The performance of activated sludge exposed to arsanilic acid and amprolium

hydrochloride in sequencing batch reactors

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Abstract

Arsanilic acid (ASA) and amprolium hydrochloride (AMP) are widely used as feed additives to control coccidial intestinal parasites and improve feed efficiency, but due to low metabolism most of the drugs are excreted by the animals unchanged and eventually end up in wastewaters. Little is known about the impacts of AMP and ASA on the performance of activate sludge in sequencing batch reactors (SBRs) as well as about their fate in wastewater treatment. In this study, the long-term performance of activated sludge in SBRs exposed to ASA and AMP was investigated. The COD removal and nitrification were not affected when the concentration of ASA or AMP was lower than 20 mg L^{-1} , but were markedly inhibited at 100 mg L^{-1} of ASA or AMP. The inhibition to of COD removal was reversible whereas the inhibition to nitrification was irreversible. Phosphate removal was not affected by the continuous exposure to ASA or AMP. ASA and AMP were very resistant to be degraded by the activated sludge in SBRs, and only a small quantity of ASA was degraded to inorganic arsenic (no more than $300 \text{ } \mu\text{g L}^{-1}$) in the form of As (III) and As (V).

Keywords: Activated sludge. Arsanilic acid (ASA). Amprolium hydrochloride (AMP).

Nutrient removal

Introduction

Large amounts of antibiotics and veterinary drugs, about 100,000-200,000 tons, are used around the world every year (Du and Liu, 2011). The usage of these compounds in China was about 25,000 tons per year (Hou et al., 2015), including more than 8000 tons as feed additives to treat infections and promote growth of animals (Ben et al., 2009). However, the majority of veterinary drugs is excreted via manure and urine without metabolism by animals (Sarmah et al., 2006). Residual veterinary drugs in wastewater, surface water and soil environments (Du et al., 2015; Gao et al., 2016; Kümmerer, 2009; Xu et al., 2007), may foster the spread of antibiotics-resistant bacteria and ultimately compromise the clinical or veterinary effectiveness of antibiotics (Bergeron et al., 2015; Boxall et al., 2003; Máthé et al., 2012; Wang et al., 2006). Amprolium hydrochloride (1-([4-Amino-2-propyl-5-pyrimidinyl] methyl)-2-methylpyridinium chloride, AMP) and arsanilic acid (4-aminophenylarsonic acid, ASA) were widely used as feed additives to control coccidial intestinal parasites and improve feed efficiency (El-Kosasy et al., 2015; Jones, 2007). The approved dosage of AMP in animal feed is around 30 mg kg⁻¹ (El-Kosasy et al., 2015), and 50-100 mg kg⁻¹ for ASA (Wang et al., 2010). The yearly consumption of organoarsenic feed additives is approximate 1000 tons in China (Wang et al., 2010).

Sequencing batch reactors (SBR) are widely used in wastewater treatment plants (WWTPs) because of the unique advantages of this process configuration. Compared to conventional activated sludge, SBR is known to be simpler and offers more operational flexibility, saving more than 60% of operating cost (Singh and Srivastava, 2011).

Furthermore, the SBR performs well for high biomass retention and organic carbon removal (Fernandes et al., 2013). Previous studies have reported that the performance of SBR was affected by the exposure to roxarsone (ROX), which is similar to ASA, and the removal of chemical oxygen demand (COD), nitrogen and phosphorus was inhibited (Guo et al., 2013; Liu et al., 2014). The SBR process with aerobic granular sludge can remove fluoroquinolones, but the efficiency was lower than expected (Amorim et al., 2014).

Physical-chemical processes have been studied for the treatment of ASA, e.g. photo-oxidation (Czaplicka et al., 2014) and common chemical oxidation processes (Wang and Cheng, 2015). 37-59% of the added arsanilic acid was degraded after 115 days of incubation under anaerobic conditions (Wang et al., 2014). Little is known about the effect of AMP and ASA on the performance of SBR as well as about the fate of the compounds in the treatment process. Therefore, the aim of this study was to evaluate the performance of activated sludge in SBR with long-term exposure to ASA and AMP and their removal during the process.

