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Simultaneous nitrification and denitrification using polypyrrole/microbial celluloses electrode in a membraneless bio-electrochemical system

Hooshyar Hossini[1], Abbas Rezaee[1*], Bita Ayati[2], Amir Hossein Mahvi[3]

[1]Environmental Health Department, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

[2]Department of Environmental Engineering, Faculty of Civil & Environmental Engineering, Tarbiat Modares University, Tehran, Iran

[3]School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

***Corresponding:** Abbas Rezaee

Tel/Fax: +98 21 82883575

Email: rezaee@modares.ac.ir

Abstract

In this study, feasibility of ammonium and total nitrogen (TN) removal from aqueous solution using simultaneous nitrification and denitrification was studied in a membraneless (single chamber) bio-electrochemical system with a novel electrode. Main objectives were synthesizing Polypyrrole/microbial celluloses (PPy/MC) composite and utilized as a novel electrode material. To determine the mechanical properties of PPy/MC, tensile test and Young's modulus were performed. Biofilm was prepared using the fabricate electrode during the first few weeks. Effective parameters such as initial ammonium concentrations (NH_4^+~15–150 mg N/L), HRT (6–72 h), carbon/nitrogen ratio (C/N~ 0–4), current intensity (2–10 mA), and pH 6.5-8.5 were evaluated. The following optimum values were obtained: HRT, 24 h; C/N, 2; electric current, 6 mA; and pH, 7–7.5 in the constant amount of 77.77 mg

N/L ammonium concentration. It can be concluded from the experimental data that under optimal conditions about 97.42 and 62.47% of ammonium and TN were removed successfully, respectively.

1. Introduction

Urbanization, industrialization, and agricultural activities of humans paved way for large quantities of contaminants to mix with the ecosystems and aquatic sources [1]. Nitrogen compounds are one among them, and ammonium/ammonia ions being the major ones. Water and wastewater containing large quantities of ammonium/ammonia ions can cause adverse effects on the human health (metabolic diseases) and environment (such as eutrophication and overgrowth of plant). However, to achieve efficient treating methods, many researches were performed. Various techniques were performed to remove nitrogen from water and wastewater, such as adsorption, ion exchange, reverse osmosis, air stripping, electrochemical and biological process [2]. The biological process was chosen because of the environmental friendly aspects. On the other hand, low growth rate and low cellular yields bacteria in conventional treatment were caused the advance treating technique be investigated. In recent years, bio-electrochemical systems (BESs) have been proposed as a potentially interesting technology for the production of energy from wastewaters[3, 4,5] and are highly efficient ones than conventional process. Depending on the usage of objects (production of energy or hydrogen), BESs were investigated in two categories including: microbial fuel cells (MFCs) and microbial electrolysis cells (MECs). Performance of bio-electrochemical system is accomplished using electrically coupling a microbial film and electrodes (anode/cathode). Electro-stimulation of this system occurs when the cellular configurations and microorganisms are exposed under electrochemical characteristics or electrical fields. This phenomenon can cause enzyme activation, biopolymer syntheses, membrane transport, and proliferation [6]. According to the literature review, bio-electrochemical treatment is introduced

as a highly efficient and promising technique for the removal of nitrogen from water and wastewater[7,8]. Researches have revealed that nitrogenous compounds can be removed from aqueous matrix simultaneously[10 ,9]. Also, similar studies have demonstrated that the simultaneous removal of carbon and nitrogen can occur during bio-electrochemical performance [11]. In case of simultaneous nitrification and denitrification, ammonium ions at the anode part is converted to nitrate, and then nitrate and other byproducts subsequently via denitrification get converted to nitrogen gas in the biocathode [11]. In the MEC process, carbon sources are degraded by microbes at the anode, and hydrogen is produced at cathode using the following equations:

$$10H_2O + 10e^- \rightarrow 5H_2 + 10OH^- \quad (1)$$

$$H_2O \rightarrow 2.5O_2 + 10H^+ + 10e^- \quad (2)$$

Clear mechanism of nitrification and denitrification in a bio-electrochemical system has not been described. But the reaction mechanism of ammonium to gaseous nitrogen and intermediates are proposed as the following equations **:**

$$NH_4^+ + \frac{3}{2}O_2 \rightarrow NO_2^- + 2H^+ + H_2O \quad (3)$$

$$NO_2^- + \frac{1}{2}O_2 \rightarrow NO_3^- \quad (4)$$

$$2NO_3^- + 2H_2 \rightarrow 2NO_2^- + 2H_2O \quad (5)$$

$$2NO_2^- + 2H_2 \rightarrow N_2O^- + 2H_2O + 2OH^- \quad (6)$$

$$N_2O + H_2 \rightarrow N_2\uparrow + 2H_2O \quad (7)$$

Recently, one of interest topic in bio-electrochemical system is development and fabrication the novel electrode material. Various criteria such as large active surface areas, excellent biocompatibility and conductivity, non-toxic for bacteria are a priority[12]. Therefore, introduce

a new electrode material can be interested and can cause changes in new studies. Cellulose is the most abundant biopolymer from renewable resource in the world and it is a basic component of all plant materials[13]. Microbial cellulose (MC) can be extracellularly synthesized into nano-sized fibrils from some strains of the bacterial genera such as Acetobacter, Agrobacterium, Gluconacetobacter, Rhizobium, and Sarcina, and this shows a desirable potential alternative to use for its specialized applications in the medical, acoustic, and other industries than plant-base cellulose[15 ,14 16, 17]. Accordingly, with regard to ultra-fine network structure of MC, it represents physical and chemical advantages over other substances, such as mechanical stability, crystallinity, and hydrophilicity[18]. These attractive characteristics made the MC to be used as conducting polymer composites. Polypyrrole is a promising intrinsically conducting polymer in optical, electronics, biological, and medical application due to its environmental stability, ease of synthesis, cytocompatibility, low oxidation potential, and electrical conductivity [19]. In this study, microbial cellulose was impregnated by polypyrrole for nitrogen removal in bio-electrochemistry system. Depositions of polypyrrole on fabrics and yarn surfaces have been widely investigated by researchers, but at among the microbial cellulose is an attractive material to be used as the insulation matrix in conducting polymer composites[18]. To the best of our knowledge and based on the literature, there is no previous report on the simultaneous nitrification and denitrification via bio-electrochemical process using conductive microbial cellulose as a biopolymer. The aim of this study is use of a conductive biopolymer as an electrode material for rapid biofilm generation and improving the simultaneous nitrification and denitrification via bio-electrochemical process.

2.Experimental

2.1. Material

All materials used in this study were of analytical grade and were used without further purification. An aqueous stock solution of ammonia (from NH_4Cl salt) was prepared in deionized distilled water. Different concentrations of ammonia were obtained by diluting the stock solution.

