Cathodic reduction characteristics of 2-chloro-4-nitrophenol in microbial electrolysis cell

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ABSTRACT

Microbial electrolysis cell (MEC) has been constructed to study the degradation characters of 2-chloro-4-nitrophenol (2C4NP) in waste water. The effects of applied voltage, initial concentration of substrate and co-matrix species on the reduction and degradation of 2C4NP were studied. Qualitative and quantitative analysis of 2C4NP residues and degradation intermediate by using UV-Vis, HPLC, HPLC/MS/MS, IC and other analytical testing techniques. The degradation mechanism of 2C4NP in MEC cathode was proposed. The results showed that electron and electroactive microorganisms would produce coupling effect and accelerate the degradation of 2C4NP under adding 0.5 V DC; Under the condition of satisfying the C/N ratio of electroactive anaerobic microorganism, the addition of organic substances such as glucose and sodium acetate which were easily degraded by microorganisms would hinder the degradation of 2C4NP in the cathode compartment. 2C4NP can be effectively degraded by adding appropriate amount of glucose as carbon source with the low C/N. 2C4NP undergoes reduction, dechlorination, denitrification and assimilation in the cathode compartment to form 2-chloro-4-aminophenol, 4-aminophenol, 2-chlorophenol, 2-chloro-4-hydroxyphenol, nitrophenol, hydroquinone, 4-hydroxyhexadienoic acid semialdehyde, valeric acid, oxalic acid and many other intermediate products. According to the degradation intermediates, the degradation mechanism of 2C4NP in the cathode compartment was presumed.

Keywords: Cathodic reduction, Degradation mechanism, Electroactive microorganism, Microbial electrolysis cell (MEC), 2-chloro-4-nitrophenol

1. Introduction

As a representatives among the chlorinated nitroaromatics, chloronitrophenols (CNPs) are extensively utilized in the fields of synthetic insecticides, fungicides, drugs, dyes, etc [1, 2]. The major sources of CNPs in the environment are prevailing coming from artificial manufacturing and use, or the degradation of their derivatives [3]. 2-chloro-4-nitrophenol (2C4NP) is considered as the commonest isomeride of CNPs that is used for the production of herbicides [4], molluscicides [5] as an intermediate material. One hand, the aqueous solution of 2C4NP has highly toxic because of their degradation-resistant property while electron withdrawing characteristics of the presence chloro and the nitro groups in CNPs. The other hand, 2C4NP has caused some surface water and sediment/soil contamination [5], severely affecting the human and animal health [6]. The removal of 2C4NP from the water/sediment/soil environment has caused great concern.

At present research, the major methods for degrading CNPs main-

ly include advanced oxidation methods (such as ozone catalytic oxidation [7, 8], photocatalysis [9, 10] and Fenton [11, 12], etc [13].), and electrochemical methods [14], and microbiological methods [2, 15]. However, there are lots of shortcomings of the advanced oxidation technology, such as high processing cost, inconvenient operation, the microbial treatment process and the problems of long reaction time, incomplete mineralization. In contrast, bioremediation processes are more cost-effective, eco-friendly and thorough tactics to remove CNPs from waste water.

Recent studies have shown that bioelectrochemical systems (BES) (including microbial fuel cells (MFC) [16-19], microbial electrolysis cells (MEC) [20].). There is the ability to remove refractory pollutants in water of those BES. These bioelectrochemical methods are attracted by more and more researchers because of its high removal efficiency, low operating cost and environmental sustainability [21, 22]. BES consists of two half-cell reactions of cathode and anode, and the principle is based on the transfer of electrons between microorganisms and electrodes. Thereby, it's accelerated

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that the directional reduction conversion of the pollutants is presented in the cathode chamber. Furthermore, on the condition of an anaerobic environment, contaminants such as nitrobenzene [23], chlorophenol [24], antibiotics [25], and polychlorinated biphenyls [26] will be reduced at a low voltage in the cathode chamber of the MEC system, and at the same time, it also can produce electricity, which can effectively reduce the consumption of external power supply [27, 28].

