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Long-term effect of the antibiotic cefalexin on methane production during waste activated sludge anaerobic digestion

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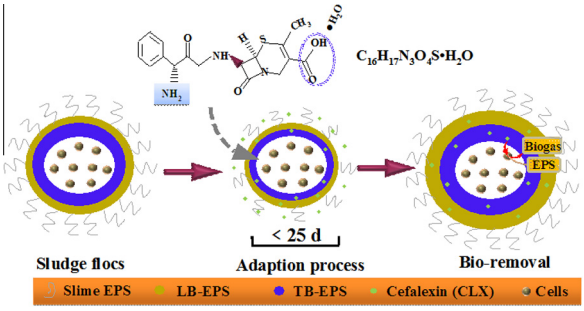
HIGHLIGHTS

- Long-term effect of cefalexin on methane production during sludge fermentation was assessed.
- Cefalexin exhibited a temporary inhibition in methane production, followed by a marked recover.
- The highest methane yield was 450 mL at 1000 mg-CLX/L after 157 days of digestion, rising by 63.8%.
- Extracellular polymeric substances protected microbes for self-growth and fermentation.

GRAPHICAL ABSTRACT



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ABSTRACT

Long-term experiments herein were conducted to investigate the effect of cefalexin (CLX) on methane production during waste activated sludge (WAS) anaerobic digestion. CLX exhibited a considerable inhibition in methane production during the initial 25 days while the negative effect attenuated subsequently and methane production recovered depending on CLX doses used (600 and 1000 mg/L). The highest methane yield reached 450 mL at 1000 mg-CLX/L after 157 days of digestion, 63.8% higher than CLX-free one. Stimulated excretion of extracellular polymeric substances (EPS) by CLX served as microbial protecting layers, creating a suitable environment for microbes' growth and fermentation. Further examination via ultraviolet visible (UV–Vis) spectra also verified the elevated slime EPS, LB-EPS and TB-EPS indicated by UV-254 in the presence of CLX. Unlike the commonly accepted adverse effect, this study demonstrated the beneficial role of CLX in methane production, providing new insights into its true environmental impacts.

1. Introduction

A high proportion of antibiotics can enter aquatic ecosystems directly, through the discharge of wastewater from the pharmaceutical industries, households and hospitals, or indirectly, by leaching and runoff of agricultural soils amended with manure from livestock (Sara et al., 2013). The release of antibiotics is becoming of considerable concern since it can cause negative effects not only on the environment but also on human health. Cefalexin (CLX), as one of the most prescribed antibiotics, belongs to the first generation cephalosporin type used in human medicine to treat aspiratory path infection, urine pathway infection and shin tissue infection and also in veterinary medicine due to its enhanced oral activity (Zhai, 2012). The widespread and overuse of CLX has lead to its detection in the aquatic environment. For

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example, the concentration of CLX present in municipal wastewater was found to be 339.4–375 ng/L (Guo et al., 2010); other studies reported by Estrada et al. (2012), as well as Saravanane and Sundararaman (2009) also showed that the effluent from a pharmaceutical drugs factory in India nearly reached 29 mg/L after treatment, suggesting that the potential effect of antibiotics in the environment requires further attention.

Anaerobic digestion (AD), a well-established technology, is the most frequently used biological process for sludge treatment (Zhen et al., 2014). Owing to heavy use of pharmaceutical drugs, high concentrations in the slurry influent to wastewater treatment plants (WWTPs) will inevitably result in the effects on the mixed population of anaerobic bacteria during the anaerobic process due to these molecules' biological residual activity (Sara et al., 2013). A recent study by Ince et al. (2013) confirmed that numbers of active bacteria and methanomicrobiales were negatively correlated with the concentrations of antibiotic oxytetracycline, in which around 50–60% reduction in biogas production were obtained with respect to the control depending on the applied doses. Over the last few years, a considerable amount of work has been done on assessing the effect of antibiotics on methane production during anaerobic digestion, however, the results about their definite functions are inconsistent, and in some cases even contradictory. Masse et al. (2000), for instance, studied the effect of six antibiotics (tylosin, lincomycin, tetracycline, sulphamethazine, penicillin and carbadox) on the psychrophilic anaerobic digestion of swine manure slurries in sequencing batch reactors (SBRs). The results indicated that presence of penicillin and tetracycline caused 35% and 25% decrease in methane output, respectively; in contrast, the slurries from pigs receiving other antibiotics did not significantly influence methane production. Similarly, Mitchell et al. (2013) obtained the significant inhibition of anaerobic digestion of cattle manure containing florfenicol at concentrations of 6.4, 36 and 210 mg/L, where biogas yield reduced by 5%, 40% and 75%, respectively; whereas sulfamethazine over the evaluated concentration range of 0.28–280 mg/L imparted no observed effect on biogas production during the 40 days' incubation. Another study by Sara et al. (2013) investigating the effect of three classes of veterinary antibiotics (danofloxacin, micospectone and ceftiofur) demonstrated that the former two showed obvious inhibition on pig manure anaerobic digestion, reducing biogas production by 10–15% and 18–23%, respectively; comparatively, ceftiofur induced a much less substantial impact mainly because of its large biodegradability. For sulfonamide, Mohring et al. (2009) did not observe any statistically significant inhibitory effect on anaerobic digestion. The above inconsistent and even contradictory findings might be attributed to differences in the types and concentrations of antibiotics used, sensitivity of analysis utilized to measure effects (Amin et al., 2006), and also durations of exposure tests.

