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How does sludge loading rate influence the growth and release of antibiotic-resistant bacteria in raw sewage?

Air

Raw sewage

Antibiotic-resistant bacteria

0.24　　　　　0.42　　　　　0.61

Sludge loading rate [kg TCOD/kg MLSS·d]
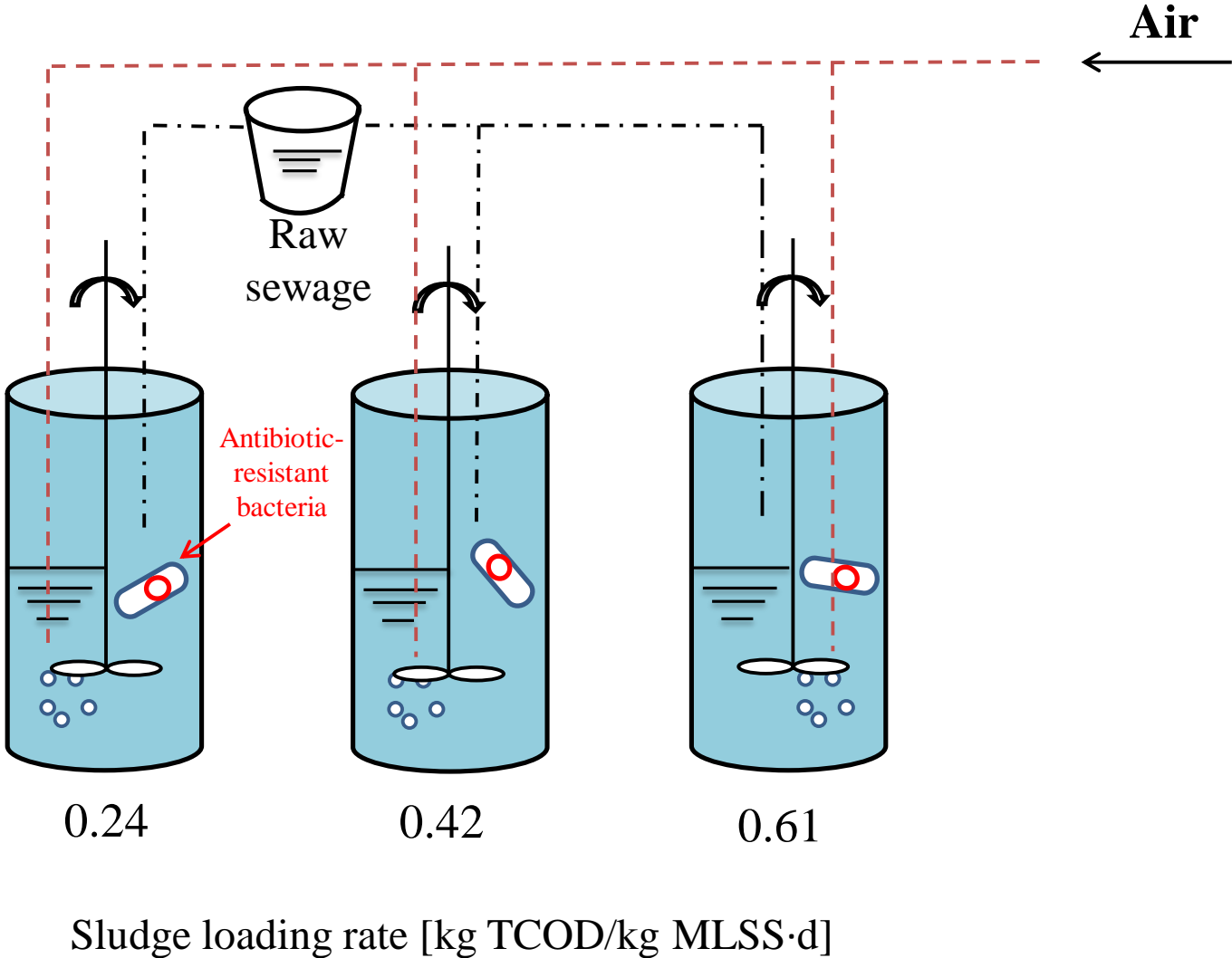
Environmental impact

This study evaluated the effects of sludge loading rate on the growth and release of six kinds of antibiotic-resistant bacteria in an activated sludge system. The results indicated that the release of most antibiotic-resistant bacteria through the effluent and biosolids were both amplified with increased sludge loading rates. Biosolids were the main pattern for all antibiotic-resistant bacteria release in the activated sludge system, which was determined primarily by their growth in the activated sludge. An activated sludge system with lower sludge loading rates was considered more conductive to controlling antibiotic resistance. These results may provide implications in assessing the fate of antimicrobial resistance in wastewater treatment system.

