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Impact of nutrient deficiency on biological sewage treatment – Perspectives towards urine source segregation

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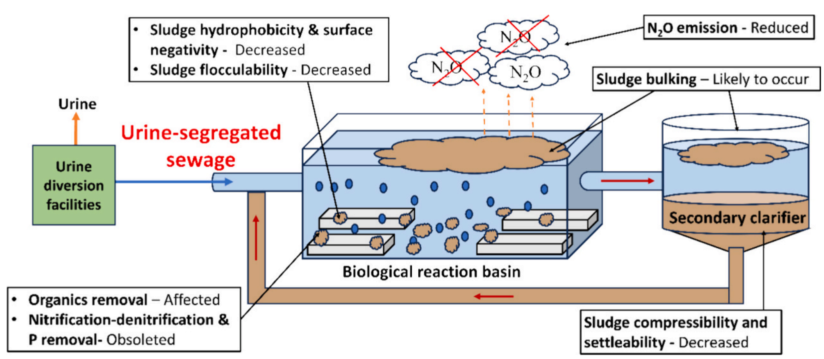
HIGHLIGHTS

- Characteristics of sewage at different urine segregation percentages were summarised.
- Organic matter, N and P removal of STPs are likely disrupted at full urine segregation.
- Sludge flocculability and settleability may decrease under urine segregation scenario.
- N_2O emissions could be reduced notably under the urine segregation scenarios.
- Treatment capacity of STP could be increased up to 48 % at urine segregation >75 %.

GRAPHICAL ABSTRACT



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ABSTRACT

Human urine contains 9 g/L of nitrogen (N) and 0.7 g/L of phosphorus (P). The recovery of N and P from urine helps close the nutrient loop and increase resource circularity in the sewage treatment sector. Urine contributes an average of 80 % N and 50 % P in sewage, whereby urine source segregation could reduce the burden of nutrient removal in sewage treatment plants (STPs) but result in N and P deficiency and unintended negative consequences. This review examines the potential impacts of N and P deficiency on the removal of organic carbon and nutrients, sludge characteristics and greenhouse gas emissions in activated sludge processes. The details of how these impacts affect the operation of STPs were also included. This review helps foresee operational challenges that established STPs may face when dealing with nutrient-deficient sewage in a future where source separation of urine is the norm. The findings indicate that the requirement of nitrification-denitrification and biological P removal processes could shrink at urine segregation above 80 % and 100 %, respectively. Organic carbon, N and biological P removal processes can be severely affected under full urine segregation. The decrease in solid retention time due to urine segregation increases treatment capacity up to 48 %. Sludge

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flocculation and settleability would deteriorate due to changes in extracellular polymeric substances and induce various forms of bulking. Beneficially, N deficiency reduces nitrous oxide emissions. These findings emphasise the importance of considering and preparing for impacts caused by urine source segregation-induced nutrient deficiency in sewage treatment processes.

1. Introduction

The global population is projected to reach 10 billion in 2064 (Vollset et al., 2020). The global fertiliser demand is expected to grow by 1.3 % annually (FAO, 2019) to meet the needs of the growing population. Fertilisers mainly comprise nitrogen (N), phosphorus (P) and potassium. The current production methods of these elements come with drawbacks and limitations. The Haber-Bosch process used for N (ammonia) production is highly energy- and greenhouse gas (GHG) intensive (FAO, 2019). P extraction from P rock reserves generates toxic by-products (phosphogypsum), causing environmental impact (El Zrelli et al., 2018). In addition, P reserves are limited and distributed unevenly across the planet, potentially causing global supply chain disturbances owing to geopolitical influences (Cordell et al., 2018). There is a pressing need to find alternative ways to attain N and P.

Increasingly stringent regulations and higher influent organics and nutrient concentrations are stressing modern sewage treatment plants (STPs) (Jimenez et al., 2015). To produce satisfactory effluent water, nutrient removal processes such as nitrification-denitrification, and biological/chemical P removal, are essential. However, these processes increase land requirements (Freguia et al., 2021), energy consumption (Wang et al., 2012), and greenhouse gas (GHG) emissions (Lu et al., 2015) of STPs. With the growing population, STP operators are forced to expand or reconstruct the existing plant to handle the increasing sewage flow. Not only does this increase the capital cost for plant reconstruction (Badeti et al., 2022), but it also amplifies the existing drawbacks of sewage treatment by consuming and emitting more energy and GHG, respectively.

Urine contains 9.0 g/L of N, 0.7 g/L of P, and 1.5 g/L of potassium (K) (Winker et al., 2009). The high nutrient concentrations render the recovery of N, P and K from urine an attractive option. For this purpose, several public urine source segregation trials have been successfully conducted around the globe (Abeysuriya et al., 2013; Blume and Winker, 2011). Subsequent urine processing technologies aimed at reprocessing urine into usable fertiliser have also been developed (Freguia et al., 2019; Liu et al., 2023), indicating that large-scale urine source segregation in the future is possible.

A key concern with urine segregation is that it may affect the performance of downstream wastewater treatment plants such as activated sludge and biological nutrient removal. Urine is the primary source of N, P and K in sewage. Although the concentrations fluctuate with sewage characteristics and the time of day, urine contributes an average of 80 % of the N, 50 % of the P, and 70 % of the K. K levels are not a concern with complete urine segregation, as the required concentration for activated sludge growth is approximately 3.0 mg/L (Burgess et al., 1999). Considering that the K concentration in municipal sewage typically ranges from 25 to 95 mg/L (Burgess et al., 1999; Lei et al., 2019), >7 mg/L of K will remain even after complete urine segregation, therefore not impacting the activated sludge performance. On the contrary, activated sludge usually requires 25 mgN/L and 5 mgP/L (assume 500 mg/L influent organic carbon) to operate effectively (Burgess et al., 1999). With typical influent N and P concentrations of 50 mgN/L and 5–10 mgP/L in municipal sewage (Henze et al., 2008), these concentrations may drop below the required levels under 100 % urine source segregation. Consequently, N and P deficiencies induced by urine source segregation can pose significant concerns for the operation of biological processes in STPs.

As urine source segregation emerges as a potential solution to address the challenges outlined, its large-scale implementation in the future appears probable. However, a comprehensive and early understanding of the negative impacts of nutrient deficiency induced by urine segregation on established STPs is lacking and remains a concern. Conducting an early and preliminary evaluation of these impacts based on available literature can be helpful in a way that informs process engineers on how to prepare for and address the challenges that may arise with urine source segregation.

To the best of our knowledge, Ekama et al. (2011) is the only review article that briefly discussed the benefits of urine source segregation from the view of reduced water consumption from toilet flushing and increased treatment capacity of STPs. Review articles focusing on the impact of nutrient deficiency on STPs are still lacking. The objective of this paper therefore aims to provide a comprehensive review and evaluation of the impacts caused by N and P deficiencies on STP performance, in the aspects of liquid (sewage treatment), solids (sludge), and gaseous (GHG emissions) phases of sewage treatment. In the last section of this manuscript, the discussed impacts were summarised and related to the operation of STPs to ensure this article benefits STP operators and engineers.

2. Characteristics of urine-segregated sewage

Urine source segregation produces urine-segregated sewage with low N and P contents, resulting in a higher C/N/P ratio than municipal sewage. Depending on the urine segregation percentage, the C/N/P ratio of resulting sewage can be higher at a higher urine segregation percentage. The characteristic of 100 % urine-segregated sewage is available in the literature (Table 1) but characteristics of urine-segregated sewage at other urine segregation percentages are generally not reported.

To understand the C/N/P of urine-segregated sewage from 0 % to 100 % urine segregation, an equation proposed by Wilsenach and van Loosdrecht (2003) (Eq. 1), characteristics of municipal sewage (Henze et al., 2008) and organic and nutrient loadings from urine (Larsen et al., 2013) were used to calculate the C/N and C/P of urine-segregated sewage at different segregation percentages, as follows.

$$N_X = (N_{00} \times V_{00} – SF \times N_{urine} \times I)/V_X \tag{1}$$

Table 1
Typical compositions of sewage with urine and without urine.

Parameters (mg/L)	Municipal sewage	Sewage without urine	Municipal sewage	Sewage without urine
Chemical oxygen demand (COD)	537	500 (7)	600	600 (0)
Total nitrogen (TN)	50	11 (77)	50	11 (80)
NH_3-N	Not reported	Not reported	31	4 (89)
Total phosphorus (TP)	8	5 (34)	6	3 (50)
C/N	11[1]	47[1]	12[1] & 19[2]	55[1] & 171[2]
C/P	67.3	101	100	200
References	Wilsenach and van Loosdrecht (2003)		Jimenez et al. (2015)	

Value in bracket means % reduction due to urine segregation compared to municipal sewage.
[1] C/N calculated using TN as N.
[2] C/N calculated using NH_3-N as N.

where, N_X and N_{00} (mg/L) are N concentrations in sewage with X % urine segregation and without urine segregation, respectively. V_X and V_{00} are the sewage flow rates with X % urine segregation and without urine segregation (L/d), *SF* is the fraction of urine segregation (0.0–1.0), N_{urine} is the human excretion of nutrients from urine (mg/person/d) and *I* is the number of individuals connected to the treatment plant.

The C/N/P of urine-segregated sewage at different segregation percentages is shown in Fig. 1. Fig. 1 has shown a similar trend to studies that simulated the C/N ratio under different urine segregation percentages using BioWin software (Badeti et al., 2021), confirming the validity of Fig. 1. By using Fig. 1, it is possible to assess the characteristics of urine-segregated sewage at various stages of urine segregation. This enables a preliminary evaluation of the impact of urine-segregated sewage on STPs, particularly in terms of N and P deficiency, using available literature data.

It should be noted that the C/N and C/P ratios of urine-segregated sewage reported in this study at different urine segregation percentages can vary due to fluctuations in the characteristics of the receiving wastewater. In the best-case scenario, nutrient levels, especially P, might not be lower than the required levels if the receiving graywater and brownwater contain substantial P. Nevertheless, a high P level (e.g., > 15 mgP/L) in municipal sewage is not always the case and full urine segregation decreases the P level to below the required level is possible. This could be even worse in STPs that receive a high fraction of industrial wastewater.

