Influence of COD/sulfate ratios on the integrated reactor system for simultaneous removal of carbon, sulfur and nitrogen

Ye Yuan, Chuan Chen, Youkang Zhao, Aijie Wang, Dezhi Sun, Cong Huang, Bin Liang, Wenbo Tan, Xijun Xu, Xu Zhou, Duu-Jung Lee and Nanqi Ren

ABSTRACT

An integrated reactor system was developed for the simultaneous removal of carbon, sulfur and nitrogen from sulfate-laden wastewater and for elemental sulfur (S0) reclamation. The system mainly consisted of an expanded granular sludge bed (EGSB) for sulfate reduction and organic carbon removal (SR-CR), an EGSB for denitrifying sulfide removal (DSR), a biological aerated filter for nitrification and a sedimentation tank for sulfur reclamation. This work investigated the influence of chemical oxygen demand (COD)/sulfate ratios on the performance of the system. Influent sulfate and ammonium were fixed to the level of 600 mg SO4^2-/L and 120 mg NH4^+-L, respectively. Lactate was introduced to generate COD/SO4^2- = 0.5:1, 1:1, 1.5:1, 2:1, 3:1, 3.5:1 and 4:1. The experimental results indicated that sulfate could be efficiently reduced in the SR-CR unit when the COD/SO4^2- ratio was between 1:1 and 3:1, and sulfate reduction was inhibited by the growth of methanogenic bacteria when the COD/SO4^2- ratio was between 3.5:1 and 4:1. Meanwhile, the Org-C/S2-/NO3- ratios affected the S0 reclamation efficiency in the DSR unit. When the influent COD/SO4^2- ratio was between 1:1 and 3:1, appropriate Org-C/S2-/NO3- ratios could be achieved to obtain a maximum S0 recovery in the DSR unit. For the microbial community of the SR-CR unit at different COD/SO4^2- ratios, 16S rRNA gene-based high throughput Illumina MiSeq sequencing was used to analyze the diversity and potential function of the dominant species.

Key words | COD/SO4^2- ratio, denitrifying sulfide removal, microbial community structure, sulfate reduction, sulfur reclamation

INTRODUCTION

Many industries (pharmaceutical companies, food-making factories, etc.) usually have high concentrations of sulfate, ammonium and carbon compounds in their effluents, which can contaminate the receiving waters, cause eutrophication and endanger life, if these emissions are not well-treated before discharge (Cadena & Peters 1998; Klein & Perera 2002).

Currently, the treatment of wastewater containing sulfur and nitrogen focuses mainly on the removal of ammonium and sulfate in separate processes. For sulfate-laden organic wastewater, sulfate reduction combined with sulfide oxidation or photosynthetic sulfur oxidation is conventionally used for sulfur removal in wastewater treatment (Buisman et al. 1990; Tang et al. 2009). Sulfate is first reduced to sulfide by sulfate-reducing bacteria (SRB) under anaerobic conditions, and the dissolved sulfide that has been produced is subsequently oxidized under appropriate operating conditions by sulfide-oxidizing bacteria or photosynthetic sulfur bacteria to produce elemental sulfur (S0). For ammonium, aerobic and anaerobic biotechnologies are the strategies that are commonly used. The traditional microbial processes of autotrophic nitrification combined with heterotrophic denitrification (such as anaerobic/anoxic (A/O) or anaerobic/anoxic/anoxic (A2/O) process) are conventionally applied for ammonium removal in wastewater treatment (Baeza et al. 2004; Ahn et al. 2007). Ammonium is first
oxidized to nitrate or nitrite under aerobic conditions, and the nitrate or nitrite is later denitrified to nitrogen gas by heterotrophic denitrifiers under anoxic conditions.

