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Simultaneous in-situ excess sludge reduction and removal of organic carbon and nitrogen by a pilot-scale continuous aerobic–anaerobic coupled (CAAC) process for deeply treatment of soybean wastewater

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ABSTRACT

A pilot-scale continuous aerobic–anaerobic coupled (CAAC) process was constructed and implemented for simultaneous excess sludge reduction and removal of organic carbon and nitrogen from the anaerobic effluent generated by an internal circulation (IC) reactor for treating soybean wastewater. When the hydraulic retention time (HRT) was shortened to 1.3 days, the average removal efficiencies for chemical oxygen demand (COD), NH_4^+–N, and total nitrogen (TN) reached 86%, 91%, and 67%, respectively. The mixed liquor suspended solids (MLSS) in the effluent generated by the CAAC process amounted to only 24 mg/L on average. The sludge production rate was estimated to be 0.1582 g MLSS/g COD, which is only 32% of that achieved by the conventional activated sludge process. The denaturing gradient gel electrophoresis (DGGE) analysis result showed that some fermentative and acetogenic bacteria were detected in anoxic and anaerobic zones. Such populations are associated with the anaerobic digestion and in-situ reduction of activated sludge.

1. Introduction

Soybean protein is basically made of soy bean and has been widely used as important healthcare product because of better nutrition and easier absorption in the body. However, substantial amounts of wastewater are generated from soybean protein processing. The concentration of organics and ammonia nitrogen in soybean wastewater is very high [1–3]. The direct discharge of this kind wastewater to watercourses causes serious ecological risks and health-related problems [4]. Therefore, improving wastewater treatment techniques and enhancing the quality of discharged water are seriously necessary.

Oxic treatments, such as conventional activated sludge treatments, can remove a large quantity of organics from wastewater, but eliminating the high-strength organics from wastewater necessitates high aeration rates. Some anaerobic technologies are important alternative methods for treatment and energy recovery from such high-strength wastewater, such as up flow anaerobic sludge blanket reactors (UASBs), anaerobic baffled reactors, and anaerobic filter reactors [1,5–7]. However, the effluents generated by anaerobically treated systems do not comply with the national drainage standard (GB 8978-1996). Technological and economic factors dictate that combining anaerobic methods with oxic measures is an appropriate approach to treating high-strength wastewater [3,8]. Some researchers have presented the effects of combined anoxic and oxic methods. Li et al. [3] established a combined UASB-anoxic–oxic treatment system (UASB-A/O) and biological aerated filter system to treat soybean protein wastewater. The discharge water has a COD of 40 mg/L, biochemical oxygen demand (BOD) of 8 mg/L, and ammonia concentration of 4 mg/L; these values are better than the current national drainage standard (a COD below 100 mg/L is required) [3]. Deng et al. [9] used a combined system consisting of an IC anaerobic reactor and sequencing batch reactor to treat swine wastewater. Other oxic processes can also be used as subsequent treatment measures for deeply treating the effluent produced by anaerobic processes, such as that the aerobic membrane bioreactor [10] and sequencing fed-batch biofilm reactor [11]. However, one of the drawbacks of these post-treatment processes is high excess sludge production. The rising cost incurred from the treatment and disposal of excess sludge and adverse environmental effects emphasize the need to reduce excess sludge production. Researchers have accordingly investigated several methods, such as lysis/cryptic growth, uncoupled metabolism, maintenance metabolism, and predation on bacteria [12–14].

Some modified bioreactors have recently been designed and investigated for excess sludge production, in which alternated aerobic and anaerobic compartments are structured. Feng et al. [15]

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achieved a 63.7% in-situ reduction of excess sludge solids using a repeated coupling of aerobic and anaerobic conditions bioreactor during wastewater treatment. In our previous studies, a CAAC process was designed on lab-scale for in-situ sludge reduction feasibility studies [16]. The sludge yield coefficient of this process was only 34.5% of that reached by the conventional active sludge process (0.5) [16]. However, the feasibility of in-situ sludge reduction of CAAC process under pilot-scale has not been studied. Microorganisms also play a key role in wastewater bioreactor processes; thus, understanding microbial communities is important in improving reactor performance [17–19].

In the present study, a pilot-scale CAAC process was implemented to investigate the feasibility to treat the effluent generated by an IC reactor for treating soybean wastewater. This research focuses on the simultaneous removal of organic carbon and nitrogen and in-situ excess sludge reduction. The microbial community structure and dynamics during the start-up period were investigated by polymerase chain reaction (PCR)-DGGE and unweighted pair-group methods with arithmetic mean (UPGMA) clustering analysis. The predominant bands were sequenced to reveal the composition of the microbial communities.

