents 5–30% of the TP [5–7]. While various biological methods such as alternating anaerobic, aerobic, and anoxic steps have been employed for nutrient removal in wastewater treatment, the complexities and costs that are associated with nutrient-specific biological processes have prompted efforts to develop low-cost and efficient alternatives. One such approach is chemical precipitation, which involves the addition of chemicals such as alum or ferric salts to induce phosphorus removal by forming insoluble complexes that can be separated from the wastewater [8]. Chemical precipitation has been widely studied and implemented due to its effectiveness in reducing phosphorus concentrations and lowering effluent nutrient levels, making it a valuable technique for sustainable wastewater treatment practices. As an example, a *Chlorella* sp. was evaluated by Li et al. [9] for its ability to remove nutrients from a highly concentrated municipal wastewater stream that is generated following the thickening of activated sludge (a raw and autoclaved medium). Algae were able to remove 80.9 and 90.8% of the TP and chemical oxygen demand (COD), respectively, from a raw medium after 14 days of batch cultivation [10]. Using anaerobic and aerobic processes at the same time reduces operating costs as compared to aerobic treatment methods alone; this also increases the efficiency of removing high-load organic matter and produces aerobic sludge without changing the pH level. On the other hand, traditional treatment plants suffer from the following problems: large space requirements, large open reactors that emit pollutants into populated areas, low process efficiencies, a large surplus of sludge, and high energy consumption. Anaerobic-aerobic treatment plants eventually lose their attractiveness because of their economic and geographic disadvantages [11]. The drawbacks of conventional anaerobic-aerobic systems have been overcome through the development of new technologies. The dual goals of resource recovery and compliance with current regulations for effluent disposal have been achieved by using an anaerobic-aerobic process with high-rate bioreactors such as upflow anaerobic sludge blankets (UASBs) or filter bioreactors, fluidized bed reactors, or membrane bioreactors [12]. A UASB reactor has proven to be a reliable technology for wastewater treatment for decades [13]. The use of pretreatments prior to aerobic treatment is widespread for a variety of industrial and municipal wastewaters [14]. It is usually activated sludge that is used to treat wastewater aerobically; this is made of a suspended suspension of mixed bacteria that is stirred and aerated before it is mixed with wastewater. Depending on the type of operation that is required, activated sludge reactors can be divided into three types: plug flow reactors, continuous stirred tank reactors, and sequencing batch reactors [15]. In aerobic post-treatment, activated sludge has been extensively used, allowing both systems to be balanced in terms of their benefits and drawbacks [16, 17]. The anaerobic and aerobic zones of a bioreactor can also be integrated to achieve a more intensive biodegradation [18]. While this technology is still in its infancy, there have been a few studies on the design and operation of integrated anaerobic-aerobic bioreactors [19]. Anaerobic-aerobic sequencing batch reactors (SBRs) and combined anaerobic-aerobic culture systems (CAACS) are categorized into four types of integrated bioreactors: (i) integrated bioreactors with a physical separation of anaerobic-aerobic zones; (ii) integrated bioreactors without physical separation; (iii) combined anaerobic-aerobic culture systems; and (iv) combined anaerobic-aerobic cultures, which are developed by utilizing the principle of limited oxygen diffusion in microbial biofilms [20]. Due to its small size, low capital cost, and excellent COD-removal efficiencies, an integrated bioreactor with a stacked configuration is an excellent choice for treating of high-strength industrial wastewaters [21]. These systems are composed of upper and lower parts; the upper part uses aerobic treatment, whereas the lower end uses anaerobic treatment [22]. There are several common characteristics of conventional anaerobic-aerobic systems, including long hydraulic retention times (HRTs), low organic loading rates (OLRs), or large areas of land or digesters [23].

The primary novelty in this article that distinguishes it from previously published research on the same subject lies in the utilization of an innovative combined anaerobic-aerobic bioreactor. The unique feature of this bioreactor is its configuration (incorporating a Kaldnes packing ratio of 90% in the anaerobic section and 30% in the aerobic section), with a specific focus on phosphorus removal from municipal wastewater and considering the influence of COD. These bioreactors offer several advantages, including low energy consumption, reduced bioreactor capacity requirements, and high efficiency in removing organic matter.