However, the chemical oxygen demand (COD), ammonium and sulfate are often discharged together in wastewater. An innovative process capable of simultaneously removing these pollutants is therefore worthy of consideration. Reyes-Avila et al. (2004), Manconi et al. (2006) and Chen et al. (2008a) developed a denitrifying sulfide removal (DSR) process that could simultaneously convert sulfide, nitrate and COD into elemental sulfur ($S^0$), nitrogen gas ($N_2$) and carbon dioxide ($CO_2$) based on the cooperative work of the autotrophic and heterotrophic denitrifiers (hNRB). Chen et al. (2008c) operated an expanded granular sludge bed denitrifying sulfide removal (EGSB-DSR) reactor at sustainable loadings of 6.09 kg S m$^{-3}$ d$^{-1}$ for sulfide, 3.11 kg N m$^{-3}$ d$^{-1}$ for nitrate and 3.27 kg C m$^{-3}$ d$^{-1}$ for acetate; for all three contaminants, the removal efficiency exceeds 93%, which is significantly higher than the values reported in the literature, and the $S^0$ conversion rate exceeds 90%. Meanwhile, the production of alkalinity in the DSR process can supplement the post-nitrification process to lower the operational cost. In addition, alkalinity can keep the pH of the suspension high to minimize release of hydrogen sulfide ($H_2S$) from the sulfate reduction and organic carbon removal unit (SR-CR) and DSR units.

Based on the above advantages of the DSR process, we recently proposed an integrated reactor system for simultaneous removal of COD, sulfate and ammonium (integrated C-S-N removal system) (Figure 1) and investigated the operating parameters for high level elemental sulfur production (Yuan et al. 2014). However, in many cases, different industrial effluents usually present various COD/$SO_4^{2-}$ ratios. It is important to investigate the factor that determines the competition between methane-producing bacteria (MPB) and SRB communities to improve the anaerobic treatment of these wastewaters. Chen et al. (2014) discussed the effects of the COD/$SO_4^{2-}$ ratio on the performance of the integrated simultaneous desulfurization and denitrification (ISDD) process, which could achieve simultaneous removal of total organic carbon (TOC), $SO_4^{2-}$ and $NO_3^-$ in a single reactor. The primary difference between the integrated C-S-N removal system and the ISDD process is that the former uses a biological aerated filter (BAF) to perform nitrification to provide nitrate for the DSR unit. The integrated C-S-N removal system can accommodate wider ratios of COD/$SO_4^{2-}$ through cooperation of three functional bioreactors. In this system, converting the majority of the sulfate into dissolved sulfide in the SR-CR unit could ensure high $S^0$ production efficiency in the DSR unit. The main objectives of this study were to: (1) explore the effects of different COD/$SO_4^{2-}$ ratios on the performance of the integrated C-S-N removal system; and (2) analyze the microbial community structure and function of the dominant species of the SR-CR unit at different operational stages.

**Figure 1** | A schematic diagram of the experimental setup of the integrated C-S-N system. (a) SR-CR, (b) DSR, (c) sulfur reclamation unit (SR) and (d) AN filter.
MATERIALS AND METHODS

Experimental setup

The integrated C-S-N removal system (Figure 1) used in this work was proposed by Yuan et al. (2014). The system consists of four units, as follows. (a) SR-CR, which uses an EGSB reactor to reduce sulfate to sulfide and remove COD via MPB and SRB. (b) DSR unit, which also uses an EGSB reactor for DSR. The autotrophic denitrifiers sulfide oxidizing bacteria (aNR-SOB) convert sulfide to elemental sulfur using nitrate recirculated from the effluent of the aerobic nitrification (AN) unit as an electron acceptor (NO$_3^-$-NO$_2^-$), while the hNRB utilize the residual volatile fatty acids (VFAs) generated from the SR-CR unit for denitrification (NO$_3^-$-N$_2$). (c) Sulfur reclamation unit (SR), which is a sedimentation tank that is used to collect elemental sulfur before the effluent of the DSR unit enters into the AN unit. (d) AN unit, which includes a BAF used to nitrify ammonia to nitrate, which is then recycled to the DSR unit for DSR.

The EGSB for the SR-CR unit used in the experimental setup is identical in appearance and size to the DSR unit. Wastewater was fed to the reactor in the up-flow mode using a peristaltic pump (Longer BT100-2J) automatically ensured stable measuring temperatures at 30 ± 1 °C. A peristaltic pump was introduced into the reactor at the column bottom. A gas-washing device collected the H$_2$S gas that was generated at the column top. The biogas flowed into the bottle through the long glass tube below the surface, and the H$_2$S in the biogas was absorbed by the gas-washing device. The liquid inside was the NaOH solution of 5 mol L$^{-1}$. The gas production rate was measured in a wet type of gas meter (SHINAGAWA WS-1A). The compositions of gas were analyzed using a gas chromatography apparatus (Agilent 6890). The BAF was 20 cm in diameter and 30 cm in height, with a working volume of 5.0 L. As support for immobilization of the biomass, 10 mm polyurethane foam cubes with an apparent density of 23 kg m$^{-3}$ and porosity near 95% were used. The supporting materials were packed to a filtration layer depth of 15 cm in the reactor. Wastewater was fed to the reactor in the up-flow mode using a peristaltic pump.

