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Cometabolic degradation of lincomycin in a Sequencing Batch Biofilm Reactor (SBBR) and its microbial community

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HIGHLIGHTS

- The lincomycin was degraded effectively in cometabolic system with glucose as growth substrate.
- *Roseovarius*, *Thiothrix*, *Halomonas*, *Ignavibacterium*, and *TM7_genus_incertae_sedis* were dominant populations.
- Functional groups of lincomycin were effectively degraded.
- Cometabolism may be synergistically caused by different functional dominant bacteria.

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ABSTRACT

Cometabolism technology was employed to degrade lincomycin wastewater in Sequencing Batch Biofilm Reactor (SBBR). In contrast with the control group, the average removal rate of lincomycin increased by 56.0% and Total Organic Carbon (TOC) increased by 52.5% in the cometabolic system with glucose as growth substrate. Under the same condition, Oxidation–Reduction Potential (ORP) was 85.1 ± 7.3 mV in cometabolic system and 198.2 ± 8.4 mV in the control group, indicating that glucose changed the bulk ORP and created an appropriate growing environment for function bacteria. Functional groups of lincomycin were effectively degraded in cometabolic system proved by FTIR and GC–MS. Meanwhile, results of DGGE and 16S rDNA showed great difference in dominant populations between cometabolic system and the control group. In cometabolic system, *Roseovarius* (3.35%), *Thiothrix* (2.74%), *Halomonas* (2.49%), *Ignavibacterium* (2.02%), and *TM7_genus_incertae_sedis* (1.93%) were verified as dominant populations at genus level. Cometabolism may be synergistically caused by different functional dominant bacteria.

1. Introduction

Due to human activities, a large quantity of antibiotics in wastewater has been discharged into environment (Phillips et al., 2010), leading to increasing antibiotics in the environment (Kummerer, 2009). As a broad-spectrum macrolides antibiotic, lincomycin was isolated in 1962 from a soil actinomycete found near Lincoln, Nebraska (Stratton, 1998) and applied into the diseases prevention and treatment in animal husbandry, capable of suppressing the activity of sensitive bacteria peptidyl transferase, stopping extension of follow-up peptide chains, and inhibiting protein syntheses in bacteria cell bodies. Besides, lincomycin has poor biodegradability because of structures such as amines, ring structures, aliphatic ethers and sulphur (Bertelkamp et al., 2014).

Technology of lincomycin degradation has been the research hotspot, for example, sorption (Wang et al., 2012), photo-fenton process (Bautitz and Nogueira, 2010), coupling photocatalytic and membrane technologies (Augugliaro et al., 2005) and solar photodegradation (Andreozzi et al., 2006) and so on, most of which belong to physical and/or chemical treatment technologies. It is discovered that, as one of the most efficient bioaugmentation ways, cometabolism can be used to remove many refractory organics economically and environmental-friendly (Nzila, 2013), such as ethyl mercaptan (Sedighi et al., 2016), trichloroethylene (Kocamemi and Cecen, 2010; Zhang and Tay, 2015), 1,2-dibromoethane (Hatzinger et al., 2015), without secondary pollution.

Cometabolism may be realized by virtue of multiple bacterial synergism (Liang et al., 2011; Rodriguez-Verde et al., 2014). At present, single bacterium such as *Ralstonia eutropha* (Sedighi et al., 2016), *Pseudomonas putida* (Lv et al., 2016), *Martelella sp* AD-3

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(Feng et al., 2012), *Pseudomonas oleovorans* DT4 (Zhou et al., 2011) is adopted for cometabolic microbial researches. However, there are only limited reports about construction of cometabolic degrading flora and compositions of microbial community.

In this research, firstly, lincomycin cometabolic biotreatment system and the control group were constructed in two parallel aerobic SBBRs, and then lincomycin degradation efficiency was investigated and microbial community was studied by PCR-DGGE and 16S rDNA amplicon pyrosequencing.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

All chemicals including lincomycin used in the experiments were analytical or reagent grades. Activated seed sludge was acquired from a municipal wastewater treatment plant. To meet the nutrition demands of microorganisms, microelements were added as below (mg/L): NH_4Cl, 145.5; KH_2PO_4, 34.33; $CoCl_2{\cdot}6H_2O$, 0.41; $MgSO_4{\cdot}7H_2O$, 12.32; $CaCl_2$, 0.55; $ZnSO_4{\cdot}7H_2O$, 0.43; $FeSO_4{\cdot}7H_2O$, 2.50; $Na_2MoO_4{\cdot}2H_2O$, 1.26 and $MnSO_4$, 0.75.

2.1.2. Bioreactor

The SBBR (Fig. 1) with 12 L of working volumes (30 cm height, 25 cm diameter), of which 40% were packed with combination packing fillings (constituted by hanging 6–7 g white semi-flexible filiform materials on each discs), was fed with artificial wastewater. Oxygen was pumped into each reactor by air pump, and temperature was adjusted by incubator.

