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INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF ENVIRONMENTAL ENGINEERING



**THE INVESTIGATION OF BIOREACTOR IN TARGETED
DITCH SYSTEM FOR THE TREATMENT OF
AGRICULTURAL DRAINAGE WATER**

Master's Thesis

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2024

ACCEPTANCE AND APPROVAL OF THE THESIS

The study entitled “**THE INVESTIGATION OF BIOREACTOR IN TARGETED DITCH SYSTEM FOR THE TREATMENT OF AGRICULTURAL DRAINAGE WATER**” prepared by **Forat Jamal Abdulaali ISMAEL**, and supervised by **Prof. Dr. Emre Burcu OZKARAOVA**, was found successful and unanimously accepted by committee members as Master thesis, following the examination on the date 29.1.2024 .

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ÖZET

TARIMSAL DRENAJ SUYUNUN ARITILMASINA YÖNELİK HEDEFLİ HENDEK SİSTEMİNDE BİYOREAKTÖRÜN İNCELENMESİ

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Atık su ve tarımsal suyun arıtılmasındaki zorluklar gerçek ve ekonomik açıdan maliyetli bir sorundur. Acil ve ekonomik açıdan önemli olan konu, atık su ve tarımsal su arıtımıyla ilgili zorlukların etkin bir şekilde ele alınmasında yatmaktadır. Nitrat ve fosfor kirliliğinin kaynaklarının başında gübre geliyor olup drenaj sularının hassas alıcı ortamlardaki etkisi nedeniyle çeşitli önlemler geliştirilmiştir. Drenaj suyunun arıtılmasının ve yeniden kullanımının önemi, özellikle göller, deltalar ve nehirler gibi alıcı ortamların su kalitesi üzerindeki etkileri göz önüne alındığında önemli görülmektedir. Bununla birlikte su kaynaklarının %70'inin tarım için kullanıldığı düşünüldüğünde drenaj sularının arıtılması ve tekrar değerlendirilmesi her geçen gün daha önemli görünmektedir. Doğa temelli çözümler (nature based solutions), maliyet etkinliği, ekolojik sürdürülebilirlik ve yenilenebilir kaynakların kullanımına atfedilen önemli potansiyelleri ve pratiklikleri nedeniyle son zamanlarda büyük ilgi görmeye başlamıştır. Bu araştırma, nitrat, amonyum ve fosfatın etkili bir şekilde uzaklaştırılması için hendek içi sistemler (inditch systems) geniş kullanım alanlarını araştırmaktadır. Çalışma kapsamında işletilen hendek içi sistemi nitrat, fosfat ve amonyum gidermek amacıyla biyofiltre ve iki adet reaktif (kalsine deniz kabuğu ve zeolite)'ten oluşan bölmeden oluşmaktadır. Kenevir kökünden oluşan biyofiltrenin sadece %94'nin üzerinde nitrat giderimi yanı sıra fosfat ($\leq\%94$) ve amonyum ($\leq\%98$.) de gerçekleştirmiştir. Kalsine deniz kabuğu ilk günlerdeki yüksek fosfat salınımı nedeniyle etkinlik gösterememiş olup kullanılan zeolit olağanüstü amonyak giderim verimleri sergilemiştir. Zeolit, neredeyse %100'lük oldukça yüksek bir arıtım verimliliği sergilemiş ve büyük kapasiteli, olağanüstü etkili bir iyileştirme işlemi olarak işlev görmüştür. Bununla birlikte laboratuvardaki sıcaklık artışlarının da dönem dönem arıtım verimini arttırdığı anlaşılmıştır. Genel olarak giderim verimini etkileyen faktörler arasında reaktif yaşı ve hidrolik bekleme süre yer almaktadır. Kapsamlı laboratuvar ve saha çalışmaları yürütmek yalnızca biyoreaktör trendleri ve uygulamalarına ilişkin anlayışımızı geliştirmekle kalmaz, aynı zamanda performanslarını optimize etmek için değerli bilgiler de sunar. Bu, Hendek içi sistem, özellikle gelişmekte olan ülkelerdeki acil zorlukların üstesinden gelmek için en pratik ve uygun maliyetli yaklaşım haline getirmektedir.

Anahtar Sözcükler: Mulç biyoreaktörleri, Tarımsal drenaj suyu, Doğa bazlı çözümler

ABSTRACT

THE INVESTIGATION OF BIOREACTOR IN TARGETED DITCH SYSTEM FOR THE TREATMENT OF AGRICULTURAL DRAINAGE WATER

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The challenges of treating wastewater and agricultural water are a real and economically costly problem. The urgent and economically significant concern lies in effectively tackling the challenges associated with wastewater and agricultural water treatment. The issue of nitrate exports through stormwater and agricultural runoff is a significant global concern due to the wide array of pollution sources involved. The significance of reclamation and reuse of drainage water cannot be overstated, particularly in relation to their effects on the water quality of receiving bodies such as lakes, deltas, and rivers.

Nature-based solutions have recently gained significant attention due to their considerable potential and practicality, which is attributed to their cost-effectiveness, ecological sustainability, and utilization of renewable resources. This research explores the wide range of bioreactor use for effectively removing nitrate, ammonium and phosphate. It highlights the suitability of different natural materials for constructing woodchip bioreactors, which in turn leads to improved outcomes in terms of removal and treatment. Furthermore, it attained exceptional ammonia removal outcomes by employing zeolite. Zeolite exhibited a fairly high removal efficiency of almost 100%, functioning as an exceptionally effective polishing process with a large capacity. Additionally, this study uncovers a significant increase in the elimination of nitrate-N as temperatures increase, accompanied by the presence of a specific temperature coefficient. Significant contributors to enhancing removal efficiency include factors such as reactive age and hydraulic retention time. Conducting comprehensive laboratory and field studies not only enhances our comprehension of In-ditch systems trends and applications but also offers valuable insights for optimizing their performance. This renders bioreactors the most practical and cost-effective approach for addressing urgent challenges, particularly in developing countries.

Keywords: Woodchip bioreactors, Agricultural drainage water, Nature-based solutions

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SYMBOLS AND ABBREVIATIONS

BMP	: Best Management Practices
EPA	: Environmental Protection Agency
WHO	: World Health Organization
HRT	: Hydraulic Retention Time
COD	: Chemical oxygen demand
DO	: Dissolved oxygen
ORP	: Oxidation Reduction Potential
DNRA	: Dissimilatory nitrate reduction to ammonium
Q	: Flow rate
USEPA	: United States Environmental Protection Agency
CEC	: Cation Exchange Capacity

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1. INTRODUCTION

Around two-thirds of the Earth's surface is covered by water, the majority of which is composed of saline seawater, accounting for around 97% of the total water volume. Merely 3% of the Earth's total water supply is classified as fresh water, while the remaining 2% is confined within ice caps and glaciers. According to the study conducted by (Tahir et al., 2020). The majority of developing nations throughout the world are plagued by a scarcity of water resources, whether it be surface water or groundwater, as well as a decline in the quality of these water supplies as a result of pollution. The effects of changing climate have a significant and negative impact on this scenario (Kusangaya et al., 2014)

According to the 2016 World Water Development Report of the United Nations (UNWWAP, 2016), there may be a 40% worldwide water shortfall by 2030. With rising temperatures owing to climate change, this startling number is expected to get worse. In 2017, the World Bank estimated that, globally 70% of freshwater is used for agricultural activity that feeds the human population of Earth. Plants, on the other hand, only absorb 5% to 30% of this water (Wallace et al., 2002)

Nitrate (NO_3) pollution of the world's water supplies has recently been identified as one of the most pressing environmental issues of our day. Nitrate contamination can come from a variety of sources, some of which are point sources and some of which are nonpoint sources. Some of these sources include the discharge of wastewater from municipal facilities, runoff from agricultural areas that use significant amounts of chemical fertilizers, and deposition from the atmosphere.

Nitrate concentrations greater than 2.0 mg/L are deleterious to several types of freshwater organisms (Isaza et al., 2020). The excessive accumulation of nitrate waste originating from agricultural watersheds has global implications for water quality, giving rise to a multitude of environmental concerns such as eutrophication, proliferation of algal blooms, and detrimental effects on aquatic life, including fish mortality (Addy et al., 2016). From 1970 to 2000, human-caused global output of reactive nitrogen grew by around 225%, surpassing all terrestrial natural production (Martin et al., 2015).

This production came from agricultural cultivation, fossil fuel burning, and the manufacture of nitrogen fertilizers. The presence of nitrogen compounds in both surface and groundwater can be linked to a variety of sources, including civilian and

industrial wastes, agriculture, and animal husbandry. Most tree species' wood contains 47% to 51% carbon (C) and less than 0.20% nitrogen (N) (Martin et al., 2015). Organic nitrogen, in particular, tends to be converted into ammonium and persists in this form under reducing circumstances. Ammonium, on the other hand, is poisonous to fish and is a major contributor to eutrophication (Randall and Tsui, 2002; Smith et al., 1999). Moreover, changes in chemical conditions or biological processes might encourage its transition into other molecules, including the creation of carcinogenic nitrosamines (Nawrocki and Andrzejewski, 2011).

Phosphorus (P) represents a notable source of nutritional pollution in water systems. Its introduction into aquatic environments occurs through various activities such as mining, industrial and agricultural operations, as well as sewage discharges. The elevated concentrations of phosphorus in water contribute to the phenomenon known as eutrophication (Hussain et al., 2011). Phosphorus is found in water and wastewater in the form of orthophosphate, polyphosphates, and organic phosphorus.

Hydrolysis and/or microbial mobilization convert polyphosphates and organic Phosphorus to orthophosphate (Weiner et al., 2012). Reminding that the majority of freshwater is used for agriculture, the management of drainage water is also taken serious. Drainage water may contain various pollutants like nitrate, ammonium, phosphate and different pesticides. To avoid the deterioration (e.g., eutrophication) of receiving water bodies (e.g., groundwater, rivers and lakes) it is required to treat contaminated water sources (e.g., wastewater, drainage water etc.).

Although there are several technologies (such as combination nitrification/denitrification systems, breakpoint ion exchange, ammonia volatilization, selective ion exchange, and selective ion exchange) for removing nutrients (e.g., nitrogen) from wastewaters, they are frequently expensive (Cameron et al., 2010). Therefore, water treatment with nature based self working systems is under continuous research.

Enhancing understanding regarding the importance of these systems, along with examining current and future technologies aimed at enhancing their management, will contribute to the formulation and implementation of water quality conservation initiatives. Due to the relative newness of the technology and the relatively lengthy expected life, there is a scarcity of information on denitrifying bioreactors treating subterranean drainage water near the end of their initial design life (Christianson et al., 2020).

Therefore, it is necessary to carry out a laboratory investigation that incorporated a continuous bioreactor for the treatment of agricultural drainage water. The reactor comprised three primary components: the biological, physical, and chemical sections, all housed within the same unit. The 357 project days serves as a successful demonstration of bioreactor systems as an effective option for treating pollution through Nature-based solutions. Through careful monitoring of the removal outcomes, we have shown that the efficiency of removal is influenced by factors such as temperature, hydraulic retention time, and flow velocity. The hemp roots exhibited exceptional efficacy in the biological section of the reactor for nitrate removal



2. LITERATURE SURVEY

2.1. Agricultural Drainage Water

The term "drainage" encompasses the methods and techniques employed to eliminate surplus water from both the surface and subsurface layers of soil (Figure 2.1). Therefore, drainage systems are used to manage agricultural drainage water. The design of a drainage system (such as cannels and/or ditches) depends on many factors such as type of agricultural irrigation method, topography, regional and climatic conditions. The composition of drainage water may change from region to region depending on water use, variations in agricultural activities, seasons and/or climatic conditions. When water from drainage passes over or through the soil, it collects various substances that are dissolved or suspended, such as salts, organic compounds, and soil particles (Kvítek et al., 2015).

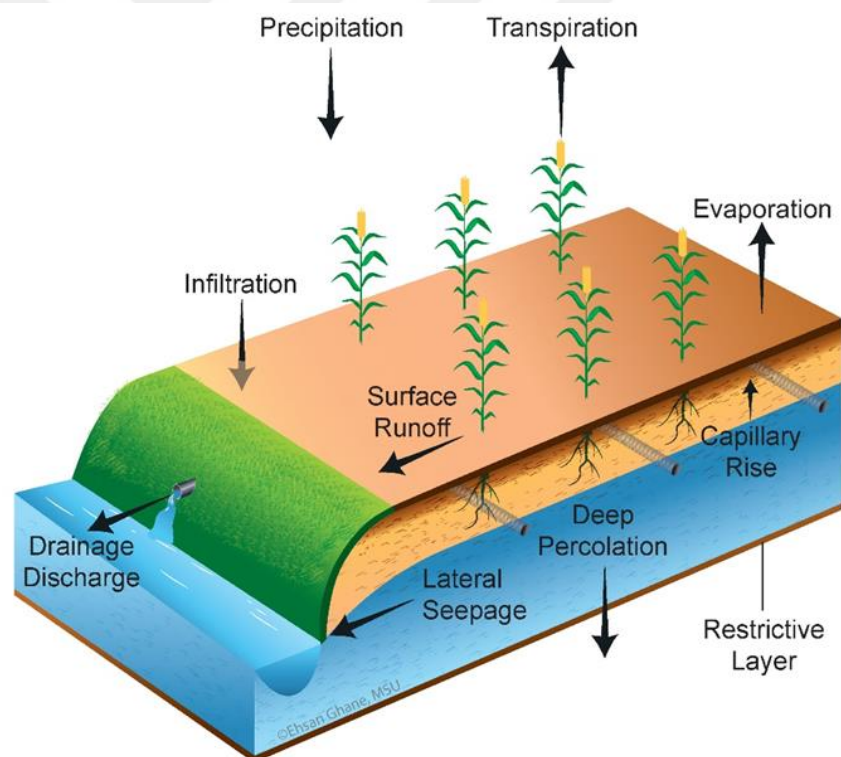


Figure 2.1 Illustrates the water cycle on a field that utilizes subsurface (tile) Drainage (Ghane 2018).

The composition of agricultural drainage water encompasses several types of wastewater that arise from activities such as crop irrigation, livestock production (e.g., piggeries), and farming practices (Ateia et al., 2015). Thus, the drained water entering the drainage ditch may sometimes more concentrated influenced from point sources or more diffused. Concentrated streams can arise from contaminating point source farming activities (e.g., husbandry, piggeries etc.), while nonpoint source pollution may be due to precipitation, surface runoff, and stream discharge. Surface runoff and stream discharge resulting from irrigation traverses from/within agricultural areas, may transport significant amounts of pesticides and fertilizers into the system (Pandey et al., 2022).

2.1.1 General Composition

The composition of drainage water is very variable depending on various reasons stated above. The primary pollutants in agricultural drainage water may be summarized as nitrogenous compounds (e.g. nitrate, ammonium etc.), phosphorus compounds (e.g. phosphate), potassium and some others like heavy metals arising from different fertilizers (organic and inorganic) and various pesticides. Contaminants are not limited to these primary compounds as agricultural activities are very broad and can be applied in different ways (intense agriculture vs organic agriculture). The concentration of pollutants changes according to the application of chemicals, intensity of other farming activities and water regimes (e.g. precipitation, irrigation). A brief summary of some important parameters can be seen in Table 1.