In this review, the literature included are studies that: (1) specifically studied the effect of urine segregation on STPs using feed sewage with C/N and C/P between 15 and 82, and 100–190, respectively; (2) did not exclusively focus on the effect of urine segregation but examined the effect of C/N and C/P on sewage treatment processes using feeds with C/N and C/P ratios in a similar range as in (1); and (3) used feeds that had a C/N and C/P above 82 and 190, respectively. This is particularly relevant because urine-segregated sewage with a C/N and C/P above 82 and 190, respectively, is possible, especially in STPs receiving industrial wastewater with high organic and low nutrient concentrations. Literature that used real and synthetic sewage that met criteria (1) to (3) were included in this review. In a few instances, we referenced studies that used other wastewater matrices (e.g. industrial wastewater) when they contributed to understanding the effects of nutrient deficiency. However, literature that used industrial wastewater with high C/N/P but also with unusually high toxic contaminants (e.g., heavy metal) was not included in this review.

Real urine-segregated sewage is currently unavailable and urine separation has so far been trialled only at building scale. Many studies therefore use synthetic sewage as the experimental medium, using recipes that have been accepted by the scientific community as representative of municipal wastewater. In addition, the majority of references cited in the paper use wastewater feeds and sludge sources that make them directly comparable to real sewage. Despite this, real urine-segregated sewage is far more complex in terms of composition and biodegradability. Therefore, the impact induced by urine segregation discussed in this review can only be considered a preliminary assessment. We recommend that future research be based on real urine-segregated sewage and STPs to address the knowledge gap of the effects of urine separation on real sewage and STP performance.

3. Effect of nutrient deficiency on the wastewater treatment performance

3.1. Organics removal

Organics and nutrients are metabolized and removed from sewage (Eckenfelder and Cleary, 2013) with the presence and absence of dissolved oxygen (DO) by microorganisms. With urine segregation where N and P become deficient, sewage bacteria start to assimilate organics as polyhydroxyalkanoate (PHA) for future use instead of using it for growth (Guo et al., 2014). This reduces the growth and reproduction rates of microorganisms. Consequently, the organics oxidation rate and the

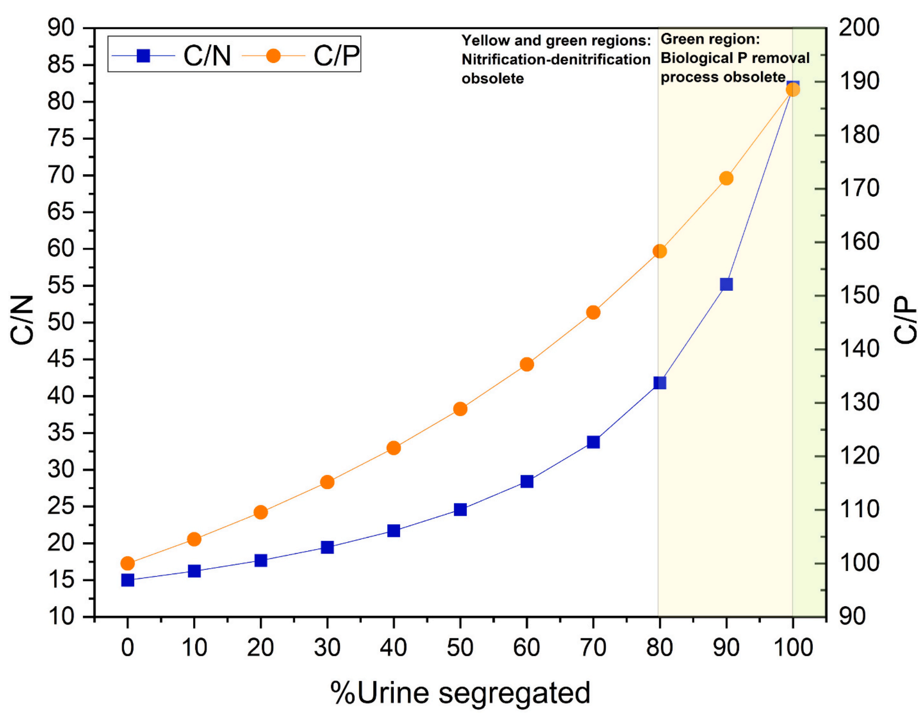


Fig. 1. C/N and C/P ratios of urine-segregated sewage at different segregation percentages. COD, TN and TP concentrations in non-urine separation municipal sewage used for calculation were 600 mgCOD/L, 40 mgN/L and 6 mgP/L, respectively, represented a C/N/P of 100:7:1. COD, TN and TP loadings from urine were 16.0, 10 and 0.93 g/p/d, respectively (Larsen et al., 2013). The details of Fig. 1 were included in supplementary materials Table S1 and S2. Yellow and green regions: the nitrification-denitrification process could be obsolete; Green region: biological P removal process could be obsolete. The details of how green and yellow regions were determined can be referred to in Sections 3.2 and 3.3, respectively.

organics removal capability of STPs are reduced (Greenberg et al., 1955; Guo et al., 2014). This is valid for aerobes as Ning et al. (2000) reported a decrease in the DO uptake rate of aerobes from 20 to 10 mgO_2/L.hr under N deficiency. Nevertheless, a lower degree of nutrient deficiency (such as urine segregation of <90 %) is unlikely to induce a notable effect on the organic removal of STPs but is significant in the cases of severe N and P deficiency (> 90 % urine segregation) (Peng et al., 2003) (Table S3). For anoxic and anaerobic bacteria, while no specific study examines the effect of nutrient deficiency on them, STPs affected by nutrient deficiency contain both anoxic and aerobic phases suggesting that nutrient deficiency is likely to equally affect both anoxic and aerobic bacteria.

Both N- and P-deficiencies can affect the organic removal of STPs. Guo et al. (2014) experimented with sequencing batch reactors with either N- (C/N 60, 90 % urine segregation) or P-deficiency (C/P 333, 100 % urine segregation). They reported a 5 % decrease in organic removal with N deficiency and a 30 % decrease in organic removal with P deficiency. The outcome of this study suggested that N deficiency affects organic removal to a lesser extent compared to P deficiency. However, Peng et al. (2003) reported that N deficiency (C/N 110, 100 % urine segregation) can also reduce organic removal notably, from 90 % to 35 %. This contradicts Guo et al. (2014) observation as both studies used the same N concentration (5 mgN/L).

The organic removal capability of STPs is closely linked to the mixed liquor-suspended solids (MLSS) concentration (Martín-Pascual et al., 2015), with higher organic removal typically achieved under higher MLSS concentrations. In Peng et al. (2003), the authors observed a continuous loss of MLSS, which was not the case in the study by Guo et al. (2014). The continuous loss of MLSS under nutrient deficiency is possible due to sludge decay and disintegration at lower cell metabolism (Nguyen et al., 2022). Nutrient deficiency-induced sludge bulking can also lead to continuous MLSS loss. The loss of MLSS might be the potential reason for the deterioration in organic removal under severe N deficiency in Peng et al. (2003). However, P deficiency was shown to decrease organic removal regardless of the presence of MLSS loss.

Fig. 2 was constructed to examine the effect of N- and P-deficiencies on organic removal using available data from the literature (Table S3), which suggests that organic removal can be affected by N and P deficiencies. P deficiency appears to have a more pronounced effect, supporting the findings reported by Guo et al. (2014). Nevertheless, it is important to note that N- and P-deficiencies can occur simultaneously

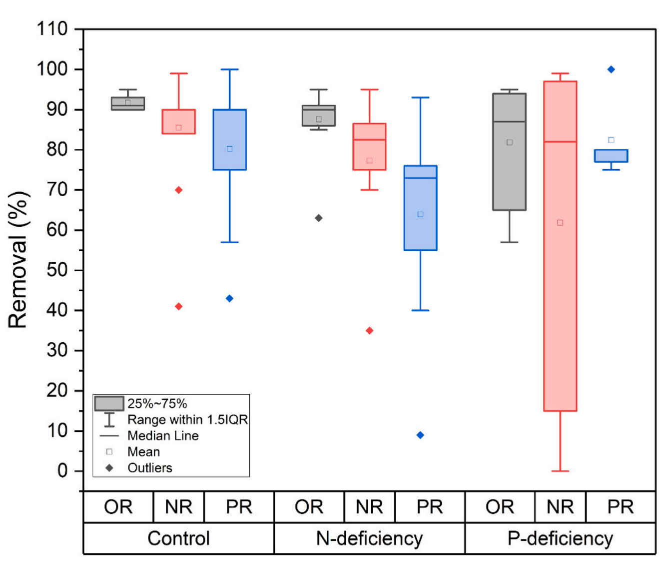


Fig. 2. Organic, N and P removals in STPs under control, N and P deficiency. OR: organic removal; NR: N removal; PR: P removal. Data used to construct this Figure was included in supplementary material Table S3.

under high urine segregation. Therefore, the compound effects illustrated in Fig. 2 should be taken into consideration. For instance, if N deficiency decreases organic removal by 10 % and P deficiency decreases it by 30 %, the latter effect would be controlling the overall impact.

Recently, Yang et al. (2022a) and Yang et al. (2022b) operated an activated sludge system with N- (C/N 50, 85 % urine segregation) and P-deficient (C/P 250, 100 % urine segregation) synthetic sewage as the feed, without any MLSS loss from the system, they observed an 85 % - 99 % organics removal efficiency during the 60 days of operation (Table S3). In the extreme scenario where either N or $P = 0$ mg/L, Gao et al. (2023) also found that microorganisms were able to survive without compromising the system's organic removal (> 90 % for all times). Similarly, no loss of MLSS was observed in these studies. These studies further support the speculation that urine segregation affects the organic removal of STPs through MLSS loss.

Yang et al. (2022a) and Yang et al. (2022b) then analysed the microbial community of activated sludge in their reactors. They showed that the population density of heterotrophs, such as Proteobacteria in activated sludge, increased from 70 % to 80 % (under N deficiency) (Yang et al., 2023) and from 5 % to 25 % (under P deficiency) (Yang et al., 2022b). At the genus level, the increase in Proteobacteria was accounted for by an increase in glycogen-accumulating organisms (GAOs) – Ca. *competibacter* and Ca. *contendobacter*, respectively. The enrichment of GAOs is possible under N- and P deficiencies due to their ability to store PHA internally (Hossain et al., 2017). GAOs are a group of bacteria that can uptake organics under all redox conditions (Hossain et al., 2017; Schroeder et al., 2008). The enrichment in GAOs might contribute to maintaining high organics removal in Yang et al. (2022a) and Yang et al. (2022b). In addition, Muszyński and Miłobędzka (2015) suggested that phosphate accumulating organisms (PAOs) can behave as GAOs under P deficiency conditions which might also aid in organics removal.

