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Detoxification of nicotine solution using Fe^0-based processes: Toxicity evaluation by *Daphnia magna* neonate and embryo assays

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ABSTRACT

Three Fe^0-based techniques were used to treat wastewater containing nicotine. *Daphnia magna* neonates and embryos were used in bioassays to evaluate the toxicity of nicotine before and after the treatments. The results show that nicotine was highly toxic to *Daphnia* neonates ($EC_{50,\ 48h}$ = 0.379 mg L^{-1}, $EC_{50,\ 72h}$ = 0.125 mg L^{-1}). Although nicotine was moderately toxic to *Daphnia* embryos after a 48 h exposure ($EC_{50,\ 48h}$ = 250.8 mg L^{-1}), nicotine was highly toxic to the embryos ($EC_{50,\ 72h}$ = 1.328 mg L^{-1}) when exposure time was extended to 72 h. Nicotine induced malformations in the second antenna, carapace, and tail spine. The Fe^0/air process was unable to effectively degrade nicotine. In contrast, the Fe^0/H_2O_2 process and micro-electrolysis efficiently removed nicotine within 30 min (90.8% and 94.6%, respectively). The chemical oxygen demand removed by micro-electrolysis (70.4%) was clearly higher than that removed by the Fe^0/H_2O_2 technique (58.1%). Micro-electrolysis maintained its effectiveness (> 81.3%) after seven reuse cycles. A spectral analysis indicated that the micro-electrolysis process effectively removed the pyridine (A_{260}) and pyrrolidine rings (A_{206}) of nicotine. The toxicity of Fe^0/H_2O_2- and micro-electrolysis-treated solutions were low (3.75% embryo mortality rate after 72 h). The above results show that micro-electrolysis is a potential approach to remove nicotine from solution.

1. Introduction

Nicotine, 3-(1-methyl-2-pyrrolidinyl)-pyridine, is a major alkaloid in tobacco and consists of pyridine and pyrrolidine rings [1]. Nicotine is often found in surface water, domestic wastewater, and tobacco wastewater [2–4], particularly, tobacco waste leachate often contains high levels of nicotine [4]. Nicotine has adverse effects on human health, including addiction, effects on the central nervous system, and promotion of cancer growth [2,4–8]. Nicotine is bio-recalcitrant and is extremely toxic to most groups of microorganisms and aquatic organisms [4,9]. Nicotine is also listed as an emerging pollutant [10].

Daphnids have an important role in the aquatic food chain, as they feed upon algae and are the main food of some fish [11]. Because of their high sensitivity to pollutants, short generation time, and ease of handling [12,13], *Daphnia* is widely used to evaluate the toxicity of chemicals, including heavy metals, pesticides, and pharmaceuticals [12,14,15]. The life-cycle of daphnids includes the embryonic, neonatal, juvenile, and adult stages. The embryonic and neonatal stages are particularly important in toxicity studies [16,17]. Several studies have reported that *Daphnia* embryos are more sensitive to several toxicants than *Daphnia* neonates [16–18]. For example, Abe, Saito, Niikura, Shigeoka and Nakano [16] used *Daphnia magna* neonates and embryos *to ev*aluate the toxicity of aniline derivatives. Their results indicated that *Daphnia* embryos were more sensitive to aniline derivatives than the juveniles in the acute immobilization assay. Our previous studies showed that *Daphnia* embryos are also more sensitive to disinfectants and plant growth regulators than neonates [17,18].

Fe^0 is considered a green material because huge quantities of iron are available as recycled material and it can effectively decompose pollutants [19–21]. Many Fe^0-based techniques, including Fe^0/air, Fe^0/H_2O_2, and Fe^0/granular activated carbon /ultrasound (micro-electrolysis) have been used to treat organic pollutants [19,22,23]. For example, the Fe^0/air method can be used to effectively degrade dye, phenolic, and ammunition wastewater [19,24].

In a Fe^0/H_2O system, Fe^0 corrodes and produce iron corrosion products, such as $Fe(OH)_2$, $Fe(OH)_3$, F_2O_3 or green rusts (Eqs. 1–4) [25]

$$Fe^0 \rightarrow Fe^{2+} + 2e^- \quad (1)$$

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$$Fe^{2+} \rightarrow Fe^{3+} + e^{-} \quad (2)$$

$$Fe^{2+} + 2OH^{-} \rightarrow Fe(OH)_2 \quad (3)$$

$$Fe^{3+} + 3OH^{-} \rightarrow Fe(OH)_3 \quad (4)$$

During the Fe^0/air treatment, iron corrodes and forms a layer of iron oxides/hydroxide generates on the Fe^0 surface [26]. The adsorption onto and co-precipitation of pollutants with iron corrosion products can remove organic pollutants [27]. Several researches also reported that the presence of oxygen and Fe^0 in the acidic solution allows for the generation of H_2O_2 (Eq. (5)) [28–30]. The H_2O_2 can react with the Fe^{2+} to form strong oxidants, such as hydroxyl radical ($OH^{\cdot}$) (Eq. (6)) and ferry (iron (VI) ion species) [30], which may also oxidize organic contaminates.

$$Fe^0 + O_2 + 2H^+ \rightarrow H_2O_2 + Fe^{2+} \quad (5)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-} \quad (6)$$

In the Fe^0/GAC system, the Fe^0 and GAC act as the anode and cathode, respectively, in the micro-electrolysis system. Numerous microscopic galvanic cells are formed spontaneously between these two electrodes. The half-cell reactions in the anoxic and acidic conditions can be represented as follows (Eqs. (7) and (8) [31,32]:

Iron anode (oxidation):

$$Fe^0 \rightarrow Fe^{2+} + 2e^{-} \quad (7)$$

Carbon cathode (reduction):

$$2H^+ + 2e^{-} \rightarrow H_{2(g)} \quad (8)$$

The Fe^0/H_2O_2 method uses the heterogeneous Fenton reaction to destroy pentachlorophenol, pharmaceutical, and olive mill by-products in wastewater [23,33,34]. Micro-electrolysis effectively treats acrylonitrile-butadienestyrene, pharmaceutical, and coal-coking wastewater [35–37]. However, studies that have used Fe^0-based techniques to treat nicotine wastewater are limited [38].

