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# Efficient treatment of azo dye containing wastewater in a hybrid acidogenic bioreactor stimulated by biocatalyzed electrolysis

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## ARTICLE INFO

### Article history:

Received 8 August 2015

Revised 26 September 2015

Accepted 11 October 2015

Available online 30 December 2015

### Keywords:

HAB (hybrid acidogenic bioreactor)

Scale-up

Azo dye

Domestic wastewater

Cyclic activated sludge system (CASS)

## ABSTRACT

In this study, a novel scaled-up hybrid acidogenic bioreactor (HAB) was designed and adopted to evaluate the performance of azo dye (acid red G, ARG) containing wastewater treatment. Principally, HAB is an acidogenic bioreactor coupled with a biocatalyzed electrolysis module. The effects of hydraulic retention time (HRT) and ARG loading rate on the performance of HAB were investigated. In addition, the influent was switched from synthetic wastewater to domestic wastewater to examine the key parameters for the application of HAB. The results showed that the introduction of the biocatalyzed electrolysis module could enhance anoxic decolorization and COD (chemical oxygen demand) removal. The combined process of HAB-CASS presented superior performance compared to a control system without biocatalyzed electrolysis (AB-CASS). When the influent was switched to domestic wastewater, with an environment having more balanced nutrients and diverse organic matters, the ARG, COD and nitrogen removal efficiencies of HAB-CASS were further improved, reaching  $73.3\% \pm 2.5\%$ ,  $86.2\% \pm 3.8\%$  and  $93.5\% \pm 1.6\%$  at HRT of 6 hr, respectively, which were much higher than those of AB-CASS ( $61.1\% \pm 4.7\%$ ,  $75.4\% \pm 5.0\%$  and  $82.1\% \pm 2.1\%$ , respectively). Moreover, larger TCV/TV (total cathode volume/total volume) for HAB led to higher current and ARG removal. The ARG removal efficiency and current at TCV/TV of 0.15 were  $39.2\% \pm 3.7\%$  and  $28.30 \pm 1.48$  mA, respectively. They were significantly increased to  $62.1\% \pm 2.0\%$  and  $34.55 \pm 0.83$  mA at TCV/TV of 0.25. These results show that HAB system could be used to effectively treat real wastewater.

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## Introduction

Recently, stricter standards for wastewater treatment and the continuous rise in energy prices have adversely affected the

balance sheet of wastewater treatment facilities and threatened the economic feasibility of wastewater treatment plants (Escapa et al. 2015). Therefore, a novel-biocatalyzed electrolysis system (BES) has emerged to solve these problems. In the

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## Nomenclature

AB	acidogenic bioreactor
HAB	hybrid acidogenic bioreactor
BES	biocatalyzed electrolysis system
CASS	cyclic activated sludge system
ARG	acid red G
HRT	hydraulic retention time
ARG-LR	acid red G loading rate
OLR	organic loading rate
COD	chemical oxygen demand
TCV	total cathode volume
TV	total volume
UASB	up-flow anaerobic sludge blanket
mWWTP	mixed Wastewater Treatment Plant
R1	HAB
R2	AB
R3	CASS followed by HAB
R4	CASS followed by AB
MLSS	mixed liquor suspended solids
MLVSS	mixed liquor volatile suspended solids
NH <sub>3</sub> -N	ammonia nitrogen
NO <sub>3</sub> -N	nitrate nitrogen
NO <sub>2</sub> -N	nitrite nitrogen
RE	removal efficiency

past decade, the biocatalyzed electrolysis system has been drawing wide interest due to its remarkable potential for many applications in wastewater treatment (Cui et al. 2014; Korneel Rabaey et al. 2010; Mu et al. 2009). Many proof-of-concept systems have been successfully tested, and achieved superior performance in treating wastewater containing recalcitrant contaminants, such as azo dye (Mu et al. 2009), chloramphenicol (Sun et al. 2013; Liang et al. 2013), p-fluoronitrobenzene (Feng et al. 2014), p-nitrophenol (Tao et al. 2013), 4-chloronitrobenzene (Yuan et al. 2015) and nitrobenzene (Wang et al. 2011). However, these experiments were usually conducted with synthetic wastewater substrates and operated in sub-liter-scale reactors for only a few weeks.

In reality, if the BES is to fulfill its potential as a full-scale wastewater treatment technology, many gaps still need to be bridged to take this concept closer to the reality (Heidrich et al. 2014). To this end, in the past two years, some researchers have started to carry out scale-up investigations of BES involving the testing mixtures of contaminants or even real wastewater, as well as extending the operating time to at least a half year (Dong et al. 2015; Heidrich et al. 2014; Ma et al. 2015; Nguyen et al. 2014; Nguyen et al. 2013). However, most of these works have relied on reactors with specialized designs, such as the sMDC (integrating a microbial desalination cell and anaerobic digestion reactor) (Zhang and Angelidaki, 2015), UASB-BES (Shen et al. 2014), rotating hanging media bioreactor (Nguyen et al. 2014), etc. Little information is known on how to combine BES on-site with an existing wastewater treatment process, which, in fact, could be beneficial in decreasing the capital cost compared to a newly constructed process, and therefore would be expected to facilitate the application of BES.

In this regard, here, two sets of 120 L/day small pilot-scale wastewater systems were constructed according to a wastewater treatment plant located in east China, which simultaneously accepts domestic wastewater and dyeing industrial wastewater as the influent. The core units of the system consisted of an acidogenic bioreactor (AB) followed by a cyclic activated sludge system (CASS). In order to integrate BES on-site, an electrode module was deployed into the AB to form a hybrid acidogenic bioreactor (HAB) in one set of systems, while the other without electrodes was operated as the control. Azo dye acid red G (ARG) was tested as the target recalcitrant contaminant, which was dissolved into synthetic or real domestic wastewater to simulate the municipal-industrial dye mixed wastewater. The objective of this research is: a) to compare the ARG and COD removal efficiencies between HAB and AB, as well as their performance under different operation and electrode module design conditions; b) to verify the feasibility of using real domestic wastewater; c) to understand the influence of integrating AB with BES on the performance of whole system in terms of decolorization efficiency, COD removal efficiency, and nitrogen removal efficiency.