Environmental Science: Processes & Impacts

ARTICLE

Sludge loading rate regulates growth and release of heterotrophic bacteria resistant to six types of antibiotics in wastewater activated sludge

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Wastewater treatment plants are considered as hot reservoirs for antimicrobial resistance. However, the fates of antibiotic-resistant bacteria during biological treatment processes and relevant influencing factors have not been fully understood. This study evaluated the effects of sludge loading rate on the growth and release of six kinds of antibiotic-resistant bacteria in an activated sludge system. The results indicated that higher sludge loading rates amplified the growths of all six types of antibiotic resistant bacteria. The release of most antibiotic-resistant bacteria through the effluent and biosolids were both amplified with increased sludge loading rate. Biosolids were the main pattern for all antibiotic-resistant bacteria release in the activated sludge system, which was determined primarily by their growth in the activated sludge. A higher sludge loading rate reactor tended to retain more antibiotic resistance. An activated sludge system with lower sludge loading rates was considered more conductive to control antibiotic resistance.

1. Introduction

Wastewater treatment plants (WWTPs) are considered as hot reservoirs for antimicrobial resistance, an emerging pollutant imposing a significant threat to public health. Although even after treatment by biological processes or disinfection technologies, high amounts of antibiotic-resistant bacteria (ARB) are still frequently detected in the effluents of WWTPs and/or subsequent aquatic environments. Munir et al. reported that more than 10^{11} CFU/d of tetracycline- and 10^{12} CFU/d of sulfonamide-resistant heterotrophic bacteria were discharged through WWTPs effluents [1]. Furthermore, even higher release loads of bacteria carrying resistance in biosolids (compared to effluents) have also been demonstrated [1,2], making WWTPs potentially the most significant emission source of antimicrobial resistance.

The fate of ARB during wastewater treatment is at the epicenter of the concerns of microbiologists and wastewater engineers interested in controlling antibiotic resistance [3]. The conditions in WWTPs are considered favorable for the proliferation of ARB. Luczkiewicz et al. observed that *E. coli* resistant to penicillines, fluoroquinolones, trimethoprim/ sulphamethoxazole and tetracycline was more prevalent in treated wastewater compared to their abundances in the influent [4]. Similarly, Morozzi et al. and Andersen reported an increase in the percentage of multiple-antibiotic resistant (MAR) coliforms following biological treatment of municipal sewage

[5,6]. Regular wastewater treatment processes and disinfection processes often determine a significant reduction in total numbers of ARB, but large amounts of them are still detected in effluents and biosolids [7–9].

Biological processes and their operating conditions (e.g., sludge loading rate, hydraulic retention time, solid retention time, biomass, dissolved oxygen concentration, etc.) have been considered to have significant effects on ARB behaviors in WWTPs. Threedeach et al. found that the percentages of bacteria resistant to most antibiotics were higher in anaerobic operations than in semi-aerobic conditions [10]. Munir et al. investigated the effects of different biological processes [including membrane biological reactors (MBR), conventional activated sludge, oxidative ditch and rotatory biological contactors] on the removal of tetracycline- and sulfadiazine- resistant heterotrophic bacteria and found that the MBR was more effective than other traditional treatment processes (from 2.6 to 7.1 log removal) [1]. Moreover, Kim et al. investigated the fate of tetracycline-resistant bacteria as a function of organic loading rate; the results indicated that increases in organic loading and growth rates resulted in higher increased concentrations and production rates of tetracycline-resistant bacteria [11].

However, the knowledge in this area is still scarce. Additional operating conditions in biological processes, especially the kind that directly determine bacterial proliferation, were rarely reported as to their effects on the fate of ARB. The sludge loading rate (food-to-microorganism ratios, F/M) was considered to be one of the most important factors that influence organics degradation and bacterial growth in the activated sludge process. In order to provide guidance concerning antibiotic resistance control in WWTPs, the effects of the sludge loading rate on ARB behaviors in biological processes need further investigation. On the other hand, the growths of ARB in activated sludge, as well as their release characteristics, were both considered important for understanding ARB fates in WWTPs. However, previous studies in this area only concentrated on single behaviors, either in activated sludge or in the effluent – causing difficulties in determining their

systematic fates in biological processes. Therefore, a comprehensive research study is urgent concerning the simultaneous growth and discharge properties of ARB.

Thus, the objective of this study is to evaluate the effect of the sludge loading rate on the fate of ARB in a bench-scale activated sludge treatment process. Heterotrophic bacteria resistant to six kinds of commonly used antibiotics, including cephalexin (CEP), erythromycin (ERY), gentamicin (GEN), sulfadiazine (SD), tetracycline (TC) and vancomycin (VAN), were studied as representative cases. Their growth and release were investigated simultaneously to evaluate their fates in an activated sludge system. Three typical sludge loading rates were tested to explore this factor on ARB behaviors.

