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Simultaneous carbon and nitrogen removal from anaerobic effluent of the cassava ethanol industry

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This study investigated the simultaneous carbon and nitrogen removal from anaerobic effluent of cassava stillage using a lab-scale integrated system consisting of an upflow anaerobic sludge blanket (UASB) reactor and an activated sludge (AS) process. Simultaneous denitrification and methanogenesis (SDM) was observed in the UASB with nitrate recirculation. Compared with the blank reactor without recirculation, the overall chemical oxygen demand (COD) removal efficiencies in the combined system with nitrate recirculation were similar (80–90%), while the TN removal efficiencies were significantly improved from 4.7% to 71.0%. Additionally, the anaerobic COD removal efficiencies increased from 21% to 40% as the recirculation ratio decreased from 3 to 1. Although the influent nitrate concentrations fluctuated (60–140 mg N/L), the nitrate removal efficiencies could be maintained at about 97% under different recirculation conditions. With the decreasing recirculation ratio from 3 to 1, the CH_4 content in biogas improved from 2% to 40% while the N_2 content reduced from 95.8% to 50.6%. The 16S rDNA sequencing results indicated that bacteria diversity in anaerobic SDM granular sludge was much higher than archaea. The effect of recirculation ratios on the bacterial and archaeal communities in SDM granular sludge could be further confirmed by the relative abundance of denitrifying bacteria.

[**Key words:** Denitrification; Methanogenesis; Recirculation ratio; Microbial communities; Cassava ethanol wastewater]

Cassava stillage (CS) is a typical high-strength organic wastewater containing high concentrations of organics, suspended solids, and nitrogen, which can lead to severe ecological and public health problems if not properly handled. Our previous study showed that CS is suitable for two-stage anaerobic treatment in which the organics are converted to methane (1). Considering the most readily degradable organics were already consumed in the first-stage anaerobic reactor with a COD removal efficiency of 90%, chemical oxygen demand (COD) removal efficiency in the second-stage anaerobic reactor was relatively low (30–40%). Moreover, high concentrations of ammonium nitrogen were produced in this system. In conventional methods, biological nitrogen is removed through the use of anoxic/oxic tanks placed in series in a system that includes recirculation. However, the high operational costs associated with the supplied aeration, the long duration of the process, and the need for an external carbon source to achieve complete denitrification have hindered the development of this type of reactor configuration (2).

To date, considerable attention has been devoted to combined anaerobic treatment technologies for the removal of carbon and nitrogen, but of potentially greater interest is the ability to combine denitrification and anaerobic digestion in an integrated process coupled to a nitrification stage (3,4). This so-called simultaneous denitrification and methanogenesis (SDM) has been investigated in single anaerobic reactors using a wide variety of substrates, ranging from synthetic high-strength organic wastewater to real industrial wastewaters (5–7).

The nature of the carbon source and the COD/NO_3^-–N ratio determine both the nitrate reduction pathway and the carbon utilization pattern, which together explain the COD and nitrate removal efficiencies (5,8). Thus, the recirculation ratio of the aerobic effluent can be expected to greatly influence SDM performance in the anaerobic reactor. Huang et al. (9) observed that a higher recirculation ratio of the anaerobic-aerobic reactors resulted in a higher TN removal efficiency (52–72%) in the treatment of piggery wastewater; but the anaerobic COD removal efficiency (83–85%) remained essentially the same. However, some other researchers observed that an increase in the recirculation ratio led to a decrease in anaerobic COD removal efficiency using anaerobic-aerobic bioreactors in series with recirculation (10,11). In the study of An et al. (12), a significant improvement in organics removal from treated synthetic municipal wastewater was achieved only for recycling ratios of 0.5 and 1; while the TOC removal efficiency decreased slightly at higher recycling ratios. Thus, although extensive research has been conducted on the effect of the recycling ratio on SDM, consistent results have not been obtained due to the different carbon sources used Moreover, the substrates in most studies are synthetic wastewater and little information is available on industrial effluents. Research is still needed to clarify basic aspects related to the interaction of the microorganisms involved and the effect of operational conditions like the COD/N ratio.

