



Investigating organic nitrogen production in activated sludge process: Size fraction and biodegradability



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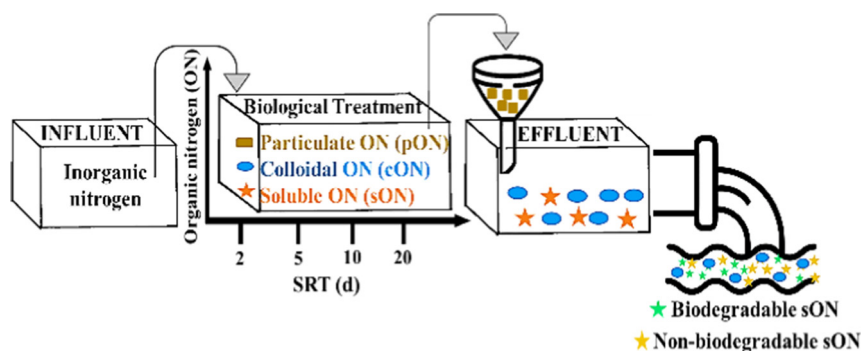
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HIGHLIGHTS

- Activated sludge produces different sizes of organic nitrogen (ON).
- Size fractions of effluent ON vary with solids retention time (SRT).
- Produced colloidal ON can be a larger fraction than produced soluble ON in effluent.
- Biodegradability of produced soluble ON decreased greatly from SRT of 10 to 20 d.

GRAPHICAL ABSTRACT



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ABSTRACT

The effect of sludge retention time (SRT) on the production of organic nitrogen (ON) fractions (particulate, colloidal and soluble) and the biodegradability of produced soluble ON in an activated sludge process was investigated. Synthetic wastewater with no ON was fed to the four laboratory-scale reactors operated at SRTs of 2, 5, 10 and 20 d, respectively. Effluent ON from each reactor was fractionated into particulate, colloidal, and soluble ON (pON, cON, and sON). The effluent total ON contained 5.7 to 11.9 mg/L pON, 3.6 to 3.8 mg/L cON, and 2.3 to 4.6 mg/L sON. cON fraction can be larger than sON fraction in the secondary effluent. Therefore, besides focusing on sON, water resource recovery facilities aiming to meet stricter effluent TN limits should also identify appropriate technologies to target cON. More than 50% of effluent sON was biodegradable under SRTs of 2, 5, and 10 d but the biodegradability decreased to 31% at 20-d SRT. Large fractions of non-biodegradable sON (69%) at SRT of 20-d could be contributed by extracellular polymeric substances and soluble microbial products, specifically biomass associated products due to endogenous respiration. Thus, sON generated at long SRTs may take longer to decompose in receiving waters.

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1. Introduction

Organic nitrogen (ON) becomes the major nitrogen fraction in the effluent for water resource recovery facilities (WRRFs) that are trying to achieve very low effluent total nitrogen (TN) limits, such as less than 5 mg/L. The ON can be as high as 80% of the effluent TN for WRRFs producing low effluent TN (Sattayatewa et al., 2009; Chen

et al., 2011; Simsek et al., 2016). It is made up of particulate (pON), colloidal (cON) and soluble (sON) fractions. Since the majority of pON is effectively removed through settling and filtration (if adopted) processes in WRRFs, cON and sON become the dominant forms of organic nitrogen in the effluent. About 45% of effluent organic nitrogen was reported as cON (Sattayatewa et al., 2010), while sON ranged between 20 and 85% of the effluent organic nitrogen (Pagilla et al., 2006, 2008; Pehlivanoglu and Sedlak, 2004; Simsek et al., 2016). Moreover, the concentrations of cON and sON combined in the final effluent can range between 0.5 and 3 mg/L, posing a major challenge in achieving low levels of TN (Sattayatewa et al., 2009; Simsek et al., 2013; Czerwionka and Mękinia, 2014; Hu et al., 2018).

Influent cON and sON are removed mainly during biological treatment processes in WRRFs. Since organic nitrogen is contributed by the influent and also produced during biological processes, controlling effluent organic nitrogen has been a challenge for WRRFs (Parkin and McCarty, 1981a, 1981b, 1981c; Pehlivanoglu-Mantas and Sedlak, 2006; Hu et al., 2018; Hu et al., 2020). Although around 1 to 2 mg/L of effluent organic nitrogen has been identified for both biological nutrient removal (BNR) and non-BNR activated sludge facilities, the relative contributions of influent-derived and process-derived organic nitrogen are not known (Pagilla et al., 2006, 2008, Sattayatewa et al., 2009, 2010).

Distinguishing between influent-derived and process-derived fractions is critical to optimize the removal of influent ON and to minimize the amount of process-derived ON. The difficulty in removing organic nitrogen often led to the use of physical-chemical methods for ON removal from secondary effluents (Randtke et al., 1978). These methods have high capital and operation costs and are not efficient in removing sON, especially the portion that is generated from the biological process (Bratby et al., 2008). Therefore, it is often desirable to minimize the generation of ON in a biological process. However, ON production is affected by the type of biological treatment employed and the types of operational conditions used (Sattayatewa et al., 2009; Liao et al., 2019; Hu et al., 2018, Hu et al., 2019; Hu et al., 2020).