2. Materials and methods

2.1 Bench-scale activated sludge system design and operation

Three parallel 500 mL conical flask were adopted as activated sludge reactors with each having a working volume of 400 mL (Fig. 1). Magnetic stirrers (Sile apparatus, Minhang Shanghai, China) were used for liquid agitation of the three reactors at a constant rate (100 rpm). Aeration in the reactors was supplied by an aquarium air pump (CX-0088, Chuangxing Electric, Guangdong, China) with a gas flow rate of 0.6–0.8 L/min. During the experiments, a Plexiglass® cap was placed over each reactor to minimize evaporative losses.

First, three reactors were seeded with a mixture of return-activated sludge and the effluent of the primary clarifier from the Shanghai WWTP. The wastewater quality is shown in Table S1. The sludge was fed with 'fresh' wastewater every day, keeping the system stirred and aerated. When each reactor achieved a constant removal of Total Chemical Oxygen Demand (TCOD), Filtered Chemical Oxygen Demand (FCOD), Turbidity, NH_4^+-N, Total Nitrogen (TN) and Total Phosphorus (TP) for a period of at least three weeks, it was considered that stable running conditions were achieved.

Second, the reactors were operated in the following plan (Table 1). The operation cycle of the reactors was 8 h, and the solids retention time (SRT) was 8 d. SBR operation consisted of four periods in each cycle: fill, react, settle and decant as presented in Table 1. To evaluate the effect of the sludge loading rate on the fate of various ARB, three different sludge loading rates [0.24 kg TCOD/(kg MLSS d) for A, 0.42 kg TCOD/(kg MLSS d) for B and 0.61 kg TCOD/(kg MLSS d) for C] were operated for at least five weeks.

Table 1 Operating schedule of sequencing batch reactors

Reactor	Hydraulic loading rate (L/d)	Sludge loading rate [kg TCOD/(kg MLSS d)]	Cycle length (h)	SRT (d)	DO (mg/L)	Operating time in a cycle (min)					Cycles (/d)
						Fill	React	Settle	Decant	Total	
A	0.36	0.24	8	8	3-4	2	448	25	5	480	3
B	0.6	0.42	8	8	3-4	2	448	25	5	480	3
C	0.96	0.61	8	8	3-4	2	448	25	5	480	3

The reactors were sampled twice a week for physical and chemical analysis according to national standard methods, to ensure that they were running under stable conditions. Regular analyses included pH, TCOD, FCOD, MLSS, Turbidity, Dissolved Oxygen (DO), NH_4^+-N, TN and TP. Infrared spectroscopy analyses (IR, Nicolet 5700, Thermo Electron Scientific Instruments Corp., U.S.A) were carried out for activated sludge samples from the two reactors to compare their extracellular polymeric substance (EPS) structures.

2.2 Detection of heterotrophic antibiotic-resistant bacteria

After five weeks of operation, samples including raw sewage, activated sludge samples, effluents of the reactors and biosolids, were analyzed for antibiotic resistance using classical spread-plating techniques. Before plating, each sample was blended for 3 min to homogenize the culture. 1 mL of each homogenized sample was removed, serially diluted, and then plated in duplicate on nutrient agar (beef extract 3 g/L, peptone 10 g/L, NaCl 5 g/L and agar 15 g/L, pH: 7.2 ± 0.2), spiked with different antibiotics. The concentrations of the six antibiotics in the agar were as follows: CEP: 16 mg/L; ERY: 8 mg/L; GEN: 16 mg/L; SD: 512 mg/L; TC: 16 mg/L; VAN: 32 mg/L. According to our previous studies [8], the antibiotic concentrations were defined as the maximum value of all minimum inhibitory concentrations (MICs) for pathogen resistance to an antibiotic listed in the CLSI (Clinical and Laboratory Standards Institute) documentation [12]. The plates were then incubated at 37 ℃ for 24 h. All samples were processed by the standard count technique. Only dilutions with 20–300 CFU per plate were used for colony enumeration. Each sample was also incubated in nutrient agar with no antibiotic added to determine total heterotrophic bacterial count (HPC) levels. Each sample were was conducted triplicate for the statistical analysis.

2.3 Data analysis

The results were analyzed using the following method.

