Investigating the inhibitory effect of phenol and cyanide in the activated sludge process employed for the treatment of petroleum wastewater

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Abstract

In this work, the inhibitory effects of cyanide, phenol and 4-nitrophenol were investigated in the activated sludge process. The inhibition of the oxidation of organic matter, nitrification and denitrification were examined in batch activity tests by measuring the specific oxygen uptake rate (sOUR), the specific ammonium uptake rate (sAUR) and the specific nitrogen uptake rate (sNUR). The tested cyanide, phenol and 4-nitrophenol concentrations were 0.2-1.7 mg/L, 4.8-73.1 mg/L and 8.2-73.0 mg/L respectively. Cyanide was highly toxic as it significantly (>40%) inhibited the activity of autotrophic, aerobic heterotrophic biomass and denitrifiers at low concentrations (<2 mgCN^-/L). The addition of 1.7 mg/L CN^- resulted in 93% inhibition of the activity of aerobic heterotrophic biomass and 58% of the sNUR, while the addition of 1 mg/L CN^- inhibited sAUR by 41%. Significant phenol and 4-nitrophenol concentrations were required to accomplish appreciable inhibition of aerobic heterotrophic biomass. Specifically, the addition of 65 mg/L phenol and 4-nitrophenol resulted in sOUR inhibition of 40% and 29% respectively. The autotrophic bacteria were more sensitive to phenol than the aerobic heterotrophs. The denitrifiers were very resistant to phenol. To conclude, phenol, 4-nitrophenol and cyanide can inhibit aerobic heterotrophs, nitrifying and denitrifying biomass.

1. Introduction

Petroleum refinery industries result in the production of significant quantities of wastewater from various processes which include desalting, vacuum distillation, hydrocracking, catalytic cracking, catalytic reforming, alkylation [1-2]. Petroleum and petrochemical wastewater contain phenols, hydrocarbons, benzene, toluene, ethylbenzene, xylene, polycyclic aromatic hydrocarbons (PAHs), spent caustic soda, cyanides, heavy metals, sulphides, sodium chloride and other substances [3-5]. Many of these substances can inhibit the biochemical reactions that take place during the biological treatment of wastewater [6].

The oil and gas industry in Kazakhstan is a major producer of wastewater in both upstream and downstream processes. In several oil refinery plants the petroleum wastewater is treated by the activated sludge process. However, the inhibitory effect of several substances could render the process problematic. Di Fabio et al. [6] examined biomass activity in a pilot scale submerged membrane bioreactor treating petrochemical wastewater. The authors determined a specific oxygen uptake rate (sOUR) for heterotrophic biomass which was on average between 7.8-11.0 mgO_2/g VSS h for the different experimental runs. The specific ammonium uptake rate (sAUR) was 0.12-0.34 mgN/gVSS and the specific nitrogen uptake rate (sNUR) was 1.03-2.70 mg N/gVSS h. The rates are much lower compared to the typical ones met in municipal wastewater treatment plants, probably due to the inhibitory effect of certain petrochemical compounds.

This work examined the inhibitory effects of cyanide, phenol and 4-nitrophenol to aerobic heterotrophic biomass, nitrifiers and denitrifiers.

2. Materials and Methods

The inhibitory effect of cyanide, phenol and 4-nitrophenol was examined in batch reactors which consisted of 1 L Erlenmeyer flasks. Activated sludge was obtained from the activated sludge recycle of a municipal wastewater treatment plant in Astana of Kazakhstan. The inhibition of aerobic heterotrophic biomass was examined by monitoring the sOUR, while inhibiting the nitrification process. Biomass was placed overnight under aeration. Then, 400 mL of activated sludge was placed in each of the 1 L Erlenmeyer flasks under continuous aeration and agitation. Temperature was maintained at 20±2^{o}C and the pH at 7.8±0.4 by adding $NaHCO_3$. Nitrification was inhibited by adding 10-12 mg/L allyl-thiourea. After 30 minutes, the biomass was placed in a BOD flask under very mild agitation without aeration and the DO was recorded with time. The slope of DO decrease with time corresponded to the endogenous oxygen uptake rate ($OUR_{endogenous}$). The biomass was returned back to the Erlenmeyer flasks where it was again maintained under agitation and continuous aeration. The reactors were spiked with specific cyanide, phenol and 4-nitrophenol concentrations. Then 250 mgCOD/L of sodium acetate was added to the reactors. At the time intervals of 10, 30 minutes after the addition of the substrate the OUR was determined following the procedure described above. The average of these values was taken as the OUR_{max}.

