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Reducing organic substances from anaerobic decomposition of hydrophytes

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Abstract Oxidation–reduction properties of surface sediments are tightly associated with the geochemistry of substances, and reducing organic substances (ROS) from hydrophytes residues may play an important role in these processes. In this study, composition, dynamics, and properties of ROS from anaerobic decomposition of *Eichhornia crassipes* (*Mart.*) *Solms*, *Potamogenton crispus Linn*, *Vallisneria natans* (*Lour.*) *Hara*, *Lemna trisulca Linn* and *Microcystis flos-aquae* (*Wittr*) *Kirch* were investigated using differential pulse voltammetry (DPV). The type of hydrophytes determined both the reducibility and composition of ROS. At the peak time of ROS production, the anaerobic decomposition of *M. flos-aquae* produced 6 types of ROS, among which 3 belonged to strongly reducing organic substance (SROS), whereas there were only 3–4 types of ROS from the other hydrophytes, 2 of them exhibiting strong reducibility. The order of potential of hydrophytes to produce ROS was estimated to be: *M. flos-aquae* > *E. crassipes* > *L. trisulca* > *P. crispus* ≈ *V. natans*, based on the summation of SROS and weakly reducing organic substances (WROS). The dynamic pattern of SROS production was greatly different from WROS. The total SROS appeared periodic fluctuation with reducibility gradually weakening with incubation time, whereas the total WROS increased with incubation time. Reducibility of ROS from hydrophytes was readily affected by acid, base and ligands, suggesting that their properties were related to these aspects. In addition to the reducibility, we believe that more attention should be paid to the other behaviors of ROS in surface sediments.

Keywords Anaerobic decomposition · Anaerobic microbe · Hydrophytes · Reducing organic substance · Voltammetry

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Introduction

Hydrophytes play an important role in aquatic ecosystems. They are the primary producers of energy substances and accumulators of nutrients in oligotrophic freshwater ecosystems. In addition, vegetation of hydrophytes is a refuge of many animal species from predation. In eutrophicated freshwater ecosystems, some of hydrophytes serve as a "scavenger" of nutrients and contaminants (Obarska and

Ozimek 2003). However, if mismanaged, hydrophytes can inversely aggravate the aquatic environment through residue decay (Kroger et al. 2007; Peng et al. 2007). Anaerobic decomposition of hydrophytes residues in surface sediments may alter the physicochemical and biological properties of sediments, resulting in delay in ecosystem restoration progress (Carpenter 2003). Therefore, the knowledge of anaerobic decomposition of hydrophytes is necessary for effective management practice of hydrophytes.

The decomposition of hydrophytes is commonly promoted by anaerobic microbes in surface sediments, followed by production of organic substances containing reducing organic substances (ROS). These ROS can directly consume oxygen transferred from upper water column (Dittmar and Lara 2001; Li et al. 2003a; Kim and Pfaender 2005), and reduce components such as manganese oxides and ferric oxides (Lovley 1995). As a result, the bottom water hypoxia of a lake is aggravated, adsorption capacity of sediments is declined, and the release of nutrients from sediments to water is accelerated (Hem 1978; Correll 1999). Therefore, the composition and properties of ROS are crucial to these processes, and it is necessary to characterize ROS from anaerobic decomposition of hydrophytes.

The production of ROS as metabolites and matrix of anaerobic microbes depends on composition of organic materials. Anaerobic microbes commonly prefer substrates similar to aerobic microbes with a low ratio of carbon to nitrogen (Carpenter and Adams 1979; Carpenter 1980; Gilbert 1998; Holmboe et al. 2001; Hume et al. 2002; de Haas et al. 2002; Bastviken et al. 2007; Esslemont et al. 2007). Some of carbohydrates, hydrocarbons, organic sulfurs and unsaturated ester etc. are either reducing substances or preferential substrates for anaerobic microbes (Allen 1994; Van Hamme et al. 2003), and the potential of organic materials to produce ROS are like related to the composition of these substances. Some of anaerobic microbes gain energy for the vital movement by transferring electrons in organic materials to organic electron acceptors such as quinone and its analogs etc. (Scott et al. 1998). These organic electron acceptors in organic materials are also responsible for production of ROS as they are decomposed (Lovley et al. 1998; Kappler et al. 2004). However, these results have been mainly obtained with terrestrial organic materials. It is possible that ROS from hydrophytes can be greatly different from those from terrestrial plants due to the difference of their components and structure.

