Regular article

# Study on poultry manure wastewater treatment by two-stage aerobic coupled process and its microbial community analysis

Chao Li [a,b,c], Zhen Zhang [a,b,c], Jiashun Cao [a,b,c,*], Yi Li [a,b,c]

[a] Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes, Ministry of Education, Hohai University, Nanjing 210098, China
[b] College of Environment, Hohai University, Nanjing 210098, China
[c] State Key Engineering Research Center of Efficient Utilization of Water Resources and Engineering Safety, Hohai University, Nanjing 210098, China

## ARTICLE INFO

## ABSTRACT

Two-stage aerobic coupled process consisted of anoxic, aerobic-1 with low DO and aerobic-2 with high DO (short for "A-LO-HO" process), was applied for post-treatment of poultry manure wastewater, which suffered by high ammonia nitrogen and high salinity. Outstanding $NH_4^+$-N removal efficiency of 99.5% was obtained, and the COD removal efficiency was about 75%, even under the severe stress. Multiple molecular technologies, include DGGE, real time PCR and high-throughput sequencing were applied to reveal the microbial community of the sludge. The reasonable distribution of the dominant bacteria *Nitrosomonas* and *Nitrobacter* would explain the synergistic effect of the partial nitrification in "A-LO" section and the completed nitrification in "HO" section. The microbial community and EPS analysis further confirmed the outstanding nitrifying ability, good salt-tolerant ability, and unaffected sedimentation property of the sludge with particular enhanced features which is acclimated for long time by the targeted coupled process and suitable for this kind of wastewater treatment.

## 1. Introduction

Increasing quantities of poultry wastes production has become one of the most critical environmental problems in terms of water, air pollution and human health [1]. The manure can be partially used for anaerobic co-digestion with other wastes for their nutrients supplement [2], which is considered as an attractive option. Even so, most of the liquid fraction of manure, as large amount of poultry manure wastewater, has to be treated [3].

Variety of different anaerobic reactors has been reported for pre-treatment of poultry manure wastewater [4], to first reduce the pollutants to a significant extent. However, more effective post-treatment technologies still need to be explored, because remained pollutants, such as high concentration of ammonia nitrogen in poultry manure wastewater, require further appropriate disposal [5].

A number of technologies have been successfully applied for the post-treatment, such as aerobic/anoxic biofilter for upflow anaerobic sludge blanket (UASB) pretreated manure wastewater [6], Fenton's oxidation as an advanced treatment for anaerobically treated poultry manure wastewater [3], and electrocoagulation technique disposing the effluent of UASB pretreated poultry manure wastewater [5].

With the increasing of poultry wastes production and strict environmental regulations, there has been a dearth of efficient post-treatment processes of poultry manure wastewater. The biological technologies usually yield satisfactory effluents and are promising [7] due to their low cost. However, as the post-treatment (after anaerobic digested), the biological treatment systems still have to face the severe challenges from the poultry manure wastewater, such as high ammonia nitrogen concentration, high total dissolved solids (TDS, corresponding high salinity) and potential pathogens risk. Therefore, only if the strong and efficient sludge (can resist the severe stress) acclimated, can the biological process exhibit satisfactory performance for poultry manure wastewater treatment.

Microorganisms play the key role in the acclimatization of the enhanced sludge. Nevertheless, the microbial community information is still lack of systematical study on manure wastewater treatment [8]. The rapid development of molecular biological technology, especial the next generation sequencing, can provide better

understandings of microbial activities and community structures, which would help to evaluate the sludge situation and optimize the operation parameters of the biological process.

Therefore, this study applied the pure biological process—consisted of anoxic, aerobic-1 with low dissolved oxygen (DO), and aerobic-2 with high DO (short for "A-LO-HO"), to act as the post-treatment process for poultry manure wastewater. And all the experiments were conducted at full-scale dimensions, based on a practical wastewater treatment plant (WWTP) which mainly takes over liquid poultry manure (after anaerobic pre-treatment), and the integrated influent was suffered from high ammonia nitrogen and high salinity. The nitrogen transformations are very complex and the nitrification (from ammonia nitrogen) mainly includes two stages:

$$NH_4^+ + 1.5O_2 = NO_2^- + 2H^+ + H_2O \tag{1}$$

$$NO_2^- + 1.5O_2 = NO_3^- \tag{2}$$
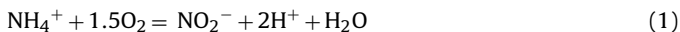
So, numerous studies on efficient denitrification have been succeeded in partial nitrification and SND via nitrite ($NO_2^-$) in biological treatment, which would save the energy (aeration during nitrification) and carbon source (denitrification requirement). The designed process "A-LO-HO" in this study is just trying to achieve high ammonia nitrogen removal of manure wastewater by multipath denitrification.

This study aims at: (1) investigate the performance (especial for $NH_4^+$-N removal) of the designed "A-LO-HO" process, as the post-treatment of liquid poultry manure wastewater, suffered from high salinity and high nitrogen; (2) provide a better evaluation of the microbial community by multiple molecular biological technologies, and obtain a more in-depth understanding on the biological mechanisms of the sludge acclimatization with enhanced features.

## 2. Material and method

### 2.1. Description of the biological process (WWTP)

The whole process based on "A-LO-HO", was particularly designed for the liquid poultry manure wastewater treatment, which was consisted of the storage tank, anoxic tank, two-stage aerobic tank with sediment, and MBR tank (Fig. 1).