## 2. Materials and Methods

### 2.1. Characteristics of Bioreactor

A laboratory-scale anaerobic treatment schematic of artificial wastewater is illustrated in Figure 1. The reactor is filled from the bottom to the top with packing that is anaerobic at the bottom and aerobic at the top. In order to analyze different retention time ratios, the anaerobic and aerobic sections were separated by a grid plate. Reactors have anaerobic zones that serve as entrances to the influent and exits from the tops of the columns (aerobic zone) [24]. In one study, Moosavi et al. found that the maximum percentage of COD removal in this pilot was 95% when the total hydraulic retention time was 9 hours (4 hours for the anaerobic section, and 5 hours for the aerobic section) [25].

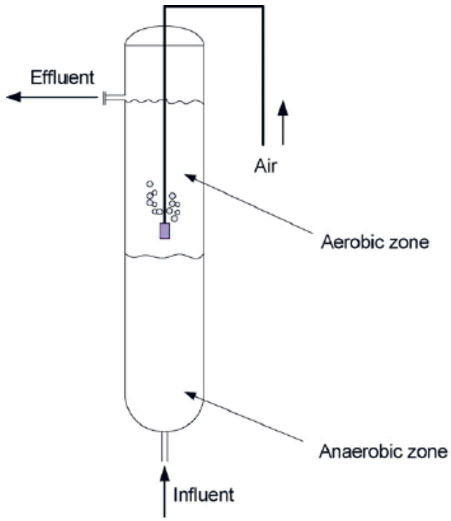


**Fig. 1.** Continuous anaerobic-aerobic reactors without physical separator between two parts
Source: [26]

A reactor that was made of plexiglass had the dimensions of 10 cm × 10 cm × 80 cm with an inlet valve located at the lowest point of the column height (5 cm from the bottom). For the phosphorus removal, the sewage first passed through the anaerobic section and then into the aerobic area. In order to investigate how the phosphorus concentrations changed in the bioreactor, outlet valves were installed on the opposite side of the reactor at 12.5-, 30.0-, 52.5-, and 75.0-cm heights. Each outlet valve contained a tube that sampled the more homogeneous liquid in the center of the column. A schematic of the bioreactor that was used for Phases 1 and 2 of this study is shown in Figures 2 and 3.

In aerobic and anaerobic reactors, the type of packing that is used has a significant impact on performance [27]. Our anaerobic and aerobic sections were equipped with carriers (Kaldnes-type) with internal diameter 21 mm, internal length 41 mm, density 96 g/cm$^3$, and surface area 480 m$^2$/m$^3$. A small Air Stone ball with a 2-inch diameter and a weight of 3.6 oz was installed in the aerobic section in order to ensure that the dissolved air concentration was 2 mg/L and that it was completely mixed. A sedimentation tank with a volume of 3 L was used to separate the sludge from the effluent and return it to the reactor. In Figure 4, the packing in the aerobic and anaerobic reactors is shown before and after the formation of a biofilm layer.