Table 2.1 Drainage water composition of different locations

Location	Nitrate (mg NO ₃ ⁻ - N/L)	Ammonia (mg/L)	Phosphorus (mg P/L)	DO (mg/L)	pH	EC (µS/cm)	Reference
United States	8.28	0.18	0.94				(Gali et al., 2012)
Portugal	5.65	1.05			7.7		(Palma et al., 2023)
China	7.36	0.93	0.42				(Shao et al., 2013)

Egypt	4.89			2.99	7.43		(Shaban et al., 2010)
Egypt	3.90	0.75	0.60		7.30	1375.0	(Allam et al., 2013)
Italy	8.80	0.10	0.20				(Lavrić et al., 2018)
Egypt	3.70			3.15	8.10	2962.5	(Nasr et al., 2016).
Egypt	12.80			7.20			Assar et al., 2019).

2.1.2 Impact of Drainage Water Discharge into Receiving Water Environment

Direct discharge of agricultural drainage water into the ecosystem may result in water quality impact, direct habitat loss or alteration and hydrologic effect. The impact of drainage water strongly depends on its composition. The number and level of pollutants present in the drainage water and the load of drainage water into the ecosystem will determine its influence on both water quality and environmental health.

Surface and subsurface drainage water from irrigated agriculture is normally degraded compared with the quality of the original water supply. Studies on agricultural drainage focused on cumulative spatial and temporal effects and in general found changes in ecosystem structure and function (Figure 2.2). Summary of these studies presented loss in biodiversity and habitats, alteration in hydrology of wetlands, streams and rivers due to agricultural drainage (Blann et al., 2009).

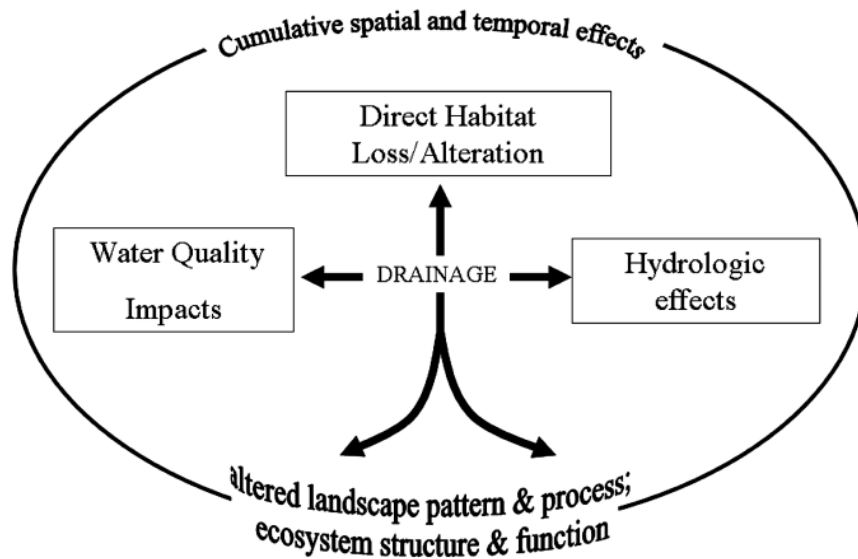


Figure 2.2 Conceptual model of the impacts of agricultural drainage water after discharge (Blann et al., 2009).

In general, the release of drainage water can cause substantial adverse effects on the water environment it flows into. These effects include the deterioration of water quality, harm to aquatic organisms, changes in physical properties, limitations on human interaction, and economic consequences.

Drainage discharge nutrients can cause eutrophication resulting in recipient bodies being more susceptible to colonies of undesirable blue-green algae. Periodic high discharge rates from artificial drainage could reduce the salinity of estuary headwaters, thereby potentially altering the estuary's ecosystem (Madramootoo et al., 1997).

2.1.3 General Removal Treatment for Nitrate, Ammonium and Phosphate

Drainage water treatment is typically seen as one of the final alternatives for managing drainage water. The rationale behind this phenomenon might be attributed to the significant financial implications and the inherent ambiguity around the attainable extent of treatment. The consideration of wastewater treatment becomes necessary in situations where alternative strategies for drainage water management prove ineffective in guaranteeing safe disposal or when it presents a financially viable option.

Treatment objectives for subsurface drainage water with elevated salinity, selenium, and other trace elements to decrease the concentration of salts and toxic

constituents to levels that are not considered hazardous, to meet the objectives of agricultural water management, to attain water quality targets in surface waters, and to lower the levels of these substances to levels that do not pose a risk to wildlife.

Drainage water can be managed according to site specific conditions in different ways including its reuse (Figure 2.3). So, the final decision determines whether treatment is needed and if needed at what level for its proper management. For example, the nature and condition of receiving water resources will clarify the treatment processes to be used for the removal of specific contaminants. The elimination of nitrate, ammonium, and phosphate from water is of utmost importance in order to avert water contamination and the process of eutrophication in aquatic ecosystems. There exist multiple methodologies for their elimination, encompassing biological, physical, and chemical approaches.

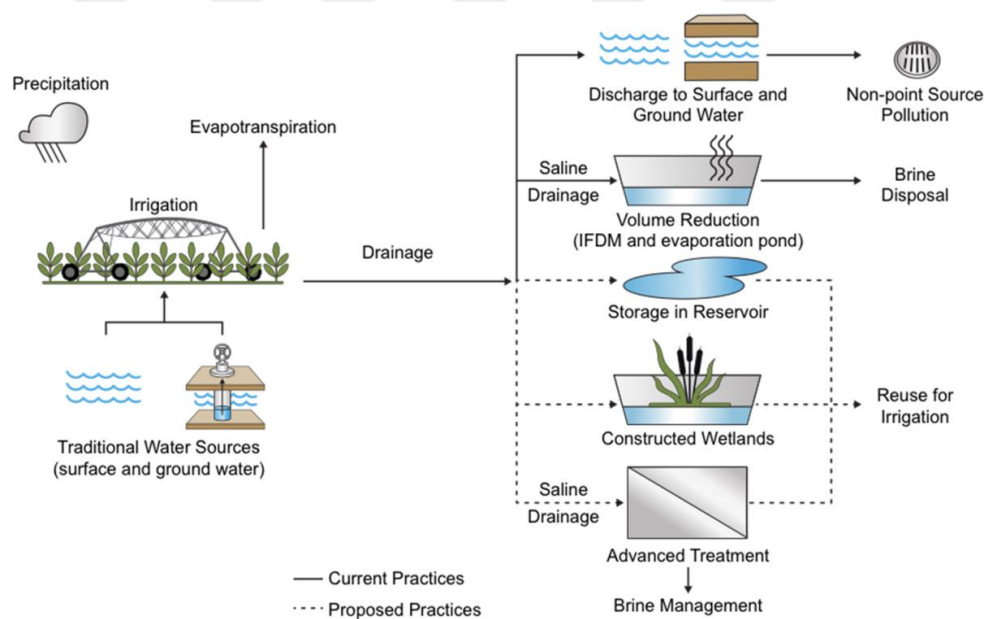


Figure 2.3 Different approaches for agricultural drainage water

(Hejase et al., 2022).

Nitrate is generally removed by biological processes, but some physicochemical processes are also used. Removal of nitrate from water is generally done with biological denitrification process. Denitrification is a microbiological

process that converts nitrate and nitrite into gaseous forms of nitrogen, primarily nitrous oxide (N₂O) and nitrogen (N₂). A wide variety of microorganisms have the ability to denitrify. Denitrification occurs as a reaction to variations in the oxygen (O₂) levels in the surrounding environment. Denitrifiers will only transition from aerobic respiration to anaerobic respiration and utilize nitrate (NO₃) and nitrite (NO₂) as an electron acceptors when there is a restriction of O₂.

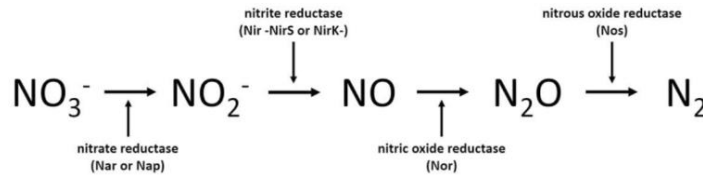


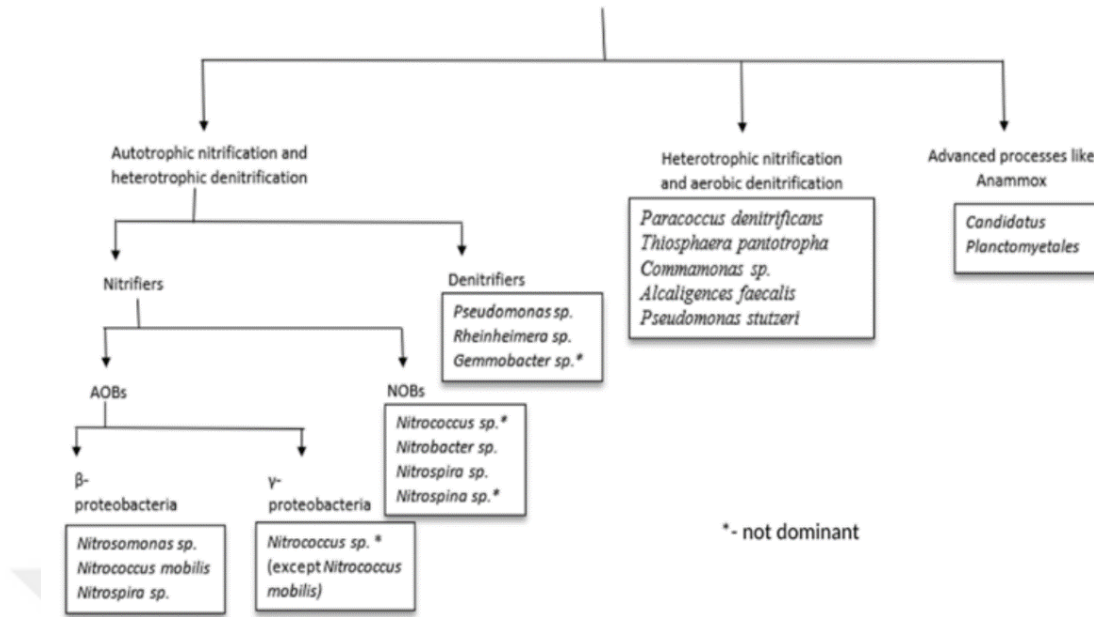
Figure 2.4 Denitrification process steps

(Adapted from Lee et al., 2023).

Nitrate is also removed very often by ion exchange, which involves anionic resins that allows replacement of an anion on resin surface with the nitrate in water. Exhausted resins will require treatment for its regeneration causing to additional control of pollutants released after regeneration process and final disposal of resins Afterall. Among other processes are electro-chemical reduction (chemical denitrification) and reverse osmosis, but these are rarely applied.

Ammonia removal with biological treatment is generally done with nitrification, but with regard of water source and its composition, other processes like Anammox is also carried out. When nitrification followed by denitrification total removal can be achieved with biological transformation processes. Nitrification is simply the oxidation of ammonia in the presence of oxygen under carbon limited conditions to nitrite and nitrate. Anammox, on the other hand, is biotransformation of ammonia to nitrogen gas under anaerobic conditions.

Classification of microbes responsible for nitrification and denitrification at a glance



*- not dominant

Figure 2.5 Classification of microorganisms involved in nitrification and denitrification (Bhattacharya et al., 2021).

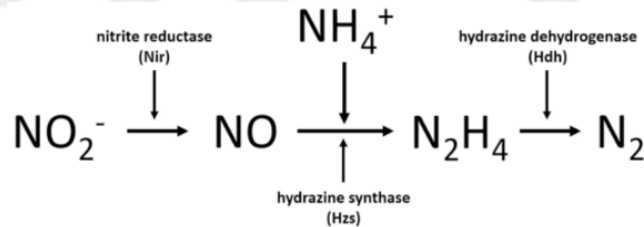


Figure 2.6 Anammox process steps (Adapted from Lee et al., 2023).

At elevated ammonia concentrations ammonia stripping is also used for its removal. Adsorption and ion exchange are often among the applied methods, preferred over precipitation (as struvite) and membrane processes such as reverse osmosis. Phosphorus removal can be based on two process steps, first of which is relying on the conversion of soluble phosphorus to solid form by biological or chemical processes, followed by another second step for the removal of solids from water by clarification. In the biological processes phosphorus accumulating organisms take part.

Conventional wastewater treatment schemes for enhanced biological phosphorus removal are based on anaerobic and aerobic treatment, followed by

clarification (Luo et al., 2018). Some treatment schemes also involve anoxic treatment steps for additional nutrient removal. Chemical precipitation is a good alternative to biological treatment, but is similarly not effective at low phosphorus concentrations.

For low level phosphorus presence like drainage water, removal processes like sorption and ion exchange are preferred. Nowadays constructed wetlands are also widely preferred as they involve multiple processes under self-working sustainable systems (Loganathan et al., 2014).

2.2 Denitrifying Woodchip Bioreactor

Denitrification is the primary microbial process responsible for eliminating NO_3^- . It involves a series of successive enzymatic reactions. Reactions that decrease the concentration of NO_3^- to NO_2^- , NO_2^- to NO , NO to N_2O and finally N_2O to N_2 . Woodchip-based denitrification systems have been found to be highly effective in reducing nitrate concentrations in agricultural runoff (Johnson et al., 2022).

2.2.1 General Principles

Biological denitrification is a purely biological process that requires an appropriate treatment system and an organic or inorganic carbon source. It contains adequate quantities of C, H, O, N, P, and S, as well as trace amounts of minerals (K, Na, Mg, Ca, and Fe) (Hiscock et al., 1991).

Bioreactors typically consist of excavated pits that are lined and filled with a solid carbon source, often woodchips. These bioreactors create an anaerobic environment that facilitates the proliferation of heterotrophic denitrifying microorganisms, which thrive in the absence of oxygen (Schipper et al., 2010).

Denitrifying bioreactors have been categorized into three distinct types according to their hydrological settings. The first type is denitrification walls, which are designed to intercept shallow groundwater flow. These walls are positioned perpendicular to the flow direction. The second type is denitrifying beds, which are designed to intercept subsurface discharge. In this case, the flow path runs along the length of the bed. The third type is denitrifying layers, which are horizontal covers made of solid carbon substrates. These layers effectively reduce nitrate loads from leachate, preventing their entry into the groundwater. Numerous scholarly

investigations have been conducted to examine denitrifying beds and their application in the treatment of diverse types of wastewaters, including agricultural tile drainage, aquaculture wastewater, stormwater, and nitrified wastewater (Moghaddam, 2023).

A denitrifying bioreactor can be described as a containment structure that contains a carbonaceous substrate, such as woodchips or maize husks. Within this structure, microorganisms facilitate the conversion of nitrate through the denitrification process, resulting in the production of gaseous nitrogen compounds, primarily dinitrogen gas (N_2). Denitrifying bioreactors have been employed for the remediation of shallow contaminated groundwater by the implementation of a permeable reactive barrier, commonly known as a 'denitrification wall', which intercepts shallow lateral flow (Rivas et al., 2020). Woodchip-based materials constitute the most prevalent of the numerous types of media utilized (Schipper and Vojvodic-Vukovic, 2001; Robertson and Merkley, 2009).