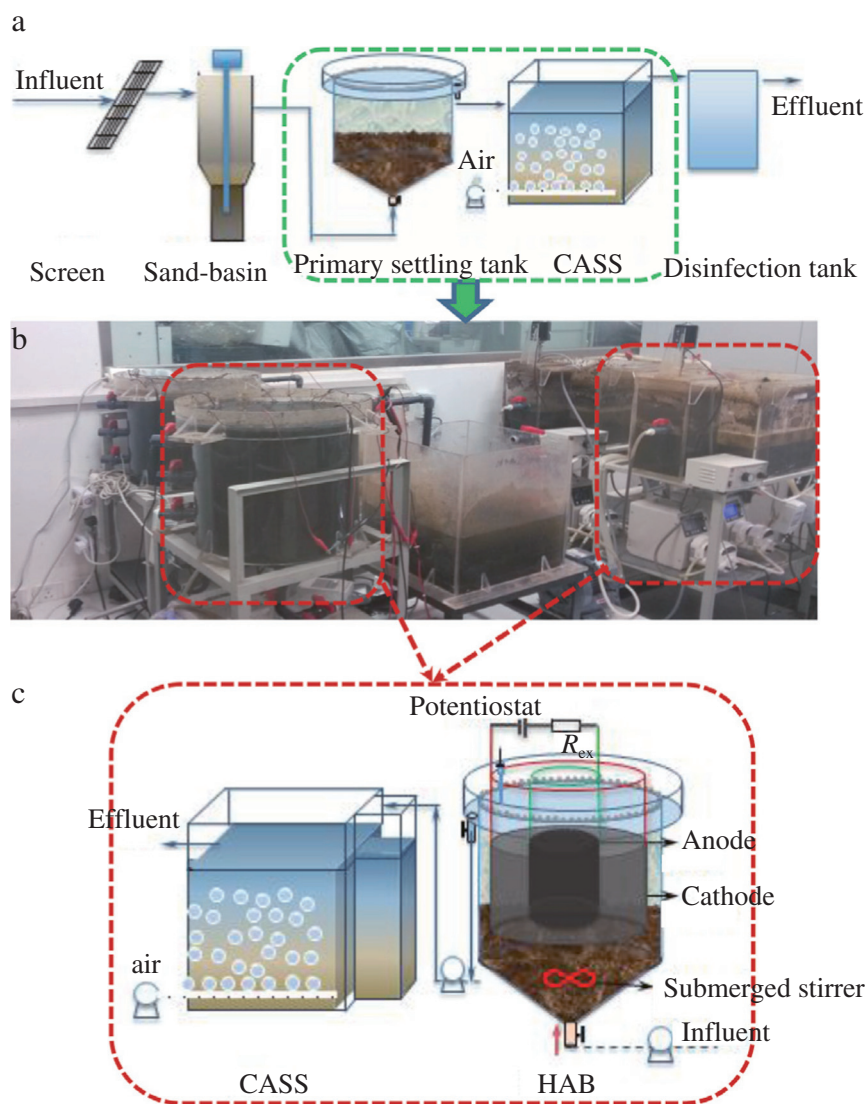
## 1. Methods and materials

### 1.1. Reactor configuration

The schematic diagram of HAB + CASS is illustrated in Fig. 1. HAB + CASS consisted of a HAB (R1) and a CASS reactor (R3). The control process included a conventional AB (R2) without BES and a CASS reactor (R4). The HAB and AB were made of plexiglass with effective volume of 30 L (dimension of 32 cm diameter × 29 cm height). The electrode modules in HAB were composed of carbon brushes (ID 3 cm × L 30 cm, Toray Industries, Inc. Japan) and arranged in a circular geometry. The cathode and anode modules, with 21 carbon brushes and 7 carbon brushes respectively, were set in the outer and inner ring, respectively. Titanium wire (1 mm diameter, Baoji city kaize metal materials Co., Ltd.) was used as current collector to connect the cathode and anode brushes. The CASS bioreactor was also made of plexiglass plates (L 40 cm × W 40 cm × H 40 cm) with effective water depth of 30 cm and working volume of 48 L. The entire bioreactor was divided into two regions, with a volume ratio of 1:10. The front region was anoxic and the back region was aerobic.

The current of  $700 \pm 10$  mA was applied in HAB with a power supply. An Ag/AgCl reference electrode (+197 mV vs. standard hydrogen electrode) was installed between the anode and the cathode to enable potential measurement. The cathode and the reference electrode were connected to a data acquisition system. An external resistor (Rex) of 10  $\Omega$  was connected in the circuit. The voltage across Rex and the cathode potential were recorded using the data acquisition system every 10 min.

All design parameters (HRT, SRT, influent wastewater characteristics etc.) of the experimental system were based on the process of a real mixed wastewater treatment plant (mWWTP) located in Jiangsu province. The influent of the mWWTP was domestic wastewater mixed with dyeing and printing wastewater and electronic industrial wastewater.



**Fig. 1 – (a) Schematic representation of the mixed wastewater treatment plant, (b) photograph of the small pilot-scale HAB-CASS system, and (c) schematic representation of the small pilot-scale HAB-CASS system. HAB: hybrid acidogenic bioreactor; CASS: cyclic activated sludge system.**

The main processes of this mWWTP were two-stage screen, sand-basin, primary settling tank, CASS and disinfection tank (Fig. 1a). In this study, we upgraded the primary settling tank as a hydrolysis acidification unit.

## 1.2. Inoculation and operation

The inoculate sludge was collected from a domestic wastewater treatment plant (1,000,000 m<sup>3</sup>/day), Beijing, China. The VSS/TSS ratio of the sludge was 70%. The synthetic water contained (in mg/L): sodium bicarbonate, 3000; glucose, 250–500; acetate, 250–500; ARG, 20–50; NaCl, 1500; NH<sub>4</sub>Cl, 400–640; yeast extract, 100; CaCl<sub>2</sub>, 16; FeCl<sub>3</sub>, 5; K<sub>2</sub>HPO<sub>4</sub>, 67; 0.5 mL trace element solution (Wolin et al. 1963; Donlon et al. 1997), and 0.5 mL vitamin solution (Wolin et al. 1963; Donlon et al. 1997). The domestic waster was collected from a domestic wastewater storage tank of a housing estate (Haidian, Beijing) with average COD of 430 mg/L, ammonium of 138 mg/L, and nitrate

and nitrite concentration less than 0.5 mg/L. The average pH was 7.5.

