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RESEARCH ARTICLE

Individual and combined inhibition of phenol and thiocyanate on microbial activity of partial nitritation

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Abstract This study evaluated the individual and interactive effect of phenol and thiocyanate (SCN^-) on partial nitritation (PN) activity using batch test and response surface methodology. The IC_{50} of phenol and SCN^- on PN sludge were 5.6 and 351 mg L^{-1}, respectively. The PN sludge was insensitive to phenol and SCN^- at levels lower than 1.77 and 43.3 mg L^{-1}, respectively. A regression model equation was developed and validated to predict the relative specific respiration rate (RSRR) of PN sludge exposed to different phenol and SCN^- concentrations. In the range of independent variables, the most severe inhibition was observed with a valley value (17%) for RSRR, when the phenol and SCN^- concentrations were 4.08 and 198 mg L^{-1}, respectively. An isobole plot was used to judge the combined toxicity of phenol and SCN^-, and the joint inhibitory effect was variable depending on the composition and concentration of the toxic components. Furthermore, the toxic compounds showed independent effects, which is the most common type of combined toxicity.

Keywords Partial nitritation · Anammox · Phenol · Thiocyanate · Toxicity · Response surface methodology

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Introduction

Reducing ammonium nitrogen concentration in wastewater effluent is one of the major goals to prevent aquatic eutrophication. In general, autotrophic nitrogen removal by combined partial nitritation (PN) and anaerobic ammonium oxidation (anammox) has attracted considerable attention in recent years as an alternative to convention nitrogen removal processes, because the PN-anammox is a cost-effective and energy-saving potential process. Nitrogen removal with PN-anammox is possible for low C/N ratio wastewater treatments, and approximately 30–40% of the overall nitrogen removal cost will be saved compared to nitrification or denitrification and does not require organic carbon source (Desloover et al. 2011). In the PN-anammox process, half of the ammonium (NH_4^+-N) is oxidized with oxygen to nitrite (NO_2^--N) by PN, which provides NO_2^--N for anammox. Subsequently, NH_4^+-N and NO_2^--N are directly converted to nitrogen gas (N_2) by anammox (Huang et al. 2016; Lotti et al. 2014).

Phenol and thiocyanate (SCN^-) are toxic compounds to most living things, and landfill leachates as well as several industries such as coke plants and refineries produce complex wastewater containing NH_4^+-N, phenol, and SCN^- (Milia et al. 2012; Morita et al. 2007). The phenol concentration ranges between 0.35 and 1040 mg L^{-1} in landfill leachate and coke wastewater (Aziz et al. 2010; Toh and Ashbolt 2002), whereas the SCN^- concentration has been reported as high as 535 mg L^{-1} (Toh and Ashbolt 2002). Satisfactory treatment of leachate is not trivial due to its high concentrations of ammonium nitrogen and toxic compounds. Thus, it is vital to study phenol/SCN^--mediated inhibition of the PN-anammox process to guide the research and application of this method to treat both nitrogen- and phenol/SCN^--containing wastewater.

Several studies have reported phenol inhibition on anammox activity or biomass (Hou et al. 2014; Jin et al. 2013b; Pereira et al. 2014; Toh and Ashbolt 2002; Yang et al. 2013; Yang and Jin 2012), and the inhibition of SCN^- on anammox has also been assessed by Chen et al. (2017a, b) and Toh and Ashbolt (2002). However, systems based on granular biomass tend to perform better in the presence of inhibitory or toxic compounds because the granule architecture creates diffusion gradients that help protect sensitive bacteria (Maszenan et al. 2011). In this sense, some studies have suggested the possibility that anammox granules acclimate in phenol-containing wastewater (Toh and Ashbolt 2002). Moreover, as the first step of the PN-anammox process, PN is the fundamental basis for a successful PN-anammox operation (Fudala-Ksiazek et al. 2014). PN sludge mainly contains two different autotrophic groups of bacteria, ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). It is well known that AOB and NOB are sensitive to environmental factors such as pH, temperature, heavy metals, and even organic solvents (Gilbert et al. 2014). However, little research has examined the effect of toxic substances (phenol and SCN^-) present in the landfill leachate or coke wastewater on the PN process or their combined toxicity. Mixture exposure is a universal phenomenon; however, assessing the potential toxic effects and quantifying the exposure risk to chemical mixtures in landfill leachate and coke wastewater still remains a major challenge. Consequently, the joint, cumulative effects of toxic mixtures might cause microbial risk. Models for predicting the effect of mixtures are urgently needed.

A certain composite design (CCD) coupled with response surface methodology (RSM) is an efficient design tool to determine the optimal conditions for a desirable response. These methods were successfully applied for wastewater treatment to optimize hydrogen production and identify the inhibitory effects of toxicants (Rastegar et al. 2011; Xing et al. 2014).

Therefore, this study aims to (1) quantify the IC_{50} of phenol and SCN^- on PN sludge with short-term effects, (2) investigate the inhibition of phenol and SCN^- on AOB and NOB, and (3) evaluate the interactive effects of phenol and SCN^- on PN sludge activity using RSM with CCD.

Materials and methods

Activated sludge and synthetic medium

The activated sludge used in this study was collected from a 6.8-L internal-loop airlift reactor operated in continuous-flow mode at 30 ± 1 °C with a hydraulic retention time of 26 h. The DO concentration was maintained at 0.8–1.3 mg L^{-1}, and the level of free ammonia in the system was between 48.4 and 95 mg L^{-1}, which is favorable for partial nitrification. The synthetic wastewater and the element solution were made according to a modified formula described by Xing et al. (2013); the feed medium was devoid of organic carbon and contained 900–950 mg NH_4^+-N L^{-1}. The system ran with an NH_4^+-N removal efficiency of approximately 43–59% with the molar ratio of NO_2^--N/NH_4^+-N in the effluent from 0.8 to 1.2, and nitrite accumulation ([NO_2^--N]/[NO_x^--N] × 100%) ranged from 44.1 to 54.5%.