The reaction rate in denitrifying bioreactors is influenced by the size and texture of the carbon source in multiple ways. When the carbon releasing potential is strong, the presence of small particle sizes will lead to elevated concentrations of organic carbon in solution over shorter periods of time. Furthermore, alongside heightened carbon concentrations, the discharge of various substances such as nitrogenous and phosphorus compounds, heavy metals, and others has been documented as pollutant swaps (Healy et al., 2012).

2.2.2 Affecting Factors:

Bioreactors for drainage systems are specifically engineered to effectively control and process agricultural subsurface drainage water. Various variables can influence the performance and efficacy of these bioreactors. The performance of individuals can be influenced by several crucial factors which may have a significant impact. The paramount of these factors is given below.

2.2.2.1 Temperature

The influence of temperature on biological processes is widely recognized, as there is a general trend of increasing rates with higher temperatures. When conducting

a comparison between denitrifying beds operating at different temperature ranges, it was observed that beds with intermediate temperatures ranging from 6 to 16.9°C, which represent the typical groundwater temperature range in the Midwest of the United States, and beds with higher temperatures exceeding 16.9°C ($p < 0.05$), displayed noticeable differences in nitrate removal efficiency. Specifically, it was evident that beds operating at temperatures below 6°C exhibited lower levels of nitrate removal, as depicted in Figure (2.7 a). Remarkably, there was no substantial differentiation detected between the high-temperature and intermediate-temperature classifications, despite a considerable proportion of the rates of bed nitrate removal displaying elevated values within the high-temperature cohort.

The relationship between temperature and the variation in effect sizes (lnR) among bed studies was shown to be statistically significant ($p < 0.1$). The linear model, as shown in Figure (2.7 b), revealed that the removal rate (N removal rate = $1.79e0.0766$) was dependent on temperature after back-transformation from variance-weighted mean effect sizes. The regression analysis yielded a Q10 value of 2.15, indicating that the removal rate increases by a factor of 2.15 for every 10°C rise in temperature. This value closely aligns with those published in previous studies on bioreactors by (Cameron and Schipper, 2010; Warneke et al., 2011b). According to (Thapa et al., 2023), the efficiency of nitrate removal is diminished when the temperature falls below 12 °C.

Full-scale woodchip bioreactors can be employed to eliminate effluent NO₃-N in freshwater recirculating fish farms. (Within the temperature range of 4.5 to 15.6 °C, as assessed in the present study) Woodchip bioreactors demonstrate consistent and annual rates of NO₃-N removal (von Ahnen, et., 2018).

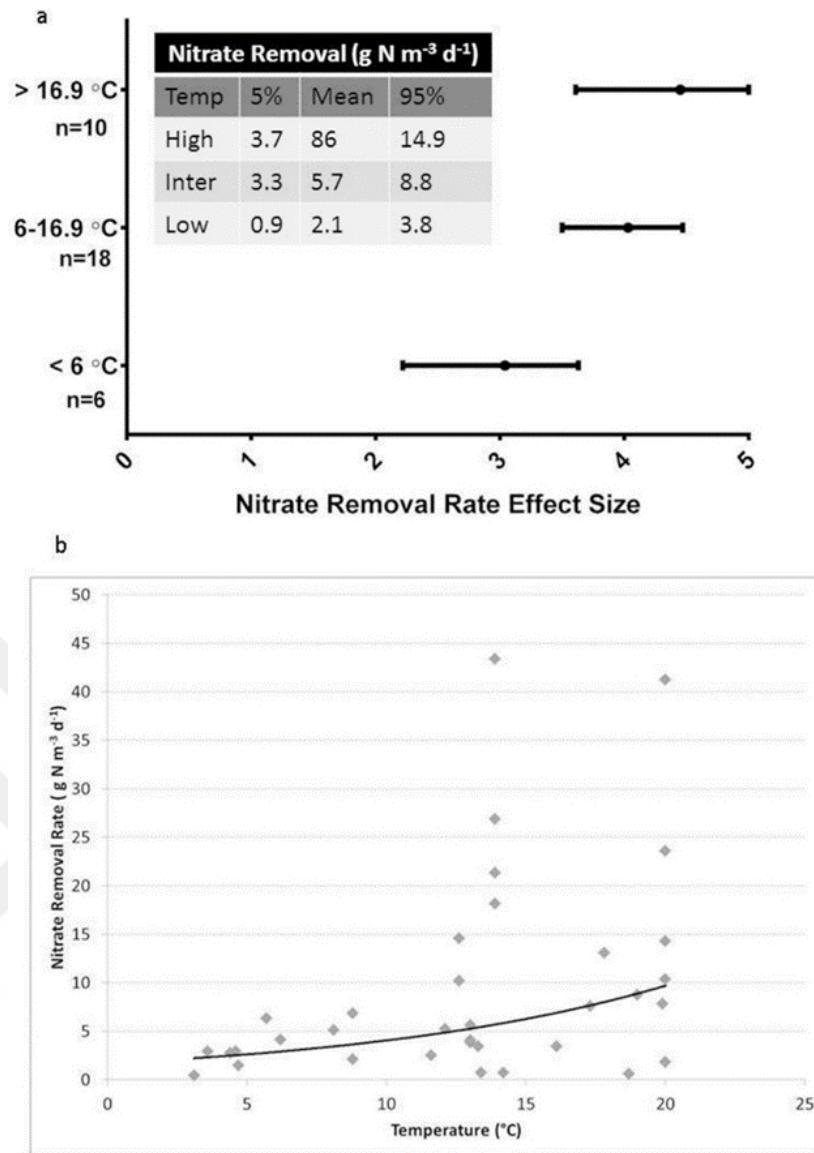


Figure 2.7 The bed size and bias-corrected 95% confidence interval depend on the mean nitrate removal rate in different temperature groups. Bed units in each analytical category are represented by the bars' n numbers. Bars with various letters indicate significant differences ($p < 0.1$). The mean nitrate removal rate ($\text{g N m}^{-3} \text{d}^{-1}$) calculated in reverse is included in the supplemental tables, along with a 95% confidence interval adjusted for bias for each category (trends remain unchanged). Addy et al. (2016) found a significant linear association (b) ($p < 0.1$) between variance-weighted mean effect sizes and temperature in nitrate clearance rates.

2.2.2.2 Dissolved Oxygen (DO)

According to the data obtained from the database, denitrification predominantly occurs when the dissolved oxygen (DO) concentration is below 2 mg/L, as illustrated in Figure (2.8 C). The highest average reduction rating (NRR) was recorded at a dissolved oxygen (DO) level of 0-0.5. Furthermore, the NRR dropped as the DO increased within the range of 0-2 mg/L. The data presented in Figure (2.8D) does not show a clear pattern for NRE and DO. The specific threshold that must be attained by DO to effectively obstruct NRR remains uncertain, as indicated by the research conducted by Warnake et al. (2011). Furthermore, there is a consensus among scholars that anaerobic environments confer benefits for the process of denitrification.

According to Knoll et al. (2020), denitrification is improbable when the dissolved oxygen (DO) concentration exceeds 5 mg L⁻¹, which indicates favourable aerobic conditions. The underlying factor is that elevated dissolved oxygen (DO) levels have the potential to promote nitrification, while concurrently impeding denitrification (Li et al., 2012). Furthermore, it should be noted that the redox reaction sequence occurring in saturated zones commences with the reduction of oxygen to water, with a Gibbs free energy (G) value of 78.5 and an oxidation-reduction potential (Eh) of +334. This sequence culminates with the reduction of nitrate, which has a Gibbs free energy (G) value of 72.3 and an oxidation-reduction potential (Eh) of +231, as reported by Rivett et al. (2008).

Organic carbon frequently functions as an electron donor, particularly in the presence of molecular oxygen (O₂). Before the utilization of molecular oxygen became prevalent, nitrate was the preferred electron acceptor. The process of converting nitrate to inert nitrogen gas is accelerated by the reduced oxygen concentration in wastewater treatment bioreactors (WBRs). Thus, consistent with the study conducted by Li et al. (2012), our results indicate that the dissolved oxygen (DO) level for nitrogen removal rate (NRR) in a whole bed reactor (WBR) should range from 0 to 0.5 mg/L.

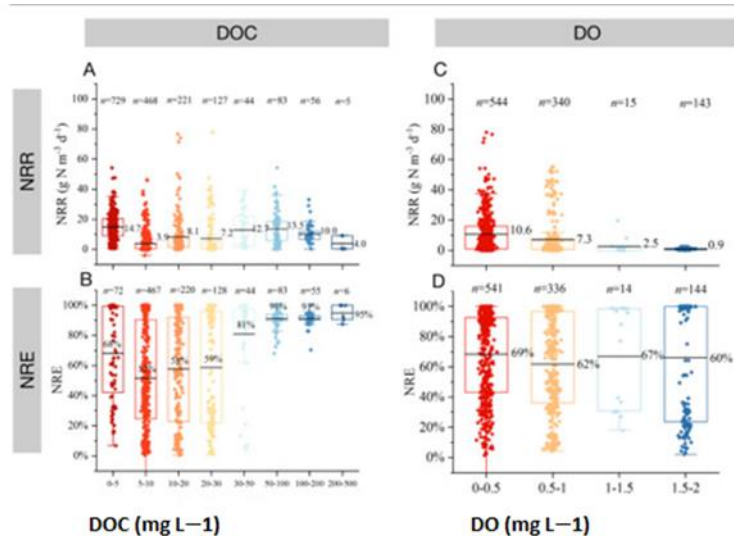


Figure 2.8 Changes in dissolved oxygen levels (Fan, et al., 2022).

2.2.2.3 Hydraulic Residence Time (HRT)

The performance of a bioreactor can be influenced by the Hydraulic Retention Time (HRT). Previous research conducted by Schipper et al. (2010a) has demonstrated that an increased duration of contact time results in a greater percentage reduction in nitrate load. The significance of a suitable retention period for denitrifying woodchip bioreactor N removal is well proven (Addy et al., 2016). Werner and Kadlec assert that the hydraulic loading rate (HLR) is a significant factor in the regulation of phosphorus (P) retention and the modulation of water flow patterns. These flow patterns, in turn, determine the distribution of hydraulic retention time (HRT) across the system (Pugliese et al., 2020).

2.2.2.4 Conductivity

Various carbon media are expected to have varying hydraulic conductivities, which is crucial for keeping effluent flowing through the bed. The deterioration of cell structure, the production of gas bubbles, secondary mineral precipitates and/or the growth of biomass can all contribute to a reduction in hydraulic conductivity over time (Cameron et al., 2010). According to Ghane et al. (2014), the hydraulic conductivity of fresh woodchips was substantially higher than that of 26-month-old woodchips.

2.2.2.5 Age of The Bioreactor

The chronological age of a bioreactor can have diverse ramifications for its design, operation, and uses. The lifespan of a functional bioreactor mostly depends on the sustainability of its carbon source. The availability of carbon over time is determined by various relevant elements, including the type of carbon and its saturation levels (Christianson et al., 2011; Ranaivoson et al., 2012). Heterotrophic bacteria obtain necessary energy for the conversion of nitrate to nitrogen gas through the electrons obtained from organic carbon, as explained by Knowles (1982).

During the early stages of operation, it is expected that woodchips may experience degradation, leading to carbon loss. However, they have shown the ability to last for up to twenty years while still retaining a reduced yet efficient rate of nitrate removal (Schipper et al., 2010). In a study conducted by Robertson et al. (2010), a thorough examination was carried out to observe the physical changes of wood particles within a denitrifying barrier over a period of time. The study's findings were highly valuable. These studies revealed clearly visible dark rings on the cross-sections of large wood particles. These rings extended from the exterior and spanned several mm, contrasting with the original lighter colour of the wood.

The process of carbon breakdown can be significantly hindered when the material remains consistently saturated (Ranaivoson, 2012; Schipper et al., 2010). A study found that woodchips positioned below the water table maintained 80% of their original carbon content throughout the year (Schipper et al., 2010).

In addition, Robertson (2010) conducted an extensive investigation lasting seven years, where a hydraulic retention time of 3 to 7 hours was maintained for a bed of woodchips. The findings of this investigation revealed limited evidence of substantial declines in removal rates over time. In a study done by Robertson et al. (2010), the nitrate removal rates of newly harvested woodchips were compared to those of woodchips that had been used in a denitrifying bioreactor for 2 and 7 years. The 7-year-old woodchip sample showed a removal rate that was 40-59% of the new woodchip sample, which is worth mentioning. Nevertheless, it was discovered that this rate fell within 75% of the woodchip sample that is 2 years old. Further investigation is warranted to assess the influence of bioreactor age on a larger scale in the field (de Oliveira et al., 2023).

2.2.2.6 pH

The pH of drainage water in a denitrification bed is crucial for maintaining water quality and can also serve as an indicator of potential alternate reactions occurring, aside from total denitrification. The ideal pH range for achieving complete denitrification is between 7.0-8.0. Lower pH values provide an environment that hinders the conversion of nitrous oxides into nitrogen gas (Knowles, 1982; Warneke et al., 2010). According to Christianson et al. (2012b), the pH values in the bioreactor have been seen to be below 7.0 during the initial weeks and months of operation, but they gradually increase over time. Extremely acidic settings (pH 5) hinder denitrification and tend to halt the denitrification cycle with the generation of nitrite or N_2O (Rivett et al., 2008).

2.3 In-Ditch Applications for Drainage Water Treatment

The phrase "drainage ditch" encompasses a wide range of channel-like depressions in a given terrain, via which water is directed away from the area and into a nearby water body (Needelman et al., 2007). Drainage ditches are typically situated in areas where surface water naturally accumulates, such as downhill slopes, however they may also receive water flow via pipelines. Drainage trenches exhibit a diverse range of dimensions and configurations, catering to a multitude of distinct objectives contingent upon their specific environmental contexts. The agricultural region possesses a distinct drainage system that has been specifically engineered to fit its needs.

2.3.1 General Principle and Applications

Audet and his friends used eight bioreactors to remove NO_3 from agricultural drainage water. The flow designs of the reactors were different (vertical upward and vertical downward), but the reactor sizes were the same. The findings provided evidence that woodchip bioreactors are effective for removing NO_3 from agricultural drainage water, but they also demonstrated that NO_3 removal efficiency may differ amongst bioreactors (Audet et al., 2021)

2.3.2 Configurations and Real-Case Application of In-Ditch Systems

In situ denitrification bioreactors are designed structures that capture polluted water, such as shallow groundwater or drainage system outputs. Natural denitrification, or the conversion of NO_3 to N_2 gas by microbial breakdown of organic carbon (C), occurs in soils and aquifers. Natural attenuation can be limited by factors such as high dissolved oxygen (DO) concentrations, poor organic C bioavailability, or short transit periods. Denitrifying bioreactors employ a range of C-rich reactive medium, resulting in optimum conditions for high denitrification rates (Schipper et al., 2010).

