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Study of atrazine degradation in subsurface flow constructed wetland under different salinity

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Abstract

To evaluate the treatment capability of subsurface flow constructed wetland (SFCW) and the effect of salinity on the degradation of atrazine, the degradation of atrazine in SFCW was studied. Under the static condition, the degradation of atrazine in SFCW followed first-order kinetics: $c = 0.09679 \exp(-0.0396t)$ (c, residue concentration, mg l^{-1}; t, retention time, d), with a half-life of approximately 17.5 days. The atrazine degradation kinetic functions were established for salinities of 1.5, 3.0, 5.0, 10.0 and 15.0 g l^{-1}, respectively, which appeared to approach first-order kinetics. The effect of salinity on the atrazine treatment efficiency showed an exponential inhibition: $\ln k = 3.204 + 0.04991C$ (k, degradation constant; C, NaCl concentration, mg l^{-1}). The attenuation of atrazine in SFCW cannot be a result of hydrolysis or sorption process. It was considered that some bacteria in the wetland system degraded atrazine into deethylatrazine (DEA) and deisopropylatrazine (DIA) and sequentially into CO_2 and H_2O. Salinity impacted on the growth of bacteria resulting in a switch of the microbial community. With the increase of salinity, Shannon–Wiener Diversity Index in the SFCW system declined. The relationship between atrazine degradation constant (k) and Shannon Index was established as shown in linear phase, $y = -0.07286 + 0.0363x$. The positive correlation between them indicated that microbial community played an important role in the atrazine degradation process.
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Keywords: Subsurface flow constructed wetland; Atrazine; Degradation; Salinity

1. Introduction

Atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine), one of the most common herbicides applied worldwide, once in the environment, is slow to break down in soil and water. The most important mechanism for the decay of atrazine involves microbial degradation (Giardi et al., 1985; Behki et al., 1993; Mirgain et al., 1993; Mandelbaum et al., 1995; Struthers et al., 1998) and chemical decomposition (Li et al., 1972; Comber, 1999; Ye Changming et al., 2001; Parra et al., 2004; Deng et al., 2005). Rapid development has been achieved in the microbial aspect since Wackett isolated and characterized a *Pseudomonas* sp. that mineralizes the s-triazine herbicide in 1995.

Constructed wetlands are often employed to treat storm runoff, polluted groundwater and surface water. As a result of storm runoff, atrazine is frequently detected as a contaminant in streams, rivers and lakes. The treatment of herbicides in constructed wetland has been studied extensively but the degradation process of atrazine affected by the environmental factors in that is not very clear (Ciba-Geige Corporation, 1994; DeLaune et al., 1997; Ghosh and Philip, 2004; Singh et al., 2004). Weaver et al. (2004) assessed the atrazine degradation in soils from a constructed wetland and reported that atrazine dissipated rapidly with a half-life of approximately 23 days, but only 10% of atrazine was mineralized to CO_2. Also, they suggested that hydrolysis of atrazine to hydroxylated metabolites was a major component of the bound residues. Michinlay and Kas-

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perek, 1999 used vertical subsurface flow wetland to decontaminate water polluted with atrazine. The mechanism of decontamination has also been shown to be biologically-based, specifically microbially-based and to reside in the rhizosphere. However, the type of microbe involved in the decontamination of atrazine is still unknown. Moore et al. (2000) studied a constructed wetland for mitigation of atrazine-associated agricultural runoff and considered that conservative buffer distances of 100–280 m would be necessary for effective runoff mitigation. Runes et al. (2003) utilized the constructed wetland to treat nursery irrigation runoff and detected the deethylatrazine (DEA), deisopropylatrazine (DIA) and Hydroxyatrazine (HA) as the residues of atrazine at the outlet. Also static water-sediment column experiments indicated that sorption was an important mechanism for atrazine loss. The common point in previous research is that constructed wetland can be regarded as a relatively effective method to decontaminate atrazine polluted water. However, the influence of various environmental factors on treatment efficiency, for instance, salinity has not been investigated in detail.