(1) Estimation of ARB growth

The parameter 'net specific growth rates (μ_{net})' was applied to determine the growth rates of various ARB. Referring to studies of Kim et al. [13], μ_{net} was calculated for each sampling date using a population balance for the sampled reactor, shown in Eq.(1).

$$\mu_{net} = (Q_{eff}X_{eff} + Q_{bio}X_{bio} - Q_{in}X_{in} + VdX/dt)/XV \quad \text{Eq. (1)}$$

where

μ_{net} is the net specific growth rate for ARB (/d)

X is the ARB concentration in the SBR mixed liquor (CFU/mL)

Q_{in}, Q_{eff}, $Q_{biosolid}$ are the daily volumes of the influent, effluent and wasted biosolids (L/d), respectively

X_{in}, X_{eff}, X_{bio} are the ARB concentrations in the influent, effluent and wasted biosolid (CFU/mL), respectively

dX/dt is the increase in bacterial counts with increased sampling dates

V is the reactor working volume (L)

The term dX/dt was calculated by measuring the difference in ARB counts between sampling dates, while the other parameters were measured directly.

(2) Estimation of release loads of ARB from the reactor

Referring to studies of Munir et al. [1], the release loads of various ARB through the effluent (RL_{eff}) and biosolid (RL_{bio}) were calculated based on their concentrations (number of CFU) in the effluent or biosolids and the discharging volume, shown in Eqs. (2) and (3), respectively. Total release loads (RL_{total}) and release load ratios ($RL_{bio/eff}$) were calculated based on the release loads in the effluent and biosolid, as shown in Eqs. (4) and (5), respectively. The ratios between the ARB release load and input load in the influent (RL/IL) were calculated based on RL_{total}, Q_{in} and X_{in}, shown in Eq. (6).

$$RL_{eff} = Q_{eff} \times X_{eff} \quad \text{Eq. (2)}$$

$$RL_{bio} = Q_{bio} \times X_{bio} \quad \text{Eq. (3)}$$

$$RL_{total} = RL_{eff} + RL_{bio} \quad \text{Eq. (4)}$$

$$RL_{bio/eff} = RL_{eff} / RL_{bio} \quad \text{Eq. (5)}$$

$$RL/IL=RL_{total}/(Q_{in}X_{in}) \quad \text{Eq. (6)}$$

(3) Statistical analysis

All statistical tests were performed using SPSS Ver.19. The student t- test was used to conduct the statistical analysis of the results (i.e., for comparison of concentration means). The null hypothesis that the ARB concentration was not different between different samples was rejected at a p-value less than or equal to 0.05.

3. Results and discussion

3.1 The prevalence of various ARB in the activated sludge system

Six kinds of antibiotic-resistant heterotrophic bacteria were detected in the raw sewage (Fig. 2). CEP-resistant bacteria were the most prevalent kind with a concentration of $(1.5 \pm 0.1) \times 10^6$ CFU/mL, and the proportion to total heterotrophic bacteria was over 50 %. VAN-, SD- and ERY-resistant bacteria followed in abundance with proportions over 40 %. By comparison, low abundances of TC- and GEN- resistant bacteria were detected in the wastewater, with proportions of 7.5 % and 6.1 %, respectively.

The prevalence of ARB in the reactor with a sludge loading rate of 0.24 kg TCOD/(kg MLSS·d) are presented in Fig. 2. All ARB abundances in activated sludge decreased slightly compared to those in the influent.The concentration of CEP-resistant bacteria was $(1.0 \pm 0.1) \times 10^6$ CFU/mL, which was still the dominant ARB in activated sludge. ERY-, VAN-, and SD-resistant bacteria followed in abundance. The concentrations of GEN- and TC-resistant bacteria also decreased in the activated sludge, with their proportions at 3.3 % and 1.8 %, respectively. All ARB abundances were significantly enriched in the biosolid samples of the reactor.

The activated sludge system significantly decreased ARB concentrations in treated effluent; nevertheless all were still detected (Fig. 2). CEP-, VAN-, SD- and ERY-resistant bacteria were still the

four dominant kinds, with concentrations of $(1.2\sim2.7) \times 10^3$ CFU/mL. Relatively low abundances of TC- and GEN- resistant bacteria were detected, with concentrations of 190 ±10 CFU/mL and 80 ± 20 CFU/mL, respectively. However, their proportions were not significantly reduced with values of 1.8% and 0.6%, respectively. The results indicated that the activated sludge treatment process was not so effective in controlling antibiotic resistance, just as many other researchers had observed [1,14].

3.2 Release of ARB through the effluent and biosolids of the activated sludge system

To investigate ARB release from the activated sludge system, release loads of six kinds of ARB through the effluent and biosolids in reactor A [0.24 kg TCOD/(kg MLSS·d)] were calculated and are presented in Fig. 3. Bacteria resistant to CEP, VAN, SD and ERY were four major kinds that the system released to the environment through both effluent and biosolids. Their release loads were up to $(0.3\sim8.3) \times 10^5$ CFU/d through the effluent and $(0.1\sim4.9) \times 10^7$ CFU/d through biosolids, respectively.

