2024년 2월

석박사학위 논문

Application of electric potential and Fe(III) to stimulate biodegradation of perfluorooctanoic acid (PFOA) by ANAMMOX granules

조선대학교 대학원

첨단에너지자원공학과

이 종 화

Application of electric potential and Fe(III) to stimulate biodegradation of perfluorooctanoic acid (PFOA) by ANAMMOX granules

아나목스 그래뉼의 과불화옥탄산 생분해 촉진을 위한 전위 및 3가 철의 적용

2024년 2월 23일

조선대학교 대학원

첨단에너지자원공학과

이 종 화

Application of electric potential and Fe(III) to stimulate biodegradation of perfluorooctanoic acid (PFOA) by ANAMMOX granules

지도교수 박 준 규

이 논문을 공학 석사학위 신청 논문으로 제출함

2023년 10월

조선대학교 대학원

첨단에너지자원공학과

이 종 화

이종화의 석사학위논문을 인준함

위원장 장 일 식 (인)

위 원 박 준 규 (인)

위 원 김 종 국 (인)

2023 년 12 월

조선대학교 대학원

Contents

List of tables

List of FIgure

Abstract

Application of electric potential and Fe(Ⅲ) to stimulate biodegradation of perfluorooctanoic acid (PFOA) by ANAMMOX granules

Jonghwa Lee
Adv. Prof. : Jun-gyu Park, Ph.D,
Dept. of Advanced Energy Resources Engineering
Graduate School of Chosun University

1940년대에 개발된 과불화화합물은 탄화수소에서 수소를 플루오린으로 치환한 인공적인 화합물이며, 탄소고리와 작용기에 따라 4,700종 이상이 존재한다. 과불화화합물의 화학적 안정성, 물과 기름에 대한 저항성, 불활성 등의 장점으로 다양한 산업분야에서 사용되고 있다. 하지만, 과불화화합물의 독성이 입증되어 여러 선진국에서는 단계적인 규제로 생산을 중단하였으나, 여전히 환경에서 검출되고 있다. 특히, 총 8개의 탄소에 플루오린으로 완전히 치환되고, 카르복실산 작용기를 가지는 과불화옥탄산은 강한 독성과 잔류성으로 스톡홀롬 협약에의한 잔류성 유기오염물질 (Persistent Organic Pollutants, POPs)로 규제되었다. 과불화옥탄산을 비롯한 과불화화합물을 제거하기 위해 다양한 방법들이 연구되었으며, 실제 폐수처리시설에서는 운전과 관리가 용이한 활성탄, 이온교환수지, 나노필터 등을 이용하여 물리화학적으로 처리하고 있다. 이러한 물리화학적인 방법들은 재생, 교체와 같은 후처리공정이 필수적으로 요구되기 때문에 낮은 경제성을 보인다. 적절한 운전조건 유지 및 관리가 동반된 생물학적처리는 물리화학적처리보다 경제적이고 환경친화적이어서 최근 과불화화합물의 생물학적처리에 관한 다양한 연구가 진행중이다. 본 연구에서는 과불화옥탄산의 생물학적처리를 위해 실제 질소제거공정에 사용되는 혐기성 암모늄 산화 (ANAMMOX) 그래뉼을 이용하여 과불화옥탄산의 생분해를 관찰하였다. 또한, anammox 박테리아의 세포외전자전달능력이 입증됨에 따라 +0.4 V vs. Ag/AgCl의 전압을 가해 미생물전기화학시스템을 적용하였으며,

anammox 박테리아의 전자 전달향상을 위해 3가 철을 주입하였다. 전압과 3가 철을 적용한 반응조에서 암모늄과 과불화옥탄산의 제거율이 각각 41.98, 50.93%로 전압과 철을 적용하지 않은 반응조 (11.52, 19.20%)에 비해 제거율이 향상되었음을 확인했다. 2가 철, 플루오린 이온 및 단쇄 과불화화합물의 분석과 전기화학적분석을 통해 과불화옥탄산의 생분해를 확인하였으며, anammox 그래뉼의 대사작용에 의한 생분해로 제거된 과불화옥탄산은 35.03%로 나타났다. 본 연구에서 anammox 그래뉼의 과불화옥탄산 분해능력, 세포외 전자전달능력, 철 환원능력을 입증했으며, 폐수처리 공정에 적용가능성을 확인했다.

1. Introduction

1.1. Research background & object

PFASs (Per- and poly-fluoroalkyl substances) are artificial carbon compounds in which hydrogen replaced with fluorine. Since 1940s, it has been used in various industries due to its chemical stability, resistance to water and oil, and inertness. Depending on the number of carbons and functional groups, they are classified into thousands of types. Among them, PFOA, which has eight carbon atoms and fifteen fluorine atoms, affects the immune system, thyroid, liver, and cancer and is regulated as a persistent organic pollutants (POPs) due to its high bioaccumulation and persistence tendencies. Although PFASs have been regulated and been forbidden from production worldwide, PFASs have been detected in water and soil, directly affecting humans, causing PFASs in the blood of 98% of Americans.

Various physicochemical methods have been used and studied to remove PFASs. granular activated carbon (GAC), ion-exchange resins, reverse osmosis (RO), and nanofiltration (NF) are used to remove PFASs with high efficiency, however these are not beneficial and eco-friendly due to the necessity of the post-treatment process. Although electrochemical oxidation and ultrasonic wave also show high removal efficiency, demand of large amount of energy Since the methods commonly used to remove PFASs are the way that separate complete removal from environment can not be achieved.

Biodegradation could be one option to overcome the bottlenecks of these physicochemical technologies. The biodegradation of PFASs with one or more hydrogen atoms has been reported more frequently than PFASs, fully substituted with fluorine (e.g., PFOA and PFOS). Although PFOA's strong C-F bond and hydrophobic layer prevent biodegradation, several microorganisms, having PFOA biodegradation capability have been reported. *Pseudomonas parafulva* strain YAB1 removed 48% of PFOA for 5 days and *Acidimicrobium sp*. strain A6 removed 63% and 60% of PFOA and PFOS at low concentrations, and 50% and 47% at high

concentrations for 100 days. Most PFASs biodegradation studies required long operating time and most PFOA biodegradation studies are conducted using strains. T hese limitations make biodegradation be challenging to apply to actual wastewater treatment processes.

Bio-electrochemical systems (BES) and Fe(Ⅲ) can be countermeasures to overcome these limitations. BES can be applied to microorganisms capable of extracellular electron transfer (EET) and improve microbial activity. Unspontaneous metabolism is achieved by applying electric potential and removes target substances more efficiently. *Acidimicrobium sp*. strain A6 also improved the PFOA removal efficiency to 76±16.2% by applying BES. The other strategy is Fe(Ⅲ). Fe(Ⅲ) acts important role that electron transfer between microorganisms and improve microbial activity. In addition, synergy of Fe(Ⅲ) and iron-reducing bacteria improve the PFOA removal efficiency.

Anammox (anaerobic ammonium oxidation) bacteria used for biological treatment for removing nitrogen in the wastewater treatment are microorganisms that have the potential for PFOA biodegradation. Anammox bacteria have the capability of EET through the C-type cytochromes protein. Moreover, anammox bacteria can reduce Fe (Ⅲ) through various pathways such as electron acceptors, electron shuttles, and siderophores, and the reduced Fe(Ⅲ), Fe(Ⅱ), is used to construct proteins such as Heme C. Therefore, this study aims to confirm anammox granules' ability to degrade PFOA as an electron acceptor and improve efficiency through BES application and Fe (Ⅲ) injection.

1.2. Research scope

This study's ultimate purpose is to confirm the possibility of PFASs biodegradation with enhanced efficiency. Therefore, this study is conducted as follows: 1) Replacing NO_2^- among the original substrates NH_4^+ (electron donor) and NO_2^- (electron acceptor) with PFOA. 2) Improving the capacity of anammox grunules' PFOA biodegradation by applying electric potential through the BES based on EET. 3) Enhanced efficiency due to improved electron transfer capacity and activity of anammox bacteria by Fe(Ⅲ) injection. These methods are applied to overcome the limitation of PFOA biodegradation and reducing the energy demand for partial nitritiation make the process more economical.

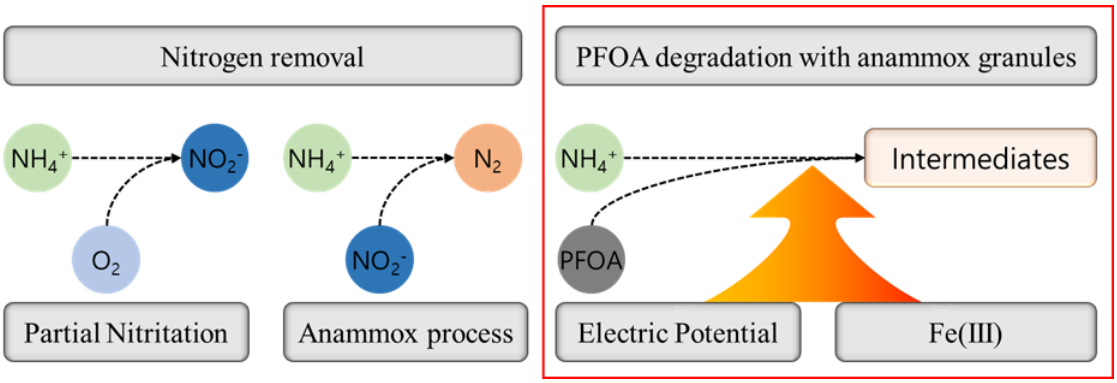


Fig. 1-1 Existing anammox process and process for PFOA degradation

2. Literature review

2.1. Theoretical framework

PFASs are artificial compounds that replace hydrogen in hydrocarbons with fluorine through electrochemical fluorination or telomerization. The strong bonding force between carbon and fluorine (105.4 kcal/mol) is accompanied by strong durability and creates structured layers in a similar way to soap. It is a useful emulsifier for producing firefighting foam and fluoropolymers (Kempisty et al., 2018; Dean et al., 2020). Due to these unique physicochemical properties, they have been made and used in various industries since the 1940s. PFASs are estimated to be between 9,000 and 12,000 types depending on the number of carbon and functional groups and can be found in more than 4,700 products, including life tools, medical devices, various household goods, and consumer goods. OECD (2018) systematically classified 4,730 species into 8 categories (Table 2-1). If more than one fluorine is present, they are called polyfluoroalkyl acids; if all carbon atoms bond with fluorine atoms, they are called perfluoroalkyl acids. Depending on the functional group, it is classified into Perfluoroalkyl carboxylic acids (PFCAs) and Perfluoroalkys sulfonic acids (PFSAs). Additionally, when more than 8 carbons are bonded, it is referred to as a long-chain, and less than that is referred to as a short-chain (Buck et al., 2011).

<Table 2-1> Classification of PFASs

Series	Structure category	Num	Rate(%)
100	Perfluoroalkyl carbonyl compounds	514	11
200	Perfluoroalkane sulfonyl compounds	629	13
300	Perfluoroalkyl phosphate compounds	23	1
400	Fluorotelomer-related compounds	1,872	40
500	Per- and poly- fluoroalkyl ether-based compounds	365	8
600	Other PFAA precursors and related compounds-perfluoroalkyl ones	314	7
700	Other PFAA precursors or related compounds-semifluorinated	746	16
800	Fluoropolymers	267	6
	Sum	4,730	100

Among them, PFOA is a PFASs in which all hydrogen is replaced with fluorine and has eight carbons, including the carboxylic acid group. Its half-life in the human body is estimated to be 2-4 years, and it is not easy to degrade in the environment. Manufacturing processes, as well as consumer use and extensive movement of water, can result in the detection of PFASs in local populations and even in remote regions (e.g., the Arctic or Antarctic) (Shin et al., 2011; MacInnis et al., 2019). The tendency for bioaccumulation throughout the food chain worsens pollution, and PFASs have already been detected in the serum of 98% of Americans (Sunderland et al., 2019). Therefore, this shows that consumers are exposed to PFASs everywhere (Trudel et al., 2008).

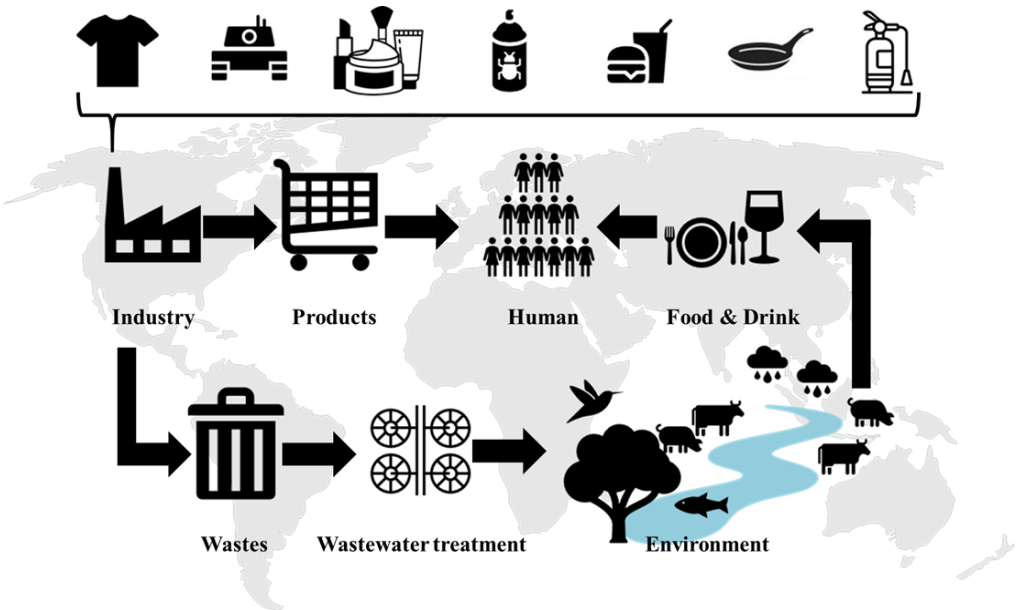


Fig. 1-2 PFASs exposure pathway

PFASs are related to various diseases such as immune function, thyroid function, liver disease, and cancer. In particular, PFOA was considered in this literature because it is toxic to mammals and is the most commonly found perfluorinated compound (Hale et al., 2017; Zareitalabad et al., 2013). PFOA is detected in water more frequently than PFOS due to its high melting point, boiling point, and solubility (Table 2-2).

<Table 2-2> Characteristic of PFOA and PFOS

Classification	PFOA	PFOS
Chemical formula	$C_8HF_{15}O_2$	$C_8HF_{17}O_3S$
Cas No.	335-67-1	1763-23-1
Molar mass	414.07 g/mol	500 g/mol
Melting point	40-50 ℃	-
Boiling point	189℃	133℃
solubility	9.5 g/L	0.68 g/L

In the main river in South Korea, PFOS was detected at only 4 sites for 5 years (2017-2021). whereas, PFOA was detected at 38 sites with high concentration. Although the enhanced method detection limit (20 ng/L to 5 ng/L) after 2020 and increasing detection frequency, PFOS detection in water was rare (Table 2-3). These exposed PFOA in aquatic system affect directly ecosystem and cause various diseases (Table 2-4).

Although it has been phased out worldwide, PFOA and their related compounds were regulated as POPs by the Stockholm Convention because they remain in the environment due to persistence, bioaccumulation by stability, and toxicity (Fenton et al., 2021; Stockholm, 2019). The United States enhanced the regulation of PFASs from 0.07 μg/L of sum of PFOA and PFOS to new indicators such as the MCL(G) and Hazard Index (USEPA, 2023). The EU has set maximum concentrations dependi ng on food categories and will regulate them at 0.5 μg/L concentrations of all PFASs compounds from January 2024 (EU, 2021). In Korea, in accordance with water quality monitoring standards for drinking, the sum of Perfluorooctanesulfonic acid (PFOS) and PFOA at 0.07 μg/L and perfluorohexane sulfonate (PFHxS) at 0.48

ug/L are regulated. In this way, many other countries regulate PFASs. Despite regulations from developed countries, new manufacturers in Asia are using PFASs. Even the detection of PFASs in Ghana, which does not manufacture PFASs, emphasizes the widespread of PFASs and is an obstacle to complete removal (Essumang et al., 2017).

<Table 2-3> Detected concentration of PFOS and PFOA in main rivers in South Korea (2017-2021) (MoE, 2021)

Main river	Sites	PFOS					PFOA				
		2017	2018	2019	2020	2021	2017	2018	2019	2020	2021
Han river	H1	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	H2	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	H3	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	H4	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	H5	N.D	N.D	N.D	N.D	N.D	N.D	N.D	22.87	N.D	N.D
Nak dong river	N1	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	N2	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	N3	N.D	N.D	N.D	N.D	N.D	97.3	56.3	86.6	49.4	42.8
	N4	N.D	N.D	N.D	N.D	N.D	60.3	25.7	56.5	N.D	9.3
	N5	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	N6	N.D	N.D	N.D	N.D	N.D	62.2	28.8	23.9	N.D	7.0
	N7	N.D	N.D	N.D	N.D	N.D	58.0	41.2	36.2	N.D	7.7
	N8	N.D	N.D	N.D	N.D	N.D	38.4	32.9	45.9	N.D	5.6
Geum gang river	G1	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	5.4
	G2	N.D	N.D	N.D	N.D	7.6	N.D	N.D	N.D	N.D	11.2
	G3	N.D	N.D	N.D	N.D	5.4	590.4	26.9	24.6	N.D	5.2
	G4	N.D	N.D	N.D	N.D	N.D	199.0	154.0	149.4	N.D	10.1
Yeong san river	Y1	N.D	N.D	N.D	N.D	8.4	8.4	N.D	N.D	N.D	N.D
	Y2	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	5.3
An seong stream	A	N.D	N.D	N.D	N.D	7.1	38.7	38.0	51.7	30.4	46.9

<Table 2-4> Diseases related with PFOA

Possible disease related with PFOA	Reference
Kidney cancer	Barry et al., 2013
Testicular cancer	Barry et al., 2013
Pregnancy-induced hypertension	Savitz et al., 2012a. Savitz et al., 2012b, Darrow et al., 2013
Thyroid disease	Winquist and Steenland 2014b
High cholesterol	Winquist and Steenland 2014a, Fitz-Simon et al., 2013
Ulcerative colitis	Steenland et al., 2013

<Table 2-5> Regulations on PFASs around the world (k-water, 2019)

Country	PFOS (μg/L)	PFOA (μg/L)	PFHxS (μg/L)	Reference
America	0.004	0.004	-	USEPA
Korea	0.07 (PFOS + PFOA)		0.48	
Canada	0.6	0.2	0.6	Health Canada
Germany	0.3	0.3	-	Drinking water commision
U.K	0.3	0.3		Drinking water inspectorate Tier 2: 0.3; Tier 3: 1.0; Tier 4: 45
Austrailia	0.07	0.56	0.07	Health based action level
EU	0.1-0.5	-	-	Water framework directive

2.2 PFASs treatment

C-F bonds, which are extremely difficult to break, are obstacles that prevent removal in the environment. Various studies have been conducted to remove PFASs using physicochemical and biological treatment technologies.

2.2.1 Physicochemical treatment

The adsorption mechanism is most commonly used to eliminate PFASs because it is highly efficient and easy to apply (USEPA, 2023). Activated carbon (AC) is an efficient method of removing PFOA due to developed pores, excellent adsorption performance, high strength, and easy regeneration. AC generally refers to the carbonization of charcoal and coal, which are raw materials, at a temperature of about 500°C and then activated at a temperature of about 900°C, and in a broad sense, refers to various types of carbon component materials with high porosity and a large specific surface area.

Currently, AC is widely used as an excellent adsorbent through its high porosity and large specific surface area. It is mainly used to remove various organic and inorganic pollutants dissolved in the gaseous state or liquid phase and is also used in various industries (Bhatnagar et al., 2013; Heidarinejad et al., 2020). When the wastewater flows, PFASs adsorb to the pores of carbon particles. AC is divided into two types depending on the size of the particles. Generally, it can be divided into GAC with an average particle size of 0.1 mm or more and powder activated carbon (PAC) with an average particle size of less than 0.1 mm. Pramanik et al. (2015) showed a 95% PFOA removal rate in 10 minutes using PAC and GAC combination. AC has a high removal efficiency but is less selective about some short-chain PFASs (Liu et al., 2020). In addition, the presence of other particles in wastewater reduces PFASs capacity.

IX resins are polymer compounds with a three-dimensional structure in which groups with ion-exchange functions are stably combined through covalent bonds and

are evenly fixed and distributed on the resin surface. IX resin removes pollutants through adsorption like AC. IX resin has high efficiency and the advantage of not relying on the length of the carbon chain, unlike AC (Kothawala et al., 2017). The efficiency of IX resin varies depending on the physical and chemical properties of the resin and various properties such as the matrix and functional groups. Many studies have shown a PFOA removal efficiency of more than 90% (Parvin et al., 2023; Dixit et al., 2021). Although, IX resin can remove PFASs with high efficiency, chemical flush process for reuse requires managing chemical liquid and is expensive.

The filtration is also commonly used to removal PFASs. The RO membrane is characterized by tiny pores (0.1 to 1 nm), and high pressure is required for the RO process, causing high expense (Cui et al., 2010). NF uses 0.5 to 2 nm pores and low operating pressure, making maintenance less expensive (Mastropietro et al., 2021). NF can remove 97-99% of PFOA, and RO also has a high PFOA removal efficiency of more than 99% (Boonya-Atichart et al., 2016; Flores et al., 2013; Tang et al., 2007). These methods, including AC, IX resin, and filtration, are widely used in actual treatment processes due to their high efficiency and applicability. However, they can remove PFASs by not degrading but separating from the water. The separate PFASs from water, which is attached to material, requires post-treatment, resulting in additional costs and contamination. Therefore, further research is need to overcome the necessary post-treatment process with adverse economic and environmental affects.