3.2. Nitrogen removal

Bacterial nitrification and denitrification are the predominant N-removal processes in STPs, resulting in dissimilation of N as N_2 gas. To a lesser extent, the assimilation of inorganic N compounds (such as ammonium (NH_4^+)) into microbial biomass (heterotrophic and autotrophic) is also involved, ca. 12 % of their biomass (Curtin et al., 2011). A higher biomass growth yield leads to a greater amount of N being assimilated by heterotrophs. The extent of N-assimilable depends on the availability of organics, which is often a limiting factor (Brown et al., 1981). Under typical sewage organic concentrations (400 mgCOD/L), approximately 20 % of the N (8 mgN/L over 40 mgN/L) can be assimilated by heterotrophs (Wang et al., 2020). At increased COD of 700 mgCOD/L, N-assimilation can increase to 55 % (Li and Irvin, 2007). For the removal of the remaining N, the presence of autotrophic nitrifiers and heterotrophic denitrifiers is necessary.

Competition for N between heterotrophs and autotrophs in STPs does occur but is uncommon because the N availability in typical sewage exceeds their total need (Jimenez et al., 2015). In fact, N is mainly removed through nitrification-denitrification in municipal sewage with a C/N ratio below 15 (Li and Irvin, 2007; Wang et al., 2020). At C/N above 20 (urine segregation of 40 %), N assimilation becomes increasingly important (Li and Irvin, 2007; We et al., 2022). Modelling works have suggested that urine segregation of >75 % results in most N being removed by assimilation by heterotrophs resulting in effluent NO_3^- concentrations decreasing to a negligible level (Badeti et al., 2021, 2022; Jimenez et al., 2015; Wilsenach and van Loosdrecht, 2003, 2004). The experimental findings of Yin et al. (2019) using aerobic granular system and high-strength wastewater support this observation. They observed the presence of NH_4^+, NO_2^- and NO_3^- in the effluent when the system operated with a C/N ratio of 20 (COD = 2000 mg/L, NH_4^+ = 100 mgN/L) but none of these were detectable when the C/N ratio increased to 50, even the NH_4^+ concentration was as high as 40 mgN/L.

The population of ammonia oxidising bacteria (AOB) and nitrite oxidising bacteria (NOB) also decreased with an increase in urine segregation due to the lack of NH_4^+ and NO_2^- as substrates. At low urine segregation (10 %), the concentration of AOB and NOB started to decrease (Jimenez et al., 2015). At C/N 25–30 (50–70 % urine segregation), the growth of AOB and NOB was suppressed and the growth of heterotrophs was promoted (Kocaturk and Erguder, 2016). At C/N ratios exceeding 60 (90 % urine segregation), the relative abundances of AOB and NOB were reduced to below the detection limit and the efficiency of nitrification decreased rapidly, proven theoretically (Jimenez et al., 2015) and experimentally (Yang et al., 2023).

Unlike AOB and NOB, the population of ammonia-oxidising archaea (AOA) in STPs could increase under urine segregation, as AOA are capable of surviving under low NH_4^+ conditions (as low as 0.18 μg/L) and in non-AOB dominant conditions (Martens-Habbena et al., 2009; Zheng et al., 2021). With a comparable NH_4^+ oxidising rate to AOB (Wright and Lehtovirta-Morley, 2023), AOA could replace the role played by AOB in STPs during urine segregation. The same goes for comammox (e.g., *Nitrospira*), which can survive and oxidize NH_4^+ and NO_2^- to NO_3^- under low substrate conditions (as low as 0.3 mg/L) (Spasov et al., 2020). This makes comammox a potential candidate to replace AOB and NOB under urine segregation scenarios in STPs. Besides, heterotrophic nitrifiers could also survive under low NH_4^+ conditions due to the ability to use simple organic-N (e.g., amino acid) to grow and produce NO_2^- to NO_3^- through secondary metabolism (Martikainen, 2022). The potential benefit of the presence of these microorganisms in STPs is that they enable the oxidation of remaining influent NH_4^+ to NO_3^- after N-assimilation by heterotrophs under urine segregation.

Denitrification reduces NO_2^- and NO_3^- to N_2 gas with organic carbon as the electron donor. Complete denitrification (>95 %) requires high organic availability (e.g., $C/N > 25$) (Badia et al., 2019). As such, urine segregation above 60 % is sufficient to achieve complete denitrification, according to Fig. 1. For STPs that need additional carbon for complete denitrification, 60 % urine segregation could be the threshold that no external carbon is needed. At urine segregation higher than 80 %, most N can be assimilated by heterotrophs (Badeti et al., 2021; Li and Irvin, 2007) and the NO_3^- produced from oxidation of remaining NH_4^+ by AOA and comammox is insignificant (Badeti et al., 2021; Wang et al., 2020) and could meet the discharge standard in most countries (ARMCANZ and ANZECC, 1997; E.U·W, 1991).

Overall, nitrification-denitrification process could be obsolete at the point where urine segregation produces sewage with NH_4^+ concentration of 6–8 mgN/L. Taking 30 mgN/L NH_4^+ without urine segregation as example, an 80–90 % urine segregation is sufficient (Table S2) and could referred to as the general threshold level for the obsolescence of the nitrification-denitrification process for STPs. This threshold can be increased or decreased depending on influent N concentration.

3.3. Phosphorus removal

Phosphorus can be removed through chemical precipitation or biological P removal by PAOs in activated sludge. A small portion of P (10–13 %) can also be assimilated by microorganisms for growth and reproduction (Brown et al., 2022; Szeląg et al., 2021). P removal through chemical precipitation is not within the scope of this review and therefore not included. To allow for biological P removal, the presence of enriched populations of PAOs is necessary. At typical sewage C/P of <50 the relative abundance of PAOs is around 3–5 % in activated sludge, and is sufficient to remove >80 % P in the activated sludge system (Qiu et al., 2019; Yang et al., 2023). Common PAOs in municipal STPs include *Candidatus accumulibacter, Tetrasphaera, Dechloromonas,* and *Defluviicoccus* (Qiu et al., 2019).

In urine-segregated sewage, P concentration decreases while the organic carbon concentration remains high, which increases the C/P ratio of sewage. The high C/P ratio can decrease the relative abundance of PAOs in sludge (Yang et al., 2023). Qiu et al. (2019) examined the relative abundance of PAOs (mainly *Candidatus accumulibacter* and *Tetrasphaera*) in STPs that handled sewage with different C/P ratios and showed STPs that handled sewage with C/P 55 have PAOs abundance between 2.8 % – 4.4 % (no urine segregation scenario) and 1.2–1.8 % in STPs that handle sewage with C/P of 76 (20 % urine segregation). This suggested that the PAOs population can be reduced at the early stage of urine segregation. In addition, GAOs – the competitor of PAOs – can be enriched (Chu et al., 2021; Law et al., 2016; Yang et al., 2023) when C/P is above 50. Enriched GAOs often deteriorate the biological P removal process in the STPs (Law et al., 2016). The combined effects of reduced P and enriched GAOs could be the reasons for the notable decrease in the PAOs population at the early stage of urine segregation.

At C/P 130 (urine segregation ~60 %), Muszyński and Miłobędzka (2015) reported that the population of PAOs decreased by 76 % in an aerobic granule system. At C/P 200 ($P = 2$ mg/L) (~100 % urine segregation), Zeng et al. (2003) reported that PAOs were outcompeted by GAOs and the population of PAOs became negligible due to the unsupportive P concentration. This was also observed in a modelling study using the Activated sludge Model (Jimenez et al., 2015). At this point, the biological P removal process could become obsolete due to the minimum PAOs population, and the remaining P can be removed through P-assimilation. Nevertheless, this highly depends on the influent P concentration and full urine segregation is mostly required for the obsolescence of biological P removal process. For high-strength sewage that has high P even after full urine segregation (e.g., > 2mgP/L), the biological P removal process could remain as PAOs are still thriving.

3.4. Crossover-impacts of nitrogen and phosphorus deficiencies

Nitrogen and P deficiencies can have crossover impacts on the P and N removal process in STPs, respectively. These include impacts on the main functional bacteria that carry out N and P removal in STPs (Fig. 3).

Polyphosphate-accumulating organisms can use DO as an electron acceptor under aerobic conditions, and NO_2^- and NO_3^- under anoxic conditions, to oxidize PHA, replenish glycogen, and gain energy for cell growth (Guo et al., 2018; Zhao et al., 2023). Therefore, N deficiency is not likely to affect PAOs significantly, as although NO_2^- and NO_3^- may be limited, DO is generally not limited in most STPs. However, many studies suggested the opposite. For example, under constant MLSS concentration (to exclude the effect of P assimilation) and grow-supportive P concentration, Guo et al. (2014) demonstrated that N-free conditions deteriorated P removal in an activated sludge system from 57 % to 3 %. Yang et al. (2023) also indicated that the relative abundance of PAOs decreased from 1.5 % to 0.2 % under N deficiency, suggesting that N deficiency can suppress the growth of PAOs. This could also be observed from Fig. 2 that N deficiency reduced P removal notably. The reasonable explanation for this could be the lack of survival N requirements, thus unable to undergo cell synthesis.

The growth of biomass requires both N and P. When P concentrations are low, the demand for N is reduced, which in turn decreases the sludge's potential to assimilate N. However, under full urine segregation, P concentration is not likely to affect the growth and function of nitrifiers, as 0.05 mgP/L and 0.2 mgP/L are required for AOB and NOB, respectively (Nowak et al., 1996; Zhang et al., 2023). Given that P concentrations under full urine segregation are around 2.9 mgP/L (Table S2), P does not appear to be a limiting factor for nitrification in this scenario.