2.2. Microbial cellulose production

Acetobacter xylinum was obtained from the National Institute of Technology and Evaluation (NITE), in Japan. Hestrin–Schramm culture medium was used for the production of MC membrane [16]. The harvested microbial cellulose membranes were boiled in 2% sodium dodecyl sulfate ($CH_3(CH_2)_{11}OSO_3Na$) and 4% NaOH solutions in a boiling water bath. The MC membranes were then rinsed in distillated water until their pH became neutral. The MC sheet was then put into the drier at 70°C for 24 h in an electric oven prior to use.

2.3. MC/PPy preparation

For the preparation of MC/PPy, the method used by Muller et al. (2011) was considered [19]. The MC was cut into 6×15 cm pieces and used as a base in the polymerization. Prepared MC sheet with a 2 mm diameter was coated with polypyrrole (PPy) through surface polymerization. The MC sheet was immersed in pyrrole aqueous solution with concentrations varying from 0.03 mol L^{-1} at 25°C. Then, this solution was exposed to MC for 10 min under stirring. Iron chloride hexahydrate ($FeCl_3.6H_2O$) as oxidant agent was used in 2:1 of molar ratio with pyrrole. The surface polymerization of MC was carried out for 4 h. Finally, after completing polymerization, the white MC sheet appeared black [20]. To remove the excessive PPy, byproducts and residues of the polymerization reaction from surface, black BC was rinsed with pyrrole aqueous solution and distillated water. For electrode preparation, the MC/PPy composite sheet was dried at 60°C [19].

2.4.Electrodes fabrication

Steel mesh (mesh 18) was used as a conductive frame to minimize electrical resistance. The steel mesh was cut into 6×15 cm pieces similar to MC sheets and was cleaned to remove impurity and grease using acetone solution and distillated water. Then, the MC/PPy composite sheet was covered by the steel mesh, bilaterally.

2.5. Nitrifying bacteria

Inoculum mass for the growth and enrichment of nitrifying bacteria was collected from returned activated sludge of a wastewater treatment plant in Tehran, Iran. For enrichment, the following synthetic medium was used: 0.3 g/L KH_2PO_4, 1 g/L $Na_2HPO_4.12H_2O$, 0.1–0.5 g/L NaCl, 2 g/L $NaHCO_3$, 0.1 g/L $MgSO_4.7H_2O$, and 0.1–0.4 g/L NH_4Cl.

2.5.Denitrifying bacteria

For biofilm generation, the sludge (from returned activated sludge) was collected from a wastewater treatment plant in Tehran, Iran. The feed wastewater was prepared by dissolving certain amount of KNO_3 as a nitrogen source, and bicarbonate/acetate as a carbon source for autotrophic/heterotrophic denitrification. C/N ratio (stoichiometric carbon/NH_4^+-N) was adjusted to about 1.5 in the primary operation. The pH of the influent was normally around 7.5. The following synthetic growth medium was used: 0.3 g/L KH_2PO_4, 1 g/L $Na_2HPO_4.12H_2O$, 0.1 g/L NaCl, 2 g/L $NaHCO_3$, 0.1 g/L $MgSO_4.7H_2O$, 10 mM acetate, and trace elements were provided using tap water[21].

2.6.Bio-electrochemical systems start up and operation

A 2 L glassy vessel (with effective volume of 1.8 L) was considered for bio-electrochemical system. The schematic of bio-electrochemical cell with related compartments is illustrated in Fig. 1. The anode and cathode were placed vertically at a fixed distance of 5.5 cm without any separated membrane. A DC power supply was set for the startup process. Flow rate and hydraulic retention time (HRT) were maintained at approximately 0.9 L/min and 24 h, respectively. The temperature of the bio-electrochemical cell was adjusted using an online

thermometer. The enriched nitrifying/denitrifying bacteria for the biofilm formation were inoculated in to the reactor with a primary MLSS of about 7000 mg/L (approximately, about 20% of reactor). The bio-electrochemical reactor was operated in a batch wise mode as the following: feeding by a peristaltic pump (Hidolf, Germany) for about 18 min, reaction (depending on HRT), and discharge step. Schematic plan of single-chambered bio-electrochemical reactor with related compartments is shown in Fig. 1. Frist, the reactor was launched with an ammonium load of 38.88 mg, N/L (50 mg NH_4^+/L), 5 mA, C/N 2 with a HRT 24 h for 3 weeks. The bio-electrochemical system was fed with a growth medium containing (in term of g/L) 0.45 Na_2HPO_4, 0.15 KH_2PO_4, 0.1 $MgSO_4.7H_2O$, 0.015 $CaCl_2.7H_2O$, and 1 mL/L trace nutrients solution of 1.5 $FeCl_3 \cdot 6H_2O$, 0.15 H_3BO_3, 0.03 $CuSO_4 \cdot 5H_2O$, 0.18 KI, 0.12 $MnCl_2 \cdot 4H_2O$, 0.06 $Na_2MoO_4 \cdot 2H_2O$, 0.12 $ZnSO_4 \cdot 7H_2O$, 0.15 $CoCl_2 \cdot 6H_2O$, and 10 mM acetate. According to Table 1, the main parameters such as effect of ammonium concentrations, HRT, C/N ratio, applied current, and effect of initial pH were analyzed. This table is considered for operating condition in the bio-electrochemical process.

2.7.Analysis

Samples were analyzed according to the standard methods for water and wastewater. To determine the ammonium content, the Phenate method at λ_{max} 640 nm was used. The nitrate was measured at λ_{max} 220 and 275 nm, spectrophotometrically. Also, the nitrite content was analyzed by colorimetric method using sulfanilamide and naphthylethylendiamine dihydrochloride reagents at λ_{max} 543 nm. TN removal was calculated by the remaining nitrate, nitrite, and ammonium. To determine the mechanical properties of the bacterial cellulose sheet, the ASEM method D 636 (Instron tensile test) was used. The oxidation reduction potential and dissolved oxygen were carried out using an ORP meter (ORPmeter,

Eutch) and DOmeter (Hach, USA). Scanning electron microscope (SEM) image was taken by X' Pert MPD Philips Holland.

3.Result and Discussion

3.1. Mechanical properties of PPy/MC

For the PPy/MC to be used as a biofilm electrode base, it is imperative that the mechanical properties are evaluated. Tensile test is one of the essential criteria for estimating the strain of a mater. Based on the tensile testing, Young's modulus is calculated to find the resistance of a material to deform edits. As can be seen in Fig. 2a, b, the stress–strain curve and its initial linear region are illustrated. The slope from linear region (Fig b) has been used to estimate the Young's modulus. Accordingly, ultimate tensile strength (UTS) for PPy/MC composite was 0.09 mm. The UTS is the maximum load the specimen sustains during the tensile test. The mechanical properties of the PPy/MC sheets such as Young's modulus, tensile strength, and elongation at break are shown in Table 3. It can be found that the Young's modulus of PPy/MC composite is obtained at 30 MPa. This result demonstrates that the PPy/MC composite is a resistant material as an electrode against force stress in solution.