On the other hand, compared to the effluent, significantly higher loads of all ARB were released through biosolids. For ERY- and CEP-resistant bacteria, the release load in biosolids was up to 100-fold compared to the value in the effluent. Similarly, the values were 46.2, 78.3, 44.2 and 56.0 for VAN-, SD-, TC- and GEN- resistant bacteria, respectively. These results imply that in a typical activated sludge system, the biosolids is the major pattern for the ARB release. Similar conclusions were also reported by Munir et al., who found that more than 10^{14} and 10^{15} CFU/d of TC- and SD-resistant heterotrophic bacteria were released into environment through biosolids in the WWTPs, while their release loads through the effluent were below 10^{12} and 10^{13} CFU/d [1].

3.3 Effects of sludge loading rate on the release of ARB from the activated sludge system

The release loads of six kinds of ARB in the effluents and biosolids of the reactors of various sludge loading rates are shown in Fig. 4. Significantly higher release loads for all ARB were detected in the effluent when the sludge loading rate increased from 0.24 to 0.61 kg TCOD/(kg MLSS·d). Especially, for GEN- resistant bacteria, their release loads in higher sludge loading rate system were up to11.9-fold of reactor A. The results are probably related to much higher hydraulic load of the higher sludge loading rate system.

Similarly, ARB release loads through the biosolids was also amplified with increased sludge loading rate. Data indicated that ARB release in the biosolids of reactor C was approximately 3.7~6.0-fold of that in reactor A. It was considered that, in the activated sludge system with good settleability (i.e., SVI = 50–150 mL/g), the bacterial release loads in the biosolids would be positively related to their concentrations in activated sludge. This hypothesis was confirmed by most ARB behaviors in the study (Fig. S1). When sludge loading rate increased from 0.24 to 0.42 and 0.61 kg TCOD/(kg MLSS·d)), releases of most ARB (CEP, VAN, SD, ERY and TC) in biosolids varied in accordance with their abundances in the activated sludge.

The release load ratio (ARB release in biosolids/ effluent) was introduced to assess the release contribution from biosolids and effluent. Considering the release pattern of antibiotic resistance, it was found that the biosolids were always the main channel for ARB release in both of the three reactors (Fig. 5). When the sludge loading rate increased to 0.61 kg TCOD/(kg MLSS·d), the release load ratio (biosolids/effluent) for all ARB decreased significantly from 56.0~110.7 to 21.7~56.2,, indicating more ARB were released through the effluent. However, most ARB were still released through the biosolids. Therefore, the potential risk brought by ARB release through the biosolids, especially in the low sludge loading rate reactor, was more severe than the effluent and should receive more attention. The development of biological treatment processes with less wasted biosolids, such as the MBR and oxidation ditch processes, might contribute to the minimization for ARB release in WWTPs.

The total release load of antibiotic resistance within the activated sludge system were also greatly

influenced by sludge loading rate. The total release loads of most ARB (expressed by RL_{total}) increased significantly when the sludge loading rate was up to0.61 kg TCOD/(kg MLSS·d) (Table S2). However, considering the three reactors received different loads of ARB in the raw sewage, which would influence their release in the effluent and biosolids, the total release load might not be suitable to evaluate the impact of sludge loading rate on ARB release in activated sludge system. To minimize the interference of input difference of ARB in the raw sewage, another index RL/IL (total release load/input load of the raw sewage) was introduced and calculated in Fig. 6. It was observed that the value was always less than 1 in all of the three

3.4 Effect of the sludge loading rate on the growth rates of various ARB

It had been demonstrated above that the release of antibiotic resistance in the system was significantly influenced by ARB abundances in activated sludge, which were further determined by their growth characteristics. Therefore, the growth rates of the six kinds of ARB were also explored. The growth rate (expressed by μ_{net}) of all ARB was observed to be prompted significantly when sludge loading rate increased from 0.24 to 0.42 kg TCOD/(kg MLSS·d) (Fig. 7). Further increase of sludge loading rate still resulted in the amplification of most ARB growth, but to a slighter extent.

The increase of ARB growth rates with operating condition was also observed previously. Kim et al. found that increases in the organic loading rate resulted in amplification of tetracycline resistance in activated sludge reactors loaded with typical municipal background tetracycline concentrations (1 μg/L), and those receiving influent augmented with 250 μg/L tetracycline [11].