Materials and methods

2.1 Chemicals

AMP and ASA were purchased from Sigma Aldrich (Steinheim, Germany) and Alfa Aesar (Heysham, UK), respectively. Methanol and acetonitrile were purchased from CNW Technologies (Düsseldorf, Germany) with HPLC grade. Other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd with analytical grade.

2.2 Synthetic wastewater

The synthetic wastewater contained (per liter of water): 445.2 mg $C_{12}H_{22}O_{11}$ (sucrose), 96 mg NH_4Cl , 28.22 mg $K_2HPO_4 \cdot 3H_2O$, 5.14 mg KH_2PO_4 (COD: N: P =100: 5: 1), 100 mg $MgSO_4 \cdot 7H_2O$, 0.83 mg $FeCl_3 \cdot 6H_2O$, 7.5 mg $CaCl_2$, 0.25 mg $Al_2(SO_4)_3 \cdot 18H_2O$, 0.11 mg $ZnSO_4 \cdot 7H_2O$, 0.05 mg $MnSO_4 \cdot H_2O$, 0.05 mg $CoCl_2 \cdot 6H_2O$, 0.05 mg $NiCl_2 \cdot 6H_2O$, 0.05 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.05 mg H_3BO_4 , and 0.05 mg $CuCl_2$.

2.3 Set up and operation of the SBRs

Three parallel SBR reactors (R0, R1 and R2) each with working volume of 3 L were used in this experiment. The height and inner diameter of the reactor were 48.5 cm and 10 cm, respectively. Activated sludge from a municipal wastewater treatment plant (Hefei, China) was used as the inoculum. After washing with tap water, the activated sludge was equally divided into three parallel reactors. The system was operated in cycles by an automatic timer to control pumps for influent, aeration, and effluent discharge. The volume exchange ratios of three SBRs were all set at 70%. The SBRs were operated for two cycles per day at room temperature. Each cycle lasted for 12h, with 10 min for feeding, 480 min for aeration, 180 min for settling, 5min for effluent discharge, and 45 min for idling. Hydraulic retention time (HRT) and sludge retention time (SRT) were 17 h and 16 d, respectively. The mixed liquor suspended solids (MLSS) was $3447 \pm 351 \text{ mg L}^{-1}$, and dissolved oxygen (DO) in aerobic reaction period was $3.4 \pm 0.3 \text{ mg L}^{-1}$. The pH of inflow was maintained at 7.5 ± 0.3 by dosing 0.5 M NaOH or 0.5 M HCl.

2.4 Experimental procedure

Three identical reactors run simultaneously and seven phases were segmented for each reactor, as listed in Table 1. One reactor was used as the blank control (R0) which was free of ASA and AMP in the influent. The other two reactors (R1 and R2) were continuously spiked with ASA and AMP in the influent, which were used to evaluate the response of the activated sludge.

2.5 Activity batch experiments

Biomass activity was determined through ex-situ experiments. Activated sludge was taken from the reactor at phase VI and IV. A blank control was always conducted throughout the experiments. Nitrification and denitrification rates were evaluated by specific ammonium uptake rate (sAUR) and specific nitrate uptake rate (sNUR), respectively. The phosphorus uptake and release rate (sPUR, sPRR) were also determined for assessing the activity of phosphate accumulating organisms in aerobic and anaerobic conditions. In every test, 400 mL of activated sludge was washed with tap water and then placed in flasks. The temperature and pH were maintained at 25 ± 2 °C and 7.5 ± 0.3 , respectively. DO was kept at 4.0 ± 0.5 mg L⁻¹ in aeration phase. Ammonium (20 mg L⁻¹), nitrate (10 mg L⁻¹) and phosphorus (10 mg L⁻¹) were added to flasks for activity tests. Samples were collected from flasks every 10-20 min and the ammonium, nitrite, nitrate and phosphorus were measured. The sAUR, sNUR, sPUR and sPRR were determined by linear fitting of their concentrations over time by the constant concentration of volatile suspended solids (VSS).