PN activity with batch tests

Batch assays were performed in triplicate, and the activity of PN sludge was measured using the respiration rate. The respirometric tests were used because the respiration rate of activated sludge is reduced in the presence of toxic substances and they provided an experimental assessment of the oxygen uptake rate (OUR). A Strathtox respirometer (Strathkelvin Strathtox, Scotland, UK) was used to measure the toxicity of trade effluents entering the wastewater treatment plant. This equipment was based on the respirometry applications in the biomedical field and used six oxygen electrodes simultaneously. The use of six oxygen electrodes allowed the respiration rate of a control sample of the same sludge mixed with five other different concentrations of toxic compounds. Tests were carried out in six 20-mL glass tubes. The tubes were kept stirred with a magnetic stir bar in a water bath of Strathtox unit. Toxic compounds at different concentrations were added to five testing glass tubes each with a total volume of 18 mL. After reaching the constant temperature of 25 °C, 2 mL of activated sludge was quickly added to each tube and oxygen electrodes were inserted into the tubes for recording the respiration rate values. The test was stopped as soon as the dissolved oxygen content of the mixed liquor in the tube with the fastest respiration rate had fallen to near zero. The concentration of each component is listed in Table 1.

The specific respiration rate (SRR) was calculated according to Eq. (1) and was used to express PN sludge activity.

$$SRR = \frac{OUR}{VSS} \times 10^{-3} \quad (1)$$

in which OUR is the oxygen uptake rate (mg L^{-1} h^{-1}) and VSS is the volatile suspended solids (g L^{-1}).

The relative specific respiration rate (RSRR), which was calculated according to Eq. (2), was used to express the relative PN sludge activity with toxic compounds at different concentrations compared to the control.

$$\mathrm{RSRR} = \frac{SRR_e}{SRR_c} \times 100\% \quad (2)$$

in which SRR_e represents the SRR of the experimental groups (with inhibitors or toxic compounds added during the experiment), and SRR_c represents the SRR of the control group.

Table 1 The compositions of the batch tests for respirometric calculations

Objective	Concentration (mg L^{-1})					
	NH_4^+-N	KH_2PO_4	$MgSO_4{\cdot}7H_2O$	$CaCl_2$	Trace elements I and trace elements II (mL L^{-1})	Phenol/SCN^-
Short-term effects of phenol on PN sludge	100	27.0	300	136	1.25	0, 2.50, 5.00, 7.50, 10.0, 12.5
Short-term effects of SCN^- on PN sludge	100	27.0	300	136	1.25	0, 200, 400, 600, 800, 1000

Composition of trace elements I (g L^{-1}): EDTA, 5.00; $FeSO_4{\cdot}7H_2O$, 9.14

Composition of trace elements II (g L^{-1}): EDTA, 15.00; H_3BO_4, 0.014; $ZnSO_4{\cdot}7H_2O$, 0.43; $MnCl_2{\cdot}4H_2O$, 0.99; $CuSO_4{\cdot}5H_2O$, 0.25; $NaMoO_4{\cdot}2H_2O$, 0.22; $NiCl_2{\cdot}6H_2O$, 0.21; $CoCl_2{\cdot}6H_2O$, 0.24

PN partial nitritation

The respiration inhibition ratio (RIR) was calculated as follows:

$$RIR = 100\% - \mathrm{RSRR} \tag{3}$$

Inhibition of AOB and NOB

Selective inhibitors of AOB and NOB were used to test the variable activity responses of AOB and NOB to phenol and SCN^-. Allylthiourea (ATU) was selected to inhibit AOB (Wang and Gu 2014), and $NaClO_3$ was chosen as an NOB inhibitor in this study.

First, the original specific oxygen uptake rate (SOUR) was tested and recorded as α. After 3 min, as the DO level decreased, $NaClO_3$ was added at a concentration of approximately 2.13 g L^{-1}, and the SOUR was measured and recorded as β. Three minutes later, ATU was added to each glass tube at a final concentration of approximately 5 mg L^{-1} to suppress AOB metabolism, and γ represented the SOUR at this point. As described above, the AOB activity was defined as $(\beta - \gamma)$, and the NOB activity was $(\alpha - \beta)$.

The interactive effects of phenol and SCN^-

A CCD was used to study the interactive effects of phenol and SCN^- on the PN activity response. Design Expert software version 8.0.6.1 (STAT-EASE Inc., Minneapolis, USA) was used to design the CCD experiment, to perform the regression analysis of the experimental data, and to plot the response surface and contour plots. In the experiment, both variables were assessed at five different coded levels, marked $-\alpha$, −1, 0, +1, and $+\alpha$. The ranges and levels of the independent input variables for the phenol and SCN^- concentrations were set as listed in Table 2 based on the results from PN activity with batch tests. The RSRR was selected as the dependent output variable. The CCD applied in this study is presented in Table 3, which provides the experimental conditions and their responses. As the response of the PN sludge activity, the RSRR (Y) was modeled as a second-order polynomial equation, showing the mathematical relationship between the independent variables phenol and SCN^- and Y, as Eq. (4) presents.

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i<j}^{n} \sum_{j=1}^{n} \beta_{ij} X_i X_j \tag{4}$$

in which Y is the predicted response, n is the number of factor variables, X_i and X_j are the coded levels of the independent variables, β_0 is the offset term, β_i is the ith linear coefficient, β_{ii} is the iith quadratic coefficient, and β_{ij} is the ijth interaction coefficient.

The model obtained from regression analysis was used to generate the response surface and contour plots. The coefficient of determination R^2 was used to express the quality of the polynomial model equation fit, and the significance was analyzed using an F test in the same program. A t test was used to test the significance of the regression coefficient (Xing et al. 2014). An analysis of variance (ANOVA) was used to evaluate the interaction between the process variables and the response, and $p < 0.05$ was considered statistically significant.