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In the light of the above results, there would be several advantages to the simultaneous removal of carbon and nitrogen from the first-stage anaerobic effluent of cassava wastewater. The main objective of the study presented below was to use an anaerobic-aerobic system with recirculation to explore SDM in a first-stage anaerobic effluent generated during the industrial production of ethanol from cassava. The effect of the recirculation ratio on SDM was evaluated under continuous operation mode. In addition, the microbial communities of the SDM granular sludge were identified by polymerase chain reaction followed by denaturing gradient gel electrophoresis.

MATERIALS AND METHODS

Inoculum and substrate Anaerobic granular sludge acquired from a full-scale mesophilic UASB treating citric acid wastewater was used as inoculum. The total and volatile suspended solids (TSS and VSS) concentrations in the seed sludge were approximately 56.2 and 45.7 g/L, respectively. Excess sludge obtained from the secondary sedimentation tank of the Quyang sewage treatment plant (Shanghai, China) was seeded into the AS reactor.

The anaerobic CS effluent used as substrate was obtained directly from the full-scale continuous stirred tank reactor used in the first-stage anaerobic digestion process carried out once a month at the Taicang cassava ethanol plant (Jiangsu, China). The quality of the anaerobic CS effluent fluctuated at different operational mode. The residual soluble COD concentration ranged from 4240 mg/L to 7650 mg/L, and total nitrogen (296−765 mg/L) was mostly NH_4^+−N (116−578 mg/L). With average pH of 8, the anaerobic CS effluent contained large amounts of volatile fatty acids. After their collection, the anaerobic CS effluents were stored in a refrigerator at 4 °C until needed.

Reactor systems and operation Two sets of reactor systems were operated with/without recirculation for comparison. The reactor system consisted of two perspex reactors connected in series, as shown in Fig. 1, an anaerobic UASB reactor (hydraulic retention time, HRT = 24 h without consideration of recirculation) for digestion and denitrification, and an AS reactor for nitrification.

The UASB reactor (2 L) was a cylindrical tube with a conical-shaped bottom, a reaction-zone height of 0.28 m, an inner diameter of 70 mm, and a working volume of 1.2 L. The operational temperature was automatically maintained at about 35 °C in a heated water bath by recirculating heated water through a water jacket attached to the reactor. A three-phase separator was installed at the top of the UASB reactor to separate the biogas from the mixed liquor and to retain suspended particles in the reactor. Biogas was collected in gas-tight aluminum bags. An overflow line was situated above the three-phase separator, which was connected to the AS reactor (a cylindrical tank with a total volume of 3 L). A sequencing batch reactor (SBR) was operated as the AS reactor in intermittent operating mode with HRT of 20−25 h, and the working volume of the SBR changed in the range of 2.10−2.95 L when the system was operated with recirculation. Aeration was provided through an air module pump device after 15 min of inflow, with an aeration rate of 0.3−0.5 L/min. The dissolved oxygen content was kept at 0.5−6.0 mg/L. Continuous mixing was carried out using a magnetic stirring device. The AS reactor was operated at ambient temperature (20−30 °C) without temperature control. The pH of all reactors was uncontrolled.

The anaerobic CS effluent was supplied into the feed port of the UASB reactor by a peristaltic pump, with timers used to change the flow rate. The reactors were operated under continuous operation mode after an initial start-up period during which they were fed semi-continuously. When the COD removal efficiency and gas production in the UASB reactor reached steady state, the digested overflow from the top of the anaerobic UASB reactor was transferred to the AS reactor. A certain portion of the nitrified effluent from the AS reactor was recirculated by a peristaltic pump directly to the UASB reactor. For this study, experiments were conducted at recirculation ratios of 3, 2, and 1. As shown in Fig. 1, the raw influent flow rate of the system (Q) was 0.05 L/h. The flow rates of the recirculated nitrified effluent were 0.15 L/h, 0.10 L/h and 0.05 L/h at recirculation ratios of 3, 2, and 1, respectively. Consequently, the flow rates into UASB reactor were 0.20 L/h, 0.15 L/h and 0.10 L/h at recirculation ratios of 3, 2, and 1. The organic loading rate (OLR) of the UASB reactor ranged from 6.78 to 8.08 kg COD/(m^3 d) and the nitrogen-loading rate (NLR) ranged from 0.12 to 0.25 kg NO_x^-−N/(m^3 d) throughout the operation, considering the variation in influent wastewater characteristics and recirculation ratios. Effluent wastewaters from the UASB and AS reactors were collected in closed containers and stored in a refrigerator at 4 °C until further analysis.