Nitrification rate was determined by measuring the specific ammonium uptake rate (sAUR). Biomass (400 mL) was placed into a 1 L Erlenmeyer flask under continuous aeration (DO>4 mg/L). The inhibitors (cyanide, phenol, 4-nitrophenol) were spiked at fixed concentrations. Then ammonium chloride (NH_4Cl) solution was added to the biomass at a concentration of 30 mgN/L. The biomass was kept at room temperature (20±2^{o}C), the DO concentration was maintained >4 mg/L and the pH within the range of 7.8±0.4. At fixed time intervals 10-15 mL of biomass was collected and immediately centrifuged and filtered through Whatman 0.45 μm membrane filters. The filtrate was measured for its NO_3-N and NO_2-N concentration. The rate of increase of the sum of NO_2-N and NO_3-N with time was taken to calculate AUR.

The denitrification potential of biomass can be assessed by measuring the specific nitrogen uptake rate (sNUR). Biomass (400 mL) was placed inside a 1 L Erlenmeyer flask under mild agitation (no aeration). The inhibitors (cyanide, phenol, 4-nitrophenol) were spiked at fixed concentrations. Sodium nitrate ($NaNO_3$) solution was added to the biomass so that the initial NO_3-N concentration was approximately 40 mgN/L; sodium acetate was also added as carbon source to the batch reactor (250 mgCOD/L). The biomass was maintained at room temperature (20±2^{o}C) and the pH at7.8±0.4. At fixed time intervals 10-15 mL of biomass was collected, and was immediately centrifuged and filtered (Whatman membrane filters 0.45 μm). The filtrate was measured for its NO_3-N concentration. The nitrogen uptake rate is given by the slope of nitrate decrease with time (mgNO_3-N/L h).

The OUR, AUR and NUR results were normalized to the VSS concentration and thus the specific OUR (sOUR mgO_2/gVSS h), specific AUR (sAUR mgN/gVSS h) and specific NUR (sNUR mg N/g VSS h) were determined. The results are reported to the reference temperature of 20^{o}C using the following temperature correction equations:

$$sOUR_{20} = sOUR_T / 1.09^{(T-20)}$$

$$sAUR_{20} = sAUR_T / 1.026^{(T-20)}$$

$$sNUR_{20} = sNUR_T / 1.026^{(T-20)}$$

Table 1 summarizes the concentrations at which the inhibitors were added in the batch reactors for the different experiments.

Table 1: Tested cyanide, phenol and 4-nitrophenol concentrations for each activity test

	sOUR	sAUR	sNUR
Cyanide (mg/L)	0.00 (Control)	0.00 (Control)	0.00 (Control)
	0.42	0.19	0.42
	0.85	0.49	0.83
	1.69	0.97	1.66
Phenol (mg/L)	0.00 (Control)	0.00 (control)	0.00 (Control)
	8.50	4.81	7.99
	43.03	9.71	40.98
	65.05	48.69	62.50
		73.14	
4-nitrophenol	0.00 (Control)	0.00 (Control)	0.00 (Control)
	8.33	9.52	8.20
	42.64	48.25	41.98
	64.90	72.97	63.95

The parameters of pH, total and volatile suspended solids (TSS, VSS), chemical oxygen demand were determined using standard methods [7]. The parameters of ammonium (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N) were determined by ion chromatography.