Table 2 Experimental ranges and levels of the independent test variables

Variable	Low axial ($-\alpha$)	Low factorial (−1)	Center point (0)	High factorial (+1)	High axial ($+\alpha$)
Phenol (mg L^{-1})	0.600	1.24	2.80	4.36	5.00
SCN^- (mg L^{-1})	51.0	87.5	175.5	263.5	300

Table 3 Experimental plan showing the different experimental sets for the CCD

Run	Experimental sets				RSRR (%)	
	Phenol (mg L^{-1})	Coded value	SCN^{-1} (mg L^{-1})	Coded value	Experimental value	Model predicted
1	2.80	0	175.5	0	27.8	27.5
2	2.80	0	175.5	0	25.7	27.5
3	4.36	+1	263.5	+1	29.5	27.1
4	2.80	0	300	$+\alpha$	42.6	47.1
5	1.24	−1	87.5	−1	86.3	89.1
6	2.80	0	175.5	0	26.5	27.5
7	5.00	$+\alpha$	175.5	0	24.1	22.8
8	2.80	0	175.5	0	31.2	27.5
9	1.24	−1	263.5	+1	71.1	67.2
10	2.80	0	175.5	0	27.8	27.5
11	2.80	0	51.0	$-\alpha$	79.6	74.7
12	0.60	$-\alpha$	175.5	0	82.1	83.0
13	4.36	+1	87.5	−1	39.8	44.1

The experiments were designed (Table 4) to validate the regression model equation obtained from the CCD experiment. The RSRR tests at phenol concentrations of 1.5, 3.0, and 4.5 mg L^{-1} with varying SCN^- concentrations were used to evaluate the effect of SCN^- on PN sludge activity. The validation experiments were performed in triplicate.

Assessment of the combined toxicity of phenol and SCN^-

Independent, additive, synergistic, and antagonistic effects describe the four main types of combined toxicities (Ding et al. 2015). Isobole plots (IP) are considered a simple and straightforward method for characterizing the combined toxicities of bicomponent mixtures (Ding et al. 2015).

Analytical methods and mathematical model

VSS levels were determined using standard methods (APHA, AWWA, AEF, 2012). The statistical tests were performed with the *F* test for ANOVA.

The modified non-competitive inhibition model (Eq. (5) was used to represent the inhibitory characteristics of the toxic compounds in the activated sludge (Jin et al. 2013a), and the model was fit to the experimental data using the minimum squared errors method.

$$RIR = \left(1 - \frac{1}{1 + \left(s / k_a\right)^b}\right) \times 100\% \quad (5)$$

in which *RIR* is the inhibition response, *s* is the concentration of phenol or SCN^- in the experiment, k_a is the IC_{50} value of phenol or SCN^- on PN sludge, and *b* is the fitting parameter.

Results and discussion

Individual toxicity

The acute toxicities of phenol and SCN^- individually under fixed initial substrate level conditions were obtained.

Table 4 Experiments to validate the regression model equation

Experimental set	Phenol (mg L^{-1})	SCN^- (mg L^{-1})	RSRR (%)	
			Model predicted	Experimental value
1	1.5	70	91.0	87.8
2	3.0	70	60.5	61.4
3	4.5	140	25.7	26.4
4	1.5	140	61.2	60.1
5	3.0	210	24.0	21.7
6	4.5	210	19.0	16.8

The modified non-competitive inhibition model described the acute-term effects of phenol on PN sludge activity expressed with RIR. Accordingly, the RSRR decreased as the phenol concentrations increased, and the IC_{50} of phenol on PN sludge was 5.60 mg L^{-1} ($RIR = 100\ \% \times (1 - 1/(1 + (s/5.60)^{1.91}))$($R^2 = 0.9772$)). In a study by Liu et al. (2005), nitrifying sludge tolerated the toxicity of an initial phenol concentration below 10 mg L^{-1}, although a partial irreversible disruption of the nitrification was observed when the exposure to phenol was above 15 mg L^{-1}. However, large differences were observed in a pure culture experiment to simultaneously remove toxic pollutants. In a pure culture study by Lauchnor et al. (2011), the ammonia oxidation rates were inhibited by 50% when exposed to 5.27 mg L^{-1} phenol, whereas in a study using adapted activated sludge to treat coke wastewater, significant nitrification inhibition was observed at a phenol concentration of 200 mg L^{-1} (Kim et al. 2008). The tremendous differences between the IC_{50} levels might be caused by variations in the microorganism species, the sludge structure, or the operating conditions.

The toxic effect of SCN^- on PN sludge was initially apparent as the concentrations increased. During the acute exposure batch tests, the IC_{50} of SCN^- on PN sludge was 351 mg L^{-1} ($RIR = 100\ \% \times (1 - 1/(1 + (s/351)^{1.05}))$($R^2 = 0.8792$)). An inhibitory effect on nitrification was observed when the concentration of SCN^- was above 200 mg L^{-1} as well as increased ammonia loading (Kim et al. 2008). However, in this study, 200 mg L^{-1} SCN^- inhibited specific respiration by approximately 44.0%.

The sensitivity of PN sludge activity was characterized by the IC_{10} (concentration inhibiting the activity by 10%). Concentrations below this were regarded as relatively safe, and the IC_{10} values were obtained using the modified non-competitive inhibition model mentioned above. Accordingly, the IC_{10} of phenol and SCN^- on PN sludge were 1.77 and 43.3 mg L^{-1}, respectively, suggesting the peak concentrations of PN sludge were not inhibited when treating wastewater containing phenol and SCN^-. Moreover, considering the practical concentration of phenol and SCN^- in industrial effluents such as coke wastewater and landfill leachate (Table 5), the concentration of phenol in coke wastewater is generally above 15 mg L^{-1} and ranges between 0.35 and 33 mg L^{-1} in landfill leachate. Thus, serious inhibition of PN sludge occurred during coke wastewater treatment, whereas slight inhibition was observed during the treatment of landfill leachate. However, the diversity of the sludge structure and the acclimatization of the microorganisms contribute to sludge tolerance in unfavorable conditions (Cho et al. 2014; Kim et al. 2013).

Phenol and SCN^- inhibition of AOB and NOB

By adding ATU and $NaClO_3$, selective inhibitors of AOB and NOB, the inhibition of phenol and SCN^- on AOB and NOB was quantified.