3.2. Experimental set up

Primary startup of bio-electrochemical process at certain conditions was performed for about 3 weeks, and ammonium removal efficiency and TN were controlled daily. At the end of this period, ammonium removal became up to 78%, but TN removal efficiency was below than 27% (data not shown). This end point was selected when the ammonium removal percentage arrived to variation below than 5% (a quasi-steady-state condition). During start up, biofilm was formed on PPy/MC/SSM electrodes. As can be shown in Fig. 3a–h, SEM and graphical images of rinsed, polymerized bacterial cellulose, abiotic-electrode, biofilm-electrode, and

attached bacteria are illustrated. During startup period repaid generation of biofilm onto PPy/MC sheets was observed. Graphical image (after biofilm generation in Fig.1) is illustrated bioelectrode preparation.

3.3. Influence of ammonium concentration

Different concentrations of ammonium can affect the biological activities and internally ohmic resistane during bioelectrochemical nitrification and denitrification. An increase in ammonium concentreation stimulates the growth of nitrifing bacteria. This fact was observerd when the sequence analysis of *Nitrosomonas eutropha* C-91 (SSU rRNA sequence) at the increased concentration of ammonium was investigated [22]. In addition, ammonium concentration gradient will influence the charge transport in MFC performance [23]. On the other hand, the amount of converted ammonium from a water/wastewater depends on the mass transfer coefficient. The mass transfer is one of the main phenomena affecting the performance of a biological process (Bishop et al., 1997). The mass transfer of a pollutant or nutrient into the biofilm happens by two main mechanisms. Thesis mechanisms are advetion-movement and diffusion-movement, such that the concentration gradients affect the diffusion mechanism.

As can be seen in Fig. 4a, the effect of different concentrations on ammonium removal and TN removal efficiency was investigated with six influent concentrations in the bio-electrochemical reactor, 15.55, 31.11, 46.66, 77.77, 116.66, and 155.56 mg NH_4^+-N/L. The bioelectrochemical reactor was operated for about 28 days after the start-up period. During different ammonia loading stage, overally a significant decrease in ammonium removal and TN were observed (except at low ammonium concentrations). Mean removal percentage was determined for 20–200 mg/L of ammonium around 97.96±0.19%, 95.6± 3.47%, 94.66±4.03%, 90.07±5.95%, 69.07±3.09%, and 63.91±5.34%, respectively. The related TN

removal percentage was 18.37±1.5%, 23.21±2.37%, 29.4±6.2%, 37.92±3.94%, 38.95±8.62%, and 42.84±5.67%, respectively. A lower TN removal efficency during early days can happen by a small population of nitrite oxidizing bacteria and accumulation of nitrite. The nitrite accumulation was observed at about 10–27 mg/L from operating first day of experiments till 28th day of operation. Because of low specific growth rate (low growth yield) and the small energy gain, small TN removal by nitirte acumulation occurs. Accumulation phenomenon was associated with inhibition of denitrifying bacteria by nitrite intermediate (hydroxyl amine, NH_2OH) [24], and this can be affected on TN removal percentage. Also, the growth of ammonia and nitrite oxidizing bacteria in lower ammonium concentrations as supernatant source is limited. However, dissolved oxygen levels in liquid matrix become saturated and denitrification pathway will be impaired. Moreover, environmental condition variables known to affect nitrification rates include toxicity, temperature, salinity, amounts of dissolved oxygen, pH, and ammonium ion availability [25]. The ORP and the final pH of ammonium loading stages are represented in Fig. 4b. The variation of dissolved oxygen (DO) ORP and pH were recored at about 1.2-1.5 mg/L (data not shwon), 7.2–8.34 and 150–269 mV, respectively. These parameters have been considered as indicators for nitrification/denitrification. An ORP more than 123 (DO=1 mg/L) till 208 mV for nitrification was suggested by Li and Irvin, 2007[26], and also they reported a ORP about 173–175 mV is indicative a dissolved oxygen level from 3 to 5.5 mg/L. similar research have been demostrated that the nitrification fails when ORP values were lower than 50–150 mV [27]. Generally, there is an inverse relationship between pH and ORP, regardless of the oxidant type (such as oxygen) or concentration and this trend can be seen from the results. The pH value can affect the reductive reaction of oxygen as demonstrated in the following equation:

$$O_2 + 2H_2O + 4e^- \leftrightarrow 4OH^- \qquad (8)$$

3.4. Kinetic study

One of the affecting parameters that can have large effects on a biological process is a provision of an adequate time between the biomass/biofilm and the substrate mater. Fig. 5a displays the effect of reaction time on bio-electrochemical nitrification and denitrification. The performance of the ammonium removal system was tested at HRT of 6, 12, 18, 24, 32, 48, and 72 h. Depending on HRTs, from 6 to 72 h, the average ammonium and TN removal percentage were determined around 10.62 ±1.5%, 29.58±3.37%, 45.66±3.6%, 77.37± 7.03%, 98.62±0.56%, 98.76±0.57%, 99.89±0.09% and 2.09±0.68%, 9.58±2.12%, 23.65±2.38%, 39.4±1.25%, 55.82±7.21%, 67.71±0.56%, 70.91±0.85% respectively. It can be seen from Fig. 5a that the large part of ammonium and TN has been removed at a 24 h HRT. When the HRT was higher than 24 h, >90% ammonium removal efficiency with 34% TN removal were provided. From the results it can be found that a long HRT is required to treat the higher amounts of ammonium concentrations. This fact can occur due to very slow growth of autotrophic nitrifiers [28]. In case of *Nitrobacter* sp. (as dominant sp. of nitrite oxidizing bacteria) generation times has been reported about 18 and 69 h [29], and this can provide a low cell yield. On the other hand, during short HRTs, low partial ammonia converts to nitrite and other intermediates, and it implies that the denitrifying bacteria access is limited to electron sources. As a result from Fig. 5a, improving nitrogen depletion is observed with a sufficient hydraulic retention time.

Nitrogen removal kinetic during nitrification and denitrification is shown in Fig. 5b. In order to evaluate Michaelis–Menten's kinetics was used. According to this equation, ammonia oxidation process resembles a first-order reaction (Eq. 10) [30]:

$$LnC_t = -kt + LnC_0 \qquad (9)$$

where C_0 and C_t are the ammonium/TN concentrations (mg/L) at the beginning and at the end of each experiment, t is the time in terms of hour (h), k is ammonium/TN removal rate constant, and it is determined using the slop of kinetic plot (1/h).