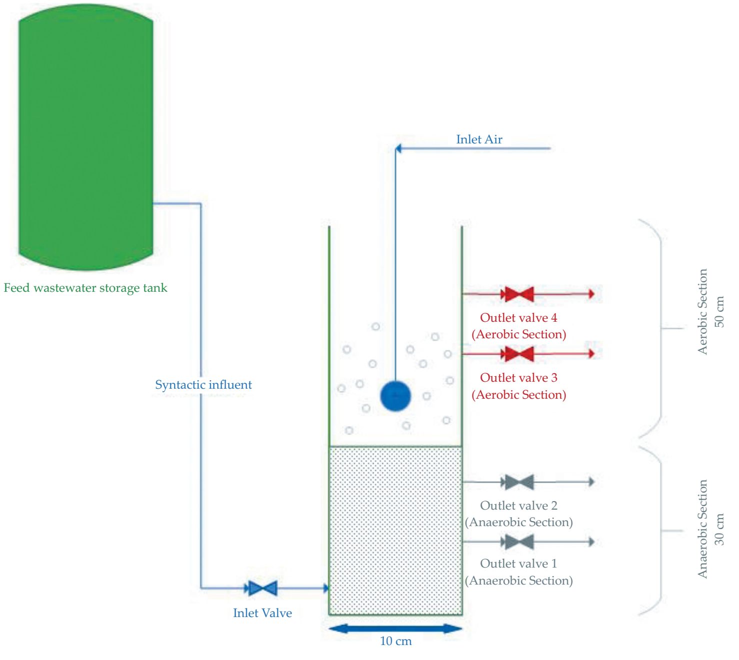


**Fig. 2.** Schematic of bioreactor in Phase 1

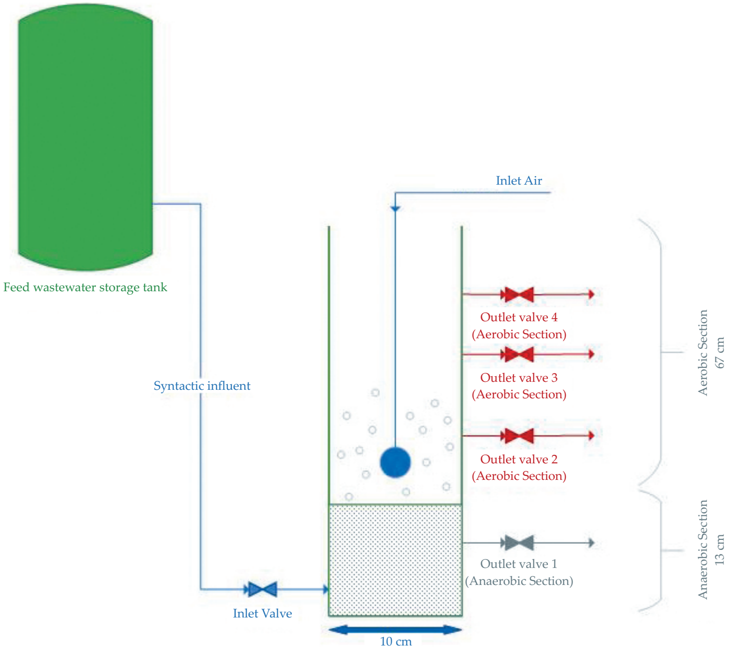


**Fig. 3.** Schematic of bioreactor in Phase 2

a) b)

**Fig. 4.** Before (a) and after (b) biofilm formation

## 2.2. Setup

### Batch Period

The first batch of the MBBR reactor was started with return sludge from the Ekbatan Treatment Plant in Tehran (MLSS = 2700 mg/L; COD = 420 mg/L). To achieve this, the initial batch of the MBBR reactor used return sludge from the Ekbatan Treatment Plant. The system operated as a batch process for 1.5 months to allow for biofilm formation on the outer surface of the packing. During this time, two separate reactors (one aerobic, and one anaerobic) were utilized to treat the sludge. The anaerobic reactor was sealed to minimize aeration and was fed every ~3–4 days, the dissolved oxygen was checked with the DO meter to make sure that the aeration was almost zero, and the aerobic reactor featured continuous adjustments in aeration, temperature, pH levels, and feed for six weeks. After forming the biofilm and achieving the desired sludge quality in both the aerobic and anaerobic phases, the two parts were transferred to the main reactor in order to create a combined aerobic and anaerobic system. The chemical oxygen demand (COD) of the feed was determined based on the COD of the reactor and proportionally adjusted to it.

### Continuous Period

To initiate the continuous-operation phase, the sludge was moved to the primary reactor once the biofilm had developed on the packing material. In order to ensure system stability and enable uninterrupted processes, a retention time of three days was established for the feedstock in the aerobic reactor. Tests were conducted for three different initial phosphorus concentrations (12.8, 32.0, and 44.8 mg/L), two different volumes for each section, and four chemical oxygen demands (CODs) (500, 1000, 1200, and 1400 mg/L). In the first test, 500 mg/L of COD and 12.8 mg/L of phosphorus were tested, followed by 32.0 and 44.8 mg/L of phosphorus. The plate was moved between the anaerobic and aerobic sections in order to perform all of these steps. The process of running all 24 tests took approximately 4 months.