2. Materials and methods

2.1. Experimental system

Experiments were carried out in a pilot-scale CAAC process (as shown in Fig. 1), which was constructed next to the IC reactor for deeply treating the soybean wastewater. The process consisted of four main bodies and two buffer banks. The four main bodies are the moving bed biofilm reactor (MBBR) (zone 1), anoxic zone (zone 2), anaerobic zone (zone 3), and aerobic zone (zone 4). This pilot-scale CAAC reactor is made of carbon steel with the preservative treatment and has a total liquid volume of 8 m^3 (MBBR: 2 m^3). Suspended carriers (cross inside and longitudinal fins outside) are placed in the MBBR with a packing ratio of 30% (vol/vol). Zones 2–4 are divided into three equal compartments by two internal baffles. A diving mixer is placed at the bottom of zone 2. Zones 3 and 4 are filled with industrial slag as the fixed carrier with packing ratios of 100% and 80%, respectively. The industrial slag has a diameter of 3 cm to 5 cm, and a porous structure with a porosity of 50–60%. The aeration devices are fixed at the bottom of zones 1 and 4. The pilot-scale CAAC process was continuously operated for 83 days. When the HRT was shorted from 2.6 days to 1.3 days, the treatment capacity of the system gradually increased from 2.5 $m^3\ day^{-1}$ to 5 $m^3\ day^{-1}$. The average dissolved oxygen (DO) concentrations in the MBBR, anoxic zone, anaerobic zone, and aerobic zone were 3.4, 1.1, 0.5, and 6.6 mg/L on average, respectively. The DO was measured in the upper 20 cm of water in anaerobic and aerobic zones because the block of carriers of fixed bed in these zones. The operating temperature applied in the CAAC process was kept constant at 25–30 °C.

2.2. Characteristics of wastewater

The feed wastewater used in this investigation was the anaerobic effluent produced by the IC reactor mentioned before for treating soybean wastewater in a soybean protein processing plant located in Y City, China. The characteristics (average values) of the raw influent wastewater are as follows: pH, 7.70–8.55; COD, 182.1–1151.4 mg/L; BOD, 108.2–805.7 mg/L; suspended solids (SS), 247–603 mg/L; TN, 108.0–271.0 mg/L; NH_4^+–N, 106.3–270.6 mg/L; total phosphorus (TP), 56.0–79.0 mg/L; COD/TN, 0.9–4.9 (average, 2.3). In order to obtain the biofilm on carriers in MBBR in a short period and immediately start the reactor, 1.06 kg glucose was added to the MBBR every day as carbon source during the first 18 days.

2.3. Sludge seeding

The activated sludge for seeding was collected from the secondary settling tank of an anoxic–oxic (A/O) bioreactor in the same soybean protein processing plant. The A/O bioreactor was used to uninterruptedly treat the anaerobic effluent generated by the IC reactor in the plant. The sludge from the secondary settling tank was domesticated by the wastewater generated by the IC reactor. The sludge concentration of each compartment was 500 mg/L on average after being inoculated into the pilot-scale CAAC process.

2.4. Analytical methods

Samples from different sampling sites along the flow direction were obtained through centrifugation at 8000 × g for 10 min. The supernatants were used to measure COD, NO_2^-–N, NO_3^-–N, NH_4^+–N, TN, MLSS, and TP through standard methods [20]. The DO was measured using a DO electrode connected to a DO meter (JPBJ-608, Shanghai Precision & Scientific Instrument Co., China). The pH was measured using a pH meter (HI8424, Hanna Co., Italy). To evaluate the nitrification performance of the process, the nitrification efficiency (%) was calculated by Eqs. (1) and (2).

$$Nitrification\ efficiency(\%) = \frac{(NH_4^+ - N)_{\text{inf luent}} - (NH_4^+ - N)_{effluent}}{(NH_4^+ - N)_{\text{inf luent}}} \times \frac{r_N}{r_A} \times 100 \quad (1)$$

$$r_N = r_A - r_B \quad (2)$$

where r_N is the nitrification rate of the process (g-N $m^{-3}\ day^{-1}$); r_A is the removal rate of ammonia nitrogen (g-N $m^{-3}\ day^{-1}$); r_B is the amount of NH_4^+–N removal rate by biomass assimilation (g-N $m^{-3}\ day^{-1}$).

2.5. DNA extraction and PCR amplification

Sludge samples were obtained from each zone at different operational periods. Immediately upon sampling, the sampled sludge was preserved in 50% ethanol and stored at −20 °C prior to analysis. To extract DNA from the sludge samples, the direct lysis method was used, following Zhou et al. [21]. The crude extract was purified using Wizard PCR preps (Promega, USA) according to the manufacturer's instructions [22]. The DNA extract was stored at −20 °C until analysis.

PCR amplification was performed in a 30 μL PCR mixture containing 1.5 U Taq DNA polymerase, 3 μL of 10 buffers, 50 μM of each primer, 200 μM dNTP, and 10 ng of purified DNA extract. The primer sets GC (CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G) + P1 (5′- ACT CCT ACG GGA GGC AGC AG -3′) and P2 (5′- ATT ACC GCG GCT GCT GG -3′) were used for the direct amplification of the 16S rRNA gene sequences from the purified DNA [23]. The PCR amplification conditions used for primers P1–P2 and GC + P1–P2 were as follows: 95 °C for 8 min, 35 cycles of 95 °C for 40 s, 55 °C for 40 s, 72 °C for 1 min, and 72 °C for 10 min. The PCR products were analyzed by agarose (1.1% [wt/vol]) gel electrophoresis with a 1TAE buffer that contained 1.5 mg/mL GoldView.

2.6. DGGE analysis and cloning of DGGE band sequences

DGGE analysis was performed with a DCode mutation detection system (Bio-Rad, USA). The PCR products were