With regard to linear equations of ammonium/TN removal and their slopes, the kinetic constants were calculated about 0.1085 and 0.0131 per hour. High correlation coefficients (R^2) indicate the good fitting between model kinetic and experimental ammonium/TN data.

3.5. Influence of effective factors

To evaluate the influence of C/N ratio, a synthetic wastewater with C/N range of 0, 0.5, 1, 2, and 4 were considered by adjusting sodium bicarbonate as the inorganic carbon source. The ammonium and TN removal efficiency under a condition of 77.77 mg N/L of ammonium, 24 h HRT, and an electric current of 5 mA in the bio-electrochemical cell are shown in Fig. 6a. The results show that when C/N balance is available, nitrifying and denitrifying bacteria operate successfully. It is evident that the balanced C/N ratio was set at 2. At the C/N ratio of 2, a desirable condition for nitrogen removal (ammonium ~ 81.71%, TN ~ 46.65%) via nitrification and denitrification pathways was provided while in poor C/N ratio overall nitrogen removal declined. This can accomplish from lower amount of available carbon source (Eqs. 1 and 2, carbon dioxide in case of autotrophic bacteria). Aside from improving the ammonia removal efficiency with increasing C/N, a ratio more than 2 have caused an accumulation in nitrification by products, and consequently loss in nitrogen removal was observed. As a result of an insufficient C/N ratio, improper denitrification can occur, while a high C/N ratio may cause accumulation of nitrite or extra production of nitrous other than nitrogen gas [1]. In case of bicarbonate or carbonate as carbon sources, there is an increase in pH with an increase in C/N ratios. Reaction of sodium bicarbonate in aqueous solution is shown in Eqs. 11 and 12.

$$NaHCO_3 + H_2O \leftrightarrow H_2CO_3 + OH^- \quad (10)$$

$$H_2CO_3 \leftrightarrow CO_2 + H_2O \quad (11)$$

With regard to this, alkalinity occurs. However, increase in pH occurs with higher dosage of bicarbonate with an increase in C/N ratios. In return, higher pH contents can block nitrification and denitrification pathway by accumulation of nitrite. Okhravi (2015) has reported that with an increasing C/N in biofilms, a competition between heterotrophic and nitrifying bacteria occurs for dissolved oxygen and space, and this fact can be decreased on nitrifying population [31]. Due to lower growth rate and yield of nitrifying bacteria (including AOB and NOB) rather than heterotrophic and denitrifiers bacteria are more vulnerable by effects of this competition [32]. Ballinger et al. (2002) performed a single sludge reactor fed with C/N ratios of 2 and 5, and they reported a desirable activity of AOB at C/N ratio of 2, whereas they were not detected in both activities of AOB and heterotrophic bacteria at the C/N ratio of 5 due to their competition [33]. An optimum C/N ratio of 1 was reported by Zhao et al. (2012) using mixotrophic denitrifiers[34]. As can be seen in Fig 6 b different ranges of electric current intensity (2, 4, 6, 8, and 10 mA) and 77.77 mg N/L of ammonium, 24 h HRT, and C/N 2 were investigated, and ammonium and TN removal rate (%) was also determined. The results demonstrated that nitrogen could be removed successfully by increasing current intensity from 2 to 6 mA, and then loss in efficiency was observed at 10 mA. The ammonium and TN removal percentage were around 49.81, 64.94, 98.01, 92.62, 65.90%, and 16.43, 19.24, 59.44, 35.86, 27.67%, respectively. A significant ammonium removal efficiency was achieved for 6 mA, and also, the highest TN removal rate was provided. Similarly, it occurred due to the inactivation of nitrifying and denitrifying population in high electrical current. Many researches have investigated the effect of current intensity and their magnitude on bioelectrochemical nitrogen removal. A varied applied current intensity was used from 0 to 1000 mA [35, 36]. Accordingly, lower ranges of current intensity was always below 30 mA

and this has been achieved as a optimum applied electrical charge. Adding to this, this space has been associated with adverse effects. Calzia et al. (2009) studied the structural modification of proteins by direct electric current and reported that when the voltage was higher than 3.5 V, enzyme activity failed[37]. These results confirmed that the higher electrical current (or an increase in applied voltage) can destroy nitrifying and denitrifying enzymes and consequently impair nitrogen depletion. On the other hand, decrease in TN removal efficiency by increasing the current intensity may arise as a result of excess hydrogen gas production in cathode. Wan et al. (2010) reported that when the electrical current was higher than 30 mA, cathode was saturated by hydrogen gas and hydrogenotrophic denitrifiers could not use hydrogen gas as an electron donor[38]. Also, Islam and Suidan (1998) represented that at high current intensity hydrogen gas inhibits denitrification pathway[39]. The pH is a prominent affecting key factor on biological processes and achieving optimum value between two bacteria community is necessary. For this intent, a pH range of 6.5–8.5 was tested. Under certain conditions (77.77 mg N/L of ammonium in influent, 24 h HRT, C/N 2, and electric current of 6 mA) the influence of initial pH on nitrogen removal rate is shown in Fig. 6c. At a constant condition, and initial pH values of 6.5, 7.0, 7.5, and 8.5, ammonium and TN removal efficiency were 42.81, 78.67, 97.42, 61.3% and 2.43, 38.77, 62.47, 36.65, 12.06%, respectively. It can be concluded from the pH data that a slight alkaline (pH 7.5) condition has better simultaneous for ammonium and TN removal. The AMO enzyme is activated in the presence of large concentrations (20 mM) of nitrite, and more inactivity happens under alkaline than under acidic conditions [40]. When the pH value is higher than 8.6, nitrite accumulation occurs [41]. This result confirms that a narrow pH range of about 7–7.5 is essential for a permanent nitrification and denitrification. Researches have also proposed that optimal pH for bio-electrochemical nitrogenous compound reduction is between 7 and 8 [42].

Conclusion

This study addressed the simultaneous nitrification and denitrification using PPy/MC electrodes in a bio-electrochemical system. With regard to the Young's modulus of PPy/MC composite, it has been demonstrated that the PPy/MC is a resistant material as an electrode against force stress. Using fabricate electrode, we saw a biofilm generation during first few weeks. Nitrogen depletion was most effective at HRT 24 h, C/N 2, electric current 6 mA, and pH 7–7.5 for 77.77 mg N/L of ammonium concentration. It can be concluded that the ammonium and TN were removed successfully with PPy/MC composite as a novel electrodes in bio-electrochemical system.