4. Effect of nutrient deficiency on sludge

Hitherto, no studies have specifically examined the effect of urine source segregation on the characteristics of activated sludge. Modelling studies that predict the impact of urine segregation were unable to reveal the changes on sludge with software solely. However, it is

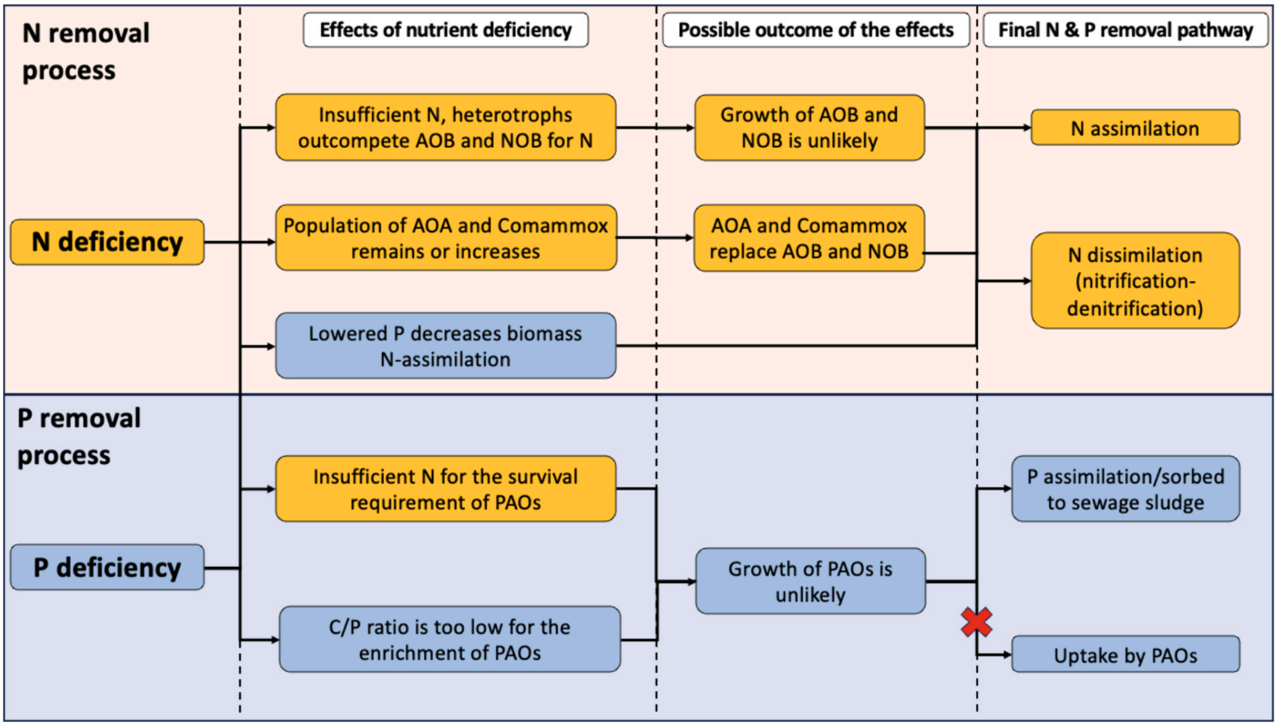


Fig. 3. Effect of N and P deficiencies on biological N and P removal. (Orange: impacts caused by N deficiency; blue: impacts caused by P deficiency). AOB: ammonium-oxidising bacteria. AOA: ammonia-oxidising archaea, NOB: nitrite-oxidising bacteria. PAOs: polyphosphate-accumulating organisms.

possible to assemble insights into the impacts of N and P deficiency on sludge based on the results from studies that involved different C/N/P ratios. In the following discussion, the term nutrient deficiency (or N and P deficiency) refers to urine segregation of 100 % (unless otherwise specified) as most of the referenced studies experimented using C/N and C/P higher than 100 and 200, respectively.

While referring to the following section, it should be noted that urine segregation induces both N and P deficiencies simultaneously. Therefore, if N deficiency, for example, induces effect A while P deficiency induces effect B, A and B are likely to appear simultaneously.

4.1. *Effects of nutrient deficiency on extracellular polymeric substance*

4.1.1. *Total extracellular polymeric substance*

An adequate concentration (~15 to 150 mg/g suspend solids (SS)) of EPS is beneficial for sludge flocculation and sedimentation (Hoa et al., 2003; Shin et al., 2000) (Tables 2 & 3). Excessive production of EPS, can, however, become a barrier to sludge flocculation through increased electrostatic repulsion and decreased hydrophobic interaction (Liu et al., 2010; Melo et al., 2022), hence reducing sludge settleability (Durmaz and Sanin, 2003). EPS is produced and secreted during microbial metabolism. Slowing the metabolism of microorganisms can therefore affect EPS production and subsequent EPS concentrations in the sludge (Yang et al., 2022a; Yang et al., 2022b).

Under nutrient deficiency, the metabolism of microorganisms is lowered due to the lack of adequate nutrients for growth. This results in a decrease in EPS secretion and sludge EPS concentration. According to Guo et al. (2014), activated sludge grown in synthetic sewage with C/N/P of 100:10:5 have EPS concentration between 120 and 160 mg/g volatile suspended solids (VSS) while sludge grown with C/N 60 or C/P 100 (90 % or 55 % urine segregation) have EPS concentrations between 75 and 100 and 60–105 mg/gVSS, respectively. Hoa et al. (2003) also suggested that activated sludge grown with typical sewage (C/N/P: 100:5:1–100:7:1) had a total EPS range between 77 and 84 mg/gSS, whereas sludge grown with N- (C/N 33–100, urine segregation >65 %) and P-deficient sewages (C/*P* > 200, urine segregation >100 %) had a total EPS decreased to 70–76 mg/gSS, and 55–69 mg/gSS, respectively. Sludge grown under both N and P deficiencies had the lowest total EPS (38 mg/gSS). One could expect that the total EPS of activated sludge can decrease by 6–30 % after urine segregation.

However, there were conflicting reports, such as Ye et al. (2011), suggesting that N and P-deficiencies have a negligible effect on total EPS production. Further, Geyik and Çeçen, (2016), Durmaz and Sanin (2003) and Peng et al. (2012) suggest that nutrient deficiency can even increase EPS production (Tables 2 & 3).

4.1.2. *Protein and polysaccharide extracellular polymeric substance*

Extracellular polymeric substance in sewage sludge is composed of proteins, polysaccharides, nucleic acids, glycolipids, and phospholipids (Czaczyk and Myszka, 2007; Di Martino, 2018), with proteins (PN-EPS), polysaccharides (PS-EPS) and humic acid being the principal components of sewage sludge EPS (Ye et al., 2011). In this review, only PN- and PS-EPS were discussed as most studies were focused only on these components.

Protein-EPS is highly hydrophobic, owing to its hydrophobic side groups (Liao et al., 2001; Urbain et al., 1993). Increases in PN-EPS increase sludge floc hydrophobicity and hydrophobic interaction between microorganisms, substrate, and sludge through hydrogen bonding (Guo et al., 2016; Yang et al., 2022a). PS-EPS are hydrophilic and negatively charged polymers due to the presence of carboxyl and *o*-acetylated groups (Durmaz and Sanin, 2003; Yang et al., 2022b). PS-EPS can also act as the 'backbone' for sludge flocculation. Hence, an adequate PS-EPS concentration (typically <25 mg/g SS) (Hoa et al., 2003) is beneficial for sludge flocculation. Presence excessively could reduce sludge hydrophobicity and increase the surface negativity of sludge, which demotes sludge aggregation (Durmaz and Sanin, 2003).

When nutrients are at typical levels, PN-EPS is often the main EPS component (relative to PS-EPS) in activated sludge, about 1.5–5 times higher in concentration than PS-EPS (Tables 2 & 3). A PN/PS-EPS ratio of 2–3 appears to benefit sludge settleability and flocculability (Durmaz and Sanin, 2003; Guo et al., 2014; Shin et al., 2000), and a ratio below this could lead to floc destabilisation due to excessive PS-EPS (Shin et al.,

Table 2
Physiochemical characteristics and the EPS concentration of sludge under N deficiency.

Imitated urine segregation percentage	C/N	Physiochemical characteristics of sludge/floc: Parameters	Before/control	After	Changes	EPS (mg/g SS): Parameters	Before/control	After	References
	∞	SVI (mL/g): Filamentous bulking: Viscous bulking:	40–125 Present, minor Absent	40–130 Present, minor Absent	☒ = =	Total EPS: PN-EPS: PS-EPS: PN/PS:	126.9 104.1 18.2 5.7	99.3 75 19.3 3.9	Guo et al. (2014)
	∞	SVI (mL/g): Sludge size (μm): Others: • Hydrophobicity • Surface negativity (Zeta Potential)	40 157 57 % −12 mV	55 287 30 % −14 mV	☒ ☑ ☒ ☒	PN-EPS: PS-EPS: PN/PS:	50 15 2.6	48 30 1.5	Yang et al. (2022a)
	∞	SVI (mL/g): Filamentous bulking:	30–40 Absent	40–70 Present	☒ ☒	PN-EPS: PS-EPS: PN/PS:	50 20 2.5	45 35 1.3	Yang et al. (2023)
	460	SVI (mL/g): Filamentous bulking:	75 Present, minor	192.3 Present, major	☒ ☒	Total EPS:	12.9	52.9	Hoa et al. (2003)
~100 %	455	SVI (mL/g): Filamentous bulking: Sludge form:	95–120 Absent Floc	400–730 Present, major Disperse	☒ ☒ ☒	N/A			Amanatidou et al. (2016)
	158	Others: • Surface negativity	−0.26 meq/ gVSS	−0.23 meq/ gVSS	☑	PN-EPS: PS-EPS: PN/PS:	50 40 1.25	40 25 1.6	Zhu et al. (2015)
	106	SVI (mL/g): Viscous bulking: Sludge form:	90 Absent Floc	458 Present, major Disperse	☒ ☒ ☒	N/A			Peng et al. (2003)
	100	SVI (mL/g): Floc size (μm): Sludge form:	65 190 Floc	160 70 Disperse	☒ ☒ =	Total EPS: PN-EPS: PS-EPS: PN/PS: TB-EPS: LB-EPS:	140 160 39 4.1 130 6	140 143 41 3.5 130 6	Ye et al. (2011)
	100	SVI (mL/g):	69.8	115.8	☒	Total EPS: PN-EPS: PS-EPS: PN/PS:	84.4 6.4 33.1 0.19	70.2 4.6 37.9 0.12	Hoa et al. (2003)
95 %	75	SVI (mL/g): Floc size (μm):	61.3 63	64.2 98	☒ ☑	Total EPS: PN-EPS: PS-EPS: PN/PS: TB-EPS: LB-EPS:	13.5 14.8 5.4 2.8 11.2 2.3	18.1 14.1 2.9 4.8 17.1 1	Peng et al. (2012)
90 %	60	SVI (mL/g): Filamentous bulking: Sludge form:	108 Present, minor Floc	62 Present, minor Floc	☑ = =	Total EPS: PN-EPS: PS-EPS: PN/PS:	126.9 104.1 18.2 5.7	93.7 74.9 15.7 4.8	Guo et al. (2014)
85 %	50	SVI (mL/g): Floc size (μm): Other: • Hydrophobicity • Surface negativity (Zeta Potential)	30–35 157.2 57 % −12 mV	30 290.4 52 % −9 mV	= ☑ ☒ ☑	PN-EPS: PS-EPS: PN/PS:	50 15 3.3	90 40 2.3	Yang et al. (2022a)
85 %	50	SVI (mL/g): Floc size (μm):	18 475	23 550	☒ ☑	PN-EPS: PS-EPS: PN/PS: TB-EPS: LB-EPS:	14 7 2 17.5 3.5	17 16 1 28 5.5	Yin et al. (2019)
85 %	43	SVI (mL/g): Filamentous bulking: Viscous bulking: Sludge form: Other: • Hydrophobicity • Surface negativity	64 Absent Absent Floc 75 % −0.2 meq/ gVSS	133 Absent Present Disperse 65 % −0.7 meq/ gVSS	☒ = ☒ ☒ ☒ ☒	Total EPS: PN/PS:	47 1.2	60 0.4	Durmaz and Sanin (2003)
70 %	32	Others: • Surface negativity	−0.26 meq/ gVSS	−0.24 meq/ gVSS	☑	PN-EPS: PS-EPS: PN/PS:	50 40 1.25	45 30 1.5	Zhu et al. (2015)
70 %	30	SVI (mL/g): Floc size (μm): Filamentous bulking:	58 2500 Absent	33 2400	☑ ☒ ☒	PN-EPS: PS-EPS: PN/PS:	168 106 1.58	212 99 2.14	Kocaturk and Erguder (2016)[1]