The IC_{50} values for phenol on AOB and NOB were 4.52 and 7.56 mg L^{-1}, respectively, as shown in Fig. 1a. The results show that AOB is more sensitive to phenol stress than NOB, and this phenomenon is caused by a DO concentration imbalance in the bulk liquid. Phenol reduced the NH_4^+-N removal rate, and the consumption of nitrite was not affected (Toh and Ashbolt 2002), as demonstrated by the substrate consumption results. Dyreborg and Arvin (1995) demonstrated that the pseudo-critical concentration of phenol was 3.7 mg L^{-1} in a pure culture of nitrifying microorganisms. As Morita et al. (2007) reported, 5.0 mg L^{-1} phenol completely inhibited the ammonia oxidation capability of suspended *Nitrosomonas europaea* cells.

Table 5 Phenol and SCN^- levels in landfill leachate and coke wastewater and the removal method used

	NH_4^+-N (mg L^{-1})	Phenol (mg L^{-1})	SCN^- (mg L^{-1})	Treatment method	Test conditions	References
Coke wastewater	5100	50–550	535	Anammox	Long-term operation, adaption	Toh and Ashbolt (2002)
	330	333	184	NA		
	50–500	~15	10–200	Nitrification	Batch tests	Kim et al.(2008)
	182–259	342–487	96–146	MBBR	Batch tests and long-term operation	Li et al. (2011)
	47–110	282–562	242–721	Nitrification	Batch response surface analysis tests	Cho et al. (2014)
	92–118	198–249	NA	Nitritation	Long-term operation	Hou et al. (2014)
Landfill leachate	130–2150	0.35–5.25	NA	NA	Results from different leachate compositions	Aziz et al. (2010)
	530 ± 10	2.6 ± 0.4	NA	Fenton treatment	–	Kochany and Lipczynska-Kochany (2009)
	710–2000	33 ± 3	NA	Chemical and biological oxidation processes	Chemical and biological oxidation	Klauson et al. (2015)

NA not available, *MBBR* moving bed biofilm reactor

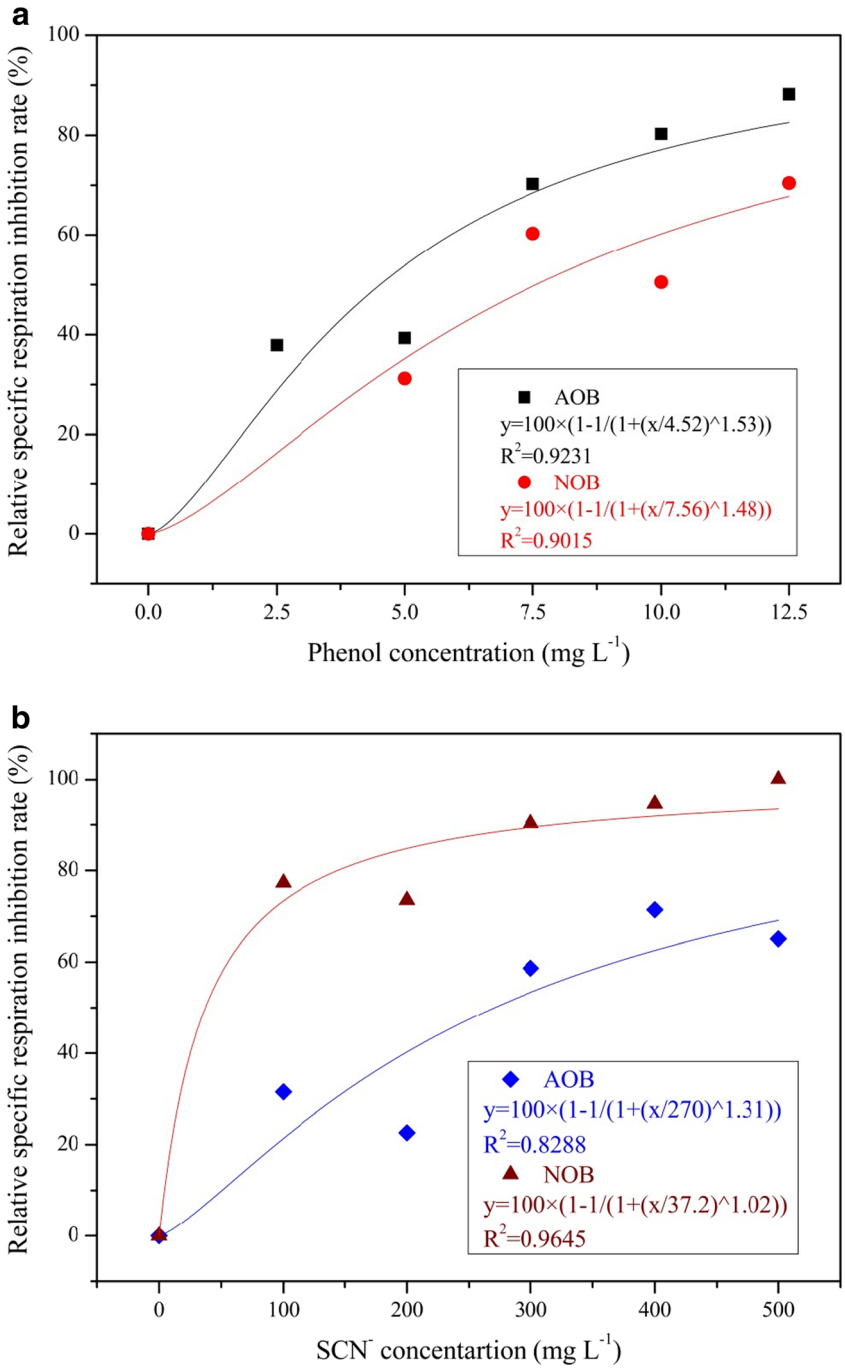

Fig. 1 Inhibition of different phenol (**a**) and SCN^- (**b**) concentrations on the activity of AOB and NOB

In the presence of SCN^-, the IC_{50} values for AOB and NOB were 270 and 37.2 mg L^{-1}, respectively, as shown in Fig. 1b. Both AOB and NOB were sensitive to SCN^- exposure, which might be caused by the SCN^- inhibition of several enzyme systems and Mg^{2+} ATPases (Gould et al. 2012). NOB is relatively more sensitive to thiocyanate than AOB.