Shanghai, located along the coast of the East China Sea and the southern banks of the mouth of the Yangtze River, is hit by the salt tide periodically. During the salt tide period, the behaviors of microorganisms in natural conservative buffer zones are greatly affected due to the high salinity and decontamination would also be impacted due to the changes of salinity. To gain more knowledge on the effect of salinity on the atrazine degradation in wetlands, in this work, we evaluated this in a constructed wetland system. Also the influence caused by salinity changes as well as shift of the microbial community in this environment was studied.

2. Materials and methods

2.1. Constructed wetland setup

Two series of subsurface flow constructed wetland (SFCW) located at Tongji University, Shanghai, were established in September 2005 and were operated in two static scenarios separately as follows: atrazine degradation with salinity changes and characterization of microbial communities in SFCW during that process.

For the first scenario, the static batch consisted of 3 beds ($0.3 \times 0.3 \times 0.5\ m^3 = 45$ l), namely, M1, M2 and M3, operating in parallel, which were filled with gravel (8–15 mm rounded). Each bed was planted with four plants of *Typha angustifolia*. The schematic diagram of the bed is shown in Fig. 1.

To study the microbial community in the constructed wetland during the atrazine degradation, four beds, namely, W1, W2, W3 and W4, same as the M-series, operated in batch mode were established. The substrate and plant in the batch were the same as the M-series.

Synthetic wastewater was used throughout the studies, as shown in Table 1.

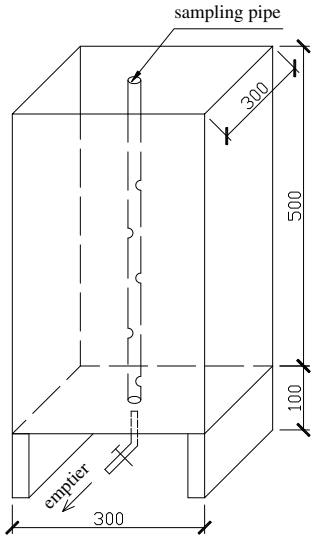

Fig. 1. The schematic diagram of batch reactor M (W)-series.

Table 1
Characteristics of inflow in SFCW experiments

Components	Concentration (mg l^{-1})	Components	Concentration (mg l^{-1})
Starch	16	Yeast	50
Glucose	60	$MgSO_4 \cdot 7H_2O$	158
NH_4Cl	148	$MgCl_2 \cdot 6H_2O$	150
KH_2PO_4	87	$CaCl_2 \cdot 2H_2O$	500
KNO_3	360	$FeCl_2 \cdot 4H_2O$	21
$NaHCO_3$	100	EDTA · 2Na · $2H_2O$	40
Atrazine	0.1		

2.2. Experimental methods

2.2.1. Incubation and sampling

2.2.1.1. Atrazine half-life. Incubation: synthetic wastewater (20 l) was initially added into each of the three beds (M1–M3), respectively. The treated wastewater was discarded from the batches, and replaced by an equal volume of fresh wastewater with higher atrazine concentration every three days. Operation was continued for 30 days to ensure that the plants get an atrazine polluted environment where atrazine concentration was increased from 0.01 mg l^{-1} to a final 0.1 mg l^{-1}.

Sampling: after the incubation phase, 20 l synthetic wastewater with atrazine concentration of 0.1 mg l^{-1} was added into beds, respectively. On the 1st, 3rd, 5th, 8th, 10th, 15th, 20th, 30th and 45th day, respectively, 150 ml liquid was collected as sample for HPLC detection of atrazine and its metabolites, using the peristaltic pump from the sampling pipe at 200 mm point from the upmost of the gravel layer. The redox potential, pH and electrical conductivity at the sampling point were monitored throughout the study to take reliable data. The redox potential at a different height in the sampling pipe was maintained between −80 and −150 mV during the incubation period to ensure the anaerobic condition in the batch.