Considering the challenges associated with reducing the effluent ON, there is an imperative need to optimize the operational conditions to minimize process-derived organic nitrogen generation. Sludge retention time (SRT), food to microorganisms ratio (F/M), and chemical oxygen demand (COD) to nitrogen ratio (COD/N) are some of the critical operational parameters that are commonly used in process optimization for WRRFs. During the adjustments to these parameters in full-scale WRRFs, the focus is given at TN and less attention is paid toward ON, mainly due to lack of direct measurement of ON.

Studies have reported the influences of SRT (Simsek et al., 2016; Parkin and McCarty, 1981b) and COD/N (Hu et al., 2018) on ON production during biological treatment. Simsek et al. (2016) fed primary effluent to a chemostat and did not find any correlation between SRT and effluent sON. Hu et al. (2018) fed secondary effluent to a denitrifying filter and noticed maximum effluent sON at the carbon to nitrogen (C/N) ratio of 3 and no impact on effluent sON for higher C/N ratios (4, 5 and 6). The presence of ON in the influent and fluctuation in its concentration did not allow these studies to clearly estimate the actual production of ON in the biological process. There was a study by Sattayatewa et al. (2010) that fed laboratory-based sequencing batch reactors with ammonia only as the nitrogen source to eliminate influent ON. They reported that the majority of the effluent organic nitrogen was soluble, probably due to the use of synthetic influent feed. However, they did not examine the impact of SRT on ON production and its fractions (pON, cON and sON). Although considerable attention has been given to the generation of sON in biological treatment, surveys showed the presence of significant amounts of cON in full-scale WRRF effluents (Pagilla et al., 2008; Sattayatewa et al., 2010; Pagilla et al., 2011; Czerwionka et al., 2012; Czerwionka and Mękinia, 2014).

As described above, pON is susceptible to sedimentation filtration (if adopted) whereas both cON and sON could be found in the final effluent (Sattayatewa et al., 2010). Since effluent organic nitrogen could be the

majority nitrogen fraction in very low effluent TN (<3 mg/L), identifying the contribution of cON versus sON fractions to effluent ON from influent or biological processes is very important. To understand the N fractions better, Czerwionka and Mękinia (2014) evaluated secondary effluent concentrations in eight full-scale BNR activated sludge systems in Poland. They reported an average sON concentration of 0.5 to 1.3 mg/L (12 to 45% of effluent total ON) and average cON concentration was 0.7 to 1.9 mg/L (35 to 44% of effluent total ON). Therefore, the size-fractionation of process-derived ON fractions is necessary to determine the contribution of cON and sON to the treatment plant to meet stricter effluent TN permit limits. The obtained size fractionation information can help the WRRF target the removal of specific fractions after the activated sludge process. For example, employing chemical precipitation and filtration can help in sON removal, whereas anaerobic and anoxic compartments can help reduce effluent cON fractions (due to influent dilution and recirculated mixed liquor providing more surface area for the influent colloids to adsorb) (Arnaldos and Pagilla, 2010; Czerwionka et al., 2012).

Although several studies have investigated the ON fractions in biological treatment processes (Pagilla et al., 2008; Mękinia et al., 2009; Sattayatewa et al., 2009; Pagilla et al., 2011; Czerwionka et al., 2012), so far none has examined the effect of SRT on effluent ON fractions in an activated sludge process. Thus, this study aims to investigate the production of pON, cON and sON in a bench-scale activated sludge process at different SRTs. To eliminate the role of influent-derived ON, batch bioreactors were fed with ammonia only as a nitrogen source. Effluent ON production and its fractions were measured for different SRTs (2, 5, 10 and 20 days) corresponding to F/M (1.2, 0.8, 0.5 and 0.2). Besides, the effluent COD from each SRT was also fractionated into particulate, colloidal and soluble fractions. Finally, effluent from each SRT was examined for biodegradable and non-biodegradable fractions of sON.

2. Materials and methods

2.1. Synthetic wastewater composition

Synthetic wastewater (SWW) recipe was modified from Nagaoka et al. (1996) to mimic a medium-strength domestic wastewater composition. The composition included glucose (450 mg COD/L), ammonium chloride (50 mg N/L), orthophosphate using monopotassium phosphate and disodium phosphate (5 mg P/L), calcium chloride (11 mg/L), magnesium sulfate heptahydrate (17 mg/L), ferric chloride hexahydrate (4 mg/L) and sodium bicarbonate (350 mg/L). Trace metals including zinc chloride (0.2 mg/L), nickel chloride (1.5 mg/L), manganese chloride (2 mg/L) and cobalt chloride (3 mg/L) were added. Vitamins were contributed by RPMI 1640 solution (Sigma-Aldrich, St. Louis, Missouri).