## 3. Results and Discussion

Figures 5–8 illustrate how the percentages of the TP changed with input COD values during the first initial retention time (Phase 1); i.e., when Valve 2 produced an anaerobic effluent. The retention time ratio between the anaerobic and aerobic sections of the bioreactor could be adjusted by moving the retaining lattice plate between the two sections. The anaerobic section extended up to the second outlet valve, while the aerobic section extended up to the third and fourth outlet valves. Each outlet valve was measured to determine the rate of phosphorus removal.

As can be seen in Figure 5, the removal efficiency increased by passing the influent through the bioreactor. The elimination percentage was raised smoothly in all three of the line graphs; the best result was achieved for the inlet concentration ratio of COD : N : P = 100 : 5 : 2, which was 82.09% for the anaerobic section and 94.62% for the whole bioreactor. This meant that the combined reactor performed well in removing phosphorus from artificial waste with the same composition as with domestic sewage. In the next part of this study (Fig. 6), the feed COD was increased to 1000 mg/L; unfortunately, this change made the condition of the system (synthetic wastewater composition, dissolve oxygen, etc.) unstable, and the final results showed a significant fluctuation. By increasing the phosphorus concentration, the final removal was 46.47% for COD : N : P = 100 : 5 : 5 and 48.29% for COD : N : P = 100 : 5 : 7. It was concluded that the combined anaerobic-aerobic system was not suitable for domestic waste with an inlet of COD = 1000 mg/L. Figure 7 indicates the result for COD = 1200 mg/L, which indicate a 60% phosphorus removal for COD : N : P = 100 : 5 : 7 on Valve 3, while the effluent with the domestic concentration resulted in a 52.22% removal on the final outlet (Valve 4). Due to a broken pilot plate prior to the commencement of this particular test, the plant had to be restarted; this led to inadequate stabilization. In the final part of Phase 1 of this study, the removal range raised slightly in all cases (as shown in Figure 8); the best results was achieved for COD : N : P = 100 : 5 : 7, with a 29.75% removal. The emphasis is that this pilot performed well for inlet COD = 500 mg/L (Fig. 5) yet did not provide remarkable results for the rest of the cases. To sum up, the best percentage of phosphorus removal in COD = 500 mg/L was 94.62%, in COD = 1000 mg/L – 48.29%, in COD = 1200 mg/L – 60.54%, and in COD = 1400 mg/L – 29.75%. This indicated that the system performed well at low COD values.

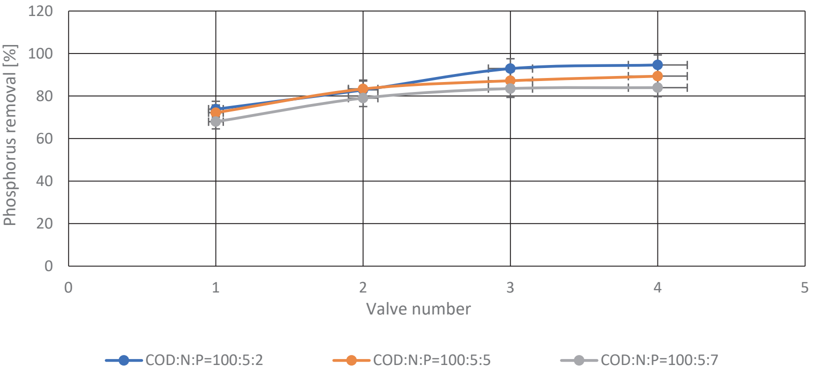


**Fig. 5.** Illustration of phosphorus-removal percentage in reactor with COD = 500 mg/L – Valve 2 represents anaerobic section's outlet

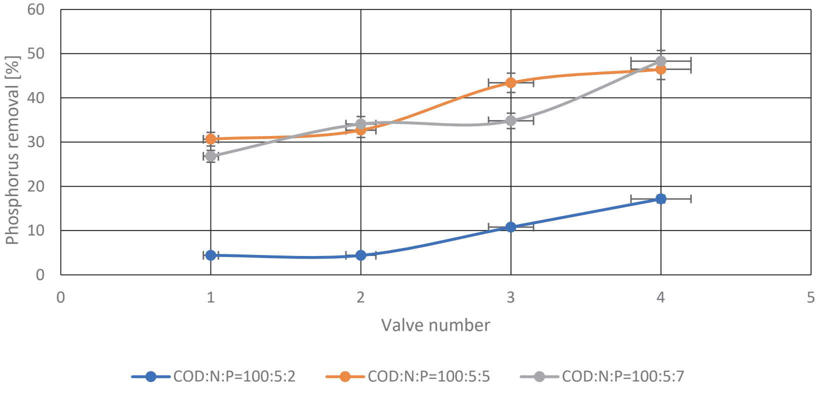


**Fig. 6.** Illustration of phosphorus-removal percentage in reactor with COD = 1000 mg/L – Valve 2 represents anaerobic section's outlet