Table 2
Operational conditions of the study of the effect of salinity on the degradation of atrazine

Salinity (g l^{-1})	Conductivity	pH	Temperature (°C)
0	2.77–2.94	6.58–6.9	22.5–25.2
1.5	5.13–5.42	6.54–6.69	22.0–25.5
3	8.38–8.77	6.57–6.69	26.1–30.0
5	12.05–12.58	6.61–6.81	27.5–31.1
10	23.1–25.6	6.57–6.8	28.5–31.0
15	33.4–35	6.54–6.71	27.4–31.5

2.2.1.2. Atrazine degradation under different salinity. The concentration of NaCl added into the system was to represent the salinity in the environment. Operational conditions under different salinity are shown in Table 2. The conductivity increased with the increasing of salinity and the water temperature was kept between 22 °C and 31 °C.

Incubation: to help the plant adapt a high salty environment gradually, a longer incubation procedure was performed in the M-series batch. Initially 20 l synthetic wastewater (atrazine concentration: 0.1 mg l^{-1}) containing 0.1 g l^{-1} NaCl was added into the three beds (M1–M3) after the half-life experiment and the water was emptied and replaced every four days. The concentration of NaCl was increased gradually every time the water was replaced until the target salinity of 1 g l^{-1} was reached.

Sampling: While the incubation was finished, the batches were drained and replaced by 20 l fresh synthetic wastewater with 0.1 mg l^{-1} atrazine and 1 g l^{-1} NaCl. The same sampling procedure was followed as Section 2.2.1.1. to detect atrazine concentration change and its metabolites.

Also, atrazine degradation processes under the other salinities (1.5, 3, 5, 10 and 15 g l^{-1} in sequence) were performed following the same procedure described above sequentially.

2.2.1.3. Atrazine metabolites and microbial community characteristics. The same atrazine and salinity incubation processes were employed until the atrazine concentration of each bed (W1–W4) was 1.0 mg l^{-1} and the salinity of W1, W2 and W3 reached 10, 5 and 1.5 g l^{-1}, respectively (W4 was fed with the same strength of wastewater as the other reactors but without salinity). Then, 20 l water with atrazine concentration of 0.1 mg l^{-1} was added into each bed, respectively, to observe the effect of salinity on the microbial community in the batch. Eighteen days later (approximately half-life) 1 l gravels near to plant rhizospheres of each batch were collected, brushed and washed with sterilized water, respectively. The mixing liquid was collected and centrifuged at 5000 rpm for 5 min. The sediment was stored in a refrigerator at −70 °C for DNA extraction.

2.3. Analysis methods

2.3.1. HPLC procedures

Routine analysis of concentrations of atrazine and its metabolites in the liquid phase was performed by centrifugation, solid phase extraction, and HPLC analysis. Liquid samples (150 ml) were collected and centrifuged for 10 min at 6000 rpm. A 100 ml of the clear supernatant was passed through a 3-ml ENVI-18 SPE column (Supelo) preconditioned by eluting with 10 ml methanol followed by 10 ml of D.I. water, and then washed with 5 ml of D.I. water. Atrazine and its metabolites were eluted from the SPE column with 5 ml of methanol (Merck).

ProStar high-performance liquid chromatograph (VARIAN) was employed to analyze the sample. Concentrations of atrazine and its metabolites (DIA, DEA, HA, Dr Ehrenstorfer GmbH) were determined with a 250 mm × 4.6 mm × 5 mm C18 reverse-phase column (Shimazu, Japan). The injection volumes were 20 μl, the flow rate was 1 ml min^{-1}, the compounds were detected at 215 nm and the mobile phase was programmed to 5%–100% acetonitrile (B) gradient with D.I. water (A), as follows: 5%B (hold on for 2 min) $\overset{8\ min}{\rightarrow}$ 25%B $\overset{8\ min}{\rightarrow}$ 70%B $\overset{7\ min}{\rightarrow}$ 100%B (the figure above the arrow represents gradient program time).