2.2. Inoculum source

Mixed liquor suspended solids (MLSS) were collected from a WRRF treating municipal wastewater located in Moorhead, MN, United States and used as an inoculum for the experimental work. The treatment facility removes organic carbon through a high purity oxygen activated sludge process which is operated at an SRT of 3 d. The treatment facility receives an average flow of 15,000 m³/day.

2.3. Experimental setup

Four laboratory-scale chemostat reactors were set up with an initial total suspended solids (TSS) concentration of 1800 mg/L and operated in parallel. The reactors were set up by mixing the MLSS collected from the Moorhead WRRF with lab-grade de-ionized water. Fig. 1 shows the bench-scale experimental setup employed for this study. In parallel, the four reactors were fed with SWW using peristaltic pumps operated at different flow rates to achieve 2, 5, 10 and 20 days of hydraulic residence times (HRT). Since the reactors were operated



Fig. 1. Bench-scale experimental setup for chemostat operation on day 1 (top) and day 20 (bottom). SWW – synthetic wastewater water; Eff – effluent; SRT – solids retention time.

as chemostat with no wasting or recycling of sludge, HRT would be equivalent to SRT at steady-state conditions. Mixing and aeration were achieved by magnetically stirring the sludge mixture and by supplying air through a coarse bubble diffuser, respectively. Sludge inside the reactors was magnetically stirred to avoid sludge settling or the creation of dead zones within the reactors.

Dissolved oxygen (DO) concentration was maintained between 2 and 4 mg/L. pH in the reactors was maintained between 7.2 and 7.8 using NaHCO_3 and HCl. Effluent from each reactor was analyzed daily for TSS, volatile suspended solids (VSS), chemical oxygen demand (COD) and nitrogen species (ammonia, nitrite, nitrate and TN). Steady-state conditions were assumed to be achieved when less than 10% variation was observed in the concentrations of effluent quality parameters (TSS, VSS, COD and ammonia) consecutively for five days. At steady state, effluent samples were fractionated and analyzed for particulate, colloidal and soluble fractions of COD and nitrogen species.

2.4. Fractionation technique

Effluent samples were separated into particulate, colloidal and soluble fractions using different size fractionation techniques to analyze for COD and nitrogen species. Initially, the unfiltered effluent sample was analyzed for a total fraction (T). Samples were then filtered through a

1.2 μm pore-size glass microfiber filter (GF/C, Whatman Inc., Kent, UK) to obtain colloidal plus soluble fraction (C + S) in the filtrate (the particulate fraction was retained on the filter).

Conventional filtration technique using a 0.45 μm pore size membrane filter to obtain soluble fraction can contain colloidal fraction that ranges between 0.1 and 1.0 μm diameter. Hence, the flocculation-filtration technique (Mamaï et al., 1993) was employed to remove both particulate and colloidal fractions from the effluent. Zinc sulfate (ZnSO_4) and sodium hydroxide (NaOH) were added to the effluent sample to flocculate colloids and particulates followed by filtration with a 0.45 μm pore size cellulose acetate membrane filter (PALL Co., Port Washington, NY, USA) to obtain true soluble (S) fraction. Particulate (P) and colloidal (C) fractions were calculated using the following equations:

$$\text{Particulate fraction (P)} = T - (C + S) \quad (1)$$

$$\text{Colloidal fraction (C)} = T - (S + P) \quad (2)$$

where, T = total fraction, C = colloidal fraction, S = soluble fraction and P = particulate fraction.

At each fractionation step, samples were analyzed for COD and nitrogen species.

2.5. Analytical methods

All samples were analyzed in triplicates. TSS and VSS measurements were performed according to Standard Methods (APHA et al., 2005). Inorganic and total nitrogen species were measured using the HACH kits (TNT 830, 832 and 833 for ammonia, TNT 839 and 840 nitrite, TNT 835 and 836 for nitrate and; TNT 826, 827 and 828 for TN). COD measurements were also performed using HACH kits (TNT 821 and 822). HACH DR 5000 spectrophotometer was used for all absorbance measurements. Total organic nitrogen was calculated by subtracting the sum of all inorganic nitrogen species (ammonia, nitrite and nitrate) from total nitrogen. Bench top meters were used to continuously monitor the pH (model 250A+, Thermo Scientific Orion) and DO (model 850 Thermo Scientific Orion).

2.6. Biodegradability assay

The biodegradable sON (bsON) was measured following the method developed by Khan et al. (2009). The bsON bioassay was performed in triplicates for all samples. A blank control was included by using deionized distilled water as a sample. The difference in sON reduction of the sample and the control during the incubation was bsON as shown in Eq. (3):

$$\text{bsON (mg/L)} = (\text{sON}_i - \text{sON}_f) - (\text{sON}_{b_i} - \text{sON}_{b_f}) \quad (3)$$

where sON_i and sON_f are sON before and after the incubation for effluent samples; and,

sON_{b_i} and sON_{b_f} are sON before and after incubation for control.