In this paper, we started the MEC cathode chamber as the reaction system, together with 2-chloro-4-nitrophenol was used as the target pollutant. The effects of applied voltage, initial concentration of substrate and common substrate type on the degradation of 2-chloro-4-nitrophenol in the cathode chamber were also investigated. According to the qualitative and quantitative analysis results of intermediate products, the mechanism of 2-chloro-4-nitrophenol degradation was discussed. This study can provide theoretical basis and basic parameters for the treatment of CNPs in wastewater by microbial electrolysis process.

2. Materials and Methods

2.1. Materials and Instruments

Materials: 2-chloro-4-nitrophenol AR (97% Purity) are from Xiya Reagent Research Center, China, methanol ($\geq 99.5\%$ LC Luda Chemical Reagent Factory, China), potassium dihydrogen phosphate ($\geq 99.5\%$ AR Kermel, China), glucose (AR Kermel, China), sodium acetate (97% AR Kermel, China), urea ($\geq 99.0\%$ AR Fuchen, China), the concentration measurement of Cl^- and NO_3^- were high-purity ($\geq 99.5\%$ LC) analytical reagents from Shanghai Chemicals Co., China. High-performance liquid chromatography (HPLC) grade HCOOH and methanol solvent were used for collecting the HPLC and HPLC/MS/MS.

Instruments: constant temperature shaker (RE-52A Shanghai Yarong Biochemical Instrument Factory, China), high speed centrifuge (3-18 SiGmA, Germany), high performance liquid chromatography (Agilent 1200 Agilent, USA), and Mass spectrometer (Agilent 6538, Q-TOF Agilent, USA).

2.2. Experimental Methods

2.2.1. Preparation of nutrient solution

(1) Anode nutrient solution (1 L): glucose 0.5 g, urea 0.057 g, K_2HPO_4 0.158 g, NaHCO_3 0.5 g, trace element solution 1 mL (FeSO_4 2 g/L, CoSO_4 0.15 g/L, NiSO_4 0.5 g/L, ZnSO_4 0.05 g/L, CuSO_4 0.04 g, MnSO_4 0.5 g/L, H_3BO_3 0.05 g/L, $(\text{NH}_4)_2\text{MoO}_4$ 0.05 g/L); pH = 7.0.

(2) Cathodic nutrient solution

① Nutritional solution with glucose as primary matrix (1 L): glucose 1 g, urea 0.057 g, K_2HPO_4 0.158 g, NaHCO_3 0.5 g, 2C4NP 0.03 g, trace element solution 1 mL (FeSO_4 2 g/L, CoSO_4 0.15 g/L, NiSO_4 0.5 g/L, ZnSO_4 0.05 g/L, CuSO_4 0.04 g/L, MnSO_4 0.5 g/L, H_3BO_3 0.05 g/L, $(\text{NH}_4)_2\text{MoO}_4$ 0.05 g/L); pH = 7.0.

② Nutrient solution with sodium acetate as primary matrix (1 L): Sodium acetate 1 g, urea 0.057 g, 2C4NP 0.03 g, trace

element solution 1 mL (FeSO_4 2 g/L, CoSO_4 0.15 g/L, NiSO_4 0.5 g/L, ZnSO_4 0.05 g/L, CuSO_4 0.04 g/L, MnSO_4 0.5 g/L, H_3BO_3 0.05 g/L, $(\text{NH}_4)_2\text{MoO}_4$ 0.05 g/L); pH = 7.0.

③ Nutrient solution with 2-chloro-4-nitrophenol as a single substrate (1 L): urea 0.057 g, dipotassium hydrogen phosphate 0.158 g, sodium bicarbonate 0.5 g, 2C4NP (1 ~ 30 mg/L), trace element solution 1 mL (FeSO_4 2 g/L, CoSO_4 0.15 g/L, NiSO_4 0.5 g/L, ZnSO_4 0.05 g/L, CuSO_4 0.04 g/L, MnSO_4 0.5 g/L, H_3BO_3 0.05 g/L, $(\text{NH}_4)_2\text{MoO}_4$ 0.05 g/L); pH = 7.0.

2.2.2. Experimental device

The bioelectrochemical experimental was carried out in a two-chamber MEC as a reactor, which was separated by a proton exchange membrane. The schematic structure of the reactor is shown in Fig. 1. The anode and cathode chambers were preformed at a volume of 1,000 mL and the working volume of 500 mL. Both the materials of anode and cathode electrode are carbon brushes (diameter: 3 cm, length: 4 cm, model: CTN0310), which one hand was connected to the spiral titanium wire, and the other hand connecting wire is touched to the power source.