Atrazine and its metabolites standard stock solution with a concentration of 1.0 g l^{-1} were prepared and diluted into 5.0, 4.0, 1.0, 0.5, 0.2 and 0.1 mg l^{-1}, respectively with methanol (LC). The analysis process was performed as mentioned above. Standard curves showed high correlations ($R^2 > 0.999$).

2.3.2. PCR amplification

DNA was extracted using 3s DNA isolation kit for Environmental Samples (Shanghai Shengnengbocai, China). The primers, F341GC and R518, (Shanghai Shenggong Biological Tech Co., Ltd.) located at V3 region of the 16S rRNA genes were utilized to assess bacterial community diversity. PCR amplification was performed with a P × 2 thermal cycler (Thermo Elemental, USA). PCR amplification program included: 94 °C, 10 min; 35 cycles consisting of 94 °C 45 s, 60 °C 45 s, 72 °C 45 s; 72 °C 10 min, finally.

2.3.3. DGGE analysis

The PCR products of the approximately 250 bp were analyzed by parallel DGGE. The denaturant defined as 100% at full strength was equal to 7 M urea plus 40% v v^{-1} formamide. An 8% (wt/vol) acrylamide gel with a vertical gradient of 35%–60% of the full strength denaturant was used to analyze the PCR products. Gels were run for 4–5 h at 150 V and 60 °C with the DcodeTM transgenation detection system (Bio-Rad Co. Ltd.). DNA was visualized after ethidium bromide staining (15 min) by UV transillumination and was photographed and analyzed with a Smart Viewer gel imaging system. All the samples were treated in the same way twice to testify the reproducibility of the result.

The above PCR and DGGE procedures were replicated three times.

2.3.4. Shannon–Wiener Diversity Index analysis

Shannon–Wiener Diversity Index (Shannon index in short) is an analysis method often used in biology/ecology. It measures the rarity and commonness of the species in a community. Here, Shannon index was employed to measure microbial community diversity in the designed constructed wetland.

3. Results and discussions

3.1. Atrazine half-life in SFCW

Under static experimental conditions, atrazine half-life in SFCW was approximately 16 days, as shown in Fig. 2. With initial concentration of 0.1 mg l^{-1}, atrazine decreased to 0.053, 0.054 and 0.056 mg l^{-1} in M1, M2 and M3, respectively after 15 days. The degradation kinetics followed first-order kinetics using the Model: exp2PMod1 procedure (OriginPro 7.5). The degradation equation: $y = 0.09679 \quad \exp(-0.0396t) \quad (C_0 = 0.09676 \quad (0.001457), k_0 = 0.0396 \ (0.002086) \ d^{-1}$ (mean values, standard error given in parentheses), $R^2 = 0.983$) was established using the procedure and the half-life 17.5, was calculated from the first-order degradation rate constant.

3.2. Salinity effect on the degradation of atrazine

The degradation kinetics of atrazine under different salinity followed first-order kinetics. With the increase of salinity, the efficiency of the degradation of atrazine dropped, presented as decreased degradation kinetic constants.

The relationship between salinity and $-\ln k$ appeared linearly: $-\ln k = 3.20378 + 0.04991c$ (c, NaCl concentration, mg l^{-1}), as shown in Fig. 3, which indicated that salinity inhibited atrazine degradation process in the constructed wetland. Studies by Nitisoravut and Klomjek, 2005 also reported that the effect of salinity on BOD removal appeared to approach exponential phase in the