The operation was divided into four stages as shown in Table 1. At stage I, R1 (700 ± 10 mV applied) and R2 were fed with synthetic wastewater. The influent acid red G concentration was 50 mg/L and HRT was kept at 24 hr. Once 70% ARG removal efficiency was achieved and the current was stable, the reactors were started up. At stage II, HRT was shortened from 24 to 16, 10 and 6 hr in sequence. Correspondingly, the flow rates of influent containing 50 mg/L ARG were 30, 45, 72 and 120 L/day, respectively. At stage III, the ratio of the cathode and total electrode volume (TCV/TV) in R2 was increased from 0.15 to 0.25 by adding 14 carbon brushes to the cathode module. Simultaneously, a stirrer was installed for R1 and R2 to test the performance under stirring and without stirring. At stage IV, the influent feeding was switched to domestic wastewater containing ARG. R1 and R2 operated with stirring and HRT was kept at 6 hr.

Table 1 – Operational conditions at different stages.

Stage	Phase	Time (day)	Substrate	Reactor	HRT <sup>c</sup> (hr)	TCV/TV <sup>d</sup>	Operation mode	Power supplied <sup>g</sup> (V)	ARG <sup>h</sup> (mg/L)	ARG LR <sup>i</sup> (g ARG/(L·m <sup>3</sup> ))	COD <sup>g</sup> (mg/L)	OLR <sup>k</sup> (g COD/(L·m <sup>3</sup> ))
I	1	0–30	sWW <sup>a</sup>	R1 & R2	24	0.15	×	0.7	48.9 ± 4.8	48.9 ± 4.8	969.1 ± 42.1	969.1 ± 42.1
	2	31–43	sWW	R1 & R2	24	0.15	×	0.7	44.6 ± 1.9	44.6 ± 1.9	953.4 ± 57.5	953.4 ± 57.5
	3	44–54	sWW	R1 & R2	16	0.15	×	0.7	40.7 ± 2.4	61.1 ± 3.6	1376.5 ± 66.3	1376.5 ± 66.3
II	4	55–67	sWW	R1 & R2	10	0.15	×	0.7	43.0 ± 2.5	103.1 ± 6.1	867.8 ± 53.9	2082.7 ± 129.4
	5	68–79	sWW	R1 & R2	6	0.15	×	0.7	41.3 ± 1.8	165.2 ± 7.3	839.7 ± 43.8	3358.8 ± 175.2
	6	80–95	sWW	R1 + R3 & R2 + R4	6	0.25	✓ <sup>f</sup>	0.7	42.1 ± 1.4	168.4 ± 5.6	754.7 ± 71.3	3018.9 ± 285.2
III	7	96–101	sWW	R1 + R3 & R2 + R4	6	0.25	×	0.7	40.6 ± 1.7	162.4 ± 6.8	756.5 ± 71.6	3025.9 ± 286.5
	8	102–109	sWW	R1 + R3 & R2 + R4	6	0.25	×	0.7	22.2 ± 1.6	88.9 ± 6.3	489.6 ± 31.8	1958.4 ± 127.3
	9	110–117	sWW	R1 + R3 & R2 + R4	6	0.25	✓	0.7	23.1 ± 1.5	92.6 ± 6.0	445.4 ± 28.1	1781.8 ± 112.7
IV	10	118–123	dWW <sup>b</sup>	R1 + R3 & R2 + R4	6	0.25	✓	0.7	13.2 ± 0.5	53.0 ± 2.1	441.9 ± 30.8	1767.5 ± 123.1
	11	124–129	dWW	R1 + R3 & R2 + R4	6	0.25	✓	0.7	27.1 ± 1.0	108.4 ± 4.1	426.8 ± 29.1	1707.3 ± 116.7
	12	130–134	dWW	R1 + R3 & R2 + R4	6	0.25	✓	0.7	43.3 ± 2.2	173.3 ± 9.0	434.8 ± 34.9	1739.3 ± 139.6

R1 is the hybrid acidogenic bioreactor, R2 is the hydrolysis acidification tank, R3 is the CASS reactor connected with R1, and R4 is the CASS reactor connected with R2.

<sup>a</sup>: sWW means synthetic wastewater, <sup>b</sup>: dWW means domestic wastewater, <sup>c</sup>: h, i, j, k: the parameters for HAB and HAT, <sup>g</sup> and <sup>h</sup> are parameters for HAB.

×: means R1 and R2 operated without stir, ✓: means R1 and R2 operated with stir.

HRT: hydraulic retention time; TCV/TV: total cathode volume/total volume; ARG: acid red G; ARG LR: acid red G loading rate; COD: chemical oxygen demand; OLR: organic loading rate.

CASS reactors (R3 and R4) connected with R1 and R2 were operated under the same conditions during stages III and IV. The running cycle lasted for 6 hr (1.5 hr for inflow, 4.0 hr for microporous aeration, 1.0 hr for sedimentation and 1.0 hr for discharge) with 100% sludge reflux ratio. Both influent and effluent volumes in a running cycle were 19.2 L, which means both influent and effluent had a ratio of 40%. Biomass in CASS was 3500 mg/L and the dissolved oxygen was 4 mg/L.

### 1.3. Chemicals and analytical methods

#### 1.3.1. Chemicals

Acid red G (ARG) was commercial purity grade (Aladdin Industrial Corporation, China). All other chemicals used were analytical grade.

#### 1.3.2. Analysis

The water quality parameters including ARG, COD, MLSS, MLVSS, NH<sub>3</sub>-N, NO<sub>3</sub>-N, NO<sub>2</sub>-N, pH values, DO concentration, conductivity and temperature were tracked. Liquid samples taken from HAB were immediately filtered through a 0.22 μm filter.

The UV–Vis spectra of ARG were recorded from 300 to 900 nm using a UV–Vis spectrophotometer (T6 New Century, Purkinje General, China). ARG was quantified at the wavelength of 560 nm. COD was measured using commercially available colorimetric COD test kits (100–1000 mg COD/L, Lian-hua Tech. Co., Ltd., China) according to manufacturer's instructions (Lian-hua Tech. Co., Ltd., China). MLSS, MLVSS, NH<sub>3</sub>-N, NO<sub>2</sub>-N, and NO<sub>3</sub>-N were determined according to standard methods (APHA 1998). The pH value was measured using a portable pH meter (Five Easy Plus, Mettler Toledo, Inc. USA). DO, temperature and conductivity were measured by a Multi 3420 portable multi parameter water quality analyzer (Wissenschaftlich-Technische Werkstätten GmbH, Germany) DO, temperature and conductivity probe.