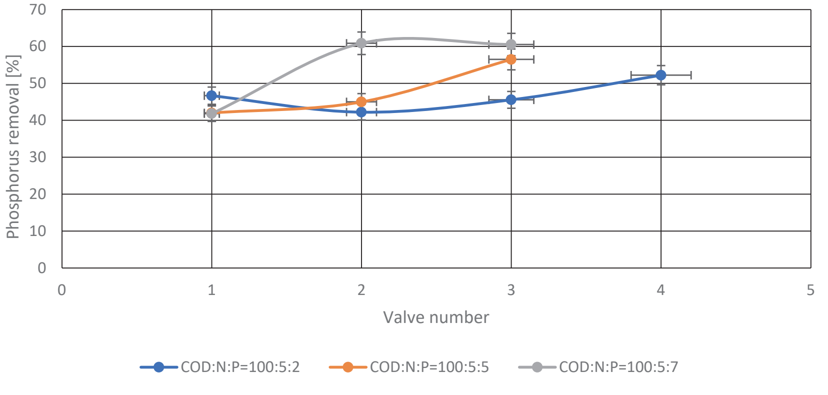


**Fig. 7.** Illustration of phosphorus-removal percentage in reactor with COD = 1200 mg/L – Valve 2 represents anaerobic section's outlet

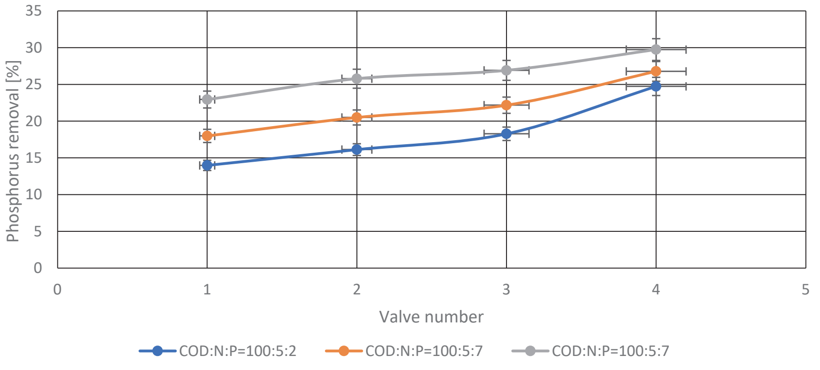


**Fig. 8.** Illustration of phosphorus-removal percentage in reactor with COD = 1400 mg/L – Valve 2 represents anaerobic section's outlet

At the secondary retention time (Phase 2), the anaerobic portion of the process was located up to outlet Valve 1, whereas the aerobic portion was located in Valves 2 through 4. Each test consisted of sampling all outlet valves to observe the trends in the phosphorus removal throughout the reactor. As integrated bioreactors must have a long lengths to maintain anaerobic and aerobic conditions, they are more like plug flow reactors than CSTRs. In other words, the relatively small reactor volume makes microorganism growth more challenging, which leads to sludge being drained from the system faster; this results in a washout [28]. The percentage of phosphorus removal increased in the reactor. Figure 9 illustrates the performance of the combined bioreactor in phosphorus removal for COD = 500 mg/L in the second phase, which provided higher residual time for the aerobic section as compared to the previous case. As can be seen, 98.65% of the phosphorus was removed for COD : N : P = 100 : 5 : 2; by increasing the inlet phosphorus to COD : N : P = 100 : 5 : 5 and COD : N : P = 100 : 5 : 7, the removal fell dramatically (44.68 and 32.27%, respectively). By increasing the COD to 1000 mg/L (Fig. 10), the phosphorus removal was not extended from 30% in the anaerobic section and 45% in the whole unit; the best result and was achieved for inlet COD : N : P = 100 : 5 : 5. Despite the low removal efficiency, it raised constantly through the bioreactor and made its behavior predictable. A comparison of the results in Figures 10 and 11 indicate that, if the inlet COD = 1200 mg/L, the best result was provided for COD : N : P = 100 : 5 : 7 (36.45%); this means that the reactor's performance was almost the same as in the previous case (COD = 1000 mg/L – Fig. 10). In the last part of this study, the inlet COD was increased to 1400 mg/L (as shown in Figure 12); by omitting the 91.87% removal for COD : N : P = 100 : 5 : 7, this was significantly higher as compared to the other outcomes in this situation. This indicated that this was not very reliable, as it lacked predictability. The rest of the data also indicated the low performance of the bioreactor in inlet COD more than in COD same as municipal wastewater. Further data analysis also indicated that the bioreactor has underperformed, resulting in higher inlet COD levels than that of the municipal wastewater.