Results and Discussion

Table 2 summarizes the maximum and endogenous sOUR, the sAUR and the sNUR that was obtained for the control experiments in which inhibitors were not added. As expected, the values are within the range of activity values typically met in municipal wastewater treatment plants [8-9].

Table 2: Activity of biomass used in the control experiments

Parameter	Average value ± standard deviation
$sOUR_{endogenous}$ 20°C (mgO_2/gVSS h)	4.0 ± 0.7
$sOUR_{max}$ 20°C (mgO_2/gVSS h)	22.7 ±5.7
sAUR (mgN/gVSS h)	1.9 ± 1.0
sNUR (mgN /gVSS h)	2.7 ± 0.4

Figure 1 shows the sOUR inhibition caused by cyanides, phenol and 4-nitrophenol to the aerobic heterotrophic biomass. Cyanide is much more toxic than phenol and 4-nitrophenol to aerobic heterotrophic biomass. Specifically, CN^- concentrations of 0.85 mg/L and 1.69 mg/L resulted in sOUR inhibition of 82% and 93% respectively. On the contrary, phenol and 4-

nitrophenol concentrations in the range of 8.3-8.5 mg/L resulted in limited (<20%) sOUR inhibition. Very high phenol concentrations up to 65 mg/L resulted in 40% sOUR inhibition. In the case of 4-nitrophenol such concentration resulted in even lower sOUR inhibition. The results showed that the inhibition of sOUR followed the order cyanide >> phenol > 4-nitrophenol.