Non-biodegradable sON (nbsON) was calculated using the following equation:

$$\text{nbsON (mg/L)} = \text{sON} - \text{bsON} \quad (4)$$

3. Results and discussion

3.1. Performance of reactors at different SRTs

This subsection discusses the basic reactor performances observed during the experiments whereas the following two subsections highlight the main findings pertaining to ON fractions and sON biodegradability. Initially, each reactor for SRTs 2, 5, 10 and 20 d was seeded with 1800 mg/L-TSS of mixed liquor, which gave dark color to each

reactor. Upon achieving steady-state, the mixed liquor appearance inside each reactor changed from dark brown to light brown (Fig. 1). Differences in the reactor appearances indicated faster washout at shorter SRTs compared to longer SRTs. All the reactors were fed with 50 mg/L NH_4Cl (no organic nitrogen in the feed). Nitrification increased with increasing SRT as expected (Fig. S1 in Supplementary data (SD)). Partial nitrification observed at lower SRTs could be due to the presence of nitrifiers in the MLSS, which was used as a seed in the reactors. MLSS was collected from the aeration basin of Moorhead WRRF, and the past operational data of the plant indicated the presence of nitrifiers in the mixed liquor, which was evident by the significant removal of ammonia in the aeration basin (Simsek et al., 2016).

All reactors were fed with 450 mg sCOD/L in the form of glucose with no cCOD and pCOD in the SWW. During steady state, the majority of influent sCOD was transformed into pCOD (76 to 86% pCOD/TCOD), suggesting that influent COD was readily consumed by the biomass in all the reactors (Fig. 2). Effluent pCOD decreased significantly from SRT of 2 d to 5 d, after which it did not change much (201.2, 140.5, 141.1, 143.4 mg/L). At SRT of 2 d, maximum washout was expected (1.0, 0.4, 0.2 and 0.1 L/d was the influent flowrate) because of which the rate of hydrolysis in the floc matrix was restricted (Jimenez et al., 2007), thus leading to a higher concentration of pCOD in the effluent. The higher concentration of pCOD in the effluent due to washout at SRT of 2 d is also supported by effluent VSS concentration (195, 110, 98 and 104 mg/L VSS) which was the highest for SRT of 2 d and did not change much at SRT of 5, 10 and 20 d confirming that the maximum amount of biomass discharged at SRT of 2 d. The colloidal fraction of COD was the smallest out of the three fractions investigated in the effluent of each reactor (2 to 9% cCOD/TCOD). The sCOD fraction drop from SRT of 2 d to 10 d (44.6, 29.2, 16.3 mg/L sCOD), after which it went up at SRT of 20 d (25.9 mg/L sCOD). The increase in effluent sCOD at SRT of 20 d is attributed to the endogenous respiration occurring in the reactor due to starvation.

Overall, influent COD was not completely degraded in any reactor leaving approximately 37 to 57% COD in the effluent (Fig. S2 in SD). It is important to mention that laboratory-scale chemostat reactors were operated in this study and could only partially degrade the influent COD (450 mg/L) due to their limited capability. From a different perspective, this finding of incomplete COD degradation was consistent with suggestions by Hu et al. (2020). They investigated process-derived sON formation for the purpose of modeling at both bench and full scales. They operated activated sludge systems at SRT of 20 d under different concentrations of MLSS (1000, 2000 and 3000 mg/L) and ammonia-N (10, 20 and 30 mg N/L). They suggested that

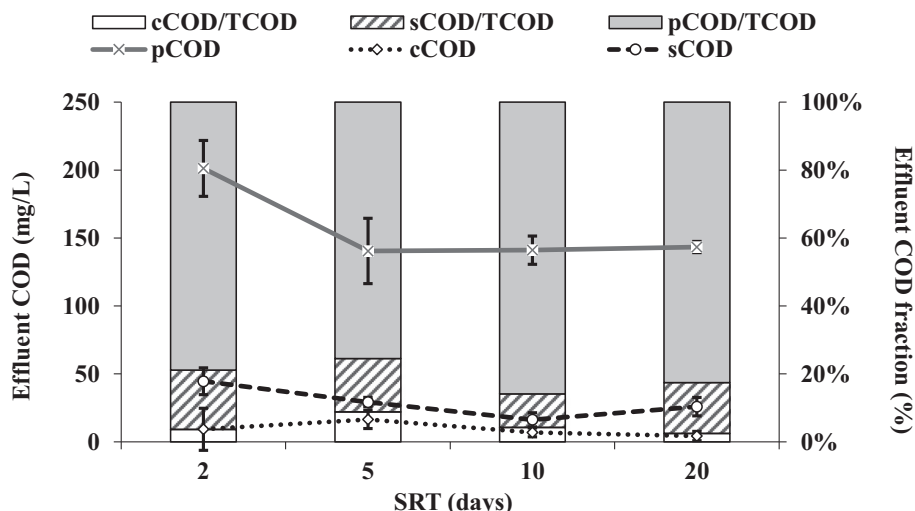


Fig. 2. Fractions of effluent total COD (TCOD) under SRTs 2, 5, 10 and 20 d.