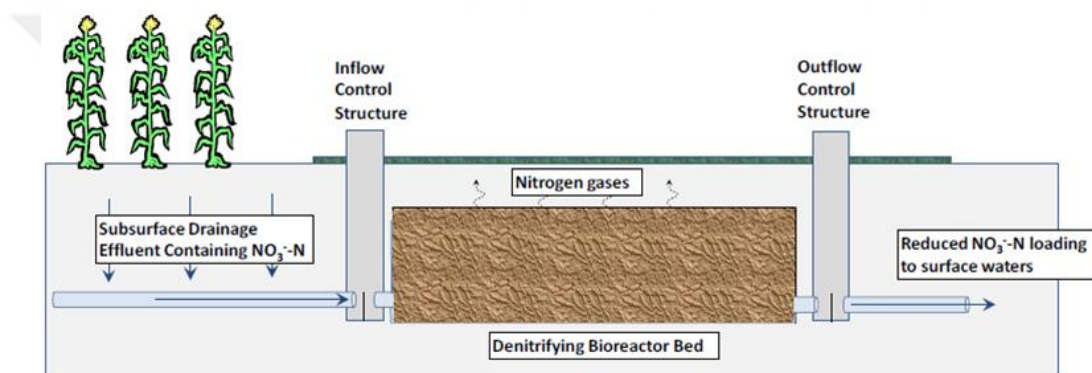


Figure 2.9 Schematic of denitrification bioreactor for agricultural drainage (adapted from Christianson et al., 2012).

A bioreactor with a length of 92 m was installed within a trench on a privately owned farm located in Crisfield, Maryland, during the month of December in the year 2015. The bioreactor consisted of three separate segments, each measuring 27, 38, and 27 m in length, respectively. These segments were specifically constructed to accommodate hydraulic retention times that ranged from 4.6 to 11.5 hr. It is worth mentioning that there was a discrepancy in the excavation depths of the ditch bottom between the upstream and downstream ends. Specifically, the depth below the bed elevation was 0.6 m at the upstream end and 0.3 m at the downstream end. In order to delineate the sections, three wooden embankments were implemented, with heights of 91, 76, and 61 cm, respectively. These embankments were afterwards filled with

woodchips to depths of 46, 30, and 15 cm, correspondingly. In order to stabilize the woodchips, a layer of 57 stone with a thickness ranging from 10 to 15 cm was utilized for each part. It is worth noting that wire or plastic mesh might also be employed as viable alternatives to fulfil the same objective.

In addition, a flow collecting manifold was implemented upstream of each wooden berm. This manifold consisted of two sections of perforated tile, each measuring 1.5 m in length and 10 cm in diameter, connected by a 'T' connector. The water from each section of the bioreactor was sent through the drainage system into a 76-m unperforated tile with a diameter of 10 cm. Significantly, in the event that a berm's capacity is exceeded, any surplus water would be directed into the subsequent section, so guaranteeing uninterrupted treatment. Water samples were obtained from the point of entry and exit of the bioreactor in a systematic manner over a period of four months. The analysis of these samples primarily focused on the amounts of $\text{NO}_3\text{-N}$, as flow data were not available. Under conditions of base flow, it has been approximated that a range of 10% to 30% of the water bypasses the bioreactor. This percentage increases to above 50% during periods characterized by high flows.

The construction procedure unveiled diverse levels of complexity and labour intensity among the three distinct types of bioreactors employed. The wall made of sawdust, which can be installed quickly, proved to be the most economically efficient option in terms of labour hours. On the other hand, the in-ditch design, although comparatively efficient, necessitated a greater amount of labour, particularly in terms of mitigating the risk of ditch overtopping. In conclusion, the design of the ditch diversion bioreactor, which is similar to tile drainage bioreactors, incorporates a wider range of knowledge. However, its implementation may require the removal of land from agricultural production, which is consistent with previous practices (Christianson et al., 2013a; USDA-NRCS, 2015).

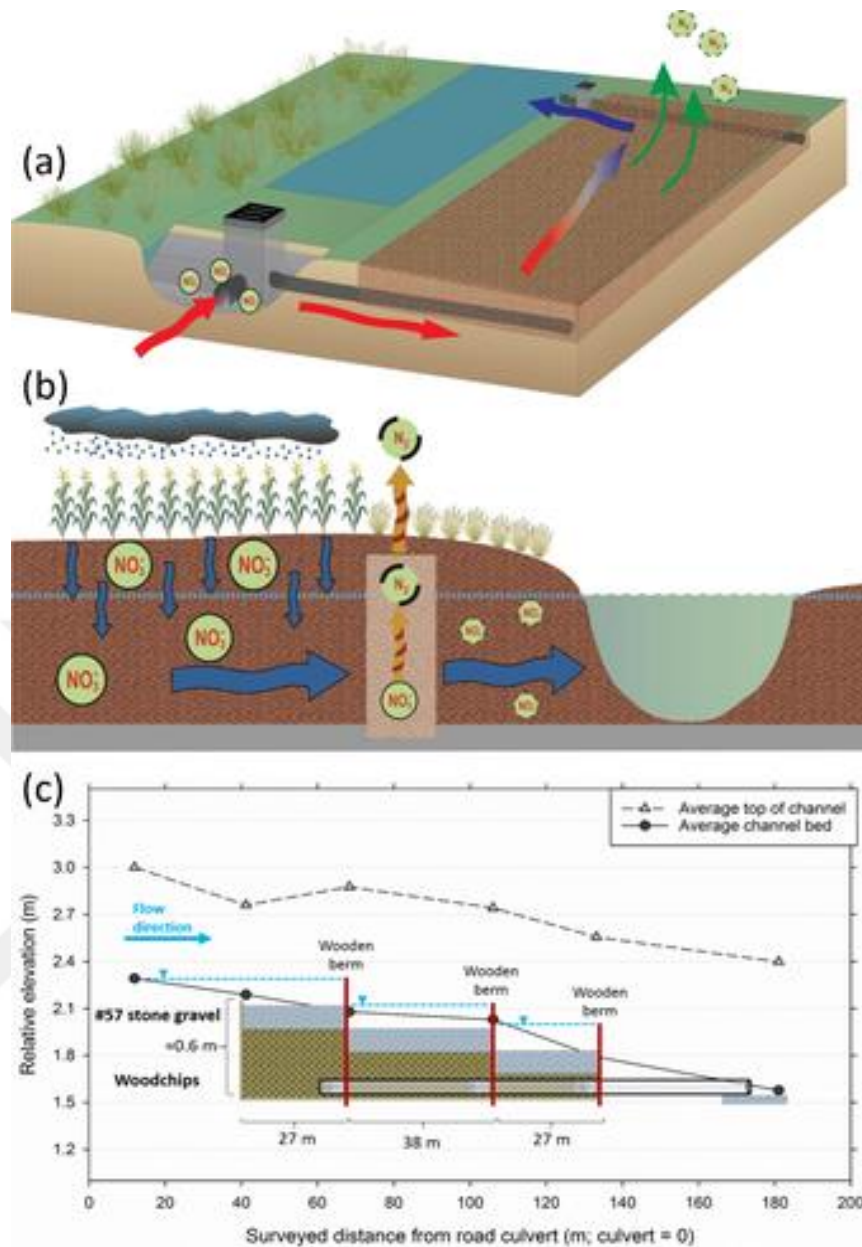


Figure 2.10 The mid-Atlantic region conducted an examination of three distinct types of bioreactors used for ditch treatment. These include: (a) a bioreactor that diverts the flow of the ditch; (b) a denitrification wall made of sawdust; and (c) a schematic representation of an in-ditch bioreactor. The road culvert was selected as a survey reference due to its practicality and the fact that the x and y scales varied. According to the study conducted by (Christianson et al., 2017).

2.4 Advantages and Disadvantages of In-Ditch Systems

Bioreactors in-ditch systems are renowned for their efficacy in eliminating nitrates from agricultural subsurface drainage water. Tile drainage systems in this context regularly achieve an average annual nitrate load reduction rate of around 33% (Bock et al., 2018). Self-sustainability is a notable characteristic that contributes to its efficacy. These bioreactors rely on the carbon content present in the wood chips as an essential supply of nutrients for denitrifying microorganisms. Bioreactors in-ditch systems possess an innate potential to independently sustain and facilitate denitrification processes.

Bioreactors in-ditch systems are preferred not just for their performance but also for their cost-effectiveness. Compared to alternate denitrification practices, these systems provide a cost-effective solution (Christianson 2017). The technology's simplicity further amplifies its allure. Constructing and operating these systems is simple, use an underground network of drainage pipes called tile lines. A fraction of the effluent is sent through a layer of wood chips, where bacteria play a crucial role in the denitrification process, transforming nitrate into gas.

The benefits of denitrification beds over possible alternatives include the ability to remove more than 99% of nitrate (Robertson et al., 2005; Schipper et al., 2010a), a relatively small footprint, limited maintenance compared to more expensive and complicated treatment options, and a lower installation cost (Cameron et al., 2010).

Although bioreactors in-ditch systems have their advantages, it is important to acknowledge that they may face certain obstacles. The occurrence of sedimentation and blockage can lead to potential disruptions in their functionality (Bock et al., 2018). However, in general, bioreactors in-ditch systems are highly successful and economically efficient options for eliminating nitrates from agricultural subsurface drainage water.

2.5 Previous Studies About

The Potential Use of In-Ditch Bioreactors as Best Management Practices (BMPs) An example of a potential ditch best management practices (BMP) is an in-ditch bioreactor, alternatively referred to as a bioreactor "sock" or an organic channel barrier. This approach operates on a smaller scale, employing the concepts of a

denitrifying bioreactor (DNBR). Similar to how DNBRs facilitate denitrification in tile-drained systems, in-ditch bioreactors offer comparable advantages within a ditch drainage system. In-ditch bioreactors are composed of a carbon-based substrate, often woodchips, which are enclosed within a plastic netting. The dimensions of the bioreactor are designed to match the width of the ditch bottom. The in-ditch bioreactor undergoes microbial degradation of organic carbon material, leading to the creation of an anoxic environment when it becomes saturated with water (Schipper et al., 2010).

Multiple studies on inditch bioreactors have shown evidence supporting their effectiveness in reducing dissolved oxygen (DO) concentrations from inflow to outflow, particularly in situations of low flow (Pfannerstill et al., 2016; USDA-NRCS, 2020). Previous research has investigated the implementation of in-ditch bioreactors in two distinct settings: ditches that get agricultural runoff and roadside ditches that receive road runoff (Liu et al., 2015; Pfannerstill et al., 2016; Plier, 2018; USDA-NRCS, 2020; Maxwell et al., 2022). The results of these studies are succinctly presented in Table 2. Numerous investigations pertaining to in-ditch bioreactors have consistently demonstrated their efficacy in the removal of NO₃-N from runoff. Moreover, these studies have uniformly recommended the pursuit of additional research endeavours aimed at enhancing the design of such bioreactors.

Table 2.2 This summary presents the findings on the percentages of nutrient removal seen in several experiments conducted on in-ditch bioreactors.

Study	Bioreactor Substrate	NO₃ Reduction	P Reduction
USDA-NRCS (2015)	Woodchip	30%	NR
Pfannerstill et al. (2016)	Woodchip	28%	0%
Plier (2018)	Woodchip	25%	NR
Liu et al. (2015) ^[a]	Rice straw	95%	Low
Liu et al. (2015) ^[a]	Pine sawdust	35%	NR

Liu et al. (2015) ^[a]	Activated carbon with sand	10%	NR
Maxwell et al. (2022)	Woodchips	-1%	NR

Based on a report published by the United States Department of Agriculture's Natural Resources Conservation Service (USDA-NRCS) in 2020, the implementation of in-ditch bioreactors within rural roadside ditches has exhibited noteworthy efficacy in mitigating the levels of NO₃-N (nitrate) in agricultural runoff. These bioreactors have achieved an average reduction of 30% in nitrate concentrations. The focal ditch under investigation was situated in the northern region of Pennsylvania and served as a recipient of runoff from a 40-acre farmland characterized by mixed land use. This agriculture was subject to biannual manure applications. Two bioreactor "socks" were strategically placed in a sequential manner within the ditch, employing polyester debris netting and a mixture of hardwood woodchips. During the summers of 2018 and 2019, a systematic collection of water samples was conducted from multiple locations, including above, between, and below two bioreactor socks. These samples were then analysed for NO₃-N, total P (phosphorus), and metals.

In order to assess the efficacy of the bioreactors, a rating scale has been designed to quantify the flow rate at both the intake and outflow of the bioreactors. This was achieved by monitoring and analysing depth and flow data. The aforementioned data played a crucial role in the estimation of the hydraulic retention time (HRT) within the ditch, which exhibited a range of 40 min to more than 1.5 hr during the duration of the study. Significantly, the effectiveness of nitrate removal was dependent on the flow rate in the ditch and significantly dropped during periods of high flow that surpassed the capacity of the bioreactors. In contrast, it was observed that a reduction in dissolved oxygen (DO) occurred when water flowed through the bioreactors under low-flow circumstances. This finding provides evidence in favor of the idea that in-ditch bioreactors can establish an environment that promotes denitrification.

Based on the findings, the research suggests that the inclusion of in-ditch bioreactors should be contemplated as a prospective conservation measure within the jurisdiction of the Natural Resources Conservation Service (NRCS), or as a

supplementary component to the established NRCS Denitrifying Bioreactor (605) standard (USDA-NRCS, 2015).

Recent studies have provided insights into the efficiency of in-ditch bioreactors as a financially viable mechanism for nutrient removal, while minimizing disruption to the bottom of the ditch. Prior research has underscored the potential of the interventions; nevertheless, a recent empirical inquiry carried out in Illinois (Maxwell et al., 2022) has cast doubt on their overall efficacy. The present investigation revealed that the implementation of in-ditch bioreactors had a negligible effect on nutrient concentration, as evidenced by an average decrease of just -1% in nitrate ($\text{NO}_3\text{-N}$) concentration. The restricted extraction seen can be ascribed to inadequate hydraulic connectivity between the bioreactor and the water column, principally resulting from the lack of mechanisms to induce water flow through the bioreactor and the buildup of silt atop it. However, the pore water samples extracted from the bioreactor demonstrated indications that it was operating as designed, with reduced levels of dissolved oxygen and $\text{NO}_3\text{-N}$ concentrations in comparison to the surface water. The aforementioned findings highlight specific constraints in hydraulic connectivity linked to in-ditch bioreactors, which could potentially impact their effectiveness as a Best Management Practices (BMP).

It is imperative to acknowledge that a primary benefit of these bioreactors lies in their inherent simplicity and cost-effectiveness. The implementation of adjustments aimed at improving efficiency may result in increased costs or construction complexity, hence potentially altering the overall cost-effectiveness of the project.

3. MATERIALS AND METHOD

3.1 Materials

3.1.1 Substrate

According to Salentijn et al. (2015), hemp, scientifically referred to as *Cannabis sativa* L., is a crop of considerable agricultural importance, possessing numerous applications in the industrial domain. Das et al. (2017) observed that this particular crop demonstrates exceptional versatility, as it serves as a valuable source for a range of industrial products. These products include textiles and construction materials derived from its fibre, food products, and oil extracted from its seeds. Moreover, hemp has noteworthy characteristics, including remarkable gas permeability and antimicrobial capabilities, which have led to its reassessment as a legitimate crop variety, as emphasized by (Branca et al., 2017) and (Tang et al., 2017).

The experiment employed the hemp plant's root as the substrate delivering organic carbon for denitrification process. The approximately 3 mm chipped hemp roots underwent a two-week immersion in a sealed container stored in the refrigerator before use.



Figure 3.1 Chopped hemp submerged in water for its saturation.

3.1.2 Synthetic Agricultural Drainage Water

Tap water mixed with chemical components ($\text{NaCl} = 10 \text{ mg/L}$; ($\text{NaNO}_3 = 0.1800 \text{ mg/L}$; ($\text{NH}_4)_2 \text{SO}_4 = 0.0367 \text{ mg/L}$) was used in laboratory studies a feed water supply.