The treatment capacity of the WWTP is 7200 m³/d. The working volumes of the three compartments (A, LO and HO) in the system were approximately 600, 4800, and 4800 m³, respectively. The influent rate was 300 m³/h, which resulted in a hydraulic retention time (HRT) of 2 h, 16 h and 16 h respectively in the A, LO and HO zones. In the study period, DO was maintained at $0.9 \pm 0.2$ mg/L and $5.5 \pm 0.5$ mg/L for LO and HO respectively. The mixed liquor suspended solids (MLSS) were controlled at 4000–6000 mg/L, and the solid residence time (SRT) was $9 \sim 12$ days. The temperature range was $24 \sim 30$ °C during the study period. The reflux from sediment-1 back to the anoxic tank is fixed at 100% to achieve denitrification (Fig. 1). Whereas, the reflux from sediment-2 back to anoxic tank is alternative, according to nitrogen pollutants pressure of the influent.

The specific conditions of the WWTP are listed in Table 1.

The whole process based on "A-LO-HO" (Fig. 1 and Table 1) mainly targeted efficient nitrogen removal of the manure wastewater by two-stage denitrification system ("A-LO" and "HO"). The wastewater (was mainly taken up by liquid poultry manure after pre-treatment anaerobiclly) from the storage tank moved into the anoxic tank and then to the aerobic-1 (together can be considered as a common A/O process) to remove organics and nitrogen. The additional aerobic-2 tank followed was to enhance nitrification (Fig. 1), and the membrane bio-reactor (MBR, operating aperiodically) at last guaranteed the satisfactory effluent. The inlet of the system

is: pH $\approx$ 8.3, $COD_{Cr} \approx 5800$ mg/L, $NH_4^+$-N $\approx 2365$ mg/L, and is to meet the "Discharge standard of pollutants forlivestock and poultry breeding (GB18596-2001)", with pH = 6.0~9.0, $COD_{Cr} \leq 400$ mg/L, $NH_4^+$-N $\leq 80$ mg/L.

### 2.2. Characterization and analysis of water quality

Stable operation during a recent continuous 3-month period was assessed by characterizing the water quality. COD, $NH_4^+$-N, TN, pH and Total Dissolved Solids (TDS) were estimated every day, according to the guidelines of Standard Methods [9].

### 2.3. Characterization of extracellular polymeric substances (EPS) of the sludge

The EPS extraction process was performed according to the protocol described [10]. The protein, carbohydrates and humic acids contents were determined referred to the previous reports [11,12].

Excitation-emission matrix (EEM) fluorescence spectroscopy was further used to analyze the EPS by the fluorescence spectrophotometer (Hitachi, F7000), which includes the following fluorescence techniques: (i) excitation (EX) and emission (EM) were both fixed at $200 \sim 600$ nm, (ii) synchronous scan at a constant offset wavelength 5 nm between excitation and emission.

### 2.4. Bacterial community analysis

The bacterial community of the main bio-tanks was evaluated and analyzed by PCR-DGGE, real time PCR and high-throughput sequencing technology (Miseq).

#### 2.4.1. PCR-DGGE analysis

Sludge samples (300 μg, of precipitated sludge) were collected from each bio-tank, and the total genomic DNA was extracted by a DNA extraction kit (Fasta, MP).

PCR was performed using primers 518r (ATTACCGCGGCT-GCTGG) and 338f (CCTACGGGAGGCAGCAG, with GC-clamp) under the following conditions: denaturation at 94 °C for 5 min; 30 cycles of 94 °C for 30 s, 58 °C for 30 s and 72 °C for 45 s; and a final extension step at 72 °C for 10 min.

The PCR products were then subjected to denaturing gradient gel electrophoresis (DGGE) using 18 cm × 18 cm polyacrylamide gels (10% (w/v)) with a thickness of 0.75 mm. The gels were prepared with denaturing gradients of 35–65% gel. Electrophoresis was conduct in 1 × TAE buffer at 90 V for 14.5 h at 60 °C. Gels were photographed using Kodak 1D Image Analysis Software after ethidium bromide (EB) staining.

#### 2.4.2. Real time PCR assay

Quantification of nitrifying bacteria was characterized by *amoA* genes targeted real-time PCR. The primar pair amoA-1F (GGGGTTTCTACTGGTGGT) and amoA-2R (CCCCTCKGSAAAGC-CTTCTTC) were used. And the real-time PCR conditions for *amoA* genes were: 3 min at 94 °C, followed by 40 cycles 30 s at 94 °C, 30 s at 55 °C, 45 s at 72 °C (also for data acquisition step) [13]. One last step from 55 to 95 °Cwith an increase of 0.2 deg/s was added to obtain a specific denaturation curve for the real-time PCR assays.

Plasmids (pEASY-T1 Cloning Kit, Transtaq) containing the *amoA* genes which were cloned to DH5α were used to draw standard curves ($r^2 = 0.991$ with amplification efficiency of 95.6%).

#### 2.4.3. High-throughput sequencing (Miseq)

High-throughput sequencing of the 16S rRNA gene (Miseq, Illumina) was conducted for further analysis of the key 4 sludge samples (for influent, anoxic, aerobic-1 and 2, i.e., the "A-LO-HO"