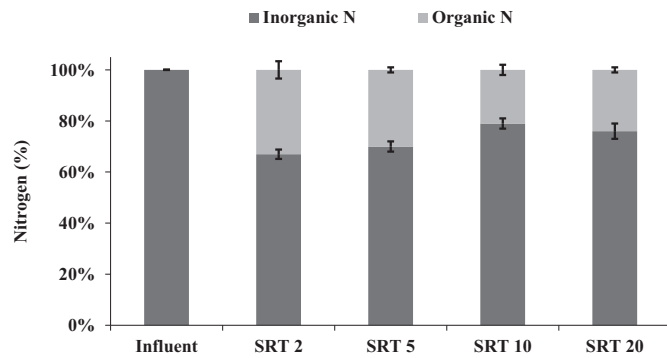


Fig. 3. Nitrogen mass balance for influent and effluent from each reactor.

carbonaceous compounds were excreted during the microbial metabolism because of which COD (influent SWW contained 300 mg/L COD) was not completely degraded (Hu et al., 2020).

Results from this study showed that SRT influences the influent COD degradation efficiency in an activated sludge process. SRT can be used to control both settleable (pCOD) and non-settleable (cCOD and sCOD) fractions. Operating reactors at higher SRTs (>5 d) can help in reducing the non-settleable fractions, which usually contribute toward final effluent in a WRRF. Therefore, both colloidal and soluble COD fractions should be the target for the advanced wastewater treatment technologies applied after the activated sludge process in a treatment facility.

3.2. Effect of SRT on effluent ON fractions

Overall, the total ON (TON)/TN ratio declined with increasing SRT (36%, 30%, 21% and 24% at SRTs 2, 5, 10 and 20 d, respectively) as displayed in Fig. 3. This result suggests that operating an activated sludge process at lower SRTs (2 and 5 d) generates a larger fraction of TON/TN, which would be degraded further if operated at longer SRTs (10 to 20 d). Effluent ON fractions under different SRTs are presented in Fig. 4. Organic nitrogen was produced in all the reactors when no ON was fed in the influent which is consistent with past studies (Parkin and McCarty, 1981b; Pehlivanoglu-Mantas and Sedlak, 2006; Urgan-Demirtas et al., 2008; Pagilla et al., 2011, Hu et al., 2020). For effluent TON, the highest contribution was from pON (45–60% of TON) followed by cON (18–31% of TON) and sON (19–25% of TON). For

effluent TON, the highest contribution was from pON (45–60% of TON) followed by cON (18–31% of TON) and sON (19–25% of TON).

Pagilla et al. (2008) examined nitrifying plants in the U.S., and BNR plants in Poland, to understand the fate of sON through different treatment trains. Secondary effluent samples were fractionated into cON and sON fractions for the plants in the U.S. and pON, cON and sON for the Polish plants by filtering the samples through different pore-sized (0.1, 0.22, 0.3, 0.45 and 1.2 μm) membrane filters. sON ranged 56–95% and cON was 5–44% of TON for the effluent from 3 different WRRFs in the U.S. All three fractions, sON (19–62% of TON), cON (21–62% of TON) and pON (17–27% of TON), were observed for the effluent from 4 different WRRFs in Poland. Effluent sON (19–25% of TON) and cON (18–31% of TON) fractions obtained from this study are relatively closer to the fractions observed for Polish plants compared to plants in the U.S. The effluent pON (17–27% of TON) from Poland was much smaller than that reported in this study (45–60% pON of TON). It is important to note that the reported fractions from the Poland WRRFs were obtained from the effluent of secondary clarifiers, which further remove particulates and in turn the pON.

Effluent sON fraction range obtained from the U.S. WRRFs was higher than that from the Polish facilities as well as this study. Pagilla et al. (2008) suggested that higher fractions of sON were contributed by different types of secondary treatment processes employed by the WRRFs in the U.S. The effluent sON samples in the U.S. WRRFs were determined strictly by 0.45 μm pore-size membrane filtration unlike filtration-flocculation method used in this study, which is expected to further remove colloidal fractions. Overall, effluent cON fraction range contributed by the U.S. WRRFs was large; however, the highest (44% cON of TON) fraction was contributed by one facility whereas the other two facilities contributed smaller fractions (5 and 14% cON of TON). The cON fractions in these facilities were reduced due to the use of coagulant in the secondary clarifiers (Pagilla et al., 2008). The cON fractions obtained from this study fall within the range reported by the U.S. WRRFs.