The composition and properties of ROS from hydrophytes are important parameters to evaluate their environmental impacts. Reducibility of ROS can be described by their redox potentials (Ding 1996; Liu et al. 1997), which involves the standard potential and activity of ROS. Acid–base reaction is generally coupled with transfer of electrons, and the response of ROS to acid or base is also an important factor to govern the extent of redox reaction (Contreras et al. 2007; Servais et al. 2007). Some ROS are natural ligands, and their complexation behavior probably affects their own reducibility (Li et al. 2008). Anaerobic decomposition of hydrophytes produces a mixture of ROS with different reducibility with various amount (Yu and Zhang 1984). It is difficult to characterize the reducibility of a single substance using a mixed potential. Because ROS are considerably labile, but not necessarily soluble, they cannot easily quantified by traditional methods such as chemical oxygen demand (COD), biological oxygen demand (BOC), dissolved organic carbon (DOC), and even total carbon (TC). Therefore, knowledge on the total of ROS from hydrophytes is very limited.

Voltammetry has been introduced to soil and aquatic science for multi-element determination and species analysis (Yu 1985; Yu and Ji 1993; Kissinger and Heineman 1996). The advantage of this method over other methods is that a substance can be characterized qualitatively and quantitatively even when their stoichiometry is unknown in a complex system. This study is to investigate the characteristics of ROS from anaerobic decomposition of hydrophytes using differential pulse voltammetry (DPV), and to compare the difference in reducibility of ROS from various hydrophytes.

Materials and methods

Preparation of organic materials

Hydrophyte species *Eichhornia crassipes* (*Mart.*) *Solms*, *Potamogenton crispus Linn*, *Vallisneria natans* (*Lour.*) *Hara*, *Lemna trisulca Linn* and

Microcystis flos-aquae (*Wittr*) *Kirch* were chosen in this experiment. These hydrophytes were gathered from the Dianchi Lake and the Yuehu Lake in China at senescence stage. *P. crispus* and *L. triculca* were collected in May, 2005, *E. crassipes* and *M. flos-aquae* were sampled in October, 2005, and *V. natans* was in November, 2005. To simplify the research, the hydrophytes samples were rinsed with distilled water to remove dust or mineral attachment. After the free water was separated, the samples were rapidly dried at 30°C in vacuum, and ground into powdery forms. The organic powder was kept at 4°C until experiment was conducted.

Preparation of mixed anaerobic microbes

A fresh surface sediment sample of 20.0 g (sampled from Dianchi Lake) was transferred into a brown bottle, mixed in the dark after addition of incubation solution, and then incubated at 25 ± 1°C for 30 day under free from oxygen. The supernatant served as a source of mixed anaerobic microbes.

Anaerobic incubation of organic materials

The powdery organic material (10.0 g) was placed into a 1,200 ml incubation vessel with double-distilled water to form a suspension. Prior to inoculation, the suspension was deaerated for 1 h with oxygen-free nitrogen and a vacuum pump. After adding 100.0 ml supernatant containing mixed anaerobic microbes (obtained as described above), the suspension of organic materials was anaerobically incubated in the dark at 25 ± 1°C. The changes in pH and DOC of the anaerobic solution were recorded (Table 1).

Instruments

A pHS-3C meter (Xinkong Medical Apparatus Co., Ltd., Jiangyan, China) was used to adjust pH of incubation solutions. A PAR-174 polarograph (Princeton Applied Research) equipped with an X–Y recorder (2000-Recorder, Houston) was employed for the determination of ROS with differential pulse mode. A glass carbon electrode, a platinum-wire, and a Ag/AgCl electrode were used in the three-electrode electrochemical cell as working electrode, counter electrode, and reference, respectively.

To prevent possible oxidation of produced ROS during sampling, a specific sampler was designed (Fig. 1). The operation procedures were as following: (1) prior to sampling, the measure vessel and electrochemical cell were flushed with pure nitrogen to replace oxygen, while the valve connected to the enclosed electrochemical cell remained closed (valve 4); (2) the valves connected to culture chamber (valve 1, 2 and 3) was opened, and the peristaltic pump was turned on; (3) as about 50 ml of anaerobic solution was obtained, the valve 2 was turn off; (4) then valve 4 was opened and rotation direction of peristaltic pump was changed. Finally, a given volume of anaerobic solution in the culture chamber was transferred into an electrochemical cell. Because each culture chamber was equipped with a sampler, the oxidation of ROS was avoided during the interval sampling.