Two possibilities might account for ARB amplification with an increased sludge loading rate. First, antibiotic-resistant populations present in the influent responded favorably to the growth conditions applied and achieved higher growth rate under high sludge loading rate. Because of the significantly higher hydraulic loading rate in reactor B and C (Table 1), most ARB in activated sludge

reactors, indicating most ARB tended to lose resistance after treated by activated sludge. However, the values in reactor B (0.06~0.20) and reactor C (0.04~0.17) were still significantly higher than reactor A (0.02~0.11). The data implied that, although antibiotic resistance was easy to be lost in the complex matrix, a reactor with higher sludge loading rate would retain more antibiotic resistance, thus resulting in more ARB released. This might be connected to ARB activity in various reactors. A high sludge loading rate provided more nutrients for ARB growth, causing more ARB were released. From this aspect, an activated sludge system operated at a lower sludge loading rate would be more conducive to controlling antibiotic resistance.

were surrounded by an environment with a higher antibiotic concentration, which was generally believed to promote antimicrobial resistance [13, 15, 16]. Second, more frequent transfer of ARGs among bacteria might be occurred in a higher sludge loading rate system, since nutrient was reported to be essential for horizontal gene transfer (HGT) [17].

However, ARB growth was not greatly lifted when the sludge loading rate increased from 0.42 to 0.61 kg TCOD/(kg MLSS·d), indicating considerable superfluous nutrient just promoted the growth rate slightly. According to Monod Equation[18] (Eq. 7) which described bacterial growth in function of substrate concentration, bacterial growth rate increased rather slowly with abundant nutrient concentration and eventually achieved a maximum rate (μ_{max}). Here the sludge loading rate in reactor C [0.61 kg TCOD/(kg MLSS·d)] represented a considerable high level in traditional activated sludge process, which is generally 0.2~0.4 kg BOD/(kg MLSS·d). Therefore, reactor C provided excess nutrients for ARB growth and the growth rates were not greatly lifted compared to reactor B.

$$\mu = \frac{\mu_{max}S}{K+S} \qquad \text{Eq. (7)}$$

μ—bacterial growth rate

μ_{max}—maximum rate of bacterial growth

K—half-saturation value

S—substrate concentration

In addition, it should be noted that the μ_{net} of TC- and GEN- resistant bacteria had negative values in the reactor of 0.24 kg TCOD/(kg MLSS·d). Concerning the concentrations of the two ARB were significantly lower than the other four types, it seemed that in a nutrient-insufficient environment, ARB with low abundance (e.g., < 10%) hardly kept their resistance and probably lost the ARGs. This might be caused by many possibilities, such as some ARB in the raw sewage hardly surviving the activate sludge. The decrease of ARGs transfer frequency due to the nutrients lack might also account for the result. In addition, the extracellular polymeric substance (EPS) was also reported for their characteristic of influencing antibiotic resistance [19, 20]. In our experiment, a significant change of EPS composition with different sludge loading rates was observed (Fig. S2). Further study on the mechanism that ARB obtain, maintain and dissipate resistance in activated sludge is proposed, so as to better understand how the sludge loading rate influences the growth of various ARB.

The releases of ARB were largely determined by their growth in the activated sludge. The growth rates of most ARB were promoted with increased sludge loading rate, thus resulting in an increase of their abundances in the activated sludge and the subsequent promotion of their releases through the effluent and biosolids. By comparison, activated sludge system with a low sludge loading rate limited the growth of ARB and the transfer of ARGs, thus leading to the decrease of antibiotic release. In general, the activated sludge treatment process was an important source for antibiotic resistance release into the environment. The findings of this study, which investigated the behaviors of six kinds of ARB in effluent/biosolids and the activated sludge, emphasize the relationships between their release and growth. The activated sludge process with a relatively low sludge loading rate and wasted biosolids volume was considered to be advantageous in antibiotic resistance control. Further studies on the comparison and optimization of other treatment processes and more operating conditions are proposed, so as to better minimize the potential risk of antibiotic resistance.

4. Conclusions

In this study three activated sludge treatment processes were constructed to evaluate the effects of the sludge loading rate on the releases and growth rates of six kinds of ARB. The biosolids were the principal pattern for all ARB release in the activated sludge system. The release load ratio (biosolids/effluent) was 44.2~110.7 under 0.24 kg TCOD/(kg MLSS·d) and 21.7~56.2 under 0.61 kg TCOD/(kg MLSS·d). More ARB were released through effluent when the sludge loading rate increased.

The growth rates of most ARB were promoted greatly with increased sludge loading rate from 0.24 to 0.42 kg TCOD/(kg MLSS·d) but did not change significantly with the further increase of sludge loading rate. The growth of most ARB in activated sludge largely determined their releases through the effluent and biosolids.

The release loads for all ARB in the effluent and biosolids were both significantly amplified when the sludge loading rate increased from 0.24 to 0.61 kg TCOD/(kg MLSS·d). The total release loads for ARB were also significantly lifted with increased sludge loading rate. A lower sludge loading rate was considered more conducive to the control of antibiotic resistance in activated sludge.

