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Evaluation of performance and microbial community in a two-stage UASB reactor pretreating acrylic fiber manufacturing wastewater

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ABSTRACT

A two-stage UASB reactor was employed to pretreat acrylic fiber manufacturing wastewater. Mesophilic operation (35 ± 0.5 °C) was performed with hydraulic retention time (HRT) varied between 28 and 40 h. Mixed liquor suspended solids (MLSS) in the reactor was maintained about 8000 mg/L. The results showed COD and sulfate removal could be kept at 51% and 75%, respectively, when the HRT was no less than 38 h. Sulfate reduction mainly occurred in the acidification-stage reactor while methane production mainly occurred in the methane-stage reactor. The size of granule formed in the acidification-stage reactor ranged between 1 and 5 mm while the largest size of granule in the methane-stage reactor ranged from 0.5 to 2 mm. Compared to microbial populations in the acidification-stage reactor, the microbial diversity in methane-stage reactor was more abundant. In the acidification-stage reactor, the *Syntrophobacter sulfatireducens* devoted to both sulfate reduction and acetate production.

1. Introduction

Acrylic fiber is one of the major synthetic fibers commonly used in the mass production of clothing. The chemical synthesis of acrylic fiber is carried out by polymerization of the acrylonitrile (AN) monomers. The quantity of acrylic fiber manufacturing wastewater is inclined to increase with the increment of acrylic fiber used, and the components of wastewater discharged are complicated and variable. The biodegradability of the acrylic fiber manufacturing wastewater was very low: the ratio of BOD_5/COD was 0.1–0.2, and there were some biorefractory organic pollutants in the wastewater (Zhang et al., 2003). For wastewater treatment, most acrylic fiber manufacturing companies had adopted a conventional biological treatment (Cheng et al., 2004) or biological treatment followed by physicochemical methods of neutralization, coagulation and sedimentation (Young et al., 2005). Ultrafiltration (UF) and reverse osmosis (RO) were also employed to treat acrylic fiber manufacturing wastewater, and the separation characteristics of wastewater were investigated with the variations of pressure and temperature (Lee et al., 2006).

Anaerobic treatment with its particular advantages (Cheng et al., 2010) has become a viable technology and has been most commonly used. Among them, the up-flow anaerobic sludge blanket (UASB) reactor attains successful applications due to its simple design; easy construction and maintenance; low operating cost; and ability to withstand fluctuations in pH, temperature, and influent substrate concentration (Vasileios and Alexandros, 2007; Cronin and Lo, 1998; Alvarez et al., 2006). Two-stage UASB could control the acidification in the first reactor and prevent the overloading and/or inhibition of methanogenesis in the second reactor (Prawit et al., 2011). Although UASB reactors are being increasingly used for the treatment of various high-strength industrial wastewaters, many problems are encountered: granulation may not occur and the seeded granular sludge may get washed out (Revanuru and Mishra, 2008).

The development of the molecular biology tools has contributed to the detection, quantification, and identification of the microbial communities involved in the wastewater treatment. Cloning and sequencing of 16S rRNA gene fragments provide information about the phylogeny of the microorganisms. In addition, fluorescent in situ hybridization (FISH) technique is an appropriate tool for comparison and quantification, respectively, of the changes in the microbial composition in the bioreactor (Daz et al., 2006). However, studies on the molecular identification of the microbial communities in anaerobic treatment processes based on cloning and analysis of the nucleotide sequences of genes encoding the 16S ribosomal RNA (16S rRNA) are quite limited (Nadais et al., 2010).

In this work, a two-stage UASB reactor was employed to pretreat acrylic fiber manufacturing wastewater and the molecular biotechnology was applied to study the microbial population. The aims of the work were as follows: (a) to test the performance of simultaneous COD and sulfate removal in the two-stage UASB reactor pretreating acrylic fiber manufacturing wastewater; (b) to

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evaluate the methanogenic archaea and anaerobic bacteria by constructing 16S rDNA clone libraries and phylogenetic analyses; (c) to quantify the sulfate-reducing bacteria (SRB) and archaea by fluorescent in situ hybridization (FISH).

2. Methods

2.1. Wastewater characteristics

The acrylic fiber manufacturing wastewater was collected from a synthetic-fiber factory located at the city of Ningbo, China. The wastewater quality was quite complicated because it consisted of acrylonitrile unit, vinyl acetate unit, oligomers, DMAc, EDTA and sulfate as Table 1 showed.

Table 1
Characteristics of acrylic fiber manufacturing wastewater.