Operation of the system

There are three stages (I–III) in the present test. The operational parameters are listed in Table 1. Synthetic

<table>
<thead>
<tr>
<th>Stage</th>
<th>Time (day)</th>
<th>HRT (h)</th>
<th>COD/SO$_4^{2-}$ ratio</th>
<th>pH</th>
<th>InSR-CR SO$_4^{2-}$ (mg L$^{-1}$)</th>
<th>OutSR-CR SO$_4^{2-}$ (mg L$^{-1}$)</th>
<th>InSR-CR NH$_3$ (mg L$^{-1}$)</th>
<th>OutSR-CR NH$_3$ (mg L$^{-1}$)</th>
<th>InDSR TOC (mg L$^{-1}$)</th>
<th>OutDSR TOC (mg L$^{-1}$)</th>
<th>InDSR COD (mg L$^{-1}$)</th>
<th>OutDSR COD (mg L$^{-1}$)</th>
<th>InDSR NH$_3$ (mg L$^{-1}$)</th>
<th>OutDSR NH$_3$ (mg L$^{-1}$)</th>
<th>InDSR N$_2$ (mg L$^{-1}$)</th>
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<th>OutAN N$_2$ (mg L$^{-1}$)</th>
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<td>I</td>
<td>1–20</td>
<td>12–24</td>
<td>0.67</td>
<td>7.4</td>
<td>23</td>
<td>11</td>
<td>6.1</td>
<td>2.3</td>
<td>12</td>
<td>11</td>
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<tr>
<td>II</td>
<td>21–35</td>
<td>18</td>
<td>0.80</td>
<td>7.4</td>
<td>20</td>
<td>11</td>
<td>6.1</td>
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<td>III</td>
<td>36–55</td>
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<td>0.80</td>
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Stoichiometric ratio of TOC:COD was 2.4:0.02.
wastewater consisting of 600 mg L$^{-1}$ SO$_4^{2-}$, 120 mg L$^{-1}$ NH$_4^+$ and lactate made COD/SO$_4^{2-}$ = 0.5:1, 1:1, 1.5:1, 2:1, 2.5:1, 3:1 and 4:1. One milliliter of trace element solution was added to 1 L of feed, with the compositions listed in Chen et al. (2008b). Bicarbonate was used to maintain the influent pH at 8.5 ± 0.3. When the pH of sulfate reduction is between 8 and 9, sulfide is in an ionized state (Yuan et al. 2014).

The SR-CR unit was initially inoculated with 5 L of activated sludge, which was obtained from the Taiping Wastewater Treatment Plant (Harbin, China). The DSR granular sludge and the nitrifying biomass cultivated by Yuan et al. (2014) were used to inoculate the DSR unit and the BAF, respectively. The optimum reflux ratio (3:1) was used from the BAF to the DSR unit (Yuan et al. 2014).

### Chemical analysis

The sulfide, COD, total suspended solids and volatile suspended solids were determined according to Standard Methods (APHA 2005). As dissolved sulfide ions interfere with the COD measurements, TOC instead of COD in the effluent of the SR-CR unit was analyzed with a TOC analyzer (Shimadzu TOC-VCPH) to determine the organic strength of the samples. VFA concentrations and species were determined using a high-performance liquid chromatography (HPLC) system (Shimadzu LC-10A). The concentrations of nitrate, ammonium and sulfate in the collected liquor samples following 0.45 μm filtration were measured by an ion chromatography apparatus (Dionex ICS-3000 PCD-33A) equipped with a conductivity detector and an IonPac AG4A AS4A-SC 4 mm analytical column. The compositions of gas (N$_2$, N$_2$O, CO$_2$ and CH$_4$) were analyzed using a gas chromatography apparatus (Agilent 6890). Elemental sulfur analysis was performed following the sulfide method (Jiang et al. 2009). The S$^0$ conversion rate was calculated according to the description by Yuan et al. (2014).