(*continued on next page*)

Table 2 (*continued*)

Imitated urine segregation percentage	C/N	Physiochemical characteristics of sludge/floc				EPS (mg/g SS)			References
		Parameters	Before/ control	After	Changes	Parameters	Before/ control	After	
40 %	20	SVI (mL/g): Floc size (μm): Filamentous bulking:	58 2500 Absent	Filamentous growth is present, but no bulking 30 1500 Absent	☑ ☒ =	PN-EPS: PS-EPS: PN/PS:	168 106 1.58	235 96 2.45	Kocaturk and Erguder (2016)[1]

[1] Study conducted with aerobic granule system. SVI: Sludge volume index; *Total EPS: data from the sum of polysaccharide and protein extracellular polymeric substances (PS- and PN-EPS). ☑: indicates beneficial changes (e.g., better flocculation, sedimentation or increased floc size); ☒: indicates undesirable changes (e.g., worsened flocculation, sedimentation or decreased hydrophobicity); =: indicates neutral changes/unchanged.

Table 3
Physiochemical characteristics and the EPS concentration of sludge under P deficiency.

Imitated urine segregation percentage	C/P	Physiochemical characteristics of sludge/floc				EPS (mg/g SS)			Reference
		Parameters	Before/ control	After	Changes	Parameters	Before	After	
~100 %	∞	SVI (mL/g): Filamentous bulking: Sludge form:	108 Present, minor Floc	153 Present, major Disperse	☒ ☒ ☒	Total EPS PN-EPS PS-EPS PN/PS	126.9 104.1 18.2 5.7	84.6 64 17 3.8	Guo et al. (2014)
	∞	SVI (mL/g):	35	70	☒	PN-EPS PS-EPS PN/PS	50 18 2.7	40 40 1	Yang et al. (2023)
	∞	SVI (mL/g): Sludge size (μm): Sludge form: Other: • Hydrophobicity • Surface negativity (zeta potential)	43 178 Floc 46 % −10 mV	39 160 Floc 32 % −16 mV	☑ ☒ = ☒ ☒	PN-EPS PS-EPS PN/PS	35 13 2.7	30 25 1.2	Yang et al. (2022b)
	600	SVI (mL/g):	155	180	☒	PN-EPS PS-EPS PN/PS	48 18 2.67	27 25 1.08	Sponza (2002)
	300	SVI (mL/g): Filamentous bulking: Sludge form:	108 Present, minor Floc	80 Absent Floc	☑ ☑ =	Total EPS PN-EPS PS-EPS PN/PS	126.9 104.1 18.2 5.7	91.4 61.8 25.8 2.39	Guo et al. (2014)
	200	SVI (mL/g):	69.8	66.2	☑	Total EPS: PN-EPS: PS-EPS: PN/PS:	84.4 6.4 33.1 0.19	54.9 5.48 32.3 0.17	Hoa et al. (2003)

SVI: Sludge volume index; *Total EPS: data from the sum of polysaccharide and protein extracellular polymeric substances (PS- and PN-EPS). ☑: indicates beneficial changes (e.g., better flocculation, sedimentation or increased floc size); ☒: indicates undesirable changes (e.g., worsened flocculation, sedimentation or decreased hydrophobicity); =: indicates neutral changes/unchanged.

2000; Yang et al., 2023).

Under N deficiency where N availability becomes unsupportive for growth, microorganisms tend to: (1) utilise the N contained in PN-EPS through endogenous respiration, which reduces the concentration of PN-EPS (Yang et al., 2022a); (2) reduce the secretion of PN-EPS to conserve energy (Yang et al., 2022a; Ye et al., 2011); (3) store harvested organics in the form of PS-EPS because of the 'substrate storage' function of PS-EPS for future use (Yang et al., 2022b) and (4) increase the PS-EPS production to compensate for the reduced PN-EPS secretion (Depraetere et al., 2015) to maintain the sludge stability because the energy required for glycogen synthesis is lower than for protein synthesis (Yang et al., 2022a).

Under P deficiency, the production of PN-EPS remains unaffected in most cases since N is still available (Guo et al., 2014; Yang et al., 2022b). However, the production of PS-EPS exhibits an increasing trend, possibly because: (1) P deficiency triggers the overexpression of the *AfeI* gene and increases the production of acyl-homo-serine lactone (AHL) (a molecule related to quorum sensing in bacterial cells). Increased concentration of AHL triggers the over-production of PS-EPS (Farah et al., 2005), and (2) the harvested organics are preferentially used for PS-EPS secretion by microorganisms (for substrate storage purposes) instead of for growth, as P is limiting cellular reproduction (Yang et al., 2022b).

Overall, activated sludge under N deficiency tends to have PN-EPS concentration decrease and PS-EPS concentration increase, while activated sludge under P deficiency increases its PS-EPS and has PN-EPS remains unchanged. These ultimately decrease the PN/PS-EPS ratio which could end up affecting the sludge flocculability and settleability (Yang et al., 2022a; Yang et al., 2022b). Interestingly, nutrient deficiency tends to increase or decrease a particular type of EPS instead of all types of EPS in sludge, resulting in a relatively stable total EPS concentration of sludge (Tables 2 & 3). This helps explain why multiple studies suggested that total EPS remains unchanged under nutrient deficiency, which has been discussed in Section 4.1.1.

4.1.3. *Loosely and tightly bound extracellular polymeric substances*

In activated sludge, approximately 60 % of EPS in sludge are TB-EPS and 30 % are LB-EPS, with 10 % as humic substances (Guo et al., 2016). TB-EPS is relatively stable and aids in maintaining the structure of sludge while LB-EPS acts as the primary surface for cell attachment and flocculation (Li and Yang, 2007). The concentration of LB-EPS in sludge can change according to the surrounding environment but not the case for TB-EPS (Li and Yang, 2007). As such, nutrient deficiency could mainly impact LB-EPS instead of TB-EPS (Ye et al., 2011).

This could be observed in Ye et al. (2011) in which LB-EPS of sludge increased while TB-EPS showed negligible changes after exposed to N-deficient sewage. A similar investigation was also carried out by Gao et al. (2023) to examine the impact of N- and P-free sewage on an activated sludge. They found that the concentration of LB-EPS increased by 41 % and 32 %, respectively after 49 days in an N and P-free environment. Peng and colleagues also suggested that N deficiency decreased LB-EPS by 30 % and TB-EPS by 2.3 % (Peng et al., 2012). Based on these outcomes, it can be concluded that nutrient deficiency is likely to alter the concentration of LB-EPS in sludge. However, whether nutrient deficiency results in an overall increase or decrease of LB-EPS is still unclear.

4.2. Effects of nutrient deficiency on the physical properties and morphology of sludge

4.2.1. Sludge flocculation

Sludge flocculation is critical for settling and clarification. According to the extended Derjaguin Landau Verwey Overbeek (XDLVO) theory, interactive forces such as attractive Lifshitz van der Waals force, repulsive or attractive electrostatic double-layer force, and Lewis acid-base interactions (hydrophobic interaction) (van Oss, 2003) are involved in the sludge flocculation processes (Bayoudh et al., 2009). Among these, hydrophobic and electrostatic interactions are the main forces that govern sludge flocculation (Yang et al., 2022b).

As discussed in Section 4.1.2, nutrient availability can decrease the PN/PS-EPS ratio of sludge. This results in a decrease in sludge hydrophobicity and increases electrostatic repulsion between sludge, rendering sludge flocculation difficult. This observation was frequently reported by previous studies. According to Ye et al. (2011), Peng et al. (2003), and Durmaz and Sanin (2003), sludge tends to grow in a dispersed form rather than as flocs under nutrient deficiency. Yang et al. (2022b) also showed the sludge aggregation capability decreased from 62 % (no nutrient deficiency) to 48 % and 38 % under N and P deficiency, respectively. The authors explain that this is because nutrient deficiency increases the activation energy for sludge flocculation.

In addition, the growth of EPS-producing microorganisms that are beneficial for sludge flocculation like *Defluviicoccus, Phaeodactylibacter,* and *Flavobacterium* can be suppressed by P deficiency (Pishgar et al., 2019; Wang et al., 2021; Yang et al., 2022b), whilst microorganisms (e.g., *Chloroflexi*) that can disrupt sludge flocculation through degrading sludge EPS were shown enriched under deficiency (Speirs et al., 2019; Yang et al., 2023), results in further decrease in sludge flocculability.

4.2.2. Sludge floc size, density and settleability

When nutrients are unsupportive for the metabolism of microorganisms, cell autolysis and endogenous decay occur (Nguyen et al., 2022) and result in sludge flocs disintegration. Sludge decay and disintegration can be more severe as one goes deeper into the sludge flocs, since nutrient diffusion into the core of the floc is very limited, especially under nutrient deficiency (Schmidt and Ahring, 1996). Combined with deteriorated sludge flocculation, large floc is less likely to be formed under nutrient deficiency (Yang et al., 2022b; Ye et al., 2011).