The effluent cON concentration was almost similar in each reactor (3.6, 3.8, 3.7 and 3.8 mg/L). On comparing effluent cON with sON concentrations, except at SRT of 2 d (3.6 mg/L cON < 4.6 mg/L sON) the concentrations of cON were always larger than sON in all other reactors (SRT 5 d: 3.8 > 3.2 mg/L, 10 d: 3.7 > 2.3 mg/L, and 20 d: 3.8 > 3.1 mg/L). This finding suggests that cON is the larger fraction generated in an activated sludge process when operating at SRT between 5 and 20 d. However, when operating at smaller SRT of 2 d, sON is the

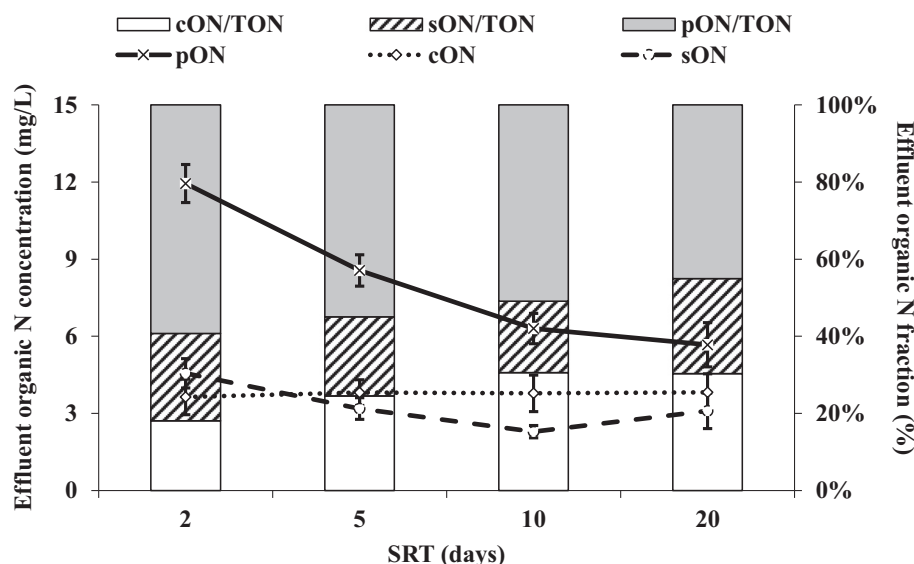


Fig. 4. Concentration (left axis) and fractions (right axis) of effluent total organic N (TON) under SRTs 2, 5, 10 and 20 d.

larger fraction of effluent TON. It is possible that the rate of hydrolysis (pON to cON) or cell lysis increases with increasing SRT causing a reduction in pON fraction and increasing the cON fraction (Pagilla et al., 2006; Mękinia et al., 2009). However, the rate of hydrolysis of pON to cON must be different from the hydrolysis of cON to sON because unlike cON fraction which increased with increasing SRT, sON fraction declined with increasing SRT (Fig. 4). Pagilla et al. (2011) reported negligible effluent cON while operating a bench-scale SBR which was fed with SWW (27.9 mg/L-N and 170.3 mg/L-COD) for an SRT of 10 d. The SWW did not contribute any organic N to the reactors. While acknowledging that full-scale plants generate effluent cON fractions, they mentioned that effluent cON was absent in their bench-scale SBR reactor because it was fed with SWW with no suspended solids. However, they suggested a further investigation on effluent cON generation (Pagilla et al., 2011).

Fig. 4 displays a decreasing trend in sON concentration from SRT of 2 d to 10 d (4.6, 3.2, 2.3 mg/L) after which an increase was observed at SRT of 20 d (3.1 mg/L). These results are different from the results reported by both Hu et al. (2018) and Simsek et al. (2016), that investigated the effect of SRT on sON degradation using bench-scale reactors. Hu et al. (2018) found that effluent sON decreased (4.9, 4.4, 3.7 and 3.6 mg/L) with increasing SRT (5, 13, 26 and 40 d), whereas Simsek et al. (2016) reported no trend between SRT (0.3, 0.7, 2, 3, 4, 5, 7, 8 and 13 d) and effluent sON concentration (minimum sON was 4.75 mg/L at SRT 0.3 d and maximum sON was 8.08 mg/L at SRT 4 d). The absence of a trend between sON concentration and SRT was expected by Simsek et al. (2016) due to fluctuation in the influent TN.

The magnitude of sON concentrations observed in this study is comparable with results concluded by Hu et al. (2018). Also, the decreasing trend of effluent sON with increasing SRT observed in this study is similar with a trend reported by Hu et al. (2018) study. Unlike this study wherein chemostat reactors were operated, sequencing batch reactors were employed by Hu et al. (2018). The working volume (2 L) and the initial mixed liquor suspended solids concentrations (1800 mg/L) of the reactors were similar in both studies. In addition, no sludge was recirculated or reinoculated in both studies. On the other hand, sON concentrations reported in this study as well as Hu et al. (2018) were smaller than those reported by Simsek et al. (2016). Although no trend was observed between SRT and effluent sON, a more conclusive trend was reported between effluent sON biodegradability (effluent biodegradable sON/effluent sON) and SRT by Simsek et al. (2016). Effluent sON biodegradability decreased with increasing SRT until SRT of 8 d and then increased slightly on increasing the SRT to 13 d. This finding highlighted higher generation of non-biodegradable sON at longer SRTs, showcasing the importance of SRT in controlling biodegradable

fraction of sON in the effluent. The difference in the effluent sON concentrations reported by Simsek et al. (2016) and this study is attributed to different characteristics of influent wastewater and fluctuation in influent TN concentration.