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Figure and Table captions

Fig.1. Schematic plan of single chamber bioelectrochemical system (a) [1– DC power supply, 2- Aerator pump, 3– Bioreactor, 4– Electromotor , 5– Propeller, 6– Diffuser, 7– Peristaltic pump, 8– Feeding Tank, 9- Discharge Value , 10– Anode, 11– Cathode, 12– Steel Mesh, 13–

Ammonia oxidizing Bacteria, 14– Polypyrrole Layer, 15– Microbial Cellulose, 16– Denitrifying Bacteria], and MC/PPy/SSM electrodes after and before biofilm generation (b).

Fig.2. Stress–strain (a) and its linear section (b) curve for PPy/BC composite sheet.

Fig 3a–h. SEM and graphical images of electrode and their materials; Graphical image of rinsed and polymerized MC (a), polymerized MC SEM (b) raw Stainless Steel Mesh surface SEM (c) MC/PPy/SSM electrode SEM (d), biofilm SEM (e and f), and attached bacterial on electrode (g and h).

Fig.4. Chemical variations tracking for bioelectrochemical nitrification and denitrification, effect of different concentrations of ammonium (a), end point data of pH and ORP changes.

Fig.5. Effect of hydraulic retention time (HRT) (a) and nitrogen removal kinetic (b) on bioelectrochemical nitrification and denitrification.

Fig.6. Effect of main parameters on bioelectrochemical nitrification and denitrification, C/N ratio (a), Electrical current (b), and and pH (c).

Table 1. Performance of bioelectrochemical system for nitrogen removal.

Table2. Operating condition in the bioelectrochemical process.

Table 3. Representative mechanical properties of PPy/BC composite

Table 1.

Bio-electrochemical reactor type	objectives	wastewater	Nitrogen removal	References
Microbial fuel cell (MFC)	simultaneous removal of carbon and nitrogen	synthetic wastewater	NH_4^+-N removal rate 97.4%, TN removal 97.3%	[11]
Microbial fuel cell (MFC)	promote nitrification denitrification	synthetic wastewater	ammonium > 86.9%, nitrogen removal 86.9%, nitrogen removal rate 3.39mg N/L/h	[43]
Microbial fuel cell (MFC)	organic matter and nitrogen removal	synthetic domestic wastewater	nitrate removal 294 g $NO3^-$/N/m3 d	[44]
Microbial electrolysis cell (MEC)	Nitrate removal	synthetic wastewater	nitrate removal 92.7%	[2]
Microbial fuel cell (MFC)	nitrite removal, decreases the energy demand and the carbon requirements	synthetic domestic	nitrite removal 37 ± 5%, Nitrogen removal rate 135 g N/m^3/d	[45]
Microbial electrolysis cell (MEC)	Nitrate removal	drinking water	NO_3^--N removal 90% to 100%,	[36]
Microbial electrolysis cell (MEC)	Nitrate removal	synthetic wastewater	Denitrification efficiency 84%	[46]
Microbial fuel cells (MFCs)	simultaneous nitrification and ,denitrification without extra energy input	Synthetic wastewater (acetate and ammonium)	ammonia removal > 96.8%	[10]
Microbial fuel cell(MFCs)	Simultaneous carbon and nitrogen removal	piggery wastewater using	nitrogen removal rate 0.194 kg N/m3/d	[9]

Table2.

Operation parameter	Operation type	Ammonium Conc.		HRT	C/N ratio	Electrical current	pH	Temp.
		$mg.NH_4^+/L$	mg.N/L	h	-	mA	-	^oC
Influence of NH_4^+ conc.	Batch	20-200	15.5-155.5	24	2	5	~7.8	-
Influence of HRT	Batch	100	77.76	8-72	2	5	~7.8	Controlled (23 ± 2°C)
Influence of C/N ratio	Batch	100	77.76	24	0-4	5	~7.8	Controlled (23 ± 2°C)
Influence of Electrical	Batch	100	77.76	24	2	2-10	~7.8	Controlled (23 ± 2°C)
Influence of initial pH	Batch	100	77.76	24	2	6	6.5-8.5	Controlled (23 ± 2°C)

Table 3.

Young's modulus (MPa)	Breaking strain (%)	Ultimate Tensile Strength
30	60.229	0.090