### High-throughput 16S rRNA gene pyrosequencing and data analysis

The details of the DNA extraction, 16S rRNA gene polymerase chain reaction (PCR) amplification and Illumina MiSeq sequencing, and data analysis are available in the Supplementary Material (available online at http://www.iwaponline.com/wst/071/533.pdf).

### RESULTS AND DISCUSSION

#### Effects of different COD/SO$_4^{2-}$ ratios on the performance of the system

In stage I (1–35 days), the SR-CR unit was initially operated at a hydraulic retention time (HRT) of 24 h (1–20 days) and achieved 94% TOC removal efficiency. However, only 76% of SO$_4^{2-}$ was reduced to dissolved sulfide (S$^2^-$) (Figure 2(a)). The minimal COD requirement for sulfate reduction is 1 g of COD consumed per 1.5 g of SO$_4^{2-}$ reduced (COD/SO$_4^{2-}$ (g/g) = 0.67) (McCartney & Oleszkiewicz 1993). The initial COD/SO$_4^{2-}$ ratio was 0.5:1, indicating an insufficient reduction potential in the SR-CR unit.

During the same period, the DSR unit was connected to the SR-CR unit and received its effluent. The AN unit operating at a >95% rate of HN$_3^+$ conversion to NO$_3^-$ can provide approximately 90 mg NO$_3^-$ L$^{-1}$ for the DSR unit (Figure 2(d) and Table 1). In the DSR unit (1–20 days), the TOC, NO$_3^-$ removal efficiency and rate of S$^2^-$ conversion to S$^0$ reached 99%, 10.75% and 67%, respectively (Figure 2(c)). A lower TOC/NO$_3^-$ ratio in the DSR unit led to accumulation of nitrite (71.13 mg NO$_3^-$ L$^{-1}$), which can affect the rate of S$^2^-$ conversion to S$^0$ (Table 1). Reyes-Avila et al. (2004) proposed that the DSR process is achieved by converting NO$_3^-$ → NO$_2^-$ via the autotrophic denitrification (S$^2^-$ → S$^0$) pathway (1) and converting NO$_2^-$ → N$_2$ via the heterotrophic denitrification (acetate → CO$_2$) pathway (2). At TOC/NO$_3^-$ – N ratio < 1.26:1, the NO$_2^-$ will be accumulated gradually because of the loss of function of hNRB. The accumulation of nitrite in the DSR unit will decrease the activity of autotrophic denitrifiers, causing a lower S$^0$ production efficiency (Chen et al. 2008c).

\[
S^2^- + NO_3^- + H_2O \rightarrow S^0 + NO_2^+ + 2OH^- , \quad (1)
\]

\[
3CH_3COO^- + 8NO_2^- + H_2O \rightarrow 6CO_2 + 4N_2 + 11OH^- . \quad (2)
\]

When the COD/SO$_4^{2-}$ ratio was increased to 1:1 (21–35 days), the efficiency of SO$_4^{2-}$ reducing to S$^2^-$ was increased to 96% in the SR-CR unit. Piña-Salazar et al. (2011) operated a sulfate-reducing down-flow fluidized bed
reactor at COD/\(\text{SO}_4^{2-}\) ratios ranging from 0.67 to 2.5 and also confirmed that sulfate removal efficiency is improved with an increase in the COD/\(\text{SO}_4^{2-}\) ratio. The TOC removal efficiency was reduced to 78%, and there was no methane produced in the SR-CR unit at this stage. Meanwhile, the DSR unit achieved a TOC of 99%, a NO\(_3^-\) removal efficiency of 80% and a S\(^2^-\) conversion to S\(^0\) of 96.8%. In this stage, the entire system could achieve 99% COD, 76–94% SO\(_4^{2-}\) and 95% HN\(_3^-\) removal efficiency. The rate of S\(^2^-\) conversion to S\(^0\) was increased from 67 to 96% with the improvement of the COD/\(\text{SO}_4^{2-}\) ratio from 0.5:1 to 1:1.

