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A novel methanotrophic co-metabolic system with high soluble methane monooxygenase activity to biodegrade refractory organics in pulping wastewater

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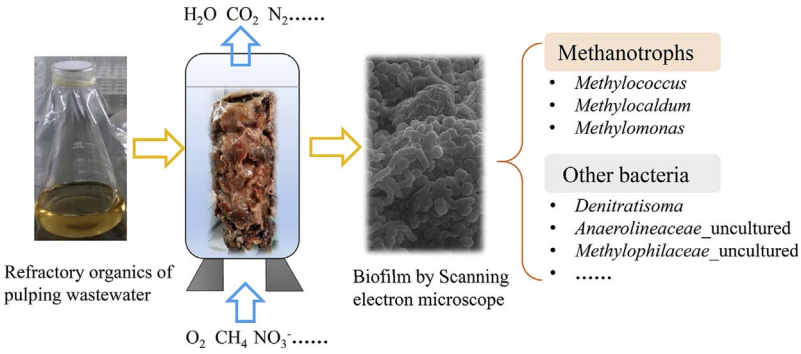
GRAPHICAL ABSTRACT



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ABSTRACT

Pulping wastewater still contains massive refractory organics after biotreatment, with high colority, low biodegradability, and lasting biotoxicity. To eliminate refractory organics in pulping wastewater, a methanotrophic co-metabolic system in a gas cycle Sequencing Batch Biofilm Reactor (gcSBBR) seeded by soil at a ventilation opening of coal mine was quickly built on the 92nd day. The removal rate of COD, colority and TOC was 53.28%, 50.59% and 51.60%, respectively. Analysis of 3D-EEM indicated that glycolated protein-like, melanoidin-like or lignocellulose-like, and humic acid-like decreased by 7.85%, 5.02% and 1.74%, respectively. Moreover, this system exhibited high activity of soluble methane monooxygenase (sMMO) and mmoX encoding sMMO reached up to 7.89×10^5 copies/μL. Methanotrophs, namely, *Methylocaldum* (8.28%), *Methylococcus* (6.06%) and *Methylomonas* (0.07%), were detected by 16S rRNA sequencing. And other bacteria were dominated by *Denitratisoma*, *Anaerolineaceae*_uncultured and *Methylophilaceae*_uncultured. Refractory organics was biodegraded through the synergy among microorganisms, and a postulated synergy pathway was put forward.

1. Introduction

Pulping wastewater has huge discharges and a high concentration, and contains complicated pollutants (Thompson et al., 2001). Aerobic and anaerobic processes are adopted to degrade biodegradable organics, but there were still have massive refractory organics in the effluents, exhibiting high colority, low biodegradability and lasting biotoxicity. Various physical and chemical methods, such as chemical oxidation (Pirkanniemi et al., 2007), coagulation–flocculation (Wong et al., 2006), membrane filtration (Mänttäri et al., 2008), adsorption

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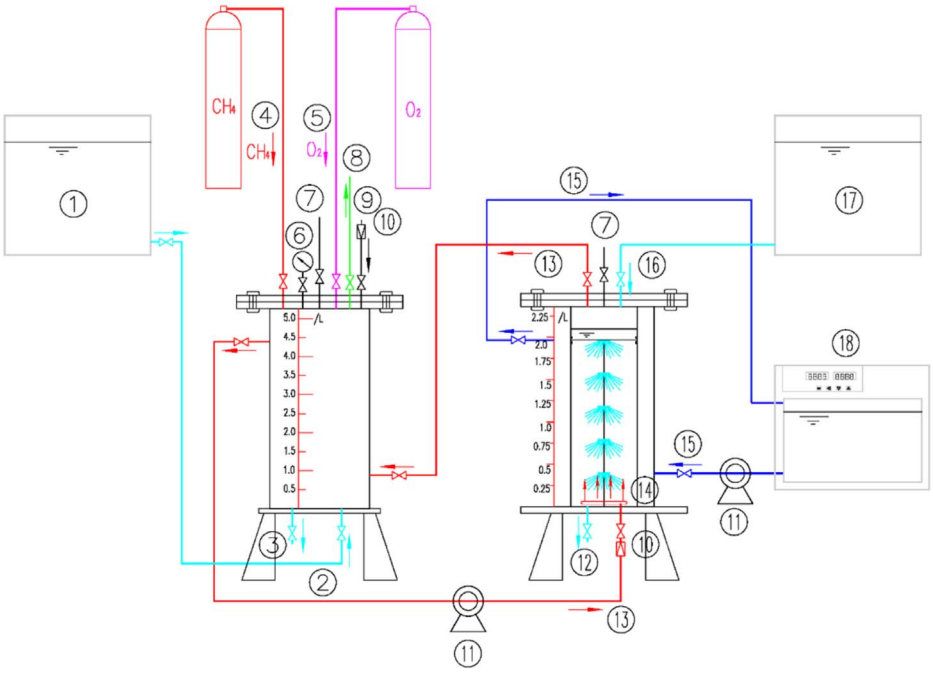