Analysis of dissolved organic carbon

In the present work, the total of dissolved organic carbon (DOC) was measured with the procedure similar to COD determination (Qi et al. 2002); i.e.,

Table 1 pH and DOC dynamics of anaerobic decomposition solutions

Time (day)	pH					DOC μeq g^{-1} DW				
	L	E	V	P	M	L	E	V	P	M
0	7.04	7.34	6.22	7.87	6.51	30.3	16.8	51.8	44.3	41.6
4	5.84	5.95	5.17	6.27	6.27	44.9	30.3	51.8	66.8	72.2
8	5.94	5.53	4.94	5.74	6.47	62.1	42.6	83.5	87.3	88.5
10	5.89	5.52	4.93	5.50	6.57	61.7	46.5	89.4	90.1	88.9

L, E, V, P and M represent *L. trisulca*, *E. crassipes*, *V. natans*, *P. crispus* and *M. flos-aquae*; DOC denotes dissolved organic carbon; DOC is mean of triplicate

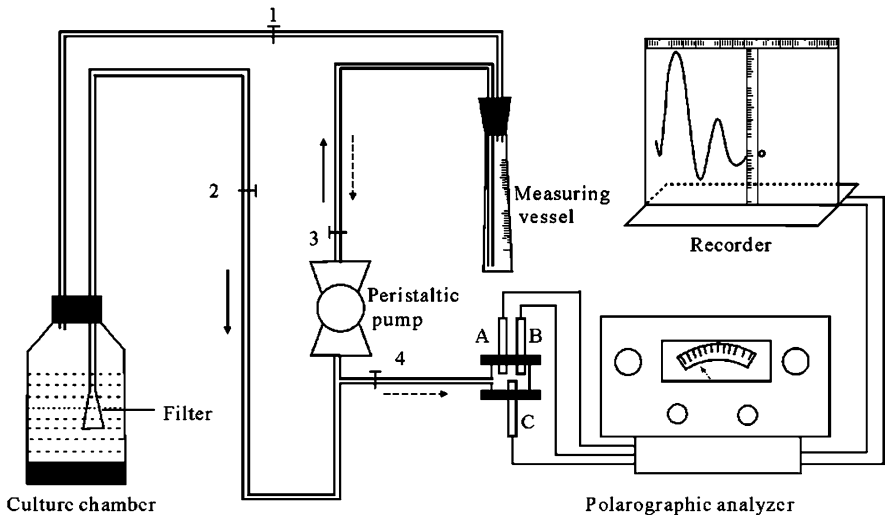

Fig. 1 Schematic diagram of a system designed for sampling and determination of organic reducing substances. *A*, *B* and *C* denote glass carbon, reference and Ag/AgCl electrodes, respectively

the filtrate of anaerobic incubation solution containing DOC was firstly oxidized with acidic potassium dichromate at a high temperature, and the amount of DOC was determined by the amount of potassium dichromate consumed. Although this method might include constitutes such as ferrous and ammonium in DOC, their contribution to DOC was neglected due to the low content in plant bodies. In the analysis, triplicate was used.

Voltammograms for organic reducing substances

At a certain incubation interval, the anaerobic incubation solution of 20 ml was transferred to electrochemical cell with 5.0 ml of 1.0 M ammonium acetate served as indifferent electrolyte. The operating conditions to obtain voltammograms included scan speed of 2 mV s^{-1}, pulse voltage of 50 mV, and current range of 0.05–0.2 mA. During the sampling and determination, the upper space of the electrochemical cell was flushed with pure nitrogen to prevent ROS from oxidation.

Because ROS can function as ligands, 2 ml of 0.5 M ethylenediamine tetraacetic acid (EDTA) was added as chelating agent to dissociate the complexes containing ROS. Considering the acid–base property of ROS, the indifferent electrolyte was altered to either 2.0 ml of 1.0 M sodium hydroxide or 2.0 ml of 5.0 M sulphuric acid.

Standard calibrated curve

To quantify the unknown reducing substances, Fe(II) was used as a standard substance, and determined by DPV. The stock solution of Fe(II) was prepared through dissolving ferrous ammonium sulphate in distilled water with small amount of ascorbic acid to prevent ferrous ion from oxidizing. The standard calibrated curve of Fe(II) was achieved by the series of diluted Fe(II) stock solution and corresponding peak current of ferrous ion on valtommograms. To increase the sensitivity of Fe(II) determination, 2, 2'-dipyridine was added.