Unlike pCOD concentration, which did not decrease much after SRT of 5 d (201.2, 140.5, 141.1 and 143.4 mg/L), effluent pON dropped gradually with increasing SRT (11.9, 8.6, 6.3 and 5.7 mg/L). Increasing the SRT could lead to higher hydrolysis of pON into cON and sON, hence the drop in pON concentration as hypothesized by Pagilla et al. (2006). However, similar to pCOD/TCOD, pON/TON was also the largest fraction in the effluent of each reactor. Unlike WRRFs wherein the activated sludge processes are followed by secondary clarifiers, the bench-scale reactors operated in this study were chemostats (SRT = HRT) i.e. concentration of N components will be the same inside the reactor and in the effluent, because the collected effluent was not separated from the solids. Secondary clarifiers remove a major fraction of particulates via activated sludge settleability (Pagilla et al., 2008). As a result, the effluent pON concentrations achieved in this study via chemostat reactors will always be higher than effluent pON from WRRFs.

This study shows that operating at different SRTs can affect the generation of different fractions of effluent ON in an activated sludge process. However, it should be noted that contribution of effluent cON fraction can be larger than sON in the secondary effluent TON. This is crucial because WRRFs can then identify appropriate technologies for further lowering effluent TN. For instance, polymer addition to water containing colloidal material enhances flocculation, thus removing colloidal particles from the solution via sedimentation. Therefore, considering the generations of sON and cON fractions during AS process, treatment technologies employed at WRRFs to meet stricter effluent TN limits should target these fractions.

3.3. Effect of SRT on effluent sON biodegradability

The goal behind examining the biodegradability of effluent sON was to determine the fraction of sON generated in the activated sludge system that is biodegradable and likely be reduced with more prolonged treatment time in a chemostat reactor wherein HRT is equal to SRT. The biodegradability assay quantified the bsON and nbsON fractions of the effluent sON from each reactor (Fig. 5). More than 50% of effluent sON was bsON at the SRTs of 2 d (58%), 5 d (62%) and 10 d (53%), whereas effluent sON at SRT of 20 d was the least biodegradable (31%). Although no specific trend was observed between SRT and sON biodegradability, larger fractions of sON at lower SRTs (2 d and 5 d) were biodegradable, and the biodegradability decreased with

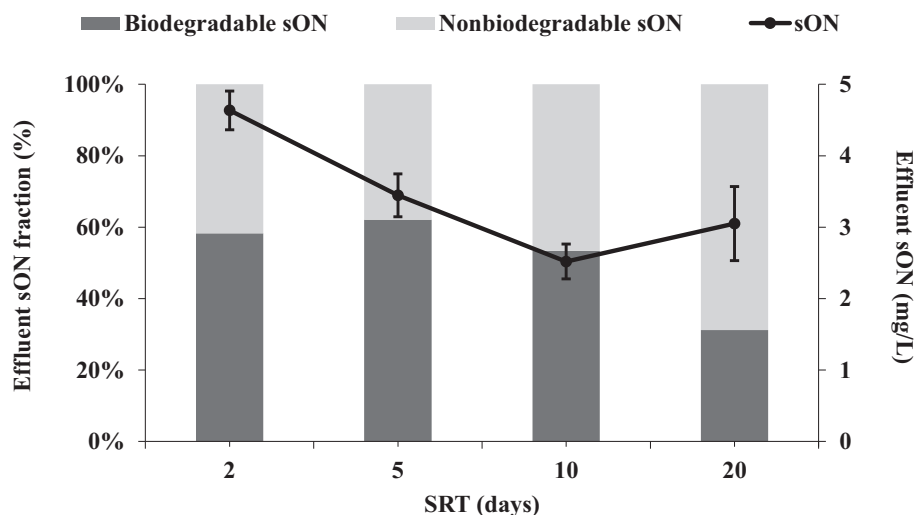


Fig. 5. Fractions of biodegradable and non-biodegradable sON obtained under different SRTs.

increasing SRT (10 d and 20 d). Operating the reactor at SRT of 10 d, generated the least amount of sON (2.29 mg/L), which was partially biodegradable (53%). At SRT of 20 d, a larger concentration of sON (3.10 mg/L) was generated when compared with SRT of 10 d and was highly non-biodegradable (69%). Operating reactors at longer SRTs enhanced nitrifiers and provided a longer time for the biomass to degrade sON fraction in the reactor. However, it is important to note that in this study the chemostat reactors were operated without wasting or recycling any sludge which means that during longer SRTs such as SRT of 20 d, endogenous decay is expected. A F/M ratio of 0.2 (mg COD/mg VSS d) corresponded with SRT of 20 d, indicating that the effluent sludge was old and perhaps decayed due to starvation, thus releasing non-biodegradable endogenous residues.