We were pretreated the carbon brushes by sequentially washing with “deionized water-acetone-ethanol” and heating in a nitrogen atmosphere muffle furnace at 450°C for 30 min before using it; And the proton exchange membrane was pretreated by immersing for 30 min in each step of “ H_2O_2 (5%) at $80^\circ\text{C} \rightarrow \text{H}_2\text{SO}_4$ (5%) at $80^\circ\text{C} \rightarrow$ deionized water”.

Fig. 1. Schematic of microbial electrochemical reactor.

2.2.3. MEC reactor startup

(1) Electro-electricity sludge domestication

The sludge from the secondary settling tank of the municipal sewage treatment plant was added into the MEC reactor containing the cathode or the cation culture liquid, and the cathode chamber was blown off with N_2 for 30 min to form an anaerobic environment. The MEC reactor was placed in a shaker incubator, the cultured and acclimated was carried out at 25°C and 160 rpm without external power supply, the culture solution was changed after from 72 h to 96 h, and this process was repeated 6 times.

The MEC reaction device was carried out by passing 0.5 V DC, and the content of COD in the cathode chamber of the MEC reactor

was monitored at 0 h, 12 h, 24 h, 48 h, and 72 h. While the removal rate of COD in the water reached more than 85%, and the color of the sludge in the zone was gradually changed from dark brown to taupe and granular, it means that the electro-chemical sludge was domesticated successfully.

(2) Domestication of MEC cathodic reducing bacteria

After domesticating, the 2-chloro-4-nitrophenol degrading bacteria were domesticated in the double-chamber MEC reactor with placed in the shaker. The initial concentration of 2-chloro-4-nitrophenol was divided into five batches of 2.0, 5.0, 10.0, 20.0, 30.0 mg/L for domestication, and the time of transformation in each gradient was limited to 72 h. The concentration was acclimated until it is stable when the gradient degradation efficiency was not obvious.

(3) Startup of MEC reaction device

The effects of applied voltages, initial concentrations and different matrices were allowed to study the degradation of 2C4NP in the dual chamber MEC cell which was placed in a shaker at 60 rpm and the cathode chamber was purged with high purity nitrogen to maintain an anaerobic environment after domesticating the 2-chloro-4-nitrophenol degrading bacteria. Water samples collected at regular intervals (6 h each time) were at 3000 r/min for 5 min. and stored in a 4°C refrigerator for analysis after filtered through a 0.22 µm filter.

2.3. Sample Analysis

UV-vis spectra of 4-NP degradation after both photocatalytic and BAF were scanned on a UV-vis spectrophotometer (UV-vis 2501 PC; Shimadzu Japan).

HPLC and HPLC/MS/MS were used to analyze the residual amount of 2C4NP and degradation intermediates in the reaction solution.

The concentration of 2C4NP was measured by high-performance liquid chromatograph (HPLC, Agilent 1200) equipped with an Agilent ZORBAX SB-C18 column (stainless steel 150*4.6 mm) and a UV detector, and a solution of methanol : water (V/V, 40:60) was used as mobile phase. The mobile phase was filtered and sonicated in order to remove dissolved gas and the flow rate of the mobile phase for the analysis was kept at 1.23 mL/min under 10.7 MP a pressure and column temperature of 303 K using $\lambda = 295$ nm for UV detection. Aliquots of 20 µL were injected manual into the HPLC to determine the concentration of 2C4NP. Reagent grade standards were used to calibrate the HPLC.

The metabolites were identified by HPLC/MS/MS (Agilent 6538 Q-TOF System) equipped with an ESI source. As for the HPLC condition in HPLC/MS/MS testing, MeOH: 0.1% methanoic acid = 70:30 was used as mobile phase and a flow rate of 0.2 mL/min without a separation column. Full scale MS spectra both in negative modes in the mass range between 50 and 500 m/z was recorded.