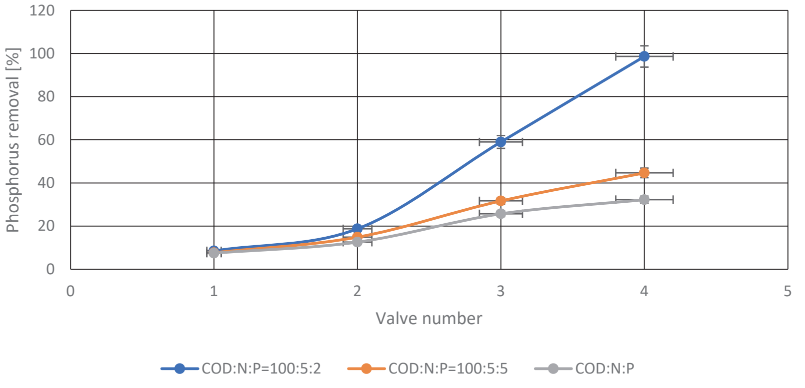


**Fig. 9.** Illustration of phosphorus-removal percentage in reactor with COD = 500 mg/L – Valve 1 represents anaerobic section's outlet

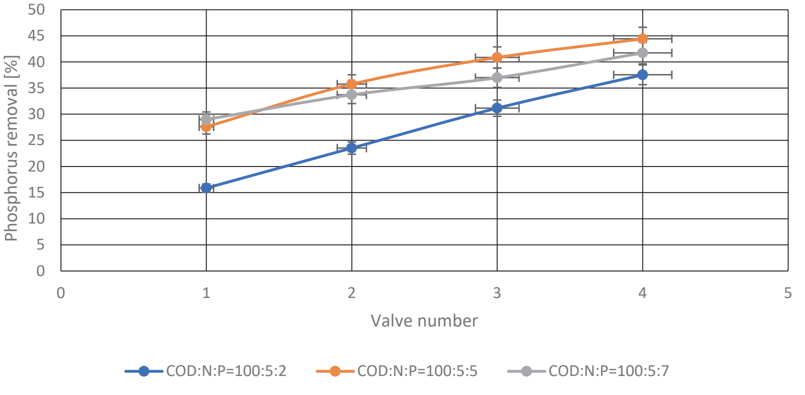


**Fig. 10.** Illustration of phosphorus-removal percentage in reactor with COD = 1000 mg/L – Valve 1 represents anaerobic section's outlet

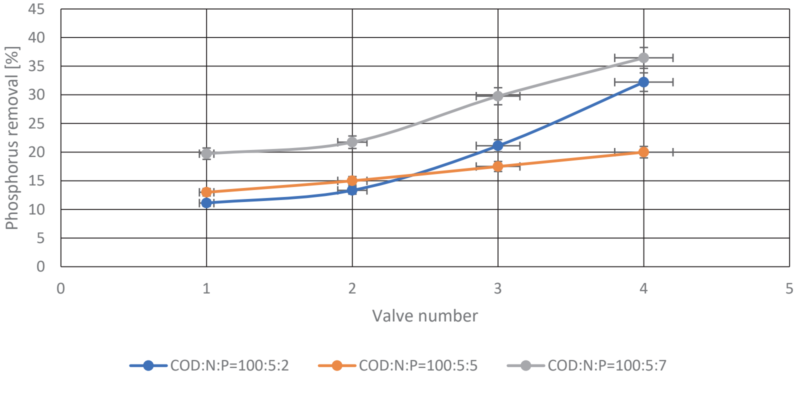


**Fig. 11.** Illustration of phosphorus-removal percentage in reactor with COD = 1200 mg/L – Valve 1 represents anaerobic section's outlet