In stage II (36–85 days), when the influent COD/\(\text{SO}_4^{2-}\) ratio of the SR-CR reactor was increased to 1.5:1 (36–55 days), the TOC removal efficiency increased from 45 to 78% and the sulfate reduction rate reached 94%. The reactor gradually began to produce methane, and the production of methane reached 0.065 m\(^3\) m\(^{-3}\) d\(^{-1}\) (Figure 2(b)). Lactate could be degraded to acetate easily by acid-forming bacteria. VFAs in the effluent of the SR-CR consisted mainly of acetate, which accounted for 95% of the TOC. When the COD/\(\text{SO}_4^{2-}\) ratio was further increased to 2:1 (56–70 days) and 3:1 (71–85 days), the TOC removal efficiency gradually increased to 87% and the sulfate reduction efficiency was still maintained at 95%. This result is in agreement with the study of Velasco et al. (2008), who reported 87% ethanol removal and 94% sulfate efficiency at a COD/\(\text{SO}_4^{2-}\) ratio of 2.5:1 in an up-flow anaerobic sludge blanket reactor. In addition, the production of methane improved from 0.065 to 0.523 m\(^3\) m\(^{-3}\) d\(^{-1}\). Meanwhile, the DSR unit achieved 95% TOC, 96% NO\(_3^-\) removal efficiency and 96% S\(^2^-\) conversion to S\(^0\) (Figure 2(c)). In this stage, the influent TOC/NO\(_3^-\) ratio of the DSR unit was approximately 1:1, which was an appropriate ratio for maximum sulfur production (Reyes-Avila et al. 2004; Chen et al. 2008b).

In this stage, the entire system could maintain approximately 95% removal efficiencies of COD, SO\(_4^{2-}\) and HN\(_3^-\) and a 96% rate of S\(^2^-\) conversion to S\(^0\). This result is also in agreement with the results of the previous study of Yuan et al. (2014), who reported 98% COD, 98% sulfate and 78% nitrogen removal efficiency at a COD/\(\text{SO}_4^{2-}\) ratio of 1.4:1 in this system.

In stage III (86–115 days), as the COD/\(\text{SO}_4^{2-}\) ratio increased from 3.5 to 4, the TOC removal efficiency increased from 88 to 95%, whereas sulfate reduction efficiency decreased from 66 to 40%. The production of methane increased considerably from 0.639 to 0.813 m\(^3\) m\(^{-3}\) d\(^{-1}\). McCartney & Oleszkiewicz (1993) also observed that sulfate could not achieve full reduction when the COD/\(\text{SO}_4^{2-}\) ratio was higher than 3.0. Meanwhile, the corresponding removal efficiency for NO\(_3^-\) in the DSR unit increased from 79 to 40%. However, the TOC removal efficiency remained at 95%. The rate of S\(^2^-\) conversion to S\(^0\)
decreased considerably from 72 to 36%. In this stage, the entire system could achieve 99% COD, 66–40% SO$_2^-$/C0$_4^-$ and 95% HN$_3^+$ removal efficiency. The rate of S$^{2-}$ conversion to S$^0$ was decreased from 79 to 40% with an increase in the COD/SO$_2^-$ ratio from 3.5 to 4. The insufficient sulfate reduction in the SR-CR unit led to a lower rate of S$^{2-}$ conversion to S$^0$ in the DSR unit.

**Microbial community structure and dominant species analysis**

SR1 (sample name), SR2 and SR3 were sampled from stage I (Day 35), stage II (Day 85) and stage III (Day 115), respectively. The sequence numbers in the three samples of SR1, SR2 and SR3 were 18,840, 21,290 and 23,860, respectively. The relative operational taxonomic units (OTUs) number comparison of the three samples was SR1 > SR2 > SR3 (Figure 3). The rarefaction curves and the estimated species richness YY for each sample indicated that the relative diversity comparison of the three samples was SR3 > SR2 > SR1.

To understand the microbial community structure and the succession in the SR-CR unit at the three stages, the classified OTUs were analyzed at the family level (Figure 4). Meanwhile, to explain the key role of the dominant functional species, the classified OTUs were analyzed at the genus level (Table 2).