Theory of voltammetry

Voltammetry is a tool commonly used to determine reduction and oxidation (redox) properties of substances (Bard and Faulkner 1980). When a reducing substance is oxidized on a solid polarized electrode, it can be described as following:

$$\mathrm{R} - ne \leftrightarrows \mathrm{O} \tag{1}$$

where R denotes reducing state of a substance, and O represents its oxidizing state. The current produced can be calculated as:

$$i = nFAm_0(C_0^* - C_0) \tag{2}$$

in which n, F, A and m_0 denote the number of electrons that reducing substance loses, Faraday

constant, electrode surface area, and transferring constant, respectively. C_0^* stands for the concentration of reducing substance in bulk solution, and C_0 represents the concentration of reducing substances on electrode surface. As scan voltage increases, the reducing substance on electrode surface is oxidized. As a result, the concentration of reducing substance decreases. When the concentration approaches zero, the current through electrode surface reaches the maximum.

$$i_l = nFAm_0C_0^* \tag{3}$$

where i_l is referred as limitation current, which depends on the concentration of reducing substance in bulk solution. From Eq. 3, the concentration of reducing substance can be determined.

The peak potential of reducing substance can be deduced. On the basis of concentrations of both reducing and oxidizing states of a substance on electrode surface, the potential controlled by the substance is given by Nernst equation:

$$E = E_o^* + \frac{RT}{nF} \ln \frac{[O]}{[R]} \tag{4}$$

where [O] and [R] represent the concentrations of substance in oxidizing and reducing states. The meanings of n and F are same as Eq. 2. R and T denote the thermodynamic constant and temperature in K.

When the concentrations of both oxidizing and reducing states of the substance in Eq. 4 are substituted by their currents, the Eq. 4 is changed to:

$$E = E_o^* + \frac{RT}{nF} \ln \frac{m_R}{m_o} + \frac{RT}{nF} \ln \frac{(i_i - i)}{i} \tag{5}$$

where i is instant current through the electrode surface.

When $i = i_l/2$, the Eq. 5 is changed as following:

$$E = E_o^* + \frac{RT}{nF} \ln \frac{m_R}{m_o}. \tag{6}$$

This indicates that the potential of reducing substance on electrode surface is a constant. The potential is referred as peak potential of a substance. Based on Eq. 5, peak potential is a characteristic value of reducing substance, which depends on standard potential of a substance and transferring constants of both its oxidizing and reducing states. According to peak potential, the reducibility of various reducing substances can be obtained.

Results

Reducing organic substances from anaerobic decomposition of hydrophytes

To compare the differences of several hydrophytes in producing ROS, we chose incubation time between 5 and 7 days when the types of ROS and their amount was the most. Based on the number and potential of current peaks on the voltammogram, the types of ROS and their reducibility appeared to be dependent on the types of hydrophytes (Fig. 2). The solution of *M. flos-aquae* incubated for 5 day showed six ROS peak potentials, and they were −0.32, 0.00, 0.17, 0.47, 0.57 and 0.82 V (marked as E_1, E_2, E_3, E_4, E_5 and E_6 on a voltammgram). These unidentified