The advanced oxidation process (AOP) is a pollutant treatment process using a strong oxidizer as an intermediate product, including electrochemical oxidation and ultrasonic waves. In electrochemical oxidation, strong hydroxyl radicals adsorbed on the surface of insoluble electrodes decompose pollutants using electron transfer in the anode. The degradation mechanism is outlined through chemical reactions, beginning with an electron transfer from PFASs to the anode, forming PFASs radicals (Eq. 1-2). These radicals degrade into shorter-chain PFASs with subsequent elimination of $-CF_2-$ units. For PFOA degradation, two main pathways are described. PFOA radical reacts with hydroxyl (•OH) radicals in one pathway, generating

intermediates that lose -CF_2- units, eventually forming simpler compounds (Eq. 3-5). In the other pathway, •C_7F_{15} radicals react with oxygen to create reactive radicals that further react with each other or with oxygen, leading to degradation products and shorter-chain PFASs (Eq. 6-10) (Deng et al., 2021).

$$C_nF_{2n+1}COO^- \rightarrow \cdot C_nF_{2n+1}COO + e^- \quad \text{(Eq.1)}$$

$$C_nF_{2n+1}COO \rightarrow \cdot C_nF_{2n+1} + CO_2 \quad \text{(Eq.2)}$$

$$\cdot C_7F_{15} + \cdot OH \rightarrow C_7F_{15}OH \quad \text{(Eq.3)}$$

$$C_7F_{15}OH \rightarrow C_6F_{14}COF + HF \quad \text{(Eq.4)}$$

$$C_6F_{14}COF + H_2O \rightarrow C_6F_{13}COOH + F^- \quad \text{(Eq.5)}$$

$$\cdot C_7F_{15} + O_2 \rightarrow C_7F_{15}OO \cdot \quad \text{(Eq.6)}$$

$$C_7F_{15}OO \cdot + RCOO \cdot \rightarrow C_7F_{15}O \cdot + RCO \cdot + O_2 \quad \text{(Eq.7)}$$

$$C_7F_{15}O \cdot + HO_2 \cdot \rightarrow C_7F_{15}OH + O_2 \quad \text{(Eq.8)}$$

$$C_7F_{15}O \cdot \rightarrow \cdot C_6F_{13} + COF_2 \quad \text{(Eq.9)}$$

$$COF_2 \rightarrow CO_2 + 2HF \quad \text{(Eq.10)}$$

Zhuo et al. (2017) showed a PFOA removal efficiency of 92.1% using electrochemical oxidation with PbO_2 electrodes, and Lin et al. (2018) achieved a PFOA removal efficiency of more than 99.9% using porous Ti_4O_7 ceramic electrodes. Numerous results depend on the type, temperature, pH, and electrodes and clearly show high removal efficiency.

In ultrasonic wave, it vibrates the water molecule with high velocity, reducing pressure. The bubbles are formed at low pressure, and when they recover the original pressure, they burst and generate shock waves. This phenomenon is called a cavitation bubble. This cavitation bubble emits tremendous energy, forming a local high-temperature and high-pressure nearby and forming hydroxyl radicals. The generated radical can effectively remove PFOA. Uriakhil et al. (2021) showed 79% removal efficiency using ultrasonic sound, and Xiong et al. (2023) showed 100% efficiency. Though showing high efficiency, AOP is also chanllenging to use in actual processes because it physicochemically degrades PFOA using electrical forces that require much energy.

2.2.2 Biological treatment

Biological treatment of PFOA can be a solution because numerous microorganisms in the water system can degrade organics if the biological community keeps stable. As numerous species of microorganisms are studied to remove trace pollutants, the possibility of removal by microorganisms cannot be ruled out. In the case of PFASs with one or more hydrogen atoms, the degradation of various microorganisms has been reported. The strong C-F bond and hydrophobic layer of PFOA, which replaced all hydrogens with fluorine, act as a factor that inhibits biological treatment, making it difficult to expect efficient degradation. However, it has been reported that some microorganisms can degrade PFOA. (Kucharzyk et al., 2017; Zhang et al., 2022). Huang and Jaffé (2019) introduced *Acidimicrobiaceae* sp. strain A6 for PFOA biodegradation. They operated reactors for 100 days and reported 63% and 50% PFOA degradation efficiencies when the initial concentrations of PFOA were 0.1 and 100 mg/L, respectively. Ruiz-Urigüen et al. (2022) confirmed that the PFOA degradation efficiency can be improved by applying BES with an insoluble electron acceptor (electrode) instead of Fe(Ⅲ), which is known as the main electron acceptor for *Acidimicrobiaceae* sp. strain A6. The PFOA degradation with BES reached to 76±16.2% and this result demonstrates the EET and PFOA biodegradation ability of *Acidimicrobiaceae* sp. strain A6. besides, *Pseudomonas parafulva* YAB1 showed a 32.4% degradation efficiency at a 500 mg/L PFOA concentration when PFOA was used as the only carbon source and improved the PFOA degradation efficiency by 48.1% when 1 g/L of glucose as an exogenous carbon source was added to the reactor (Yi et al., 2016). Tang et al. (2022) studied the PFOA degradation by inoculating anammox granules for 100 days. During the initial step of reactor operation, the rapid degradation rate of PFOA resulted from hydrophobic interactions between PFOA and sludge. After 30 days of operation, the average PFOA degradation efficiency was 18.82±2.24%. Most research about PFOA degradation used a strain, which is a variant or subtype of microorganisms. Although unique genetic characteristic of strain make them be possible to degrade

PFOA, it is difficult to apply actual wastewater treatment process. Furthermore, long-term demand compared with efficiency is the main obstacle for efficient treatment. According to Kang et al. (2023), addition of iron oxide and iron-reducing bacteria improve the PFOA removal and BES can enhance the electron transfer of microorganisms. Therefore, Fe(Ⅲ) injection and BES can be considered as a method to overcome these limitations of biological treatment.

In this study, the PFOA biodegradation based on the following four competitiveness of anammox granules: being applied in the actual wastewater treatment, capability to degrade PFOA, ability of use Fe(Ⅲ) and improving activity, and BES applicable by EET capability.

2.3 Bio-electrochemical system (BES)

BES is an electrochemical system that applies microbial metabolism, and the reaction occurs through microorganisms attached to electrodes to improve redox reactions by integrating microorganisms or biocatalysts with electrochemical methods. Microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) represent BES. In the MFCs, microorganism oxidizes organic matter through Spontaneous redox reactions, and the organic matter is oxidized at the anode, releasing protons and electrons. The emitted electrons move to the cathode through an external circuit, generating current and converting chemical energy into electric power.

In the MECs, unspontaneous microbial metabolism occurs supplied with an external voltage. The organic matter in the anode chamber is oxidized to CO_2 and electrons during microbial metabolism. The electrons transfer to the electrode and the generated protons transfer to the cathode chamber through the electrolyte. In the cathode chamber, electrons and protons generate hydrogen or react with other species to generate biofuels, such as methane (Badwal et al., 2014).

Not all microorganisms can apply to BES, only microorganisms that have the capability of EET can apply to BES. In particular, *Geobacter* and *Shewanella* species are most known as EET-capable microorganisms, and frequently used

microbial models in BES. EET is usually carried out through four paths: C-type cytochromes, nanowire, electron shuttle, and electron transfer in biofilms. C-type cytochromes are heme-containing proteins, mainly in bacteria and archaea, and are considered the most important factor in electron transport. Nanowires are electrically conductive pili in the Fe(Ⅲ) reduction process by *G.sulfurrenducens*, known as electron transfer strategies (Reguera et al., 2005). It extends to tens of micrometers and contacts solid electron acceptors or bacterial partners, with varying electrical conductivity depending on diameter or length (Strycharz-Glaven et al., 2011). Electron shuttles should be soluble, stable, reusable, environmentally friendly, and have adequate oxidation potential. The electron shuttle is generated by secretion in bacteria, and the current generation may stimulate shuttle secretion (Rabaey et al., 2004). In addition, some other redox chemicals distributed in the environment can also be used as electron shuttles. It has disadvantages such as higher overvoltage and lower diffusion coefficient than direct electron transfer (Torres et al., 2010). Electron transfer in biofilms consists of numerous microbial cells densely accumulated and spatially distributed in extracellular polymeric substances (EPS). A complex electron network, including various electron transport components, is estimated in a current-generating biofilm.

BES through various EET pathways shows positive results. Applying BES in anaerobic digestion helps stable processes by interacting with biological activities and electrochemical reactions (Park et al., 2020). In addition, various nitrogen-removing microorganisms have also been proven to improve efficiency through BES application and can be operated without existing substrates, which is a promising technology (Khanthong et al., 2023). Therefore, applying BES in various processes can enable stable operation and be eco-friendly and economical.

BES usually uses a two-electrode system or a three-electrode system. The two-electrode system uses two electrodes: a working electrode and a reference electrode. Unlike the 3-electrode, there is no counter electrode, and the working electrode acts as a counter electrode. Two-electrode systems are simple and easy to use, but they can cause unwanted side reactions when current flows through the cell, making them less accurate. Additionally, as current flows to the reference

electrode, the zero point may fluctuate due to changes in the solution components within the reference electrode.

In contrast, in a three-electrode system with a working, counter, and reference electrode, current flows to the counter electrode in the opposite direction to the working electrode, helping to maintain electrical neutrality. In other words, it helps the smooth flow of electrons and prevents charge imbalance. Because the voltage of the working electrode is accurately controlled with respect to the reference electrode, it is used for purposes such as investigating the mechanism of electrochemical reaction, analyzing it, and obtaining other electrochemical parameters. Due to the anammox bacteria has the EET ability, enhanced activity of anammox granules through applying BES can be expected. Therefore, this study was conducted applying BES to anammox granules, and use a three-electrode system for accurate analysis.

2.4 Anammox

Nitrogen from agricultural, sewage, and industrial wastewater pollutes groundwater and surface water through various pathways. Since nitrogen causes eutrophication of the water system and acts as a pollutant in water quality and aquatic ecosystems, the treatment of nitrogen is essential. Instead of the physicochemical method, More effective and cost-effective biological treatment methods are commonly used to remove nitrogen from wastewater (Huang et al., 2018).

Anammox bacteria play a significant role in nitrogen cycling worldwide and are crucial for sustainable wastewater treatment. Anammox is a reaction that occurs under anaerobic conditions, using hydrazine (N_2H_4) and hydroxylamine (NH_2OH) as intermediates to oxidize ammonium to nitrogen gas, with nitrate serving as the electron acceptor (Eq. 11) (Jetten et al., 1998). Three enzymes directly participate in the anammox reaction through a series of redox reactions. Initially, nitrite is reduced to nitric oxide, catalyzed by the nitrite reductase enzyme (Nir). The generated nitric oxide is then catalyzed by the hydrazine synthase enzyme (HZS) to form hydrazine.

Subsequently, hydrazine is converted to nitrogen gas by the hydrazine dehydrogenase enzyme (HDH) (de Almeida et al., 2015; Gao et al., 2014). These enzymatic reactions are crucial for the efficient removal of ammonium from wastewater and contribute to sustainable waste treatment processes.

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 2N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad \text{(Eq.11)}$$

In the nitrogen removal process using anammox granules, the partial nitritaion process that oxidizes NH_4^+ to NO_2^- is required. The partial nitritation process is performed by aeration and possesses a lot of the overall expense. Anammox bacteria can utilize insoluble extracellular electron acceptors based on EET. In other words, the partial nitration process required to produce the existing electron acceptor NO_2^- is not necessary for the anammox with BES process. Anammox reaction occurs through the EET function, which performs various functions, including electron transfer, oxidation-reduction catalysis, gas detection, and transport in Heme protein contained in C-type Cytochromes protein present inside microorganisms (Shaw et al., 2020). BES improves the activity, increasing nitrogen removal efficiency. In addition, in EET-dependent anammox processes, complete NH_4^+ oxidation to N_2 is possible without the accumulation of NO_2^- and NO_3^-. EET-dependent anammox processes are essential for energy-efficient wastewater treatment as they can occur at low voltages (0.3-0.6 V vs. SHE) (Eq. 12-14).

Anodic reaction: $2NH4+ \rightarrow N2 + 8H+ + 6e-$ $E0 = +0.275$ V vs. SHE (Eq. 12)
Cathodic reaction: $6H+ + 6e- \rightarrow 3H2$ $E0 = -0.028$ V vs. SHE (Eq. 13)
Overall reaction: $2NH4+ \rightarrow N2 + 3H2 + 2H+$ $E0 = +0.247$ V vs. SHE (Eq. 14)

Iron is another way to increase the efficiency of anammox bacteria. Fe(Ⅲ) is mostly insoluble, and bacteria that use insoluble electron acceptors are known to transfer electrons to cytochrome in the extracellular membrane during the electron transfer process of respiration instead of transporting electron acceptors into cells

(Myers et al., 1992). Anammox granule can reduce Fe(Ⅲ) to Fe(Ⅱ) through their various utilization abilities of Fe(Ⅲ) as electron acceptors, electron shuttles, and siderophores, thus improving their activity. Fe(Ⅱ), the final product of Fe(Ⅲ) reduction, can be used to organize proteins, such as Heme C, to increase the activity of mixed culture anammox granules (Chen et al., 2014; Lis et al., 2015; Zhao et al., 2014). Therefore, the application of BES, and Fe(Ⅲ) injection are expected to have a positive effect on anammox granules on the economics and the environment.

3. Research methodology

3.1 Reactor set-up

Eight acrylic single-chamber bio-electrochemical reactors with a total volume of 1 L (11 cm length × 10.3 cm width × 9 cm height) were used for PFOA degradation of anammox granules. The working volume of each chamber was 400 mL. Three electrodes were used to check the microbial electrochemical PFOA degradation of anammox granules: working electrode, counter electrode, and reference electrode. A carbon brush (D 3 cm × H 6 cm) was used as both the working and counter electrode materials, and an Ag/AgCl electrode (CHI111, CH Instruments, Inc., Texas, USA) was used as the reference electrode. A potential of +0.4 V vs. Ag/AgCl was applied to the working electrode using a μStat 8000 Multi Potentiostat/Galvanostat (Metrohm, Herisau, Switzerland). All reactors were kept at room temperature (25± 1℃) during experimental periods. Nitrogen gas purging was performed in all reactors to eliminate the effect of oxygen and to provide completely anaerobic conditions before starting the main experiments. The detailed reactor configuration and characteristics are shown in Fig. 3-1.

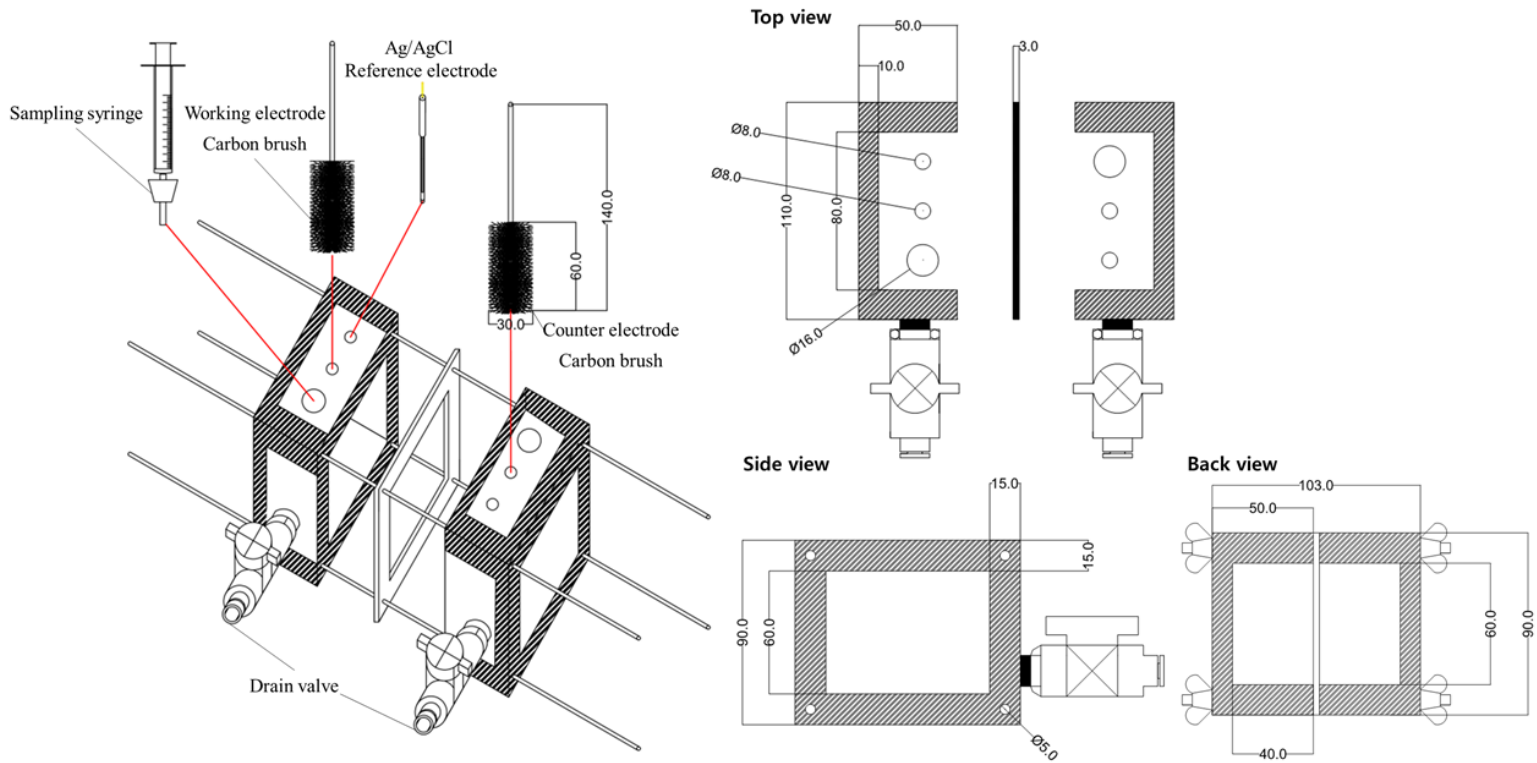


Fig. 3-1 Schematic diagram of single-chamber bio-electrochemical batch reactor with three-electrodes system

3.2. Experimental design and condition

The experiment was conducted to confirm the degradability of PFOA in anammox granules under eight different conditions. The reactor was indicated by inoculation (A(O and X)), applying electric potential (A(O and X)-P), Fe(Ⅲ) injection (A(O and X)-Fe), and both electric potential and Fe(Ⅲ) (A(O and X)-Fe+P). Two control reactors, A(O)-ctrl and A(X)-ctrl, were installed without the addition of Fe(Ⅲ) and electric potential for scientific comparison. PFOA and NH_4^+ were added to all reactors to monitor whether anammox granules could utilize PFOA as an electron acceptor or intermediate. Four reactors were also injected with Fe(Ⅲ) to confirm anammox bacteria's enhancement of PFOA degradation efficiency. Additionally, four reactors were applied with a voltage of +0.4 V vs. Ag/AgCl to analyze the effect of electric potential. The details in reactors set-up are presented in Table 3-1. Without injecting the existing electron acceptor NO_2^-, 30 mg/L of PFOA was injected into all reactors to confirm whether PFOA could be used as final or/and terminal electron acceptors in the anammox metabolism. Eight reactors were operated for scientific comparison and discussion. A(X)- reactors were operated using an abiotic configuration (pure electrochemical reactor), and A(O)- reactors were operated using anammox granules inoculum. A total of 150 mg/L Fe_2O_3 were injected into A(O and X)-Fe and -Fe+P reactors to confirm its synergetic interaction with Fe(Ⅲ) and electric potential. A multi Potentiostat/Galvanostat (μStat 8000, Metrohm, Herisau, Switzerland) was introduced for four bio-electrochemical reactors (A(O and X)-P and -Fe+P reactors) and electric potential of +0.4 V vs. Ag/AgCl was applied to working electrode in each reactor.

<Table 3-1> Experimental conditions for all reactors

Reactors	ANAMMOX	PFOA	Fe(Ⅲ)	Potential
A(X)-ctrl	-	O	-	-
A(X)-Fe	-	O	O	-
A(X)-P	-	O	-	O
A(X)-Fe+P	-	O	O	O
A(O)-ctrl	O	O	-	-
A(O)-Fe	O	O	O	-
A(O)-P	O	O	-	O
A(O)-Fe+P	O	O	O	O

3.3. Electrochemical analysis and calculations

The generated current was measured using μStat 8000 multi-potentiostat/ Galvanost at (Metrohm, Herisau, and Switzerland). Coulombic efficiency (CE) was calculated to confirm the activity and electron balance of the reaction using the generated current, and the removed concentration and Eq. (15) was used for the calculation.

$$CE(\%) = \frac{\sum_{0}^{t} I_{measured} \times t}{n \times F \times Q \times (NH_{4_{input}}^{+} - NH_{4_{out\,put}}^{+})} \quad \text{(Eq. 15)}$$

where, $I_{measured}$ is the total current generated during the entire operating time (t); F is the Faraday constant (96,485 C/mol); n is the number of electrons transferred when 1 mol of NH_4^+ is oxidized (3 per NH_4^+); and Q is the reactor volume per total operating time (t). $NH_4^+{}_{input}$ and $NH_4^+{}_{output}$ are the initial and final NH_4^+ concentrations (M), respectively.

Cyclic Voltammetry (CV) is an essential tool in electrochemical systems that provides information about electron transfer interactions between microorganisms and electrodes during microbial growth and metabolism (Fricke et al., 2008). CV increases at a constant rate at the initial voltage over time, and returns to the initial voltage at the final voltage. (a)-(b) section is where reduction occurs, and the (b)-(c)

section is where oxidation occurs (Fig. 3-2). The peak (i_{pc}) generated when the voltage is applied in the negative direction indicates the reduction potential (E_{pc}) and the peak (i_{pa}) generated when the voltage is applied in the positive direction indicates the oxidation potential (E_{pa}) (Fig. 3-3). That is, the voltage of the peak means an overpotential required for the redox of reactants. The higher scan rate, the higher the current can be generated due to the effect of the reaction at a previous voltage, and the higher current determines the higher reaction rate. The area of a CV graph also has the same meaning. After 14 days of operation, the bulk solution in each reactor was replaced with a new solution, with the same properties as the initial solution, and CV analysis was performed. For CV, the response current was measured by applying an electric potential from -1.0 to 1.0 V vs. Ag/AgCl at a rate of 1 mV/s using 8000 multi potentiostat/ Galvanostat (Metrohm, Herisau, Switzerland).