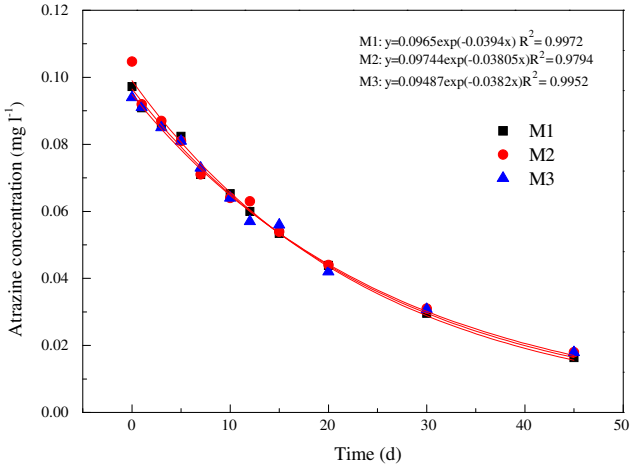

Fig. 2. Atrazine half-life in SFCW.

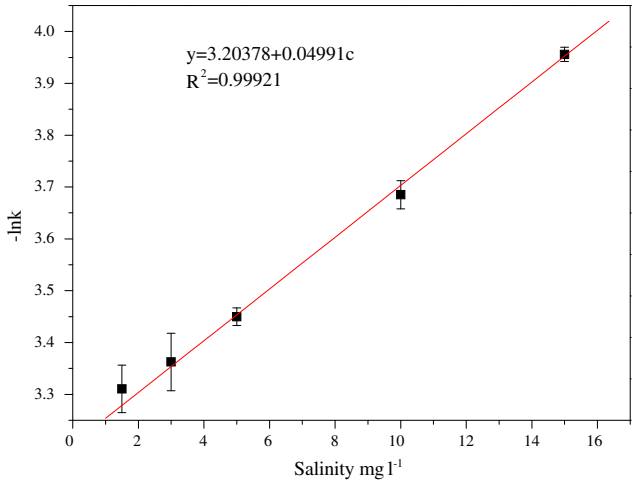

Fig. 3. The relationship between the salinity and $-\ln k$.

constructed wetland. The same restraining effect showed that salinity inhibited the metabolism of microorganisms in the wetland environment, which may be critical for the proper functioning and maintenance of the system.

3.3. Atrazine disappearance and its metabolites in SFCW under different salinity

According to the mechanism of atrazine disappearance, adsorption, hydrolysis and biodegradation processes were the three main processes in SFCW.

According to the analysis of the correlation between sorption coefficient *Kaf* and the physical and chemical properties of the soils, organic matter content of soils was the dominant factor in the adsorption. Hydrogen bonding was found to be the common mechanism in the absorption of atrazine (Yang et al., 2000). In our experiments, neither organic material nor hydrogen bonding existed in the gravel which belongs to inorganic substance. Therefore, sorption did not play any role during the atrazine degradation process. The literatures suggested that humic acid and clay mineral could catalyze the hydrolysis of atrazine to the dechlorinated 2-hydroxy compound. Especially in acid or strongly acidic soil, proton could catalyze the hydrolysis process (Prosen and Zupaneie-Kralj, 2005). Namely, the decrease of pH would promote atrazine hydrolysis (Liu, 2006). Consequently, atrazine hydrolysis hardly occurred in our experimental condition (neutral environment and little humic acid), which was further demonstrated by the experimental results (data not shown).

As mentioned above, biodegradation was the main process for the disappearance mechanism of atrazine in the designed SFCW. DIA and DEA were detected in chromatograms. This shows that atrazine removal from the system was due to biodegradation, which was supported by the occurrence of dealkylated intermediate product in the residues. Less than 5% of added herbicide accumulated as DEA and DIA (data not shown). No other primary

metabolite (hydroxy analogue) was observed in any of the samples of reactors W1, W2 and W3, which was partially attributed to the neutral soil where hydrolysis occurred unfavorably, and partly to the unfavorable environment in which OHA cannot be formed under microbial conditions. It was indicated that more atrazine must have degraded into other compounds. Meanwhile, with respect to glucose as external carbon in synthetic wastewater, reduction in observed atrazine concentration might be due to the occurrence of co-metabolism process compared with the case of readily biodegradable substrate that is not available. However, according to other researchers, the removal of atrazine by virtue of co-metabolism is limited (Chung et al., 1996; Ghosh and Philip, 2004).

