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Review

# The effect and biological mechanism of COD/TN ratio on nitrogen removal in a novel upflow microaerobic sludge reactor treating manure-free piggery wastewater

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## HIGHLIGHTS

- An upflow microaerobic sludge reactor treating low COD/TN ratio piggery wastewater.
- Effect and biological mechanism of influent COD/TN ratio being investigated.
- Nitrifiers, hetero- and auto- trophic denitrifiers all thrived in the reactor.
- The nitrifiers and denitrifiers restricted by the increased influent COD/TN ratio.
- An influent COD/TN ratio less than 0.70 ensured a TN removal over 80% in the reactor.

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## ABSTRACT

A novel upflow microaerobic sludge reactor (UMSR) was constructed to treat manure-free piggery wastewater with high $NH_4^+$-N concentration and low COD/TN ratio, and the effect and biological mechanism of COD/TN ratio on nitrogen removal were investigated at a constant hydraulic retention time of 8 h and 35 °C. The results showed that the UMSR could treat the wastewater with a better synchronous removal of COD, $NH_4^+$-N and TN. The microaerobic UMSR allowed nitrifiers, and heterotrophic and autotrophic denitrifiers to thrive in the flocs, revealing a multiple nitrogen removal mechanism in the reactor. Both the nitrifiers and denitrifiers would be restricted by an influent COD/TN ratio more than 0.82, resulting in a decrease of TN removal in the UMSR. To get a TN removal over 80% with a TN load removal above 0.86 kg/($m^3$·d) in the UMSR, the influent COD/TN ratio should be less than 0.70.



**Contents**



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## 1. Introduction

With the increased demand for pork, more and more intensive pig farms are developed, especially in the developing countries like China, resulting in an increasing discharge of piggery wastewater (Zhao et al., 2014). Characteristics of piggery wastewater vary greatly with manure collection methods mainly including urine-free manure (UFM), combined manure with urine (CMU) and soaked manure with urine (SMU) (Wang and Guo, 2009). As a traditional mode, UFM collection method is still widely used in China and the piggery flushed wastewater is defined as manure-free piggery wastewater. Collection of manure and flushed water results in an increased ammonium ($NH_4^+$-N) and a decreased chemical oxygen demand (COD) in the manure-free piggery wastewater that is identified by low C/N ratio (Zhao et al., 2014). Discharge of the piggery wastewater into aquatic systems without treatment will cause serious eutrophication (Yamamoto et al., 2008). Therefore, effective nitrogen removal from the manure-free piggery wastewater is necessary to meet a certain emission standard, such as the Discharge Standard of Pollutants for Livestock and Poultry Breeding in China (MEPPRC, 2001).

The traditional nitrogen removal process is a combination of aerobic nitrification and anaerobic denitrification catalyzed by autotrophs and heterotrophs, separately (Kuenen and Robertson, 1994). Generally, as electron donor, carbon source in denitrification is essential for the reduction of nitrate and nitrite to gaseous nitrogen ($N_2$ and/or $N_2O$) (Islas-Lima et al., 2004). Theoretically, 2.86 g COD/g N is needed for the traditional nitrogen removal via nitrate reduction without assimilation, while 1.72 g COD/g N needed for denitrification via nitrite (Akunna et al., 1992). It has been reported that a C/N ratio less than 3.4 could inhibit the growth of denitrifying bacteria (Kuba et al., 1996), and the beneficial ratio to denitrification was 6–8 (Obaja et al., 2003). It is a challenge to remove nitrogen from the manure-free piggery wastewater due to the high $NH_4^+$-N concentration and low C/N ratio (Zhao et al., 2014). Dosing carbon source or decreasing $NH_4^+$-N concentration through physical chemistry methods have been used to gain a feasible C/N ratio for the subsequent biological nitrogen removal from piggery wastewater, but extra treatment cost and complexity of equipment management would be caused as a result (Guštin and Marinšek-Logar, 2011).