Several studies have suggested that sludge flocs can become larger under nutrient deficiency (Tables 2 & 3). Larger sludge flocs under nutrient deficiency might be explained by excessive filamentous bacteria proliferation (the details of filamentous bacteria proliferation due to nutrient deficiency will be discussed in the following section), in which filaments extend from floc into the bulk liquid, increasing the overall size of sludge floc.

On the other hand, cell decay and filamentous proliferation can increase the porosity of the sludge flocs, reducing their density. Schmidt and Ahring (1996) explained that cell decay caused by limited nutrient diffusion leads to the formation of hollow and loosely structured sludge flocs that contain a high fraction of ‘void space’, which reduces their density. For filamentous bacteria proliferation, studies have suggested that the sludge usually exhibits higher porosity compared to non-filamentous bacteria-proliferated sludge (Banti et al., 2017, 2020; Nguyen et al., 2022), due to the formation of pores associated with the extension of filaments (Martins et al., 2003). Lee (1972) and Yang et al. (2022b) also demonstrated that both N and P deficiencies can result in low-density loose sludge, with long filaments extending from it. In addition, PAOs were also shown helpful in forming dense and stronger flocs due to their slow-growing characteristic. Their population reduction under P deficiency could decrease floc density and stability (Larsen et al., 2006).

Overall, nutrient deficiency tends to promote sludge characteristics that are not conducive to good settleability, such as reduced flocculation, decreased floc density and increased porosity, filamentous bacteria proliferation, and excessive growth of PS-EPS. The combination of these effects could result in poor sludge settleability and compressibility in STPs. This can be observed from Tables 2 & 3 that most studies showed that N and P deficiencies led to an increase in sludge volume index (SVI). Poor sludge settleability can lead to a notable loss of MLSS. This reduces bacterial diversity and richness of functional bacteria (Yang et al., 2023). This might not be a concern since functional bacteria such as AOB, NOB and PAOs are likely unsustainable due to the lack of substrate. However, this could affect organic removal, as MLSS is closely related to organic removal in STPs, as discussed in Section 3.1.

4.2.3. Sludge bulking

Sludge bulking can be separated into two categories: filamentous bulking and non-filamentous bulking (also known as viscous bulking). Both filamentous and viscous bulking can be triggered by nutrient deficiency (Durmaz and Sanin, 2003; Li et al., 2014).

4.2.3.1. Filamentous bulking. The presence of filamentous bulking during nutrient deficiency can be explained by combining several theories that are related to nutrient availability such as kinetic selection (Chudoba et al., 1973), diffusion selection (Martins et al., 2003) and quorum sensing (QS) (Shi et al., 2022) theories.

Kinetic selection theory suggests that floc-formers have lower substrate affinity (higher K_s values) and specific growth rates but higher maximum specific growth rates (μ_{max}) than filamentous bacteria (Chudoba et al., 1973). Diffusion selection theory proposes that filaments have the following advantages: (1) larger surface-to-volume ratio than floc-formers (thus higher substrate uptake rate) and (2) higher outward growth velocity because floc-formers usually grow in three dimensions (or directions) while filaments grow only in two dimensions (Martins et al., 2003; Pipes, 1968; van Loosdrecht et al., 1995). QS theory proposes that filaments are capable of releasing acyl-homo-serine lactone (AHL) and the concentration of AHL increases with their population density. When population density increases (due to environmental factors such as low DO and nutrient deficiency), the AHL concentration will increase and accelerate filamentous bacteria proliferation (Shi et al., 2022).

By integrating these theories, filamentous proliferation during nutrient deficiency can be explained as follows. At high nutrient levels, the nutrient diffusion rate from the external to the inner part of the sludge floc is high due to the high nutrient gradient (Guo et al., 2014). The nutrient levels in the inner part of the sludge floc are supportive of the growth of both floc-formers and filaments. Given that nutrients are not the limiting factor, the μ_{max} of floc-formers (higher) and filaments (lower) becomes the limiting factor, and this provides a competitive advantage to floc-formers. The low population density of filamentous bacteria produces an insufficient concentration of AHL to trigger the ‘acceleration proliferation’ mode of filaments. In addition, a high nutrient level throughout the sludge floc demotivates the filaments from protruding from the core of the sludge floc. At sufficiently high nutrient levels, filamentous bulking is unlikely (stage 1 in Fig. 4).

Under nutrient deficiency, the nutrient levels in the bulk solution decrease. This reduces the nutrient gradient between the inner and external parts of the sludge floc, reducing the nutrient diffusion rate and the concentration of nutrients in the deeper part of the floc. Once the nutrients become limiting inside the sludge floc (stage 2 in Fig. 4), the maximum growth rate of floc-formers and filaments is no longer the limiting factor, but their respective substrate affinity and specific growth rate are. Since the nutrient affinity rate of filaments is higher than floc-formers due to the larger surface-to-volume ratio, as well as the higher specific growth rate of filaments at the low nutrient concentration, they outcompete the floc-formers.

Meanwhile, both types of microorganisms are forced to grow outward to compete for the substrate in bulk solution. However, because of the higher outward growth velocity, filaments are likely to outcompete floc-formers and protrude from flocs. Here, it can be speculated that nutrient uptake by protruded filaments would further decrease nutrient availability near the floc particle, leading to an even lower nutrient level that can diffuse into the floc. At this stage, the population density of filaments could approach the threshold level at which the released AHL concentration is sufficient to up-regulate the gene related to 'accelerated proliferation mode' (Shi et al., 2022), and thus filamentous density increases more rapidly (stage 3).

Nevertheless, it should be noted that most of these theories were previously examined with respect to organic substrates (sucrose, glucose, and long-chain volatile acid), instead of nutrients. Whether filamentous growth triggered by nutrient deficiency follows the same mechanisms suggested above needs to be further verified because filamentous proliferation during nutrient deficiency has not been universally observed. For example, Guo et al. (2014) showed that only P-free conditions can induce filamentous growth, while neither N-free, N-deficient nor P-deficient (C/N 60 & C/P 300, urine segregation 100 %) could. On the contrary, Yang et al. (2023) suggested that only an N-free medium could induce filamentous growth, with no filamentous proliferation observed under P-free, N-deficient, or P-deficient conditions. More results on the filamentous growth under different C/N/P can be found in Tables 2 and 3.

4.2.3.2. Viscous bulking. The occurrence of viscous bulking is related to the excessive secretion of PS-EPS under sub-optimal conditions (Bakos et al., 2022; Mesquita et al., 2011). Under typical conditions, the produced insoluble PS-EPS can encapsulate cells and prevent dispersed growth. During nutrient deficiency, PS-EPS concentration can be increased. This is because low nutrient availability triggers the release of AHL which mediates the over-expression of the *pep* gene that is responsible for PS-EPS production (Shi et al., 2022). When PS-EPS is present in excess, the viscosity of sludge increases remarkably, and the compaction between cells is hindered (Bakos et al., 2022), decreasing sludge flocculation. The hydrodynamic resistance also increases, which decreases sludge settleability (Burger et al., 2017) and causes viscous bulking.

5. Effects of nutrient deficiency on greenhouse gas emissions

The sewage treatment process emits considerable amounts of greenhouse gases (GHGs) (Bao et al., 2016). About 5 % of the global total nitrous oxide (N_2O) (Tian et al., 2020) and 5–8 % methane (CH_4) (Ocko et al., 2021) are released from the wastewater sector. Both GHGs are major contributors to global warming (IPCC, 2006). The CO_2 emitted from STPs is regarded as a biogenic carbon emission, which will be balanced by carbon uptake by plants through photosynthesis before harvest and therefore is excluded from the GHG inventory (IPCC, 2006). As such, most studies have focused on the emission of N_2O and CH_4 only (Bao et al., 2016; Foley et al., 2010; Law et al., 2012). For this reason, the

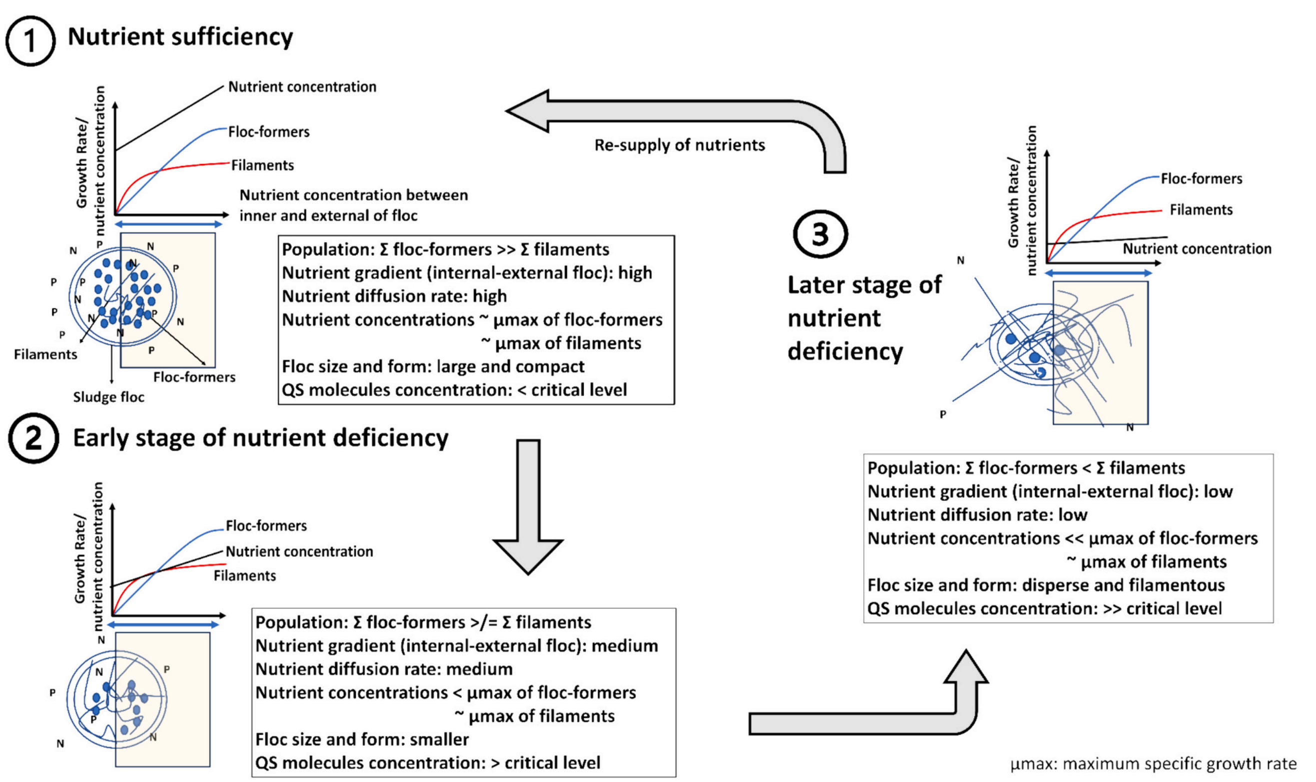


Fig. 4. Mechanism inducement of filamentous bacteria proliferation in flocs at different stages of nutrient deficiency based on kinetic selection, diffusion selection, and quorum sensing theories. Graphs in each stage compare the specific growth rate of floc forming and filamentous bacteria to nutrient concentration profiles within the floc.

emission of CO_2 is not considered in the following discussion.