Acknowledgement

Notes and References

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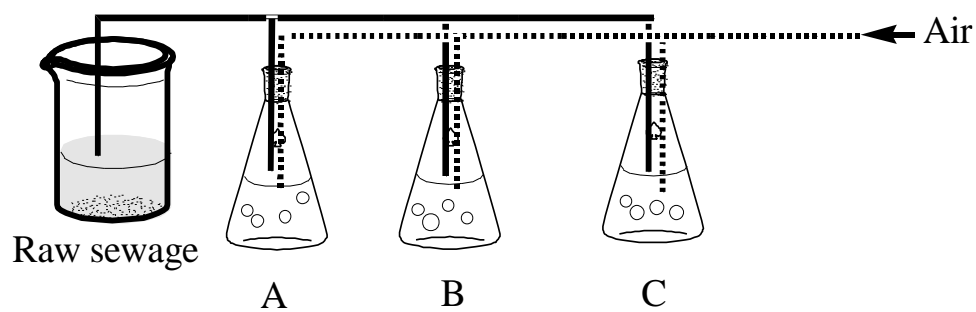
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Fig. 1. Schematic diagram of the two reactors used in study.
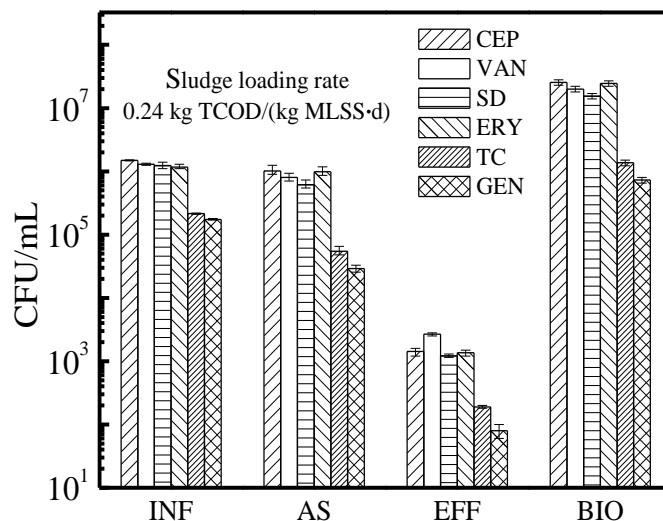
Fig. 2. Abundances of six ARB at four sampling points in the reactor [sludge loading rate = 0.24 kg TCOD/(kg MLSS·d)]. INF, AS, EFF and BIO represent raw sewage, activated sludge, effluent and biosolids, respectively. CEP, VAN, SD, ERY, TC and GEN represent cephalexin-, vancomycin-, sulfadiazine-, erythromycin-, tetracycline- and gentamicin- resistant heterotrophic bacteria, respectively.
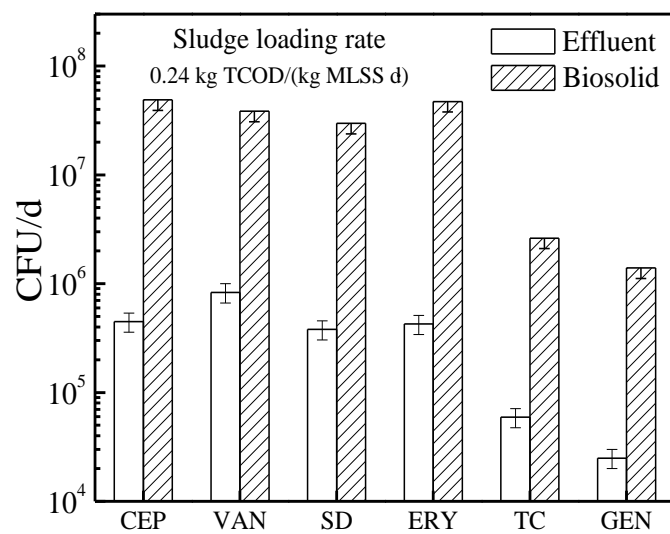
Fig. 3. Release loads of six kinds of ARB in effluents and biosolids of the activated sludge system at of