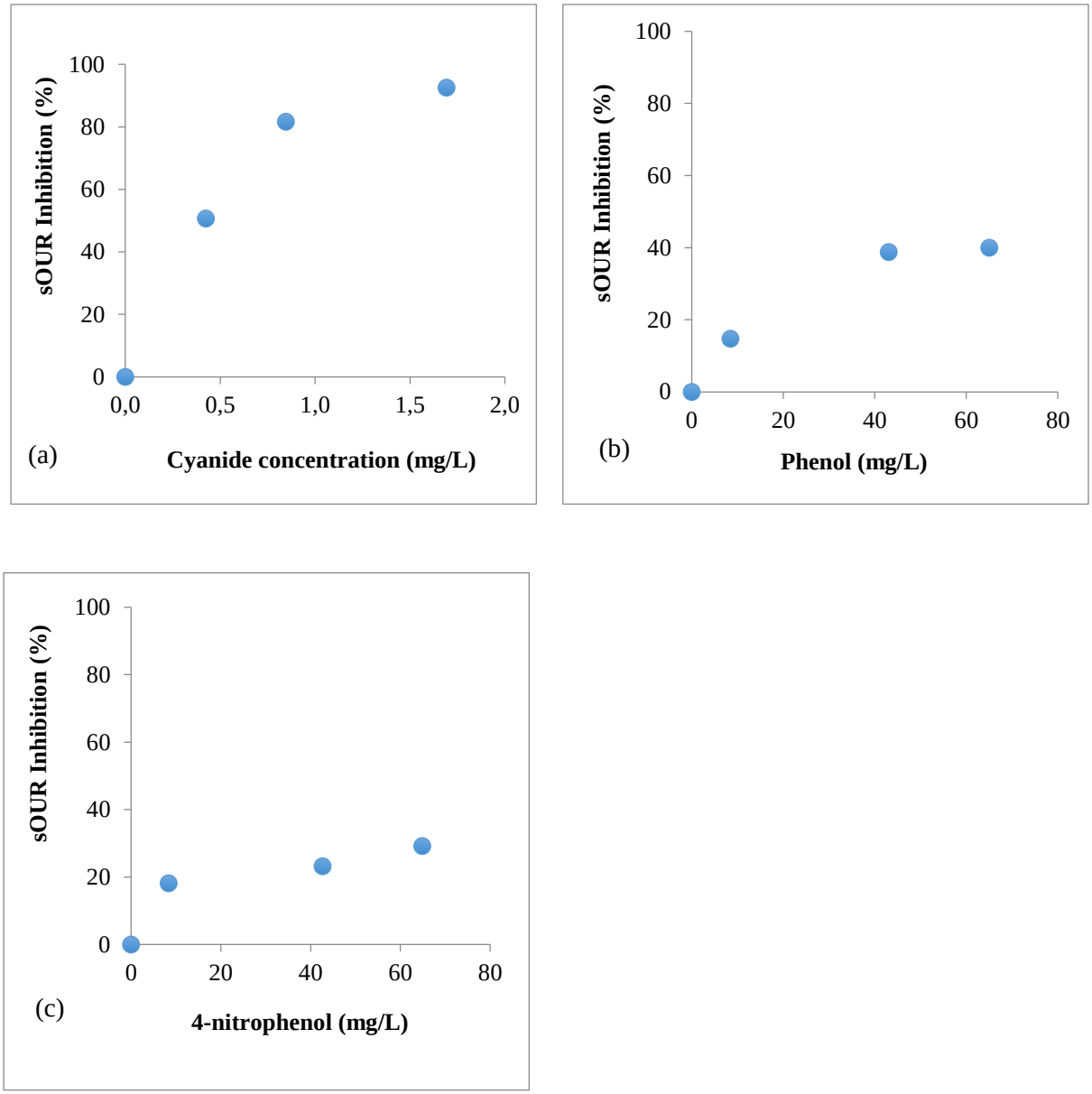

Figure 1. Inhibition caused by (a) cyanide, (b) phenol and 4-nitrophenol to aerobic heterotrophic biomass (sOUR)

Figure 2 shows the sAUR inhibition caused by cyanides, phenol and 4-nitrophenol to the nitrifying biomass. The cyanide inhibited the sAUR even at low concentrations. Surprisingly, the sAUR was inhibited less than the sOUR for similar CN^- concentrations. In the case of phenol and 4-nitrophenol even relatively low concentrations of 9.5-9.7 mg/L resulted in significant (43% and 60%) inhibition of sAUR. In the case of phenol and 4-nitrophenol the autotrophic biomass was more sensitive to toxic compounds than the aerobic heterotrophic

biomass. The results showed that the inhibition of sAUR followed the order cyanide >> phenol > 4-nitrophenol.