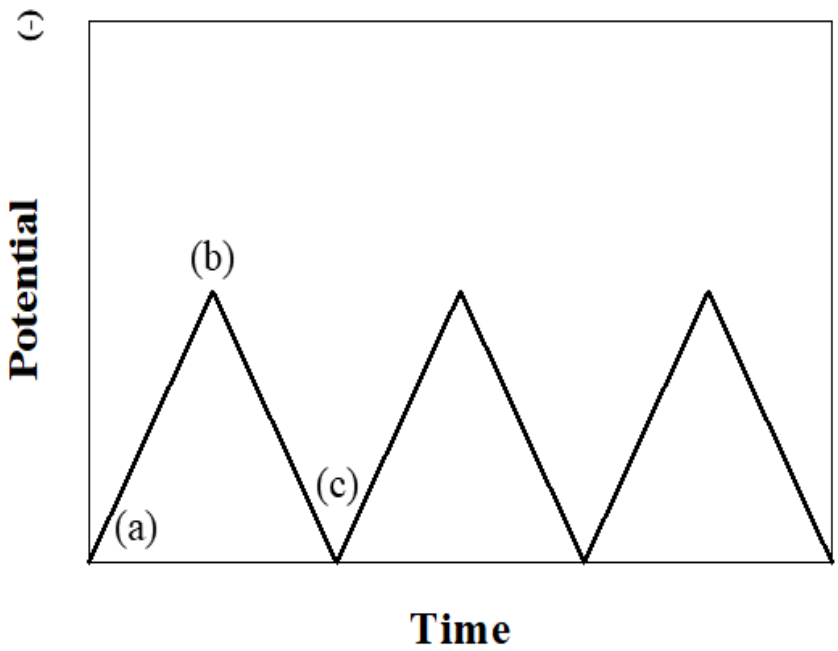


Fig. 3-2 Potential changes with times at CV

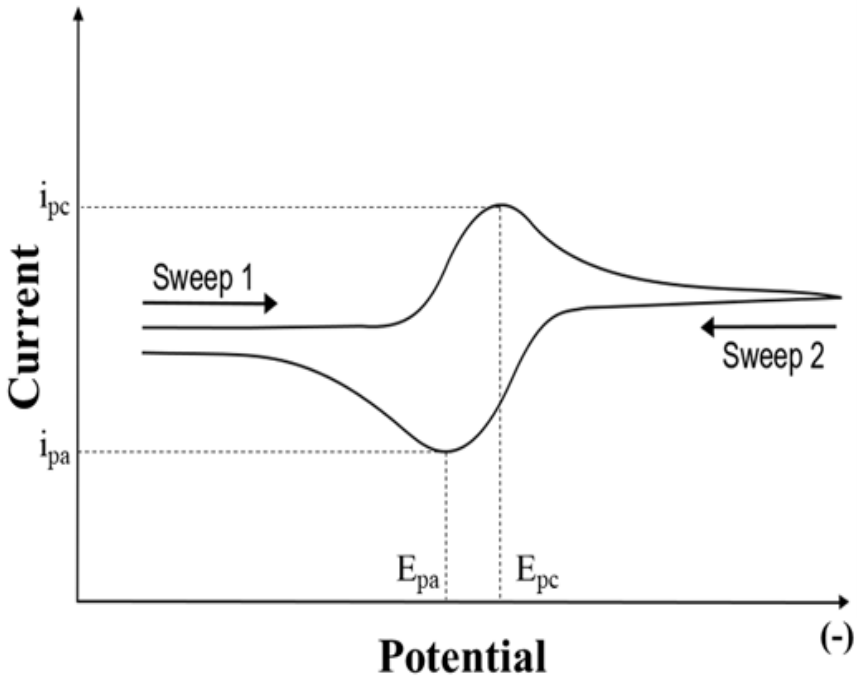


Fig. 3-3 The current change with potential at CV

3.4. Analytical methods

A total of 5 mL bulk solution in each reactor was sampled every two days. pH was measured using an Orion Star A211 pH meter (Thermo Fisher Scientific, Waltham, MA, USA). The concentration of NH_4^+ and Fe^{2+} was measured according to standard methods (Rice et al., 2012). NO_2^-, NO_3^-, and F^- concentrations were measured using ion chromatography (SDV50A, Youngling Co., Korea) with an absorbance detector (UV725S, Younglin Co., Korea). The concentrations of PFOA and its intermediate byproducts were measured using liquid chromatography-MS-MS (Shimadzu Corp., Japan). Separation was accomplished using a Phenomenex Gemini NX-C18 analytical column (150 mm length × 2.0 mm internal diameter, particle size 5 μm) (Phenomenex, Torrance, CA, USA). A delay column was installed between the mixer and sample injector to separate impurities that may have existed in the chromatographic system. The mobile phase consisted of 2 mM ammonium acetate in

water (mobile phase A) and methanol (mobile phase B) delivered at a flow rate of 0.35 mL/min. The gradient program was initially set at 20% B and then ramped up to 98% B over 6 min, followed by an isocratic hold at 98% B for 9 min. At 9.01 min, the gradient returned to 20% B and held until 11 min. The total run time for each injection was 11 min, the sample injection volume was set at 5.0 μL, and the column temperature was maintained at 40℃. DNA extraction, polymerase chain reaction, pyrosequencing, and MiSeq pipeline for analyzing the microbial community in the inoculum anammox granules were conducted according to the methodologies of Park et al. (2021).

4. Results

4.1. Microbial communities in anammox granules

Based on the results of the microbial community structure analysis of inoculated mixed culture anammox granules, uncultured anammox bacteria (LC192376), *Fimbriimonas ginsengisoli*, and *Comamonas granuli* were dominant species, showing their compositions of 30.87, 11.34, and 5.79%, respectively. As a result of 16S rRNA analysis, uncultured anammox (LC192376) showed 99.55% similarity in nucleotide sequence with anammox bacteria. This made it clear that anammox bacteria grew as dominant species in the mixed culture anammox granules inoculum and was dominant in nitrogen removal pathways. *Fimbriimonas ginsengisoli* is a Fe (Ⅲ) and sulfate reducing microorganism found in soil and contains Siroheme, an iron-chelated modified tetrapyrrole protein with crucial roles in a variety of biological functions. (Beas et al., 2022). Tang et al. (2018) confirmed the presence of the genus *Fimbriimonas* in the lab scaled anammox reactor and confirmed that this bacterium was rich in BCG (Bacterial Communication Gene). They also reported that bacteria with abundant BCG were more likely to interact with bacteria with the same functional properties, indicating potential communication-related interactions between anammox bacteria besides co-substrate utilization. *Comamonas granuli,* which belong to the family *Comamonadaceae,* possess the capability of reducing nitrate to nitrite in anammox granules (Zhang et al., 2016). The genus *Fimbriimonas* and the family *Comamonadaceae* mediate the nitrogen cycle coupled with iron redox cycling under anaerobic conditions (Bao et al., 2017; Zhang et al., 2016). In addition, based on their high abilities of redox reactions and electron transfer, they were often enriched in BES for nitrogen removal under complete anaerobic conditions (Wang et al., 2022; Feng et al., 2016). Therefore, the microbial community structure results support that inoculated mixed culture anammox granules were enriched with anammox bacteria. Fe(Ⅲ) and electric potential applied in this study can be involved in nitrogen removal and PFOA degradation through interaction with anammox bacteria.

<Table 4-1> Bacterial population distribution in inoculated mixed culture anammox granules

Class	Order	Family	Genus	Species	Rate
Fimbriimonadia	*Fimbriimonadales*	*Fimbriimonadaceae*	*Fimbriimonas*	*Fimbriimonas ginsengisoli*	11.34%
Anaerolineae	*Aggregatilineales*	*Aggregatilineaceae*	*Aggregatilinea*	*Aggregatilinea lenta*	8.96%
Chitinophagia	*Chitinophagales*	*Chitinophagaceae*	*Filimonas*	*Filimonas zeae*	6.90%
Betaproteobacteria	*Burkholderiales*	*Comamonadaceae*	*Comamonas*	*Comamonas granuli*	5.79%
Betaproteobacteria	*Burkholderiales*	*Burkholderiaceae*	*Zeimonas*	*Zeimonas arvi*	4.59%
Holophagae	*Holophagales*	*Holophagaceae*	*Geothrix*	*Geothrix fermentans*	4.50%
Alphaproteobacteria	*Hyphomicrobiales*	*Rhizobiaceae*	*Ensifer*	*Ensifer adhaerens*	3.16%
Ignavibacteria	*Ignavibacteriales*	*Ignavibacteriaceae*	*Ignavibacterium*	*Ignavibacterium album*	2.53%
Gammaproteobacteria	*Xanthomonadales*	*Xanthomonadaceae*	*Thermomonas*	*Thermomonas koreensis*	1.97%
Clostridia	*Eubacteriales*	*Clostridiaceae*	*Clostridium*	*Clostridium huakuii*	1.83%
Chitinophagia	*Chitinophagales*	*Chitinophagaceae*	*Sediminibacterium*	*Sediminibacterium goheungense*	1.79%
Betaproteobacteria	*Nitrosomonadales*	*Nitrosomonadaceae*	*Nitrosomonas*	*Nitrosomonas europaea*	1.73%
Flavobacteriia	*Flavobacteriales*	*Flavobacteriaceae*	*Amniculibacterium*	*Amniculibacterium aquaticum*	1.68%
Gammaproteobacteria	*Xanthomonadales*	*Xanthomonadaceae*	*Stenotrophomonas*	*Stenotrophomonas acidaminiphila*	1.66%
Nitrospira	*Nitrospirales*	*Nitrospiraceae*	*Nitrospira*	*Nitrospira moscoviensis*	1.65%
Gammaproteobacteria	*Moraxellales*	*Moraxellaceae*	*Acinetobacter*	*Acinetobacter baumannii*	1.39%
Chitinophagia	*Chitinophagales*	*Chitinophagaceae*	*Parasediminibacterium*	*Parasediminibacterium paludis*	1.35%
Deltaproteobacteria	*Desulfuromonadales*	*Geobacteraceae*	*Geobacter*	*Geobacter sulfurreducens*	1.33%
Caldilineae	*Caldilineales*	*Caldilineaceae*	*Litorilinea*	*Litorilinea aerophila*	1.13%
Gammaproteobacteria	*Xanthomonadales*	*Xanthomonadaceae*	*Thermomonas*	*Thermomonas haemolytica*	1.11%
Acidimicrobiia	*Acidimicrobiales*	*Acidimicrobiaceae*	*Aciditerrimonas*	*Aciditerrimonas ferrireducens*	1.09%
Planctomycetia	*Gemmatales*	*Gemmataceae*	*Limnoglobus*	*Limnoglobus roseus*	1.07%
Betaproteobacteria	*Rhodocyclales*	*Rhodocyclaceae*	*Azovibrio*	*Azovibrio restrictus*	1.06%
Anaerolineae	*Anaerolineales*	*Anaerolineaceae*	*Bellilinea*	*Bellilinea caldifistulae*	0.98%
Caldilineae	*Caldilineales*	*Caldilineaceae*	*Caldilinea*	*Caldilinea aerophila*	0.97%

Anaerolineae	*Anaerolineales*	*Anaerolineaceae*	*Thermomarinilinea*	*Thermomarinilinea lacunifontana*	0.83%
Thermomicrobia	*Sphaerobacterales*	*Sphaerobacteraceae*	*Sphaerobacter*	*Sphaerobacter thermophilus*	0.82%
Bacilli	*Bacillales*	*Bacillaceae*	*Neobacillus*	*Neobacillus cucumis*	0.65%
Alphaproteobacteria	*Hyphomicrobiales*	*Hyphomicrobiaceae*	*Hyphomicrobium*	*Hyphomicrobium facile*	0.52%
Gammaproteobacteria	*Xanthomonadales*	*Xanthomonadaceae*	*Pseudoxanthomonas*	*Pseudoxanthomonas japonensis*	0.47%
Gammaproteobacteria	*Xanthomonadales*	*Xanthomonadaceae*	*Stenotrophomonas*	*Stenotrophomonas maltophilia*	0.44%
Rubrobacteria	*Gaiellales*	*Gaiellaceae*	*Gaiella*	*Gaiella occulta*	0.42%
Blastocatellia	*Blastocatellales*	*Blastocatellaceae*	*Stenotrophobacter*	*Stenotrophobacter terrae*	0.42%
Planctomycetia	*Pirellulales*	*Pirellulaceae*	*Lignipirellula*	*Lignipirellula cremea*	0.40%
Betaproteobacteria	*Burkholderiales*	*Comamonadaceae*	*Pelomonas*	*Pelomonas puraquae*	0.33%
Acidobacteriia	*Bryobacterales*	*Bryobacteraceae*	*Paludibaculum*	*Paludibaculum fermentans*	0.31%
Actinomycetia	*Corynebacteriales*	*Nocardiaceae*	*Rhodococcus*	*Rhodococcus ruber*	0.27%
Alphaproteobacteria	*Hyphomicrobiales*	__	*Pseudorhodoplanes*	*Pseudorhodoplanes sinuspersici*	0.26%
Alphaproteobacteria	*Rhodospirillales*	*Azospirillaceae*	*Azospirillum*	*Azospirillum lipoferum*	0.23%
Gammaproteobacteria	*Chromatiales*	*Thioprofundaceae*	*Thioprofundum*	*Thioprofundum lithotrophicum*	0.23%
Deltaproteobacteria	*Myxococcales*	*Kofleriaceae*	*Haliangium*	*Haliangium tepidum*	0.18%
Alphaproteobacteria	*Hyphomicrobiales*	*Hyphomicrobiaceae*	*Hyphomicrobium*	*Hyphomicrobium aestuarii*	0.17%
Thermoleophilia	*Solirubrobacterales*	*Solirubrobacteraceae*	*Solirubrobacter*	*Solirubrobacter ginsenosidimutans*	0.16%
Deinococci	*Deinococcales*	*Deinococcaceae*	*Deinococcus*	*Deinococcus aerius*	0.13%
Betaproteobacteria	*Burkholderiales*	*Alcaligenaceae*	*Castellaniella*	*Castellaniella defragrans*	0.12%
Alphaproteobacteria	*Hyphomicrobiales*	*Bradyrhizobiaceae*	*Afipia*	*Afipia carboxidovorans*	0.12%
Caldilineae	*Caldilineales*	*Caldilineaceae*	*Caldilinea*	*Caldilinea tarbellica*	0.11%
Gemmatimonadetes	*Gemmatimonadales*	*Gemmatimonadaceae*	*Roseisolibacter*	*Roseisolibacter agri*	0.11%
Others	-	-	-	-	1.79%

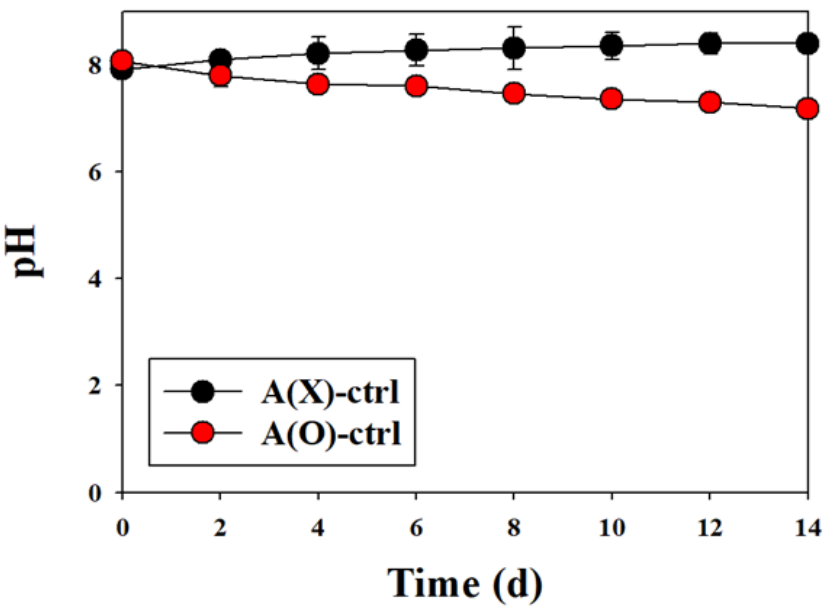
4.2. Anammox performance

4.2.1. pH

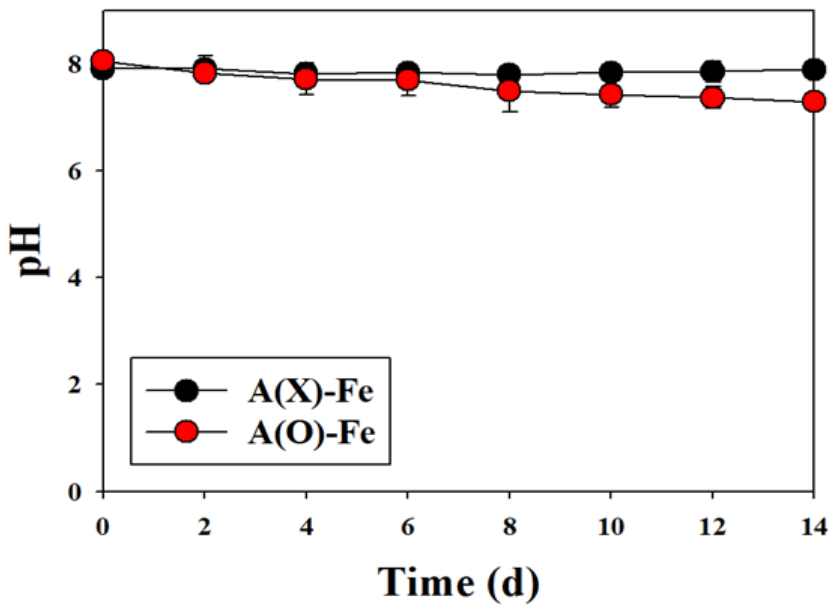
The pH is a significant parameter that can show whether microbial metabolism occurs and optimum pH is an essential condition for microbial growth. Since anammox bacteria are sensitive to pH changes, pH control is important during process operation. The optimum pH used for wastewater treatment was reported in the range of 6.7-8.3 (Strous et al., 1999). The pH, a critical parameter for assessing the anammox process, exhibited fluctuations in all reactors except for the control group. Prior to the commencement of the experimental run, the pH of all reactors was adjusted to fall within the optimal range of 7.8-8.0. This range is known to be conducive to the growth of mixed culture anammox granules, as established in previous research (Tomaszewski et al., 2017). According to Eq. 11, the anammox reaction with NO_2^- as an electron acceptor leads to pH increase due to the consumption of 0.13 moles of protons per mole of NH_4^+. However, in scenarios where NH_4^+ undergoes oxidation without NO_2^-, potential oxidation reactions (Eq. 12-14) result in proton production, subsequently causing a pH decrease (Li et al., 2016; Shaw et al., 2020). Depicted in Fig. 4-1, the pH trends in all reactors highlight that the A(O)- reactors, which were inoculated with mixed culture anammox granules, experienced a decrease in pH to the range of 7.1-7.4 by the end of the 14 days. In contrast, the A(X)- reactors, where mixed culture anammox granules were absent, demonstrated minimal fluctuations in pH.

The presence of Ammonium oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB), and ferric ion-dependent ammonium oxidation (FEAMMOX) bacteria within the granules suggest that anammox bacteria do not solely cause pH changes. However, the microbial community supports that the dominant species, anammox bacteria, plays a significant role in the primary reaction.

(a)


pH
Time (d)
A(X)-ctrl
A(O)-ctrl
0
2
4
6
8
10
12
14

(b)


pH
Time (d)
A(X)-Fe
A(O)-Fe
0
2
4
6
8
10
12
14

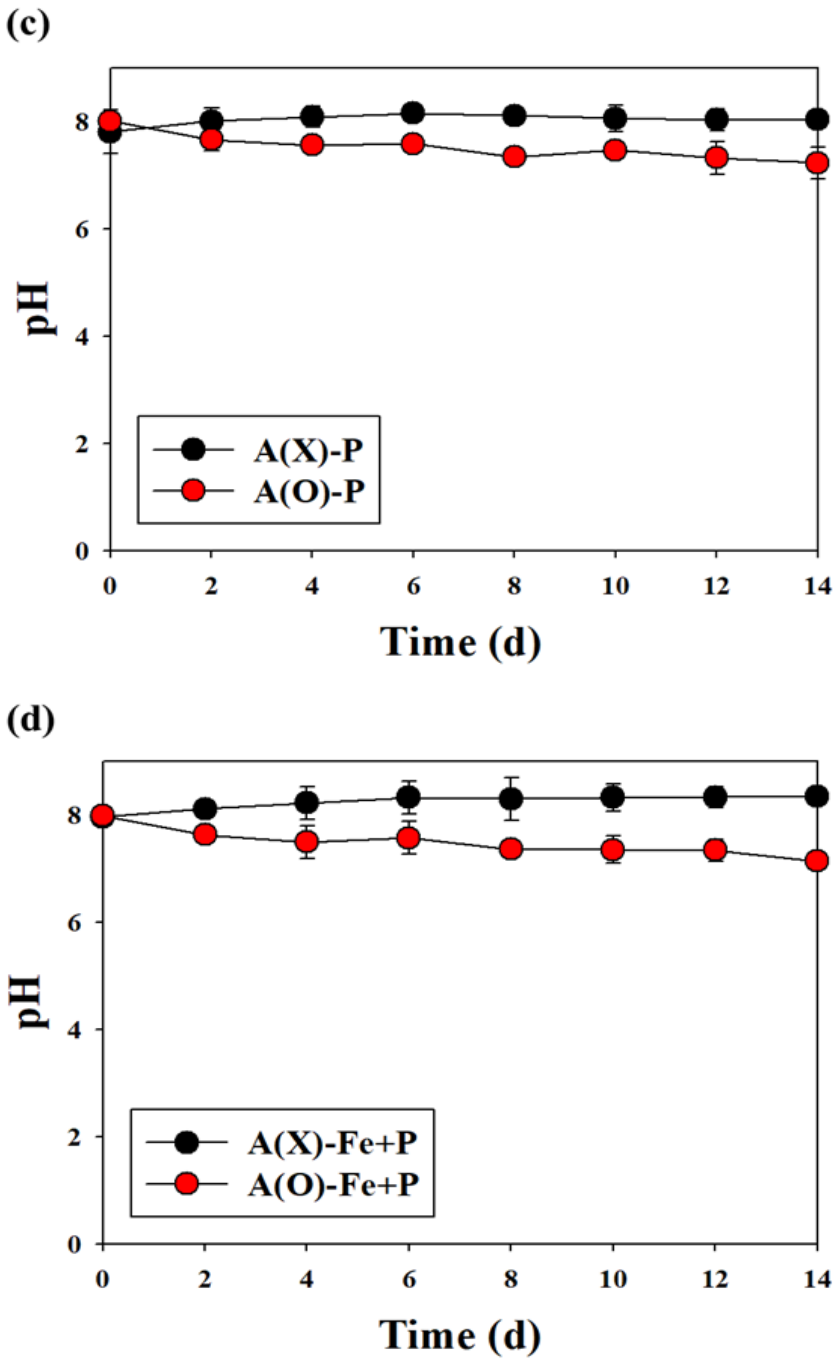
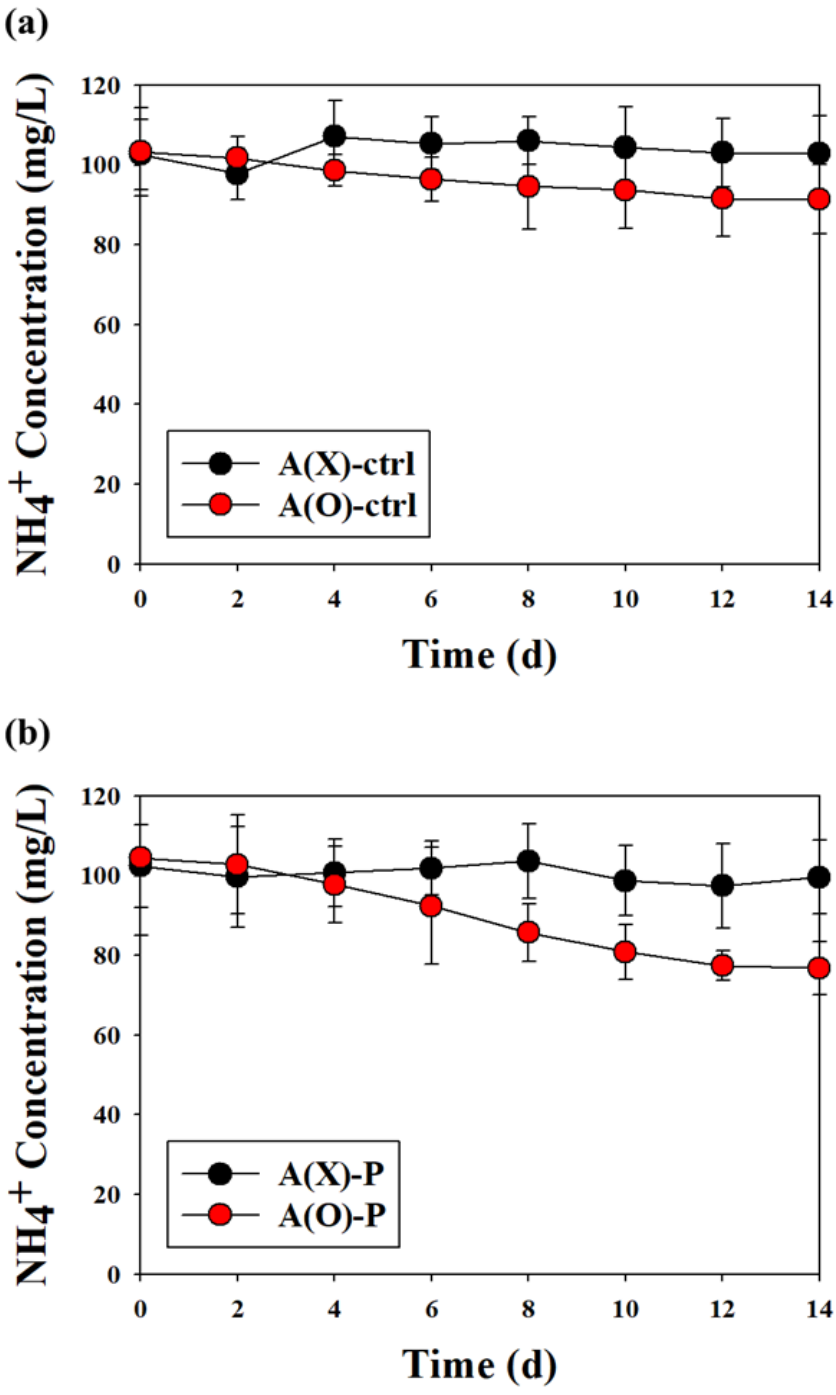