RSM experiment and statistical analysis

Thirteen experiments were performed randomly to minimize the interference of uncontrolled variables on the obtained responses. The quadratic multinomial regression model is summarized in Eq. (6):

$$\begin{aligned} \mathrm{RSRR} = {} & 197 - 44.6 \times [\mathrm{Phenol}] - 0.892 \times [SCN^-] + 8.94 \\ & \times 10^{-3} \times [\mathrm{Phenol}] \times [SCN^-] + 5.25 \\ & \times [\mathrm{Phenol}]^2 + 2.16 \times 10^{-3} \times [SCN^-]^2 \end{aligned} \quad (6)$$

Statistical tests involving the reduced quadratic models for the RSRR were performed with the F test for the ANOVA analysis, and the results are shown in Table 6.

Table 6 ANOVA on the response surface quadratic model

Source	Sum of squares	*df*	Mean square	*F* value	*p* value
Model	7109.45	5	1421.89	86.62	<0.0001
A	3617.79	1	3617.79	220.40	<0.0001
B	757.11	1	757.11	46.12	0.0003
AB	6.00	1	6.00	0.37	0.5644
A^2	1124.45	1	1124.45	68.50	<0.0001
B^2	1943.3	1	1943.30	118.39	<0.0001
Residual	114.90	7	16.41		
Lack of fit	94.93	3	31.64	6.34	0.0533
Pure error	19.97	4	4.99		
Cor total	7224.35	12			
SD	4.05	R^2	0.9841		
Mean	45.57	Adj R^2	0.9727		
CV%	8.89	Pred R^2	0.9022		
PRESS	706.26	Adeq Precision	24.085		

Accordingly, the intercept coefficient (β_0), the linear coefficient (β_i), the quadratic coefficient (β_{ii}), and the interaction coefficient (β_{ij}) all exhibited significant effects. Furthermore, the influence of the individual operational parameters on the RSRR followed the order of phenol > SCN^-, and both of the linear coefficients (−44.6 and −0.892) were below zero, indicating that they negatively influence (inhibiting or toxic) the RSRR. The model was highly significant, with an *F* value of 86.62 and probability values of less than 0.05 for a 95% confidence interval. A "Pred R^2" of 0.9022 is in reasonable agreement with the "Adj R^2" of 0.9727. "Adeq Precision" measures the signal-to-noise ratio, and the calculated value of 24.085 was greater than 4, suggesting adequate signal, meaning that the model can be used to navigate design space (Xing et al. 2014).

Good convergence between the experimental and predicted values are also depicted in Fig. 2a, and the predicted values versus the actual values almost graphed on the line "$y = x$." In Fig. 2b, a random distribution was observed for the residual plots for the models and the RSRR dataset, and the absolute value of the internally studentized residuals remained low. Moreover, the six additional experiments (Table 5) confirmed that the RSRR experimental values are very close to the predictive model values. Based on these results, the model is suitable for the design space.

Interactive effects of phenol and SCN^- on PN sludge activity

Three-dimensional response surface plots and two-dimensional contour plots were constructed to get a better visualization of the levels of the independent variables (phenol and SCN^-). The output of the predictive model for RSRR is shown in Fig. 3a (three-dimensional response surface) and Fig. 3b (two-dimensional contour plots).

It can be concluded from Fig. 3a that the RSRR increased slightly when the concentration of phenol and SCN^- were below 0.79 and 54.6 mg L^{-1}, respectively. However, the RSRR decreased with further increases in the phenol and SCN^- concentrations. The minimum RSRR was identified as the surface confined to the smallest curve of the contour plot. In the range of independent variables, the most severe inhibition was observed with a valley value (17%) for the RSRR when the phenol and SCN^- concentrations were 4.08 and 198 mg L^{-1}, respectively.

The shape of the contour reflects the intensity of the interaction between the factors, and an ellipse represents a significant interaction, whereas a circle indicates insignificance (Chen et al., 2014). The phenol and SCN^- contours are elliptical, indicating that the interaction between phenol and SCN^- is a mostly significant interaction. In the perturbation study, phenol played a dominant role when its concentration was below 2.8 mg L^{-1} and the concentration of SCN^- did not exceed 175.5 mg L^{-1}; however, when the phenol concentration was above 2.8 mg L^{-1} and SCN^- exceeded 175.5 mg L^{-1}, SCN^- was the more toxic compounds. These findings agree with the sharp curve for phenol and SCN^-. In Fig. 3b, for a constant phenol concentration of 0.6 mg L^{-1} and a concentration range for SCN^- of 51–300 mg L^{-1}, the RSRR response was maintained above 81% and only slightly fluctuated. However, when the concentration of SCN^- was fixed at 51 mg L^{-1}, the RSRR response ranged between 64 and 124% (Fig. 3b).