2.2. Methods

2.2.1. Experimental procedure

SBBRs were firstly seeded by the sludge (10 g/L TSS), and then, cycled in an operational mode of "influent 0.5 h – reaction 22.5 h – sedimentation 0.5 h – effluent 0.5 h". Hydraulic retention time (HRT) was 22.5 h, and exchange ratio of volumes approximated 1. The temperature was kept constant at 30 °C ± 0.5 °C, without any pH control.

Under the same circumstances, reactor A and B were adopted for the microbial degradation tests. Specifically, reactor A was set as cometabolic biotreatment system with 3.5 ± 0.5 mg/L DO and 0.2 kgCOD/(m^3·d) lincomycin plus 1.0 kgCOD/(m^3·d) glucose as growth substrate and reactor B was set as the control group solely with 3.5 ± 0.5 mg/L DO and 0.2 kgCOD/(m^3·d) lincomycin. Differences between two reactors were determinated during the typical stable cycles, and then, PCR-DGGE and 16S rDNA amplicon pyrosequencing were used to analyse the microbial communities of the two systems.

2.2.2. Wastewater quality analysis

Lincomycin concentration was measured using an ultraviolet (UV)–visible spectrophotometer (DR5000; Hach, USA) with an absorption wavelength of 276 nm. Linear relation between lincomycin concentration (C) and absorbance value (A) was $C = 410.36\,A$, $R^2 = 0.9999$ (C:0–400 mg/L). TOC was analyzed by UV-light catalytic oxidation (Elementar Liqui TOC, Germany), and parameters of DO and temperature were monitored by a DO meter (HQ30d; Hach, USA).

2.2.3. FTIR and GC–MS analysis for wastewater composition

Effluent and lincomycin (analytical reagent, AR) were collected for Fourier transforms infrared spectroscopy (FTIR) study. Effluent of 100 ml was frozen to solid state and powdered by vacuum freeze drier firstly; then the mixture of the powder and KBr was ground by pestle and mortar in infrared light, and pressed into slice under $(5–10) \times 10^7$ Pa. The spectra were collected using FTIR system (NicoletiN10, USA) equipped with diffuse reflectance accessory in a range of 400–4000 cm^{-1}. All spectra were plotted using the same scale on the transmittance axis.

Major compositions of effluent were identified by GC–MS analyzer (Agilent 5975C/7890, USA). Effluent of 150 ml were pretreated with liquid–liquid extraction by isopyknic dichloromethane under acidic, neutral and alkaline conditions, respectively. The extracted organic solvents were mixed and added with anhydrous sodium sulfate for 24 h, and finally condensed at 3–5 mL with a rotatory evaporator.

ADB-5 (30 m × 0.25 mm × 0.25 μm) was used under the following conditions: 1 μL at a split ratio, 5:1; injection temperature, 250 °C; carrier gas, helium (1.0 mL/min); temperature program

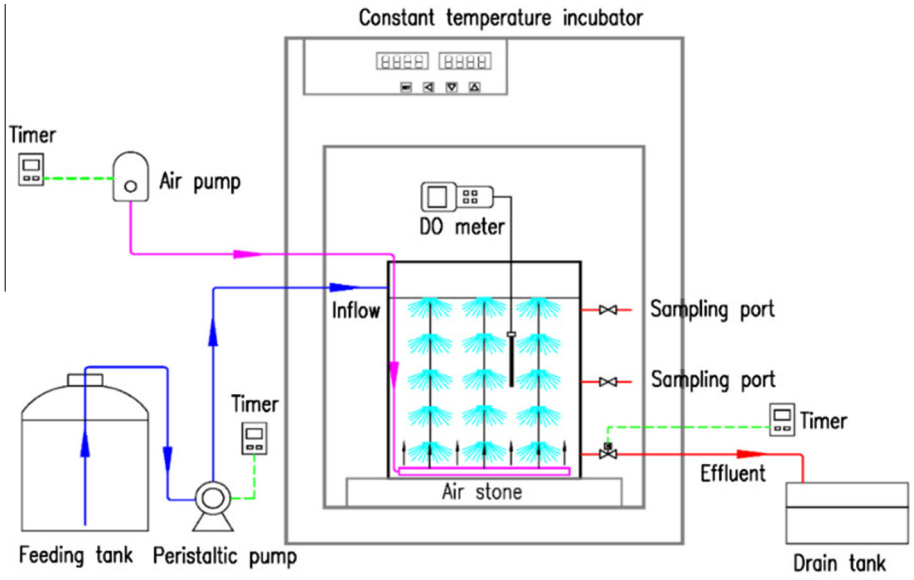

Fig. 1. Experimental equipment of SBBR.