Fig. 4-1 Change in pH (a) A(O/X)-ctrl, (b) A(O/X)-Fe, (c) A(O/X)-P, (d) A(O/X)-Fe+P reactors

4.2.2. Nitrogen removal and ferric ion

NH_4^+ is an essential substrate for anammox bacteria. Anammox bacteria use NH_4^+ as an electron donor and NO_2^- as an electron acceptor. However, PFOA was injected instead of NO_2^- to confirm its role as an electron acceptor. NH_4^+ removal was observed in all A(O)- reactors over 14 days duration. Fig. 4-2 illustrates the NH_4^+ removal from the reactors within this timeframe. The NH_4^+ was not removed in reactors without anammox granules during the 14 days. However, in reactors with anammox granules, the removal efficiencies varied. The A(O)-ctrl reactor had a removal efficiency of 11.52%, while A(O)-Fe, P, Fe+P reactors had removal efficiencies of 34.05, 26.51, and 41.98%, respectively. Reactors injected with Fe(Ⅲ) exhibited notably greater NH_4^+ removal efficiency than other reactors. In particular, the reactor injected with Fe(Ⅲ) and applied a potential of 0.4 V vs. Ag/AgCl showed the most notable NH_4^+ removal. These findings underscore the substantial enhancement in anaerobic NH_4^+ removal achieved by applying electric potential. Likewise, the injection of Fe(Ⅲ) alongside the application of electric potential enhanced the electron transfer rate of mixed culture anammox granules in conditions without NO_2^-. This outcome aligns with a previous study that observed a 50.38 and 38.8% increase in anaerobic NH_4^+ removal through a BES-Fe(Ⅲ) reactor compared to single chamber BES and Fe reactors (T.-t. Zhu et al., 2021). Similarly, Ruiz-Urigüen et al. (2019) reported a significantly enhanced NH_4^+ removal efficiency in the BES (+0.3 V vs. Ag/AgCl) with Fe(Ⅲ) of the feammox bacteria, *Acidimicrobiaceae* sp. A6. The synergistic effects of BES and feammox process likely contribute to the improved NH_4^+ removal observed in this study.


(a)
NH4+ Concentration (mg/L)
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
Time (d)
A(X)-ctrl
A(O)-ctrl
(b)
NH4+ Concentration (mg/L)
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
Time (d)
A(X)-P
A(O)-P

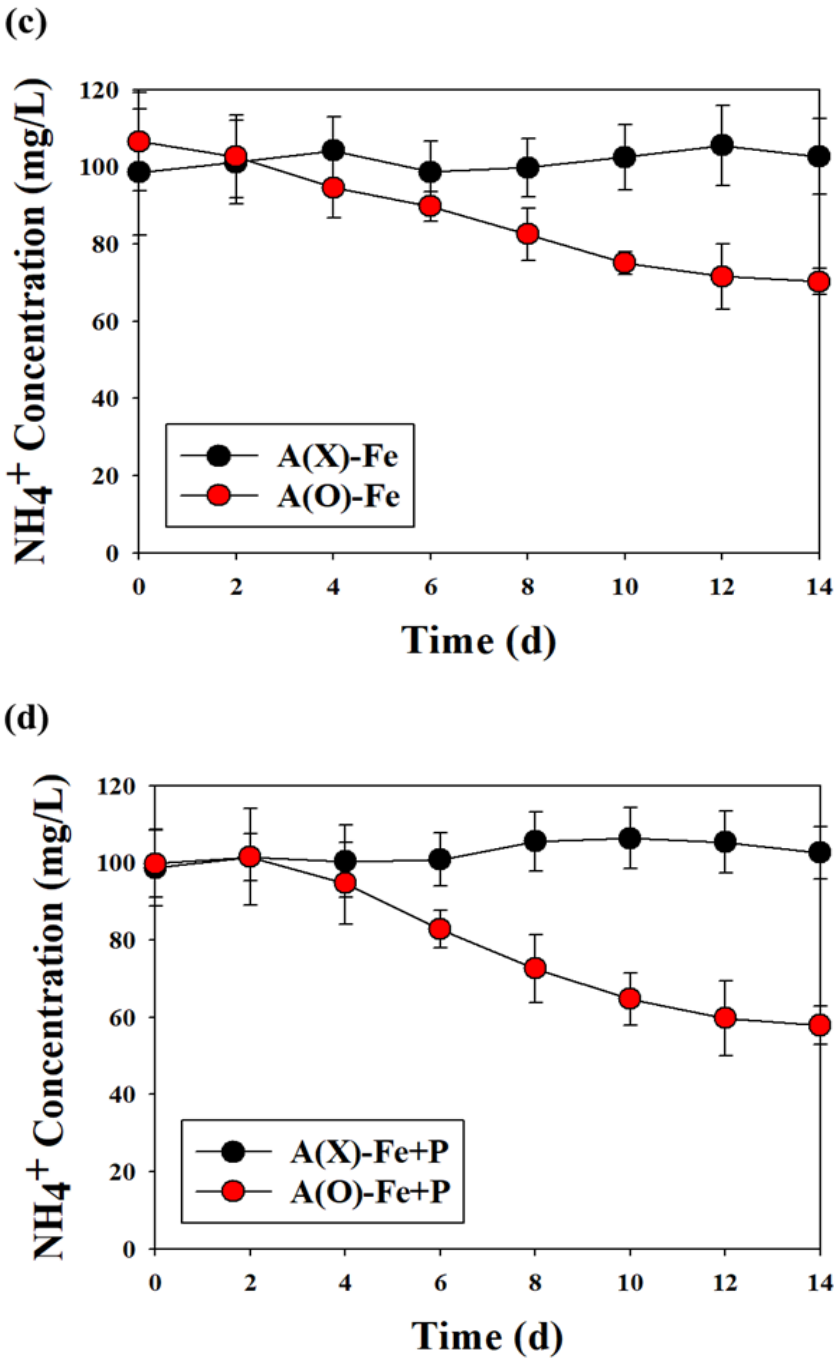


Fig. 4-2 Change in NH4+ concentration (a) A(O/X)-ctrl, (b) A(O/X)-Fe, (c) A(O/X)-P, (d) A(O/X)-Fe+P reactors

The Fe(Ⅱ) concentrations were measured to explore the involvement of Fe(Ⅲ) reduction in enhancing anammox granules electron transfer rates and changes in reactor. Fig. 4-3 illustrates the change in Fe(Ⅱ) concentration across all reactors over the 14 days. The final Fe(Ⅱ) concentration in reactors A(O)-Fe+P and A(O)-Fe increased to 58.5 and 31.4 mg/L, respectively. No Fe(Ⅱ) was detected except for A(O)-Fe and A(O)-Fe+P reactors. The presence of detectable Fe(Ⅱ) provides evidence that mixed culture anammox granules can utilize Fe(Ⅲ) as a final electron acceptor and an intermediate electron shuttle when supported by electric potential. Apart from anammox bacteria, multiple Feammox reactions are believed to occur within anammox sludge. These reactions enable NH_4^+ oxidation in an anoxic atmosphere, producing NO_2^- and NO_3^- (Eq. 16-18) (J. Zhu et al., 2021)

$$3Fe(OH)_3 + 5H^+ + NH_4^+ \rightarrow 3Fe^{2+} + 9H_2O + 0.5N_2$$

$$\triangle_r G_m = -245 \text{ kJ mol}^{-1} \text{ (Eq. 16)}$$

$$6Fe(OH)_3 + 10H^+ + NH_4^+ \rightarrow 6Fe^{2+} + 16H_2O + NO_2^-$$

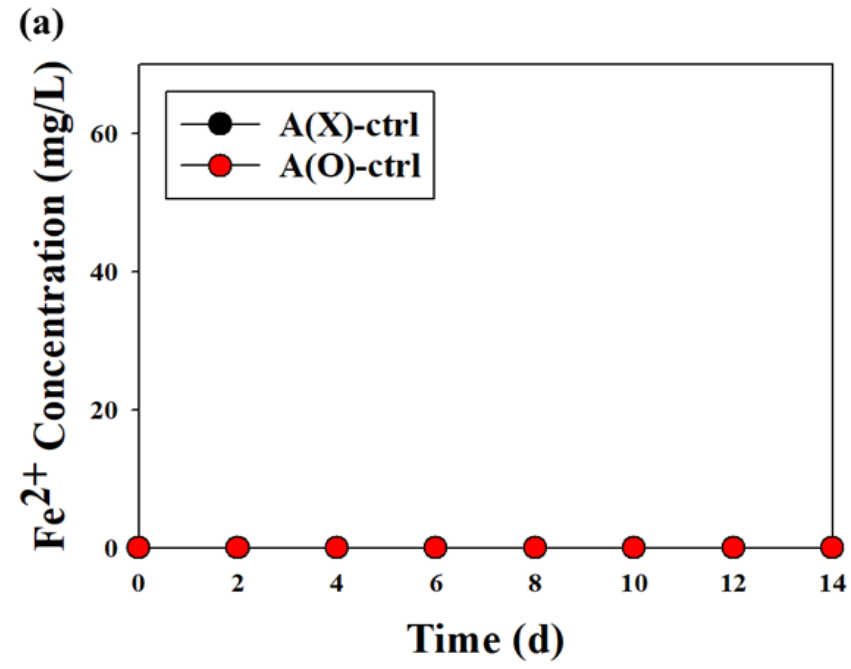
$$\triangle_r G_m = -164 \text{ kJ mol}^{-1} \text{ (Eq. 17)}$$

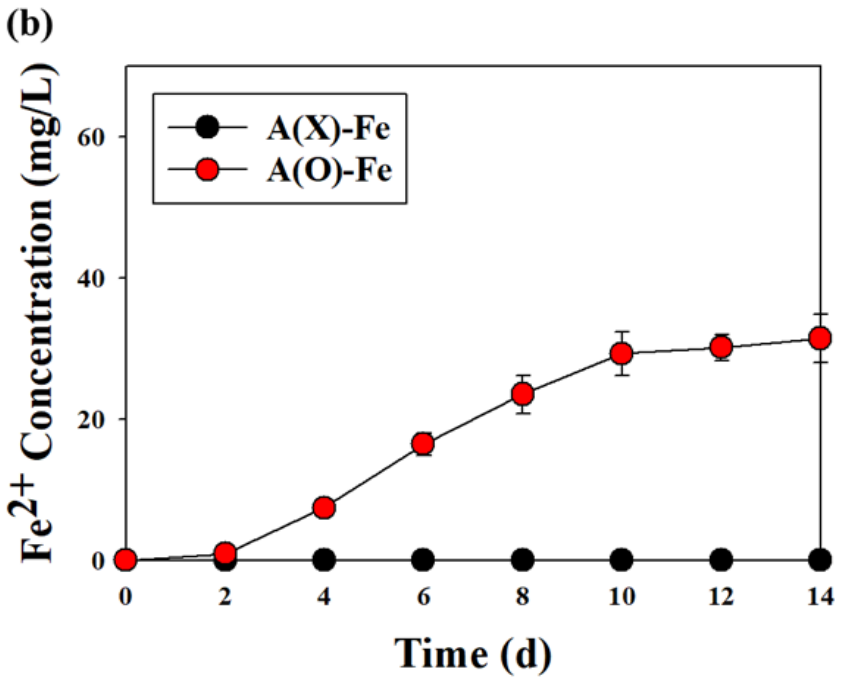
$$8Fe(OH)_3 + 14H^+ + NH_4^+ \rightarrow 8Fe^{2+} + 21H_2O + NO_3^-$$

$$\triangle_r G_m = -207 \text{ kJ mol}^{-1} \text{ (Eq. 18)}$$

In these reactions the Fe(Ⅲ) (hydr) oxides serve as electron acceptors, converting to Fe(Ⅱ) under anaerobic conditions and generating N_2 (Ding et al., 2014). Notably, although some NH_4^+ residue is oxidized to NO_3^- and NO_2^-, the most efficient pathway yields N_2 (J. Zhu et al., 2021). The addition of Fe(Ⅲ) could have improved electrochemical characteristics by enriching iron-reducing bacteria (FeRB). Enhanced NH_4^+ removal might be attributed to NO_2^- generated from the feammox process driving anoxic NH_4^+ removal (T.-t. Zhu et al., 2021). However, as NH_4^+ was oxidized, NO_3^- levels in the reactors increased. Fig. 4-4 portrays the increasing NO_3^- concentrations in all reactors. Notably, the A(X)-Fe+P reactor with applied potential exhibited higher NO_3^- accumulation than other reactors. This phenomenon can be attributed to various factors. Prior studies also noted NO_3^- accumulation in BES system with anoxic NH_4^+ oxidation. The application of anode potential likely influenced the NH_4^+ to NO_2^- process,

compensating for the absence of NO_2^- and promoting anammox with increased effluent NO_3^-. Furthermore, feammox can generate NO_3^- through Fe(Ⅲ) reduction and NH_4^+ oxidation, contributing to NO_3^- accumulation (Wan et al., 2022). Similarly, it's been reported that AOB and feammox bacteria in mixed culture anammox granules can produce NO_3^- under iron dependent conditions without NO_2^- (Li et al., 2018). Despite this, comparing A(O)-Fe and A(O)-ctrl reactors, NO_2^- production from feammox bacteria supported by Fe(Ⅲ) may have had a lesser impact on NH_4^+ removal, given similar NO_3^- concentrations. Overall, this study demonstrates that both Fe(Ⅲ) and applied electric potential, acting as electron acceptors and/or intermediate electron shuttles, can support anammox bacteria in oxidizing NH_4^+ without the need for NO_2^-. These positive effects of anammox metabolism could expediate NH_4^+ removal and replace NO_2^- as an electrode (insoluble material) electron acceptor alongside PFOA and Fe(Ⅲ). Nevertheless, the NH_4^+ removal efficiency witnessed in this investigation was notably lower than the outcomes reported in other studies that achieved considerably higher removal rates through the utilization of BES. This disparity could potentially be attributed to the concentrations of PFOA.


(a)
A(X)-ctrl
A(O)-ctrl
Fe2+ Concentration (mg/L)
0
20
40
60
0
2
4
6
8
10
12
14
Time (d)


(b)
A(X)-Fe
A(O)-Fe
Fe2+ Concentration (mg/L)
0
20
40
60
0
2
4
6
8
10
12
14
Time (d)

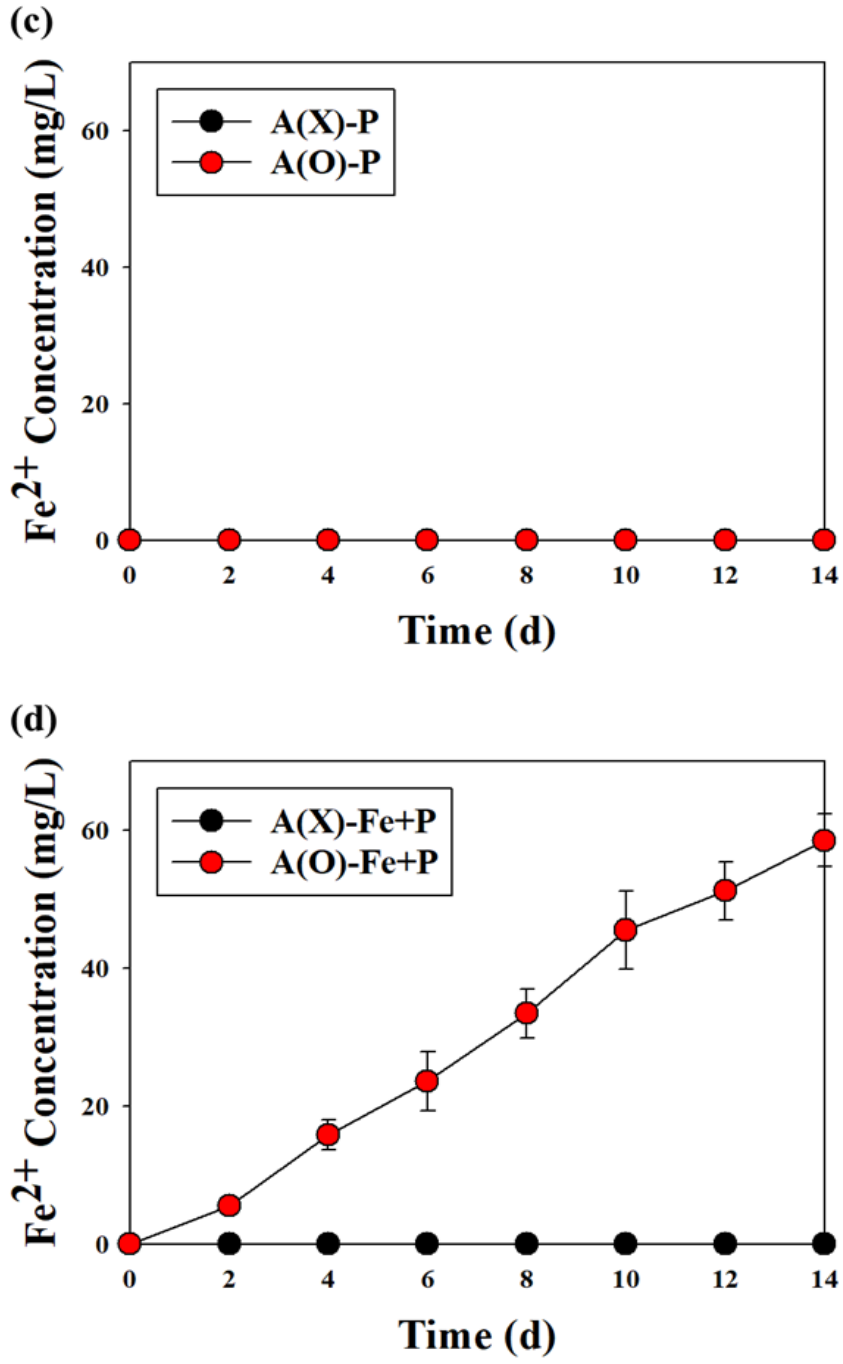
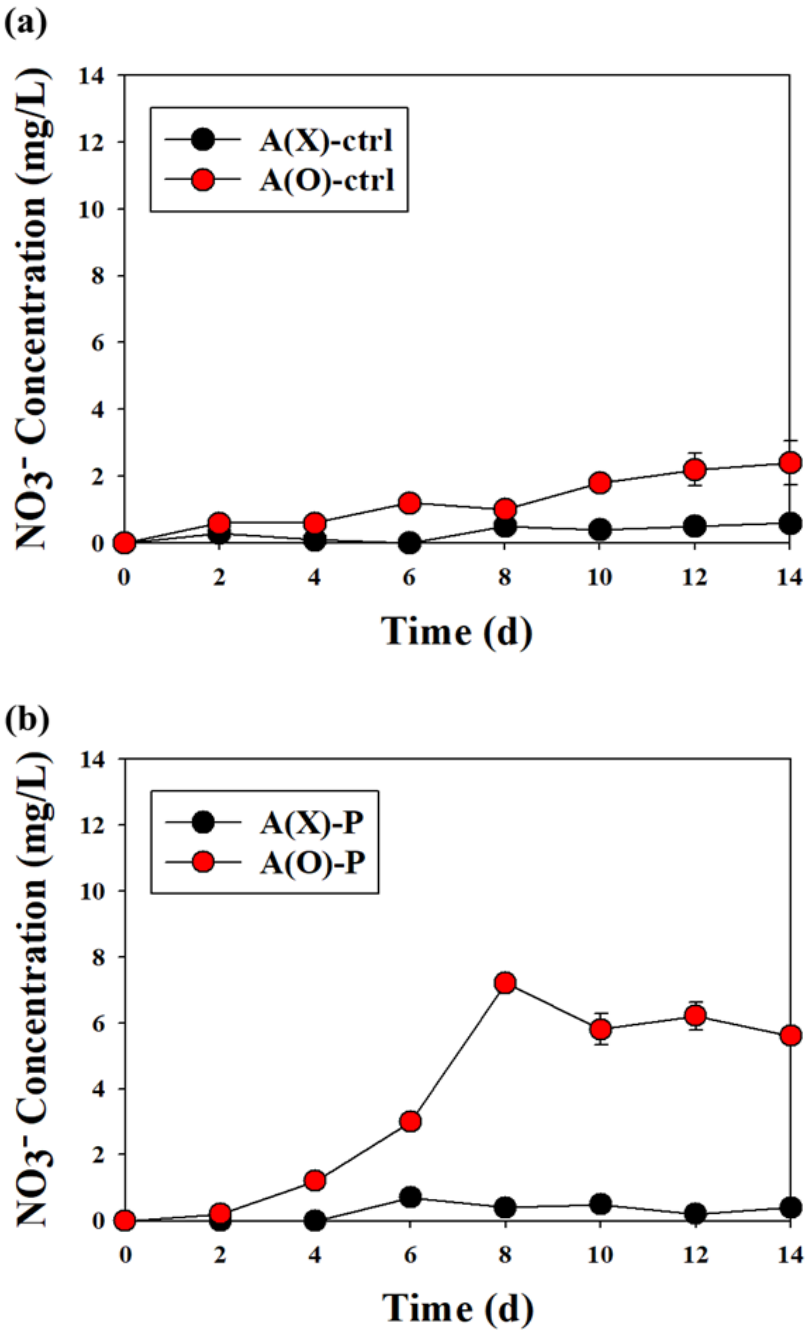