The concentrations of Cl^- and NO_3^- were monitored by ion chromatography (ICS-2500, Dionex, USA) equipped with an electrical conductivity detector (ECD), on an anionic exchange column (Ion Pack AS18, 2*250 mm). Aliquots of 20 µL were injected into the IC, and carried with a mobile phase containing 1.7 mmol/L NaHCO_3

+ 1.8 mmol/L Na_2CO_3 solution with a flow rate of 0.25 mL/min. Quantitative analysis was performed using an external standard method.

3. Results and Discussion

3.1. Analysis of 2C4NP Cathodic Reduction Degradation

3.1.1. UV-Vis analysis of 2C4NP cathodic reduction degradation

Fig. S1. shows a UV-Vis absorption spectra corresponding during the transformation of 2C4NP to different MEC reaction time intervals (0 h, 6 h, 12 h, 24 h, 48 h, 72 h). The spectra correspond to MEC cathodic reduction degradation of 30 mg/L 2C4NP solutions were used the microorganisms that were acclimated and centrifuged by the 2C4NP as the strain, and the 2C4NP as the sole matrix nutrient solution. 2C4NP displayed three UV absorption bands at wavelengths 224 nm, 265.5 nm, and 403.5 nm. The band at 265.5 nm and 403.5 nm completely disappeared within 72 h of treatment, furthermore, the intensity of the band at 403.5 nm decreased significantly during 48 h. These results indicate that 2C4NP can be effectively degraded in the MEC cathode chamber, and it can be complete degrading after 72 h.

3.1.2 HPLC analysis of 2C4NP cathodic reduction degradation

With the addition of 0.5 V DC to the reaction and the initial concentration of 2C4NP was 30 mg/L, the HPLC analysis of the intermediate product of 2C4NP at 4 samples (0 h, 12 h, 48 h, 72 h) is shown in Fig. S2. The 0 h and 12 h sample clearly reveal that the retention time of 2C4NP peak was 17.16 min, contrasting the result of UV-vis that the consumption of 2C4NP in the sample during 0 h from 12 h were used to bacterial growth [29] and the peak intensity decreased gradually with the extension of reaction time. Furthermore, more small peaks appear at the retention time 1.466 min, 1.741 min, 2.048 min, 2.680 min, respectively, which indicated that many intermediate products have been generated in the metabolic process. In the sample of 48 h, 2C4NP was completely degraded as the peak at 17.16 min disappeared, and a strong intermediate peak appeared at 2.044 min. With the time proceeded to 72 h, the peak intensity of the main intermediate product decreased, indicating that the primary intermediate product was further degraded to another. Both the UV-vis and HPLC analysis showed that 2C4NP was effectively degraded in the MEC cathode chamber, and the analysis of HPLC shown the presence of the index compound 2C4NP drawn from 0 h and 12 h to bacterial growth, whereas the presence of the intermediate product was indicated in the samples drawn from 48 h and 72 h.

3.2. Effect of Applied Voltage on the Degradation of 2-chloro-4-Nitrophenol

The microorganisms which were domesticated and centrifuged at the cathode of the MEC by 2C4NP were used as the degrading bacteria, and the 2C4NP was used as a sole matrix nutrient solution. When the initial concentration is 30 mg/L, the degradation results of 2C4NP under different applied voltages (0, 0.25, 0.5, 0.75, 0.9 and 1.5 V) are shown in Fig. 2. It can be seen from Fig. 2 that in the anaerobic environment and under the addition of 0.25 ~ 1.5 V DC,

Fig. 2. Effect of different applied voltages on the degradation of 2C4NP.

the degradation rate of 2C4NP was significantly higher than that without the external power supply. At the initial stage of degradation reaction (0 ~ 12 h), the degradation of 2C4NP was slow under various voltage conditions, and there was no significant difference in degradation efficiency. A better degradation effect could be discovered in the range of the applied voltage to 0.5 ~ 0.75 V, and the degradation rate of the applied voltage is too low (< 0.5 V) or too high (> 0.9 V).