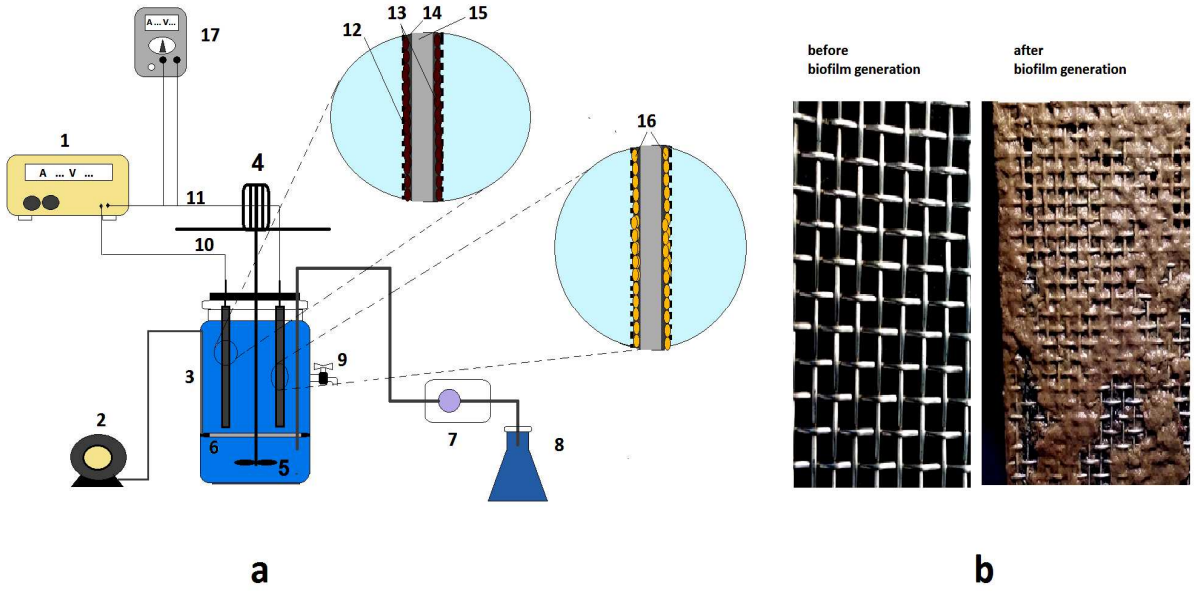
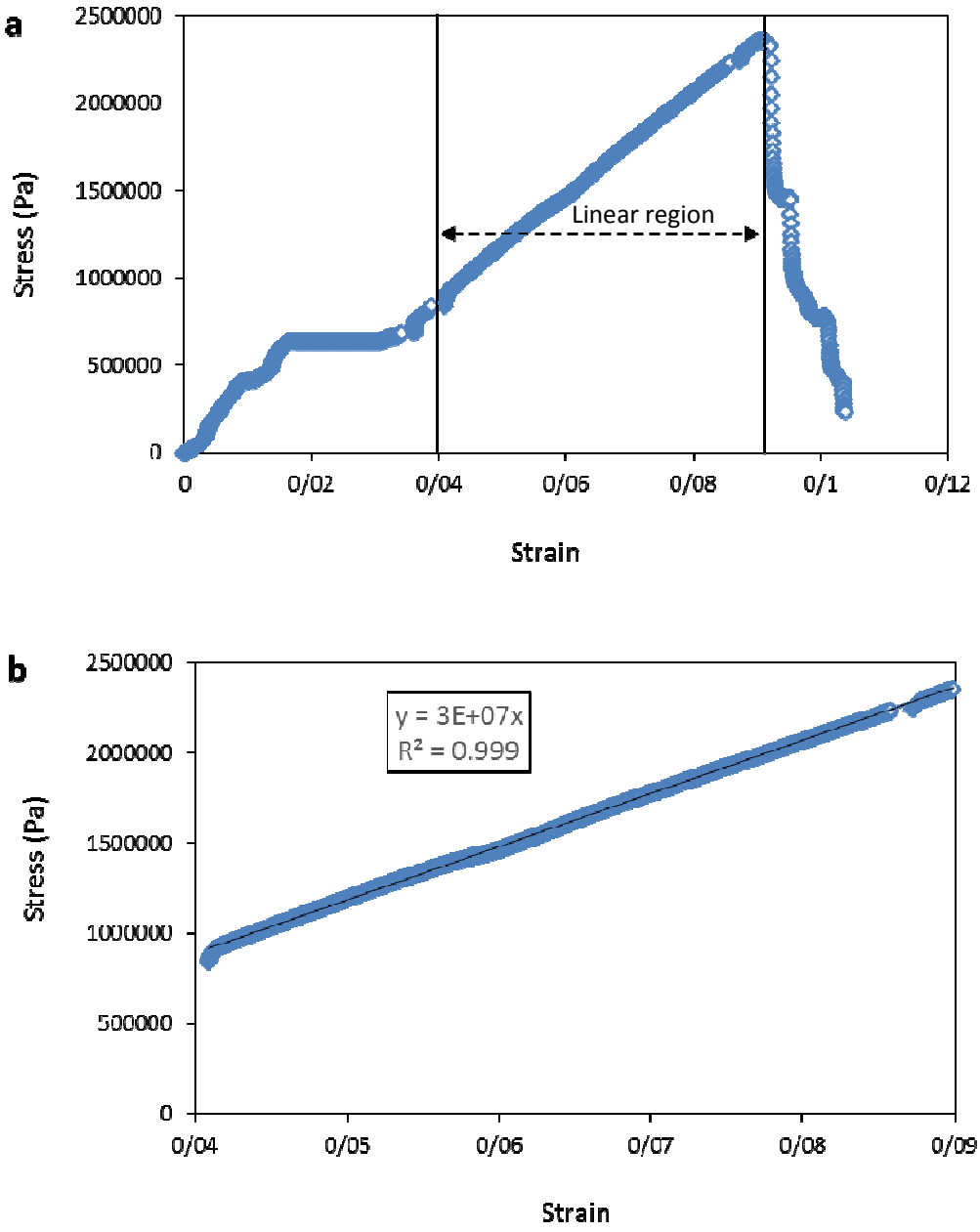


Fig.1.



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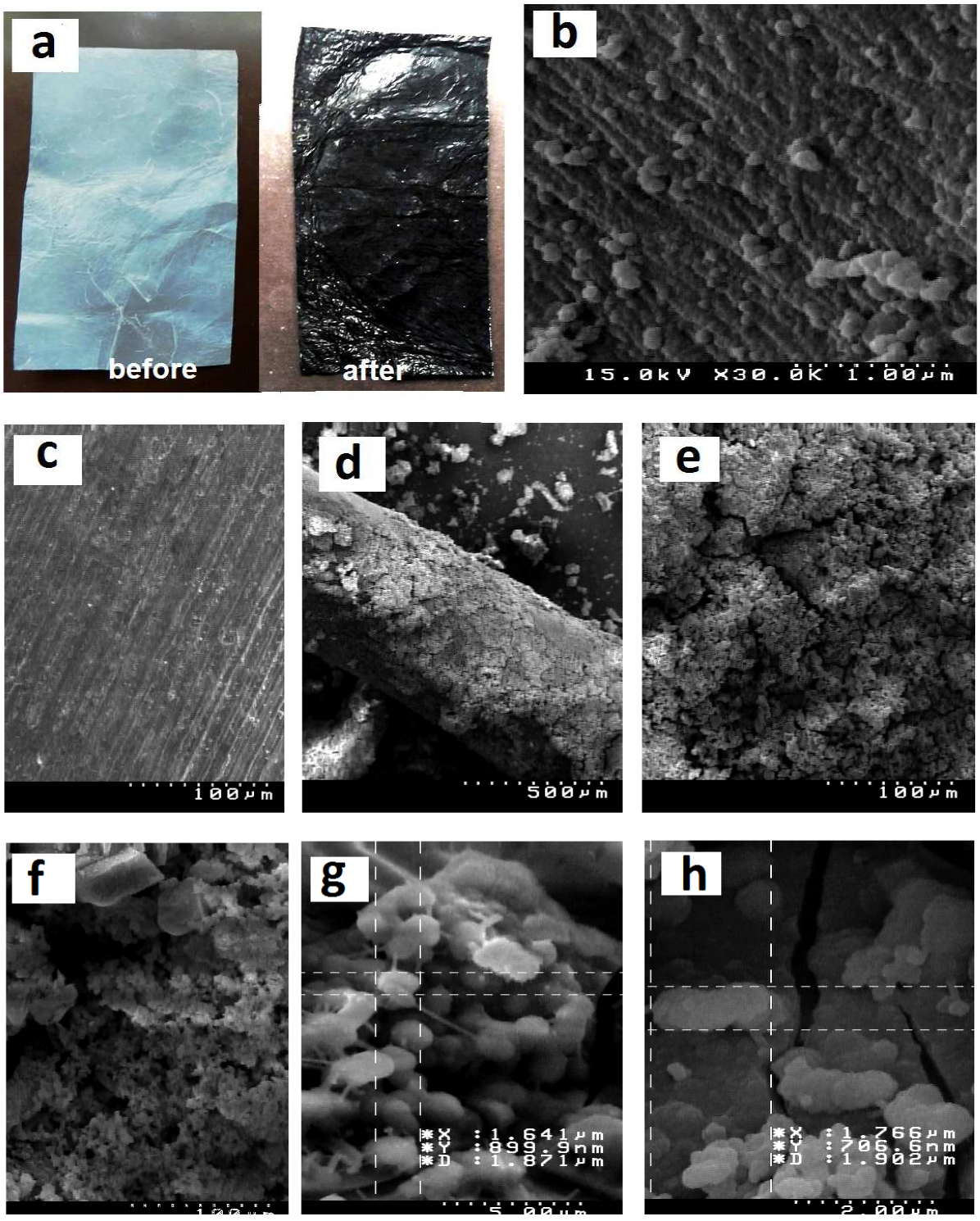


Fig 3 .