2.6 Analytical methods

Liquid sample was immediately filtered through a cellulose acetate filter (0.22 μm pore-size) for the analysis of nitrate, nitrite and residual drugs. MLSS, VSS, COD, $\text{NH}_4^+\text{-N}$, $\text{PO}_4^{3-}\text{-P}$ were determined according to the standard methods (APHA, 2005). Nitrate and nitrite were analyzed using ion chromatograph with conductivity detector (DIONEX, ICS-90, USA).

A high performance liquid chromatograph (HPLC, 1260 infinity, Agilent Technologies) with UV detector was used to measure the concentrations of ASA and AMP at 270 nm. The Wondasil C18 column (4.6 mm $\times$ 250 mm, 5 μm , GL Sciences Inc., Japan) was applied for the separation of ASA and AMP, and the mobile phase composed of acetonitrile, water and formic acid (25:75:1, V/V/V) at a flow rate of 1.0 mL min⁻¹.

Concentrations of total arsenic and total inorganic arsenic were measured with an atomic fluorescence spectrometer (AFS-8220, Titan Beijing LTD, China, (Zhang et al., 2014)). The arsenic species were determined by a high performance liquid chromatography–hydride generation–atomic fluorescence spectrometer (HPLC–HG–AFS, SAP-10, Titan Beijing LTD, China, (Shi et al., 2014)).

3. Results and discussion

3.1 Impacts on SBRs Performance

3.1.1 COD removal

Fig. 1 depicts the COD removal efficiency in three SBRs exposed to ASA and AMP under different phases. The phase I was used to acclimate activated sludge and the COD

removal efficiency in each reactor eventually reached around 95%. During the phases II to IV, the COD removal efficiency was not markedly influenced by the spiked ASA and AMP, indicating that the mineralization of organic matter was not affected when ASA and AMP concentrations were below 20 mg L⁻¹. However, from the beginning of phase V, the COD removal efficiencies in R1 and R2 were slightly lower than that in R0. Moreover, during phase VI, a sharp decline of COD removal efficiencies in R1 and R2 occurred. At the end of phase VI, about 95% of COD removal was achieved in R0 while only 70% was obtained in R1 and R2. The microbial activity was significantly inhibited when the concentrations of ASA and AMP reached 100 mg L⁻¹. In phase VII, ASA and AMP addition was stopped, and effective COD removal in R1 and R2 was reestablished. These results are similar to those reported in a previous study (Liu et al., 2014), where COD removal decreased from 89% to 46% when roxarsone concentration increased from 0 to 80 mg L⁻¹.

3.1.2 Removal of nitrogen and phosphate

The removal of nitrogen and phosphate in various phases is shown in Fig. 2. At the end of phase I, the removal of NH₄⁺-N and PO₄³⁻-P in each reactor were all up to 99% and 60%, respectively. The concentration of NO₃⁻-N in the effluent was approximately 0.54 mM and NO₂⁻-N was below 0.01 mM. From the phase II to V, ammonia was consumed in each cycle and ammonium and nitrite were barely detected in the effluent of each reactor. Nitrate concentrations in the effluent of each SBR exposed to ASA and AMP were at the similar level with that in R0, indicating that nitrification was not affected by the spiked ASA and AMP.