3.4. Microbial community and distribution in SFCW under different salinity

3.4.1. PCR-DGGE results

Banding patterns for the 16S rRNA DGGE-PCR amplicons are presented in Fig. 4, where W1, W2, W3 and W4 had the salinity of 10, 5, 1.5 and 0 g l^{-1}, respectively. Total microbial communities of each sample were separated clearly in chosen gradient scope. Gels for other sampling times showed similar DGGE patterns (data not shown). Electrophoresis analysis was replicated twice under the same condition and the result had good reproducibility. Bands of each sample might differ in number, mobility and light intensity.

Each sample showed a variety of electrophoresis bands, which indicated that abundant microbial population existed in the SFCW system. The difference apparent in the number of the band of each sample resulted from microbial diversity. Special bands appeared in the DGGE profile, marked as 1, 2 and 3 with the salinity of 10 g l^{-1}. On the other hand, special bands also emerged under low salinity (1.5 and 0 g l^{-1}), marked as 4, 5, 6 and 7. Salinity impacted the growth of microorganism and induced a switch in microbial community structure. Some bacteria that predominated under low salinity had difficulties surviving in the high salinity environment where simultaneously halophilic microorganisms could be incubated. Common bands marked as 8 and 9 appeared in each sample, which could be attributed to some identical microbial communities occurring in these batches. It was considered that certain microbial community could survive in different salinity environments. However, the type of microbe which might be involved in the decontamination of atrazine is not known.

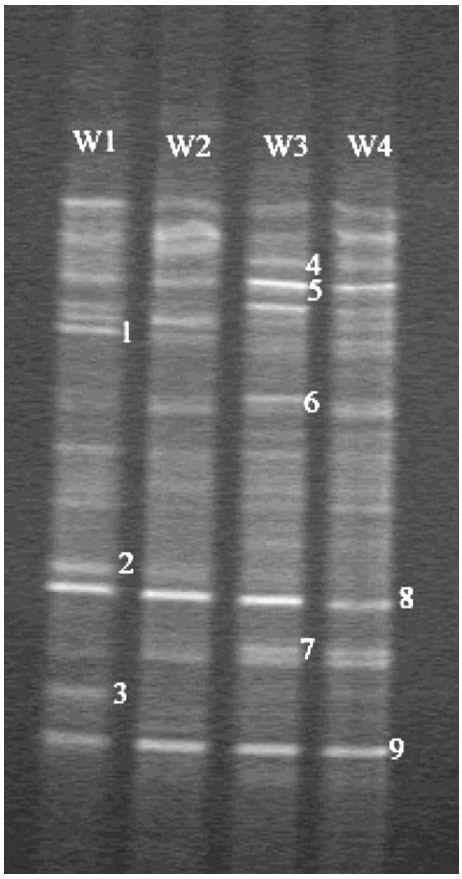

Fig. 4. DGGE bacterial profile in SFCW with atrazine under different salinity.

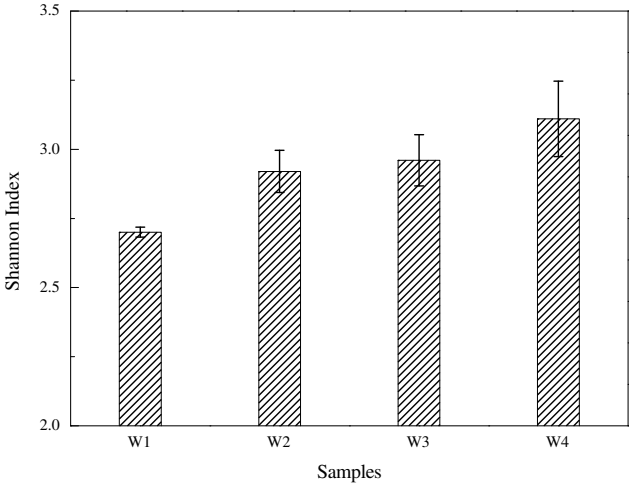

Fig. 5. Shannon Index of each sample in W1–W4.