All data were obtained at the steady-state of each operation condition. Steady-state was defined as sustained biogas production within ±15% deviation. Each experimental run lasted at least 2 weeks under constant operating conditions. The average values of the data obtained under steady-state conditions were used for further calculations.

Analytical methods The collected liquid samples were centrifuged at 11,000 rpm for 10 min and then filtered through 0.45-μm filters to determine their soluble components. Total and soluble chemical oxygen demand (TCOD and SCOD), TSS, VSS, total nitrogen, and pH were analyzed in accordance with standard methods (13). The concentration of total organic carbon (TOC) was determined using a TOC analyzer (TOC-VCPN, Shimadzu).

Nitrate, nitrite, and ammonia were analyzed by ion chromatography in a system equipped with two conductivity detectors and two sets of columns (Dionex ICS-3000). Anions were separated and eluted on an IonPac AG11−HC (4 × 50 mm) guard column and an IonPac AS11-HC (4 × 250 mm) analytical column utilizing an eluent of 18 mM KOH at an isocratic flow rate of 1.2 mL/min. Cations were analyzed on an IonPac CG12A (4 × 50 mm) guard column and an IonPac CS12A (4 × 250 mm) analytical column utilizing an eluent of 20 mM methanesulfonic acid at an isocratic flow rate of 1.0 mL/min. Auto-suppression mode was used during the detections.

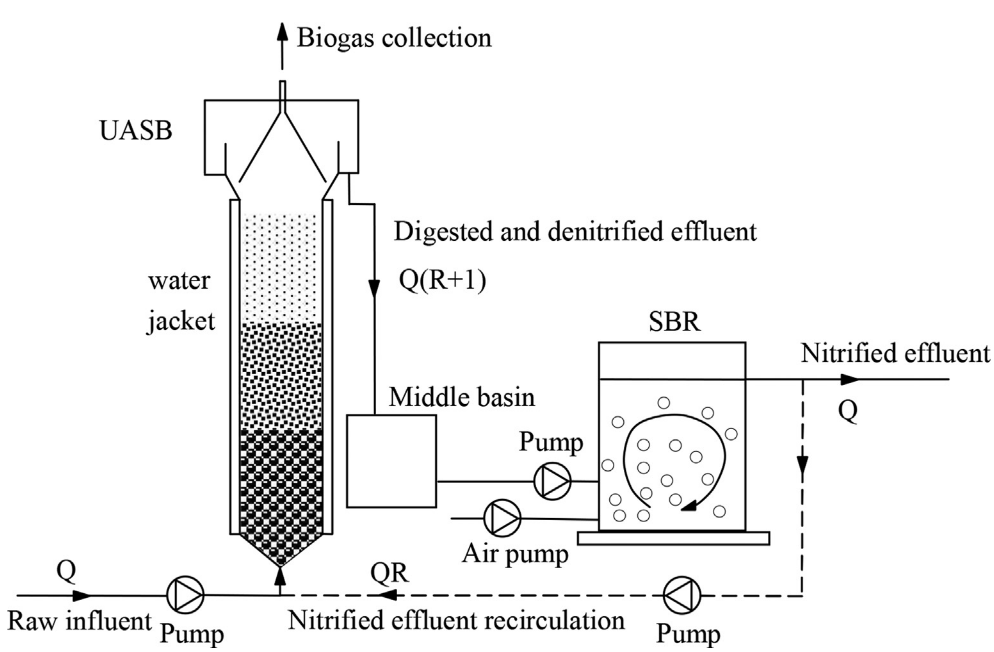

FIG. 1. Schematic diagram of lab-scale experimental setup. Q, the raw influent flow rate of the system; R, recirculation ratio.