3.1.3 Reactive Used in the Experimental Set-up

3.1.3.1 Shells

Mussel or oyster shells are known to have high sorption attitude in removing inorganic ions such as phosphate (Popović et al., 2023). The adsorption capacity of shells especially increase after proper calcination, in which CaCO_3 component of shell is CaO (Chen et al., 2013; Yamashiro et al., 2023).

The shell of mussels from the family Cardiidae were used as the filling material in the second section in order to remove phosphorus from the drainage water. The mussel shells obtained from Samsun Atakum (north of Turkey) were ground, sifted, and calcinated before undergoing preliminary examination (Gülmüş, 2022).

All mussel shells were subjected to calcination in the system at a temperature of 900°C for a duration of 3 hr at the Department of Ceramic and Glass of the Ondokuz Mayıs University's Faculty of Fine Arts. This calcination study described in detail by (Martins et al., 2017) involved the use of sieved particles with sizes ranging from 1.18 to 3 mm.



Figure 3.2 Shells after the calcination process.

3.1.3.2 Zeolites

Zeolites are aluminosilicates that are hydrated and have a crystalline structure. These structures contain channels and/or cavities with very small dimensions, ranging from 0.3 to 1.5 nm in diameter. Zeolites have a ubiquitous presence in our daily lives, as they are extensively utilised as sorbents, ion exchangers in detergents, and catalysts in various industrial processes, including oil refining, petrochemistry, and the production of chemicals and fine chemicals. A multitude of zeolite structures have been documented, resulting in a broad range of possibilities in terms of their pore sizes, dimensionalities of channel systems, and compositions (Reedijk et al., 2013).

Zeolites provide a diverse array of uses due to their distinct porous structure, capability for ion exchange, and ability to adsorb substances. They are widely employed in several industries due to their effectiveness in procedures involving separation, purification, and catalysis. Their appeal lies not only in their functionality but also in their environmentally friendly nature, attributed to their non-toxicity and safe operation (Reedijk et al., 2013). The zeolite from the Manisa region that aims the

elimination of ammonium, supplied from ROTA Madencilik.

Table 3.1 Properties of zeolite used in bioreactor system.

MINERAL CONTENT	Percentage
	(%)
Clinoptiloid	90-95
Cristobalite	0-5
Tridymite	0-5
CHEMICAL CONTENT	Percentage
	(%)
SiO ₂	65-72
Al ₂ O ₃	10-12
CaO	2.4-3.7
K ₂ O	2.5-3.8
Fe ₂ O ₃	0.7-1.9
MgO	0.9-1.2
Na ₂ O	0.1-0.5
LOI***	9-14
MnO	0-0.08
Cr ₂ O ₃	0-0.01
P ₂ O ₅	0.02-0.03
SiO ₂ /Al ₂ O ₃	5.4-7.2
PHYSICAL PROPERTIES	Structural Properties of Zeolite (Physical)
Appearance	Ivory white
Smell	None
Porosity	45-50%
Hardness	2-3
Odor	None
Water absorption	42-50%
Plasticity	Minar
Oil absorption (mL/100 gr)	57
Corrosion (mg/100 gr)	87
Single point surface area	43-47 m ² /gr
Micropore area	10-12 m ² /gr
Mesopore area	28-30 m ² /gr
Effective pore diameter	4 angstroms
Solubility	None
pH	7.0-8.0
Softening	1150 °C
Melting	1300 °C
Bulk density	0.6-0.8 gr/cm ³

Real density	2.2-2.4 gr/cm ³
CATION EXCHANGE CAPACITY	(CEC)
Total CEC	1.5-2
CATIONS	Primary exchangeable cations

Rb, Li, Cs, NH₄, Na, Ca, Ag, Cd, Pb, Zn, Ba, Sr, Cu, Hg, Mg, Fe, Co, Al, Cr

3.1.3.3 Experimental set-up

The bioreactor was fabricated using a x m diameter and 2 m long PVC pipe, brought to an open canal shape by cutting some part of upper part. The reactive were placed into three distinct portions. The initial segment of the open pipe was densely packed with hemp root-derived wood chips, exhibiting a size range spanning from 1.18 mm to 3 mm. The second segment was filled with an abundance of seashells, which exhibited a range of sizes approximately equivalent to 1.18-2 mm. The third and concluding segment was containing zeolite.

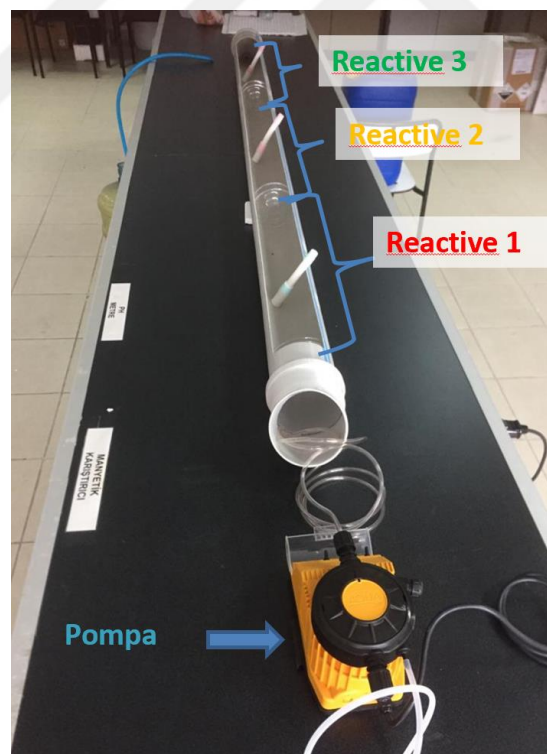


Figure 3.3 Partitions created in a continuous system.

The system was feeded from a tank containing synthetic drainage water with a flow rate of 12.5 mL/min. The experiments were conducted at ambient temperature.

3.2 Apparatus

The apparatuses used during the study can be seen below.

3.2.1 Electronic laboratory equipment

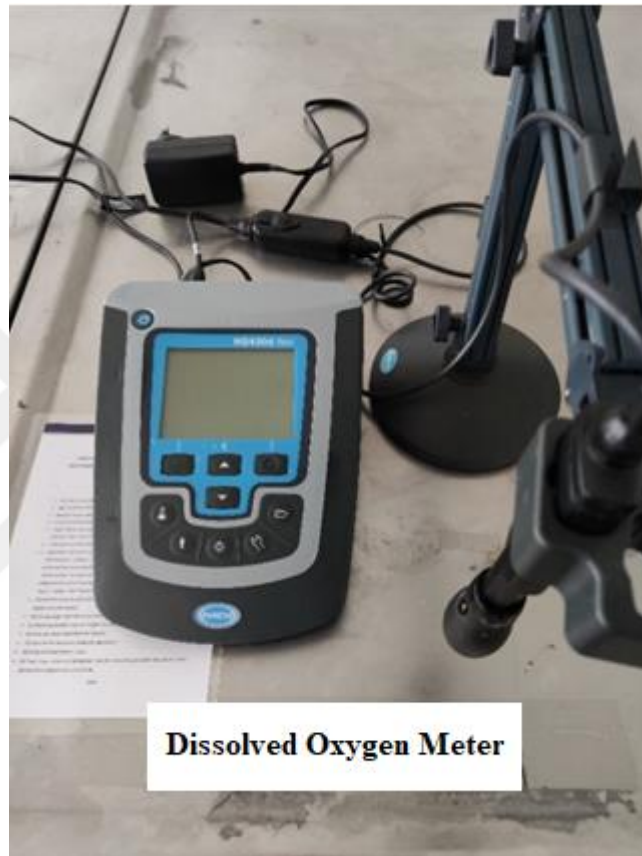


Figure 3.4 Dissolved Oxygen Meters (HACH HQ430d Flexi).

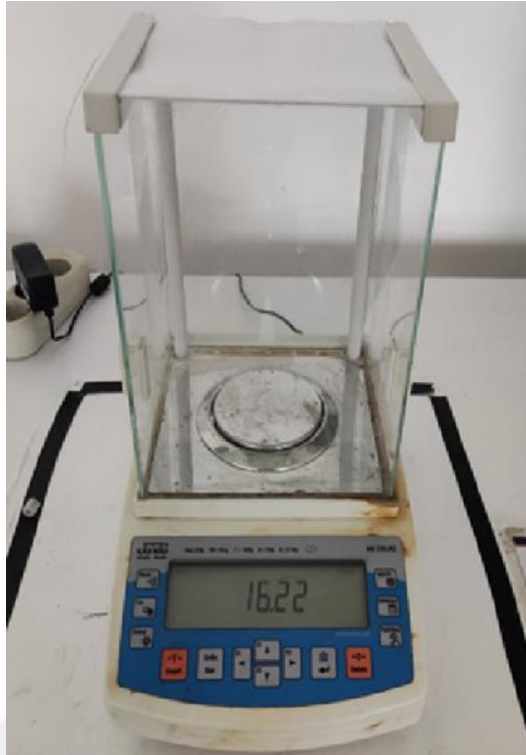


Figure 3.5 Analytical Weighing Scale (RADWAG AS 220.R2).



Figure 3.6 Thermarator Device (Hach Lange LT200).



Figure 3.7 Spectrophotometer (HACH LANGE DR 6000).



Figure 3.8 Distilled Water Device (NUVE ND4).

3.2.2 Sample Collection and Analysis

Upon making the solution and introducing it into the water tank, the water is

subsequently propelled through the pump, propelling it at a flow rate of 20%. Consequently, the water proceeds through the initial section of the reactor, known as the biological component, which is filled with cannabis.

Following a duration of around 8 hr, the initial section becomes fully immersed, causing the water to transition to the subsequent section, known as the physical component of the reactor, which is filled with Shells.

Following an additional duration of roughly 8 hr, the physical segment of the reactor becomes fully immersed, causing the water to flow into the chemical section and subsequently into the third half of the reactor, which is occupied by zeolite.

Around 70 mL of the sample is filtered for elemental analysis using the spectrophotometer. Approximately 250 mL of liquid is extracted from each compartment that is placed between two segments in the set-up. This is done twice a week, at a consistent time and ambient temperature of the laboratory. Four samples were extracted at regular intervals during the course of the experiment (357 day), Entrance, Port 1, Port 2, Exit.



Figure 3.9 The bioreactor with its four components.

3.2.2.1 pH and Electrical Conductivity (EC) Measurement

The pH and electrical conductivity (EC) measurements were conducted using the Thermo Scientific-Orion Star A215 equipment. Measurements were conducted

using the ROSS Ultra Triode in conjunction with the DuraProbe 4-cell conductivity sensor and an epoxy-body pH/ATC electrode.

3.2.2.2 Dissolved Oxygen Measurement

The concept of dissolved oxygen refers to the presence of oxygen molecules in a liquid medium, typically water. The HACH-HQ430d flexi gadget was utilised to conduct the measurements. The measurement was conducted using a luminescent/optical dissolved oxygen electrode.

3.2.2.3 Oxidation-Reduction Potential

The measurement tool is utilized to quantify the intensity of chemical reactions involving oxidation and reduction in an aqueous solution or any other chemical system. With ORP GEL-FILLED PROBE (STD, 1m) with luminescent/optical dissolved oxygen electrode type measurement was made.

3.2.2.4 Nitrate (NO₃) Analyses

The programme selected for analysis on the HACH Lange DR6000 spectrophotometer was 353 N, specifically designed for measuring Nitrate MR PP at a wavelength of 400 nm.

3.2.2.5 Nitrite (NO₂) Analyses

The programme "373 M, Nitrite HR PP 585 nm" was chosen from the saved programmes in the HACH Lange DR6000 spectrophotometer. HACH's Ferrous Sulphate programme was implemented in accordance with the guidelines outlined in Method 8153.

3.2.2.6 Ammonium (NH₃) Analyses

The programme used for analysis on the HACH Lange DR6000 spectrophotometer was 380 N, specifically for measuring Ammonia Ness at a wavelength of 425 nm. The procedure was conducted in accordance with HACH's USEPA Nessler programme Method 8038.

3.2.2.7 COD Analyses

The solutions, calibration range, and techniques can be found in the APHA/AWWA/WPCF (1998) guidelines. To measure, begin by adding 0.9 mL of dichromate and 2.1 mL of sulfuric acid to the tubes, 1.5 mL of the sample was added and agitated by inverting. Subjecting to a two-hour digestion process at a temperature of 148°C the tubes, anticipated to undergo cooling following the procedure, are positioned collectively within the HACH LANGE DR6000 apparatus. Measurements were conducted at a wavelength of 600 nm for high range COD (100-900 ppm). For Low range COD (10-90 ppm), low range reagents were used and measurements were conducted at 420 nm (APHA/AWWA/WPCF, 1998), (Figure 3.10) Calibration curve.

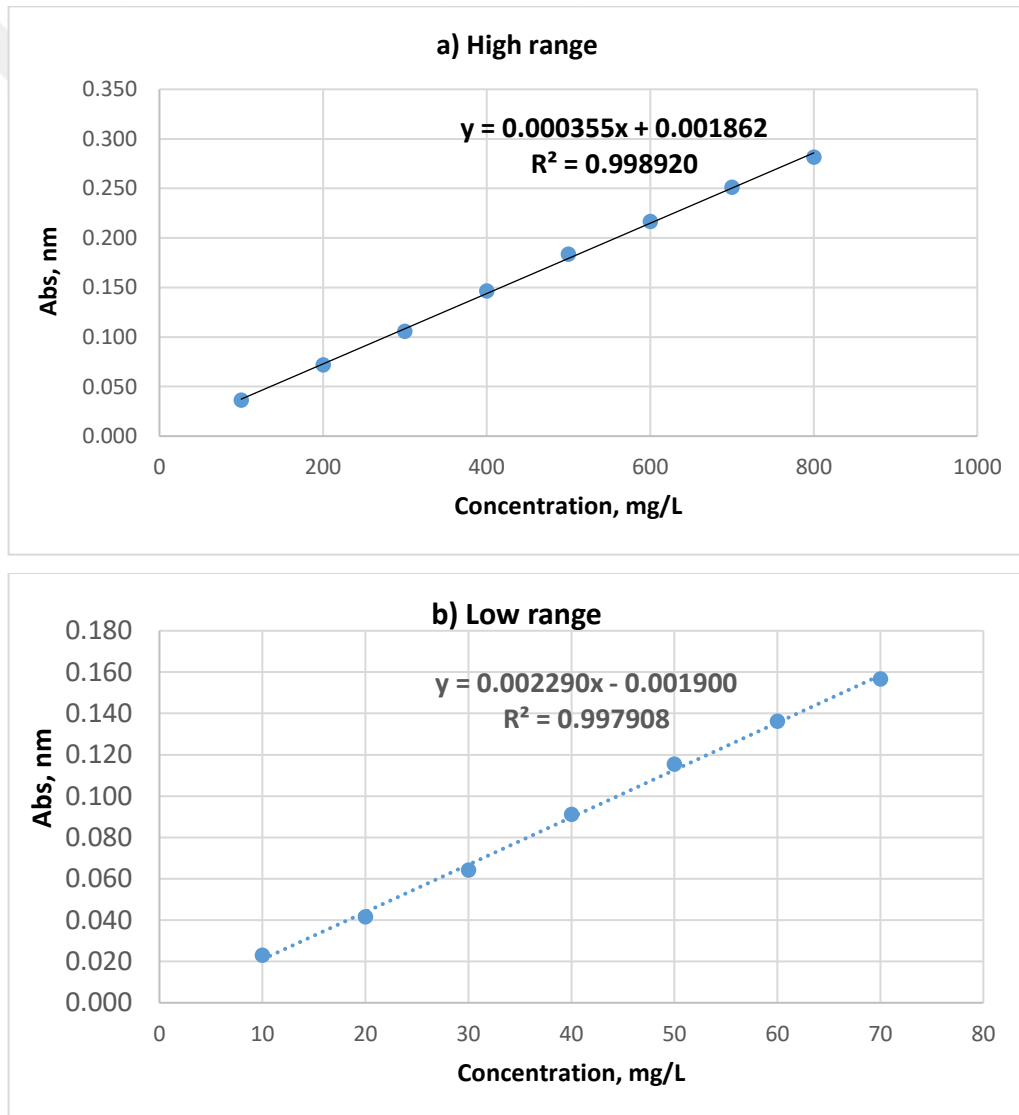


Figure 3.10 COD calibration curves (a) High range and (b) Low range

3.2.2.7 Chemicals Used

- (HACH) Nitriver Cat. 2106169
- (HACH) Nitriver Cat. 2107569
- (HACH) Nessler Cat. 2119449
- (HACH) PhosVer 3. 2106069
- Sodium chloride (NaCl) - (99.0% purity)
- Sodium nitrate (NaNO₃) - (99.0% purity)
- Ammonium sulfate ((NH₄)₂SO₄) - (99.0% purity)
- Mono potassium phosphate (KH₂PO₄) - (99.0% purity)

3.2.3 Calculations

3.2.3.1 Calculation of Pollutant Removal Efficiencies

To determine the pollutant removal throughout the study, the following equation is used: The stated simple yield calculation was using in (Equation 1)

$$\% \text{ Removal} = \frac{(C_o - C_e)}{C_o} \times 100 \quad (\text{Eq.1})$$

C_o = Concentration of pollutant initially present in water (mg/L)

C_e = The concentration of pollutant in the water after treatment (mg/L)

4. RESULTS

The results of three consecutive treatment steps are evaluated in two different approaches. Initially the impact of reactive within each unit/segment on the individual contaminant is interpreted. Secondly, the change in monitored parameters are evaluated for each individual treatment unit.