#### 1.3.3. Calculation

ARG and COD removal efficiencies (RE: %) were calculated based on the difference between influent and effluent concentrations:

$$RE_{AR1} = \frac{C_{in-AR1} - C_{eff-AR1}}{C_{in-AR1}} \times 100\% \quad (1)$$

$$RE_{COD} = \frac{C_{in-COD} - C_{eff-COD}}{C_{in-COD}} \times 100\% \quad (2)$$

where  $C_{in-AR1}$  (mg/L) and  $C_{in-COD}$  (mg/L) are the influent ARG and COD concentration, respectively;  $C_{in-AR1}$  (mg/L) and  $C_{eff-COD}$  (mg/L) are the effluent ARG and COD concentration, respectively. The current was calculated from the external resistance using Ohm's law. The volumetric current density was reported based on the total cathode volume (Cui et al. 2012; Cui et al. 2014).

## 2. Results and discussion

### 2.1. Start-up and batch operation

The start-up period of R1 and R2 was operated in batch mode and lasted for 30 days. The loading rate of synthetic wastewater



was  $\sim 50 \text{ g-ARG}/(\text{m}^3 \cdot \text{day})$  and  $1 \text{ kg-COD}/(\text{m}^3 \cdot \text{day})$ . Following inoculation for 15 days, the current of HAB increased from 0 to  $31.9 \text{ mA}$  (Fig. 2a). Simultaneously, the anode and cathode potentials decreased to  $(419 \pm 11) \text{ mV}$  and  $(1128 \pm 12) \text{ mV}$ , respectively, which would be thermodynamically favorable to ensure the oxidation of glucose on the anode and reduction of ARG on the cathode.

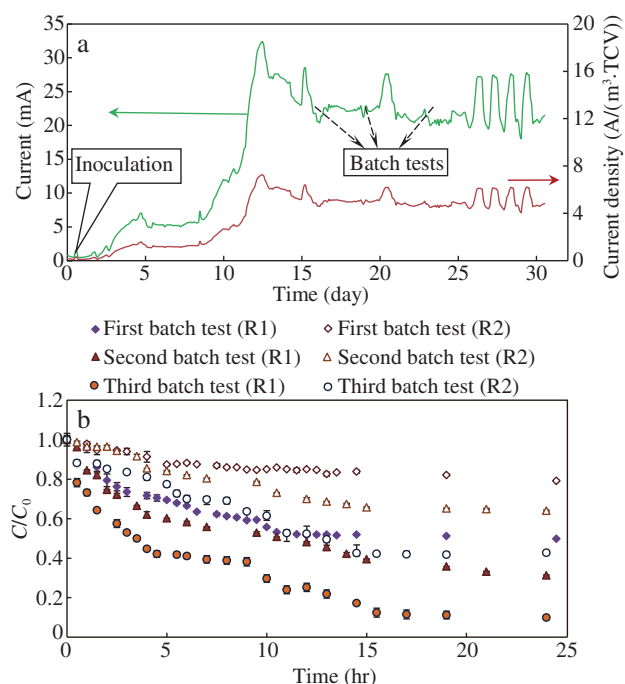
In order to further evaluate the performance of R1 and R2 during start-up, two batch tests were respectively conducted on day 16 and day 20 (Fig. 2b). Though high current densities were observed in R1, both reactors obtained poor ARG removal efficiencies. From day 25–28, both reactors were subjected to new batch tests (third batch test) at the same operating condition. It was observed that the decolorization rate of R1 was obviously faster than that of R2. About 60% ARG removal efficiency was obtained in R1 at 5 hr, while only 30% ARG was removed in R2. It took 9 hr for R1 to remove 70% ARG but 15 hr for R2. The ARG removal efficiencies of R1 and R2 after 24 hr reached 88.8% and 75%.

## 2.2. Performance and optimization of R1 and R2

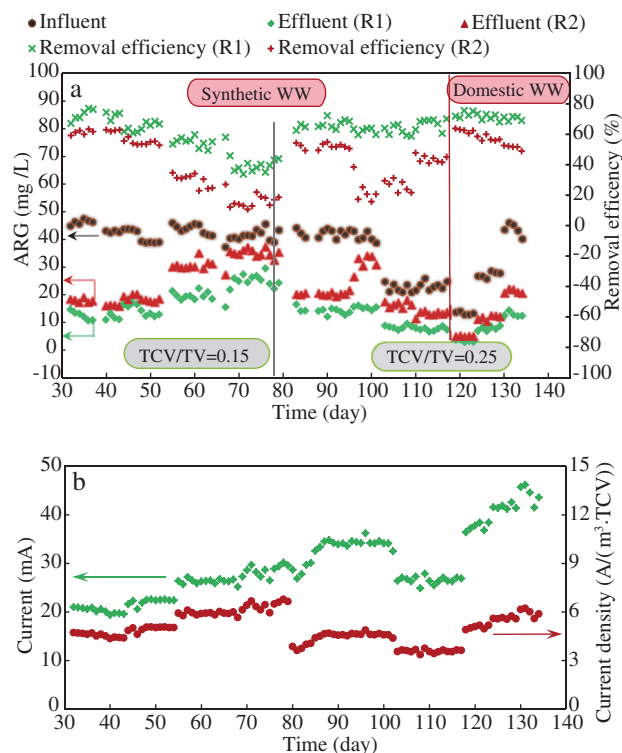
From stage II to stage IV, R1 and R2 were operated in continuous mode under different conditions (Table 1). The variation of ARG concentration, ARG removal efficiencies, current and current density is shown in Fig. 3.