Fig. 4-3 Change in Fe2+ concentration (a) A(O/X)-ctrl, (b) A(O/X)-Fe, (c) A(O/X)-P, (d) A(O/X)-Fe+P reactors


(a)
A(X)-ctrl
A(O)-ctrl
NO3- Concentration (mg/L)
Time (d)
(b)
A(X)-P
A(O)-P
NO3- Concentration (mg/L)
Time (d)

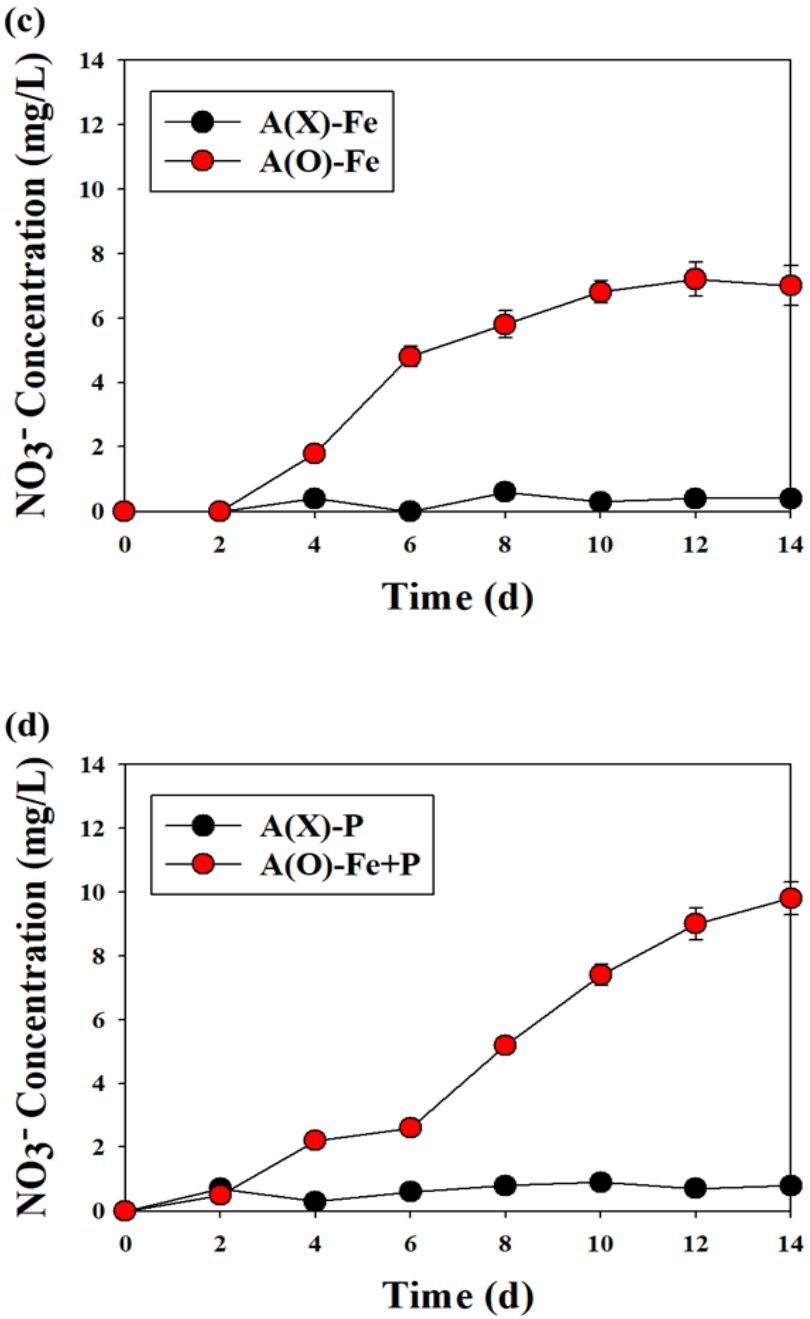


Fig. 4-4 Change in NO3- concentration (a) A(O/X)-ctrl, (b) A(O/X)-Fe, (c) A(O/X)-P, (d) A(O/X)-Fe+P reactors

4.3. PFOA degradation & byproducts

Although the biodegradation pathway isn't clearly demonstrated, various pathways are proposed in physicochemical fields. According to Density Functional Theory (DFT), when additional electrons attack Perfluoroalkyl carboxylic acids (PFCAs), the C-F bond is stretched, which promotes the breaking of the C-F bond. In the case of photochemical degradation, H/F exchange which replaces fluorine in PFOA with hydrogen, and chain shortening, are proposed. In the H/F exchange, the added electrons drop one fluorine ion from PFOA, and hydrogen is attached (Eq. 19-20). After two times of H/F exchanges, chain shortening is performed through a photochemical reaction of Eq. 21-22. The second proposed pathway is Decarboxylation-Hydroxylation-Elimination-Hydrolysis (DHEH) mechanism. DHEH, which degrades PFOA through these four steps, is proposed as the most likely mechanism for PFCAs chain shortening and accompanying F^- release (Eq. 23-26) (Bentel et al., 2019).

$$C_7F_{15}COO^- + e_{aq}^- + \rightarrow \ \cdot C_7F_{14}COO^- + F^- \quad \text{(Eq. 19)}$$

$$\cdot C_7F_{14}COO^- + H_2O \rightarrow \ \cdot C_7F_{14}HCOO^- + OH^- \quad \text{(Eq. 20)}$$

$$C_7F_{13}H_2COO^- + h\nu \rightarrow \ \cdot C_6F_{13} + \ \cdot COO^- + CH_2 \quad \text{(Eq. 21)}$$

$$\cdot C_6F_{13} + \cdot COO^- \rightarrow C_6F_{13}COO^- \quad \text{(Eq. 22)}$$

$$C_7F_{15}COO^- + e_{aq}^- + \rightarrow \ \cdot C_7F_{15} + COO^- \quad \text{(Eq. 23)}$$

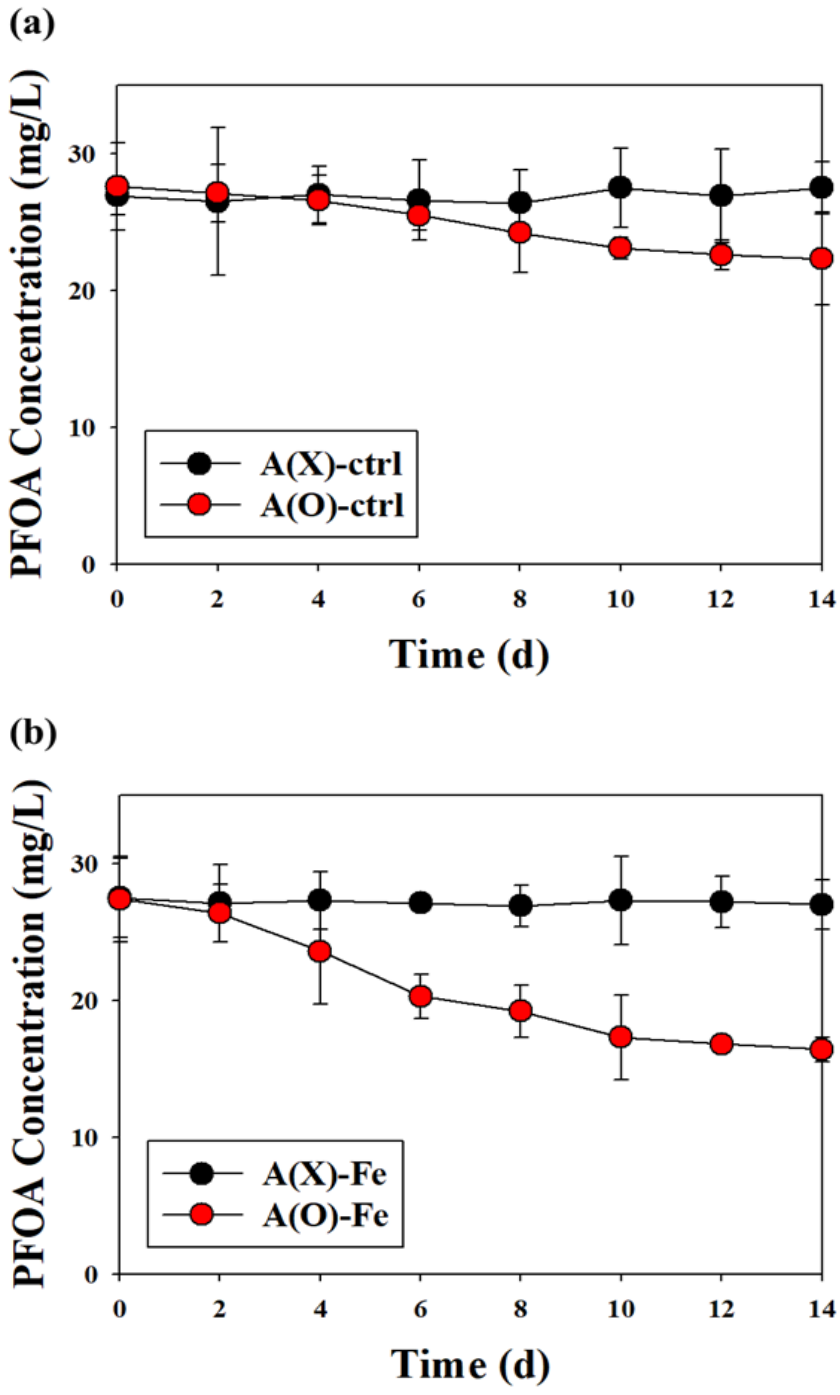
$$\cdot C_7F_{15} + H_2O \rightarrow C_7F_{15} + H^+ \quad \text{(Eq. 24)}$$

$$C_7F_{15}OH \rightarrow C_6F_{13}COF + H^+ + F^- \quad \text{(Eq. 25)}$$

$$C_6F_{13}COF + H_2O \rightarrow C_6F_{13}COO^- + 2H^+ + F^- \quad \text{(Eq. 26)}$$

Therefore, it can be assumed that biological treatment also degrades PFOA by eliminating fluorine ions and generating perfluoroalkyl acids (PFAA) due to additional electrons. The PFOA concentration in each reactor was measured to confirm the PFOA degradation efficiency of the anammox granules and quantify the byproducts of PFOA degradation, such as F^-, perfluorohexanoic acid (PFHxA, $C_6HF_{11}O_2$), perfluoroheptanoic acid (PFHpA, $C_7HF_{13}O_2$), hexafluorobutyric acid (HFBA, $C_4HF_7O_2$), and

perfluoropentanoic acid (PFPeA, $C_5HF_9O_2$). There was no change in the PFOA concentration in A(X)- reactors, but it decreased in A(O)-reactors. A total of 40.15, 36.16, 50.93, and 19.20% of PFOA were degraded in the A(O)-Fe, A(O)-P, A(O)-Fe+P, and A(O)-ctrl reactors over 14 days, respectively (Fig. 4-5). The A(O)-ctrl reactor confirmed the degradation of PFOA despite its lowest efficiency. This supports previous results suggesting that mixed culture anammox granules could degrade PFOA (Tang et al., 2022).


(a)
PFOA Concentration (mg/L)
30
20
10
0
A(X)-ctrl
A(O)-ctrl
0
2
4
6
8
10
12
14
Time (d)
(b)
PFOA Concentration (mg/L)
30
20
10
0
A(X)-Fe
A(O)-Fe
0
2
4
6
8
10
12
14
Time (d)

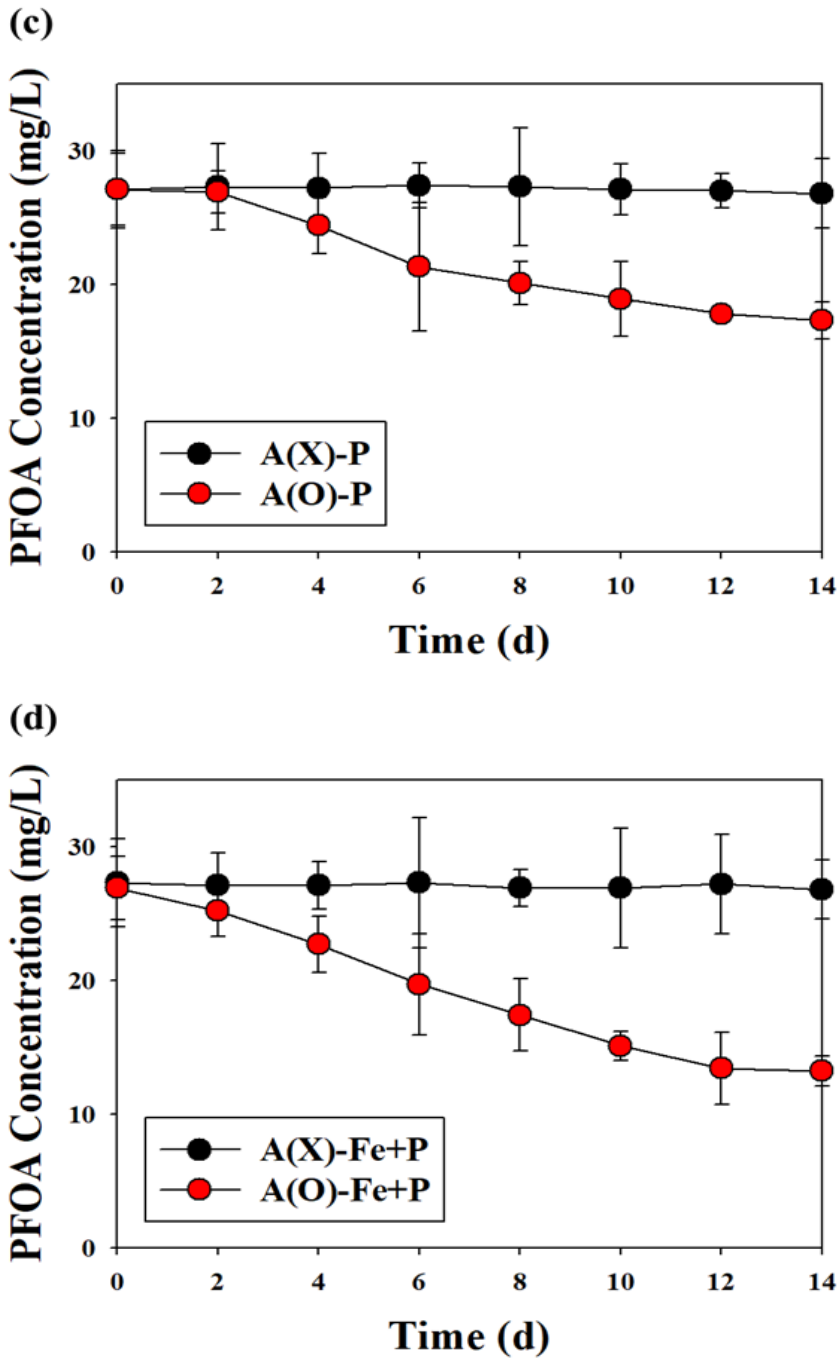


Fig. 4-5 Change in PFOA concentration (a) A(O/X)-ctrl, (b) A(O/X)-Fe, (c) A(O/X)-P, (d) A(O/X)-Fe+P reactors

Huang and Jaffe (2019) tracked PFOA removal with fluorine of PFOA, fluorine of PFAAs, and Fluorine balance using detected F^- and showed high accuracy. Various studies have proposed degradation mechanisms involving decarboxylation, and biodegradation is also believed to cause PFOA attacks of e^-, release of F^-, and formation of short-chain PFAAs, as suggested by photochemical or electrochemical degradation (Trojanowicz et al., 2018; L. Yang et al., 2020). Therefore, F^- and PFAAs can show clear evidence of PFOA biodegradation. Lenka et al. (2021) reported that a decrease in the PFOA concentration might be due to the adsorption of PFOA onto the inner cell and electrode surface through hydrophobic interactions, given that PFOA is a long-chain PFASs with hydrophobic characteristics and strong affinity for organic matter. This supports the possibility of PFOA attachment on anammox granules and electrode surface through electric adsorption. In this study, F^- was detected in proportion to the removed PFOA, with 2.51, 2.06, 3.98, and 0.55 mg/L in the A(O)-Fe, A(O)-P, A(O)-Fe+P, and A(O)-ctrl reactors, respectively. Further, PFAAs, PFOA intermediates, were also detected, balancing the PFOA footprints (Table 4-2 and Fig. 4-7-4-10), and the feasible pathway of PFOA biodegradation is simply indicated in Fig. 4-6. The 0.398, 0.355, 0.496, and 0.192 mM of fluorine are present in the removed PFOA from the A(O)-Fe, A(O)-P, A(O)-Fe+P, and A(O)-ctrl reactors, respectively. The total sum of fluorine in the four types of PFAA and detected fluorine is 0.174, 0.137, 0.291, and 0.037 mM, accounting for 43.65, 38.65, 58.67, and 19.29% of the balance. The 19.29% of fluorine balance in the A(O)-ctrl reactor suggests that only 0.037 mM (1.022 mg/L) of PFOA was degraded by the metabolism of anammox granules, and 0.155 mM (4.278 mg/L) was removed by adsorption. Since the same concentration of anammox granules was injected into all biotic reactors, 4.278 mg/L of PFOA was removed by adsorption in all biotic reactors. Except for the removed PFOA by adsorption, the PFOA biodegradation efficiency by metabolism of the anammox granules in each reactor is 24.5, 20.4, 35.0, and 3.7% in the A(O)-Fe, A(O)-Fe+P, and A(O)-ctrl reactors, respectively.

In addition, the possibility of the presence of other intermediate substances cannot be excluded due to the mixture of various species. Anammox bacteria was the dominant species of mixed culture anammox granules used in this study. Following that species,

various heterotrophic bacteria, such as *Aggregatilinea lenta* (8.96%), *Filimonas zeae* (6.90%), *Geothrix fermentans* (4.50%), and others, were the subdominant species (Table 4-1). Heterotrophic bacteria are believed to produce several shorter-chain PFAAs because they can further break the C-C bonds of polyfluorinated alkyl substances (Huang & Jaffé, 2019; Ruiz-Urigüen et al., 2022). Because PFOA degradation of many microorganisms has been reported, tracking F^- released from PFOA entirely is complex. However, when the PFOA degradation tendency and F^- mass balance were analyzed, injection of electric potential and Fe(Ⅲ) favored efficient PFOA degradation by the mixed culture anammox granules. Based on these results, it can be believed that the mixed culture anammox granules serve efficient PFOA degradation when Fe(Ⅲ) and electric potential are applied under NO_2^- absent NH_4^+ oxidation conditions.