5.1. Nitrous oxide

Nitrous oxide can be formed during nitrification and denitrification processes. In nitrification, nitrous oxide is a by-product generated by AOB during the oxidation of hydroxylamine (NH_2OH) and via nitrifier denitrification processes. In denitrification, it is an obligate intermediate from nitric oxide reduction by heterotrophic denitrifiers (Duan et al., 2021). Due to the multiple production pathways, N_2O generation and emission in wastewater treatment are regulated by many operational parameters. For example, a suboptimal DO level, e.g., below 1.5 mg/L or above 3.5 mg/L, promotes the formation of N_2O through nitrifier denitrification (Soler-Jofra et al., 2021), or hydroxylamine oxidation (Caranto et al., 2016), respectively. The presence of high NO_2^- concentration (>5 mg N/L), or high NH_4^+ (>10 mgN/L) will promote N_2O generation via nitrifier denitrification pathway (Zhao et al., 2023), or NH_2OH oxidation pathway (Duan et al., 2021), respectively.

Denitrification can also be a notable source of N_2O emissions in STPs (Gruber et al., 2022), particularly when denitrification is interrupted by the depleted organic carbon (Law et al., 2012), or when N_2O reductase is inhibited, by e.g., DO shock (Otte et al., 1996), sudden pH decrease (Pan et al., 2012) or low copper availability (Paraskevopoulos et al., 2006).

The effect of nutrient deficiency on N_2O emission has not yet been experimentally assessed. The only relevant study was Badeti et al. (2021), in which the authors examined the impact of urine segregation on N_2O emission via BioWin simulation software. The software predicted that 0.16 kg N_2O would be released per day without urine segregation. With 90 % urine segregation, N_2O emission was reduced by 98 % to 0.003 kg N_2O/day. A life cycle assessment conducted by Hilton et al. (2021) also suggested that 0.003 kg N_2O/person/year could be avoided with 70 % urine segregation. While these studies agreed that urine segregation can reduce N_2O emission, the modelling study could not provide any insight that could be used to justify the biochemical process that lead to the reduction of N_2O emission.

Nevertheless, the effects of N deficiency on N_2O emissions in STPs can be evaluated based on the available evidence, summarised in Table 4, as follows: (1) N_2O emissions can be reduced due to the strong correlation of N_2O emissions with N influent loading, where higher N influent loading result in increased N_2O emissions and vice versa (IPCC et al., 2019); (2) most of the N is removed through assimilation rather than dissimilation pathways that lead to N_2O generations (i.e., nitrification and denitrification); (3) minimal nitrification results in minimal NO_2^- production and reducing the chance of N_2O generation via nitrifier denitrification (Guo et al., 2017); (4) N deficiency decreases the bulk NH_4^+ concentration which in turn decreases the likelihood of high NH_4^+-induced N_2O generation from the hydroxylamine oxidation pathway; (5) a higher C/N ratio due to urine segregation will likely support a complete denitrification process, which is a sink for N_2O generated from wastewater treatment; These effects would be able to markedly mitigate N_2O emissions from STPs. Reducing influent N load as a possible way to reduce N_2O emissions was previously suggested by Zheng et al. (2015) in the wastewater sector, and Hu et al. (2012) in the aquaculture sector, indicating the possibility of reducing N_2O emissions in the sewage treatment sector under N deficiency.

For P deficiency, reduced P levels can decrease the growth of AOB and NOB since P is necessary for their growth. Consequently, the nitrification process in STPs can be hindered, thereby impacting the nitrification-denitrification process, which can subsequently reduce the emission of N_2O under nutrient deficiency.

5.2. Methane

Methane is mainly formed from the anaerobic fermentation process that takes place in the sewer (Guerrero et al., 2011; Liu et al., 2015). Around 1 % of the chemical oxygen demand in sewage will be converted

Table 4
Summary of the effect of nutrient deficiency on GHG emissions in STPs.

GHG	Emission pathway	Typical criteria needed for emission pathway to occur	How does nutrient deficiency affect the emission pathway?	Can this GHG be reduced under nutrient deficiency?
N_2O	Hydroxylamine oxidation	High DO and high NH_4^+ accumulation	Most N will be assimilated by heterotrophs. Only a minimal amount of N will undergo nitrification.	Highly probable
	Nitrifier denitrification	Low DO & NO_2^- accumulation	The disrupted nitrification process will reduce the likelihood of NO_2^- accumulation. Urine segregation will also reduce the level of NH_4^+ accumulation.	
	Interrupted heterotrophic denitrification	DO shock, pH shock, low copper availability	If most N is assimilated by heterotrophs, the presence of heterotrophic denitrification can be minimized. These criteria should not affect N_2O emissions anymore.	
		Low C/N, insufficient organic carbon for complete denitrification	The high C/N ratio promotes complete denitrification, which is a sink for N_2O generated.	
CH_4	Anaerobic fermentation	Anaerobic condition	CH_4 production can be increased with the increases of C/N, but unsure under nutrient deficiency.	Unsure, more study is required

to CH_4 in the sewer, according to Daelman et al. (2012). CH_4 is subsequently released into the atmosphere either at the exit of the sewer pipe (Guisasola et al., 2008) or emitted during the aeration phase of STPs (Daelman et al., 2012). This is evident in the high CH_4 emissions at the initial stage of the treatment process (Liu et al., 2014). Subsequent production of CH_4 in the primary clarifier is possible (Song et al., 2023) but the aerobic phase of STPs remains a topic of debate.

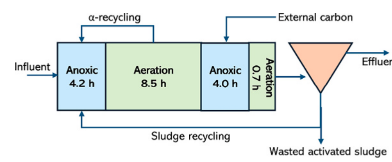
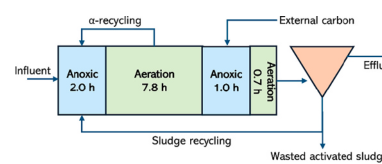
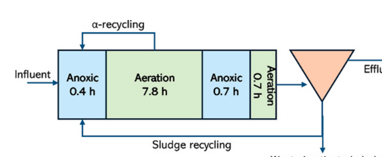
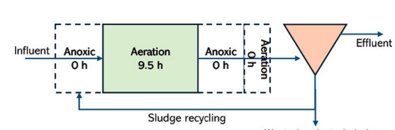
Nitrogen deficiency can increase CH_4 formation under anaerobic conditions. According to Syaichurrozi and Sumardiono (2013), the maximum CH_4 production under C/N 57 was 108 mL/g COD, while CH_4 production under C/N 86 was 139 mL/g COD, corresponding to a 30 % increase in CH_4 production. The authors explained that anaerobic reactors usually have alkaline pH (>9) which can change the speciation of NH_4^+ to NH_3 and inhibit anaerobes, a phenomenon that is more pronounced at high N concentrations. For P deficiency, Boonsawang et al. (2014) showed that an increase in C/P from 100 to 200 can increase CH_4 production by 67 % from 0.036 to 0.167 L/CH_4/$gCOD_{removed}$ (Boonsawang et al., 2014).

To the best of our knowledge, there is no evidence to suggest that CH_4 production and emission in aerobic STPs is affected by nutrient deficiency and relevant studies are rare at this stage. Therefore, further studies are required to clarify this matter and explore the potential influence of nutrient deficiency on CH_4 production in aerobic STPs, as well as in sewers.

6. Impacts urine segregation on the operation of existing sewage treatment plants

The discussion hereafter summarises the impacts of urine segregation at different segregation percentages on the performance and operation of existing STPs, using outcomes from modelling studies (BioWin and Activated Sludge-Anaerobic Digester model) (Freguia et al., 2021; Jimenez et al., 2015) and discussion in previous sections. Subsequently, potential beneficial changes in plant operation resulting from urine segregation are discussed. Only changes involving the operation of STPs are discussed here, whereas infrastructure changes or whole-plant redesigns are not covered. All operating values (e.g., SRT & HRT) were obtained from a single study (Freguia et al., 2021), which modelled STPs using a four-stage Bardenpho configuration in BioWin. The maximum specific growth rate of heterotrophs and nitrifiers was assumed to be constant by the model throughout the urine segregation scenarios. Although nutrient-limited conditions that decrease biomass growth were suggested a few decades ago (Grau, 1991), whether urine segregation approaches the threshold of limiting biomass growth, or at what stage of urine segregation this threshold occurs, remains unknown at

Table 5
Changes in the operational parameters of STPs during various urine segregation percentages. All values for operation parameters were obtained from Freguia et al. (2021). The MLSS concentration in all scenarios was fixed at 3500 mg/L.