4.1. Treatment Capabilities of Reactive on Individual Contaminant Removal

4.1.1. Nutrients

For the removal of nitrate, heterotrophic denitrification supported by carbon release from the hemp in the bioreactor was chosen. As can be seen in Figure (4.1) within the very first day nitrate nitrogen dropped approximately below 0.5 NO₃-N/L representing removal efficiencies approaching 90%. After 130-days, nitrate levels in effluent started to increase approaching inlet concentrations but still delivering removal efficiencies of about 10%. Considering all three treatment steps, trends in nitrate nitrogen concentration are the same. Therefore, it can be stated that seashell and zeolite is contributing at minimal level as expected.

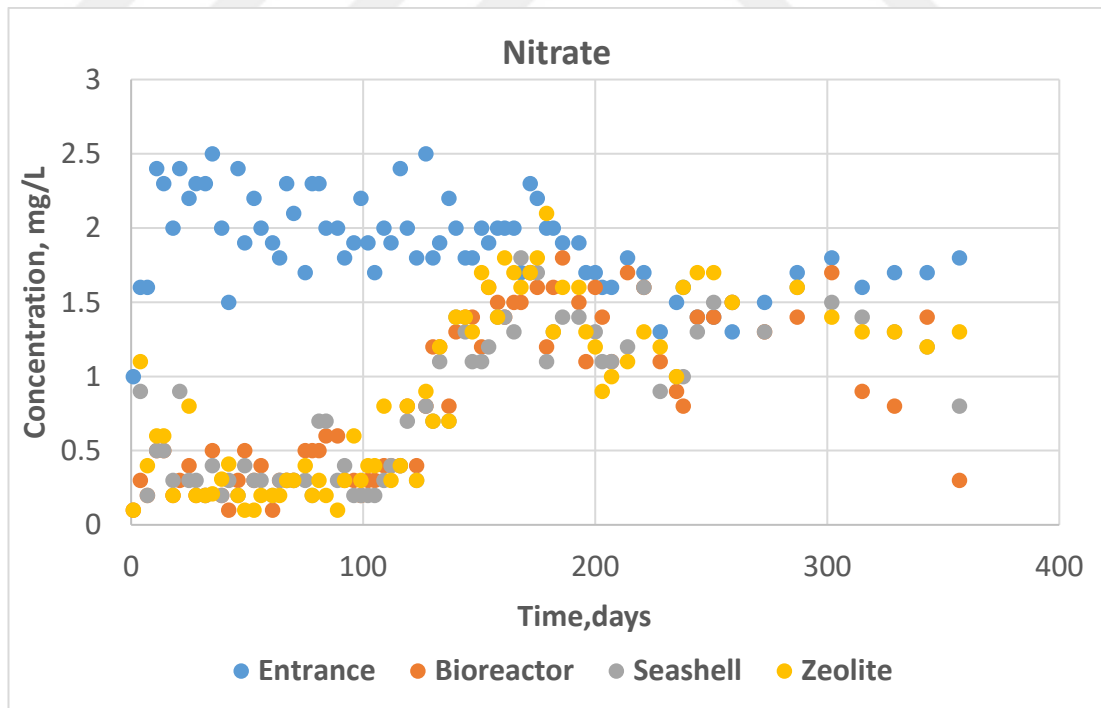


Figure 4.1. Change in nitrate concentration with time for different treatments.

Regarding the ammonium concentrations an increase in concentration approaching 0.9 mg NH₄-N/L at the exit of the bioreactor was observed, which was explained by leaching of ammonium from the hemp in the first treatment segment. Similar findings indicating leaching of contaminants from substrates have been monitored in similar studies (Özkaraoğlu et al., 2019). This influence was also reflected by data of initial days from the seashell and zeolite segments representing.

Ammonium nitrogen levels continued to decline in all units. So, ammonium seems to be removed by all processes reflected by the lower concentration observed at the exit of bioreactor and continuing with seashell and zeolite. Zeolite reflected the highest removal efficiency ~ (100%), working like a very high capacity polishing step, which was quite stable, shown remarkable efficacy in removing substances, Throughout the entire duration of the reactor's operation, spanning from the commencement to the conclusion of the experiment seashell was also very effective in removing ammonium nitrogen.

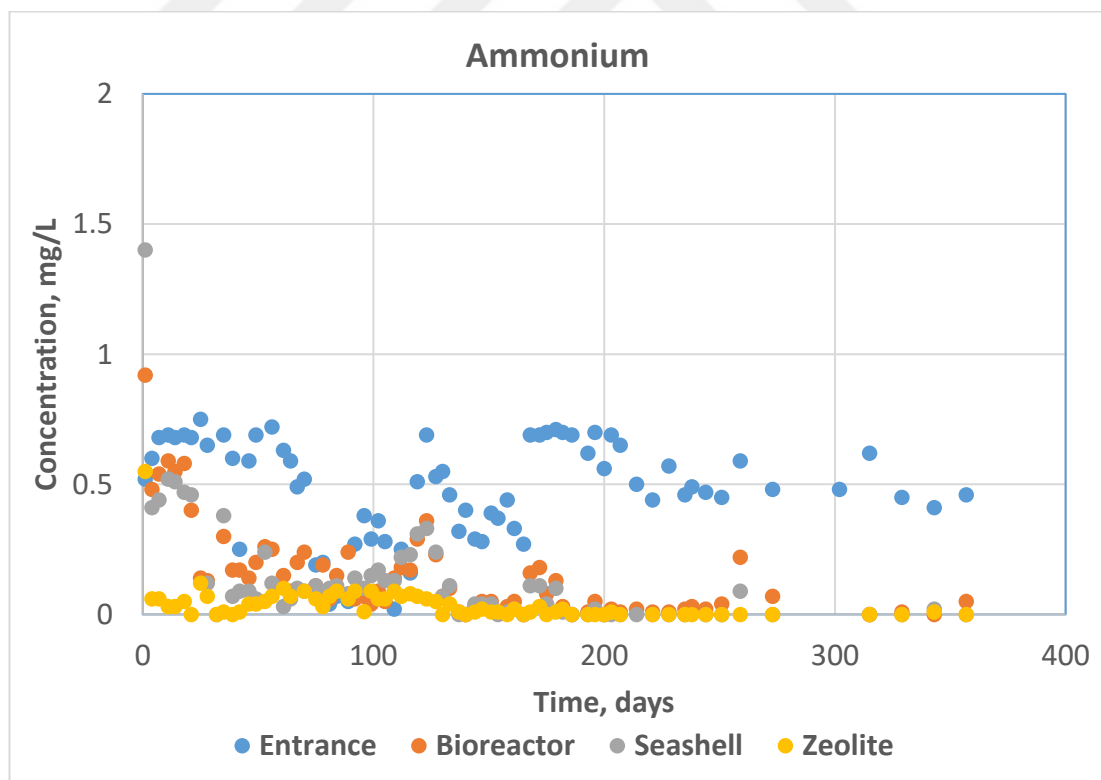


Figure 4.2. Change in ammonium concentration with time for different treatment.

At the beginning of the reactor's operation, there was a high release of phosphorus over 2.5 mg PO₄ /L in the bioreactor section (by the Hemp). However, after 7 days, the reactor started effectively removing the phosphate and continued to do so until the experiment concluded.

During the initial 130-days period, the seashell continued to releasing phosphate, but after 100 days, it started to stop releasing. Specifically, there was a complete absence of phosphate elimination in the Seashell as a result of the initial increase as mentioned in previous studies (Peinemann et al., 2019).

Regarding the zeolite, initially, there was a release of phosphate after approximately 200 days. The released levels ceased and approached the input levels derived from the seashell. This release persisted at a consistent level until the conclusion of the experiment. Based on the experimental data, it can be concluded that some phosphate removal took place in the zeolite segment.

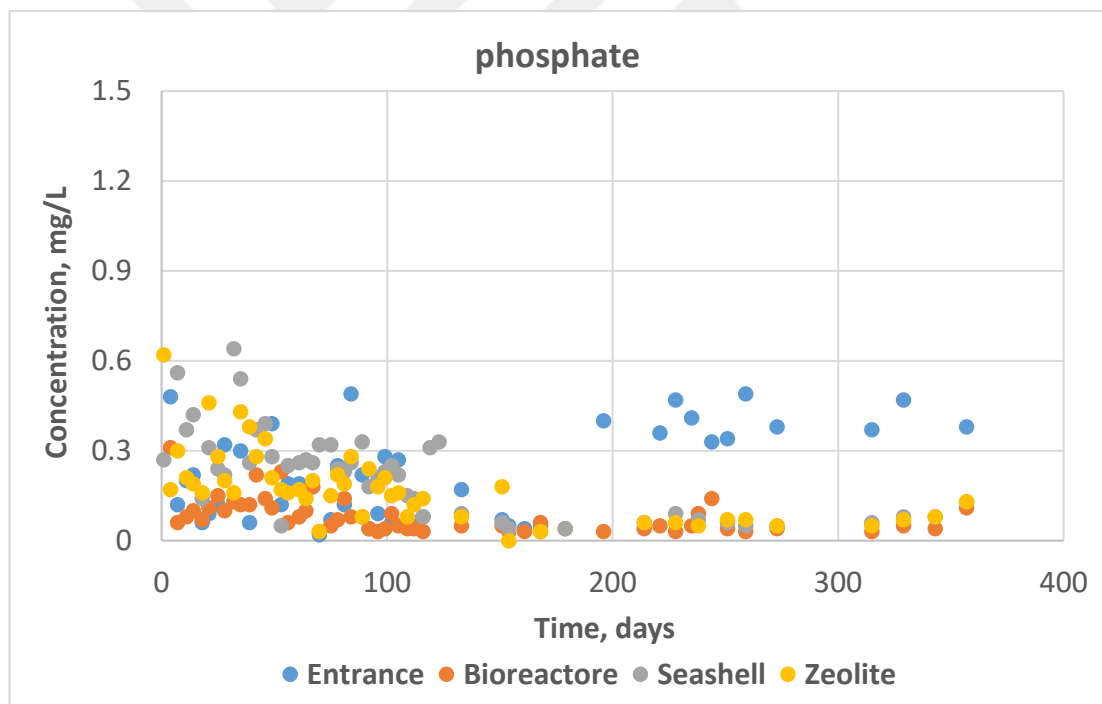


Figure 4.3. Change in phosphate concentration with time for different treatments.

4.1.2 Dissolved Oxygen (DO)

Based on the observed data, the dissolved oxygen rate in the inlet varied between

8 - 10.39 mg O₂/L, with 10.39 mg O₂/L being the highest recorded value. Furthermore, throughout the trial, the dissolved oxygen level at the inlet remained consistently higher than in the rest of the bioreactor.

The woodchip bioreactor section exhibited the lowest concentration of dissolved oxygen due to the oxygen consumption by bacteria present in the bioreactor (Schipper et al., 2005). Bacteria progressively transform phosphorus and carbon compounds and ammonium by utilizing oxygen during various processes in bioreactor (Sanchez, 2020). In the seashell segment an increase in dissolved oxygen level was observed which was even higher in the final zeolite segment. There is no definite explanation for this increasing trend but simple transport of solute in system may have caused oxygenation (Figure 4.4).

The higher oxygen levels during the initial weeks especially recorded in the bioreactor segment were attributed to an adaptation phase where activity of microorganism might be lower, such as ammonium transforming microorganisms. This impact seems also to influence the trend in the seashell segment.

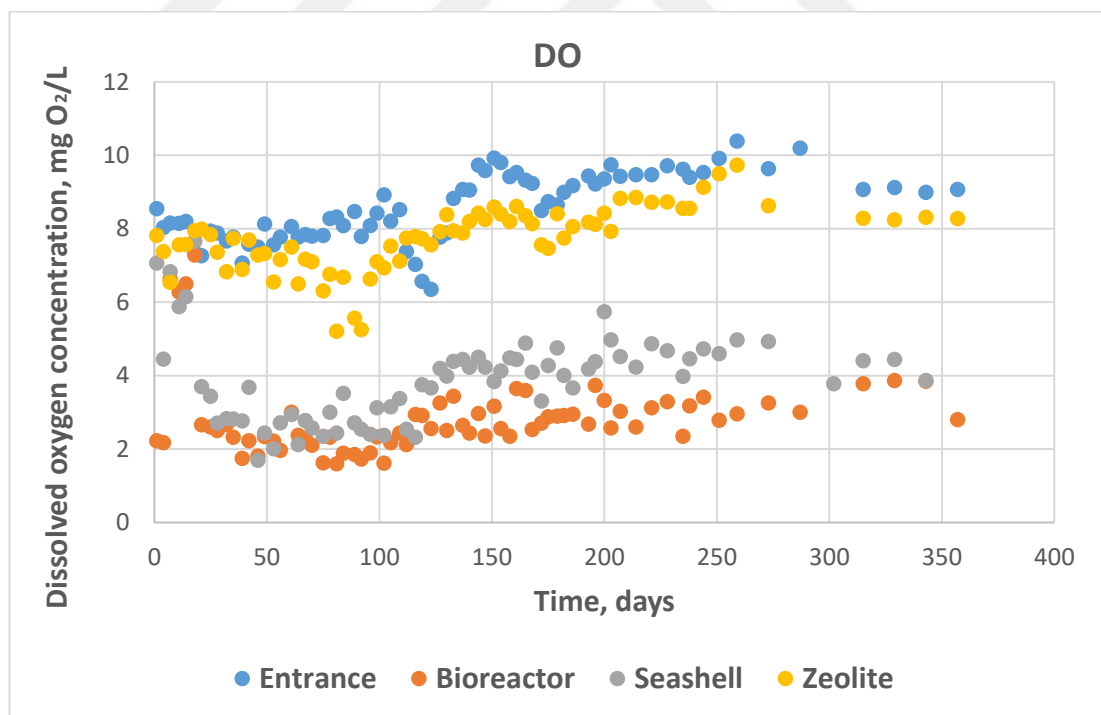


Figure 4.4. Change in dissolved oxygen concentration with time for different treatments.