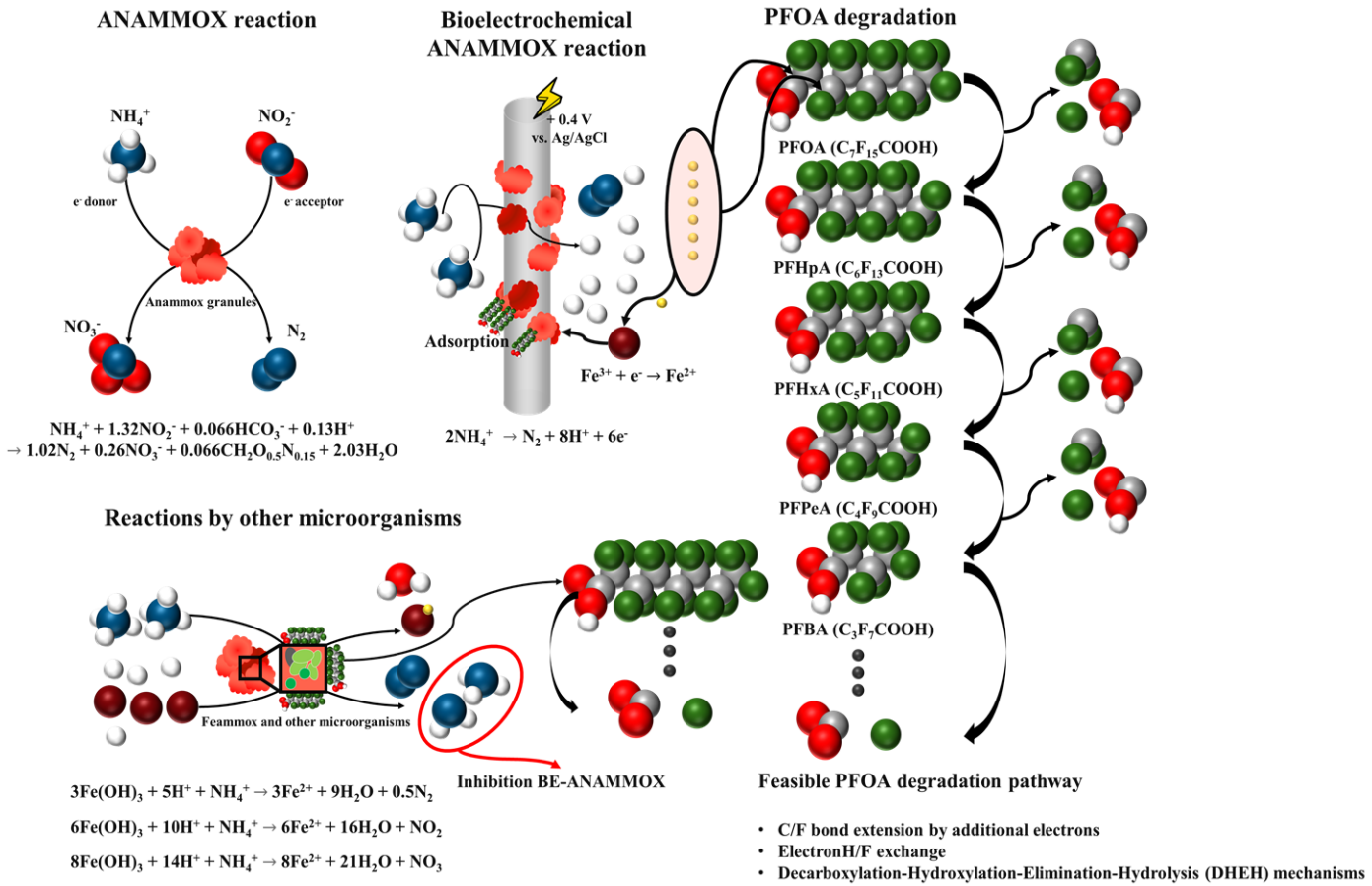
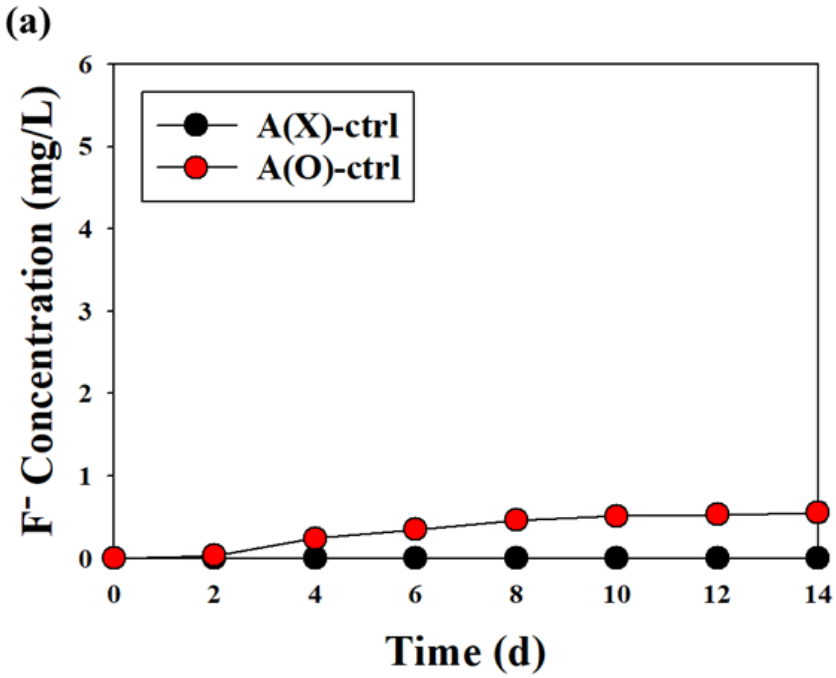
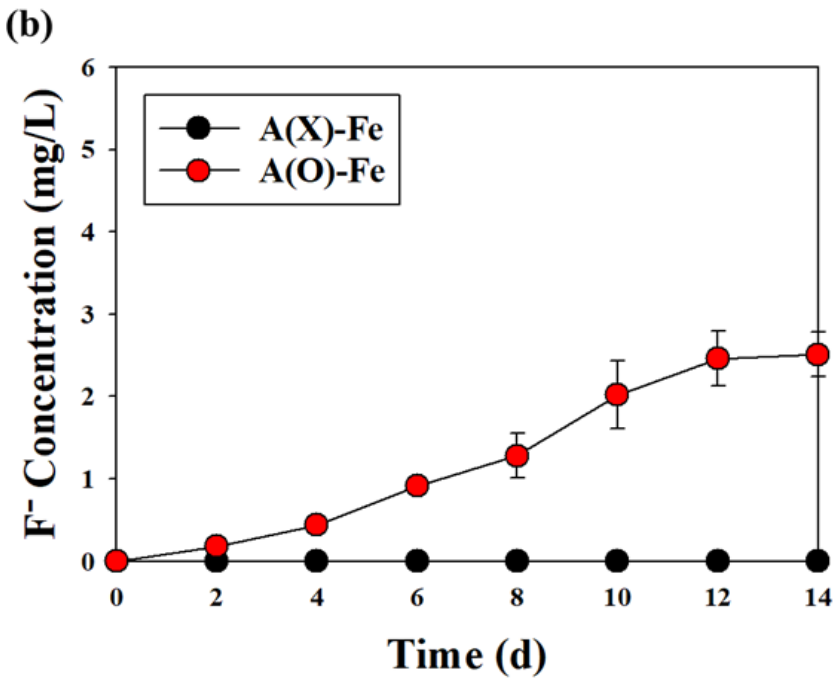


Fig. 4-6 The feasible pathway of PFOA degradation

<Table 4-1> Fluorine mass balance for PFOA degradation

Reactors	F in removed PFOA (mM)	F in PFHxA (mM)	F in PFHpA (mM)	F in PFPeA (mM)	F in HFBA (mM)	F^- (mM)	Detected total F (mM)	Total F / removed PFOA (%)
A(X)-ctrl	0	0	0	0	0	0	0	0
A(X)-Fe	0	0	0	0	0	0	0	0
A(X)-P	0	0	0	0	0	0	0	0
A(X)-Fe+P	0	0	0	0	0	0	0	0
A(O)-ctrl	0.192	0.007	0.001	0	0	0.029	0.037	19.29
A(O)-Fe	0.398	0.029	0.01	0.002	0	0.132	0.174	43.65
A(O)-P	0.355	0.022	0.005	0.002	0	0.108	0.137	38.65
A(O)-Fe+P	0.496	0.05	0.027	0.004	0	0.209	0.291	58.67


(a)
A(X)-ctrl
A(O)-ctrl
F- Concentration (mg/L)
Time (d)
0
1
2
3
4
5
6
0
2
4
6
8
10
12
14


(b)
A(X)-Fe
A(O)-Fe
F- Concentration (mg/L)
Time (d)
0
1
2
3
4
5
6
0
2
4
6
8
10
12
14

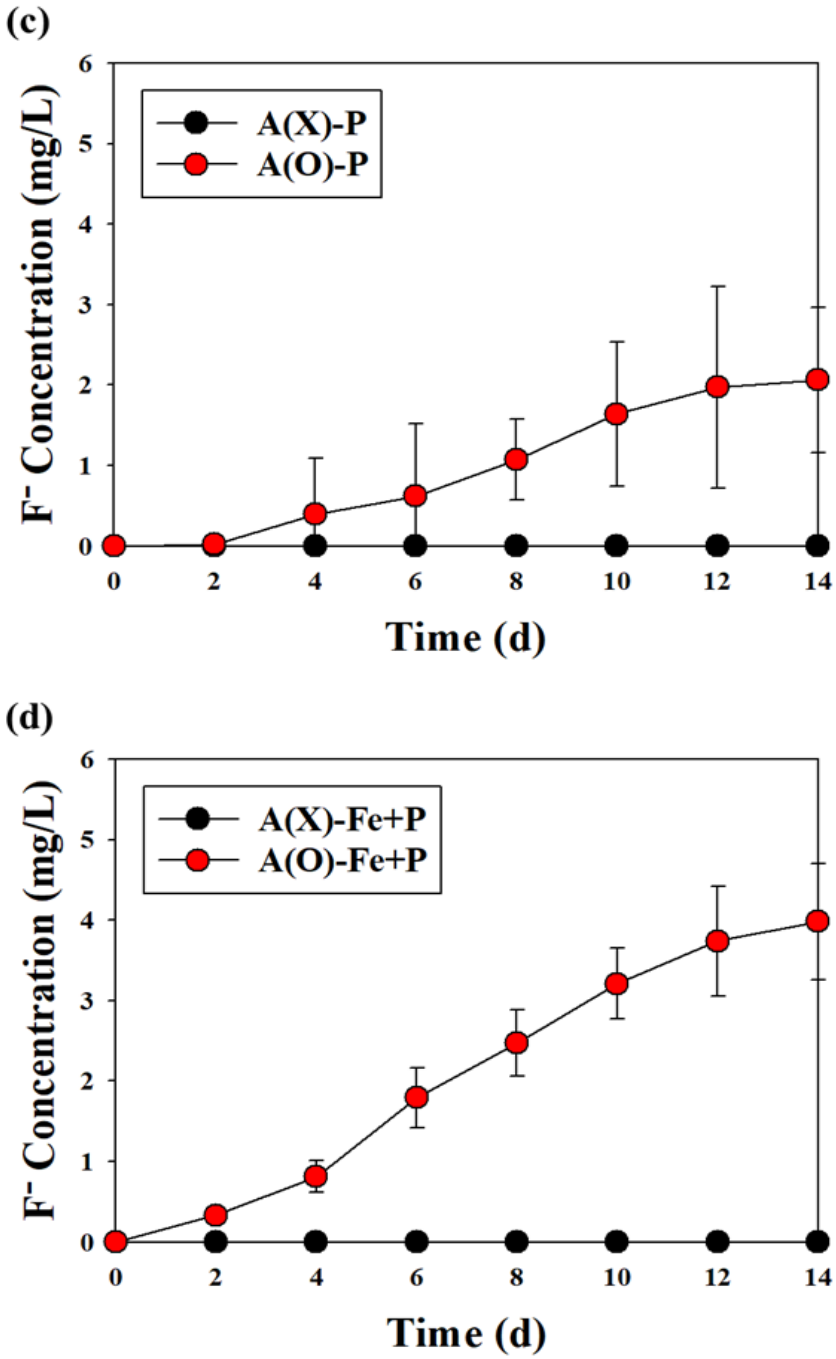
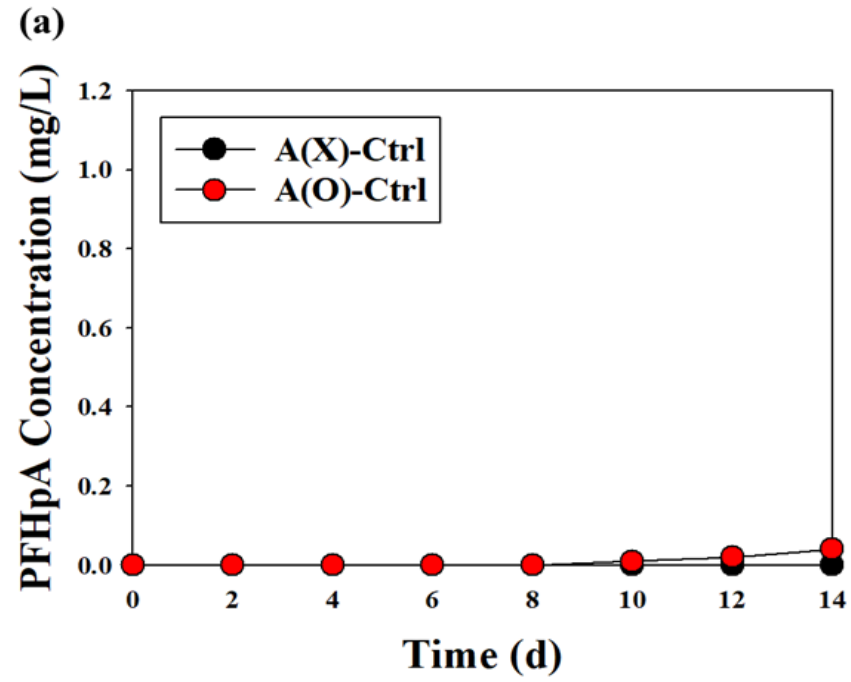
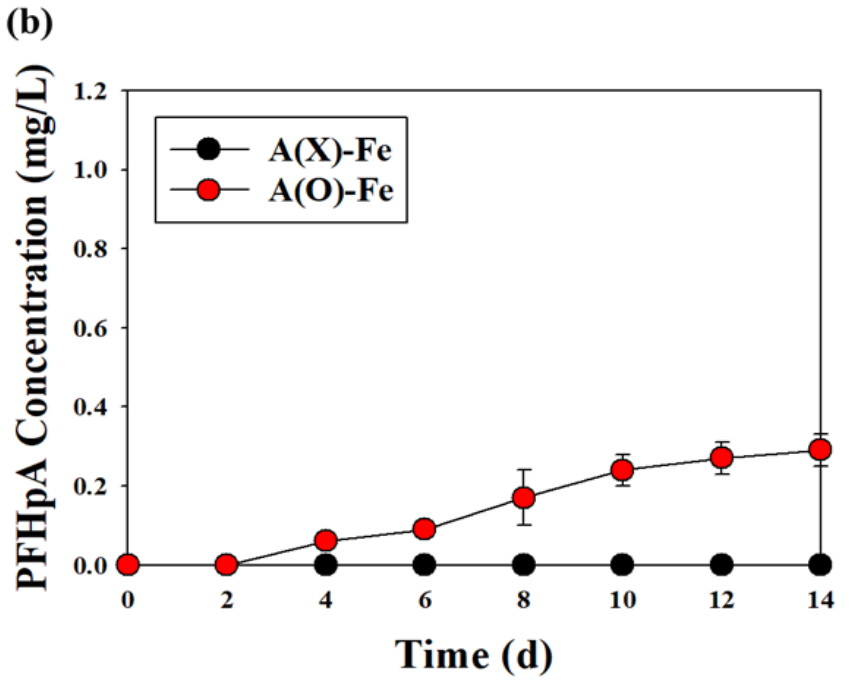


Fig. 4-7 Change in Fluorine ion concentration (a) A(O/X)-ctrl, (b) A(O/X)-Fe, (c) A(O/X)-P, (d) A(O/X)-Fe+P reactors


(a)
PFHpA Concentration (mg/L)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
A(X)-Ctrl
A(O)-Ctrl
0
2
4
6
8
10
12
14
Time (d)


(b)
PFHpA Concentration (mg/L)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
A(X)-Fe
A(O)-Fe
0
2
4
6
8
10
12
14
Time (d)

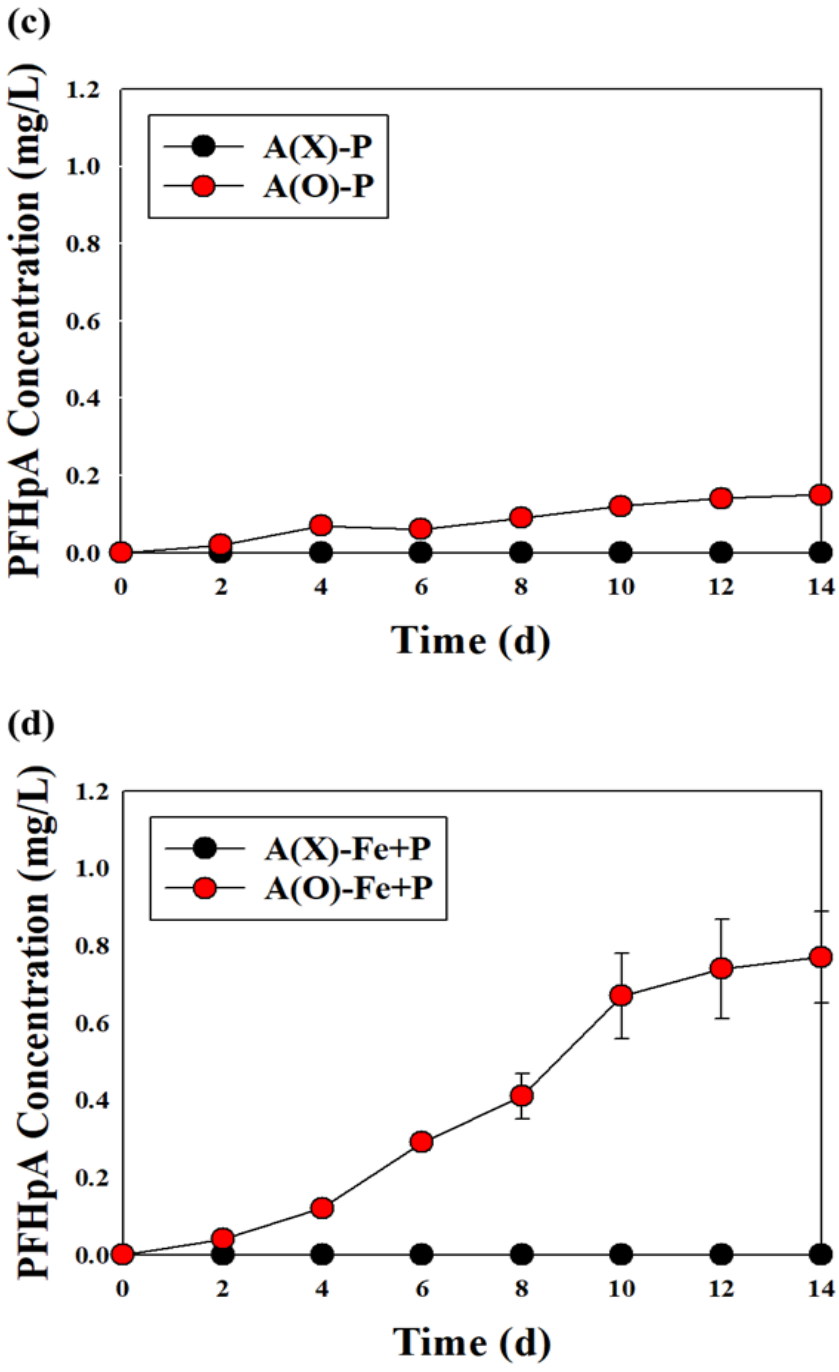
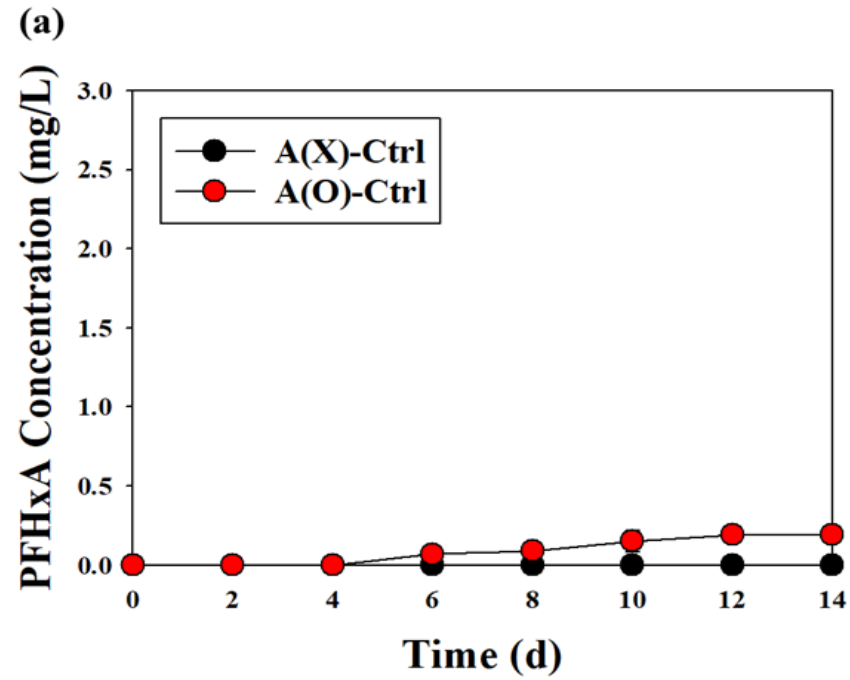
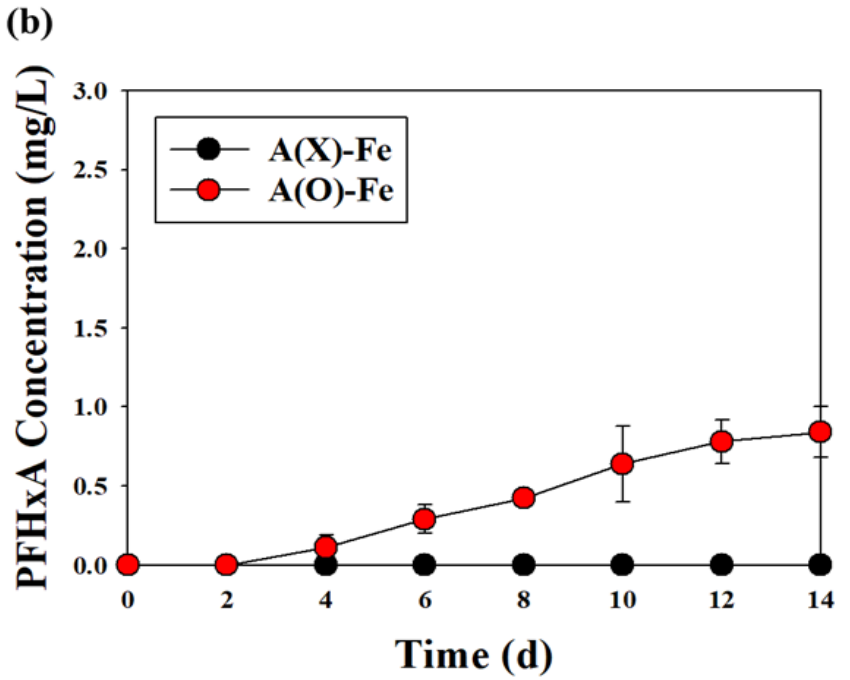


Fig. 4-8 Change in PFHpA concentration (a) A(O/X)-ctrl, (b) A(O/X)-Fe, (c) A(O/X)-P, (d) A(O/X)-Fe+P reactors


(a)
PFHxA Concentration (mg/L)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
A(X)-Ctrl
A(O)-Ctrl
0
2
4
6
8
10
12
14
Time (d)


(b)
PFHxA Concentration (mg/L)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
A(X)-Fe
A(O)-Fe
0
2
4
6
8
10
12
14
Time (d)