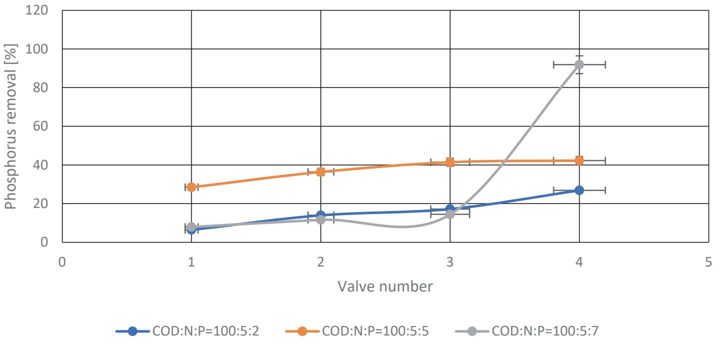


**Fig. 12.** Illustration of phosphorus-removal percentage in reactor with COD = 1400 mg/L – Valve 1 represents anaerobic section's outlet

To conclude, the highest percentages of removed phosphorus were nearly 99, 45, 37, and 43% for CODs of 500, 1000, 1200, and 1400 mg/L, respectively. By comparing these four graphs, it is clear that the best removal efficiency was achieved at COD = 500 mg/L.

Table 1 compares some sequencing batch configurations for TP-removal with the combined anaerobic-aerobic system that was used in Phases 1 and 2 of this study.

As indicated in Table 1, the conventional anaerobic-aerobic system with volumes that ranged from 5 to 10 L and hydraulic retention times (HRTs) of 12 to 24 hours demonstrated an efficiency of over 80% for phosphorus removal. In contrast, the integrated bioreactor that was utilized in this study (with a volume of approximately 8 L and the mentioned HRT) achieved TP-removal efficiencies of 94.62 and 98.65% in Phases 1 and 2, respectively, for synthetic wastewater with properties that were similar to municipal wastewater (inlet COD = 500 mg/L).

Earlier studies documented the effectiveness of sequential anaerobic-anoxic or anaerobic-aerobic systems for P-removal. Liu et al. [34], for example, revealed an efficiency of about 94.33% in removing phosphorus from municipal wastewater when using an anaerobic-aerobic-anoxic sequencing batch reactor (AOA-SBR). Furthermore, Brown et al. [35] found that increasing the anaerobic hydraulic retention time from 0.5 to 2 hours increased the phosphorus-removal range from 40 to 82%. As the retention times were increased to 3 hours, the phosphorus-removal efficiency decreased in the anaerobic and anoxic sections. In another study, Mahvi et al. [36] announced that a combined upflow sludge bed-filter (USBF) system (investigated the presence of phosphorus in agricultural and industrial wastewater, reduced these two parameters, and set the wastewater standard to the level of irrigation or drainage to water sources. By using a hybrid system such as USBF, it achieved a 55% phosphorus removal when compared to other traditional wastewater treatment methods [36]. Meanwhile, this study compacted both sections into one pilot experiment (making it cost-effective and requiring a low footprint) and examined its effectiveness in removing phosphorus by considering higher inlet COD concentrations as a side effect.