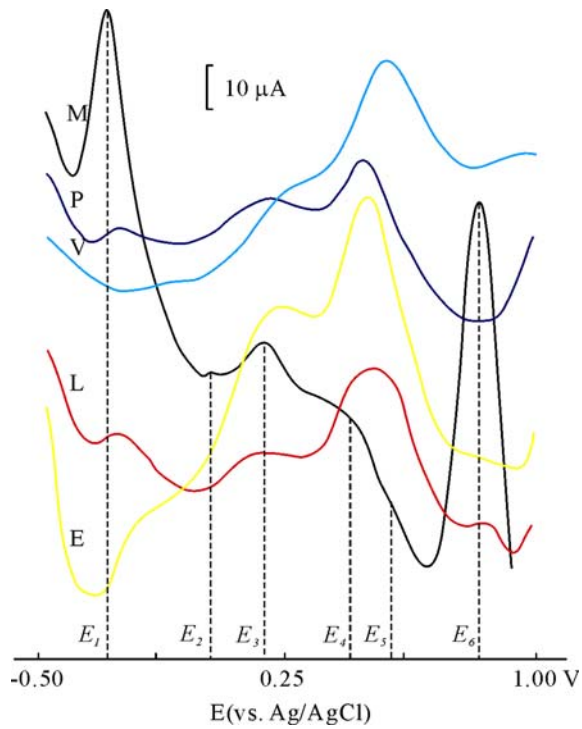

Fig. 2 Voltammograms for anaerobic incubation solution of hydrophytes. *M*, *P*, *V*, *L* amd *E* represent *M. flos-aquae*, *P. crispus*, *V. natans*, *L. trisulca* and *E. crassipes*, respectively. *V. natans* and *E. crassipes* were incubated for 7 day and the others were for 5 day

reducing substances were named as $S_{-0.32}$, $S_{0.00}$, $S_{0.17}$, $S_{0.47}$, $S_{0.57}$, and $S_{0.82}$, respectively. Because peak potential of 0.35 V (vs. Ag/AgCl saturated electrode) is believed to distinguish strongly reducing substances from weakly reducing substances (Ding and Wang 1993), $S_{-0.32}$, $S_{0.00}$, and $S_{0.17}$ belonged to strongly reducing organic substances (SROS), and the others were weakly reducing organic substances (WROS). When *V. natans* was decomposed for 7 day, $S_{-0.18}$, $S_{0.17}$ and $S_{0.53}$ were detected, in which $S_{-0.18}$ and $S_{0.17}$ were SROS. For all other hydrophytes, 3–4 types of ROS were produced, but 5 peak potentials were observed.

Interestingly, the same ROS were observed in anaerobic solutions of different hydrophytes. The results seemed considerably different from those obtained with terrestrial plants (Li et al. 2003b; Wu 1989). $S_{0.17}$ and $S_{0.47}$ could be originated from almost all the chosen hydrophytes, except for *V. natans*, in which $S_{0.47}$ was missing. However, $S_{-0.28}$ appeared only from *L. trisulca* and *P. crispus*. Because the kind and population of anaerobic microbes in the initial inoculation and the incubation conditions were identical, the occurrence of the same reducing substances was probably attributed to similar substrates in hydrophytes tissues or metabolites of anaerobic microbes.

The difference of ROS in composition can be reflected on the amount of various ROS. Being in direct proportion to peak current in DPV, the concentrations of ROS can be estimated. Based on the voltammgram (Fig. 2), the peak current values are given in Table 2. It was found that the peak currents of various ROS were greatly different even for the same ROS. For example, while *V. natans* was decomposed for 5 day, the peak current of $S_{0.53}$ was 25.0 μA, the highest among three types of ROS, indicating the highest concentration. The second highest was 3.0 μA of $S_{0.17}$. The concentration of $S_{-0.18}$ was negligible due to its low peak current. However, as for *M. flos-aquae*, the highest peak current was 84.2 μA of $S_{0.82}$, and the second highest was 51.0 μA of $S_{-0.32}$, followed by $S_{0.17} > S_{0.47} > S_{0.00} > S_{0.57}$. For other hydrophytes, the composition difference in the amount of ROS was also observed. It can be concluded that the types of hydrophytes were responsible for not only the types and reducibility of ROS, but also the amount of these substances.

To further compare the characteristics of ROS, the sums of SROS and WROS were calculated (Table 2). For *M. flos-aquae*, the total peak currents of SROS and WROS were 61.4 and 90.1 μA. In another word, each dry weight (DW) of *M. flos-aquae* could produce 24.1 μeq of SROS and 35.3 μeq of WROS. For *L. trisulca*, the estimated amounts of SROS and WROS were 3.6 and 11.7 μeq g^{-1} DW. Similarly, the estimated amounts of SROS for *P. crispus*, *E. crassipes* and *V. natans* were 3.4, 7.0 and 1.1 μeq g^{-1} DW, and their WROS were 7.4, 15.1 and 9.8 μeq g^{-1} DW. Considering the total potential of different hydrophytes in producing ROS (the summation of total SROS and WROS), the order was *M. flos-aquae* > *E. crassipes* > *L. trisulca* > *P. crispus* ≈ *V. natans*.