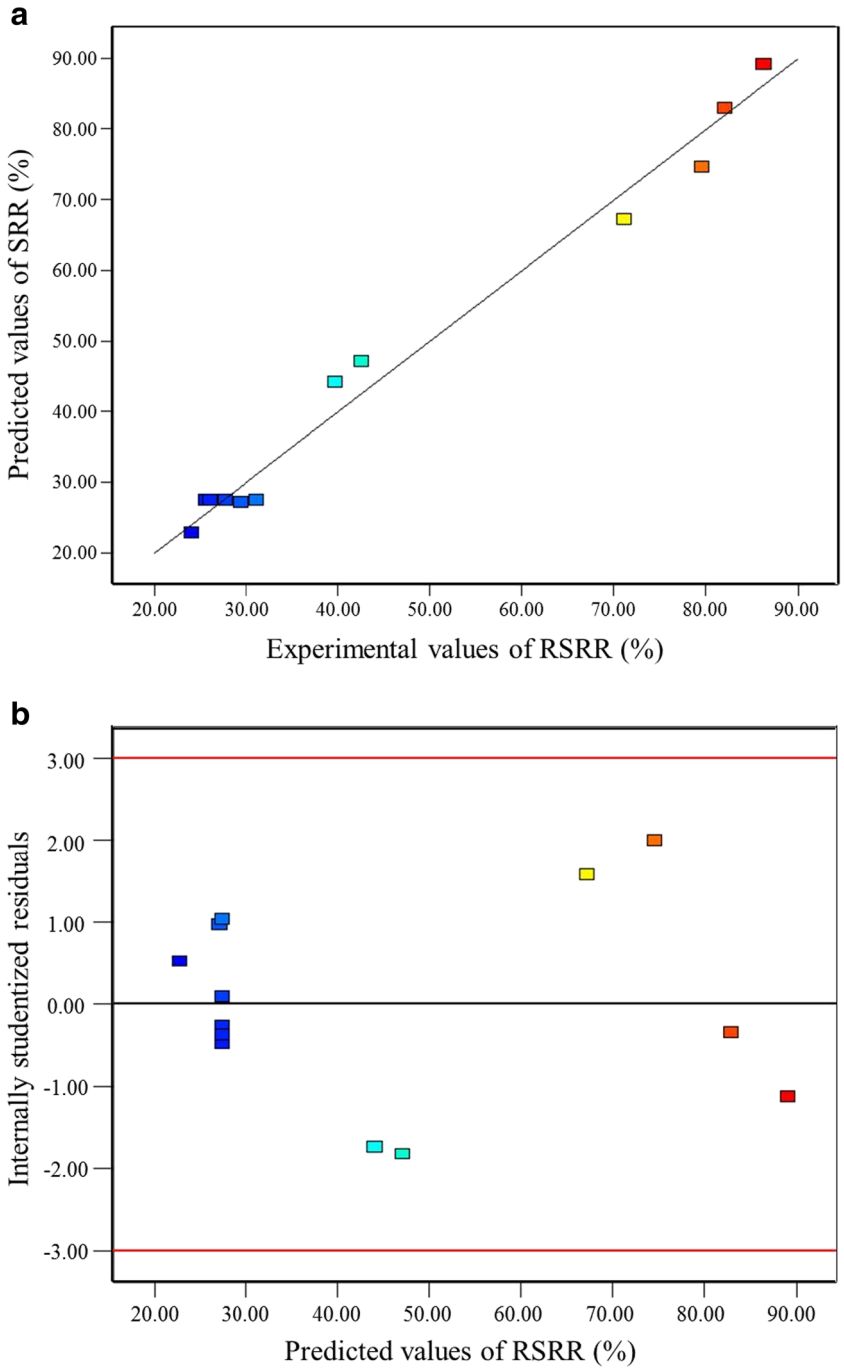

Fig. 2 Predicted versus actual values for RSRR (**a**) and internally studentized residuals in the residuals versus the predicted (**b**)

Because the joint toxicities of multicomponent mixtures are usually different from the toxicities of the individual chemicals (Ding et al. 2015), an IP was used to judge the combined toxicity of phenol and SCN^- as shown in Fig. 4. The joint toxic effect of phenol and SCN^- shifted from synergistic to additive, then to independent and finally to antagonistic with increasing concentrations. Accordingly, they shared more space independently.

Although both phenol and SCN^- exhibited toxic effects, the sensitivity of PN sludge does not necessarily mean that the activity of PN sludge is absent in a reactor treating substances that inhibit PN. For low concentrations of phenol and SCN^-, the effective concentration of the inhibitor can be lowered by absorption, precipitation, chelation, and biodegradation (Vázquez et al. 2006). As the concentration of phenol and SCN^- increased, the absorption sites reached saturation, and an additive effect was observed. No cross-inhibition of