### 2.2.1. Effect of HRT

In order to study the influence of HRT on ARG and COD removal, R1 and R2 were operated continuously with synthetic wastewater under four HRT conditions (24, 16, 10 and 6 hr) at stage II. As the influent ARG concentrations fluctuated between  $37.0$  to



**Fig. 2 – (a) Current and current density profiles of hybrid acidogenic bioreactor during the start-up, (b) performance of ARG removal in batch operation.**



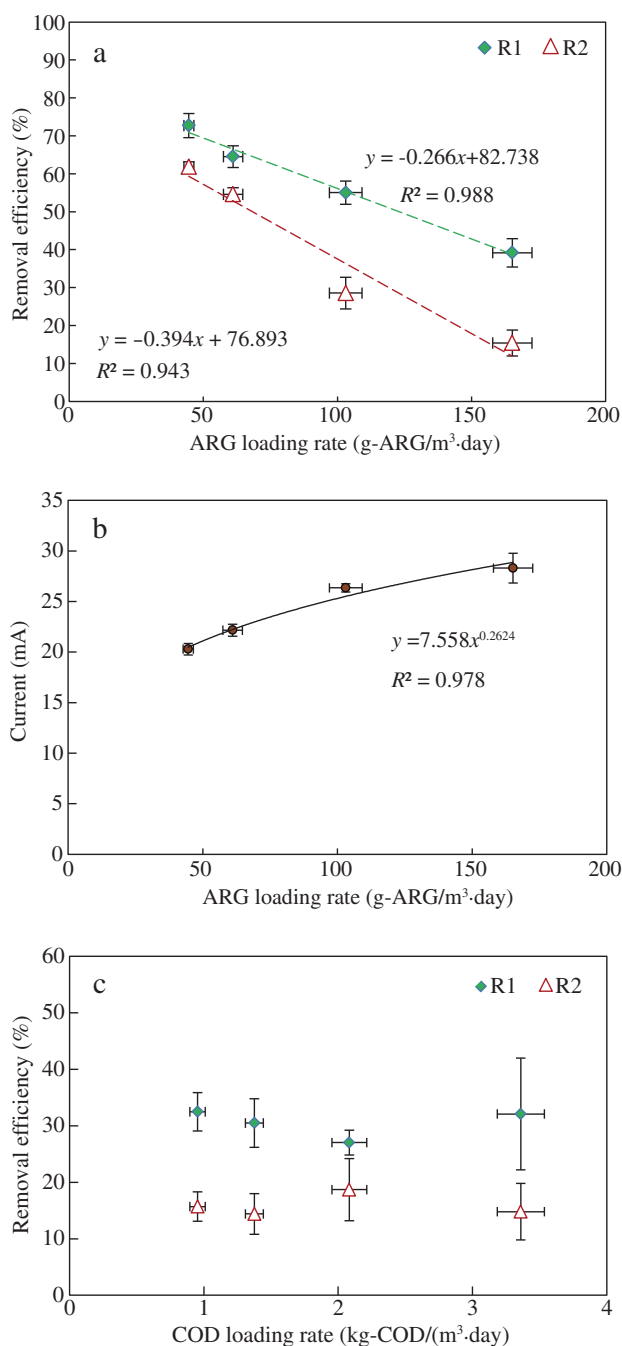
**Fig. 3 – (a) Performance of ARG removal of continuous feeding period, (b) performance of current and current density of HAB during continuous feeding period. HAB: hybrid acidogenic bioreactor.**

$47.2 \text{ mg/L}$ , the corresponding ARG loading rates were  $44.6 \pm 1.9$ ,  $61.1 \pm 3.6$ ,  $103.1 \pm 6.1$  and  $165.2 \pm 7.3 \text{ g-ARG}/(\text{m}^3 \cdot \text{day})$ . As shown in Fig. 4a, the ARG removal efficiency of both R1 and R2 declined with the shortening of HRT. At HRT of 24 hr, the highest ARG removal efficiency ( $72.7 \pm 3.2\%$ ) was obtained in R1, which was 10% higher than that in R2 ( $61.8 \pm 1.3\%$ ). The difference of performance between R1 and R2 was more prominent at shorter HRT, e.g.,  $64.5\% \pm 2.9\%$  vs.  $54.6\% \pm 1.7\%$  at HRT of 16 hr,  $55.0\% \pm 3.1\%$  vs.  $28.6\% \pm 4.2\%$  at HRT of 10 hr and  $39.2\% \pm 3.7\%$  vs.  $15.4\% \pm 3.4\%$  at HRT of 6 hr.

A significant linear relationship between HRT and ARG removal rate could be observed in the two reactors as expressed Eq. (3).

$$\begin{cases} y_{R1} = -0.266x_{R1} + 82.738 & R^2 = 0.988 \\ y_{R2} = -0.394x_{R2} + 76.893 & R^2 = 0.943 \end{cases} \quad (3)$$

where  $y_{R1}$  and  $y_{R2}$  represent the ARG removal efficiency of R1 and R2 (%), and  $x_{R1}$  and  $x_{R2}$  represent the ARG loading rate ( $\text{g-ARG}/(\text{m}^3 \cdot \text{day})$ ), respectively. The regression coefficients  $R^2$  of the two models were 0.988 and 0.943. A higher slope was obtained for R2 in linear equations for ARG removal efficiency and ARG loading rate. This result implied that HAB presented a greater advantage in comparison with AB at low HRT and high ARG loading rate. In HAB, the external voltage can provide external electrons for ARG reduction, while AB decolorization takes place only via conventional anaerobic biological methods. In the anaerobic biological decolorization removal methods, an electron donor is required to create the necessary reductive conditions. The reductant usage for HAB is considerably smaller than in AB, and



**Fig. 4 – (a) ARG removal efficiency with variation of ARG-LR, (b) current of HAB with variation of ARG-LR, (c) COD removal efficiency with variation of OLR. ARG: acid red G; HAB: hybrid acidogenic bioreactor; ARG-LR: acid red G loading rate, COD: chemical oxygen demand.**

very limited organic substrate is required for the HAB decolorization of ARG.

Variation of HRT did not affect COD removal significantly. As shown in Fig. 4c, the COD removal efficiency of R1 fluctuated from  $27.0\% \pm 2.2\%$  to  $32.5\% \pm 3.4\%$  with HRT shortening from 24 to 6 hr, which remained higher than that of R2 ( $14.4\% \pm 3.6\%$  to  $18.7\% \pm 5.5\%$ ).

The current and current density in HAB presented an increasing tendency with the increase of ARG loading rate

under different HRTs (Table 2 and Fig. 4b), reaching  $28.30 \pm 1.48$  mA and  $6.36 \pm 0.33$  A/(m<sup>3</sup>·TCV) at HRT of 6 hr, respectively. Interestingly, an exponential function relationship was observed between current and ARG loading rate. The power function is shown in Eq. (4).

$$y = 7.558x^{0.262} \quad R^2 = 0.978 \quad (4)$$

where  $y$  represents the current (mA) of HAB, and  $x$  represents the ARG loading rate (g-ARG/(m<sup>3</sup>·day)), respectively.