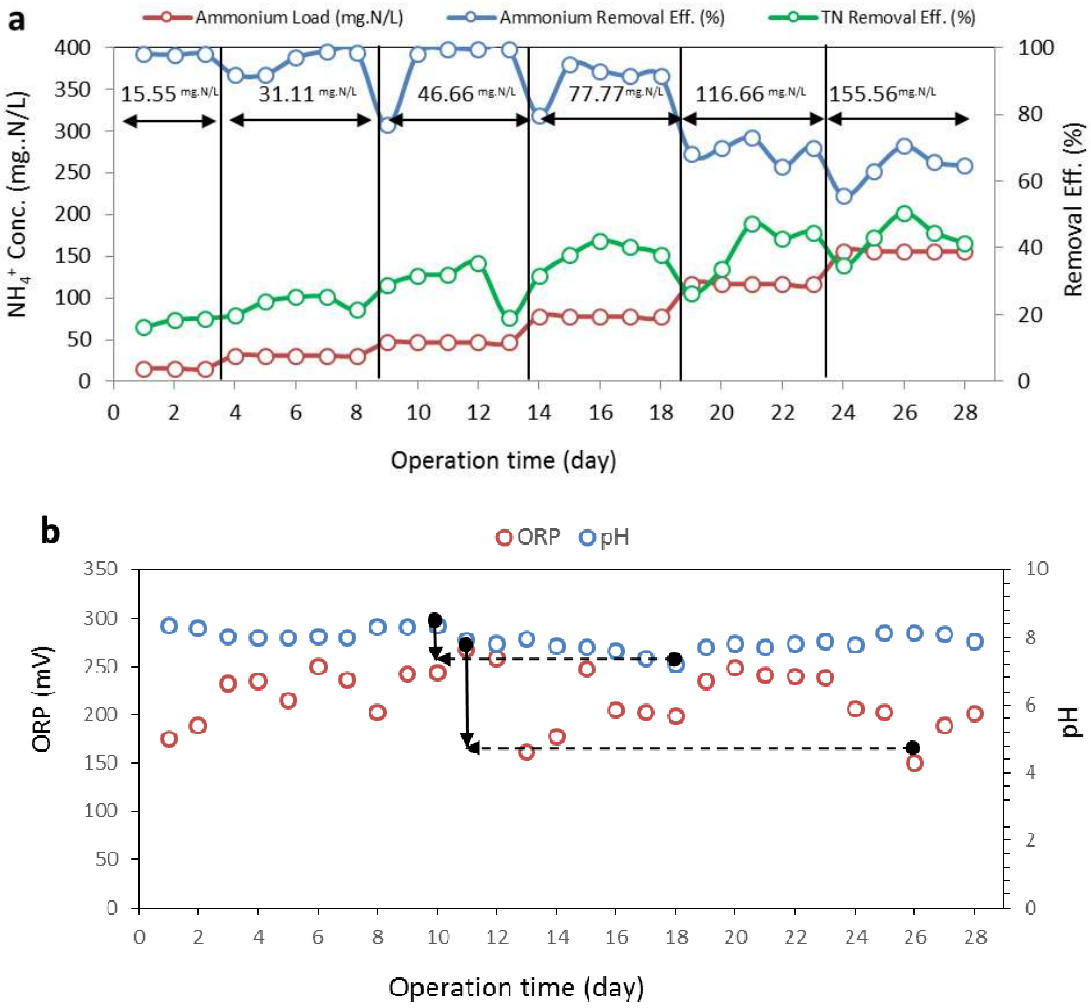


Fig.4.