In the initial stage of the reaction (0 ~ 12 h), the domesticated and isolated microorganisms have an growth and adaptation process to 2C4NP, and the appropriate amount of applied voltage has little effect on the microbial activity. In the 12th h, when the applied voltage was in the range of 0.25 ~ 1.5 V, the degradation rate was between 26.4% and 29.8%, than it was only 19.0% under 0 V impressed voltage. With the reaction time prolonged, the coupling effect of electro-microorganisms was strengthened. On the one hand, the activity of electro-microorganisms was improved. On the other hand, electrons could directly attack 2C4NP adsorbed on the surface of microorganisms through the rapid transfer of microbial coenzymes, causing dechlorination, denitrated, or ring cleavage of 2C4NP [2, 3, 28]. In the samples of 36 h, the degradation efficiency of 2C4NP attained to more than 92% under the conditions of various applied voltages, while the degradation efficiency of 2C4NP was 85.9% when no voltage was applied. The degradation rate of 2C4NP rapidly increased to reached 92.2% at 24 h in the range of 0.5 V ~ 0.75 V applied voltage, which was 8.0% ~ 10% higher than other applied voltages and more than 20% to the un-applied voltage. The study of various applied voltages shows that the microorganisms acclimated by the 2C4NP at the cathode of the MEC and the direct current of 0.5 V would make a good coupling effect between microorganisms and electrons. And when the initial concentration of 2C4NP was 30 mg/L, 2C4NP would be completely degraded in the cathode chamber after 60 h.

3.3. Effect of Different Initial Concentrations on the Degradation of 2C4NP

We had also studied the effect of different initial concentrations (1 mg/L, 10 mg/L, and 30 mg/L) of 2C4NP on the function of

removal it by the degraded microorganisms at the applied voltage of 0.5 V.

As shown in Fig. 3, the removal rate of 2C4NP in the 1 mg/L and 10 mg/L was higher than 30 mg/L at the pre-reaction period (0 ~ 12 h), which the degradation rates at 6 h were 11.83%, 26.74%, 6.73%, and 28.92%, 65.45%, 29.89% at 12 h, respectively. It's noticeable that the the removal rate of 2C4NP in initial concentration of 10 mg/L at short-term was more than doubled to 1 mg/L and 30 mg/L, which we consider that the domesticated and isolated microorganisms have an adaptation process to growth, the higher or lower the initial concentration of 2C4NP, the longer the adaptation time, and it's the same as our result above. And the concentration of 10 mg/L was easier to accommodate for the strains. In other words, the growth of anaerobic microorganisms has an adapted range of carbon to nitrogen ratio. On the contrary, in the middle and late stage of the reaction (≥ 12 h), the degradation rate of 2C4NP increased with the increase of the initial concentration. When the initial concentration was 1 mg/L, the degradation rate of 2C4NP almost unchanged. But at the 30 mg/L initial concentration, 2C4NP could completely degraded at 72 h, while it increased slowly, even didn't change after 24 h at the 1 mg/L, 30 mg/L, and the final degradation rate of its were only 37% at 1 mg/L, 85% at 10 mg/L respectively. We deem that the main influencing factor of 2C4NP degradation in the cathode on the late is the discrepancy of C/N ratio. While the initial concentration of 1 mg/L, 10 mg/L, the C/N of the reaction solution is 0.59 and 0.71, the degradation rate of 2C4NP increased slowly, even didn't change. But when the initial concentration of 2C4NP reached 30 mg/L, the initial C/N rose to 0.95, and the 2C4NP could be completely degraded after 72 h.

The experimental results show that the microorganisms acclimated by the 2C4NP at the MEC cathode have a suitable C/N ratio under the condition of adding 0.5 V direct current. The electrical microbial activity is lower with the nethermore C/N ratio, and the lower initial concentration would result in 2C4NP incompletely degraded in the anaerobic cathode chamber. In order to completely degrade 2C4NP, the C/N ratio in the initial solution of the reaction should be greater than 0.95.

Fig. 3. Effects of Different initial concentrations on the degradation of 2C4NP.