4.1.3 Oxidation Reduction Potential (ORP)

The ORP data indicated a consistently steady trend in relation to the water inflow into the reactor (Entrance part). Regarding the bioreactor, there was a distinct inconsistency due to microbial transformation reactions. During the initial operation of the reactor, the ORP values fluctuated between 100 mV and eventually dropped to -100 mV on the 28th day. This value remained steady until the 100th day. Subsequently, the values ascend and persist with positive magnitudes until the conclusion of the experiment as depicted in the (Figure 4.5). ORP values substantially increased from one segment to the other, reflecting the impact of transport within the system. So as microbial reactions decreased ORP reflected a rising trend (Catianis et al., 2021). The values of seashell and zeolite were close after 100th day throughout the duration of the experiment.

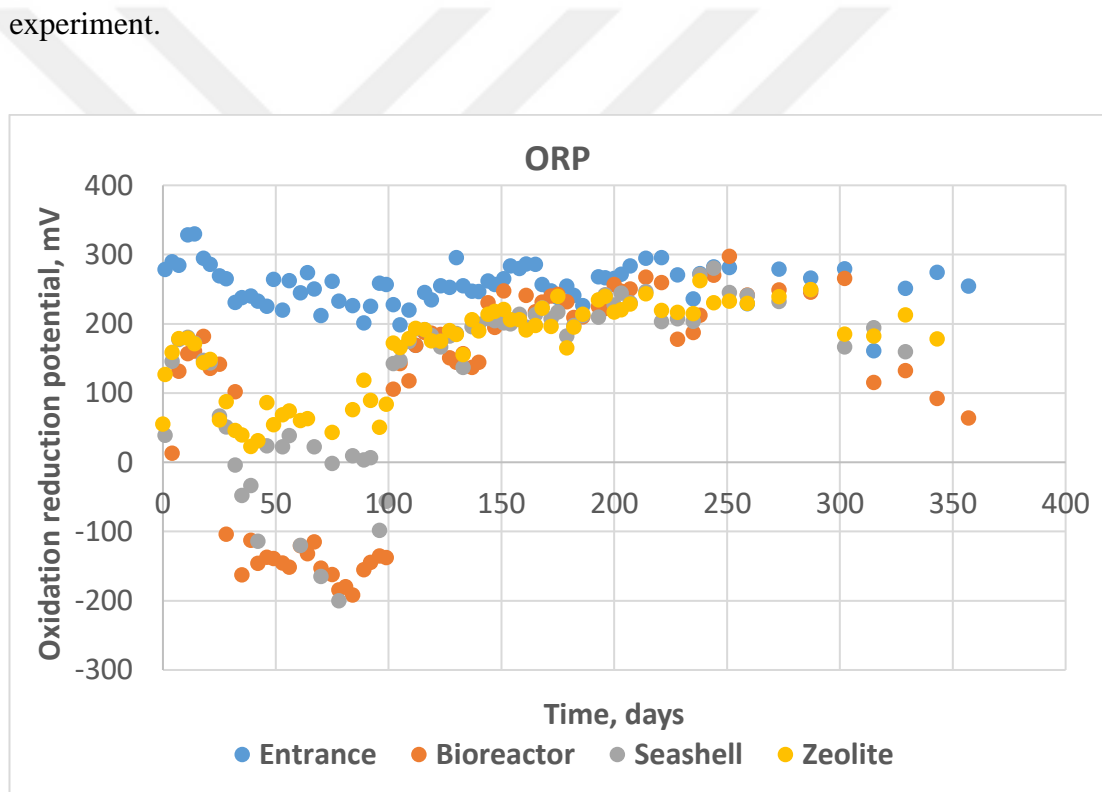


Figure 4.5 Change in ORP concentration with time for different treatments.

4.1.4 Chemical Oxygen Demand

Hemp releases organic materials to provide nourishment for heterotrophic denitrifying microorganisms. Following the 100th day, the release begins to decline,

indicating reduced COD concentrations and leading to elevated levels of nitrate, dissolved oxygen (DO), and oxidation-reduction potential (ORP) in the solution.

The COD data of seashell and zeolite show a minimal decrease, suggesting that some COD is removed through adsorption, without leaving any remaining compounds throughout time. This suggests that the materials are non-contaminating and devoid of any negative side effects.

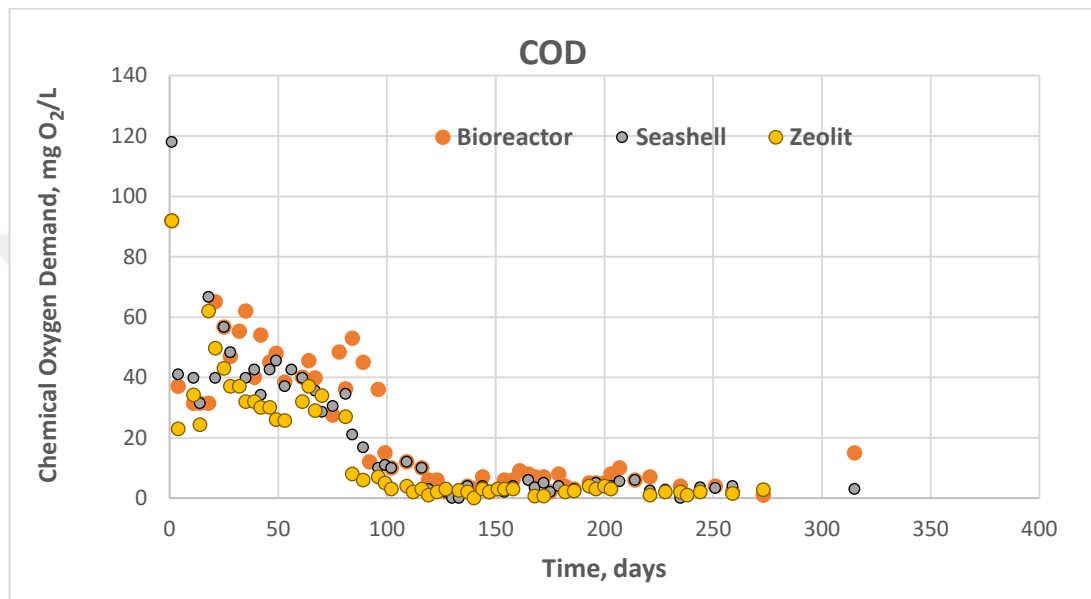


Figure 4.6 Change in COD concentration with time for different treatments.

4.1.5 pH and EC Evaluation

The observed data shows that the levels of pH electrical conductivity (EC) were relatively stable throughout the experiment and constant. Initially, the pH and electrical conductivity values exhibited a modest increase during the experiment, but subsequently reduced and reached a stable state after the 14th day (Figure 4.7 and 4.8).

Regarding all data pH was found to be lower in the bioreactor segment, followed by seashell and zeolite segments. Lower pH values may be attributed to the microbial transformations generating hydrogen ions (Özkaraova et al., 2019). The pH level in seashell improved and matched entrance water level may be because of some calcareous compound dissolution as seashell is known to be primarily composed of carbonates (Ismail et al., 2021). Similarly, pH of the solution taken at the exit of zeolite segment was representing the highest level among all three segments. Trends in EC

were about the same as pH.

Upon analysing the pH and values in the bioreactor, it was seen that within the initial month, the pH climbed by approximately 1.3 units within initial days, reaching pH 8, but then decreased to pH 6.4 at about the 90th day reflecting the most active period of reactor (Figure 4.7). As the dissolved oxygen level begins to increase, it seems that the pH level is also starting to rise. Regarding EC data in (Figure 4.8), the level first rose as a result of dissolution from infill materials in the system, but then stabilised at approximately 2300 mS. Conversely, EC levels are primarily influenced by the tap water quality.

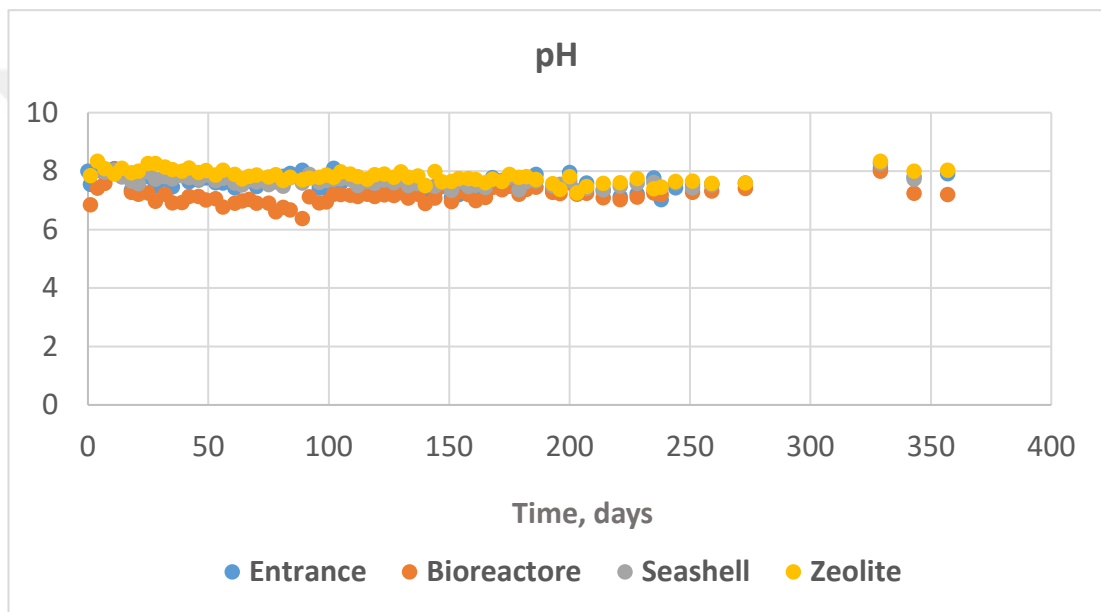


Figure 4.7. Change in pH concentration with time for different treatments.

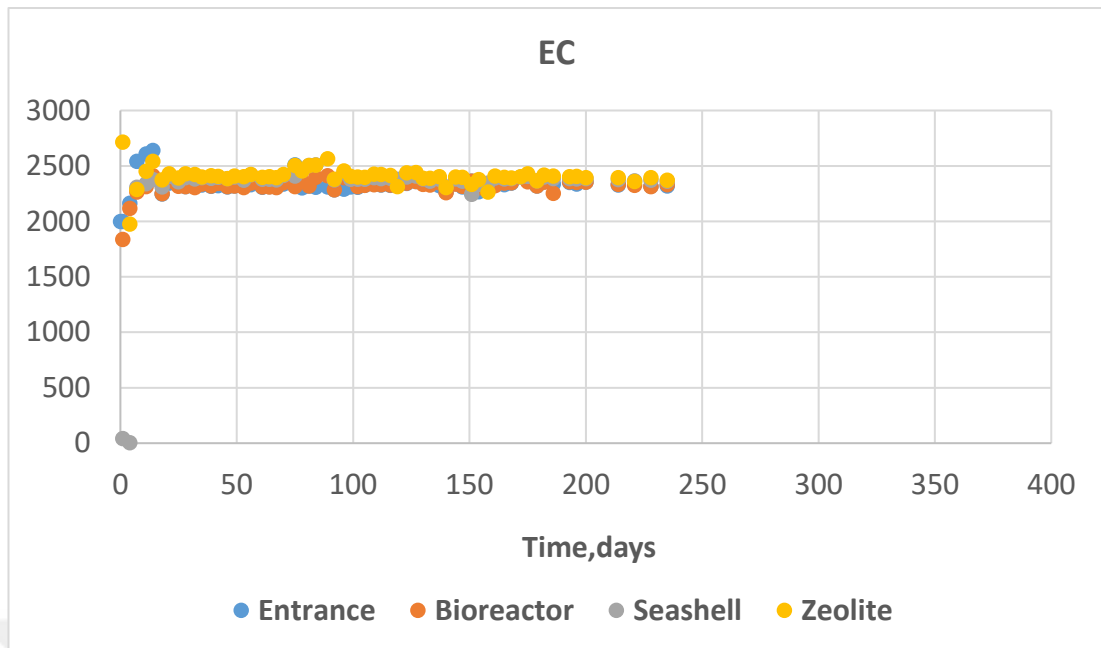


Figure 4.8. Change in EC concentration with time for different treatments.

4.2 Treatment Capabilities of Treatment Segments

4.2.1. Biofilter Removal Efficiency

The biofilter is the part for biotransformation of the reactor, as it contains hemp roots, as previously stated. Hemp is used as a substrate in the initial compartment of the system to remove nitrate-nitrogen ($\text{NO}_3\text{-N}$) from agricultural drainage water. This section consists of stems and roots that serve this purpose. Examination of samples collected from the weir, located at the exit of the reagent compartment, reveals variations in the observed concentrations of pollutants, as shown in (Figure 4.9). The observed fluctuations indicate the operation of the heterotrophic denitrification process, thereby verifying the presence of nitrate removal. The initial concentration of nitrate in the feeding tank, ranging from 1.8-2.4 mg $\text{NO}_3\text{-N/L}$, decreases significantly by 70-90% to levels between 0.1-0.6 mg $\text{NO}_3\text{-N/L}$.

According to the preliminary reactor experiment, the amount of time spent within the compartment was around 8 hr. Within the initial days of this experiment, there is a significant decrease in nitrate concentration to a range of 0.1-0.3 mg $\text{NO}_3\text{-N/L}$ approaching removal efficiencies above 90%. The significant decrease highlights the quick stimulation of denitrifying microorganisms, demonstrating their

effectiveness. The lack of intentional inoculation in the process demonstrates the strength of the native organisms found in the hemp.

Regarding the fluctuations in nitrate the impact of temperature needs to be mentioned. Removal efficiencies in nitrate definitely increased when temperature rises. Based on a comprehensive three-year investigation of a bioreactor, the researchers have concluded that the primary determinant of nitrate removal is the water temperature (David et al., 2016).

Following 130 days of the reactor working at its highest efficiency, the rates at which nitrates are being removed gradually decline, potentially due to the colder temperatures during the winter season, as suggested by previous studies (Fan 2022; Maxwell 2020). However, on day 357 of the experiment, the removal rates begin to increase once more due to increase in temperature.

In the early phase of the experiment, there was an increase in $\text{NH}_4\text{-N}$ concentration in output, which was attributed to the presence of hemp in the system. The increase in concentration is ascribed to the process of ammonium dissolution (leaching) into the solution. These incidents are similar to findings made with similar organic substances, which showed a decrease over time due to the washing effect. An analysis of changes over time reveals a decrease in the concentration of ammonium, which may decrease to levels as low as 0.1 mg $\text{NH}_4\text{-N/L}$ (Figure 4.9). The effectiveness of exchange varies depending on the concentration, although it can reach levels of up to 80%-90%, indicating consistent elimination. Essentially, although the in-trench bioreactor was originally designed for nitrate removal, it is evident that it also effectively removes ammonium, despite its primary purpose.