At SRT of 20 d, only 31% of the produced sON was biodegradable. With minimum biodegradable fraction produced, this observation sounds promising from an effluent quality perspective (to be discharged in water bodies). However, several studies have found that endogenous residue undergoes slow biodegradation (Jones et al., 2007; Laera et al., 2005; Lubello et al., 2009; Ramdani et al., 2010). This suggests that the effluent sON from SRT of 20 d could degrade even further. On examining the active biomass fraction and the endogenous decay rate under aerobic conditions, Ramdani et al. (2012) found that the active biomass fraction (based on VSS/TSS) reduced from 68% to 59% from SRT of 5.2 d to 10.4 d. In contrast, the endogenous decay rate increased from 32% to 41%. Therefore, SRT affects both active biomass fraction as well as endogenous decay rate. Results from this study are agreeable with the previous study (Ramdani et al., 2012) suggesting that endogenous decay rate may increase with increasing SRT. This agreement is based on the effluent sON concentration (2.2 and 3.1 mg/L sON) and active biomass fraction (0.90 and 0.60 VSS/TSS) at SRT of 10 d and 20 d. On increasing the SRT from 10 d to 20 d, active biomass fraction declined significantly (90% to 60%) which can be attributed to increased endogenous decay, thus increasing the effluent sON concentration.

Simsek et al. (2016) reported the increase in effluent nbsON at longer SRTs being contributed by soluble microbial products (SMPs) and extracellular polymeric substances (EPSs). SMPs include organic material such as proteins, which are released by biomass. SMPs can be categorized as i) utilization associated products (UAPs), associated with biomass growth and ii) biomass associated products (BAPs), associated with biomass decay (Ni et al., 2011). EPSs include proteins and are subject to hydrolysis and contribute to BAPs (Aquino and Stuckey, 2004). Several studies conducted in the past provided several conclusions about the contributions made by EPSs and SMPs. For example, more EPSs were produced by biomass under stressful conditions (Aquino and Stuckey, 2004), higher amounts of SMPs were produced at higher SRTs, and UAPs decreased while BAPs increased with increasing SRT (Ni et al., 2010). These results suggest that at SRT of 20 d, large fractions of nbsON could be contributed by EPSs and SMPs, specifically BAPs. Ni et al. (2010) reported higher accumulation of BAPs than UAPs when a continuous flow activated sludge reactor was operated and simulated at an SRT of 20 d under famine condition. This finding by Ni et al. (2010) further confirms the presence of BAPs at SRT of 20 d due to low availability of substrate. Effluent BAPs can be targeted post biological treatment via techniques such as membrane filtration, adsorption using granular activated carbon, coagulation and filtration (Kiser et al., 2010; Ni et al., 2011).

SRT affects the non-biodegradability of produced sON due to changes in the endogenous residue characteristics (e.g. production and degradation of EPSs and SMPs). SMP fractions (UAPs and BAPs) and their biodegradability are known to change with SRT (Jarusutthirak and Amy, 2006; Ni et al., 2010). Therefore, based on the results obtained from this study as well as past studies, it is inferred that SRT and (non-) biodegradability of sON are related irrespective of the source of sON (influent-derived or process-derived). Increasing the SRT enhances the non-biodegradability of sON. This finding is in agreement with a previous study (Simsek et al., 2016) which

investigated the biodegradability of effluent sON under different SRTs (0.3, 0.7, 2, 3, 4, 5, 7, 8 and 13 d) in a chemostat reactor that was fed with primary treated wastewater with approximately 13 mg/L of sON. Larger fractions of nbsON in the effluent were observed at longer SRTs (Simsek et al., 2016). Therefore, operating an aerobic reactor at an SRT of 20 d or longer will generate sON that will be recalcitrant in receiving waters for a longer period of time. The WRRFs attempting to achieve lower TN in the final effluent should be aware about the production and recalcitrant nature of sON, especially when operating under longer SRTs.

4. Conclusions

The results from this study show that pON, cON and sON were generated in an AS process when the influent contained no ON. This study concludes that SRT affects each fraction of organic nitrogen along with the biodegradability of effluent sON. The results reveal that besides effluent sON, which has already garnered attention in the past two decades, effluent cON also deserves attention from the research community because it contributes a significant fraction to effluent total organic nitrogen in the biological process. The current study demonstrated sON and cON production for a simple aerobic system. Depending on the biological treatment processes configuration, generation of organic nitrogen and its fractions will vary. Therefore, WRRFs should account for the ON production, particularly when they aim to reduce more TN by increasing the SRT. Considering the bioavailability of sON in natural waters, effluent sON produced at different SRTs should be investigated with mixed algal culture for sON uptake because past studies have shown that influent sON is highly bioavailable at longer SRTs. This study focused on aerobic production of sON, similar research under anoxic and anaerobic conditions at different SRTs should be investigated. Examining how much of the produced sON under different SRTs is contributed by SMP and EPS can further enhance our understanding on the relative importance of these process-derived sON sources. This recommendation is extended to anoxic and anaerobic processes associated with BNR.

CRediT authorship contribution statement

Ruchi Joshi: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Murthy Kasi:** Conceptualization, Writing – review & editing. **Tanush Wadhawan:** Conceptualization, Writing – review & editing. **Eakalak Khan:** Conceptualization, Project administration, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.145695>.

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