3.4. Effect of Co-matrix Species on the Degradation of 2C4NP

Co-metabolism is a relatively unique metabolic method [30-32]. Some organic substances which are difficult to biodegrade can be initiated or promoted the degradation of those substances by adding other substances what are easily degraded by microorganisms such as glucose, ethanol, and sodium acetate [3, 33]. To study the effect of cometabolism for the biodegradation of 2C4NP by the microbes in the anaerobic MEC cathode chamber, the carbon source with different nutrient solution was added to the reactor: First, 2-chloro-4-nitrophenol as a single matrix nutrient solution (30 mg/L); Second, a common-based nutrient solution with glucose as a primary matrix (wherein 2C4NP was 30 mg/L); Third, sodium acetate as a primary matrix nutrient solution (wherein 2C4NP was 30 mg/L), externally connected DC 0.5 V, and the degradation rate of 2-chloro-4-nitrophenol with the time was shown in Fig. 4. A reverse result with Min [3] that the carbon source with different nutrient solution of glucose and sodium acetate can inhibit 2C4NP degradation. Although the degradation efficiency of 2C4NP by adding glucose as a co-substrate wasn't obvious lower than 2C4NP as the only substrate, the degradation rate was significantly reduced by adding sodium acetate. Under the condition of single matrix, glucose and sodium acetate as co-substrate, 2C4NP degradation rate was 53.37%, 29.89% and 9.16%, respectively, at 12 h, when the reaction was 24 h, the degradation rate of 2C4NP was 97.31%, 95.53%, 23.50%. In the case of 2C4NP as single matrix and glucose as the co-substrate, 2C4NP was completely degraded in the cathode chamber after 60 h and 72 h, respectively. However, adding sodium acetate as the co-substrate, 2C4NP could not be completely degraded in the cathode chamber for 84 h. The experimental results show that the co-substrate is not conducive to the degradation of 2C4NP in the anaerobic MEC cathode chamber.

Under the experimental conditions, the microorganisms acclimated by the 2C4NP at the MEC cathode can be used as the only carbon source to degrade the 2C4NP in the anaerobic cathode compartment. When glucose and sodium acetate are added to the cathode chamber, respectively, 2C4NP and glucose or sodium acetate have a competitive relationship and inhibitional effect (sodium acetate > glucose > 2C4NP) in the utilization of electroactive microorganisms. The results also show that under the condition

Fig. 4. Effect of co-matrix species on the degradation of 2C4NP.

of satisfying the carbon-nitrogen ratio in anaerobic microorganisms, adding organic substances such as glucose and sodium acetate will hinder the degradation of 2C4NP; furthermore, an appropriate amount of glucose can be added as a carbon source to adjust the lower nitrogen ratio so that 2C4NP can be effectively reduced and degraded in the cathode chamber.

3.5. Analysis of Intermediate Products of 2C4NP Degradation in the EMC

3.5.1. HPLC/MS/MS qualitative analysis of intermediates

The optimum conditions with 30 mg/L of 2C4NP as a single matrix nutrient solution, and applied 0.5 V DC were used to study the intermediates of 2C4NP degraded by the strains in the EMC via HPLC/MS/MS analyses. The results of HPLC/MS/MS analysis of the reaction solution in which 2C4NP was degraded for 24 h are shown in Fig.5 and Fig. 6.

Fig. 5. Total ion current map of 2C4NP degradation products.

There were many intermediate products formed during the degradation of 2C4NP (Fig. 5), the analysis results of TIC by MS/MS are shown in Fig. 6 and Table S1. Several molecular ion peaks such as its fragments at m/z 142.9475 was 2-chloro-4-aminophenol, and 143.1067 was chlorohydroquinone (CHQ) [2, 3, 30-35], 141.0528 possibility was γ -hydroxymuconic semialdehyde [33, 35], 138.0187 could be 4-Nitrophenol, 126.9953 was 2-Chlorophenol, 108.0202 was 4-Aminophenol, 109.0278 was hydroquinone (HQ) [2, 3, 30-35], 101.0606 and 88.9769 were valeric acid and oxalic acid, respectively, etc, which detected at 1.284 ~ 1.293 min, 1.364 ~ 1.373 min, 1.247 ~ 1.256 min, 2.892 ~ 2.902 min, 1.231 ~ 1.245 min, 1.116 ~ 1.125 min, 1.113 ~ 1.122 min, 1.175 ~ 1.186 min and 1.139 ~ 1.147 min in TIC, respectively. The results in Table S1 indicate that under the coupling of electricity and anaerobic microorganisms, 2C4NP was reduced and assimilated to form many intermediates.