After examining the phosphate values, it is evident that there is an initial increase in the first few days, followed by a subsequent decrease in the input value (Figure 4.9). The elevated phosphate levels are attributed to the release of phosphorus constituents from the hemp as it moves through the trench-type reactor. While phosphorus was initially present, the levels subsequently dropped below the initial value. Afterward, removal efficiencies over 50% were achieved. Although the lowest efficiency was observed in removing phosphorus by the biofilter, the presence of a simultaneous purification system enhanced phosphate removal overall. The removal of phosphorus was relatively consistent until the conclusion of the experiment.

Upon analyzing the oxygen level in the reactor samples, it was observed that within the initial month, the concentration of O_2 dropped about 6 mg/L, indicating the presence of aerobic transformations. Hemp releases organic components resulting in oxygen consumption by microbes. During the 127 days of the experiment, the level of oxygen in the reactor stayed at approximately 2 mg O_2 /L, as it was utilized. As the organic matter and ammonium concentrations in the solute reduces, oxygen consumption likewise reduces, resulting in a transition to aerobic conditions. Similar alterations to these modifications transpire when there are variations in oxygen reduction potential values. Over time, it increased slightly after that until the end of the experiment.

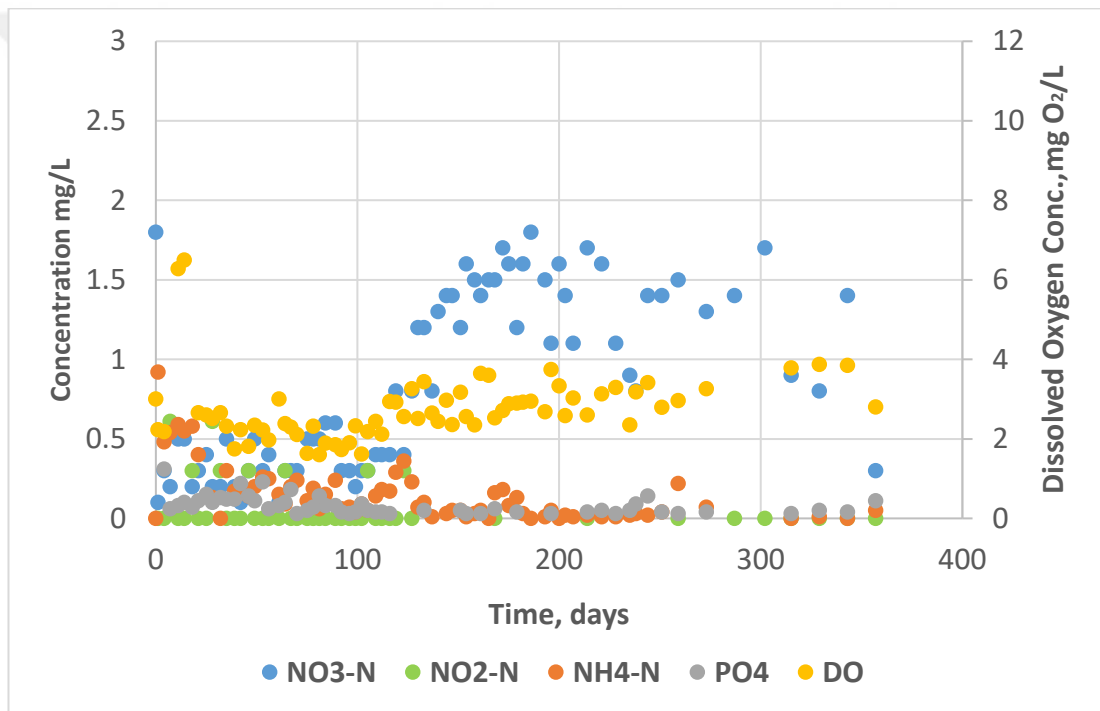


Figure 4.9. Change in pollution concentration with time.

4.2.2 Seashell Removal Efficiency

The efficiency of calcined seashell was evaluated considering the output concentrations of the bioreactor, which serves as the input data for the seashell segment. The reactive compartment, which is made of shell material, does not show any noticeable nitrate removal. It passes through remaining unchanged at similar

concentration levels within the secondary reagent compartment (Figure 4.10).

Interestingly, the calcined mussel shells in the reagent compartment show ammonium removal. The removal continued throughout the early months and showed a tendency to increase afterwards from time to time. The long-term investigations conducted in the mussel reagent section have highlighted its capability in removing ammonium, despite the lack of early expectations. Ammonium removal continued stably until the end of the experiment.

Calcined mussel shells demonstrated exceptional performance in the batch study. Martins et al. (2017) also reported higher phosphorus removal from saltwater with calcined oyster shell when compared with natural shell. However, noticeable differences became apparent when comparing the behavior of artificially created phosphate solutions under batch reactor conditions and the salty drainage water that flowed through the bioreactor in terms of their composition under continuous flow conditions. To assess the effectiveness of this Reactive 2, it is necessary to consider the composition of the water that flows out of bio-reactive compartment (Reactive 1), also considering the drainage water that enters the system.

The hemp in the bioreactor, consists of water-soluble phosphorus compounds, as well as a variety of organic chemicals, that were released. These dissolved components were adsorbed by the calcined seashell, because of this initial high concentration available surface the calcined seashell was potentially depleted and thus its ability to adsorb didn't further continue. Over time, there was a transition from the retention (adsorption) process to the release (desorption) process. As is clear in the data, it was found that the output values exceeded their corresponding input values throughout the experiment's duration of 357 days. This can be better recognized in Section 4.4.

The oxygen levels in the samples obtained from the reactor exhibit a similarity to those found in the bioreactor. An analogous alteration was noted. Essentially, it takes approximately 2-4 days for oxygen levels to reach a peak related to inhibition in aerobic microorganisms. Decrease in oxygen level continued reaching about 2 mg O₂/L.

It is acknowledged that DO reaches certain levels and then reverts to aerobic conditions once more. Oxygen exhibited a gradual and steady upward trend,

maintaining a state of equilibrium, following a span of 123 days, there was a little upward trend in the oxygen level, which persisted until it reached a plateau and subsequently declined again after 343 days, towards the conclusion of the experiment.

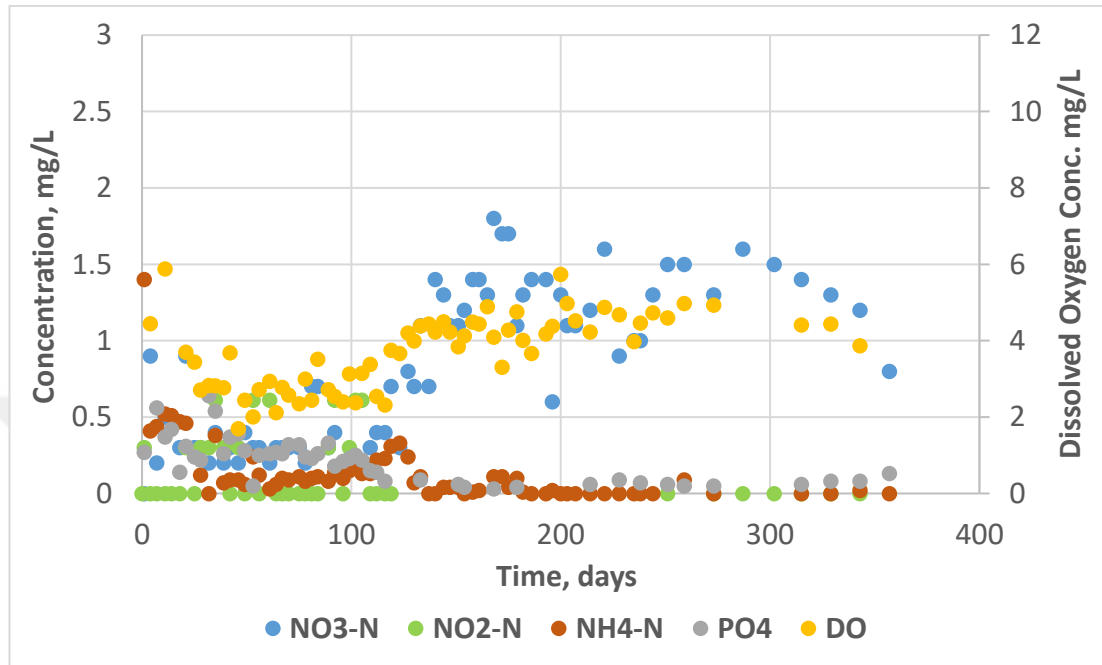


Figure 4.10. Change in pollution concentration with time.

4.2.3 Zeolite Removal Efficiency

The efficiency of zeolite was evaluated considering the output concentrations of the seashell, which serves as the input data for the zeolite segment. The nutrients remaining in the drainage water that enters the final reactive compartment experiences significant retention or transformation within this system.

Initial days, no nitrate removal was observed in this section. However, a gradual reduction in nitrate concentration was recorded persisting until 90th day. Subsequently, the nitrate levels increased outside the inlet, indicating to some release of nitrate from both the seashell and zeolite reactive zones. No significant reduction in nitrates was observed for the entire duration of the experiment using zeolite.

There was a significant elimination of ammonium within the zeolite segment over the first few months, presenting a sustained high ammonium removal capacity of zeolite due to its presence in high amounts. However, due to elevated ammonium

concentration its effectiveness of removal showed a decreasing pattern within the year. The initial increased ammonium level is thought to be release by the hemp. The concentration initially fell from 0.5 mg NH₄-N/L to 0.01 mg NH₄-N/L and subsequently reached a stable state, staying at around 0.1 mg NH₄-N/L until the end of the experiment.

Zeolite exhibited a marginal reduction in phosphate levels especially after its release by the seashell for the initial month, however, this high removal capacity did not persist. Zeolite did not have a very significant impact in removing phosphate in general, but some removal was seen during the operation of the reactor. The concentration initially fell from 0.6 mg PO₄/L to approximately 0.1 mg PO₄/L until the end of the experiment.

Upon analyzing the oxygen level in the samples collected from the reactor, it was noted that there were no significant decreases in oxygen levels at the combined reactor exit (Figure 4.11). In general, the dissolved oxygen concentration was found to increase from about 6 mg O₂/L to about 9 mg O₂/L.

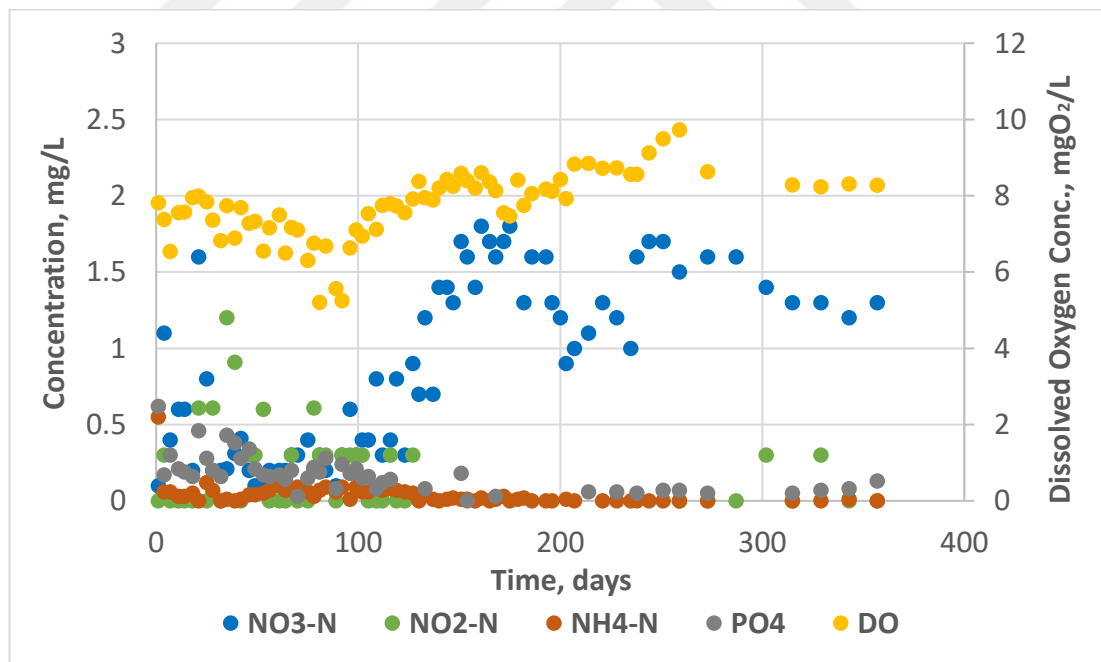


Figure 4.11. Change in pollution concentration with time.

5. CONCLUSION AND RECOMMENDATIONS

The project aimed to treat agricultural drainage water before reaching and polluting potential water resources to protect the integrity of water infiltrating into groundwater and surface water bodies, such as rivers, lakes, and deltas to avoid the impact caused by these pollutants.

The evaluation of observed data from this study showed high treatment efficiencies in this investigation, it was clear that they showed significantly high rates of elimination. Specifically, the hemp in the biofilter has been identified as the primary filling material responsible for this reported effectiveness. Hemp demonstrated exceptional efficacy over time in removing nutrients, despite the release of phosphate and ammonium. Significantly, the calcined seashell exhibited elevated phosphate concentrations in solution, however, the complex nature of these solutions made them inadequate causing to ineffective phosphorus removal. Conversely, zeolite has shown notable success in removing ammonium, further highlighting its efficiency.

The experiment results proved the correlation between temperature and removal efficiency in the bioreactor section of the reactor. It was observed that the removal rate of both nitrates and phosphates exhibited an upward trend over the summer season, coinciding with the rise in temperatures.

An extensive examination exposes the enduring and self-regulatory characteristics of biological processes, especially in terms of their efficacy. The prolonged effectiveness of this process is mostly related to the abundance of microorganisms present in hemp roots, which expedite the process and guarantee its continued efficiency for a long time. This observation highlights the ongoing inquiry into the possible use of hemp for comparable purposes, indicating the need for further exploration into its utilization. Furthermore, it is crucial to continue the search for alternative reagents that can effectively remove phosphate and have a longer lifespan without being quickly used up. Zeolite is a valuable support for physicochemical processes, highlighting its importance and potential usefulness in such applications.

Based on the experiment and the findings from the seashell segment it is evident that there was no significant improvement in treatment. Therefore, removing it from the reactor would enhance productivity and efficiency, both the bioreactor segment and the zeolite segment can be extended in length.

It is critical to include developing countries with limited budgets and challenging climate circumstances in the chance to conserve their resources, which can only be accomplished by implementing efficient and cost-effective solutions. The inditch system offers these countries a low-cost way to ease pressure on their water resources while also purifying the water for reuse in several areas. The integrated filtration system incorporates environmentally friendly components that are easily recyclable once the system has reached its end of life.



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Publications:

1. Forat Jamal Abdulaali ISMAEL, Emre Burcu OZKARAOVA, 9th International Erciyes Conference on Scientific Research on 17 July 2023 “Trends in Denitrifying Woodchip Bioreactors “