Dynamics of reducing organic substances

The dynamics of ROS from anaerobic decomposition of hydrophytes was also investigated using *M. flos-aquae* as an example. Our results showed that the types, reducibility and amount of various ROS changed with incubation time (Table 3). At the 1st day, both $S_{0.17}$ and $S_{1.00}$ were produced. However, they vanished at the 3rd day, accompanied with the occurrence of $S_{0.47}$. The results also concluded that these substances were organic. At the 5th day, the types of ROS were increased to 6. The production of these ROS must be a result of anaerobic microbe activities, because both $S_{-0.32}$ and $S_{0.00}$ exhibited extremely strong reducibility and could be readily oxidized in the atmosphere. With incubation time increasing, the types of reducing substances decreased. At the 7th and 9th day, the types of reducing substances were 4, and they further decreased to 3 at the 17th day.

The change of SROS summation appeared periodically with incubation time. At the 1st day, the total SROS was 5.5 μeq g^{-1} DW, but it diminished to a negligible extent at the 3rd day. However, at the 5th day, it was the highest at 24.1 μeq g^{-1} DW, whereas at the 7th day, it decreased to 1.0 μeq g^{-1} DW. The fluctuation of SROS amount could also be observed at the 9th day and 17th day.

The total WROS seemed to be related to incubation time. The amount of WROS gradually increased with incubation time, except for an abrupt peak time of ROS production. For example, at the 1st day, the

Table 2 Composition and reducibility of ROS from anaerobic decomposition of several hydrophytes

Hydrophytes	ROS						SROS			WROS			Total		
							No.	T_i	Con	No.	T_i	Con	No.	T_i	Con
V. natans	$S^a_{-0.18}$	$S_{0.17}$	$S_{0.53}$				2[c]		1.1[e]	1		9.8	3		10.9
	tr	3.0[b]	25.0					3.0[d]			25.0			28.0	
M. flos-aquae	$S_{-0.32}$	$S_{0.00}$	$S_{0.17}$	$S_{0.47}$	$S_{0.57}$	$S_{0.82}$	3		24.1	3		35.3	6		59.4
	51.0	1.1	9.3	5.9	tr	84.2		61.4			90.1			151.5	
P. crispus	$S_{-0.28}$	$S_{0.17}$	$S_{0.47}$				2		3.4	1		7.4	3		10.8
	3.3	5.4	19.0					8.7			19.0			27.7	
L. trisulca	$S_{-0.28}$	$S_{0.17}$	$S_{0.47}$	$S_{0.84}$			2		3.6	2		11.7	4		15.4
	4.7	4.7	26.5	3.4				9.4			29.9			39.3	
E. crassipes	$S_{-0.19}$	$S_{0.17}$	$S_{0.47}$				2		7.0	1		15.1	3		22.1
	5.4	12.6	38.5					18.0			38.5			56.5	

[a] Name of reducing substance, reducibility, of which is labeled with peak potential (V) at subscript

[b] Peak current of reducing substances (μA)

[c] Types of ROS

[d] Total current (μA)

[e] Content of ROS (μeq g^{-1} DW); tr means trance; ROS, SROS and WROS denote reducing organic substances, strongly organic reducing substances, and weakly organic reducing substances, respectively

Table 3 Dynamics of ROS from anaerobic decomposition of *M. flos-aquae*

Time/day	ROS (μeq g^{-1} DW)								T_{SROS}	T_{WROS}	Total
	$S_{-0.32}$	$S_{0.00}$	$S_{0.17}$	$S_{0.31}$	$S_{0.47}$	$S_{0.57}$	$S_{0.82}$	$S_{1.00}$			
1	n	n	5.5	n	n	n	n	6.8	5.5	6.8	12.3
3	n	n	tr	n	11.4	n	n	n	tr	11.4	11.4
5	20.3	tr	3.8	n	2.8	tr	28.7	n	24.1	35.3	59.4
7	tr	n	1.0	n	2.6	n	15.2	n	1.0	17.8	18.9
9	tr	n	7.9	9.40	n	n	27.9	n	17.4	27.9	45.4
17	n	n	2.0	n	2.9	n	39.5	n	2.0	42.5	44.5

tr means trace; n represents no present; t naerobic decompositionganic carbon; T_{SROS} and T_{WROS} denote the total content of strongly organic reducing substances and weakly reducing substances, respectively

total WROS was mainly contributed by $S_{1.00}$, and its amount was 6.8 μeq g^{-1} DW. However, at the 3rd, 7th, 9th and 17th day, the total WROS were 11.4, 17.8, 27.9 and 42.5 μeq g^{-1} DW, respectively. The production of WROS might be dependent on potential substrates. At the initial stage of decomposition, the release of a large amount of soluble and labile organic carbon might have stimulated anaerobic microbes (Table 1). Correspondingly, the amount of WROS increased rapidly. With the consumption of labile organic carbon and a part of labile WROS, the production of WROS merely depended on the stable organic carbon.