However, after increasing the concentration of ASA and AMP to 100 mg L⁻¹ (phase VI), as shown in Fig. 2, the removal efficiency of NH₄⁺-N in R1 and R2 immediately decreased from 99.0% and 99.1% to 64.5% and 66.3%, respectively. The effluent NO₃⁻-N concentration in R1 and R2 declined sharply from 0.56 mM and 0.54 mM to 0.22 mM and 0.22 mM, respectively, while the effluent NO₂⁻-N concentration in R1 and R2 increased to 0.19 mM and 0.06 mM, respectively. Activity test results were shown in Table 2. The sAUR of Exp.4 (3.02 mg N g⁻¹ VSS h⁻¹) and Exp.5 (1.40 mg N g⁻¹ VSS h⁻¹) was much lower than that of Exp.1 (5.23 mg N g⁻¹ VSS h⁻¹). The results indicated that the nitrification process was affected by dosing 100 mg L⁻¹ AMP or ASA. However, in the following days of phase VI, the removal efficiency of NH₄⁺-N in R1 and R2 gradually recovered from the inhibition of ASA and AMP. But the effluent NO₃⁻-N concentration in R1 and R2 was suppressed at around 0.21 mM and 0.46 mM, respectively, which was lower than R0. Moreover, the effluent NO₂⁻-N concentration in R1 and R2 was maintained around 0.36 mM and 0.14 mM, respectively. This result indicated that nitrification bioactivity in R1 and R2 was lower than that in R0. (Katipoglu-Yazan et al., 2013) have already reported that high concentration of erythromycin and tetracycline (200 mg L⁻¹) inhibited the activity of nitrifiers, leading to the blockage of nitrification. In phase VII, the COD removal efficiency was recovered, but the effluent NO₃⁻-N and NO₂⁻-N concentrations were almost unchanged, indicating that the inhibition of ASA and AMP to nitrification was an irreversible process. Furthermore, it can be clearly observed in Fig.2b and 2c that the influence of ASA to nitrification bioactivity was more serious than AMP. The effluent NO₃⁻-N concentration in

R1 was obviously lower than that in R2 at the end of phase VI, while the effluent NO_2^- -N concentration in R1 was much higher than that in R2, which might be due to the different toxicity of ASA and AMP to nitrifying microorganisms, including ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB).

In addition, the phosphate removal was not affected during the operation of SBRs during phases II to VII, indicating that the activity of phosphate accumulating organisms was not influenced by the feeding of ASA and AMP. Activity tests of sPUR and sPRR also confirmed this result. However, a decline of phosphate removal was reported by (Amorim et al., 2014) after prolonged exposure of activate sludge to fluoroquinolones.

3.2 Removal of AMP and ASA

3.2.1 AMP and ASA removal

Figs. 3 and 4 show the profile of AMP and ASA removal during the phases. At the beginning of phases II to VI, AMP and ASA concentrations after feeding were lower than the theoretical values in the reactor, most probably due to adsorption. Afterwards, dissolved AMP and ASA concentration gradually increased in the reactor, and then reached a stable value, in which the influent and effluent concentrations of AMP and ASA were almost the same. This result indicated that activated sludge reached the maximum adsorption capacity, and AMP and ASA were resistant to be degraded by activated sludge during SBR. Similar results for other antibiotics have been reported in previous studies (Amorim et al., 2014; Ferreira et al., 2016). However, a small amount of inorganic arsenic was detected by atomic fluorescence spectrometry in the effluent in R1, indicating that the degradation of ASA

occurred in the reactor. Based on the mass balance analysis of arsenic, the difference between total arsenic concentration in influent and effluent was far greater than that of the concentration of inorganic arsenic (Table 3). Therefore, the loss was mainly due to the absorption of ASA to activated sludge, and only small amount of ASA was degraded into inorganic arsenic. After stopping the feeding of AMP or ASA in phase VII, a gradual decrease of AMP and ASA concentration was observed, and eventually AMP and ASA could not be detected in the effluent.