**Table 1.** Sequencing batch configurations for P-removal

| Reactor type | Volume [L] | Temperature [°C] | Wastewater source | Influent COD/P [g COD/g P] | HRT [h] | MLSS [mg/L] | P-removal efficiency [%] | Reference |
|---|---|---|---|---|---|---|---|---|
| Anaerobic-aerobic | 7 | 20 | synthetic | 13 | 12 | – | 100 | [29] |
| Anaerobic-anoxic (with biofilm) | 10 | 22 ±2 | synthetic | 22 | not described | – | 79 ±6 | [30] |
| SBR+EBPR | 10 | 20 ±1 | synthetic | 40 | 16 | – | 95–97 | [31] |
| Anaerobic-aerobic | 5.4 | 16 ±0.5 | synthetic | 400/8 | 24 | – | >90 (DO = 1 mg/L) | [32] |
| Anaerobic-anoxic | 6 | 16–21 | synthetic | 40 | not described | – | 91.60 | [33] |
| This study | | | | | | | | |
| Combined anaerobic-aerobic bioreactor/Phase 1 | anaerobic: 3 | 37 | synthetic | COD = 500 (COD : N : P = 100 : 5 : 2) | anaerobic: 2.5 | anaerobic: 1220 | 94.62 | – |
| | aerobic: 4.5 | | | | aerobic: 3.75 | aerobic: 1030 | | |
| Combined anaerobic-aerobic bioreactor/Phase 2 | anaerobic: 1.25 | 37 | synthetic | COD = 500 (COD : N : P = 100 : 5 : 2) | anaerobic: 1.042 | anaerobic: 1220 | 98.65 | – |
| | aerobic: 6.25 | | | | aerobic: 5.21 | aerobic: 1030 | | |

When comparing the results that are depicted in Figure 5 (Phase 1, inlet COD = 500 mg/L, maximum TP-removal in anaerobic section: 82.36%) and Figure 12 (Phase 2, inlet COD = 1400 mg/L, maximum TP-removal in anaerobic section: 28.45%), it is evident that the phosphorus-removal rates in the anaerobic section decreased substantially as the concentrations of the inlet COD increased.

Moreover, increases in the residence times in the anaerobic sections resulted in significant decreases in phosphorus removal. This could be attributed to various factors; one possible reason was that extended residence times in the anaerobic sections allowed for the growth and proliferation of phosphorus-releasing bacteria. These bacteria have the ability to release phosphorus back into the wastewater, counteracting the intended removal process. Additionally, longer residence times in the anaerobic sections may have led to increased fermentation and the production of volatile fatty acids (VFAs). VFAs can be utilized as an energy source by phosphorus-accumulating bacteria in the subsequent aerobic sections; in turn, this promotes greater phosphorus uptake during the aerobic stage.

The anaerobic section's output enters the aerobic section; therefore, the low phosphorus-removal percentage is not important because the phosphorus leaves the aerobic section at the end of the system. Since avoiding the aeration of the anaerobic and aerobic section interface is impossible, the middle part usually operates anoxically. Additionally, the phosphorus that is stored in the biological treatment reactor is removed simultaneously with the disposal of part of the biomass. The majority of the phosphorus is expected to be removed through the aerobic processes for several reasons. First, aerobic conditions facilitate the growth and activity of phosphorus-accumulating bacteria, enabling the efficient uptake and storage of phosphorus. Secondly, the availability of dissolved oxygen in aerobic environments acts as an electron acceptor, aiding in the breakdown of complex phosphorus compounds and promoting the release of soluble forms for microbial uptake. Additionally, the use of an aerobic biomass (such as activated sludge or biofilms) further enhances the phosphorus-removal capabilities. These microbial communities possess specific metabolic pathways and enzymatic activities that contribute to the phosphorus's uptake and storage. Moreover, systems with an anaerobic stage preceding an aerobic stage allow for the reaeration of phosphorus-rich sludge, leading to additional removal during the aerobic process.

## 4. Conclusion

In summary, this paper argues the performance of integrated anaerobic-aerobic systems in TP removal at different HRT and influent concentrations. Based on the current findings, this pilot system could provide a viable alternative to conventional systems that have such disadvantages as large space requirements and high energy consumption. It removes 94% of the phosphorus from synthetic wastewater in

Phases 1 and 2, which is similar to municipal wastewater. In other cases, however, the increasing inlet COD causes pilots to encounter fluctuation and shock, and the phosphorus-removal rate does not reach more than 60%. It should be noted that increasing the HRT in Phase 2 only slightly enhances the phosphorus removal (which could be omitted). In the future, additional attempts may prove to be quite beneficial to the literature.

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**Competing interests**

The authors declare no competing interests.

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