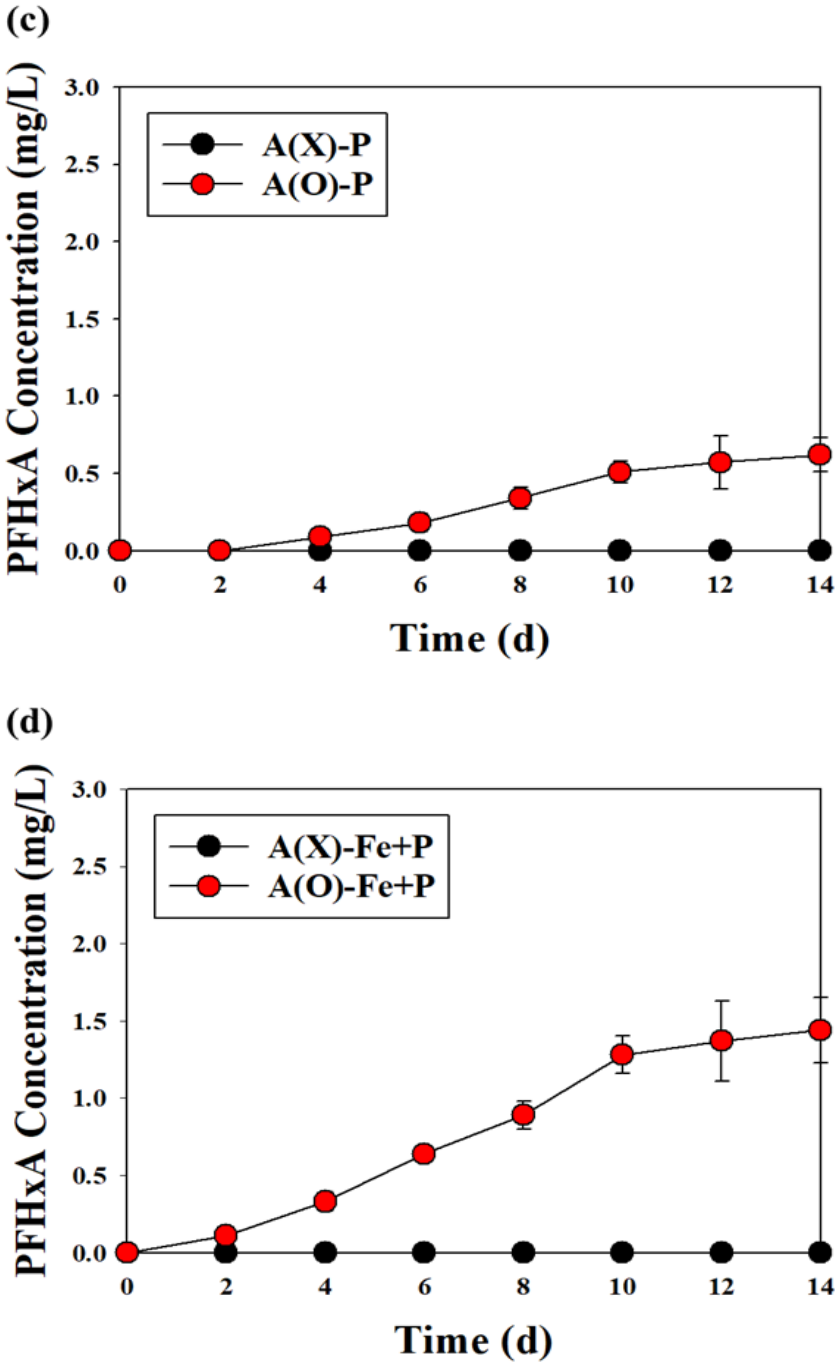
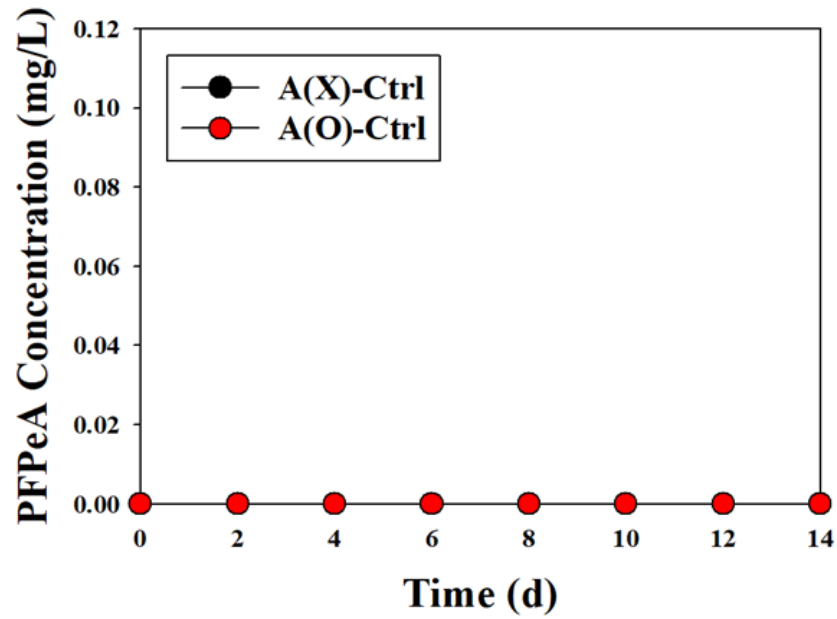
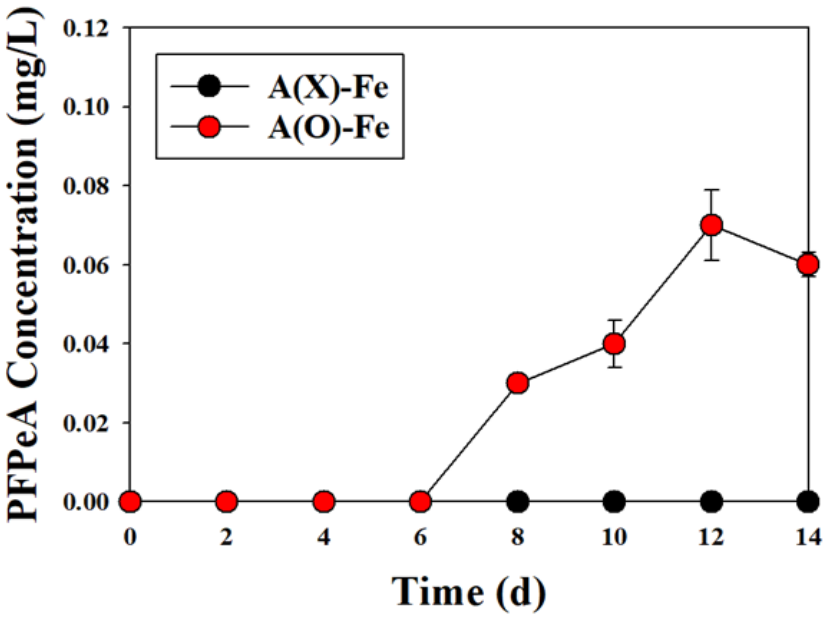


Fig. 4-9 Change in PFHxA concentration (a) A(O/X)-ctrl, (b) A(O/X)-Fe, (c) A(O/X)-P, (d) A(O/X)-Fe+P reactors

(a)


PFPeA Concentration (mg/L)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
A(X)-Ctrl
A(O)-Ctrl
0
2
4
6
8
10
12
14
Time (d)

(b)


PFPeA Concentration (mg/L)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
A(X)-Fe
A(O)-Fe
0
2
4
6
8
10
12
14
Time (d)

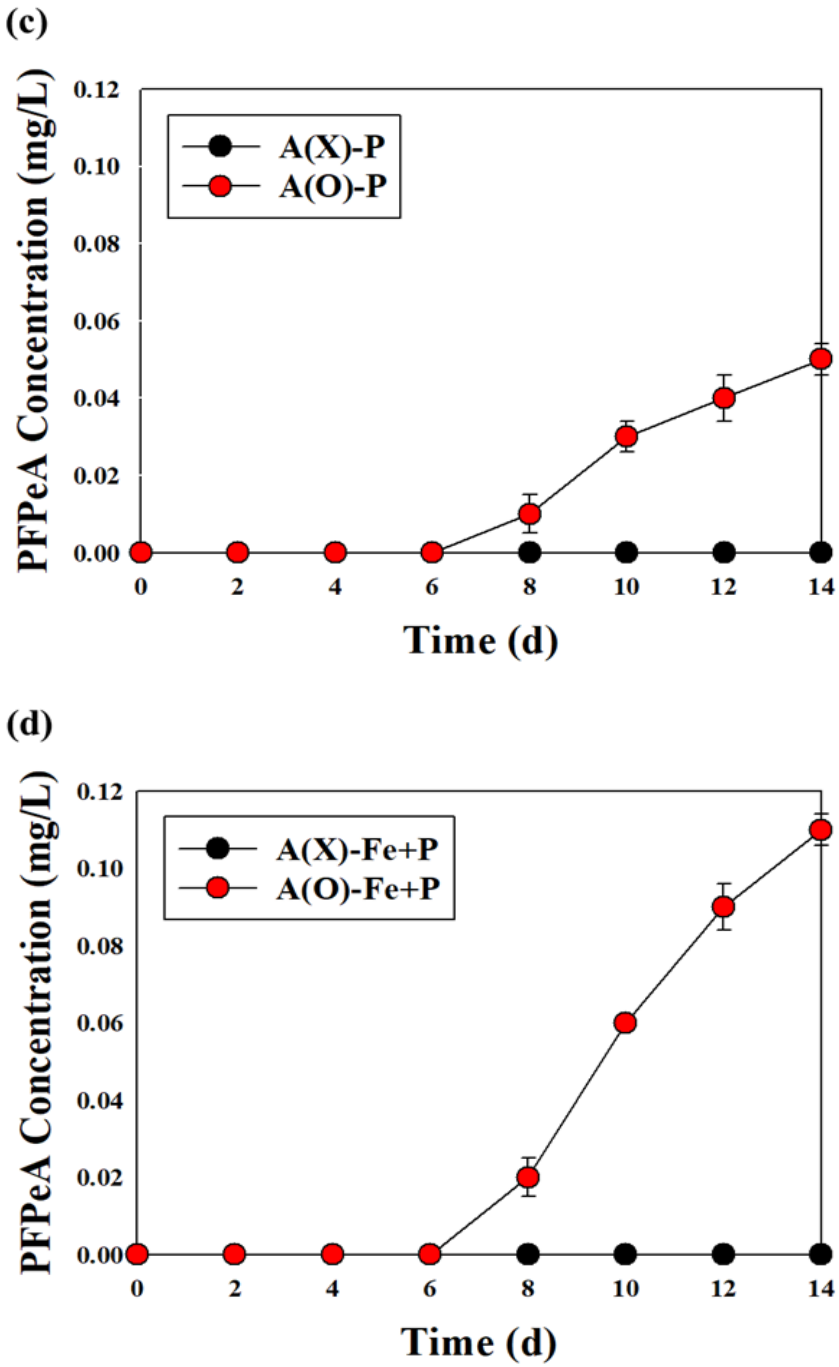


Fig. 4-10 Change in PFPeA concentration (a) A(O/X)-ctrl, (b) A(O/X)-Fe, (c) A(O/X)-P, (d) A(O/X)-Fe+P reactors

4.4. Bio-electrochemical properties

The current of the reactors to which the voltage was applied was measured over time (Fig. 4-11). No current was generated in A(X)-P and A(X)-Fe+P reactors. Conversely, the current was generated in A(O)-P and A(O)-Fe+P reactors. The initial current generated in the A(O)-P and A(O)-Fe+P reactors was 258.7 and 282.72 μA/s, respectively. Both reactors generated the highest current after 3 days, with currents of 509.05 and 562.12 μA/s, respectively. Subsequently, the reactors exhibited a decreasing trend after generating a constant current. After 14 days, 281.22 and 331.95 μA/s currents were generated in the A(O)-Fe and A(O)-Fe+P reactors, respectively. It is clear that Fe(Ⅲ) and an electric potential efficiently generated a current through electron transfer and that both contributed to simultaneous NH_4^+ oxidation and PFOA degradation.

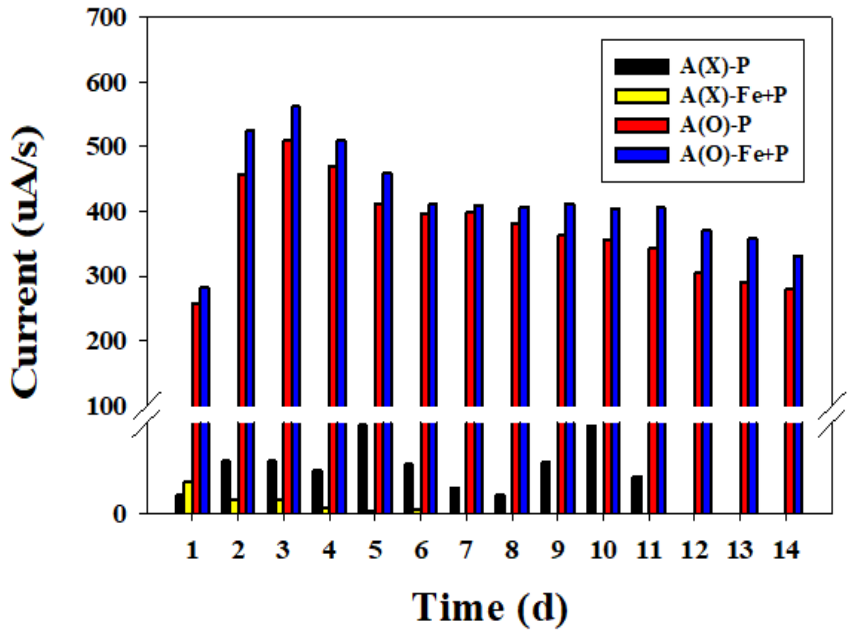


Fig. 4-11 Results of current generation

Assuming that 3 mol of electrons are released when 1 mol of NH_4^+ is oxidized and that electrons are released only by NH_4^+ oxidation of the anammox granules, 175.64 and 141.79% of CE were calculated in A(O)-P and A(O)-Fe+P reactors (Fig. 4-12). Shaw et al. (2020) reported that the results of the BES experiment using *Ca. Brocadia* and *Ca. Scalindua* showed 87.8±3.2% of CE, which was lower than the results of this study. It indicates the various reactions caused by numerous microbial species (AOB, NOB, feammox) within the anammox granules, which means that anammox bacteria cannot lead to the overall reaction. Osset-Álvarez et al. (2022) showed a CE of 108±83% when NH_4^+ was used as a substrate and 175±5% when NO_2^- was used as a substrate in a nitrogen removal experiment using anoxic BES. The side reactions that consume current without affecting the voltage can cause over 100% of CE. NO_3^- detection in this study was produced by a complex reaction of AOB, NOB, and feammox bacteria, and these unexpected reactions due to a number of species in anammox granules could cause high CE results. Detection of Fe (Ⅱ) in the A(O)-Fe+P reactor, resulted in lower CE than the A(O)-P reactor due to its role as an electron acceptor for Fe(Ⅲ) and relatively more PFOA degradation and PFAA production are also believed to have played the same role.

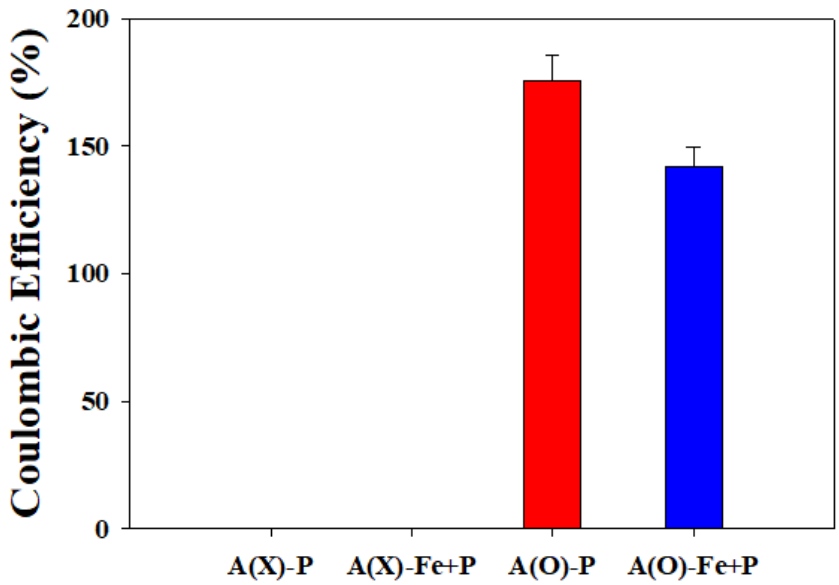


Fig. 4-12 Coulombic efficiency of the reactors with the voltage applied

The electrochemical activity was measured using CV (Fig. 4-13). No remarkable peaks were formed in the A(X)-P and A(X)-Fe+P reactors. In contrast, the A(O)-P and A(O)-Fe+P reactors generated the meaningful current. In the A(O)-P reactor, the oxidation peak of a 24.562 mA current was formed at 0.005 V vs. Ag/AgCl, and the reduction peak of -25.687 mA was formed at -0.775 V vs. Ag/AgCl. Conversely, in the at A(O)-Fe+P reactor, the oxidation peak of 27.32 mA was formed at -0.073 V vs. Ag/AgCl, and the reduction peak of -27.295 mA at -0.742 V vs. Ag/AgCl. The formation of a high current means a high reaction rate and a low overvoltage indicates the reaction's spontaneity. Therefore, the formation of oxidation peaks at 0.005 and -0.073 V vs. Ag/AgCl (24.562, 27.32 mA, respectively) in the A(O)-P reactor and the A(O)-Fe+P reactor is a means to compare the spontaneity and reaction rate of the two reactors. In addition, the larger CV graph area of the A(O)-Fe+P reactor indicates that active electron transfer occurred. Electrochemical analysis results do not support the whole of the results. However, the current generation and EET function by removing NH_4^+ and PFOA are proved again, and the spontaneity and reaction rate of the anammox granules are improved with the injection of Fe(Ⅲ).

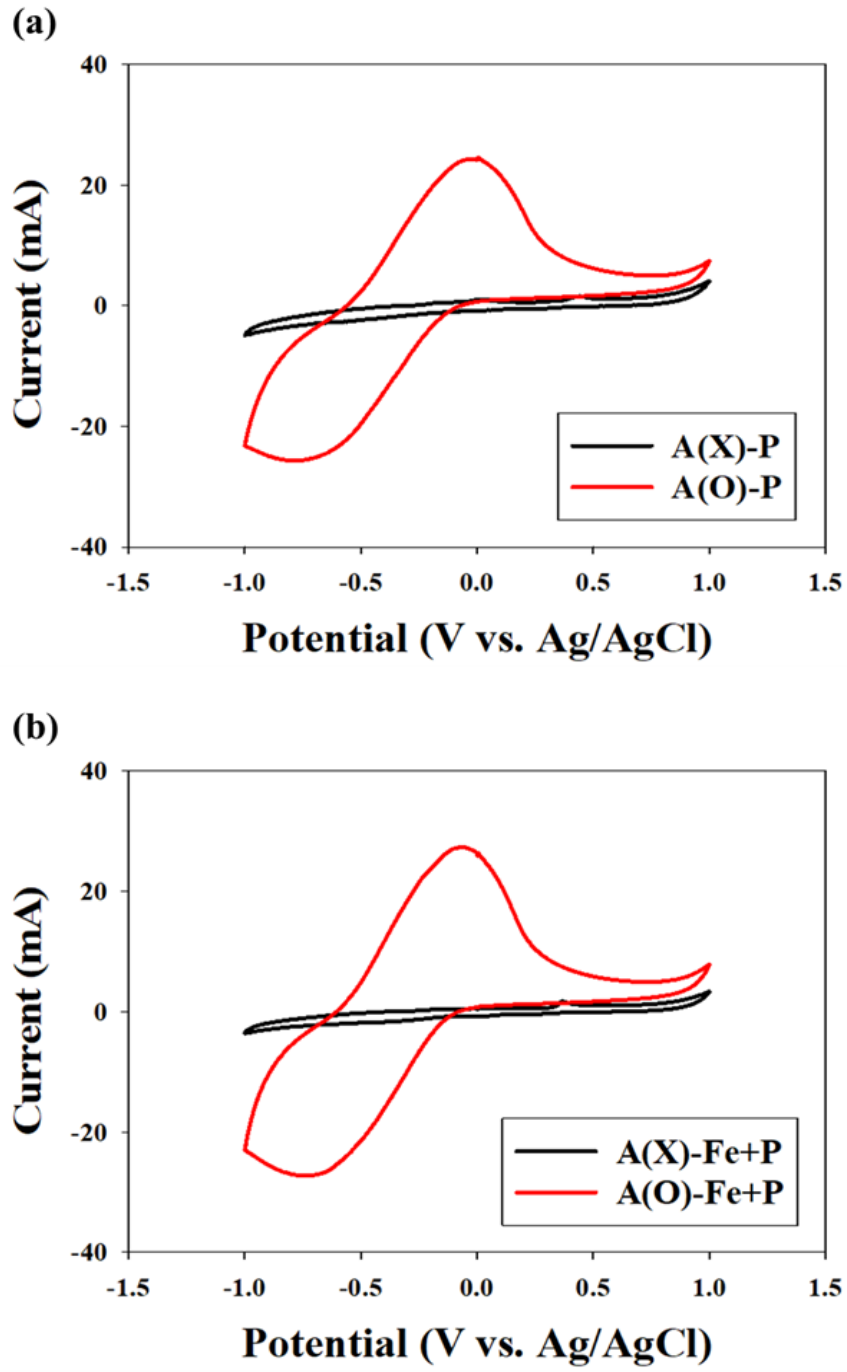


Fig. 4-13 CV results of (a) A(X/O)-P (b) A(X/O)-Fe+P reactors

5. Conclusion

This study aimed to present a new technique for achieving higher applicability and better performance of PFOA biodegradation. The significant results showed the possibility of PFOA degradation by mixed culture anammox granules with injection of Fe(Ⅲ) and an electric potential. The degradation efficiency for NH_4^+ and PFOA showed the same tendency, followed by the A(O)-Fe+P, A(O)-Fe, A(O)-P, and A(O)-ctrl reactors. The A(O)-Fe+P reactor, which was operated with electric potential and Fe(Ⅲ), reached PFOA and NH_4^+ removal efficiency of 50.93 and 41.98%, respectively, for 14 days. The removal of PFOA, caused by biodegradation by anammox granules was 35.03%. The results and balance of the F^-, and PFOA intermediates concentrations highlighted efficient PFOA degradation when Fe(Ⅲ) was added and an electric potential was applied. Moreover, the results of the current, CE, and CV curves showed the possibility of electric potential application improving the PFOA degradation. This Fe (Ⅲ)-supported BES is expected to be economically viable and widely applicable because the mixed culture anammox granules efficiently degraded PFOA with ammonium removal under a nitrite absent environment.