3.2.2 ASA biotransformation

Organoarsenic compounds, like ASA and roxarsone (ROX), can be slowly degraded to inorganic arsenic under anaerobic conditions (Sierra-Alvarez et al., 2010; Stolz et al., 2007). Generally for living organisms, inorganic arsenic species are more toxic than organic forms. At the last cycle from phase II to IV, small amounts of As (III) and As (V) were detected (Table 3). At the end of phase IV, As (III) and As (V) concentrations were $19.53 \pm 2.24 \mu\text{g L}^{-1}$ and $70.15 \pm 1.93 \mu\text{g L}^{-1}$, respectively. But in phase V and VI, increase of As (III) and As (V) occurred, which might be due to the high level of feeding ASA. The concentrations of As (III) and As (V) finally reached $41.73 \pm 3.00 \mu\text{g L}^{-1}$ and $250.92 \pm 39.23 \mu\text{g L}^{-1}$, respectively.

4. Conclusions

In this study, the performance of activated sludge in SBRs exposed to ASA and AMP was evaluated. The COD removal and nitrification were not affected by $< 20 \text{ mg L}^{-1}$ of

ASA and AMP, but markedly affected by 100 mg L⁻¹ of ASA and AMP exposure. The inhibition to nitrification was irreversible but the inhibition to COD removal was reversible. ASA and AMP were very resistant to be degraded during wastewater treatment process, and only a small amount of ASA was degraded to inorganic arsenic in the form of As (III) and As (V). The result from this study will provide useful information for the treatment of wastewater containing ASA or AMP from animal farm or pharmaceutical plant treatment.

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References

- Amorim, C.L., Maia, A.S., Mesquita, R.B., Rangel, A.O., van Loosdrecht, M.C., Tiritan, M.E., Castro, P.M., 2014. Performance of aerobic granular sludge in a sequencing batch bioreactor exposed to ofloxacin, norfloxacin and ciprofloxacin. *Water Res.* 50, 101-113.
- APHA, 2005. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington, D.C.
- Ben, W., Qiang, Z., Pan, X., Chen, M., 2009. Removal of veterinary antibiotics from

- sequencing batch reactor (SBR) pretreated swine wastewater by Fenton's reagent. *Water Res.* 43, 4392-4402.
- Bergeron, S., Boopathy, R., Nathaniel, R., Corbin, A., LaFleur, G., 2015. Presence of antibiotic resistant bacteria and antibiotic resistance genes in raw source water and treated drinking water. *Int. Biodeterior. Biodegrad.* 102, 370-374.
- Boxall, A.B.A., Kolpin, D.W., Halling-Sørensen, B., Tolls, J., 2003. Peer reviewed: Are veterinary medicines causing environmental risks? *Environ. Sci. Technol.* 37, 286A-294A.
- Czaplicka, M., Bratek, Ł., Jaworek, K., Bonarski, J., Pawlak, S., 2014. Photo-oxidation of p-arsanilic acid in acidic solutions: Kinetics and the identification of by-products and reaction pathways. *Chem. Eng. J.* 243, 364-371.
- Du, J., Fan, Y., Qian, X., 2015. Occurrence and behavior of pharmaceuticals in sewage treatment plants in eastern China. *Front. Environ. Sci. Eng.* 9, 725-730.
- Du, L., Liu, W., 2011. Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review. *Agron. Sustainable Dev.* 32, 309-327.
- El-Kosasy, A.M., Hussein, L.A., Magdy, N., Abbas, M.M., 2015. Sensitive spectrofluorimetric methods for determination of ethopabate and amprolium hydrochloride in chicken plasma and their residues in food samples. *Spectrochim. Acta, Part A* 150, 430-439.
- Fernandes, H., Jungles, M.K., Hoffmann, H., Antonio, R.V., Costa, R.H., 2013. Full-scale sequencing batch reactor (SBR) for domestic wastewater: performance and diversity of