Autotrophic denitrification without any carbon sources required is considered a cost-effective alternative to traditional denitrification process (Strous et al., 1999a). In the process, nitrite is reduced to $N_2$ and/or $N_2O$ mainly with ammonium, elemental sulfur and/or Fe(0) as electron donors instead of organic carbon (Strous et al., 1999a). In addition, the autotrophic nitrogen removal process has a lower sludge production compared with heterotrophic processes (Molinuevo et al., 2009). Thus, autotrophic denitrification is considered to be feasible for treating ammonium rich wastewaters with low C/N ratio, such as the manure-free piggery wastewater (Molinuevo et al., 2009).

A bioprocess for treating organic wastewater with a dissolved oxygen (DO) ranged from 0.3 to 1.0 mg/L is normally identified a microaerobic treatment process (Hu et al., 2005; Zitomer, 1998). The co-existence of aerobe, anaerobe and facultative aerobe in the microaerobic condition is favorable for the simultaneous removal of nitrogen and carbon with low cost (Zheng and Cui, 2012). Though microaerobic treatment processes have been introduced to treat municipal wastewater, few researches on treating low C/N ratio wastewater could be found (Chu et al., 2006; Zheng and Cui, 2012). A novel upflow microaerobic sludge reactor (UMSR) has been constructed to treat manure-free piggery wastewater without extra carbon source supplied (Meng et al., 2015a). With a COD/TN ratio of about 0.84 in the raw wastewater and a TN loading rate of 1.10 kg/($m^3$·d), the average COD, $NH_4^+$-N and TN removal reached 0.72, 0.76 and 0.94 kg/($m^3$·d), respectively, in the UMSR. Though autotrophic anaerobic ammonium oxidation (anammox) was derived as the main mechanism for $NH_4^+$-N and TN removal in the microaerobic system, the biological mechanism for the simultaneous removal of COD, $NH_4^+$-N and TN was not known exactly by now (Meng et al., 2015a). Furthermore, COD, $NH_4^+$-N and COD/TN ratio in the manure-free piggery wastewater varies significantly with breeding seasons (Zhao et al., 2014), and change of the wastewater quality, especially the COD/TN ratio, would have a significant impact on the biological nitrogen removal process (Ni et al., 2012). However, few researches could be found to investigate the effect of COD/TN ratio on microaerobic processes (Zhang et al., 2015).

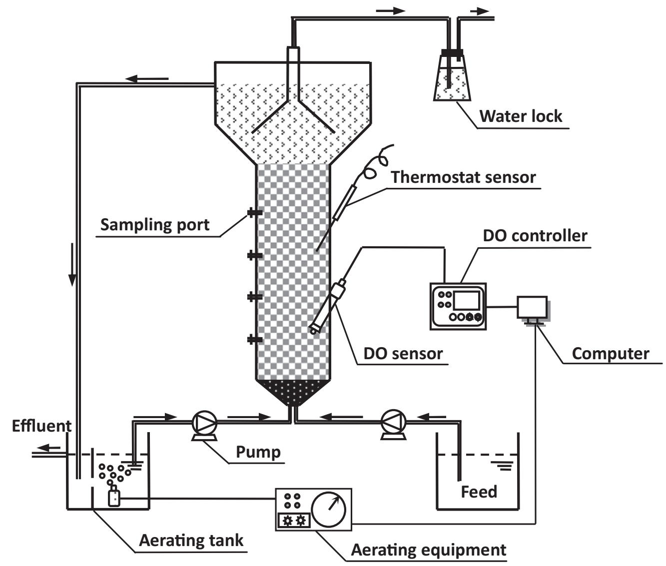


**Fig. 1.** Schematic representation of the lab-scale UMSR process.

In the present research, the UMSR was continually operated for another 84 days by stages to investigate the effect of influent COD/TN ratio on carbon and nitrogen removal in microaerobic condition. To provide comprehensive insights into the biological mechanism for the simultaneous removal of COD, $NH_4^+$-N and TN, functional microbial populations in the flocs under different COD/TN was also investigated using Illumina Miseq platform.

## 2. Methods

### 2.1. The microaerobic treatment system

As shown in Fig. 1, the UMSR in the microaerobic treatment process was a 0.5-meter-high plexiglass column with a working volume of 4.9 L. A 0.5 L circular cone was attached to the bottom of the col-