6. Reference

1. Badwal, S. P., Giddey, S. S., Munnings, C., Bhatt, A. I., Hollenkamp, A. F. (2014). Emerging electrochemical energy conversion and storage technologies. Frontiers in chemistry, 2, 79.
2. Barry, V., Winquist, A., Steenland, K. (2013). Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. Environmental health perspectives, 121(11-12), 1313-1318.
3. Bao, P., Li, G. X. (2017). Sulfur-driven iron reduction coupled to anaerobic ammonium oxidation. Environmental Science & Technology, 51(12), 6691-6698.
4. Beas, J. Z., Videira, M. A., Saraiva, L. M. (2022). Regulation of bacterial haem biosynthesis. Coordination chemistry reviews, 452, 214286.
5. Bentel, M. J., Yu, Y., Xu, L., Li, Z., Wong, B. M., Men, Y., Liu, J. (2019). Defluorination of per-and polyfluoroalkyl substances (PFASs) with hydrated electrons: structural dependence and implications to PFAS remediation and management. Environmental science & technology, 53(7), 3718-3728.
6. Bhatnagar, A., Hogland, W., Marques, M., Sillanpää, M. (2013). An overview of the modification methods of activated carbon for its water treatment applications. Chemical Engineering Journal, 219, 499-511.
7. Boonya-Atichart, A., Boontanon, S. K., Boontanon, N. (2016). Removal of perfluorooctanoic acid (PFOA) in groundwater by nanofiltration membrane. Water Science and Technology, 74(11), 2627-2633.
8. Buck, R. C., Franklin, J., Berger, U., Conder, J. M., Cousins, I. T., De Voogt, P., ... van Leeuwen, S. P. (2011). Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integrated environmental assessment and management, 7(4), 513-541.
9. Chen, H., Yu, J.-J., Jia, X.-Y., Jin, R.-C. (2014). Enhancement of anammox performance by Cu (II), Ni (II) and Fe (III) supplementation. Chemosphere, 117, 610-616.
10. Commission Regulation (EU) 2021/1297 of 4 August 2021 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council as

regards perfluorocarboxylic acids containing 9 to 14 carbon atoms in the chain (C_9-C_{14} PFCAs), their salts and C_9-C_{14} PFCA-related substances (Text with EEA relevan ce)

11. Cui, Z. F., Jiang, Y., Field, R. W. (2010). Fundamentals of pressure-driven membra ne separation processes. In Membrane technology (pp. 1-18). Butterworth-Heinemann.
12. Darrow, L. A., Stein, C. R., Steenland, K. (2013). Serum perfluorooctanoic acid and perfluorooctane sulfonate concentrations in relation to birth outcomes in the Mid-Ohi o Valley, 2005–2010. Environmental health perspectives, 121(10), 1207-1213.
13. de Almeida, N. M., Neumann, S., Mesman, R. J., Ferousi, C., Keltjens, J. T., Jette n, M. S., Kartal, B.,van Niftrik, L. (2015), "Immunogold localization of key metabol ic enzymes in the anammoxosome and on the tubule-like structures of Kuenenia stut tgartiensis", Journal of bacteriology, 197(14), 2432-2441.
14. Dean, W. S., Adejumo, H. A., Caiati, A., Garay, P. M., Harmata, A. S., Li, L., Ro driguez, E. E., Sundar, S. (2020). A Framework for Regulation of New and Existin g PFAS by EPA. Journal of Science Policy & Governance, 16(1), 1-14.
15. Deng, Y., Liang, Z., Lu, X., Chen, D., Li, Z., Wang, F. (2021). The degradation m echanisms of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFO S) by different chemical methods: A critical review. Chemosphere, 283, 131168.
16. Ding, L.-J., An, X.-L., Li, S., Zhang, G.-L., Zhu, Y.-G. (2014). Nitrogen loss throu gh anaerobic ammonium oxidation coupled to iron reduction from paddy soils in a chronosequence. Environmental science & technology, 48(18), 10641-10647.
17. Dixit, F., Dutta, R., Barbeau, B., Berube, P., Mohseni, M. (2021). PFAS removal b y ion exchange resins: A review. Chemosphere, 272, 129777.
18. Efficient sorption and removal of Perflouroalkyl Substances(PFASs) on appliable sor bents in drinking water treatment plant 2019.01 korea water resources corporation.
19. Essumang, D. K., Eshun, A., Hogarh, J. N., Bentum, J. K., Adjei, J. K., Negishi, J., Nakamichi, S., Habibullah-Al-Mamun, M., Masunaga, S. (2017). Perfluoroalkyl ac ids (PFAAs) in the Pra and Kakum River basins and associated tap water in Ghana. Science of the Total Environment, 579, 729-735.
20. Feng, Q., Song, Y. C., Bae, B. U. (2016). Influence of applied voltage on the perf ormance of bio-electrochemical anaerobic digestion of sewage sludge and planktonic

microbial communities at ambient temperature. Bioresource technology, 220, 500-508.
21. Fenton, S. E., Ducatman, A., Boobis, A., DeWitt, J. C., Lau, C., Ng, C., Smith, J, S., Roberts, S. M. (2021). Per‐and polyfluoroalkyl substance toxicity and human hea lth review: Current state of knowledge and strategies for informing future research. Environmental toxicology and chemistry, 40(3), 606-630.
22. Fitz-Simon, N., Fletcher, T., Luster, M. I., Steenland, K., Calafat, A. M., Kato, K., Armstrong, B. (2013). Reductions in serum lipids with a 4-year decline in serum pe rfluorooctanoic acid and perfluorooctanesulfonic acid. Epidemiology (Cambridge, Mas s.), 24(4), 569.
23. Flores, C., Ventura, F., Martin-Alonso, J., Caixach, J. (2013). Occurrence of perfluor ooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in NE Spanish surface wat ers and their removal in a drinking water treatment plant that combines conventional and advanced treatments in parallel lines. Science of the Total environment, 461, 61 8-626.
24. Fricke, K., Harnisch, F., Schröder, U. (2008). On the use of cyclic voltammetry for the study of anodic electron transfer in microbial fuel cells. Energy & Environmenta l Science, 1(1), 144-147.
25. Gao, F., Zhang, H., Yang, F., Li, H., Zhang, R. (2014). "The effects of zero-valent iron (ZVI) and ferroferric oxide (Fe_3O_4) on anammox activity and granulation in ana erobic continuously stirred tank reactors (CSTR)", Process Biochemistry, 49(11), 1970 -1978.
26. Hale, S. E., Arp, H. P. H., Slinde, G. A., Wade, E. J., Bjørseth, K., Breedveld, G. D., Straith, B, F., Moe, K, G., Jartun, M., Høisæter, Å. (2017). Sorbent amendment as a remediation strategy to reduce PFAS mobility and leaching in a contaminated s andy soil from a Norwegian firefighting training facility. Chemosphere, 171, 9-18.
27. Heidarinejad, Z., Dehghani, M. H., Heidari, M., Javedan, G., Ali, I., Sillanpää, M. (2020). Methods for preparation and activation of activated carbon: a review. Enviro nmental Chemistry Letters, 18, 393-415.
28. Huang, J., Kankanamge, N. R., Chow, C., Welsh, D. T., Li, T., Teasdale, P. R. (20 18), "Removing ammonium from water and wastewater using cost-effective adsorbent s: A review", Journal of Environmental Sciences, 63, 174-197.

29. Huang, S., Jaffé, P. R. (2019). Defluorination of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) by *Acidimicrobium* sp. strain A6. Environmental sc ience & technology, 53(19), 11410-11419.
30. Jetten, M. S., Strous, M., Van De Pas-Schoonen, K. T., Schalk, J., Van Dongen, U. G., Van De Graaf, A. A., Logemann, S., Muyzer, G., van Loosdrecht, M, C., Kuen en, J. G. (1998). The anaerobic oxidation of ammonium. FEMS Microbiology revie ws, 22(5), 421-437.
31. Kang, Y., Guo, Z., Ma, H., Wu, H., Zhang, J. (2023). Enhanced Removal of Perflu orooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) in Constructed Wetl ands: Iron Cycling and Microbial Mechanisms. ACS ES&T Water, 3(2), 287-297.
32. Kempisty, D. M., Xing, Y., Racz, L. (Eds.). (2018). Perfluoroalkyl substances in the environment: theory, practice, and innovation. CRC Press.
33. Khanthong, K., Jang, H., Kadam, R., Jo, S., Lee, J., Jungyu, P. (2023). Bioelectroc hemical system for nitrogen removal: Fundamentals, current status, trends, and challe nges. Chemosphere, 139776.
34. Kothawala, D. N., Köhler, S. J., Östlund, A., Wiberg, K., Ahrens, L. (2017). Influe nce of dissolved organic matter concentration and composition on the removal effici ency of perfluoroalkyl substances (PFASs) during drinking water treatment. Water re search, 121, 320-328.
35. Kucharzyk, K. H., Darlington, R., Benotti, M., Deeb, R., Hawley, E. (2017). Novel treatment technologies for PFAS compounds: A critical review. Journal of environme ntal management, 204, 757-764.
36. Lenka, S. P., Kah, M., Padhye, L. P. (2021). A review of the occurrence, transform ation, and removal of poly-and perfluoroalkyl substances (PFAS) in wastewater treat ment plants. Water research, 199, 117187.
37. Li, X., Huang, Y., Liu, H.-w., Wu, C., Bi, W., Yuan, Y., Liu, X. (2018). Simultan eous Fe (III) reduction and ammonia oxidation process in Anammox sludge. Journal of Environmental Sciences, 64, 42-50.
38. Li, Y., Xu, Z., Cai, D., Holland, B., Li, B. (2016). Self-sustained high-rate anammo x: from biological to bioelectrochemical processes. Environmental Science: Water Re search & Technology, 2(6), 1022-1031.

39. Lin, H., Niu, J., Liang, S., Wang, C., Wang, Y., Jin, F., Luo, Q Huang, Q. (2018). Development of macroporous Magnéli phase Ti4O7 ceramic materials: As an efficie nt anode for mineralization of poly-and perfluoroalkyl substances. Chemical Engineer ing Journal, 354, 1058-1067.
40. Lis, H., Shaked, Y., Kranzler, C., Keren, N., Morel, F. M. (2015). Iron bioavailabili ty to phytoplankton: an empirical approach. The ISME journal, 9(4), 1003-1013.
41. Liu, L., Liu, Y., Gao, B., Ji, R., Li, C., Wang, S. (2020). Removal of perfluoroocta noic acid (PFOA) and perfluorooctane sulfonate (PFOS) from water by carbonaceous nanomaterials: A review. Critical Reviews in Environmental Science and Technology, 50(22), 2379-2414.
42. MacInnis, J. J., Lehnherr, I., Muir, D. C., St. Pierre, K. A., St. Louis, V. L., Spenc er, C., De Silva, A. O. (2019). Fate and transport of perfluoroalkyl substances from snowpacks into a lake in the High Arctic of Canada. Environmental science & tech nology, 53(18), 10753-10762.
43. Mastropietro, T. F., Bruno, R., Pardo, E., Armentano, D. (2021). Reverse osmosis a nd nanofiltration membranes for highly efficient PFASs removal: overview, challenge s and future perspectives. Dalton Transactions, 50(16), 5398-5410.
44. Ministry of Environment (MOE), A report on the operation results of the persistent organic pollutant monitoring network, pp. 277~286, 374. (2021)
45. Myers, C. R., Myers, J. M. (1992). Localization of cytochromes to the outer membr ane of anaerobically grown Shewanella putrefaciens MR-1. Journal of bacteriology, 1 74(11), 3429-3438.
46. OECD. (2018). Toward a new comprehensive global database of per- polyfluoroalkyl substances (PFASs): Summary report on updating the OECD 2007 list of per and p olyfluoroalkyl substances (PFASs). Series on Risk Management No. 39. OECD. http s://www.oecd.org/chemicalsafety/portal-perfluorinated-chemicals/
47. Osset-Álvarez, M., Pous, N., Chiluiza-Ramos, P., Bañeras, L., Balaguer, M. D., Pui g, S. (2022). Unveiling microbial electricity driven anoxic ammonium removal. Biore source Technology Reports, 17, 100975.
48. Park, J. G., Jiang, D., Lee, B., Jun, H. B. (2020). Towards the practical application of bioelectrochemical anaerobic digestion (BEAD): Insights into electrode materials, r

eactor configurations, and process designs. Water Research, 184, 116214.

49. Park, J.-G., Lee, B., Heo, T.-Y., Cheon, A.-I., Jun, H.-B. (2021). Metagenomics app roach and canonical correspondence analysis of novel nitrifiers and ammonia-oxidizin g archaea in full scale anaerobic-anoxic-oxic (A_2/O) and oxidation ditch processes. B ioresource technology, 319, 124205.
50. Parvin, S., Hara-Yamamura, H., Kanai, Y., Yamasaki, A., Adachi, T., Sorn, S., Hon da, R., Yamamura, H. (2023). Important properties of anion exchange resins for effi cient removal of PFOS and PFOA from groundwater. Chemosphere, 341, 139983.
51. Per- and Polyfluoroalkyl Substances National Primary Drinking Water Regulation htt ps://www.regulations.gov/
52. Pramanik, B. K., Pramanik, S. K., Suja, F. (2015). A comparative study of coagulat ion, granular-and powdered-activated carbon for the removal of perfluorooctane sulfo nate and perfluorooctanoate in drinking water treatment. Environmental technology, 3 6(20), 2610-2617.
53. Rabaey, K., Boon, N., Siciliano, S. D., Verhaege, M., Verstraete, W. (2004). Biofue l cells select for microbial consortia that self-mediate electron transfer. Applied and environmental microbiology, 70(9), 5373-5382.
54. Reguera, G., McCarthy, K. D., Mehta, T., Nicoll, J. S., Tuominen, M. T., Lovley, D. R. (2005). Extracellular electron transfer via microbial nanowires. Nature, 435(70 45), 1098-1101.
55. Rice, E. W., Bridgewater, L., Association, A. P. H. (2012). Standard methods for th e examination of water and wastewater (Vol. 10). American public health associatio n Washington, DC.
56. Ruiz-Urigüen, M., Shuai, W., Huang, S., Jaffé, P. R. (2022). Biodegradation of PFO A in microbial electrolysis cells by *Acidimicrobiaceae* sp. strain A6. Chemosphere, 292, 133506.
57. Ruiz-Urigüen, M., Steingart, D., Jaffé, P. R. (2019). Oxidation of ammonium by Fe ammox *Acidimicrobiaceae* sp. A6 in anaerobic microbial electrolysis cells. Environm ental Science: Water Research & Technology, 5(9), 1582-1592.
58. Secretariat of the Stockholm Convention. All POPs listed in the Stockholm Conventi on. (2019).https://www.pops.int/Home/tabid/2121/Default.aspx

59. Savitz, D. A., Stein, C. R., Elston, B., Wellenius, G. A., Bartell, S. M., Shin, H. M., Vieir, V, M., Fletcher, T. (2012a). Relationship of perfluorooctanoic acid exposu re to pregnancy outcome based on birth records in the mid-Ohio Valley. Environme ntal health perspectives, 120(8), 1201-1207.
60. Savitz, D. A., Stein, C. R., Bartell, S. M., Elston, B., Gong, J., Shin, H. M., Welle nius, G. A. (2012b). Perfluorooctanoic acid exposure and pregnancy outcome in a hi ghly exposed community. Epidemiology (Cambridge, Mass.), 23(3), 386.
61. Shaw, D. R., Ali, M., Katuri, K. P., Gralnick, J. A., Reimann, J., Mesman, R., van Niftrik, L., Jetten, M. S., Saikaly, P. E. (2020). Extracellular electron transfer-depen dent anaerobic oxidation of ammonium by anammox bacteria. Nature communication s, 11(1), 2058.
62. Shin, H. M., Vieira, V. M., Ryan, P. B., Detwiler, R., Sanders, B., Steenland, K., Bartell, S. M. (2011). Environmental fate and transport modeling for perfluorooctano ic acid emitted from the Washington Works Facility in West Virginia. Environmenta l science & technology, 45(4), 1435-1442.
63. Steenland, K., Zhao, L., Winquist, A., Parks, C. (2013). Ulcerative colitis and perflu orooctanoic acid (PFOA) in a highly exposed population of community residents and workers in the mid-Ohio valley. Environmental health perspectives, 121(8), 900-905.
64. Strous, M., Kuenen, J. G., Jetten, M. S. (1999). Key physiology of anaerobic ammo nium oxidation. Applied and environmental microbiology, 65(7), 3248-3250.
65. Strycharz-Glaven, S. M., Snider, R. M., Guiseppi-Elie, A., Tender, L. M. (2011). O n the electrical conductivity of microbial nanowires and biofilms. Energy & Environ mental Science, 4(11), 4366-4379.
66. Sunderland, E. M., Hu, X. C., Dassuncao, C., Tokranov, A. K., Wagner, C. C., All en, J. G. (2019). A review of the pathways of human exposure to poly-and perfluor oalkyl substances (PFASs) and present understanding of health effects. Journal of ex posure science & environmental epidemiology, 29(2), 131-147.
67. Tang, C. Y., Fu, Q. S., Criddle, C. S., Leckie, J. O. (2007). Effect of flux (transm embrane pressure) and membrane properties on fouling and rejection of reverse osm osis and nanofiltration membranes treating perfluorooctane sulfonate containing waste water. Environmental science & technology, 41(6), 2008-2014.

68. Tang, X., Guo, Y., Jiang, B., Liu, S. (2018), Metagenomic approaches to understand ing bacterial communication during the anammox reactor start-up. Water research, 13 6, 95-10.
69. Tang, L., Su, C., Fan, C., Li, R., Wang, Y., Gao, S., Chen, M. (2022). Long-term effect of perfluorooctanoic acid on the anammox system based on metagenomics: Pe rformance, sludge characteristic and microbial community dynamic. Bioresource techn ology, 351, 127002.
70. Tomaszewski, M., Cema, G., Ziembińska-Buczyńska, A. (2017). Influence of temper ature and pH on the anammox process: a review and meta-analysis. Chemosphere, 1 82, 203-214.
71. Torres, C. I., Marcus, A. K., Lee, H. S., Parameswaran, P., Krajmalnik-Brown, R., Rittmann, B. E. (2010). A kinetic perspective on extracellular electron transfer by a node-respiring bacteria. FEMS microbiology reviews, 34(1), 3-17.
72. Trojanowicz, M., Bojanowska-Czajka, A., Bartosiewicz, I., Kulisa, K. (2018). Advan ced oxidation/reduction processes treatment for aqueous perfluorooctanoate (PFOA) a nd perfluorooctanesulfonate (PFOS)−a review of recent advances. Chemical Engineeri ng Journal, 336, 170-199.
73. Trudel, D., Horowitz, L., Wormuth, M., Scheringer, M., Cousins, I. T., Hungerbühle r, K. (2008). Estimating consumer exposure to PFOS and PFOA. Risk Analysis: An International Journal, 28(2), 251-269.
74. Uriakhil, M. A., Sidnell, T., Fernandez, A. D. C., Lee, J., Ross, I., Bussemaker, M. (2021). Per-and poly-fluoroalkyl substance remediation from soil and sorbents: A rev iew of adsorption behaviour and ultrasonic treatment. Chemosphere, 282, 131025.
75. USEPA. 2023. Best Available Technologies and Small System Compliance Technolo gies Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water. EPA-822-P-23-009.
76. Wan, L., Liu, H., Wang, X. (2022). Anaerobic ammonium oxidation coupled to Fe (III) reduction: Discovery, mechanism and application prospects in wastewater treatm ent. Science of The Total Environment, 818, 151687.
77. Wang, C., Xing, Y., Zhu, X., Yuan, X., Qu, J., Zheng, H., Jin, D. (2022). Synergis tic degradation of o-chlorophenol in aerobic microbial fuel cells with a coupled phot

ocatalytic-bioelectrochemical anode. Journal of Power Sources, 543, 231830.

78. Winquist, A., Steenland, K. (2014a). Perfluorooctanoic acid exposure and thyroid dis ease in community and worker cohorts. Epidemiology, 255-264.
79. Winquist, A., Steenland, K. (2014b). Modeled PFOA exposure and coronary artery d isease, hypertension, and high cholesterol in community and worker cohorts. Environ mental health perspectives, 122(12), 1299-1305.
80. Xiong, X., Shang, Y., Bai, L., Luo, S., Seviour, T. W., Guo, Z., Ottosen, L, D We i, Z. (2023). Complete defluorination of perfluorooctanoic acid (PFOA) by ultrasonic pyrolysis towards zero fluoro-pollution. Water Research, 235, 119829.
81. Yang, L., He, L., Xue, J., Ma, Y., Xie, Z., Wu, L., Huang, M., Zhang, Z. (2020). Persulfate-based degradation of perfluorooctanoic acid (PFOA) and perfluorooctane su lfonate (PFOS) in aqueous solution: Review on influences, mechanisms and prospect ive. Journal of hazardous materials, 393, 122405.
82. Yi, L., Chai, L., Xie, Y., Peng, Q., Peng, Q. (2016). Isolation, identification, and d egradation performance of a PFOA-degrading strain. Genet. Mol. Res, 15(2), 235-24 6.
83. Zareitalabad, P., Siemens, J., Hamer, M., Amelung, W. (2013). Perfluorooctanoic aci d (PFOA) and perfluorooctanesulfonic acid (PFOS) in surface waters, sediments, soil s and wastewater–A review on concentrations and distribution coefficients. Chemosp here, 91(6), 725-732.
84. Zhang, Z., Sarkar, D., Biswas, J. K., Datta, R. (2022). Biodegradation of per-and po lyfluoroalkyl substances (PFAS): A review. Bioresource technology, 344, 126223.
85. Zhao, R., Zhang, H., Li, Y., Jiang, T., Yang, F. (2014). Research of iron reduction and the iron reductase localization of anammox bacteria. Current microbiology, 69(6), 880-887.
86. Zhang, Q., Ji, F., Xu, X. (2016). Effects of physicochemical properties of poly-ε-ca prolactone on nitrate removal efficiency during solid-phase denitrification. Chemical Engineering Journal, 283, 604-613.
87. Zhu, J., Li, T., Liao, C., Li, N., Wang, X. (2021). A promising destiny for Feamm ox: From biogeochemical ammonium oxidation to wastewater treatment. Science of The Total Environment, 790, 148038.

88. Zhu, T.-t., Zhang, Y.-b., Liu, Y.-w., Zhao, Z.-s. (2021). Electrostimulation enhanced ammonium removal during Fe (III) reduction coupled with anaerobic ammonium oxi dation (Feammox) process. Science of The Total Environment, 751, 141703.
89. Zhuo, Q., Xiang, Q., Yi, H., Zhang, Z., Yang, B., Cui, K., Bing, X., Xu, Z., Lian g, X., Guo, Q., Yang, R. (2017). Electrochemical oxidation of PFOA in aqueous sol ution using highly hydrophobic modified PbO2 electrodes. Journal of Electroanalytica l Chemistry, 801, 235-243.