- microbial communities. *Bioresour. Technol.* 132, 262-268.
- Ferreira, V.R.A., Amorim, C.L., Cravo, S.M., Tiritan, M.E., Castro, P.M.L., Afonso, C.M.M., 2016. Fluoroquinolones biosorption onto microbial biomass: activated sludge and aerobic granular sludge. *Int. Biodeterior. Biodegrad.* 110, 53-60.
- Gao, J., Huang, J., Chen, W.W., Wang, B., Wang, Y.J., Deng, S.B., Yu, G., 2016. Fate and removal of typical pharmaceutical and personal care products in a wastewater treatment plant from Beijing: a mass balance study. *Front. Environ. Sci. Eng.* 10, 491-501.
- Guo, Q., Liu, L., Hu, Z., Chen, G., 2013. Biological phosphorus removal inhibition by roxarsone in batch culture systems. *Chemosphere* 92, 138-142.
- Hou, J., Wang, C., Mao, D., Luo, Y., 2015. The occurrence and fate of tetracyclines in two pharmaceutical wastewater treatment plants of Northern China. *Environ. Sci. Pollut. Res.* 23, 1722-1731.
- Jones, F.T., 2007. A broad view of arsenic. *Poult. Sci.* 86, 2-14.
- Kümmerer, K., 2009. Antibiotics in the aquatic environment – A review – Part I. *Chemosphere* 75, 417-434.
- Katipoglu-Yazan, T., Pala-Ozkok, I., Ubay-Cokgor, E., Orhon, D., 2013. Acute impact of erythromycin and tetracycline on the kinetics of nitrification and organic carbon removal in mixed microbial culture. *Bioresour. Technol.* 144, 410-419.
- Liu, H., Wang, G., Ge, J., Liu, L., Chen, G., 2014. Fate of roxarsone during biological nitrogen removal process in wastewater treatment systems. *Chem. Eng. J.* 255,

500-505.

Máthé, I., Benedek, T., Táncsics, A., Palatinszky, M., Lányi, S., Márialigeti, K., 2012.

Diversity, activity, antibiotic and heavy metal resistance of bacteria from petroleum hydrocarbon contaminated soils located in Harghita County (Romania). *Int. Biodeterior. Biodegrad.* 73, 41-49.

Sarmah, A.K., Meyer, M.T., Boxall, A.B., 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere* 65, 725-759.

Shi, L., Wang, W., Yuan, S.J., Hu, Z.H., 2014. Electrochemical stimulation of microbial roxarsone degradation under anaerobic conditions. *Environ. Sci. Technol.* 48, 7951-7958.

Sierra-Alvarez, R., Cortinas, I., Field, J.A., 2010. Methanogenic inhibition by roxarsone (4-hydroxy-3-nitrophenylarsonic acid) and related aromatic arsenic compounds. *J. Hazard. Mater.* 175, 352-358.

Singh, M., Srivastava, R.K., 2011. Sequencing batch reactor technology for biological wastewater treatment: a review. *Asia-Pac. J. Chem. Eng.* 6, 3-13.

Stolz, J.F., Perera, E., Kilonzo, B., Kail, B., Crable, B., Fisher, E., Ranganathan, M., Wormer, L., Basu, P., 2007. Biotransformation of 3-nitro-4-hydroxybenzene arsonic acid (roxarsone) and release of inorganic arsenic by *Clostridium* species. *Environ. Sci. Technol.* 41, 818-823.

Wang, H.-L., Hu, Z.-H., Tong, Z.-L., Xu, Q., Wang, W., Yuan, S., 2014. Effect of arsanilic

- acid on anaerobic methanogenic process: Kinetics, inhibition and biotransformation analysis. *Biochem. Eng. J.* 91, 179-185.
- Wang, L., Cheng, H., 2015. Birnessite (δ -MnO₂) mediated degradation of organoarsenic feed additive p-arsanilic acid. *Environ. Sci. Technol.* 49, 3473-3481.
- Wang, P.L., Zhao, G.L., Tian, J., Su, X.O., 2010. High-performance liquid chromatography inductively coupled plasma mass spectrometry based method for the determination of organic arsenic feed additives and speciation of anionic arsenics in animal feed. *J. Agric. Food Chem.* 58, 5263-5270.
- Wang, Y., Leung, P.C., Qian, P.Y., Gu, J.D., 2006. Antibiotic resistance and plasmid profile of environmental isolates of *Vibrio* species from Mai Po Nature Reserve, Hong Kong. *Ecotoxicology* 15, 371-378.
- Xu, W.H., Zhang, G., Li, X.D., Zou, S.C., Li, P., Hu, Z.H., Li, J., 2007. Occurrence and elimination of antibiotics at four sewage treatment plants in the Pearl River Delta (PRD), South China. *Water Res.* 41, 4526-4534.
- Zhang, F.-F., Wang, W., Yuan, S.-J., Hu, Z.-H., 2014. Biodegradation and speciation of roxarsone in an anaerobic granular sludge system and its impacts. *J. Hazard. Mater.* 279, 562-568.