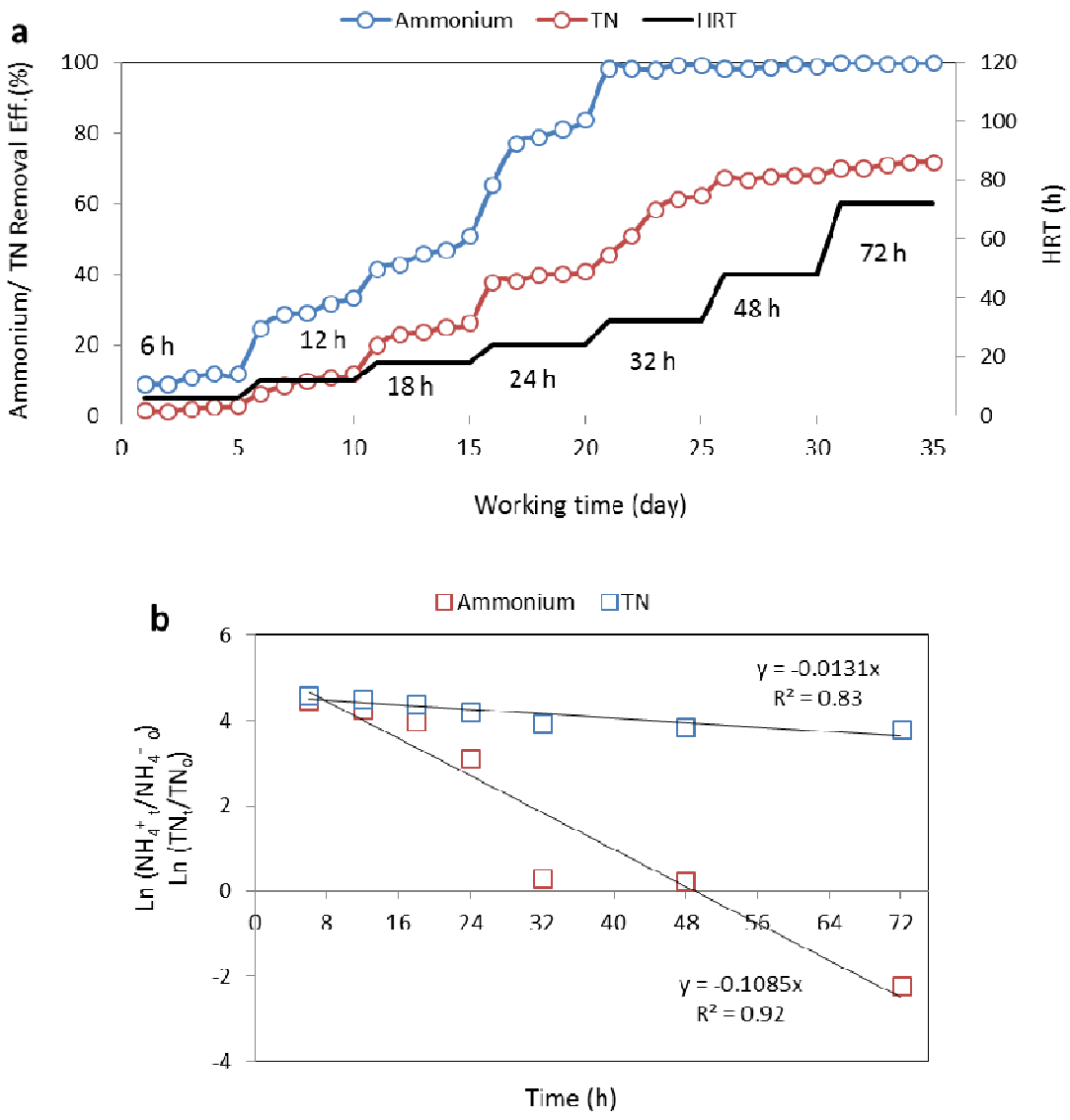
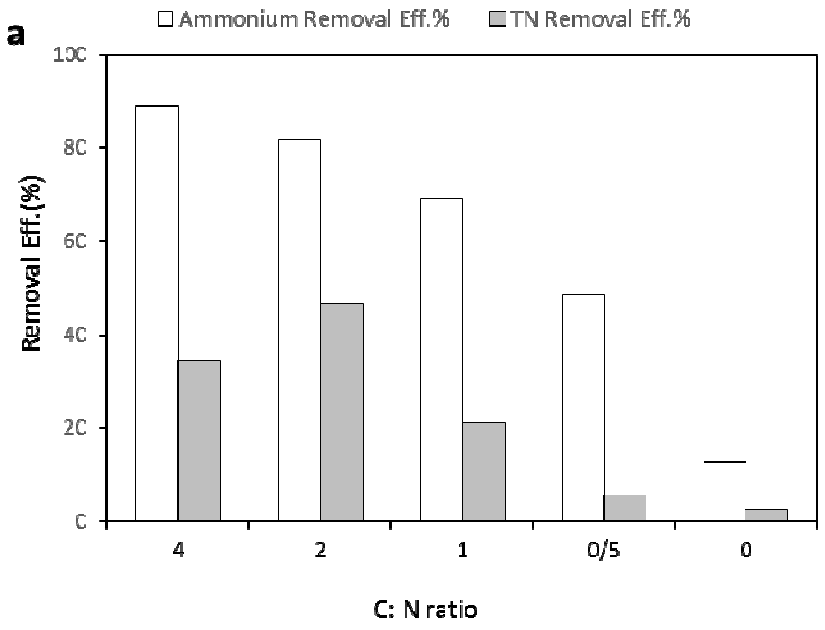
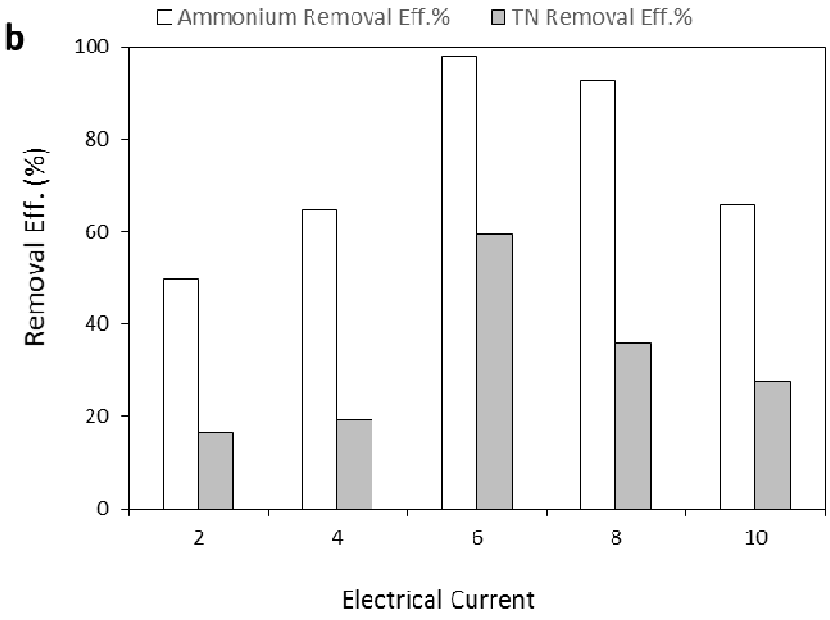


Fig.5.





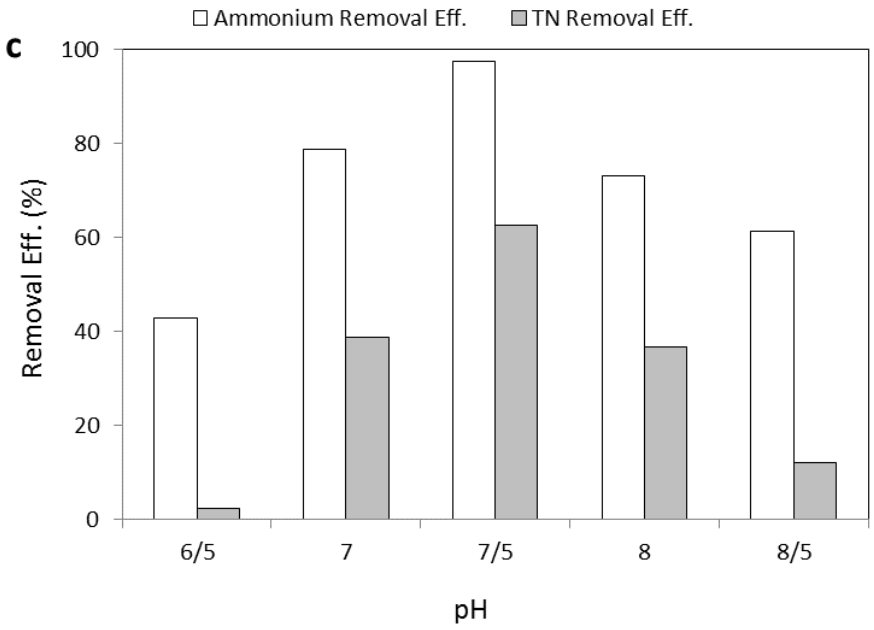


Fig.6.