① Water tank ② Water inflow ③ Water effluent ④ CH_4 inflow ⑤ O_2 inflow ⑥ Piezometer ⑦ Ventilation tube ⑧ Sampling port ⑨ Air inflow ⑩ One-way inlet valve ⑪ Peristaltic pump ⑫ Effluent and Sampling port ⑬ Cycle gas pipe ⑭ Air stone ⑮ Cycle hot water pipe ⑯ NMS medium inflow ⑰ Feeding tank ⑱Constant temperature electric water bath

Fig. 1. Experimental equipment of methanotrophs.

(Tao et al., 2006), have been applied to degrade the refractory organics in pulping wastewater. However, they are not environmental friendly and economically feasible. Besides, bio-enhancement technologies such as especial bacteria (Chandra et al., 2007), bio-augmentation (Yu & Mohn, 2002), and fungal consortium (Malaviya & Rathore, 2007) are also researched. But they are inapplicable at present, due to consecutive replenishment, low stability, single function and high prices.

Methanotrophs are widely distributed in sewage sludge (Ho et al., 2013), coal mine (Han et al., 2009), lake sediments (Rahalkar et al., 2009), freshwater pond (Costello et al., 2002) and so on. Methane can be utilized by methanotrophs as the sole carbon and energy source. It is firstly converted into methyl alcohol with the catalysis of methane monooxygenase (MMO) generated by methanotrophs (Semrau et al., 2010); then methyl alcohol is converted into formaldehyde, for the follow-up metabolism of microorganism (Kalyuzhnaya et al., 2015). There are two kinds of MMOs (Hanson & Hanson, 1996): particulate MMO (pMMO) in cytoplasm and soluble MMO (sMMO) in intracytoplasmic membranes, of which pMMO is rather common in almost all the known methanotrophs, and sMMO appears only in some methanotrophs in the presence of less Cu^{2+} (Nielsen et al., 1997).

It is founded that sMMO generated by methanotrophs can catalyze the oxidation of carbon substrates and oxidize many refractory organics, for example, bentazone (Hedegaard et al., 2018); trichloroethylene (TCE) (Shukla et al., 2009); benzene, xylene and toluene (BTX) (Hesselsoe et al., 2005); and alicyclic, aromatic and heterocyclic compounds (Colby et al., 1977). Basically, sMMO can oxidize alkanes with even 8-C atoms, as well as ethers, cyclic alkanes, and aromatic hydrocarbons and so on; while pMMO no more than 5-C atoms (Burrows et al., 1984). Based on the non-specific degradation property of sMMO, a methanotrophic system could be used to remove refractory organics in pulping wastewater. Besides, in the previous studies, methanotrophs could be enriched in a petri dish, or reactors like fluidized bed reactor (Pfluger et al., 2011), membrane bioreactor (Kampman et al., 2014), membrane biofilm reactor (Modin et al., 2010), and so on.

As a clean, inexpensive and sustainable energy source, methane is the main constituent of natural gas, biogas and oilfield gas. It can be also largely generated during the anaerobic treatment of pulping wastewater. Thus, we tried to utilize methane to build a methanotrophic co-metabolic system in a gcSBBR; and applied this system to degrade those residual refractory organics in the pulping wastewater after the routine bio-treatment. Then, mechanisms of the system was researched by analyzing the metabolites and microbial population structures. By this way, this research was aimed to provide a new way to biodegrade refractory organics in pulping wastewater.