Properties of ROS from anaerobic decomposition of hydrophytes

To understand the effect of acid–base and complexation on both species and reducibility of ROS, we further studied ROS from *M. flos-aquae* and *P. crispus* incubated for 5 day (Fig. 3). The results showed that the addition of EDTA changed the peak potential of $S_{-0.32}$, suggesting that the original substance was reducing compound with complexng ability through its ligand (curves 1 and 3 in Fig. 3a). The addition of base shifted the peak potential of original substance to −0.40 V, and the peak current

significantly decreased, whereas the addition of acid caused the current peak to disappear (as described by curves 2 and 4 in Fig. 3a). These results indicated that this reducing substance was extremely vulnerable, attested by its disappearance at the 7th day. The current peaks of $S_{0.00}$ and $S_{0.17}$ vanished under EDTA addition, indicating that both compounds were complexes, and that complexation had enhanced their solubility. In either acidic or basic conditions, these peak potentials remained constant, but peak currents were greatly affected, demonstrating that their solubility was pH-dependant. The peak of $S_{0.47}$ could be alkali because of its increase in peak current in acidic condition and disappearance in basic condition. The increase of peak current induced by EDTA addition suggested the restriction of central ions to solubility. The weakly reducing substance of $S_{0.82}$ seemed a complex containing reducing group, since EDTA shifted its peak potential to 0.88 V. Reducibility of this substance could be intensified under both acidic and basic conditions as peak potential was shifted to 0.66 V (showed in curves 2 and 4 Fig. 3a). The effect of various electrolytes on $S_{0.57}$ was insignificant due to their lower concentrations in the anaerobic solution.

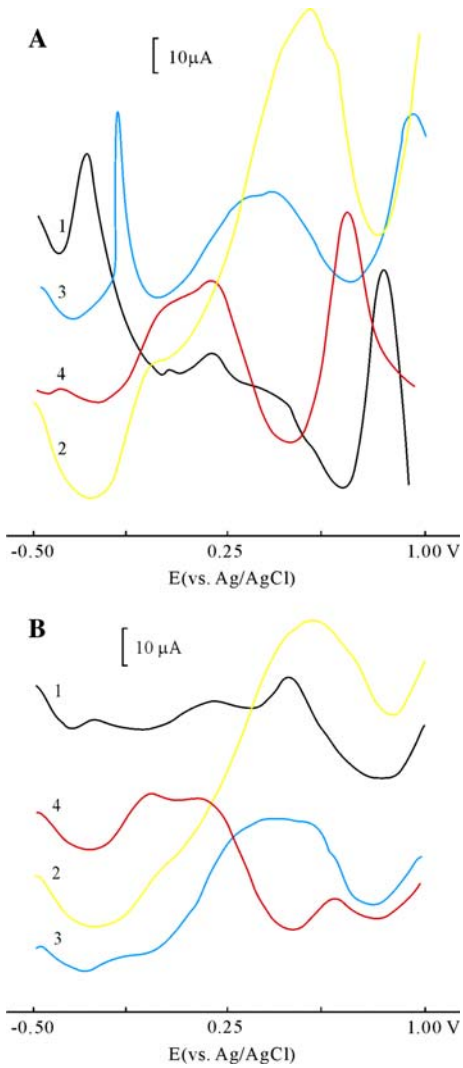

Fig. 3 Voltammograms for ROS in different electrolytes. **a** and **b** denote *M. flos-aquae* and *P. crispus*. *Curves 1*, *2*, *3* and *4* were obtained in original solution, acid-added, EDTA-added and base-added solutions, respectively

The voltammogram of anaerobic solution of *P. crispus* in different electrolytes indicated that $S_{-0.28}$ was a complex (curves 1 and 3 in Fig. 3b). EDTA shifted its peak potential from −0.28 to −0.20 V, inferring that the reducing group might participate in the formation of complexes. The reducing group was sensitive to both acid and base according to the changes in peak potential and current. Base converted peak potential to −0.07 V with increased peak current. For both $S_{0.17}$ and $S_{0.47}$, their properties observed were similar to those obtained with the anaerobic incubation solution of *M. flos-aquae*, indicating that they were probably the same substance. A new current peak occurred on the voltammograms as base and EDTA were used as indifferent electrolyte, but this peak did not exist in acidic solution, demonstrating the reducing substance had weak acidity.