0.24 kg TCOD/(kg MLSS·d).
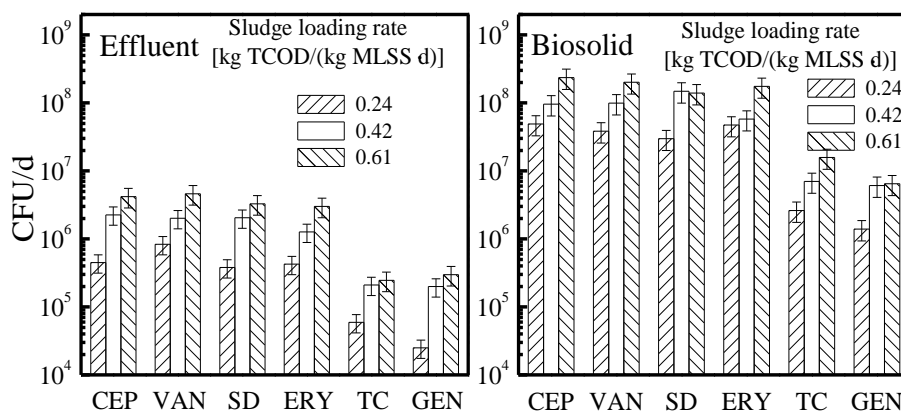
Fig. 4. Release load of six kinds of ARB in the effluents and biosolids of the three reactors with sludge loading rates of 0.24, 0.42 and 0.61 kg TCOD/(kg MLSS·d), respectively.
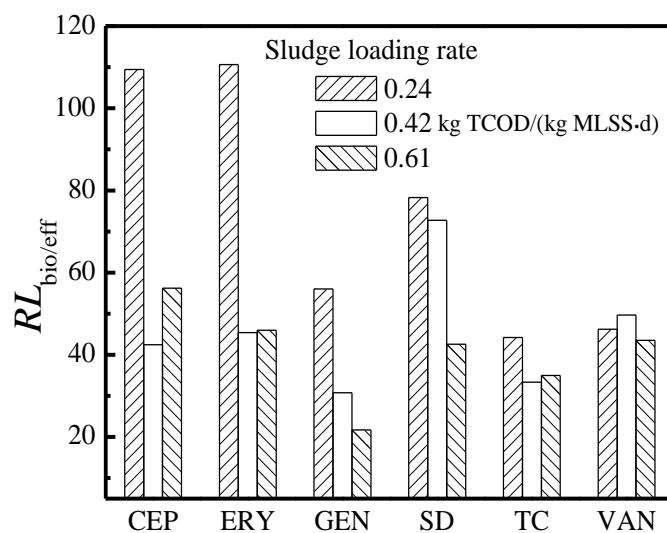
Fig. 5. The release load ratios ($RL_{\text{bio/eff}}$) of various ARB in activated sludge system of different sludge loading rates [0.24, 0.4 and 0.61 kg TCOD/(kg MLSS•d)].
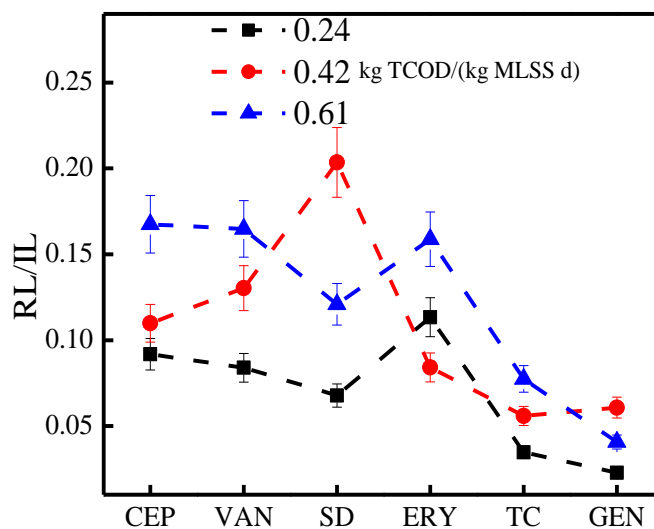
Fig. 6. The ratio curve between the release load and the influent load (RL/IL) of various ARB in activated sludge system at different sludge loading rates [0.24, 0.4 and 0.61 kg TCOD/(kg MLSS•d)].
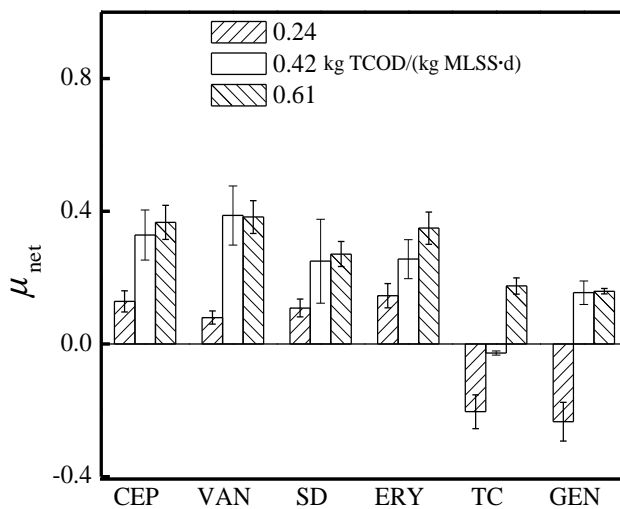
Fig.7. Net specific growth rates (μ_{net}) of various ARB in activated sludge system of three different sludge loading rates.