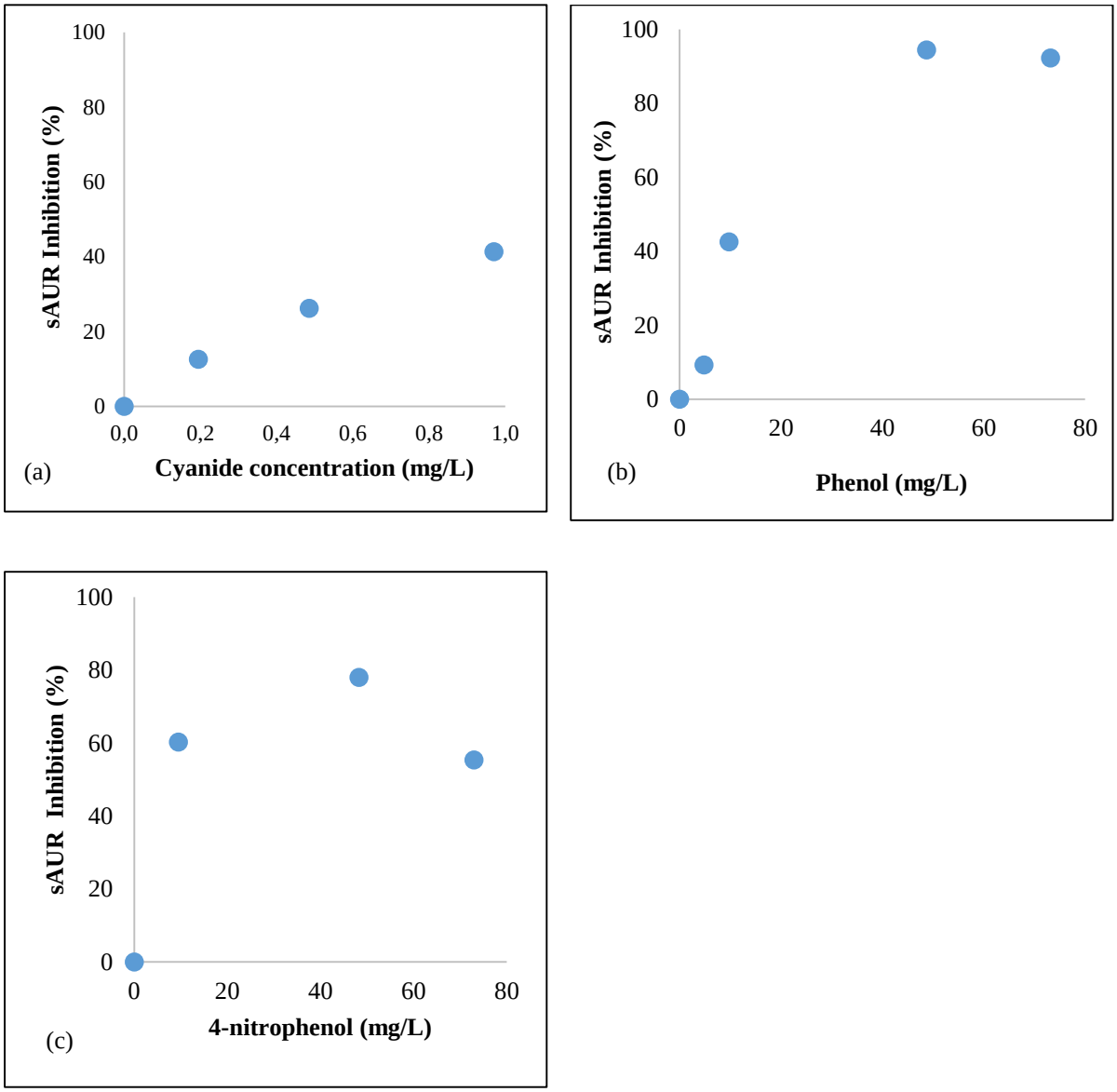

Figure 2. Inhibition caused by (a) cyanide, (b) phenol and (c) 4-nitrophenol to nitrifying biomass (sAUR)

Figure 3 shows the sNUR inhibition caused by cyanides, phenol and 4-nitrophenol to the denitrifying biomass. Cyanide resulted in significant inhibition of sNUR even at low CN^- concentration. The addition of 1.66 mg/L CN^- resulted in sNUR inhibition of 58%. The denitrifiers showed very good resistance to phenols but were completely inhibited at high (>40 mg/L) 4-nitrophenol concentrations. The results showed that the inhibition of sAUR followed the order 4-nitrophenol >> phenol.