To compare the curves 2 in Fig. 3a, b, a similar complicated current peak occurred at 0.52 V when strong acid served as indifferent electrolyte. This might be due to the weak acidity of some ROS. Strong acid could promote the formation of reducing substance molecules. As these ROS molecules participated in electrode reaction, the transferring mass process was changed, and excess potentials were required (Bard and Faulkner 1980). Therefore, several current peaks were overlapped, forming the complicated pattern.

Discussion

Anaerobic decomposition of hydrophytes was coupled with production of ROS. According to their

reducibility, ROS were classified into SROS and WROS. Most of SROS occurred at the initial stage of the decomposition as their reducibility declined with incubation time, whereas WROS existed during the whole decomposition process. We also found that the amount of SROS was greatly lower than that of WROS, and, to a great extend, the type of hydrophytes was responsible for the types, amount and reducibility of ROS. The potential of hydrophytes to produce ROS was single-cell aquatic plant > phytoplankton > submerged plant. Most ROS hardly existed in their free states, and were readily affected by acid, base, and chelating agents.

Advantage of voltammetry over other techniques in distinguishing SROS

The SROS from hydrophytes exhibited multiplicity and extremely strong reducibility. Their peak potentials were usually less than 0.17 V, and the total six types were observed. These findings should be attributed to the introduction of voltammetry. SROS were readily oxidized by oxidants or degraded by anaerobic microbes. If these substances were investigated through common separation techniques, the protection of those compounds from oxidation would be a challenge. Because the composition of soluble organic carbon from the decomposition of hydrophytes is greatly complex, and SROS merely account for a small parts of them (as given in Table 1, 2), direct separation techniques might result in extremely low separation efficiency. Another disadvantage of direct separation techniques is the change of the species of ROS during the processes. As shown in Fig. 3, most of ROS appeared in a combination with other substances rather than in their free states. The change of medium environments in separation processes might further reduce their stability, resulting in a significant change in characteristics of those compounds. Because of limited knowledge of the stoichiometry and properties of SROS currently, the use of voltammetry shall be preferred for the investigation of SROS. It overcomes some disadvantages of direct separation techniques by in situ and on-line monitoring, and effectively protects SROS from oxidation. Of course, the significant disadvantage of voltammetry is that it cannot definitely identify the stoichiometry of SROS.

Geochemical significance of ROS

One of typical characteristics of sediments is anoxic because of impedance of oxygen supply from atmosphere. As residues of hydrophytes deposit on the surface sediments, especially after bloom of blue algal in an eutrophicated lake, the oxidation–reduction potential of sediments decreases abruptly, followed by the enhanced geochemistry cycle of some of substances such as phosphate, nitrogen and iron etc. on the sediments water interface. Thus, the role of ROS in these processes shall be of geochemical significance. As shown in Table 1 and 3, the total ROS from *M. flos-aquae* almost accounts for 15.8–82.2% of DOC of anaerobic decomposition solution. SROS among ROS can rapidly reduce electron acceptors to decrease redox potential of sediments (Carpenter 1980). Consequently, adsorption capacity of sediments is decreased, and release rates of nutrients and contaminants to water column are accelerated. A typical example has been found that the concentration of phosphate in overlaying water column was elevated after algal bloom (Andersen and Jensen 1991). It has been reported that the reduction of ferric and manganese oxides is generally inhibited by accumulation of produced Fe(II) and Mn(II) on the surface of oxides (Royer et al. 2002). However, complexation of ROS can improve this situation, because the complexation can facilitate the departure of Fe(II) and Mn(II) from the surface of oxides (Fig. 3). As a result, the adsorption capacity of sediments is further decreased. Another function of ROS to enhance the geochemistry cycle of substances in sediments is the competition for adsorption sites on the surface of sediment particles. For example, anaerobic sediments generally exhibit strong ability to sorb phosphate than aerobic sediments, and yet the concentration of phosphate interstitial water was also generally higher (Patrick and Hhalid 1974). Interpretation to the results is possibly attributed to the presence of ROS (Guppy et al. 2005), which may promote phosphate desorption from the surface of sediment particles because of their high affinity.

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