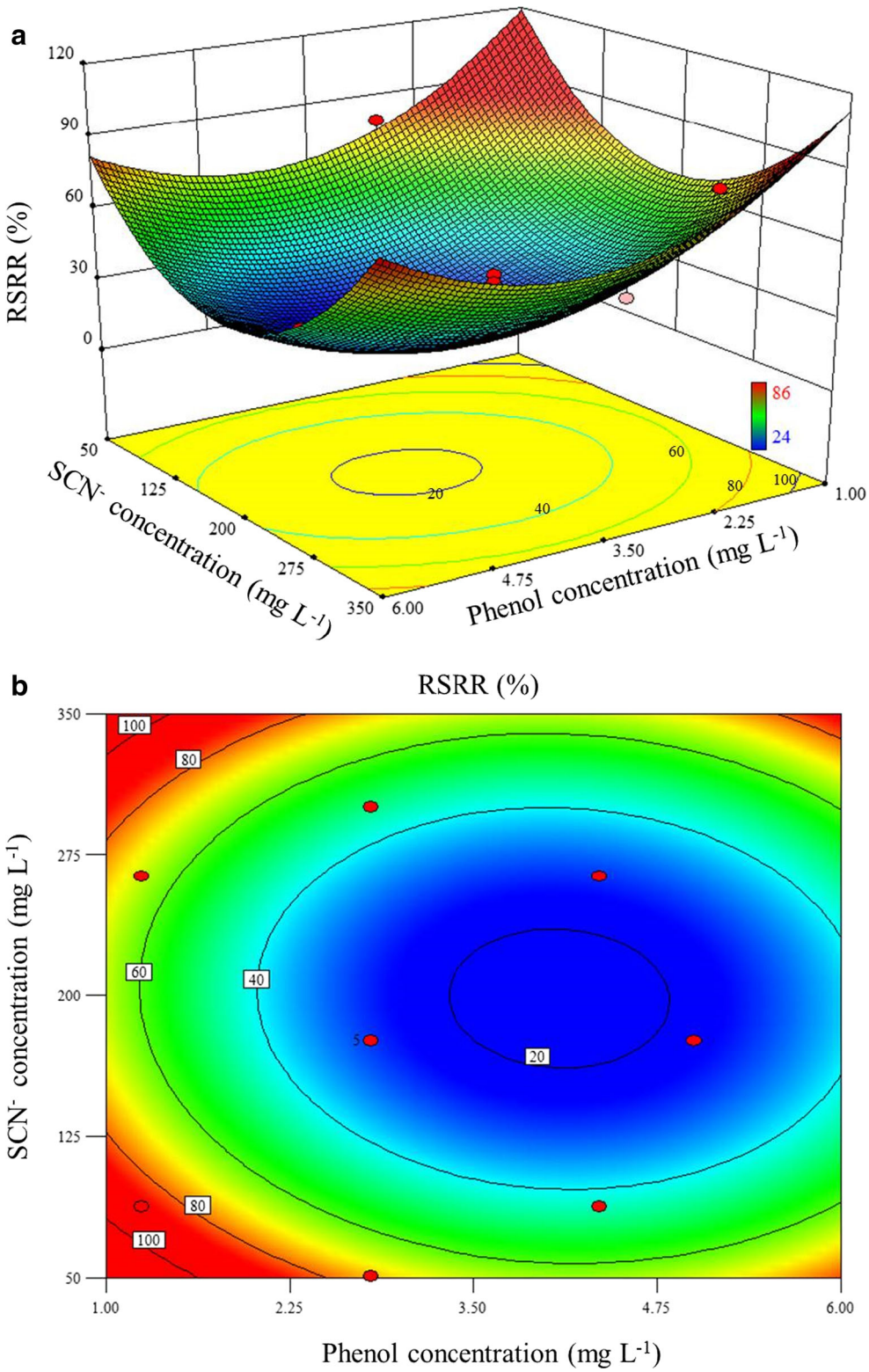

Fig. 3 Three-dimensional (**a**) and contour plots (**b**) for the response variable (RSRR) with respect to the independent variables (phenol and SCN^-)

PN sludge activity was apparent in the independent area, and when the phenol concentration exceeded 5.95 mg L^{-1}, the presence of SCN^- aggravated the toxic effects on PN sludge.

In a PN process for treating wastewater from coke plants, refineries, and landfill leachates, some major compounds present in the wastewater would cause an inhibitory effect on microbial activity in activated sludge. Particularly, phenol most seriously inhibits PN in activated sludge; the influx of toxic compounds into the process should be controlled below their threshold concentrations.

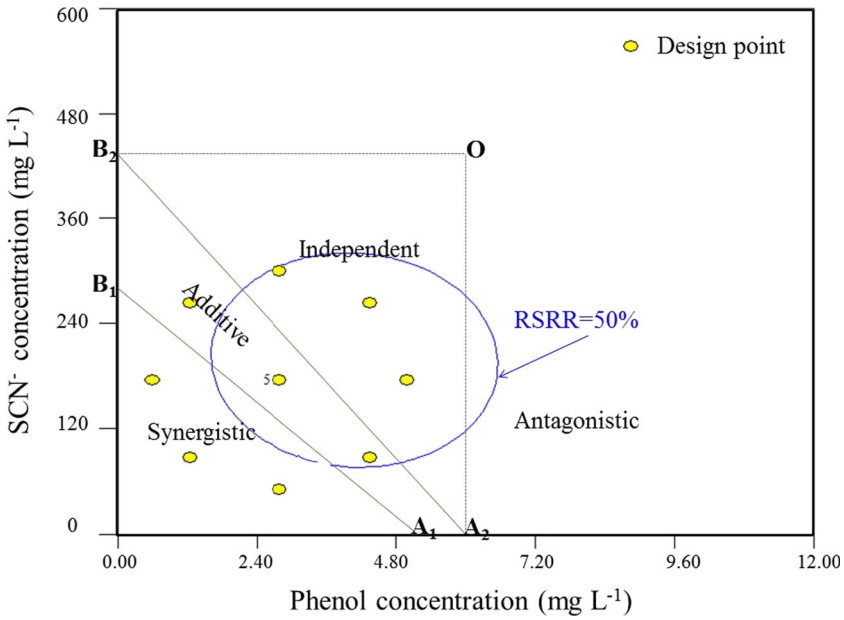

Fig. 4 Equivalent effect picture of the joint toxicity of phenol and SCN^-. A_1 and A_2 represent the 95% confidence intervals for the IC_{50} of phenol, and B_1 and B_2 represent the 95% confidence intervals for the IC_{50} of SCN^-

Conclusions

The IC_{50} values of phenol and SCN^- on PN sludge were 5.60 and 351 mg L^{-1}, respectively. The relatively sustainable phenol and SCN^- concentrations for PN sludge were 1.77 and 43.3 mg L^{-1}, respectively. Based on batch tests, AOB is more sensitive to phenol than NOB, whereas NOB is more seriously suppressed by SCN^-. The RSM analysis indicated a significant interaction between phenol and SCN^-. The most severe inhibition, resulting in an RSRR of 17.0%, occurred when the concentrations of phenol and SCN^- were 4.08 and 198 mg L^{-1}, respectively. The joint inhibition effect of phenol and SCN^- tended to vary based on the different concentration of the toxic components.

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References

APHA, AWWA, AEF (2012) Standard methods for the examination of water and wastewater, 22nd ed. American Public Health Association, Washington, DC, USA.

Aziz SQ, Aziz HA, Yusoff MS, Bashir MJ, Umar M (2010) Leachate characterization in semi-aerobic and anaerobic sanitary landfills: a comparative study. J Environ Manag 91:2608–2614

Chen QQ, Chen H, Zhang ZZ, Guo LX, Jin RC (2017a) Effects of thiocyanate on granule-based anammox process and implications for regulation. J Hazard Mater 321:81–91

Chen QQ, Sun FQ, Guo Q, Shen YY, Zhu WQ, Jin RC (2017b) Process stability in an anammox UASB reactor with individual and combined thiocyanate and hydraulic shocks. Sep Purif Technol 173: 165–173

Chen H, Yu JJ, Jia XY, Jin RC (2014) Enhancement of anammox performance by cu (II), Ni (II) and Fe (III) supplementation. Chemosphere 117:610–616

Cho KH, Kim JO, Kang S, Park H, Kim S, Kim YM (2014) Achieving enhanced nitrification in communities of nitrifying bacteria in full-scale wastewater treatment plants via optimal temperature and pH. Sep Purif Technol 132:697–703

Desloover J, De Clippeleir H, Boeckx P, Du Laing G, Colsen J, Verstraete W, Vlaeminck SE (2011) Floc-based sequential partial nitritation and anammox at full scale with contrasting N_2O emissions. Water Res 45:2811–2821

Ding S, Wu J, Zhang M, Lu H, Mahmood Q, Zheng P (2015) Acute toxicity assessment of ANAMMOX substrates and antibiotics by luminescent bacteria test. Chemosphere 140:174–183