3.5.2. Cl^- and NO_3^- ion concentrations change during the reaction

30 mg/L of 2C4NP was applied as a single matrix nutrient solution under the condition of 0.5 V DC. The residual concentration of 2C4NP in the degradation process of MEC cathode, the change of Cl^- and NO_3^- ion concentration are shown in Fig.7. In addition to the degradation of 2C4NP, concomitant accumulation of chloridion was also observed in the bioelectrochemical treatment, together

Fig. 6. HPLC/MS/MS analysis of 2C4NP degradation intermediates.

Fig. 7. Variation of 2C4NP, chloride ion and nitrate concentration in the degradation process with reaction time.

with the NO_3^- ion concentration gradually increased [33] in the first 60 h, and then decreased slowly.

Under the coupling of electricity and anaerobic microorganisms, 2C4NP undergoes reduction, dechlorination, denitrification and assimilation to be degraded, and the removal pathways of $-Cl$ and $-NO_2$ were mainly the channels: with the action of the dehalogenase [33], the $-Cl$ on the 2C4NP and the degraded intermediate (such as 2C4AP, etc.) was removed from the benzene ring or other groups; and $-NO_2$ was directly oxygenated removed from the benzene ring by *Pseudomonas* [34]. On the other hand, *Bacillus* was capable of catalyzing the transformation of $-NO_2$ to $-NH_2$ on the benzene ring [34], and NO_2^- was directly transformed to NH_4^+ by anaerobic bacteria, or the $-NH_2$ was removed from the benzene ring by the action of a deaminase to form NH_4^+ .

3.6. Discussion on the Mechanism of 2C4NP Degradation In MEC Cathode

According to HPLC, HPLC/MS/MS and IC analysis results of 2C4NP cathode degradation products, it can be seen that 2C4NP can be

Fig. 8. Possible degradation mechanism of 2C4NP in the cathode chamber.

reduced, dechlorinated, denitrified, and assimilated to complete mineralization at the cathode of the MEC. The metabolic pathways of 2C4NP for microorganism treatments were studied [2, 3, 29-35], and several degradation pathways were surmised by our experiment. These ways shown in Fig. 8 include (i) the 2C4NP degradation was initiated by the oxidative release of nitro group to the formation of CHQ and followed by reductive release of chloro group to form HQ [3, 31, 33]; (ii) the 2C4NP degradation was initiated by the release of nitro group into the 2-Chlorophenol and occurred oxidation to produce HQ; (iii) the 2C4NP degradation was initiated by the release of chloro group into the 4-Nitrophenol and occurred reduction to produce 4-Aminophenol, then the oxidative release amino to form HQ [33]; finally, the ring cleavage of HQ to the formation of γ -hydroxymuconic semialdehyde [2, 3, 32, 33], followed the γ -hydroxymuconic semialdehyde was disintegrated into valeric acid, oxalic acid and ethyl alcohol, which the end to the formation of CO_2 and H_2O [2, 3, 33-35].

4. Conclusion

MEC was a more reasonable and effective system compared with the traditional anaerobic sludge system. The external voltage supply enhanced the 2C4NP microbial degradation, higher or too lower external voltage was not considered conducive to the removal of 2C4NP. The complete degradation was obtained when the initial concentration of 30 mg/L 2C4NP. Multiple carbon sources would affect and inhibit the degradation rate of 2C4NP. The intermediates after degraded were analyzed by LC/MS/MS, which showed that 2-chloro-4-aminophenol, 4-aminophenol, 2-chlorophenol 2-chloro-4-hydroxyphenol, nitrophenol, hydroquinone, 4-hydroxyhexadienoic acid semialdehyde, valeric acid, oxalic acid and many other intermediate products were produced in the course of decomposition. We presumed three decomposition pathways of the 2C4NP by the microorganisms in the EMC system.

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Author Contributions

Q.Y. (M.S. student) performed the experiment, wrote and revised the manuscript. W.X. (M.S. student) drafted and revised the manuscript. D.H. (Professor) provided research ideas and designed experimental. C.L. (M.S. student) performed the experiment and drafted the manuscript. Q.Y. (M.S. student), T.G. (M.S. student) and Q.W. (M.S. student) collected, cleaned and analyzed data.

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