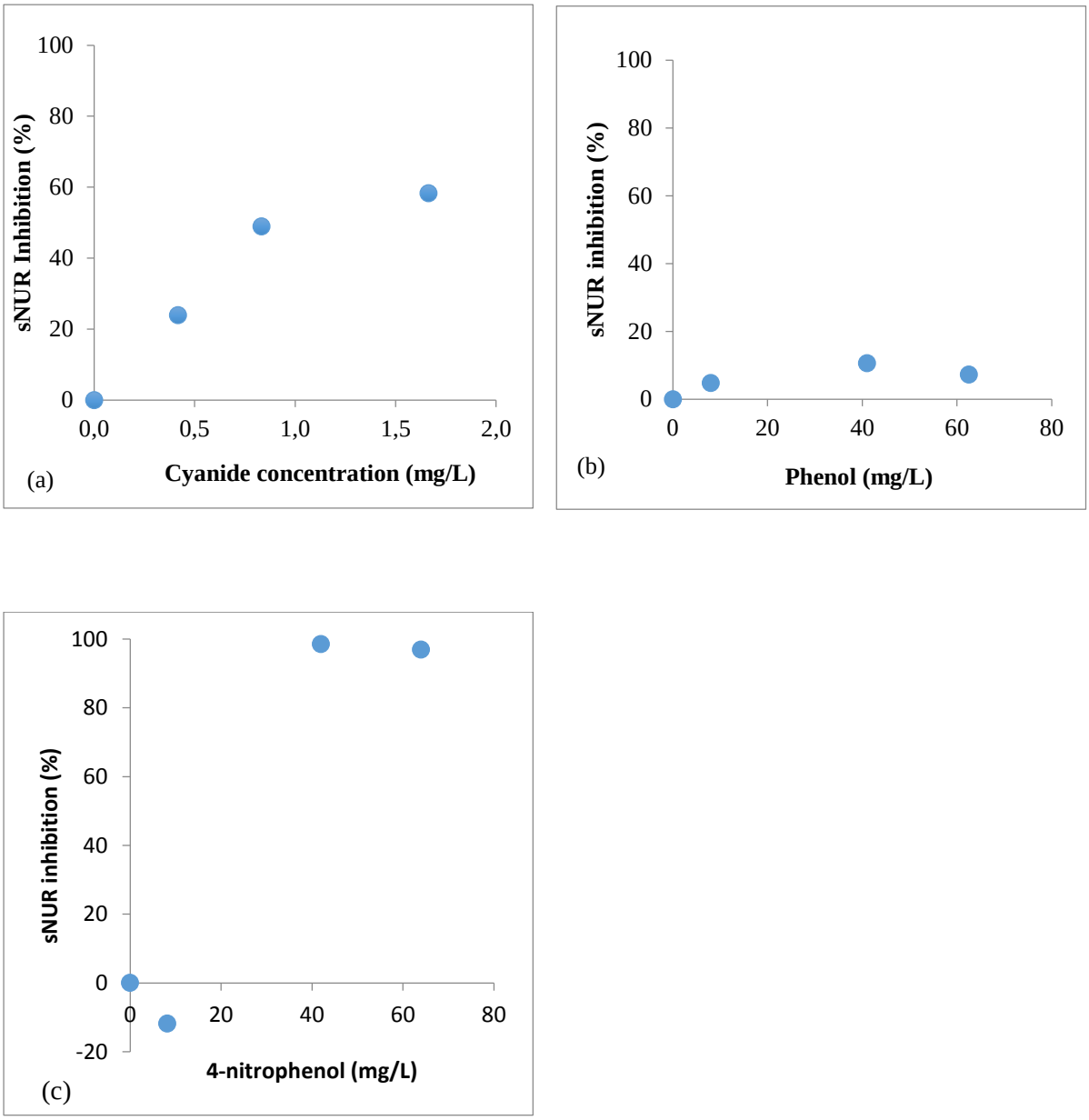

Figure 3. Inhibition caused by (a) cyanide, (b) phenol and (c) 4-nitrophenol to denitrifying biomass (sNUR)

For cyanide the inhibition order for the different types of bacteria followed the order: sOUR > sNUR > sAUR. For phenol the order was sAUR > sOUR > sNUR. For 4-nitrophenol the inhibition order was sNUR > sAUR > sOUR.

Conclusion

Cyanide was highly toxic as it significantly (>40%) inhibited sOUR, sAUR and sNUR at low concentrations (< 2 mg/L). The denitrifiers were the most resistant to phenol. Significant phenol and 4-nitrophenol concentrations (> 40 mg/L) were required to accomplish appreciable

inhibition (>40%) of aerobic heterotrophic biomass. The sOUR was inhibited more than the sAUR by cyanide. 4-nitrophenol was much more toxic compared to phenol for denitrifiers.

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