3.4.2. Microbial diversity index and degradation kinetics

As shown in Fig. 5 Shannon–Wiener Index of each sample in W1–W4, which originated from ecological analysis and presents microbial diversity, was calculated and compared. The relationship between Shannon Index and salinity appeared to follow certain rules. Opposite to the increase of salinity, Shannon Index tended to decline from 3.1 at 0 g l^{-1} to 2.7 at 10 g l^{-1}. It indicated that salinity inhibited the growth of some bacteria directly leading to a decrease of the Shannon Index.

Atrazine degradation kinetics, along with Shannon Index, showed a degressive trend with increasing salinity. The relationship between Atrazine degradation kinetic constant k and Shannon Index indicated a linear relationship:

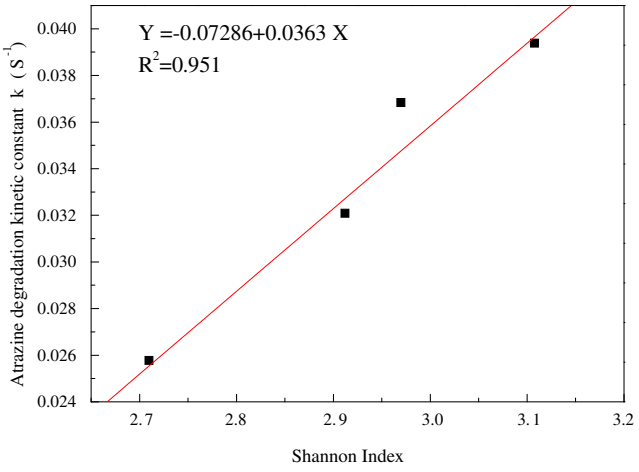

Fig. 6. Relationship between Shannon Index and degradation kinetic constant k.

$y = -0.07286 + 0.0363x$ ($R^2 = 0.951$), as shown in Fig. 6. The value of k increased with an increase in the microbial Shannon Index. It was postulated that atrazine degradation in SFCW was performed by the cooperation of different microbial communities. The increase of salinity depressed microorganism activity and therefore caused poor degradation efficiency.

4. Conclusion

1. In the static experiment (neutral pH and water temperature kept at 22–30 °C) atrazine half-life in SFCW was equal to 17.5 days. Degradation process indicated a reasonable first-order kinetic fit: $c = 0.09679\ \exp(-0.0396t)$ (c, atrazine residue concentration, mg l^{-1}; t, retention time, d).
2. The increase of salinity in SFCW could inhibit the degradation of atrazine. Degradation kinetic formula, which approached first-order kinetics, was found under different salinity. The relationship between salinity and degradation kinetic constant k was $\ln k = -3.20378 - 0.04991c$ (c, NaCl concentration, mg l^{-1}). It was considered that the inhibiting effect of salinity on atrazine degradation appeared to approach exponential phase.
3. The attenuation of atrazine in SFCW might not result from hydrolysis or sorption process. Biodegradation was the main process for the disappearance mechanism of atrazine in the designed SFCW. A small amount of metabolites was present as DIA and DEA. It was more likely that more atrazine degraded into other metabolites. Additionally, OHA was not detected during the experiment.
4. Salinity played an important role in the growth of microorganisms resulting in the switch of microbial population in the experiments. Microbial Shannon Index declined along with the increase of salinity. The relationship between atrazine degradation kinetic constant k and microbial Shannon Index under different salinity appeared to approach linear phase: $y = -0.07286 + 0.0363x$. The positive correlation between them indicated that microbial community played an important role in the atrazine degradation process.

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