Dyreborg S, Arvin E (1995) Inhibition of nitrification by creosote-contaminated water. Water Res 29:1603–1606

Fudala-Ksiazek S, Luczkiewicz A, Fitobor K, Olanczuk-Neyman K (2014) Nitrogen removal via the nitrite pathway during wastewater co-treatment with ammonia-rich landfill leachates in a sequencing batch reactor. Environ Sci Pollut Res 21:7307–7318

Gilbert EM, Agrawal S, Karst SM, Horn H, Nielsen PH, Lackner S (2014) Low temperature partial nitritation/anammox in a moving bed biofilm reactor treating low strength wastewater. Environ Sci Technol 48:8784–8792

Gould WD, King M, Mohapatra BR, Cameron RA, Kapoor A, Koren DW (2012) A critical review on destruction of thiocyanate in mining effluents. Miner Eng 34:38–47

Hou B, Han H, Jia S, Zhuang H, Zhao Q, Xu P (2014) Effect of alkalinity on nitrite accumulation in treatment of coal chemical industry wastewater using moving bed biofilm reactor. J Environ Sci 26:1014–1022

Huang X, Sun K, Wei Q, Urata K, Yamashita Y, Hong N, Hama T, Kawagoshi Y (2016) One-stage partial nitritation and anammox in membrane bioreactor. Environ Sci Pollut Res 23:1–14

Jin RC, Yang GF, Zhang QQ, Ma C, Yu JJ, Xing BS (2013a) The effect of sulfide inhibition on the ANAMMOX process. Water Res 47:1459–1469

Jin RC, Zhang QQ, Yang GF, Xing BS, Ji YX, Chen H (2013b) Evaluating the recovery performance of the ANAMMOX process following inhibition by phenol and sulfide. Bioresour Technol 142: 162–170

Kim YM, Park H, Cho KH, Park JM (2013) Long term assessment of factors affecting nitrifying bacteria communities and N-removal in a full-scale biological process treating high strength hazardous wastewater. Bioresour Technol 134:180–189

Kim YM, Park D, Lee DS, Park JM (2008) Inhibitory effects of toxic compounds on nitrification process for cokes wastewater treatment. J Hazard Mater 152:915–921

Klauson D, Kivi A, Kattel E, Klein K, Viisimaa M, Bolobajev J, Velling S, Goi A, Tenno T, Trapido M (2015) Combined processes for wastewater purification: treatment of a typical landfill leachate with a combination of chemical and biological oxidation processes. J Chem Technol Biotechnol 90:1527–1536

Kochany J, Lipczynska-Kochany E (2009) Utilization of landfill leachate parameters for pretreatment by Fenton reaction and struvite precipitation—a comparative study. J Hazard Mater 166:248–254

Lauchnor EG, Radniecki TS, Semprini L (2011) Inhibition and gene expression of *Nitrosomonas europaea* biofilms exposed to phenol and toluene. Biotechnol Bioeng 108:750–757

Li HQ, Han HQ, Du MA, Wang W (2011) Removal of phenols, thiocyanate and ammonium from coal gasification wastewater using moving bed biofilm reactor. Bioresour Technol 102:4667–4673

Liu YQ, Tay JH, Ivanov V, Moy BYP, Yu L, Tay STL (2005) Influence of phenol on nitrification by microbial granules. Process Biochem 40: 3285–3289

Lotti T, Kleerebezem R, Hu Z, Kartal B, Jetten M, Van Loosdrecht M (2014) Simultaneous partial nitritation and anammox at low temperature with granular sludge. Water Res 66:111–121

Maszenan A, Liu Y, Ng WJ (2011) Bioremediation of wastewaters with recalcitrant organic compounds and metals by aerobic granules. Biotechnol Adv 29:111–123

Milia S, Cappai G, Perra M, Carucci A (2012) Biological treatment of nitrogen-rich refinery wastewater by partial nitritation (SHARON) process. Environ Technol 33:1477–1483

Morita M, Kudo N, Uemoto H, Watanabe A, Shinozaki H (2007) Protective effect of immobilized ammonia oxidizers and phenol-degrading bacteria on nitrification in ammonia- and phenol-containing wastewater. Eng Life Sci 7:587–592

Pereira AD, Leal CD, Dias MF, Etchebehere C, Chernicharo CAL, de Araujo JC (2014) Effect of phenol on the nitrogen removal performance and microbial community structure and composition of an anammox reactor. Bioresour Technol 166:103–111

Rastegar S, Mousavi S, Shojaosadati S, Sheibani S (2011) Optimization of petroleum refinery effluent treatment in a UASB reactor using response surface methodology. J Hazard Mater 197:26–32

Toh S, Ashbolt N (2002) Adaptation of anaerobic ammonium-oxidising consortium to synthetic coke-ovens wastewater. Appl Microbiol Biotechnol 59:344–352

Vázquez I, Rodríguez J, Marañón E, Castrillón L, Fernández Y (2006) Simultaneous removal of phenol, ammonium and thiocyanate from coke wastewater by aerobic biodegradation. J Hazard Mater 137: 1773–1780

Wang YF, Gu JD (2014) Effects of allylthiourea, salinity, and pH on ammonia/ammonium-oxidizing prokaryotes in mangrove sediment incubated in laboratory microcosms. Appl Microbiol Biotechnol 98: 3257–3274

Xing BS, Guo Q, Zhang ZZ, Zhang J, Wang HZ, Jin RC (2014) Optimization of process performance in a granule-based anaerobic ammonium oxidation (anammox) upflow anaerobic sludge blanket (UASB) reactor. Bioresour Technol 170:404–412

Xing BS, Ji YX, Yang G-F, Chen H, Ni WM, Jin RC (2013) Start-up and stable operation of partial nitritation prior to ANAMMOX in an internal-loop airlift reactor. Sep Purif Technol 120:458–466

Yang GF, Guo XL, Chen SX, Liu JH, Guo LX, Jin RC (2013) The evolution of Anammox performance and granular sludge characteristics under the stress of phenol. Bioresour Technol 137:332–339

Yang GF, Jin RC (2012) The joint inhibitory effects of phenol, copper (II), oxytetracycline (OTC) and sulfide on Anammox activity. Bioresour Technol 126:187–192