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Review

# Control of carbon and ammonium ratio for simultaneous nitrification and denitrification in a sequencing batch bioreactor

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**Abstract**

This study shows how the carbon and nitrogen (C/N) ratio controls the simultaneous occurrence of nitrification and denitrification in a sequencing batch reactor (SBR). Data demonstrated that a low C/N ratio resulted in a rapid carbon deficit, causing an unbalanced simultaneous nitrification–denitrification (SND) process in SBR. When the initial COD/$NH_4^+$-N ratio was adjusted to 11.1, the SND-based SBR achieved complete removal of $NH_4$-N and COD without leaving any $NO_2^-$-N in the effluent. The nitrogen removal efficiency decreases gradually with increasing ammonium-loading rate to the SND–SBR system. Altogether, data showed that appropriate controls of carbon and nitrogen input are required to achieve an efficient SND–SBR. An established SND technology can save operation time and energy, and might replace the traditional two-stage biological nitrification and denitrification process.


*Keywords:* Carbon source; Heterotrophic nitrification; Oxidation–reduction potential (ORP); Simultaneous nitrification and denitrification (SND)

**Contents**



## 1. Introduction

The traditional biological process used in wastewater treatment to achieve nitrogen removal involves separate aerobic and anaerobic phases that are generally carried out in separate bioreactors or by different aeration intervals

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(Metcalf and Eddy, 1991). However, Ferguson (1994) proposed that heterotrophic microorganisms may be involved in nitrification and simultaneous aerobic denitrification. Some heterotrophic nitrifiers have been reported to denitrify nitrite ($NO_2^-$) and nitrate ($NO_3^-$) aerobically (Robertson et al., 1988; Zart and Eberhard, 1998). Other studies show that nitrification and denitrification occur concurrently in a single reactor under aerobic conditions (von Münch et al., 1996; Zeng et al., 2003), which is often referred to as the simultaneous nitrification and denitrification (SND) process. The SND process represents a significant advantage over the conventional separated nitrification and denitrification processes.

SND occurs naturally inside microbial biofilms and flocks due to the oxygen gradient that establishes across the biomass (von Münch et al., 1996). Nitrifiers are active in areas of high dissolved oxygen (DO) concentration, whereas denitrifiers are active in areas of very low DO concentration. The uneven distribution of DO inside the biomass allows simultaneous proliferation of nitrifying and denitrifying bacteria.

On the other hand, some heterotrophic bacteria, e.g., *Alcaligenes faecalis* (van Niel et al., 1992) and *Thiosphaera pantotropha* (Robertson and Kuenen, 1988), are capable of performing SND by using organic substrates aerobically as sources of carbon and energy to convert ammonium ($NH_4^+$) aerobically into nitrogen gas (Stouthamer et al., 1997). Pathways of heterotrophic SND metabolism are different from those of autotrophic nitrifiers and heterotrophic denitrifiers. Ammonium is hydroxylated to hydroxylamine ($NH_2OH$) by ammonium monooxygenase under aerobic conditions. Subsequently, hydroxylamine is oxidized to nitrite by hydroxylamine oxidase. Finally, nitrite is directly transformed into $N_2$. The SND process is also termed ‘‘aerobic deammonification’’ (Bock et al., 1995; van Loosdrecht and Jetten, 1998).

Hellinga et al. (1998) designed an SND-based reactor in which aerobic heterotrophic bacteria removed nitrite produced from ammonia by *Nitrosomonas* in the same reactor. Robertson and Kuenen (1988) studied the nitrification and denitrification ability of *T. pantotropha*, and observed that this facultative anaerobic and autotrophic sulfur bacteria can perform simultaneous heterotrophic nitrification and aerobic denitrification by using $O_2$ and $NO_3^-$ simultaneously. Their observation implies that *T. pantotropha* is capable of carrying out SND. Pochana and Keller (1999) investigated the efficiency of nitrogen removal from wastewaters by an SND-based sequencing batch reactor (SBR). They observed that higher DO concentrations enhanced nitrification rates. Simultaneously, high DO concentrations inhibited the denitrification process, causing an accumulation of nitrite and nitrate in the reactor. On the other hand, at lower DO concentrations the nitrification process was slowed down and the denitrification process was enhanced. Therefore, the DO level is a factor critical to the SND process; it must be maintained at an appropriate level in the SND reactor in order to reach a balanced equilibrium between the nitrification and denitrification processes.

Under either oxygen-limiting or oxygen-free conditions, *Nitrosomonas europaea* transformed $NH_4^+$ into $NO_2^-$, which was simultaneously transformed to nitrous oxide ($N_2O$) and $N_2$ (Shrestha et al., 2002). von Münch et al. (1996) observed that an efficient SND process occurred at a DO concentration lower than $0.5\,mg\,L^{-1}$. The importance of maintaining a low DO concentration was confirmed by Pochana and Keller (1999), who showed that $NH_4^+$, $NO_2^-$, $NO_3^-$, and soluble COD were removed in 4 h under DO conditions between 0.3 and $0.8\,mg\,L^{-1}$. This suggests that some heterotrophic nitrifiers have the ability to denitrify under low oxygen conditions to affect an SND reaction. When *Paracoccus denitrificans* was grown under conditions favoring the coupled heterotrophic nitrification–denitrification reaction, a small increase in the maximum microbial growth rate and a drastic reduction in cell yield were observed compared to cultures that were not growing under SND conditions (Stouthamer et al., 1997). Similar results related to other pure cultures have also been reported by van Niel et al. (1992) and Robertson et al. (1988).

The available literature shows that under suitable conditions, the SND process will occur in the wastewater system. Wastewater treatment based on an SND–SBR process would represent a simpler biological nitrogen removal system than the conventional two-step nitrification and denitrification process. However, the conditions that may result in the occurrence of an efficient SND-based SBR process are not yet well established. In this investigation, an SND-based SBR process was operated to resolve some of the conditions under which significant SND activities can occur.

## 2. Materials and methods

### 2.1. Composition of the synthetic wastewater

A synthetic wastewater was used for investigating the SND at different influent COD/$NH_4^+$-N (C/N) ratios and ammonium-loading rates with the formula listed in Table 1. The optimal COD/nitrate ratio to support conventional biological denitrification was reported to be 6.0–8.0 by Chiu and Chung (2003). Zhao et al. (1999) suggested that heterotrophic nitrifiers, which can coexist with autotrophic nitrifiers under a wide range of conditions, play a significant role in the oxidation of ammonia at a BOD/N ratio of 6.9 or greater. Therefore, three C/N ratios of a synthetic wastewater (6.3, 11.1, and 19.7) were tested in this study by adjusting the sodium acetate dosage. In order to evaluate the influence of $NH_4^+$-N loading on the nitrification efficiency of the SND–SBR system, quantities of ammonium chloride ($NH_4Cl$) and sodium acetate were adjusted at different levels in three different operational phases during the experimental period. Concentrations of relevant constituents contained in the synthetic wastewater are summarized in Table 1. The high $NH_4^+$-N content was used to simulate the inorganic nitrogen variation in domestic effluents.

### 2.2. Operational conditions of the SND-based SBR system

A schematic diagram of the experimental SBR system comprised of two reactors is shown in Fig. 1. Each reactor was a rectangular acrylic tank,