The power function relationship between current and ARG loading indicated that with the increase of ARG loading rate, the current did not show a linear increase. Several reasons may have caused the nonlinear relationship between current and ARG loading rate, such that there were other electron acceptors (mostly H<sup>+</sup>) besides azo dye involved in current generation, higher pollutant concentrations inhibited the activity of microbes, and microbes presented a lower current generation efficiency.

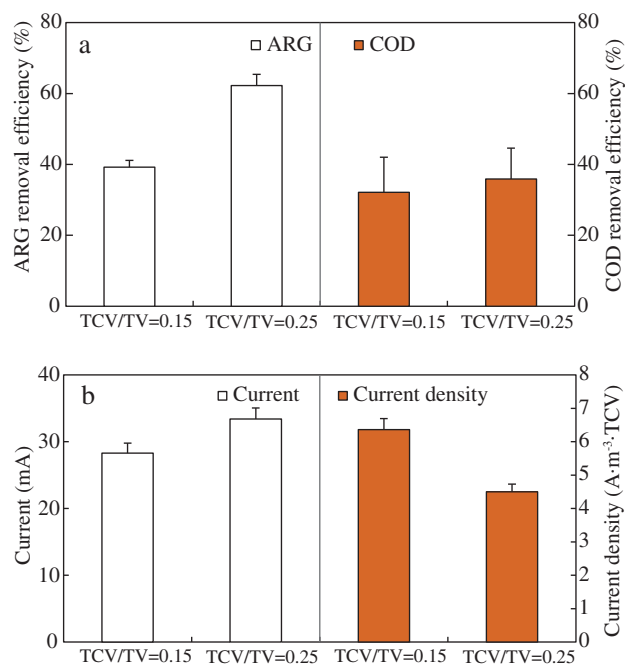
#### 2.2.2. Effect of cathode volume

The cathode in BES determines the reductive reactions and thus played the main role in azo dye removal. Cathode volume is a very important parameter of the cathode and would be expected to affect the rate of reductive reactions and current significantly. In this work, to investigate the effect of cathode volume on the performance of HAB, the cathode module volume was enlarged by adding 12 carbon brushes. The ratio of cathode volume to total electrode volume (TCV/TV) increased from 0.15 to 0.25 at HRT of 6 hr and ARG loading rate of 160 g-ARG/(m<sup>3</sup>·day). As shown in Fig. 5a, the ARG removal rate was correspondingly improved and raised from  $39.2\% \pm 3.7\%$  to  $62.2\% \pm 2.0\%$ . However, COD removal rate did not present an increasing trend with the rise of TCV/TV. For the current and current density, the TCV/TV was increased to 0.25 and an obvious increasing trend of current was observed, while the volumetric current density (based on total cathode volume) presented a downward trend (Fig. 5b). The current was greatly increased from  $28.30 \pm 1.48$  mA to  $34.55 \pm 0.83$  mA, and the corresponding current density decreased from  $6.36 \pm 0.33$  to  $4.66 \pm 0.11$  A/(m<sup>3</sup>·TCV) when TCV/TV rose to 0.25, respectively.

**Table 2 – Current and current density of HAB at different stages.**

Stage	Phase	Current (mA)	Current density (A/m <sup>3</sup> TCV)
II	2	$20.29 \pm 0.57$	$4.56 \pm 0.13$
	3	$22.16 \pm 0.60$	$4.98 \pm 0.14$
	4	$26.35 \pm 0.40$	$5.93 \pm 0.09$
	5	$28.30 \pm 1.48$	$6.36 \pm 0.33$
III	6	$33.39 \pm 1.66$	$4.50 \pm 0.23$
	7	$34.55 \pm 0.83$	$4.66 \pm 0.11$
	8	$27.40 \pm 2.24$	$3.69 \pm 0.30$
	9	$26.50 \pm 0.53$	$3.57 \pm 0.07$
IV	10	$37.72 \pm 0.72$	$5.08 \pm 0.10$
	11	$41.68 \pm 0.51$	$5.62 \pm 0.07$
	12	$44.32 \pm 1.87$	$5.97 \pm 0.25$

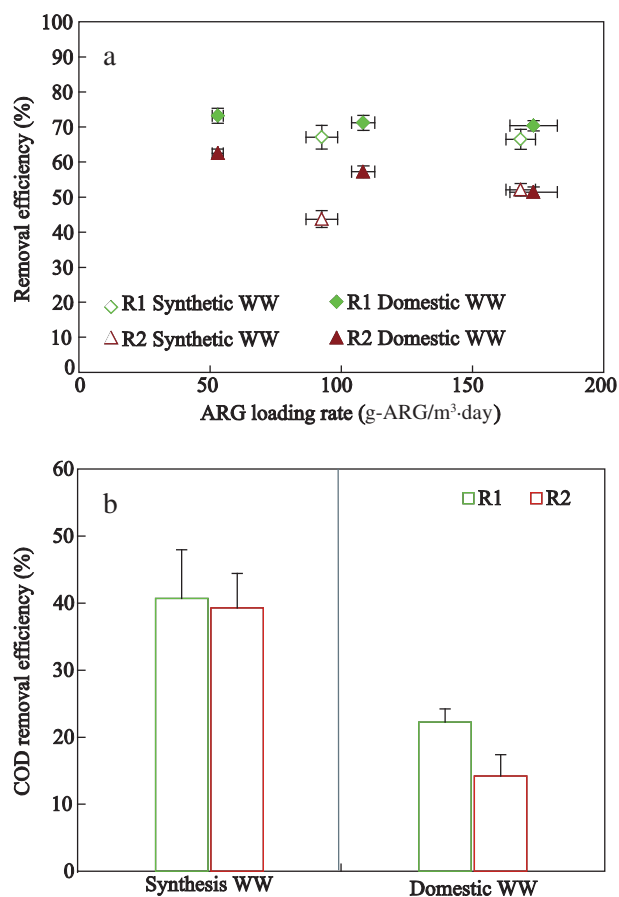
HAB: hybrid acidogenic bioreactor.