In stage I, sample SR1 exhibited relative abundances for the Desulfomicrobiaceae (6.06%), Desulfobulbaceae (7.98%) and Methanobacteriaceae (1.4%) families in the SR-CR unit (Figure 4). *Desulfovibrio* (6.06%), *Desulfobulbus* (3.18%) and *Desulfocapsa* (4.73%) species were enriched in the SR-CR unit and are capable of reducing sulfate to sulfide (Barton & Fauque 2003; Prato-Garcia et al. 2013) (Table 2). *Methanobacterium* (1.01%) and *Methanosphaera* (0.34%) species only exhibited obviously lower abundance (Table 2). Choi & Rim (1991) studied the methane-producing potential and sulfate-reducing efficiency at various COD/SO$_2^-$ ratios. When the COD/SO$_2^-$ ratio was less than 1.0, the SRB were dominant, and no methane was produced in the course of the test. Wang et al. (2009) also found that there was no methane production in the process of treating synthetic saline wastewater (265 mg COD L$^{-1}$, 500 mg SO$_2^-$/C0$_4^-$ L$^{-1}$, COD/SO$_2^-$ = 0.53).

In stage II, the relative abundances for the Desulfomicrobiaceae (9.98%), Desulfobulbaceae (10.04%) and Methanobacteriaceae (9.97%) families were increased with an increase in the COD/SO$_2^-$ ratio from 1:1 to 3:1 (Figure 4). *Desulfovibrio* (9.98%), *Desulfobulbus* (4.82%) and *Desulfoptocapsa* (5.07%) species were obviously dominant in the SR-CR unit. *Methanobacterium* (7.67%) and *Methanosphaera* (2.3%) species became enriched, and methane was produced gradually in this stage (Table 2).

In stage III, the relative abundances for Desulfomicrobiaceae (4.53%) and Desulfobulbaceae (2.44%) were decreased with an increase in the COD/SO$_2^-$ ratio from 3.5:1 to 4:1 (Figure 4). However, Methanobacteriaceae (21.04%) began to exhibit obviously higher abundance. *Methanobacterium* (15.03%) and *Methanosphaera* (5.7%) species were obviously dominant in the SR-CR unit. *Desulfovibrio* (4.53%), *Desulfobulbus* (1.14%) and *Desulfoptocapsa* (1.05%) species sharply decreased in this stage (Table 2). McCartney & Oleszkiewicz (1996) also observed that sulfate could not achieve full reduction when the COD/SO$_2^-$ ratio was higher than 3.0. Koster et al. (1986) and Harada et al. (1994) also found that the...
MPB were insensitive to the toxicity of sulfide and gradually gained the ability to compete for electron donors with the increasing COD/\(\text{SO}_4^{2-}\) ratio. Our study also corroborated the finding that COD was removed gradually by the MPB with an increase in the COD/\(\text{SO}_4^{2-}\) ratio. The performance of the DSR unit is greatly affected by insufficient sulfate reduction efficiency in the SR-CR unit.

### CONCLUSIONS

The effects of different COD/\(\text{SO}_4^{2-}\) ratios on the performance of the integrated C-S-N removal system were investigated. This study provided insights into microbial community succession and the functional role of the dominant species in the SR-CR unit under various COD/\(\text{SO}_4^{2-}\) ratios. When the COD/\(\text{SO}_4^{2-}\) ratio was between 1:1 and 3:1, the SR-CR unit could find a balance between the sulfate-reducing efficiency and the methane-producing efficiency. The SR-CR unit could thereby provide appropriate concentrations of TOC and dissolved sulfide for the DSR unit to realize a maximum S\(^0\) conversion efficiency. When the COD/\(\text{SO}_4^{2-}\) ratio was higher than 3.5:1 or lower than 1:1, the SR-CR unit was found to exhibit insufficient sulfate reduction efficiency. The performance of the DSR unit is greatly affected by insufficient sulfate reduction in the SR-CR unit.

### ACKNOWLEDGEMENTS

We gratefully acknowledge the support from the National High-tech R&D Program of China (863 Program, Grant No. 2011AA060904), the National Science Foundation for Distinguished Young Scholars (Grant No. 51225802), the Science Fund for Creative Research Groups of the National Natural Science Foundation of China (Grant No. 51121062), the National Key Technology Research and Development Program of the Ministry of Science and Technology of China (2010BAC67B02) and from the National Natural Science Foundation of China (Grant Nos 51176037 and 51308147).

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First received 18 August 2014; accepted in revised form 16 December 2014. Available online 29 December 2014.