In this study, the effectiveness of three Fe^0-based techniques (Fe^0/air, Fe^0/H_2O_2, and micro-electrolysis) to remove nicotine from water was investigated. *Daphnia* neonates and embryos were employed in bioassays to evaluate the toxicity of the nicotine solution before and after the treatments.

2. Materials and methods

2.1. Chemicals

Nicotine (CAS number 54-11-5, $C_{10}H_{14}N_2$, M.W. = 162.23 g mol^{-1}, 99% purity, Fig. 1) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Zero-valent iron (analytical grade, 99% purity, 300 mesh) was obtained from Shimakyu Chemical (Osaka, Japan) and was used as received. H_2O_2 (30% purity) was obtained from Hayashi Pure Chemical Ind. Ltd. (Tokyo, Japan). A commercial coconut-based GAC (4.76 mm diameter, 2 mm length, Hong-Yu, Taiwan) was used as received.

2.2. Daphnia test

2.2.1. Neonate acute toxicity tests

The *Daphnia* neonate acute toxicity test was described in our previous study [4]. *D. magna* is cultured parthenogenetically at Chung-Shan Medical University, Taiwan. Randomly selected neonates from the cultures (< 24 h old, ≧third-brood) were used to assess *Daphnia* neonatal acute toxicity to nicotine. Four replicates with five neonates per replicate at different nicotine concentrations (0, 0.0625, 0.125, 0.25, 0.5, and 1 mg L^{-1}) were used to estimate the EC_{50} of nicotine to the *Daphnia* neonates [39]. The control set only contained simulated high hardness medium without nicotine (nicotine concentration = 0 mg L^{-1}). Tests were performed in 50 mL of a simulated high hardness medium. The composition of the simulated high hardness medium were 0.77 mM $CaCl_2{\cdot}2H_2O$, 0.46 mM $MgSO_4$, 0.01 mM K_2HPO_4, 0.20 mM $NaNO_3$, 1.50 mM $NaHCO_3$, 0.11 mM $Na_2SiO_3{\cdot}9H_2O$, 0.39 mM H_3BO_3, 0.08 mM KCl, and conductivity of 720 μmho cm^{-1} (25 °C). The solution pH was adjusted to 7.4–4.5 [40]. The glass beakers containing *Daphnia* neonates were placed in a constant growth chamber (temperature 20 °C ± 2 °C, 16-h/8-h light/dark cycle). Immobility was used as the endpoint for measuring acute toxicity; daphnids showing no movement within 15 s after gentle stirring were considered immobile. The EC_{50} values were calculated by probit analysis [18] based on nominal concentrations.

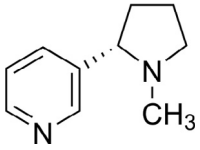

Fig. 1. Molecular structure of nicotine (3-(1-methyl-2-pyrrolidinyl)-pyridine).

Table 1
Nicotine 48 and 72 h acute toxicity EC_{50} (mg L^{-1}) by *Daphnia* neonates and embryos (mean ± SD) (n = 20).

	48 h	72 h
Neonate EC_{50} (95% CI)	0.379 ± 0.020 (0.207–0.651)	0.125 ± 0.010 (0.058–0.230)
Embryo EC_{50} (95% CI)	250.8 ± 1.5 (75.5–853.7)	1.328 ± 0.090 (0.759–2.280)
Embryo LOEC[a] (95% CI)	40.9 ± 0.1 (0.2–99.1)	0.406 ± 0.020 (0.022–0.725)

[a] Lowest observed effect concentration.

2.2.2. Embryo acute toxicity tests

The simulated high hardness medium was also used in the embryo toxicity test [40]. After the *Daphnia* eggs precipitated to the bottom of the well, the medium in each well was removed carefully and replaced with 300 μL of fresh simulated high-hardness medium containing nicotine at different concentrations. The embryo toxicity test is according to the US EPA method [39]. Four replicates with five eggs per replicate at different nicotine concentrations (0, 62.5, 125, 250, 500, 1000 mg L^{-1}) were used for embryo acute toxicity tests. The control set only contained simulated high hardness medium without nicotine (nicotine concentration = 0 mg L^{-1}). The plates were maintained under a 16-h/8-h light/dark cycle at 20 °C ± 2 °C and covered to minimize loss of the culture medium. The eggs were inspected daily to examine their development, and the incidence of premature death was recorded. Premature death included undeveloped embryos or lysed embryos compared with the control set. The endpoint was defined as the ratio of live neonates hatched to the original number of exposed *D. magna* embryos. The hatched *D. magna* were examined for gross morphological abnormalities under a low-magnification microscope (SMZ800; Nikon, Japan). Experiments were repeated four times. The 48 and 72 h EC_{50} values and 95% confidence limits were estimated by probit analysis [18].

2.2.3. Embryo developmental teratogenic assay

The nicotine concentrations for the embryo developmental teratogenic assay were chosen according to the embryo 48 h- and 72 h-lowest observed effect concentrations of nicotine, which were based on the data from the embryo acute toxicity test. The control set only contained simulated high hardness medium without nicotine (nicotine concentration = 0 mg L^{-1}). Because the nicotine may accumulate in the *Daphnia* embryo with exposure time, the observation time was extended to 72 h. The embryo development abnormalities were compared