**Fig. 5 – (a) Influence of HAB TCV/TV on ARG and COD removal efficiencies, (b) influence of HAB TCV/TV on current and current density. HAB: hybrid acidogenic bioreactor; TCV/TV: total cathode volume/total volume; ARG: acid red G; COD: chemical oxygen demand.**

### 2.2.3. Effect of substrate

At stage IV, the influent was switched from synthetic wastewater to domestic wastewater to investigate the adaptability of HAB to real wastewater. Two ARG loading rates (ARG-LR = 100 and 160 g-ARG/(m<sup>3</sup>·day)) were investigated. As shown in Fig. 6, the ARG decolorization efficiency of R1 was 71.2% ± 2.1% at ARG-LR of 100 g-ARG/(m<sup>3</sup>·day). It was not obviously affected by higher ARG-LR, and was as high as 70.4% ± 1.5% at ARG-LR of 160 g-ARG/(m<sup>3</sup>·day). Compared with the results of synthetic wastewater feeding (66.5% ± 2.9% and 67.1% ± 3.4% at ARG-LR of 100 and 160 g-ARG/(m<sup>3</sup>·day), respectively), the ARG removal rate was improved by the HAB. Correspondingly, the current and current density also increased and reached 44.32 ± 1.87 mA and 5.97 ± 0.25 A/(m<sup>3</sup>·TCV) at ARG-LR of 160 g-ARG/(m<sup>3</sup>·day), which were much higher than those obtained with synthetic wastewater feeding (33.39 ± 1.66 mA and 4.50 ± 0.23 A/(m<sup>3</sup>·TCV)) (Table 2). However, COD removal efficiency sharply dropped. It decreased from 40.7% ± 7.3% to 22.3% ± 2.0% in R1 and from 39.2% ± 5.1% to 14.2% ± 3.2% in R2 at HRT of 6 hr when the substrate was switched from synthetic wastewater to domestic wastewater (Fig. 6b). The reason for this was due to the more complex organics in the domestic wastewater, which were difficult to degrade under anaerobic conditions. Higher ARG removal rate and lower COD removal rate in the domestic wastewater might be due to the more balanced nutrients and diverse organic matters in the domestic wastewater, which can provide better conditions for microbes to degrade azo dye. In addition, the intermediate products of ARG decolorization were very hard to degrade under anaerobic conditions (Stolz 2001). Therefore, more ARG degradation means that more intermediate products contribute to COD, as the intermediate

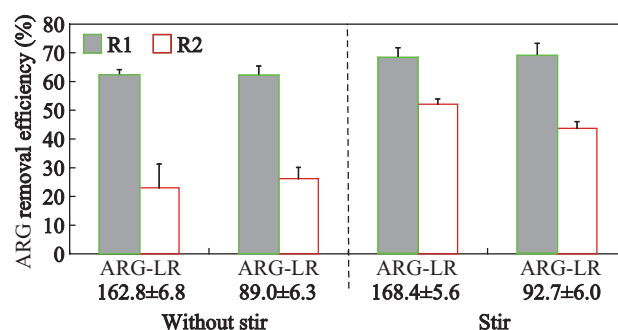


**Fig. 6 – (a) Influence of substrate and ARG-LR on ARG removal efficiencies, (b) influence of substrate on COD removal efficiencies. ARG-LR: acid red G loading rate; ARG: acid red G; COD: chemical oxygen demand.**

product-derived COD still remained in the wastewater (Korneel Rabaey et al. 2003; Singh et al. 2007).

### 2.2.4. Effect of stirring

Stirring could promote mass transfer, microbial growth rate and biomass (Zambrano et al. 2015). In order to improve the performance of R1 and R2, a stirrer was installed in both reactors at stage III as illustrated in Fig. 7.



**Fig. 7 – Variation of ARG removal efficiency under different operation modes. ARG: acid red G. ARG-LR: acid red G loading rate.**

The ARG removal rate of R2 was improved greatly by stirring. It reached  $43.7\% \pm 2.4\%$  at the ARG loading rate of  $\sim 90 \text{ g-ARG}/(\text{m}^3 \cdot \text{day})$ , which was much higher than that without stirring ( $26.2\% \pm 3.9\%$ ). It further increased to  $52.1\% \pm 1.8\%$  with ARG loading rate increasing to  $\sim 160 \text{ g-ARG}/(\text{m}^3 \cdot \text{day})$ . However, this trend was not observed in R1 in stirring mode. The ARG removal rate in R1 only grew by 7% compared with that without stirring. The reasons for this phenomenon maybe concluded as follows: (1) R1 had reached its maximum capacity for removing ARG when operated as piston flow reactor (PFR), so that stirring could not remove more ARG from R1; (2) sludge was distributed throughout the reactor, and sludge that gathered in the carbon fiber led to an increase in the inner resistance of R1 and reduced the biocatalysis electrolysis efficiency of the reactor.