Figure Captions

Fig. 1- COD removal efficiency in three SBRs in different phases

Fig. 2- Performance of three SBRs in different phases (a) NH_4^+ -N removal, (b) Effluent NO_2^- -N concentration, (c) Effluent NO_3^- -N concentration, (d) PO_4^{3-} -P removal

Fig. 3- Influent and effluent AMP concentrations in SBR in different phases

Fig. 4- Influent and effluent ASA concentrations in SBR in different phases

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Table 1

The spiked concentrations of ASA and AMP in three SBRs

Phase	Days	Influent (mg L ⁻¹)				Cycle time (h)
		R0		R1	R2	
		ASA	AMP	ASA	AMP	
I	0-29	0	0	0	0	12
II	30-49	0	0	5	5	12
III	50-69	0	0	10	10	12
IV	70-89	0	0	20	20	12
V	90-109	0	0	50	50	12
VI	110-139	0	0	100	100	12
VII	140-159	0	0	0	0	12

Table 2

Activity test parameters obtained in different experiments.

Batch experiments	VSS	sAUR	sNUR	sPUR	sPRR
	(g L ⁻¹)	(mg N g ⁻¹ VSS h ⁻¹)		(mg P g ⁻¹ VSS h ⁻¹)	
Exp.1 (Blank)	2.03 ± 0.24	5.23 ± 1.01	1.92 ± 0.44	1.31 ± 0.24	0.54 ± 0.11
Exp.2 (50 mg L ⁻¹ AMP)	1.97 ± 0.21	6.92 ± 1.22	2.08 ± 0.37	1.36 ± 0.11	0.40 ± 0.17
Exp.3 (50 mg L ⁻¹ ASA)	1.98 ± 0.15	4.35 ± 0.54	2.11 ± 0.51	1.23 ± 0.21	0.49 ± 0.04
Exp.4 (100 mg L ⁻¹ AMP)	2.02 ± 0.17	3.02 ± 0.31	2.00 ± 0.09	1.44 ± 0.23	0.59 ± 0.16
Exp.5 (100 mg L ⁻¹ ASA)	2.06 ± 0.31	1.40 ± 0.33	1.88 ± 0.08	1.34 ± 0.34	0.51 ± 0.20

Table 3

Influent and effluent concentrations of total arsenic and effluent inorganic arsenic concentration in the last cycle of each phase

Phase	Cycle	Total Arsenic (influent) (mg L ⁻¹)	Total Arsenic (effluent) (mg L ⁻¹)	As (III) (effluent) (µg L ⁻¹)	As (V) (effluent) (µg L ⁻¹)
II	40	1.73 ± 0.01	1.72 ± 0.13	14.83 ± 0.28	32.71 ± 2.25
III	40	3.33 ± 0.02	3.34 ± 0.01	16.78 ± 3.00	41.30 ± 1.39
IV	40	6.42 ± 0.12	6.39 ± 0.10	19.53 ± 2.24	70.15 ± 1.93
V	40	16.68 ± 0.14	16.66 ± 0.07	24.03 ± 1.77	113.55 ± 12.10
VI	60	34.95 ± 0.18	35.48 ± 0.13	41.73 ± 3.00	250.92 ± 39.23

Graphical abstract

Nitrification steps exposed to antibiotics