Obviously, although numerous investigations have been carried out to explore the effect and fate of different types of antibiotics in anaerobic treatment, up to date, the questions on whether and/or how antibiotics, particularly CLX will affect sludge anaerobic digestion still remain unknown. Moreover, most of current studies were mainly focused on the short-term response of anaerobic process to antibiotics (Beneragama et al., 2013; Shi et al., 2011; Mitchell et al., 2013), and the useful information on the long-term toxic impacts of CLX is definitely limited (Estrada et al., 2012; Zhai, 2012). It is therefore of considerable importance to make additional effort for better understanding the long-term effect and exact role of CLX on/in digestion efficiency and stability of anaerobic digestion process. Hence, the main objective of this study was to explore the potential influence of the presence of CLX on methane production from WAS during anaerobic digestion. The inhibitory effect of CLX was assessed by monitoring methane production, variations of extracellular polymeric substances (EPS), and accumulation of individual volatile fatty acids (VFAs). Removal of CLX was evaluated based on measured bulk phase concentrations of CLX.

2. Methods

2.1. Test materials

Antibiotic cefalexin (CLX) used in this study was purchased from Wako (99% purify), Japan. Chemical molecular formula of CLX is $C_{16}H_{17}N_3O_4S.H_2O$; the molecular weight is 365.40 g/mol; and the solubility is 1790 mg/L. The waste activated sludge (WAS) was withdrawn from the secondary sedimentation tank of a municipal WWTP in Sendai, Japan. The sludge samples were transferred immediately to the laboratory and stored at 4 °C in order to maintain sample freshness. The seed sludge was collected directly from discharged sludge from a 5-L lab-scale completed stirred treatment reactors (CSTR) operated at 35 ± 1 °C in our lab. The reactor was made of plexiglass and controlled to 35 ± 1 °C by water jackets and heaters. The feed sludge was comprised of thickened WAS. Feeding and drawing pumps were operated 6–12 times per day by using a timer-controller. The inoculum was degassed and incubated in a water bath (35 ± 1 °C) (BT 100, Yamato, Japan) under anaerobic condition by 5–7 days until methane content reached about 55%. The principle characteristics of the WAS and seed sludge are given in Table 1.

2.2. Biochemical methane potential (BMP) assay

Biochemical methane potential (BMP) assay (Shi et al., 2011; Zhen et al., 2014) was conducted in the series of 120 mL sealed glass serum bottles to evaluate the potential effect of CLX on WAS anaerobic digestion. The effective working volume was 75 mL. The seed sludge to WAS ratio was 2:1 (volume:volume). To determine dose–response of methane output to CLX and provide the possible guides for addressing the CLX-related issues in case of emergency, the investigations of a broad range of antibiotic concentrations were performed herein. CLX was initially negligible in the WAS samples and was added into assays with the final concentrations of 0, 50, 200, 400, 600, 1000, and 2000 mg/L. The pH of all samples was then carefully neutralized to around 7.0 before the start-up of reactors. A control with 75 mL of inoculums was conducted to determine the biogas production from endogenous inspiration. After being sealed with butyl rubber stoppers secured with aluminum crimp, the bottles were flushed with high-purity nitrogen gas at the flow rate of 0.5 L/min for about 2 min to ensure an anaerobic atmosphere before the beginning of fermentation. After that, all bottles were placed in a water bath (BT 100, Yamato,

Table 1
Characteristics of excess sludge and seed sludge used in this study.

Items	Seed sludge	WAS
pH	6.90–6.91	6.44–6.47
Moisture content (%)	84.81	90.43
TSS (g/L)	72.14	21.00
VSS (g/L)	67.16	4.76
TCOD (mg/L)	94890.26	30374.18
SCOD (mg/L)	14591.76	27.32
NH_4^+-N (mg/L)	1369.18	22.34
TPS (mg/L)	24064.87	4385.68
SPS (mg/L)	1439.81	–
TPN (mg/L)	23670.67	8111.93
SPN (mg/L)	2273.32	1194.21

TSS: total suspended solids; VSS: volatile suspended solids; TCOD: total chemical demand oxygen; SCOD: soluble chemical demand oxygen; TPN: total protein; SPN: soluble protein; TPS: total polysaccharide; SPS: soluble polysaccharide.