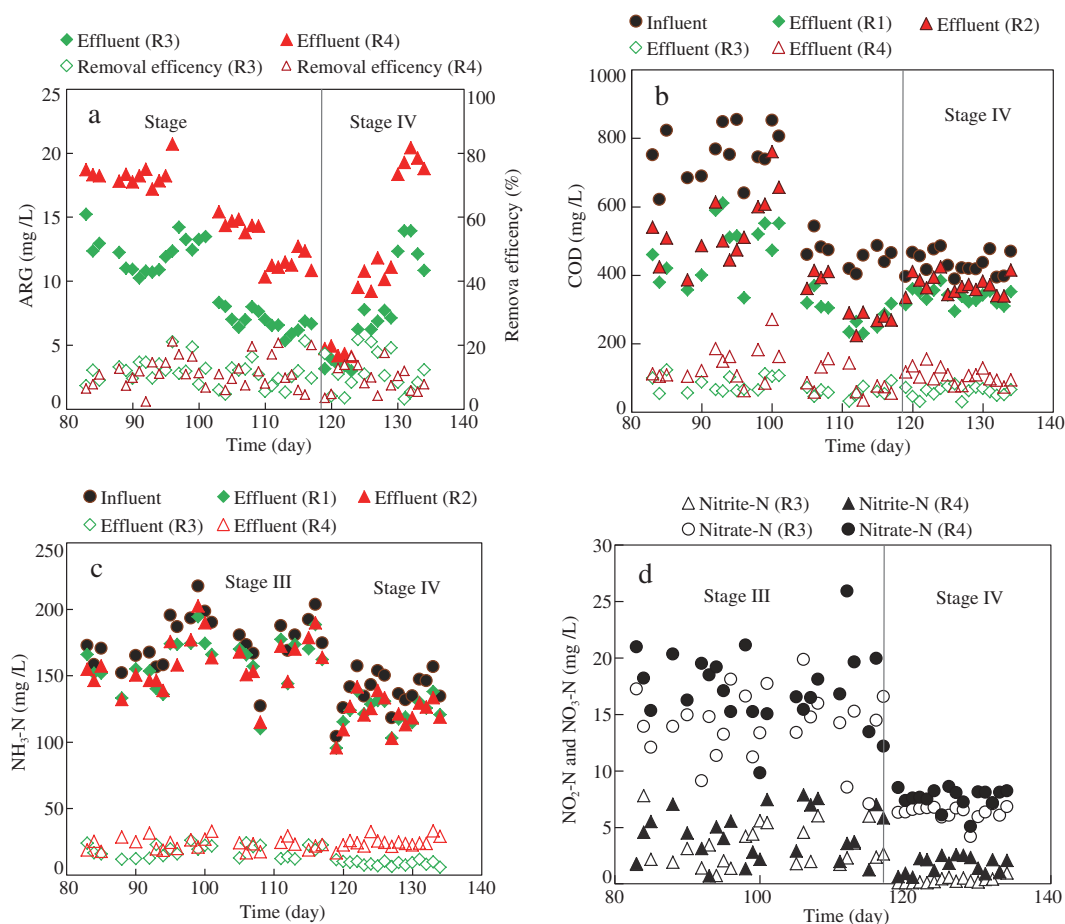
### 2.3. Performance of aerobic system

To further remove the residual contaminants from the effluent of the anaerobic reactor, a CASS was connected with R1 and R2 from Day 80 on. The HRTs of R1, R2 and CASS were all 6 hr. The ARG, COD and nitrogen removal performance of the combined anaerobic-aerobic system were monitored during stage III and stage IV.

As shown in Fig. 8a, ARG decolorization was not obvious in R3 and R4. The ARG in the effluent of R1 and R2 was poorly removed, by  $9.9\% \pm 3.4\%$  in R3 and  $10.0\% \pm 3.7\%$  in R4, with

synthetic wastewater feeding. Similar results ( $10.7\% \pm 5.6\%$  for R3 and  $9.6\% \pm 4.3\%$  for R4) were obtained when domestic wastewater was used as substrate. The low ARG removal in CASS was due to the fact that the azo bond could not be reduced in the aerobic system (O'Neill et al. 2000; Çınar et al. 2008). Thus, the contribution of CASS to ARG decolorization was limited. However, CASS played an important role in COD removal as illustrated in Fig. 8b. The average COD concentration was  $398.1 \pm 106.4 \text{ mg/L}$  in the effluent of R<sub>1</sub>, and it significantly decreased to  $73.8 \pm 23.3 \text{ mg/L}$  in that of R3 at stage III with synthetic wastewater as feed. Correspondingly, the COD removal efficiency was increased from 80.7% to 89.9%. At stage IV, the results were not affected by the change of influent type. The average COD removal efficiency was 82.2% with domestic wastewater as feed. R4 presented an inferior COD removal performance: 73.86% and 71.41% COD removal at stage III and stage IV, respectively. Also, the HAB-CASS combined system presented a superior COD removal efficiency compared to that of the AB-CASS system,  $87.3\% \pm 3.6\%$  versus  $79.1\% \pm 7.1\%$ .

In this study, long sludge retention time provided good retention of slow growing ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) in the CASS reactor for nitrification (Ma et al. 2013). As Fig. 8c shows, a small amount of  $\text{NH}_3\text{-N}$  was removed in R1 (10.0%) and R2 (10.3%); however, excellent removal of  $\text{NH}_3\text{-N}$  was achieved in both CASS reactors. At stage III and stage IV,  $88.5\% \pm 3.3\%$  and  $92.7\% \pm$



**Fig. 8 – Removal of (a) ARG, (b) COD, (c)  $\text{NH}_3\text{-N}$ , (c)  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  in the combined anoxic-aerobic system. ARG: acid red G; COD: chemical oxygen demand;  $\text{NH}_3\text{-N}$ : ammonia nitrogen;  $\text{NO}_2\text{-N}$ : nitrite nitrogen;  $\text{NO}_3\text{-N}$ : nitrate nitrogen.**



1.7% of  $\text{NH}_3\text{-N}$  were removed in R3, respectively. R4 eliminated  $85.3\% \pm 3.3\%$  and  $79.7\% \pm 2.6\%$   $\text{NH}_3\text{-N}$  at stages III and VI. When fed with synthetic wastewater, R3 and R4 effluent ammonium-nitrogen concentrations were 18.3 and 23.3 mg/L, respectively. However, the effluent ammonium-nitrogen concentrations of R3 sharply dropped to 8.8 mg/L when fed with domestic wastewater, while the R4 effluent still remained at a high level (24.8 mg/L). The effluent  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  of R3 and R4, as demonstrated in Fig. 8d, at stage IV, showed that lower  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  effluent were obtained than at stage III.

### 3. Conclusions

The combined system of HAB-CASS was a new attempt in the scaling up of BES. The results indicated that it was efficient for azo dye wastewater treatment, and even for more species of refractory wastewaters, at a total scale of 120 L/day. The model azo dye (ARG) removal efficiencies were significantly improved by 10% in HAB compared with that in AB. Domestic wastewater was more favorable for HAB, which implied the practical applicability of HAB. Larger cathode volume/total volume ( $\text{TCV}/\text{TV} = 0.25$ ) for HAB led to higher current and azo dye removal efficiency, indicating that the cathode played an important role in azo dye decolorization.

### Acknowledgments

This work was financially supported by the Ministry of Environmental Protection of the People's Republic of China (Major Science and Technology Program for Water Pollution Control and Treatment) (No. 2014ZX07204-005), the National Natural Science Foundation of China (Nos. 51222812, 31370157, 21407164, 51508551), the China Postdoctoral Science Foundation (No. 2015M580140), the National Science Foundation for Distinguished Young Scholars (No. 51225802), and Hundred Talents Program of the Chinese Academy of Sciences (No. 29BR2013001).

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