	Unit	Range	Average
Acrylonitrile	mg/L	2.99–3.51	3.33
Vinyl acetate	mg/L	0.028–0.038	0.033
Oligomers	mg/L	201.8–218.5	208.3
DMAc	mg/L	85–115	100
pH	–	3.0–3.8	3.5
COD	mg O_2/L	4378–4611	4528
NH_4^+-N	mg N/L	71–88	85
SO_4^{2-}	mg S/L	2061–2262	2158
PO_4^{3-}	mg P/L	0.015–0.021	0.018

2.2. Experimental set-up and start-up

Fig.1 showed a flow chart of two-stage UASB reactor used in this study. Continuous operation was carried out during the whole experiment. The volume of acidification-stage reactor and methane-stage reactor were 11 and 30 L, respectively. Temperature control was accomplished by water bath with water recirculation through the reactor's double jacket. The study was conducted at mesophilic condition at 35(±0.5) °C. The seed sludge was inoculated from a full-scale UASB reactor treating brewing wastewater. NaOH was added to make influent pH value at 6.5. The MLSS concentration in the two-stage UASB reactor was maintained about 8000 mg/L by extracting excess sludge.Fig.1 Schematic diagram of the two-stage UASB reactor treating acrylic fiber manufacturing wastewater

2.3. Sample collection and preparation

Samples were withdrawn from the liquid media at the beginning and at the end of each treatment period. They were centrifuged at 6000 rpm for 30 min to remove microorganisms from the liquid medium. Clear supernatants were analyzed for COD, sulfate contents according to standard methods (APHA, 1999). Samples were analyzed in triplicate and average values were reported. ORP and pH measurements were done by using the relevant probes and analyzers. Biomass concentrations were determined by filtering the washed salt-free samples through 0.45 μm membrane filter and drying in an oven at 105 °C to constant weight.

The cooling extraction method, which is described as follows, was applied for the extraction of the extracellular polymeric substances (EPS) from the sludge. For the EPS analysis, 2 mL of sludge were centrifuged, removed of supernatant, added with 10 ml of 0.85% NaCl solution and 60 mL formalin. The EPS in this mixed liquor was extracted with ultrasonication for 300 s while being cooled in ice water. After being centrifuged at 12,000 rpm for 30 min, the supernatant was analyzed for polysaccharide and protein, which were regarded as the main parts of EPS materials. Polysaccharide was determined by a sulphuric acid-anthrone method and protein was analyzed according to the Lowry Folin method (Lowry et al., 1951).

2.4. Construction of 16S rDNA clone libraries and phylogenetic analysis

Total DNA templates were extracted from the sludge samples of the acidification-stage reactor and methane-stage reactor. Community 16S rDNA genes were amplified with bacterial primer set EUB27F (5′-GAGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-CTAC GGCTACCTTGTTACGA-3′). PCR was performed with 1 μl of DNA (diluted 10 times from sample), 0.2 μM of both primers, 250 μg of bovine serum albumin solution (DingGuo, China), 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, each dNTP at a concentration of 0.2 mM, 1.25 IU of Taq polymerase (TianGen, China), and distilled deionized water (ddH_2O) was added to each reaction to reach a total volume of 50 μL. DNA amplification was performed using a thermal program consisting of a initial denaturation at 95 °C for 5 min, 32 cycles of 95 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min 30 s, and a final extension at 72 °C for 10 min. PCR products were verified by electrophoresis. Amplified DNA from three separate reactions was pooled and purified using a kit (Fermentas, Canada). The purified amplicons were then cloned by using a pMD18-T vector (Takara, Japan). The PCR-amplified products of positive recombinants were digested with the restriction enzyme HaeIII (Takara, Japan) at 37 °C for 4 h, electrophoresed in 1.5% agarose gels at 200 V for 20 min and stained with ethidium bromide. Clones with similar banding patterns were grouped together, and one representative clone from each group was chosen for sequencing. The obtained sequences of ca. 1500 bp 16S rDNA were compiled and compared to available rDNA gene sequences in GenBank using the NCBI BLAST program. A neighbor-joining tree with the Jukes-Cantor method was conducted with bootstrapping (1000 replicates) using the

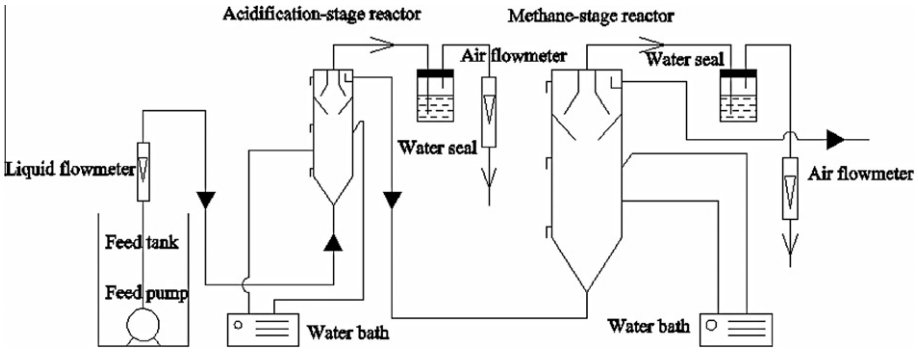

Fig. 1. Schematic diagram of the two-stage UASB reactor treating acrylic fiber manufacturing wastewater.