Urine segregation	STPs operation parameters		Treatment train after changes (values in each process are the respective HRT)
0 %	• Organic removal: > 95 % • Nitrification-denitrification as primary N removal. • Nitrifier population: 150 mgCOD/L • AOB & NOB growth rate: 0.9 & 0.7 d^{-1} • 600 g/p/d carbon is required for complete denitrification. • Biological P-removal as primary P-removal (not illustrated in the Figure on the righthand side). • PAOs population: 850 mgCOD/L • PAOs growth rate: 0.95 d^{-1} • Filamentous bulking: not likely • Sludge sedimentation: good • Sludge form: floc	**Operation parameters of baseline scenario:** • HRT & SRT: 17 h & 9 days • α-recycling ratio: 7 • Aeration power: 100 % • Pumping power: 100 % • Treatment capacity increases due to HRT and SRT: 0 % • Sludge production rate: 48 kg VSS/EP/d	
25 %	• Organic removal: > 95 % • Nitrification-denitrification as primary N removal. • Nitrifier population: 105 mgCOD/L • AOB & NOB growth rate: 0.9 & 0.7 d^{-1} • 300 g/p/d carbon is required for complete denitrification. • Biological P removal as primary P removal (not illustrated in the Figure on the righthand side). • PAOs population: 600 mgCOD/L • PAOs growth rate: 0.95 d^{-1} • Filamentous bulking: not likely • Sludge sedimentation: good • Sludge form: floc	**Operation parameters if the beneficial changes were made:** • HRT & SRT: 11 h & 6 days • α-recycling ratio: 6 • Aeration power: 91 % • Pumping power: 96 % • Treatment capacity increases due to SRT and HRT: 36 % • Sludge production rate: 49 kg VSS/EP/d	
50 %	• Organic removal: > 95 % • Nitrification-denitrification as primary N removal. • Nitrifier population: 45 mgCOD/L • AOB & NOB growth rate: 0.9 & 0.7 d^{-1} • External carbon is not required for complete denitrification. • Biological P removal as primary P removal (not illustrated in the Figure on the righthand side). • PAOs population: 300 mgCOD/L • PAOs growth rate: 0.95 d^{-1} • Filamentous bulking: not likely • Sludge form: floc • Sludge sedimentation: good • Sludge EPS: starts to decrease	**Operation parameters if the beneficial changes were made:** • HRT & SRT: 10 h & 5 days • α-recycling ratio: 2 • Aeration power: 79 % • Pumping power: 36 % • Treatment capacity increases due to SRT and HRT: 48 % • Sludge production rate: 52 kg VSS/EP/d	
> 75 %	• Organic removal: 5 % - 65 % reduced. • N-assimilation as primary N removal. • Nitrifier population: negligible • AOB & NOB growth rate: 0.9 & 0.7 d^{-1} • External carbon is not required for complete denitrification. • P-assimilation as primary P removal. • PAOs population: negligible • PAOs growth rate: 0.42 d^{-1} • Filamentous bulking: likely • Sludge sedimentation: bad • Sludge disintegration and MLSS loss can happen. • Sludge form: likely to be dispersed.	**Operation parameters if the beneficial changes were made:** • HRT & SRT: 10 h & 5 days • α-recycling ratio: 0 • Aeration power: 73 % • Pumping power: 12 % • Treatment capacity increases due to SRT and HRT: 48 % • Sludge production rate: 52 kg VSS/EP/d	

this time. Therefore, it is reasonable that the modelling studies referred to in this section use the same kinetic constants as in regular sewage.

The baseline scenario (0 % urine segregation) is shown in Table 5. At 25 % urine segregation, organic removal is largely unaffected. Nitrification-denitrification remains the main N-removal process, but the populations of AOB and NOB decrease by 30 % due to reduced NH_4^+ and NO_2^- concentrations, respectively (Jimenez et al., 2015). This offers potential beneficial changes: (1) HRT of primary anoxic and aerobic, and secondary anoxic tanks can be shortened from 4.2 to 2.0 h, 8.5 to 7.8 h and 4 to 1 h, respectively; and (2) recycling streams from aerobic to anoxic tanks (α-recycling) could be reduced from 7 to 6 due to lower NO_3^- aerobic effluent concentration, though the addition of external carbon is still required to achieve complete denitrification in peak scenarios (Jimenez et al., 2015) and (3) the SRT could be reduced from 9 to 6 days.

If the above changes were made, the power requirement of the aerator and pump would be reduced by 9 % and 4 %, respectively. The sludge production at a constant influent rate would increase slightly (1.2 %) due to the increased aerated fraction of the bioreactors. The ratio of HRT to SRT is roughly constant as a consequence of nearly unchanged sludge production, as this analysis was done at constant MLSS concentration. Both HRT and SRT are reduced by ~35 %, leading to a potential increase in treatment capacity, whereby approximately 36 % of additional sewage flow could be treated without modification of the treatment train of the existing STP. P removal in STPs mainly occurs through biological P removal (not illustrated in Table 5, refer to Section 3.3). Based on the analysis of the literature offered above in Sections 4.2.1 to 4.2.2, the flocculation and sedimentation of sludge at this stage can be expected to be as effective as in the baseline scenario, as the C/N/P ratio is still close to municipal sewage. Filamentous proliferation is not likely to occur.

At 50 % urine segregation, the portion of N removed through assimilation is similar to that removed by nitrification-denitrification. The nitrifier population drops by 57 % due to the further decreased NH_4^+ and NO_2^- concentrations. Because a large portion of N is assimilated by biomass, denitrification requirements are reduced. At this stage, the following beneficial changes can be made: (1) The α-recycling ratio can be further decreased to 2 (from 6 at 25 % urine segregation), resulting in a decrease in the NO_3^- to COD ratio in the anoxic tank, along with an increase in COD concentration due to the reduced dilution by the recycle stream, which increases the organic concentration in the anoxic tank (Freguia et al., 2021). This renders the addition of external carbon no longer required for complete denitrification, even at peak scenarios (Jimenez et al., 2015). (2) The HRT of primary and secondary anoxic tanks can be decreased further from 2 h and 1 h at 25 % urine segregation to 0.4 h and 0.7 h, respectively. No change could be made on the primary and secondary aerobic tanks as their HRTs remain unchanged and (3) SRT can be further reduced from 6 to 5 days (Table 5), resulting in further increases in treatment capacity to a total of 48 % from the baseline scenario. Overall, the lowered nitrification-denitrification requirement reduces the power requirement for the aerator and recycle pump to 79 % and 36 % from the baseline scenario, respectively. Sludge production also increases from 49 at 25 % urine segregation to 52 kg VSS/equivalent population (EP)/d as more COD is being oxidised aerobically rather than anoxically.

At urine segregation of >75 %, the organic removal of STPs may be affected by loss of MLSS due to sludge disintegration and the presence of filamentous and non-filamentous bulking (Peng et al., 2003). The effluent suspended solids may also increase due to the poor sludge settleability. The continuous loss of MLSS, combined with low substrates can reduce functional bacteria (such as nitrifiers & PAOs) and the diversity of the microbial community (Yang et al., 2023) but this does not seem to be a concern in urine segregation scenarios since these bacteria are likely to be unviable due to the lack of substrates. Nevertheless, heterotrophs that could survive under low N and P (e.g., GAOs) conditions can be enriched through microbial reshuffling (Yang et al., 2023) but this process could be slow. Hence, the operator of STPs could temporarily retain and recirculate the effluent sludge until the performance of the STPs becomes stable.

At this stage, most N can be removed via N-assimilation. The population of conventional nitrifiers (AOB & NOB) can become negligible due to low bulk NH_4^+ concentration but this might not be the case for AOA and comammox. Production of NO_2^- and NO_3^- from remaining NH_4^+ by AOA and comammox is still possible but the concentration is likely to be very minor. This results in potential beneficial changes: (1) shutting down the α-recycling as no denitrification process is required anymore, and (2) diverting the influent sewage to the primary aerobic tank directly without needing to pass through anoxic tank. However, treatment capacity does not further increase as the process becomes controlled by aerobic oxidation of organics. The SRT remains unchanged and the treatment increase is capped at 48 %. Overall, if the above changes were made, the power requirement of the pump would be reduced to only 12 % of the baseline scenario due to zero α-recycling (only return activated sludge flow). The power requirement of the aerator also decreases to 73 % due to obsolete nitrification.

For STPs that have biological P-removal capability, the population of PAOs becomes negligible due to the low bulk P concentration and the slowed growth rate (Jimenez et al., 2015). The remaining P removal could solely depend on P-assimilation by activated sludge if the influent P concentration is not at a high level (e.g., > 15 mgP/L).

7. Conclusion

The demand for the transition from a linear towards a circular economy has increased considerably in recent years. In the sewage treatment sector, urine source segregation is viewed as an important approach to achieve this, as it would facilitate nutrient recovery from the source-segregated urine and reduce the nutrient load to established STPs. Depending on the extent, high urine segregation can induce nutrient deficiency at STPs and affect operation in different ways. In this review, we showed that urine segregation of below 100 % is unlikely to affect the organic removal in STPs but is possible at 100 % due to the loss of MLSS in the bioreactor. Putative functional bacteria like nitrifiers (e.g., AOB & NOB) and PAOs can be decreased as soon as urine segregation starts. Urine segregation of above 80 % and 100 %, respectively is likely to have these unviable. However, AOA and comammox are likely to survive even under full urine segregation. The requirement of biological N and P removal processes can be shrunk at urine segregation above 80 % and 100 %, respectively. As a result, the HRT and SRT of STPs can reduced and the treatment capacity of STPs can increase by up to 48 % in terms of receiving more sewage without treatment train modification. We also highlighted that sludge flocculation and settleability can deteriorate due to the shift in the EPS components and surface properties of sludge flocs (hydrophobicity and negativity). Different forms of bulking can also be induced by nutrient deficiency. Beneficially, N_2O emissions can be reduced notably since the N load in sewage influent is drastically reduced. Finally, when aerobic becomes the sole process in STPs due to process simplification, sludge production can increase, which could increase CH_4 production during anaerobic digestion for power generation. This would result in reduced dependence on the power grid.

Overall, from the view of the sewage treatment sector, urine source segregation could help us move towards achieving net-zero emissions by reducing the energy consumption for the aeration for nitrification, the chemical dosing for P removal, and the reduction in GHG emissions, particularly N_2O, as outlined in Sustainable Development Goal (SDG) 13 - climate action. The lowered water consumption for toilet flushing under the urine segregation scenario could help achieve SDG 6 – water and sanitation, and the N and P recovered from source-segregated urine could be used for fertiliser production in a circular economy to meet SDG 12 – sustainable consumption and production.

CRediT authorship contribution statement

Chee Xiang Chen: Writing – original draft, Visualization, Formal analysis, Conceptualization. **Veera Koskue:** Writing – review & editing, Validation. **Haoran Duan:** Writing – review & editing, Validation. **Li Gao:** Writing – review & editing. **Ho Kyong Shon:** Writing – review & editing, Funding acquisition. **Gregory J.O. Martin:** Writing – review & editing, Supervision. **George Q. Chen:** Writing – review & editing, Supervision. **Stefano Freguia:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have speared to influence this work.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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